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Antibacterial Activity of the Essential Oils of Sudanese Accessions of Basil (*Ocimum basilicum* L.)

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Abstract: This study is concerned with the evaluation of antibacterial activity of the essential oils of the most commonly grown ornamental-type basils (five types) as well as that of wild-type basil which spontaneously grows in large pure stands during the rainy season. The essential oils of all six basil types showed strong antibacterial activity against *E. coli*, *Staphylococcus aureus* and *Salmonella typhimurium*. This activity was dose-dependent. Calculated LD_{50} values varied between 40 and 325 μL of crude essential oil/well, for the three bacteria, using the agar well diffusion method. The crude essential oil of wild Sudanese basil, assayed by the disc diffusion method, had three TLC-separated compounds which were active against *Salmonella typhimurium*. One of these was identified as geraniol, a major constituent of the essential oil. Thus basil essential oil has potential clinical or food applications as an antibacterial agent.

Key words: Basil (*Ocimum basilicum*), essential oil, antibacterial, *E. coli*, *Staphylococcus aureus*, *Salmonella typhimurium*

INTRODUCTION

Aromatic plants are used the world over as flavours, as well as folk medicines according to local beliefs. Essential oils are currently of much research interest in view of their biological activities including bacterial and antifungal effects (Burt, 2004; Omidbeygi *et al*., 2007; Tzortzakis and Economakis, 2007; Bakkali *et al*., 2008) antitrypansomal (Santoro *et al*., 2007; Politio *et al*., 2007; Hussain *et al*., 2008) antiviral (Sinico *et al*., 2005) antioxidant (Juntochote *et al*., 2006) insecticidal (Cetin and Yanikoglu, 2006) and other activities (Skandamis and Nycha, 2001; Lin *et al*., 2006; Gandomi *et al*., 2009; Randrianarivelo, 2009).

The potential use of essential oils as clinical antibiotics or as food additives against microbial spoilage is appealing because of their long and safe use as natural products. Development of bacterial resistance to synthetic antimicrobial agents and the side-effects associated with their use favour essential oils for alternative or complementary use. Research efforts to increase the weather efficacy of essential oils continue. For example, Rosato *et al*. (2007) reported a synergist effect of the essential oil of pelargonium and its chemical components on the efficacy of norfloxacin antibiotic, when used in combination. The essential oil of *Helichrysum italicum* reduced the multidrug resistance of four bacteria, with geraniol, a component of the oil, significantly increasing the antibacterial effects of β-lactam, quinolones and chloram phenicol antibiotics (Lorenzi *et al*., 2009). Similar synergistic effects between antibiotics and other natural products (plant lattices) hoped to decrease antibiotic dose and therefore side-effects is well documented (Giordani *et al*., 2005). Hemaiswarya *et al*. (2008) reviewed the research on synergism between natural products and clinical antibiotics.

The practical problems facing the use of essential oils as antimicrobial food additives is their larger amounts that need be used compared with synthetic alternatives, resulting in imparting unwanted flavor to the food. In some instances no such problem arose, as in the work reported on the use of oregano oil with meat (Oussalah *et al*., 2006). To overcome the problem of unwanted flavor several reports exploited the synergism, in antibacterial action, between combinations of different crude essential oils or their components, in order to reduce the amounts of essential oil used as food additive (Kim *et al*., 2006; Gutierrez *et al*., 2008).

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The genus *Ocimum* of the family Labiatae (Lamiaceae) is widely distributed in tropical, subtropical and temperate zones of the world (Gupta, 2006). *Ocimum basilicum* is a major essential oil-producing species belonging to the genus, with its oil showing a great diversity of chemical structure (Grayer *et al*., 1996).

We have previously reported on the considerable variability of chemical composition of the essential oil of several accessions of indigenous and introduced accessions of *O. basilicum* grown in Sudan (Abduelrahman *et al*., 2009; Nour *et al*., 2009a). We also reported on some biological activities of the essential oils of some of these accessions including mosquito larvicidal activity (Nour *et al*., 2009b) and adult mosquito repellency (Nour *et al*., 2009c). The results reported here are on the antibacterial properties of some basil accessions, especially against *Salmonella typhimurium*.

