Advancement in Medicinal Plant Research
Vol. 4(4), pp. 122-128, December 2016
ISSN: 2354-2152
Full Length Research Paper

Antibacterial activity of clove oil against some microorganisms at Khartoum State

Zahra O. Fagere[1*] and Aisha Zoheir Al Magboul[2]

[1]Medical and Health Studies Board, University of Khartoum. Khartoum, Sudan.
[2]Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), P.O. Box 2404, National Center for Research, Khartoum, Sudan.

Accepted 29 November, 2016

ABSTRACT

Microorganisms have increased and that may cause some health problems, so it has become necessary to find out new antibacterial agents. This is a descriptive cross-sectional study aiming to evaluate the antibacterial activity of the essential oil of *Syzygium aromaticum* against selected bacteria at Khartoum State, Sudan. The essential oil of *S. aromaticum* was obtained by hydrodistillation technique using Clevenger's apparatus and was screened for *in vitro* antibacterial activity against four standard bacteria two Gram positive: *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923) and two Gram negative: *Eschericia coli* (ATCC 25922) and *Psuedomonas aeruginosa* (ATCC 27853), using the cup plate agar diffusion method and the micro-dilution method. The essential oil of *S. aromaticum* dissolved in methanol (1:10) showed high activity (28 and 22 mm) against Gram positive bacteria (*S. aureus* and *B. subtilis*) and (26 and 21 mm) against Gram negative bacteria (*E. coli* and *P. aeruginosa*). The minimum inhibitory concentration of the essential oil of *S. aromaticum* against Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) was 0.78 mg/ml and against the Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) was 1.5 mg/ml. The minimum bactericidal concentrations of the essential oil of *S. aromaticum* against (*B. subtilis*, *S. aureus and E. coli* were 3.125 mg/ml and *P. aeruginosa*) was 6.25 mg/ml. The result of antibacterial activity of the essential oil was compared with the activity of reference drugs. The essential oil of the *S. aromaticum* possessed antibacterial activity against the Gram positive and Gram negative organisms which are responsible for several diseases. It is recommended that pharmacological, toxicological and clinical studies be carried out on the essential oils of the clove *Syzygium aromaticum* to assess its safety, therapeutic efficacy and potential for commercial utilization.

Keywords: *In vitro*, antibacterial activity, essential oil, *Syzygium aromaticum*, Khartoum State.

*Corresponding author. E-mail: zozo101990@gmail.com.

INTRODUCTION

Essential oils of plants and their other products from secondary metabolism have had a great usage in folk medicine, food flavoring, fragrance, and pharmaceutical industries (Alma et al., 2004).

Essential oils are aromatic volatile oily hydrophobic liquid concentrates that are extracted from plant material, such as flowers, buds, seeds, leaves, twigs, bark, wood, fruits, roots and whole plant. These essential oils are highly complex mixtures of 20 to 60 volatile compounds, albeit some may contain more than 100 different components (Djilani and Dicko, 2012; Ibrahium et al., 2013).

These essential oils contain a variety of volatile molecules such as terpenes, terpenoids and phenol derived aromatic and aliphatic compounds, which might have bactericidal, antiviral, and fungicidal consequences (Akthar et al., 2014). Terpenoids are the primary constituents of the essential oils responsible for the aroma and flavor (Nuzhat and Vidyasagar, 2013).

Essential oils have been traditionally used for treatment of infections diseases all over the world for centuries (Rios and Recio, 2005). Today the use of essential oils is

a growing market and there are a considerable range of applications. The oils are used in the food, beverages industry, as fragrances in perfumes and cosmetics, but the oils also cover a broad spectrum of biological activity which has lead to an increased interest among researchers. In recent years there has been extensive research to explore and determine the antimicrobial activity of essential oils. All oils tested to date have displayed some antimicrobial activity and some have been shown to be more effective than others (Bergkvist, 2007). By tradition, it has been used in food preservation as flavoring and antimicrobial substance (Velluti et al., 2003).

