CHEMICAL AND BIOLOGICAL STUDIES ON THE PIGMENTS OF TOMATO (*Lycopersicon esculentum* Mill.) AND LITCHI (*Litchi chinensis* Sonn.) OF BANGLADESH

Thesis submitted

By

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AUGUST 2016

DADICATED

TO

MY BELOVED PARENTS

DECLARATION BY THE CANDIDATE

This is hereby declared that the work presented in the thesis entitled 'Chemical and Biological Studies on the Pigments of Tomato (*Lycopersicon esculentum* Mill.) and Litchi (*Litchi chinensis* Sonn.) of Bangladesh' is original in nature and to the best of my knowledge; it has not been submitted earlier in part or full to any other University or Institute for award of any other degree or diploma.

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AIM OF THE WORK

Tomato and Litchi are the most popular vegetables and fruits respectively all over the world .Tomato (*Lycopersicon esculenttum* Mill.) is a member of the genus *Lycopersicon* under the family of *Solanaceae* whereas Litchi (*Litchi chinensis* Sonn.) is the most renowned of a group of edible fruits belonging to family of *Sapindaceae* a tropical to subtropical crop that originated in South-East Asia. Tomato grows in winter season whereas Litchi grows in summer season.

Literature survey reveals that both tomato and litchi contain vitamin A, C, anthocyanin, and carotenoids with the exception of lycopene which only was found in Tomato [1]. Both these are grow abundantly in Asia. Tomatoes are popularly used as sauce, tomato fruit juice, Jelly etc. Both these also contain chemicals like sugar, vitamin C, other vitamins, poly-phenols, anthocyanins, and flavonoid compounds [2].

Epidemiological studies have shown that consumption of raw tomato and its tomato based products is associated with a reduced risk of cancer and cardiovascular diseases [3, 4]. The pericarp, seed and pulp of litchi contain large quantities of phenolic compounds, which are potential sources of natural antioxidants as well as antimicrobial activities [5]. Both are well known to have enormous medicinal effect as on liver, heart and also as stomach tonic [6]. These are widely taken by people in our country. Both these fruit have food and medicinal values. Moreover there are no reports of any chemical investigation and biological activities on the pigments of these vegetables and fruit grown in Bangladesh.

A literature review has revealed that further thorough investigation is necessary on these fruits. So phytochemical study and biological activities have been under taken with a keen interest for isolation and characterization of different compounds from them which may enable us to obtain interesting scientific findings elaborating the theme of this genus along with its chemosystematics. The biological interest of these compounds is for antioxidant and antimicrobial activity.

Above all, attempts were made to develop the methods of extraction using different solvents, identification, purification, stability, applications and evaluate the biological activities of compounds from tomato and litchi. The analysis of pure compounds will be

achieved by chromatographic methods such as TLC silica plates and column chromatography.

The main focus will be on solvent extraction, followed by methods of identification, purification and to evaluation of the nutritional potential of the various by-products of these fruits. Moreover, the antioxidant and antimicrobial activities were assessed. The antioxidant activities were evaluated using Ferric (Fe^{3+}) reducing antioxidant power (FRAP) assay and antimicrobial activities were evaluated by applying agar disc diffusion method using bacterial and fungal cultures [216; 217].

Abstract

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important widely consumed vegetables of the Solanaceae family, of Bangladesh whereas Litchi (*Litchi chinensis* Sonn.) is one of the most beautiful as well as uncommon sub-tropical evergreen tree belonging to the Sapindaceae family and sub-family *Nepheleae*, native to Southeast Asia, is fast becoming popular throughout the world because of its attractive appearance and delicious taste.

Tomatoes and their products such as tomato pulp, ketchup, juice and sauce are important source of micronutrients; these contain certain minerals (notably potassium) and carboxylic acids, including ascorbic, citric, malic, fumaric and oxalic acids. All of these are essential for health because of their antioxidant and antimicrobial activities in plants as well as in animals and humans.

Among vegetables, *Lycopersicon esculentum* are rich sources of carotenoids – principally lycopene, followed by β-carotene, lutein, phytofluene, phytoene and tocophrols are also present. Lycopene the most abundant pigment (60-64 %) is mainly responsible for the characteristic deep-red colour of ripe tomato fruits and tomato products. Because of the presence of long-chain conjugated double bonds, lycopene has been reported to possess antioxidant and antimicrobial activity and is superior to lutein or β - carotene.

Numerous epidemiological studies have suggested that a large consumption of raw tomato and tomato based products may experience a decreased risk for developing certain cancers such as prostate cancer, lung and stomach cancer, and cardiovascular diseases. The antioxidant and antimicrobial capacity, together with provitaminic properties typical of other tomato carotenoids has drawn attention towards widely consumed fruits over years. The second most important carotenoid is β-carotene, which represents about 7% of the total carotenoid content.

Preliminary trials were carried out before to proceed for Soxhlet extraction these involve use of ethyl acetate (EtOAc) as solvent for extraction. Moreover different solvents i.e. ethyl acetate (100%), di-mixture (acetone: pet ether, 1:1, v/v) and tri-mixture (n-hexane: acetone: ethanol, 2:1:1, v/v/v) were employed. Ethyl acetate was the best solvent for

extracting of *trans*- and *cis*-isomers of lycopene and β-carotene. The consumption of tomato would exert nutritional, biological and health benefits by virtue of their antioxidant, antimicrobial and anti-inflammatory activity. Attempts were taken into account to explain the scientific basis for the medicinal and nutritional benefits of these fruits. The phytochemical contents, antioxidant and antimicrobial activity were also assessed. The *in-vitro* antimicrobial activity of the test microorganism was performed by agar disc diffusion method by measuring the diameter of zone of inhibition in terms of millimeter with a calibrated scale.

The isolation, purification and identification of chemical structure of the isolated lycopene and β - carotene involve the use of thin layer chromatography (TLC), vacuum liquid chromatography (VLC), column chromatography and spectroscopic methods. The tomato extract described in this application is the ethyl acetate (100%), acetone: pet ether (1:1, v/v) and n-hexane: acetone: ethanol (2:1:1, v/v/v) extracts of ripe tomato fruits with lycopene content ranging from 17 to 29 mg/100 g. The lycopene content in tomato differs with the variety, geographic location, technique of cultivation, soil condition and degree of ripeness of tomato fruits.

The significant antimicrobial activity of active extracts was evaluated using bacterial cultures of *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Escherichia* coli (*E. coli*), *Streptococcus pneumonia* (*S. pneumonia*), and fungal cultures of *Aspergillus niger* (*A. niger*), *Aspergillus flavus* (*A. flavus*), *Candida albicans* (*C. albicans*) was compared with the Ciprofloxacin and Ketoconazole as standard antimicrobes. Chloroform extract showed more antibacterial and antifungal activities and most of the extracts executed moderate to good antimicrobial activity against the tested micro-organisms.

The various extracts of tomato were active against the entire tested microorganisms for anti-bacterial activity with the range of Minimum Inhibitory Concentration **(**MIC) values for *S. aureus* (MIC: 17-33 µg /ml), *E. coli* (MIC: 16-32 µg /ml) *S. pneumonia* (MIC: 17-33 µg /ml) and *P.aeruginosa* (MIC: 15-39 µg /ml) and for anti-fungal activity with the range of MIC values for *A. niger* (MIC: 17-34 µg/ml), *A. flavus* (18-37 µg/ml) and *C. albicans* (16-35 µg/ml).

Fruits and vegetables are known to contain a variety of different antioxidant compounds such as ascorbic acid, tocopherol, glutathione and carotenoids, which may all contribute to protection against oxidative damage. General phytochemical screening of the *Litchi chinensis* revealed the presence of alkaloids, steroids, phenolic compounds, tannins, saponins and fatty acids. These phenolic compounds belong to the class of anthocyanins, flavanol or flavonoids and are antioxidant compounds remains in the pericarp, seeds and flowers of litchi fruits. In general, phenolic acids and flavonoids exhibit powerful antioxidant activities that inhibit key enzymes in mitochondrial respiration, offer protection against coronary heart diseases, and have anti-inflammatory, antitumor and antimicrobial activities. That's why *Litchi chinensis* has wide folk medicinal uses.

The extraction was carried out using dried powder of litchi fruits pericarp (LFP) with distilled methanol (MeOH) in a soxhlet apparatus. The MeOH extract was concentrated in vacuo. These concentrated mass was treated with pet ether. The pet ether fraction was examined by TLC (silica gel: pet ether, 90:10) showed the presence of five spots of which two were violet, two pink and one green. The presence of pink colour spot was thought to be an indication of the presence of either steroid or fatty acid material or both. However its Salkowski and Liberman-Burchard reaction gave positive results confirming the presence of steroids. Upon VLC and crystallization of the fraction T-7 and purified it to mini column chromatography and the light yellowish fraction TS-2 was left undisturbed at room temperature gave white sharp needle crystals. The crystals were dissolved in chloroform and transferred to a vial marked SS-I. Its TLC study showed a single spot with the R_f value of 0.38. After recrystallization isolated compound was characterized by UV, IR, ^{1}H-NMR, ^{13}C-NMR, DEPT-135 spectrum and it was identified Stigmasterol.

This is the first report so far of occurrence and details spectroscopic description of this compound from litchi fruit pericarp (LFP).

The antibacterial activities of litchi fruit parts both pulp and waste (peel and seeds) extracted using two different solvents (ethanol and acetone), were evaluated against medically important bacteria i.e. *E. coli, P. aeruginosa, S. aureus, and B. subtilis***.** The antibacterial activity of litchi pulp and waste were compared with Bacitracin as standard antibiotic. The results revealed that litchi waste ethanolic extract exerted the strongest

inhibition against all tested bacterial strains compared to other extracts while pulp acetonic extract showed no effect against most of tested bacteria.

Extracted Stigmasterol was further used to determine its antimicrobial activity. The antimicrobial activity ranged from 12 mm to 23.5 mm for the Stigmasterol and 19.5 mm to 27.5 for Gentamicin against *E. coli, P.aeruginosa, S. aureus and K. pneumoniae*. In comparison with standard antibacterial compound Gentamicin (20 µg /mL) it was found that purified Stigmasterol (20 µg /mL) has antimicrobial activity almost equivalent to the standard.

The antioxidant activities were found in the Litchi Fruits Pericarp (LFP) approximately 15% of total fruits. Possibly these are due to the presence of phenolics, flavonoids and ascorbic acids. To achieve the antioxidant activity LFP was treated with preheated Charcoal to make them free from oily and colouring materials. This cleaned mixture contains powerful antioxidant compounds, such as ascorbic acid, phenolic acids and flavonoids which are capable of blocking the harmful effects of free radicals in the body. Antioxidant activities of phenolic and flavonoid compounds of LFP of litchi extracts were evaluated using Ferric (Fe^{3+}) reducing antioxidant power (FRAP) assay activity.

Keywords

Antioxidant, Lycopene, Antimicrobial activity, Carotenoids, Pigment, Flavonoids, Phytochemical, Stigmasterol, Fatty acids, Antibacterial activity, Antifungal activity

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1.1 General Introduction

Tomato is a winter seasonal vegetable and is cultivated in most of the districts of Bangladesh. It is green when it is raw and it is red or yellow when it is ripe. The tomato is a familiar ingredient of our everyday meals, yet its significance extends beyond nutrition to both agriculture and bioengineering. Natural colored pigments from plant products have drawn great attention worldwide. These pigments display various colors and are made up of different phytochemicals commonly found in the food matrix such as orange (β-carotene), yellowish-green (lutein), green (chlorophyll), and blue-purple (anthocyanin) dark reddish-brown (lycopene).

Tomatoes and tomato products are rich in food components that are antioxidant and considered to be a source of carotenoids, in particular lycopene and phenolic compounds [7; 8; 9; 10; 11]. Fruits and vegetables are the major sources of an assortment of carotenoids for the human diet. Some dietary carotenoids, such as β-carotene, are converted to vitamin-A contribute significantly to meeting nutritional requirements, whereas non-provitamin-A carotenoids purportedly target diverse biological functions by a range of mechanisms that affect health and disease risk [1].

Lycopene belongs to a group of naturally-occurring pigments known as carotenoids and it is a natural constituent of red fruits and vegetables and of certain algae and fungi.The major colouring principle of lycopene extract from tomato is all-trans-lycopene, however, minor amounts of cis-isomers and other carotenoids and related substances including β-carotene, phytofluene, phytoene and tocopherols are also present. Lycopene in tomatoes and tomato products consists predominantly of all-trans-lycopene (35-96% of the total lycopene content) and low levels of cis-lycopenes (01-22% of the total lycopene content) [12]. All-trans-lycopene may be converted to its cis configuration during food processing. Several reports have demonstrated that the cis isomers of lycopene are absorbed into the body more easily and play a more important part in biological function than all-trans-lycopene.

In a recent study, tomatoes ranked first as a source of lycopene (71.6%), second as a source of vitamin-C (12.0%), pro-vitamin-A, carotenoids (14.6%) and β-carotene (17.2%), and third as a source of vitamin-E (6.0%). Chemically, a tomato fruit contains water, lipid waxes, lycopene pigments, and distinctive polysaccharide and polyester

biopolymers. Tomato fruit colour became darker and the ratio of red to green colour increased during the ripening process. This red colored pigment was first discovered in the tomato by Millardet in 1876. It was later named lycopene by Schunck. The other sources of lycopene include watermelon, pink grape fruit, guava and papaya.

In a landmark paper from the early 1980s, Doll and Peto estimated that up to 30% of cancer deaths could be attributed to nutritional factors equal number deaths as can be attributed to tobacco use [13]. There is consistent evidence in the literature that increased consumption of fruits and vegetables is protective against cancers of the stomach, esophagus, lung, oral cavity/pharynx, endometrium, pancreas and colon [14] and results of various studies suggest that lycopene plays a role in the prevention of different health issues, cardiovascular disorders, digestive tract tumors and in inhibiting prostate carcinoma cell proliferation in humans [15].

It is clear that tomatoes and tomato products particularly lycopene and β-carotene are food colours, good source of antioxidants, provitaminic properties as well as decreased risk for developing certain cancers and evaluation of economic costs and simplicity and availability of extraction methods are very necessary. Several methods and solvents have been used to extract carotenoids in particular lycopene soxhlet extraction was carried out using ethyl acetate and identification of lycopene and β-carotene include TLC, column comatography and different spectrophotometry methods. A wide variety of organic solvents can be used as the mobile phase, and the column can be operated under isocratic or gradient elution conditions. The various compounds eluted can be detected using UV–Vis spectrophotometry.

1.2 Phytochemicals

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. “Phyto” comes from the Greek word for “plant”, and so phytochemicals are essential chemical compounds that can be found in plant foods like fruits, vegetables, beans and whole grains. There are more than thousand known Phytochemicals. These various chemicals give foods their colour, taste, smell and to protect itself but recent research demonstrates that they can protect humans against diseases. For example, carotenoids are the pigments that are responsible for deep red,

dark orange and yellow colour in foods while anthocyanins provide the various shades of red, purple, and blue found in fruits and vegetables. Flavonoids are responsible for the yellow, orange, and red shades in foods. They are not essential nutrients and are not required by the human body for sustaining life but serve various functions in plants, helping to protect the plant's vitality. For example, some phytonutrients protect the plant from UV radiation while others protect it from insect attack.

1.2.1 How do Phytochemicals Work in the Body?

There are many phytochemicals and each works differently and appears to have significant physiological effects in the body. Whether they are acting as antioxidants, mimicking hormones, stimulating enzymes, interfering with DNA replication, destroying bacteria, or binding to cell walls, they seem to work to curb the onset of diseases such as cancer and heart disease. There are many phytochemicals and each works differently. These are some possible actions:

- **Antioxidant Property** - Most phytochemicals have strong antioxidants activity and protect our cells against oxidative damage and reduce the risk of developing certain types of cancer, which is a normal process the body uses to produce energy. Phytochemicals may also enhance the body's ability to detoxify chemicals, slow or stop growth of cancer cells and even kill cancer cells.
- **Hormonal Activity** – Help to regulate hormones. Isoflavones was found in soy, imitate human estrogens and help to reduce menopausal symptoms and osteoporosis.
- **Stimulation of Enzymes** - Indoles are found in cabbages which stimulate enzymes that make the estrogen less effective and could reduce the risk for breast cancer. Other phytochemicals, which are interfering with enzymes, are protease inhibitors, terpenes, etc.
- **Interference with DNA Replication** - Prevent DNA damage and help with DNA repair. Saponins found in beans interfere with the replication of cell DNA, thereby preventing the multiplication of cancer cells. Capsaicin, found in hot peppers, protects DNA from carcinogens.
- **Antimicrobial Activity** - The phytochemical allicin from garlic has anti-bacterial properties, seems to work by preventing bacteria from sticking to places they should

not be or preventing blood cells from sticking together and flowing freely. Also, some phytochemicals may reduce inflammation that occurs in the walls of arteries.

- **Physical Property** - There are phytochemicals which physically bind to the cell walls and preventing the adhesion of pathogens to human cell walls. Such as Proanthocyanidins are responsible for the anti-adhesion properties of cranberry and Consumption of cranberries will reduce the risk of urinary tract infections.

1.2.2 Botanical Features of Tomato (*Lycopersicon esculentum*)

Kingdom	:	Plant
Order	:	Tub florae
Family	:	*Solanaceae*
Genus	:	*Lycopersicon*
Species	:	*Lycopersicon esculentum*

Figure 1.1 A bisection of tomato (*Lycopersicon esculentum*).

Scientific Classification

Kingdom	:	Plantae
(unranked)	:	Angiosperms
(unranked)	:	Eudicots
(unranked)	:	Asterids
Order	:	Solanales
Family	:	*Solanaceae*
Genus	:	Solanum
Species	:	*S. lycopersicum*

Binomial name

Solanum lycopersicum L.

Synonyms

Lycopersicon lycopersicum
Lycopersicon esculentum

1.3 Classification of Tomato

There are around 7,500 tomatoes varieties grown for various purposes. Heirloom tomatoes are becoming increasingly popular. There are five types' tomatoes available in Bangladesh. They are:

a. Manik
b. Ratan
c. Marglobe
d. Roma V-f
e. BARI-tomato-4

1.4 Soil for Cultivation

Sand or fertile clay and all kinds of soil are suitable for tomato cultivation. But sandy loamy soil is the best for tomato production. The month of October and November is the best time of filling the soil and souring the seeds in land. The shape and size of mother and heaper 20 cm x 20 cm x 20 cm and 30 cm x 30c m x 30 cm . The distance between two tomato plants is 45 cm (Interdish-11 project)

1.5 Botanical Traits

Lycopersicon esculentum plant have a compact habit an intermediate number of steam and grow between 1 to 3 feet high depending on the climate and growing condition. The leaves are ovate slight smooth and measure 3 inches long and 2 inches wide .The flowers have yellowish corollas with no spots and purple anthers. The pods are borne erect and measure 2 inches long 1 inch wide. Immature pods are greenish yellow maturing to bright red or yellow. The height of the plants depends on climate with the plants growing the largest in warmer parts of the country .We get 50 to 100 tomatoes per plants (Interdish-11 project).

1.6 Tomatoes as a Source of Lycopene

Lycopene can be obtained from natural sources or synthesized chemically. Tomatoes have one of the highest known concentrations of natural lycopene and most people get much of the lycopene in their diet from tomato products. Other plants known to contain high concentrations of lycopene can also be utilized. The distribution of lycopene in the tomato fruit is not uniform. The skin of the tomato fruit contains high levels of lycopene, comprising an average of 37% of the total fruit lycopene content or 3- to 6-fold higher than in whole tomato pulp [16].

About 12 mg of lycopene per 100 g fresh weight was found in tomato skin, while the whole tomato fruit contained only 3.4 mg per 100 g fresh weight [17]. The outer pericarp constitutes the largest amount of total carotenoids and lycopene, while the locule contains a high proportion of carotene [18]. Tomatoes and processed tomato products (juice, sauce, soup, pizza and spaghetti sauce) constitute the major sources and accounts for more than 85% of all the dietary sources of lycopene. The chemical composition of the tomato fruit depends on its genetics, ripeness and the conditions under which it was cultivated. Tomatoes and tomato products are rich in antioxidant compounds and are considered an important source of carotenoids, in particular lycopene, ascorbic acid and phenolic compounds [19]. **(Table 1.1)**

Table 1.1 Tomato composition given in USDA* Food Composition Data, value per 100 g [19]

Nutrient	Value	Nutrient	Value
Water (%)	95	Potassium (mg)	237
Protein (g)	0.88	Sodium (mg)	51
Total lipid (fat)(g)	0.20	Zn, Cu, Mg (mg)	0.10 - 100
Carbohydrate (g)	3.92	Vitamin C, total ascorbic acid (mg)	12.7
Fibre total dietary (g)	1.20	Thiamine (mg)	0.037
Calcium (mg)	10	Niacin (mg)	0.594
Iron (mg)	0.27	β-carotene (μg)	449
Magnesium (mg)	11	α-carotene (μg)	101
Phosphorus (mg)	24	Lycopene (μg)	2573

*USDA: United States Department of Agriculture

Regular consumption of tomatoes is reported to have enormous health benefits including reducing various types of cancers and cardiovascular diseases, reducing cholesterol, improving vision, maintaining the gut, lowering hypertension, alleviating diabetes and preventing urinary tract infections and gallstones [20].

1.7 Lycopene Chemistry

Lycopene is a highly unsaturated 40 carbon acyclic hydrocarbon containing 11 conjugated and 2 unconjugated double bonds arranged in all *trans*- configuration in tomatoes; the most thermodynamically stable form. The acyclic structure of lycopene makes it more soluble in organic solvents such as chloroform, hexane, benzene, methylene chloride, acetone and petroleum ether. Its molecular weight is 536. 89, Chemical Abstract Service (CAS) number is 502-65-8, and chemical formula is $C_{40}H_{56}$ melting point is 172–175ºC with 89.45% carbon and 10.51% hydrogen. Structure of lycopene is shown in Figure 1.2.

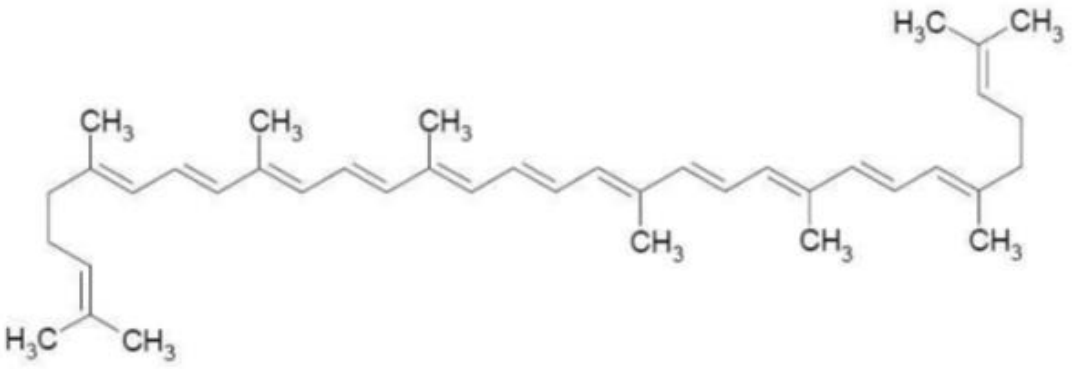


Figure 1.2 Chemical structure of lycopene.

1.8 Chemical Composition of Lycopene

Lycopene extract from tomato contains carotenoids (5-15% w/w) as well as non-carotenoid components. The carotenoid fraction of the tomato extract consists mainly of lycopenes, of which ~86% is all-*trans*-lycopene, ~6% is 5-*cis*-lycopene, ~2% is 9-*cis*-lycopene and ~2% is 13-*cis*-lycopene, and ~4% are other carotenoids.

Lycopene is an unsaturated acyclic hydrocarbon. It contains 13 double bonds, of which 11 are conjugated. The chemical name of lycopene is 2,6,10,14,19,23,27,31-octamethyl-2,6,8,10,12,14,16,18,20,22,24,26,30-dotriacontatridecaene. Common names include *Ψ, Ψ*- carotene, all-*trans*-lycopene, and (all-E)-lycopene. Amongst the isomers of lycopene (Figure 1.3), 5- *cis* lycopene has been found to be the most stable isomer (followed by all *trans*, 9 *cis*, 13 *cis*, 15 cis, 7 *cis* and 11 *cis*) and with highest antioxidant properties (followed by 9 *cis*, 7 *cis*, 13 *cis*, 11 *cis* and all *trans*- isomer).

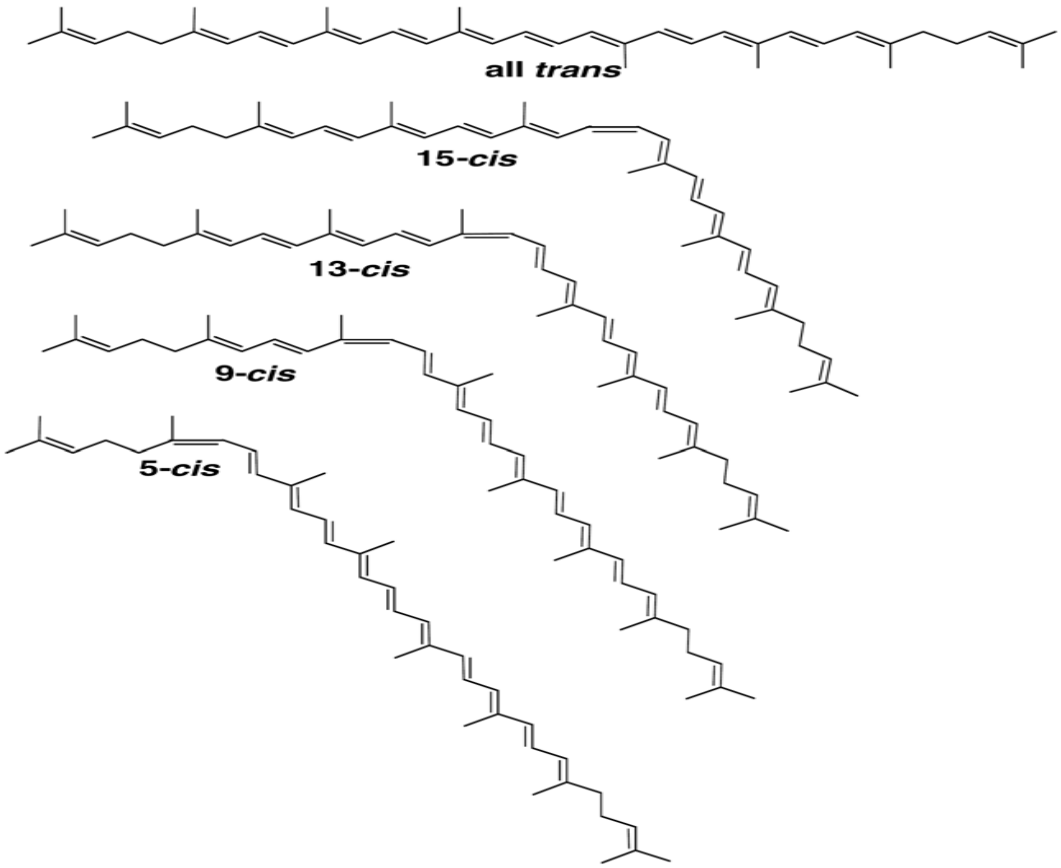


Figure 1.3 All *trans* and *cis*- isomeric forms of lycopene [21].

1.9 Stability of Lycopene

Lycopene occurs in the all-*trans* and various *cis* configurations. Naturally-occurring lycopene consists predominantly of all-*trans*-lycopene. For example, lycopene present in red tomato fruits typically contains 94-96% of all-*trans*-lycopene [12]. However, lycopene may undergo *trans*-to-*cis* isomerization during tomato processing or preparation of tomato-based meals, thus increasing the proportion of *cis* isomers. The chemical structure of lycopene, particularly the long chain of conjugated carbon-carbon double bonds, predisposes lycopene to isomerization and degradation upon exposure to light, heat, and oxygen [22] and the subsequent loss of its colouring properties [23**;** 24].

Lycopene present in the tomato extract was shown to be stable when stored at 4^0C and at room temperature over a period ranging from 18 to 37 months. When used as a food colour, lycopene remained stable in the food matrix under appropriate storage conditions. Lycopene stability depends on the particular food to which it is added, as well as on the production process.

1.10 Lycopene Absorption, Transportation and Distribution in Human Body

1.10.1 Absorption

As a fat soluble compound, absorption of lycopene is similar to other lipid soluble compounds and is absorbed across gastro intestinal tract via a chylomicron mediated mechanism and is released into lymphatic system for transport to the liver. In the stomach and duodenum, lycopene will separate from the food matrix and subsequently dissolve in the lipid phase [25]. Prior to absorption, the lipid phase will form droplets, resulting from the reaction with bile salts and pancreatic lipases. Then, it enters the duodenum and appears as the multi-lamellar lipid vesicles [26]. Finally, the lipid vesicles will absorb into small intestine via passive or diffusion process [27]. Additionally, there are *in vitro* studies suggested that the intestinal absorption of lycopene was aided by the participation of a specific epithelial transporter [28; 29].

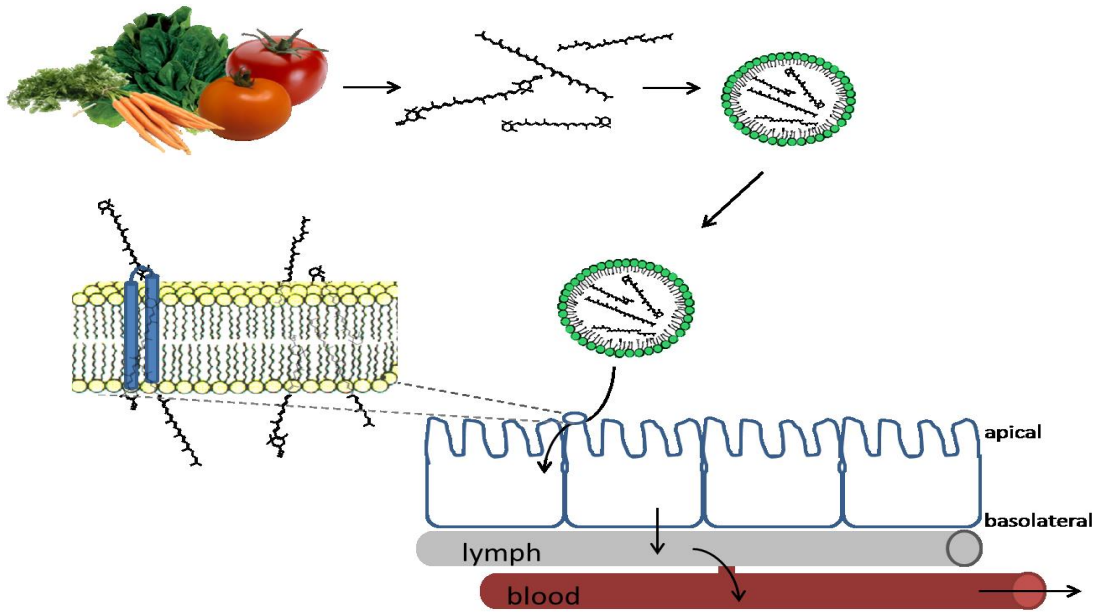


Figure 1.4 Schematic of carotenoid absorption [30].

1.10.2 Transportation and Bioavailability

Isomerization of lycopene affects its absorption efficiency, *Cis*-isomers are produced during processing and cooking of tomato products, in addition, some isomerization may occur in the gastrointestinal tract, especially in the environment of the stomach [31]**.** After the uptake by intestinal mucosa, lycopene will be parceled into triacylglycerol-rich

chylomicrons and will be secreted into lymph transport system, and lastly transferred to the liver [32**]**.

Perhaps, all-*trans*-lycopene, a long linear molecule, may be less soluble in bile acid micelles. In contrast, *cis*-isomers of lycopene may move more efficiently across plasma membranes and preferentially incorporate into chylomicrons [33]. Lycopene is prone to accumulate in the lipophilic compartments of membrane or lipoprotein [34]. It is transported by plasma lipoproteins and the distribution depends on its chemical structure. As a hydrophobic compound, lycopene is found at the lipophilic part of lipoproteins which is the core of the lipoprotein [18], while other polar carotenoids can be found at the surface of lipoproteins. Therefore, lycopene is mostly transported by low density lipoproteins, while other oxygenated carotenoids are transported by both low density and high density lipoprotein [18]. In addition, *cis* isomers of lycopene were reported to have higher ability to be incorporated in lipoprotein and other protein compared to all *trans*-isomer due to the shorter chain length [35].

1.10.3 Distribution

The distribution of lycopene in human organs and plasma has been reported higher concentrations of lycopene are found in the liver, adrenal and reproductive tissues (ten times higher than other tissues) [36]. The concentrations were within the range of 0.2–21.4 nmol/g tissue [37]. Studies have reported that lycopene concentration was highest in human testes, followed by adrenal gland > liver > prostate > breast > pancreas > skin > colon > ovary > lung >stomach >kidney > fat tissue > cervix [38; 39].

Table 1.2 Distribution of lycopene in human tissues [40-42]

Tissue	**Lycopene (nmol/g wet weight)**	**Tissue**	**Lycopene (nmol/g wet weight)**
Adipose	0.2 – 1.3	Testis	4.3 – 21.4
Adrenal	1.9 - 21.6	Lung	0.2 - 0.6
Brainstem	Not detectable	Ovary	0.3
Breast	0.8	Prostate	0.8
Colon	0.3	Skin	0.4
Liver	1.3 - 5.7	Stomach	0.2

1.11 Tomatoes as a Source of Carotenoids Pigments

Among vegetables tomatoes and tomato products are rich sources of carotenoids- principally lycopene, followed by β-carotene and lutein. Carotenoids are pigments ubiquitous in nature and responsible for the bright yellow to dark red colour of vegetal products. Carotenoids usually contain 9 to 11 conjugated double bonds, but may contain as few as 3 and as many as 15 [43]. One function of carotenoids in plants is to help aid in photosynthesis, mainly by absorbing light and protecting against photosensitization [44]. Since carotenoids are highly unsaturated pigments, many possible *cis*/*trans*- isomers are possible, both of which have biological implications, depending on the carotenoid of interest. In raw, red tomatoes, approximately 95% of the lycopene present is in the all-*trans*- form [45]. In contrast, *cis*-isomers account for 58-73% of total lycopene in serum, and a surprisingly high 79-88% of total lycopene in benign or malignant prostate tissue [42].

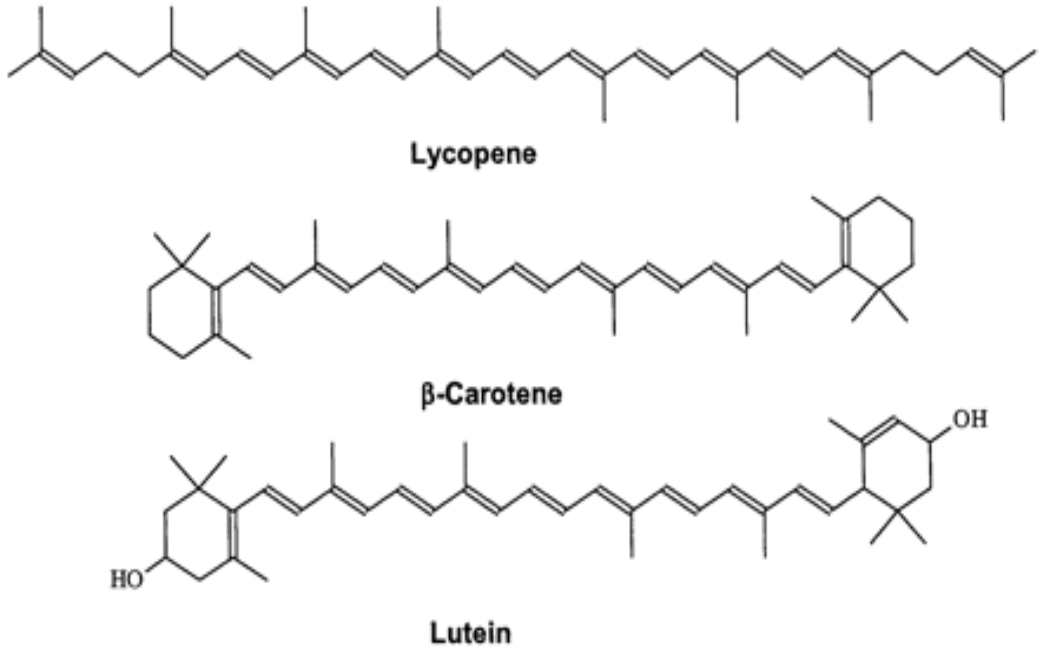


Figure 1.5 Structures of major carotenoids in tomatoes.

1.12 Tomatoes as a Source of Mineral

Significant influence on K, Ca, and Na or Mg contents in tomato fruits depends on the growing method and cultivar. Organic tomatoes contain significantly greater concentrations of minerals [47]. We found higher concentrations of P, K, Ca and Mg in organic tomatoes where as in conventionally grown tomato contain greater concentrations of Zn, Fe and Cu [48].

Potassium concentrations were similar to those (191.42–236.54 mg 100 gm-1) [49]. Calcium concentrations (15.97– 23.13 mg 100 g^{-1}) were higher in the reported literature [50]. Significantly greater concentrations of Ca and Mg in organic tomatoes also represented [44]. Magnesium concentrations in organic (17.36-22.22 mg 100 g^{-1}) and conventional tomato (18.75-19.16 mg 100 g^{-1}) were higher than those found (10.30–11.88 mg 100 g^{-1}) [50], but similar to those found in a comparable study [49].

1.13. Toxicity Studies

Lycopene is generally considered safe, non toxic and consumption is usually without known side effects. Excessive carotenoid intake have been reported in a middle aged woman who had prolonged and excessive consumption of tomato juice, her skin and liver were coloured orange-yellow and she had elevated levels of lycopene in her blood. After three weeks on a lycopene-free diet her skin colour returned to normal [51].

Part-B

1.14. General Introduction

Since the beginning of human civilization, medicinal plants have been used by man kind for its therapeutic value. Nature has been a source of medicinal agent for thousands of years and an impressive number of modern drugs have been isolated from natural sources. In the last few decades, natural fruits, fruit extract and seeds have received much attention as sources of bioactive substances such as antioxidants, antimutagens, antimicrobial and anticarcinogens [52; 53]. One of such natural source is litchi (*Litchi chinensis*).

The litchi is a seasonal food in our country which grows well in Bangladesh. Litchi fruit pericarp (LFP) accounts for approximately 15% by weight of the whole fresh fruit and contains significant amounts of sugar, vitamin-C, other vitamins, anthocyanin and poly phenolic compounds which are usually discarded as a waste in the process [55; 56], therefore litchi LFP tissues may be considered an important source of dietary flavonoids [54]. In addition, the peel and seed are rich in antioxidants such as ascorbic acid, phenolic compounds including gallic acid, flavonoids (procyanidin B4, procyanidin B2 and epicatechin), and anthocyanins (cyaniding 3-rutinoside, cyanidin-3-glucoside, quercetin 3-rutinoside and quercetin 3-glucoside)[103]. The phenolics of LFP have been confirmed to have antioxidant, anticancer [57], immunomodulatory [58] activities also comprised of significant amount of flavonoids. Thus, LFP tissues can be used as a readily accessible source of natural antioxidants and has been considered a new source of pharmaceuticals and food industry.

The fruit, recognized as a source of vitamins, minerals and fibre, is nutritionally important to our diet. In recent years, greater attention has been given to these foods, since evidence suggests that regular consumption of fruit is associated with reduced mortality and the morbidity of some chronic diseases [59; 60], as they contain in addition to nutrients, bioactive substances such as vitamins and secondary metabolites, which are capable of carrying out pharmacological activities.

Litchi is considered as one of the best fruits due to its high nutritive value, sweet-acidic taste, excellent aroma, and bright red colour of its peel. Medicinally fruit of litchi is tonic

to heart, brain, and liver; allays thirst; very whole some to the body. The flowers are used in form of decoction as gargle for throat infections. The seeds in Malay Peninsula are used by Chinese as anodyne and are prescribed in various neuralgic disorders and in orchitis [46].

Phenolic compounds have been accepted to be possible chemopreventive and treatment agents for cancer [85; 104; 106; 115]. Polyphenols are obtained mainly from plants, and some have been regarded as forming part of a healthy diet for many years, such as tea, soybean, pomegranate, and pine nuts [148].
Litchi seeds have been analyzed and were found to possess rich amounts of polyphenols and exhibit strong antioxidant and inflammatory activities [162; 190]. Recently, several studies by our research group and others have further revealed that litchi seed extract exhibits anti-cancer activity towards colorectal, liver, lung, and cervical cancer [162; 197].

Stigmasterol is a phytosterol well spread in plants and animals as well as fungi, and has structural similarity to cholesterol. The most important benefit for these secondary metabolites is their enrolment amongst the health promoting constituents of natural foods which contains them. In fact, the European Foods Safety Authority [61] recommends consuming about 1.5 - 2.4 g/day of phytosterols and/or stanols in order to reduce blood cholesterol. The antibacterial activities of stigmasterol hass been reported in many reports [62-64]. Research has indicated that stigmasterol may be useful in prevention of certain cancers, including ovarian, prostate, breast, and colon cancers. It also possesses potent antioxidant, hypoglycemic and thyroid inhibiting properties [65].
Stigmasterol has been investigated for its pharmacological prospects such as antiosteoarthritic, antihypercholestrolemic, cytotoxicity, antitumor, hypoglycaemic, antimutagenic, antioxidant, anti-inflammatory and CNS effects. The antibacterial activities of stigmasterol have been reported in many reports [62-64].

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases [198]. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections [203]. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by

pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity [219; 228].

The purpose of this study is to identify and characterize the bioactive principles from LFP. To the best of our knowledge, for the first time we have isolated Stigmasterol from LFP. A very negligible work on antioxidant and antimicrobial activities of *Litchi chinensis* LFP and seeds has so far been reported, so the present study is designed to evaluate the antioxidant and antimicrobial activity of LFP extract. The antimicrobial activity of isolated Stigmasterol and LFP extract using different solvents was evaluated by using agar diffusion method [216; 217] and the antioxidant activities were evaluated using Ferric (Fe^{3+}) reducing antioxidant power (FRAP) assay activity.

1.15 History of Litchi

Tropical Bangladesh is enriched with numerous kinds of fruits, plants, herbs and creepers and most of them have medicinal value. It contains a delicious red fruit, which started unofficial records over 2000 years back, first within the north exotic rain forests as well as mountain forests of Southern China, Malaysia, and northern Vietnam [66]. Another member of the sub-family 'Anshphal' (*Euphoria longana Lam.*) also grows in Bangladesh, mostly in backyards. It bears longan type but small sized fruits of less commercial value.

Litchi are extensively grown in China, and also elsewhere in Brazil, South-East Asia, Pakistan, Bangladesh, India, southern Japan, and more recently in California, Hawaii, Texas, Florida, the wetter areas of eastern Australia and sub-tropical regions of South Africa, Israel and also in the states of Sinaloa and San Luis Potosí (specifically, in La Huasteca) in Mexico. They require a warm subtropical to tropical climate that is cool but also frost-free or with only very slight winter frosts not below -4°C, and with high summer heat, rainfall, and humidity. Growth is best on well-drained, slightly acidic soils rich in organic matter.

Litchi production has a long and illustrious history having been praised and pictured in Chinese literature from the earliest known record in 1059 A.D. Cultivation spread over the years through neighboring areas of southeastern Asia and offshore islands. Late in the 17th Century, it was carried to Burma and, 100 years later, to India. It arrived in the West Indies in 1775, was being planted in greenhouses in England and France early in the 19th

Century, and Europeans took it to the East Indies. It reached Hawaii in 1873 and Florida in 1883, and was conveyed from Florida to California in 1897. In recent times, Australia has turned into a main manufacturer of litchies within Queensland as well as New South Wales. We may still find villages in Southern China today along with litchi trees which are over 1000 years old! There is certainly several proof to point out that wild litchi might have additionally originated from northern mountain parts of Vietnam.

No authentic documentation on the history of cultivation of litchi in Bangladesh is available. However, it is believed that litchi came from Burma to Bangladesh sometime in the early 19th century. Chinese varieties along with Indian cultivars like Mujaffarpuri and Bombai were introduced in the early 20th century from West Bengal through the efforts of nurserymen and plant lovers. Litchi is mainly cultivated in the backyard (2-3 plants), or in very small orchards (15-20 plants) adjacent to the homesteads. Litchi grows almost all over Bangladesh but the main areas of cultivation are Jessore, Rajshahi, Rangpur, Dinajpur, Khulna, and Dhaka, Kushtia, Sylhet and Chittagong districts.

1.16 Background

Litchi is botanically designated *Litchi chinensis* Sonn. *(Nephelium litchi* Cambess) and widely known as litchi and regionally as *lichi, lichee, laichi, leechee* or lychee, has over 2,000 species and 150 genera. Litchi is a highly priced, popular and major table fruit in Bangladesh. It comes to market in the months of May-June when the market is full of other fresh fruits, particularly mango and jackfruit. *Litchi chinensis* has been reported to have anti-inflammatory, antioxidant, antimicrobial and antidiabetic activities. Litchi has moderate amounts of polyphenols, which is higher than several other fruits analyzed, such as grapes and apples [67]. The regular consumption of fruits and vegetables has long been associated with the prevention of many chronic diseases, including cardiovascular disease and cancer, as evidenced by the results of clinical trials and epidemiological studies [68].

Litchi fruit is round or oval with a single seed in the middle of the fruit, juicy, sweet and slightly sour [69]. The fruit of litchi comprises a white translucent edible aril surrounded by a pericarp. The pericarp of litchi has been the focus of studies associated with fruit size, coloration, cracking and shelf life. Substantial changes in color are often evident during fruit maturation and can provide an important indication of maturity and quality in many fruit species [70]. As a tissue of litchi plant, litchi leaf has been found to be a good source of phenolics, including flavonoids and lignans, which exhibit diverse health benefits like antioxidation and anticancer effects. Litchi chinensis has generally received

much attention because of pulp and waste (including seeds and pericarp) of fruit for antibacterial, antioxidant activity, total flavonoid and total phenolic contents. In litchi a red color on the fruit surface is commercially desirable, although some litchi cultivars do not have a strong red coloration, mainly due to slow chlorophyll degradation in the pericarp. High concentrations of chlorophylls in the pericarp not only mask the red fruit surface color that is provided by anthocyanins, but also slow their biosynthesis [71]. Chlorophyll breakdown contributes to a stronger coloration during fruit ripening as the photosynthetic pigment absorbs much of the incident red light, thus reducing the degree of phytochrome control of anthocyanin biosynthesis [72].

Anthocyanins are examples of flavonoids, the biosynthetic pathways of which have been extensively studied not only because they result in the production of red, blue and black plant pigments, but also in the contexts of their diverse roles in UV protection and pathogen defense, as well as their nutritional value in the human diet [73]. The red pericarp color of litchi fruit is known to result from anthocyanin accumulation [74], and the pericarp also contains an abundance of phenolic compounds (51 to 102 g/ kg dry weight), which inhibit fat acid oxidation and act as free radical scavengers [75]. Chlorophyll degradation, anthocyanin accumulation, increase in membrane permeability, and cell wall disassembly have all been suggested to coincide with the onset of litchi maturation [76], but the specific factor(s) that triggers this important transition in litchi fruits is still unknown.

1.17 Botanical Features of Litchi

Figure 1.6 Fruits of *Litchi chinensis* Sonn.

Scientific classification

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Sapindales
Family	:	*Sapindaceae*
Genus	:	*Litchi* Sonn.
Species	:	*L. chinensis*

Binomial name

Litchi chinensis Sonn.

1.18 Description

Litchi chinensis is an evergreen tree; handsome, dense, round-topped, slow-growing that is frequently less than 10 m (33 ft) tall, sometimes reaching more than 28 m (92 ft). In present times, it is widely cultivated in most tropical countries of the world including Bangladesh. Litchi tree has been popular for thousands of years and combines oriental style with tropical beauty and offers one of the most delicious fruits that a gardener can harvest. Lush, dark green foliage adds great contrast to the litchi fruits that hang in

bunches like giant, rose colored grapes. The lychee tree starts giving yield from the age of 5 years and the yield increases until they are 20 to 30 years old. In Bangladesh 60-70 years old litchi trees were found to give satisfactory yield. At present the total area under lychee cultivation is about 4,800 hectares and total annual production is about 12,800 MT.

The litchi bears fleshy fruits that are up to 5 cm long and 4 cm wide approximatelyof 20 gm. The fruit has a soft jelly like, white, translucent pulp having a mildly sweet flavor and taste. The fruit is covered by a pink to dark red, rough textured, thin skin that can be peeled away easily. The flesh is white and soft and juices very easily. It contains a single dark brown seed with a smooth skin. The fresh fruit has a "delicate, whitish pulp" with a floral smell and a fragrant, sweet flavor. Since this perfume-like flavor is lost in the process of canning, the fruit is usually eaten fresh [77]. Fruit quality of Bombai, China-3 and Bedana varieties is the best among the available land races.

Fruits mature in 80–112 days, depending on climate, location, and cultivar. Fruit are highly variable, depending on the cultivar. They can be round, ovoid or heart-shaped, and from 2.0 to 3.5 cm in diameter. The skin can be smooth or rough with distinct protuberances, thick or thin, and pink-red, bright red or purple-red. The flesh or aril is an out growth of the outer cells of the seed coat (outer integument), and in good cultivars may comprise 80 percent of fruit weight. Many cultivars can be distinguished by their flavour and aroma. Some cultivars have a high proportion of aborted seeds and thus a high flesh recovery. They are popular in the market-place, especially in Asia. There are a few cultivars that produce nearly seedless fruit, although the fruit usually weigh less than 10 gm. The variety wise average yields of 15-20 years old good litchi trees are given below.

Table 1.3 Variety wise average yield/plants (nos) of different litchi

Sl. No.	Variety	Yield/plant(nos)
1.	Bombai	7000-8000 (nos)
2.	Madrajie	6000-7000 (nos)
3.	China-3	4000-5000 (nos)
4.	Bedana	2000-3000 (nos)

The recently released BARI Lichu-3 resembles China-3 in size, shape, quality and taste and the production of good variety litchi in Bangladesh is as good as in India, but the national average yield of litchi is far less.

1.19 Varieties

There are numerous varieties of litchi in our country. These are Rajshahi, Bombai, Madrajie, Mongalbari, Kadmi, Kalipuri. Muzaffarpuri, Bedana and China-3. The greatest mouth watering is really a variety known as 'Bedana' is considered the best variety but gives the poorest yield. Bombai is the oldest high yielding variety in the country growing in different areas of Bangladesh. Bedana and China-3, introduced in the 1950, are now cultivated successfully in different parts of Bangladesh. Bedana, China-3, and Rajshahi local contain more edible portion, with high quality pulp and the fruit size in these varieties is bigger with attractive skin colour.

The average yield per litchi plant is approximately 3,000 fruits. The variety is confined to the Dinajpur district, northwest Bangladesh, which falls in agro-ecological zone-1. Good quality litchi is produced in the northwest region of Bangladesh covering 16 districts, hill tracts consisting of three districts and in Jessore, Tangail and Dhaka. Bangladesh Agricultural Research Institute (BARI) was released three varieties such as BARI Lichu-1, BARI Lichu-2 and BARI Lichu-3 recently. Among the released varieties BARI Lichu-3 is considered the best in respect of fruit size, pulp, colour and yield. The variety closely resembles China-3 variety. If proper management practices like fertilizer and irrigation management are adopted, the yield of good quality litchi like Bedana and China-3 can be increased substantially.

1.20 Taxonomy

Litchi chinensis was described and named by French naturalist Pierre Sonnerat in his Voyage aux Indes orientales et à la Chine, fait depuis 1774 jusqu'à 1781 (1782). There are three subspecies, determined by flower arrangement, twig thickness, fruit, and number of stamens.

Litchi chinensis subsp. chinensis is the only commercialized lychee. It grows wild in southern China, Bangladesh northern Vietnam, and Cambodia. It has thin twigs, flowers typically have six stamens, fruit are smooth or with protuberances up to 2 mm.

Litchi chinensis subsp. philippinensis (Radlk.) Leenh. It is common in the wild in Philippines and Papua New Guinea and rarely cultivated. It has thin twigs, six to seven stamens, and long oval fruit with spiky protuberances up to 3 mm [78].

Litchi chinensis subsp. javensis. It is only known in cultivation, in Malaysia and Indonesia. It has thick twigs, flowers with seven to eleven stamens in sessile clusters, smooth fruit with protuberances up to 1 mm. [66; 79]

The best variety litchi is china-3 [80] China-3 litchi fruit is spherical. Seeds are smaller than any kind of litchi [81]. This fruit are available in Bangladesh However, in the northern Bogra, Rajshahi and Dinajpur [82] region are famous for litchi fruit. China-3 litchi is quite large in size, and the size of litchi is particularly popular for relish [83].

LITERATURE REVIEW

2.1 Introduction

Tomato is one of the most important local market vegetable in Bangladesh. The crop is mainly grown by small scale farmers in most arable areas in Bangladesh. Tomatoes have one of the highest known concentrations of natural lycopene and most people get much of the lycopene in their diet from tomato products. Lycopene is an important biological compound and has received great interest in the past decade because of its important role in preventing chronic diseases such as atherosclerosis, skin cancer and prostate cancer [84]. The human body cannot produce lycopene so it must be obtained from food sources [18].

Lycopene, the major carotenoid in tomato fruit, is a powerful antioxidant, anti-inflammatory and also has an antimicrobial property and has generated much attention because of the linkage between lycopene-rich diets and lower risks of certain cancers, heart disease, and age-related disease. Although most phenolic compound in tomato fruit have disappeared at maturity, fully ripened fruit contains modest quantities of quercitin-3-0-rutinoside, which like other flavonols is a powerful antioxidant associated with reduced cancer risk. [6].

Tomatoes contain a wide variety of antioxidants including vitamin E, ascorbic acid, carotenoids, flavonoids and phenolic compounds. The scientific name for the tomato is *Lycopersicon esculentum*, so its major antioxidant has been named lycopene. It is also one of the most abundant non-vitamin analogues present in human blood from food consumption [86]. Carotenoids are natural antioxidants which protect the cells of the body from the harmful effects of oxidation due to free radicals [87]**.** It is the most abundant carotenoid in tomatoes, followed by β -carotene, γ -carotene, phytoene and other minor carotenoids.

Carotenoids are a class of pigments ranging from yellow to red in color and are found ubiquitously in plants (both edible and non-edible) and photosynthetic microorganisms. They are the most widely distributed pigments in nature [88]. Additionally, the carotenoid biosynthetic pathway also produces abscisic acid, a critical plant hormone involved in plant growth and response to environmental stress [89]. Some carotenoids have provitamin A activity (e.g. β-carotene, α-carotene, and β-cryptoxanthin; those carotenoids with an unsubstituted β-ionone ring) while most do not (e.g. lycopene, lutein,

zeaxanthin). A relationship between carotenoids and cancer risk was first observed in 1981 [90].

The antioxidant capacity, together with provitaminic properties typical of other tomato carotenoids [12], has drawn attention towards this widely consumed fruit over many years. Tomatoes and tomato products contain many carotenoids, such as γ-carotene, neurosporene, phytofluene and phytoene. Numerous epidemiological studies have demonstrated a relationship between carotenoid intake and reduction in risk of developing degenerative diseases. There have been correlations between carotenoid levels and breast cancer [91], colorectal cancer [92, 93], prostate cancer [94] and lung cancer [95], just to mention a few.

Litchi (*Litchi chinensis* Sonn.), is considered as one of the best fruits due to its high nutritive value, sweet-acidic taste, excellent aroma, and bright red colour of its peel and is fast becoming popular throughout the world because of its attractive appearance and are favored by consumers for their delicious taste and attractive red pericarp [96]. Medicinally fruit of litchi is tonic to heart, brain, and liver; allays thirst; very whole some to the body. It is well known that phenolic compounds exist in both free and bound forms in plant cells, and that the free phenolic compounds are solvent extractable. In contrast, the bound phenolic compounds, which are covalently bound to the plant matrix, cannot be extracted into water or aqueous/organic solvents mixtures [97].

The pericarp, seed and pulp of litchi contain large quantities of phenolic and flavonoids compounds, which are potential sources of natural antioxidants. The health effects of substantially increasing fruit consumption have been associated with the beneficial antioxidant activity of phenolic compounds in the fruit. Litchi fruit pericarp (LFP) tissues and is comprised of significant amount of dietary flavonoids as well as steroid can be used as a readily accessible source of natural antioxidants [98].

It is from epidemiology that we have gained an understanding of the benefits of fruits and vegetables in the diet. There is consistent evidence in the literature that increased consumption of fruits and vegetables is protective against cancers of the stomach, esophagus, lung, oral cavity/pharynx, endometrium, pancreas and colon [99]. Carotenoids, glucosinolates, phenolics, polyphenols, phytoestrogens, sulfides and thiols are all categories of plant-based compounds believed to have beneficial phytonutrient effects [100].

The number of infections which are caused by multi drug resistant gram positive and gram negative pathogens and viruses are life threatening for human being. Infections

caused by these organisms pose a serious challenge to the scientific community and need for an effective therapy has lead for novel antimicrobial agents. The health effects of substantially increasing fruit consumption have been associated with the beneficial antioxidant activity of phenolic compounds in the litchi fruit [101]. The pericarp, seed and pulp of litchi contain large quantities of phenolic compounds, which are potential sources of natural antioxidants [102].

Recent work has been carried out, aiming to evaluate the nutritional potential of the by-products of litchi fruit, with initial results indicating that the peel and seed have high energy and nutritional potential [103]. In addition, the peel and seed are rich in antioxidants such as ascorbic acid, phenolic compounds including gallic acid, flavonoids (procyanidin B4, procyanidin B2and epicatechin), and anthocyanins (cyaniding 3-rutinoside, cyanidin-3-glucoside, quercetin 3-rutinoside and quercetin 3-glucoside). Pharmacological studies indicate that the by-products of the litchi have various effects including anti-inflammatory, anti-hyperlipidemic, anti-hyperglycemic, hepatic and cardioprotective, as well as having high antioxidant activity [59; 105].

