A study of the inhibitory effect of some plant oils on the growth of *Staphylococcus aureus* and *Proteus spp. in vitro*

By

Lec.Zainab Adnan Hatem

Lec.Jinan Abdul-Amir Sabeeh Al-Hussaini

College of Vet. Medicine/Al-Qadissiyia University

Summery

The present *in vitro* study was undertaken to evaluate the antimicrobial efficacy of essential oils of nine medicinal plants including: *Prunus dulcisvar*, *Nigella sativa*, *Elettaria cardamomum*, *Eugenia caryophyllus*, *Linum usitatissimum*, *Allium sativum*, *Brassica nigra*, *Mentha piperita*, and *Sesamum indicum* against the growth of two pathogenic microorganisms: two; *Staphylococcus aureus* (gram-positive bacteria) and *Proteus spp.* (gram-negative bacteria). The method employed to test the antimicrobial efficacy was the Agar Well Diffusion Technique.

The essential oil of *Nigella sativa* exhibited maximum activity (at $p<0.05$) against *Staphylococcus aureus* with mean diameter of zone of inhibition; 48.11±2.10 mm, followed by 26±0.37 mm for *Eugenia caryophyllus*, 16.77±2.07 mm for *Sesamum indicum*, 15.77±0.32 mm for *Mentha piperita*, 14.11±0.53 mm for *Elettaria cardamomum*, 13.77±0.87 mm for *Brassica campestris* which represented the lowest value among the positive results of essential oils, where as essential oils of *Prunus dulcisvar*, *Linum usitatissimum* and *Allium sativum* showed no activity against growth of *Staphylococcus aureus*. On the other hand, *Proteus spp.* was sensitive only to the essential oils of *Elettaria cardamomum* and *Allium sativum* with mean diameter of zone of inhibition; 19±0.78 mm, 10.44±0.33 mm respectively, but it was resistant to the other test essential oils. This study had also been depended on the use of nine standard antibiotics as a positive control for each of the test microorganism, they included; Lomefloxacin, Erythromycin, Amoxicillin, Sparfloxacin, Pipemidic acid, Ciprofloxacin, Novobiocin, Cefprozil and Piperacillin. *Staphylococcus aureus* was sensitive to the first seven antibiotics and resistant to Cefprozil and *Piperacillin*, in contrast, Proteus spp. was sensitive to Piperacillin only.

Introduction

The research for components with antimicrobial activity has gained increasing importance in recent times due to growing worldwide concern about the alarming increase in the rate of infection by antibiotic-resistant microorganisms (1).

Bacteria are very adaptable organisms because of their very short generation time (as little as 15 to 20 minutes for some species under ideal conditions) and their propensity for sharing genetic information-even among different species of bacteria. The presence of an antibiotic may kill most of the bacteria in an environment but the resistant survivors can eventually re-establish themselves and pass their resistance genes on to their offspring and often to other species of bacteria. Both medical and veterinary uses of antibiotics have resulted in the appearance of resistant strains of bacteria which may cause disease that are difficult to treat (2, 3).

Also the problem posed by the high cost, adulteration and increasing toxic side effects of antibiotic coupled with their inadequacy in diseases treatment found more especially in the developing countries cannot be over emphasized (4). However, there has also been a rising interest in the research for natural products from plants for the discovery of new antimicrobial agents in the last three decades and in recent times (5, 6).

More so, many of these plants have been known to synthesize active secondary metabolites such as phenolic compounds found in essential oils (7, 8, 9) with established potent antimicrobial activities which indeed was formed the basis for their applications in some pharmaceuticals, alternative medicines and natural therapies (10, 11). Santos et al., 1995 (12) remarked the World Health Organization has indeed recognized medicinal plants as the best source for obtaining a variety of synthetic drugs. No doubts, some studies have identified and isolated the main active ingredients in the plants responsible for this antimicrobial activity.

The purpose of this study was to investigate the antimicrobial efficacy of essential oils of four known medicinal plants; ***Prunus dulcisvar, Nigella sativa, Elettaria cardamomum, Eugenia caryophyllus, Linum usitatissimum, Allium sativum, Brassica nigra, Mentha***

piperita, and ***Sesamum indicum*** against the growth of *Staphylococcus aureus* and *Proteus spp.* in order to prove the folkloric claims.

Materials and methods

Selection of medicinal plant materials:

Essential oils of eight medicinal plants including: *Prunus dulcisvar* (Bitter almond oil, HEMANI-IRP), *Nigella sativa* (Black cumin oil, HEMANI-IRP), *Elettaria cardamomum* (Cardamom oil, HEMANI-IRP), *Eugenia caryophyllus* (Clove oil, HEMANI-IRP), *Linum usitatissimum* (Flax seed oil, Ashams for oil-ROI), *Allium sativum* (Garlic pearls, RANBAXY-India), *Brassica nigra* (Mustard oil, HEMANI-IRP), *Mentha piperita* (Peppermint oil, El-Captain Company-Egypt), *Sesamum indicum* (Sesame oil, HEMANI-IRP) where utilized in this study. All these plant oils were purchased from the local market and identified at the National Iraqi Institute for Herbs, Baghdad, Iraq.

Antibiotics:

Nine standard antibiotics had been chosen according to their broad-spectrum activity used as positive control against the test microorganisms (*Staphylococcus aureus* and *Proteus spp.)*, they include: LOM 10 (Lomefloxacin-10 mcg), E 15 (Erythromycin-15 mcg), Ax 25 (Amoxicillin-25 mcg), CIP 5 (Ciprofloxacin-5 mcg), CPR 30 (Cefprozil 30 mcg), PI 20 (Pipemidic acid-20 mcg), PRL 100 (Piperacillin-100 mcg), SPX 5 (Sparfloxacin-5 mcg), NV 30 (Novobiocin-30 mcg) (Bioanalyse) ®.

