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Antibacterial Activity of Essential oils Isolated from Eucalyptus globulus Labill and Eugnia caryophyllata Thunbery (Family Myrtaceae): A comparative study.

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Abstract :

The essential oils of *Eucalyptus globulus* Labill and *Eugnia caryophyllata* Thunbery analyzed by Thin Layer Chromatography TLC. The results showed that TLC plates separated five compounds from E. globulus oils and four compounds from *E. caryophyllata* oils with different Rf values. The obtained essential oils from leaves E. globulus and flower buds *E. caryophyllata* were tested on *Escherichia coli* and *Staphylococcus aureus*, by using agar well diffusion method, to determine its antibacterial activities. The strongest effected was noted in E. globulus oils against S. aureus and E.coli with diameter of zone of inhibition (25 mm and 23 mm respectively). While the *E. caryophllata* oils showed the least effect on *S. aureus* and *E. coli* in compare with *E. globulus* oils with diameter of zone of inhibition (20 mm and 18 mm respectively). We concluded that the essential oils obtained from the leaves of E. globulus offer significant antibacterial activity toward *E.coli* and *S. aureus*.

Introduction:

The essential oils are a complex mixture of volatile compounds synthesized as secondary metabolites within aromatic and medicinal plants provide them with characteristic odor, flavor and a number of other properties (4, 12 and 21). These oils obtained physically by pressing or distillation from a whole or a part of plant of known taxonomic origin (28, 33). However, environmental conditions, collection period, dehydration methods, storage conditions and isolation methods determine their chemical constituent (20). Essential oil possesses antibacterial properties, with neither reported resistance after prolonged exposure to pathogenic bacteria and no side effects on human health. On the opposite side, continuous evolution of bacterial resistance to currently available antibiotics necessitates the search for novel and more effective antibacterial compounds. Hence, these essential oils can be promising remedy for bacterial diseases (26). *Eucalyptus globulus* Labill. (Family: Myrtaceae). Tree tall, aromatic, evergreen, leaves containing essential oil of commercial importance used in perfumery and pharmaceutical industry (6). Eucalyptus plant is native of Australia; however, many species found all over the world (24). Several Eucalyptus species are well known in Folk medicine. It is used against several upper respiratory tract infections and influenza viruses (2). Eucalyptus possesses several medicinal properties such as: anti-inflammatory, antibacterial, antimalarial, pain alleviation, reducing blood pressure and used for chest problems and skin rashes (22, 23 and 35). Its essential oil is obtained by aqueous distillation of fresh leaves, which occurs as light yellow fluid (16). This essential oil comprises of variety of volatile monoterpene which includes: 1, 8-cineole, citronellal, limonene, linalool and αterpinene (6). 1, 8-cineole (eucalyptol), p-cymene and α-pinene are the main responsible component of the essential oil in Eucalyptus for antimicrobial activity (32). Eugnia caryophyllata Thunbery (Family: Myrtaceae). Its common name is clove. Tree is an evergreen, aromatic; height is ranged from 10-20 m. It is native of Indonesia and tropics area, particularity in tropical America and Australia (30). This plant is volatile oils abundant that mediated several medicinal properties, such as: anti-inflammatory, analgesic, antipyretic, antifungal, antiviral and antibacterial (7, 19).

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This essential oil is a colorless or light yellowish fluid obtained from extracted dried flower buds (30). Eugenol (C10H12O2), eugenylacetate and β-caryophyllene are the major constituent of the essential oil responsible for its medicinal properties. Eugenol (4-allyl-2-methoxyphenol) comprises 70-90% by weight (10, 18), Eugenol acetate (>17%) and cariofilen (>12%), 1,8-cineole (0.1%), linalool (0.2%), β-caryophyllene (9%) α –copaene (1.2%), α -humulene (3.5%), β-cadinene (0.5%), α-copaen (0.1%), $^{\delta}$-cadinene (3.6%), eugenyl acetate (4.2%), epizonarene (0.1%), α -muurolene (0.1%), in addition, clove contains tannins, flavonoids, triterpenoids (8, 15). Terpene compounds found in a wide range of clove oil and responsible for its odor and taste (27, 31). The aim of this study is to evaluate the antibacterial activity of the essential oils of these two plants and analyze them by TLC searching for a possible new drugs more effective medicine.

Materials and Methods:

Plant material:

The plant materials of E.globulus (fresh leaves) collected from a garden in Najaf, Iraq. While the plant materials of E.caryophllata (flower buds) obtained from local market in Najaf. The identification and authentication of the plants materials was done by Botany Taxonomy Department, Kufa University.

Extraction of the essential oils:

The plant materials were extracted by steam distillation method, using "Clevenger apparatus" to extract its essential oils (14). The distillation was done for 4 hours and the oil was collected separately in airtight containers that were dried over anhydrous sodium sulphate (3). The oil was stored in a freezer at -4 C0 for further use. The Dimethyl Sulfoxide (DMSO) was used to prepare different concentrations of the oils.

Thin Layer Chromatography (TLC):

The essential oils, components were separated by TLC, Silica gel F254. Aluminum plates (20x20 cm) were used and Silica plates were activated by heating in an oven for 5 min at 100 C0 . The sample was loaded at one end of the TLC plate by capillary tube then entered in TLC Jar that contained appropriate solvent system, Hexane: Chloroform (6:4). When the solvent phase reached the top, the plate air-dried and checked the separated compounds by UV visible (254 nm), rate of Retention factor (Rf) values were recorded by calculated from below equation:

Rf = distance a compound moves / distance solvent front (14).

