Biological Activities of Essential Oils and Methanol Extracts of Five *Ocimum* Species against Pathogenic Bacteria

Soumen SAHA[1,2], Tarak Nath DHAR[2], Chandan SENGUPTA[2] and Parthadeb GHOSH[1]

[1]Department of Botany, Cytogenetics and Plant Biotechnology Research Unit, University of Kalyani, Nadia, West Bengal, India, [2]Department of Botany, Microbiology Research Unit, University of Kalyani, Nadia, West Bengal, India

Abstract

Saha S., Dhar T.N., Sengupta C., Ghosh P.D. (2013): **Biological activities of essential oils and methanol extracts of five *Ocimum* species against pathogenic bacteria**. Czech J. Food Sci., **31**: 194–202.

The essential oils and methanol extracts of *Ocimum basilicum* L., *Ocimum kilimandscharicum* Guerke, *Ocimum gratissimum* L, *Ocimum canum* Sims, and *Ocimum tenuiflorum* L. (green type) were examined for their potential antibacterial activities. The chemical composition of essential oils of *Ocimum* species was analysed by GC-MS. The inhibitory effects of essential oils and methanol extracts were studied on two Gram-positive (*Bacillus subtilis, Micrococcus luteus*) and five Gram-negative (*Pseudomonas aeruginosa, Shigella dysenteriae, Escherichia coli, Vibrio cholera,* and *Shigella flexneri*) bacteria using disc-diffusion method. Minimum inhibitory concentration (MIC) was assessed by micro broth dilution method. The antibacterial test results showed that the essential oils of *Ocimum basilicum* L., *Ocimum kilimandscharicum* Guerke, and *Ocimum gratissimum* L. strongly inhibited the growth of all of the microorganisms studied, especially of the Gram-negative strains, whereas other two essential oils showed moderate activities. The result may suggest that the essential oils of *Ocimum* possess compounds with antibacterial activities, and therefore could be used as natural preservative ingredients in food and/or pharmaceutical industries.

Keywords: antibacterial activity; essential oil; GC-MS; *Ocimum* species

Medicinal and aromatic plants (MAPs) have been used for centuries as remedies for human diseases because they contain components of therapeutic value. It has been estimated by WHO that 80% of the population, the majority of this in the developing countries, still rely on plant-based medicine for primary health care needs (WHO 1993). For this purpose, various strategies have been developed, e.g. biological screening, isolation, as well as clinical trials for a variety of plants.

Following the advent of modern medicine, herbal medicine suffered a setback, but during the last two or three decades the advances in phytochemistry and in the identification of the plant compounds, providing effective against certain chronic diseases and emergence of multidrug resistant bacteria.

This awakening has led to a sudden demand for herbal medicine. Worldwide as well as in the developing countries, the most humans died due to infectious bacterial diseases (Nathan 2004). The bacterial organisms including both Gram positive and Gram negative ones are the main cause of severe infections in humans, because they have the ability to survive in harsh conditions due to their multiple environmental habitats (Ahameethunisa & Hopper 2010). Nowadays, multiple drug resistance is developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases (Gupta *et al*. 2008). In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-

Support by the University Grant Commission (UGC), New Delhi, Govt. of India, Project No. F14-2(SC)/2007/(SA-III).

suppression, and allergic reactions (Werner *et al*. 1999). On the other hand, environmental safety is a major concern in many countries, and the application of synthetic agrochemicals runs the risk of causing unacceptable environmental damage, such as health hazards to humans, toxicity to useful non-targeted animals and environmental pollution. The use of natural products would be a helpful way to reduce this risk, and also the situation requires searching for new antimicrobial substances. The screening of the active compounds from plants has lead to the discovery of new medicinal drugs which show efficient protection and treatment roles against various diseases, including cancer (Kumar *et al*. 2004; Sheeja & Kuttam 2007). Therefore, there is a need to develop alternative antimicrobial drugs from medicinal plants for the treatment of infectious diseases.