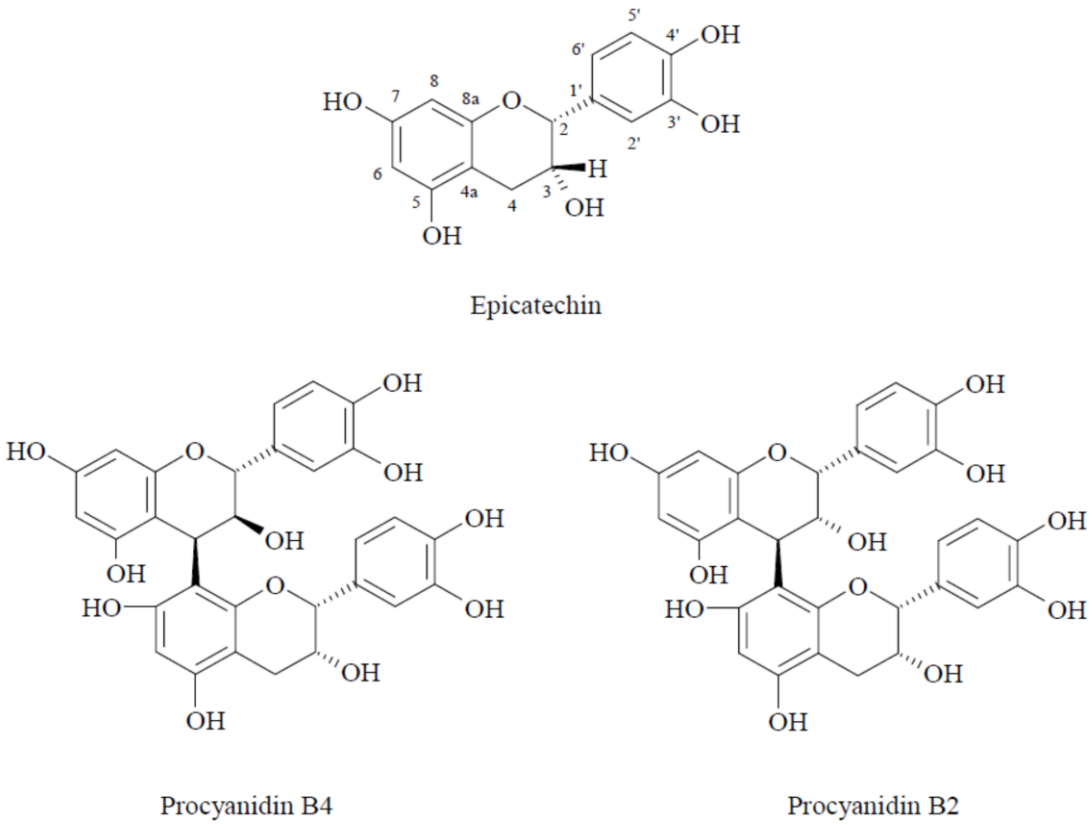


Figure 2.1 Three major flavonoids from pericarp tissues of Huaizhi cv. litchi [55]

2.2 Biological Functions of Anthocyanin and Flavonoid Pigments

Interest in the nutritional and antioxidant properties of tomatoes has traditionally focused on the carotenoid components lycopene and β-carotene, and antioxidant vitamins like vitamin C. Anthocyanins are an important phytonutrient and in red wine are thought to confer a health benefit. Anthocyanins have received particular attention because of very strong antioxidant activity exhibited by anthocyanin-rich fruits.

Flavonoids are present in plants as mixture and it is very rare to find only a single soavonoid component in a plant tissue because they are mixtures of different flavonoid classes. Flavonoid content in fruits and vegetable seems to be related to available N [107]. Plants with limited N accumulate more flavonoids than those that are well-supplied. The coloured anthocyanins in plants are almost invariably accompanied by colorless flavones or flavonols are recent research has established that the flavoner are important co-pigments of anthocyanin colour in floral tissues. Interestingly, yellow flavonoids and anthocyanins did not follow the pattern of total phenolics. For instance, the concentration of yellow flavonoids was 70% higher in organic fruits when compared to fruits from conventional growing system, but only at the harvesting stage, which is consistent with similar observations previously [108].

Anthocyanins belong to a wider class of phenolic compounds and are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium or flavylium salts [3; 6]. The differences between individual anthocyanins relate to the number of hydroxyl groups, the nature and number of sugars attached to the molecule, the position of this attachment and the nature and number of aliphatic or aromatic acids attached to the sugars in the molecule [111; 112]. Anthocyanins are an important phytonutrient [109] and in red wine are thought to confer a health benefit. Anthocyanins have received particular attention because of very strong antioxidant activity exhibited by anthocyanin-rich fruits. Grapes [110], blueberries, blackberries, raspberries and cherries [113] all these exhibit high antioxidant capacity in comparison to other fruits and vegetables.

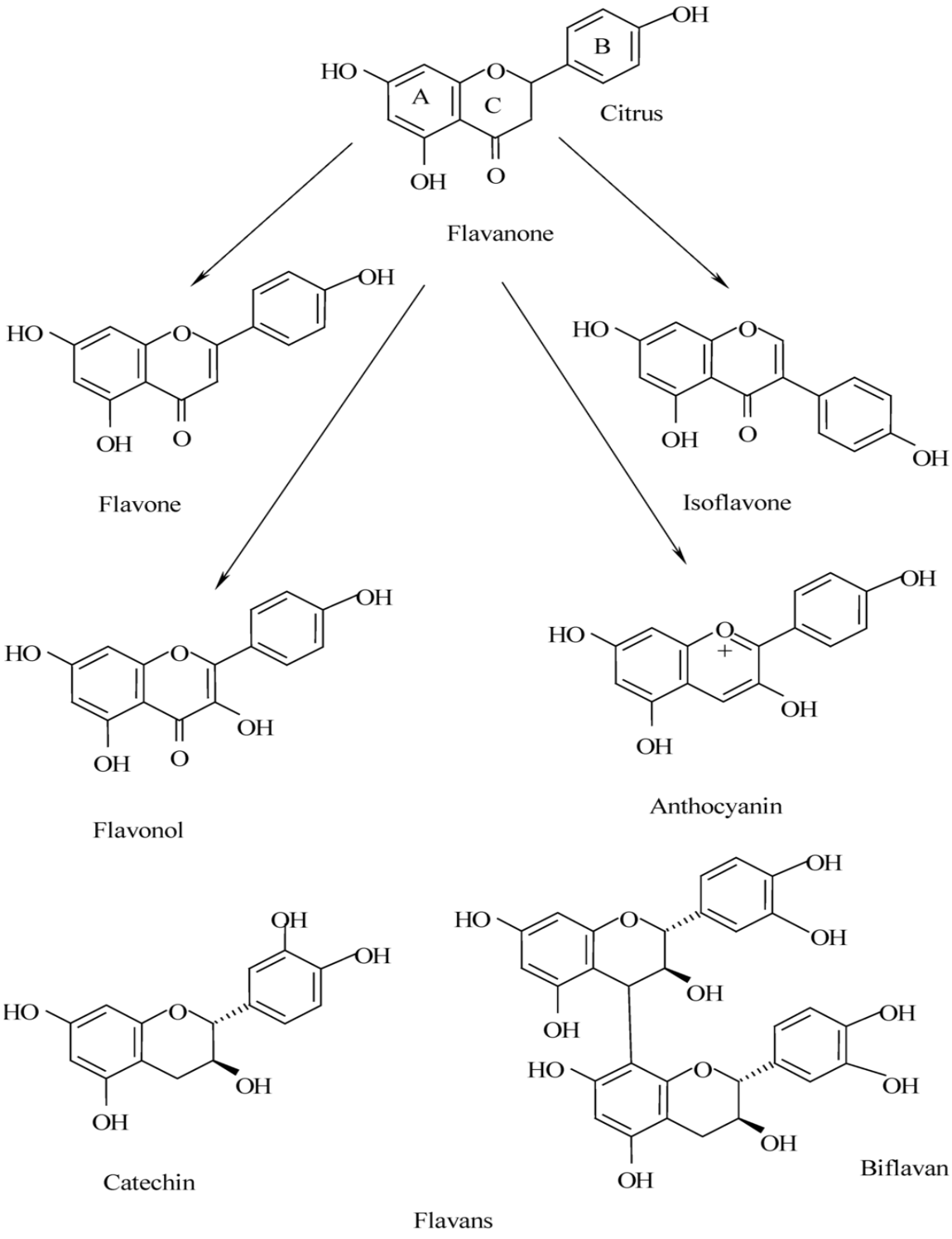


Figure1. Molecular structures of flavonoids adapted from Peterson and Dwyer (arrows indicate biosynthetic path) [3].

The concentration in anthocyanins was lower in the fruits from organic farming at all three stages of fruit development [97]. These discrepancies indicate that organic farming had the effect of modifying the levels of transcripts or the activities of enzymes

controlling intermediary steps of the biosynthetic pathway of phenolic compounds. Mixture of anthocyaunine is also the rule, particularly in the flowers of ornamental plants and any one flower tissue may contain up to ten different pigments. Classification of flavonoid type in a plant tissue is based initially on a steady of solubility properties and color reactions.

Table 2.1 Important classes of flavonoids and their biological significance [98]

class	number of known members	biological significance (so far as known)
anthocyanin(s)	250	red and blue pigments
chalcons	60	yellow pigments
aurones	20	yellow pigments
flavones	350	cream-coloured pigments of flowers
flavonols	350	feeding repellents (?) in leaves
dihydrochalcons	10	some taste bitter
proanthocyanidins	50	astringent substances
catechins	40	some have properties like those of tannins
biflavonoids ?	65	?
isoflavonoids	15	oestrogen effect, toxic for fungi

The basic structure of flavonoids is derived from the C_{15} body of flavone. They differ from other phenolic substances in the degree of oxidation of their central pyran ring and very fundamentally, also in their biological properties. While some classes (the flavonones, for example) are colourless, the members of other classes (the anthocyanes, for example) are always coloured and known as pigments of flowers or other plant parts. Anthocyanes are normally red or yellow; their colour is pH-dependent. Blue pigments are achieved by chelate formation with certain metal ions (Fe^{III} or Al^{III}, for example).

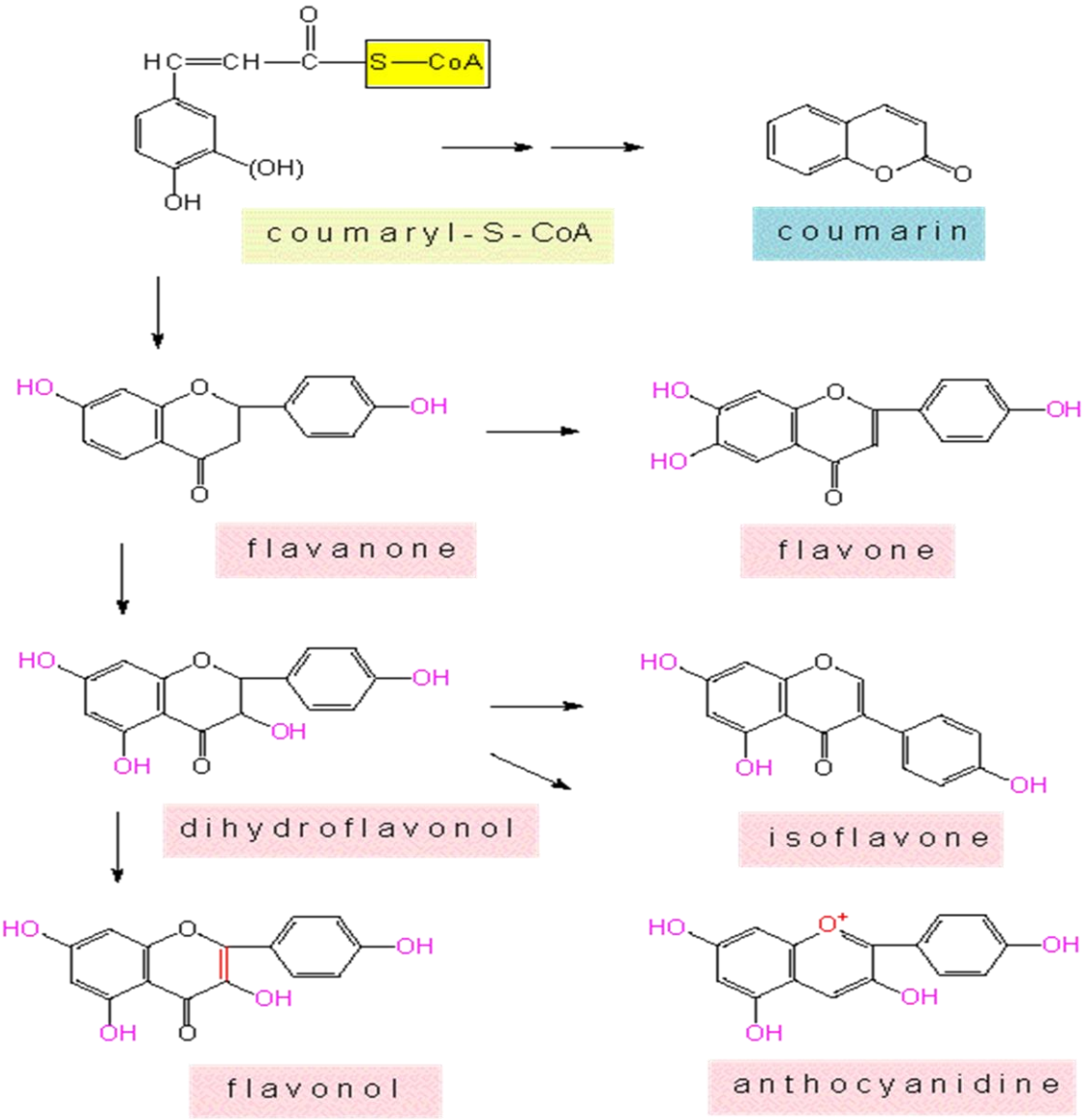


Figure 2.3 Isolation of flavonol and anthocyanidine

Plant tissue is hydsolysed with HCl (conc) .The cooled solution is extracted for UV spectrum .The five common substances- Apigenin , Lutelin, Kaempferol, Quercetin and Myricetin are readily separated and identification can be confirmed by spectral measurements.

Table 2.2 Spectral characteristic of main flavonoid classes

Principal maxima (nm)	Subsidiary maxima (with relative inten)(nm)	Indication
475-560	275 55%	Anthocyanins
390-430	240-270 32%	Aurones
365-390	240-260 30%	Chalcones
350-390	300 40%	Flavonols
250-270		Flavones and biflabonyls
330-350	Absent	Flavones and
250-270		Flavononols
275-290		Iso flavones
255-265	310-330 30%	
	310-330 25%	

2.3 Nutritional Composition of Tomatoes

2.3.1 Nutritional Value

Tomato is one of the most popular of vegetables, used as juice, soup, puree, ketchup or paste. In terms of human health, tomato fruit provide significant quantities of β-carotene, a provitamin A carotenoid and ascorbic acid [113]. These health protective effects have been widely attributed to the presence of key antioxidants such as lycopene, β -carotene, vitamin C, quercetin glycosides, naringenin chalcone and chlorogenic acid.

2.3.2 Sugars

Food is the source of energy. It's used as fruits and vegetable. Sugars and organic acids are important for the enzymatic determination of glucose, fructose, sucrose, L-malate and citrate in tomato. The micro plate assays were used to compare some compound available in tomato to study the compartmentation of sugar's and acids between pericarp and Locular tissue [114].

2.3.3 Fatty Acids

High performance liquid chromatography is a modern technique for the identification, separation and quantitative determination of a trace constituent in a mixture of compounds. This technique can also be applied for the analysis of fatty acids. Fatty acid

identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using CSW 1.7 software and expressed in relative percentage of each fatty acid.

2.3.4 Tocopherols

Tomato antioxidants include carotenoids such as β-carotene, a precursor of vitamin A, and mainly lycopene, which is largely responsible for the red color of the fruit, vitamins such as ascorbic acid and tocopherols, and phenolic compounds such as flavonoids and hydroxycinnamic acid derivatives [19; 116-119]. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response, using the internal standard method, and tocopherols contents were further expressed in mg per 100 g of dry fresh (fw).

2.3.5 Ascorbic acid

Vitamin-C of tomato fruits accounts for up to 40% of the recommended dietary allowance for human beings. Farm management skills combined with site-specific effects contribute to high vitamin-C levels, and the choice of variety significantly influences the content of ascorbic acid [120]. The variation in vitamin- C content in tomatoes depends mainly on environmental conditions. Exposure to light is a favorable factor for ascorbic acid accumulation [121; 122]. Therefore, it is important to compare organic and conventional foods that are planted and harvested during the same season of the year and that originate from regions with similar incidence of solar radiation. Ascorbic acid content in organically fertilized tomatoes ranges between 29% and 31% [107; 123], which is higher than the results obtained from tomatoes that were fertilized with mineral solutions. Similarly, ascorbic acid content in tomatoes cultivated with an organic substrate was higher than hydroponically cultivated tomatoes [124]. Many citations from literature confirm that tomatoes coming from organic cultivation procedures present higher vitamin-C content than fruits from conventional cultivation [125; 126]. It was also found that fertilizer that was rich in soluble nitrogen (N) could cause a decrease in the ascorbic acid content, probably for indirect reasons, since the nitrogen supply increased the plants' leaf density, which promoted shading over the fruits.

2.3.6 Carotenoids

The typical color changes during tomato ripening from green to red are associated with chlorophyll breakdown and the synthesis of carotenoid pigments due to the transformation of chloroplasts to chromoplasts [127]. Pigment synthesis in tomato is closely related to the initiation and progress to ripening and red color of the fruit results from the accumulation of lycopene [128], so that lycopene has been suggested as a good indicator of the level of ripening. Lycopene is considered the predominant carotenoid of tomato fruit (80-90%), followed by β-carotene (5-10%) [129]. Tomatoes grown organically contained substantial amounts of lycopene when ripened to firm red or soft red stages. About half of the total lycopene found in soft red tomatoes was present in pink tomatoes and 70 percent in light-red fruit. Fruit picked at unripe stages (breaker through light red) gained as much or more lycopene as those picked at the firm or soft red stages. Results indicate that fruit could be harvested well before full visible red color without loss of lycopene [130]. Tomatoes grown by the conventional or organic agricultural practices did not show any significant difference in the carotenoid content [123].Thus, the absence of any difference between the organic and conventional tomatoes could be due to the control over the ripening, transportation and storage conditions [131].

2.3.7 Phenolics

The levels of some phenolic compounds are known to be higher in organic fruit. Plants create phenolic compounds for many reasons, but a major reason is to make plant tissues less attractive to herbivores, insects and other predators. Accordingly, it is important to sort out if higher levels of phenolic compounds affect the taste of organic fruits and vegetables when compared to conventionally grown produce [132]. The organic growing system affects tomato quality parameters such as nutritional value and phenolic compound content. Interestingly, yellow flavonoids and anthocyanins did not follow the pattern of total phenolics. For instance, the concentration of yellow flavonoids was 70% higher in organic fruits when compared to fruits from conventional growing system, but only at the harvesting stage, which is consistent with similar observations previously [133]. Flavonoid content in tomatoes seems to be related to available N [107]. Plants with limited N accumulate more flavonoids than those that are well-supplied.

2.4 Biological Functions and Health Benefit of Tomato

Tomatoes are important not only because of the large amount consumed, but also because of their almost all kinds of health benefits and nutritional contributions to humans. One of the most well-known tomato eating benefit is its' Lycopene content. Lycopene is a vital anti-oxidant that helps in the fight against cancerous cell formation as well as other kinds of health complications and diseases. They are in fact; a highly versatile health product and protective effects of lycopene have been shown on oxidative stress, cardiovascular disease, hypertension, atherosclerosis, cancers, diabetes and others.

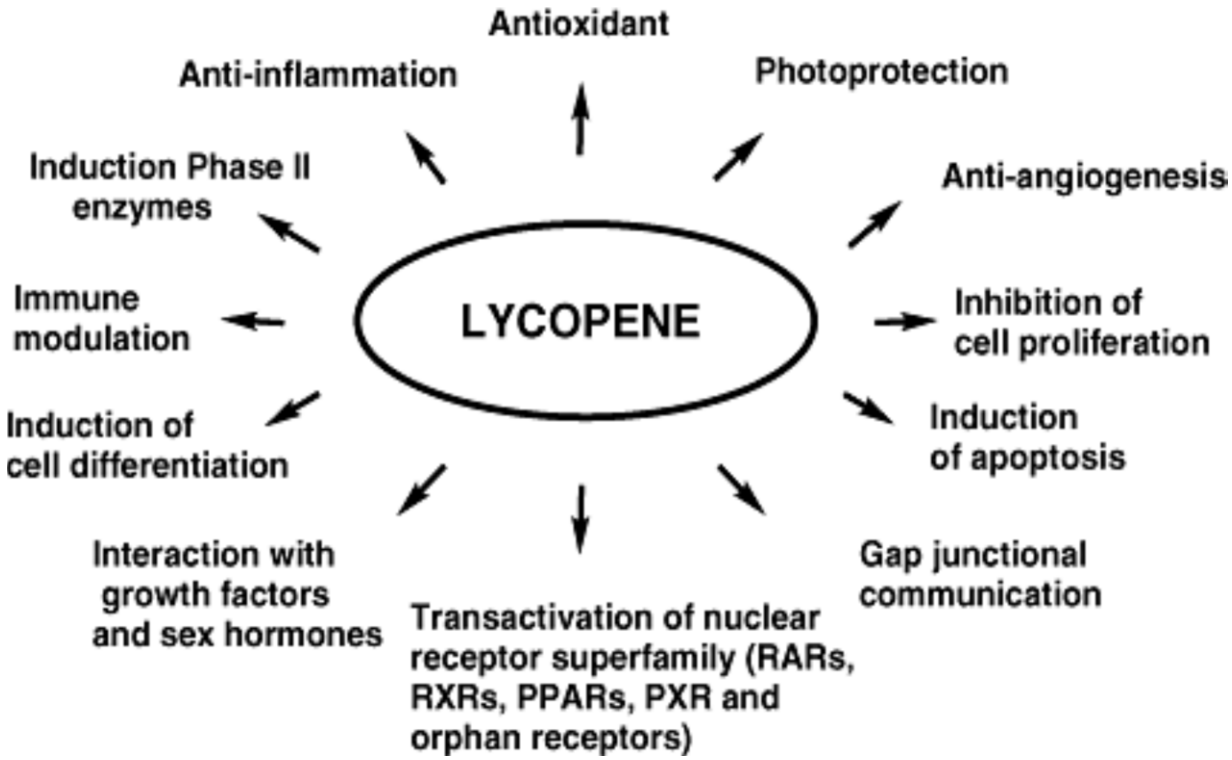


Figure 2.4 Biological functions of lycopene [134].

Lycopene is the acyclic 40 carbon carotenoid molecule that is the biosynthetic precursor to β -carotene is a strong absorber of visible light, with its long chain conjugated double bonds. Because of its acyclic form, lycopene has no vitamin A activity. There is more health benefits derived from eating a tomato given bellow:

2.4.1 Tomato Prevents Several Types of Cancer

Cancer has emerged as a major public health problem around the world. The beneficial effect of lycopene is a natural antioxidant that works effectively to slow the growth of

cancerous cells. The antioxidative properties of lycopene was significantly diminished the DNA damage in prostate tissues [135]. The protective effects were highest for more advanced or aggressive prostate cancer [136]. Protective effects were also achieved with increased consumption of lycopene-rich diet [135; 137].

2.4.2 Tomatoes Preventing Skin Damage

Carotenoids may play an impotent role in protection of the skin against oxidative damage. The sun emits ultraviolet (UV) radiation from approximately 100-400nm, which can be further divided into UVA (315-400 nm), UVB (250-315 nm and UVC (~100-280 nm) [138]. UVB light appears to be much more effective in producing cancer in animals compared to UVA light, while most UVC light is filtered out by the atmosphere [139]. Photooxidative damage can affect lipids proteins and DNA and is involved in skin aging, erythema, photodermatoses and skin cancer [140]. It is hypothesized that carotenoids act as photoprotectants [141], free radical quenchers [142] and antioxidants [143], all of which may play a role in protecting the skin against UV-induced damage. Tomatoes contain a high level of lycopene, which is used as a natural sunscreen and provide protection against UV rays.

2.4.3 Tomato Maintains Strong Bones

Tomatoes contain a considerable amount of calcium and Vitamin K. Both of these nutrients are essential in strengthening and performing minor repairs on the bones as well as the bone tissue. Vitamin K activates osteocalcin, the major non-collagen protein in bone. Osteocalcin mineralizes calcium molecules inside of the bone.

2.4.4 Antimicrobial Activity of Tomato

Tomatoes contain a variety of phytochemicals, including carotenoids like lycopene(highest concentration -85%), phytoene, phytofluene and the provitamin A, carotenoid (β-carotenoid), polyphenols including quercetin, kaempferol, naringenin, neutriants like folate vit-C, vit-E, vit-K vit-B, phosphorus,sulphur potassium calcium,iron (significant quantities),sugars like aldoses, ketoses, disaccharides, polysaccharides mainly starch, proteins and amino acids, enzyme polyphenol oxidase,phytosterol like cholesterol, sitosterol and small quantities of fats. All of these are known to contribute significantly to the antioxidant activity of tomato fruit [144, 145]. These health protective effects have been widely attributed to the presence of key

antioxidants such as lycopene, β-carotene, vitamin C, quercetin glycosides, naringenin chalcone and chlorogenic acid.

Lycopene, the major carotenoid in tomato fruit, is a powerful antimicrobial property and has generated much attention because of the linkage between lycopene-rich diets and lower risks of certain cancers, heart disease, and age-related disease. This because lycopene, the red pigment of tomato, is a tetraterpene assembled from eight isoprene units composed entirely of carbon and hydrogen, containing 11 conjugated and two nonconjugated carbon-carbon double bonds [146; 147] which mainly responsible for antibacterial and antifungal properties.

2.4.5 Antioxidant Activity of Tomato

Researchers have been interested for many years in the health benefits of carotenoids, particularly β-carotene as a result of its pro-vitamin A and antioxidant activity. Lycopene is one of the most potent antioxidants. Its singlet-oxygen-quenching ability is twice that of β-carotene and ten times higher than that of α-tocopherol [136].

Tomatoes contain a great deal of Vitamin A and Vitamin C. This is primarily because these vitamins and β -carotene work as antioxidants to neutralize harmful free radicals in the blood. As an antioxidant, it traps reactive oxygen species, increasing the overall antioxidant potential and reducing the oxidative damage to lipids (lipoproteins, membrane lipids), proteins (important enzymes) and DNA (genetic material), thereby lowering oxidative stress. This reduced oxidative stress leads to reduced risk of cancer, cardiovascular heart disease and other chronic diseases. Free radicals in the blood stream are dangerous because it may lead to cell damage. Remember, the redder the tomato you eat is, the more beta-carotene it contains. In addition, you also want to keep in mind that cooking destroys the Vitamin C, so for these benefits, the tomatoes need to be eaten raw.

2.4.6 Tomatoes reducing atherosclerosis, control cholesterol levels and heart disease

Because of the Vitamin B and potassium in tomatoes, they are effective in reducing cholesterol levels and lowering blood pressure. Lycopene may be helpful in people with high cholesterol, atherosclerosis or coronary heart disease, possibly due to its antioxidant properties. Lycopene prevents oxidation of low density lipoprotein (LDL) cholesterol and reduces the risk of arteries becoming thickened and blocked. Most published studies in this area used tomato juice as a treatment. Drinking two to three 8-oz (200 mL) glasses

of a processed tomato juice would provide more than the 40 mg lycopene per day recommended for reducing LDL cholesterol [149].

2.4.7 Tomato good for Hair

Tomato antioxidants include carotenoids such as β-carotene, a precursor of vitamin A, and mainly lycopene, vitamins, tocopherols, and phenolic compounds such as flavonoids and hydroxycinnamic acid derivatives. The Vitamin A in tomatoes works perfectly to keep your hair shiny and strong. In addition, it also does wonders for our eyes, skin, bones and teeth.

2.4.8 Tomato good for Kidneys

Tomatoes without seeds to our daily diet have been proven in some studies to reduce the risk of kidney stones. Lycopene accounts for 50% of the carotenoids found in human serum, with particularly high concentrations in the prostate gland, adrenal glands, skin, liver and kidneys.

2.4.9 Tomato Reducing Blindness

The Vitamin A found in tomatoes is fantastic for improving your vision. In addition, eating tomatoes is one of the best foods to eat to prevent the development of night blindness. 0.2 mg/kg b.w. daily *In vivo*–rats Cataract Significant delayed in the onset and progression of galactose cataract and reduced the incidence of selenite cataract [**150**]. Age-related macular degeneration (ARMD) is the most common form of blindness in elderly people in the Western world. Lycopene is the only micro-nutrient whose serum level is shown to be inversely related to the risk of ARMD.

2.4.10 Tomato good for Diabetics

Tomatoes are packed full of the valuable mineral known as chromium. It works effectively to help diabetics keep their blood sugar levels under better control. Lycopene is closely related to various metabolic complications, especially diabetes. Serum lycopene is inversely associated with type-2 diabetes and impaired glucose metabolism. The fact is proven by Coyne *et al.* [151] that plasma glucose and fasting insulin concentrations decreased significantly with increase in serum lycopene. Besides, Polidori *et al.* [152] found that plasma lycopene were significantly lower in very old diabetic

patients as compared to controls, while significant inverse correlations were found between age and lycopene.

2.4.11 Tomato Protects from Cell Damage

Tomatoes contain a wide variety of antioxidants including vitamin E, ascorbic acid, carotenoids, flavonoids and phenolic compounds. Carotenoids are natural antioxidants which protect the cells of the body from the harmful effects of oxidation due to free radicals [153]. Antioxidants travel through the body, neutralizing dangerous free radicals that otherwise damage cells and cell membranes.

2.4.12 Tomato Inhibiting Cancer Cells

Lycopene has a protective effect against stomach, colon, lung and skin cancers. Free radicals in the body can damage DNA and proteins in the cells and tissues, resulting in inflammation which may lead to cancer. Hence, the antioxidant properties of lycopene in eliminating free radicals may reduce the risk of cancer [154]. Research in breast, lung and endometrial cancers has shown that lycopene is even more effective than the other bright vegetable carotenoids, α- and β-carotene in delaying the cell cycle progression from one growth phase to the next, thus inhibiting growth of tumour cells.

Lycopene also plays a role in modulating intercellular communication by regulating irregular pathways that may be associated with cancer [155]. People with a higher intake of lycopene have been shown to have a reduced risk of developing cervical and breast cancer [149]. Multiple studies have investigated whether intake of tomatoes or tomato-based products helps prevent digestive tract cancers, including oral, pharyngeal, oesophageal, gastric, colon, and rectal cancer.

The function of carotenoids in humans may or may not be related to their antioxidant function. Lycopene has been shown to be the most effective singlet oxygen quencher *in vitro* [156]. Lycopene, carotenoids, and carotenoids in mixtures can also inhibit the formation of oxidation byproducts, like thiobarbituric acid-reactive substances [157]. However, there is some evidence that purified carotenoids given at supraphysiologic doses in people who already have high levels of oxidation (e.g. smokers) can be detrimental [158; 159]. *In vivo* studies have shown that a tomato rich diet can decrease LDL sensitivity to oxidative damage [21; 160-161]. In addition, serum thiobarbituric acid-reactive substances were decreased in serum of those supplemented with a lycopene rich tomato product for one week [163].

Carotenoids and lycopene may provide health benefits in their ability to modulate immune function. A recent study has shown that adding tomatoes to a high fat meal can modulate post-prandial induction of an inflammatory cascade [164]. A study of 106 overweight or obese women showed that serum levels of both IL-8 and TNF-α post-intervention and IL-6 was decreased in the obese cohort [165].

2.5 Biosynthesis of Carotenoid Pigments in Plants

There are over 600 carotenoids found in nature and they are generally colourful orange, red, and yellow pigments synthesized by photosynthetic plants, bacteria, and fungi in plants, carotenoids are synthesized in plastids via the 1-deoxy-D-xylulose-5-phosphate pathway rather than the mevalonic acid pathway of cholesterol biosynthesis. Tomatoes primarily contain carotenes with C_{40} structures with a series of conjugated double bonds. These double bonds confer electron delocalization, *cis-trans* isomerization, light absorption, and consequently pigmentation. The carotenoids present in plant foods occur in several different isomeric configurations. In plants, phytoene is generally synthesized as the 15-*cis* isomer and the intermediates leading to 7, 9, 7′, 9′-*tetracis*-lycopene (prolycopene) are present in different *cis* conformations. Prolycopene is then isomerized by an isomerase, CrtISO, to yield all-*trans*-LYC. The presence of other LYC isomers in tomatoes or tomato food products is thought to be derived from isomerization induced by heat, light, or chemical reactions.

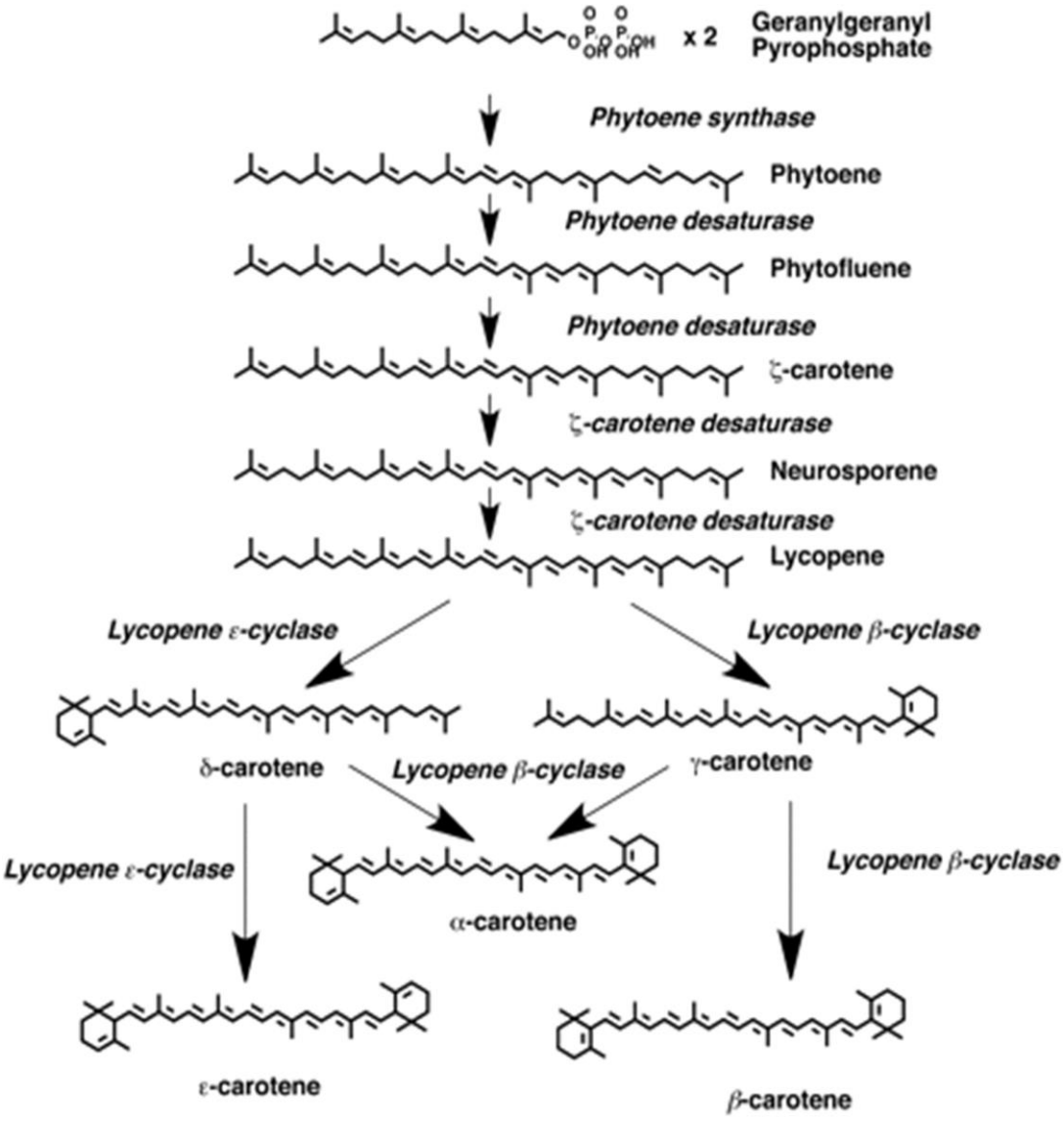


Figure 2.5 Carotenoid biosynthetic pathway found in plants [24].

2.6 Nutritional Composition of Litchi

Fresh whole litchi contains a total 72 mg of vitamin-c per 100 grams of fruit, an amount representing 86% of the Daily Value (DV) [166]. On average, about 9 litchis will provide the daily requirement of this vitamin to an adult. A cup of litchi fruit provides, among other minerals, 14% Daily Value (DV) of copper, 9% DV of phosphorus, and 6% DV of potassium (for a 2000-calorie diet). Like most plant-based foods, litchi is low in saturated fat and sodium and is cholesterol free. It provides many B-complex vitamins and is a good source of fiber as well as remnants of vitamins E and K.

For phenolic composition, flavan-3-ol monomers and dimers were the major compounds representing about 87% of total polyphenols that declined in content during storage or

browning [167]. Cyanidin-3-glucoside represented 92% of total anthocyanins [167] which also declined with storage or browning. Small amounts of malvidin-3-glucoside were also found.

It is a slow growing, medium-sized evergreen tree with dense, round-topped foliage, and smooth, gray, brittle trunk and branches. Litchis are not only eye-catching plant during spring when its huge spray of flowers adorns the tree but also a stunning sight for fruit lovers when its tree totally covered with beautiful berries.

Litchi has sweet taste, and fragrant flavor that everyone from children to elderly delighted to savor. Its outer skin is rough leathery rind or peel, featuring pink color. Its peel can be detached easily in the ripe berries. Inside, its flesh consists of edible portion or aril that is white, translucent, sweet, and juicy. Litchi is hot by constitution so do not consume excessively. It might lead to fainting spells or skin rashes.

It includes minerals which fall in between are usually potassium, phosphorous, magnesium, manganese, iron, selenium, calcium and zinc. Litchi fruit consists of polyphenols which have been discovered to assist battle weight problems as well as liver damage which can be brought on by Type 2 diabetes.

Table 2.3 Nutritional benefit of litchi per 100 g of litchi fruit

Nutrient	**Units**	**Value per 100 g**
Proximates		
Carbohydrate, by difference	g	16.53
Energy	kcal	66
Fiber, total dietary	g	1.3
Protein	g	0.83
Sugars, total	g	15.23
Total lipid (fat)	g	0.44
Water	g	81.76
Minerals		
Calcium, Ca	mg	5
Iron, Fe	mg	0.31
Magnesium, Mg	mg	10
Phosphorus, P	mg	31
Potassium, K	mg	171
Sodium, Na	mg	1
Zinc, Zn	mg	0.07
Vitamins		
Folate, DFE	mcg_DFE	14
Niacin	mg	0.603
Riboflavin	mg	0.065
Thiamin	mg	0.011
Vitamin B-6	mg	0.100
Vitamin C, total ascorbic acid	mg	71.5
Vitamin E (alpha-tocopherol)	mg	0.07
Vitamin K (phylloquinone)	µg	0.4
Lipids		
Fatty acids, total saturated	g	0.099
Fatty acids, total monounsaturated	g	0.120
Fatty acids, total polyunsaturated	g	0.132

2.7 Biological Functions and Health Benefits of Litchi

Litchi fruits are low in calories, contains no saturated fats or cholesterol, but rich in dietary fiber, which, can be very important for individuals who are concerned about their excess body weight. Litchi is loaded with vitamin C, providing 19% of the recommended daily value in one serving. Other nutritive ingredients in litchi include high levels of B vitamins, such as vitamin B6, as well as potassium (which helps help control heart rate and blood pressure and stave off strokes and heart disease), thiamin, niacin, folate, and copper (which produces red blood cells, maintains healthy bones, prevents thyroid problems, and anemia).

All these are vital for maintaining carbohydrate, protein, and fat metabolization. However, consume litchis in moderation because they contain fructose which may be harmful to your health in excessive amounts. Litchi fruit has lots of the anti-oxidant, Vitamin C as well as the important mineral Potassium. In addition to its fairly sweet as well as tangy taste, Litchi has substantial health advantages.

2.7.1 Oligonol

Oligonol, a trade marked ingredient from litchi fruit. Research studies suggest that oligonol, a low molecular weight polyphenol found abundantly in litchi fruit. Litchi contains oligonol, a short-chain polyphenol under preliminary evaluation for its potential biological properties [168; 169]. Oligonol has been found to have several anti-oxidant, anti-influenza virus actions. In addition, it helps improve blood flow in organs, reduce weight, and protect skin from harmful UV rays. [170].

2.7.2 Prevents Cancer Cells Growth

Litchi consist of a lot more β-carotene as compared to carrots which act as anti-oxidant combat against most cancers.Several epidemiological studies report that β-carotene in delaying the cell cycle progression from one growth phase to the next, thus inhibiting growth of tumor cells and reduced risk of cancer. Litchi is used as natural cancer treatment by preventing the development of cancer cells as retain the flavones quercetin as well as kaempferol as effective substances in cutting the expansion of cancer cells.

2.7.3 Promoted Heart Health & Lower the Risk of Heart Disease

Litchi fruit has got the second-highest degree of polyphenols, nearly 15% a lot more polyphenols as compared to grapes which usually promoted heart health, of all fruits examined. Cardiovascular disease signifies just like cardiac illness although not coronary

disease. Coronary disease is the term for problems of the blood vessels as well as heart, whilst cardiovascular disease is the term for only the heart. The anti-oxidant offers numerous health advantages, which includes improving immunity, slowing down the advancement of cataracts, as well as blocking cardiovascular disease as well as most cancers possibly due to its antioxidant properties.

2.7.4 Reduces Colds and Sore Throat and Boost up the Immune System

Litchi, like citrus fruits, is an excellent source of **vitamin-C** which includes 40% a lot more vitamin-C as compared to orange juice; 100 g fresh fruits provide 71.5 mg or 119% of daily-recommended value. Studies suggest that consumption of fruits rich in vitamin-C helps human body develop resistance against infectious agents and scavenge harmful, pro-inflammatory free radicals. Foods full of vitamin-C assist to increase the human body's immunity. They assist the body to build up a greater resistance to bacterial infections. If our immune system is vulnerable then we have problems with the various types of illnesses just like cold flu, a sore throat and so on. Frequent usage of l litchi fruit can easily avoid regular common colds, flu virus as well as a sore throat. It may also help to ease inflammation and pain.

2.7.5 Control Excessive Body Weight

Fruits supply the important vitamins which the body requires. There are numerous fruits which can be consumed to help you shed weight. It has no saturated fats or cholesterol, but composes of good amounts of dietary fiber, vitamins, and antioxidants. Litchi also carries a very good amount of minerals like potassium and copper. Consuming fruits which contain a great deal of water will fill up our stomach, decreasing the requirement for higher calorie food can be quite essential for people who are worried about their excessive body weight.

2.7.6 Beneficial for Gastrointestinal Tract

Litchi fresh fruit is astringent and is utilized for intestinal tract problems and also to be free from the body of intestinal worms. Ingested in moderate amounts, the litchi is said to relieve coughing and to have a beneficial effect on gastralgia, tumors and enlargements of the glands. Litchi flesh is an excellent antacid which deal with higher acidity, nausea as well as dyspepsia.

2.7.7 Contain High Copper and Potassium

Litchi also carries a very good amount of minerals like potassium and copper. Potassium is an important component of cell and body fluids help control heart rate and blood pressure; thus, it offers protection against stroke and coronary heart diseases. Copper is required in the production of RBC (Red Blood Cells).

2.7.8 Prevents Constipation

Litchi contain soluble fiber, they are able to assist with digestion and stop bowel problems and they assist in keeping the stomach free from toxic compounds which help clean the colon. In case of young kids, bowel problems might result from becoming scared or even slowing down a bowel movement as soon as the desire is felt, which could in addition appear in grown-ups.

2.7.9 Reduces Anemia

Litchi contains minerals like potassium and copper. Copper is required in the production of red blood cells. Litchi is definitely the abundant supply of copper that is significantly required for the development of RBC (Red Blood Cells). Anemia is regarded as the typical problem of the blood. Because of lack of nutrition a serious anemia is additionally brought on by malaria in several areas of Africa and other developing countries, particularly in very poor locations.

2.7.10 Provide Anti-Stress Nutrition

Litchi is a very good source of B-complex vitamins such as thiamin, niacin, and folates. These vitamins are essential since they function by acting as co-factors to help the body metabolize carbohydrates, protein, and fats. A single serving of lychee can easily provide an abundant supply of vitamin B6, one of the so-called anti-stress nutritional vitamins.

2.7.11 Provide Minerals for Healthy Bones

Litchi carries a very good amount of minerals like potassium, copper and phosphorus. Litchi fruits contain abundant with copper along with zinc, copper boosts the effectiveness of Vitamin D, which usually increases the assimilation of calcium. Litchi can also be a wealthy source of phosphorus as well as magnesium which will help to support powerful bones, as well as the trace minerals copper as well as manganese.

2.8 Sterols

Sterols are one group of steroids that have been isolated from all major groups of living organisms which are lipids having common erhydrocyclopentanophenanthrene skeleton (Figure 2.6). Compounds which produce Diel's hydrocarbon on selenium dehydrogenation (3/-methyl-1,2-cyclopentenophen anthrene) are known as steroids.

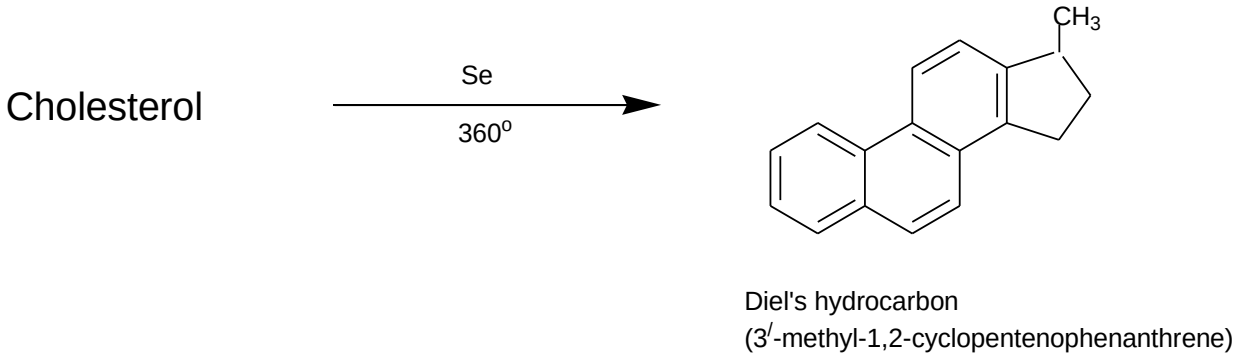


Figure 2.6 Diel's hydrocarbon on selenium dehydrogenation of sterol

A steroid is an organic compound with four rings arranged in a specific configuration. Examples include the dietary lipid cholesterol, the sex hormones estradiol and testosterone [172; 176-179] and the anti-inflammatory drug dexamethasone [173] The occurrence and biosynthesis of steroids in animals have been thoroughly investigated and are well understood that in higher animals cholesterol (Fig. 2.8) is the major sterol while cholesterol or mixtures of 27-, 28-, or 29-carbon sterols occur in lower animals [171]. The 28- and 29-carbon sterols of lower animals appear to be derived from their diet [171; 173]. The majority of the phytosterols possess an alkyl side chain at C-24 and one or more double bonds in a ring or the side chain. In higher plants~-sitosterol is the major sterol in most species investigated and is often found together with varying amounts of stigmasterol and campesterol (Figure 2.8).

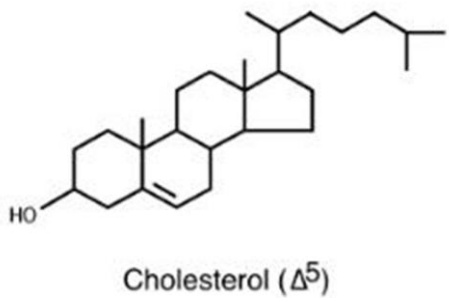


Figure 2.7 Chemical structure of cholesterol

Cholesterol is characterized by a double bond at position [174], and no alkyl group on the side chain. At least 100 different sterols have been identified in plants (e.g. sitosterol, stigmasterol and spinasterol) Figure 2.8.

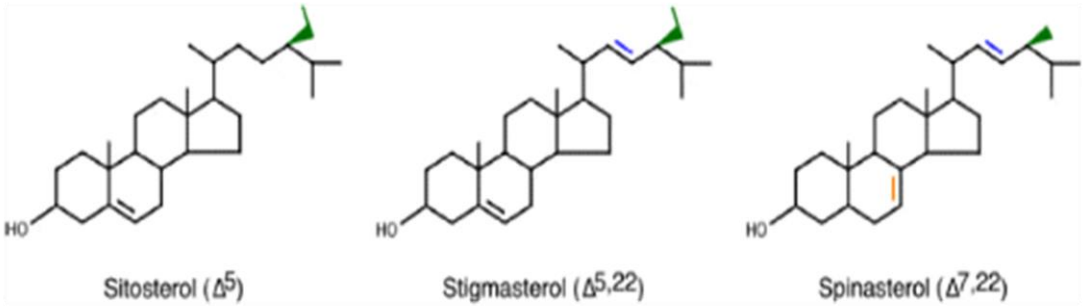


Figure 2.8 Chemical structure different sterols

2.8.1 Chemistry of Stigmasterol

The chemical name of stigmasterol is, (3S, 8S, 9S, 10R, 13R, 14S, 17R)-17-[(E, 2R, 5S)-5-ethyl-6-methylhept-3-en-2-yl]-10, 13-dimethyl-2, 3, 4, 7, 8, 9, 11, 12, 14, 15, 16, 17-dodecahydro-1Hcyclopenta[a]phenanthren-3-ol. Stigmasterol has been isolated and its presence was confirmed by Salkowski and Liebermann Burchard reaction [176] and structure of stigmasterol was elucidated by IR and NMR. As it is non-polar in nature, it is isolated from various parts of the plants by extracting with solvents which are higher in the ellutropic series i.e., non-polar solvents only.

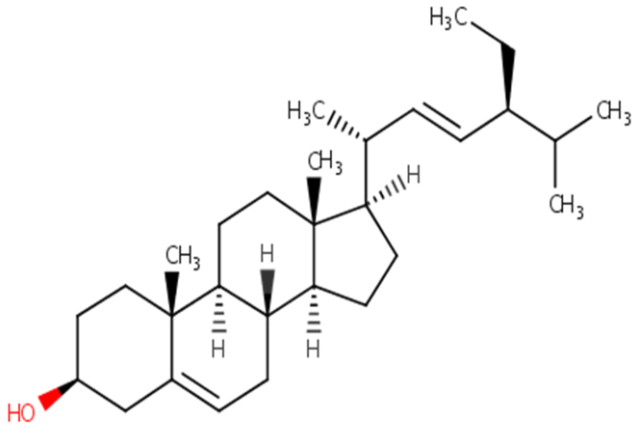


Figure 2.9 Structure of Stigmasterol ($C_{29}H_{48}$)

It has been found in the petroleum ether extract of aerial parts of Ageratum conyzoides (Asteraceae) [177], Calotropis gigantean [178], root and aerial part of Desmodium gangeticum [179], seeds of Terminalia chebula [185], petroleum ether extract of aerial parts of Byrophyllum pinnatum [181], petroleum ether extract of woody stem of Abelmoschus manihot [182], hexane extract of leaves of Pandanus amaryllifolius [183].

2.8.2 Biosynthesis of Stigmasterol

Biosynthesis of phytosterol has so far been reported to be mainly from the classical mevalonate pathway, i.e. the biosynthesis of phytosterols via the novel deoxy-xylulose pathway [175]. Diagrammatic representation of the pathway is shown in figure 2.9.

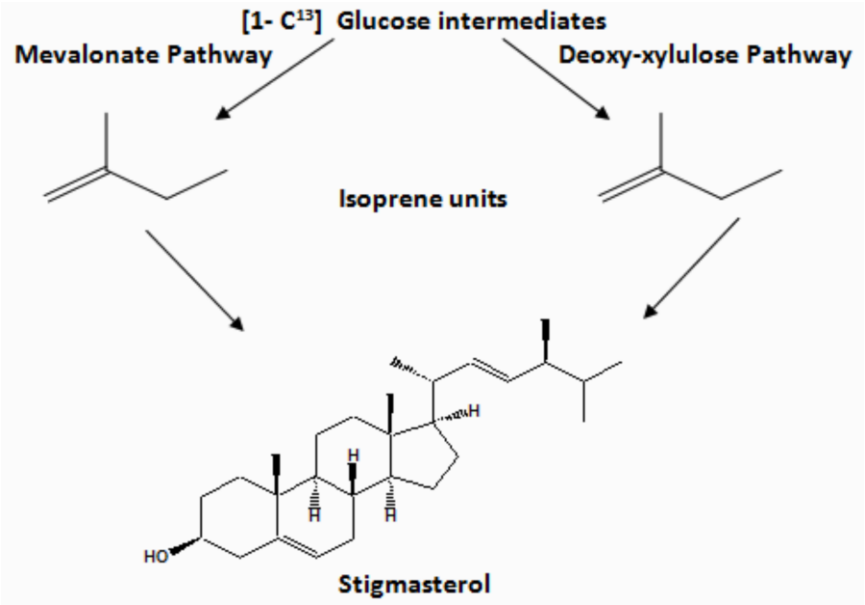


Figure 2.10 Diagrammatic representation of biosynthetic pathway of Stigmasterol in *croton sublyratus*

2.8.3 Biological functions of Stigmasterol

2.8.3.1 Anti-hypercholestrolemic Activity

It was found that stigmasterol has significant effect on serum cholesterol comparable with the antihypercholestrolemic activity of β-sitosterol. So, it is clear that saturation of the side chain, at least at C-22 is important for antihypercholestrolemic activity [184]. Stigmasterol also found to compete with cholesterol for intestinal absorption and thus lower the plasma concentration of cholesterol. It was reported to inhibit cholesterol biosynthesis via inhibition of sterol Δ24-reductase in human Caco-2 and HL-60 cell lines thus suppressing hepatic cholesterol [185].

2.8.3.2 Cytotoxicity

Stigmasterol was found to be Cytotoxic to *Spodoptera litura* cells and its action was more marked in comparison to the other active constituents of the plant namely, friedelin and rotenone [186] Stigmasterol cytostatic activity against Hep-2 and McCoy cells was determined and showed high degree of inhibition when compared with 6-Mercaptopurine against both cultures [187]

2.8.3.3 Anti-tumor

Stigmasterol which markedly inhibited the tumor promotion in the two-stage carcinogenesis experiments [188] Stigmasterol was found to inhibit the lyase activity of DNA polymerase β and also potentiate the inhibitory effect of the anti-cancer drug bleomycin in cultured A549 cells. These actions were a result of an inhibition of DNA repair synthesis [189]

2.8.3.4 Antioxidant Activity

Stigmasterol present in LFP showed decrease in hepatic lipid peroxidation and increase in the activities of catalase, superoxide dismutase and glutathione thereby suggesting its antioxidant property [65].

2.8.3.5 Antimicrobial Activity

Among the several phytochemicals, Stigmasterol is mainly known for its anti-diabetic and anti-cancer and several other medicinal activities. It is an important nutrient in the diet meal, hydrophobic and soluble in organic solvents and considered as a good biomarker due to its biological activity as well as antimicrobial activity.

2.8.3.6 Anti-inflammatory Activity

Pet ether of LFP was found to contain stigmasterol and found to reduce carrageenan induced paw oedema and also inhibited ear oedema induced by 12-O-tetradecanoylphorbol acetate (TPA) after topical application [191], by single and multiple application of phlogistic agent and was found to reduce oedema. It also exerts a significant topical anti-inflammatory action [192].

2.8.3.7 CNS activities

The petroleum ether extract of LFP on preparative TLC gave a fraction which upon IR study revealed that the compound has structural similarity with stigmasterol derivatives and this showed significant analgesic activity as it significantly reduced the number of writhes and stretches induced in mice by 1.2% acetic acid solution. Pretreatment with these fractions caused substantial protection against strychnine- and leptazol- induced convulsions [193].

METHODOLOGY

3.1 Methods of Phytochemical Screening

The phytochemical analysis is the detection, isolation, characterization and identification of the chemical components or ingredients present in the fruits and natural products are of diverse and varied nature. They usually include simple hydrocarbons and other classes of compounds to accomplish this task by different methods. So there are a large number of different physiochemical methods and physiochemical techniques have to be employed to study of them. Some of them are given below:

3.1.1 General Methods

The chemical investigation of a plants, vegetables and fruits can be divided as per following major steps:

a. Collection and proper identification, and preservation of plant materials
b. Preparation of the plant sample
c. Extraction processes
d. Fractionation and isolation of compounds
e. Structure characterization of purified compounds

3.1.2 Collection and Preservation of the Plant Sample

At first with the help of a comprehensive literature review a plant was selected for investigation and then the whole plant/plant part(s) was collected from an authentic source. Tomato (*Lycopersicon esculentum* Mill.) and Litchi (*Litchi chinensis* Sonn.) were collected from Mirpur-12 bazaar, in the month of April-May 2015 near Mirpur Cantonment, Dhaka. The samples may contain soil, dust, dirt etc. So the sample was washed with running water to remove these particles. These collected samples were kept in a poly bag and stored in cool, dark and dry place before experimentation.

3.1.3 Chemicals and Solvents

Analytical grade chemicals and solvents were used in these experiments. These were either meant for laboratory use or analytical reagent grade, n-Hexane, DCM, Pet-Ether,

ethyl acetate commercial ethanol (absolute alcohol), Chloroform, methanol was produced from Care & co, Darshana. Few other solvents and reagents used in these experiments were purchased from E.Merk (Germany), BDH (England) and E.Merk (India).

3.1.4 Distillation of the Solvents

The commercial grade solvents (petrol, ethyl acetate, chloroform and methanol) were distilled in a glass distillation set before using in the experimentation. Pet ether (b.p 40-60)^{0}C was obtained by distilling petrol. Distilled solvents were used through the investigation.

