World Journal of Medical Sciences 4 (2): 104-111, 2009
ISSN 1817-3055

Sensitivity of Multi-drug Resistant *Pseudomonas aeruginosa* Isolated from Surgical Wound-infections to Essential Oils and Plant Extracts

[1,2]*Wagih A. El-Shouny and* [1]*Sulaiman Magaam*

[1]Faculty of Medical Sciences, Hodeidah University, Yeman
[2]Department of Biology, Microbiology Section, Faculty of Sciences, Tanta University, Egypt

Abstract: In this study, five different Gram-negative bacteria were isolated from surgical specimens and minced meat, then biochemically identified as *Pseudomonas aeruginosa*. The results of disk sensitivity testing for the isolated *P. aeruginosa* showed that the five tested isolates were multiple-resistant to 6 antibiotics; amoxicillin, augmentin, cephotaxime, clarthromycin, co-trimoxazole and nitrofurantoin. The most effective drugs were gentamicin, imipenem, polymyxin and ofloxacin. The antimicrobial activities of four different essential oils against the multi-drug resistant *P. aeruginosa* isolates were investigated. Black seed, cinnamon and thyme oils showed high inhibition of bacterial growth. Inhibition zones of about 30 to 40 mm were recorded for *P. aeruginosa* (Isolates No. 1, 2 and 3). Lower sensitivity was observed for isolates number 4 and 5, where the inhibition zones of their growth ranged from 25 to 30 mm using the tested essential oils. However, olive oil exhibited the lowest inhibitory effect against all tested isolates. The efficacy of some plant extracts; green ginger, thyme, black tea, green tea and cinnamon were tested against the multi-drug resistant *P. aeruginosa* isolates. The ginger extract was the most effective antimicrobial agent, followed by cinnamon, thyme, green tea and black tea. Thus, ginger extract recorded inhibition zones ranged from 30 to 40 mm for the five tested isolates of *P. aeruginosa.* All tested bacterial isolates were sensitive to ginger extract at MIC ranged from 0.4 to 80 mg/ml. Cinnamon oil inhibited the bacterial growth at minimum concentrations of 0.64 to 0.96 mg/ml. The MICs of thyme oil against all tested isolates of *P. aeruginosa* reached 0.32 - 0.48 mg/ml.

Key words: *Pseudomonas aeruginosa* · Antibiotics resistance · Pyocyanin · Black seed · Cinnamon · Thyme · Ginger · Tea · Oil · Antimicrobial

INTRODUCTION

The problems associated with hospital infections caused by *Pseudomonas aeruginosa* have become increasingly evident. The ability of this opportunistic human pathogen to acquire resistance to a broad range of antibiotics has made effective therapy more difficult. Several recent investigations have dealt with the problem of antibiotic resistance in *P. aeruginosa* [1-3]. A superinfection in adults with cystic fibrosis chronically colonized by *P. aeruginosa* was reported [4]. Multidrug resistant *P. aeruginosa* strain caused an outbreak in a neurosurgery ward [5]. Furthermore, *P. aeruginosa* and *Acinetobacter baumannil* infections were recorded in the healthcare setting [6]. An alarming increase in bacterial strains resistant to existing antimicrobial agents demands a renewed effort to seek agents effective against pathogenic bacteria resistant to current antimicrobials.

Essential oils are natural products extracted from vegetal materials, which are characterized with their antibacterial [7-9], antifungal [10], antioxidant and anti-carcinogenic properties [11]. Most of the antimicrobial activity in essential oils from spices and culinary herbs; "cinnamon leaf, thyme, clove, black seed, oregano, olive and tea tree" appears to be associated with phenolic compounds [7-9, 12-15].