Microorganisms:

Microorganisms used were standard strains of gram-positive bacteria: *Staphylococcus aureus* and gram-negative bacteria: *Proteus spp.* These strains were obtained from the Laboratory of Microbiology at the College of Veterinary Medicine, Al-Qadisiya University and all were identified and confirmed at the Central Laboratory of Health, Baghdad, Iraq.

Antibacterial activity:

Inhibition of microbial growth was tested by using the agar well diffusion method (13). All the test microorganisms were subcultured in nutrient broth media (HIMEDIA Laboratories, Mumbai-India) which was prepared by dissolving 13 gm of nutrient broth in 1000 ml of distilled water, shaked well and heated for several minutes using water bath at a temperature of 80ºC to ensure complete dissolving, then sterilized for 15 minutes at 15 lb pressure in an autoclave. Nutrient broth media was later poured into two sterile test tubes at average of 10 ml of media for each tube, after that several colonies of each bacteria were picked by sterile inoculating loop and inoculated in its own test tube containing 10 ml of sterile nutrient broth, after mixing well, the tubes were incubated at 37ºC for 24 hours to produce bacterial suspensions that revealed by the presence of turbidity. As well as, Mueller Hinton Agar (HIMEDIA Laboratories, Mumbai-India) which is a growth media used for testing antibiotics and the chosen plant oils susceptibility of the test microorganisms was prepared by dissolving 38 gm of Mueller Hinton agar in 1000 ml distilled water, shaked, heated, and sterilized by autoclave in a similar way to the preparation of nutrient broth. This media was poured aseptically at 45ºC into sterilized Petri plates (two plates were used for each three plant oils for each of the test microorganisms besides six plates for the chosen antibiotic discs distributed as three discs over each plate for each bacteria, so that the final number of Petri plates used in this study was eighteen plates). After complete solidification, 4 Wells of 5 mm diameter were bored into each plate with sterile un-drawn Pasteur pipette and plugs were removed with sterile tips (14) (plates which were used for antibiotic study were left without boring). A sterile cotton swab was dipped into the bacterial suspension produced by *Staphylococcus aureus* to be inoculated on the Mueller Hinton agar surface by streaking of the swab over its. This step was repeated with the other bacterial suspension resulted from *Proteus spp.* on its own plates. Finally and after the inoculums were dried, 0.1 ml of each plant oil at a concentration of 100% was poured into the wells of its inoculated plates by using micropipette. The plates were kept at room temperature for 15 minutes to allow the extracts to seep into the media before incubation. On the other hand, using an ethanol dipped and flamed forceps, the antibiotic discs were

aseptically placed over their seeded agar plates sufficiently separated from each other to avoid overlapping of inhibition zones.

All these plates were incubated in an upright position at 37ºC for 24 hours. The diameter of inhibition or no inhibition zones produced by each plant oil was measured in mm with the aid of ruler, three readings were recorded for each zone and average zone size was taken. The values were given as mean ± standard deviation. The data were analyzed by ANOVA test with least significant differences (LSD) at a significant level of ($P<0.05$) by using SPSS (Version 10).

Results and Discussion

One gram-positive bacteria; *Staphylococcus aureus* and one gram-negative bacteria; *Proteus spp.* were used in the present study. The results of *in vitro* antimicrobial activity of essential oils of *Prunus dulcisvar*, *Nigella sativa*, *Elettaria cardamomum*, *Eugenia caryophyllus*, *Linum usitatissimum*, *Allium sativum*, *Brassica nigra*, *Mentha piperita* and *Sesamum indicum* are presented in Table (1), and Figure (1).

The essential oil of *Nigella sativa* exhibited the maximum activity (at $p<0.05$) against growth of *Staphylococcus aureus* on agar plate surface with mean diameter of zone of inhibition **48.11±2.10** mm; followed by **26±0.37** mm for *Eugenia caryophyllus*, **16.77±2.07** mm for *Sesamum indicum*, **15.77±0.32** mm for *Mentha piperita*, **14.11±0.53** mm for *Elettaria cardamomum*, **13.77±0.87** mm for *Brassica nigra* which represented the lowest value among the positive results of essential oils, where as essential oils of *Prunus dulcisvar*, *Linum usitatissimum* and *Allium sativum* showed no activity against growth of *Staphylococcus aureus*. See Figure (2, 3, 4).

On the other hand, *Proteus spp.* was sensitive to the essential oils of *Elettaria cardamomum* (cardamom showed strong antimicrobial activity against *Proteus spp*.) and *Allium sativum*,with mean diameters of zone of inhibition were **19±0.78** mm **, 10.44±0.33** mm respectively, but it was resistant to the other test essential oils (*Prunus dulcisvar*, *Eugenia caryophyllus*, *Linum usitatissimum*, *Brassica campestris*, *Mentha piperita* and *Sesamum indicum*). See Figure (5,6).

The present study had also been depended on the use of nine standard antibiotics as a positive control for each of the test microorganism (Table 2). Lomefloxacin was the strongest among the used antibiotics in producing significant inhibitory effect (at $p<0.05$) against the growth of *Staphylococcus aureus* with mean diameter of zone of inhibition; **29.44±0.41** mm, followed by Erythromycin; **23.22±0.46** mm, Amoxicillin; **21.77±0.36** mm, Sparfloxacin; **21.55±0.44** mm, Pipemidic acid; **20.66±0.37** mm, Ciprofloxacin; **19.88±0.42** mm, and Novobiocin; **19±0.4** mm that revealed the lowest antibacterial activity in comparison to the other antibiotics used in the study, where as Cefprozil and Piperacillin were resisted by *Staphylococcus aureus*. *Proteus spp.* was sensitive only to Piperacillin among all of the used antibiotics, the mean diameter of zone of inhibition was **14.33±0.28** mm. while it was resistant to all of the other used antibiotics (Lomefloxacin, Erythromycin, Amoxicillin, Sparfloxacin, Pipemidic acid, Ciprofloxacin, Novobiocin, and Cefprozil).

Table (1): Inhibition zones (mm) of *Staphylococcus aureus* growth produced by tested plant oils in culture media.

