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

Ali A. Shareef
*Biology Department, Education College, Basrah University*
Received 1-6-2011, Accepted 23-11-2011

**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 µg/ml and 2-80 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 were acetic acid octyl ester (49.46 %), 1-octanol (15.37 %), 1,6-octadien-3-ol, 3,7-dimethyl (6.64 %), acetic acid trimethyl-bicyclo, hept-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 growth is in east and south east of Asia to Australia. Cinnamon is an evergreen tree reaching about nine meters in height 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) consists 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].

Test organisms: -

*Staphylococcus aureus* and *Escherichia 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 $1 \times 10^5$ cell/ml (0.1 optical density on 540 nm wavelength). 100 µl of a 24 h broth culture of test bacteria, wells of 6 mm diameter each were made and filled with 100 µl 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 10^6 cell/ml were spotted on each plate supplement with varying concentrations (80, 40, 35, 30, 25, 20, 15, 10, 5, 3, and 1.0 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-Albaat 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: 1st 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-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-propanol (5.39%) and Acetic acid, 1,7,7-trimethylbicyclo[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

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

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

<table>
<thead>
<tr>
<th>MIC</th>
<th>Bacteria</th>
<th>cinnamon (µg/ml/)</th>
<th>frankincense (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Escherichia coli</td>
<td>64</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Staphylococcus aureus</td>
<td>64</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Klebsiella pneumoniae</td>
<td>128</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Brucella sp.</td>
<td>128</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Proteus sp.</td>
<td>128</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas aeruginosa</td>
<td>128</td>
<td>-</td>
</tr>
</tbody>
</table>

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

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

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

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

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

Fig. (1) Gas Chromatography-Mass Spectrum of Cinnamon essential oil.
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 Chromatography-Mas Spectrum of Cinnamon essential oil.
Component (1) GC-MS of bicyclo[3.1.0]hex-2-ene,2-methyl-5-(1-methylrthyl)

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

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

Component (3) GC-MS of 1-Octanol

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

Component (5) GC-MS of Butane,1-bromo-3-methyl
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 Chromtography-Mas Spectrum of Frankincense essential oil.

Discussion
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 Acetac 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],while 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

67
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 µg/ml. Babu *et al.* (2011) [40] who reported that *Campylobacter jejuni* 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 it is 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 Sal vat *et al.* (2004) [44], plant extracts with MICs less than or around 0.5 mg/ml (500 µg/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 indicates 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 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].

References
4-D.Crow. Vedic Society, the botany, culture, and therapeutic use. (2006).
36- N.Sanla- Ead,A.Jangchud,V.Chonhenchob, and P.Suppakul. The 15 th IAPRI World Conference on packing.(ND).
تقييم الفعالية ضد الجرثومية للزيوت الأساسية لنباتي البستنج

Cinnamomum sp. وظل Boswellia sp.

علي عبد شريف
قسم علوم الحياة/كلية التربية/جامعة البصر

الخلاصة

عزلت و شخصت الزيوت الطيارة لنباتي البستنج و Staphylococcus aureus و Escherichia coli و Proteus sp. كíveis للبرمائي Pseudomonas aeruginosa المثبط الأدنى MIC (64-128 ميكرو جرام/مل) لنبات الدارسين. استخدمت تقنية كروماتوغرافيا الغاز/الإشعاع الكتلة GC/MS لتشخيص مكونات الزيوت الطيارة لنباتي البستنج. و أوضح بأن المكونات الرئيسية للزيوت الطيارة لنبات الدارسين وهي Eugenol (7.1% و (13.58%) Acetic acid, octyl ester (41.62%) Cinnamaldehyde (2.64% 1-octanol و pregnane-11,20dione,3,17-dihydroxy (3.46%) و (4.04%) Coumarin (15.37%) 1-octanol و Acetic acid octyl ester (49.46%) و (1-Octadien-3-ol,3,7-dimethyl (6.64% و (1.6-octadien-3-ol,3,7-dimethyl ester (2.28% و propane,1-bromo و Acetic acid trimethyl-bicyclo,hept-2-y1 (6.64% و (1.6-octadien-3-ol,3,7-dimethyl ester (1.1% و). 

الكلمات المفتاحية: نباتي البستنج والبستنج، الزيوت الطيارة، الفعالية ضد بكتيرية و MIC و وظيف الكتلة