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## Evaluation of antibacterial activity of essential oils of *Cinnamomum* sp. and *Boswellia* sp.

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### Abstract

Essential oils of cinnamon (*Cinnamomum* sp.) and frankincense (*Boswellia* sp.) have been investigated for their antibacterial activity against six bacterial species including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Brucella* sp., *Klebsiella pneumoniae*, and *Proteus* sp. The minimum inhibitory concentration (MIC) of these essential oils were determined using an agar dilution method. MICs of *Cinnamomum* sp., and *Boswellia* sp. essential oils ranged from 64-128  $\mu\text{g/ml}$  and 2-80  $\text{mg/ml}$  respectively. GC-MS technique was used for constituent analysis of these oils. The composition of *Cinnamomum* sp. oil was dominated by cinnamaldehyde (41.62 %), acetic acid, octyl ester (13.58 %), eugenol (7.1 %), coumarin (4.04 %), pregnane-11,20dione, 3,17-dihydroxy (3.46 %), and 1-octanol (2.64 %). The main constituents of *Boswellia* sp. oil was acetic acid octyl ester (49.46 %), 1-octanol (15.37 %), 1,6-octadien-3-ol, 3,7-dimethyl (6.64 %), acetic acid trimethyl-bicyclohept-2-yl ester (2.28 %), and propane, 1-bromo (1.1 %).

**Key words** : *Cinnamomum* sp. and *Boswellia* sp. essential oils, antibacterial activity, MIC, GC-MS.

## Introduction

*Cinnamomum* Shaeffer is sometimes called true cinnamon or Ceylon cinnamon belonging to the family Lauraceae. Its grow in east and south east of Asia to Australia. Cinnamon is an evergreen tree reaching about nine meters in high and it is covered with a smooth, pale bark[1]. Cinnamon can be used as spice because of its sweet flavoring and spicy characteristics, and it also plays an important role in pharmacological effects such as: anti-inflammation, antimicrobial, antioxidant, antidiabetes type 2 antispasmodic, anti-ulcer, and cytotoxic properties [2].

The genus *Boswellia* (family Burseraceae) consist of many species widespread thought the world. It includes approximately 23 species of small trees that grow mainly in Arabia, on eastern coast of Africa and India. Olibanum is a natural oleo-gum resin that exudes from tapping in the bark of *Boswellia* trees [3]. Therapeutic value of *Boswellia* sp. resin and essential oil is immune – enhancing, antibacterial, antifungal, antiviral, antiseptic wound healing, anti-inflammatory, and anti cancer properties [4].

## Materials and Methods :-

### Preparation of essential oils :-

Dried bark of *Cinnamomum* sp. and oleo –gum resin of *Boswellia* sp. were purchased from local retail markets, then were grounded using a grinder into a fine powder, then they were kept in dark bottles until the day of use.

### Volatile oils extraction :-

35g of finely ground cinnamon and frankincense were hydro distilled in 375 ml of DW. Then essential oils were collected and extracted from water using n-hexane in separation funnel. Hexane fractions were poured into an rotary evaporator flask and concentrated by vacuum evaporator until all of the hexane was completely evaporated, leaving the absolute oils [11].

Essential oils are odorous principles which are stored in special plant cells eg. glands, glandular hairs, oil ducts and resin ducts. They may occur in flowers, fruits, leaves, roots, wood, stems bark and saps. These oils are responsible for the distinctive aromas associated with individual plant species [5]. Essential oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties [6],[7]. Some oils have been used in cancer treatment [8]. Some other oils have been used in food preservation, aromatherapy, and fragrance industries [9].

Essential oils are a rich source of biologically active compounds. There has been an increased interest in looking at antimicrobial properties of extracts from aromatic plants particularly essential oils. Therefore, it is reasonable to expect a variety of plant compounds in these oils with specific as well as general antimicrobial activity and antibiotic potential[10]. The aim of the present study is to evaluate the antibacterial activity of *Cinnamomum* sp. and *Boswellia* sp. essential oils, and identification of chemical composition of these essential oils

### Test organisms :-

*Staphylococcus aureus* and *Escheria coli*, *Brucella* sp., *Pseudomonas aeruginosa*, *Proteus* sp. and *Klebsilla pneumonia* were obtained from microbiology laboratory /College of Education.

