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Ministry of Higher Education and Scientific Research
University of Mustansiriyah
College of Pharmacy

SCREENING FOR ANTIBACTERIAL COMPOUNDS FROM IRAQI MEDICINAL PLANT OF THE FAMILY PINACEAE

A Thesis submitted to the Pharmacognosy and medicinal plants department and to the College of Pharmacy in Al-Mustansiriyah University in Partial Fulfillment of the Requirements for the Degree of Master of Science in pharmacy (Pharmacognosy and medicinal plants)

by
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2013 **1434**

بسم الله الرحمن الرحيم

﴿ وَقُلْ رَبِّ أَدْخِلْنِي مُدْخَلَ
صِدْقٍ وَأَخْرِجْنِي مُخْرَجَ
صِدْقٍ وَاجْعَلْ لِي مِنْ لَدُنْكَ
سُلْطَاناً نَصِيراً ﴾

صدق الله العظيم
سورة الأسراء اية (٨٠)

DEDICATION

TO …

ALL MY FAMILY

MY DEAR PARENTS

AND MY LOVED HUSBAND AND

DAUGTERS

WHO OFFERED ME LOVE & CARE AS

WELL AS THE INSPIRATION NECESSARY

TO SHAPE MY ACADEMIC LIFE THE WAY

IT IS NOW

RASHA

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Also, I would like to thank Mrs. Fitua Aziz, Mrs. Samara, Mrs. Buthaina and all my friends, colleagues and all the people who assisted me and who I forget to mention for their help, encouragement during hard times, understanding and for the useful time that we spent in the discussions and sharing ideas.

I am grateful to the Head and the staff of the Pharmacy College/ University of Al-Mustansyria for their support.

Finally, I may not forget the patience and the help of my family to succeed in this work; I would like to thank them for their confidence in me over the years. I will not forget their kindness, supports, patience and encouragement to reach this stage of education.

I pray to God to enable me showing them my graceful gratitude.

Rasha Eldalawy

Certificate

We certify that this thesis **(Screening for antibacterial compounds from Iraqi medicinal plant of the family Pinaceae)** was prepared under our supervision at the department of Pharmocognacy and medicinal plants/ College of Pharmacy/ Al-Mustansiriyah University as a partial fulfillment of the requirements for the degree of M.Sc. in Pharmacy (Pharmacognosy and Medicinal plants)

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Abstract

Pine tree exudates are regarded as a remedy of wounds in traditional medicine. This plant contains terpenes like abietic acid and isopimaric acid; also it contains lignans like nortrachelogenin. All these active constituent posses a variety of medical importance such as antibacterial activity and this is important because some of important bacteria have resistant to some antibiotic and this resistant extend to super resistant strain to some antibiotics. The development of bacterial resistant strains is resulting in currently used antibiotic agents failing to end many bacterial infections. The search for compounds which can be combined with antibiotics in the treatment of drug resistant infections may be an alternative to overcoming the problem of resistance in bacteria. Crude extracts of medicinal plants stand out as veritable sources of potential resistance modifying agents .The hexane and acetone/water fraction of *Pinus halepensis Mill* and *Cedrus libani A. Richard were* assayed against eight different bacteria of G+ve and G-ve using agar

diffusion method. The zones of inhibition were determined and compared with the wide spectrum antibacterial ciprofloxacin as a positive control.

The Gass chromatography/ Mass Spectroscopy analysis of the plants showed that the terpene fraction of both plants contain abietic acid which is responsible for the antibacterial activity, while pinene present only in the terpene fraction of *C. libani A.* Also the GC/MS analysis of the hydrophilic part of both plants showed the presence of nortrachelogenin.

Both plants showed potent antibacterial activity; the most sensitive bacteria for the hydrophilic part of *P. halepensis M.* was *Salmonella typhi*, while *Streptococcus pneumoniae* is the most sensitive bacteria for *C. libani A.* The hexane fraction exhibit more potent antibacterial activity than the hydrophilic fraction, the most sensitive bacteria for the terpene fraction of *P. halepensis M.* was *Klebsiella pneumonia* while *Proteus vulgaris* is the most sensitive bacteria for the terpene fraction of *C. libani A.*

List of abbreviation

C.	Cedrus
Cfu	Colony forming units
DMAPP	Dimethylallyl diphosphate
EDL	Enterodiol
ENL	Enterolactone
EtOH	Ethanol
CVD	Cardiovascular disease
DCM	Dichloromethane
DIR	Dirigent protein
Fpp	farnesyl pyrophosphate
GC/MS	Gass chromatography/Mass spectroscopy
Gpp	geranyl pyrophosphate
GRAS	Generally recognized as save
HCl	Hydrochloric acid
HMR	Hydroxymatieresinol
HPLC	High Performance Liquid Chromatography
hr	hour
hrs	hours
IPP	isopentenyl diphosphate
M^+	molecular ion

MAT	Matieresinol
MDR	Multidrug resistance
MEP	methylerythritol phosphate
µg	microgram
Min.	minutes
µm	micrometer
MVA	mevalonic acid
M/Z	Mass/ Charge
NADPH	Nicotinamide adenine dinuclutide phosphate
OM	Outer membrane
PPARγ	Peoxisome proliferator activator receptorγ
R	Resistance
R_f	Retention factor
RND	Resistance nodulation devision
SEC	Secoisoresinol
Spp	species
Subsp.	Subspecies
Syk	Spleen tyrosine *kinase*
TLC	Thin layer chromatography
Var.	Variety

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1. Introduction

1.1 Background

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [1].

Such a fact is cause for concern, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-resistant. Consequently, new infections can occur in hospitals resulting in high mortality [2]. The development of bacterial resistant strains is resulting in currently used antibiotic agents failing to end many bacterial infections. For this reason the search is ongoing for new antimicrobial agents, either by the design and synthesis of new agents, or through the search of natural sources for as yet undiscovered antimicrobial agents [3].

The World Health Organization reports that at least 75 - 95% of the world populations of developing countries were chiefly relay on traditional medicines and major part of traditional therapies involves the use of plant extract products or their active constituents [4]. Traditional medicine usage is a common practice in developed and developing countries at the primary

healthcare level [5]. Pinaceae family contains plants such as pine, cedar, abies, used in folk medicine [6], and it was found that their methanol, acetone, and chloroform extract known to have antibacterial activity [7].

1.2 Bacterial resistance to Antibiotic

The treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance to antimicrobial agents. Antimicrobial agents are often categorized according to their principal mechanism of action [8]. Such as:-

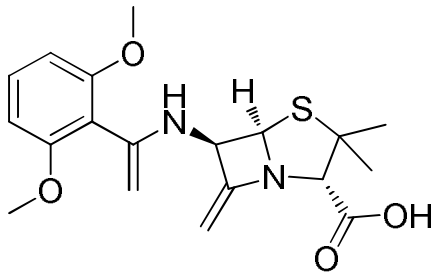
1- Interference with cell wall synthesis (e.g. β-lactams agents like methicillin (**1**).

2- Inhibition of protein synthesis (macrolides erythromycin (**2**) and tetracyclines (**3**).

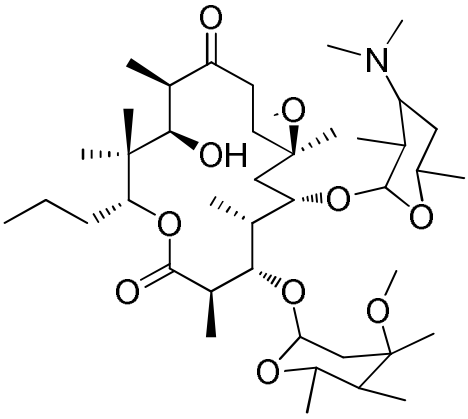
3- Interference with nucleic acid synthesis (ex. Ciprofloxacin (**4**).

4- Inhibition of a metabolic pathway (trimethoprim-sulfamethoxazole (**5**).

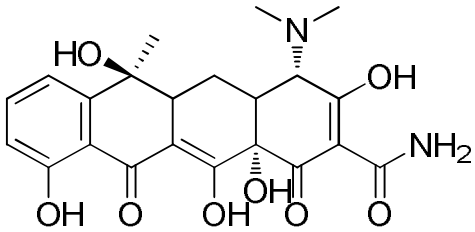
5- Disruption of bacterial membrane structure (polymyxins (**6**) [9].



Methicillin **1**



Erythromycin **2**



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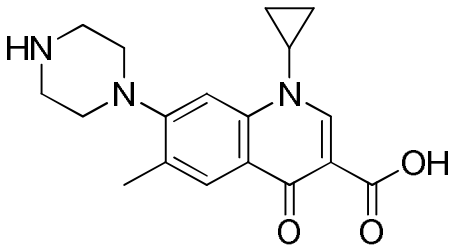
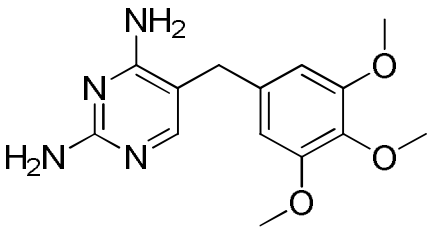
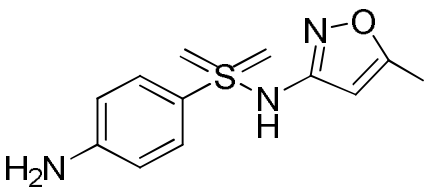
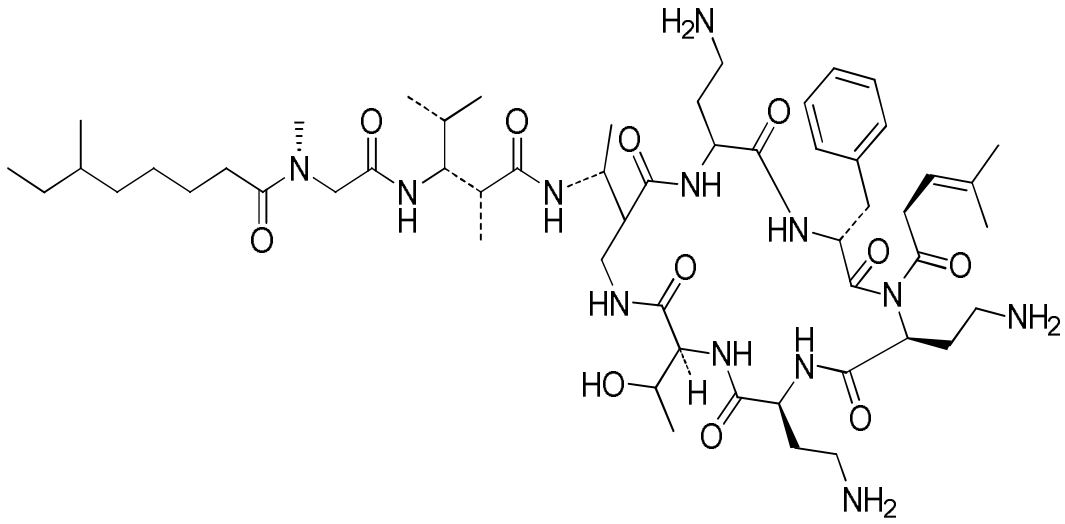


Ciprofloxacin **4**





Trimetoprim **5**



Polymexin **6**

Antibiotics are a major tool utilized by the health care industry to fight bacterial infections; however, bacteria are highly adaptable creatures and are capable of developing resistance to antibiotics. Consequently, decades of antibiotic use, or rather misuse, have resulted in bacterial resistance to many modern antibiotics. This antibiotic resistance can cause significant danger and suffering for many people with common bacterial infections, those firmly treated with antibiotics [10].

Biochemical mechanisms for antimicrobial resistance full into three major categories:-

1- Production of hydrolytic or modifying enzyme.

2- Alteration of targets such that they are no longer susceptible to antibacterial action.
3- Modifying of target accessibility, including permeability barrier and energy dependant antibiotic efflux pump [11].

Emergence of resistance to multiple antimicrobial agents in pathogenic bacteria has become a significant public health threat as there are fewer, or even sometimes no, effective antimicrobial agents available for infections caused by these bacteria. Gram-positive and Gram-negative bacteria are both affected by the emergence and rise of antimicrobial resistance e.g. multidrug-resistant carbapenemase-producing *Klebsiella pneumoniae* and *Acinetobacter* spp.) [12].

Even those Certain Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, and quinolone-resistant *Streptococcus pneumoniae* have achieved the status of "superbugs", in that there are few or no antibiotics available for therapy against these pathogens. Only a few classes of novel antibiotics have been introduced in the past 40 years [13].

Multidrug resistance in bacteria may be generated by one of two mechanisms:-

First: - these bacteria may accumulate multiple genes, each coding for resistance to a single drug, within a single cell. This accumulation occurs typically on resistance (R) plasmids.

Second: - multidrug resistance may also occur by the increased expression of genes that code for multidrug efflux pumps, extruding a wide range of drugs [14].

Efflux mechanisms, both drug-specific and multidrug, are important determinants of intrinsic and/or acquired resistance to these antimicrobials in important human pathogens. Multidrug efflux mechanisms are generally chromosome-encoded, with their expression typically resultant from mutations in regulatory genes, while drug-specific efflux mechanisms are encoded by mobile genetic elements whose acquisition is sufficient for resistance [15].

Efflux pumps are transport proteins involved in the extrusion of toxic substrates from within cells into the external environment. These proteins are found in both Gram-positive and -negative bacteria as well as in eukaryotic organisms. Pumps may be specific for one substrate or may transport a range of structurally dissimilar compounds (including antibiotics of multiple classes); such pumps can be associated with MDR [16].

Among these, most pumps located in the cytoplasmic membrane and pump out drugs rapidly into the periplasm, because the drugs can penetrate back into cytosol frequently by spontaneous diffusion. Only the RND pumps (and a few exceptional pumps) exist in a tripartite form traversing both the OM and the inner membrane in addition to the RND pump protein located in the inner membrane allows the bacteria to pump out drug molecules directly into the external medium. This is a huge advantage for bacteria, because the drug in the medium has to cross the low permeability OM in order to re-enter the cells, in contrast to the drug molecules in the periplasm that can penetrate easily the high permeability inner membrane [17].

The ability of some chemical compounds (called MDR inhibitors or resistance modifying agents) to modify the resistance phenotype in bacteria by working synergistically with antibiotics *in vitro* has been observed. The search for such compounds which can be combined with antibiotics in the treatment of drug resistant infections may be an alternative to overcoming the problem of resistance in bacteria. Crude extracts of medicinal plants stand out as veritable sources of potential resistance modifying agents [18]. MDR inhibitors can reach their objective by different strategies:-

1- By-passing efflux activity: improving the molecular design of old antibiotics to reduce their efflux**.**

2- Direct action on the permeability of the bacterial cell envelope: decreasing the efficacy of the membrane barrier.
3- Blocking the efflux capacity of bacterial cell: alteration of pump function [19].

Iraq has a huge number of natural products, in different artisanal preparations, mainly from plants, have been used by traditional populations to cure diseases. Despite some of these plants have been studied, many of them are waiting to have their compounds chemically characterized and investigated their pharmacodynamics properties, this work deal with two of important Iraqi medicinal plant from Pinaceae family.

A major constitutive defence of particular importance in the Pinaceae is resin-producing and storing structures, effective especially against bark beetles and associated fungi, Inducible defence mechanisms involve cell wall alterations (lignification, suberization), production of lytic enzymes (chitinases, glucanases) and antimicrobial compounds (phenols, stilbenes, lignans, flavonoids, terpenoids). To date, targeted studies have identified a set of genes induced by biotic stress and encoding peroxidases, a defensin, chitinases, a β-1, 3-glucanase, a chalcone synthase and a family of dirigent (DIR) proteins [20].

