TAXONOMY, PROPAGATION AND CHEMICAL PROPERTIES OF SELECTED ANTICANCEROUS PLANTS OF BANGLADESH.

A
DISSERTATION SUBMITTED
TO
THE UNIVERSITY OF DHAKA
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
BOTANY (PLANT TAXONOMY)

AUGUST 2017

By

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Dedicated
To My
Beloved Parents, Husband
And
Respected Teachers

DECLARATION

I hereby declare that the work presented in this thesis entitled "**Taxonomy, propagation and chemical properties of selected anticancerous plants of Bangladesh.**" is the result of my own investigation. I further declare that this thesis has not been submitted in any previous application for the award of any other academic degree in any university. All sources of information have been specifically acknowledged by referring to the authors.

August, 2017

(Nahid Sultana)
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CERTIFICATE

*This is to certify that the research work presented in this dissertation entitled "**Taxonomy, propagation and chemical properties of selected anticancerous plants of Bangladesh.**" is the outcome of the original work carried out by **Nahid Sultana** in the Plant Taxonomy and Herbal Medicine Laboratory, Department of Botany and the Laboratory of Pharmaceutical Chemistry, Department of Pharmacy, University of Dhaka under our supervision.*

This is further certified that the style and contents of this dissertation is approved for submission in fulfillment of the requirements for the degree of Doctor of Philosophy in Botany (Plant Taxonomy).

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ACKNOWLEDGEMENTS

All praises and gratitude belong to the Almighty "ALLAH" the most merciful, Who enabled me to complete this work.

The author has the honour to acquaint candidly her profound respect and deepest gratitude to her supervisor Professor Dr. Md. Abul Hassan, Department of Botany and co-supervisor Professor Dr. Md. Abdur Rashid, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka for their continuous supervision, encouragement, valuable advice and useful suggestions throughout the progress of the work and also for their constant help in the preparation of the manuscript.

The author is indebted to Dr. Md. Abul Bashar, Chairman, Department of Botany, University of Dhaka to allow me to use the chemicals for my research work.

I am grateful to the Chairman, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka to allow me to work in the Laboratory and utilize instrument facilities.

I express my sincere and heartfelt gratitude to Dr. Md. Oliur Rahman, Professor, Department of Botany, University of Dhaka for his enormous help, suggestions and continuous support during the research period. Sincere thanks are also due to Dr. Momtaz Begum and Dr. Mohammad Zashim Uddin, Professor, Department of Botany, University of Dhaka for their valuable help and suggestions.

I convey my cordial thanks to Ronok Zahan, Assistant Professor, Department of Pharmacy, University of Rajshahi for her enormous help and co-operation.

I express my heartfelt thanks to Faiza Tahia, Lecturer, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka for her help and co-operation in many ways.

I wish to acknowledge Prime Minister's Office for financial support under "Prime Ministers research and higher education supporting fund" to continue my research work.

I can never forget the friendly mentor, Dr. Mohammad Rashedul Haque, Associate Professor, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka. I also grateful to Bushra, Safayat, Rajon, Adib, Sufian and Sajib for their enormous co-operation.

I express my sincere gratitude to Dr. Sheikh Shamimul Alam, Professor and Syeda Sharmeen Sultana, Assistant Professor, Department of Botany, University of Dhaka for their valuable suggestions.

The author is thankful to Dr. Hosne Ara, Director-in-Charge, Bangladesh National Herbarium for allowing to work in the Bangladesh National Herbarium. Special thanks to

Naymur Rahman, Scientific Officer, Bangladesh National Herbarium for his valuable help.

It is my pleasant duty to thank Md. Aman Ullah, Sr. Herbarium Assistant, Department of Botany, University of Dhaka who assisted me in various ways.

Thanks are due to Mirza Zulfikar Ali, Technical Officer, Md. Azam Khan, Sr. Laboratory attendant and Md. Abdus Sobhan Mondal, Laboratory attendant, Department of Botany, University of Dhaka for their assistance and co-operation in various ways.

I wish to express my special thanks to my parents, husband and other members of my family who have been a constant source of inspiration during the course of my research work. It will be unfair, if I do not express my gratitude to my son Muhammad Uzair Morshed who missed me a lot at a time when he needed me most.

The Author

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ABSTRACT

This dissertation explains the identification of the anticancerous plants of Bangladesh and their taxonomic enumeration, propagation experiment of some selected anticancerous plants and chemical and biological investigation of two anticancerous plants of Bangladesh, namely *Aphanamixis polystachya* (Wall.) R. N. Parker and *Oroxylum indicum* (L.) Kurz.

Oroxylum indicum belongs to the family Bignoniaceae is a deciduous tree, characterized by compound leaf, long terminal raceme, zygomorphic flowers, campanulate calyx, 5 stamens, axile placentation, sword-like fruits, winged seeds. *Aphanamixis polystachya* belongs to the family Meliaceae is an evergreen tree characterized by compound leaf, sweet-scented flowers, reddish calyx, waxy petals, 6 stamens, 3-lobed stigmas, obovoid fruits and plano-convex seeds.

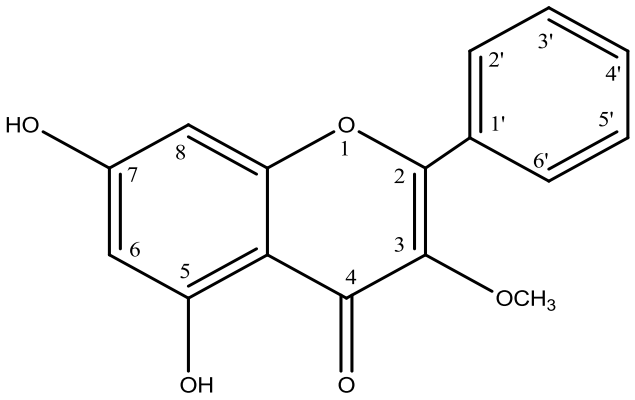
Over 3000 plant species have been used in one way or another for cancer treatment. In Bangladesh, a total of 220 plant species have been identified having anticancerous properties through literature survey.

Propagation experiment of 10 species were carried out. Among them, the highest germination rate (100%) was observed in *Plumbago zeylanica* and the lowest germination rate (10%) was noticed in *Abrus precatorius*. In case of *Vitex trifolia*, seeds were not germinated, however, this plant species was propagated by stem cutting process. Out of the five stem cuttings, all five came out to leafy branches in *Vitex trifolia*.

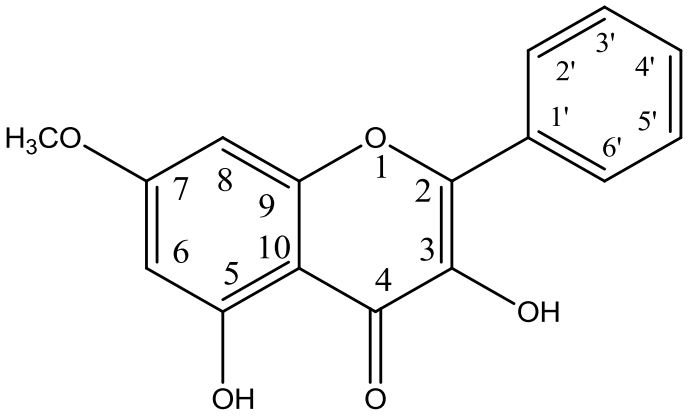
A thorough and systematic study has been carried out in order to isolate compounds having anticancer properties. The study describes the isolation and characterization of compounds from the root bark of *O. indicum*, and bark and leaves of *A. polystachya*. In addition, the crude methanol extract of the root bark of *O. indicum*, and bark and leaves of *A. polystachya* and its different soluble partitionates i.e. petroleum ether, DCM (dichloromethane), chloroform, ethyl acetate and aqueous soluble fractions were obtained by Kupchan partitioning subjected to screenings for anticancer activities.

Chromatographic techniques are the most useful in the isolation and purification of compounds from plant extracts. The advent of relatively new chromatographic media, e.g. Sephadex (Gel filtration chromatography) has improved the resolution of separation. Gel filtration chromatography was carried out using Lipophilic Sephadex LH-20 as stationary phase different composition of solvent system as mobile phase.

Four compounds, *viz.*, 5,7-dihydroxy-3-methoxyflavone (1), 7-methoxy-3,5 dihydroxyflavone (2), 5,7-dihydroxyflavone (Chrysin, 3) and 3,4',5,7-tetrahydroxyflavonol (Kaempferol, 4) from the root bark of *O. indicum* and one compound - Stigmasterol (5) from the leaves of *A. polystachya* were isolated. The structure of the purified compounds were elucidated by extensive analysis of their high resolution ^{1}H spectroscopic data as well as by comparison with published values.



5,7-dihydroxy-3-methoxyflavone (1)



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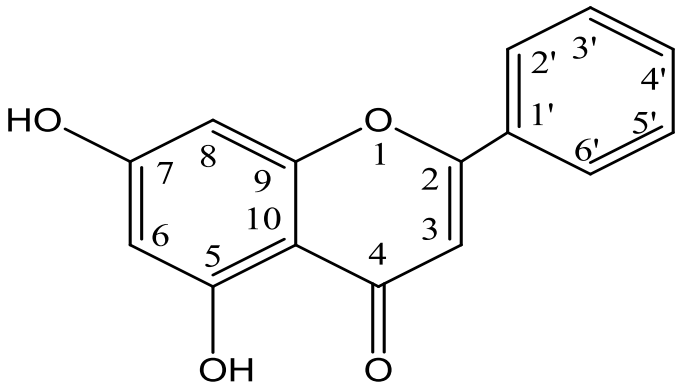

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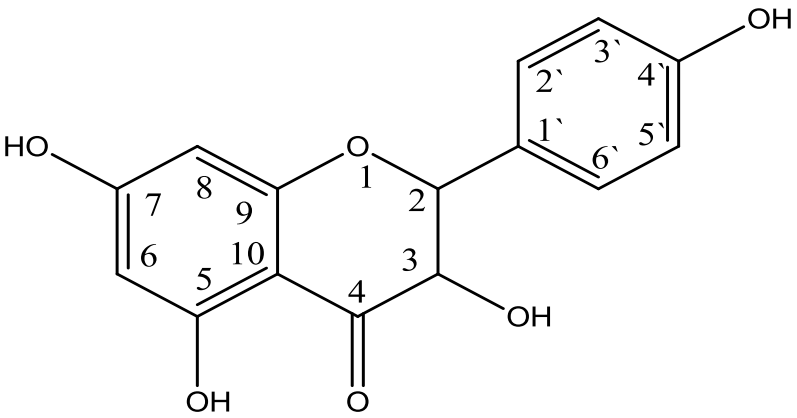
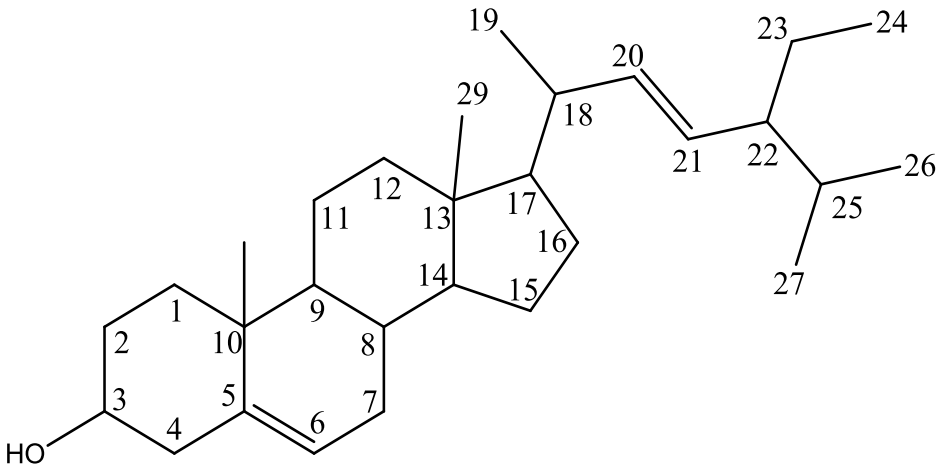


3, 4',5,7-tetrahydroxyflavonol (Kaempferol, 4)



Stigmasterol (5)

The extractives of root bark of *O. indicum* and bark and leaves of *A. polystachya* were subjected to various biological screenings such as anticancer, brine shrimp lethality, antimicrobial and antioxidant activities.

The crude extract and aqueous soluble fraction of the root bark of *O. indicum* and chloroform soluble fraction of bark of *A. polystachya* was found to have significant anticancer activity by a *in vivo* test. Ehrlich's ascites carcinoma cells were inoculated in Swiss Albino mice. Number of viable cells after the administration of plant extracts were counted and compared to that of standard group of bleomycin.

In the brine shrimp lethality bioassay, the highest cytotoxicity have been noticed in the chloroform and dichloromethane soluble fraction for *O. indicum.* In *A. polystachya*, chloroform soluble fraction of bark and petroleum ether soluble fraction of leaves showed highest cytotoxic activity.

In case of microbiological investigation, dichloromethane soluble fraction of *O. indicum* and chloroform soluble fraction of bark and crude methanolic extract of leaves of *A. polystachya* showed maximum zone of inhibition which is 16 mm, 11 mm and 10 mm respectively, while other fractions showed weak to moderate activity.

The antioxidant activity was evaluated in terms of total phenolic content and DPPH free radical scavenging activity. The highest phenolic content was found in crude methanolic extracts (15.75 mg of GAE/gm of extractives) of *O. indicum* and bark of *A. polystachya (*29.25 mg of GAE/gm of extractives) and chloroform soluble fraction of leaves of *A. polystachya* (26 mg of GAE/gm of extractives), respectively.

Significant free radical scavanging activity was showed by crude methanolic extracts in both *O. indicum* (IC_{50} value 9.29 μg/ml) and bark of *A. polystachya* (IC_{50} value 5.36 μg/ml) and chloroform soluble fraction of leaves of *A. polystachya* (IC_{50} value 8.41 μg/ml) respectively .

Therefore, considering the potential bioactivity, these plant materials can further be studied extensively to find out their occult efficacy and to rationalize their uses as traditional medicines.

CHAPTER 1

INTRODUCTION

Take medicines for your ills. The Almighty Allah created no ailment but established for it an antidote, except old age. When the antidote is applied, the patient will recover with Allah's Permission (Al Bukhari, 582). So, we the best creation of Allah, should continue our search for antidote of each and every ailment. In ancient time human being were fully dependent on medicinal plants to treat their diseases, and in modern time we are still largely dependent on medicinal plants for our drug of choice. Perhaps only 20% of our total plant species on the planet have only been screened for their medicinal properties. On the other hand not all the medicines now prescribed are perfectly safe, effective and side effect free. Therefore, there is a need to screen more and more plant species to establish new, potent, effective and safe drugs, especially for **cancer** treatment because plant drugs have less side effects than synthetic drugs. When a patient and a drug come from the same or similar environment, the drug should act more effectively and safely on the patient. As cancer is spreading rapidly among people of all classes and all ages in Bangladesh, search for better anticancer drug from our own plant species should remain continued.

1.1 Cancer

Cancer or malignant neoplasm is a disease where a group of cells show uncontrolled growth (division beyond the normal limits), invasion (intrusion on and destruction of adjacent tissues), and metastasis (spread to other locations in the body through lymph or blood). These three malignant properties of cancers make a distinction them from benign tumours which are self-limited, and do not invade or metastasize. Most cancers form a tumour except leukemia.

1.2 Causes of cancer

The causes of cancer or neoplasm are many. They are summarized below:

1.2.1 Mutation (Chemical carcinogens)

The substances that cause DNA mutations are known as mutagens and mutagens that cause cancers are known as carcinogens. Among them, tobacco smoking causes 90% of lung cancer (Sasco *et al.*, 2004, Biesalski *et al.*, 1998). Tobacco also causes lung, larynx, head, neck, stomach, bladder, kidney, oesophagus and pancreas cancers (Kuper *et al.*, 2002).

Prolonged exposure to asbestos fibers is associated with mesothelioma (O'Reilly *et al.*, 2007).

Many mutagens are also carcinogens, but some carcinogens are not mutagens. Alcohol is such a chemical carcinogen but not a mutagen (Seitz *et al.*, 1998).

1.2.2 Mutation (Ionizing radiation)

Ionizing radiation, such as radon gas can cause cancer. Prolonged exposure to ultraviolet radiation from the sun can cause melanoma and other skin cancers (English *et al.*, 1997). Radio-frequency radiation from mobile phones can also causes cancer, but there is little evidence (Feychting *et al.*, 2005).

1.2.3 Viral or bacterial infection

Some cancers can be caused by infection with pathogens (Pagano *et al.*, 2004). Many cancers create from a viral infection, especially in animals such as birds, but also in humans, as viruses are responsible for 15% of human cancers worldwide. The viruses related with human cancers are human papilloma virus, hepatitis B and hepatitis C virus, Epstein-Barr virus and human T-lymphotropic virus. Hepatitis viruses are responsible for liver cancer. Epstein-Barr virus also known as human herpes virus, causes several types of cancers especially it targets lymphocytes. Human papilloma virus is the source of cervical, anal, oropharyngeal, vaginal, vulvar and penile cancers, and human T-lymphotropic virus is responsible for T-cell leukemia.

Besides virus, bacteria are also responsible for causing cancers. The most prominent example is *Helicobacter pylori* which causes gastric cancer (Peter and Beglinger, 2007).

1.2.4 Hormonal imbalances

Some hormones play a role in the development of cancer by promoting cell proliferation. They are important agents in sex-related cancers, such as cancer of the breast, endometrium, prostate, ovary and testis and also of thyroid cancer and bone cancer (Henderson *et al.*, 2000).

1.2.5 Immune system dysfunction

Immune deficiency conditions are also associated with increased risk of malignancy (Mellemkjaer *et al.*, 2002).

1.2.6 Heredity

Most categories of cancer are non-hereditary. Therefore, a number of recognized syndromes of cancer with a hereditary component, often a defective tumour suppressor allele.

1.2.7 Diet and exercise

30-35% of cancer deaths are associated with diet, physical inactivity and obesity (Kushi *et al.*, 2006). Some specific foods are linked to specific cancers. A high-salt diet is related to gastric cancer. Aflatoxin B1, a frequent food contaminant, causes liver cancer. Betel nut chewing can cause oral cancer (Park *et al.*, 2008).

1.3 Prevention

Cancer prevention is defined as active measures to reduce cancer threat. Between 70% to 90% of common cancers are due to environmental factors and therefore potentially preventable (Wu *et al.,* 2016). More than 30% of cancer deaths could be prevented by avoiding eight risk factors including:

1. Tobacco smoking,
2. Excessive alcohol use,
3. Low diet in fruit and vegetables,
4. Limited physical exercise,
5. Human papilloma virus infection (unsafe sex),
6. Urban air pollution,
7. Domestic use of solid fuels and
8. Contaminated injections (Hepatitis B and C) [Danaei *et al.,* 2005].

1.4 Food and cancer

The National Cancer Institute (NCI-USA) estimates that about one-third of all cancer deaths may be diet related. The following foods have the ability to help prevent cancer and some can even help inhibit cancer cell growth or reduce tumour size (Table 1).

Table 1. Cancer fighting foods.

Name of the plants	Responsible compounds	Types of cancer
1. Carrots	Beta carotene	Lung, mouth, throat, stomach, intestine, bladder, prostate and breast cancer
2. Chili peppers	Capsacin	Stomach cancer
3. Cruciferous vegetables- broccoli, cauliflower, cabbage	Lutein and zeaxanthin	Prostate cancer
4. Flax	Lignan	Block or suppress cancerous changes

Table 1. Contd.

Name of the plants	Responsible compounds	Types of cancer
5. Garlic	Allium compounds (dialyl sulfides)	Increase the activity of immune cells that fight against cancer and indirectly help break down cancer causing substances
6. Oranges and lemons	Limonene	Break down cancer causing substances
7. Grapes	Biflavonoids	Work as cancer preventives
8. Green and yellow leafy vegetables	--	Stomach cancer
9. Mushroom	Polysaccharides, especially lentinan	Help the body fight cancer and build the immune system
10. Nut	Quercetin and kaempferol	Suppress the growth of cancers
11. Papayas	Vitamin C	Reduce absorption of cancer causing nitrosamines
12. Seaweed and sea vegetables	Beta-carotene, protein, vitamin $B_{12,}$ fiber and chlorophyll as well as chlorophylones - important fatty acids	Breast cancer
13. Sweet potatoes	Contain many anticancer properties including beta-carotene	Protect DNA in the cell nucleus from cancer causing chemicals outside the nuclear membrane
14. Teas: green tea and black tea	Polyphenols (catechins)	Prevent cancer cells
15. Tomatoes	Lycopene	Reduced risk of breast, prostate, pancreas and mouth cancer
16. Turmeric	Enzyme cyclo-oxygenase 2 (COX-2)	Colon cancer

Source: https://www. cancure. org/12-links-page/37-cancer-fighting-foods-spices

1.5 Cancer treatment

Cancer can be treated by-

- Surgery
- Chemotherapy
- Radiation therapy
- Immunotherapy
- Monoclonal antibody therapy

The selection of therapy depends upon the location and grade of the tumour and the stage of the disease. Among these treatments, chemotherapy is the main treatment available for spreading malignant diseases. Chemotherapy is the treatment of cancer with drugs that can destroy cancer cells.

1.6 Cancer medicines

The medicines used in the treatment of cancer are depicted in Table 2.

Table 2. Medicines of cancer.

Medicine	Generic name	Brand name	Types of cancer
1. Tamoxifen	Tamoxifen	Soltamox	Breast cancer
2. Carboplatin	Carboplatin	Paraplatin	Ovarian cancer
3. Adriamycin	Doxorubicin	Adriamycin	Breast cancer, bladder cancer, Kaposi's sarcoma, lymphoma, and acute lymphocytic leukemia
4. Cytoxan	Cyclophosphamide	Cytoxan and Neosar	Lymphoma, leukemia, neuroblastoma, ovarian cancer, eye and breast cancer
5. Adrucil	Fluorouracil	Adrucil	Colon, rectum, breast, stomach and pancreas cancer
6. Etoposide	Etoposide	Etopophos and Toposar	Lung cancer and testicular tumours
7. Cosmegen	Dactinomycin	Cosmogen	Wilms' tumour, childhood rhabdomyosarcoma, Ewing's sarcoma and metastatic, nonseminomatous testicular cancer

Table 2. Contd.

Medicine	Generic name	Brand name	Types of cancer
8. Ethyol	Amifostine	Ethyol	Ovarian, head and neck cancer
9. Leukeran	Chlorambucil	Leukeran	Chronic lymphocytic leukemia, Hodgkin's lymphoma, and non-Hodgkin's lymphoma
10. Vincasar	Vincristine	Vincasar PFS	Certain types of cancer (Leukemia, Hodgkin's disease, non-Hodgkin's lymphomas, neuroblastoma)
11. Fludara	Fludarabine	Fludara	Chronic lymphocytic leukemia, non-Hodgkin's lymphoma, acute myeloid leukemia, and acute lymphocytic leukemia
12. Hycamtin	Topotecan	Hycamtin	Ovarian, lung, and cervical cancer
13. Ifex solution	Ifosfamide	Ifex	Testicular cancer
14. Mustargen	Mechlorethamine	Mustargen	Hodgkin's disease (Stages III and IV), lymphosarcoma, chronic myelocytic or chronic lymphocytic leukemia, polycythemia vera, mycosis fungoides, and bronchogenic carcinoma
15. Velban	Vinblastin	Velban	Hodgkin's disease, lymphoma, testicular, breast and uterine cancer
16. Bleomycin	Blenoxane	Bleomycin	Hodgkin's disease, non-Hodgkin's, testicular, ovarian and cervical cancer

Source: https://www.drugs.com/condition/malignant-disease.html

1.7 Cancer medicines from plant source

The cancer medicines which are derived from plant source are given in Table 3.

Table 3. List of the plant drugs to treat cancer (After Bhanot *et al.*, 2011).

Name of the drugs	Name of the plants	Family	Types of cancer
Taxol	*Taxus brevifolia* Nutt.	Taxaceae	Ovarian, breast and lung cancers
Vincristine, vinblastine, vinleurosine, vinrosidine	*Catharanthus roseus* (L.) G. Don	Apocynaceae	Leukemia, lymphoma, breast, lung, pediatric solid cancers, renal cancer
Camptothecin	*Camptotheca acuminate* Decne.	Nyssaceae	Colon, ovarian and lung cancers
Etoposide (epipodophyllotoxin)	*Podophyllum peltatum* L. and *P. emodi* Wall. *ex* Hook. f. & Thomson	Berberidaceae	Small-cell lung carcinoma

Taxus brevifolia, *Camptotheca acuminata* and *Podophyllum peltatum* and *P. emodi* do not grow in Bangladesh. They are not also suitable for cultivation in Bangladesh. *Catharanthus roseus*, though cultivated in gardens as an ornamental herb, not feasible for extraction of the alkaloids. Therefore, our own plants should be tried for novel anticancer drugs.

1.8 Objectives of the present study

The present study has been undertaken in order to achieve the following objectives:

1. Exploring and listing of plants having anticancerous properties available in Bangladesh.
2. Propagation of selected anticancerous plants of Bangladesh.
3. Isolation and characterization of the chemical compounds of *Oroxylum indicum* (L.) Kurz and *Aphanamixis polystachya* (Wall.) R.N. Parker.
4. Investigating anticancerous activity of *Oroxylum indicum* and *Aphanamixis polystachya*.
5. Exploring biological activities including cytotoxicity, antimicrobial investigation and antioxidant properties of *Oroxylum indicum* and *Aphanamixis polystachya*.

CHAPTER 2

ANTICANCER PLANTS OF BANGLADESH AND THEIR TAXONOMIC ENUMERATION

Plants that have therapeutic properties or apply beneficial pharmacological effects on the animal body are known as medicinal plants. Plants, plant parts and plant products, particularly those with medicinal properties, are always used as principal components or ingredients of various traditional medicines. The number of plants with medicinal properties included in the *materia medica* of traditional medicinein this subcontinent at present stands at about 2000 (Chopra *et al.*, 1958). A total of 747 medicinal plants have so far been enlisted as growing in Bangladesh (Yusuf *et al.*, 2009). This number of the indigenous medicinal plants is in the increase with the discovery and introduction of newer plants everyday (Ghani, 2003).

In the 1950s, scientists started scientifically investigating natural organisms as a source of potential anticancer substances (Cragg and Newman, 2005). Plants have been used in medication for their natural uncontaminated properties. Thus, researcher wanted to develop the potential drugs for diseases including cancer from plant extracts. Many plant species are already being used to treat or prevent increase of cancer (Greenwell and Rahman, 2015).

Hartwell (1982) has reported that over 3000 plant species have been used as cancer treatments. In Bangladesh, a total of 220 plant species are identified having anticancerous properties through literature survey. Important anticancerous plants available in Bangladesh are provided below along with their valid name, photographs and synonym (where available), family name, local name (where available), a crisp description, distribution, parts used, active compound and type of the tested cancer cells (where available) and references.

1. **Abrus precatorius** L., Syst. Nat. ed. 12(2): 472 (1767). [Photo 1]
   Synonym: Glycine abrus L. (1753).
   Family: Fabaceae.
   Local name: Kunch.

Description: A perennial climber with slender and tough branches; leaves compound; corolla pink or white; fruits pod; seeds ovoid, scarlet with a black spot at the hilum.
Distribution outside Bangladesh: Cosmopolitan in the tropics, often planted.
Distribution in Bangladesh: It is common throughout the country.
Part used: Seeds.
Active compound: Abrin.
Type of the tested cancer cells: BALB/c and CBA strain mouse spleens.
Reference: Kaufman and McPherson, 1975.

2. **Acacia catechu** (L. f.) Willd., Sp. Pl. 4: 1079 (1806).
Synonym: Mimosa catechu L. f. (1782).
Family: Mimosaceae.
Local names: Khair, Khair Babul.
Description: A medium-sized, deciduous tree; bark reddish-brown; leaves compound; flowers creamy-white; fruit a pod, dark chocolate-brown to reddish-brown or blackish when dry; seeds 3-10 per pod.
Distribution outside Bangladesh: Sub-Himalayan tracks from Punjab to Sikkim, Sri Lanka, Pakistan to South East Asia, including Myanmar and Southern China.
Distribution in Bangladesh: It is distributed in the northern districts especially in Rajshahi and Pabna.
Part used: Seeds.
Active compound: Lectin.
Type of the tested cancer cells: Leukocytes and mononuclear cells.
Reference: Agarwal and Agarwal, 1990.

3. **Acacia nilotica** (L.) Delile subsp. **indica** (Benth.) Brenan, Kew Bull. 12: 84 (1957). [Photo 2]
Synonyms: Mimosa nilotica L. (1753), *Acacia arabica* (Lamk.) Willd. (1806).
Family: Mimosaceae.
Local name: Babla.
Description: A tree; leaves pinnately compound; flowers bright yellow, fragrant; fruits strap-shaped; seeds black.
Distribution outside Bangladesh: India, Pakistan, Sri Lanka, Nepal, Bhutan, Egypt, Arabia, tropical Africa and Indonesia, cultivated in Malaysia.
Distribution in Bangladesh: It is planted especially in the northern districts.
Parts used: Aerial parts.
Active compound: Gamma-sitosterol.

Type of the tested cancer cells: Dalton's ascitic lymphoma induced solid and ascitic tumour model.
Reference: Velusamy *et al.*, 2016.

4. **Acalypha indica** L., Sp. Pl. 2: 1003 (1753). [Photo 3]
Synonym: Ricinocarpus indicus O. Kuntze (1891).
Family: Euphorbiaceae.
Local name: Muktajhuri.
Description: A small herb; leaves long petiolate; inflorescence spicate, axillary; fruits trilobed; seeds grey, with a whitish hilum.
Distribution outside Bangladesh: Throughout the hotter parts of India, Sri Lanka, Africa and the Philippines.
Distribution in Bangladesh: It is found throughout the country.
Parts used: Aerial parts.
Active compound: L-quebrachitol.
Type of the tested cancer cells: NCIH187-small cell lung cancer.
Reference: Sanseera *et al.*, 2012.

5. **Acanthus ilicifolius** L., Sp. Pl. 2: 639 (1753). [Photo 4]
Synonym: Dilivaria ilicifolia (L.) Juss. (1789).
Family: Acanthacease.
Local names: Hargoza, Harkuch-kanta.
Description: A mangrove shrub or sub-shrub; leaves spini-tipped; corolla bluish-purple; fruit a capsule; seeds 4 per capsule, softly wrinkled.
Distribution outside Bangladesh: Indian Peninsula, Pakistan, Sri Lanka, Malaysia, the Philippines, Australia, and the adjoining areas.
Distribution in Bangladesh: Satkhira, Khulna, Bagerhat, Pirojpur, Barguna, Patuakhali, Bhola, Noakhali, Chittagong and Cox's Bazar districts.
Parts used: Root and leaves.
Type of the tested cancer cells: MCF-7 and PA-1 cell lines.
Reference: Smitha *et al.*, 2014.

6. **Achyranthes aspera** L., Sp. Pl. 1: 204 (1753). [Photo 5]
Synonyms: Cyathula geniculata Lour. (1790), *Achyranthes aspera* L. var. *rubro-fusca* Wight (1852).
Family: Amaranthaceae.
Local names: Apang, Bilaikhamchi, Upatlengra.
Description: A herb or undershrub; inflorescence terminal and lateral spike; flowers greenish; seeds black.
Distribution outside Bangladesh: In tropical and warmer regions of the World.
Distribution in Bangladesh: This plant grows wild in all parts of the country.

Part used: Leaves.
Type of the tested cancer cells: Pancreatic cancer cell lines.
Reference: Nataru *et al.*, 2014.

7. **Acorus calamus** L., Sp. Pl.: 324 (1753). [Photo 6]
Synonyms: Acorus calamus var. *vulnaris* L. (1753), *Acorus calamus* var. *verus* L. (1753).
Family: Araceae.
Local names: Bach, Mithabach.
Description: Perennial herb, rootstock stout; leaves linear; inflorescence on a leaf-like peduncle; perianth oblong; fruit a berry; seeds obconical.
Distribution outside Bangladesh: North and Central America, Europe and Asia.
Distribution in Bangladesh: Rajshahi, Chittagong and Cox's Bazar districts, and also planted in many gardens.
Parts used: Whole plant.
Reference: Funde, 2015.

8. **Aeginetia indica** L., Sp. Pl.: 632 (1753).
Synonym: Orobanche aeginetia L. (1753).
Family: Orobanchaceae.
Description: A leafless herb; scape purplish-red; flower solitary; corolla purple with fine darker venation; fruit a partially 2-valved capsule; seeds numerous, yellowish-white.
Distribution outside Bangladesh: India, Nepal, Sri Lanka, Myanmar, China, Japan and the Philippines.
Distribution in Bangladesh: Chittagong, Dhaka and Mymensingh districts.
Part used: Seeds.
Active compound: 55 kDa protein.
Type of the tested cancer cells: Syngeneic Meth-A tumour bearing BALB/c mice.
Reference: Ohe *et al.*, 2001.

9. **Agave americana** L., Sp. Pl.: 323 (1753). [Photo 7]
Family: Agavaceae.
Local names: Shatabdi Udvid, Shatabarshi Udvid.
Description: A herb; flowers pale yellow; perianth tube funnel-shaped; seeds black.
Distribution outside Bangladesh: A native of Mexico, naturalized in the Mediterranean region, India and Pakistan.

Distribution in Bangladesh: This plant is cultivated in gardens throughout the country.
Part used: Leaves.
Type of the tested cancer cells: Human cell line of ovarian teratocarcinoma.
Reference: Manoharan and Kaur, 2013.

10. **Ageratum conyzoides** L., Sp. Pl.: 839 (1753). [Photo 8]
Family: Asteraceae.
Local names: Ochunti, Fulkuri.
Description: An annual aromatic herb; inflorescence capitulum; corolla white, light pink or whitish-blue; cypsela oblong.
Distribution outside Bangladesh: A native of South America, now widely spread throughout warm countries of the world.
Distribution in Bangladesh: It is a common weed of waste places, especially in moist situations.
Parts used: Whole plant.
Type of the tested cancer cells: Leukemic (Jurkat), prostate (LNCap), breast (MCF-7) and normal prostate (PNT2) cell lines.
Reference: Acheampong *et al.*, 2015.

11. **Alangium salviifolium** (L. f.) Wangerin in Engl., Pflanzenr. 4 (220b): 9 (1910). [Photo 9]
Synonyms: Grewia salvifolia L. f. (1781), *Alangium decapetalum* Lamk. (1783).
Family: Alangiaceae.
Local names: Ankora, Akarkanta.
Description: A small to medium-sized tree; flowers white, sweet-scented; corolla reflexed; fruits red or black when ripe.
Distribution outside Bangladesh: Africa, India, Sri Lanka, China, Thailand, the Philippines and Malaysia.
Distribution in Bangladesh: It is common in Dhaka and the adjoining areas and also in Chittagong forest areas.
Parts used: Stem and leaves.
Type of the tested cancer cells: Dalton's ascitic lymphoma.
Reference: Nataru *et al.*, 2014.

12. **Albizia lebbeck** (L.) Benth. & Hook., Lond. J. Bot. 3: 87 (1844). [Photo 10]
Synonyms: Mimosa lebbeck L. (1753), *Mimosa sirisa* Roxb. (1832).
Family: Mimosaceae.
Local names: Kala-koroi, Siris.

Description: A large deciduous tree; bark brownish-grey or sometimes almost black; inflorescence yellowish-brown, pubescent; leaves bipinnately compound; flowers greenish to yellowish-white; fruits strap-shaped; seeds 6-12 per pod, light brown.
Distribution outside Bangladesh: Native to tropical Asia, Africa and northern Australia and one of the best known trees of India, Myanmar, Pakistan, Sri Lanka, China and Malaysia.
Distribution in Bangladesh: This species occurs in most parts of the country.
Part used: Leaves.
Type of the tested cancer cells: Dalton's lymphoma ascites (DLA) bearing mice.
Reference: Padamanabhan *et al.*, 2016.

13. **Allamanda cathartica** L., Mant. Pl. 2: 214 (1771). [Photo 11]
Family: Apocynaceae.
Local names: Harkakra, Alakanada, Kalkephul.
Description: An evergreen, scandent shrub; leaves 3-5 in a whorl; flowers bright yellow; corolla funnel-shaped; fruit a spiny capsule.
Distribution outside Bangladesh: A native of tropical America, largely grown as ornamental and sometimes naturalized in the most zone including India, Myanmar, Nepal, Pakistan and Sri Lanka.
Distribution in Bangladesh: It is found throughout the country.
Part used: Root.
Active compound: Allamandin.
Reference: Evans, 2009.

14. **Allium sativum** L., Sp. Pl. 1: 297 (1753).
Family: Liliaceae.
Local name: Rashun.
Description: An erect herb, aerial pseudo-stem is formed by sheathing leaf bases; leaves simple; inflorescence umbel; fruits seedless.
Distribution outside Bangladesh: Garlic is believed to have been originated from Central Asia (Tien Shan) where its wild ancestor *A. longicuspis* Regd is endemic.
Distribution in Bangladesh: It is cultivated throughout the country during the winter.
Part used: Aged garlic extract.
Active compound: S-allylmercaptocysteine.
Type of the tested cancer cells: MBT2 murine bladder carcinoma model.
Reference: Riggs *et al.*, 1997.

15. **Aloe vera** (L.) Burm. f., Fl. Ind.: 83 (1768). [Photo 12]
Synonyms: Aloe perfoliata var. *vera* L. (1753), *Aloe barbadensis* Miller (1768).
Family: Aloaceae.
Local names: Ghritakanchan, Ghritakumari, Musabbar.
Description: A xerophytic herb, succulent; perianth orange or red; flowering and fruiting scarce.
Distribution outside Bangladesh: Tropics and subtropics. Nowadays, it is cultivated commercially in the United States, Mexico, the Caribbean, Middle East, Australia, Thailand and south Kalimantan.
Distribution in Bangladesh: It is cultivated commercially in the northern districts and also in many gardens as an ornamental and medicinal plant.
Part used: Leaves.
Active compound: Aloe-emodin.
Reference: Nataru *et al.*, 2014.

16. **Alpinia galanga** (L.) Sw., Obs. Bot.: 6 (1791).
Synonyms: Maranta galanga L. (1762), *Languas vulgare* Koen. (1783).
Family: Zingiberaceae.
Local names: Kulinjan, Kulanjan.
*Description:*A tall perennial rhizomatous herb; stem leafy; flowers fragrant; petals pale green; fruits yellowish-green to orange-red on ripening.
Distribution outside Bangladesh: India, Indo-China, Indonesia, Malaysia, the Philippines and Sri Lanka.
Distribution in Bangladesh: Mostly in greater Sylhet district. Also planted in Dhaka University Botanic Garden.
Part used: Root.
Type of the tested cancer cells: Adenocarcinoma of human prostate cell line.
Reference: Nataru *et al.*, 2014.

17. **Alstonia macrophylla** Wall. *ex* G. Don, Gen. Syst. 4: 87 (1837)
Synonyms: Alstonia costata Wall. (1829), *Alstonia acuminata* Miq. (1869).
Family: Apocynaceae.
Local name: Chhatim.
Description: A tall tree; leaves 3-4 in a whorl; flowers white; follicles pendulous.
Distribution outside Bangladesh: Cambodia, China, India, Indonesia, Malaysia, New Guinea, the Philippines and Thailand.
Distribution in Bangladesh: It is introduced as a forest tree.
Part used: Bark.
Active compound: Indole alkaloids.

Type of the tested cancer cells: Two human lung cancer cell lines, MOR-P (adenocarcinoma) and COR-123 (large cell carcinoma).
Reference: Keawpradub *et al.,* 1999.

18. **Alstonia scholaris** (L.) R. Br., Mem. Wern. Nat. Hist. Soc. 1: 76 (1811). [Photo 13]
Synonyms: Echites scholaris L. (1767), *Nerium tinctorium* Perr. (1824).
Family: Apocynaceae.
Local names: Chhatim, Chaitan.
Description: A tall tree; leaves 5-10 in a whorl; flowers greenish-white; follicles pendulous; seeds obtuse at both ends.
Distribution outside Bangladesh: India, Sri Lanka, Southern China, Malesia, Queensland and the Solomon Islands.
Distribution in Bangladesh: It is cultivated throughout the country, and occurs naturally in the forests in eastern parts of the country.
Part used: Leaves.
Active compound: Alkaloids and triterpenes.
Type of the tested cancer cells: A549 cell line.
Reference: Feng *et al.*, 2013.

19. **Amaranthus spinosus** L., Sp. Pl. 1: 991 (1753). [Photo 14]
Family: Amaranthaceae.
Local names: Kanta-nutia, Kanta Miris, Khuira Kanta.
Description: A spinescent herb; inflorescence usually green; fruits rugose; seeds black.
Distribution outside Bangladesh: Throughout India, Sri Lanka and common in all tropical countries of the world.
Distribution in Bangladesh: This plant grows wild in all parts of the country.
Part used: Leaves.
Type of the tested cancer cells: Ehrlich ascites carcinoma in Swiss albino mice.
Reference: Nataru *et al.,* 2014.

20. **Amaranthus tricolor** L., Sp. Pl. 1: 989 (1753). [Photo 15]
Synonyms: Amarnthus tristis L. (1753), *Amarnathus melanchlicus* L. (1753), *Amaranthus polygamus* L. (1755), *Amaranthus gangeticus* L. (1759).
Family: Amaranthaceae.
Local names: Kankanotey, Denga, Lal Sak.
Description: A herb; stem stout, branches angular; leaves green or variably purplish; inflorescence green to crimson; seeds brown.

Distribution outside Bangladesh: Widespread in the tropics of both Old and New Worlds.
Distribution in Bangladesh: It is found as a weed in northern and central parts of the country.
Parts used: Stem and leaves.
Active compound: Galactosyl diacylglycerols 1-3.
Type of the tested cancer cells: Human AGS (gastric), CNS (central nervous system; SF-268), HCT-116 (colon), NCI-H460 (lung), and MCF-7 (breast) cancer cell lines.
Reference: Nataru *et al.*, 2014.

21. **Ananas comosus** (L.) Merr., Interpr. Herb. Amboin.: 133 (1917). [Photo 16]
Synonyms: Bromelia comosa L. (1754), *Ananas sativus* (Lindl.) Schult. f. (1830).
Family: Bromeliaceae.
Local name: Anarash.
Description: A herb; leaves spiny, parallel-veined; inflorescence a terminal spike having a cone like bunch of bluish flowers.
Distribution outside Bangladesh: A native of tropical and sub-tropical America. It is now also introduced in other tropical countries, and is extensively cultivated in Hawaii, Mexico, Cuba, the West Indies, Formosa, Sri Lanka, India, China, Taiwan, the Philippines, Singapore and Queensland (Australia).
Distribution in Bangladesh: It is widely cultivated in greater Sylhet and the Chittagong Hill Tracts and the Madhupur forests in Tangail and Mymensingh districts.
Part used: Stem.
Active compound: Bromelain.
Type of the tested cancer cells: P-388 leukaemia, sarcoma (S-37), Ehrlich ascitic tumour (EAT), Lewis lung carcinoma (LLC), MB-F10 melanoma and ADC-755 mammary adenocarcinoma.
Reference: Nataru *et al.*, 2014.

22. **Andrographis paniculata** (Burm. f.) Wall. *ex* Nees in Wall., Pl. As. Rar. 3: 116 (1832). [Photo 17]
Synonym: Justicia paniculata Burm. f. (1768).
Family: Acanthaceae.
Local names: Kalomegh, Kalmegh.
Description: An erect or sometimes suberect, annual herb; corolla white or pale with deep pink or deep purplish-violet markings inside at the base of the lower lip, upper lip notched; seeds yellowish-brown.

Distribution outside Bangladesh: India, Sri Lanka and the West Indies.
Distribution in Bangladesh: It is found throughout the country, particularly in cultivated form.
Part used: Leaves.
Type of the tested cancer cells: HeLa cell line.
Reference: Ali *et al.,* 1996.

23. **Anisomeles indica** (L.) O. Kuntze, Rev. Gen.: 512 (1891). [Photo 18]
Synonyms: Nepeta indica L. (1753), *Anisomeles ovata* R. Br. (1811).
Family: Lamiaceae.
Local name: Gobura.
Description: An aromatic herb; stem tetrangular; corolla pink; nutlets obovate with a triangular white marking at the base.
Distribution outside Bangladesh: India, Sri Lanka to Malay Peninsula, Archipelago, China and the Philippines.
Distribution in Bangladesh: It is common in most of the districts.
Part used: Leaves.
Active compound: Apigenin, ovatodiolide, β-sitosterol and acteoside.
Type of the tested cancer cells: 12-O-tetradecanoylphorbol-13-acetate (TPA) induced human breast adenocarcinoma MCF-7 cells.
Reference: Nataru *et al.,* 2014.

24. **Annona muricata** L., Sp. Pl.: 536 (1753). [Photo 19]
Family: Annonaceae.
Local name: Muri-ata.
Description: A small tree; petals thick, cordate; fruits dark green, spines curved and fleshy.
Distribution outside Bangladesh: A native of tropical America, but is now grown in most tropical countries.
Distribution in Bangladesh: It is grown in home gardens.
Part used: Leaves.
Type of the tested cancer cells: 7,12-dimethylbenza(α) anthracene/Croton induced skin papillomagenesis.
Reference: Nataru *et al.,* 2014.

25. **Annona reticulata** L., Sp. Pl.: 537 (1753). [Photo 20]
Family: Annonaceae.
Local names: Nona, Nona-ata, Ata.
Description: A small tree; petals arranged in 2 series, outer petals triqetrous, inner petals minute or absent; fruits reddish-brown when ripe; seeds black.

Distribution outside Bangladesh: Widely cultivated in Old and New World tropics.
Distribution in Bangladesh: It is a common homestead fruit plant and found all over the country.
Part used: Root.
Type of the tested cancer cells: A-549 (Human lung carcinoma), K-562 (Human chronic myelogenous leukemia bone marrow), HeLa (Human cervix) and MDA-MB (Human adenocarcinoma mammary gland) cancer cell lines.
Reference: Nataru *et al.*, 2014.

26. **Annona squamosa** L., Sp. Pl.: 537 (1753). [Photo 21]
Family: Annonaceae.
Local names: Sharifa, Sitaphal.
Description: A small tree; petals pale yellow with deep purple spot inside at the base; fruits yellowish-green; seeds dark brown to black.
Distribution outside Bangladesh: The species is widely distributed throughout tropical South America. It is also grown in Thailand, the Philippines, Malaysia and the Indian subcontinent.
Distribution in Bangladesh: It is cultivated all over the country.
Part used: Seeds.
Active compound: Acetogenin.
Type of the tested cancer cells: Histiocytic tumour cell line, AK-5 in rat.
Reference: Pardhasaradhi *et al.*, 2004.

27. **Aphanamixis polystachya** (Wall.) R. N. Parker, Ind. For. 57: 486 (1931). [Photo 22]
Synonyms: Sphaerosacme polystachya Wall. (1829), *Aphanamixis timorensis* A. Juss. (1830), *Amoora rohituka* (Roxb.) Wight & Arn. (1833), *Amoora timorensis* (A. Juss.) Wight & Arn. *ex* Steud. (1840).
Family: Meliaceae.
Local names: Pitraj, Royna, Tiktaraj.
Description: A medium-sized evergreen tree; flowers sweet-scented; petals cream to yellow or bronze, sometimes tinged red; fruits yellowish at first, pink or red at maturity.
Distribution outside Bangladesh: Sri Lanka, India, Nepal, Pakistan, Bhutan and Myanmar.
Distribution in Bangladesh: Chittagong, Cox's Bazar, Gazipur, Mymensingh, Sherpur, Tangail and Sylhet districts, and the Chittagong Hill Tracts.
Part used: Stem bark.
Active compound: Amooranin.

Type of the tested cancer cells: Human colon carcinoma cell line.
Reference: Cheppail *et al.*, 2006.

28. **Aquilaria agallocha** Roxb., Fl. Ind. 2: 422 (1820).
Family: Thymelaeaceae.
Local name: Agar.
Description: A large evergreen tree; bark whitish; flowers campanulate, densely villous inside.
Distribution outside Bangladesh: North East hilly regions of India.
Distribution in Bangladesh: The plants grow in Maulvi Bazar forests, cultivated occasionally by the Forest Department as well as by private owners in Sylhet region.
Part used: Stem bark.
Active compound: 1,3-dibehenyl-2-ferulyl glyceride and 12-0-n-deca-2,4,6-trienoylphorbol-13-acetate.
Type of the tested cancer cells: Eagles' carcinoma of the nasopharynx (KB) and P388 lymphocytic leukemia.
Reference: Gunasekera *et al.*, 1981.

29. **Arachis hypogaea** L., Sp. Pl. 2: 741 (1753). [Photo 23]
Family: Fabaceae.
Local names: Cheena Badam, Badam.
Description: A trailing or prostrate hairy herb; petals yellow; fruit a pod, torulose but not jointed.
Distribution outside Bangladesh: Originated in South America in very ancient time. Now found in all tropical and subtropical countries.
Distribution in Bangladesh: It is cultivated in many places especially in *Char* areas of the country, as Rabi and Kharif crop.
Parts used: Leaves and seeds.
Active compound: Resveratrol.
Type of the tested cancer cells: Mouse xenograft models of human neuroblastoma and human colorectal cancer cells.
Reference: Velusamy *et al.*, 2016.

30. **Argemone mexicana** L., Sp. Pl.: 508 (1753). [Photo 24]
Family: Papaveraceae.
Local names: Baroshialkanta, Shialkanta, Siakanta.
Description: A glabrous, glaucous, robust herb with yellow juice; leaves thistle-like, spinous; flowers yellow; seeds globose, netted.

Distribution outside Bangladesh: Indigenous to tropical America, Mexico and the West Indies; naturalized throughout Indian subcontinent.
Distribution in Bangladesh: This plant is available in most of the districts in fallow lands.
Part used: Seeds.
Type of the tested cancer cells: Cervix cancer (ME180, SiHa), leukemia (HL60, K562), lung cancer (A549), breast cancer (MCF7, MDA-MB-468), prostate cancer (PC3, DU145), hepatoma (HEP G2), colon cancer (HT29, Colo205), ovarian cancer (A2780, Ovkar-3) and oral cancer cell lines (AW13516).
Reference: Patil *et al.*, 2014a.

31. **Argyreia nervosa** (Burm. f.) Boj., Hort. Maurit.: 224 (1837). [Photo 25]
Synonyms: Convolvulus nervosus Burm. f. (1768). *Argyreia speciosa* Sweet (1827).
Family: Convolvulaceae.
Local names: Bara Dudhi, Hris Gandha.
Description: A large twiner containing viscid milky juice; stem densely whitish or fulvous-pubescent; corolla pinkish-purple to lavender with darker throat; fruits yellowish-brown; seeds brownish.
Distribution outside Bangladesh: India and Myanmar.
Distribution in Bangladesh: Dhaka, Noakhali, Patuakhali and Sylhet districts.
Part used: Leaves.
Type of the tested cancer cells: Ehrlich ascites carcinoma.
Reference: Sharma *et al.*, 2015.

32. **Aristolochia indica** L., Sp. Pl.: 960 (1753). [Photo 26]
Synonym: Aristolochia lanceolata Wight (1858).
Family: Aristolochiaceae.
Local name: Ishwarmul.
Description: A twinner, woody at the base; flowers irregular; perianth ovoid; seeds deltoid.
Distribution outside Bangladesh: India, Nepal and Sri Lanka.
Distribution in Bangladesh: Dhaka, Tangail, Mymensingh, Rajshahi districts and in the Sundarbans.
Part used: Whole plant.
Active compound: Aristolochic acid.
Type of the tested cancer cells: 4-nitroquinoline 1-oxide induced oral cancer in Albino rats.
Reference: Nataru *et al.*, 2014.

33. **Artabotrys hexapetalus** (L. f.) Bhandari, Baileya 12: 149 (1965). [Photo 27]
Synonyms: Annona hexapetala L. f. (1781), *Artabotrys odoratissima* R. Br. *ex* Ker-Gawl. (1820), *Uvaria odoratissima* Roxb. (1832).
Family: Annonaceae.
Local name: Kanthali Champa.
Description: A large evergreen shrub, climbing by the hooked peduncles; petals yellowish-green to bright yellow; ripe carpels yellow and fragrant.
Distribution outside Bangladesh: Native to China, now widely cultivated in the tropics and subtropics.
Distribution in Bangladesh: The plant is widely grown as a homestead tree.
Parts used: Stem, bark and root.
Active compound: Aporphine alkaloids, liriodenine and atherospermidine.
Type of the tested cancer cells: Human KB, A-549 lung carcinoma and HCT-8 colon tumour, murine P-388 and L-1210 lymphocytic leukemia.
Reference: van Valkenburg and Bunyapraphatsara, 2002.

34. **Asclepias curassavica** L., Sp. Pl.: 215 (1753). [Photo 28]
Family: Asclepiadaceae.
Local names: Moricha, Kakturi, Ban Karpas.
Description: An erect perennial herb; corolla bright crimson, corona adnate to the staminal column; pollinia present.
Distribution outside Bangladesh: A native of the West Indies, naturalized and growing wild in the East Asian tropics including Bangladesh, India, Malaysia, Myanmar and Thailand.
Distribution in Bangladesh: It is found in many parts of the country.
Part used: Leaves.
Type of the tested cancer cells: Dalton's lymphoma ascites cells (DLA).
Reference: Aiswarya and Ramana, 2016.

35. **Asparagus racemosus** Wild., Sp. Pl. 2: 152 (1799). [Photo 29]
Family: Liliaceae.
Local name: Shatamuli.
Description: A perennial, slender, scandent shrub with reflexed spines; root tuberous; leaves scale-like, cladode present; flowers white and sweet-scented.
Distribution outside Bangladesh: India, Sri Lanka, Pakistan, Nepal, Bhutan, Malaysia, Australia and tropical Africa.
Distribution in Bangladesh: Sal forests of Dhaka, Gazipur, Mymensingh and Sherpur districts.
Parts used: Leaves and roots.
Active compound: Steroids.

Type of the tested cancer cells: UOK 146 renal cell carcinoma cell line.
Reference: Verma *et al.*, 2014.

36. **Azadirachta indica** A. Juss., Mem. Mus. Hist. Nat. Paris 19: 221, t. 13 (1832). [Photo 30]
Synonym: Melia azadirachta L. (1753).
Family: Meliaceae.
Local name: Neem.
Description: A medium-sized to large evergreen to semi-deciduous tree; leaves imparipinnate; inflorescence fragrant.
Distribution outside Bangladesh: Madagascar, tropical Asia to Australia.
Distribution in Bangladesh: This species is planted and also naturalized throughout the country.
Part used: Leaves.
Type of the tested cancer cells: HeLa cell line.
Reference: Chowdhury *et al.*, 2009.

37. **Bacopa monnieri** (L.) Pennell, Proc. Acad. Nat. Sci. Philadelphia 98: 94 (1946). [Photo 31]
Synonyms: Lysimachia monnieri L. (1756), *Gratiola monnieri* L. (1759), *Herpestis monnieri* Benth. (1835), *Bacopa monnieria* (L.) Wettst. (1891).
Family: Scrophulariaceae.
Local names: Brammi, Brammi Shak.
Description: An annual glabrous herb; stem creeping; corolla white, purple or blue; seeds yellowish-brown.
Distribution outside Bangladesh: Widespread in the tropics and subtropics.
Distribution in Bangladesh: It is found in most of the districts, especially in coastal districts.
Parts used: Whole plant.
Active compound: Saponin and flavonoid.
Type of the tested cancer cells: Cervix (ME180, SiHa), leukemia (HL60, K562), ovarian (A2780, Ovkar-3), breast (MCF-7, MDA-MB-468, MDA-MB-435, MDA-MB-231, ZR-75-1, BT-474), prostate (PC3, DU145), colon (HT29, Colo205), lung (A549), hepatoma (HEPG2) and oral (AW13516) cancer cell lines.
Reference: Patil *et al.*, 2014b.

38. **Bauhinia purpurea** L., Sp. Pl. 1: 375 (1753). [Photo 32]
Synonyms: Bauhinia coromandeliana DC. (1825), *Bauhinia triandra* Roxb. (1832), *Phanera purpurea* (L.) Benth. (1852).
Family: Caesalpiniaceae.

Local names: Devakanchan, Karalli, Gandi, Kanchan, Raktakanchan.
Description: A shrub to medium-sized tree; flowers deep pink or mauve; fruits sword-shaped; seeds brown.
Distribution outside Bangladesh: Bhutan, India, Myanmar, Nepal, Pakistan, Sri Lanka and Thailand.
Distribution in Bangladesh: This species is found almost throughout the country.
Parts used: Whole plant.
Type of the tested cancer cells: Hepatocarcinogenesis in Wistar rats.
Reference: Nataru *et al.*, 2014.

39. **Bauhinia racemosa** Lamk., Encycl. Meth. 1: 390 (1785).
Synonyms: Bauhinia parviflora Vahl (1794), *Piliostigma racemosa* (Lamk.) Benth. (1852).
Family: Caesalpiniaceae.
Local names: Jhinjera, Kosundra, Kanchnal, Banarj, Banraji.
Description: A small tree; leaves reniform; flowers white or fading-yellow; fruits turgid; seeds black.
Distribution outside Bangladesh: Cambodia, China, India, Laos, Malay Peninsula, Pakistan, Sri Lanka, Timor and Vietnam.
Distribution in Bangladesh: Chittagong and Sylhet districts (Khan *et al.*, 1996).
Part used: Stem.
Type of the tested cancer cells: Ehrlich ascites carcinoma.
Reference: Manoharan and Kaur, 2013.

40. **Bauhinia variegata** L., Sp. Pl.: 375 (1753). [Photo 33]
Synonyms: Bauhinia candida Ait. (1789), *Phanera variegata* (L.) Benth. (1852).
Family: Caesalpiniaceae.
Local names: Rakta Kanchon, Lal-kanchon, Vaga-kanchon.
Description: A medium-sized tree; flowers fragrant, purple, pink or white; fruits slightly curved.
Distribution outside Bangladesh: Bhutan, China, India, Myanmar, Nepal, Pakistan and Sri Lanka.
Distribution in Bangladesh: This species is found all over the country.
Part used: Stem.
Type of the tested cancer cells: Liver cancer cell, epithelial larynx cancer and human breast cancer.
Reference: Chanda and Nagani, 2013.

41. **Belamcanda chinensis** (L.) Red., Lilac. 3, t. 121 (1805). [Photo 34]
Synonyms: Ixia chinensis L. (1753), *Pardanthus chinensis* Ker-Gawl. (1805).
Family: Iridaceae.
Description: A herb with creeping rootstock; flowers opening in the forenoon and withering by midday, orange or scarlet spotted; seeds shining black.
Distribution outside Bangladesh: A native of China, cultivated and locally naturalized in many tropical and subtropical countries.
Distribution in Bangladesh: It is cultivated in gardens.
Parts used: Root and rhizome.
Type of the tested cancer cells: PC3, MGC-803, Bcap-37, MCF-7 and HepG2 cell lines.
Reference: Larbie and Abboah-Offei, 2014.

42. **Benincasa hispida** (Thunb.) Cogn. in DC., Monog. Phan. 3: 513 (1881). [Photo 35]
Synonyms: Cucurbita hispida Thunb. (1784), *Benincasa cerifera* Savi (1818).
Family: Cucurbitaceae.
Local name: Chalkumra.
Description: A robust, annual, hispid, climbing herb, tendrils present; male flower yellow, female flower yellow-brown.
Distribution outside Bangladesh: Tropical and subtropical countries of the world. Laos, Vietnam, Cambodia and India are the centres of greatest diversity of this species.
Distribution in Bangladesh: This species is usually occurs throughout the country in kitchen gardens.
Part used: Stem.
Active compound: Flavonoid (mixture of 7,7"-dimethyllanaraflavone and 7"-methylagathisflavone).
Type of the tested cancer cells: HT-29 colon adenocarcinoma, NCl-H460 non-small cell lung carcinoma, MCF-7 breast cancer cell, OVCAR-3 ovarian adenocarcinoma cells, and RXF-393 renal cell carcinoma.
Reference: Pradhan *et al.*, 2009.

43. **Beta vulgaris** L., Sp. Pl. 1: 222 (1753).
Family: Chenopodiaceae.
Local names: Beet, Palak.
Description: A succulent herb; flowers greenish; perianth 5-partite; fruit a nut; seeds kidney-shaped, brown.
Distribution outside Bangladesh: Worldwide.
Distribution in Bangladesh: The plant is sparsely cultivated as a winter crop.

Parts used: Root and leaf juice.
Type of the tested cancer cells: Skin and lung cancer.
Reference: Chanda and Nagani, 2013.

44. **Bidens pilosa** L., Sp. Pl.: 832 (1753).
Synonym: Bidens chinensis auct. *non* Willd., Hook. f. (1881).
Family: Asteraceae.
Description: A herb, leaves imparipinnately compound; ray-florets few, disc-florets yellow; fruits black.
Distribution outside Bangladesh: India, Pakistan and Afghanistan.
Distribution in Bangladesh: It was reported to occur in Bangladesh but no locality was mentioned (Hajra *et al.,* 1995), now occurs under plantation in Dhaka University Botanic Garden, originally collected from Sajek of Hill Tracts.
Parts used: Whole plant.
Type of the tested cancer cells: Cervix carcinoma, nasopharyngeal epidermal carcinoma cancer cell lines.
Reference: Chanda and Nagani, 2013.

45. **Bixa orellana** L., Sp. Pl.: 512 (1753). [Photo 36]
Synonym: Bixa katagensis Delpierre (1790).
Family: Bixaceae.
Local names: Belati Haldi, Latkan.
Description: A large shrub or small tree; petals white; capsules spiny, dark brownish; seeds triangular.
Distribution outside Bangladesh: A native of tropical America, widely naturalized pantropically but not indigenous.
Distribution in Bangladesh: It is found throughout the country.
Part used: Seed.
Type of the tested cancer cells: Radiation induced chromosomal aberration in Swiss albino mice.
Reference: Nataru *et al.,* 2014.

46. **Blumea lacera** (Burm. f.) DC. in Wight., Contrib. Bot. Ind.: 14 (1834). [Photo 37]
Synonym: Conyza lacera Burm. f. (1768).
Family: Asteraceae.
Local names: Barokukshim, Barosaksang, Kukurshunga.
Description: An erect annual herb; inflorescence a capitulum; flowers yellow, pappus white.

Distribution outside Bangladesh: India, Sri Lanka, China, Malaysia, Australia and tropical Africa.
Distribution in Bangladesh: It is common in all parts of the country.
Part used: Leaves.
Active compound: Steroidal glycoalkaloid.
Type of the tested cancer cells: Breast cancer (MCF-7) cell line.
Reference: Akter *et al.*, 2015.

47. **Boerhaavia diffusa** L., Sp. Pl. 1: 3 (1753). [Photo 38]
Synonyms: Boerhaavia repens L. (1753), *Boerhaavia coccinea* Mill. (1768), *Boerhaavia paniculata* Rich. (1792), *Boerhaavia adscendns* Willd. (1797).
Family: Nyctaginaceae.
Local name: Punarnova.
Description: A perennial creeping or climbing herb; flowers umbelliform clusters; perianth campanulate.
Distribution outside Bangladesh: Tropical and subtropical Asia, Africa, America and Australia.
Distribution in Bangladesh: It grows all over the country.
Parts used: Whole plant.
Type of the tested cancer cells: Cervical cancer (SiHa) cell line.
Reference: Venkatajothi, 2017.

48. **Bolboschoenus maritimus** (L.) Palla subsp. **affinis** (Roth) T. Koyama, Brittonia 31: 284 (1979).
Synonyms: Scirpus maritimus L. (1753), *Scirpus affinis* Roth (1817), *Scirpus maritimus* L. var. *affinis* (Roth) C.B. Clarke (1893).
Family: Cyperaceae.
Description: Rhizomatous perennial herb; leaves basal or cauline, blades linear; spikelet pale yellowish to pale brownish.
Distribution outside Bangladesh: Widely distributed all over the tropical and temperate regions of the world.
Distribution in Bangladesh: On the banks of the river Padma and also in the districts of Satkhira, Bagerhat and greater Sylhet districts.
Type of the tested cancer cells: Leukemia cell.
Reference: Hwang *et al.*, 1980.

49. **Butea monosperma** (Lamk.) Taub. in Engl. & Prantl, Nat. Pflanz. 3(3): 366 (1894). [Photo 39]
Synonyms: Erythrina monosperma Lamk. (1788), *Butea frondosa* Roxb. (1795).

Family: Fabaceae.
Local name: Polash.
Description: A small to medium-sized deciduous tree; petals reddish-orange; fruits yellowish-brown when ripe; seeds dark brown.
Distribution outside Bangladesh: India, Pakistan, Sri Lanka, Nepal, Myanmar, Indonesia, Thailand, Indo-China and introduced in New Guinea.
Distribution in Bangladesh: It grows wild in the forests of Dhaka and Mymensingh districts, also planted as an ornamental tree in most of the districts of the country.
Part used: Flowers.
Type of the tested cancer cells: Hepatoma cell line.
Reference: Choedon *et al.*, 2010.

50. **Caesalpinia bonduc** (L.) Roxb., Fl. Ind. 2(2): 362 (1832).
Synonyms: Guilandinia bonduc L. (1753), *Guilandinia bonducella* L. (1762), *Caesalpinia bonducella* (L.) Fleming (1810).
Family: Caesalpiniaceae.
Local names: Nata, Jhagragota, Lalkanta.
Description: A climber or scrambling bush or shrubby tree; leaves paripinnately compound; flowers yellow; fruits covered with sharp prickles.
Distribution outside Bangladesh: China, Hong Kong, India, Malay Peninsula, Myanmar, Nepal, Singapore, Sri Lanka and Taiwan.
Distribution in Bangladesh: This species is found throughout the country.
Type of the tested cancer cells: Carcinoma cell line.
Reference: Nataru *et al.*, 2014.

51. **Caesalpinia pulcherrima** (L.) Swartz, Obs. Bot. Ind. Occ.: 166 (1791). [Photo 40]
Synonym: Poinciana pulcherrima L. (1753).
Family: Caesalpiniaceae.
Local names: Radhachura, Chhoto-krisnachura.
Description: A shrub; flowers orange-yellow, red to rosy-red, center of limb crimson, red or golden-red; fruits pod; seeds brown.
Distribution outside Bangladesh: Native of South America and cultivated throughout the tropical countries.
Distribution in Bangladesh: This species is planted throughout the country.
Part used: Bark.
Type of the tested cancer cells: Head and neck cancer (HNSCC4 and HNSCC31), oral carcinoma (KB) and osteosarcoma (HOS) cell lines.
Reference: Pankaj *et al.*, 2011.

52. **Cajanus cajan** (L.) Millsp., Publ. Field. Mus. Nat. Hist. Bot. Ser. 2: 53 (1900). [Photo 41]
Synonyms: Cytisus cajan L. (1753), *Cajanus indicus* Spreng. (1826).
Family: Fabaceae.
Local names: Arhar, Arual.
Description: A shrub; leaves pinnately trifoliolate; corolla bright yellow with reddish-brown lines; fruits yellow or green, striped with maroon or purplish-black; seeds brown when dry.
Distribution outside Bangladesh: Native of tropical Africa, widely distributed in India, Pakistan, New Guinea and other tropical countries.
Distribution in Bangladesh: It is widely cultivated throughout the country.
Parts used: Root.
Active compound: Beta carotene.
Reference: Velusamy *et al.*, 2016.

53. **Calendula officinalis** L., Sp. Pl.: 921 (1753). [Photo 42]
Family: Asteraceae.
Local name: Calendula.
Description: A herb; ray-florets 2 to many-seriate, female, disc-florets bisexual, many-seriate, corolla of ray-florets yellow.
Distribution outside Bangladesh: A native of southern Europe. It is now cultivated in most countries of the world as an ornamental plant.
Distribution in Bangladesh: It is cultivated in gardens in winter.
Part used: Flowers.
Type of the tested cancer cells: Human and murine tumour cell lines.
Reference: Nataru *et al.*, 2014.

54. **Calophyllum inophyllum** L., Sp. Pl. 1: 513 (1753). [Photo 43]
Synonyms: Balsamaria inophyllum Lour. (1790), *Calophyllum bintagor* Roxb. (1832), *Calophyllum blumei* Wight (1840).
Family: Clusiaceae.
Local names: Kath Champa, Sultan Champa, Punang.
Description: A medium-sized tree; stem angular, exuding golden-yellow latex; flowers white, sweet-scented; fruits yellow or greenish, becomes pale-brown when dry.
Distribution outside Bangladesh: Native of East Africa, distributed in India, Sri Lanka, Malaya, Polynesia, New Caledonia, Madagascar to Australia and the Pacific.

Distribution in Bangladesh: Naturally occurs in Sundarbans regions, sometimes planted here and there as an ornamental plant.
Parts used: Aerial parts.
Active compound: 4-phenyl-coumarins.
Type of the tested cancer cells: Epstein-Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-ace-tate in Raji cells.
Reference: Nataru *et al.*, 2014.

55. **Calotropis gigantea** (L.) R. Br. in Ait. f. Hort. Kew. ed. 2, 2:78 (1811). [Photo 44]
Synonym: Asclepias gigantea L. (1753).
Family: Asclepiadaceae.
Local name: Boro Akand.
Description: A large shrub or small tree; corolla white, lilac or purple, corona present, coronal scales fleshy; pollinia present.
Distribution outside Bangladesh: China, India, Indonesia, Malaysia, Myanmar, Nepal and Thailand.
Distribution in Bangladesh: It is common and occurs throughout the country.
Part used: Leaves.
Active compound: Calotropain.
Type of the tested cancer cells: Human epidermal carcinoma of the nasopharynx.
Reference: van Valkenburg and Bunyapraphatsara, 2002.