The genus *Ocimum* involves economically the most important medicinal and aromatic herbs, undershrubs or shrubs in the world. It belongs to the family Lamiaceae, subfamily Ocimoideae, and comprises more than 30 species distributed in tropical and subtropical regions of Asia, Africa, and Central and South America (Paton 1992). Traditionally, the genus *Ocimum* is widely used for the treatment of various ailments including rheumatism, paralysis, epilepsy, high fever, diarrhea, sunstroke, influenza, gonorrhea, mental illness, abdominal pains, colds, coughs, measles, and has also antipyretic, antihelmentic, stomatic, anti-emetic, and antimalarial effects (Caceres *et al*. 1990; Obeng-Ofori *et al*. 1998; Nyarko *et al*. 2002; Ezekwesili *et al*. 2004). It is also a source of aroma compounds and essential oils containing biologically active constituents that possess insecticidal (Deshpande *et al*. 1997), nematicidal (Chaterjee *et al*. 1982), and fungistatic properties (Reuveni *et al*. 1984). The active compounds present as volatile oil from the leaves consist mainly of eugenol, thymol, citrol, geraniol, camphor, linalool l, and methyl cinnamate (Charles & Simon 1992; Jirovetz & Buchbauer 2001; Mondello *et al*. 2002; Vina & Murillo 2003; Padalia & Verna 2011; Singh *et al*. 2011; Verma *et al*. 2011). The seeds contain oil composed of fatty acids and sitosterol. The roots contain sitosterol and three triterpenes A, B, and C. Additionally, they also contain rosmarinic acid, thymol, methyl chavicol and, citral etc. (Dhar *et al*. 1968), and vitamins C, A, and minerals like calcium, zinc, and iron (Anbarasu & Vijayalakshmi 2007), as well as chlorophyll and many other phytonutrients. Recent interest in *Ocimum* has resulted from its inhibitory activity against HIV-I reverse transcriptase and platelets aggregation induced by collagen and ADP (adenosine 5'-disphosphate) (Okazaki *et al*. 1998; Yamasaki *et al*. 1998). However, the antimicrobial activity of *Ocimum* essential oil against microorganisms has been investigated by some researchers (Prasad *et al*. 1986; Nakamura *et al*. 1999; Adebolu & Oladimeji 2005; Adigüzel *et al*. 2005; Moghaddam *et al*. 2011; Verma *et al*. 2011) using different techniques and their investigations mostly covered one individual or two species. Unfortunately, the published data on the former subject are difficult to compare, because the chemical composition of essential oils is known to vary with the local climate, harvest period, and environmental conditions (Vieira & Simon 2000), and is also dependent on the type of solvent used in the extraction procedure (Cu *et al*. 1989). Therefore, the present study is aimed at evaluating the *in vitro* antibacterial activity of essential oils and methanol extracts of five species of *Ocimum*, namely *Ocimum basilicum* L., *Ocimum kilimandscharicum* Guerke, *Ocimum gratissimum* L., *Ocimum canum* Sims., and *Ocimum tenuiflorum* L. (green type) against the selected microorganisms.

MATERIAL AND METHODS:

Plant material collection. Fresh leaves of *Ocimum basilicum* L., *Ocimum kilimandscharicum* Guerke, *Ocimum gratissimum* L., *Ocimum canum* Sims., and *Ocimum tenuiflorum* L. (green type) were collected from medicinal and aromatic plant garden, Department of Botany, University of Kalyani, Kalyani, West Bengal, India, in August of 2011 (Table 1), which is located at 22°57'N latitude, 88°22'E longitude with an average altitude of 9.75 m a.s.l. The taxonomic identification of the plant material was confirmed by Dr. G. G. Maity, Taxonomy and Plant systematic Unit, Department of Botany, University of Kalyani. The voucher specimens (143, 148, 149, 150, and 163 KUH, respectively) were deposited and preserved at the Department of Botany, University of Kalyani, Kalyani, and West Bengal, India, for reference.

Preparation of methanolic plant extracts. Each collected plant material was dried in the shade and ground in a grinder with a 2 mm diameter mesh. The dried and powdered leaves (100 g) were succes-

Table 1. Plants and plant parts used

No.	Scientific name	Common name	Family	Parts used
1.	*Ocimum basilicum* L.	sweet basil		
2.	*Ocimum kilimandscharicum* Guerke.	camphor basil		
3.	*Ocimum gratissimum* L.	shrubby basil	Lamiaceae	dried leaves
4.	*Ocimum canum* Sims.	hoary basil		
5.	*Ocimum tenuiflorum* L. (green type)	holy basil		

sively extracted with 500 ml of methanol (1:5 w/v) using a Soxhlet extractor for 72 h at a temperature not exceeding the boiling point of the solvent (Lin *et al*. 1999). The extracts were filtered using Whatman filter paper (No. 1) and then concentrated in vacuo at 40°C using a rotary evaporator (Buchi Rotavapor R-200; Buchi Labortechnik AG, Flawil, Switzerland). The extracts were then lyophilised and kept in the dark at +4°C until tested.

Extraction of the essential oils. The air-dried and ground aerial parts of the plants collected were submitted to water distillation for 2.5 h using a Clevenger-type apparatus (Clevenger *et al*. 1928)). The essential oils obtained were dried over anhydrous sodium sulphate and, after filtration, stored at +4°C until tested and analysed.

