Chemical and Biological Studies of Some *Litsea* Species Available in Bangladesh

Dissertation submitted to the Faculty of Pharmacy, Dhaka University in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Pharmaceutical Chemistry

By

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Declaration

I do hereby declare that the resources personified in this thesis entitled " **Chemical and Biological Studies of Some *Litsea* Species Available in Bangladesh**" inscribed for submission to the University of Dhaka, Dhaka, Bangladesh for the Degree of Doctor of Philosophy in Pharmaceutical Chemistry are the original research works of mine and have not been previously submitted for the award of any Degree.

(Israt Jahan Bulbul)
Signature of the candidate

Certificate

This is to certify that the things and resources included in this thesis are the original research work conducted by Israt Jahan Bulbul, Reg. no. 50(New), session 2017-2018, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka, Bangladesh. The thesis contains no material previously published or written by another person except when due reference is made in the text of the thesis.

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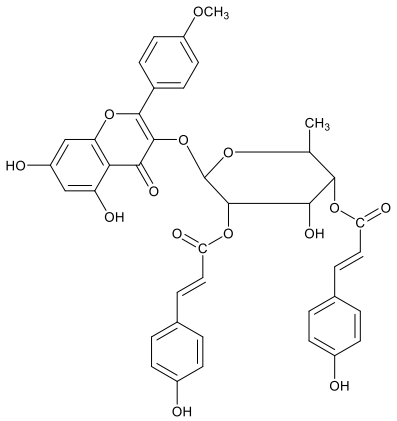
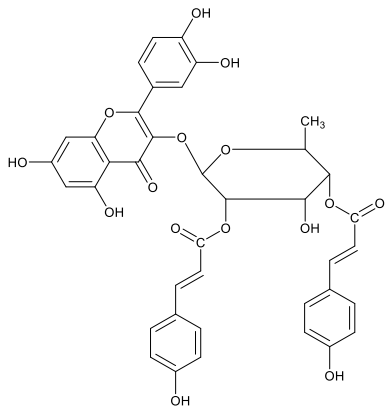
Abstract

In order to discover new phytochemicals as well as searching for evidence based information of traditional therapeutic uses of medicinal plants, four species of the genus *Litsea* belonging to the Lauraceae family were selected in this study. The selected plants are *Litsea glutinosa* Lour., *L. monopetala* Roxb., *L. deccanensis* Gamble. and *L. lancifolia* Hook. f.

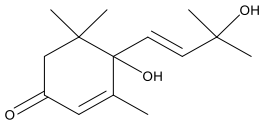
Two compounds were revealed from *L. glutinosa* and they are 4′-*O*-methyl-(2″,4″,-di-*E*-p-coumaroyl)-afzelin (LGC-26, **95**) and quercetin-3-*O*-(2″,,4″,-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside [or, 5′-hydroxyl-(2″,4″-di-*E*-*p*-coumaroyl) afzelin] (LGC-45-3, **96**). Both of the compounds were reported for the first time from *Litsea* species. Five compounds were isolated and purified from the leaf extract of *L. monopetala* and characterized as vomifoliol (LML 363-1, **97**), α-amyrin (LML 309, **98**), β-amyrin (LML 301, **99**), *(E)*-6,7,8,9,10,11-hexahydro-8,17:10,16-di(metheno)dibenzo-[*h,l*][1]oxa[5] azacyclotridecine-1,4-diol (LML 339-1, **100**) and (*Z*)-1,2,3,4,5,6-hexahydro-8,11-etheno-2,13:4,12di(metheno)benzo[*h*][1]-oxa[5]aza-cyclopentadecine (LML 339-2, **101**) by ^{1}H and ^{13}C NMR, COSY, HSQC, HMBC spectral data analysis. All these five compounds are reported for the first from *L. monopetala*, while compounds **101** and **101** appear to be new compounds. The obtained ^{1}H NMR spectral data and the comparison with the reference value helped us to characterize lupeol (LDC-10-3, **102**) and a mixture (4:1 ratio) of β-sitosterol and stigmasterol (LDC-10-2, **55** & **56**) from *L. deccanensis* and β-sitosterol (LLC-10-1, **55**) from *L.lancifolia*. Lupeol has been isolated from *L. deccanensis* for the first time.



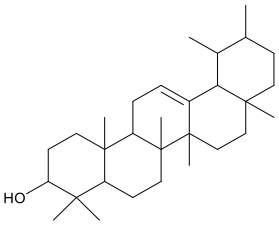
4′-*O*-methyl (2″,4″-di-*E*-*p*-coumaroyl) afzelin (**95**)



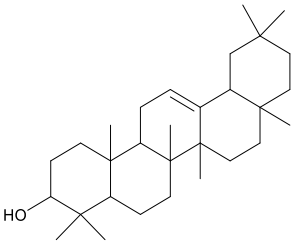
Quercetin3-*O*-(2″,4″-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside [**or** 5′-hydroxyl-(2″,4″-di-*E*-*p*-coumaroyl) afzelin] (**96**)



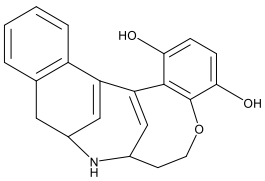
Vomifoliol (**97**)



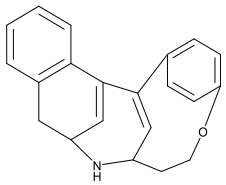
α- amyrin (**98**)



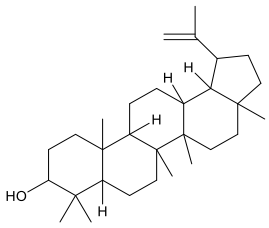
β- amyrin (**99**)



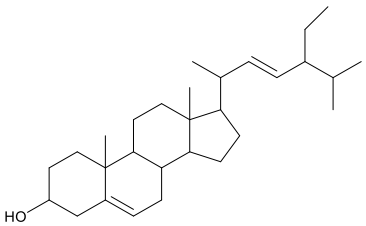
(E)-6,7,8,9,10,11-hexahydro-8,17:10,16-di(metheno)dibenzo-[*h*,*l*][1]oxa[5]azacyclotridecine-1,4-diol (**100**)



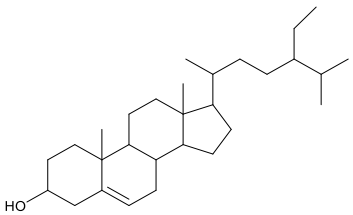
(*Z*)-1,2,3,4,5,6-hexahydro-8,11-etheno-2,13:4,12-di(metheno)benzo-[*h*][1]oxa[5]azacyclopentadecine (**101**)



Lupeol (**102**)



Stigmasterol (**56**)



β-Sitosterol (**55**)

The crude extracts of *L. glutinosa*, *L. monopetala*, *L. deccanensis* and *L. lancifolia* were evaluated for biological activities through *in-vitro* and *in-vivo* screenings. For antidiarrheal activity test, 100, 200 and 400 mg/kg bw methanol extract of *L. glutinosa* (MELG), *L. monopetala* (MELM), *L. deccanensis* (MELD) and *L. lancifolia* (MELL) were administered in two animal models where 0.5 ml castor oil was used for diarrhea induction and 3 mg/kg bw loperamide was used as standard drug. In all the groups treated with MELG, MELM, MELD and MELL extracts wet feces number, total number of feces and total weight of the foecal output were decreased significantly ($p<0.05$) with rising of doses. The maximum peristaltic inhibition was observed 32.36%, 22.52%, 26.26% and 33.22% for 400 mg/kg by MELG, MELM, MELD and

MELL extracts respectively. The peristaltic indices were 59.0%, 79.0%, 59.1% and 63.0% for 400 mg/kg of MELG, MELM, MELD and MELL respectively compared to the control (90.0%) and standard (66.7%) groups. For all the plant extracts the percentage inhibition of gastrointestinal motility and peristalsis index were comparable to the standard.

In analgesic activity test, MELG, MELM, MELD and MELL on acetic acid-induced writhing in mice at two different doses (100 and 200 mg/kg bw) showed significant reduction of squirming ($p<0.001$, $p<0.01$ and $p<0.05$) in a dose dependent manner as paralleled to control. In the second animal model (Eddy's hot plate method), pain was induced by heat and analgesia was assessed by counting the time required for the initiation of the reaction. Out of the four plants, all the plants at 100, 200 and 400 mg/kg doses, increased the latency time. The pain-relieving activity data (formalin method) are presented as licking and biting time in seconds at early- and late-phases of treatment with plant extracts. In both the early- and late-phase, reaction time for licking and biting hind paw were decreased with the increment of the doses (from 100 mg/kg bw to 200 mg/kg bw) but in the late phase (20-30 min) the reaction time was decreased significantly ($p < 0.05$) with the increment of doses for all the studied plant extracts as well as standard indomethacin at 10 mg/kg bw.

The effects of MELG, MELM, MELD and MELL on blood glucose level in streptozocin (STZ) induced diabetic rats were found to drop the blood glucose level (BGL) significantly ($p<0.05$) after 7^{th} days of treatment with the plant extracts at 300 and 500 mg/kg/day doses. Percentage inhibition of blood glucose level for MELG, MELM, MELD and MELL were comparable with that of standard metformin and they are 66.69%, 57.06%, 68.16% and 69.33% respectively at 500 mg/kg/day dose as compared to the untreated diabetic control group.

Hole cross test was performed to investigate the possible neuropharmacological effects (CNS stimulant or depressant) of MELG, MELM, MELD and MELL and all the extracts at two different doses (300 and 500 mg/kg bw) found to reduce locomotion in the test animals and to decrease the passing number through the hole in between the hole cross chamber by the animals in a dose dependent manner.

Three fractions of four different species of *Litsea* were studied for antimicrobial activity by disc diffusion method. The results of antimicrobial activity test of different fractions showed mild to moderate activity for *L. monopetala,* very good activity for *L. lancifolia,* moderate to good activity for *L. glutinosa* and mild to very good antimicrobial activity for *L. deccanensis* against the microorganisms selected for this study.

The quantities of phenolic compounds were found in ethyl acetate fraction of *L. glutinosa* (103.04±0.06), followed by ethyl acetate fraction of *L. lancifolia* (79.94±0.07). Among the plants *L. lancifolia* and *L. glutinosa* have shown very good total phenolics compared to *L. deccanensis* and *L. monopetala.* In DPPH free radical scavenging activity test, the IC_{50} for pet-ether, chloroform and ethyl acetate fractions of *L. deccanensis* were 31.75 µg/ml, 24.62 µg/ml and 31.04 µg/ml, respectively. All the values are comparable with that of ascorbic acid (31.66 µg/ml). For *L. lancifolia* 63.97 µg/ml pet-ether, 65.91 µg/ml chloroform and 80.46 µg/ml ethyl acetate extractives were required for 50% scavenging of free radicals. The effective concentrations for pet-ether, chloroform and ethyl acetate fractions of *L. glutinosa* were measured as 25.19 µg/ml, 37.90 µg/ml and 67.41 µg/ml, respectively. The IC_{50} values were 31.94 µg/ml, 24.91 µg/ml and 31.10 µg/ml for pet-ether, chloroform and ethyl acetate fractions of *L. monopetala*, respectively. From results, it may be proposed that three different extractives of *L. deccanensis*, *L. lancifolia*, *L. glutinosa and L. monopetala* were able to exhibit the free radical scavenging activity compared to ascorbic acid, a potent antioxidant compound.

The docking simulation was conducted against aldose reductase (AKR1B1) protein model with the purified compound **95** and **96** by using Auto Dock Vina software. Compound **95** exposes the higher negative binding affinity (-9.8 kcal/mol) as compared to the compound **96** with binding affinity (-9.4 kcal/mol) for the interaction of the target protein aldose reductase (AKR1B1). Compound **95** exhibited strong connection with eleven hydrophobic bonds, hydrogen bonds and one other bond while compound **96** developed stable interactions by three hydrogen bonds, and eleven hydrophobic bonds. During investigating interaction pattern, binding affinity, and best binding poses of the compounds it can be proposed that both structures might be promising inhibitors against aldose reductase (AKR1B1) protein.

Molecular docking analysis of isolated compounds **95** and **96** (−9.4, and −8.9 kcal/mol, respectively) against human pancreatic alpha amylase showed promising docking affinity. Compound **95** formed polar contacts with Tyr-151, Thr-163, Arg-195, Asp-197, His-201, and His-299 residues and compound **96** showed polar contacts with Gln-63, Arg-195, Asp-197, and His-299 residues. These findings suggest that these compounds are promising inhibitors of human pancreatic alpha amylase.

The docking results of vomifoliol clearly indicate that it is a better candidate as an analgesic agent. Vomifoliol **(97)** is a potent binder (-4.9 kcal/mol) to COX-2 than indomethacin (-1.1 kcal/mol) indicating that it is supposed to have better analgesic action.

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Chapter 1: Introduction

1.1 Rationale of the work

People have been using plants for therapeutic purposes for about 4000 years. Egyptian papyrus, ancient Ayurvedic and Unani manuscripts and Chinese writings mentioned the use of herbs and all of them exist as evidence of using herbs as medicine for long time. Treatment using herbs and plants is becoming very popular worldwide due to minimum or no side effects, absent or low toxicity, comprehensive biodegradability, easy availability as compared with the insufficient supply, unaffordable treatment cost, severe side effects with synthetic drugs. According to WHO, 80% of the total population worldwide depend on 21,000 plant species for various aspects of their major health care requirements (Lucy and Edger, 1990). As estimated, in developed countries like the United States, plant drugs contributed 25%, while it is 80% of the total drugs in fast developing countries like India, China and Bangladesh (Khan, 2016). Furthermore, medicinal plants play a very significant role in the economy of countries like Bangladesh.

In Bangladesh, many of the medicinal plants such as black cumin, garlic, aloe, neem, tulsi, turmeric, clove, cardamom and ginger are well-known home remedies for their uses in numerous common diseases. Traditional healers of Bangladesh are giving many plant drugs very effectively for treating diarrhea, constipation, pain and fever, gout and arthritis, asthma, cough, urinary disorder, menstrual disorder, hypertension, diabetes, leucorrhoea etc (Ahmmed *et al.*, 2017). Though for about two decades, there has been a remarkable increase in the study of plant medicine; however, there is still a noteworthy lack of research based data in this field. Therefore, our study focused on searching evidence based information of selected medicinal plants by pharmacological studies (Zohora *et al.*, 2016; Kumar and Bhowmic, 2010).

In the first spans of the past century, the use of the natural substances had been replaced with the modern synthetic medicines but continuous study of searching for evidenced based data for the pharmacological studies as well as isolation and characterization of new novel bioactive chemical constituents from plants has made remarkable progress in growing the knowledge about plants. Considering plants as the prime source of structurally and therapeutically important compounds, our aim is to carry out

pharmacological and chemical studies of some *Litsea* species available in Bangladesh. Plants possess various therapeutic activities because they are considered as enormous resources of therapeutically active phytoconstituents or secondary metabolites that can lead new drug discovery (Mishra and Tiwari, 2011; Rey-Ladino *et al.,* 2011; Cragg and Newman, 2005; Haefner, 2003; Butler, 2004). Over the past two decades, there is a continuous study of searching of new bioactive chemical constituents from plants, but several plants still remain as the novel source of structurally important compounds that lead to the development of innovative drugs. So, there is huge scope to search for new novel compounds from medicinal plants.

In the Lauraceae family, *Litsea* is the second largest genus. Among more than 300 species there are 12 species of *Litsea* are recorded in Bangladesh (Ara *et al.,* 2007). Most of them are in usage by different groups of people of Bangladesh. Considering *Litsea* genus as the prime source of structurally and therapeutically important compounds, our aim is to carry out pharmacological studies of crude extracts and different partitionates of some *Litsea* species available in Bangladesh as well as isolation and characterization of bioactive principles from these plants.

1.2 General introduction

Bangladesh is bio-geographically located in a favorable location in between the Indo-China and the Indo-Malayan sub region. Due to its distinctive biophysical site, deltaic freshwater and a huge sea, nature provides our country with a rich variety of plant species (Nishat *et al.,* 2002; Barua *et al.,* 2001). Biodiversity gifted us 6000 different plant species among which about 500 species are claimed as medicinal plants (Ghani, 2003).

1.3 Definition of medicinal plant

Medicinal plant refers to plants (herb, shrub or tree) consumed in any forms (fresh/ dried/ decoction etc) to cure ailments, prevent disease and maintain good health.

1.4 Historical documents of medicinal plants

The first identified medical document is the clay tablets by Sumerian that were recorded about 4000-years earlier. Ebers Papyrus is the document of plants having hundreds of

remedies by the ancient Egyptians which was documented about 3500 year ago. The Rig Veda (4500-1600 BC), the 'Charaka Samhita' were the documents of the use of medicinal plants in the Indian subcontinent (Chauhan *et al.*, 2020). There are records of using herbals by the king Hammurabi of Babylon (1800 B.C.) (Tawalare *et al.*, 2021). The Chinese emperor Shen Nung included 300 herbs in a book called 'Pen Tsao' (Bretschneider, 1895). The Greek physician, Hippocrates (460-370 B.C.), developed pharmacopeia and he compiled the information of 300-400 medicinal plants in a book called 'Materia Medica'. The Dioscorides' 'De Materia Medica' (1st century Ad) includes the information of 600 medicinal plants (Staub *et al.*, 2016). Nicholus Culpepper transcribed *A Physical Directory* in 1649 and later on 'The English Physician' which was the manual for the ordinary people to use for health care (Culpeper, 2014). The *U.S. Pharmacopeia* was first published in 1820 and was revised in 1906 (Hershenson, 1964). A new door has opened with the purification of the pharmacologically active phytoconstituents from medicinal plants in the beginning of the 19th century which advanced more with the development of the formulation of the purely synthetic drugs based on medicinal plants in the mid of the 19th century (Petrovska, 2012).

1.5 Importance of secondary metabolites or phytoconstituents obtained from plants

Plants produce lots of secondary metabolites as waste products or bi-products, which are not required for their primary need but for their protection and survival, that is they are necessary for the secondary need of the plants. Many secondary metabolites from plants have important therapeutic purposes. Among them the vital bioactive compounds of medicinal plants are alkaloids, flavonoids, glycosides, tannins and phenolic compounds (Tiwari and Rana, 2015; Makkar *et al.*, 2007).

1.5.1 Alkaloids and their uses

Generally most of the alkaloids have prominent pharmacological activity. Some of the examples of therapeutically important alkaloids are as follows

- Cocaine (*Erythroxyllum coca*) and morphine (*Papaver somniferum*) have antipyretic and analgesic activities (Vogel and Vogel, 1997).
- Emetine (*Cephalis ipecacuanha*) is used as antiviral, antiparasitic, anticancer and

contraceptive. Also, it is reported to regulate several genes (Akinboye and Bakare, 2011).

- Atropine, hyoscine and hyoscyamine (*Datura stramonium, Hyoscyamus niger, Atopa belladonna*) antispasmodic and anodyne and midriatic effects (Luduena and Branin, 1966).
- Quinine, cinchonine (*Cinchona ledgiriana, C. calisaya, C. succirubra*) are used in the treatment of malaria (Ainley and King, 1938).
- Codeine, noscopine (*Papaver somniferum*) are used as antitussive (Bellville *et al.*, 1958).
- Caffine (*Camellia sinensis*), strychnine and brucine (*Strychnos nux-vomica*) have CNS stimulant effects (Cappelletti *et al.*, 2015; Washizu *et al.*, 1961).
- Theobromine and theophylline (*Camellia sinensis*) are respiratory stimulants and have diuretic effects (Kennedy, 2021; Dorfman and Jarvic, 1970; Rieg *et al.*, 2005).
- Ergotamine and ergometrine (*Claviceps purpurea*) stimulate uterine smooth muscle and act as oxytocic (Ma *et al.*, 2018).
- Physostigmine and pilocarpine (various genus of *Pilocarpus*) act as ophthalmic cholinergic (Pinheiro *et al.*, 2018).
- Reserpine, rescinnamine and dcscipidine (*Rauwolfia serpentina*) possess hypotensive effects (Lemieux *et al.*, 1956).
- Vinblastine and vincristine (*Catharanthus roseus*) have an anticancer effect (Rai *et al.*, 2014).

1.5.2 Glycosides and their uses

- Digoxin, digitoxin, gitoxin (*Digitalis purpurea*), K-strophanthin (*Strophanthus* genus), scillaren (*Urginia maritima*) are cardiac muscle stimulators and used for congestive heart failure (Patel, 2016).
- Salicin (Willow or Salix bark) has analgesic and anti-inflammatory properties (Shah *et al.*, 2016).
- Sennoside (*Cassia angustifolia*), cascaroside (*Cascara sagrda*), aloin and barbaloin (*Aloe vera* and *Aloe barbadensis*) possess laxative effects (Sakulpanich and Gritsanapan, 2009).

- Sinigrin (*Brassica nigra*), sinalbin (*Brasica alba*) act as local irritants (Eib *et al.*, 2020).
- Rutin, rutoside (Bitter orange peel, Lemon peel) keep elasticity of blood vessels (Ganeshpurkar and Saluja, 2017).
- Aloin (*Aloe vera* and *Aloe barbadensis*), glycyrrhizin (*Glycyrrhiza glabra*) have anti-inflammatory activity (Xiao *et al.*, 2022).

1.5.3 Volatile oils (Terpenoids and phenylpropanoids) containing plants and their uses

Terpenoids and phenylpropanoids are the chief phytoconstituents of volatile oils which afford several biological properties and can be used for various health problems by traditional healers (Raut and Karuppayil, 2014). Some such biological activities are mentioned below

- Black pepper (*Piper nigram*), Cinnamon (*Cinnamomum zeylanicum*), Sandalwood (*Santalum* genus) have wound healing properties.
- Herbs such as Chamomile (*Matricaria chamomilla*), Ajwain (Trachyspermum ammi), Basil (*Ocimum basicicum*), Cardamom (*Elettaria cardamomum*), Coriander (*Coriandrum sativum*), Fennel (*Foeniculum vulgare*), Peppermint (*Mentha piperita*) and Spearmint (*Mentha spicata*), Cinnamon (*Cinnamomum zeylanicum*), Ginger (*Zingiber officinale*) and Turmeric (*Curcuma longa*) promote good blood circulation can be used as cardiac stimulants.
- Sandalwood (*Santalum* genus) and Cinnamon (*Cinnamomum zeylanicum*) are used to stop bleeding as they act as astringents.
- Basil (*Ocimum basicicum*), Fennel (*Foeniculum vulgare*), Thyme (*Thymus vulgaris*), Mint (*Mentha piperita*), Oregano (*Origanum vulgare*), Rosemary (*Salvia rosemarinus*) have carminative property.
- Cardamom (*Elettaria cardamomum*), Coriander (*Coriandrum sativum*), Peppermint (*Mentha piperita*), Cloves (*Syzygium aromaticum*) and Turmeric (*Curcuma longa*) possess appetizing qualities.
- Turmeric (*Curcuma longa*) and some other herbs have antibiotic properties.
- Ginger (*Zingiber officinale*) and Cloves (*Syzygium aromaticum*) are used as expectorants.

1.6 Application of medicinal plants against diseases

1.6.1 Medicinal plants and their phytochemicals for the treatment of infectious diseases

In the last 100 years scientists from different fields have been exploring plants with antimicrobial usefulness (Anand *et al.,* 2019). Isolation, purification and characterization of thousands of phytochemicals with their inhibitory effects on several types of microorganisms proved medicinal plants effective for prevention and treatment of communicable diseases. The medicinal plants also may offer significant possibility for the development of new novel antibacterial treatments and adjunct usages. Simple Phenols (epicatechin and catechol), Phenolic acids (Cinnamic acid), Quinones (Hypericin), Flavonoids (Chrysin), Flavonols (Totarol), Tannins (Ellagitannin), Terpenoids, Alkaloids (Berberine), essential oils (Capsaicin), Lectins and polypeptides and Polyacetylenes were reported to have antimicrobial activity (Ferdes, 2018; Rathore *et al.,* 2011; Cowan, 1999).

1.6.2 Medicinal plants and their phytochemicals having anticancer activity

The vinblastine and vincristine from *Catharanthus roseus* (Apocynaceae), paclitaxel from *Taxus brevifolia* (Taxaceae), camptothecin from *Camptotheca acuminate* (Nyssaceae), epipodophyllotoxin from *Podophyllum emodi* and *P. peltatum* Linnaeus (Berberidaceae), homoharringtonine from *Cephalotaxus harringtonia* (Cephalotaxaceae), elliptinium and a ellipticine derivative from *Bleekeria vitensis* have been isolated from plants and have reported to exhibit anticancer activity (Cragg and Newman, 2005; Potmeisel, 1995; Kantarjian *et al.,* 1996).

1.6.3 Medicinal plants and their antioxidant potential

Highly reactive free radicals that are produced as a waste product or byproduct of metabolism can damage DNA; sometimes can be the principal factor to induce cancer. Furthermore, free radical results in several neurodegenerative disorders, such as Parkinson's disease, Alzheimer's disease, cataracts, heart diseases and aging. Some endogenous antioxidants formed in the body as well as some exogenous antioxidants obtained from foods and medicinal plants scavenge these free radicals. Carotenoids, vitamins A, E and C are some antioxidants obtained from diet whereas phenols,

phenolic acids, tannins, flavonoids are antioxidants derived from medicinal plants (Brahmachari and Gorai, 2006).

1.7 The plant family: Lauraceae

Lauraceae is a flowering plant family. The laurels of this family contains about 2850 identified species in 45 genera all-inclusive Most of the laurels are dicotyledons and mainly grow in hot temperate and tropical areas, particularly South America and Southeast Asia. Most of them are aromatic trees or shrubs. The genus *Cassytha* is exceptional in the lauraceae and its members are parasitic vines (Christenhusz and Byng 2016). The fruits of the laurels are one-seeded with a hard layer called drupes, the endocarp which is very thin, adjoining the seed so the fruit look like a one-seeded berry. In some species (Genera *Oreodaphne and Ocotea*) the fruits are enclosed in a cup-shaped cupule and in some species (*Lindera*), the fruit has an enlarged fleshy structure below the fruit called hypocarpium.

1.7.1 Genera of the family Lauraceae; Recent taxonomic revisions

*Adenodaphne, Actinodaphne, Aiouea, Alseodaphnopsis, Alseodaphne, Aniba, Beilschmiedia, Aspidostemon, Cassytha, Caryodaphnopsis, Chlorocardium, Cinnamomum, Cinnadenia, Clinostemon, Cryptocarya, Damburneya, Dehaasia, Dicypellium, Dodecadenia, Endiandra, Endlicheria, Eusideroxylon, Hexapora, Hypodaphnis, Kubitzkia, Laurus, Licaria, Lindera, **Litsea**, Machilus, Mespilodaphne, Mezilaurus, Nectandra, Neocinnamomum, Neolitsea, Nothaphoebe, Ocotea, Paraia, Parasassafras, Persea, Phoebe, Phyllostemonodaphne, Pleurothyrium, Potoxylon, Potameia, Pseudocryptocarya, Sassafras, Rhodostemonodaphne, Sextonia, Sinopora, Syndiclis, Triadodaphne, Umbellularia, Williamodendron, Urbanodendron, Yasunia.*

1.7.2 Importance of Lauraceae family

Many species of the family Lauraceae are valued for perfumes and spices in cooking due to its aroma and richness of essential oils. Bay leaves are popularly used as spice in many American, European and Asian cuisines. Some species have therapeutic importance and possess important phytoconstituents of medicinal value. There are some genera of certain commercial value: *Cinnamomu, Laurus, Lindera, Persea, Sassafras* etc. Many species are in danger of extermination as an effect of habitat conversion,

over-exploitation and overcutting. Contrariwise, some species are considered as hostile aggressors in some areas. Such as, *Cinnamomum camphora*, is so invasive that it is declared as a weed in South Africa.

1.7.3 Ecology

The species of Lauraceae family are common on alluvial places but also on hill tracks. The plants of the species are fast growing and generally grow on sandy soils, but may also on limestone. Lauraceae fruits are a significant food source for birds; thereby they leave the seeds in a suitable condition for germination. Seed dispersal is also supported by other animals like arboreal rodents, monkeys, porcupines, fishes and opossums. Certain mites create their home in some species of the Lauraceae family; some have a mutualistic, commensalistic or parasitic relationship with insects like.

In southern and northern hemisphere of the wolrd at tropical region to mild temperate regions trees of the laurel family occure abundantly. Some members are pantropical in lowland, in Africa and in Afromontane forest. Some species of this family are prevalent to countries such as Sudan, Tanzania, Cameroon, Congo and Uganda. Some relict (A surviving remnant) species of the family occur in temperate areas of world's southern and northern hemispheres. These plants are adjusted to heavy rainfall as well as humidity and they have leaves that are covered with a wax layer, providing a glossy look.

The leaves have an acuminate apex with an oval shape leaf allowing them to shed water in spite of the humidity and to continue the transpiration. Some species have adjusted to tough environments in semiarid climates, but they depend on advantageous circumstances of the soil, for example, periodic groundwater flows, perennial aquifers, or occasionally flooded forests in sand with scarce nutrients.

Many species have adjusted to swampy environments by growing aerial roots that for adaptations. Paleobotanists, a branch of Botany have suggested Lauraceae family initiated about 174±32 million years back, while others do not rely on this. But, fossil flowers ascribed to this family arise in 100.5 and 93.9 million years ago (Mya) (Lübbe, 1991).

1.7.4 Plant genus: Litsea

In the Lauraceae family, *Litsea* is a large genus which is the second larger one than the *Octea*. The species of *Litsea* genus form a vital element of tropical forests. Among more than 300 species, most of them are found in tropical Asia and a few species are habitat in Australia, the islands of the Pacific and in Central and North America (Mabberley, 2008; Bhuinya *et al.*, 2010).

The recently published provincial revisions are for China with 74 species; Peninsular Malaysia with 54 species; Nepal with 11 species; Borneo with 22 *Litsea* species and in Bangladesh, there are 11 species of *Litsea* as recorded by the Ministry of Environment and Forest (Van der Werff, 2001; Huang *et al.*, 2008; Pendry, 2011).

More than 407 phytochemicals of various types including alkaloids, sesquiterpenes, terpenoids, fatty acids, flavonoids, lactones, lignans etc. were reported from *Litsea* species. Many of them possess important pharmacologic activities as antimicrobial, antidiarrheal, analgesic, insecticidal, antioxidant, anti-HIV, anti-inflammatory etc (Wang *et al.*, 2016).

1.8 Plant review *Litsea glutinosa* (Lour.) C.B.Rob.

1.8.1 Description of the plant L. glutinosa

Litsea glutinosa (Family Lauraceae) is a deciduous or evergreen, small- to medium-sized tree of 3-20 m tall. Its leaves are alternate, the shape is elliptical to oblong-elliptical and the size of the leaves is 3.5-10 × 1.5-11 cm with a velvety or glabrous surface. Flowers are small yellowish that have 8-20 stamens. Fruits are globular with 8 mm diameter. (Puhua *et al.*, 2008).

1.8.2 Common names and synonyms

Bengali: কুকুরচিতা; **English:** Indian laurel; **English/Australia:** brown bollygum, bolly beech, brown bollywood, soft bollygum; brown beech; **Afrikaans:** Indiese lourier; **French:** litsée glutineuse, avocat marron; **French/Mauritius:** bois d'oiseau; **Tagalog:** sablot puso-puso; **Vietnamese:** bời lời đỏ; **Chinese:** 潺槁木姜子; **Thai**: หมูทะลวง.

Synonyms

Litsea laurifolia (Jacq.), *Sebifera glutinosa* Lour., *Tetranthera laurifolia* Jacq.

1.8.3 Distribution of L. glutinosa

L. glutinosa is indigenous to all over Asia, containing China, Bhutan, India, Nepal, Myanmar, Vietnam, Thailand, the Philippines, Bangladesh, Hong Kong, Indonesia, Malaysia, Laos, Cambodia, Papua New Guinea, the Solomon Islands. It is also native to Northern Territory, Western Australia, Cape York Peninsula, southern end and north-east Queensland (Huang *et al.*, 2008; Chowdhury *et al.*, 2008; Australian Tropical Rainforest Plants, 2010).

L. glutinosa is familiarized on the islands of Mauritius, La Réunion Island, Mayotte Island, the Seychelles Islands and the Comoros Islands of French Territory of Mayotte. It is also introduced on Grand Terre Islands in the Pacific to New Caledonia, KwaZulu-Natal State in South Africa (Madagascar), and in South America (Brazil) (Meyer, 2001; Jacq and Hladik, 2005).

1.8.4. Taxonomical classification

Taxonomical classification	***Litsea glutinosa* (Lour.) C. B. Rob. [Lauraceae]**
• **Domain: Eukaryota** • **Kingdom: Plantae** • **Phylum: Tracheophyta** • **Class: Magnoliopsida** • **Order: Laurales** • **Family: Lauraceae** • **Genus: *Litsea*** • **Species: *Litsea glutinosa***	

Figure 1.1: Taxonomical classification *Litsea glutinosa* (Lour.) C. B. Rob.

1.8.5 Traditional uses of L. glutinosa

Litsea glutinosa is a fast-growing tree with several purposes. In ethnomedicine, roots, bark, leaves and seeds of *L. glutinosa* are considered as therapeutically important. Its leaves and bark are used for diarrhea and dysentery, and its root paste is applied as

poultice for bruises and sprains in India (Das *et al.*, 2013). Chopped and soaked leaves are used as plaster in the Northern Philippines. *L. glutinosa* is a tree with low density wood, so it is used as fuel wood (Rabena, 2008). In China, the seed oil is used for making soap (Puhua *et al.*, 2008). In Mayotte (Indian Ocean) *L. glutinosa* is used as a fodder tree to feed 93% of the cattle there (Aubriot, 2011).

1.8.6 Literature review of L. glutinosa

1.8.6.1 Biological literature review of L. glutinosa

A crude methanol extract of *L. glutinosa* leaves was reported to have thrombolytic, anti-inflammatory, analgesic and antipyretic activities (Bhowmick *et al.* 2014); methanol extract of the bark exhibited antibacterial activity against both gram-negative and gram-positive bacteria (Mandal *et al.* 2000) and methanol extract of bark was also reported to have potent hepatoprotective action against liver damage in rats induced by paracetamol and CCl_4 (Ghosh *et al.*, 2016); the mucilage from the leaves was reported to exhibit anti-diabetic and antioxidant activities (Palanuvej *et al.* 2009); while the berry oil is used by some traditional and tribal healers in the treatment of rheumatism.

Figure 1.2: *Litsea glutinosa* leaves and fruits

1.8.6.2 Chemical literature review of L. glutinosa

Several alkaloids for example isoboldine, actinodaphnine, liriodenine, laureliptine, n-methyl actinodaphnine, laurotetanine, boldine, n-methyllaurotetanine, laurolitsine, litsine, litseferine and glutinosine A (Jin *et al.,* 2018; Wu *et. al.,* 2017 and Das *et al.,* 2013); Flavonoid such as 2',5,7-trihydroxy-6-methoxyflavone 2'-*O*-beta-D-glucopyranoside (Wang *et al.,* 2010); sesquiterpenes like β-Caryophyllene, Caryophyllene oxide and monoterpenes such as *(E)*-β-Ocimene, *(Z)*-β-Ocimene (Choudhury *et al.,* 1996) have been reported from this plant previously.

1.9 Plant review of *Litsea lancifolia* (Roxb.) Hook.f.

1.9.1 Description of the plant L. lancifolia

The name *L. lancifolia,* comes from the Chinese word litse (LIT-see-a) or *Ii* which means small or little of the family: Lauraceae. *L. lancifolia* is a small tree, leaves are 7.6-20.3 cm long, elliptic oblong or lanceolate with acute or acuminate apex. Flowers grow in umbels of leaf-axils; they may be solitary or clustered. Fruits are globules with a diameter of 1.27 cm.

1.9.2 Common names and synonyms of L. lancifolia

Chakma tribes of Bangladesh: জুডিজায়লা; **Nepal:** Kali pahenlo; **Mizo:** Hnahpawte; **Malay:** Medang; **Sabah:** Medang Kikisang.

Synonyms

Litsea stocksii Hook. fil.; *Litsea josephii* S.M. Almeida.

1.9.3 Distribution of L. lancifolia

L. lancifolia Hook.f. is distributed in Peninsular Malaysia (Malaysia), India (Assam), Himalaya (Nepal to Bhutan), China and the hill track regions (the south-eastern area) of Bangladesh.

1.9.4 Taxonomical classification

Taxonomical classification	*Litsea lancifolia* (Roxb.) Hook.f. [Lauraceae]
• **Domain:** ***Eukaryota*** • **Kingdom:** ***Plantae*** • **Phylum:** ***Tracheophyta*** • **Class:** ***Magnoliopsida*** • **Order:** ***Laurales*** • **Family:** ***Lauraceae*** • **Genus:** ***Litsea*** • **Botanical name:** ***Litsea lancifolia*** **Hook.f.**	

Figure 1.3: Taxonomical classification of *Litsea lancifolia* (Roxb.) Hook.f.

Figure 1.4: *Litsea lancifolia* leaves and flowers

1.9.5 Traditional uses of L. lancifolia

In Rangamati, Bangladesh warm root extract of *L. lancifolia* (Roxb.) Hook. f. is used by Chakma tribals to treat diarrhea (Yusuf *et al.* 2009).

1.9.6 Literature review of L. lancifolia

1.9.6.1 Biological literature review of L. lancifolia

L. lancifolia was reported for its anti-diabetic, analgesic, CNS depressant, anti-oxidant, antimicrobial (Alsawalha *et al.*, 2019; Bulbul *et al.*, 2020).

1.9.6.2 Chemical literature review of L. lancifolia

Several alkaloids are reported by Sulaiman *et al.* (2011) and Li *et al.* (2008) including boldine, norboldine, litseferine, juziphine, lancifoliaine, phanostenine, actindaphnine. Steroid such as β-sitosterol as well as some other compounds including aristotetralone, 4'-methylenedioxyflavan-3-ol, 5,7-dimethoxy-3', dehydrodiisoeugenol, β-hydroxybenzoic acid, dihydrodehydro diconiferyl alcohol and vanillin were also reported from this plant (Syazreen, 2012).

1.10 Plant review of *Litsea deccanensis* Gamble.

1.10.1 Description of the plant L. deccanensis

Litsea deccanensis (Lauraceae), the name of plant comes from the Chinese word litse (LIT-see-a) or *Ii*, means small or little and (day-kahn-NEN-sis) means from Deccan peninsula, India. It is an evergreen small tree with spirally arranged leaves. Leaves are elliptic to oblong to sub ovate-elliptic in shape, 4-9 x 9-15 cm in size and the apex is obtuse, acute or obtusely acuminate. Flowers grow in umbels or in leaf-axils. There are up to 25 flowers in an umbellue, each flower contains 25-30 stamens. Fruits are berries of 6 mm in diameter with a single seed.

1.10.2 Common names and synonyms of L. deccanensis

Deccan litsea, Deccan tallow laurel, Ganapaty tree. **Bangla:** গনপাতা**; Marathi:** Chikna, Kurak; **Telegu:** Narramamidi; **Malayalam:** Mala-poenna, Pathali.

Synonyms:

Litsea quinqueflora; Tetranthera tomentosa; Litsea tomentosa; Actinodaphne quinqueflora.

1.10.3 Taxonomical classification

Taxonomical Classification	*Litsea deccanensis* Gamble. [Lauraceae]
• **Domain: *Eukaryota*** • **Kingdom: *Plantae*** • **Phylum: *Tracheophyta*** • **Class: *Magnoliopsida*** • **Order: *Laurales*** • **Family: *Lauraceae*** • **Genus: *Litsea*** • **Botanical name: *Litsea deccanensis* Gamble.**	

Figure 1.5: Taxonomical classification *Litsea deccanensis* Gamble.

1.10.4 Distribution of L. deccanensis

This species is found in Chattogram, Bangladesh, Pakistan, Western India, Sri Lanka, Deccan Peninsula, Myanmar, Philippines, Malaysia, Thailand, Indo-China, South China.

Figure 1.6: *Litsea deccanensis* leaves, fruites and flower

1.10.5 Traditional uses of L. deccanensis

Traditional healers of Kottayam district in Kerala use *L. deccanensis* for the remedy of inflammatory disorders. In Andhra Pradesh, leaves of this plant are used in chest pain.

Traditionally this plant is used for sprained or swollen joints such as ankles or knees, sickle cell anemia, scabies and gastric acidity (Henk, 2001).

1.10.6 Literature review of L deccanensis

1.10.6.1 Biological literature review of L. deccanensis

Methanol extract *L. deccanensis* was reported to have cardioprotective effect in animal models, *in-vitro* antioxidant and reducing activities (Kumar *et al.*, 2011a; Kumar *et al.*, 2011b).

1.10.6.2 Chemical literature review of L. deccanensis

The essential oil from *L. deccanensis* leaf was reported to have about 40 compounds among which caryophyllene epoxide, β-caryophyllene, germacra-3,9,11-triene, α-humulene, bicyclogermacrene and limonene are the major volatile constituents (Irulandi *et al.*, 2016). GC-MS study of *L. deccanensis* by Kumar *et al.* (2011a) reported the presence of quassin, stigmasterol, squalene, vitamin E and oleic acid in the extract. Several aporphine alkaloids including isocorydine, corytuberine, dicentrine, nordicentrine, boldine, norboldine, and magnoflorine have been isolated and characterized by Gupta and Bhakuni (1989).

1.11 Plant review of *L. monopetala* Roxb. Pers.

1.11.1 Description of the plant L. monopetala

Litsea monopetala Roxb. Pers. is a small evergreen tree of 18 m tall in the family lauraceae. Elliptic leaves of *L. monopetala* are arranged alternately; the size is 9-24 cm by 5-11 cm, with rounded apex. Flowers are yellow with 6 tepals and the numbers of stamens are 9-12. Fruit is 0.7-1.2 cm long and the shape is oblong to ellipsoid.

1.11.2 Common names and synonyms of L. monopetala

Bengali: বড় কুকুরচিতা; **Hindi**: Gwa, Meda, Singraf, Jangli-rai-am, Katmarra; **Manipuri**: Tumitla; **Tamil**: muchaippeyetti, maidalagadil, picinpattai; **Marathi**: ranamba; **Mizo:** nauthak; **Telugu**: meda, chiru mamidi, naara, nara maamidi, narachettu; **Kannada**: hemmadi, gajapippali, kainji; **Khasi**: dieng sohtyllap, dieng soh pho ski; **Assamese**: muga, sualu; **Nepali:** Kutmira.

Synonyms: *Litsea polyantha, Tetranthera monopetala, Tetranthera alnoides*

1.11.3 Distribution of L. monopetala

L. monopetala is available in the sal forests, hilly and village areas of Bangladesh. Besides Bangladesh, its area extends to India, Burma, Nepal and southwest China.

Figure 1.7: *Litsea monopetala* leaves and fruits

1.11.4 Taxonomical classification

Taxonomical Classification	***L. monopetala* Roxb. Pers. [Lauraceae]**
• **Kingdom: Plantae** • **Phylum: Tracheophyta** • **Class: Magnolipsida** • **Order: Laurales** • **Family: Lauraceae** • **Genus: *Litsea*** • **Species: *monopetala*** • **Botanical name: *Litsea monopetala***	

Figure 1.8: Taxonomical classification *L. monopetala* Roxb. Pers.

1.11.5 Traditional uses of L. monopetala

Traditional uses of *L. monopetala* leaves include treating arthritis; powdered bark for diarrhea; roots for bruises and seeds for rheumatism. *L. monopetala* has long been used traditionally for treating dislocation, fracture, gonorrhea and skin disease also (Ghose and Sinha, 2010).

1.11.6 Literature review of L. monopetala

1.11.6.1 Biological literature review of L. monopetala

Both the leaves and bark extracts are reported to have antioxidant, analgesic, antimicrobial, hypoglycemic, antidiarrheal and CNS depressant activities (Ferdous *et al.*, 2018; Bulbul *et al.*, 2020).

1.11.6.2 Chemical literature review of L. monopetala

Eugenol, chalcone (Ghose and Sinha, 2010) have been reported from the bark extract; α-caryophyllene alcohol, caryophyllene oxide, tricosane, humulene oxide and pentacosane from flower oil; capric acid, nonanol and decanal from fruit oil and tridecanol, tridecanal, myristic acid and tetradecanal from bark oil were revealed from *L. monopetala* (Choudhury *et al.*, 1997).

1.12 Ethnomedicinal properties and phytoconstituents of *Litsea glutinosa, L. lancifolia, L. deccanensis and L. monopetala*

Ethnomedicinal properties deliver thorough information about local medicinal plants. Ethnomedicinal properties discuss the pharmacological properties and phytoconstituents of medicinal plants and they serve as valuable raw materials for traditional and modern medicines. This segment includes recent scientific reports of traditionally used medicinal plants.

Table 1.1: Distribution, usable plant parts and traditional uses of *Litsea glutinosa, L. lancifolia, L. deccanensis* and *L. monopetala*

Species	Distribution	Usable parts	Traditional uses	Ref.
Litsea glutinosa (Lour.) C.B. Rob.	China, Bangladesh, Myanmar, Sri Lanka, India and Malaysia	Leaves, Bark, Fruits, Roots	Mild astringent and demulcent for diarrhea; for poulticing bruises and sprains; the seed oil for rheumatism	Mandal *et al.* (2000)
L. monopetala (Roxb.) Pers	Nepal, India, Bangladesh, Burma, China	Leaves, Bark, Roots, Trunk	Leaves, bark and roots for skin diseases, boil, gonorrhoea etc.; leaves for arthritis and diarrhea.	Ghosh and Sinha (2010)
L. deccanensis Gamble	India (Andhra Pradesh state)	Leaves	Chest pain	Kumar *et al.* (2011)
L. lancifolia (Roxb.) Hook. f.	Bangladesh, Bhutan, China, Cambodia, India, Indonesia, Hong Kong, Laos, the Philippines, Nepal.	Leaves, Bark, Roots, Seeds	Leaves and bark are used for diarrhea; root paste is applied as poultice for bruises and sprains in India	Das *et al.* (2013)

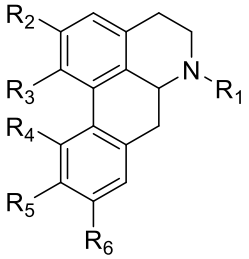
Table 1.2: Usable plant parts and reported biological activities of *Litsea glutinosa, L. lancifolia, L. deccanensis* and *L. monopetala*

Species and usable parts	Biological activities	References
L. glutinosa (Lour.) C.B. Roxb. Leaves, Bark, Fruits, Roots	Thrombolytic, analgesic, anti-inflammatory, antipyretic, antibacterial, anti-diabetic (type II), antioxidant, hepatoprotective activities	Bhowmick *et al.* (2014); Palanuvej *et al.* (2009); Mandal *et al.* (2000); Ghosh *et al.* (2016)
L. monopetala (Roxb.) Pers Leaves, Bark, Roots, Trunk	Antioxidant, antimicrobial, analgesic, ypoglycemic, CNS depressant, antidiarrheal activities	Ferdous *et al.* (2018); Bulbul *et al.* (2020); Hasan *et al.* (2016); Ghosh and Sinha (2010)
L. deccanensis Gamble; Leaves	Antioxidant and reducing capacities, cardioprotective effect in rat models.	Kumar *et al.*, (2011a); Kumar *et al.*, (2011b)
Litsea lancifolia (Roxb.) Hook. f. Leaves, Bark, Roots, Seeds	Anti-diabetic, analgesic, anti-inflammatory, antimicrobial, CNS depressant, antioxidant activities.	Alsawalha *et al.*, (2019); Bulbul *et al.*, (2020)

Table 1.3: Usable plant parts and identified phytoconstituents of *Litsea glutinosa*

Species and Usable parts	Identified Phytoconstituents	References
L. glutinosa (Lour.) C.B. Rob. Barks and leaves	***Alkaloids:*** Boldine (**1**), Isoboldine (**2**), Nor-boldine (**3**); Laurelliptine (**4**), Laurolitsine (**5**), Laurotetanine (**6**), N-Methyllaurotetanine (**7**), Actinodaphnine (**8**), N-Methylactinodaphnine (**9**), Litseferine (**10**), Litsine (**11**), Glutinosine A (**12**), Liriodenine (**13**), Litsine (**14**), Litseglutine B (**15**), Liriodenine (**16**). ***Flavonoids:*** Kaempferol 3-O-b-d-glucopyranoside (astragalin) (**17**), Kaempferol 7-glucoside (**18**), Quercitrin (**19**), Naringin (**20**), Naringerin (**21**), Pelargonidin 3-glucoside (**22**), Pelargonidin 5-glucoside (**23**), 2',5,7-trihydroxy-6-methoxyflavone 2'-O-beta-D-glucopyranoside (**24**), Epicatechin (**25**), Glutin (**27**). ***Monoterpenes:*** *(E)*-β-Ocimene (**28**), *(Z)*-β-Ocimene (**29**). ***Sesquiterpenes:*** (6R,7E,9R)-9-Hydroxy-megastigma-4,7-dien-3-one 9-O-β-D-glucopyranoside (**30**), Spinoside A (**31**), Alangionoside E (**32**), Blumenol C Glucoside (**33**), Euodionoside A (**34**), Euodionoside F (**35**), Euodionoside G (**36**), Roseoside (**37**, Apocynoside I (**38**), Apocynoside II (**39**), β-Caryophyllene (**40**), Caryophyllene oxide (**41**) ***Diterpenoids:*** Trans-phytol (**42**) ***Lignans:*** (7′S,8R,8′S)-4,4′,9-trihydroxy-3,3′,5-trimethoxy-9′-*O*-β-D-xylopyranosyl-2,7′-cyclolignan (**43**), (-)-Lyoniresinol (**44**), (-)-Isolariciresinol-9′-*O*-β-Dxylopyranoside (**45**), (7′R,8S,8′R)-Nudiposide (**46**), (7′S,8R,8′S)-Lyoniresinol (**47**), Sioriside (**48**), Glochidioboside (**49**), [(2R,3S)-2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-5-(3-Hydroxypropyl)- 7-methoxy-1-benzofur-an-3-yl]methyl β-Dglucopyranoside (**50**), (7′R,8′R)-3,5′-Dimethoxy-9,9′-dihydroxy-4,7′-epoxylignan-4'-β-D-glucopyranoside (**51**), (7′R,8′S)-Dihydrodehydrodiconifenylalcohol-9'-O-β-D-xylopyranoside (**52**), Pinoresinol-3-O-β-D-glucopyranoside (**53**), (-)-Isolariciresinol-5′-methoxy-9′-O-β-Dxylopyranoside (**54**).	Hart *et al.* (1969); Tewari *et al.* (1972); Yang *et al.* (2005); Jin *et al.* (2018); Ji *et al.* (2019); Wang *et al.* (2010); Mohan *et al.* (1975); Choudhury *et al.* (1996); Agrawal *et al.* (2013); Wang *et al.* (2012); Mohan and Pathak (1975); Pan *et al.* (2010)

	Steroids: β-Sitosterol (**55**), Stigmasterol (**56**). ***Butanolides and butanolactones:*** Litsealactone C (**57**), Litsealactone D (**58**), Litsealactone G (**59**), (3R,4S,5S)-2-hexadecyl-3-hydroxy-4-methylbutanolide (**60**).	
L. lancifolia (Roxb.) Hook. f.	***Alkaloids:*** Lancifoliaine (**61**), Juziphine (**62**), Phanostenine (**63**), Lancifolianine (**64**), Boldine (**1**), Norboldine (**3**), Actindaphnine (**8**), N-Methylactinodaphnine (**9**), Litseferine (**10**). ***Steroids:*** β-sitosterol (**55**). ***Flavonoids:*** 4'-methylenedioxyflavan-3-ol,5,7-dimethoxy-3',β-hydroxybenzoic acid (**65**) ***Lignans:*** Aristotetralone (**66**); Dihydrodehydrodiconiferyl alcohol (**67**) ***Phenyl propanoids:*** Dehydrodiisoeugenol (**68**); Vanillin (**69**)	Sulaiman *et al.* (2011); Li *et al.* (2008); Yang *et al.* (2008)
L. deccanensis Gamble.	***Alkaloids:*** Boldine (**1**); Isocorydine (**70**); Corytuberine (**71**); Dicentrine (**72**); Nordicentrine (**73**); Magnoflorine (**74**); Laurolitsine (**5**). ***Terpenes:*** α-humulene (**75**); Caryophyllene epoxide (**76**); β-Caryophyllene (**40**); Bicyclogermacrene (**77**); germacra-3,9,11-triene (**78**); Squalene (**79**); Quassin (**80**) ***Steroids:*** Stigmasterol (**56**)	Gupta and Bhakuni (1989); Irulandi *et al.* (2016); Kumar *et al.* (2011a)
L. monopetala Roxb.	***Alkaloids:*** Actinodaphnine (**8**) ***Fatty acids:*** Capric acid (**81**); Myristic acid (**82**) ***Others:*** 5-Methylchalcone (**83**); eugenol (**84**); Caryophyllene oxide (**85**); α-caryophyllene alcohol (**86**); Humulene oxide (**87**); Tricosane (**88**); Pentacosane (**89**); Nonanol (**90**); Decanal (**91**); Tridecanol (**92**); Tridecanal (**93**); Tetradecanal (**94**)	Dutta (1968); Ghosh and Sinha (2010); Choudhury *et al.* (1997)



	R1	R2	R3	R4	R5	R6
1	Me	OH	OMe	H	OMe	OH
2	Me	OMe	OH	H	OMe	OH
3	H	OH	OMe	H	OMe	OH
4	H	OMe	OH	H	OMe	OH
5	H	OH	OMe	H	OMe	OH
6	H	OMe	OMe	H	OMe	OH
7	Me	OMe	OMe	H	OMe	OH
70	Me	OMe	OMe	OH	OMe	H
71	Me	OMe	OH	OH	OMe	H

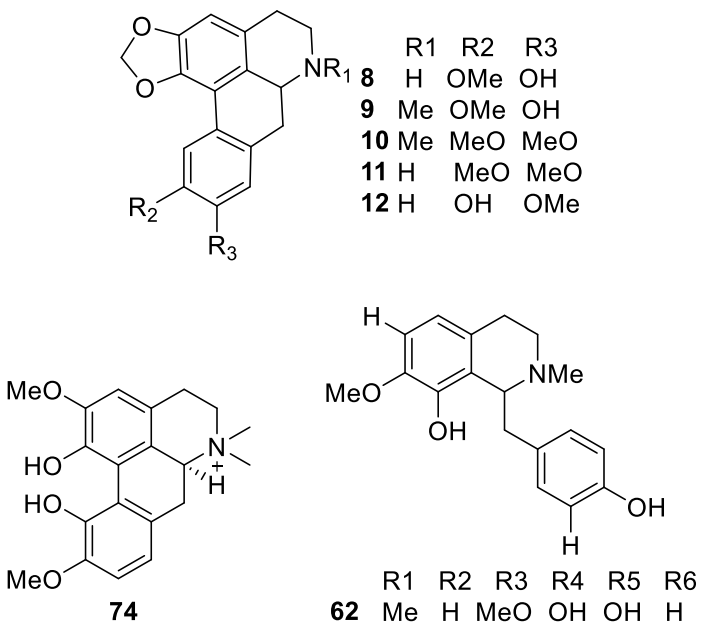


Figure 1.9: Structures of some alkaloids reported from *Litsea glutinosa, L. lancifolia, L. deccanensis* and *L. monopetala*

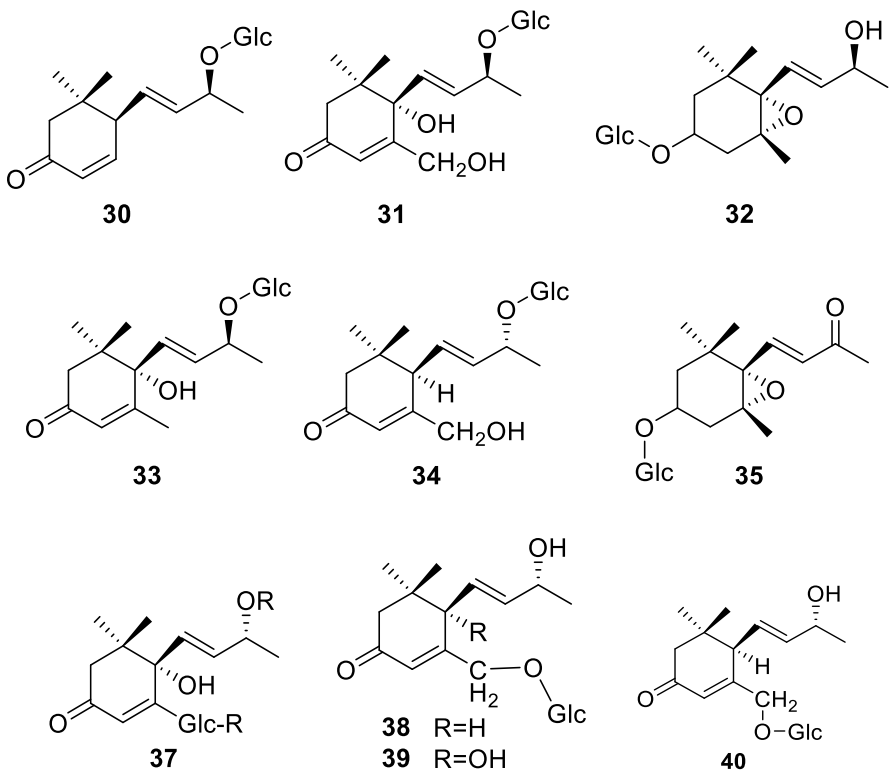


Figure 1.10: Structures of some sesquiterpenes reported from *Litsea glutinosa, L. lancifolia, L. deccanensis* and *L. monopetala*

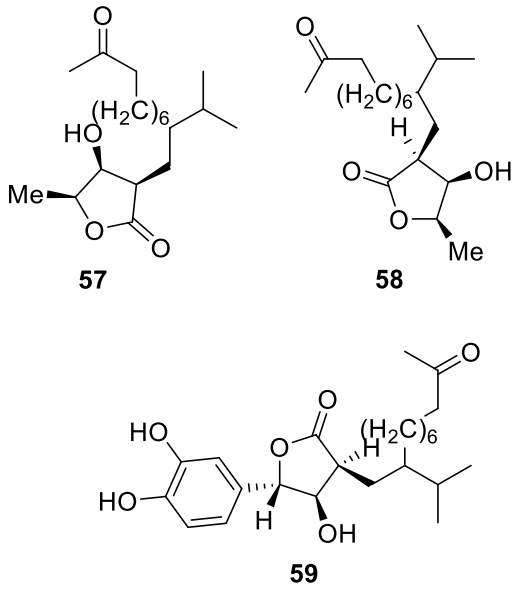


Figure 1.11: Structures of some butenolactones reported from *Litsea glutinosa, L. lancifolia, L. deccanensis* and *L. monopetala*

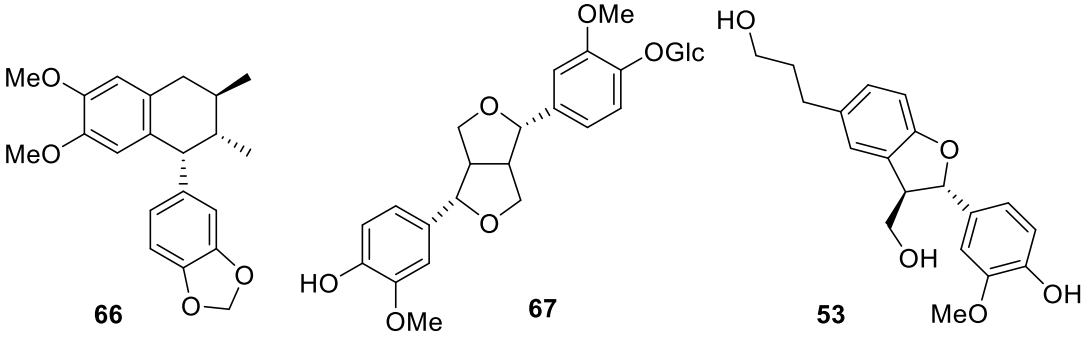


Figure 1.12: Structures of some lignans reported from *Litsea glutinosa, L. lancifolia, L. deccanensis* and *L. monopetala*

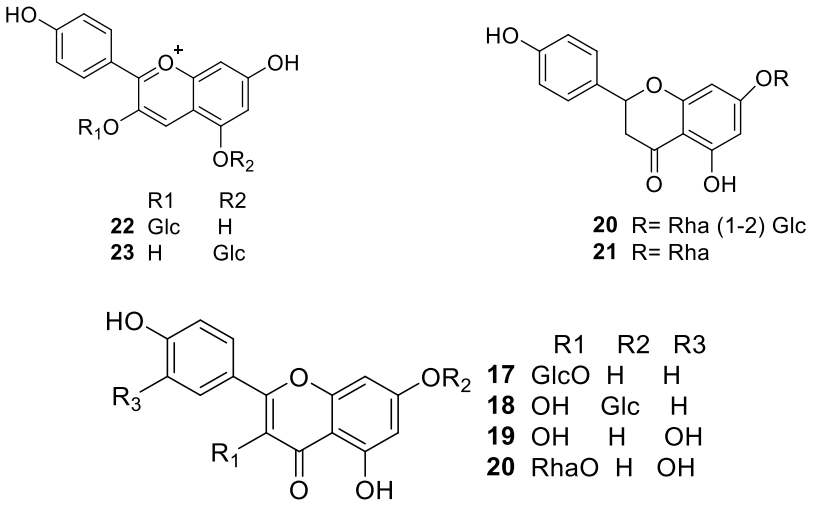


Figure 1.13: Structures of some flavonoids reported from *Litsea glutinosa, L. lancifolia, L. deccanensis* and *L. monopetala*

Chapter 2: Materials and Methods

2.1 Introduction

The present study has been divided into two parts, including pharmacological screenings and phytochemical investigations.

