Original Research Article

Evaluation of Antimicrobial Activity of *Commiphora myrrh* against standard bacterial strains and clinical isolates with chemical analysis profiling

ABSTRACT

Aims : The objective of present study was to investigate the chemical analysis biological activates of commiphora myrrh the chemical analysis of myrrh was analyzed several ways.

Study design: where wet digestion was used to estimate the concentrations of a number of chemical elements in it, which are of great importance to humans and are also attributed to many of its medicinal uses.

Place and Duration of Study: clinical isolates from wound infections obtained from laboratory of marjan hospital Hilla city Iraq during period Fed. 2020 which include four E.coli ,4.s.aureus and 4 psendomonas aurogenosa. All clinical isolates were classified and by laboratory of marjan hospital :Hilla city Iraq .

Methodology: In order to know the nature of the groups present in it, in addition to the quality of the organic materials, FTIR analysis and G.C analysis were used by using the ethanolic extract, where some of the organic materials within their compositions in identified.

Results: The antimicrobial potential of ethanolic extracts of myrrh were studied against many standard strains of gram positive and gram negative bacteria and(12) clinical isolated from patients with wound infections obtained from the bacteriology section of the clinical microbiology laboratory of marjan hospital Hill city/Iraq during period feb.2020 to Nov.2020. the clinical isolates include(4) isolates of staphylococcus aureus, (4) isolates of E.coli and (4) isolates of psendomonas auroginosa , and it was confirmed using the usual methods of diagnosis. The broth dilution method was used for determination of the MICs of (minimal inhibitiory concentration) of myrrh extract against pathogens under study. Six concentration(80 ,60 ,30 ,12 ,6 ,3 mg/mL)of myrrh extracts were tested.

Conclusion: The result revealed that the highest acticity was against S.aureus at concentration (80 ,60 ,30 mg/mL) which showed completely inhibition of the growth (100%). While the gram-negative bacteria E.coli and P.auroginosa the concentration (80 – 60 mg/mL) showed 100% inhibition in contrast the concentration (12 ,6 ,3 mg/mL) showed no avtivity of myrrh extract against all pathogens under study. The result indicates that myrrh is an antibacterial agent that can be used in the future by making appropriate doses

Key words: myrrh ,S.auras ,E.coli , p.aerginosa , myrrh extraction , myrrh chemical analysis

