Study of Chemical Composition and Antibacterial Activity of
Rosmarinus officinalis and Eucalyptus spathulata Hook Extracts

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Abstract
The chemical composition and antibacterial activity of Rosmarinus officinalis and Eucalyptus spathulata leaves extracts, which were prepared by steam distillation and extraction by ethanol 95% for two hours were studied against the Staphylococcus aureus and Pseudomonas aeruginosa by using agar well diffusion method. The results showed that the alcoholic extracts gave a percentage of dry weight of crude substance of Rosemary leaves was 31% and 40.25% for eucalyptus leaves. The results also, showed that the primary chemical composition of rosemary leaves extract contains alkaloids, flavonoids, resins, phenol glycosides, saponins, tannins, terpene, cineol and α-pinene. The chemical constituents of eucalyptus leaves extract include alkaloids, flavonoids, glycosides, phenol, coumarins, saponins, tannins, steroids and terpene. The antibacterial activity showed that both leaves extracts were effective against both bacteria. The minimum inhibitory concentration (MICs) of rosemary extract were 0.5 mg/ml for S. aureus and 1.0 mg/ml for P. aeruginosa and the MICs of eucalyptus leaves extract were 0.25 mg/ml for S. aureus and 1.0 mg/ml for P. aeruginosa.
Introduction
The compounds that have natural biological activity have drawn attention for the control of human diseases of microbial origin (Smith et al., 2002). Essential oils are volatile compounds of plant secondary metabolism and may act as phytotoxic agents (Faliero et al., 1999). These compounds also have insecticide, antifungal and antibacterial activities, which are important for food preservation and the control of human and plant diseases of microbial origin (Pattanaik et al., 1996).

*Rosemarinus officinalis* (rosemary) is a blue–flowered plant that grows wild around the Mediterranean coast. It is an anti-catarrhal, anti-infectious and improves memory. Its oil 0:1 may be beneficial for skin condition and may help and support the immune system (Covelier et al., 1996).

*Eucalyptus spathulata* hook belongs the myrtales (Myrtaceae), the major constituent is the volatile oil eucalyptol (1, 8–cineol) (Robbers and Tyler, 1999). The bluish-green leaves carry the medicinal properties as anti-septic, anti bacterial, expectorant, anti inflammatory and for topical arthritis (Fischer and Susanne, 1996).

The objectives of this study were to determine the chemical composition of both leaves extracts of *Rosemary officinalis* and *Eucalyptus spathulata* and their antibacterial effects against the growth of *S.aureus* and *P. areuginosa*.

**Materials and methods:**

**Raw material preparation**

Alcoholic extracts of *Rosmarinus officinalis* and *Eucalyptus spathulata* were prepared. The leaves were separated and placed in the shadow inside a well-ventilated room. The dried leaves were grounded to a fine powder in a domestic mixer for 10 second, the particle size distribution was determined with a vibratory sieve shaker. The ground particles were stored under vacuum and maintained in freezer at -20 C° until use (Fehri et al., 1994).

**Extraction by ethanol 95%:**
To 18 gm of dry plant powder, 150 ml of ethanol 95% was added, left on magnetic stirrer for 2 hours, the whole mixture was filtered firstly by medical gauze followed by centrifugation at 3000 rpm/min for 15 second. The supernatant was collected and put in earthen bowl to dryness by oven at 40 C°, the crude extract was also extracted by hydro distillation for six hours using a cleveinger type distillater.

The oils obtained were dried using anhydrous sodium sulfate and stored in tightly closed dark vials at 4 C° until use (Fehri et al., 1994).

**Chemical composition study:**

The chemical composition of both leaves extracts was determined by using many procedures such as: (Shihata, 1951, Gessman, 1962, Harborne, 1973, AL-khazragi, 1991, 1993, and AL-maisrey, 1999) to detect alkaloids, glycosides, flavonoids, phenol, resins, taninns, saponins, steroids, terpen and coumarins.

1- Taninns:
10 gm. of plant powder boiled with 50 ml distilled water, solution filtered, left to be cold and then it divided in two parts, to the first part 1% lead acetate was added and to the second 1% ferric chloride, the appearance of white gel precipitate indicates the presence of tannis in part I and the appearance of bluish
green in part II also refers to the presence of tannins (Shihata, 1951)

2- Glycosides

1 ml from plant watery extract was added to 5 ml of Bundact reagent in test tube, boiled in water bath to 100 °C for 5 seconds, after cooling the tubes, the appearance of red precipitate refers to the presence of sugars (1993

3- Resins:

Ethyl alcohol 95% was added to 5 gm. of plant powder and put in water bath at 100 °C for 2 seconds after filtration 100 ml of distilled water was added to filtrate and mixed with HCl 4%, the appearance of clear turbidity indicate the presence of resins (Shihata, 1951)

4- Phenols:

1 ml of 1% FeCl₃ in distilled water was added to 1 ml of planed extract, the appearance of green or green bluish color refers to the presence of phenolic compound (Harborne, 1973)

5- Flavonoids: Two solutions were prepared:

Solution I: prepared by dissolving 10 gm. of plant powder in 5 ml ethanol 95% then filtered

Solution II: prepared by adding 10 ml from ethanol 50% to 10 ml of potassium hydroxide 50%, then equal amounts from solution I and II were mixed, the appearance of yellow color refers to presence of flavonoid (Al-Khazragi, 1991)

6- Saponins:

Plant watery extract was shacked strongly in tube, the persistent froth after shacking refers to the presence of saponins. (Shihata, 1951)

To 3 ml from watery extract of each plant in tube, 2 ml of Marquis’s Reagent was added, then shacked, the presence of granules leady color refers to alkaloid (Harborne, 1973)

8- Steroids and Terpen:

According to (Al-Maisrey, 1999) 1ml of watery extract of each plant was added to 1-2 ml chloroform and also added few drops of acetic anhydrite and then a drop of sulfuric acid was added to it, the appearance of brown color refers to the presence of terpen, if it forms after a very short time a dark blue color refers to the presence of steroids.

9- Coumarins:

Alcoholic extract of each plant was put in tube, ethanol alcohol was added to it, tube covered by filter paper moistened with diluted NaOH and boiled in water bath for a few seconds, filter paper then exposed to U.V. light, the appearance of greenish yellow color refers to the presence of coumarins (Geissman, 1962)

Anti bacterial activity of plant extracts:

Agar well diffusion method was used to determine the activity of each plant extract in vitro.

Four pure colonies of each the bacteria P. aeuginosa and S. aureus were suspended separately into 4 ml of nutrient broth and incubated for 2-4 hrs at 37 °C. The turbidity of inoculums was standardized with Macfarland tube No. (one) containing (1.5x10⁸) cfu/ml. The suspended cells (0.1 ml) were streaking on the N.agar,four wells were made in Nutrient agar plate using a sterile cork
borer (6mm) with micro pipette, 200 micro liters of each plant extract was poured in three wells and a sterile N. broth was poured in the fourth well as a control. The plates were incubated at 37 C° for 18 hrs, then the diameters of inhibition zones were measured. (Mahmood, et. al, 1989).

Minimum inhibitory concentrations (MICs) of plant extracts:
The inoculum was an overnight culture of each bacterial species in Mueller–Hinton broth diluted in the same media to a final concentration of approx. 10^8 cfu/ml. Ten mg of both Extracts were dissolved separately in 100 ml dimethyl sulfoxide (DMSO) (10% w/v of final volume) and diluted with Muller–Hinton broth to a concentration of 2 mg/ml. Further 1:2 serial dilutions were performed by addition of the same broth to reach a final concentration with in a range of 1.92 to 0.015 mg/ml. One hundred micro liters of each dilution was placed into the well plates and sterility control was also carried out (growth control contained Mueller-Hinton broth + DMSO). Each test and growth control wells was inoculated with five µl of bacterial suspension (10^8 cfu / ml). All experiments were performed in triplicate and the micro dilution trays were incubated at 37 C° for 24 hrs. MICs values were defined as the lowest concentration of each extract which completely inhibit the microbial growth. The results were expressed in mg / ml (Genena et. al., 2008).

Results: -
The alcoholic extracts of two types of leaves showed that the yield of dry weight of crude substance gave a percentage of 40.25% of Eucalyptus leaves and 31% of Rosemary leaves.

The results of extraction of both plants leaves were showed that the Rosemary leaves extract contain Alkaloids, Glycosides, Phenol, Resins, Flavonoids, Tannins, Terpen and α-Pinene,while the Eucalyptus leaves extract contain: Alkaloid, Flavonoids, Coumarins, Phenol, Resins, Steroids, Saponins, Tannins and Terpen. Table (1).

Table (2), figures (1),( 2), ( 3 )and ( 4 ) were showed the antibacterial activity of plant leaves extracts. The results indicated that the rosemary extracts showed antibacterial activity mainly against gram-positive bacteria (S. aureus) and also exhibited an effect against gram-negative bacteria (P. aeruginosa). The mean of inhibition zone diameters of alcoholic extract of rosemary were (18 ± 0.8) mm for S. aureus and (12 ± 0.6) mm for P. aeruginosa. The alcoholic extract of Eucalyptus gave a high rate of growth inhibition for S. aureus with the mean of inhibition zone diameter of (22 ± 0.5) mm and (15 ± 0.8) for P. aeruginosa.

The MICs values were defined as the lowest concentration which completely inhibit microbial growth and expressed in mg/ml.

