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

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Abstract

The chemical composition and antibacterial activity of *Rosmarinus officinalis* and *Eucalyptus spathualata* leaves extracts, which were prepared by steam distillation and extraction by ethanol 95% for two hours were studied against the *Staphylococcus aureus* and *Pseudomonas areuginosa* 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 alkaliods, 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. areuginosa* and the MICs of eucalyptus leaves extract were 0.25 mg/ml for *S. aureus* and 1.0 mg/ml for *P. areuginosa* .

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 phytoprotective 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 spathualata*. 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 clevenger 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 , الشيخلي وجماعتـــه 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 taninns (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 (الشيخلي و جماعته,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% $FeCl_3$ 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)

7- Alkaloids:

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 moisted 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 ($1.5x10^8$) 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.

Table1: Determination of chemical composition of *Rosmarinus officialis* and *Eucalyptus sapthulata* leaves extracts

The plants	The chemical composition								
	Tannis	glycosides	Resins	Phenol	Flavonoids	Saponins	Alkaloids	Steroid and Terpen	coumarins
Rosmarinus officialis	+	+	+	+	+	-	+	+	-
Eucalyptus sapthulata	+	+	+	+	+	+	+	+	+

(+): present

(-): absent

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

The leaves extract	The inhibition zone diameters (mm) (Mean ± SE)	
	S. aureus	*P. aeruginosa*
Rosemary leaves extract	18 ± 0.8	12 ± 0.6
Eucalyptus leaves extract	22 ± 0.5	15 ± 0.8

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

The alcoholic extracts	MICs (mg/ml)	
	S. aureus	*P. aeruginosa*
Rosemary leaves	0.5	1.0
Eucalyptus leaves	0.25	1.0

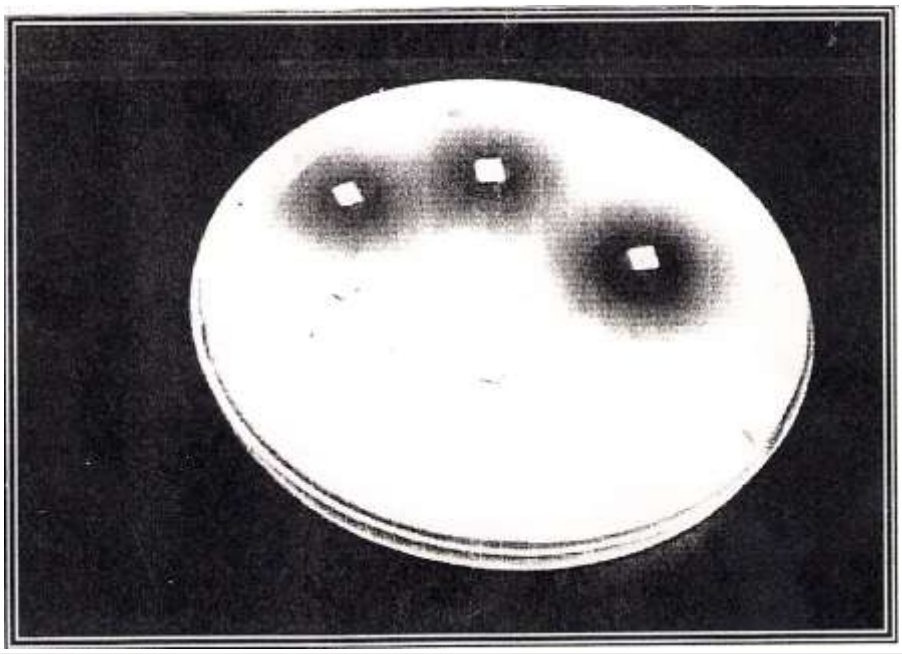

Figure1: The inhibitory effect of Rosemary extract on the growth of *Staph. aureus*