Clove belongs to a tree *Eugenia caryophyllata* (*Syzygium aromaticum*), is used as a spice in almost all the world's fare. It has a very major role in spice trade and is highly appreciated for their therapeutic properties. Cloves are an excellent source of manganese. They are also a very good source of dietary fiber, vitamin C, vitamin K, and Ω-3 fatty acids and a good source of magnesium and calcium. Cloves consist of a significant amount of proteins, iron, carbohydrates, calcium, phosphorus, potassium, sodium and hydrochloric acid. The most important constituent of clove is the phenyl-propene eugenol due to which it has strong characteristic aroma. Major parts of clove consist of eugenol comprises 70 to 90% and remaining 15% consist of dry weight (Shobana and Naidu, 2000). Molds, yeast and bacterial growth could be inhibited by the application of clove essential oil (Burt, 2004). Microorganisms like *Alternaria* sp*., Aspergillus* sp*., Canninghamella* sp*., Lactobacillus* sp*. Fusarium* sp*., Clostridium* sp; *Mucor* sp*., Salmonella* sp*., Penicillium* sp*.* and *Bacillus* sp*.* could be repressed by using clove essential oil (Soliman and Badeaa, 2002). The cloves are antimutagenic, anti-inflammatory, antioxidant, antiul-cerogenic, antithrombotic and antiparasitic. The essential oil extracted from the dried flower buds of clove are used for acne, warts, scars and parasites (Miyazawa and Hisama, 2003).

Clove (*Syzygium aromaticum* L. Merrill and Perry) is one of the most valuable spices that have been used from centuries as food preservative and for many medicinal purposes. Cloves are native of Indonesia but nowadays are cultivated in several parts of the world (Cortés-Rojas et al., 2014). Flower bud have many medicinal proprieties like antiviral, antimicrobial, antifungal general stimulating, hypertensive aphrodisiac, light stomachic, carminative , anesthetic useful in cataract (Di Paoli et al., 2007; Politeo et al., 2010; Koba et al., 2011; Machado et al., 2011).

The oil of cloves has been used in a variety of health conditions including indigestion, generalized stress, parasitic infestations, cough, toothaches, headache, and blood impurities. In fact, the expert pane German Commission recently approved the use of its essential oil as a topical antiseptic and anesthetic (Hänse and Sticher, 2007). It has also been used for nausea and vomiting, while in tropical Asia, it has been given to treat such diverse infections as malaria, cholera and tuberculosis (Chevaillier, 2001). The main objective of the study is to evaluate the antibacterial activity of essential oil of *S. aromaticum* against selected bacteria.

MATERIALS AND METHODS

Plant materials

Clove (*Syzygium aromaticum*) was bought from Alyhya Mole (Khartoum, Jabraa). They were identified and authenticated by the taxonomist Mr. Yaha S. Mohammed (Medicinal and Aromatic Plants and Traditional Medicine Research Institute, Khartoum, Sudan (MAPIMRI). The specimens were deposited at the Herbarium of the Institute (Figure 1).

Preparation of essential oil

The oil of the tested plant Clove (*Syzygium aromaticum*) was obtained by Hydrodistillation technique using Clevenger's apparatus. 100 g from the tested plant material were placed in 1 L round bottom flask and distilled water was added and mixed thoroughly. The contents of the flask were boiled gently for 4 h until the volatile oil has been distilled. The crude volatile oil was 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 oil was decanted into brown glass bottle and kept in the refrigerator until needed for analysis.

Test microorganisms

The essential oil of *S. aromaticum* was tested against four standard bacteria two Gram positive: *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923) and two Gram negative: *Eschericia coli* (ATCC 25922), and *Psuedomonas aeruginosa* (ATCC 27853), using the cup plate agar diffusion method, and the micro-dilution method. The bacterial strains used in the study were obtained from the Department of Microbiology, (MAPTMRI) and National Health Laboratory of Khartoum in Sudan. The bacterial cultures were maintained on nutrient agar and incubated at 37°C for 18 h and then used for the antimicrobial test.

In vitro testing of extracts for antibacterial activity

The cup-plate agar diffusion method described in Kavanagh, (1972) was used adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. One ml of the standardized bacterial stock suspension (between 10^8 and 10^9 CFU/ml) was thoroughly mixed with 100 ml of molten sterile nutrient agar which was maintained at 45°C. 20 ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dish plates. The agar was left to set and in all of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed. Each cup was filled with 0.1 ml sample of the essential oil using an automatic microliter pipette, and thereafter the extracts were allowed to diffuse at room temperature for two hours. The plates were then incubated in an upright position at 37°C for 18 h. Two replicates were carried out for each extract against each of the test organisms. Simultaneously positive control involving the addition of methanol instead of the oil in methanol was carried out separately after incubation the diameters of the resultant growth

Figure 1. *Syzygium aromaticum* (Linn) Merr. and Perry.

inhibition zones were measured and averaged. The mean values were tabulated.

