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Evaluation of the Phytochemical and Biological Properties of *Albizia lebbeck* Flowers Growing in Saudi Arabia

تقييم الخصائص الكيميائية والبيولوجية لأزهار نبات الألبيزيا لبخ النامي في المملكة العربية السعودية

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Thesis presented

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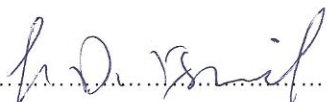
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Summary

Albizia lebbeck L. (الألبيزيا لبخ) is a member of the legume family (Fabaceae) which is the third largest family of flowering plants with more than 18,000 described species. *A. lebbeck* L. is among the most important tree species imported many years ago from India and well adapted to the harsh environmental conditions of the central part of Saudi Arabia. It is used for furniture, flooring and a variety of agricultural tools. The cut bark yields a reddish-brown gum used as a substitute for gum Arabic obtained from *Acacia senegal*. *A. lebbeck* L. has many synonyms such as Acacia, koko, shack-shack, whistling-bean, Sarenh and woman's tongue.

The current literature revealed that some plants belonging to the genus *Albizia* have possessed medicinal values. Previous phytochemical investigation of different species belonging to genus *Albizia* afforded different classes of secondary metabolites such as saponins, terpens, alkaloids and flavonoids. Some bioactive compounds were isolated and identified from genus *Albizia* e.g. triterpenoid saponins (julibroside J_{29}, julibroside J_{30}, julibroside J_{31}), novel macrocyclic alkaloids (budmunchiamines A, B and C) and two flavonol glycosides (quercitrin and isoquercitrin) which showed different biological activities such as anti-tumor, antiplatelets aggregation and bactericidal activities.

Reviewing the current literature for the importance of the plant *Albizia lebbeck* growing worldwide revealed many biological interests, but nothing was reported concerning the species cultivated in Saudi Arabia. This study was dedicated to the biological investigations of some phytochemical metabolites in *A. lebbeck*. Preliminary biological evaluation of different extracts of the titled species has been performed and revealed the presence of secondary metabolites exhibiting antibacterial, estrogenic, anti-inflammatory, analgesic and antipyretic activities.

The phytochemical investigation of *A. lebbeck* cultivated in Saudi Arabia resulted in isolation and identification of one novel β- lactam derivative; albactam, in addition to seven knwon compounds; β- Amyrin, 11α, 12α-oxidotaraxerol, 1-*O*-β-D-glucopyranosyl-[(2*S*, 3*S*, 4*R*, 8*E*)-2-[(2′*R*)-hydroxyhexadecanoylamino]-8-tetra-cosene-1, 3, 4-triol, [(2*S*,

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3S, 4R, 8E)-2-[(2'R)-hydroxyhexadecanoylamino]-8-tetra-cosene-1, 3, 4-triol, 3, 7-dimethyloct-1-en-3, 6, 7-triol, rutin and kaempferol-3-O-rutinoside. 11α, 12α-oxidotaraxerol, 3, 7-dimethyloct-1-en-3, 6, 7-triol, ceramide and its glycoside are reported for the first time from the species *lebbeck*.

The new compound, albactam, was subjected to further biological evaluation and it showed anti-aggregatory activity against adenosine diphosphate and arachidonic acid-induced guinea-pigs' platelets aggregation *in vitro* at doses 208 µg/ml and 172 µg/ml respectively, whereas yields of other compounds were low.

Therefore, this study is represented in five parts:

- Part 1: The introductory part that includes a review of taxonomic classification of *Albizia lebbeck*, folkloric uses and biological activities. Furthermore, the isolation of chemical constituents from *A. lebbeck* are covered in this part.
- Part 2: Materials and methods that includes all materials, equipments and techniques used in this study. In addition, the details of the biological evaluation methods, isolation and characterization of isolates are covered. Moreover, detailed preliminary phytochemical screening tests are also presented.
- Part 3: Results and discussion includes characterization and structure elucidations of the isolated compounds are listed.
- Part 4: Detailed biological screening results for antimicrobial, antipyretic, analgesic, estrogenic and anti-inflammatory activities are presented in the biological investigation part.
- Part 5: Includes a list of references found at the end of this thesis.

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List of Abbreviations

δ	Chemical shift value
^{13}C NMR	Carbon 13-nuclear magnetic resonance
^{1}HNMR	proton nuclear magnetic resonance
cm	Centimeter
cm^{-1}	Reciprocal of Centimeter
$CDCl_3$	Deuterated chloroform
DEPT	Distortionless Enhancment by Polarization Transfer
ESI	Electron Spray Ionization
Fig.	Figure
Hr	Hours
Hz	Hertz
IR	Infrared
J	Nuclear spin-spin coupling constant
MS	Mass spectrum
$[M]^+$	Molecular ion peak
m/z	Mass/ charge ratio
mp	Melting point
nm	Nanometer
ppm	parts per million (10^{-6})
R_f	Relative to solvent front
SRB	Sulforhodamine B protein binding dye
TLC	Thin layer chromatography
µg/ml	Micro-gram per milliliter
CFU	Colony forming units

IC_{50}	Inhibitory concentration 50%
LC_{50}	lethal concentration 50%
MBC	Minimum bactericidal concentration
MIC	Minimum inhibitory concentration
R*t*	Retention time
HMQC	Heteronuclear Multiple Quantum Correlation
HMBC	Heteronuclear Multiple Bond Correlation

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Introduction

Since ancient times, nature has been an important source of medicinal agents. This fact is illustrated by the large number of natural products currently in use in medical practice. The value of natural products in this regard can be assessed using three criteria: first, the rate of introduction of new chemical entities of wide structural diversity including natural products serving as templates for semi-synthetic and total synthetic analogues. Secondly, the number of diseases treated or prevented by these natural substances. Thirdly, their frequent use in treatment of diseases. In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants. This interest in drugs of plant origin is due to several reasons, namely, the frequent inefficiency of conventional medicine, possible development of side effects of synthetic drugs, and that a large percentage of the world's poor population doesn't have access to conventional pharmacological treatment. In addition, the long history of use of folk medicine suggests that "natural" products are usually harmless. The plant kingdom offers a unique and renewable resource for the discovery of potential new drugs and important leads against various pharmacological targets including pain, cancer, HIV/AIDS, Alzheimer's and malaria.

Reviewing the current literature for the importance of the plant *Albizia lebbeck* L. growing worldwide revealed many biological interests, but nothing was reported concerning the study of the species growing in Saudi Arabia. As a part of the efforts to explore therapeutic agents from local plants, the current phytochemical and biological studies on some extracts and isolated compounds from *A. lebbeck* were carried out. *A. lebbeck* is among the most important tree species that were imported many years ago from India and well adapted to the harsh environmental conditions of the central part of Saudi Arabia. Being cultivated, it is possible to obtain sustainable amounts of many valuable bioactive components discovered from this species.

A-Taxonomy

Family *Fabaceae*:

The legume family (Fabaceae) is the third largest family of flowering plants with more than 18,000 described species. It is surpassed in size only by the orchid family (Orchidaceae) with about 20,000 species and the sunflower family (Asteraceae) with about 24,000 species [Parrotta, 2002]. The family includes herbs, shrubs, trees and vines distributed throughout the world, especially the tropical rain forest. The fruit is technically called a legume or pod. It is composed of a single seed-bearing carpel that splits open along two seams. Legume fruits come in an enormous variety of shapes and sizes, including indehiscent pods that do not split open [Parrotta, 2002]. The leaves are usually alternate and compound. Most often they are even- or odd-pinnately compound (e.g. *Caragana* and *Robinia* respectively), often trifoliate (e.g. *Trifolium*, *Medicago*) and rarely palmately compound (e.g. *Lupinus*), in the Mimosoideae and in the commonly bipinnate Caesalpinioideae (e.g. Acacia, Mimosa). They always have stipules, which can be leaf-like (e.g. *Pisum*), thorn-like (e.g. *Robinia*) or be rather inconspicuous. Leaf margins are entire or, occasionally, serrate. Both the leaves and the leaflets often have wrinkled pulvini to permit nastic movements. In some species, leaflets have evolved into tendrils (e.g. *Vicia*).

Many species have leaves with structures that attract ants that protect the plant from herbivore insects (a form of mutualism). Extrafloral nectaries are common among the Mimosoideae and the Caesalpinioideae, and are also found in some Faboideae (e.g. *Vicia sativa*). In some Acacia, the modified hollow stipules are inhabited by ants [Hélène *et al.*, 2006].

The flowers always have five generally fused sepals and five free petals. They are generally hermaphrodite, and have a short hypanthium, usually cup shaped. There are normally ten stamens and one elongated superior ovary, with a curved style. They are usually arranged in indeterminate inflorescences. Fabaceae are typically entomophilous plants (i.e. they are pollinated by insects), and the flowers are usually showy to attract pollinators [Hélène *et al.*, 2006].

Fruits of legume family have ovaries which most typically develop into legumes. A legume is a simple dry fruit that usually dehisces (opens along a seam) on two sides. A common name for this type of fruit is a "pod", although that can also be applied to a few other fruit types. A few species have evolved samarae, loments, follicles, indehiscent legumes, achenes, drupes, and berries from the basic legume fruit [Gurcharan, 2004].

Many Fabaceae host bacteria in their roots within structures called root nodules. These bacteria, known as rhizobia, have the ability to take nitrogen gas (N_2) out of the air and convert it to a form of nitrogen that is usable to the host plant (NO_3- or NH_3). This process is called nitrogen fixation. The legume, acting as a host, and rhizobia, acting as a provider of usable nitrate, form a symbiotic relationship [Gurcharan, 2004].

There are three subfamilies of the legume family which are Subfamily Papilionoideae, Subfamily Caesalpinioideae and Subfamily Mimosoideae. Members of the subfamily Mimosoideae have flowers with radial symmetry, small, inconspicuous corollas and numerous, showy stamens. The flowers are typically in many-flowered heads or spikes. This subfamily includes *Acacia* (wattle), *Albizia* (silk tree), *Samanea* (monkeypod), *Prosopis* (mesquite) and *Calliandra* (powder puff) [Parrotta, 2002].

Genus *Albizia* L.:

The genus *Albizia* comprises approximately 150 species, mostly trees and shrubs native to tropical and subtropical regions of Asia and Africa. Leaves are bipinnate with leaflets in numerous pairs or larger in fewer pairs. Petiolar glands are conspicuous. Flowers are in globose heads or spikes. Stamens elongate, usually white. Corolla is funnel-shaped, connate beyond the middle. Fruit is broadly linear indehiscent or 2-valved, valves not twisted [Migahid, 1989].

Albizia lebbeck L.:

Albizia lebbeck is a fast-growing, medium-sized deciduous tree with a spreading umbrella-shaped crown of thin foliage and smoothish, finely fissured, grayish-brown bark. Depending on site conditions, annual height growth ranges from 0.5 to 2.0 m; on good sites, individual trees attain an average height of 10 to 15 m. The species grows well

from sea level to 1500 m on sites receiving between 500 and 2500 mm^3 annual rainfall and tolerates both light frosts and drought. While it grows poorly on heavy clay soils, it tolerates saline sites. The tree grows best on moist, well-drained soils. *A. lebbeck,* a valued timber species within its native Asian range, was previously exported to Europe under the trade name East Indian walnut. It is used for furniture, flooring and a variety of agricultural implements. The cut bark yields a reddish-brown gum that is used as a substitute for gum Arabic obtained from *Acacia senegal*. In its native range, the species is sometimes planted as a shade tree in coffee, tea, cardamom, and cacao plantations. It is also used as a host for the lac insect.

Flowers usually appear with new leaves over an extended period beginning at the end of the dry season; in the Caribbean region this season occurs between April and September. Flowering can occur on trees as young as 10 months. The fragrant, cream-colored flowers develop on lateral stalks in rounded clusters 5 to 7.5 cm across the many threadlike, spreading, whitish-to-yellow stamens tipped with light green, borne at the ends of lateral stalks 4 to 10 cm long. The fruits, flattened pods 10 to 20 cm long and 2.5 to 3.8 cm broad, are produced in large numbers and each contains several seeds. Immature pods are green, turning straw-colored on maturity, usually 6 to 8 months after flowering. The dry pods remain on the tree well into the following season. Seeds are released from the mature, dehiscent pods while still attached to the tree or from windblown pods that later split open or decompose. Under natural conditions, insect attack is responsible for high seed predation.

The mature pods may be collected by hand from the ground or low branches or clipped with pruning poles. Seeds are easily extracted from the pods by hand or by crushing the pods and winnowing. *A. lebbeck* seeds are small, oblong, approximately 9 by 7 mm long and broad, compressed, and light brown in color with a smooth, hard testa. Seeds average from 7,000 to 11,000 per kg. Seeds can be stored for up to 5 years in sealed containers at room temperature with only moderate reduction in percentage viability.

A. lebbeck L. (الألبيزيا لبخ) has many synonyms such as Acacia, Acacia amarilla, Aroma francesa, Barba de Caballero, black ebony, East Indian walnut, Forestina, Guarmuche, koko, lebbek, lebbek albizia, shack-shack, singer-tree, siris-tree, tcha-tcha, tibet-tree, vieille fille, West Indies-ebony, whistling-bean and woman's-tongue [Parrotta, 2002].

Fig. (1): *Albizia lebbeck* tree

Fig. (2): *A. lebbeck* flowers

Fig. (3): *A. lebbeck* pods

Fig.(4): *A. lebbeck* leaves, pods & flowers

Taxonomic classification of *Albizia lebbeck :*

The taxonomists classify the plant as follows:

 kingdom : *Plantae*, division: *Magnoliophyta*, class: *Magnoliopsida*, subclass: *Rosidae*, order: *Fabales*, family: *Fabaceae*, subfamily: *Mimosoideae*, genus: *Albizia*, and species: *lebbeck.*

B- Folkloric uses and biological activities of Albizia species:

The current literature revealed that some plants belonging to genus *Albizia* have great medicinal values. Recently, an antitumor triterpene saponin julibroside J_{28} was isolated from the stem bark of *A. julibrissin* and displayed significant antitumor activity *in vitro* against PC-3M-1E8, Bel-7402, and HeLa cancer cell lines at 10μM assayed by SRB method [Hong *et al*., 2005].

A. lebbeck is a tree well known in the Indian subcontinent for its range of uses. *A. lebbeck* is used in Indian traditional system and folk medicine as well to treat several inflammatory pathologies such as asthma, arthritis and burns [*Ayurvedic Pharmacopoeia of India,* 2001]. *A. lebbeck* inhibited the passive cutaneous anaphylaxis and mast cell degranulation in rat. Moreover, it could protect the sensitized guinea pig from antigen-induced anoxic convulsion [Baruach *et al*., 1997]. Recently, it was found that the alcoholic extract of *A. lebbeck* has antihistaminic property, by neutralizing the histamine directly or due to corticotrophic action as evidenced by raising cortisol levels in plasma [Babu *et al*., 2009].

It is also reported in Indian folk medicine that *A. lebbeck* has antiseptic, antidysentric and anti-tubercular activities [*Ayurvedic Pharmacopoeia of India,* 2001]. Moreover, Saponins of *A.lebbeck* have been claimed to be useful in treatment of Alzheimer's and Parkinson's diseases [Sanjay, 2003].

The bark has acrid taste and its extract showed antimicrobial activity. The active constituents of the bark extract were anthraquinone glycosides that cause the leakage of the cytoplasmic constituents [Ganguli and R.M. Bhatt, 1993]. It has also immunomodulatory effect [Baruah, *et al*., 2000]. In addition, It is used for bronchitis, leprosy, paralysis and helminth infections [*Ayurvedic Pharmacopoeia of India,* 2001]. Oral administration of a saponin isolated from *A. lebbeck* bark at the dose level of 50 mg/kg per day to male albino rats showed a significant decrease in the weight of testes, epededymides seminal vesicle and ventral prostate [Gupta *et al*., 2005].

Leaves have been claimed to have anticonvulsant activity [Kasture *et al.*, 1996] and nootropic effect [Chintawar *et al.*, 2002] which may be due to the presence of certain important compounds like alkaloids and flavanoids. Moreover, the aqueous extract of *A. lebbeck* leaves showed antioxidant activity in diabetic rats [Resmi *et al.*, 2006]. The saponins of the seeds of *A. lebbeck* exhibited antiovulatory properties. The seeds had anti-fertility effect on male rats [Singh *et al.*, 1991] and antidiarhoeal activity studied on conventional rodents models of diarrhea [Besra *et al.*, 2002]. In addition, the flowers are being commonly used to treat anxiety, depression and insomnia in traditional Chinese medicine [King *et al.*, 2007].

Clinical study of herbal and ayurvedic preparations of *A. lebbeck* is carried out individually by B. Mukhopadhyay, Iyenger and Chandra [Mukhopadhyay *et al.*, 1992] and results show that these preparations are safe and effective.

Treatment of allergic conjunctivitis by oral and local application of *A. lebbeck* has been studied. This clinical study was done on 60 cases of various types of allergic conjunctivitis to assess the role of *A. lebbeck* in the form of eye drops and capsule for a period of 60 days for treatment and further 90 days for follow up. Significant results were observed [Mukhopadhyay *et al.*, 1992].

The decoction of herbal combination of (*Ashatoda vasica* (malabar nut); *Coleus forskholii*; *Albizia lebeck*; *Croton tiglium*; *Picrorrhiza kurroa*; *Tylophora indica/asthmatica* (Indian ipecac)) was administered to 14 patients suffer from asthma in a dose of 30 ml three times or two times daily and significant improvement in peak expiratory flow rate and eosinophil count were observed after 28 days of treatment. All patients showed clinical improvement in their symptoms of breathlessness, cough and wheezing. The combination is effective in the prophylaxis of asthma [Iyenger *et al.*, 1994].

C- Some bioactive compounds isolated from Genus *Albizia*:

The genus *Albizia* has been known to contain many classes of bioactive compounds. It includes bioactive saponins, alkaloids, flavonoids…etc. The following are some representative examples of these bioactive compounds isolated from genus *Albizia*.

1- Saponins:

Two active saponins, Albiziatrioside A and B **(1&2)** were isolated using bioassay-guided fractionation of a methanolic extract of *A. subdimidiata.* Both isolated compounds showed significant cytotoxicity against the A2780 cell line [Abdel-Kader *et al*., 2001].

Three new anti-tumor triterpenoid saponins, julibroside J_{29} **(3)**, julibroside J_{30} **(4)** and julibroside J_{31} **(5)**, were isolated from the stem bark of *A. Julibrissin* [Zheng *et al*., 2005]. In addition, three new oleanane-type triterpene saponins **(6-8)**, named grandibracteosides A−C, were isolated from the methanolic extract of leaves of *A. grandibracteata.* The crude extract and the pure compounds showed significant inhibitory activity against KB and MCF7 tumor cell lines *in vitro*. The compounds are glycosides of acacic acid acylated by an *o*-aminobenzoyl unit [Sabrina *et al*., 2005].

Moreover, three new saponins were isolated from the bark of *A. procera*. They were characterized as 3-*O*-[β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl] echinocystic acid **(9)**, 3-O-[α-L-arabinopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)-2-acetamido-2-deoxy-→D-glucopyranosyl] echinocystic acid **(10)** and 3-*O*-[β-D-xylopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 6)-2-acetamido-2-deoxy-β-D-glucopyranosyl] acacic acid lactone **(11).** The saponin **(9)** exhibited cytotoxicity against HEPG2 cell line with IC_{50} 9.13 µg/ml [Melek *et al*., 2007].

Three new oleanane type triterpene saponins, albizosides A-C **(12-14)**, were isolated from the stem bark of *A. chinensis*. These compounds showed cytotoxic activity against a small panel of human tumor cell lines as well as hemolytic activity against rabbit erythrocytes [Rui *et al*., 2009].

A new oleanane-type saponin coriariosides A **(15),** along with known saponin was isolated from the roots of *A. coriaria*. The compound tested for cytotoxicity against two

colorectal human cancer cells and showed activity against the HCT 116 (IC$_{50}$ 4.2 µM) and HT-29 (IC$_{50}$ 6.7 µM) cell lines [Note *et al.*, 2009]. Structures are shown in Fig. 5.

2- Alkaloids:

Novel macrocyclic alkaloids (budmunchiamines A, B and C **(32-34)** were isolated from *A. amara.* They were also found to have antiplatelets aggregation and bactericidal activity [Mar *et al.*, 1991].

Two new macrocyclic spermine alkaloids **(35&36)** were isolated as a mixture from the leaves of *A. inopinata*. Preliminary studies indicated that the compounds shown a posible pharmacological depresor activity on the central nervous system [De Assis *et al.*, 1999].

In addition, bioassay guided fractionation of the crude methanol extracts of the stem bark and leaves of *A. adinocephala* led to the isolation of two new bioactive spermine alkaloids, budmunchiamines L4 **[37]** and L5 **[38].** The extracts were found to inhibit the malarial enzyme plasmepsin II [Ovenden *et al.*, 2002].

Moreover, the alkaloidal fraction of the methanolic extract of *A. gummifera* exhibited strong activity against chloroquine sensitive (NF54) and resistant (ENT30) strains of *Plasmodium falciparum* with IC$_{50}$ of 0.16 ± 0.05 and 0.99 ± 0.06 µg/ml, respectively. Five known spermine alkaloids **(39-43)** were isolated from the alkaloidal fraction [Rukunga *et al.*, 2007].

3- Flavonoids:

A novel flavonol glycoside was isolated from the acetone soluble fraction of ethanolic extract of stem of *A. procera* which was identified as 5,2', 4'-trihydroxy-3,7,5'-trimethoxyflavonol-2'-*O*-β-D-galactopyranosyl-(1→4)-*O*-β-D-glucopyranoside **(50).** The acetone sol. fraction showed moderate anti-inflammatory action on albino rats by using non-imunological carrageenan induced hind paw edema method [Yadava and Tripathi, 2000].

Two flavonol glycosides, quercitrin **(51)** and isoquercitrin **(52)** were isolated from the flowers of *A. julibrissin* and showed sedative activity. Both compounds **(50)** and **(51)**

increased pentobarbital-induced sleeping time in dose-dependent manner in mice. These results supported the use of the flowers of this plant as a sedative agent in oriental traditional medicine [Kang *et al*., 2000].

In addition, a new biologically active flavonol glycoside 3, 5, 4'-trihydroxy, 7, 3'-dimethoxy-3-*O*-β-D-glucopyranosyl-(1→4)-α-L-xylopyranoside **(53)** was isolated from the chloroform-soluble fraction of the seeds of *A. julibrissin*. The antibacterial activity of the chloroform-soluble fraction of the methanolic extract was fairly active against gram positive and gram negative bacteria [Yadava and Reddy, 2001].

Oxygen radical absorbance capacity (ORAC) values showed that the methanolic extracts of *A. julibrissin* foliage displayed antioxidant activity. The analysis confirmed the presence of three compounds in *A. julibrissin* foliage methanolic extract: an unknown quercetin derivative, hyperoside (quercetin-3-*O*-galactoside) **(54),** and quercitrin (quercetin-3-*O*-rhamnoside) **(55)** [Lau *et al*., 2007].

4- Phenolic compounds:

Two phenolic glycosides (albibrissinosides A **(58)** and B **(59)** were isolated from the stem bark of *A. julibrissin*. The albibrissinoside B were found to be a radical scavenger on the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical [Jung *et al*. 2004].

D-Chemical constituents of Genus *Albizia*:

Phytochemical investigation of the different species belong to genus *Albizia* afforded different classes of secondary metabolites such as triterpenes, triterpenoidal saponins, flavonoids, alkaloids, lipids and amino acids…etc.

A lot of phytochemical investigation has been carried out on this species. Moreover, the flowers on steam distillation gave colorless, sweet-smelling oil (4.3% w/v) [Kumar *et al*., 2007].

The following are some representative examples for different classes of secondary metabolites isolated from genus *Albizia*:

1- Saponins:

Genus *Albizia* has been known to contain substantial amounts of saponins. Lebbekanin E (**16**) was isolated from *A. lebbeck* and its structure determined on the basis of its IR spectrum and hydrolysis products [Varshney, Pal and Vyas, 1976]. Three saponins were also isolated from the seeds of *A. lucida*. Their structures were established as 3-*O*-[β-D-xylopyranosyl (1→2)- α-L-arabinopyranosyl (1→6)][β-D-glucopyranosyl (1→2)]-β-D-glucopyranosyl echinocystic acid (**17**); 3-*O*-[α-L-arabinopyranosyl (1→6)][β-D-glucopyranosyl (1→2)]- β-D-glucopyranosyl echinocystic acid (**18**) and 3-*O*-[β-D-xylopyranosyl (1→2)-β-D-fucopyranosyl (1→6)-2- acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid (**19**) characterized as methyl esters [Orsini, pelizzoni and Verotta, 1991]. In addition, three main saponins were isolated from the bark of *A. lebbeck* and named albiziasaponins A, B and C (**20-22**) [Bikas *et al*., 1995].

Also, the stem bark of *A. gummifera* yields oleanane saponins: vitalboside-A (**23**) and vitalboside-A 2'- methylglucuronate (**24**) [Rukunga and waterman, 2001]. Moreover, albiziahexoside (**25**), a new hexaglycosylated saponin, was isolated from the leaves of *A. lebbeck* [Minoru *et al*., 2003]. Two new oleanane-type triterpene saponins, adianthifoliosides A (**26**) and B (**27**), were also isolated from a ethanolic extract of roots of *A. adianthifolia.* The two compounds were characterized as glycosides of acacic acid acylated by an *O*-hydroxybenzoyl unit [Haddad *et al*., 2003]. Structures are in Fig. (25).

Table 1: Distribution of some saponins in the Genus *Albizia*

Species	Investigated part	Compound	Biological activity	Ref.
A. subdimidiata	Whole plant	Albiziatrioside A (**1**) and B (**2**)	cytotoxicity against the A2780 cell line	[Abdel-Kader *et al*., 2001].
A. Julibrissin	The stem bark	Julibroside J_{29}(**3**), J_{30} (**4**) and J_{31} (**5**)	Anti-tumor	[Zheng *et al*., 2006]
A. grandibracteata	Leaves	grandibracteosides A−C (**6-8**)	significant inhibitory activity against KB and MCF7 tumor cell lines in vitro	[Sabrina *et al*., 2005]
A. procera	The stem bark	3-*O*-[β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl] echinocystic acid (**9**), 3-*O*-[α-L-arabinopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)-2-acetamido-2-deoxy-→D-glucopyranosyl] echinocystic acid (**10**) and3-*O*-[β-D-xylopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1	The saponin (**9**) exhibited cytotoxicity against HEPG2 cell line with IC_{50} 9.13 µg/ml	[Melek et al., 2007]

Table 1 *Cont.*

		→ 6)-2-acetamido-2-deoxy-β-D-glucopyranosyl] acacic acid lactone (11)		
A. chinensis	The stem bark	albizosides A-C (12-14)	cytotoxic activity against a small panel of human tumor cell lines as well as hemolytic activity against rabbit erythrocytes	[Rui *et al.*, 2009].
A.coriaria	The roots	Coriariosides A (15)	The compound tested for cytotoxicity against two colorectal human cancer cells and showed activity against the HCT 116 (IC$_{50}$ 4.2 μM) and HT-29 (IC50 6.7 μM) cell lines	[Note *et al.*, 2009]
A. lebbeck	Whole plant	Lebbekanin E (16)		[Varshney, Pal and Vyas, 1976]
	The stem bark	albiziasaponins A, B and C (20-22)		[Bikas *et al.*, 1995]
	The leaves	Albiziahexoside (25)		[Minoru *et al.*, 2003]

Table 1 *Cont.*

A. *lucida*	The seeds	3-*O*-[β-D-xylopyranosyl (1→2)-α-L-arabinopyranosyl (1→6)][β-D-glucopyranosyl (1→2)]-β-D-glucopyranosyl echinocystic acid(**17**); 3-*O*-[α-L-arabinopyranosyl (1→6)][β-D-glucopyranosyl (1→2)]- β-D-glucopyranosyl echinocystic acid (**18**) and 3-*O*-[β-D-xylopyranosyl (1→2)-β-D-fucopyranosyl (1→6)-2- acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid (**19**) characterized as methyl esters		[Orsini, pelizzoni and Verotta, 1991]
A. *gummifera*	The stem bark	vitalboside-A (**23**) and vitalboside-A 2'-methylglucuronate (**24**)		[Rukunga and waterman, 2001]
A. *adianthifolia*	Roots	Adianthifoliosides A (**26**) and B (**27**)		[Haddad *et al.*, 2003]
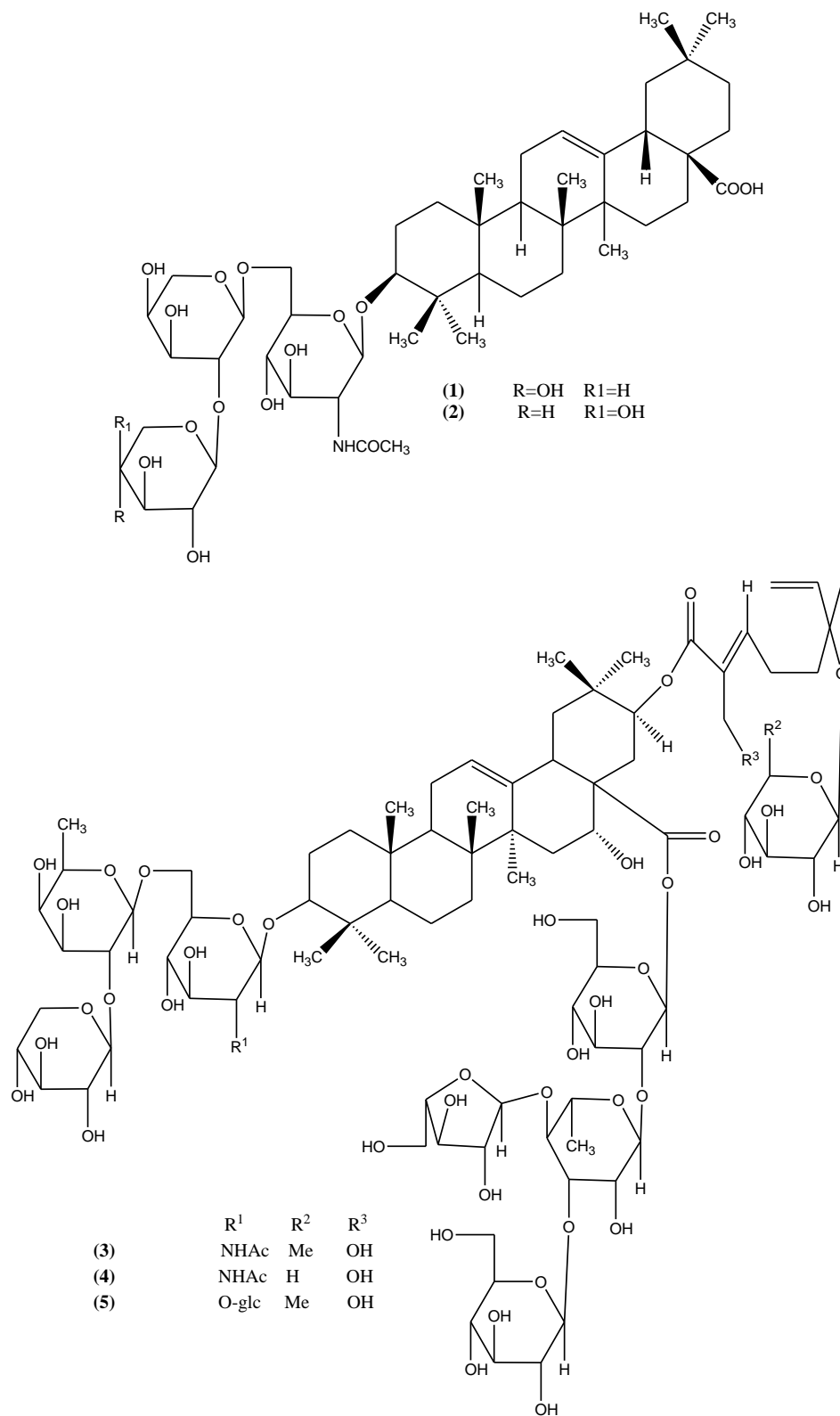
16

	R	R1
(1)	R=OH	R1=H
(2)	R=H	R1=OH

	R^1	R^2	R^3
(3)	NHAc	Me	OH
(4)	NHAc	H	OH
(5)	O-glc	Me	OH

Fig. (5): Structures of some saponins (1-5) isolated from genus *Albizia*

R= Fructose and xylose (2-1)

	R1	R2	R3
(6)	H	H	Xylose
(7)	Glucose	H	Xylose
(8)	H	Glucose	xylose

(9) R= β-D-Xylp-(1-2)-α-L-Arap
(10) R= α-L-Arap-(1-2)-β-D-Fucp

Fig. (5): Structures of some saponins (6-10) isolated from genus Albizia (*Cont*.)

(11)

	R[1]	R[2]	R3
(12)	Me	H	S
(13)	Me	Glu	S
(14)	H	Glu	H

Fig. (5): Structures of some saponins (11-14) isolated from genus Albizia (*Cont*.)

