

Full Length Research Paper

Antimicrobial activity and chemical analysis of some edible oils (Clove, Kalonji and Taramira)

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Received 31 January, 2014; Accepted 21 October, 2014

Antimicrobial activity of oils of *Syzigium aromaticum* (Clove), *Nigella sativa* (Kalonji) and *Eruca sativa* Miller (Taramira) was checked against bacteria and fungi by agar well diffusion assay. It was found that Gram negative bacteria were more sensitive to these oils as compared to Gram positive bacteria. *Klebsiella pneumonia*, *Aspergillus flavus* and *Cunninghamella* were found to be more sensitive organisms showing large zones of inhibition. Taramira showed highest antifungal potential as compared to other tested oils. Further, oils were also checked in combination with each other and with antibiotics but no significant results were obtained. Activity units of Kalonji, Taramira and Clove oils were found to be 160, 20 and 160 AU/ml, respectively. Clove and Kalonji oils have higher MICs (that is, 1:4) as compared to Taramira oil (that is, 1:2). Effects of *S. aromaticum* and *N. sativa* oils on growing cells of *K. pneumonia* were found to be bactericidal. Moreover oils were chemically analyzed to determine their composition where moisture content was found to be 9.22% for Clove, 7.79% for Kalonji and 12.25% for Taramira. Acid values of Clove, Kalonji and Taramira oils were found to be 0.418, 1.673 and 0.334%, respectively whereas iodine values of Kalonji and Taramira oils were found to be 23.173 and 41.950 ml, respectively. Current study was focused to investigate the antibacterial and antifungal potential of natural edibles that is, Clove, Kalonji and Taramira oils in order to treat infectious diseases caused by bacteria and fungi. Taramira can be used to treat fungal infections as it exhibits maximum activity against tested fungi. Thus we can conclude that these oils can be used as bio control agents to treat bacterial and fungal infections.

Key words: Antimicrobial activity, Clove, Kalonji, Taramira, chemical analysis.

INTRODUCTION

Many developed as well as developing countries are facing problems related to infectious diseases that cause mortality and morbidity (Lewis et al., 2006). Antibiotics

can cause alterations in intestinal microecology leading to pathogen colonization and over growth (Sullivan et al., 2001; Hooker et al., 1988). Overuse of antibiotics,

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incomplete course of treatment and horizontal transfer of resistant genes among bacterial species reduce the effectiveness of antibiotics (Amit et al., 2006; Aibinu, 2007). Earlier study described that the administration of antibiotics may cause disturbance of gut microbiota (Clayton et al., 2006).

All these factors forced the researchers to search for alternative antimicrobial substances from different sources including plants and plant based products (Mandal et al., 2010).

Natural products have been an integral part of ancient traditional medicine systems for example, Chinese and Egyptian (Sarker and Nahar, 2007). *Nigella sativa* is a small herbaceous plant, one of the Ranunculaceae, contains 40% saponin (melanthin) and up to 1.4% volatile oil (Chevallier, 1996).

Traditional medicine uses *N. sativa* seed and its oil for the treatment of different illnesses including bronchial asthma in adults. These seeds have been used traditionally for long time in the Middle East, Northern Africa and South Asia for the treatment of various diseases (Brutis et al., 2000). It is used as laxative, carminative and intestinal antiprotozoal drug (Amin, 1990). Their crude extracts (Ali et al., 2001; Mouhajir et al., 1999) and essential oil (Halwani et al., 1999) possess antibacterial.

Clove oil can be obtained from the flower buds of *Syzygium aromaticum*, family Myrtaceae. Its active ingredient 'eugenol' bears antimicrobial properties against many bacteria like *Campylobacter jejuni*, *Salmonella enteritidis*, *L. monocytogenes*, *Escherichia coli* and *Staphylococcus aureus* (Beuchat, 2000; Cressy et al., 2003) and inhibits the growth of molds and yeasts (Matan et al., 2006). Clove oil is used as a food flavoring agent (Zheng et al., 1992), as a medicine for the treatment of asthma and different allergies (Kim et al., 1998), and as an antiseptic in medical dental practices (Cai and Wu, 1996). Lee and Shibamoto (2001) reported that Clove oil has antioxidant activity and can also be used as an anti-carcinogenic agent. *Eruca sativa*, also known as rocket or arugula, is an edible plant. It is an annual plant growing to 20-100 cm tall and their flowers are 2-4 cm in diameter.

It is rich in vitamin C and potassium. *E. sativa* Miller grows in Middle East, India and Pakistan as a minor oil crop and it is used for the preparation of traditional medicines (Flanders and Abdul Karim, 1985). Their seeds can yield oil up to 35% (Yadava et al., 1998) and 27% of protein (Flanders and Abdul Karim, 1985). It resists drought conditions, possesses the ability of salt tolerance (Shannon and Grieve, 1999) and commonly used as animal feed in Asia (Kim and Ishii, 2006). Current study was focused to investigate the antibacterial and antifungal potential of natural edible oils that is, Clove, Kalonji and Taramira oils in order to treat infectious diseases caused by bacteria and fungi.