Microorganisms / Essential oils	*Staphylococcus aureus*	*Proteus spp.*
Bitter almond oil	**0±0 A**	**0±0 A**
Black cumin oil	**48.11 2.10 B**	**0±0 A**
Cardamom oil	**14.11±0.53 C**	**19±0.78 B**
Clove oil	**26±0.37 D**	**0±0 C**
Flax seed oil	**0±0 E**	**0±0 C**
Garlic oil	**0±0 E**	**10.44±0.33 D**
Mustard oil	**13.77±0.87 F**	**0±0 E**
Peppermint oil	**15.77±0.32 F**	**0±0 E**
Sesame oil	**16.77±2.07 F**	**0±0 E**

* Different capital letters mean significant changes for vertical values at level ($p<0.05$).

* Results were expressed as mean ± SE.

Table (2): Inhibition zones (mm) of *Staphylococcus aureus* growth produced by antibiotic drugs in culture media when used as positive control.

Microorganisms / Antibiotics	*Staphylococcus aureus*	*Proteus spp.*
LOM	**29.44±0.41 A**	**0±0 A**
E	**23.22±0.46 B**	**0±0 A**
AX	**21.77±0.36 C**	**0±0 A**
SPX	**21.55±0.44 C**	**0±0 A**
PI	**20.66±0.37 C**	**0±0 A**
CIP	**19.88±0.42 C**	**0±0 A**
NV	**19±0.4 CA**	**0±0 A**
CPR	**0±0**	**0±0 A**
PRL	**0±0**	**14.33±0.28 B**

* Different capital letters mean significant changes for vertical values at level ($p<0.05$).

* Results were expressed as mean ± SE.

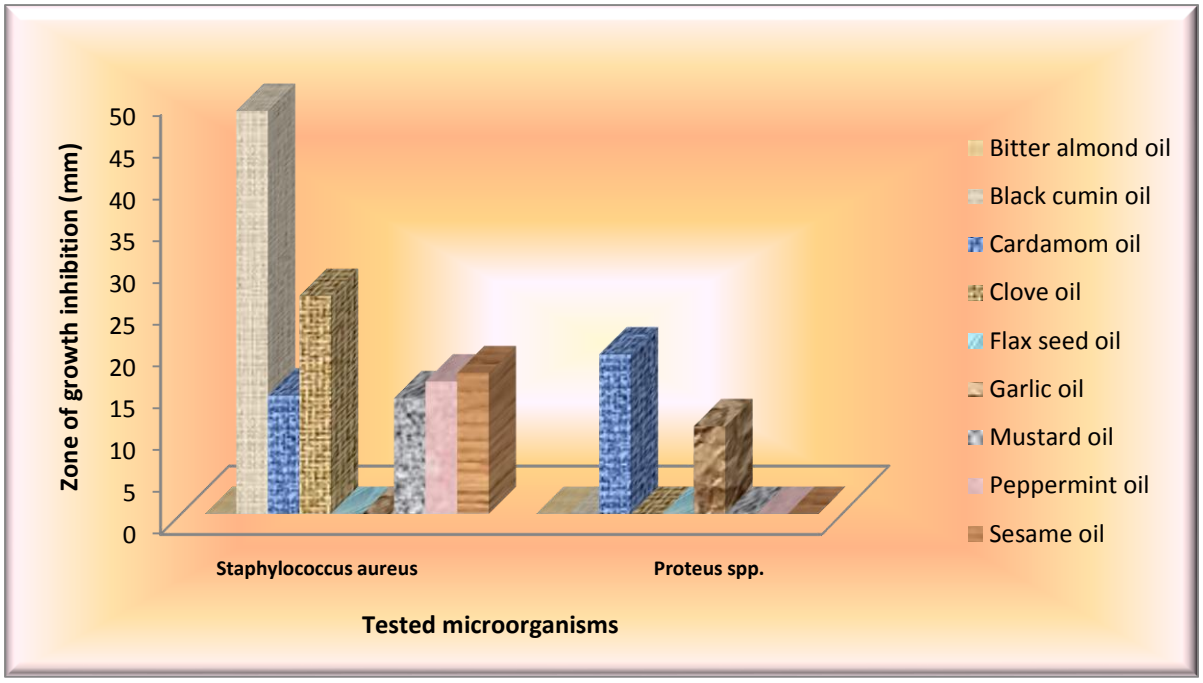

Figure (1): Inhibition zones of *Staphylococcus aureus* and *Proteus spp.* exhibited by tested plant oils.

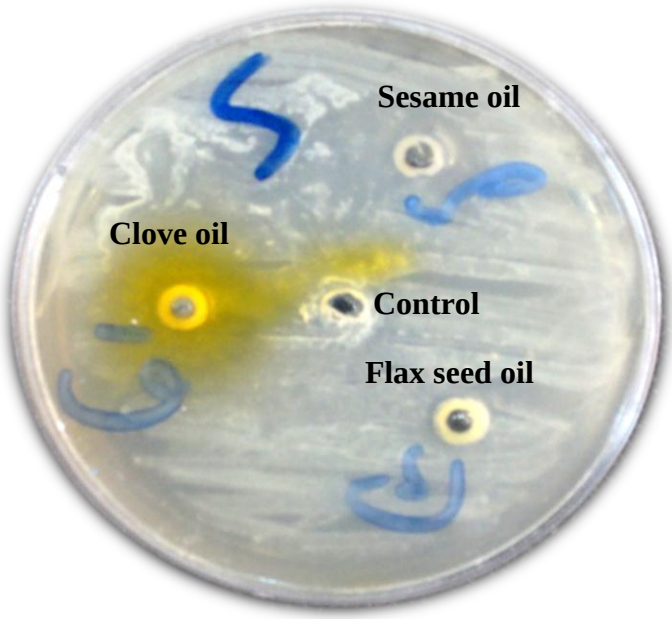

Figure (2): Zones of inhibition produced by Sesame, Clove, and Flax seed oils against growth of *Staphylococcus aureus.*