The *in-vivo* studies of crude methanol extracts *of L. glutinosa, L. monopetala, L. deccanensis* and *L. lancifolia* have been carried out in various experimental animal models while several biological potentials have been investigated through various *in-vitro* studies (Figure 2.1).

Different fractions of the plants were subjected for phytochemical studies to isolate and purify compounds following different chromatographic techniques and they have been characterized by ^{1}H NMR, ^{13}C NMR, COSY, HSQC and HMBC spectral data.

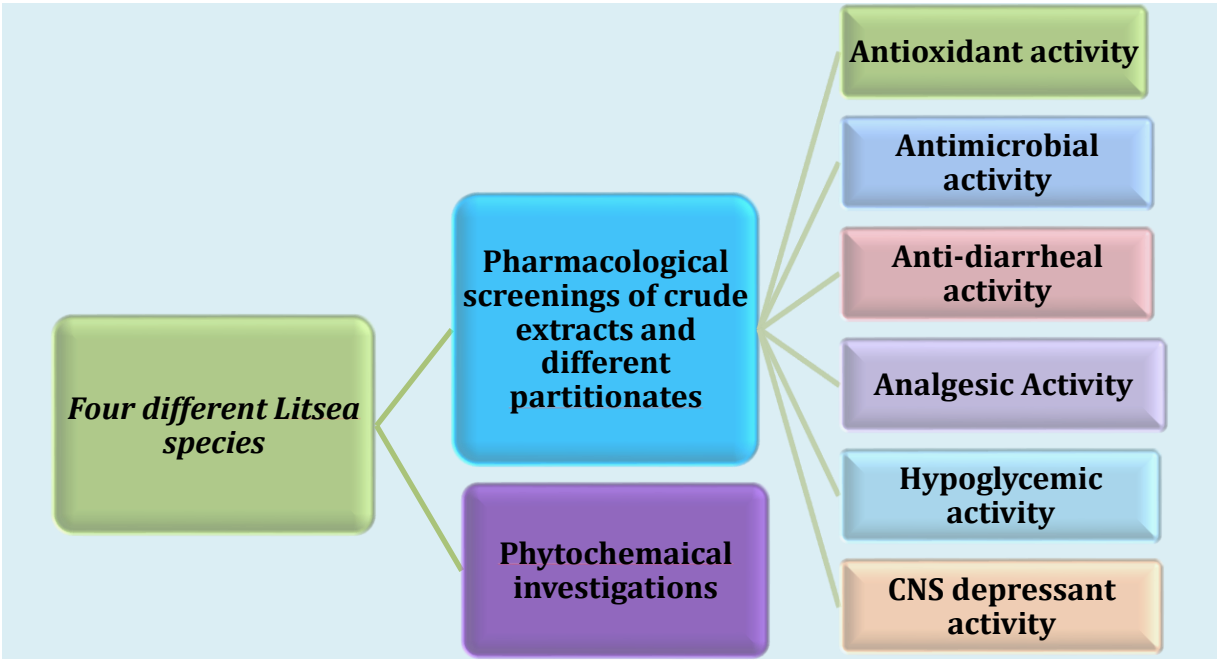


Figure 2.1: Present study protocol

2.2 Collection and Identification

L. glutinosa, L. monopetala, L. lancifolia leaves and *L. deccanensis* bark were collected from Chittagong hilly area of Bangladesh. All the plant samples were identified by a taxonomist of Bangladesh National Herbarium, Mirpur-1, Dhaka-1216 and an accession number was provided for each plant sample such as, *L. glutinosa* (Acc. No.

DACB-37904), *L. monopetala* (Acc. No. DACB-38437), *L. lancifolia* (Acc. No. DACB-35164) and *L. deccanensis* (Acc. No. DACB-35517).

2.3 Cold extraction of the plant materials

After collecting, washing with water and drying for some days, all the plant samples were ground to coarse powder. Then the crushed plant samples (800 g to 1000 g) were macerated in methanol (at room temperature) for 7 to 10 days with intermittent shaking. The extracts of all the plant samples were obtained by filtration through cotton plug and then through filter papers followed by evaporation by using a rotary evaporator to remove excess solvent. Excess of methanol from the extracts was evaporated to get dry mass and retained in the refrigerator for further studies.

2.4 Solvent-solvent partitioning of crude extract

Solvent-solvent partitioning was done using different solvents with increasing polarity following the procedure by Kupchan which was revised by Van Wagenen *et al.*, (1993). In this method 5 gm of the crude methanol extract was dissolved in aqueous methanol (10%). Then it was partitioned with petroleum ether, followed by chloroform and ethyl acetate. This partitioning process is shown in figure 2.2 and this process was repetitive with 5gm methanol extract every time. After evaporation of the solvent, the yields of different partitionates obtained from methanolic extract of *L. glutinosa*, *L. monopetala, L. deccanensis* and *L. lancifolia* were measured and mentioned in table 2.1.

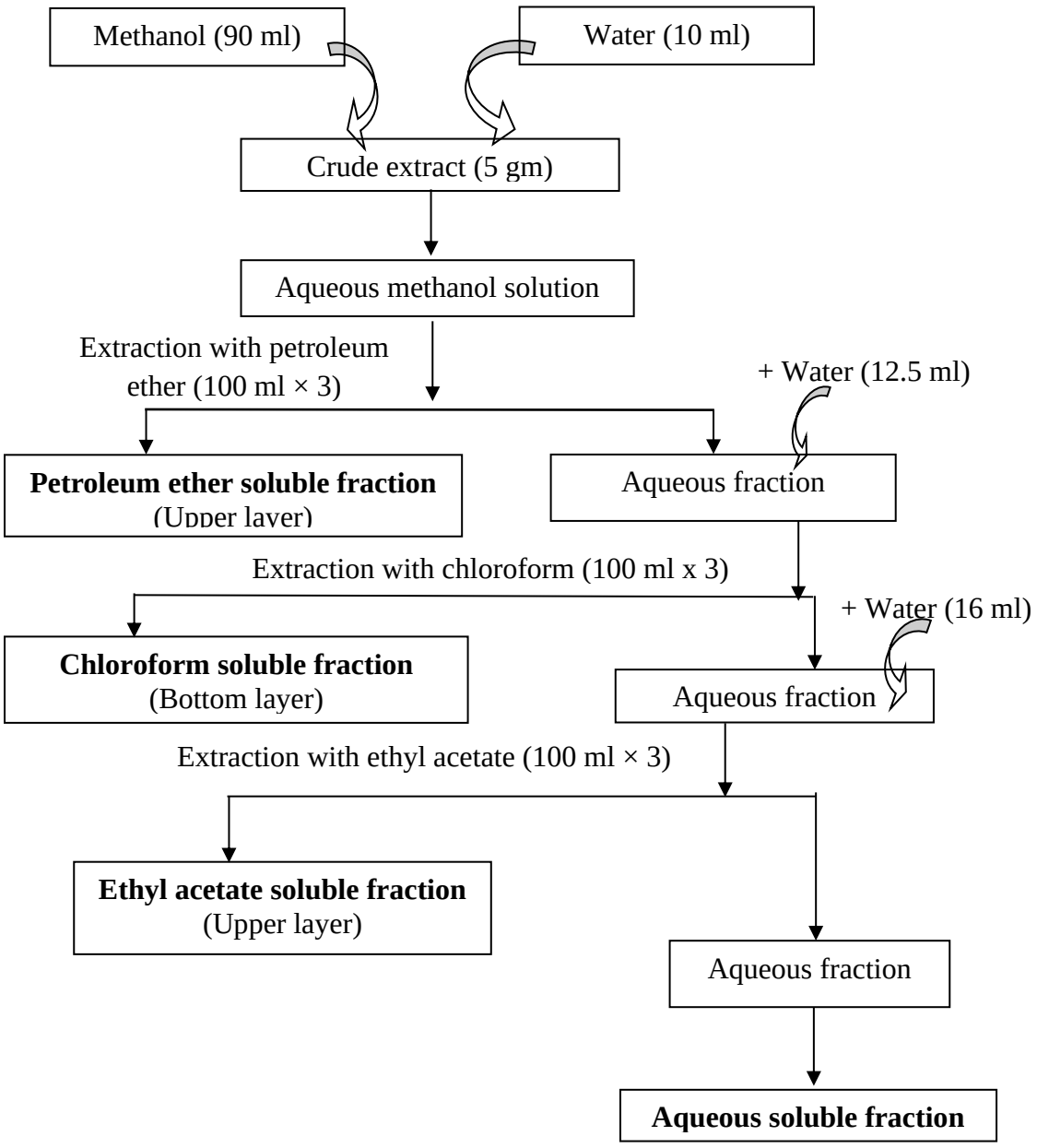


Figure 2.2: Modified Kupchan partitioning method

Table 2.1: Yields of the crude methanolic extracts and the partitionates of MELM, MELD, MELG and MELL

Crude extracts and yields (gm)	Fractions	Yields (gm)
Methanol extract of *L. monopetala* (MELM): 20.6 gm	Petroleum ether soluble fraction (LMPE)	4.7
	Chloroform soluble fraction (LMC)	4.5
	Ethyl acetate soluble fraction (LME)	2.2
	Aqueous soluble fraction (LMAq)	5.2
Methanol extract of *L. deccanensis* (MELD): 17.5 gm	Petroleum ether soluble fraction (LDPE)	4.1
	Chloroform soluble fraction (LDC)	3.5
	Ethyl acetate soluble fraction (LDE)	2.5
	Aqueous soluble fraction (LDAq)	4.4
Methanol extract of *L. glutinosa* (MELG): 17.7 gm	Petroleum ether soluble fraction (LGPE)	3.2
	Chloroform soluble fraction (LGC)	3.7
	Ethyl acetate soluble fraction (LGE)	1.9
	Aqueous soluble fraction (LGAq)	3.9
Methanol extract of *L. lancifolia* (MELL): 19.7 gm	Petroleum ether soluble fraction (LLPE)	3.9
	Chloroform soluble fraction (LLC)	3.2
	Ethyl acetate soluble fraction (LLE)	2.1
	Aqueous soluble fraction (LLAq)	6.5

2.5 Phytochemical investigation

Different partitionates of four different species of *Litsea* namely *L. monopetala*, *L. glutinosa*, *L. lancifolia* and *L. deccanensis* of lauraceae family were selected for chemical investigation.

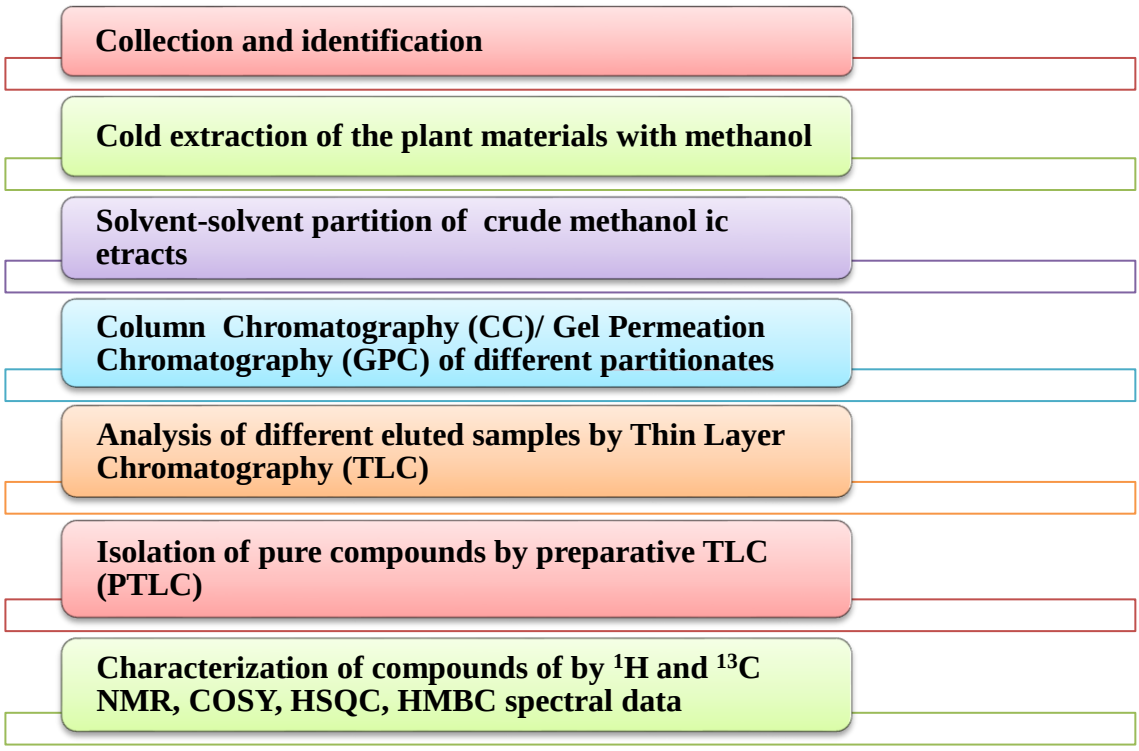


Figure 2.3: Flow diagram of the steps of phytochemical investigation of different fractions

2.5.1 Phytochemical investigation of chloroform soluble fraction of *L. glutinosa* (LGC)

Gel permeation chromatography (GPC) for LGC

Chloroform soluble fraction of *L. glutinosa* (LGC) was selected for gel permeation chromatography (GPC) followed by the analysis of different column washings by thin layer chromatography (TLC). Then pure compounds were isolated by preparative TLC (PTLC) and characterized by modern spectroscopic methods. Gel permeation chromatography (GPC) is a type of size-exclusion chromatography which separates bioactive phytoconstituents on the basis of their molecular size. The stationary phase comprises of Sephadex LH-20 (porous beads).

Step1: Column packing in GPC for LGC

For suitable swelling dried sephadex LH-20 was drenched in a mixture of n-hexane-dichloromethane- methanol at 2:5:1 ratio for about 12 hours. Then, the slurry of sephadex was added into a glass column 55 cm in height and 1.1 cm in diameter. To

ensure a compact packing of the column the solvent system was run several times through the column.

Step2: Solvent systems used as mobile phases in GPC for LGC

Elution was started with a mixture of n-hexane-dichloromethane-methanol at 2:5:1 ratio and the polarity of the solvent system were increased as shown in the table 2.2.

Table 2.2: Solvent systems used as mobile phases in GPC column for LGC along with their eluted fractions

Fraction no	Solvent Systems	No. of test tubes	Volume collected (mL)
1	n-hexane-dichloromethane-methanol (2:5:1)	(1-18)	60
2	Dichloromethane-methanol (9:1)	(19-23)	25
3	Dichloromethane-methanol (1:1)	(24-28)	25
4	100% methanol	(29-35)	40

Step3: Loading the sample onto the packed column for LGC

320 mg of the chloroform fraction of *L. glutinosa* was dissolved in the same solvent mixture as used to pack the column and later applied on top of the packed column by a Pasteur pipette.

Step 4: Eluting several sample fractios

Depending upon the different solvent systems several sample fractions were eluted and collected in 35 test tubes, each containing about 5ml of eluted samples. Then each testube was analysed by thin layer chromatography (TLC) and then purify by preparative TLC (PTLC).

Step 5: Column cleaning after eluting several sample fractions in GPC for LGC

After eluting all those sample fractions, the column was cleaned with the mixtures of the same solvents but in decreasing polarity and thus prepared for the next sample. The solvent systems for washing were listed in following table 2.3.

Table 2.3: Solvent systems used for washing the GPC column for LGC

Serial no.	Solvent Systems	Volume used (mL)
1	100% Methanol	50
2	Dichloromethane: Methanol (1:1)	50
3	Dichloromethane: Methanol (9:1)	50
4	n-Hexane: Dichloromethane: Methanol (2:5:1)	80

Step 6: Analysis of GPC fractions of chloroform extract of L. glutinosa by TLC

Different fractions of GPC column (table 2.2) were screened by TLC, observed the spots under UV light as well as by spraying with vanillin/sulfuric acid reagent, then by heating at 100-110 ºC for about 10 minutes. Depending on the activities of the spots on the TLC plate, samples of the similar spots were mixed and each mixture was subjected for Preparative TLC (PTLC) to purify the compounds.

2.5.2 Phytochemical investigation of chloroform soluble partitionate of *L. monopetala* (LMC)

Column chromatography (CC) for LMC

Plant: *L. monopetala* leaf

Fraction selected for column chromatography: Chloroform soluble fraction of methanolic extract (LMC)

Column Size: 38"

Packed about: 24"

Sample weight: 4.32 gm

Step 1: Packing of column with column grade silica gel for LMC

Column was packed with column grade silica in 100% petroleum ether. For good packing, the solvent was allowed to drain for several times to settle the stationary phase for ensuring a tightly packed column.

Step 2: Selection of the solvent systems for the column chromatography (CC) of LMC

Petroleum ether with ethyl acetate of increasing polarities was selected for the column chromatography of chloroform partitionate of *L. monopetala* as mentioned in table 2.4.

Step 3: Analysis of different eluted sample fractions by Thin Layer Chromatography (TLC)

The eluted sample fractions of each test tube were analyzed by thin layer chromatography (TLC) on silica gel 60 F254 (Mark Germany) coated on aluminum sheet and the mobile phase was chloroform and methanol (9:1). After running the mobile phase through the loaded TLC plate, it was air dried, visualized under UV-lamp and marked the spots in both the short and long wavelengths, sprayed with vanillin-sulfuric acid, then heated the plate at 100-110 ºC for 5-10 minute.

Step 4: Mixing the eluted sample fractions showing similar spots observed by thin layer chromatography (TLC)

The eluted sample fractions of the similar spots were mixed (Table 2.5) and screened by preparative thin-layer chromatography (PTLC). Some of the selected fractions were further separated by Sephadex columns.

Step 5: Gel Permeation Chromatography (GPC) of selected eluted sample fractions

The eluted samples of 15, 16 and 17 fractions, corresponding the number of test-tubes from 343-362 were mixed together and subjected to a Sephadex column. The eluted samples of 18, 19 and 20 fractions, corresponding the number of test-tubes from 363-379 were mixed together and subjected to another Sephadex column. The mobile phases were selected as mentioned in the table 2.6.

Step 6: Isolation of pure compounds by Preparative Thin Layer Chromatography (PTLC)

A number of compounds were isolated and purified from different fractions and sub-fractions using preparative TLC over silica gel.

Table 2.4: Solvent systems used for the column chromatography (CC) of chloroform soluble fraction of *L. monopetala* (LMC)

Fraction no.	Solvent systems	No. of test-tubes collected	Vol. collected (ml)
1	Pet. ether -100%	1-30	150
2	Pet. ether-Ethyl acetate (99.5 : 0.5)	31-50	100
3	Pet. ether-Ethyl acetate (99 : 1)	51-70	100
4	Pet. ether-Ethyl acetate (98.5 : 1.5)	71-90	100
5	Pet. ether-Ethyl acetate (98 : 2)	91-110	100
6	Pet. ether-Ethyl acetate (97.5 : 2.5)	111-130	100
7	Pet. ether-Ethyl acetate (97 : 3)	131-150	100
8	Pet. ether-Ethyl acetate (96 : 4)	151-170	100
9	Pet. ether-Ethyl acetate (95 : 5)	171-190	100
10	Pet. ether-Ethyl acetate (92.5 : 7.5)	191-210	100
11	Pet. ether-Ethyl acetate (90 : 10)	211-230	100
12	Pet. ether-Ethyl acetate (87.5 : 12.5)	231-250	100
13	Pet. ether-Ethyl acetate (85 : 15)	251-270	100
14	Pet. ether-Ethyl acetate (82.5 : 17.5)	271-290	100
15	Pet. ether-Ethyl acetate (80 : 20)	291-310	100
16	Pet. ether-Ethyl acetate (75 : 25)	311-330	100
17	Pet. ether-Ethyl acetate (70 : 30)	331-350	100
18	Pet. ether-Ethyl acetate (50 : 50)	351-370	100
19	Pet. ether-Ethyl acetate (25 : 75)	371-390	100
20	Ethyl acetate 100%	391-410	100
21	Ethyl acetate-Methanol (99 : 1)	411-430	100
22	Ethyl acetate-Methanol (98 : 2)	431-450	100
23	Ethyl acetate-Methanol (95 : 5)	451-470	100
24	Ethyl acetate-Methanol (90 : 10)	471-500	150

Table 2.5: Mixing of the eluted sample fractions showing similar spots and their yields

Fraction no.	No. of test-tubes showing similar spots	Amount yield (mg)
1	1-100	500
2	101-150	200
3	151-174	100
4	175-209	100
5	210-224	100
6	235-255	100
7	256-274	100
8	275-283	100
9	284-289	100
10	290-300	100
11	301-320	100
12	321-326	100
13	327-337	100
14	338-342	100
15	343-348	200 (Sephadex column)
16	349-355	200 (Sephadex column)
17	356-362	200 (Sephadex column)
18	363-367	100
19	368-374	100
20	375-379	200 (Sephadex column)
21	380-388	100
22	389-399	100
23	400-439	100
24	440-450	200
25	451-500	500

Table 2.6: The solvent systems selected as mobile phases in Gel Permeation Chromatography (GPC)

Serial no.	Solvent Systems
1	n-hexane-DCM-Methanol (2:5:1)
2	Methanol-DCM (1:9)
3	Methanol-DCM (1:1)
4	100% Methanol
5	Methanol-DCM (1:1)
6	Methanol-DCM (1:9)
7	n-hexane-DCM-Methanol (2:5:1)

***DCM= Dichloromethane**

2.5.3 Phytochemical investigation of chloroform soluble fraction of *L. deccanensis* (LDC): *Gel permeation chromatography (GPC)*

To separates bioactive phytoconstituents from the chloroform soluble fraction of *L. deccanensis* Sephadex LH-20 column was used.

Step1: Column packing of and sample application in GPC for LDC

After packing a glass column (55 cm in height and 1.1 cm in diameter) with Sephadex (LH-20), 320 mg of the chloroform soluble fraction of *L. deccanensis* in a mixture of n-hexane-dichloromethane-methanol (2:5:1) was loaded onto the packed column.

Step 2: The mobile phases in GPC for LDC

A mixture of n-hexane-dichloromethane-methanol (2:5:1) was selected as starting mobile phase, then the polarities of the solvent system were increased as methanol-dichloromethane (10:90) followed by methanol-dichloromethane (50:50) and 100% methanol.

Step3: Analysis of GPC fractions of LDC by TLC

The eluted GPC fractions (Table 2.7) were screened by TLC, followed by spraying with vanillin/sulfuric acid reagent, then by heating at 100-110 ºC for about 10 minutes. On the basis of the spot's characteristics on the TLC plate, samples of the similar spots were mixed and subjected for preparative TLC (PTLC) to purify the compounds.

Table 2.7: The mobile phases in GPC column for LDC along with their eluted fractions

Fraction no	Mobile phases	No. of test tubes	Volume collected (mL)
1	n-hexane- dichloromethane-methanol (2:5:1)	(1-20)	60
2	Dichloromethane:-Methanol (9:1)	(21-30)	30
3	Dichloromethane-Methanol (1:1)	(31-40)	30
4	100% Methanol	(41-50)	40

2.5.4 Phytochemical investigation of chloroform fraction of *L. lancifolia* (LLC): Gel permeation chromatography (GPC)

Step 1: Column packing of and sample application in GPC for LLC

As mentioned earlier, in gel permeation chromatography for LLC the column was filled with Sephadex (LH-20), was drenched in a mixture of n-hexane-dichloromethane-methanol (2:5:1) followed by the application of 320 mg of the chloroform soluble fraction of *L. glutinosa* in the same solvent mixture.

Step 2: The mobile phases and the eluted GPC fractions in GPC for LLC

Solvent systems with increasing polarities n-hexane- dichloromethane-methanol (2:5:1), dichloromethane-methanol (9:1), dichloromethane-methanol (1:1) and 100% methanol were used as mobile phases. Different fractions of GPC column were collected in 45 test tubes as mentioned in the table 2.8.

Step3: Analysis of GPC fractions of chloroform extract of L. lancifolia* (LLC) *by TLC

To detect compounds from different eluted fractions of GPC column (Table 2.8), spotting samples on TLC plate, spraying with vanillin/sulfuric acid reagent, then heating at 100-110 ºC for about 10 minutes was carried out.

Depending on the characteristics of the spots on the TLC plate, some samples were subjected for preparative TLC (PTLC) to purify the compounds.

Table 2.8: The mobile phases in GPC column for LLC along with their eluted fractions

Fraction no	Mobile phases	No. of test tubes	Volume collected (mL)
1	n-hexane- dichloromethane-methanol (2:5:1)	(1-18)	60
2	Dichloromethane-Methanol (9:1)	(19-26)	30
3	Dichloromethane-Methanol (1:1)	(27-34)	30
4	100% Methanol	(35-45)	40

2.6 Pharmacological Screening

- ❑ Antidiarrheal activity of crude methanol extracts
  - ❑ Castor oil-induced antidiarrheal activity
  - ❑ Gastrointestinal motility test by using barium sulfate meal
- ☐ Analgesic activity test of crude methanol extracts
  - ☐ Peripheral analgesic activity test, analgesia by acetic acid
  - ☐ Central analgesic activity test by Eddy's hot plate method
  - ☐ Formalin-induced paw licking and biting test
- ❑ *In-vivo* evaluation of hypoglycemic activity of methanol extracts
- ❑ CNS depressant activity of crude methanol extracts
- ❑ Antimicrobial activity test of different partitionates by disc diffusion method
- ❑ Antioxidant activity of different partitionates
  - ❑ Determination of total phenolic content
  - ❑ DPPH assay
- ❑ Molecular docking of pure compounds
  - ❑ Molecular docking of LGC-26 (**95**) and LGC-45-3 (**96**) with human aldose reductase for its antidiabetic property
  - ❑ Molecular docking of LML-363-13 (**97**) for its analgesic and antidiabetic activity

2.6.1 Antidiarrheal activity of crude methanol extracts

2.6.1.1 Castor oil induced anti-diarrheal activity

Principle

In developing countries among different gastroentrological diseases, diarrhoea is one of the foremost causes of infant's death (Mandeville *et al.*, 2009). And due to easy availability and low cost, people of these countries rely on natural drugs especially plant drugs are being used to treat diarrhoea. This study was performed according to Awouters *et al.* (1978) by using pure analytical grade castor oil for the induction of diarrhea. The objective of this study includes the evaluation of the antidiarrheal effects of the methanol extracts of *Litsea glutinosa*, *L. monopetala*, *L. deccanensis* and *L. lancifolia* in mice models. The antidiarrheal activity was evaluated by measuring % inhibition of diarrheal feces, total fecal output and gastrointestinal motility and by measuring peristaltic indices. In all the animal models castor oil was used to induce diarrhea and loperamide as standard. In the experiments three different doses as 100, 200, and 400 mg/kg of methanol extracts of these four *Litsea* species were used.

Experimental design

Animal grouping and dosing

For each extract in both models, animals were allocated into five groups (each group containing five mice) as follows

Group I: Control, indicated to administer only vehicle (10 ml/kg bw distilled water)
Group II: Standard control, indicated to administer Loperamide (3 mg/kg bw)
Group III, IV and V: Treatment controls, indicated to administer three different doses (100, 200 and 400 mg/kg bw respectively)

Extracts coding for different doses

The codes for 100, 200 and 400 mg/kg bw of the methanol extract of *L. glutinosa* were MELG_1, MELG_2 and MELG_3; the codes were MELM_1, MELM_2 and MELM_3 for *L. monopetala;* for *L. deccanensis* the codes were MELD_1, MELD_2 and MELD_3 and for *L. lancifolia* the codes were MELL_1, MELL_2 and MELL_3.

Anti-diarrheal activity test by castor oil induced diarrhea

Step 1: This study was performed using Swiss albino mice that were kept fasted for 18 h before commencing the experiment and only water was given.

Step 2: Three doses (100, 200 and 400 mg/kg) of four plant extracts (MELD, MELL, MELM and MELG) and 3 mg/kg loperamide as standard were administered orally to each group.

Step 3: One hour after administering plant extracts and standard, 0.5 ml castor oil was given orally to every mouse for the induction of diarrhea.

Step 4: Then each mouse was taken in a separate cage with a white paper on the floor to count and observe the stools (both dry and diarrheal stool), total number of stool and total fecal weight for subsequent four hours. White paper was reformed hourly.

Step 5: Then, % inhibition of wet defecation (diarrheal inhibition) and % of fecal output (% FOP) were calculated by using the following formulas

1) % inhibition of wet defecation (diarrheal inhibition) = (Wfc-Wft) /Wfc ×100

Where, Wfc: Mean wet feces of control group

Wft: Mean wet feces of treatment group (test samples / standard drug)

2) % of fecal output (% FOP) = FWt /FWc × 100

Where, FWt = Mean fecal weight of treatment group (test samples / standard drug)

FWc = Mean fecal weight of control group

2.6.1.2 Gastrointestinal motility test by using barium sulfate meal

This study was accomplished by the method of Chatterjee (1993) and Mazumdar *et al.* (2015) and explored the gastrointestinal motility persuaded by castor oil. To evaluate the gastrointestinal motility, the distance travelled by intestinal content through the intestine was measured. White barium sulphate as meal facilitates the measurement of the distance in this method.

Step 1: This study was performed using Swiss albino mice that were kept fasted for 18 h before commencing the experiment and only water was given and were gathered as negative control, standard control and treatment control groups.

Step 2: Three doses (100, 200 and 400 mg/kg bw) of four plant extracts (MELD, MELL, MELM and MELG) and 3 mg/kg bw loperamide as standard were administered orally to each group.

Step 3: One hour after administering plant extracts and standard, 0.5 ml pure grade castor oil was given orally to every mouse for the induction of diarrhea.

Step 4: After one hour of castor oil administration, 1 ml of barium sulphate suspension (5%) was administered orally by gavage to all mice.

Step 5: After 30 minutes of barium sulphate gavage, all the animals were sacrificed and subjected to isolate the small intestine for every mouse. Then the full length of the intestine as well as the intestinal length traveled by the barium sulphate meal was measured by using a centimeter scale.

By using the following formulas the percentage of inhibition of the gastrointestinal motility and peristaltic index were calculated.

1) % inhibition of the gastrointestinal motility = $(DT_C – DT_T)/ DT_C \times 100$

Where, DT_C = Mean distance traveled by the control group

DT_T: Mean distance traveled by the test group.

2) Peristalsis index = Distance traveled by barium sulfate meal /Length of small intestine × 100

Statistical analysis

The Graph Pad Prism 8, a statistical software was used to accomplish the statistical analysis and the values are represented as mean ± SEM (n=5). To relate multiple groups, one-way analysis of variance (ANOVA) followed by a Dunnet test were done. Values were stated to be statistically significant at $p<0.05$, $p<0.01$, $p<0.001$.

2.6.2 Analgesic activity test of crude methanol extracts

2.6.2.1 Peripheral analgesic activity test, analgesia by acetic acid

Principle

The analgesic test by counting writhing in mice was accomplished according to the method of Koster (1959). In this method, 0.7% v/v acetic acid was administered to the investigational animals through intra-peritoneal root to produce pain sensation. In consequence, the animals twist and curl their body because of pain sensation. This twist and curl response of the body is named as "writhing". Writhing is continued until the animals feel pain. Every writhing as well as half writhing is totalled as an sign of pain sensation. An analgesic drug or plant extracts with the phytoconstituents having analgesic activity are theoretical to reduce the number of writhing of animals. And in this method, the writhing inhibition of standard and test samples is compared with control. As standard, NSAID can be used and in the present study, Indomethacin was used as the standard. Though acetic acid-induced writhing experiment is not a specific model for analgesia (anticholinergic, antihistaminic and some other agents indicate activity in this test) it is a commonly used for analgesic screening and involves histaminic and cholinergic receptor located in peritoneal cavity.

Experimental design

Extracts coding for different doses

The codes for 100 and 200 mg/kg bw of the methanol extract of *L. glutinosa* were MELG_1 and MELG_2; the codes were MELM_1 and MELM_2 for *L. monopetala;* for *L. deccanensis* the codes were MELD_1 and MELD_2 and for *L. lancifolia* the codes were MELL_1 and MELL_2.

Animal grouping and dosing

For each extract in all the experimental models, animals were allocated into four groups (each group contains four mice) as follows

Group I: Control, indicated to administer only vehicle i.e. saline water, tween 80

Group II: Standard control, indicated to administer Indomethacin, 10 mg/kg bw

Group III: Treatment control indicated to administer 100 mg/kg bw

Group IV: Treatment control indicated to administer 200 mg/kg bw

Procedure of inducing writhing by acetic acid

Step 1: At zero hour of the experiment, saline water to group I, indomethacin (10 mg/kg) to group II and test samples (100 and 200 mg/kg bw) to group III, IV were administered orally by means of a feeding needle.

Step 2: After 30 minutes of the treatment, 0.7% v/v acetic acid was administered through intraperitoneal route to each animal of all the groups. The 30 minutes' interval of the peroral administration of standard and methanol extracts of plants and acetic acid was administered intraperitonealy to ensure appropriate absorption of the orally administered samples.

Step 3: After 5 minutes of the intraperitoneal administration of acetic acid, the total number of twists or writhings was counted for every mouse for 30 minutes.

Step 4: The total number of acetic acid-induced squirms in the mice of test groups' i.e. selected plant extracts treated mice was compared with individuals in the control and standard group mice.

Counting of writhing

Each mouse of control and treatment groups was observed for counting the writhing number individually in 30 minutes of time period after five minutes of i.p. administration of 0.7% v/v acetic acid. Complete squirming or writhing was not always taking place by the experimental animals, sometimes they started to squirm or twist but they could not complete. This type of incomplete writhing was considered as half writhing and two half writhing were considered as one complete writhing.

2.6.2.2 Central analgesic activity test by Eddy's hot plate method

Principle

The method was formerly established by Woolfe and MacDonald (1944). They developed the method depending on the basis that the paws of mice or rats are sensitive to heat at 55ºC temp, which does not damage the skin. The response may be jumping, paws withdrawal from the hot surface or paws licking. This original method was

updated by Eddy and Leimback (1953) which was further modified by Toma *et al.* (2003).

In this method, the investigational animals are placed on the hot plate maintained at temperature at 55˚C. Consequently, the mice will lick their paw or jump or show any response due to the effect of the surface of the hot plate. A substance with pain relieving activity is supposed to reduce the response (paw licking or jumping) time of animals. The responses produced with the test plant extracts are compared with the control as well as standard. As standard, tramadol, was used which produces central analgesia by generating O-desmethyltramadol as a metabolite which act on μ-opoid receptor.

Extracts coding for different doses

The codes for 100, 200 and 400 mg/kg bw of the methanol extract of *L. glutinosa* were MELG_1, MELG_2 and MELG_3; the codes were MELM_1, MELM_2 and MELM_3 for *L. monopetala;* for *L. deccanensis* the codes were MELD_1, MELD_2 and MELD_3 and for *L. lancifolia* the codes were MELL_1, MELL_2 and MELL_3.

Animal grouping and dosing

For each extract in all the experimental mece models, mice were allocated into four groups (each group contains four mice) as follows

Group I: Control group, indicated to administer only vehicle i.e. saline water, tween 80
Group II: Standard control group, indicated to administer tramadol, 10 mg/kg bw
Group III: Treatment control group indicated to administer 100 mg/kg bw
Group IV: Treatment control group indicated to administer 200 mg/kg bw
Group V: Treatment control group indicated to administer 400 mg/kg bw

Procedure of Eddy's hot plate method

Step 1: At zero-hour saline water to group I, tramadol (10 mg/kg bw, p. o.) to group II and three different doses of each plant extract were administered to group III, IV and V orally by means of a feeding needle.

Step 2: One hour after peroral administration of plant extract at three different doses and standard tramadol, the experimental animals were positioned on the hot plate with

maintained temperature at 55˚± 0.5 C. To avoid damage of the paw the animals were not kept for more than 15 s. A stopwatch was used to note the reaction time as well as the form of response.

Step 3: The showed response was noted as the reaction time which was measured after 30, 60, 120 and 180 min following peroral administration of 100, 200 and 400 mg/kg of each of the extract to different groups.

Step 4: Then mean values of the reaction times were determined and the % of pain inhibition was calculated by using the equation mentioned below:

% of pain inhibition = (drug latency – baseline latency)/ baseline latency × 100

2.6.2.3 Central analgesic activity test, formalin-induced paw licking in mice

Principle

In this method, pain is induced by chemical formalin which is given to the sub plantar region, hind paw of the investigational animals. Consequently, a pain sensation is induced in animals and the animals produce responses in the form of paw licking. An analgesic substance is ecpected to decrease the pain sensation as well as paw licking of the animals within the given time frame for the experiment which is compared to the experimental control group (Tjolsen et al., 1992). The reduced number of paw licking in the animals of positive control groups (treatment groups) was compared with those of the control group. As positive control, indomethacin, a NSAID was used as standard.

Extracts coding for different doses

The codes for 100 and 200 mg/kg bw of the methanol extract of *L. glutinosa* were MELG_1 and MELG_2; the codes were MELM_1 and MELM_2 for *L. monopetala;* for *L. deccanensis* the codes were MELD_1 and MELD_2 and for *L. lancifolia* the codes were MELL_1 and MELL_2.

Animal grouping and dosing

For each extract in all the experimental models, animals were allocated into four groups (each group contains four mice) as follows

Group I: Control group, indicated to administer only vehicle i.e. saline water, tween 80

Group II: Standard control group, indicated to administer indomethacin, 10 mg/kg bw
Group III: Treatment control group indicated to administer 100 mg/kg bw
Group IV: Treatment control group indicated to administer 200 mg/kg bw

Preparation of standard and crude methanol extracts of two different concentrations

Sodium chloride salt (0.9 gm) was weighed, dissolved in distilled water and made the volume up to 100 ml. For preparing indomethacin as a standard solution at the dose of 10 mg/kg bw, required amount of indomethacin was dissolved in 2 ml of saline water. Each 0.5 ml contains 10 mg/kg bw of indomethacin and 0.5 ml was given to each mouse orally.

To prepare the crude extract at dose of 100 and 200 mg/kg bw, required amount of extract was triturated with a small amount of suspending agent tween-80 and then added saline water to get suspension of the extract. Each 0.5 ml contains 100 mg/kg or 200 mg/kg bw of extract and 0.5 ml was given to each mouse of group III and group IV respectively.

Procedure of formalin induced paw licking test

Step 1: At zero-hour saline water to group I, indomethacin (10 mg/kg bw) to group II and two different doses of each plant extract were administered to group III and IV peroral by means of a long feeding needle.

Step 2: After 30 minutes of oral administration of standard indomethacin as well as two different doses of each plant extracts, 2% formalin was administered to every animal of all the groups. The 30 minutes' interval of the peroral administration of standard and methanol extracts of plants and formalin was administered underneath the skin of the hindpaw to ensure appropriate absorption of the orally administered samples.

Step 3: The total time of licking and biting the particular formalin injected paw by the experimental mice was recorded by using stopwatch.

Step 4: Depending upon the type of response as described earlier by Tjolsen *et al.* (1992), the response was measured in two different phases of rigorous licking periods stated as early phase and late phase. The time that mice spent for licking or biting their

injected paws or legs at early phase (0–5 min after formalin injection) and the late phase (20–30 min after formalin injection) was documented for further data analysis. The latency of paw licking in the first 5 minutes indicates response to neurogenic pain while the latency of paw licking in between 20-30 minutes indicates inflammatory pain.

The % inhibition of paw biting and licking was calculated by the formula:
$(PL_C - PL_T)/(PL_C) \times 100$;
Where, PL_C denotes the mean value of paw licking of the control group; and
PL_T denotes the mean value of paw licking of the treated group.

2.6.3 Hypoglycemic activity test of crude methanol extracts

Principle

Diabetes is a chronic metabolic disease that reduces or stop glucose metabolism and consequently reduces energy production from glucose. Both the genetic and environmental factors increase insulin deficiency as well as insulin resistnce which ultimately increase blood glucose level (glucose toxicity) and induce diabetes. It may develop from diverse pathogenic mechanisms, but hyperglycemia is the only result of this disorder (Brunton *et al.,* 2011). Diabetes leads to several severe complications blood vessels and heart disease (stroke, atherosclerosis and high bllod pressure), nerve damages (peripheral neuropathy), Eye damage (blindness, cataract and glaucoma), chronic kidney disease, increase susceptibility to infectious diseases, slow healing, dementia etc. (Katzung, 2015). In this method streptozocin (STZ) is used to induce diabetes. STZ is a glucosamine-nitrosourea that is used to treat β cell carcinoma of pancreas. After diabetes induction treatment with standard metformin as well as methanol extracts of selected plants reduce the blood glucose level and that was measured to evaluate antihyperglycemic activity.

Experimental design

Extracts coding for different doses

The codes for 300 and 500 mg/kg bw of the methanol extract of *L. glutinosa* were MELG_1 and MELG_2; the codes were MELM_1 and MELM_2 for *L. monopetala;* for *L. deccanensis* the codes were MELD_1 and MELD_2 and for *L. lancifolia* the codes were MELL_1 and MELL_2.

Animal grouping and dosing

For each extract in all the experimental models, animals were allocated into four groups (each group contains six mice) as follows

Group I: Normal control group, indicated to administer only vehicle i.e. saline water and Tween 80

Group II: Untreated diabetic control group, indicated to administer nothing

Group III: Standard control group, indicated to administer metformin HCl 50 mg/kg bw

Group IV: Treatment control group indicated to administer 300 mg/kg bw MELD

Group V: Treatment control group indicated to administer 500 mg/kg bw MELD

Group VI: Treatment control group indicated to administer 300 mg/kg bw MELL

Group VII: Treatment control group indicated to administer 500 mg/kg bw MELL

Group VIII: Treatment control group indicated to administer 300 mg/kg bw MELG

Group IX: Treatment control group indicated to administer 500 mg/kg bw MELG

Group X: Treatment control group indicated to administer 300 mg/kg bw MELM

Group XI: Treatment control group indicated to administer 500 mg/kg bw MELM

Procedure

Step 1: All the animals were divided into non-diabetic (Group I) and diabetic (Group II-Group XI) groups. Animals of diabetic group (Group II - Group XI) were subjected to diabetic induction with streptozotocin (STZ).

Step 2: On the day 1 of 5 consecutive days, all foods were removed from the cage except water 4 hr prior to administration of STZ treatment, for all groups. Then the required amount of STZ was calculated (40 mg/kg/bw) for every animal and dissolved in sterile normal saline solution and given 0.5 ml to each animal through intra-peritoneal route. Then return the animals in the cages with normal food, water and 10% sugar solution.

Step 3: After 5 days of STZ administration, blood was withdrawn from the tail vein and the blood glucose level (BGL) was tested by a digital glucometer. Animals with above 8.0 mmol/L BGL were chosen for this study.

Step 4: The required amount of standard and selected plants extracts were calculated rendering the animals' body weight and dissolved in saline water. 0.5 ml solution

contains the desired amount of standard and selected plants extracts and each animal was fed 0.5 ml test solution.

Step 5: Peroral administration of standard and selected plants extracts for seven consecutive days the blood glucose level (BGL) was tested at 3rd, 5th and 7th day of the tratment (Saleh *et al.,* 2013). % inhibition of blood sugar was calculated by the equation mentioned below:

% Inhibition of Blood Glucose Level (BGL) = $(BGL_{dc} - BGL_t)/ BGL_{dc} \times 100$,
BGL_{dc} = Mean blood glucose level of diabetic control
BGL_t = Mean blood glucose level of treatment

2.6.4 CNS depressant activity test of crude methanol extracts

Principle

In the current study, the possible neuropharmacology (CNS stimulant or depressant) of methanol extract of *L. deccanensis* (MELD), *L. lancifolia* (MELL), *L. glutinosa* (MELG) and *L. monopetala* (MELM) was investigated in comparison with the diazepam administered to experimental standard group as well as normal control group. This experiment was performed by means of hole cross method which was approved according to Takagi *et al.* (1971). Here, a divider was fixed in the mid of chamber having a dimensions of 30×20×14 cm. A 3 cm diameter hole was made at 7.5 cm height in the center of the hole cross chamber. The crossing number through the hole from one chamber to the other by the mouse was totaled for a period of 3 minutes at 0, 30, 60, 90 and 120 minutes after oral administration of the crude extracts of the studied plants.

Experimental design

Extracts coding for different doses

The codes for 300 and 500 mg/kg bw of the methanol extract of *L. glutinosa* were MELG_1 and MELG_2; the codes were MELM_1 and MELM_2 for *L. monopetala;* for *L. deccanensis* the codes were MELD_1 and MELD_2 and for *L. lancifolia* the codes were MELL_1 and MELL_2.

Animal grouping and dosing

For each extract in all the experimental models, animals were allocated into four groups (each group contains six mice) as follows

Group I: Control group, indicated to administer only vehicle i.e. saline water, tween 80

Group II: Standard control group, indicated to administer Diazepam 1 mg/kg bw

Group III: Treatment control group indicated to administer 300 mg/kg bw MELD

Group IV: Treatment control group indicated to administer 500 mg/kg bw MELD

Group V: Treatment control group indicated to administer 300 mg/kg bw MELL

Group VI: Treatment control group indicated to administer 500 mg/kg bw MELL

Group VII: Treatment control group indicated to administer 300 mg/kg bw MELG

Group VIII: Treatment control group indicated to administer 500 mg/kg bw MELG

Group IX: Treatment control group indicated to administer 300 mg/kg bw MELM

Group X: Treatment control group indicated to administer 500 mg/kg bw MELM

Procedure

Step 1: Sodium chloride salt (0.9 gm) was weighed and then it was added to the distilled water and mixed properly. The final volume of saline water was made 100 ml.

Step 2: The crude methanol extracts were administered at the doses of 300 and 500 mg/kg bw. Required amount of different extracts of *Litsea* species were measured and triturated unidirectional way with the addition of small amount of suspending agents (Tween-80) in separate containers. After proper mixing of extracts and suspending agent, normal saline water was slowly added in those containers.

Step 3: At zero hour, 1% Tween-80 in saline water orally to Group I and 1 mg/kg bw diazepam was given through intra-peritoneal root to Group II. Then sample suspensions of four different species of *Litsea* at the doses of 300 and 500 mg/kg bw were administered orally by using a feeding needle to each mouse of Group III to Group X.

Step 5: The passage number of every mouse of different groups through the hole in between the two chambers was counted for about 3 min period on 0, 30, 60, 90 and 120 min after treatment with the doses of 300 and 500 mg/kg bw of the methanol extract of *L. glutinosa, L. monopetala, L. deccanensis* and *L. lancifolia.*

2.6.5 Antimicrobial screening of different partitionates by disc diffusion method

Principle of disc diffusion method

Antimicrobial resistance results in the morbidity and mortality due to the failure of infectious disease treatment and these days, antibiotic resistance has become a severe global problem. Inappropriate use, unnecessarily prescribing, availability of antibiotics as OTC are responsible for antibiotic resistance. So, plants having antimicrobial activities may be used as alternatives to reduce the inappropriate or unnecessary use of antibiotics. Disc diffusion method is a technique to determine antimicrobial activity of test materials including plant extracts. In this method, nutrient agar medium is seeded with the different test microorganisms and placed on petridishes, paper discs made with double layered filter papers (5 mm diameter) were impregnated with standard and test samples. Standard antibiotic (Ciprofloxacin/Griseofulvin) discs as positive control and blank discs are used as negative control. The patridishes are retained at 4°C for about 16 to 24 hours expecting proper diffusion of antimicrobial agents. Then the plates are inverted and incubated at 37°C for 24 hours. The test materials having antimicrobial properties prevent growth of microorganisms in the media that surrounds the filter paper discs and thus produce a clear area called zone of inhibition. Then diameter (mm) of the zone of inhibition is measured to determine antimicrobial potential of the test samples. This method was performed three times, then calculates the mean and compared with negative control and positive control. (Barry, 1976; Bauer *et al.*, 1966).

Experimental procedure

The experiment was completed by the method as illustrated by Bauer *et al.* (1966)

Preparation of the medium

To make the necessary amount of the medium, the required quantity of medium was taken and distilled water was added to it to make the needed volume in a conical flask. The medium was heated first gently in a water bath to make a clear solution, then, 5 ml medium was taken in a 10 ml screw cap test tube to prepare slant. The capped test tubes were then sterilized by autoclaving at 121° C and at 15-lbs./sq. inch pressure for 20 minutes. For preparing fresh cultures of microorganisms that slants were used and employed for antimicrobial or sensitivity study.

Sterilization procedure

With the aim of avoiding slightest contamination or cross contamination test organisms with other organisms, the antimicrobial test was performed in Laminar Hood and entire safeguard was well-maintained. Before one hour of working UV light of Laminar Hood was switched on to sterilize the area within the Laminar Hood. Glasswares, micropipette tips, forceps, cotton, blank discs and petridishes were sterilized by autoclaving at 121 ºC and at 15-lbs./sq. inch pressure for 20 minutes.

Subculture preparation

In order to prepare fresh cultures test organisms were taken from fresh pure cutures and transferred to the freashly prepared agar slants by using sterilized platinum loop uder the Laminar Hood in the aseptic area. Then the inoculated agar slants were incubated at 37 ºC for 24 hrs for optimal growth of microbial strains.

Preparation of the test plate

Each organism was transferred from the freashly prepared subculture to test tube specified for that organism which contains 10 ml of warm, sterilized and melted agar medium by a sterilized platinum loop. A uniform suspension of the organism was prepared by shaking the test tube in between the palms of hand and transferred to a petridish immediately. While a clockwise and anticlockwise rotation of the petridish was performed to ensure homogeneous mixture of the test organism. This method was repeated for every organism and the whole process was performed in the aseptic area uder the Laminar Hood.

Preparation of discs

Blank, standard and sample discs were prepared for antimicrobial screening. (Bayer *et al.*,1966). The use of blank discs or discs without sample (only with vehicles) ensure the negative effect of solvent even after drying. Standard discs or discs with embedded standard antibiotics ensure the known effect to compare with the unknown response of the test samples. Sample discs implanted with test samples were prepared by soaking sample solution followed by drying.

Diffusion and incubation

The Blank, standard and sample discs were placed on the agar plates which was previously inoculated with test mirorganisms. Then they were kept at 4 ºC in a refrigerator for 16 to 24 hrs for efficient diffusion of the antimicrobial potential from the discs to the agar media surrounding the discs. After that, the petridishes were upturned and kept at 37 ºC to incubate for 24 hrs for the optimum microbial growth.

2.6.6 Antioxidant activity test of different partitionates

2.6.6.1 Determination of total phenolic content

Total phenolic content of three different extractives of *L. glutinosa, L. monopetala, L. deccanensis* and *L. lancifolia* were determined by the method of Singleton and Rossi, 1965 which involve the use of Folin-Ciocalteu reagent (FCR) as oxidizing agent while Gallic acid as standard. This method was then further amended by Skerget *et al.* (2005) and Majhenic *et al.* (2007).

Principle

Polyphenols are the common antioxidant natural products of medicinal plants which can be measured by Folin–Ciocalteu Reagent (FCR). This reagent actually determines the reducing capacity of a sample. The FCR is supposed to contain heteropolyphosphotungstates - molybdates. When it is added in an ionized phenolic solution FCR readily oxidizes the ionized phenols. After incubation, the oxidation will be completed and the yellow colour of FCR will become blue, possibly due to $(PMoW_{11}O_{40})_4$. This color change will be measured with a UV Visible spectrophotometer at 760 nm and the absorbance value will be used to calculate the total phenolic content of the sample solution (Harbertson and Spayd, 2006).

Experimental Procedure

Step1: Three different extractives of *L. glutinosa, L. monopetala, L. deccanensis* and *L. lancifolia* were diluted to attain the concentration of 1 mg /ml. Gallic acid standard solutions were prepared at 20, 40, 60, 80 and 100 mg/L concentration by serial dilution. Folin-ciocalteu reagent was diluted 10 times with distilled water and 7.5% sodium carbonate solution was prepared.

Step 2: In a test tube 0.5 ml of each partition of plant extracts or different concentrations of standard solution was taken. Then 2.5 ml Folin – ciocalteu reagent (FCR) and 2.5 ml of sodium carbonate (7.5% w/v) were added into every test tube.

Step 3: Then the test tubes were incubated at 23 ± 2°C for 20 minutes in order to complete the reaction. The absorbance of each solution was then taken at 760 nm by using UV spectrophotometer. The absorbance for a blank solution with all reagents excluding the plant extracts or the standard solution was taken also.

Step 4: The TPC was estimated by using the standardization curve for gallic acid and then the results were stated as mg of GAE/g of extract, that is, the gallic acid equivalent per gram of dry weight of each extract.

2.6.6.2 DPPH assay for antioxidant activity

Principle

The free radical scavenging activity of the plant extracts was assessed by the method of Brand-Williams *et al.,* 1995. The basis of the method is the reduction of DPPH (1,1-diphenyl-2-picrylhydrazyl), a stable free radical. This free radical DPPH has an odd electron which reacts with antioxidants and becomes paired off and reduced to the DPPHH. Reduction from DPPH (purple colour) to DPPHH (yellow colour) by the presence of antioxidants results a reduction of absorbance. This decrease in absorbance produced by DPPH free radical scavenging reaction has been extensively used to check the ability of medicinal plant extracts to have free radical scavengers or antioxidant potential. The absorbance is taken at 517 nm by using UV-spectrophotometer while methanol or ethanol is used as a solvent and ascorbic acid is used as a standard.

This method was evidently presented near about 50 years ago by Marsden Blois (Blois, 1958) which was followed by a number of researchers (Kim *et al.*, 2002; Zhu *et al.*, 2002). Lately, an updated method has been introduced by Brand-Williams and his colleagues (Brand-Williams *et al.,* 1995) which has been employed by several groups of researchers (GO ′ mez-Alonso *et al.,* 2003; Lebeau *et al.,* 2000; Yepez *et al.,* 2002).

Experimental procedure

Step 1: 0.004% DPPH solution was prepared in 95% methanol. For protection from light by the test tubes were covered with aluminum foil the process was performed in a dark place. Three different extractives of *L. glutinosa, L. monopetala, L. deccanensis* and *L. lancifolia* were mixed with 95% methanol to make the stock solution (4 mg/40ml).

Step 2: The obtained concentration of the sample solution (termed as stock solution) was 100 µg/ml. From this stock solution 2 ml, 4 ml, 6 ml, 8 ml and 10 ml were withdrawn into five test tubes and serially diluted with methanol to get 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml respectively.

Step 3: Then freshly prepared 100 µl DPPH solution was added in each of these test tubes containing three different extractives of *L. glutinosa, L. monopetala, L. deccanensis* and *L. lancifolia* and after 20 minutes, the absorbance was taken at 517 nm using a spectrophotometer.

Step 4: 100 µl DPPH solution was taken in 10 ml methanol and the absorbance was investigated immediately at 517nm to get control reading. The DPPH solution in 95% methanol without sample solution was used as blank.

Step 5: As positive control ascorbic acid was used in this study. % scavenging of the DPPH free radical was calculated by the equation mentioned as below.

% inhibition of DPPH free radical scavenging = $[1-(Ab_S/Ab_C)] \times 100$
Here, Ab_C = absorbance of control, Ab_S = absorbance of sample solution.

Then, IC_{50} values for standard and samples were calculated from the graph obtained by plotting % inhibitions of DPPH radical scavenging against corresponding concentration. The concentration of the samples required for 50% scavenging of stable DPPH radical is defined as IC_{50}.

2.6.7 Molecular docking of pure compounds

2.6.7.1 Molecular docking of LGC-26 (95) and LGC-45-3 (96) against human aldose reductase for its anti-diabetic property

Human aldose reductase (AKR1B1, AR) is a crucial enzyme that plays an important role for catalyzing the reduction of glucose to sorbitol when glucose concentrations are high. Certainly, AKR1B1 overstimulation is associated with diabetes related secondary complication (Accumulation of sorbitol in eye/nerve may cause retinopathy of peripheral neuropathy). To overcome this problem researchers are searching for new AKR1B1 inhibitors but not become successful because of undesirable side effects and/or poor pharmacokinetic properties of those. Human AKR1B1, AR is a possible therapeutic target for treating diabetes related secondary complications as its inhibition reduces the conversion of glucose to sorbitol in hyperglycemic conditions.

So, the use of aldose reductase inhibitor may be a noble therapeutic strategy to reduce diseases accompanying with hyperglycemia. Two different compounds were isolated, purified and characterized as LGC-26 **(95)** as 4′-*O*-methyl(2 ″,4 ″-di-*E*-*p*-coumaroyl)afzelin and LGC-45-3 **(96)** as quercetin3-*O*-(2′′,4′′-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside from the chloroform fraction of *L. glutinosa.* As crude methanol extract of *L. glutinosa* showed very good anti-diabetic property, these pure compouds are expected to possess anti-diabetic and aimed for molecular docking against human aldose reductase (AKR1B1, AR)

Computaional Methods

Ligand preparation

The preliminary geometries of the synthesized drug molecules were drawn in Gaussian 09 program package. The strcutures were optimized using Quantum mechanics (QM) calculations were conducted to optimize. Gaussian 09 program package was applied for all quantum calculations. Semiempirical PM6 method was used for optimization in harmonic approximation. The imaginary frequencies were absent during vibrational frequencies calculation (Crespo *et al.*, 2018; Zhang *et al.*, 2013).

Molecular docking

In the study, synthesized drugs were utilized for molercular docking against antidiabetic target aldose reductase (AKR1B1**)**. The protein structure (PDB ID: 4JIR) was retrieved from Protein Data Bank (PDB). The drug-protein interactions and binding affinities of the drug compounds were investigated via Auto Dock Vina protocol. During docking, the grid box was set around the residues Trp20, Tyr48, Met109, His110, Trp111, Phe122, Trp219, Leu300 of aldose reductase (AKR1B1**)** which were commonly interacting with previous inhibitor compounds within the vina search space including center X= -7.78 Å, Y= 7.12 Å, and Z= 18.85 Å and dimensions were X: 26.15 Å, Y: 21.94 Å , and Z: 28.40 Å. covering desired binding site residues in the protein. BIOVIA Discovery Studio version 4.5 was the software to visualize the non-covalent interactions in the drug-protein complex.