MATERIALS AND METHODS

Microorganisms and culture conditions

Eighteen (18) bacterial and eight fungal cultures were collected from Reference Culture Collection Laboratory (RCCL) of Department of Microbiology, University of Karachi to check the antimicrobial activity. Their purity was checked on the basis of standard microbiological characteristics as per Bergey's Manual of Determinate Bacteriology 9th edition by Holt (1994). Bacterial cultures were maintained in nutrient agar (NA) and fungal cultures were maintained in Sabraud dextrose agar (SDA) slants at 4°C.

Crude oils and standard antibiotics

S. aromaticum (Clove), *N. sativa* (Kalonji) and *E. sativa* Miller (Taramira) oils were purchased locally from markets in Karachi, Pakistan. Equal volume of absolute methanol was added into oils in order to get better diffusion (Pretorius et al., 2003). Standard antibiotics ampicillin, streptomycin, chloramphenicol and cefamezin were used for antibacterial activity and nystatin was used for antifungal activity.

Standardization of microorganisms

Duly isolated colonies from 18-24 h culture were picked and transferred into tube containing 3 ml phosphate buffer saline pH 7 (Roopashree et al., 2008). Density of the suspension was adjusted to 0.5 McFarlane standards.

Screening for antimicrobial activity

Agar well diffusion method was followed for screening of antibacterial and antifungal activities of oils. Briefly, following standardization and making lawn on Muller Hinton agar (for antibacterial activity) and SDA (for antifungal activity); wells were cut in agar and 30 µl of methanol diluted oils (1:1) were placed. 30 µl of methanol was placed in a well serving as a control. MHA plates were incubated at 37°C for 24 h and SDA plates were incubated at ambient temperature for 48-72 h and observed for the zones of inhibition around each well as described by Adwan et al. (2008).

Determination of combined effect

In order to determine the combined effect of oil with oil, equal volume of two oils were mixed and incubated for 1 h prior to determine antibacterial and antifungal activities as described earlier. Plates were incubated at 37°C for 24 h (for bacteria) and at ambient temperature for 48-72 h (for fungi). Same protocol was followed in order to determine the combined effect of oils with antibiotics.

Determination of activity unit (AU/ml)

Modified method of Mayr-Hartings et al. (1972) was followed to determine the activity units of oils on MHA medium by agar well diffusion method. Oils were serially diluted by using methanol and transferred to wells made on plates seeded with standardized inoculum of *Klebsiella pneumoniae*. Activity unit (AU/mL) is defined as the reciprocal of the last serial dilution demonstrating inhibitory

activity after an incubation period.

Determination of minimum inhibitory concentration (MIC)

In order to determine MIC, oils were serially diluted by using methanol and 20 µl of standardized inoculum of *Klebsiella pneumoniae* was transferred to each tube, incubated at 37°C for 24 h to observe turbidity (CLSI, 2011). MIC is defined as the reciprocal of the last serial dilution demonstrating no turbidity after an incubation period.

Mode of action of oils

Modified method of Bhunia et al. (1988) was followed in order to evaluate mode of action of screw pressed Clove and Kalonji oils on the viable count of log phase cells. Briefly, sensitive culture was inoculated into BHI broth and incubated at 37°C under shaking condition for 2 h to get the log phase culture. 100 µl of this log phase culture was then transferred in the tube containing screw pressed oil and incubated at 37°C. After every hour, 500 µl of sample was taken out and optical density at 600 nm was measured. Control was run simultaneously by using BHI broth.

Chemical analysis

Clove, Kalonji and Taramira oils were chemically analyzed simultaneously in three parallel reactions as described by American Oil Chemists Society (AOCS, 1998):

Moisture content

Oil was placed in hot oven at 105°C for 2 h followed by reweighing. Difference in weight indicates moisture content.

Free fatty acids (FFA) and acid value (AV)

Oil samples were filtered through filter paper in order to remove any debris. Equal volume of diethyl ether was mixed with alcohol and neutralized with 0.1 N NaOH in the presence of phenolphthalein. Oil was dissolved in neutral solvent and titrated with aqueous 0.1 N NaOH till the appearance of pink color.

Calculation:

FFA values are usually reported as oleic acid; however, in oils such as coconut and palm kernel, FFA is reported in terms of lauric acid or palmitic acid, respectively.

For oleic acid, FFA was calculated as follows:

$$\text{FFA as \% oleic acid} = \frac{\text{ml NaOH} \times \text{NaOH normality} \times 28.2}{\text{weight of sample (g)}}$$

Acid value was calculated as follows:

FFA × 1.99 for oleic acid
FFA × 2.81 for lauric acid
FFA × 2.19 for palmitic acid

Iodine value (IV)

Oil samples were filtered through filter paper and placed in water

bath at 70°C. Cyclo-hexane was added and dissolved into the oil followed by Wijs' solution. Flask was sealed and placed in dark for 1 to 2 h. 10% potassium iodide (KI) solution and distilled water were added. Titration was done with 0.1 N sodium thiosulphate till the disappearance of yellow-brown color.