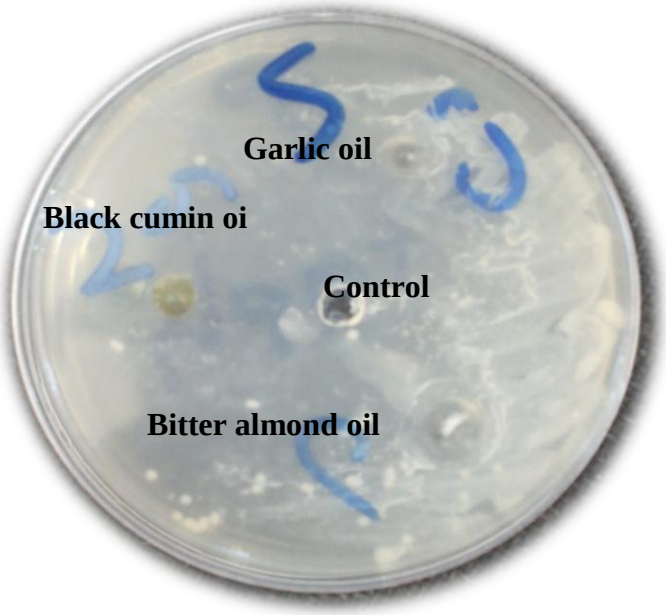

Figure (3): Zones of inhibition produced by Garlic, Black cumin and Bitter almond oils against growth of *Staphylococcus aureus.*

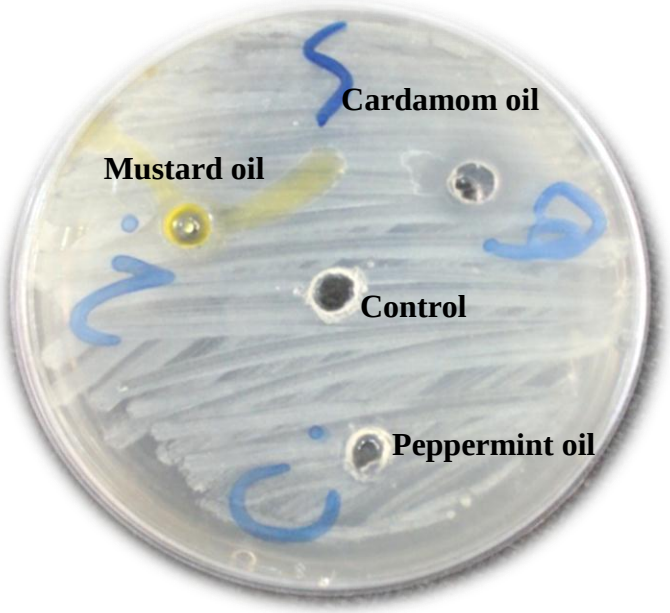

Figure (4): Zones of inhibition produced by Sesame, Clove, and Flax seed oils against growth of *Staphylococcus aureus*.

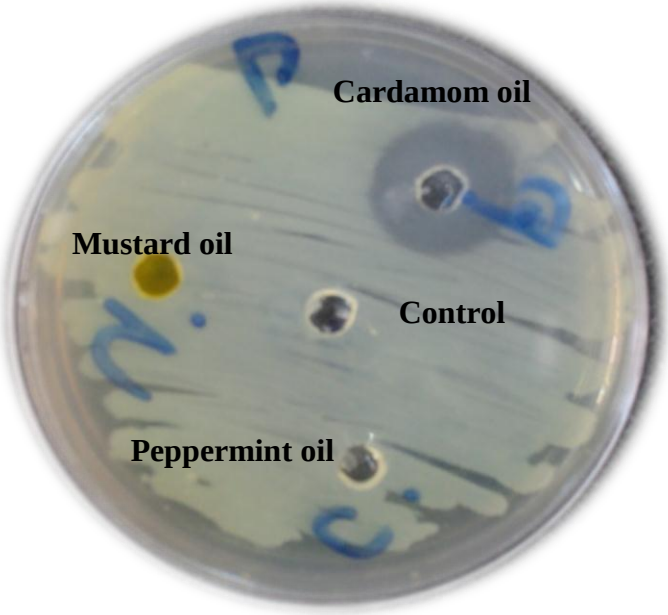

Figure (5): Zones of inhibition produced by Cardamom oil against growth of *Proteus spp.*

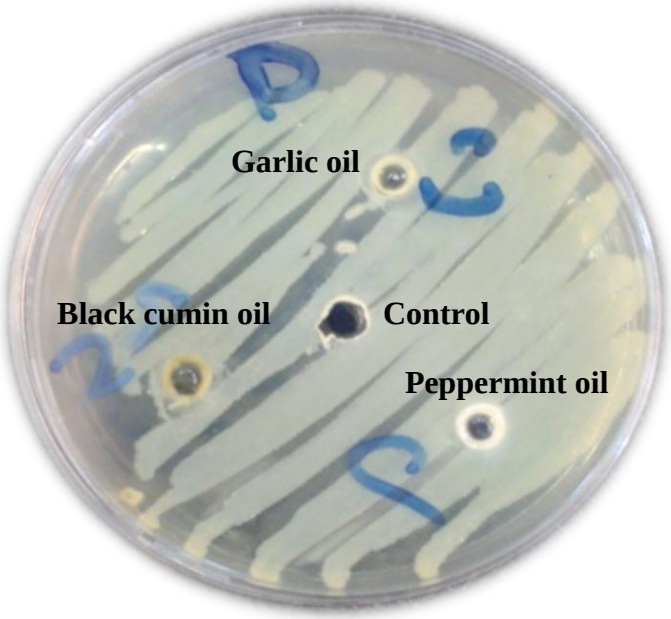

Figure (6): Zones of inhibition produced by Garlic oil against growth of *Proteus spp*.