### Antibacterial activity :-

Antibacterial activity of essential oils were extract from cinnamon and frankincense were evaluated for their antibacterial activity by agar well diffusion method. Petri- dishes with 20 ml of Mueller – Hinton agar were prepared, inoculated with  $1 \times 10^6$  cell/ml (0.1 optical density on 540 nm wavelength). 100  $\mu$ l of a 24 h broth culture of test bacteria. wells of 6 mm diameter each were made and filled with 100  $\mu$ l of essential oils. The inoculated

plates were incubated for 24 h at 37°C . After incubation , the diameters of inhibition zone were measured in mm [12].

#### **Minimum inhibitory Concentration (MIC):-**

Essential oils of cinnamon and frankincense were tested to determine the minimal inhibitory concentration (MIC) for each bacteria tested in the present study were grown on nutrient broth medium for 6 h . After then 100µl of 10<sup>6</sup> cell/ml were spotted on each plate supplement with varying concentrations (80,40, 35, 30, 25, 20, 15, 10, 5, 3,and1.0 mg/ml), and (128,64,32,16,8,4,2,1,0.5,and 0.25 µg/ml) of the essential oils. The plates were incubated at 37°C for 24 h .The MICs were determined as the lowest concentration of oil inhibiting

visible growth of each organism on the agar plate [13].

#### **Gas Chromatography Mass Spectroscopy :-**

Essential oils of cinnamon and frankincense were isolated and identified by using analytical gas chromatography mass spectrum (GC/MS) in Al- Albait University , Water, Environment and Arid Region Research Center (WEARRC) / Central Labs Jordan .The reaction conditions was : injection temperature 150 C , detector temperature 250 C , and columns as follow : 1<sup>st</sup> 5 minutes temperature 60 C then temperature was increased in rate of 30 C /minute to 250 C , then the temperature was constant at this temperature for 15 minutes. The total flow rate is 1 ml/minute , and the column pressure was 40 PSI .

## **Results**

### **Antibacterial activity**

The antibacterial activity of cinnamon and frankincense essential oils against six bacterial species are summarized in table 2 . The results obtained in this study concluded that the bacterial species tested against the essential oil of cinnamon oil , *Staphylococcus aureus* was found to be highly sensitive to it is action followed by *Escherichia coli* , *Pseudomonas aeruginosa*, *Proteus sp.*, *Klebsiella pneumoniae* ,and *Brucella sp.*,while frankincense essential oil showed moderate antibacterial activity against *Proteus sp.* , *Staphylococcus aureus* and *Escherichia coli*. Both Gram - positive and Gram negative bacteria were found to be sensitive to the potent of cinnamon and frankincense essential oils .

#### **Minimum Inhibitory Concentration (MIC) :-**

Microbial susceptibilities to the tested oils are shown in table 2. All the bacterial species were all susceptible to cinnamon

essential oil, while three species were resistant to frankincense essential oil.

#### **Gas Chromatography (GC/MS) analysis :-**

GC/MS analysis of cinnamon essential oil identified nine phytochemicals as constituents of these cinnamaldehyde was the major compound (41.62%) followed by acetic acid 1- octyl acetate (13.58%) , Eugenol (7.1%),coumarin (4.49%) , Pregnane- 11, 20 - dione,3,17-dihydroxy(3.46%).

Frankincense essential oil had eight phytochemicals as consistent of these :acetic acid octyl ester (49.46%) , followed by 1-octanol (15.37%) 1,6- octadien -3-ol,3,7-dimethyl(6-64%) , 2-propenal (5.39%) and Acetic acid,1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester(2.88%). Remaining chemical compounds were in trace amount . The major components and their retention time are summarized in tables (4 , 5).

**Table (1) : Antibacterial activity of cinnamon and frankincense essential oils**

Bacteria	Inhibition zone diameter (mm)	
	cinnamon	Frankincense
<i>Escherichia coli</i>	25	11
<i>Staphylococcus aureus</i>	26	14
<i>Klebsiella pneumoniae</i>	19	0
<i>Brucella sp.</i>	18	0
<i>Proteus sp.</i>	19	20
<i>Pseudomonas aeruginosa</i>	23	0

**Table (2) Minimum inhibitory concentration (MIC) of cinnamon and frankincense essential oils .**

MIC	Bacteria	cinnamon ( $\mu\text{g/ml}$ )	frankincense (mg/ml)
	<i>Escherichia coli</i>	64	80
	<i>Staphylococcus aureus</i>	64	25
	<i>Klebsiella pneumoniae</i>	128	-
	<i>Brucella sp.</i>	128	-
	<i>Proteus sp.</i>	128	2
	<i>Pseudomonas aeruginosa</i>	128	-