Soluble starch was added and then titration was continued until the disappearance of blue/brown color. Blank was run with cyclo-hexane simultaneously. Calculation was done by using following formula.

Calculation:

$$\text{IV} = \frac{(\text{ml Na}_2\text{S}_2\text{O}_3 \text{ for blank} - \text{ml Na}_2\text{S}_2\text{O}_3 \text{ for sample}) \times \text{Na}_2\text{S}_2\text{O}_3 \text{ normality} \times 12.69}{\text{sample weight (g)}}$$

RESULTS

Antimicrobial activity

S. epidermidis was the most resistant organism among Gram positive bacteria and showed zone of inhibition only against streptomycin with 27 mm diameter. *Klebsiella pneumoniae* was found to be more resistant organism among gram negative bacteria and showed no zone of inhibition against any of the standard antibiotics (Tables 1, 2, 3 and 4). Moreover, maximum antibacterial activity was achieved by Clove and Taramira oils against *E. coli* 5014 and by Kalonji against *K. pneumoniae*.

Antifungal activity of Clove, Kalonji and Taramira oils by using agar well method is depicted in Table 5. Kalonji oil showed no antifungal activities while Clove showed comparatively low antifungal activities against tested fungi. Taramira exhibited maximum activity against *Nigrosporum* with 40 mm diameter of zone of inhibition and it was found that this oil possessed anti dermatophytic activity.

Combined effect

In current study, oils were also checked in combination with each other and with antibiotics but no significant results were obtained. Combination of oils with each other and with antibiotics antagonized the antimicrobial potentials of the tested oils (Figure 1).

Activity units and MIC

It was found that Clove and Kalonji oils have higher activity units and MICs as compared to Taramira oil against *Klebsiella pneumoniae* as shown in Figures 2, 3 and Table 6.

Mode of action

Effects of screw pressed *S. aromaticum* (Clove) and N.

Table 1. Antibacterial activity of *Syzigium aromaticum* (Clove), *Nigella sativa* (Kalonji) and *Eruca sativa* Miller (Taramira) oils and rice bran (against gram positive bacteria).

Organism	Zones of inhibition (in mm)			
	T	K	C	M
<i>Staphylococcus aureus</i>	-	15	14	12
<i>Staphylococcus epidermidis</i>	12	16	25	20
<i>Bacillus subtilis</i>	9	18	15	15
<i>Bacillus cereus</i>	-	-	-	-
<i>Bacillus cereus31</i>	14	20	20	16
<i>Streptococcus faecalis</i>	28	25	35	20
<i>Micrococcus luteus</i>	21	-	-	-
<i>Corynebacterium hoffmonii</i>	27	-	15	-
<i>Corynebacterium xerosis</i>	19	14	24	-

C = Clove oil, K = Kalonji oil, T = Taramira oil, M = diluted methanol (- control), - = no zone of inhibition.

Table 2. Antibacterial activity of *Syzigium aromaticum* (Clove), *Nigella sativa* (Kalonji) and *Eruca sativa* Miller (Taramira) oils and rice bran (Against gram negative bacteria).

Organism	Zones of inhibition (in mm)			
	T	K	C	M
<i>Escherichia coli 5014</i>	22	15	26	-
<i>Escherichia coli</i>	20	-	21	-
<i>Enterobacter aerogenes</i>	10	-	-	-
<i>Klebsiella pneumoniae</i>	20	18	20	-
<i>Salmonella typhi</i>	20	14	21	-
<i>Salmonella para typhi A</i>	15	-	20	-
<i>Salmonella para typhi B</i>	-	-	-	-
<i>Pseudomonas aeruginosa</i>	18	-	18	-
<i>Proteus vulgaris</i>	20	12	-	-

C = Clove oil, K = Kalonji oil, T = Taramira oil, M = diluted methanol (- control), - = no zone of inhibition.

sativa (Kalonji) oils on growing cells of *K. pneumoniae* were found to be bactericidal as inhibition of the microbial cells was observed (Figures 4 and 5). Clove oil showed maximum inhibition of *K. pneumoniae* upto 4 h as compared to Kalonji oil.

Chemical analysis of oils

Oils were chemically analyzed to determine their composition as shown in Table 7. It was found that Taramira oil has higher moisture content as compared to other tested oils.

Free fatty acid is defined as the percentage by weight of free acid groups in the oil whereas acid value is defined as the weight (mg) of KOH required to neutralize

Table 3. Standard antibiotic activity against gram positive bacteria

Organism	Zones of inhibition (in mm)			
	Ce	CH	A	S
<i>Staphylococcus aureus</i>	38	-	-	28
<i>Staphylococcus epidermidis</i>	-	-	-	27
<i>Bacillus subtilis</i>	25	10	-	29
<i>Bacillus cereus</i>	23	18	11	30
<i>Bacillus cereus31</i>	30	21	13	30
<i>Streptococcus faecalis</i>	30	15	-	17
<i>Micrococcus luteus</i>	40	23	12	40
<i>Corynebacterium hoffmonii</i>	40	-	-	32
<i>Corynebacterium xerosis</i>	47	17	19	40

Ce= Cefamezin, CH= Chloramphenicol, A= Ampicillin, S= Streptomycin, - = no zone of inhibition.