Medicinal plants are important elements of traditional medicine in virtually all cultures. Many investigators have demonstrated the antimicrobial activity of the constituents of some higher plants. Tea consumption provides protection against bacterial infection [16, 17]; and also against viral infection [18, 19]. The prophylactic effect of green tea drinking on certain forms of cancer and why tea extracts have been traditionally used in "alternative medicine" as anticarcinogenic/antibiotic agents could be also explained by Navarro-Perán *et al.*

Corresponding Author: Wagih A. El-Shouny, Faculty of Medical Sciences, Hodeidah University, Yeman

[20]. The extract of the olive leaves inhibited a variety of microorganisms [21]. This coincides with the natural ability of olive tree to protect itself from microbial attack using a variety of antimicrobial substances. The antibacterial activity of the aqueous and ethanolic extracts of garlic and ginger was observed [22]. The antibacterial enhancement of combinations of ethanol extracts and/or methanol extracts of *Rhus coriaria* and *Thymus vulgaris* against a clinical isolate of multiple drug resistant *P. aeruginosa* was recorded [23]. The antifungal efficacy of carnation, cinnamon, garlic and thyme tested as powders and/ or extracts was reported [10, 24]. In diseases of microbial origin, the plants function as a result of antimicrobial activity against the causative agents [25].

The objective of the present study was to evaluate the antibiotic resistance of *P. aeruginosa* isolated from clinical specimens; especially after surgical operations of some patients attending El-Olafey Hospital, Hodeidah Governorate, Republic of Yemen. The antibacterial effects of some essential oils and plant extracts towards the multi-drug resistant isolates of *P. aeruginosa* were investigated.

MATERIALS AND METHODS

Bacteria: Four selected blue-pigment producing bacteria were isolated using swabs from surgical wounds of patients attending El-Olafey Hospital, Hodeidah Governorate, Republic of Yemen. One isolate producing greenish-pigment was obtained from minced meat. Each isolate was subjected to standard microbiological and biochemical techniques for identification in the Microbiology Laboratory, Medical Laboratories Department, Faculty of Medical Sciences, Hodeidah University, Yemen. Thus, the morphological and biochemical tests included the motility, Gram-staining, growth at a temperature range of 5-42°C, pyocyanin production, oxidase reaction, indole test, gelatin liquefication, carbohydrate "glucose, lactose and sucrose" fermentation on triple sugar iron agar slants; hydrogen sulphide production [26].

Culture Media and Maintainance of Bacteria: Nutrient agar, blood agar, Mac Conkey agar and Müller Hinton agar were used for the growth of *P. aeruginosa* and production of pyocyanin. All bacterial isolates were maintained on nutrient agar slants at 4°C with monthly transfers.

Antimicrobial Agents: The isolated *P. aeruginosa* were tested for their sensitivity to some clinically used antibiotics. Then, the selected multi-drug resistant isolates were further tested for their sensitivity to essential oils as well as a number of plant extracts. The antibiotics and their concentrations, oils as well as plant extracts and their preparation methods are mentioned in the following:

Antibiotics: Amoxicillin 10 μg, augmentin 30 μg, cephotaxime 30 μg, clarthromycin 15 μg, co-trimoxazole 25 μg, gentamicin 10 μg, imipenem 10 μg, polymyxin 10 μg, nitrofurantoin 300 μg, ofloxacin 5 μg were used to determine the antibiotic sensitivity of the isolated *P. aeruginosa*.

Essential Oils: Black seed (*Nigella sativa* L.), cinnamon (*Cinnamomum burmannil* L.), olive (*Olea europaea* L.) and thyme (*Thymus vulgaris* L.) oils were purchased from local retail markets and stored in full dark vials at 4°C. The oils were tested for their antimicrobial activities against some clinical and food contaminating isolates of *P. aeruginosa*.

Plant Extracts: Some plant extracts of green ginger (*Zingiber officinale* L.), cinnamon (*Cinnamomum burmannil* L.) and thyme seeds (*Thymus vulgaris*), black and green teas (*Camellia sinesis* L.) were tested for their antimicrobial activities. The plant materials were purchased from local retail markets. The ginger was washed, peeled, cut into pieces and ground using an electric blender. 25 g of the ground material were soaked in 100 ml of hot sterile water and allowed to stand for 24 hours. The dry plant materials of thyme seeds, black tea, green tea and cinnamon (10 g each) were extracted with 100 ml of hot water. The crude extracts were obtained by filtration through Whatman No. 2 filter paper under suction. All extracts were collected in dark bottles and stored at 4°C when not in use.

Antibiotic Sensitivity Test: Disk sensitivity testing was performed by the modified Kirby-Bauer single-disk technique on Müller Hinton agar with the tested antibiotics [27]. The agar plates were seeded with 1 ml of test culture corresponding to 10^6 of cells in nutrient broth. After the inoculum has been dried, antibiotic discs were applied to inoculated medium with sterile forceps and pressed down gently to ensure even contact. Plates were incubated for 24 h at 37°C and antibiotic resistance was interpreted by diameter of inhibition zones around the antibiotic discs [28].