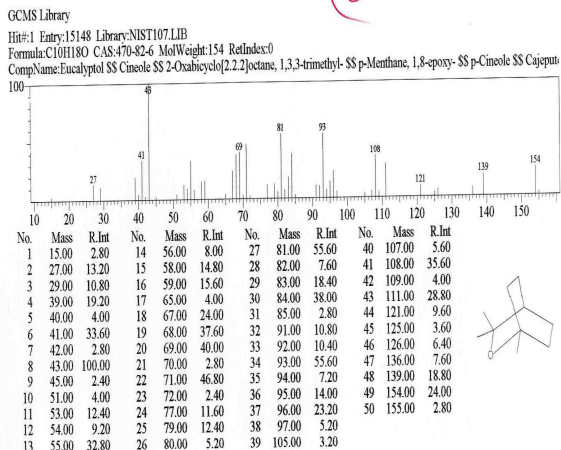
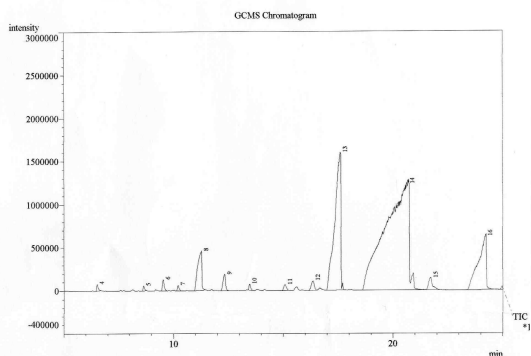
**Table (3) Results of GC/MS analysis of the essential oil of cinnamon**

Peak	Retention time	Intensity %	Component	m.wt
1	9.525	0.32	Eucalyptol(Cineole)	154
2	11.269	2.64	1-Octanol	130
3	12.325	0.66	1,6-octadien -3-ol,3,7-dimethyl	154
4	16.353	0.45	3- cyclohexene-1- methanol	154
5	17.617	13.58	Acetic acid,octyl ester	172
6	20.673	41.62	Cinnamaldehyde	132
7	24.213	7.1	Eugenol	164
8	26.945	4.04	Coumarin	146
9	52.879	3.46	Pregnane- 11, 20 – dione,3,17-dihydroxy	348

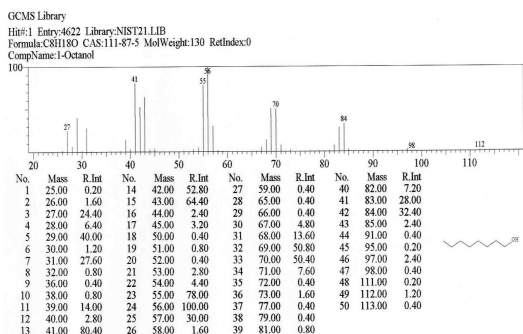
**Table (4) Results of GC/MS analysis of the essential oil of frankincense .**

Peak	Retention time	intensity %	Component	m.wt
1	6.563	0.77	Bicyclo[3.1.0]hex-2-ene,2-methyl-5-(1-methyl)	136
2	9.597	1.1	1-bromo ,Propane	122
3	12.231	15.37	1-Octanol	130
4	13.16	6.64	1,6-octadien-3-ol,3,7-dimethyl	154
5	16.258	1.32	Butane,1-bromo-3-methyl	150
6	18.348	49.46	Acetic acid, octyl ester	172
7	19.674	5.39	2-propenal,3-phenyl	132
8	20.927	2.88	Acetic acid,1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester	196

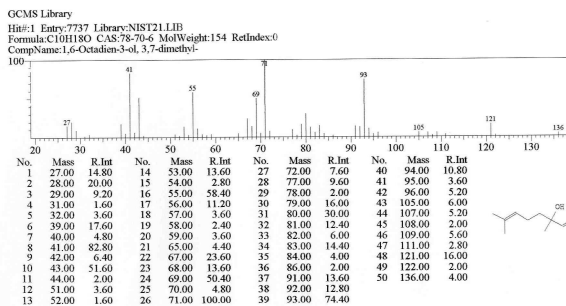
Data Acquired by : Muhammad H.M.Masad  
 Acquisition Date : Tue 11.08.2009  
 Sample Type : Unknown  
 Sample Name : AD  
 Sample ID# : AD  
 Data File : C:\GCMS\solution\essential oil\AD-Hisham.gsd  
 Method File : C:\GCMS\solution\essential oil\esson油.gsm  
 Report File :  
 Tuning File : C:\GCMS\solution\System\Tune\2-8-06.gsf