R₁=

R₂=

R₃=

R₄=

(15)
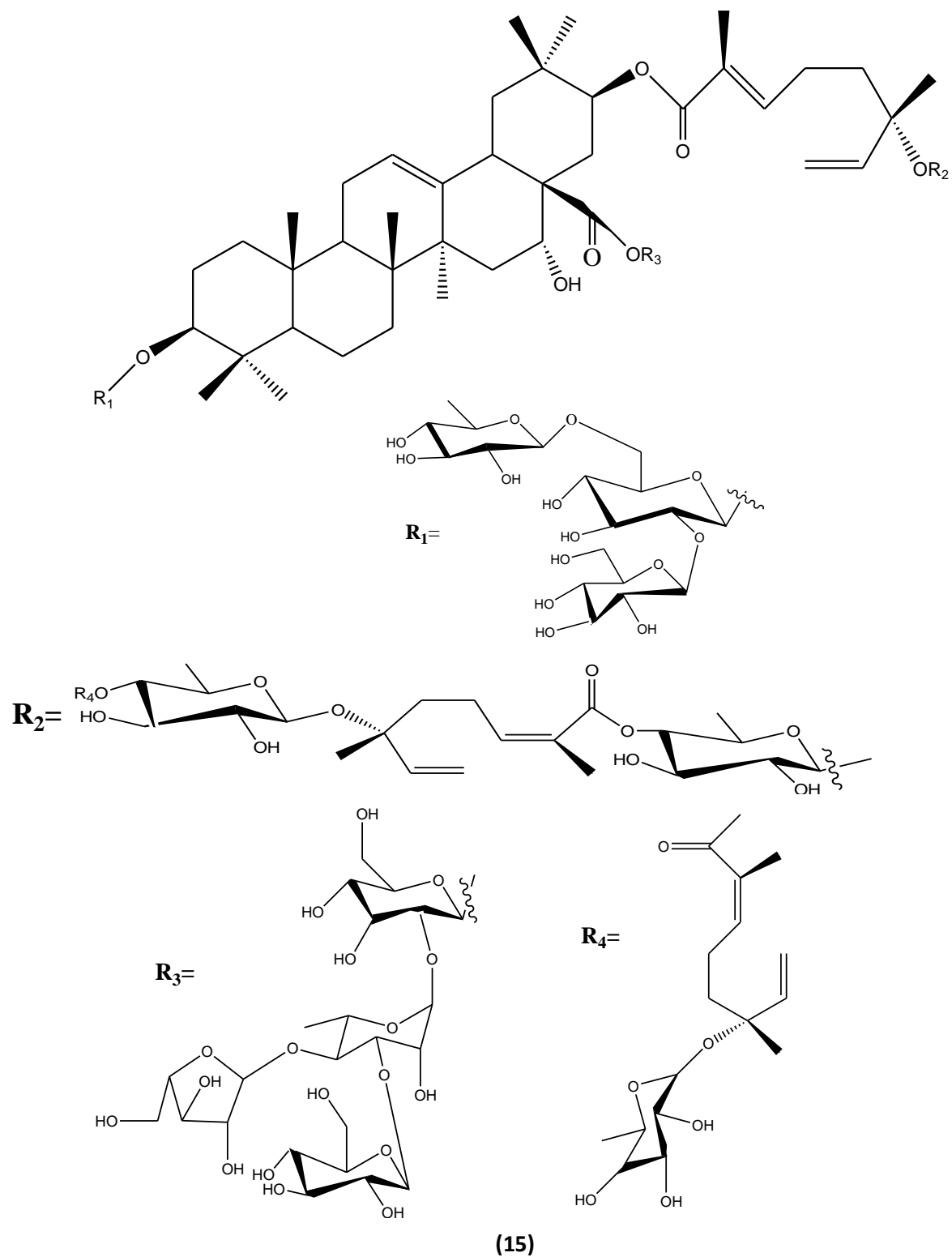
Fig. (5): Structures of some saponins (15) isolated from genus Albizia (*Cont*.)
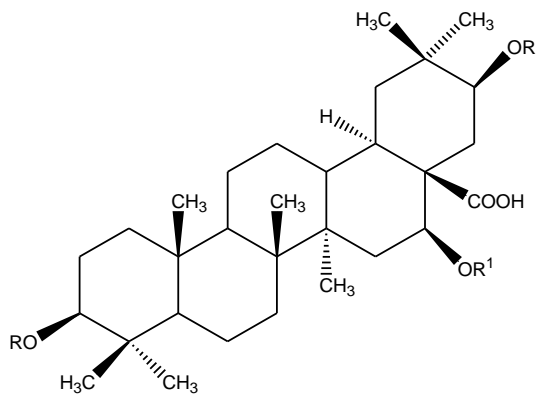
(16) R and/or R^1 = glucose, arabinose, xylose and rhamnose
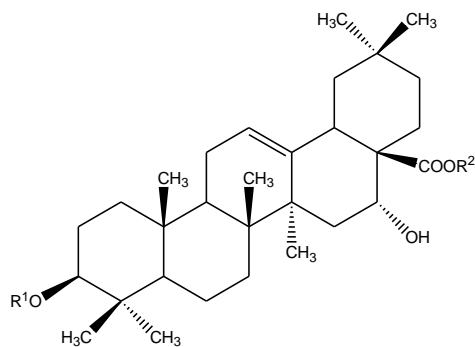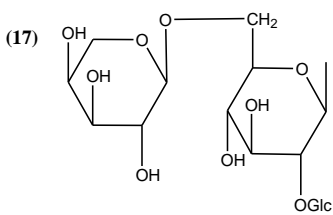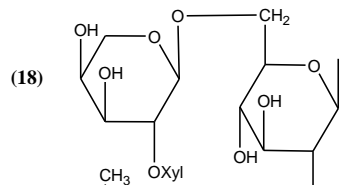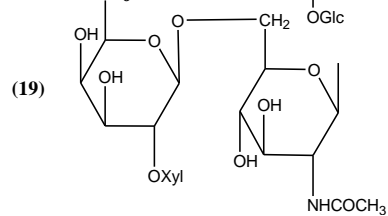
	R^1	R^2
(17)		H
(18)		H
(19)		CH3

Fig. (5): Structures of some saponins (16-19) isolated from genus Albizia (*Cont*.)
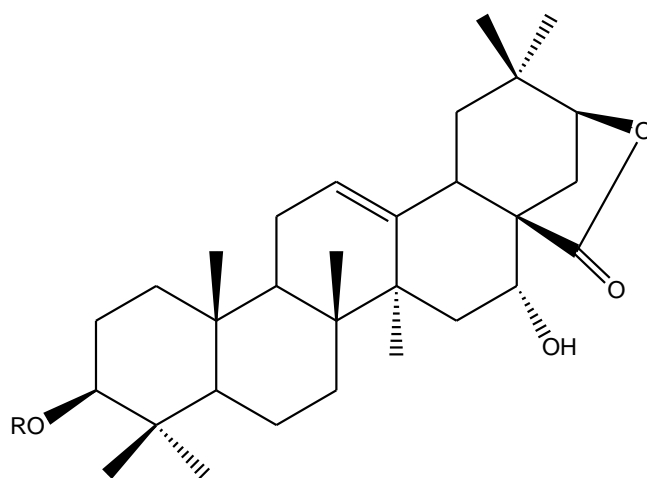
(20) R=β-glucopyranosyl-α-arabinosyl–β- xylopyranosyl
(21) R=β-glucopyranosyl-α-arabinosyl-β-glucopyranosyl
(22) R= β-xylopyranosyl-α-arabinosyl-β-glucopyranosyl-β-glucopyranosyl
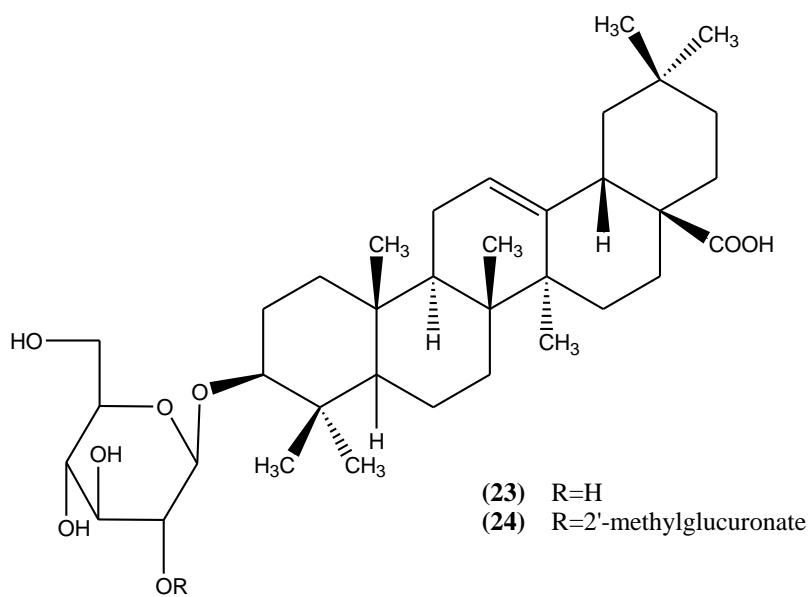
(23) R=H
(24) R=2'-methylglucuronate

Fig. (5): Structures of some saponins (20-24) isolated from genus Albizia (*Cont*.)

(25)

(26) R= H
(27) R= β-D-glucopyranosyl
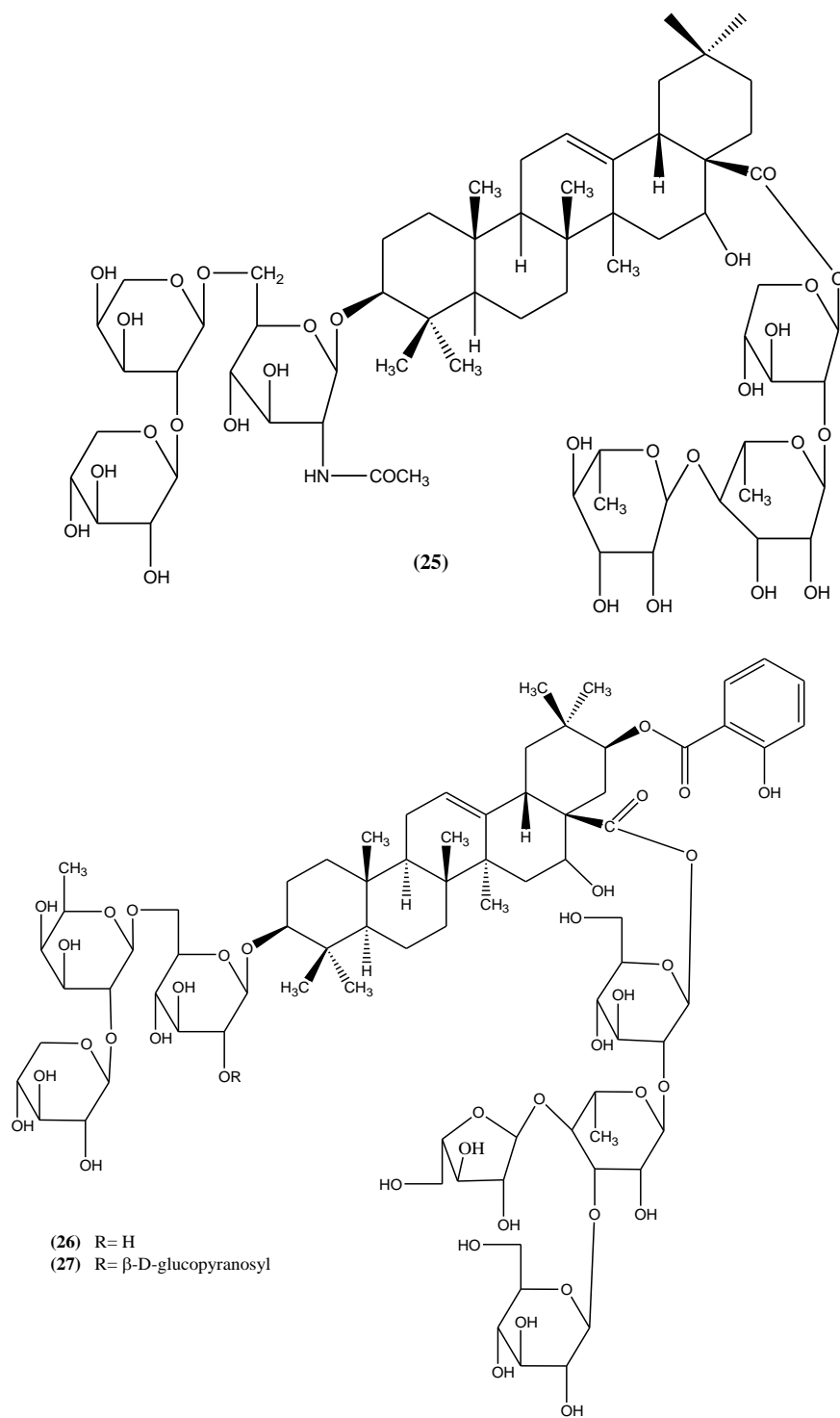
Fig. (5): Structures of some saponins (25-27) isolated from genus Albizia (*Cont*.)

2- Terpenes:

Occurrence of triterpene aglycones was reported in some species of *Albizia*. A new monoterpene conjugated triterpene from the stem bark of *A. julibrissin* was isolated. The new terpene was identified as 21-[4-(ethylidene)-2-tetrahydrofuranmethacryloyl] mechaerinic acid **(28)** [Woo and Kang, 1984]. In addition, lupeol **(29)** and acacic acid lactone **(30)** were isolated from *A. versicolor* [Rukunga and Waterman, 2001].

Moreover, the stem bark of *A. gummifera* has yielded three triterpenes, lupeol **(29),** lupenone **(31)** and vitalboside-A **(23)** [Rukunga and Waterman, 2001].

Table 2: Distribution of some terpenes in Genus *Albizia*

Species	Investigated part	Compound	Ref.
A. julibrissin	the stem bark	21-[4-(ethylidene)-2-tetrahydrofuranmethacryloyl] mechaerinic acid **(28)**	[Woo and Kang, 1984]
A. versicolor	Whole plant	Lupeol **(29)** and acacic acid lactone **(30)**	[Rukunga and Waterman, 2001]
A. gummifera	The stem bark	lupenone **(31)**	[Rukunga and Waterman, 2001]
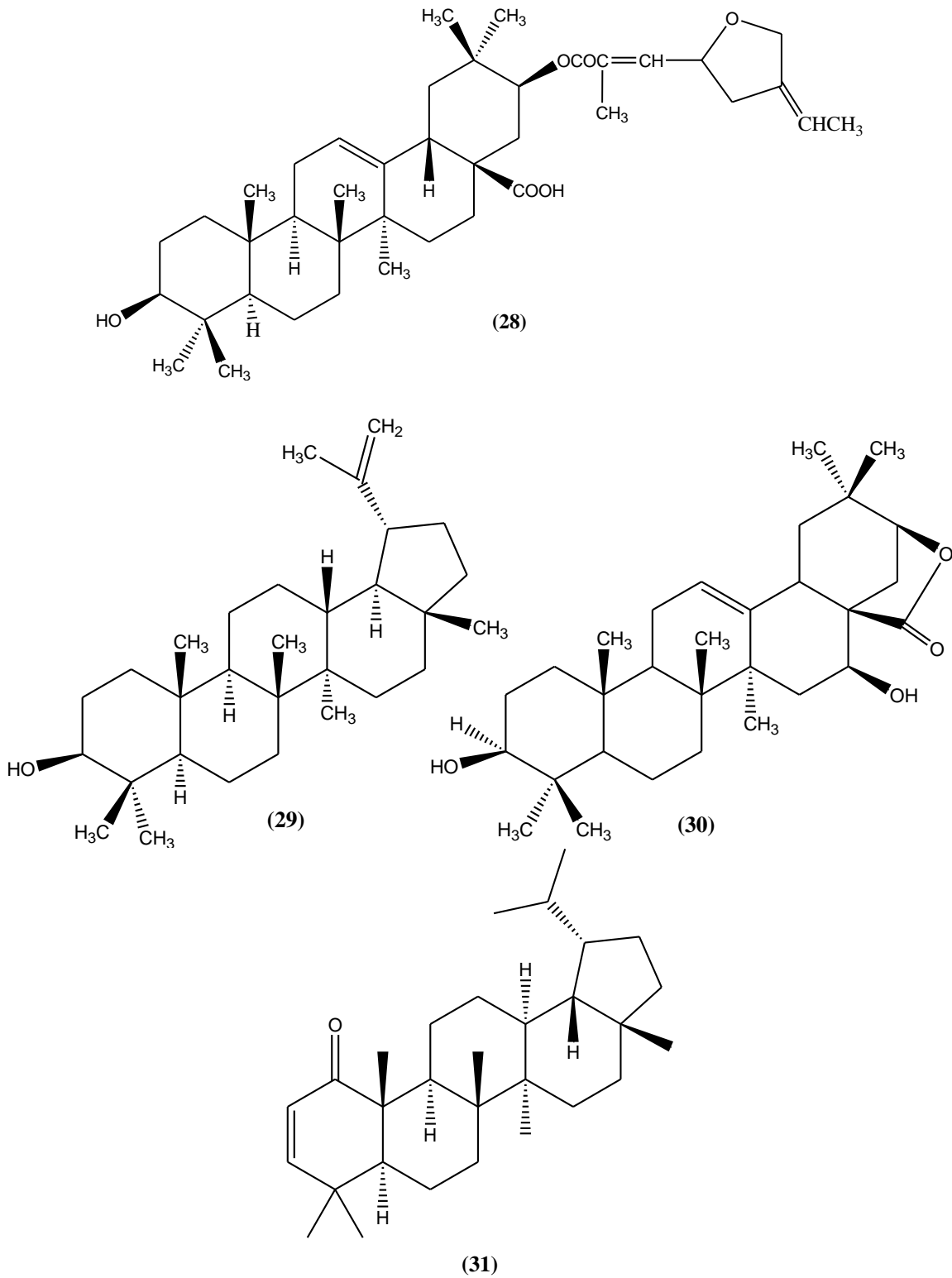
Fig. (6): Structures of some terpenes (28-31) isolated from genus Albizia

3- Alkaloids and nitrogenous compounds:

Spermine-type alkaloids are characteristic for genus *Albizia*. They are characterized by the presence of a macrocyclic lactam ring, formed by combination of the base with long-chain fatty acids or cinnamic acid and its derivatives. The natural polyamines putrescine, spermidine and spermine are common bases reported to have several important functions in animals, plants and microorganisms. In plants their involvement in organ development, flowering, fruit ripening, senescence and stress responses is reported [Hesse *et al*., 2001].

A novel macrocyclic spermidine alkaloid, albizzine A **(44)** was isolated from stem bark of *A. myriophylla* [Ito *et al*., 1994].

Three macrocyclic spermine alkaloids, budmunchiamines L1-L3 **(45-47)** were isolated from the methanol extract of seeds of *A. lebbeck* [Misra, Dixit and Wagner, 1995].

In addition, a new ceramide and its glycoside were isolated from the flower of *A. julibrissin*. Their structures were established as (2S, 3S, 4R, 8E)-2-[(2'R)-hydroxyhexadecanoylamino-8-tetra-cosene-1, 3, 4-triol **(48)** and 1-*O*-β-D-glucopyranosyl-(2S, 3S, 4R, 8E)-2-[(2'R)-hydroxyhexadecanoylamino-8-tetra-cosene-1, 3, 4-triol **(49)** on basis of chemical and spectroscopic studies [Kang *et al*., 2006].

Table 3: Distribution of some alkaloids and nitrogenous compounds in Genus *Albizia*

Species	Investigated part	Compound	Biological activity	Ref.
A. amara	Whole plant	Budmunchiamines A (**32**), B (**33**) and C (**34**)	Have antiplatelets aggregation and bactericidal activity	[Mar *et al*., 1991].
A. inopinata	The leaves	The trivial names felipealbizine A (**35**) and felipealbizine B (**36**))	Possible pharmacological depresor activity on the central nervous system.	[De Assis *et al*., 1999].
A. adinocephala	the stem bark and leaves	Budmunchiamines L4 (**37**) and L5 (**38**)	Inhibit the malarial enzyme plasmepsin II.	[Ovenden *et al*., 2002].
A. gummifera	Whole plant	Five known spermine alkaloids (**39-43**).	Exhibited strong activity against choroquine sensitive (NF54) and resistant (ENT30) strains of *Plasmodium falciparum* with IC $_{50}$ of 0.16 ± 0.05 and 0.99 ± 0.06 μg/ml, respectively	[Rukunga *et al*., 2007].

Table 3. *Cont.*

A. *myriophylla*	Bark	Albizzine A **(44)**		[Ito *et al.*, 1994].
A. *lebbeck*	Seeds	Budmunchiamines L1-L3 **(45-47)**		[Misra, Dixit and Wagner, 1995]
A. *julibrissin*	The flower	(2S, 3S, 4R, 8E)-2-[(2'R)-hydroxyhexadecanoylamino-8-tetra-cosene-1, 3, 4-triol **(48)** and 1-*O*-β-D-glucopyranosyl-(2S, 3S, 4R, 8E)-2-[(2'R)-hydroxyhexadecanoylamino-8-tetra-cosene-1, 3, 4-triol **(49)**		[Kang *et al.*, 2006].

(32) n= 10
(33) n= 8
(34) n= 12

(35)

(36)
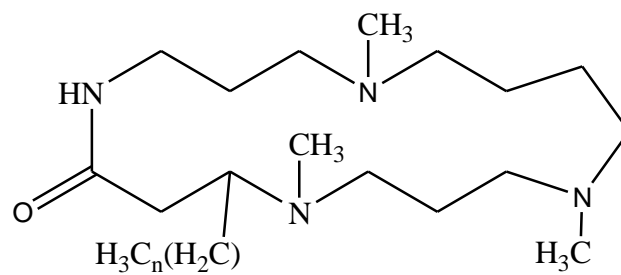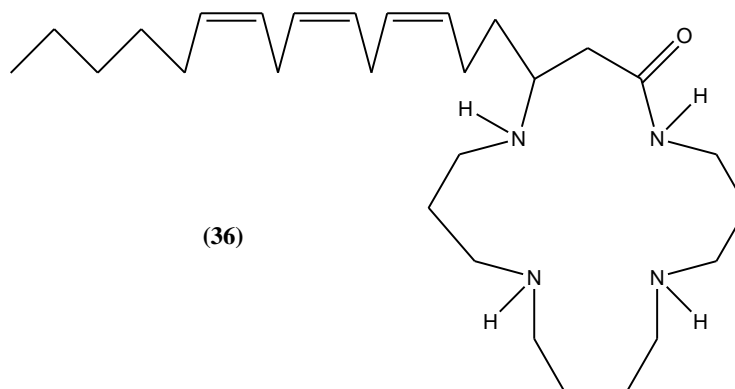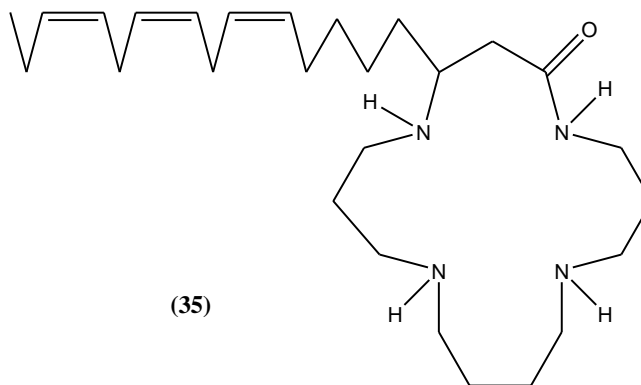
Fig. (7): Structures of some nitrogenous compounds (32-36) isolated from genus *Albizia*
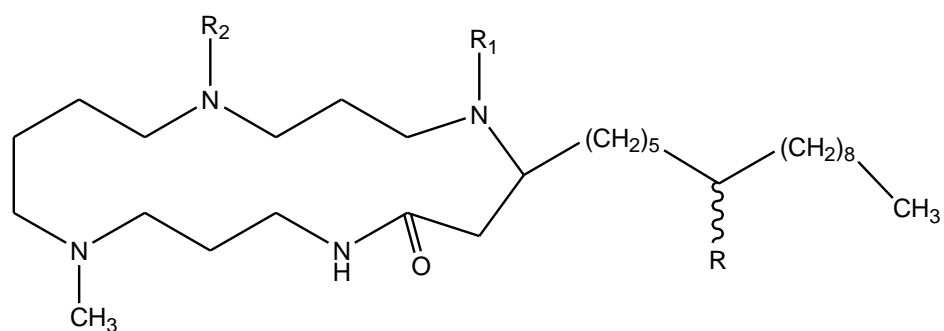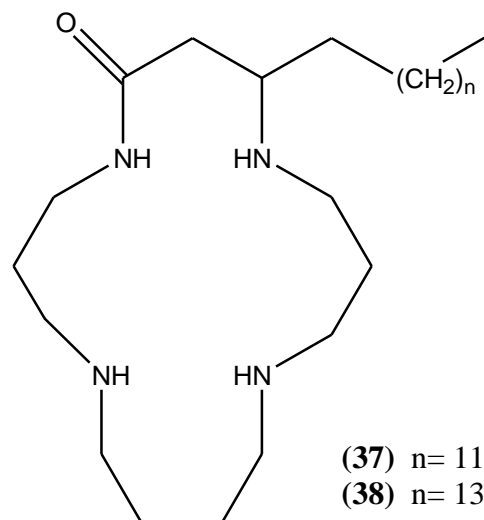
	R	R₁	R₂
(39)	H	CH3	CH3
(40)	OH	CH3	CH3
(41)	H	H	CH3
(42)	OH	H	CH3
(43)	H	CH	H
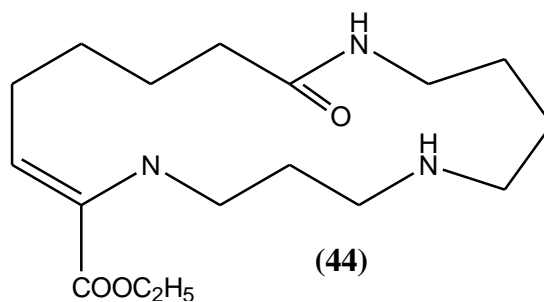
Fig. (7): Structures of some nitrogenous compounds (37-44) isolated from genus *Albizia* (*Cont.*)

	n	X
(45)	8	H2
(46)	6	H2
(47)	3	O
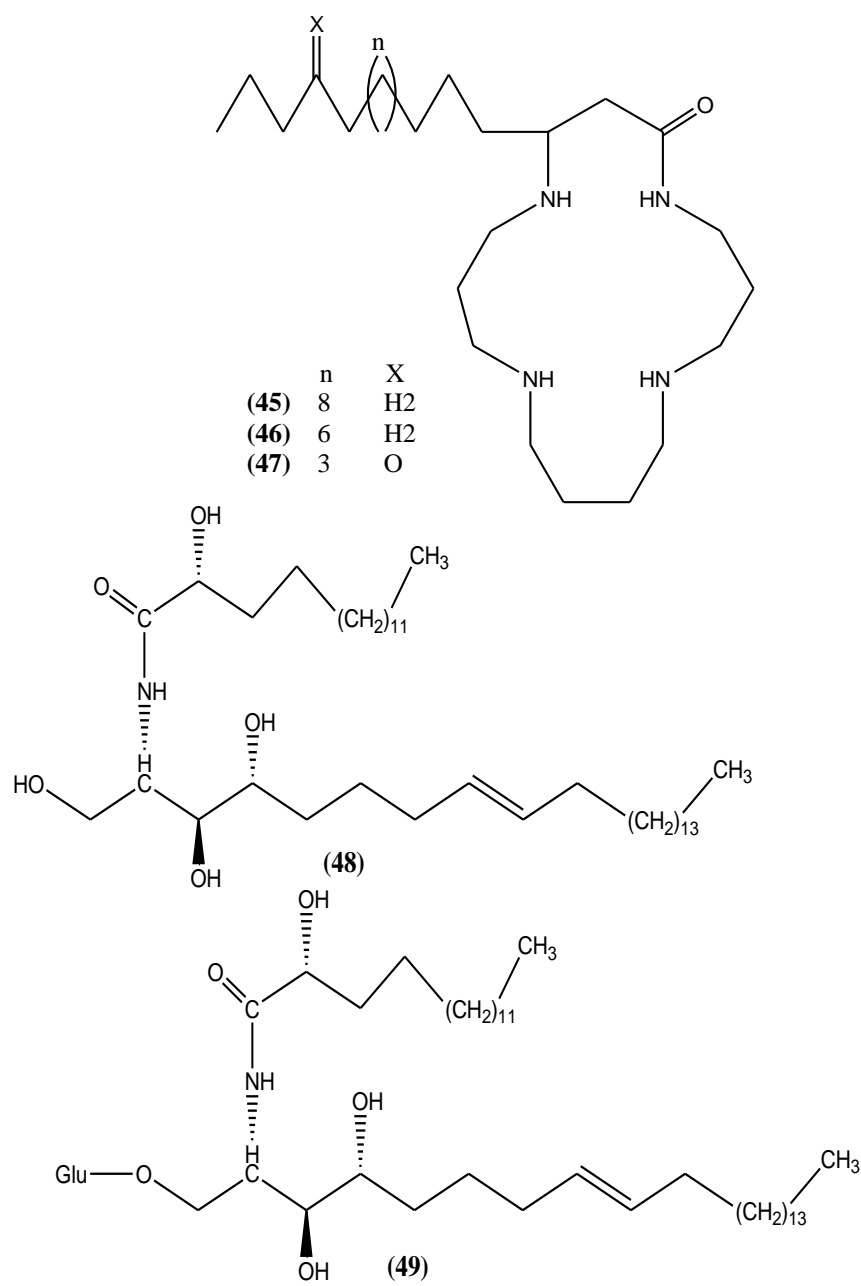
Fig. (7): Structures of some nitrogenous compounds (45-49) isolated from genus *Albizia*
(Cont.)

4- Flavonoids:

Two new tri-*O*-glycoside flavonols, quercetin and kaempferol 3-*O*-α-rhamnopyranosyl (1→6)-β-glucopyranosyl (1→6)-β-galactopyranosides **(56&57)**, were identified from the leaves of *A. lebbeck* [El-Mousallamy, 1998].

Table 4: Distribution of some flavonoids in Genus *Albizia*

Species	Investigated part	Compound	Biological activity	Ref.
A. procera	The stem	5,2', 4'-trihydroxy-3,7,5'-trimethoxyflavonol-2'-*O*-β-D-galactopyranosyl-(1→4)-*O*-β-D-glucopyranoside **(50)**	Moderate anti-inflammatory action on albino rats.	[Yadava and Tripathi, 2000].
A. julibrissin	The flowers	quercitrin **(51)** and isoquercitrin **(52)**.	Sedative activity.	[Kang *et al*., 2000].
	The seeds	3, 5, 4'-trihydroxy, 7, 3-dimethoxy-3-*O*-β-D-glucopyranosyl-α-L-xylopyranoside **(53)**	Antibacterial and antifungal.	[Yadava and Reddy, 2001].
	Foliage	Hyperoside (quercetin-3-*O*-galactoside **(54),** and quercitrin (quercetin-3-*O*-rhamnoside) **(55)**	Antioxidant activity	[Lau *et al*., 2007].
A. lebbeck	The leaves	Quercetin and kaempferol 3-*O*-α-rhamnopyranosyl (1→6)-β-glucopyranosyl (1→6)-β-galactopyranosides **(56&57)**		[El-Mousallamy, 1998].
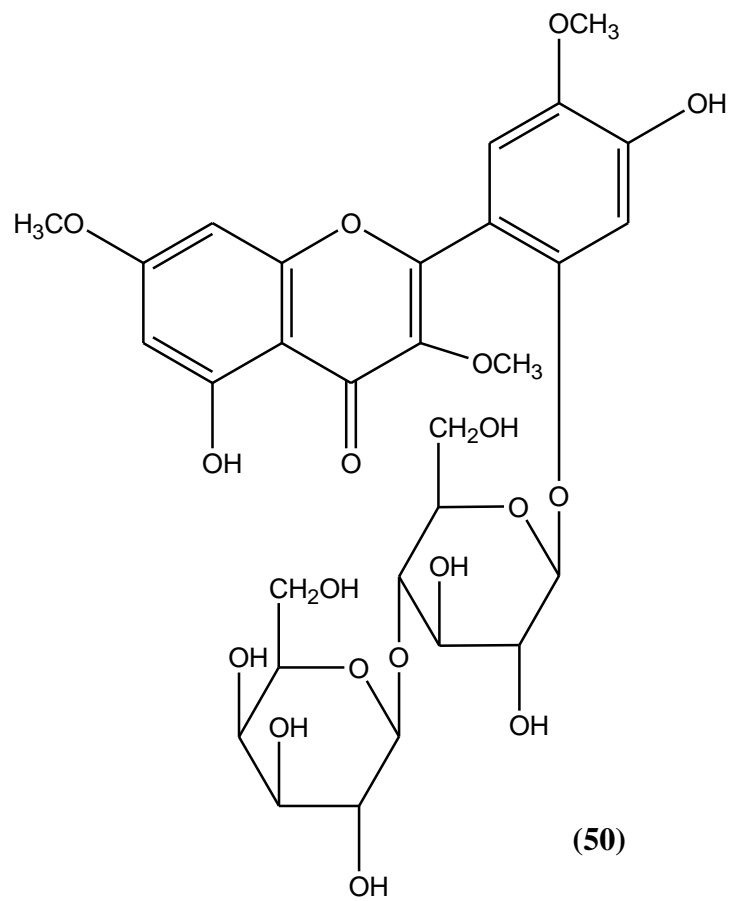
Fig. (8): Structures of some flavonoids (50) isolated from genus *Albizia*

(51) R= α-L-Rhamnopyranose

(52) R= β-D-Glucopyranose

(53)
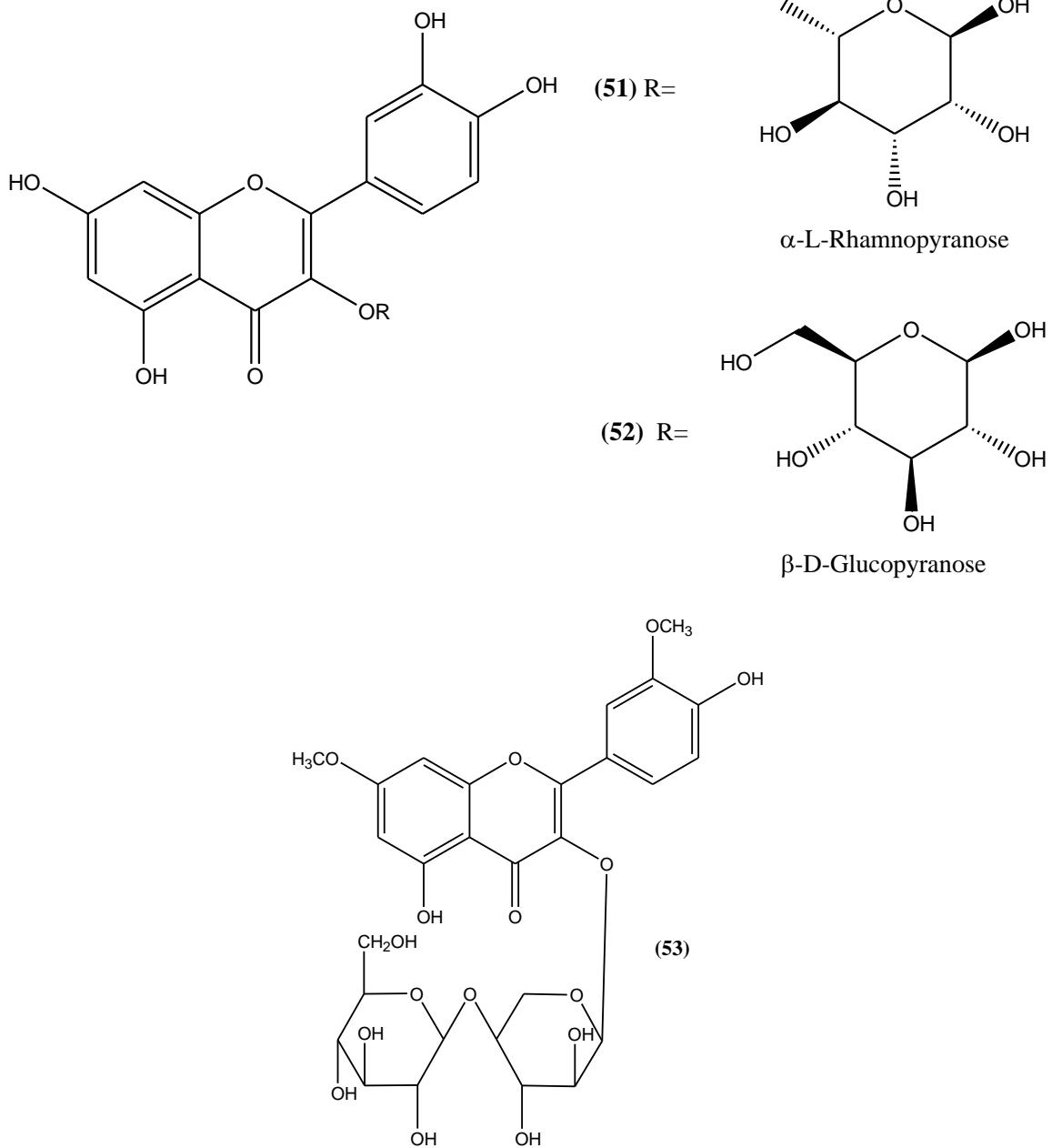
Fig. (8): Structures of some flavonoids (51-53) isolated from genus *Albizia (Cont.)*

(54)

(55)

(56)

O-α-rhamnopyranosyl(1→6)-β-glucopyranosyl(1→6)-β-galactopyranoside

(57)

O-α-rhamnopyranosyl(1-6)-β-glucopyranosyl(1-6)-β-galactopyranoside
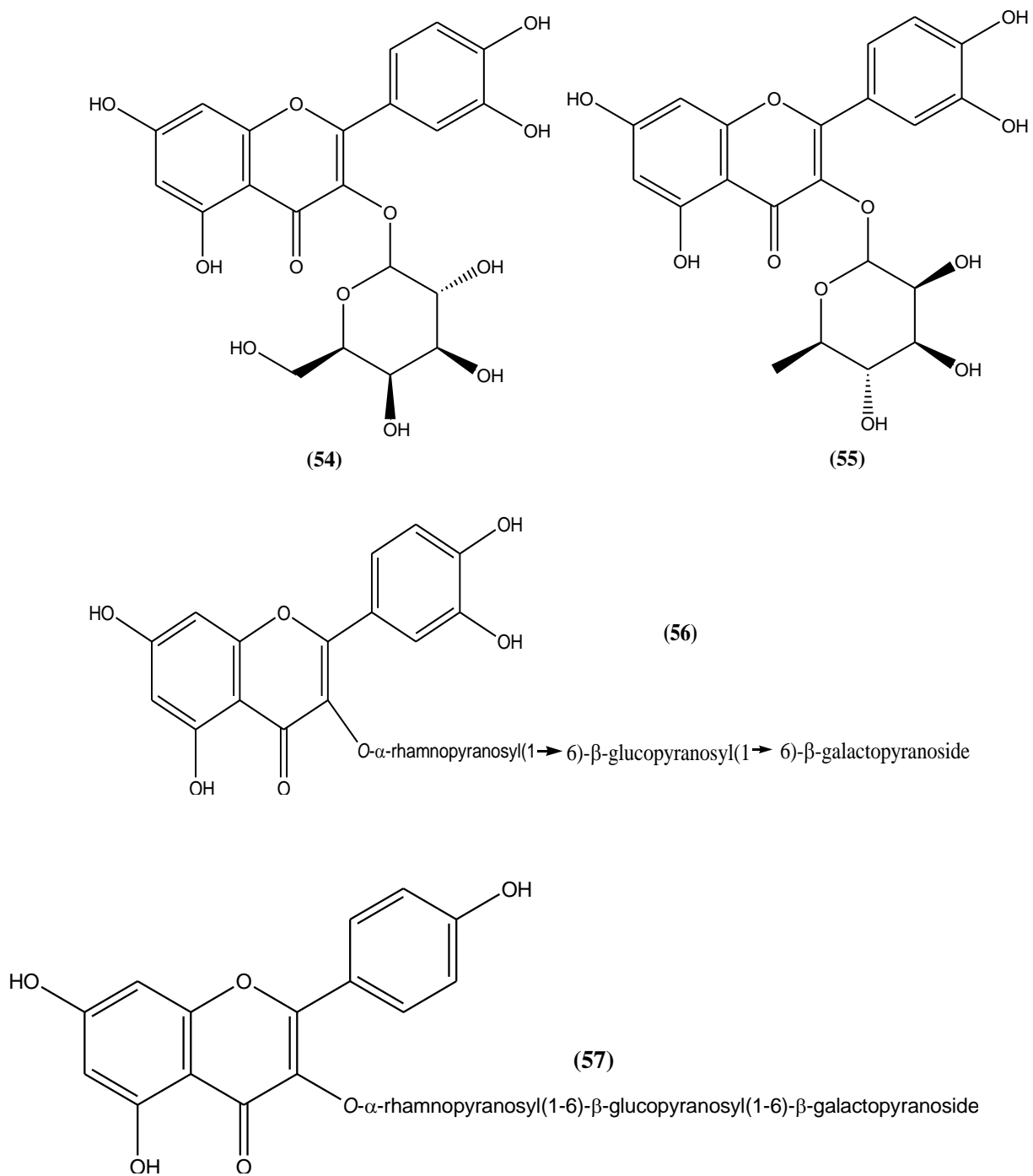
Fig. (8): Structures of some flavonoids (54-57) isolated from genus *Albizia(Cont.)*

5- Phenolic compounds:

Four new glycosides **(60-63)** and icariside E_5 **(64)**, were isolated from the dried stem bark of *A. julibrissin*. These were determined to be 3,4,5-trimethoxyphenol 1-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside **(60),** vomifoliol 3'-*O*-β-D-apifuranosyl-(1→6)-β-D-glucopyranoside **(61)**, (+)-lyoniresinol 9'-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside **(62),** (+) − lyoniresinol 4, 9'- di-*O*-β-D- glucopyranoside **(63)** and [Higuchi *et al*., 1992].