Essential oils are well known in traditional medicine as antiseptic and antimicrobial agents. Gram-positive bacteria were shown to be more sensitive to the spice essential oils than gram-negative bacteria.

Black cumin oil showed high activity against *Staphylococcus aureus* (The widest inhibition zone), and no activity against *Proteus* spp. This confirms the study of Agarwal et al., 1979 (15) and Salman et al., 2005 (16), who reported that the oil inhibited growth of S. aureus where as proteus spp. was insensitive. Our observation is also in accordance with the study of Toama et al., 1974 (17) who reported the volatile oil obtained by steam distillation of fixed oil showed marked antibacterial activity against *Staphylococcus aureus*. Activity against *S. aureus* has also been reported with the fixed oil (18), crude oil (19) and extracts (20). A number of compounds derived from plants often show considerable activity against gram +ve bacteria but not against gram-ve species. gram-negative bacteria have an effective permeability barrier, comprised of the outer membrane, which restricts the penetration of amphipathic compounds, and multidrug resistance pumps that extrude toxins across this barrier. It is possible that the apparent ineffectiveness of plant antimicrobials is largely due to the permeability barrier (16). The Antimicrobial activity of *Nigella sativa* oil may be attributed to the presence of Thymoquinone16, Thymohydroquinone10 and Thymol2 in the essential oil, all of which have been shown to possess Antimicrobial activity (21).

The essential oil extracted from the dried flower buds of *Eugenia caryophyllus* is used as a topical application to relieve pain and to promote healing (22). Several constituents of *Eugenia caryophyllus* had been identified mainly eugenol, eugenyl acetate, β-caryophyllene, 2-heptanone (23), acetyl eugenol, α-humolene, methyl salicylate, isoeugenol, methyl eugenol (24), phenyl propranoides, dehydrodieugenol, trans-confireryl aldehyde, biflorin, kaempferol, rhamnocitrin, myricetin, gallic acid, ellagic acid and oleanolic acid (25). The main constituents of essential oil are phenylpropanoides such as carvacrol, thymol, eugenol and cinnamaldehyde (22). The antimicrobial activity of *Eugenia caryophyllus* had been studied by several authors (26). The spectrum of activity is fairly broad, with action against gram-positive and gram-negative rods and cocci, yeast and fungi (27). The present study revealed strong antimicrobial activity of *Eugenia caryophyllus* essential oil against *Staphylococcus aureus* where as *Proteus spp*. was resistant. Our findings agreed with those found by some workers; Rams, 1999 (28), Nzeako et al., 2006 (29), Saeed and Tariq, 2008 (30), Ali et al., 2009 (31), but differ from them in producing positive results against the growth of *Proteus spp*. A possible explanation for these diverse results is the fact that *Eugenia caryophyllus* oil composition is variable depending on the region and season that it is collected (32). Consequently, the active compounds may not present in sufficient quantities or quality. The mechanism of antimicrobial action of *Eugenia caryophyllus* oil, though not completely understood, seems to be complex and may vary according to its composition. The compounds known to have antimicrobial action are mainly the flavonoids and cinamic acids (33).

The results of our study had also shown the antimicrobial activity of *Sesamum indicum* oil. *Staphylococcus aureus* growth was sensitive and significantly inhibited by *Sesamum indicum* oil with no efficacy on *Proteus spp.* growth. *Sesamum indicum* contained mainly essential oils such as aromatic phenolic compounds-sesamol, sesaminol, sesamin, carboxylic acids and other classes of compounds including fatty acids like palmitic acids, arachidonic acid, arachidic acid, stearic acid, myristic acid, oleic acid, linoleic acid, thiazole, pyrroles, disulphide and aldehyde (34). Many studies had proven the antimicrobial effect of *Sesamum indicum* oil and leaves extract as general. Bankole et al., 2007 (35) and Asokan et al., 2009 (36) had investigated significant inhibitory effect of *Sesamum indicum* oil against the growth of *Staphylococcus aureus*, but there is no any available study about the antibacterial efficacy of *Sesamum indicum* oil against the growth of *Proteus spp*. The mechanism by which *Sesamum indicum* oil cause bacterial growth inhibition is not well known. *Sesamum indicum* oil has three lignans; sesamin, sesamolin, and sesaminol that have antioxidant properties, it has increased polyunsaturated fatty acids and reduced the lipid peroxidation (37). The other possible mechanism might be the saponification or the soap making process that occurs as a result of the alkali hydrolysis of fat (38). The unsaponifiable fraction, a class of substances not found in the fats (sesamin or sesamolin) can probably protect against infection by its antioxidant property. These mechanisms could have been the reason for the reduction of microorganism growth, but more studies have to be done to check and prove the antimicrobial effect of compounds of *Sesamum indicum* oil (36).

Peppermint oil showed activity against *Staphylococcus aureus*, however, the test gram-negative bacteria (*Proteus spp*.) was found to be resistant. Positive results obtained by the present study against *Staphylococcus aureus* was exactly resembling to those recorded by Gupta et al., 2008 (39). The antimicrobial activity of peppermint oil is due to the presence of terpenoids-menthol, menthone, 1,8-cineole, methyl acetate, methofuran, isomenthone, limonene, b-pinene, a-pinene, germacrene-d, trans-sabinene hydrate and pulegone (40).

Cardamom seed oil obtained naturally from dried ripe seeds of Elettaria cardamomum. Major components in the oil were 1,8-cineole, *α*-terpineol, DL-limonene, nerolidol, 4-terpineol, *δ*-terpineol, *δ*-3-carene, *β*-myrcene, germacrene D, *α*-terpinene and longifolenaldehyde (41). In this study, essential oil of cardamom seed displayed variable degrees of antimicrobial activity on the tested microorganisms. *Proteus spp.* was found to be more sensitive strain than *Staphylococcus aureus*, the widest inhibition zone was formed around *Proteus spp.* Present study is in agreement with Norajit et al., 2007 (42) and Patil and Kamble, 2011 (43) regarding inhibition effect of cardamom oil against *Staphylococcus aureus* besides some of the gram-negative bacteria but not *Proteus spp.* Antimicrobial characteristics of the herbs are due to various chemical compounds including volatile oils, alkaloids, tannins and lipids that are presented in their tissue (44). The inhibitory effect of cardamom detected in the present study may be due to the presence of the major component, γ-terpinene in its oil (42).