Antimicrobial Activity of Oils and Plant Extracts: The antibacterial activity of essential oils and plant extracts was assayed by agar wells diffusion. Agar plates were prepared using Müller Hinton agar. The plates were seeded with 1 ml of test culture corresponding to 10^6 of cells in nutrient broth. Wells of 3 mm in diameter were made using a sterile cork borer in solidified agar and 20 µl of the test oils or plant extracts were added to the wells. Plates were left for one hour at 4°C and then incubated for 24 h at 37°C. Wells without oils were considered as controls. Inhibition zones were measured in mm and three replicates were averaged [29].

Minimum Inhibitory Concentrations (MIC) of Oils and Plant Extracts: The most active oils were tested for their MICs for the individual *P. aeruginosa* strains by the agar well diffusion method on Müller Hinton agar with an inoculum of ca. 10^6 cfu/plate. Wells of 3 mm in diameter were made using a sterile cork borer. The essential oils diluted in absolute methanol to test concentrations (0.08 - 1.44 mg/ml) were added to the wells (20 µl) and the same volume of ethanol (20 µl) was used as a control. Plates were left for one hour at 4°C and then incubated for 24 h at 37°C. The diameter (mm) of inhibition zones of the oils was measured [30, 31]. The MIC of the selected plant extract was similarly determined using different concentrations of 0.4 to 80 mg/ml [32, 33]. The experiments were performed in triplicate.

RESULTS AND DISCUSSION

Isolation and Identification of Bacteria: In this study, five different bacteria were isolated from clinical specimens (surgical wounds and pus) and minced meat on nutrient agar (Table 1). The isolates appeared as actively motile, gram-negative short rods and grew at a temperature range of 5-42°C, optimally at 37°C. Pyocyanin was produced on nutrient agar, blood agar, Mac Conkey agar and Müller Hinton agar. Oxidase reaction was positive, indole was not produced and gelatin was liquefied in 18 hr, producing a greenish-yellow colour. Glucose, lactose and sucrose were not fermented on triple sugar iron agar slants; no hydrogen sulphide or other gas was produced, but the slant surface became slightly alkaline. Thus, the morphological and biochemical tests indicated the belonging of the five isolates to the *Pseudomonas aeruginosa* [27].

Antibiotic Sensitivity Test: Table 2 gives the results of disk sensitivity testing for the *P. aeruginosa* strains isolated. The five strains tested were multiple-resistant to 6 antibiotics; amoxicillin, augmentin, cephotaxime, clarthromycin, co-trimoxazole and nitrofurantoin. The most effective drugs were gentamicin, imipenem, polymyxin and ofloxacin. The sensitivity of the tested bacterial isolated showed variable levels to some potent antibiotics.Many researchers reported investigated the drug resistance of bacteria. *P. aeruginosa* strains and members of Enterobacteriaceae tested were resistant to amikacin, gentamicin, carbenicillin, tobramycin, ampicillin and cephotaxime [34]. *P. aeruginosa* is inherently resistant to a wide variety of the commonly used antibiotics due to the synergy between multi-drug efflux systems or a type 1 AmpC β-lactamase and low outer membrane permeability [1]. A paralleled correlation between bacterial plasmids, outer membrane proteins with antibiotic resistance was observed [2]. Our findings correspond to previous reports concerning the efficacy of imipenem against isolates of Enterobacteriaceae and *P. aeruginosa* [3, 34]. Aminoglycosides are bactericidal agents mainly reserved for the treatment of sepsis due to coliforms and other Gram negative aerobic bacilli. Side effects include hypersensitivity reactions, ototoxicity and nephrotoxicity [26]. As gentamicin is a member of this group, although of its effectiveness against the herein tested *P. aeruginosa* isolates, unfortunately it could not be safely recommended for further use *in vivo* treatments. Ofloxacin is belonging to quinolines and has side effects

Table 1: Origin of *Pseudomonas aeruginosa* isolates, colour of the colonies on nutrient agar and growth in nutrient broth at different temperatures

			Growth	
Isolate No.	Origin	Colour	5°C	42°C
P. aeruginosa 1	Surgical wound	Blue	+	+
P. aeruginosa 2	Bone surgical wound	Blue	+	+
P. aeruginosa 3	Abdominal surgical wound	Blue	+	+
P. aeruginosa 4	Pus	Greenish Blue	+	+
P. aeruginosa 5	Minced meat	Greenish	+	+

The presence of greenish to blue colour indicated the production of pyocyanin pigment. All isolates grew at 24, 30, 37 and 40°C at pH 7.2.