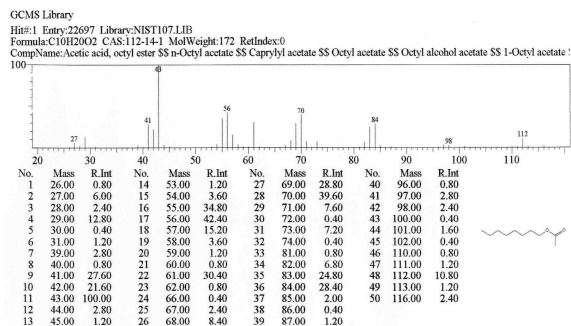
Component (1) GC-MS of Eucalyptol



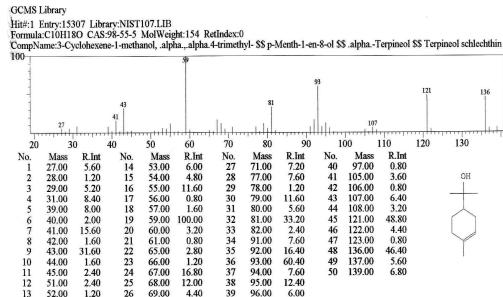
Component (2) GC-MS of 1-octanol



Component(3) GC-MS of 1,6-Octadien-3-ol, 3,7-dimethyl



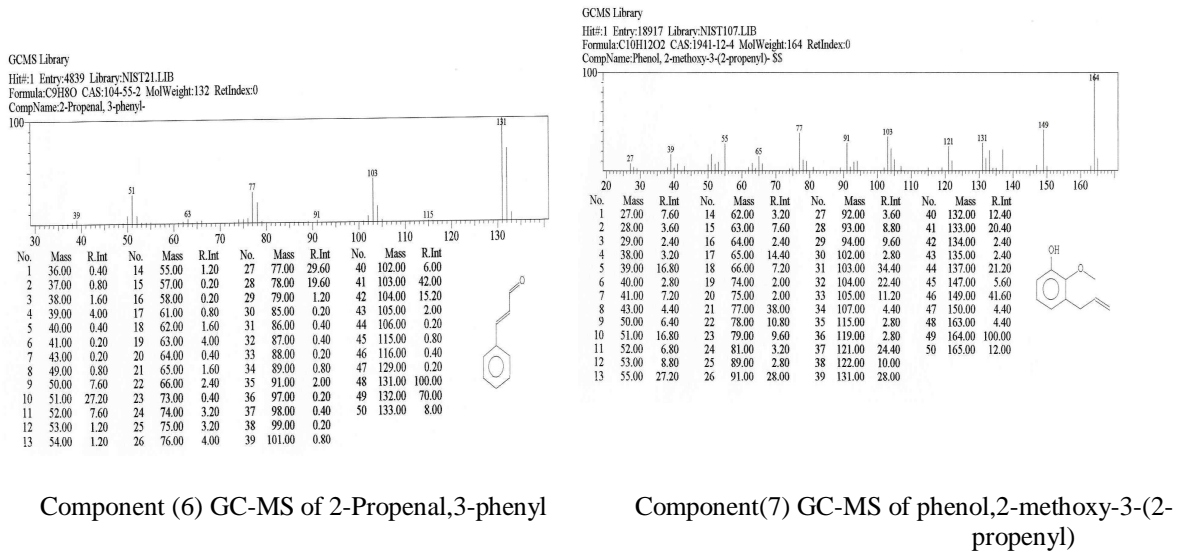
Component (4) GC-MS of 3-Cyclohexene-1-methanol