1. INTRODUCTION

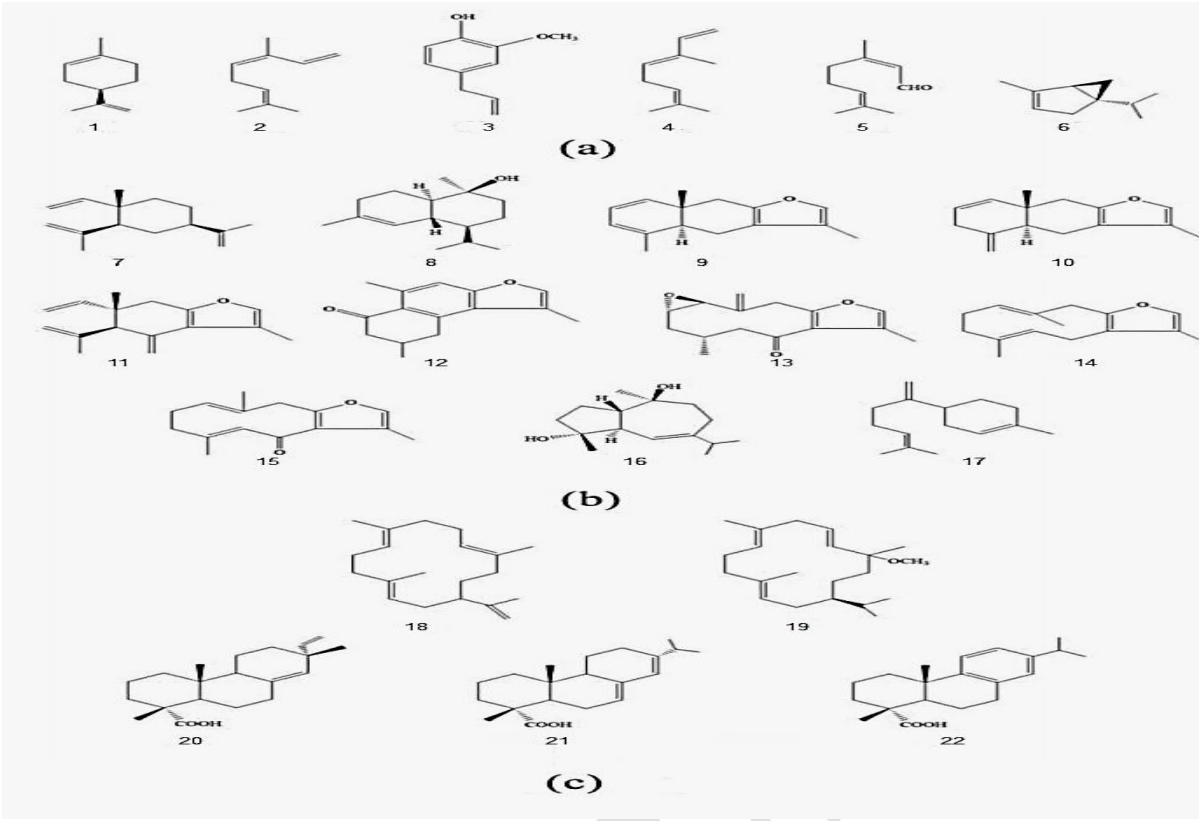
Myrrh is one of the important and widely used medicinal plants, as it was used by the ancient Egyptians as medicine (E.S.H. El Ashry1 et al 2003). It has an economically and culturally ,valuable product obtain from commiphora myrrh tree. The natural plant product used in several pharmaceuticals industries ,cosmetics and others .there is many local applications in medicinal ,hygienic and insecticide (C A Michie and E Cooper 1991). It is widely used in traditional medicines for treatments of wide variety of infectious disease. In more recent times, myrrh has been used as a medical antiseptic, as it was used in the mouth when there are cases of ulcers and infections (Lumir O. Hanuša et al 2005). Scientific classification of myrrh. It belong to kingdom: Plantae

,division : magnoliophyta class:magnoliopsida :order: Spindale's ,family burseraceae :genus commphorra (L. O. Hanuš et al 2008). It is also a small tree with a short true myrrh plant produced by C. Myrrha. Different types of trees are also found in many places such as southern Arabia and northeastern Africa .Myrrh is reddish brown resinous material (Tucker ,O. 1986). The raw myrrh resin and its essential oil upon combustion produce bitter smoke and phenol. Myrrh can be used as antimicrobial ,antifungal and somewhat antiviral ,immune stimulant ,bitter ,circulatory stimulant ,anti-inflammatory and antispasmodic (Elashry E, 2003). myrrh a popular traditional natural medicine (Dolara,p 2000). As for the chemical composition of myrrh from a basic water-soluble gum and an alcohol-soluble resin (Fatani AJ 2010). Among the uses of myrrh is as an analgesic and is also used to clean wounds, as it was used for this purpose for more than 2000 years until the discovery of morphine (Mahboub.M and Mohanned.T 2016).

Myrrh is one of the ancient and important medicines because of its use by the ancient Egyptians on a large scale. Recent studies also showed that the extracts of the myrrh plant have great efficacy against various types of pathogens, and many of the compounds that were isolated from this plant were found to have antibacterial activity and proven It is through strong inhibition with minimum inhibitory concentration (MIC) values ranging from 0.2-2.8 Mg/ml (- Rhaman MM 2008 , Bo Cao, et al 2019). Some isolated compounded like sesquiterpenoids inhibited the different pathogens growth with (MIC) ranging between (4-256 Mg/ml) . The aim of the present studs was to evaluate the effect of ethanolic extracts of C.Myrrh as antibacterial against gram positive and gram negative pathologies isolated from patients. it tends not to dissolve very well in water (A.Pequreul et al 1993), Myrrh contains a 2-8% volatile oil (myrrhol), 23-40% resin (myrrhin), 40-60% gum, and a bitter principle10-25% (Sarah, and Mahmoud ,M.E 2008). . The gum contains polysaccharides and proteins, while the volatile oils consist of steroids, sterols and terpenes. Furanosesquiterpenes are also derived from the distinctive aroma of myrrh (Lina,A.N et al 2006). The main Chemical compounds of Myrrh (Obam.L.C, et al 2007).

