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Antibacterial Screening of fixed and volatile oils extracted from *Syzygium aromaticum* (clove)

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Abstract

The fixed oil and volatile oil of *Syzygium aromaticum* (clove) were screened for antimicrobial activities against some pathogens viz., *Escherichia coli, Pseudomonas aeruginosa Staphylococcus aureus ,Acinetobacter sp. and Klebsiella pneumonia.*The fixed oil and volatile oils extracted were found to produce significant inhibition against all the pathogens. Fixed oil extract were observed to be more active than volatile oil extract as compared to the standard drug. Finally, the present study suggests that both fixed and volatile extracted from *S.aromaticum* have potential activity as source of natural antioxidant.

Key-Words: *Syzybium aromaticum* (Clove), Anti-microbial activity, Fixed and Volatile oils

Introduction

Syzygium aromaticum (L.) commonly called clove, which belongs to the family *Myrtaceae*, is an important aromatic spice. Clove is commercially cultivated in India, Madagascar, Sri Lanka, Indonesia and the south of China. Now-a days it also cultivated in Bangladesh in a small scale. The clove oil especially has been used by traditional folk healers as well as by modern pharmacists and dentists in alleviating the symptoms associated with toothache and dental decay. Also in the treatment of skin ulcers [1].

The clove herbal tea is prepared by boiling and steeping the dried clove buds in water. This tea is seen as a cure for problems such as nausea and as an aid to eliminating excess gas in the stomach and the intestines. Clove bud oil has biological activities, such as antibacterial, antifungal, and antioxidant properties, and are used traditionally as flavoring agent and antimicrobial material in food, It is clear that common spices *S. aromaticum* has broad spectrum pharmacological effects against various microorganism as well as in treatment of different health problems in human beings. Chemicals present in *S. aromaticum* have significant effect against cancer, cardiovascular rich factors, and as antidibetic/antioxidant, [2]. And also shows antibacterial properties against food borne pathogens (S. aureus, P. aeruginosa, E. coli) [3].

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Oil of Clove showed maximum antifungal activity against Aspergillus flavus, A. niger, A. terreus, A. oryzae, A. fumigatus, Fusarium moniliforme, F. solani and Penicillium fungal species [4]. With other 16 medicinal plants, clove used in the treatment of Jaundice in the Satra culture people that related to their livelihoodas well as socio-economic and spiritual aspect [5].

Ample experimental and epidemiological studies support the involvement of oxidative stress in pathogenesis and progression of many diseases. It is quite known that oxygen, indispensable for maintaining life, sometimes becomes toxic and results in the generation of most aggressive agents such as reactive oxygen species (ROS). The high reactivity of ROS can trigger a host of disorders in biological systems. Endogenous antioxidant enzymes are responsible for preventing and neutralizing the free radical induced damages of tissues. Oxidative stress is an outcome of imbalance between ROS production and antioxidant defences, which in turn evokes a series of events deregulating the cellular functions [6]. Antioxidant is a substance that has the ability to delay the oxidation of a substrate by inhibiting the initiation or propagation of oxidizing chain reactions caused by free radicals. It playsimportant roles to prevent fats and oils from becoming rancid and protects human body fromfree radicals. Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertbutylhydroquinone (TBHQ) and propyl

gallate (PG) have been widely used around the world for decades. However, they are being scrutinized for possible toxic and carcinogenic effects. As a result, an intense new area of research has been developed concerning the search for identification and characterization of naturally occurring antioxidants. Natural antioxidants are more ideal as food additives, not only for their free radical scavenging properties, but also on the belief that natural products are healthier and safer than synthetic ones; thus they are more readily acceptable to the modern consumers [7].

Numerous aromatic, spicy and medicinal plants have been examined for their antioxidative potential [8]. Herbs and spices that are usually used to flavour dishes are among the tremendous sources of phenolic compounds, which have been reported to show good antioxidant activity, Chemical constituents with antioxidant activity found in high concentrations in plants determine their considerable role in the prevention of various degenerative diseases [9 -10]. The purpose of this study is to evaluate the Antibacterial properties of the fixed and volatile oils extracted from the buds of Syzygium aromaticum (clove).

Material and Methods

Plant material

The buds of S. aromaticum of good quality were obtained from a local market, Benghazi, Libya,2012.