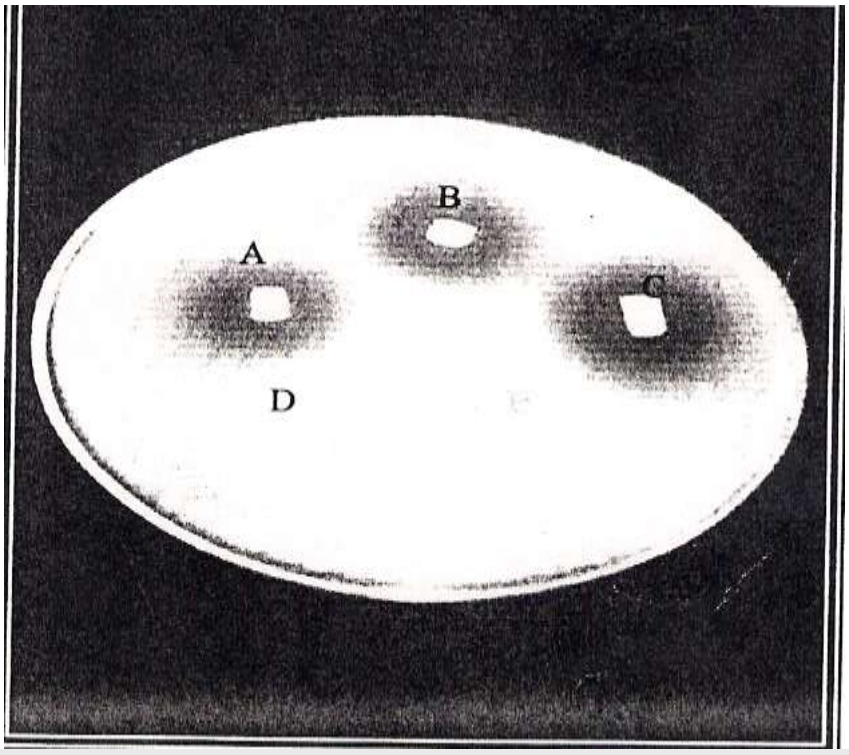

Figure 2: The inhibitory effect of Rosemary extract on the growth of *P. aeruginosa*

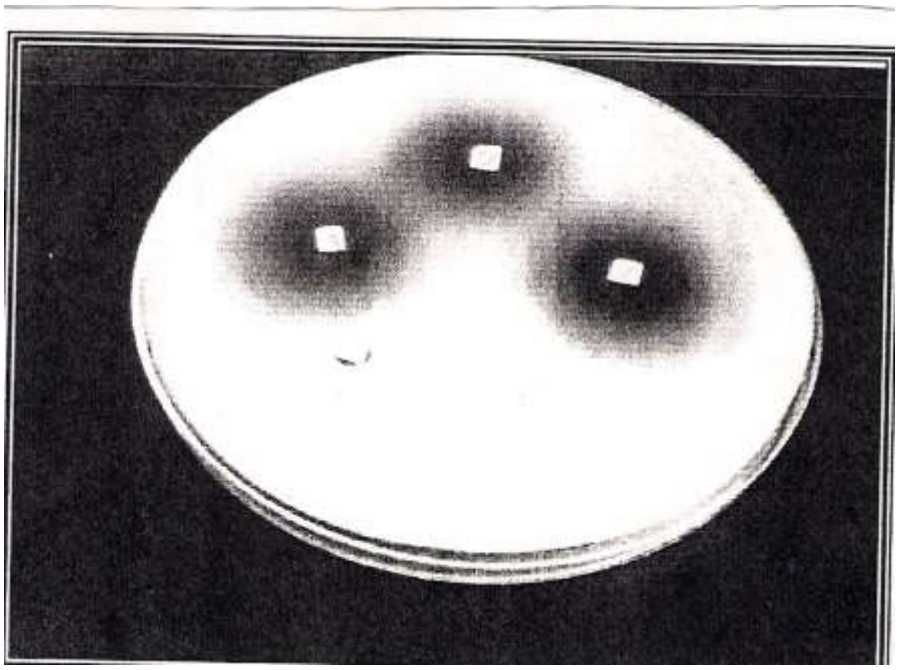

Figure 3: The inhibitory effect of Eucalyptus extract on the growth of *Staph. aureus*