The results for MICs are shown in table (2). It was indicated that the both extracts showed antibacterial activity against gram positive and gram-negative bacteria and these effects against gram positive was more efficient than that presented against the gram negatives. Since higher MICs values was obtained with gram-negative bacteria.
### Table 1: Determination of chemical composition of *Rosmarinus officinalis* and *Eucalyptus sapthulata* leaves extracts

<table>
<thead>
<tr>
<th>The plants</th>
<th>The chemical composition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tannins</td>
</tr>
<tr>
<td><em>Rosmarinus officinalis</em></td>
<td>+</td>
</tr>
<tr>
<td><em>Eucalyptus sapthulata</em></td>
<td>+</td>
</tr>
</tbody>
</table>

(+) present  
(-) absent

### Table 2: The activity of plant extracts against *S. aureus* and *P. aeruginosa*.

<table>
<thead>
<tr>
<th>The leaves extract</th>
<th>The inhibition zone diameters (mm) (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>Rosemary leaves extract</td>
<td>18 ± 0.8</td>
</tr>
<tr>
<td>Eucalyptus extract</td>
<td>22 ± 0.5</td>
</tr>
</tbody>
</table>

### Table 3: The MICs of alcoholic extracts of leaves against *S. aureus* and *P. aeruginosa*.

<table>
<thead>
<tr>
<th>The alcoholic extracts</th>
<th>MICs (mg/ml)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>Rosemary leaves</td>
<td>0.5</td>
</tr>
<tr>
<td>Eucalyptus leaves</td>
<td>0.25</td>
</tr>
</tbody>
</table>
Figure 1: The inhibitory effect of Rosemary extract on the growth of *Staph. aureus*

Figure 2: The inhibitory effect of Rosemary extract on the growth of *P. aeruginosa*
Figure 3: The inhibitory effect of Eucalyptus extract on the growth of *Staph. aureus*

Figure 4: The inhibitory effect of Eucalyptus extract on the growth of *P. aeruginosa*
Discussion:

The pharmacological action of crude drug is determined by the nature of its constituents, such as alkaloids, terpenoids, flavonoids, glycosides and tannins (Mukerjee, 2002).

The difference in the purity and strength of the crude drugs may be due to quantitative and qualitative difference in the active principles as well as the nature of drugs, geographical location, processing of drug, storage, the amount and nature of active constituents is not constant throughout the year, and the age of plant (Horonok, 1992).

The results were showed that the rosemary leaves extract contains: alkaloids, flavonoids, resins, phenol, terpene and indicated that Eucalyptus leaves extract contained: alkaloids, glycosides, tannins, phenol, resin, flavonoids, saponin and terpene, these results came in agreement with that mentioned by (Babayi et.al., 2004). The differences in composition have been reported (Sacchetti et.al., 2005), these differences could be attributed to climatic effect on the plant (Gachkar et.al., 2007). Besides, many factors should be considered when observing differences between studies as: genotypic and environmental differences within species, extraction technique used and the two plants were belonged different types.

The antibacterial activity of rosemary and eucalyptus leaves extracts indicated that the rosemary extract showed antibacterial activity mainly against G+ bacteria (Weckesser et.al., 2007). Simillar behavior was reported by Panizzi et.al., (1993).

The rosemary extracts contained α-pinenene, Camphor and Carvacrol, which are caused antimicrobial activity in rosemary (Burt, 2004). The results also showed that the alcoholic extract of eucalyptus leaves exhibited inhibition effect for bacterial growth in vitro which came in agreement with that was mentioned by Vigo et.al., (2004).

In order to compare the effectiveness of both extracts on bacterial growth we applied the notation used by Benjilali et.al., (1984) that the degree of effectiveness of both extracts on both bacteria was approximately similar.

المصادر العربية:

References


Covelier, M.E.; Richard, H.; Berset, C. 1996. Antioxidative activity and phenolic composition of pilot plant and commercial extracts of sage and...


دراسة التركيب الكيميائي والفعالية ضد جرثومي لمستخلص أوراق إكليل الجبل والصفصاف

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الخلاصة:
تم دراسة التركيب الكيميائي لمستخلص أوراق إكليل الجبل والصفصاف محضرين بالتقطر البخاري والاستخلاص بالكحول الالكيل 95% لمدة ساعتين، وفعاليتها ضد الجرثومي لكلا المستخلصين على نمو المكورات العنقودية الذهبية والنزعة الزنجارية باستخدام فحص الانتشار بالحفر. اشارت نتائج التحليل الكيميائي بأن نسبة استخلاص أوراق الجبل من المادة الجافة بلغت حوالي 31% و 40.25% في أوراق الكالبيوس، واظهرت النتائج بأن التركيب الكيميائي لمستخلص أوراق إكليل الجبل قد احتوى على الفلغونات والفلوسيتيدات، الزيتون، الفينول، السبان، البطري، السينول، والفا-بنين، بينما احتوى مستخلص أوراق الصفصاف على الفلغونات، الفلغونات، الكلوسيتيدات، الفينول، الكواردر، السبان، السبان، البطري، والفا-بنين. اشارت نتائج فحص الفعالية ضد الجرثومي إلى أن كل المستخلصين أظهروا فعالية مضادة للجراثيم المدروسة. بلغ أقل تركيز مثبت من مستخلص إكليل الجبل لنمو جرثوم المكورات العنقودية 0.5 ملغم/مل و 1.0 ملغم/مل بالنسبة للنسبة الزنجارية في حين بلغ أقل تركيز مثبت مستخلص أوراق الصفصاف لنمو جرثوم المكورات العنقودية 0.25 ملغم/مل و 1.0 ملغم/مل لجرثومة النزعة الزنجارية.