Bacteria used

Bacteria were taken from the laboratory of microbiology in Banghazi medical center, which know as multi drag resistant bacteria .The bacteria used were Escherichia coli (MDR)ATCC, Staphylococcus aureus (MDR) ATCC, Pseudomonas aeruginosa (MDR) ATCC, Klebsiella pneumonia (MDR) and Acinetobacter sp (MDR) . Other bacteria were not multi drag resistant such as Escherichia coli, Staphylococcusaureus and Pseudomonas aeruginosa. The organisms were isolated and identified by standard methods, and identification confirmed by using phonex. The organisms were then subcultured and maintained on nutrient agar slants.

Sample preparation

Extraction of fixed oil

The fixed oil from the powdered buds of *S. aromaticum* (100g) was extracted with lightpetroleum ether (40-60 C°) in a soxhlet apparatus for about 4h and the solvent was removed by rotary vacuumevaporator.

Extraction of volatile oil

The dry powdered buds of *S. aromaticum* (200 g) were subjected to hydrodistillationusing Clevenger apparatus for 6h for isolation of volatile oils separately [11]. The oil samples werestored at 7°C in air-tight containers after drying them over anhydrous sodium sulfate and filtered before analysis.

Antibacterial activities of fixed and volatile oilsextracts from the *Syzygium aromaticum*

In this study diluted fixed and volatile oils were used . The diluted fixed and volatile oils were prepared by using Dimethyl Sulfoxide (DMSO) to obtain 4%(v/v), 6%(v/v) and 8%(v/v) concentrations . DMSO was used as negative control. A screening assay using well diffusion [12] . Muller Hinton agar plates were inoculated by rubbing sterile cotton swabs after immerse 100µ l bacterial suspensions on plates (over night cultures grown at 37°C on nutrient agar and adjusted to 0.5 McFarland in sterile saline) over the entire surface of the plate. After inoculation 9 mm diameter wells were cut into the surface of the agar using a sterile cork borer. Different concentrations (4, 6 and 8 %) were added to the wells . Plates were incubated at 37°C for 24 h. Control wells contained solvent DMSO. Zones of inhibition were measured by using ruler .The diameter of zones was recorded. Each assay was carried out in triplicate. The antibacterial assay plates were incubated at 37°C for 24hr. The effect of fixed and volatile oils on the tested bacteria was compared with the sensitivity of the same bacteria to five antibiotics Colisti sulphate, Amicacin, Amoxycillin, gentamicin and sulphamethoxazole trimethoprim (60µg/ml) [12-14].

Results and Discussion

Antibacterial activity assay

The results of the well diffusion test revealed that the fixed and volatile oils extracts of clove showed different degrees of growth inhibition, depending upon the bacterial strains (Table 1) (figure1,2,3,4,5,6,7,8)fixed oil extract were observe to be more active than volatile oil extract. As compare to the standard (Table 2). This studied that the oils rich in phenolic constituent such as eugenol has the highest antioxidant activity against bacterial. [15]reported that the eugenol and thymol have an antioxidant property, in fact, eugenol found in these oils , act on the cell membrane increasing its permeability. [16] reported the antibacterial effects of hydrous, methanolic and ethanolic extracts of clove, cinnamon, sage, thyme and rosmarinus on Gram- positive and Gram- negative bacteria . The results showed that all of these plants had antibacterial action on methicillin-resistant Staphylococcus aureus (MRSA) and Bacillus subtilis, but they were weakly active against Gram-negative bacteria such as Pseudomonas *aeruginosa* and enteropathogenic *Escherichia coli* [17]. Gram-negative bacteria are more resistant to antibiotics than the Gram-

positive bacteria due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism [18].

Conclusion

It is observed that the fixed oil of clove buds at all concentration from "4% to 8%" has higher activities as Antibacterial than the volatile oil. Results clearly indicate that further purification of this extract can leads to isolation of potent antibacterial compound.