56. **Calotropis procera** (Ait.) R. Br. in Ait. f., Hort. Kew. ed. 2, 2: 78 (1811).
Synonyms: Asclepias procera Ait. (1789), *Calotropis hamiltonii* Wight (1834), *Calotropis heterophylla* Wall. *ex* Wight (1834), *Calotropis wallichii* Wight (1834).
Family: Asclepiadaceae.
Local name: Akand.
Description: A large shrub, young branches and leaves beneath covered with a white floccose tomentum; corolla pink above and white at the base, corona and pollinia present.
Distribution outside Bangladesh: Widely distributed in tropical and subtropical Africa, Asia including Middle East, the West Indies and Mascarene Islands.
Distribution in Bangladesh: Common in greater Rajshahi district.
Part used: Leaves.
Active compound: Calotropain.

Type of the tested cancer cells: Human epidermal carcinoma of the nasopharynx.
Reference: van Valkenburg and Bunyapraphatsara, 2002.

57. **Calycopteris floribunda** (Roxb.) Lamk., Enc. Meth. Bot. Suppl. 2: 41 (1811).
Synonym: Getonia floribunda Roxb. (1798).
Family: Combretaceae.
Local name: Guicha Lata.
Description: A diffuse or scandent shrub, with drooping branches, young branchlets rusty villous; flowers yellowish-green; fruits densely villous.
Distribution outside Bangladesh: India, Myanmar, Malaysia and Thailand.
Distribution in Bangladesh: Greater Chittagong and Sylhet districts and the Chittagong Hill Tracts.
Part used: Flowers.
Active compound: Calycopterone (Biflavonoid).
Type of the tested cancer cells: Leukemia, colon cancer, melanoma and renal cancer cell lines.
Reference: Wall *et al.*, 1994.

58. **Cannabis sativa** L., Sp. Pl.: 1027 (1753). [Photo 45]
Synonym: Cannabis indica Lamk. (1783).
Family: Cannabaceae.
Local names: Bhang, Ganja, Siddhi.
Description: An erect, aromatic annual herb; leaves palmately lobed.
Distribution outside Bangladesh: North West Himalayas and Central Asia.
Distribution in Bangladesh: In Naogaon district, once it was cultivated under Government supervision, but the plant grows wild in all districts west of Jamuna (Ahmed *et al.*, 2008).
Parts used: Flowers and leaves.
Active compound: Cannabinoid.
Type of the tested cancer cells: HeLa cervical carcinoma, Lewis lung carcinoma.
Reference: de Padua *et al.*, 1999.

59. **Cardiospermum halicacabum** L., Sp. Pl.: 366 (1753). [Photo 46]
Family: Sapindaceae.
Local names: Phutka, Lataphutki, Kopalphutki, Kanphutki.
Description: A climbing herb; stem deeply 5-sulcate; petals white to creamy with yellowish margin; fruits 3-lobed, green, reddish at the base; seeds black.
Distribution outside Bangladesh: Weed of the tropics and sub-tropics.

Distribution in Bangladesh: The species occurs almost throughout the country.
Part used: Leaves.
Type of the tested cancer cells: Lung A-549 carcinoma cell lines.
Reference: Nataru *et al.*, 2014.

60. **Carthamus tinctorius** L., Sp. Pl.: 830 (1753). [Photo 47]
Family: Asteraceae.
Local names: Kusumphul, Kusum, Kajhira.
Description: A pubescent herb; florets all bisexual, orange-red; fruits 4-angled.
Distribution outside Bangladesh: A native of the Old World.
Distribution in Bangladesh: It is cultivated in many parts of the country.
Parts used: Flowers.
Type of the tested cancer cells: Human colon cancer (SW 620 cell line).
Reference: Nataru *et al.*, 2014.

61. **Cassia fistula** L., Sp. Pl. 1: 377 (1753). [Photo 48]
Synonyms: Cathartocarpus fistula (L.) Pers. (1805), *Cassia rhombifolia* Roxb. (1832).
Family: Caesalpiniaceae.
Local names: Sonali, Sonalu, Bandar Lathi.
Description: A deciduous tree; leaves compound; inflorescence pendulous raceme; flowers bright yellow; fruit a pod, woody.
Distribution outside Bangladesh: Throughout the tropics.
Distribution in Bangladesh: It is a very common ornamental tree, planted along roadsides and gardens for beautification. Occurs naturally in Sal forests of Dhaka, Mymensingh, Dinajpur and Comilla districts.
Part used: Flowers.
Active compound: Rhein.
Type of the tested cancer cells: Colon cancer cell line.
Reference: Duraipandiyan *et al.*, 2012.

62. **Cassia roxburghii** DC., Prodr. 2: 289 (1825).
Synonym: Cassia marginata Roxb. (1832).
Family: Caesalpiniaceae.
Local names: Lal-golapi Sonalu, Lal Cassia.
Description: A small tree, branches drooping; leaves paripinnately compound; flowers deep pink to terracotta-red; fruit a pod.
Distribution outside Bangladesh: India, New Guinea, Pakistan and Sri Lanka.
Distribution in Bangladesh: This species has been recorded from Chittagong and Rangpur districts (Khan *et al.*, 1996).

Part used: Seeds.
Active compound: Lectin.
Type of the tested cancer cells: Leukocytes and mononuclear cells.
Reference: Agarwal and Agarwal, 1990.

63. **Casuarina equisetifolia** Forst., Char. Gen.: 103, t. 52 (1776).
Synonym: Casuarina muricata Roxb. (1832).
Family: Casuarinaceae.
Local name: Jhau.
Description: A spreading tree; flowers unisexual, cones elliptic, form woody bracts enclosing the mature fruits; fruits samaroid.
Distribution outside Bangladesh: Indigenous to the sea coast of New South Wales in Australia, but later introduced in various countries.
Distribution in Bangladesh: Dhaka, Cox's Bazar and Jessore districts.
Part used: Bark.
Reference: Shafiq *et al.*, 2014.

64. **Catharanthus roseus** (L.) G. Don, Gen. Hist. 4: 95 (1837). [Photo 49]
Synonyms: Vinca rosea L. (1759), *Lochnera rosea* (L.) Reichb. (1828).
Family: Apocynaceae.
Local name: Nayantara.
Description: A perennial herb or sub-shrub; flowers white or pink; corolla salver-shaped; fruits follicle.
Distribution outside Bangladesh: A native of Madagascar, widely cultivated and naturalized in the tropics and subtropics of both hemispheres.
Distribution in Bangladesh: It is grown in many gardens as an ornamental plant and also cultivated for medicinal use.
Parts used: Aerial parts.
Active compound: Vincristine and vinblastine.
Type of the tested cancer cells: Breast cancer (MCF) cell line and Ehrlich ascites carcinoma (EAC) tumour model.
Reference: Ruskin and Aruna, 2014.

65. **Cayratia trifolia** (L.) Domin, Biblioth. Bot. 89: 371 (1927). [Photo 50]
Synonyms: Vitis trifolia L. (1753), *Cissus carnosa* Lamk. (1783), *Cissus crenata* Vahl (1794).
Family: Vitaceae.
Local names: Amallat, Amal Lata, Anol Lata.
Description: A climber, tendrils usually branched; leaves pinnately trifoliate; flowers greenish-white; fruits fleshy.
Distribution outside Bangladesh: India, Myanmar and Sri Lanka.
Distribution in Bangladesh: Chittagong, Rangamati and Dhaka districts.

Parts used: Whole plant.
Active compound: Epifriedelanol.
Type of the tested cancer cells: Ovarian cancer cell line.
Reference: Perumal *et al.*, 2016.

66. **Celastrus monospermus** Roxb., Fl. Ind. 2: 394 (1824).
Synonyms: Celastrus championii Benth. (1851), *Celastrus hindsii* Benth. (1851).
Family: Celastraceae.
Description: An evergreen, scandent shrub; flowers white to greenish-white; seeds yellowish to reddish-brown.
Distribution outside Bangladesh: India and China.
Distribution in Bangladesh: The species rarely occurs in the Sylhet district (Kanjilal *et al.*, 1934).
Active compound: Maytenfolone-A.
Type of the tested cancer cells: Hepatoma (HEPA-2B) and nasopharynx carcinoma (KB).
Reference: Kuo and Kuo, 1997.

67. **Centella asiatica** (L.) Urban in Mart., Fl. Braz. 11(1): 187 (1879).[Photo 51]
Synonym: Hydrocotyle asiatica L. (1753).
Family: Apiaceae.
Local name: Thankuni.
Description: A perennial herb; stem creeping; lamina reniform; flowers usually 3; petals white to rose-tinged.
Distribution outside Bangladesh: Tropics and subtropics of the Old and New Worlds.
Distribution in Bangladesh: It grows naturally in all parts of the country.
Part used: Leaves.
Active compound: Asiatic acid.
Type of the tested cancer cells: Benzo(a)pyrene induced lung tumour nodules in mice.
Reference: Hamid *et al.*, 2016.

68. **Centipeda minima** (L.) A. Br. & Aschers., Ind. Sem. Hort. Berol. App.: 6 (1867).
Synonyms: Artemisia minima L. (1753), *Cotula minima* (L.) Willd. (1803).
Family: Asteraceae.
Local names: Machitti, Hachuti, Mechuta, Nakchikni.
Description: A prostrate annual herb; flowers yellowish; fruit cypsela.

Distribution outside Bangladesh: Afghanistan, Australia, Pacific Islands and throughout tropical Western Asia.
Distribution in Bangladesh: Chittagong, Noakhali, Comilla, Sylhet, Sunamganj and Jessore districts, and the Chittagong Hill Tracts.
Parts used: Aerial parts.
Active compound: 2β-(Isobutyryloxy) florilenalin (a sesquiterpene lactone).
Type of the tested cancer cells: Human nasopharyngeal carcinoma CNE cell line.
Reference: Su *et al.*, 2009.

69. **Cerbera odollam** Gaertn., Fruct. 2: 193. t. 124 (1791). [Photo 52]
Synonyms: Cerbera manghas L. (1753), *Tanghinia odollam* (Gaertn.) G. Don (1837).
Family: Apocynaceae.
Local names: Dabur, Dhakur.
Description: A small or medium-sized tree; corolla with a yellow eye; fruit green.
Distribution outside Bangladesh: The coasts of Sri Lanka, India, Malaysia and Indonesia.
Distribution in Bangladesh: The coasts of Chittagong and Cox's Bazar districts.
Part used: Leaves.
Active compound: 17 βH-neriifolin.
Type of the tested cancer cells: Two breast cancer cell lines (T47D and MCF7), two ovarian cancer cell lines (SKOV3 and CaOV3) and a normal (Vero) cell line.
Reference: Syarifah *et al.*, 2011.

70. **Cestrum nocturnum** L., Sp. Pl.: 191 (1753). [Photo 53]
Family: Solanaceae.
Local name: Hasna-hena.
Description: A branched shrub; flowers greenish-white, night blooming, sweet-scented; fruit a spongy berry; seeds boat-shaped, black.
Distribution outside Bangladesh: A native of West Indies.
Distribution in Bangladesh: It is planted throughout the country.
Part used: Leaves.
Active compound: Flavonol glycosides and steroidal saponins.
Type of the tested cancer cells: Human oral squamous cell carcinoma (HSC-2) cells and normal human gingival fibroblasts.
Reference: Mimaki *et al.*, 2001.

71. **Chylocalyx perfoliatus** (L.) Hassk. *ex* Miq., Fl. Ned. Ind. 1(1): 1012 (1858). [Photo 54]

Synonyms: Polygonum perfoliatum L. (1759), *Persicaria perfoliatum* (L.) Gross (1919), *Ampelygonum perfoliatum* (L.) Roberty & Vautier (1964).
Family: Polygonaceae.
Local name: Kanta Tokpata.
Description: An annual herb; perianth light green; fruits firstly green, then turning pinkish to reddish and finally becoming blue.
Distribution outside Bangladesh: Bhutan, China, Eastern India, Japan, Korea, Nepal, the Philippines and Taiwan.
Distribution in Bangladesh: Rangpur, Khulna, Sylhet and Dhaka districts.
Parts used: Whole plant.
Active compound: Polysaccharides.
Type of the tested cancer cells: Human lung carcinoma A549 cell line.
Reference: Lai and Li, 2016.

72. **Cicer arietinum** L., Sp. Pl. 2: 738 (1753). [Photo 55]

Family: Fabaceae.
Local names: Boot, Chhola, Chana, Boot Kalai.
Description: An annual herb; leaves imparipinnate; flowers white, greenish, pink or blue; seeds rough, white, yellow or black.
Distribution outside Bangladesh: C. arietinum was originated in Turkey and carried to the Indian subcontinent before 200 BC. Now cultivated in many parts of the tropics.
Distribution in Bangladesh: It is cultivated throughout the country.
Part used: Seeds.
Active compound: C-25 protein.
Type of the tested cancer cells: Oral cancer cell and normal cell.
Reference: Al-Snafi, 2016.

73. **Clausena excavata** Burm. f., Fl. Ind.: 87, t. 29, 2 (1768).

Synonyms: Amyris sumatrana Roxb. (1832), *Cookia graveolens* Wight & Arn. (1834), *Clausena punctata* (Roxb.) Wight & Arn. *ex* Steud. (1840).
Family: Rutaceae.
Local name: Pan-karpur.
Description: An aromatic shrub; leaves imparipinnate; petals pale green to yellowish-white; fruit greenish-white when young, pink when mature.
Distribution outside Bangladesh: South and South East Asia, Southern China, Southern Taiwan, the Philippines and New Guinea.
Distribution in Bangladesh: Forests of Sylhet and Chittagong districts.

Parts used: Stem and root bark.
Active compound: Clausenamine-A and its analogues.
Type of the tested cancer cells: Leukemia, nonsmall cell lung cancer, colon cancer, melanoma, ovarian and renal cancer and breast cancer cell lines.
Reference: Zhang and Lin, 2000.

74. **Cleome gynandra** L., Sp. Pl. 2: 671 (1753).
Synonyms: Gynandropsis pentaphylla DC. (1824), *Gynandropsis gynandra* (L.) Briq. (1914).
Family: Capparaceae.
Local names: Sada Hurhuria, Arkahuli.
Description: An annual herb; leaves palmately compound; flowers white or tinged with purple; seeds blackish-brown.
Distribution outside Bangladesh: Sri Lanka to South East Asia, Malaysia, Africa and America.
Distribution in Bangladesh: Dhaka, Gaibandha, Kushtia and Rajshahi districts.
Parts used: Whole plant.
Type of the tested cancer cells: Human epidermal carcinoma of the nasopharynx.
Reference: van Valkenburg and Bunyapraphatsara, 2002.

75. **Cleome viscosa** L., Sp. Pl. 2: 672 (1753). [Photo 56]
Synonym: Polanisia viscosa DC. (1824).
Family: Capparaceae.
Local names: Halde Hurhure, Hurhuria.
Description: A herb, all parts more or less viscid; leaves 3- or 5-foliolate; flowers yellow; seeds red-brown.
Distribution outside Bangladesh: Native to tropical and wamer parts of India and distributed throughout the world.
Distribution in Bangladesh: It is found throughout the country.
Part used: Bark.
Type of the tested cancer cells: Ehrlich ascites carcinoma.
Reference: Manoharan and Kaur, 2013.

76. **Cocos nucifera** L., Sp. Pl.: 1189 (1753). [Photo 57]
Family: Arecaceae.
Local names: Narikel, Daab.
Description: A tall perennial palm; leaves pinnatisect; inflorescence covered by spath; fruits trigonous, green or yellowish.

Distribution outside Bangladesh: Tropical and subtropical world.
Distribution in Bangladesh: It is cultivated all over the country.
Part used: Husk fiber.
Active compound: Catechins, epicatechins and condensed tannins.
Type of the tested cancer cells: Leukemia cell line K562.
Reference: Koschek *et al.*, 2007.

77. **Codiaeum variegatum** (L.) A. Juss., Euph. Tent.: 33, t. 9 (1824). [Photo. 58]
Synonyms: Croton variegatus L. (1753), *Codiaeum chrysosticton* Spreng. (1826).
Family: Euphorbiaceae.
Local name: Patabahar.
Description: An evergreen shrub; male flowers greenish or yellowish-white; fruits reddish-brown.
Distribution outside Bangladesh: Pantropical.
Distribution in Bangladesh: It is cultivated throughout the country as an ornamental.
Part used: Leaves.
Active compound: Alkaloids.
Reference: Larbie and Abboah-Offei, 2014.

78. **Coix lachryma-jobi** L., Sp. Pl. ed.1: 972 (1753). [Photo 59]
Synonym: Coix lacryma L. (1759).
Family: Poaceae.
Local names: Gurgor, Kalokunch, Tasbi, Kaich Gota.
Description: A coarse annual grass; leaf blades linear-lanceolate; inflorescence all bisexual on flattened peduncles (a female spike and a male spike).
Distribution outside Bangladesh: Throughout the tropics, and naturalized in many warm countries.
Distribution in Bangladesh: It occurs throughout the country.
Parts used: Whole seed, endosperm and hull.
Type of the tested cancer cells: Human colon adenocarcinoma.
Reference: Manosroi *et al.*, 2016.

79. **Colocasia esculenta** (L.) Schott in Schott & Endlicher, Melet. Bot.: 18 (1832). [Photo 60]
Synonyms: Arum esculenta L. (1753), *Colocasia nymphaeifolia* (Vent) Kunth (1841), *Colocasia antiquorum* Schott (1832).
Family: Araceae.

Local name: Kachu.
Description: Perennial herb; basal part of spathe green and upper part yellow; male flowers cream, female flowers green.
Distribution outside Bangladesh: Pantropical.
Distribution in Bangladesh: It is very common and found throughout the country.
Parts used: Root and stem.
Type of the tested cancer cells: Human breast adenocarcinoma (MCF-7).
Reference: Wei *et al.*, 2011.

80. **Coriandrum sativum** L., Sp. Pl. 1: 256 (1753).
Synonyms: Coriandrum majus Gouan (1762), *Coriandrum diversifolium* Gilib. (1782), *Coriandrum globosum* Salisb. (1796), *Selinum coriandrum* E.H. Krause (1904).
Family: Apiaceae.
Local names: Dhonay, Dhonia.
Description: An annual herb; leaves pinnately compound; corolla white or pale pink.
Distribution outside Bangladesh: Coriander is native to the Mediterranean regions. Now it is cultivated worldwide, sometimes naturalized.
Distribution in Bangladesh: It is cultivated all over the country as a spice and culinary herb.
Part used: Leaves.
Type of the tested cancer cells: Human colon cancer HT-29 cell lines.
Reference: Nithya and Sumalatha, 2014.

81. **Crinum asiaticum** L., Sp. Pl.: 419 (1753). [Photo 61]
Family: Liliaceae.
Local names: Bara Kanur, Nagdal, Sukhdarshan.
Description: A perennial herb; flowers white, fragrant at night; seeds round.
Distribution outside Bangladesh: India, Sri Lanka and Nepal.
Distribution in Bangladesh: Sundarbans and coastal areas of Chittagong, and also planted in gardens.
Part used: Leaves.
Active compound: Alkaloid.
Type of the tested cancer cells: Potato disc crown gall tumour and mouse tumour cell line (P388 D_1).
Reference: Ahmad, 1996.

82. **Crotalaria juncea** L., Sp. Pl.: 714 (1753).
Synonyms: Crotalaria benghalensis Lamk. (1786), *Crotalaria fenestrata* Sims (1817), *Crotalaria tenuifolia* Roxb. (1832).
Family: Fabaceae.
Local names: Shonpat, Shon.
Description: An annual herb; corolla bright yellow; fruit velvety; seeds reniform.
Distribution outside Bangladesh: Pantropical.
Distribution in Bangladesh: The plant is cultivated in all districts.
Part used: Leaves.
Reference: Velusamy *et al.*, 2016.

83. **Crotalaria sessiliflora** L., Sp. Pl. ed. 2: 1004 (1763).
Synonyms: Crotalaria anthylloides Lamk. (1786), *Crotalaria nepalensis* Link (1822), *Crotalaria eriantha* Sieb. & Zucc. (1843).
Family: Fabaceae.
Description: An erect annual herb; corolla blue; fruits black when mature; seeds cordate, shiny, yellowish.
Distribution outside Bangladesh: From Pakistan through India, South East Asia to the Philippines and New Guinea, northwards to China, Taiwan and Japan.
Distribution in Bangladesh: It was reported from Dhaka district (Datta and Mitra, 1953).
Active compound: Monocrotalline alkaloid.
Type of the tested cancer cells: Walker carcinoma 256, sarcoma 180 in rats.
Reference: Huang *et al.*, 1980.

84. **Crotalaria spectabilis** Roth, Nov. Pl. Sp.: 341 (1821). [Photo 62]
Synonym: Crotalaria sericea Retz. (1789).
Family: Fabaceae.
Local names: Pipli-jhunjan, Jhunjhuni-ghati.
Description: A robust annual undershrub; corolla yellow; fruits brown when mature; seeds cordate, brown.
Distribution outside Bangladesh: Pantropical.
Distribution in Bangladesh: It occurs throughout the country.
Active compound: Monocrotaline.
Reference: Evans, 2009.

85. **Croton tiglium** L., Sp. Pl.: 1004 (1753).
Family: Euphorbiaceae.

Local names: Jaypal, Jaiphal, Jamalgota.
Description: A shrub or small tree; petals narrower than the sepals in male flowers and petals absent in female flowers; fruits dull yellow; seeds greyish-brown.
Distribution outside Bangladesh: Cambodia, China, Hong Kong, India, Indonesia, Japan, Malaysia, Myanmar, the Philippines, Sri Lanka and Thailand.
Distribution in Bangladesh: Chittagong, Gazipur and Sylhet districts.
Part used: Seeds.
Active compound: Phorbol-12-tiglate-13-decanoate.
Type of the tested cancer cells: Leukemia cell.
Reference: Hwang *et al.*, 1980.

86. **Cucumis melo** L., Sp. Pl. ed. 1: 1011 (1753).
Synonyms: Cucumis acidus Jacq. (1771), *Cucumis utilissimus* Roxb. (1832).
Family: Cucurbitaceae.
Local names: Bangi, Futi.
Description: A robust, annual, climbing herb; stem prostrate; corolla yellow in male flowers, female flowers solitary; fruits variable in size, shape, colour, odour and taste.
Distribution outside Bangladesh: Cultivated all over the tropical and temperate regions of the world.
Distribution in Bangladesh: This species is cultivated throughout the country.
Parts used: Whole plant.
Type of the tested cancer cells: Skin cancer (Melanoma).
Reference: Al-Shawi, 2015.

87. **Cullen corylifolium** (L.) Medic., Vorles. Churpf. Phys. Ges. 2: 381 (1787). [Photo 63]
Synonym: Psoralea corylifolia L. (1753).
Family: Fabaceae.
Local names: Lata Kosturi, Babchi, Buchkidana.
Description: A herb or undershrub; leaves 1-foliolate or rarely trifoliolate; petals yellow.
Distribution outside Bangladesh: Throughout the Indian sub-continent.
Distribution in Bangladesh: Chapai Nawabgonj and Rajshahi districts.
Part used: Seeds.
Active compound: Psoralen and isopsoralen.

Type of the tested cancer cells: Human oral carcinoma line KB, KBv200 (the vincristine resistance subline of KB), human erythroleukemia cell K562 and K562/ADM (the doxorubicin resistance subline of K562).
Reference: Wang *et al.*, 2008.

88. **Curculigo orchioides** Gaertn., Fruct. 1: 63. t. 16 (1788).
Synonym: Curculigo brevifolia Dryand. (1811).
Family: Liliaceae.
Local name: Talmuli.
Description: A herb; flowers yellow, the lowest bisexual, all the rest male; seeds black.
Distribution outside Bangladesh: Subtropical Himalayas from Kumaon eastwards, Khasia Hills, Tripura and Manipur of India and Java of Indonesia.
Distribution in Bangladesh: Dhaka, Sylhet and Chittagong districts.
Part used: Root.
Type of the tested cancer cells: Breast cancer cell line.
Reference: Chanda and Nagani, 2013.

89. **Curcuma amada** Roxb., Asiat. Res. 11: 341 (1810).
Family: Zingiberaceae.
Local name: Amada.
Description: Leafy rhizomatous herb, rhizome pale yellow inside, smells like green mango; corolla whitish.
Distribution outside Bangladesh: India.
Distribution in Bangladesh: One species was reported from Purana Paltan in Dhaka City in 1946 (Yusuf, 1999), also found in northern districts.
Part used: Whole plant.
Active compound: Amadannulen.
Type of the tested cancer cells: Ehrlich ascites carcinoma.
Reference: Manoharan and Kaur, 2013.

90. **Curcuma longa** L., Sp. Pl. 1: 2 (1753). [Photo 64]
Synonyms: Amomum curcuma Jacq. (1776), *Kua domestica* Medic. (1790), *Stissera curcuma* Giseke (1792), *Curcuma domestica* Valet. (1918).
Family: Zingiberaceae.
Local names: Halud, Haldi.
Description: A rhizomatous herb, rhizome orange-yellow inside; leaves 5-7 in number; inflorescence spike, fertile bracts white to light green; corolla tube light yellow; labellum roughly square.
Distribution outside Bangladesh: Cultivated throughout the tropics.

Distribution in Bangladesh: It is cultivated throughout the country.
Part used: Rhizome.
Active compound: Curcumin.
Type of the tested cancer cells: Benzo[α]pyrene induced forestomach tumours in Swiss mice and methyl-(acetoxymethyl)-nitrosamine induced oral mucosal tumours in Syrian golden hamsters.
Reference: Azuine and Bhide, 1994.

91. **Curcuma zedoaria** (Christm.) Rosc. in Trans Linn. Soc. London 8: 354 (1807). [Photo 65]
Synonyms: Amomum latifolia Lamk. (1692), *Curcuma officinalis* Salisb. (1747), *Amomum zedoaria* Christm. (1779), *Curcuma zerumbet* Roxb. (1810).
Family: Zingiberaceae.
Local names: Shoti, Failla.
Description: A rhizomatous herb, rhizome light yellow inside; leaves 4-6 in number; inflorescence spike, fertile bracts pale green; corolla white.
Distribution outside Bangladesh: Bhutan, India, Indonesia and Malaysia.
Distribution in Bangladesh: This species is fairly common.
Part used: Rhizome.
Active compound: Isocurcumenol.
Type of the tested cancer cells: Human and murine cancer cells.
Reference: Lakshmi *et al.*, 2011.

92. **Cuscuta chinensis** Lamk., Encycl. 2: 229 (1786).
Family: Cuscutaceae.
Local name: Swarnalata.
Description: A twining parasite; stem golden-yellow; corolla lobes shorter than tube; capsule enclosed by corolla.
Distribution outside Bangladesh: India, Sri Lanka, Eastwards to Australia and Weswards to North Central States of America.
Distribution in Bangladesh: Hooker recorded this species from Sylhet in 1885, since then no other collection has been made.
Part used: Whole plant.
Active compound: Cuscutic resinoside A, a resin glycoside.
Type of the tested cancer cells: Human acute lymphoblastic leukemia cell line.
Reference: Zeraati *et al.*, 2010.

93. **Cyclea barbata** Miers, Contrib. Bot. 3: 237 (1871). [Photo 66]
Synonym: Cyclea peltata sensu Miq. (1858).
Family: Menispermaceae.

Local name: Patalpur.
Description: A slender climber; stem herbaceous or woody; male flowers light green to light yellow, petals in female flower more or less reniform.
Distribution outside Bangladesh: India (Assam), Myanmar (type), Thailand, South Vietnam and Indonesia.
Distribution in Bangladesh: Chittagong, Comilla, Cox's Bazar and Sylhet districts.
Part used: Root.
Active compound: Tetrandrine.
Reference: Evans, 2009.

94. **Daucus carota** L., Sp. Pl.: 242 (1753). [Photo 67]
Synonym: Daucus gingidum L. (1753).
Family: Apiaceae.
Local name: Gazor.
Description: An erect herb; tap root fleshy, reddish, reddish-violet or yellow, rarely yellowish-orange; leaves 8-12; flowers small.
Distribution outside Bangladesh: Worldwide.
Distribution in Bangladesh: It is cultivated all over the country as a vegetable.
Part used: Oil extract. There are claims to be completely cured from cancer by taking much fresh juice per day for about three months.
Reference: Shebaby *et al.*, 2013.

95. **Deeringia amaranthoides** (Lamk.) Merr., Int. Rumph. Herb. Amb.: 211 (1917).
Synonyms: Achyranthes amaranthoides Lamk. (1785), *Deeringia celosoides* R. Br. (1810).
Family: Amaranthaceae.
Local names: Gholemouni, Golamohani.
Description: A scandent, sometimes scrambling shrub; flowers greenish-yellow; tepals pale green or yellowish, white-margined; seeds reniform.
Distribution outside Bangladesh: India (common in tropical Himalayas and Assam), Bhutan, Myanmar, extending to Malaysia, China and Australia.
Distribution in Bangladesh: Sylhet, Maulvi Bazar and Chittagong districts and the Chittagong Hill Tracts.
Part used: Fruits.
Reference: Pandey and Tripathi, 2014.

96. **Dendrobium nobile** Lindl., Gen. Sp. Orch. Pl.: 24 (1830). [Photo 68]
Synonyms: Dendrobium coerulescens Wall. (1838), *Dendrobium lindleyanum* Griff. (1851), *Callista nobilis* (Lindl.) O. Kuntze (1891).
Family: Orchidaceae.
Description: Plant epiphytic or lithophytic; inflorescence arising from the nodes; flowers waxy, fragrant; sepals and petals white at the base, pinkish-mauve above, lip maroon at the base, margin yellow or white with mauve to purple.
Distribution outside Bangladesh: India, Myanmar, Bhutan, China, Thailand and Vietnam.
Distribution in Bangladesh: The species was reported from Dhaka city (Seidenfaden, 1985).
Part used: Aerial parts.
Active compound: Phenanthrenes (4,7-dihydroxy-2-methoxy-9,10-dihydrophenanthrene and denbinobin).
Type of the tested cancer cells: A549 (human lung carcinoma), SK-OV-3 (human ovary adenocarcinoma), and HL-60 (human promyelocytic leukemia) cell lines.
Reference: Lee *et al.*, 1995.

97. **Dillenia indica** L., Sp. Pl.: 535 (1753). [Photo 69]
Synonym: Dillenia speciosa Thunb. (1791).
Family: Dilleniaceae.
Local name: Chalta.
Description: A medium-sized to large tree; flowers pendent; fruits enclosed by enlarged fleshy sepals.
Distribution outside Bangladesh: Tropical Himalayas, India, Sri Lanka, Myanmar, Thailand, Cambodia and Vietnam to Malaysia.
Distribution in Bangladesh: It is found in all forests.
Part used: Fruits.
Active compound: Betulinic acid.
Type of the tested cancer cells: Human leukaemic cell lines U937, HL60 and K562.
Reference: Nataru *et al.*, 2014.

98. **Dioscorea bulbifera** L., Sp. Pl.: 1033 (1753).
Family: Dioscoreaceae.
Local names: Amda Lata, Rata Alu, Pagla Alu.
Description: A twining herb; stem angled, bulbils abundant; leaf blade cordiform; flowers whitish to pale pink, fragrant.

Distribution outside Bangladesh: India, Nepal, Bhutan, Malaysia and Africa to the farthest Islands of the Pacific.
Distribution in Bangladesh: Dhaka, Noakhali, Chittagong, Cox's Bazar, Bandarban, Rangamati and Khagrachari districts. Also cultivated in different parts of the country.
Part used: Rhizome.
Active compound: Kaempferol-3,5-dimethyl ether, catechin, myricetin, quercetin-3-O-galactopyranoside, myricetin-3-O-galactopyranoside, caryatin, myricetin-3-O-glucopyranoside and diosbulbin.
Type of the tested cancer cells: JB6 mouse epidermal cells.
Reference: Gao *et al.,* 2002.

99. **Diospyros malabarica** (Desr.) Kostel., Allg. Med.-Pharm. Fl. 3: 1099 (1834). [Photo 70]
Synonyms: Diospyros embryopteris Pers. (1807), *Diospyros peregrina* Guerke (1891).
Family: Ebenaceae.
Local names: Gab, Deshi Gab.
Description: A small to medium-sized evergreen tree; flowers whitish, scented; fruit yellowish when ripe.
Distribution outside Bangladesh: India, Sri Lanka, Myanmar, Thailand, Malaysia and Indonesia.
Distribution in Bangladesh: It is cultivated in homesteads throughout the country.
Part used: Aerial parts.
Type of the tested cancer cells: Human epidermoid carcinoma of the nasopharynx.
Reference: Lemmens and Bunyapraphatsara, 2003.

100. **Diospyros montana** Roxb., Pl. Corom. 1: 37 (1795). [Photo 71]
Synonyms: Diospyros cordifolia Roxb. (1795), *Diospyros calcarea* Fletcher (1937).
Family: Ebenaceae.
Local name: Bon Gab.
Description: A small tree; flowers white; fruits yellow when ripe; seeds black.
Distribution outside Bangladesh: India, Sri Lanka, Myanmar, Cambodia, Laos, Vietnam, Thailand, the Philippines and tropical Australia.
Distribution in Bangladesh: It was planted along roadsides and in village groves in many districts and near *Hindu* temples.
Active compound: Diospyrin.

Type of the tested cancer cells: Ehrlich ascites carcinoma.
Reference: Lemmens and Bunyapraphatsara, 2003.

101. **Duchesnea indica** (Andr.) Focke in Engler & Prantl, Nat. Pflanzenfam. 3(3): 33 (1888).
Synonyms: Fragaria indica Andr. (1807), *Potentilla indica* (Andr.) Wolf (1904).
Family: Rosaceae.
Local name: Jongli Strawberry.
Description: A stoloniferous, prostrate herb; stem creeping with runners; flowers yellow; fruits bright red, kidney-shaped.
Distribution outside Bangladesh: Afghanistan, subtropical Himalaya, India, east to China and Japan, and Malaysia.
Distribution in Bangladesh: Dinajpur, Munshiganj and Sylhet districts.
Parts used: Whole plant.
Type of the tested cancer cells: Sarcoma 180 (S180) tumour bearing mice.
Reference: Lili *et al.*, 2013.

102. **Eclipta alba** (L.) Hassk., Pl. Jav. Rar.: 528 (1848). [Photo 72]
Synonyms: Verbesina alba L. (1753), *Verbesina prostrata* L. (1753), *Cotula alba* L. (1767), *Eclipta prostrata* (L.) Mant. (1771).
Family: Asteraceae.
Local names: Kesaraj, Kesuti, Kalokeshi, Bhangra, Bhimraj.
Description: A herb; stem reddish or brick-red; ray-florets white, disc-florets white.
Distribution outside Bangladesh: Central and South America, now common and cosmopolitan in all warm countries.
Distribution in Bangladesh: It is found all over the country.
Part used: Leaves.
Type of the tested cancer cells: HepG2, C6 glioma and A498 cell lines.
Reference: Nataru *et al.*, 2014.

103. **Elephantopus scaber** L., Sp. Pl. 2: 814 (1753).
Synonym: Elephantopus scaber L. var. *typicus* Koster (1935).
Family: Asteraceae.
Description: A perennial herb; stem dichotomously branched; leaves radical and cauline; flowers pinkish-red.
Distribution outside Bangladesh: Cosmopolitan in the tropics.
Distribution in Bangladesh: Chittagong, Comilla, Noakhali, Sylhet and Maulvi Bazar districts, and the Chittagong Hill Tracts.
Parts used: Whole plant.

Active compound: Deoxyelephantopin.
Type of the tested cancer cells: Walker 256 carcinosarcoma.
Reference: de Padua *et al.*, 1999.

104. **Embelia ribes** Burm. f., Fl. Ind.: 62, t. 23 (1768).
Synonyms: Embelia glandulifera Wight (1848), *Ribesiodes ribes* (Burm. f.) O. Kuntze (1891).
Family: Myrsinaceae.
Local names: Bakul Lata, Biranga.
Description: A large scandent or scrambling shrub; flowers small, greenish or white; fruits bluish-black, wrinkled.
Distribution outside Bangladesh: South East Asia, Sri Lanka, India, Thailand, Vietnam, Northwards to South China, Malaysia, Indonesia and Singapore.
Distribution in Bangladesh: It was reported to occur in Sylhet district.
Part used: Fruits.
Active compound: Embelin.
Reference: de Padua *et al.*, 1999.

105. **Emilia sonchifolia** (L.) Candolle in Wight, Contr. Bot. India.: 24 (1834). [Photo 73]
Synonym: Cacalia sonchifolia L. (1753).
Family: Asteraceae.
Local names: Mechitra, Sadimudi, Sadusi.
Description: An erect herb; stem glaucous; leaves radical and cauline; corolla purple; pappus hairy.
Distribution outside Bangladesh: Asia and tropical Africa.
Distribution in Bangladesh: It is found all over the country.
Part used: Whole plant.
Active compound: Terpene.
Type of the tested cancer cells: Dalton's lymphoma, Ehrlich ascites carcinoma and mouse lung fibroblast (L-929) cells.
Reference: Shylesh *et al.*, 2005.

106. **Entada rheedii** Spreng., Syst. Veg. 2: 325 (1825). [Photo 74]
Synonyms: Mimosa entada L. (1753), *Entada monostachya* DC. (1825), *Entada scandens* auct. *non* Benth. (1842), *Entada phaseoloides* auct. *non* (L.) Merr. (1918).
Family: Mimosaceae.
Local names: Gila, Gilagachh, Pangra.

Description: A woody climber, overtopping tallest tree; leaves pinnately compound; petals white; fruit a pod, exocarp and endocarp woody; seeds large.
Distribution outside Bangladesh: Africa, tropical Asia, Australia and a small part of the Pacific, Malay Peninsula, Indonesia, the Philippines and New Guinea.
Distribution in Bangladesh: Gazipur, Tangail, Bandarban and Panchagarh districts and the Chittagong Hill Tracts.
Part used: Seed kernels.
Active compound: Saponin.
Type of the tested cancer cells: Walker 256 carcinosarcoma in rats.
Reference: Liu *et al.,* 1972.

107. **Erythrina variegata** L., Diss. Herb. Amb. Amoen. Acad. 4: 122 (1754). [Photo 75]
Synonyms: Erythrina picta L. (1753), *Erythrina indica* Lamk. (1786), *Erythrina indica* Lamk. var. *alba* Blatter and Mill. (1929).
Family: Fabaceae.
Local names: Mandar, Madar, Paltemadar, Parijat.
Description: A small to medium-sized tree; leaves trifoliolate; corolla bright red; seeds kidney-shaped, red or brown.
Distribution outside Bangladesh: India, Sri Lanka, Myanmar, Malesia and Polynesia.
Distribution in Bangladesh: It is found throughout the country.
Part used: Seeds.
Active compound: Lectins with proteinase inhibiting properties.
Type of the tested cancer cells: Raji (Burkitt lymphoma), BALL1 (acute B lymphoblastic leukemia), Molt4 (acute T lymphoblastic leukemia), HL-60 (acute myelogenous leukemia), Jurkat (acute T lymphoblastic leukemia), and RPMI8402 (acute T lymphoblastic leukemia) cell lines.
Reference: Ohba *et al.,* 1998.

108. **Excoecaria agallocha** L., Syst. Nat. ed. 10. 2: 1288 (1759).
Synonyms: Commia cochinchinensis Lour. (1790), *Excoecaria camettia* Willd. (1805), *Excoecaria affinis* Endl. (1833), *Stillingia agallocha* (L.) Baill. (1858).
Family: Euphorbiaceae.
Local names: Gengwa, Genwa, Gewa.
Description: A deciduous shrub or small tree; flowers yellow, fragrant; seeds ovoid-globose.

Distribution outside Bangladesh: India, and Sri Lanka to Formosa and throughout Malaysia to Northern Australia and the Pacific.
Distribution in Bangladesh: Coastal regions of Bagerhat, Chittagong, Cox's Bazar and Satkhira districts.
Part used: Resinous wood.
Active compound: Diterpenes.
Type of the tested cancer cells: Epstein-Barr virus early antigen (EBV-EA) in Raji cells.
Reference: Konoshima *et al.*, 2001.

109. **Ficus racemosa** L., Sp. Pl.: 1060 (1753). [Photo 76]
Synonyms: Ficus glomerata Roxb. (1798), *Ficus goolerea* Roxb. (1832), *Covellia glomerata* Miq. (1848).
Family: Moraceae.
Local names: Jagyadumur, Dumur.
Description: A small to medium-sized tree; inflorescence hypanthodia; figs pyriform, rosy-red.
Distribution outside Bangladesh: India, Pakistan, Sri Lanka, South China, Myanmar, Thailand, Malaysia and Indonesia to North Australia.
Distribution in Bangladesh: This species occurs in almost all districts.
Part used: Fruits.
Type of the tested cancer cells: MCF7 human breast cancer cell line.
Reference: Gavhane *et al.,* 2016.

110. **Fissistigma polyanthum** (Hook. f. & Thom.) Merr. in Philipp. Journ. Sc. Bot. 15: 135 (1919).
Family: Annonaceae.
Synonym: Melodorum polyanthum Hook. f. & Thom. (1855).
Description: A woody climber; branches dark brown; petals orange-red; fruits berry, silky hairy; seeds in 2 rows, shiny.
Distribution outside Bangladesh: India.
Distribution in Bangladesh: Sylhet and Cox's Bazar districts.
Reference: Lemmens and Bunyapraphatsara, 2003.

111. **Foeniculum vulgare** Miller, Gard. Dict. ed. 8. no. 1 (1768).
Synonyms: Anethum foeniculum L. (1753), *Foeniculum capillaceum* Galib. (1782), *Foeniculum officinale* Allioni (1785).
Family: Apiaceae.
Local name: Pan-mohuri.
Description: A robust, glaucous, aromatic herb; petals yellow; fruits light green to yellow-brown.

Distribution outside Bangladesh: It is probably originated from southern Europe and the Mediterranean. Now it is cultivated throughout the world and naturalized in many places.
Distribution in Bangladesh: It is cultivated in Rajbari, Noakhali, Tangail, Jamalpur, Kurigram and Panchgarh districts.
Part used: Whole plant.
Active compound: Limonene.
Reference: de Guzman and Siemonsma, 1999.

112. **Glycosmis pentaphylla** (Retz.) A. DC., Prodr. 1: 538 (1824). [Photo 77]
Synonyms: Limonia pentaphylla Retz. (1788), *Limonia arborea* Roxb. (1788), *Glycosmis arborea* (Roxb.) A. DC. (1824).
Family: Rutaceae.
Local names: Datmajani, Matkila.
Description: An evergreen shrub or small tree; petals creamy-white; fruits cream to crimson-red or pinkish when ripe; seeds round to plano-convex.
Distribution outside Bangladesh: South and South East Asia, the Philippines, southern China and Australia.
Distribution in Bangladesh: It is found all over the country.
Parts used: Leaves and stem.
Active compound: Arborinine (alkaloid).
Type of the tested cancer cells: Crown gall tumours produced by *Agrobacterium tumefaciens* in a potato disc bioassay.
Reference: Quader *et al*., 1999.

113. **Glycine max** (L.) Merr., Interpr. Rumph. Herb. Amboin.: 274 (1917).
Synonyms: Phaseolus max L. (1753), *Glycine hispida* (Moench) Maxim. (1873), *Soja max* (L.) Piper (1914).
Family: Fabaceae.
Local names: Gari Kalai, Soya Bean.
Description: A herb; leaves 3-foliolate; corolla white or lilac; fruits recurved, 3-4 seeded.
Distribution outside Bangladesh: India, Pakistan, Nepal, Myanmar, China and East Asia.
Distribution in Bangladesh: It is cultivated in many districts.
Part used: Seeds.
Active compound: Aglycones.
Type of the tested cancer cells: Osteoblast cell line.
Reference: Velusamy *et al.*, 2016.

114. **Gossypium arboreum** L., Sp. Pl.: 693 (1753). [Photo 78]
Synonyms: Gossypium indicum Medic. (1784), *Gossypium obtusifolium* Roxb. *ex* G. Don (1831), *Gossypium nanking* Meyen (1834).
Family: Malvaceae.
Local names: Karpas, Rui, Tula.
Description: A shrub or small tree; young branches, petioles and pedicels densely covered with hairs; corolla pale yellow, rarely red or purple.
Distribution outside Bangladesh: Throughout the tropics and subtropics of the world.
Distribution in Bangladesh: This species has two varieties, var. *arboreum* is planted in the plains throughout the country while var. *cernum* is cultivated in the hilly areas of the Chittagong Hill Tracts, in lieu of *Jhum* cultivation.
Part used: Seeds.
Active compound: Lectin.
Type of the tested cancer cells: Leukocytes and mononuclear cells.
Reference: Agarwal and Agarwal, 1990.

115. **Gossypium herbaceum** L., Sp. Pl.: 693 (1753). [Photo 79]
Synonym: Gossypium frutescens Lasteyrie (1808).
Family: Malvaceae.
Local name: Karpas Tula.
Description: A herb or undershrub; twigs and young parts hairy; corolla yellow with crimson centre.
Distribution outside Bangladesh: It is indigenous to the tropics and subtropics of the Old World where it is cultivated.
Distribution in Bangladesh: It is cultivated in fields and gardens throughout the country.
Type of the tested cancer cells: Salmonella/ microsomal system in the presence of picronolic acid or benzo[a]pyrene.
Reference: Lee and Lin, 1988.

116. **Hedyotis diffusa** Willd., Sp. Pl. 1: 566 (1797).
Synonym: Oldenlandia diffusa (Willd.) Roxb. (1832).
Family: Rubiaceae.
Description: An erect or semi-prostrate herb; flowers solitary; hypanthium globose; seeds narrowly winged.
Distribution outside Bangladesh: Sri Lanka, India, Nepal, Bhutan, Indonesia and Malaysia.
Distribution in Bangladesh: Dhaka and Comilla districts.
Part used: Whole plant.

Type of the tested cancer cells: Non-small cell lung cancer cell lines, A549 and NCIH460, the human pancreatic cell line MiaPacA-2, human embryonic kidney cell line HEK293 and human epidermoid carcinoma cell line KB-C2 and KB-3-1.
Reference: Deng *et al.*, 2013.

117. **Helianthus annuus** L., Sp. Pl.: 904 (1753). [Photo 80]
Family: Asteraceae.
Local name: Surjamukhi.
Description: A tall herb; lamina cordate; inflorescence capitulum; ray-florets neuter, disc-florets bisexual.
Distribution outside Bangladesh: A native of North America. But now it is cultivated in France, Russia, Egypt, Turkey, Germany and Italy. In Pakistan and India, it is grown in the gardens for ornamental purposes.
Distribution in Bangladesh: It is mainly grown in the gardens for ornamental purposes, but now-a-days, it is also cultivated in the field as an edible oil-yielding plant.
Part used: Seeds.
Active compound: Coumarin, tannins and selenium.
Type of the tested cancer cells: Rhabdomysarcoma (RD) and murine L20B cell line.
Reference: Al-Jumaily *et al.*, 2013.

118. **Heliotropium indicum** L., Sp. Pl. 1: 130 (1753). [Photo 81]
Synonym: Heliotropium velutinum DC. (1845).
Family: Boraginaceae.
Local names: Hatisur, Hatisura.
Description: A hirsute herb; inflorescence curved; flowers white or pale violet-blue; corolla salver-shaped; fruits bluntly 4-ribbed.
Distribution outside Bangladesh: A native of tropical America, now widespread in all tropical regions of the world.
Distribution in Bangladesh: It occurs throughout the country.
Part used: Leaves.
Active compound: Indicine-N-oxide.
Type of the tested cancer cells: W-256 carcinosarcoma in rats and leukemia L-1210 in mice.
Reference: Kugelman *et al.*, 1976.

119. **Hemidesmus indicus** (L.) R. Br. in Ait. f., Hort. Kew. ed. 2, 2: 75 (1811).
Synonyms: Periploca indica L. (1753), *Asclepias pseudo-sarsa* Roxb. (1824).

Family: Asclepiadaceae.
Local name: Anantamul.
Description: A prostrate or slightly twining undershrub; coronal scales knob-shaped; seeds black with white hairs.
Distribution outside Bangladesh: India, Malaysia, Myanmar, Pakistan and Sri Lanka.
Distribution in Bangladesh: It is found in dry forest areas of central and southeastern parts of the country.
Part used: Root.
Type of the tested cancer cells: HepG2 cell line.
Reference: Hafidh *et al.,* 2009.

120. **Hibiscus rosa-sinensis** L., Sp. Pl.: 694 (1753). [Photo 82]
Family: Malvaceae.
Local names: Joba, Raktajoba.
Description: A shrub; flowers solitary; epicalyx segments 5-8; corolla usually red, rose-yellow; fruit not set in Bangladesh.
Distribution outside Bangladesh: Probably a native of China, planted as an ornamental throughout the tropical and subtropical regions of the world.
Distribution in Bangladesh: It is planted in most flower gardens all over the country.
Part used: Leaves.
Active compound: Triterpenes and flavonoids.
Type of the tested cancer cells: Carcinoma of breast (MCF-7 cells), liver (HepG2 cells), lung (NCI-H23) and colon (HT-29) cancer cells.
Reference: Basha *et al.,* 2013.

121. **Hibiscus sabdariffa** L., Sp. Pl.: 695 (1753). [Photo 83]
Synonym: Hibiscus digitatus Cav. (1787).
Family: Malvaceae.
Local names: Mesta Pat, Chukair.
Description: A herb; flowers solitary; epicalyx segments 8-12; corolla yellow or purplish-yellow with a dark purple centre; seeds reniform.
Distribution outside Bangladesh: Probably of African origin and domesticated in ancient times. Cultivated in tropical and subtropical countries of the world.
Distribution in Bangladesh: It is cultivated in all parts of the country.
Part used: Leaves
Type of the tested cancer cells: Human prostate cancer cell line.
Reference: Chiu *et al.,* 2015.

122. **Holarrhena antidysenterica** (L.) Wall. *ex* Decne., Prodr. 8: 413 (1844). [Photo 84]

Synonyms: Nerium antidysentericum L. (1753), *Echites antidysenterica* Roxb. *ex* Flem. (1810), *Echites pubescens* Buch.-Ham. (1822), *Chonemorpha antidysenterica* G. Don (1837), *Holarrhena pubescens* Wall. *ex* G. Don (1837), *Holarrhena codaga* G. Don (1848).

Family: Apocynaceae.

Local names: Kurchi, Kuruj.

Description: A small to medium-sized tree; flowers white; follicles pendulous.

Distribution outside Bangladesh: India, Myanmar, Nepal, Pakistan and Sri Lanka.

Distribution in Bangladesh: It occurs almost throughout the country.

Part used: Leaves.

Type of the tested cancer cells: Fourteen human cancer cell lines-A-549, COLO-205, DU-145, HeLa, HEP-2, IMR-32, KB, MCF-7, NCI-H23, OVCAR-5, SiHa, SK-N-MC, SW-620 and ZR-75-1 from nine different tissues (breast, colon, cervix, CNS, lung, liver, oral, ovary and prostate).

Reference: Sharma *et al.,* 2014.

123. **Hydrocotyle sibthorpioides** Lamk., Enc. 3: 153 (1769). [Photo 85]

Synonym: Hydrocotyle rotundifolia Roxb. (1814).

Family: Apiaceae.

Description: A herb; stem creeping; leaves reniform; petals greenish-white.

Distribution outside Bangladesh: Tropical Asia, Africa and Australia, introduced into the New World.

Distribution in Bangladesh: It grows naturally in almost all districts.

Parts used: Whole plant.

Reference: Babu *et al.,* 1995.

124. **Hygrophila schulli** (Buch.-Ham.) M. R. and S. N. Almeida, Journ. Bomb. Nat. Hist. Soc. 83 (Suppl.): 221 (1986). [Photo 86]

Synonyms: Bahel schulli Buch.-Ham. (1824), *Barleria auriculata* Schum. (1827), *Hygrophila spinosa* T. Anders. (1860), *Hygrophila auriculata* (Schum.) Heine (1962).

Family: Acanthaceae.

Local names: Kanta Kalika, Talmakhna.

Description: An erect, unbranched herb; stem thorny; flowers purplish-blue.

Distribution outside Bangladesh: Indo-China, Myanmar, India, Nepal, Sri Lanka, Pakistan and Tropical Africa.

Distribution in Bangladesh: This species is widely distributed throughout the country.
Part used: Root.
Type of the tested cancer cells: Ehrlich ascites carcinoma and sarcoma-180 induced mice.
Reference: Mazumdar *et al.*, 1997.

125. **Hyptis suaveolens** (L.) Poit., Ann. Nat. Hist. 7: 472 (1806). [Photo 87]
Synonym: Ballota suaveolens L. (1759).
Family: Lamiaceae.
Local names: Gonged Tulsi, Bilati Tulsi, Tokma.
Description: A sweet smelling herb; stem tetrangular; flowers violet; nutlets blackish-brown.
Distribution outside Bangladesh: It is native of tropical America, but now naturalized in Africa, South Asia including Thailand, Malaysia, the Philippines and Formosa.
Distribution in Bangladesh: Hilly areas of Chittagong and the Chittagong Hill Tracts and other parts of the country.
Part used: Leaves.
Reference: Ghani, 2003.

126. **Ichnocarpus frutescens** (L.) R. Br., Mem. Wern. Soc. 1: 62 (1811). [Photo 88]
Synonyms: Apocynum frutescens L. (1764), *Echites frutescens* Wall. (1829), *Ichnocarpus ovatifolius* A. DC. (1844), *Ichnocarpus frutescens* (L.) R. Br. var. *pubescens* Kurz (1877), *Ichnocarpus volubilis* Merr. (1922).
Family: Apocynaceae.
Local names: Paralita Lata, Dudhi Lata, Shyamalata.
Description: A large shrub; leaves rusty pubescent beneath; flowers greenish-white, fragrant; corolla salver-shaped.
Distribution outside Bangladesh: Australia, China, India, Kashmir, Myanmar, Malaysia, Nepal, Sri Lanka and Pakistan.
Distribution in Bangladesh: It is common throughout the country.
Part used: Root.
Active compound: Two triterpenes-α amyrin and ursolic acid.
Type of the tested cancer cells: MCF-7 (Human breast cancer cell line), BEL-7402 (Human hepatocellular carcinoma cell line), SPC-A-1 (Human lung cancer cell line) and SGC-7901 (Human gastric cancer cell line).
Reference: Nataru *et al.*, 2014.

127. **Impatiens balsamina** L., Sp. Pl.: 938 (1753). [Photo 89]
Synonym: Impatiens cornuta L. (1753).
Family: Balsaminaceae.
Local name: Dupati.
Description: A herb; flowers white or pink; fruits densely tomentose; seeds globose.
Distribution outside Bangladesh: Probably native of India and parts of South East Asia and found throughout the tropics, subtropics and temperate zone as an ornamental.
Distribution in Bangladesh: It is found in all districts.
Parts used: Whole plant.
Type of the tested cancer cells: Salmonella/ microsomal system in the presence of picronolic acid or benzo[a]pyrene.
Reference: Lee and Lin, 1988.

128. **Indigofera tinctoria** L., Sp. Pl. 2: 751 (1753). [Photo 90]
Synonyms: Indigofera indica Lamk. (1789), *Indigofera sumatrana* Gaertn. (1791), *Indigofera tinctoria* L. var. *macrocarpa* DC. (1825).
Family: Fabaceae.
Local name: Nil.
Description: A shrub; branches more or less angular; corolla pink; fruits straight or slightly curved.
Distribution outside Bangladesh: Cultivated throughout the tropics.
Distribution in Bangladesh: It is found in most parts of the country.
Parts used: Aerial parts.
Reference: Bhanot *et al.*, 2011.

129. **Ipomoea batatus** (L.) Lamk., Tabl. Encycl. 1: 465 (1791).
Synonym: Convolvulus batatus L. (1753).
Family: Convolvulaceae.
Local names: Misti Aloo, Lomba Aloo, Ranga Aloo.
Description: A trailer; root white or pink; stem prostrate; corolla campanulate to funnel-shaped, pale violet.
Distribution outside Bangladesh: A native of America, now cultivated throughout the tropics and subtropics.
Distribution in Bangladesh: It is cultivated throughout the country.
Part used: Root tuber.
Active compound: 4-Ipomeanol.
Reference: Evans, 2009.

130. **Ixora javanica** DC., Prodr. 4: 487 (1830). [Photo 91]
Family: Rubiaceae.
Local name: Rangan.
Description: A shrub; flowers yellow, pink, orange or red; fruits rarely seen.
Distribution outside Bangladesh: India, Indo-China and Malaysia.
Distribution in Bangladesh: Chittagong and Cox's Bazar districts.
Part used: Flowers.
Active compound: Ferulic acid.
Type of the tested cancer cells: Dalton's lymphoma, K-562 and lymphocytes.
Reference: Nair and Panikkar, 1990.

131. **Jatropha curcas** L., Sp. Pl. 2: 1006 (1762). [Photo 92]
Family: Euphorbiaceae.
Local names: Baghverenda, Banbherenda, Sadajeol.
Description: A soft-wooded shrub or small tree; leaf blade cordate; male flower greenish-yellow; fruits green, yellowish or black; seeds black.
Distribution outside Bangladesh: It is native to tropical America. Bhutan, China, Hong Kong, India, Malay Peninsula, Pakistan, Thailand and Taiwan.
Distribution in Bangladesh: This species is found in most parts of the country.
Part used: Root extract.
Type of the tested cancer cells: Human colon adenocarcinoma (HT-29) cell line and human hepatocyte (Chang cell).
Reference: Oskoueian *et al.*, 2011.

132. **Jatropha gossypifolia** L., Sp. Pl.: 1066 (1753). [Photo 93]
Synonyms: Adenoropium elegans (Pohl) Muell.-Arg. (1826), *Jatropha gossypifolia* L. var. *elegans* (Pohl) Muell.-Arg. (1866).
Family: Euphorbiaceae.
Local names: Lalbherenda, Laljeol.
Description: A soft-wooded erect shrub; leaf blade cordate; male flowers reddish-purple; fruits rounded-trilobate; seeds pale greyish-brown.
Distribution outside Bangladesh: A native of South America, also found in India, Myanmar, Pakistan and the West Indies. It is introduced into the Old World tropics.
Distribution in Bangladesh: This species is found throughout the country.
Parts used: Whole plant or stem.
Active compound: Jatrophone.
Reference: Evans, 2009.

133. **Justicia procumbens** L., Sp. Pl.: 15 (1753).
Synonyms: Rostellaria procumbens (L.) Nees (1832), *Rostellularia procumbens* (L.) Nees (1847), *Ecbolium procumbens* (L.) O. Kuntze (1891).
Family: Acanthaceae.
Local name: Chilpi.
Description: A branched herb; flowers pink; seeds more or less ovate.
Distribution outside Bangladesh: India, Pakistan, Sri Lanka, Malaysia and Australia.
Distribution in Bangladesh: Chittagong district.
Parts used: Whole plant.
Active compound: Justicidin-A and Diphyllin.
Type of the tested cancer cells: P-388 lymphocytic leukemia growth in BDF_1 male mice and 9-KB (human nasopharyngeal carcinoma) cell culture assay.
Reference: Fukamiya and Lee, 1986.

134. **Kigelia africana** (Lamk.) Benth. in Hook., Nigir. Fl.: 463 (1849).
Synonyms: Bignonia africana Lamk. (1785), *Crescentia pinnata* Jacq. (1789), *Tanaecium pinnatum* Willd. (1801), *Kigelia pinnata* (Jacq.) Rev. (1838), *Kigelia aethiopica* (Fenzyl) Decne. (1845).
Family: Bignoniaceae.
Local names: Jhar Fanoos, Shell Brikma.
Description: A large spreading tree; leaves usually in whorls of 3, pinnately compound; corolla deep chocolate-red, corolla opens in the evening, release a unpleasant smell.
Distribution outside Bangladesh: A native of tropical Africa (Mozambique), and also found in India, Vietnam, Thailand, Malaysia, Indonesia and the Philippines.
Distribution in Bangladesh: A few large trees are found near Carmaichael College in Rangpur town. Smaller plants are found in Dhaka University campus.
Part used: Stem bark.
Active compound: Norviburtinal, lapachol.
Type of the tested cancer cells: Melanoma and renal carcinoma.
Reference: Lemmens and Bunyapraphatsara, 2003.

135. **Lantana camara** L. var. **aculeata** (L.) Moldenke and Moldenke in Dassanayake and Fosberg., Rev. Handb. Fl. Ceylon 4: 225 (1985). [Photo 94]
Synonyms: Lantana camara L. (1753), *Lantana aculeata* L. (1753), *Lantana scabrida* Soland *ex* Ait. (1789), *Lantana maxima* Tourn. (1876).
Family: Verbenaceae.
Local names: Lantana, Guay Ganda, Punchphuli, Karnaphuli.

Description: A prickly shrub; flowers mostly yellow, turning to red, later on scarlet; fruits fleshy, shining dark green but black when ripe.
Distribution outside Bangladesh: A native of tropical America and naturalized in many tropical and subtropical regions.
Distribution in Bangladesh: It is found throughout the country.
Parts used: Root and leaves.
Active compound: Verbascoside.
Reference: de Padua *et al.*, 1999.

136. **Leonurus sibiricus** L., Sp. Pl. 2: 584 (1753). [Photo 95]
Family: Lamiaceae.
Local name: Roktodron.
Description: A stout herb; stem 4-angled; inflorescence of axillary whorls; lower lip dull red, often white; nutlets triquetrous.
Distribution outside Bangladesh: India, Myanmar, tropical Asia, Africa and America.
Distribution in Bangladesh: It is found almost throughout the country.
Parts used: Above ground parts.
Type of the tested cancer cells: Pregnancy-dependent mammary tumours.
Reference: Nagasawa *et al.*, 1990.

137. **Lindernia procumbens** (Krocker) Philcox, Taxon 14: 30 (1965).
Synonyms: Anagalloides procumbens Krocker (1790), *Vandellia erecta* Benth. (1835), *Vandellia pyxidaria* (L.) Maxim. (1875).
Family: Scrophulariaceae.
Local name: Bakpuspa.
Description: A small herb; corolla white, pink to purple; capsules globose; seeds reticulate.
Distribution outside Bangladesh: Afghanistan, China, India, Indonesia, Japan, Kazakhistan, Laos, Nepal, Pakistan, Russia, South Europe, Tajikistan, Thailand and Vietnan.
Distribution in Bangladesh: This species is available all over the country.
Parts used: Whole plant.
Active compound: 2 oleanane-type triterpene saponins, linderniosides A and B.
Reference: van Valkenburg and Bunyapraphatsara, 2002.

138. **Lobelia radicans** Thunb., Trans. Linn. Soc. 2: 330 (1794). [Photo 96]
Synonym: Lobelia chinensis Lour. (1790).
Family: Campanulaceae.
Description: A prostrate herb; flowers solitary; corolla green with pink marks, rose or rosy-white; fruits obconical.

Distribution outside Bangladesh: India, China, Japan and Indonesia.
Distribution in Bangladesh: Gazipur and Mymensingh districts, planted in Dhaka University garden.
Parts used: Whole plant.
Active compound: Lobelanidine, lobeline and lobelanine.
Reference: Chen *et al*., 2014.

139. **Lonicera japonica** Thunb., Fl. Jap.: 89 (1784). [Photo 97]
Family: Caprifoliaceae.
Local name: Sada Lanichera.
Description: A scandent shrub; flowers paired; corolla 2-lipped, opening white and fading yellow.
Distribution outside Bangladesh: China, Japan, Korea and cultivated in temperate regions.
Distribution in Bangladesh: Although this species was recorded from Bangladesh, the exact locality was not cited (Dey, 2006).
Part used: Stem.
Active compound: 7,7"-dimethyllanaraflavone, agathisflavone and 7"-methylagathisflavone.
Type of the tested cancer cells: HT-29 colon adenocarcinoma, NCl-H460 non-small cell lung carcinoma, MCF-7 breast cancer cell, OVCAR-3 ovarian adenocarcinoma cells, and RXF-393 renal cell carcinoma.
Reference: Pradhan *et al.*, 2009.

140. **Ludwigia hyssopifolia** (G. Don) Exell apud A. and R. Fernandes, Garcia de Orta. 5: 471 & 474, t. 2 (1957). [Photo 98]
Synonyms: Jussiaea linifolia Vahl (1798), *Jussiaea hyssopifolia* G. Don (1832), *Jussiaea fissendocarpa* Haines (1920).
Family: Onagraceae.
Description: A herb, woody at the base, sometimes undershrub or trailing; stem angled; flowers yellow; seeds paler brown to brown.
Distribution outside Bangladesh: West tropical Africa, South East Asia, Sri Lanka, South West in Peninsular India, up to 1000 m extending Northwards to Assam and upper Myanmar, East to Taiwan, throughout Malaysia and Northern Australia, Polynesia and tropical America.
Distribution in Bangladesh: It is distributed all over the country as weed.
Parts used: Whole plant.
Active compound: Piperine.
Type of the tested cancer cells: Agrobacterium tumefaciens-induced crown gall tumour formation in potato disc.

Reference: Das *et al.*, 2007.

141. **Macrosolen cochinchinensis** (Lour.) Van Tiegh., Bull. Soc. Bot. France. 41: 122 (1894).
Synonyms: Loranthus cochinchinensis Lour. (1790), *Loranthus globosus* Roxb. (1832), *Loranthus ampullaceus* Roxb. (1832).
Family: Loranthaceae.
Local names: Chota Banda, Rema, Renda.
Description: A parasitic shrub; petals greenish-yellow, tip purplish; fruits green, yellow and dark violet.
Distribution outside Bangladesh: From Sikkim Himalayas, west and south westwards up to South China, Malay Peninsula, the Philippines and Indonesia.
Distribution in Bangladesh: It occurs throughout the country as parasitic epiphyte.
Parts used: Leaves and stem.
Active compound: L-asparaginase.
Reference: Lemmens and Bunyapraphatsara, 2003.

142. **Mallotus philippensis** (Lamk.) Muell.-Arg., Linnaea 34(1): 196 (1865). [Photo 99]
Synonyms: Croton philippense Lamk. (1786), *Croton punctatus* Retz. (1789), *Rottlera tinctoria* Roxb. (1802), *Echinus philippinensis* Baill. (1866), *Mallotus reticulatus* Dunn (1908).
Family: Euphorbiaceae.
Local names: Kamalaguli, Kamela, Kingur, Sinduri.
Description: A shrub or small tree; young shoot, leaves and inflorescence pubescent; fruits 3-lobed, sometimes 4-lobed; seeds black.
Distribution outside Bangladesh: Widespread from West Himalaya and Sri Lanka to China and throughout South East Asia to East Australia and Malesia.
Distribution in Bangladesh: This species is found in most parts of the country.
Part used: Fruits.
Reference: Lemmens and Bunyapraphatsara, 2003.

143. **Mangifera indica** L., Sp. Pl. 1: 200 (1753). [Photo 100]
Family: Anacardiaceae.
Local name: Aam.
Description: A medium to large-sized tree; petals creamish to pinkish; fruits skin yellowish-green when ripe.
Distribution outside Bangladesh: Native to tropical Asia, particularly the Assam-Myanmar region. It is planted throughout the semi-arid to sub-humid

tropics and subtropics and has become naturalized in many parts of the tropical world.
Distribution in Bangladesh: It is cultivated throughout the country. High quality mangoes are grown in Chapai Nawabganj and Rajshahi districts.
Parts used: Fruit, bark and leaves.
Type of the tested cancer cells: Lung cancer.
Reference: Chanda and Nagani, 2013.

144. **Manihot esculenta** Crantz, Inst. 1: 167 (1766). [Photo 101]
Synonyms: Jatropha manihot L. (1753), *Janipha manihot* (L.) Kunth (1817), *Manihot utilissima* Pohl (1827), *Manihot edule* A. Rich. (1853), *Manihot manihot* (L.) Cockerell (1899).
Family: Euphorbiaceae.
Local names: Kasava, Simul-alu.
Description: A shrub; bark reddish; leaves palmately 3-9 lobed; male flowers greenish, tinged orange and crimson; seeds slightly triangular.
Distribution outside Bangladesh: It is widely cultivated in the tropics of both hemispheres. It is native to tropical Brazil.
Distribution in Bangladesh: This species is cultivated by different ethnic communities in different parts of the country.
Part used: Shoot.
Type of the tested cancer cells: Breast cancer cell lines.
Reference: Hafidh *et al.,* 2009.

145. **Marsdenia tenacissima** (Roxb.) Moon, Cat. Pl. Ceylon: 21 (1824).
Synonym: Asclepias tenacissima Roxb. (1819).
Family: Asclepiadaceae.
Local names: Jitti, Chitti, Siti.
Description: A twining shrub; stem and branches slightly stout and densely tomentose; coronal scales 5; pollinia solitary in each anther sac.
Distribution outside Bangladesh: China, Eastern Himalaya, India, Myanmar, Nepal and Thailand.
Distribution in Bangladesh: Bandarban, Chittagong, Rangamati and Tangail districts.
Part used: Stem.
Active compound: Tenacigenin B ester derivatives, 11α-O-tigloyl-12β-O-acetyltenacigenin B, 11α,12β-di-O-tigloyltenacigenin B, 11α-O-2-methylbutanoyl-12β-O-tigloyltenacigenin B, and 11α-O-(2-methylbutanoyl)-12βO-benzoyltenacigenin B.

Type of the tested cancer cells: KB-3-1, HeLa, HepG2 and K562 cells to paclitaxel treatment.
Reference: Zhu *et al.*, 2014.