MATERIALS AND METHODS

Plant material (seed sources): Seeds of basil accessions used in these studies were obtained from different parts of Sudan and directly sown on ridges at the farm of the Faculty of Agricultural Sciences, University of Gezira, Wad Madeni, Sudan. Sowing was done on Feb. 20, 2005. Watering and weeding were carried out as necessary. No chemicals (fertilizers or others) were applied. Taxonomic identification of plants was performed by Prof. O. Elzaki of the National Centre for Research, Department of Biology, Khartoum, Sudan. Specimens of seeds and herbaceous parts of the plant were deposited (ref. No. NCR/05/2/199). The accessions were given numbers. Initially ornamental basil accessions were used as sources of leaf or flower essential oils. These were referred as IF, IIF, IIIL, IVL and VL, the letters referring to flower and leaf organs. Subsequent experiments used wild basil leaf.

Essential oil extraction: Essential oils were obtained from fresh leaf and flowers of basil plants by steam distillation.

Chromatography: Thin layer chromatography was used to separate essential oil components using the solvent systems: Benzene/ ethyl acetate (95:5) or n-hexane/ethyl acetate/ methanol (79:17:4) and used vanillin-sulfuric acid, anisaldehyde-sulfuric acid for detection reagents.

Preparative TLC (0.5 mm layers) was used to separate the essential oil of wild basil for subsequent assay of antibacterial activity. After development the plates were covered in such a way revealing a narrow area for application of the detection reagent. Corresponding areas uncontaminated with reagent are scraped, eluted with chloroform/ methanol (2:1) and applied to bioassay disc.

Spectroscopy: A Perkin Elmer Fourier Transform Infra Red (FTIR) spectrophotometer model 1600 FT-IR was used. Spectra were measured in chloroform for the frequencies 1000 to 4000 cm^{-1}.

Bioassay of bacterial activity: Essential oils of selected basil accessions were evaluated for antibacterial activity using the well or disc diffusion methods on nutrient agar media. Calculations of LD_{50} were determined by using the modified agar-well diffusion method (Okeke *et al*., 2001). Wells are made in nutrient agar plates used cork borer (5 mm). Inoculums of bacteria *E. coli*, *Staphylococcus aureus* and *Salmonella typhimurium* were thoroughly mixed with molten media before pouring. Inoculated plates were left to solidify at room temperature. Then 0.05 mL of the essential oil solution was placed in each well made in inoculated plates. Similarly each plate carried a blank well with solvent (methanol) only saved as a control. Different concentrations of basil oil were tested. All plates were incubated for 48-72 h, at 25±2°C. Zones of inhibition round the wells were measured in mm. In diffusion disc method was made in accordance with NCCLS guidelines M23-A2 and M37-A2 (Miller *et al*., 2003), discs were saturated with 20 µL undiluted oil (100%) or oil diluted with dichloromethane down to 10% (oil in solvent). In the control treatment discs were saturated only with pure solvent. The disc and Petri-dish diameter were 0.5 and 10 cm, respectively.

The bacterial species used were obtained from the Institute of Nuclear Medicine, University of Gezira, Sudan.

RESULTS AND DISCUSSION

Antibacterial activities of the crude essential oils of basils: Table 1 and Fig. 2A shows that the crude essential oils of five Sudanese accessions of ornamental basil exhibited marked growth inhibitory effects on the three bacterial species tested, namely, *E. coli*, *Staphylococcus aureus* and *Salmonella typhimurium*. The agar well diffusion method was used at this stage. Both leaf and flower essential oils were active, with the bacterial growth inhibition reaching 100% value with most oils and bacterial treatments. *Salmonella typhimurium* showed greater sensitivity to basil leaf and flower essential oils. Antibacterial activity was close-dependent and calculated LD_{50} values varied between 40 and 325 µL of oil/well.

Another method, the disc diffusion method, showed similar results of inhibition for *E. coli* and *Staphylococcus aureus* when the essential oil of wild Sudanese basil was tested (Fig. 1, 2B). A clear dose dependent inhibition of the two bacteria was observed (Fig. 1). This wild accession, which abundantly grows as an annual during

Table 1: Antibacterial activity of the crude essential oils of five ornamental basil accessions against three bacteria