Table 5: Distribution of some phenolic compounds in Genus Albizia

Species	Investigated part	Compound	Biological activity	Ref.
A. julibrissin	The stem bark	Albibrissinosides A **(58)** and B **(59)**	Radical scavenger on the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical	[Jung, *et al*. 2004].
A. julibrissin	The stem bark	3,4,5-trimethoxyphenol 1-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside **(60),** vomifoliol 3'-*O*-β-D-apifuranosyl-(1→6)-β-D-glucopyranoside **(61),** (+)-lyoniresinol 9'-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside **(62),** (+) − lyoniresinol 4, 9'- di-*O*-β-D-glucopyranoside **(63)** and icariside E_5 **(64)**		[Higuchi, *et al*., 1992].

	R1	R2
(58)	OCH₃	OCH₃
(59)	OH	H
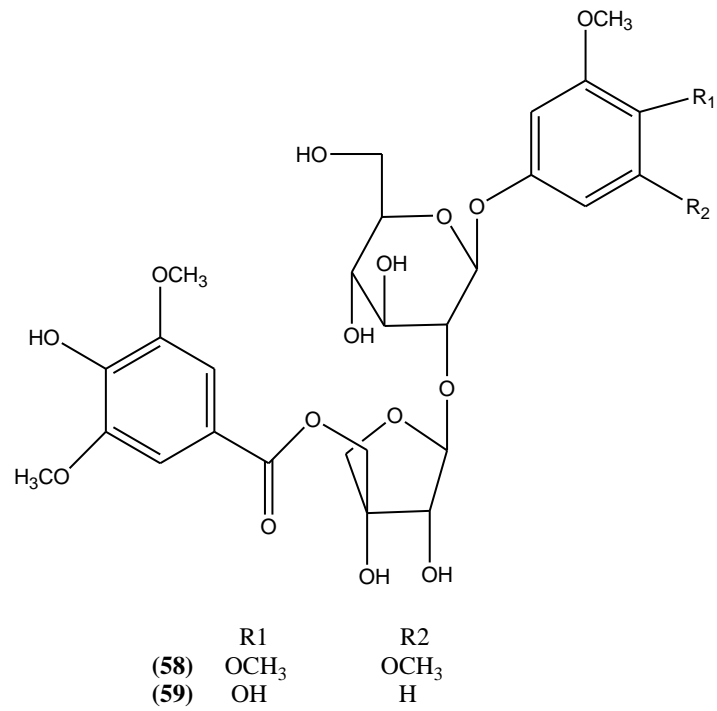
Fig. (9): Structures of some phenolic compounds (58&59) isolated from genus *Albizia*

(60)

(61)

	R1	R2
(62)	-Glc---Glc	H
(63)	- Glc	-Glc

(64)
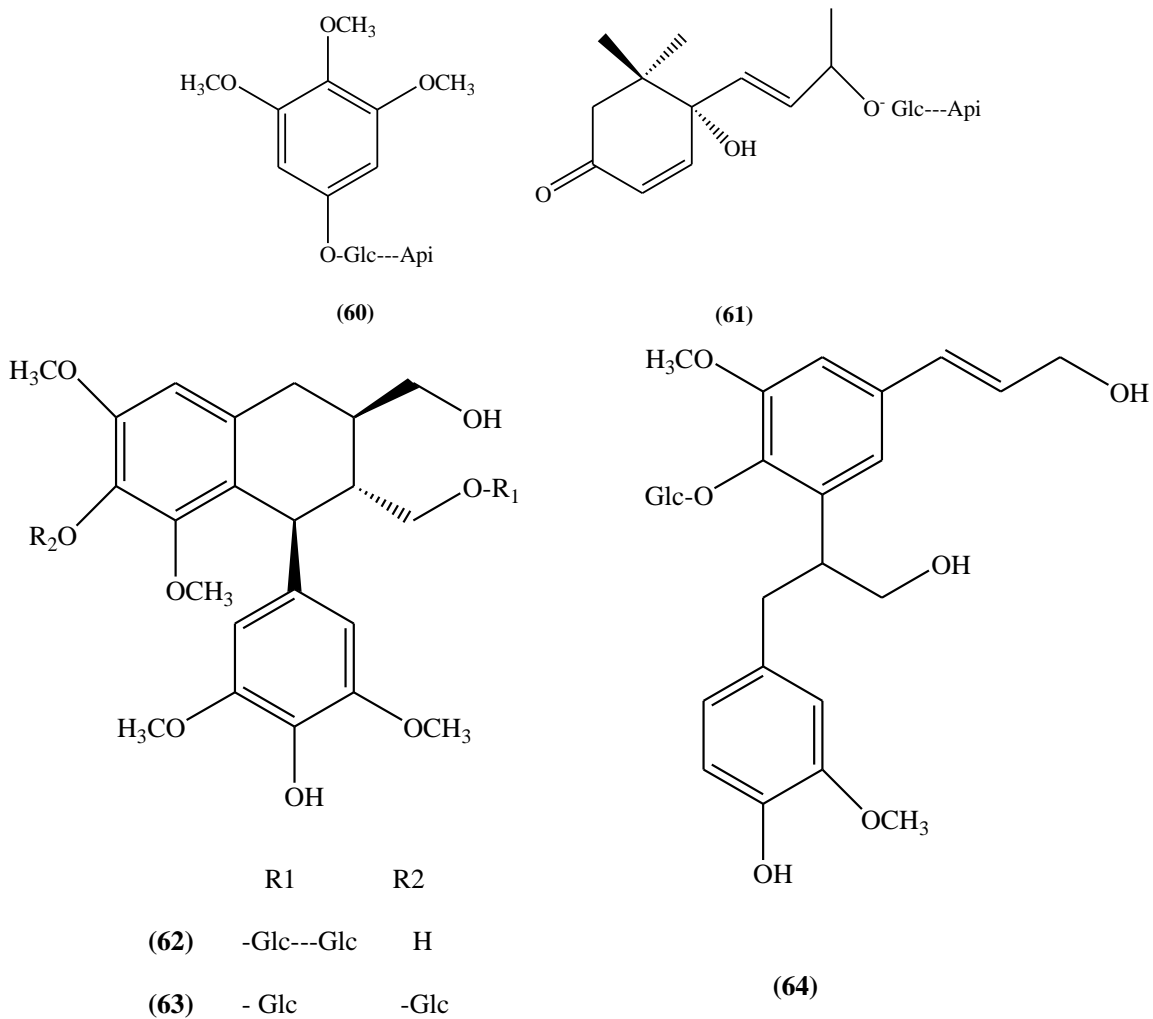
Fig. (9): Structures of some phenolic compounds (60-64) isolated from genus *Albizia (Cont.)*

EXPERIMENTAL

Materials, equipments and techniques

A) Materials

I- Plant material

The dried inflorescence of *A. lebbeck* was collected from Al-Ta'awon district, Riyadh, Saudi Arabia in Spring 2008. The plant was identified and kindly authenticated by Professor Dr. Ahmad Alfarhan, Department of Botany, College of Science, King Saud University. A voucher specimen was deposited at the Pharmacognosy Department, College of pharmacy, King Saud University.

II- Solvents

n- Hexane, petroleum ether, dichloromethane, chloroform, ethyl acetate, acetone, methanol and ethanol that used in the extraction and chromatographic steps are of analytical grade. Methanol HPLC grade is used for spectroscopic purposes (GPR™, BDH limited poole, England).

III- Reagents

1) Dragendorff's spray reagent [Kritchvesky and Tepper, 1968].
2) 25% sulfuric acid – methanol spray reagent (universal reagent).
3) Anisaldehyde spray reagent:
   Anisaldehyde (0.5 ml), glacial acetic acid (10 ml), methanol (85 ml) and concentrated sulfuric acid (5 ml) were mixed in the same sequence. The reagent was sprayed on TLC plates, which were then heated with a hot air gun until color development was complete [Kritchvesky and Tepper, 1968].

4) Baljet's reagent: 95% picric acid and 5% NaOH.
5) Acids: Hydrochloric acid (HCl), sulfuric acid (H_2SO_4), acetic acid (CH_3COOH).

IV- Chromatographic materials

1- Thin layer chromatographic plates (TLC Pre-coated silica gel 60 F_{254} Merck and pre-coated RP-18 Merck, Germany). Different solvent systems were used in different ratios such as: petroleum ether-ethyl acetate, chloroform-acetone, dichloromethane-methanol, hexane-ethyl acetate, hexane-acetone, dichloromethane-acetone.

2- Glass columns of different sizes and dimensions.

3- Column chromatography (CC) was performed using normal phase silica gel 60, particle size 0.04 - 0.063 mm, reversed phase silica gel (RP-18) (E. Merck, Germany) and Diaion (HP-20).

4- Glass jars, 10x20x24 cm, fitted with covers, were used for TLC (Camag).

5- Capillary tubes for application of the test solution to TLC plates.

6- Spray bottles, 100 ml capacity.

7- Glassware such as flasks, funnels, beakers, vials and glass rods.

B) Apparatus, Equipments and techniques

1-Rotary vacuum evaporator

Evaporation of solvents was done at 40°C under reduced pressure, using Buchi® rotatory evaporator (model R-210) with vacuum pump (V-850) and water chiller (Switzerland).

2-Ultraviolet lamp

The ultraviolet lamp used in visualizing TLC plates was a Mineralight® device, multiband UV-254 / 366 run obtained from UVP, Inc. (USA).

3- Fraction collector

Fractions were collected by using a fraction collector instrument (model Foxy Jr), Teledyne Isco company (USA).

4-Ultraviolet spectral analysis (Hitachi Japan)

Ultraviolet absorption spectra were obtained in spectroscopic methanol on a Unicum Heyios UV -Visible Spectrophotometer.

5-Infrared spectral analysis

Infrared spectra were generally obtained in potassium bromide (KBr) discs using Perkin-Elmer, FTIR, model, 1600 spectrophotometer, USA.

6-Mass spectrophotometer analysis

The electron impact ionization (EIMS) mass spectra were obtained on a solid probe using Shimadzu QP-class-500. Also, HPLC (Agilent 1200) connected to mass detector (Agilent 6410 QQQ).

7-Melting Point Determination

Melting points were carried out using Thermosystem FP800 Metler with central processor supplied with a mettler FP 81 MBC cell apparatus.

8-Nuclear Magnetic Resonance (NMR)

^{1}H and ^{13}C NMR spectra including DEPT experiments (1D experiments) were obtained on the Bruker AM 500 spectrometer (Germany) operating at 500 MHz (^{1}HNMR) and 125 MHz (^{13}CNMR) in spectroscopic grade $CDCl_3$, CD_3OD, pyridine-d_5 and DMSO-d_6. The chemical shifts values are expressed in δ (ppm) units using (TMS) as an internal standard and the coupling constants (J) are expressed in Hertz (Hz). Standard pulse sequences were used for generating COSY, HMQC and HMBC spectra (2D experiments) [Silverstein, 1991].

C) Biological Evaluation

Crude extracts of *A. lebbeck* ; *n*-hexane, dichloromethane, ethyl acetate, *n*-butanol as well as the total alcohol extract of *Albizia lebbeck* were tested in preliminary screening assays for the following activities: antimicrobial, antipyretic, analgesic and estrogenic.

1-Screening for antimicrobial activity

Crude extracts of *A. lebbeck*; tested for antimicrobial activity; were evaluated using the following strains of bacteria, Gram-positive bacteria: *Bacillus subtilis* (ATCC 26633), *Staphylococcus aureus* (ATCC 25923), Gram – negative bacteria: *Escherichia coli* (ATCC 25922), *Psuedomonas aeruginosa* (ATCC 27853) and the acid fast stain bacteria: *Mycobacterium smegmatis* (ATCC 35797), these bacteria were obtained using American type of culture collection (ATCC) standard. Stock cultures were maintained at 4°C on slants of tryptic soy broth supplemented with 5 g/l yeast extract and 15 g/l Agar agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to flasks of Mueller–Hinton broth (MHB) that were incubated at 37°C for 24h. The cultures were diluted with fresh Mueller–Hinton broth to achieve optical densities corresponding to 2.0 x 106 CFU/ml for bacteria [Rasool, *et al*., 2008] & [National Committee for Clinical Laboratory Standards, 1997].

- Broth microdilution method

A broth microdilution method was used to determine the Minimal Bactericidal Concentration (MBC) in mg/ml [Clinical and Laboratory Standards Institute (CLSI), 2006]. All tests were performed in Mueller- Hinton broth (MHB) supplemented with Tween – 80 detergent to a final concentration of 0.5% (v/v). Geometric dilutions ranging from 0.1 to 5 mg/ml of the crude extracts and/or pure compounds were prepared in a 96 – well microtitre plate. Overnight broth cultures of each strain were prepared and the final concentration in each was adjusted to 2.0 x 106 CFU/ml. Petri dishes were kept at 4°C for 2h. Bacteria were incubated at 37°C for 24h. The MIC was defined as the lowest concentration of test sample that resulted in a complete inhibition of visible growth. The microbial growth was indicated by the presence of a white "pellet" on the well bottom. To determine MBC, broth was taken from the well that showed no visible growth and

was inoculated in MHA for 24h at 37°C for bacteria. MBC was defined as the lowest concentration of test sample at which 99.9% of the microorganisms were killed. All determinations were performed in triplicate and two growth controls consisting of MHB medium with 0.5% Tween-80 were included. Amphotericine B and gentamycin (30 μg/disc) served as positive controls [Rasool, *et al.*, 2008] & [National Committee for Clinical Laboratory Standards, 1997].

2. Screening for antipyretic activity

Six male albino mice, weighing 20–25 g, were fasted overnight before the experiments. Pyrexia will be induced by a subcutaneous injection of 20% w/v brewer's yeast suspension (10 ml/kg) into the animal's dorsum region. Seventeen hours after the injection, the rectal temperature of each rat was measured using a digital thermometer. Only rats that showed an increase in temperature of at least 0.7^{o}C were used for experiments. A dose of 1g/kg of each extract, except *n*-butanol extract which was administered in dose of 0.25 g/kg, was administered intraperitoneally, and the temperature measured at 15, 30, 60, 120 and 180 minutes after injection. Water ad libitum was used as a negative control and aspirin (200 mg/kg) was used as a positive control [Yongna, *et al.*, 2005].

3. Screening for analgesic activity
- Hot plate method

Male albino white mice, weighing 20–25 g, were used. They were gently placed on a hot plate thermostatically maintained at 55 ^{o}C. [Leighton *et al.*, 1987]. The time sec at which the animals displayed nociceptive responses exhibited as licking the front paws or fanning (blowing) the hind paws was recorded and the animals were removed from the plate. A cut-off time of 40 sec was used to avoid damage of the paws. Crude extracts (500 mg/kg) and a reference analgesic drug, aspirin (200 mg/kg) were administered [El-Tahir, 2007].

4. Screening for estrogenic activity

Two groups each of three immature female albino rats, weighing 15 and 30 g, were housed under a temperature and light controlled room. They were maintained in a well ventilated animal quarter. They had free access to water and commercially available food. To determine estrogenic activity, a dose of 500 mg/kg/day of each crude extract was administered intraperitoneally in 1 ml saline for 3 consecutive days and on the 4th day animals were sacrificed and uteri were removed and weighed. Normal saline was used as a negative control and 17-ß-estradiol (0.32 μg/animal/day) was used as a positive control [Kumar and Pakrasi, 1995].

5. Screening for Anti-inflammatory activity

- Carrageenan-Induced Inflammation

Carrageenan-induced rat hind paw oedema was induced following the method of [Duncan *et al*., 1993]. Initially the volumes of the hind paws of male Wistar rats, weighing 150-200 g, were measurd using hydroplethysmograph (Model 7150, Ugo Basile, Caemerio, Italy). For this purpose each paw was marked at the level of the lateral malleoulus and then dipped gently into the 0.45% NaCl fluid in the chamber of plethysmograph. This instrument measures the volume of the paw in ml. Then 0.1 ml of carregeenin (2% w/v in sterile saline) was injected in one paw under the planter aponeurosis. The paw volume was then measured hourly for 4-5 hours. Inflammation (or oedema) was expressed as volume (in ml) increase above the original volume of the paw or as a percentage increase in the paw volume.

Animals were then injected (i.p) with various doses of the crude extracts 15-30 min. before injection of carrageenan. Then carrageenan was injected as described above and the paw volume determined hourly for 4-5 hours thereafter. The influence of the treatment on the induced inflammation was evaluated [El-Tahir, 2007].

PHYTOCHEMICAL STUDY

I-   Preliminary phytochemical screening

The inflorescences of *A. lebbeck* were collected from Al-Ta'awon district, Riyadh, Saudi Arabia. They were air dried, milled, weighed (700 gm), and extracted by maceration with 70% ethanol for 3 days with occasional shaking. This process was repeated until complete exhaustion of the inflorescences (until clear solution appears and no residue on a watch glass) [Skoog, Holler and Nieman, 1992]. The alcohol extract was then concentrated to dryness under reduced pressure at 40°C using a rotary vaccum evaporator to produce the alcoholic extract (95 gm).

The total alcohol extract was then dissolved in water/alcohol (20:80) and partitioned with ethylacetate giving two fractions; ethyl acetate soluble part **(A)** and ethyl acetate insoluble part **(B).** The two fractions were then separately evaporated to dryness. A portion of each fraction was subjected to preliminary phytochemical screening tests and the remaining part was weighed and kept in the refrigerator for further chemical investigation (Scheme 1).

Albizia lebbeck flowers (500 g)

Extracted by EtOH (3L.X3),

40 g of dried total alcohol extract

Partitioned with EtOAc

EtOAc soluble part (11 gm)

EtOAc insoluble part (29 gm)

CC-I | CC, Silica gel using
n-hex. -acetone gradient,

Dissolved in small amount of H_2O
and least amount of MeOH and applied to
Diion HP20 CC, eluted by H_2O/MeOH mix.

CC-V

% of n-.hex/ Acetone

20%

90%

40% MeOH

60% MeOH

80%

Sub fr. 30-41

Sub fr. 191-209

Sub fr. 13-17

Sub fr. 18-24

Sub fr. 25-30

CC-II

Si. gel CC, using
n- hex/acetone gradient

CC, Si. gel using
CH_2Cl_2/MeOH /H_2O gradient.

CC-III

Si. gel cc, using CH_2Cl_2/MeOH/H_2O
gradient

CC-VIII

CC-VI

30% MeoH

CC-VII

9% Acetone

11%
Acetone

10% MeoH

15% MeoH

3% MeoH

35% MeOH

30% MeOH

AL -1

AL-2

Sub fr. 42-44

AL-4

AL-6

AL-7

Subfr. 129-141

CC-IV | 7% MeoH

AL-5

MPLC using
RP-18 CC MeOH/
H_2O,
gradient

CC-IX

AL-3

AL-8

(scheme 1) Extraction, Seperation and Purification Procedure of Albizia lebbeck flowers

Preliminary phytochemical screening tests:

A portion of each of ethyl acetate soluble and ethyl acetate insoluble fractions was subjected to preliminary phytochemical screening for the presence of flavonoids, saponins, tannins, alkaloids, anthraquinones, carbohydrates and/or glycoside, cardiac glycoside and triterpenes and/or sterols. The results are summarized and shown in table 6:

- Test for saponins (Froth test): The dried extract was dissolved in water, transferred into a test tube and shaken vigorously, then left to stand for 10 minutes, when a thick persistent froth appears indicating the presence of saponins [Onwukaeme *et al.*, 2007].

- Test for alkaloids: 50 mg of extract was dissolved in 50 mls of methanol in a water bath for 20 minutes; the extract was then filtered off and allowed to cool. Two ml of the extract was poured into test tubes. The Dragendorff's or Mayer's reagent was added to the tube and the presence or absence of colors or any precipitates was noted [Onwukaeme *et al.*, 2007].

- Test for Flavonoids: Each fraction was treated with dil. NaOH, followed by the addition of diluted HCl, solubility and color were noted. A yellow solution with NaOH, which turns colorless with dil HCl confirms the presence of flavonoids [Onwukaeme *et al.*, 2007].

- Test for tannins (Ferric chloride test solution): aqueous extract was treated with a 15% ferric chloride test solution. A blue color indicates condensed tannins; a green color indicates hydrolysable tannins [Onwukaeme *et al.*, 2007].

- Test for anthraquinone derivatives (Borntrager's test): To 2 mls of the extract, 1ml of dilute (10 %) ammonia was added and the mixture was shaken. A pink-red color in the ammoniacal (lower) layer shows anthracene derivatives [Onwukaeme *et al.*, 2007].

- Test for Carbohydrates and/or glycosides (Molisch's test): 1 ml of the extract solution was pipetted into a test tube; 3 drops of Molisch reagent (2 g α-naphthol dissolved in 20 mls EtOH 96%) are added to the extract solution. After mixing, 1ml of concentrated sulfuric acid was added to the wall of test tube. A positive test for

carbohydrates is indicated by a violet ring forming at the interface between the denser sulfuric acid and the less dense test solution above [Foulger, 1931].

- Test for sterols and/or triterpenes (Liebermann-Burchard test): 1 ml of glacial acetic acid was added to 1 ml chloroform and cooled to 0°C, then one drop of concentrated sulphuric acid was added to the cooled mixture followed by the extract. The solution was observed for a blue, green, red or orange color that changes with time [Harborne, 1984].

- Test for cardiac glycosides (Baljet test): 95%picric acid, 5 % NaOH. [Handa *et al*., 1986].

Table 6: Phytochemical screening results of the flowers of *A. Lebbeck*:

Constituents tested	Reagent used	Ethyl acetate soluble fraction	Ethyl acetate insoluble fraction
Saponins	Froth test	-	+
Alkaloids and nitrogenous compounds	Drangendorff's	+	+
Flavonoids	NaOH	+	+
Tannins	Fe Cl$_3$	-	+ (Blue)
Carbohydrates and / or glycosides	Molisch's test	+	+
Sterols and/or triterpenes	Liebermann's test, Burchard	+	+
Anthraquinones	Borntrager's test	-	-
Cardiac glycosides	Baljet test	-	-

II-   Extraction and fractionation

The two different fractions **(A and B)** of flowers of *A. lebbeck* were subjected to further chemical investigations. Eight compounds from these fractions designated as AL-1 to AL-8 were isolated and identified.

A- Investigation of the ethyl acetate soluble fraction:

Preliminary phytochemical screening of this fraction showed the presence of alkaloids, flavonoids, tannins, carbohydrates and/ or glycosides, terpenes and/ or sterols. So, an attempt to isolate and identify some of these compounds has been done.

1- Thin layer chromatography:

Part of the ethyl acetate soluble fraction was examined by TLC on silica gel F_{254} chromatoplate using different solvent systems as mobile phases. The developed plates were air-dried and visualized in daylight and under UV lights at 254 nm and 366 nm, before and after spraying with anisaldehyde spray reagent. It was found that *n*-hexane – acetone (7.5:2.5) was the solvent system of choice and revealed at least three major spots in addition to four minors.

EtOAC sol. fraction
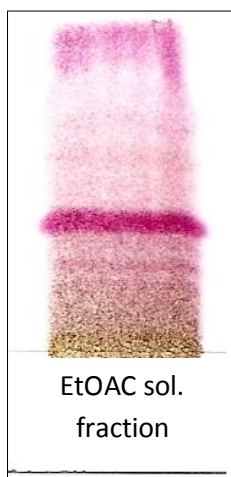
Fig. (10): TLC plate of ethyl acetate soluble fraction using system *n*-hexane – acetone (7.5:2.5)

The R_f values and color responses are listed in Table. 7

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2- <u>Chromatographic fractionation</u>:

The ethyl acetate soluble fraction was subjected to a column chromatography (550 gm silica gel, 150 x 4 cm) (column No.1) packed by wet method with *n*- hexane only. Elution was started with *n*- hexane and polarity was gradually increased with acetone and washed with methanol. Fractions (100 ml of each) were collected. The collected fractions (209 fractions) were then concentrated under reduced pressure at 40° C using rotary evaporator to dryness, and then monitored by TLC using solvent systems *n*-hexane-acetone of different ratios. The plates were sprayed with anisaldehyde spray reagent followed by heating. Similar fractions were pooled together then evaporated to dryness under reduced pressure. Fractions (30-41) eluted by *n*-hexane: acetone (8:2) in on pooling and concentration afforded (46 mg) of a yellow oily residue and fractions (191-209) eluted by 90% acetone on pooling and concentration also afforded (2.7 gm) of yellow residue.

Table 7: Results of TLC of the ethyl acetate soluble fractions:

Spot No.	R_f^* values	Color (Anisaldehyde/ Sulphuric acid spray reagent)					
		Day light		UV Light			
				254 nm		366 nm	
		Before spray	After spray	Before spray	After spray	Before spray	After spray
1	0.2	colorless	Bluish black	Violet	Black	Violet	Blue
2	0.26	colorless	Bluish black	Black	Black	Blue	Red
3	0.37	No color	Red	Dark	Dark	Violet	Red
4	0.51	colorless	Faint violet	Black	Black	Black	Red
5	0. 57	colorless	Faint violet	Black	Black	Red	Red
6	0.91	colorless	Red	Black	Black	Red	Red
7	0.94	colorless	Red	Black	Black	Red	Red

* *n*-hexane: acetone (75:25)

Isolation of compounds AL1 and AL2:

Repeated column chromatography (2.3 gm silica gel, 20 x 2.5 cm column No. 2) of fractions (30-41 of column No.1) eluted by *n*-hexane –acetone gradually yielded:

AL1: (fractions 16-21 of column No.2, 22 mg) isolated as a white fine crystals eluted with 9% acetone in *n*-hexane.

AL2: (fractions 33-41 of column No.2, 15 mg) isolated as a white fine crystals eluted with 11% acetone in *n*-hexane.

TLC of fractions (16-21) using *n*-hexane – acetone (75:25) as a mobile phase showed one major spot with (R_f = 0.37). It gives a violet color when sprayed with anisaldehyde reagent. Also, TLC of fractions (33-41) using *n*-hexane – acetone (75:25) as a mobile phase showed one major spot with (R_f = 0.26). It gives a violet color when sprayed with anisaldehyde reagent. The R_f values and color responses were cited in Table. 8

16-21	33-41
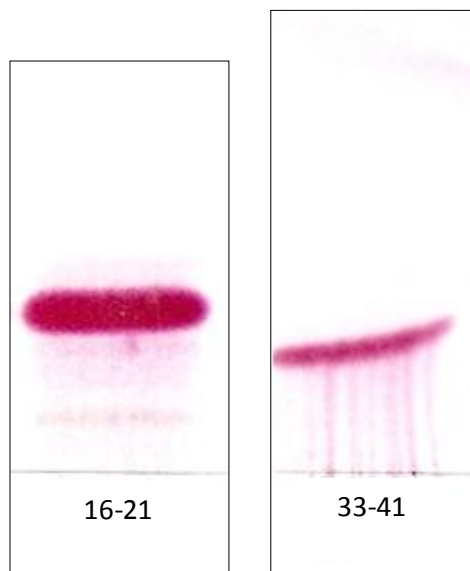
Fig. (11): TLC plates of AL-1 & Al-2 using system *n*-hexane – acetone (75:25)

Table 8: TLC results of compounds AL-1 & AL-2:

Spot No.	R_f * value	Color					
		Day light		UV Light			
				254 nm		366 nm	
		Before spray	After spray	Before spray	After spray	Before spray	After spray
AL-1	0.37	No color	Red	Dark	Dark	Violet	Red
AL-2	0.26	No color	Bluish black	Black	Black	Blue	Red

* *n*-hexane: acetone (75:25)

Isolation of compound AL3:

Fractions (191-209 of column No.1) were subjected to further column chromatography (150 gm silica gel, 100 cm x 4 cm) (column No. 3) packed by wet method with dichloromethane. Gradient elution was done using dichloromethane, methanol and water. Fractions (143 fractions, 100 ml each) were collected, then concentrated under reduced pressure at 40° C using a rotary evaporator until dryness. Fractions were monitored by TLC using the following systems: dichloromethane (100 %), dichloromethane / methanol (97:3), (95:5), (90:10), (85:15), (80:20) and dichloromethane / methanol / water (90: 10:1), (85: 15: 1.5) and (80: 20: 2). TLC of these fractions yielded AL3 & AL4 as follows:

Fractions (42-44 of column No.3) eluted with dichloromethane / methanol / water (90: 10: 1) on pooling and concentration afforded (27 mg) of an amorphous white powder. TLC of this powder using dichloromethane / methanol (93: 7) showed two major spots after spraying with anisaldehyde/H_2SO_4 , one was violet with R_f = 0.48 and another

was brown with $R_f = 0.51$ in addition to two minor black spots. Therefore, the fractions were subjected to further chromatographic purification using column chromatography (1.5 gm silica gel, 20 x 2.5 cm) (column No.4) packed by wet method with *n*- hexane only. Elution was started with *n*- hexane and polarity was gradually increased with acetone and washed by methanol. Fractions (21-22) on pooling and concentration afforded (10 mg) of white amorphous powder. TLC of this powder using dichloromethane / methanol (97:3) showed one major spot of $R_f = 0.2$.

Isolation of compound AL4:

Fraction no. 69 of column No. 3, 60mg, were isolated as white fine needles eluted with dichloromethane / methanol / water (85: 15: 1.5). TLC of this fraction using dichloromethane / methanol (90: 10) showed two major spots, one was violet with $R_f = 0.18$ and another was brown with $R_f = 0.20$. Further crystallization from methanol gave one major spot with $R_f = 0.18$.
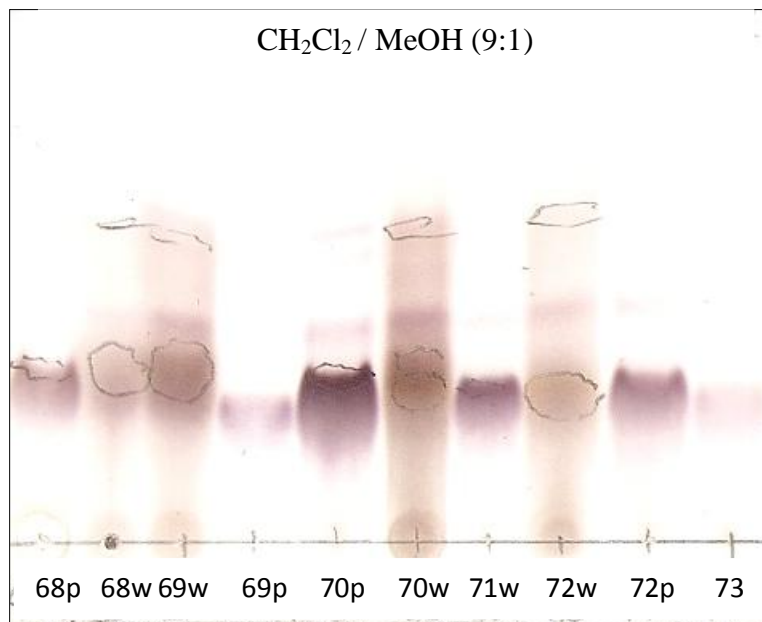
Fig. (12): TLC plate of some fractions of column no.3 using system CH_2Cl_2 / MeOH (9:1)

Table 9: TLC results of compound AL-3& AL-4:

Spot No.	R_f value	Color					
		Day light		UV Light			
				254 nm		366 nm	
		Before spray	After spray	Before spray	After spray	Before spray	After spray
AL-3[*]	0.2	No color	violet	No Color	Dark brown	No color	red
AL-4[**]	0.18	No color	violet	Dark	brown	Violet	Dark red

*Dichloromethane: methanol (93:7)

**Dichloromethane: methanol (90:10)
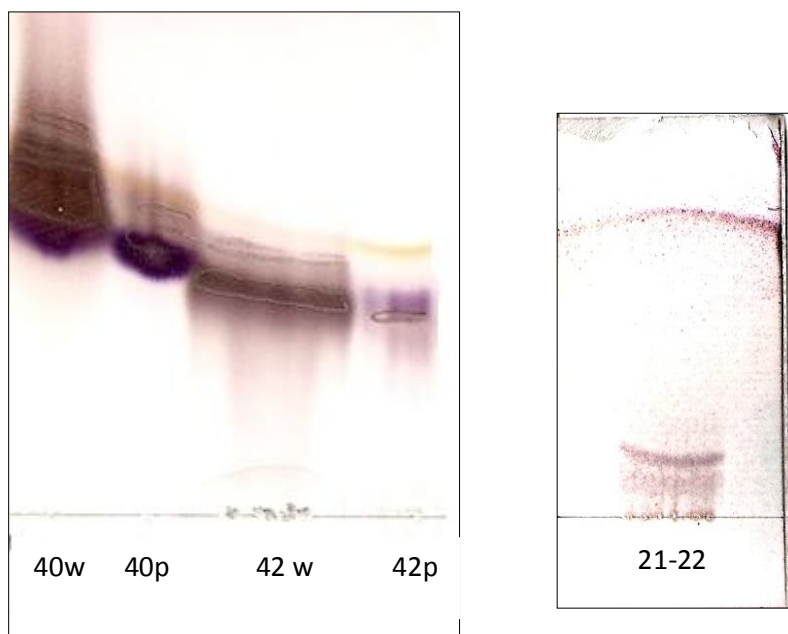
Fig. (13): TLC plates of compound AL-4 using system CH_2Cl_2 / MeOH (90:10)

B- Investigation of the ethyl acetate Insoluble fraction:

Preliminary phytochemical examination of this fraction showed the presence of alkaloids, flavonoids, tannins, Saponins, carbohydrates, glycosides, terpenes and/ or sterols. So, an attempt to isolate and identify some of these compounds has been done.

The fraction was subjected to Diaion HP_2O column chromatography (column No.5) packed by wet method with water. The elution started with water and the polarity was decreased gradually with methanol to acetone. Fractions (250 ml each) were collected. The collected fractions (45 fractions) were then concentrated under reduced pressure at 40°C using rotary evaporator to dryness, and then examined by TLC using solvent systems dichloromethane-methanol of different ratios. The plates were sprayed with anisaldehyde spray reagent followed by heating. Similar fractions were added together then evaporated to dryness using rotary evaporator.

Column fractions (13-17) eluted with 40% methanol in water on pooling and concentration afforded (300 mg) of a dark residue and fractions (18-24) eluted with 60% methanol in water on pooling and concentration also afforded (400 mg) of a dark yellow residue. In addition, fractions (25-30) eluted with 80% methanol in water on concentration also afforded (3.4 gm) of a dark residue. Screening of these fractions on TLC encouraged us to do further chromatographic investigation. Chromatogram of these fractions is shown in Fig. 14.
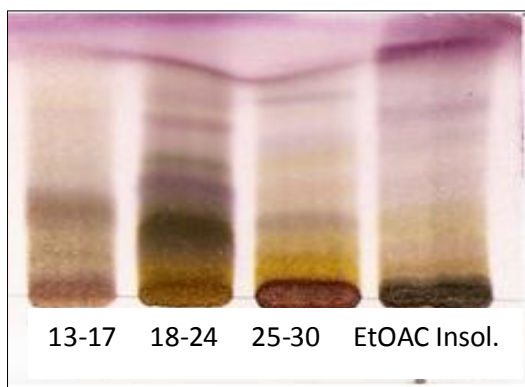
Fig. (14): TLC plate of ethyl acetate insoluble fraction using system CH_2Cl_2: MeOH (80:20)

Isolation of compound AL5:

Fractions (13-17 of column No.5) eluted with 40% MeOH in water from HP_2O Diaion column, were subjected to further column chromatography (15 gm silica gel, 60 cm x 2.5 cm) (column No.6) packed with wet method with dichloromethane only. Elution was started with dichloromethane and polarity was gradually increased with methanol and water. Fractions (50 ml each) were collected. Fractions (78-84) (column No.6) eluted by dichloromethane / methanol / water 70:30:3 on pooling and concentration afforded (60 mg) of white amorphous powder. TLC of this powder using dichloromethane / methanol (75:25) showed one major spot of ($R_f = 0.13$). TLC plate of this plate is shown in Fig. 15.