The seeds of *Brassica nigra* (mustard oil) have important medicinal uses such as in treatment of rheumatism and joint pains, indurations of the liver and spleen, throat tumors and as a laxative (45). Mustard oil like peppermint oil, also demonstrated preferentially to produce slight inhibitory activity against *Staphylococcus aureus* (46) as compared to *Proteus spp.* Phytochemical analyses as done by Obi et al., 2009 (46) revealed that the oil extract of mustard seeds contain tannins, alkaloids and flavonoids which have been reported to be responsible for antimicrobial properties in some plant extracts and as a result, it could serve as antimicrobial agents for the treatment of microbial infections.

There is extensive literature on the antibacterial effects of fresh garlic juice, aqueous and alcoholic extracts, lyophilized powders, steam distilled oil and other commercial preparations of garlic. Tested gram-negative bacteria *Proteus spp*. was sensitive to garlic oil, where as gram-positive *staphylococcus aureus* was resistant. This is in agreement with the work of Onwuliri and Wonang, 2005 (47), An et al., 2009 (48), Ayaz and Alpsy, 2007 (49), but disagreed with those found by Olubunmi et al., 2010 (50), Deresse, 2010 (51), and Durairaj et al., 2009 (52). They demonstrated the inhibitory effect of aqueous extract of garlic on *Staphylococcus aureus* along with *Proteus spp*. Garlic yields allicin - a powerful antibiotic (53), it also contains Allin, Ajoene, enzymes, vitamin B, minerals and flavonoids. Garlic consists of not less than 200 components, these include antioxidants, the volatile oils, (allin, allicin and ajoene) consisting of sulfur, enzymes (allinase, peroxidase and miracynase), carbohydrates (sucrose, glucose), mineral (germanium, selenium, Zinc), amino acids like cysteine, glutamine isoleucine and methionine, bi-flavonoids like quercetin and cyanidin and allistatin I and II,C,E and A vitamins and niacin, B1,B2 vitamins and beta carotene (54).

Feldberg et al., 1988 (55) showed that allicin exhibits its antimicrobial activity mainly by immediate and total inhibition of RNA synthesis, although DNA and protein syntheses are also partially inhibited, suggesting that RNA is the primary target of allicin action. The structural differences of the bacterial strains may also play a role in the bacterial susceptibility to garlic constituents. The lipid content of the membranes will have an effect on the permeability of allicin and other garlic constituents. On the basis of this hypothesis, it is interesting to recall the difference in susceptibility we observed between gram-negative *Proteus spp*. and gram positive Staphylococcus aureus to garlic extract (56).

Bitter almond oil and Flax seed oil also used in this study, but they had produced negative activity against both microorganisms.

References

(1) Davis P. H. (1982): "Flora of Turkey and East Eagean Island, Edinburg". Edinburg University Press, 7: 947.

(2) Barton M. D. (1998): " Does the use of antibiotics in animals affect human health". Austral. Vet. J., 76(3): 177-180.

(3) Khachatourians G. G. (1998): "Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria". Can. Med. Assoc. J., 159: 1129-1136.

(4) Shariff Z. U. (2001): " Modern herbal therapy for common ailments. nature pharmacy series". (Vol. 1), Spectrum Books Limited, Ibadan, Nigeria in Association with Safari Books (Export) Limited, United Kingdom, 9-84.

(5) Nascimento G. F., Juliana L., Paulo C. F., Giuliana L. (2000): " Silva Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria". Brazilian J. of Microbiology, 31: 247-256.

(6) R´ıos J. L., Recio M. C. (2005): " Medicinal plants and antimicrobial activity". J. of Ethnopharmacology, 100: 80-84.

(7) Hammer K. A., Carson C. F., Riley T. V. (1999): "Antimicrobial activity of essential oils and other plant extracts". J. Applied Microbiol., 86: 985- 990.

(8) Cox S. D., Mannnn C. M., Markham J. L. (2000): "The mode of antimicrobial action of the essential oil of Melaleuca alternifolia. J. Applied Microbiol., 88: 170-175.

(9) Dorman H. J., Deans S. G. (2000): "Antimicrobial agents from plants: antibacterial activity of plant volatile oils". J. Applied Microbiol., 88: 308-316.

(10) Reynolds J. E. (1996): "Martindale-the extra pharmacopia". 31st ed., Royal Pharmaceutical Society of Great Britain, London.

(11) Lis-Balchin M., Deans S. G.(1997): "Bioactivity of selected plant essential oils against *Listeria monocytogenes".* J. of Applied Bacteriology, 82: 759-762.

(12) Santos P. R., Oliveira A. C., Tomassini T.C. (1995): "Controlemicrobiógico de produtos fitoterápicos". Rev. Farm. Bioquím., 31: 35-38.

(13) Saeed, S. And Tariq, P. (2005): "Antibacterial activities of *Mentha piperita, Pisum sativum and Momordica charantia"*. Pakistan J. Of Botany, 37(4): 997-1001.

(14) Dorman H. G. D., Deans S. G. (2000): "Antimicrobial Agents from Plants: Antibacterial Activity of Plant Volatile Oils". J. Appl. Microbiol., 88:308-316.

(15) Agarwal R., Kharya M. D., Shrivastava R. (1979): " Antimicrobial and anthelmintic activities of the essential oil of *Nigella sativa* Linn". Indian J. Exp. Biol., 17(11): 1264-1265.

(16) Salman M. T., Chan R. A., Shukla I. (2005): "In vitro antimicrobial activity of Nigella sativa oil agains t multi-drug resistant bacteria". Dept. of Pharmacology, Jawaharlal Nehru Medical College, Aligarh Muslim University, India, mtariqsalman@gmail.com.