Table 4. Standard antibiotic activity against Gram negative bacteria

Organism	Zones of inhibition (in mm)			
	Ce	CH	A	S
<i>Escherichia coli 5014</i>	40	-	-	26
<i>Escherichia coli</i>	32	-	-	26
<i>Enterobacter aerogenes</i>	-	12	-	22
<i>Klebsiella pneumoniae</i>	-	-	-	-
<i>Salmonella para typhi A</i>	28	-	-	22
<i>Salmonella para typhi B</i>	28	-	-	28
<i>Salmonella typhi</i>	15	-	-	30
<i>Pseudomonas aeruginosa</i>	-	-	-	25
<i>Proteus vulgaris</i>	-	-	-	19

Ce = Cefamezin; CH = Chloramphenicol; A = Ampicillin; S = Streptomycin; - = no zone of inhibition.

free acid groups. Furthermore, Iodine value of Kalonji and Taramira oils was found to be 23.173 and 41.950 ml, respectively.

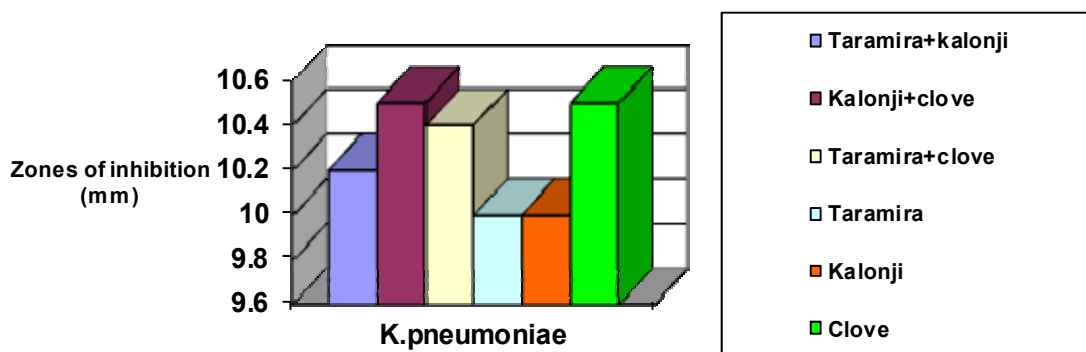
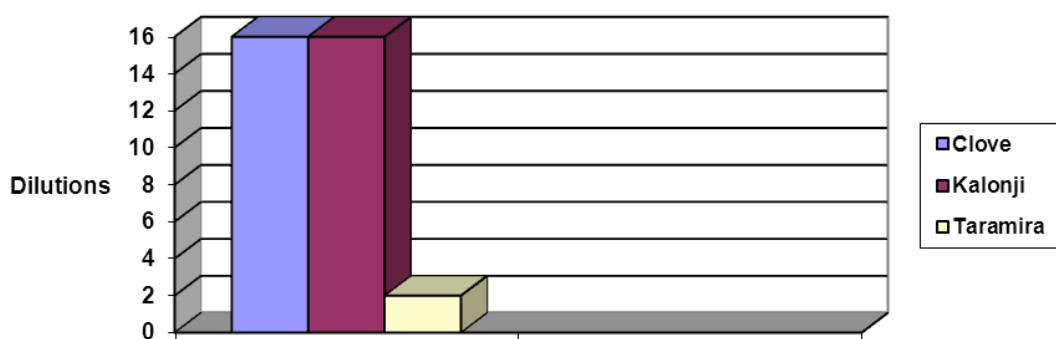
DISCUSSION

Increasing antimicrobial resistance is a growing threat to human health and is mainly a consequence of overuse of antibiotics. Administration of antibiotics greatly disrupts the intestinal microflora, there is always a chance that the patient will become colonized by resistant organisms and develop serious infections. Current study was focused to investigate the antibacterial and antifungal potential of natural edible oils that is, *S. aromaticum* (Clove), *N. sativa* (Kalonji) and *E. sativa* Miller (Taramira) oils in order to treat infectious diseases caused by bacteria and fungi. To the best of our knowledge, limited studies have focused on antimicrobial potential of Taramira oil. In the early experiments, we also used rice bran in order to

Table 5. Antifungal activity of standard antibiotic (Nystatin) and *Syzigium aromaticum* (Clove), *Nigella sativa* (Kalonji) and *Eruca sativa* Miller (Taramira) oils

Organism	Zones of inhibition (in mm)				
	K	C	T	M	N
<i>Penicillium</i>	-	-	20	-	20
<i>T. rubrum</i>	-	-	-	-	16
<i>T. mentagrophyte</i>	-	20	16	-	20
<i>M. canis</i>	-	-	25	-	17
<i>M. gypsum</i>	-	-	34	-	20
<i>Nigrosporum</i>	-	-	40	-	20
<i>Cunninghamella</i>	-	-	24	-	22
<i>A. niger</i>	-	-	20	-	18
<i>A. flavus</i>	-	-	20	-	18

C = Clove oil; K= Kalonji oil; T= Taramira oil; M= Diluted methanol (- control); N= Nystatin (+ control); - = no zone of inhibition.