146. **Melastoma malabathricum** L., Sp. Pl. 1: 390 (1753). [Photo 102]
Synonyms: Melastoma affine D. Don (1823), *Melastoma polyanthum* Blume (1831), *Melastoma royenii* Blume (1831), *Melastoma ellipticum* Naud. (1849), *Melastoma scabrum* Ridl. (1918).
Family: Melastomataceae.
Local names: Ban-tezpata, Datranga, Lutki.
Description: A shrub; petals pink to mauve or purple; fruits dark purple.
Distribution outside Bangladesh: South East Asia and across Malesia to New Guinea, the Philippines and North Australia.
Distribution in Bangladesh: It is found throughout the country, especially in the hilly areas of greater Sylhet, Chittagong, Dhaka, Mymensingh and Tangail districts, and the Chittagong Hill Tracts.
Part used: Flower.
Reference: Bhanot *et al.*, 2011.

147. **Melia azedarach** L., Sp. Pl. 1: 384 (1753).
Synonyms: Azedarach deleteria Medic. (1787), *Melia sempervirens* (L.) Sw. (1788), *Melia dubia* Cav. (1789), *Melia composita* Willd. (1799), *Melia australis* Sweet (1830), *Azedarach sempervirens* (L.) O. Kuntze (1891).
Family: Meliaceae.
Local names: Goranim, Kawanim, Mahanim, Poma.
Description: A moderate-sized tree; petals white to lilac or bluish; fruits yellowish-brown; seeds brown.
Distribution outside Bangladesh: India, Pakistan, Nepal, Sri Lanka, tropical China, Malaysia, Indonesia, the Philippines, New Guinea, Australia and the Solomon Islands.
Distribution in Bangladesh: This species occurs in most of the districts.
Part used: Leaves.
Reference: Chanda and Nagani, 2013.

148. **Merope angulata** (Willd.) Swingle, Journ. Wash. Acad. Sci. 5: 420 (1915).
Synonyms: Paramignya angulata (Willd.) Kurz (1875), *Paramignya longispina* Hook. f. (1875).
Family: Rutaceae.
Local name: Bon-lebu.
Description: A shrub or small tree; flowers fragrant; petals white; fruits green to yellowish when ripe.

Distribution outside Bangladesh: Eastern India and South East Asia.
Distribution in Bangladesh: Chakaria Sundarbans.
Type of the tested cancer cells: KB, LE and PS388 cell lines.
Reference: Bowen and Lewis, 1978.

149. **Mimosa pudica** L., Sp. Pl. 1: 518 (1753). [Photo 103]
Synonym: Mimosa aspirata Blanco (1837).
Family: Mimosaceae.
Local names: Lajjabati, Sarminda.
Description: A prostrate herb, branches prickly; leaves pinnately compound; corolla pink; fruits dark brown with bright brown prickly bristle margin when dry.
Distribution outside Bangladesh: Pantropical weed of South American origin, distributed to all the tropical countries of the world.
Distribution in Bangladesh: It is a common weed growing all over the country.
Parts used: Aerial parts.
Active compound: 6-glycosylflavone, L-Mimosine.
Type of the tested cancer cells: MCF-7 breast cancer cell line and lymphoma Daudi cells.
Reference: Velusamy *et al.*, 2016.

150. **Mirabilis jalapa** L., Sp. Pl. 1: 177 (1753). [Photo 104]
Family: Nyctaginaceae.
Local names: Sandhyamalati, Krishnakali.
Description: A herb; flowers fragrant, red, magenta, pink, yellow or white, sometimes flowers of different colour on the same plant; perianth funnel-shaped; fruits black when mature.
Distribution outside Bangladesh: A native of South America. Widely cultivated and found as an escape in many tropical areas.
Distribution in Bangladesh: It is a common plant found in gardens.
Part used: Seeds.
Active compound: Protein.
Reference: Watthanachaiyingcharoen *et al.*, 2010.

151. **Momordica charantia** L., Sp. Pl. 2: 1009 (1753). [Photo 105]
Synonyms: Momordica indica L. (1754), *Momordica elegans* Salisb. (1796).
Family: Cucurbitaceae.
Local name: Karala.
Description: A climbing herb; leaves reniform or suborbicular; male flower solitary, yellow, in female flower calyx and corolla as in the male.

Distribution outside Bangladesh: The plant is both wild and cultivated in tropical countries but it is abundant in the Indo-Malayan regions. It was domesticated first possibly in Eastern India and Southern China.
Distribution in Bangladesh: This species is cultivated throughout the country.
Parts used: Ripe fruit.
Active compound: Momordin.
Type of the tested cancer cells: Rat prostate adenocarcinoma and murine lymphoma.
Reference: Jilka *et al.*, 1983.

152. **Morinda citrifolia** L., Sp. Pl. 1: 176 (1753).
Synonyms: Morinda bracteata Roxb. (1824), *Morinda citrifolia* L. var. *bracteata* (Roxb.) Hook. f. (1880).
Family: Rubiaceae.
Local names: Ach, Banach, Barachand, Haldi Kachu, Hardi.
Description: A shrub or small tree; stem 4-angled; corolla infundibular, white; fruits smooth and glossy on fleshy heads.
Distribution outside Bangladesh: Sri Lanka, India, Pakistan, Myanmar, Malaysia, China, Australia and Pacific islands.
Distribution in Bangladesh: Bhola, Chittagong, Cox's Bazar, Dhaka, Dinajpur, Manikganj, Noakhali, Rajshahi and Tangail districts.
Part used: Fruit juice.
Active compound: Polysaccharide rich substance.
Type of the tested cancer cells: 7,12-dimethylbenz(a)anthracene (DMBA)-DNA adduct formation in different organs of female SD rats and male C57 B1-6 mice.
Reference: Wang and Su, 2001.

153. **Moringa oleifera** Lamk., Encycl. 1(2): 398 (1785). [Photo 106]
Synonyms: Moringa pterygosperma Gaertn. (1791), *Moringa polygona* DC. (1825), *Moringa zeylanica* Pers. (1830).
Family: Moringaceae.
Local names: Sajna, Sojne.
Description: A small tree; leaves tripinnately compound; corolla white; fruits pendulous; seeds winged.
Distribution outside Bangladesh: It is indigenous to Indian subcontinent. It is naturalized in many African countries.
Distribution in Bangladesh: This plant is found all over the country, planted mainly for its green fruits.
Part used: Leaves.
Active compound: Gallic acid, quercetin and kaempferol.

Type of the tested cancer cells: Hepatocarcinoma (HepG2), colorectal adenocarcinoma (Caco-2) and breast adenocarcinoma (MCF-7).
Reference: Charoensin, 2014.

154. **Morus alba** L., Sp. Pl. 2: 986 (1753). [Photo 107]
Synonyms: Morus indica L. (1753), *Morus atropurpurea* Roxb. (1832), *Morus morettiana* Jacq. *ex* Burr. (1873).
Family: Moraceae.
Local names: Tunt, Tut.
Description: A small to medium-sized tree; flowers unisexual; fruits pinkish to dark purple when ripe.
Distribution outside Bangladesh: Native of China, now widely cultivated in temperate and tropical regions.
Distribution in Bangladesh: It is cultivated throughout the country, particularly in northern districts.
Part used: Root bark.
Active compound: Morusinol (flavone).
Reference: de Padua *et al.*, 1999.

155. **Murdannia loriformis** (Hassk.) Rolla Rao and Kamm., Bull. Bot. Surv. Ind. 3: 393 (1961).
Synonyms: Aneilema loriforme Hassk. (1852), *Aneilema terminalis* Wight (1853), *Aneilema nudiflorum* R. Br. var. *terminalis* (Wight) C.B. Clarke (1881).
Family: Commelinaceae.
Description: A leafy herb; stem angular; petals bluish or purplish; fruits 3-celled; seeds triangular.
Distribution outside Bangladesh: India (Khasia Hills and Kerala), Sri Lanka and extending eastwards through Indonesia up to China.
Distribution in Bangladesh: In the foothills of Khasia in Sylhet district.
Parts used: Whole plant.
Active compound: Glycosphingolipid (1β-O-D-glucopyranosyl-2-(2'-hydroxy-6'-ene-cosamide)-sphingosine.
Type of the tested cancer cells: Human breast, lung, colon and liver cancer cell lines.
Reference: Jiratchariyakul *et al.*, 1998.

156. **Nerium oleander** L., Sp. Pl. 1: 209 (1753). [Photo 108]
Synonyms: Nerium indicum Mill. (1786), *Nerium odorum* Soland (1789), *Nerium odoratum* Lamk. (1823).

Family: Apocynaceae.
Local names: Karabi, Rakta Karabi.
Description: A shrub; leaves usually 3 in a whorl; flowers red or white, fragrant; seeds with a tuft of hairs at one end.
Distribution outside Bangladesh: China, India, Japan and Mediterranean to Persia.
Distribution in Bangladesh: It is grown in gardens throughout the country.
Parts used: Leaves and flowers.
Type of the tested cancer cells: Lung cancer cells, A549, PC3 and DU145 cell lines.
Reference: Larbie and Abboah-Offei, 2014.

157. **Nigella sativa** L., Sp. Pl. 2: 584 (1753). [Photo 109]
Synonym: Nigella indica Roxb. (1824).
Family: Ranunculaceae.
Local names: Kala-jeera, Mugrala.
Description: A stout herb; leaves decompound; flower single; petals bluish or purplish or white; seeds black.
Distribution outside Bangladesh: South Europe, North Africa and South East Asia.
Distribution in Bangladesh: It is cultivated in many districts.
Part used: Seed.
Type of the tested cancer cells: Colon cancer.
Reference: Chanda and Nagani, 2013.

158. **Ocimum basilicum** L., Sp. Pl. 2: 597 (1753). [Photo 110]
Synonym: Ocimum caryophyllatum Roxb. (1832).
Family: Lamiaceae.
Local name: Babui Tulsi.
Description: A herb; stem quadrangular; corolla white, pubescent outside; nutlets brownish-black, mucilaginous when wet.
Distribution outside Bangladesh: Throughout South Asia reaching eastwards up to China, Formosa and Polynesia.
Distribution in Bangladesh: It is found occasionally in kitchen gardens.
Part used: Leaves.
Active compound: Ursolic acid.
Type of the tested cancer cells: Human cancer cell lines HT-144, MCF-7, NCLH-460 and SF-268 cell lines.
Reference: Nataru *et al.,* 2014.

159. **Ocimum gratissimum** L., Sp. Pl. 2: 1197 (1753). [Photo 111]
Family: Lamiaceae.
Local name: Raam Tulsi.
Description: A shrub; leaves pubescent on both surfaces; nutlets brown, not mucilaginous when wet.
Distribution outside Bangladesh: In the tropics of Africa, America and Asia.
Distribution in Bangladesh: It is found almost all over the country.
Parts used: Stem and leaves.
Type of the tested cancer cells: Breast cancer.
Reference: Chanda and Nagani, 2013.

160. **Ocimum tenuiflorum** L., Sp. Pl. 2: 597 (1753). [Photo 112]
Synonyms: Ocimum sanctum L. (1757), *Geniosporum tenuiflorum* (L.) Merr. (1921).
Family: Lamiaceae.
Local names: Kalo Tulsi, Tulsi.
Description: An aromatic herb; stem quadrangular, hairy, purplish; petals white, often purplish; nutlets pale brown or reddish with small markings.
Distribution outside Bangladesh: Throughout the Old tropics, extending from Arabia to Malay Peninsula, China and Japan, up to Pacific Islands and Australia.
Distribution in Bangladesh: It is cultivated throughout the country.
Type of the tested cancer cells: HeLa cell line.
Reference: Ali *et al.,* 1996.

161. **Ophiorrhiza mungos** L., Sp. Pl.: 178 (1753). [Photo113]
Family: Rubiaceae.
Local names: Gandhanakuli, Kalashona.
Description: A herb or undershrub; corolla white, infundibular; fruits reddish to black.
Distribution outside Bangladesh: Sri Lanka, Bhutan, Nepal, India, Myanmar and Malaysia.
Distribution in Bangladesh: Chittagong district and the Chittagong Hill Tracts.
Parts used: Aerial parts.
Active compound: 10-hydroxy-camptothecin.
Type of the tested cancer cells: Neck, head, colon, lung, ovarian and cervical cancer.
Reference: van Valkenburg and Bunyapraphatsara, 2002.

162. **Oroxylum indicum (**L.) Kurz, Fl. Brit. Burm. 2: 237 (1877). [Photo 114]
Synonyms: Bignonia indica L. (1753), *Bignonia pentandra* Lour. (1790), *Calosanthes indica* (L.) Blume (1826).
Family: Bignoniaceae.
Local names: Thona, Kanaidingi, Sonapatha, Shona.
Description: A small to medium-sized tree; leaves bi- or tripinnately compound; corolla red, nocturnal; fruits boat-shaped, sword-like; seeds winged.
Distribution outside Bangladesh: India, Myanmar, Thailand, China, Cambodia, Malaysia, Indonesia and the Philippines.
Distribution in Bangladesh: It is found in most parts of the country.
Part used: Fruits.
Active compound: Baicalein.
Type of the tested cancer cells: HL-60 cell line.
Reference: Roy *et al.*, 2007.

163. **Paederia foetida** L., Mant. 1: 52 (1767).
Synonyms: Paederia tomentosa Blume (1826), *Paederia sessiliflora* DC. (1832), *Paederia ovata* Miq. (1869), *Paederia foetida* L. var. *microcarpa* Kurz (1877).
Family: Rubiaceae.
Local names: Badali, Gandha Bhadali, Gandha Bhaduli, Gandhal, Madhu Lata.
Description: A twining creeping plant; flowers pink or blue; fruits yellow to reddish-brown.
Distribution outside Bangladesh: India, Nepal, Indo-China, Myanmar, Malaya, Thailand and Indonesia.
Distribution in Bangladesh: It occurs throughout the country.
Part used: Leaves.
Active compound: Paederoside.
Type of the tested cancer cells: Human epidermoid carcinoma of the nasopharynx.
Reference: van Valkenburg and Bunyapraphatsara, 2002.

164. **Peltophorum pterocarpum** (DC.) Backer *ex* K. Heyne, Nutt. Pl. Ned.-Ind. ed. 2. 2: 755 (1927). [Photo 115]
Synonyms: Inga pterocarpa DC. (1825), *Caesalpinia inermis* Roxb. (1832), *Peltophorum inerme* Roxb. (1832), *Peltophorum roxburghii* G. Don (1832), *Peltophorum ferrugineum* (Decne.) Benth. (1864).
Family: Caesalpiniaceae.
Local names: Halud Krishnachura, Aurunjyoti.

Description: A large tree; leaves bipinnately compound; flowers golden-yellow, sweet-scented; fruits shield-shaped, reddish-brown; seeds light brown.
Distribution outside Bangladesh: Native of Andaman's Coast. It is found in Australia, Cambodia, Indonesia, Malaysia, Singapore, Sri Lanka, Thailand and Vietnam.
Distribution in Bangladesh: This species is planted throughout the country, especially in cities.
Part used: Flowers.
Active compound: Terrestribisamide (bisamide alkaloid).
Type of the tested cancer cells: COLO320 colorectal adenocarcinoma cell line.
Reference: Raj *et al.*, 2013.

165. **Persicaria minor** (Huds.) Opiz, Seenam, Rosplin, Kbeteny, Ceske: 72 (1852).
Synonyms: Polygonum minus Huds. (1762), *Polygonum kawagoeanum* Mak. (1914), *Persicaria kawagoeana* (Mak.) Nakai (1926).
Family: Polygonaceae.
Local name: Chhoto-bishkatali.
Description: A small prostrate herb; flowers red, rarely white; tepals 5; nuts black, shining, strongly biconvex to orbicular, rarely trigonous.
Distribution outside Bangladesh: Britain, China, India, Indonesia, Japan, Nepal, the Philippines, Siberia, Sri Lanka and Vietnam.
Distribution in Bangladesh: It is found throughout the country.
Parts used: Aerial parts.
Type of the tested cancer cells: Human cervical carcinoma.
Reference: van Valkenburg and Bunyapraphatsara, 2002.

166. **Persicaria viscosa** (Buch.-Ham. *ex* D. Don) Nakai, Rigakkai 24: 300 (1926). [Photo 116]
Synonym: Polygonum viscosum Buch.-Ham. *ex* D. Don (1825).
Family: Polygonaceae.
Local name: Athalo Bishkatali.
Description: A herb, the entire plant surface is covered with white hairs; ochrea tubular; tepals crimson to red; nuts trigonous, black.
Distribution outside Bangladesh: China, Japan, Nepal and North East India.
Distribution in Bangladesh: Chittagong, Cox's Bazar, Maulvi Bazar, Panchagarh and Sylhet districts.
Parts used: Aerial parts.
Active compound: Tannins, coumarin, quercetin, kaempferol.

Type of the tested cancer cells: Ehrlich ascites carcinoma (EAC) in Swiss albino mice.
Reference: Alam *et al.*, 2014.

167. **Phyla nodiflora** (L.) Greene, Pittonia 4: 46 (1899). [Photo 117]
Synonyms: Verbena nodiflora L. (1753), *Verbena capitata* Forssk. (1775), *Lippia nodiflora* (L.) Rich. (1803).
Family: Verbenaceae.
Local names: Bhuiokra, Karoghar, Bakkam.
Description: A tough creeping herb; stem prostrate; flowers white, rarely pinkish; fruits ovate.
Distribution outside Bangladesh: Tropical and subtropical regions of the world.
Distribution in Bangladesh: It occurs all over the country.
Parts used: Leaves and stem.
Type of the tested cancer cells: Breast cancer cell line (MCF7).
Reference: Lin *et al.*, 2013.

168. **Phyllanthus emblica** L., Sp. Pl. 2: 982 (1753). [Photo 118]
Synonyms: Emblica officinalis Gaertn. (1790), *Dichelactina nodicaulis* Hance (1852).
Family: Euphorbiaceae.
Local names: Amloki, Amla, Ambolati, Awla.
Description: A tree, male flowers yellowish-green; fruits succulent, greenish or yellowish-white; seeds chestnut-brown.
Distribution outside Bangladesh: Cambodia, China, Hong Kong, India, Indonesia, Laos, Malaysia, the Philippines, Sri Lanka and Southern America.
Distribution in Bangladesh: This species is found throughout the country.
Part used: Dry fruits.
Type of the tested cancer cells: Liver cancer.
Reference: Chanda and Nagani, 2013.

169. **Physalis angulata** L., Sp. Pl. 1: 183 (1753). [Photo 119]
Family: Solanaceae.
Local name: Fotka.
Description: A herb; stem quadrangular; flowers greenish-chocolate; petals pale yellow, with or without dark spots; fruits with 10 distinct angles, enveloped in the bladder-like enlarged calyx.
Distribution outside Bangladesh: Native to tropical America, now distributed pantropically, including Malesia.

Distribution in Bangladesh: It occurs throughout the country.
Parts used: Whole plant.
Active compound: Physalin F.
Type of the tested cancer cells: Five human cancer cell lines: HA22T (hepatoma), HeLa (cervix uteri), KB (nasopharynx), Colo-205 (colon) and Calu-1 (lung) and three animal cancer cell lines: H1477 (melanoma), Hep-2 (laryngeal) and 8401 (glioma).
Reference: Chiang *et al.*, 1992.

170. **Piper betle** L., Sp. Pl. 1: 28 (1753). [Photo 120]
Synonyms: Chavica betle (L.) Miq. (1844), *Piper pinguispicum* C. DC. & Koord. (1909).
Family: Piperaceae.
Local names: Pan, Tambuli.
Description: A stout twinning climber; sepals and petals absent; fruit a fleshy drupe.
Distribution outside Bangladesh: A native to Malaysia, widely spread in tropical regions of the world.
Distribution in Bangladesh: It is extensively cultivated as a cash crop throughout the country.
Part used: Leaves.
Active compound: Epigallocatechin gallate, catechin, genistein and quercetin (Phenolic compounds).
Type of the tested cancer cells: Human lung cancer cell line (A549).
Reference: PadmaPriya and Poonguzhali, 2015.

171. **Piper longum** L., Sp. Pl. 1: 29 (1753). [Photo 121]
Synonyms: Piper latifolium Hunter (1809), *Chavica roxburghii* Miq. (1844).
Family: Piperaceae.
Local names: Pipla, Pipla-mul, Pipul, Pipul Morich.
Description: A herb; shoot prostrate; flowers white or pinkish-white; sepals and petals absent; fruits pungent.
Distribution outside Bangladesh: Widely cultivated in India and Sri Lanka but infrequently elsewhere, including South East Asia.
Distribution in Bangladesh: It is found in all areas of the country.
Part used: Fruits.
Active compound: Piperine.
Type of the tested cancer cells: Dalton's lymphoma ascites (DLA) cells and Ehrlich ascites carcinoma (EAC) cells.
Reference: Nataru *et al.*, 2014.

172. **Pisum sativum** L., Sp. Pl.: 727 (1753). [Photo 122]
Family: Fabaceae.
Local names: Motor, Motor-shuti.
Description: A climbing herb; stem weak; leaves pinnately compound; corolla usually white, pink and purple; fruit 2-valved.
Distribution outside Bangladesh: Cosmopolitan.
Distribution in Bangladesh: It is cultivated throughout the country.
Parts used: Root, fruit and seed.
Active compound: Asperagenase enzyme.
Type of the tested cancer cells: L20B tumour cell line.
Reference: Velusamy *et al.*, 2016.

173. **Plumbago zeylanica** L., Sp. Pl. 1: 151 (1753). [Photo 123]
Family: Plumbaginaceae.
Local names: Chita, Chitrak.
Description: A herb or undershrub; flowers white; fruits 5-furrowed; seed solitary.
Distribution outside Bangladesh: South Asia, Malaysia and Hawaii, also found in the tropics of the Old World.
Distribution in Bangladesh: Dhaka, Jamalpur and Panchagarh districts.
Part used: Root.
Active compound: Plumbagin (naphthoquinone).
Type of the tested cancer cells: Dalton's ascitic lymphoma.
Reference: Kavimani *et al.*, 1996.

174. **Polyalthia longifolia** (Sonn.) Thw., Enum. Pl. Zeyl.: 398 (1864). [Photo 124]
Synonyms: Uvaria longifolia Sonn. (1782), *Unona longifolia* (Sonn.) Dunal (1817).
Family: Annonaceae.
Local name: Debdaru.
Description: A tall tree; flowers light green; ripe carpels purple; seed solitary, pinkish or yellowish-white.
Distribution outside Bangladesh: India and Sri Lanka.
Distribution in Bangladesh: It is found in all parts of the country.
Part used: Stem bark.
Active compound: Clerodane diterpenes.
Type of the tested cancer cells: A-549 (human lung carcinoma), MCF-7 (human breast carcinoma), and HT-29 (human colon adenocarcinoma).
Reference: Zhao *et al.*, 1991.

175. **Psidium guajava** L., Sp. Pl. 1: 470 (1753). [Photo 125]
Synonyms: Psidium pyriferum L. (1753), *Psidium pomiferum* L. (1762), *Psidium cujavillus* Burm. f. (1768).
Family: Myrtaceae.
Local names: Piyara, Sabri Aam.
Description: A small tree; flowers white; fruits crowned by the calyx limb, green or yellowish-green when ripe; seeds subreniform, reddish-brown.
Distribution outside Bangladesh: India, Myanmar and other tropical countries.
Distribution in Bangladesh: It is found throughout the country.
Part used: Young branch.
Type of the tested cancer cells: Human colon cancer cell line.
Reference: Manoharan and Kaur, 2013.

176. **Punica granatum** L., Sp. Pl. 1: 472 (1753). [Photo 126]
Family: Punicaceae.
Local name: Dalim.
Description: A shrub or low tree; petals scarlet, wrinkled; fruits yellowish; seeds angular.
Distribution outside Bangladesh: Balkans to Himalayas. Cultivated in a different place in the tropics and subtropics.
Distribution in Bangladesh: This species is found throughout the country.
Part used: Peel of fruits.
Type of the tested cancer cells: Prostate carcinoma cell.
Reference: Chanda and Nagani, 2013.

177. **Raphanus sativus** L., Sp. Pl. 2: 669 (1753).
Synonym: Raphanus caudatus L. (1767).
Family: Brassicaceae.
Local name: Mula.
Description: A herb; roots fleshy, white, pink, red or black; petals purple, pink or sometimes white.
Distribution outside Bangladesh: Native to the Mediterranean region, cultivated worldwide.
Distribution in Bangladesh: It is cultivated all over the country.
Part used: Sprouts.
Active compound: 4-methylthio-3-butenyl isothiocyanate (raphasatin).
Type of the tested cancer cells: Three human colon carcinoma cell lines (LoVo, HCT-116 and HT-29).
Reference: Papi *et al.,* 2008.

178. **Rhinacanthus nasutus** (L.) Kurz, J. Asiat. Soc. Beng. Pt. 2, Nat. Hist. 39: 79 (1870).
Synonyms: Justicia nasuta L. (1753), *Rhinacanthus communis* Nees (1832).
Family: Acanthaceae.
Local names: Palak Jui, Jui-pana.
Description: An undershrub; corolla white or light bluish-tinged; seeds black.
Distribution outside Bangladesh: India, Sri Lanka, Indonesia and Madagascar.
Distribution in Bangladesh: It grows almost all parts of the country.
Part used: Root.
Active compound: Rhinacanthin C.
Type of the tested cancer cells: Human cervical carcinoma HeLa S3 cells.
Reference: Hafidh *et al.*, 2009.

179. **Rhus succedanea** L., Mant. Pl. Alter.: 221 (1771).
Synonyms: Rhus acuminata DC. (1825), *Rhus pubigera* Blume (1826), *Rhus succedanea* L. var. *acuminata* Hook. f. (1876), *Toxicodendron succedanea* (L.) Moldenke (1946).
Family: Acanthaceae.
Local names: Kakrasinghi, Kakrasing.
Description: A middle-sized tree; leaves imparipinnate; flowers light green or yellowish; fruits shining yellow or tan-brown.
Distribution outside Bangladesh: Pakistan, India, Nepal, Bhutan, Myanmar, Thailand, Vietnam, China and Japan.
Distribution in Bangladesh: It was recorded from Sylhet district.
Part used: Fruits.
Active compound: Hinokiflavone.
Type of the tested cancer cells: KB cell line.
Reference: Lin *et al.*, 1989.

180. **Ricinus communis** L., Sp. Pl. 2: 1007 (1753). [Photo 127]
Family: Euphorbiaceae.
Local names: Bherenda, Reri, Venna.
Description: A shrubby or tree-like; male flowers yellowish-green, female flowers purplish; seeds shiny, greyish or silvery.
Distribution outside Bangladesh: This plant is widely cultivated throughout the tropics, subtropics and temperate regions of the world. This species is native to North East tropical Africa.
Distribution in Bangladesh: It is found throughout the country.
Part used: Seeds.
Active compound: Ricin.

Type of the tested cancer cells: Colon cancer cell lines.
Reference: Padala, 2014.

181. **Ruellia tuberosa** L., Sp. Pl. 2: 635 (1753). [Photo 128]
Family: Acanthaceae.
Local name: Chatpoty.
Description: A herb; stem 4-angled; flowers bluish-violet; fruits oblong; seeds orbicular.
Distribution outside Bangladesh: A native of tropical America. It is introduced and naturalized in Africa and South East Asia.
Distribution in Bangladesh: It occurs in most of the areas of the country.
Part used: Bark.
Reference: Bhanot *et al.*, 2011.

182. **Salvia plebeia** R. Br., Prodr. Fl. Nov. Holl.: 500 (1810). [Photo 129]
Family: Lamiaceae.
Local name: Bhui-tulsi.
Description: An aromatic herb; stem obtusely 4-angled; corolla white; nutlets brown, mucilaginous when wetted.
Distribution outside Bangladesh: Afghanistan, India, and extending eastwards up to Malaysia, China and Australia.
Distribution in Bangladesh: It is found in different parts of the country.
Parts used: Aerial parts.
Type of the tested cancer cells: B16 melanoma and P815 mastocytoma.
Reference: Um *et al.*, 1996.

183. **Saraca indica** L., Mant. Pl. 1: 98 (1767). [Photo 130]
Synonyms: Saraca bijuga Prain (1897), *Saraca harmandiana* Pierre (1899), *Saraca pierreana* Craib (1928).
Family: Caesalpiniaceae.
Local names: Ashok, Asoka.
Description: A tree; leaves paripinnately compound; flowers apetalous, orange; fruits oval to oblong-lanceolate.
Distribution outside Bangladesh: South Asia including India, Indonesia, Laos, Malaysia, Myanmar, Sri Lanka and Vietnam.
Distribution in Bangladesh: It is usually planted as an ornamental plant throughout the country.
Part used: Leaves.
Type of the tested cancer cells: Ehrlich ascites carcinoma.
Reference: Manoharan and Kaur, 2013.

184. **Sarcolobus globosus** Wall., Asd. Res. 12: 577, t. 4 (1816).
Family: Asclepiadaceae.
Local names: Baoli Lata, Baoli Phal, Baoni Lata, Haroya.
Description: A twining shrub; stem and branches stout; follicles globose.
Distribution outside Bangladesh: India, Indo-China, Myanmar, Thailand and Malaysia.
Distribution in Bangladesh: Along the coasts of Chittagong, Cox's Bazar and Khulna districts including the Sundarbans.
Part used: Seeds.
Active compound: Two alkaloids and four glycosides.
Type of the tested cancer cells: HeLa and HEP 2.
Reference: Jabbar and Khan, 1979.

185. **Sauropus androgynus** (L.) Merr., Philipp. Bur. For. Bull. 1: 30 (1903).
Synonyms: Clutia androgyna L. (1767), *Sauropus albicans* Blume (1825), *Sauropus sumatranus* Miq. (1860), *Sauropus parviflorus* Pax and Hoffm. (1922).
Family: Euphorbiaceae.
Description: A shrub; flowers reddish-green; fruits white; seeds triquetrous, black.
Distribution outside Bangladesh: Cambodia, China, Hong Kong, India, Indonesia, Laos, Malaysia, New Guinea, the Philippines, Sri Lanka, Thailand and Vietnam.
Distribution in Bangladesh: This species is found in Sylhet district.
Part used: Root.
Type of the tested cancer cells: Breast cancer cell lines.
Reference: Hafidh *et al.*, 2009.

186. **Scoparia dulcis** L., Sp. Pl. 1: 116 (1753). [Photo 131]
Synonyms: Gratiola micrantha Nutt. (1822), *Scoparia grandiflora* Nash (1896).
Family: Scrophulariaceae.
Local names: Bondhone, Bandhuni.
Description: An erect herb; leaves arranged in whorls; corolla white; seeds dull brown.
Distribution outside Bangladesh: Throughout the tropics and subtropics.
Distribution in Bangladesh: This species is common throughout the country.
Parts used: Aerial parts.
Active compound: Scopadulcic acid B.

Type of the tested cancer cells: 12-O-tetradecanoylphorbol-13-acetate (TPA) enhanced phospholipid synthesis in cultured cells and also suppressed the promoting effect of TPA on skin tumour formation in mice.
Reference: Nishino *et al.*, 1993.

187. **Semecarpus anacardium** L. f., Suppl. Pl.: 182 (1781). [Photo 132]
Synonyms: Anacardium officinarum Gaertn. (1788), *Anacardium latifolium* Lamk. (1789), *Semecarpus latifolius* Pers. (1805).
Family: Anacardiaceae.
Local names: Bela, Bhela, Beda, Bhelatuku.
Description: A small to medium-sized tree; leaves crowded at the end of branches; petals greenish-white; fruits purplish-black when ripe.
Distribution outside Bangladesh: India and northern Australia.
Distribution in Bangladesh: Sylhet and Mymensingh districts and the Chittagong Hill Tracts and Madhupur National Park and Madhupur forest areas.
Part used: Dry fruits.
Type of the tested cancer cells: Acute myeloblastic leukemia, chronic myelogenic leukemia, breast adenocarcinoma, cervical epithelial carcinoma and colon carcinoma cancer cell lines.
Reference: Chanda and Nagani, 2013.

188. **Senna alata** (L.) Roxb., Fl. Ind. 2: 349 (1832). [Photo 133]
Synonym: Cassia alata L. (1753).
Family: Caesalpiniaceae.
Local name: Dadmardan.
Description: A soft wooded shrubby plant; leaves paripinnately compound; flowers bright yellow; fruits turned black when ripe.
Distribution outside Bangladesh: Pantropical.
Distribution in Bangladesh: It is found in most districts.
Part used: Leaves.
Reference: de Padua *et al.*, 1999.

189. **Senna tora** (L.) Roxb., Fl. Ind. 2: 340 (1832). [Photo 134]
Synonyms: Cassia tora L. (1753), *Cassia humulis* Colladon (1816).
Family: Caesalpiniaceae.
Local names: Teraj, Araj, Chakunda, Kalkasham.
Description: A herb or undershrub; leaves paripinnately compound; flowers yellow; fruit a pod, straight or curved.
Distribution outside Bangladesh: Bhutan, India, Malaysia, Nepal, Pakistan, the Philippines and Thailand.

Distribution in Bangladesh: The species is commonly found all over the country.
Part used: Leaves.
Type of the tested cancer cells: Human cervical cancer cell lines (HeLa).
Reference: Nataru *et al.,* 2014.

190. **Smilax glabra** Wall. *ex* Roxb., Fl. Ind. ed. 3: 792 (1832).
Family: Smilacaceae.
Description: A climber; in male flowers perianth obtusely trigonous, pale greenish, in female flowers perianth pale, tepals incurved; fruits blueish-black at maturity.
Distribution outside Bangladesh: India, Myanmar, Cambodia, Laos, Vietnam, Thailand, China and Taiwan.
Distribution in Bangladesh: It is found in Sylhet district.
Part used: Rhizome.
Type of the tested cancer cells: HepG2 cell line.
Reference: Hafidh *et al.,* 2009.

191. **Solanum nigrum** L., Sp. Pl. 1: 186 (1753).
Family: Solanaceae.
Local names: Tit-begun, Kakmachi.
Description: A herb; flowers campanulate; corolla white or rarely tinged with purple; fruits dull purple-black; seeds light brown.
Distribution outside Bangladesh: India, Pakistan and Sri Lanka.
Distribution in Bangladesh: Although this species was recorded by most of the previous workers as a common weed, its occurrence in Bangladesh is doubtful, may be quite rare (Hassan, 2008).
Parts used: Fruits and leaves.
Active compound: Steroidal glycoalkaloids.
Type of the tested cancer cells: Breast (MCF-7) and liver (HepG-2) cancer cell lines.
Reference: El-Hawary *et al.,* 2015.

192. **Solanum virginianum** L., Sp. Pl. 1: 187 (1753). [Photo 135]
Synonyms: Solanum surattense Burm. f. (1768), *Solanum xanthocarpum* Schrad. & Wendl. (1795).
Family: Solanaceae.
Local name: Kantakari.

Description: A prickly herb; flowers pentamerous; corolla purplish-blue to violet; fruits white with green markings when young but light yellow or whitish when ripe.
Distribution outside Bangladesh: India, Pakistan, Sri Lanka, Myanmar and extending to South East Asia and tropical Australia.
Distribution in Bangladesh: It occurs in all parts of the country.
Parts used: Whole plant.
Active compound: Solasodine.
Reference: Roshy *et al.*, 2012.

193. **Solidago virgaurea** L., Sp. Pl.: 880 (1753).
Family: Asteraceae.
Description: An erect herb; inflorescence capitulum; outer florets 1-seriate, female, disc florets many-seriate, corolla of outer florets yellow.
Distribution outside Bangladesh: A native of north temperate regions of the world.
Distribution in Bangladesh: It is cultivated in the gardens.
Parts used: Whole plant.
Active compound: Triterpenoid saponins-virgaureasaponin E.
Type of the tested cancer cells: Sarcoma 180 and in the syngeneic DBA/2-MC.SC-1 fibrosarcoma tumour model.
Reference: Plohmann *et al.*, 1997.

194. **Stereospermum suaveolens** (Roxb.) DC., Prodr. 9: 211 (1838).
Synonyms: Bignonia suaveolens Roxb. (1832), *Tecoma suaveolens* G. Don (1838).
Family: Bignoniaceae.
Local names: Parul, Kam-sonalu, Batsil.
Description: A medium-sized tree; leaves unipinnate and imparipinnately compound; corolla pale or dark purple, fragrant; fruits dark grey or purple.
Distribution outside Bangladesh: India and Sri Lanka.
Distribution in Bangladesh: Madhupur and the Chittagong Hill Tracts forests.
Active compound: Lapachol.
Reference: Evans, 2009.

195. **Streblus asper** Lour., Fl. Cochinch. 2: 615 (1790). [Photo 136]
Synonyms: Trophis aspera Retz. (1789), *Streblus lactescens* Blume (1918), *Diplothorax tonkinensis* Gagnep. (1928).
Family: Moraceae.
Local names: Sheora, Asshaora, Harbi, Harbon, Hekra.

Description: A small tree, branches often drooping, the lower branches prostrate; male flowers fragrant, female flowers green; fruits yellow to orange; seeds greyish-white.
Distribution outside Bangladesh: Bhutan, Cambodia, China, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, the Philippines, Sri Lanka, Thailand and Vietnam.
Distribution in Bangladesh: It is found throughout the country.
Part used: Root bark.
Active compound: Cardiac glycosides.
Type of the tested cancer cells: KB cell line.
Reference: Fiebig *et al.,* 1985.

196. **Symplocos lucida** (Thunb.) Sieb. and Zucc., Fl. Jap. 1: 55, t. 24 (1835).
Synonyms: Laurus lucida Thunb. (1784), *Symplocos japonica* DC. (1844), *Symplocos phyllocalyx* C.B. Clarke (1882), *Symplocos warburgii* Brand (1901).
Family: Symplocaceae.
Local name: Bhauri.
Description: A shrub or tree; flowers often tripartite below; fruits ellipsoid to rarely orbicular; seeds horse-shoe shaped.
Distribution outside Bangladesh: India, Nepal, Bhutan, Myanmar, Thailand, Malay Peninsula, Indonesia, China, Vietnam, Japan and the Philippines.
Distribution in Bangladesh: This plant was reported from Bangadesh without citing any locality (Nayar and Majumdar, 1990).
Parts used: Leaves and stem.
Reference: Lemmens and Bunyapraphatsara, 2003.

197. **Tacca plantaginea** (Hance) Drenth, Blumea 20: 391 (1972). [Photo 137]
Synonym: Schizocapsa plantaginea Hance (1881).
Family: Taccaceae.
Description: A herb; leaves rosulate, involucral bracts 4, arranged in 2 pairs; flowers light green; fruits triangular.
Distribution outside Bangladesh: South China, Vietnam and Laos.
Distribution in Bangladesh: This species has been collected from Rema-Kalenga Wildlife Sanctuary in Habiganj district, now well maintained in Dhaka University Botanic garden.
Parts used: Whole plant.
Active compound: Taccalonolides.
Type of the tested cancer cells: P 388 leukemia cells.
Reference: Chen *et al.*, 1997.

198. **Tamarindus indica** L., Sp. Pl. 1: 34 (1753). [Photo 138]
Synonyms: Tamarindus occidentalis Gaertn. (1878), *Tamarindus officinalis* Hook. f. (1878).
Family: Caesalpiniaceae.
Local names: Ambli, Amli, Tentul, Tentuli.
Description: A large tree; leaves paripinnately compound; flowers pale or golden-yellow; fruits oblong, light brown, sour in taste; seeds reddish-brown to blackish-brown.
Distribution outside Bangladesh: Probably native of tropical Africa, widely cultivated in different parts of the world.
Distribution in Bangladesh: It occurs throughout the country.
Parts used: Leaves, fruit, flower and seed.
Active compound: Limonene, tannin and geraniol.
Reference: Velusamy *et al.*, 2016.

199. **Tephrosia purpurea** (L.) Pers., Syn. Pl. 2: 329 (1807). [Photo 139]
Synonyms: Galega purpurea L. (1753), *Cracca purpurea* L. (1753), *Tephrosia purpurea* (L.) Pers. var. *diffusa* Roxb. (1832), *Tephrosia hamiltonii* Drumm. *ex* Gamble (1918).
Family: Fabaceae.
Local name: Bon-neel.
Description: A herb; flowers purplish or pinkish; fruits linear, slightly curved.
Distribution outside Bangladesh: South Asia, tropical Africa and Australia.
Distribution in Bangladesh: It occurs throughout the country.
Part used: Root.
Type of the tested cancer cells: Oral squamous cell carcinoma.
Reference: Chanda and Nagani, 2013.

200. **Terminalia arjuna** (Roxb. *ex* DC.) Wight & Arn., Prodr.: 314 (1834). [Photo 140]
Synonyms: Pentaptera arjuna Roxb. *ex* DC. (1828), *Terminalia urjan* Royle (1835).
Family: Combretaceae.
Local names: Arjun, Arjuna, Kahu.
Description: A medium-sized to large tree; flowers yellowish-white or pale yellow; fruit a 5-winged nut.
Distribution outside Bangladesh: India, Sri Lanka and Malay Peninsula.
Distribution in Bangladesh: It is commonly found throughout the country, mostly planted.
Part used: Leaves.

Active compound: Triterpenoid.
Type of the tested cancer cells: K562 leukemic cell line.
Reference: Moulisha *et al.*, 2010.

201. **Terminalia chebula** Retz., Obs. Bot. 5: 31 (1788). [Photo 141]
Synonyms: Myrobalanus chebula Gaertn. (1790), *Terminalia tomentella* Kurz (1873).
Family: Combretaceae.
Local names: Haritoki, Gol Haritoki.
Description: A medium to large tree; flowers dull-white to yellowish; fruits pale greenish-yellow, turning blackish when dry; seed solitary, lanceolate.
Distribution outside Bangladesh: India, Malaysia, Myanmar, Sri Lanka and Thailand.
Distribution in Bangladesh: The plant is found in different forests.
Part used: Fruit pericarp.
Active compound: Chebulinic acid.
Type of the tested cancer cells: Colo205, Hop62, HT29, SiHa, MIA-PA-CA-2, DWD, T24, PC3, A549, ZR-75-1, A2780, DU145, MCF7 and K562.
Reference: Manoharan and Kaur, 2013.

202. **Thevetia peruviana** (Pers.) K. Schum., Pflanzenfam. 4(2): 159 (1895). [Photo 142]
Synonyms: Cerbera peruviana Pers. (1805), *Cerbera thevetia* L. (1753), *Thevetia neriifolia* Juss *ex* Steud. (1841).
Family: Apocynaceae.
Local names: Halde Karabi, Kalki Phul, Kanai Phul.
Description: A large shrub or small tree; flowers yellow or orange, occasionally white; fruits shining black.
Distribution outside Bangladesh: Native of tropical America. It is cultivated and naturalized in the tropics.
Distribution in Bangladesh: It is planted throughout the country.
Parts used: Stem and leaves.
Reference: Bhanot *et al.*, 2011.

203. **Tiliacora acuminata** (Lamk.) Hook. f. and Thoms., Fl. Ind. 1: 187 (1855). [Photo 143]
Synonyms: Menispermum acuminatum Lamk. (1797), *Tiliacora racemosa* Colebr. (1822).
Family: Menispermaceae.
Local names: Tiliakora, Tiliakoru, Bhag-lata.

Description: A climbing shrub or liana; leaves papyraceous; male flowers yellow; drupes red.
Distribution outside Bangladesh: India, Nepal, Upper Myanmar and Sri Lanka.
Distribution in Bangladesh: Bagerhat, Dhaka, Faridpur, Khulna, Kushtia and Rajshahi districts.
Part used: Root.
Type of the tested cancer cells: Acute myeloblastic leukemia, chronic myelogenic leukemia, breast adenocarcinoma and cervical epithelial cancer cell lines.
Reference: Chanda and Nagani, 2013.

204. **Tinospora cordifolia** (Willd.) Hook. f. and Thoms., Fl. Ind. 1: 184 (1855). [Photo 144]
Synonyms: Menispermum cordifolium Willd. (1806), *Cocculus convolvulaceus* DC. (1817).
Family: Menispermaceae.
Local names: Ghora-gulancha, Nim-gulancha.
Description: A woody climber, leafless at the time of flowering; leaves broadly cordate; male flowers yellow, in female flowers petals broadly spathulate; fruits red.
Distribution outside Bangladesh: India, Myanmar and Sri Lanka.
Distribution in Bangladesh: Barisal, Chittagong, Comilla, Dhaka and Dinajpur districts.
Parts used: Whole plant.
Type of the tested cancer cells: Skin cancer cells.
Reference: Hafidh *et al.*, 2009.

205. **Trigonella foenum-graceum** L., Sp. Pl.: 777 (1753). [Photo 145]
Family: Fabaceae.
Local name: Methi.
Description: An aromatic herb; leaves pinnately trifoliolate; petals yellowish-white; fruits linear.
Distribution outside Bangladesh: Throughout tropical Asia, Africa, Pakistan, Europe, Arabia and Ethiopia.
Distribution in Bangladesh: It is cultivated in many places of the country.
Part used: Seeds.
Active compound: Lignin.
Reference: Velusamy *et al.*, 2016.

206. **Triumfetta rhomboidea** Jacq., Enum. Syst. Pl.: 22 (1760). [Photo 146]
Synonyms: Bartramia indica L. (1753), *Triumfetta bartramia* L. (1759).
Family: Tiliaceae.
Local name: Bon Okra.
Description: A herb or undershrub; flowers golden-yellow; fruits tomentose.
Distribution outside Bangladesh: South East Asia, tropical and subtropical India, Sri Lanka, Malay Peninsula, China, Africa and America.
Distribution in Bangladesh: It is found throughout the country as a weed.
Part used: Leaves.
Type of the tested cancer cells: Ehrlich ascites carcinoma.
Reference: Nataru *et al.*, 2014.

207. **Triphasia trifolia** (Burm. f.) P. Wils., Torreya 9: 33 (1909).
Synonym: Triphasia aurantifolia Lour. (1790).
Family: Rutaceae.
Local name: Cheeninarangi.
Description: A small tree; leaves trifoliolate; flowers solitary, fragrant; petals white; fruits reddish when ripe; seeds green.
Distribution outside Bangladesh: Probably a native of South East Asia, now it is naturalized and cultivated in many tropical and subtropical regions.
Distribution in Bangladesh: This species is found in the forests of Sylhet district.
Type of the tested cancer cells: KB, LE and PS388 cell lines.
Reference: Bowen and Lewis, 1978.

208. **Tylophora indica** (Burm. f.) Merr., Philipp. J. Sci. 19: 373 (1921). [Photo 147]
Synonyms: Cynanchum indicum Burm. f. (1768), *Asclepias asthmatica* L. f. (1781), *Tylophora asthmatica* (L. f.) Wight and Arn. (1834).
Family: Asclepiadaceae.
Local names: Antamul, Anantamul.
Description: A tall twining shrub; corolla greenish-yellow with 7-8 brown stripes; seeds ovate.
Distribution outside Bangladesh: Tropical and subtropical Asia.
Distribution in Bangladesh: It occurs in many parts of the country.
Part used: Leaves.
Active compound: Tylophorine.
Type of the tested cancer cells: Human umbilical vein endothelial cells *in vitro* and Ehrlich ascites carcinoma tumour *in vivo*.
Reference: Nataru *et al.*, 2014.

209. **Typhonium flagelliforme** (Lodd.) Blume, Rumphia 1: 134 (1837). [Photo 148]

Synonyms: Arum flagelliforme Lodd. (1819), *Typhonium cuspidatum* (Blume) Decaisne (1834).

Family: Araceae.

Local name: Ghechu.

Description: Small tuberous or cormous herb; spathe greenish-white and pinkish; inflorescence surrounded by persistent spathe tube.

Distribution outside Bangladesh: Indo-China, tropical Asia, East Malesia to Australia (Queensland).

Distribution in Bangladesh: It is found throughout the country.

Parts used: Whole plant.

Type of the tested cancer cells: Human breast cancer (MCF-7) cell line.

Reference: Nobakht *et al.,* 2014.

210. **Urginea indica** (Roxb.) Kunth, Enum. Pl. 4: 333 (1843). [Photo 149]

Synonyms: Scilla indica Roxb. (1832), *Scilla coromandeliana* Roxb. (1832), *Urginea coromandeliana* (Roxb.) Hook. f. (1892), *Urginea wightiana* Hook. f. (1892).

Family: Liliaceae.

Local names: Jongli Piyaj, Kanda, Shamudra Piyaj.

Description: A perennial bulbous herb; perianth greenish-white; fruits brownish-yellow; seeds black.

Distribution outside Bangladesh: India, Nepal, Myanmar and Africa.

Distribution in Bangladesh: Only found in the coastal area from Cox's Bazar to Teknaf.

Part used: Bulb.

Type of the tested cancer cells: ER positive and negative breast cancer cell lines (MCF7) and cathepsin B and MMP-9 enzyme inhibition activity.

Reference: Bevara *et al.,* 2012.

211. **Vernonia cinerea** (L.) Less., Linnaea 4(1): 291 (1829). [Photo 150]

Synonyms: Conyza cinerea L. (1753), *Vernonia albicans* auct. *non* DC. (1931).

Family: Asteraceae.

Local names: Kuksim, Chhatokuksim, Shial Lata, Shialmutra, Dankuni.

Description: A herb; flowers purplish or pinkish, sometimes violet-blue; fruits obscurely 3-5 angular.

Distribution outside Bangladesh: It grows all over tropical Asia, Africa, Arabia, West Indies, South America, tropical Australia and Polynesia.

Distribution in Bangladesh: It is found all over the country.
Parts used: Whole plant.
Active compound: Vernolide-A.
Type of the tested cancer cells: C57BL/6 mice B16F-10 melanoma cells and K-562 cells.
Reference: Nataru *et al.*, 2014.

212. **Vitex negundo** L., Sp. Pl. 2: 638 (1753). [Photo 151]
Synonym: Vitex paniculata Lamk. (1788).
Family: Verbenaceae.
Local names: Baro-nishinda, Nishinda.
Description: A large shrub to small tree; leaves 3-5 foliolate; flowers slightly scented, purplish-blue or bluish; corolla hypocrateriform; fruits purple-black when ripe.
Distribution outside Bangladesh: India, Nepal, Bhutan, Indo-China, West Asia, North Africa, Malaysia and Myanmar.
Distribution in Bangladesh: It is found throughout the country.
Part used: Leaf juice.
Type of the tested cancer cells: Dalton's ascitic lymphoma (DAL) in Swiss albino mice and mouse lung fibroblast (L-929) cells.
Reference: Sharma and Kayande, 2009.

213. **Vitex trifolia** L. f., Suppl.: 293 (1781).
Synonyms: Vitex indica Mill. (1768), *Vitex triphylla* Royle (1839).
Family: Verbenaceae.
Local name: Choto Nishinda.
Description: A shrub, sometimes a small tree; leaves 3-foliolate; flowers purplish-blue; corolla hypocrateriform; seeds black when ripe.
Distribution outside Bangladesh: Widespread from Afghanistan to India, Myanmar, Malaya, Indonesia, New Guinea, Nepal, Australia, New Caledonia, China, the Philippines, Japan, Madagascar and Sri Lanka.
Distribution in Bangladesh: In forests of Cox's Bazar, Teknaf and other coastal areas.
Part used: Leaves.
Type of the tested cancer cells: Human breast cancer (MCF-7) and Vero cell line.
Reference: Garbi *et al.*, 2015.

214. **Wattakaka volubilis** (L. f.) Stapf in Curtis, Bot. Mag. Sub t. 8979 (1923).
Synonyms: Asclepias volubilis L. f. (1781), *Hoya viridiflora* R. Br. (1811), *Wattakaka viridiflora* (R. Br.) Hassk. (1857), *Dregea volubilis* (L. f.) Hook. f. (1883), *Marsdenia volubilis* (L. f.) T. Cooke (1904).
Family: Asclepiadaceae.
Local names: Titakunga, Madhumalati, Nakchickna.
Description: A large twining shrub; stem and branches stout or slightly woody; inflorescence drooping; corolla greenish-yellow.
Distribution outside Bangladesh: Throughout tropical and subtropical Asia.
Distribution in Bangladesh: It occurs almost throughout the country.
Part used: Stem.
Active compound: Dregeosides (glycosides).
Type of the tested cancer cells: Ehrlich ascites carcinoma and melanoma B-16 cell lines.
Reference: Yoshimura *et al.,* 1983.

215. **Withania somnifera** (L.) Dunal in DC., Prodr. 13(1): 453 (1852).
[Photo 152]
Synonym: Physalis somnifera L. (1753).
Family: Solanaceae.
Local name: Asvagandha.
Description: An undershrub, thinly woolly; corolla greenish; fruits yellow or orange-yellow; seeds reniform, yellowish-brown.
Distribution outside Bangladesh: Mediterranean region, and South Africa.
Distribution in Bangladesh: Mostly cultivated in Bangladesh.
Parts used: Root and leaves.
Type of the tested cancer cells: Cervical, breast, lung and ovary cancer cell line.
Reference: Manoharan and Kaur, 2013.

216. **Woodfordia fruticosa** (L.) Kurz, J. Asiat. Soc. Beng. 40(2): 56 (1871).
[Photo 153]
Synonyms: Lythrum fruticosum L. (1759), *Grislea tomentosa* Roxb. (1795), *Woodfordia floribunda* Salisb. (1806).
Family: Lythraceae.
Local names: Dhatriphul, Ragkat.
Description: A large shrub; floral tube light red, red-orange, or deep red, greenish basally; seeds reddish-brown.
Distribution outside Bangladesh: Bhutan, China, India, Indonesia, Laos, Myanmar, Nepal, Pakistan and Thailand.

Distribution in Bangladesh: Chittagong, Cox's Bazar and Dhaka districts, and the Chittagong Hill Tracts.
Part used: Dried flowers.
Active compound: Woodfordin A, B and C.
Type of the tested cancer cells: Sarcoma 180 cell line.
Reference: Yoshida *et al.*, 1990.

217. **Xanthium indicum** Koen. *ex* Roxb., Fl. Ind. 3: 601 (1832). [Photo 154]
Synonyms: Xanthium orientale L. (1753), *Xanthium strumarium* L. (1753).
Family: Asteraceae.
Local names: Ghagra, Hagra, Khagra, Chhotoghagra, Ban-okra, Bichhaphal, Baksala.
Description: A pubescent herb; leaves broadly ovate; inflorescence capitulum, male capitulum globose, female capitulum ovoid, covered with spines; fruit a rostra.
Distribution outside Bangladesh: India, Malaysia and Indonesia.
Distribution in Bangladesh: It is found all over the country.
Part used: Leaves.
Active compound: Xanthatin.
Type of the tested cancer cells: Trypanosoma brucei and leukemia HL-60 cell.
Reference: Nibret *et al.*, 2011.

218. **Zanthoxylum rhetsa** (Roxb.) DC., Prodr. 1: 728 (1825). [Photo 155]
Synonyms: Fagara rhetsa Roxb. (1820), *Zanthoxylum budrunga* (Roxb.) DC. (1824), *Zanthoxylum limonella* (Dennst.) Alston (1931).
Family: Rutaceae.
Local names: Bazinali, Bajna, Kantahorina, Tambol.
Description: A medium-sized small tree; leaves paripinnate or imparipinnate; petals white or pale yellow; fruits orange or reddish-yellow when ripe; seeds bluish-black.
Distribution outside Bangladesh: South and South East Asia, the Philippines and southern Papua New Guinea.
Distribution in Bangladesh: In the forests of Chittagong, Cox's Bazar, Sylhet, Dhaka, Gazipur and Mymensingh districts, and the Chittagong Hill Tracts.
Part used: Bark.
Active compound: Lignan and columbamine (alkaloid).
Type of the tested cancer cells: B16-F10 melanoma cancer and normal human dermal fibroblast (HDF) cell lines
Reference: Santhanam *et al.*, 2016.

219. **Zingiber officinale** Rosc., Trans. Linn. Soc. Lond. 8: 348 (1807).[Photo 156]
Synonym: Amomum zingiber L. (1753).
Family: Zingiberaceae.
Local name: Ada.
Description: A small rhizomatous herb; petals creamy-yellow; labellum dark purple with creamy-yellow blotches.
Distribution outside Bangladesh: Widely cultivated throughout tropical Asia.
Distribution in Bangladesh: It is cultivated throughout the country.
Parts used: Leaves and rhizomes.
Active compound: Quercetin.
Type of the tested cancer cells: Human breast carcinoma cell.
Reference: Manoharan and Kaur, 2013.

220. **Ziziphus mauritiana** Lamk., Encycl. Method. Bot. 3: 319 (1789).
Synonyms: Rhamnus jujube L. (1753), *Ziziphus jujube* (L.) Gaertn. (1788).
Family: Rhamnaceae.
Local names: Kul, Boroi.
Description: A small to medium-sized tree; flowers greenish-yellow; fruits green when young, yellowish to reddish when ripe.
Distribution outside Bangladesh: May be originated in the Middle East or in the Indian subcontinent. Now cultivated throughout the tropics and subtropics.
Distribution in Bangladesh: It is cultivated throughout the country.
Parts used: Stem bark and fruit.
Type of the tested cancer cells: Human melanoma cell.
Reference: Bhanot *et al.*, 2011.

Photographs of some anticancerous plants of Bangladesh

Photo 1. *Abrus precatorius* L.

Photo 2. *Acacia nilotica* (L.) Delile subsp. *indica* (Benth.) Brenan

Photo 3. *Acalypha indica* L.

Photo 4. *Acanthus ilicifolius* L.

Photo 5. *Achyranthes aspera* L.

Photo 6. *Acorus calamus* L.

Photo 7. *Agave americana* L.

Photo 8. *Ageratum conyzoides* L.

Photo 9. *Alangium salviifolium* (L. f.) Wangerin

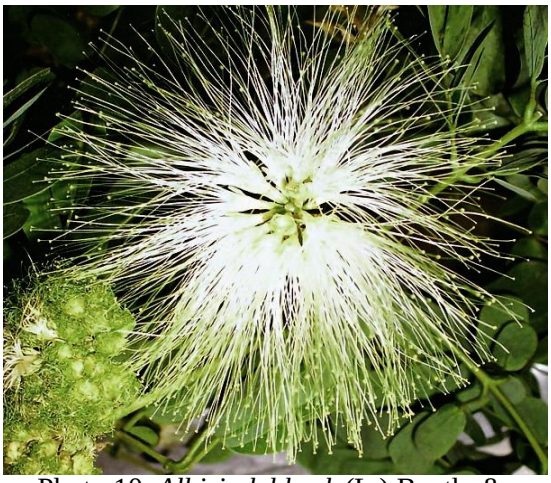


Photo 10. *Albizia lebbeck* (L.) Benth. & Hook.

Photo 11. *Allamanda cathartica* L.

Photo 12. *Aloe vera* (L.) Burm. f.

Photo 13. *Alstonia scholaris* (L.) R. Br.

Photo 14. *Amaranthus spinosus* L.

Photo 15. *Amaranthus tricolor* L.

Photo 16. *Ananas comosus* (L.) Merr.

Photo 17. *Andrographis paniculata* (Burm. f.) Wall. *ex* Nees

Photo 18. *Anisomeles indica* (L.) O. Kuntze

Photo 19. *Annona muricata* L.

Photo 20. *Annona reticulata* L.

Photo 21. *Annona squamosa* L.

Photo 22. *Aphanamixis polystachya* (Wall.) R. N. Parker

Photo 23. *Arachis hypogaea* L.

Photo 24. *Argemone mexicana* L.

Photo 25. *Argyreia nervosa* (Burm. f.) Boj.

Photo 26. *Aristolochia indica* L.

Photo 27. *Artabotrys hexapetalus* (L. f.) Bhandari

Photo 28. *Asclepias curassavica* L.

Photo 29. *Asparagus racemosus* Willd.

Photo 30. *Azadirachta indica* A. Juss.

Photo 31. *Bacopa monnieri* (L.) Pennell

Photo 32. *Bauhinia purpurea* L.

Photo 33. *Bauhinia variegata* L.

Photo 34. *Belamcanda chinensis* (L.) DC

Photo 35. *Benincasa hispida* (Thunb.) Cogn.

Photo 36. *Bixa orellana* L.

Photo 37. *Blumea lacera* (Burm. f.) DC.

Photo 38. *Boerhaavia diffusa* L.

Photo 39. *Butea monosperma* (Lamk.) Taub.

Photo 40. *Caesalpinia pulcherrima* (L.) Swartz

Photo 41. *Cajanus cajan* (L.) Millsp.

Photo 42. *Calendula officinalis* L.

Photo 43. *Calophyllum inophyllum* L.

Photo 44. *Calotropis gigantea* (L.) R. Br.

Photo 45. *Cannabis sativa* L.

Photo 46. *Cardiospermum halicacabum* L.

Photo 47. *Carthamus tinctorius* L.

Photo 48. *Cassia fistula* L.

Photo 49. *Catharanthus roseus* (L.) G. Don

Photo 50. *Cayratia trifolia* (L.) Domin

Photo 51. *Centella asiatica* (L.) Urban

Photo 52. *Cerbera odollam* Gaertn.

Photo 53. *Cestrum nocturnum* L.

Photo 54. *Chylocalyx perfoliatus* (L.) Hassk. *ex* Miq.

Photo 55. *Cicer arietinum* L.

Photo 56. *Cleome viscosa* L.

Photo 57. *Cocos nucifera* L.

Photo 58. *Codiaeum variegatum* (L.) A. Juss.

Photo 59. *Coix lachryma-jobi* L.

Photo 60. *Colocasia esculenta* (L.) Schott

Photo 61. *Crinum asiaticum* L.

Photo 62. *Crotalaria spectabilis* Roth.

Photo 63. *Cullen corylifolium* (L.) Medic.

Photo 64. *Curcuma longa* L.

Photo 65. *Curcuma zedoaria* (Christm.) Rosc.

Photo 66. *Cyclea barbata* Miers

Photo 67. *Daucus carota* L.

Photo 68. *Dendrobium nobile* Lindl.

Photo 69. *Dillenia indica* L.

Photo 70. *Diospyros malabarica* (Desr.) Kostel.

Photo 71. *Diospyros montana* Roxb.

Photo 72. *Eclipta alba* (L.) Hassk.

Photo 73. *Emilia sonchifolia* (L.) DC.

Photo 74. *Entada rheedii* Spreng.

Photo 75. *Erythrina variegata* L.

Photo 76. *Ficus racemosa* L.

Photo 77. *Glycosmis pentaphylla* (Retz.) A. DC.

Photo 78. *Gossypium arboreum* L.

Photo 79. *Gossypium herbaceum* L.

Photo 80. *Helianthus annuus* L.

Photo 81. *Heliotropium indicum* L.

Photo 82. *Hibiscus rosa-sinensis* L.

Photo 83. *Hibiscus sabdariffa* L.

Photo 84. *Holarrhena antidysenterica* (L.) Wall. *ex* Decne.

Photo 85. *Hydrocotyle sibthorpioides* Lamk.

Photo 86. *Hygrophila schulli* (Buch.-Ham.) M. R. & S. N. Almeida

Photo 87. *Hyptis suaveolens* (L.) Poit.

Photo 88. *Ichnocarpus frutescens* (L.) R. Br.

Photo 89. *Impatiens balsamina* L.

Photo 90. *Indigofera tinctoria* L.

Photo 91. *Ixora javanica* DC.

Photo 92. *Jatropha curcas* L.

Photo 93. *Jatropha gossypifolia* L.

Photo 94. *Lantana camara* L. var. *aculeata* Moldenke and Moldenke

Photo 95. *Leonurus sibiricus* L.

Photo 96. *Lobelia radicans* Thunb.

Photo 97. *Lonicera japonica* Thunb.

Photo 98. *Ludwigia hyssopifolia* (G. Don) Exell apud A. and R. Fernandes

Photo 99. *Mallotus philippensis* (Lamk.) Muell.-Arg.

Photo 100. *Mangifera indica* L.

Photo 101. *Manihot esculenta* Crantz

Photo 102. *Melastoma malabathricum* L.

Photo 103. *Mimosa pudica* L.

Photo 104. *Mirabilis jalapa* L.

Photo 105. *Momordica charantia* L.

Photo 106. *Moringa oleifera* Lamk.

Photo 107. *Morus alba* L.

Photo 108. *Nerium oleander* L.

Photo 109. *Nigella sativa* L.

Photo 110. *Ocimum basilicum* L.

Photo 111. *Ocimum gratissimum* L.

Photo 112. *Ocimum tenuiflorum* L.

Photo 113. *Ophiorrhiza mungos* L.

Photo 114. *Oroxylum indicum* (L.) Kurz

Photo 115. *Peltophorum pterocarpum* (DC.) K. Heyne

Photo 116. *Persicaria viscosa* (Buch.-Ham. *ex* D. Don) Nakai

Photo 117. *Phyla nodiflora* (L.) Greene

Photo 118. *Phyllanthus emblica* L.

Photo 119. *Physalis angulata* L.

Photo 120. *Piper betle* L.

Photo 121. *Piper longum* L.

Photo 122. *Pisum sativum* L.

Photo 123. *Plumbago zeylanica* L.

Photo 124. *Polyalthia longifolia* (Sonn.) Thw.

Photo 125. *Psidium guajava* L.

Photo 126. *Punica granatum* L.

Photo 127. *Ricinus communis* L.

Photo 128. *Ruellia tuberosa* L.

Photo 129. *Salvia plebeia* R. Br.

Photo 130. *Saraca indica* L.

Photo 131. *Scoparia dulcis* L.

Photo 132. *Semecarpus anacardium* L. f.

Photo 133. *Senna alata* (L.) Roxb.

Photo 134. *Senna tora* (L.) Roxb.

Photo 135. *Solanum virginianum* L.

Photo 136. *Streblus asper* Lour.

Photo 137. *Tacca plantaginea* (Hance) Drenth

Photo 138. *Tamarindus indica* L.

Photo 139. *Tephrosia purpurea* (L.) Pers.

Photo 140. *Terminalia arjuna* (Roxb. *ex* DC.) Wight & Arn.

Photo 141. *Terminalia chebula* Retz.

Photo 142. *Thevetia peruviana* (Pers.) K. Schum.

Photo 143. *Tiliacora acuminata* (Lamk.) Hook. f. & Thoms.

Photo 144. *Tinospora cordifolia* (Willd.) Hook. f. & Thoms.

Photo 145. *Trigonella foenum-graceum* L.

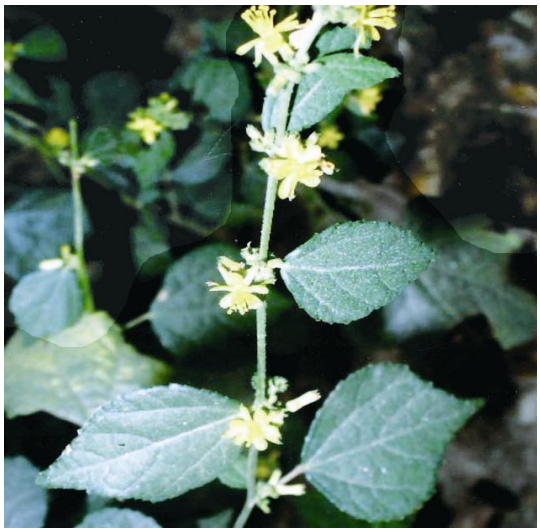


Photo 146. *Triumfetta rhomboidea* Jacq.

Photo 147. *Tylophora indica* (Burm. f.) Merr.

Photo 148. *Typhonium flagelliforme* (Lodd.) Blume

Photo 149. *Urginea indica* (Roxb.) Kunth

Photo 150. *Vernonia cinerea* (L.) Less.

Photo 151. *Vitex negundo* L.

Photo 152. *Withania somnifera* (L.) Dunal

Photo 153. *Woodfordia fruticosa* (L.) Kurz

Photo 154. *Xanthium indicum* Koen. *ex* Roxb.

Photo 155. *Zanthoxylum rhesta* (Roxb.) DC.

Photo 156. *Zingiber officinale* Rosc.

CHAPTER 3

REPRODUCTIVE BIOLOGY OF SOME SELECTED ANTICANCEROUS PLANTS OF BANGLADESH

3.1 Introduction

Study on the reproductive biology of flowering plants includes flower structure, anthesis, receptive capacity of stigmas, mode of pollination and the pollinators. Production of fruits and seeds, successful seed germination and growing up of the seedlings to mature plants, dormancy, viability and suitable timing of sowing and germination, vegetative propagation by rhizome, sucker, stolon, stem cutting or by other parts of the plants. It also may require embryological work. However, here only seed germination experiments were done, also tried for other suitable methods of propagation where seeds do not germinate.

Seed germination may be defined as resumption of metabolic activity and growth of an embryo, resulting in the rupture of the seed coat and emergence of the young plant. Germination involves complex biochemical, physiological and morphological changes within the seed and depends upon seed viability, breaking of dormancy and suitable environmental conditions (Sadhu, 1989). Definite environmental conditioned may be required to break dormancy and other conditions are often required to allow germination after dormancy is broken (Bewley, 1997). Seeds of many species require days, weeks or months at low temperatures to break dormancy (Vleeshouwers *et al.*, 1995), whereas others require warm temperatures for after-ripening to germinate when tolerant conditions arrive (Baskin and Baskin, 1972).

Out of 220 plant species listed as anticancer plants, seed germination experiments on 10 plant species were performed.

3.2 Materials

Earthen tubs (12")

Compost soils

Seeds collected from mature fruits and stored in natural condition at normal temperature

3.3 Method

Earthen tubs (12") were taken and filled with sandy loam soil and compost (1:1). Seeds were sown to a depth of about 1 cm in the earthen tubs. The tubs were kept in semi-shade and watered every day.