GC-MS and GC-FID analysis. GC-MS analyses of the essential oils were performed on a Hewlett-Packard gas chromatograph, model 6890, equipped with a FID, using HP-5MS capillary column (30 m length × 0.25 mm *i.d.* × 0.25 µm film thickness) (Agilent Technologies, Santa Clara, USA). The injector was set at 250°C and the detector at 280°C, respectively. The oven temperature of the chromatogram was raised from 60°C to 280°C, respectively, at the heating rate of 10°C/min, and was then isothermally held for 5 minutes. The injector volume was 1.0 µl. The solvent delay was 2 min and it was injected in a split ratio of 1:10. Helium was used as the carrier gas at 15 P.S.I. inlet pressure. The essential oil constituents were identified by the comparison of their GC retention indices (RI) and mass spectra with those of the authentic standard compounds. Quantification of the relative amounts of the individual components was performed according to the area percentage method (Telci *et al*. 2006) for each plant.

Antibacterial activity

Test microorganism. The methanol extracts and essential oils were individually tested against a panel of microorganisms including Gram-positive *Bacillus subtilis* (MTCC 441), *Micrococcus luteus* (MTCC 2522), Gram-negative *Pseudomonas aeruginosa* (MTCC 741), *Shigella dysenteriae* (Clinical isolate), *Escherichia coli* (MTCC 443), *Vibrio cholera* (MTCC 3904), and *Shigella flexneri* (MTCC 1457). All the bacterial strains except *Shigella dysenteriae* were obtained from the Institute of Microbial Technology, Chandigarh, India. The reference strains of bacteria were maintained on nutrient agar (HiMedia, Mumbai, India) slants at 4°C with a subculture period of 30 days.

Preparation of McFarland standard. The turbidity standard was prepared by mixing 0.5 ml of 1.75% (w/v) $BaCl_2 \cdot 2H_2O$ with 99.5 ml of 1% $H_2SO_4 \cdot BaSO_4$ (v/v). The standard was put into screw cap test tube to compare the turbidity. The bacterial cultures of the selected strains were grown overnight and were subsequently mixed with physiological saline. Turbidity was corrected by adding sterile saline until McFarland 0.5 $BaSO_4$ turbidity standard 10^8 Colony Forming Unit (CFU) per ml was achieved. These inocula were used for seeding of the nutrient agar.

Disc-diffusion assay. The essential oils and methanol extracts were dissolved in DMSO (dimethylsulfoxide) to a final concentration of 30 mg/ml and sterilised through filtration using 0.45 µm membrane filters. The antibacterial tests were then carried out by disc diffusion method (Murray *et al*. 1995) using 100 µl of the suspension containing 10^8 CFU/ml of bacteria on nutrient agar. The discs (6 mm in diameter) were impregnated with 10 µl of essential oil or 30 mg/ml extracts (300 µg/disc) placed on the inoculated agar. Negative controls were prepared using DMSO. Gentamicin (10 µg per disc) was used as positive reference standard to determine the sensitivity of each bacterial species tested. The inoculated plates were incubated at 37°C for 24 hours. The antibacterial activity was evaluated by measuring the zone of inhibition, the diameters of these zones being measured in millimeters against the test organisms.

Determination of minimum inhibitory concentration. The minimal inhibitory concentration (MIC) values were followed with the bacteria strains sensitive to the essential oils and/or extracts in the disc diffusion assay. The inocula of the bacterial strains were prepared from 12 h broth cultures and the suspensions were adjusted to 0.5 McFarland standard turbidity. The essential oils and extracts of *Ocimum* ssp. dissolved in 10% DMSO, were first diluted to the highest concentration (500 µg/ml) to be tested, and then serial twofold dilutions were made in order to obtain a concentration range from 7.8 to 500 µg/ml in 10 ml sterile test tubes containing the nutrient broth. MIC values of the extracts against bacterial strains were determined based on the micro well dilution method as previously described (Sokmen *et al*. 2004). The 96-well plates were prepared by dispensing into each well 95 µl of the nutrient broth and 5 µl of the inoculum. A volume of 100 µl from the stock solutions of *Ocimum* spp. essential oils and extracts initially prepared at the concentration of 500 µg/ml was added into the first wells. Then, 100 µl from their serial dilutions was transferred into six consecutive wells. The last well containing 195 µl of the nutrient broth without the compound and 5 µl of the inoculum on each strip was used as negative control. The final volume in each well was 200 µl. The plate was covered with a sterile plate sealer and then incubated at the appropriate temperature 37°C for 24 hours. The bacterial growth was determined by absorbance measured at 600 nm using the ELx 800 universal microplate reader (Biotek Instrument Inc., Winooski, USA) and confirmed by plating 5 µl samples from clear wells on nutrient agar medium. The MIC was defined as the lowest concentration of the compound to inhibit the growth of microorganisms. Each test in this study was repeated at least twice.

