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Antimicrobial, Antioxidant activities and GC/MS analysis of clove oil (*Syzygium aromaticum*)

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Abstract

Introduction: Essential oils are volatile, complex, strong-smelling natural compounds formed by aromatic plants as secondary metabolites. Usually, it is obtained by steam or hydrodistillation.
In nature, essential oils play an important role in the protection of plants, Essential oils can be synthesized by all plant organs, i.e. buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood or bark, and are stored in secretory cells, cavities, canals, epidemic cells or glandular trichomes.
Method: Extraction of Clove Oil Steam hydro-distillation is selected as a method to isolate clove oil, the antimicrobial and antioxidant activity was detected, and the chemical content of clove oil. Clove oil was tested against five standard bacteria strains (Bacillus subtilis, Staphylococcus aurous, Pseudomonas aeruginosa. salmonella typhi, Escherichia coli), and two fungi (Asperiglus nigur, Candida albicans) using the cup-plate agar diffusion method. Also tested was the antioxidant effect using DPPH. The chemical constituents of clove oil were also detected using GC/MS. Clove oil showed high activity against Salmonella typhi, Staphylococcus aurous, Pseudomonas aeruginosa, Escherichia coli, Asperiglus nigur and Bacillus subtilis
Result: Clove Oil extraction high activity against all types of bacteria under study (*Bacillus subtilis*, *Staphylococcus aurous*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*), and it had high activity against *Aspergillus nigur*, while it had low activity against *Candida albicans* at a concentration of 100 mg/ml.. interestingly, the antioxidant activity of clove oil was higher compared to Propyl gallate (Standard). Chemical detection by GC/MS showed the presence of 4 compounds. The main compounds in clove oil were Eugenol (58.38) and Caryophyllene (34.87).

Kayword: Antimicrobial, Antioxidant, GC/MS, clove oil, *Syzygium aromaticum*.
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Introduction: Essential oils are volatile, complex, strong-smelling natural compounds formed by aromatic plants as secondary metabolites. Usually, it is obtained by steam or hydro-distillation (1). In nature, essential oils play an important role in the protection of plants. They also may attract some insects to promote the dispersion of pollens and seeds or keep away other undesirable insects. Thus, EOs can play a role in mediating the interactions of plants with the environment (2). Essential oils can be synthesized by all plant organs, i.e. buds, flowers, leaves, stems, twigs, seeds, fruits, roots,transplacental transfer from the mother usually following acute maternal infection. If

congenital toxoplasmosis occurs early in pregnancy, it may lead to severe damage or abortion.(4) Though the infection is frequently asymptomatic or mild and self-limiting (fever, agitation, lymph- wood or bark, and are stored in secretory cells, cavities, canals, epidemic cells or glandular trichomes (1). Clove (*Syzygium aromaticum* L.) Merril. & Perry, is a member of the family Myrtaceae. The clove of commerce is its dried unopened flower buds. Whole and ground cloves are used to enhance the flavor of meat and rice dishes. They are highly valued in medicine as a carminative and stimulant and are said to be a natural anthelmintic (3). Clove oil has been listed as a ‘‘Generally Regarded As Safe’’ substance by the United States Food and Drug Administration when administered at levels not exceeding 1500 ppm in all food categories (4). Clove oil its antimicrobial properties, antioxidant, analgesic, anesthetic, anti-inflammatory, and insecticidal activity It is also used for preventing degenerative diseases because of its antioxidant effects. Clove bud oil is a common healing agent for wounds and burns in traditional medicine (5). The major constituents of the clove essential oils are eugenol, β-caryophyllene, α-humulene and humulene epoxide. These constituents are known to possess antibacterial antifungal and anti-carcinogenic properties. According to these various biological activities, clove oils find uses in toothpaste, mouth washes, soaps and other cosmetic items (6).

The purpose of this research is to investigate Antimicrobial, Antioxidant activities and GC/MS profiling of clove oil (*Syzygium aromaticum*).

Material and Methods: Plant Collection: The sample of clove was purchased from Omdurman local market. clove used was identified and authenticated at the herbarium of the botany department. Faculty of Science and Technology, Omdurman Islamic University.

Oil Extraction: Extraction of Clove Oil Steam hydro-distillation is selected as a method to isolate clove oil. A hundred grams of dried clove flowerswere put in a steam flask. The steam distillation was conducted several times such as 3, 4, 5, and 6h. The time started to be counted when the first drop of distillate comes out.

