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Antimicrobial Activity of *Syzygium aromaticum* and *Citrus aurantifolia* Essential Oils Against Some Microbes in Khartoum, Sudan

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Abstract

Traditional plants representing rich sources of pharmacological compounds have always been of great interest to scientists, which have been already used as a traditional treatment for several diseases such as microbial infections are major public health problems in the developed and developing countries. This study was carried out to investigate antimicrobial activity of (*Syzygium aromaticum)* and lemon (*Citrus aurantifolia*) against standard microorganism. performed by cup-plate agar diffusion method against Five standard bacteria species: two Gram-positive Bacteria *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923), Three Gram-negative bacterial strains including *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella typhi* (6539), one standard fungal strains, *Candida albicans* (ATCC 7596). The oil solution results of *S. aromaticum* exhibited inhibitory effects against most of the tested organisms with the zone of inhibition ranging from (10 to 32 mm). The most active effect observed against *C. albicans* (ATCC 7596) (32 mm) on the other hand the lemon oil peels showed antimicrobial activities against all tested microbes (11 - 20 mm) the highest inhibition zone was observed against *B. subtilis* (NCTC 8236) (20 mm) in 100 mg/ml concentration for each test control positive Gentamicin and Nystatin with zone of inhibition ranging from (14 to 35 mm) against all strains tested. These findings provide scientific evidence to support the traditional medicinal uses of these extracts and indicate a promising potential of these plants for medicinal purposes.

Keywords: *Antimicrobial; Essential Oil; Citrus aurantifolia; Syzygium aromaticum; Sudan*

Introduction

Clove (*Syzygium aromaticum*) is a tall evergreen plant with leathery leaves usually cultivated in coastal areas at maximum altitudes of 200m above the sea level, it contains different bioactive compounds such as volatile oil (15 - 20%), oil (5%), and phenol propanoids (60 - 90%). They also contain trace amounts of tannin complex, gum and resin, and glycosides of sterols [1-3]. Jirovetz., *et al*. [4] which clarified its history of culinary and medicinal use as control nausea and vomiting, cough, diarrhea, dyspepsia, flatulence, stomach distension and gastro intestinal spasm, relieve pain, cause uterine contractions and stimulate the nerves [5-9]. In addition, the cloves are highly antiseptic [10] antimutagenic [11], anti-inflammatory [12], antioxidant [13], antiulcerogenic [14,15], antithrombotic [16], antifungal [17,18], antiviral [19] and anti-parasitic [20], beside its use as a perfume and food flavouring [21,22], as a medicine for the treatment of asthma, rheumatoid arthritis, acne, warts, scars and various allergic disorders [12], as an analgesic, anti-spasmodic, and as a general antiseptic in medical dental practices [23].

On the other hand, clove oil is toxic to human cells [24]. Also its esteemed as a flavouring agent and also used as a spice for scenting, chewing tobacco and an ingredient of betel chew. If ingested or injected in sufficient quantity, it has been shown to cause life threatening complications, including Acute Respiratory Distress Syndrome, Fulminant Hepatic Failure and Central Nervous System disorder. The lethal oral dose is 3.752 g/Kg body weight [25-27].

Lemon (*Cit. aurantifolia*), is an evergreen tree, growing to over 6m in height with toothed leaves and the citrus fruit (lemon) is oval, small, and green to yellow [28,29].

Citrus fruits in general contain sugars, polysaccharides, organic acids, lipids, carotenoid (pigment), vitamins, minerals, flavonoids, bitter limonoids, and volatile components [30]. The lemon is a good source of potassium 145 mg per 100g fruit, bioflavonoids, and vitamin C 40 to 50 mg/100g, twice as much as oranges [28,28], which reflecting its activity as antioxidant [31] and anticancer [32]. Lemon juice and lemon oil have been evaluated for antimicrobial action. The oil shows some bacteriostatic and antiviral action thought to be due to citral and linalool content [31,33]. Lemon has been shown to inhibit the growth of *Aspergillus* mold [34] and to inactivate rabies virus [35], they have been used to disinfect drinking water [36].

So, the main objective of the study is to evaluate the antimicrobial activity of the essential oil of *S. aromaticum* against some standard microorganism.

Materials and Methods

Plants materials

The Clove (Syzygium aromaticum) and lemon (Citrus aurantifolia), was collected from Khartoum central Sudan during the period from April to July 2016, and the plants was kindly identified and authenticated by Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI) in Khartoum, Sudan. and kept in the herbarium until oil extraction steps.

Plants preparation

The oils of the tested plants were obtained by Hydrodistillation technique using Clevenger's apparatus. 100g from the tested plants material were placed in 1L round bottom flask and distilled water was added and mixed thoroughly. The contents of the flask were boiled gently for 4h until the volatile oil has been distilled. The crudes volatile oils were transferred by means of a pipette into a separate brown glass bottle. Anhydrous sodium sulphate was added agitated gently to absorb the water and the clear oils were decanted into brown glass bottles and kept in the refrigerator until needed for analysis.

Bacteria strains

The standard bacterial and fungal strains used in this study were obtained from the Department of Microbiology National Research Centre, Khartoum, Sudan. The bacterial cultures were maintained on nutrient agar and incubated at 37°C for 18h and then used for the antimicrobial test.

The oil solution was tested against Five standard bacteria species: two Gram-positive Bacteria *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923), Three Gram-negative bacterial strains *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella typhi* (6539), one standard fungal strains, *Candida albicans* (ATCC 7596) using the cup-plate agar diffusion method. The study was conducted in the microorganism's lab, Department of Microbiology, Faculty of Pharmacy and Medical Laboratory Sciences, International University of Africa, Khartoum, Sudan.