Ten seeds per tub were sown at different times of the year to record dormancy, suitable period of germination, percentage of germination, type of seeds, viability and nature of germination.

In case of *Oroxylum indicum,* fruits were collected from Dhaka University Botanic Garden. Germination tests of seeds presented here, were done with one year old seeds as the experimental documents from fresh seeds were lost. Seeds were dried in the sun and stored in plastic containers in the laboratory at the room temperature for future use.

Firstly the seeds were washed with 70% alcohol and distilled water. Then germination tests were performed in the petridishes (8.8 cm diameter) in the laboratory with distilled water. Ten seeds were placed on Whatman No. 1 filter paper moistened with distilled water. The filter paper was not allowed to dry and the dishes were slowly watered by wash bottle when necessary. Number of seeds germinated in each day was counted. The seeds were kept in the petridishes for 7 days to germinate. Percentage of germination was calculated after 7 days and 14 days.

3.4 Results

3.4.1 Seed germination experiment of *Abrus precatorius* L.

Seeds sown immediately after collection in April (21.04.2012) took about 5 days to germinate, whereas seeds sown in July (23.07.2012) took about 7 days to germinate, seeds sown in October (23.10.2012) took about 10 days to germinate and sown in January (24.01.2013) took about 90 days to germinate (Table 4). The study indicates that there is no dormancy period.

Photo 157. Seed germination experiment of *Abrus precatorius* L.

Table 4. Results of seed germination experiment in *A. precatorius* L.

Date of seeds collected	Date of seeds sown	No. of seeds sown	Date of seeds germination	No. of seeds germinated	Days taken to germinate	% of germination
21.04.2012	21.04.2012	10	26.04.2012	1	c. 5	10
	23.07.2012	10	30.07.2012	1	c. 7	10
	23.10.2012	10	03.11.2012	1	c. 10	10
	24.01.2013	10	25.04.2013	1	c. 90	10

In all the cases percentage of germination was 10% (Photo 157). In all the four sets sown only one seed germinated indicating the low rate of germination. Therefore the rate of germination remains uniform throughout the year. However, early sowing may be suggested as it took less time for germination. Seeds remain viable even after 1 year of collection. Nature of germination was hypogeal. 100% of the seedlings survived.

3.4.2 Seed germination experiment of *Aphanamixis polystachya* (Wall.) R. N. Parker

Photo 158. Seed germination experiment of *Aphanamixis polystachya* (Wall.) R. N. Parker

Seeds sown immediately after collection in March (14.03.2012) took about 26 days to germinate, whereas seeds sown in late June (30.06.2012) also took 26 days to germinate but percentage of germination was only 30% indicating starting of loss of viability (Table 5). The study indicates that there is no dormancy period or if any very short.

Table 5. Results of seed germination experiment in *A. polystachya* (Wall.) R. N. Parker.

Date of seeds collected	Date of seeds sown	No. of seeds sown	Date of seeds germination	No. of seeds germinated	Days taken to germinate	% of germination
08.03.2012	14.03.2012	10	09.04.2012	7	c. 26	70
	30.06.2012	10	27.07.2012	3	c. 26	30
	22.09.2012	10	Not germinated	--	--	--
	27.12.2012	10	Not germinated	--	--	--

The percentage of germination was highest (70%) when sown in March (2012) just after collection. Suitable period for germination might be March-April as from the seed sown in late June only 30% germinated.

Viability starts losing after three months after collection. Seeds first sown in March (2012), germinated after 26 days and percentage of germination was 70%. Secondly seeds sown in June (2012), also germinated after 26 days and percentage of germination was 30% (Photo 158). Seeds when sown in

September (2012) and then again in December (2012), did not germinate. So, the seeds loss viability rapidly. Nature of germination was hypogeal. 100% of the seedlings survived.

3.4.3 Seed germination experiment of *Boerhaavia diffusa* L.

The results of seed germination in *B. diffusa* is shown in Table 6:

Photo 159. Seed germination experiment of *Boerhaavia diffusa* L.

Table 6. Results of seed germination experiment in *B. diffusa* L.

Date of seeds collected	Date of seeds sown	No. of seeds sown	Date of seeds germination	No. of seeds germinated	Days taken to germinate	% of germination
17.12.2012	27.12.2012	10	15.04.2013	7	c. 100	70
	27.03.2013	10	10.06.2013	7	c. 70	70
	27.06.2013	10	20.09.2013	6	c. 90	60
	20.12.2013	10	22.03.2014	7	c. 90	70

Seed germination in *B. diffusa* revealed that seeds sown immediately after collection in December (17.12.2012) took about 100 days to germinate, whereas seeds sown in March (27.03.2013) took about 70 days to germinate and seeds sown in June (27.06.2013) and December (20.12.2013) also took about 90 days to germinate (Photo 159). So there at least 4 months dormancy period.

Percentage of germination was more or less uniform (60-70%) as indicate when sown at different intervals throughout the year. Seeds remain viable after 1 year of collection. Nature of germination was hypogeal. 100% of the seedlings survived.

3.4.4 Seed germination experiment of *Calotropis procera* (Ait.) R. Br.

Seeds sown immediately after collection in June (09.06.2012) took about 9 days to germinate, whereas seeds sown in September (22.09.2012) took about 15 days to germinate but percentage of germination was only 40% indicating starting of loss of viability (Table 7). The study indicates that there is no dormancy period or if any very short.

Photo 160. Seed germination experiment of *Calotropis procera* (Ait.) R.

Table 7. Results of seed germination experiment in *C. procera* (Ait.) R. Br.

Date of seeds collected	Date of seeds sown	No. of seeds sown	Date of seeds germination	No. of seeds germinated	Days taken to germinate	% of germination
02.06.2012	09.06.2012	10	18.06.2012	8	c. 9	80
	22.09.2012	10	06.10.2012	4	c. 15	40
	27.12.2012	10	Not germinated	--	--	--
	27.04.2013	10	Not germinated	--	--	--

Percentage of germination was highest (80%) when sown in June (2012) just after collection. Suitable period for germination was June (2012).

Viability starts losing after three months when collection. Seeds first sown in June (2012), germinated after 9 days and percentage of germination was 80%

(Photo 160). Secondly seeds sown in September (2012), germinated after 15 days and percentage of germination was 40%. Seeds when sown in December (2012) and then again in April (2013), seeds did not germinate. So the seeds seeds loss viability rapidly. Nature of germination was hypogeal. 100% of the seedlings survived.

3.4.5 Seed germination experiment of *Jatropha gossypifolia* L.

The results of seed germination in *J. gossypifolia* is shown in Table 8:

Photo 161. Seed germination experiment of *Jatropha gossypifolia* L.

Table 8. Results of seed germination experiment in *J. gossypifolia* L.

Date of seeds collected	Date of seeds sown	No. of seeds sown	Date of seeds germination	No. of seeds germinated	Days taken to germinate	% of germination
26.05.2012	27.05.2012	10	28.09.2012	8	c. 120	80
	22.09.2012	10	16.10.2012	3	c. 24	30
	27.12.2012	10	Not germinated	--	--	--
	27.04.2013	10	Not germinated	--	--	--

Seed germination in *J. gossypifolia* revealed that seeds sown immediately after collection in May (27.05.2012) took about 120 days to germinate, whereas seeds sown in September (22.09.2012) took about 24 days to germinate. So there is at least 3 months dormancy period.

Percentage of germination was highest (80%) when sown in May (2012). Suitable period for germination was May (2012) to September (2012). Viability starts losing after four months after collection. Seeds first sown in May (2012), germinated after 120 days and percentage of germination was 80% (Photo 161). Secondly seeds sown in September (2012), germinated after 24 days and percentage of germination was 30%. Seeds when sown in December (2012) and then in April (2013), seeds did not germinate. So the seeds loss viability rapidly. Nature of germination was hypogeal. 100% of the seedlings survived.

3.4.6 Seed germination experiment of *Leonurus sibiricus* L.

Photo 162. Seed germination experiment of *Leonurus sibiricus* L.

Seeds sown immediately after collection in April (28.04.2012) took about 130 days to germinate, whereas seeds sown in July (29.07.2012) took about 75 days to germinate (Table 9). The study indicates that there is at least 3 to 4 months dormancy period.

Table 9. Results of seed germination experiment in *L. sibiricus* L.

Date of seeds collected	Date of seeds sown	No. of seeds sown	Date of seeds germination	No. of seeds germinated	Days taken to germinate	% of germination
28.04.2012	28.04.2012	10	22.08.2012	4	c. 130	40
	29.07.2012	10	15.09.2012	2	c. 75	20
	22.11.2012	10	Not germinated	--	--	--
	27.03.2013	10	Not germinated	--	--	--

Percentage of germination was highest (40%) when sown in April (2012). Suitable period for germination was April (2012) to August (2012).

Viability starts losing after four months after collection. Seeds first sown in April (2012), germinated after 130 days and percentage of germination was 40%. Secondly seeds sown in July (2012), germinated after 75 days and percentage of germination was 20% (Photo 162). Seeds when sown in November (2012) and then in March (2013), did not germinate. So the seeds loss viability rapidly. Nature of germination was hypogeal. 100% of the seedlings survived.

3.4.7 Seed germination experiment of *Oroxylum indicum* (L.) Kurz

The results of seed germination in *O. indicum* is depicted in Table 10:

Table 10. Results of seed germination experiment in *O. indicum* (L.) Kurz.

Date of seeds sown	No. of seeds sown	Date of seeds germination	No. of seeds germinated	Days taken to germinate	% of germination
07.07.2014	10	13.07.2014	2	6	80%
		14.07.2014	1	7	
		17.07.2014	3	10	
		19.07.2014	1	12	
		20.07.2014	2	13	

From the experiment it revealed that seeds even after one year of storage remain viable for germination (so the seeds are orthodox). Seeds took 6-13 days for germination in average c. 10 days. Germination rate was as high as 80% (Photo 163) and the germination was epigeal.

The self sown seeds also germinated under and near parent plants.

Photo 163. Seed germination experiment of *Oroxylum indicum* (L.) Kurz

3.4.8 Propagation by stem cutting process of *O. indicum* (L.) Kurz

Ten stem cuttings which were cut in the joint of internodes and sown in individual tub in 10.11.2014. They were sown in 12" earthen tubs and a small amount of soil was put on the cut surface. So that water loss be minimized through cut surface and the stem do not dry up. A little leaf branches were seen from the stem cuttings in 13.12.14. Out of ten stem cuttings, eight came out to leafy branches (Photo 164).

Photo 164. Stem cutting process of *Oroxylum indicum* (L.)

3.4.9 Seed germination experiment of *Plumbago zeylanica* L.

Seeds sown immediately after collection in February (10.02.2012) took about 34 days to germinate, whereas seeds sown in May (26.05.2012) took about 11 days to germinate. So there might be at least 1 month dormancy period (Table 11).

Photo 165. Seed germination experiment of *Plumbago zeylanica* L.

Table 11. Results of seed germination experiment in *P. zeylanica* L.

Date of seeds collected	Date of seeds sown	No. of seeds sown	Date of seeds germination	No. of seeds germinated	Days taken to germinate	% of germination
10.02.2012	10.02.2012	10	14.03.2012	8	c. 34	80
	26.05.2012	10	06.06.2012	10	c. 11	100
	22.08.2012	10	Not germinated	--	--	--
	22.12.2012	10	Not germinated	--	--	--

Percentage of germination was highest (100%) when sown in May (2012). Suitable period for germination was May (2012) to June (2012).

Viability starts losing after three months after collection. Seeds first sown in February (2012), germinated after 34 days and percentage of germination was 80%. Secondly seeds sown in May (2012), germinated after 11 days and percentage of germination was 100% (Photo 165). Seeds when sown in August (2012) and then in December (2012), seeds did not germinate. So the seeds loss viability rapidly. Nature of germination was hypogeal. 100% of the seedlings survived.

3.4.10 Seeds germination experiment of *Ricinus communis* L.

Seeds sown immediately after collection in March (28.03.2012) took about 120 days to germinate, whereas seeds sown in June (30.06.2012) took about 14 days to germinate and when sown in September (22.09.2012) took about 15 days to germinate (Table 12). The study indicates that there is at least 4 months dormancy period.

Photo 166. Seed germination experiment of *Ricinus communis* L.

Table 12. Results of seed germination experiment in *Ricinus communis* L.

Date of seeds collected	Date of seeds sown	No. of seeds sown	Date of seeds germination	No. of seeds germinated	Days taken to germinate	% of germination
23.03.2012	28.03.2012	10	20.07.2012	1	c. 120	10
	30.06.2012	10	14.07.2012	4	c. 14	40
	22.09.2012	10	06.10.2012	3	c. 15	30
	22.01.2013	10	Not germinated	--	--	--
	20.04.2013	10	Not germinated	--	--	--

Percentage of germination was highest (40%) when sown in June (2012). Suitable period for germination was June (2012) to July (2012).

Viability starts losing after six months after collection. Seeds first sown in March (2012), germinated after 120 days and percentage of germination was 10%. Secondly seeds sown in June (2012), germinated after 14 days and percentage of germination was 40% and thirdly sown in September (2012), germinated after 15 days and percentage of germination was 30% (Photo 166). Seeds when sown in January (2013) and then again in April (2013), seeds did not germinate. So, the seeds loss viability. Nature of germination was hypogeal. 100% of the seedlings survived.

3.4.11 Seed germination experiment of *Vitex trifolia* L.

The results of seed germination in *V. trifolia* is displayed in Table 13:

Table 13. Results of seed germination experiment in *V. trifolia* L.

Date of seeds collected	Date of seeds sown	No. of seeds sown	Date of seeds germination	No. of seeds germinated	Days taken to germinate	% of germination
02.02.2012	07.02.2012	10	Not germinated	--	--	--
	10.05.2012	10	Not germinated	--	--	--
	10.08.2012	10	Not germinated	--	--	--
	11.11.2012	10	Not germinated	--	--	--

From the germination experiment (Table 12), it was evident that in case of *V. trifolia*, seeds did not germinate. This plant species was propagated by stem cutting process.

3.4.12 Stem cutting process of *V. trifolia* L.

Five stem cuttings of about equal size (24 cm long) were taken. Each cutting bore about 4-5 nodes. They were planted in 12" earthen tubs in February (07.02.2013). New leaves appeared from the nodes in February (25.02.2013) [Photo 167]. All the five stem cuttings producing leafy branches established themselves.

Photo 167. Stem cutting process of *Vitex trifolia* L.

CHAPTER 4
PREVIEW OF TWO SELECTED ANTICANCEROUS PLANTS OF BANGLADESH

4.1 Introduction

The ability to manufacture a wide variety of chemical compounds that are used to achieve important biological functions, and to look after against attack from predators such as insects, fungi and herbivorous mammals is called herbal medicine. Many of these phytochemicals have beneficial effects on long-standing health when consumed by humans, and can be used to effectively treat human diseases.

Plants are the excellent sources for the detection of pharmaceutical compounds and medicines. Natural products could be probable drugs for humans or live stock species and also these products as well as their analogues can proceed as intermediates for synthesis of useful drugs (Makkar *et al.*, 2009). Plants have been used as a source of medicine from very early age of human civilization, moreover as pure compounds or as standardized extracts (De Pasquale, 1984).

Plants produce diverse types of compounds. Many currently existing pharmaceuticals are derived from the secondary metabolites produced by plants such as opium, aspirin, cocaine and atropine. The phytochemicals are applied mostly for protective and remedial purposes. About 25% of the drugs approved worldwide which come from plants, 121 such active compounds are use currently. Among 252 drugs well thought-out as basic and essential by the World Health Organization (WHO), 11% are utterly of plant origin and a considerable number are synthetic drugs obtained from natural precursors (Rates, 2001).

It is estimated that a total of 5000 species have been studied for medical use (Payne *et al.*, 1991). The NCI (National Cancer Institute, USA) has tested more than 50,000 plant samples for anti-HIV and 33,000 samples for anti-tumour activity (Rates, 2001). The potential use of higher plants as a source of new drugs is still inadequately explored. Of the estimated 250,000 (Ayensu and De Filipps, 1978) 500,000 (Schultes, 1972) plant species, only about 6% have been screened for biological activity, 15%

have been evaluated phytochemically (Verpoorte, 2000) and only about 0.75% herbal drugs have been studied in clinical trials (Ali, 2009). However, the remaining are still unexposed. Between the years 1957 and 1981, the NCI screened around 20,000 plant species from Latin America and Asia for anti-tumour activity, but even these were not screened for other pharmacological activities (Hamburger and Hostettman, 1991).

The main concern is to investigate for potential drugs against tumours, viruses and cardiovascular and tropical diseases. Bioactive plant derived compounds through publications in the last few years, are anti-tumour drugs, antibiotics, drugs active against tropical diseases, contraceptive drugs, anti-inflammatory drugs, immunomodulators, kidney protectors and drugs for psychiatric use (Hamburger and Hostettman, 1991).

Vegetables and fruits include a number of phytochemicals that have antioxidative, antimutagenic and anticarcinogenic effects (Kusamran *et al.*, 1998; Nakamura *et al.*, 1998), making plants useful for treating atherosclerosis, cancer and other diseases in humans. Non-contamination vegetables and fruits become accepted among consumers and plants being used as daily vegetables have increased in many countries (Tukan *et al.*, 1998), but there is minute information about their prospective risk to human health.

World Health Organization (WHO) characterizes medicinal plants as a herbal research which is produced by set up plant materials into various processes. These comprise extraction, fractionation, purification, concentration, or other physical or biological processes (Manoharan and Kaur, 2013). Various substances in plants stated cytotoxic and genotoxic activities and explain correlation with the occurrence of tumours (Ames, 1983). So, consideration on the health benefits and potential toxicity of these plants is important (Yen *et al.*, 2001).

Therefore, the rationality of the present study designated the innovative of developing herbal medicines, which needs a systematic research on indigenous medicinal plants for the benefit of the humanity. Consequently, the present study was aimed at -

- Isolating and characterizing the chemical constituents,
- Exploring the anticancerous activity,

- Studying different pharmacological toxicological and microbiological profile of the crude extracts and its partitionate,
- Discovering the possibility of developing new drug candidate from these plants for the treatment of various diseases.

4.2 Plant Preview

4.2.1 Family: Meliaceae A. L. de Jussieu (1789).

Trees or shrubs. Leaves usually exstipulate, spirally arranged, pinnate, leaflets usually entire. Inflorescence thyrsoid, racemose or spicate, sometimes reduced to fascicles or solitary flower. Flowers hermaphrodite, more usually unisexual, with well-developed rudiments of opposite sex. Calyx usually lobed, sometimes with discrete sepals. Petals 3-7 (-14), usually in 1 whorl, green, white, cream, pink, violet or yellow. Stamens usually partially or completely united by a tube with or without lobes, anthers 3-10, usually in 1 whorl. Ovary 1-6 locular, ovules 1-many in each locule. Fruit a capsule, berry or drupe. Seeds with fleshy aril or sarcotesta or a combination of these, endosperm usually absent.

Known as "The Mahogany Family" the Meliaceae consists of 51 genera and about 550 species and is widely distributed in tropical and sub-tropical areas, with relatively few species in temperate regions. In Bangladesh, this family is represented by 16 genera and 28 species (Ahmed *et al.*, 2009).

4.2.1.1 Genus: Aphanamixis Blume (1825).

Trees. Leaves imparipinnate, leaflets opposite. Inflorescence axillary to supra-axillary. Male flowers in panicles, female and hermaphrodite in long spikes or racemes. Calyx deeply 5-lobed, lobes imbricate. Petals 3, imbricate, united below with base of the staminal tube. Staminal tube globose to deeply cyathiform. Ovary 3-4 locular, each locule with collateral to superposed ovules; styles short; stigmas conical to truncate. Fruit a loculicidal capsule, 2-3 valved, valves 1-2 seeded. Seeds arillate, cotyledons plano-convex.

4.2.1.2 Species: Aphanamixis polystachya (Wall.) R.N. Parker, Ind. For. 57: 486 (1931).

Classification: Classification of *Aphanamixis polystachya* (L.) Kurz proposed by Arthur Cronquist (1981)

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Sapindales

Family: Meliaceae

Genus: *Aphanamixis*

Species: *Aphanamixis polystachya* (Wall.) R. N. Parker

Synonyms: *Aglaia polystachya* Wall. in Roxb., Fl. Ind. 2: 429 (1824), *Aphanamixis timorensis* A. Juss. (1830), *Amoora polystachya* (Wall.) Wight & Arn. *ex* Steud. in Nomencl. ed. 2(1): 78 (1840); *Amoora rohituka* (Roxb.) Wight & Arn., Cat. Indian Pl. 24 (1833); *Amoora timorensis* (A. Juss.) Wight & Arn. *ex* Steud. in Nomencl. ed. 2(1): 78 (1840).

Local names: *Baiddiraj, Pitraj, Royna, Tiktaraj.*

English name: Amoora.

Description: Medium-sized evergreen tree, bark reddish-brown. Leaves 6-10 jugate, red when young, petiolate, leaflets oblong to elliptic-oblong, entire, cuspidate-acuminate, sub-coriaceous. Inflorescence more or less supra-axillary. Flowers sweet-scented, complete, bisexual, zygomorphic. Sepals 5, reddish. Petals 3, cream to yellow or bronze, sometimes tinged red, waxy. Staminal tube nearly as long as petals, cream-coloured; anthers 6. Carpels 3, syncarpus; styles stout; stigmas 3-lobed. Fruit a capsule, obovoid, yellowish at first, pink or red at maturity, 3-valved (Photo 168). Seeds 1-3, plano-convex, covered with brownish-red or orange oily aril. *Flowering and fruiting:* February - May.

Habitat: Lowlands and hill forests, including seasonally flooded forests and secondary forests.

Distribution: India, Pakistan, Nepal, Bhutan, Myanmar and Sri Lanka. In Bangladesh, it occurs in the forests of Chittagong, Cox's Bazar, Gazipur, Mymensingh, Sherpur, Tangail and Sylhet districts, and the Chittagong Hill Tracts.

Economic uses: The timber is used for house construction and is suitable for furniture, boat ribs, vehicle bodies and plywood manufacture. Seeds oil is used as a liniment in rheumatism and as a dressing for wounds. Bark is used for treatment of rheumatism, cold and pain in the chest. Fruits are said to be poisonous (Ahmed *et al.*, 2009).

A. A branch with fruit **B. Leaves**

Photo 168. Aphanamixis polystachya (Wall.) R.N. Parker.
A. a branch with fruit; B. leaves.

Ethnobotanical information: In India, the tribal people *Lodhas* prescribe the aril of the seeds with honey to the children for treatment of enlargement of spleens and livers. The *Santal* ethnic peole use wood extract in the treatment of cancerous wounds. In the Moluccas, the mashed leaves in a water solution are used to control diseases of paddy (Ahmed *et al*., 2009).

4.2.1.3 Reported biological activities of *Aphanamixis polystachya* (Wall.) R.N. Parker

Some reported biological activities of *Aphanamixis polystachya* are presented in Table 14.

Table 14. Some reported biological activities of *Aphanamixis polystachya* (After Mishra *et al.*, 2014).

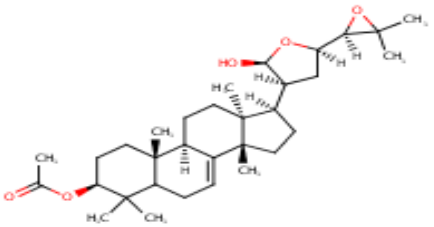
Sl. No.	Functional properties	Plant part	Solvent extract	References
1.	Antioxidant	Fruits	*n*-hexane, ethyl acetate , methanol	Apu *et al.* (2013)
2.	Anticancer	Stem, fruits	Dichloromethane, ethyl acetate, *n*-hexane, methanol	Rabi (1996), Habib *et al.* (2011)
3.	Insecticidal, antifeedant	Seed	Petroleum ether, acetone, ethanol	Talukder and Howse (1993)
4.	Laxative	Stem bark	Petroleum ether, methanol, dichloromethane	Chowdhury and Rashid (2003)
5.	Thrombolytic activity	Fruits	*n*-hexane, ethyl acetate, methanol	Apu *et al.* (2013)
6.	Antimicrobial	Stem bark	Petroleum ether, chloroform, ethanol, hydro methanol	Shaikh *et al.* (2012)

4.2.1.4 Previously isolated phytochemicals from *Aphanamixis polystachya* (Wall.) R.N. Parker

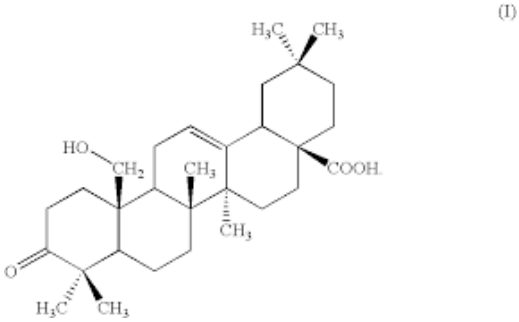
Aphanamixinin, aphanamixin, aphanamixolin, aphanamixolide, aphananin, aphanamixol, amoorinin, prieurianin, amooranin, dammer-(20:21)-ene-(24:25)-epoxy-3β-O-α-Lrhamnopyranosl-(1-4)-b- D-xylopyranoside, 1,5 dihydroxy-6,7,8-trimethoxy-2-methyl-3-O-β-D-xylopyranoside, naringenin 7,4'-dimethylether-5-O-α-L-rhamnopyranoside, poriferastrol-3-rhamnoside, betulin3 b-O-β-D-xylopyranoside, 8-C-methyl-5,7,3',4' tetrahydroxyflavone-3-O-β-D-xylopyranoside, β-sitosterol, stigmasterol, fatty acids and tannins (Fig. 1).

Fruit shell contains triterpenes aphanamixin. Bark contains tetra nortriterpene, aphanamixinin. Leaves contain diterpene, alcohol, aphanamixol and β-sitosterol. Seed yields a limonoid, rohitukin, polystachin and other an alkaloid, a glycoside and a saponin. a chromone and three flavonoid glycosides have been reported from the root (Saboo *et al.*, 2014).

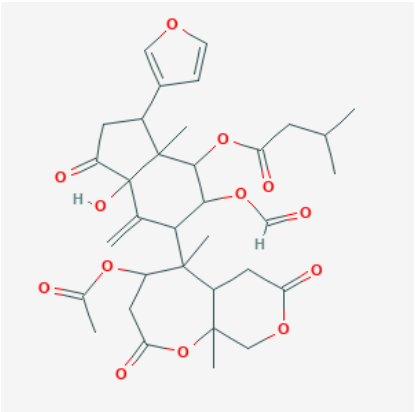
Some reported compounds are as follows:



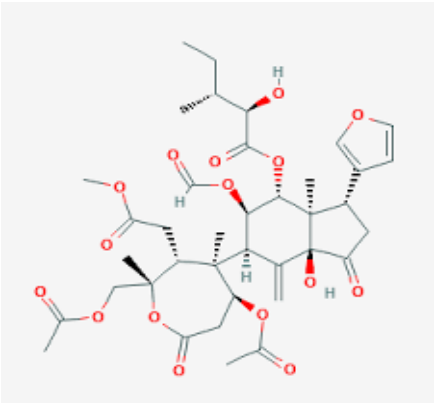
Aphanamixin



Amooranin



Rohitukin



Prieurianin

Fig. 1. Compounds isolated from *Aphanamixis polystachya.*

4.2.2 Family: Bignoniaceae A. L. de Jussieu (1789).

Trees, shrubs or very often woody vines. Leaves opposite or sometimes whorled, rarely alternate, simple or compound. Flowers usually large and showy, mostly in inflorescence, bracteate and bracteolate, bisexual. Calyx of united sepals, campanulate, truncate or 2-5 lobed. Corolla of united petals, campanulate or tubular, usually 5-lobed. Stamens usually 4, didynamous, the fifth one small and staminodal or absent, anthers opening longitudinally. Carpels 2, syncarpous, ovary superior, usually 2-lobed. Fruit a bivalve, septicidal or loculicidal capsule, sometimes fleshy and indehiscent. Seeds in most cases winged.

This family is known as "The Trumpet Creeper Family". The family Bignoniaceae consists of more than 100 genera and c. 800 species, mainly tropical in distribution. In Bangladesh, this family is represented by 15 genera and 17 species (Ahmed *et al*., 2008).

4.2.2.1 Genus: Oroxylum Vent. (1808).

Deciduous trees. Stem glabrous, less branched. Leaves large, 2-3 pinnae, all nodes articulated, leaflefts entire. Inflorescence a terminal raceme. Flowers large, nocturnal. Calyx campanulate, persistent. Corolla of 5 petals, basally united into a tube. Stamens 5. Fruit a capsule, flat, boat or ovoid-shaped, septicidally dehiscent. Seeds thin, discoid, broadly winged, wing hyaline.

4.2.2.2 Species: Oroxylum indicum (L.) Kurz ., Fl. Bri. Burm. 2: 237 (1877).

Classification: Classification of *Oroxylum indicum* (L.) Kurz proposed by Arthur Cronquist (1981)

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Scrophulariales
Family: Bignoniaceae
Genus: *Oroxylum*
Species: *Oroxylum indicum* (L.) Kurz

Synonyms: *Bignonia indica* L., Sp. Pl. 2: 625 (1753), *Bignonia pentandra* Lour., Fl. Cochinch. 2: 379 (1790), *Calosanthes indica* (L.) Blume, Bijdr.: 761 (1826).

Local names: *Kanaidingi, Sonapatha, Thona, Shona.*

English names: Midnight Horror, Broken Bones Plant, Indian Trumpet Flower.

Description: Small to medium-sized deciduous tree. Leaves shortly-petioled, opposite, bi- or tripinnately compound, leaflets broadly ovate, entire. Inflorescence long terminal raceme. Flowers bisexual, complete, zygomorphic, pentamerous. Calyx campanulate, gamosepalous, blackish-purple, caudate-acuminate, fleshy. Corolla with 5 petals, petals united into a tube, lobes 5, upper 3 lobes sub-equal, lower 2 slightly smaller, pinkish-red. Stamens 5, exserted, 4 sub-equal, the 5^{th} shorter; anther 2-celled. Carpels 2, syncarpous, ovary 2-celled; style 1; stigma 2-lobed; placentation axile. Fruit a capsule, flat, boat-shaped, sword-like (Photo 169). Seeds many, winged. *Flowering and fruiting:* June - March.

Habitat: Secondary forests and thickets.

Distribution: India, Myanmar, Thailand, China, Cambodia, Malaysia, Indonesia and the Philippines. In Bangladesh, it is found in most parts of the country.

Economic uses: The bitter bark is used as an astringent and tonic, widely employed for intestinal complaints, diarrhoea and dysentery. A decoction of the dried root bark or stem bark is used in the treatment of urticaria, jaundice, asthma, throat, laryngitis, hoarseness, gastralgia, diarrhoea and dysentery. They have antimicrobial properties against both gram-positive and gram-negetive bacteria. Barks and fruits are also used in tanning and dying (Ahmed *et al*., 2008).

Ethnobotanical information: The *Chakmas* of Chittagong Hill Tracts in Bangladesh cook young pods and flowers as vegetable. Throughout South-east Asia the same use has also been recorded. The leaves are used in anti-rheumatic baths (Ahmed *et al*., 2008).

A. Habit

B. A flower

C. Root bark

D. Fruit and seed (inset)

Photo 169. Oroxylum indicum (L.) Kurz with its different parts.

A. habit; B. a flower; C. root bark; D. fruit and seed (inset).

4.2.2.3 Reported biological activities of *Oroxylum indicum* (L.) Kurz

Some reported biological activities of *Oroxylum indicum* are given in Table 15.

Table 15. Some reported biological activities of *Oroxylum indicum* (After Deka *et al.*, 2013).

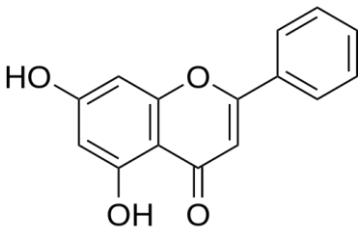
Sl. No.	Functional properties	Plant parts	Solvent extract	References
1.	Antioxidant	Stem, stem bark, leaves, root, root bark, fruits	Ethyl acetate, methanol, ethanol and chloroform	Gupta *et al*. (2008), Mishra *et al*. (2010)
2.	Antimicrobial	Root bark	Ethyl acetate and methanol	Uddin *et al*. (2003)
3.	Antiulcer	Root bark	Ethanol, petroleum ether, *n*-butanol	Khandhar *et al*. (2006)
4.	Anti-inflammatory	Leaves	Aqueous	Laupattarakasem *et al*. (2003)
5.	Anti-hepatotoxic	Leaves	Ethanol	Tenpe *et al*. (2009)
6.	Anticancer	Fruit, Stem bark	Ethanol, Aqueous and methanol	Roy *et al*. (2007), Tepsuwan *et al*. (1992)
7.	Immunomodulatory	Root bark	*n*-butanol	Zaveri *et al*. (2006)
8.	Gastroprotective	Root bark	Alcoholic and *n*-butanol	Zaveri and Jain (2007)
9.	Antimutagenicity	Fruit	Methanol	Nakahara *et al*. (2002)

4.2.2.4 Previously isolated phytochemicals from *Oroxylum indicum* (L.) Kurz

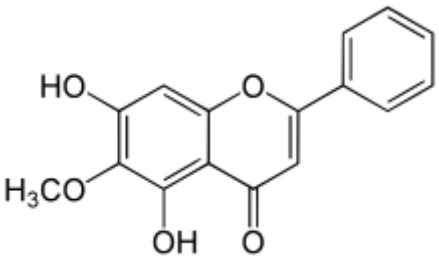
The *Oroxylum indicum* contains number of compounds such as phenols, tannins, alkaloids, flavonoids and saponins. Stem bark and leaves contain flavonoids namely chrysin, oroxylin-A and baicalein, oroxyloside methyl ester and chrysin-7O- methyl glucoside. Seeds contain ellagic acid. Yan *et al*. (2011) reported nineteen different compounds isolated from seeds. Root bark contains chrysin, baicalein, biochanin-A, and ellagic acid (Fig. 2). Oroxylin A, chrysin, triterpene carboxylic acid and ursolic acid are found in fruits. Besides β-Sitosterol, scuttellarien, baicalein-7-Odiglucoside, baicalein-7-Oglucoside, scutellarein-7O-glucopyranoside, aequinetin, chrysin-6-C-β-Dglucopyranosyl-8C-α-L arabinopyranoside, pinocembrin, pinobanksin, lupeol, 2α-Hydroxyl lupeol, echinulin, adenosine, dimethyl sulfone present in different parts of *O. indicum*. Despite twenty seven compounds were reported there is still lack of

knowledge on details of chemical constituents present in different parts of *O. indicum* (Deka *et al.*, 2013).

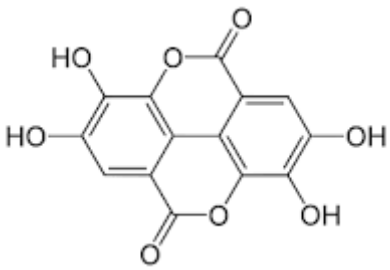
Some reported compounds are as follows:



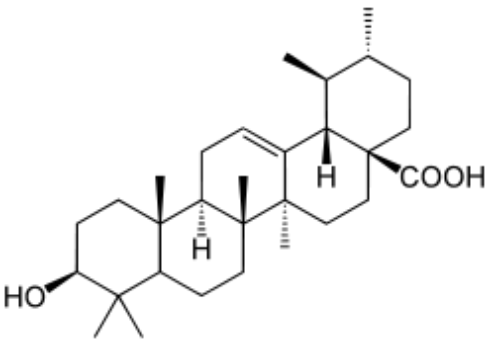
Chrysin



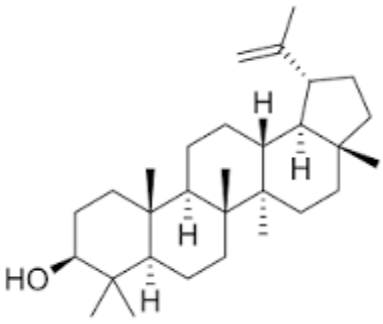
Oroxylin-A



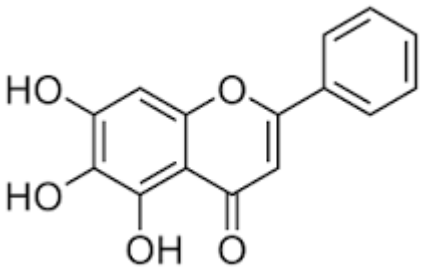
Ellagic acid



Ursolic acid



Lupeol



Baicalein

Fig. 2. Compounds isolated from *Oroxylum indicum*.

CHAPTER 5

MATERIALS AND METHODS

5.1 Materials

Materials and machines used in different methods of chemical investigation are shown in Table 16.

Table 16. Materials and machines used in the present study.

Methods	Materials and machines
Drying	Knife, Paper, Pot, Tray Dryer
Grinding	Grinding machine
Solvent Distillation	Distillation machine, Funnel, Ammonium foil, Amber bottle
Extraction	Amber bottle, Methanol
Filtration (After extraction)	Large funnel, Funnel stand, 500 ml beaker, Cotton, Spatula
Filtration (After PTLC)	Pasteur pipette, Cotton, Vial, EA (Ethyl Acetate)
Rotary Evaporation	Rotary evaporator, Filtrate, 500 ml beaker, Methanol, Cotton
Partitioning	500 ml separating funnel, Funnel stand, 500 ml beaker, 100 ml beaker, Marker pen, Ethyl Acetate, Petroleum Ether, Chloroform, DCM, Water, Tissue paper, Rubber band
Column Chromatography (CC)	Silica column, Column stand, Column grade silica, Sephadex, Cotton, Test tube, Test tube stand, Tissue paper, Rubber band, Marker pen, Air Dried sample, Petroleum Ether, Ethyl Acetate, Methanol
Vacuum Liquid Chromatography (VLC)	VLC Column, Column stand, VLC grade silica, Cotton, Sand, Filter paper, 100 ml beaker, Tissue paper, Rubber band, Marker pen, Electronic pump, Air dried sample, Ethyl Acetate, Petroleum Ether, Methanol
Thin Layer Chromatography (TLC)	TLC Plate, Spotter, Pencil, TLC jar, Ethyl Acetate, Petroleum Ether, Toluene, Chloroform, Methanol, Acetic Acid, UV light, Spray reagent, Dryer
Preparative Thin Layer Chromatography (PTLC)	TLC plate, PTLC jar, Spotter, Pencil, Ethyl Acetate, Petroleum Ether, Toluene, Chloroform, Methanol, UV light, Spray reagent, Dryer
NMR Spectroscopy	NMR machine, Pure compound, Vial

5.2 Methods

5.2.1 Drying

Drying is a mass transfer process in which water or another solvent is removed from a solid, semi-solid or liquid by evaporation. In this investigation process drying was performed in several steps including:

- Sun drying of the collected plant
- After washing any glassware in tray dryer
- Air drying of solvent after partitioning
- Air drying of the solvent after rotary evaporation
- Air drying of the solvent after VLC (Vacuum Liquid Chromatography)
- Air drying of the solvent after CC (Column Chromatography)
- Drying of TLC plate after spraying spray reagent in tray dryer

5.2.2 Grinding

Grinding is an abrasive machining process that uses grinding wheel as the cutting tool. In this process large plant parts are grinded to produce powder to increase the surface area. In the whole investigation process grinding was only used after collection and drying of the plant samples. The dried plant samples were grinded to coarse powder using high capacity grinding machine.

5.2.3 Solvent distillation

Distillation is a method of separating mixtures based on differences in volatility of components in a boiling liquid mixture. Distillation is a physical separation process, and not a chemical reaction. In this chemical and biological investigation process, distillation was used to purify the solvents used in the whole process (Photo 170). Solvents which were distilled are given in Table 17.

Photo 170. Solvent distillation

Table 17. Various solvents and amount distilled.

Solvents	Amount
Methanol	17 L
Petroleum Ether	16 L
Ethyl Acetate	10 L
Chloroform	7.5 L
Dichloromethane	1.5 L
Toluene	1.5 L
n-Hexane	1.5 L

5.2.4 Extraction

Extraction is the crucial step in the analysis of medicinal plants, because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization.

In this investigation process, the powdered plant materials were submerged in a suitable solvent or solvent systems in an air-tight flat bottomed container for several days, with occasional shaking and stirring. The major portion of the extractable compounds of the plant material was dissolved in the solvent during this time and hence extracted as solution.

5.2.5 Filtration

Filtration is a process in which a solid is separated from a fluid (liquid or gas) through a semi-permeable membrane. Filter paper or cotton can be used as semi-permeable membrane. The whole mixture was then filtered through fresh cotton plug (Photo 171).

Photo 171. Filtration process

5.2.6 Rotary evaporation

After filtration and solvent-solvent partitioning, the solvents of various fractions were evaporated by a rotary evaporator. A rotary evaporator is a device used in chemical laboratories for the efficient and gentle removal of solvents from samples by evaporation (Photo 172).

Photo 172. Rotary evaporation

5.2.7 Solvent-solvent partitioning of crude extract

The crude extract were diluted with sufficient amount of aqueous alcohol (90%) and then gently shaken in a separating funnel with almost equal volume of a suitable organic solvent which is immiscible with aqueous alcohol. The mixture was kept undisturbed for several times for separation of the organic layer from the aqueous phase. The materials of the crude extract were partitioned between the two phases depending on their affinity for the respective solvents (Photo 173). The organic layer was separated and this process was carried out thrice for maximum extraction of the samples.

Photo 173. Solvent-solvent partitioning of crude extract

After separating of the organic phase, the aqueous phase thus obtained was successively extracted with other organic solvents, usually of the increasing polarity. The solvents used in this purpose were DCM (dichloromethane), chloroform, ethyl acetate, petroleum ether and methanol. Finally, all the fractions (organic phases as

well as the aqueous phase) were collected separately and evaporated to dryness. These fractions were used for isolation of compounds.

5.2.8 Chromatographic technique

Chromatographic technique is the most useful in the isolation and purification of compounds from plant extracts. The advent of relatively new chromatographic media e.g. Sephadex and Polyamide, have improved the range of separations that can be performed easily.

5.2.8.1 Column Chromatography (CC)

Column Chromatography is the most common separation technique based on the principle of distribution (partition/adsorption) of compounds between a stationary and mobile phase. In this case, a normal Chromatographic column was packed with silica gel (kiesel gel 60, mesh 70-230). A slurry of silica gel in a suitable solvent was added into a glass column of appropriate height and diameter. When the desired height of adsorbent bed was obtained, a few hundred mili-litre of solvent was run through the column for proper packing of the column (Photo 174).

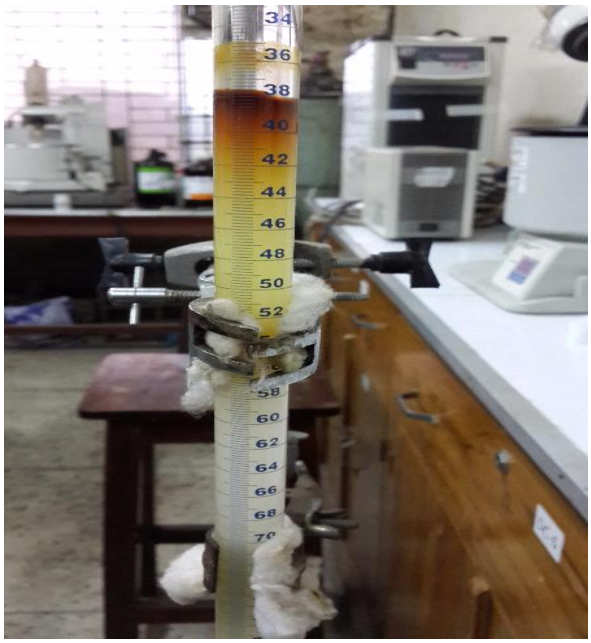


Photo 174. Column Chromatography (CC)

After packing, the sample to be separated was applied as a concentrated solution in a suitable solvent or the sample was adsorbed onto silica gel (kiesel gel 60, mesh 70-230), allowed to dry and subsequently applied on top of the adsorbent layer. Then the column was developed with suitable solvent mixtures of increasing polarity. Elute was collected in test tubes.

A silica column was used to separate and isolate the compounds of the bark of *Aphanamixis polystachya* and a Sephadex column was used to isolate and separate the

compounds of the leaves of *Aphanamixis polystachya* and root bark of *Oroxylum indicum*. Silica gel used as stationary phase and different % combination of PE (petroleum ether) and EA (ethyl acetate) were used as mobile phase.

5.2.8.2 Vacuum Liquid Chromatography (VLC)

Vacuum Liquid Chromatography is a relatively recent separation technique which involves short column chromatography under reduced pressure, the column being packed with fine VLC grade silica (kiesel gel 60H). In this case, the column was packed with silica gel (kiesel gel 60H) under vacuum. The size of the column and the height of the adsorbent layer are dependent upon the amount of extract to be analyzed.

Photo 175. Vacuum Liquid Chromatography

The column was initially washed with a non-polar solvent (petroleum ether) to facilitate compact packing. The sample to be separated was adsorbed onto silica gel (kiesel gel 60, mesh 70-230), allowed to dry and subsequently applied on top of the adsorbent layer (Photo 175). The column was then eluted with a number of organic solvents of increasing polarity and the fractions were collected in 100 ml beakers. Different % combination of PE and EA were used as solvent system.

5.2.9 Thin Layer Chromatography (TLC)

Ascending one-dimensional thin layer chromatographic technique is used for the initial screening of the extracts and column fractions and checking the purity of isolated compounds.

Cylindrical glass chambers (TLC tank) with airtight lid were used for the development of chromate plates.

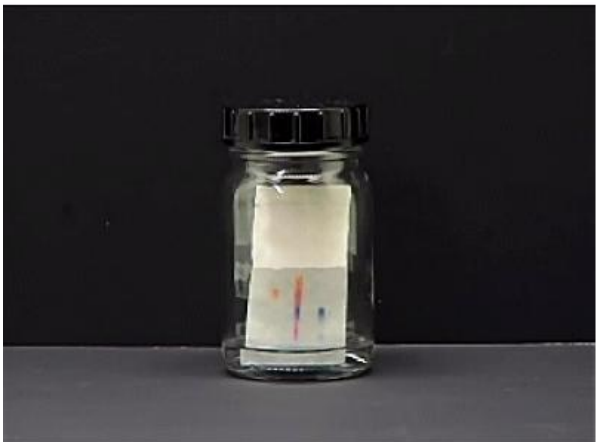


Photo 176. Thin Layer Chromatography (TLC)

The selected solvent system was poured in sufficient quantity into the tank (Photo 176). The tank was then made airtight and kept for few minutes to saturate the internal atmosphere with the solvent vapor. A small amount of dried extract was dissolved in a suitable solvent to get a solution (approximately 1%, Harborne, 1976; Touchstone and Dobbins, 1978). A small spot of the solution was applied on the activated silica plate with a capillary tube just 1 cm above the lower edge of the plate. The spot was dried with a hot air blower and a straight line was drawn 2 cm below the upper edge of the activated plate which marks the upper limit of the solvent flow.

The spotted plate was then placed in the tank in such a way as to keep the applied spot above the surface of the solvent system and the cap/lid was placed again. The plate was left for development. When the solvent front reaches up to the given mark, the plate was taken out and air-dried. The properly developed plates were viewed under UV light of various wave lengths as well as treated with suitable reagents to detect the compounds (Photo 177).

Photo 177. UV light

5.2.10 Preparative Thin Layer Chromatography (PTLC)

The principle of preparative thin layer chromatography is same as that of TLC. Preparative thin layer chromatographic technique was used for final purification of the compounds.

5.2.11 NMR Spectroscopy

NMR spectroscopy was used at the final step of isolation. After finishing PTLC, the pure compounds in the vials were sent to BCSIR (Dhaka), London and Malaysia for NMR spectroscopy. In NMR spectroscopy, $CDCl_3$, was used as solvent and 500 mega Hz was used as applied magnetic field.

For chemical investigation including solvent distillation, extraction, filtration, rotary evaporation, solvent-solvent partitioning of crude extract, chromatographic technique, thin layer chromatography, preparative thin layer chromatography and NMR spectroscopy, Faisal (2012) was followed.

CHAPTER 6

EXPERIMENTAL CHEMICAL

6.1 Chemical investigation of *Oroxylum indicum* (L.) Kurz and *Aphanamixis polystachya* (Wall.) R. N. Parker

6.1.1 Collection, identification and preparation of the plant material

The bark and leaves of *Aphanamixis polystachya* (Wall.) R. N. Parker were collected in June, 2012 from Narsingdhi district and root bark of *Oroxylum indicum* (L.) Kurz was collected in June, 2014 from Gazipur district.

These plants were identified in Dhaka University Salar Khan Herbarium (DUSH), and voucher specimen (Accession no. 42994 for *A. polystachya* and 42995 for *O. indicum*) have been maintained in Bangladesh National Herbarium and DUSH for future reference.

After proper washing, the experimental plant parts were sun dried for several days. The dried plant parts were then grounded to coarse powder using high capacity grinding machine.

6.1.2 Extraction of the plant material

650 gm of each of the powered material was taken in a clean, amber coloured bottle (3 liters) and soaked in 2 liters of methanol. The container with its content was kept for a period of 10 days accompanied by occasional shaking and stirring.

6.1.3 Filtration

The whole mixture was then filtered using fresh cotton plug. The volume of the filtrate was then reduced using a rotary evaporator. The weight of the crude extract was 30 gm for root bark of *O. indicum,* and 35 gm for leaf and 25 gm for bark of *A. polystachya.*

6.1.4 Solvent-solvent partitioning of crude extract

Solvent-solvent partitioning was performed using the protocol designed by Kupchan and modified by Van Wagenen *et al*. (1993). The crude extract (5 gm) was dissolved

in 10% aqueous methanol. It was extracted with petroleum ether, then with chloroform and finally with ethyl acetate. The whole partitioning process is schematically shown in Fig. 3.

This process was repeated for several times and after evaporation the weight of the different fractions obtained is mentioned in Table 18 and 19. All the four fractions were evaporated to dryness (Table 18 and 19) and were used for further analysis.

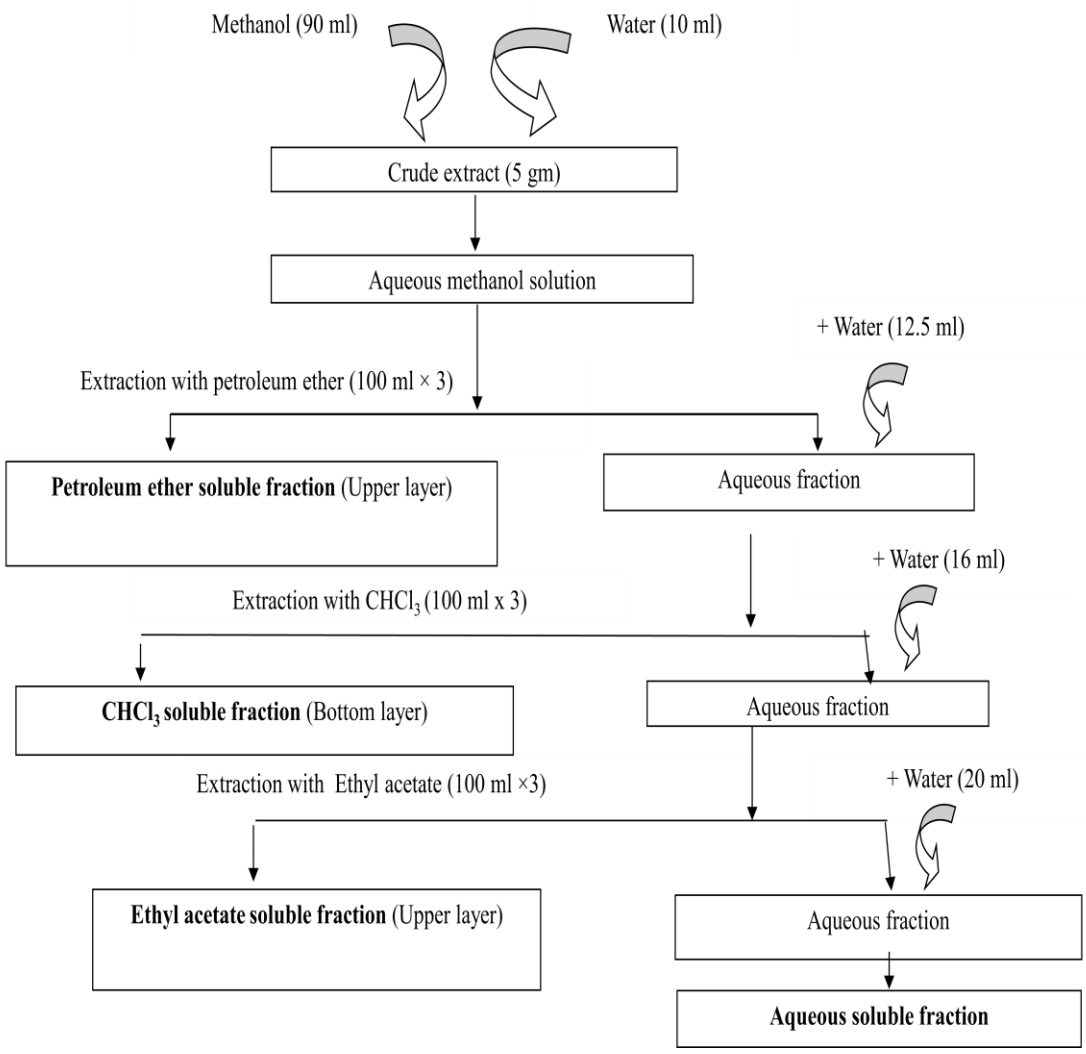


Fig. 3. Schematic representation of the modified Kupchan Partitioning of methanolic crude extract.

Table 18. Amount of partitionates obtained from methanolic extract of root bark of *O. indicum.*

Plant part	Sample code	Fraction	Weight (gm)
Root bark of *Oroxylum indicum*	PESF	Petroleum ether soluble fraction	1.6
	DCMSF	Dichloromethane soluble fraction	1.0
	CHSF	Chloroform soluble fraction	0.5
	EASF	Ethyl acetate soluble fraction	0.4
	AQSF	Aqueous soluble fraction	1.3

Table 19. Amount of partitionates obtained from methanolic extract of bark and leaves of *A. polystachya.*

Plant part	Sample code	Fraction	Weight (gm)
Bark of *Aphanamixis polystachya*	PESF	Petroleum ether soluble fraction	0.75
	CHSF	Chloroform soluble fraction	0.55
	EASF	Ethyl acetate soluble fraction	0.40
	AQSF	Aqueous soluble fraction	2.50
Leaves of *Aphanamixis polystachya*	PESF	Petroleum ether soluble fraction	0.85
	CHSF	Chloroform soluble fraction	0.65
	EASF	Ethyl acetate soluble fraction	0.30
	AQSF	Aqueous soluble fraction	2.65

6.2 Gel Filtration Chromatography (Sephadex) of DCM (dichloromethane) fraction of root bark of *O. indicum*

300 mg of extract was subjected to gel filtration chromatography using lipophilic sephadex LH-20. In this process, lipophilic sephadex LH-20 was used as stationary phase and different solvent system was used as mobile phase (Table 20).

Table 20. Gel Filtration Chromatography (Sephadex) using different solvent systems.

Serial no.	Solvent systems
1	n-Hexane : DCM : Methanol = 2: 5: 1
2	10% Methanol : DCM = 5: 45
3	50% Methanol : DCM = 25: 25
4	100% Methanol
5	50% Methanol : DCM = 25: 25
6	10% Methanol : DCM = 5: 45
7	n-Hexane : DCM : Methanol = 2: 5: 1

DCM = Dichloromethane

6.2.1 Analysis of GFC (Gel Filtration Chromatography) fractions by Thin Layer Chromatography (TLC)

The elutes of GFC fractions were numbered and successively spotted on TLC plates and chromatograms were developed using different solvent systems. The plates were then examined under UV light followed by spraying with 1% vanillin in sulphuric acid reagent. A number of compounds were detected, which were purified from different sub-fractions employing preparative TLC.

6.2.2 Compounds isolated from *O. indicum*

Four compounds were isolated from root bark of *O. indicum*. All compounds were isolated from DCM fraction by gel filtration chromatography over Sephadex-LH20. The compounds were named AR-O17, AR-O18, AR-O23 and AR-O30, respectively (Table 21).

Table 21. Compounds isolated from the root bark (DCM fraction) of *Oroxylum indicum* by gel filtration chromatography.

Beaker/ Test tube	Solvent system	Compound code	Compounds	R_f value	Amount found
Test tube 12	1% Me in $CHCl_3$	AR-O17	5,7-dihydroxy-3-methoxy flavone	0.7	2.5 mg
Test tube 18	3% Me in $CHCl_3$	AR-O18	7-methoxy-3,5-dihydroxyflavone	0.5	4.2 mg
Test tube 20	3% Me in $CHCl_3$	AR-O23	5,7-dihydroxy flavone (chrysin)	0.6	2.3 mg
Test tube 30	3% Me in $CHCl_3$	AR-O30	Kaempferol-3,4',5,7-tetrahydroxy flavonol	0.7	2.7 mg

Me = Methanol; $CHCl_3$= Chloroform

6.3 Vacuum Liquid Chromatography (VLC) of chloroform fraction of leaves of *A. polystachya*

Method developed by Pelletier *et al*. (1986) was followed in this study. Petroleum ether and ethyl acetate were used as solvent systems. The solvent systems employed for VLC analysis of chloroform fraction are presented in Table 22.

Table 22. Different solvent systems used for VLC analysis of chloroform fraction.

Beaker number	Solvent systems	Volume collected (ml)
1	Petroleum ether 100%	150
2	2.5% EA in PE	150
3	5% EA in PE	150
4	7.5% EA in PE	150
5	10% EA in PE	150
6	12.5% EA in PE	150
7	15% EA in PE	150
8	17.5% EA in PE	150
9	20% EA in PE	150
10	25% EA in PE	150

Table 22. Contd.

Beaker number	Solvent systems	Volume collected (ml)
11	30% EA in PE	150
12	35% EA in PE	150
13	40% EA in PE	150
14	50% EA in PE	150
15	75% EA in PE	150
16	Ethyl acetate 100%	150
17	10% MeOH in EA	150
18	15% MeOH in EA	150
19	MeOH 100%	150

EA = Ethyl acetate; PE = Petroleum ether; MeOH = Methanol

6.3.1 Column Chromatography (Sephadex) of the VLC Beakers 12-16

The beakers which showed positive result in screening process were beaker no. 12-16. The extracts from the beakers 12-13 and beakers 14-16 were mixed together in two beakers. Each resulted in 300 mg of extract. This amount was subjected to column chromatography (Sephadex LH-20) [Table 23].

Table 23. Gel Filtration Chromatography (Sephadex) using different solvent systems.

Serial No.	Solvent systems
1	n-Hexane : DCM : Methanol = 2: 5: 1
2	10% Methanol : DCM = 5: 45
3	50% Methanol : DCM = 25: 25
4	100% Methanol
5	50% Methanol : DCM = 25: 25
6	10% Methanol : DCM = 5: 45
7	n-Hexane : DCM : Methanol = 2: 5: 1

DCM = Dichloromethane

6.3.2 Column Chromatography (Silica) of chloroform fraction of bark of *A. polystachya*

In column chromatography, petroleum ether and ethyle acetate were used as solvent systems (Table 24).

Table 24. Column chromatography (Silica) of chloroform fraction.

Test tube	Solvent systems	Volume collected (ml)
--	Petroleum ether 100%	900
--	1% EA in PE	900
--	2% EA in PE	900
1-26	3% EA in PE	900
27-45	4% EA in PE	450
46-64	5% EA in PE	450
65-83	7% EA in PE	450
84-101	9% EA in PE	450
102-120	12% EA in PE	450
121-139	15% EA in PE	450
140-157	17% EA in PE	450
158-175	20% EA in PE	450
176-195	25% EA in PE	450
196-215	27.5% EA in PE	450
216-237	30% EA in PE	450
238-258	32.5% EA in PE	450
259-276	35% EA in PE	450
277-294	37.5% EA in PE	450
295-311	40% EA in PE	450
312-329	45% EA in PE	450
330-345	50% EA in PE	450
346-366	60% EA in PE	450
367-388	70% EA in PE	450
389-409	75% EA in PE	450

Table 24. Contd.

Test tube	Solvent systems	Volume collected (ml)
410-428	Ethyl acetate 100%	450
429-447	2% MeOH in EA	450
448-465	5% MeOH in EA	450
466-483	10% MeOH in EA	450
484-501	20% MeOH in EA	450
502-513	50% MeOH in EA	450
514-524	Methanol 100%	450

EA = Ethyl acetate; PE = Petroleum ether; MeOH = Methanol

6.3.3 Analysis of VLC (Vacuum Liquid Chromatography), GFC (Gel Filtration Chromatography) and Column Chromatography (Silica) fractions by Thin Layer Chromatography (TLC)

The elutes of VLC, GFC and CC fractions were numbered and successively spotted on TLC plates and chromatograms were developed using different solvent systems. The plates were then examined under UV light and then sprayed with 1% vanillin in sulphuric acid reagent. A number of compounds were detected, which were purified from different sub-fractions employing preparative TLC.

6.3.4 Compounds isolated from *A. polystachya*

One compound was isolated from leaves of *A. polystachya*. The compound is named AR-AL25, respectively (Table 25).

Table 25. Compound isolated from the leaves ($CHCl_3$ fraction) of *Aphanamixis polystachya* by gel filtration chromatography.

Beaker/ Test tube	Solvent systems	Compound code	Compound	R_f value	Amount found
Test tube 25	15% EA in PE	AR-AL25	Stigmasterol	0.5	2.5 mg

CHAPTER 7

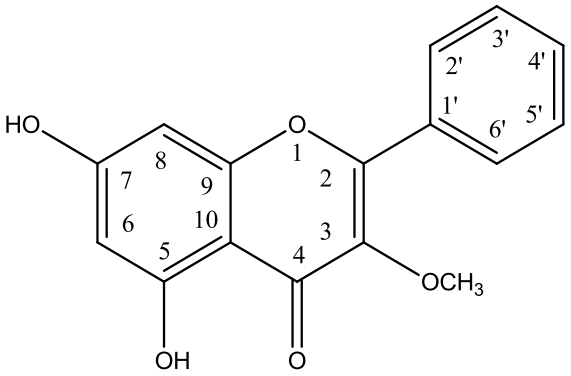
RESULTS AND DISCUSSION OF CHEMICAL INVESTIGATION

7.1 Characterization of AR-017 as 5,7-dihydroxy-3-methoxyflavone (1)

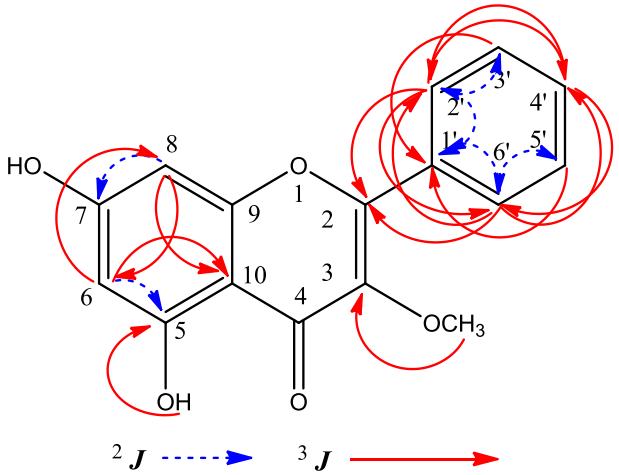
AR-017 was isolated from the dichloromethane soluble fraction of methanolic extract of root bark of *Oroxylum indicum* (L.) Kurz as yellowish crystals. Spraying the developed plate with vanillin-sulfuric acid followed by heating at 105-110°C for few minutes gave a yellow coloured spot. The compound was found to be soluble in chloroform, methanol and ethyl acetate. The R_f value of the compound was 0.7 ($CHCl_3$-MeOH, 99:1) on silica gel PF_{254} plate which is identical to that observed for 5,7-dihydroxy-3-methoxyflavone (**1**) (Kalff and Robinson, 1925).

The ^{13}C NMR (75 MHz, $CDCl_3$) spectrum of the compound (Fig. 7 & 8) showed 13 signals appropriate16 carbons in a flavone moiety. Therefore, it had one methoxy (-OCH_3) at δ 60.9, and seven methines (CH) (δ 93.5, 105.3, 129.6 × 2, 131.9 × 3) and eight quaternary carbons including one carbonyl (C=O) group at δ 183.0.

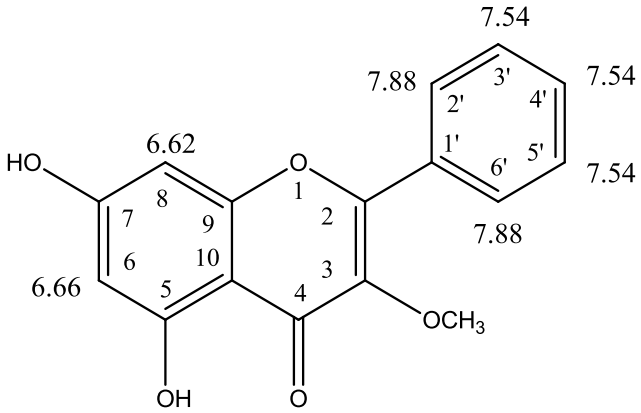
The ^1H NMR (300 MHz, $CDCl_3$) of AR-017 (Fig. 5 & 6) displayed two sharp singlets at δ 4.05 (3H) and 13.01 (1H) which confirmed the presence of a -OCH_3 and a chelated -OH group, respectively. The broad singlet at δ 7.88 integrated for two protons were assigned to the meta coupled protons at C-2' and C-6' position on B ring of flavone nucleus. Therefore, the broad singlet at δ 7.54 that integrated for 3 protons can be assigned to the C-3', C-4' and C-5' protons of the B ring. The singlets at δ 6.66 and 6.62 were assigned to the C-6 and C-8 protons and these were confirmed by the two and three bond HMBC correlations with C-5, C-7 and C-8, C-10 respectively (Fig. 9 & 10). The location of C-2' and C-6' protons was also established by their proton to carbon C-2, C-1', C-4' correlations. The proton to carbon correlations of C-3', C-4' and C-5' confirmed their position on B ring of the flavone nucleus. The placement of -OCH_3 group at C-3 position was confirmed from the HMBC correlation with its respective carbon (Fig. 9 & 10). Therefore, the structure of AR-017 was established as 5,7-dihydroxy-3-methoxyflavone (**1**) [Fig. 4]. The complete ^1H and ^{13}C NMR assignments of AR-017 has been unambiguously resolved by using HMBC spectral data as shown in Table 26.



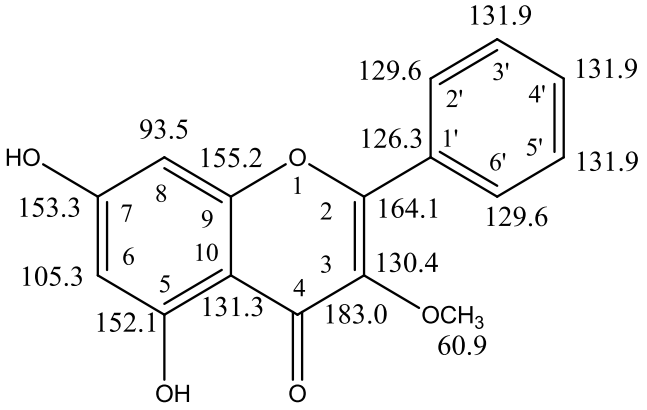
Structure of 5,7-dihydroxy-3-methoxyflavone (**1**)



Key HMBC correlations observed for 5,7-dihydroxy-3-methoxyflavone (**1**)



^{1}H NMR assigment of 5,7-dihydroxy-3-methoxyflavone (**1**)



^{13}C NMR assigment of5,7-dihydroxy-3-methoxyflavone (**1**)

Fig. 4. Structure of 5,7-dihydroxy-3-methoxyflavone (1).

Table 26. ^{1}H ($CDCl_3$, 300 MHz), ^{13}C ($CDCl_3$, 75 MHz) and HMBC spectral data of 5,7-dihydroxy-3-methoxyflavone.