Accession/ plant part	Oil doses (µL mL^{-1})	Microorganism		
		E. coli	*Staphylococcus aureus*	*Salmonella typhimurium*
I (Flower)	33.3	26.0 (65%)	08.0 (20%)	12.0 (30%)
	50	30.0 (75%)	10.0 (25%)	20.5 (51.3%)
	100	-	-	-
	500	40.0 (100%)	21.0 (52.5%)	40.0 (100%)
II (Flower)	33.3	17.0 (42%)	15.0 (38%)	18.0 (45%)
	50	25.0 (62%)	20.0 (50%)	28.0 (70%)
	100	25.0 (62%)	25.0 (62%)	34.0 (85%)
	500	30.0 (75%)	40.0 (100%)	40.0 (100%)
III (Leaf)	33.3	15.0 (38%)	0.0 (0)	20.0 (50%)
	50	20.0 (50%)	09.0 (22.5%)	25.5 (63.8%)
	100	20.5 (51.3%)	14.0 (35%)	28.5 (71.3%)
	500	40.0 (100%)	40.0 (100%)	40.0 (100%)
IV (Leaf)	33.3	12.0 (30%)	0.0 (0%)	0.0 (0%)
	50	16.5 (41.3)	0.0 (0%)	10.0 (25%)
	100	21.0 (52.5)	20.5 (51.3%)	21.0 (52.5%)
	500	40.0 (100%)	32.5 (81.3%)	40.0 (100%)
V20 (Leaf)	33.3	09.0 (22.5%)	0.0 (0%)	0.0 (0%)
	50	13.0 (32.5%)	10.0 (25%)	0.0 (0%)
	100	22.5 (56.3%)	16.0 (40%)	24.0 (60%)
	500	31.5 (78.8%)	23.0 (57.5%)	40.0 (100%)

The values show bacterial inhibition as growth zone diameter, in mm and % inhibition of bacterial growth (bracketed). 0: No inhibitions, -: Not determine

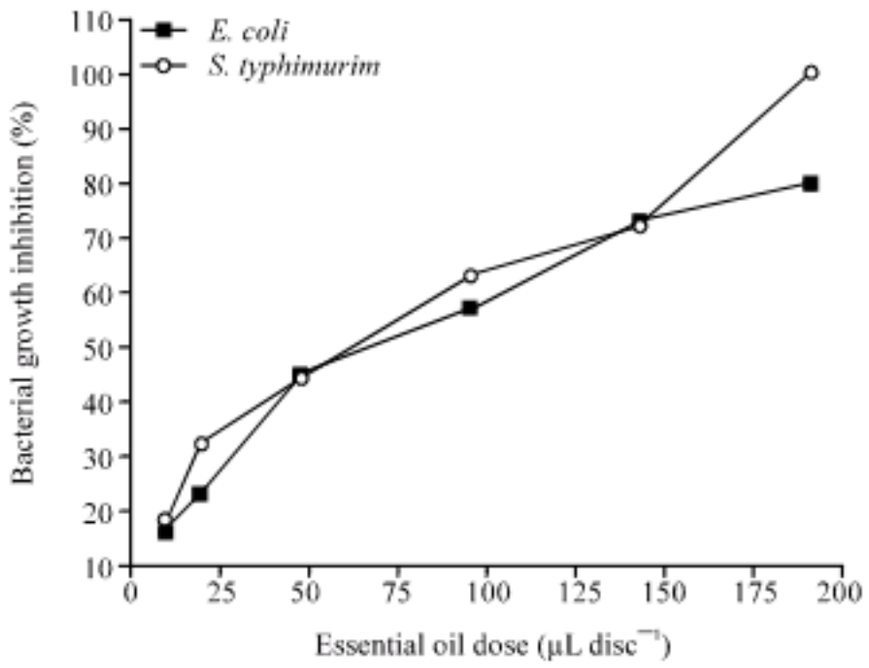

Fig. 1: Growth inhibitory effect of the essential of wild basil against *E.coli* and *S. typhimurium*. The disc diffusion method was used (data of Table 2)

the rainy season in Sudan, is believed to be of uniform essential oil-chemical composition (Abduelrahman *et al*., 2009). Further work directed towards elucidation of the active antibacterial ingredient was carried out using this wild basil accession. Figure 2A and B show typical bacterial inhibition, using the well and disc diffusion methods of bioassay.