Antimicrobial activity : 3.2.2.1.1Tested bacteria and fungi : Five standard bacteria and two fungi species were obtained from Microbiology lab, Omdurman Islamic University. They were gram positive (G+ve): *Bacillus subtilis*, *Staphylococcus aurous* and gram negative (G-ve): *Pseudomonas aeruginosa*, *salmonella typhi*, *Escherichia coli*, and fungi. *Asperiglus nigur* and *Candida albicans*. The bacteria and fungi were activated and cloned three successive times in nutrient agar and stored on nutrient agar slants at 4°C. Subsequent cultivation and tests were done on nutrient agar medium.

Preparation of bacterial suspensions : One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37º C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 108-109c.f.u/ml. The suspension was stored in a refrigerator at 4° C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable

counting technique (7). Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes on drop of the appropriate dilutions were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

Preparation of fungal suspension: The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100ml of sterile normal saline, and the suspension were stored in the refrigerator until used.

In vitro testing of extracts for antibacterial activity : The cup-plate agar diffusion method as described by Kavanagh, 2014 (8) 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 108 and 109 CFU/ml) was thoroughly mixed with 100 ml of molten sterile Mueller Hinton agar (HiMedia, 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) was cut using a sterile corkborer and agar discs were removed. Each cups were filled with 0.1 ml of the methanolic extract using an automatic microliter pipette, and thereafter the extract was allowed to diffuse at room temperature for two hours. The plates were then incubated in an upright position at 37°C for 24h. Two replicates were carried out for each extract against each of the tested microorganisms. After incubation the diameters of the resultant growth inhibition zones were measured and averaged. The mean values were tabulated.

In vitro testing of extracts for Antifungal activity: The same method as for bacteria was adopted. Instead of nutrient agar, Sabouraud dextrose agar was used. The inoculated medium was incubated at 25 ºC for two days for the *Candida albicans*.

Antioxidant activity: DPPH radical scavenging assay: The DPPH free radical scavenging activity Principle: The antioxidant activity was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. Experiments were carried out according using the method of Shimada et al., 1992 (9), with slight modification. Active samples can reduce the stable radical DPPH to the yellowcolored diphenyl- picrylhydrazine.

Assay: Test samples were allowed to react with 2.2 di (4-tretoctylphenyl)-1-picrylhydrazyl stable free radical (DPPH) for half an hour at 37oc in 96-wells plate. The concentration of DPPH was kept at (300µM). the test sample was dissolved in DMSO

while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multi plate reader spectrophotometer. Percentage of radical scavenging activity of the sample was determined in comparison with a DMSO treated control. All tests were conducted in triplicate. $DPPH\ radical\ scavenging$ **(%) = 100−{(**$Ac-At$**)/**Ac**} × 100**

Where, At= Absorbance value of test compound; Ac=Absorbance value ofcontrol.

Chromatographic analysis:

Gas Chromatography Mass Spectrometry (GC MS) analysis: GC-MS technique was used in this study to identify the phytocomponents present in the most active fractions. The tested extracts were analyzed by GC-MS using Shimadzu Mass Spectrometer-2010 series. 1 µL of sample was injected in GC-MS equipped with a split injector.

The MS was operated in the electron ionization (EI) mode (70 eV). Helium was employed as the carrier gas and its flow rate was adjusted to 1.2 mL/min.

The analytical column connected to the system was an Rtx-5 capillary column (length-30 m × 0.25 mm i.d., 0.25µm film thickness). The column head pressure was adjusted to 93.9 kPa. Column temperature programmed from 110 °C (7 min) to 200 °C at 10 °C/min and from 200-280 °C at 5 °C/min withhold time 0 and 9 min respectively.

A solvent delay of 4.50 min was selected. Theinjector temperature was set at 250 °C. The GC-MS interface was maintained at 280 °C. The MS was operated in the ACQ mode scanning from m/z 40 to 550.0. In the full scan mode, EI mass spectra in the range of 40–550 (m/z) were recorded at electron energy of 70 eV. Compounds were identified by comparing mass spectra with library of the National Institute of Standard and Technology (NIST), USA/Wiley.

Results and Discussion: Antimicrobial activity:

The antimicrobial activity of clove oil was studied in different concentrations (100, 50, 25, 12.5 and 6.2 mg/ml against standard bacterial and fungal. The antibacterial and antifungal potential of clove oil was assessed in terms for inhibition zone of bacterial growth. The results of the antibacterial and antifungal activities are presented in **Table (1)** and **Figure (1)**. Clove oil showed high activity against all types of bacteria under study (*Bacillus subtilis*, *Staphylococcus aurous*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*), and it had high activity against *Aspergillus nigur*, while it had low activity against *Candida albicans* at a concentration of 100 mg/ml. The study obtained by (10) showed that clove oil has an inhibitory and bactericidal effect against S. aureus, E. coli, L. monocytogenes and S. Typhimurium. In addition, the results obtained by (11) showed that the petroleum ether extract of cloves has high efficacy against the standard strain of *Streptococcus oralis, Streptococcus parasanguinis*, *Streptococcus mutans*, and *Streptococcus mutans* that cause dental caries.