1.3 Pinaceae

1.3.1Classification:-

- **Kingdom:** Plantae
- **Division:** Pinophyta
- **Class:** Pinopsida
- **Order Type:** Gymnosperm
- **Order:** Coniferales (Pinales)
- **Family:** Pinaceae
- **Family Common Name:**Pine Family
- **Genus:** *pinus, cedrus, Larix, Abies, Cathaya, Keteleeria, Picea, Pseudotsuga, Tsuga ,Nothotsuga,* Pseudolarix [21].
- **Species:** like:- *Pinus halepensis* and *Cedrus libani*

Pinaceae are trees or rarely shrubs, evergreen or deciduous, monoecious. Branchlets often dimorphic: long branchlets with clearly spirally arranged. Sometimes scale like leaves [22], foliage leaves either borne on long shoots or clustered tightly on short shoots, and pollen cones (microsporangiate strobili) bearing spirally arranged [23]. The family is characterized by the presence of linear leaves (needles), ovulate cones with independent cone scales (each of which has two ovaries), bisaccate pollen, a specialized proembryogeny, and an absence of biflavonoids [24].

Members of the Pinaceae are of major economic importance as producers of most of the world's softwood timber. Additionally, they are sources of pulpwood, naval stores (e.g., tar, pitch, turpentine, etc.), essential oils, and other forest products. All members of the family, especially pines, are of varying importance to wildlife for food and cover. Many species, including most of the genera, are grown as ornamentals and shelter-belt trees and for revegetation [25].

Pinaceae are the largest family of the world's conifer which contain 615 species and classified into eight families of which 231 species belong to the family pinaceae, fig (1.1) [26].

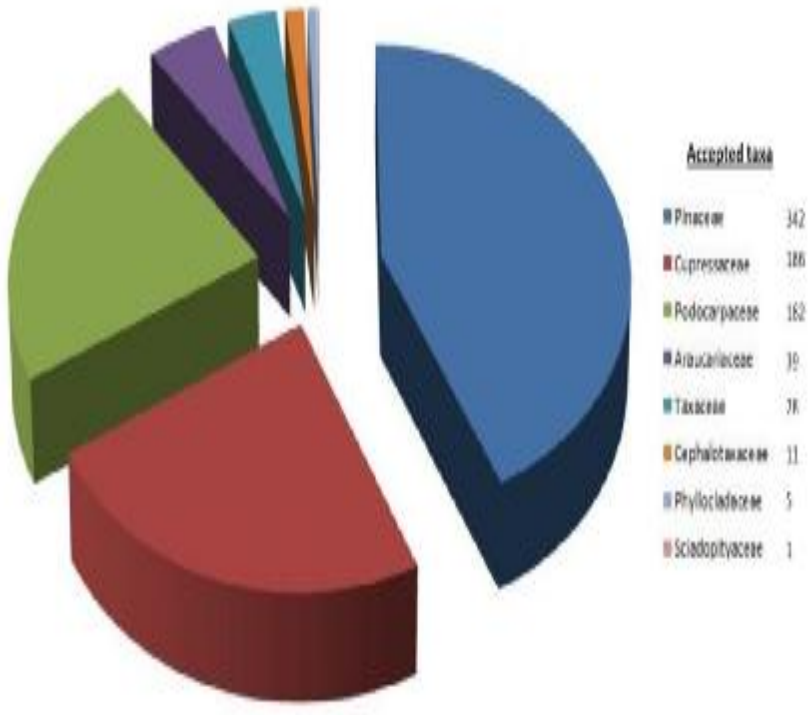


Fig. 1.1: Classification of conifer

The family Pinaceae contains a total of eleven commonly accepted genera [27]. The presence or absence of short shoots, and the petiole and pulvinus form, culminating in the subfamily arrangements outlined below [28, 29]:-

- Subfamily Pinoideae: *Pinus*

Shoots and foliage strongly dimorphic. Strobili carried on long shoots.

- Subfamily Laricoideae Melch. & Werd.: *Larix, Pseudolarix, Cedrus*

Shoots dimorphic, foliage monomorphic. Strobili carried on short shoots.

- Subfamily Abietoideae Pilger: *Abies, Cathaya, Keteleeria, Picea, Pseudotsuga, Tsuga ,Nothotsuga*

Shoots not or only weakly dimorphic, foliage monomorphic. strobili carried on long shoots [30].

1.3.2 Pinus

Pines are native to most of the northern hemisphere , and have been introduced throughout most temperate and subtropical regions of the world, where they are grown as timber and cultivated as ornamental plants in parks and gardens[31].

Genus *Pinus* is important and very often a dominant component of the vegetation over large parts of the northern hemisphere. They play important ecological roles and they have enormous economic value [32].

The poreal forest of which pinus are an important component, play a significant role in determining regional and global climate. For example the presence of forest in these northern latitudes makes the high reflectance of snow, leading to warmer winter temperature than would be the case if tree were absent [33]. The pine genus is the largest in the family, with 115 species [34]

Subdivided into three groups based on cone, seed and leaf characters:-

- Subgenus Pinus (the yellow or hard pines).
- Subgenus Strobes (the white or soft pines).
- A third subgenus Ducampopinus (the foxtail or pinyon group) [23].

Human have harvested pines and their product for thousands of years [30]. The usages of pines are very common among local people, especially wood is used to build house, cellar, etc. Wood and cones are collected for firewood. Various products are obtained from pinus species. Resin, turpentine, pine oil, tar, etc. besides pine wood has very different uses, because of this it has first -order importance in trade [35].

Pines are commercially among the most important of species used for timber and wood pulp in temperate and tropical region of the world; this is

due to their fast growing softwood that can be planted in relatively dense stands, and because their acidic decaying needles inhibit the growth of other competing plants [36].

Drugs obtained from pinus species have various ethnomedicinal usages; they are used as antiseptic, tonic, expectorant, especially in respiratory and urinary system disorder and externally against rheumatic pain and skin disease, also they have usage in dye, paper and leather industries [37].

Medicinal utilization of pines can be listed below in nine groups [38]:-

1- Human treatment: non detailed data.
2- Panacea: mention as cure all".
3- Skin disease: abscess, abdominal ptosis, burn, chopped foot and hand, contusion, callus, cut, dermatitis, erysipelas, fungal infection, lesion, psoriasis, warts, and wound.
4- Respiratory system diseases: asthma, bronchitis, common cold, cough, expectorant, flu, lung disease, pneumonia, and tuberculosis.
5- Digestive system disease: carminative, colitis, diarrhea, and gastritis, hemorrhoids, indigestion, laxative, liver disease, stomach disease, and ulcer.
6- Urinary system disease.
7- Analgesic: abdominal pain, anodyne, rheumatic pain, stomachache, toothache, and waist pain.
8- Endocrine system disease: diabetic, goiter.

9- Others: antimicrobial, antiseptic, aphrodisiac, anthelmintic, fatigue, fracture, hygienic for teeth, inflammatory disease, internal disease, lactogogue, parapraxia, sedative, and snake bites.

1.3.3 Cedrus

Evergreen, tall, and monoecious trees. Crown broad with erect or bent top. Branches not in whorls. Bark on young trees smooth and gray, eventually furrowed and scaly dark gray. Shoots of two kinds: long terminal shoots bearing solitary and spirally arranged needles, and short shoots with tufts of needles, long cone-like inflorescences, reddish, and ovate, composed of numerous scales and surrounded by needles at the base. Cones erect, ovate to cylindrical, 5-10 cm long, maturing in the second or third year [39].

Cedars share a very similar cone structure with the firs (*Abies*) and were traditionally thought to be most closely related to them, but molecular evidence supports a basal position in the family [40].

There are five taxa of *Cedrus*, assigned according to taxonomic opinion to between one and four different species: [41, 42, 43, 22, 44, 45, 46, and 47].

- Deodar or Deodar Cedar, *C. deodara* (syn. *C. libani* subsp. *deodara*). Western Himalaya. Leaves bright green to pale glaucous green, 25–60 mm; cones with slightly ridged scales.

- Lebanon Cedar or Cedar of Lebanon *C. libani*. Cones with smooth scales; two (or up to four) subspecies:
  - Lebanon Cedar *C. libani* subsp. *libani*. Mountains of Lebanon, western Syria and south-central Turkey. Leaves dark green to glaucous blue-green, 10–25 mm.
  - Turkish Cedar *C. libani* subsp. *stenocoma*. Mountains of southwest Turkey. Leaves glaucous blue-green, 8–25 mm.
- Cyprus Cedar *C. brevifoli* (syn. *C. libani* subsp. *brevifolia*, *C. libani* var. *brevifolia*). Mountains of Cyprus. Leaves glaucous blue-green, 8–20 mm.
- Atlas Cedar *C.atlantica* (syn. *C.libani* subsp. *Atlantica)* Atlas mountains in Morocco & Algeria. Leaves dark green to glaucous blue-green, 10–25 mm.

Cedars are very popular ornamental trees, widely used in horticulture in temperate climates where winter temperatures do not fall below about −25 °C. The Turkish Cedar is slightly hardier, to −30 °C or just below. Extensive mortality of planted specimens can occur in severe winters where temperatures do drop lower [48].

Cedar wood and cedar oil are known to be a natural repellent to moths, references to beneficial effects of cedarwood (wood, leaf, bark) go back to the ancient Greeks – Dioscorides mentions ‘cedar’ oil used to preserve dead bodies. The Bible has several references to Cedar wood, but it is not always

clear if either of these sources refer to Cedar of Lebanon *C. libani A.* or to Juniper species such as *Juniperus phoenicia.* but, under 'uses', the oil is only listed in the monograph as an expectorant, with application in catarrhal conditions of the upper respiratory tract only being indicated as unproven[49]. *C. libani A.* takes various forms such as the symbolical and the woody forms. The Symbolical form is a perennial tree having a short and wide trunk, thick branches extended horizontally and making up a broad corona with a pyramidal shape, present in the Lebanese flag. Leaves of conical form are longer and sharper than those of the symbolic form, and the color of leaves becomes bluish green. The lower lateral branches grow in a more active way than the upper branches up to 20 m; for this reason, lower lateral branches normally are cut giving the typical "umbrella like" appearance [50].

It has been shown that chloroform, acetone and methanol extracts of the leaves, resins, cones and fruit of *C. libani A.* inhibited development of nine out of ten bacteria (but not *E. coli*) although the role of the essential oil content for these findings is not clear[7].

Ethnobotanical surveys revealed that *C. libani A.* have been used to promote wound healing in folk medicine. The experimental study revealed that *C. libani A.* display remarkable wound healing and anti-inflammatory activities [51].

Today, traditional people produce wood extract, called *katran*, from *C. libani A.*, and use it to protect wooden structures against insects and fungi, to fight parasites and bacteria, and to heal wounds and cure various diseases in humans and domestic animals, both internally and externally[52]. Furthermore, the cones of *C. libani A.* possess anti-ulcerogenic remedies for anti-*Helicobacter pylori* activity [53].

The ethanol extracts derived from cones and leaves of *C. libani* A. and essential oil obtained from the wood inhibit the growth of herpes simplex virus-1 *in vitro* [54]. Moreover, research showed that essential oils and same ethanol extracts derived from cones and wood of *C. libani A.* possess anti-diabetic activity [55].

1.4 Chemical constituent of cedrus and pine

1.4.1 Terpens

Terpenes are not only the largest group of plant natural products, comprising at least 30,000 compounds, but also contain the widest assortment of structural types. Hundreds of different monoterpene (C10), sesquiterpene (C15), diterpene (C20) and triterpene (C30) carbon skeletons are known. Natural products chemists have long marveled at the structural diversity of terpenes and speculated on its biosynthetic basis.

The wealth of terpene carbon skeletons can be attributed to an enzyme class known as the terpene synthases. These catalysts convert the acyclic prenyl

diphosphates and squalene into a multitude of cyclic and acyclic forms. The chief causes of terpene diversity are the large number of different terpene synthases and the fact that some terpene synthases produce multiple products [56]. They play important roles in direct and indirect plant defense against herbivores and pathogens, in reproduction by attraction of pollinators and seed disseminators, and in plant thermotolerance. Apart from their importance in plant physiology and ecology, volatile terpenoids are also used as natural flavor and aroma compounds and have beneficial impact on humans as health promoting compounds [57].

As shown in Fig. 1.2, terpenoids are derived from the universal C5 precursor isopentenyl diphosphate (IPP) and its allylic isomer dimethylallyl diphosphate (DMAPP), which in higher plants are generated from two independent pathways located in separate intracellular compartments. In cytosol, IPP is derived from the long-known mevalonic acid (MVA) pathway that starts with the condensation of acetyl-CoA. In plastids, IPP is formed from pyruvate and glyceraldehydes 3-phosphate. This MVA-independent pathway, also called methylerythritol phosphate (MEP) pathway after the key intermediate (MEP), was discovered only in the last 10 years [58].

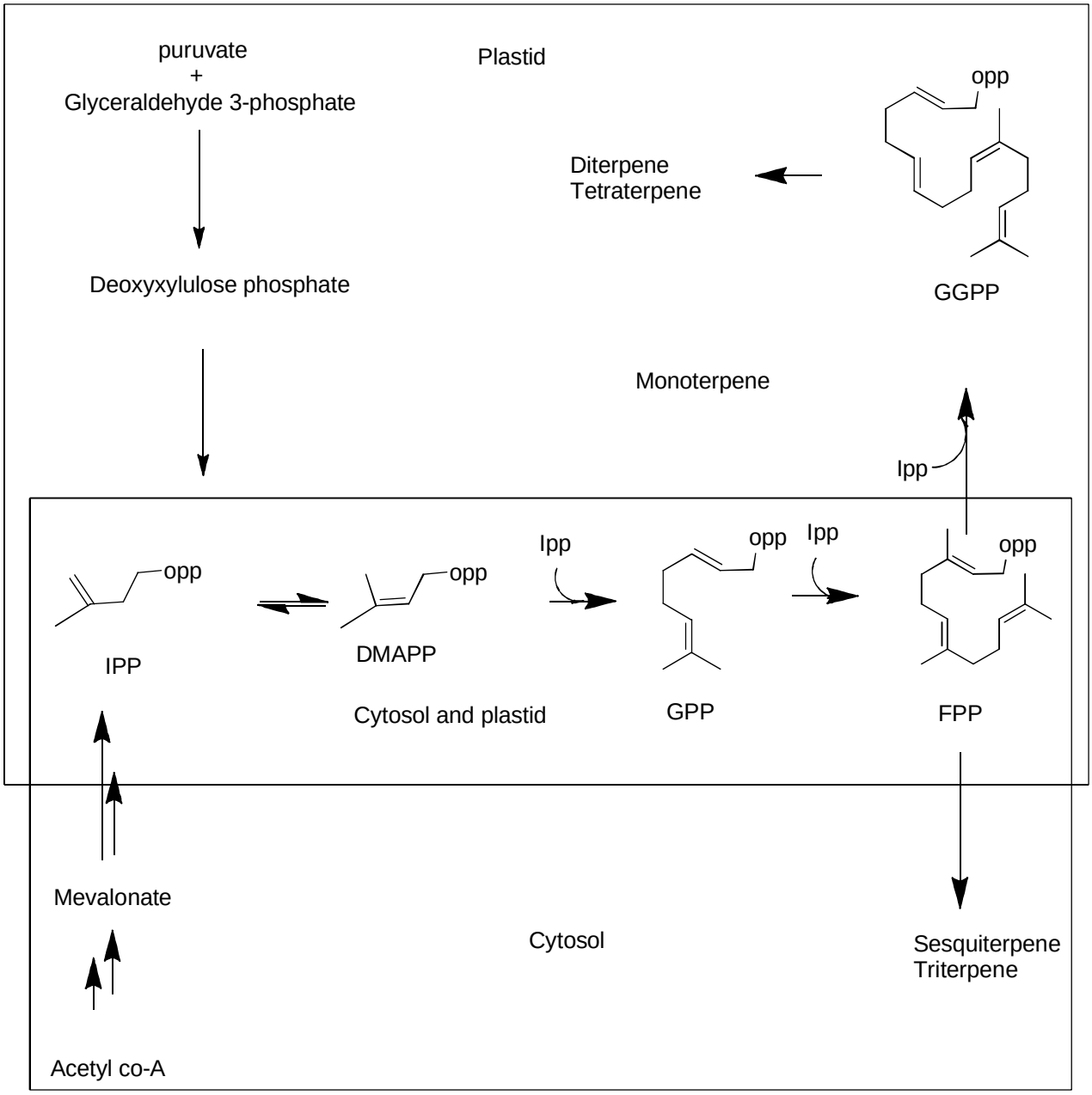


Fig. 1.2: Biosynthetic pathway of terpene in plant cells.