3.1.5 Evaporation

All evaporations were carried out under reduced pressure using a Buchi rotary evaporator at a bath temperature of 45^{0}C. The residual solvent in the extracts and compounds were removed under high vacuum.

3.1.6 Freeze-Drying

All freeze-drying were performed with a Varian 801 models LY-3-TT and HESTOSIC (Hetolab equipment Denmark) freeze-dryer. Organic solvents were completely removed by evaporation and using a drying pumps before freeze-drying. The aqueous extracts and fractions of the samples were first frozen in round-bottomed flasks in a methanol freezer (Hetofrig Cd5, Hetobirkero, Denmark) at -30^0 to -40^{0}C and finally the materials were subjected to freeze-drying operation.

3.1.7 Oven

All glass apparatus were dried and anhydrous sodium sulphate was stored inside an oven.

3.1.8 Preparation of Extracts

Fresh sample is one of the necessary conditions for fruit analysis in various cases. The fruits and vegetables were collected in fresh condition and washed with water to remove mud and dust particles. The samples were cut into small pieces, dried under sun and then dried in an oven at reduced temperature in the oven at 40^{0}C. The dried samples were grind to powder by a grinder. The powder was then stored in air–tight container with marking for identification and kept in cool, dark and dry place for future use.

3.1.9 Extraction Procedures

For successful and reliable screening test the extract should be properly prepared before subjecting it to various qualitative chemical tests. Extraction is carried out in two ways:

- Cold extraction
- Hot extraction

3.1.9.1 Cold Extraction

In cold extraction the powdered plant materials is submerged in a suitable solvent or solvent systems in an air-tight flat bottomed container for several days, with occasional shaking and stirring. Whatever material is used the fruit constituents must be exhaustively extracted from the sample by a suitable solvent or solvent mixture. The major portion of the extractable compounds of the plant material will be dissolved in the solvent during this time and hence extracted as solution. One of the most important and fundamental considerations in designating procedure is the selection of a proper extracting solvent. The commonly used solvents for routine screening of constituents include.

a. Steam extract
b. Petroleum ether extract
c. Ethyl acetate extract
d. Methanol extract
e. The extracts were collected by different solvents and filtered

The filtrate then concentrated by a "Rotavapour" under reduced pressure.

3.1.9.2 Hot Extraction

In hot extraction the powdered material is successively extracted to exhaustion in a soxhlet at elevated temperature with several solvents of increasing polarity. The sample materials extracted in a soxhlet apparatus with pet ether (boiling point, 40^0-60^0C), then with dichloromethane (DCM), then ethyl acetate (EtOAc) and lastly with methanol (MeOH). All the extracts were filtered individually and then concentrated by Buchi rotary evaporator at 40^0-60^0C under reduced pressure.

3.1.10 Solvent-Solvent Partitioning of Crude Extract

The crude extract is diluted with sufficient amount of aqueous alcohol (90%) and then gently shaken in a separating funnel with almost equal volume of ethyl acetate which is immiscible with aqueous alcohol. The mixture is kept undisturbed for several hours for

separation of the organic layer from the aqueous phase. The crude extract mixture will be partitioned between the two phases depending on their affinity for the respective solvents. The organic layer is separated and this process is carried out thrice for maximum extraction of the samples. After separating of the organic phase, the aqueous phase thus obtained is successively extracted with other organic solvents, usually of the increasing polarity (such as carbon tetrachloride, DCM, chloroform, EtOAc and butanol etc). Finally, the entire fraction (organic phases as well as the aqueous phase) are collected separately and evaporated to dryness. These fractions are used for isolation of the compounds.

3.1.11 Detection

The ascending technique in glass jars or tanks were used to develop TLC plates. A sufficient amount of suitable solvent system was poured into glass jar or tank. It was covered by watch glass to achieve saturation. The solvent at the bottom of the tank must not be above the line of spot where the sample solution was applied to the plate. As the solvent rises upward, the plate becomes moisten, and then it was taken out and dried. The solvent front was allowed to travel well before the silica coated surface.

3.1.12 Visualization

The compounds on the TLC plates were examined by various methods such as:

- Spray Reagent
- Vanillin-Sulfuric acid
- Dragendirff's Reagent
- Ferric Chloride/EtOH

3.1.12.1 Spray Reagents

Different types of spray reagents were used depending upon the nature of the compounds expected to be present in the fractions or the crude extracts.

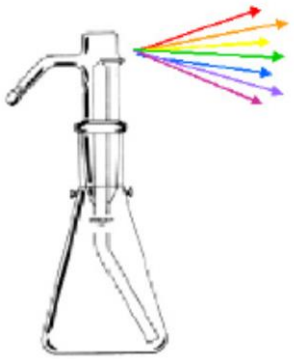


Figure 3.1 Vanillin–sulphuric acid Spray

3.1.12.2 Vanillin-Sulphuric Acid Reagent

The developed plates were sprayed with 1% vanillin in concentrated sulfuric reagent followed by heating the plates to 100^0C for 10 minutes. Steroids, terpenoids and lignin's etc. were visualized either as a violet or pink spot.

3.1.12.2.1 Dragendroff's Reagent

The presence of an alkaloid was detected by the appearance of an orange red spots on spraying the developed plate with Dragendroff's reagent.

3.1.12.2.2 Ferric Chloride/EtOH Reagent

Some of the phenolic compounds were detected by spraying the plates with ferric chloride (5% ferric chloride in absolute EtOH) reagent.

3.1.13 Preparation of the Reagents

3.1.13.1 Vanillin-Sulphuric Acid Reagent

400 mL sulfuric acid and 100 mL absolute alcohol were mixed in a beaker which was kept in an ice-bath after that 0.025 g vanillin was added to the mixture of alcohol and sulfuric acid. This cooled solution was used for spraying the plates.

3.1.13.2 Dragendroff's Reagent

- **SOLUTION-1:** 0.6 g of bismathnitrate was dissolved in 02 mL of water.
- **SOLUTION-2:** 0.6 g of potassium iodide was dissolved in 10 mL of water.

These two solutions were mixed with 22 mL of dilute HCl (7 mL of cone. HCl and 15 mL of water) and the mixture was diluted with 400 mL of water. The developed plates

were sprayed with dragendroff's reagent. Alkaloid was detected by the appearance of orange red spot.

3.1.14 Isolation of Compounds

Pure compounds were extracted from samples and then the fractionated extracts were isolated using different chromatographic and other techniques. A brief and general description of these is given below:

3.1.14.1 Chromatographic Techniques

Chromatography has been defined as primarily a separation process which is for the separation of essentially molecular mixtures. It depends upon the redistribution of molecules of the mixture between two or more phases. It is modern technique for the separation of extracted compounds (test sample) into individual pure ones. Various types of chromatographic techniques were used, such as Vacuum Liquid Chromatography (VLC), Column Chromatography (CC) and Thin Layer Chromatography (TLC).

3.1.14.1.1 Thin Layer Chromatography (TLC)

Ascending one-dimensional TLC technique was used for the initial screening of the extracts and column fractions and checking the purity of isolated compounds. For the latter purpose commercially available pre coated silica gel (kiesel gel 60 PF_{254}) plates were usually used. For initial screening, TLC plates were made on glass plates with silica gel (kiesel gel 60 PF_{254}).

A number of glass plates measuring 6 cm x 2 cm or 10 cm x 05 cm were thoroughly washed and dried in an oven. The dried plates were then swabbed with acetone-soaked cotton in order to remove any fatty residue. To make the slurry required amount of silica gel 60 PF_{254} and appropriate volume of distilled water (2 mL/gm of silica gel) were mixed in shaken. The slurry is then evenly distributed over the plates using TLC spreader. After air dried the coated plates are subjected to activation by heating in an oven at 110^0 C for 30 minutes [194]. Table- shows the amount of silica gel required for preparing plates of varying thickness. Two types TLC plates were used throughout the experiment:

- Precoated TLC plates: 0.2 mm thin coatings of silica gel on glass plates or aluminum sheets were used.
- Manually prepared silica gel coated glass plates were used.

Table 3.1 Amount of Silica Gel Required for Preparing TLC Plates of Various Thicknesses

Size (cm x cm)	Thickness (mm)	Amount of silica gel/plate (gm)
20 x 05	0.3	0.9
	0.4	1.2
	0.5	1.5

3.1.14.1.2 Preparation of Plates

TLC plates were prepared by spreading a film of an aqueous slurry (gel: water = 1: 2 w/v) of silica gel G-60 PF_{254} (E, Merck) over the entire surface of the glass plates (06 cm x 12 cm) by means of spreader. This thickness of the silica gel layer was 0.2 mm. The plates were dried in the air and finally activated by heating at 110^0C for an hour followed by cooling at room temperature for few hours.

3.1.14.1.3 Sample Application (Spotting the Plates)

The TLC plates were spotted with a small amount of crude extract by using a narrow glass capillary tube (two to three times). It was washed with either acetone or ethanol before each sample was applied.

3.1.14.1.4 Solvent Systems

The solvents of different polarity were used for TLC are given below:

- n-Hexane
- Pet-Ether
- Dichloromethane
- Ethyl acetate
- Chloroform
- Methanol
- Pet-Ether: Dichloromethane (in different ratio)
- Pet-Ether: Ethyl acetate (in different ratio)
- Dichloromethane: Methanol (in different ratio)
- Chloroform: Methanol (in different ratio)
- Butanol Saturated with water

3.1.14.1.5 Preparation of TLC Tank and Development of the Plates

The ascending technique was used in glass jars or beakers to develop TLC plates. A sufficient amount of suitable solvent system was poured into glass beaker. For saturation beaker was covered with a lid and kept undisturbed for a certain period. A filter paper was usually introduced in the beaker to promote the saturation process. Then the plate was placed in the beaker in such a way that it was inclined and the lower edge immersed in the selected mobile phase but the solvent level at the bottom of the beaker must not be above the line of the place where the sample solution was applied to the plate. As the solvent rises, the plate becomes moistened. When the solvent front moves almost near the end of the plats, the plate was taken out and dried [195]. The solvent front must not be allowed to travel beyond the end of the silica-coated surface.

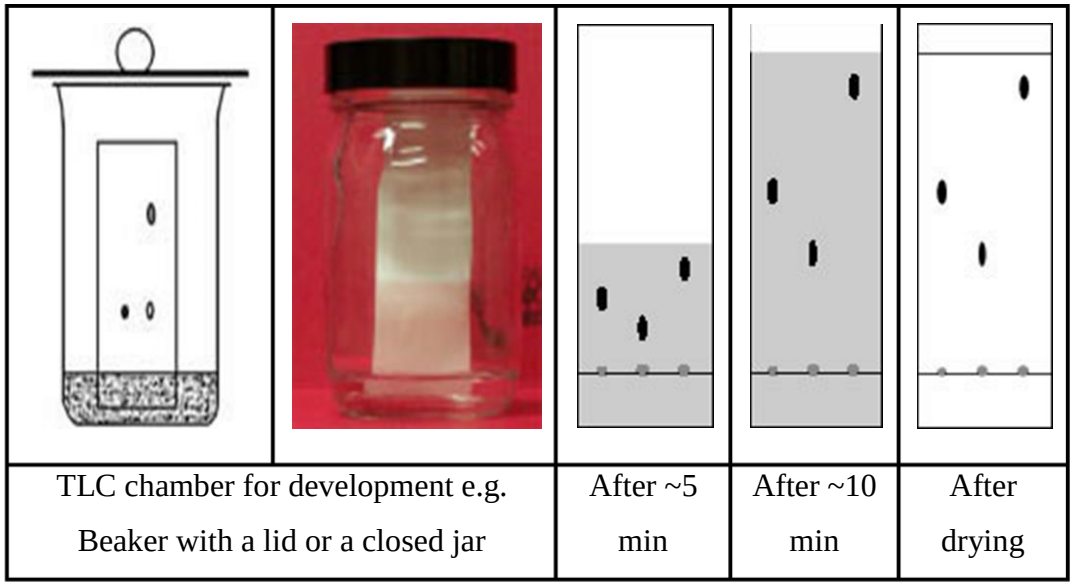


Figure 3.2 TLC Chamber developments of plates.

3.1.14.1.6 Detection of Spots

For the location of the separated components, the plates were examined using following methods:

- The plates were exposed to iodine vapor for several minutes.
- The plates were sprayed with vanillin-sulfuric acid regent (1.0%) followed by heating in an oven at 120^0 C for 15 minutes.

3.1.14.1.7 The R_f Value

Retardation factor (R_f) is the ratio of the distance between the distance travel by substance to the distance travel by the solvent.

$$R_F = \frac{\text{distance travel by a substance}}{\text{distance travel by a solvent}}$$

$$R_F = \frac{y}{x} \text{ [13]}$$

Usually, the R_f value is constant for any given compound and it corresponds to a physical property of that compound [195].

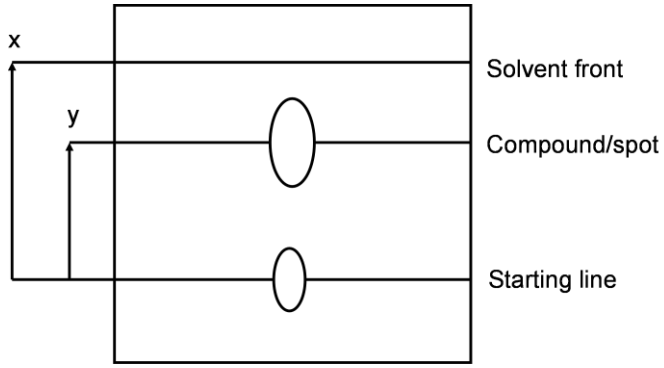


Figure 3.3 A Plate for the Calculation of R_f Value.

3.1.14.2 Vacuum Liquid Chromatography (VLC)

The concept of VLC is a relatively recent development in the field of chromatography separation. With a reduced pressure, the column is packed with fine TLC grade silica gel. These techniques were applied for the concept of the VLC is a relatively recent development in the field of chromatography separation [196]. It is a column chromatography under fractionating the crude extracts in order to obtain either a pure compound or mixture of less number of compounds.

A glass column of about 40 cm length having a system fitted with a water pump and collecting tube at the bottom was taken (Figure 3.4). Silica gel 60-GF_{254} was used as adsorbent. This slurry was packed into hard cake under an applied vacuum to give a column 3.15 cm in diameter and about 7.0 cm in height. The mixture, which was to be separated, was dissolved in a minimum volume of suitable solvent. The solution was then either adsorbed into small amount over the packing material in the column under a reduced pressure or over layered on the top of the packed column or applied as a solution with a pipette into the top of the column. Gradient elution of increasing polarity was

taken until the more polar components of the mixture were eluted. These elutes were collected manually in the fractions of different amounts and then the individuals was monitored by TLC. This chromatography technique requires fewer amounts of eluting solvent, minimum quantity of solid adsorbent and short time.

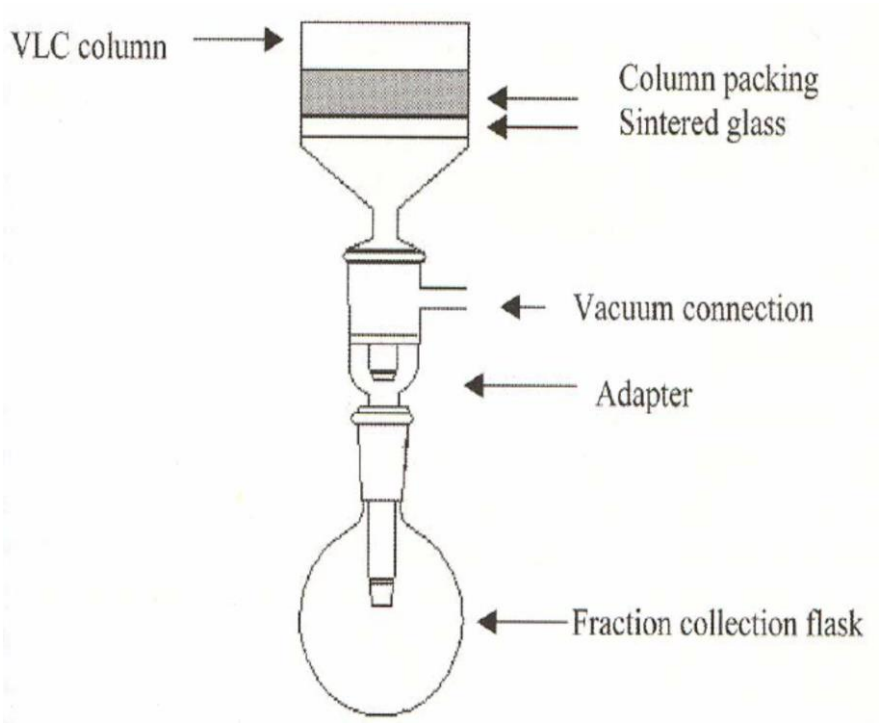


Figure 3.4 Vacuum Liquid Chromatography Techniques.

3.1.14.3 Column Chromatography

Column Chromatography is the most common separation technique based on the principle of distribution or partition or adsorption of compounds between a stationary and mobile phase. For normal phase column chromatography, silica gel of particle size (kiesel gel 60) 230-400 mesh from (Merck) was used and separation was performed by gravitational flow with solvents of increasing polarity. Slurry of silica gel in a suitable solvent is added into a glass column of appropriate height and diameter. When the desired height of adsorbent bed is obtained, a few hundred milliliter of solvent is run through the column for proper packing of the column.

After packing, the sample to be separated is applied as a concentrated solution in a suitable solvent or the sample is adsorbed onto silica gel (kiesel gel 60, mesh 230-400), allowed to dry and subsequently applied on top of the adsorbent layer. Then the column is eluted with suitable solvent mixtures of increasing polarity. Elutes are collected either in test tubes or in conical flasks.

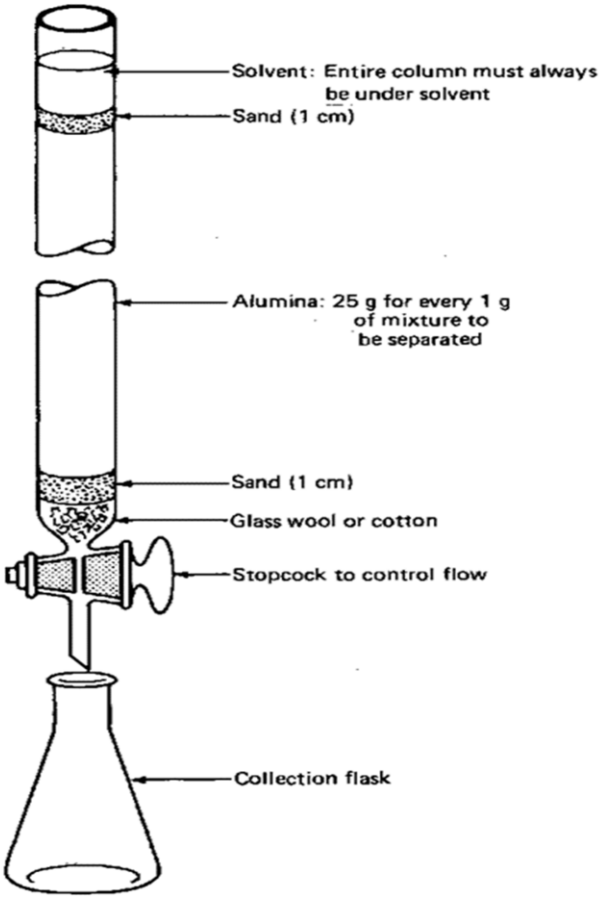


Figure 3.5 Various Parts of a Column.

3.1.14.3.1 Procedure for Micro Scale Flash Column Chromatography

In micro scale flash chromatography, the column does not need either a pinch clamp or a stopcock at the bottom of the column to control the flow, nor does it need air–pressure connections at the top of the column. Instead, the solvent flows very slowly through the column by gravity until we apply air pressure at the top of the column with an ordinary Pasteur pipet bulb.

3.1.14.3.2 Preparation of Column for Micro Scale Operation

A Pasteur pipet was plugged with a small amount of cotton to prevent the adsorbent from leaking. The Pasteur pipet was filled with the slurry of column grade silica gel with a stream of solvent using a dropper. It was ensured that the "sub column" is free from air bubbles by recycling the solvents several times. The samples were applied at the top of the column. Elution was started with petroleum ether followed by increasing polarity.

3.1.15 Solvent Treatment

Solvent treatment is a process by which a compound consisting of the major portion of a mixture of compounds can be purified utilizing selective solvent washing. Initially, a solvent or a solvent mixture in which the desired compound is practically insoluble and other components are soluble is chosen. The undesired components are separated with repeated washing with this solvent or solvent mixture. If required other solvent or solvent mixture can be used until a pure compound is obtained.

3.1.16 Crystallization

Crystallization was employed as a final purification process .The solvent in which the compound was dissolved in a minimum volume of solvent in hot condition and was left undisturbed for crystallization. Sometimes a mixture of solvents was also used. The compound was dissolved in a suitable solvent and then a solvent in which the compound was insoluble, was gradually until cloudiness developed in the solution.

3.1.17 Refluxing

For the esterification of fatty acid, a refluxing system was employed. The system consists of a condenser and a pear shaped flask being placed on a hot water bath.

3.1.18 Spectroscopic Techniques

3.1.18.1 Ultra-Violet Spectroscopy (UV-Vis)

Ultra-violet spectra were recorded using the Shimadzu UV- 160A spectrophotometer. A small amount of the samples of different compounds were dissolved in chloroform or pyridine separately. The solution was taken in quartz cell (01 cm x 01 cm) to record the spectrum. The main bands (λ max) were recorded as wavelength (nm).

3.1.18.2 Infra-Red Spectroscopy (IR)

A shimadzu IR-470 spectrophotometer was used for recording infra-red spectrum. Major bands (ν max) were recorded in wave number (cm^{-1}) and sample analyzed as KBr pellets.

3.1.18.3 Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectra of pure sample were recorded by ^{1}H-NMR (400MHZ) and ^{13}C-NMR spectrometer. The spectra were recorded using $CDCl_3$ with tetramethyl silane (TMS) as standard reference.

3.1.18.4 Melting Point Determination

Melting point (m.p) was determined by using an electro thermal melting point apparatus. Extra care was given for perfect determination of melting point.

Figure 3.6 Electro thermal melting point apparatus.

EXPERIMENTAL

4.1 Collection of Sample

The fruit *Lycopersicon esculentum* is known as tomato was collected from Mirpur-12 bazaar, in the month of April-May 2015 near Mirpur Cantonment, Dhaka.

4.2 Sample Preparation

The fruit may contain soil, dust, dirt etc. So the sample was washed with running water to remove these particles. These were cut into small pieces with knife and squeezed by hands to obtain aqueous juice. This juice was filtrated using cleaned thin cloth. The filtrate was extracted with ethyl acetate (EtOAc) in a separatory funnel and shaken several times and kept undisturbed for 20 minute. Two separate layers were formed. Upper layer EtOAc extract and the lower layer is aqueous part. Similarly the pulp is subsequently extracted using EtOAc and both the extracts were concentrated in vacuo. This procedure was repeated. The concentrated extract was examined by TLC (silica gel as stationary phase and toluene: hexane as mobile phase (1:19, v/v), NMR and UV-Vis spectrum.

The residue was left after extracted using EtOAc and was kept submerged in a conical flask with 50 mL aqueous methanol solution or 1 kg of tomato paste was kept submersed in 500 mL aqueous MeOH (80% MeOH and 20% H_2O) solution for 24 hours and filtered. The filtrate was concentrated to 100 mL and labeled **as Le-MeE**. This concentrated methanolic aqueous part was subjected to classical tests:

4.3 Classical Test of Aqueous Methanolic Extract of *Lycopersicon esculentum*

This extract was subjected to the classical tests for the detection of the presence or absence of the following:

- Unsaturation
- Carbonyl group
- Phenolic group
- Acetyl group
- Amino acid
- Sugar.

4.4 Classical Test of Aqueous Methanolic Extract (Le-MeE)

4.4.1 Test for Unsaturation

4.4.1.1 Baeyer's Test

The aqueous methanolic extract 10 mL was taken in a test tube to which dilute $KMnO_4$ solution was added. The disappearance of the purple colour of dilute $KMnO_4$ solution was observed which indicate the presence of unsaturation in the aqueous methanolic extract.

Sample + $KMnO_4$ $\rightarrow$ Colourless (+Ve) [9]

4.4.1.2 Br_2 –Water Test

Br_2- Water in CCl_4 was added to 10 mL sample solution .The red colour of Br_2-Water becomes fade. It indicates the presence of unsaturation in the aqueous methanolic extract.

Sample + Br_2 + H_2O + CCl_4 $\rightarrow$ Colourless (+Ve) [8]

4.4.2 Test for Carbonyl Group

The aqueous methanolic extract 10 mL was treated with a few mL of DNP (2, 4-dinitrophenylhydrazine) and yellow precipitate was formed. This test excluded the possibility of the presence of an aldehydic moiety or an aldehydic compound in the aqueous methanolic extract. A positive response to the DNP test and a positive response for unsaturation obviously indicate the presence of unsaturated molecule in the aqueous methanolic extract.

4.4.3 Test for Phenolic OH

The aqueous methanolic extract 10 mL was taken in a test tube to which 2 mL of freshly prepared ethanolic $FeCl_3$ solution was added and light violet color appeared. This test confirmed the presence of phenolic compound in the aqueous methanolic extract.

4.4.4 Test for Carboxylic Acid

The aqueous methanolic extract 10 mL was taken in a test tube and hot sodium bicarbonate ($NaHCO_3$) was added. The bubble was evolved. So carboxylic acid group is present.

4.4.5 Test for Acetyl Group

4.4.5.1 Iodoform Test

The sample solution 10 mL was taken in a test tube to which 2 mL of I_2 in KI solution was added and warmed in a hot water bath for 10 minutes then aqueous NaOH solution was added drop wise until the disappearance of I_2 colour and creamy yellow precipitate deposited at the bottom of the test tube. This test indicates the presence of either CH_3CH_2O- or CH_3CO- or both.

4.4.6 Test for Triterpenoid and Steriod

4.4.6.1 The Salkowski Reaction

A solution of 2 mL methanolic extract was dissolved in 2 mL of chloroform and 2 mL of concentrated sulphuric acid and shaken gently develops a red colour in the chloform layer which indicates the presence of steroid in the sample.

4.4.6.2 The Liebermann - Burchard Reaction

A solution of fatty sample was dissolved in chloroform when treated with few drops of concentrated sulfuric acid and 2-3 drops of acetic anhydride. Solution turned violet blue and finally greenish colour [18]. This indicates the presence of triterpenes and sterols in the sample.

4.4.7 Test for Flavonoid

A portion of methanolic extract of tomato was heated with 10 mL of ethyl acetate over a steam bath for 5 minutes. The mixture was filtered and 4 mL of the filtrate was shaken with 1 mL of dilute ammonia solution. Yellow colour was observed indicating the presence of flavonoids in tomato.

4.4.8 Test for Alkaloid

About 50 mg of solvent free extract was treated with little quantity of dilute HCl and filtrated. The filtrate was tested carefully with various alkaloid reagents.

4.4.8.1 Dragendoffr's Reagent

The filtrate 1 mL was treated with few drops of Dragendoffr's (potassium bismuth iodide solution) reagent. The formation of a prominent brick red or pink colour precipitate indicates the presence of alkaloids.

4.4.8.2 Mayer's Reagent

The filtrate 1 mL was treated with few drops of Mayer's (potassium mercuric iodide solution) reagent along the sides of the test tube. The formation of yellow or brownish yellow colour precipitate indicates the presence of alkaloids.

4.5 Identification of Fatty Acid

4.5.1 Isolation of Fatty Acids

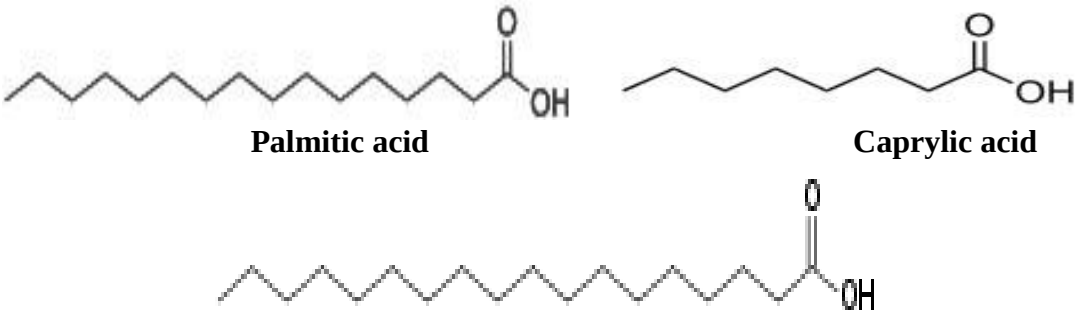
Lycopersicon esculentum 100 g was kept submerged in 50 mL pet ether. The pet ether extract of *Lycopersicon esculentum* was taken in a pear-shaped flask. Potassium hydroxide in methanol (0.5M 10 mL) was added to it and shaken vigorously. The mixture was refluxed for about 30 minutes and the solution was extracted in their esterified form with minutes in a boiling water bath. The mixture was then evaporated in vacuo to dryness. About 8 mL distilled water was added to the dried mass and the pH was maintained to 2.5 by adding ortho-phosphoric acid, then the fatty acids of hexane part was taken in another conical flask and anhydrous Na_2SO_4 was added to it, to make it free from water. Then the mixture was filtered and taken in another pear-shaped flask and evaporated to dryness.

4.5.2 Identification and Quantification

Borontrifluoride-methanol (BF_3-MeOH) complex was added to the dry mass. The mixture refluxed in a boiling water bath for about 8-10 minutes. The mixture was then evaporated in a Rotavapor until dryness. The dried mass was treated with hexane and the hexane part was subjected to gas liquid chromatography (GLC). Individual fatty acid ester was identified by comparing its retention time with those of the peak area of all fatty acid esters. Properties of GLC report of fatty acid:

- A sharp peak at retention time = 3.50
- A sharp peak at retention time = 12.53
- A sharp peak at retention time = 15.31

Peak at retention time at 3.50, 12.53 and 15.31 reveals the presence of palmitic acid (CH_3-$(CH_2)_{14}$-COOH), caprylic acid (CH_3-$(CH_2)_6$-COOH) and steric acid (CH_3-$(CH_2)_{16}$-COOH respectively.



Palmitic acid **Caprylic acid**

Steric acid

Figure 4.1 Different fatty acids from litchi fruits pericarp

4.6 Identification of Amino Acid

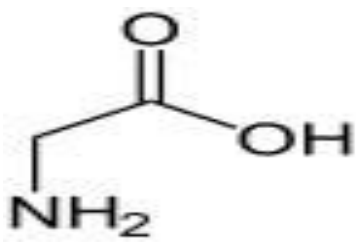
4.6.1 Paper Chromatography

R. spirit extract was applied on a Whatmann no-1 paper with standard mixture. The paper was run in BAW (n-BuOH: OHAc: H_2O = 4:1:1) solvent for 24 hours. The paper was then taken out, dried and sprayed with ninhydrin (triketohydrindene hydrate) solution and four different characteristic coloured spots were detected that matched with the standard Glycine, Alanine, Glutamic acid and Glutamine. Inference: So R. Spirit extract may contain Glycine, Alanine, Glutamine and Glutamic acid.

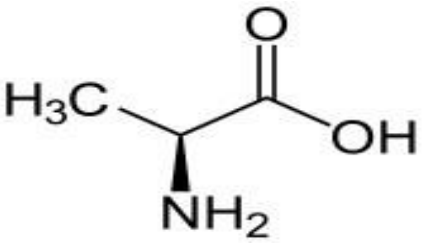
Table 4.1 Ninhydrin colours of different amino acids

Ser. No.	Amino acid	Colour of standard	Sample
1	Glycine	Pink/Red Violet	
2	Alanine	Violet	
3	Glutamic acid	Violet	Light Violet
4	Glutamine	Violet	

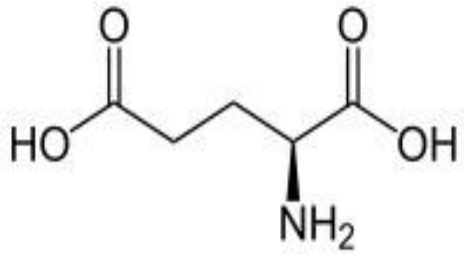
Solvent: BAW=n-BuOH: HOAc: H_2O = 4:1:1



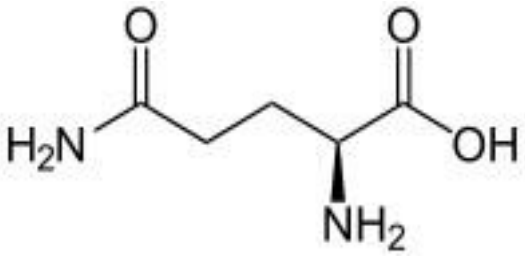
Glycine



Alanine



Gultamic acid



Glutamine

Figure 4.3 Different amino acids from litchi fruits pericarp

4.7 Tests for Glucose

The aqueous methanolic extract 10 mL was taken in a test tube to which 2 mL of 1:1 Fehling's solution-I and Fehling's solution-II was added when red precipitate appeared confirming the presence of glucose molecules (monosaccharide) in the aqueous methanolic extract .

4.7.1 Identification of Glucose

4.7.2 Paper Chromatography

Aqueous methanolic extract was applied on a Whatmann no-1 paper with standard mixture containing glucose, arabinose, maltose and xylose. This paper was run in BEW (n-BuOH: EtOH: H_2O, 40:11:19) solvent for 24 hours. The paper was taken out from the tank, dried in air and treated with $AgNO_3$ followed by washing with 2% NaOH and 10% $Na_2S_2O_3$ successively. Two distinct spots were observed which corresponds to the standard glucose, and arabinose.

4.7.3 Estimation of Glucose in the Collected Sample

Preparation of Stock Solution

Raw tomato fruits 200 g were cut into small fragments. It was kept submerged in 100 mL aqueous MeOH (80% MeOH and 20% H_2O) solution for 24 hours in a conical flask and filtered. The filtrated was used as stock solution.

Preparation of Standard 100 mL Glucose Solution

Weight of the standard glucose = 0.605 g

So the concentration of the standard glucose solution = (0.605/100) g/mL

$= 6.05 \times 10^{-3}$ g/mL

Table 4.2 Standardization of Fehling solution by standard glucose Solution (Volume of Fehling solution taken per titration 10 mL)

No. of Observation	Burette Reading (mL)			Mean Volume(mL)
	IBR	FBR	DBR	
01	0.0	9.5	9.5	9.5
02	9.5	18.9	9.4	
03	18.9	28.4	9.5	

Calculation:

1 mL glucose solution contain $= 6.05 \times 10^{-3}$ g glucose.

So 9.5 mL glucose solution contain = $6.05 \times 10^{-3} \times 9.5$ g glucose

$= 5.7475 \times 10^{-2}$ g glucose.

10 mL Fehling solution neutralized by 5.7475×10^{-2} g glucose

10 mL Fehling solution = 5.7475×10^{-2} g glucose

Estimation of Glucose in the Sample

100 mL volumetric flask was filled up to the mark by sample solution.

Table 4.3 Estimation of glucose in litchi pericarp by standardization Fehling solution.

No. of Observation	Burette Reading (mL)			Mean Volume (mL)
	IBR	FBR	DBR	
01	0.0	14.8	14.8	14.8
02	14.8	29.7	14.9	

Each titration taken 10 mL Fehling solution

14.8 mL sample solution = 10 mL Fehling solution = 5.7475×10^{-2} g glucose.

So 14.8 mL sample solution contain = 5.7475×10^{-2} g glucose.

100 mL sample solution contain = 0.3883 g glucose.

Results

200 g raw tomato contain = 0.3883 g glucose.

So 01 kg raw tomato contain = 1.94 g glucose.

So the amount of glucose in the sample is 1.94 g/kg.

4.8 Estimation of Vitamin-C in the Sample

Vitamin-C (ascorbic acid) rapidly reduces iodine to iodide. A procedure for estimation of vitamin-C is to generate excess iodine (by the reaction of iodate with iodine), which then reacts with ascorbic acid. The excess iodine is titrated with standard thiosulphate solution. The chemical structure of vitamin-C is given below:

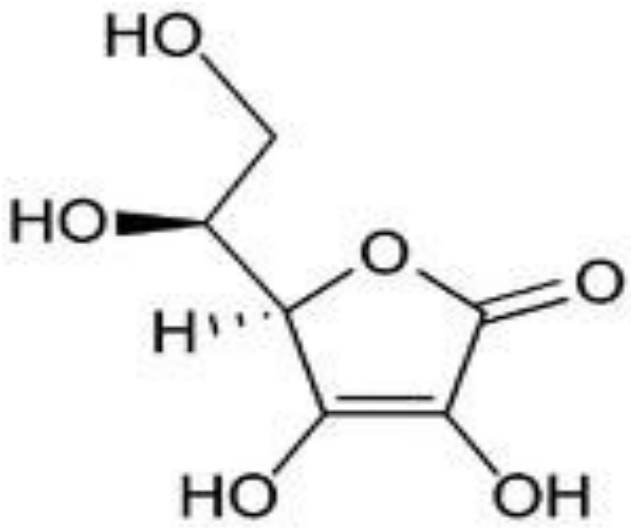


Figure 4.4 Chemical structure of vitamin-C in litchi fruits pericarp

Reactions

$4KI + KIO_3 + 2H_2SO_4 \rightarrow KIO + 2I_2 + 2K_2SO_4 + 2H_2O$

I_2 + Ascorbic acid → Addition product

$I_2 + 2S_2O_3^{-2} \rightarrow 2I^- + S_4O_6^{2-}$

So, 1 mole KIO_3 = 2 moles I_2

1 mole I_2 = 1 mole ascorbic acid

1 mole I_2 = 2 mole $S_2O_3^{2-}$

Experimental Data

Preparation of Stock Solution

About 200 g of the immature tomato was weighed, chopped and kept submerged in a mixture of aqueous ethanol (80 mL) and water (20 mL) for 02 days. Then the solution was filtered and used for estimation of ascorbic acid.

Preparation of Approximately 0.001 M Potassium Iodate Solution:

The molecular weight of KIO_3 is 214.00

So, to prepare 0.001M solution in a 250 mL volumetric flask we need,

(250x0.001x214.00)/1000 g of KIO_3 = 0.0535 g of KIO_3

Taken amount of KIO_3 = 0.0554 g

So, concentration of KIO_3 solution = (Taken amount/ to be taken) x0.01M

= (0.0554/0.0535) x 0.001 M = 0.00103 M

Preparation of approximately 0.008 M thiosulphate solution:

The molecular weight of $Na_2S_2O_3.5H_2O$ is 248.20

So, to prepare 0.008 M solution in a 500 mL volumetric flask we need,

(500x0.008x248.20)/1000 g of $Na_2S_2O_3$

= 0.9928 g of $Na_2S_2O_3$

Taken amount of $Na_2S_2O_3$ = 0.9941 g

So, concentration of $Na_2S_2O_3$ solution = (Weight taken/to be taken)x0.008 M

= (0.9941/0.9928)x0.008 M

= 0.00801 M

4.8.1 Estimation of Vitamin-C in Tomato (*Lycopersicon esculentum*)

Procedure

About 07 mL of 0.25 M sulphuric acid was added to 20 mL of the vitamin-C containing sample solution; 0.5 g of potassium iodide and 50 mL of standard potassium iodate solution was also added to this solution. Finally the solution was back titrated with standard thiosulphate solution using starch as indicator just before the end point (up to the pale yellow colour observed).

Table 4.4 Determination of required volume of $Na_2S_2O_3$ solution to titrate excess iodine

No. of Observation	Burette reading of $Na_2S_2O_3$ solution (mL)		Difference (mL)	Average volume (mL)
	IBR	FBR		
1	0.00	22.40	22.40	22.40
2	22.40	44.90	22.50	

Calculation:

Concentration of KIO_3 solution is 0.00103 mol/L

So, 50 mL of KIO_3 solution contain (0.00103x50)/1000 mole of KIO_3

$= 5.15x10^{-5}$ mole of KIO_3

So, the no. of moles of KIO_3 is $5.15x10^{-5}$

So, the no. of moles of total iodine = $5.15\ x10^{-5}x02$ mole

$= 1.03x10^{-4}$

Concentration of $Na_2S_2O_3$ solution is 0.00801mol/L

Volume of $Na_2S_2O_3$ solution required is 22.40 mL

So, the no. of moles of $Na_2S_2O_3$ is $22.40\ x\ 10^{-3}x0.00801$

$=1.794\ x10^{-4}$

No. of moles of iodine reacted with $Na_2S_2O_3$ = $1.794\ x\ 10^{-4}/2$

$= 8.971\ x10^{-5}$

So, the no. of moles of iodine reacted with ascorbic acid is

$= 1.03x10^{-4} – 8.971x10^{-5}$ mole

$= 9.402x10^{-6}$ mole

So, no. of moles of ascorbic acid is $9.402x10^{-6}$ mole

$= 9.402x10^{-6}$ mole x176 g

$= 16.547x10^{-2}$ g = 1.6547 mg

So, 20 mL of the sample solution contain = 1.6547 mg of ascorbic acid

So, 100 mL of the sample solution contain = (1.6547x100)/20 mg of ascorbic acids

= 8.273 mg of ascorbic acid

So, 200 g of immature Tomato contain 8.273 mg of ascorbic acid.

Results

200 g of immature Tomato contain = 8.273 mg of ascorbic acid.

So, 01 kg of immature Tomato contain = 41.36 mg of ascorbic acid.

4.9 Extraction, Isolation and Investigation of Compounds from *Lycopersicon esculentum*

Conventional solvent was used to extract lycopene and β-carotene from tomato paste of *Lycopersicon esculentum*. Sample was protected from heat, light and moisture uptake.

4.9.1 Extraction of Lycopene and β-Carotene from Tomato Paste

4.9.1.1 Preliminary trials

A first trial was carried out to extract lycopene from tomato paste, About 15 g of tomato pastes were taken from fresh ripe fruits which separated manually and cut into small pieces with knife and squeezed by hands to obtain aqueous juice. This juice was filtrated using cleaned thin cloth. The filtrate was extracted using 50 mL of ethyl acetate (EtOAc) in a separatory funnel and shaken several times and kept undisturbed for 20 minute. Two separate layers were formed. Upper organic layer was EtOAc extract and the lower layer is aqueous part.

Figure 4.5 Extraction of tomato paste using ethyl acetate (EtOAc) solvent.

Similarly the pulp is subsequently extracted using 50 mL of EtOAc and both the extracts were concentrated in vacuo. This procedure was repeated. To confirm the lycopene the separated fraction run on TLC and labeled **as Le-EAE**. TLC was carried out using silica gel plates as stationary phase and toluene: hexane (1:19, v/v) as mobile phase and confirmed the separated fraction sample was Lycopene.

The residue was left after extracted using EtOAc and was kept submerged in a conical flask with 50 mL aqueous methanol solution or 1 kg of tomato paste was kept submersed in 500 mL aqueous MeOH (80% MeOH + 20% H_2O) solution for 24 hours and filtered. The filtrate was concentrated to 100 mL and labeled **as Le-MeE**. This is used as stock solution. The whole extraction process is shown below:

4.9.1.2 EXTRACTION SCHEME OF TOMATO FRUITS PASTE

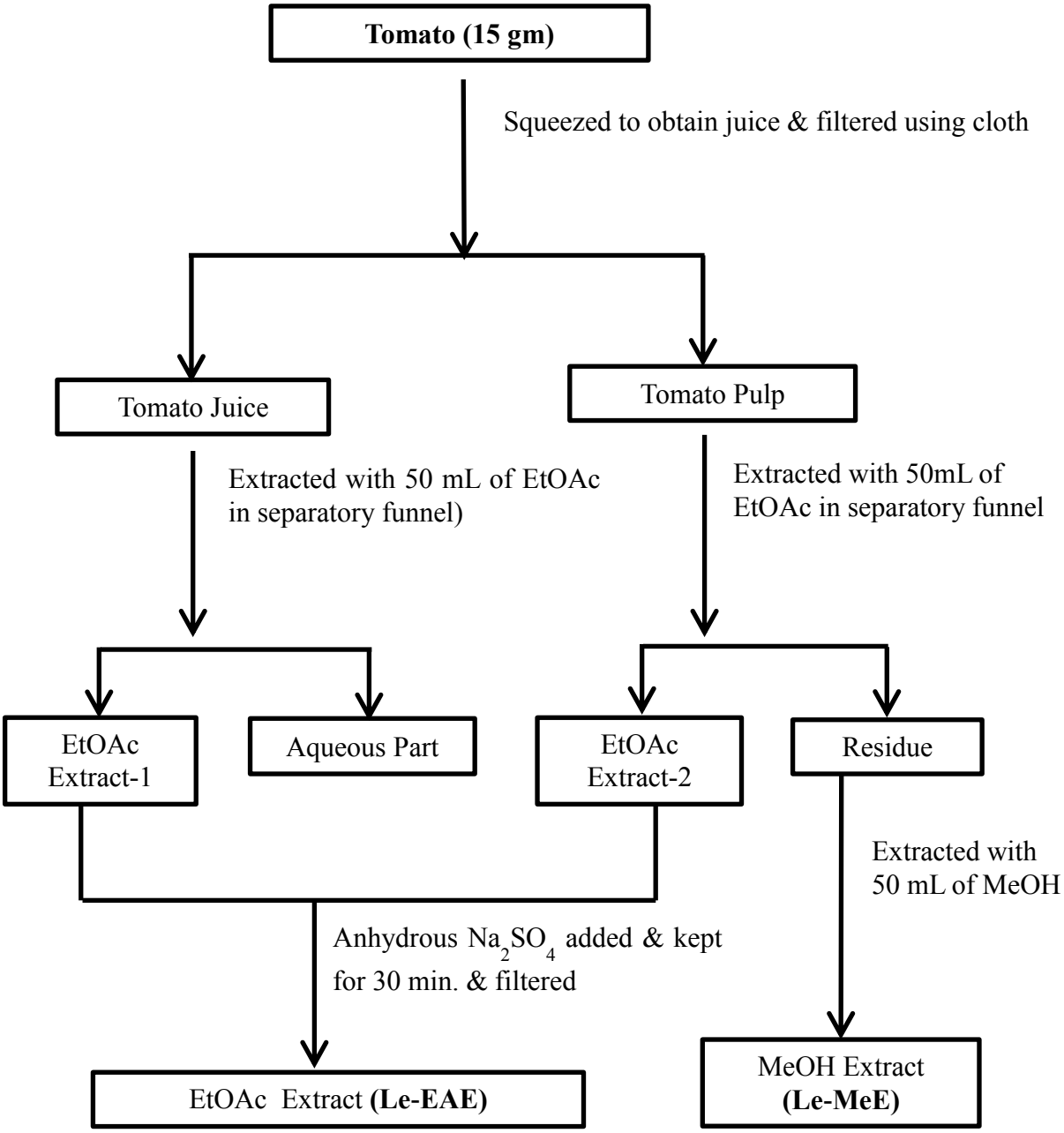


Figure 4.6 Schematic diagram of the phytochemical investigation of tomato paste using ethyl acetate (EtOAc) solvent (**Trial-01)**

A second trial was carried out to extract lycopene from tomato paste 15 g (wet weight) using 100 ml of di-mixture solvent (acetone: pet ether, 1:1, v/v) in conical flasks and mixed using a glass rod. Pet ether with a low boiling point was used as it is non-polar and can dissolve most of the carotenoid residue.

Figure 4.7 Extraction of tomato paste using pet ether: acetone (1:1, v/v) solvent

The flasks were placed in a shaking water bath for 20-min at 37°C and 10 mL of distilled water was added. The samples were transferred to centrifuge tubes and centrifuged for 10-min at 4000 rpm. The samples separated into two layers (Figure 4.7), which were removed using a pipette. To analyze the extracted lycopene from the tomato paste samples thin layer chromatography were used.

4.9.1.3 EXTRACTION SCHEME OF TOMATO FRUITS

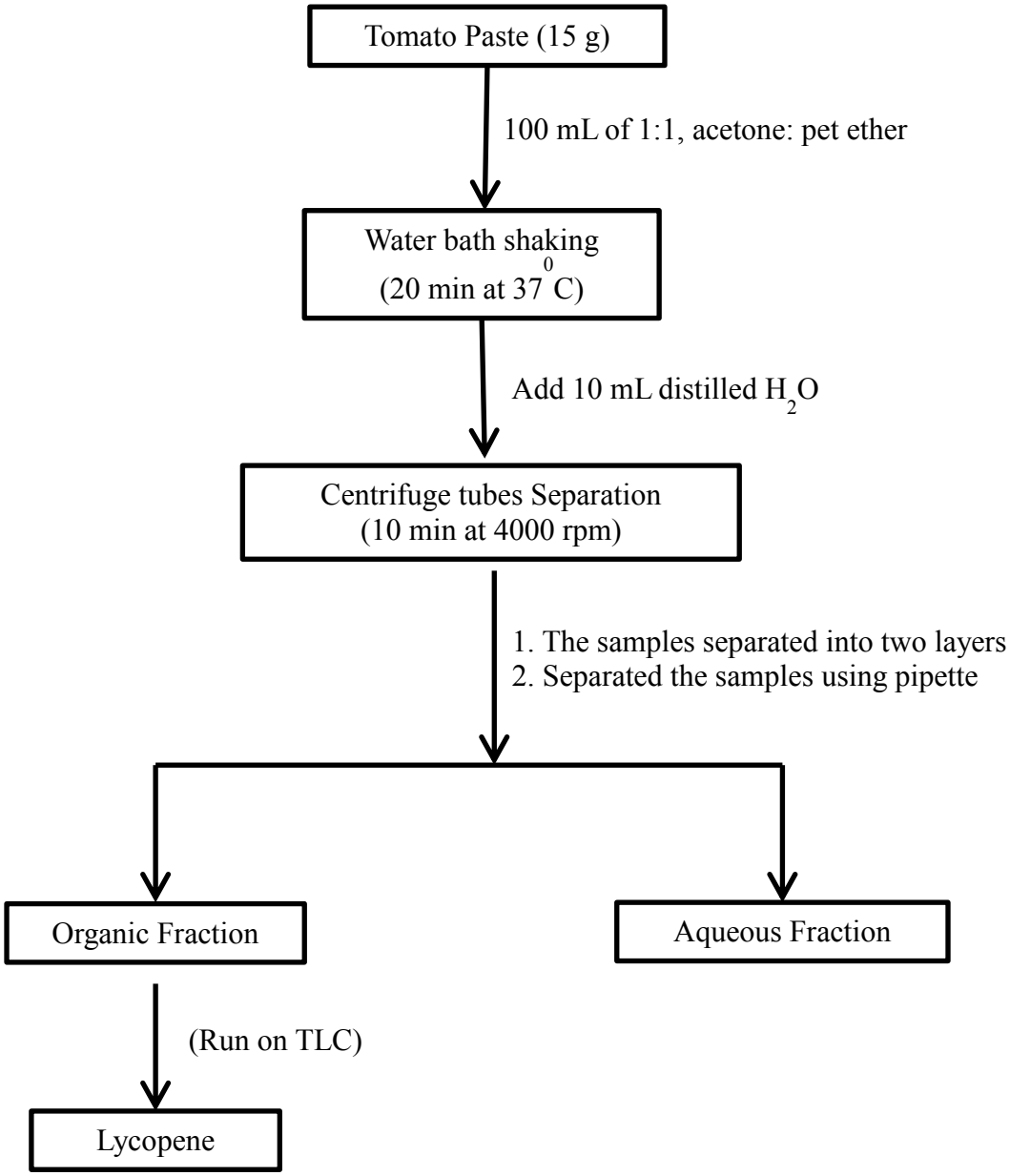


Figure 4.8 Schematic diagram of the phytochemical investigation of tomato paste using pet ether: acetone (1:1, v/v) solvent (**Trial-02)**

A third trial was also carried out to extract lycopene and other carotenoids (β-carotenoids) from the tomato paste with a tri-mixture of n-hexane: acetone: ethanol (50:25:25, v/v/v). 100 mL of tri-mixture solvent was added to 15 g of *Lycopersicon esculentum* paste (wet weight) sample in a conical flask and mixed with a stirrer for 20 min at 37°C.

4.9.1.4 EXTRACTION SCHEME OF TOMATO FRUITS

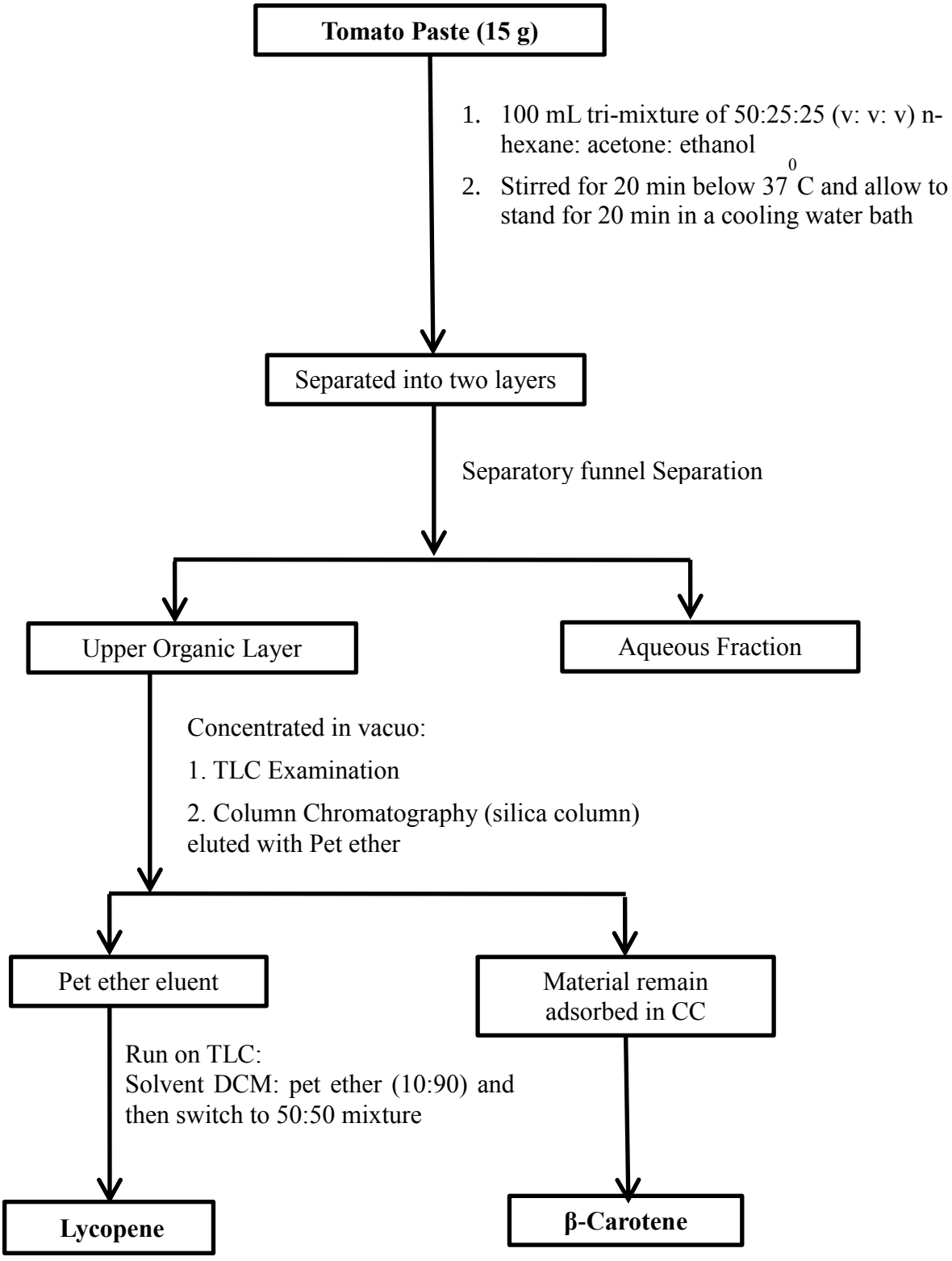


Figure 4.9 Schematic diagram of the phytochemical investigation of tomato paste using n-hexane: acetone: ethanol (1:1:1, v/v/v) solvent (**Trial-03)**

Then stand for 20 min in a cooling water bath, the solution was separated into two layers. Separating funnel was used to separate these layers. Upper organic layer was used to separate β-carotene and lycopene. Upper organic layer contains lycopene and β-carotene (Trial-03) (Figure 4.10).

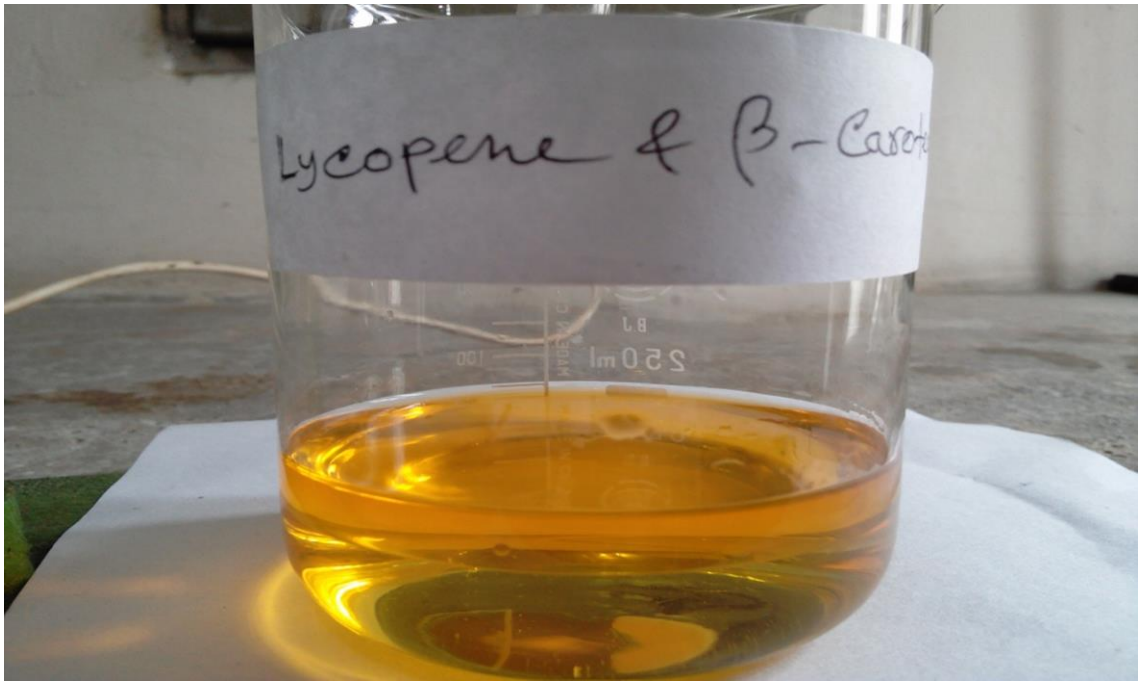


Figure 4.10 Extraction of lycopene and β-carotene from tomato paste using n-hexane: acetone: ethanol (1:1:1, v/v/v) solvent

The carotenoid mixture was applied to the silica column. Column chromatography is a two phase system, the stationary phase is a column of adsorbent (silica gel) and the mobile phase (pet ether) is a liquid eluent. 20 g of silica gel mix with pet ether, swirl until obtained homogenous slurry and added silica mixture using a funnel into the burette to make a silica column.