ADMET properties

The significant ADMET properties for the synthesized compounds were explored via admetSAR server (Cheng *et al.,* 2012). ADMET profiles encompass absorption, distribution, metabolism, excretion, and toxicity properties of a drug molecule. To minimize the risk of attrition for potential drug development the understanding pharmacokinetic properties, toxicity, and potency of drugs is indispensable. The SMILE file formats of the compounds were retrieved from the BIOVIA Discovery Studio version 4.5 for the analysis. The various properties of the synthesized drug molecules (e.g., human intestinal absorption (HIA), Caco-2 permeability, cytochrome P450 enzyme inhibition level, and P glycoprotein inhibitor (PGI), AMES toxicity, carcinogens**)** were considered for the analysis.

2.6.7.2 Molecular docking of LGC-26 (95) and LGC-45-3 (96) against alpha amylase for its anti-diabetic activity

Preparation of macromolecule

The protein target, which was retrieved from the RCSB Protein Data Bank (PDB ID: 3BAJ), served as docking receptor. All of the bound ligands and water molecules were removed from the active site of the receptor before docking.

Molecular docking analysis using AutoDock Vina

The molecular docking studies were carried out using AutoDockTools (ADT) and AutoDockVina programs (Trott and Olson, 2010). Standard protocol was used while docking the compounds against the active site of protein (PDB ID: 3BAJ). The grid box was constructed using 32, 30, and 24, pointing in x, y, and z directions, respectively, with a grid point spacing of 1.0 Å. The center grid box is of 9.412 Å, 18.615 Å and 43.422 Å. Nine different conformations were generated for each ligand scored using AutoDockVina scoring functions and ranked according to their binding energies. The conformations with the most favorable (least) free binding energy were chosen for analyzing the interactions between the target receptor and ligands.

2.6.7.3 Molecular docking of LML 363-13 (97) against COX-2 for its analgesic and against AMPK against anti-diabetic activity

Protein preparation

The coordinate of the crystal structure of cycloygenase-2 (PDB ID: 5IKT) bound to a co-crsytal tolfenamic acid (PDB ID: TLF) was obtained from protein dara bank (PDB) (Orlando and Malkowski, 2016). The resolution of the crystal was 2.45 Å which is within the acceptable limit in terms of the quality of the crystal. In the crystal structure, there were a bunch of water molecules along with some other compounds.

These compounds and water molecules were removed from the structure using PyMol (Schrodinger, 2008), visualization software leaves a "free" protein. The crystal structure of the enzyme was a dimer two chains namely A and B. Since the two chains were identical, only chain A was kept for docking purpose Also, the coordinate of the active site of the protein was established using the PyMol "active site" command which identified the amino acid residues within 5 Å of tolfenamic acid. It was assumed that the site at which tolfenamic acid was bound represented the active site of the enzyme. The "free" protein was then energy minimized using SwissPDB viewer since it appeared to contain multiple conformation for some residues. Such treatment repairs distorted geometries by moving atoms to release internal constraints.

The crystal structure of AMP-activated protein kinase (PDB ID: 4ZHX) bound to AMP with a resolution of 2.99 Å was retrieved from protein dara bank (PDB) (Langendorf *et al.*, 2016). This protein was "cleaned" and energy minimized in the same way as

described above. Since this protein is a heteromer, no chains were removed. Only small molecules and water were deleted.

Ligand preparation

Vomifolil, indomethacin and metformin structures were downloaded from Pubchem and energy optimized using the structure building and minimization tool of Chimera 1.14. The energy minimization procedure was based on force field method where MM2 force field parameters were utilized. Such energy minimization treatment prior to docking is essential to get rid of the impact of any possible unfavorable bond lengths, bond angles, torsion angles, or unfavorable non-bonded interactions.

Docking

Vomifoliol **(97)** indomethacin and metformin were docked to cycloygenase-2 (COX-2) and AMP-activated protein kinase (AMPK) using AutoDock Vina protocol (Trott and Olson, 2010). Indomethacin and metformin were controls for COX-2 and AMPK respectively. The molecular docking approach using AutoDock Vina protocol predicted the binding affinity and the interaction of the molecules with these proteins. The binding affinities of the drugs were measured in kcal/mol unit and sorted according to the higher negative values, which imply the best binding affinities. The docking grid box was set around the COX-2 enzyme active site where the center was X = 40.32, Y = 25.37, and Z = 240.14 and the dimensions were X: 25.00, Y: 18.73, and Z: 19.92. The grid box center for AMPK active site was X = 157.90, Y = -42.50, and Z = 68.25 and the dimensions were X: 30.07, Y: 25.00, and Z: 33.92. The molecular interactions of the drugs as predicted by docking simulation were analyzed in BIOVIA Discovery Studio Visualizer (Biovia, 2017)

Chapter 3: Results and Discussions

Phytochemical Investigations

3.1 Compounds isolated, purified and characterized from L. glutinosa

The chloroform soluble partitionate was subjected to gel permeation chromatography (GPC) over Sephadex LH-20 and LGC-26 and LGC-45-3 were isolated as pure. ^{1}H and ^{13}C NMR, COSY, HSQC, HMBC spectral data allowed to characterize these compounds as 4′-*O*-methyl (2″,4″-di-*E*-*p*-coumaroyl) afzelin (LGC-26, **95**) and quercetin3-*O*-(2″,4″-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside (LGC-45-3, **96**). Both the compounds are reported here for the first time from *Litsea* species which have previously been isolated from *Machilus litseifolia* (Li *et al.*, 2019), *Lindera akoensis* (Huang *et al.*, 2017) and *Mammea longifolia* (Rao *et al.*, 2002).

3.1.1 Characterization of LGC-26 as 4′-O-methyl (2″,4″-di-E-p-coumaroyl) afzelin **(95)**

Compound LGC-26 (**95)** was isolated as a yellowish white amorphous powder. Its ESIMS showed a pseudo-molecular ion peak [M+Na^{+}] at *m/z* 761.100 which indicated a molecular formula of $C_{40}H_{34}O_{14}$ (Figure 3.16 & 3.17). The ^{1}H NMR spectrum demonstrated a 4′-*O*-methyl kaempferol unit, a rhamnopyranosyl moiety and two *trans*-*p*-coumaroyl units at C-2″ and at C-4″ of the rhamnopyranosyl unit.

For the 4′-*O*-methyl kaempferol unit the ^{1}H NMR (400 MHz; CD_3OD) spectrum displayed two singlets at δ 6.21 (1H, s, H-6), and 6.39 (1H, br. s, H-8); two doublets at δ 7.91 (2H, *J* = 8.4 Hz, H-2′/6′) and 7.18 (2H, *J* = 8.4 Hz, H-3′/5′) and a methoxy group at δ 3.86 (3H, s) for the C-4′ position (Figure 3.4 & 3.5, Table 3.1). In addition, a rhamnopyranosyl moiety was identified by signals at δ 5.70 (1H, br. s, H-1″), 5.52 (1H, m, H-2″), 4.15 (1H, m, H-3″), 4.95 (1H, dd, *J* = 10.0, 9.6 Hz, H-4″), 3.28 (1H, m, H-5″) and a doublet at δ 0.83 (1H, *J* = 6.4 Hz, H-6″) (Figure 3.6, Table 3.1). The presence of two *trans*-*p*-coumaroyl units with trans-configuration were identified from signals at δ 7.55 (1H, d, *J* = 16.0 Hz, H-7‴′), 6.26 (1H, d, *J* = 16.0 Hz, H-8‴′), 7.68 (1H, d, *J* = 16.0 Hz, H-7‴) and 6.40 (1H, d, *J*=16.0 Hz, H-8‴). The aromatic ring protons for *p*-coumaroyl unit-A were identified by the doublets at δ 7.50 (2H, *J* = 8.4 Hz, H-2‴/6‴) and 6.83 (2H, *J* = 8.4 Hz, H-3‴/5‴) while the aromatic ring protons for *p*-coumaroyl

unit-B were ascertained by the doublets at δ 7.48 (2H, *J* = 8.4 Hz, H-2‴″/6‴″) and 6.79 (2H, *J* = 8.4 Hz, H-3‴″/5‴″) (Figure 3.7 & 3.8, Table 3.1).

Furthermore, five sets of COSY correlations between H-1" and H-2" at δ 5.70 and 5.52, between H-2" and H-3" at δ 5.52 and 4.15, between H-3" and H-4" at δ 4.15 and 4.95, between H-4" and H-5" at δ 4.95 and 3.28 and between H-5" and H_3-6" at δ 3.28 and 0.83, revealed the presence of a rhamnopyranosyl moiety in **95** (Figure 3.1 & Figure 3.10).The COSY NMR spectral data demonstrated correlations between H-7"" and H-8"" at δ 7.55 and 6.21, H-7"' and H-8"' at δ 7.68 and 6.40, between H-3"'/5"' and H-2"'/6"' at δ 7.50 and 6.83; between H-3""/5"" and H-2""/6"" at δ 7.48 and 6.79 confirmed the presence of two coumaroyl units in LGC-26 isolated from *L. glutinosa* (Figure 3.1 & 3.9).

The HSQC spectral data provided important information to reveal the structure. In the case of rhamnopyranosyl moiety, the HSQC spectrum showed cross peaks for H2"- C2" correlation at δ_H 5.52/ δ_C 71.7, connectivity peaks for H4"- C4" at δ_H 4.95/ δ_C 73.3, observed H3"- C3" correlation at δ_H 4.15/ δ_C 67.0, interactions for H5"- C5" observed at δ_H 3.28/ δ_C 68.4 and cross peaks for H6"- C6" correlation at δ_H 0.83/ δ_C 16.3 for rhamnopyranosyl moiety in LGC-26 (Figure 3.14). The HMBC correlations from δ_H 7.91 (H-2′/6′) to δ_C 157.6 (C-2) as well as δ_H 3.46 (OCH_3) to δ_C 162.2 (C-4′) ascribed the presence of kaempheride skeleton. The correlation from δ_H 5.70 (H-1") to δ_C 133.7 (C-3) indicated the glycosidic linkage between C-1" of the rhamnopyranosyl moiety and C-3 of kaempherol unit. The HMBC correlations from δ_H 5.52 (H-2") to δ_C 166.9 (>C=O) and δ_H 4.95 (H-4") to δ_C 167.0 (>C=O) confirmed two *trans-p*-coumaroyl units to be positioned at C-2" and C-4" of the rhamnopyranosyl unit. The connections between the 4′-*O*-methyl kaempferol unit with the rhamnopyranosyl unit as well as between the rhamnopyranosyl unit with two *trans-p*-coumaroyl units were established by HMBC correlations as depicted in figure 3.2 & 3.15.

The assignment for LGC-26 (**95**) has been completed depending on its ^{1}H NMR and ^{13}C NMR spectral data (Table 3.1, Figure 18). Thus, LGC-26 was identified as 4′-O-methyl-(2″,4″-di-*E-p*-coumaroyl) afzelin. The compound was previously reported from *Machilus litseifolia* (Li *et al.*, 2019) and *Lindera akoensis* (Huang *et al.*, 2017). This is the first report of its occurance from *Litsea* species.

Table 3.1: Comparison between the ^{1}H NMR (400 MHz; MeOD) and ^{13}C NMR (100 MHz; MeOD) spectral data of LGC 26 and 4′-*O*-methyl (2″,4″-di-*E*-*p*-coumaroyl) afzelin (Li *et al.*, 2019)

LGC 26: 4′-*O*-Methyl (2″, 4″-di-*E*-*p*-coumaroyl) afzelin			**4′-*O*-Methyl-(2″-Z- p-coumaroyl-4″-*E*-*p*-coumaroyl) afzelin**		
Position	**δ_C**	**δ_H, mult (*J* in Hz)**	**Position**	**δ_C**	**δ_H, mult (*J* in Hz)**
2	157.6, C		2	158.9, C	
3	133.7, C		3	135.0, C	
4	177.9, C		4	-	
5	161.9, C		5	163.2, C	
6	98.6, CH	6.21, s	6	99.7, CH	6.23, br.s
7	164.7, C		7	165.7, C	
8	93.5, CH	6.39, br.s	8	94.5, CH	6.41, br.s
9	157.3, C		9	158.5, C	
10	104.6, C		10	105.7, C	
1′	122.4, C		1′	123.6, C	
2′/6′	130.5, CH	7.91, d (8.4)	2′/6′	131.5, CH	7.90
3′/5′	114.0, CH	7.18, d (8.4)	3′/5′	115.1, CH	7.19
4′	162.2, C		4′	163.4, C	
7′	54.7, CH_3	3.86, s	7′	55.9, CH_3	3.86, s
Rha					
1″	98.0, CH	5.70, br.s	1″	99.3, CH	5.61, d (1.6)
2″	71.7, CH	5.52, m	2″	72,4, CH	5.50, dd (3.2, 1.6)
3″	67.1, CH	4.15, m	3″	68.0, CH	4.16, dd (9.9, 3.0)
4″	73.3, CH	4.95, dd (10.0, 9.6)	4″	74.3, CH	4.87, (overlapping)
5″	68.4, CH	3.28, m	5″	69.5, CH	3.41, m
6″	16.3, CH_3	0.83, d (6.4)	6″	17.5, CH_3	0.85, d (6.3)
Coum-A					
1‴	125.8, C		1‴	127.0, C	
2‴/6‴	129.9, CH	7.50, d (8.4)	2‴/6‴	130.9, CH	7.50
3‴/5‴	114.5, CH	6.83, d (8.4)	3‴/5‴	116.5, CH	6.84
4‴	160.0, C		4‴	161.4, C	
7‴	146.1, CH	7.68, d (16.0)	7‴	145.7, CH	6.91, d (12.9)
8‴	113.3, CH	6.40, d (16.0)	8‴	115.5, CH	5.85, d (12.9)
>C=O	166.8, C		9‴	166.8, C	
Coum-B					
1⁗	125.7, C		1⁗	127.4, C	
2⁗/6⁗	130.0, CH	7.48, d (8.4)	2⁗/6⁗	133.5, CH	7.66
3⁗/5⁗	115.5, CH	6.79, d (8.4)	3⁗/5⁗	115.5, CH	6.77
4⁗	160.1, C		4⁗	160.0, C	
7⁗	145.5, CH	7.55, d (16.0)	7⁗	146.6, CH	7.57, d (15.9)
8⁗	113.6, CH	6.26, d (16.0)	8⁗	114.7, CH	6.27, d (15.9)
>C=O	167.0, C		9⁗	168.2, C	

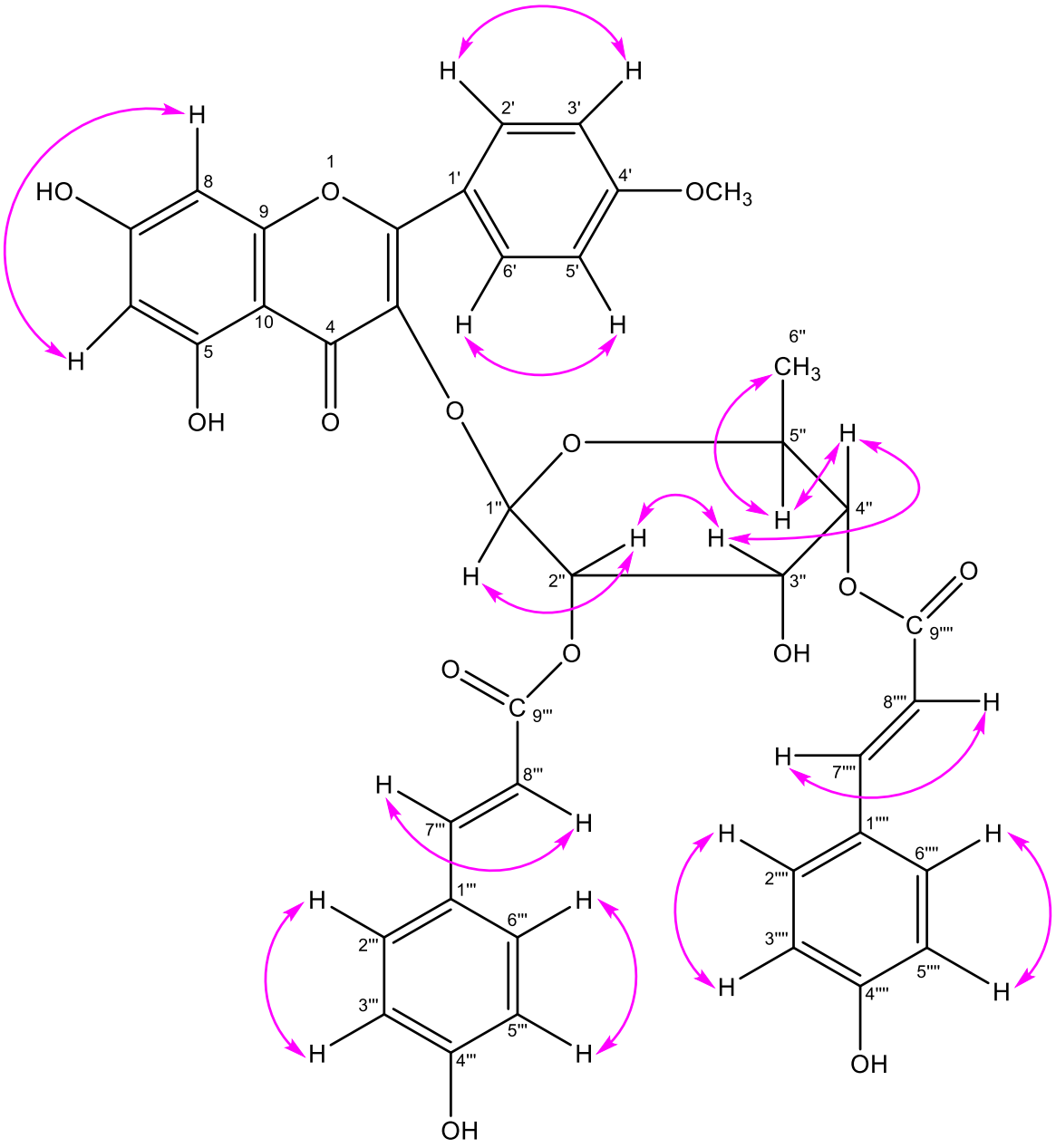


Figure 3.1: Key COSY correlations observed in LGC-26 [4′-*O*-methyl-(2″,4″-di-*E*-*p*-coumaroyl) afzelin, 95]

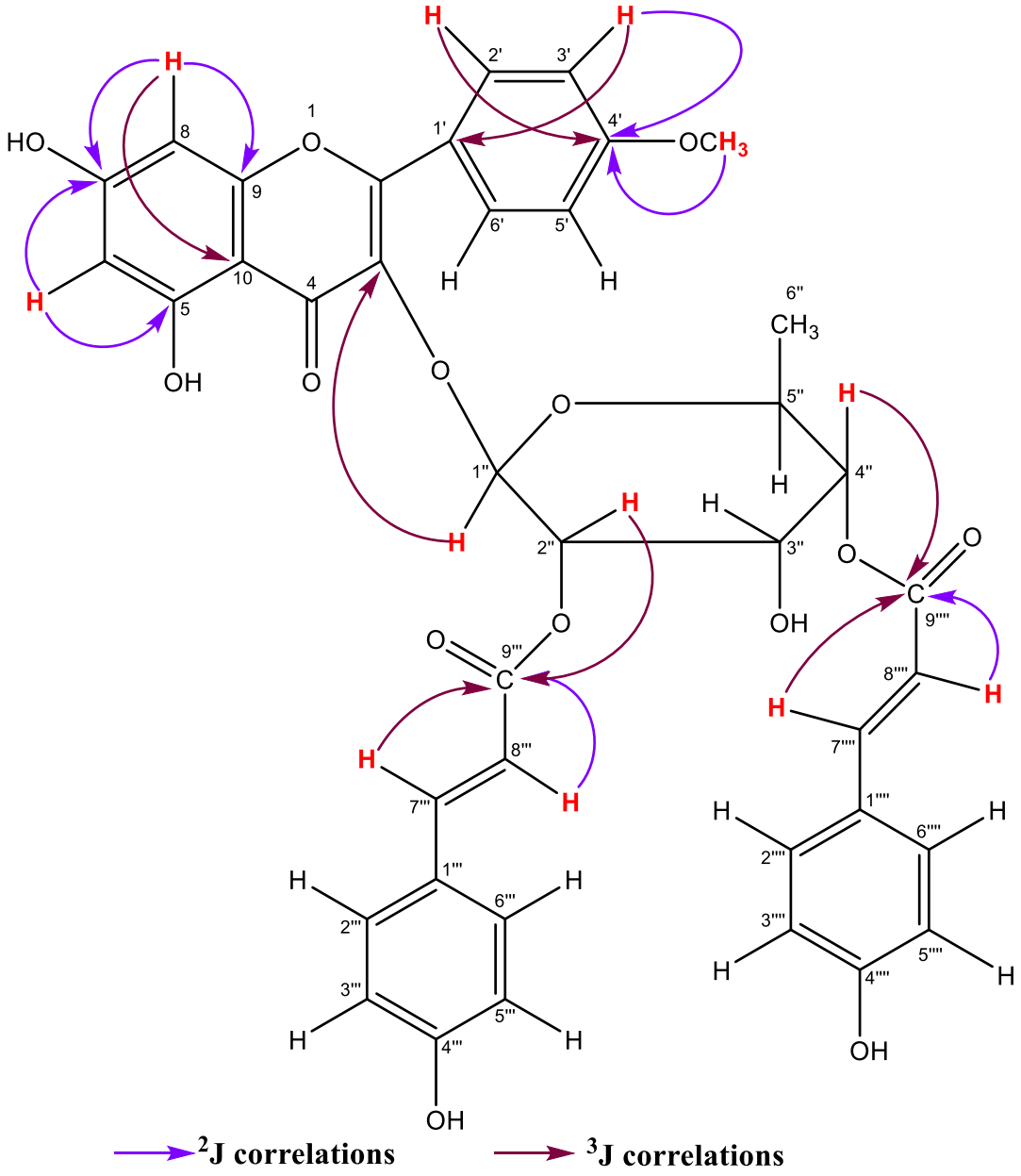


Figure 3.2: Key HMBC correlations observed in LGC-26 [4′-*O*-methyl-(2″,4″-di-*E*-*p*-coumaroyl) afzelin, 95]

Figure 3.3: Partially expanded ^{1}H NMR (400 MHz; MeOD) spectrum of LGC-26 as 4′-*O*-methyl(2′′,4′′-di-*E*-*p*-coumaroyl) afzelin, 95.

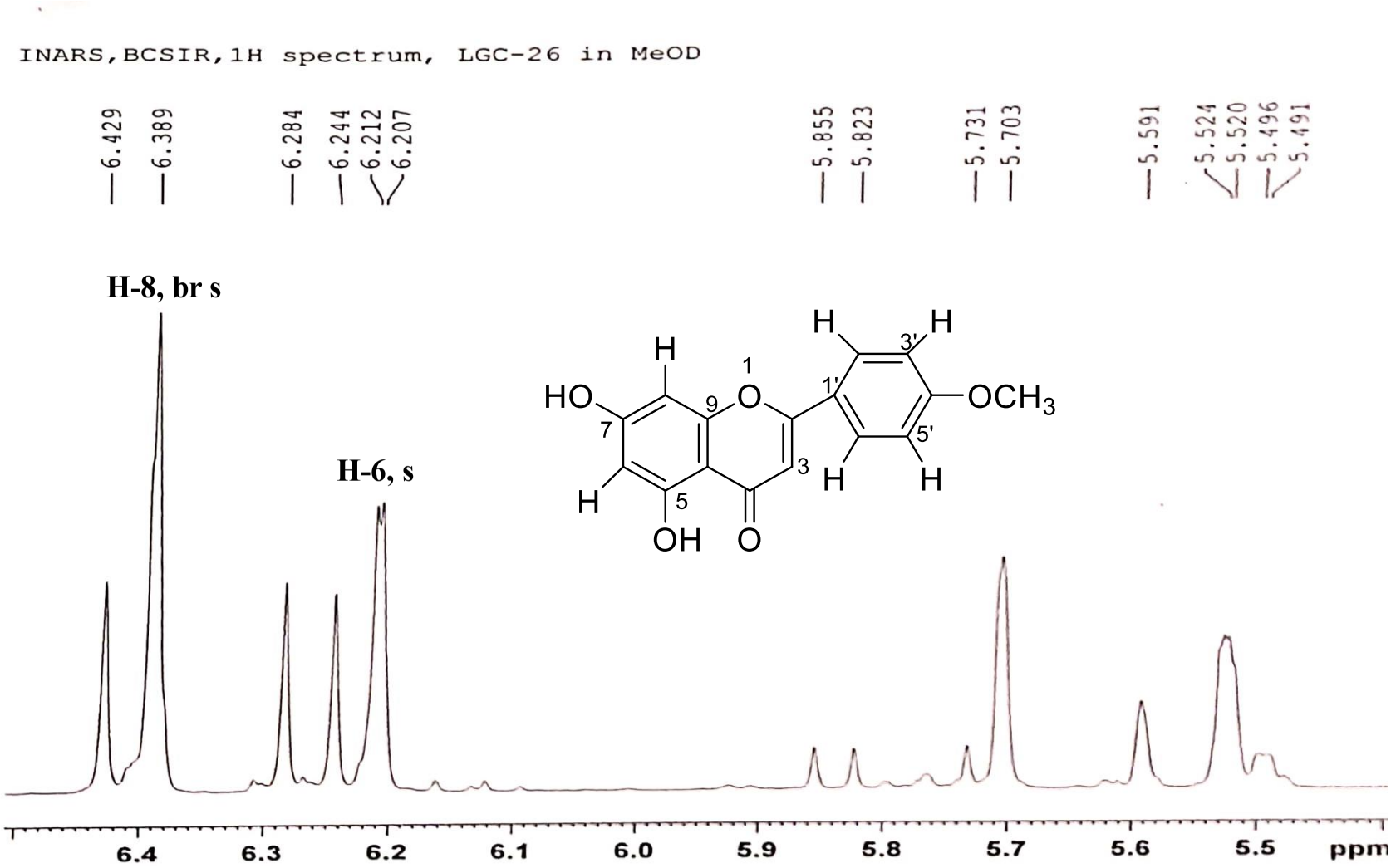


Figure 3.4: Partially expanded ^{1}H NMR (400 MHz; MeOD) spectrum of LGC-26 as4´-*O*-methyl(2´´,4´´-di-*E*-*p*-coumaroyl)afzelin, 95.

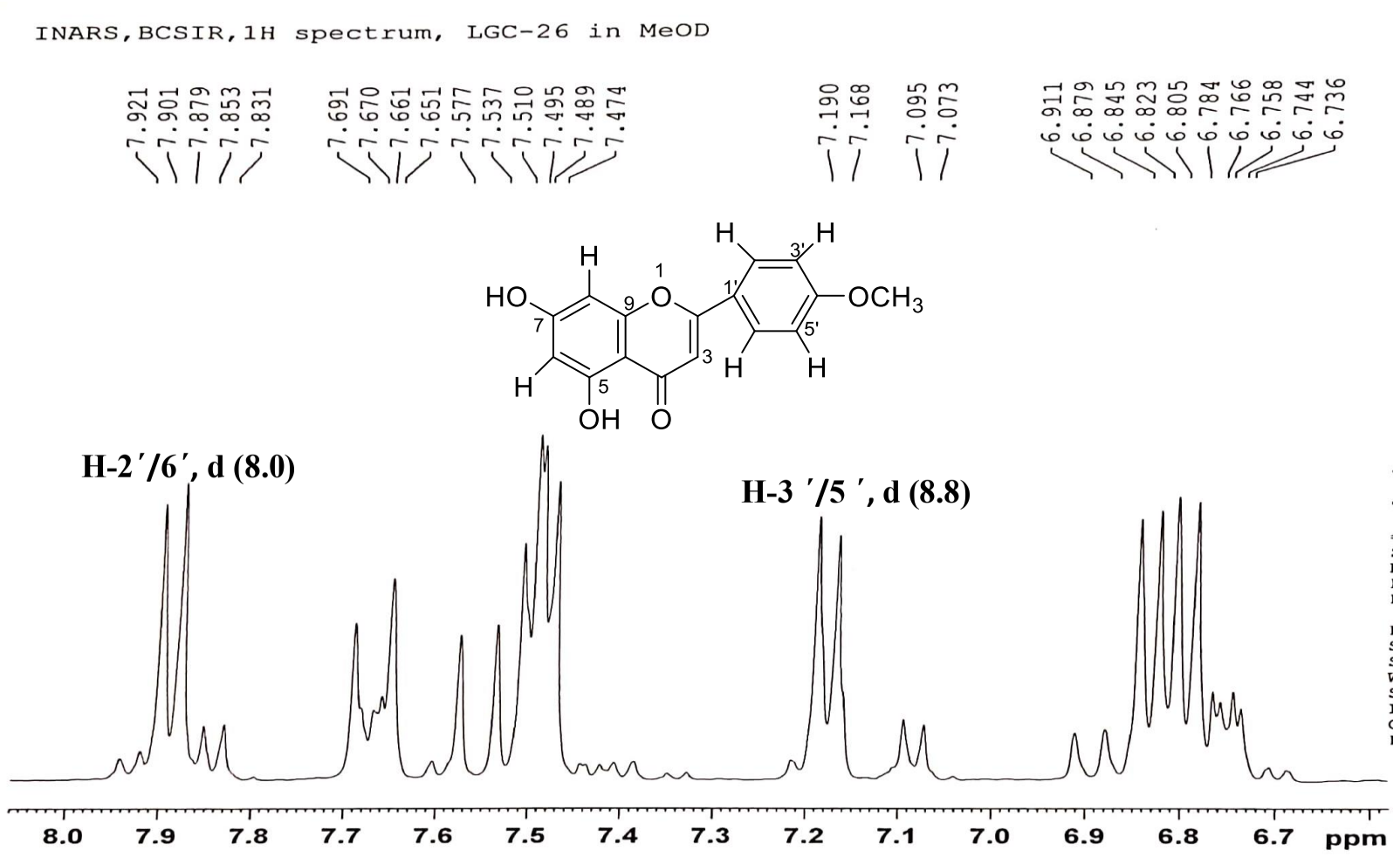


Figure 3.5: Partially expanded ^{1}H NMR (400 MHz; MeOD) spectrum of LGC-26 as 4′-*O*-methyl(2′′,4′′-di-*E*-*p*-coumaroyl)afzelin, 95.

Figure 3.6: Partially expanded ^{1}H NMR (400 MHz; MeOD) spectrum of LGC-26 as 4′-*O*-methyl(2′′,4′′-di-*E*-*p*-coumaroyl)afzelin, 95.

Figure 3.7: Partially expanded ^{1}H NMR (400 MHz; MeOD) spectrum of LGC-26 as 4′-*O*-methyl(2′′,4′′-di-*E*-*p*-coumaroyl)afzelin, 95.

Figure 3.8: Partially expanded ^{1}H NMR (400 MHz; MeOD) spectrum of LGC-26 as 4′-*O*-methyl(2′′,4′′-di-*E*-*p*-coumaroyl)afzelin, 95.

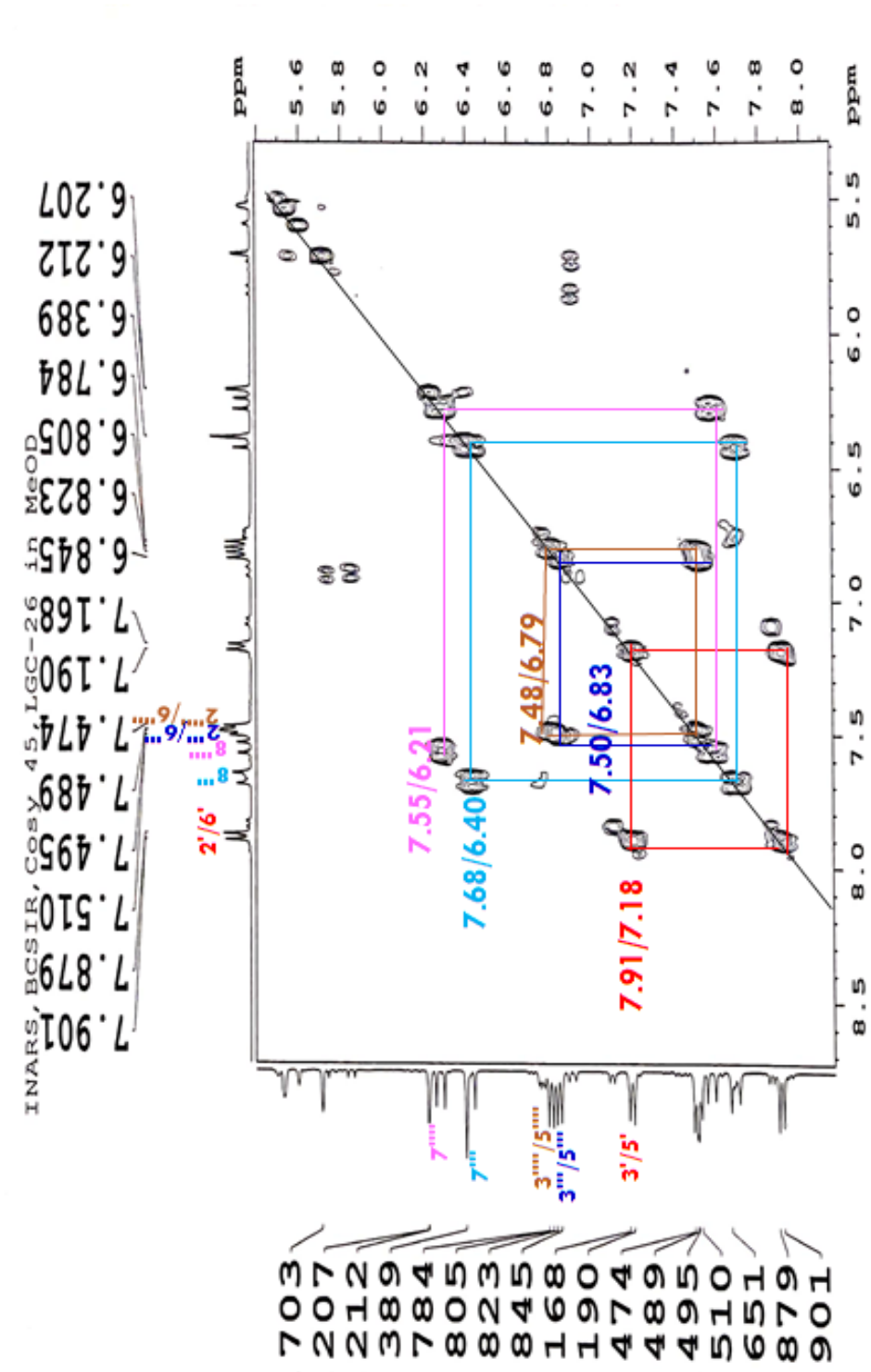


Figure 3.9: COSY NMR spectrum of LGC-26 as 4′-*O*-methyl(2′′,4′′-di-*E*-*p*-coumaroyl)afzelin, 95 in MeOD.

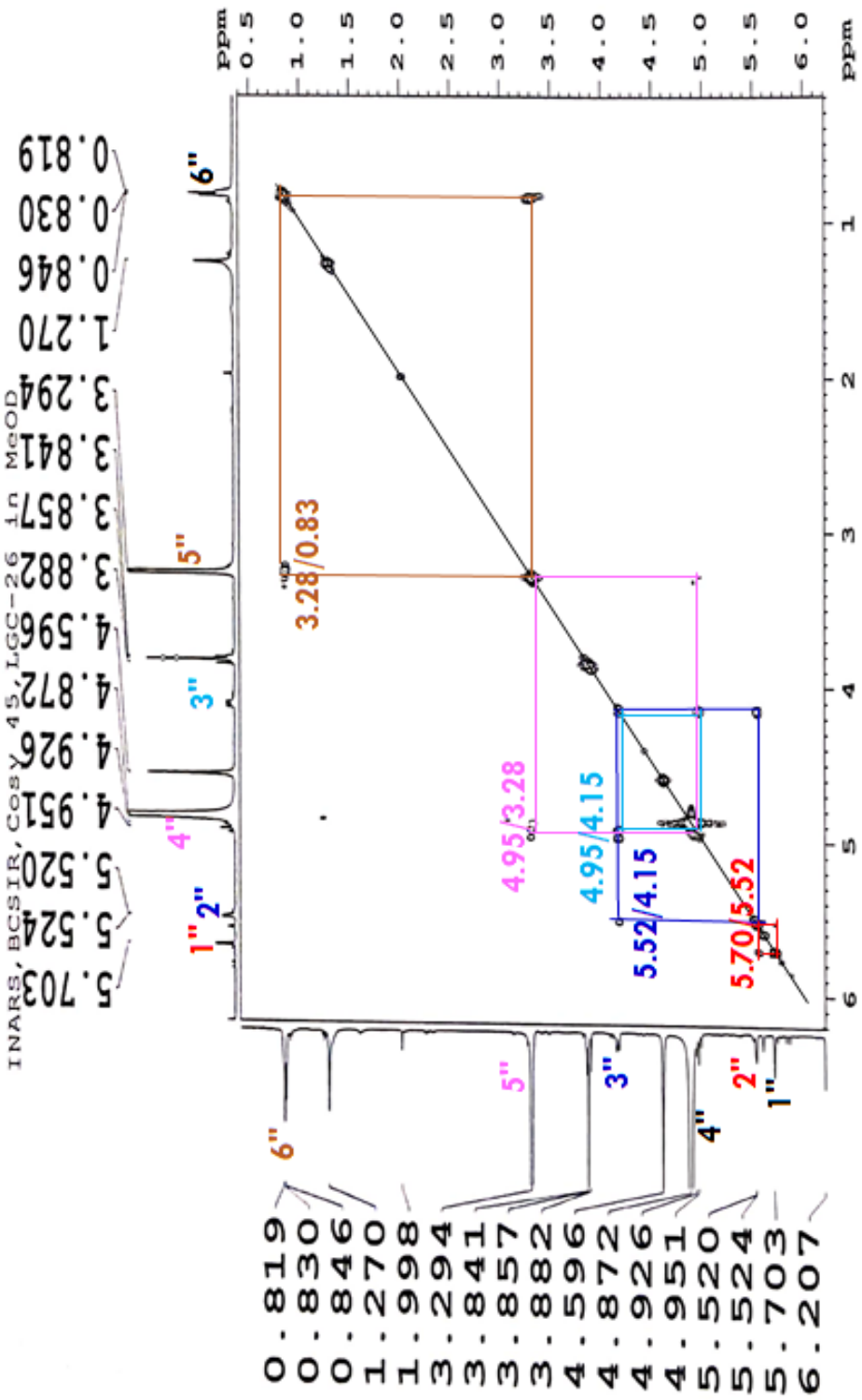


Figure 3.10: COSY NMR spectrum of LGC-26 as 4′-*O*-methyl(2′′,4′′-di-*E*-*p*-coumaroyl)afzelin, 95 in MeOD.

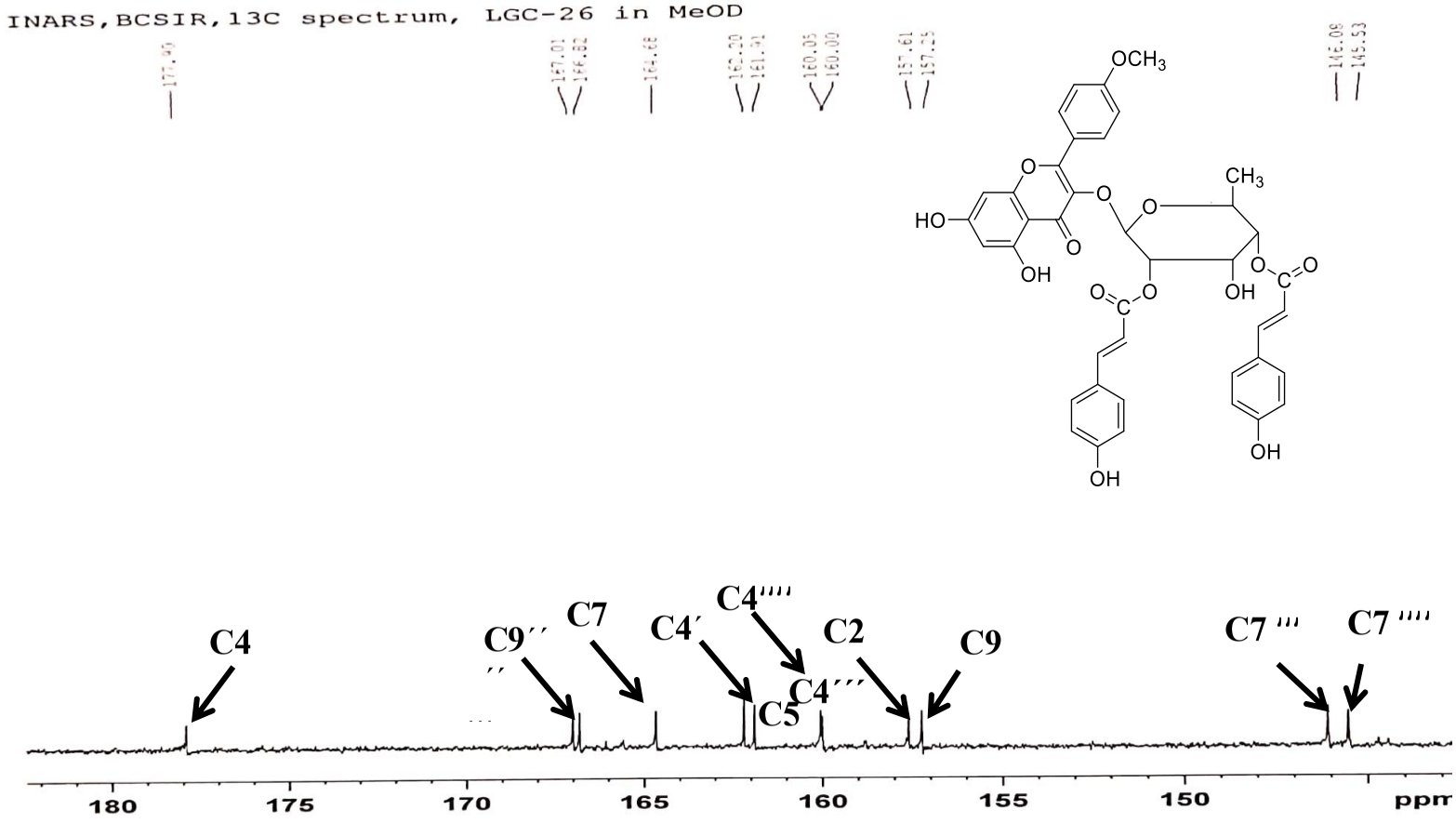


Figure 3.11: Partially expanded ^{13}C NMR (100 MHz; MeOD) spectrum of LGC-26 as 4′-*O*-methyl(2′′,4′′-di-*E*-*p*-coumaroyl)afzelin, 95.

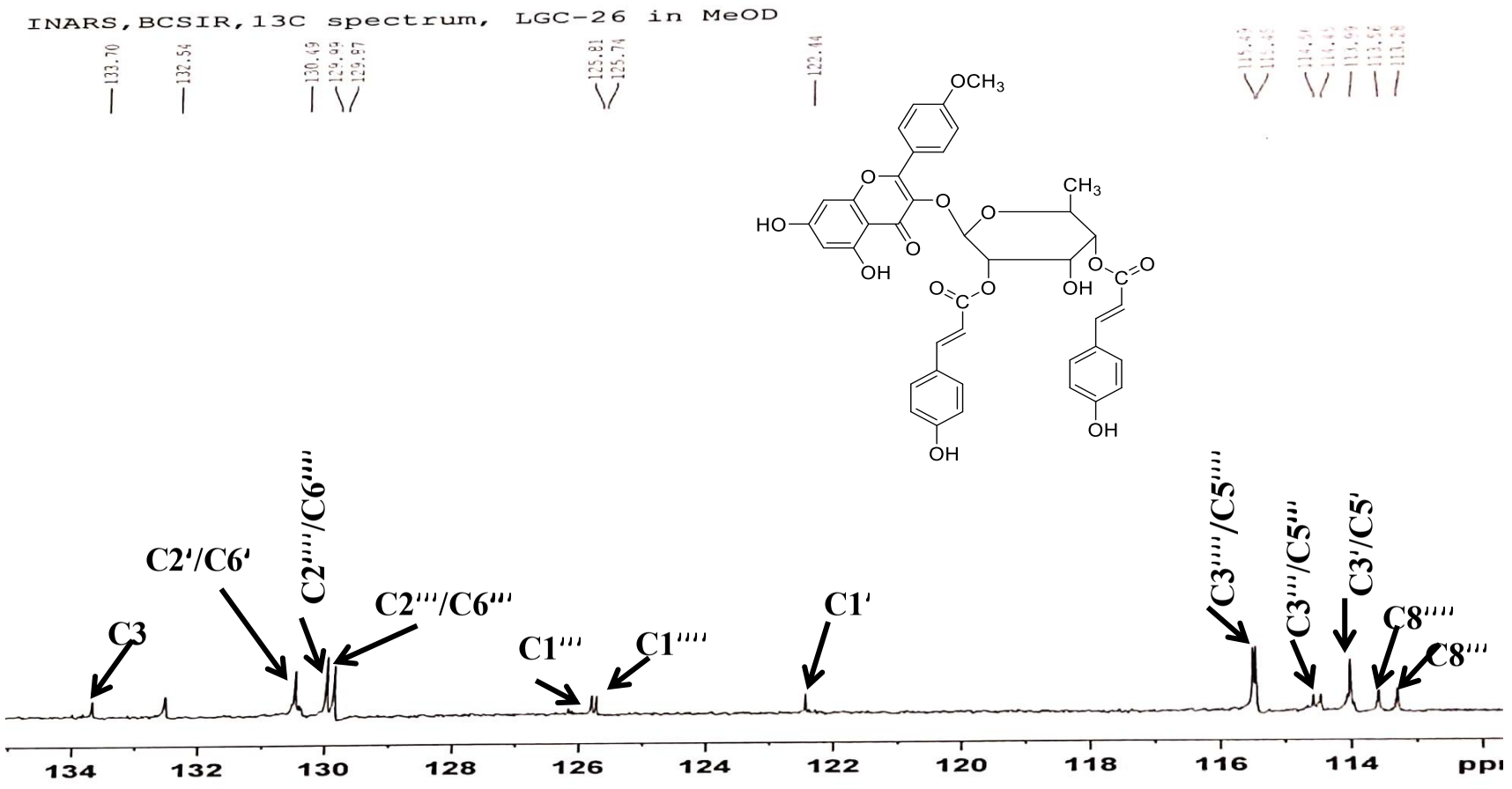


Figure 3.12: Partially expanded ^{13}C NMR (100 MHz; MeOD) spectrum of LGC-26 as 4′-*O*-methyl(2′′,4′′-di-*E*-*p*-coumaroyl)afzelin, 95.

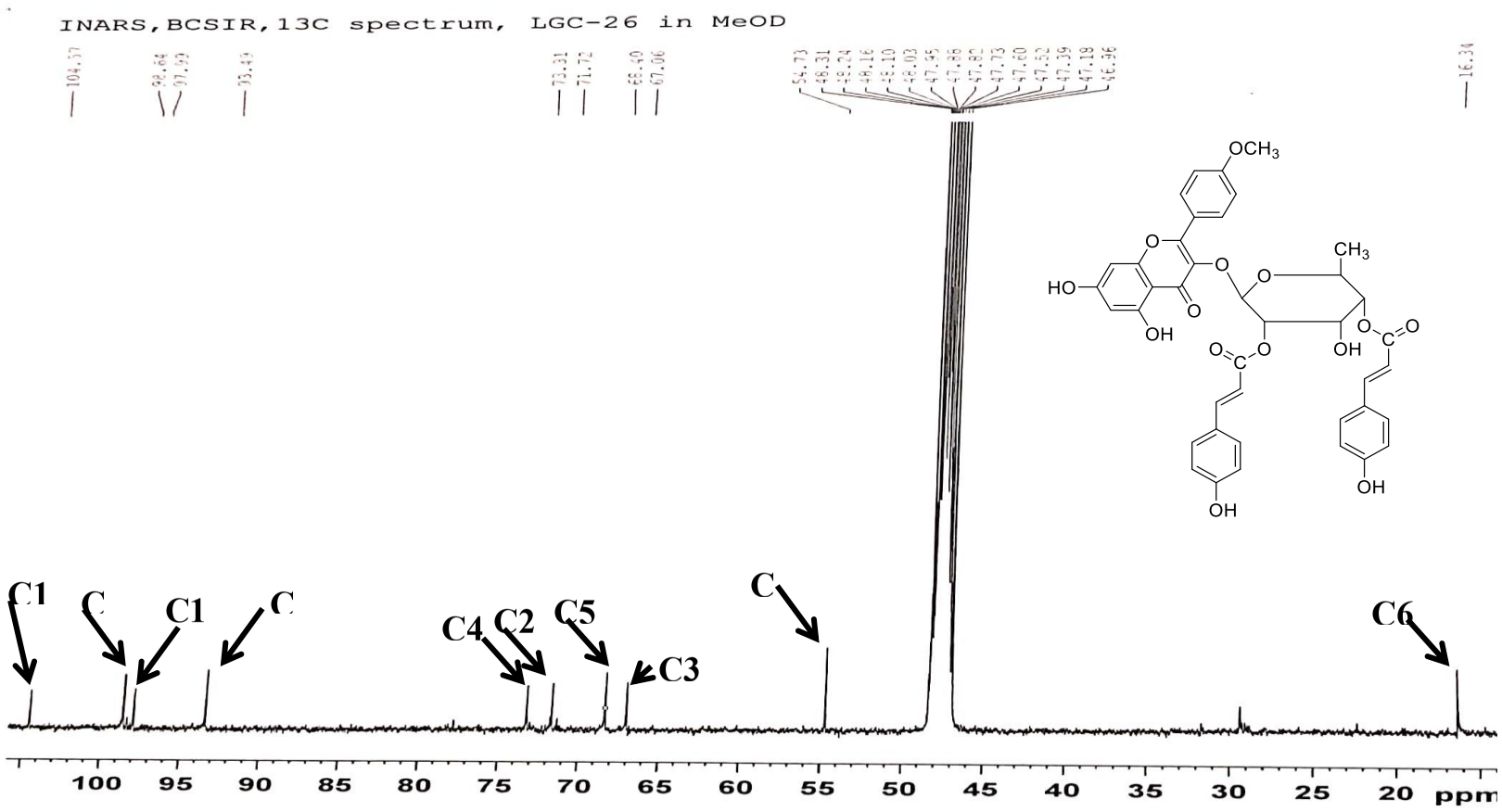


Figure 3.13: Partially expanded ^{13}C NMR (100 MHz; MeOD) spectrum of LGC-26 as 4′-*O*-methyl(2′′,4′′-di-*E*-*p*-coumaroyl)afzelin, 95.

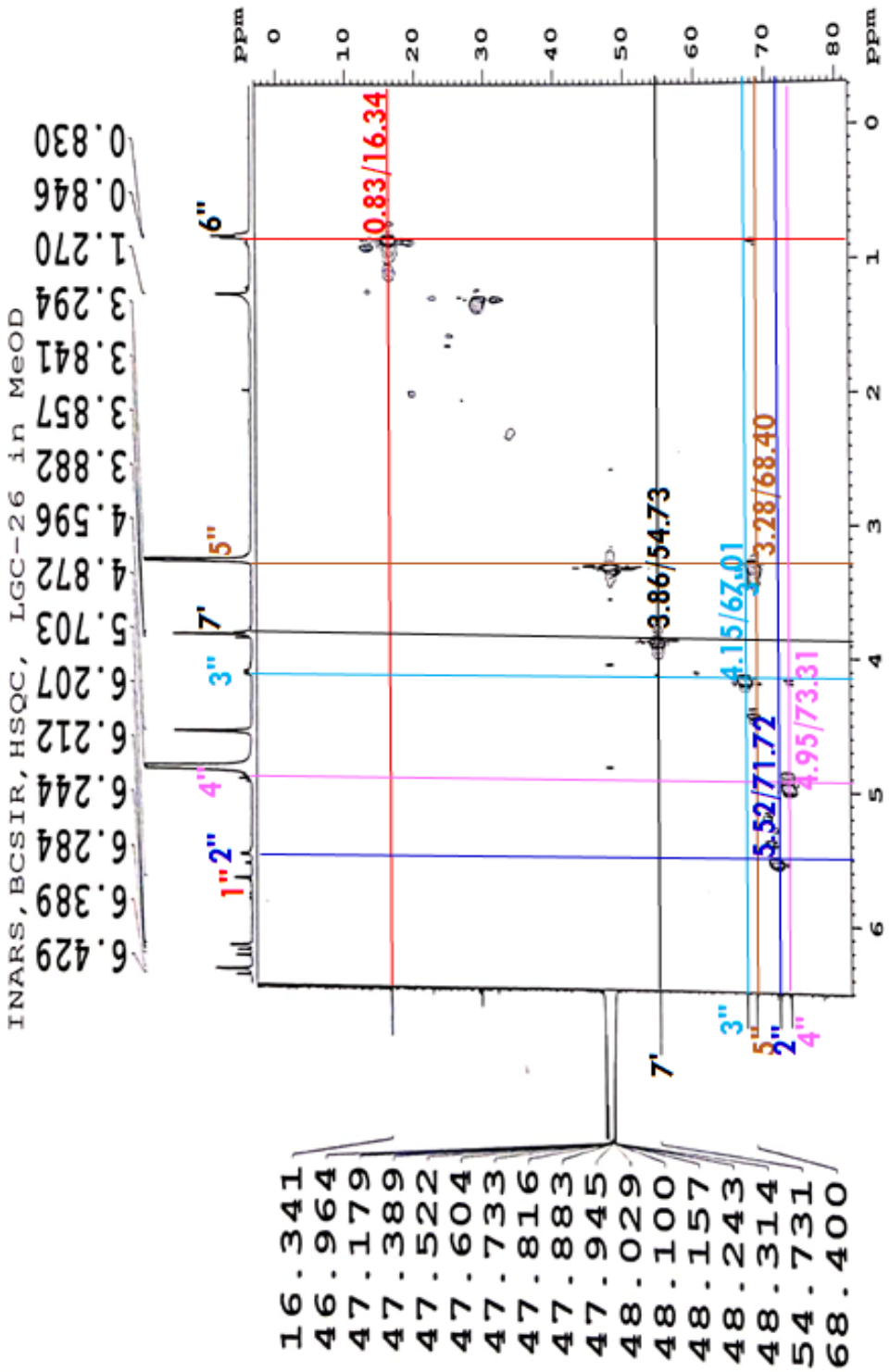


Figure 3.14: HSQC NMR spectrum of LGC-26 as 4′-*O*-methyl(2′′,4′′-di-*E*-*p*-coumaroyl)afzelin, 95 in MeOD.

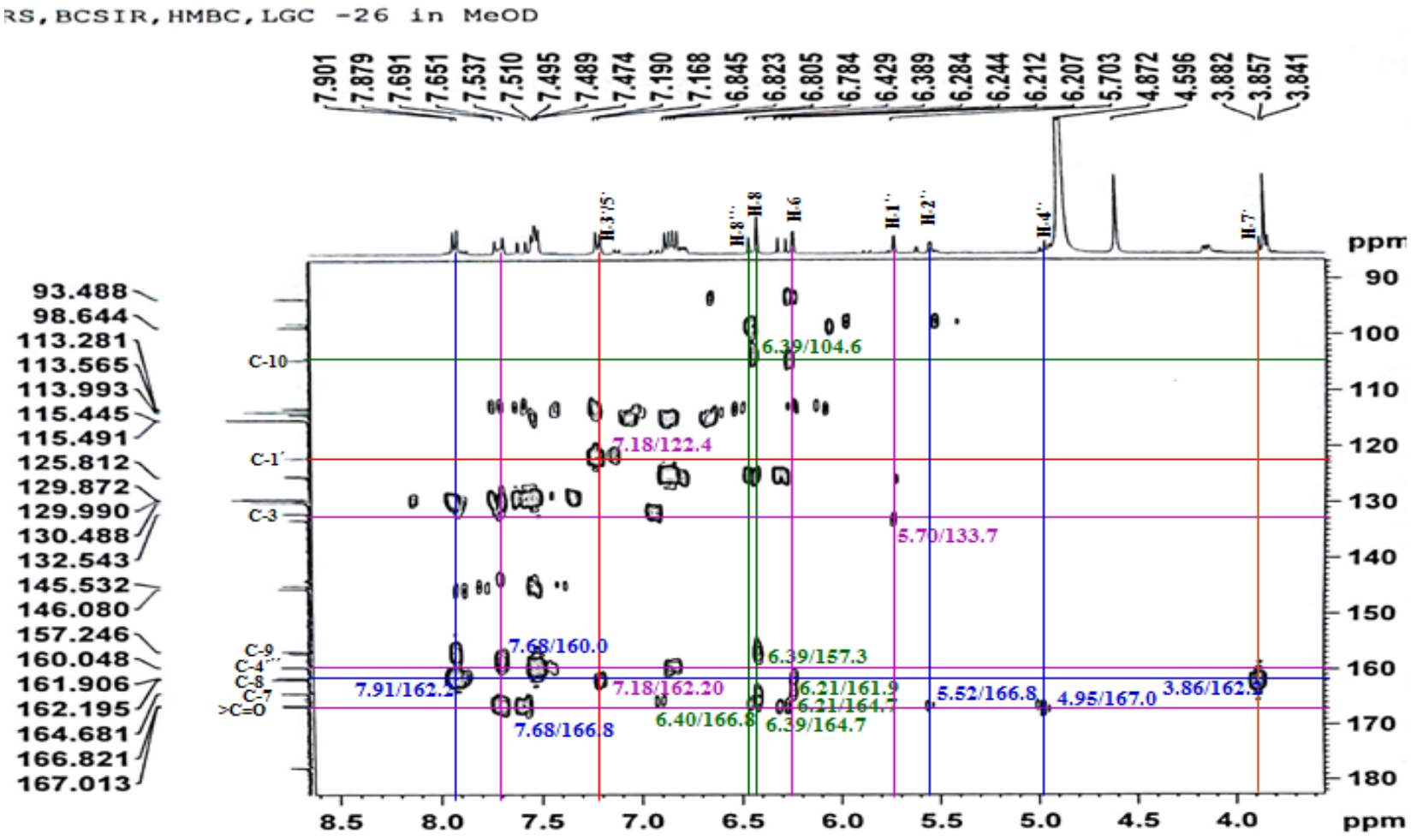


Figure 3.15: HMBC NMR spectrum of LGC-26 as 4′-*O*-methyl(2′′,4′′-di-*E*-*p*-coumaroyl)afzelin, 95 in MeOD.