66-68 69-77 78-79 80-84 85

Fig. (15): TLC plate of compound AL-5 using system CH_2Cl_2 / MeOH (75: 25)

Isolation of compound AL6:

Fractions (18-24 of column No.5) eluted with 60% MeOH in water from HP_2O Diaion column, were subjected to further column chromatography (15 gm silica gel, 60 cm x 2.5 cm) (column No.7) packed with wet method with dichloromethane only. Elution was started with dichloromethane and polarity was gradually increased with methanol and water. Fractions (50 ml each) were collected. Fractions (45-46, column No.7) eluted with dichloromethane / methanol (97:3) on pooling and concentration afforded (5 mg) of oily residue. TLC of this powder using dichloromethane / methanol (97:3) showed one major spot of ($R_f = 0.24$).
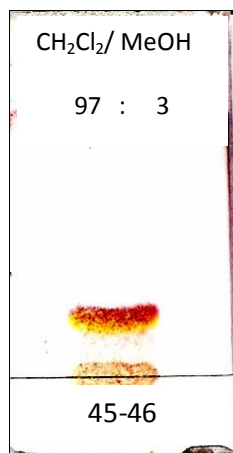
Fig. (16): TLC plate of compound AL-6 using system CH_2Cl_2 / MeOH (97:3)

Isolation of compound AL7:

Further chromatographic purification of fractions (25-30 of column No.5) eluted with 80% MeOH in water from HP_2O Diaion column, was achieved by further column chromatography (170 gm silica gel, 100 cm x 4 cm) (column No.8) packed with wet method with dichloromethane only. Gradient elution analysis was done using dichloromethane and polarity was gradually increased with methanol and water. Fractions (150 ml each) were collected. Fractions (155-156) (column No.8) eluted with dichloromethane / methanol / water (65:35:3.5) on pooling and concentration afforded (30 mg) of yellow amorphous powder. TLC of this powder using normal phase plate and solvent system dichloromethane / methanol (75: 25) showed one major spot of (R_f =0.19) The purity of this spot was proven by respotting on (RP_{18} plate). TLC plate of this fraction is shown in Fig. 17.
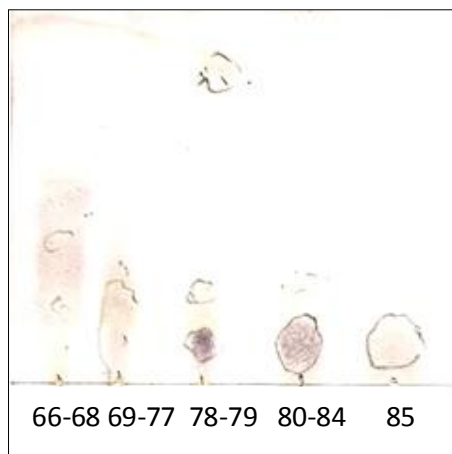

Fig. (17): TLC plate of compound AL-7 using system CH_2Cl_2 / MeOH (75:25)

Isolation of compound AL8:

Sub-fractions (129-141 of Column No. 8) eluted with dichloromethane / methanol / water 70:30:3.0, were subjected to further purification using [MPLC column, Rp$_{18}$, Lobar 310-25 Lichroprep R$_P$ (40-53 μm] (column No.9). Elution was started with water and polarity was gradually decreased with methanol. Fractions (50 ml each) were collected . Sub-fractions (45-49) (column No.9) eluted with 55% methanol on pooling then concentration and recrystalization afforded (45 mg) yellow needle crystals. TLC of these fractions using normal phase plate and solvent system dichloromethane / methanol (80: 20) showed one major spot (R$_f$ =0.21). TLC plate of this fraction is shown in Fig. 18.

45-49

Fig. (18): TLC plate of compound AL-8 using system CH$_2$Cl$_2$ / MeOH (80:20)

Table 10: TLC results of compounds AL5-AL-8:

Spot No.	R$_f$ value	Color					
		Day light		UV Light			
				254 nm		366 nm	
		Before spray	After spray	Before spray	After spray	Before spray	After spray
AL-5*	0.17	No color	Bluish Red	Dark	Dark brown	Blue	violet
AL-6**	0.24	No color	Orange Red	Dark	Red	Violet	violet
AL-7*	0.19	Yellow	Yellow	Dark	yellow	Black	yellow
AL-8***	0.21	Pale yellow	Yellow	Dark	yellow	Black	yellow

*Dichloromethane: methanol (75:25)

**Dichloromethane: methanol (92:8)

***Dichloromethane: Methanol (80:20)

Results & Discussion

Characterization and structure elucidation of compound AL1

Compound AL1 was isolated as white crystalline needles, melting point 193°-195°C, from the ethyl acetate soluble fraction. It gave a positive result (green color) with Libermann-Burchard's test, indicating its steroidal or triterpenoidal nature. The IR spectrum (KBr) revealed presence of hydroxyl group (O-H) (a broad absorption at 3460 cm^{-1} and absorption of C-O stretching at 1055 cm^{-1}) and presence of vinyl double bond (a weak absorption at 1625 cm^{-1} and the absorption of the vinyl C-H stretching at $3030 cm^{-1}$).

The EI mass spectrum of trimethyl silane derivative (TMSi) (Fig. 19) indicated molecular ion peak at m/z 498 calculated for $C_{34}H_{61}SiO$ and other significant fragments at m/z 483 (M-CH$_3$), 408 (M-TMSiOH), 393 (M-TMSiOH and CH$_3$), 279 (M- C*[a]DE rings), 218 (M- ABC* rings), 205 (M- ABC* rings and C-11 moiety) and 203 (M-ABC* rings and C-28 moiety). These significant peaks strongly indicated Δ^{12}-triterpenoidal structure that underwent a retro-Diels-Alder reaction to form fragments containing the ABC*-rings and the C*ED-rings (The suggested fragmentation pattern is shown in Fig. 20).

The ^1H and ^{13}C NMR spectral data presented in Table 11 showed triterpenoidal CH$_3$ and CH$_2$ signals from δ_H 0.72-2 with thirty carbon resonances. The ^{1}HNMR spectrum (Fig. 21) showed eight methyl groups resonating at δ_H 0.72, 0.76, 0.8, 0.8, 0.87, 0.9, 0.93 and 1.07 together with the ^{13}C NMR signals of eight carbons at δ_C 15.1 (C-25), δ_C 15.2 (C-24), δ_C 16.8 (C-26), δ_C 23.7 (C-30), δ_C 25.9 (C-27), δ_C 28.1 (C-23), δ_C 28.4 (C-28) and δ_C 33.3 (C-29); two of the carbon signals resonated in the olefinic region at δ_C 145.2 (C-13) and δ_C 121.8 (C-12) and one oxygenated carbon resonated at δ_C 79.0 assigned for (C-3). DEPT 135° experiment (Fig. 22) revealed the presence of ten methylene groups in addition to thirteen methyl and/or methine groups.

The correlations between each proton signal and the directly attached carbon was achieved and confirmed by HSQC experiment (Fig. 23). HSQC spectra correlated the

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[a] C* indicates the presence of only a portion of ring C

signal of (C-3) to its directly connected proton signal at δ_H 3.15. Moreover, HSQC correlations confirmed the assignment of the olefinic proton H-12 at δ_H 5.12. The HMBC experiment was useful in the final structure elucidation of compound AL-1 where two and three bond correlations were observed from H-12 (δ_H 5.12) to C-9 (δ_C 47.7), C-11(δ_C 23.5), C-14 (δ_C 41.7) and C-18 (δ_C 47.3) so the location of the double bond between (C-12) and (C-13) was confirmed. In addition, three bonds correlations were observed from H-1, H-2 and H-5 to C-3 confirmed the position of hydroxyl group -OH at C-3. The high coupling constant of the proton at δ_H 3.15 (*dd,* 11.5, 5 Hz) confirmed the beta configuration of Hydroxyl group at C-3.

NMR spectral data, TLC and m.p of compound AL-1 were in good agreement with that reported for β- Amyrin [Abbas, *et al*., 2009] and [Tanakaa and Matsunaga, 1989]. So, the identification of the AL-1 was confirmed to be β- Amyrin.
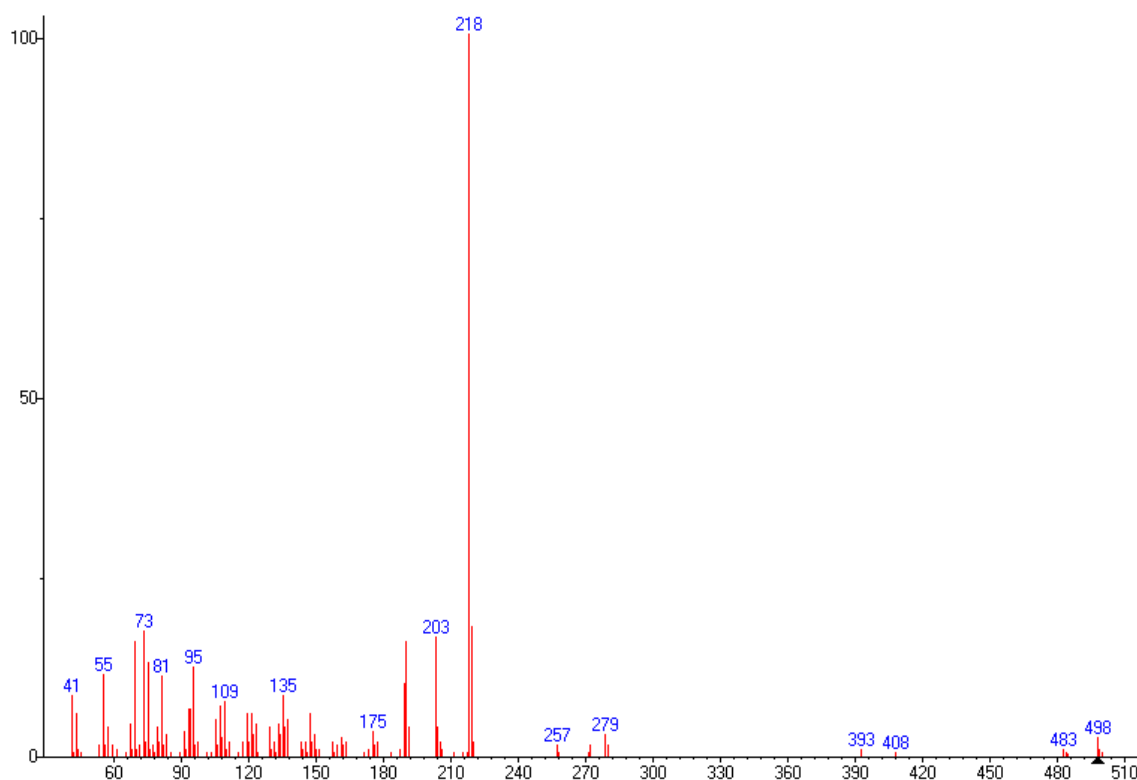
Fig. (19): The EI MS spectrum of trimethyl silane derivative of AL-1
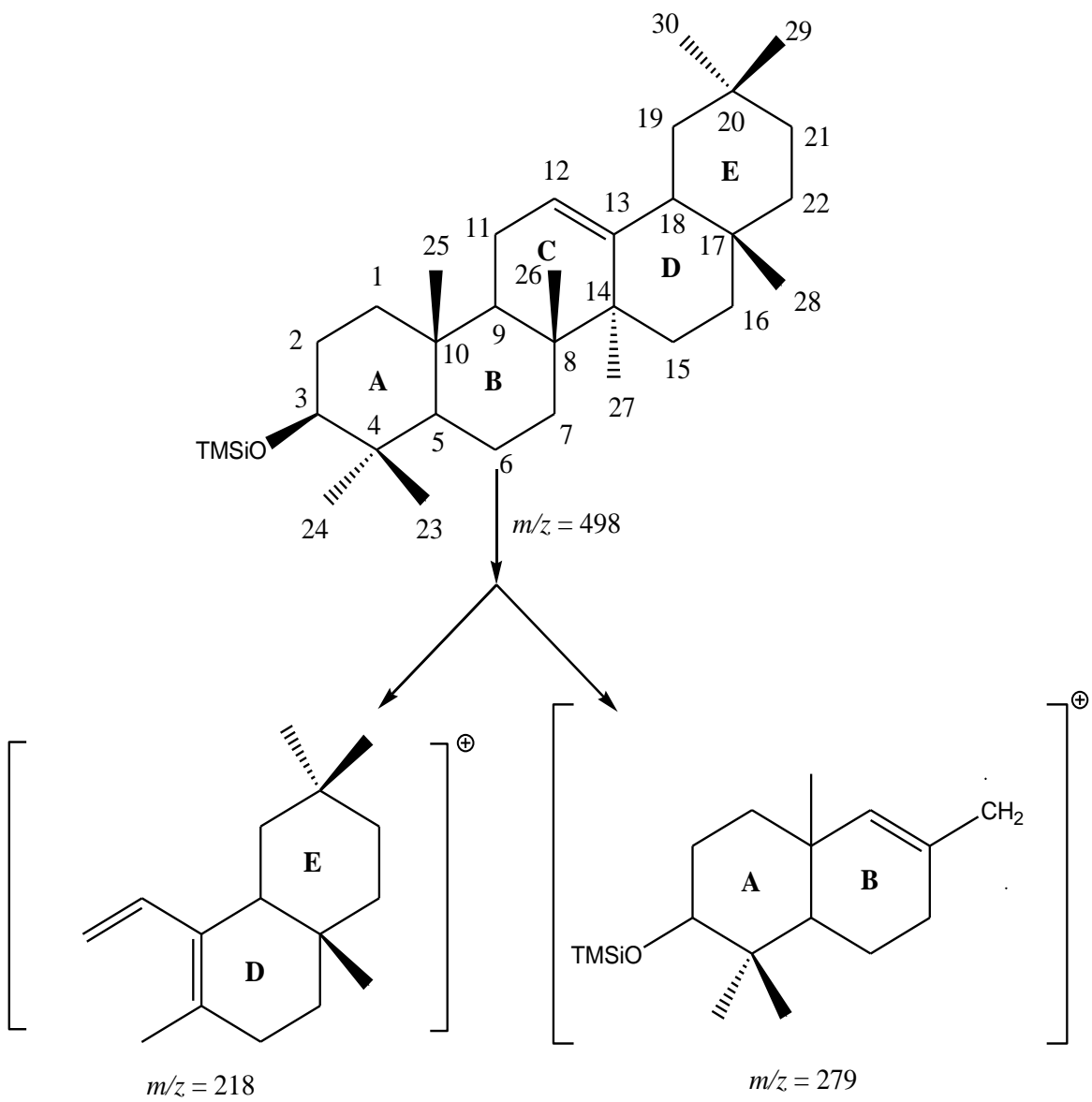
Fig. (20): The suggested fragmentation pattern of AL-1
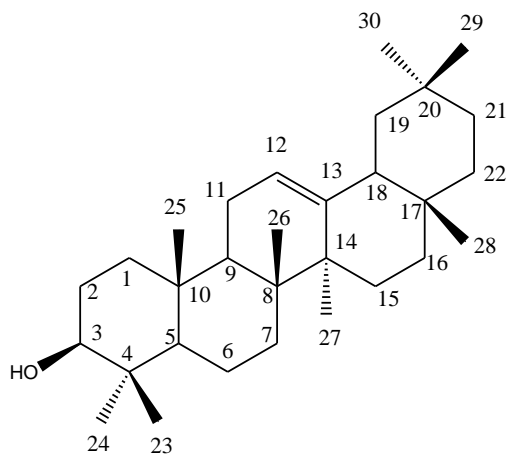
Structure of compound AL-1

Table 11: ^{1}H and ^{13}C NMR Assignment of Compound AL-1 (500 MHz, CDCl$_3$)

Position	δ ^{1}H (Multiplicity, J in Hz)	δ ^{13}C	δ ^{13}C of reference compound[#] CDCl$_3$ at 22.63 MHz
1	1.41 (*t*,4)	38.8	38.7
2	1.54 (*m*)	27.3	27.3
3	3.15 (*dd*, 11.5, 5)	79.0	79.0
4	-	39.8	38.8
5	1.27 (*m*)	55.2	55.3
6	1.33* (*m*)	18.4	18.5
7	1.29 (*t*,5.5)	32.7	32.8
8	-	38.6	38.7
9	1.45 (*t*, 4)	47.7	47.7
10	-	37.2	37.6
11	1.80 (*m*)	23.5	23.6
12	5.12 (*t*, 8.5)	121.8	121.8
13	-	145.2	145.1

Table 11 *Cont.*

14	-	41.7	41.8
15	1.70 (*t*, 5)	26.2	26.2
16	1.93 (*t*, 5)	26.9	27.0
17	-	32.5	32.5
18	1.90 (*t*, 3.5)	47.3	47.3
19	1.60 (*d*, 3.5)	46.9	46.9
20	-	31.1	31.1
21	1.32 *	34.8	34.8
22	1.39 (*t*, 5)	36.9	37.2
23	0.76 (s)	28.1	28.2
24	0.87 (s)	15.2	15.5
25	0.72 (s)	15.1	15.6
26	0.90 (s)	16.8	16.9
27	1.07 (s)	25.9	26.0
28	0.93 (s)	28.4	28.4
29	0.80(s)	33.3	33.3
30	0.80 (s)	23.7	23.7

[#] [Tanakaa and Matsunaga, 1989]

* Overlapped signal
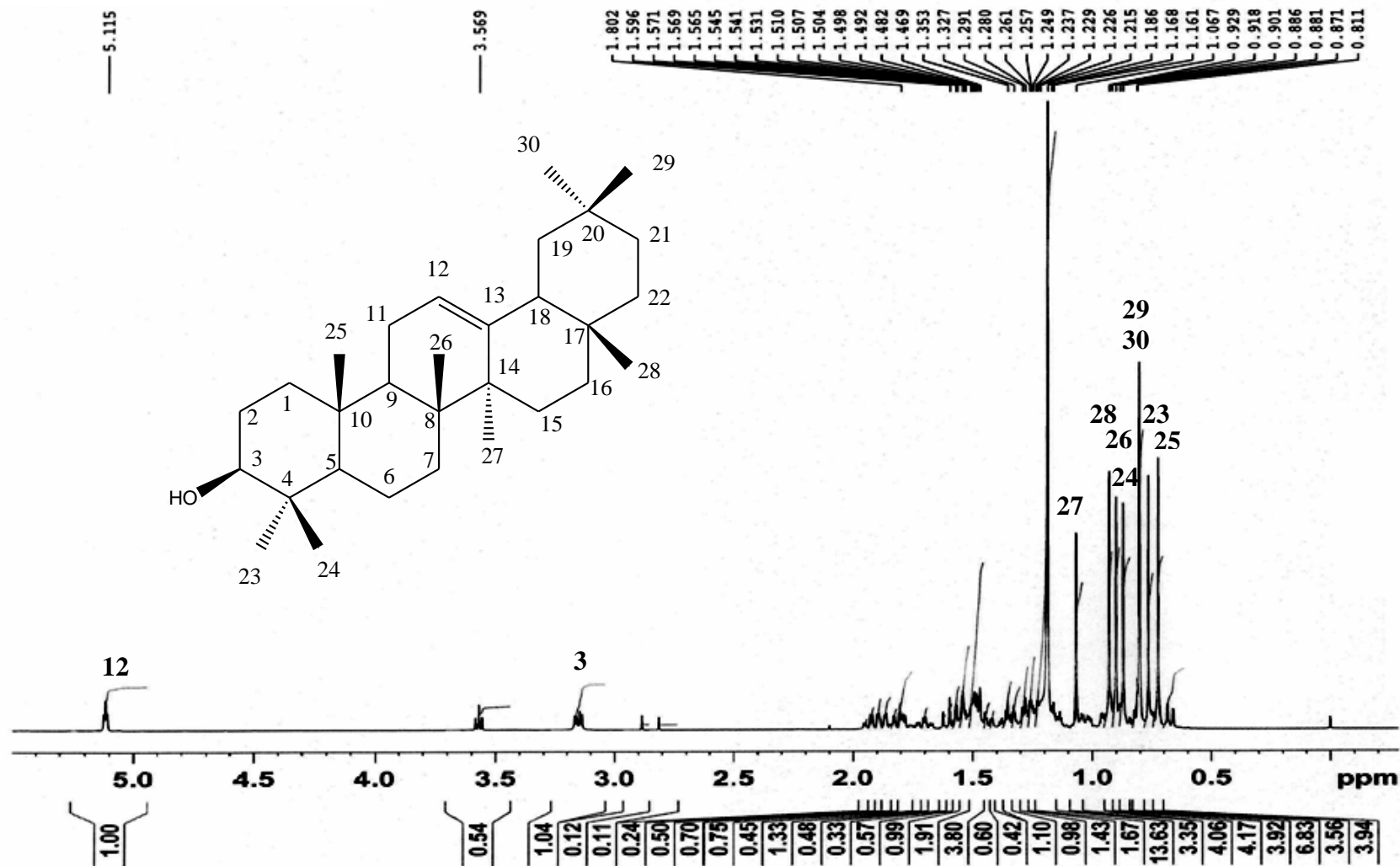
Fig. (21): The ^{1}HNMR spectrum of compound AL-1
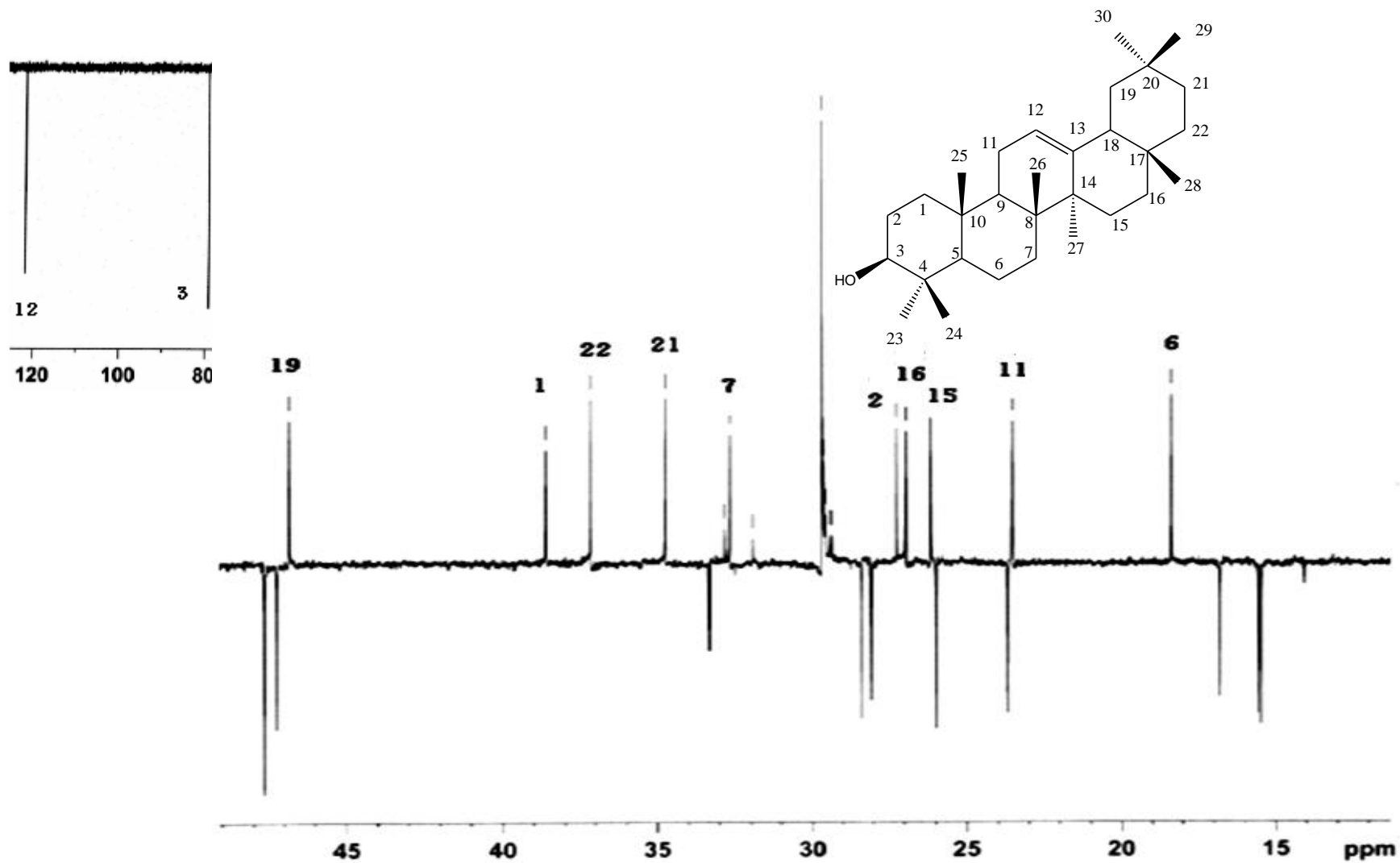
Fig. (22): Dept 135° spectrum of compound AL-1
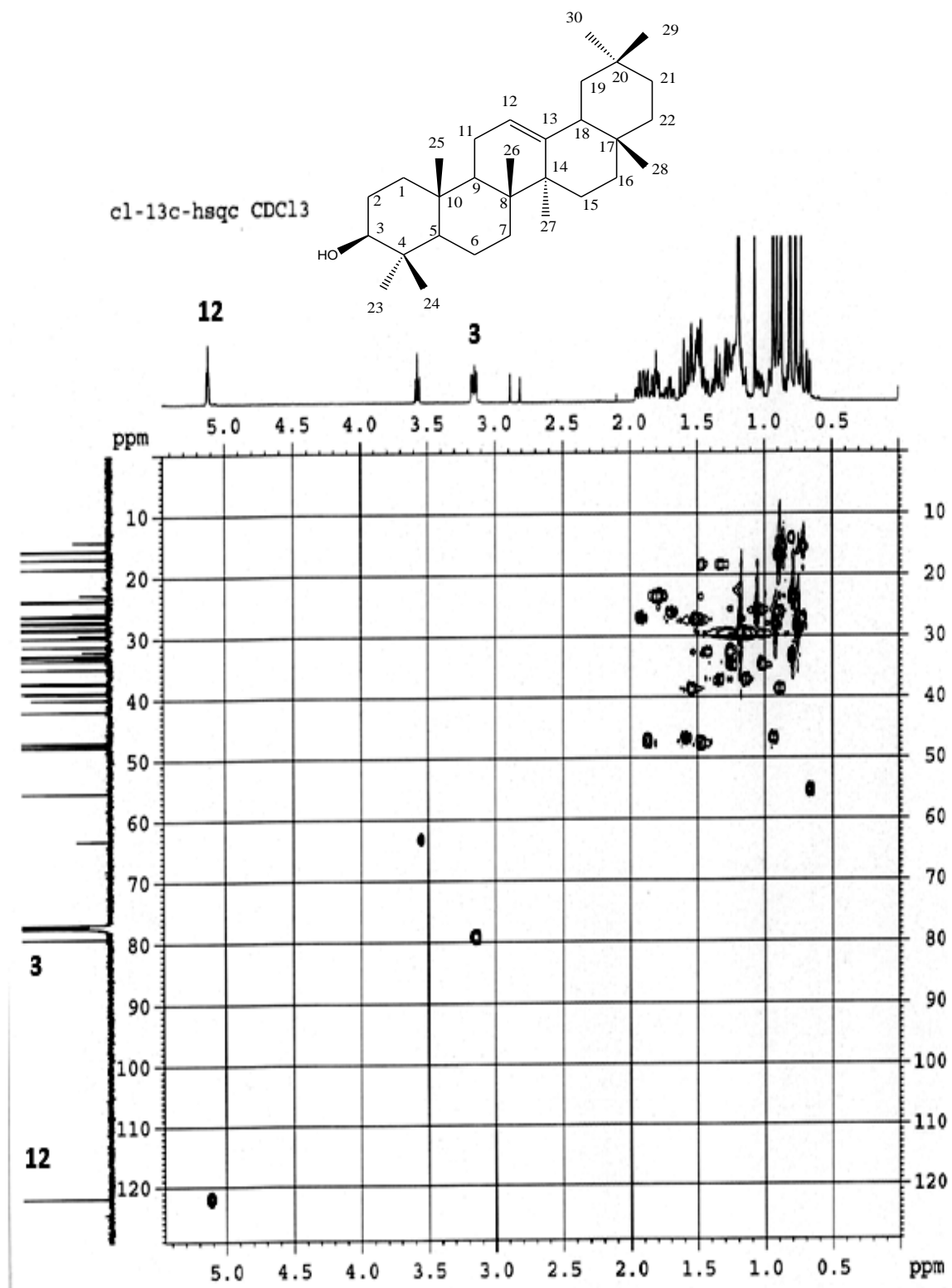
Fig. (23): HSQC spectrum of compound AL-1
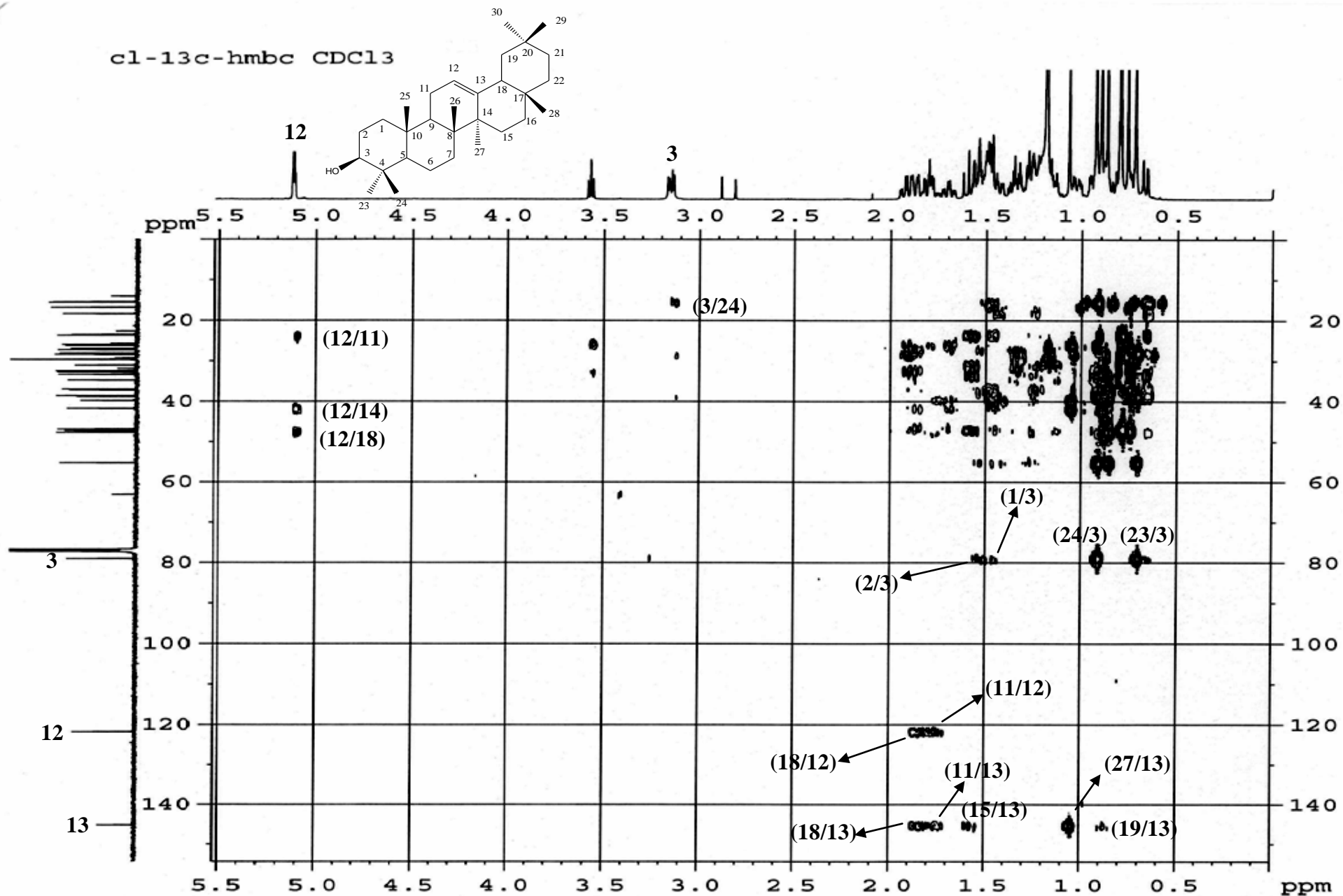
Fig. (24): HMBC spectrum of compound AL-1

Characterization and structure elucidation of compound AL-2

Compound AL-2 was isolated as white crystalline needles, melting point 425 ^{o} - 427 ^{o} C, from the ethyl acetate soluble fraction. It gave a positive result (green color) with Libermann-Burchard's test, indicating its steroidal or triterpenoidal nature. The IR spectrum (KBr) revealed presence of a hydroxyl group (O-H) (a broad absorption at 3510 cm^{-1} and absorption of C-O stretching at 1150 cm^{-1}) and presence of double bond (a weak absorption at 1630 cm^{-1} and the absorption of a vinyl C-H stretching at 3030 cm^{-1}).

The mass spectrum showed molecular ion peak [M]$^{+}$ at *m/z* 440 calculated for $C_{30}H_{48}O_2$ in addition to other significant fragments at *m/z* 422 (M-H_2O), 404 (M-2H_2O) and 299 (Retro Diels-Alder fragment confirming position of double bond. Fragmentation pattern is suggested in (Fig. 25).
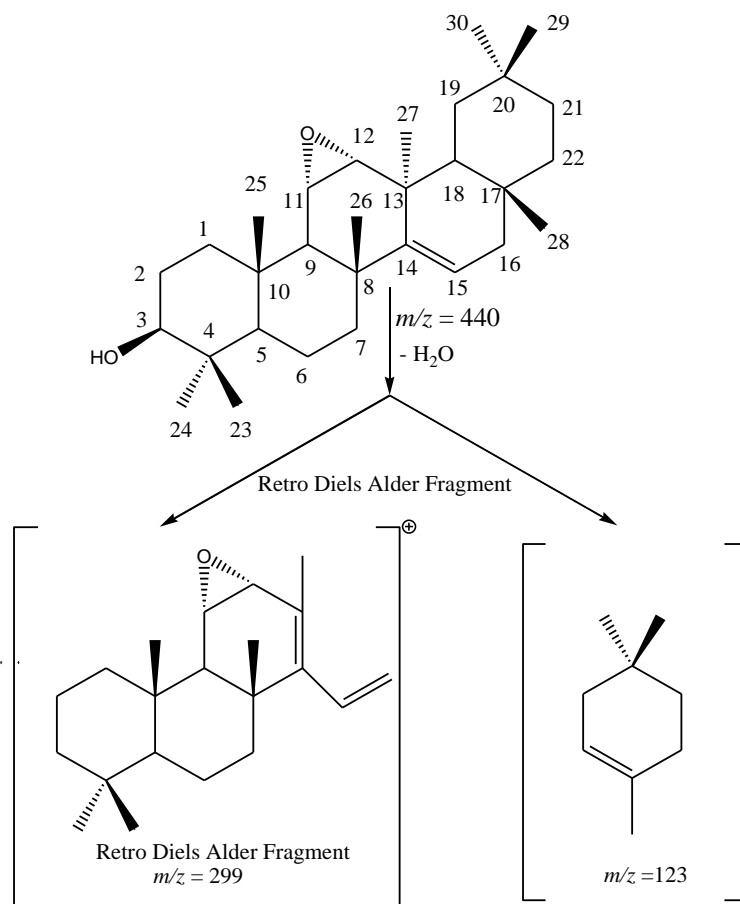
Fig. (25): Fragmentation pattern of compound AL-2

The ^{1}H and ^{13}C NMR spectral data presented in Table 12 showed characteristic triterpenoidal CH_3 and CH_2 signals from δ_H 0.85-2.1 and thirty carbon resonances. The DEPT 135° experiment (Fig. 28) represented eight methylene groups and fifteen methyl and/or methine groups.