RESULTS AND DISCUSSION

Essential oil composition

The percent chemical composition of the essential oils of five species of *Ocimum,* namely *O. basilicum*, *O. kilimandscharicum*, *O. gratissimum*. *O. canum*, and *O. tenuiflorum* (green type) is given in Table 2 in the order of the retention times of the constituents. The yields of the essential oils obtained from dry leaves were 1.77, 1.92, 1.63, 1.81, and 1.73% (v/w), respectively. The main constituents of *O. basilicum* were geraniol (34.89%), citral (23.51%), linalool (2.21%), and eugenol (1.33%); *O. kilimandscharicum* – camphor (21.65%), eugenol (9.65%), cineole (2.07%), and citral (1.23%); *O. gratissimum* – eugenol (47.45%), citronellal (3.56%), cineole (1.97%), geraniol (1.52%), and vanillin (1.52%); *O. canum* – camphor (3.47%), cineole (1.44%), vanillin (1.03%), and *O. tenuiflorum* (green type) – eugenol (8.81), citronellal (1.44%), and vanillin (1.16%), respectively. The essential oil composition of *O. basilicum*, *O. gratissimum*, and

Table 2. Percentage composition of the essential oils of five species of *Ocimum* cultivated in West Bengal

Compound	Retention indices[a] (RI) (min)	Essential oil (%)[b]				
		O. basilicum L.	*O. kilimandscharicum* Guerke	*O. gratissimum* L.	*O. canum* Sims.	*O. tenuiflorum* L. (green type)
α-Pinene	4.76	0.23	0.98	0.12	–	0.17
Camphor	4.87	0.64	21.65	0.15	3.47	0.05
Citral	5.26	23.51	1.23	0.52	0.33	0.22
Geraniol	5.76	34.89	0.49	1.52	0.14	0.83
Cineole	5.83	0.05	2.07	1.97	1.44	–
β-Pinene	5.92	0.19	1.02	0.18	0.13	0.09
Citronellal	5.96	0.59	0.51	3.56	0.79	1.44
Eugenol	6.57	1.33	9.65	47.45	0.32	8.81
Vanillin	6.72	0.27	0.30	1.52	1.03	1.16
Linalool	7.41	2.21	0.23	0.12	–	0.02

[a]identification of oil components was based on their relative retention indices (retention times) with those of authentic standards; [b]quantitative estimation was done by analysis of FID area percent data

Table 3. Antibacterial activity of the essential oil and methanol extract of *Ocimum* ssp. against the bacterial strains tested based on disc diffusion method

Microorganism	Inhibition zone in diameter (mm) around the discs impregnated with 10 µl of essential oils and extracts (300 µg/disc)										
	essential oil (10 µl/disc)[a]					MeOH extracts[b]					gentamicin (10 µg/disc)
	E1	E2	E3	E4	E5	M1	M2	M3	M4	M5	
B. subtilis (MTCC 441)	22	24	18	15	22	8	8	7	[c]–	–	26
M. luteus (MTCC 1541)	20	16	16	14	18	–	–	–	–	–	26
P. aeruginosa (MTCC 741)	23	21	22	15	14	–	–	–	–	–	13
S. dysenteriae (clinical isolate)	16	16	15	18	–	–	–	–	–	–	18
E. coli (MTCC 443)	16	17	21	–	9	10	8	8	–	–	30
V. cholera (MTCC 3904)	22	16	21	18	18	10	7	–	–	–	17
S. flexneri (MTCC 1457)	18	18	15	–	21	–	–	–	–	–	27

E1–E5 – essential oils: EI – *Ocimum basilicum* L.; E2 – *Ocimum kilimandscharicum* Guerke; E3 – *Ocimum gratissimum* L.; E4 – *Ocimum canum* Sims; E5 – *Ocimum tenuiflorum* L. (green type); M1–M5 – methanol extracts: M1– *Ocimum basilicum* L.; M2 – *Ocimum kilimandscharicum* Guerke; M3 – *Ocimum gratissimum* L.; M4 – *Ocimum canum* Sims; M5 – *Ocimum tenuiflorum* L. (green type); – not active; [a]inhibition zone in diameter (mm) around the discs impregnated with 10 µl of essential oil; [b]inhibition zone in diameter (mm) around the discs impregnated with extracts (300 µg/disc)

O. kilimandscharicum obtained from plants grown in northern India, was found to be rich in *O. basilicum* methyl chavicol and linalool (21.9%); *O. gratissimum* eugenol (72.2%), 1.8-cineole (7.6%), germacrene D (2.7%) and β-caryophyllene (1.7%); and *O. kilimandscharicum* camphor (64.9%), limonene (8.7%), in accordance with previous report (Padalia & Verma 2011). Similar results were found with *O. gratissimum* and *O. kilimandscharicum* in sub tropical India (Verma *et al.* 2011). In the oils obtained from the aerial part of *O. basilicum* grown in Colombia and Bulgaria, linalool and methyl cinnamate were reported as the major components of volatile oils, respectively (Jirovetz & Buchbauer 2001; Vina & Murillo 2003). It is interesting that the oils extracted from *Ocimum basilicum* L. collected from Bangladesh contain linalool and geraniol as their main constituents (Mondello *et al.* 2002), and the authors concluded that the oil composition could be dependent on the climatic conditions. The chemical composition of essential oils of *Ocimum* species shows a large interspecies variability and, within the same species, it seems to depend on the genetic characteristics of the plant and on the conditions under which it has grown. In the present study, our findings on the major components of *O. kilimandscharicum* and