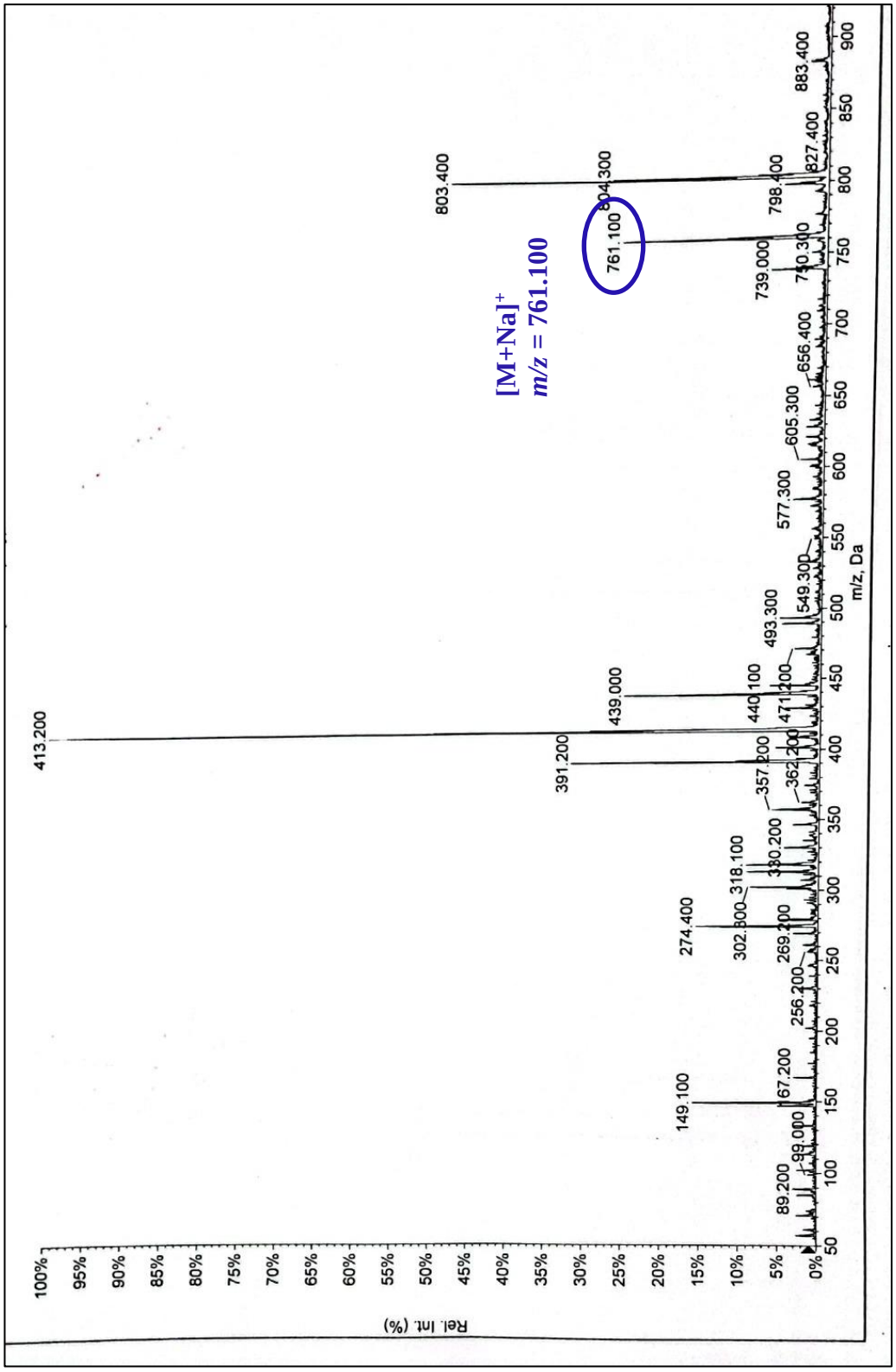


Figure 3.16: ESI Mass spectrum of LGC-26 as 4´-*O*-methyl(2´´,4´´-di-*E*-*p*-coumaroyl)afzelin, 95.

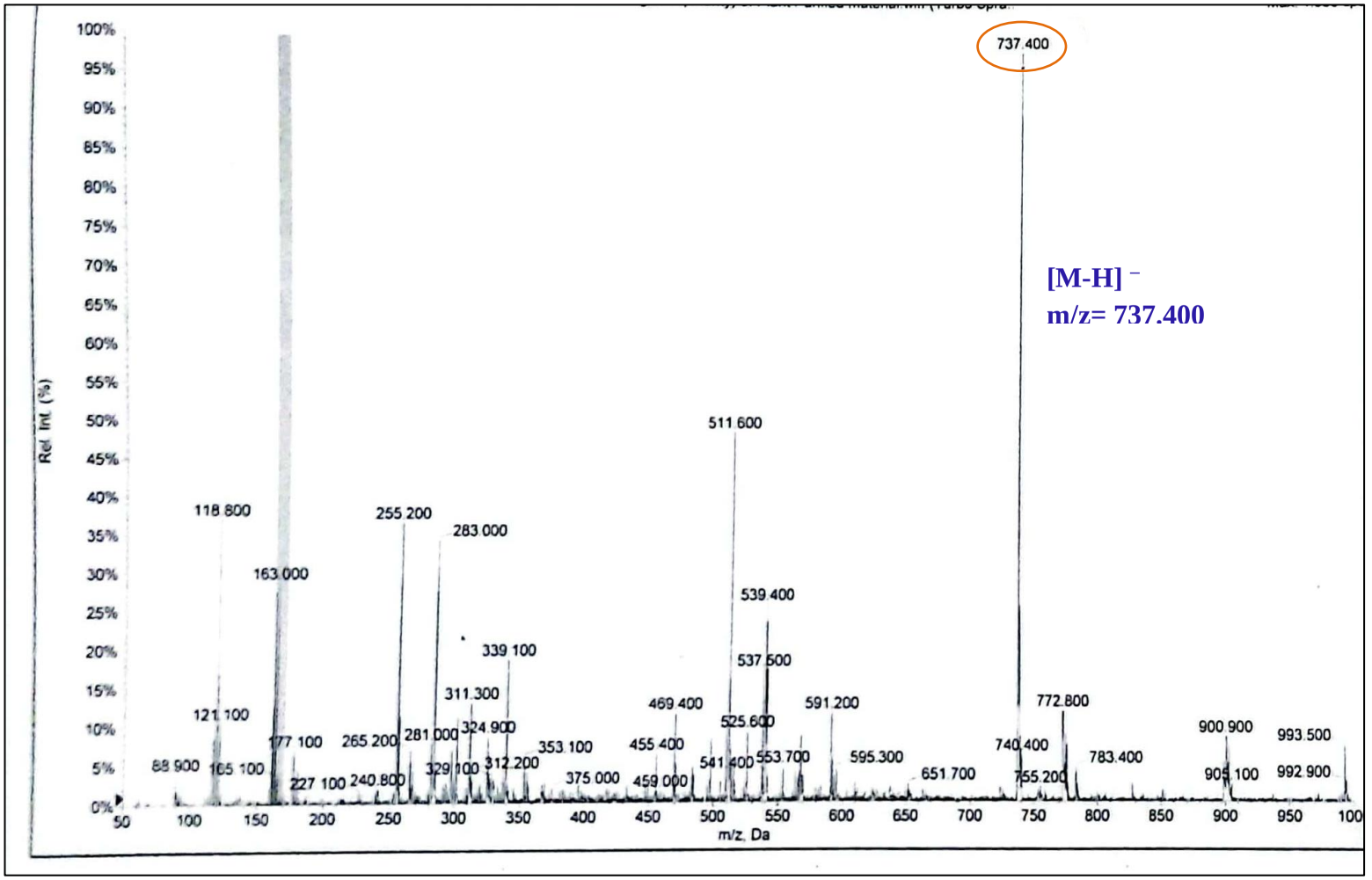


Figure 3.17: ESI Mass spectrum of LGC-26 as 4′-*O*-methyl(2′′,4′′-di-*E*-*p*-coumaroyl)afzelin, 95.

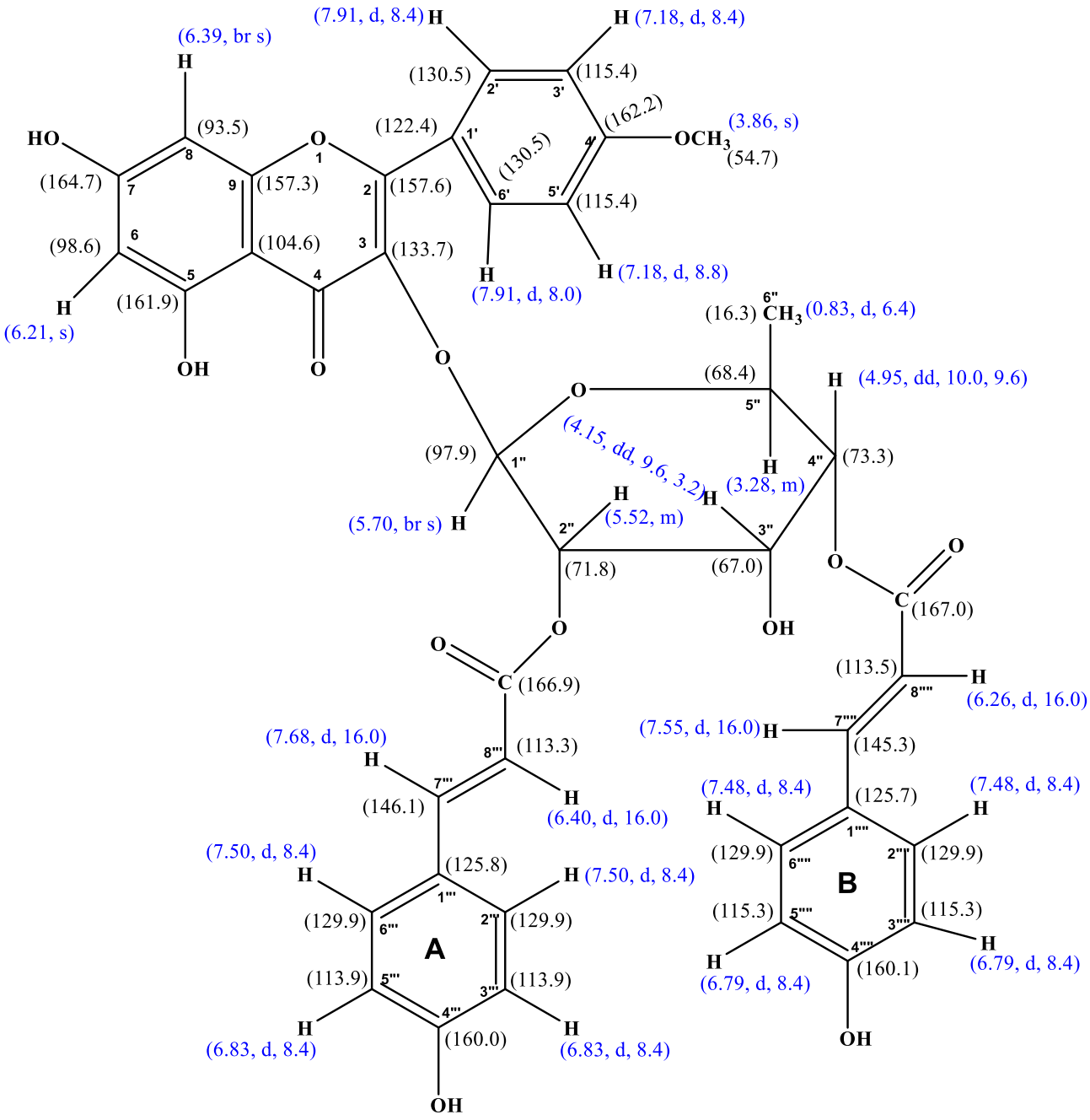


Figure 3.18: Complete ^{1}H and ^{13}C NMR spectral assignment of LGC-26 [4′-*O*-methyl-(2″,4″-di-*E*-*p*-coumaroyl) afzelin, 95]

3.1.2 Characterization of LGC-45-3 as quercetin-3-O-(2′′,4′′-di-E-p-coumaroyl)-α-L-rhamnopyranoside **(96)**

Compound LGC-45-3 was isolated as a yellowish white powder. Its ESIMS showed a pseudo-molecular ion peak [M+Na^+] at *m/z* 763.007 which indicated a molecular formula of $C_{39}H_{32}O_{15}$ (Figure 3.29 & 3.30).

The ^{1}H NMR (400 MHz) spectra of LGC-26 (**95**) and LGC-45-3 (**96**) were almost identical, which suggested that LGC-45-3 is a derivative of the former one (LGC-26). The B-ring protons in LGC-26 were evident as an AA′ and BB′ pattern, whereas, in LGC-45-3 the trisubstituted B-ring protons appeared as an *ortho* (δ 7.01, *J* = 8.0 Hz), *ortho-meta* (δ 7.33, *J* = 8.0, 2.0 Hz) and *meta* (δ 7.40, *J* = 2.0 Hz) coupled protons. In addition, the methoxyl group signal observed for LGC-26 could not be seen in ^{1}H NMR spectrum of LGC-45-3. This demonstrated that the methoxyl group was replaced by a hydroxyl group in the latter one. The presence of only B-ring proton signals and lack of methoxyl group resonance also ascertained that the remaining carbon has hydroxyl moiety (Figure 3.21 & 3.31).

The ^{1}H NMR (400 MHz) spectrum demonstrated signals assignable to a 4′,5′-dihydroquercetin unit, a rhamnopyranosyl moiety and two *trans-p*-coumaroyl units at C-2″ and at C-4″ of the rhamnopyranosyl unit. The ^{1}H NMR spectrum displayed resonances for the 4′,5′-dihydroquercetin moiety as four doublets at δ 6.22 (1H, *J* = 2.0 Hz, H-6), 6.40 (1H, *J* = 2.0 Hz, H-8), 7.01 (1H, *J* = 8.0 Hz, H-3′), and 7.40 (1H, *J* = 2.0 Hz, H-6′) and a double-doubles at δ 7.33 (1H, *J* = 8.0, 2.0 Hz, H-2′) (Table 3.2, Figure 3.31).

In addition, a rhamnopyranosyl moiety was identified by signals at δ 5.77 (1H, s, H-1″), 5.57 (1H, br. s, H-2″), 4.21 (1H, dd, *J* = 9.6, 3.6 Hz, H-3″), 4.95 (1H, dd, *J* = 10.0, 9.6 Hz, H-4″), 3.32 (m, H-5″), and 0.87 (d, *J* = 6.4 Hz, H-6″) (Table 3.2, Figure 3.22). Two p-coumaroyl units with trans-configuration were clearly evident from signals at δ 7.62 (1H, d, *J* = 16.0 Hz, H-7‴′), 6.34 (1H, d, *J* = 15.8 Hz, H-8‴′), 7.71 (1H, d, *J* = 16 Hz, H-7‴), and 6.45 (1H, d, *J* = 15.8 Hz, H-8‴) as demonstrated in table 3.2 and figure 3.23.

The COSY NMR spectrum displayed the expected correlations between H-2" and H-3" at δ 5.57 and 4.21, between H-3" and H-4" at δ 4.21 and 4.95, between H-4" and H-5"

at δ 5.00 and 3.32 and between H-5" and H_3-6" at δ 3.32 and 0.87. These five correlations demonstrated the presence of a rhamnopyranosyl moiety in LGC-45-3 (Figure 3.22). Furthermore, COSY correlations between H-7"" and H- 8"" at δ 7.62 and 6.74, between H-7"' and H-8"' at δ 7.71 and 6.45, between H-3"'/5"' and H-2"'/6"' at δ 7.52 and 6.82, between H-3""/5"" and H-2""/6"" at δ 7.58 and 6.86 confirmed the presence of two coumaroyl units in LGC-45-3 (Figure 3.20 & 3.27).

The assignment for LGC-45-3 (**96**) has been completed depending on its ^{1}H NMR and ^{13}C NMR spectral data and comparison with the closely related compound **95** (Page 76). Thus, LGC-45-3 was identified as quercetin-3-*O*-(2′′,4′′-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside. It can also be named as 5′-hydroxyl-(2″,4″-di-*E*-*p*-coumaroyl) afzelin (Figure 3.31). Although the compound was previously been reported from *Machilus litseifolia* (Li *et al*., 2019) and *Mammea longifolia* (Rao *et al.*, 2002), this is the first report of its occurance from *Litsea* species.

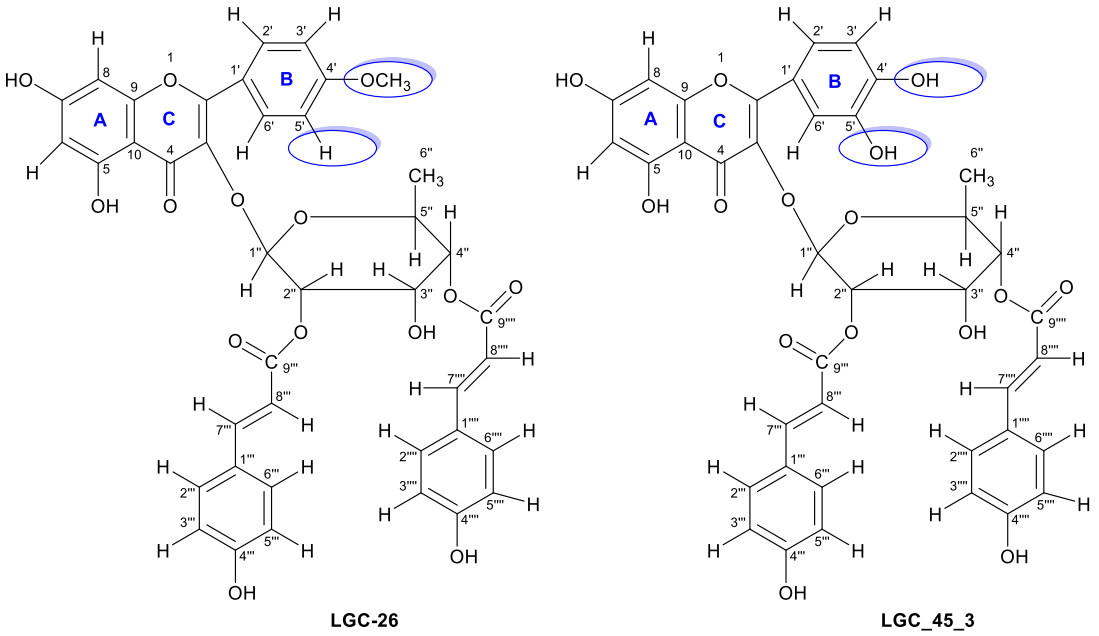


Figure 3.19: Comparison of the structures; LGC-26 (95) and its derivative LGC-45-3 (96)

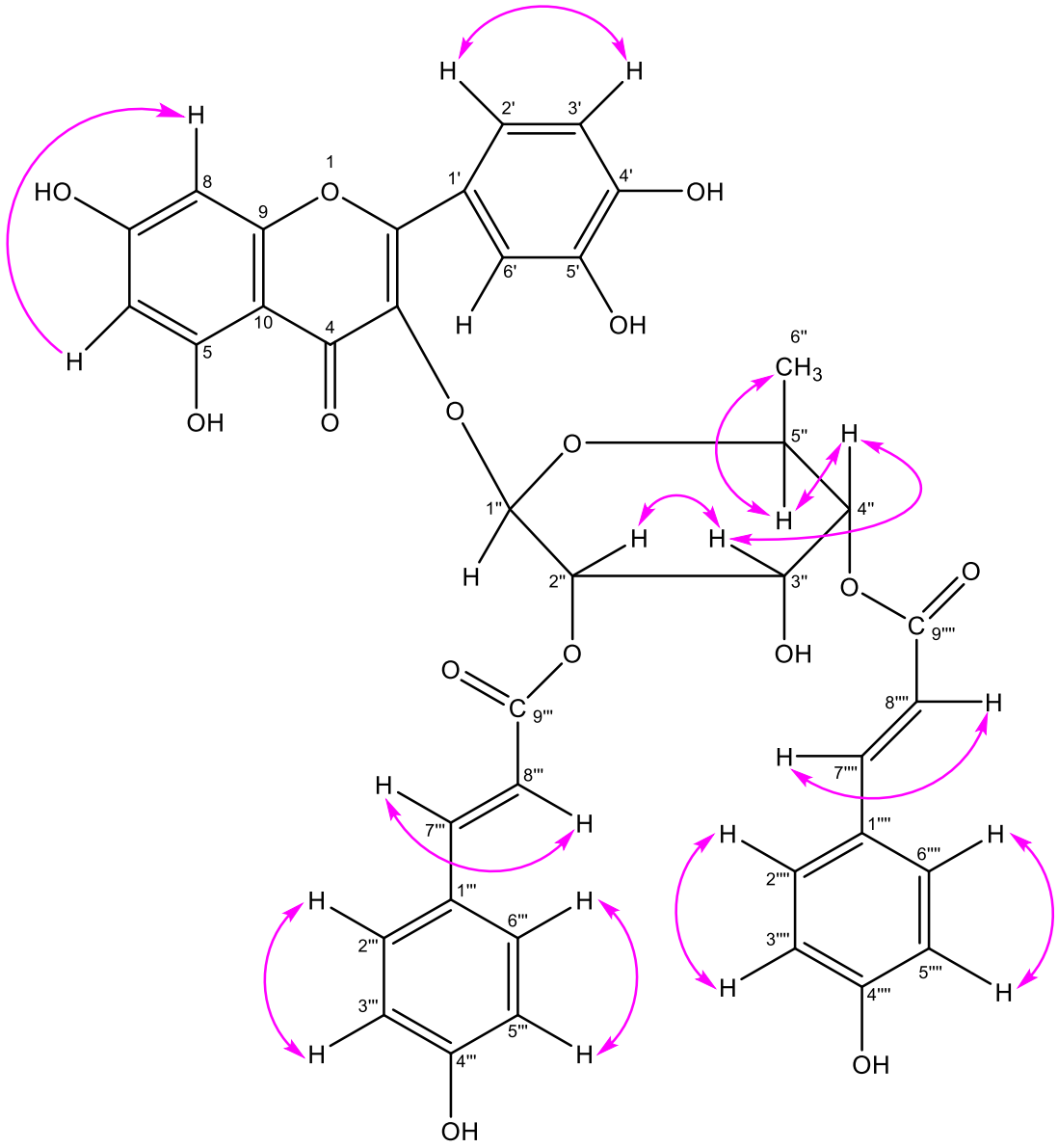


Figure 3.20: Key COSY correlations observed in LGC-45-3 [Quercetin3-*O*-(2´´,4´´-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside (96)

Table 3.2: Comparison between the ^{1}H NMR (400 MHz; MeOD) and ^{13}C NMR (100 MHz; MeOD) spectral data of LGC-45-3 (96) and LGC-26 (95)

*LGC-45-3: Quercetin3-O-(2′′,4′′-di-E-p-coumaroyl)-α-L-rhamnopyranoside (**96**)*			*LGC-26: 4′-O-Methyl (2′′, 4′′-di-E-p-coumaroyl) afzelin (**95**)*		
Position	**δ_C**	**δ_H, mult (*J* in Hz)**	**Position**	**δ_C**	**δ_H, mult (*J* in Hz)**
2	158.06, C		2	157.61, C	
3	132.41, C		3	133.70, C	
4	177.78, C		4	177.9, C	
5	176.83, C		5	161.91, C	
6	99.00, CH	6.22, d (2.0)	6	98.64, CH	6.21, s
7	-		7	164.68, C	
8	93.55, CH	6.40, d (2.0)	8	93.49, CH	6.39, br.s
9	157.25, C		9	157.25, C	
10	104.30, C		10	104.57, C	
1′	121.43, C		1′	122.44, C	
2′	148.69, CH	7.33, dd (8.0, 2.0)	2′/6′	130.49, CH	7.91, d (8.4)
3′	147.92, CH	7.01, d (8.0)	3′/5′	113.99, CH	7.18, d (8.4)
4′	-		4′	162.20, C	
6′	-	7.40, d (2.0)	-		
			7′	54.73, CH3	3.86, s
Rha					
1′′	99.04, CH	5.77, s	1′′	97.99, CH	5.70, br.s
2′′	71.74, CH	5.57, br.s	2′′	71.72, CH	5.52
3′′	67.08, CH	4.21, dd (9.6, 3.6)	3′′	67.06, CH	4.50
4′′	73.40, CH	5.00, dd (10.0, 9.6)	4′′	73.31, CH	4.95, dd (10.0, 9.6)
5′′	68.37, CH	3.32, m	5′′	68.40, CH	3.28
6′′	16.30, CH3	0.87, d (6.4)	6′′	16.34, CH3	0.83, d (6.4)
Coum-A					
1′′′	125.83, C		1′′′	125.81, C	
2′′′/6′′′	129.97, CH	7.58, d (8.8)	2′′′/6′′′	129.87, CH	7.50, d (8.4)
3′′′/5′′′	115.43, CH	6.86, d (8.8)	3′′′/5′′′	114.45, CH	6.83, d (8.4)
4′′′	159.97, C		4′′′	160.00, C	
7′′′	146.04, CH	7.71, d (16.0)	7′′′	146.08, CH	7.68, d (16.0)
8′′′	113.31, CH	6.45, d (15.8)	8′′′	113.28, CH	6.40, d (16.0)
>C=O	166.85, C		>C=O	166.82, C	
Coum-B					
1′′′′	125.83, C		1′′′′	125.74, C	
2′′′′/6′′′′	129.97, CH	7.52, d (8.4)	2′′′′/6′′′′	129.99, CH	7.48, d (8.4)
3′′′′/5′′′′	115.43, CH	6.82, d (8.4)	3′′′′/5′′′′	115.45, CH	6.79, d (8.4)
4′′′′	161.87, C		4′′′′	160.05, C	
7′′′′	145.64, CH	7.62, d (16.0)	7′′′′	145.53, CH	7.55, d (16.0)
8′′′′	113.59, CH	6.34, d (15.8)	8′′′′	113.56, CH	6.26, d (16.0)
>C=O	nd		>C=O	167.01, C	

Figure 3.21: Partially expanded ^{1}H NMR (400 MHz; MeOD) spectrum of LGC-45-3 as quercetin3-*O*-(2′′,4′′-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside (96).

Figure 3.22: Partially expanded ^{1}H NMR (400 MHz; MeOD) spectrum of LGC-45-3 as quercetin3-*O*-(2'',4''-di-*E*-*p*-coumaroyl)-*α*-L-rhamnopyranoside (96).

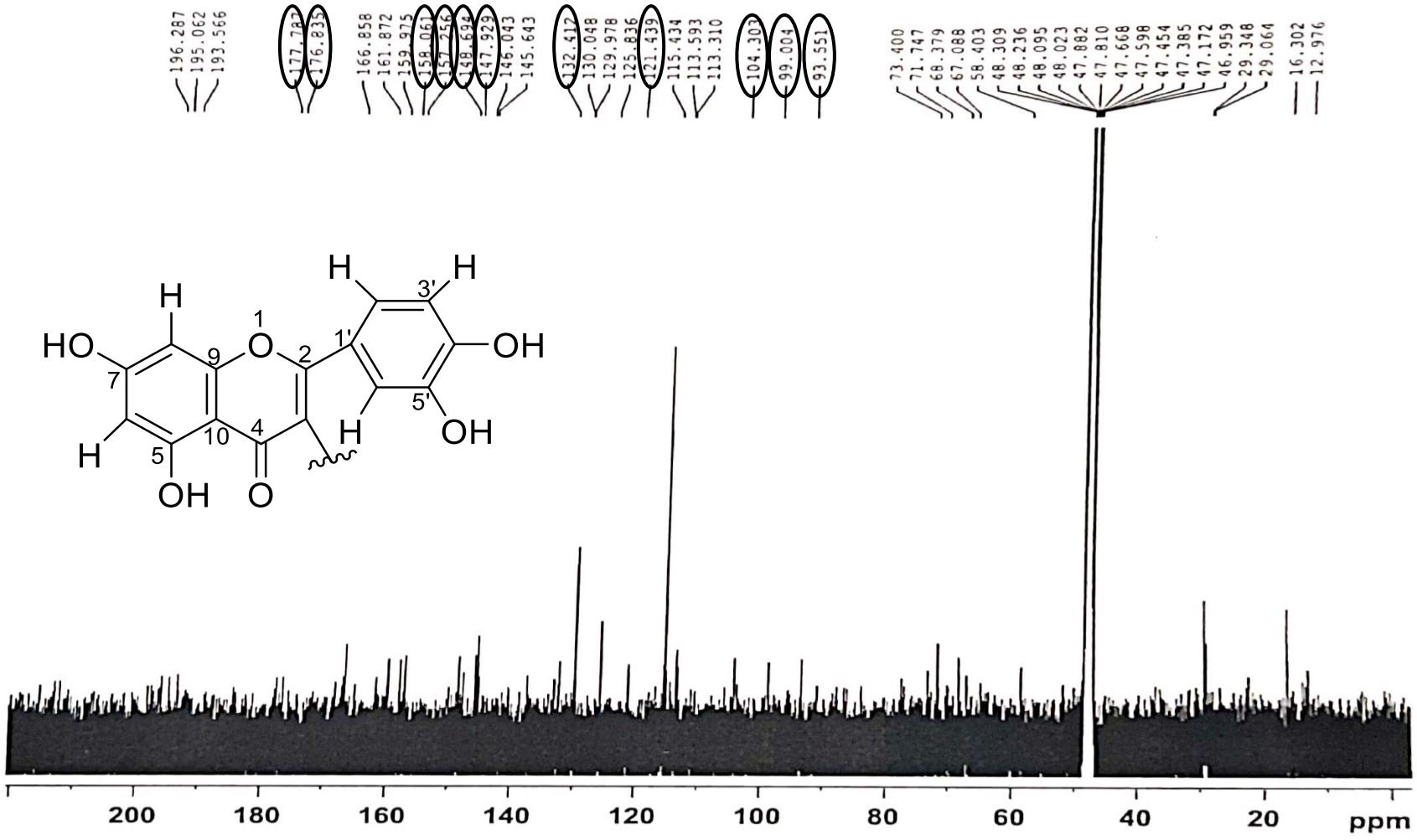


Figure 3.23: Partially expanded ^{13}C NMR (100 MHz; MeOD) spectrum of LGC-45-3 as quercetin3-*O*-(2´´,4´´-di-*E*-*p*-coumaroyl)-*α*-L-rhamnopyranoside (96).

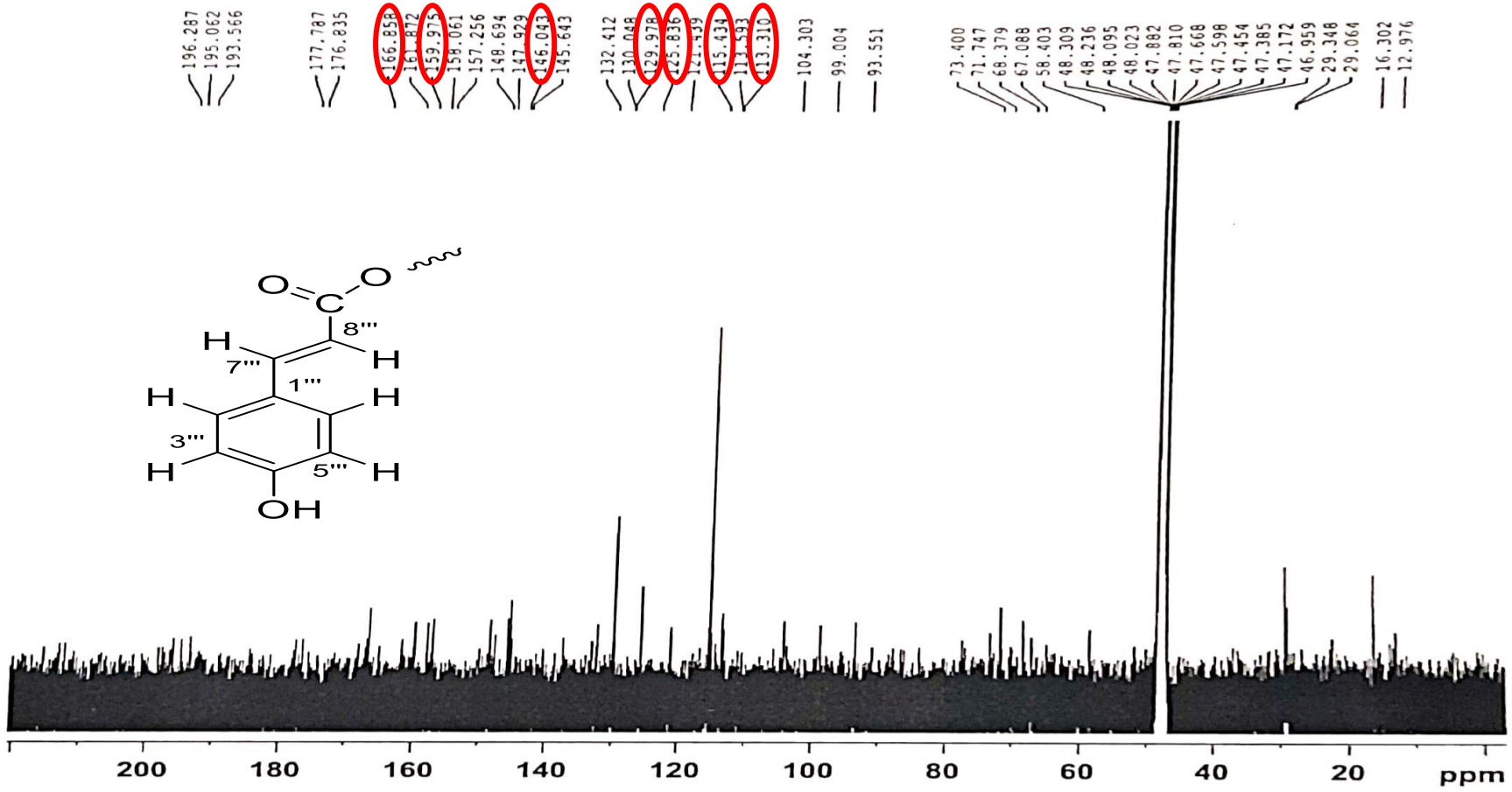


Figure 3.24: Partially expanded ^{13}C NMR (100 MHz; MeOD) spectrum of LGC-45-3 as quercetin3-*O*-(2´´,4´´-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside (96).

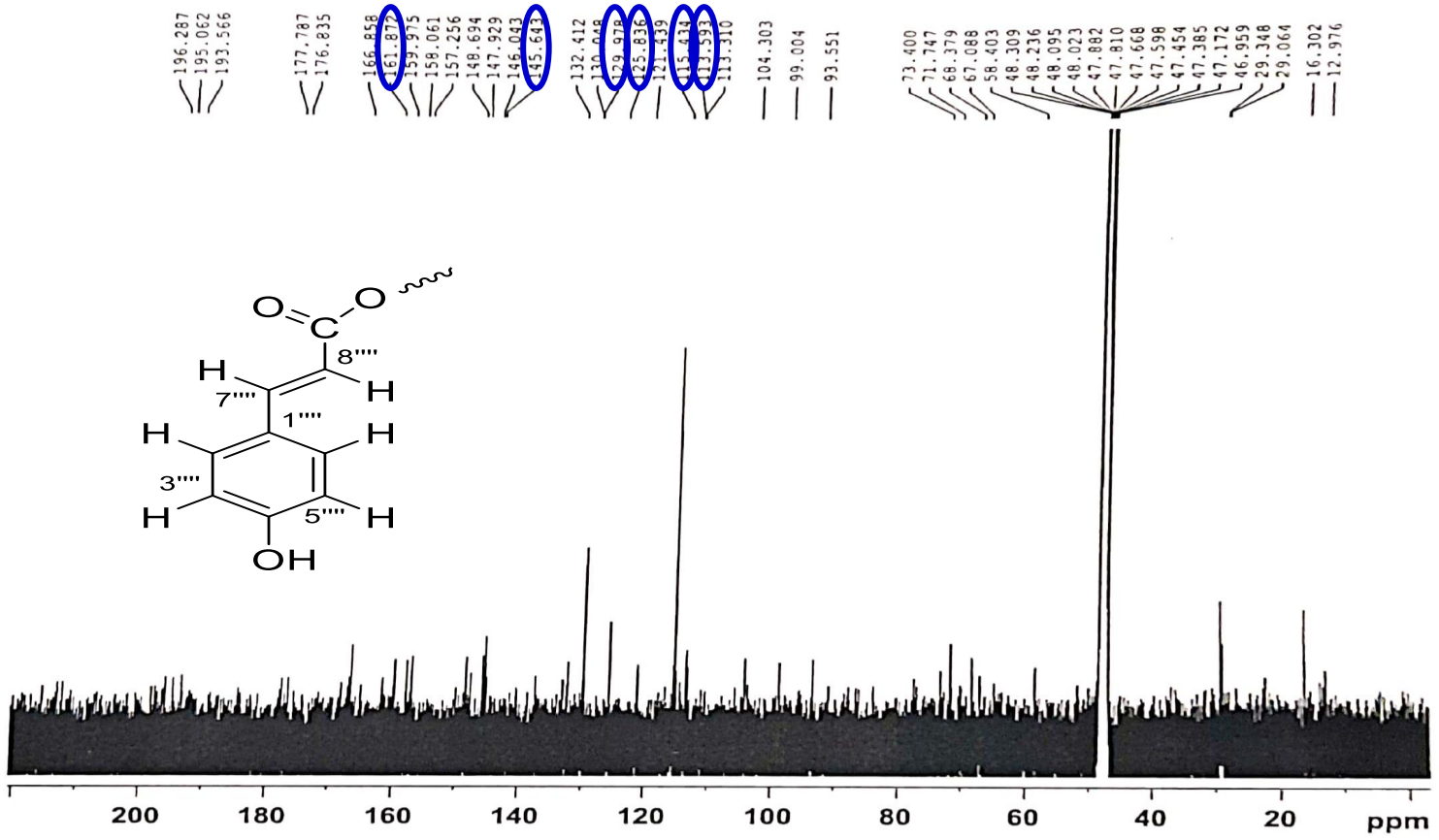


Figure 3.25: Partially expanded ^{13}C NMR (100 MHz; MeOD) spectrum of LGC-45-3 as quercetin3-*O*-(2´´,4´´-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside (96).

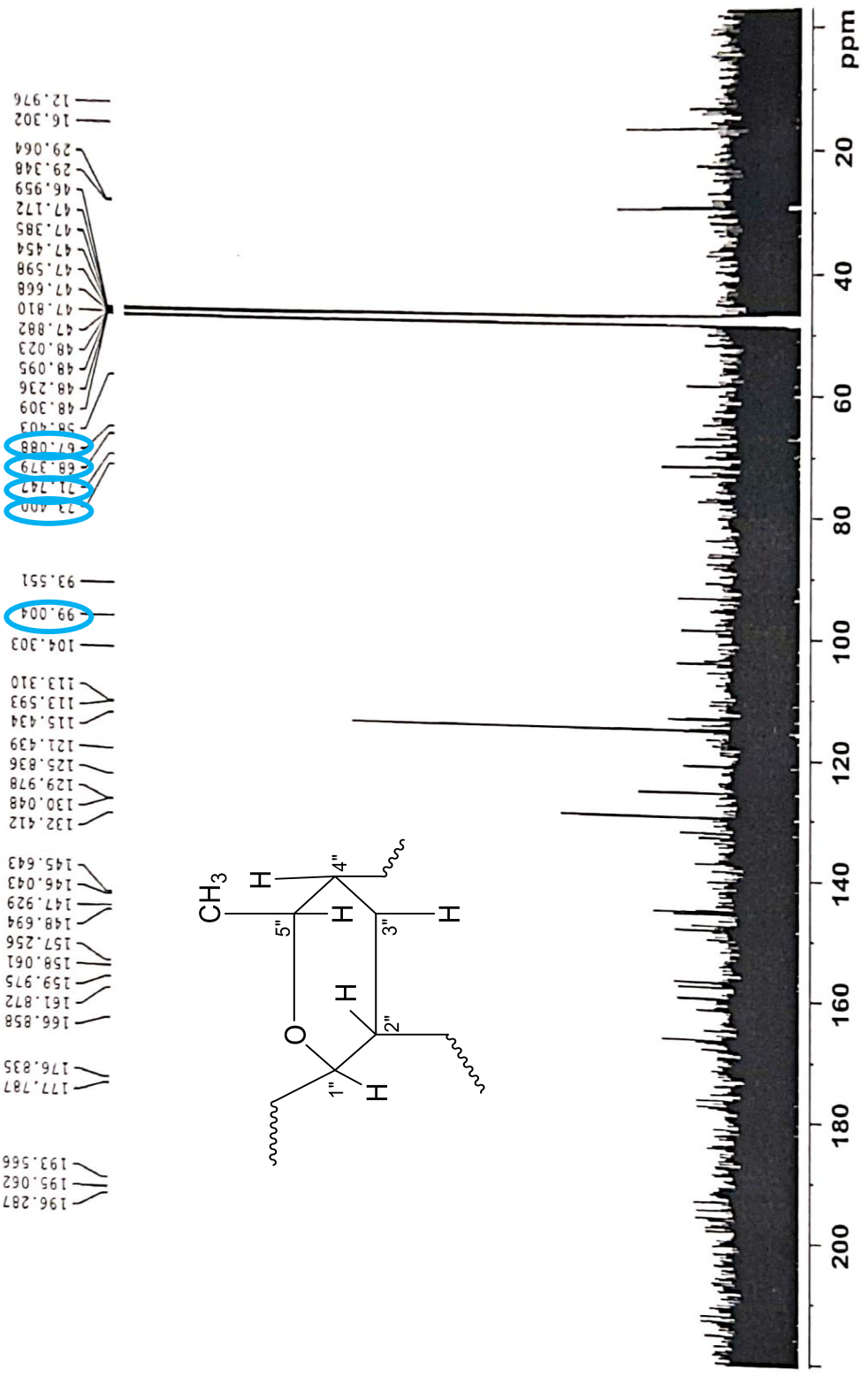


Figure 3.26: Partially expanded ^{13}C NMR (100 MHz; MeOD) spectrum of LGC-45-3 as quercetin3-*O*-(2′′,4′′-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside (96).

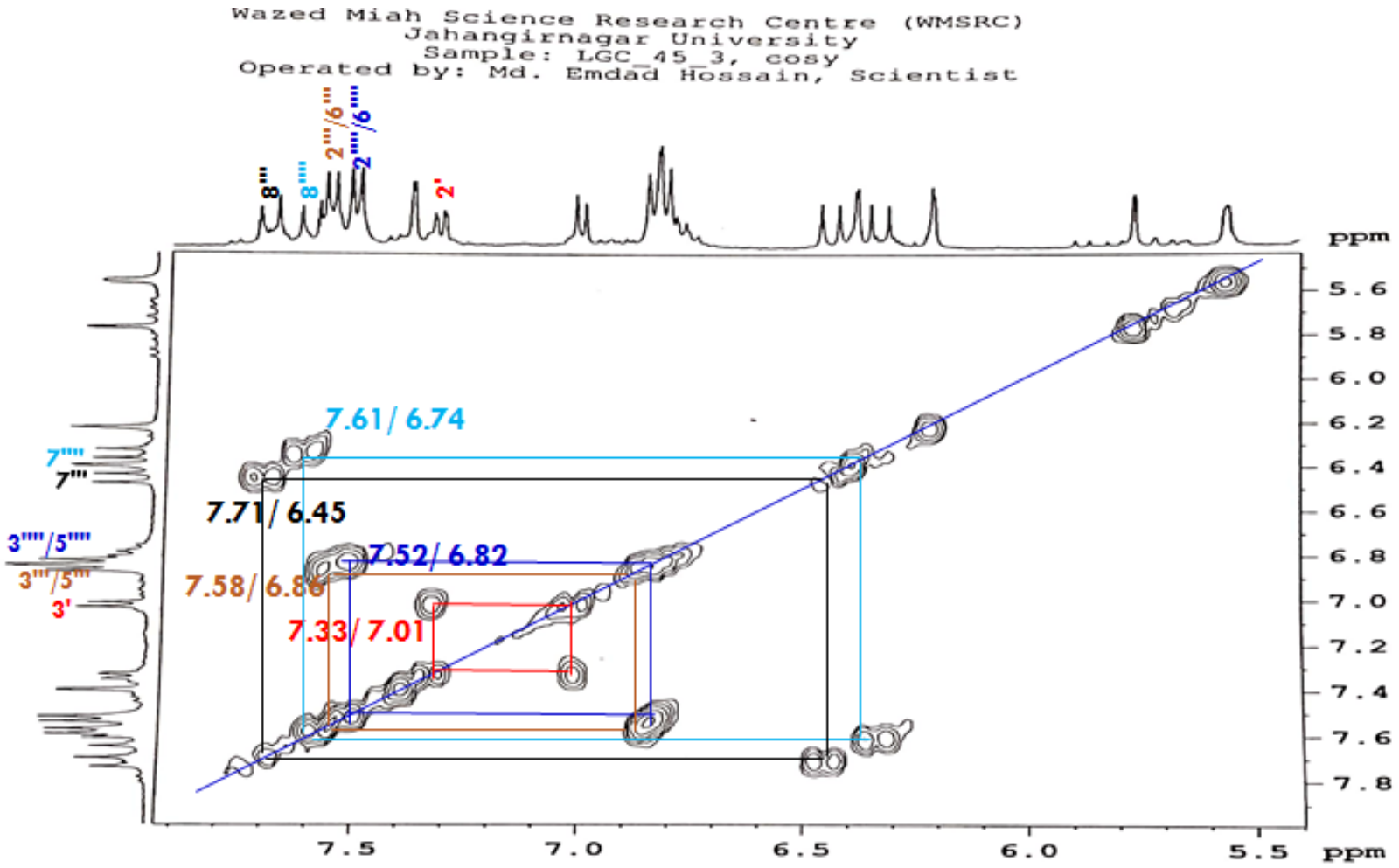


Figure 3.27: COSY NMR spectrum of LGC-45-3 as quercetin3-*O*-(2′′,4′′-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside (96) in MeOD.

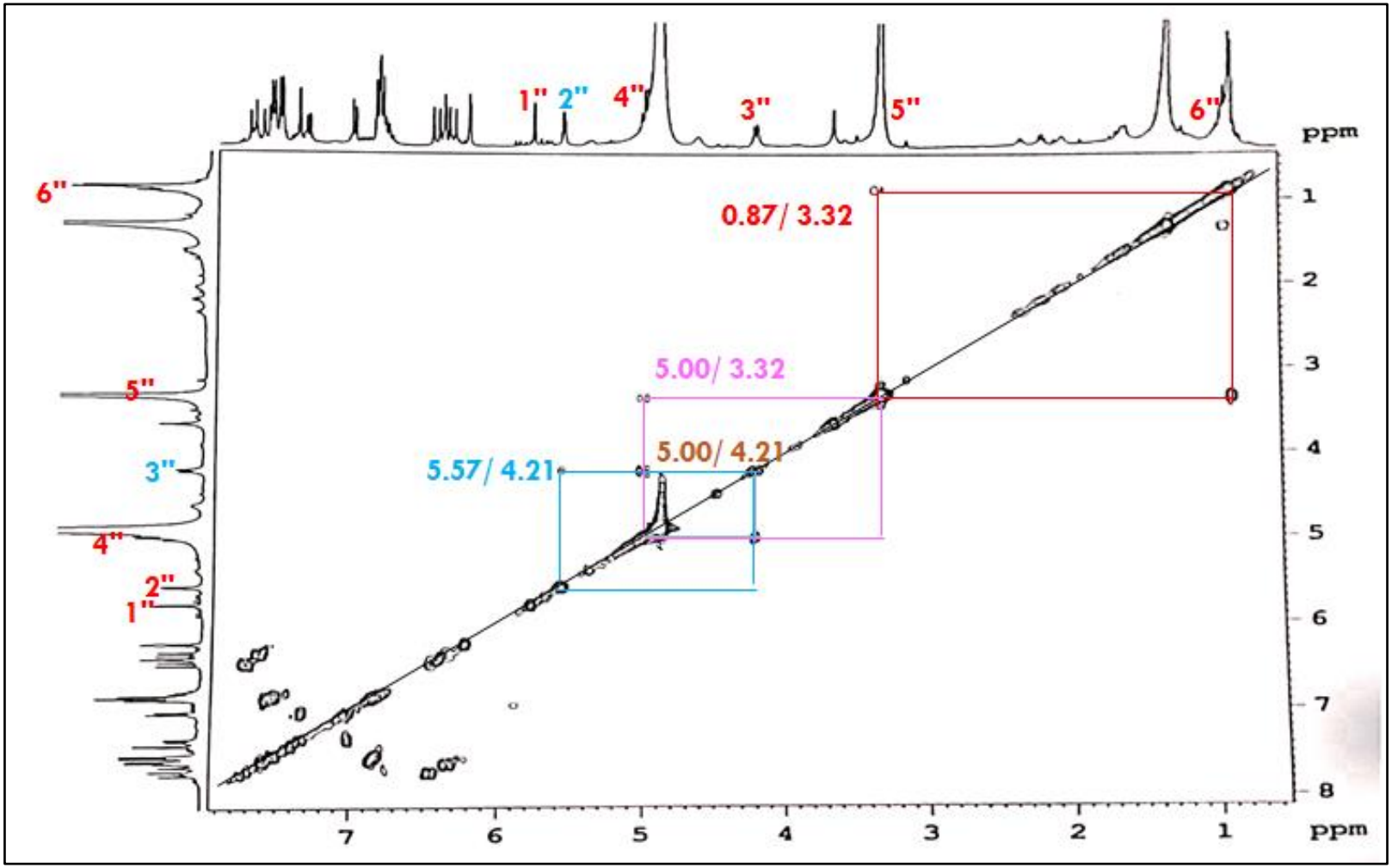


Figure 3.28: COSY NMR spectrum of LGC-45-3 as quercetin3-*O*-(2´´,4´´-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside (96) in MeOD.

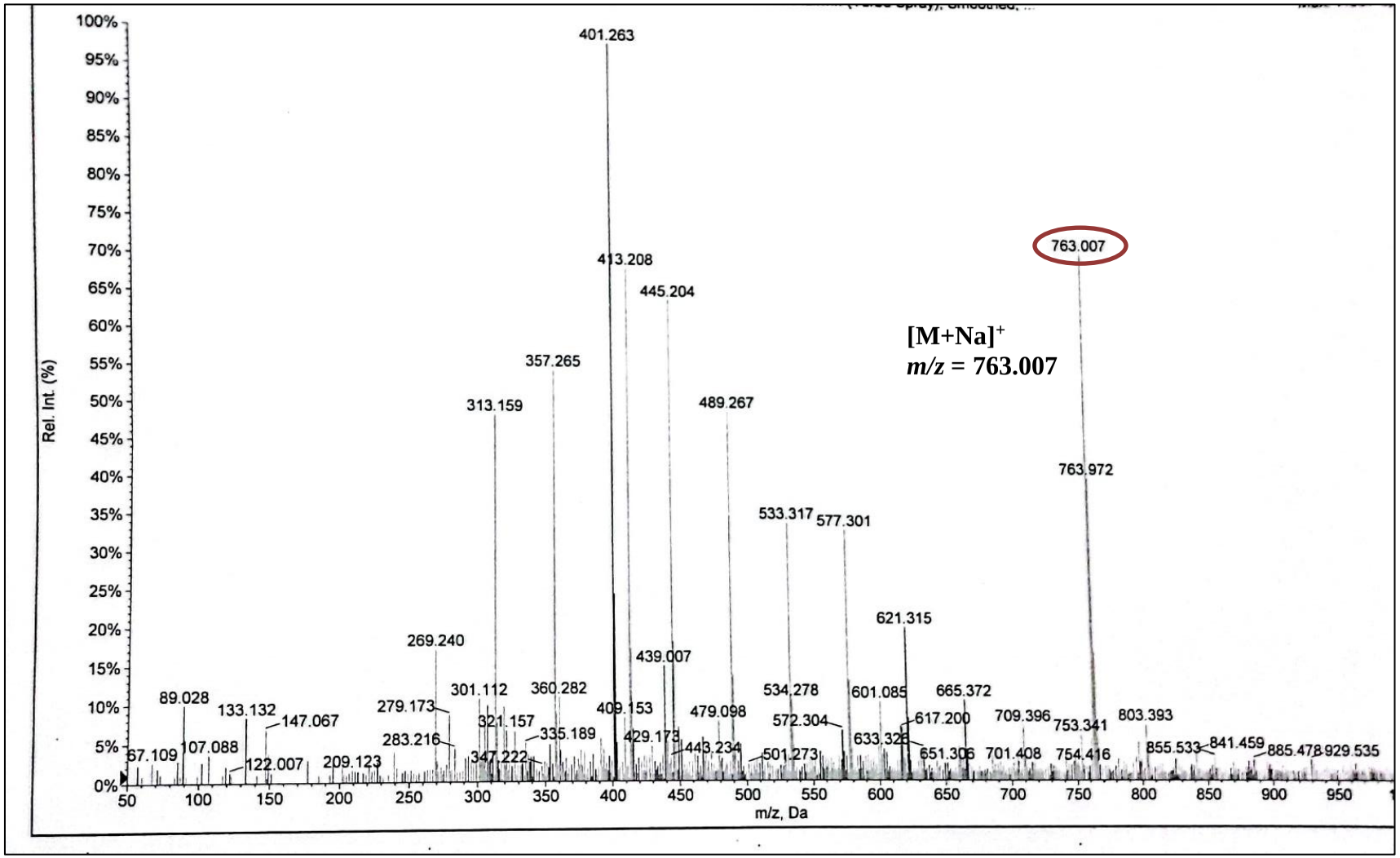


Figure 3.29: Mass spectrum of LGC-45-3 as quercetin3-*O*-(2´´,4´´-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside (96).

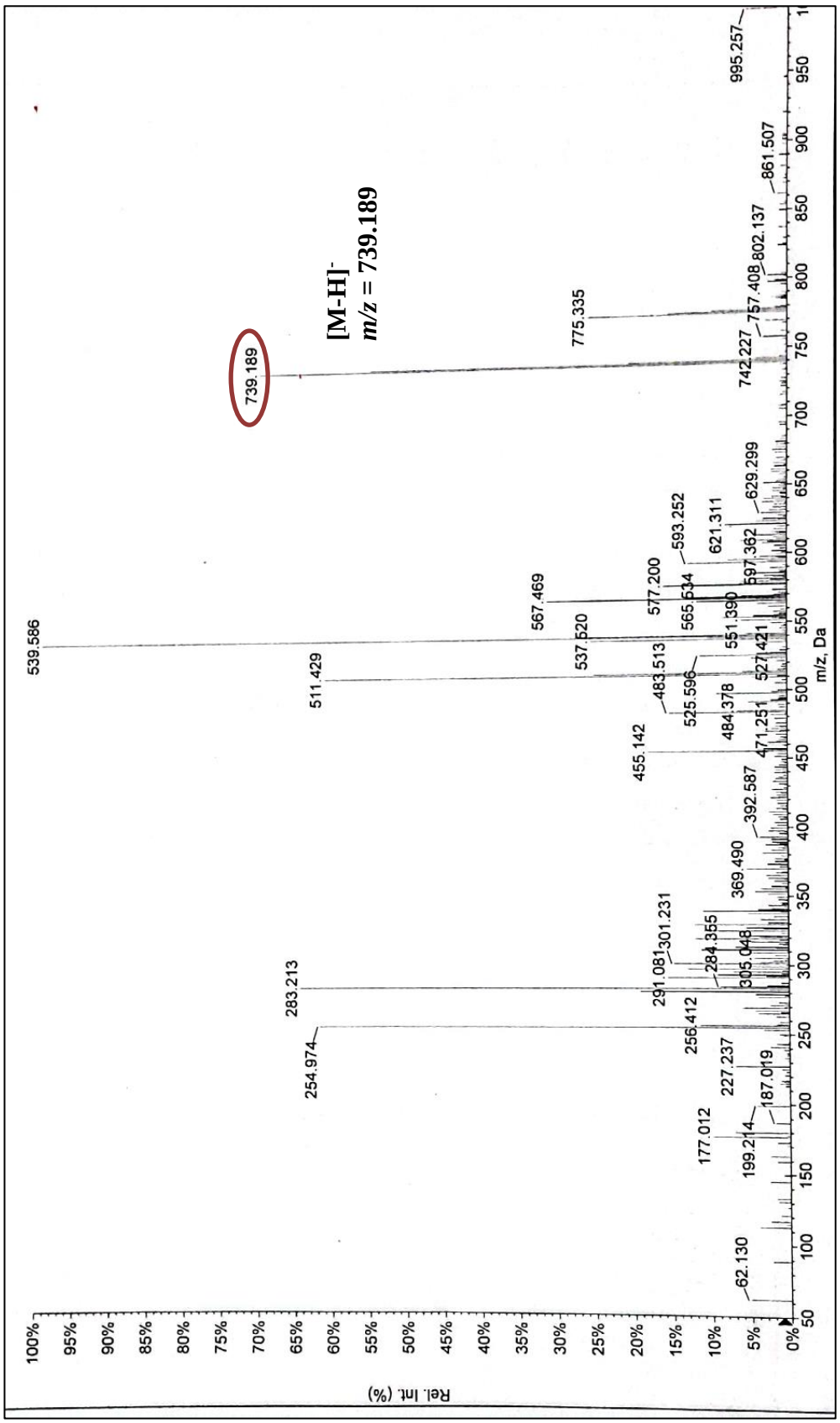


Figure 3.30: Mass spectrum of LGC-45-3 as quercetin3-*O*-(2′′,4′′-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside (96).

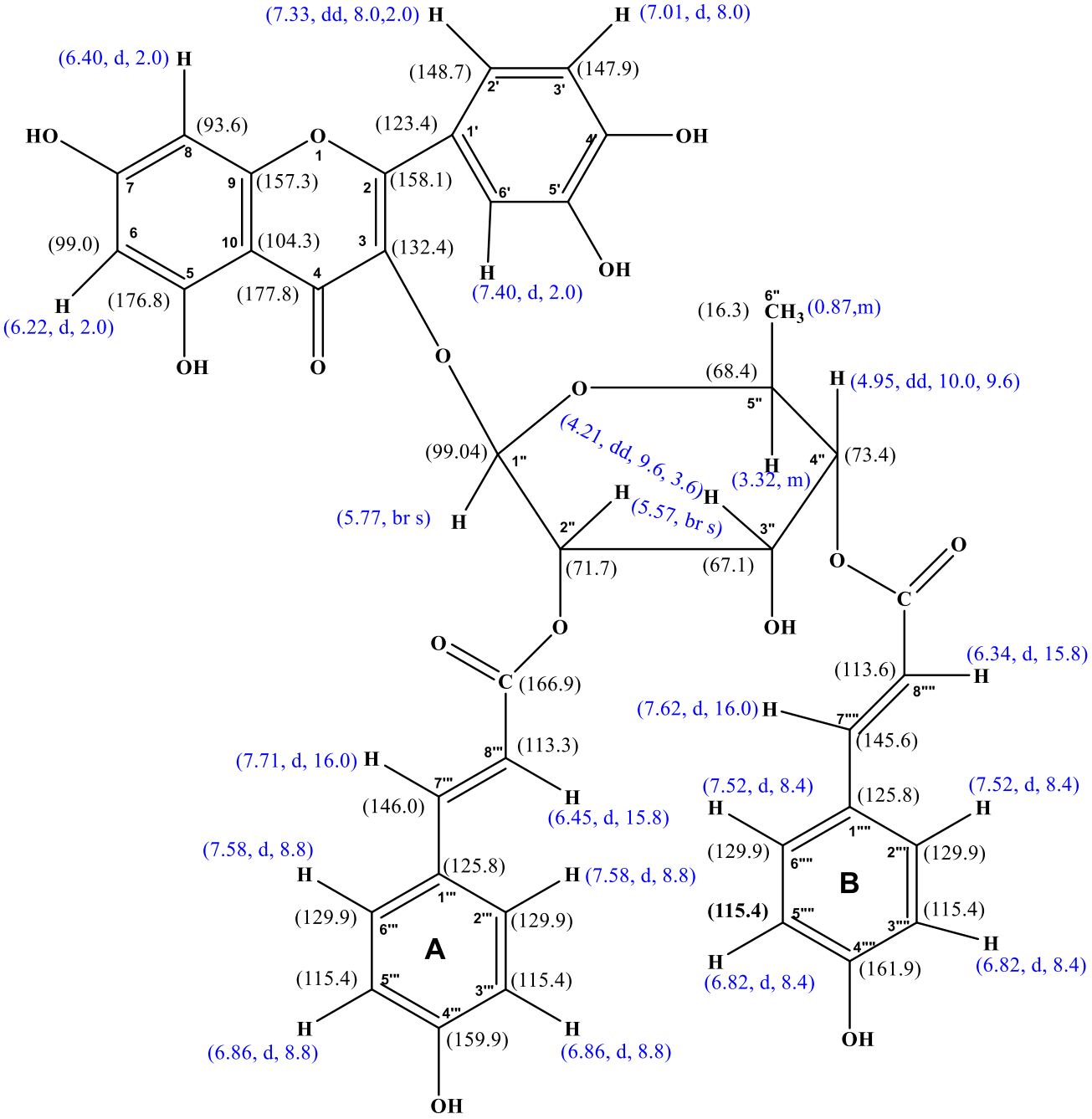


Figure 3.31: Complete ^{1}H and ^{13}C NMR spectral assignment of LGC-45-3 [Quercetin3-*O*-(2´´,4´´-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside (96)

3.2 Compounds isolated, purified and characterized from *L. monopetala*

A total of five compounds were isolated and purified from the leaf extract of *L. monopetala* by column chromatography following Sephadex LH-20 column by size exclusion chromatography. On the basis of ^{1}H and ^{13}C NMR, COSY spectral data, the compounds were characterized as vomifoliol (LML 363-1, **97**), α-amyrin (LML 301, **98**), β-amyrin (LML 309, **99**), *(E)*-6,7,8,9,10,11-hexahydro-8,17: 10,16di(metheno) dibenzo[h,l][1]oxa[5] azacyclotridecine-1,4-diol (LML 339-1, **100**) and *(Z)*-1,2,3,4,5,6-hexahydro-8,11-etheno-2,13:4,12di(metheno)benzo[h][1]oxa[5] azacyclopentadecine (LML 339-2, **101**)

3.2.1 Characterization of LML-363-13 as vomifoliol (97)

Compound LML 363-13 (**97)** was isolated as a white crystalline compound. Its electrospray ionisation mass spectrum (ESIMS) showed a pseudo-molecular ion peak [M+Na^{+}] at *m/z* 247 which indicated a molecular formula of $C_{13}H_{20}O_3$ (Figure 3.39). The ^{1}H-NMR and ^{13}C NMR spectral data of LML 363-13 were compared with the corresponding NMR data reported for vomofoliol (Zhang *et al.*, 2021; Maria *et al.*, 2013, Hammami *et al.*, 2004) and were found to be identical with the published values (Table 3.3).