The upper organic layer carotenoid mixture was applied to the top of the column in a narrow band using pet ether. The eluent flows down through the column by gravity of action. Pet ether with a low boiling point and dichloromethane (DCM) was used to pass through the column to separate the β-carotene and lycopene (Figure 4.13 and 4.14).

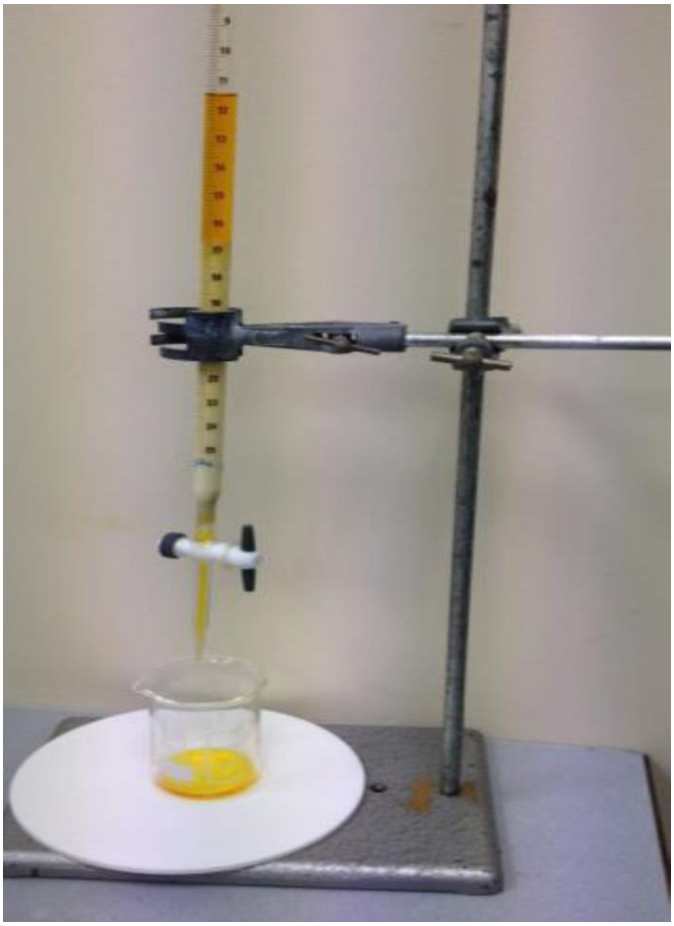


Figure 4.11 Separation of lycopene and ß-carotene using Silica Column Chromatography

When pet ether was passed through the column, carotenoids remain in the narrow band at the top of the column then the solvent was changed to 10:90 ratios DCM: pet ether. After that solvent is switched to a 50:50 mixture of DCM: pet ether to speed up the elution of the lycopene band, which is collected in a separate beaker.

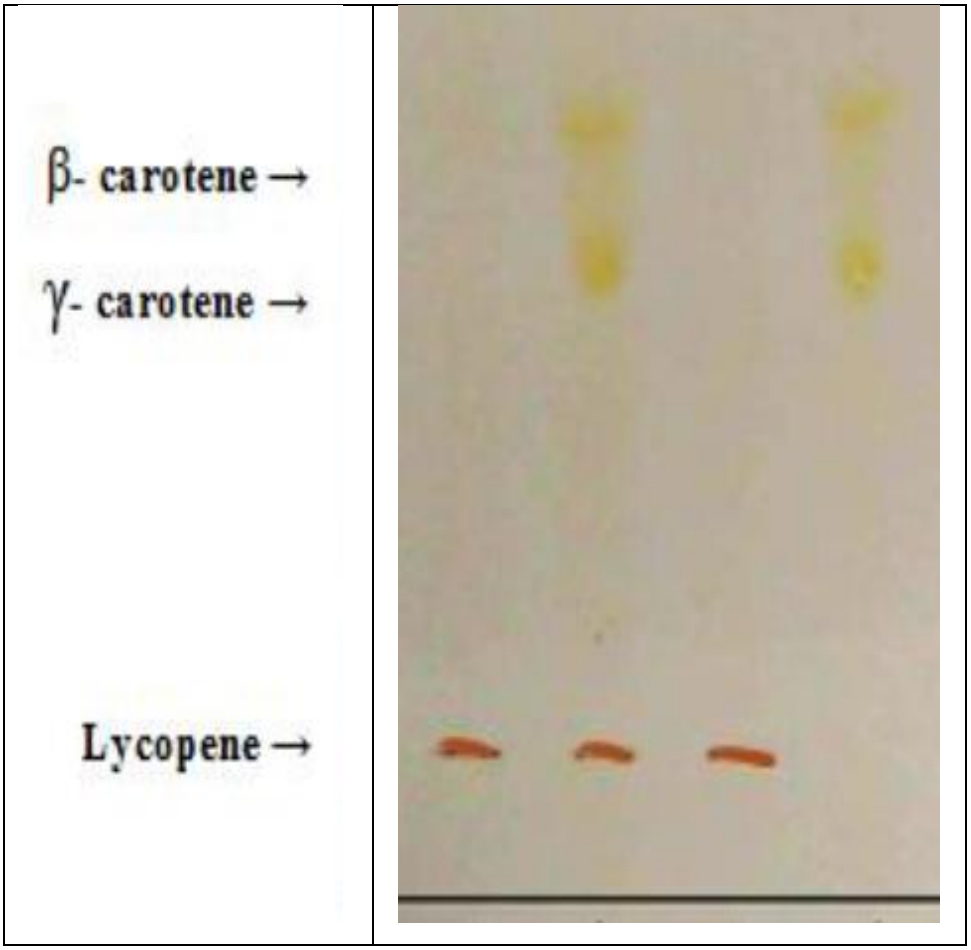


Figure 4.12 TLC of extracted lycopene & carotenoids. The plates were developed with toluene: hexane (1:19, v/v). (a) Standard lycopene (Sigma). (b) Separated lycopene & carotenoids using EtOAc extract. (c) Separated lycopene and (d) Separated carotenoids from silica column chromatography.

To confirm the lycopene and β-carotene the separated fractions run on TLC using silica gel plates and confirmed the separated fraction samples were lycopene and β-carotene and marked as **Le-TME**.

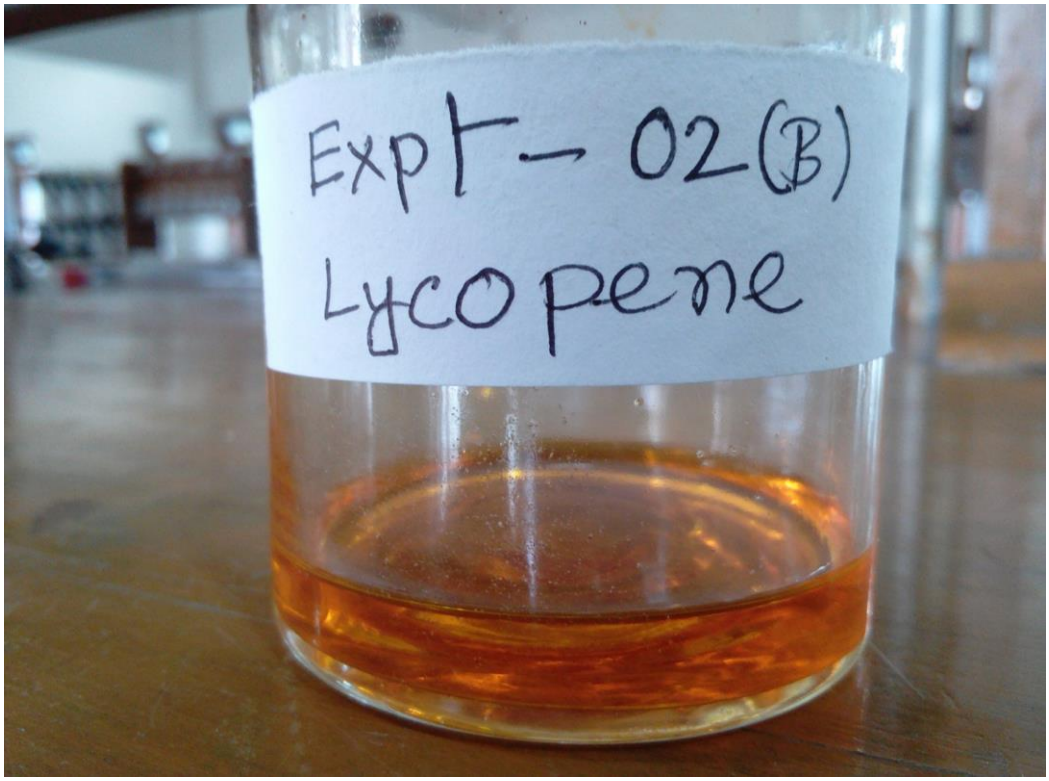


Figure 4.13 Separated lycopene from Silica Column

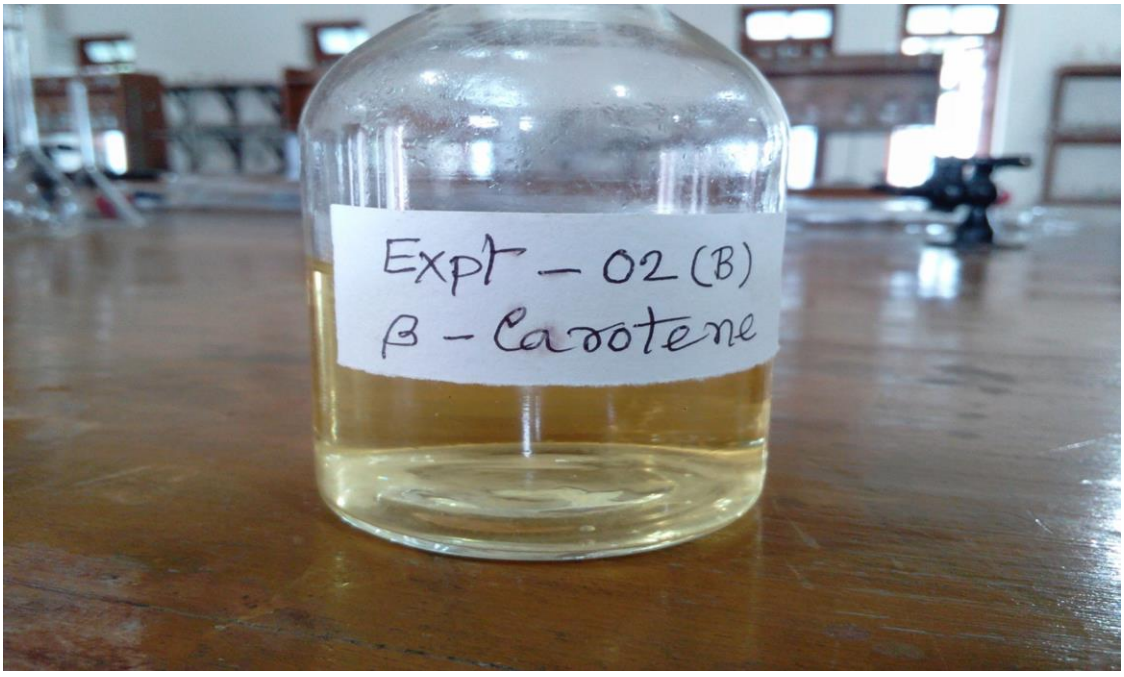


Figure 4.14 Separated β-Carotene from Silica Column

4.10 Soxhlet Extraction

For this research, the soxhlet extraction method was used to extract lycopene from tomato paste. To extract lycopene accurately weigh 25 g of samples and were cut into small pieces with knife and smashed by hands to make paste. The paste was placed in a porous cellulose thimble for soxhlet extraction. Thimble was made up with fine cloth and care must be taken to fit its size with the soxhlet.

The thimbles were placed in the soxhlet extraction unit and extracted for 20 hours with 150 mL of EtOAc solvent. Boiling chips were added to solvent-filled boiling flask and controlled the boiling point of the solvent (EtOAc boiling point is 77^0 C).

Figure 4.15 Soxhlet Extraction of tomato paste using ethyl acetate (EtOAc)

Once the solvent was boiling at a steady rate, the boiling flask was covered with aluminium foil to minimize heat loss. Extractions were done in the dark to minimize lycopene degradation and run for 20 hours to allow the refluxes. The amount of solvent remaining in the boiling flask was measured to determine how much solvent evaporated during the extraction process. A known volume (approximately 2 ml) of the concentrate solvents were removed by pipette for lycopene analysis and marked as **Le-SEAE**. The remaining solvent was then evaporated in vacuo. An allowance was made for the sample that had been removed for lycopene determination. The dried concentrate containing the extracted lycopene was weighed.

4.11 Identification of Lycopene

Lycopene was determined by thin layer chromatography (TLC). Silica gel was used as adsorbent and toluene: n-hexane (1:19, v/v) mobile phase.

4.11.1 Thin Layer Chromatography (TLC)

Silica plates were used as adsorbent for the stationary phase to confirm purity of the extracted lycopene in each lycopene-extracted solvent. Solvent were used as the mobile phase on the TLC plate is toluene-hexane (1:19 v/v). 20 μL of the extract was applied with a pipette as a spot on the TLC plate (Figure 4.16), and the plate was placed in the mobile phase, which rising up by capillary action.

Carotenoids are unstable and sensitive to oxygen, light, heat and acids. Therefore all TLC procedures were carried out as rapidly as possible. The developing chamber was covered with foil paper because carotenoids are sensitive to light and the carotenoid zones faded rapidly due to oxidative breakdown.

The TLC results were photographed and documented, and the retention factor (Rf) for lycopene was calculated by the following formula:

$$R_f = \frac{\text{distance travel by a substance}}{\text{distance travel by a solvent}}$$

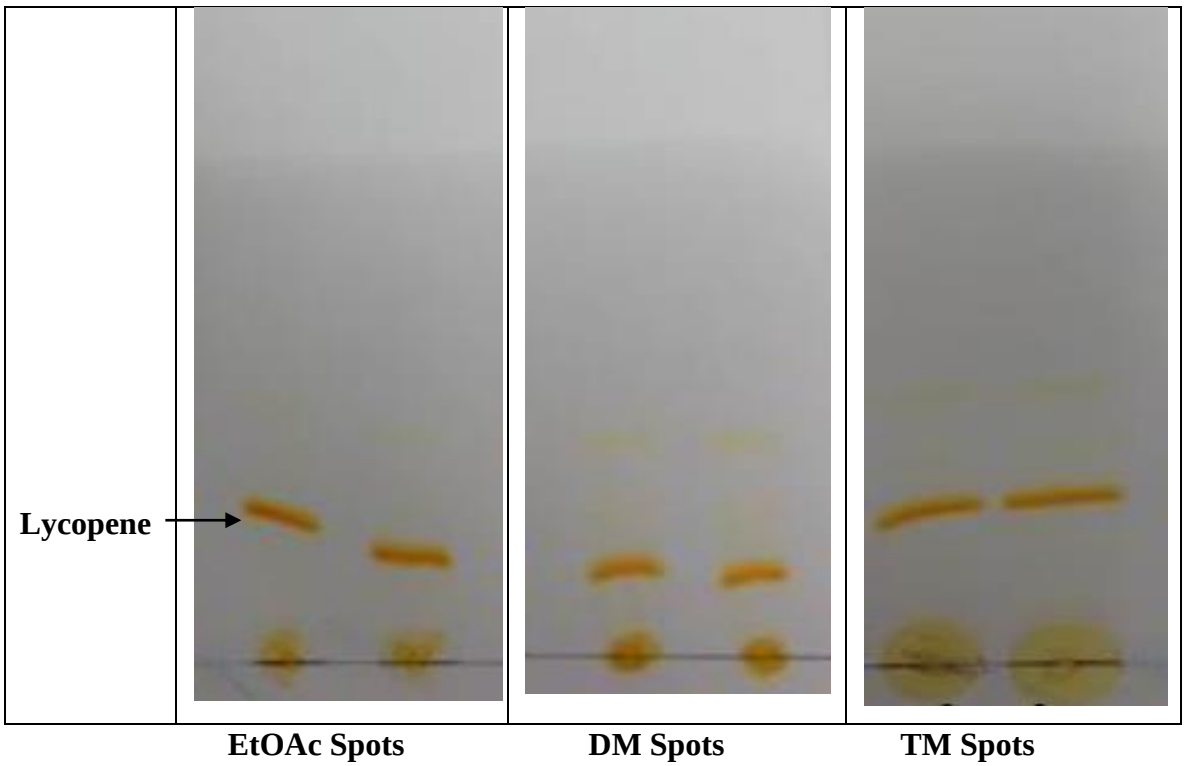


Figure 4.16 TLC Silica plates with drop of lycopene extracts using EtOAc, di-mixture (DM) and tri-mixture (TM) solvents.

Figure 4.17 Lycopene extracted from EtOAc extract

4.12 Analysis of Extracted Lycopene

Various analytical methods have been developed to measure and analysis of lycopene content in food and biological samples. These include IR, ultraviolet-visible (UV-Vis) spectrophotometry, liquid chromatography (LC), vacuum liquid chromatography (VLC), thin layer chromatography (TLC) [22].

UV-VIS spectrophotometry is more convenient, faster and less expensive than HPLC analysis and large numbers of samples can be processed in a relatively short time. However, UV-VIS spectrophotometry cannot detect very small quantities of lycopene (less than 1 μg) whereas HPLC can detect quantities as small as 1 μg.

4.13 Soxhlet Extraction

The extract from soxhlet extraction of lycopene from tomato paste with ethyl acetate was reddish (Figure 4.15). This indicated that ethyl acetate extracted all carotenoids including lycopene. Its boiling point is 77°C and it is miscible with water and ethanol. Because of its low toxicity compared to the other organic solvents, it is used in pharmaceutical preparations, food additives and fragrances. It has been approved for use in food products by the U.S. Food and Drug Administration and biodegrades completely into CO_2 and water [199]. The results show that ethyl acetate is an excellent solvent for extracting of *trans*– and *cis*-isomers of lycopene and β-carotene.

4.14 Properties and Spectral Characteristics of Ethyl Acetate (EtOAc), Di-mixture (acetone: pet ether) Tri-mixture (hexane: acetone: ethanol) and Soxhlet Extraction Extracts of *Lycopersicon esculentum*

To isolate lycopene (ψ, ψ-carotene) from tomato paste (*Lycopersicon esculentum)* preliminary trials were performed. Soxhlet extractions were conducted using ethyl acetate solvent to determine the amount of lycopene in tomato paste. During analysis the sample was protected from heat, light and moisture uptake and stored individually. The extract was examined separately.

4.14.1 Determining Optimum Wavelength by UV-Vis Spectra

The UV-Vis absorption of lycopene extracted from the tri-mixture and a standard lycopene (Sigma New Zealand) was measured in the range 250 to 600 nm. The UV-Vis spectra of the lycopene standard had the maximum absorption peaks at 513, 480, 455 and nm reported [201; 202] also used UV-Vis to identify lycopene extracted from tomato paste using 2:1:1 hexane, ethanol, and acetone and the optical density of the hexane extract were measured at 480 nm. Kun et al. (2006) [202] also used UV-Vis to identify lycopene extracted from tomato paste using 2:1:1 hexane, ethanol, and acetone. The optical density of the hexane extract was measured at 480 nm. The UV-Vis spectra of the lycopene extracted from tomato paste using n-hexane, ethanol, and acetone (2:1:1) was measured at 480 nm [202].

4.14.1.1 Spectral Characteristics of Le – DME

a) Physical state : Deep Yellowish

b) Solubility : Soluble in acetone & pet ether insoluble in water

c) R_f : 0.34 and 0.56

d) Melting Point : 174^0C

e) Amount : 17 mg/100 g

4.14.1.2 UV-Vis spectrum of Le – DME (Figure: 4.18) $\lambda_{Max/nm}$

$\lambda_{Max/nm}$	**Absorbance**
662	0.059
481	3.778
470	3.651
383	2.048

4.14.1.3 IR spectrum of Le – DME (Figure: 4.19): V_{max} cm^{-1}

3350	-OH Stretching (H-bonded)
2920 & 2850	aliphatic C-H Stretching
1710	>C= O Stretching
1455-1365	$-CH_2-$, $-CH_3$ Bending

4.14.1.4 Spectral Characteristics of Le –TME

a) Physical state : Light Yellowish

b) Solubility : Soluble in n-hexane, acetone & ethanol insoluble in water.

c) R_f : 0.34 and 0.56

d) Melting Point : 175^0 C

e) Amount : 21 mg/100 g

4.14.1.5 UV-Vis spectrum of Le –TME (Figure: 4.20) $\lambda_{Max/nm}$

$\lambda_{Max/nm}$	**Absorbance**
502	0.515
470	0.706
446	0.612
362	0.902
346	0.920

4.14.1.6 IR spectrum of Le–TME (Figure: 4.21): V_{max} cm^{-1}

3320	-O-H Stretching (H-bonded)
2910	aliphatic C-H Stretching
1655	>C=C< Skeleton vibration
1455	$-CH_2-$ Bending
1375	$-CH_3$ Bending
1300-1000	C-O Stretching

4.14.1.7 Spectral Characteristics of Le-EAE and Le –SEAE

a) Physical state : Reddish

b) Solubility : Soluble in EtOAc and insoluble in water

c) R_f : 0.34 and 0.56

d) Melting Point : 174^0C

e) Amount : 29 mg/100 g

4.14.1.8 UV-Vis spectra of Le-EAE and Le –SEAE (Figure: 4.22) $\lambda_{Max/nm}$

$\lambda_{Max/nm}$	**Absorbance**
516	0.523
484	0.714
458	0.620
375	0.910
359.5	0.928

4.14.1.9 IR spectra of Le-EAE and Le –SEAE (Figure: 4.23): V_{max} cm^{-1}

3400	O-H	Stretching (H-bonded)
1725	>C= O	Stretching
1650	>C=C<	Skeleton vibration
1455	$-CH_2-$	Bending
1380	$-CH_3$	Bending

Part-B

4.15 Collection of Sample

The fruit *Litchi chinensis* was collected from Mirpur-12 bazaar, in the month of April-May 2015 near Mirpur Cantonment, Dhaka. The collected sample may contain soil dust, dirt etc. So the sample was washed with running water to remove these particles.

4.16 Extraction

Fully ripened fresh fruits with brightly coloured red skins were selected and divided manually into three parts:

a. Litchi Fruit Pericarp (LFP)
b. Litchi Pulp
c. Litchi Seed.

LFP about 15 g was taken in a test tube with stopper and kept submersed under 100 mL aqueous MeOH (20% H_2O + 80% MeOH) at room temperature. After 72 hours the extracts were decanted into clean test tubes marked as **Lc-MeE-I.** These solutions used as stock solution.

4.17 Classical Test of Aqueous Methanolic Extract of Litchi (Lc-MeE-I)

The extracts were subjected to the classical tests for the detection of the presence or absence of the following:

- Unsaturation
- Phenolic group
- Carbonyl group
- Carboxylic acid group
- Acetyl group
- Sugar

4.17.1 Test for Unsaturation

4.17.1.1 Baeyer's Test

Litchi (pericarp, pulp and seed) 10 mL of each part aqueous methanol extract was taken in a test tubes and few drops of very dilute $KMnO_4$ solution added. The disappearance of the purple colour of dilute $KMnO_4$ solution was observed which indicates the presence of unsaturation or aldehydic compounds in each part of litchi.

Sample + $KMnO_4$ → Colourless (+Ve) [9]

4.17.1.2 Br_2 –Water Test

Br_2- Water in CCl_4 Was added to the aqueous methanol of each part of litchi. Red colour of Br_2- Water becomes fade which indicates the presence of unsaturated carbons in the aqueous methanol extract.

Sample + Br_2 + H_2O + CCl_4 → Colourless (+Ve) [8]

4.17.2 Test for Phenolic OH

Litchi (pericarp, pulp and seed) aqueous methanol extract 10 mL of each part was taken in a test tubes to which 02 mL of freshly prepared ethanolic $FeCl_3$ solution was added when greenish yellow precipitate for pulp, greenish deep blue precipitate for skin and greenish blue precipitate for seed colour appeared. This test confirmed the presence of phenolic compound in the aqueous methanol extract of litchi's each part.

4.17.3 Test for Carbonyl Group

The aqueous methanol extract of Litchi (pericarp, pulp & seed) 10 mL of each part was treated with a few mL of DNP (2, 4-dinitrophenylhydrazine) and 02 drops of concentrated HCl was added when orange red precipitate observed for pulp, no precipitate observed for skin and seed . No yellow precipitate was formed. This test excluded the possibility of the presence of an aldehydic moiety or an aldehydic compound in the aqueous methanol extract. A negative response to the DNP test and a positive response for unsaturation obviously indicate the presence of unsaturated molecule in the aqueous methanolic extract

4.17.4 Test for Carboxylic Acid

Aqueous methanol extract of Litchi (pulp, pericarp & seed) 10 mL of each part was added with sodium bicarbonate ($NaHCO_3$) in the test tubes. The bubbles were evolved in both of pulp and pericarp but in seed no bubble was evolved. So carboxylic acid group is present in both pulp and pericarp but absent in seed of Litchi.

4.17.5 Test for Acetyl Group

4.17.5.1 Iodoform Test

Cold distillation aqueous methanol extract 10 mL of each part was taken in test tubes to which 02 mL of I_2 in KI solution was added and warmed in a hot water bath for 10 minutes then aqueous NaOH solution was added drop wise until the disappearance of I_2 colour . Creamy yellow precipitate deposited at the bottom of the test tube, indicates the presence of either CH_3CH_2O- or CH_3CO- or both in Litchi pulp. Pericarp & seed not form these types of colour.

4.17.6 Test for Sugar

Litchi (pericarp, pulp and seed) cold distilled aqueous methanol extract 10 mL of each part was taken in test tubes to which 2 mL of 1:1 Fehling's solution-I and Fehling's solution-II added. The reddish precipitate appeared for each part of aqueous methanol extract which indicate the presence of sugar molecules (monosaccharide) in litchi.

4.17.7 Identification of Sugars

4.17.7.1 Paper Chromatography

Aqueous methanolic extract of Litchi (pulp, pericarp and seed) were applied on a Whatmann no-1 paper with standard mixture containing glucose, arabinose, maltose and xylose. This paper was then run in BEW (n-BuOH:EtOH:H_2O = 40:11:19) solvent for 24 hours. The paper was taken out from the tank, dried in the air then treated with $AgNO_3$ and finally washed with 2% NaOH and 10% $Na_2S_2O_3$ successively. Three distinct spots corresponding to the standard glucose, arabinose and galactose were observed in Litchi pulp, glucose and galactose were observed in litchi pericarp and glucose and arabinose was observed in litchi seed.

4.18 Preliminary Phytochemical Screening of MeOH Extracts of Litchi Fruit Pericarp (LFP)

Litchi Fruit Pericarp (LFP) powder 200 g was kept submersed in 100 mL aqueous MeOH (80% MeOH + 20% H_2O) solution for 24 hours and filtered. The filtrate was concentrated to 20 mL and labeled **Lc-MeE-II**. This concentrated methanolic extract was subjected to various phytochemical screening. Methanolic extract mainly contain medicinally active secondary metabolites. These metabolites are alkaloids, glycosides, tannins, and terpenoides etc. so, methanolic extract of LFP was subjected to different chemical tests to detect the presence of following constituents.

4.18.1 Qualitative Determination

4.18.1.1 Test for Tannin

About 0.5 g of dried powdered sample was boiled in 20 mL of water in a test tube and then filtered. A few drops of freshly prepared $FeCl_3$ (0.1% w/v) solution was added and brownish green colour was observed which indicate the presence of tannin in the sample.

4.18.1.2 Test for Phlobatannin

Methanolic extract was boiled with aqueous HCl (1%w/v) in a test tube and the red precipitate deposited at the bottom of the test tube which indicate the presence of phlobatannins.

4.18.1.3 Test for Saponin

Saponins are glycosides of both triterpens and sterols [200]. They are surface active agents with soap like properties. About 02 g of dried powdered sample was boiled with 20 mL of distilled water in a water bath and filtered. The filtrate (about 10 mL) was mixed with 5 mL distilled water and shaken vigorously for a stable persistent froth. The froth was mixed with 3 drops of olive oils and shaken vigorously then emulsion was formed which indicate the presence of saponin in the sample.

4.18.1.4 Test for Flavonoid

A portion of the powdered sample was heated with 10 mL of ethyl acetate over a steam bath for 3 minutes and filtered and the 4 mL filtrate was shaken with 1 mL of dilute ammonia solution. Yellow colour was observed which indicate the presence of flavonoid in the sample.

4.18.1.5 Test for Triterpenoid and Steriod

4.18.1.5.1 The Liebermann - Burchard Reaction

A solution of fatty sample was dissolved in chloroform and a few drops of concentrated sulfuric acid were added to it followed by addition of 2-3 drops of acetic anhydride. Solution turned violet blue and finally green [197]. This indicates the presence of triterpenes and sterols in the sample.

4.18.1.5.2 The Salkowski Reaction

A solution of 2 mL methanolic extract was dissolved in was 2 mL of chloroform and 2 mL of concentrated sulphuric acid and shaken gently develops a red colour in the chloroform layer which indicates the presence of steroid in the sample [197].

4.18.1.6 Test for Alkaloid

About 50 mg of solvent free extract was treated with little quantity of dilute HCl and filtrated. The filtrate was tested carefully with various alkaloid reagents.

4.18.1.6.1 Dragendoffr's Reagent

About 1 mL of extract was treated with few drops of Dragendoffr's (potassium bismuth iodide solution) reagent. The formation of a prominent brick red or pink colour precipitate indicates the presence of alkaloids.

4.18.1.6.2 Mayer's Reagent

About 1 mL of extract treated with few drops of Mayer's (potassium mercuric iodide solution) reagent along the sides of the test tube. The formation of yellow or brownish yellow colour precipitate indicates the presence of alkaloids.

4.18.1.7 Test for Cardiac Glycoside

4.18.1.7.1 Keller- Killani Test

Methanolic extract about 5 mL was treated with 2 mL of glacial acetic acid containing 1-2 drops of freshly prepared $FeCl_3$ solution. The mixture was then poured into a test tube containing 1 mL of concentrated H_2SO_4 solution. A brown ring at the interphase indicates the presence of deoxy sugar, charecteristics of cardenolides. A violet ring may appear bellow the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Table 4.5 Qualitative analysis of phytochemical of litchi fruits pericarp (LFP)

ser	plants	Alkaloid	Tannin	Saponin	Flavonoid	Steroid	Terpenoid	Cardiac Glycoside
1	LFP	+	+	+	+	+	--	--

4.18.2 Quantitative Determination of Chemical Constituency of LFP

4.18.2.1 Alkaloid Determination

In a 100 mL conical flask 0.5 g of LFP powdered sample was taken and acetic acid (20 mL in 10%) ethanol was added. It was covered and allows standing for 12 hours. The mixture was filtered and the extract was concentrated on water bath to one-fourth of the original volume. Concentrated $Al(OH)_3$ was added drop wise to the extract until the precipitation complete. The whole solution was allowed to stand and the precipitate was collected and washed with dilute NH_4OH solution and then filtered. The residue is the alkaloid, which was dried and weighed [2]. The results are given in Table-4.6.

4.18.2.2 Flavonoid Determination

The LFP powdered sample 1g was extracted repeatedly with aqueous methanol (20 ml in 80%) at room temperature. The whole solution was filtered through Whatmann no-1 paper. The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed to a constant weight. The results are given in Table-4.6.

4.18.2.3 Saponin Determination

The LFP powdered sample 5 g were put into a conical flask and 25 mL 20% aqueous ethanol was added. The samples were concentrated over a hot water bath for 4 hours with continuous stirring at about 55^0 C. The mixture was filtered and the residue re-extracted with 50 mL 20% aqueous ethanol. The combined extracts were reduced to 10 mL over water bath at about 90^0 C. The concentrated extract was transferred into a separatory funnel and 5 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the whether layer discarded. The purification process was repeated. About 15 mL of n-butanol was added and extracts were washed with 3 mL aqueous NaCl (5%) twice. The remaining solution was heated on a water bath. After evaporation the samples were dried in an oven to a constant weight; the saponin content calculated as percentage. The results are given in Table-4.6.

Table 4.6 Amount (% on dry powder basic) of Crude Alkaloid, Flavonoid and Saponin on the Litchi Fruit Pericarp (LFP) Investigated

Ser	Plants	Alkaloids (%)	Flavonoids (%)	Saponin (%)
1	Litchi Fruit Pericarp (LFP)	0.96	33.24	2.65

4.19 Identification of Fatty Acids in Litchi Fruit Pericarp

4.19.1 Isolation of Fatty Acids

100 g of litchi was kept submerged in 50 mL pet ether. The pet ether extract of litchi was taken in a pear-shaped flask. Potassium hydroxide in methanol (0.5M 10 mL) was added to it and shaken vigorously. The mixture was refluxed for about 30 minutes and the solution was extracted in their esterified form with minutes in a boiling water bath. The mixture was evaporated and 10 mL distilled water was added to it. The pH of the solution was kept at 2.5 by adding ortho-phosphoric acid and extracted with hexane .The hexane part was taken in a conical flask and anhydrous Na_2SO_4 was added to it to make it completely free from water. The solution was then filtered and the hexane part was dried.

4.19.2 Identification

20 mL of borontrifluoride-methanol (BF_3-MeOH) complex was added to the dry mass. The mixture refluxed in a boiling water bath for about 10 minutes. The mixture was then evaporated in vacuo until dryness. The dried mass was treated with hexane and the hexane part was subjected to gas liquid chromatography (GLC). Individual fatty acid ester was identified on the basis of the retention time with the fatty acids in the experimental sample and comparing them with those of the standard of the pure substance and the following fatty acids were identified. Properties of GLC report of fatty acid:

Table 4.7 Standard Retention Time (RT) of different methyl esters of different fatty acids from GC chromatogram

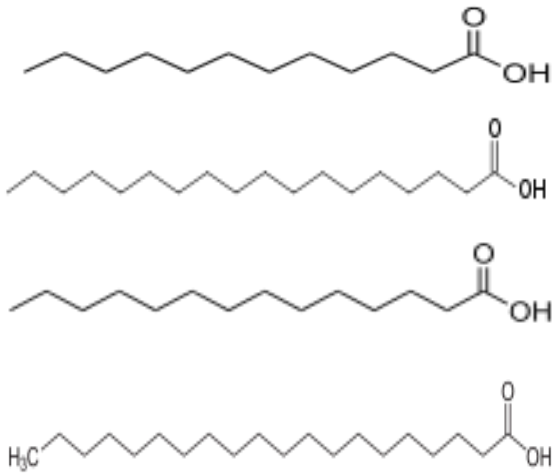
Standard	
RT(min)	**Fatty acid**
9.27	Lauric acid
15.36	Oleic acid
15.61	Stearic acid
18.58	Arachidic acid
23.74	Lignoceric acid

Table 4.8 Relative percentages of free fatty acids (saturation) in litchi pericarp

RT(min)	Fatty acid	Relative Percentages (%)
9.50	Myristic acids	35.07
15.69	Stearic acid	24.55
23.50	Lignoceric acid	21.98

Table 4.9 Relative percentages of free fatty acids (unsaturation) in litchi pericarp

RT(min)	Fatty acid	Relative Percentages (%)
9.25	Lauric acid	Trace
18.59	Arachidic acid	1.08



Lauric acid

Stearic acid

Myristic acid

Arachidic acid

Figure 4.47 Structures of different fatty acids identified in litchi fruits pericarp of *Litchi Chinensis*

4.20 Identification of Amino Acids in Litchi Fruit Pericarp

4.20.1 Paper Chromatography

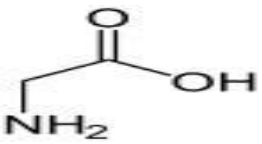
R. spirit extract was applied on a Whatmann no-1 paper with standard mixture. The paper was run in BAW (n-BuOH: OHAc:H_2O = 4:1:1) solvent for 24 hours. The paper was then taken out, dried and sprayed with ninhydrin (triketohydrindene hydrate) solution and four different characteristic coloured spots were detected that matched with the standard.

Aqueous alcoholic extract of Litchi pulp, seed and pericarp were applied on a Whatmann no-1 Paper with standard. The paper was run in BAW solvent for 24 hours. Then sprayed with ninhydrin (triketohydrindene hydrate) solution and four different characteristic spots were found that matched with the standard.

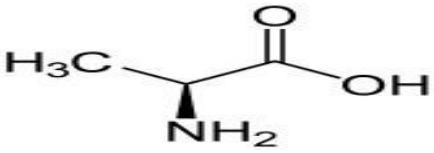
Table 4.10 Ninhydrin colours of different amino acids

Amino acid	**Colour**	**Litchi Pericarp**
Glycine	Pink/Red Violet	Pink
Alanine	Violet	
Glutamic acid	Violet	Light Violet
Glutamine	Violet	

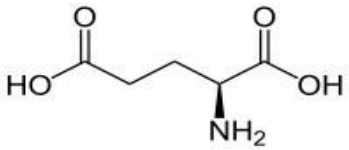
Solvent: BAW = nBuOH-HOAc.H_2O (4:1:1)



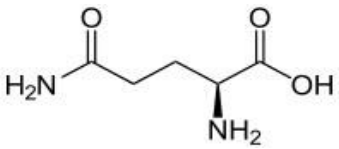
Glycine



Alanine



Glutamic acid



Glutamine

Figure 4.48 Different amino acids identified in litchi fruits pericarp of *Litchi chinensis*

4.21 Estimation of Glucose in Litchi Fruit Pericarp

Preparation of Glucose Solution and Standardization of Fehling Solution

About 0.525g of dry glucose was taken in a 100 mL volumetric flask, dissolved in water and made up to mark. 10 mL of freshly prepared Fehling solution was taken in a 250 mL conical flask and added with 50 mL distilled water. The solution was heated to boiling and glucose solution was added from a great until the blue color of the solution just disappeared. This will give a value of the volume of the glucose solution was required.

To obtain the exact value, the solution was repeated and 1-2 mL of excess glucose solution was added to reaction vessel. This excess glucose solution is required for complete reduction. The solution was heated to boiling for 2 minutes. Without removal of the flame, 3-5 drops of 1% aqueous solution of methyl blue was added to the conical flask. The titration was completed by adding glucose solution drop wise until the colour of the methyl blue just disappears. The titration was repeated until consistent values were obtained.

Experimental Data

Preparation of Standard 100 mL Glucose Solution

Weight of the standard glucose = 0.525 g

So, concentration of standard glucose solution = (525/100) g/mL.

$$= 5.25\times10^{-3}\ \text{g/mL}$$

Table 4.11 Standardization of Fehling solution by standard glucose solution (Volume of Fehling solution taken per titration 10 mL)

No. of Observation	Burette reading of standard glucose solution (mL)		Difference (mL)	Average volume (mL)
	IBR	FBR		
1	0.00	10.70	10.70	10.70
2	10.70	21.70	10.70	

Calculation

1mL glucose solution contains = 5.25×10^{-3} g glucose

10.70 mL glucose solution contains = 5.25×10^{-3} x 10.7g glucose

= 0.05617 g glucose

Preparation of sample solution

For estimation of glucose in Litchi pulp, about 10 g were weighed, chopped and kept submerged in a mixture of aqueous ethanol (80 mL ethanol and water 20 mL) for a few days. Then the solution was filtered and the filtrate was used for glucose estimation

Table 4.12 Estimation of glucose in litchi pericarp by standard Fehling solution (Volume of Fehling solution taken per titration 10 mL)

No.of Observation	Burette reading of standard glucose solution (mL)		Difference (mL)	Averrage volume (mL)
1	IBR	FBR	6.5	6.5
	0.00	6.5		

Calculation

6.5 mL sample solution = 10.0 mL Fehling solution = 0.05617 g glucose

So, 6.5 mL sample solution contain = 0.05617 g glucose

100 mL sample solution contain = (0.05617 x100)/6.5 g glucose

= 0.8641 g glucose

So, 1000 mL sample solution contain = (0.8641x1000)/100 g glucose

= 8.641 g glucose

So, the amount of glucose in the sample is 8.641 g/L

10 g of the Litchi pericarp were taken to prepare 100 mL sample solution.

So, 10 g of Litchi pericarp contain 0.8641 g glucose

So, 100 g Litchi pericarp contain (0.8641x100)/10g glucose = 8.641 g glucose

Results

The amount of glucose in Litchi pericarp is 8.641g/L

100 g Litchi pericarp contain 8.641 g glucose

4.22 Estimation of Vitamin-C in Litchi Fruits Pericarp

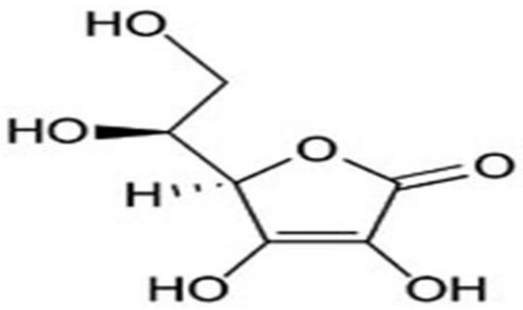


Figure 4.49 Chemical structure of Vitamin-C (ascorbic acid)

Ascorbic acid rapidly reduces iodine to iodide. A procedure for estimation of vitamin- C is to generate excess iodine from the reaction of iodate with iodide which then reacts with ascorbic acid. The excess iodine is then titrated standard thiosulphate solution.

Reaction

$4KI+KIO_3 + 2H_2SO_4 \longrightarrow KIO+2I_2+2K_2SO_4+2H_2O$

I_2 + Ascorbic acid $\longrightarrow$ Addition product

$I_2 + 2S_2O_3 \longrightarrow 2I^- + S_4O_6^{2-}$

So,1mole KIO_3 = 2 moles of I_2

And 1mole I_2 = 2 moles of $S_2O_3^{2-}$

Preparation of 0.001M KIO_3 Solution

The molecular weight of KIO_3 = 214.00

So to prepare 0.001 M solution in a 250 volumetric flask it is required

(250x0.001x214)/1000g 0f KIO_3 = 0.0535g 0f KIO_3

So, weight taken = 0.0537g of KIO3

Preparation of 0.008 M Sodium Thiosulphate Solution

The molecular weight $Na_2S_2O_3.5H_2O$ = 248.20

So to Prepare of 0.008 M solution in a 500 mL volumetric flask required = (500x0.008x248.2)/1000 g 0f $Na_2S_2O_3.5H_2O$ = 0.9928 g

So, weight taken = 0.9928 g of $Na_2S_2O_3.5H_2O$

Preparation of Litchi pericarp sample

About 50g of the Litchi pericarp was weighed, chopped and kept submerged in a mixture of aqueous EtOH (80mL ETOH+ 20mL H2O) for a few days. Then the solution was filtered and used for estimation of ascorbic acid.

Procedure:

About 7.0 mL of 0.25 M H_2SO_4 was added to 20 mL of the vitamin-C containing sample solution. 0.5 g of KI and 50 mL of standard KIO_3 solution was also added to this solution. Finally the solution was back titrated with standard thiosulphate solution using starch as indicator just before the end point (when the solution is a pale yellow colour).

Table 4.13 Determination of the volume of thiosulphate solution required to titratation of excess iodine

No.of observation	Burette reading of standard glucose solution (mL)		Difference (mL)	Average volume (mL)
1	IBR	FBR	19.8	19.8
	0.00	19.8		

Calculation:

Concentration of KIO_3 solution = 0.001 mL/L

So, 50 mL of KIO_3 solution contains = (0.001x50)/1000 moles of KIO_3

$= 5.0x10^{-5}$ moles of KIO_3

So, the no. moles of KIO_3 $= 5.0x10^{-5}$

So, the no. of moles of total iodine $= 5.0x10^{-5} \times 2$

$= 1.0x10^{-4}$

Concentration of $Na_2S_2O_3$ solution = 0.008 mol/L

Volume of $Na_2S_2O_3$ solution required = 19.8 mL

So, no. moles of $Na_2S_2O_3$ is = $19.8x10^{-3}$ x0.008

$= 1.584x10^{-4}$

No. of moles of iodine reacted with $Na_2S_2O_3$ = $1.584x10^{-4}/2$

$= 7.92x10^{-5}$

So, no. of moles of iodine reacted with ascorbic acid = $(1.0x10^{-4} - 7.92x10^{-5})$ moles

$= 20.80x10^{-6}$ moles

So, no. of moles in ascorbic acid is = $20.80x10^{-6}$ moles

$= 20.80x10^{-6}$ x 176 g

$= 4.16x10^{-3}$ g

= 3.6608 mg

So, 20 mL of the sample solution contain = 3.6608 mg ascorbic acid

100 mL of the sample solution contain = (3.6608x100)/20 = 18.304 mg ascorbic acid

So, 50 g Litchi pericarp contain =18.304 mg ascorbic acid

100 mg Litchi pericarp contain = (18.304 x100)/50 mg ascorbic acid

= 36.6 mg ascorbic acid

Results

100g of Litchi pericarp contain 36.6 mg of Vitamin-C

4.23 Metal Ions Test

Litchi fruit pericarp 3 gm of dry powdered was taken in a porcelain disc and burnt to make it ash. Then the ash was powdered by mortar and dissolved in 20 mL distilled H_2O and filtered. The filtrate was collected in a conical flask for metal ions test.

4.23.1 Flame Test

Elements	**K^+**	**Ca^{2+}**
Colour in oxidation flame	Lilac/violet	Brick red

Results

Each part of litchi fruit pericarp gave positive result for K^+ and Ca^{2+}. K^+ ions are good for heart and Ca^{2+} ions are good for bones. Both of these nutrients are essential in strengthening and performing minor repairs on the bones as well as the bone tissue.

4.23.2 Test for Fe^{3+}

Sample solution + K_3 [Fe (CN) $_6$] = K Fe [Fe (CN) $_6$]

(Brown solution)

Litchi fruit pericarp gave brown colour, which indicates the presence of Fe^{3+} ion in each part of litchi fruit.

4.23.3 Test for Ca^{2+}

Sample solution + ammonium oxalate = Calcium oxalate

(White ppt)

Litchi fruit pericarp gave white precipitate, which indicates the presence of Ca^{2+} ion in each part of litchi fruit.

4.23.4 Test for Mg^{2+}

Sample solution + sodium pyroantimonate = pink colour

Litchi fruit pericarp gave pink colour, which indicates the presence of Mg^{2+} ion in each part of litchi fruit.

Plants	**K^+**	**Ca^+**	**Fe^+**	**Mg^+**
Litchi Pericarp	+	+	+	+

4.24 Isolation and Investigation of Compounds from Litchi Fruit Pericarp (LFP)

4.24.1 Soxhlet Extraction

The Litchi Fruit Pericarp (LFP) was separated manually from fresh ripe fruits of *Litchi chinensis* and cut into small pieces. The LFP was dried in open air under the sun and finally dried in an oven at 40^0 C. The dried LFPs were powdered in a blander machine to yield 250 g sample. These powdered materials were divided into ten parts i.e. 25 g each. The 25 g LFP powder was placed in a porous cellulose thimble. Then the thimbles were placed in the soxhlet extraction unit and extracted in a soxhlet apparatus for 20 hours with 150 mL of distilled methanol (MeOH). Thimble was made up with fine cloth and care must be taken to fit its size with the soxhelt. Boiling chips were added to solvent-filled boiling flask and controlled the boiling point of the solvent. The flask with the solvent was heated, evaporated into the condenser, and condensed to a liquid, which trickled into the extraction chamber containing the sample. The extraction was continued until no residue was left upon evaporation of a few milliliter of the fresh extract. This procedure was repeated until all extraction is completed. The extract about 300 mL was filtered through Whatmann no-1 filter paper and the filtrate was collected and marked as **MELFP**.

4.24.2 Identification of Compound

The compound was determined by TLC. Silica gel was used as adsorbent and 100% Pet ether as mobile phase.

4.24.3 Thin Layer Chromatography (TLC)

Silica plates were used as adsorbent for the stationary phase to confirm purity of the extracted compound in each extracted solvent. Solvent were used as the mobile phase on the TLC plate is 100% Pet ether. 20 μL of the extract was applied with a pipette as a spot on the TLC plate, and the plate was placed in the mobile phase, which rising up by capillary action.

A TLC study of methanolic extract of LFP (**MELFP)** showed the presence of five spots of them three were yellow spots and two were violet spots when kept in an iodine chamber. One of these spots showed blue florescence spot in UV light. Upon spraying with vanillin –sulphuric acid followed by heating in an oven at 110^0 C for 10 minutes showed the presence of seven spots of them three were violet, two were pink, one was yellowish and one was blackish. The presence of pink colour spots was thought to be an indication of the presence of either steroid or fatty acid material or both. However, its Salkowski and Libermann-Burchard reaction gave positive result confirming the presence steroids. The concentrated methanol extract was then taken for identification of fatty acids. It also gave positive result. The extract about 300 mL of **MELFP** was concentrated in vacuo.

EXTRACTION SCHEME OF LITCHI FRUITS PERICARP (LFP)

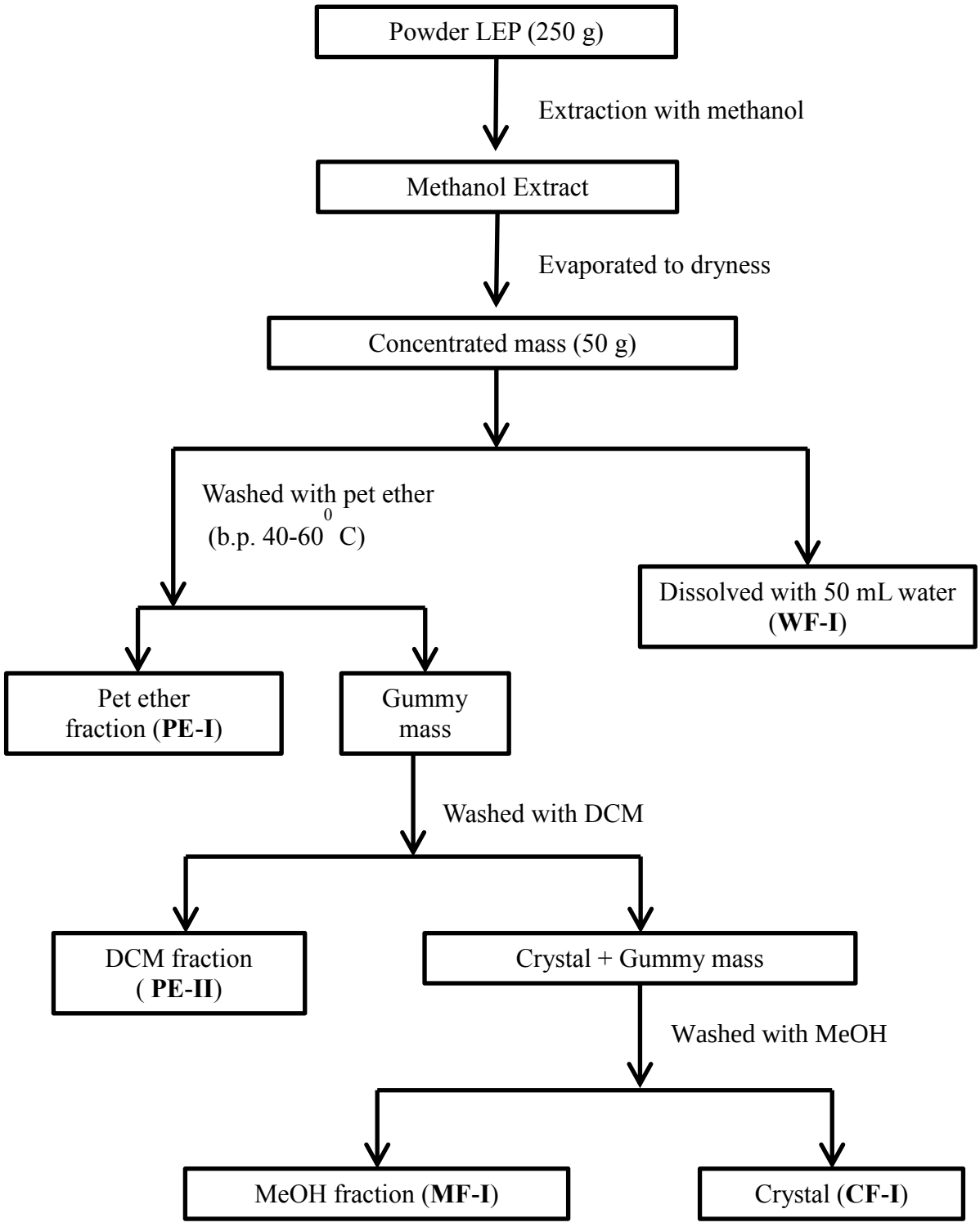


Figure 4.50 Schematic diagram of the phytochemical investigation of litchi pericarp

4.24.4 The Fraction PE-I

The methanol extract was concentrated by evaporator and washed upon adding pet either which then separates this fraction; marked as **PE-I** for TLC study.

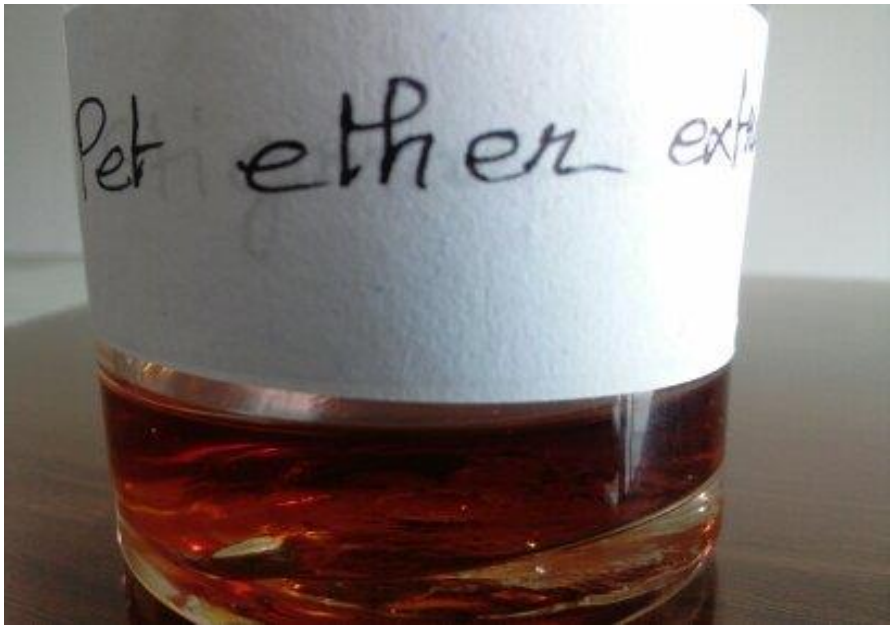


Figure 4.51 Pet ether washed fraction of concentrated MeOH extract of LFP powder

The TLC study of pet either fraction showed the presence of five spots when kept in an iodine chamber being visible on spraying with vanillin-sulphuric acid followed by heating in an oven at 110^0 C for 10 minutes. Of these five spots two were violet, two pink spots and one green. The presence of pink colour was thought to be an indication of the presence of either steroid or fatty acid material or both. However, its Salkowski and Libermann-Burchard reaction (Chapter-4.18.1.5.1-2) gave positive indication confirming the presence of steroids.

4.24.5 The Fraction PE-II

These were left for few days. At the bottom of the conical flasks solid particles settled down with gummy mass. The solid particles and gummy mass were then washed using DCM solution and the washed DCM solution was put in a test tube. The test tube was marked as **PE-II**.

4.24.6 The Fraction MF-I

The solid particles (crystal and gummy mass) were again washed with MeOH and warmed in a hot water bath for 20 minute. The washed MeOH solution were put in another test tube and marked as **MF-I.**

4.24.7 The Fraction CF-I

The fraction gave very little amount of white crystals. But the collected crystals could not dissolve in pet ether, ethyl acetate, chloroform and methanol solvents at room temperature or even on heating. The melting point of the compound was not sharp. It started to melt at 94^0 C and at last melted at 110^0 C. Its IR spectrum was taken and showed in Figure 4.24. The crystals were put in a vial marked as **CF-I**.

4.24.8 The Fraction WF-I

This aqueous fraction was transferred to a separating funnel and mixed with 50 mL pet ether (1:1) and shaken vigorously. No compounds were settled down from the mother liquor and thus the fraction was not studied further.