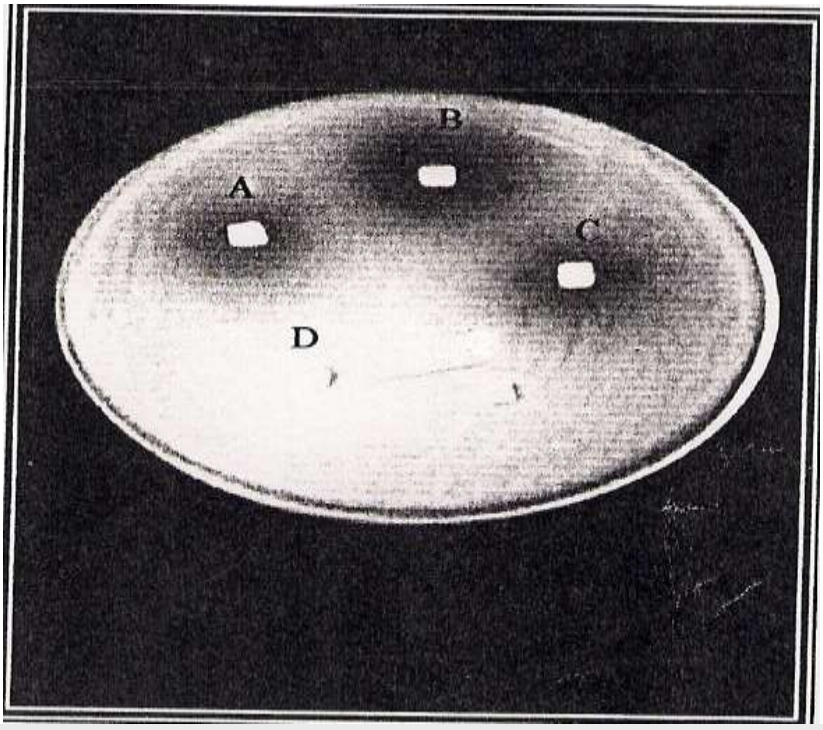

Figure 4: The inhibitory effect of Eucalyptus extract on the growth of *P. aeruginosa*

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-pinene, 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 :

الشيخلي عبد الستار ؛ الغراوي فريال حسن و الفياض حسن ١٩٩٣. الكيمياء التحليلية، الجامعة المستنصرية.

References

AL-khazragi, S**.** M**.** 1991**.** Biopharmacology. Study of Artemision Herba**,** Alba**.** M.Sc. thesis, college of pharmacology, university of Baghdad**.**

AL-Maisrey, M**.** 1999**.** Effect of oil and alcoholic extract of *Azdirachata indica* on some pathogenic fungi of plant**,** M.Sc. thesis. College of science, university of Baghdad**.**

Babayi**,** H**.;** Kolo**,** I**.;** Okogun**,** J.I**.** and liah, U.J.J. 2004. The antimicrobial activities of methanolic extract of *Eucalyptus camaldulensis* and *terminalia catappa* against some pathogenic microorganisms**.** Biokemistri, 76(2):106-111**.**

Benjilali, B**.;** Tantaoui -Elaraki, A.; Ismaili-Alaoui, M.and Ihlal, M. 1984. Method to study antimicrobial effects of essential oils: Application to the antifungal activity of six Moroccan essences. J. Food prot. 47:752-784**.**

Burt, S. 2004. Essential oils**:** their antibacterial properties and potential applications in foods-a review. Int. J. of food microbio., utrecht, 94 (3):223-253**.**

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

rosemary. J. Am. oil. chem. Soc. 73: 645-665.

Faleiro, L.; Miguel, G.M.; Gurrero, C.A.; Brito, J.M. 1999. Antimicrobial activity of esseatial oils of *Rosmarinus officinali, L. Thymus mastichina (L). L.SSP mastichina* and *Thymus albicans* hufmanns e link - Pharmacognosy, pharmacology, phytomedicines, Toxicology. Acta Hort. 501. ISHS.

Fehri, B.; Aiache, J.; Memmi, A.; Korbi, S. and lamaison, J. 1994. Hypotension, Hypoglycemia. Hypouricemia recorded after repeated administration of equeous leaf extract of Oleaeuropeal. J. pharm. Belg. 49 (2): 8 – 101.

Fischer-Rizzi, S. 1996. Medicine of the Earth.Rudra press, 2(2):234-238.

Gachkar, L. 2007. Chemical and biological characteristics of *Cuminum cyminum* and *Rosmarinus officinalis* essential oils .Food chemistry, Tehran. 102:898-904.

Geissman, T.A. 1962 .Chemistry of flavonoid compound. MC, Millanco., New York.

Genena, A.K.;Hense, H.; Junior, A.S.; Desouza, S.M. 2008. Rosemary *(Rosmarinus officinalis)* - a study of the composition, antioxidant and antimicrobial activities of extracts obtained with super critical carbon dioxide. Gienc. Tecnol. Aliment., Campinas., 28(2): 463-469.