The ^{1}H-NMR spectral data (400 MHz, $CDCl_3$) of LML 363-13 revealed two doublets at 2.23 (1H, *J* = 17.0 Hz, H-3a) and at δ 2.43 (1H, *J* = 17.0 Hz, H-3b) and a quartet at δ 4.40 (1H, *J* = 6.4 Hz, H-9. Three olefinic proton signals at δ 5.83 (1H, d, *J* = 5.2 Hz, H-7), 5.77 (1H, d, *J* = 15.6 Hz, H-8) and 5.89 (1H, br s, H-5) and four signals at δ 1.00 (1H, s, H-11), 1.07 (1H, s, H-12), 1.28 (1H, d, *J* = 6.4 Hz, H-10) and 1.88 (1H, s, H-13) for four methyl groups were observed in the ^{1}H-NMR spectrum (Table 3.3, Figure 3.34, 3.35 & 3.36).

The ^{13}C NMR spectral data (100 MHz, $CDCl_3$) demonstrated a total of 13 signals corresponding to four quaternary, four methine, four methyl and one methylene carbons including a carbonyl carbon at δ 198.0. A signal at δ 68.1 (CH unit) suggested the attachment of hydroxyl group to this carbon (Table 3.3, Figure 3.37). The ^{1}H-^{1}H COSY NMR spectral data confirmed LML 363-13 as vomifoliol. In the ^{1}H-^{1}H COSY spectrum, the cross peak at δ 5.89/1.88 was due to the long-range coupling between the olefinic H-5 with the methyl H_3-13. The cross peaks at δ 5.89/2.23 correlating H-5/H-

3a, 5.77/4.40 corresponding to H-8/H-9, 4.40/1.28 for H-9/H-10, 2.43/1.07 due to interactions between H-3b/H-12 and 2.43/2.23 correlating H-3b/3a supported to confirm the structure of LML 363-13 as vomifoliol (Figure 3.33 and 3.38).

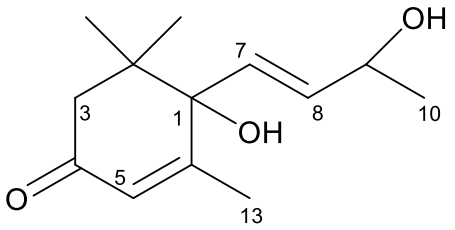


Figure 3.32: Structure of LML 363-13 as vomifoliol (97)

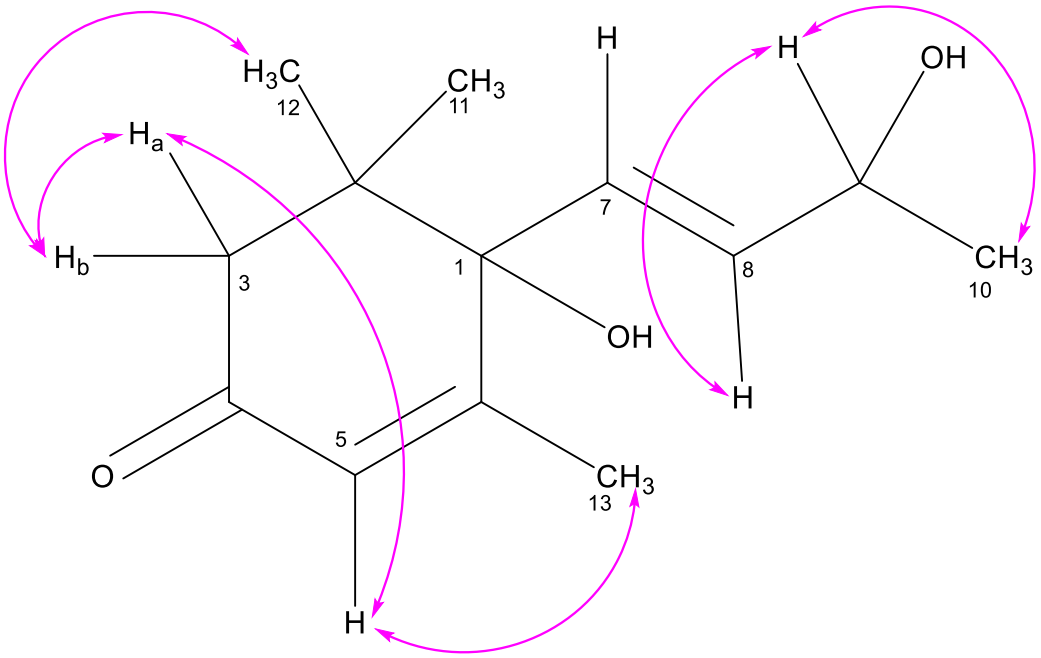


Figure 3.33: Key COSY correlations observed in LML 363-13 as vomifoliol (97)

Table 3.3: Comparison between the ^{1}H NMR (400 MHz; $CDCl_3$) and ^{13}C NMR (100 MHz; $CDCl_3$) spectral data of LML-363-13 (97) and vomifoliol (Hammami *et al.*, 2004).

	LML-363-13 (97)		Vomifoliol	
Position	**δ_C**	**δ_H (*J* in Hz)**	**δ_C**	**δ_H (*J* in Hz)**
1	78.1		78.9	
2	41.2		41.0	
3a	49.7	2.23, d (17.0)	49.6	2.25, d (16.9)
3b		2.43, d (17.0)		2.45, d (16.9)
4	198.0		195.5	
5	127.0	5.89, br s	127.9	5.90, br s
6	162.7		162.2	
7	129.0	5.79, d (15.6)	129.0	5.81, d (14.0)
8	135.8	5.87, dd (15.6, 5.2)	135.7	5.84, d (14.5)
9	68.1	4.40, q (6.4)	68.1	4.41, m
10	24.1	1.28, d (6.4)	23.7	1.29, d (6.4)
11	23.3	1.00, s	24.0	1.01, s
12	22.9	1.07, s	23.0	1.08, s
13	18.9	1.88, s	18.8	1.89, s

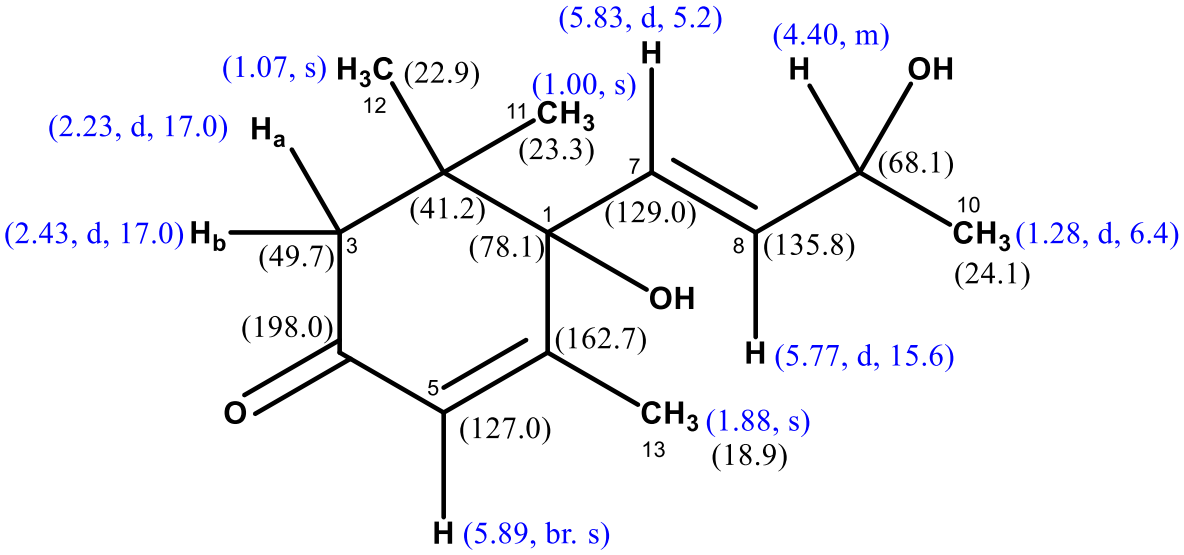


Figure 3.34: Complete ^{1}H and ^{13}C NMR spectral assignment of LML 363-13 as vomifoliol (97)

Figure 3.35: Partially expanded ^{1}H NMR (400 MHz; $CDCl_3$) spectrum of LML-363-13 as vomifoliol (97).

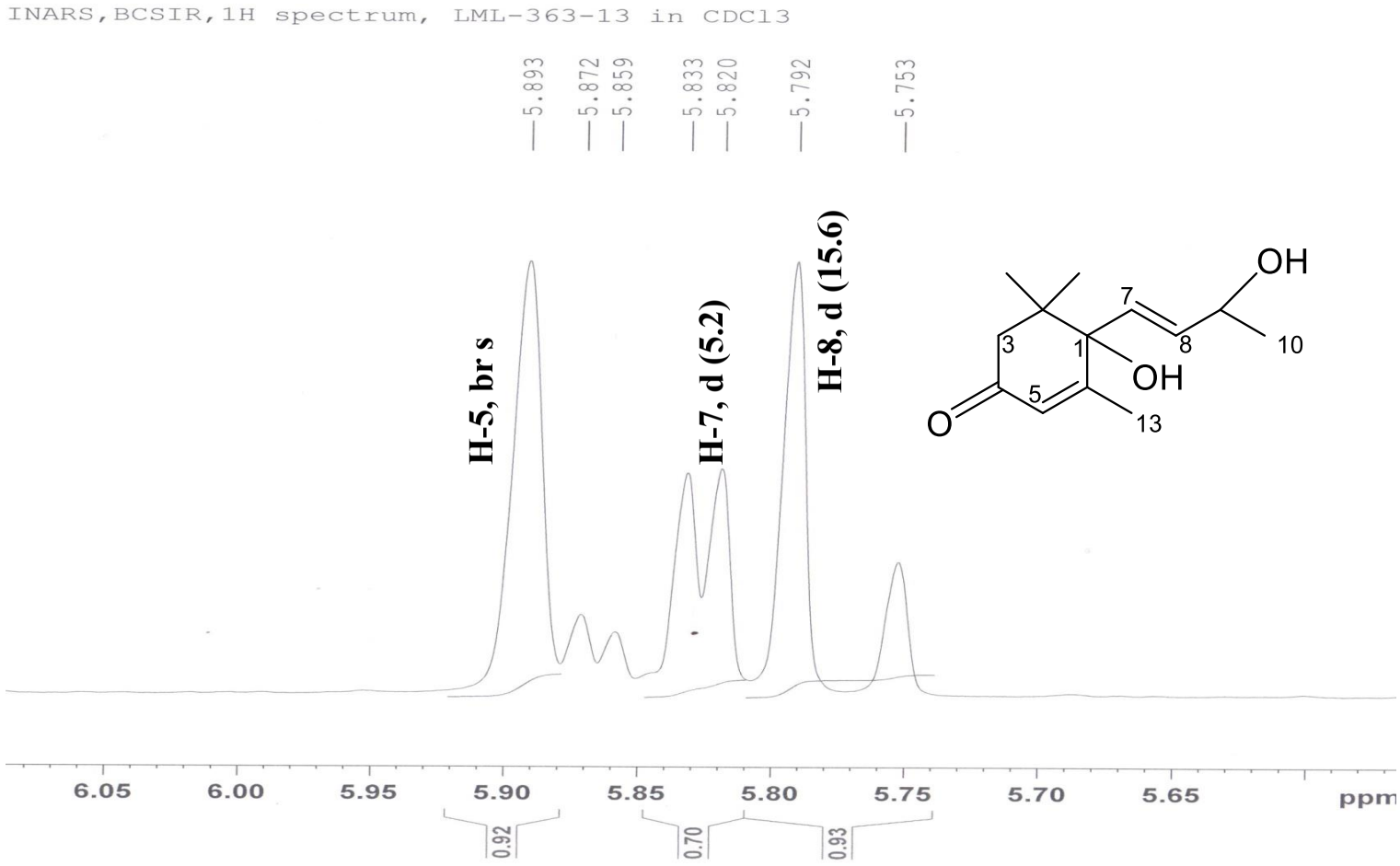


Figure 3.36: Partially expanded ^{1}H NMR (400 MHz; $CDCl_3$) spectrum of LML-363-13 as vomifoliol (97).

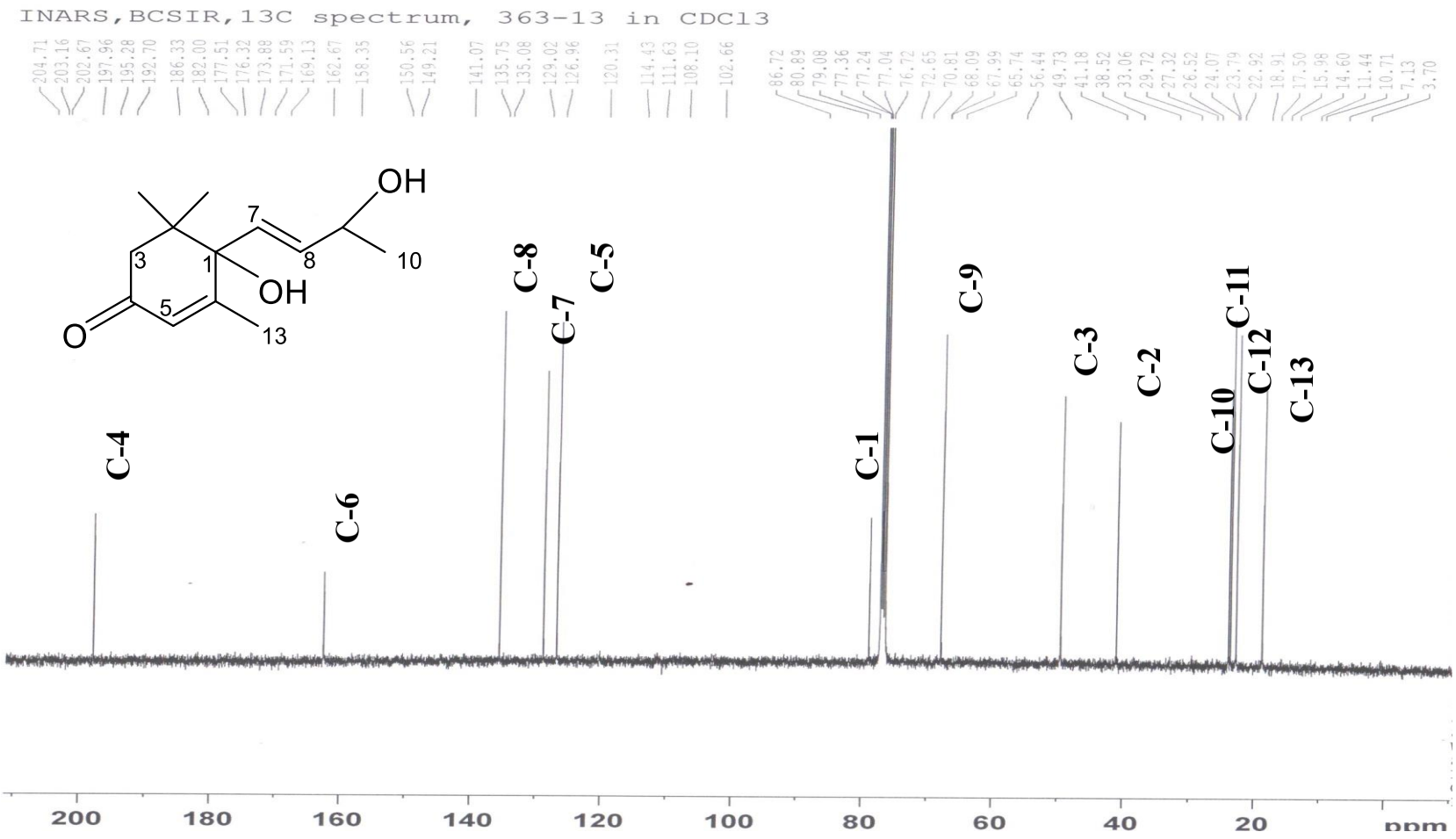


Figure 3.37: Partially expanded ^{1}C NMR (100 MHz; $CDCl_3$) spectrum of LML-363-13 as vomifoliol (97) in $CDCl_3$

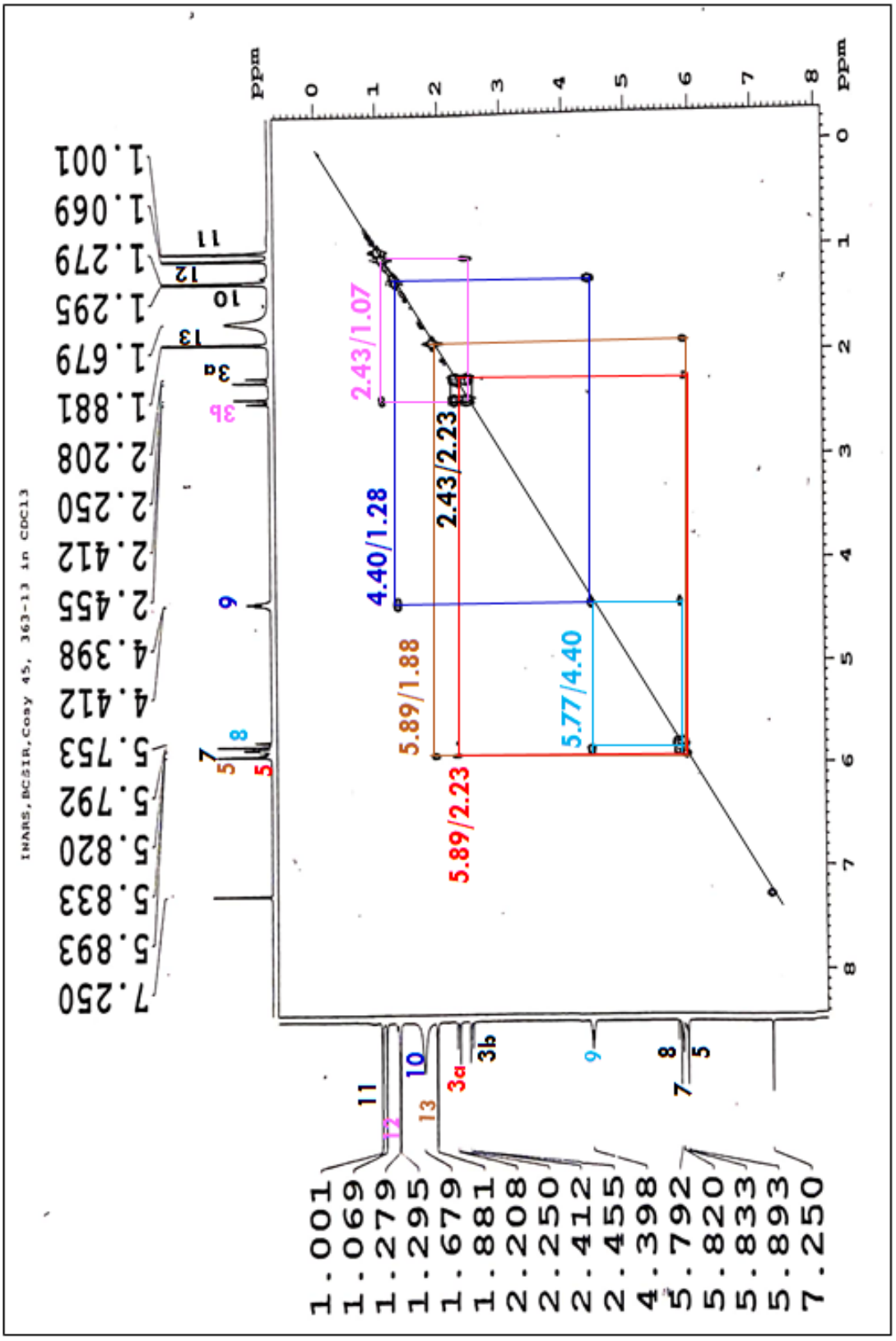


Figure 3.38: COSY NMR spectrum of LML-363-13 as vomifoliol (97) in $CDCl_3$

Designated Reference Institute for Chemical Measurements (DRiCM)

Bangladesh Council of Scientific and Industrial Research (BCSIR)
Dr. Qudrat-i-Khuda Road, Dhanmondi, Dhaka-1205

MS Data Spectrum

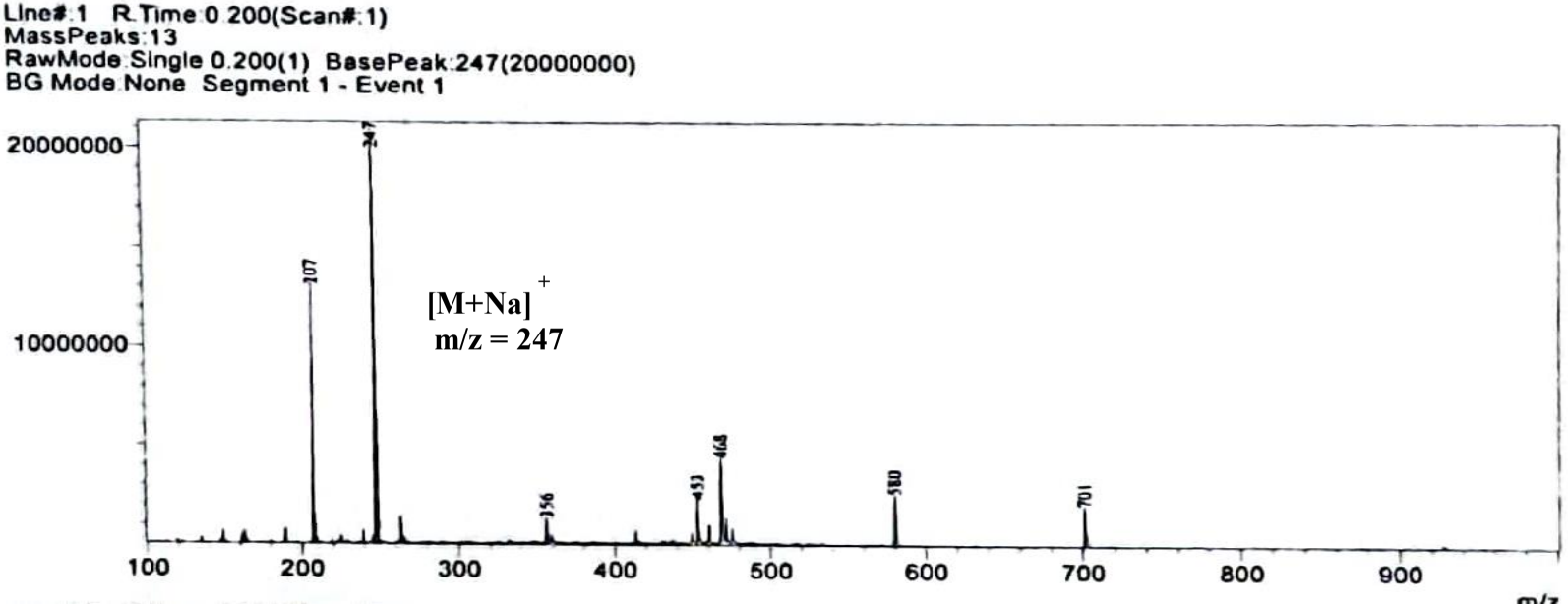


Figure 3.39: ESI Mass spectrum of LML-353-13 as vomifoliol (97).

3.2.2 Characterization of LML-309 as α-amyrin (98) from L. monopetala

LML 309 was obtained as colorless solid.^{1}H NMR (400 MHz, $CDCl_3$) revealed the major signals at δ 3.21 (1H, dd, *J*=10.8, 4.4 Hz, H-3), 5.25 (1H, br s, H-12), 1.08 (3H, s, H_3-27), 0.98 (3H, s, H_3-26), 0.95 (3H, s, H_3-28), 0.93 (3H, s, H_3-25), 0.79 (3H, d, *J* = 8.8 Hz, H_3-30), 0.90 (3H, s, H_3-23), 0.85 (3H, d, *J* = 6.4 Hz, H_3-29), 0.92 (3H, s, H_3-24) (Table 3.4).

^{1}H-NMR spectrum of LML-309 revealed the presence of several signals between 0.76 and 1.08 (Table 3.4) which are attributed to methyl, overlapping methylenes and methine protons typical of triterpenes. The signal observed at δ 5.25, which is a broad singlet is typical of an olefinic proton (H-12); that at δ 3.21 (*J* =10.8, 4.4 Hz) corresponds to the oxymethine proton typical of hydrogen at C-3 of triterpenes (Figure 3.41). Apart from this, six singlets and two doublets, each of three proton intesity were observed at low field between δ 0.78 and 1.08, which confirmed the presence of eight methyl groups in LML-309 (Figure 3.42). The methyl doublets were seen at δ 0.79 (3H, d, *J* = 8.8 Hz, H_3-30) and 0.85 (3H, d, *J* = 6.4 Hz, H_3-29) confirmed the structure as α-amyrin type triterpenoid. The spectrum also showed six methyl singlets at δ 1.08 (3H, s, CH_3-27), 0.98 (3H, s; CH_3-26), 0.95 (3H, s; CH_3-28), 0.93 (3H, s; CH_3-25), 0.90 (3H, s; CH_3-23) and 0.92 (3H, s; CH_3-24). Thus, the structure of LML-309 (**98**) was solved as α-amyrin, the identity of the compound as α-amyrin was further confirmed by comparison of its spectral data with reported values (Sharker *et al.*, 2013).

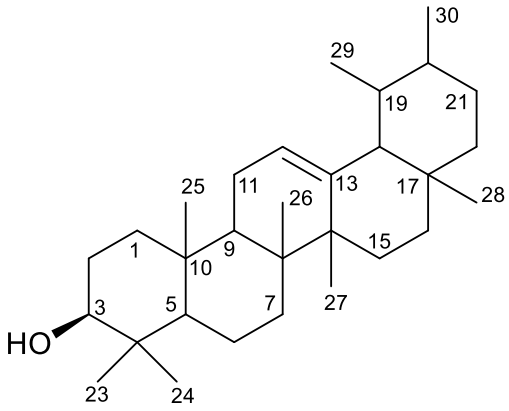


Figure 3.40: Structure of LML 309 as α-amyrin (98)

Tble 3.4: Comparison between the ^{1}H NMR (400 MHz; $CDCl_3$) spectral data of LML 309 and α-amyrin (Sharker *et al.*, 2013)

	LML-309	α-amyrin
Position	**δ_H (*J in Hz*)**	**δ_H (*J in Hz*)**
3	3.21 (dd, 10.8, 4.4)	3.22, m
12	5.25, br s	5.19, t (4.0)
23	0.90, s	0.80, s
24	0.92, s	0.79, s
25	0.93, s	1.00, s
26	0.98, s	1.01, s
27	1.08, s	1.08, s
28	0.95, s	0.96, s
29	0.86, d (6.4)	0.88, br. s
30	0.79, d (8.8)	0.91, br. s

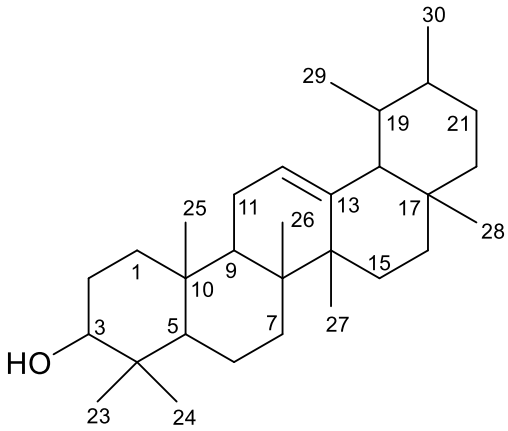


Figure 3.40: Structure of LML 309 as α-amyrin (98)

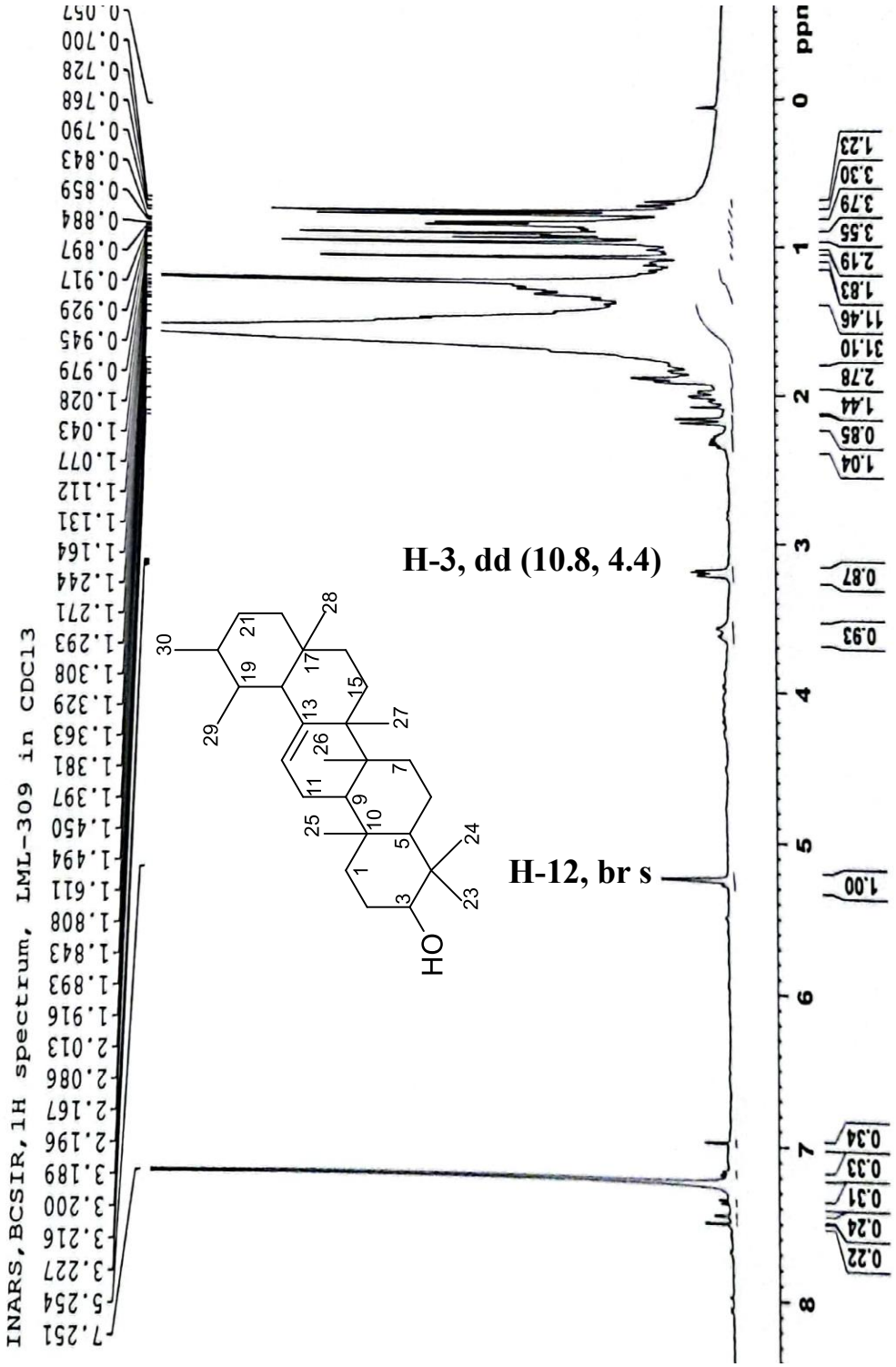


Figure 3.41: Partially expanded ^{1}H NMR (400 MHz; $CDCl_3$) spectrum of LML 309 as α-amyrin (98).

Figure 3.42: Partially expanded ^{1}H NMR (400 MHz; $CDCl_3$) spectrum of LML 309 as α-amyrin (98).

3.2.3 Characterization of LML-301 as β-amyrin (99) from L. monopetala

LML 301 (**99**) was obtained as colorless crystal. The ^{1}H NMR (400 MHz, $CDCl_3$) spectral data revealed the major peaks at δ 3.21 (1H, dd, *J* = 4.0, 10.4 Hz), 5.25 (1H; d, *J* = 5.4 Hz), 0.87 (3H, s, H_3-23), 0.78 (3H, d, *J* =7.2 Hz, H_3-24), 0.92 (3H, s, H_3-25), 1.08 (3H, s, H_3-26), 1.13 (3H, s, H_3-27), 0.95 (3H, s, H_3-28), 0.84 (3H, s, H_3-29), 0.90 (3H, s, H_3-30) (Table 3.5).

The ^{1}H NMR spectrum of LML 301 showed eight methyl group resonances at δ 0.79, 0.78, 0.98, 1.08, 1.13, 0.95, 0.92 and 0.90 which could be assigned to H_3-23, H_3-24, H_3-25, H_3-26, H_3-27, H_3- 28, H_3-29 and H_3-30, respectively of an oleanane- type triterpenoid carbon skeleton (Figure 3.45). A characteristic triplet at δ 5.25 (*J* = 5.4 Hz) was attributed to H-12. This again suggested an olean-12-ene-type carbon skeleton. On the other hand, a one proton double doublet at δ 3.21 (1H, d, *J* = 10.4, 4.0 Hz) could be ascribed to the typical oxymethine proton at C-3 of the pentacyclic triterpene (Figure 3.44). The above spectral features are in close agreement to those observed for β- amyrin. Thus, compound LML 301 was characterized as β-amyrin. This identity was further confirmed by direct comparison of its ^{1}H NMR spectrum with that recorded for β-amyrin (Dias *et al.,* 2011) as well as by co-TLC with an authentic sample.

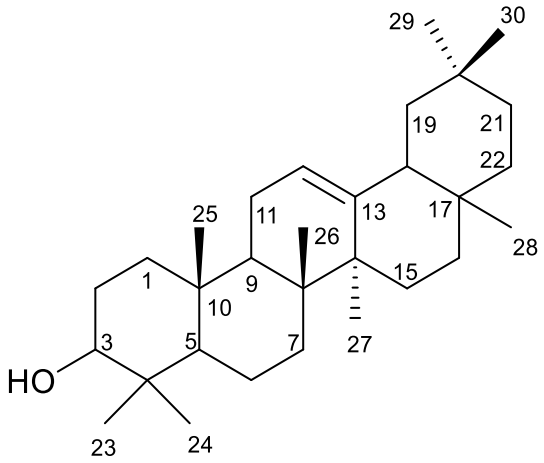


Figure 3.43: Structure of LML 301as β-amyrin (99)

Table 3.5: Comparison between the ^{1}H NMR (400 MHz; $CDCl_3$) spectral data of LML 301 (99) and β-amyrin (98) [Dias *et al.*,2011]

	LML 301 (99)	β-amyrin (98)
Position	**δ_H (*J in Hz*)**	**δ_H (*J in Hz*)**
3	3.21 (dd, *J* = 4.0, 10.4)	3.22, m
12	5.25, d (5.4)	5.18, t (4.0)
23	0.79, s	0.80, s
24	0.78, s	0.79, s
25	0.98, s	1.00, s
26	1.08, s	1.01, s
27	1.13, s	1.07, s
28	0.95, s	0.96, s
29	0.92, s	0.91, s
30	0.90, s	0.88, s

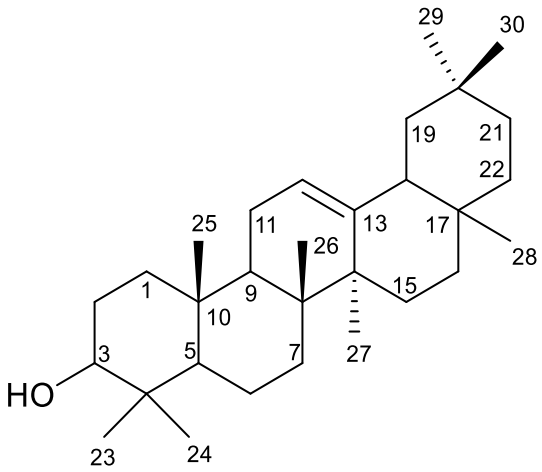


Figure 3.43: Structure of LML 301 as β-amyrin (99)

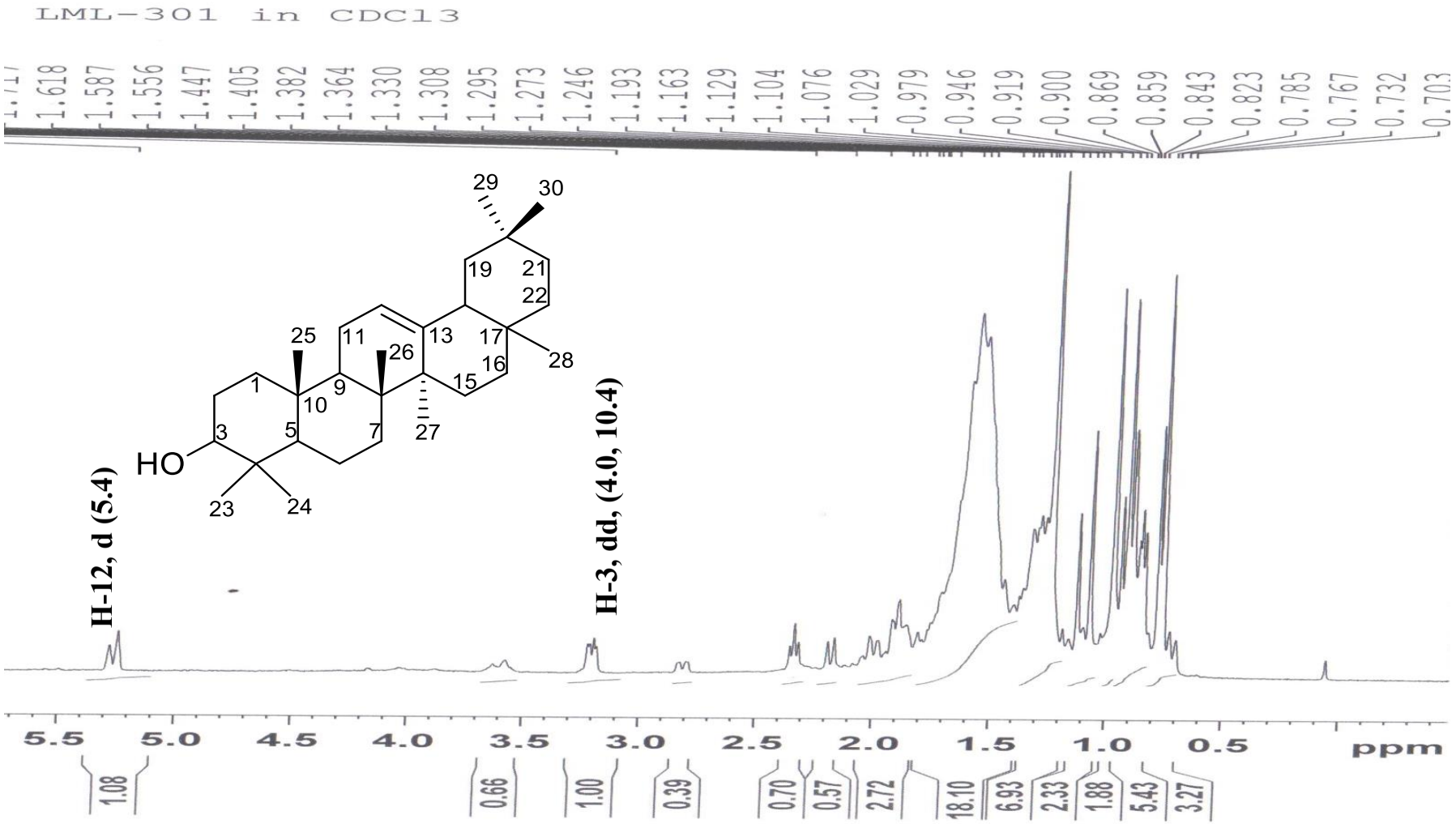


Figure 3.44: Partially expanded ^{1}H NMR (400 MHz) spectrum of LML 301 as β-amyrin (99).

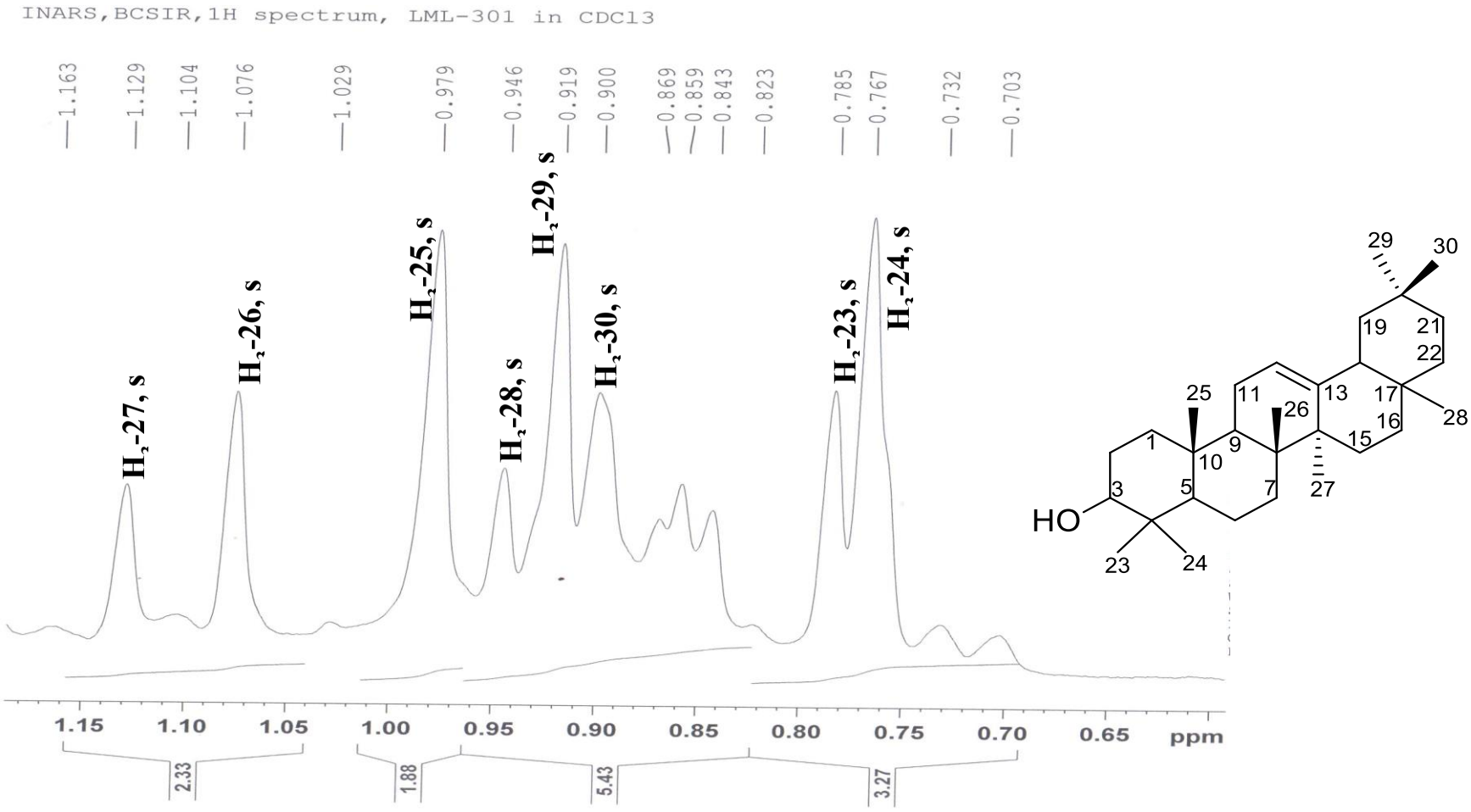


Figure 3.45: Partially expanded ^{1}H NMR (400 MHz; $CDCl_3$) spectrum of LML 301 as β-amyrin (99).

3.2.4 Characterization of LML-339.1 as (E)-6,7,8,9,10,11-hexahydro-8,17:10,16 di(metheno)dibenzo[h,l][1]oxa[5]azacyclotridecine-1,4-diol **(100)** *from L. monopetala*

The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound LML-339.1 displayed well resolved signals at δ 2.0-8.0. Careful analysis of signals revealed that there are three sets of methylene protons at δ 3.80 (1H, dd, *J* =11.2, 5.0 Hz), 3.92 (1H, dd, *J* = 11.2, 3.2 Hz)]; δ 3.19 (1H, dd, *J* = 11.0, 5.6 Hz), 3.04 (1H, dd, *J* = 11.0, 5.4 Hz) and δ 2.74 (2H, br. s) for three methylene groups. Signals for two methine protons attached to N at δ 4.74 (1H, q, *J* = 7.2 Hz) and 4.34 (1H, br. s) and two olefenic protons at δ 6.72 (1H, d, *J* = 6.8 Hz) and 5.90 (1H, d, *J* = 8.4 Hz) were also observed. Two sets of aromatic ring protons at δ 7.70 (1H, d, *J* = 7.8 Hz), 7.43 (1H, td, *J* = 7.8, 7.2 Hz), 7.51 (1H, dd, *J* = 7.2, 7.2 Hz) & 7.25 (1H, m) for ring A and δ 7.15 (1H, d, *J* = 7.2 Hz) & 7.06 (1H, d, *J* = 6.8 Hz) for ring B have been seen in the ^{1}H NMR spectrum of LML-339.1 (Table 3.6, Figure 3.48-3.50). The number and splitting of aromatic protons suggested two aromatic nucleus. Extensive analysis of COSY correlations allowed us to find out all the expected correlation between different spin systems as shown in figure 3.47 & 3.51. Combining all the spectral data allowed to tentatively identify as *(E)*-6,7,8,9,10,11-hexahydro-8,17:10,16di(metheno) dibenzo[h,l][1]oxa[5] azacyclotridecine-1,4-diol (**100**) (Figure 3.46). Thus, LML-339.1 was characterized as a new compound. However, additional spectral data acquisition is in progress to confirm its structure.

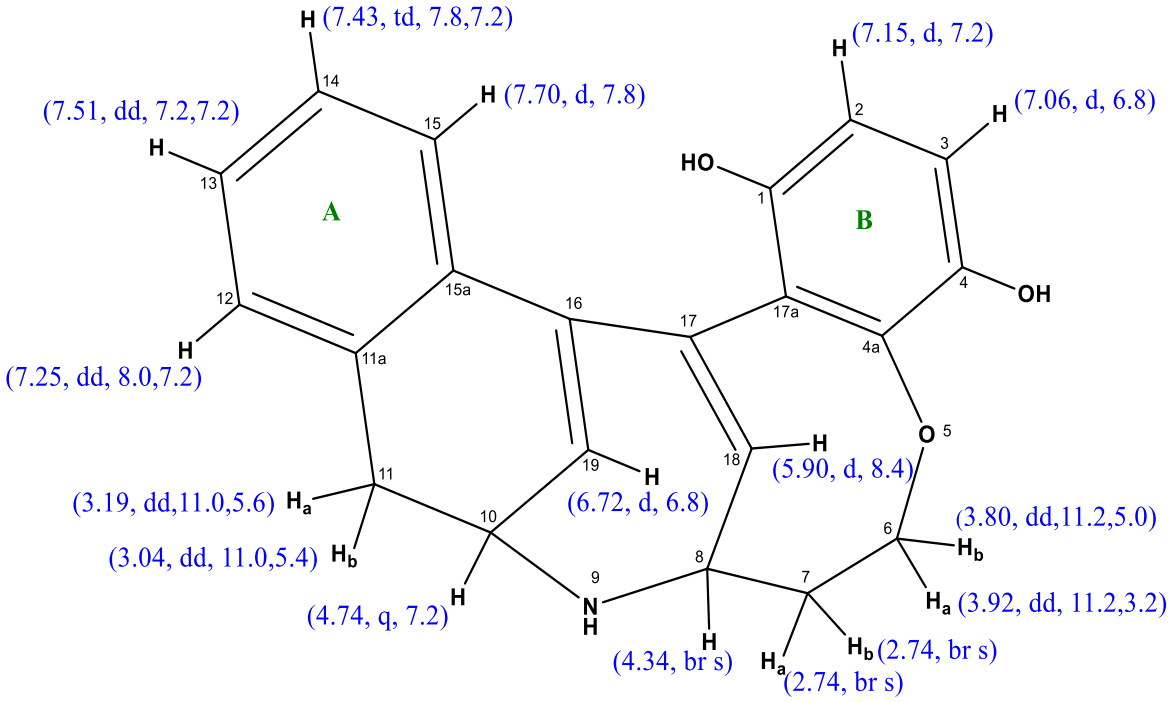


Figure 3.46: Structure of LML-339.1 as *(E)*-6,7,8,9,10,11-hexahydro-8,17:10,16 di(metheno)-dibenzo[h,l][1]oxa[5]azacyclotridecine-1,4-diol (100)

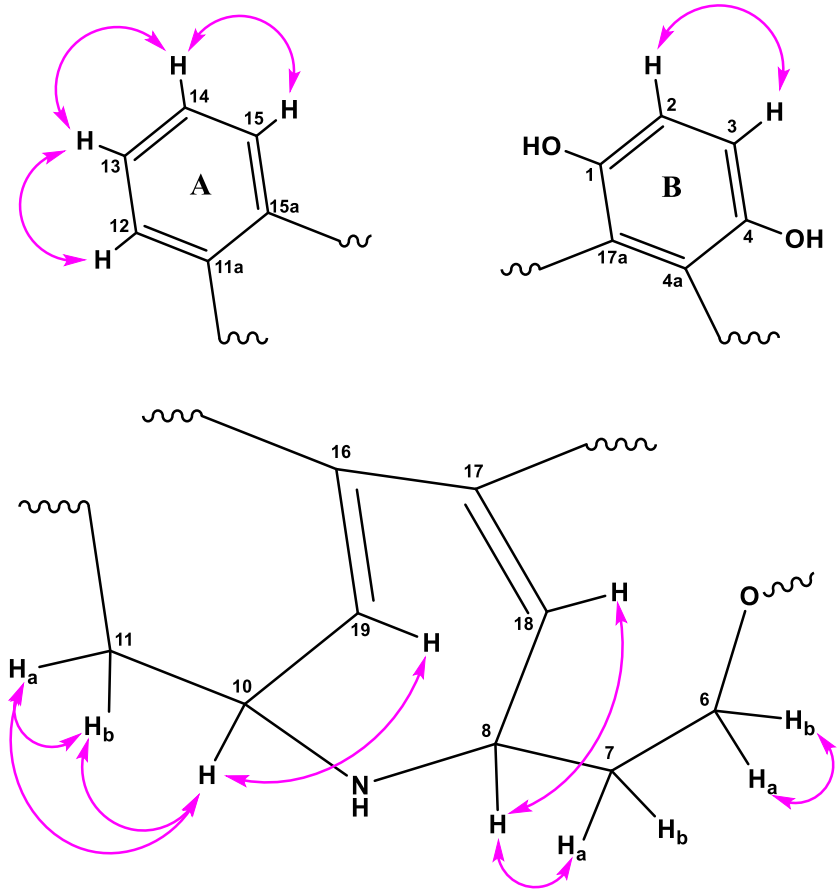


Figure 3.47: Key COSY correlations observed in LML-339.1

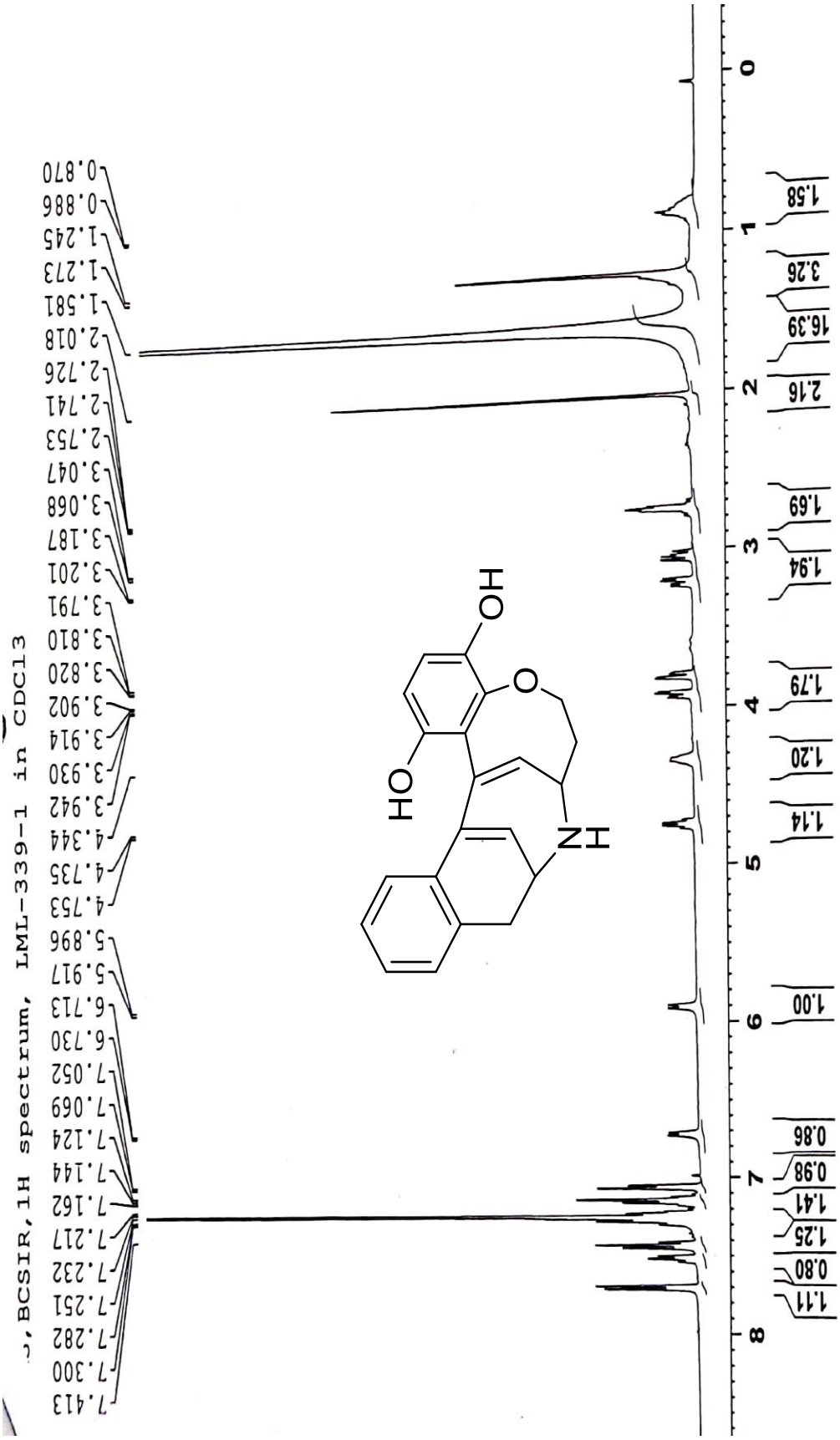


Figure 3.48: Partially expanded ^{1}H NMR (400 MHz; $CDCl_3$) spectrum of LML-339.1 as *(E)*-6,7,8,9,10,11-hexahydro-8,17:10,16 di(metheno)dibenzo[h,ll][1]oxa[5]azacyclotridecine-1,4-diol (100).

Figure 3.49: Partially expanded ^{1}H NMR (400 MHz; $CDCl_3$) spectrum of LML-339.1 as *(E)*-6,7,8,9,10,11-hexahydro-8,17:10,16 di(metheno)dibenzo[h,ll][1]oxa[5]azacyclotridecine-1,4-diol (100).

Figure 3.50: Partially expanded ^{1}H NMR (400 MHz; $CDCl_3$) spectrum of LML-339.1 as *(E)*-6,7,8,9,10,11-hexahydro-8,17:10,16 di(metheno)dibenzo[h,l][1]oxa[5]azacyclotridecine-1,4-diol (100).