Table 4. The MIC values of essential oils of the five species of *Ocimum* against the bacterial strains tested in microdilution assay (µg/ml)

Microorganism	Essential oil				
	O. basilicum	*O. kilimandscharicum*	*O. gratissimum*	*O. canum*	*O. tenuiflorum* (green type)
B. subtilis (MTCC 441)	15.62	15.62	62.50	62.50	62.50
M. luteus (MTCC 1541)	31.25	62.50	125	125	125
P. aeruginosa (MTCC 741)	62.50	62.50	125	250	250
S. dysenteriae (clinical isolate)	125	250	250	125	nt
E. coli (MTCC 443)	15.62	31.50	31.50	nt	500
V. cholera (MTCC 3904)	31.25	125	125	125	250
S. flexneri (MTCC 1457)	15.62	62.50	125	nt	62.50

nt – not tested

O. gratissimum oils were in agreement with the previous report (Verma *et al.* 2011). According to the literature, the major compounds concerned of *O. basilicum*, *O. canum*, and *O. tenuiflorum* (green type) are different. The observed differences may be due to the different environmental and genetic factors, different chemotypes, and the nutritional status of the plants as well as other factors that can influence the oil compositions.

Antibacterial activity (Disc diffusion test)

The *in vitro* antibacterial activities of the five species of *Ocimum* essential oils and methanol extracts against the microorganism employed and their potentials were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and the zones diameters, MIC values being showed in Tables 3 and 4. According to the result given in Table 3, the essential oils of *O. basilicum*, *O. kilimandscharicum*, and *O. gratissimum* had a great potential of antibacterial activities (inhibition diameters ranged from 15–24 mm) against all 7 bacteria tested, whereas those of *O. canum* and *O. tenuiflorum* had substantial activities (inhibition diameters ranged from 9 mm to 22 mm) against 5 and 6 bacteria tested. On the other hand, the fractions of the methanol extracts of *O. basilicum*, *O. kilimandscharicum*, and *O. gratissimum* were also found to be effective against *B. subtilis, E. coli,* and *V. cholera* out of 7 bacterial species examined (inhibition diameters ranged from 7–10 mm), respectively, probably due to the presence of similar compounds in these methanol fractions, whereas those of *Ocimum canum* Sims and *Ocimum tenuiflorum* L. plants showed no antibacterial activities (Table 3). This disagreement can be explained by that the better extraction of antimicrobial compounds from various medicinal plants may require different solvents. When compared to the methanol extracts, the essential oils exhibited a stronger and broader activity as compared to the methanol extract tested. Based on these results of chemical composition of the essential oils, it is possible to conclude that the antibacterial nature of the essential oils studied is apparently related to their high phenolic contents, particularly oxygenated terpenoids and phenolic terpenes, and this finding is in agreement with previous reports (Burt 2004; Gallucci *et al.* 2009; Bassolé & Juliani 2012). This claim is further supported by our findings indicating high contents of terpenoids such as citral, geraniol, eugenol, and camphor in the oils (Table 2). The findings in this study support the observations of some other researchers about *Ocimum* species containing some substances with antibacterial properties (Prasad *et al.* 1986; Nakamura *et al.* 1999; Adebolu & Oladimeji 2005; Adigüzel *et al.* 2005; Moghaddam *et al.* 2011; Verma *et al.* 2011). However, it is difficult to compare the data with the literature because several variables influence the results, such as the environmental and climatic conditions of the plant and the choice of the extraction method and antimicrobial test. Moreover, standard criteria for the evaluation of the plant activity are missing and therefore the results obtained by different authors are widely different (Recio *et al.* 1989; Vanden Burghe *et al.* 1991). This is the first study to provide data about the extracts and essential oils of the five species of *Ocimum* plants possessing potential antibacterial activities as evaluated against seven microorganisms. The results indicate that the essential oils of *Ocimum* species can be used as a natural source that may lead to their use as safe alternatives to synthetic antimicrobial drugs. In addition, the data in the present study support the use of *Ocimum* species as additives in foods, and as traditional remedies for the treatment of infectious diseases.