A terpene is classified according to the number of isoprene unit (a), and carbon atoms (b), and is identified by the notation a: b, Fig. 1.3 e.g. monoterpens (2:10), sesquiterpens (3:15), diterpens (4:20), sestrpens (5:25),

triterpens (6:30), carotenoids(8:40), and rubber (>100:>500). Terpene may be lipopohilic or hydrophilic, volatile or non volatile, cyclic or acyclic [59].

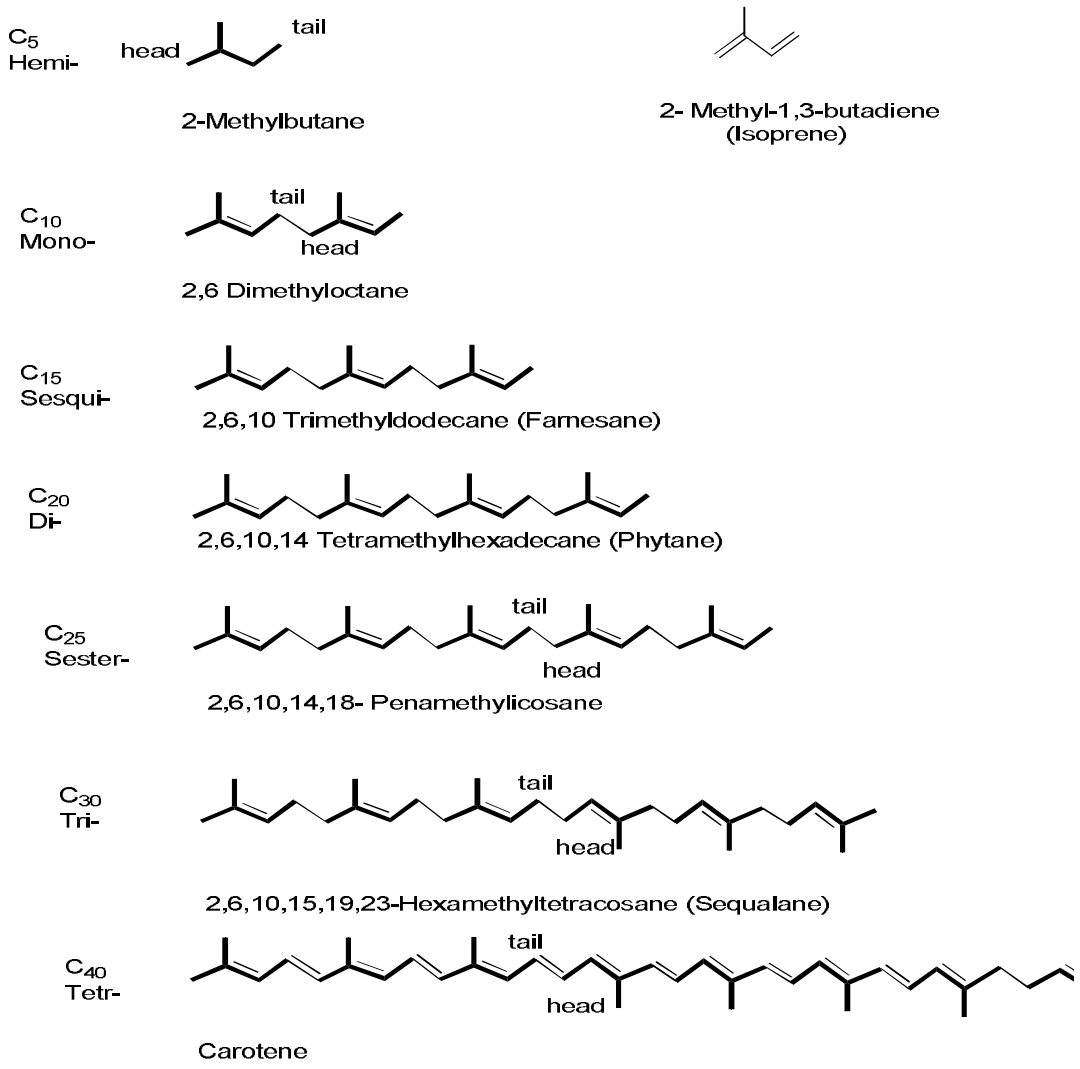
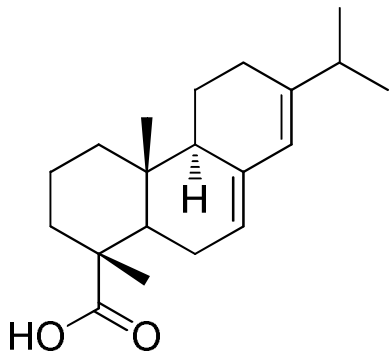


Fig. 1.3: Parent hydrocarbons of terpenes (isoprenoid)

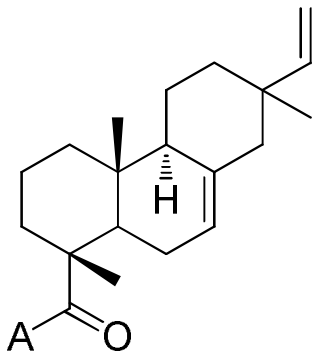
The Pine contains a variety of terpenoid compounds in the barks, woods, leaves, and cones, particularly in the characteristic oleoresins of the resin canals or vesicles. The bulk of the volatile portion of the stem and leaf oleoresins is usually a complex mixture of monoterpenes. These impart

much of the characteristic fragrance associated with Pinaceae. Terpenoid and hydrocarbon profiles of the oleoresins or turpentines (the steam-distillable portion) often show significant differences among species and have been widely used in chemosystematics of the Pinaceae [23].

As a major part of their constitutive and inducible defensive repertoire, conifers produce an abundant and complex mixture of terpenoids in the form of oleoresin secretions and volatile emissions [60]. The biological activity of natural abietane- acids has been reviewed antimicrobial, antiulcer and cardiovascular activities are the most representative for this class of diterpenoids Abietic acid (**7**) and isopimaric acid (**8**) has shown antiallergic anti-inflammatory, phytoalexin-like and anticonvulsant activities[61]. Isopimaric acid and abietic acid has shown antibacterial activity against multidrug resistant and methicillin resistance *Staphylococcus aureus* [62].



abietic acid **7**



isopimaric acid **8**

1.4.1.1 Abietic acid

Abietic acid has an aromatic diterpene structure with three rings, three chiral carbon atoms, two conjugated C=C bonds, and a reactive carboxyl group. Due to this structure, abietic acid and its derivatives show biological activity and intriguing chirality. These compounds have found applications

in such areas as the manufacturing paper, printing inks, adhesives, and synthetic rubber [63]. Abietic acid is used as an ingredient in pharmaceutic aids and in the manufacture of soaps, waxes, also assist the growth of lactic and butyric acid bacteria, and inhibit the activity of prostate testosterone 5α-reductase that is useful in the treatment of androgen dependant disease such as begnin prostatic hyperplasia[64].

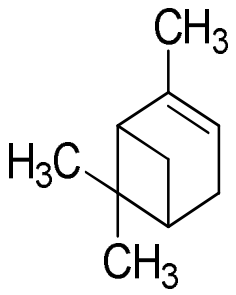
Abietic acid, inhibited 5-lipoxygenase, since the lipoxygenase pathway leads to the biosynthesis of leukotrienes this result supports the view that abietic acid may be used in the treatment of allergic reactions [65].

Abietic acid activates peroxisome proliferator-activated receptor-γ (PPARγ) in RAW264.7 macrophages and 3T3-L1 adipocytes to regulate gene expression involved in inflammation and lipid metabolism [66]. Abietic acid and its derivatives used for inhibiting the growth of cancer cell or treating a cancer, preferably, reducing the tumor size of the cancer [67].

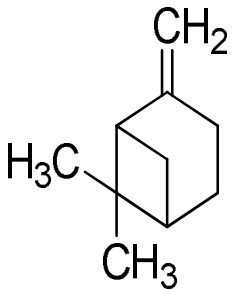
abietic acid exerts *in-vivo* anti-inflammatory activity after oral or topical administration and has partial ability to prevent the production of some inflammatory mediators. This compound significantly inhibited oedema after oral or topical administration. In addition Non-toxic concentrations of abietic acid inhibited prostaglandin E_2 (PGE_2) production in lipopolysaccharide-treated macrophages, whereas nitrite, tumour necrosis factor α and interleukin-1β production were only weakly affected by this diterpene and it failed to modify leukotriene C_4 production [68].

1.4.1.2 Pinene

Pinene (α and β pinene (**9**) **,** (**10**) represent the two major compounds of the essential oil of turpentine [69].



σ–pinene **9**



β–pinene **10**

Volatile pinenes of turpentine enter the body through inhalation but also through the skin, with a good correlation between the level of

contamination of particular body parts and the potential body exposure [70].The ability of volatile pinenes to penetrate through the skin, the low irritancy potential and the inclusion in the list of the substances that are Generally Recognized as Safe (GRAS), make it possible to use them as a support to increase the absorption of various chemicals. They are used, for example, for enhanced neuroleptic drug absorption [71].

The turpentine respiratory sessions considerably increase the capacity of the organism to transform the xenobiotics at the hepatic level, by increasing the activity of the NADPH cytochrome C reductase and the 7-ethoxycoumarine de-ethylase [72].

Pinene reported to have, antinociceptive[73], antioxidant, anticholinesterase and anti-inflammatory that it inhibit the enzyme cycloxygenase, an activity that may be of particular relevance to anti-inflammatory treatment of Alzahiemer disease, α-pinene(**9**) have demonstrated weak (significant) inhibition of eicosanoid synthesis, though there may be more potent constituents present in minute quantities in the essential oil[74]. Pinenes also show antifungal properties, especially on *Candida* spp. When acting on yeast, they were found to inhibit mitochondrial respiration, the proton pump activity and K+ transport, and to increase membrane fluidity, they also exhibit pest-destroying properties against the protozoon *Plasmodium berghei*, insecticidal properties against lice and the mosquito Anopheles

Aedes aegypti as well as an antiseptic effect on oral normal flora. In general, they exert a considerable antibacterial effect, especially on a methicilline-resistant *S. aureus* and other Gram-positive and Gram-negative bacteria .Without α-pinenes (**9**), but with other terpenes, β-pinenes (**10**) present antiradical activity. They belong to the essential oils used against the osteoclast activity (they thus play a protective role against osteoporosis) [69].

Alpha-pinene (**9**) is a broad-spectrum enzyme inductor of the phenobarbital type, with certain quantitative differences thus under the effect of alpha-pinene **(9)** treatment. A moderate increase was found in the amount of microsomal protein and cytochrome P 450, a marked increase in the metabolism of both aminopyrine (substrate of type I) and aniline (substrate of type II), together with an increase in the butylisocyanide-absorption maxima of both haemoproteins involved in the reaction [75]. Pinene exhibits *in vitro* cytotoxic activity against Hep-G2 and SK-Mel-28 human tumor cell lines [76].

1.4.2 Lignan

Lignans are a large group of natural products characterized by the coupling of two C6C3 units at the β-position in the propane side chain as shown in fig 1.4 [77].

Lignan biosynthesis has been found to be closely related to other phenolic cmpounds but distinct from those of other phenylpropanoids, such as norlignans, lignins and neolignans. The diversity exhibited by lignans and the similarity of their biosynthesis to those of other phenylpropanoids piqued our scientific interest in the origination and evolution of phenylpropanoids biosynthesis in vascular plants, and a central theme in plants science, hence, lignan may be a good subject for studying the evolution of plant secondary metabolite[78].

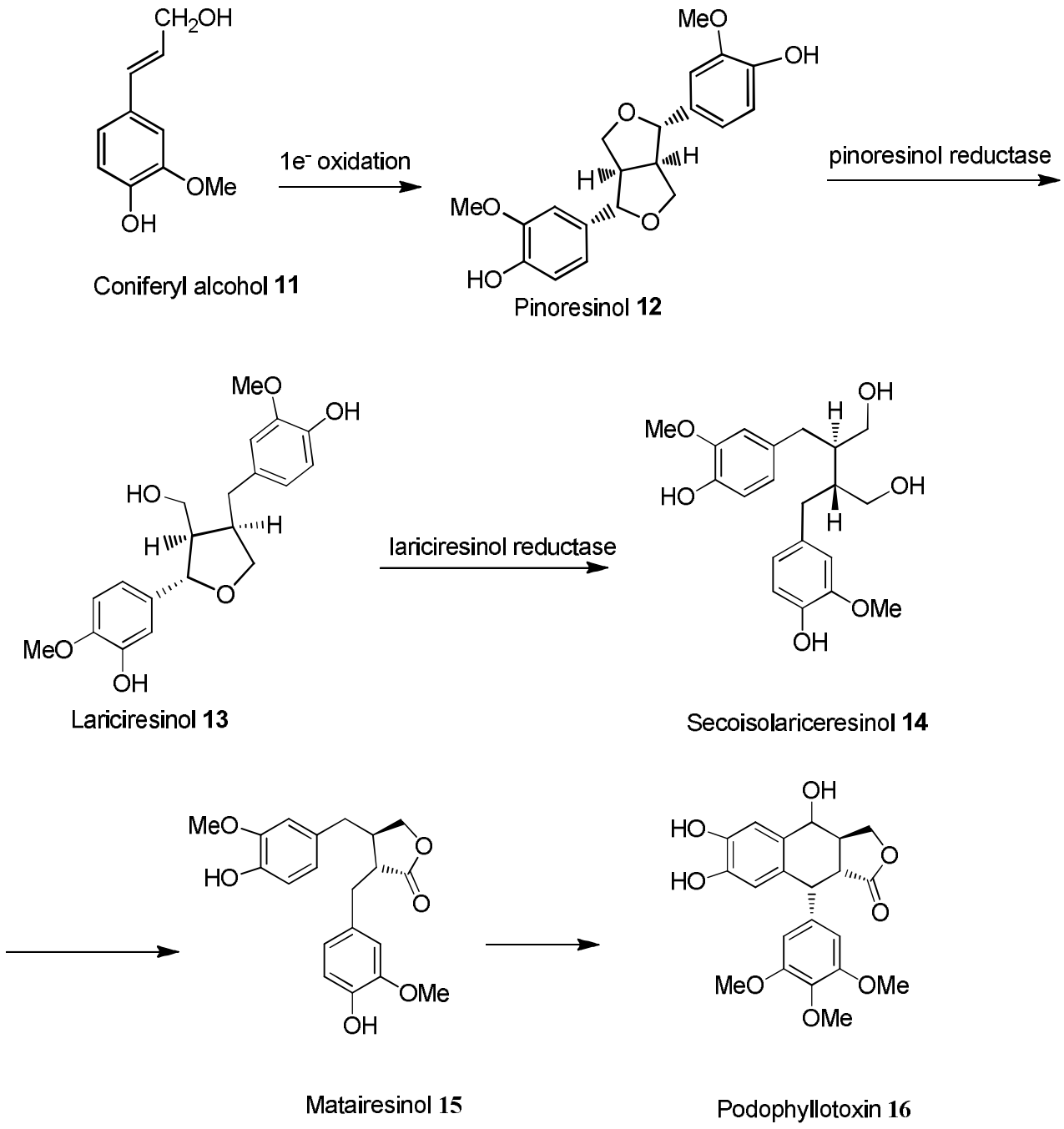
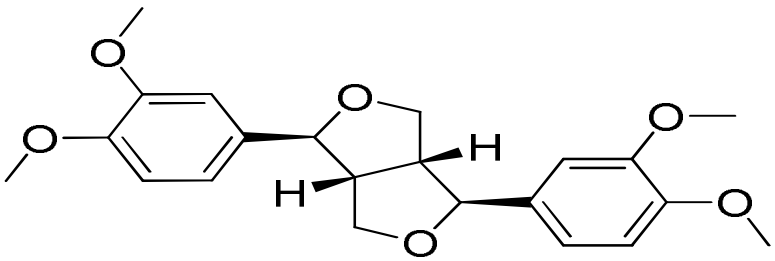


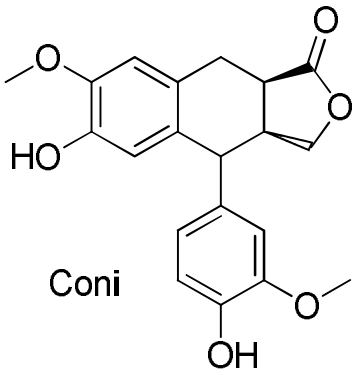
Fig. 1.4 Biosynthesis of lignans

Plant lignans was first identified in 19th Century from woody tissues of trees. Several hundreds of lignans have been documented since then in roots, stems, cereals, oil seeds, nuts, legumes and fruits. Nowadays, with the growing interest towards nutraceuticals, plant lignans are becoming important therapeutically active class of compounds because of their putative beneficial health effect such as antitumor, and antioxidant. Both estrogenic and antiestrogenic activity and protection against coronary heart disease [79]. Lignin also has antimitotic, and antiviral properties, as well as unique stereochemical properties [78].