Table (1):Main Chemical Compounds of Myrrh

No.	Chemical Class	Chemical Name	Plant Sources
1	Monoterpenes	limonene	*Commiphora quadricincta*
2		cis-β-ocimene	*Commiphora quadricincta*
3		eugenol	*Commiphora mukul*
4		trans-β-ocimene	*Commiphora tenuis*
5		(2Z)-3,7-dimethyl-2,6-octadienal	*Commiphora tenuis*
6		α-thujone	*Commiphora tenuis*
7	Sesquiterpenes	β-elemene	*Commiphora myrrha*
8		T-cadinol	*Commiphora guidottii*
9		furanocudesma-1,3-diene	*Commiphora myrrha*
10		lindestrene	*Commiphora myrrha*
11		curzerene	*Commiphora myrrha*
12		myrrhone	*Commiphora myrrha; Commiphora opobalsamum*
13		rel-1S,2S-epoxy-4R-furanogermacr-10(15)-en-6-one	*Commiphora myrrha*
14		furanodiene	*Commiphora myrrha*
15		1(10), 4-furanodien-6-one	*Commiphora erythraea*
16		guaianediol	*Commiphora opobalsamum*
17		β-bisabolene	*Commiphora guidottii*
18	Diterpenoids	(dl)-cembrene A	*Commiphora mukul*
19		(1E,4E,8E)-4,8,14-trimethyl-11-(1-methylethyl)-14-methoxycyclotetradeca-1,4,8-triene	*Commiphora mukul*
20		sandaracopimaric acid	*Commiphora myrrha*
21		abietic acid	*Commiphora myrrha*
22		dehydroabietic acid	*Commiphora myrrha*