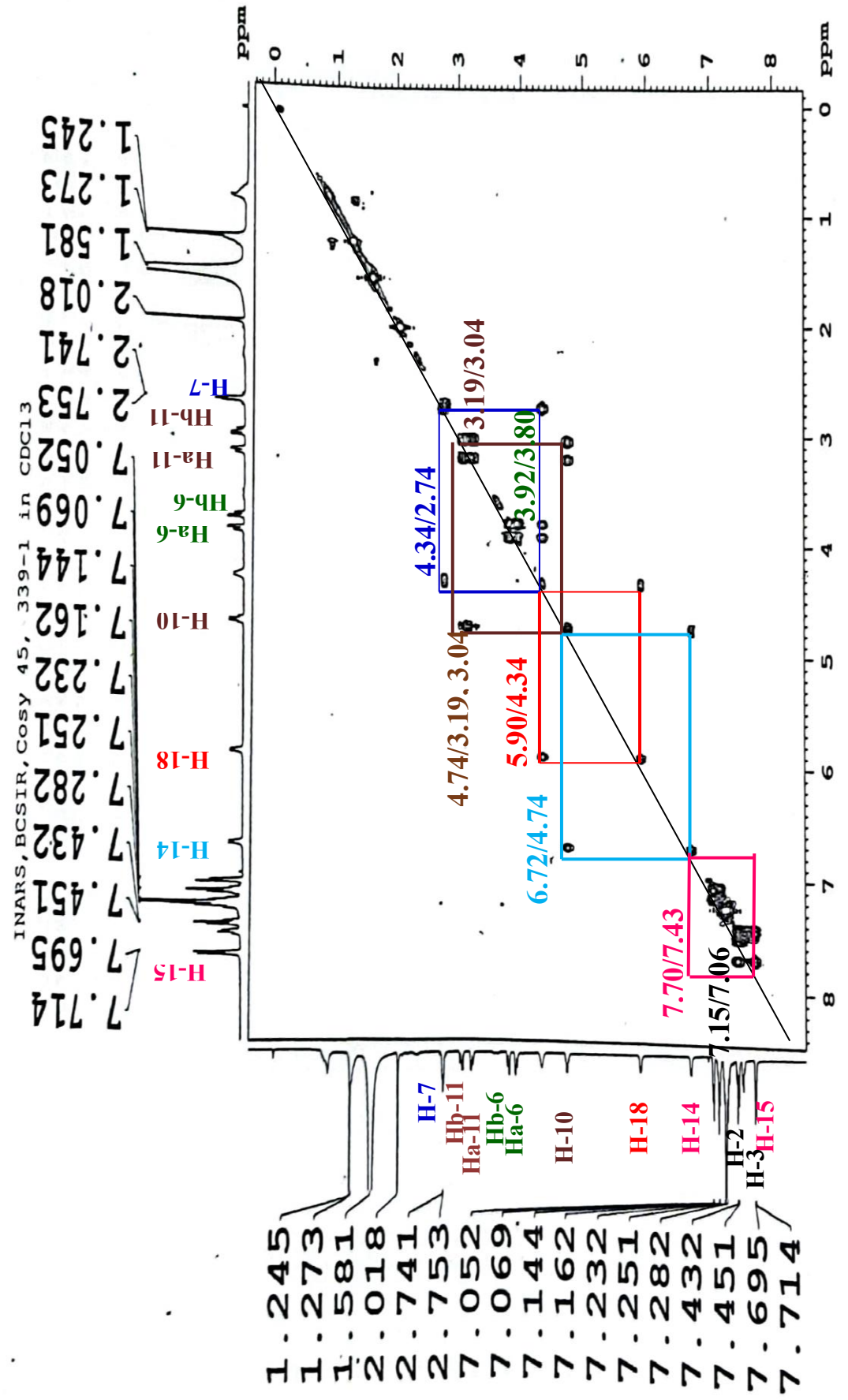


Figure 3.51: COSY NMR spectrum of LML -339.1 as *E*)-6,7,8,9,10,11-hexahydro-8,17:10,16 di(metheno)dibenzo[h,l][1]oxa[5]azacyclotridecine-1,4-diol (100) in $CDCl_3$

3.2.5 Characterization of LML-339.2 as (Z)-1,2,3,4,5,6-hexahydro-8,11-etheno-2, 13:4,12-di(metheno)benzo[h][1]oxa[5] azacyclopentadecine **(101)** *from L. monopetala*

The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound LML-339.2 displayed well resolved signals at δ 2.0-8.0. Careful analysis of signals revealed that there are three sets of protons at δ [4.54 (1H, d, *J* =11.6 Hz); 4.02 (1H, dd, *J* = 11.6, 3.2 Hz)], δ [3.27 (1H, dd, *J* = 11.0, 6.4 Hz); 3.22 (1H, dd, *J* = 11.0, 6.8 Hz)] and δ [2.98 (1H, dd, 11.0, 6.4 Hz); 2.90 (1H, dd, 11.0, 8.4) for three methylene groups. Signals for two methine protons attached to N at δ 4.91 (1H, q, *J* = 6.4 Hz) and δ 4.60 (1H, br. s) and two olefenic methine protons at δ 6.65 (1H, d, *J* = 8.0 Hz) and δ 6.55 (1H, d, *J* = 6.4 Hz) were also observed. Two sets of aromatic protons at δ 7.68 (1H, d, *J* = 7.2 Hz), 7.65 (1H, d, *J* = 7.2 Hz), 7.38 (1H, dd, *J* = 8.0, 7.2 Hz) & 7.30 (1H, t, *J* = 7.2 Hz) for ring A and δ 7.28 (2H, d, *J* = 6.8 Hz) & 7.21 (2H, d, *J* = 6.8 Hz) for ring B (Table 3.6, Figure 3.54-3.57). The number and splitting of aromatic protons suggested two aromatic nucleus. Extensive analysis of COSY correlation allowed us to find out all the expected correlation between different spin systems as shown in figure 3.53 & 3.58. Combining all the spectral data allowed to tentatively identify as *(Z)*-1,2,3,4,5,6-hexahydro-8,11-etheno-2, 13:4,12-di(metheno)benzo[h][1]oxa[5] azacyclopentadecine (**101**) (Figure 3.52). Thus, LML-339.2 was characterized as a new alkaloid. However, additional spectral data acquisition is in progress to confirm its structure.

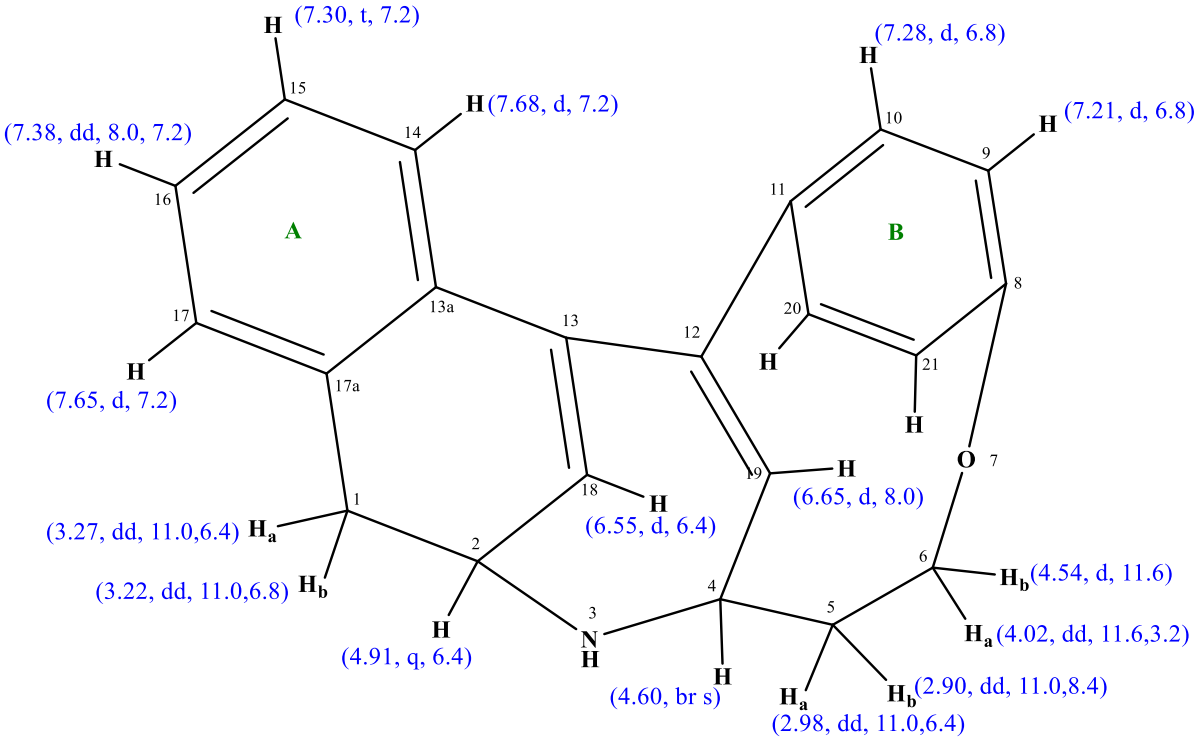


Figure 3.52: Structure of LML-339.2 as *(Z)*-1,2,3,4,5,6-hexahydro-8,11-etheno-2, 13:4,12-di(metheno) benzo[h][1]oxa[5] azacyclopentadecine (101)

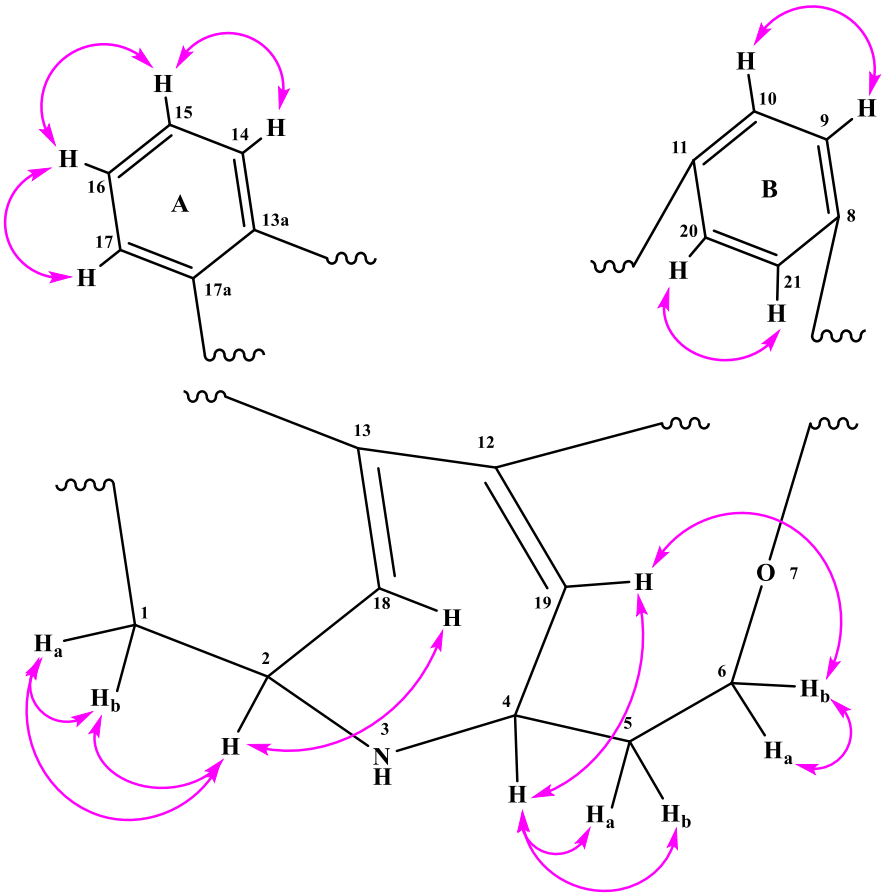


Figure 3.53: Key COSY correlations observed in LML-339.2

Table 3.6: Comparison between the ^{1}H NMR (400 MHz; $CDCl_3$) spectral data of LML-339.1(100) and LML-339.2 (101)

LML-339.1		LML-339.2	
Position	**δ_H, mult (Jin Hz)**	**Position**	**δ_H, mult (Jin Hz)**
2	7.15, d (7.2)	1	3.27, dd (11.0, 6.4) 3.22, dd (11.0, 6.8)
3	7.06, d (6.8)	2	4.91, q (6.4)
6	3.80, dd (11.2, 5.0) 3.92, dd (11.2, 3.2)	4	4.60, br s
7	2.74, br s 2.74, br s	5	2.98, dd (11.0, 6.4) 2.90, dd (11.0, 8.4)
8	4.43, br s	6	4.54, d (11.6) 4.02, dd (11.6, 3.2)
10	4.74, q (7.2)	9	7.21, d (6.8)
11	3.19, dd (11.0, 5.6) 3.04, dd (11.0, 5.4)	10	7.28, t (11.2, 6.8)
12	7.25, dd (7.2, 8)	14	7.68, d (7.2)
13	7.51, dd (7.2, 7.2)	15	7.30
14	7.43. td (7.8, 7.2)	16	7. 38, dd (8, 7.2)
15	7.70, d (7.8)	17	7.64, d (7.2)
18	5.90, d (8.4)	18	6.55, d (6.4)
19	6.72, d (6.8)	19	6.65, d (8.0)

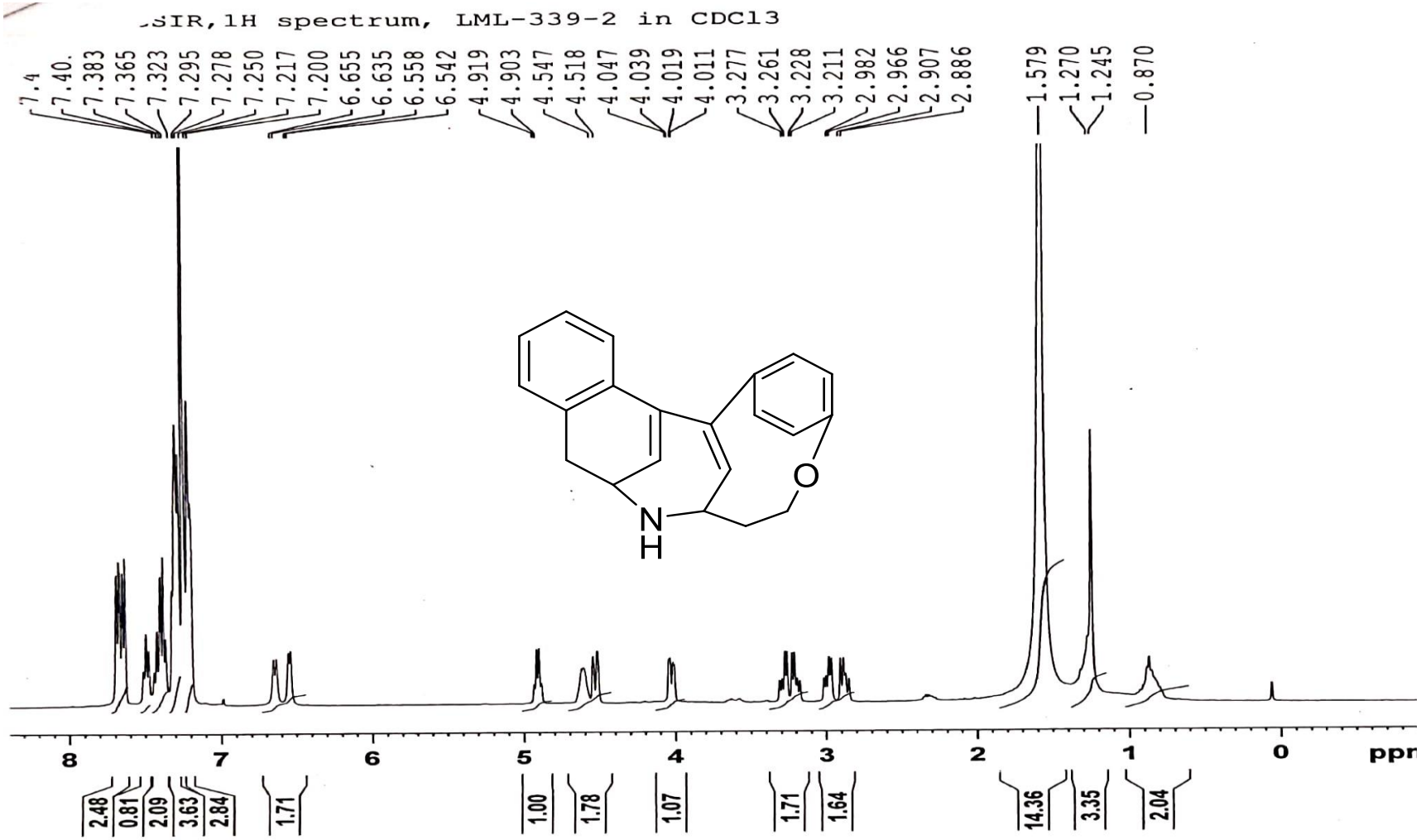


Figure 3.54: Partially expanded ^{1}H NMR (400 MHz; $CDCl_3$) spectrum of LML-339.2 as *(Z)*-1,2,3,4,5,6-hexahydro-8,11-etheno-2, 13:4,12-di(metheno)benzo[h][1]oxa[5] azacyclopentadecine (101).

Figure 3.55: Partially expanded ^{1}H NMR (400 MHz; $CDCl_3$) spectrum of LML-339.2 as *(Z)*-1,2,3,4,5,6-hexahydro-8,11-etheno-2, 13:4,12-di(metheno)benzo[h][1]oxa[5] azacyclopentadecine (101).

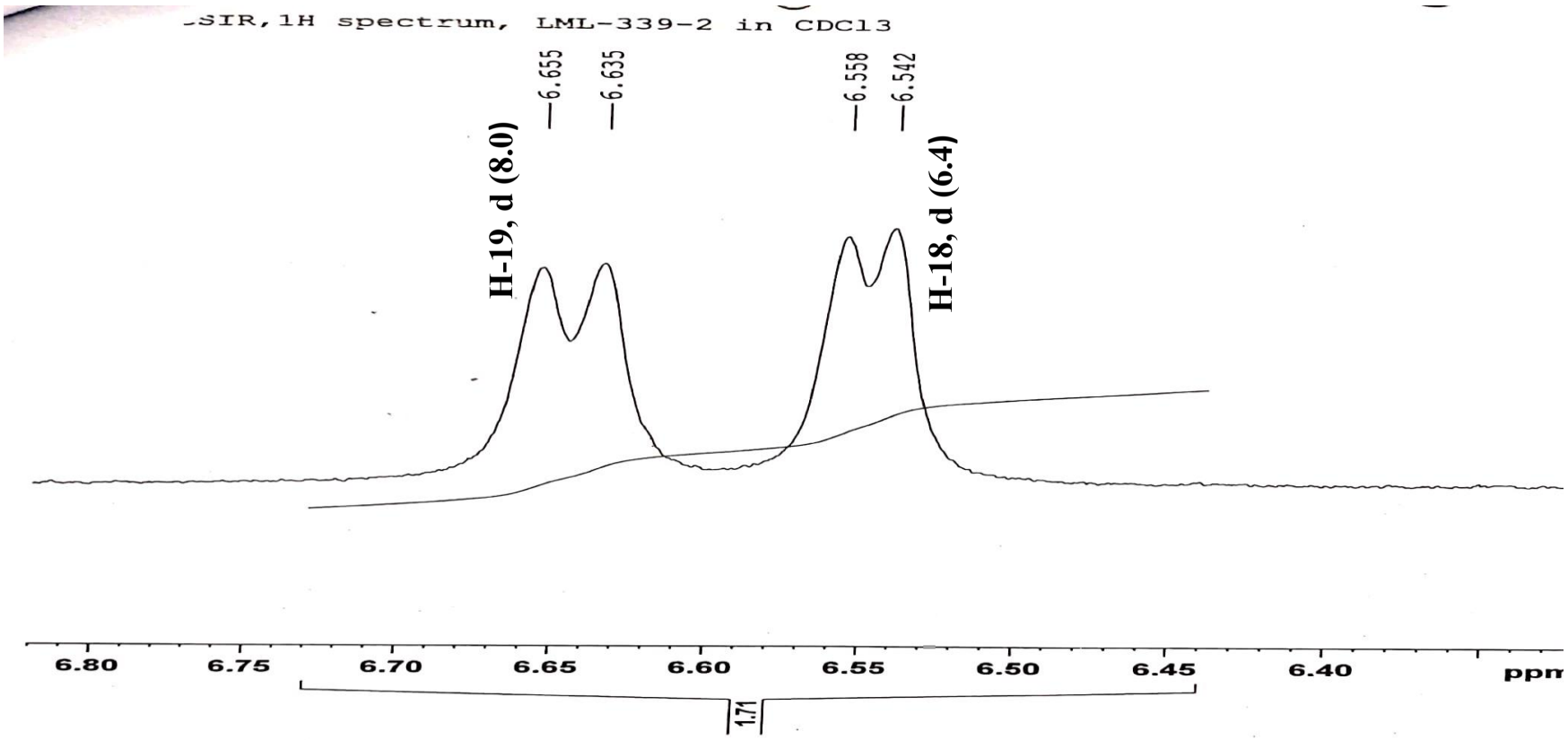


Figure 3.56: Partially expanded ^{1}H NMR (400 MHz; $CDCl_3$) spectrum of LML-339.2 as *(Z)*-1,2,3,4,5,6-hexahydro-8,11-etheno-2, 13:4,12-di(metheno)benzo[h][1]oxa[5] azacyclopentadecine (101).

Figure 3.57: Partially expanded ^{1}H NMR (400 MHz; $CDCl_3$) spectrum of LML-339.2 as *(Z)*-1,2,3,4,5,6-hexahydro-8,11-etheno-2, 13:4,12-di(metheno)benzo[h][1]oxa[5] azacyclopentadecine (101).

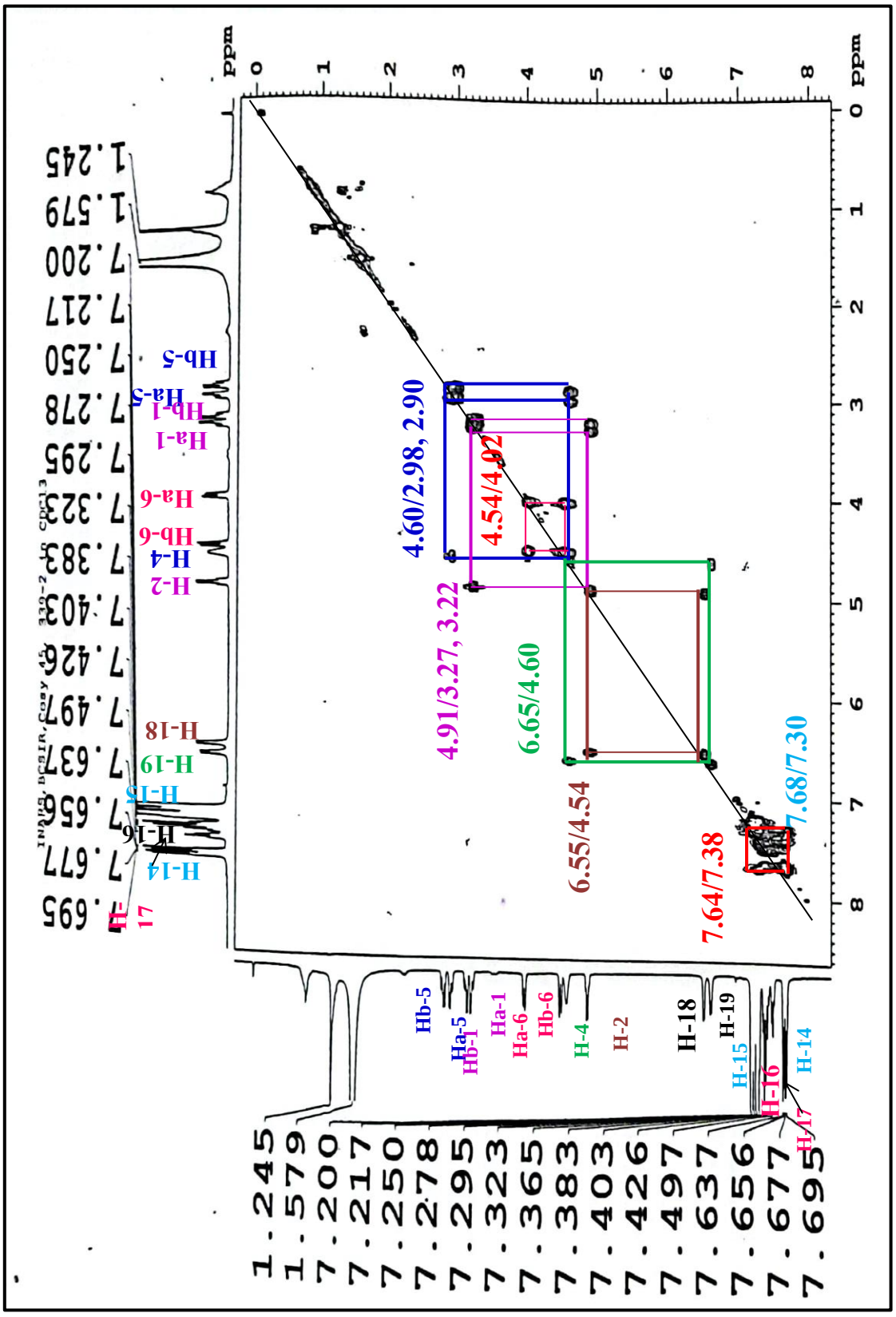


Figure 3.58: COSY NMR spectrum of LML-339.2 as *(Z)*-1,2,3,4,5,6-hexahydro-8,11-etheno-2, 13:4,12-di(metheno)benzo[h][1]oxa[5] azacyclopentadecine (101) in $CDCl_3$

3.3 Compounds from L. deccanensis

The chloroform soluble material of *L. deccanensis* was subjected for gel permeation chromatography (GPC) over Sephadex LH-20. Compounds LDC-10-3 and LDC-10-2 were isolated from test tube no 10 (Eluted fraction of Sephadex column) by PTLC method and obtained as pure. The obtained ^{1}H NMR spectral data and the comparison with the reference spectral data helped us to characterize LDC-10-3 as lupeol (**102**) and LDC-10-2 as a mixture of β-sitosterol (**55**) and stigmasterol (**56**). Lupeol was identified in *L. deccanensis* for the first time.

3.3.1 Characterization of LDC-10-3 as lupeol (102) from L. deccanensis

Lupeol (**102**), a pentacyclic triterpenoid, was isolated from the chloroform soluble fraction of *L. deccanensis.* The compound (6 mg) appeared as white needles. The major peaks for LDC-10-3 (**102**) are δ 4.71 (1H, br. s, Ha-29), 4.59 (1H, br. s, Hb-29), 3.19 (1H, dd, *J* = 11.2, 4.4 Hz, H-3), 2.43 (m), 0.97 (3H, s, H_3-23), 0.79 (3H, s, H_3-24), 0.85 (3H, s, H_3-25), 1.05 (3H, s, H_3-26), 0.99 (3H, s, H_3-27), 0.81(3H, s, H_3-28) and 1.70 (3H, s, H_3-30) (Table 3.7).

The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **102** showed a double doublet (*J* = 1.2, 4.4 Hz) of one proton intensity centered at δ 3.19 typical for an oxymethine proton at C-3 of a triterpene skeleton. The splitting pattern and *J* values of this proton confirmed the β (beta) orientation of the C-3 oxygenated substituent. The spectrum displayed two broad singlets at δ 4.71 and 4.59 (1H each) assignable to the vinylic protons at C-29 (Figure 3.61). It also showed seven singlets for methyl protons at δ 0.97, 0.79, 1.05, 0.85, 0.99, 0.81 including a methyl on a vinylic moiety at 1.70 (3H each) assignable to the methyl group protons at C-4 (H_3-23, H3-24), C-8 (H_3-26), C-10 (H_3-25), C-14 (H_3-27), C-17 (H_3-28) and C-20 (H_3-30), respectively (Figure 3.62). On this basis, compound **102** was characterized as lupeol. The identity of **102** was further confirmed by comparing its spectral data with previously reported values (Ragasa *et al.*, 2015) as well as co-TLC with an authentic sample of lupeol, previously isolated in our laboratory.

These assignments for ^{1}H NMR spectral data of LDC-10-3 (**102**) from *L. deccanensis* are good agreement to agree the structure as lupeol (Ragasa *et al.,* 2015) as demonstrated in the table 3.7.

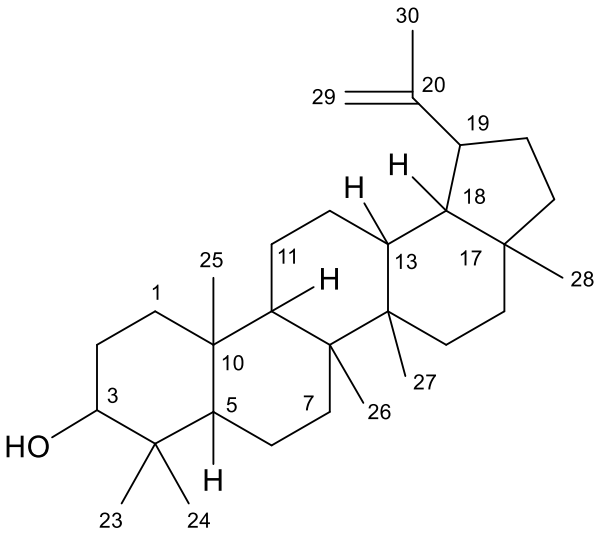


Figure 3.59: Structure of LDC-10-3 as lupeol (102)

Table 3.7: Comparison between the ^{1}H NMR (400 MHz; $CDCl_3$) spectral data of LDC-10-3 (102) and Lupeol (Ragasa *et al.*, 2015)

		LDC-10-3 (102)	Lupeol
Position	**H**	**δ_H**	**δ_H**
3	CH	3.19, dd (11.2, 4.4)	3.16
19	CH	2.43, m	2.43
23	CH_3	0.97, s	0.96, s
24	CH_3	0.79, s	0.79, s
25	CH_3	0.85, s	0.89, s
26	CH_3	1.05, s	1.13, s
27	CH_3	0.99, s	1.01, s
28	CH_3	0.81, s	0.83, s
29	CH_2	4.59, 4.71 (br. s)	4.57, 4.69 (d, *J*= 1.9 Hz)
30	CH_3	1.70, s	1.67, s

(4

Figure 3.60: Partially expanded ^{1}H NMR (400 MHz; $CDCl_3$) spectrum of LDC-10-3 as lupeol (102).

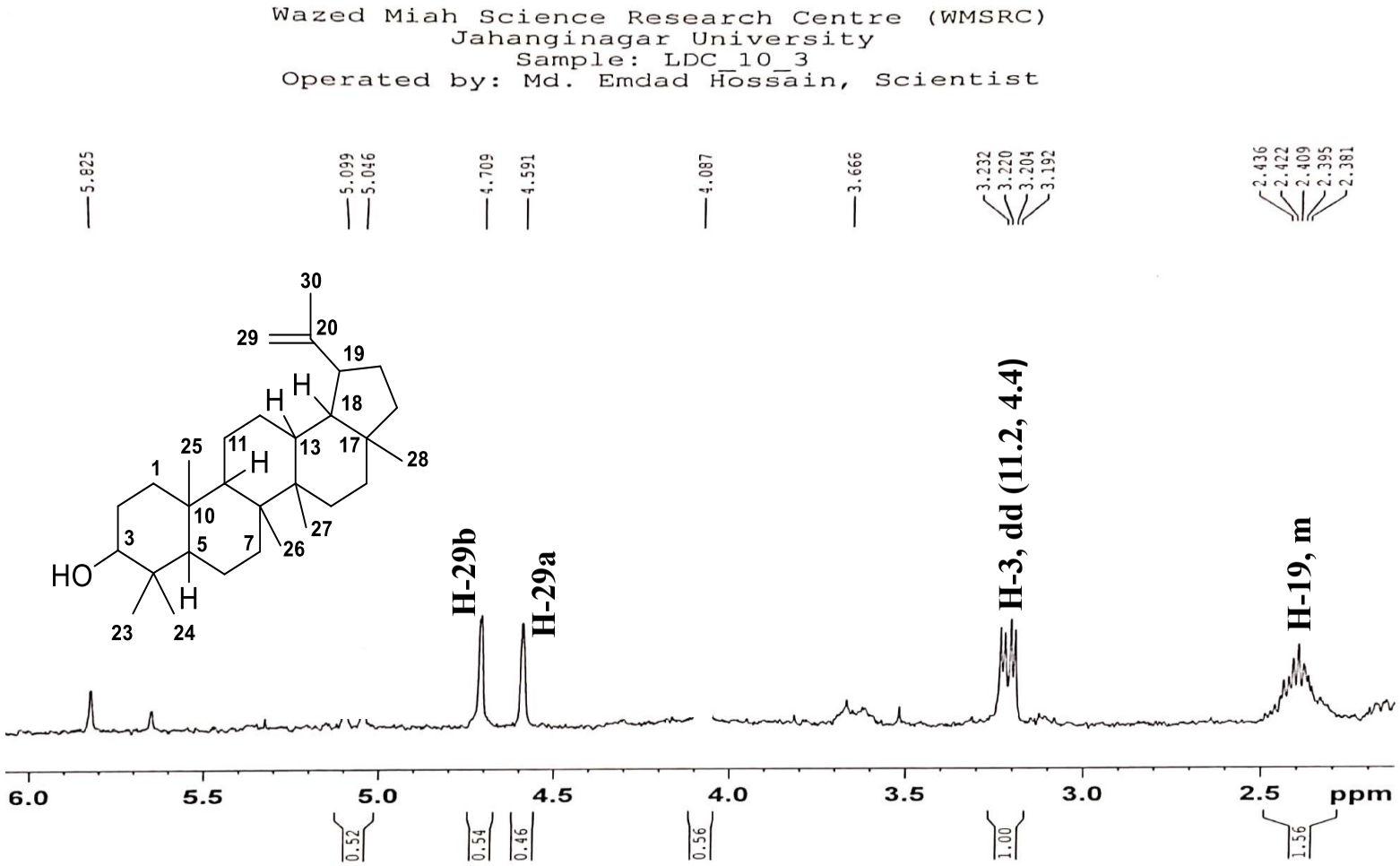


Figure 3.61: Partially expanded ^{1}H NMR (400 MHz; $CDCl_3$) spectrum of LDC-10-3 as lupeol (102).

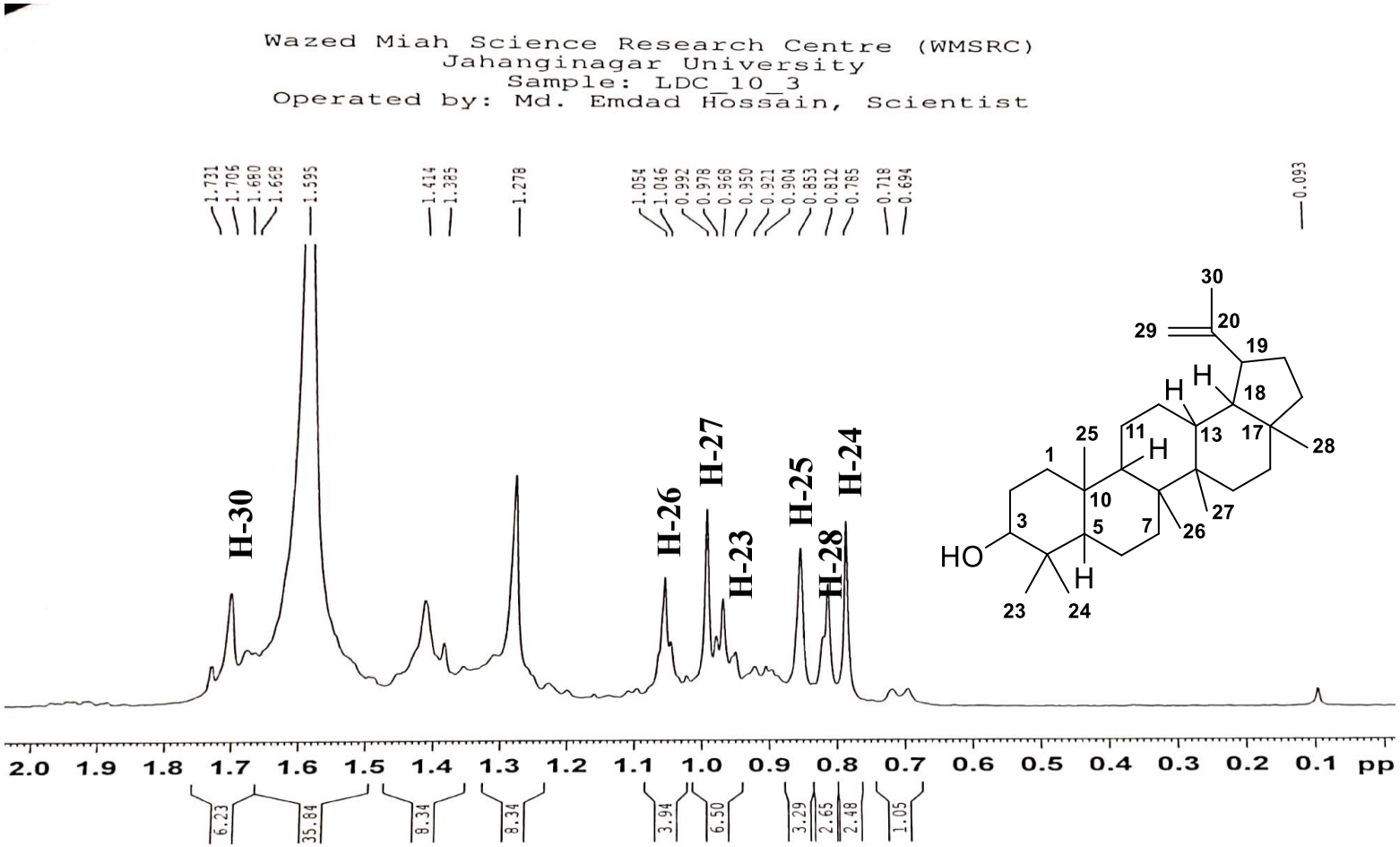


Figure 3.62: Partially expanded ^{1}H NMR (400 MHz; $CDCl_3$) spectrum of LDC-10-3 as lupeol (102).

3.3.2 Characterization of LDC-10-2 as a mixture (4:1) of β-sitosterol (55) and stigmasterol (56)

LDC-10-2 was isolated from the GPC fraction as a white powder. The ^{1}H NMR spectrum of LDC-10-2 showed several methyl groups suggesting a mixture of triterpenoids, compound **55** and **56**. The ^{1}H NMR (400 MHz, $CDCl_3$) spectral data revealed the major peaks at δ 3.51 (1H, m, H-3), 5.37 (1H, br s, H-6), 0.72 (3H, s, H_3-18), 1.03 (3H, s, H_3-19), 5.18 (1H, dd, *J* = 8.4, 15.2 Hz, H-22), 5.04 (1H, dd, *J* = 8.8, 15.2 Hz, H-23), 0.95 (3H, d, *J* = 6.4 Hz, H_3-21), 0.83 (3H, t, *J* = 1.6 Hz, H_3-26), 0.82 (3H, d, *J* = 2.4 Hz, H_3-27) and 0.86 (3H, d, *J* = 7.6 Hz, H_3-29) (Figure 3.64-3.66).

The ^{1}H NMR spectral data of this compound displayed a multiplet at δ 3.51 which conforming to the H-3 position of a sterol. The characteristic signal at δ 5.37 (1H, br s, H-6) signifying the incidence of H-6 olefinic proton of the steroidal skeleton (Figure 3.65). The spectrum showed two singlets at δ 0.70, and 1.03 corresponding to the protons of two tertiary methyl groups at C-18 and C-19, respectively. Two doublets at δ 0.82 (*J* = 2.4 Hz) and 0.86 (*J* = 7.6 Hz) are indicating H_3-27 and H_3-29 protons, while a triplet at δ 0.83 (*J* = 1.6 Hz) demonstrating the presence of H_3-26 proton (Figure 3.66). All the features of the ^{1}H NMR spectral data of LDC-10-2 are in near agreement with the data by Chaturvedula, 2012 and identified as β-sitosterol (Chaturvedula and Prakash, 2012).

In the ^{1}H NMR spectrum of LDC-10-2 two double-doublets at δ 5.18 (*J* = 8.4, 15.2) and 5.04 (*J* = 8.8, 15.2) were revealed which are suggestive of trans coupling with the olefenic protons and vicinal coupling with neighbouring methine protons. The presence of these two olefinic protons at C-22 and C-23 is characteristic of stigmasterol (Chaturvedula and Prakash, 2012) (Table 3.8, Figure 3.65). Thus, LDC-10-2 is a mixture of two compounds in the ratio of 4:1 as evident from the ^{1}H NMR spectral analysis.

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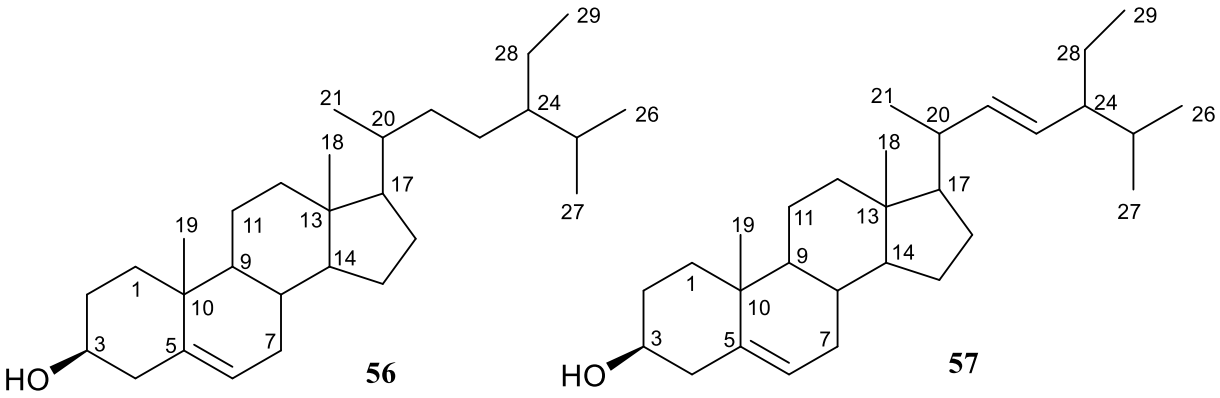

Figure 3.63: Structure of LDC-10-2 as a mixture (4:1 ratio) of β-sitosterol (55) and stigmasterol (56)

Table 3.8: Comparison between the ^{1}H NMR (400 MHz; $CDCl_3$) spectral data of LDC-10-2 (55 & 56, a mixture at 4:1 ratio) and β-sitosterol and stigmasterol (Chaturvedula and Prakash, 2012)

Position	LDC-10-2 δ_H, mult (*J* in Hz)		Stigmasterol δ_H, mult (*J* in Hz)	β-Sitosterol δ_H, mult (*J* in Hz)
	Stigmsterol (56)	β-Sitosterol (55)		
3	3.55, 1H, m	3.55, 1H, m	3.51, tdd (1H, 4.5, 4.2, 3.8)	3.53, tdd (1H, 4.5, 4.2, 3.8)
6	5.37, 2H, br s	5.38, 1H, br s	5.31, t (1H, 6.1)	5.36, t (1H, 6.4)
18	0.72, 3H, s	0.70, 3H, s	0.71 (s, 3H)	0.68 (s, 3H)
19	1.03, 3H, s	1.03, 3H, s	1.03 (s, 3H)	1.01 (s, 3H)
21	0.95, 3H, d (6.4)	0.95, 3H, d (6.4)	0.91, d (3H, 6.2)	0.93, d (3H, 6.5)
22	5.18, 2H, dd (8.4, 15.2)	-	5.14 (m, 1H)	-
23	5.04, 2H, dd (8.8, 15.2)	-	4.98 (m, 1H)	-
26	0.83, 3H, t (1.6)	0.83, 3H, t (1.6)	0.82 (d, 3H, 6.6)	0.83, d (3H, 6.4)
27	0.82, 3H, d (2.4)	0.82, 3H, d (2.4)	0.80 (d, 3H, 6.6)	0.81, d (3H, 6.4)
29	0.86, 3H, d (7.6)	0.86, 3H, d (7.6)	0.83 (t, 3H, 7.1)	0.84, t (3H, 7.2)

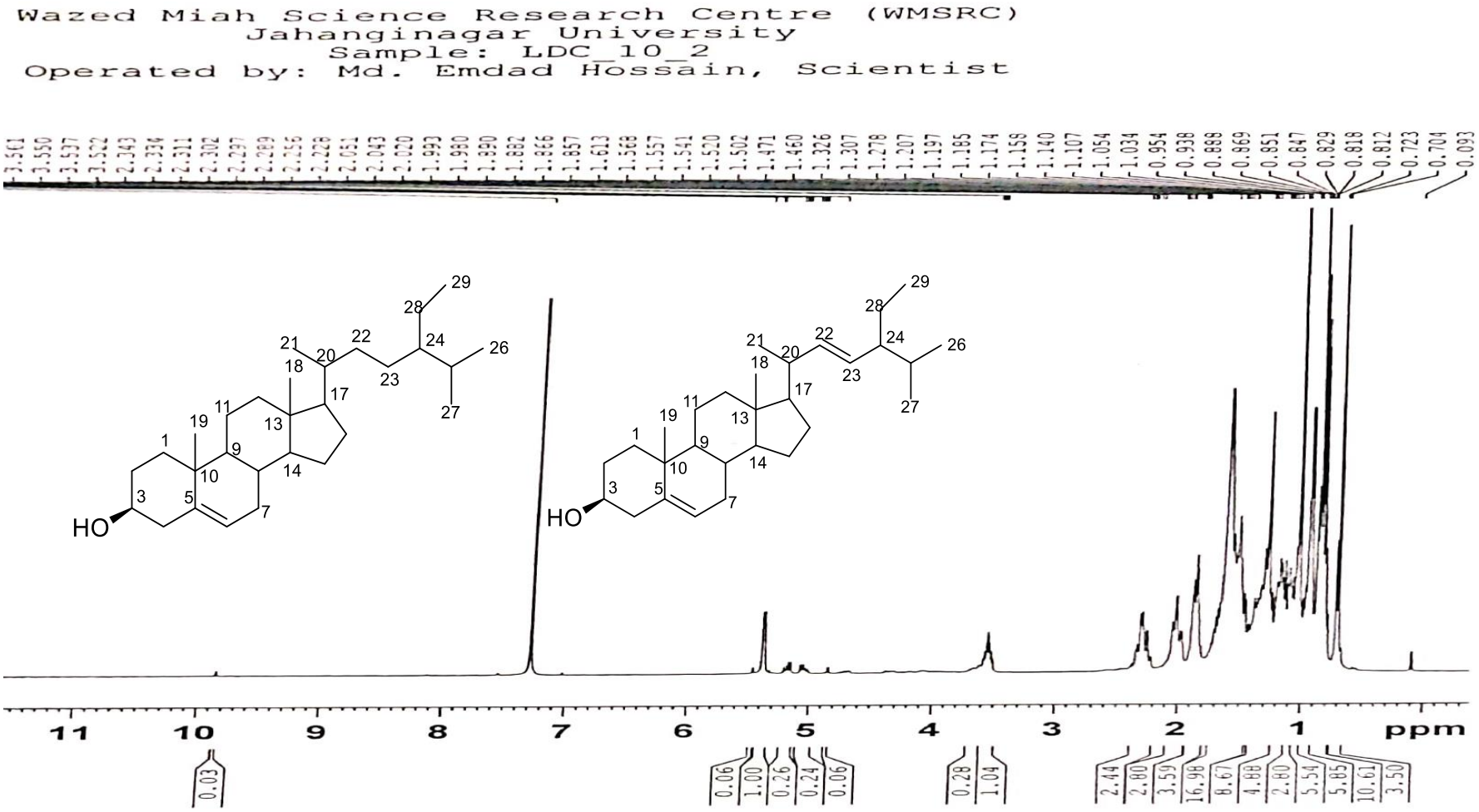

Figure 3.64: Partially expanded ^{1}H NMR (400 MHz; $CDCl_3$) spectrum of LDC-10-2 as β-sitosterol (55) and stigmasterol (56).

Figure 3.65: Partially expanded ^{1}H NMR (400 MHz; $CDCl_3$) spectrum of LDC-10-2 as β-sitosterol (55) and stigmasterol (56).

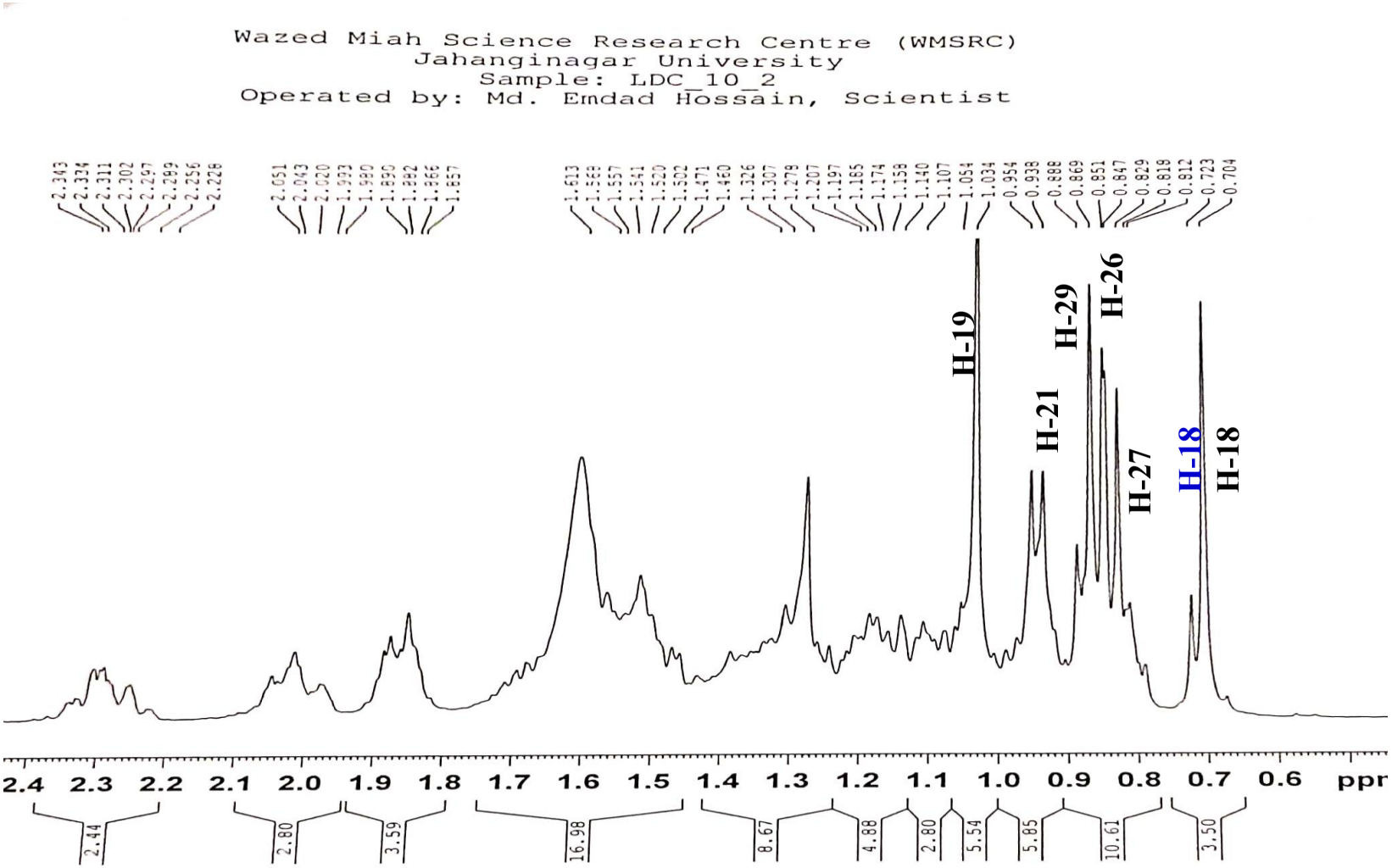

Figure 3.66: Partially expanded ^{1}H NMR (400 MHz; $CDCl_3$) spectrum of LDC-10-2 as β-sitosterol (55) and stigmasterol (56).

3.4 Compounds from L. lancifolia

Chloroform soluble partitionate of *L. lancifolia* was subjected for gel permeation chromatography (GPC) over Sephadex LH-20 and LLC-10-1 was isolated as pure. The obtained ^{1}H NMR spectral data and the comparison with the reference value of ^{1}H NMR spectrum helped us to characterize LLC-10-1 as β-sitosterol (**56**) from *L. lancifolia*.

3.4.1 Characterization of LLC-10-1 as β-sitosterol (56)

LLC-10-1 was isolated from the GPC fraction as colourless crystal. Spraying the developed plate withvanillin-sulfuric acid gave a purple coloured spot when the plate was heated at 110 ^{o}C for several minutes. The compound was found to be soluble in chloroform.

The ^{1}H NMR (400 MHz, $CDCl_3$) spectral data of this compound displayed a multiplet at δ 3.56, the position and multiplicity of which was indicative to H-3 of a steroid nucleus. The typical olefinic H-6 of the steroidal skeleton was evident due to the presence of a broad singlet at δ 5.38 (Figure 3.69). The spectrum also revealed two singlets at δ 0.70, and 1.03 (3H each) assignable to two tertiary methyl groups at C-13 (H_3-18) and C-10 (H_3-19), respectively.

The ^{1}H NMR spectrum showed two doublets centered at δ 0.82 (3H, J = 8.0 Hz) and 0.85 (3H, J = 8.4 Hz) which could be attributed to the methyl groups (H_3-27 and H_3-26) at C-25. The doublet at δ 0.95 (3H, J = 6.4 Hz) was assignable to methyl group (H_3-21) at C-20. On the other hand, the triplet of three proton intensity at δ 0.86 (3H, J = 8.0 Hz) could be assigned to the primary methyl group (H_3-29) attached to C-28 (Figure 3.70). All the features of the ^{1}H NMR spectral data of LLC-10-1 are in near agreement with the data by Chaturvedula, 2012 and identified as β-sitosterol (Chaturvedula and Prakash, 2012).

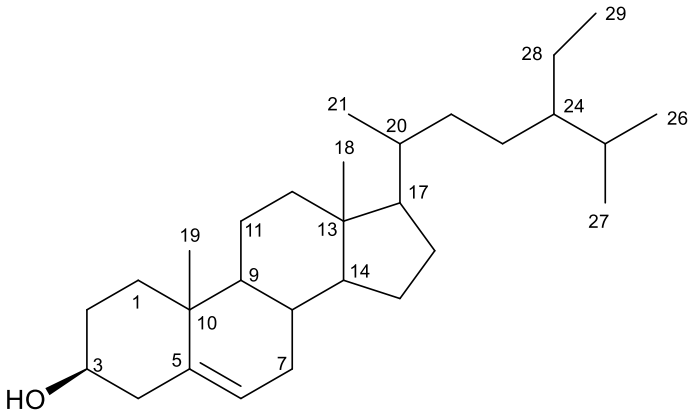

Figure 3.67: Structure of LLC-10-1 as β-sitosterol (55)

Table 3.9: Comparison the ^{1}H NMR (400 MHz; $CDCl_3$) spectral data of LLC-10-1 (56) and β-itosterol (Chaturvedula, 2012)

Position	LlC-10-1 δ_H, mult (*J* in Hz) β-Sitosterol	β-Sitosterol Chaturvedula, 2012 δ_H, mult (*J* in Hz)
3	3.56, 1H, m	3.53, 1H, tdd (4.5, 4.2, 3.8)
6	5.38, 1H, br s	5.36, 1H, t (6.4)
18	0.70, 3H, s	0.68, 3H, s
19	1.03, 3H, s	1.01, 3H, s
21	0.95, 3H, d (6.4)	0.93, 3H, d (6.5)
26	0.85, 3H, d (8.4)	0.83, 3H, d (6.4)
27	0.82, 3H, d (8.0)	0.81, 3H, d (6.4)
29	0.86, 3H, t (8.0)	0.84, 3H, t (7.2)

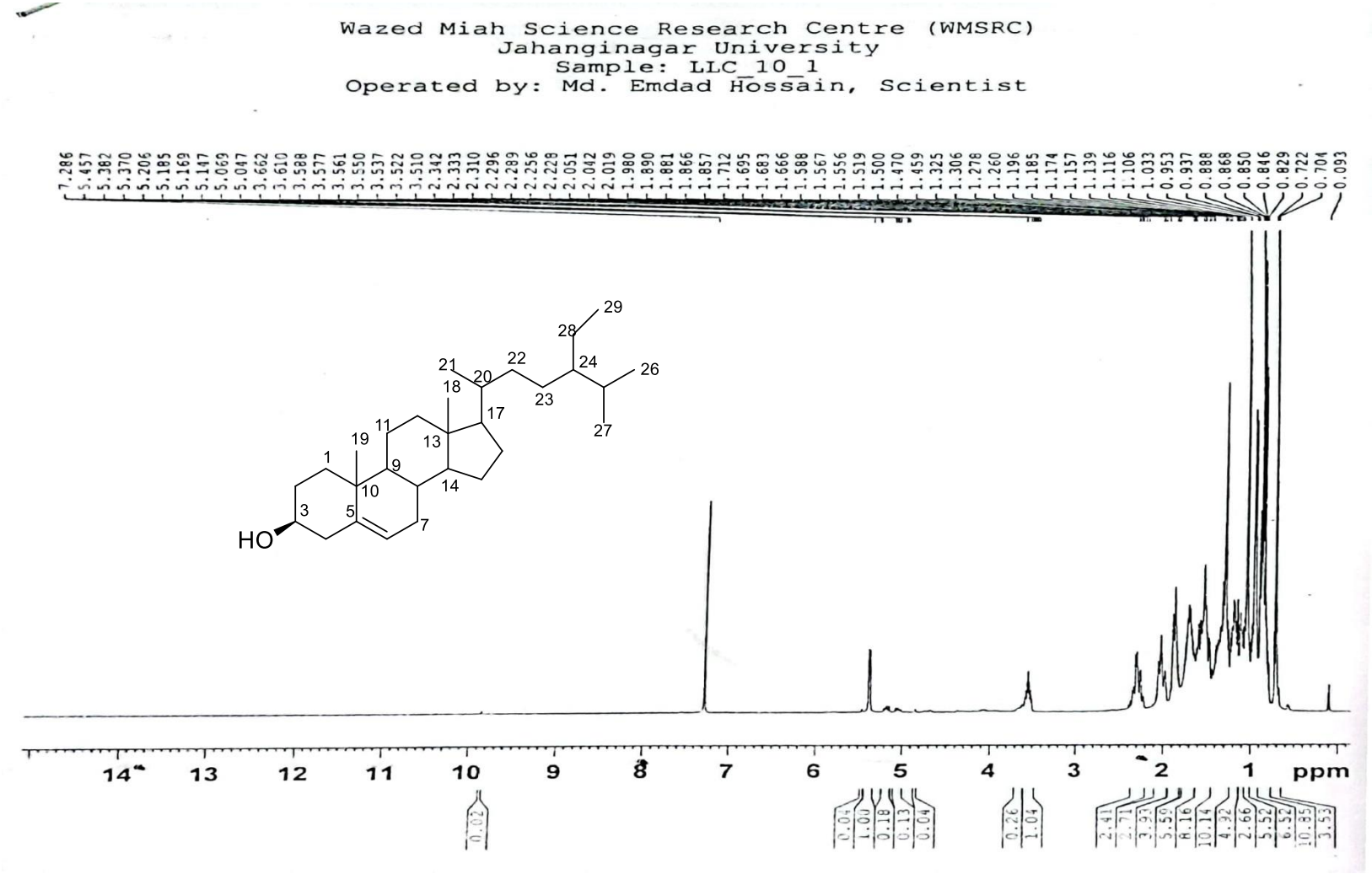

Figure 3.68: Partially expanded ^{1}H NMR (400 MHz; $CDCl_3$) spectrum of LLC-10-1 as β-sitosterol (55).