Minimum inhibitory concentration

All the essential oils tested were subjected to MIC studies against all the microorganisms. The results in Table 4 interpreted as the lowest concentrations that inhibit the visible microbial growth. The maximal inhibition zones and MIC values for bacterial strains, which were sensitive to the essential oils of the five species of *Ocimum*, were in the range of 9–24 mm and 15.62–500 μg/ml (Table 4). Based on these results, it is possible to conclude that the essential oils have a stronger and broader spectrum of antimicrobial activity as compared to the methanol extracts tested. This observation confirmed the evidence given in a previous study reporting that the essential oils from medicinal plants contain more antimicrobial substances than other extracts such as water, methanol, ethanol, and hexane exstracts (Ahmad *et al.*1998; Eloff 1998). Our results also indicated in the present study that

the *O. basilicum*, *O. kilimandscharicum*, and *O. gratissimum* essential oils were more active against all pathogenic bacteria including Gram-negatives ones than the other two essential oils, probably owing to the high levels of phenolic compounds in the former ones (Table 2).

According to a number of studies, the Gram-positive bacteria are more sensitive to essential oil than Gram-negative bacteria. A possible explanation may reside in the possession of an outer membrane, surrounding the cell wall of Gram-negative bacteria, thus it is logical to expect that these bacteria wll be less susceptible to the antibacterial activity of essential oil. This outer membrane may restrict the diffusion of hydrophobic compounds through its lipopolysaccharide covering, presenting a barrier to the penetration of numerous antibiotic molecule, and is also associated with the enzymes in the periplasmic space, which are capable of breaking down the molecules introduced from the outside (Nikaido 1994; Gao *et al.* 1999). Gram-positive bacteria do not have such an outer membrane and cell wall structure. The antibacterial substances can easily destroy their bacterial cell wall and cytoplasmic membrane and cause leakage of the cytoplasm and its coagulation (Kalemba & Kunicka 2003). However, the current findings show a remarkable activity against all gram-negative bacteria. The antibacterial activities of *O. basilicum*, *O. kilimandscharicum*, and *O. gratissimum* leaves extracts essential oils may be due to high contents of tannins and phenolic constituents. The most active constituents (essential oils) rich in phenolic compounds are widely reported to possess high levels of antimicrobial activity (Prasad *et al*. 1986; Nakamura *et al.* 1999; Dorman & Deans 2000), which has been confirmed and extended in the present study, although antimicrobial activities of phenolic compounds may involve multiple modes of action. The mode of action of antimicrobial agents also depends on the type of microorganisms and is mainly related to their cell wall structure and the outer membrane arrangement. Medicinal plants contain complex phenolics and the mechanism of action of each phenolic compound against various bacteria is also very complicated (Burt 2004). Therefore, it is necessary to investigate further and understand the relationship between the antibacterial activity and chemical structure of each phenolic compound in the extracts tested.

CONCLUSION

The growing tendency for replacing synthetic additives with natural ones has brought about great interest in the evaluation of antimicrobial properties of the plants products in both academical and industrial fields because of their relatively safe status, wide acceptance by consumers, and their exploitation for potential multipurpose functional use. To the best of our knowledge, this is the first report on the *in vitro* comparative evaluation of the antibacterial activities of the essential oils and methanol extracts of five species of *Ocimum*. The essential oils of *Ocimum* species proved to possess interesting properties, emerging from both their chemical composition and from the evaluation of their *in vitro* biological activities. However, it is very difficult to attribute the biological effect of a total essential oil to one or a few active principles, because in addition to the major compounds, also minor compounds may make a significant contribution to the oil activity. From the results given above, we could infer that *Ocimum* essential oils, indicating strong antibacterial activities, are very important botanical dietary supplements that can be freely used in the food industry as culinary herbs.

Acknowledgement. The authors are grateful to DST-FIST programme, Govt. of India, Department of Botany, University of Kalyani for Instrumental facilities.

References

Adebolu T.T., Oladimeji S.A. (2005): Antimicrobial activity of leaf extracts of *Ocimum gratissimum* on selected diarrhoea causing bacteria in southwestern Nigeria. African Journal of Biotechnology, **4**: 682–684.

Adigüzel A., Güllüce M., Sengül M., Öğütcü H., Sahün F., Karaman U. (2005): Antimicrobial effects of *Ocimum basilicum* (Labiatae) extract. Turkish Journal of Biology, **29**: 155–160.

Ahameethunisa A.R., Hopper W. (2010): Antibacterial activity of *Artemisia nilagirica* leaf extracts against clinical and phytopathogenic bacteria. BMC Complementary and Alternative Medicine, **10**: 6.

Ahmad I., Mehmood Z., Mohammad F. (1998): Screening of some Indian medicinal plants for their antimicrobial properties. Journal of Ethnopharmacology, **62**: 183–193.

Anbarasu K., Vijayalakshmi G. (2007): Improved shelf life of protein-rich tofu using *Ocimum sanctum* (tulsi)

extracts to benefit Indian rural population. Journal of Food Science, **72**: M300-05.