**Figure 1.** Combined effect of oils (1:1) against *Klebsiella pneumoniae*.**Figure 2.** Activity unit (AU/ml) of Clove, Kalonji and Taramira oils against *Klebsiella pneumoniae*.

assess its antimicrobial spectrum but unfortunately we did not get any significant results.

It was found that Gram negative bacteria and fungi were more sensitive to all the tested oils. Bii et al. (2010) reported good activity of methanol extracts of *Prunus africana* against some bacterial and fungal strains. In our

study, *K. pneumoniae*, *A. flavus* and *Cunninghamella* were found to be more sensitive microorganisms showing larger zones of inhibition, and mode of action of Clove and Kalonji oils were bactericidal. *Klebsiella* sp. causes wound, respiratory and catheter related infections while *A. flavus* and *Cunninghamella* are considered as

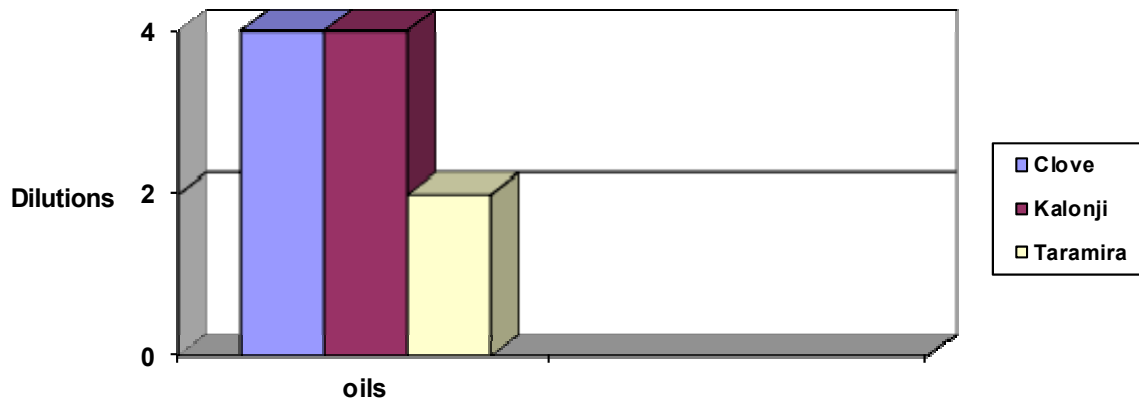


Figure 3. MICs of Clove, Kalonji and Taramira oils (against *Klebsiella pneumoniae*).

Table 6. Activity unit and MICs of Clove, Kalonji and Taramira oils (Against *Klebsiella pneumoniae*).

Oil	Activity unit (AU/ml)	MIC
Clove	1:16	1:4
Kalonji	1:16	1:4
Taramira	1:2	1:2

Table 7. Chemical analysis of *Syzigium aromaticum* (Clove), *Nigella sativa* (Kalonji) and *Eruca sativa Miller* (Taramira) oils.

Chemical analysis	Clove	Kalonji	Taramira
Moisture content (%)	9.22	7.79	12.25
Free fatty acids (%)	0.21	0.841	0.168
Acid value (%)	0.418	1.673	0.334
Iodine value (ml)	ND	23.173	41.950

ND= Not done.

pathogenic fungi. Infections caused by *E. coli*, *K. pneumoniae*, *Salmonella typhi* and *P. aeruginosa* may be treated with these oils as they are more active against gram negative bacteria. *P. aeruginosa* is an opportunistic human respiratory pathogen and has high degree of resistance to many antibiotics (Pearson et al., 2000). *P. aeruginosa* has distinct properties which enable the bacteria to colonize and infect Cystic Fibrosis lung (Matsui et al., 2006). One of the study determined antimicrobial sensitivity of Clove oil against some Gram negative bacteria including *E. coli*, *Klebsiella pneumoniae*, *Salmonella paratyphi*, Gram positive bacterium *S. aureus* and a fungus *Candida albicans* where Clove oil showed a broad spectrum activity (Ayoola et al., 2008). Number of studies reported antibacterial activity of *N. sativa* seeds. Their seeds contain tannins, which can be extracted by methanol

(Eloff, 1998) and have reported antimicrobial properties (Scalbert, 1991).

Recent work also focused to test these oils for their anti dermatophytic activities as different studies focused antifungal potentials of Clove oils (Soliman and Badea, 2002; Velluti et al., 2003; Feng and Zheng, 2007; Lopez-Malo et al., 2007). Taramira can be used to treat fungal infections and it may be used as anti-dandruff as it exhibits maximum activity against tested fungi.