Table 2: Sensitivity of different isolates of *Pseudomonas aeruginosa* to antibiotics as determined by disk diffusion on Müller-Hinton agar

	Sensitivity of *P. aeruginosa* isolates				
Tested antibiotics	Pa 1	Pa 2	Pa 3	Pa 4	Pa 5
Amoxicillin 10 µg	R	R	R	R	R
Augmentin 30 µg	R	R	R	R	R
cephotaxime 30 µg	R	R	R	R	R
Clarthromycin 15 µg	R	R	R	R	R
Co-trimoxazole 25 µg	R	R	R	R	R
Gentamicin 10 µg	S*	S	S*	S	S
Imipenem 10 µg	S	S	S	S	S
Polymyxin 10 µg	S	S	S	S	S
Nitrofurantoin 300 µg	R	R	R	R	R
Ofloxacin 5 µg	S	S*	S	S	S

Pa: *P. aeruginosa,* R: resistant, S: sensitive, S*: Highly sensitive.

Table 3: Antibacterial activities of essential oils against multi-drug resistant strains of *Pseudomonas aeruginosa* grown on Müller-Hinton plates (assayed by agar-wells diffusion)

	Inhibition zone (mm) of *P. aeruginosa* strains (1-5)				
Tested oils	Pa 1	Pa 2	Pa 3	Pa 4	Pa 5
Black seed	40	35	35	30	35
Cinnamon	40	35	35	34	35
Olive	33	33	30	26	29
Thyme	35	35	35	28	36

Pa: *P. aeruginosa* Each value is the mean of three readings

in treating bacterial infections in the children [26]. Therefore, it is conditionally prescribed although its potency recorded in this investigation. The increase in resistance to antibiotics along with the adverse side effects associated with the conventional treatments led researchers to investigate other options including essential oils [3, 8, 9] and plant extracts [22, 23, 32] in treating the multi-drug resistant infections.

Antibacterial Activity of Oils: Data presented in Table (3) indicated the antimicrobial activities of four different essential oils against the multi-drug resistant *P. aeruginosa* isolates. Black seed, cinnamon and thyme oils showed high inhibition of bacterial growth. Inhibition zones of about 30 to 40 mm were recorded for *P. aeruginosa* (Isolates No. 1, 2 and 3). Lower sensitivity was observed for isolates number 4 and 5, where the inhibition zones of their growth ranged from 25 to 30 mm using the tested essential oils. However, olive oil exhibited the lowest inhibitory effect against all tested isolates.

The antibacterial activities of the black seed fixed oils against food spoilage and/or pathogenic and lactic acid bacteria was reported [7]. Therefore, the oil of black seed may be used in food as a preservative. The oils at 2.0% concentration in absolute methanol were more effective than the other concentrations (0.5% and 1.0%). The antibacterial effects may be closely related to their high percentage of thymoquinone, p-cymene and carvacrol. The antimicrobial activity of black cumin oil against multi-drug resistant bacteria from clinical isolates including *S. aureus, P. aeruginosa* and others was recorded [9]. The antimicrobial activity of this oil may be attributed to the presence of thymoquinone, thymohydroquinone and thymol in the oil "all of which possessed antimicrobial activity". However, another finding was not completely coincided with the herein mentioned data. Thus, both Saudi *Nigella sativa* oil and whole seed exhibited fairly good antimicrobial activity against most of Gram-positive bacteria and some of Gram-negative ones. On contrary, the study was unable to achieve any degree of susceptibility to *P. aeruginosa, E. coli* and *Candida albicans* even with double or four times increase in volumes of oil or whole seed added [34]. This variation in antimicrobial activities reported in the different investigations could be explained by the use of the active

Table 4: Antibacterial activities of plant extracts against multi-drug resistant strains of *Pseudomonas aeruginosa* grown on Müller-Hinton plates (assayed by agar-wells diffusion)

	Inhibition zone (mm) of *P. aeruginosa* strains (1-5)				
Tested plant extracts	Pa 1	Pa 2	Pa 3	Pa 4	Pa 5
Green ginger	40	40	40	30	40
Thyme	32	35	35	25	36
Black tea	30	35	35	28	35
Green tea	30	35	37	28	36
Cinnamon	35	40	37	26	37

Pa: *P. aeruginosa*. Each value is the mean of three readings

principle compound, designated thymoquinone, utilized in the different studies or possibly to some ecological factors related to the plant species.