Position no.	δ_H (mult, *J* in Hz)	δ_c	δ_c (Kalff and Robinson, 1925)	HMBC	
				^{2}J	^{3}J
1	-	-	-	-	-
2	-	164.1	164.1	-	-
3	-	130.4	130.4	-	-
4	-	183.0	183.0	-	-
5	-	152.1	152.1	-	-
6	6.66 (br s)	105.3	105.2	C-5 (152.1)	C-8 (93.5), C-10
7	-	153.3	153.2	-	-
8	6.62 (br s)	93.5	93.5	C-7 (153.3)	C-6 (105.3), C-8 (93.5), C-10 (131.3)
9	-	155.2	155.2 (8a)	-	-
10	-	131.3	131.3 (4a)	-	-
1'	-	126.3	126.3	-	-
2'	7.88 (br s)	129.6	129.1	C-1' (126.3), C-3' (131.9)	C-2 (164.1), C-1' (126.3), C-4' (131.9), C-6' (129.6)
3'	7.54 (br s)	131.9	131.9	C-2' (129.6)	C-1' ((126.3)
4'	7.54 (br s)	131.9	131.9	-	C-2' (129.6), C-6' (129.6)
5'	7.54 (br s)	131.9	131.9	C-6' (129.6)	C-1' (126.3)
6'	7.88 (br s)	129.6	129.1	C-5' (131.9)	C-2 (164.1), C-1' (126.3), C-2' (129.6), C-4' (131.9)
OH-5	13.01 (s)	-		-	-
OMe-3	4.05 (s)	60.9	60.1	-	C-3 (130.4)

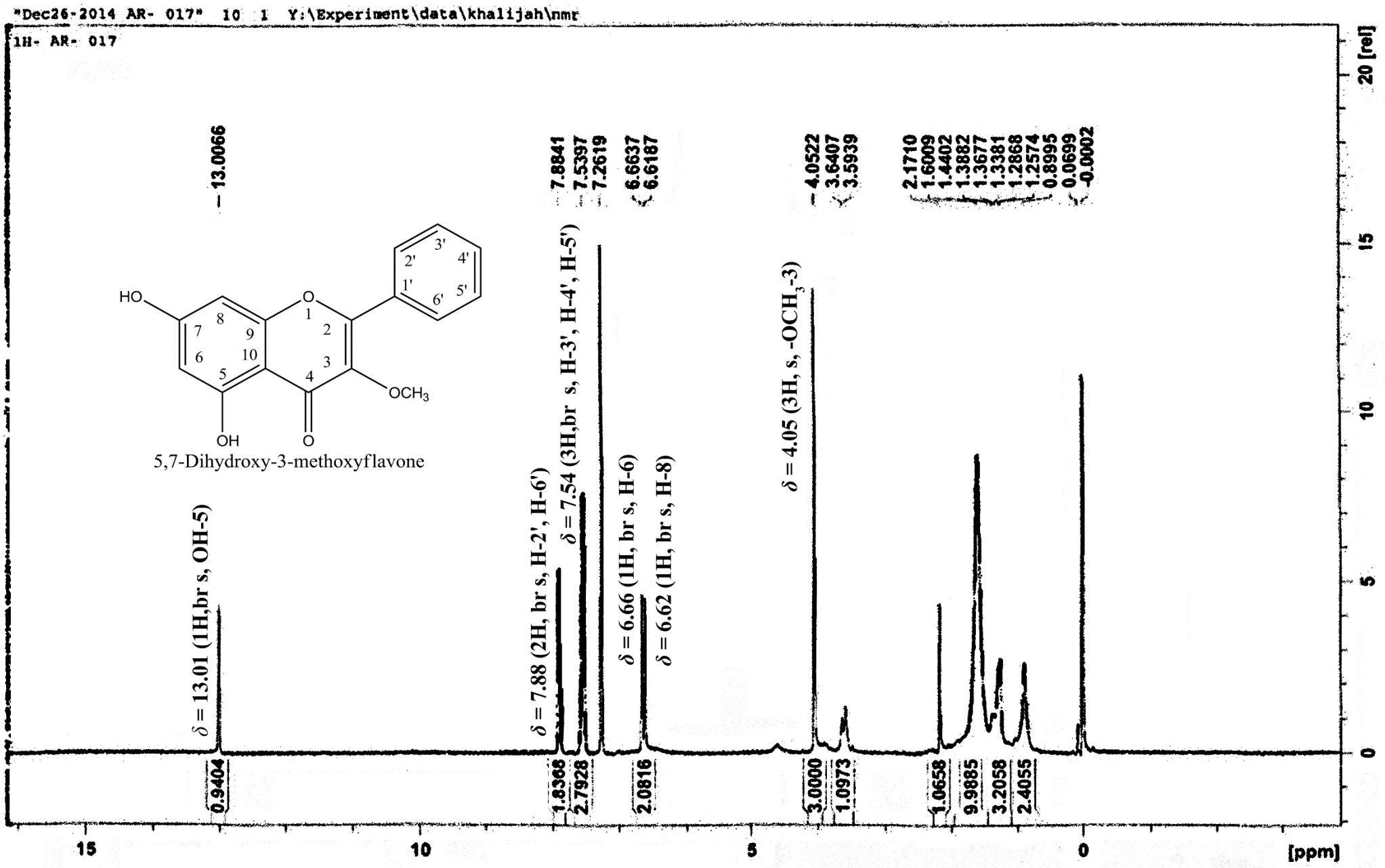


Fig. 5. ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of 5,7-dihydroxy-3-methoxyflavone (1).

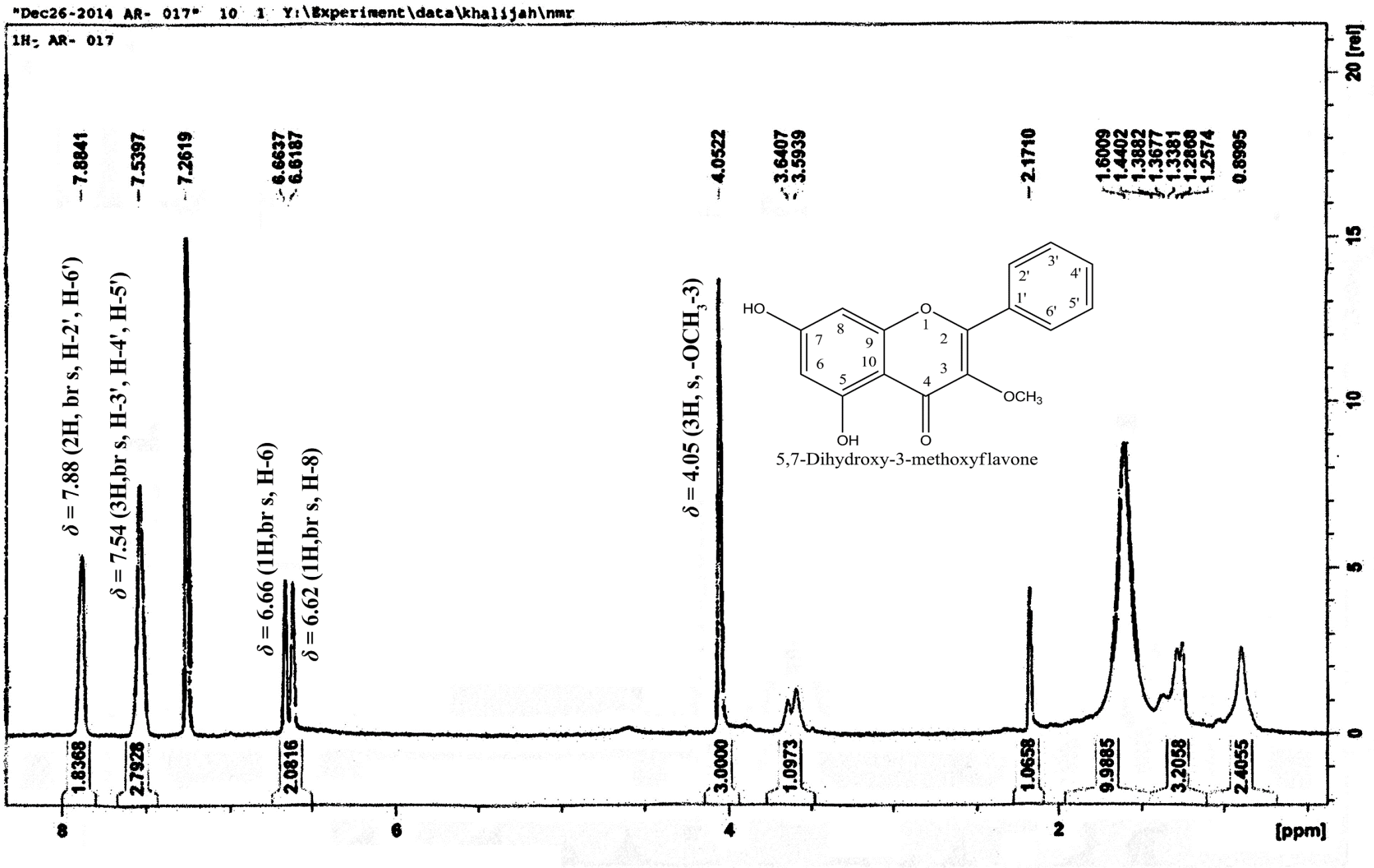


Fig. 6. Partial expansion of ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum of 5,7-dihydroxy-3-methoxyflavone (1).

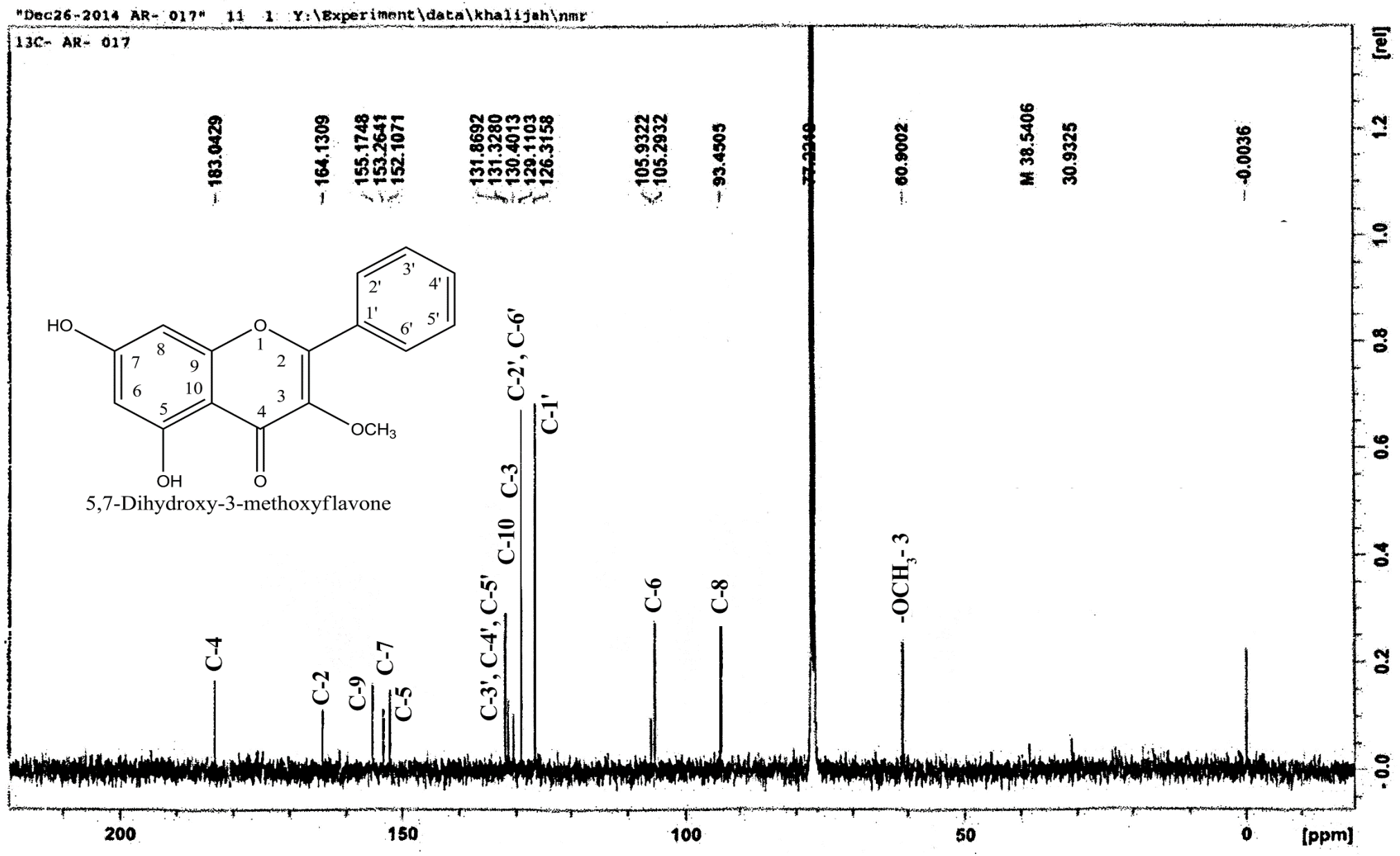


Fig. 7. ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of 5,7-dihydroxy-3-methoxyflavone (1).

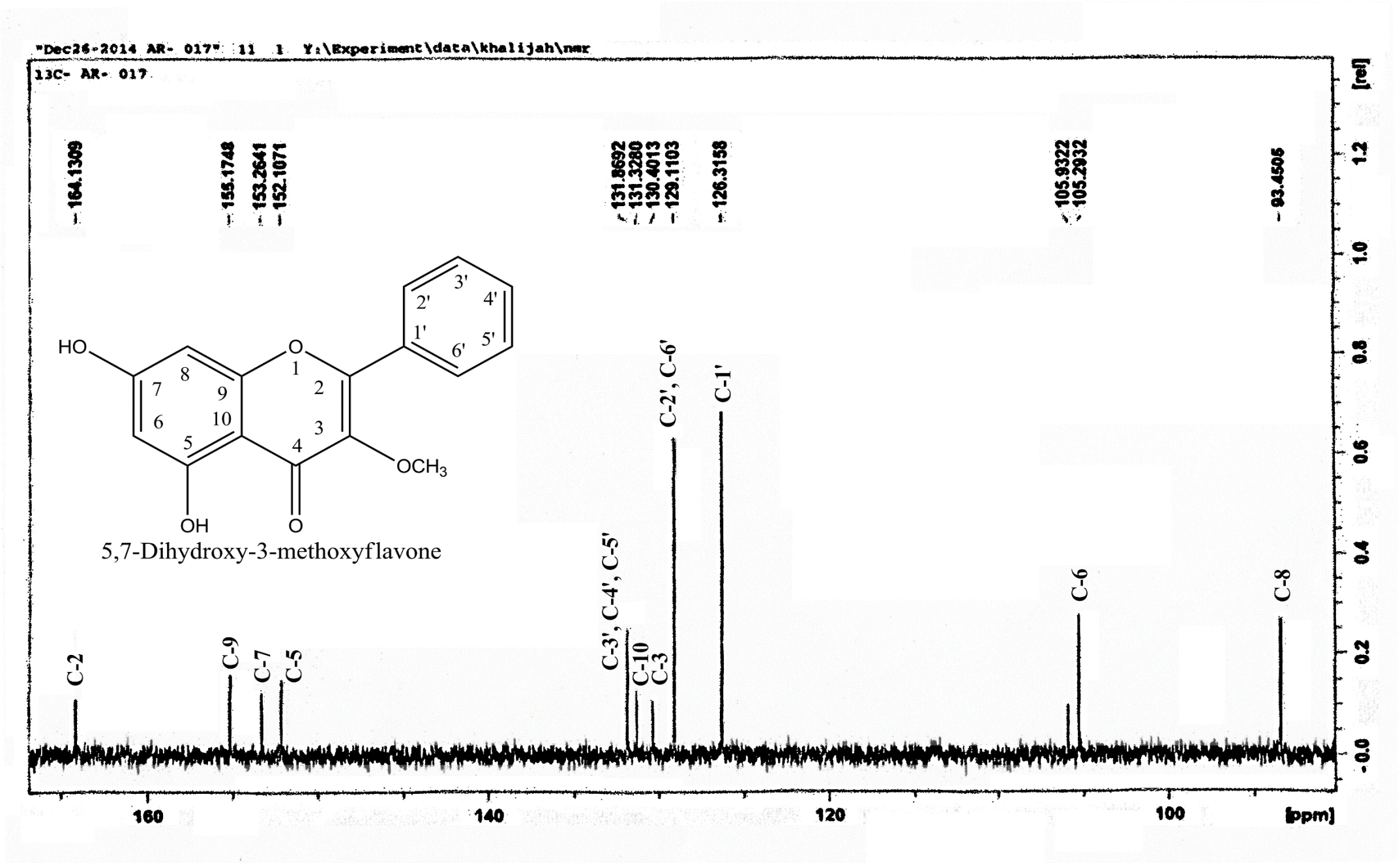


Fig. 8. Partial expansion of ^{13}C NMR ($CDCl_3$, 75 MHz) spectrum of 5,7-dihydroxy-3-methoxyflavone(1).

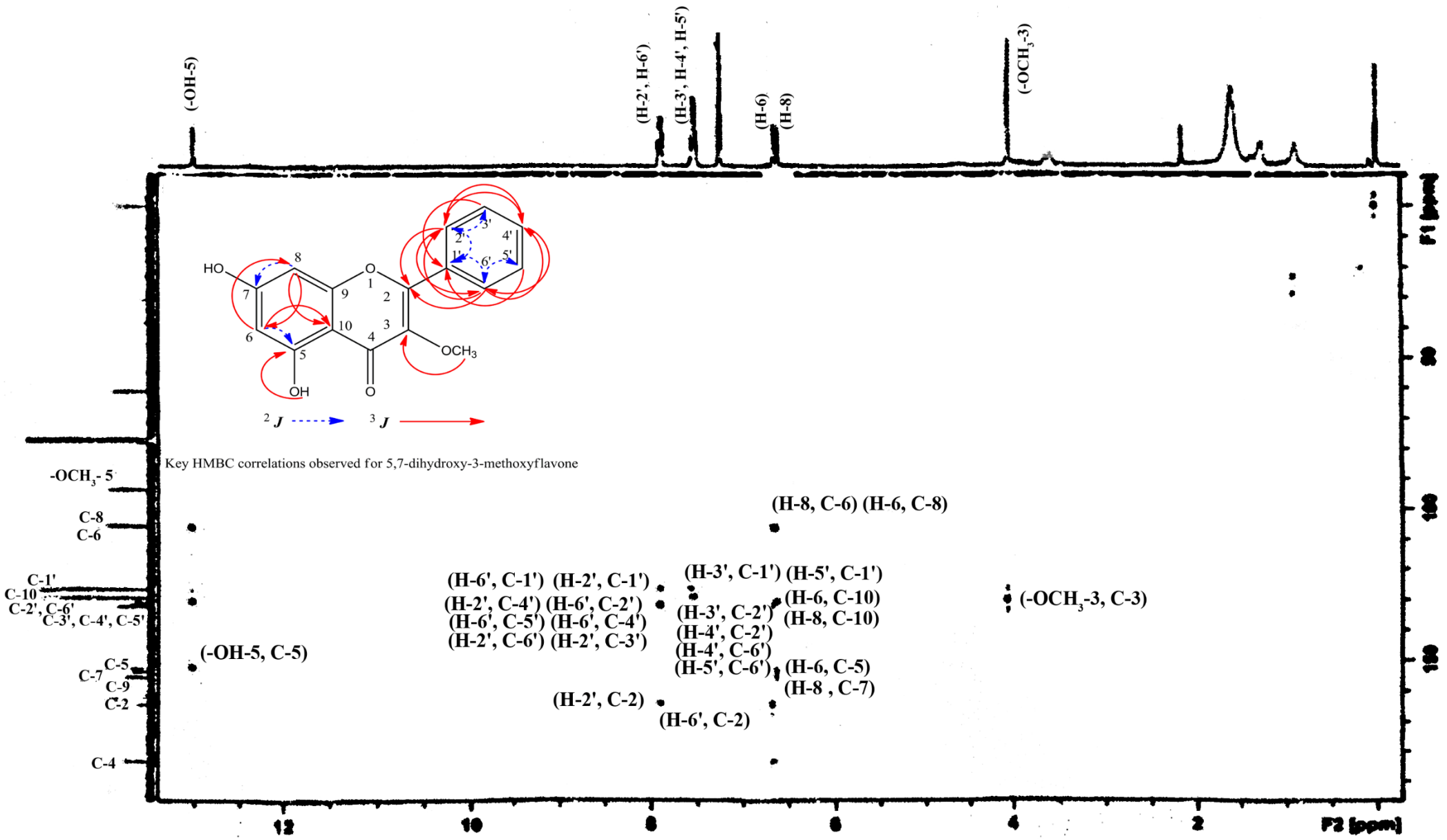


Fig. 9. HMBC spectrum of 5,7-dihydroxy-3-methoxyflavone (1).

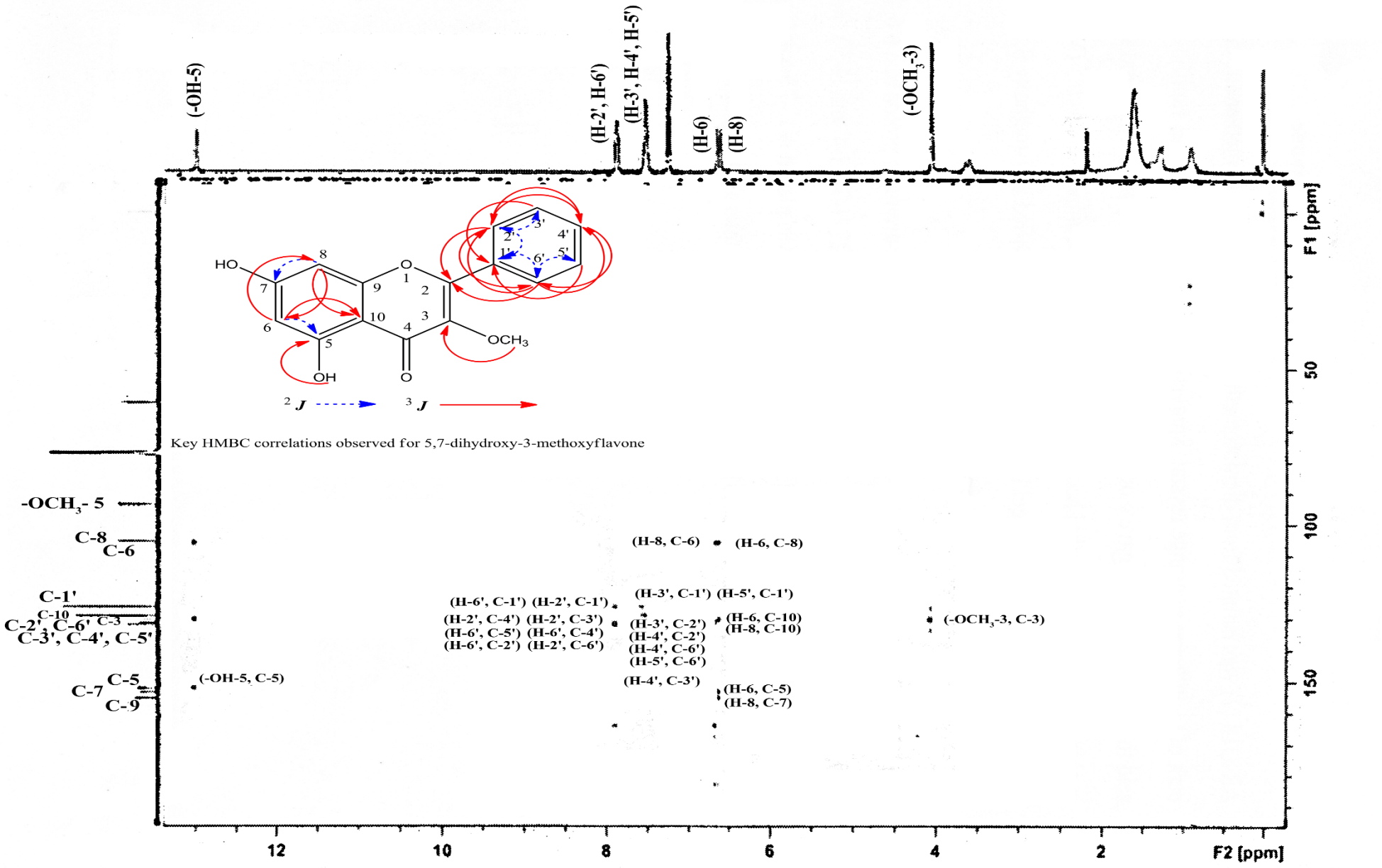


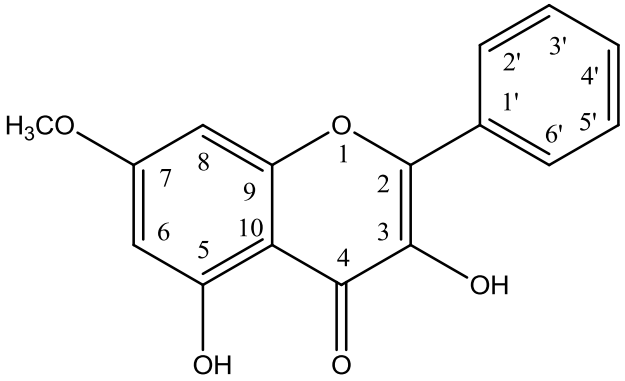
Fig. 10. Partial expansion of HMBC spectrum of 5,7-dihydroxy-3-methoxyflavone (1).

7.2 Characterization of AR-O18 as 7-methoxy-3,5 dihydroxyflavone (Izalpinin, 2)

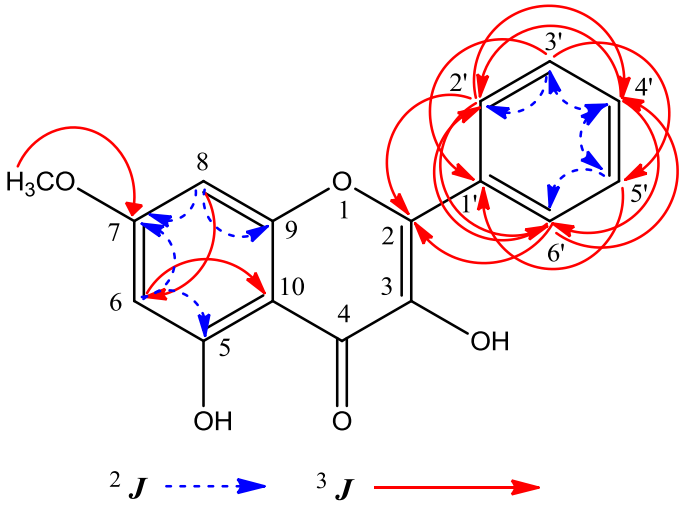
The isolated compound AR-O18 was obtained as orange crystals from the dichloromethane soluble fraction of a methanol extract of root bark of *O. indicum*. It afforded orange colour on developed TLC plate upon spraying with vanillin-sulfuric acid followed by heating at 105 - 110°C temperature for 5 minutes. The compound showed solubility in chloroform, methanol and ethyl acetate. The R_f value for the compound was calculated as 0.5 in $CHCl_3$-MeOH (97: 3) solvent system.

The ^{13}C NMR (75 MHz, CD_3OD) spectrum (Fig. 14 & 15) of AR-O18 exhibited 13 signals for 16 carbons which could be attributed for the characteristics of flavone nucleus. Therefore, the flavone had one methoxyl (-OCH_3) at δ 59.5, seven methines (CH) (δ 94.1, 104.6, 126.0, 128.8 × 2, 131.2, 131.7) and eight quaternary carbons including one carbonyl group at δ 182.9.

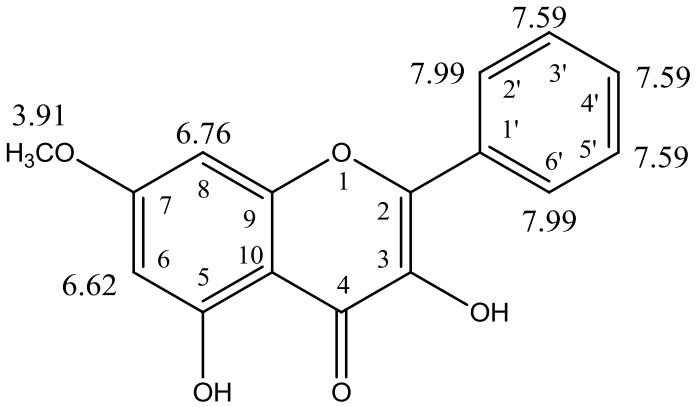
The ^1H NMR (300 MHz, CD_3OD) spectrum (Fig. 12 & 13) of the compound AR-O18 demonstrated a sharp singlet at δ 3.95 that integrated for 3 protons can be attributed to a -OCH_3 group. The ^1H NMR spectrum displayed a broad singlet for two meta-coupled aromatic protons which can be localized at C-2' and C-6' position on the B ring of the flavone moiety. The three aromatic protons of another broad singlet at δ 7.59 can be assigned to the protons at C-3', C-4' and C-5' position on B ring. The braod singlets at δ 6.76 and 6.62 can be ascribed to the C-8 and C-6 protons on ring A, respectively. The placement of the -OCH_3 group at C-7 position was established through a ^3J HMBC correlation from 3.91 to 128.8 with the respective carbon (Fig. 16). The assignments of other protons were also confirmed from the ^3J and ^2J HMBC correlation as showed in Table 27 and the structure of AR-O18 was unambiguously assigned for 7-methoxy-3,5 dihydroxyflavone (Izalpinin**, 2**) [Fig. 11].



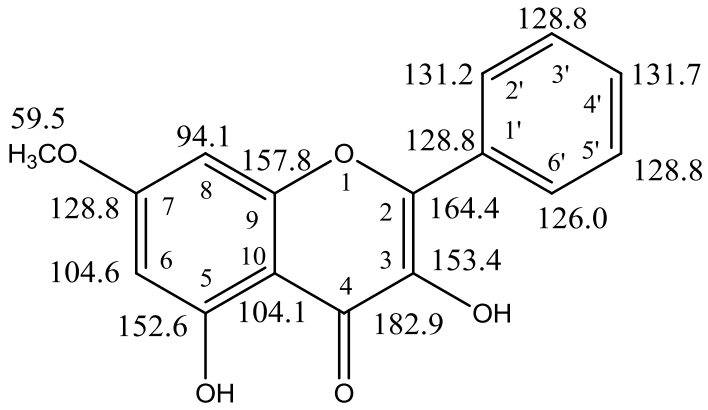
Structure of 7-methoxy-3,5 dihydroxyflavone (**2**)



Key HMBC correlations observed for 7-methoxy-3,5 dihydroxyflavone (**2**)



^{1}H NMR assignment of 7-methoxy-3,5 dihydroxyflavone (**2**)



^{13}C NMR assignment of 7-methoxy-3,5 dihydroxyflavone (**2**)

Fig. 11. Structure of 7-methoxy-3,5 dihydroxyflavone (Izalpinin, 2).

Table 27. ^{1}H (CD_3OD, 300 MHz), 13^{C} (CD_3OD, 75 MHz) and HMBC spectral data of 7-methoxy-3,5 dihydroxyflavone (Izalpinin, 2).

Position no	δ_H (mult, *J* in Hz)	Reference (Asakawa, 1971)	δ_c	HMBC	
				^{2}J	^{3}J
1	-		-	-	-
2	-		164.4	-	-
3	-		153.4	-	-
4	-		182.9	-	-
5	-		152.6	-	-
6	6.62 (br s)	6.38	104.6	C-5 (152.6), C-7 (128.8)	C-10 (104.1)
7	-		128.8	-	
8	6.76 (br s)	6.50	94.1	C-9 (157.8)	C-6 (104.6), C-7 (128.8)
9	-		157.8	-	
10	-		104.1	-	
1'	-		128.8	-	
2'	7.99 (br s)	7.52	131.2	-	C-2 (164.4), C-4' (131.7),C-6' (126.0)
3'	7.59 (br s)	7.52	128.8	C-2' (131.2),C-4' (131.7)	C-1' (128.8),C-5'(128.8)
4'	7.59 (br s)	7.52	131.7	C-3' (128.8), C-5' (128.8)	C-2' (131.2), C-6' (126.0)
5'	7.59 (br s)	7.52	128.8	C-4' (131.7),C-6' (126.0)	C-1' (128.8)
6'	7.99 (br s)	7.52	126.0	-	C-2 (164.4), C-2' (131.2), C-4' (131.7)
OMe-7	3.91 (3H, s)	3.83	59.5	-	C-7 (128.8)

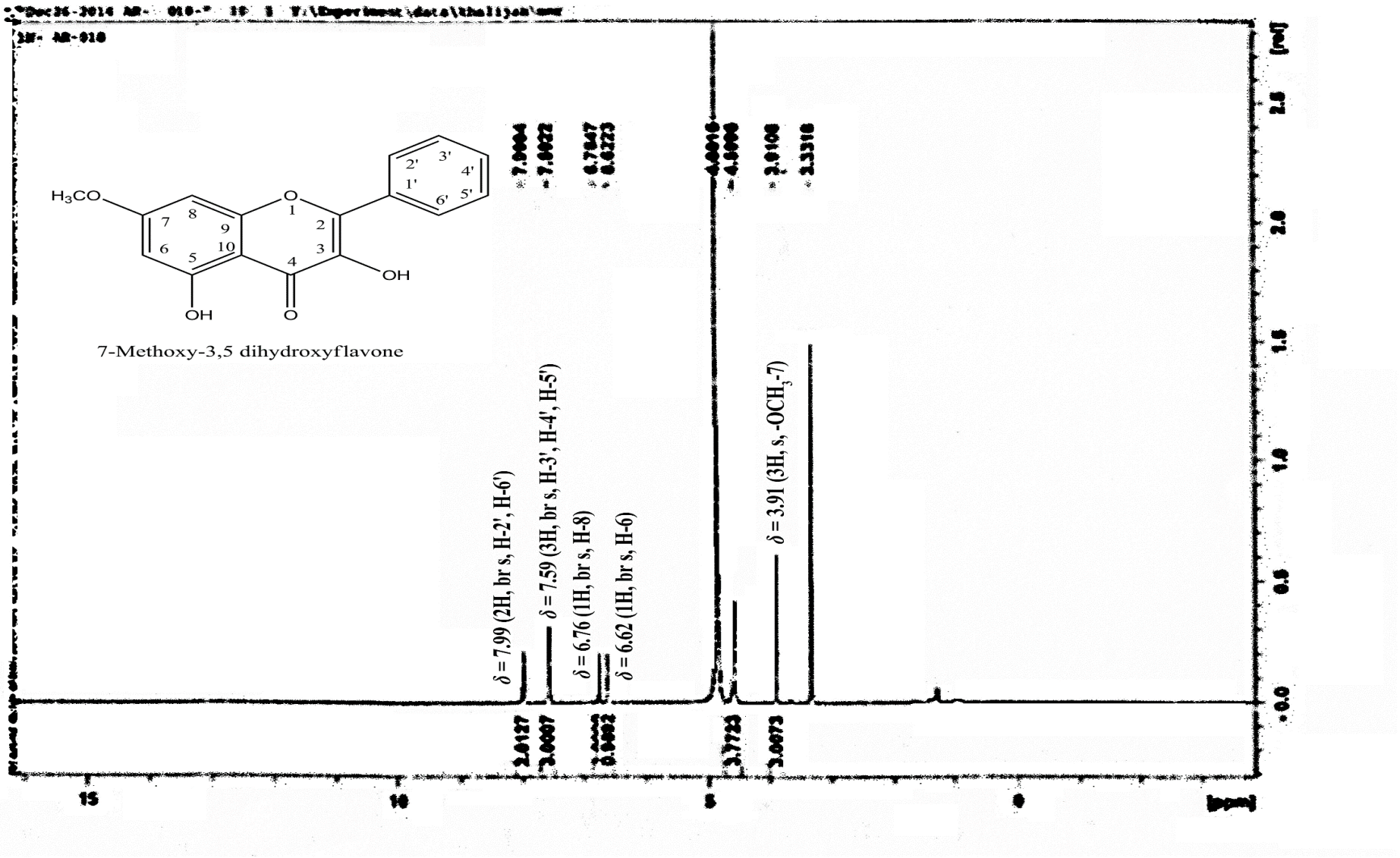


Fig. 12. ^{1}H NMR (300 MHz, CD_3OD) spectrum of 7-methoxy-3,5 dihydroxyflavone (Izalpinin, 2).

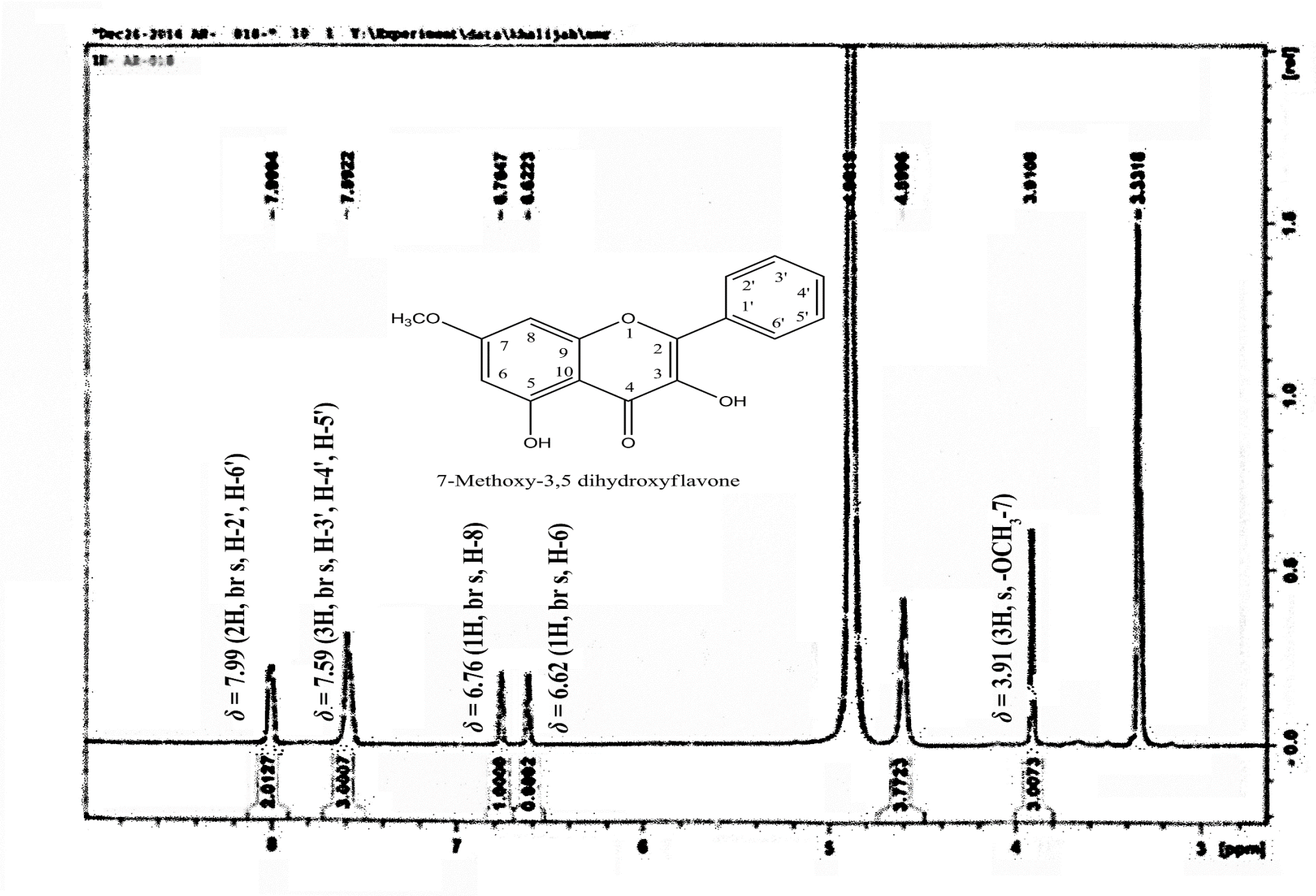


Fig. 13. Partial expansion of ^{1}H NMR (300 MHz, CD_3OD) spectrum of 7-methoxy-3,5 dihydroxyflavone (Izalpinin, 2).

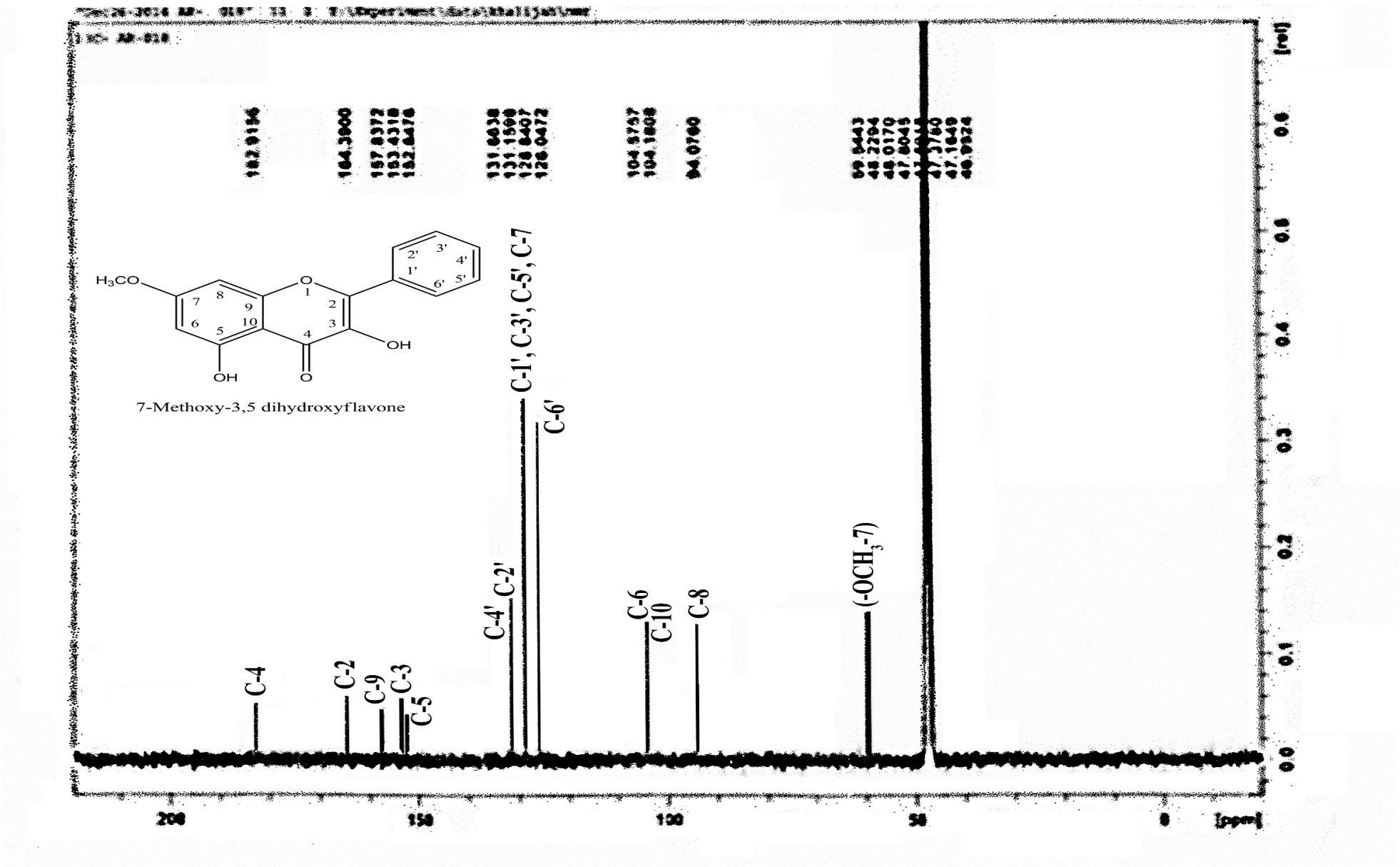


Fig. 14. ^{13}C NMR (75 MHz, CD_3OD) spectrum of 7-methoxy-3,5 dihydroxyflavone (Izalpinin, 2).

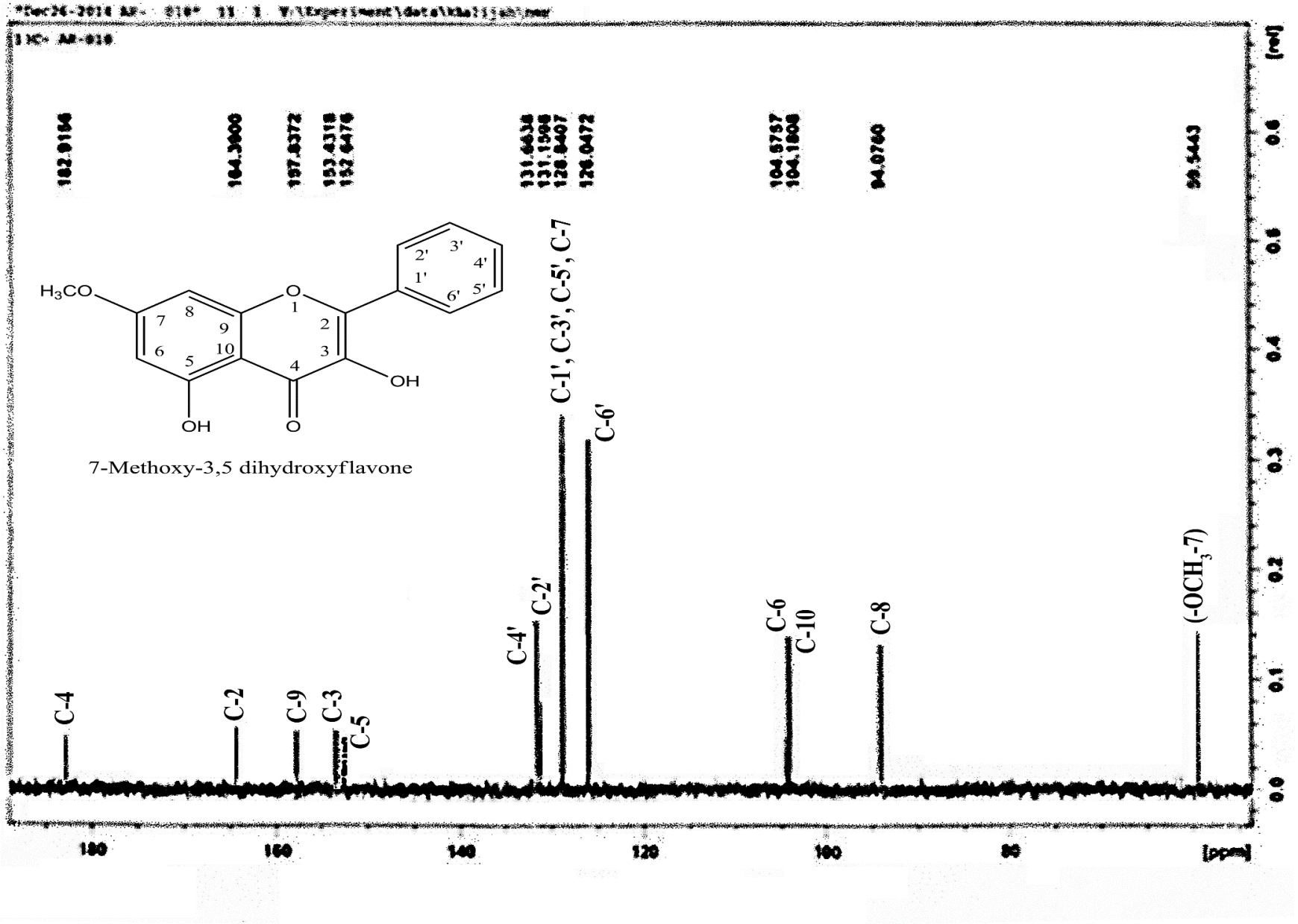


Fig. 15. Partial expansion of ^{13}C NMR (75 MHz, CD_3OD) spectrum of 7-methoxy-3,5 dihydroxyflavone (Izalpinin, 2).

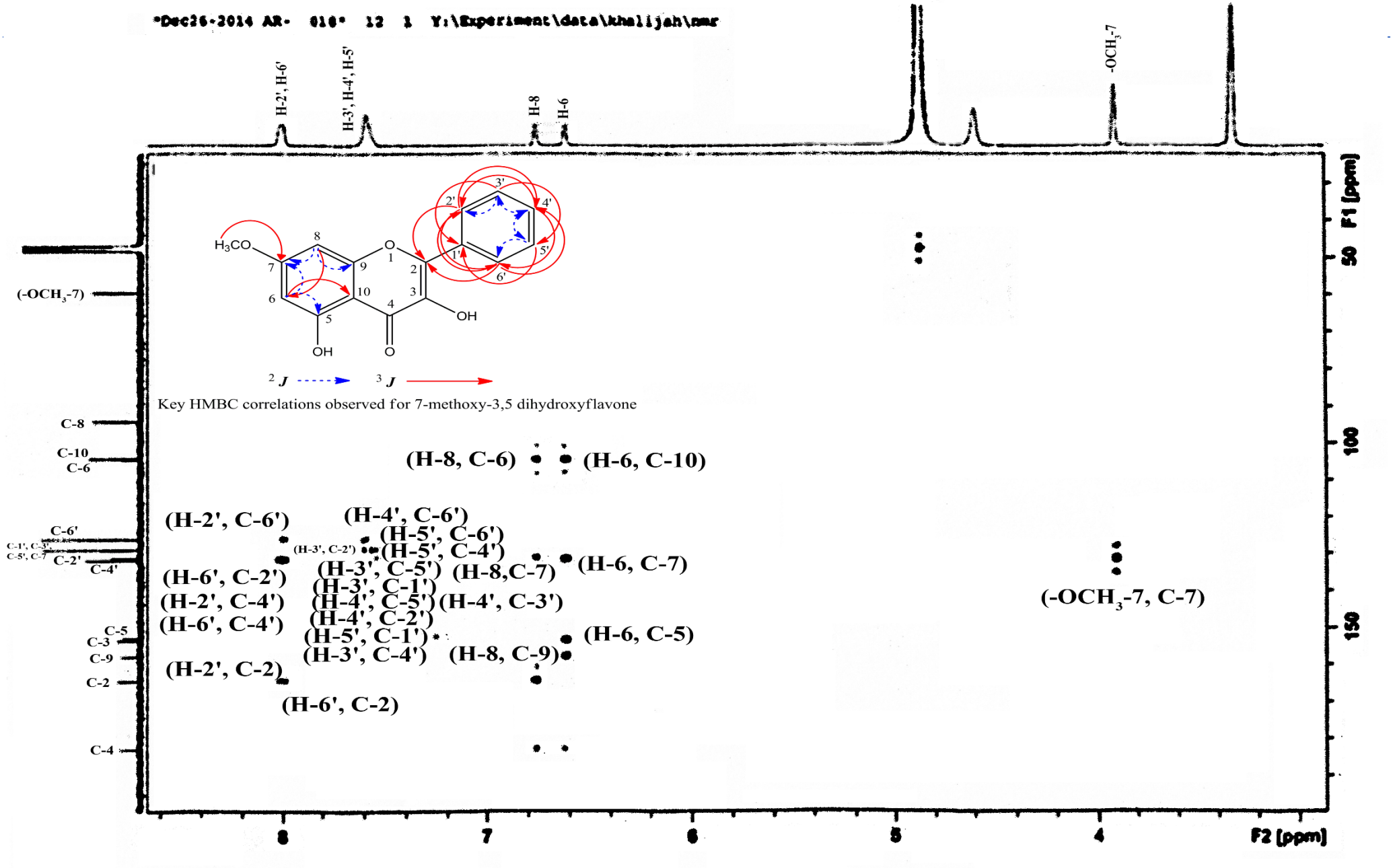
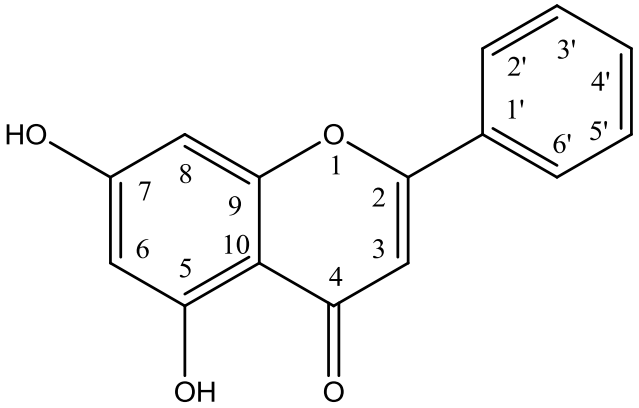


Fig. 16. HMBC spectrum of 7-methoxy-3,5 dihydroxyflavone (Izalpinin, 2).

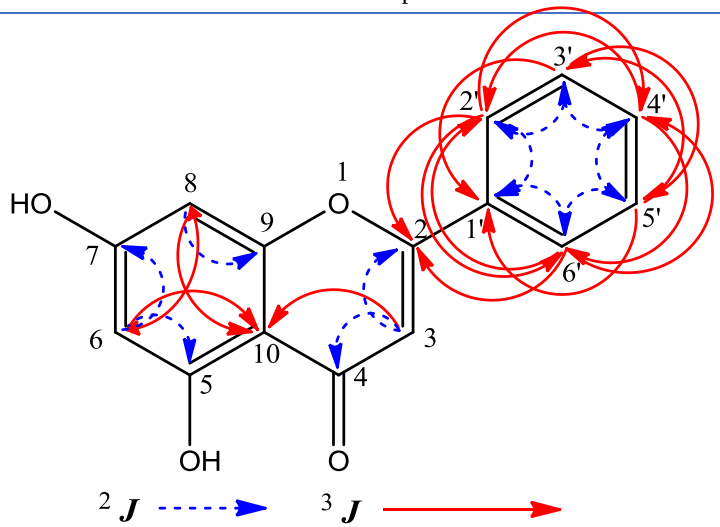
7.3 Characterization of AR-O23 as 5,7-dihydroxyflavone (Chrysin, 3)

AR-O23 was obtained as yellow crystals form the dichloromethane soluble fraction of methanol extract of root bark of *O. indicum*. The compound gave yellowish spot on developed TLC plate upon spraying with vanillin-sulfuric acid followed by heating at 105-110°C for few minutes. The compound was soluble in chloroform, ethyl acetate and methanol. The R_f value [(0.6, $CHCl_3$-MeOH (97:3)] on silica gel PF_{254} of the compound was identical to that observed for 5,7-dihydroxyflavone (Chrysin) [Maungjunburee and Mahabusarakam, 2010].

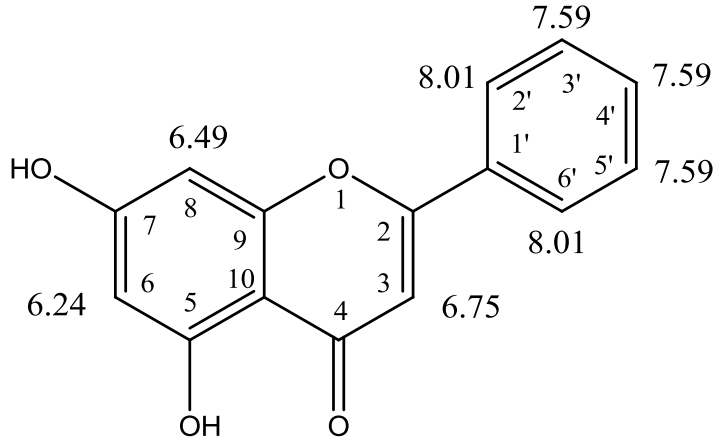
The ^{13}C NMR spectrum (75 MHz, CD_3OD) of AR-O23 displayed the signals typical for flavonoid (Fig. 20 & 21). The spectrum exhibited 13 signals, including six quaternary (δ 103.9, 131.2, 161.9, 164.2, 168.8) together with one carbonyl (δ 182.4) and eight methines (94.0, 99.3, 104.6, 126.0 × 2, 128.8 × 2,131.6) carbons. The ^1H NMR spectrum (300 MHz, CD_3OD) of the compound **3** (Fig. 18 & 19) demonstrated three sharp singlets at δ 6.24 (1H), 6.49 (1H) and 6.75 (1H) confirming the presence of two aromatic protons on ring A and one proton on ring C, respectively. A broad singlets δ 7.59 for three aromatic protons allowed to assign three protons at 3**',** 4' and 5' position on B ring of flavone skeleton**.** The broad signal at δ 8.01 that integrated for two protons could be assigned to the aromatic protons H-6' and H-2' of B ring. The position of the H-3**',** H-4' and H-5' was further confirmed from the two and three bond HMBC correlations with C-1', C-2**',** C-3', C-4', C-5' (Fig. 22). The correlations of H-3, H-2**',** H-6' protons to C-2 supported the position of H-3, H-2', H-6'. In addition, the assignment of H-6 and H-8 protons was confirmed from their three bond HMBC correlations with C-7 and C-10 respectively (Fig. 22). Therefore, the structure of AR-O23 was unambiguously solved as 5,7- dihydroxyflavone (Chrysin, **3**) [Fig. 17] which has been previously reported from (*O. indicum*). The complete set of ^1H and ^{13}C NMR spectral resonances of AR-O23 have been presented in Table 28.



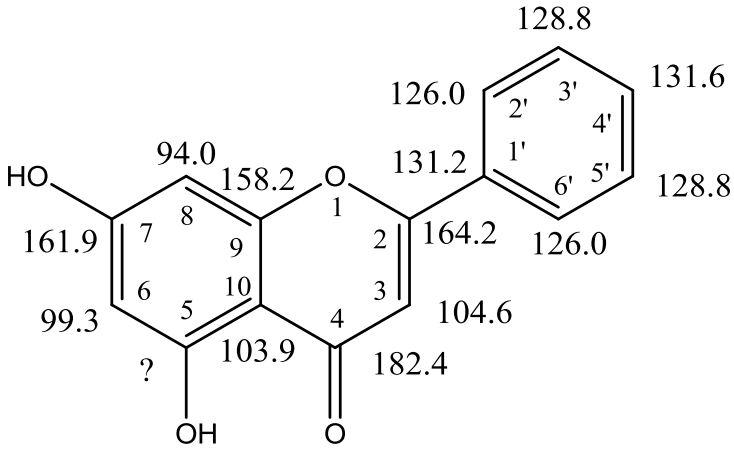
Structure of 5,7- dihydroxyflavone (Chrysin, **3**)



Key HMBC correlations observed for 5,7- dihydroxyflavone (**3**)



^{1}H NMR assignment of 5,7- dihydroxyflavone (**3**)



^{13}C NMR assignment of 5,7- dihydroxyflavone (**3**)

Fig. 17. Structure of 5,7-dihydroxyflavone (Chrysin, 3).

Table 28. ^{1}H (CD_3OD, 300 MHz), ^{13}C (CD_3OD, 75 MHz) and HMBC spectral data of 5,7-dihydroxyflavone (Chrysin, 3).

Position no	δ_H (mult, *J* in Hz)	δ_c	δ_c (Maungjunburee and Mahabusarakam, 2010)	HMBC	
				^{2}J	^{3}J
1	-	-	-	-	-
2	-	164.2	163.6	-	-
3	6.75 (1H, s)	104.6	105.5	C-2 (164.2), C-4 (182.4)	C-10 (103.9)
4	-	182.4	182.4	-	-
5	-	168.8	164.4	-	-
6	6.24 (1H, s)	99.3	99.7	C-5 (168.8), C-7 (161.9)	C-8 (94.0), C-10 (103.9)
7	-	161.9	162.1	-	-
8	6.49 (1H, s)	94.0	94.3	C-7 (161.9), C-9 (158.2)	C-6 (99.3), C-10 (103.9)
9	-	158.2	158.0 (8a)	-	-
10	-	103.9	105.5 (4a)	-	-
1'	-	131.2	133.5	-	-
2'	8.01 (br s)	126.0	126.2	C-1' (131.2)	C-2 (164.2) C-6' (126.0), C-4' (131.6)
3'	7.59 (s)	128.8	128.6	C-2' (126.0), C-4' (131.6)	C-1' (131.2), C-5' (128.8)
4'	7.59 (s)	131.6	131.6	C-3' (128.8), C-5' (128.8)	C-2' (126.0), C-4' (131.6)
5'	7.59 (s)	128.8	128.6	C-4' (131.6), C-6' (126.0)	C-1' (131.2), C-3' (128.8)
6'	8.01 (br s)	126.0	126.2	-	C-2 (164.2), C-1' (131.2), C-2' (126.0), C-4' (131.6)

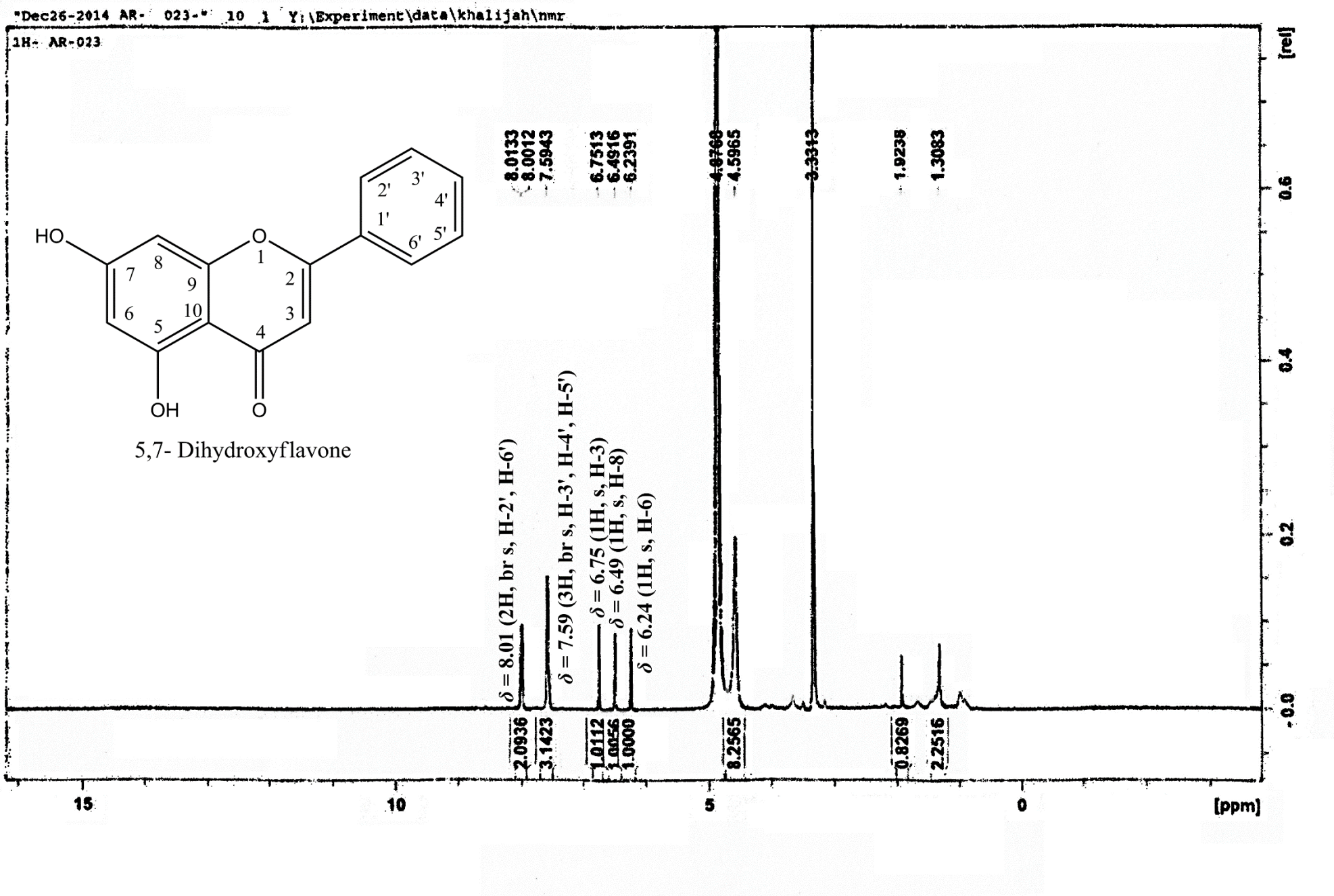


Fig. 18. ^{1}H NMR (300 MHz, CD_3OD) spectrum of 5,7-dihydroxyflavone (Chrysin, 3).

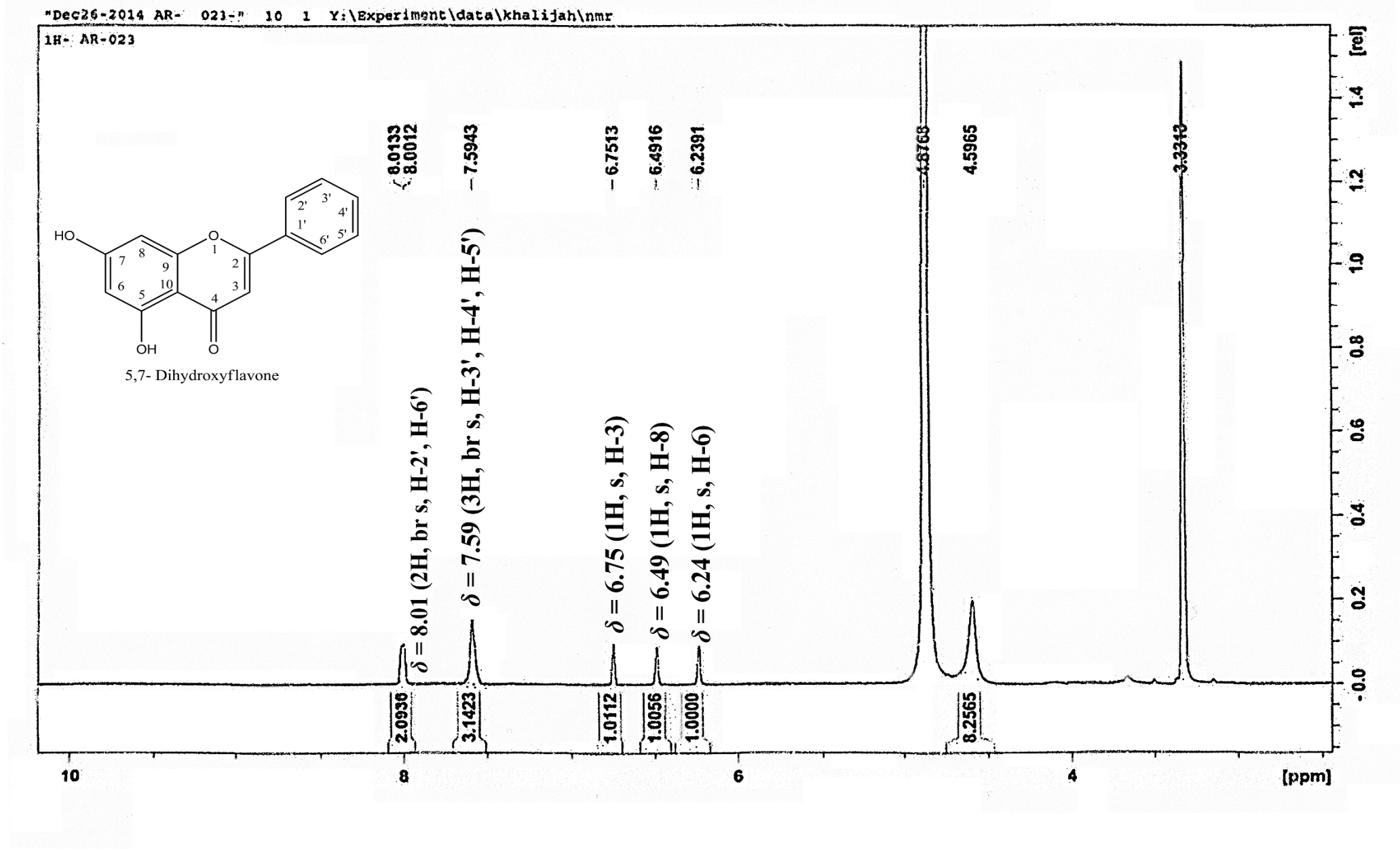


Fig. 19. Partial expansion of ^{1}H NMR (300 MHz, CD_3OD) spectrum of 5,7-dihydroxyflavone (Chrysin, 3).

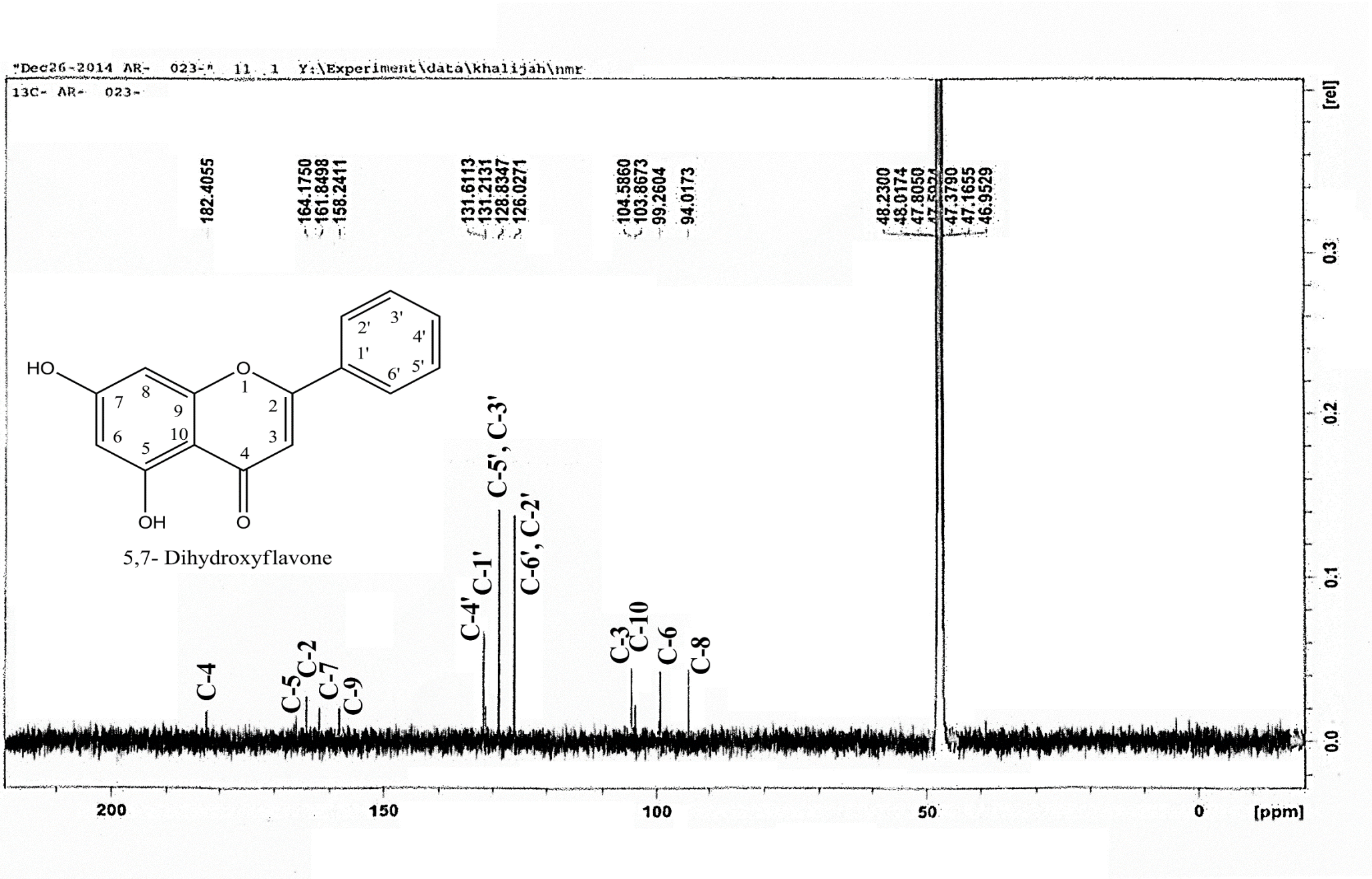


Fig. 20. ^{13}C NMR (75 MHz, CD_3OD) spectrum of 5,7-dihydroxyflavone (Chrysin, 3).