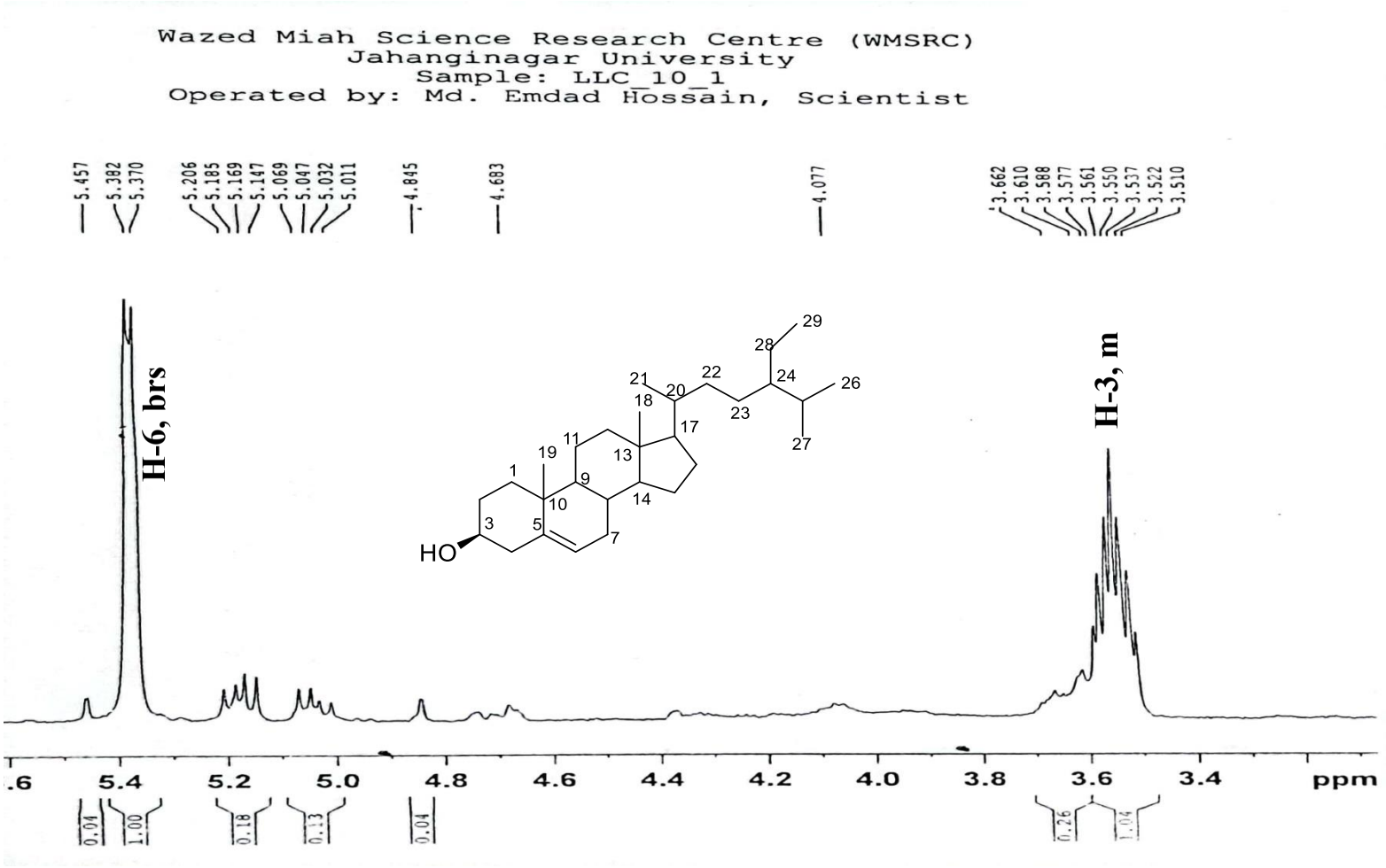

Figure 3.69: Partially expanded ^{1}H NMR (400 MHz; $CDCl_3$) spectrum of LLC-10-1 as β-sitosterol (55).

Figure 3.70: Partially expanded ^{1}H NMR (400 MHz; $CDCl_3$) spectrum of LLC-10-1 as β-sitosterol (55).

Results of Pharmacological Investigation

3.5 Antidiarrheal activity of crude methanolic extracts

3.5.1 Castor oil induced antidiarrhoeal activity

In all the experimental treatment groups (treated with standard loperamide and methanolic extracts) the number of wet feces, total number of feces and total weight of the foecal output were calculated and found reduced with rising of doses. The standard reduced both the total number of wet feces (0.25±0.25) and total number of dry and wet feces (2±0.7) significantly ($p < 0.05$) at a dose of 10 mg/kg bw dose as compared with control.

For methanol extract of *L. deccanensis* (MELD), all the doses reduced the total number of feces significantly at ($p < 0.05$) and % inhibition of wet defecation were 37.10%, 40.32% and 43.55% at 100, 200 and 400 mg/kg bw doses, respectively. In case of diarrheal feces the highest dose (400 mg/kg bw) showed a significant reduction. Percentage of fecal output was decreased from 46.62% to 40.14% with the increasing of doses for *L. deccanensis.*

In case of methanol extract of *L. lancifolia* (MELL) total number of diarrheal feces inhibited for all the doses but was found significantly at 400 mg/kg bw dose and % inhibition of wet defecation were 38.71%, 40.32% and 45.16% at 100, 200 and 400 mg/kg bw doses, respectively. Percentage of fecal output was decreasing from 74.02% to 62.27% with the increasing of doses for *L. lancifolia.* For methanol extract of *L. glutinosa* (MELG) total number of diarrheal feces was reduced but the results were insignificant and % inhibition of wet defecation were 14.52%, 16.13% and 32.26% at 100, 200 and 400 mg/kg bw doses, respectively. Percentage of fecal output was decreased from 81.14% to 64.06% with the increasing of doses for *L. glutinosa.*

Methanol extract of *L. monopetala* (MELM) showed significant reduction of wet feces (9±0.71) at 400 mg/kg bw dose ($p < 0.05$) and % inhibition of wet defecation were 33.87%, 37.10% and 41.94% at 100, 200 and 400 mg/kg bw doses, respectively. Percentage of fecal output was decreased from 55.87% to 46.26% with increasing of doses for *L. monopetala.*

Wet feces were markedly reduced by the studied plant extracts and maximum diarrheal inhibition were shown by MELD, MELL, MELG and MELM at 400 mg/kg bw where % inhibition of wet defecation were 43.55%, 45.16%, 32.26% and 41.94% respectively. Moderate inhibition of fecal output was shown by MELD, MELL, MELG and MELM extracts at 400 mg/kg bw dose and that were 40.14%, 62.27%, 64.06%, 46.26% respectively (Table 3.10, Figure 3.71).

Table 3.10: Effect of the methanol extracts of *L. glutinosa* (MELG), *L. monopetala* (MELM), *L. deccanensis* (MELD) and *L. lancifolia* (MELL) on castor oil induced diarrhea in mice

Treatment Groups	Dose (mg/kg, p.o)	Total number of feces	Total number of wet feces	% inhibition of wet defecation	% of Fecal Output
Control	-	22.5±1.85	15.5±2.02		
Loperamide	3	2±0.71*	0.25±0.25*	98.39	12.28
MELG_1	100	19.5±1.041	13.25±1.32	14.52	81.14
MELG_2	200	17.5±0.65	13±1.41	16.13	77.58
MELG_3	400	15.5±3.66*	10.5±3.23	32.26	64.06
MELM_1	100	17±0.71	10.25±0.95	33.87	55.87
MELM_2	200	14±1.0*	9.75±0.63	37.10	52.31
MELM_3	400	14±1.68*	9±0.71*	41.94	46.26
MELD_1	100	12.25±2.06*	9.750±1.60	37.10	46.62
MELD_2	200	10.25±1.70*	9.25±1.89	40.32	39.86
MELD_3	400	9.75±1.49*	8.75±1.65*	43.55	40.14
MELL_1	100	16.25±1.03	9.5±0.28	38.71	74.02
MELL_2	200	16.75±1.32	9.250±1.11	40.32	70.11
MELL_3	400	14.75±0.85*	8.5±0.5*	45.16	62.27

All values are stated as mean ± SEM (n = 5); One way ANOVA test were carried out for data analysis, Here, * values are statistically significant at $p < 0.05$

3.5.2 Gastrointestinal motility by using barium sulphate meal

In this test, the gastrointestinal motility was reduced dose dependently by methanol extracts of *L. deccanensis* (MELD), *L. lancifolia* (MELL), *L. glutinosa* (MELG) and *L. monopetala* (MELM). The maximum peristaltic inhibition was observed 26.26%, 33.22%, 32.36% and 22.52% for 400 mg/kg bw of MELD, MELL, MELG and MELM extracts, respectively. The peristaltic indices were 59.1%, 63.0%, 59.0% and 79.0% for 400 mg/kg bw of MELD, MELL, MELG and MELM extracts, respectively compared to control (90.0%) and standard (66.7%). For all the plant extracts % inhibition of gastrointestinal motility and % peristalsis index are comparable to standard (Table 3.11, Figure 3.72).

Table 3.11: Effect of the methanol extracts of *L. glutinosa* (MELG), *L. monopetala* (MELM), *L. deccanensis* (MELD) and *L. lancifolia* (MELL) on gastrointestinal motility in mice

Treatment Groups	Dose (mg/kg, p.o)	Length (cm) of small intestine	Distance (cm) traveled by Barium Sulphate	% of Inhibition	Peristalsis Index (%)
Control	-	53.1±7.12	47.943±4.06		90.3
Loperamide	3	52.070±1.80	34.73±1.59	27.56	66.7
MELG_1	100	50.165±1.10	35.242±2.66	26.49	70.3
MELG_2	200	51.435±2.67	32.933±2.31	31.31	64.0
MELG_3	400	54.928±3.38	32.428±6.66	32.36	59.0
MELM_1	100	48.135±1.74	41.225±2.39	14.01	85.6
MELM_2	200	48.955±0.62	39.688±3.57	17.22	81.1
MELM_3	400	47.05±1.29	37.148±3.64	22.52	79.0
MELD_1	100	47.158±3.31	44.45±1.80	7.29	94.3
MELD_2	200	47.568±2.98	37.748±1.66	21.26	79.4
MELD_3	400	59.860±2.87	35.353±5.06	26.26	59.1
MELL_1	100	48.013±2.10	35.268±6.14	26.44	73.5
MELL_2	200	53.658±2.10	32.385±9.53	32.45	60.4
MELL_3	400	50.8±1.56	32.018±7.42	33.22	63.0

All values are stated as mean ± SEM (n = 5); One way ANOVA test were carried out for data analysis, here, * values are statistically significant at $p < 0.05$

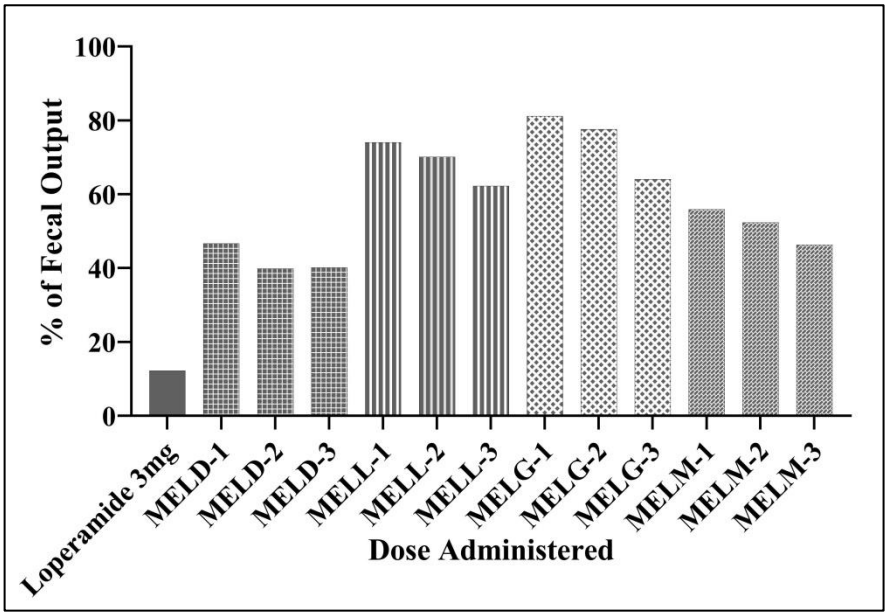

Figure 3.71: Percentage of fecal output of methanol extracts of *L. deccanensis* (MELD), *L. lancifolia* (MELL), *L. glutinosa* (MELG) and *L. monopetala* (MELM) on castor oil induced diarrheal mice.

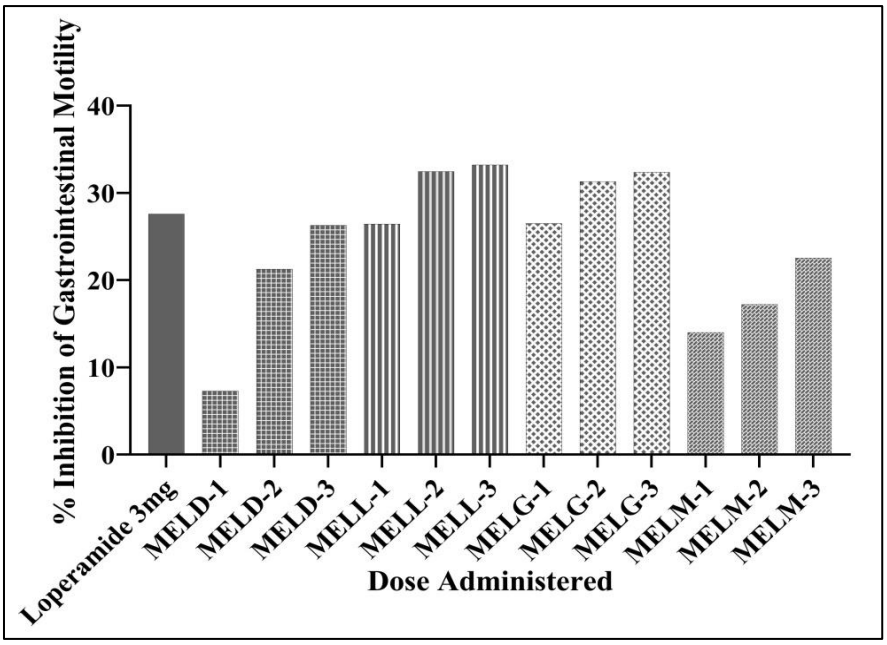

Figure 3.72: Percentage inhibition of gastrointestinal motility of methanol extracts of *L. deccanensis* (MELD), *L. lancifolia* (MELL), *L. glutinosa* (MELG) and *L. monopetala* (MELM) using barium sulfate meal.

3.6 Analgesic activity of crude methanolic extracts

3.6.1 Peripheral analgesic activity (Acetic acid induced writhing method)

The analgesic effect of *L. deccanensis*, *L. lancifolia*, *L. glutinosa* and *L. monopetala* was investigated in three different animal models of analgesia. In the first animal model (acetic-acid induced writhing model), pain is induced by intra-peritoneal administration of acetic acid and analgesic activity was evaluated by counting number of writhing. The obtained data revealed from both the doses of methanol extract of *L. deccanensis* (MELD) showed a decrease of pain sensation induced by acetic acid in a dose dependent manner and the results are highly significant with $p<0.001$. The % pain inhibition for 100 mg/kg bw of MELD was measured as 37.28% and it was measured as 79.66% for 200 mg/kg bw. In case of methanol extract of *L. lancifolia* (MELL), both the doses showed significant ($p<0.01$) pain reduction with % inhibition of 69.45% and 77.96% for 100 mg/kg and 200 mg/kg bw, respectively when compared untreated control group.

Table 3.12: Analgesic Activity of methanol extract of *L. deccanensis* (MELD), *L. lancifolia* (MELL), *L. glutinosa* (MELG) and *L. monopetala* (MELM) by acetic acid induced writhing test on animal model.

Treatment groups	Dose (mg/kg, p.o.)	No. of writhing	% of inhibition
Control		14.75±5.56	
Indomethacin	10	3.00±2.16***	79.66 %
MELD_1	100	9.25±1.26***	37.28 %
MELD_2	200	3.00±2.94***	79.66 %
MELL_1	100	4.50±1.29**	69.45 %
MELL_2	200	3.25±3.59**	77.96 %
MELG_1	100	8.63±1.49**	41.52 %
MELG_2	200	4.50±1.29*	69.49 %
MELM_1	100	9.75±2.75*	33.89 %
MELM_2	200	9.00±2.16*	38.98 %

All the values are stated as mean ± STDEV. (Where, n=4); significance at ***$p<0.001$, **$p<0.01$, *$p<0.05$ as compared to control

Methanol extract of *L. glutinosa* (MELG) showed significant ($p<0.05$) protection from pain with 41.52% and 69.49% for the doses of 100 mg/kg and 200 mg/kg bw, respectively. Both the doses of methanol extract of *L. monopetala* (MELM) showed significant ($p<0.05$) lowering of the pain sensation with 33.89% and 38.98% for the doses of 100 mg/kg bw and 200 mg/kg bw, respectively. Table 3.14 shows the effects of the methanol extract of *L. deccanensis* (MELD), *L.lancifolia* (MELL), *L. glutinosa* (MELG) and *L. monopetala* (MELM) on acetic acid-induced writhing in mice at the doses of 100 and 200 mg/kg bw Both the doses of extracts showed significant reduction of squirming ($p<0.001$, $p<0.01$ and $p<0.05$) as compared to control in a dose dependent manner. The percent inhibition of writhing for standard indomethacin at the dose of 10 mg/kg bw was 79.66%, while for the plant extracts maximum percent inhibitions were observed at 200 mg/kg bw and they were 79.66%, 77.96%, 69.49% and 38.98% for MELD, MELL, MELG and MELM, respectively (Table 3.12, Figure 3.73).

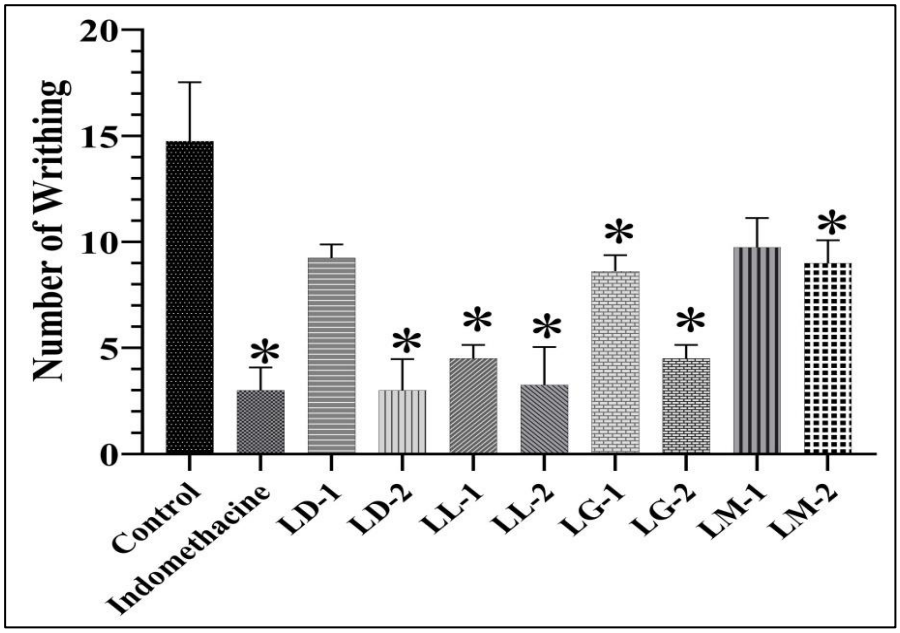

Figure 3.73: Analgesic activity of methanol extract of *L. deccanensis* (MELD), *L. lancifolia* (MELL), *L. glutinosa* (MELG) and *L. monopetala* (MELM) by acetic acid induced writhing test on animal model. All the values are stated as mean ± STDEV. (n=4); (*p<0.001, **p<0.01, *p<0.05 as compared to control).**

3.6.2 Central analgesic activity (Hot plate method)

The analgesic effect of *L. deccanensis*, *L. lancifolia*, *L. glutinosa* and *L. monopetala* was investigated in three different animal models of analgesia. In the second animal model (Eddy's hot plate method), pain is induced by heat and analgesia was assessed by counting the time required for the initiation of the reaction. The effects of analgesia, as reaction time for each mouse, for methanol extracts of *L. deccanensis*, *L. lancifolia*, *L. glutinosa* and *L. monopetala* were shown by the tables 3.13-3.17.

When various doses (100, 200 and 400 mg/kg bw) were given to animals and were subjected to induce pain by heat, animals produced an increased reaction time in a dose dependent manner compared to the control group. The pain-relieving activity data (Hot plate method) were presented as reaction time in seconds at 0, 30, 60, 120 and 180 min after treatment with standard tramadol 10 mg/kg bw and plant extracts the studied *Litsea* species.

The present research discloses a moderate analgesic activity of all methanol extracts of the four different species when compared to control group but the results were insignificant. The table 3.18 represents the percentage of analgesic activity of methanol extract of *L. deccanensis* (MELD), *L. lancifolia* (MELL), *L. glutinosa* (MELG) and *L. monopetala* (MELM)

Table 3.13: Reaction times for each mouse in seconds at different times of normal control group and standard control group.

Reaction time in seconds at time (min) of **Normal control group**						Reaction time in seconds at time (min) of **Standard (Tramadol 10mg/kg)**					
Mice no	T-0	T-30	T-60	T-120	T-180	Mice no	T-0	T-30	T-60	T-120	T-180
1	6.1	5.46	5.58	4.92	5.01	1	3.3	4.77	8.16	8.97	8.52
2	5.4	2.75	4.32	3.4	3.18	2	4	7.5	5.53	8.51	8.89
3	5	2.35	2.14	4.42	2.95	3	6.2	4.6	9.57	4.44	5.06
4	3.6	2.6	6.58	3.24	3.23	4	3.37	5.27	7.63	8.85	5.41
5	5.2	4.06	6.23	4.02	5.9	5	2.31	5.88	5.21	5.53	8.81
Mean	5.06	3.444	4.97	4	4.054	Mean	3.836	5.604	7.22	7.26	7.338
STDEV	0.92	1.31	1.80	0.70	1.32	STDEV	1.45	1.17	1.84	2.12	1.93

Table 3.14: Reaction times for each mouse in seconds at different times of treatment groups treated with 100 mg/kg, 200 mg/kg and 400 mg/kg bw doses of MELD

	Reaction time in seconds at time (min) of **100 mg/kg** of MELD					Reaction time in seconds at time (min) of **200 mg/kg** of MELD					Reaction time in seconds at time (min) of **400 mg/kg** of MELD				
Mice no	T-0	T-30	T-60	T-120	T-180	T-0	T-30	T-60	T-120	T-180	T-0	T-30	T-60	T-120	T-180
1	4.4	2.85	4.12	4.79	5.9	4.46	3.4	3.68	5.17	4.57	3.55	4.28	4.07	6.54	6.29
2	2.7	2.42	3.7	5.88	4.36	2.38	2.74	4.79	4.84	6.69	3.91	3.87	3.15	6.78	4.84
3	3.9	5.5	3.09	4.35	2.39	2.21	3.25	3.58	3.29	3.49	3.04	1.89	6.95	3.32	5.05
4	3.19	2.63	3.89	3.38	5.02	5.21	3.04	3.89	5	5.22	5.02	3.75	3.77	4.64	8.08
5	3.07	3.2	4	4.11	5.72	4.33	4	4.81	5.34	3.99	3.52	4.84	6.3	4.57	4.12
Mean	3.45	3.32	3.76	4.50	4.68	3.72	3.29	4.15	4.73	4.79	3.81	3.73	4.85	5.17	5.68
SD	0.69	1.25	0.41	0.92	1.42	1.34	0.47	0.60	0.83	1.24	0.74	1.11	1.67	1.46	1.55

Table 3.15: Reaction times for each mouse in seconds at different times of treatment groups treated with 100 mg/kg, 200 mg/kg and 400 mg/kg bw doses of MELL

	Reaction time in seconds at time (min) of **100 mg/kg** of MEL.L					Reaction time in seconds at time (min) of **200 mg/kg** of MEL.L					Reaction time in seconds at time (min) of **400 mg/kg** of MEL.L				
Mice no	T-0	T-30	T-60	T-120	T-180	T-0	T-30	T-60	T-120	T-180	T-0	T-30	T-60	T-120	T-180
1	2.26	3.6	4.44	2.42	4.09	5.22	5.74	4.04	4.11	4.07	2.36	5.61	5.74	6.95	6.67
2	3.16	3.7	3.9	4.92	4.19	2.23	3.41	3.42	5.67	4.92	2.8	4.18	6.85	6.1	6.67
3	2.43	3.28	3.38	3.87	3.71	4.61	2.73	5.78	4.86	4.56	6.08	3.92	5.67	5.27	8.25
4	3.84	3.2	3.08	5.64	4.77	3.81	3.71	3.92	5.3	5.61	4.21	4.64	5.02	6.96	6.44
5	4.78	3	3.86	3.72	4.17	3.21	6.29	5.19	3.15	5.22	4.96	4.83	5.45	5.34	5.13
Mean	3.29	3.36	3.73	4.11	4.19	3.82	4.38	4.47	4.62	4.88	4.08	4.64	5.75	6.12	6.63
SD	1.04	0.29	0.52	1.23	0.38	1.17	1.55	0.98	1.01	0.59	1.53	0.65	0.68	0.83	1.11

Table 3.16: Reaction times for each mouse in seconds at different times of treatment groups treated with 100 mg/kg, 200 mg/kg and 400 mg/kg bw doses of MELG.

	Reaction time in seconds at time (min) of **100 mg/kg** of MEL.G					Reaction time in seconds at time (min) of **200 mg/kg** of MEL.G					Reaction time in seconds at time (min) of **400 mg/kg** of MEL.G				
Mice no	T-0	T-30	T-60	T-120	T-180	T-0	T-30	T-60	T-120	T-180	T-0	T-30	T-60	T-120	T-180
1	1.64	2.5	2.42	2.92	3.22	5.99	4.44	3.02	2.44	3.05	4.07	4.86	4.06	5.38	5.55
2	4.79	4.33	4.46	4.91	4.21	5.02	6.34	6.81	6.97	6.08	3.72	3.15	4.25	4.63	5.67
3	2.12	2.15	2.67	3.9	4.17	3.7	4.08	2.86	5.46	5.28	3.44	4.45	5.24	5.48	5.59
4	2.04	2.3	2.57	2.89	4.24	2.29	4.2	5.17	5.65	6.28	4.17	4.8	4.35	4.17	4.46
5	4.92	4.61	4.01	3.33	5.37	2.62	3.12	4.42	4.51	5.16	5.2	5.06	6.04	5.7	5.48
Mean	3.10	3.18	3.23	3.59	4.24	3.92	4.44	4.46	5.01	5.17	4.12	4.46	4.79	5.07	5.35
SD	1.61	1.19	0.94	0.84	0.76	1.57	1.18	1.63	1.68	1.28	0.67	0.77	0.83	0.65	0.50

Table 3.17: Reaction times for each mouse in seconds at different times of treatment groups treated with 100 mg/kg, 200 mg/kg and 400 mg/kg bw doses of MELM

	Reaction time in seconds at time (min) of **100 mg/kg** of MEL.M					Reaction time in seconds at time (min) of **200 mg/kg** of MEL.M					Reaction time in seconds at time (min) of 4**00 mg/kg** of MEL.M				
Mice no	T-0	T-30	T-60	T-120	T-180	T-0	T-30	T-60	T-120	T-180	T-0	T-30	T-60	T-120	T-180
1	5.2	3.33	3.85	3.72	4.36	2.06	2.31	4.36	5.13	4.21	3.62	3.25	3.03	3.77	4.02
2	1.97	2.59	2.87	3.06	4.28	3.69	3.63	3.69	3	5.41	3.88	4.01	5.98	5.79	6.53
3	3.4	3.86	2.17	3.3	4.19	3.98	3.99	4.27	3.7	4.71	2.49	3.39	3.39	3.81	4.97
4	2.63	3.74	3.47	3.72	6.9	3.42	4.72	3.85	3.8	5.71	3.09	3.43	3.23	3.93	4.02
5	1.81	2.21	3.98	3.96	3.34	2.67	3.91	4.23	5.36	4.57	2.55	3.41	3.35	3.6	5.5
Mean	3.00	3.15	3.27	3.55	4.61	3.16	3.71	4.08	4.20	4.92	3.13	3.50	3.80	4.18	5.01
SD	1.38	0.72	0.75	0.36	1.34	0.79	0.88	0.29	1.01	0.62	0.62	0.29	1.23	0.91	1.06

In control group, the animals were untreated and only allowed for the vehicle, so the latency time was very short, while the latency time for the animals treated with standard (tramadol 10 mg/kg) increased significantly ($p < 0.05$) at 30 mins, 60 mins, 120 mins and 180 mins of the study period. The % pain inhibition for tramadol 10 mg/kg bw was 81.23%.

Out of the four plants all the plants at 100, 200, and 400 mg/kg bw doses, increased the latency time but was not significant. In the present study, methanol extract of *L deccanensis* (MELD) revealed 15.56%, 18.27% and 40.25% pain inhibition at 180 mins at 100, 200 and 400 mg/kg bw doses respectively. For methanol extract of *L. lancifolia* (MELL) the percent inhibition at 100 mg/kg, 200 mg/kg and 400 mg/kg doses were 3.46%, 20.49% and 39.01%, respectively. Methanol extract of *L. glutinosa* (MELG) showed 4.93%, 22.72% and 32.10% pain inhibition with the increasing of dose. At 100, 200 and 400 mg/kg bw doses of methanol extract of *L. monopetala* (MELM) produced 13.83%, 21.48% and 23.70% inhibitory effect on central pain sensation.

At 180 minute time period of pain sensation, percent inhibition were 40.25%, 39.01%, 32.1% and 23.7% for methanol extracts of *L. deccanensis, L. lancifolia, L. glutinosa* and *L. monopetala*, respectively, while for standard pentazocine hydrochloride it was 81.23%. Maximum analgesia was revealed for 400 mg/kg bw of all the plant extracts. Percent protection was observed in a dose dependent manner, for methanol extracts of *L. deccanensis* they were 15.56%, 18.27% and 40.25%, for *L. lancifolia* they are 3.46%, 20.49% and 39.01%, for *L. glutinosa* they are 4.93%, 22.72% and 32.1% while for *L.*

monopetala they were 13.83%, 21.48% and 23.7% as stated in the table 3.18 and figure 3.74.

Table 3.18: Effect of *Litsea deccanensis* (MELD), *L. lancifolia* (MELL), *L. glutinosa* (MELG) and *L. monopetala* (MELM) on heat-induced pain in mice models.

Treatment groups	Dose (mg/kg), p.o.	Reaction time in seconds at time (min)					% inhibition at 180 min
		0	30	60	120	180	
Control	-	5.06±0.92	3.44±1.31	4.97±1.80	4.00±0.70	4.05±1.32	
Tramadol	10	3.84±1.45	5.06±1.17*	7.22±1.84*	7.26±2.12*	7.34±1.93*	81.23
MELD_1	100	3.45±0.69	3.32±1.25	3.76±0.41	4.50±0.92	4.68±1.42	15.56
MELD_2	200	3.72±1.34	3.29±0.47	4.15±0.60	7.65±0.83	4.79±1.24	18.27
MELD_3	400	3.81±0.74	3.73±1.11	4.85±1.67	5.17±1.46	5.68±1.55	40.25
MELL_1	100	3.29±1.04	3.36±0.29	3.73±0.52	4.11±1.23	4.19±0.38	3.46
MELL_2	200	3.82±1.17	4.38±1.55	4.47±0.98	4.62±1.01	4.88±0.59	20.49
MELL_3	400	4.08±1.53	4.64±0.65	5.75±0.68	6.12±0.83	6.63±1.11	39.01
MELG_1	100	3.1±1.61	3.18±1.19	3.23±0.94	3.59±0.84	4.24±0.76	4.93
MELG_2	200	3.92±1.57	4.44±1.18	4.46±1.63	5.01±1.68	5.17±1.28	22.72
MELG_3	400	4.12±0.67	4.46±0.77	4.79±0.83	5.07±0.65	5.35±0.50	32.10
MELM_1	100	3.00±1.38	3.15±0.72	3.27±0.75	3.55±0.36	4.61±1.34	13.83
MELM_2	200	3.16±0.79	3.71±0.88	4.08±0.29	4.20±1.01	4.92±0.62	21.48
MELM_3	400	3.13±0.62	3.50±0.29	3.8±1.23	4.18±0.91	5.01±1.06	23.70

All the values are stated as mean ± STDEV. (Where, n=5); significance at ***p<0.001, **p<0.01, *p<0.05 as compared to control

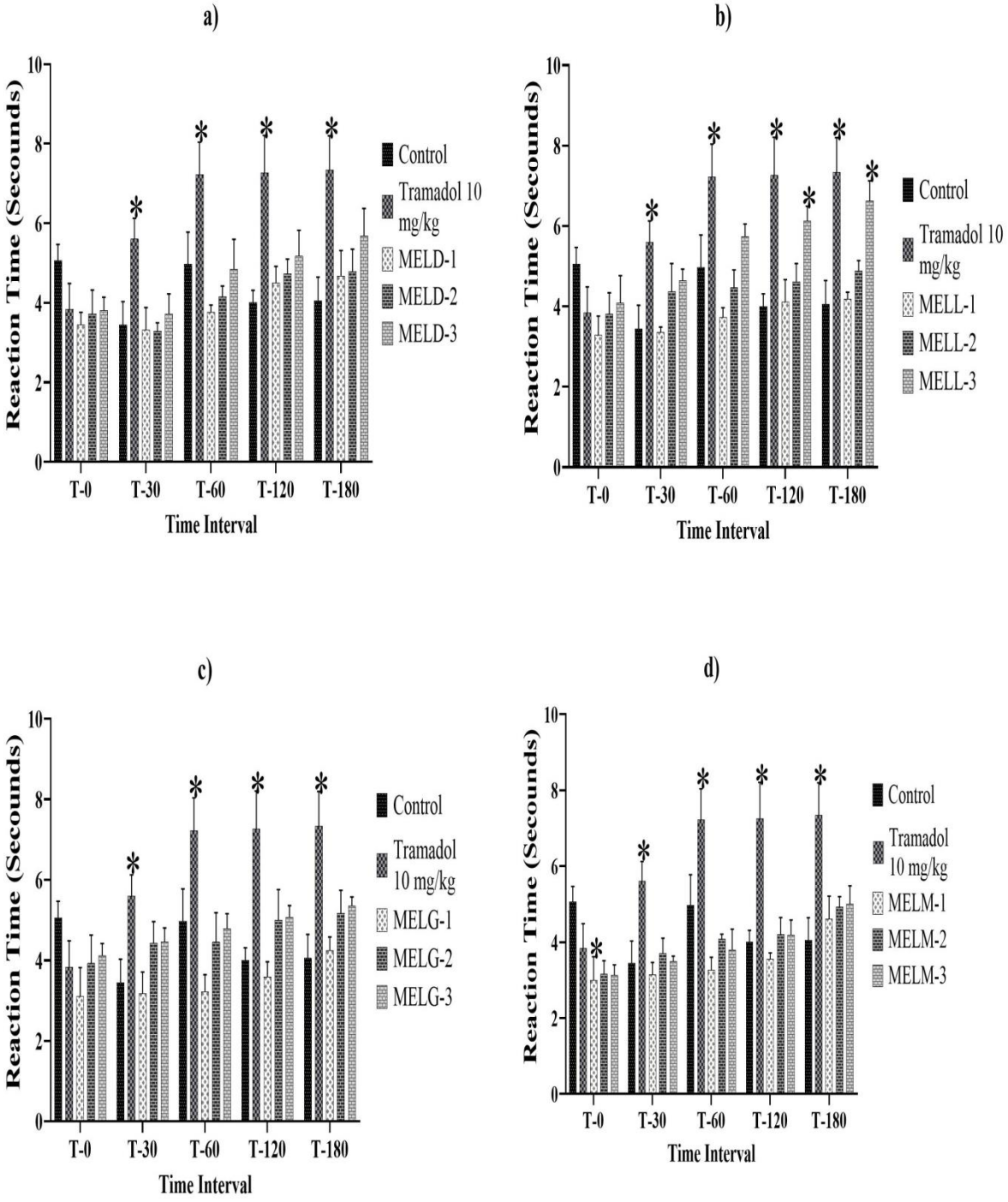

Figure 3.74: Reaction time in seconds at different time (min) of treatment groups that were treated with 100 mg/kg, 200 mg/kg and 400 mg/kg bw doses of a) methanol extract of *L. deccanensis* (MELD); b) methanol extract of *L. lancifolia* (MELL); c) methanol extract of *L. glutinosa* (MELG); and d) methanol extract of *L. monopetala* (MELM) on heat-induced pain (Eddy's hot plate) in mice models.

3.6.3 Central analgesic activity (Formalin induced paw licking method)

The analgesic effect of *L. deccanensis*, *L. lancifolia*, *L. glutinosa* and *L. monopetala* was investigated in three different animal models of analgesia. In the third model (formalin induced paw leaking test), pain was induced by formalin and pain inhibition effect was investigated by calculating the total time of leaking and biting their paws.

To investigate analgesia by using formalin test is advantageous because it includes responses in two different phases named as “an early phase” and “a late phase”. The early phase indicates neurogenic while the late phase indicates inflammatory pain (Hunskaar and Hole, 1987) and the by using these models the studied plant extracts may also can be classified as neurogenic or inflammatory pain reliever. The effects of analgesia for methanol extracts of *L. deccanensis*, *L. lancifolia*, *L. glutinosa* and *L. monopetala* were showen by the table 3.19-3.22. When both the doses (100 and 200 mg/kg bw) were given to animals and were subjected to induce pain by injecting 2% formalin to their hind paw. Animals produced a decreased licking and biting time in a dose dependent manner compared to the control group.

The pain-relieving activity data (Formalin method) were presented as licking and biting time in seconds at early and late phases after treatment with standard indomethacin 10 mg/kg bw and plant extracts of the studied *Litsea* species. The paw licking time was significantly reduced in the inflammatory (late) than as neurogenic (early) phase, which indicates the plants may reduce inflammatory pain.

In both the early phase and late phase, reaction times for licking and biting hind paw were decreased with the increment of the doses (from 100 mg/kg bw to 200 mg/kg bw), but in the late phase (20-30 min) the reaction time decreased significantly ($p < 0.05$) with the increment of doses for all the studied plant extracts as well as standard indomethacin at 10 mg/kg bw.

Table 3.19: Reaction times for licking and biting hind paw by each mouse in seconds at two different time phases of normal, standard and treatment groups that were treated with 100 mg/kg and 200 mg/kg bw doses of MELD

	Normal		Standard		MELD 100 mg/kg		MELD 200 mg/kg	
Mice	(0-5) min	(20-30) min	(0-5) min	(20-30) min	(0-5) min	(20-30) min	(0-5) min	(20-30) min
1	121.00	86.00	81.00	41.00	92.00	123.00	99.00	61.00
2	131.00	79.00	69.00	53.00	92.00	152.00	66.00	87.00
3	74.00	145.00	91.00	57.00	113.00	122.00	71.00	22.00
4	21.00	123.00	84.00	22.00	106.00	125.00	74.00	86.00
5	118.00	124.00	96.00	68.00	66.00	120.00	127.00	69.00
Mean	103.00	111.00	84.00	48.00	93.00	104.00	87.00	69.00
STDEV	0.02	0.02	0.01	0.01	0.01	0.04	0.02	0.02

Table 3.20: Reaction times for licking and biting hind paw by each mouse in seconds at two different time phases of normal, standard and treatment groups that were treated with 100 mg/kg and 200 mg/kg bw doses of MELL

	Normal		Standard		MELL 100 mg/kg		MELL 200 mg/kg	
Mice	(0-5) min	(20-30) min	(0-5) min	(20-30) min	(0-5) min	(20-30) min	(0-5) min	(20-30) min
1	121.00	86.00	81.00	41.00	55.00	40.00	52.00	16.00
2	131.00	79.00	69.00	53.00	45.00	34.00	56.00	42.00
3	74.00	145.00	91.00	57.00	76.00	5.00	67.00	38.00
4	21.00	123.00	84.00	22.00	90.00	25.00	80.00	24.00
5	118.00	124.00	96.00	68.00	88.00	64.00	72.00	3.00
Mean	103.00	111.00	84.00	48.00	70.00	33.00	65.00	24.00
STDEV	0.02	0.02	0.01	0.01	0.01	0.02	0.01	0.01

Table 3.21: Reaction times for licking and biting hind paw by each mouse in seconds at two different time phases of normal, standard and treatment groups that were treated with 100 mg/kg and 200 mg/kg bw doses of MELG

	Normal		Standard		MELG 100 mg/kg		MELG 200 mg/kg	
Mice	(0-5) min	(20-30) min	(0-5) min	(20-30) min	(0-5) min	(20-30) min	(0-5) min	(20-30) min
1	121.00	86.00	81.00	41.00	58.00	35.00	59.00	34.00
2	131.00	79.00	69.00	53.00	87.00	73.00	64.00	42.00
3	74.00	145.00	91.00	57.00	37.00	15.00	68.00	20.00
4	21.00	123.00	84.00	22.00	118.00	93.00	61.00	47.00
5	118.00	124.00	96.00	68.00	45.00	29.00	64.00	90.00
Mean	103.00	111.00	84.00	48.00	69.00	49.00	63.00	46.00
STDEV	0.02	0.02	0.01	0.01	0.02	0.02	0.002	0.02

Table 3.22: Reaction times for licking and biting hind paw by each mouse in seconds at two different time phases of normal, standard and treatment groups that were treated with 100 mg/kg and 200 mg/kg bw doses of MELM

	Normal		Standard		MELM 100 mg/kg		MELM 200 mg/kg	
Mice	(0-5) min	(20-30) min	(0-5) min	(20-30) min	(0-5) min	(20-30) min	(0-5) min	(20-30) min
1	121.00	86.00	81.00	41.00	62.00	6.00	18.00	34.00
2	131.00	79.00	69.00	53.00	44.00	18.00	64.00	15.00
3	74.00	145.00	91.00	57.00	87.00	8.00	29.00	00.00
4	21.00	123.00	84.00	22.00	78.00	7.00	67.00	00.00
5	118.00	124.00	96.00	68.00	79.00	32.00	102.00	00.00
Mean	103.00	111.00	84.00	48.00	70.00	14.00	56.00	9.00
STDEV	0.02	0.02	0.01	0.01	0.01	0.01	0.02	0.01

Table 3.23: Effect of methanol extract of *L. deccanensis* (MELD), methanol extract of *L. lancifolia* (MELL), methanol extract of *L. glutinosa* (MELG) and methanol extract of *L. monopetala* (MELM) on chemical-induced pain (formalin) in mice models.

Treatment groups	Dose (mg/kg, p.o.)	Paw licking at Early Phase (0-5 min)		Paw licking at Late Phase (20-30 min)	
		Time spent to lick or bite (sec)	Percentage (%) inhibition	Time spent to lick or bite (sec)	Percentage (%) inhibition
Control	-	103.00 ± 0.02		111.00 ± 0.02	
indomethacin	10	84.00 ± 0.01	18	48.00 ± 0.01	57
MELD_1	100	93.00 ± 0.01	10	104.00 ± 0.04	6
MELD_2	200	87.00 ± 0.02	16	69.00 ± 0.02	38
MELL_1	100	70.00 ± 0.01	32	33.00 ± 0.02*	16
MELL_2	200	65.00 ± 0.01	37	24.00 ± 0.01*	78
MELG_1	100	69.00 ± 0.02	33	49.00 ± 0.02*	56
MELG_2	200	63.00 ± 0.02	39	46.00 ± 0.02*	59
MELM_1	100	70.00 ± 0.01	32	14.00 ± 0.01*	87
MELM_2	200	56.00 ± 0.02	46	9.00 ± 0.01*	92

All the values are stated as mean ± STDEV. (Where, n=5); significance at *p<0.05 as compared to control

Licking and biting reactions for normal control group were lasted for 103.00 ± 0.02 at early phase while the responses at late phase lasted for 111.00 ± 0.02 s. For standard indomethacin the response time was measured 84.00 ± 0.01 s at early phase and 48.00 ± 0.01 s at late phase. For *L. deccanensis* (MELD), the response time (69.00 ± 0.02 s) was significantly decreased by the treatment with 200 mg/kg bw at the late phase ($P < 0.05$). For *L. lancifolia* (MELL), both the doses significantly decreased the biting and licking response at the late phase ($P < 0.05$) and they were 33.00 ± 0.02 and 24.00 ± 0.01 s for the doses 100 mg/kg to 200 mg/kg bw, respectively.

Both the doses of *L. glutinosa* (MELG) reduced the reaction time significantly at the late phase and they were 49.00 ± 0.02 and 46.00 ± 0.02 s for the doses 100 mg/kg to 200 mg/kg bw, respectively. Among the plants the most promising effects were observed by *L. monopetala* (MELM). The response time were measured as 14.00 ± 0.01s and 9.00 ± 0.01 s for the doses 100 mg/kg to 200 mg/kg bw, respectively.

At the early phase, the percent inhibitory effects for MELD were 10% and 16%; for MELL were 32% and 37%; for MELG were 33% and 39% and for MELM pain inhibition were 32% and 46% at 100 mg/kg and 200 mg/kg bw, respectively. On the other side, at the late phase the percent protection for MELD were 6% and38%; for MELL were 16% and 78%; for MELG were 56% and59% and for MELM were 87% and 92% at 100 mg/kg and 200 mg/kg bw respectively. Among four plants *L. monopetala* showed the maximum pain inhibition compared to *L. deccanensis*, *L. lancifolia* and *L. glutinosa* (Table 3.23, Figure 3.75).

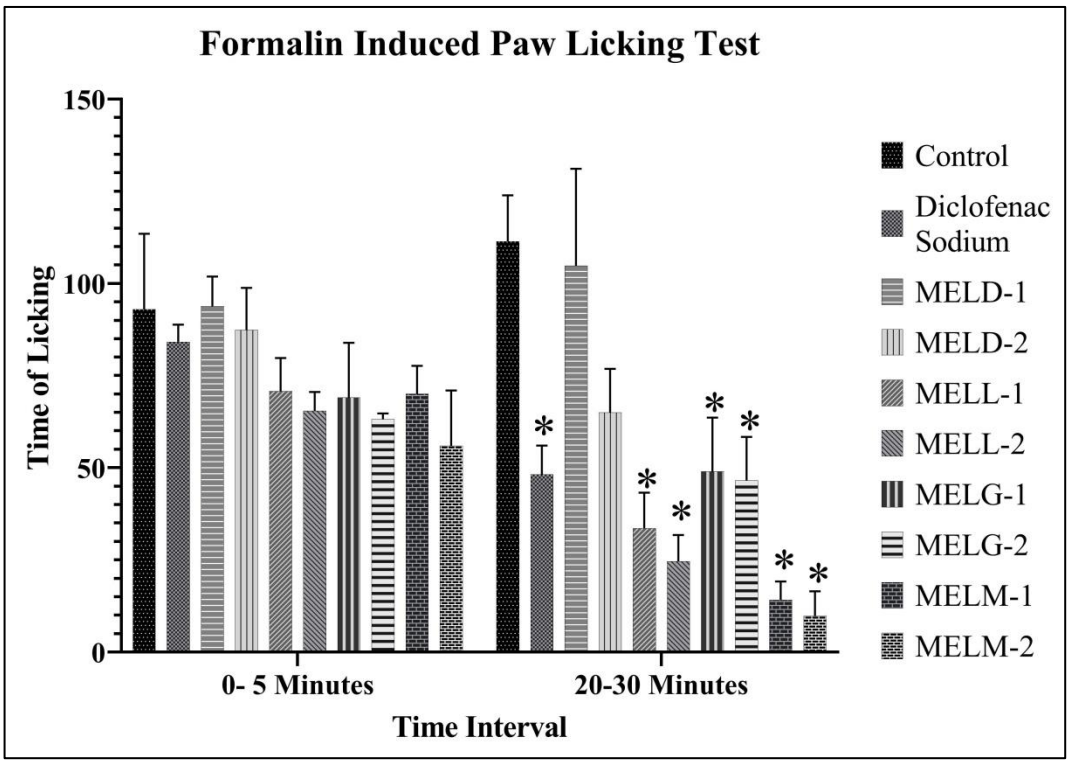

Figure 3.75: Effect of *Litsea deccanensis* (MELD), *L. lancifolia* (MELL), *L. glutinosa* (MELG) and *L. monopetala* (MELM) on chemical-induced pain (formalin) in mice models. All the values are stated as mean ± STDEV. (Where, n=4); significance at *p<0.05 as compared to control

3.7 Hypoglycemic activity of crude methanol extracts

Methanol extract of *L. deccanensis* (MELD), *L. lancifolia* (MELL), *L. glutinosa* (MELG) and *L. monopetala* (MELM) were evaluated for its *in-vivo* hypoglycemic activity in streptozotocin (STZ) induced Swiss Albino mice as compared to the control group and the standard group treated with metformin, a hypoglycaemic drug to verify

its effect. Administration of STZ at multiple low doses (40 mg/kg bw) for 5 days resulted an increase in blood glucose level in mice diabetic group. Blood glucose level increased significantly to the untreated diabetic control group and on 7^{th} day of the treatment it was 16.3±3.15 mmol/liter.

All the test groups including control, untreated diabetic control, treated with standard and extracts at two different doses were subjected to determine blood glucose level at 3^{rd}, 5^{th} and 7^{th} days. In this study, treatment with the standard metformin at 50 mg/kg bw reduced blood glucose level significantly ($p < 0.01$) compared to the control group and the % diabetic inhibition was calculated as 73.83%.

For methanol extract of *L. deccanensis* (MELD), hyperglycemia was reduced with the treatment with both the doses and the results were found significant ($p < 0.05$) at 5^{th} and 7^{th} days of the treatment and after 7 days the blood sugar level became normal. In case of methanol extract of *L. lancifolia* (MELL), 500 mg/kg bw of MELL reduced blood glucose level from 11.2±0.12 to 5.00 ±0.20 mmol/liter; 300 mg/kg bw of MELL reduced blood glucose level from 9.8±0.52 to 5.00 ±0.20 mmol/liter and both the results were significant at $p < 0.01$. Methanol extract of *L. glutinosa* (MELG) at a dose of 300 mg/kg bw showed significant ($p < 0.05$) reduction of blood glucose level on 7^{th} day but 500 mg/kg bw resulted more significant ($p < 0.01$) reduction after 7 days treatment with MELG. For methanol extract of *L. monopetala* (MELM), both 300 mg/kg and 500 mg/kg bw doses reduced blood glucose level, significantly ($p < 0.05$) on the 7^{th} day of the treatment.

The effects of MELD, MELL, MELG and MELM on blood glucose level in STZ induced diabetic rats were shown in the table 3.24 which represented that the blood glucose level was significantly decreased ($p < 0.05$) on 7^{th} day for all the plant extracts at both the doses. Parcentage (%) inhibition of blood glucose level for MELD, MELL, MELG and MELM were comparable with that of standard metformin and they were 68.16%, 69.33%, 66.69% and 57.06% respectively at the dose of 500 mg/kg/day bw as compared to the untreated diabetic control group (Figure 3.76).

Table 3.24: Hypoglycemic effect of methanol extract of *L. deccanensis* (MELD), *L. lancifolia* (MELL), *L. glutinosa* (MELG) and *L. monopetala* (MELM) on STZ induced diabetic mice

Groups	Dose (mg/kg, p.o.)	Blood glucose level (mmol/liter)			
		1st Day	3rd Day	5th Day	7th Day
Control (non-diabetic)	-	5.20± 0.17	5.01± 0.13	5.50± 0.35	4.87±0.26
Untreated diabetic control	-	8.0±0.53	9.0±0.71	13.6±1.02	16.3±3.15
Metformin HCl	50	12.46±0.67	5.53±0.27**	4.46±0.14**	4.26±0.32**
MELD_1	300	11.07±1.03	8.47 ±0.72	7.00±0.41*	6.10 ±0.43*
MELD_2	500	10.07±0.43	7.83 ±0.72	6.00±0.33*	5.20 ±0.03**
MELL_1	300	9.8±0.52	7.00 ±0.62	6.55±0.35*	6.00 ±0.27**
MELL_2	500	11.2±0.12	6.50 ±0.62	5.25±0.05**	5.00 ±0.20**
MELG_1	300	10.2±1.02	8.02±0.12	7.53±0.32	6±0.12*
MELG_2	500	11.2±0.82	7.22±0.52	6.22±0.32*	5.43±0.12**
MELM_1	300	12.85 ±1.63	10.8 ±1.2	8.15 ±.77	7.6 ±0.44*
MELM_2	500	11.5±0.63	9.3 ±0.94	7.7 ±0.54	7.0 ±0.75*

All the values are stated as mean ± STDEV. (Where, n=5); significance at ***$p < 0.001$, **$p<0.01$, *$p<0.05$ as compared to control

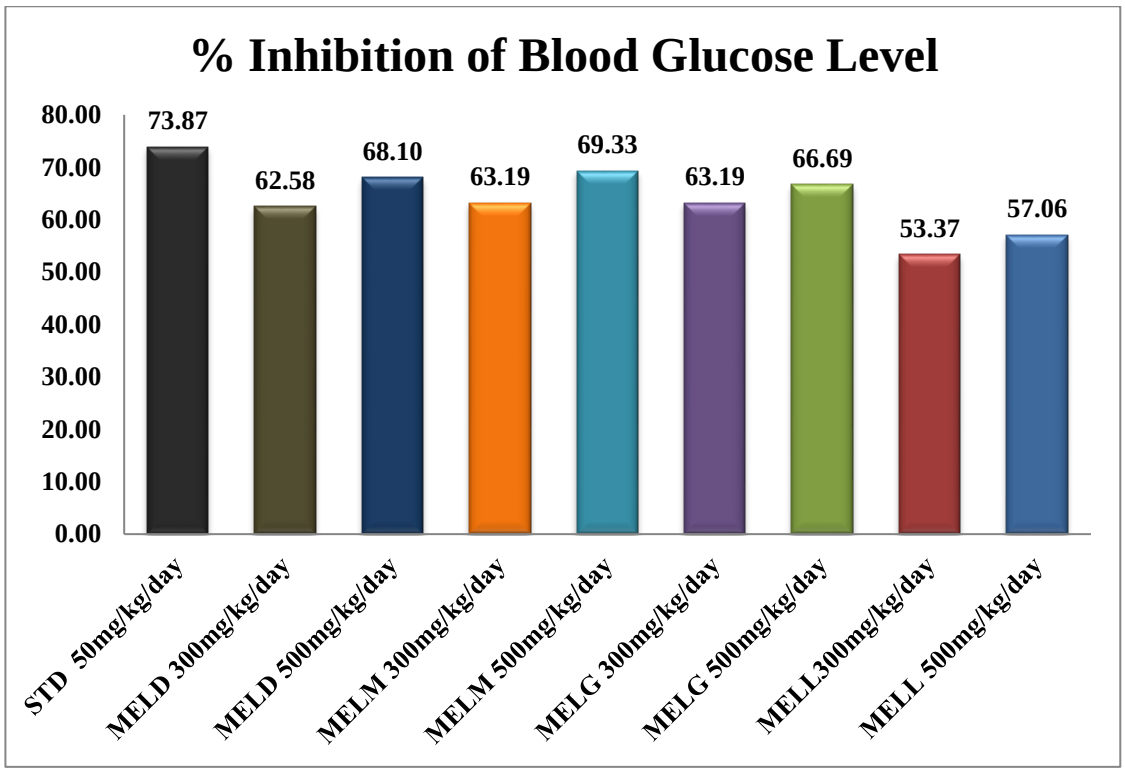

Figure 3.76: % inhibition of blood glucose levels of standard Metformin and MELD, MELL, MELG and MELM at both the doses as compared to untreated diabetic control.

3.8 CNS depressant activity of crude methanol extracts

This study was performed to investigate the possible neuropharmacology (CNS stimulant or depressant) of methanol extract of *L. deccanensis* (MELD), *L. lancifolia* (MELL), *L. glutinosa* (MELG) and *L. monopetala* (MELM) in comparison with the diazepam as standard group as well as normal control group. All the extracts at two different doses (300 and 500 mg/kg bw) of *L. deccanensis* , *L. lancifolia,, L. glutinosa,* and *L. monopetala* were found to reduce locomotion in the test animals and to decrease the passing number through the hole in between the hole cross chamber by the animals in a dose dependent manner (Table 3.25).

The number of movements by mice from one chamber to other was alike at 0 to 120 min study period for the control group. For standard diazepam at 1 mg/kg bw the number of movements was 10.75±0.96 at 0 min and 1.75±0.96 at 120 min. In case of methanol extract of *L. deccanensis* (MELD), this number decreased from 8.25±0.96 to 4.75±0.96 for 300 mg/kg bw dose while for 500 mg/kg bw dose, this number decreased from 7.25±0.50 to 3.25±0.96. The movement number for methanol extract of *L.*

lancifolia (MELL) was measured to decrease from 8.00±0.82 to 4.00±0.82 and 8.50±0.58 to 2.50±0.58 for 300 and 500 mg/kg bw, respectively. Mice treated with methanol extract of *L. glutinosa* (MELG) at two different doses (300 and 500 mg/kg bw) showed a significant decrease in movement through the hole from 5.50±0.58 to 2.50±0.58 for 300 mg/kg and 4.50±1.29 to 0.75±0.96 for 500 mg/kg bw. In case of methanol extract of *L. monopetala* (MELM), the movement number decreased from 8.75±0.96 to 3.50±1.00 for 300 mg/kg dose while for 500 mg/kg bw dose, this number decreased from 6.75±1.71 to 2.25±1.26 (Figure 3.77).

Table 3.25: CNS effect of methanol extract of *L. deccanensis* (MELD), *L. lancifolia* (MELL), *L. glutinosa* (MELG) and *L. monopetala* (MELM) on hole cross test.

Groups	Dose (mg/kg,) and route	Number of movements				
		0 min	30 min	60 min	90 min	120 min
Control	-	13.50±2.38	14.00±2.58	14.25±1.71	14.00±2.16	13.50±0.58
Diazepam	1; i.p.	10.75±0.96	5.50±1.29	4.50±1.73	3.00±1.41	1.75±0.96
MELD_1	300; p.o.	8.25±0.96	7.25±0.96	6.25±0.96	5.25±0.50	4.75±0.96
MELD_2	500; p.o.	7.25±0.50	6.25±0.50	5.50±0.58	4.50±1.29	3.25±0.96
MELL_1	300; p.o.	8.00±0.82	6.25±0.96	5.50±0.58	4.75±0.50	4.00±0.82
MELL_2	500; p.o.	8.50±0.58	5.00±0.82	4.25±0.96	3.50±0.58	2.50±0.58
MELG_1	300; p.o.	5.50±0.58	4.25±0.50	3.50±0.58	3.00±0.82	2.50±0.58
MELG_2	500; p.o.	4.50±1.29	3.25±0.96	2.00±0.82	1.75±0.96	0.75±0.96
MELM_1	300; p.o.	8.75±0.96	6.25±0.96	5.00±0.82	3.75±0.50	3.50±1.00
MELM_2	500; p.o.	6.75±1.71	5.00±1.41	4.00±1.83	4.00±1.41	2.25±1.26

All the values are stated as mean ± STDEV. (Where, n=5); *p<0.05 as compared to control

In this study, the revealed data indicated that the methanol extract of *L. deccanensis*, *L. lancifolia*, *L. glutinosa* and *L. monopetala* decreased locomotor activity significantly ($p<0.05$) with the increasing of dose. Analysis of locomotor activity is an important parameter to measure the level CNS excitability (Mansur *et al.*, 1980) and a decreased in locomotor activity is meticulously related to the CNS depressant effect (Ozturk *et al.*, 1996).