Bassolé I.H.N., Juliani H.R. (2012): Essential oils in combination and their antimicrobial properties. Molecules, **17**: 3989–4006.

Burt S. (2004): Essential oils: their antibacterial properties and potential applications in foods – a review. International Journal of Food Microbiology, **94**: 223–253.

Caceres A., Cano O., Samayoa B., Aguilar L. (1990): Plants used in Guatemala for the treatment of gastrointestinal disorders. 1. Screening of 84 plants against enterobacteria. Journal of Ethnopharmacology, **30**: 55–73.

Charles D.J., Simon J.E. (1992): Essential oil constituents of *Ocimum kilimandscharicum* Guerke. Journal of Essential Oil Research, **4**: 125–128.

Chaterjee A., Sukul N.C., Laskal S., Ghoshmajumdar S. (1982): Nematicidal principles from two species of Lamiaceae. Journal of Nematology, **14**: 118–120.

Clevenger J.E. (1928): Apparatus for determination of volatile oil. Journal of the American Pharmaceutical Association, **17**: 346–349.

Cu J.Q., Perineau F., Deltnas M., Gaset A. (1989): Comparison of the chemical composition of carrot seed essential oil extracted by different solvents. Flavour and Fragrance Journal, **4**: 225–231.

Deshpande R.S., Tipnis H.P. (1997): Insecticidal activity of *Ocimum basilicum* L. Pesticides, **12**: 21–28.

Dhar M.L., Dhar M.M., Dhawan B.N., Mehrotra B.N., Roy C. (1968): Screening of Indian plants for biological activity, Part I. Indian Journal of Experimental Biology, **62**: 32–247.

Dorman H.J.D., Deans S.G. (2000): Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology, **88**: 308–316.

Eloff J.N. (1998): Which extract should be used for the screening and isolation of antimicrobial components from plants? Journal of Ethnopharmacology, **60**: 1–8.

Ezekwesili C.N., Obiora K.A., Ugwu O.P. (2004): Evaluation of anti-diarrhoeal property of crude aqueous extract of *Ocimum gratissimum* L. (Labiatae) in rats. Biokemistri, **16**: 122–131.

Gallucci M.N., Oliva M., Casero C., Dambolena J., Luna A., Zygadlo J., Demo M. (2009): Antimicrobial combined action of terpenes against the food-borne microorganisms *Escherichia coli, Staphylococcus aureus* and *Bacillus cereus.* Flavour and Fragrance Journal, **24**: 348–354.

Gao Y., Van Belkum M.J., Stiles M.E. (1999): The outer membrane of Gram negative bacteria inhibits antibacterial activity of brochocin-C. Applied and Environmental Microbiology, **65**: 4329–4333.

Gupta C., Garg P.A., 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.

Jirovetz L., Buchbauer G. (2001): Analysis, chemotype and quality control of the essential oil of new cultivated basil (*Ocimum basilicum* L.) plant from Bulgaria. Scientia Pharmaceutica, **69**: 85–89.

Kalemba D., Kunicka A. (2003): Antibacterial and antifungal properties of essential oils. Current Medicinal Chemistry, **10**: 813–829.

Kumar R.A., Sridevi K., Kumar N.V., Nanduri S., Rajagopal S. (2004): Anticancer and immunostimulatory compounds from *Andrographis paniculata*. Journal of Ethnopharmacology, **92**: 291–295.

Lin J., Opoku A.R., Geheeb-Keller M., Hutchings A.D., Terblanche S.E., Jager A.K., Van Staden J. (1999): Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and anti-microbial activities. Journal of Ethnopharmacology, **68**: 267–274.

Moghaddam A.M.D., Shayegh J., Mikaili P., Sharaf J.D. (2011): Antimicrobial activity of essential oil extract of *Ocimum basilicum* L. leaves on a variety of pathogenic bacteria. Journal of Medicinal Plants Research, **5**: 3453–3456.

Mondello L., Zappia G., Cotroneo A., Bonaccorsi I., Chowdhury J.U., Usuf M. (2002): Studies on the chemical oil-bearing plants of Bangladesh. Part VIII. Composition of some *Ocimum* oils *O. basilicum* L. var. *purpurascens*; *O. sanctum* L. green; *O. sanctum* L. purple; *O. americanum* L., citral type; *O. americanum* L., camphor type. Flavour and Fragrance Journal, **17**: 335–340.

Murray P.R., Baron E.J., Pfaller M.A., Tenover F.C., Yolke R.H. (1995): Manual of Clinical Microbiology. 6th Ed. ASM, Washington.

Nakamura C.V., Nakamura T.U., Bando E., Melo A.F.N., Cortez D.A.G., Filho B.P.D. (1999): Antibacterial activity of *Ocimum gratissimum* L. essential oil. Memórias do Instituto Oswaldo Cruz, **94**: 675–678.