Determination of minimum inhibitory concentrations (MIC) by agar well diffusion method

The minimum inhibitory concentrations of the oil was determined on solid media (Nutrient agar) using the method adopted by Kavanagh (1972).The ranges of concentrations were100, 50, 25, 12.5,6.125, 3.5, 1.56 and 0.78 mg/ml. MIC is the least concentration of antimicrobial agent that completely inhibits the growth.

Minimum Bactericidal Concentrations (MBC_s)

In addition to the solid medium diffusion procedure, the micro plate bioassay (micro dilution) was used, as recommended by NCCLS (1990), for determination of minimum bactericidal concentration (MBC). The MBC was defined as lowest concentration of plant extract that kills bacteria after incubation for 20 h at 37°C.

Into each well 100 µl of nutrient broth inoculated with the bacteria inoculum prior to the essay. An aliquot (100 µl) of the plant extract was added in first well. 50, 25, 12.5 and 6.125 mg/ml of the plant extract were prepared in 16 well of microtitre plate, including one growth control (NB + Bacterial inoculum) and one sterility control (NB + test plant extract). The contents of the wells were mixed and micro plates were incubated at 37°C for 24 h. The MBC was determined by sub-culturing from each test concentrations in the plates were incubated at 37°C for 24 h.

Antibacterial activity of reference drugs against standard microorganisms

In the present work, two antibacterial drugs (Ciprofloxacin and Gentamicin) were tested at different concentrations obtained by taking 0.1 g of powdered drug and dissolved in 100 ml sterile distilled water to give a concentration of 1000 µg/ml followed by serial dilutions to give concentrations of 40, 20, 10 and 5 µg/ml. These drugs were tested against reference bacteria, that is, *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*.

RESULTS

The antibacterial activity of the essential oil from dried flower buds of Clove (*Syzygium aromaticum* (Linn.)) Merr. and Perry. family (Myrtaceae) showed variable activity against Gram positive and Gram negative organisms (Table 1 and Figures 2, 3 and 4).

The minimum inhibitory concentration of the essential oils of (*S. aromaticum)* was determined against the standard organisms (*Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*) using the cup plate agar diffusion method and the results were summarized in Table 2 as mg/ml. The essential oil of *S. aromaticum* showed that 1.5 mg/ml was the lowest concentration to inhibit the growth of all organisms tested against Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*).

The minimum bactericidal concentration of the essential oil of *S. aromaticum* was determined against the standard organisms (*Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*) using the micro dilution method.

The results were summarized in Table 3 as mg/ml. The MBC of the essential oil of *S. aromaticum* was 3.125 mg/ml against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* while 6.25 mg/ml against *Pseudomonas aeruginosa*.

Comparison of observation given in Tables 2 and 4 showed that the *Syzygium aromaticum* dissolved in methanol inhibited *S. aureus*, *E. coli* and *P. aeruginosa* higher than 40 µg/ml Ciprofloxacin and *B. subtilis* similar to 40 µg/ml Ciprofloxacin. It inhibited *S. aureus* almost similar to40 µg/ml Gentamicin and inhibited *E. coli* higher than 10 µg/ml Gentamicin and *P. aeruginosa* similar to 10 µg/ml Gentamicin.

Table 1. Screening of *Syzygium aromaticum* against the standard bacteria.

Family/ Plant name/ Vernacular name	Part used	Yield%	Solvent	Tested organisms used			
				B. s	***S. a***	***E. c***	***P***
Myrtaceae/ *Syzygium aromaticum*/ Goronful	Dried flower buds	3.25	Methanol	22	28	26	21

Key: Interpretation of results: MDIZ (mm) : >18 mm: Sensitive, 14 to 18 mm: Intermediate: <14 mm: Resistant. Concentration used 100mg/ml at 0.1 ml/cup. *B. s = Bacillus subtilis, S. a = Staphylococcus aureus, E. c = E. coli, P = Pseudomonas aeruginosa.*

Figure 2. Zones inhibition of antibacterial activity of clove oil (*Syzygium aromaticum)* against *Staphylococcus aureus* by concentration used 10%.