Component(5) GC-MS of Acetic acid,octyl ester

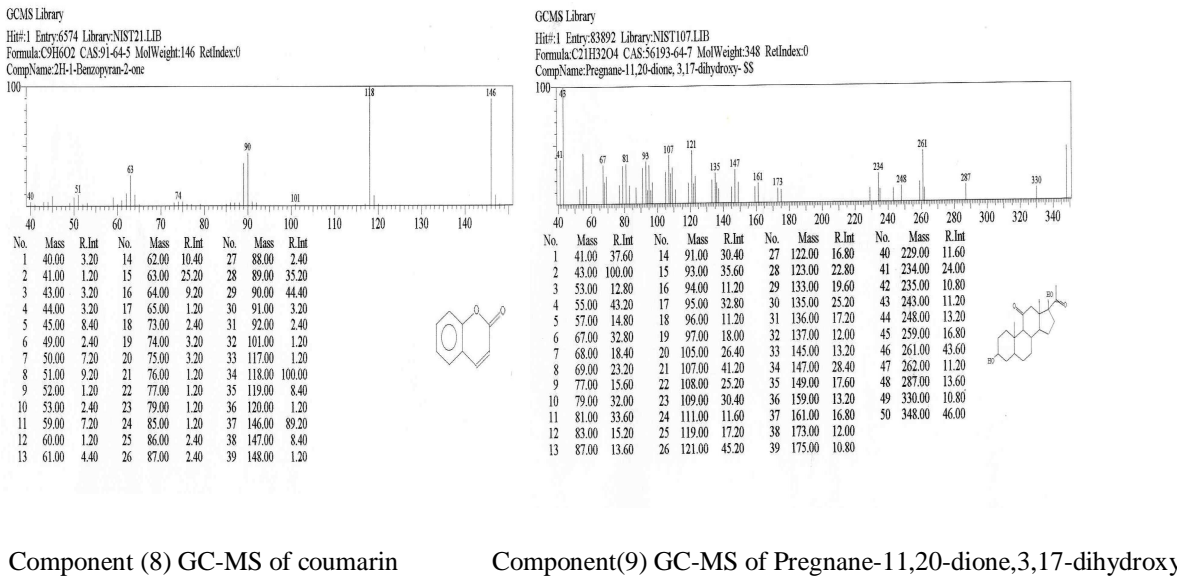
Fig.(1) Gas Chromatography-Mas Spectrum of Cinnamon essential oil.

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Component (6) GC-MS of 2-Propenal,3-phenyl

Component(7) GC-MS of phenol,2-methoxy-3-(2-propenyl)



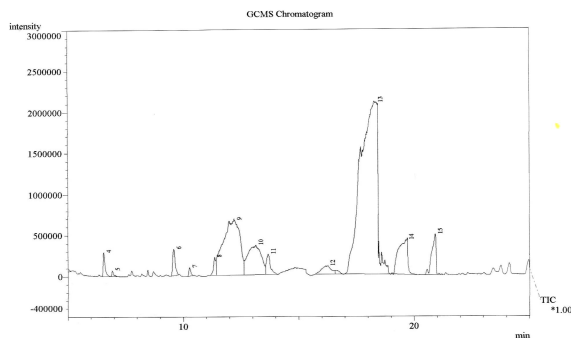
Component (8) GC-MS of coumarin

Component(9) GC-MS of Pregnane-11,20-dione,3,17-dihydroxy

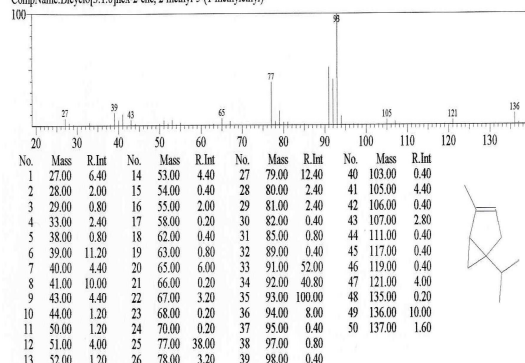
Fig.(2) Gas Chromtography-Mas Spectrum of Cinnamon essential oil.



Data Acquired by : Muhammad H.M.Massad  
 Acquisition Date : Mon 10/08/2009  
 Sample Type : Unknown  
 Sample Name : AB  
 Sample ID# : AB  
 Data File : C:\GCMSolution\essential oil\AB-Hisham.gpd  
 Method File : C:\GCMSolution\essential oil\essen.ol.gpm  
 Report File :  
 Tuning File : C:\GCMSolution\System1\Tune12-8-06.gct

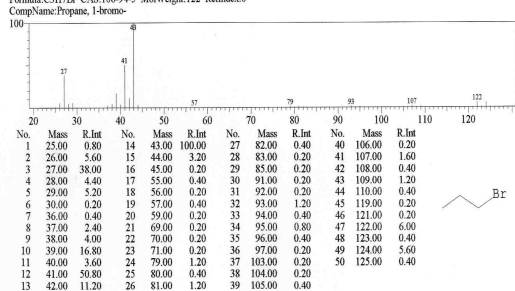


GCMS Library  
 Hit#1 Entry:5398 Library:NIST21.LIB  
 Formula:C10H16 CAS:2867-5-2 MolWeight:136 RetIndex:0  
 CompName:Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylrthyl)-