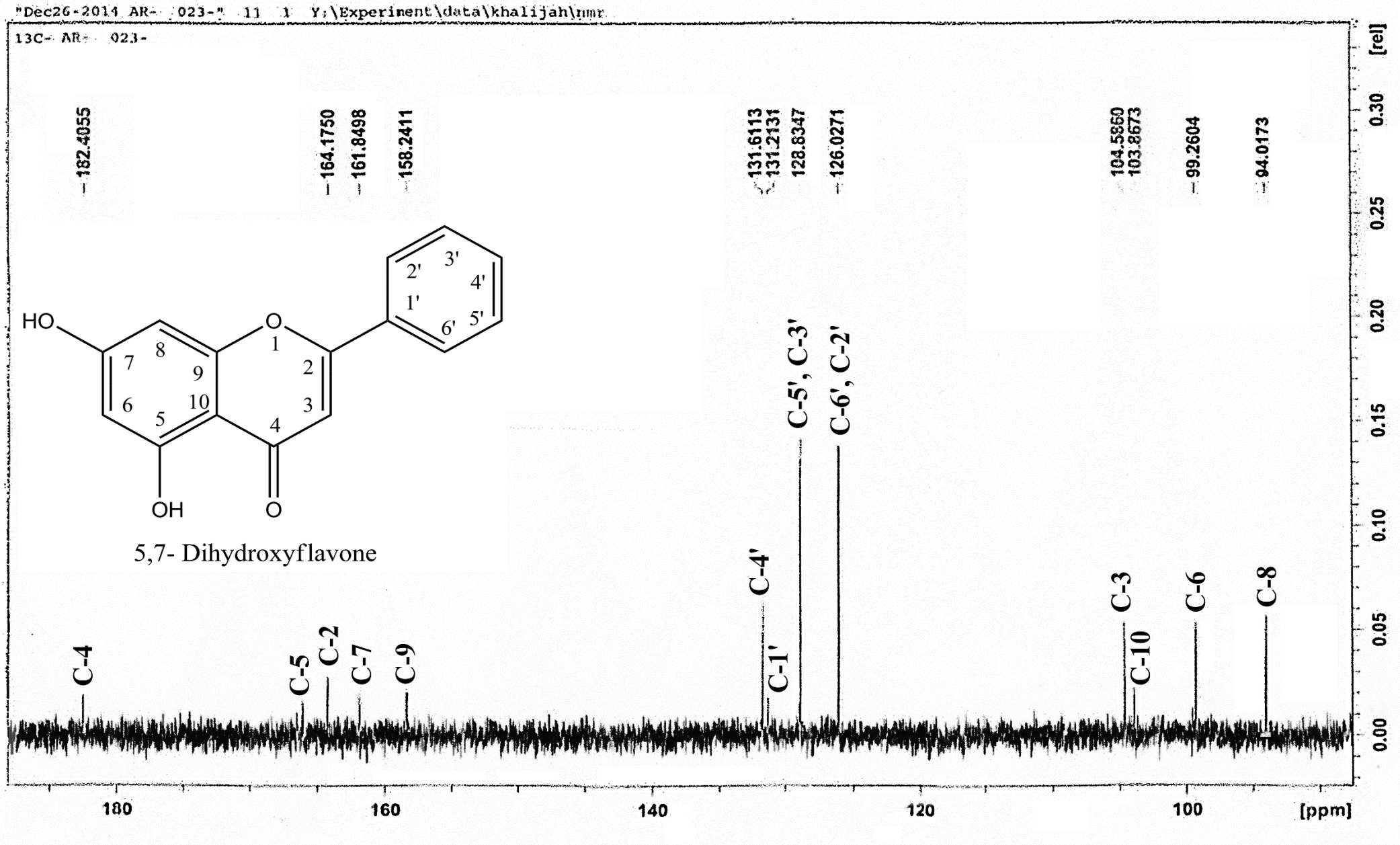


Fig. 21. Partial expansion of ^{13}C NMR (75 MHz, CD_3OD) spectrum of 5,7-dihydroxyflavone (Chrysin, 3).

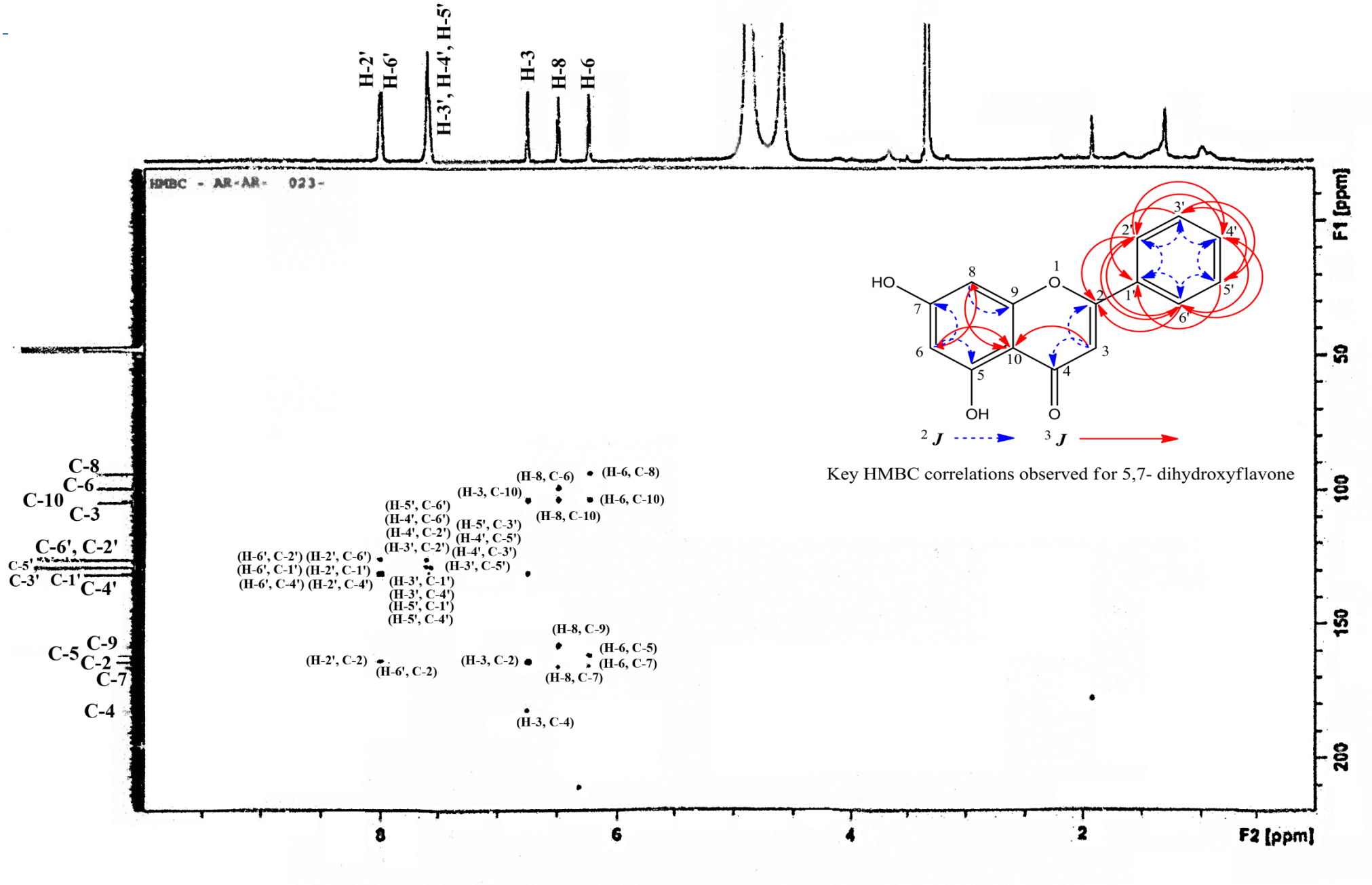
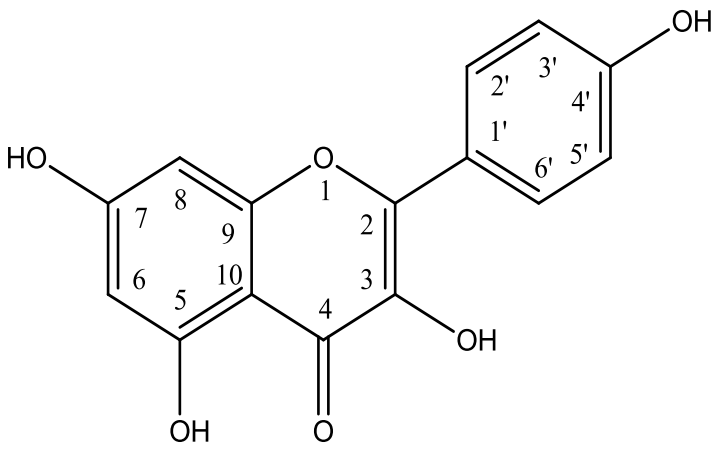


Fig. 22. HMBC spectrum of 5,7-dihydroxyflavone (Chrysin, 3).

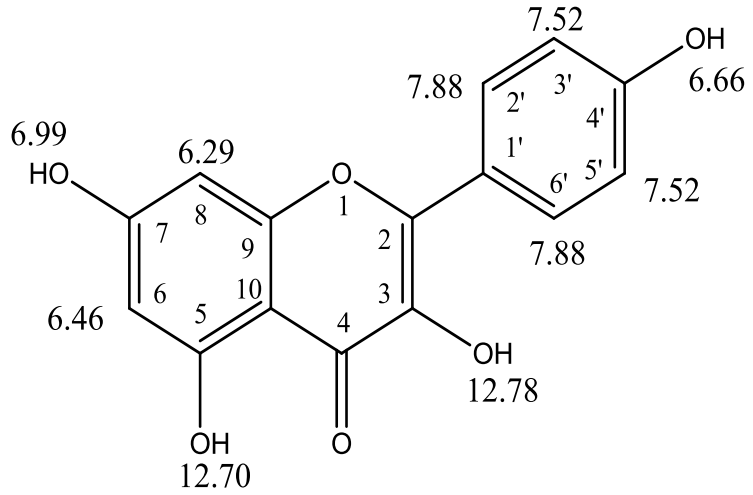
7.4 Characterization of AR-O30 as 3,4',5,7-tetrahydroxyflavonol (Kaempferol, 4)

Compound AR-O30 was isolated as yellowish crystals from the dichloromethane soluble fraction of methanolic extract of root bark of *O. indicum*. It provided yellow coloured spot after spraying the developed TLC plate with vanillin-sulfuric acid followed by heating at 105-110°C for few minutes. The calculated R_f value [0.7 in $CHCl_3$-MeOH (97:3)] over silica gel (PF_{254}) was identical to that reported for Kaempferol (3,4',5,7-tetrahydroxyflavonol) by Islam *et al*. (2010).

The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of AR-O30 (Fig. 24 & 25) displayed characteristic signals appropriated for a flavonol moiety. The doublets (J = 8.4 and 6.8) centered at 7.52 and 7.88 ppm (2H, J = 6.8 Hz) can be attributed to the ortho-coupled aromatic protons located at C-2' to C-6' and C-3' to C-5' position on B ring of the flavonol moiety. In addition, the singlets at 6.46 (1H) and 6.29 (1H) ppm could be assigned for H-6 and H-8 respectively on ring A. The broad singlets of δ 6.99 and 6.66 were therefore assigned to the -OH group protons at 5 of C-7 and C-4' position, respectively. The downfield sharp singlet at δ 12.78 (1H) could be assigned to the chelated hydroxyl group at C-5. Comparing to the afore-mentioned data with the values described for Kaempferol from the *O. indicum* (Islam *et al*., 2010) the structure of AR-030 was established as 3,4',5,7-tetrahydroxyflavonol (Kaempferol, 4)[Fig. 23]. The complete set of ^{1}H NMR assignment have been demonstrated in Table 29.



3,4',5,7- Tetrahydroxyflavonol (Kaempferol, **4**)



^{1}H NMR assignment of 3,4',5,7- tetrahydroxyflavonol (Kaempferol, **4**)

Fig. 23. Structure of 3,4',5,7-tetrahydroxyflavonol (Kaempferol, 4).

Table 29. ^{1}H ($CDCl_3$, 400 MHz) spectral data of 3, 4', 5, 7-tetrahydroxyflavonol (Kaempferol, 4).

Position no	δ_H (mult, *J* in Hz)	δ_H (mult, *J* in Hz) (Islam *et al*., 2010)
1	-	
2	-	
3	-	
4	-	
5	-	
6	6.46 (1H, s)	6.46(s)
7	-	
8	6.29 (1H, s)	6.27 (s)
9	-	
10	-	
1'	-	
2'	7.88 (2H, d, *J*= 6.8)	7.88 (d)
3'	7.52 (2H, d, *J*= 8.4)	7.53(d)
4'	-	
5'	7.52 (2H, d, *J*= 8.4)	
6'	7.88 (2H, d, *J*= 6.8)	
OH-5	12.78 (1H, s)	12.76 (s)
OH-7	6.99(1H, s)	
OH-4'	6.66 (1H, s)	

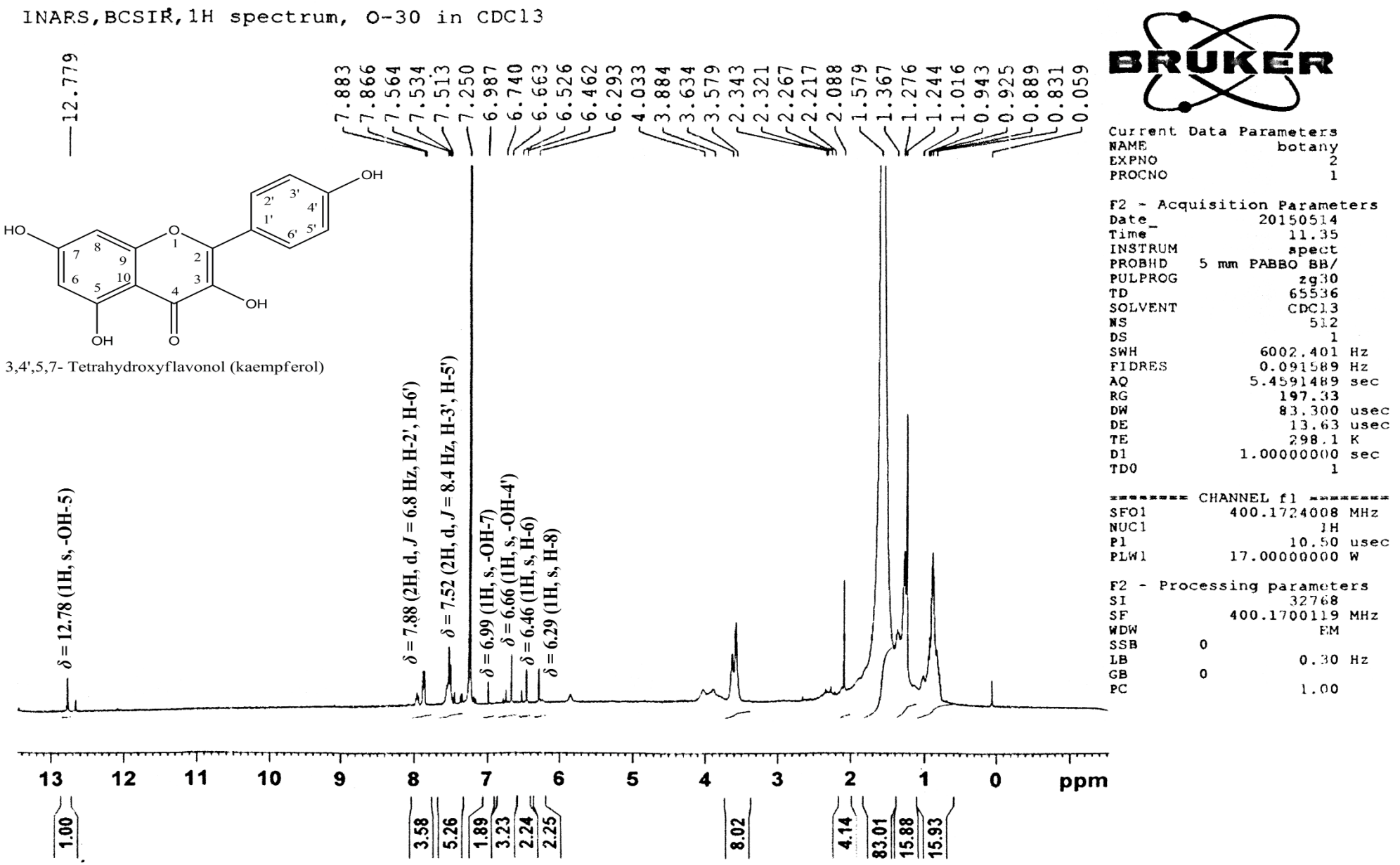


Fig. 24. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of 3,4',5,7-tetrahydroxyflavonol (Kaempferol, 4).

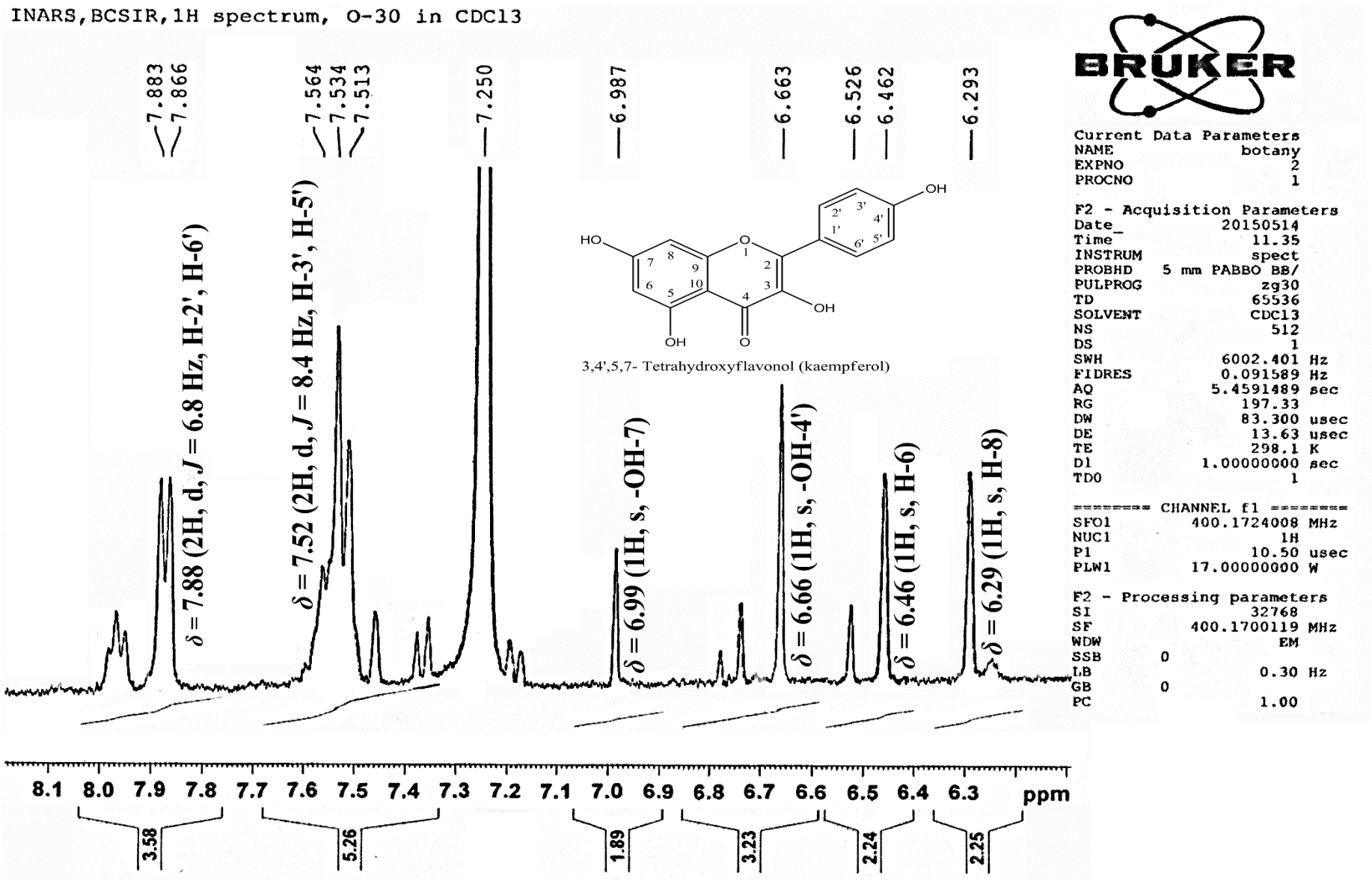


Fig. 25. Partial expansion of ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of 3,4',5,7-tetrahydroxyflavonol (Kaempferol, 4).

7.5 Characterization of AR-AL25 as Stigmasterol (5)

AR-AL25 was isolated from chloroform soluble fraction of methanolic extract of leaves of *A. polystachya* as colourless liquid. Spraying the developed plate with vanillin-sulphuric acid followed by heating gave a green coloured spot. The compound was found to be soluble in chloroform, petroleum ether and ethyl acetate. The R_f value of the compound was 0.5 (Ethyl acetate: petroleunm ether = 15:85) on silica gel PF_{254} plate which is identical to that observed for Stigmasterol (Khan, 1991).

The ^{1}H NMR spectrum (500 MHz, CDCl3) of AR-AL25 (Table 30 and Fig. 27-29) revealed a one proton multiplet at δ 3.52, the position and multiplicity of which was indicative of H-3 of the steroid nucleus. The typical H-6 of the steroidal skeleton was evident as a multiplet at δ 5.32 that integrated for one proton.

The olefinic protons H-22 and H-23 appeared as characteristic downfield signals at δ 5.12 and 5.01, respectively in the ^{1}H NMR spectrum. Both signals were observed as double doublets (J = 15.2 Hz, 8.8 Hz) and (J = 15.2 Hz, 8.4 Hz) respectively, which is indicative of *trans* coupling with the olefinic proton and vicinal coupling with neighboring methane proton.

The spectrum further revealed signals at δ 1.00 (3H) assignable to the protons of two tertiary methyl groups at C-10, respectively. These ^{1}H NMR spectral features are in close agreement with data published for Stigmasterol (5). Therefore AR-AL25 was identified as Stigmasterol (5) [Fig. 26].

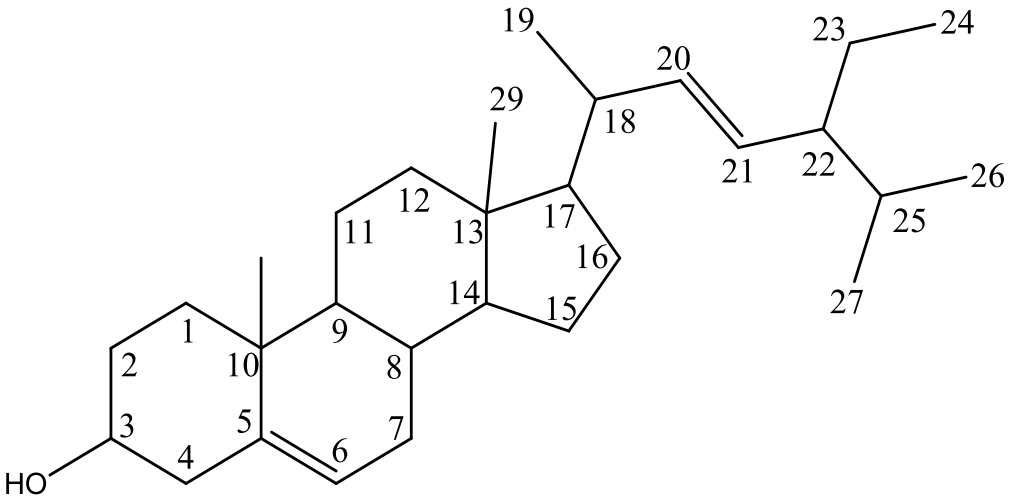


Fig. 26. Structure of Stigmasterol (5).

Table 30. Comparison between the ^{1}H NMR spectral data of AR-AL25 (400 MHz) and Stigmasterol (Khan, 1991) in $CDCl_3$.

Position	AR-AL25 δ_H in ppm in $CDCl_3$	Stigmasterol δ_H in ppm in $CDCl_3$
H-3	3.52 m	3.52 m
H-6	5.32 m	5.33 m
Me-10	1.00 s	1.00 s
H-20	0.92 s	0.90 s
H-22	5.12 dd (J = 15.2, 8.8 Hz)	5.15 dd
H-23	5.01 dd (J = 15.2, 8.4 Hz)	5.03 dd

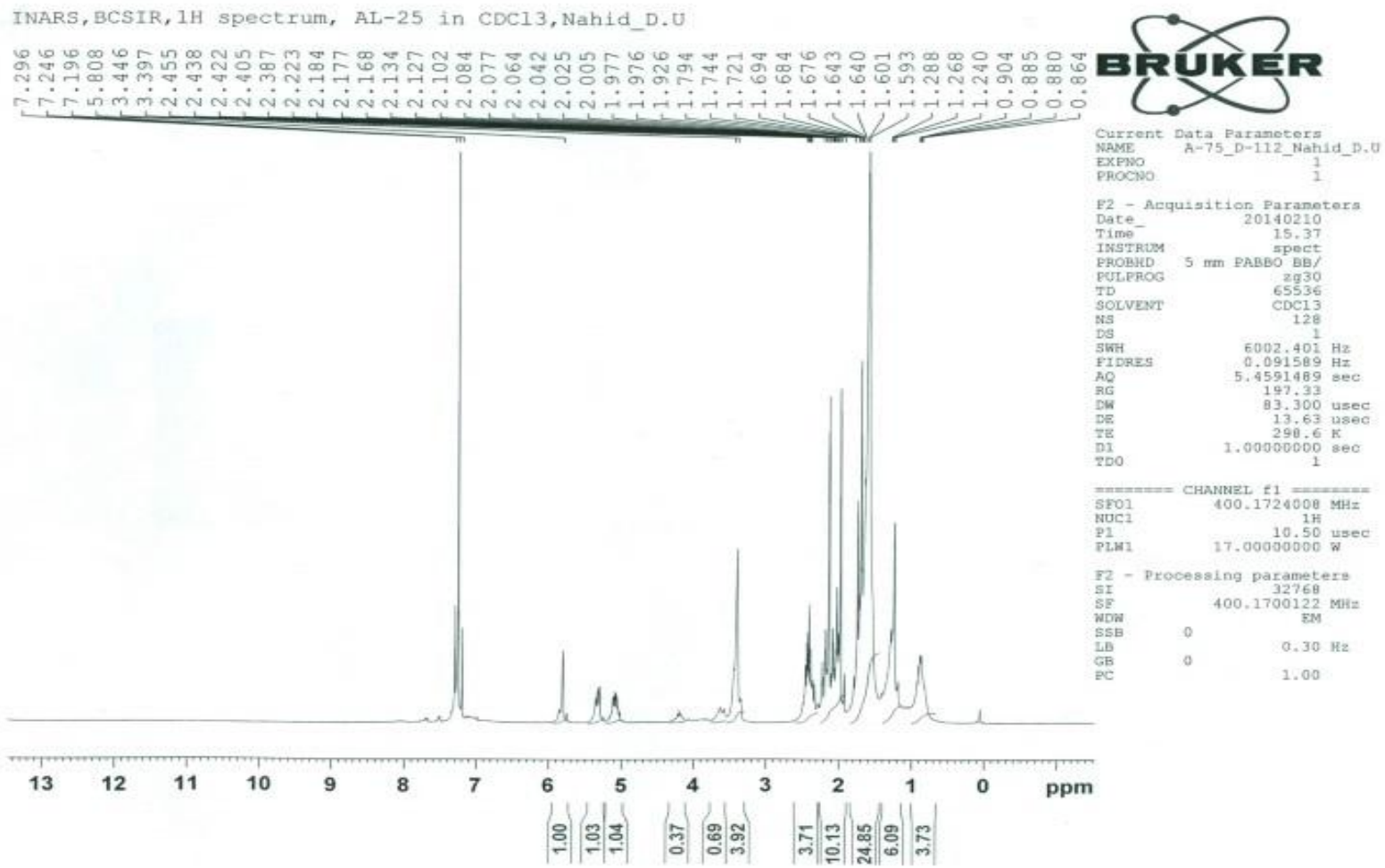


Fig. 27. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of Stigmasterol (5).

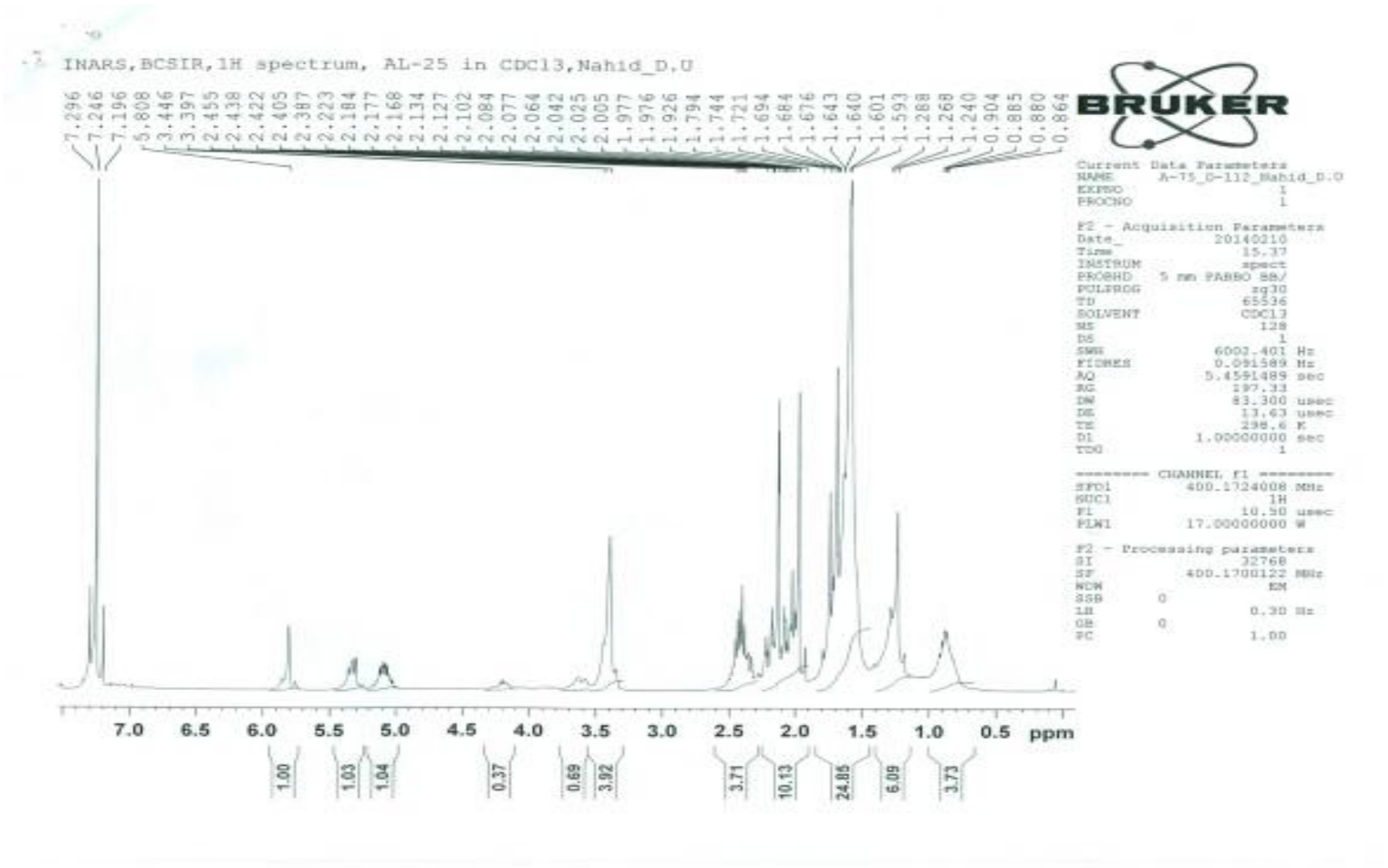


Fig. 28. ^{1}H NMR spectrum of AR-AL25 (6): Stigmasterol.

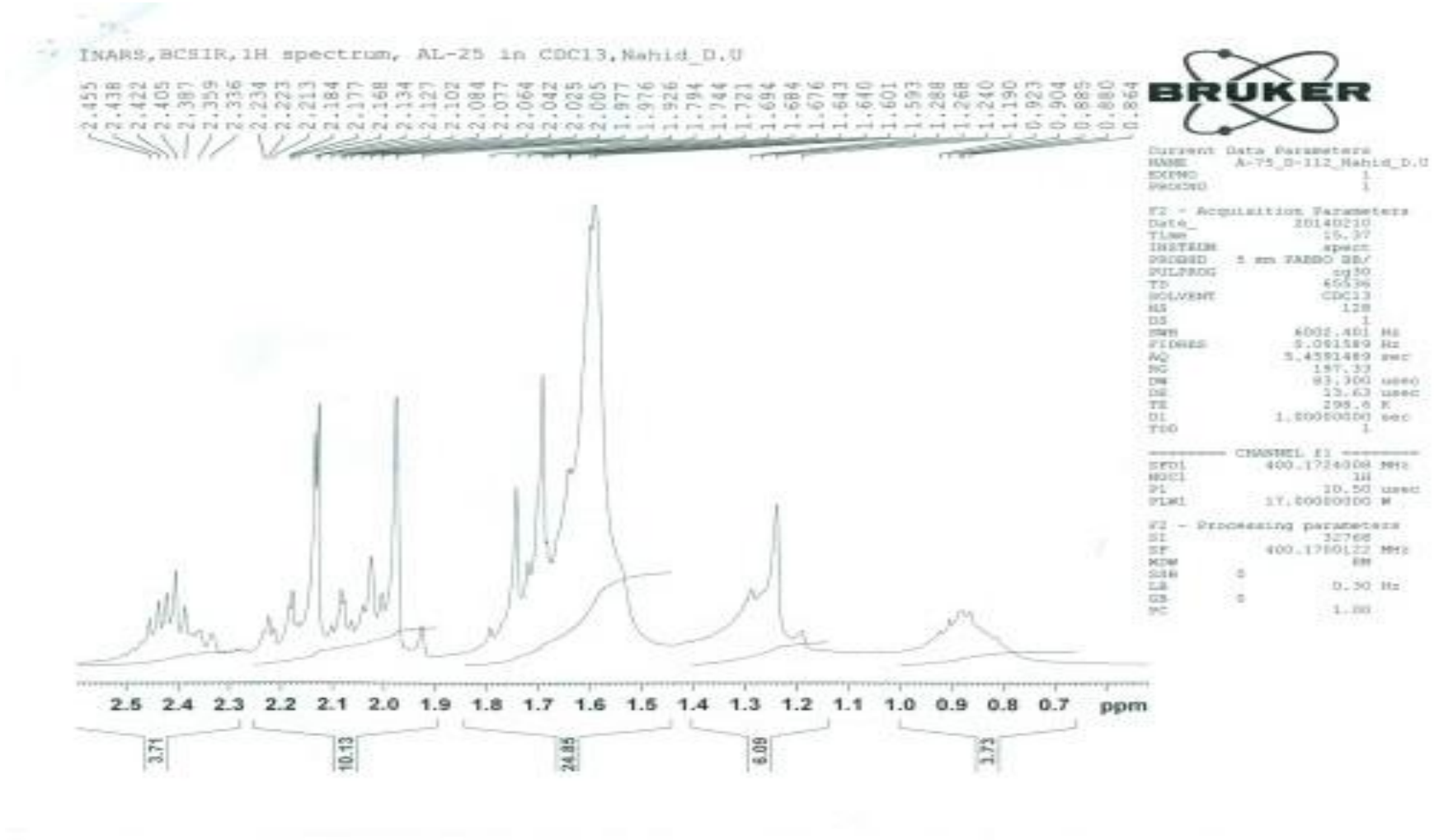


Fig. 29. Partial expansion of ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of Stigmasterol (5).

CHAPTER 8

ANTICANCER ACTIVITY OF SELECTED PLANTS

8.1 Introduction

Screening is a investigation by which it can be determined within short time and with equally minute effort whether the bacterial metabolites have any toxic effect on cancer cells or not. An important part of developing a new anticancer drug is the testing of the potentiality of the new compound against animal tumours both *in vivo* and *in vitro*. In *in vitro* tests, determination is done to see whether the compound has any effect against neoplasm in the living system or not. In *in vivo* tests, determination is done not only to study the effects of the drug on animals (bearing transplanted tumour) but also on the host, including its toxicity and therapeutic index.

8.2 Materials and Methods

8.2.1 Instruments and apparatus

8.2.1.1 Microscope: Samples on the haemocytometer were visualized with the help of a binocular microscope under magnification of 10x, 40x and 100x.

8.2.1.2 Haemocytometer: Tumour cells were counted with a Neubauer Haemocytometer. The subsequent cell concentration per ml was determined by using the following formula:

$$\text{No. of cells per ml} = \frac{\text{The average count per square} \times \text{Dilution factor}}{\text{Depth of fluid under cover slip} \times \text{Area counted}}$$

$$= \frac{\text{The average count per square} \times \text{Dilution factor}}{(0.1\text{ mm}) \times (1\text{ mm})^2}$$

$$= \frac{\text{The average count per square} \times \text{Dilution factor}}{(0.1) \times (1\text{ mm})^3}$$

$$= \frac{\text{The average count per square} \times \text{Dilution factor}}{(0.1) \times 10^{-3}\text{ ml}}$$

$$= \text{The average count per square} \times \text{Dilution factor} \times 10^4$$

8.2.2 Experimental animal

Swiss Albino mice of 5-7 weeks old, weighing 20-26 gram were collected from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B), Mohakhali, Dhaka.

8.2.2.1 Mice vital statistics

• Scientific name: *Mus musculus*

• Life span: 2-3 years

• Potential life span: 4 years

• Desirable environmental temperature range: 18-27°C

• Desirable relative humidity range: 30-70%

• Age at onset of puberty: 28-40 days

• Estrus (heat) cycle length: 4-5 days

• Estrus length (period during which female is receptive to male for copulation): 12 hours

• Gestation (pregnancy) period: 19-21 days

• Weaving age: 21-28 days

8.2.2.2 Animal care

a. Cage: Mice were kept in iron cages with saw dust and straw bedding which was changed once a week regularly. Standard mouse diet (recommended and prepared by ICDDRB) and adequate water were given.

b. Temperature, light and humidity: The room temperature was maintained around 25-32°C and a controlled 14 hours day light and 10 hours dark were maintained in the laboratory (animal house).

8.2.3 Experimental tumour model

Transplantable tumour (Ehrlich Ascites Carcinoma) was used in this experiment. The initial inoculums of EAC cells was provided by the Indian Institute of Chemical Biology (IICB), located at Kolkata, India. The EAC cells were thereafter propagated in our laboratory bi-weekly intraperitoneal (i. p.) injections of $2\times l0^6$ cells, freshly drawn from a donor Swiss albino mouse bearing 6-7 days old ascites tumour suspended in 0.3 ml sterile saline solution.

8.2.3.1 Ehrlich Ascites Carcinoma (EAC)

Ehrlich Ascites Carcinoma (EAC) cells are being used in cancer research worldwide. In 1907, Ehrlich located this tumour in the mammary gland of a white mouse and thus the tumour was named after him. The present form of EAC cells has been developed by Loewenthal and John from one of the several lines of mammary gland origin. External surface of EAC cells is covered with a thin section. The membrane matrix of Ehrlich ascites tumour cells is especially strong. Extensive studies on the morphology of normal and cancer cells have shown that both the surface and intracellular membranes have 'unit membrane' structure- a bimolecular lipid leaflet lined on both sides of protein or polysaccharide material. EAC cell being cancer cells also possess the same type of structure. Tumour can be grown subcutaneously as solid form, but the present ascitic form is produced by infecting tumour cell suspension into the mouse peritoneal cavity. The ascitic tumour develops as a milky white fluid containing large rounded tumour cells. One million of tumour cells multiply to yield about 25-100 million tumour cells/ml. Host carrying such a tumour survives for about 14-30 days.

8.2.4 Transplantation of ascitic tumour

Ascitic fluid was drawn out from different tumour bearing Swiss albino mice at the respective log-phases of tumour cells. A 3 ml syringe filled with 20 gauge needle was used for this tumour cell aspiration. The freshly drawn fluid was diluted with normal saline (0.98% NaCl solution) and the number of tumour cells was adjusted to approximately 2×10^6 cells/ml by counting the cell number with the help of a haemocytometer. The viability of tumour cells was observed by trypan blue dye

(0.4%) exclusion assay. Cell sample showing above 90% viability were used for transplantation. Tumour suspension of 0.1 ml was injected intraperitoneally (i. p.) to each Swiss albino mouse (Photo 178). Strict aseptic condition was maintained throughout the transplantation process.

8.2.5 Determination of cell growth inhibition with the peel extract (*in vivo*)

In vivo cell growth inhibition was determined by the method followed by Rana and Khanam (2002). To determine the cell growth inhibition of the peel extract, eight groups of Swiss albino mice (6 in each group) weighing 20-25 gm were used. For therapeutic evaluation 2×10^6 EAC cells in every mouse were inoculated into each group of mice on day "0". Treatments were started after 24 hours of tumour inoculation and continued for five days. Three groups of mice received test extract (Crude methanolic extract and aqueous soluble fraction of root bark of *O. indicum* and chloroform soluble fraction of *A. polystachya*) at the dose of 10 mg/kg (i. p). The other three groups received at the dose of 20 mg/kg (i. p). Standard group was received bleomycin at the dose of 0.3 mg/kg (i. p) and group eight was used as control (Photo 179). Mice in each group were sacrificed on day six and the total intraperitoneal tumour cells were harvested by normal saline (0.98%). Viable cells were first identified by using trypan blue and then counted by a haemocytometer. Total number of viable cells in every animal of the treated groups was compared with those of control (EAC treated only) group.

8.2.6 The equation for calculation of % of cell growth inhibition (Antineoplastic activity)

For experimental mice (Treated with extracts or drug)

The average count (cell) per square = X_1……..X_n

Dilution factor = Z

Cells/ml = $X_1 \times Z \times 10^4$ (for X_1 mice)

Cells/ml = $X_n \times Z \times 10^4$ (for X_n mice)

$$X = \frac{X_1 + \ldots\ldots..+ X_n}{n}$$

For control mice

The average count (cell) per square = N_1……..N_n

Dilution factor = Z

Cells/ml = $N_1 \times Z \times 10^4$ (for N_1 mice)

Cells/ml = $N_n \times Z \times 10^4$ (for N_n mice)

$$N = \frac{N_1 + \ldots\ldots..+N_n}{n}$$

$$\% \text{ of cell growth inhibition} = \frac{N - X}{N} \times 100$$

8.3 Results

The crude extract and aqueous soluble fraction of the root bark of *Oroxylum indicum* was found to have significant anticancer activity in Ehrlich's Ascites Carcinoma Test by calculating % of cell growth inhibition (40.46% and 68.09%) and the chloroform soluble fraction of the bark of *Aphanamixis polystachya* was also found to have significant anticancer activity (64.80%) with comparison to standard bleomycin (89.0%) using an *in vivo* test (Table 31).

Table 31. % of cell growth inhibition for different samples of studied species.

Name of the plants	Fractions	% of cell growth inhibition found
Oroxylum indicum	Crude methanol extract	40.46
	Aqueous extract	68.09
Aphanamixis polystachya	Chloroform extract	64.80
Standard bleomycin		89.00

Photo 178. Transplantation of ascitic tumour.

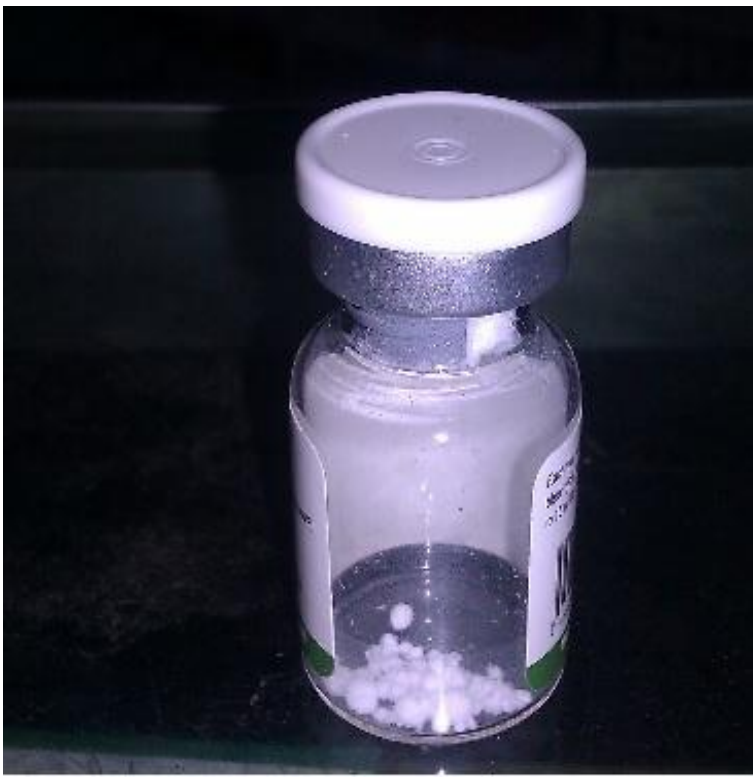


Photo 179. Bleomycin.

CHAPTER 9

BRINE SHRIMP LETHALITY BIOASSAY

9.1 Introduction

Bioactive compounds are always toxic to living body at some higher doses and rationalize the statement that 'Pharmacology is simply toxicology at higher doses and toxicology is simply pharmacology at lower doses'. Brine shrimp lethality bioassay is a rapid and broad bioassay for the bioactive compound of the natural and synthetic origin (McLaughlin, 1990; Persoone *et al*., 1980). By this method, natural product extarcts, fractions as well as the pure compounds can be tested for their bioactivity. In this method, *in vivo* lethality in a simple zoological organism (Brine shrimp nauplii) is used as a positive view for screening and fractionation in the discovery of new bioactive natural products.

This bioassay shows cytotoxicity as well as a broad range of pharmacological activities such as antimicrobial, antiviral, pesticidal and anti-tumour etc. of the compounds (Meyer *et al*., 1982).

Brine shrimp lethality bioassay technique places better-quality to other cytotoxicity testing procedures because it is a rapid process, inexpensive and requires no special equipment or aseptic technique. It develops a large number of organisms for statistical validation and a relatively small amount of sample. Furthermore, contrasting other methods, it does not require animal serum.

9.2 Principle

Brine shrimp eggs are hatched in simulated sea water to get nauplii. By the addition of calculated amount of dimethylsulphoxide (DMSO), desired concentration of the test sample is prepared. The nauplii are counted by visual inspection and are taken in vials containing 5 ml of simulated sea water. Then samples of different concentrations are added to the premarked vials through micropipette. The vials are then left for 24 hours. Survivors are counted after 24 hours (Meyer *et al*., 1982).

9.3 Materials

- *Artemia salina* leach (brine shrimp eggs)
- Sea salt (NaCl)
- Small tank with perforated dividing dam to hatch the shrimp
- Lamp to attract shrimps
- Pipettes
- Micropipette
- Glass vials
- Magnifying glass
- Test tubes
- Test samples of experimental plants

9.4 Experimental Procedure

9.4.1 Preparation of seawater

38 gm sea salt (pure NaCl) was weighed, dissolved in one litre of distilled water and filtered off to get clear solution.

9.4.2 Hatching of brine shrimps

Artemia salina leach (brine shrimp eggs) collected from pet shops was used as the test organism. Seawater was taken in the small tank and shrimp eggs were added to one side of the tank and then this side was covered. One day was allowed to hatch the shrimp and to be matured as nauplii. Constant oxygen supply was given through the hatching time. The hatched shrimps were attracted to the lamp through the perforated dam and they were taken for experiment. With the help of a pasteur pipette 10 living shrimps were added to each of the test tubes containing 5 ml of seawater.

9.4.3 Preparation of test samples of the experimental plant species

All the test samples (Tables 33 & 42) were taken in vials and dissolved in 200 µl of pure dimethylsulfoxide (DMSO) to get stock solutions. Then 100 µl of solution was taken in the first test tube containing 5 ml of simulated seawater and 10 shrimp nauplii. Thus, final concentration of the prepared solution in the first test tube was 400 µg/ml. Then a series of solutions of varying concentrations were prepared from the

stock solution by serial dilution method. In every case, 100 µl sample was added to test tube and fresh 100 µl DMSO was added to vial. Thus different concentrations were found in the different test tubes (Table 32).

Table 32. Test samples with concentration values after serial dilution.

Test Tube No.	Concentration (µg/ml)
1	400.0
2	200 .0
3	100 .0
4	50 .0
5	25 .0
6	12.5
7	6.25
8	3.125
9	1.5625
10	0.78125

9.4.4 Preparation of control group

Control groups are used in cytotoxicity study to validate the test method and ensure that the results obtained are only due to the activity of the test agent and the effects of the other possible factors are nullified. Usually two types of control groups are used-

i) Positive control

ii) Negative control

9.4.4.1 Preparation of the positive control group

Positive control in a cytotoxicty study is a widely accepted cytotoxic agent and the result of the test agent is compared with the result obtained from the positive control. In the present study vincristine sulphate was used as the positive control. Measured amount of the vincristine sulphate was dissolved in DMSO to get an initial concentration of 20 µg/ml from which serial dilutions are made using DMSO to get

10 µg/ml, 5 µg/ml, 2.5µg/ml, 1.25 µg/ml, 0.625 µg/ml, 0.3125 µg/ml, 0.15625 µg/ml, 0.078125 µg/ml and 0.0390 µg/ml. Then the positive control solutions were added to the premarked vials containing ten living brine shrimp nauplii in 5 ml simulated seawater to get the positive control groups.

9.4.4.2 Preparation of the negative control group

100 µl of DMSO was added to each of three premarked glass vials containing 5 ml of simulated seawater and 10 shrimp nauplii to use as control groups. If the brine shrimps in these vials show a rapid mortality rate, then the test is considered as invalid as the nauplii died due to some reason other than the cytotoxicity of the compounds.

9.4.5 Counting of nauplii

After 24 hours, the vials were inspected using a magnifying glass and the number of survivors were counted. The per cent (%) mortality was calculated for each dilution. The concentration-mortality data were analyzed statistically by using linear regression using a simple IBM-PC program. The effectiveness or the concentration-mortality relationship of plant product is usually expressed as a median lethal concentration (LC_{50}) value. This represents the concentration of the chemical that produces death in half of the test subjects after a certain exposure period.

Table 33. Test samples of root bark of *Oroxylum indicum.*

Plant part	Sample code	Test Samples	Calculated amount (mg)
Root bark of *O. indicum*	MEROI	Methanolic extract of root bark of *O. indicum*	4.0
	PESF	Petroleum ether soluble fraction	4.0
	CHSF	Chloroform soluble fraction	4.0
	DCMSF	Dichloromethane soluble fraction	4.0
	EASF	Ethyl acetate soluble fraction	4.0
	AQSF	Aqueous soluble fraction	4.0

9.5 Results of the test samples of *O. indicum*

The methanolic extract of root bark of *O. indicum* (MEROI) and the different partitionate, i.e. pet-ether soluble fraction (PESF), chloroform soluble fraction (CHSF), dichloromethane soluble fraction (DCMSF), ethyl acetate soluble fraction (EASF) and aqueous soluble fraction (AQSF) were subjected to brine shrimp lethality bioassay following the procedure of Meyer *et al.*, (1982). The lethality of the extractives to brine shrimp was determined and the results are presented in Tables 35-40 as well as in Figures 31-36.

The lethal concentration LC_{50} of the test samples after 24 hr. was obtained by a plot of percentage of the shrimps died against the logarithm of the sample concentration (toxicant concentration) and the best-fit line was obtained from the curve data by means of regression analysis.

Table 34. Effect of Vincristine sulphate (positive control) on shrimp nauplii.

Conc. (µg/mL)	Log_{10} conc.	% Mortality	LC_{50}
0	-	0	0.413
0.7813	-0.1072	30	
1.5625	0.19382	50	
3.125	0.49485	50	
6.25	0.79588	60	
12.5	1.09691	70	
25	1.39794	80	
50	1.69897	80	
100	2	90	
200	2.30103	100	
400	2.60206	100	

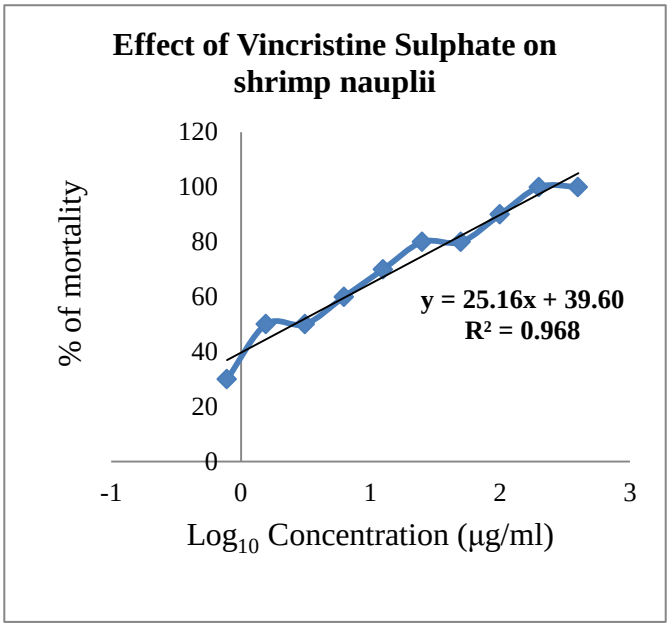


Fig. 30: Plot of % mortality and predicted regression line of VS.

Table 35. Effect of methanolic extract of root bark of *O. indicum* (MEROI) on shrimp nauplii.

Conc. (µg/mL)	Log_{10} conc.	% of mortality	LC_{50}
0	-	0	1.817
0.78125	-1.1072	0	
1.5625	0.19382	0	
3.125	0.49485	10	
6.25	0.79588	10	
12.5	1.09691	20	
25	1.39794	30	
50	1.69897	40	
100	2	60	
200	2.30103	70	
400	2.60206	90	

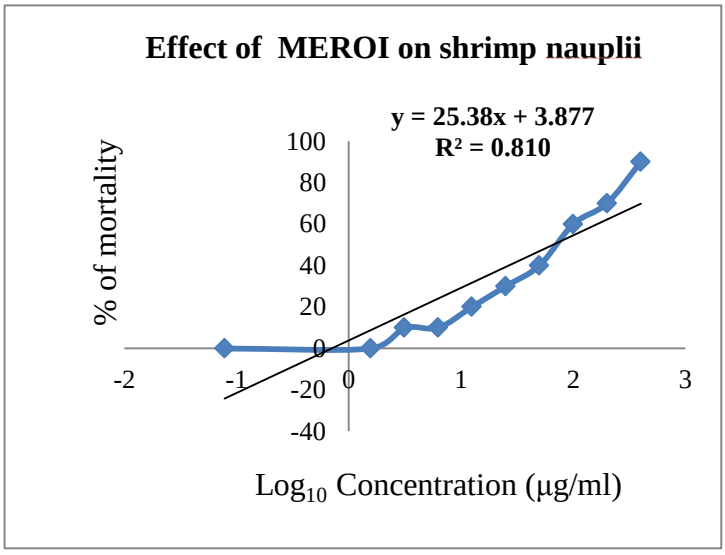


Fig. 31: Plot of % mortality and predicted regression line of MEROI.

Table 36. Effect of pet-ether soluble fraction of the methanolic extract (PESF) of *O. indicum* on shrimp nauplii.

Conc. (µg/mL)	Log_{10} conc.	% of mortality	LC_{50}
0	-	0	1.183
0.78125	-1.1072	10	
1.5625	0.19382	10	
3.125	0.49485	20	
6.25	0.79588	30	
12.5	1.09691	30	
25	1.39794	50	
50	1.69897	70	
100	2	80	
200	2.30103	90	
400	2.60206	100	

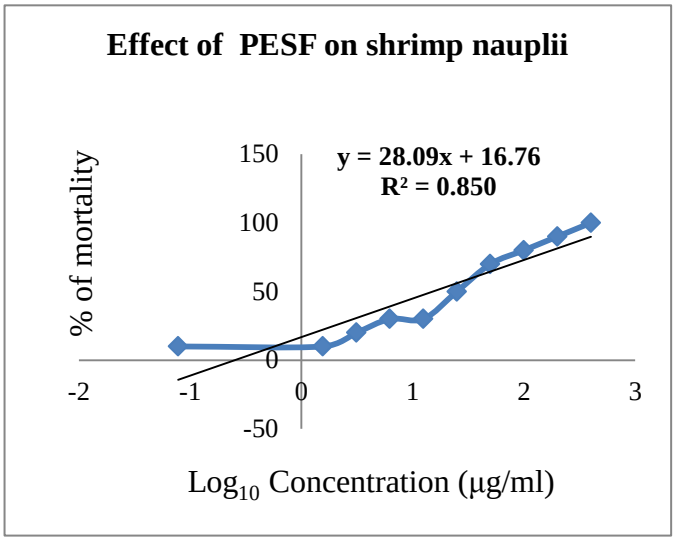


Fig. 32. Plot of % mortality and predicted regression line of PESF.

Table 37. Effect of chloroform soluble fraction of the methanolic extract (CHSF) of *O. indicum* on shrimp nauplii.

Conc. (µg/mL)	Log_{10} conc.	% of mortality	LC_{50}
0	-	0	0.633
0.78125	-1.1072	20	
1.5625	0.19382	20	
3.125	0.49485	30	
6.25	0.79588	30	
12.5	1.09691	70	
25	1.39794	80	
50	1.69897	100	
100	2	100	
200	2.30103	100	
400	2.60206	100	

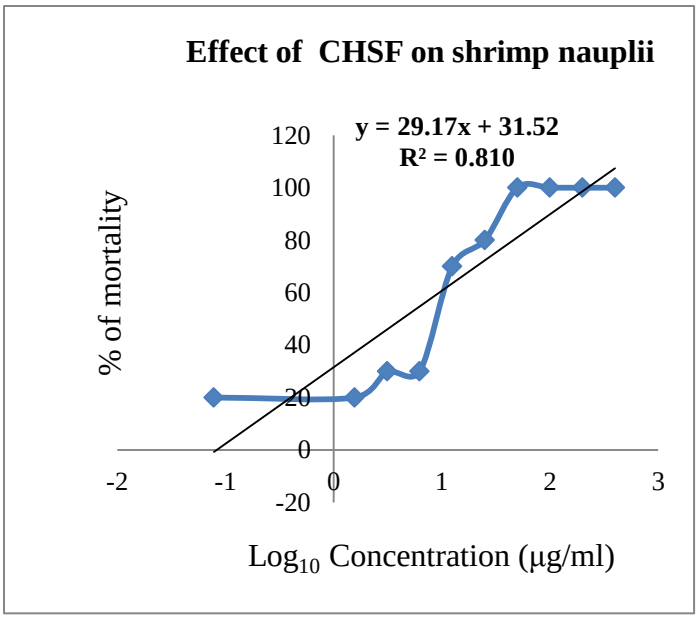


Fig. 33: Plot of % mortality and predicted regression line of CHSF.

Table 38. Effect of dichloromethane soluble fraction of the methanolic extract (DCMSF) of *O. indicum* on shrimp nauplii.

Conc. (µg/mL)	Log_{10} conc.	% of mortality	LC_{50}
0	-	0	0.677
0.78125	-1.1072	20	
1.5625	0.19382	20	
3.125	0.49485	20	
6.25	0.79588	30	
12.5	1.09691	70	
25	1.39794	80	
50	1.69897	100	
100	2	100	
200	2.30103	100	
400	2.60206	100	

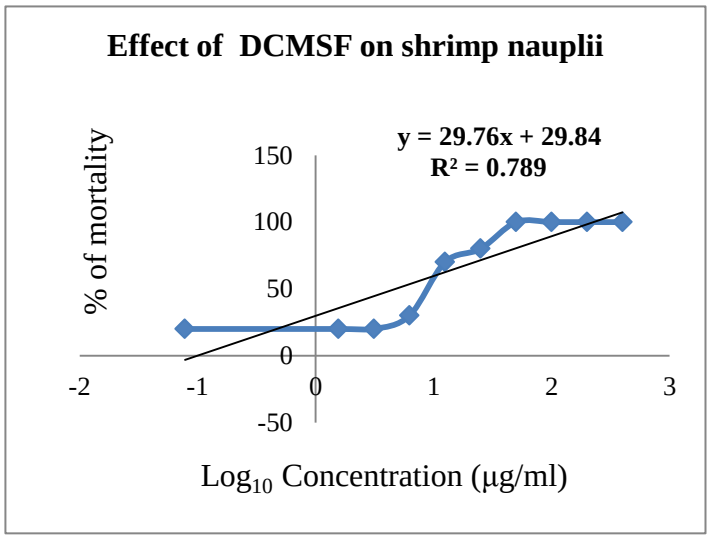


Fig. 34. Plot of % mortality and predicted regression line of DCMSF.

Table 39. Effect of ethyl acetate soluble fraction of the methanolic extract (EASF) of *O. indicum* on shrimp nauplii.

Conc. (µg/mL)	Log_{10} conc.	% of mortality	LC_{50}
0	-	0	2.120
0.78125	-1.1072	0	
1.5625	0.19382	0	
3.125	0.49485	0	
6.25	0.79588	10	
12.5	1.09691	10	
25	1.39794	30	
50	1.69897	50	
100	2	40	
200	2.30103	60	
400	2.60206	80	

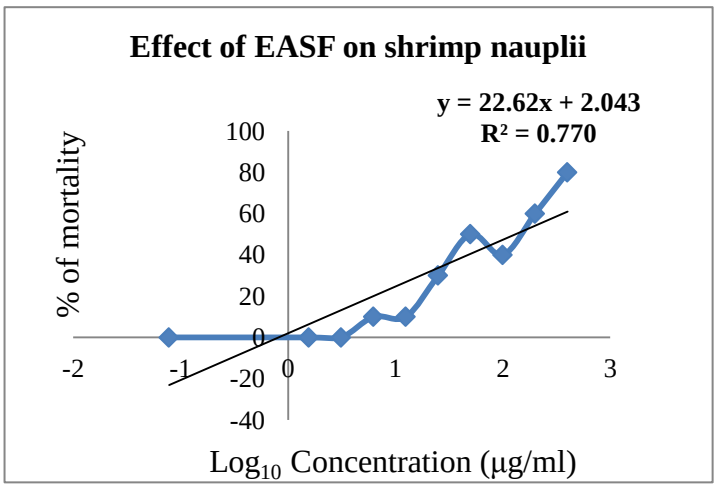


Fig. 35. Plot of % mortality and predicted regression line of EASF.

Table 40. Effect of aqueous soluble fraction of the methanolic extract (AQSF) of *O. indicum* on shrimp nauplii.

Conc. (µg/mL)	Log_{10} conc.	% of mortality	LC_{50}
0	-	0	2.576
0.78125	-1.1072	0	
1.5625	0.19382	0	
3.125	0.49485	20	
6.25	0.79588	20	
12.5	1.09691	50	
25	1.39794	40	
50	1.69897	30	
100	2	30	
200	2.30103	40	
400	2.60206	60	

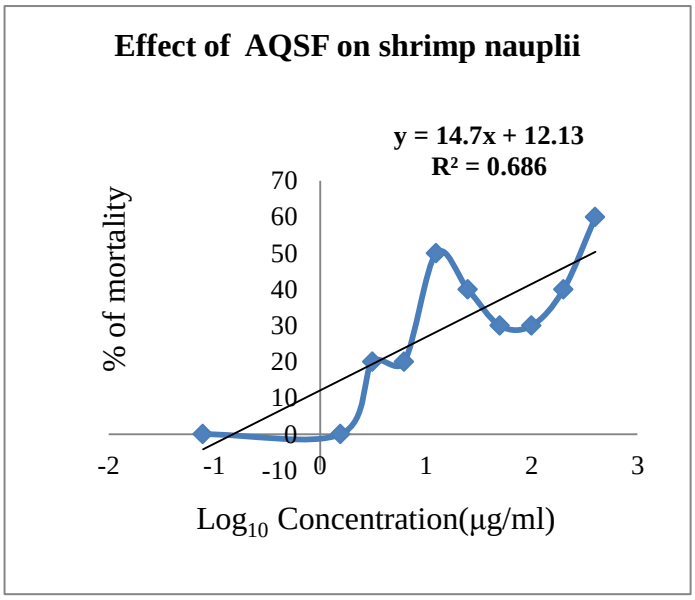


Fig. 36. Plot of % mortality and predicted regression line of AQSF.

Vincristine sulphate (VS) was used as positive control and the LC_{50} was found 0.413 ± 0.55 µg/ml for VS (Table 34 and Fig. 30). Compared with the negative control VS (positive control) gave significant mortality and the LC_{50} values of the different extractives were compared to this positive control.

The LC_{50} values of MEROI, PESF, CHSF, DCMSF, EASF and AQSF were found to be 1.817 ± 0.23 µg/ml, 1.183 ± 0.32 µg/ml, 0.633 ± 0.17 µg/ml, 0.677 ± 0.15 µg/ml, 2.120 ± 0.73 µg/ml and 2.576 ± 0.45 µg/ml respectively (Table 41 and Fig. 37). CHSF and DCMSF showed significant lethality whereas MEROI, PESF, EASF and AQSF showed moderate activity.

Table 41. LC_{50} values of the test samples of *O. indicum*.

Test samples	Regression line	R^2	LC_{50} (µg/ml)
VS	y = 25.16x + 39.60	0.968	0.413 ± 0.55
MEROI	y = 28.68x + 31.09	0.826	1.817 ± 0.23
PESF	y = 27.95x + 35.92	0.852	1.183 ± 0.32
CHSF	y = 29.17x + 31.52	0.810	0.633 ± 0.17
DCMSF	y = 29.76 x + 29.84	0.789	0.677 ± 0.15
EASF	y = 22.62x + 2.043	0.770	2.120 ± 0.73
AQSF	y = 14.7x + 12.13	0.686	2.576 ± 0.45

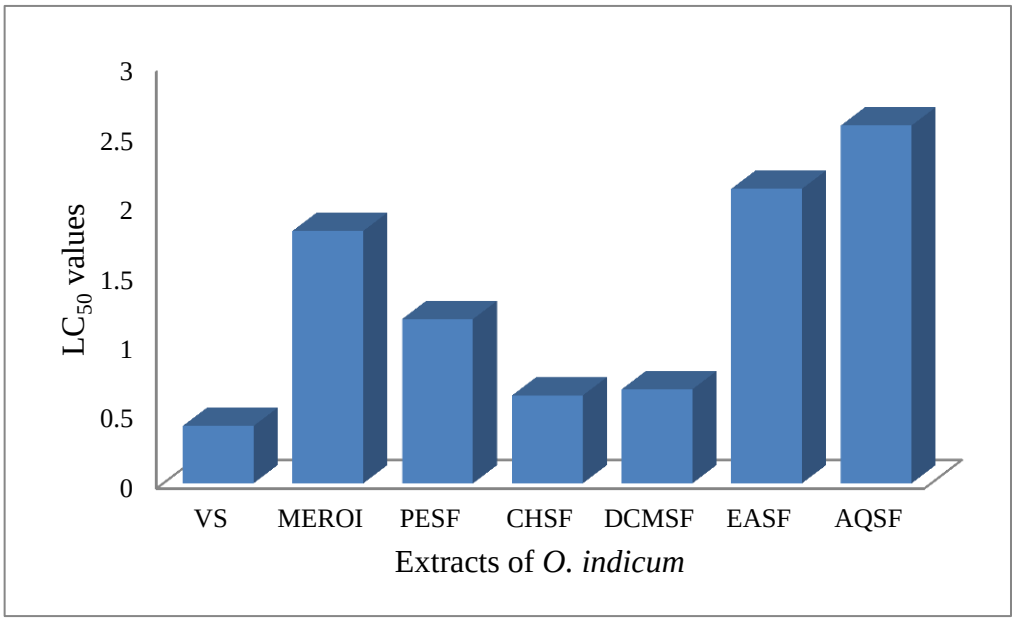


Fig. 37. LC_{50} values of the different extractives of *O. indicum*.