The antibacterial activity of the crude essential oil of *Ocimum basilicum* has been reported against the meat spoilage bacterium, *Pseudomonas putide* (Oussalah *et al*., 2006), disease-causing organisms of the

Table 2: Growth inhibitory effects of TLC separated constituents of the essential oil of wild basil assayed against *S. typhimurium*

TLC spot number	1	2	3	4	5	6	7	8
% growth inhibition (*S. typhimurium*)	0.0	0.0	33.3	0.0	27.8	35.6	0.0	0.0

The TLC spots, numbered from near the solvent front region downwards were scraped, in the amounts present as their natural proportions and eluted as described in Material and Methods

genera *Staphylococcus*, *Enterococcus* and *Pseudomonas* (Opalchenova and Obreshkova, 2003), pathogens of the genera *Enterococcus*, *Escherichia*, *Listeria* and *Staphylococcus* (Carovic-Stanko *et al*., 2009). Hussain *et al.* (2008) reported activity of the oil against food borne species of Bacillus and Pasteurella.

Despite the strong bacterial growth-inhibitory activity of the essential oil of *Ocimum basilicum* reported by several authors, others reported very weak or no activity (Shan *et al*., 2007; Runyora *et al*., 2009). Runyora *et al.* (2009) reasoned that the weak antibacterial activity they observed with the *O. basilicum* essential oil was due to the lack of active ingredients like geraniol and linalool in the sample tested. *Ocimum basilicum* showed immense variability in essential oil chemical composition (Carovic-Stanko *et al*., 2009; Abduelrahman *et al*., 2009). Accordingly reported biological activity would be expected to vary, not only depending on the plant chemotype, but seasonal variation in chemical composition of the oil was reported within the same plant type (Hussein *et al*., 2008). Similarly reported MIC or LD50 values vary depending on factors related to the plant, bacterial strain and bacterial growth media (Opalchenova and Obreshkova, 2003; Nazer *et al*., 2005; Burt, 2004; Rosato *et al*., 2007; Bagamboula *et al*., 2004).

Determination of the chemical component (s) of basil oil, active against *S. typhimurium*: Thin layer chromatography (TLC) separation of the essential oil of wild basil resulted in eight anisaldehyde-positive components. The eight components were eluted from the silica gel using chloroform/methanol (2:1) and appropriately tested, separately, against *S. typhimurium*, using the disc diffusion method. For each component all the amount scraped off four TLC plates was eluted. The TLC spots or bands were assigned numbers (1-8) of decreasing R_f. Only three of the TLC-separated compounds were active against *S. typhimurium* (Table 2). These had the numbers 3, 5 and 6. The objective was to isolate and identify the active antibacterial components of basil essential oil and no attempt was made to determine the amounts of the eluted components.

Aliquots of the eluates of the active TLC spots were subjected to FTIR spectroscopic analysis. TLC spot 5 could not be identified. The TLC spot 3 was tentatively