23	Triterpenoids	β-amyrin	*Commiphora kua; Commiphora confuse*
24		cycloartan-24-ene-1α,2α,3β-triol	*Commiphora opobalsamum*
25		3-epi-α-amirone	*Commiphora myrrha*
26		mansumbinone	*Commiphora myrrha*
27		3,4-seco-mansumbinoic acid	*Commiphora molmol*
28		mansumbinoic acid	*Commiphorakua*
29		myrrhanone A	*Commiphora mukul*
30		myrrhanone B	*Commiphora mukul*
31		3β-hydroxydammar-24-ene	*Commiphora confuse*
32		3β-acetoxycycloartan-24-ene-1α,2α-diol	*Commiphora opobalsamum*
33	Steroids	(Z)-guggulsterone	*Commiphora mukul*
34		(E)-guggulsterone	*Commiphora mukul*
35		guggulsterone-M	*Commiphora wightii*
36		guggulsterol-Y	*Commiphora wightii*
37		pregn-4-ene-3,16-dione	*Commiphora mukul*
38		β-sitosterol	*Commiphora sphaerocarpa*
39	Lignans	erlangerins A	*Commiphora erlangeriana*
40		erlangerins B	*Commiphora erlangeriana*
41		(+)-sesamin	*Commiphora mukul*
42		picropolygamain	*Commiphora incisa*
43		polygamain	*Commiphora incisa*
44		diayangambin	*Commiphora mukul*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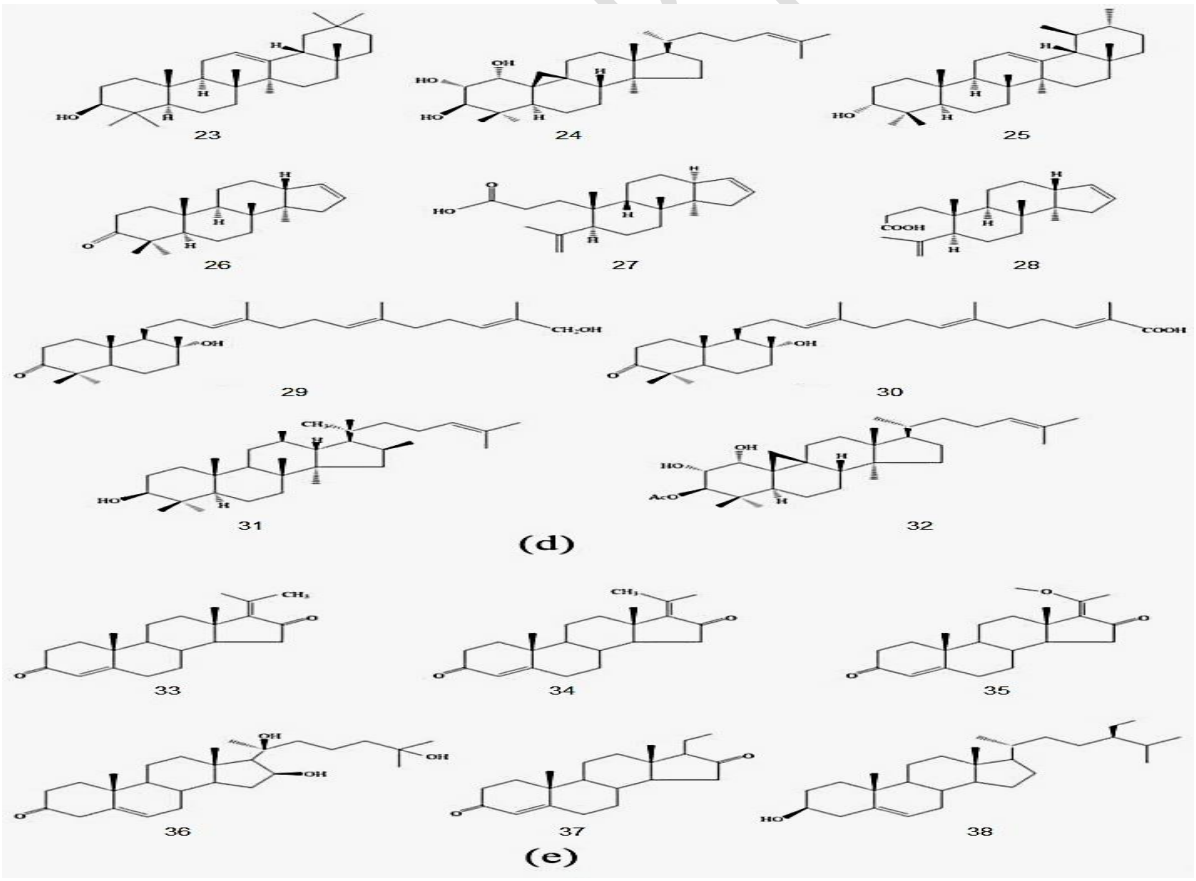
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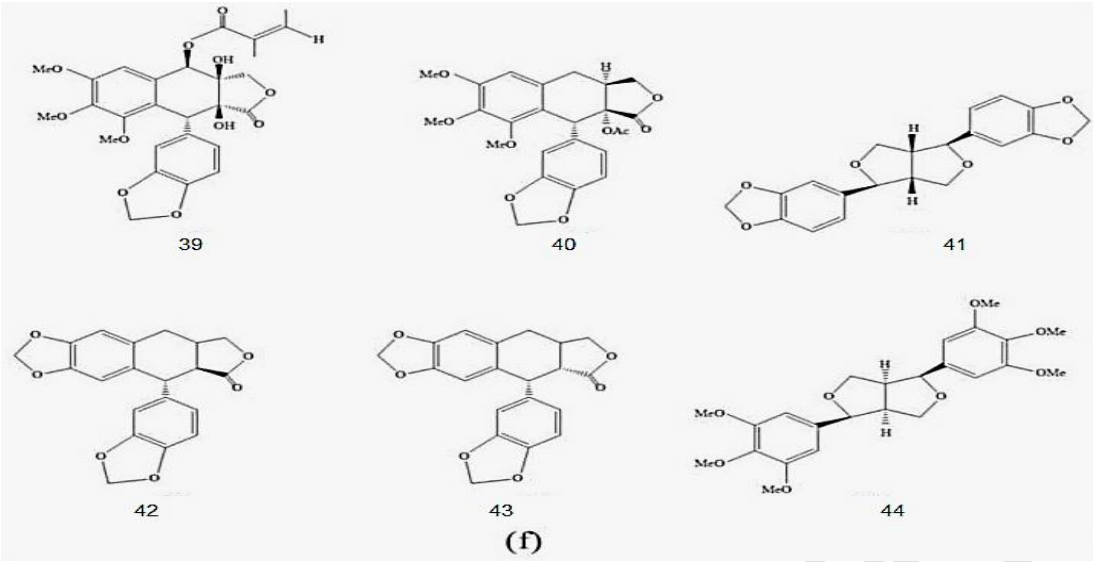