Table 42: Test samples of bark and leaves of *Aphanamixis polystachya.*

Plant part	Sample code	Test Samples	Calculated amount (mg)
Bark of *A. polystachya*	MEBAP	Methanolic extract of bark of *A. polystachya*	4.0
	PESF	Petroleum ether soluble fraction	4.0
	CHSF	Chloroform soluble fraction	4.0
	EASF	Ethyl acetate soluble fraction	4.0
	AQSF	Aqueous soluble fraction	4.0
Leaves of *A. polystachya*	MELAP	Methanolic extract of leaves of *A. polystachya*	4.0
	PESF	Petroleum ether soluble fraction	4.0
	CHSF	Chloroform soluble fraction	4.0
	AQSF	Aqueous soluble farction	4.0

9.6 Results of the test samples of bark and leaves of *A. polystachya*

The methanolic extract of bark of *A. polystachya* (MEBAP) and the different partitionate, i.e. pet-ether soluble fraction (PESF), chloroform soluble fraction (CHSF), ethyl acetate soluble fraction (EASF) and aqueous soluble fraction (AQSF) were concerned to brine shrimp lethality bioassay following the procedure of Meyer *et al.*, (1982). The lethality of the extractives to brine shrimp was determined and the results are given in Tables 43-47 as well as in Figures 38-42.

Vincristine sulphate (VS) was used as positive control and the LC_{50} was found 0.413 ± 0.55 μg/ml for VS (Table 34 and Fig. 30). Compared with the negative control VS (positive control) gave significant mortality and the LC_{50} values of the different extractives were compared to this positive control.

Table 43. Effect of methanolic extract of bark of *A. polystachya* (MEBAP) on shrimp nauplii.

Conc. (µg/mL)	Log_{10} conc.	% of mortality	LC_{50}
0	-	0	2.462
0.78125	-1.1072	0	
1.5625	0.19382	0	
3.125	0.49485	10	
6.25	0.79588	20	
12.5	1.09691	20	
25	1.39794	30	
50	1.69897	40	
100	2	40	
200	2.30103	50	
400	2.60206	60	

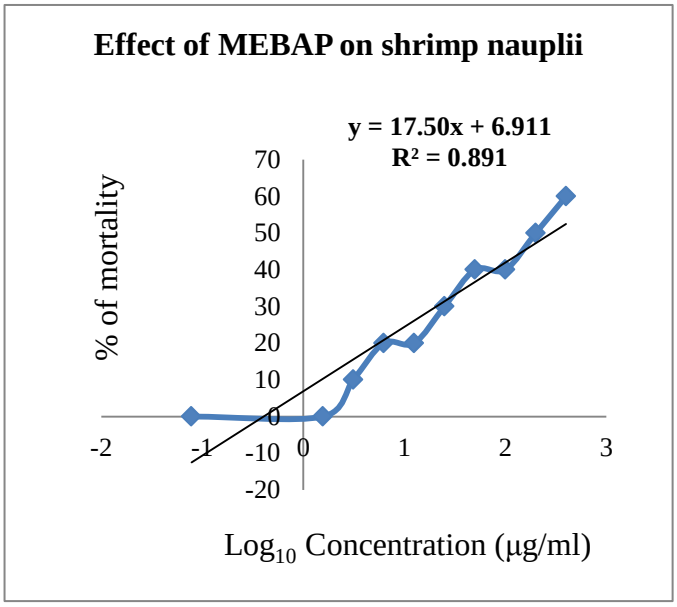


Fig. 38. Plot of % mortality and predicted regression line of MEBAP.

Table 44. Effect of pet-ether soluble fraction of the methanolic extract (PESF) of bark of *A. polystachya* on shrimp nauplii.

Conc. (µg/mL)	Log_{10} conc.	% of mortality	LC_{50}
0	-	0	1.392
0.78125	-1.1072	10	
1.5625	0.19382	20	
3.125	0.49485	30	
6.25	0.79588	40	
12.5	1.09691	30	
25	1.39794	40	
50	1.69897	60	
100	2	60	
200	2.30103	80	
400	2.60206	80	

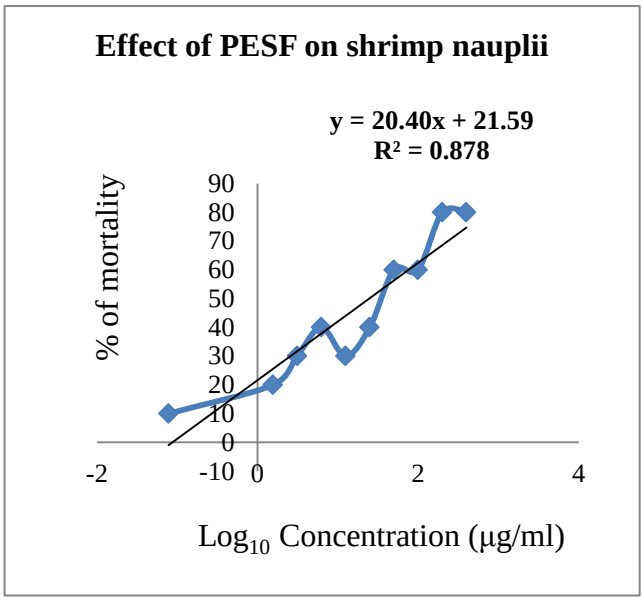


Fig. 39. Plot of % mortality and predicted regression line of PESF.

Table 45. Effect of chloroform soluble fraction of the methanolic extract (CHSF) of bark *A. polystachya* on shrimp nauplii.

Conc. (µg/mL)	Log_{10} conc.	% of mortality	LC_{50}
0	-	0	0.677
0.78125	-1.1072	20	
1.5625	0.19382	40	
3.125	0.49485	40	
6.25	0.79588	50	
12.5	1.09691	50	
25	1.39794	50	
50	1.69897	70	
100	2	90	
200	2.30103	100	
400	2.60206	100	

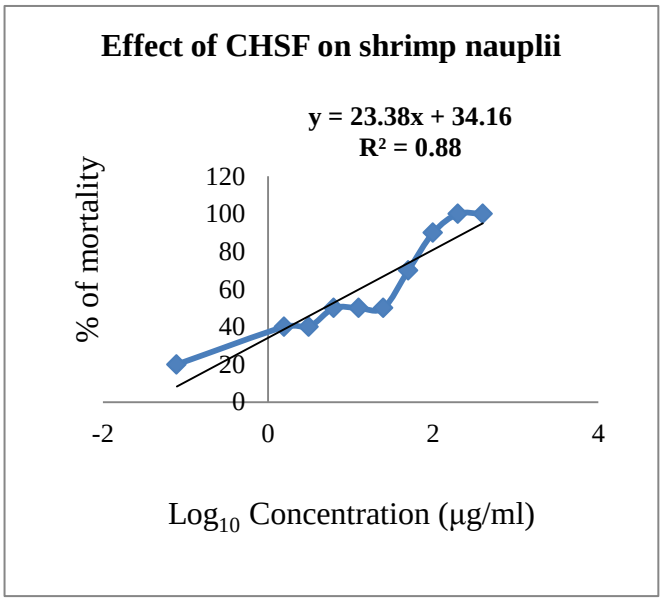


Fig. 40. Plot of % mortality and predicted regression line of CHSF.

Table 46. Effect of ethyl acetate soluble fraction of the methanolic extract (EASF) of bark of *A. polystachya* on shrimp nauplii.

Conc. (µg/mL)	Log_{10} conc.	% of mortality	LC_{50}
0	-	0	2.036
0.78125	-1.1072	0	
1.5625	0.19382	10	
3.125	0.49485	10	
6.25	0.79588	20	
12.5	1.09691	20	
25	1.39794	20	
50	1.69897	30	
100	2	50	
200	2.30103	70	
400	2.60206	80	

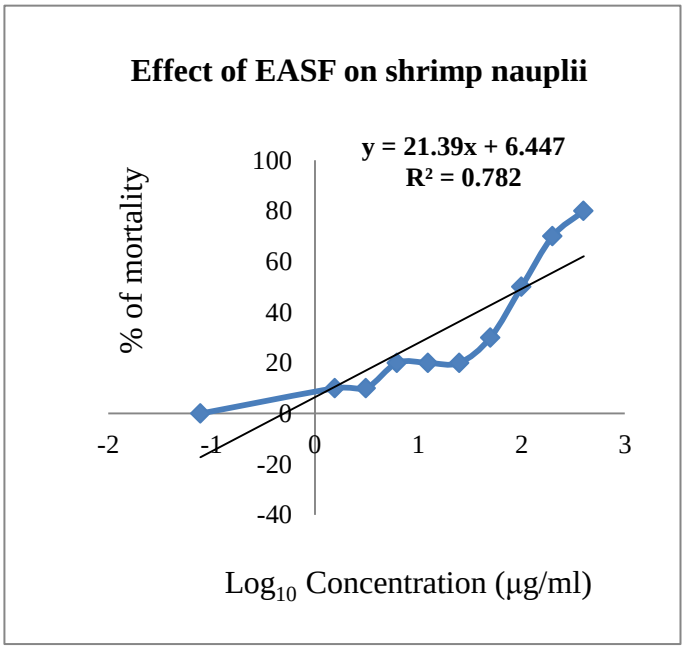


Fig. 41. Plot of % mortality and predicted regression line of EASF.

Table 47. Effect of aqueous soluble fraction of the methanolic extract (AQSF) of bark of *A. polystachya* on shrimp nauplii.

Conc. (µg/mL)	Log_{10} conc.	% of mortality	LC_{50}
0	-	0	2.112
0.78125	-1.1072	0	
1.5625	0.19382	0	
3.125	0.49485	10	
6.25	0.79588	10	
12.5	1.09691	20	
25	1.39794	30	
50	1.69897	30	
100	2	50	
200	2.30103	60	
400	2.60206	80	

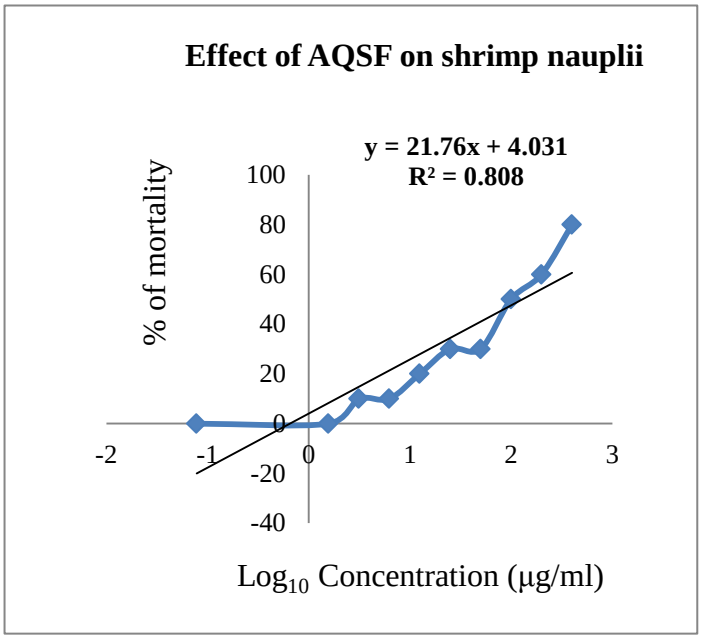


Fig. 42. Plot of % mortality and predicted regression line of AQSF.

The LC_{50} values of MEBAP, PESF, CHSF, EASF and AQSF were found to be 2.462 ± 0.14 µg/ml, 1.392 ± 0.21 µg/ml, 0.677 ± 0.64 µg/ml, 2.036 ± 0.41 µg/ml and 2.112 ± 0.59 µg/ml respectively (Table 48 and Fig. 43). CHSF showed significant lethality whereas MEBAP, PESF, EASF and AQSF showed moderate activity.

Table 48. LC_{50} values of the test samples of bark of *A. polystachya*.

Test samples	Regression line	R^2	LC_{50} (µg/ml)
VS	y = 25.16x + 39.60	0.968	0.413 ± 0.55
MEBAP	y = 17.50x + 6.911	0.891	2.462 ± 0.14
PESF	y = 20.40x + 21.59	0.878	1.392 ± 0.21
CHSF	y = 23.38x + 34.16	0.880	0.677 ± 0.64
EASF	y = 21.39x + 6.447	0.782	2.036 ± 0.41
AQSF	y = 21.76x + 4.031	0.808	2.112 ± 0.59

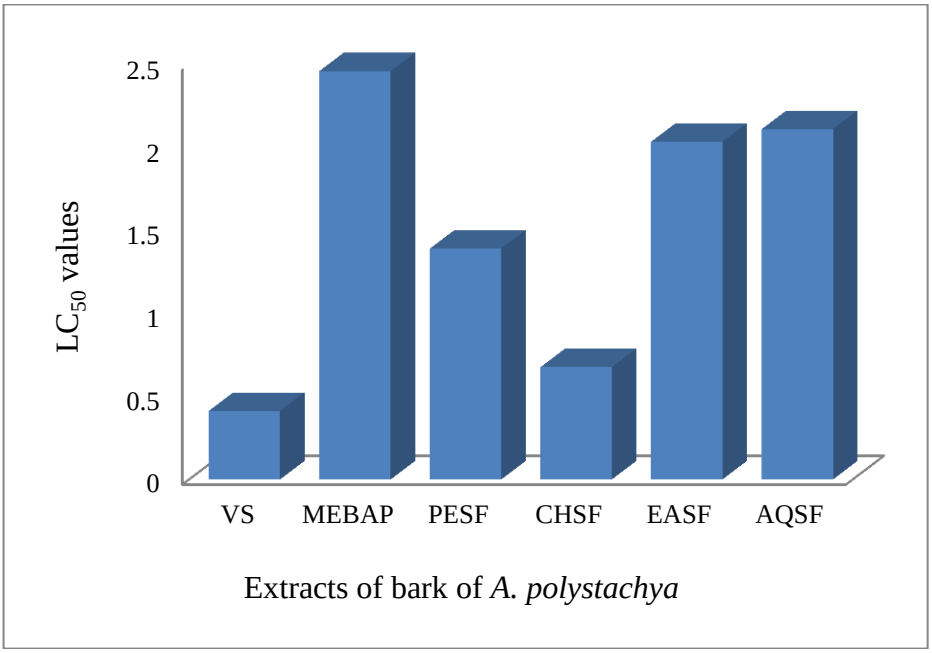


Fig. 43. LC_{50} values of the different extractives of bark of *A. polystachya.*

The methanolic extract of leaves of *A. polystachya* (MELAP) and the different partitionate, i.e. pet-ether soluble fraction (PESF), chloroform soluble fraction (CHSF) and aqueous soluble fraction (AQSF) were focused to brine shrimp lethality bioassay. The lethality of the extractives to brine shrimp was determined and the results are given in Tables 49-52 and Figures 44-47.

Table 49. Effect of methanolic extract of leaves of *A. polystachya* (MELAP) on shrimp nauplii.

Conc. (µg/mL)	Log_{10} conc.	% of mortality	LC_{50}
0	-	0	1.334
0.78125	-1.1072	0	
1.5625	0.19382	20	
3.125	0.49485	30	
6.25	0.79588	40	
12.5	1.09691	50	
25	1.39794	50	
50	1.69897	60	
100	2	60	
200	2.30103	70	
400	2.60206	80	

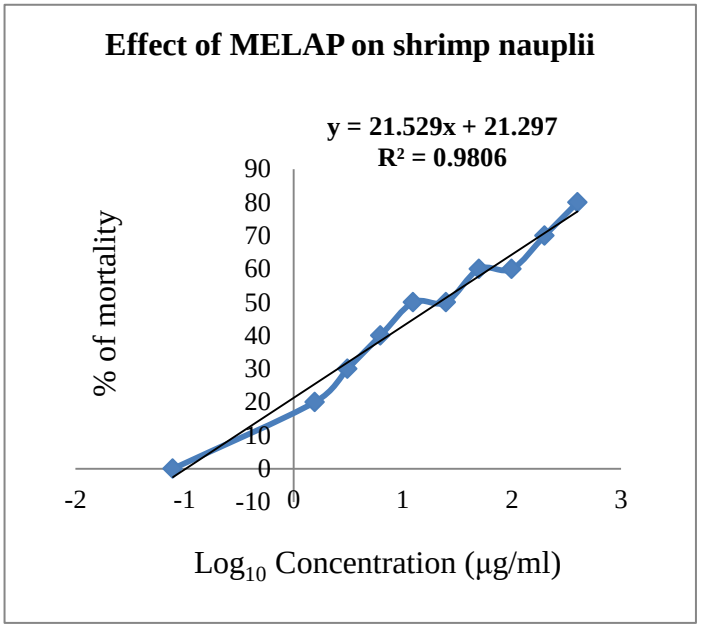


Fig. 44. Plot of % mortality and predicted regression line of MELAP.

Table 50. Effect of pet-ether soluble fraction of the methanolic extract (PESF) of leaves of *A. polystachya* on shrimp nauplii.

Conc. (µg/mL)	Log_{10} conc.	% of mortality	LC_{50}
0	-	0	0.682
0.78125	-1.1072	10	
1.5625	0.19382	20	
3.125	0.49485	30	
6.25	0.79588	50	
12.5	1.09691	60	
25	1.39794	80	
50	1.69897	90	
100	2	100	
200	2.30103	100	
400	2.60206	100	

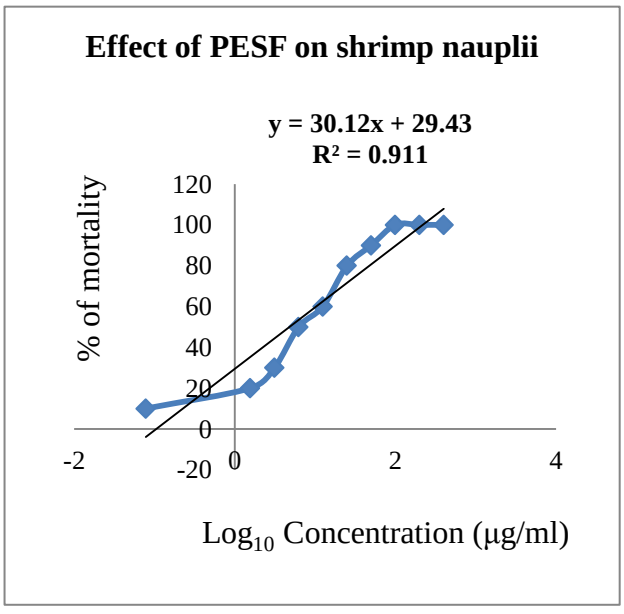


Fig. 45. Plot of % mortality and predicted regression line of PESF.

Table 51. Effect of chloroform soluble fraction of the methanolic extract (CHSF) of leaves of *A. polystachya* on shrimp nauplii.

Conc. (µg/mL)	Log_{10} conc.	% of mortality	LC_{50}
0	-	0	1.216
0.78125	-1.1072	0	
1.5625	0.19382	10	
3.125	0.49485	20	
6.25	0.79588	40	
12.5	1.09691	30	
25	1.39794	50	
50	1.69897	70	
100	2	70	
200	2.30103	90	
400	2.60206	100	

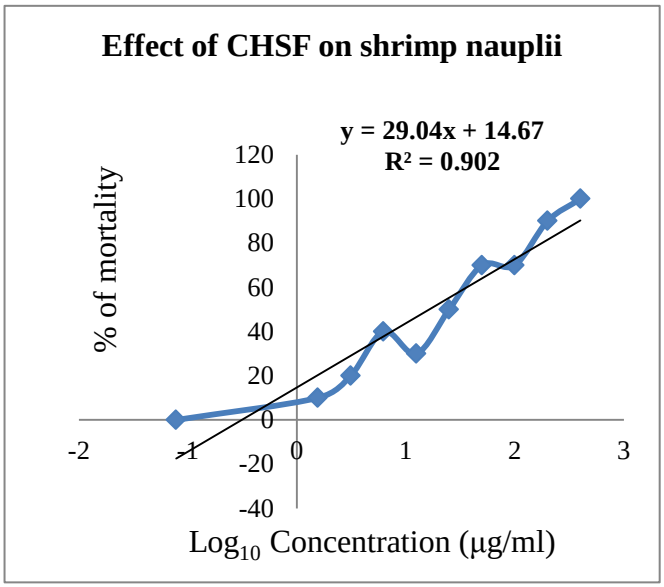


Fig. 46. Plot of % mortality and predicted regression line of CHSF.

Table 52. Effect of aqueous soluble fraction of the methanolic extract (AQSF) of leaves of *A. polystachya* on shrimp nauplii.

Conc. (µg/mL)	Log_{10} conc.	% of mortality	LC_{50}
0	-	0	1.667
0.78125	-1.1072	10	
1.5625	0.19382	20	
3.125	0.49485	30	
6.25	0.79588	30	
12.5	1.09691	40	
25	1.39794	40	
50	1.69897	50	
100	2	50	
200	2.30103	60	
400	2.60206	80	

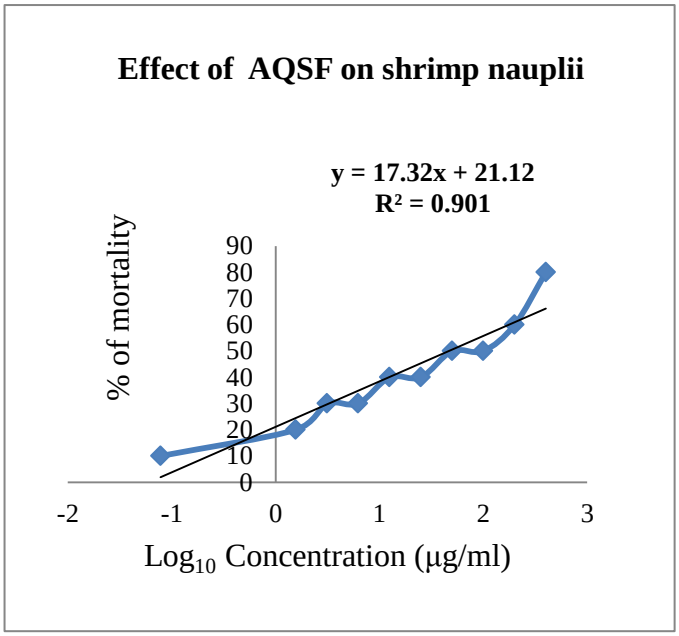


Fig. 47. Plot of % mortality and predicted regression line of AQSF.

The LC_{50} values of MELAP, PESF, CHSF and AQSF were found to be 1.334 ± 0.91 μg/ml, 0.682 ± 0.25 μg/ml, 1.216 ± 0.62 μg/ml and 1.667 ± 0.07 μg/ml respectively (Table 53 and Fig. 48). PESF showed significant lethality whereas MEBAP, CHSF and AQSF showed moderate activity.

Table 53. LC_{50} values of the test samples of leaves of *A. polystachya.*

Test samples	Regression line	R^2	LC_{50} (μg/ml)
VS	y = 25.16x + 39.60	0.968	0.413 ± 0.55
MELAP	y = 27.45x + 35.49	0.888	1.334 ± 0.91
PESF	y = 27.18x + 35.80	0.889	0.682 ± 0.25
CHSF	y = 30.62x + 7.857	0.865	1.216 ± 0.62
AQSF	y = 23.66x + 37.85	0.855	1.667 ± 0.07

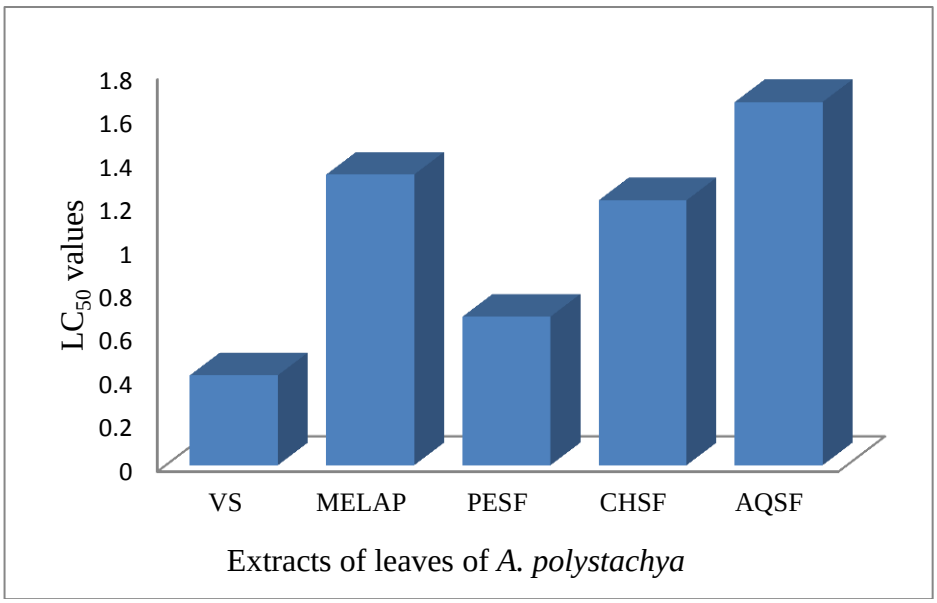


Fig. 48. LC_{50} values of the different extractives leaves of *A. polystachya.*

Bioactive compounds are almost always toxic at higher dose. Thus, *in vivo* lethality in a simple zoological organism can be used as a convenient informant for screening and fractionation in the discovery of new bioactive natural products.

In the present bioactivity study all the crude extracts, pet-ether, chloroform, dichloromethane, ethyl acetate and aqueous soluble fractions of methanolic extract showed positive results indicating that the test samples are biologically active. Each of the test samples showed different mortality rates at different concentrations. Plotting of log of concentration versus per cent mortality for all test samples showed an approximate linear correlation. From the graphs, the median lethal concentration (LC_{50}, the concentration at which 50% mortality of brine shrimp nauplii occurred) was determined for the samples.

From the results of the brine shrimp lethality bioassay, it can be well predicted that the crude extract and partitionate fractions possess cytotoxic principles. Compared with positive control (VS) the cytotoxicity exhibited by the extractives indicated that further bioactivity guided investigation can be done to find out potent antitumour and pesticidal compounds from this plant.

CHAPTER 10
ANTIMICROBIAL SCREENING

10.1 Introduction

Infectious disease is one of main causes of death worldwide relating for approximately one-half of all deaths in tropical countries. In developing nations, it is not surprising to see these statistics, but what may be significant is that infectious disease mortality rates are actually increasing in developed countries, such as the United States. Death from infectious disease, ranked 5th in 1981, has become the 3rd leading cause of death in 1992, an increase of 58%. It is estimated that infectious disease is the underlying cause of death in 8% of the deaths occurring in the US (Pinner *et al*., 1996). This is alarming that it was once believed that we would remove infectious disease by the end of the millennium. The increases are accredited to increases in respiratory tract infections and HIV/AIDS. Other contributing factors are an increase in antibiotic resistance in nosicomial and community acquired infections. In addition, the most spectacular increases are occurring in the 25-44 year old age group (Pinner *et al*., 1996).

These negative health tendency call for a changed interest in infectious disease in the medical and public health communities and renewed strategies on treatment and prevention. It is this last solution that would include the development of new antimicrobials (Fauci, 1998).

The antimicrobial screening, being the first stage of antimicrobial drug research is performed to establish the susceptibility of various fungi and bacteria to any agent. This test measures the ability of each test sample to inhibit the *in vitro* fungal and bacterial growth. This ability may be estimated by Disc diffusion method, Serial dilution method and Bioautographic method.

But there is no standardized method for expressing the results of antimicrobial screening (Ayafor *et al*., 1982). Some investigators use the diameter of zone of inhibition and/or the minimum weight of extract to inhibit the growth of

microorganisms. However, a large number of factors *viz*., the extraction methods, inoculum volume, culture medium composition (Bauer *et al.,* 1966), pH, and incubation temperature can control the results.

Among the above mentioned techniques the disc diffusion (Bauer *et al.,* 1966) is a widely accepted *in vitro* investigation for preliminary screening of test agents which may possess antimicrobial activity. It is essentially a quantitative or qualitative test indicating the sensitivity or resistance of the microorganisms to the test materials. However, no distinction between bacteriostatic and bactericidal activity can be made by this method (Roland, 1982).

10.2 Principle of Disc Diffusion method

In this classical method, antibiotics diffuse from a confined source through the nutrient agar gel and create a concentration gradient. Dried and sterilized filter paper discs (6 mm diameter) containing the test samples of known amounts are placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic (Ciprofloxacin) discs and blank discs are used as positive and negative control. These plates are kept at 4°C for 24 hours to allow maximum diffusion of the test materials to the surrounding media (Barry, 1976). The plates are then inverted and incubated at 37°C for 24 hours for optimum growth of the organisms. The test materials having antimicrobial property inhibit microbial growth in the media surrounding the discs and thereby yield a clear, distinct area defined as **zone of inhibition**. The antimicrobial activity of the test agent is then determined by measuring the diameter of zone of inhibition expressed in millimeter (Bauer *et al.,* 1966; Barry, 1976).

In the present study the crude extracts as well as fractions were tested for antimicrobial activity by disc diffusion method.

10.3 Experimental Design

10.3.1 Apparatus and reagents

Filter paper discs	Autoclave
Nutrient agar medium	Laminar air flow hood
Petridishes	Spirit burner
Sterile cotton	Refrigerator
Micropipette	Incubator
Inoculating loop	Chloroform
Sterile forceps	Ethanol
Screw cap test tubes	Nose mask and hand gloves

10.3.2 Test organisms

The bacterial strains used for the experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka. Both gram positive and gram negative organisms were taken for the test and they are listed in Table 54.

Table 54. List of gram positive and gram negative bacteria.

Gram positive bacteria	Gram negative bacteria
Bacillus cereus	*Escherichia coli*
Bacillus megaterium	*Salmonella* Paratyphi
Bacillus subtilis	*Salmonella* Typhi
Sarcina lutea	*Shigella boydii*
Staphylococcus aureus	*Shigella dysenteriae*
	Pseudomonas aeruginosa
	Vibrio mimicus
	Vibrio parahemolyticus

10.3.3 Test materials

The test materials used in this study were given in Table 55.

Table 55. List of test materials.

Plant part	Sample code	Test Sample
Root bark of *O. indicum*	MEROI	Methanolic extract of root bark of *O. indicum*
	PESF	Petroleum ether soluble fraction
	CHSF	Chloroform soluble fraction
	DCMSF	Dichloromethane soluble fraction
	EASF	Ethyl acetate soluble fraction
	AQSF	Aqueous soluble fraction
Bark of *A. polystachya*	MEBAP	Methanolic extract of bark of *A. polystachya*
	PESF	Petroleum ether soluble fraction
	CHSF	Chloroform soluble fraction
	EASF	Ethyl acetate soluble fraction
	AQSF	Aqueous soluble fraction
Leaves of *A. polystachya*	MELAP	Methanolic extract of leaves of *A. polystachya*
	PESF	Petroleum ether soluble fraction
	CHSF	Chloroform soluble fraction
	AQSF	Aqueous soluble fraction

10.3.4 Composition of culture medium

Nutrient agar medium is the most frequently used and also used in the present study for testing the sensitivity of the organisms to the test materials and to prepare fresh cultures. The nutrient agar medium consists of the following compositions.

Nutrient agar medium

Ingredients	*Amount*
Bacto peptone	0.5 gm
Sodium chloride	0.5 gm
Bacto yeast extract	1.0 gm

Bacto agar	2.0 gm
Distilled water	100 ml
pH	7.2 + 0.1 at 25^0C

10.3.5 Preparation of the medium

To prepare required volume of this medium, calculated amount of each of the constituents was taken in a conical flask and distilled water was added to it to make the required volume. The contents were heated in a water bath to make a clear solution. The pH (at 25^0C) was adjusted at 7.2-7.6 using NaOH or HCl. 10 ml and 5 ml of the medium was then transferred in screw cap test tubes to prepare plates and slants respectively. The test tubes were then capped and sterilized by autoclaving at 15-lbs. pressure at 121^0C for 20 minutes. The slants were used for making fresh culture of bacteria and fungi that were in turn used for sensitivity study.

10.3.6 Sterilization procedure

In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precautions were highly maintained. UV light was switched on one hour before working in the Laminar Hood. Petridishes and other glassware were sterilized by autoclaving at a temperature of 121^0C and a pressure of 15-lbs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized by UV light.

10.3.7 Preparation of subculture

In an aseptic condition under laminar air cabinet, the test organisms were transferred from the pure cultures to the agar slants with the help of a transfer loop to have fresh pure cultures. The inoculated strains were then incubated for 24 hours at 37^0C for their optimum growth. These fresh cultures were used for the sensitivity test.

10.3.8 Preparation of the test plate

The test organisms were transferred from the subculture to the test tubes containing about 10 ml of melted and sterilized agar medium with the help of a sterilized transfer loop in an asceptic area. The test tubes were shaken by rotation to get a uniform suspension of the organisms. The bacterial suspension was immediately transferred to the sterilized petridishes. The petridishes were rotated several times clockwise and anti-clockwise to assure homogenous distribution of the test organisms in the media.

10.3.9 Preparation of discs

Measured amount of each test sample (Tables 56 & 58) was dissolved in specific volume of solvent (Chloroform or methanol) to obtain the desired concentrations in an asceptic condition. Sterilized metrical (BBL, Cocksville, USA) filter paper discs were taken in a blank petridish under the laminar hood. Then discs were soaked with solutions of test samples and dried.

Table 56. Preparation of sample Discs of *Oroxylum indicum.*

Plant part	Code	Dose μg/disc	Required amount for 20 disc (mg)
Root bark of *O. indicum*	MEROI	400	8.0
	PESF	400	8.0
	CHSF	400	8.0
	DCMSF	400	8.0
	EASF	400	8.0
	AQSF	400	8.0

Standard Ciprofloxacin (30 μg/disc) discs were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of produced by the test sample. Blank discs were used as negative controls which ensure that the residual solvents (left over the discs even after air-drying) and the filter paper were not active themselves.

10.3.10 Diffusion and incubation

The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria. The plates were then kept in a refrigerator at 4^0C for about 24 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 37^0C for 24 hours.

10.3.11 Determination of the zone of inhibition

The antimicrobial potency of the test agents are measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition (Photo 180). After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale (Photo 181).

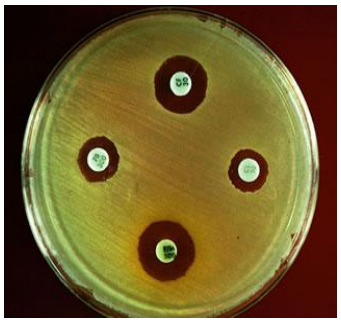


Photo 180. Clear zone of inhibition.

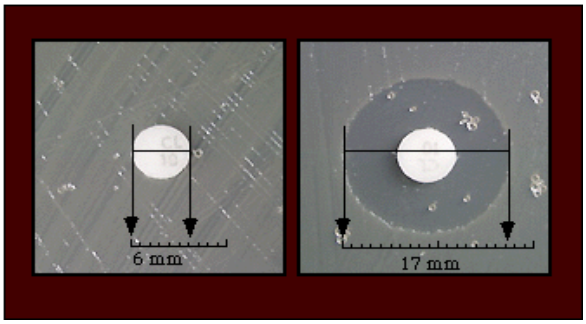


Photo 181. Determination of clear zone of inhibition.

10.4 Results of *in vitro* antimicrobial screening of root bark of *Oroxylum indicum*

The methanolic extract of root bark of *O. indicum* (MEROI) and different partitionates i.e. pet-ether soluble fraction (PESF), chloroform soluble fraction (CHSF), dichloromethane soluble fraction (DCMSF), ethyl acetate soluble fraction (EASF) and aqueous soluble fraction (AQSF) were subjected to antimicrobial screening with a concentration of 400 μg/disc in every case. Among the extractives, MEROI exhibited mild to moderate antimicrobial activity and EASF and AQSF showed no antibacterial activity. The results are given in the Table 57.

Table 57. Antimicrobial activity of test samples of root bark *O. indicum.*

Test microorganisms	Diameter of zone of inhibition (mm)						
	MEROI	PESF	CHSF	DCMSF	EASF	AQSF	Ciprofloxacin
Gram positive bacteria							
Bacillus cereus	7.0	11.0	12.0	9.0	--	--	42.0
B. megaterium	9.0	9.0	10.0	10.0	--	--	39.0
B. subtilis	10.0	15.0	15.0	16.0	--	--	45.0
Staphylococcus aureus	--	--	--	--	--	--	43.0
Sarcina lutea	8.0	7.0	10.0	10.0	--	--	45.0
Gram negative bacteria							
Escherichia coli	8.0	8.0	9.0	9.0	--	--	43.0
Pseudomonas aeruginosa	10.0	12.0	13.0	12.0	--	--	43.0
Salmonella Paratyphi	9.0	8.0	14.0	10.0	--	--	40.0
S. Typhi	7.0	10.0	10.0	9.0	--	--	40.0
Shigella boydii	7.0	8.0	8.0	7.0	--	--	35.0
Sh. dysenteriae	--	10.0	15.0	15.0	--	--	42.0
Vibrio mimicus	12.0	15.0	13.0	14.0	--	--	45.0
V. parahemolyticus	7.0	7.0	12.0	9.0	--	--	40.0

The dichloromethane soluble fraction exhibited the highest inhibition against microbial growth having zone of inhibition ranged from 7.0 mm to 16.0 mm. The maximum zone of inhibition produced by DCMSF was found to be 16.0 mm against

B. subtilis. This partitionate also showed antimicrobial activity against *Sh. dysenteriae* (15.0 mm), *Vibrio mimicus* (14.0 mm) and *Pseudomonas aeruginosa* (12.0 mm).

Chloroform soluble fraction also exhibited significant inhibition against microbial growth having zone of inhibition ranged from 8.0 mm to 15.0 mm. This fraction displayed highest inhibitory activity against *B. subtilis*, *Sh. dysenteriae* (having zone of inhibition of 15.0 mm in both cases), *Salmonella* Paratyphi (14.0 mm), *Pseudomonas aeruginosa* and *Vibrio mimicus* (having zone of inhibition of 13.0 mm in both cases), *Bacillus cereus,* and *V. parahemolyticus* (having zone of inhibition of 12.0 mm in both cases).

Pet-ether soluble fraction also demonstrated significant inhibition against microbial growth having zone of inhibition ranged from 7.0 mm to 15.0 mm. This fraction showed highest inhibitory activity against *B. subtilis* and *Vibrio mimicus* (having zone of inhibition of 15.0 mm in both cases), followed by *Pseudomonas aeruginosa* (12.0 mm), and *Bacillus cereus* (11.0 mm).

Table 58. Preparation of sample Discs of *Aphanamixis polystachya*.

Plant parts	**Code**	**Dose** µg/disc	**Required amount for 20 disc** (mg)
Bark of *A. polystachya*	MEBAP	400	8.0
	PESF	400	8.0
	CHSF	400	8.0
	EASF	400	8.0
	AQSF	400	8.0
Leaves of *A. polystachya*	MELAP	400	8.0
	PESF	400	8.0
	CHSF	400	8.0
	AQSF	400	8.0

10.5 Results of *in vitro* antimicrobial screening of bark and leaves of *A. polystachya*

The methanolic extract of bark of *A. polystachya* (MEBAP) and different partitionates i.e. pet-ether soluble fraction (PESF), chloroform soluble fraction (CHSF), ethyl acetate soluble fraction (EASF) and aqueous soluble fraction (AQSF) were focused to antimicrobial screening with a concentration of 400 μg/disc in every case. Among the extractives, MEBAP, EASF exhibited mild to moderate antimicrobial activity against more or less all the test organisms ranged from 7.0 mm to 8.0 mm. The results are given in the Table 59.

Table 59. Antimicrobial activity of test samples of bark of *A. polystachya.*

Test microorganisms	Diameter of zone of inhibition (mm)					
	MEBAP	PESF	CHSF	EASF	AQSF	Ciprofloxacin
Gram positive bacteria						
Bacillus cereus	--	--	--	8.0	--	42.0
B. megaterium	--	--	--	7.0	--	45.0
B. subtilis	7.0	10.0	11.0	8.0	8.0	40.0
Staphylococcus aureus	8.0	8.0	9.0	7.0	7.0	39.0
Sarcina lutea	--	--	--	--	--	43.0
Gram negative bacteria						
Escherichia coli	--	--	--	8.0	--	45.0
Pseudomonas aeruginosa	--	--	--	--	--	43.0
Salmonella Paratyphi	--	8.0	7.0	7.0	--	41.0
S. Typhi	8.0	8.0	7.0	8.0	--	43.0
Shigella boydii	8.0	8.0	7.0	8.0	--	40.0
Sh. dysenteriae	8.0	7.0	8.0	7.0	--	40.0
Vibrio mimicus	7.0	--	9.0	8.0	--	40.0
V. parahemolyticus	7.0	7.0	7.0	7.0	--	42.0

The chloroform soluble fraction exhibited mild or moderate inhibition against microbial growth having zone of inhibition ranged from 7.0 mm to 11.0 mm. The maximum zone of inhibition produced by CHSF was found to be 11.0 mm against *B. subtilis*. This partitionate also showed antimicrobial activity against *Staphylococcus aureus* and *Vibrio mimicus* (having zone of inhibition of 9.0 mm in both cases), and *Sh. dysenteriae* (8.0 mm).

Pet-ether soluble fraction also displayed mild or moderate inhibition against microbial growth having zone of inhibition ranged from 7.0 mm to 10.0 mm. This fraction demonstrated highest inhibitory activity against *B. subtilis* (10.0 mm), *Staphylococcus aureus*, *Salmonella* Paratyphi, *S.* Typhi and *Shigella boydii* (having zone of inhibition of 8.0 mm in all cases).

The methanolic extract of leaves of *A. polystachya* (MELAP) and different partitionates i.e. pet-ether soluble fraction (PESF), chloroform soluble fraction (CHSF) and aqueous soluble fraction (AQSF) were issued to antimicrobial screening with a concentration of 400 μg/disc in every case. Among the extractives, PESF and CHSF exhibited mild to moderate antimicrobial activity against more or less all the test organisms ranged from 7.0 mm to 9.0 mm and AQSF showed no antimicrobial activity. The results are given in the Table 60.

Table 60. Antimicrobial activity of test samples leaves of *A. polystachya.*

Test microorganisms		Diameter of zone of inhibition (mm)			
	MELAP	PESF	CHSF	AQSF	Ciprofloxacin
Gram positive bacteria					
Bacillus cereus	8.0	8.0	8.0	--	42.0
B. megaterium	8.0	8.0	9.0	--	45.0
B. subtilis	10.0	--	--	--	40.0
Staphylococcus aureus	--	--	9.0	--	39.0
Sarcina lutea	8.0	7.0	8.0	--	43.0
Gram negative bacteria					
Escherichia coli	9.0	8.0	8.0	--	45.0
Pseudomonas aeruginosa	7.0	--	--	--	43.0
Salmonella Paratyphi	8.0	7.0	9.0	--	41.0
S. Typhi	--	--	8.0	--	43.0
Shigella boydii	8.0	8.0	7.0	--	40.0
Sh. dysenteriae	--	--	7.0	--	40.0
Vibrio mimicus	8.0	7.0	8.0	--	40.0
V. parahemolyticus	8.0	--	--	--	42.0

The crude methanolic extract revealed the highest inhibition against microbial growth having zone of inhibition ranged from 7.0 mm to 10.0 mm. The maximum zone of inhibition produced by MELAP was found to be 10.0 mm against *B. subtilis.* This partitionate also showed antimicrobial activity against *Escherichia coli* (9.0 mm).

The results of *in vitro* microbial screening of both *O. indicum* and *A. polystachya* indicated that DCMSF, CHSF and PESF of *O. indicum*, CHSF and PESF of bark and MELAP of leaves of *A. polystachya* possesses better antimicrobial activity and these can be further studied to explore potent antimicrobial agents.

CHAPTER 11

EVALUATION OF ANTIOXIDANT ACTIVITY

11.1 Introduction

Majority of the diseases/disorders are mainly associated to oxidative stress due to free radicals (Gutteridge, 1995). Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals, and also by acting as oxygen scavengers (Büyükokuroğlu *et al*., 2001; Shahidi *et al.*, 1992). There is an growing interest in the antioxidant effects of compounds derived from plants, which could be relevant in relation to their nutritional prevalence and their role in health and diseases (Steinmetz and Potter, 1996; Aruoma, 1998; Bandoniene *et al*., 2002; Pieronia *et al.*, 2002; Couladis *et al.*, 2003). A number of reports on the isolation and testing of plant derived antioxidants have been described during the past decade (Shahidi *et al.*,1992; Velioglu *et al.*, 1998; Pietta *et al.*, 1998). The medicinal properties of plants have been demonstrated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability (Auudy *et al*., 2003).

Different synthetic antioxidant such as *tert*-butyl-1-hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG) and tert-butylhydroquinone (TBHQ) used as food additives to increase self life are known to have not only toxic and carcinogenic effects and humans (Ito *et al*.,1986; Wichi,1988), but also abnormal effects on enzyme systems (Inatani *et al*., 1983). Therefore, the interest in natural antioxidant, especially of plant origin, has greatly increased in recent years (Jayaprakasha and Jaganmohan, 2000). Plant polyphenols have been studied mostly because of the possibility that they might underlie the protective effects afforded by fruit and vegetable intake against cancer and other chronic diseases (Elena *et al*., 2006).

The purpose of this study was to evaluate different extractives of root bark of *Oroxylum indicum*, and bark and leaves of *Aphanamixis polystachya* as new potential sources of natural antioxidants and phenolic compounds.

11.2 Assays for total phenols

The antioxidative effect is mainly due to phenolic components, such as flavonoids (Pietta, 1998), phenolic acids, and phenolic diterpenes (Shahidi *et al.,* 1992). The phenolic compounds exert their antioxidant properties by redox reaction, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994). Many phytochemicals possess significant antioxidant capacities that may be associated with lower incidence and lower mortality rates of cancer in several human populations (Velioglu *et al*., 1998).

11.2.1 Principle

In the alkaline condition phenols ionize completely. When Folin-Ciocalteu reagent is used in this ionized phenolic solution the reagent will readily oxidize the phenols. Usual colour of Folin-Ciocalteu reagent is yellow and after the oxidation process the solution becomes blue. The intensity of the colour change is measured in a spectrophotometer at 760 nm. The absorbance value reflects the total phenolic content of the compound (Harbertson and Spayd, 2006).

$$\text{Phenols} + Na_2CO_3 \xrightarrow[\text{ionization}]{\text{Complete}} \text{Ionized phenols}$$

$$\text{Ionized phenols} + \underset{\text{reagent}}{\text{Folin-Ciocalteu}} \xrightarrow{\text{Oxidation}} \underset{\textbf{(Blue)}}{\text{Folin-Ciocalteu reagent complex}}$$

(**Yellow**)

11.2.2 Materials and Methods

Total phenolic content of *O. indicum* and *A. polystachya* extractives was measured employing the method described by Skerget *et al*. (2005) involving Folin-Ciocalteu reagent as oxidizing agent and gallic acid as standard (Majhenic *et al*., 2007).

11.2.2.1 Materials

- Folin-Ciocalteu reagent (10 X diluted)
- Na_2CO_3 solution (7.5 %)
- *tert*-butyl-1-hydroxytoluene (BHT)
- Ascorbic acid
- Methanol
- Chloroform
- Carbon tetrachloride
- n-hexane
- Distilled water
- UV-spectrophotometer
- Vial
- Beaker (100 and 200 ml)
- Test tube
- Pipette (1 ml)
- Pipette (5 ml)
- Micropipette (50-200 µl)

11.2.2.2 Composition of Folin-Ciocalteu reagent

SL. No.	Component	Per cent
1	Water	57.5
2	Lithium Sulfate	15.0
3	Sodium Tungstate Dihydrate	10.0
4	Hydrochloric Acid ≥ 25%	10.0
5	Phosphoric Acid 85% solution in water	5.0
6	Molybdic Acid Sodium Dihydrate	2.5

11.2.2.3 Standard curve preparation

Gallic acid was used as standard. Different gallic acid solution were prepared having a concentration ranging from 100 µg /ml to 0 µg /ml. 2.5 ml of Folin-Ciocalteu reagent (diluted 10 times with water) and 2 ml of Na_2CO_3 (7.5% w/v) solution was added to

0.5 ml of gallic acid solution. The mixture was incubated for 20 min at room temperature. After 20 min the absorbance was measured at 760 nm. After plotting the absorbance in ordinate against the concentration in abscissa a linear relationship was obtained which was used as a standard curve for the determination of the total phenolic content of the test samples.

11.2.2.4 Sample preparation

2 mg of the extractives was taken and dissolved in the distilled water to get a sample concentration of 2 mg/ml in every case. The samples of *Oroxylum indicum* and *Aphanamixis polystachya* along with their concentration for the total phenolic content measurement are given in the Tables 61 and 64, respectively.

Table 61. Test samples for total phenolic content determination of *Oroxylum indicum*

Plant part	Sample code	Test Samples	Conc. (mg/ml)
Root bark of *O. indicum*	MEROI	Methanolic extract of root bark of *O. indicum*	2.0
	PESF	Petroleum ether soluble fraction	2.0
	CHSF	Chloroform soluble fraction	2.0
	DCMSF	Dichloromethane soluble fraction	2.0
	EASF	Ethyl acetate soluble fraction	2.0
	AQSF	Aqueous soluble fraction	2.0

11.3 Total phenolic content analysis

To 0.5 ml of extract solution (conc. 2 mg/ml), 2.5 ml of Folin-Ciocalteu reagent (diluted 10 times with water) and 2.0 ml of Na_2CO_3 (7.5 % w/v) solution was added in a beaker. The mixture was incubated for 20 minutes at room temperature. After 20 minutes the absorbance was measured at 760 nm by UV-spectrophotometer and using the standard curve prepared from gallic acid solution with different concentration, the total phenols content of the sample was measured (Fig. 49). The phenolic contents of the sample were expressed as mg of GAE (gallic acid equivalent) / gm of the extract.

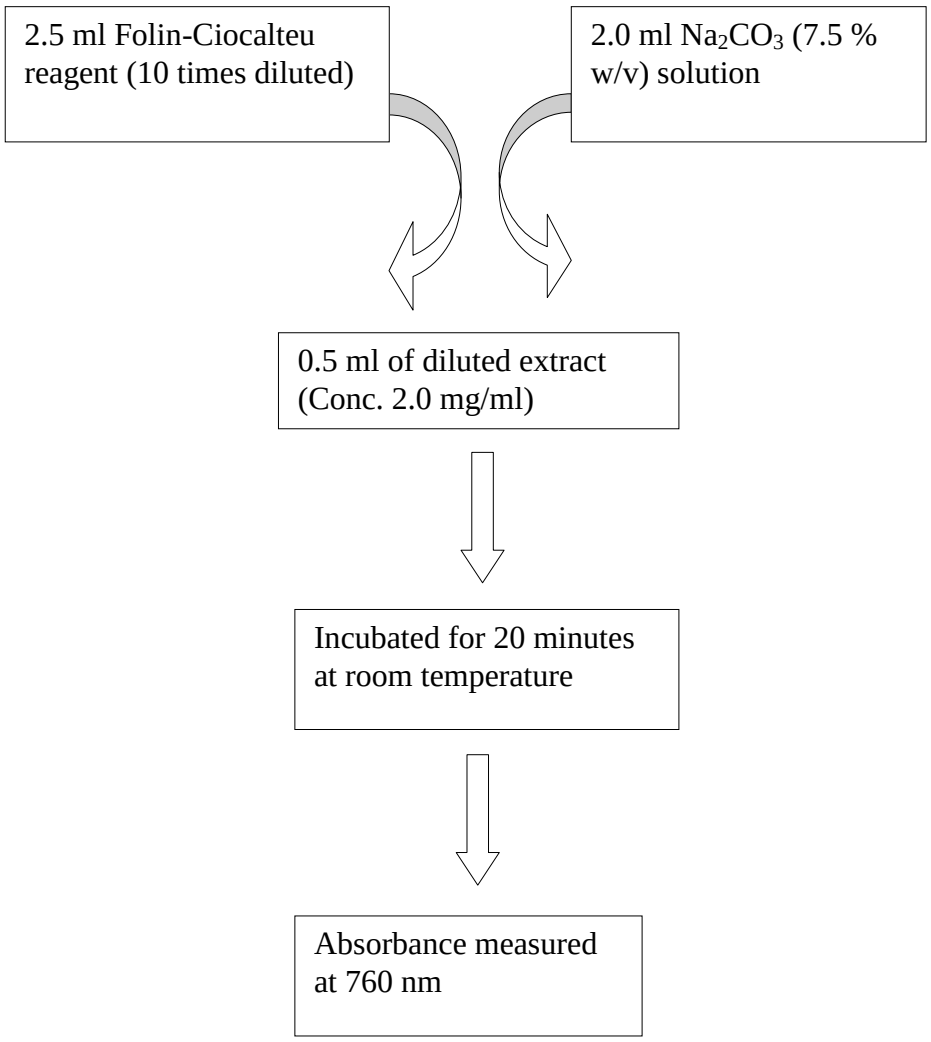


Fig. 49. Schematic representation of the total phenolic content determination.

11.4 Results of the test samples of *Oroxylum indicum*

11.4.1 Total phenolic content (TPC)

The methanolic extract of root bark of *O. indicum* (MEROI) and different partitionates i.e. pet-ether soluble fraction (PESF), chloroform soluble fraction (CHSF), dichloromethane soluble fraction (DCMSF), ethyl acetate soluble fraction (EASF) and aqueous soluble fraction (AQSF) were subjected to total phenolic content determination. Based on the absorbance values of the various extract solutions, reacted with Folin-Ciocalteu reagent and compared with the standard solutions of gallic acid equivalents (Table 62 and Fig. 50), results of the colorimetric analysis of the total phenolics are given in Table 63 and Fig. 51. Total phenolic content of the samples are expressed as mg of GAE (gallic acid equivalent) / gm of extractives).

Table 62. Standard curve preparation by using gallic acid.

SL. No.	Conc. of the Standard (µg/ml)	Absorbance	Regression line	R^2
1	100	0.800	y = 0.008x + 0.007	0.998
2	50	0.423		
3	25	0.215		
4	12.5	0.123		
5	6.25	0.047		
6	3.125	0.034		
7	1.5625	0.022		
8	0.78125	0.007		
9	0.3906	0.003		
10	0	0.000		

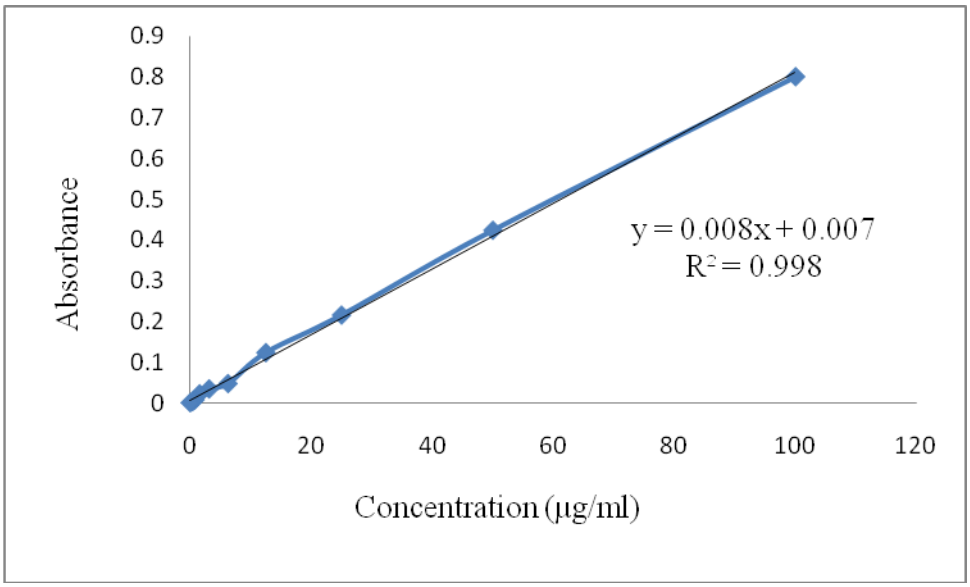


Fig. 50. Standard curve of gallic acid for total phenolic determination.

Table 63. Total phenolic content of root bark of *O. indicum*.

Plant part	Sample Code	Extracts	Total phenolic content (mg of GAE/gm of extractives)
Root bark of *O. indicum*	MEROI	Methanolic extract	15.75 ± 0.34
	PESF	Petroleum ether soluble fraction	4.0 ± 0.25
	CHSF	Chloroform soluble fraction	12.25 ± 0.52
	DCMSF	Dichloromethane soluble fraction	7.25 ± 0.61
	EASF	Ethyl acetate soluble fraction	5.75 ± 0.37
	AQSF	Aqueous soluble fraction	6.75 ± 0.44

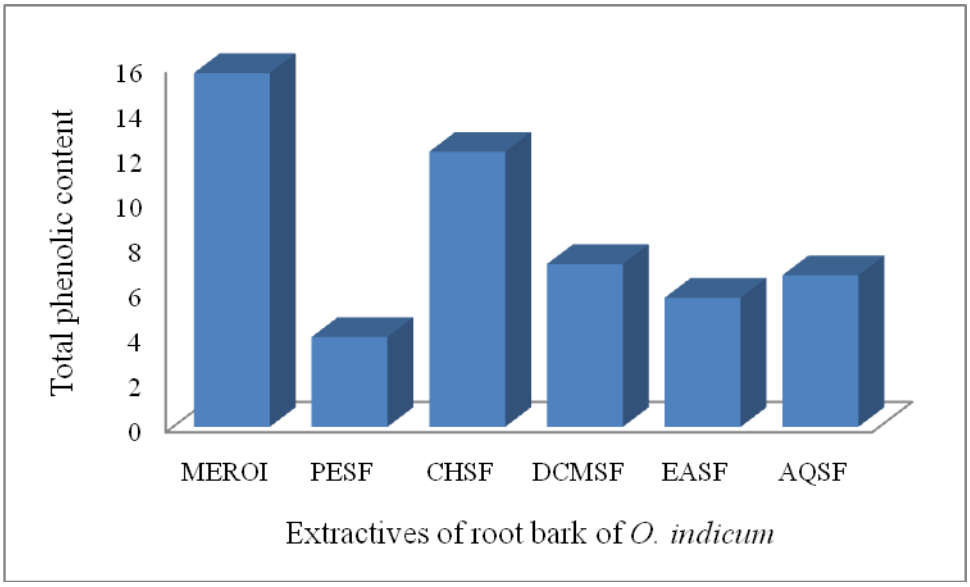


Fig. 51. Total phenolic content (mg of GAE/gm of extractives) of different extractives of root bark of *O. indicum*

The amount of total phenolic content differs in different extractives and ranged from 4.0 ± 0.25 mg of GAE / gm of extractives to 15.75 ± 0.34 mg of GAE / gm of extractives of *O. indicum* (Table 63). Among all extractives of *O. indicum*, the highest phenolic content was found in MEROI (15.75 ± 0.34 mg of GAE / gm of extractives) followed by CHSF (12.25 ± 0.52 mg of GAE / gm of extractives). Significant amount of phenolic content was also present in DCMSF (7.25 ± 0.61 mg of GAE / gm of extractives) and AQSF (6.75 ± 0.44 mg of GAE / gm of extractives).

Table 64. Test samples for total phenolic content determination of *Aphanamixis polystachya*.

Plant parts	Sample code	Test Samples	Conc. (mg/ml)
Bark of *A. polystachya*	MEBAP	Methanolic extract of bark of *A. polystachya*	2.0
	PESF	Petroleum ether soluble fraction	2.0
	CHSF	Chloroform soluble fraction	2.0
	EASF	Ethyl acetate soluble fraction	2.0
	AQSF	Aqueous soluble fraction	2.0
Leaves of *A. polystachya*	MELAP	Methanolic extract of leaves of *A. polystachya*	2.0
	PESF	Petroleum ether soluble fraction	2.0
	CHSF	Chloroform soluble fraction	2.0
	AQSF	Aqueous soluble fraction	2.0

11.5 Results of the test samples of *Aphanamixis polystachya*

11.5.1 Total phenolic content (TPC) of bark of *A. polystachya*

The methanolic extract of bark of *A. polystachya* (MEBAP) and different partitionates i.e. pet-ether soluble fraction (PESF), chloroform soluble fraction (CHSF), ethyl acetate soluble fraction (EASF) and aqueous soluble fraction (AQSF) were focused to total phenolic content determination. Based on the absorbance values of the various extract solutions, reacted with Folin-Ciocalteu reagent and compared with the standard solutions of gallic acid equivalents (Table 62 and Fig. 50), results of the colorimetric analysis of the total phenolics are depicted in Table 65 and Fig. 52. Total phenolic content of the samples are expressed as mg of GAE (gallic acid equivalent)/ gm of extractives.

Table 65. Total phenolic content of bark of *A. polystachya.*

Plant part	Sample Code	Extract	Total phenolic content (mg of GAE/gm of extractives)
Bark of *A. polystachya*	MEBAP	Methanolic extract	29.25 ± 0.75
	PESF	Petroleum ether soluble fraction	4.12 ± 0.46
	CHSF	Chloroform soluble fraction	18.87 ± 0.37
	EASF	Ethyl acetate soluble fraction	8.5 ± 0.62
	AQSF	Aqueous soluble fraction	16.0 ± 0.73

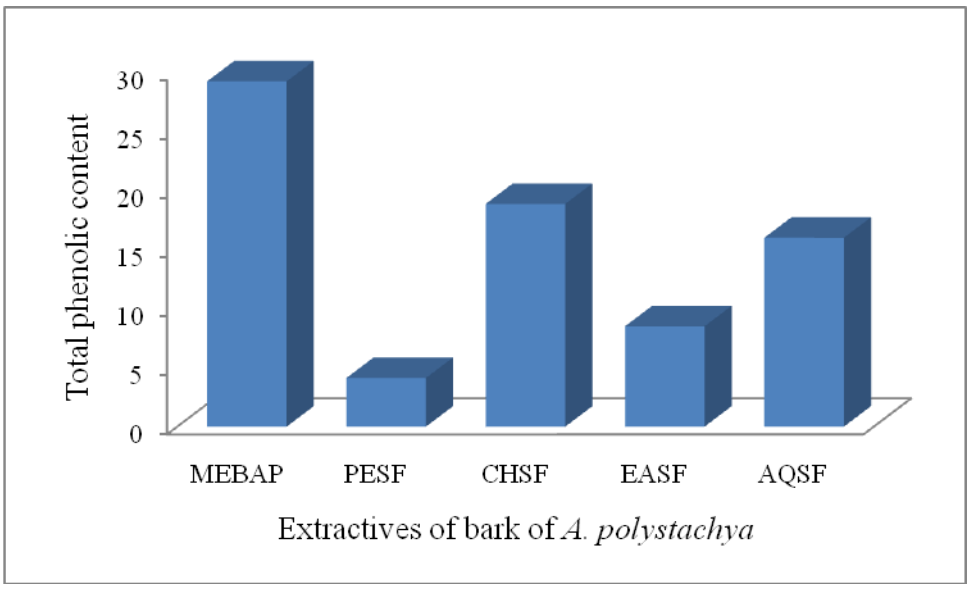


Fig. 52. Total phenolic content (mg of GAE/gm of extractives) of different extractives of bark of *A. polystachya.*

The amount of total phenolic content differs in different extractives and ranged from 4.12 ± 0.46 mg of GAE / gm of extractives to 29.25 ± 0.75 mg of GAE / gm of extractives of bark of *A. polystachya* (Table 65). Among all extractives of bark of *A. polystachya* the highest phenolic content was found in MEBAP (29.25 ± 0.75 mg of GAE / gm of extractives) followed by CHSF (18.87 ± 0.37 mg of GAE / gm of extractives). Significant amount of phenolic content was also present in AQSF (16.0 ± 0.73 mg of GAE / gm of extractives).

11.5.2 Total phenolic content (TPC) of leaves of *A. polystachya*

The methanolic extract of leaves of *A. polystachya* (MELAP) and different partitionates i.e. pet-ether soluble fraction (PESF), chloroform soluble fraction (CHSF) and aqueous soluble fraction (AQSF) were issued to total phenolic content determination. Based on the absorbance values of the various extract solutions, reacted with Folin-Ciocalteu reagent and compared with the standard solutions of gallic acid equivalents (Table 62 and Fig. 50). The total phenolic content of different extractives of leaves of A. polystachya are presented in Table 66 and Fig. 53.

Table 66. Total phenolic content of leaves of *A. polystachya.*

Plant part	Sample Code	Extracts	Total phenolic content (mg of GAE/gm of extractives)
Leaves of *A. polystachya*	MELAP	Methanolic extract	12.87 ± 0.27
	PESF	Petroleum ether soluble fraction	22.25 ± 0.39
	CHSF	Chloroform soluble fraction	26.0 ± 0.54
	AQSF	Aqueous soluble fraction	25.12 ± 0.72

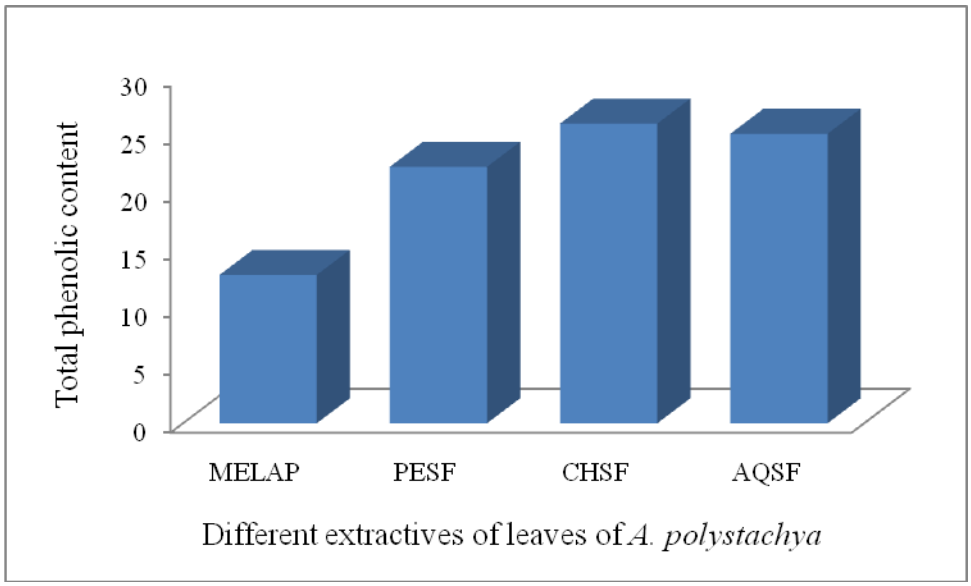


Fig. 53. Total phenolic content (mg of GAE/gm of extractives) of different extractives of leaves of *A. polystachya.*

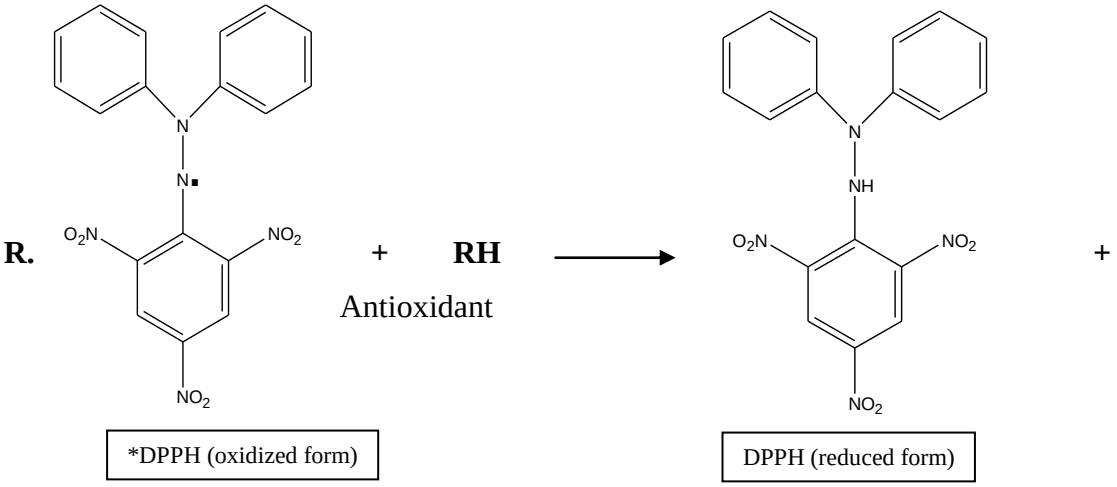
The amount of total phenolic content differs in different extractives and ranged from 12.87 ± 0.0.27 mg of GAE / gm of extractives to 26.0 ± 0.54 mg of GAE / gm of extractives of leaves of *A. polystachya* (Table 66). Among all extractives of leaves of *A. polystachya,* the highest phenolic content was found in CHSF (26.0 ± 0.54 mg of GAE / gm of extractives) followed by AQSF (25.12 ± 0.72 mg of GAE / gm of extractives). Significant amount of phenolic content was also observed in PESF (22.25 ± 0.39 mg of GAE / gm of extractives).

11.6 Antioxidant activity: DPPH assay

11.6.1 Principle

The free radical scavenging activities (antioxidant capacity) of the plant extracts on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) were estimated by the method of Brand-Williams *et al.* (1995).

2 ml of a methanol solution of the extract at different concentration were mixed with 3 ml of a DPPH methanol solution (20 µg/ml). The antioxidant potential was assayed from the bleaching of purple coloured methanol solution of DPPH radical by the plant extract as compared to that of *tert*-butyl-1-hydroxytoluene (BHT) and ascorbic aid (ASA) by UV spectrophotometer.



* DPPH = 1,1-diphenyl-2-picrylhydrazyl

11.6.2 Materials and Methods

DPPH was used to evaluate the free radical scavenging activity (antioxidant potential) of various compounds and medicinal plants (Choi *et al.,* 2000; Desmarchelier *et al.,* 1997).