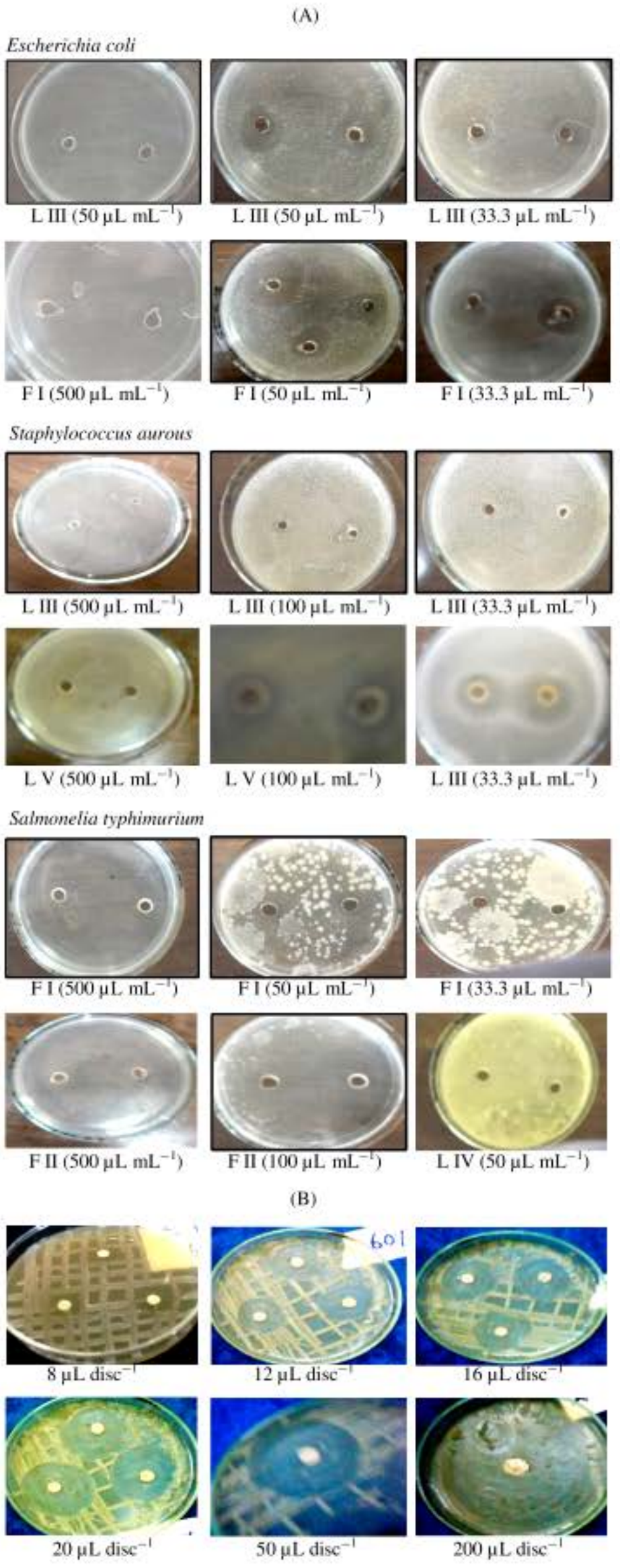

Fig. 2: (A, B) The growth inhibitory effect of different concentration of crude essential oils of different basil accessions on the growth of *Escherichia coli, Staphylococcus aurous* and *Salmonella typhimurium*. Using the (A) well method or the (B) disc method. The higher concentration (500 µL well^{-1}) in Fig. 1 completely inhibited the growth of the *Escherichia coli*, *Staphylococcus aurous* and *Salmonella typhimurium*

identified as eucalyptol. The TLC spot 6 was positively identified as geraniol. In comparison with authentic geraniol it had similar thin layer chromatographic mobility; similar Rt on GLC analysis and similar FTIR absorption spectrum. However, we have purified authentic geraniol by TLC, eluted the pure compound and tested it against *S.typhimurium*. It was active.

Geraniol, mostly obtained as a commercial standard rather the being was reported to be active against a No. of bacterial species (Boyanova and Neshev, 1999; Kishore *et al*., 2007).

Lorenzi *et al*. (2009) reported the synergistic effect of the compound when used in combination with standard antibiotics against drug resistant species of *Enterobacter*, *Escherichia*, *Pseudomonas* and *Acinetobacter*. Geraniol also, shown to be active against *Salmonella typhimurium* (Kim *et al*., 2006).

Thus gerianol, a major component of the essential oil of wild Sudanese basil (Abduelrahman *et al.,* 2009) is one of three components active against *S. typhimurium*. However, the two other unidentified components which showed antibacterial activity were present as minor components as visually judged by TLC spot size. This contrasts with the held view that antibacterial activities of essential oils are predominantly related to their main components (Oussalah *et al*., 2006).

CONCLUSIONS

Thus geraniol, a major component of the essential oil of wild Sudanese basil is one of three components active against *S.typhimurium*. However, the two other unidentified components which showed antibacterial activity were present as minor components as visually judged by TLC spot size. This contrasts with the held view that antibacterial activities of essential oils are predominantly related to their main components. Further work is underway to identify the other two ingredients of basil oil active against *S.typhimurium*.

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REFERENCES

Abduelrahman, A.H.N., S.A. Elhussein, N.A. Osman and A.H. Nour, 2009. Morphological variability and chemical composition of essential oils from nineteen varieties of basil (*Ocimum basilicum* L.) growing in Sudan. Int. J. Chem. Technol., 1: 1-10.

Bagamboula, C.F., M. Uyttendaele and J. Debevere, 2004. Inhibitory effects of thyme and basil essential oils, carvacrol, thyme, estragol, linalool and p-cymene towards *Shigella zonnei* and *S. flexneri*. Food Microbiol., 21: 33-42.