The ^{1}H NMR spectrum (Fig. 26) showed eight methyl groups resonating at δ_H 0.85, 0.85, 0.89, 0.99, 1.02, 1.02, 1.1 and 1.1 together with the ^{13}C NMR of eight methyl carbons at δ_C 16.9 (C-24), δ_C 15.4 (C-25), δ_C 19.5 (C-30), δ_C 27.0 (C-26), δ_C 27.9 (C-23), δ_C 29.9 (C-28), δ_C 30.2 (C-27), and δ_C 33.6 (C-29) ; two of the carbon signals resonated in the olefinic region at δ_C 157.21 (C-14) and δ_C 118.86 (C-15) and an oxygenated carbon resonating at δ_C 78.9 was assigned for (C-3) (Fig. 27). The presence of two oxygenated carbons (δ_C 53.7 and δ_C 58.3) and two protons with ^{1}H NMR signals at δ_H 3.14 (*t*, *J*= 10.5, 5.5 Hz) and δ_H 2.82 (*d*, *J*= 5.5 Hz) confirmed an epoxy group present at (C-11) and (C-12). The two double doublets at δ_H 5.57 (*J*= 8.5, 3.5 Hz) and δ_H 3.26 (*J*= 11.5, 5.5Hz) were assigned to olefinic (H-15) and oxygenated H-3α protons respectively.

Table 12: ^{1}H and ^{13}C NMR Assignment of Compound AL-2 (500 MHz, CDCl$_3$)

Position	δ [1]H (Multiplicity, *J* in Hz)	δ [13]C	δ ^{13}C of reference compound[*] at 100 MHz in CDCl$_3$
1	1.70 (*m*) 2.00 (*d*,3.0)	38.3	38.2
2	1.69 (*m*)	26.9	26.9
3	3.26 (*dd*, 11.5, 5.5)	78.9	79.0
4	-	38.7	38.7
5	0.76 (*d*,2.0)	54.6	54.7
6	1.28 (*m*)	18.9	18.8
7	1.33 (*m*)	33.2	33.2
8	-	38.9	38.9
9	1.72 (*d*,5.0)	51.9	52.0

Table. 12 *Cont.*

10	-	37.5	37.5
11	3.14 (*t*, 10.5, 5.5)	53.7	53.6
12	2.82 (*d*,5.5)	58.3	58.3
13	-	36.6	36.6
14	-	157.2	157.1
15	5.57 (*dd*, 8.5, 3.5)	118.9	118.9
16	1.18 (*d*, 3.5)	35.3	35.2
17	-	35.4	35.4
18	1.21 (*s*)	48.1	48.1
19	1.37 (*d*, 4.0) 2.11 (*t*)	40.4	40.3
20	-	28.7	28.7
21	1.25 (*d*, 3.0)	36.6	36.6
22	1.23 (*m*) 1. 90 (*t*)	38.2	38.2
23	0.99 (*s*)	27.9	27.9
24	0.85 (*d*, 2.0)	16.9	17.0
25	0.89 (*s*)	15.4	15.4
26	1.1 (*s*)	27.0	27.1
27	1.1 (*s*)	30. 2	30.2
28	0.85 (*d*, 2.0)	29.9	29.7
29	1.02 (*s*)	33.6	33.7
30	1.02 (*s*)	19.5	19.6

[Ibrahim& Ali, 2007].

The HMBC experiment (Fig. 29) confirmed the positions of hydroxyl group at C-3, epoxy structure at C-11&C-12 and a double bond at C-14. Two and three bond correlations were observed from H-3 to C-1, C-2, C-4 and C-5; from H-11 to C-9, C-10 and C-12; and from H-12 to C-9, C-11, C-13 and C-14.

The spectral data of compound AL-2 was in a good agreement with the reported data of 11α, 12α-oxidotaraxerol [Ibrahim& Ali, 2007].

The above data collectively indicated that the compound AL-2 is 11α, 12α-oxidotaraxerol. Literature review showed that this is the first time to isolate this compound from the genus *Albizia*.

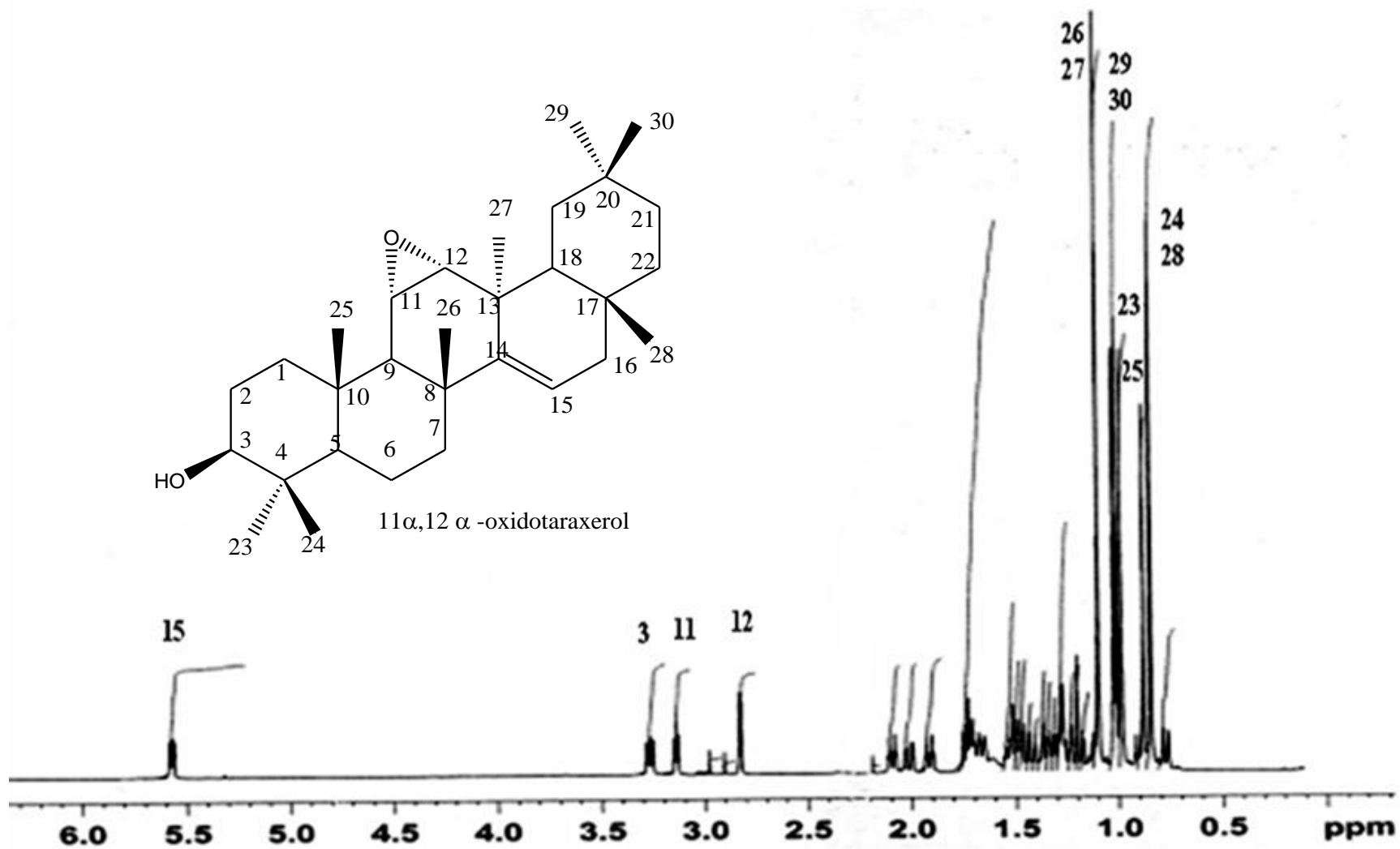11α,12 α -oxidotaraxerol
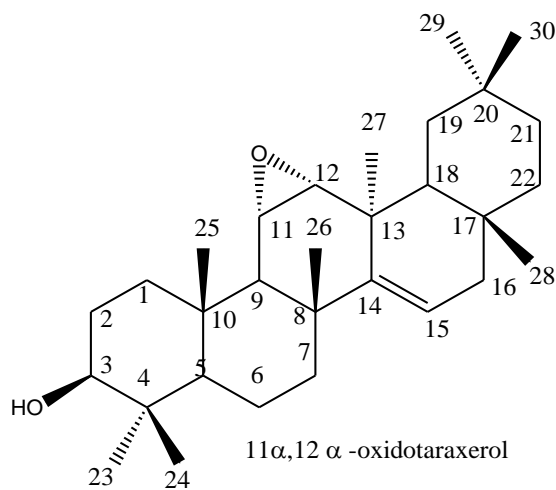
Structure of compound AL-2
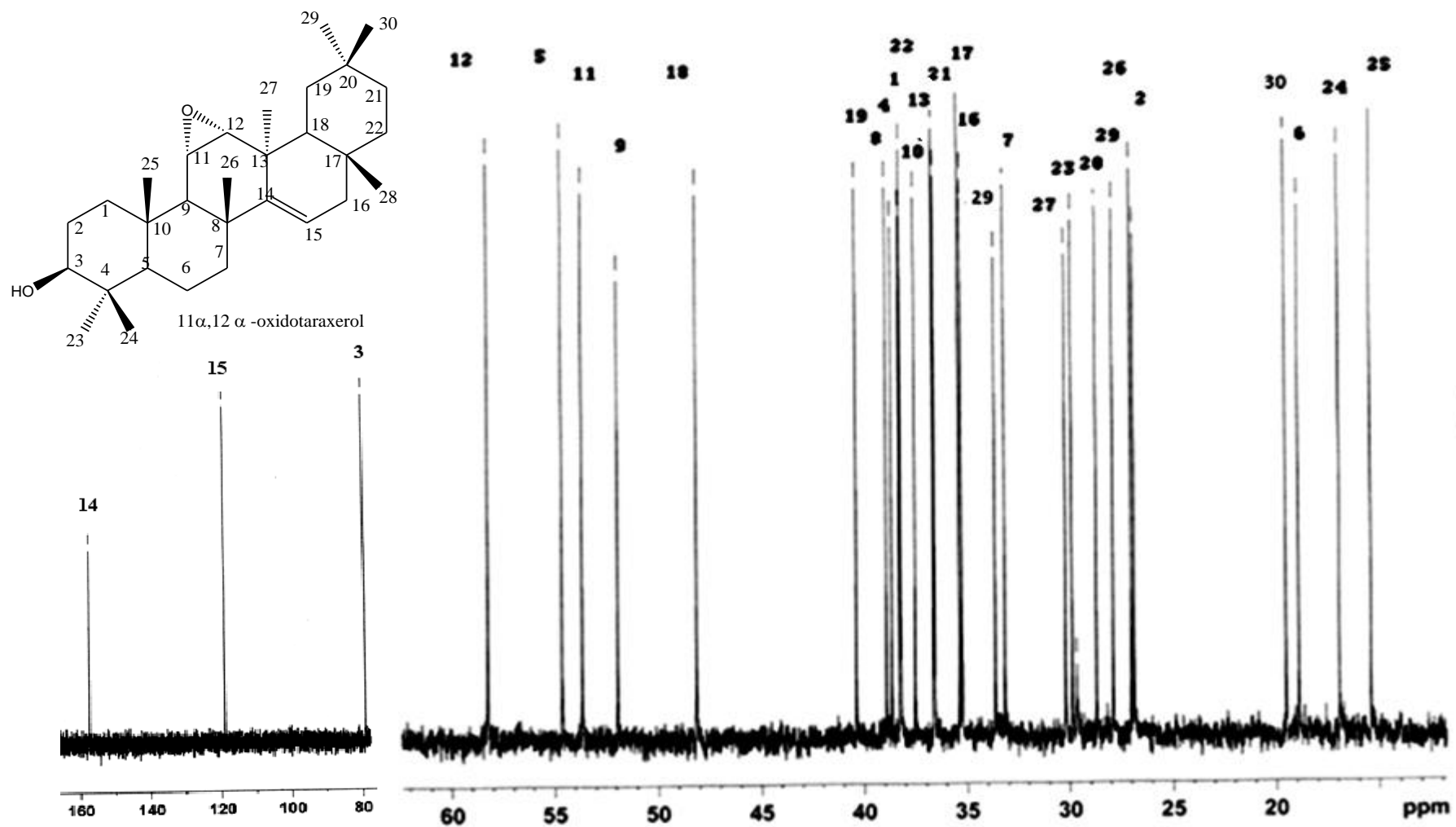
Fig. (26): ^{1}H NMR spectrum of compound AL-2

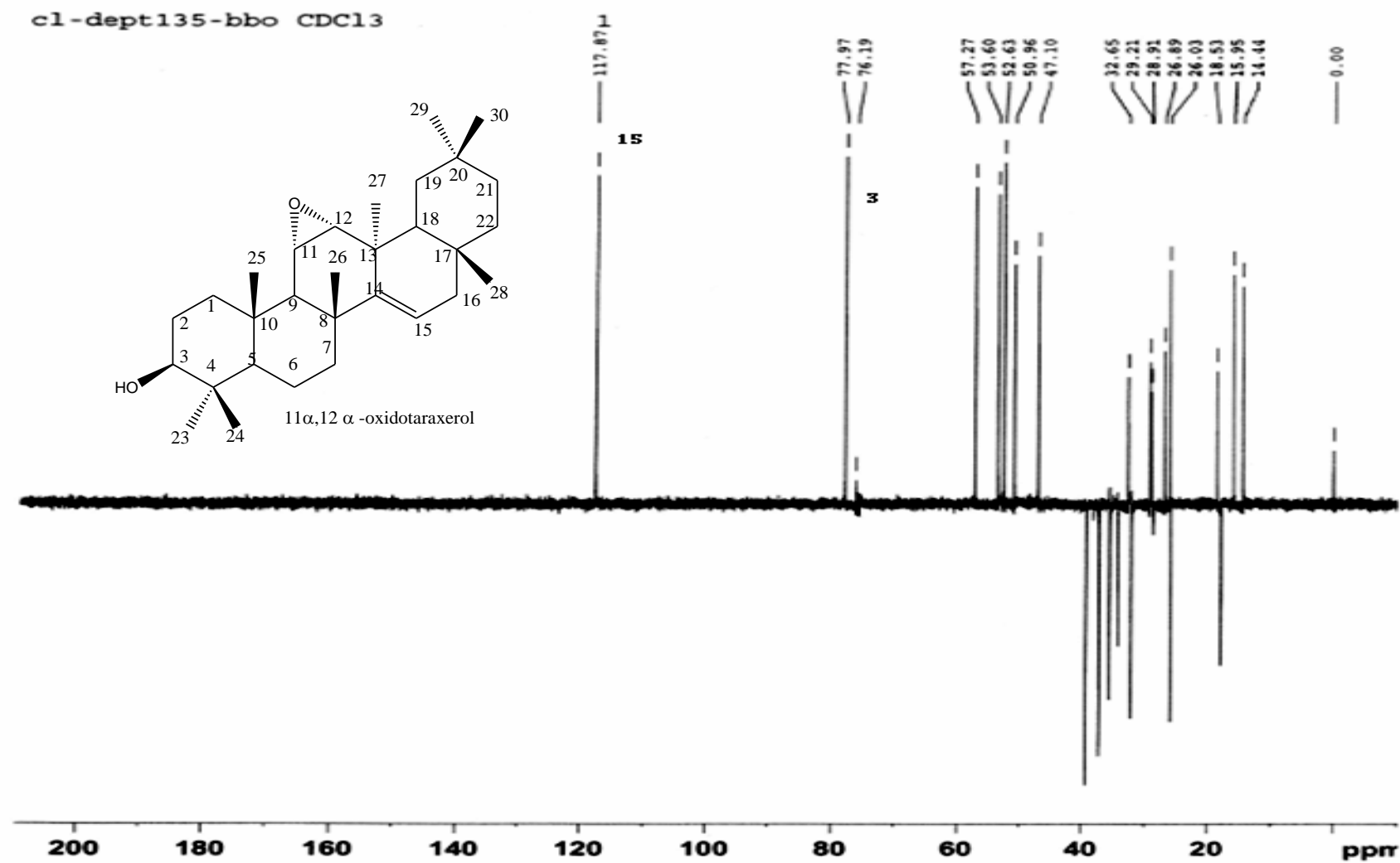Fig. (27): ^{13}C NMR spectra of compound AL-2
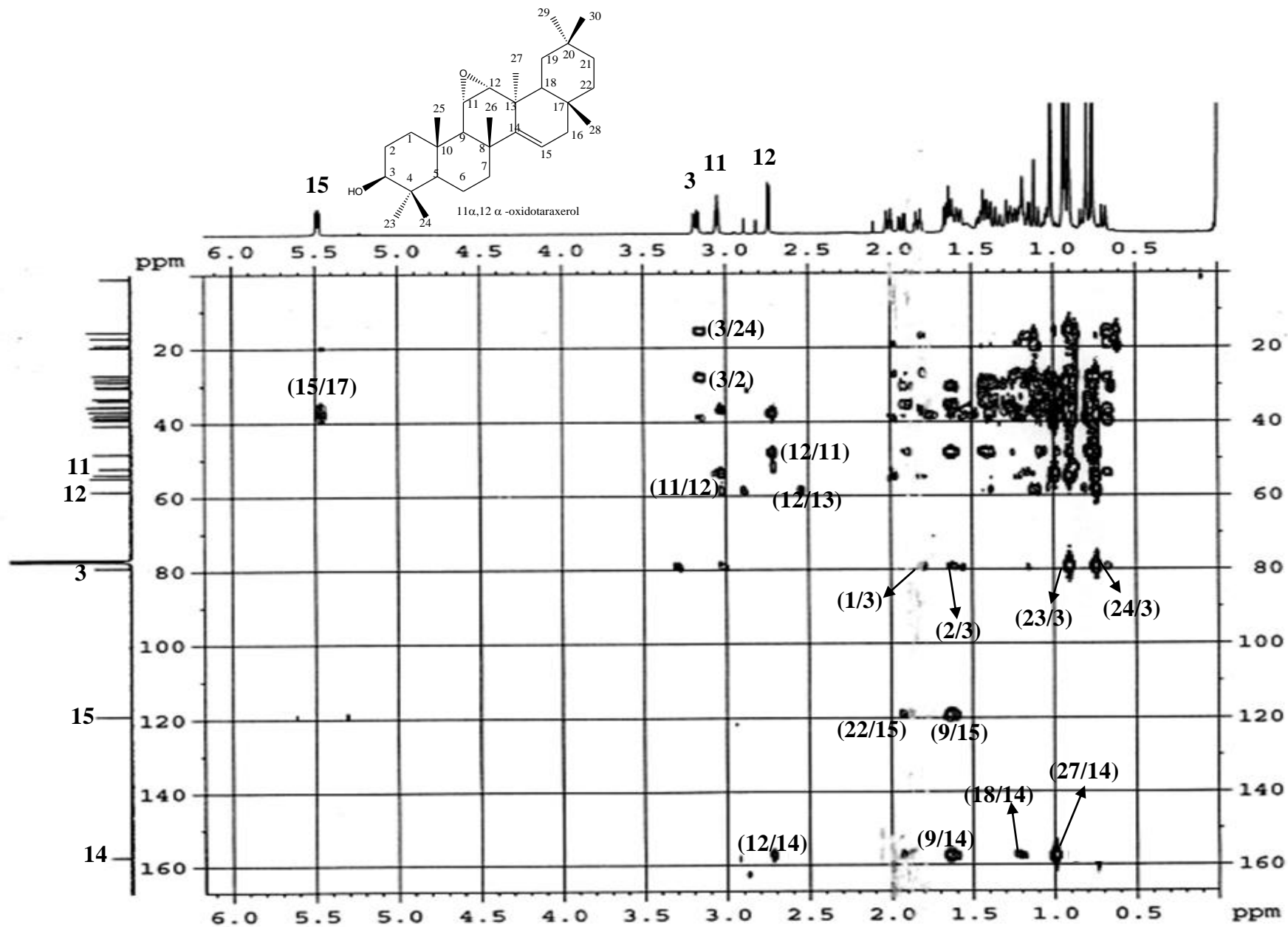
74

Fig. (28): DEPT 135° of compound AL-2

Fig. (29): HMBC spectrum of compound AL-2

Characterization and structure elucidation of compound AL-3

Compound AL-3 was isolated as white fine needles from alcohol, mp 141–142 $^{\circ}$C from the ethyl acetate soluble fraction. This compound gave red spot with ninhydrin reagent suggesting an amide. The IR spectrum showed typical absorption broad band of (-OH & -NH) at 3200-3500 cm^{-1}; C-H stretching of olefenic bond at 3100 cm^{-1} and a carbonyl absorption at 1650 cm^{-1} suggesting an amide group. These absorption bands implied the presence of hydroxyl, amide and olefenic bond functionalities.

The positive FAB-MS spectrum of AL-3 showed molecular ion peak at *m/z* 654 [M + H]$^{+}$, respectively suggesting presence of one N atom corresponding to molecular formula $C_{40}H_{79}NO_5$. In the EI-MS, the molecular ion peak of long chain base at *m/z* 398 [M - $C_{16}H_{31}O_2$]$^{+}$ in addition to fragment ions at *m/z* 356 [M - H_2O–$C_{20}H_{39}$]$^{+}$ and *m/z* 338 [M - $2H_2O$–$C_{20}H_{39}$]$^{+}$ showed that the LCB (long chain base) moiety possessed 24 carbons, containing a double bond.

The ^{1}H and ^{13}C NMR spectral data (Table 13) showed amide linkage proton *N*-**H** at δ_H 8.57(*d*, *J*= 9.0 Hz), carbonyl carbon at (δ_C 175.75) and trans double bond at δ_H 5.52 (2H), (δ_C 130.7 and 130.8). In H^1 NMR (Fig. 30) two long chain aliphatic moieties signals were detected from δ_H 0.8-2.3. Positions of amide group and hydroxyl groups were determined based on COSY correlations of (H-2). In the ^{1}H–^{1}H COSY spectrum, the signal at δ_H 8.57 (*N*-**H)** gave a cross-peak with the signal at δ_H 5.11 (H-2) which, in turn, showed cross-peaks with methylene protons (H-1) at δ_H 4.50 and δ_H 4.40 and oxymethine δ_H 4.33 (H-3). The latter correlated with the signal at δ_H 4.29 (H-4). The *trans* (*E*) configuration of the double bond was proved by the large vicinal coupling constant (δ_H 15.5 Hz) as well as by the chemical shifts (δ_C 32.6, C-7; δ_C 33.6, C-10) of the methylene carbon adjacent to the olefinic carbon, which was reported at δ_C 27 in (*Z*) isomer and at δ_C 33 in (*E*) isomer [Kang *et al*., 2007]. The chemical shift of H-2 (δ_H 5.11) and the carbon chemical shifts at δ_C 62.5 (C-1), 53.5 (C-2), 77.2 (C-3), 73.5 (C-4), 175.8 (C-1') and 73.0 (C-2') were almost identical with those of the reported data of (2*S*, 3*S*, 4*R*, 2'*R*)-phytosphingosine moieties. The chemical shift of H-2 signal and the ^{13}C chemical shifts of C-1–C-4, C-1' and C-2' of sphingosine (**65**) were useful for the determination of

the stereochemistry of the phytosphingosine moiety at positions (2*S*, 3*S*, 4*R*, 2'*R*) [Kang *et al*., 2007].

The ^{1}H and ^{13}C NMR spectral data of Compound AL-3 were in good agreement with that reported for [(2*S*, 3*S*, 4*R*, 8*E*)-2-[(2'*R*)-hydroxyhexadecanoylamino]-8-tetra-cosene-1, 3, 4-triol which previously isolated from *Albizia Julibrissin* [Kang *et al*., 2007].

The previous data collectively indicated that compound AL-3 is [(2*S*, 3*S*, 4*R*, 8*E*)-2-[(2'*R*)-hydroxyhexadecanoylamino]-8-tetra-cosene-1, 3, 4-triol and considered aglycone of AL-4. It is first time to be isolated from *A. lebbeck.*

Sphingosine (**65**)
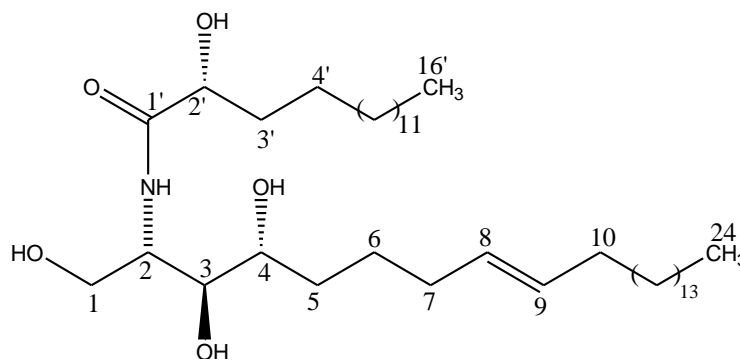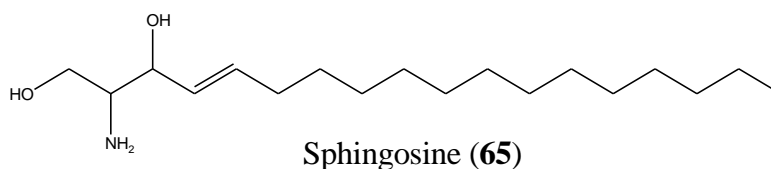
Structure of compound AL-3

Table 13: ^{1}H and ^{13}C NMR Assignment of Compound AL-3 (500 MHz, Pyr.-d$_5$) & Compound AL-4 (CD$_3$OD)

Position	δ ^{1}H (Multiplicity, *J* in Hz) Compound AL-3 (500 MHz, Pyr.-d$_5$)	δ ^{13}C Compound AL-3 (500 MHz, Pyr.-d$_5$)	δ ^{1}H (Multiplicity, *J* in Hz) Compound AL-4 (500 MHz, CD$_3$OD)	δ ^{13}C Compound AL-4 (500 MHz, CD$_3$OD)
1	4.5 (*dd*, 10.5, 4.5) 4.4 (*dd*, 10.5, 4.5)	62.5	3.78 (*dd*, 10.5, 4.5) 3.50 (*dd*, 10.5, 4.5)	71.7
2	5.11 (*m*)	53.5	4.20 (*m*)	51.7
3	4.33 (*m*)	77.2	3.17* (*m*)	75.6
4	4.29 (*m*)	73.5	3.15* (*m*)	70.9
5	1.97 (*m*)	34.6	1.19* (*m*)	30.7
6	1.79 (*m*)	27.1	1.23* (*m*)	23.7
7	1.95 (*m*)	32.6	1.97 (*m*)	32.8
8	5.52 (*dt*, 15.5, 5.5)	130.7	5.32 (*m*)	131.4
9	5.52 (*dt*, 15.5, 5.5)	130.8	5.32 (*m*)	131.6
10	2.05 (*m*)	33.6	2.05 (*m*)	33.1
11-21	1.29-1.4* (*m*)	30.1-30.8	1.21-1.4 (*m*)	30.5-32.8
22	1.93 (*m*)	33.8	1.61 (*m*)	33.7
23	1.43 (*m*)	23.4	1.52 (*m*)	23.7
24	0.85 (*t*, 7)	14.7	0.8 (*t*, 6.5)	14.5
NH	8.57 (*d*, 9)	-	-	-
1'	-	175.8	-	174.7
2'	4.62 (*dd*, 3.5,7.5)	73.0	3.49 (*m*)	72.9
3'	2.23 (*m*) 2.21(*m*)	36.2	1.64 (*m*)	35.7

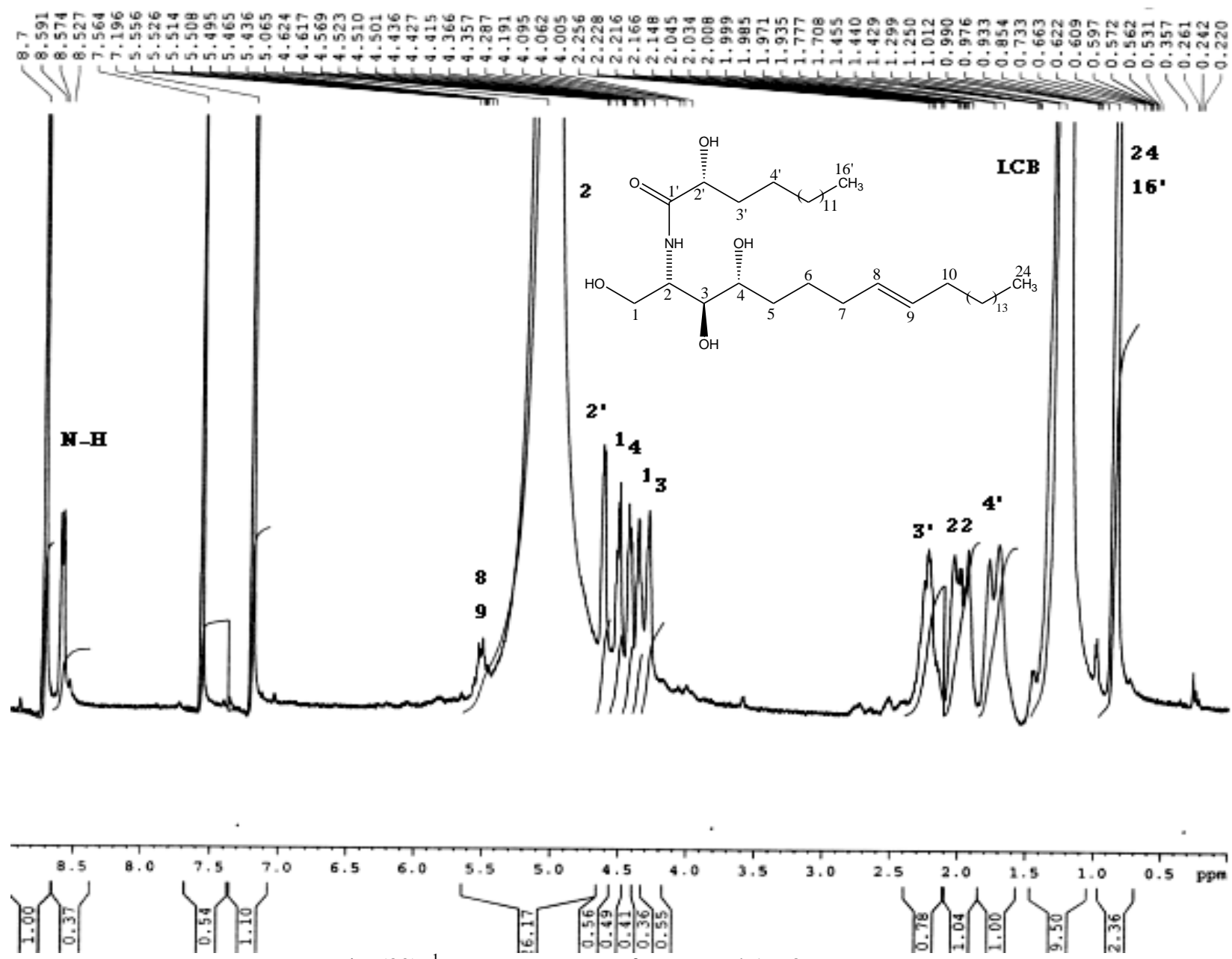|				
4'	1.71(*m*)	26.3	1.29 (*m*)*	27.2
5'-13'	1.29-1.4* (*m*)	30.1-30.8	1.21-1.4* (*t*)	30.5-32.8
14'	1.23*(*m*)	33.8	1.21*(*m*)	30.4
15'	1.27* (*m*)	23.3	1.32* (*m*)	26.1
16'	0.85 (*t*, 7)	14.7	0.8 (*t*,7)	14.5
1"	-	-	4.75 (*d*, 8.5)	104.7
2"	-	-	3.45 (*m*)	75.1
3"	-	-	3.28* (*m*)	77.9
4"	-	-	3.2* (*m*)	71.6
5"	-	-	3.91(*m*)	78.0
6"	-	-	3.77 (*s*)	62.7

* Overlapped signals

Fig. (30): ^{1}H NMR spectrum of compound AL-3

Fig. (31): ^{13}C NMR spectrum of compound AL-3

Characterization and structure elucidation of compound AL-4

Compound AL-4 was isolated as white fine needles from alcohol, mp 207–208 °C from the ethyl acetate soluble fraction. AL-4 has mostly similar basic features regarding color with ninhydrin and IR data. The FAB-MS of compound AL-4 showed molecular ion peak at m/z 816 [M + H]$^+$. The odd molecular weight (m/z 815) indicated the presence of N- atom, corresponding to molecular formula $C_{46}H_{89}NO_{10}$. In addition, the EI-MS spectrum showed a fragment at m/z 635, indicating the loss of a glucose moiety.

The ^{1}H and ^{13}C NMR spectral data were similar to those of compound Al-3 except for the presence of glucose moiety signals at δ_C 104.7, 75.0, 77.9, 69.3, 78.0 and 62.7 in the ^{13}C NMR spectrum (Fig. 33) and anomeric proton at δ_H 4.75 (d, $J = 8.5$ Hz) in the ^{1}H NMR spectrum (Fig. 32) confirming that compound AL-4 is the glycoside of compound AL-3.

The ^{1}H and ^{13}C NMR spectral data of compound AL-4 were in good agreement with that reported for 1-O-β-D-glucopyranosyl- [(2S, 3S, 4R, 8E)-2-[(2'R)-hydroxyhexadecanoylamino]-8-tetra-cosene-1, 3, 4-triol.which was previously isolated from *Albizia Julibrissin* [Kang *et al*., 2007].