(17) Toama M. A., El-Alfy T. S., El-Fatatry H. M. (1974): "Antimicrobial Activity of the Volatile Oil of *Nigella sativa* Linneaus Seeds". Antimicrobial Agents Chemotherapy, 6(2): 225-226.

(18) Farrag H. A. (2000): "Effect of gamma radiation on the bacterial flora of *Nigella sativa* seeds and its oil constituents; Acta Pharm, 2000, 50, 197-207.

(19) Halwani R., Habbal M. Z., Abdelnoor A. M. (1999): "The antibacterial effect of some constituents of *Nigella sativa* oil". Arab J. of Pharmaceutical Sc., 1(1): 87-96.

(20) Ali O., Basbulbul G., Aydin T. (2007): "Antimitotic and antibacterial effects of the *Nigella sativa* L. Seed". Adnan Menderes U¨niversitesi, Fen-Edebiyat Faku¨ ltesi Biyoloji Bo¨lu¨mu¨ Aydin, Turkey, 60 (3): 270-272.

(21) Halawani E. (2009): "Antibacterial Activity of Thymoquinone and Thymohydroquinone of *Nigella sativa* L. and Their Interaction with Some Antibiotics". Advances in Biological Research 3(5-6): 148-152.

(22) Chaieb K., Hajlaoui H., Zmantar T., Nakbi K. A., Rouabhia M., Mahdouani K., Bakhrouf A. (2007a): "The chemical composition and biological activity of essential oil, *Eugenia cryophyllata* (*Syzygium aromaticum* L. Myrtaceae): a short review". Phytother. Res., 21(6): 501-506.

(23) Chaieb K., Zmantar T., Ksouri R., Hajlaoui H., Mahdouani K., Abdelly C., Bakhrouf A. (2007b): "Antioxidant properties of essential oil of *Eugenia caryophyllata* and its antifungal activity against a large number of clinical *Candida* species". Mycosis, 50(5): 403-406.

(24) Yang Y.C., Lee S. H., Lee W.J., Choi D. H., Ahn Y.J. (2003) : "Ovicidal and adulticidal effects of *Eugenia cryophyllata* bud and leaf oil compounds on *Pediculus capitis*". J. Agric. Food Chem., 51(17): 4884-4888.

(25) Cai L., Wu C. D. (1996): "Compounds from *Syzygium aromaticum* possessing growth inhibitory activity against oral pathogens". J. Nat. Prod., 59(10): 987-990.

(26) Wagner G.N., Singer T. D. (2003): "Scott McKinley Aquaculture Research, 34(13): 1139-1146.

(27) Burt S. A. (2003): "Reinders Letters in Applied Microbiology". 36(3): 162-167.

(28) Rams T. E. (1999). "J. of Applied Microbiology"., 86(6): 985-990.

(29) Nzeako B. C., Al-Kharousi S. N., Al-Mahrooqui Z. (2006): "Antimicrobial Activities of Clove and Thyme Extracts". Sultan Qaboos Univ. Med. J., 6(1): 33-39.

(30) Saeed S. and Tariq P. (2008): " *In vitro* antibacterial activity of clove against gram-negative bacteria". Pak. J. Bot., 40(5): 2157-2160.

(31) Ali H. S., Kamal M., Mohamed S. B. (2009): "*In vitro* clove oil against periodontopathic bacteria". J. Sc. Tech., 10(1).

(32) Chami N., Bennis S., Chami A., Aboussekhra A., Remmal A. (2005): "Oral Microbiology and Immunology". 20(2): 106-111.

(33) Walsh S.E., Maillard J. Y., Russell A. D., Catrenich **C. E.,** Charbonneau D. L., Bartolo R.G. (2003): "J. of Applied Microbiology". 94(2): 240-247.

(34) Laj S., Bankole M. A., Ahmed T., Bankole M. N. (2007): "Antibacterial and antifungal activities of crude extracts of *Sesame radiatum* against some common pathogenic microorganisms". J. of Pharmacol. and Therap., 6 (2): 165-170.

(35) Bankole M. A., Shittu L. A., Ahmed T. A., Bankole M. N., Shittu R. K., Kpela T., Ashiru O. **A.** (2007): **"**Synergistic Antimicrobial Activities of Phytoestrogens in Crude Extracts of Two Sesame Species Against Some Common Pathogenic Microorganisms". Afr. J. Tradit. Complement, Altern. Med., 4(4): 427-433.

(36) Asokan S., Emmadi P., Chamundeswari R. (2009): "Effect of oil pulling on plague induced gingivitis: A randomized controlled triple-blind study". Indian J. of Dental Research, 20(1): 47-51.

(37) Sankar D., Sambandam G., Rao R., Pugalendi K. V.(2005): "Modulation of blood pressure, lipid profiles and redox status in hypertensive patients taking different edible oils". Clin. Chim. Acta., 355:97-104

(38) "Ambika Shanmugam. Lipids In: Fundamentals of biochemistry for medical students" (2001) 7 th ed. Kartik Offset Printers, 4-50.

(39) Gupta C., Garg A. B., Uniyal R. C., Kumari A. (2008): "Antimicrobial activity of some herbal oils against common food-borne pathogens". African Journal of Microbiology Research, 2: 258-261.

(40) Ahmed I., Beg A. Z. (2001): "Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multidrug resistant human pathogens". J. Ethnopharmacol., 74: 113-123.

(41) Balaji S., Chempakam B. (2008): "Mutagenicity and Carcinogenicity Prediction of Compounds from Cardamom (*Elettaria cardamom* Maton.)". Indian Institute of Spices Research, Ethnobotanical Leaflets, 12: 682-689.

(42) Norajit K., Laohakunjit N., Kerdchoechuen O. (2007): "Antibacterial Effect of Five Zingiberaceae Essential Oils". *Molecules* , *12*: 2047-2060.