Bakkali, F., S. Averbeck and M. Idaomar, 2008. Biological effects of essential oils: A review. Food Chem. Toxicol., 46: 446-475.

Boyanova, L. and G. Neshev, 1999. Inhibitory effect of rose oil products on *Helicobacter pylori* growth *in vitro*: Preliminary report. J. Med. Microbial, 48: 705-706.

Burt, S.A., 2004. Essential oils: Their antibacterial properties and potential applications in foods: A review. Int. J. Food Microbiol., 94: 223-253.

Carovic-Stanko, K., S. Orlic, O. Politeo, F. Strikic, I. Kolak, M. Milos and Z. Satovic, 2009. Composition and antibacterial activities of essential oils of seven *Ocimum* taxa. Food Chem., 119: 196-201.

Cetin, H. and A. Yanikoglu, 2006. A study of the larvicidal activity of *Origanum* (Labiatae) species from southwest Turkey. J. Vector Ecol., 31: 118-122.

Gandomi, H., A. Misaghi, A. Basti, S. Bokaei, A. Khosravi, A. Abbasifar and A.J. Javan, 2009. Effect of *Zataria multiflora* Boiss. Essential oil on growth and aflatoxin formation by *Aspergillus flavus* in culture media and cheese. Food Chem. Toxicol., 47: 2397-2400.

Giordani, R., P. Regli, J. Kaloustian, C. Mikail, L. Abou and H. Portugal, 2005. Antifungal effect of various essential oils against *Candidaalbicans*. Potentiation of antifungal action of amphotericin B by essential oil from *Thymus vulgaris*. Phytotherapy Res., 18: 990-995.

Grayer, R., G. Kite, F. Goldstone, S. Bryan and E. Putievsky, 1996. Infraspecific taxonomy and essential oil chemotypes in sweet basil (*Ocimum basilicum* L.). Phytochemistry, 43: 1033-1039.

Gupta, S.C., 2006. Genetic analysis of some chemotypes in *Ocimum basilicum* var. *Glabratum*. Plant Breeding, 112: 135-140.

Gutierrez, J., C. Barry-Ryan and P. Bourke, 2008. The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. Int. J. Food Microbiol., 124: 91-97.

Hemaiswarya, S., A.K. Kruthiventi and M. Doble, 2008. Synergism between natural products and anti biotics against infectious diseases. Phytomedicine, 15: 639-652.

Hussain, A.L., F. Anwar, S.T.S. Sherazi and R. Przybylski, 2008. Chemical composition, an antioxidant and antimicrobial activity of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. Food Chem., 108: 986-995.

Juntochote, T., E. Berghofer, S. Siebenhandl and F. Bauer, 2006. The antioxidative properties of Holy basil and Galangal in cooked ground pork. Meat Sci., 72: 446-456.

Kim, J.M., M. Marshall, J.A. Cornell, J.F. Freston and C.I. Wei, 2006. Antibacterial activity of carvacrol, citral and geraniol against *Salmonella typhimurium* in culture medium and on fish cubes. J. Food Sci., 60: 1364-1368.

Kishore, G.K., S. Pande and S. Harish, 2007. Evaluation of essential oils and their components for broad-spectrum antifungal activity and control of late leaf spot and crown Rot diseases in peanut. Plant Dis., 91: 375-379.

Lin, A.L., N. Shangari, T.S. Chan, D. Remirez and P.J. Obrien, 2006. Herbal monoterpenes alcohols inhibit propotol metabolism and prolong anesthesia time. Life Sci., 79: 21-29.

Lorenzi, V., A. Muselli, A.F. Bernarolini, L. Berti, J. Pages, L. Amaral and J. Bolla, 2009. Geraniol restores antibiotic activities against multidrug-resistant isolates from Gram-negative. Antimicrobial Agents Chemother., 53: 2209-2211.

Miller, R.A., R.D. Walker, A. Baya, K. Clemens and M. Coles *et al*., 2003. Antimicrobial susceptibility testing of aquatic bacteria: quality control disk diffusion ranges for *Escherichia coli* ATCC 25922 and *Aeromonas salmonicida* subsp. *Salmonicida* ATCC 33658 at 22 and 28°C. J. Clin. Microbiol., 41: 4318-4323.

Nazer, A.I., A. Kobilinsky, J. Tholozan and F. Dubois-Brissonnet, 2005. Combination of food antimicrobials at low levels to inhibit the growth of Salmonella sv. Typhimurium: A synergistic effect. Food Microbiol., 22: 391-398.