4.24.9 Fractionation of Extract 'PE-I' by Vacuum Liquid Chromatography (VLC):

The pet ether fraction marked '**PE-I**' about 50 mL was concentrated in vacuo and was subjected to VLC over TLC grade silica gel 'Merck $60PF_{254}$' previously washed with pet ether, chloroform, acetone, ethyl acetate and methanol successively to remove the adhering plasticizer. The column was first eluted with 100% pet ether (b.p. 40-60^0C) and then eluted with mixtures of pet ether and increasing amount of ethyl acetate and finally with methanol. The eluents were collected in an amount of about 20 mL in a series of test tubes. The solvent system used for eluting the VLC column was recorded below:

Table 4.14 The solvent systems used successively for eluting the VLC column

Solvent	Percent	Amount (mL)	Test tube no.
Pet ether (40^0 - 60^0C)	100%	50 mL	1-4
EtOAc in Pet ether	1%	50 mL	5-9
"	5%	25 mL	10-11
"	10%	25 mL	12-18
"	20%	400 mL	19-37
"	25%	250 mL	38-48
"	30%	150 mL	49-56
"	40%	25 mL	57-58
"	50%	200 mL	59-76
"	60%	25 mL	77-80
"	70%	150 mL	81-91
"	80%	25 mL	92-94
"	90%	25 mL	95-96
EtOAc	100%	25 mL	97-98
$CHCl_3$	100%	25 mL	99-100
MeOH in $CHCl_3$	5%	25 mL	101-102
"	10%	50 mL	103-106
"	20%	25 mL	107-108
"	30%	50 mL	109-110
MeOH	100%	100 mL	Conical flask

The pet ether extract elutes from VLC was monitored by TCL. The fractions of similar TCL behavior were combined together and were designated as per following Table 4.15.

Table 4.15 Pet ether extract elutes from VLC was monitored by TCL

Ser No	Fraction No.	Test Tube No.
1	T-1	1-4
2	T-2	5-9
3	T-3	10-14
4	T-4	15-21
5	T-5	22-23
6	T-6	24-29
7	T-7	30-35
8	T-8	36-46
9	T-9	47-58
10	T-10	59-71
11	T-11	72-84
12	T-12	85-98
13	T-13	99-110

Table 4.16 TCL behavior of the VLC fractions

Fraction no.	Solvent system	No. of Spots (After spraying with vanillin-sulphuric acid & heating at 110^0C)	Colour of the spots before spray	Colour of the spots after spray	R_f value
T-1	100% PE	1 spot + tailing	Yellow & blue in UV light	Violet	0.70
T-2	95% PE +5% EA	1 spot	Yellow	Violet	0.63
T-3	90% PE +10% EA	3 Spots very close + tailing	Yellow Colourless	Violet Greenish	0.81 0.67
T-4	90% PE +10% EA	1 spot + long tailing	Yellow	Violet	0.65
T-5	90% PE +10% EA	1 spot + tailing	Yellow	Violet	0.66
T-6	80% PE +20% EA	2 spots + tailing	Light green	Violet	0.47 0.39
T-7	70% PE +30% EA	2 spots + tailing	Yellow & blue in UV light	Pink & greenish	0.51 0.38
T-8	70%PE +30%EA	1 spot + tailing	Colourless	Black	0.55
T-9	60%PE +40%EA	1 spot + long tailing	Creamy	Blackish	0.60
T-10	50%PE +50%EA	1 spot + long tailing	Colourless	Violet	0.65
T-11	40%PE +60%EA	Tailing	-	-	-
T-12	20%PE +80%EA	Long tailing	-	-	-
T-13	100%EA	Long tailing	-	-	-

The fractions were concentrated using rotary evaporator under reduced pressure bellow at 60^0C and then kept undisturbed at room temperature. Upon standing undisturbed at room temperature solid was appeared in different conical flasks.

4.24.9.1The Fraction T-1

Fraction T-1 was yellow in colour. Upon standing undisturbed at room temperature no compounds were settled down from the mother liquor and thus the fraction was not studied further.

4.24.9.2 The Fraction T-2

Fraction T-2 was also colourless. Upon standing undisturbed at room temperature no solid was appeared at the bottom of the conical flask. The mother liquor was transferred in a vial marked as **CF-II** and its TLC study (95% PE+5% EA) showed a single spot on spraying with vanillin-sulphuric acid reagent followed by heating in an oven. However it's IR was taken and revealed that it was a fat like compound (Figure 4.25).

4.24.9.3 The Fraction T-3

Fraction T-3 was light yellow in colour. Upon standing undisturbed at room temperature little amount 1.4 mg of Fat like solid was appeared at the bottom of the conical flask. The solid was washed with pet ether and then dissolved in DCM and liquid taken in a vial marked as **CF-III**. Its TLC study in the solvent (90% PE+10% EA) showed one violet spot with tailing on spraying with vanillin-sulphuric acid reagent and R_f value was 0.65. Its IR was taken and showed in figure 4.26.

4.24.9.4 The Fraction T-4

Fraction T-4 was yellowish in colour. Upon standing undisturbed at room temperature little amount of gummy solid was appeared at the bottom of the conical flask. The mother liquor was decanted and the solid was dissolved with ethyl acetate. Its TLC study in solvent (90% PE+10% EA) showed tailing being visible on spraying with vanillin-sulphuric acid reagent.

4.24.9.5 The Fraction T-5

Fraction T-5 was yellow in colour. No compounds were settled down from the mother liquor and thus the fraction was not studied further.

4.24.9.6 The Fraction T-6

Fraction T-6 was light green in colour. Upon standing undisturbed at room temperature little amount of gummy solid appeared at the bottom of the conical flask. The mother liquor was decanted and the solid was dissolved with ethyl acetate. Its TLC study in solvent (80% PE+20% EA) showed tailing being visible on spraying with vanillin-sulphuric acid reagent.

4.24.9.7 The Fraction T-7

Fraction T-7 was yellow in colour upon standing undisturbed at room temperature for several weeks little amount of yellowish solid appeared at the bottom of the conical flask. It was dissolved in pet ether. A TLC study in solvent (70% pet ether: 30% ethyl acetate) presence of one spot showed in UV light and the two spots and little tailing showed on spraying with vanillin- sulphuric acid reagent. Further these fractions were concentrated about 25 mL & subjected to column chromatography by column grade silica gel; Merck $60PF_{254}$ which was previously washed with pet ether successively to remove the adhering plasticizer. The column was first eluted with 100% pet ether (b.p. 40^0-60^0C) and then eluted with mixtures of pet ether and increasing amount of ethyl acetate and finally with methanol. The eluents were collected in an amount of about 30 mL in a series of test tubes. The solvent system used for eluting the mini column was recorded bellow:

Table 4.17 The solvent system used successively for eluting the mini column

Solvent	Percent	Amount (mL)	Test tube no.
Pet ether (40^0-60^0 C)	100%	50 mL	S-1&S-2
EtOAc in Pet ether	1%	25 mL	S-3
“	2%	25 mL	S-4
“	3%	25 mL	S-5
MeOH	100%	50 mL	S-6 & S-7

Table 4.18 TCL behavior of the mini column fractions

Fraction No.	Test tube no.	Solvent system	No. of Spots(After spraying with vanillin - sulphuric acid & heating at 110^0C)	Colour of the spots before spray	Colour of the spots after spray	R_f value
TS-1	S-1 & S-2	100% PE	1 spot + tailing	Yellow & blue spot in UV light	Violet	0.42
TS-2	S-3	98% PE + 2% EA	1 spot + tailing	light yellow	Violet	0.38
TS-3	S-4 & S-5	97% PE + 3% EA	tailing	-	-	-
TS-4	S-6	95% PE + 5% EA	tailing	-	-	-
TS-5	S-7	85% PE + 15% EA	tailing	-	-	-

4.24.9.7.1 The Fraction TS-1:

Fraction TS-1 was yellow in colour. Upon standing undisturbed at room temperature no crystals were appeared at the bottom of the test tube and thus the fraction was not studied further.

4.24.9.7.2 The Fraction TS-2:

Fraction TS-2 was light yellowish in colour. It was left undisturbed at the room temperature gave white sharp needle types crystals at the sides of the test tube. The crystals were dissolved in $CHCl_3$ (2 mL x 3) and transferred in a vial **Marked as SS-I** for further recrystallization. Its TCL study (98% pet ether: 2% ethyl acetate) showed a single spot on spraying with vanillin-sulphuric acid reagent followed by heating in an oven at 110^0C for 10 minutes. However after recrystallization it's IR (Figure 4.28), ^{1}H-

NMR (Figure 4.29, 4.29(a), 4.30, 4.30(a), 4.31, 4.32 4.33 and 4.33(a), ^{13}C-NMR (Figure 4.34 and 4.35), DEPT-135 (Figure 4.36, 4.37 and 4.38) spectrum were taken and revealed that it was a Sterol type Compound.

4.24.9.7.3 Other Fractions (TS-3, TS-4, & TS-5):

These fractions didn't yield any crystals in the test tube. Therefore, these fractions were not studied further.

4.24.9.7.4 The Fraction T-8:

Fraction T-8 was colourless. Upon standing undisturbed at the room temperature didn't gives any crystal and thus the fraction was not studied further.

4.24.9.7.5 The fraction T-9:

It was a creamy type yellow coloured substance when concentrated gave two spots on spraying with vanillin-sulphuric acid and also I_2 active. No compounds settled down from the mother liquor and thus the fraction was not studied further.

4.24.9.7.6 The Fractions T-10, T-11, T-12 & T-13:

These fractions didn't yield any crystals in the test tube. Therefore, these fractions were not studied further.

4.25 Properties of the Compounds Isolated from MeOH Extract of *Litchi Chinensis*

4.25.1 Properties of CF-I

Physical state	: crystal solid
Solubility	: crystals could not dissolve in any solvents at room temperature and even on heating
R_f value	: nil
Amount	: ~ 8.2 mg

4.25.2 Spectral Characteristics

IR spectra of **CF-I** Figure 4.24: $\bar{\nu}_{max}$ cm^{-1} (in KBr pellet)

3300	O-H Stretching (H-bonded)
2900	C-H stretching (aliphatic)
1650	>C=C< stretching
1455	-CH_2- bending in aliphatic compound

1375	-CH_3 bending in aliphatic compound
1300-1000	C-O stretching
900-690	Aromatics (out of-plane bend)

4.25.3 Properties of CF-II

Physical state	: Colorless liquid (with significant smell)
Solubility	: Nil
R_f value	: 0.62
Amount	: ~ 1.3 mL

4.25.4 Spectral Characteristics

IR spectra of **CF-II** Figure 4.25): $\bar{\nu}_{max}$ cm^{-1} (in KBr pellet)

2900	C-H Stretching (aliphatic)
1635	>C=C< Stretching
1595	-NH- bend
1440	-CH_2- bending in aliphatic compound
1375	-CH_3- bending in aliphatic compound

Also significant peaks at 965 and 875

4.25.5 Properties of CF-III

Physical state	: White solid
Solubility	: DCM
R_f value	: 0.45
Amount	: ~ 1.75 mg

4.25.6 Spectral Characteristics

IR spectra of **CF-III** (Fig.4.26): $\bar{\nu}_{max}$ cm^{-1} (in KBr pellet)

3300	O-H Stretching (H-bonded)
2900	C-H Stretching (aliphatic)
1720	Carboxylic acid
1635&1610	>C=C<Stretching

Also significant peaks at1540, 1455, 965 and 875

4.26 Properties of the Compounds Isolated from Petroleum Extract of *Litchi chinensis*

4.26.1 Characterization of Compound SS-I:

Physical state	:	white crystal
Solubility	:	Soluble in chloroform
Rf value	:	0.38
Melting point	:	154 -157.2^{0}C
Amount	:	11.74 mg

4.26.2 Spectroscopic Characteristics

4.26.2.1 UV-Vis Spectrum Study

The UV-Vis Spectrum is shown in **Figure 4.27**

$\lambda_{Max/nm}$	**Absorbance**
296 nm	0.91

4.26.2.2 IR Spectroscopic Study (Figure 4.28)v^{-}_{max}: cm^{-1} (in KBr pellet)

The IR Spectrum is shown in **Figure 4.28;** v^{-}_{max}: cm^{-1} (in KBr pellet). The compound **'SS-I'** had importance frequencies of absorption at 3350, 2920, 2850, 1655, 1455, 1365, 1040, 965 cm^{-1}.

IR spectra of compound SS-I (Figure 4.28)

v^{-}_{max} **cm^{-1}**	
3350	-OH Stretching (H-bonded)
2920 & 2850	C-H stretching (aliphatic)
1655	>C=C< Skeleton vibration
1455	$-CH_2-$ Bending
1365	$-CH_3$ Bending
965	Steroidal in nature

Its IR spectrum showed a strong absorption band at 3350 cm^{-1} indicative of a hydroxyl group (-OH). The sharp absorption bands at 2920 and 2850 cm^{-1} were demonstrative of aliphatic C-H stretching. The absorption bands at 1455 and 1365 cm^{-1} indicated the $-CH_2-$ and $-CH_3$ bending vibrations respectively. Absorption bands at 1655 cm^{-1} is suggestive for the presence of >C=C< stretching. The band at 965 cm^{-1} was indicative of its steroidal in nature [204].

^{1}H- NMR Spectrum (400 MHz, $CDCl_3$) Study, Figure 4.29, 4.29(a), 4.30,4.30(a), 4.31, 4.32, 4.33, and 4.33(a)

The ^{1}H- NMR Spectrum (400 MHz, $CDCl_3$) was shown in **Figure 4.29, 4.29(a), 4.30, 4.30(a), 4.31, 4.32, 4.33, and 4.33(a)**. The compound **SS-I** has important chemical shift value (δ) at 5.33 (d), 5.16(dd), 5.04(dd), 3.51(m), 0.85(d), 1.00 (s).

Summary of ^{1}H- NMR Spectrum (400MHz, $CDCl_3$); (Figure 4.29 and 4.29(a)

δ_H value in ppm	**Assignment/Assesment**
0.69(s)	Methyl protons at C-18
0.797(s, J=6.2 Hz)	Methyl proton at C-29
0.845(d, J=3.2Hz)	Methyl proton at C-26
0.815(d, J=4.4Hz)	Methyl proton at C-27
1.00(s)	Methyl proton at C-19
3.51(m, J=4.4 Hz)	Oxymethine proton at C-3
5.04(dd, J=13.3, 8.0 Hz)	Trans olefinic protons
5.156(dd, J=13.3, 8.0 Hz)	attached to C-22
5.334(d, J=2.4 Hz)	Olefinic proton at C-6

Table 4.19 ^{1}H-NMR chemical shift data for the structure of identified compound '**SS-I'**

No. of protons	Chemical shift value (δ)	
	Experiment value (δ)	Reported value (δ)
H-6	5.334(d)	5.33(d)
H-22	5.156(dd, J=13.3,8.0 Hz)	5.16(dd, J=15.0,8.4 Hz)
H-23	5.04(dd, J=13.2, 8.0 Hz)	5.02(dd, J=15.0, 8.4 Hz)
H-3	3.51(m, J=4.4 Hz)	3.52(m)
19-H_3	1.00(s)	1.01(s)
21-H_3	1.001(d, J=6.4 Hz)	1.02(d, J=6.4 Hz)
26-H_3	0.85(d, J=3.2 Hz)	0.85(d, J=3.4 Hz)
27-H_3	0.845(d, J=4.4 Hz)	0.82(d, J=4.3 Hz)
29-H_3	0.797(s, J=6.2 Hz)	0.80(s, J=6.0 Hz)
18-H_3	0.69(s, J=7.2 Hz)	0.69(s, J=7.2 Hz)

^{13}C-NMR Spectrum (400MHz, $CDCl_3$) (Figure 4.34 and 4.35)

The ^{13}C- NMR Spectrum '400 MHz, $CDCl_3$' was shown in **Figure 4.34 and 4.35**. The compound **SS-1** has important chemical shift value (δ) at 140.801, 138.328, 128.5, 121.737, 71.856, 56.9, 56.024, 42.35, 37.305, 31.951, 31.710, 36.2, 29.716, 21.24, 21.114, 19.422 and 12.257. So the signals are at:

$\sim\delta$c = 140.801 ppm (C- 5)

$\sim\delta$c = 140.801 ppm (C- 10) | Three quaternary carbons

$\sim\delta$c = 42.350 ppm (C- 13)

$\sim\delta$c = 71.856 ppm was assigned for methylene carbon (C- 3).

$\sim\delta$c = 138.328 ppm (C- 22) | $>C=C<$ carbons

$\sim\delta$c = 129.341 ppm (C- 23)

$\sim\delta$c = 121.737 ppm (C- 6) | $>C=C<$ carbons (Phytosterol, John Goad).

$\sim\delta$c = 140.801 ppm (C- 5)

Table 4.20 ^{13}C-NMR Chemical shift data for the structure of identified compound '**SS-I**' [237]

No. of Carbon	Types of Carbon	Value of "δc"	
		Observed value "δc"	Reported value "δc" [237]
1	$=CH_2$	37.305	37.162
2	$=CH_2$	31.710	31.502
3	=CH-	71.856	71.074
4	$=CH_2$	*38.00	37.9
5	=CH	140.801	139.573
6	$=CH_2$	121.737	117.475
7	=CH-	29.716	29.659
8	=C=	40.492	40.281
9	=CH-	50.221	49.47
10	=C=	*34.00	34.241
11	$=CH_2$	21.24	21.563
12	$=CH_2$	39.732	39.482
13	=C=	42.350	43.308
14	=CH-	*55.8	55.113
15	$=CH_2$	22.705	23.028
16	$=CH_2$	*28.5	28.507
17	=CH-	56.024	55.862
18	$-CH_3$	12.080	12.061
19	$-CH_3$	*14.00	13.048
20	-CH-	40.49	40.823
21	$-CH_3$	21.114	21.094
22	=CH-	138.328	138.174
23	=CH-	129.341	129.462
24	=CH-	51.276	51.263
25	=CH-	31.951	31.888
26	$-CH_3$	*21.00	21.382
27	$-CH_3$	19.422	19.008
28	$-CH_3$	*25.40	25.405
29	$-CH_3$	12.257	12.250

*This observed δc value measured manually

Analysis by DEPT-135 (Figure 4.36, 4.37 and 4.38)

The DEPT-135 was shown in **Figure 4.36, 4.37 and 4.38:** The DEPT-135 gave

Positive signals at 138.326, 129.340, 121.736, 11.898, 21.096, 51.225 etc;

Negative signals at 42.351, 39.832, 39.737, 37.308, 21.244, 31.912, 29.724, 25.427 etc.

No peak at 140.801, 42.26 and 36.6.

Combining DEPT-135 and ^{13}C-NMR chemical shift data it is clear that different types of carbon was found in the structure of isolated compound.

4.27 Isolation and Characterization of Compounds from DCM Extract (PE-II) of Pericarp

The DCM extract **(PE-II)** was subjected to TLC screening to observe the type of compounds present in the extract. The whole portion of the DCM extract was subjected to column chromatography for rapid fractionation. The column fractions were screened by TLC to find out interesting fractions.

4.27.1 Column Chromatography (CC) of DCM Extract (PE-II)

The column was packed with fine silica gel (Kieselgel 60, mesh 70-230) was used as the packing material. Slurry of silica gel in n-hexane was added into a glass column having the length and diameter of 62 cm and 04 cm respectively. When the desired height of the adsorbent bed was obtained, the column was washed with few hundred milliliter of n-hexane to facilitate proper packing. The sample was prepared by adsorbing 3.5 gm of methanol soluble extract into silica gel then allowed to dry and subsequently applied on the top of the adsorbent layer. The column was then eluted with n-hexane, followed by mixtures of n-hexane and DCM of increasing polarity, then by DCM and finally with DCM and methanol mixtures of increasing polarity. Solvent systems used as mobile phases in the analysis of n-hexane soluble extract were listed in Table 3.9. A total of 19 fractions were collected.

Table 4.21 Different Solvent System Used for CC Analysis of DCM Extract

Fraction no.	Solvent Systems	Volume Collected (mL)
1	n-hexane + DCM (80 : 20)	100 + 100
2	n-hexane + DCM (70 : 30)	100 + 100
3	n-hexane + DCM (65 : 35)	100 + 100
4	n-hexane + DCM (60 : 40)	100 + 100
5	n-hexane + DCM (55 : 45)	100 + 100
6	n-hexane + DCM (50 : 50)	100 + 100
7	n-hexane + DCM (45 : 55)	100 + 100
8	n-hexane + DCM (40 : 60)	100 + 100
9	n-hexane + DCM (35 : 65)	100 + 100
10	n-hexane + DCM (30 : 70)	100 + 100
11	n-hexane + DCM (25 : 75)	100 + 100
12	n-hexane + DCM (15 : 85)	100 + 100
13	n-hexane + DCM (10 : 90)	100 + 100
14	DCM 100%	100 + 100
15	DCM + Methanol (98 : 2)	100
16	DCM + Methanol (97 : 3)	100
17	DCM + Methanol (90 : 10)	100
18	DCM + Methanol (50 : 50)	100
19	Methanol 100%	100

4.27.2 Analysis of Column Fractions by TLC

All the column fractions were screened by TCL under UV light and a number of similar fractions were mixed together and identified them by new code that is summarized in Table 4.22.

Table 4.22 List of New Fraction Codes

Ser No.	Column Fraction	New Code
1	1-3	CC-1
2	4-5	CC-2
3	6-8	CC-3
4	9-10	CC-4
5	10-12	CC-5
6	13-17	CC-6
7	18-19	CC-7

4.27.3 Attempt of Purification and Characterization of the Fractions

All the CC fractions were screened by TLC under UV light and by spraying with vanillin-sulfuric acid reagent followed by heating at 110^0 C. Depending on the TLC behavior fractions CC-1, CC-4, CC-5 and CC-6 were selected for further investigation.

Analysis of Fraction CC-1

Fraction CC-1 left undisturbed at the room temperature for several days. A white needle shaped crystal 40 mg was obtained. The crystals were separated from the mother liquor and washed with different solvents. Its TLC study (pet ether: EtOAc , 80: 20) showed three spots of which two were found to be UV active and other UV inactive. The UV inactive spots were identified by using vanillin-sulfuric acid spray reagent. The latter compound was separated by preparative thin layer chromatography (PTLC).

Preparation of Thin Layer Chromatography

The crystals (~25 mg) were dissolved in chloroform to make a solution. This solution was used to separate the component by TLC using the solvent (pet ether: EtOAc, 80: 20). After drying, the plate was covered with a paper except an edge and the uncovered part was sprayed with vanillin-sulfuric acid reagent and heated with hot air blower. Reddish pink color was observed on the edge, which became violet coloured on heating for long time. Then the plate was marked accordingly.

Separation and Elution of the Identified Band

The band was separated with the help of a clean spatula and was kept in a cleaned beaker. The ethyl acetate solution was then added to the beaker and shaken for several minutes. An elution tube was cleaned and dried then plugged with cotton and fixed with a stand. The content of the beaker was then introduced into the elution tube. The filtrate was collected in a beaker. Then another 30 mL of ethyl acetate was passed through the elution tube and the filter was collected in the beaker. After removal of the solvent, white crystal was obtained which was marked as **SS-II**.

Column Chromatography (CC) of Combined Column Fractions of (CC-4, CC-5 and CC-6)

Fraction CC-4, CC-5 and CC-6 left undisturbed at the room temperature for several days. These fractions didn't yield any crystals in the test tube. Therefore, these fractions were not studied further.

4.27.4 Properties of the Compound Isolated from DCM Extract

Characterization of SS-II

IR Spectrum (Figure 4.39) : Similar to **SS-I**

^{1}H-NMR Spectrum (400 MHz, $CDCl_3$) (Figure 4.40) : Similar to **SS-I**

5.1 Introduction

Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern [205]. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens [206]. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases [207].

Tomato (*Lycopersicon esculentum*) is one of the most important vegetables worldwide because of its high consumption, year round availability and large content of health related components [208]. The consumption of tomatoes has been proposed to reduce the risk of several chronic diseases such as cardiovascular diseases and certain types of cancer and especially prostate cancer [198; 209]. In addition, tomato consumption leads to decreased serum lipid levels and low density lipoprotein oxidation [210].

Lycopene, the major carotenoid in tomato fruit, is a powerful antioxidant, anti-inflammatory and also has an antimicrobial property and has generated much attention because of the linkage between lycopene-rich diets and lower risks of certain cancers, heart disease, and age-related disease. Although most phenolic compound in tomato fruit have disappeared at maturity, fully ripened fruit contains modest quantities of quercitin-3-0-rutinoside, which like other flavonols is a powerful antioxidant associated with reduced cancer risk. [211].

Litchi (*Litchi chinensis*) is becoming popular widely and cultivated throughout the world because of its attractive appearance, delicious taste and possible health benefits [212]. The number of infections which are caused by multi drug resistant gram positive and gram negative pathogens and viruses are life threatening for human being. Infections caused by these organisms pose a serious challenge to the scientific community and need for a effective therapy has lead for novel antimicrobial agents.

Litchi fruit pericarp (LFP) and seed accounts for approximately 50% of the whole fresh fruit and contains significant amounts of phenolics and flavonoids which are usually discarded as a waste in the process by both industry and consumers [213; 214]. The phenolics and flavonoids of LFP, seed and pulp have been confirmed to have antioxidant, anticancer as well as antimicrobial activities and have been considered a

new source of pharmaceuticals and food industry [104; 105]. Pharmacological studies indicate that the by-products of the litchi have various effects including anti-inflammatory, anti-hyperlipidemic, anti-hyperglycemic, hepatic and cardio protective, as well as having high antioxidant and antimicrobial activity [104; 105; 215].

Recent work has been carried out; aiming to evaluate the nutritional potential of the by-products of litchi fruit and the antibacterial activities of litchi fruit parts extracted using different solvents. Stigmasterol was extracted from LFP and has been investigated for its biological prospects i.e. antimicrobial and antioxidant activities. Extracts of litchi pulp and waste and extracted Stigmasterol were tested for antibacterial activity against four strains of pathogenic bacteria using agar disk diffusion method.

5.2 Determination of Antimicrobial Studies of Various Extracts of *Lycopersicon esculentum*

5.2.1 Introduction

Acetone, methanol and chloroform extracts of tomato paste revealed the presence of reducing sugar, pentoses, hexose, disaccharides, starch, glycogen, proteins and amino acids, sterols, carotinoids, flavaoids, and polyphenols using standard procedure [213; 214]. Bacterial cultures *S. aureus, S. pneumonia, P. aeruginosa, E. coli* and fugal culture of *A. niger, A. flavus, C. albicans* were purchased from the culture collection of the department of microbiology, University of Dhaka, Bangladesh. All the microorganisms were maintained at 4^0C on nutrient agar slants.

5.2.2 Extraction Procedure

Ripe tomatoes were mashed to prepare paste and weigh 20 gm of tomato paste into a 250 mL round bottomed flask. Then add 50 mL of methanol and 60 mL of dichloromethane. Heat the mixture under reflux for 5 min on stem-bath with frequent shaking. Filter the mixture under suction and transfer the filtrate to a separatory funnel. Wash this mixture containing lycopene with three portions of 150 mL each sodium chloride. Dry the organic larger over anhydrous magnesium sulfate. Filter and evaporate most of the solvent in vacuum without heating.

The tomato fruit paste was extracted using methanol, acetone and chloroform and tested for antibacterial and antifungal activities.

5.2.3 Antibacterial and Antifungal Activity Assay

The antibacterial property was evaluated by using agar diffusion method [216; 217]. The sterilized medium was inoculated 100 µl of microbial suspension 108 cfu/mL (108 colony forming unit per milliliter) of the microorganism was spread onto Mueller Hinton agar in Petridishes to give a depth of 3-4 mm paper disc. The paper disc (Whatmann number 1) impregnated with the test compounds (50, 100, 150 µg/mL in dimethyl formamide) was placed on the solidified medium and dried at 40^0C. The plates were pre-incubated for 1 hour at room temperature and incubated at 37^0C for 18-24 hour for antibacterial and antifungal activities respectively. The concentrations of Ciprofloxacin (100 µg/disc) and Ketoconazole (100 µg/disc) used as a standard and the extracts of acetone, methanol and chloroform were used as follows; 150, 100, 50 µg/mL in dimethyl formamide (DMF). The bacterial inhibition was determined by measuring the diameter of the clear zone of inhibition of growth around each disc and recorded as diameter of inhibition zone in millimeter [218].

5.2.4 Minimum Inhibitory Concentration (MIC)

The MIC was determined by agar streak dilution method. A stock solution of the synthesized compounds (100µg/mL) in Dimethyl formamide (DMF) was prepared and graded quantities of the test compounds were incorporated in specified quantities of molten nutrient agar medium. A specified quantity of the medium containing the compounds was poured into a Petridishes to give a depth of 3-4 mm and allowed to solidify. Suspension of the micro-organism were prepared to contain approximately 108 cfu/ mL and applied to plates with serially diluted compounds in Dimethyl formamide to be tested and incubated at 37^0C for 18-24 hours for bacteria and fungi.

MIC was considered to be the highest dilution i.e. lowest concentration of the test substance exhibiting no visible growth of bacteria on the plate. The observed MIC is represented in table.

Table 5.1 Antibacterial results of various (methanol, acetone and chloroform) extracts and Ciprofloxacin against the test microorganisms

Bacteria	**Diameter of zone of inhibition (mm)**									**Ciprofloxacin** (100 µg/disc)	
	150 µg/mL			**100 µg/ mL**			**50 µg/ mL**			Zone of inhibition	MIC
	AE	ME	CE	AE	ME	CE	AE	ME	CE		
S. aureus	18.5	22.5	21	17	20	20.5	16	16.5	18	38	0.2
E. coli	20	21	22.5	19	19.5	21	17	17	18.5	38	0.2
P. aeruginosa	19	19	22.5	17	17	20.5	16.5	15.5	19	37	0.2
S. pneumonia	19.5	21.5	22	17.5	19	20	16.5	17.5	19	38	0.2

Note: Acetone Extract-AE, Methanol Extract-ME and Chloroform Extract-CE Control: DMF, Standard – Ciprofloxacin

Table 5.2 Antifungal results for various (methanol, acetone and chloroform) extracts and Ketoconazole against the test microorganisms

Fungi	**Diameter of zone of inhibition (mm)**									**Ketoconazole** (100 µg/disc)	
	150 µg/ mL			**100 µg/ mL**			**50 µg/ mL**			Zone of inhibition	MIC
	AE	ME	CE	AE	ME	CE	AE	ME	CE		
A. niger	18.5	22	23	15.5	18.5	20.5	15.5	17.5	19	38	6.1
A. flavus	18	20.5	21	17	19	21	14	18	18.5	35	6.1
B. albicans	18.5	21	22	17.5	17.5	19.5	14.5	16	18	36	6.1

Note: Acetone Extract-AE, Methanol Extract-ME and Chloroform Extract- CE Control: DMF, Standard – Ketoconazole

Table 5.3 Minimum Inhibition Concentration (MIC) of various (methanol, acetone and chloroform) extracts (bacteria & fungi)

Extracts	Minimum Inhibition Concentration (µg/ mL)						
	S. aureus	*E. coli*	*P. aeruginosa*	*S. pneumonia*	*A. niger*	*A. flavus*	*B. albicans*
AE	33	32	15	31	34	37	32
ME	36	31	32	33	17	36	35
CE	17	16	39	17	25	18	16

5.2.5 Discussion on Antimicrobial Assay

Investigation of antibacterial activity of various extracts on some of the isolated microbes shows that the tomatoes have an antibacterial properties when compare with Ciprofloxacin an antibacterial drug and antifungal activities when compare with Ketoconazole an antifungal drug based on their MIC parameter (Table 5.1, 5.2 and 5.3). Inhibition effects of various (methanol, acetone and chloroform) tomato extracts were noticeable and possess both antibacterial and antifungal activity. These activities are due to presence of phytoconstituents mainly phenolics, flavaoids and Sterols (Chapter 4.18.1.5) compounds. It is to be mention here that chloroform Extract showed more antibacterial as well as antifungal activities. Lycopene, the red pigment of tomato, is a tetraterpene assembled from eight isoprene units composed entirely of carbon and hydrogen, containing 11 conjugated and two nonconjugated carbon-carbon double bonds [136]. Lycopene-rich diet reduces the incidence of cancers and heart diseases and possesses antibacterial and antifungal properties [146, 220]. Lycopene exerts potent antifungal activity against *C. albicans* by causing significant damage to the cell membrane [221].

5.3 Determination of Antimicrobial Activities of Litchi Fruit Parts Extracted Using two Different Solvents

5.3.1 Introduction

The antimicrobial activities of litchi fruit parts extracted using two different solvents (ethanol and acetone) of both *Litchi chinensis* pulp and waste (including seeds and pericarp). Extracts were tested for antibacterial activity against four strains of pathogenic bacteria using agar diffusion method. These inhibited the growth of *E. coli*, *P. aeruginosa, S.aureus* and *B. subtilis*.

5.3.2 Disc Diffusion Assay

Antimicrobial activity of the tested extracts was determined using sensitivity of different bacterial strains to various concentrations of each extract in terms of zone of inhibition using a modified Kirby-Bauer disc diffusion method [5]. Briefly, 100 µl of microbial suspension (108 cells/ mL for bacteria) was spread onto Mueller Hinton agar in Petri plates corresponding to the broth in which they were maintained. An aliquot 10 µl of each extract was pipetted on a sterile Whatmann number-1, 5 mm paper disc on the agar surface. Bacitracin used as standard antibacterial agent served as positive control for antimicrobial activity. The plates were inverted and incubated for 18-24 hours at 37°C. A total of four bacterial pathogens were tested including *E. coli, P.aeruginosa, S. aureus, and B. subtilis*. The bacterial inhibition was determined by measuring the diameter of the clear zone of inhibition of growth around each disc and recorded as diameter of inhibition zone in millimeter.

Table 5.4 Antimicrobial activity of litchi pulp and waste extracts against the test microorganisms

Micro organisms	Gram reaction	Diameter of zone of inhibition (mm)				
		Bacitracin	**PEE(%)**	**PAE**	**WEE(%)**	**WAE(%)**
E. coli	G-	20.5	00	00	10.5(51)	09(44)
P. aeruginosa	G-	18.5	08(43)	00	11.5(62)	10(54)
S. aureus	G+	14.5	00	00	10.5(72)	8.5(58)
B.subtilis	G+	17	09(53)	00	12(70)	10.5(62)

Note: PEE: Pulp Ethanolic Extract; PAE: Pulp Acetoneic Extract; WEE: Waste Ethanolic Extract; WAE: Waste Acetoneic Extract.

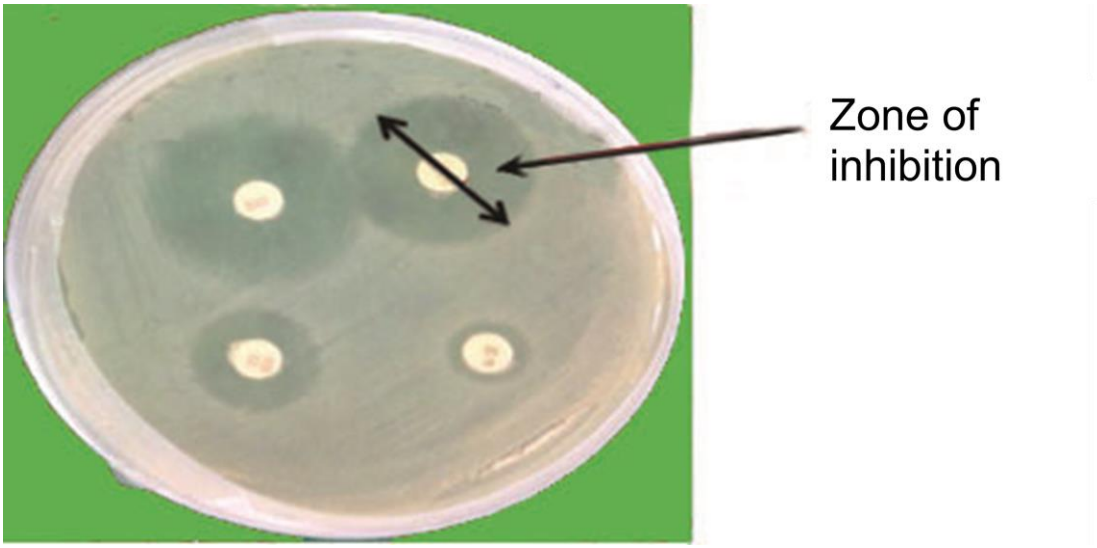


Figure 5.1 Antimicrobial activity of Bacitracin

5.3.4 Discussion on Antimicrobial Assay

The results of antimicrobial activity of litchi pulp and waste extracts are presented in Table 5.4. The antibacterial activity of these extracts was compared with bacitracin used as standard antibiotic. Ethanolic extract of waste showed the highest inhibition over acetonic extracts. The percent of inhibition of the growth of *E. coli, P.aeruginosa, S. aureus and B. subtilis* was equal to 51, 62, 72 and 70% respectively.

The results revealed that litchi waste ethanolic extract exerted the highest inhibition against all tested bacterial strains compared to other extracts while pulp acetonic extract

showed no effect against most of tested bacteria and waste acetoneic extract showed moderately inhibited all four microbes. It is worthy to note that the results revealed that both litchi waste extracts have positive effect on all tested bacteria.

5.4 Determination of Antimicrobial Activity of Stigmasterol Isolated from Litchi Fruit Pericrp (LFP) of Litchi (*Litchi chinensis Sonn.*)

5.4.1 Intruduction

The purpose of this work was to evaluate the antimicrobial activity of the extracted Stigmasterol from LFP. Extracted Stigmasterol was tested for antibacterial activity against four strains of pathogenic bacteria using agar diffusion method. Stigmasterol is the phytosterol with chemical structure similar to the cholesterol. It is an important nutrient in the diet meal, hydrophobic and soluble in organic solvents and considered as a good biomarker due to its antioxidant and antimicrobial activities.

5.4.2 Test Micro-organisms

A total of four bacterial pathogens were tested including *E. coli, P. aeruginosa, S. aureus,* and *K. pneumoniae.*

5.4.3 Disc Diffusion Assay

Antimicrobial activity of the extracted Stigmasterol from LFP was determined by the agar disk diffusion method of protocol given by Doughari et al. [222] with little modification. Gentamicin was used as a standard antimicrobial agent. *E. coli, P. aeruginosa, S. aureus and K. pneumoniae* were used as the test organisms. An aliquot 20 μg/mL of extracted Stigmasterol and Gentamicin was pipetted on a sterile Whatmann number-1, 5 mm paper disc on the agar surface. All the plates were incubated at 37^0 C for 24-48 hour. Antimicrobial activity was determined by measuring the zone of inhibition in millimeters [223]. In comparison with standard antibacterial compound Gentamicin (20 μg /mL); it was found that purified Stigmasterol (20 μg /mL) has antimicrobial activity almost equivalent to the standard.

Table 5.5 Antimicrobial activity of stigmasterol and gentamicin against the test microorganisms

Test Organisms	Diameter of zone of inhibition (mm)	
	Purified Stigmasterol	**Gentamicin**
E. coli	23.50	27.50
S. aureus	19.50	23.00
K. pneumoniae	17.00	19.50
P. aeruginosa	12.00	20.50

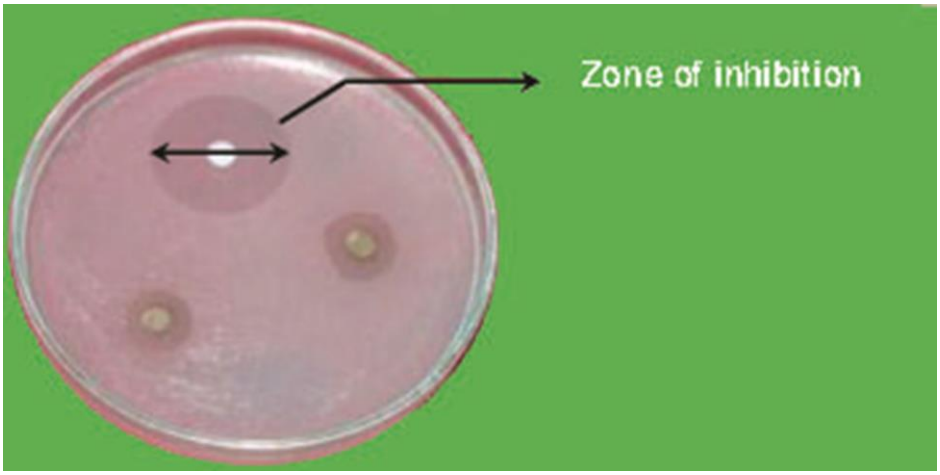


Figure 5.2 Antimicrobial activities of extracted Stigmasterol and Gentamicin

5.4.4 Discussion on Antimicrobial Assay

The antimicrobial activity of the purified Stigmasterol was evaluated and the results are summarized in the Table-5.5 and Figure-5.2. The antimicrobial activity, determined by measuring the 'inhibition zone' around the disc, ranged from 12 mm to 23.5 mm for the Stigmasterol and 19.5 mm to 27.5 for Gentamicin. Thus, the antimicrobial activity of the Stigmasterol was comparable to the standard Gentamicin. Hence Stigmasterol is having higher antimicrobial activity against *E. coli* followed by *S. aureus, K. pneumoniae* and *P. aeruginosa* at lower concentration. Thus these results prove the antimicrobial potential of Stigmasterol from LFP and further, it can serve as an alternative chemical to treat the bacterial diseases.

5.5 Determination of Antioxidant Activity of Litchi Fruits Pericarp (LFP) of Litchi (*Litchi chinensis*)

5.5.1 Introduction

Polyphenols are naturally occurring compounds widely found in the fruits, vegetables, cereals and other plants, which contribute to the colour, flavor and defend a plant from ultraviolet rays or pathogens [224]. The antioxidant activity found in the LFP of litchi is high, and is mainly due to phenolics, flavonoids and ascorbic acids. These bioactive compounds consider being the sources of natural antioxidants and having antioxidant activity which is capable of blocking the harmful effects of free radicals in the body.

To achieve the antioxidant activity LFP was treated with preheated Charcoal to make them free from oily and colouring materials. This cleaned mixture contains powerful antioxidant compounds, such as ascorbic acid, phenolic acids and flavonoids. The antioxidant properties of *Litchi chinensis* extract was evaluated using Ferric (Fe^{3+}) reducing antioxidant power assay activity

5.5.2 Ferric (Fe^{3+}) Reducing Antioxidant Power (FRAP) Assay

Antioxidant activity of methanolic extracts of Litchi fruit pericarp (LFP) of litchi determined by using Charcoal treatment. In this treatment 3 gm of preheated charcoal was added to the methanol extract (180 mL) and shaken occasionally for half an hour. Then the Charcoal treated methanol extract was filtered through Whatmann no-1 filter paper and the filtrate marked as **F-1** (filtrate-1). Filtrate-1 was concentrated (45 mL) in vacuo. This concentrated mass marked as **CM-I**. After filtration, the residue part was treated with aqueous ethyl alcohol (EtOH) in a conical flask marked as **F-2 (**filtrate-2) and again concentrated in vacuo which was marked as **CM-II.**

5.5.3 Properties and Spectral Characteristics of CM-I (MeOH Extract) and CM-II (EtOH Extract)

Properties of CM-I

Physical state	: reddish gummy solid.
Solubility	: Partially soluble in Ethyl acetate and Chloroform
and	completely soluble in methanol
R_f value	: 0.56 {ethyl acetate: pet ether (1:1)}
Amount	: ~ 5 mg (crude)

Spectral Characteristics of CM-I

UV spectra of **CM-I** (Fig.4.44)

$\lambda_{max/nm}$	Absorbance
288	0.989

UV spectra of **CM-I** with 3 drops of $NaOH_{(aq)}$ (Figure 4.45):

$\lambda_{max/nm}$	Absorbance
301	1.134

Spectral Characteristics of CM-II

UV spectra of **CM-II** (Figure 4.46)

$\lambda_{max/nm}$	Absorbance
289	0.953

5.5.4 Investigation of CM-I (MeOH Extract)

About 1 mL of **CM-I** was dissolved in ethanol and treated with a few drops of freshly prepared ethanolic $FeCl_3$ solution. The colour of the solution changes to violet which indicates the compound **CM-I** as phenolic in nature. Again in the earlier solution a few drops of NH_3 solution was added and observed that initial colour changes to green and then to reddish brown. This experiment suggests that the phenolic compound has donated an electron (e^-) to the Fe^{3+} (Ferric) and converted it to Fe^{2+} (Ferrous). The antioxidant properties of phenolic compounds were arises from their high reactivity as hydrogen or electron donors and from the ability of polyphenol-derived radicals to stabilize and delocalize the unpaired electron or from their ability to chelate transition metal ions (*i.e.* cause termination of the Fenton reaction).

Reaction: $\mathbf{Fe^{3+} + e^- = Fe^{2+}}$

The formation of $Fe^{2+}$is a necessary condition for the absorption of Iron and formation of Hb (Hemoglobin) in the living organism. Therefore, the phenolic compound **CM-I** is thought to have the role of iron absorption. This experiment suggests that the phenolic compound present in the litchi pericarp possesses the antioxidant property.

The methanol extract of LFP of litchi was used to investigate Fe^{3+} to Fe^{2+} transition to measure the reducing capacity of LFP. The reducing capacity of a compound may serve

as a significant indicator of its potential antioxidant activity. Various mechanisms such as binding of transition metal ion catalysts, decomposition of peroxides, prevention of chain initiation, prevention of continued hydrogen abstraction and radical scavenging are attributed to the antioxidant activities of the antioxidants.

5.5.5 Investigation of CM-II (EtOH)

In the second experiment **CM-II** was treated with freshly prepared ethanolic $FeCl_3$ solution and was kept for 72 hours at room temperature then 05 mL of ethyl acetate (EtOAc) was added. After that TLC was taken and studies showed that the spots did not correspond with those of the **CM-II**. This study indicates that the Fe^{3+} present in the fruits pericarp upon keeping for long time is thought to break down the phenolic compounds and converted its colour (fade).

This experiment presents a challenge to identify the break down products upon long time interaction of Fe^{3+} (usually present in the fruit pericarp) with the phenolic compounds.

5.5.6 Discussion on Antioxidants Assay

Anti-oxidants may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid per-oxidation and other mechanisms [225]. This research work demonstrates that daily consumption of litchi might be helpful in preventing or suppressing the progress of various oxidative stress related disease.

Part-A

6.1 Phytochemical Screening

The Tomato (*Lycopersicon esculentum*) is one of the most important widely consumed vegetables of the *Solanaceae* family. General phytochemical screening of the tomato paste revealed the presence of different functional groups such as unsaturation, carbonyl group, acetyl group, carboxylic acid, phenolic compounds etc., and phytochemicals such as steroids, flavonoids, alkaloids, different fatty acids, amino acids, glucose and ascorbic acid was present in this tomato paste (Chapter 4.3- 4.7).

Three fatty acid ester compounds were identified with retention time at 3.50, 12.53 and 15.31 which was compared with standard of fatty acid and reveals the presence of plamitic acid, caprylic acid and steric acid respectively from the pet ether extract. Four different characteristic pink coloured spots were detected which was compared with standard of amino acids and reveals the presence of glycine, alanine, glutamine and glutamic acid in R. spirit extract. Two sugar compounds such as glucose and arabinose were isolated from methanolic extract of *Lycopersicon esculentum* (Chapter 4.5-4.7). The amount of glucose and vitamin-C in aqueous methanolic extract was estimated experimentally and found 1.94 g/kg and 4.136×10^{-1} g/kg respectively [204] (Chapter 4.7- 4.8).

6.2 Extraction and Isolation of Lycopene and β-Carotene from *Lycopersicon esculentum*

Extraction of carotenoids (lycopene and β-carotene) was done on the preliminary trial basis and gave the results of suitable solvent/solvent mixture and the optimum method/methods which we applied mainly for this soxhlet extraction process.

The soxhlet method was selected for this research because of the low cost, multiple extractions with recycled solvent, controlled temperature and condensation conditions, and the system operates under vacuum. Recycling the solvent minimizes the amount of solvent required. These features make the soxhlet extraction method most suitable for extracting lycopene from tomato paste in controlled laboratory conditions.

6.2.1 Preliminary Trials

A trial was done using 100% ethyl acetate solvent (EtOAc) to extract lycopene from the tomato paste of *Lycopersicon esculentum*. The sample was separated into two layers. Upper organic layer containing the lycopene and a lower aqueous water layer (Figure 4.5). Upper organic layer was separated and the presence of lycopene was confirmed by thin layer chromatography using silica gel plates and toluene: hexane (1:19, v/v) as mobile phase and by UV-Vis, IR spectrum (Figure 4.22-4.23).

A second trial was done using a di-mixture of acetone: pet ether (1:1, v/v) solvent. When distilled water was added and the solution centrifuged, it separated into an upper organic layer containing the lycopene and a lower aqueous water layer (Figure 4.7). The presence of lycopene in the upper layer was confirmed by thin layer chromatography and by UV-Vis, IR spectrum (Figure 4.18-4.19).

The third trial was done using a tri-mixture of hexane: acetone: ethanol (2:1:1, v/v/v) to extract lycopene and other carotenoids (β-carotenoids) from the tomato paste. Separating funnel was used to separate these layers. The upper layer contains lycopene and β-carotene (Figure 4.10) which was further separated using a silica column (Figure 4.11) and to confirm the lycopene and β-carotene the separated fraction samples run on thin layer chromatography (Figure 4.12) followed by UV-Vis, IR spectrum (Figure 4.20-4.21). Petroleum ether with a low boiling point and dichloromethane was used to pass through the column to separate the β-carotene and lycopene (Figure 4.13-4.14).

6.2.2 Soxhlet Extraction

Soxhlet extraction method was carried out to extract lycopene from the tomato paste of *Lycopersicon esculentum* using 100% ethyl acetate solvent. Since the ethyl acetate (EtOAc) solvent was the best solvent (results from preliminary trials) and because of its low toxicity compared to the other organic solvents, it is also used in pharmaceutical preparations, food additives and fragrances. It is to be mention here that EtOAc has been approved for use in food products by the U.S. Food and Drug Administration and biodegrades completely into CO_2 and water [199] that's why it has been used for this extraction method (Chapter 4.10; Figure 4.15). At the end of the extraction remaining

solvent was then evaporated in vacuo to get the dried concentrate containing extract which was then weighed to get crystal lycopene.

6.3 Thin Layer Chromatography (TLC)

TLC using silica gel plates as stationary phase and toluene: hexane (1:19, v/v) were used as mobile phase. Lycopene when using in toluene-hexane (1:19, v/v) mobile phase appears as a reddish orange band and the β-carotene fraction has a light yellowish band (Figure 4.12).

The crude carotenoid fraction gave a reddish orange spot (R_f value was 0.34) and a yellow (R_f value was 0.56) spot on TLC silica plates using a mobile phase toluene-hexane. Lycopene is an acyclic compound. These compounds tend to crystallize on TLC plates, resulting in poor resolution. Including toluene or dichloromethane in the mobile phase keeps these compounds in solution without affecting the chromatographic separation.

Thin layer chromatography analysis is cheaper than HPLC and multiple samples can be analyzed simultaneously on the same plate under the same conditions. However, it is less accurate than HPLC, and only indicates the presence of lycopene, rather than the amount.

6.3.1 Analysis and Characterization

The colour of lycopene and β-carotene are due to the double bonds in their structure (lycopene has more double bonds than β-carotene). The β-carotene was separated as a distinct yellow band from the reddish-orange lycopene. The β-carotene (non-polar) was not as strongly bound to the silica and eluted before the lycopene. Lycopene gives a red-orange band and due to its high degree of unsaturation, eluted after yellowish β-carotene pigments.

Thin layer chromatography using silica gel plates confirmed the fractions were lycopene and β-carotene and was used to isolate and purify extracted lycopene and β-carotene and compare the samples with standards lycopene (Sigma, New Zealand) (Figure 4.12). The β-carotene dicyclic (upper spot), γ-carotene (monocyclic) the middle spot and lycopene (acyclic) lower spot (Figure 4.12) appeared on the TLC plate. Separated carotenes can easily be distinguished by their characteristic colour. Because of its strong colour, lycopene is easily detected on TLC.

6.4. Determining Optimum Wavelength by IR and UV-Vis Spectrum

6.4.1 Spectral Characteristics of Di-mixture Extract (Le-DME)

Lycopene extract from tomato is Reddish orange viscous liquid. It is soluble in ethyl acetate and n-hexane, partially soluble in ethanol and acetone, and insoluble in water. R_f was calculated from TLC and found 0.34 and 0.56 respectively. The melting point was determined at 174^0C. The amount of lycopene was 17 mg per 100 g of tomato paste.

The UV-Vis spectra of Le–DME (Figure 4.18):

The UV spectrum exhibited absorbance **($\lambda_{Max/nm}$)** at 662, 481, 470 and 383 nm. The absorbance ($\lambda_{Max/nm}$) at 662, 481 and 470 nm was indicative of *trans*-lycopene and at 383 nm indicative of *cis*-lycopene [169; 226].

The IR spectrum of Le –DME (Figure 4.19):

The IR spectrum exhibited a sharp peak at 3350 cm^{-1} indicative of the presence of -OH group (H-bonded). A sharp peak at 2920 and 2850 cm^{-1} indicative of aliphatic -C-H stretching for -C-H or H >C=C groups respectively. The adsorption bend at 1710 cm^{-1} were indicative of (>C=O) carbonyl group. The band at 1455 and 1365 cm^{-1} was demonstrative of $-CH_2-$ and $–CH_3$ bending.

6.4.2 Spectral Characteristics of Tri-mixture Extract (Le –TME)

Lycopene extract from tomato is Reddish orange viscous liquid. It is soluble in ethyl acetate and n-hexane, partially soluble in ethanol and acetone, and insoluble in water. R_f was calculated and found 0.34 and 0.56. The melting point was determined at 175^0C. The amount of lycopene is 21 mg per 100 g of tomato paste.

The UV-Vis spectra of Le–TME (Figure 4.20):

The UV spectrum exhibited absorbance **($\lambda_{Max/nm}$)** at 502, 470, 446, 362 and 346 nm. The absorbance ($\lambda_{Max/nm}$) at 502, 470 and 446 nm was indicative of *trans*-lycopene and at 362 and 346 nm indicative of *cis*-lycopene [169; 226].

The IR spectrum of Le –TME (Figure 4.21):

The IR spectrum exhibited a sharp peak at 2900 cm^{-1} indicative of the presence of -C-H Stretching. A sharp peak at 1725 cm^{-1} indicative of aliphatic >C=O Stretching O-H Stretching (H-bonded). The band at 1650 cm^{-1} were indicative of carbon-carbon

(>C=C<) double bond skeletal units [230]. The band at 1455 and 1380 cm^{-1} were demonstrative of -CH_2- bending and -CH_3 bending vibrations respectively [232].

6.4.3 Spectral Characteristics of Soxhlet Extraction (Le –SEAE) and Le – EAE

Lycopene extract from tomato is Dark-red viscous liquid. It is soluble in ethyl acetate and n-hexane, partially soluble in ethanol and acetone, and insoluble in water. R_f was calculated and found 0.34 and 0.56. The melting point was determined at 174^0C. The amount of lycopene is 29 mg per 100 g of tomato paste.

The UV-Vis spectra of Le–SEAE and Le – EAE (Figure 4.22):

The UV spectrum showed absorbance **($\lambda_{Max/nm}$)** at 516, 484, 458, 375 and 359.5 nm. The absorbance ($\lambda_{Max/nm}$) at 516 and 484 nm was indicative of *trans*-lycopene, and at 458 indicative of β-carotene [232] and at 375 and 359.5 nm indicative of *cis*-lycopene [169; 226]

IR spectra of Le –SEAE and Le-EAE (Figure 4.23): V_{max} cm^{-1}.

The IR spectrum of **Le –SEAE and Le – EAE** exhibited a sharp peak at 3400 cm^{-1} indicative of aliphatic -OH group stretching. The adsorption bend at 1725 – 1700 cm^{-1} and 1650 $cm^{-}1$ were indicative of (>C=O) carbonyl group and carbon –carbon (>C=C<) bond respectively. The band at 1455 and 1380 cm^{-1} was demonstrative of -CH_2- and -CH_3 bending respectively [232].

6.5 Analysis of Peaks from UV-Vis Spectrum

The UV-Vis absorption of lycopene extracted from the ethyl acetate (100%), di-mixture {acetone: pet ether, 1:1 (v/v)}, tri-mixture (hexane: acetone: ethanol, 2:1:1, v/v/v) and soxhlet extraction using ethyl acetate (100%) compared with a standard lycopene (Sigma New Zealand) was measured in the range 250 to 600 nm. The UV-Vis spectra of the lycopene standard absorption peaks at 455, 480, and 513 nm reported by Seifi et al. [201] (Figure 6.1).

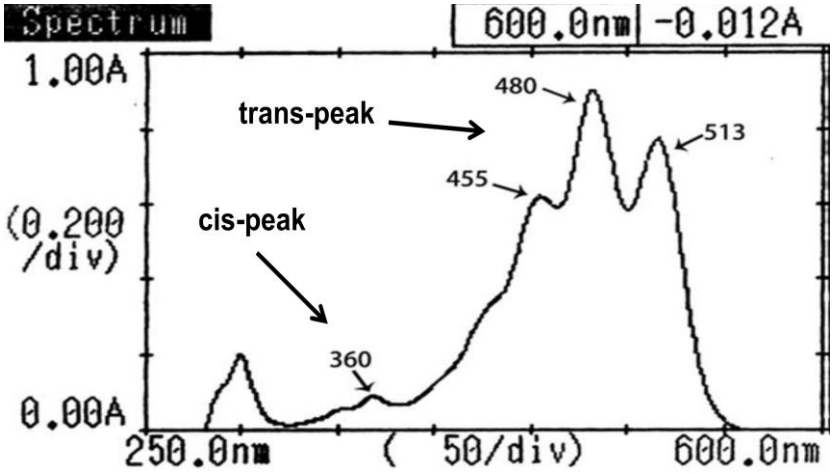


Figure 6.1 UV-Vis absorption spectra (250 to 600 nm) of 10μg/mL lycopene in dichloromethane [201]

Kun et al. [202] also used UV-Vis to identify lycopene extracted from tomato paste using 2:1:1 hexane, ethanol, and acetone. The optical density of the hexane extract was measured at 480 nm.

Different solvents in preliminary trials were tested. The UV-Vis spectrum of the lycopene extracted from tomato paste using EtOAc (100%) was sharp absorption peaks at 516, 484, 458, 375 and 359.5 nm (Figure 4.22). On the other hand lycopene extracted from tomato paste using di-mixture (acetone: pet ether, 1:1) and tri-mixture (n-hexane: acetone: ethanol, 2:1:1) solvents showed absorption peaks at 662,481, 470 and 383 nm (Figure 4.18) and 502, 470, 446, 362 and 346 nm (Figure 4.20) respectively. The UV-Vis spectrum of the lycopene was reported and the measured maximum absorption peaks at 513,480, 455 and 360 nm [201] at 480 nm [202] and at 472 nm [227]. The results show that EtOAc was the best solvent for extracting of *trans*-and *cis*-isomers of lycopene and β-carotene (Figure 4.22 and Figure 6.1). Ethyl acetate (EtOAc) solvent shows excellent UV-Vis sharp absorption peaks for lycopene indicating that no other components or contaminants were present compared with a standard. This solvent was favored because of its low cost, low toxicity, and agreeable odour compared to the other organic solvents.