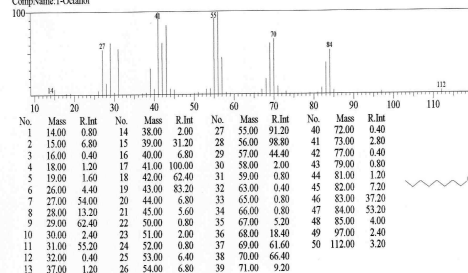
Component(1) GC-MS of bicyclo[3.1.0]hex-2-ene,2-methyl-5-(1-methylrthyl)

GCMS Library  
 Hit#1 Entry:3594 Library:NIST21.LIB  
 Formula:C3H7Br CAS:106-94-5 MolWeight:122 RetIndex:0  
 CompName:Propane, 1-bromo-



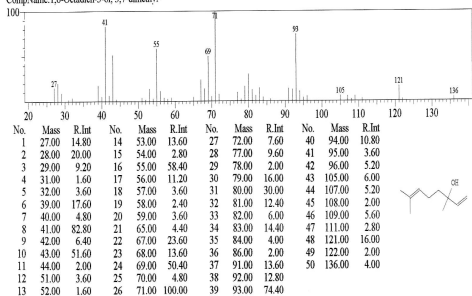
Component (2) GC-MS of Propane ,1-bromo

GCMS Library  
 Hit#1 Entry:4621 Library:NIST21.LIB  
 Formula:C8H18O CAS:111-87-5 MolWeight:130 RetIndex:0  
 CompName:1-Octanol



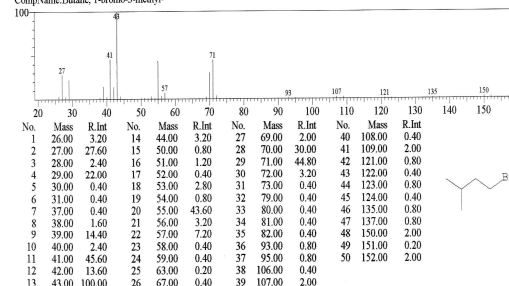
Component(3) GC-MS of 1-Octanol

GCMS Library  
 Hit#1 Entry:7737 Library:NIST21.LIB  
 Formula:C10H18O CAS:78-70-6 MolWeight:154 RetIndex:0  
 CompName:1,6-Octadien-3-ol, 3,7-dimethyl-



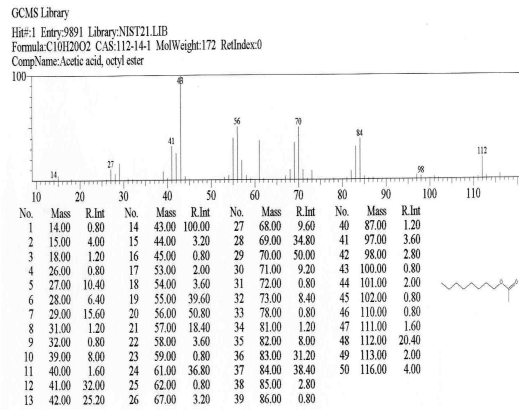
Component (4) GC-MS of 1,6-Octadien-3-ol,3,7-dimethyl

GCMS Library  
 Hit#1 Entry:6918 Library:NIST21.LIB  
 Formula:C4H9Br CAS:107-82-4 MolWeight:150 RetIndex:0  
 CompName:Butane, 1-bromo-3-methyl-