Chemical composition of some chemical compounds in myrrh:(a).Pentacyclic triterpenoids (b) tetracyclic triterpenoids (c) macrocyclic diterpenoids(d) essential oils

2. MATERIALS AND METHODS

1) extraction : Myrrh was obtained from spice shop (saudia Arabic)
2) evaluate the antimicrobial activity : The evaluation of antimicrobial activity of myrrh was done against many standard strains of gram positive and gram negative bacteria and clinical isolates from wound infections obtained from laboratory of marjan hospital Hilla city Iraq during period Fed. 2020 which include four E.coli ,4.s.aureus and 4 psendomonas aurogenosa. All clinical isolates were classified and by laboratory of marjan hospital :Hilla city Iraq .
Standard isolates *: Staphylococcus aurous NCTC 6571 , S. aurous ATCC 29213 , E.coli NCTC 5933 ,Pseudomonas auroginosa NCTC 6750 , Bacillus subtilize PCI 219, Klebsiella preumoniae ATCC 6308.*
All bacterial isolates were subjected to microscopically examination to ensure their purity and morphology and staining properties using gram staining technique.

2.1. Preparation Test Organisms

One ml of 24 hrs broth culture of the test bacteria were aseptically distributed on nutrient agar slant then incubated at 37C for 24hrs. the bacterial growth was harvested and washed off with sterile normal saline: produce suspension containing 10^{10} colony forming unit (CFU)/ml. then stored at 4C till used (Zhae.X et al 2018).

2.2. Determination of Antimicrobial Activity:

Serial dilution of myrrh extract test was prepared in methanol as dilution medium since it was devoid of any antimicrobial activity.

2.3. Determination of MIC:

All test strains were subjected susceptibility test by standard agar dilution method according to the current guidelines of the clinical laboratory standard institute. Plates containing concentration of (3 ,6 ,12 , 30 ,60 and 80 mg/ml) in Muller hunt on (MHA) agar were prepared bacterial suspensions was adjusted to McFarland standard 10 ML of (1.5×10^6) CFU of bacterial suspension spotted on to the Surface of MHA .it was allowed to spread on the agar for 10min ,then incubated at 37˚C for 24 and 48 hrs. plate were read against a dark ,nonreflecting back ground. Growth was interpreted positive if confluent. Weak if a haze forms which is difficult to read and number of colonies formed there are >10 and as negative if no growth occurred. Test strains were considered resistant if the growth was positive or weak or if more than one colony was observed.

2.4. Chemical Analysis

1- wet digestion: Nitric acid was added to the sample and left for a day. The solution was then heated on a hot plate at (120 °C). After that, hydrogen peroxide was gradually added and with several additions to the heated solution until it became colorless. The last solution was taken and diluted with hydrochloric acid (Zhae.X et al 2018).

2- Fourier transform infrared spectroscopy analysis (FT-IR): Important to determine the functional groups in the myrrh the measurement used.
3- GC–MS analysis : The phytochemical analysis of ethanolic extract was performed on a GC-MS equipment (Syed Rizwan et al 2017).

3. Result and Discussion

Clinical isolates : Twelve clinical isolates was obtained from marjan hospital laboratory to ensure their purity and identification we started with microscopic examination using gram stain technique (Syed Rizwan et al 2017). Determination of MIC against standard bacteria were shown in table(1). The ethanolic extract of Myrrh had inhibitory effect on standard bacteria at concentration of 80mg/ml (100 %)., 60mg/ml (100%),30 mg/ml(100%) while 12mg/ml showed weak growth

Table(2) minimal inhibit concentration of ethanolic extract myrrh against standard