Antibacterial assay:

The essential oils obtained from the E.caryophyllata (flower bud oil) and *E. globulus* (leave oil) were used to study their antibacterial activities, using Agar Well Diffusion method (11). Two species of bacteria were selected as target, Gram-negative bacteria (*Escherichia coli*) and Gram-positive bacteria (*Staphylococcus aureus*). Mueller–Hinton Agar (MHA) was poured in Petri dishes (Three Replication), after solidification. Suspension of the tested bacteria (1x106 CFU/ml) was spread onto media plates by sterile cotton swabs after 15 min. Four wells were made in plates with sterile borer (8 mm). The concentrations of essential oils prepared by mixing a definite amount of the oils with different volumes of Dimethyl

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Sulphoxide DMSO (1:1, 1:2, 1:3) to get the concentrations (50%, 33.3%, 25% respectively). 100 µl from each concentration of oils were loaded in the wells and control DMSO was loaded in a fourth well. The plates were left at the room temperature for 10 minutes to allow spreading the essential oils into the agar. The plates incubated at 37 C0 for 24 hours. The bacterial growth was determined by measuring the diameters of inhibition zones, measured in millimeters (mm), around each well in plates.

Results and discussion:

Thin layer chromatography (TLC):

Analysis of essential oils components through TLC gives distinct bands with different compounds. TLC plates separated four compounds from E. caryophyllata essential oils. They have detected according to Rf value for four spots (0,08, 0.38, o.55, 0.58 respectively). On other side, TLC plates separated five compounds from E. globulus essential oils with different Rf value for spots (0.14, 0.27, 0.48, 0.50, 0.52 respectively), (Table 1).

Table 1: Separation of compounds by TLC in E. globulus and E. caryophyllata essential oils.

Essential oil	Spots	Rf
E.globulus	1	0.14
	2	0.27
	3	0.48
	4	0.50
	5	0.52
E.caryophyllata	1	0.08
	2	0.38
	3	0.55
	4	0.58

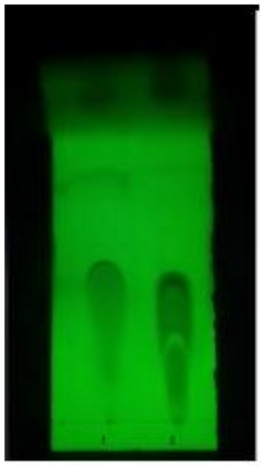

Figure 1: TLC Plate to detect essential oils. 1- E. caryophyllata 2- E.globulus

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Antibacterial activity:

In this study, agar well diffusion method was used to evolution the antibacterial activity of the E.globulus and E. caryophllata essential oils. The leaves essential oils of E.globulus showed good antibacterial activity against the Staphylococcus aureus and Escherichia coli with zone diameter of inhibition (25 mm and 23 mm respectively) in oil´s concentration 50%, while in concentration 25% was 18 mm against S.aureus and 19 mm against E.coli (Table 2). The effectiveness of antibacterial activity E. globulus essential oils attributed to the presence mixture of monoterpenes and oxygenated monoterpenes (1). As well as, present 1,8- cineol, linalool and pinocarveol. The 1,8- cineol and linalool are well-known substance with pronounced antimicrobial properties (34). The flower buds essential oils of E. caryophllata permitted antibacterial activity against S.aureus with inhibition zone diameter of 20 mm while E.coli inhibition zone diameter of 18 mm in oil´s concentration 50%, but concentration 25% the inhibition zone is 16 mm and 15 mm against S.aureus and E.coli respectively (Table 2). Eugenol is the main constituent for essential oils of E. caryophyllata responsible for antibacterial effect and other medicinal properties (17, 25 and 30). The compound Eugenal is capable of proteins denaturation and reacts with phospholipids of the cell membranes to alters its permeability (5). Both E.globulus and E.caryophyllata essential oils types showed good activity against both Gram-negative and Gram-positive bacteria. Hydrophobicity of essential oils components enable them to partition in the lipids of the bacterial cell membrane and mitochondria disturbing the structure and rendering them more permeable. This will result in ions leakage and other cell contents (29). Extensive cell contents loss or draining out of critical molecules and ions will eventually lead to death of the cell (9). The results showed that Gram-negative bacteria were the least sensitive to the action of E. globulus and E. caryophyllata essential oils than Gram-positive bacteria. The outer membrane that surrounding the cell wall in Gram-negative bacteria restricts the diffusion of hydrophobic compounds through its lipopolysaccharide layer (13).

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Table 2: Antibacterial activities of different concentration of E. globulus and E. caryophyllata essential oils.

Plants	Oils concentrations	Inhibition zone diameter (mm)	
		E.coli	*S.aureus*
E. globulus	50%	23	25
	33.3%	22	23
	25%	19	18
E. caryophyllata	50%	18	20
	33.3%	17	18
	25%	15	16
Control /DMSO		-	-

(1) (2)

Figure 2: Antibacterial activities of E. globulus essential oils as determined by zone of inhibition. 1- E. coli 2- S. aureus

(3) (4)

Figure 3: Antibacterial activities of E. caryophyllata essential oils as determined by zone of inhibition. 3- E. coli 4- S. aureus

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Conclusion:

The essential oils from the leaves of E. globulus have good antibacterial activity compared to that of flower buds of E. caryophllata. Hence, it is of medicinal importance and can be used as antibacterial agent.

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