11.6.2.1 Materials

1,1-diphenyl-2-picrylhydrazyl	UV-spectrophotometer
tert-butyl-1-hydroxytoluene (BHT)	Beaker (100 and 200 ml)
Ascorbic acid	Amber reagent bottle

Distilled water	Test tube
Methanol	Light-proof box
Chloroform	Pipette (5 ml)
Carbon tetrachloride	Micropipette (50-200 µl)
n-hexane	

11.6.2.2 Control preparation for antioxidant activity measurement

Ascorbic acid (ASA) and *tert*-butyl-1-hydroxytoluene (BHT) was used as positive control. Calculated amount of ASA and BHT were dissolved in methanol to get a mother solution having a concentration 1000 µg/ml. Serial dilution was made using the mother solution to get different concentration ranging from 500.0 to 0.977 µg /ml.

11.6.2.3 Test sample preparation

Calculated amount of different extractives were measured and dissolved in methanol to get the mother solution (Conc. 1000 µg/ml). Serial dilution of the mother solution gave different concentration ranging from 500.0 to 0.977 µg /ml which were kept in the marked flasks.

11.6.2.4 DPPH solution preparation

20 mg DPPH powder was weighed and dissolved in methanol to get a DPPH solution having a concentration 20 µg/ml. The solution was prepared in the amber reagent bottle and kept in the light proof box.

11.6.2.5 Assay of free radical scavenging activity

2.0 ml of a methanol solution of the sample (extractives/control) at different concentration (500 µg/ml to 0.977 µg/ml) were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml). After 30 min reaction period at room temperature in dark place the absorbance was measured at 517 nm against methanol as blank by UV spetrophotometer (Fig. 54).

Inhibition of free radical DPPH in percent (I%) was calculated as follows:

$$\mathbf{(I\%) = (1 - A_{sample}/A_{blank}) \times 100}$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test material).

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration.

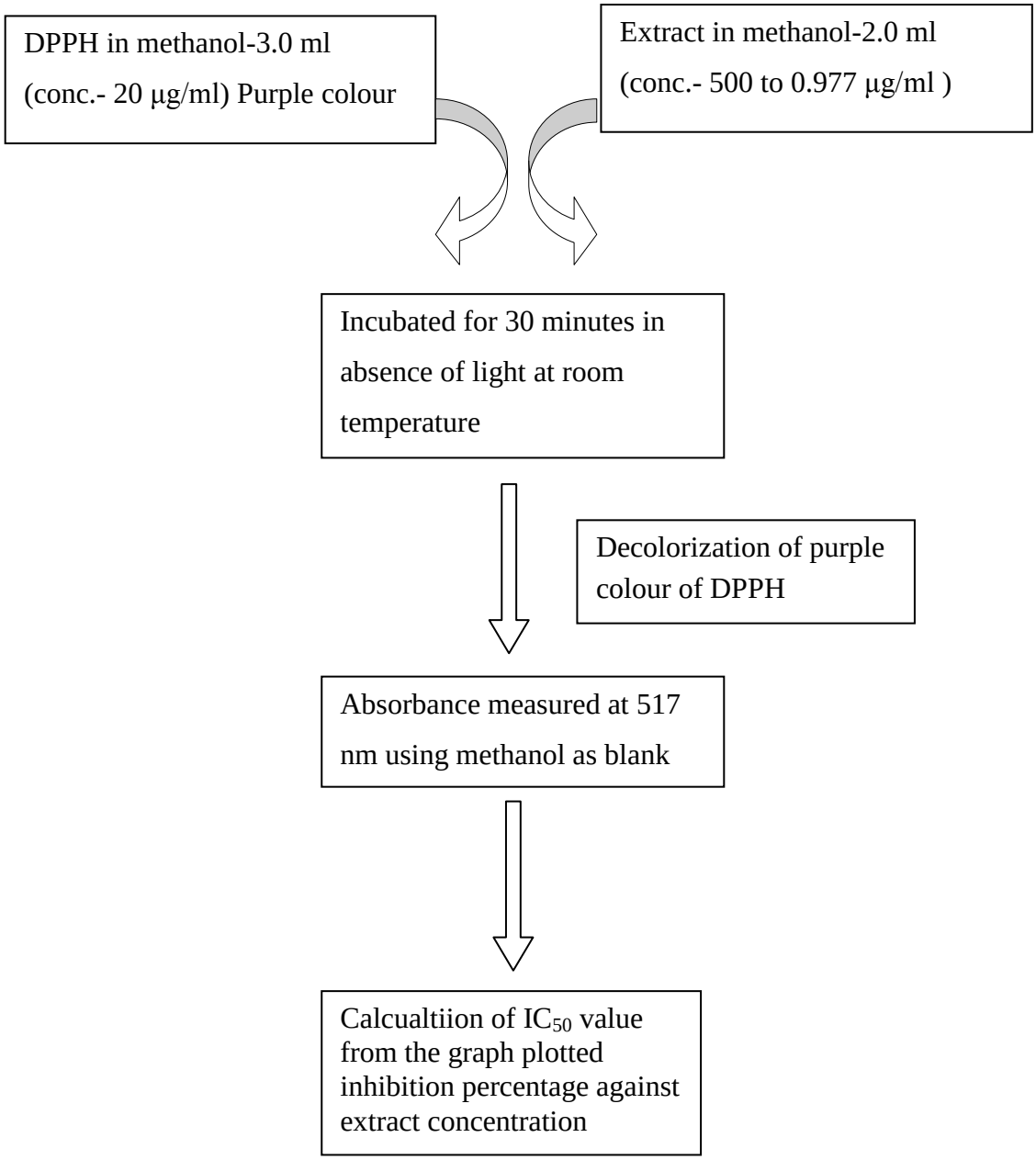


Fig. 54. Schematic representation of the method of assaying free radical scavenging activity.

The experiments were performed thrice and the the results were expressed as mean ± SD in every cases.

11.7 Results of test samples of free radical scavenging activity (DPPH)

11.7.1 Results of different extractives of root bark of *Oroxylum indicum*

The methanolic extract of root bark of *O. indicum* (MEROI), and different partitionates i.e. pet-ether soluble fraction (PESF), chloroform soluble fraction (CHSF), dichloromethane soluble fraction (DCMSF), ethyl acetate soluble fraction (EASF) and aqueous soluble fraction (AQSF) were subjected to free radical scavenging activity by the method of Brand-Williams *et al.* (1995) [Tables 69-74 and Fig. 57-62]. Here, *tert*-butyl-1-hydroxytoluene (BHT) and ascorbic acid (ASA) was used as reference standard (Table 67 and 68 as well as in Fig. 55 and 56).

Table 67. IC_{50} value of ascorbic acid (ASA).

Absorbance of the blank	Conc. (µg/ml)	Absorbance of the extract	% inhibition	IC_{50}
0.352	500	0.005	98.57	2.06
	250	0.006	98.29	
	125	0.015	95.73	
	62.5	0.024	93.18	
	31.25	0.068	80.68	
	15.625	0.098	72.15	
	7.813	0.139	60.51	
	3.906	0.186	47.15	
	1.953	0.175	50.28	
	0.977	0.193	45.17	

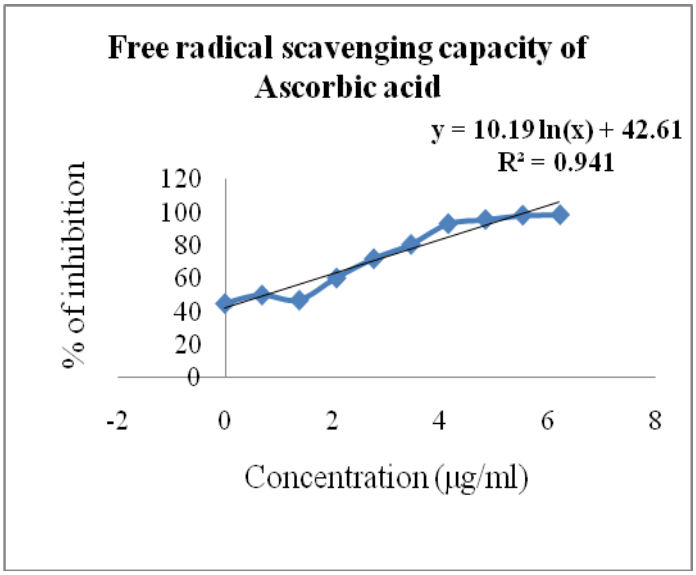


Fig. 55. IC_{50} value of ascorbic acid.

Table 68. IC_{50} value of *tert*-butyl-1-hydroxytoluene (BHT).

Absorbance of the blank	Conc. (µgm/ml)	Absorbance of the extract	% inhibition	IC_{50}
0.352	500	0.018	94.88	15.02
	250	0.068	80.68	
	125	0.097	72.44	
	62.5	0.135	61.64	
	31.25	0.159	54.82	
	15.625	0.175	50.28	
	7.813	0.206	41.47	
	3.906	0.225	36.07	
	1.953	0.238	32.38	
	0.977	0.287	18.46	

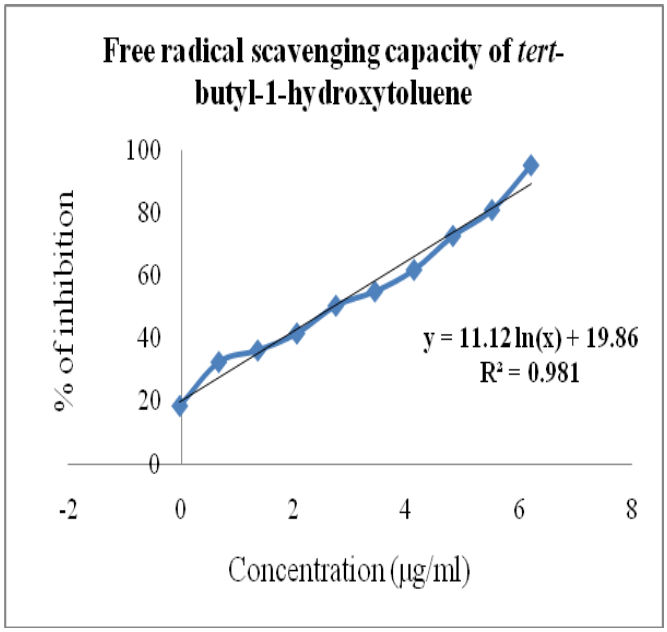


Fig. 56. IC_{50} value of *tert*-butyl-1-hydroxytoluene.

Table 69. IC_{50} value of methanolic extract of root bark of *O. indicum* (MEROI).

Absorbance of the blank	Conc. (µgm/ml)	Absorbance of the extract	% inhibition	IC_{50}
0.352	500	0.040	88.63	9.29
	250	0.023	93.46	
	125	0.014	96.02	
	62.5	0.013	96.30	
	31.25	0.010	97.15	
	15.625	0.149	57.67	
	7.813	0.170	51.70	
	3.906	0.240	31.81	
	1.953	0.306	13.06	
	0.977	0.321	8.80	

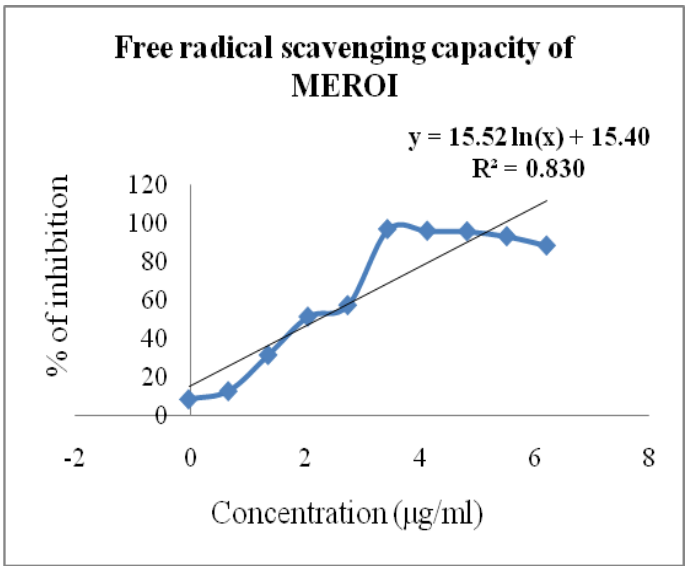


Fig. 57. IC_{50} value of methanolic extract of root bark of *O. indicum* (MEROI).

Table 70. IC_{50} value of petroleum ether soluble fraction (PESF) of methanolic extract of root bark of *O. indicum*.

Absorbance of the blank	Conc. (µgm/ml)	Absorbance of the extract	% inhibition	IC_{50}
0.352	500	0.073	79.26	37.11
	250	0.031	91.19	
	125	0.132	62.5	
	62.5	0.173	50.85	
	31.25	0.239	32.10	
	15.625	0.302	14.20	
	7.813	0.266	24.43	
	3.906	0.285	19.03	
	1.953	0.333	5.39	
	0.977	0.335	4.82	

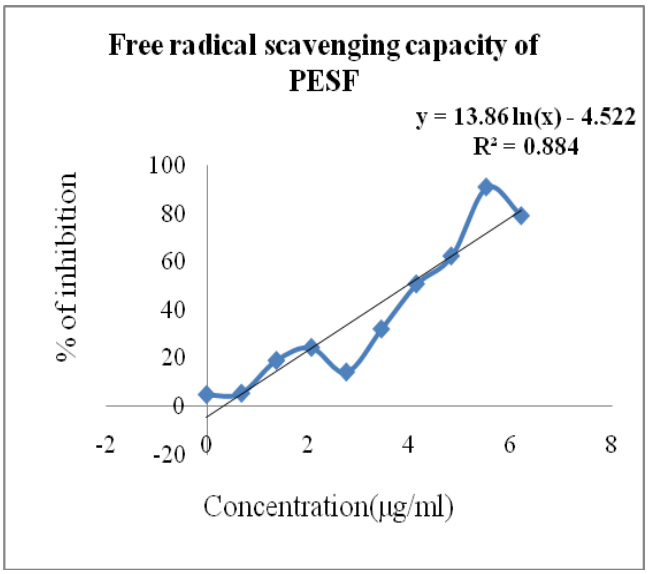


Fig. 58. IC_{50} value of petroleum ether soluble fraction (PESF) of root bark of *O. indicum*.

Table 71. IC_{50} value of chloroform soluble fraction (CHSF) of methanolic extract of root bark of *O. indicum*.

Absorbance of the blank	Conc. (µgm/ml)	Absorbance of the extract	% inhibition	IC_{50}
0.352	500	0.023	93.46	11.07
	250	0.016	95.45	
	125	0.016	95.45	
	62.5	0.017	95.17	
	31.25	0.052	85.22	
	15.625	0.169	51.98	
	7.813	0.207	41.19	
	3.906	0.253	28.12	
	1.953	0.301	14.48	
	0.977	0.314	10.79	

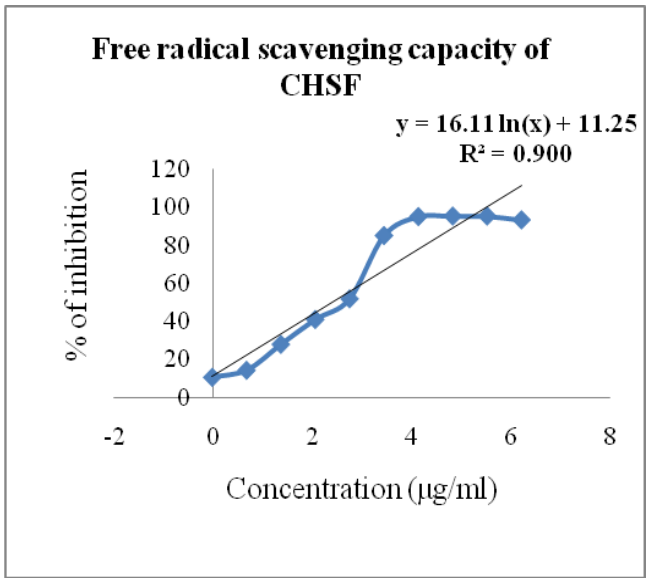


Fig. 59. IC_{50} value of chloroform soluble fraction (CHSF) of root bark of *O. indicum*.

Table 72. IC_{50} value of dichloromethane soluble fraction (DCMSF) of methanolic extract of root bark of *O. indicum*.

Absorbance of the blank	Conc. (µgm/ml)	Absorbance of the extract	% inhibition	IC_{50}
0.352	500	0.040	88.63	12.18
	250	0.023	93.46	
	125	0.014	96.02	
	62.5	0.013	96.30	
	31.25	0.019	94.60	
	15.625	0.169	51.98	
	7.813	0.210	40.34	
	3.906	0.299	15.05	
	1.953	0.306	13.06	
	0.977	0.321	8.80	

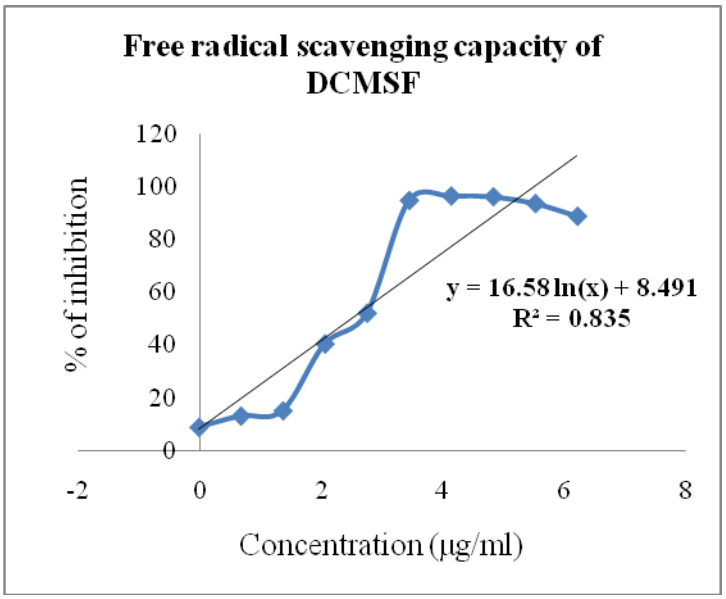


Fig. 60. IC_{50} value of dichloromethane soluble fraction (DCMSF) of root bark of *O. indicum*.

Table 73. IC_{50} value of ethyl acetate soluble fraction (EASF) of methanolic extract of root bark of *O. indicum*.

Absorbance of the blank	Conc. (µgm/ml)	Absorbance of the extract	% inhibition	IC_{50}
0.352	500	0.024	93.18	24.09
	250	0.016	95.45	
	125	0.042	88.06	
	62.5	0.096	72.72	
	31.25	0.187	46.87	
	15.625	0.265	24.71	
	7.813	0.249	29.26	
	3.906	0.281	20.17	
	1.953	0.325	7.67	
	0.977	0.325	7.67	

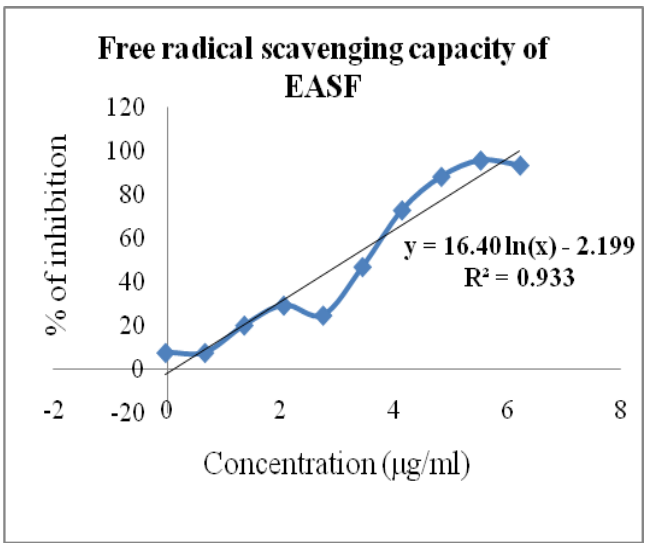


Fig. 61. IC_{50} value of ethyl acetate soluble fraction (EASF) of root bark of *O. indicum*.

Table 74. IC_{50} value of aqueous soluble fraction (AQSF) of methanolic extract of root bark of *O. indicum*.

Absorbance of the blank	Conc. (µgm/ml)	Absorbance of the extract	% inhibition	IC_{50}
0.352	500	0.023	93.46	16.94
	250	0.016	95.45	
	125	0.045	87.21	
	62.5	0.079	77.55	
	31.25	0.092	73.86	
	15.625	0.199	43.46	
	7.813	0.253	28.12	
	3.906	0.290	17.61	
	1.953	0.301	14.48	
	0.977	0.314	10.79	

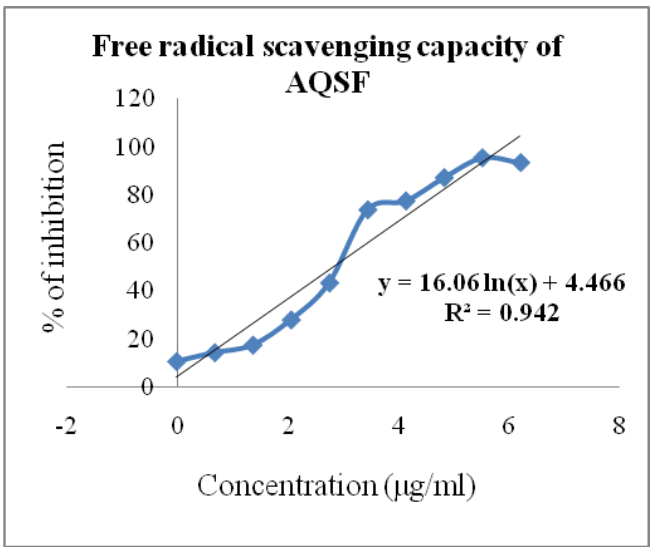


Fig. 62. IC_{50} value of aqueous soluble fraction (AQSF) of root bark of *O. indicum*.

In this investigation, MEROI showed the highest free radical scavenging activity with IC_{50} value 9.29 ± 0.28 µg/ml for root bark of *O. indicum* (Table 69). At the same time the CHSF, DCMSF and AQSF also exhibited strong antioxidant potential having IC_{50} value 11.07 ± 0.41, 12.18 ± 0.63 and 16.94 ± 0.58 µg/ml, respectively. EASF and PESF also revealed moderate scavenging activity having IC_{50} values 24.09 ± 0.91 µg/ml and 37.11 ± 0.39 µg/ml, respectively (Tables 73 and 70).

IC_{50} values of the standard ASA and BHT, and different extractives of root bark of *O. indicum* are presented in Table 75 and Fig. 63.

Table 75. IC_{50} values of the standard and partitionates of *O. indicum*.

Plant part	Sample code	Test Sample	IC_{50} (µg/ml)
Root bark of *O. indicum*	MEROI	Methanolic extract of root bark of *O. indicum*	9.29 ± 0.28
	PESF	Petroleum ether soluble fraction	37.11 ± 0.39
	CHSF	Chloroform soluble fraction	11.07 ± 0.41
	DCMSF	Dichloromethane soluble farction	12.18 ± 0.63
	EASF	Ethyl acetate soluble fraction	24.09 ± 0.91
	AQSF	Aqueous soluble fraction	16.94 ± 0.58
ASA (Ascorbic acid) (standard)			2.06 ± 0.14
BHT (*tert*-butyl-1-hydroxytoluene)			15.09 ± 0.17

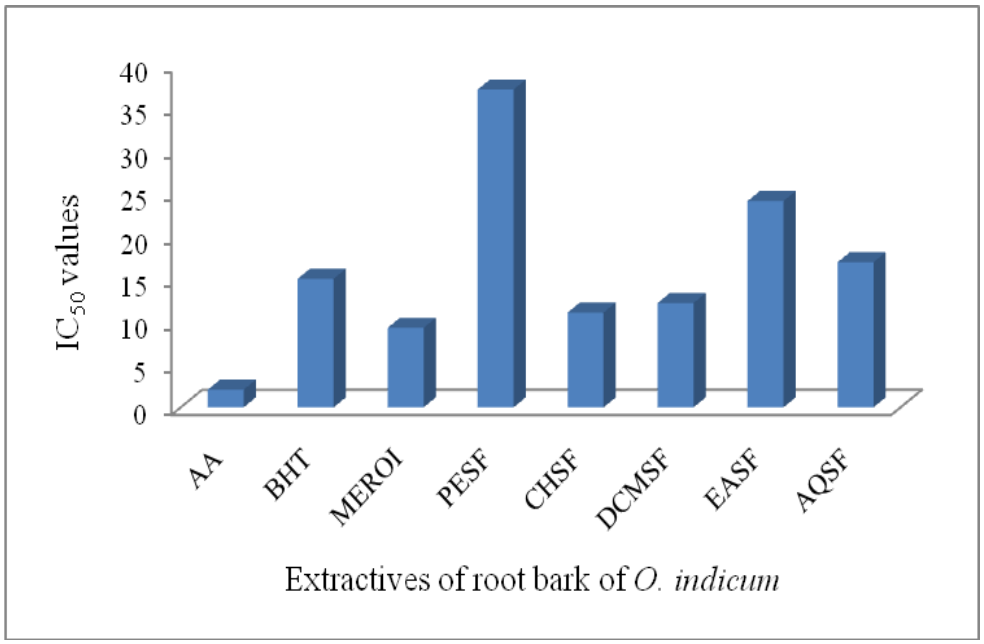


Fig. 63. IC_{50} values of the standard and partitionates of *O. indicum*.

11.7.2 Results of different extractives of bark and leaves of *Aphanamixis polystachya*

The methanolic extract of bark of *A. polystachya* (MEBAP), and different partitionates i.e. pet-ether soluble fraction (PESF), chloroform soluble fraction (CHSF), ethyl acetate soluble fraction (EASF) and aqueous soluble fraction (AQSF) were focused to free radical scavenging activity by the method of Brand-Williams *et al.* (1995) [Tables 76-80 and Fig. 64-68]. Here, *tert*-butyl-1-hydroxytoluene (BHT) and ascorbic acid (ASA) was used as reference standard (Tables 67, 68 and Fig. 55, 56).

Table 76. IC_{50} value of methanolic extract of bark of *A. polystachya* (MEBAP).

Absorbance of the blank	Conc. (µgm/ml)	Absorbance of the extract	% inhibition	IC_{50}
0.352	500	0.051	85.51	5.36
	250	0.028	90.04	
	125	0.018	94.88	
	62.5	0.016	95.45	
	31.25	0.079	77.55	
	15.625	0.122	65.34	
	7.813	0.162	53.97	
	3.906	0.191	45.73	
	1.953	0.220	37.5	
	0.977	0.283	19.60	

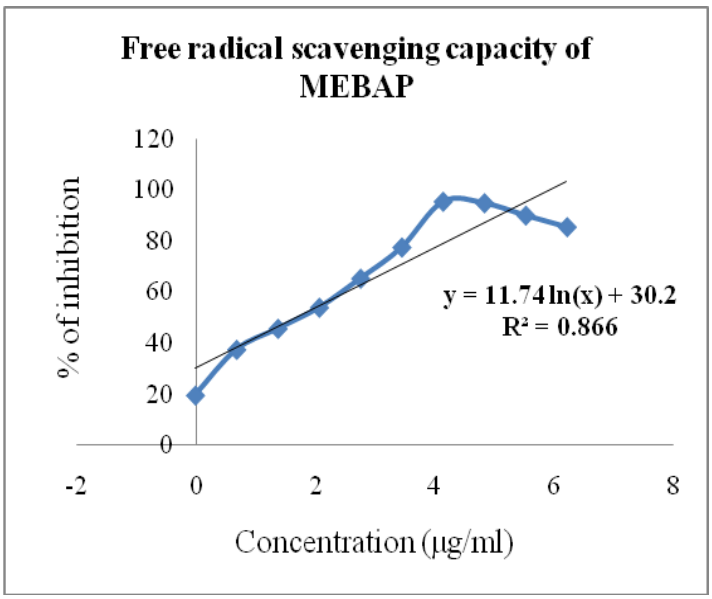


Fig. 64. IC_{50} value of methanolic extract of bark of *A. polystachya* (MEBAP).

Table 77. IC_{50} value of petroleum ether soluble fraction (PESF) of methanolic extract of bark of *A. polystachya*.

Absorbance of the blank	Conc. (µgm/ml)	Absorbance of the extract	% inhibition	IC_{50}
0.352	500	0.064	81.81	29.07
	250	0.025	92.89	
	125	0.110	68.75	
	62.5	0.163	53.69	
	31.25	0.234	33.52	
	15.625	0.244	30.68	
	7.813	0.247	29.82	
	3.906	0.252	28.40	
	1.953	0.260	26.13	
	0.977	0.268	23.86	

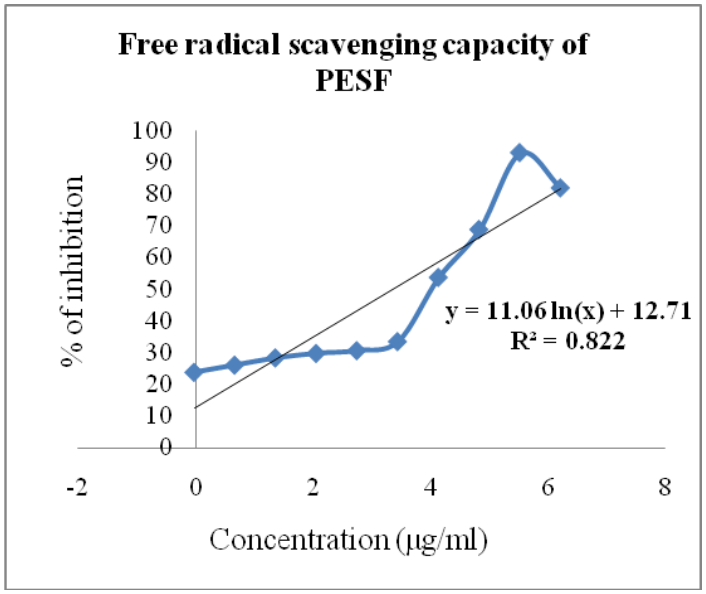


Fig. 65. IC_{50} value of petroleum ether soluble fraction of bark of *A. polystachya* (PESF).

Table 78. IC_{50} value of chloroform soluble fraction (CHSF) of methanolic extract of bark of *A. polystachya*.

Absorbance of the blank	Conc. (µgm/ml)	Absorbance of the extract	% inhibition	IC_{50}
0.352	500	0.017	95.17	8.58
	250	0.009	97.44	
	125	0.019	94.60	
	62.5	0.057	83.80	
	31.25	0.145	58.80	
	15.625	0.157	55.39	
	7.813	0.189	46.30	
	3.906	0.215	38.92	
	1.953	0.250	28.97	
	0.977	0.266	24.43	

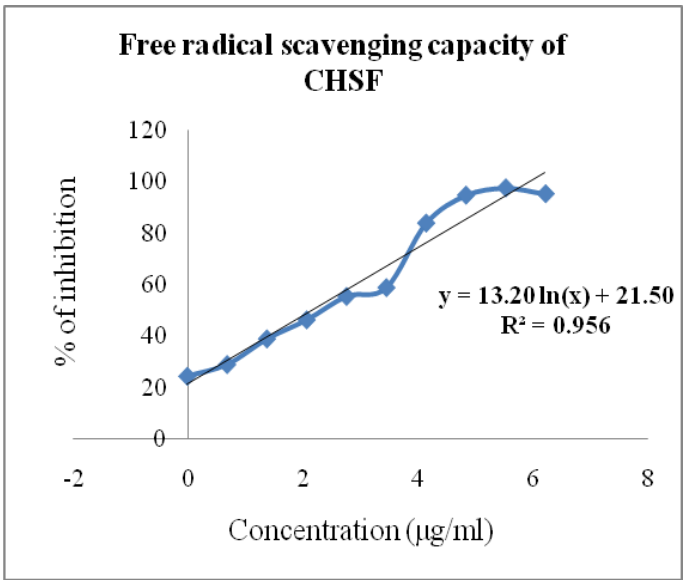


Fig. 66. IC_{50} value of chloroform soluble fraction of bark of *A. polystachya* (CHSF).

Table 79. IC_{50} value of ethyl acetate soluble fraction (EASF) of methanolic extract of bark of *A. polystachya*.

Absorbance of the blank	Conc. (µgm/ml)	Absorbance of the extract	% inhibition	IC_{50}
0.352	500	0.151	57.10	14.29
	250	0.071	79.82	
	125	0.045	87.21	
	62.5	0.052	85.22	
	31.25	0.175	50.28	
	15.625	0.193	45.17	
	7.813	0.197	44.03	
	3.906	0.220	37.5	
	1.953	0.250	28.97	
	0.977	0.270	23.29	

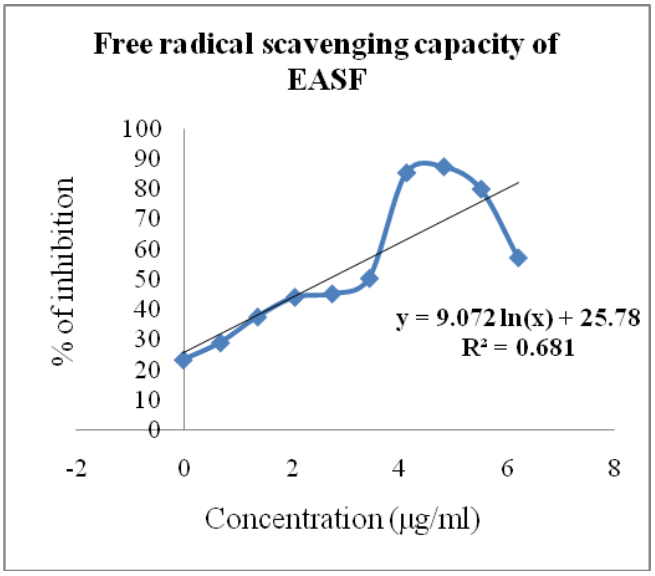


Fig. 67. IC_{50} value of ethyl acetate soluble fraction of bark of *A. polystachya* (EASF).

Table 80. IC_{50} value of aqueous soluble fraction (AQSF) of methanolic extract of bark of *A. polystachya*.

Absorbance of the blank	Conc. (µgm/ml)	Absorbance of the extract	% inhibition	IC_{50}
0.352	500	0.121	65.62	9.29
	250	0.062	82.38	
	125	0.045	87.21	
	62.5	0.052	85.22	
	31.25	0.156	55.68	
	15.625	0.181	48.57	
	7.813	0.190	46.02	
	3.906	0.210	40.34	
	1.953	0.223	36.64	
	0.977	0.251	28.69	

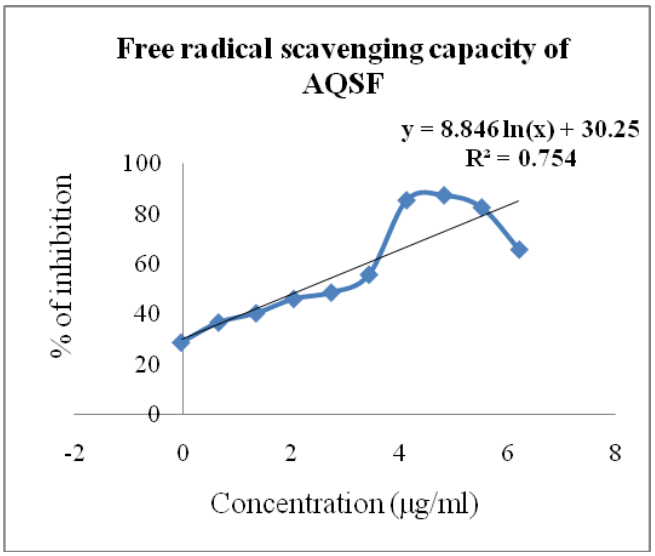


Fig. 68. IC_{50} value of aqueous soluble fraction of bark of *A. polystachya* (AQSF).

The study revealed that MEBAP showed the highest free radical scavenging activity with IC_{50} value 5.36 ± 0.85 µg/ml for bark of *A. polystachya* (Table 76). At the same time the CHSF and AQSF also exhibited strong antioxidant potential having IC_{50} value 8.58 ± 0.29 and 9.29 ± 0.71 µg/ml, respectively. EASF and PESF also revealed moderate scavenging activity having IC_{50} values 14.29 ± 0.45 µg/ml and 29.07 ± 0.51 µg/ml, respectively (Tables 79 and 77).

IC_{50} values of the standard and different extractives of bark of *A. polystachya* are shown in Table 81 and Fig. 69.

Table 81. IC_{50} values of the standard and partitionates of bark of *A. polystachya.*

Plant part	Sample code	Test Samples	IC_{50} (µg/ml)
Bark of *A. polystachya*	MEBAP	Methanolic extract of bark of *A. polystachya*	5.36 ± 0.85
	PESF	Petroleum ether soluble fraction	29.07 ± 0.51
	CHSF	Chloroform soluble fraction	8.58 ± 0.29
	EASF	Ethyl acetate soluble fraction	14.29 ± 0.45
	AQSF	Aqueous soluble fraction	9.29 ± 0.71
ASA (Ascorbic acid) (standard)			2.06 ± 0.14
BHT (*tert*-butyl-1-hydroxytoluene)			15.02 ± 0.17

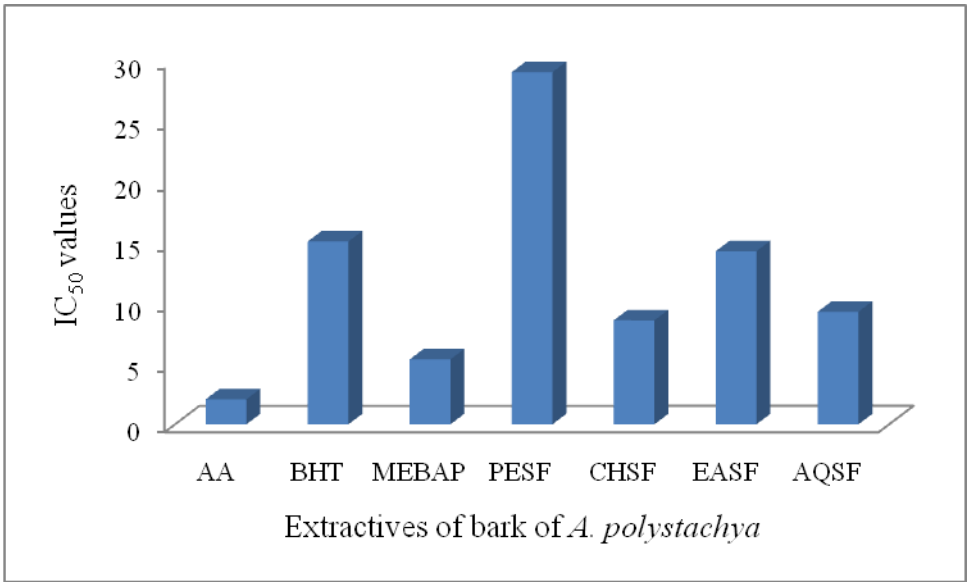


Fig. 69. IC_{50} values of the standard and partitionates of bark of *A. polystachya*

The methanolic extract of leaves of *A. polystachya* (MELAP), and different partitionates i.e. pet-ether soluble fraction (PESF), chloroform soluble fraction (CHSF) and aqueous soluble fraction (AQSF) were issued to free radical scavenging activity in Tables 82-85 as well as in Figures 70-73. Here, *tert*-butyl-1-hydroxytoluene (BHT) and ascorbic acid (ASA) was used as reference standard (Tables 67, 68 and Fig. 55, 56)

Table 82. IC_{50} value of methanolic extract of leaves of *A. polystachya* (MELAP).

Absorbance of the blank	Conc. (µgm/ml)	Absorbance of the extract	% inhibition	IC_{50}
0.352	500	0.019	94.60	20.90
	250	0.106	69.88	
	125	0.125	64.48	
	62.5	0.132	62.50	
	31.25	0.168	52.27	
	15.625	0.184	47.72	
	7.813	0.197	44.03	
	3.906	0.220	37.50	
	1.953	0.279	20.73	
	0.977	0.310	11.93	

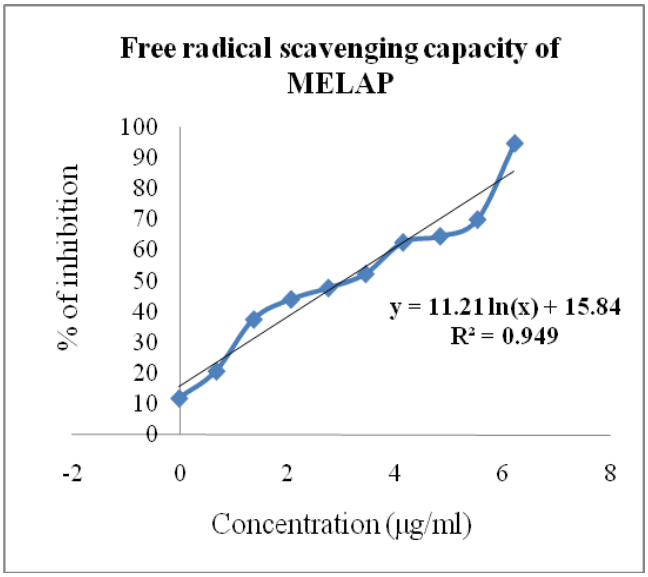


Fig. 70. IC_{50} value of methanolic extract of leaves of *A. polystachya* (MELAP).

Table 83. IC_{50} value of petroleum ether soluble fraction (PESF) of methanolic extract of leaves of *A. polystachya.*

Absorbance of the blank	Conc. (µgm/ml)	Absorbance of the extract	% inhibition	IC_{50}
0.352	500	0.021	94.03	15.79
	250	0.027	92.32	
	125	0.029	91.76	
	62.5	0.102	71.02	
	31.25	0.119	66.19	
	15.625	0.169	51.98	
	7.813	0.215	38.92	
	3.906	0.265	24.71	
	1.953	0.313	11.07	
	0.977	0.319	9.37	

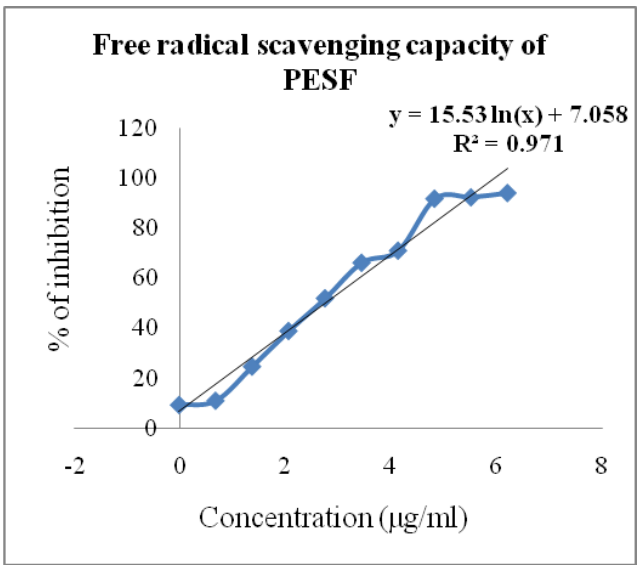


Fig. 71. IC_{50} value of petroleum ether soluble fraction of leaves of *A. polystachya* (PESF).

Table 84. IC_{50} value of chloroform soluble fraction (CHSF) of methanolic extract of leaves of *A. polystachya*.

Absorbance of the blank	Conc. (µgm/ml)	Absorbance of the extract	% inhibition	IC_{50}
0.352	500	0.064	81.81	8.41
	250	0.075	78.69	
	125	0.087	75.28	
	62.5	0.091	74.14	
	31.25	0.103	70.73	
	15.625	0.113	67.89	
	7.813	0.175	50.28	
	3.906	0.191	45.73	
	1.953	0.239	32.10	
	0.977	0.290	17.61	

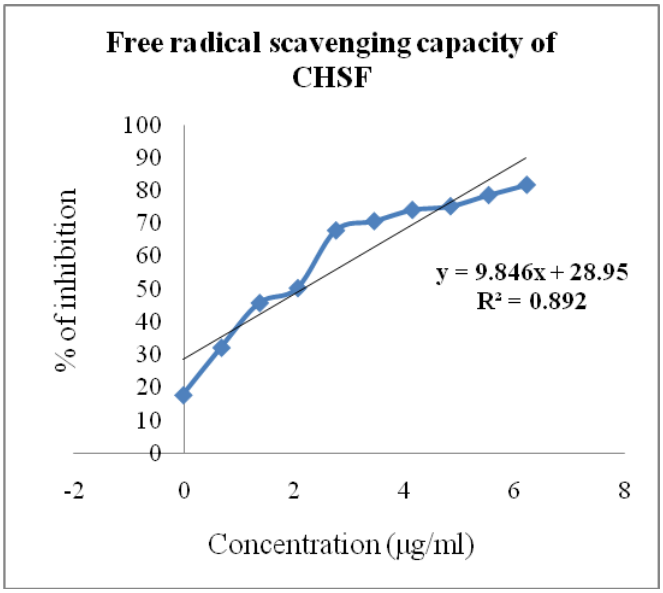


Fig. 72. IC_{50} value of chloroform soluble fraction of leaves of *A. polystachya* (CHSF).

Table 85. IC_{50} value of aqueous soluble fraction (AQSF) of methanolic extract of leaves of *A. polystachya*.

Absorbance of the blank	Conc. (µgm/ml)	Absorbance of the extract	% inhibition	IC_{50}
0.352	500	0.071	79.82	11.02
	250	0.079	77.55	
	125	0.092	73.86	
	62.5	0.099	71.87	
	31.25	0.109	69.03	
	15.625	0.119	66.19	
	7.813	0.181	48.57	
	3.906	0.196	44.31	
	1.953	0.252	28.40	
	0.977	0.310	11.93	

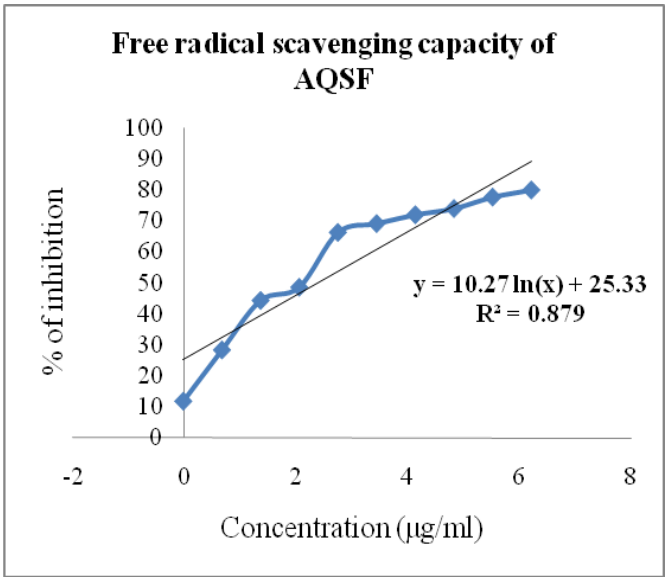


Fig. 73. IC_{50} value of aqueous soluble fraction of leaves of *A. polystachya* (AQSF).

In this study, CHSF showed the highest free radical scavenging activity with IC_{50} value 8.41 ± 0.83 µg/ml for leaves of *A. polystachya* (Table 84). At the same time the AQSF and PESF also exhibited strong antioxidant potential having IC_{50} value 11.02 ± 0.62 and 15.79 ± 0.59 µg/ml, respectively. MELAP also revealed moderate scavenging activity having IC_{50} values 20.90 ± 0.36 µg/ml, respectively (Table 82).

IC_{50} values of standard and different extractives of leaves of *A. polystachya* are depicted in Table 86 and Fig. 74.

Table 86. IC_{50} values of the standard and different partitionates of leaves of *A. polystachya.*

Plant part	Sample code	Test Samples	IC_{50} (µg/ml)
Leaves of *A. polystachya*	MELAP	Methanolic extract of leaves of *A. polystachya*	20.90 ± 0.36
	PESF	Petroleum ether soluble fraction	15.79 ± 0.59
	CHSF	Chloroform soluble fraction	8.41 ± 0.83
	AQSF	Aqueous soluble fraction	11.02 ± 0.62
ASA (Ascorbic acid) (standard)			2.06 ± 0.14
BHT (*tert*-butyl-1-hydroxytoluene)			15.02 ± 0.17

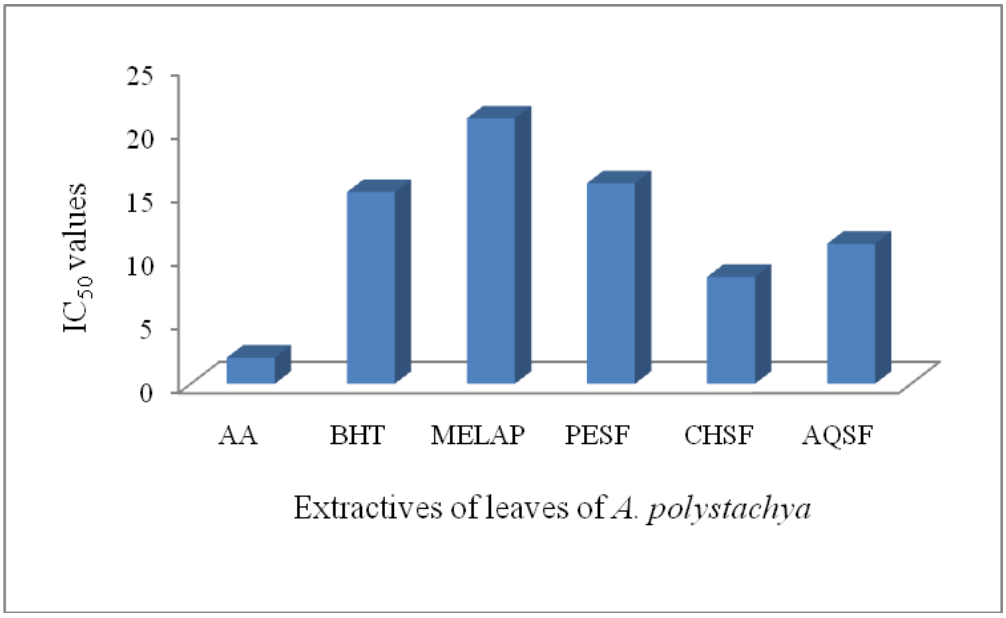


Fig. 74. IC_{50} values of the standard and partitionates of leaves of *A. polystachya*.

CHAPTER 12
GENERAL DISCUSSION

The present study revealed that the crude extract and aqueous soluble fraction of the root bark of *Oroxylum indicum* (L.) Kurz and the chloroform soluble fraction of the bark of *Aphanamixis polystachya* (Wall.) R.N. Parker was found to have significant anticancer activity in Ehrlich's Ascites Carcinoma Test by calculating per cent of cell growth inhibition (40.46%, 68.09% and 64.80%) with comparison to standard bleomycin (89.0%) using an *in vivo* test.

Naveen *et al.* (2012) examined petroleum ether hot extract (PHO) of *Oroxylum indicum* for apoptosis induction in estrogen receptor ER-negative (MDA-MB-231) and ER-positive (MCF-7) breast cancer cells by cellular DNA fragmentation ELISA. They proved that apoptosis induction was more capable in the MDA-MB-231 cells. Roy *et al.* (2007) studied baicalein, isolated from methanolic extract of the fruits of *O. indicum* for *in vitro* effects of baicalein on the viability and induction of apoptosis in the HL-60 cell line. The cell viability after treating with baicalein for 24 h was evaluated by counting viable cells using trypan blue staining. The results indicated that baicalein had anti-tumour effects on human cancer cells. Lambertini *et al.* (2004) investigated *O. indicum* extracts and showed anti-proliferative activity on MCF7 and MDA-MB-231 breast cancer cell lines. *In vivo* genotoxic activity and cell proliferative activity were observed in the stomach mucosa of male F344 rats by *in vivo* short-term methods after oral administration of a nitrosated *O. indicum* Vent (OiV) fraction, which was found to be mutagenic without S9 mix to *Salmonella typhimurium* TA98 and TA100. These results indicated that the nitrosated OiV fraction had genotoxic and cell proliferative activity in the pyloric mucosa of rat stomach *in vivo* (Tepsuwan *et al.,* 1992). The present study demonstrated that crude methanolic extract and aqueous soluble fraction of the root bark of *O. indicum* showed significant anticancer activity in Ehrlich's Ascites Carcinoma Test by calculating % of cell growth inhibition (40.46% and 68.09%) with comparison to

standard bleomycin (89.0%) using an *in vivo* test. The cell viability was counted by using trypan blue dye.

Rabi and Banerjee (2009) revealed that methyl-25-hydroxy-3-oxoolean-12-en-28-oate (AMR-Me) isolated from the stem bark of *Amoora rohituka* (Synonym of *Aphanamixis polystachya*) had the role of telomerase in mediating the growth suppression of human acute lymphoblastic leukemic CEM cells by AMR-Me. They exhibited that AMR-Me inhibited the growth and viability of CEM cells, induced apoptosis, and *in vivo* antitumour activity of AMR-Me was determined using mice inoculated with Dalton's lymphoma ascites tumour cells. Further Rabi *et al.* (2007) explained that 25-hydroxy-3-oxoolean-12-en-28-oic acid (Amooranin-AMR) isolated from the stem bark of *Amoora rohituka* exhibited potent inhibitory effect on survival of human breast carcinoma MDA-468, breast adenocarcinoma MCF-7 cells compared to breast epithelial MCF-10A control cells, while the present study demonstrated that the chloroform extract of stem bark of *A. polystachya* showed anticancer activity in Ehrlich ascites carcinoma in Swiss albino mice by analyzing % of cell growth inhibition. Jagetia and Venkatesha (2005) examined the ethanolic extract of *A. polystachya* (APE) on Swiss albino mice transplanted with Ehrlich ascites carcinoma (EAC) and exposed to various doses of γ-radiation. The best effect of APE and radiation was observed for 6 Gy γ-radiation whereas our present study showed the chloroform soluble fraction of the stem bark of *A. polystachya* had significant anticancer activity in Ehrlich's Ascites Carcinoma Test in Swiss albino mice by calculating % of cell growth inhibition (64.80%) with comparison to standard bleomycin (89.0%) using an *in vivo* test. Habib *et al.* (2011) showed that ethyl acetate and dichloromethane soluble fraction of stem of *Amoora rohituka* possesse antitumour activity against Ehrlich's ascites carcinoma (EAC) in mice. The present study also revealed that chloroform soluble fraction of stem bark of *A. polystachya* exhibited antitumour activity against Ehrlich's ascites carcinoma (EAC) in mice and was found consistent with Habib *et al.* (2011).

The present study isolated four compounds (flavonoids) from root bark of DCM fraction of *O. indicum* by gel filtration chromatography over Sephadex LH-20. They

were named as AR-O17 (5,7-dihydroxy-3-methoxy flavone), AR-O18 (7-methoxy-3,5-dihydroxyflavone), AR-O23 [5,7-dihydroxy flavone (Chrysin)] and AR-O30 (Kaempferol-3,4',5,7-tetrahydroxy flavonol).

Keying (2005) determined bicalin in *O. indicum* by reversed-phase high-performance liquid chromatography (RP-HPLC), whereas the present study isolated four flavonoids by gel filtration chromatography over Sephadex LH-20. Chen *et al.* (2005) separated a mixture of three standard flavonoids and Chen *et al.* (2003) found four flavonoids [chrysin, bicalein, baicalein-7-O-glucoside, baicalein-7-O-diglucoside (Oroxylin B) and one unknown flavonoid] from seeds of *O. indicum* by high- speed analytical counter-current chromatography and preparative high-speed counter-current chromatography (HSCCC). Teshima *et al.* (1996) isolated phenylethanoids and cyclohexylethanoids from methanolic extract of fruit of *O. indicum* by column chromatography followed by HPLC (high-performance liquid chromatography). The compound p-coumaric acid from the bark was isolated by Gaitonde and Sapre (1989), oroxindin (flavones glucuronide) from ethanol extract of seeds by Nair and Joshi (1979), aloe-emodin (anthraquinone derivatives) from the leaves by Dey *et al.* (1978) and flavonoids (oroxylin-A, chrysin, baicalein, scutellarein, scutellarein 7-rutinoside and baicalein 7-glucuronide) from the stem bark of *O. indicum* were isolated by Subramanian and Nair (1972), however the present study describes four flavonoids (5,7-dihydroxy-3-methoxy flavones, 7-methoxy-3,5-dihydroxyflavone, chrysin and kaempferol) from root bark of *O. indicum.*

Regarding several pharmacological activities of *O. indicum* were carried out. Babu *et al.* (2006) demonstrated antibacterial activity of C(7) modified chrysin analogues against a panel of susceptible and resistant gram-positive and gram-negative organisms. Ali *et al.* (1998) conducted antimicrobial activity of dichloromethane extracts of stem bark and root and also some flavonoids (baicalein, chrysin and lapachol) against gram-positive and gram-negative bacteria. Radhika *et al.* (2011) showed antibacterial activity of alcoholic extracts of stem and root using agar well diffusion method. Naveen *et al.* (2012) established cytotoxic effects of *O. indicum* on human breast cancer cells. Recently antioxidant, antimicrobial, cytotoxic and

apoptotic studies of bark extracts from *O. indicum* have been investigated (Moirangthem *et al.*, 2013). Yan *et al.* (2011) explored antioxidant activity of flavonoids by DPPH and ORAC assay, where Mishra *et al.* (2010) investigated *in vitro* antioxidant potential of different parts of *O. indicum*. Antioxidant activity using DPPH assay of flavonoids from *O. indicum* have been displayed by Deka *et al.* (2014).

The present study revealed significant cytotoxic activities of crude methanolic extract, pet-ether, chloroform, dichloromethane, ethyl acetate and aqueous soluble fraction of root bark of *O. indicum* with LC_{50} values 1.817 ± 0.23, 1.183 ± 0.32, 0.633 ± 0.17, 0.677 ± 0.15, 2.120 ± 0.73, 2.576 ± 0.45 μg/ml, respectively. In the brine shrimp lethality bioassay, the chloroform soluble fraction of root bark of *O. indicum* displayed the highest cytotoxic potential with LC_{50} value 0.633 ± 0.17 μg/ml as compared to 0.413 ± 0.55 μg/ml for vincristine sulphate. Previous study showed the cytotoxic activities of petroleum ether hot extract exhibited significantly higher cytotoxicity in MDA-MB-231 (cancer cells) when compared to WRL-68 cells (non-tumour cells) [Naveen *et al.*, 2012], whereas this study explained highest cytotoxicity of chloroform soluble fraction of *O. indicum* with LC_{50} value 0.633 ± 0.17 μg/ml as compared to 0.413 ± 0.55 μg/ml for vincristine sulphate by brine shrimp lethality bioassay. Moirangthem *et al.* (2013) stated that petroleum ether, dichloromethane and methanol extract of stem bark revealed cytotoxicity against HeLa cells and pet-ether soluble fraction showed maximum cytotoxicity, whereas the present study exhibited cytotoxic activities of crude methanolic extract, pet-ether, chloroform, dichloromethane, ethyl acetate and aqueous soluble fraction of root bark of *O. indicum* and chloroform fraction showed highest cytotoxicity in brine shrimp lethality bioassay.

The extractives of root bark of *O. indicum* when screened for antibacterial activity against five gram-positive and eight gram-negative bacteria at a concentration of 400 μg/ disc, the test samples revealed mild to moderate inhibitory activity against the pathogens having zone of inhibition ranging from 7.0-16.0 mm, with the highest inhibition of bacterial growth by the dichloromethane soluble fraction (16.0 mm)

against *Bacillus subtilis*. On the other hand, the aqueous soluble fraction exhibited no antimicrobial activity and its other fraction demonstrated antimicrobial activity. The inhibitory activity of the extractives was compared with ciprofloxacin as standard in both cases.

Results on antibacterial properties of *O. indicum* have been found inconsistent with that of Babu *et al.* (2006) where they showed that C(7) modified chrysin analogues of *O. indicum* exhibited significant antibacterial activity against a group of susceptible and resistant gram-positive and gram-negative organisms. The present study revealed mild to moderate antibacterial activity of different fractions of root bark of *O. indicum* against five gram-positive and eight gram-negative bacteria. Ali *et al.* (1998) displayed antimicrobial activity of dichloromethane extracts of stem bark and root, and flavonoids (baicalein, chrysin and lapachol) against gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) and against *Candida albicans* whereas the present study demonstrated antibacterial activity of different extracts of root bark of *O. indicum.* Among different fractions, dichloromethane soluble fraction exhibited maximum zone of inhibition (16.0 mm) against *B. subtilis*. This fraction also showed antibacterial activity against *Shigella dysenteriae*, *Vibrio mimicus* and *Pseudomonas aeruginosa*. Moirangthem *et al.* (2013) showed that among different extracts, only methanolic extracts (MeOH) inhibited both bacteria and fungi and Radhika *et al.* (2011) expressed that alcoholic extracts of stem and root exhibited antibacterial activity, and the stem extract had more antibacterial activity. In contrast, the present study revealed mild to moderate antibacterial activity of different extracts in root bark of *O. indicum.*

The total phenolic content of the extractives of root bark of *O. indicum* was found in the range of 4.0 ± 0.25 to 15.75 ± 0.34 mg of GAE/gm of extractives, with the highest amount of phenolics (15.75 ± 0.34 mg of GAE/gm of extractives) being observed in the crude methanol extract. In the DPPH free radical scavenging assay, the crude methanol extract of root bark of *O. indicum* revealed maximum free radical scavenging activity (IC_{50} = 9.29 ± 0.28 µg/ml) when compared to ascorbic acid (IC_{50} = 2.06 ± 0.25 µg/ml). Moirangthem *et al.* (2013) demonstrated that the crude

methanol extract of stem bark of *O. indicum* expressed the highest antioxidant activity (IC_{50} = 22.7 μg/ml), whereas the present study displayed that the crude methanol extract of root bark contained the highest antioxidant activity (IC_{50} = 9.29 ± 0.28 μg/ml). Yan *et al.* (2011) investigated flavonoids which were isolated from aqueous ethanolic extract of the seed and Deka *et al.* (2014) isolated flavonoids (chrysin, scutellarein, baicalein) from the ethyl acetate extract of stem bark. Mishra *et al.* (2010) explored that methanol extract of different parts (root, root bark, stem, stem bark, leaves and fruits) of *O. indicum* showed potent antioxidant activity in DPPH assay, whereas the present work exhibited that different solvent extracts (crude methanol, pet-ether, dichloromethane, chloroform, ethyl acetate and aqueous fractions) of root bark of *O. indicum* showed free radical scavenging activity.

The present study revealed isolation of one compound from leaves of chloroform fraction of *Aphanamixis polystachya* by gel filtration chromatography over Sephadex LH-20, namely AR-AL25 (Stigmasterol). Ragasa *et al.* (2014) determined α-copaene, squalene, polyprenol, β-sitosterol, lutein and β-carotene from dichloromethane extracts, and Cai *et al.* (2012) isolated Aphanamixoid A, a limonoid from leaves of *A. polystachya*. Zhang *et al.* (2014) separated six new diterpenoids named Aphanamixins A-F, nemoralisin and nemoralisin C. Wu *et al.* (2013) found four new diterpenes, *viz.* Aphanaperoxides E-H, Rabi *et al.* (2007) showed novel triterpenoid- 25-hydroxy-3-oxoolean-12-en-28-oic acid and Rabi *et al.* (2003) isolated amooranin, Chowdhury *et al.* (2003a) isolated guaiane-derived sesquiterpenoids, 6β, 7β-epoxyguai-4-en-3-one and 6β, 7β-epoxy-4β, 5-dihydroxyguaiane from petroleum ether extracts, Chakrabortty *et al.* (1969) displayed Aphanamixinin from the stem bark of *A. polystachya.* The present investigation demonstrated the compound stigmasterol from chloroform fraction of leaves of *A. polystachya* by gel filtration chromatography over Sephadex LH-20. Rohitukine, a chromone alkaloid was isolated by Kumara *et al.* (2014). Zhang *et al.* (2013) showed seven new prieurianin-type limonoids Aphapolynins C-l (1-7), and a new aphanamolide-type limonoid, Aphanamolide B from fruits. Daulatabad and Jamkhandi (1997) showed a keto fatty acid, 7-keto-octadec-cis-11-enoic acid from seed oil of *A. polystachya.*

Several studies were made on pharmacological activities of *A. polystachya*. Apu *et al.* (2013) carried out phytochemical screening and *in vitro* evaluation of pharmacological activities of fruit extracts of *A. polystachya*, while Leo *et al.* (2011) demonstrated cytotoxic effects of *Amoora rohituka* on breast and pancreatic cancer cells. Talukder and Miyata (2002) paid attention on *in vivo* and *in vitro* toxicities of seed extracts of *Pithraj* (*Aphanamixis polystachya*) and *Neem* (*Azadirachta indica*) against rice green leaf hopper. Recently chemical composition, and antimicrobial activity of leaf and fruit oils from *Amoora rohituka* (=*Aphanamixis polystachya*) have been investigated (Aboutabl *et al.*, 2000; Chowdhury *et al.*, 2003b).

The present investigation revealed significant cytotoxic activities of crude methanolic extract, pet-ether, chloroform, ethyl acetate and aqueous soluble fraction of bark of *A. polystachya* with LC_{50} values 2.462 ± 0.14, 1.392 ± 0.21, 0.677 ± 0.64, 2.036 ± 0.41, 2.112 ± 0.59 μg/ml, respectively. In the brine shrimp lethality bioassay, the chloroform soluble fraction of bark of *A. polystachya* displayed the highest cytotoxic potential with LC_{50} value 0.67 ± 0.64 μg/ml as compared to 0.413 ± 0.55 μg/ml for vincristine sulphate. In the case of leaf the crude methanolic extract, pet-ether, chloroform and aqueous soluble fraction showed cytotoxic activities with LC_{50} values are 1.334 ± 0.91, 0.682 ± 0.25, 1.216 ± 0.62, 1.667 ± 0.07 μg/ml, respectively. The highest cytotoxic potential with LC_{50} value 0.68 ± 0.25 μg/ml was found in pet-ether soluble fraction of leaves of *A. polystachya*. Previous study showed the cytotoxic activities of crude methanolic extract, pet-ether and chloroform soluble fraction of *A. polystachya* with LC_{50} values were 11.0, 10.36 and 16.45 μg/ml, respectively by brine shrimp lethality bioassay (Majumder *et al.*, 2014). In the present study, the mass potent cytotoxic activity of the extractives could be explained by the variable nature and amount of phyto-constituents with change of geographical location, condition of soil etc. Rabi *et al.* (2002) showed cytotoxicity of amooranin and its derivatives isolated from stem bark against MCF-7 and HeLa cells, whereas this work was limited to the cytotoxic activities of bark and leaves of *A. polystachya* only in brine shrimp lethality bioassay.

The extractives of bark of *A. polystachya* when screened for antibacterial activity against five gram positive and eight gram negative bacteria at a concentration of 400 µg/ disc, the test samples revealed mild to moderate inhibitory activity against the pathogens having zone of inhibition ranging from 7.0-11.0 mm, with the highest inhibition of bacterial growth by the chloroform soluble fraction (11.0 mm) against *Bacillus subtilis*. Likewise, the extractives of leaves of *A. polystachya* revealed similar inhibitory activity against the test pathogens having zone of inhibition ranging from 7.0-10.0 mm, with the highest inhibition of bacterial growth of the crude methanol extract (10.0 mm) in *B. subtilis*. On the other hand, the aqueous soluble fraction exhibited no antimicrobial activity and its other fraction demonstrated antimicrobial activity. The inhibitory activity of the extractives was compared with ciprofloxacin as standard in both cases.

Results obtained from this present investigation revealed that antibacterial properties of *A. polystachya* was found consistent with that of Chowdhury *et al.* (2003b), where they showed that stem bark of *A. polystachya* exhibited the antibacterial activity. Aboutabl *et al.* (2000) presented potential antibacterial activity in leaf and fruit oil of *Amoora rohituka*, and Mishra *et al.* (2014) exhibited significant antimicrobial activity in stem bark of *A. polystachya*. In contrast, the present study revealed mild to moderate antibacterial activity in bark and leaves of *A. polystachya*.

The total phenolic content of the extractives of bark of *A. polystachya* was found in the range of 4.12 ± 0.46 to 29.25 ± 0.75 mg of GAE/gm of extractives, with the highest amount of phenolics 29.25 ± 0.75 mg of GAE/gm of extractives, being observed in the crude methanol extract. In the DPPH free radical scavenging assay, the crude methanol extract of bark of *A. polystachya* revealed maximum free radical scavenging activity (IC_{50} = 5.36 ± 0.85 µg/ml) when compared to ascorbic acid (IC_{50} = 2.06 ± 0.14 µg/ml).

Among the extractives of leaves of *A. polystachya*, the total phenolic content was in the range of 12.87 ± 0.27 to 26.0 ± 0.54 mg of GAE/gm of extractives. In this case, the maximum phenolic content (26.0 ± 0.54 mg of GAE/gm of extractives) was

observed in the chloroform soluble fraction. Among the test samples of leaves of *A. polystachya*, the chloroform soluble fraction demonstrated the highest free radical scavenging activity (IC_{50} = 8.41 ± 0.83 μg/ml).

Finally, it is clearly evident from the present study that root bark of *O. indicum,* and the bark and leaves of *A. polystachya* have significant cytotoxic, free radical scavenging, mild to moderate antibacterial properties. *O. indicum* and *A. polystachya* is used in the treatment of cancer. Findings obtained from this present investigation justify the traditional uses of these plants. Therefore, the plants are good candidates for further chemical investigation to isolate the active constituents.

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