Nathan C. (2004): Antibiotics at the crossroads. Nature, **431**: 899–902.

Nikaido H. (1994): Prevention of drug access to bacterial targets permeability barriers and active efflux. Science, **264**: 382–388.

Nyarko A.K., Asare-Anane H., Ofosuhene M., Addy M.E. (2002): Extract of *Ocimum canum* lowers blood glucose and facilitates insulin release by isolated pancreatic beta-islet cells. Phytomedicine, **9**: 346–351.

Obeng-Ofori D., Reichmuth C.H., Bekele A.J., Hassanali A. (1998): Toxicity and protectant potential of camphor, a major component of essential oil of *Ocimum kilimandscharicum*, against four stored product beetles. International Journal of Pest Management, **44**: 203–209.

Okazaki K., Nakayama S., Kawazoe K., Takaishi Y. (1998): Antiaggregant effects on human platelets of culinary herbs. Phytotherapy Research, **12**: 603–605.

Padalia R.C., Verma R.S. (2011): Comparative volatile oil composition of four *Ocimum* species from northern India. Natural Product Letters, **25**: 569–575.

Paton A. (1992): A synopsis of *Ocimum* L. (Labiatae) in Africa. Kew Bulletin, **47**: 405–437.

Prasad G., Kumar A., Singh A.K., Bhattacharya A.K., Singh K., Sharma V.D. (1986): Antimicrobial activity of essential oils of some *Ocimum* species and clove oil. Fitoterapia, **57**: 429–432.

Recio M.C., Rios J.L., Villar A. (1989): Antimicrobial activity of selected plants employed in the Spanish Mediterranean area. Part II. Phytotherapy Research, **3**: 77–80.

Reuveni R., Flelsher A., Putievsky E. (1984): Fungistatic activity of essential oils from *Ocimum basilicum* chemotypes. Journal of Phytopathology, **110**: 20–22.

Sheeja K., Kuttan G. (2007): Activation of cytotoxic T lymphocyte responses and attenuation of tumor growth *in vivo* by *Andrographis paniculata* extract and andrographolide. Immunopharmacology and Immunotoxicology, **29**: 81–93.

Singh S.K., Anand A., Verma S.K., Siddiqui M.A., Mathur A., Saklani S. (2011): Analysis of phytochemical and antioxidant potential of *Ocimum kilimandscharicum* Linn. International Current Pharmaceutical Research Journal, **3**: 40-46.

Sokmen A., Gulluce M., Akpulat H.A., Daferera D., Tepe B., Polissiou M., Sokmen M., Sahin F. (2004): The *in vitro* antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic *Thymus spathulifolius*. Food Control, **15**: 627–634.

Telci I., Bayram E., Yilmaz G., Avci B. (2006): Variability in essential oil composition of Turkish basils (*Ocimum basilicum* L.). Biochemical Systematic and Ecology, **34**: 489-497.

Vanden Berghe D.A., Vlietinick A.J. (1991): Screening methods for antibacterial and antiviral agents from higher plants. In: Dey P.M., Harborne J.B. (eds): Methods in Plant Biochemistry. Academic Press, London: 47–67.

Verma R.S., Bisht P.S., Padalia R.C., Saikai D., Chauhan A. (2011): Chemical composition and antibacterial activity of essential oil from two *Ocimum* spp. grown in sub-tropical India during spring-summer cropping season. Journal of Traditional Medicines, **6**: 211–217.

Vieira R.F., Simon J.E. (2000): Chemical characterization of basil (*Ocimum* spp.) found in the markets and used in traditional medicine in Brazil. Economic Botany, **54**: 207–216.

Vina A., Murillo E. (2003): Essential oil composition from twelve varieties of basil (*Ocimum* spp) grown in Colombia. Journal of Brazilian Chemical Society, **14**: 744–749.

Werner F., Okemo P., Ansorg R. (1999): Antibacterial activity of East African Medicinal plants. Journal of Ethnopharmacology, **60**: 79–84.

WHO (1993): Summary of WHO guidelines for the assessment of herbal medicines. Herbal Gram, **28**: 13–14.

Yamasaki K., Nakano M., Kawahata T., Mori H., Otake T., Ueba N., Oishi I., Inami R., Yamane M., Nakamura M., Murata H., Nakanishi T. (1998): Anti-HIV-1 activity of herbs in Labiatae. Biological and Pharmaceutical Bulletin, **21**: 829–833.

Received for publication June 11, 2012
Accepted after corrections August 22, 2012

Corresponding author

Prof. Parthadeb Ghosh, Ph.D., University of Kalyani, Cytogenetics and Plant Biotechnology Research Unit, Department of Botany, 741235, Nadia, West Bengal, India; E-mail: pdgbot@yahoo.co.in