"So far this is a new dimension in the research field of solvent extraction i.e. preliminary trials may be carried out before to proceed for Soxhlet extraction. This helped to select the suitable, optimum solvent/solvents and method/methods for extraction to obtain compounds. These receive support from the sharp peaks"

The identification of *cis*-carotenoids was based on the UV spectrum which exhibits a new maximum around 330-350 nm (*cis*-peak), the intensity of which depends on the localization of the *cis*-double bond and is greatest when the double bond is located near or at the center of the chromophore [226]. Published research showed that all-*trans* lycopene accounts for 79 to 91% and *cis* lycopene isomers for 9 to 21% of total lycopene in tomatoes, tomato paste, and tomato soup [42; 229].

6.6 Stability of Carotenoids

Lycopene exists as different isomers such as 15-*cis*-, 13-*cis*-, 9-*cis*-, and all-*trans*-lycopene. The predominant isomer is all-*trans* in fresh tomato but is isomerized from trans- to the *cis*-form by heat, light and some chemical reactions [228]. The chemical structure of predisposes lycopene, particularly the long chain of conjugated carbon-carbon (>C=C<) double bonds, to isomerization and degradation upon exposure to light, heat and oxygen [22] and the subsequent loss of its colouring properties [24].

Lycopene is susceptible to chemical changes such as oxidation followed by degradation or isomerization when exposed to light, heat and oxygen. Experiment was done with a keen interest to know the degradation of lycopene in three different conditions. Lycopene stability of tomato paste was assessed by UV-Vis spectrum.
Extract-I was kept in refrigerator at 4^0C, **extract-II** was kept in room temperature without exposure of light and heat and **extract-III** was exposed to light, heat and in an open air for several weeks. The UV-Vis spectrum was taken after several weeks and the stability data graph is provided in Figures 4.41, 4.42 and {4.43, 4.43(a), 4.43(b)}.

The UV-Vis spectrums in figures 4.41, 4.42 and {4.43, 4.43(a), 4.43(b)} of different conditions were examined. The UV spectrum of lycopene of extract-I (Figure 4.41) and extract-II (Figure 4.42) was sharp peaks that means lycopene did not degraded in the solvent but it was remarkable to note in case of extract-III figures {4.43, 4.43(a), 4.43(b)} which was faded from its original colour and showed only lines without peaks or bands (UV taken four times). It was anticipated that the lycopene long chain of conjugated carbon-carbon (>C=C<) double bonds, to isomerization and degradation upon exposure to light, heat and oxygen [22] and the subsequent loss of its colouring properties [24] and this would render tomato extract ineffective as food colour. Lycopene is more stable in a tomato matrix, compared to isolated, purified or in solvents.

6.7 Antimicrobial Activities of Various (methanol, acetone and chloroform) Extracts of Tomato

6.7.1 Antimicrobial Assay

Standard drug Ciprofloxacin, Ketoconazole were used as a standard and Bacterial cultures *S. aureus, S. pneumonia, P. aeruginosa, E. coli* and fugal culture of *A. niger, A. flavus, C. albicans* were used to determine the antimicrobial activities of various extracts. The tomato fruit paste was extracted using methanol, acetone and chloroform and tested for antibacterial activities compare with Ciprofloxacin and antifungal activities compare with Ketoconazole based on their MIC parameter (Table 5.1, 5.2 and 5.3).

The antibacterial activity was evaluated by using agar diffusion method [216; 217]. The bacterial inhibition was determined by measuring the diameter of the clear zone of inhibition of growth around each disc and recorded as diameter of inhibition zone in millimeter [218]. The concentrations of Ciprofloxacin (100 μg/disc) and Ketoconazoloe (100 μg/disc) used as a standard and the extracts of acetone, methanol and chloroform were used as follows; 150, 100, 50 μg/mL in dimethyl formamide (DMF) to determine the antibacterial and antifungal activities (Table 5.1 and 5.2). MIC was considered to be the highest dilution i.e., lowest concentration of the test substance exhibiting no visible growth of bacteria on the plate. The results of antimicrobial activities and MIC were shown in Table 5.3. The pictorial views of antibacterial and antifungal activities are shown below:

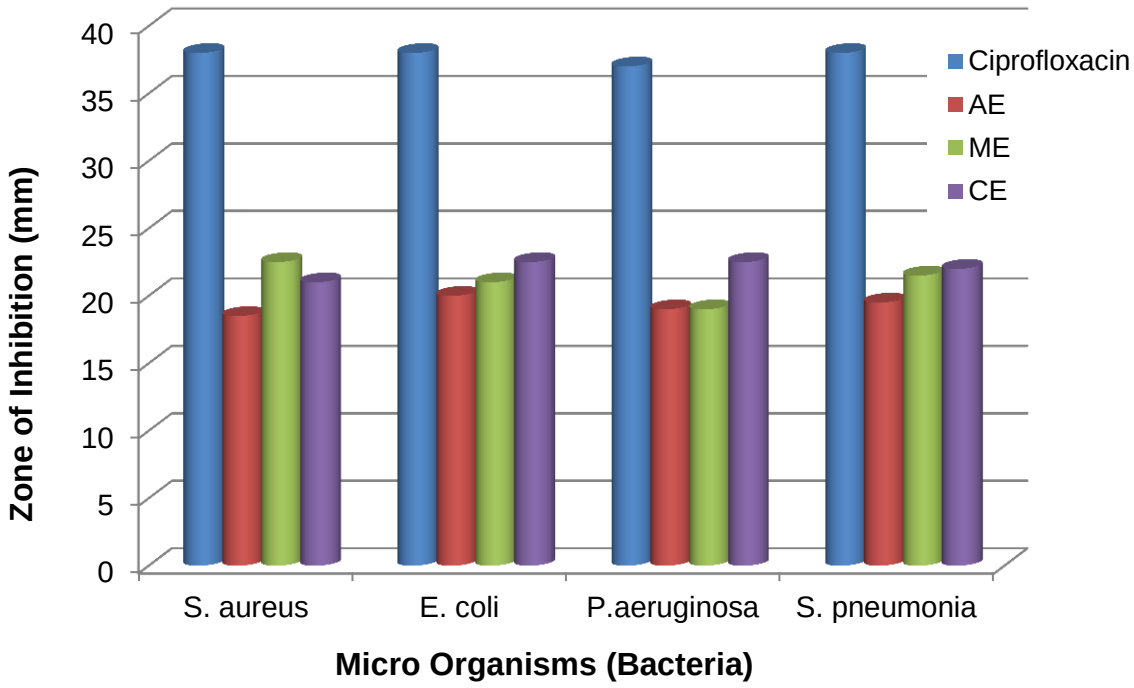


Figure 6.2 Vertical Graph of Comparison of Antibacterial Activity when 150 mg Various Extracts was and Ciprofloxacin (100 µg/disc) and Administered against the Test Microorganisms

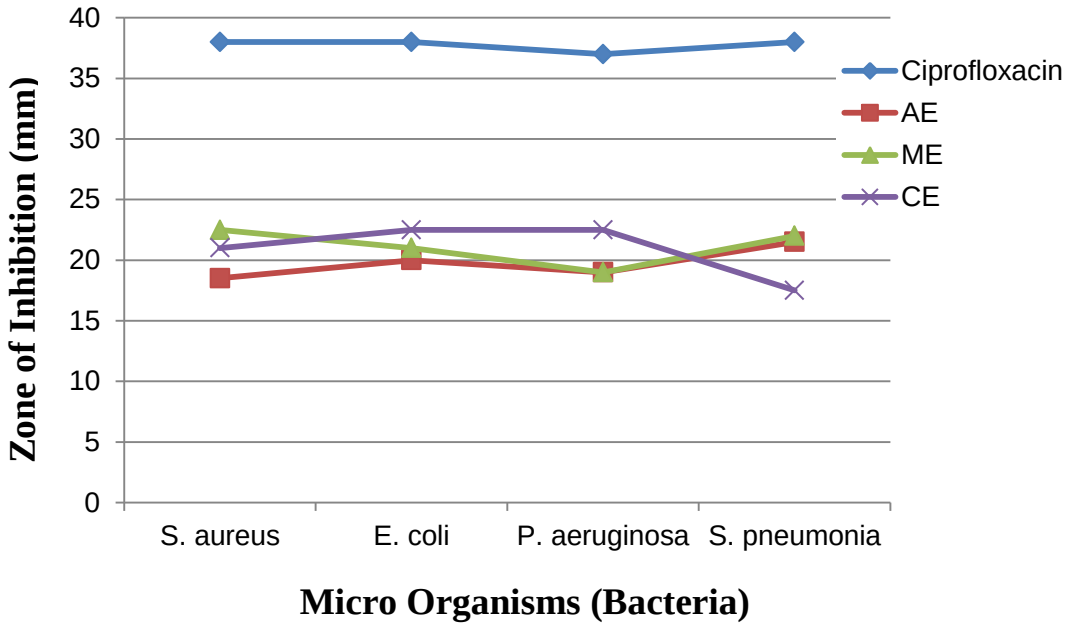


Figure 6.3 Horizontal Graph of Comparison of Antibacterial Activity when 150 mg Various Extracts was and Ciprofloxacin (100 µg/disc) and Administered against the Test Microorganisms

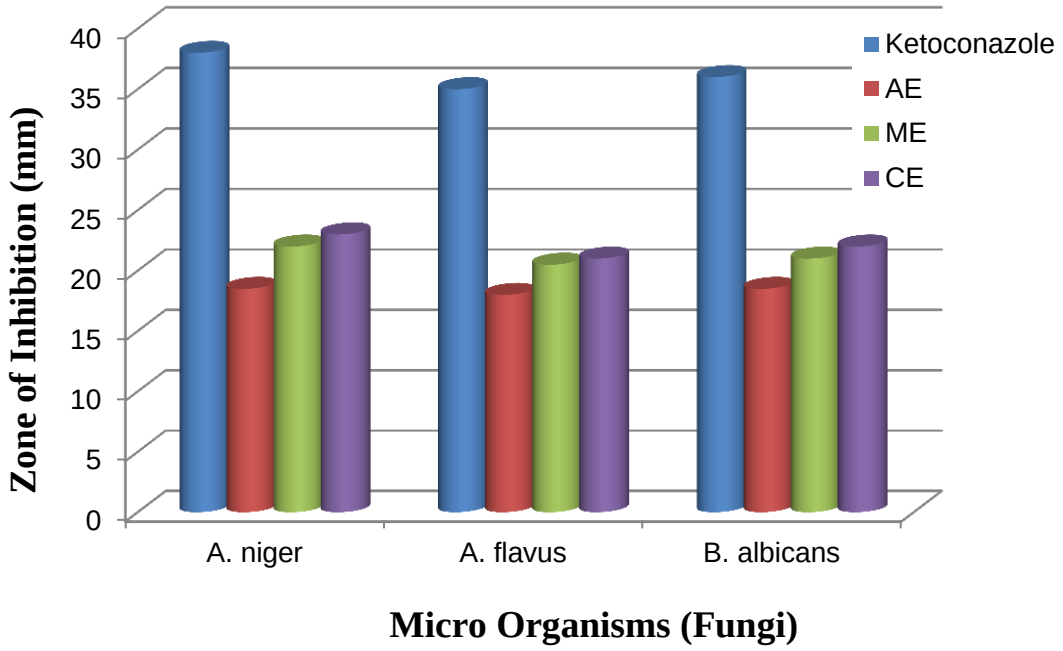


Figure 6.4 Vertical Graph of Comparison of Antifungal Activity when 150 mg Various Extracts was and Ciprofloxacin (100 µg/disc) and Administered against the Test Microorganisms

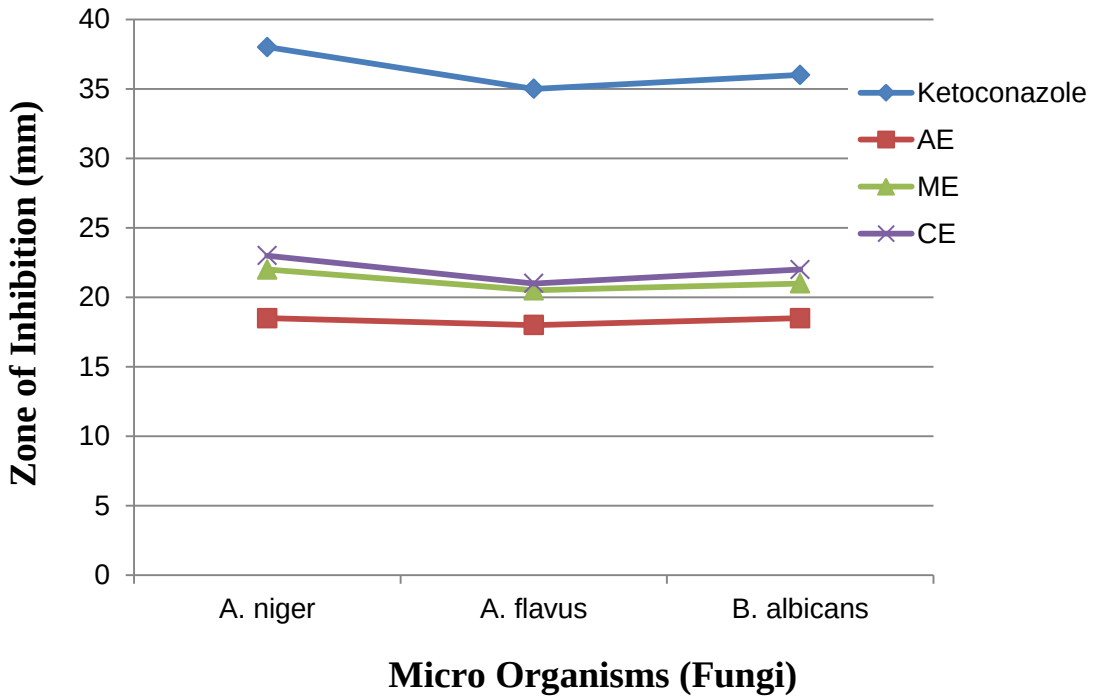


Figure 6.5 Horizontal Graph of Comparison of Antifungal Activity when 150 mg Various Extracts was and Ciprofloxacin (100 µg/disc) and Administered against the Test Microorganisms

6.7.2 Evaluation of Antimicrobial Assay

Inhibition effects of various tomato paste extracts were noticeable and found that most of the extracts executed moderate to good antimicrobial activity (Table 5.1 and 5.2) against the tested micro-organisms. Antimicrobial activities are due to presence of phytoconstituents mainly lycopene, Sterols and phenolic compounds (Chapter 4.4.6). It is to be mention here that chloroform Extract showed more antibacterial as well as antifungal activities. Lycopene extracts possesses antibacterial and antifungal properties [219; 220] and have potent antifungal activity against *C. albicans* by causing significant damage to the cell membrane [221].

This research established the fact that tomatoes can be used to treat diverse diseases and its antimicrobial effects on some selected clinical isolates confirmed it usefulness as antibiotic in the treatment of infectious diseases. So it is expected that tomatoes possess pharmacological properties which if properly harness can be used in the management of diseases.

Part-B

6.8 Phytochemical Screening

The Litchi (*Litchi chinensis*) is the most renowned of a group of edible fruits belonging to family, *Sapindaceae*. General phytochemical screening of litchi fruit pericarp (LFP) revealed the presence of different functional groups such as unsaturation, carbonyl group, acetyl group, carboxylic acids and phenolic compounds (Chapter 4.17) and phytochemicals such as steroids, flavonoids, alkaloids, different fatty acids, amino acids, glucose, tannins, phlobatannins, saponin and ascorbic acid (Chapter 4.18).

The amount of crude alkaloid, flavonoid, and saponin in LFP were estimated experimentally and given bellow: (Chapter 4.18.2) (Table 4.6).

Ser	**Plants**	**Alkaloids (%)**	**Flavonoids (%)**	**Saponin (%)**	**Glucose (g/kg)**	**Vitamin-C (g/kg)**
1	LFP	0.96	33.24	2.65	49.15	3.66

The qualitative analysis of metal ions of LFP was estimated experimentally (Chapter 4.23).

Ser	**Plants**	**K^+**	**Ca^{2+}**	**Fe^{3+}**	**Mg^{2+}**
1	LFP	+	+	+	+

Three fatty acid ester compounds were identified with retention time at 35.07, 24.55 and 21.98 which compared with standard of fatty acid and reveals the presence of myristic acid, stearic acid, lignoceric acid, lauric acid and arachidic acid respectively from the pet ether extract. Four different characteristic pink coloured spots were detected that matched with standard amino acids and reveals the presence of glycine, alanine, glutamine and glutamic acid in alcoholic extract of litchi (Chapter 4.20).

6.9 Isolation and Investigation of Compounds from Litchi Fruit Pericarp (LFP)

6.9.1 Soxhlet Extraction

The LFP powder was placed in a porous cellulose thimble. Then the thimbles were placed in the soxhlet extraction unit and extracted for 20 hours with 150 mL of distilled

methanol (MeOH). The MeOH extract was concentrated in vacuo. The concentrated mass was treated with pet ether. These pet ether fractions were examined by TLC.

6.9.2 Identification of Compound

The pet ether fractions 20 µL was applied with a pipette as a spot on the TLC plate, and the plate was placed in the mobile phase, which rising up by capillary action. TLC study of pet ether fractions were indicated the presence of five spots of which three were yellow and two violet spots when kept in an iodine chamber. One of these spots showed blue florescence spot in UV light. Upon spraying with vanillin –sulphuric acid followed by heating in an oven at 110^0C for 10 minutes showed the presence of seven spots of which three were violet, two pink spots, one yellowish and one blackish. The presence of pink colour spots was thought to be an indication of the presence of either steroid or fatty acid material or both. However, its Salkowski and Libermann-Burchard reaction gave positive result confirming the presence of steroids.

6.9.3 Characterisation of Compound CF-I

The compounds CF-I (8.2 mg) was isolated as white crystalline solid from the MeOH extract of Litchi pericarp. The collected crystals could not dissolve in any solvents (petroleum ether, ethyl acetate, chloroform and methanol) at room temperature and even on heating. The melting point of the compound was not sharp. It started to melt at 94^0C and at last melted at 110^0C.

IR Spectroscopic Study of CF-I Crystal

The IR spectrum of the compound CF-I (Figure-4.24) was showed an absorption band at 3320 cm^{-1} indicative of –OH group. The band at 2910, 1455,1375 cm^{-1} was due to the presence of aliphatic C-H stretching of either or both-CH_3,-CH_2- or >CH- group [233]. The peak at 1655 cm^{-1} was suggestive of the presence of >C=C< group. The peak at 1455 cm^{-1} was due to the presence of –CH_2- group. A lot of peaks at 1000 to 1300 indicated C-O stretching like alcohols, ethers, esters, carboxylic acids or anhydrides. The other peaks at 690 to 900 cm^{-1} for aromatics (out of plane bend). From the above discussion, it was evident that the compound **CF-I** either a phenolic compound likes flavonoids or anthocyanins.

6.9.4 Characterisation of Compound CF-II

The compounds **CF-II** (1.3 mL) was isolated as white colourless liquid (with significant smell) from the concentrated pet ether extract of litchi pericarp upon vacuum liquid chromatography and crystallization of the fraction T_2. Its TLC study indicated the presence of a single spot (R_f was 0.63) over silica gel (GF254) using pet ether: ethyl acetate (95:5) solvent as the mobile phase. The spot was visualized as violet colour upon spraying with vanillin-sulfuric acid followed by heating in an oven at 110^0C for 10 minutes.

IR Spectroscopic Study of CF-II Crystal

The IR spectrum (Figure-4.25) showed an absorption band at 2900 cm^{-1} was for C-H stretching. The band at 1635 cm^{-1} was due to >C=C< stretching. The broad bands in the range 1640-1560 cm-1 were indicated N-H bend in primary amines. The band at 1440 and 1375 cm^{-1} were due to the presence of both $–CH_3$, $–CH_2$- group. The other band at (900-740) cm^{-1} was due to C-H (out of plane) aromatic stretching.

6.9.5 Characterisation of Compound CF-III

The compounds **CF-III** (1.75 mg) was isolated as white colourless light yellow oily substance from the concentrated pet ether extract of the litchi pericarp upon vacuum liquid chromatography and crystallization of the fraction T-3. The solid was washed with pet ether and dissolved in DCM. Its TLC study over silica gel using pet ether: ethyl acetate (80%:20%) solvent showed one violet spots with tailing on spraying with vanillin-sulfuric acid reagent. R_f value was 0.65. It gives mixture type compounds.

IR Spectroscopic Study of CF-III Crystal

The IR spectrum (Figure-4.26) showed a tiny absorption band at 3030 cm^{-1} indicative of the unsaturation of the compound. The band at 2900 and 1350 cm^{-1} was indicative of $–CH_3$ groups while the bands at 2850 and 1450 cm^{-1} was diagnostic of $–CH_2$- groups. An intense absorption band at 1720 cm^{-1} indicated the presence of a carboxylic acid group. This argument was supported by the presence of bands at 1220, 1160 and 1050 cm^{-1} for C-O stretching. From the above discussion, it was predicted that the compound **CF-III** was either an unsaturated fatty acid or a lactone.

6.9.6 Characterisation of Compound SS-I

The compounds **SS-I** (8.7 mg) was isolated as pink-yellowish substance from the concentrated pet ether (b.p. 40-60^{0}C) extract of the litchi pericarp upon vacuum liquid chromatography and crystallization of the fraction T-7 and purified by mini column chromatography. The fraction TS-2 was light yellowish in colour and gave white sharp needle type crystals inside the test tube. Its TLC study over silica gel using pet ether: ethyl acetate (97%:3%) solvent showed a violet colour single spot on spraying with vanillin-sulfuric acid reagent followed by heating in an oven at 110^{0}C for 10 minutes. Its chloroform solution on treatment with few drops of concentrated sulphuric acid produced red colour indicating its steroidal nature [204].

IR Spectroscopic Study of SS-I (Figure 4.28): $_{Vmax}$ cm^{-1}(in KBr pellet)

The compound **SS-I** had important frequencies of absorption at 3350, 2920, 2850, 1655, 1455, 1365, 1040,965 cm^{-1}.

The IR spectrum (Figure-4.28) showed strong absorption band at 3350 cm^{-1} indicative of a hydroxyl group (-OH) . The sharp absorption bands at 2920 and 2850 cm^{-1} were demonstrative of aliphatic C-H stretching. The absorption bands at 1455 and 1365 cm^{-1} indicated the -CH_2- and -CH_3 bending vibrations respectively [230]. Absorption band at 1655 cm^{-1} suggestive for the presence of >C=C< stretching. The band at 965 cm^{-1} was indicative of its steroidal nature [204].

UV-Vis Spectroscopic Study

The UV-Vis spectrum (Figure 4.27) of compound SS-I showed $\boldsymbol{\lambda_{Max/nm:}}$ 296 and absorbance at 0.91

^{1}H-NMR Spectroscopy (400MHz, $CDCl_3$), Figure 4.29, 4.29(a), 4.30,4.30(a), 4.31, 4.32, 4.33, and 4.33(a)

The compound **SS-1** has important chemical shift value (δ) at 5.334 (d), 5.16(dd), 5.04(dd), 3.514(m), 0.846(d) and1.002 (s)

The ^{1}H-NMR (400 MHz in $CDCl_3$) spectrum of the isolated compound **SS-I** had two singlet's (δ 0.691, 1.002, 1x 2CH_3) of 3H proton intensity each, two doublets (δ 0.846, 0.819, 2x CH_3), a triplet (δ 0.838, 1x CH_3) and a 3H doublet (δ 1.021, 1x CH_3)

typical for a steroidal type compound [216]. The doublet of multiplet at δ 3.514 (J=9.6, 5.2, 4.4) is suggestive of an oxymethine proton flanked by two methylene groups of a cyclo-hexane ring system [216] and the higher coupling constant (J = 9.6) is indicative of its β-orientation. Its placement at C-3 of the ring system is supported by the biogenetic ground [234]. Two doublets of doublets (2dd, 1H each) at δ 5.04 and δ 5.178 with coupling constant 15.2 and 8.8 are exhibitive of trans- olefinic proton plus adjacent methane protons. A 1H broad doublet at δ 5.334 (J=2.36 Hz) is indicative of an olefinic proton at C-6 [216]. The presence of the doublet bonds at C-6 and C-22 receives support from the mass peak at m/e 271 formed by the allylic cleavage and m/e 300 arising from the retro-Diels- Alder fragmentation process [235].

Table 6.1 ^{1}H-NMR Chemical Shift Data for the Structure of the Identified Compound SS-I

No. of protons	Chemical shift value(δ)		Assessment
	Experiment value(δ)	Reported value(δ)	
H-6	5.334(d)	5.33(d)	Olifinic proton
H-22	5.156(dd,J=13.3,8.0Hz)	5.16(dd,J=15.0, 8.4 Hz)	trans olifinic protons
H-23	5.04(dd,J=13.3,8.0Hz)	5.02(dd,J=15.0, 8.4 Hz)	
H-3	3.51(m,J=4.4)	3.52(m)	Oxymethine proton
19-H_3	1.00(s)	1.01(s)	Methyl proton
21-H_3	0.91(d, J=6.4 Hz)	1.02(d, J=6.4 Hz)	
26-H_3	0.85(d,J=3.2 Hz)	0.85(d, J=3.4 Hz)	Methyl proton
27-H_3	0.84(d, J=4.4 Hz)	0.82(d, J=4.3 Hz)	Methyl proton
29-H_3	0.797(s, J=6.2 Hz)	0.80(s, J6.0 Hz)	Methyl proton
18-H_3	0.69(s, J=7.2 Hz)	0.69(s, J=7.2 Hz)	Methyl proton

On the basis of the foregoing discussion, the compound **SS-I** may have the following structure:

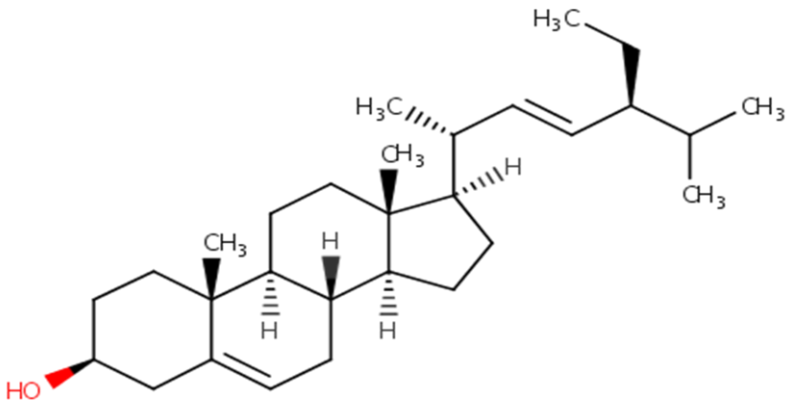


Figure 6.6 Structure of SS-I Showing ^{1}H-NMR Spectrum data

^{13}C-NMR Spectroscopy (400MHz, $CDCl_3$), (Figure 4.34 and 4.35)

The ^{13}C- NMR Spectrum '400 MHz, $CDCl_3$' was shown in **Figure 4.34 and 4.35**. The compound **SS-I** has important chemical shift value (δ) at 140.801, 138.328, 129.341, 128.5, 121.737, 71.856, 56.9, 56.024, 42.35, 37.305, 31.951, 31.710, 36.2, 29.716, 21.24, 21.114, 19.422 and 12.257.

The ^{13}C-NMR spectrum of the isolated compound **SS-I** revealed the presence of 29 carbon signals. Amount of the isolated compound **SS-I** was not sufficient, so all peaks did not observed this spectrum. But manually those peaks were measured from these spectrums. The signals at δc=140.801 (C-5),$\sim$ δc=34.2 (C-10), manually measured, δc = 42.35 (C-13) were assigned to three quaternary carbons respectively. The signal at δc 71.856 ppm was assigned for methylene carbon C-3. The signals at $\sim$ δc= 138.328 ppm and $\sim$ δc=129.341 ppm was for the >C=C< carbons of C-22 and C-23 [236] and also the signals at$\sim$ δc = 121.73 ppm and $\sim$ δc=140.801 ppm was typical for the >C=C< carbons of C-5 and C-6 [236] (Figure 4.34 and 4.35).

Table-6.2 ^{13}C-NMR spectrum data of the isolated compound SS-I with reported value "δc" [237]

No. of Carbon	Types of Carbon	Value of "δc"	
		Observed value "δc"	Reported value "δc" [237]
1	$=CH_2$	37.305	37.162
2	$=CH_2$	31.710	31.502
3	=CH-	71.856	71.074
4	$=CH_2$	*38.00	37.9
5	=CH	140.801	139.573
6	$=CH_2$	121.737	117.475
7	=CH-	29.716	29.659
8	=C=	40.492	40.281
9	=CH-	50.221	49.47
10	=C=	*34.00	34.241
11	$=CH_2$	21.240	21.563
12	$=CH_2$	39.732	39.482
13	=C=	42.350	43.308
14	=CH-	*55.80	55.113
15	$=CH_2$	22.705	23.028
16	$=CH_2$	*28.50	28.507
17	=CH-	56.024	55.862
18	$-CH_3$	12.080	12.061
19	$-CH_3$	*14.00	13.048
20	-CH-	40.492	40.823
21	$-CH_3$	21.114	21.094
22	=CH-	138.328	138.174
23	=CH-	129.341	129.462
24	=CH-	51.276	51.263
25	=CH-	31.952	31.888
26	$-CH_3$	*21.00	21.382
27	$-CH_3$	19.422	19.008
28	$-CH_3$	*25.40	25.405
29	$-CH_3$	12.257	12.250

*This observed δc value measured manually

^{13}C-NMR spectroscopic data of the isolated compound **SS-I** was identified as Stigmasterol having the following structure:

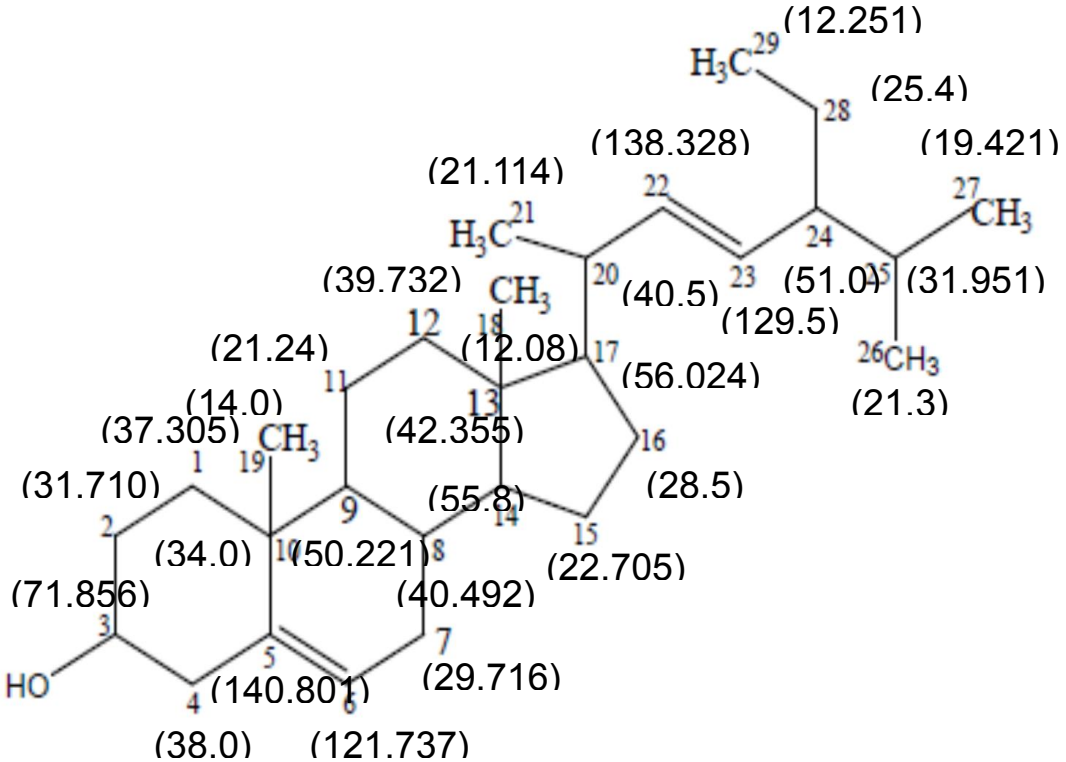


Figure 6.7 Structure of SS-I Showing ^{13}C-NMR Spectrum data

"To the best of our knowledge for the first time we have isolated Stigmasterol from this pericarp of Litchi (LFP)"

Finally, combining IR, UV-Vis, ^{1}H-NMR and ^{13}C-NMR, DEPT-135 spectroscopic data value the isolated compound SS-I from LFP was identified as Stigmasterol having the following structure:

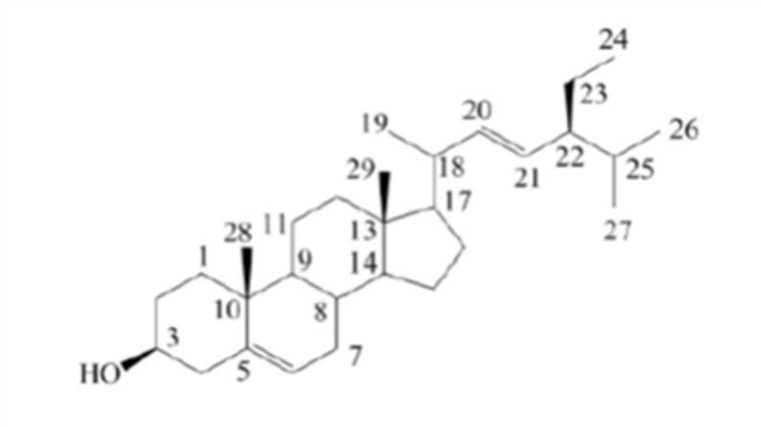


Figure 6.8 Structure of Stigmasterol.

Aqueous methanolic extraction shows the presence of phenolic compounds. These phenolic compounds belong to the class of anthocyanins, flavanol or flavonoids. All of these phenolic compounds are firmly believed to have efficiency as anticancer, antioxidant and antimicrobial activities. These chemical materials stuff may be used as food colourant. Therefore this work suggests that more intensive research has to be focused on the different parts of the Litchi's, in order to exploit its potential.

6.10 Properties of the compounds isolated from PE-II of *Litchi Chinensis*

The Elute from column chromatography of DCM extracts were monitored by TLC and gave various fractions. After systematic analysis a white crystal was found from these fractions. It was designated as SS-2. Observing IR and ^{1}H – NMR spectral data of the compound SS-2, it was decided that this compound was similar to Stigmasterol which found from the pet ether extract SS-I.

6.11 Antimicrobial Activities of Litchi Fruit Parts Extracted Using two Different Solvents

Antimicrobial Assay

Antimicrobial activities of Litchi fruit parts extracted using two different solvents i.e. ethanolic and acetonic extracts of both litchi pulp and waste (including seeds and pericarp) was determined in terms of zone of inhibition using a modified Kirby–Bauer disc diffusion method [217]. The tested bacterial pathogens were *E. coli, P.aeruginosa, S. aureus, and B. subtilis*. An aliquot 10 µl of each extract was pipetted on a sterile Whatmann number-1, 5 mm paper disc on the agar surface. Bacitracin used as standard antibacterial agent served as positive control for antimicrobial activity. The results of antimicrobial activity of litchi pulp and waste extracts are presented in Table 5.4. The pictorial views of antibacterial activities are shown below:

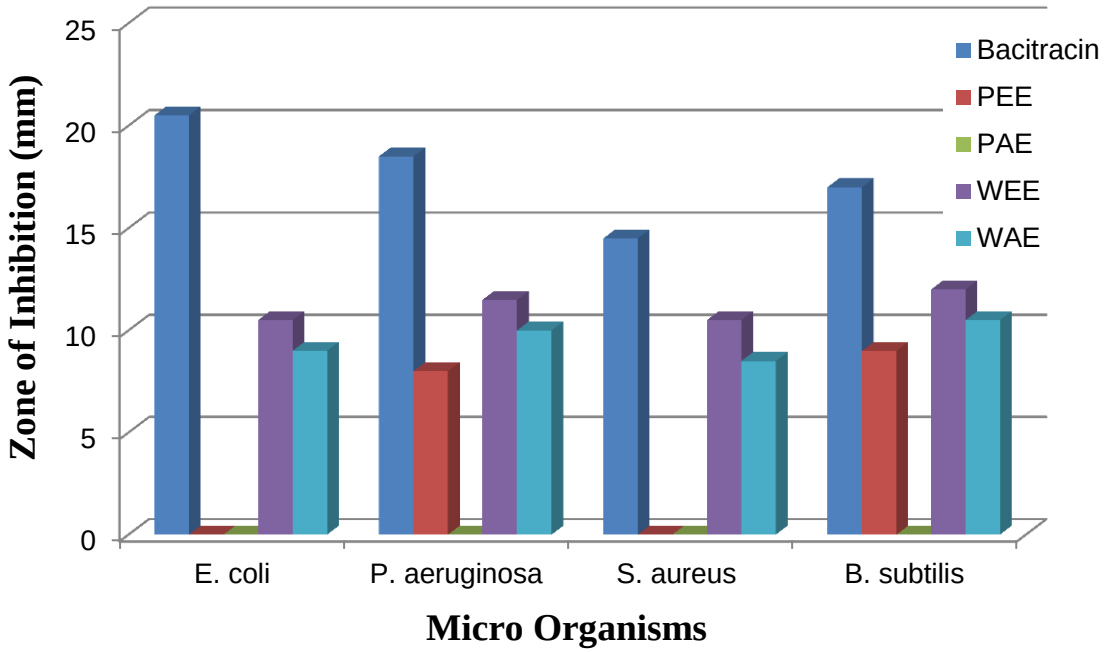


Figure 6.9 Vertical Graph of Comparison of Antimicrobial Activity of Bacitracin and Ethanolic and Acetonic Extracts of Both Litchi Pulp and Waste against the Test Microorganisms.

Note: PEE: Pulp Ethanolic Extract; PAE: Pulp Acetoneic Extract; WEE: Waste Ethanolic Extract; WAE: Waste Acetoneic Extract.

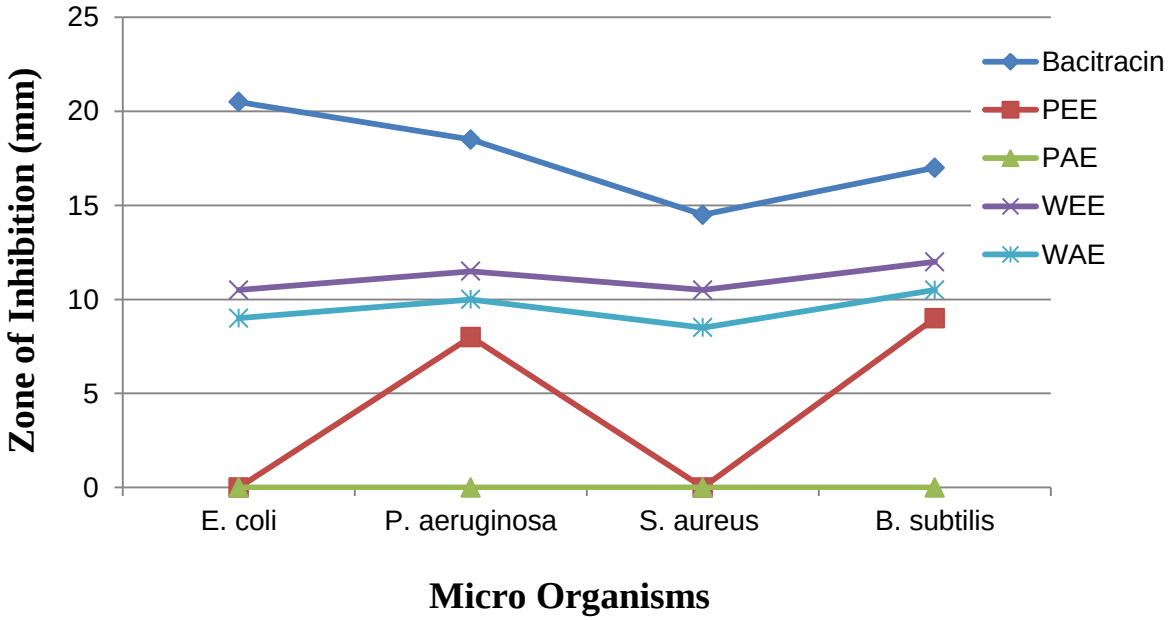


Figure 6.10 Horizontal Graph of Comparison of Antimicrobial Activity of Bacitracin and Ethanolic and Acetonic Extracts of Both Litchi Pulp and Waste against the Test Microorganisms.

Evaluation of Antimicrobial Assay

The antibacterial activity of litchi pulp and waste were compared with bacitracin and waste ethanolic extract showed the highest inhibition over acetonic extracts. It inhibited the growth of *E. coli, P.aeruginosa, S. aureus and B. subtilis* with inhibition percentages equal to 51, 62,72 and 70% respectively (Table 5.4) and waste acetoneic extract showed moderately inhibited all four microbes.

The results revealed that litchi waste ethanolic extract exerted the strongest inhibition against all tested bacterial strains compared to other extracts while pulp acetonic extract showed no effect against most of the tested bacteria. It is worthy to note that the results revealed that both litchi waste extracts have positive effect on all tested bacteria.

6.12 Antimicrobial Activity of Stigmasterol Isolated from Litchi Fruit Pericrp (LFP)

Antimicrobial Activity Assay

Antimicrobial activity of the extracted Stigmasterol from LFP was determined by the agar disk diffusion method of protocol given by Doughari et al. [222] with little modification. Gentamicin was used as a standard antimicrobial agent and *E. coli, P. aeruginosa, S. aureus and K. pneumoniae* used as the test organisms. Antimicrobial activity was determined by measuring the "zone of inhibition" in millimeters [223]. In comparison with standard antibacterial compound Gentamicin (20 μg /ml) it was found that purified Stigmasterol (20 μg /ml) has antimicrobial activity almost equivalent to the standard. The results of antimicrobial activity of Stigmasterol and Gentamicin are presented in Table 5.5. The pictorial views of antibacterial activities are shown below:

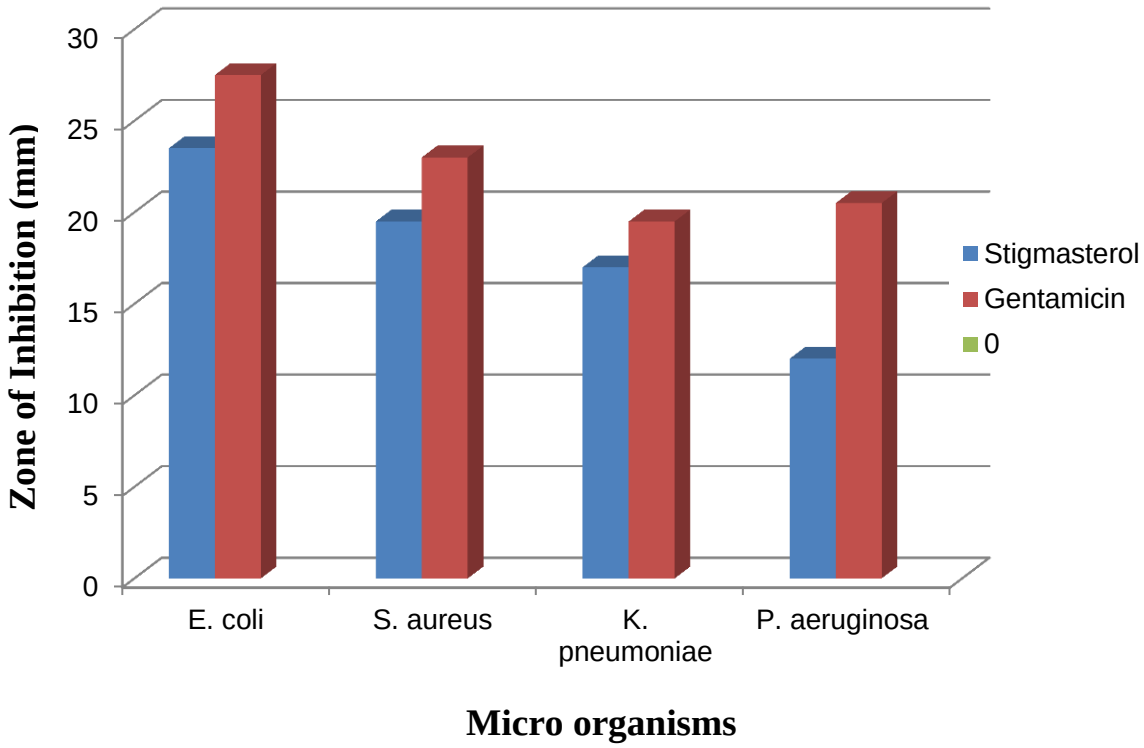


Figure 6.11 Vertical Graph of Comparison of Antimicrobial Activity of Gentamicin and Purified Stigmasterol of LFP against the Test Microorganisms.

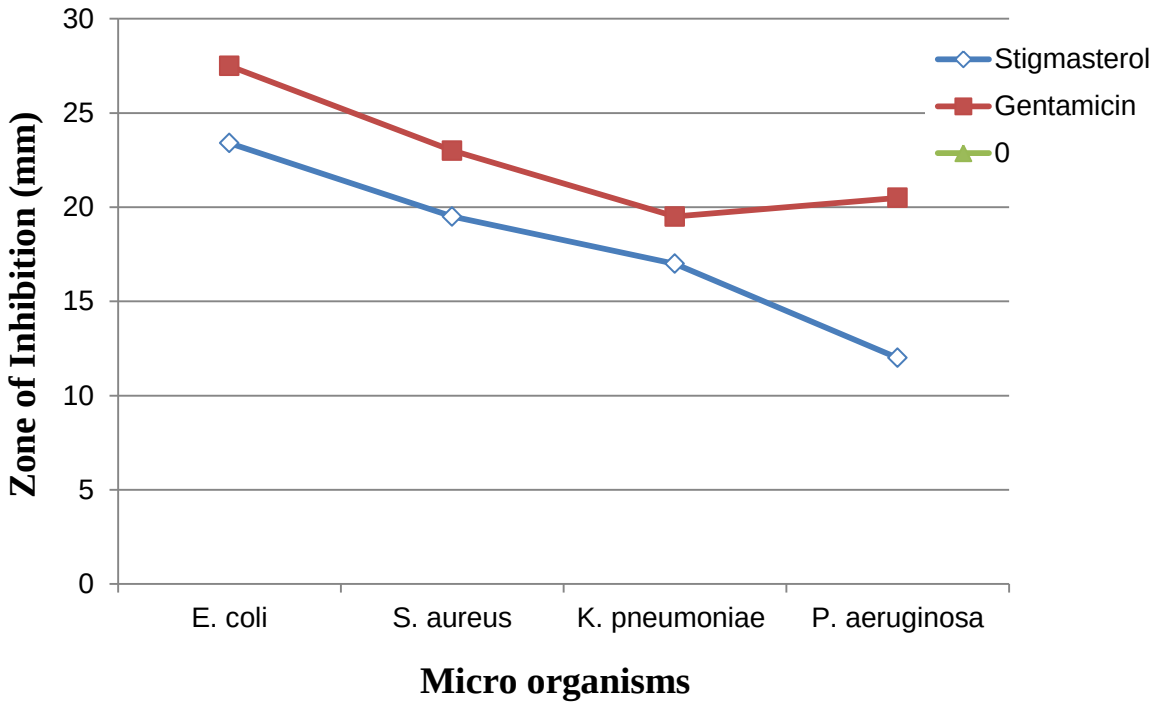


Figure 6.12 Horizontal Graph of Comparison of Antimicrobial Activity of Gentamicin and Purified Stigmasterol of LFP against the Test Microorganisms

Discussion on Antimicrobial Assay

The antimicrobial activity ranged from 12 mm to 23.5 mm for Stigmasterol and 19.5 mm to 27.5 for Gentamicin in table 5.5 and figure 5.2. The antimicrobial activity of the Stigmasterol was comparable to the standard Gentamicin and found its showing higher antimicrobial activity against *E. coli* followed by *S. aureus*, *P. aeruginosa* and *K. pneumoniae* at lower concentration. This study contributes to establish Stigmasterol as potent antimicrobial agent at lower concentration against a wide range of bacteria and serve as an alternative chemical to treat the bacterial diseases. However, still more scientific evaluation and clinical trials are required to establish its therapeutic efficacy.

6.13 Antioxidant Activity of Litchi Fruits Pericarp (LFP) Extracts of Litchi (*Litchi chinensis*)

Antioxidant Activity Assay

To achieve the antioxidant activity LFP was treated with preheated Charcoal to make them free from oily and colouring materials. This cleaned mixture contains powerful antioxidant compounds, such as ascorbic acid, phenolic acids and flavonoids. The antioxidant properties of *Litchi chinensis* extract was evaluated using Ferric (Fe^{3+}) reducing antioxidant power (FRAP) assay. The fraction CM-I (Chapter 5.5) was investigated and the presence of phenolic type compound was confirmed by chemical test (4.17.2), TLC and UV-Vis Spectrum. Antioxidant properties of this phenolic compound was observed from the test of Fe^{3+} (Ferric) to Fe^{2+} (Ferrous) conversion. The formation of Fe^{2+} is a necessary condition for the absorption of Iron and formation of Hb (Hemoglobin) in the living organism. Therefore, the phenolic compound (CM-I) is thought to have the role of iron absorption.

The antioxidant properties of phenolics likely arise from their high reactivity as hydrogen or electron donors and from the ability of polyphenol-derived radicals to stabilize and delocalize the unpaired electron or from their ability to chelate transition metal ions (i.e. cause termination of the Fenton reaction). The fraction CM-II was investigated for the breakdown of phenolic compound (converted to fade colour) by TLC and showed that the spots did not correspond with those of the CM-I upon standing 72 hours at room temperature and showed the absorption peak at $\lambda_{max/nm}$ = 289 nm (Figure 4.46).

UV-Vis spectrum study

The UV-Vis spectrum of CM-I (Figure 4.44 and 4.45) showed the absorption peaks at $\lambda_{max/nm}$ = 288 nm for CM-I and $\lambda_{max/nm}$ = 301 nm for CM-I with few drops of NaOH were for the free radical absorption of phenolic compound. The increase in absorbance after adding NaOH indicative of methanolic extract of LFP contain phenolic compound. In presence of NaOH the density of the electron in the benzene ring increases due to the resonance and gave higher UV absorption peak. This experiment suggests that the phenolic compound present in the litchi pericarp possesses the antioxidant property. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

CONCLUSION

Tomatoes (*Lycopersicon esculentum*) contain a wide variety of antioxidants including vitamin E, ascorbic acid, carotenoids, flavonoids and phenolic compounds. Conventional solvent extraction methods were used to extract carotenoids (lycopene and β-carotene) pigments from tomato paste. Different solvents were tested in preliminary trials. Ethyl acetate (EtOAc) had the best extraction and efficiency over di-mixture of acetone: pet ether (1:1, v/v) and tri-mixture of n-hexane: acetone: ethanol (2:1:1, v/v/v) solvents. The UV-Vis spectrum had sharp peaks for lycopene (*trans*- and *cis*-) and carotenoids (β-carotene) indicating no other components or contaminants were present.

Soxhlet extraction was the selected method for this research because it requires less solvent and shorter extraction times than other methods and it is suitable for the extraction of lycopene from tomato paste. Ethyl acetate is favored because of its low cost, low toxicity, agreeable odour and optimum solvent for the extraction.

Analysis of extracted samples by TLC showed the presence of lycopene as well as γ and β-carotenoids. TLC was done to determine the purity of extracted lycopene. UV-Vis spectrum had sharp and separate peaks for *cis*- and *trans*- isomers of lycopene, and carotenoids.

So far this is a new dimension in the research field of solvent extraction i.e. preliminary trials may be carried out before to proceed for Soxhlet extraction. This helped to select the suitable solvent for extraction to obtain compounds. These receive support from the sharp peaks and with the consideration of low cost, low toxicity and optimum solvent for extraction.

Lycopene content ranging from 17 to 28 mg per 100 g was extracted from tomato paste. The amount of lycopene extract was greater than that of preliminary trials; possibly because the lycopene in the tomato paste became more bioavailable due to heating during soxhlet extraction. Tomato skins and the seeds usually eliminated during tomato processing as by-product is a valuable source of carotenoids. Extracting lycopene from tomato processing wastes is economical and these by-products could become a cheap

source of lycopene, carotenoids and natural oils for formulating new nutraceuticals, pharmaceuticals, and cosmetic products.

The UV-Vis spectrum of different conditions was examined. UV spectrum of lycopene of extract-I and extract-II had sharp peaks that mean lycopene did not degraded in the solvent and was shown to be stable at 4^0C and at room temperature but it was remarkable to note in case of extract-III which was faded from its original colour and showed only lines without peaks or bands. It was anticipated that the lycopene long chain of conjugated carbon-carbon (>C=C<) double bonds, to isomerization and degradation upon exposure to light, heat and oxygen for extract-III and the subsequent loss of its colouring properties and this would render tomato extract ineffective as food colour. Lycopene is more stable in a tomato matrix, compared to isolated, purified or in solvents.

More research is needed to improve the current methods of extraction of lycopene. Extraction could be improved by using renewable feedstock such as tomato peels and processing waste, thus reducing the amount of waste generated. Safer solvents such as ethyl lactate, which is biodegradable, should be used for the extraction.

Antibacterial activities of tomato paste extract were evaluated using three different solvents (acetone, methanol, and chloroform extracts). It was found that most of the extracts of these solvents executed moderate to good antimicrobial activity against the tested micro-organisms. The extracts were active against the entire tested microorganism for antibacterial and antifungal activity. This research established the fact that tomatoes can be used to treat diverse diseases and its antimicrobial effects on some selected clinical isolates confirmed it usefulness as antibiotic in the treatment of infectious diseases. We conclude that tomatoes possess pharmacological properties which if properly harness can be used in the management of diseases.

A TLC study of MeOH extract of LFP marked as MELFP showed the presence of five spots when kept in an iodine chamber. One of these showed blue florescence spot in UV light. Upon spraying with vanillin-sulphuuric acid followed by heating in an oven100^0C for 10 minutes seven spots were observed. The extract of MELFP was concentrated in vacuo. General phytochemical screening of LFP indicated the presence of alkaloids,

flavonoids, different fatty acids, amino acids, steroids, glucose, tannins, phlobatannins, saponins and ascorbic acids.

The extract was then treated upon adding pet ether. The pet ether fraction was marked as PE-I. TLC study of pet ether fraction showed the presence of five spots being visible on spraying with vanillin-sulphuric acid followed by heating in an oven 100^0C for 10 minutes. Of these five spots two were violet, two pink spots and one green. The presence of pink colour was thought to be an indication of the presence of ether steroid or fatty acid material or both. However, its Salkowski and Libermann-Burchard reaction gave positive result confirming the presence of steroids.

The other portions were left for few days. At the bottom of the conical flasks solid particles settled down with gummy mass. This solid particles and gummy mass were then washed with DCM. The DCM fraction was put in test tube and marked as PE-II.

The rest of the fraction gave white crystals. But the collected crystals could not dissolve in any solvent at room temperature and even on heating. The melting point of the compound was not sharp. It started to melt at 94^0C and at last melted at 110^0C. Then crystals were put in a vial marked as CF-I. The amount of the crystal was also very low. However Its IR was taken and revealed that the compound CF-I either a phenolic compound likes flavonoids or anthocyanin.

The pet ether fraction PE-I was treated by VLC using different ratio of elution's and marked as T-1 to T-13. These fractions were analyzed separately. Fraction T-2 was colourless liquid upon standing undisturbed at room temperature for several hours no solid appeared. The mother liquor was transferred in a vial marked as CF-II and TLC study showed a single spot on spraying with vanillin-sulphuric acid followed by heating. However IR was taken and revealed that it was a fat like compound.

Fraction T-3 was light yellow in colour. Upon standing undisturbed at room temperature for several hours little amount of fat like solid appeared at the bottom of the conical flask. The solid was washed with pet ether: EtOAc (90:10) marked as CF-III. Its TLC study showed one violet spot with tailing on spraying with vanillin-sulphuric acid. The R_f value was 0.65.

Fraction T-7 was yellow in colour upon standing undisturbed at room temperature for several weeks little amount of yellowish solid appeared at the bottom of the conical flask . It was washed with pet ether: EtOAc (70:30). A TLC study presence of one spot showed in UV light and the two spots and a little tailing on spraying with vanillin-sulphuric acid solvent.

Further this fraction 25 mL concentrated and subjected to a mini column chromatography. The different ratio of elutions was marked as TS-1 to TS-5. These fractions were analyzed separately. The fraction TS-2 was left undisturbed at the room temperature. It gave white crystals at the inside of the test tube. The crystals were dissolved in $CHCl_3$ (02 mL x 03) and transferred to a vial marked as SS-I. Its TLC study in solvent pet ether:EtOAc (98%:2%) showed a single spot on spraying with vanillin-sulphuric acid reagent followed by heating in an oven. However, after recrystallization Its UV-Vis, IR, ^{1}H- NMR, ^{13}C-NMR and DEPT-135 spectrum was taken and it was identified as Stigmasterol.

To the best of our knowledge for the first time we have isolated Stigmasterol from the pericarp of litchi (LFP).

Elutes from column chromatography of DCM extract were monitored by TLC and gave various fractions. After systematic analysis a white crystal was found from these fractions. It was marked as SS-2. By observing IR and ^{1}H – NMR spectral data of compound SS-2, it was decided that this compound was similar to Stigmasterol which found from the pet ether extract of SS-I.

Antibacterial activities of litchi fruit parts extract was evaluated using two different solvents (ethanolic and acetonic extracts) of both pulp and waste (including seeds and pericarp). Ethanolic extract of waste showed the highest inhibition over acetonic extract. It inhibited the growth of *E. coli, P. aeruginosa, S. aureus and B.subtilis* with inhibition percentages equal to 51, 62, 72 and 70%; respectively. Waste acetonic extract moderately inhibited all four microbes. The results of this study suggested that litchi waste and pulp extracts possess bioactive compounds having antibacterial activites which can be used in local pharmaceutical industry.