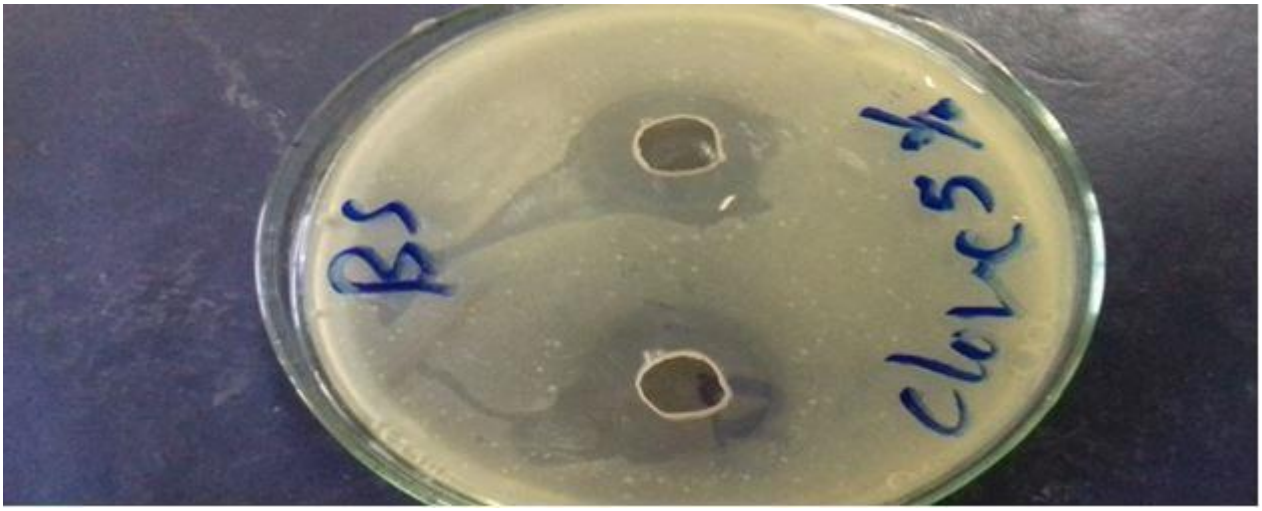

Figure 3. Zones inhibition of antibacterial activity of clove oil (*Syzygium aromaticum)* against *Bacillus subtilis* by concentration used 5%.

DISCUSSION

The essential oil of *S. aromaticum* was screened for its antibacterial activity against two Gram positive bacteria (*B. subtilis* and *S. aureus*) and two Gram negative bacteria (*E. coli* and *P. aeruginosa*) using the cup plate agar diffusion method. As can be seen from Table 1 the oil of *S. aromaticum* dissolved in methanol showed high

Figure 4. Zones inhibition of antibacterial activity of clove oil (*Syzygium aromaticum)* against *Bacillus subtilis* by concentration used 2.5%.

Table 2. Minimum inhibitory concentrations (mg/ml) of clove *Syzygium aromaticum* against the standard bacteria.

Standard microorganisms	MIC (mg/ml)							
	100	**50**	**25**	**12.5**	**6.25**	**3.125**	**1.50**	**0.78**
Bacillus subtilis	22	20	20	17	15	14	14	-
Staphyococcus aureus	28	24	20	20	18	16	15	-
Escherichia coli	26	21	17	18	16	15	11	-
Pseudomonas aeruginosa	21	20	20	22	19	17	14	-

Table 3. Minimal bactericidal concentrations (mg/ml) of *Syzygium aromaticum* against the standard bacteria.

Standard microorganisms	MBC (mg/ml)						
	100	**50**	**25**	**12.5**	**6.25**	**3.125**	**1.50**
Bacillus subtilis	-	-	-	-	-	-	+
Staphyococcus aureus	-	-	-	-	-	-	+
Escherichia coli	-	-	-	-	-	-	+
Pseudomonas aeruginosa	-	-	-	-	-	+	+

Table 4. Antibacterial activities of reference drugs against standard microorganisms.