Many lignan such as pinoresinol (**12**), secoisolaricinol (**14**), eudesmin (**17**), lariciresinol (**13**) and hydroxymatairesinol (**18**) have antibacterial and antifungal activity [80, 81].



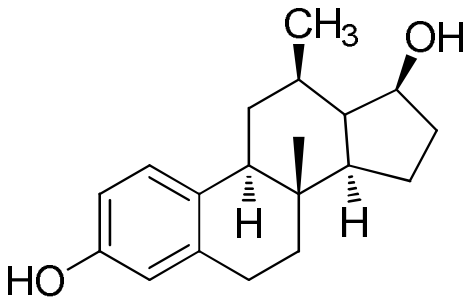
Eudesmin **17**



Hydroxymatairesinol **18**

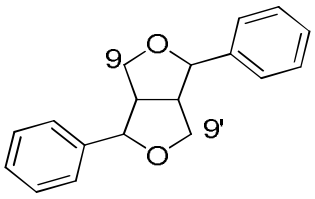
lignan intake may decrease the risk for cardiovascular disease (CVD) by modifying traditional risk factors as well as aortic stiffness. However, the role of dietary lignans on the vascular system is largely unknown, but it was found that higher matieresinol (**15**) intakes are associated to lower vascular inflammation and endothelial dysfunction, which could have some implications in CVD prevention [82].

The health benefits of lignans are thought to be due to estrogenic and antiestrogenic compounds due, in part, to the structural similarity to 17-b-estradiol (**19**), the behavior of the lignans depends on the biological levels of estradiol. At normal estradiol levels, the lignans act as estrogen antagonists, but in postmenopausal women (at low estradiol levels) they can act as weak estrogens, Other activities related to estrogen include the *in vivo* synthesis of 2-hydroxy estrogen, a compound that may protect against cancer and inhibit the binding of estrogen and testosterone to receptors on sex-binding globulin[83].

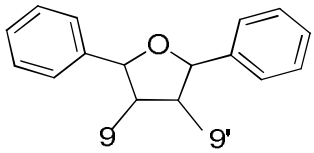


Estradiol **19**

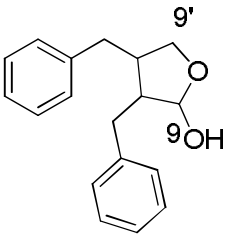
Lignans are classified in eight subgroups and among these subgroups, the furan, dibenzylbutane and dibenzocyclooctadiene lignans can be further classified in “lignans with C9 (9´)-oxygen” and “lignans without C9 (9´)-oxygen”. Fig. 1.5 displays the main classes of lignans, as well as their subgroups. It is noteworthy that, despite its structural variation, lignans also display a substantial variation on its enantiomeric composition. In this sense; these metabolites can be found as pure enantiomers and as enantiomeric compositions, including racemates [84].



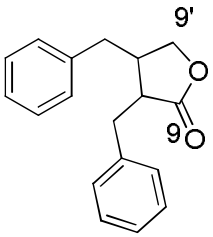
Furofuran



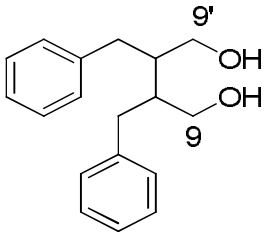
Furan without C9(9')



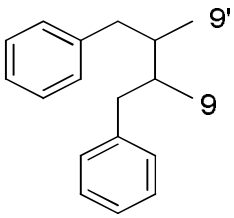
Dibenzylbutyrolactol



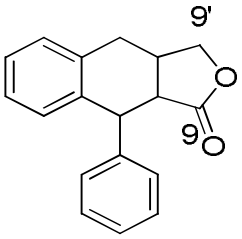
Dibenzylbutyrolactones



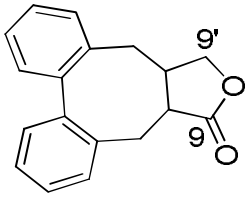
Dibenzylbutane With C9(9')- oxygen



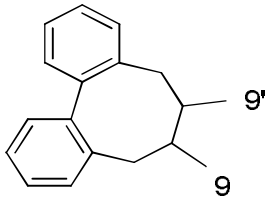
Dibenzylbutane Without C9(9')- oxygen



Aryltetralin



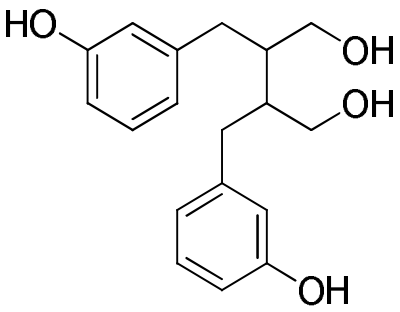
Dibenzocyclooctadienes WithC9(9')- oxygen



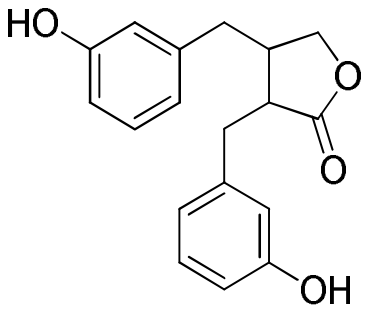
Dibenzocuclooctadienes WithoutC9(9')-oxygen

Fig. 1.5 The main classes of lignan

Historically the predominant lignan of dietary importance have been matairesinol (MAT) (**15**) and secoisoresinol (SEC) (**14**), these are converted to mammalian lignan enterodiol (**20**) (EDL) and enterolactone(**21**) (ENL) respectivally by passing through the gut and subsequent metabolism by normal flora. Plant lignans ingested as sugar conjucates then deconjucated by gastric HCl and an aerobic microbe-derived β-glycosidases and converted through a series of metabolic reactions to their respective mammalian derivatives [85].



Enterodiol 20



Enterolactone 21

Enterolactone (**21**) of which has been thought to be the major biologically active lignan, and suggested to be associated with low risk of breast cancer. In line with this, administration of plant lignans which are further metabolized to ENL, or ENL as such, have been shown to inhibit or delay the growth of mammary cancer. The mechanism of anticarcinogenic action of ENL is not yet fully understood, but there is intriguing evidence for ENL as a modulator of estrogen signaling. These findings have

generated interest in the use of lignans as components of breast cancer risk reducing functional foods [86]. Enterolactone (ENL) and enterodiol (EDL) have been suggested to be protective in both breast and colon cancer, because of their anti-oxidant, anti-proliferative, weak estrogenic/anti-estrogenic, anti-angiogenic and anti-aromatase activites. They can also inhibit 7a-hydroxylase activity and increase sex hormone binding globulin synthesis [87].

In contrast to plants, there are virtually no lignans in animal foods. Minute amounts of the enterolignans enterodiol and enterolactone are sometimes found in animal (milk products) as a result of their production by intestinal bacterial metabolism in the animals guts, but these are exceptions. Total lignan intakes vary from country to country because of different dietary sources [88].

1.5 Aims

- The aims are twofold first is to search for medicinal plants belongs to the family pinaceae found in Iraq and to extract the possible antibacterial compounds including resin acid and lignan.
- Secondely is to test the antibacterial activity of the lipophilc and hydrophilic components of the plants against Gram positive and Gram negative bacteria as well as searching for the activity against multi-drug resistance bacteria.

2. Materials and Methods

2.1 Plant material

The medicinal plants used for experiment were *P. halepensis M.* fig 2.1 *and C. libani A.* fig 2.2 while common name is pine and cedar respectively, both from the family Pinaceae. Pine and cedar were collected from the north of Iraq then the medicinal plants were authenticated by Iraqi National Herbium (Abu Ghraib). The branches of the plant without the needle were washed thoroughly by tap water to remove dust and dirt, dried by placing the clean plant material in a shade for a period of seven days at 25ºC. and chopped into small pieces.

2.2 Extraction of terpene

A sample (10 g) of chopped materials were placed in thumble and subjected to extraction in a Soxhlet extractor using hexane (200 ml) for 24 hour. The extract was filtered using Whatman filter paper No. 1 and the filterate was evaporated to dryness using rotary evaporator (Heidolph Germany) by removing hexane from the solution below 45ºC under reduced pressure, the yield is 200mg solid compound.

Fig. 2.1: *P. halepensis M.*

Fig. 2.2: *C. libani A.*

2.3 Extraction of lignan

When all the terpene have been extracted by hexane, we add another solvent for the extraction of lignan that we use 200ml of aceton /water 9/1 for 24 hr or until the solvent change to colorless, then the extract was filtered using Whatman filter paper No. 1. The filterate was evaporated to dryness using rotary evaporator by removing acetone/ water from the solution below 45ºC under reduced pressure; the yield is 1g solid compound.

2.4 Qualitative analysis

Both parts of the extract were analyzed by TLC:-

1- Hexane extract

Solvent system: - benzene/methanol 9:1.
Spray reagent: - Halphen hicked (Ccl4/phenol 2:1).
Then heating in oven [89].

2- Acetone/ water extract

Solvent system: - ethanol/ dichloromethane 79:3.
Spray reagent: - ethanol/sulphuric acid 1:1.
Then heating in oven [90].
Standard use: - free HMR prepared from HMR potassium adduct (260mg) received from Linnea company dissolved in ethanol/ water 1:1 (5µl). Sodium hydrogen sulphate (10% 4ml) was added to keep the pH (4). Ethyl acetate (10ml) was added and the phases were separated. The organic layer was dried with anhydrous sodium sulphate, filtered and evaporated in vacuo to produce free HMR (203 mg 78%).

2.5 GC/MS analysis

Both parts of the extract were analyzed by QP 2010 ultra Shimadzu GC/MS apparatus. Carrier gas was helium 99.9999٪; GC- Colum non polar.

Program temperature	Hold time	Rate
80 □ C	1 min.	12.5 ^{o}C /min.
150 □ C	1 min.	10 □ C / min.
225 □ C	1 min.	7.5 □ C / min.
300 □ C	1 min.	5 □ C / min.

MS ion source, temp. 150 □ C, Interface 280 □ C, Ion source type: - FID

Injection mode: - split

2.6 Collection of test organism and preparation of stock culture

Clinical microorganisms were received from Almustansiriyah University/ College of Science/ department of Microbiology confirmed by Gram staining and culturing in appropriate selective media.

Microorganism used in the experiment

- Gram positive bacteria:-

  Staphylococcus aureus

  Streptococcus pneumoniae

- Gram negative bacteria:-

  Escherichia coli

  Salmonella typhi

  Klebsiella pneumoniae

  Acinetobacter spp.

  Pseudomonas aeruginosa

  Proteus vulgaris

2.7 Preparation of media

The required quantities of nutrient agar (Oxoid U.K) were prepared and poured into conical flask. The flasks containing the media were plugged with cotton wool and sterilized in an autoclave. After sterilization, nutrient agar medium was poured aseptically into sterilized Petri dishes. A sterile environment was maintained during pouring to avoid contamination. The medium was allowed to solidify in Petri dishes for about an hour before placed in an inverted position (to avoid evaporation of water from the medium within the plates) in an incubator at 37ºC for 24 hrs.

2.8 Estimation of antibacterial activity

The extracts (Hexane and Watery) for the two plants were dissolved in methanol to obtain final concentration of (100, 50, 25) mg/ml and sterilized by filtration through a 0.22 Millipore filter.

The agar well diffusion method was used to determine antibacterial activity of extracts. All bacterial stock cultures were freshened by streaking using a sterile inoculation loop on nutrient agar medium plates in a laminar flow hood, then incubated at 37ºC for 24 hrs. After 24 hrs, the inoculate diluted in sterile saline solution to a final concentration of 10^6 colony forming units (cfu)/ml (adjusted to 0.5 MF standard). The diluted bacteria then spread on a Muller-Hinton agar (Oxoid U.K), six diameter wells were punched into the Muller-Hinton agar, and filled with (100, 50, and 25) mg/ml of extract, solvent (methanol) was used as a negative control while ciprofloxacin (10μg/disc) was used as a positive control.

Plates were incubated at 37°c for 18-24 hr, after overnight incubation the diameter of the zone of inhibition around the well was measured in mm and recorded for *P. halepensis M.* And *C. libani A.*

3. Results and Discussions

3.1 Lipophilic fraction

In this study hexane extract showed an efficient antibacterial activity against most of the bacteria used except *Proteus vulgaris*. This bacteria showed resistance to all concentration used. The most sensitive microorganism was *Klebsiella pneumonia*, *Pseudomonas aeruginosa and Staphylococcus aureus* (inhibition zone, 20, 20 and 19 mm respectively). *Escherichia coli* were sensitive to terpene fraction while resistant to the broad spectrum antibacterial ciproflaxacin (Fig. 3.1).