The antimicrobial activity of the purified Stigmasterol was determined by measuring the inhibition zone around the disc. It was ranged from 12 mm to 23.5 mm for the Stigmasterol and 19.5 mm to 27.5 for Gentamicin. Thus, the antimicrobial activity of the Stigmasterol was comparable to the standard Gentamicin. It has been proven to be a safe, natural and effective nutritional supplement and has shown amazing potential benefits. Thus these results prove the antimicrobial potential of Stigmasterol from LFP and further, it can serve as an alternative chemical to treat the bacterial diseases.

The methanolic extract of LFP has significant antioxidant activity which is attributed to its high phenolic and flavonoid content. The phenolic compounds may contribute directly to antioxidant action. The antioxidant properties of phenolic compounds were arises from their high reactivity as hydrogen or electron donors and from the ability of polyphenol-derived radicals to stabilize and delocalize the unpaired electron or from their ability to chelate transition metal ions. This indicated that LFP rich in phenolics have the potential to be used as functional ingredients for food and pharmaceutical applications.

Our investigation was based on the isolation and purification of different pigments from tomato paste of *Lycopersicon esculentum* and mainly the LFP of *Litchi chinensis* as well as biological activities of these extracts. So in future a separate study may carry out for pulp and seeds of litchi. Of course, more simple, new, and environment friendly sorbent such as nano and bio materials can be used in the isolation and purification of different pigments from tomato and litchi. Our findings encourage the further study and investigation of different extracts and to identify the bioactive compounds responsible for antimicrobial and antioxidant activities.

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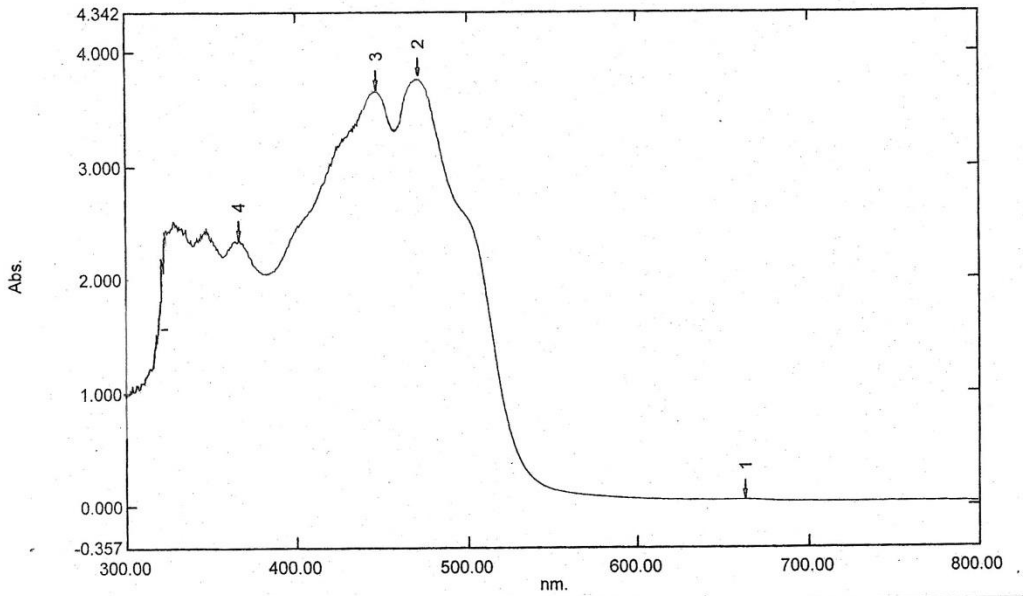

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Figure Fig 4.18 UV spectrum of di-mixture Le-DME

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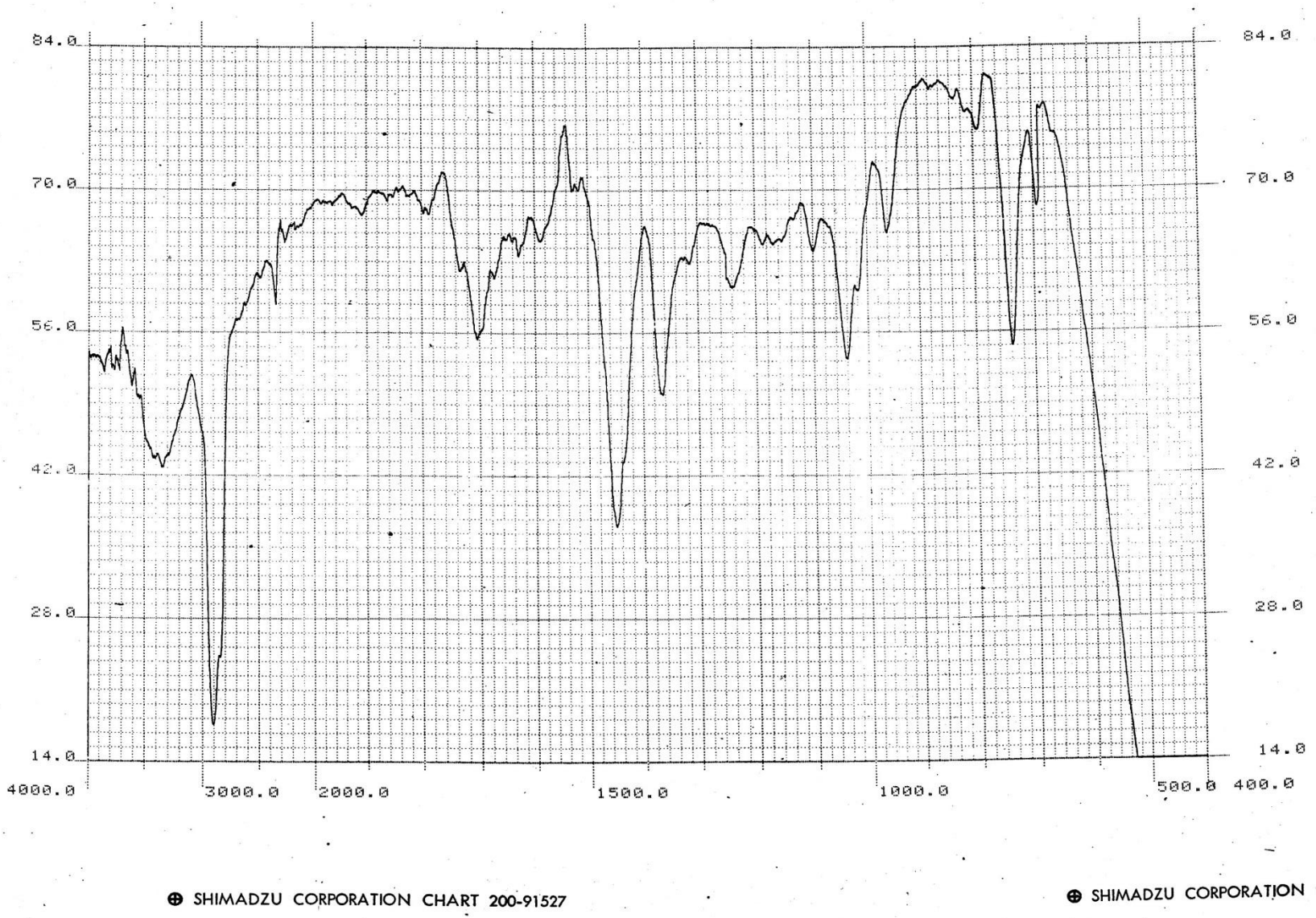

Figure 4.19 IR spectrum of di-mixture Le-DME

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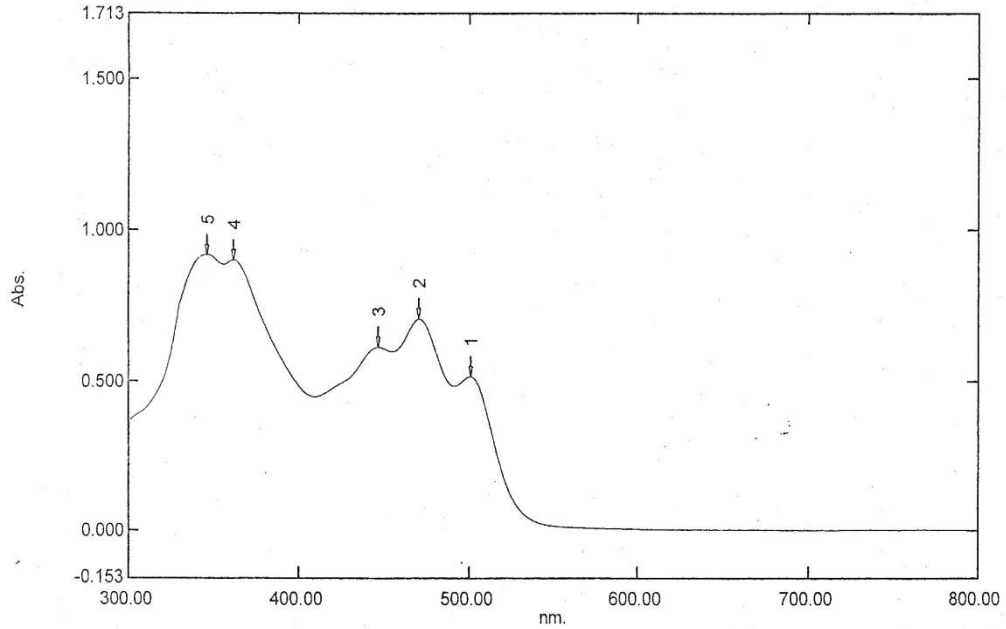

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6	V	491.00	0.482	
7	V	454.00	0.598	
8	V	409.00	0.447	
9	V	356.00	0.887	
10	V	339.00	0.908	

Figure Fig 4.20 UV spectrum of tri-mixture Le-TME

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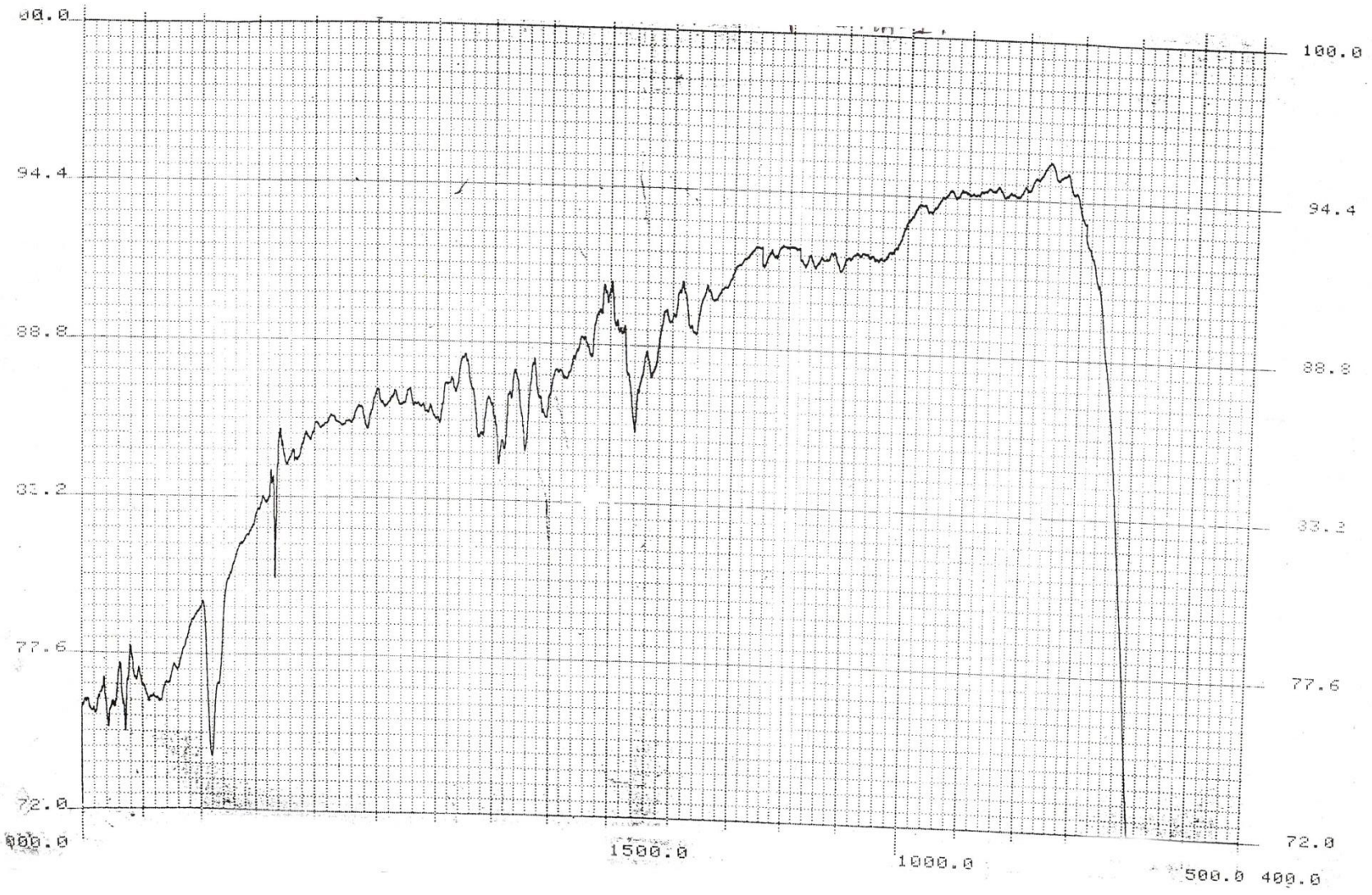

Figure 4.21 IR spectrum of tri-mixture Le-TME

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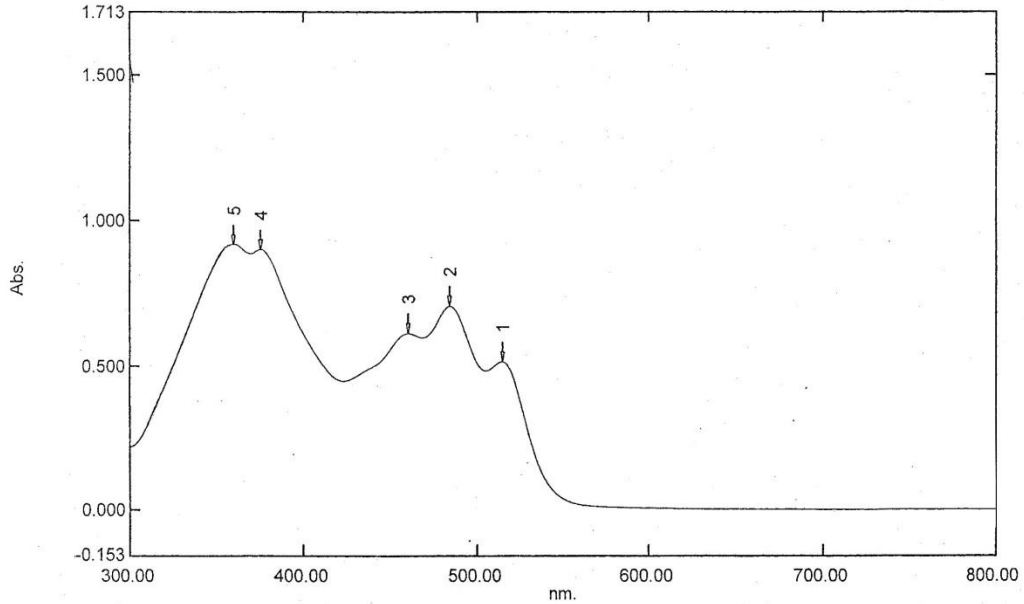

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7	↓	466.50	0.598	
8	↓	420.50	0.447	
9	↓	368.50	0.887	
10	↓	351.50	0.908	

Figure Fig 4.22 UV spectrum of EtOAc extract Le-EAE and Le-SEAE

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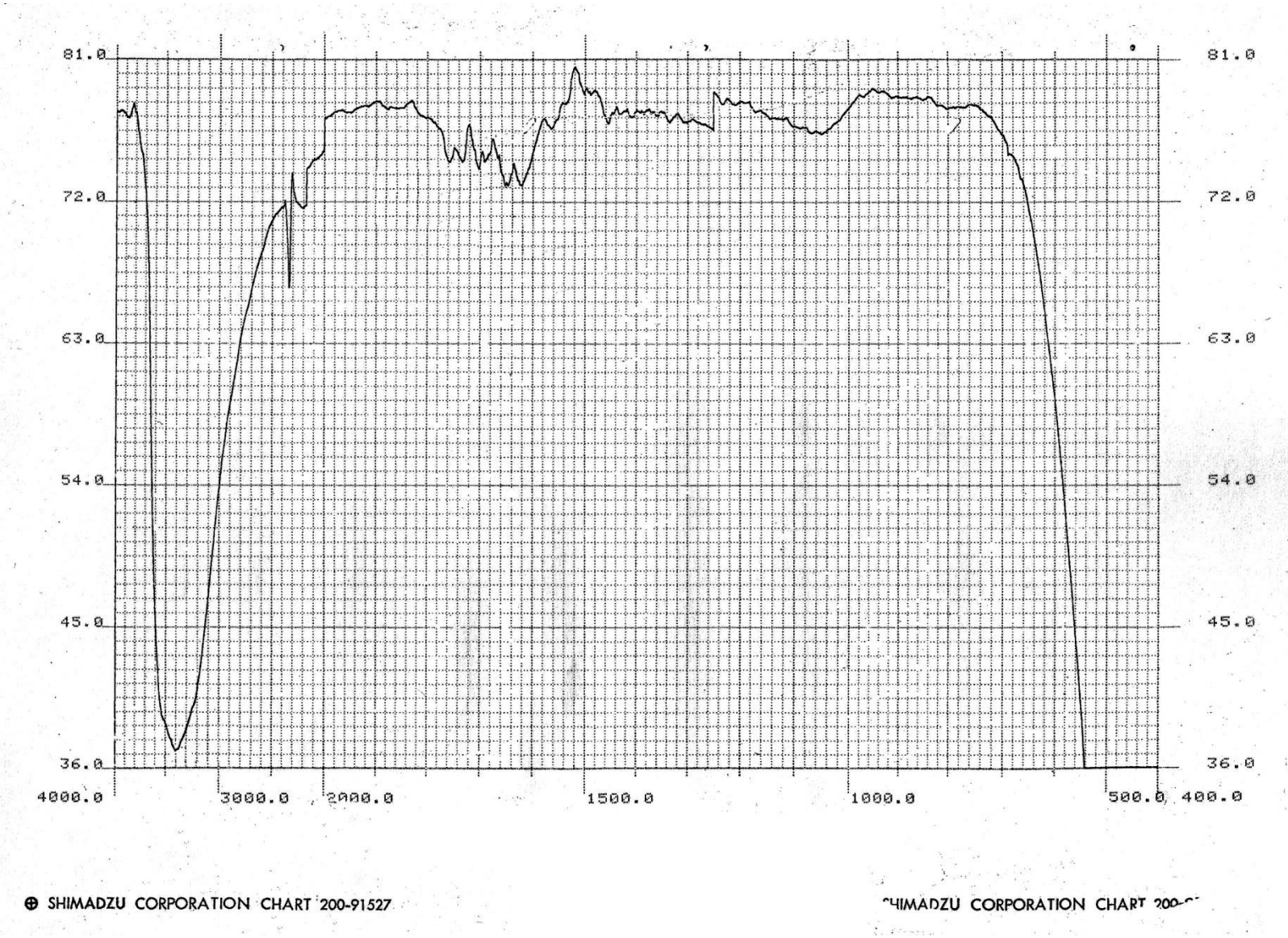


Figure Fig 4.23 IR spectrum of EtOAc extract Le-EAE and Le-SEAE

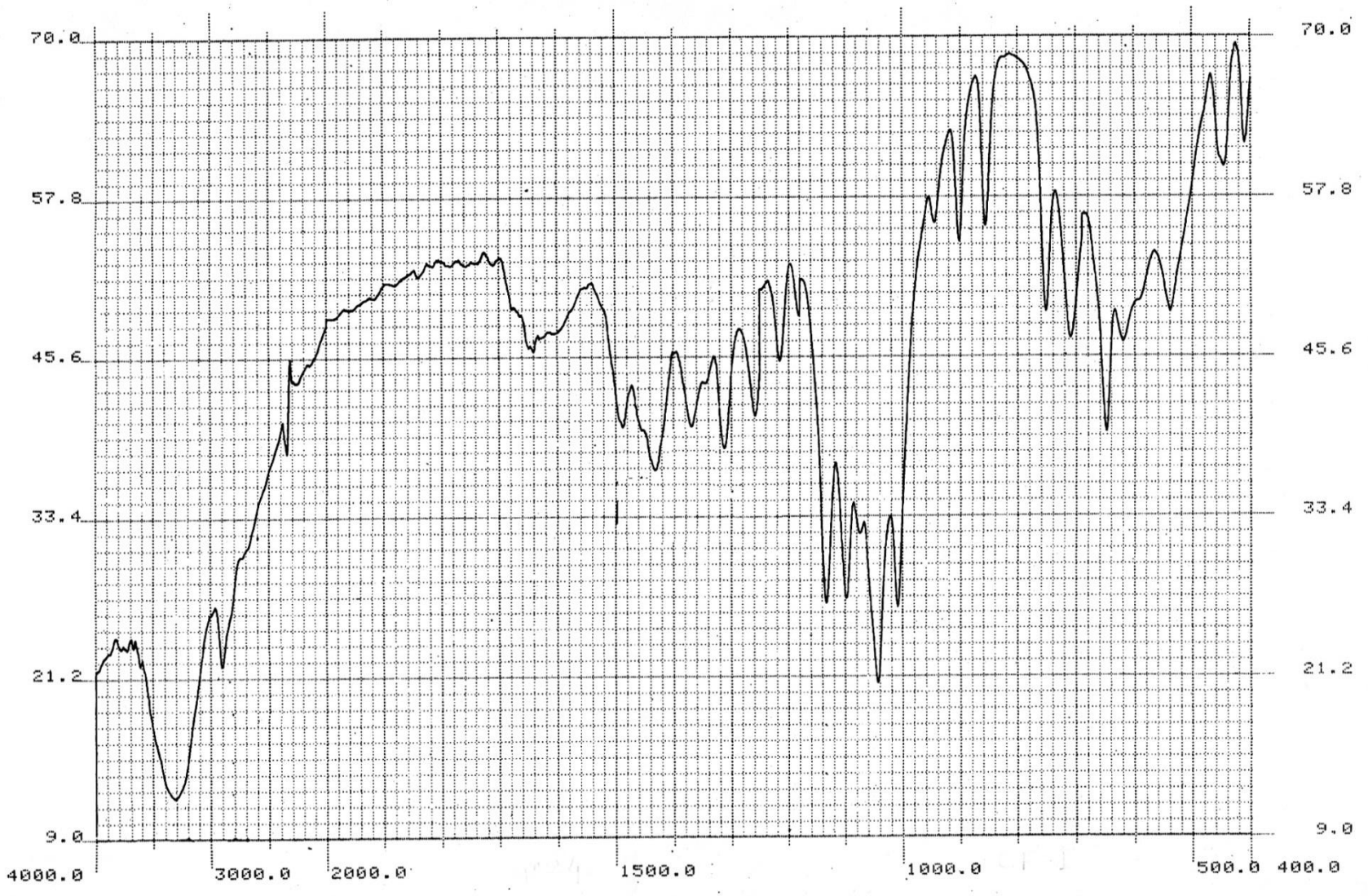


Figure 4.24 IR spectrum of fraction CF-I

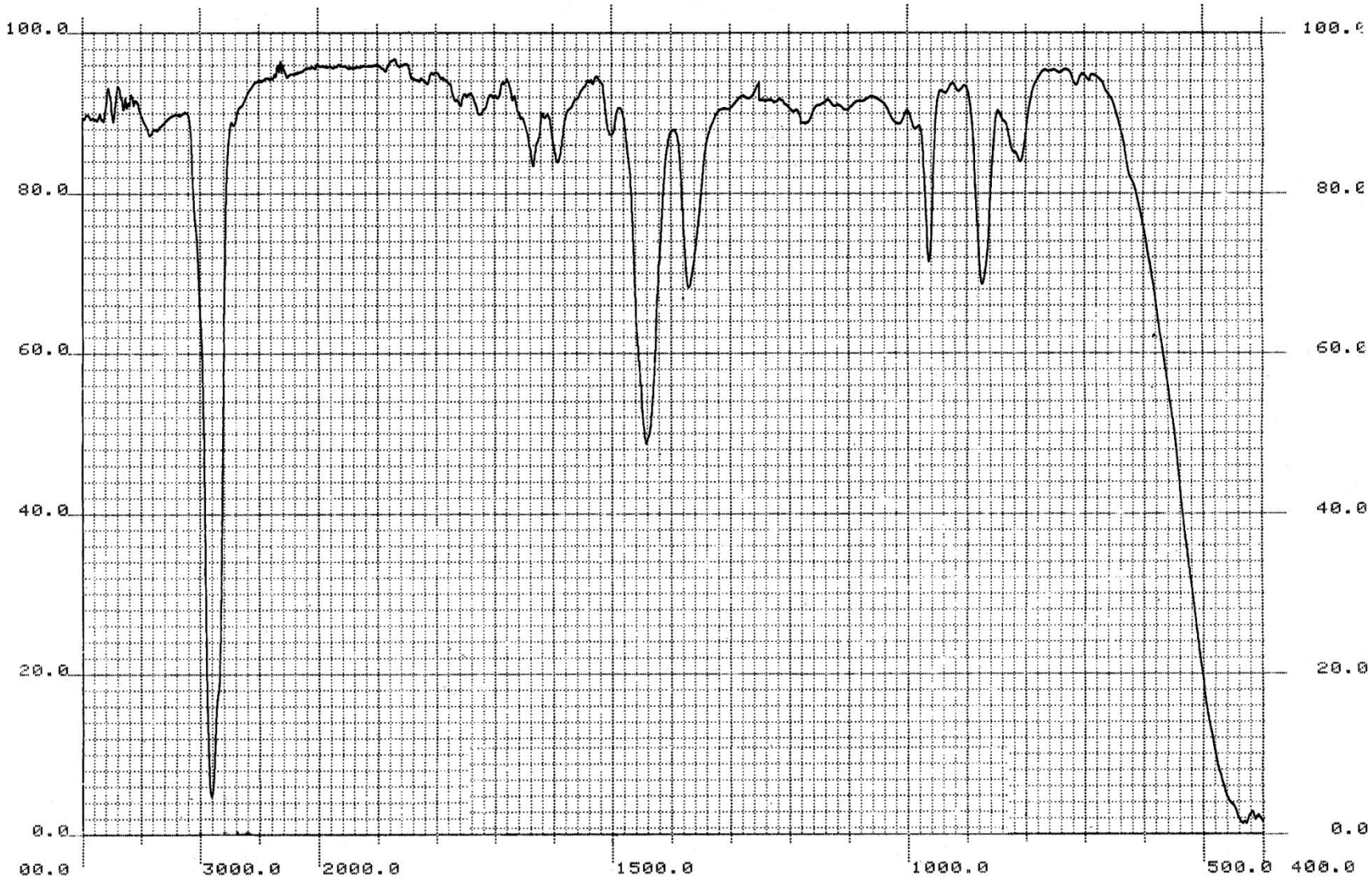


Figure 4.25 IR spectrum of fraction CF-II

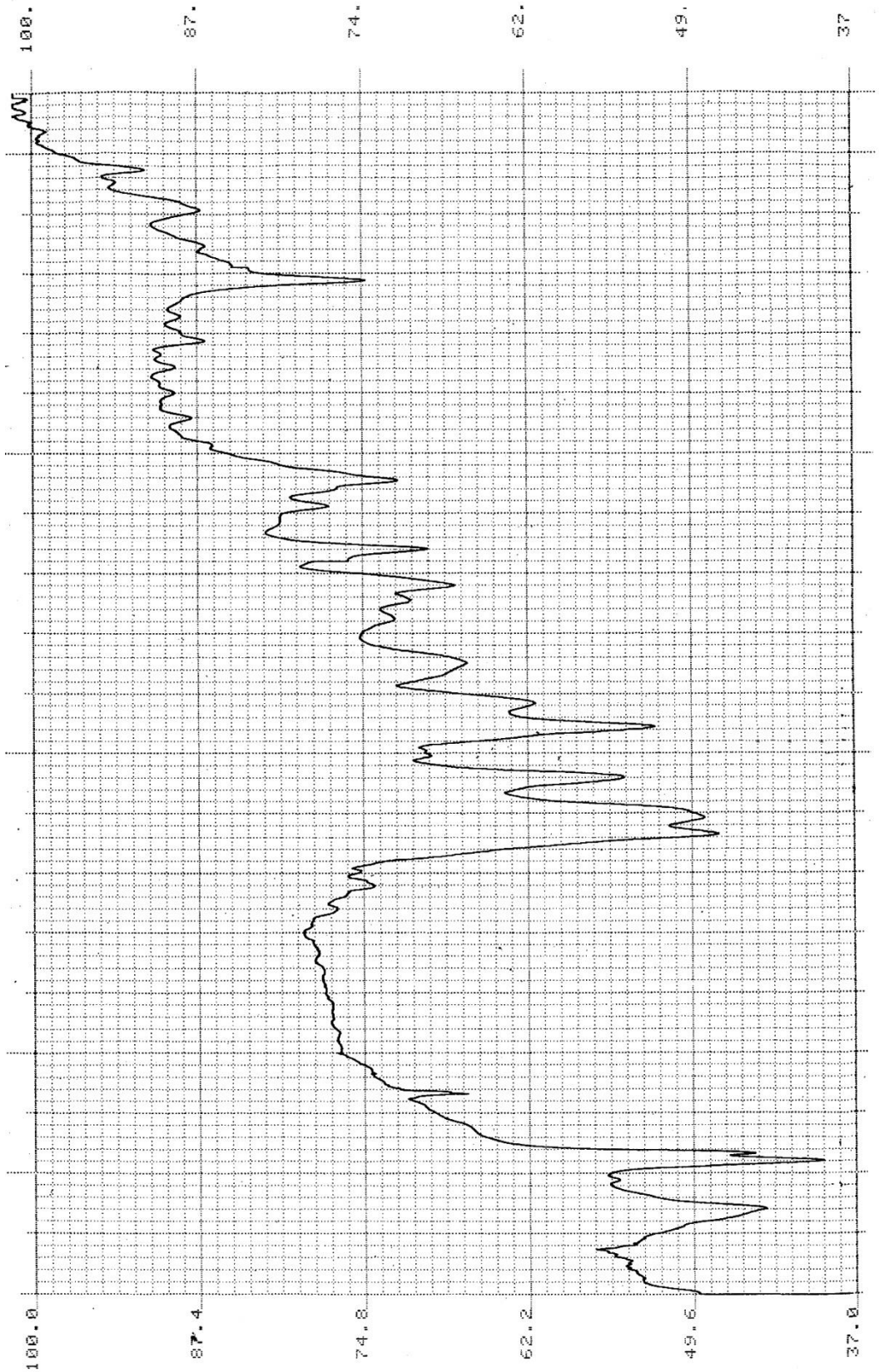
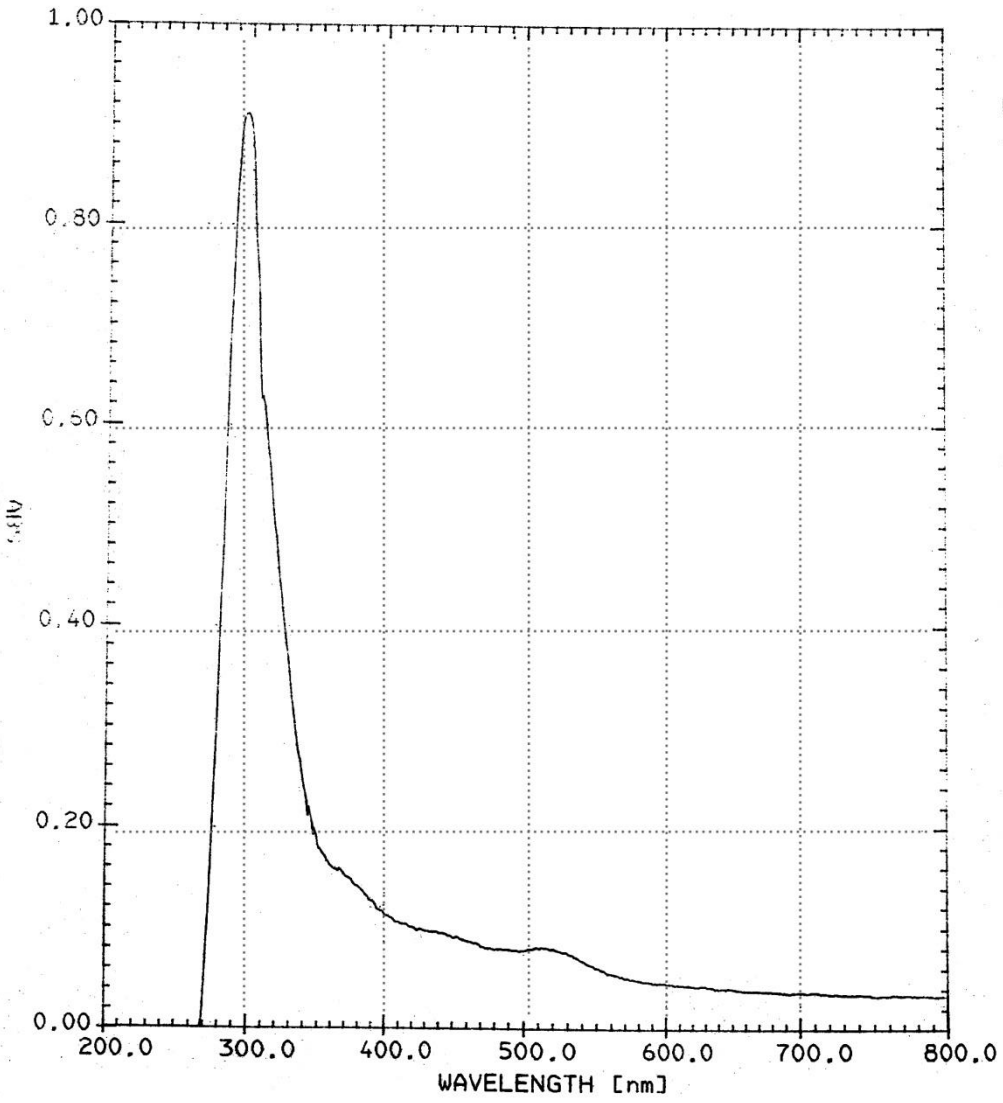


Figure 4.26 IR spectrum of fraction CF-III



*** PEAK-PICK ***

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Figure 4.27 UV spectrum of compound SS-I

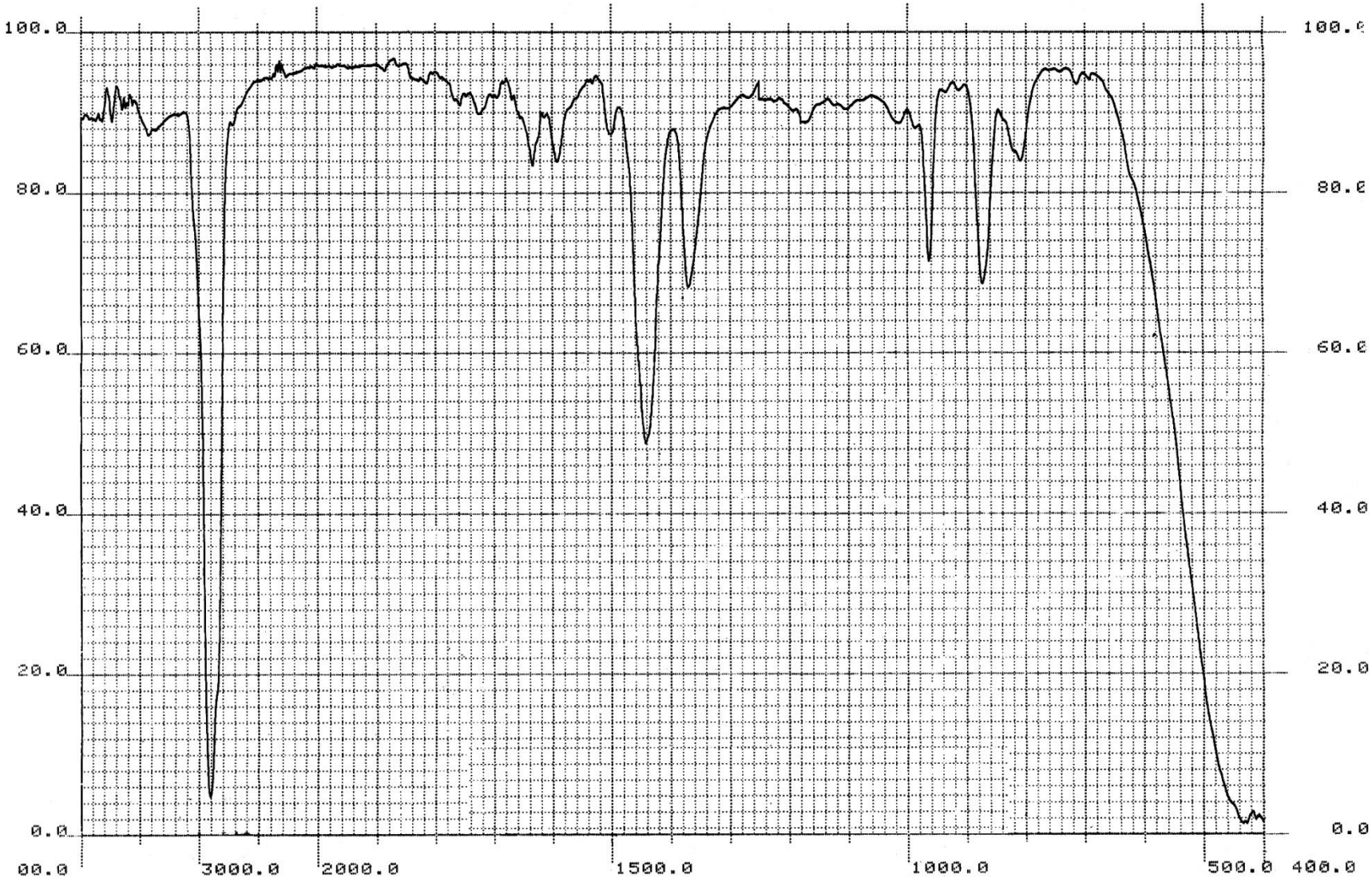

Figure 4.28 IR spectrum of compound SS-I

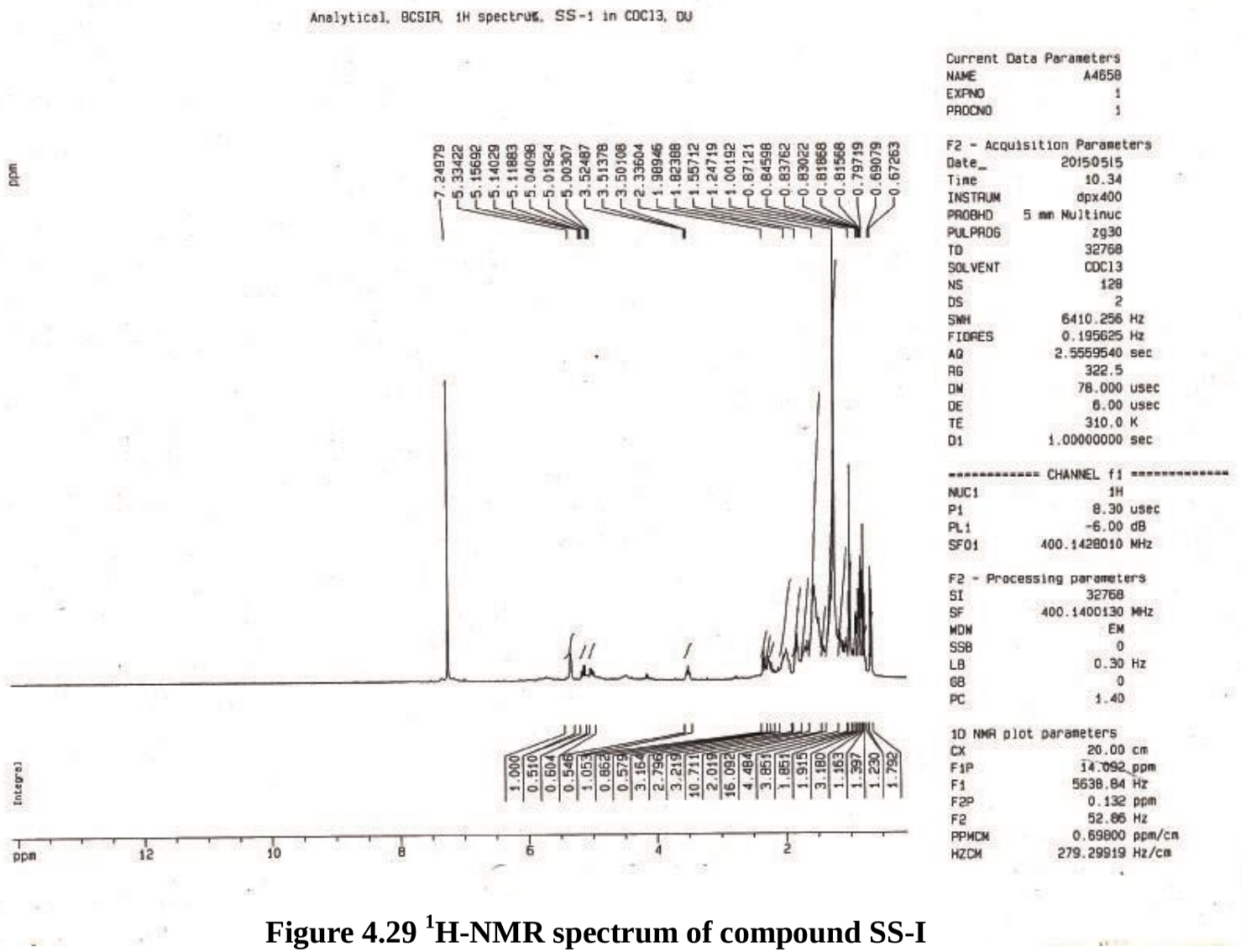

Figure 4.29 ^{1}H-NMR spectrum of compound SS-I

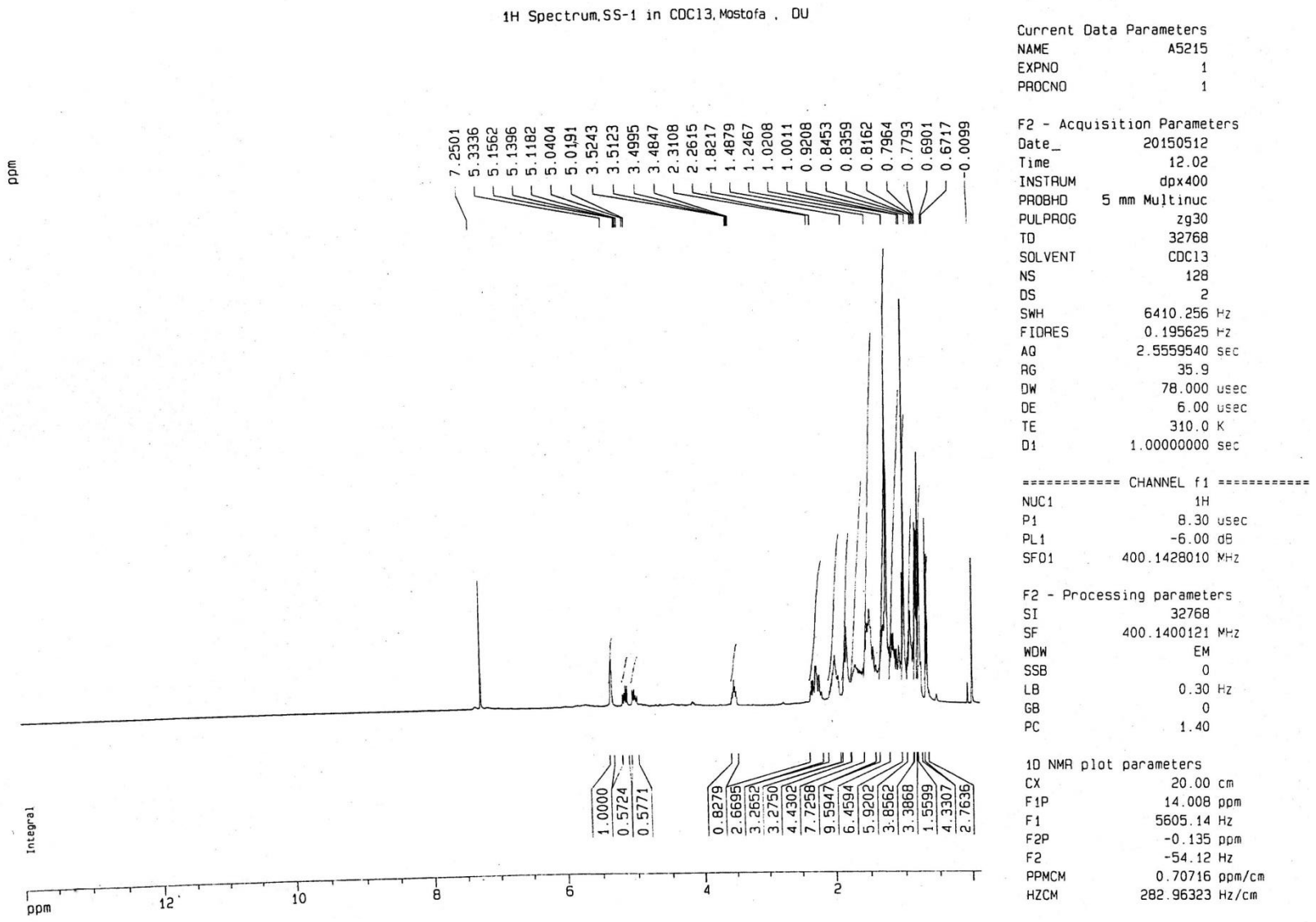

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Figure 4.29 (a) ^{1}H-NMR spectrum of compound SS-I