Drugs	Concentrations (µg/ml)	Standard microorganisms used			
		Gram positive		Gram negative	
		Bacillus subtilis	***Staphylococcus aureus***	***Escherichia coli***	***Pseudomonas aeruginosa***
Ciprofloxacin	40	22	23	20	15
	20	20	19	19	14
	10	19	17	17	-
	5	13	13	15	-
Gentamicin	40	35	29	32	23
	20	33	22	30	22
	10	30	20	17	21
	5	28	17	-	19

activity (22 to 28 mm) against *B. subtilis* and *S. aureus* and 26 to 21 mm against *E. coli* and *P. aeruginosa* though the sensitivities of the microorganisms varied. Bergkvist (2007) in contrast to our result showed that *P. aeruginosa* was the only bacterium not susceptible for the tested oil of *S. aromaticum*. Ayoola et al. (2008) found similar to our result that the volatile oil of *S. aromaticum* showed a broad spectrum of activity against *E. coli* and *S. auerus*. Previous study by Pandey and Singh (2011) and Hussein et al. (2014) demonstrated the antibacterial activity of the essential oil exhibited activity from *S. aromaticum* dried flower buds against the Gram positive *S. aureus* and the two Gram negative organisms (*E. coli* and *P. aeruginosa*). Another study by Javed et al. (2012) found similar to our result that the essential oil of *S. aromaticum* exhibited pronounced and varying degree of growth inhibition against *B. subtilus*, *S. aureus* and *E. coli*.

Therefore the essential oil of *S. aromaticum* showed high activity against all organisms tested and this may be attributed to the contents of active ingredients such as mono –and sesquiterpene hydrocarbons in the plant detected by many co-workers (Lee et al., 2009; Rodriguez et al., 2014; Koba et al., 2015; Nana et al., 2015).

On the whole, Gram positive bacteria were more active to *S. aromaticum* extract than the Gram negative bacteria. This could be due to the fact that the cell wall of Gram positive bacteria is less complex and lack the natural sieve effect against large molecule due to the small pores in their cell envelope.

The results of the present study indicated that the essential oil of *S. aromaticum* showed a huge potential to substitute the antibiotic Ciprofloxacin as antimicrobial agent for the treatment of different diseases.

CONCLUSION

The present study indicated that the essential oil of *S. aromaticum* have antibacterial activity against the tested organisms. The study also revealed that the highest inhibitory effect of the goronful extract was found against *S. aureus* and *E. coli*. Further investigations regarding the mode of action and other related pharmacological studies such as *in vivo* investigation, drug formulation and clinical trials are highly recommended.

ACKNOWLEDGEMENTS

The authors are grateful to Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), National Center for Research, Khartoum, Sudan.

REFERENCES

Akthar MS, Degaga B, Azam T, **2014**. Antimicrobial activity of essential oils extracted from medicinal plants against the pathogenic microorganisms: A review. Issue Biol Sci Pharmaceut Res, 2: 1-7.

Alma MH, Nitz S, Kollmannsberger H, Dığrak M, Efe FT lmaz N, **2004**. Chemical composition and antimicrobial activity of the essential oils from the gum of Turkish Pistachio (*Pistacia vera* L.). J. Agric Food Chem, 52: 3911-3914.

Ayoola GA, Lawore FM, Adelowotan T, Aibinu E, Adenipekun IE, Coker HAB, Odugbemi TO, **2008**. Chemical analysis and antimicrobial activity of the against fish systemic bacteria isolated from aquaculture sites. Front Agric China, 3(3): 332–336.

Bergkvist TP, **2007**. Antimicrobial activity of four volatile essential oils.Thesis of Master thesis in Pharmacy. The Pharmacy Program. University of Goteborgs.

Burt S, **2004**. Essential oils: their antibacterial properties and potential applications in foods – a review. Int J Food Microbiol, 94:223–253.

Cortés-Rojas DF, De Souza CRF, Pereira Oliveira W, **2014**. Clove (*Syzygium aromaticum*): a precious spice. Asian Pac J Trop Biomed, 4: 90-96.

Di Paoli S, Giani TS, Presta, GA, Pereira MO, Da Fonseca AD, Brandão-Neto J, Medeiros, AD, Santos-Filho, SD, Bern ardo-Filho M, **2007**. Effects of clove (*Caryophyllus aromaticus* L.) on the labeling of blood constituents with Technetium-99m and on the morphology of red blood cells. Braz Arch Biol Technol, 50: 175-182.

Djilani A, **Dicko** A, **2012**. The therapeutic benefits of essential oils. INTECH Open Access Publisher.