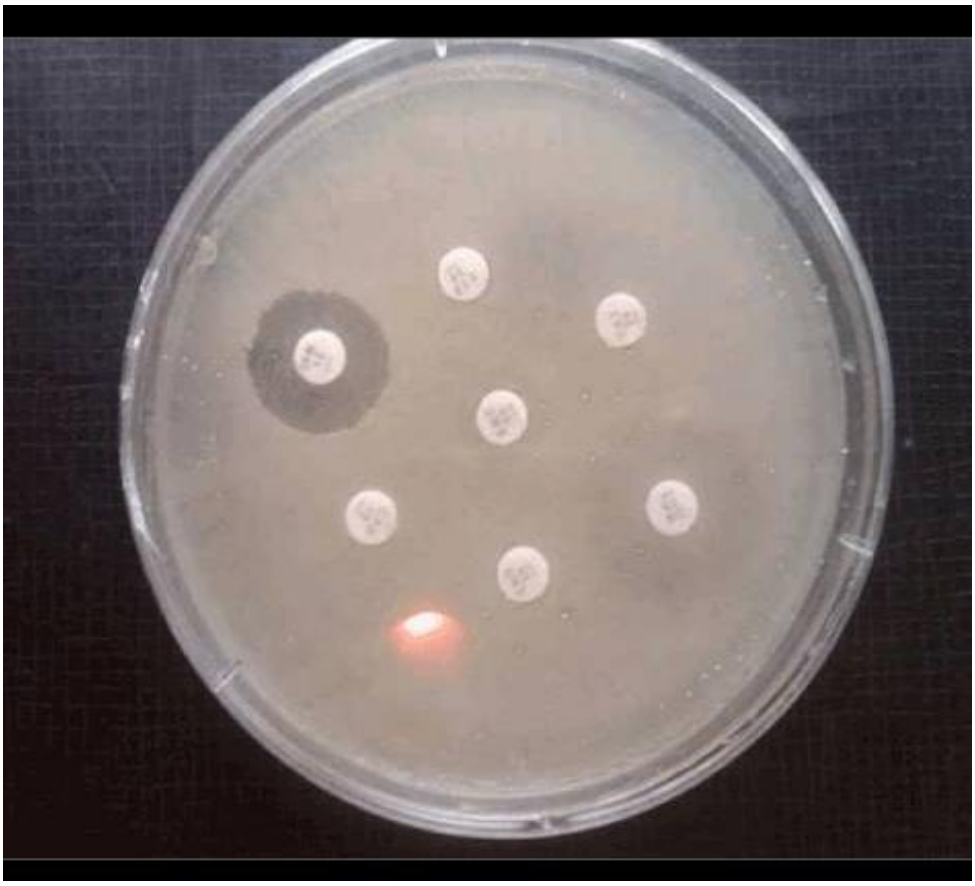


Fig. 3.1: *E. coli* exhibits positive to extract and resistant to antibiotics

The result of the current study revealed that terpene fraction of *P.halpensis M.* has antibacterial properties (Table 3.1)

Table 3.1: Antibacteriale effect of terpene of *P. halepensis* M.

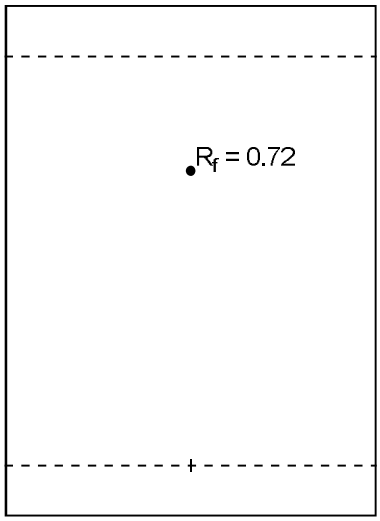
Name of organism	Diameter of zone of inhibition in millimeter (mm)				
	Conc. Of the extract in mg/ml			methanol	Ciprofloxacin 10 µg/disc
	100	**50**	**25**		
Staphylococcus aureus	19	12	11	negative	24
Streptococcus pneumoniae	17	14	12	negative	35
Proteus vulgaris	negative	negative	negative	negative	27
Esherichia coli	13	12	10	negative	negative
Klebsiella pneumoniae	20	19	14	negative	24
Pseudomonas aeruginosa	20	13	12	negative	35
Salmonella typhi	11	15	15	negative	22
Acinetobacter spp.	10	20	16	negative	26

C. libani A. terpene fraction also exhibit antibacterial activity. *Proteus vulgaris* was the most sensitive bacteria (inhibition zone 17 mm). *E. coli* was sensitive only in high concentration 100 mg/mL (Table 3.2).

Table 3.2: Antibacterial effect of terpene of *C. libani A.*

Name of organism	Diameter of zone of inhibition in millimeter (mm)				
	Conc. Of the extract in mg/ml			methanol	Ciprofloxacin 10 µg/disc
	100	**50**	**25**		
Staphylococcus aureus	14	13	12	negative	24
Streptococcus pneumoniae	negative	12	11	negative	35
Proteus vulgaris	17	16	15	negative	27
Esherichia coli	7	negative	negative	negative	negative
Klebsiella pneumoniae	negative	12	10	negative	24
Pseudomonas aeruginosa	16	12	10	negative	35
Salmonella typhi	11	12	10	negative	22
Acinetobacter spp.	17	15	13	negative	26

The antibacterial activity of the plants from the family pinaceae is attributed to the presence of the terpene acids. TLC analysis of hexane fraction show ablue spot at 0.72 which revealed to abietic acid as shown in fig. 3.2.



extract of pine
solvent system: benzene/methanol 9/1

Fig. 3.2:- TLC for the hexane extract of the plant

Gas Chromatography /Mass Spectroscopy analysis of hexane fraction revealed the presence of abietic acid M^+ 302 as a terpene which may be responsible for this anti bacterial activity in both plants. Retension time was 20 mins for each extract indicate the ocuurence of the same terpene acid in both plants (Fig. 3.3, 3.4, 3.5, 3.6). The mechanism of action of terpene is speculated to involve membrane disruption by the lipophilic compounds [91].

Abietic acid possesses a rigid lipophilic ring system and a carboxylic acid function. Bacteriolytic action of abietic acid is associated with interactions and lysis of cell membranes. Recent study revealed strong interactions between acid and model phosphatidiylcholine membranes, consistent with the insertion of the terpenoid into the membrane, with its carboxyl group in close proximity to the phospholipid carbonyl, which acted as a hydrogen-bond-acceptor group [92].

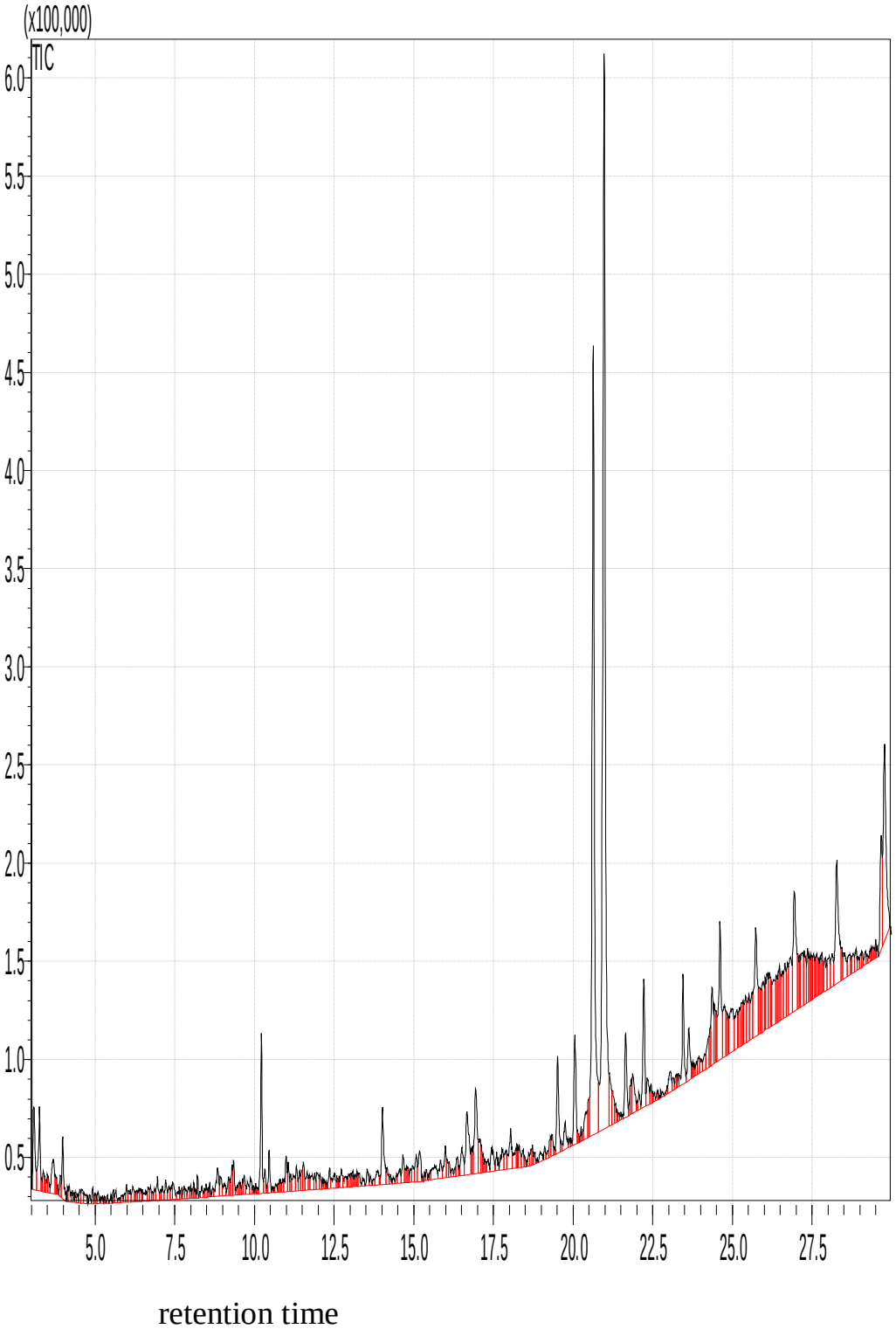
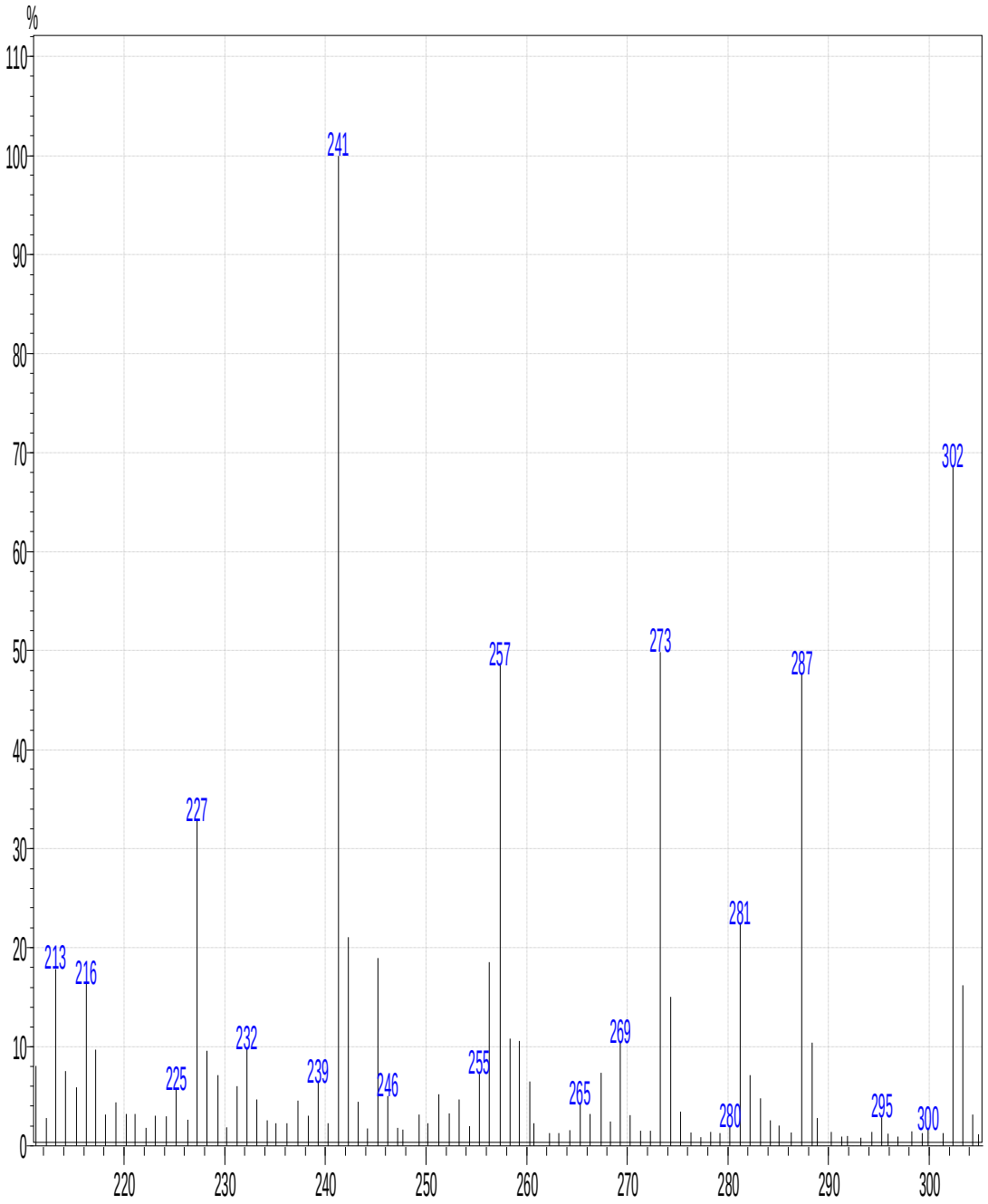


Fig. 3.3: GC/MS analysis of hexane extract of *C. libani* A.

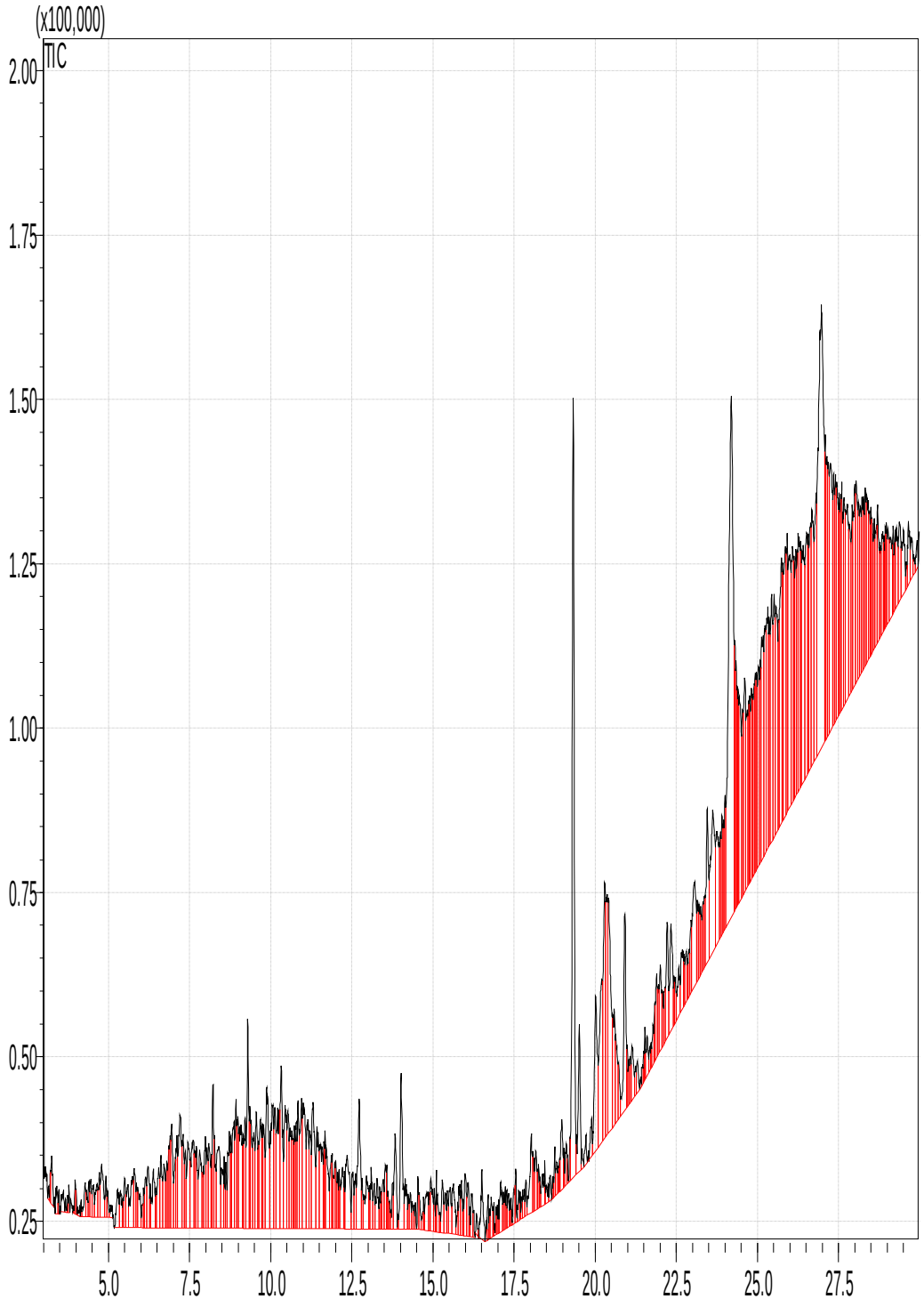
Abundance



m/z

Fig. 3.4: GC/MS of *C. libani A.* hexane extract at 20 min.