The previous data collectively indicated that the compound AL-4 is 1-O-β-D-glucopyranosyl- [(2S, 3S, 4R, 8E)-2-[(2⁻R)-hydroxyhexadecanoylamino]-8-tetra-cosene-1, 3, 4-triol. It is first time to be isolated from *A. lebbeck*.
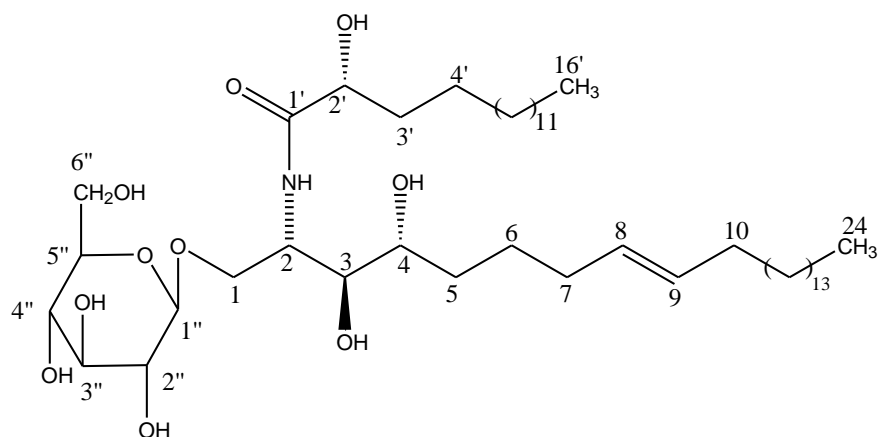
Structure of compound AL-4
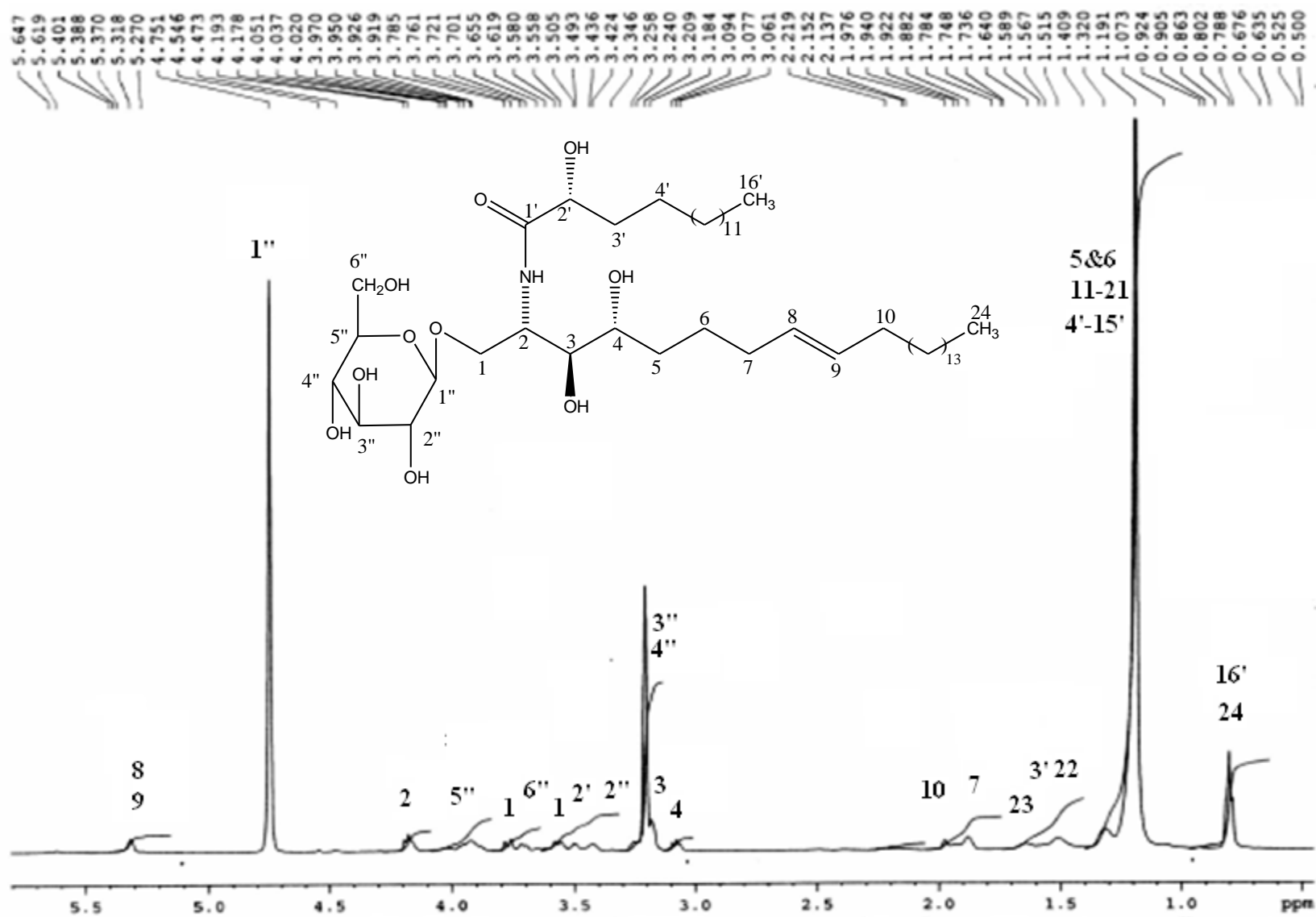
Fig. (32): ^{1}H NMR spectrum of compound AL-4
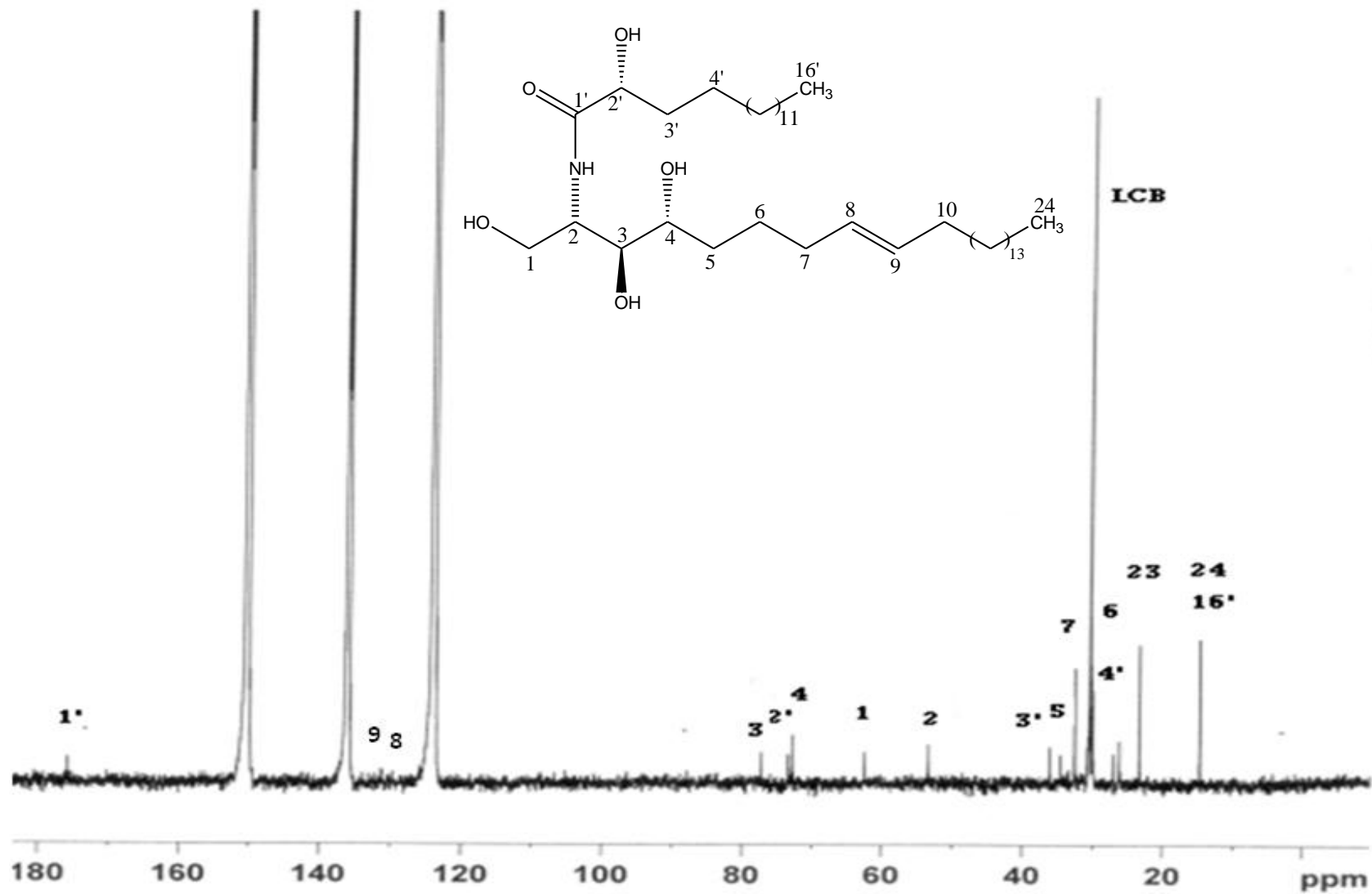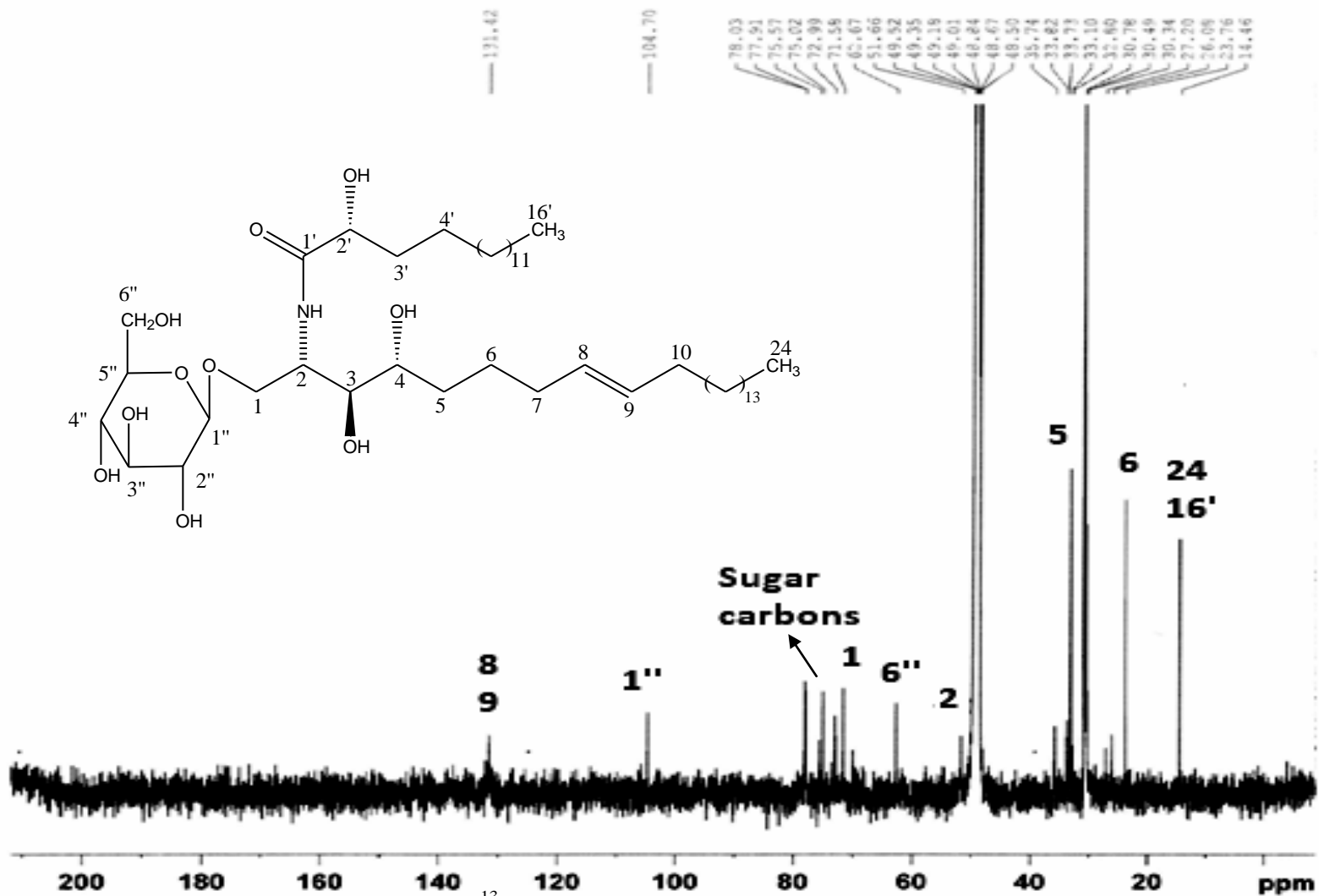
Fig. (33): ^{13}C NMR spectrum of compound AL-4
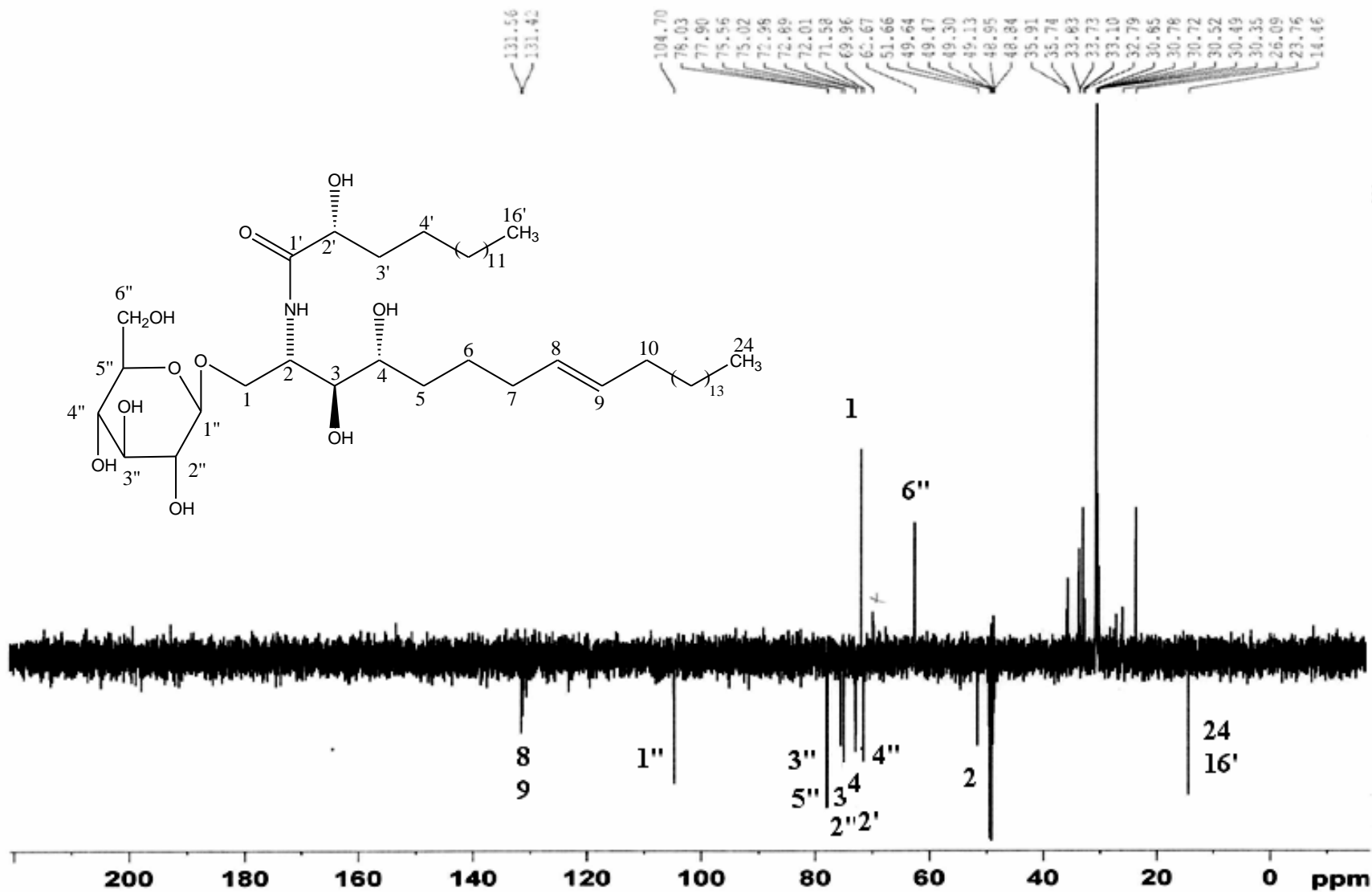
Fig. (34): DEPT 135° spectrum of compound AL-4

Characterization and structure elucidation of compound AL-5

Compound AL-5 was isolated as pale yellow fine needles, mp 142–143 $^{\circ}$C. In TLC, it gave a red spot on spraying with ninhydrin reagent suggesting an amide. The UV spectrum showed λ_{max} 224, 273, 280 and 289 nm suggesting an aromatic ring with extended conjugation.

The IR spectrum (KBr) showed typical absorption band at 3408 cm^{-1} suggesting the presence of N-H or O-H stretching and the absorption of C=O (stretching) at 1626 cm^{-1} supported the presence of an amide group. A weak absorption at 3010 cm^{-1} indicated =C-H (stretching) and medium absorption at 1605 cm^{-1} and 1504 cm^{-1} for C=C (stretching).

The ESI-Ion Trap mass spectrum indicated a *pseudo*-molecular ion peak [M+ Na]$^{+}$ at m/z 193 in addition to other fragments at m/z 157 [M- NH]$^{+}$, 117 [M-C$_2$HNO]$^{+}$, 105 [M-C$_3$HNO]$^{+}$, 91 [tropelium ion]$^{+}$ and 79 [characteristic benzenium ion ring] $^{+}$. The odd molecular weight (m/z 171) indicated the presence of one N- atom, corresponding to molecular formula C$_{11}$H$_9$NO.
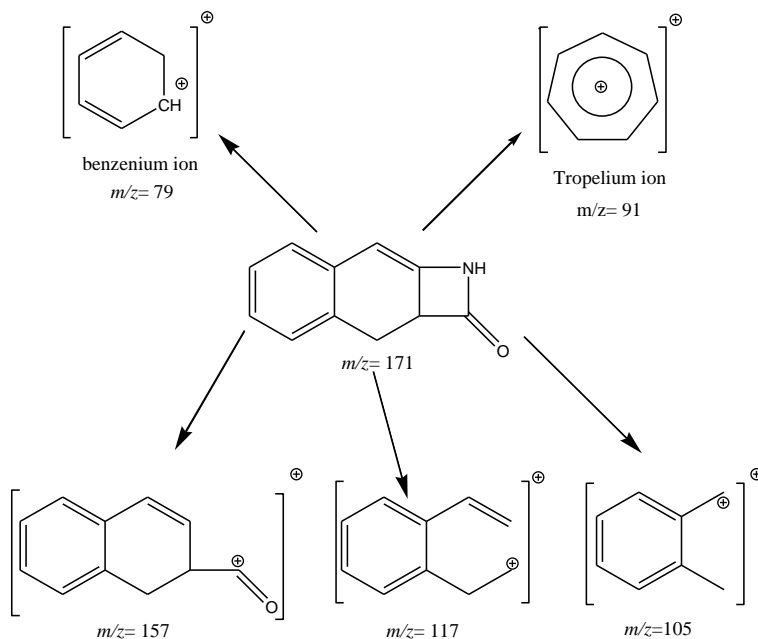
Fig. (35): Fragmentation pattern of compound AL-5

^{1}HNMR (Table 14, Fig. 36) indicated the presence of four aromatic protons at δ_H 7.02 (*t, J*= 7.5 Hz, H-7), δ_H 7.09 (*t, J*= 7Hz, H-8), δ_H 7.34 (*d, J*= 6.5Hz, H-6) and δ_H 7.60 (*d, J*= 8 Hz, H-9) suggesting *o*-disubstituted benzene. In addition, an isolated olefinic proton singlet at δ_H 7.19 (1H, *s*) was assigned to H-4 and three aliphatic protons appeared at δ_H 3.14 (*q, J*= 15.5, 9.5 Hz), δ_H 3.49 (*dd, J*= 15, 3.5 Hz) assigned for H-1 and H-1' and δ_H 3.77 (*q, J*= 9.5, 3.5 Hz) assigned for H-2.

^{13}C NMR (Table 14, Fig. 37) revealed amide carbonyl group signal at δ_C 174.5, six aromatic carbons at δ_C 112.5, δ_C 119.4, δ_C 120.1, δ_C 122.8, δ_C 128.5, and δ_C 138.4. Two olefinic carbons appeared at δ_C 109.6 (*s*) and δ_C 125.2 (*d*). Dept 135° experiment shown in (Fig. 38) represented six methane groups and one methylene group. HSQC experiment shown in (Fig. 39) confirmed the assignment of each proton to its carbon.

Positions of the amide group as well as the olefinic double bond were determined by HMBC correlations as shown in (Fig. 40). Two and three bond correlations were observed from H-1 to C-2', C-3, C-5; from H-2 to C-2' and from H-4 to C-5, C-10.

Presence of lactam ring was confirmed by HMBC correlations of carbonyl carbon with H-1 δ_H 3.14 (*q, J*= 15.5, 9.5 Hz) and δ_H H-1a 3.49 (*dd, J*= 15, 3.5 Hz).

All the previous data collectively indicated that the compound AL-5 is Albactam (2a,3-Dihydro-1*H*-naphtho[2,3-*b*]azet-2-one). It is novel compound and it is the first time to be isolated from natural source.
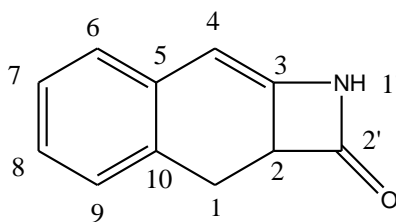
Albactam

2a,3-Dihydro-1*H*-naphtho[2,3-*b*]azet-2-one

Structure of compound AL-5

It is worthy to note the several amide-containing compounds were previously isolated from *leguminosae* species as in case of isolation of three spermidine alkaloids from the leaves of *Caesalpinia digyna* [Mahato *et al.,* 1983]. One of them; caesalpinine A (**66**), has five-membered β-lactam ring which supported the possible biosynthesis of AL-5.
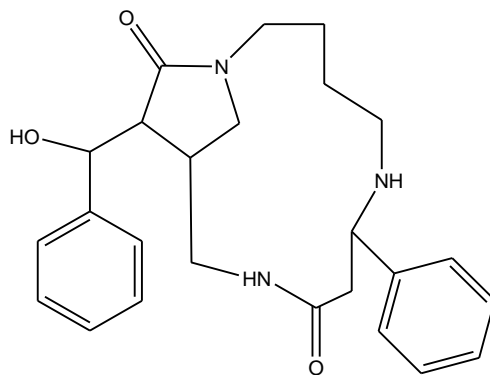
Caesalpinine A (**66**)

Albactam showed anti-aggregatory activity against adenosine diphosphate and arachidonic acid-induced guinea-pigs' platelets aggregation *in vitro* at doses 208 µg/ml and 172 µg/ml respectively (See biological evaluation chapter).

Table 14:^{1}H and ^{13}C NMR Assignment of Compound AL-5 (500 MHz, CD$_3$OD)

Position	δ [1]H (Multiplicity, J in Hz)	δ [13]C
1	3.14 (q, 15.5, 9.5) 3.49 (dd, 15.5, 3.5)	28.4
2	3.77 (q, 9.5, 3.5)	56.7
3	-	109.6
4	7.19 (s)	125.2
5	-	128.5
6	7.34 (d, 6.5)	112.5
7	7.02 (t, 7.5)	120.1
8	7.09 (t, 7)	122.8
9	7.60 (d,8)	119.4
10	-	138.4
2'	-	174.5
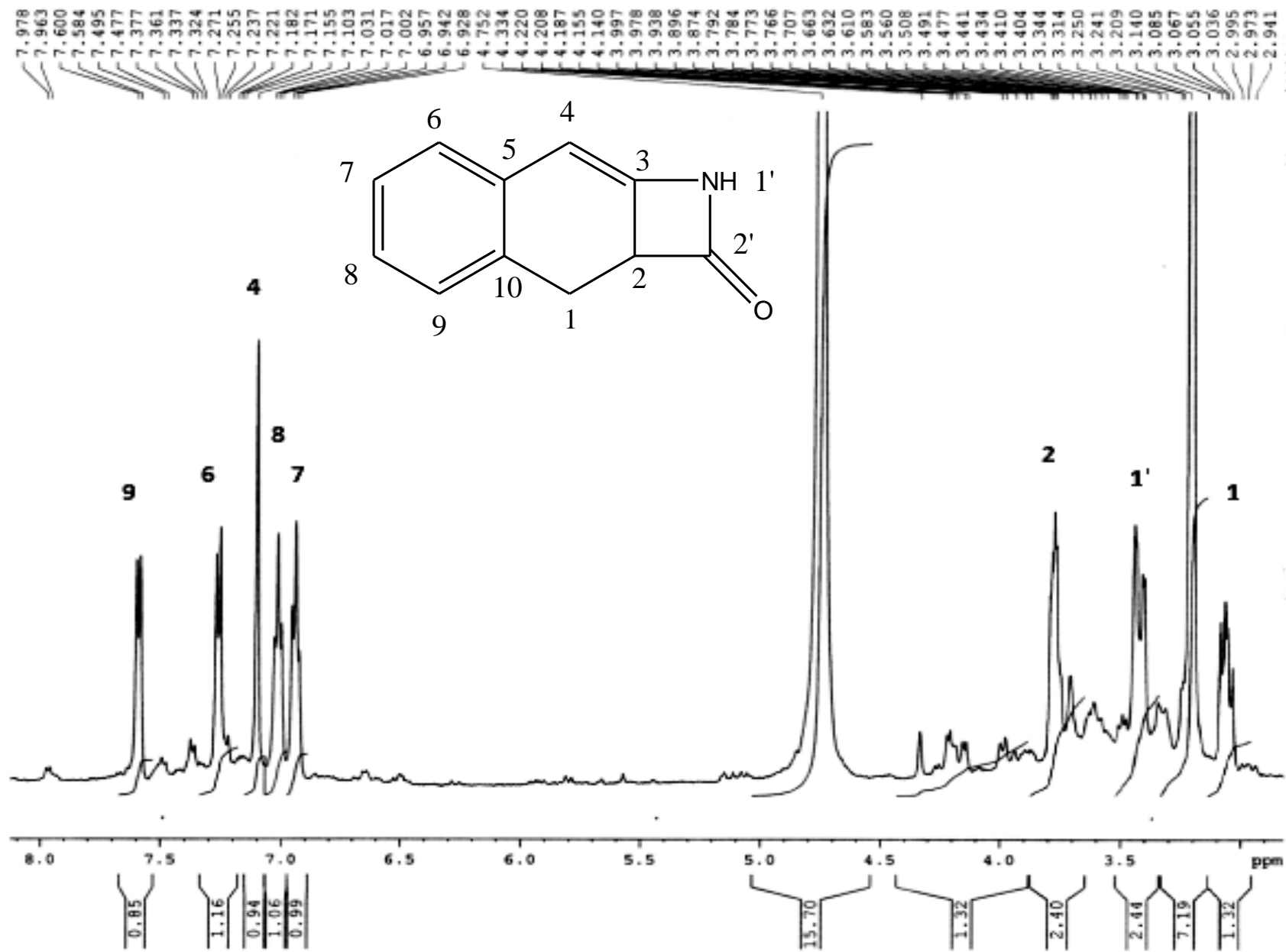
Fig. (36): ^{1}H NMR spectrum of compound AL-5
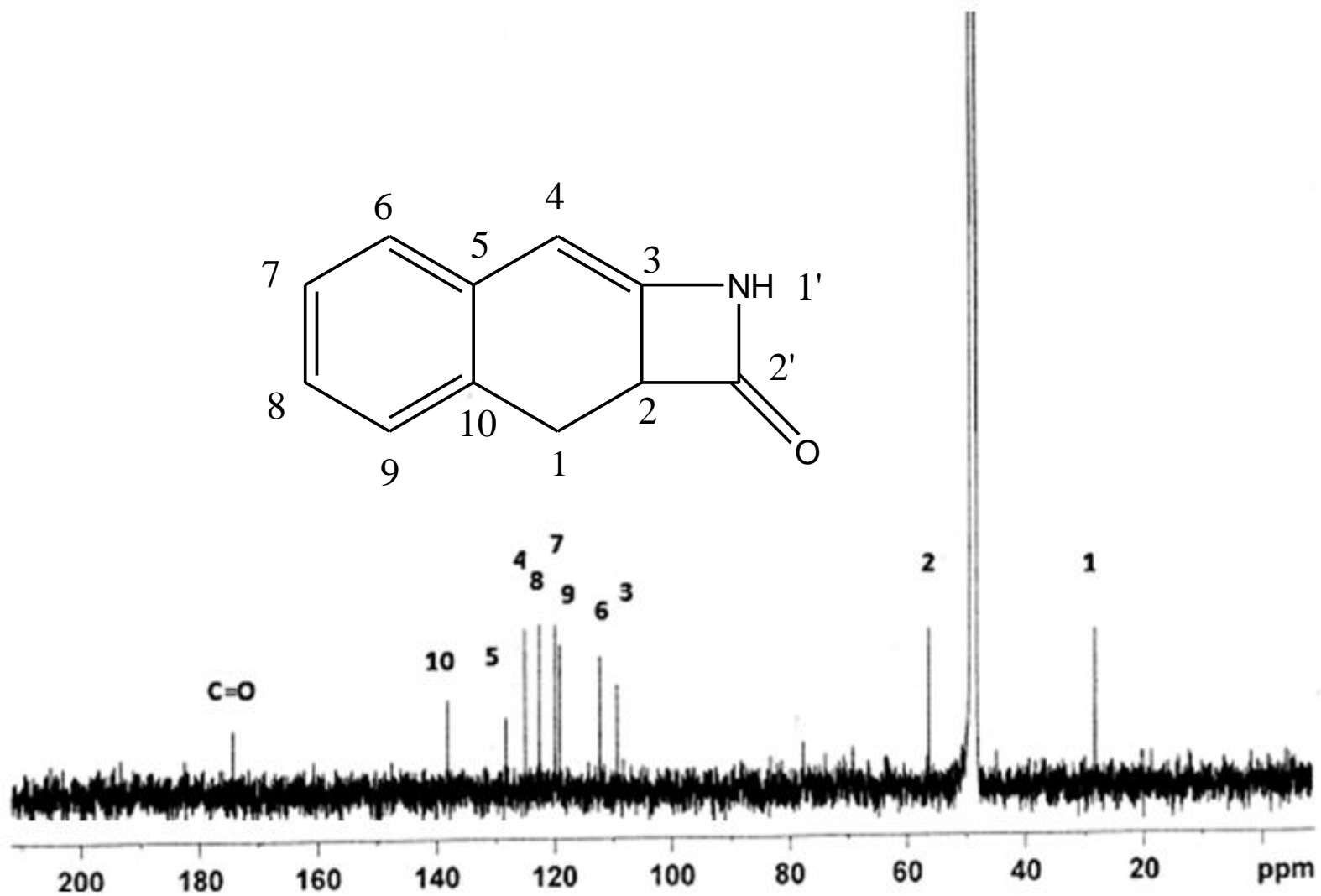
Fig. (37): ^{13}C NMR spectrum of compound AL-5
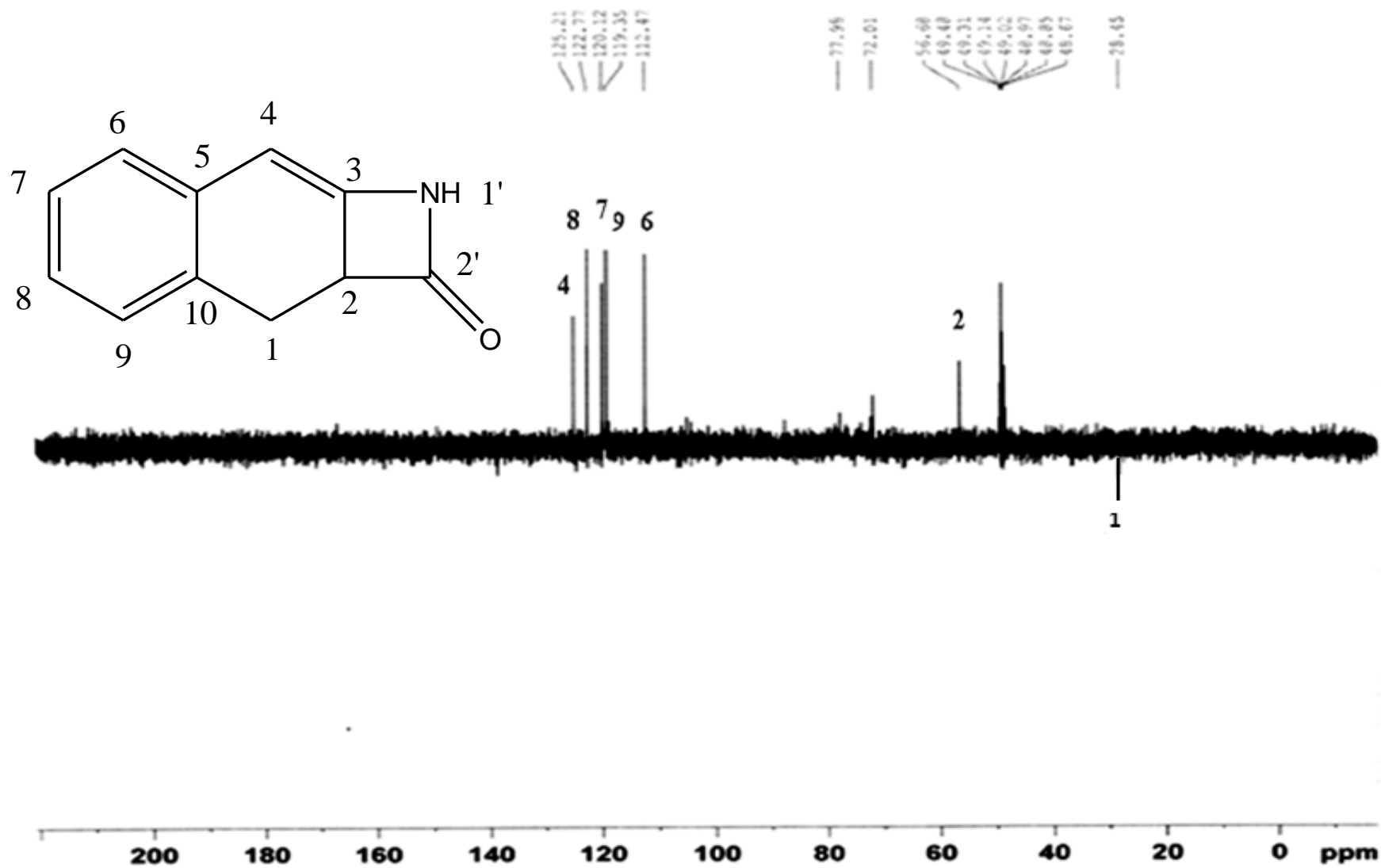
Fig. (38): DEPT 135º spectrum of compound AL-5
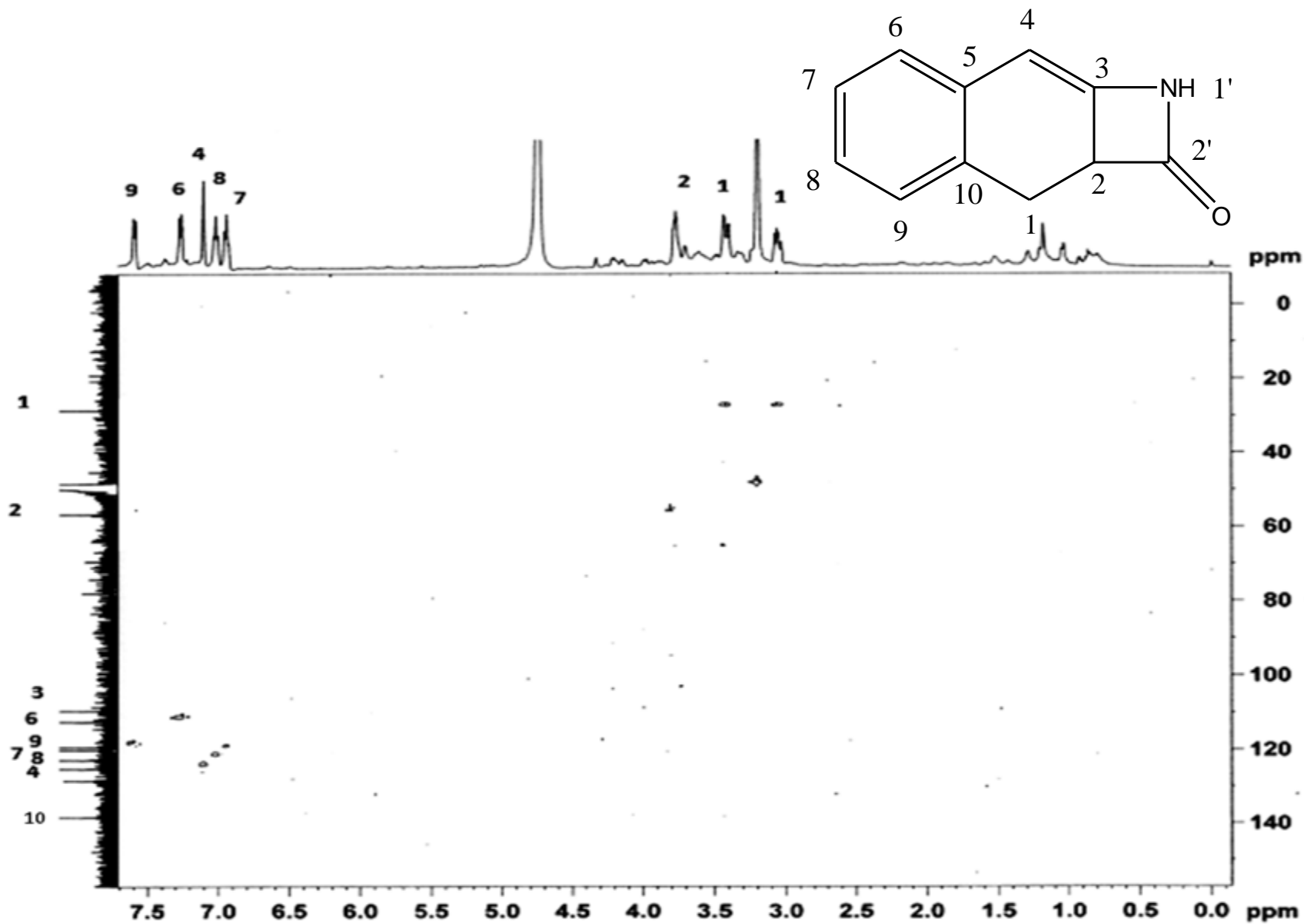
Fig. (39): HSQC spectrum of compound AL-5

Fig. (40): HMBC spectrum of compound AL-5

Characterization and structure elucidation of compound AL-6

Compound AL-6 was isolated as yellowish white fine needles, mp 54–55 $^\circ$C from the ethyl acetate insoluble fraction. The IR spectrum (KBr) indicated the presence of hydroxyl group (typical absorption band at 3600 cm^{-1} and C-O stretching band at 1150 cm^{-1}) in addition to double bond (=**C-H** st. band appeared at 3030 cm^{-1} and C=C st. band at 1630 cm^{-1}).

The ESI mass spectrum indicated a molecular ion peak [M+1]$^+$ at *m/z* 189 in addition to signals at *m/z* 173 [M-CH$_3$]$^+$, *m/z* 171 [M-H$_2$O] and *m/z* 155 [M-H$_2$O- CH$_3$]$^+$ corresponding to molecular formula C$_{10}$H$_{20}$O$_3$ that suggested a monoterpene derivative.

^{1}HNMR (Table 15, Fig. 41-43) displayed three olefinic protons at δ_H 4.96 (*dd*, *J* = 10.5, 1.5 Hz), δ_H 5.23 (*d*, *J* = 17 Hz) and δ_H 5.93 (*dd*, *J* = 17.5, 11 Hz) indicating vinyl group in addition to proton attached to oxygenated carbon at δ 3.39 (*t*). Moreover, two methyl protons appeared at δ_H 1.12 (6H, *s*). Four protons appeared at δ_H 1.33, 1.52, 1.66 and 1.84 were assigned for two methylenes at C-4 and C-5.