The cinnamon leaf oil had an excellent inhibitory effect against nine strains of bacteria. The MIC values of the leaf oils were 500 μg/ml against *K. pneumoniae* and *Salmonella* sp. The MIC of 250 μg/ml was recorded against *E. coli, P. aeruginosa, S. aureus, S. epidermidis, Enterococcus faecalis* and *Vibrio parahemolyticus*. Cinnamaldehyde possessed the strongest antibacterial activity compared to the other constituents of the essential oils [12]. Thyme oil contained two phenolic compounds, thymol (33%) and carvacrol (5.6%) and a hydrocarbon p-cymene (29%) as shown by Abo-Ghalia *et al.* [13]. The high inhibitory action of thyme oil was due to the presence of these two phenolic OH groups (thymol and carvacrol), which is quite reactive and easily forms hydrogen bonds with the active sites of target enzymes or as a H^+ carrier, depleting adenosine triphosphate pool [12, 14]. The inhibitory effect of thyme essential oil, thymol and carvacrol towards *Shigella sonnei* and *S. flexneri* was postulated [31]. The essential oils of cinnamon, thyme and clove recorded the highest antimicrobial efficacy against some multiple antibiotic resistant bacteria (*E. coli, K. pneumoniae, P. aeruginosa, S. aureus, Streptococcus pneumoniae, Bacillus subtilis*). The recorded MIC values for thyme oil ranged from 0.64 to 2.56 mg/ml, while the MIC values of cinnamon oil varied from 1.28 to 2.56 mg/ml for the different tested bacteria. The MIC values of thyme and cinnamon oils against *P. aeruginosa* were 1.28 and 2.56 mg/ml, respectively [3]. Several homeopathic substances were tested to determine activity against drug resistant strains of microorganisms. The most effective were tea tree oil, oregano oil, wintergreen oil and lemon oil. Oregano oil was determined to have the greatest antimicrobial activity with a mean MIC of o.56% v/v followed by thyme oil with a mean MIC of 2.47% v/v [8].

Antibacterial Activity of Plant Extracts: The antibacterial activity of some plant extracts; green ginger, thyme, black tea, green tea and cinnamon were tested against the multi-drug resistant *P. aeruginosa* isolates. The results (Table 4) showed that ginger extract was the most effective agent, followed by cinnamon, thyme, green tea and black tea. Thus, ginger extract recorded inhibition zones ranged from 30 to 40 mm for the five tested isolates of *P. aeruginosa*.

The aqueous and ethanolic extracts of garlic and ginger singly did not inhibit any of the test organisms; *S. aureus*, *Bacillus* spp., *E. coli* and *Salmonella* spp. The highest inhibition zone of 19 mm was observed with a combination of extracts on *S. aureus*, while *Salmonella* spp. was resistant to all the extracts except lime [22].

In her book '10 Essential Herbs' author Lalitha Thomas reported the major active ingredients in ginger are terpenes (quite similar to the chemical action of turpentine) and an oleo-resin called ginger oil. These two and other active ingredient in ginger, provide antiseptic, lymph-cleansing, circulation-stimulating and mild constipation relief qualities action that is quite effective in cleansing the system of toxins.