Relative abundance



Retention time

Fig. 3.5: GC/MS analysis of hexane extract of *P. Halepensis M.*

Abundance

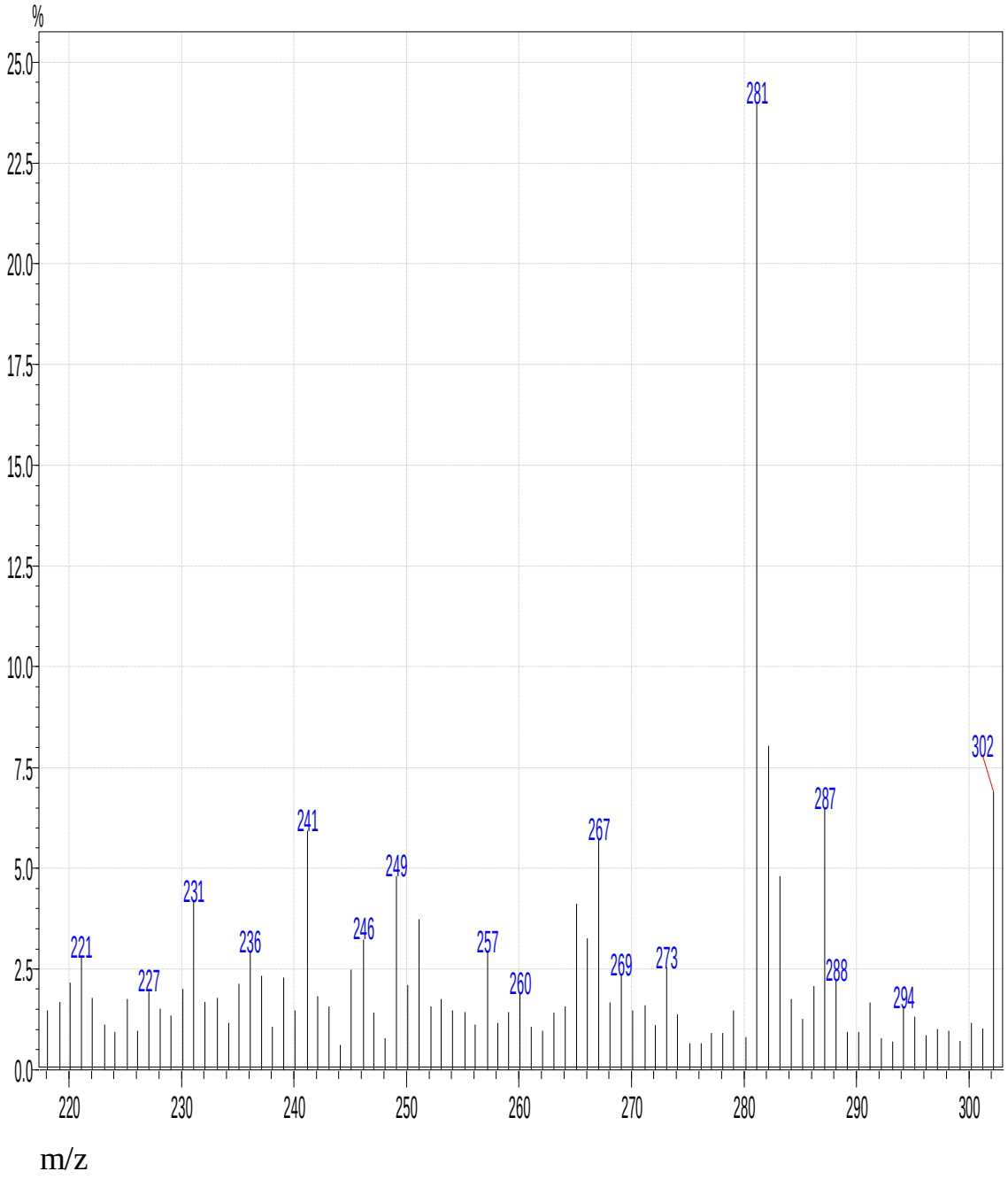


Fig. 3.6: GC/MS for hexane extract of *P. Halepensis M.* at 20 min.

Fragmentation pattern support this ion peak 302 as compared with literature (Fig. 3.7) [93].

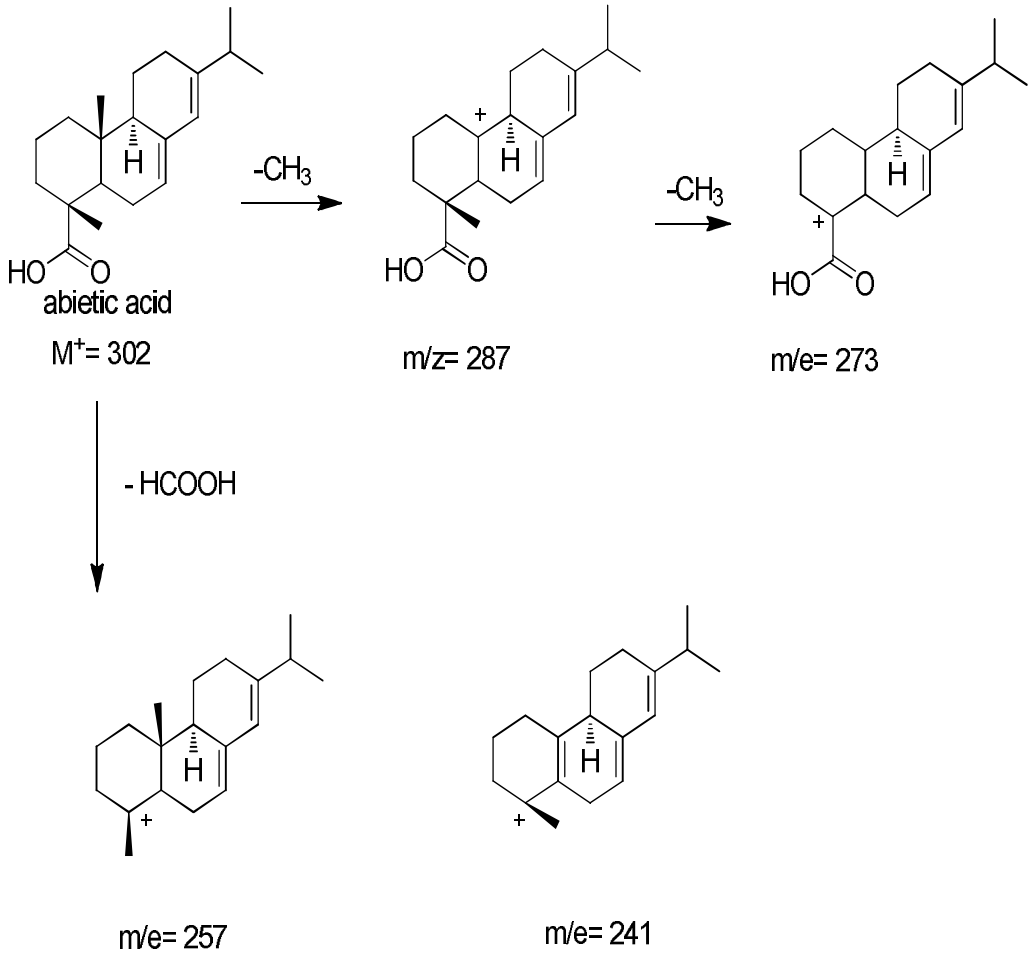


Fig. 3.7: Fragmentation pattern of abietic acid from hexane fraction

Cedrus also contain ion M^+ 137 which is not found in *pinus* indicate the presence of the antibacterial volatile oil pinene as compared with the literature (Figure 3.8) [7].

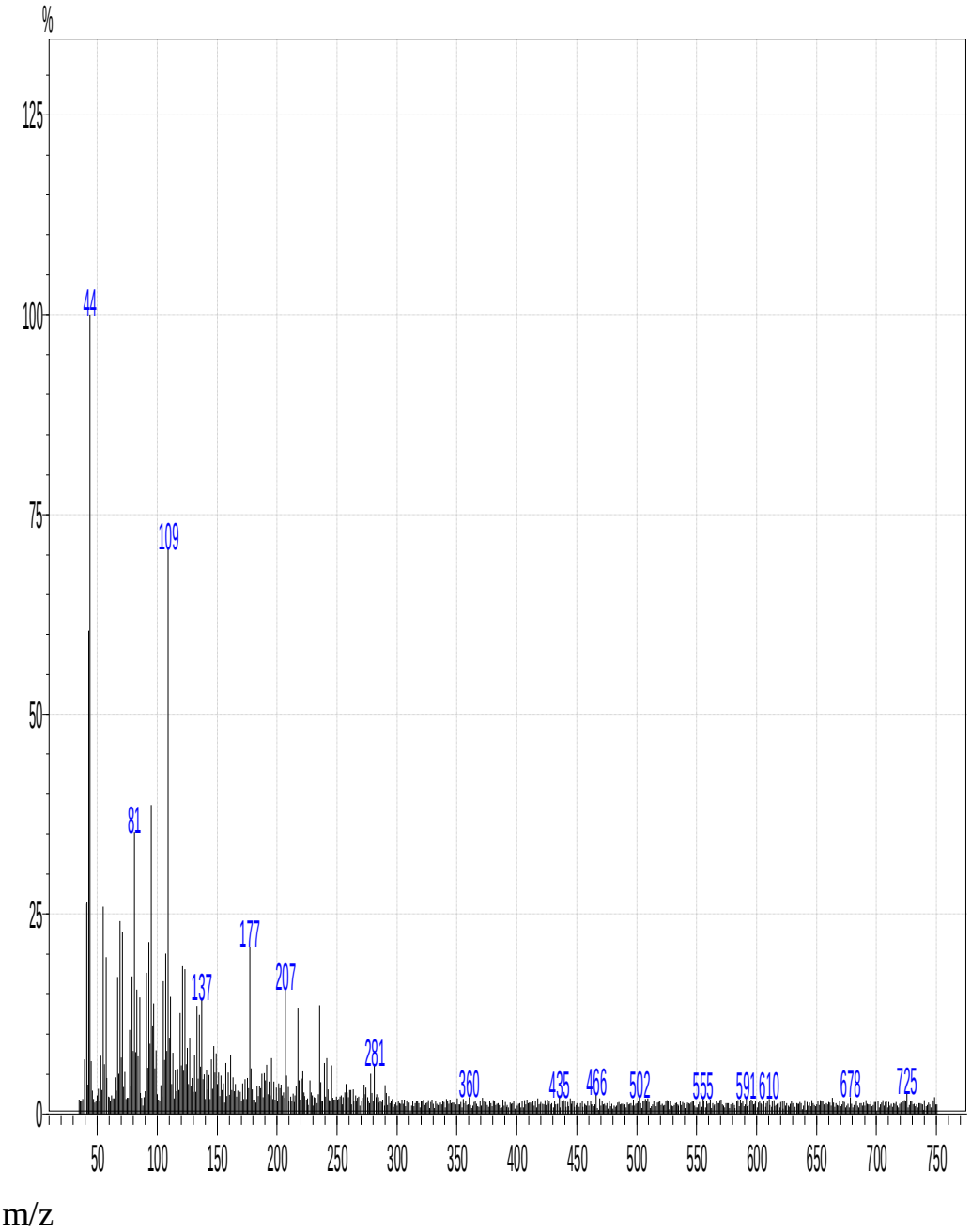


Fig. 3.8: GC/MS of *Cedrus* showing pinene volatile oil

Fragment of this oil is shown in Fig. 3.9.

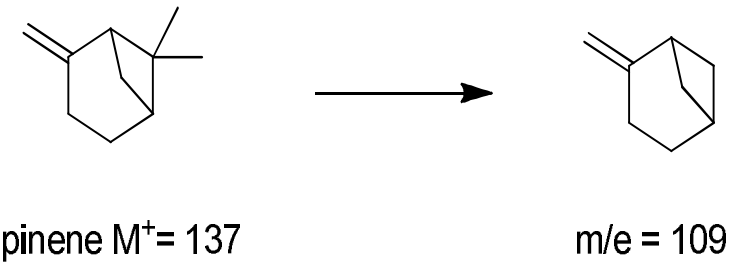


Fig. 3.9: Fragmentation of pinene from cedrus

The influence of a-pinene on the cytoplasmic membrane integrity and mitochondrial function was analyzed. It is clear that the cytoplasmic membrane was disrupted by the terpene. The vegetative cells of bacteria exhibited massive leakage of their cellular constituents when exposed to a-pinene [94].

3.2 Hydrophilic fraction

Hydrophilic part of the extract of *P. halepensis M.* also show antibacterial activity for the bacteria used in the experiment except *Escherichia coli* and *Streptococcus pneumonia*. Both of them showed resistance to all the concentration used. The most sensitive bacteria was *Salmonella typhi* (inhibition zone was 20mm) table 3.3.