(43) Patil S. D., Kamble V. A. (2011): "Antibacterial activity of some essential oils against food porne pathogen and food spoilage bacteria". International Journal of Pharma. and Bio. Sciences, 2 (3): 143-150.

(44) Agaoglui S., Dostbil N., Alemadar S. (2005): "Antimicrobial Effect of Seed Extract of Cardamom (*Elettarıa cardamomum* Maton)". YYÜ Vet. Fak. Derg., 16 (2):99-101.

(45) Gerald E. T., Williams C. (1989): "Oil Crops of the world". 2nd ed., Royal Botanic Gardens Inc. Oklahoma, pp: 341, 355, 356.

(46) Obi1 R. K., Nwanebu F. C., Ndubuisi U. U., Orji N. M. (2009): "Antibacterial qualities and phytochemical screening of the oils of *Curcubita pepo* and *Brassica nigra*". Journal of Medicinal Plants Research, 3(5): 429-432.

(47) Onwuliri F. C., and Wonang D. L. (2005): "Studies on the combined Antimicrobial action of Ginger (*Zingiber officinale* L.) and Garlic (*Allium sativum* L) on some Bacteria". Nigerian Journal of Botany, 18: 224-228

(48) An M. H., Shen Y., Zhang C. J., Cai Y., Wang R., Jiang Y. (2009): "Allicin enhances the oxidative damage effect of amphotericin B against Candida albicans". International Journal of Antimicrobial Agents, 33 (3): 258-263.

(49) Ayaz E., Alpsoy H. C. (2007): "Garlic (*Allium sativum*) and Traditional Medicine Acta Parasitology Turcica".31 (2): 145-149.

(50) Olaitan A. O., Chukwudi U. S., Margaret Y. A. (2010): "Antimicrobial potentials of some spices on beef sold in Gwagwalada market, FCT, Abuja". Department of Biological Sciences, University of Abuja, Nigeria, Academia Arena, 2(7): 15-17.

(51) Deresse D. (2010): "Antibacterial Effect of Garlic (*Allium sativum*) on *Staphylococcu aureus*: An *in vitro* Study". Asian Journal of Medical Sciences, 2(2): 62-65.

(52) Durairaj S., Srinivasa S., Lakshmanaperumalsamy P. (2009): "*In vitro* Antibacterial Activity and Stability of Garlic Extract at Different pH and Temperature". Electronic Journal of Biology, 5(1): 5-10.

(53) Prescott I. M., Harley J.P., Klein D.A. (2008): "Microbiology". 7 th ed., Mc Graw-Hill international edition.

(54) Gulson G., Erol A. (2010): "Antimicrobial effect of Garlic (*Allium sativum*) and Traditional Medicine. Journal of Animal and veterinary Advances, 9(1):1-4.

(55) Feldberg R. S., Chang S. C., Kotik A. N., Nadler M., Neuwirth Z., Sundstrom D. C., Thompson N. H: "(1988) *In vitro* mechanism of inhibition of bacterial growth by allicin. Antimicrob. Agents Chemother., 32: 1763–1768.

(56) Sivam G. P. (2001): "Protection against Helicobacter pylori and Other Bacterial Infections by Garlic". Bastyr University, Research Institute, Kenmore, Journal of Nutrition, 1106-1108.

دراسة التأثير المثبط لبعض الزيوت النباتية على نمو المكورات العنقودية الذهبية وجرثومة المتقلبات في المختبر

من قبل

م.زينب عدنان حاتم

م.جنان عبدالأمير صبيح الحسيني

كلية الطب البيطري / جامعة القادسية

الخلاصة

تضمنت الدراسة الحالية التي أجريت في المختبر تقييم الفاعلية المضادة للبكتريا لزيوت تسع من النباتات المحلية هي: اللوز المر ، الحبة السوداء ، الهيل ، القرنفل ، بذور الكتان ، الثوم ، الخردل الأسود ، النعناع ، والسمسم على نمو أثنين من الجراثيم المرضية هي المكورات العنقودية الذهبية (الموجبة لصبغة غرام) وجرثومة المتقلبات (السالبة لصبغة غرام) وقد أستخدمت تقنية الأنتشار في حفر الأكار لأجل أختبار هذه الفاعلية .

لقد أظهر زيت الحبة السوداء أقصى فاعلية (عند P<0.05) ضد جرثومة المكورات العنقودية الذهبية وبمعدل نطاق تثبيط 48.1±2.10 ملم متبوعآ بـ 26±0.37 ملم لزيت القرنفل ، 16.77±2.07 ملم لزيت السمسم ، 15.77±0.32 ملم لزيت النعناع ، 14.11±0.53 ملم لزيت الهيل ،13.77±0.87 ملم لزيت الخردل الأسود الذي مثل القيمة الأدنى من بين النتائج الأيجابية المعطاة من قبل الزيوت المختبرة. في حين لم تظهر زيوت اللوز المر وبذور الكتان والثوم أي تأثير يذكر ضد نمو جرثومة المكورات العنقودية الذهبية

من جانب آخر فإن جرثومة المتقلبات كانت حساسة فقط لزيوت الهيل والثوم مع معدل نطاق تثبيط: 19±0.78 ملم،

10.44 ±0.33 ملم على التوالي لكنها كانت مقاومة للزيوت المختبرة الاخرى .

كما أعتمدت هذه الدراسة على أستخدام تسع مضادات حياتية قياسية لكل واحدة من الجراثيم المختبرة والتي شملت: لوموفلوكساسين ، أريثرومايسين ، أموكسيسيلين ، سبارفلوكساسين ، حامض البابيميديك ، سايبروفلوكساسين ،

نوفوبايوسين ،سيفبروزيل ، بايبراسيلين . المكورات العنقودية الذهبية كانت حساسة للمضادات السبعة الأولى أعلاه ومقاومة للسيفبروزيل والبايبراسيلين. على عكس جرثومة المتقلبات التي أظهرت حساسية فقط ضد البايبراسيلين.