Hänse RO, **Sticher** A, **2007**. Nelkenöl und Eugenol in der konservierende Zahnheilkunde. Pharmakognosie–Phytopharmacie, 8. Auflage, Ed 25.8.6, Springer Medizin Verlag Heidelberg, Germany, pp. 1133-1134.

Ibrahium MI, Abd El-Ghany ME, Ammar MS, **2013**. Effect of clove essential oil as antioxidant and antimicrobial agent on cake shelf life. World J Dairy Food Sci, 8: 140-146.

Javed S, Mushtaq S, Khokhar I, Ahmad R., Haider MS, **2012**. Comparative antimicrobial activity of clove and fennel essential oils against food borne pathogenic fungi and food spoilage bacteria. Afr J Biotechnol, 11(94): 16065-16070.

Kavanagh F, **1972**. Analytical Microbiology, Vol. II, Academic Press (Pub.) New York and London. pp 11.

Koba K, Nenonene AY, Raynaud C, Chaumont J, Sanda K, **2011**. Antibacterial activities of the buds essential oil of *Syzygium aromaticum* (L.) Merr. & Perry from Togo. J Biol Active Prod Nat, 1(1): 42-51.

Lee S, Najiah M, Wendy W, Nadirah M, **2009**. Chemical composition and antimicrobial activity of the essential oil of *Syzygium aromaticum* flower bud (Clove). Afr J Microbiol Res, 2: 162-166.

Machado M, Dinis AM, Salgueiro L, Custódio JBA, Cavaleiro C, Sousa MC, **2011**. Anti-Giardia activity of *Syzygium aromaticum* essential oil and eugenol: Effects on growth, viability, adherence and ultrastructure. Exper Parasitol, 127: 32-39.

Miyazawa M, **Hisama** M, **2003**. Antimutagenic activity of phenyl-propanoides from clove (*Syzygium aromaticum*). J Agric Food Chem, 51(22): 6413-6422.

Nana WL, Eke P, Fokom R, Bakanrga-Via I, Begoude D, Tchana T, Tchameni SN, Kuate J, Menut C, Boyom FF, **2015**. Antimicrobial activity of *Syzygium aromaticum* and *Zanthoxylum xanthoxyloides* essential oils against *Phytophthora megakarya*. J Phytopathol, 163: 632–641.

NCCLS, National Committee for Clinical Laboratory Standards, **1990**. Performance standards for antimicrobial susceptibility testing (6th ed.). Approved Standards. M2-A6, Wayne, PA.

Nuzhat T, **Vidyasagar** GM, **2013**. Antifungal investigations on plant essential oils. Int J Pharm Pharmaceut Sci, 5: 19-28.

Pandey A, **Singh** P, **2011**. Antibacterial activity of *Syzygium aromaticum* (clove) with metal ion effect against food borne pathogens. Asian J Plant Sci Res, 1(2): 69-80.

Politeo O, Jukic M, Milos M, **2010**. Comparison of chemical composition and antioxidant activity of glycosidically bound and free volatiles from clove (*Eugenia caryophyllata Thunb.*). J Food Biochem, 34: 129-141.

Rios JL, **Recio** MC, **2005**. Medicinal plants and antimicrobial activity. J Ethnopharmacol, 100: 80–84.

Rodriguez OE, Sánchez R, Verde M, Núñez M, Ríos R, Chávez A, **2014**. Obtaining the essential oil of *Syzygium aromaticum*, identification of eugenol and its effect on *Streptococcus mutans*. J

Oral Res, 3(4): 218-224.
Shobana S, **Naidu** KA, **2000**. Antioxidant activity of selected Indian spices. Prostaglandins Leukot Essent Fatty Acids, 62(2): 107-110.
Soliman KM, **Badeaa** RI, **2002**. Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food Chem Toxicol, 40: 1669–1675.
Velluti A, Sanchis V, Ramos AJ, Marı'n S, **2003**. Inhibitory effect of cinnamon, clove, lemongrass, oregano and palmarose essential oils on growth and fumonisin B1 production by *Fusarium proliferatum* in maize grain. Int J Food Microbiol, 89: 145-154.

Citation: Fagere ZO, Al Magboul AZ, 2016. Antibacterial activity of clove oil against some microorganisms at Khartoum State. Adv Med Plant Res, 4(4): 122-128.