^{13}C NMR (Table 15, Fig. 44) revealed ten carbon signals with two vinylic carbons at δ_C 110.0 (**CH$_2$**) and δ_C 144.0 (**CH**) and three oxygenated carbons signals at δ_C 79.0, 78.0 and 71.0; the first one (δ_C 79.0) was assigned to quaternary carbon at C-3, the other two carbons (δ_C 78.0 and 71.0) were assigned to carbons with two hydroxyl groups at C-6 and C-7. In addition, the DEPT experiment showed two methines, three methylenes, three methyls and two quaternary signals suggested monterpene structure confirmed by Ms data.

All the above data collectively indicated that the compound AL-6 is 6, 7-dihydroxy linalool with IUPAC name: 3, 7-dimethyloct-1-en-3, 6, 7-triol [Williams *et al.*, 1980]. Reviewing the literature indicated that it is the first time to be isolated from *A. lebbeck.*
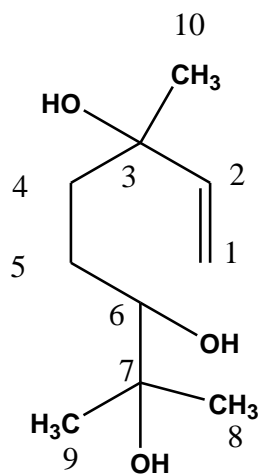
Structure of compound AL-6

Table 15: ^{1}H and ^{13}C NMR Assignment of Compound AL-6 (500 MHz, Acetone)

Position	δ ^{1}H (Multiplicity, J in Hz)	δ ^{13}C
1	4.96(*dd*,10.5, 1.5) 5.23 (*d*,17.5)	110.0
2	5.93 (*dd*, 17.5, 11.5)	144.0
3	-	79.0
4	1.66 (*m*) 1.84 (*m*)	39.8
5	1.33 (*m*) 1.52 (*m*)	24.0
6	3.39 (*t*)	78.0
7	-	71.0
8	1.12 (*s*)	25.2*
9	1.12 (*s*)	25.8*
10	1.21 (*s*)	27.7

* May be interchangeable.

Fig. (41): ^{1}H NMR spectrum of compound AL-6
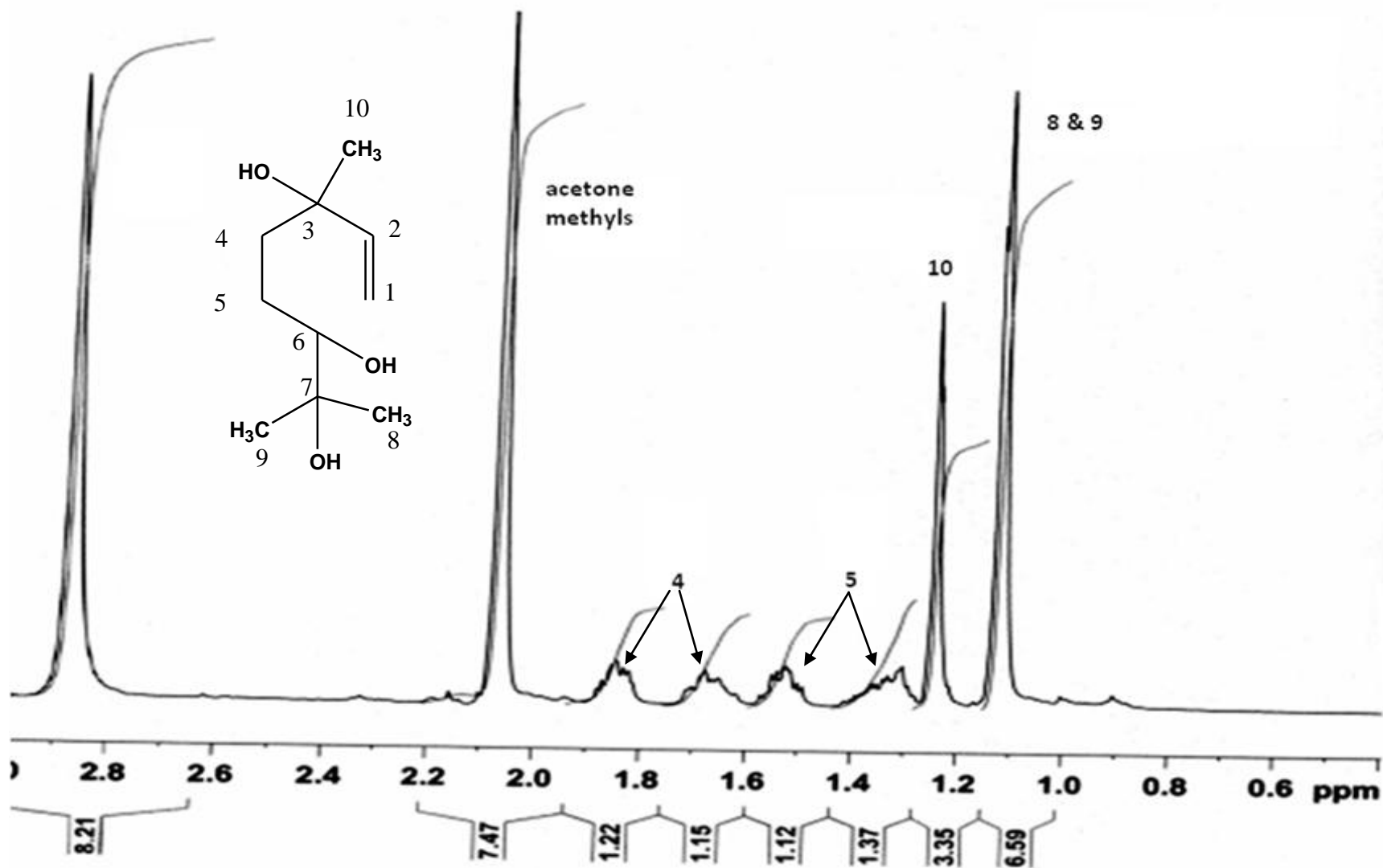
Fig. (42): expanded ^{1}H NMR spectrum (0-2.9 ppm) of compound AL-6
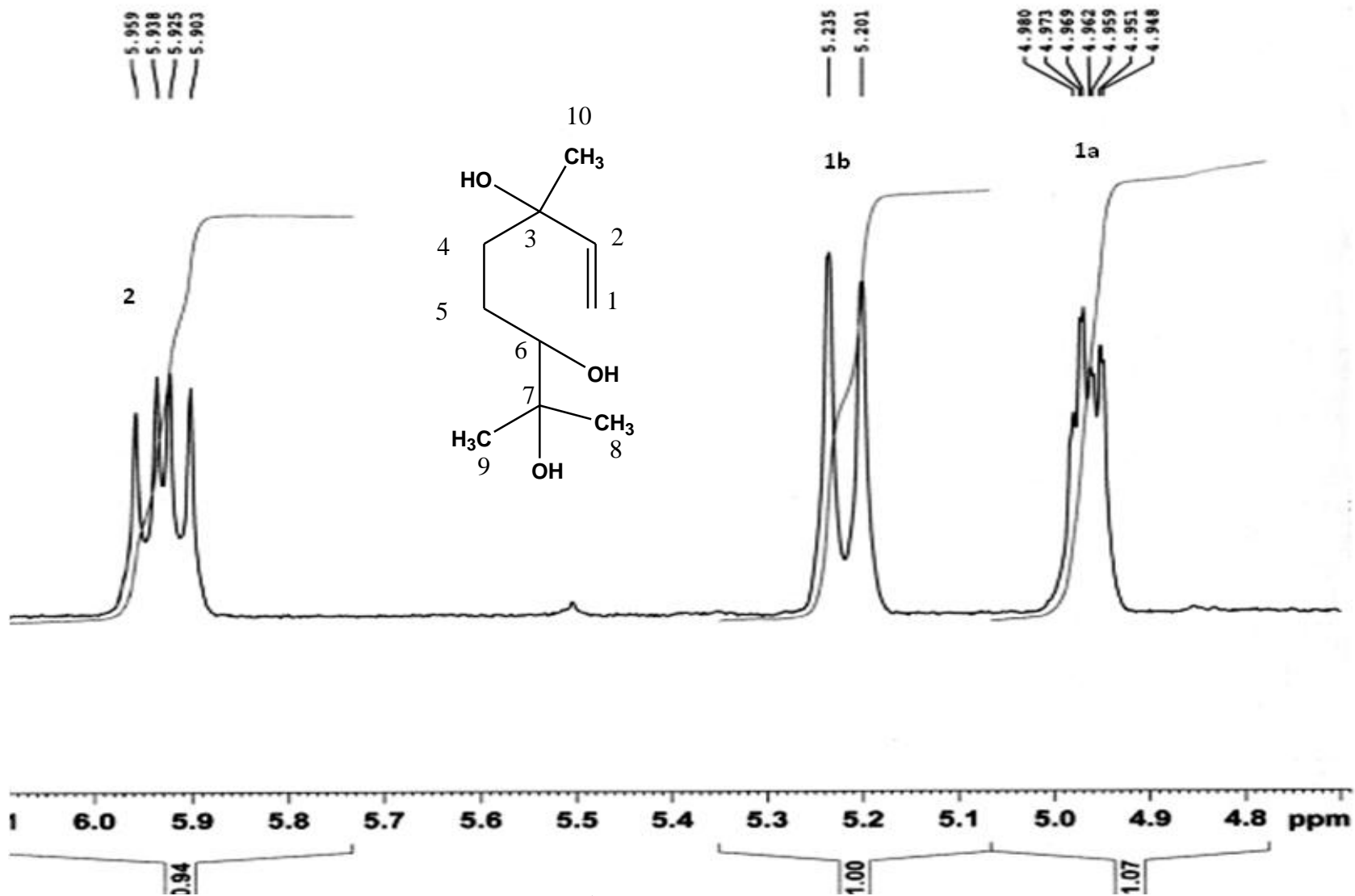
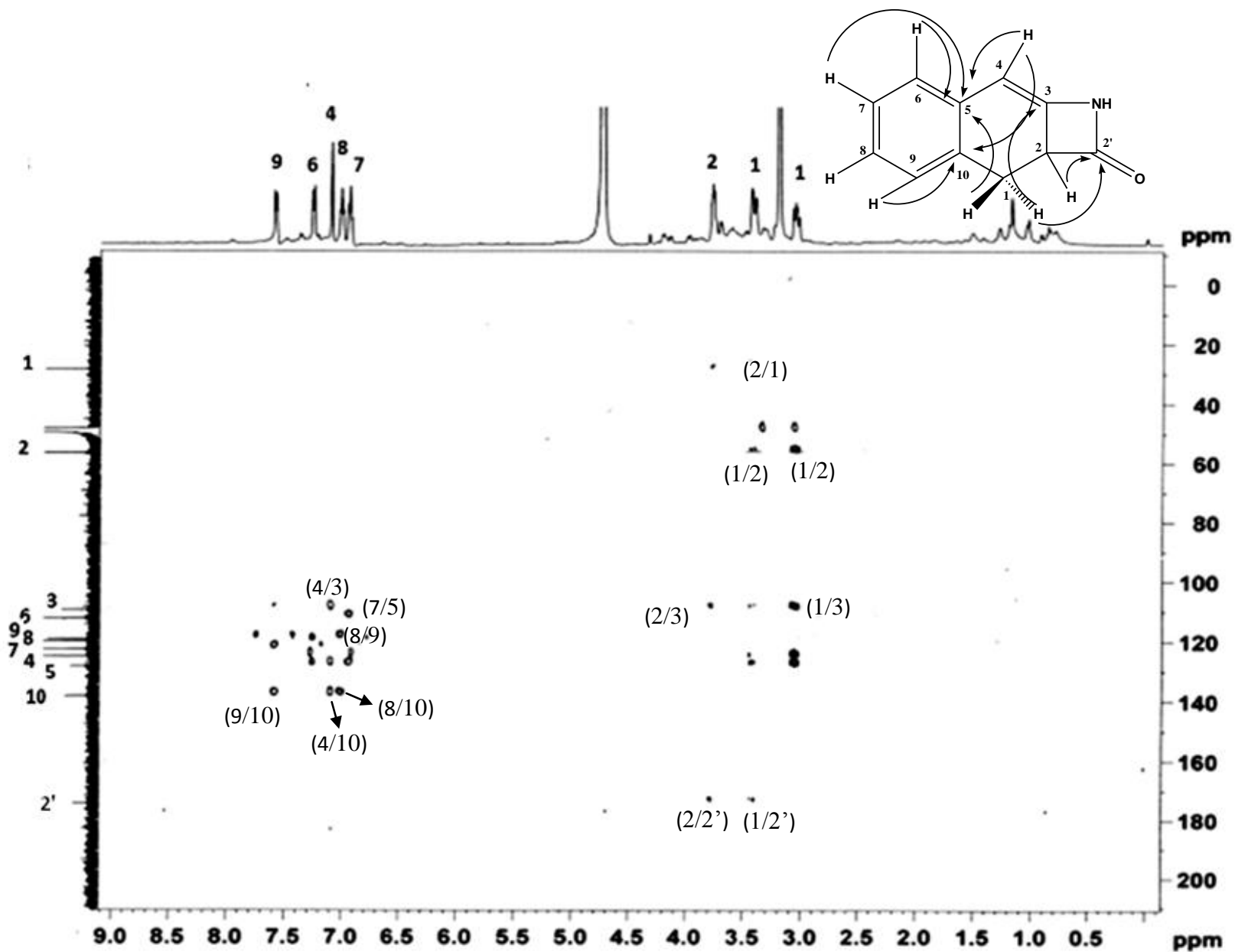Fig. (43): Expanded ^{1}H NMR spectrum (4.8-6.5 ppm) of compound AL-6
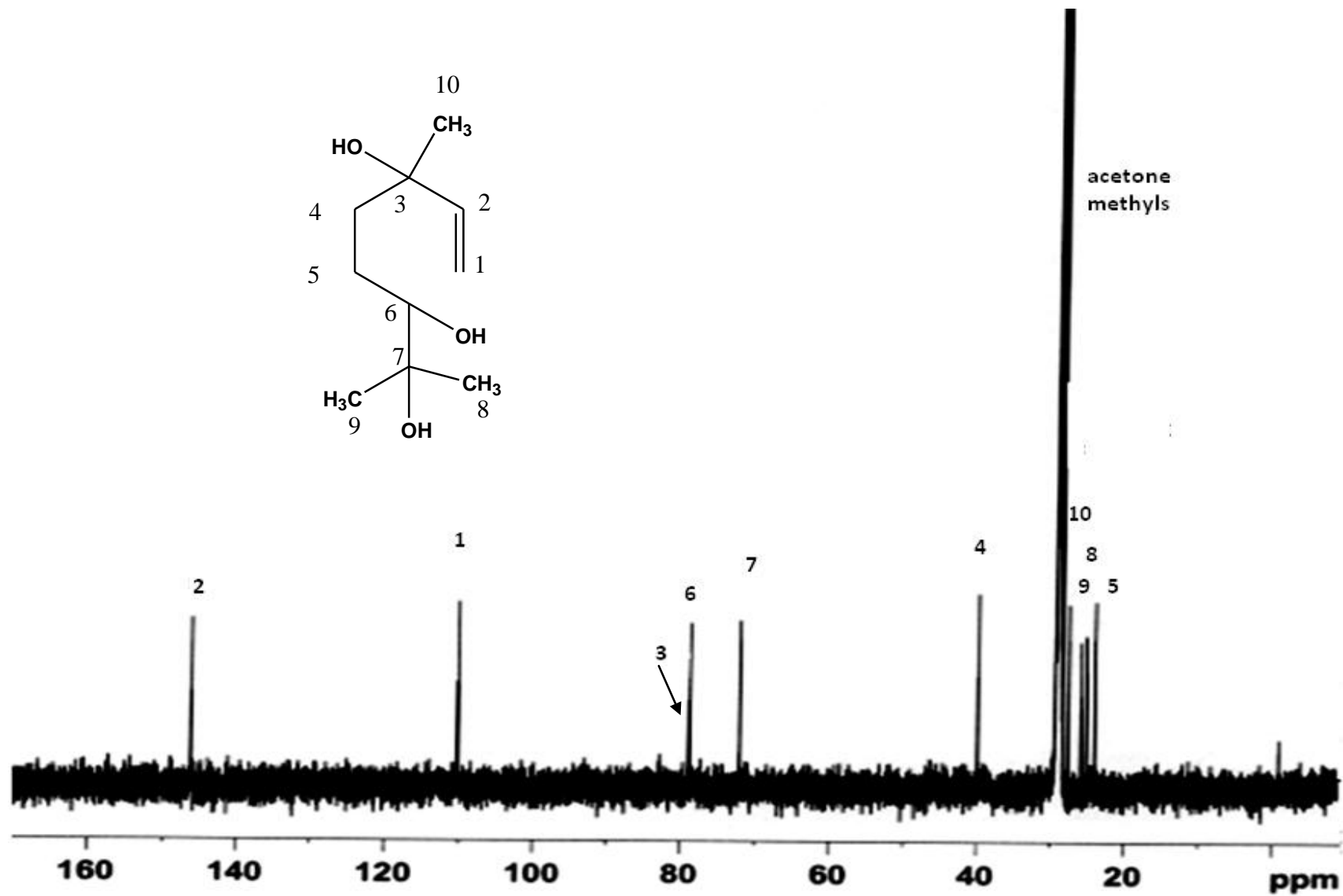
Fig. (44): ^{13}C spectrum of compound AL-6

Characterization and structure elucidation of compound AL-7

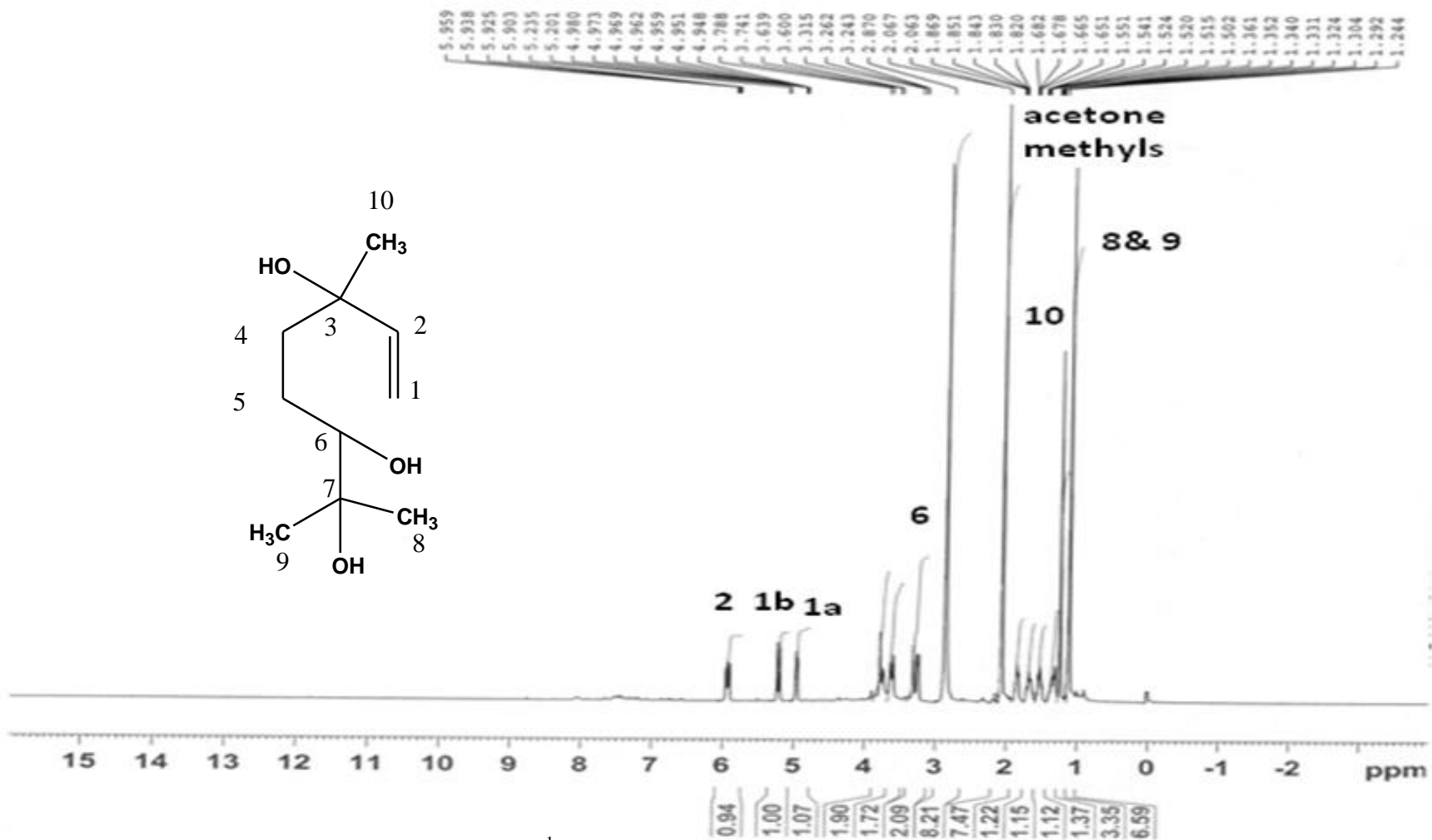Compound AL-7 was isolated as a yellowish amorphous powder from the ethyl acetate insoluble fraction and gave yellow color with NaOH. The UV spectrum showed λ_{max} at 380 and 275 nm suggesting flavonol structure. The IR spectra (KBr) of AL-7 indicated the presence of hydroxyl group –OH (a broad band at 3400 cm^{-1} and C-O stretching band at 1150 cm^{-1}); aromatic =C-H (Peaks at 3050 cm^{-1} and 1460, 1600 cm^{-1}) and conjugated carbonyl group (absorption band at 1660 cm^{-1}). The mass spectrum showed (in negative ion mode) molecular ion peak [M-1]$^-$ at *m/z* 609 corresponding to molecular formulae $C_{27}H_{30}O_{16}$.

^{1}HNMR (Table 16, Fig. 45) indicated the presence of two *meta*-coupled aromatic protons at δ_H 6.21 (*d, J*= 1.2 Hz) and δ_H 6.40 (*d, J*= 1.2 Hz) assigned to H-6 and H-8. The rest of aromatic protons were assigned for ring-B protons as a doublet at δ_H 7.70(*d, J*= 1.5 Hz) assigned to H-2', double-doublet at δ_H 7.6 (*dd, J*= 8.5, 1.5 Hz) assigned to H-6', and an *ortho*-coupled proton at δ_H 6.91 (*d, J*= 8.0 Hz) assigned to H-5'. The coupling between H-2', H-5' and H-6' signals were confirmed by COSY experiment. Two signals of anomeric protons of β–D-glucose and rhamnose appeared at δ_H 5.30 (*J*= 7.0 Hz) and δ_H 4.60 (*J*=1.5 Hz) respectively indicating the presence of glucose and rhamnose. The presence of rhamnose was further confirmed by the Signal of terminal methyl appeared as a doublet at δ 1.20 (*J*= 6.5 Hz).

^{13}C NMR (Table 16, Fig. 46) revealed 27 signals, 15 of which were typical of a flavone skeleton, and the others were assigned to rutinose moiety. Signal for carbonyl group appeared at δ_C 179.4; Five signals at δ_C 145.8, 149.8, 159.3, 166.1 and 162.9 were interpreted for the five oxygenated aromatic carbons C-3', C-4', C-5, C-7 and C-9 respectively. Anomeric carbon of glucose moiety showed signal at δ 102.4 and that of rhamnose at 104.7. A methyl signal at δ_C 17.9 confirmed presence of rhamnose. CH_2 of glucose appeared at δ_C 68.5 indicating 1→6 linkage.

The identification of the AL-7 was confirmed by comparing its TLC and spectral data (NMR) with reference sample [Aderogba, Ogundain and Eloff, 2006]. All of these data were in full agreement with those reported for Rutin [quercetin-3-*O*-rutinoside].

Rutin is the glycoside between the flavonol quercetin and the disaccharide rutinose. It was isolated previously from different natural sources. Rutin, by acting as antioxidants, exhibited beneficial effects such as anti-inflammatory, anti-allergic, anti-viral, as well as anti-cancer activity. It has also been suggested to play a protective role in liver diseases, cataracts, and cardiovascular diseases [Tapas, Sakarkar and Kakde, 2008].
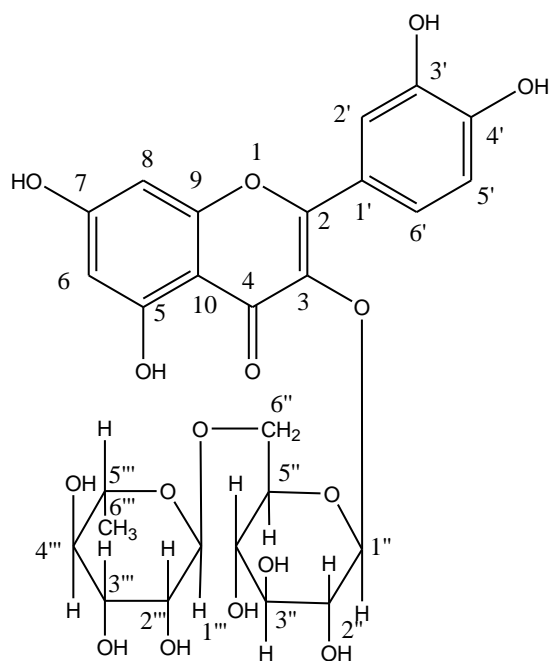
Structure of compound AL-7

Table 16: ^{1}H and ^{13}C NMR Assignment of Compounds AL-7& AL-8 (500 MHz)

Position	Compound AL-7 (CD$_3$OD)		Compound AL-8 (DMSO-d$_6$)	
	δ ^{1}H (Multiplicity, J in Hz)	δ ^{13}C	δ ^{1}H (Multiplicity, J in Hz)	δ ^{13}C
2	-	158.5	-	156.4
3	-	135.6	-	133.2
4	-	179.4	-	177.3
5	-	159.2	-	161.1
6	6.21 (*d*,1.2)	99.9	6.22 (*d*, 1.5)	98.7
7	-	166.1	-	164.2

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|				
8	6.40 (*d*,1.2)	94.8	6.42 (*d*, 1.5)	93.7
9	-	162.9	-	156.8
10	-	105.6	-	103.9
1'	-	123.1	-	120.8
2'	7.70 (*d*, 1.5)	116.0	8.00 (*d*,8.5)	130.9
3'	-	145.8	6.90 (*d*,8.5)	115.1
4'	-	149.8	-	159.8
5'	6.9 (*d*,8.5)	117.6	6.90 (*d*,8.5)	115.1
6'	7.60 (*dd*,8.5, 1.5)	123.5	8.00 (*d*,8.5)	130.9
1"	5.30 (*d*,7.0)	102.4	5.30 (*d*,7)	101.3
2"	3.90 (*m*)*	73.9	3.9 (*m*)*	74.1
3"	3.49 (*m*) *	77.1	3.49 (m) *	76.3
4"	3.40 (*m*) *	69.7	3.40 (m) *	69. 9
5"	-	75.7	-	75.7
6"	3.50 (*m*, 2H)*	68.5	3.50 (*m*, 2H)*	66.8
1'''	4.60 (*d*, 1.5)	104.7	4.39 (*d*,1.5)	100.7
2'''	3.70 (*m*)*	71.4	3.7 (*m*)*	70.3
3'''	3.48 (*m*)*	72.2	3.48 (*m*)*	70.5
4'''	3.40 (*m*)*	73.9*	3.4 (*m*)*	71.8
5'''	3.49 (m)*	78.1	3.49 (*m*)*	68.2
6'''	1.20 (*d*, 3H, 6.5)	17.9	1.10 (*d*,3H, 6.5)	17.9
2	-	158.5	-	156.4

* Overlapped signal
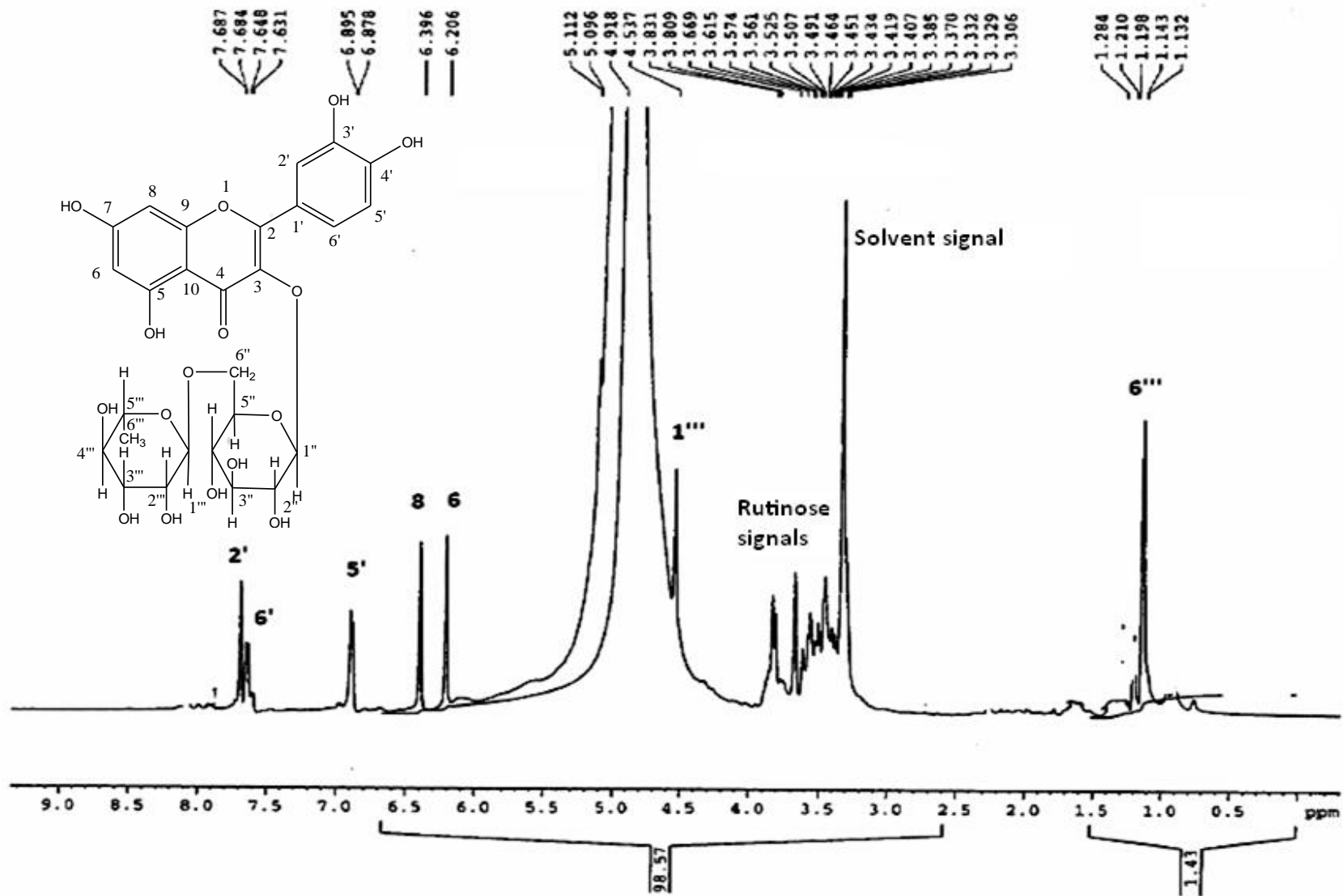
Fig. (45): ^{1}H NMR spectrum of compound AL-7
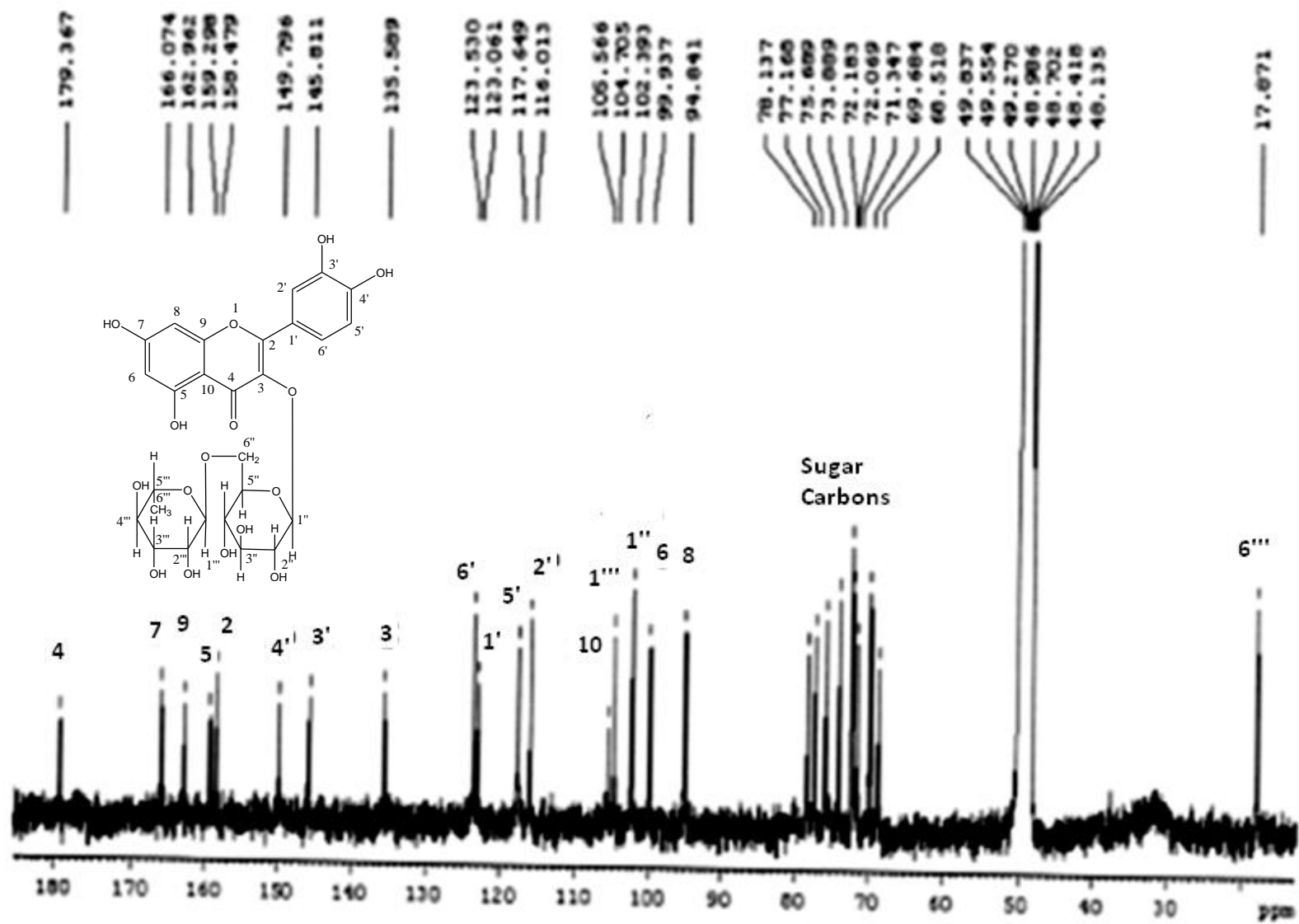
Fig. (46): ^{13}C NMR spectrum of compound AL-7

Characterization and structure elucidation of compound AL-8

Compound AL-8 was isolated as a yellowish amorphous powder from the ethyl acetate insoluble fraction. It gave yellow color with NaOH and AlCl$_3$ and UV spectra showed λ_{max}: 270 and 355 nm (MeOH) suggesting flavonol structure. The ESI-Ion Trap showed molecular ion peak at m/z 593 [M-H]$^+$in addition to signals at m/z 578 and 560 due to loss of a methyl group and methyl followed by water respectively. The IR spectra indicated hydroxyl groups (absorption band at 3380 cm^{-1}); conjugated carbonyl group (band at 1690 cm^{-1}) and aromatic rings (bands1605 and 1501 cm^{-1}).

^{1}HNMR (Table 16, Fig. 47) indicated the presence of six aromatic protons at δ_H 6.22 (H-6, J= 1.5 Hz), 6.42 (H-8, J= 1.5 Hz), 6.90 (2H, d, J= 8.5 Hz, H-3', 5'), 8.00 (2H, d, J= 8.5 Hz, H-2', 6'). The presence of an AA' BB' coupling system indicated flavonoidal structure with substituted B-ring on 4'. Two signals of anomeric protons of β–D-glucose and rhamnose appeared at δ_H 5.30 (J= 7.0 Hz) and δ_H 4.39 (J=1.5 Hz) respectively indicating the presence of glucose and rhamnose. The presence of rhamnose was further confirmed by the Signal of terminal methyl of appeared as a doublet at δ 1.10 (J= 6.5 Hz).