Standard bacteria	MIC mg/mL					
	80	60	30	12	6	**3**
S.aureus NCTC6571	NG	NG	NG	WG	G	G
S.aureus ATCC29213	NG	NG	NG	WG	G	G
E.coli NCTC 5933	NG	NG	WG	WG	G	G
Pseudomonas auroginosa NCTC 6750	NG	NG	WG	WG	G	G
Bacillus PCI subfilis 219	NG	NG	NG	WG	G	G
Klebsiella pneumonia ATCC6308	NG	NG	WG	WG	G	G

NG: no growth
WG: weak growth
G: growth

3.1. MIC for Clinical Isolates

Four isolates of S.aurous cultured on nutrient agar, showed golden yellow colonies on mannitol salt agar it changed the color of medium from red to yellow with gram stain. under microscope appeared as gram positive cocci arranged in grape like clusters knees applications ensured the identification of four isolates of clinical S.aurous received from lab. of majan hospital the results of MIC against clinical 4S.aureus showed in table (3).

Table(3): MIC of myrrh ethanolic extract against clinical S.aurous isolates

isolates	MIC mg/mL					
	80	60	30	12	6	**3**
S_1	-	-	-	+	+	+
S_2	-	-	-	+	+	+
S_3	-	-	+	+	+	+
S_4	-	-	+	+	+	+

From table (3) the results of MIC of Myrrh extract were alignonent with most clinical studies from wound swab (Zhae.X et al 2018).. The MIC of myrrh ethanolic extract against four clinical isolates of E.coli showed in table (4). The extract had inhibitory effect on the clinical E.coli at concentration 80mg/ml (100%) ,60mg/ml (100%) ,30mg/ml (50%) and there is no activity at concentration of 12gm/ml and 6mg/ml and 3mg/ml.

Table(4) minimum inhibitory conc. of ethanolic myrrh extract against clinical E.coli

isolates	MIC mg/mL					
	80	60	30	12	6	**3**
E.coli1	-	-	+	+	+	+
E.coli2	-	-	+	+	+	+
E.coli3	-	-	+	+	+	+
E.coli4	-	-	+	+	+	+

-: no growth
+: growth

Before doing the test of MIC against E.coli we ensured the isolates identified by culturing on maccokey agar medium, alarg red coloies were observed as a result of fermentation also used gram stain technique. Gram negative rods were seen (Zhae.X et al 2018).. The effect of myrrh concentration on clinical showed similar results E.coli to stap.aurous at 80 and 60 mg/ml concentration. But the growth of E.coli showed normal at 30mg/ml and 12, 6 ,3 mg/ml concentration table(4). found that.

Table(5)minimum inhibition concentration (MIC) of myrrh ethanolic extract on pseudomonas aeroginosa

isolates	Conc. of myrrh mg/mL					
	80	60	30	12	6	**3**
p.aeroginosa	–	–	+	+	+	+
p.aeroginosa	–	–	+	+	+	+
p.aeroginosa	–	–	+	+	+	+
p.aeroginosa	–	–	+	+	+	+

The inhibitory effectiveness of myrrh extract against pseudomonas auroginosa was determined in table(5). Myrrh extract had inhibitory effect on p.aeroginosa clinical isolates on macconkey agar plates pale and colonies were observed on nutrient agar plates the isolate produced blue-green pigment which diffused in the surrounding medium . The present study showed that the soluble components of myrrh were highly bactericidal against S.aurous in (80, 60 ,30)mg/ml conc. also the results showed bactericidal effect on clinical isolates of gram-negative bacteria similar activity of myrrh has been reported by many researches (Syed Rizwan et al 2017). Myrrh extract produced due to containing number of active constituents such as furan type terpenoids which were shown to possess biological activity . It was also noted in the study, that myrrh extract had a high bactericidal activity on types of standard gram positive and gram negative bacteria. The tested isolates caused variety disease. Resistance to many antibiotics quickly develops, so the observation of the bacteriostatic effect of myrrh extract makes it particularly useful. No microbial resistance against myrrh extract has been reported . According to the results of the present study following recommendation showed be done .due to the coast of antibiotics which regarded as a red problem for many people and appearance of severe antibiotic resistance to many drug this emphasizes the emphasized the need for search for anew cheap a safety antimicrobial drug. We suggested for future work study the toxicological and clinical should be carried out on many selected medicinal plants to prove their safety and therapeutic activity for commercial utilization (Syed Rizwan et al 2017).