Drug synergism between known antibiotics and bio-active plant extracts is a novel concept and could be beneficial (synergistic or additive interaction) or deleterious (antagonistic). In this study, no significant results were obtained by combination of oils with each other and with antibiotics. These results were contradictory with the fact that the combination of two agents exhibit significant synergism only if the test organism is resistant to at least one of the agents (Esimone et al., 2006). Adwan et al. (2008) determined additive interactions between antimicrobial agents and plant extracts against five strains of *S. aureus*.

Activity units and MIC values of Clove and Kalonji oils were found to be higher than Taramira oils. These results are in agreement with those found by Udomlak Sukatta et al. (2008) who demonstrated that the MIC values of Clove oil were greater than those obtained from cinnamon oil.

Chemical analysis was done to investigate the overall moisture content, presence of free fatty acids and nature of bonding in the tested oils. The results obtained from chemical analysis revealed that the Taramira oil is more unsaturated than Kalonji oil as iodine value is used to determine the level of unsaturation in oils or fats. The traditional iodine value determination method using the wigs reagent requires CCl_4 . We used cyclohexane in place of CCl_4 . Wigs reagent is iodine solution in acetic acid which provides iodine monochloride to react with double bonds. Water forces the oil into cyclohexane and the excess iodine monochloride moves into the water,

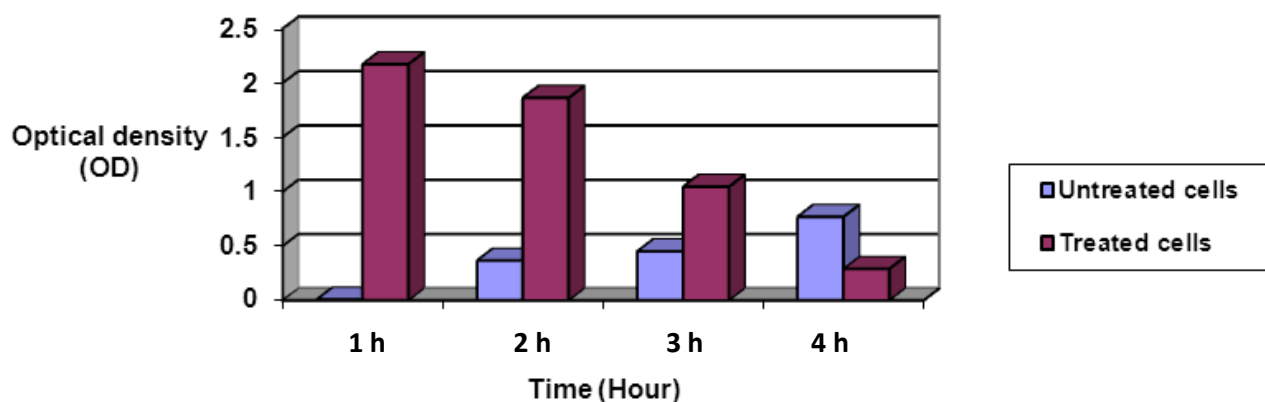


Figure 4. Effect of *Syzgium aromaticum* (Clove) oil on growing cells of *Klebsiella pneumonia*.

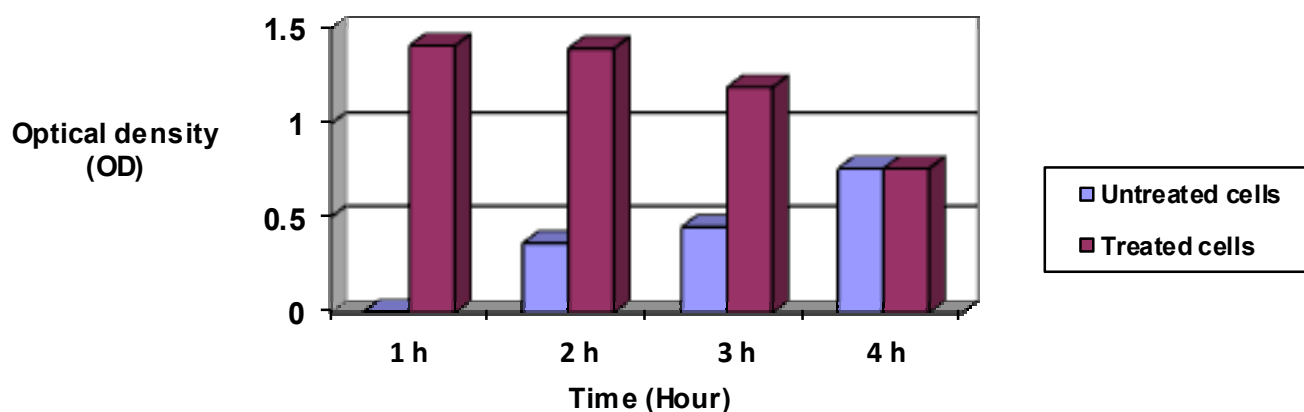


Figure 5. Effect of *Nigella sativa* (Kalonji) oil on growing cells of *Klebsiella pneumonia*.

where it is converted to I₂ and can be titrated with water soluble Na₂S₂O₃. KI solution converts excess iodine monochloride to free iodine (blue) which can be titrated to a colorless end point with Na₂S₂O₃. As compared to other tested oils Kalonji has high free fatty acid and acid values.