^{13}C NMR (Table 16, Fig. 48) revealed 27 signals, 15 of which were typical of a flavonol skeleton, and the other 12 carbons were assigned to sugar moiety. Signal for carbonyl group appeared at δ 177.3. DEPT 135° experiment of AL-8 shown in (Fig. 49) displayed eighteen methine and/or methyl groups in addition to one methylene group assigned for C-6" of glucose moiety. HMBC correlation of H-1'' to C-3 at 133.2 and the downfield shifting of C-2 and C-4 indicated that the position of attachment of glucose was C-3 [Song, *et al*., 2007]. In addition, anomeric carbons of glucose and rhamnose moieties were revealed at δ 101.3 and 100.7 respectively. The downfield shift of CH$_2$ carbon at δ 66.8 indicating that the inter glycosidic linkage was determined to be 1→6. The correlations between each proton signal and the directly attached carbon was achieved and confirmed by HMQC experiment (Fig. 50).

^{1}H and ^{13}C NMR data were consistent with those reported for kaempferol-3-*O*-rutinoside [Song, *et al*., 2007].

kaempferol-3-*O*-rutinoside is flavonol glycoside which was previously isolated from different natural sources. It is a potent inhibitor of alpha-glucosidase *in vitro* with over eight times more activity than the reference antidiabetic drug, acarbose [Habtemariam, 2011]. In addition, it has a potent antioxidant activity [Badaturugea, Habtemariam and Thomasa, 2011].
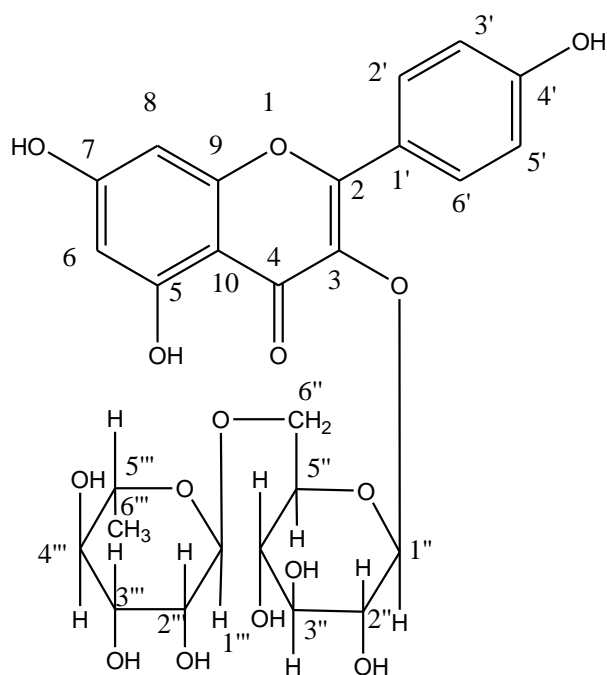
Structure of compound AL-8
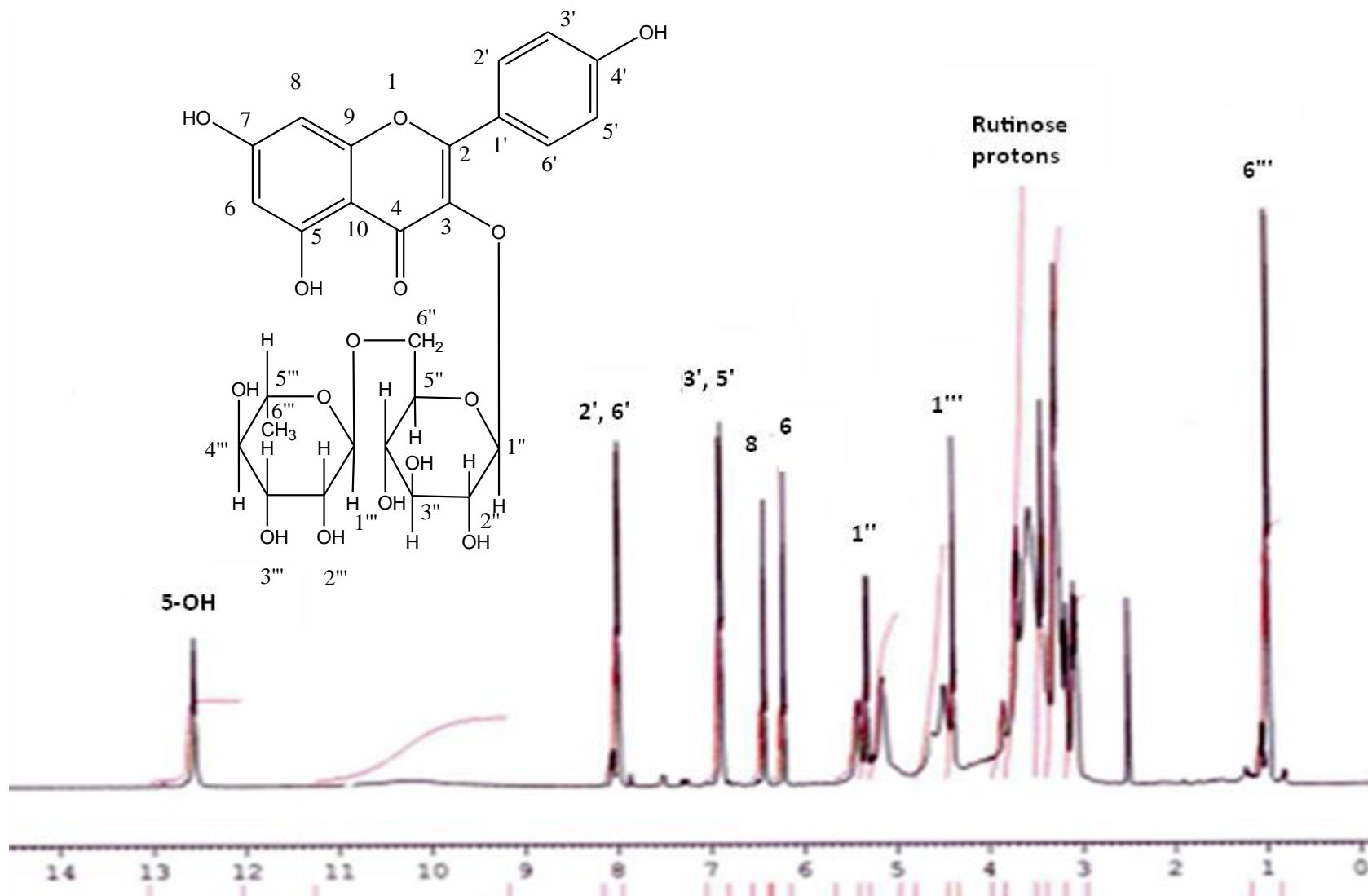
Fig. (47): ^{1}H NMR spectrum in DMSO-d_6 of compound AL-8
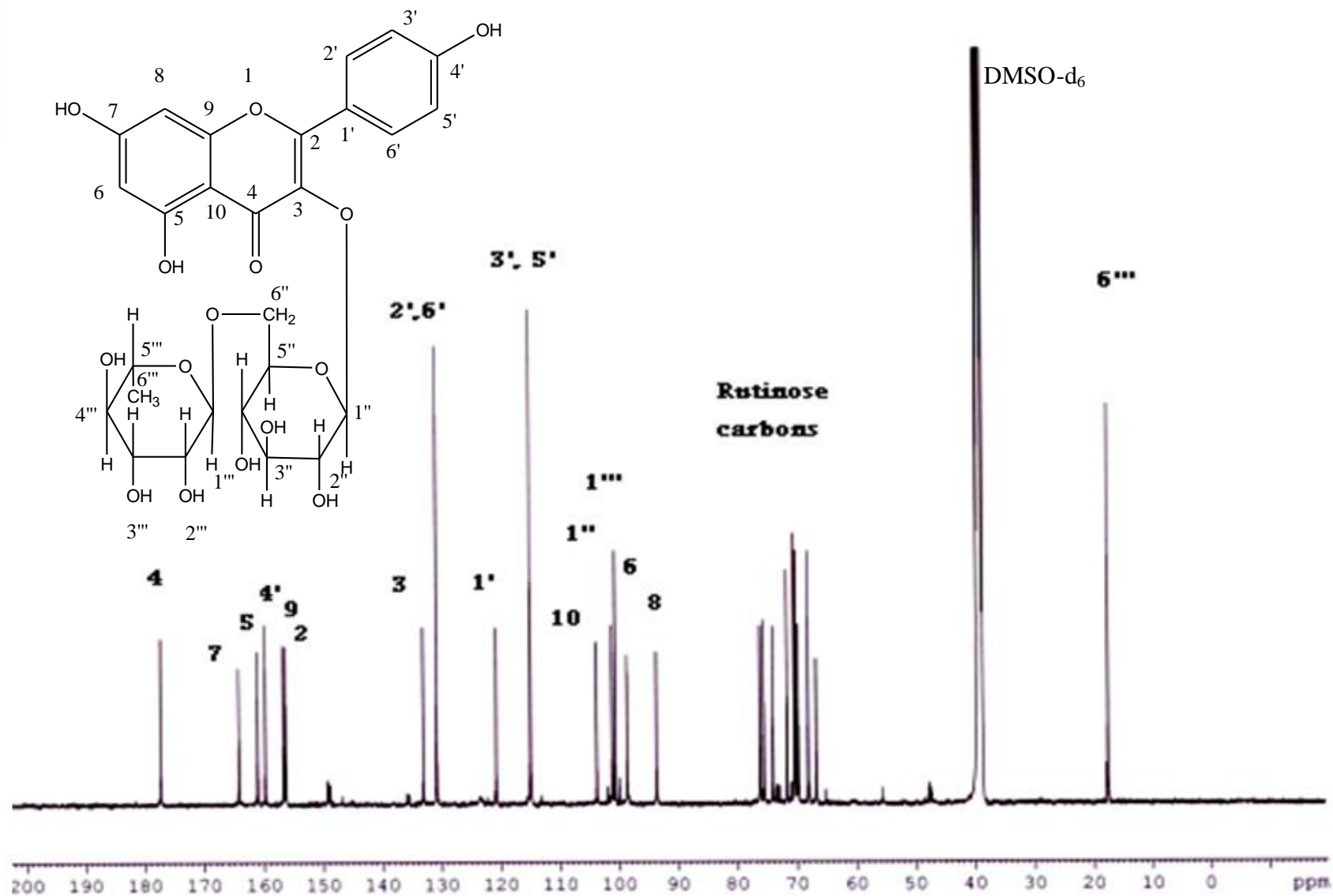
Fig. (48): ^{13}C NMR spectrum of compound AL-8

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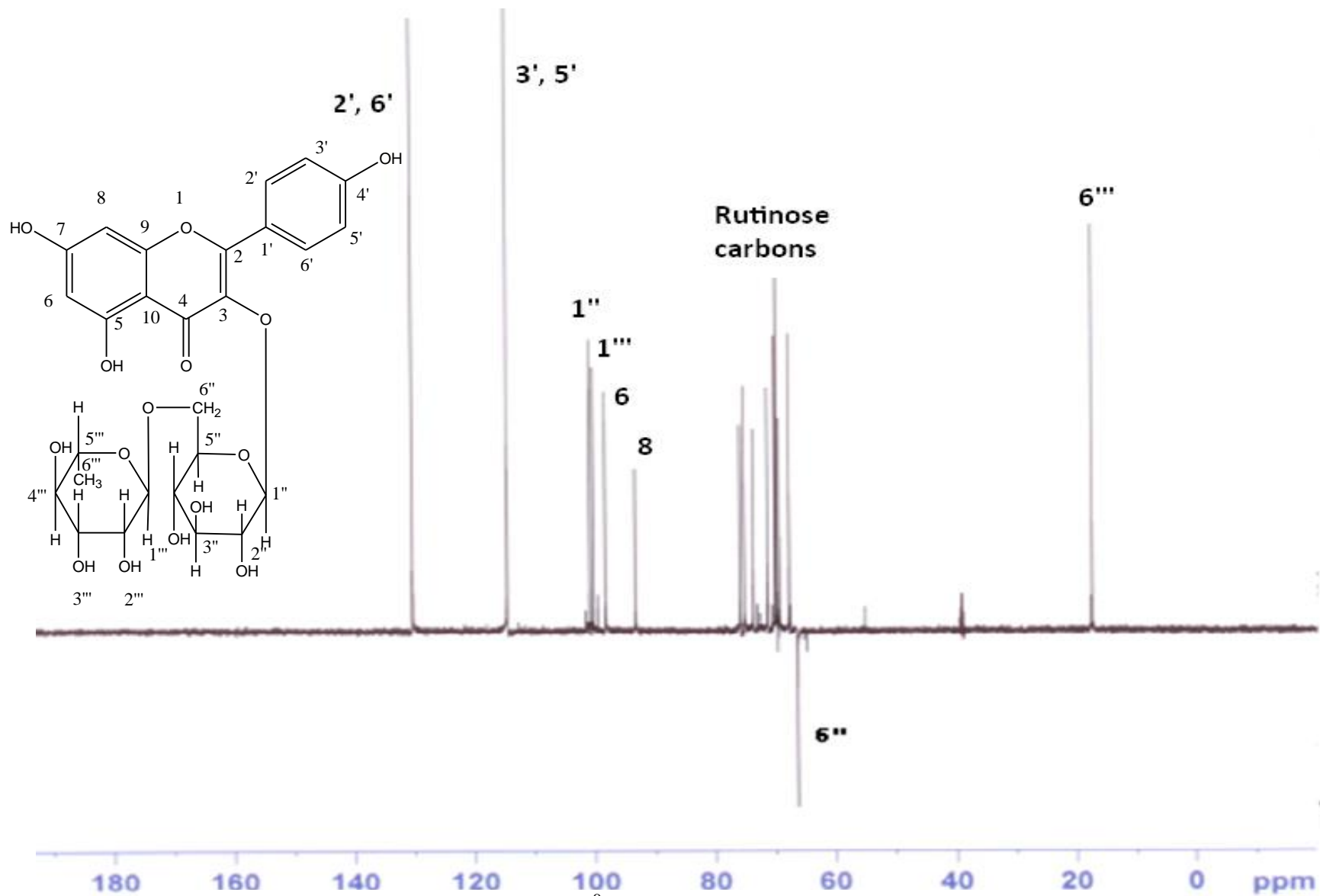
Fig. (49): DEPT 135° spectrum of compound AL-8
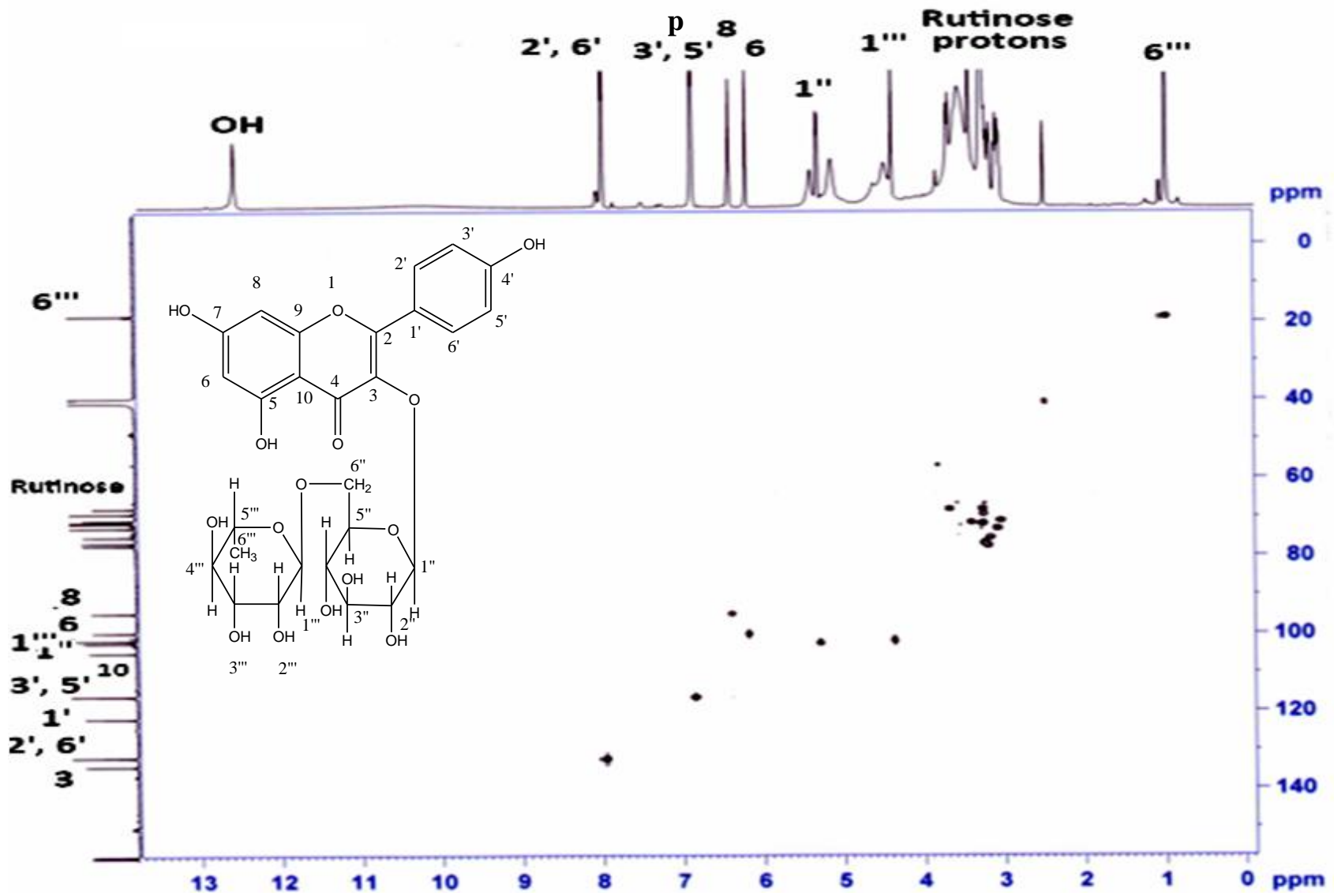
Fig. (50): HMQC spectrum of compound AL-8
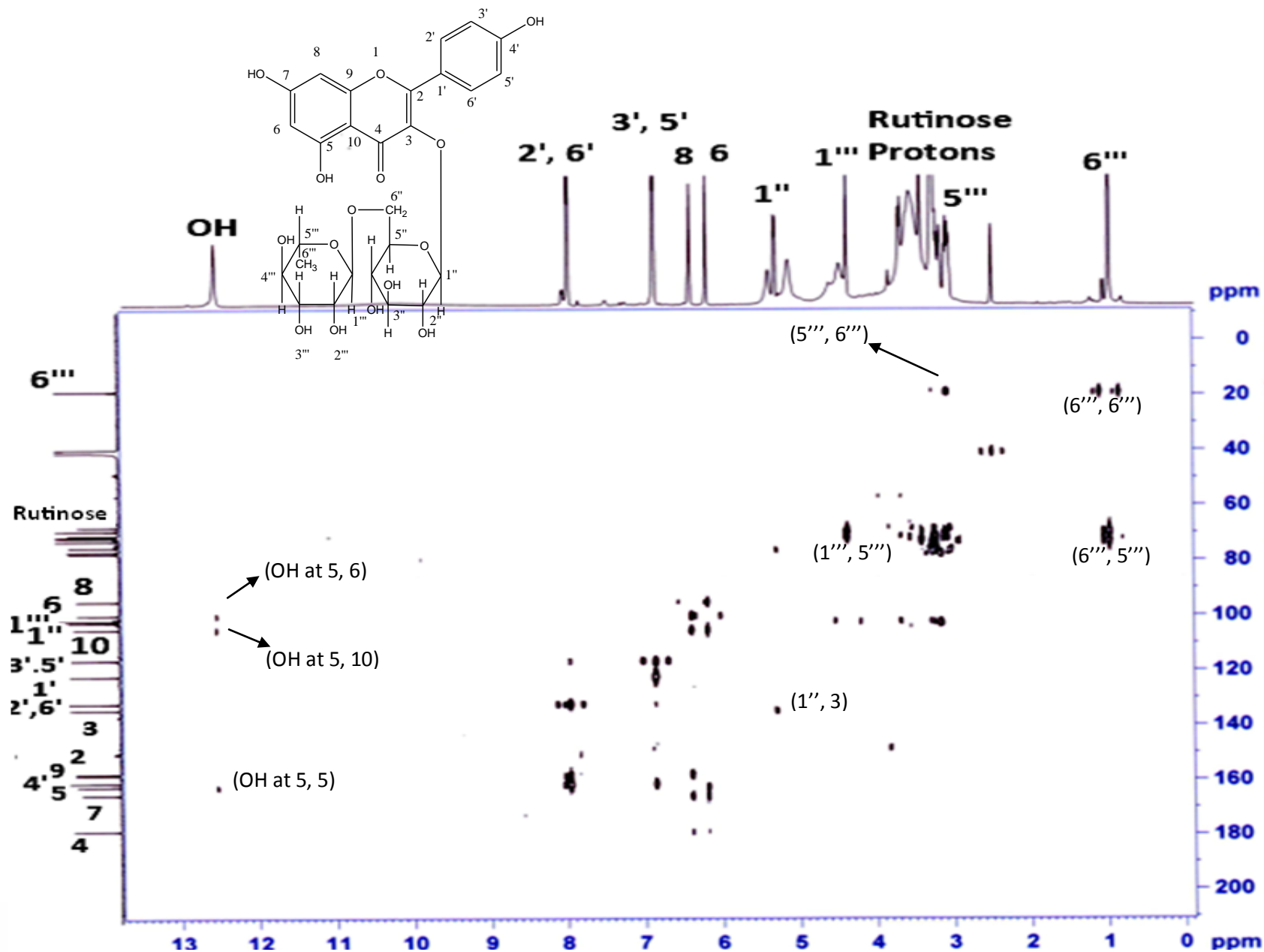
Fig. (51): HMBC spectrum of compound AL-8

Biological investigation

Five different fractions from successive extraction of *Albizia lebbeck* flowers; *n*-hexane, dichloromethane, ethyl acetate, *n*-butanol as well as the 70% total alcohol extract were subjected to biological screening tests for the following activities: antimicrobial, antipyretic, analgesic, estrogenic and anti-inflammatory and revealed the following results:

1. Antimicrobial activity:

Antimicrobial activity against two gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) was shown by the *n*-hexane fraction at a concentration of 1mg/ml. Meanwhile, this fraction was inactive against gram–negative bacteria: *Escherichia coli*, *Psuedomonas aeruginosa* and *Mycobacterium smegmatis*.

The total alcohol extract as well as dichloromethane, aqueous fraction, *n*-butanol and ethyl acetate extracts did not exhibit any activity against the tested gram-positive and gram–negative bacteria.

In addition, five isolated compounds; β- amyrin, 11α,12α-oxidotaraxerol, 1-*O*-β-D-glucopyranosyl[(2S,3S,4R,8E)-2-[(2'R)-hydroxyhexadecanoylamino]-8-tetra-cosene-1, 3, 4-triol], [(2S, 3S, 4R, 8E)-2-[(2'R)-hydroxyhexadecanoylamino]-8-tetra-cosene-1, 3, 4-triol and 5-(3, 3-Dimethyl- oxiranyl)-3-methyl-pent-1-en-3-ol were screened for antimicrobial and all of them were inactive.

All determinations were performed in triplicate and two growth controls consisting of MHB medium with 0.5% Tween-80 were included. Amphotericine B and gentamycin (30μg/disc) served as positive control [Rasool, *et al*., 2008] & [National Committee for Clinical Laboratory Standards, 1997].

Table 17: Antimicrobial Activity of *A. lebbeck* inflorescences fractions using American type of Culture Collection (ATCC) at a concentration of (1mg/ml)

Tested organism	*n*- Hexane	CH_2Cl_2	Ethyl acetate	*n*- Butanol	Aqueous	Total alcohol
Bacillus subtilis	+	-	-	-	-	-
Staphylococcus aureus	+	-	-	-	-	-
Escherichia coli	-	-	-	-	-	-
Psuedomonas aeruginosa	-	-	-	-	-	-
Mycobacterium smegmatis	-	-	-	-	-	-
Candida albicans	-	-	-	-	-	-

2. Antipyretic activity:

The antipyretic study was conducted following a published method [Yongna, *et al*., 2005] at the dose of 1g/kg, and revealed that all fractions were able to decrease basal body temperature of mice. It was found that dichloromethane and ethyl acetate fractions have significant decrease in fever, which dropped the temperature by 8 & 5 ^{o}C, respectively.

Moreover, total alcohol, *n*-butanol, aqueous and *n*-hexane extracts showed less activity than those mentioned above by dropping the temperature by 2.3, 4.7, 2.7 and 1.7 ^{o}C respectively as shown in table 18.

A dose of 1g/kg of each extract, except *n*-butanol extract which was administered in dose of 0.25 g/kg, was administered intraperitoneally, and the temperature measured at 15, 30, 60, 120 and 180 minutes after injection. Water ad libitum was used as a negative control and aspirin (200 mg/kg) was used as a positive control [Yongna, *et al*., 2005].

Table 18: Results of antipyretic study

A. *lebbeck* Extract	Dropped by (°C)
n- butanol (dose 0.25 g/kg)	2.3±0.1
Dichloromethane	8.0±0.2
Ethyl acetate	5±0.4
Total alcohol	4.7±0.1
Aqueous	2.7±0.4
n-hexane	1.7±0.1

3. Analgesic activity:

The effect of the different extracts of *A. Lebbeck* on pain sensation was tested using hot plate method [Leighton et al., 1987]. Administration of the different extracts at doses of 1g/kg I.p induced variable increases in the pain threshold in the hot plate test. The percentages in increase of pain threshold are shown in table 19.

As shown in the table. 19, a maximum of 25.7% increase was shown by the dichloromethane extract. The aqueous extract was inactive. Maximum increases in the pain threshold were observed 90 minutes after administration of each extract.

A reference analgesic drug, aspirin (200 mg/kg) were administered as a positive control [El-Tahir, 2007].

Table 19: Results of analgesic activity screening

A. *lebbeck* Extract	% increase in pain threshold
n- butanol (dose 0.25 g/kg)	14.2±3.4
Dichloromethane	25.7±1.3
Ethyl acetate	14.2±1.0
Total alcohol	15.1±0.5
Aqueous	Not active
n-hexane	8.8±0.4

4. Estrogenic activity:

Estrogens are steroid hormones with important functions in the regulation of specific sexual processes in the female. At a dose of 500 mg/kg I.P. to immature mice, only total alcohol extract caused significant increase in the ratio of weight of two uterine horns to the total body weight by 109.14%. This very high percentage indicated potent estrogenic activity of the total alcohol extract that can be point of further researches. The high estrogenic activity may be due to the pentacyclic and steroidal compounds in the plant. Whereas, the ethyl acetate extract exerted significant depression of uterine weight/ body weight ratio. Table. 20 shows the effects of different extracts of *A. lebbeck* on the uteri of immature rats.

Normal saline was used as a negative control and 17-ß-estradiol (0.32 μg/animal/day) was used as a positive control [Kumar and Pakrasi, 1995].

Table 20: Results of estrogenic activity screening

A. lebbeck Extract	% change in uterine weight/ total body ratio
n- butanol	Very toxic (Not tested)
Dichloromethane	7.8% decrease
Ethyl acetate (200 mg/kg I.P)	25.2±2.9% decrease
Total alcohol	109.141±1.1% increase
Aqueous	4.9 % increase
n-hexane	4.93% decrease

5. Anti-inflammatory activity:

Administration of the different *A. lebbeck* inflorescences extracts to rats in doses of 1 g/kg I.P (except the *n*-butanol extract 0.25 g/kg) with experimental carrageenan-induced inflammation suppressed inflammation to various degrees. The best anti-inflammatory activity was observed 2 hours after administration of carrageenan. All extracts were administered 1 hour before carrageenan. The best extract which showed anti-inflammatory activity was the dichloromethane extract 71.6% followed by the ethyl acetate extract 60.3%. These extracts contain nitrogenous compounds causing antiplatelets aggregation activity which prevent the inflammatory reactions from propagation. The results are shown in table. 21.

Table 21: Results of anti-inflammatory activity screening

A. lebbeck Extract	% suppression of inflammation 2 hours after carrageenan administration
n- butanol (200 mg/kg I.P)	Zero
Dichloromethane	71.6±4.9%
Ethyl acetate	60.3±4.2%
Total alcohol	33.9±3.7%
Aqueous	37.7±5.9%
n-hexane	50.9±1.8%

N.B. Maximum paw edema 2 hours after carrageenan intra-plantar injection was 0.53±0.05 ml.

6. Anti-platelets aggregation activity of Albactam:

Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A series of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process [El Tahir, 2007].

One of vascular changes occurs during the inflammatory response is the coagulation in which platelets aggregation has primary role. So, prevention of platelets aggregation helps in reducing inflammation. This encouraged us to test albactam for antiplatelets aggregation activity as some extracts of *A. lebbeck* flowers showed anti-inflammatory activity.

Albactam showed anti-aggregatory activity against adenosine diphosphate and arachidonic acid-induced guinea-pigs' platelets aggregation *in vitro* at doses 208 µg/ml and 172 µg/ml respectively.

The anti-aggregatory activity was proven by the following methodology:

Platelet-rich plasma (PRP) was obtained from guinea-pigs and prepared for aggregation studies [El Tahir and Williams, 1980]. Albino guinea-pigs (350-450 g) were anaesthetized with diethyl ether. Nine mls blood were collected using cardiac puncture into 12-ml plastic centrifuge tubes each containing 1 ml of 3.6-3.8 % aqueous sodium tricitrate solution. Blood was mixed gently and centrifuged at 1000 rpm for 10 min. The platelet-rich plasma (PRP) was aspirated and distributed in 1 ml plastic cuvettes. An aliquot of the PRP was centrifuged at 14330 rpm for 20 min to precipitate all platelets to get platelet-poor plasma (PPP).

Each cuvette containing PRP was inserted into a chronolog aggregometer that was calibrated such that light transmission through PRP was zero and through PPP was 100%. Each cuvette was heated (37^0C) with stirring (1000 rpm) for 2min. Then different doses of albactam in volumes of (5-20µl) were added to the PRP and their ability to aggregate the platelets was assessed. Thereafter the ability of albactam to inhibit chemically-induced aggregation was examined. For this purpose, aggregation was induced by adenosine diphosphate (ADP) (10µM) and arachidonic acid (0.5-1 µM) and each one was added to the aliquots of PRP. The concentration of the agonist that produced just irreversible aggregation was selected. PRP was then treated with various concentrations of albactam for 2-5 min. Then the aggregating agent was added and allowed to react with the platelets for 4-5min. The percentage change induced by the treatment on the agonist -induced aggregation was evaluated [El Tahir, 2007].

The different extracts of *A. lebbeck* flowers showed important biological activities such as antibacterial, analgesic, antipyretic, anti-inflammatory and estrogenic activities. These biological activities are due to different classes of secondary metabolites present in the plant such as steroidal and pentacyclic triterpenes in addition to some nitrogenous compounds. These results recommend further phytochemical & biological investigations of this plant.

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تم عمل مسح بيولوجي لكل الخلاصات المفصولة كما أن كمية مركب الألباكتام المفصولة كانت كافية بدرجة سمحت بإجراء بعض فحوص النشاط الحيوي عليه وتبين أنه له فعالية عالية ضد تجمع الصفائح الدموية للخنزير الغيني الناشيء عن ثنائي الفوسفات أوحمض الأراشيدونيك. أما باقي المركبات النقية المفصولة فقد تعذر إجراء فحوص النشاط الحيوي عليها لقلة كمياتها.

قسمت هذه الدراسة إلى خمسة أجزاء:

1 ـ المقدمة تتضمن موجزا عن التصنيف النباتي للنبات موضوع الدراسة، الاستعمالات الشعبية للنبات، والنشاطات الحيوية. علاوة على ذلك، تشتمل المقدمة على المركبات المفصولة من النبات وكذلك المركبات ذات التأثير الحيوي.

2 ـ تعريف بالأجهزة والأدوات والتقنيات المستخدمة في العمل البحثي وشرح مفصل لطرق التقييم الحيوي، وكيفية فصل المركبات والتعرف عليها. بالإضافة إلى الفحوص العقاقيرية المبدئية للكشف عن بعض المجموعات الكيميائية المعروفة مثل: القلويدات، الفلافونيدات، التربينات، والصابونين وغيرها.

3 ـ مناقشة نتائج الفصل وكيفية التعرف على المركبات المفصولة ومعرفة صيغتها الكيميائية باستخدام طرق التحاليل المختلفة مثل الأشعة فوق البنفسجية، والأشعة تحت الحمراء، و الرنين النووي المغناطيسي احادي وثنائي القطب، ومطياف الكتلة واستعراض للنتائج في جزء النتائج والمناقشة.

4 ـ استعراض لنتائج الدراسة عن النشاطات الحيوية التالية: مضاد للميكروبات، خافض للحرارة، مسكن للألم، مضادة للإستروجين، ومضاد للإلتهاب. ، علاوة على طريقة ونتيجة دراسة النشاط الحيوي للمركب الجديد ألباكتام وذلك في جزء الفحوص الحيوية.

5 ـ وقد أختتمت الرسالة بالمراجع العلمية التي تم الاستعانة بها في هذه الرسالة وعددها ثمانية وسبعون مرجعاً.

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ملخص الرسالة

ينتمي نبات الألبيزيا لبخ إلى الفصيلة البقولية، وهي ثالث أكبر فصيلة للنباتات المزهرة حيث تضم أكثر من ثمانية عشر ألف نوع. شجرة اللبخ هي واحدة من أهم أنواع الأشجار التي تم استيرادها من الهند منذ سنوات عديدة وتمكنت من التأقلم مع الأوضاع البيئية والمناخية الصعبة للمنطقة الوسطى في المملكة. يستخدم النبات في صناعة الأثاث والأرضيات كما تستخدم في العديد من الاستخدامات الزراعية. كما يفرز لحاء الشجرة صمغا ذا لون بني محمر يستخدم كبديل للصمغ العربي. نبات الألبيزيا لبخ له العديد من الأسماء المرادفة مثل أكاشيا، كوكو، شاك شاك، الحبة المصفرة و لسان المرأة وذقن الباشا.

باستعراض الدراسات العقاقيرية الحالية والسابقة تبين احتواء عدة أنواع من جنس الألبيزيا على مجموعات مختلفة من مركبات الأيض الثانوية مثل الصابونين والتربينات والقلويدات والفلافونيدات بالإضافة إلى أن بعض النباتات المنتمية لجنس الألبيزيا لها فوائد طبية، كما تبين فصل بعض المركبات الكيميائية ذات الفعالية الحيوية مثل مواد صابونية ثلاثية التربين مثل مركبات جوليبيروسيد J 29 ، J 30 ، J31 ومثل مركبات القلويدات كبيرة الحلقات مثل بودمونشيامين A و B و C وأيضا الجلوكوسيدات الفلافونيدية مثل كورسيتين وايزوكورسيتين. أظهرت هذه المركبات عدة أنواع من التأثيرات البيولوجية مثل الأثر المضاد للأورام ومنع تجمع الصفائح الدموية و كمضادات للبكتيريا.

كما أثبتت الدراسات الحالية والسابقة عن أهمية نبات الألبيزيا لبخ النامي في جميع أنحاء العالم أن له العديد من التأثيرات الحيوية الهامة إلا أنه لاتوجد أي دراسة على النوع النامي في المملكة العربية السعودية و لذلك تعتبر هذه الدراسة الأولى من نوعها في المملكة. وبإجراء الفحوص الحيوية المبدئية على مستخلصات نبات الألبيزيا لبخ النامي في المملكة تبين احتوائه على مركبات مضادة للبكتيريا، خافضة للحرارة، مسكنة للألم، ومضادة للالتهاب، ومركبات ذات تأثير مشابه للهرمونات الأنثوية (الأستروجين).

وبواسطة الدراسة العقاقيرية على نبات الألبيزيا لبخ النامي في المملكة تم فصل والتعرف على مركب جديد عبارة عن مشتق من نواة البيتا لاكتام الكيميائية وتم تسميته "ألباكتام" بالإضافة إلى سبعة مركبات معروفة هم البيتا-أميرين، 11 ألفا، 12 ألفا – أوكسيدوتراكسيرول، وسيراميد والجلوكسيد الخاص به بالإضافة إلى أحد مشتقات أحادي تربين وفلافونيد الروتين والكامبفرول -3- روتينوز.

والجدير بالذكر أن السيراميد والجلوكسيد الخاص به و11 ألفا، 12 ألفا – أوكسيدوتراكسيرول إلى جانب أحادي التيربين تم فصلهم لأول مرة من هذا النوع من أنواع اللبخ في هذه الدراسة.