Combinations of ethanol extracts and/or methanol extracts of *Rhus coriaria* and *Thymus vulgaris* (100 mg/ml each) showed an additive action (antibacterial enhancement) against a clinical isolate of multiple drug resistant *P. aeruginosa* [23]. The fact that allicin of freshly crushed garlic and thymoquinone of black cumin seeds are the major components, which have great therapeutic potential as antimicrobial agents against different pathogens of urinary tract infections including *Staphylococcus aureus* and *E. coli* species was confirmed [24]. The high significant inhibitory effect on radial fungal growth for different concentrations of carnation, cinnamon, garlic and thyme. Concentrations of 8% of powdered spices and 6% of their extracts added to the culture medium were able to cause complete growth

Table 5: Minimum inhibitory concentrations of ginger extract, cinnamon and thyme oils against multi-drug resistant strains of *Pseudomonas aeruginosa* grown on Müller-Hinton agar

	Ginger extract	Cinnamon oil	Thyme oil
Tested bacteria	MIC (mg/ml)		
P. aeruginosa 1	0.4	0.64	0.32
P. aeruginosa 2	40.0	0.64	0.32
P. aeruginosa 3	40.0	0.64	0.32
P. aeruginosa 4	80.0	0.96	0.48
P. aeruginosa 5	4.0	0.64	0.32

Each value represents three repetitions of the experiment

inhibition of the major tested fungi [10]. Olive leaves extract at low concentrations (5 mg/ml) showed significant inhibition of some fungi, Gram positive and Gram negative bacteria, with exception of *P. aeruginosa*. The high content of oleuropein and the other phenolic compounds identified in the extract might contribute for its antimicrobial properties against the tested human intestinal or respiratory tract pathogens [32].

Tea consumption provides protection against bacterial infection [16, 17] and against viral infection [18, 19]. Polyphenolic compounds present in tea may reduce the risk of a variety of illness, including cancer and coronary heart disease. Most of the polyphenols in green tea are flavanols, commonly known as catechins. In black tea, the major polyphenols are theaflavin and thearubigin [15]. It was suggested that the prophylactic effect of green tea drinking on certain forms of cancer is due to the inhibition of dihydrofolate reductase by the natural polyphynol; epigallocatechin gallate (EGCG) and it could also explained why tea extracts have been traditionally used in "alternative medicine" as anticarcinogenic/ antibiotic agents or in the treatment of conditions such as psoriasis [20].

Minimum Inhibitory Concentrations (MIC) of Oils and Plant Extracts: The essential oils and plant extract that were determined to have the greatest antibacterial effect were tested further using the agar-well diffusion method to determine minimum inhibitory concentrations. Table (5) showed the MICs of ginger extract, cinnamon and thyme oils against *P. aeruginosa* strains grown on Müller-Hinton agar. All tested bacterial isolates were sensitive to ginger extract at MIC ranged from 0.4 to 80 mg/ml. The data also indicated that cinnamon oil inhibited the bacterial growth at minimum concentrations of 0.64 to 0.96 mg/ml. The MICs of thyme oil against all tested isolates of *P. aeruginosa* reached 0.32 - 0.48 mg/ml. In this concern, previous studies showed that the cinnamon leaf oil had an excellent inhibitory effect against nine strains of bacteria. Among the tested strains, *P. aeruginosa, E. coli, S. aureus, S. epidermidis, Enterococcus faecalis* and *Vibrio parahemolyticus* were affected with MIC of 250 μg/ml [12]. The MIC values of thyme and cinnamon oils against *P. aeruginosa* were 1.28 and 2.56 mg/ml, respectively [3]. The antimicrobial activity of thyme oil against drug resistant strains of microorganisms at a mean MIC of 2.47% v/v [8].

In a conclusion, this study reflects the defect of the extensive use and misuse of antibiotics which have favoured the emergence and survival of resistant strains of microorganisms. In the treatment and control of infectious diseases, especially when caused by pathogens that are often drug resistant, sensitivity testing must be used to select effective antimicrobial drugs.

Taken together, according the pharmacological criteria of the essential oils and the plant extracts, the antimicrobial doses of thyme and cinnamon oils as well as ginger extract against *P. aeruginosa* could be adjusted singly or possibly in combination. In addition, the use of these natural antimicrobial components along with other preservation techniques, 'e.g. in combination with reduced temperature and reduced pH' may improve food safety and overall microbial quality. Furthermore, the use of extracts is recommended to achieve health benefits due to the additive and synergistic effects of phytochemicals present in whole extracts.

The tested essential oils and plant extracts possessed antimicrobial activities against all multi-drug resistant *P. aeruginosa* and may be used topically in treating various wounds, burns and serious infections. Further *in vitro* studies would be rewarding to test more pathogenic bacteria and fungi in order to recommend future use of the effective oils and plant extracts in the possible treatment of multi-drug resistant infectious microbes. *In vivo* trials are required to advocate the systemic use of the potent antimicrobials in infectious diseases.

