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In vitro antibacterial, antioxidant activity and total phenolic content of some essential oils

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Abstract

In vitro antibacterial activity of 16 essential oils was investigated by disc diffusion method against two Gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* and two Gram negative bacteria, *Shigella flexneri* and *Escherichia coli*. Oils of *Cymbopogon citratus* and *Ocimum basilicum* showed highest antibacterial activity. Gram positive bacteria were found to be more sensitive than Gram negative. Antioxidant activities were tested by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and ABTS radical cation decolourization assay while Folin-Ciocalteu method was used to determine the total phenolic content. In DPPH assay, highest antioxidant activity was observed in *O. basilicum* oil followed by *Azeratum conyzoides*, *A. marmelos* and *C. citratus*, with percent inhibition and IC_{50} value ranging from 66.11-71.93% and 14.10-17.92 µl ml^{-1} respectively. In ABTS assay, similar results were obtained but with higher percent inhibition which ranged from 67.48-76.23% and lower IC_{50} value (12.12-17.21 µl ml^{-1}). Moreover, radical scavenging activity of essential oils was lower than that observed for the synthetic antioxidant BHA and BHT. The total phenolic content of the essential oils as GAE in mg 100$µl^{-1}$ of EO was found to be highest in *O. basilicum* (0.406) oil followed by *A. conyzoides* (0.322), *A. marmelos* (0.238) and *C. citratus* (0.231). The results provide evidence that the oils of *C. citratus* and *O. basilicum* can be further recommended for treatment of infections caused by these bacterial pathogens and are potential source of natural antioxidants having appreciable amount of total phenolic content.

Key words

Antibacterial, Antibiotic susceptibility, Antioxidant, Phenolic content

Introduction

From antiquity, nature has been a rich source of remedies for relief from various ailments affecting mankind. Use of plants for treating diseases is as old as human species. Plants produce a wide variety of secondary metabolites such as vitamins, terpenoids, tannins, flavonoids, alkaloids and other metabolites, which are rich in antimicrobial and antioxidant activities (Wong *et al*., 2006; Baker *et al*., 2010). Popular observations on the use and efficacy of medicinal plants significantly contribute to disclosure of their therapeutic properties, so that they are frequently prescribed, even if their chemical constituents are not always completely known. Essential oils are volatile aromatic oils obtained by steam or hydro distillation of aromatic plant. Most essential oils are primarily composed of terpenes and their oxygenated derivatives (Ramya *et al*., 2013). Essential oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant proprieties (Burt, 2004; Kordali *et al*., 2005). Due to these properties, essential oils and essential oil blends have become an essential addition to health and wellness, and their versatile nature, accessibility and affordability makes them a safe, non-toxic addition to a person's lifestyle.

Enteric or diarrhoeal infections are major public health problems in developing countries**.** Enteric bacteria comprise of *Salmonella* sp.*, Shigella* sp., *Proteus* sp., *Klebsiella* sp., *E. coli, Pseudomonas* sp.*, Vibrio cholerae* and *Staphylococcus aureus* which are major the etiologic agents of sporadic and epidemic diarrhea both in children and adults (Tambekar and Dahikar, 2011)**.** There are many pathogenic organisms known to spoil refrigerated and ready to eat products, often leading to food poisoning. *Bacillus subtilis* is a foodborne pathogen. They are

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common soil inhabitants but may frequently contaminate food and are widely distributed in hospital environments (Yassin and Ahamd, 2012). Survival and spread of resistant bacteria is the result of consistent and broad use of antibiotics from so many years. Although the bulk of traditional antibiotics can still manage drug-resistant bacteria, many commonly used antibiotics are no longer effective (Khan and Malik, 2001). Bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. A 'threshold' hypothesis proposed that resistance could be curtailed if total antibiotic use in a particular environment stayed below a critical quantitative level (Barbosa and Levy, 2000). Limited treatment options for infections caused by such multiresistant microorganisms prompted the search for novel plant antimicrobial compounds with a broad spectrum of activity and new therapeutic strategies.

Besides being spoiled by pathogenic organisms, all refrigerated and packed food also undergo autooxidation during storage leading to formation of reactive oxygen species (Gupta and Gupta, 2011). Oxygen is one of the most essential components for living, it is also a double edged sword. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules called “free radicals". Cell damage caused by free radicals appears to be a major contributor to aging and diseases like cancer, heart disease, decline in brain function, decline in immune system etc. Humans have evolved highly complex antioxidant systems (enzymic and nonenzymic), which work synergistically and in combination with each other to protect the cells and organ systems of the body against free radical damage. Commonly used synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxy toluene (BHT) are restricted by legislative rules because they are suspected to have some toxic effects and as possible carcinogens (Feng *et al*., 2006). Due to the negative and toxic effects of synthetic antioxidants, natural phenolic antioxidants are being promoted as food preservatives and diet supplements (Gharib and Teixeira da Silva, 2012). Several reports have revealed that majority of the antioxidant activity are achieved from biochemicals such as flavonoids, isoflavones, flavones, anthocyanins, catechins and other phenolics (Alothman *et al*., 2009; Isabelle *et al*., 2010).