Harborne, J.B. 1973. Phytochemical methods, a guide to modern techniques of plant analysis. pp: 159 -165.Chapman and hall ltd. Landon.

Horonok, I. 1992 .Cultivation and processing of medicinal plants; Wiley and sons, chickester, Uk. pp; 221-235.

Mahmood, M.J., Jawed, A.J., Hassain, A.M.; AL- omeri, M. and AL-Naib, A. 1989. Invitro antimicrobial activity of *Salsola rosmarius* and *Adiantum capillus venris*. Int, J, crude. Drug. Res.27:14-16.

Mukherjee, p.k. 2002. Quality control of herbal drugs, an approach to evaluation of biotanicals, 1st edn. Busiaess Horizons, NewDelhi.

Panizzi, L. 1993. Composition and antimicrobial properties of essential oils of four Mediterranean Lamiaceae. Journal of Ethnopharmacology, Livorno / pisa, 39: 167-170.

Pattanaik, S., Subramanyam, V.R. and kole, C. 1996. Antibacterial and antifungal activity of ten essential oils invitro. Microbios, 86 (349): 237-246.

Robbers, J.E. and Tyler, V.E. 1999. Tylers herbs of choice, the therapeutic use of phytomedicines. Newyork: Haworth press, 123.

Sacchetti, G, 2005. Comparative evaluation of 11 essential oils of different origin as functional. Food chemistry, Ferrara, macas / parma, 91(4): 621-632.

Shihata, I.M. 1951. A pharmacological study of *Anagalis arvensis* .M.D.Vet. thesis, Cario.

Smith, M.A.; Perry, G. and Pryor, W.A. 2002. Causes and consequences of oxidative stress in Alzheimers disease (1, 2). Free radic. Biol. Med. 31:1049.

Vigo, E., Cepeda, A.; Galileo, O. and Perez-Fernandez , R. 2004. Invitro antiinflammatory effect of *Eucalyptus globules* and *Thymus vulgaris*. pharmacol. 56:1257.263.

Weckesser, S. 2007. Screening of plant extracts for antimicrobial activity against bacteria and yeast with dermatologycal relevance. Phytomedicine, Freiburg, 14 (7-8): 508-516.

دراسة التركيب الكيمياوي والفعالية الضد جرثوميه لمستخلص أوراق إكيل الجبل والصفصاف

عماد محمد رشيد حيدر ميري رضا كاظم جبر زبون

هيئة التعليم التقني – المعهد التقني الطبي - المنصور

الخلاصة:

تم دراسة التركيب الكيمياوي لمستخلصي اوراق إكيل الجبل (الروزماري) والصفصاف المحضرين بالتقطير البخاري والاستخلاص بالكحول الاثيلي ٩٥% لمدة ساعتين ، وفعاليتها ضد الجرثوميه لكلا المستخلصين على نمو المكورات العنقودية الذهبية والزائفة الزنجارية باستخدام فحص الانتشار بالحفر.

اشارت نتائج التحليل الكيمياوي بان نسبة استخلاص اوراق الجبل من المادة الجافة بلغت حوالي 31%و40.25% في أوراق الكالبتوس واظهرت النتائج بان التركيب الكيمياوي لمستخلص اوراق إكيل الجبل قد احتوى على القلويدات، الفلوفونات ، الكلوكسيدات، الراتنج ، الفينول ، الصابونين ، التانين ، التربين ، السينول والفا–بنين ، بينما احتوى مستخلص اوراق الصفصاف على القلويدات ، الفلوفونات ،الكلوكسيدات ،الفينول ، الكومارين ، الصابونين ، التانين ، الستيرود والتربين .

اشارت نتائج فحص الفعاليه ضد الجرثوميه الى ان كلا المستخلصين اظهرا فعالية مضادة للجراثيم المدروسه . بلغ اقل تركيزمثبط من مستخلص إكيل الجبل لنمو جرثومه المكورات العنقودية 0.5ملغم/مل و1.0 ملغم /مل بالنسبة للزائفة الزنجارية في حين بلغ اقل تركيزمثبط مستخلص اوراق الصفصاف لنمو جرثومه المكورات العنقودية 0.25 ملغم /مل و1.0 ملغم /مل لجرثومة الزائفة الزنجارية .