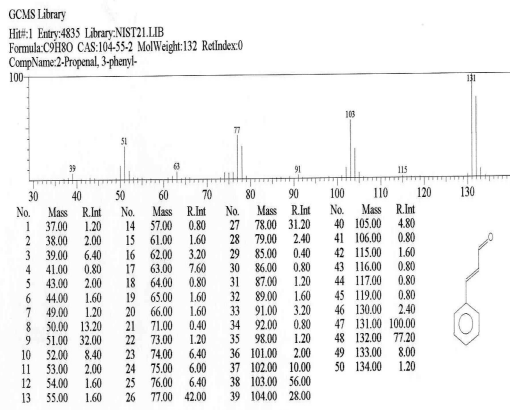


Component(5) GC-MS of Butane,1-bromo-3-methyl

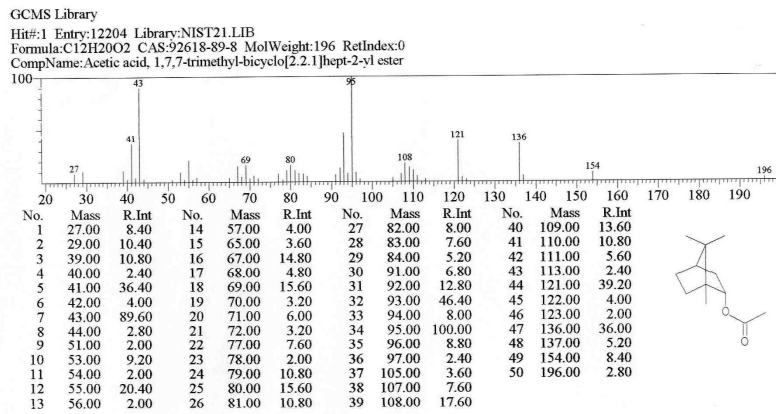
Fig.(3) Gas Chromatography-Mas Spectrum of Frankincense essential oil.



Component (6) GC-MS of Acetic acid, octyl ester



Component(7) GC-MS of 2-Propenal, 3-phenyl



Component (8) GC-MS of Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester

Fig.(4) Gas Chromatography-Mas Spectrum of Frankincense essential oil.



Plant essential oils and extracts have been used for many thousands of years [14] in food preservation, pharmaceutical, alternative medicine and natural therapies [15],[16]. Essential oils are potential source of novel antimicrobial compounds especially against bacteria pathogen [17]. The results of the antibacterial activity revealed that the essential oil of cinnamon showed high antibacterial activity against both Gram positive and Gram negative bacteria tested in the present study (table 1). The results in this study are in agreement with Bowels *et al.* (1995)[18]who recorded that *Staphylococcus aureus* was highly sensitive to cinnamon oil , whereas Helander *et al.*(1998) [19] who reported the inhibition of *Escherichia coli* O157:H7 and *Salmonella typhimurium* by the essential oil of cinnamon .Friedman *et al.* (2002) [20] who found that essential oil of cinnamon was active against *Campylobacter jejuni* and *Escherichia coli* . In another study [21] recorded that essential oil of cinnamon showed highest antibacterial activity against *Staphylococcus aureus* .Babu *et al.* (2011) [22] who found that the antibacterial activity of essential oil of cinnamon was most active against *Staphylococcus aureus* followed by *Escherichia coli* ,and *Campylobacter jejuni*.

Among the bacterial species tested against the essential oil of frankincense , the results of this study revealed that *Proteus* sp. was found to be highly sensitive to it is action followed by *Staphylococcus aureus* ,and *Escherichia coli* ,our results are in line with [23] who stated that two *Boswellia* species oleo-gum resin demonstrated presence of antibacterial activities .In another study *Boswellia papyrifera* and *B.rivae* essential oils were found to be active against Staphylococcal and *Candida albicans* biofilms [24].While [25]recorded that essential oil of *Boswellia serrata* exhibited significant inhibitory activity against *Staphylococcus aureus* OGSUTH 108, *Escherichia coli* LASUT H54 and *Proteus mirabilis* . [26]who reported that methanol extract had antibacterial activity

against methicillin resistant *Staphylococcus aureus* bacteria .[27] studied the antibacterial activity of medicinal plants from Soqotra , and he was recorded that extracts of two species belonging to the genus *Boswellia* had antibacterial activity against *Staphylococcus aureus* ,*Bacillus subtilis* , *Micrococcus flavus* , *Escherichia coli*, *Pseudomonas aeruginosa* ,and *Candida maltosa* .Raja *et al.*(2011) [28] who reported that boswellic acid had limited antibacterial activity to Gram positive bacteria .

The results of GC/MS in this study revealed that cinnamaldehyde was the major constituent of cinnamon essential oil(41.62%) followed by Acetic acid,octyl ester (13.58%),eugenol(7.1%),and coumarin(4.04%) ,this finding in agreement with the findings of the previous studies such as [29],[30], and [31]while the others were first recorded in cinnamon essential oil (table 3). Octyl ester was recorded the major component (46.46%) of frankincense essential oil recorded in the present study ,followed by Bicyclo[3.1.0]hex-2-ene,2other-methyl-5-(1-methyl)(0.77%); and ,were also recorded by other studies for example [32],[33]and[34],whereas other components were first recorded in frankincense essential oil (table 4) . This may be due to the differences in species, microclimate, soil where the trees grow, the season at which harvested, and a number of other variable. The oil is also influenced by age and storage [35].