ACKNOWLEDGEMENTS

The authors thank Mr. Morad El-Ahdal, the technician of Microbiology, Med. Lab. Dept., Fac. Med. Sci., Hodeidah Uni., Yemen for his technical cooperation during this work. Thanks also to the working group of Professor W. El-Shouny for the helps lead to the completion of the study. We are indebted to El-Olafey Hospital for permitting us swabbing some operated patients.

REFERANCES

1. Livermore, D.M., 2002. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare. Clin. Infect. Dis., 34(5): 634-640.
2. Diab, A.M., S.A. S.M. Selim El-Alfay and A.A. Abd elrahman, 2004. Plasmids and outer membrane proteins (OMPS) correlation with antibiotic resistance in bacterial eye infection. N. Egypt. J. Microbiol., 9: 152-160.
3. El-Shouny, W.A., 2006. Efficacy of some essential oils and honey types against antibiotic-resistant bacteria and fungi. El-Minia Science Bulletin, 17(1): 77-107.
4. McCallum, S.J., J. Corkill, M. Gallagher, *et al.*, 2001. Superinfection with transmissible strain of *Pseudomonas aeruginosa* in adults with cystic fibrosis chronically colonized by *P. aeruginosa.* Lancet, 358: 558-560.
5. Sekigucki, J., T. Asagi, T. Miyoshi-Akiyama, *et al.*, 2005. Multidrug resistant *Pseudomonas aeruginosa* strain that caused an outbreak in a neurosurgery ward and its aac(6)-lae gene cassette encoding a novel aminoglycoside acetyltransferase. Antimicrob. Agents Chemother, 49: 3734-3742.
6. Navon-Venezia, S., R. Ben-Aml and Y. Camell, 2005. Update on *Pseudomonas aeruginosa* and *Acinetobacter baumannil* infections in the healthcare setting. Current Option in Infectious Diseases, 18: 306-313.
7. Arici, M., O. Sagdic and U. Gecgel, 2005. Antibacterial effect of Turkish black cumin (*Nigella sativa* L.) oils. Grasas y Aceites, 56(4): 259-262.
8. Ott, J.A. and A.N. Morris, 2008. Homeopathic alternatives to conventional antibiotics. Bios., 79(2): 50-55.
9. Salman, M.T., R.A. Khan and I. Shukla, 2008. Antimicrobial activity of *Nigella sativa* Linn. Seed oil against multi-drug resistant bacteria from clinical isolates. Natural Product Radiance, 7(1): 10-14.
10. El-Mougy, N.S. and M.M. Abdel-Kader, 2007. Antifungal effect of powdered spices and their extracts on growth and activity of some fungi in relation to damping-off disease control. J. Plant Protection Res., 47(3): 267-278.
11. Teissedre, P.I. and A.I. Waterhouse, 2000. Inhibition of oxidation of human low density lipoprotein by phenolic substances in different essential oils varieties. J. Agric. Food Chem., 48: 3605-3801.
12. Chang, S.T., P.F. Chen and S.C. Chang, 2001. Antibacterial activity of leaf essential oils and their constituent from *Cinnamomum osmophyloeum.* J. Ethnopharmacol., 77(1): 123-127.
13. Abo-Ghalia, H.H., M.T. El-Mokadem, A.M. Ghanem and K.A. Shaheen, 2004. Antimicrobial activity of essential oils of some medicinal plant. N. Egypt. J. Microbiol., 9: 221-240.
14. Zambonelli, A., A.Z. D'Aulerio, A. Severi, S. Benvenuti, L. Maggi and A. Bianchi, 2004. Chemical composition and fungicidal activity of commercial essential oils of *Thymus vulgaris* L. J. Essent. Oil Res., 16: 69-74.
15. Mukhtar, H. and A. Ahmad, 2000. Tea polyphenols: Prevention of cancer and optimizing health, Am. J. Clin.Nutr. 71(suppl): 1698S-1702S.
16. Horiba, N., Y. Mackawa, M. Ito, T. Matsumoto and H. Nakamura, 1991. A pilot study of Japanese green tea as a medicament: antibacterial and bactericidal effects. J. Endod., 17: 122-124.
17. Terada, A., H. Hara and S. Nakajya *et al.* 1993. Effect of supplements of tea polyphenols on the caccal flora and caccal metabolites of chicks. Microbiol. Ecol. Health Dis., 6: 3-9.
18. Nakayama, M., M. Toda, S. Okuba and T. Shimamura, 1990. Inhibition of influenza virus infection by tea. Lett. Appl. Microbiol., 11: 38-40.
19. Tao, P., 1992. The inhibitory effects of catechin derivatives on the activities of human immunodeficiency virus reverse transcriptase and DNA polymerases. Chung Kuo I Hsueh Ko Hsueh Yuan Hsueh Pao, 14: 334-338.
20. Navarro-Perán E., J. Cabezas-Herrera, F. Garcia-Cánovas, M.C. Durrant, R.N.F. Thorneley and J.N. Rodriguez-Lopez, 2005. The antifolate activity of tea catechins, Cancer Res., 65: 2059-2064.
21. Markin, D., L. Duck and I. Berdicevsky, 2003. *In vitro* antimicrobial activity of olive leaves. Mycoses., 46: 132-136.