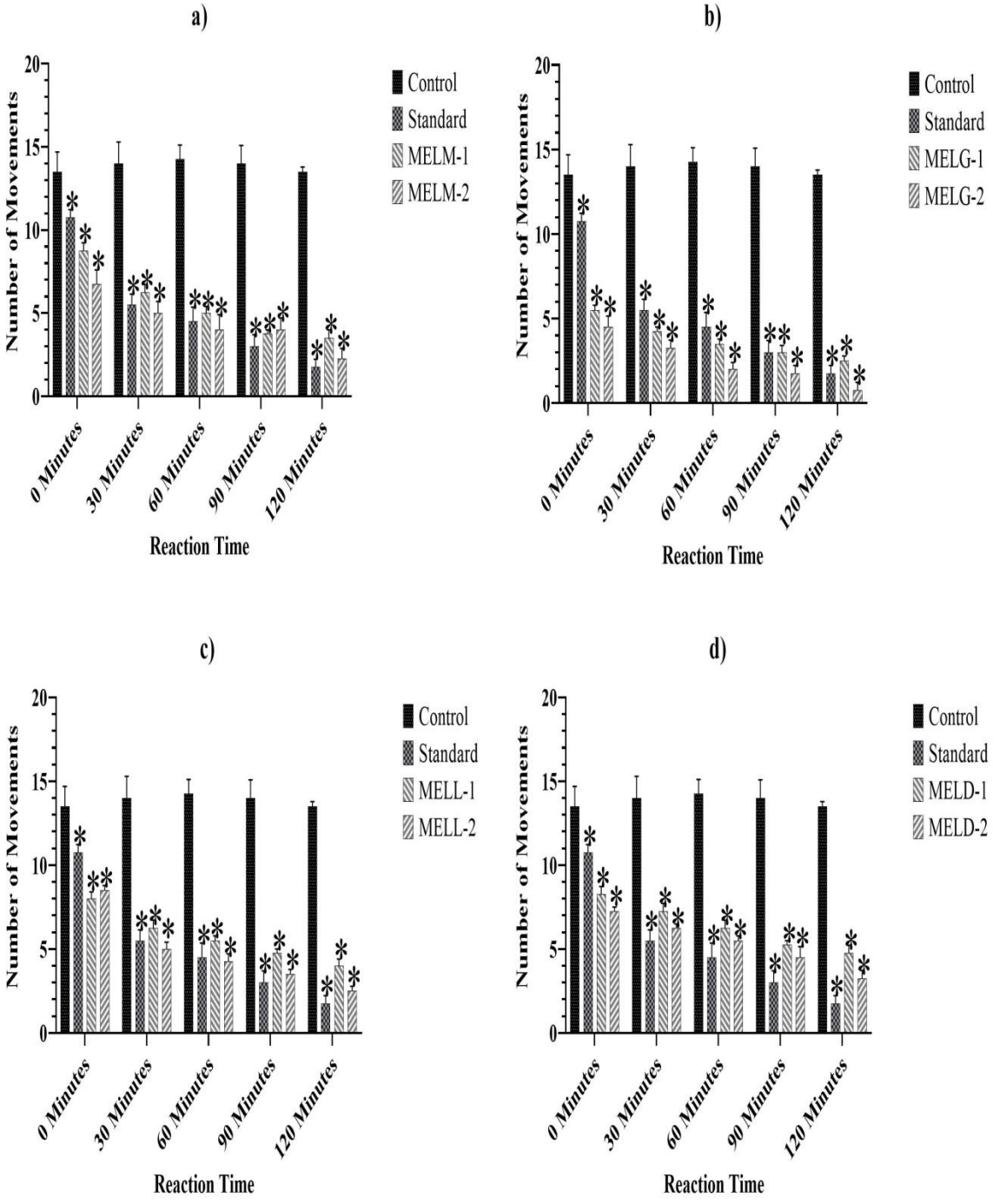

Figure 3.77: The movement through the hole in between the hole cross chamber by the animals on 0, 30, 60, 90 and 120 min after administration of two different doses (300 and 500 mg/kg bw) of the methanol extract of *L. monopetala* (a), *L. glutinosa* (b), *L. lancifolia* (c) and *L. deccanensis* (d) on hole cross test. All the values stated as mean ± STDEV. (Where, n=5); *P<0.05 as compared to control.

3.9 Antimicrobial screening of different partitionates by disc diffusion method

3.9.1 Antimicrobial activity of L. glutinosa

The results of antimicrobial activity test of different fractions of *L. glutinosa* were summarized in table 3.26. The range of zone of inhibition for petroleum ether soluble fraction was 13.00±0.82 to 18.67±0.47 mm, for chloroform fraction it was 13.00±0.82 mm to 19.00±1.41, for ethyl acetate fraction it was 12.00±0.82 to 12.00±0.82. All the fractions of *L. glutinosa* exhibited good to moderate antimicrobial activity against most of the microorganisms selected for this study. The maximum zone of inhibition exhibited by pet-ether fraction was 18.67±0.47 mm against gram negative *P. aeruginosa* and 18.33±0.47 mm against gram negative *E. coli,* the maximum activity shown by chloroform fraction was 19.00±1.41 mm against *E. coli* and for ethyl acetate fraction the maximum activity was found against *V. parahemolyticus* with 16.67±0.47 mm zone of inhibition.

Table 3.26: Zones of inhibition (mm) representing antimicrobial activity for three fractions of *L. glutinosa*; disc diameter is 5.0 mm

Microbial strain	Zone of inhibition (mm)			
	Different partitionates of methanol extract of *L. glutinosa* (MELG)			Ciprofloxacin/ Griseofulvin
	PESF	CSF	EASF	
Bacillus subtilis	14.33±1.25	15.67±0.47	14.67±0.47	27.33±0.94
Bacillus megaterium	16.33±1.25	15.33±1.25	15.33±0.94	20.00±0.82
Staphylococcus aureus	17.67±0.47	14.00±0.82	12.00±0.82	29.00±1.41
Sarcina lutea	16.67±0.94	15.33±0.47	14.67±0.47	28.33±0.47
Bacillus cereus	16.33±0.47	14.00±0.82	11.33±0.47	26.67±1.25
Escherichia coli	18.33±0.47	19.00±1.41	14.67±0.47	30.67±0.47
Vibrio mimicus	17.00±0.82	14.67±0.94	12.00±0.82	31.67±0.47
Pseudomonas aeruginosa	18.67±0.47	14.67±0.47	15.67±0.47	30.33±0.94
Salmonella paratyphi	14.33±0.47	16.33±0.47	15.67±0.47	31.00±1.41
Vibrio parahemolyticus	13.67±1.25	14.33±0.47	16.67±0.47	30.00±0.82
Shigella dysenteriae	14.33±0.94	13.67±0.94	14.67±0.94	28.67±0.94
Shigella boydii	13.00±0.82	13.33±0.47	12.33±0.47	30.33±0.47
Candida albicans	15.00±1.63	13.00±0.82	15.33±0.47	29.33±1.25
Asperagillus niger	13.00±0.82	14.67±0.47	15.00±0.82	30.67±0.94
Sacharomyeces cereveceae	15.00±1.25	14.67±0.94	13.67±1.25	30.33±0.47

Zones of inhibition of microbial growth are presented as mean±SDEV

3.9.2 Antimicrobial activity of L. monopetala

The results of antimicrobial activity test of different fractions of *L. monopetala* were summarized in table 3.27. The pet-ether fraction of *L. monopetala* showed moderate antimicrobial activity with 13.67±0.47 mm against *B. subtilis*, 13.00±0.82 mm against *B. megaterium* and 12.67±0.47 mm zone of inhibition against *V. parahemolyticus*. The chloroform fraction of *L. monopetala* revealed good antimicrobial activity against *B. subtilis* (16.00±0.82 mm) and *V. parahemolyticus* (15.33±0.47 mm).

Table 3.27: Zones of inhibition (mm) representing antimicrobial activity for three fractions of *L. monopetala*; disc diameter is 5.0 mm

Microbial strain	Zone of inhibition (mm)			
	Different partitionates of methanol extract of *L. monopetala* (MELM)			Ciprofloxacin/ Griseofulvin
	PESF	CSF	EASF	
Bacillus subtilis	13.67±0.47	16.00±0.82	15.67±0.47	31.33±0.94
Bacillus megaterium	13.00±0.82	11.33±1.25	16.33±0.47	32.00±0.82
Staphylococcus aureus	8.33±0.94	10.67±0.94	11.00±0.82	32.67±0.47
Sarcina lutea	9.00±0.82	12.33±0.47	14.33±0.47	30.33±0.47
Bacillus cereus	10.33±0.47	9.67±0.94	15.67±0.94	30.67±0.94
Escherichia coli	8.00±1.41	11.00±0.82	10.00±0.82	29.33±0.94
Vibrio mimicus	8.00±0.82	11.67±0.47	14.67±0.47	30.33±1.25
Pseudomonas aeruginosa	10.33±0.47	11.00±0.82	15.33±0.47	31.33±0.94
Salmonella paratyphi	11.67±0.94	11.67±0.47	12.67±0.47	28.33±0.47
Vibrio parahemolyticus	12.67±0.47	15.33±0.47	14.00±0.82	28.33±0.47
Shigella dysenteriae	10.00±0.82	11.33±1.25	12.67±0.94	30.67±0.94
Shigella boydii	9.00±0.82	9.67±0.47	9.67±0.47	30.33±0.47
Candida albicans	8.67±0.47	11.67±0.47	14.33±0.47	31.67±1.25
Asperagillus niger	7.67±0.94	11.33±0.94	10.00±0.82	29.67±1.25
Sacharomyeces cereveceae	10.00±0.82	11.00±0.82	10.67±0.47	28.67±0.47

Zones of inhibition of microbial growth are presented as mean±SDEV

While the chloroform fraction *L. monopetala* showed mild to moderate antimicrobial activity with 9.67±0.47 to 11.67±0.47 mm zone of inhibition against the other remaining organisms. The ethyl acetate fraction of *L. monopetala* demonstrated good activity with 15.67±0.47 mm against *B. subtilis*, 16.33±0.47 mm against *B. megaterium*,

15.67±0.94 mm against *B. cereus* and 15.33±0.47 mm zone of inhibition against *P. aeruginosa* while exhibited mild to moderate antimicrobial activity against other organisms. The range of zone of inhibition for petroleum ether soluble fraction was 7.67±0.94 mm to 13.67±0.47 mm, for chloroform fraction it was 9.67±0.47 to 16.00±0.82 mm, for ethyl acetate fraction it was 9.67±0.47 to 16.33±0.47 mm zone of inhibition. All the fractions showed mild to moderate antimicrobial activity against the selected test organisms.

3.9.3 Antimicrobial activity of L. deccanensis

The results of antimicrobial activity test of different fractions of *L. deccanensis* were summarized in table 3.28.

Table 3.28: Zones of inhibition (mm) representing antimicrobial activity for three fractions of *L. deccanensis*; disc diameter is 5.0 mm

Microbial strain	Zone of inhibition (mm)			
	Different partitionates of methanol extract of *L. deccanensis* (MELD)			Ciprofloxacin /Griseofulvin
	PESF	CSF	EASF	
Bacillus subtilis	7.67±0.47	13.67±0.47	15.67±0.47	28.67±0.94
Bacillus megaterium	8.33±0.47	9.67±0.47	15.67±0.94	30.33±0.47
Staphylococcus aureus	9.67±0.47	9.33±0.94	17.33±0.47	30.67±0.94
Sarcina lutea	11.33±1.25	10.67±0.94	18.33±0.47	29.67±0.94
Bacillus cereus	9.33±0.47	9.00±0.82	21.33±0.94	28.33±0.47
Escherichia coli	10.67±0.94	12.67±0.47	19.00±0.82	29.00±0.82
Vibrio mimicus	8.00±0.82	13.00±0.82	15.33±0.47	29.67±0.47
Pseudomonas aeruginosa	8.33±0.47	10.33±0.47	25.00±0.82	30.00±0.82
Salmonella paratyphi	9.33±0.94	9.67±0.47	16.67±0.47	29.33±0.47
Vibrio parahemolyticus	11.33±0.94	12.33±0.47	17.33±0.47	30.33±1.25
Shigella dysenteriae	9.00±0.82	13.00±0.82	13.00±0.82	30.33±1.25
Shigella boydii	9.67±0.47	12.67±0.47	10.33±0.47	30.33±0.47
Candida albicans	9.67±0.47	14.33±1.25	25.67±0.94	30.67±0.47
Asperagillus niger	10.33±0.94	14.33±0.47	23.33±0.47	29.33±0.94
Sacharomyeces cereveceae	11.33±0.47	10.33±0.47	8.33±0.47	28.67±0.47

Zones of inhibition of microbial growth are presented as mean±SDEV

The maximum zone of inhibition with mild to moderate antimicrobial activity was shown by the petroleum ether soluble fraction (PESF) of *L. deccanensis* against *S. lutea* (11.33±1.25 mm), *E. coli* (10.67±0.94 mm), *V. parahemolyticus* (11.33±0.94 mm) and *A. niger* (10.33±0.94 mm). A moderate antimicrobial activity was observed in case of chloroform fraction of *L. deccanensis* against gram positive *B. subtilis* (13.67±0.47, mm), gram negative *E. coli* (10.67±0.94, mm), *V. mimicus* (13.00±0.82, mm) and *C. albicans* (12.67±0.94 mm). The ethyl acetate fraction of *L.* deccanensis showed moderate to very good antimicrobial activity with a range of zone of inhibition from 8.33±0.47 mm to 25.00±0.82 mm. Maximum zone of inhibition was shown by the chloroform extract against gram negative *P. aeruginosa* and it was 25.00±0.82 mm. Two fungi, *A. niger* and *S, cereveceae* produced 25.67±0.94 and 23.33±0.47 mm zone of inhibition. The range of zone of inhibition for petroleum ether soluble fraction was 7.67±0.47 to 11.33±0.94 mm, for chloroform fraction it was 9.67±0.47 to 13.00±0.82 mm, for ethyl acetate fraction it was 8.33±0.47 to 25.00±0.82 mm. Among all the fractions ethyl acetate showed very good, chloroform fraction showed moderate and pet-ether fraction showed mild antimicrobial activity.

3.9.4 Antimicrobial activity of L. lancifolia

The results of antimicrobial activity test of different fractions of *L. lancifolia* were summarized in table 3.29. The petroleum ether soluble fraction (PESF) of *L. lancifolia* exhibited very good antimicrobial activity with maximum zone of inhibition 23.50±0.50 mm against *P. aeruginosa*, while produced good antimicrobial activity with 18.33±1.25 mm zone of inhibition against *S. aureus* and 17.50±1.50 mm zone of inhibition against *S. dysenteriae* and revealed mild to moderate antimicrobial activity against *B. subtilis, B. megaterium, E. coli* and *V. mimicus* with 11.00±0.82 to 14.75±0.25 mm zone of inhibition respectively. The chloroform fraction revealed very good antimicrobial activity with 16.50±0.41 to 18.50±0.41mm zone of inhibition against *B. cereus, S. paratyphi, S. boydii*, *B. subtilis* and *B. megaterium*. The chloroform soluble fraction presented mild to moderate antimicrobial activity (9.17±0.62 to 14.67±0.47 mm zone of inhibition) against *S. aureus, E. coli, S. lutea, P. aeruginosa, S. dysenteriae, V. parahemolyticus, A. niger, C. albicans, and S. cereveceae.* The ethyl acetate partitionate of *L. lancifolia* exhibited very good antimicrobial activity with 22.33±0.47 mm zone of inhibition against *E. coli*, while a moderate antimicrobial

activity with 14.17±0.24 mm zone of inhibition against *B. cereus* and 14.67±0.47mm against *B. subtilis*.

Table 3.29: Zones of inhibition (mm) representing antimicrobial activity for three fractions of *L. lancifolia*; disc diameter is 5.0 mm

Microbial strain	Zone of inhibition (mm)			
	Different partitionates of methanol extract of *L. lancifolia* (MELL)			Ciprofloxacin/ Griseofulvin
	PESF	CSF	EASF	
Bacillus subtilis	11.00±0.82	17.33±0.47	14.67±0.47	35.00±0.82
Bacillus megaterium	13.33±0.47	16.50±0.41	8.67±0.47	33.33±0.47
Staphylococcus aureus	18.33±1.25	14.67±0.47	10.17±0.24	33.33±0.47
Sarcina lutea	-	10.67±0.94	8.50±0.41	33.50±0.41
Bacillus cereus	8.17±0.62	18.33±0.47	14.17±0.24	35.33±1.25
Escherichia coli	11.75±0.75	12.83±0.85	22.33±0.47	33.00±0.82
Vibrio mimicus	14.75±0.25	-	10.67±0.94	31.83±0.24
Pseudomonas aeruginosa	23.50±0.50	9.33±0.47	-	30.67±0.47
Salmonella paratyphi	-	18.50±0.41	8.83±0.85	32.17±0.24
Vibrio parahemolyticus	-	9.33±0.47	11.67±0.47	32.50±0.41
Shigella dysenteriae	17.50±1.50	14.33±0.94	-	33.67±0.47
Shigella boydii	13.50±0.50	18.17±0.24	-	32.67±0.47
Candida albicans	-	12.67±0.94	9.50±0.41	30.67±0.47
Asperagillus niger	-	9.17±0.62	10.67±0.47	31.67±0.47
Sacharomyeces cereveceae	-	7.33±0.47	-	30.50±0.41

Zones of inhibition of microbial growth are presented as mean±SDEV

3.10 Antioxidant activity of different partitionates of crude extracts

3.10.1 Total phenol content determination

Total phenol content (TPC) in extractives were assessed by using Folin-Ciocalteu method which is defined as a colorimetric method in contrast with gallic acid as standard and the results were stated in terms of mg GAE/g dry extract. The equation from the calibration curve is given below:

$y = 0.0162x + 0.0215$, ($R^2 = 0.9985$)

y = Absorbance; x = gallic acid (mg)

Table 3.30: Standard curve preparation by using Gallic acid

S.N.	Conc. of the Standard (µg / ml)	Absorbance	Regression line	R^2
1	100	1.620		
2	50	0.866		
3	25	0.450		
4	12.5	0.253	**y = 0.0162x + 0.0215**	**0.9985**
5	6.25	0.120		
6	3.125	0.059		
7	1.5625	0.034		
8	0.78125	0.022		
9	0.3906	0.020		
10	0	0.011		

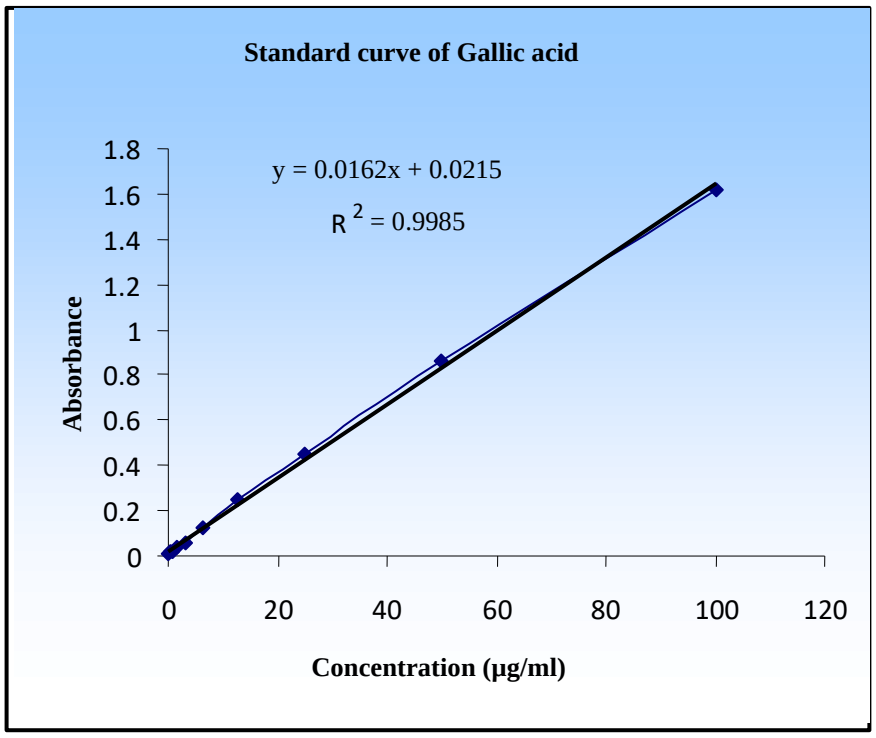

Figure 3.78: Standard curve of Gallic acid for total phenolic determination.

Table 3.31: Total phenolic content (mg of GAE / gm of extractives) of three different extractives of *L. deccanensis, L. lancifolia, L. glutinosa* and *L. monopetala*

Samples	Total phenolic content (mg of GAE / gm of extractives)	Mean±STDEV
LDPE	4.49 4.50 4.66	4.55±0.10
LDCE	3.94 3.95 3.81	3.9±0.06
LDEA	3.74 3.89 3.85	3.83±0.08
LLPE	15.96 15.98 15.96	15.97±0.01
LLCE	33.35 33.4 33.56	33.44±0.11
LLEA	79.93 80.01 79.88	79.94±0.07
LMPE	4.00 4.15 4.22	4.12±0.11
LMCE	3.94 3.92 3.89	3.92±0.03
LMEA	3.97 3.91 3.95	3.9±0.03
LGPE	22.09 22.15 22.03	22.09±0.06
LGCE	32.90 33.02 33.05	32.99±0.08
LGEA	102.99 103.01 103.11	103.04±0.06

Among four plants (*L. deccanensis*, *L. lancifolia*, *L. glutinosa* and *L. monopetala*) the total phenolic content range was found from 103.04±0.06 to 3.83±0.08 gallic acid equivalents (GAE mg/g) of dry weight of extract (Table 3.31). The range of total phenolic content for *L. deccanensis* is from 4.55±0.10 to 3.83±0.08; for *L. lancifolia* it is 79.94±0.07 to 79.94±0.07; for *L. glutinosa* it is 103.04±0.06 to 22.09±0.06 and for *L. monopetala* 4.12±0.11 to 3.9±0.03 GAE mg/g. The maximum quantities of phenolic compounds were found in ethyl acetate fraction of *L. glutinosa* (103.04±0.06), then by ethyl acetate fraction of *L. lancifolia* (79.94±0.07). Among the plants *L. lancifolia* and *L. glutinosa* have shown very good total phenolis compared to *L. deccanensis* and *L. monopetala.*

3.10.2 DPPH assay for antioxidant activity

Table 3.32: % DPPH radical scavenging activity and IC_{50} of different partitionates of *L. deccanensis*

Plant partitionate and Conc. (µg/ml)	Mean±STD	Equation and IC_{50}
LDPE 20	70.05±0.03	y = 0.5374x + 32.952 **31.75**
LDPE 40	70.77±0.15	
LDPE 60	73.45±0.11	
LDPE 80	73.95±0.17	
LDPE 100	75.27±0.13	
LDCH 20	75.56±0.17	y = 0.6586x + 33.785 **24.62**
LDCH 40	76.31±0.19	
LDCH 60	76.99±0.11	
LDCH 80	85.04±0.06	
LDCH 100	86.38±0.18	
LDEA 20	70.05±0.04	y = 0.5582x + 32.671 **31.04**
LDEA 40	70.91±0.26	
LDEA 60	71.62±0.24	
LDEA 80	73.05±0.15	
LDEA 100	74.44±0.25	

One of the widely used methods for evaluating antioxidant activity is the DPPH free radical scavenging activity on a stable free radical DPPH. In the DPPH Free radical scavenging activity, three different extractives of *L. deccanensis*, *L. lancifolia*, *L. glutinosa* and *L. monopetala* were evaluated for their antioxidant potential compared

with ascorbic acid as standard compound. The IC_{50} was calculated for each fraction of four different species of *Litsea* as well as standard ascorbic acid and précised in table 3.32 to table 3.35.

Table 3.33: % DPPH radical scavenging activity and IC_{50} of different partitionates of *L. lancifolia*

Plant partitionate and Conc. (µg/ml)	Mean±STD	Equation and IC_{50}
LLPE 20	21.56±0.19	y = 0.7625x + 1.2232 **63.97**
LLPE 40	29.62±0.21	
LLPE 60	48.70±0.34	
LLPE 80	55.69±0.07	
LLPE 100	76.03±0.41	
LLCH 20	18.47±0.32	y = 0.7166x + 2.7695 **65.91**
LLCH 40	35.37±0.28	
LLCH 60	41.50±0.56	
LLCH 80	60.35±0.70	
LLCH 100	80.40±0.85	
LLEA 20	15.51±0.37	y = 0.6194x - 0.16 **80.46**
LLEA 40	23.49±0.47	
LLEA 60	30.84±0.20	
LLEA 80	51.15±0.10	
LLEA 100	63.86±0.39	

Table 3.34: % DPPH radical scavenging activity and IC_{50} of different partitionates of *L. glutinosa*

Plant partitionate and Conc. (µg/ml)	Mean±STD	Equation and IC_{50}
LGPE 20	74.90±0.40	y = 0.6x + 34.886 **25.19**
LGPE 40	76.55±0.40	
LGPE 60	77.56±0.40	
LGPE 80	78.90±0.30	
LGPE 100	81.39±0.06	
LGCH 20	63.56±0.32	y = 0.5637x + 28.635 **37.90**
LGCH 40	65.31±0.09	
LGCH 60	67.64±0.13	
LGCH 80	69.56±0.23	
LGCH 100	74.85±0.17	
LGEA 20	45.78±0.10	y = 0.437x + 20.544 **67.41**
LGEA 40	47.63±0.04	
LGEA 60	51.61±0.06	
LGEA 80	53.71±0.07	
LGEA 100	55.62±0.12	

Table 3.35: % DPPH radical scavenging activity and IC_{50} of different partitionates of *L. monopetala*

Plant partitionate and Conc. (µg/ml)	Mean±STD	Equation and IC_{50}
LMPE 20	69.89±0.14	
LMPE 40	70.78±0.13	y = 0.5366x + 32.86
LMPE 60	71.48±0.07	**31.94**
LMPE 80	72.73±0.14	
LMPE 100	73.28±0.14	
LMCH 20	75.55±0.20	
LMCH 40	75.65±0.74	
LMCH 60	76.47±0.40	y = 0.659x + 33.581
LMCH 80	85.22±0.34	**24.91**
LMCH 100	86.29±0.06	
LMEA 20	69.81±0.27	
LMEA 40	70.83±0.23	y = 0.5567x + 32.684
LMEA 60	73.63±0.23	**31.10**
LMEA 80	73.99±0.24	
LMEA 100	74.87±0.19	

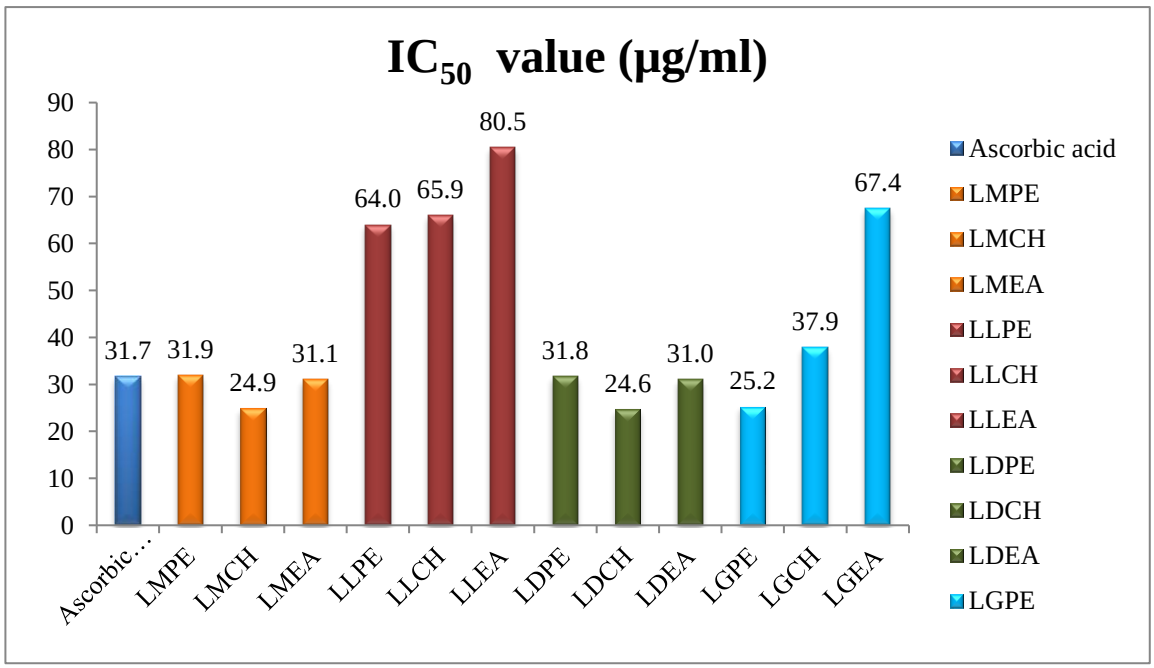

Figure 3.79: IC_{50} values of three different extractives of *L. deccanensis, L. lancifolia, L. glutinosa* and *L. monopetala* obtained by DPPH free radical scavenging activity test.

IC_{50} values for each partition of the studied plants as well as standard ascorbic acid were graphically presented in figure 3.78. The obtained results revealed the increased scavenging effect with the increased concentrations of samples.

The IC_{50} values for pet-ether, chloroform and ethyl acetate fractions of *L. deccanensis* were 31.75 µg/ml, 24.62 µg/ml and 31.04 µg/ml, respectively. All the values are comparable with that of ascorbic acid (31.66 µg/ml). For *L. lancifolia* 63.97 µg/ml pet-ether, 65.91 µg/ml chloroform and 80.46 µg/ml ethyl acetate extractives were required for 50% scavenging of free radicals. The effective concentrations to scavenge free radicals were measured as 25.19 µg/ml, 37.90 µg/ml and 67.41 µg/ml, respectively for pet-ether, chloroform and ethyl acetate fractions of *L. glutinosa*. The IC_{50} values were 31.94 µg/ml, 24.91 µg/ml and 31.10 µg/ml for pet-ether, chloroform and ethyl acetate fractions of *L. monopetala,* respectively. From results, it may be proposed that three different extractives of *L. deccanensis, L. lancifolia, L. glutinosa* and *L. monopetala* were able to reduce the free radical scavenging activity compared to ascorbic acid, a potent antioxidant compound.

3.11 Molecular docking of pure compounds

3.11.1 Molecular docking of LGC-26 (95) and LGC-45-3 (96) against human aldose reductase for its anti-diabetic property

The docking simulation was conducted against aldose reductase (AKR1B1) protein model (Figure 3.79, A) with the purified phytochemicals. The pattern of interactions and the best binding poses of drug-protein complexes were exhibited *via* molecular simulation using Auto Dock Vina software. LGC-26 (**95**) exposed the highest negative binding affinity (-9.8 kcal/mol) for the interaction of the protein and exhibits strong connection with eleven hydrophobic interactions, hydrogen bonds and one other interaction. LGC-45-3 (**96**) exerts binding affinity as -9.4 kcal/mol and develops stable interactions by three hydrogen bonds, and eleven hydrophobic bonds. During interactions, hydrophobic site played a crucial role in drug-protein interactions, contributing 79% for LGC-45-3 and 73% in LGC-26 for all interactions. In addition, hydrogen bonds contributed 21% and 20% in LGC-45-3 and 73% in LGC-26 correspondingly, while other interactions were iinvolved by 7% of the total interactions

only for LGC-26 (Figure 3.79, B). Investigating interaction pattern, binding affinity, and best binding poses of the compounds it can be proposed, both structures might be promising inhibitors against aldose reductase (AKR1B1) protein.

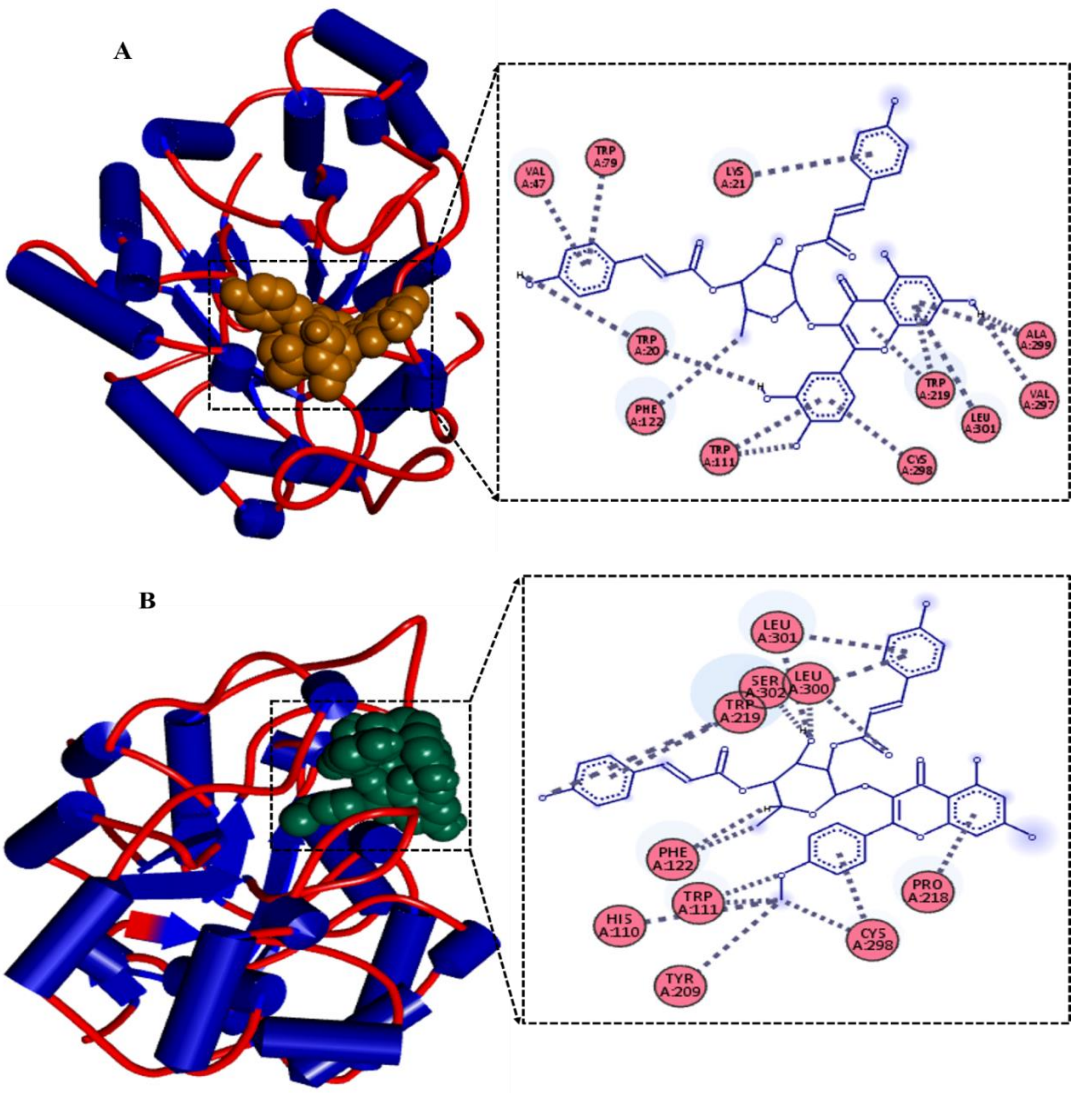

Figure 3.80: The binding poses and non-covalent interactions of (A) LGC-45-3 (96), (B) LGC-26 (95) with human aldose reductase (AKR1B1, AR; PDB ID: 4JIR) (Pose predicted by AutoDock Vina).

Table 3.36: Non-covalent interactions of LGC-45-3 (96) and LGC-26 (95) with protein (Pose predicted by AutoDock Vina)

Cpds	Binding Affinity (kcal/mol)	Hydrophobic bond	Hydrogen bond	Other
LGC-45-3 (**96**)	-9.4	Lys21,Val47,Trp79,Trp111,Phe122,Trp219,Cys298,Ala299, Leu301	Trp20,Trp111, Val297	_
LGC-26 (**95**)	-9.8	Phe122, Cys298, Trp219,Trp219, Cys298, Pro218, Leu301, His110, Trp111, Phe122, Tyr209	Trp111, Trp219, Leu300, Leu301	Cys298

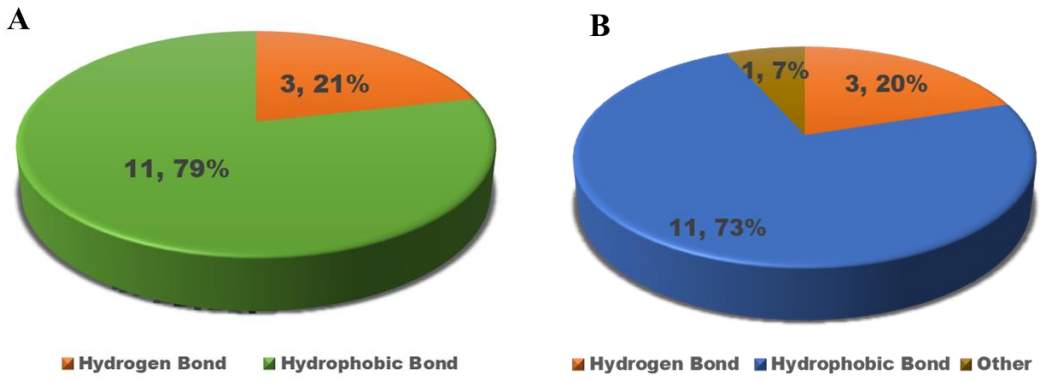

Figure 3.81: Distribution of non-covalent interactions of (A) LGC-45-3 (96) and (B) LGC-26 (95) with protein

Table 3.37: ADMET profile of the LGC-45-3 (96) and LGC-26 (95).

Compounds	HIA	Caco-2 (cm/s)	PGI	CYP450 2D6/3A4/1A2/ 2C19/2D6	AMES toxicity	Carcinogens
LGC-45-3 (**96**)	0.9113	0.7495	0.7969	No	No	No
LGC-26 (**95**)	0.9141	0.6639	No	No	Yes	No

HIA- Human intestinal absorption; PGI- P-glycoprotein inhibitor

Analysis of ADMET profiles

ADMET properties of the synthesized drug molecules are documented in Table 3.37. Analysis of pharmacokinetic and toxicity properties determines the probable success rate of therapeutic small molecules. In this study, human intestinal absorption (HIA), cytochrome inhibitor, P-glycoprotein substrate inhibition, Caco-2 permeability, AMES toxicity and carcinogens were investigated through ADMET profile analysis. The both inhibitors showed notable human intestinal absorption (HIA) and high Caco-2 permeability. In terms of parameters both compounds were found to be non-inhibitor of cytochrome P450 (CYP 450), representing their proper metabolism by CYP450. Also, the compounds were phosphorylated glycoprotein (P-gp), nontoxic and noncarcinogenic.

3.11.2 Molecular docking of LGC-26 (95) and LGC-45-3 (96) against alpha amylase for its anti-diabetic activity

Molecular docking analysis of isolated compounds showed better docking score within the active site of human alpha amylase (3BAJ) (Figure 3.81). Compounds **95** and **96** (−9.4, and −8.9 kcal/mol, respectively) showed promising docking affinity. Compound **95** formed polar contacts with Tyr-151, Thr-163, Arg-195, Asp-197, His-201, and His-299 residues and compound **96** showed polar contacts with Gln-63, Arg-195, Asp-197, and His-299 residues (Tables 3.38).

Table 3.38: Molecular docking results of LGC-26 (95) and LGC-45-3(96) against alpha amylase.

Compounds	Binding affinity (kcal/mol)	Polar contacts
LGC-26 (**95**)	-9.4	Tyr-151, Thr-163, Arg-195, Asp-197, His-201, and His-299
LGC-45-3 (**96**)	-8.9	Gln-63, Arg-195, Asp-197, and His-299

The inhibition constants (K*i*) of the compounds **95** and **96** (124, and 290 nM, respectively) were obtained from the binding energies (ΔG) using the formula: $Ki = \exp(\Delta G/RT)$, where R is the universal gas constant (1.985×10^{-3} kcal mol^{-1} K^{-1}) and T is the temperature (298.15 K). These findings suggest that these compounds are promising inhibitors of human pancreatic alpha amylase.

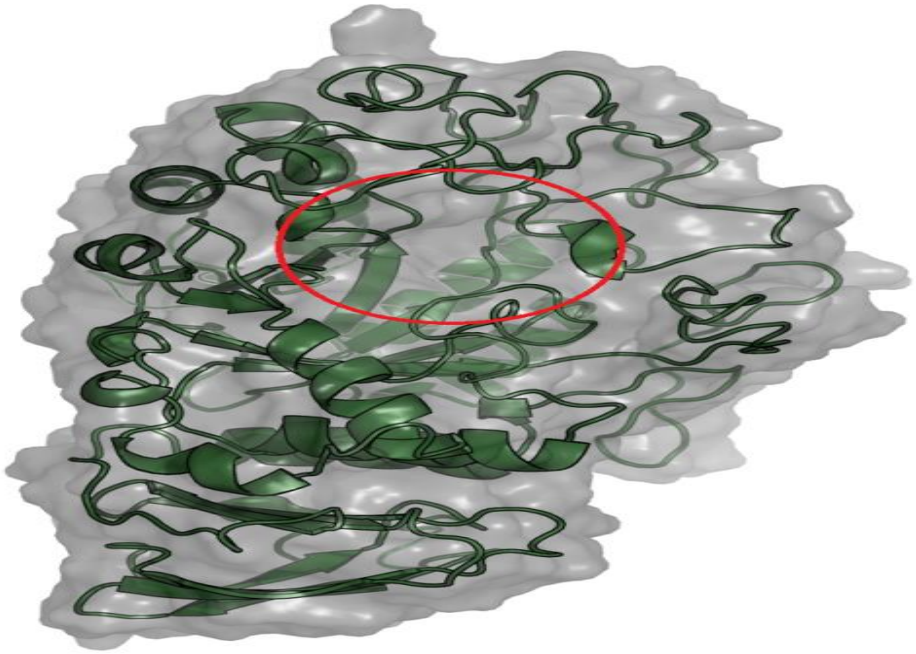

Figure 3.82: Crystal structure of human pancreatic alpha amylase (3BAJ); active site is indicated with the red circle.

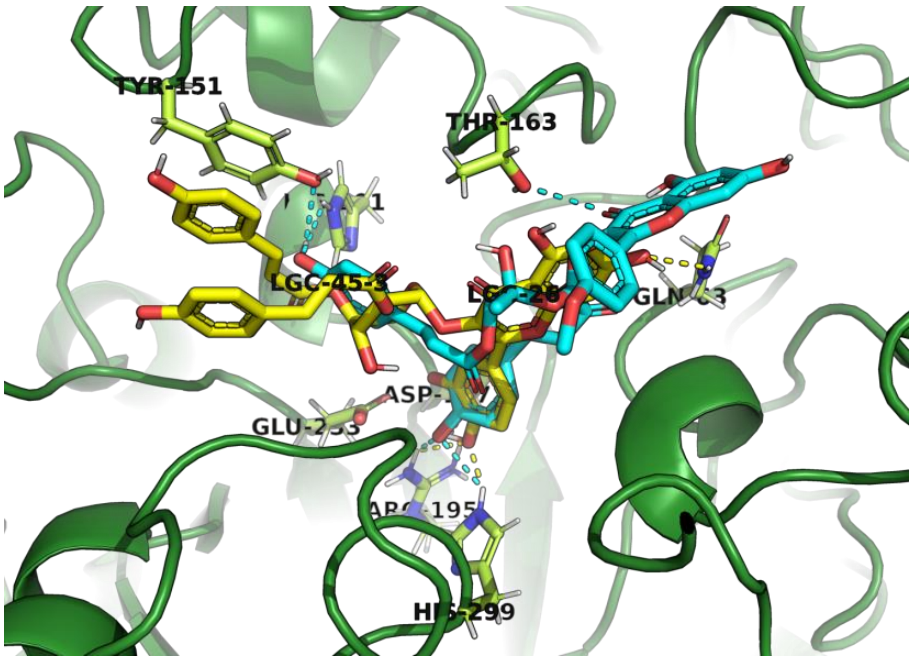

Figure 3.83: Overlay of LGC-26 (95) (cyan) and LGC-45-3 (96) (yellow) bound to the active site of human pancreatic alpha amylase (3BAJ) (green).

3.11.3 Molecular docking of LML 363-13 (97) against COX-2 for its analgesic and against AMPK against anti-diabetic activity

The docking results clearly indicate that vomifoliol (**97**) is a better candidate as an analgesic agent. Vomifoliol is a potent binder (-4.9 kcal/mol) to COX-2 than indomethacin (-1.1 kcal/mol) indicating that it is supposed to have better analgesic action. Considering the antidiabetic activity, vomifoliol is ~1.5 times more potent than metformin.

To understand the molecular details behind the docking results, interactions between drug candidate and target proteins were analyzed. Both vomifoliol and indomethacin were stabilized in their binding groove by unconventional hydrogen bonding mainly along with other types of non-covalent interactions. Although the numbers of interactions were fairly large in case of indomethacin, it appears that vomifoliol better fits into the binding site due its better shape complementarity to binding region. As expected, both metformin and vomifoliol exhibited several conventional hydrogen bonds in their binding mode. Metformin, due its guanidium like structure, also

participated in several ionic type interactions (Figure 3.83-3.86). Better binding of vomifoliol compared to metformin is difficult to explain from this type of analysis.

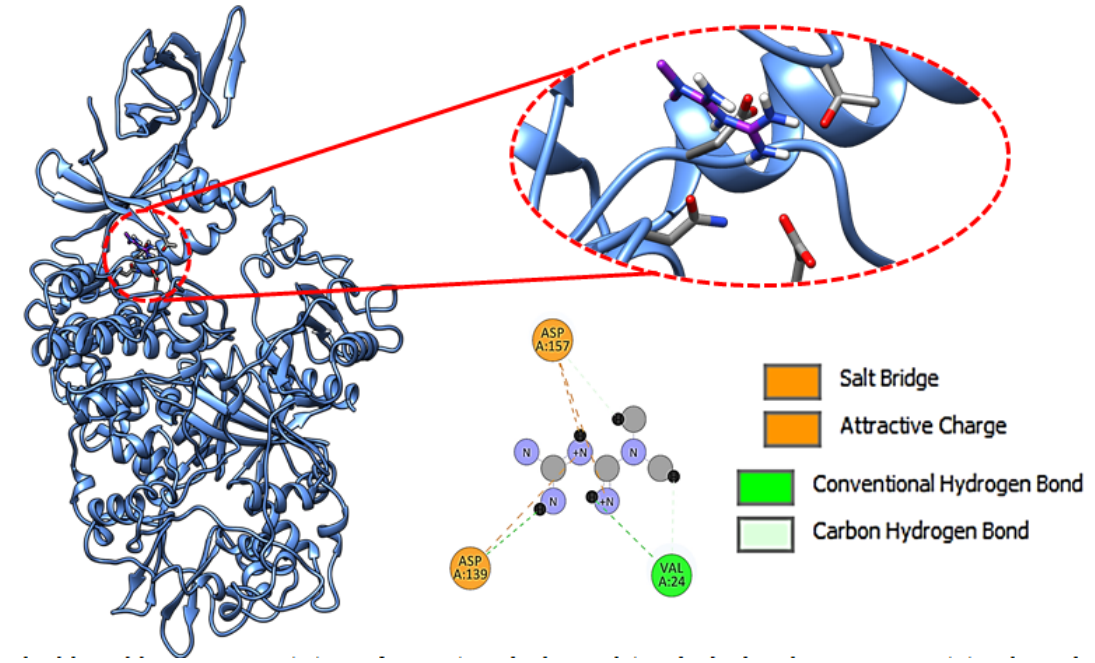

Figure 3.84: The blue ribbon is AMPK (A). Metformin (inside the circle) is docked in the active site. (B) enlarged view of the docking site. (C) Interactions between metformin and active site residues.

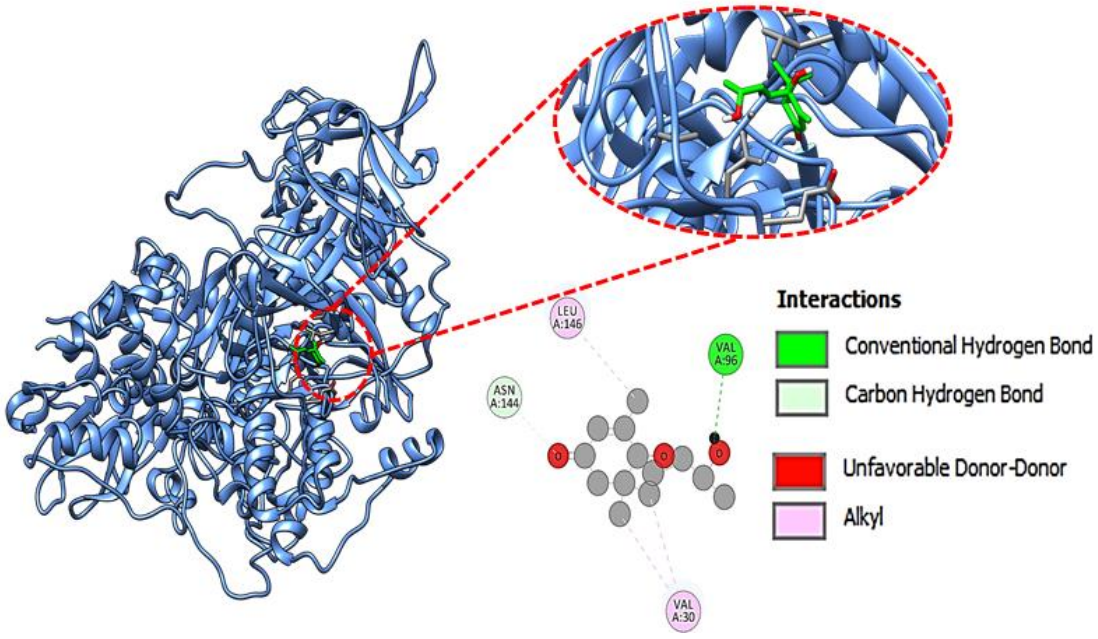

Figure 3.85: The blue ribbon is AMPK. Vomifoliol (97) is docked in the active site. (B) enlarged view of the docking site. (C) Interactions between vomifoliol and active site residues

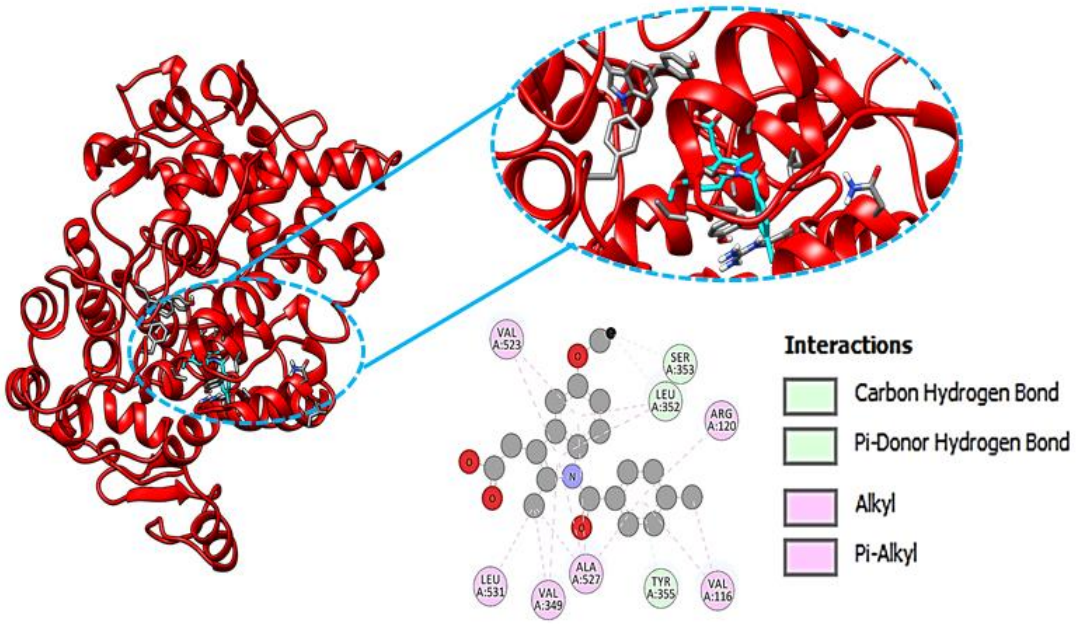

Figure 3.86: The red ribbon is COX-2. Indomethacin (inside the circle) is docked in the active site. (B) Enlarged view of the docking site. (C) Interactions between indomethacin and active site residues

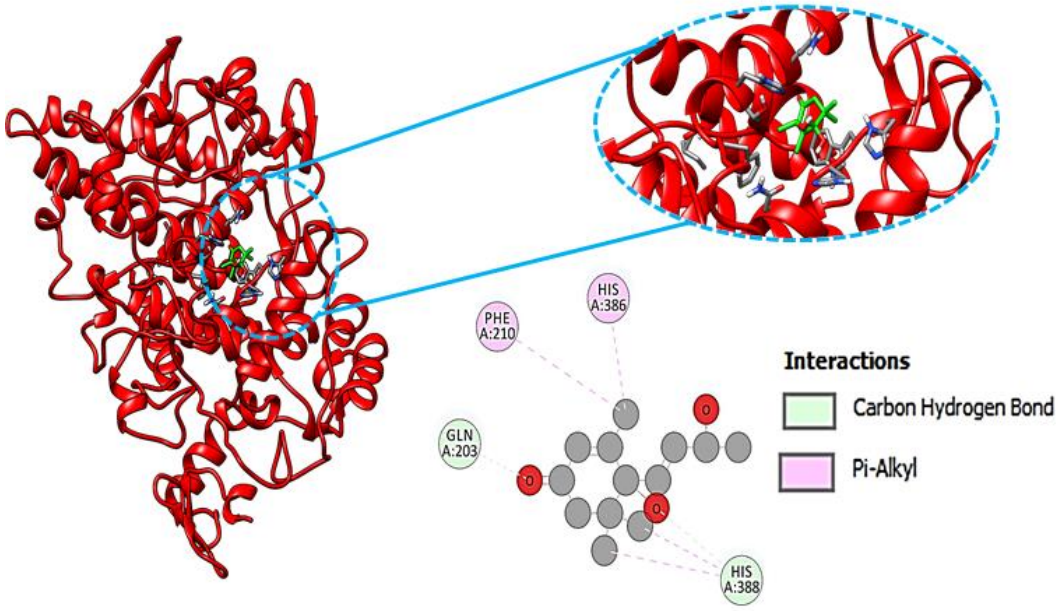

Figure 3.87: The red ribbon is COX-2. Vomifoliol (97) is docked in the active site. (B) Enlarged view of the docking site. (C) Interactions between vomifoliol and active site residues

Chapter 4: Conclusion

This thesis describes the phytochemical and pharmacological studies of medicinal plants which include isolation, purification of secondary metabolites and structure interpretation of those pure compounds as well as biological screenings to determine bioactivities of four species of genus *Litsea* belongs to the Lauraceae family. The compouds were purified by chromatographic techniques and the structures were elucidated by NMR spectroscopic studies.

A total of eleven compounds were isolated from these plants among them LML 339-1(**100**) and LML 339-2 (**101**) appear to be new compounds, LGC-26 (**95**) and LGC-45-3 (**96**) are reported for the first time from the genus *Litsea*, LML 363-1 (**97**), LML 309 (**98**), LML 301 (**99**), LDC-10-3 (**102**) are new for these selected species while LDC-10-2 (**55** and **56**) and LLC-10-1 (**55**) are reported for the first time from these *Litsea* species. LML 339-1(**100**) is characterized as *(E)*-6,7,8,9,10,11-hexahydro-8,17: 10,16di(metheno) dibenzo[h,l][1]oxa[5] azacyclotridecine-1,4-diol and LML 339-2 (**101**) as *(Z)*-1,2,3,4,5,6-hexahydro-8,11-etheno-2,13:4,12di(metheno) benzo[h]-[1]oxa[5] azacyclopentadecine. LGC-26 (**95**) and LGC-45-3 (**96**) are elucidated as 4′-*O*-methyl-(2″,4″-di-*E*-p-coumaroyl) afzelin and quercetin-3-O-(2″,4″-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside (or, 5′-hydroxyl-(2″,4″-di-*E*-*p*-coumaroyl) afzelin). LML 363-1 (**97**) is characterized as vomifoliol, LML 309 (**98**) as α-amyrin, LML 301 (**99**) as β-amyrin, LDC-10-3 (**102**) as lupeol, LDC-10-2 (**55** and **56**) as a mixture at 4:1 ratio of β-sitosterol and stigmasterol and LLC-10-1 (**55**) as β-sitosterol.

In antidiarrheal activity test, the methanol extracts of selected species of *Litsea* decreased wet feces number, total number of feces and total weight of the foecal output significantly with rising of doses. The decreased gastrointestinal motility and peristalsis index confirm the traditional use of those plants in the treatment of diarrhea.

In analgesic activity test, significant reduction of acetic acid-induced squirming or writhing and formalin induced biting or licking in mice provided us with the evidences to have pain reducing potential though Eddy's hot plate method.

In the hypoglycemic activity test, the blood glucose level in STZ-induced diabetic mice were found to decrease significantly after 7^{th} day of treatment with the plant extracts which suggests that those plants have antdibetic potential.

In CNS depressant activity test, hole cross test was performed to investigate the possible neuropharmacological (CNS stimulant or depressant) activities and all the extracts found to reduce locomotion in the test animals and to decrease the passing number through the hole in between the hole cross chamber by the animals in a dose dependent manner.

Three fractions (petroleum ether, chloroform and ethyl acetate) of four different species of *Litsea* were studied for antimicrobial activity by disc diffusion method. The results of antimicrobial activity test of different fractions showed mild to moderate activity for *L. monopetala*, very good activity for *L. lancifolia*, moderate to good activity for *L. glutinosa* and mild to very good antimicrobial activity for *L. deccanensis* agains the microorganisms selected for this study.

The maximum quantities of phenolic compounds were found in ethyl acetate soluble fraction of *L. glutinosa*, then by ethyl acetate soluble fraction of *L. lancifolia*. Among the plants *L. lancifolia* and *L. glutinosa* have shown very good total phenolis compared to *L. deccanensis* and *L. monopetala.* In DPPH free radical scavenging activity test, the IC_{50} was calculated for pet-ether, chloroform and ethyl acetate fractions of *Litsea*. From the results, it may be proposed that three different extractives of *L. deccanensis*, *L. lancifolia*, *L. glutinosa* and *L. monopetala* were able to reduce the free radical scavenging activity compared to ascorbic acid, a potent antioxidant compound.

The docking simulation was conducted against aldose reductase (AKR1B1) protein model with the purified compound **95** and **96** by using Auto Dock Vina software. Compound **95** exposes the higher negative binding affinity (-9.8 kcal/mol) compared to that of the compound **96** (-9.4 kcal/mol) for the interaction of the protein. During investigating interaction pattern, binding affinity, and best binding poses of the compounds it can be proposed that both compounds might be promising inhibitors against aldose reductase (AKR1B1) protein. Molecular docking analysis of isolated compounds **95** and **96** (−9.4, and −8.9 kcal/mol, respectively) against human pancreatic alpha amylase showed promising docking affinity and this finding suggests that these compounds are promising inhibitors of human pancreatic alpha amylase.

The docking results of vomifoliol clearly demonstrated that it is a better candidate as an analgesic agent. Vomifoliol **(97)** is a potent binder (-4.9 kcal/mol) to COX-2 than indomethacin (-1.1 kcal/mol) indicating that it is supposed to have better analgesic

action. Thus, it as clearly evident that the selected *Litsea* species (*L. deccanensis, L. lancifolia, L. glutinosa* and *L. monopetala*) contain structurally unique and biologically interesting secondary metabolites. Further studies with these plants may lead to isolation and structural characterization of more bioactive molecules which could lead to discovery of new drug candidates.

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Chapter 6: List of Publications

- Bulbul, I.J., Uddin, M.E., Nahar, N., Kuddus, M.R., Haque, M.R. and Rashid, M.A. 2021. Antidiarrheal Activity of Four Different Species of *Litsea* Available in Bangladesh. *Biomedical and Pharmacology Journal*. Sep 1; 14(3): 1259-67.
- Bulbul, I.J., Rashid, M.A. and Haque, M.R. 2020. Pharmacological Studies of Different Fractions of *Litsea monopetala* Roxb. *Bangladesh Pharmaceutical Journal*. Feb 3; 23(1): 61-4.
- Bulbul, I.J., Haque, M.R. and Rashid, M.A. 2020. Pharmacological investigations of *Litsea lancifolia* (Roxb.) Hook. F. *Bangladesh Journal of Botany*. Mar 31;49(1):179-83.