Table 3.3: Antibacterial effect of hydrophilic extract of *P. halepensis M.*

Name of organism	Diameter of zone of inhibition in millimeter (mm)				
	Conc. Of the extract in mg/ml			methanol	Ciprofloxacin 10 µg/disc
	100	**50**	**25**		
Staphylococcus aureus	14	13	12	negative	24
Streptococcus pneumoniae	negative	negative	negative	negative	35
Proteus vulgaris	11	10	9	negative	27
Esherichia coli	negative	negative	negative	negative	negative
Klebsiella pneumoniae	12	10	8	negative	24
Pseudomonas aeruginosa	12	10	9	negative	35
Salmonella typhi	20	22	18	negative	22
Acinetobacter spp.	11	10	9	negative	26

Escherichia coli also show resistance to all the concentration of the hydrophilic extract of *C. libani A.,* as well as, *Staphylococcus aureus* do as shown in table 3.4

Table 3.4: Antibacterial effect of hydrophilic extract of *C. libani A.*

Name of organism	Diameter of zone of inhibition in millimeter (mm)				
	Conc. Of the extract in mg/ml			methanol	Ciprofloxacin 10 µg/disc
	100	**50**	**25**		
Staphylococcus aureus	negative	negative	negative	negative	24
Streptococcus pneumoniae	negative	25	24	negative	35
Proteus vulgaris	negative	15	20	negative	27
Esherichia coli	negative	negative	negative	negative	negative
Klebsiella pneumoniae	negative	13	11	negative	24
Pseudomonas aeruginosa	negative	negative	13	negative	35
Salmonella typhi	negative	12	10	negative	22
Acinetobacter spp.	negative	13	23	negative	26

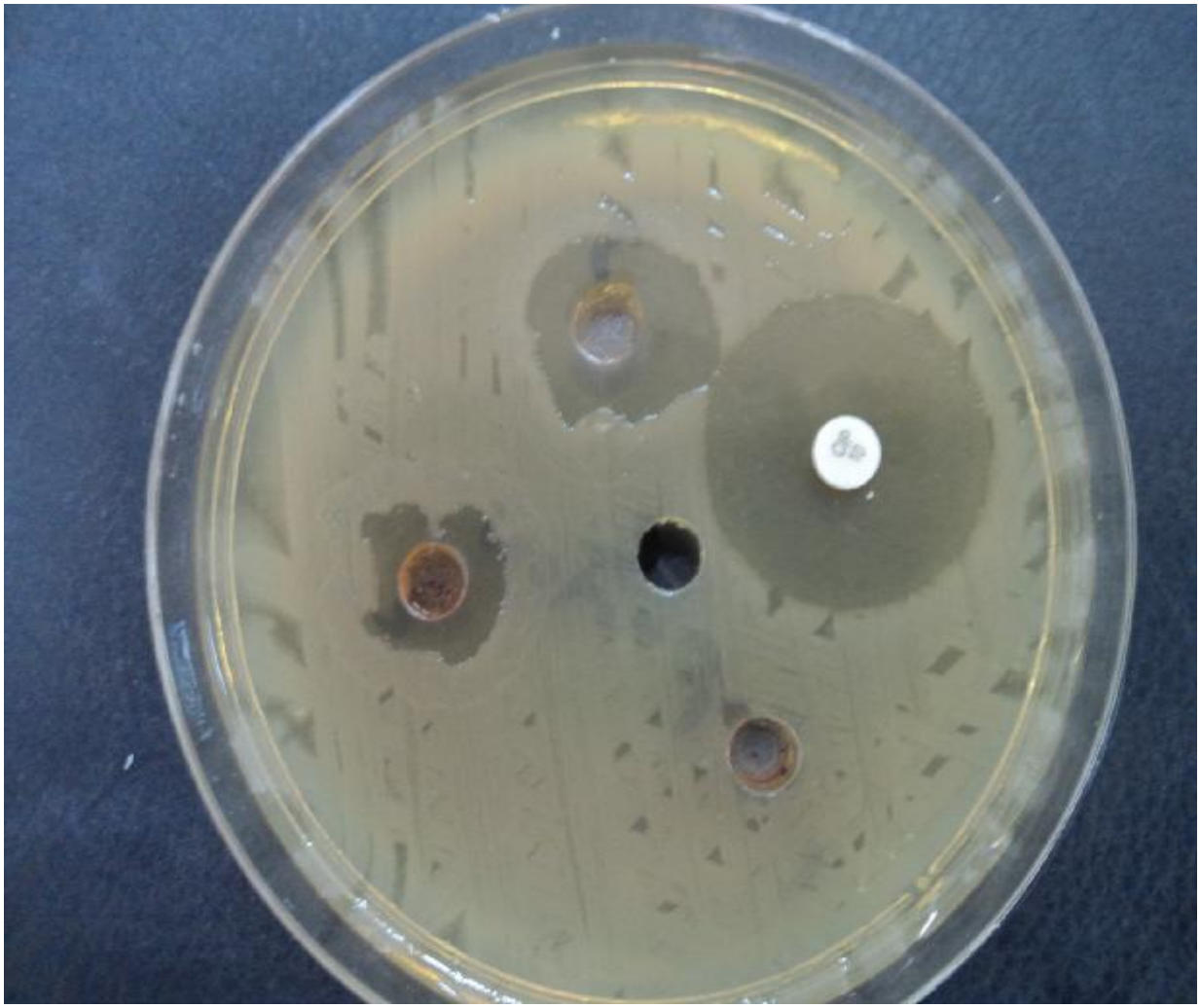


Fig. 3.10: Zone of inhibition in mm of *Cedrus* (acetone/water extract) on *Proteus vulgaris*

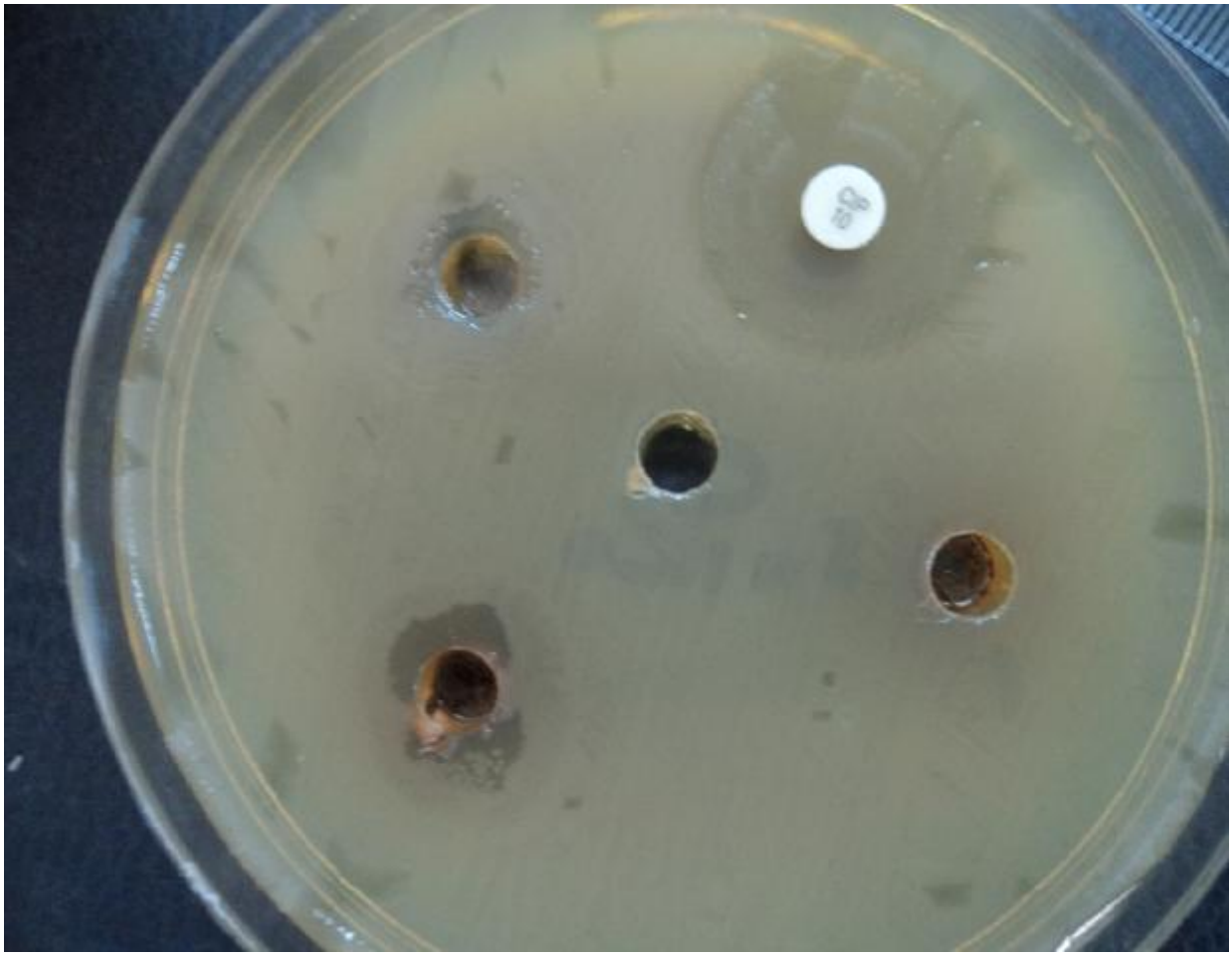
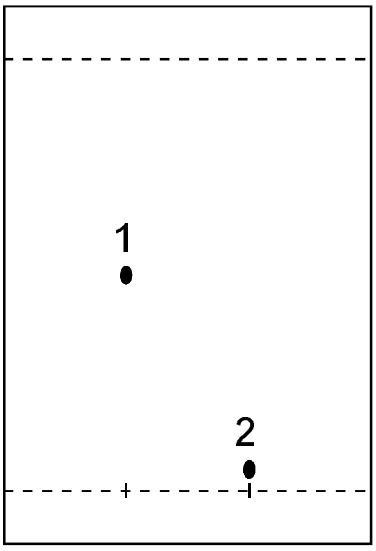


Fig. 3.11: Zone of inhibition in mm of *Cedrus* (acetone/ water extract) on *Salmonella typhi*

The antibacterial activity of acetone/water extract of pinaceae may be due to the presence of phenolic compounds particularly the lignan. The concentrated acetone/ water extract was analyzed by TLC. The analysis showed that both plants did not contain Hydroxymatieresinol (HMR) (a

compound with R_F 0.5) as compared with a standard sample of HMR. The extract contains more polar lignan than HMR fig. 3.12.

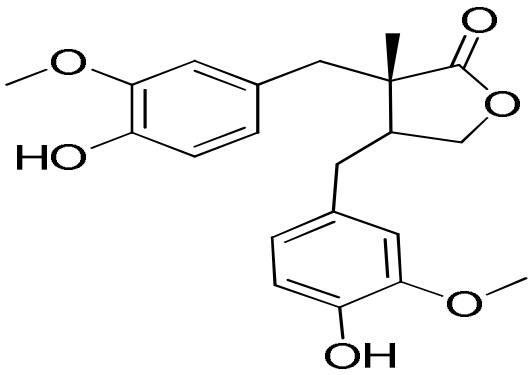


1:- HMR standard
2:- extract of pine
solven system:- EtOH/DCM 7%

Fig. 3.12: TLC for the hydrophilic extract compared with standard HMR

The most powerful technique for determining the resolution of polar lignans is GC after silylation, which is widely used to identify lignans in combination with MS. On the other hand; it is difficult to use TLC to separate polar lignans from flavonoids and stilbenes due to their similar solubility [90].

Gass chromatography /Mass Spectroscopy analysis of the extract reveals the presence of nortrachelogenin **22** M+ 374 as we can see in figure 3.13, 3.14, 3.15 and 3.16.



Nortrachelogenin **22**

The activity of lignan extract might be due to their ability to combine with bacterial cell wall and therefore, inhibiting the microbial growth. [95]

The sites and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity, also the more highly oxidized phenols the more inhibitory. The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins [96].

The resistance of the bacteria to the extract may be due to the efflux pump mechanisms which decreases the intracellular concentration of toxic compounds notably in *Staphylococcus aureus* with identified pumps and in

Gram-negative bacteria such as *Pseudomonas aeruginosa*, and *Escherichia coli* [97].