Table 1: Antimicrobial activity of Clove oil against standard organisms

Sample	Concentrations	Test organisms used Inhibition zones (mm)						
		B.s	*St.a*	*P.s*	*S.t*	*E.c*	*As.n*	*C.a*
Clove Oil	100 mg/ml	18	27	27	34	24	23	9
	50 mg/ml	-	12	12	22	-	9	-
	25 mg/ml	-	-	-	-	-	-	-
	12.5 mg/ml	-	-	-	14	14	-	-
	6.2 mg/ml	-	-	-	15	-	-	-

B.S= Bacillus subtilis; St.a: Staphylococcus aurous; Ps= Pseudomonas aeruginosa; S.t= salmonella typhi ;E.c= Escherichia coli; As.n= Asperiglus nigur; C.a= candida albicans; M. D. I. Z mm = Mean diameter of growth inhibition zones, in mm.

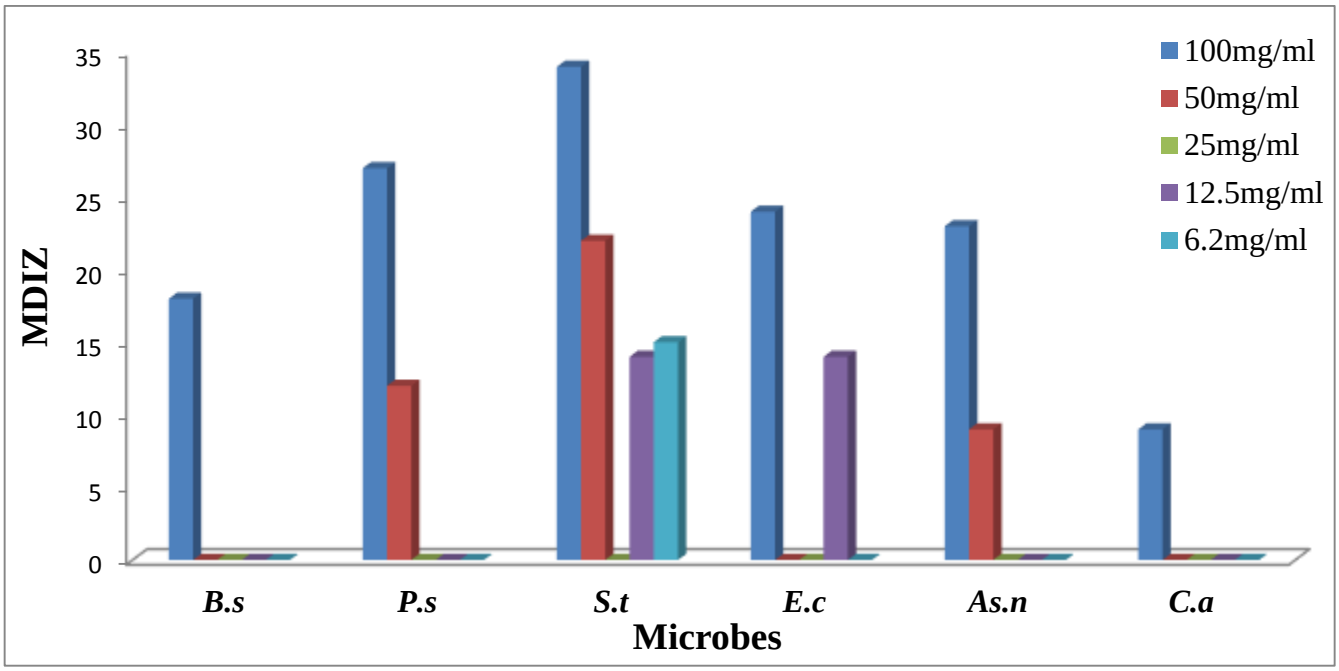

Figure (1): Antimicrobial activity of Clove oil against standard organisms

Antioxidant activity: DPPH radical scavenging assay is the most common method used in the study of the antioxidant activity of plant extracts (Table 2). Clove oil showed the highest antioxidant activity (94.7±0.003) compared to the standard npropyl gallate (91±0.01). (11) mentioned that petroleum ether extract has strong antioxidant properties. The result obtained by (4) also showed that clove oil has high activity as an antioxidant

Table (2): Antioxidant activity of Clove Oil

Sample	**Inhibition %±SD**
Clove Oil	94±0.003
Propyl gallate (Standard)	91±0.01

Gas Chromatography–Mass spectrophotometer (GC MS) of Clove oil: GC/MS chromatogram of clove oil showed the presence of four compounds representing 100% of the extract were identified and is given in **Table (3)** and Figure (2).