22. Onyeagba, R.A., O.C. Ugbogu, C.U. Okeke and O. Iroakasi, 2004. Studies on the antimicrobial effects of garlic (*Allium sativum* Linn), ginger (*Zingiber officinale* Roscoe) and lime (*Citrus aurantifolia* Linn). African J. Biotechnol., 3(10): 552-554.
23. Adwan, G., B. Abu-Shanab, K. Adwan and Abu-F. Shanab, 2006. antibacterial effects of nutraceutical plants growing in Palestine on *P. aeruginosa*. Turk. J. Biol., 30: 239-242.
24. Roy, J., D.M. Shakleya, P.S. Callery and J.G. Thomas, 2006. Chemical constituents and antimicrobial activity of a traditional herbal medicine containing garlic and black cumin. African J. Traditional, Complementary and Alternative Medicines, 3(2): 1-7.
25. Sofowora, A., 1993. Introduction to medicinal plants and traditional medicine. Spectrum books limited, 2: 8-76.
26. Collee, J.G., A.G. Fraser, B.P. Marmion and A. Simmons, 1996. Bacteria and related organisms, Mackie and McCartney, Sec. B "*Pseudomonas, Stenotrophomonas, Burkholderia*" Practical Medical Microbiology 4th Ed., Churchill Livingstone, New York, pp: 413-424.
27. Robert, S., R.L. Anders, F. Niels and E. Frabk, 2003. Evaluation of different disk diffusion/media for detection of methicillin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci. APMIS, 111: 905-914.
28. Collins, C.H., P.M. Lyne and J.M. Grange, 1998. Microbiological methods. 7th edition, Butterworth Heinemann, pp: 178-205.
29. Lis-Balchin, M., S.L. Hart, S.G. Deans and E. Eaglesham, 1995. Potential agrochemical and medicinal usage of essential oils of *Pelargonium* sp. J. Herbs Spices and Medicinal Plants, 3: 11-22.
30. Hammer, K.A., C.F. Carson and T.V. Riley, 1999: Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol., 86: 985-990.
31. Bagamboula, C.F., M. Uyttendaele and J. Debevere, 2004. Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and *p*-cymene towards *Shigella sonnei* and *S. flexneri*. Food Microbiol., 21: 33-42.
32. Pereira, A.P., I.F.R. Ferreira, F. Marcelino, *et al.*, 2007. Phenolic compounds and antimicrobial activity of olive (*Olea europaea* L. Cv. Cobrançosa) leaves. Molecules, 12: 1153-1162.
33. Bilal, N.E., A. Batouk, S. Abu-Eshy, B. Al-Ghamdi and A.A. Al-Wabel, 1996. Antimicrobial effects of *Nigella sativa* on selected microorganisms. (Preliminary report), J. Hepatol. Gastroenterol. and Inf. Dis. JHGID., 4(4): 105-111.
34. Salama, A.A., A.M.S. Hosny, A.M. Ahmady, Abdel-A.A. Hamid and A.S.M. Hussein, 2004. Susceptibility of nosocomial pathogens to certain antibiotics and biocides commonly used in Egyptian hospitals. N. Egypt. J. Microbiol., 8: 274-299.