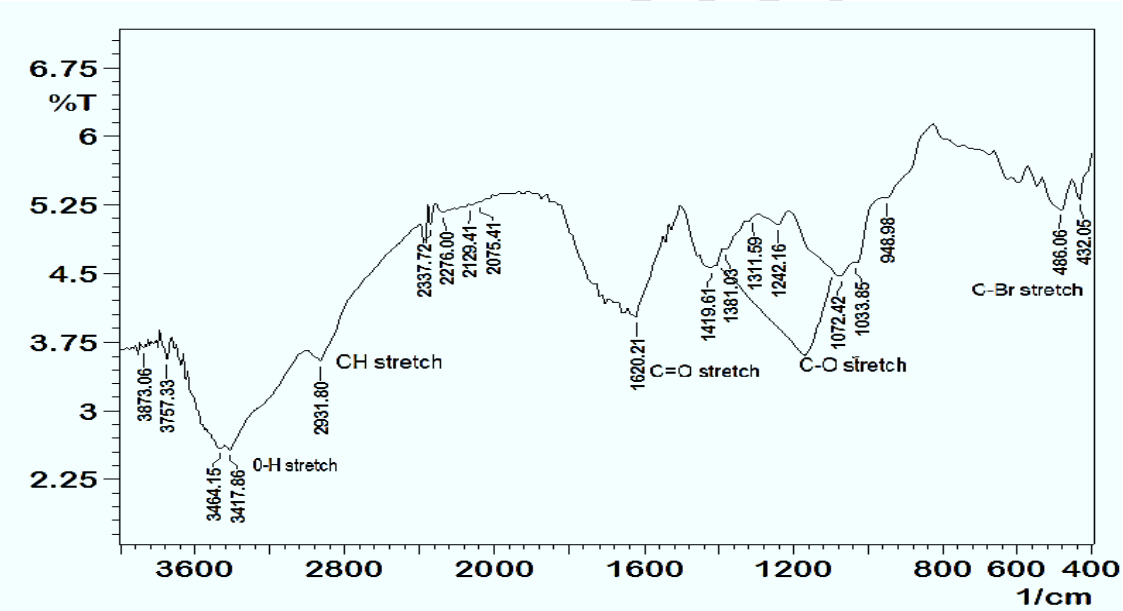
3.2. Chemical Analytics

1-It is known that chemical elements are fundamentally important in many physiological functions in humans, so an analysis of the myrrh plant was conducted to estimate some of the elements present in it, which have an important role in the human body and its functions.

Table (6)The amount of elements present in the myrrh

No	The element	The conc. (ppm)
1	Fe	0.0400
2	Al	11.5127
3	Na	0.0892
4	Mg	1.6107
5	Li	0.0114
6	Ca	183.4036
7	K	0.9013
8	Cl	18.9758
9	Mn	0.5971
10	Co	0.0703
11	Cd	0.0110
12	Zn	0.5684

FT.IR: From FTIR examination ,notice from the figures the bonds of material that appearance of (O-H stretch) bond in the wave number (2400 cm^{-1}) ,(CH stretch) bond in (2822^{-1}) ,(C=O) in (1620^{-1}) bond and (C-O) in (1520cm^{-1}) and bond (C-Br) bond in (5539^{-1})



Fig(1): IR of Material

GC technique : Using a GC technique, some of the organic compounds found in the myrrh plant were identified, as 27 organic compounds were estimated. Fig. 1 shows the chromatogram of organic constituents taken by the GC. Results indicate of the organic constituents identified in the myrrh (Syed Rizwan et al 2017).