The results of MIC of essential oil of cinnamon for various bacteria tested in the present study were in close agreement with [36]who reported that *P.aeruginosa* was not sensitive to essential oil of cinnamon ,while, *Staphylococcus aureus* was sensitive to cinnamon essential oil ,in contrast Prabuseenivasan *et al.*(2006) [37] recorded that *Pseudomonas aeruginosa* was more sensitive to cinnamon essential oil ,whereas *Staphylococcus aureus*, and *Klebsiella pneumoniae* were less sensitive to cinnamon essential oil .[38] reported that cinnamon essential oil exhibited the growth

of *Listeria monocytogenes*, *Bacillus cereus* with MIC values ranging from 1.25 to 5.0 and the lowest activity was found against *Pseudomonas aeruginosa*. In another study [39] who recorded that three strains of *Paenibacillus larvae* were sensitive to cinnamon essential oil with MIC ranging from 25-100 µ/ml. Babu *et al.* (2011) [40] who reported that *Campylobacter jejune* and *Escherichia coli* were found to be more sensitive to cinnamon essential oil. *Listeria monocytogenes* was less sensitive to cinnamon essential oil.

Among the bacterial species tested in this study against frankincense essential oil. The results revealed that *Proteus* sp., followed by *Staphylococcus aureus*, and *Escherichia coli* were found to be sensitive to its action, the present results are in line with [41], [42], and [43].

MIC results of frankincense essential oil against tested bacteria in the current study were recorded less than in cinnamon essential oil ranging between 2-80 mg/ml

According to Salvat *et al.* (2004) [44], plant extracts with MICs less than /or around 0.5mg/ml (500µg/ml) indicate good antibacterial activity. Based on this it is concluded that cinnamon essential oil followed by frankincense essential oil exhibited good antimicrobial activity against tested bacteria. However, high MIC values may indicate that active compounds in the extracts may be present in low concentrations due to the method of extraction itself. According to the results obtained from the present study, the type of functional group also had an important role in antibacterial activity. Inhibitory effect of essential oils and their components due to their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable [45] and [46]. Extensive leakage from bacterial cell of the exist of critical molecules and ions will lead to death [47].

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## تقييم الفعالية ضد الجرثومية للزيوت الاساسية لنباتي الدارسين *Cinnamomum sp.* وعلك *Boswellia sp.* البستج

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### الخلاصة

عزلت و شخصت الزيوت الطيارة لنباتي الدارسين *Cinnamomum sp.* وعلك البستج *Boswellia sp.* وقيمت فعاليتها ضد الجرثومية ضد ستة أنواع جرثومية وهي *Escherichia coli* و *Staphylococcus aureus* و *Pseudomonas aeruginosa* و *Brucella sp.* و *Klebsiella pneumoniae* و *Proteus sp.* . وقيس التركيز المثبط الأدنى (MIC) لتلك الزيوت الطيارة وتراوحت قيم ال MIC لنبات الدارسين بين 64-128 مايكرو غرام /مل و لنبات البستج 2-80 ملغرام /مل . استخدمت تقنية كروماتوغرافيا الغاز طيف الكتلة GC/MS لتشخيص مكونات الزيوت الطيارة للنباتين المدروسين . وأتضح بأن المكونات الرئيسية للزيت الطيار لنبات الدارسين وهي cinnamaldehyde (41.62 %) و acetic acid, octyl ester (13.58 %) و eugenol (7.1 %) و coumarin (4.04 %) و pregnane-11,20dione, 3,17-dihydroxy (3.46 %) و 1-octanol (2.64 %) . بينما في الزيت الطيار لنبات البستج كانت acetic acid octyl ester (49.46 %) و 1-octanol (15.37 %) و 1,6-octadien-3-ol, 3,7-dimethyl (6.64 %) و acetic acid trimethyl-bicyclo, hept-2-yl (2.28 %) و ester propane, 1-bromo (1.1 %) .

**الكلمات المفتاحية:** نباتي الدارسين والبستج، الزيوت الطيارة، الفعالية ضد بكتيرية و MIC، و طيف الكتلة .