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Table 1: Screening of Antibacterial activity of fixed and volatile oils extracted from *Syzygium aromaticum* (clove)

S/No.	Treatment	Concentration (%)	Zone of Inhibition (mm)± Standard deviation				
			EC	**PA**	**SA**	KP	AS
1.	C	-	-	-	-	-	
2.	**fixed oil extracted**	4	14±0.31	0.11 8±	±0.113	±0.0312	±0.2116
		6	17±0.26	10±0.33	±0.0416	±0.1518	±0.118
		8	18±0.22	15±0.92	8±0.261	20±0.21	±0.3518
3.	volatile oil extracted	***4***	-	-	-	-	-
		6	7±0.32	±0.054	-	4±0.15	-
		8	10±0.04	10±0.24	-	8±0.11	-

Values are expressed as Mean (X)± SD, n=3 **Abbr**. EC= *Escherichia coli*, PA = *Pseudomonas aeruginosa*,KP = *Klebsiella pneumonia*, SA= *Staphylococcus aureus*, AS= *Acinetobacter sp*, C: Control (DMSO).

Table 2: Antibiotic activity of different type of bacteria

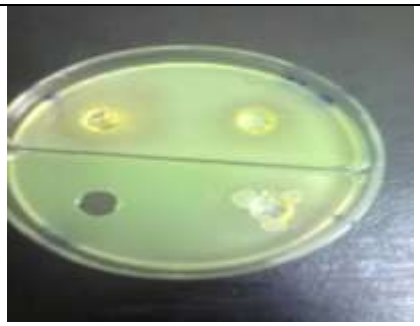
Antibiotic	Zone of Inhibition (mm)± Standard deviation				
	EC	**SA**	**PA**	AS	KP
Colisti sulphate	**-**	**±0.012**	**3±0.01**	**±0.036**	**±0.014**
Amicacin	**15±0.02**	**0.02 13±**	**9±0.01**	**-**	**±0.0412**
Amoxycillin	**-**	**3±0.01**	**-**	**-**	**±0.012**
Gentamycin	**10±0.03**	**6±0.01**	**5±0.02**	**3±0.03**	**±0.011**
Sulphmethoxazole	**3±0.12**	**19±0.03**	**-**	**±0.084**	**-**

Values are expressed as Mean (X)±SD, n=3. EC= *Escherichia coli*, PA = *Pseudomonas aeruginosa*, KP = *Klebsiella pneumonia*, SA= *Staphylococcus aureus*, AS= *Acinetobacter sp*.

Figure(1): Antibacterial activity of fixed oil against *Acinetobacter sp* bacteria at 4% ,6% and 8% concentration

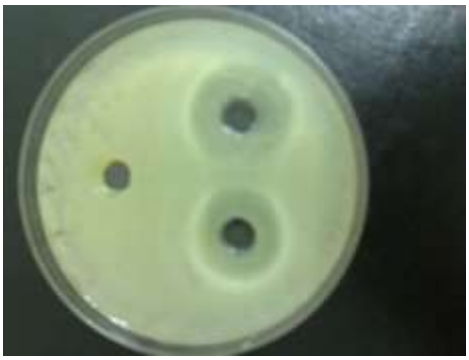
Figure(2): Antibacterial activity of fixed oil against *E.coli* bacteria at 4% ,6% and 8% concentration

Figure(3): Antibacterial activity of fixed oil against Klebsella.pneumonia bacteria at 4% ,6% and 8% concentration



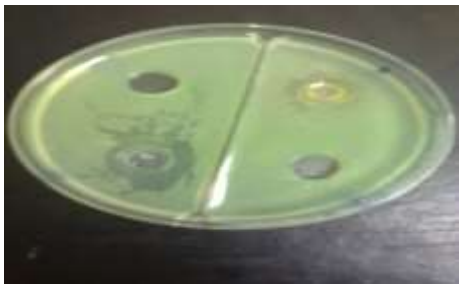
Figure(4): Antibacterial activity of fixed oil against P.aeruginosa bacteria at 4% ,6% and 8% concentration

Figure(5): Antibacterial activity of fixed oil against Staph. aureus bacteria at 4% ,6% and 8% concentration



Figure(6): Antibacterial activity of volatile oil against *E.coli*bacteria at 4% ,6% and 8% concentration

Figure(7): Antibacterial activity of volatile oil against *Klebsella.pneumonia*bacteria at 4% ,6% and 8% concentration



Figure(8): Antibacterial activity of fixed oil against *P.aeruginosa*bacteria at 4% ,6% and 8% concentration

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