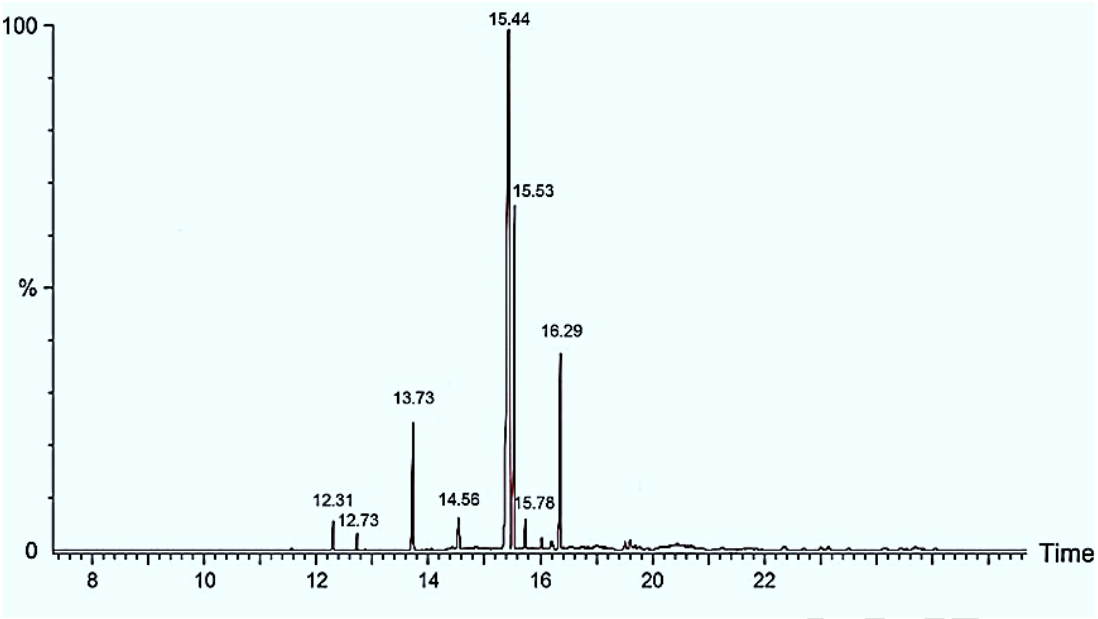
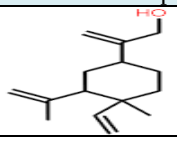
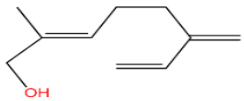
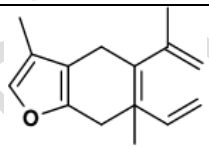
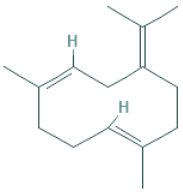
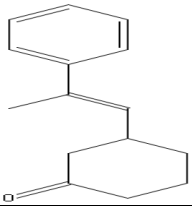
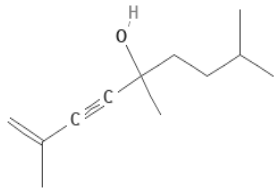
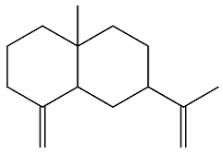
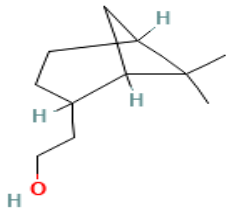


Fig.(2) the GC of myrrh ethanolic extract

Table(7) the organic compounds in the myrrh extract

NO	RT	The compound	IUPAC name
1	12.31		Elema-1,3,11(13)-trien-12-ol
2	12.73		(2*E*)-2-methyl-6-methylideneocta-2,7-dienal
3	13.73		(6S)-6-Ethenyl-3,6-dimethyl-5-prop-1-en-2-yl-5,7-dihydro-4H-1-benzofuran
4	14.56		(1E,5E)-1,5-dimethyl-8-propan-2-ylidenecyclodeca-1,5-diene
5	15.44		3-[(*E*)-2-phenylprop-1-enyl]cyclohexan-1-one

6	15.52		2,5,8-trimethylnon-1-en-3-yn-5-ol
7	15.78		(3S,4aR,8aS)-8a-methyl-5-methylidene-3-prop-1-en-2-yl-1,2,3,4,4a,6,7,8-octahydronaphthalene
8	16.29		2-(6,6-dimethyl-2-bicyclo[3.1.1]heptanyl)ethanol

CONSENT AND ETHICAL APPROVAL: Ethics committee refer that there is no plagiarism and there is no mistakes or wrong results in this work.

CONSENT
Not applicable

ETHICAL APPROVAL
Not applicable

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