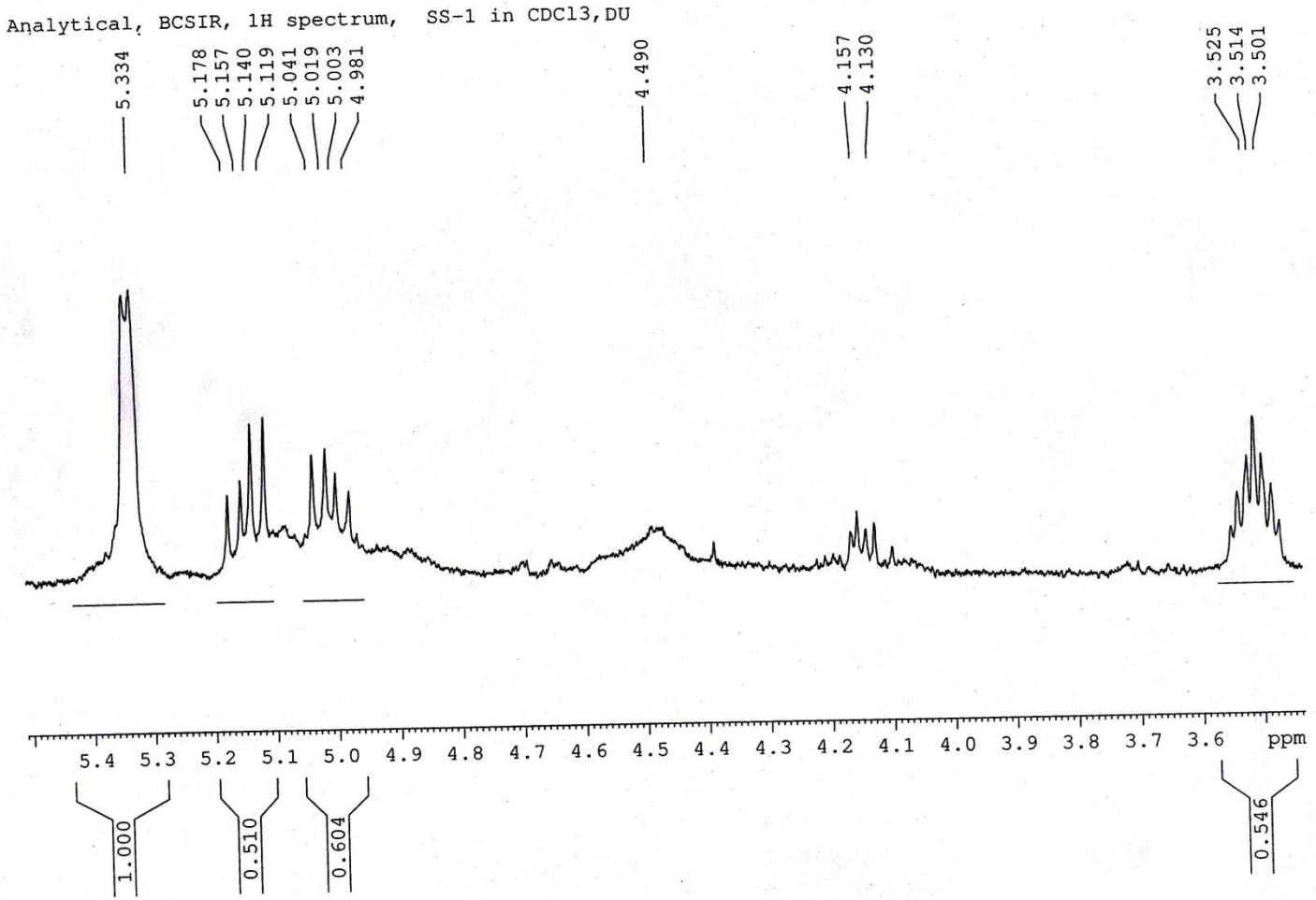


Figure 4.30 ^{1}H-NMR spectrum (expanded) of compound SS-I

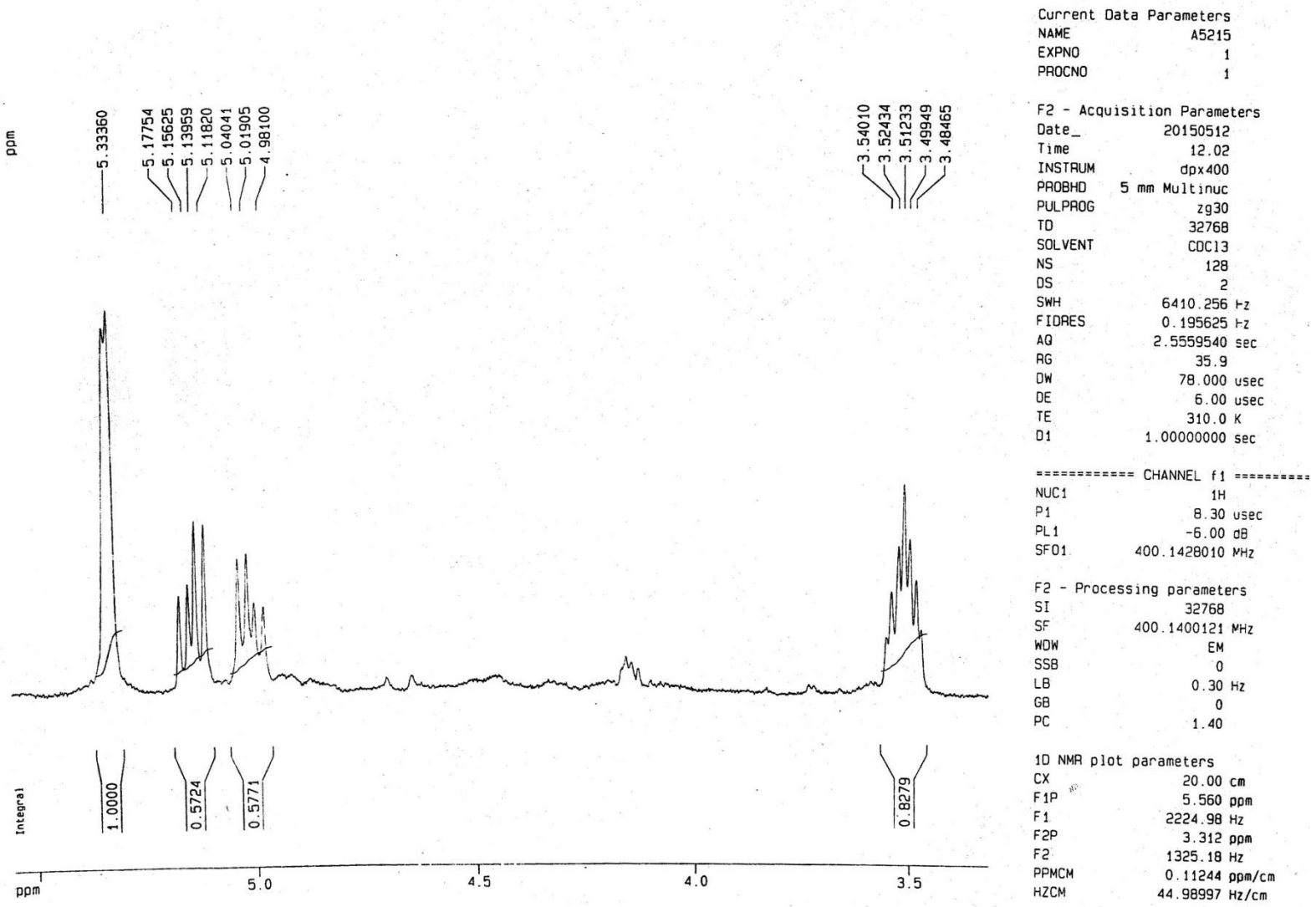


Figure 4.30 (a) ^{1}H-NMR spectrum of compound SS-I

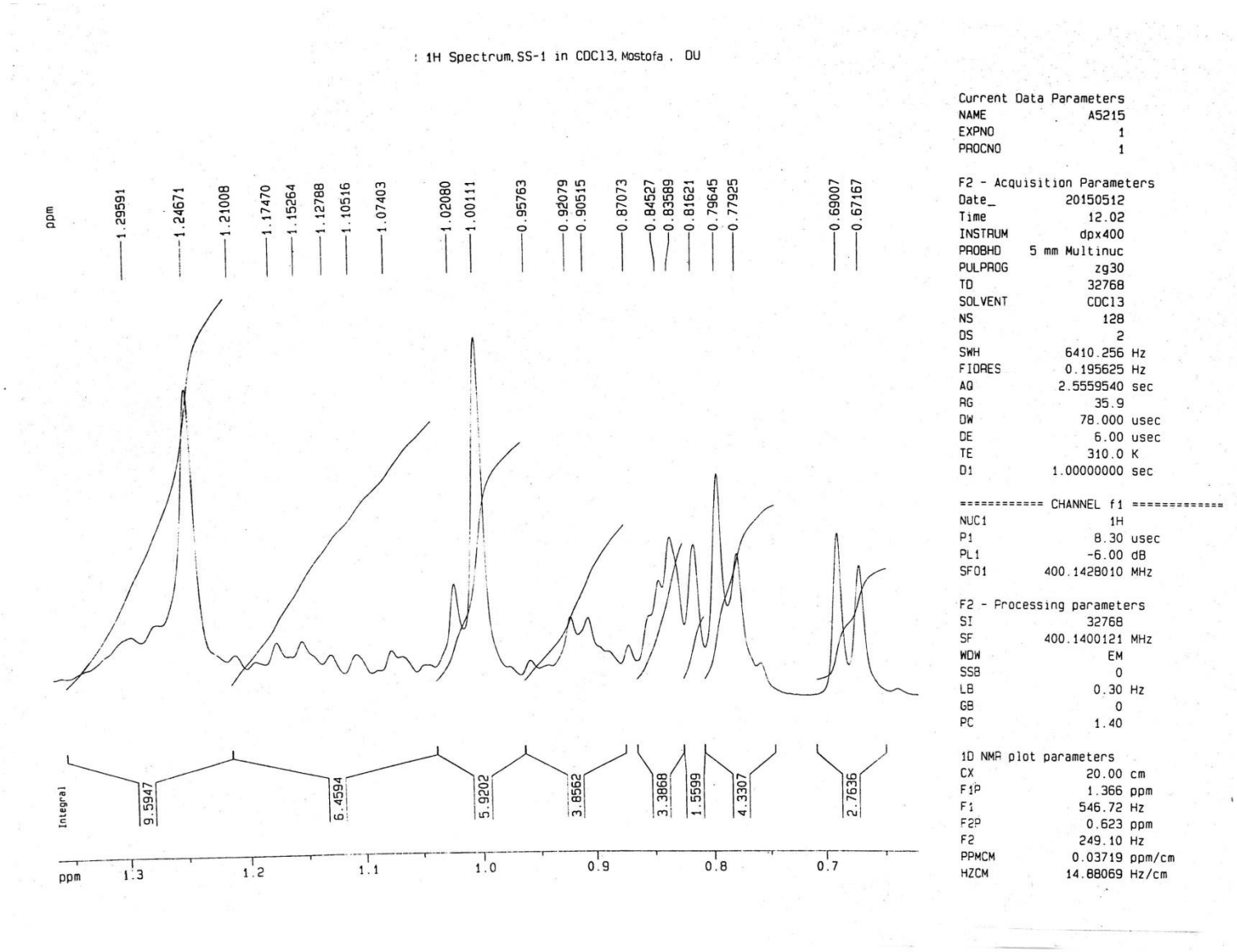


Figure 4.31 ^{1}H-NMR spectrum of compound SS-I

Figure 4.32 ^{1}H-NMR spectrum of compound SS-I

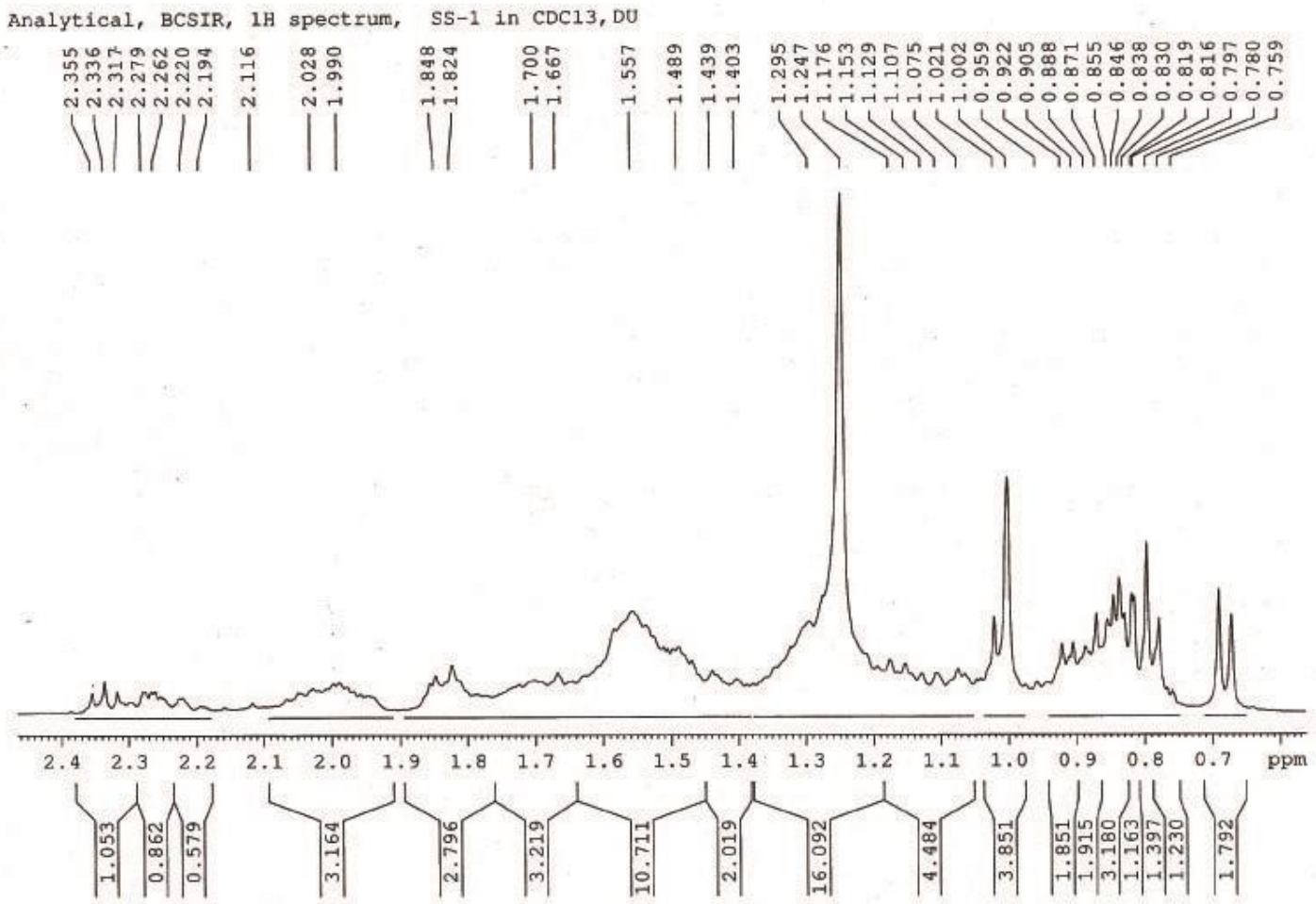


Figure 4.33 ^{1}H-NMR spectrum of compound SS-I

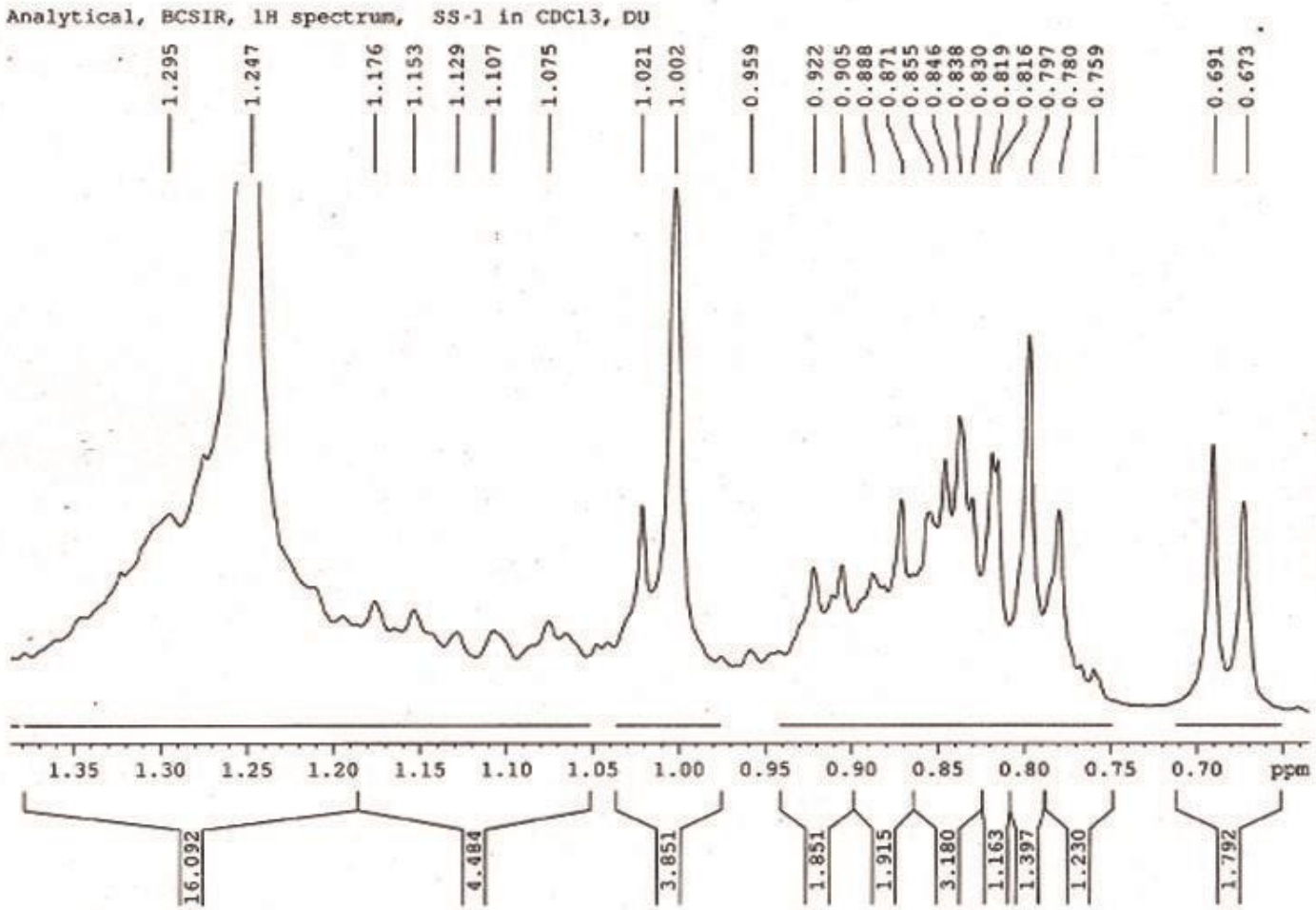
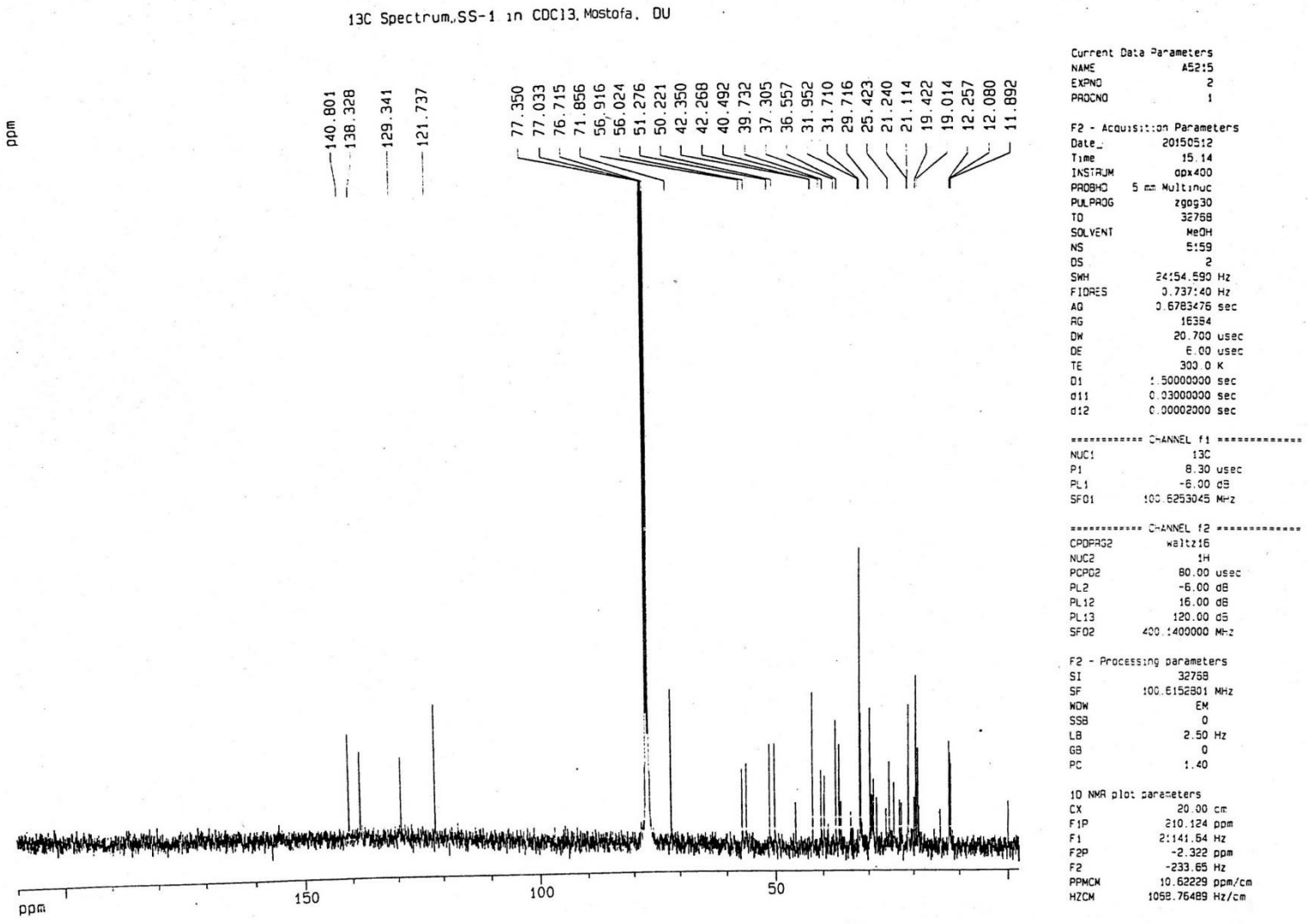


Figure 4.33 (a) ^{1}H-NMR spectrum (expanded) of compound SS-I

Figure 4.34 ^{13}C-NMR spectrum of compound SS-I



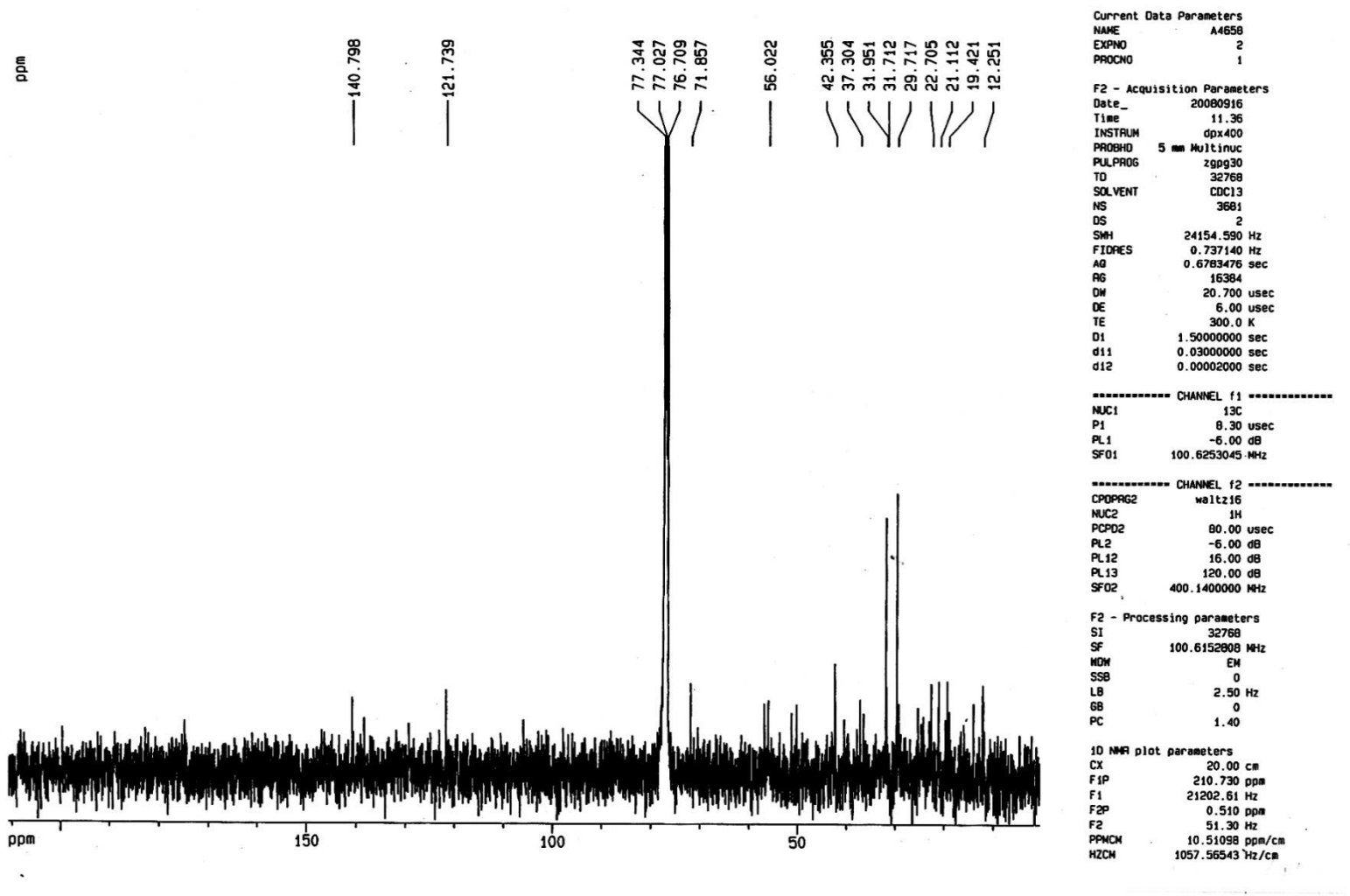
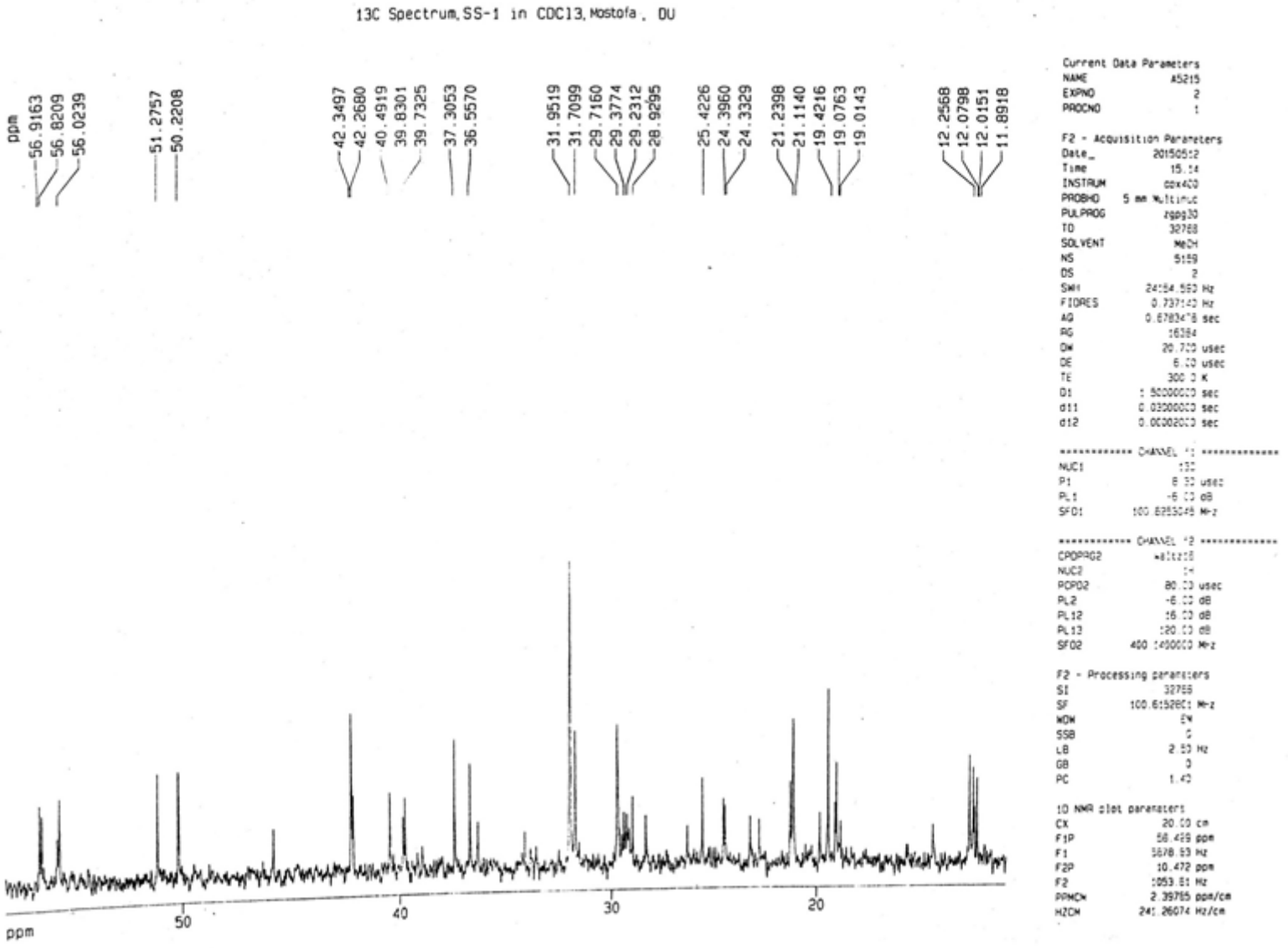


Figure 4.34 (a) ^{13}C-NMR spectrum of compound SS-I

Figure 4.35 ^{13}C-NMR spectrum of compound SS-1



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SFO1 100.6253048 MHz

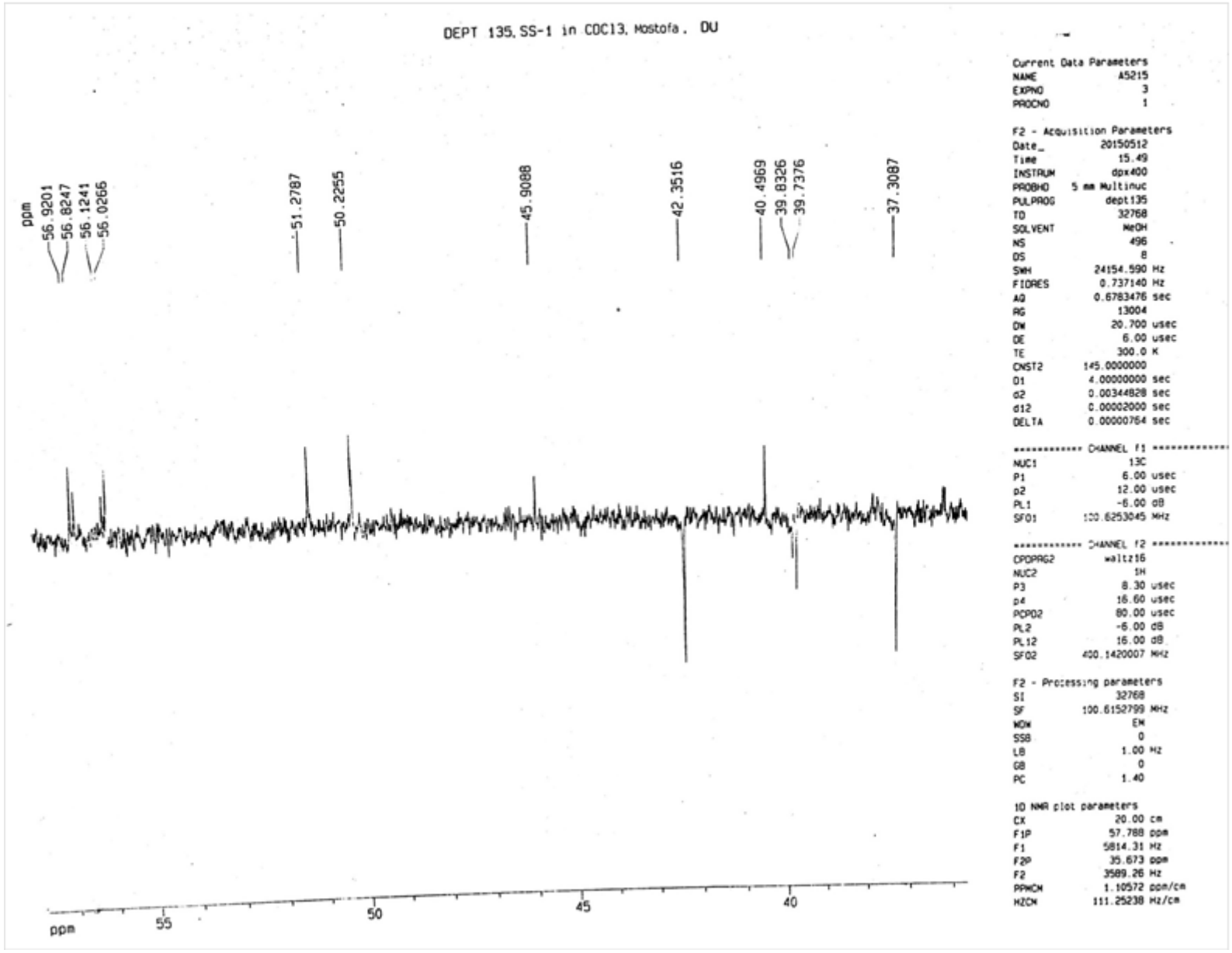
********** CHANNEL f2 **********
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -6.00 dB
PL12 16.00 dB
PL13 120.00 dB
SFO2 400.1400000 MHz

F2 - Processing parameters
SI 32768
SF 100.6152801 MHz
WDW EM
SSB 0
LB 2.50 Hz
GB 0
PC 1.40

1D NMR plot parameters
CX 20.00 cm
F1P 58.499 ppm
F1 5878.93 Hz
F2P 10.472 ppm
F2 1053.81 Hz
PPMCM 2.39765 ppm/cm
HZCM 241.26074 Hz/cm

Figure 4.36 DEPT-135 spectrum of compound SS-I

Figure 4.37 DEPT-135 spectrum of compound SS-1



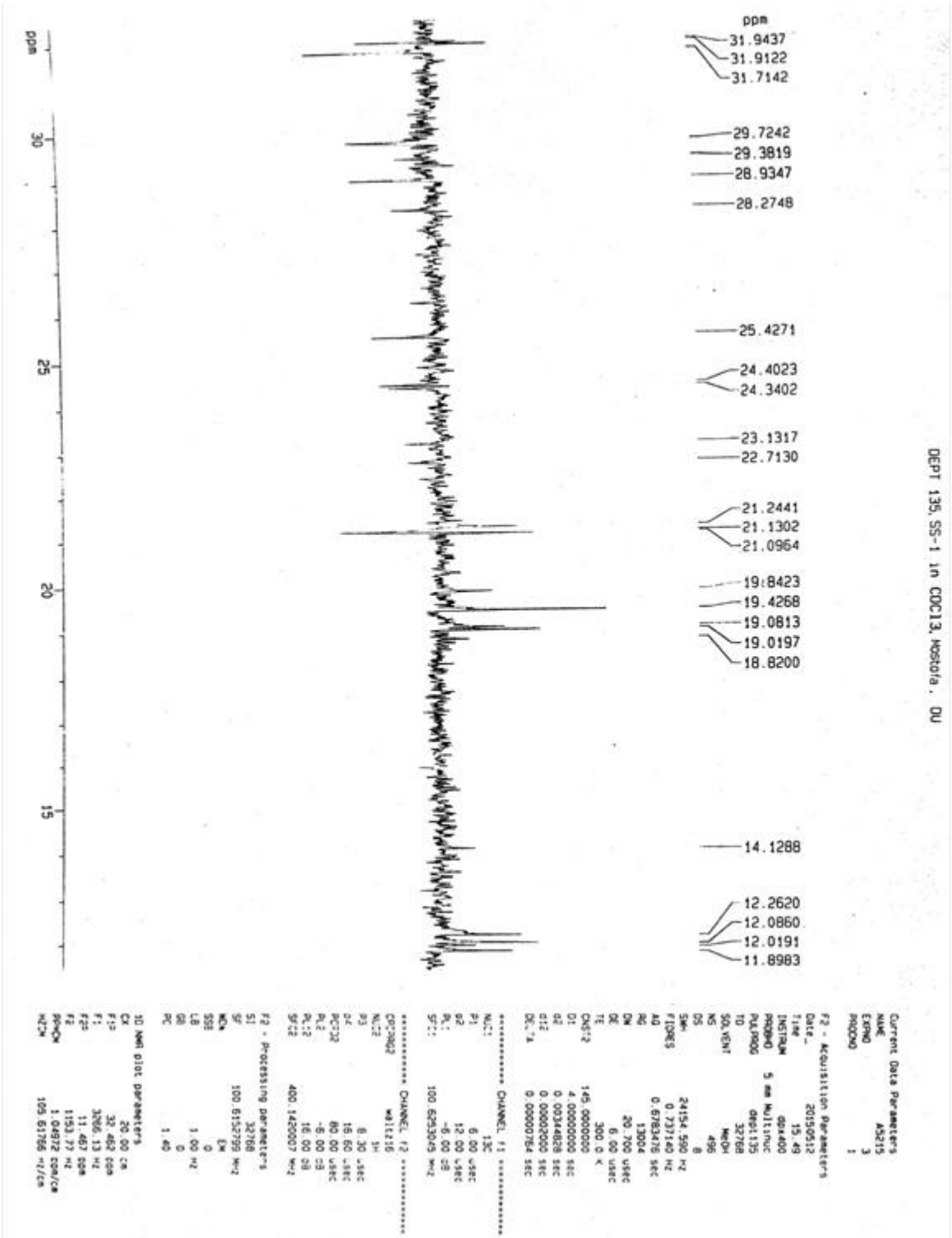


Figure 4.38 DEPT-135 spectrum of compound SS-I

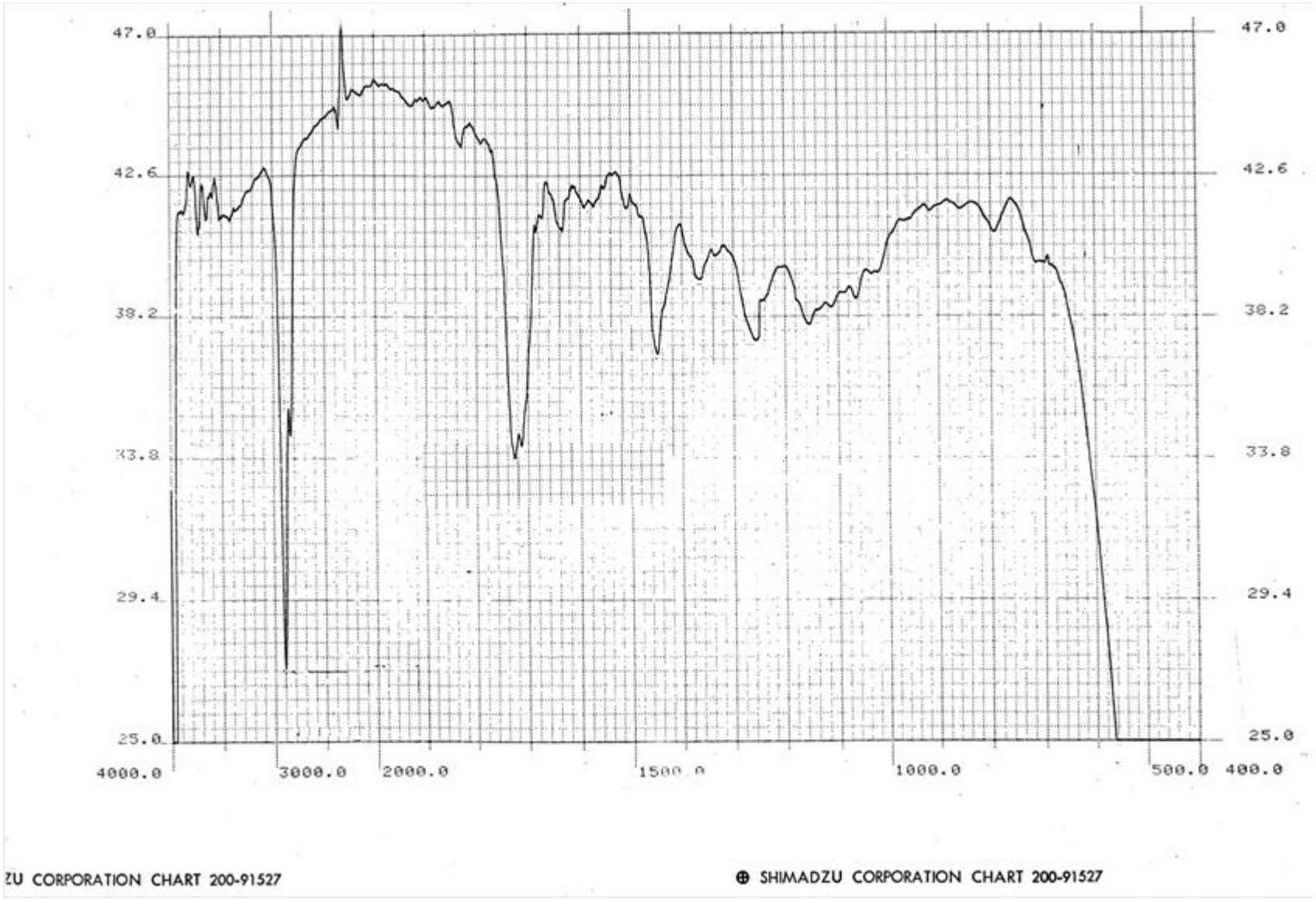
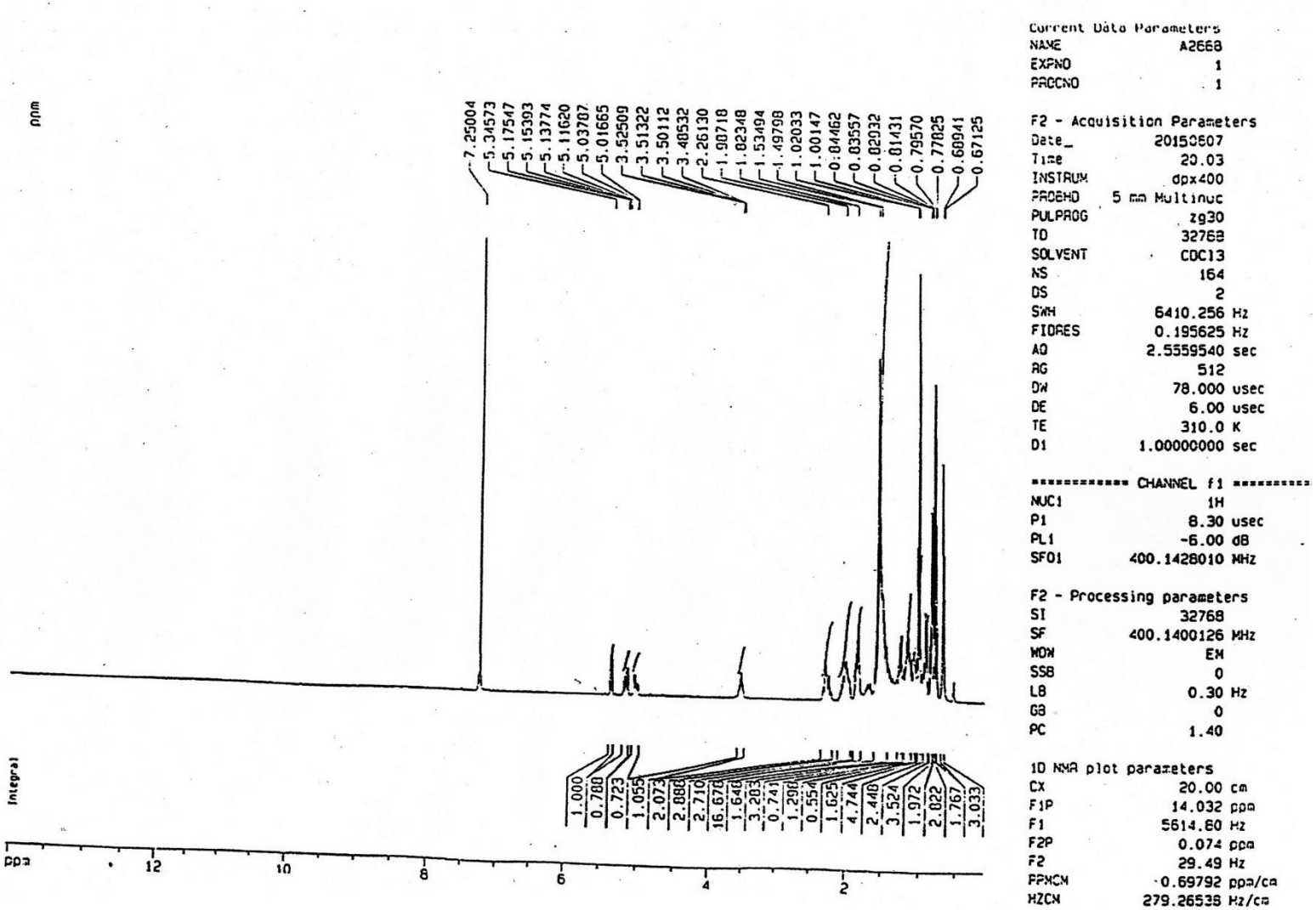


Figure 4.39 IR spectrum of compound SS-II

Figure 4.40 ^{1}H-NMR spectrum of compound SS-II



Current Data Parameters
NAME A2668
EXPNO 1
PROCNO 1

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PULPROG zg30
TD 32768
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NS 164
DS 2
SWH 6410.256 Hz
FIDRES 0.195625 Hz
AQ 2.5559540 sec
RG 512
DW 78.000 usec
DE 6.00 usec
TE 310.0 K
D1 1.00000000 sec

========== CHANNEL f1 ==========
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P1 8.30 usec
PL1 -6.00 dB
SFO1 400.1428010 MHz

F2 - Processing parameters
SI 32768
SF 400.1400126 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.40

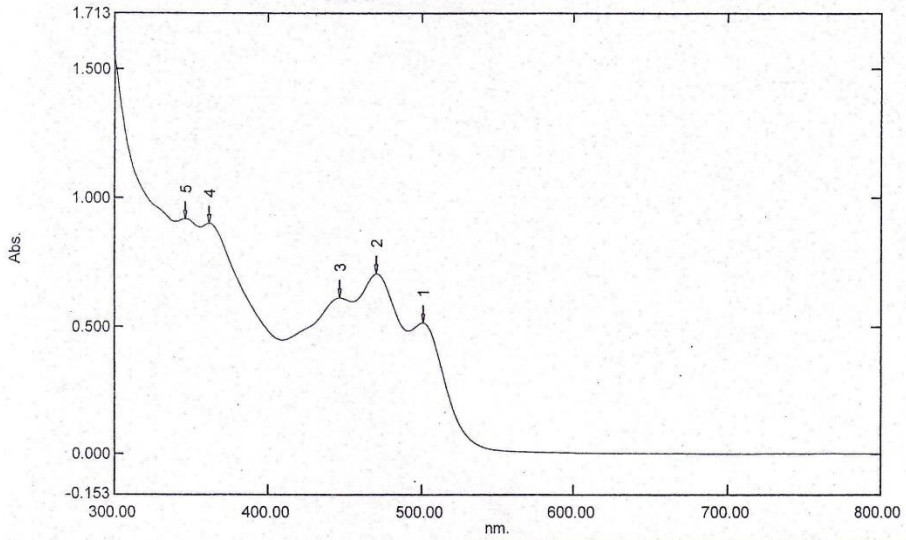
1D NMR plot parameters
CX 20.00 cm
F1P 14.032 ppm
F1 5614.60 Hz
F2P 0.074 ppm
F2 29.49 Hz
PPMCM 0.69792 ppm/cm
HZCM 279.26538 Hz/cm

Figure Fig 4.41 UV spectrum of extract-l

Spectrum Peak Pick Report

07/25/2015 12:06:05 AM

Data Set: File_159429_110447 - Data



[Measurement Properties]
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Auto Sampling Interval: Disabled
Scan Mode: Single

[Instrument Properties]
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 340.0 nm
S/R Exchange: Normal

[Attachment Properties]
Attachment: None

[Operation]
Threshold: 0.0010000
Points: 4
InterPolate: Disabled
Average: Disabled

[Sample Preparation Properties]
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

No.	P/V	Wavelength	Abs.	Description
1	↑	500.50	0.515	
2	↑	470.50	0.706	
3	↑	446.00	0.612	
4	↑	362.00	0.902	
5	↑	346.50	0.920	
6	↓	491.50	0.482	
7	↓	454.50	0.598	
8	↓	409.50	0.447	
9	↓	356.50	0.887	
10	↓	339.50	0.908	

Figure 441 UV spectrum of di-mixture extract-l of tomato paste

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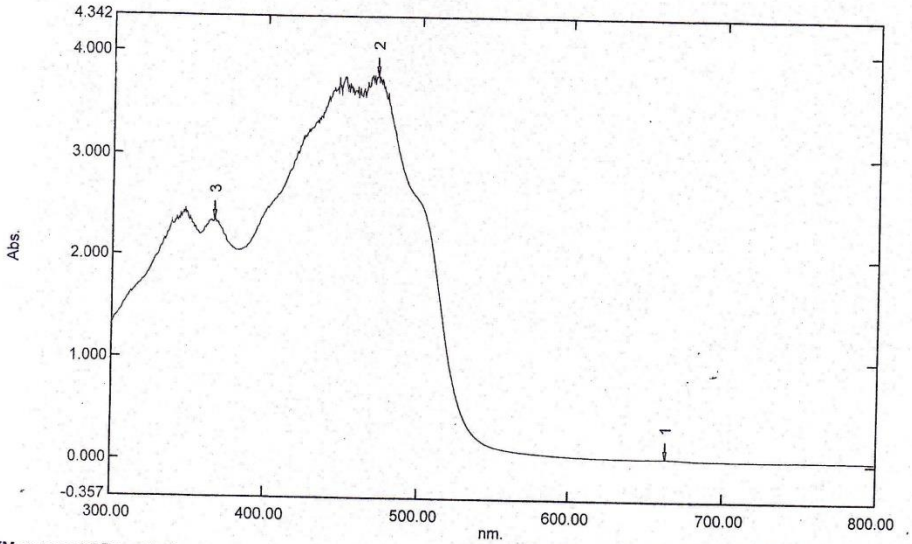
Figure Fig 4.42 UV spectrum of extract-lI

Spectrum Peak Pick Report

07/25/2015 07/25/2015 11:53:17 AM

Data Set: File_150929_112250 - Data



No.	P/V	Wavelength	Abs.	Description
1	↑	660.00	0.059	
2	↑	472.00	3.778	
3	↑	367.00	2.351	
4	↓	383.00	2.048	
5	↓	357.00	2.209	

[Measurement Properties]
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[Instrument Properties]
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Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 340.0 nm
S/R Exchange: Normal

[Attachment Properties]
Attachment: None

[Operation]
Threshold: 0.0010000
Points: 4
InterPolate: Disabled
Average: Disabled

[Sample Preparation Properties]
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

Figure 442 UV spectrum of di-mixture extract-II of tomato paste

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Figure Fig 4.43 UV spectrum of extract-lII

Spectrum Peak Pick Report

07/25/2015 12:26:20 AM

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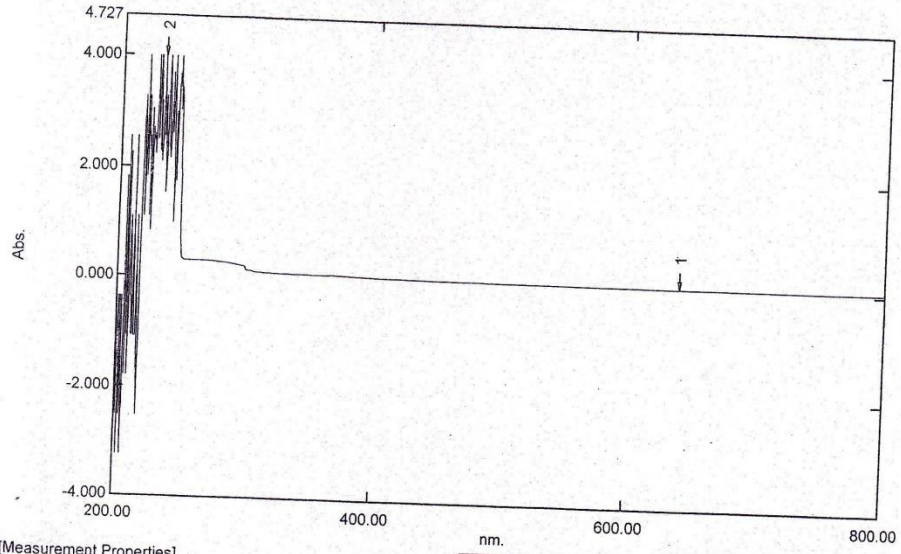

No.	P/V	Wavelength	Abs.	Description
1	⊕	640.50	0.047	
2	⊕	233.00	4.000	

[Measurement Properties]
Wavelength Range (nm.): 200.00 to 800.00
Scan Speed: Fast
Sampling Interval: 0.5
Auto Sampling Interval: Disabled
Scan Mode: Single

[Instrument Properties]
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 340.0 nm
S/R Exchange: Normal

[Attachment Properties]
Attachment: None

[Operation]
Threshold: 0.0010000
Points: 4
nterPolate: Disabled
Average: Disabled

Sample Preparation Properties]
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

Figure 443 UV spectrum of di-mixture extract-III of tomato paste

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Figure Fig 4.43 (a) UV spectrum of extract-lII

Spectrum Point Pick Report

07/25/2015 11:01:16 AM

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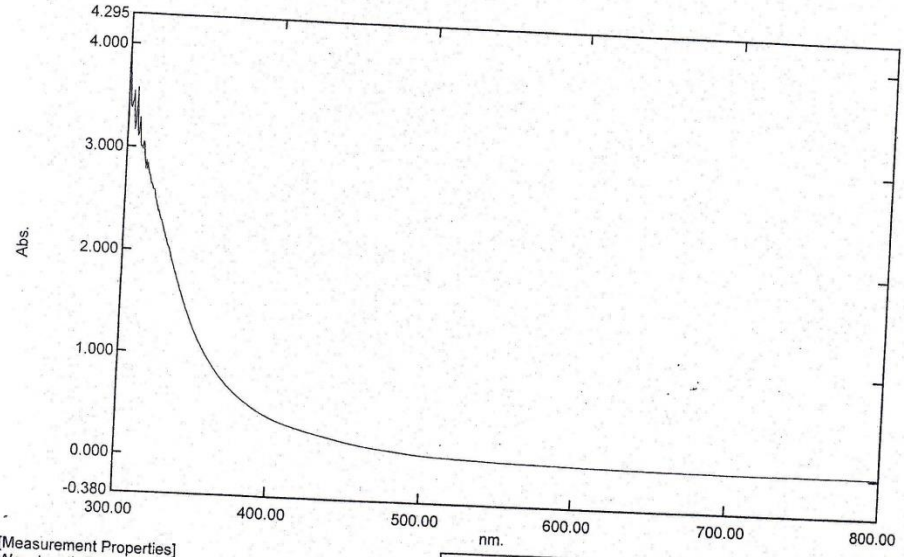

No.	Wavelength	Absorbance	Description
1			

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Auto Sampling Interval: Disabled
Scan Mode: Single

[Instrument Properties]
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
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Light Source Change Wavelength: 340.0 nm
S/R Exchange: Normal

[Attachment Properties]
Attachment: None

[Operation]
Threshold: 0.0010000
Points: 4
InterPolate: Disabled
Average: Disabled

[Sample Preparation Properties]
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

Figure 443 UV spectrum of di-mixture extract -III of tomato pulp

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Figure Fig 4.43 (b) UV spectrum of extract-lII

Spectrum Peak Pick Report 07/25/2015 12:56:03 AM

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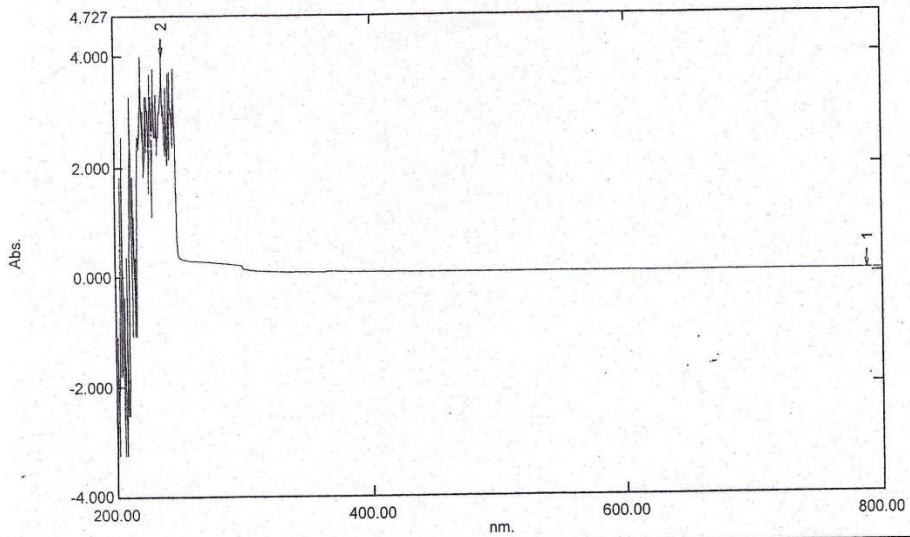

No.	P/V	Wavelength	Abs.	Description
1	↑	788.50	0.060	
2	↑	237.50	4.000	
3	↓	782.00	0.059	

[Measurement Properties]
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Scan Speed: Fast
Sampling Interval: 0.5
Auto Sampling Interval: Disabled
Scan Mode: Single

[Instrument Properties]
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 340.0 nm
S/R Exchange: Normal

[Attachment Properties]
Attachment: None

[Operation]
Threshold: 0.0010000
Points: 4
InterPolate: Disabled
Average: Disabled

[Sample Preparation Properties]
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

Figure 443 UV spectrum of di-mixture extract-III of tomato paste

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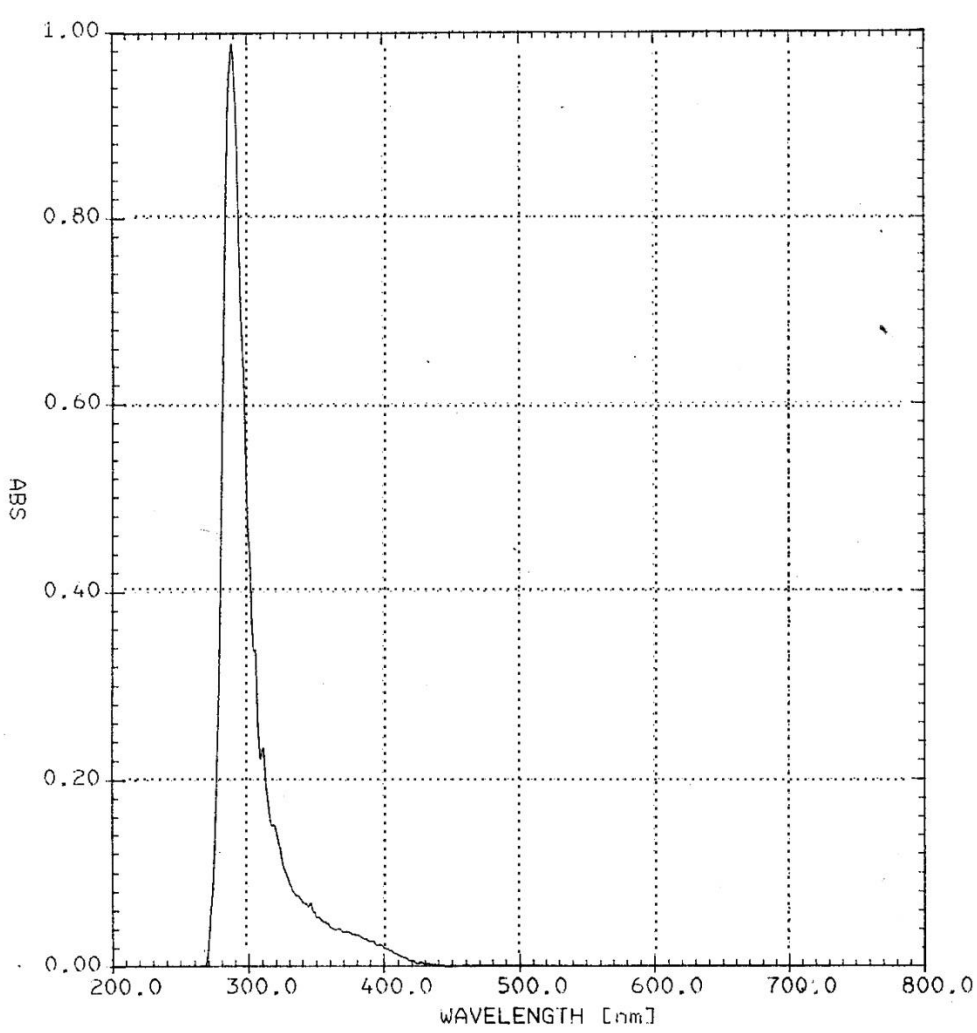

*** PEAK-PICK ***

No.	-- PEAK -- λ	ABS	-- VALLEY -- λ	ABS
1	288.0	0.989	221.0	-0.215

Figure 4.44 UV spectrum of fraction CM-I

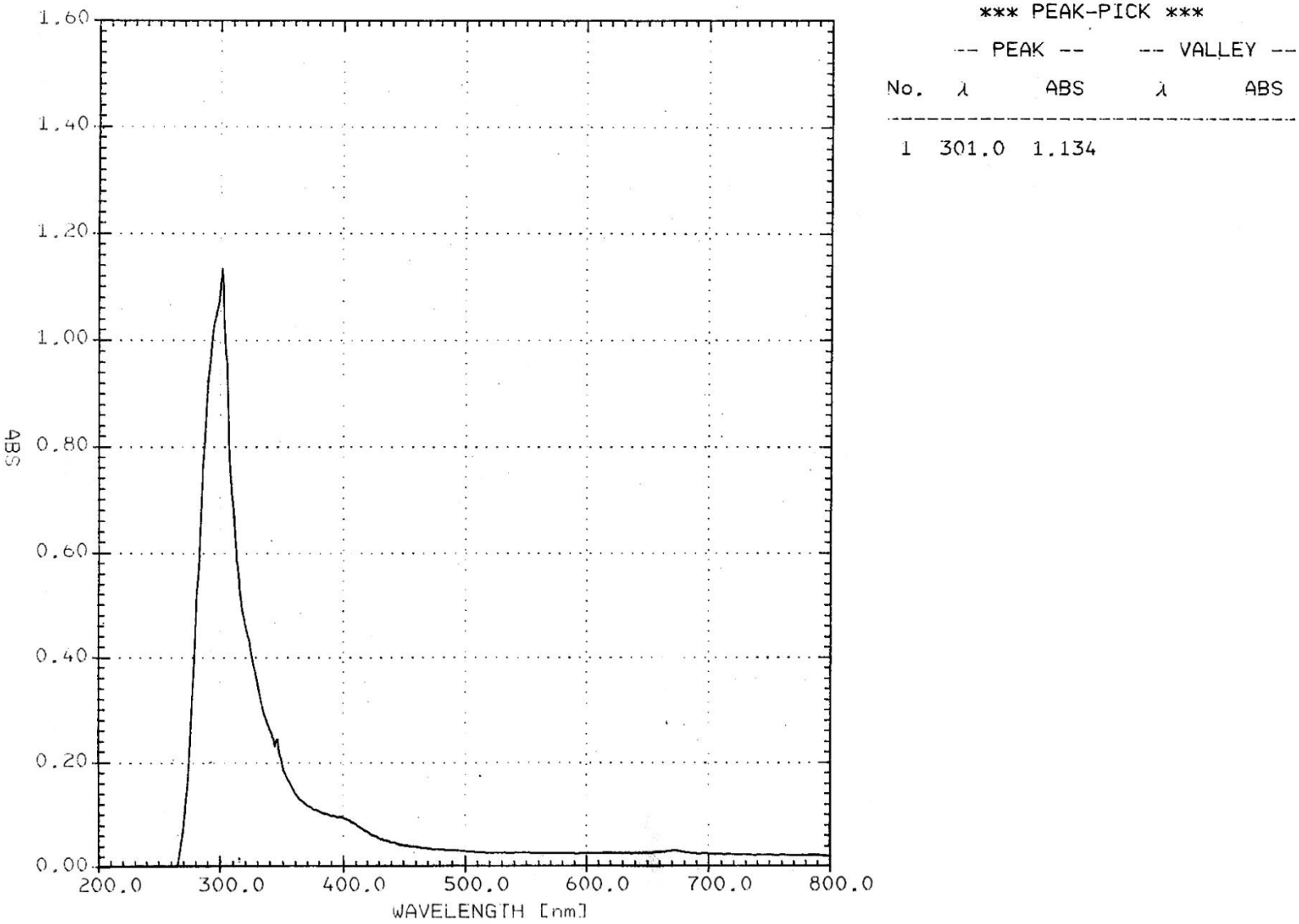

Figure 4.45 UV spectrum of fraction CM-I (with NaOH)

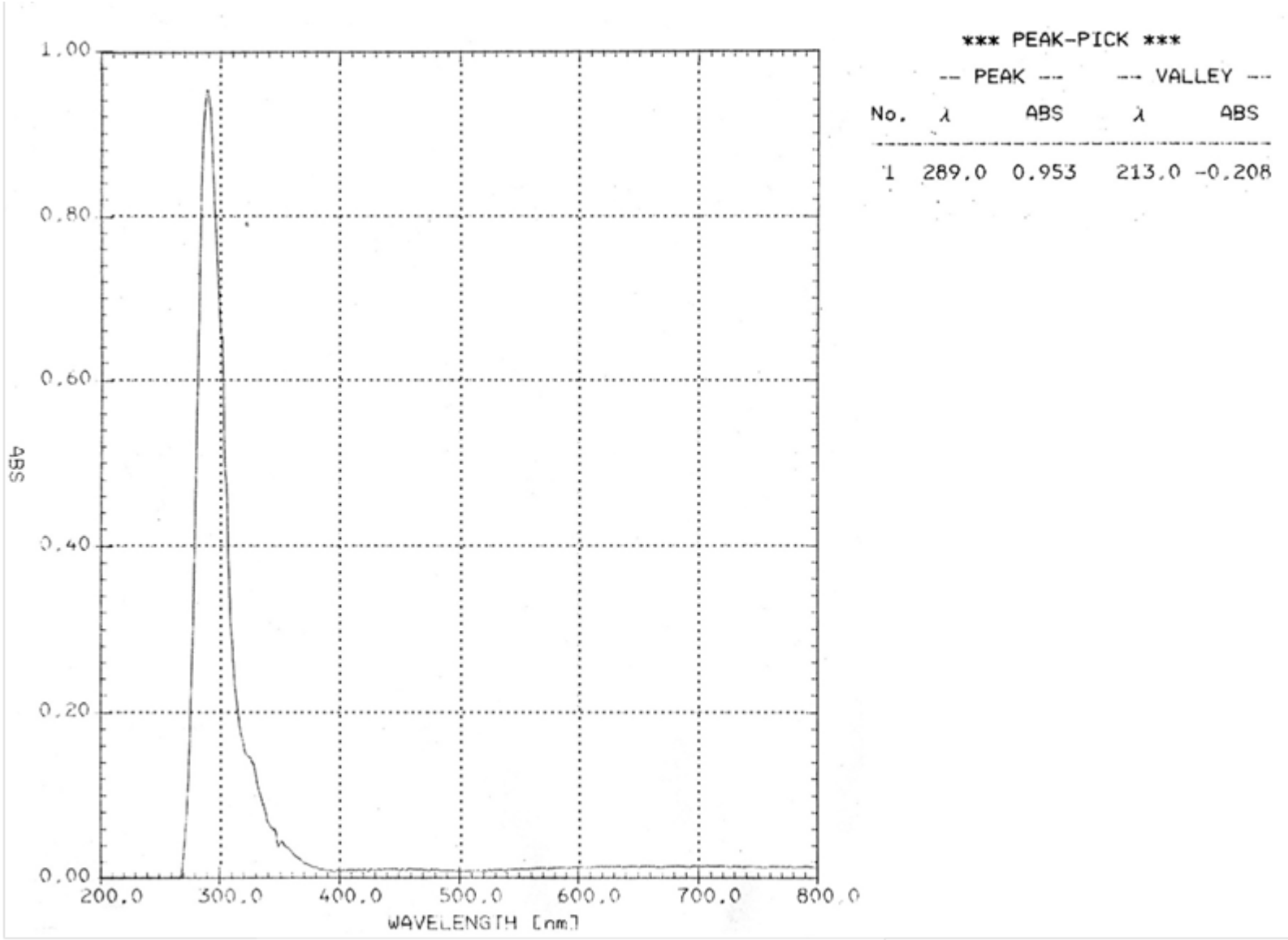

Figure 4.46 UV spectrum of fraction CM-II

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