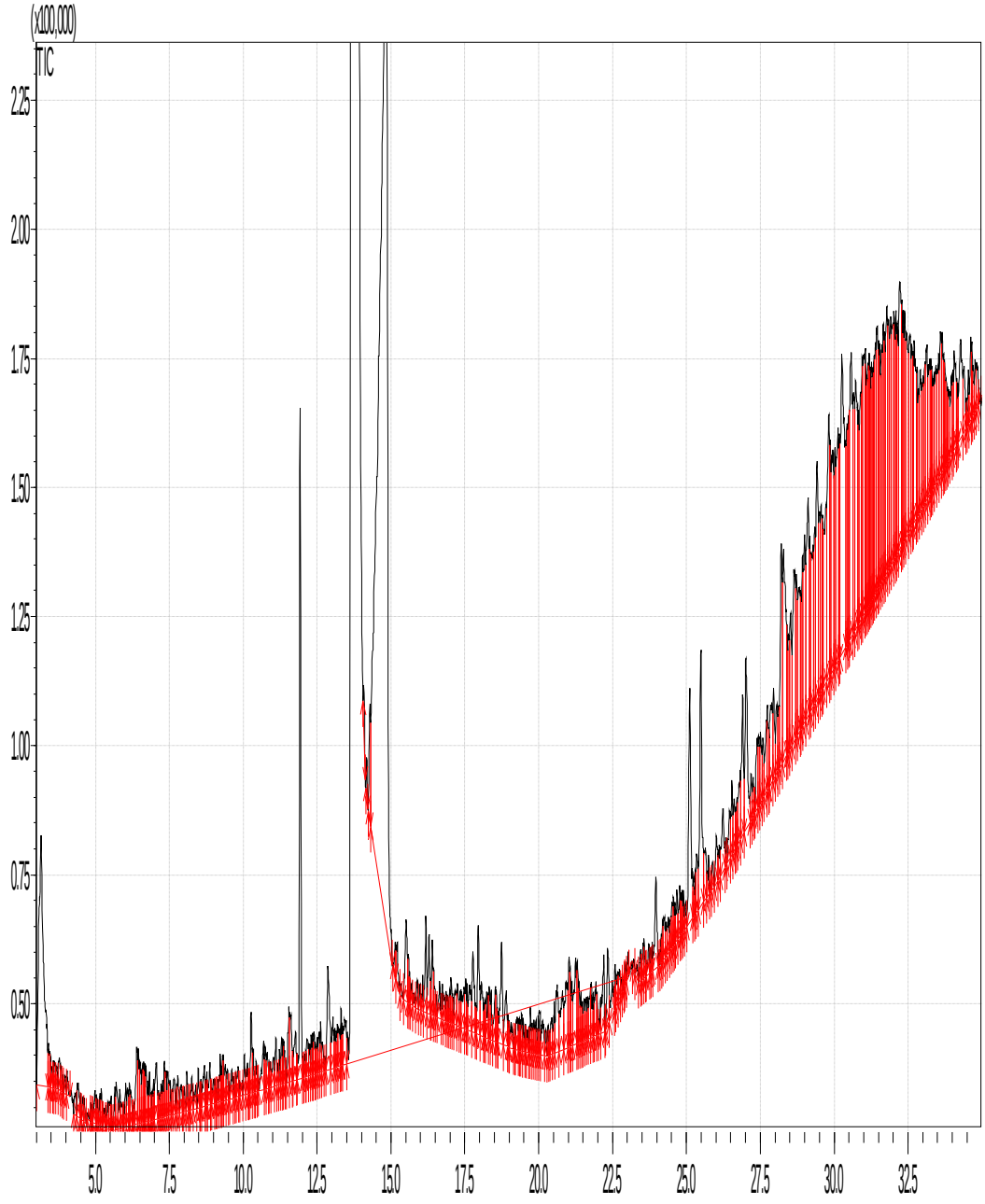


Fig. 3.13: GC/ MS analysis of acetone/water extract of *C. libani A.*

Abundance

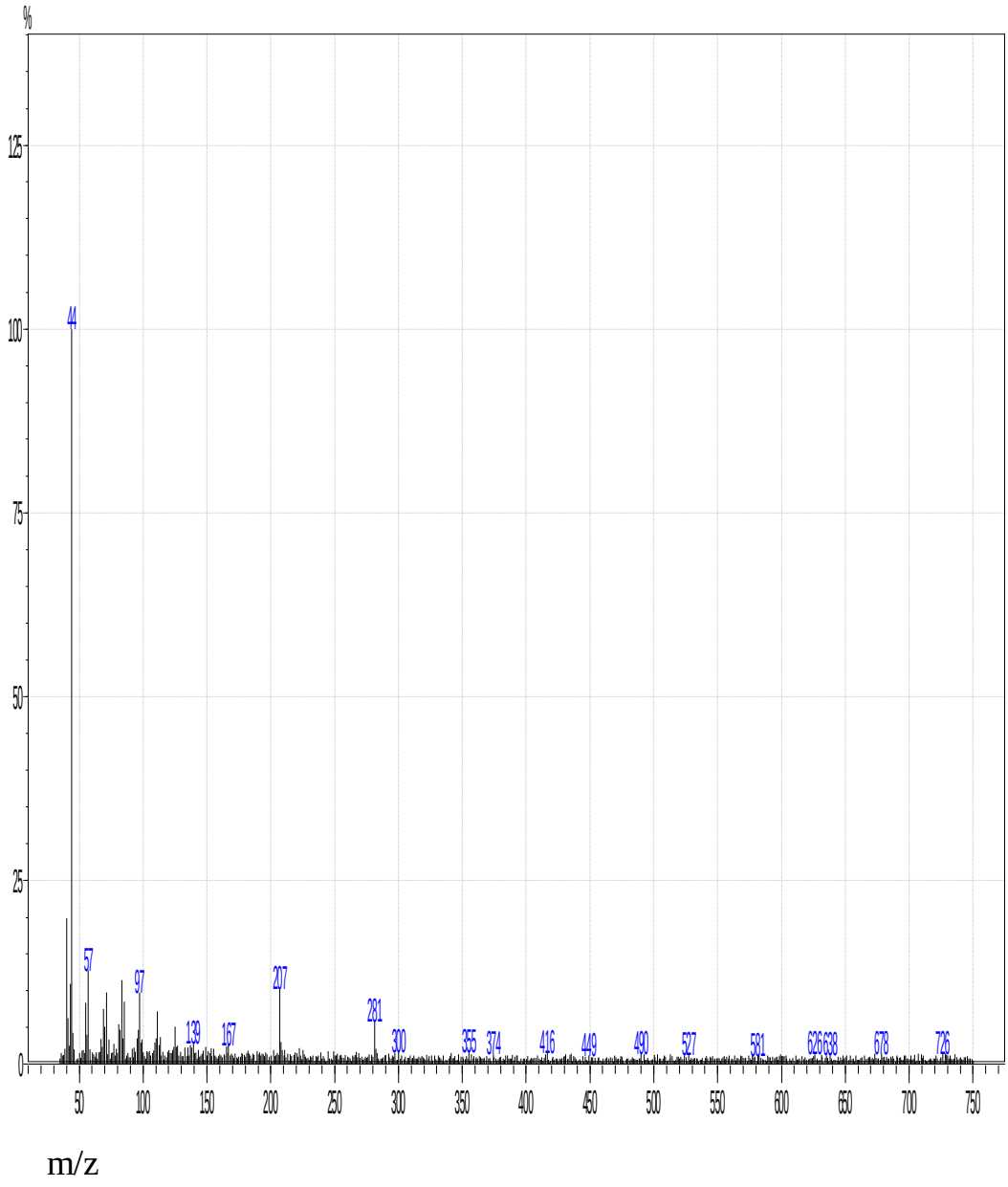
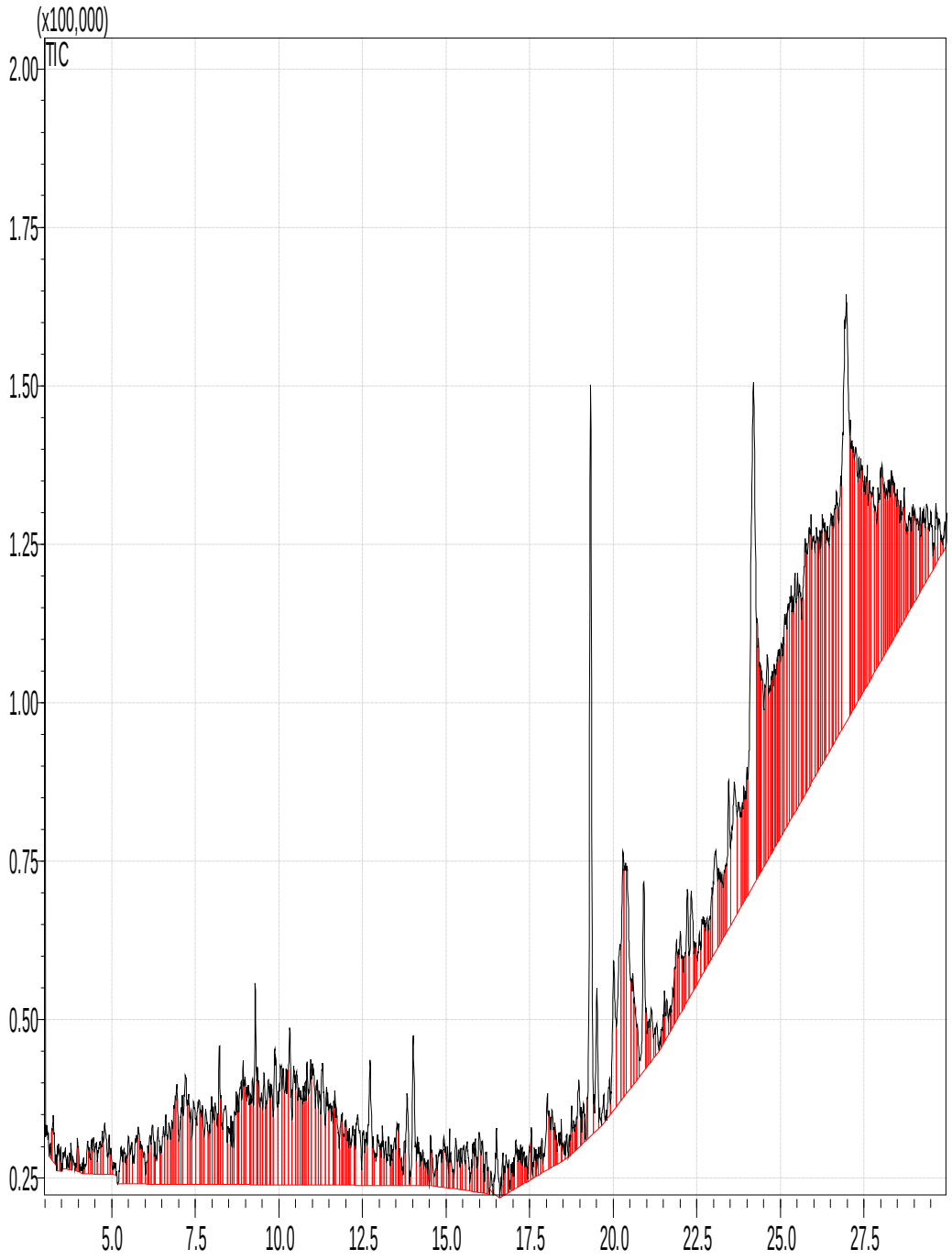


Fig. 3.14 GC/MS for acetone/water extract of *C. libani A.* at 22

R_t

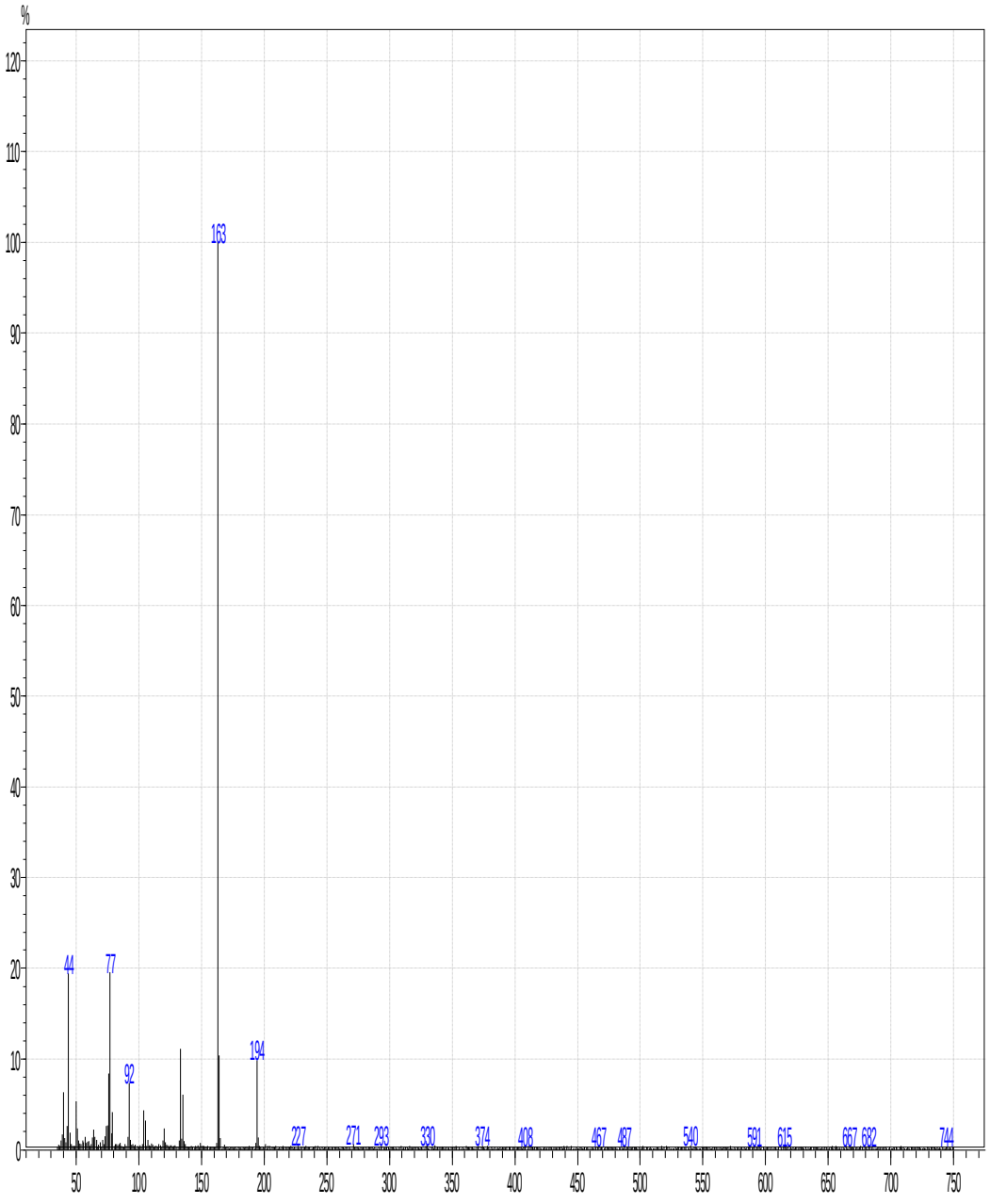
Relative abundance



m/z

Fig. 3.15: GC/MS analysis of aceton/ water extract of *P. halepensis M.*

Abundance



m/z

Fig. 3.16 GC/MS for acetone/water extract of *P. halepensis M.* at 22 R_t

The results indicated that hexane fraction exhibit more potent antibacterial activity than the aceton/ water fraction this is may be due to the presence of synergy between the compounds and other constituents of the extracts with various degrees of antibacterial activity [98].

Also in Gm-ve bacteria the nonspecific diffusion channels of OM, the porins, limit the influx of small hydrophilic agents, because their channels are quite narrow. In addition, the lipid bilayer domain of the OM is unusual in its extreme asymmetry by having the outer leaflet composed nearly exclusively of lipopolysaccharides containing only saturated fatty acid chains, Also the very low fluidity of this outer leaflet decreases the spontaneous permeation rates of hydrophobic probe molecules nearly by two orders of magnitude, when compared with the conventional phospholipid bilayers containing many unsaturated fatty acid residues [99].

3.3 Conclusions and Future recommendations

- *Pinus halepensis* and *Cedrus libani* grown in the north of Iraq but *Picea glucca* and *Pinus contorato* present in plantations of Iraq but they are doubt to be native to Iraq
- Different extract from the branches of both *Pinus halepensis* and *Cedrus libani* showed significicant activity against Gram positive and Gram negative bacteria.
- Lipophilic part show more potent antibacterial activity than hydrophilic part as well as it show activity against multidrug resistance *E. coli*.

Future work will include:-

- Modification of GC/MS analysis of the sample to investigate the occurrence of lignans and other phytochemicals which may found in the plants.
- Another goal is to use preparative HPLC to obtain nortrachelogenin which could be used to investigate the biological activities of this lignan.

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الخلاصة:-

استخدمت افرازات اشجار الصنوبر كعلاج للجروح في الطب التقليدي .هذه النباتات تحتوي على تربينات مثل حمض الأبيتيك والايزوبيمارك ، كما أنها تحتوي على قشور مثل نورتراكيلوجينين. كل هذه المكونات تمتلك مجموعة متنوعة من النشاطات الطبية واحدى هذه النشاطات هو النشاط المضاد للبكتيريا وهذا مهم جدا لأنه بالرغم من أن الصناعات الدوائية قد أنتجت عددا من مضادات حيوية جديدة في العقود الثلاثة الماضية، الا ان مقاومة الكائنات الحية الدقيقة لهذه العقاقير مازالت في تصاعد. وأن تطوير سلالات بكتيرية مقاومة للمضادات الحيوية المستخدمة حاليا سيؤدي الى فشلها في إنهاء العديد من الالتهابات البكتيرية . لذلك فأن البحث عن المركبات التي يمكن دمجها مع المضادات الحيوية في علاج الالتهابات المقاومة للأدوية قد يكون بديلا للتغلب على مشكلة مقاومة البكتيريا .وتعتبر المستخلصات النباتية الحل الامثل لتجاوز هذه المقاومة بوصفها مصادر حقيقية للمركبات المثبطة للمقاومة البكتيرية .لقد تم اختبار مستخلص الهكسان والأسيتون /ماء للصنوبر الحلبي والارز اللبناني ضد ثمانية جراثيم مختلفة من بكتريا ايجابية الجرام واخرى سلبية الجرام باستخدام طريقة الانتشار تم تحديد مناطق التثبيط بالمقارنة مع المضاد الحيوي واسع الطيف سيبروفلوكساسين كعنصر تحكم إيجابي.

أظهرت نتائج تحليل النبات بواسطة جهاز الاستشراب الغازي/ التحليل الطيفي الشامل ان المستخلص التربيني لكلا النباتين يحتوي على حمض الابيتيك الذي قد

يكون سبب التثبيط البكتيري للنبات, بينما مركب البابنين موجود فقط في الارز اللبناني .

بينما تحليل الجزء المائي لكلا النباتين اظهر وجود اللكنان نورتراكيلوجنين.

كلا النباتين اظهر نشاط تثبيطي للبكتريا المستخدمة ,أكثر الاجناس حساسية للجزء المائي للصنوبر هو السالمونيلا التيفوئيدية ,بينما كانت العقدية الرئوية اكثر الاجناس حساسية للارز اللبناني.

أظهر المستخلص المحتوي على التربينات نشاط تثبيطي للبكتريا اقوى من الجزء المائي, وكانت بكتريا الالتهاب الرئوي الكلبسيلة أكثر الاجناس حساسية للمستخلص التربيني للصنوبر الحلبي بينما كانت بكتريا البروتيوس أكثر الأجناس حساسية للمستخلص التربيني للأرز اللبناني.

جمهورية العراق
وزارة التعليم العالي و البحث العلمي
الجامعة المستنصرية
كلية الصيدلة

البحث عن مركبات مضادة للبكتريا في النباتات الطبية العراقية من العائلة الصنوبرية

رسالة مقدمة إلى فرع العقاقير والنباتات الطبية والى لجنة الدراسات العليا في كلية الصيدلة/الجامعة المستنصرية كجزء من متطلبات الحصول على شهادة الماجستير في علوم الصيدلة (العقاقيروالنباتات الطبية)

من قبل

رشا عبد الكريم عبد القادر

(بكالوريوس صيدلة ٢٠٠٧)

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