Nour, A.H., S.A. Elhussein, N.A. Osman and A.H. Nour, 2009a. Characterization and chemical composition of the fixed oil of fourteen basil (*Ocimum basilicum* L.) accessions grown in Sudan. Int. J. Chemical Technol., 1: 52-58.

Nour, A.H., S.A.A. Elhussein, N.A. Osman and H.N. Abduelrahman, 2009b. A study of the essential oils of four sudanese accessions of basil (*Ocimum basilicum* L.) against *Anopheles* mosquito larvae. Am. J. Applied Sci., 6: 1359-1363.

Nour, A.H., S.A. Elhussein, N.A. Osman and A.H. Nour, 2009c. Repellent activities of the essential oils of four sudanese accessions of Basil (*Ocimum basilicum* L.) against *Anopheles* Mosquito. J. Applied Sci., 9: 2645-2648.

Okeke, M.I., C.U. Iroegbu, E.N. Eze, A.S. Okoli and C.O. Esimaone, 2001. Evaluation of extracts of the root of *Landolphia owerrience* for antibacterial activity. J. Ethnopharmacol., 78: 119-127.

Omidbeygi, M., M. Barzegar, Z. Hamidi and H. Nafhdibadi, 2007. Antifungal activity of thyme, summer savory and clove essential oils against aspergillus flavus in liquid medium and tomato paste. Food Control, 18: 1518-1523.

Opalchenova, G. and D. Obreshkova, 2003. Comparative studies on the activity of basil-an essential oil from *Ocimum basilicum* L. against multidrug resistant clinical isolates of the genera *Staphylococcus*, *Enterococcus* and *Pseudomonas* by using different test methods. J. Microbiol. Meth., 54: 105-110.

Oussalah, M., S. Caillet, L. Saucier and M. Lacroix, 2006. Antimicrobial effects of selected plant essential oils on the growth of a *Pseudomonas putida* strain isolated from meat. Meat Sci., 73: 236-244.

Politio, O., M. Jukic and M. Milos, 2007. Chemical compositiom and antioxidant capacity of free volatile aglycones from basil (*Ocimum basilicum* L.) compared with its essential oil. Food Chem., 101: 379-385.

Randrianarivelo, R., 2009. Composition and antimicrobial activity of essential oils of *Cinnamosma fragrans*. Food Chem., 114: 680-684.

Rosato, A., C. Vitali, N. De Laurentis, D. Armenise and M. Antonietta Millilo, 2007. Antibacterial effect of some essential oils administered alone or in combination with Norfloxacin. Phytomedicine, 14: 727-732.

Runyoro, D., O. Ngassapa, K. Vagionas, N. Aligiannis, K. Graikou and I. Chinou, 2009. Chemical composition and antimicrobial activity of the essential oils of four Ocimum species growing in Tanzania. Food Chem., 119: 311-316.

Santoro, G.F., M.G. Gardoso, L.G.L. Guimaraes, L.Z. Mendonca and M.J. Soares, 2007. *Trypanosoma cruzi*: Activity of essential oils from *Achillea millefoium* L., *Syzgium aromaticum* L. and *Ocimum basilicum* L. on epimastigotes and trypomastigotes. Exp. Parasitol., 116: 283-290.

Shan, B., Y.Z. Cai, J.D. Brooks and H. Corke, 2007. The in vitro antibacterial activity of dietary spices and medicinal herbs extracts. Int. J. Microbiol., 117: 112-19.

Sinico, C., A. DeLogu, F. Lai, D. Valenti and M. Manconi *et al*., 2005. Liposomal incorporation of *Artemisia arborescens* L. essential oil and *in vitro* antiviral activity. Eur. J. Pharmaceutics Biopharmaceutics, 59: 161-168.

Skandamis, P.N. and G.J.E. Nychas, 2001. Effect of oregano essential oil on microbiological and physico-chemical attributes of minced meat stored in air and modified atmospheres. J. Applied Microbiol., 91: 1011-1022.

Tzortzakis, N.G. and C.D. Economakis, 2007. Antifungal activity of lemongrass (*Cymbopogon citrates* L.) essential oil against key postharvest pathogens. Innovative Food Sci. Emerg. Technol., 8: 253-258.