Conclusion

Present work reveals that crude oils of Clove, Kalonji and Taramira possess antimicrobial activity. Infections caused by *P. aeruginosa*, *Klebsiella* sp., *S. typhi* may be treated with these oils as they are more active against Gram negative bacteria. Taramira can be used to treat fungal infections and it may be used as anti-dandruff as it exhibits maximum activity against tested fungi. Thus we can conclude that these oils can be used as bio control agents to treat bacterial and fungal infections. However, further *in vivo* studies are required to evaluate the potential use of these oils as antimicrobial agents in tropical or oral applications.

Conflict of Interest

None of the authors declared financial and social conflict of interest regarding this study.

ACKNOWLEDGEMENTS

We are thankful to Prof. Dr. Shakeel Ahmed, Department of Microbiology, University of Karachi for his cooperation and support.

REFERENCES

- Adwan G, Mhanna M (2008). Synergistic effects of plant extracts and antibiotics on *Staphylococcus aureus* strains isolated from clinical specimens. Middle-East J. Sci. Res. 3(3):134-139.
- Aibinu I, Adenipekun T, Adelowotan T, Ogunsanya T, Odugbemi T (2007). Evaluation of the antimicrobial properties of different parts of *Citrus aurantifolia* (Lime fruit) as used locally. Afr. J. Trad. CAM. 4:185-190.
- Ali NA, Julich WD, Kusnick C, Lindequist U (2001). Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. J.