In vitro testing of oils for antibacterial and anti-fungal activity

The cup-plate agar diffusion method as described by Kavanagh [37] which was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. Aliquot of 1 ml of the standardized bacterial stock suspension (between 10^8 and 10^9 CFU/ml) was thoroughly mixed with 100 ml of molten sterile Mueller Hinton agar (HI Media, India) which was maintained at 45°C. 20 ml aliquots of the inoculated Mueller Hinton agar were distributed into sterile Petri-dish plates. The agar was left to set and in all of these plates 5 cups (8 mm in diameter) were cut using a sterile cork borer and agar discs were removed. Each cups were filled with 0.1 ml of the essential oil using an automatic microliter pipette, and allowed to diffuse at room temperature for two hours. The plates were then incu-

bated in an upright position at 37°C for 24h. Two replicates were carried out for each extracts against each of the tested microorganisms. The plates were observed for the presence of inhibition of bacterial growth that was indicated by a clear zone around the wells. The size of the zones of inhibition was measured and the antibacterial activity was expressed in terms of average diameter of the zone of inhibition in millimeters. The results were compared with the standard antibiotics.

The same method used for the antibacterial test was employed. However, the growth media used in case of fungi, was Sabouraud dextrose agar (instead of nutrient agar). The inoculated medium was incubated at 25°C for two days for *Candida albicans*.

Results and Discussion

The oily solutions of *Syzygium aromaticum* and *lemon (Citrus aurantifolia*), were screened for antimicrobial activity against five standard bacteria species: two Gram-positive Bacteria *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923), Three Gram-negative bacterial strains *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella typhi* (6539), one standard fungal strain (*Candida albicans)* (ATCC 7596) using the cup-plate agar diffusion method.

The oil solution action results of *S. aromaticum* exhibited inhibitory effects against most of the tested organisms with the zone of inhibition ranging from (10 to 32 mm) (Table 1) in length. The largest inhibition zone was observed against *C. albicans* (ATCC 7596) (32 mm), *S. aureus* (ATCC 25923) (26 mm) and *B. subtilis* (NCTC 8236) (24 mm). while in case of Gram negative highest inhibition was observed against *S. typhi* (6539), (24 mm) and *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) (22 mm) at the highest concentration checked (100 mg/ml). On the other hand, multi studies strongly supported our study showing inhibition zone extend from such as study of [38] (7 - 27 mm) at 2000 ppm was shown by *S. aromaticum* on *B. cereus* while the minimum effect of *S. aromaticum* shown on *E. coli*, other studies also supported us showing significant antimicrobial action against the tested microorganisms mainly the flavonoids and cinamic acids [39-42]. The essential oil of lemon peels showed antimicrobial activities against all tested microbes (11 - 20 mm) (Table 2). The highest inhibition zone was observed against *B. subtilis* (NCTC 8236) (20 mm), *S. aureus* (ATCC 25923) (18 mm), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) (17 mm), *S. typhi* (6539) (16 mm) and *C. albicans* (ATCC 7596) (14 mm), at the highest concentration checked (100 mg/ml). This study is compatible with many of the studies such as Ehigbai I Oikeh., *et al*., [43] and other study with same test organisms showed the peel extract has a high degree of antibacterial activity [44] may be because of the difference in the phytochemical composition in various part of the plant or may be also due to the extraction method used and/or environmental factors or difference in the genotypes of the citrus plant used. For each test control positive Gentamicin and Nystatin with zone of inhibition ranging from (14 to 35 mm) against all strains tested (Table 3) The result of minimum inhibition concentration from (Table 1 and 2) showed that 12.5 mg/ml was the lowest concentration at which all the tested microorganisms were inhibited [45].

No.	Standard microorganisms	Zone of Inhibition (mm)			
		Concentrations (mg/ml)			
		100	50	25	12.5
Tested bacteria					
1	*Bacillus subtilis*	24	19	15	10
2	*Escherichia coli*	22	17	14	12
3	*Staphylococcus aureus*	26	19	16	11
4	*Salmonella* typhi	24	18	15	11
5	*Pseudomonas aeruginosa*	22	16	11	-
Tested fungi					
6	*Candida albicans*	32	28	25	20

***Table 1**: In vitro antimicrobial activity of the essential oil of Syzygium aromaticum.*

No.	Standard microorganisms	Zone of Inhibition (mm)			
		Concentrations (mg/ml)			
		100	50	25	12.5
Tested bacteria					
1	*Bacillus subtilis*	20	18	17	15
2	*Escherichia coli*	17	15	14	11
3	*Staphylococcus aureus*	18	15	14	12
4	*Salmonella* typhi	16	15	14	12
5	*Pseudomonas aeruginosa*	17	16	15	14
Tested fungi					
6	*Candida albicans*	14	14	14	14

***Table 2**: In vitro antimicrobial activity of the essential oil of Citrus aurantifia against some microorganisms.*

Antibiotics Concentrations (µg/ml)		Gram positive		Gram negative		
	Tested bacteria					
	Mean Diameter of Growth Inhibition Zone (mm)					
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella* typhi	
Gentamicin	40	29	35	32	23	34
	20	22	33	30	22	32
	10	20	30	17	21	30
	5	17	28	-	19	27
Tested fungi (M.D.I.Z. mm)						
Nystatin	*Candida albicans*					
	50	17				
	25	14				
	12.6	-				

***Table 3:** Antibacterial and antifungal activity of reference antibiotics against standard microorganisms.*

Conclusion

The present investigation expresses that plants have great potential as antimicrobial compounds against microorganisms. Thus they can be used in the treatment of infectious diseases caused by pathogenic bacteria and as well as the essential oil of *Syzygium aromaticum* has most Biologically active compound than *Citrus aurantifolia*.

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