Quantitatively, the extract was characterized by abundance of Eugenol (58.38%) and Caryophyllene (34.87%). Eugenol is a volatile phenolic compound that is one of the main components of clove essential oil and is responsible for the aroma of cloves (12). Many studies have shown that eugenol has antimicrobial and antioxidant properties (13). caryophyllene is anatural sesquiterpene hydrocarbon present in hundreds of plant species. Caryophyllene has antimicrobial, anticancer, anti-inflammatory and antioxidant properties (14); (15).

Table (3): Phytocomponents identified of Clove Oil

Peak	R. Time	Area%	Compounds Names	Molecular Weight	Molecular Formula
1	9.929	58.38	Eugenol	164.20	$C_{10}H_{12}O_2$
2	10.829	34.87	Caryophyllene	204.35	$C_{15}H_{24}$
3	11.253	3.58	1,4,7,-Cycloundecatriene,1,5,9,9,-tetramethyl-, Z	204.35	$C_{15}H_{24}$
4	11.999	3.17	Phenol, 2-methoxy-4-(1-propenyl)-, acetate	206.24	$C_{12}H_{14}O_3$

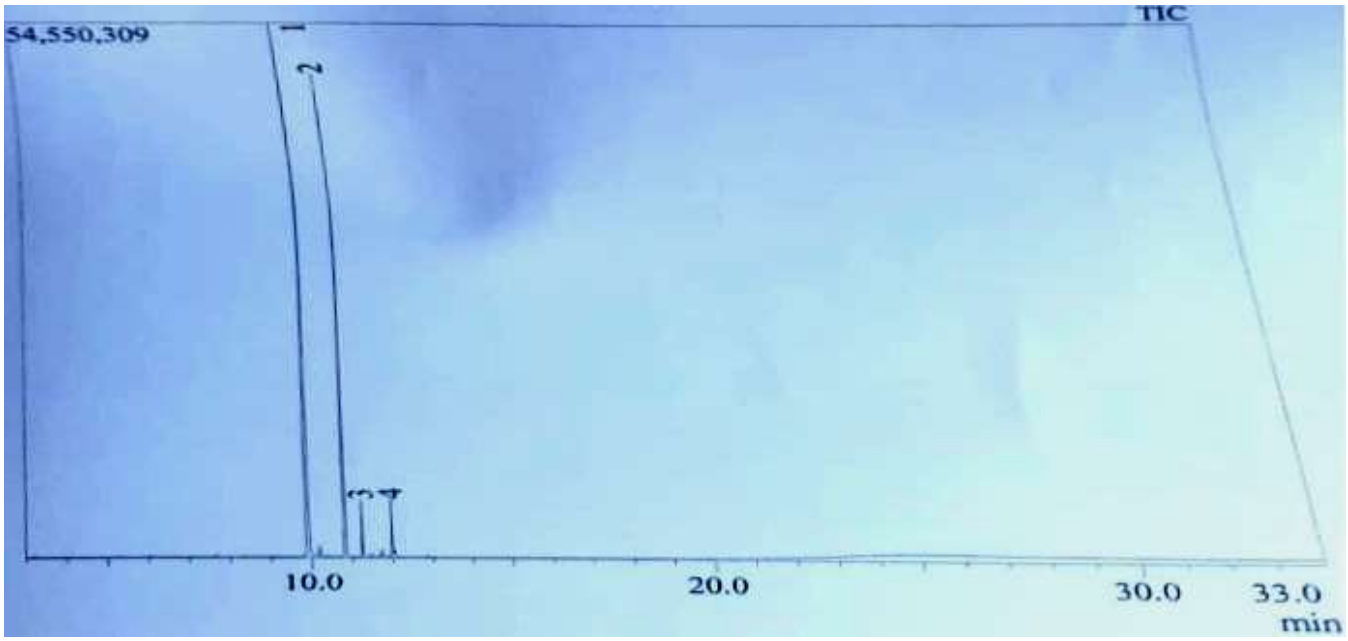

Figure (2): GC-MS chromatogram of Clove oil

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