- Ethnopharmacol. 74(2):173-179.
- Amin G (1990). Traditional herbal drugs in Iran. Iranian ministry of health publications, Tehran, 118-119.
- Amit R, Shailendra S (2006). Ethnomedicinal approach in biological and chemical investigation of phytochemicals as antimicrobials. Available at <http://www.phamainfo.net>. Accessed April 28, 2007.
- AOCS (American Oil Chemists Society) (1998). Official method Cd 8-53. Peroxide value. In official methods & Recommended Practices of the American oil chemists Society, 5th ed. (D.Firestone, ed.). AOCS, Champaign, Ill.
- Ayoola GA, Lawore FM, Adelowotan T, Aibinu IE, Adenipekun E, Coker HAB, Odugbemi TO (2008). Chemical analysis and antimicrobial activity of the essential oil of *Syzygium aromaticum* (Clove). Afr. J. Microbiol. Res. 2:162-166.
- Beuchat LR (2000). Control of foodborne pathogens and spoilage microorganisms by naturally occurring antimicrobials. In C. L. Wilson and S. Droby (Eds.), Microbial food contamination. Boca Raton, FL: CRC Press. 149-169.
- Bhunia AK, Johnson MC and Ray B (1988). Purification, characterization and antimicrobial spectrum of a bacteriocin produced by *Pediococcus acidilactici*. J. Appl. Bacteriol. 65:261-268.
- Bii C, Korir KR, Rugutt J, Mutai C (2010). The potential use of *Prunus africana* for the control, treatment and management of common fungal and bacterial infections. J. Med. Plants Res. 4(11):995-998.
- Brutis M, Bucar F (2000). Antioxidant activity of *Nigella sativa* essential oil. Phytother. Res. 14:323-328.
- Cai L, Wu CD (1996). Compounds from *Syzygium aromaticum* processing growth inhibitory activity against oral pathogens. J. Nat. Prod. 59:987-990.
- Chevallier A (1996). The encyclopedia of medicinal plants. Dorling Kindersley publishers, London, 237.
- Clayton TA, Lindon JC, Cloarec O et al. (2006). Pharmaco metabolomic phenotyping and personalized drug treatment. Nature. 440:1073-1077.
- CLSI (2011). Performance Standards for Antimicrobial Susceptibility Testing, Twenty-First Informational Supplement. CLSI document M100-S21. Clinical and Laboratory Standards Institute, Wayne, Pa, USA.
- Cressy HK, Jerrett AR, Osborne CM, Bremer PJ (2003). A novel method for the reduction of numbers of *Listeria monocytogenes* cells by freezing in combination with an essential oil in bacteriological media. J. Food Protec. 66:390-395.
- Eloff JN (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants? J. Ethnopharmacol. 60:1-8.
- Esimone CO, Iroha IR, Ibezim EC, Okeh CO, Okpana EM (2006). *In vitro* evaluation of the interaction between tea extracts and penicillin G against *Staphylococcus aureus*. Afr. J. Biotech. 5(11):1082-1086.
- Feng W, Zheng X (2007). Essential oils to control *Alternaria alternata* *in vitro* and *in vivo*. Food Control. 18:1126-1130.
- Flanders A, Abdul Karim SM (1985). The composition of seed and seed oils of Taramira (*Eruca sativa*). J. Am. Oils Chem. Soc. 62:1134-1135.
- Halwani R, Habbal MZ, Abdelnoor AM, (1999). The antibacterial effect of some constituents of *Nigella sativa* oil. Arab J. Pharmaceut. Sci. 1(1):87-96.
- Holt JG (1994) in Bergey's Manual of determinate bacteriology 9th edition by David Hendricks Bergey's.
- Hooker KD, DiPiro JT (1988). Effect of antimicrobial therapy on bowel flora. Clin. Pharm. 7:878-888.
- Kim HM, Lee EH, Hong SH, Song HJ, Shin MK, Kim SH, Shin TY (1998). Effect of *Syzygium aromaticum* extract on immediate hypersensitivity in rat. J. Ethnopharmacol. 60:125.
- Kim SJ, G Ishii (2006). Glucisnolate profile in the seeds, leaves and roots of the rocket salad (*Eruca sativa* Mill.) and antioxidant activity of intact plant powder and purified 4-methoxyglucobrassicin. Soil Sci. Plant Nutr. 52:394-400.
- Lee KG, Shibamoto T (2001). Antioxidant property of aroma extract isolated from Clove buds (*Syzygium aromaticum*). Food Chem. 74:443-448.
- Lewis K, Ausubel F (2006). Focus on antibacterials. Nature Biotech. 24(12):1453-1602.
- Lopez-Malo A, Barreto-Valdivieso J, Palou E, Martin FS (2007). *Aspergillus flavus* growth response to cinnamon extract and sodium benzoate mixtures. Food Control. 18:1358-1362.
- Mandal S, Deb Mandal M, Pal NK (2010). Synergistic anti-*Staphylococcus aureus* activity of amoxicillin in combination with *Emblica officinalis* and *Nymphae odorata* extracts. Asian Pac. J. Trop. Med. 3:711-714.
- Matan N, Rimkeeree H, Mawson AJ, Chompreeda P, Haruthaithanasan V, Parker M (2006). Antimicrobial activity of cinnamon and Clove oils under modified atmosphere conditions. J. Food Microbiol. 107:180-185.
- Matsui H, Wagner VE, Hill DB, Schwab UE, Rogers TD, Button B, Taylor RM, Superfine R, Rubinstein M, Iglewski BH, Boucher RC (2006). A physical linkage between cystic fibrosis airway surface dehydration and *Pseudomonas aeruginosa* biofilms. Proc. Natl. Acad. Sci. 103(48):18131-18136.
- Mayr-Hartings A, Hedges AJ, Barkeley RCW (1972). Methods for studying bacteriocins. In: Methods in microbiology. Ed. by J. Bergen and JR Norris. 7:315-422.
- Mouhajir F, Pedersen JA (1999). Antimicrobial thymohydroquinones of Moroccan *Nigella sativa* seeds detected by electron spin resonance. Pharmaceut. Biol. 37(5):391-395.
- NCCLS (2000). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard N Fifth Edition. NCCLS document M7-A5. NCCLS: Wayne, PA. USA.
- Pearson JP, Feldman M, Iglewski BH, Prince A (2000). *Pseudomonas aeruginosa* cell-to cell signalling is required for virulence in a model of acute pulmonary infection. Infect. Immun. 68:4331.
- Pretorius JC, Magama S, Zietsman PC (2003). Growth inhibition of plants pathogenic bacteria and fungi by extracts from selected South African plant species. S. Afr. J. Bot. 69(2):88-192.
- Roopashree TS, Dang R, Rani RHS and Narendra C (2008). Antibacterial activity of antipsoriatic herb: *Cassia tora*, *Momordica charantia* and *Calendula officinalis*. Int. J. Appl. Res. Nat. Prod. 1(3):20-28.
- Sarker SD, Nahar L (2007). Chemistry for Pharmacy Students General, Organic and Natural Product Chemistry. England: John Wiley and Sons. pp. 283-359.
- Scalbert A (1991). Antimicrobial properties of tannins. Phytochemistry 30:3875-3883.
- Shannon MC, Grieve CM (1999). Tolerance of vegetable crops to salinity. Scientia Hortic. 78:5-38.
- Soliman KM and Badea RI (2002). Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food Chem. Toxicol. 40:1669-1675.
- Sullivan A, Edlund C, Nord CE (2001). Effect of antimicrobial agents on the ecological balance of human microflora. Lancet Infect. Dis. 1:101-114.
- Udomlak Sukatta, Vichai Haruthaithanasan, Walairut Chantarapanont, Uraiwan Dilokkunanant and Panuwat Suppakul (2008). Antifungal activity of Clove and Cinnamon oil and their synergistic against postharvest decay fungi of grape *in vitro*. Kasetsart J. Nat. Sci. 42:169-174.
- Velluti A, Sanchis V, Ramos AJ, Egido J, Marin 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. J. Food Microbiol. 89:145-154.
- Yadava TP, Friedt DW and Gupta SK (1998). Oil content and fatty acid composition of Taramira (*Eruca sativa* L.) Genotypes. J. Food Sci. Technol. 35:557-558.
- Zheng GQ, Kenney PM, Lam LK (1992). Sesquiterpenes from Clove (*Eugenia caryophyllata*) as potential anticarcinogenic agents. J. Nat. Prod. 55:999-1003.