In view of the above, the present study was carried out to investigate the antibacterial potential and antioxidant properties of some essential oils.

Materials and Methods

Plant materials : Aerial parts (leaves) of 16 angiospermic aromatic plants were collected from different regions of Gorakhpur district. Leaves were plucked and packed in polythene bags. Plants were initially identified by morphological features and then confirmed from the herbarium database present in the herbarium of DDU Gorakhpur University, Gorakhpur. The scientific names and family of the plants are detailed in Table 1.

Microbial strains and preparation of inoculums : Two Gram positive bacteria *Bacillus subtilis* (MTCC No. 3053) and *Staphylococcus aureus* (MTCC No. 9542) and one two Gram negative bacteria *Shigella flexneri* (MTCC No. 9543) and *Escherichia coli* (MTCC No. 1698) were used for evaluation of antibacterial assay. Stock cultures were maintained on nutrient agar (NA) slant at 4°C and sub-cultured monthly. Working cultures were prepared by inoculating a loopful of each test microorganism in 10 ml of nutrient broth (NB) from NA slants. Broths were incubated at 37°C for 18-20 hours. The suspension was diluted with sterile distilled water to obtain approximately 10^6 CFU ml^{-1} using Mc Farland standard.

Extraction of essential oils : Air-dried aerial parts of plants (200gm) were subjected to hydrodistillation for 3 hr with distilled water (1000ml) using a Clevenger-type apparatus. Crude oil obtained was collected and dried over anhydrous sodium sulfate and stored in sealed glass vials at 4 ºC prior to analysis.

Determination of antibacterial activity : Antibacterial activity of essential oils was evaluated by disc diffusion method (Andrews, 2001a) with slight modifications. 10 ml of sterilized nutrient agar medium was poured in Petri dishes and was allowed to solidify. The plates were seeded by spreading 0.1 ml of overnight cultures (adjusted to 0.5 McFarland turbidity standards according to Andrews, 2001b) and allowed to set for 20-25 min. For screening, sterile 6mm diameter filter paper discs were impregnated with 5µl of essential oil and placed on the surface of inoculated media agar plates using sterile forceps and then gently pressed down onto the agar surface. Control sets contained only sterile disc without essential oil. Antibiotics were used as positive reference standards to determine the sensitivity of bacteria tested (Table 3). Zone of inhibition was recorded in mm. All the plates were incubated at 35-37 ºC for 24-48hr. Clear inhibition zones around the discs indicated the presence of antibacterial activity. Diameter of inhibition zones were measured in millimeters. An inhibition zone of 10mm or more was considered as high antibacterial activity.

Determination of Minimum inhibitory concentration values: Minimum inhibitory concentration value for bacterial pathogen was determined by agar dilution technique of Andrews (2001b) with slight modifications. A series of doubling dilution of oil concentrations (0.5, 1, 2, 4, 8, 16, 32 µl ml^{-1}) was prepared in Petri dishes. 10 ml of sterilized and molten nutrient agar medium was poured in each dish already containing various dilutions of oil. 0.5% (v/v) tween-80 was incorporated into agar medium to enhance solubility of oil. Agar plates were allowed to set for 30 min at room temperature. With the help of a sterilized inoculating needle, a loopful of overnight bacterial culture (adjusted to 0.5 MacFarland standard) was delivered on to the agar plate. Agar plates without oil were used as control sets. The inoculum spots

were allowed to dry at room temperature and the plates were incubated at 35-37 ºC for 24hr. MICs were determined as the lowest concentration of oil inhibiting visible growth of microorganisms on agar plate, disregarding the presence of 1 or 2 colonies.

Determination of Minimum bactericidal concentration (MBC) values : The MBC of the oil was determined as described by Mishra *et al.* (2008). The plates of MIC, which showed no visible growth that were cultured on fresh nutrient agar plates. The lowest concentration of antimicrobial agent from which bacteria do not recover on fresh medium was treated as MBC.

Antioxidant activity

DPPH free radical scavenging activity: Effect of oils on DPPH radical was estimated using method of Güllüce *et al.* (2003) with slight modifications. 0.004% of DPPH (Hi Media) was prepared in methanol and 2ml of this solution was mixed with different concentrations of oil (10, 20, 30, 40, 50 µl ml^{-1}) dissolved in methanol. Reaction mixture was vortexed thoroughly and left for 30 min. After 30min absorbance of the mixture was measured at 517 nm by UV spectrophotometer (Hitachi) against a blank (pure methanol). Control sample was also prepared as above without any oil. Butylated hydroxyanisole (BHA) and Butylated hydroxytoluene (BHT) was taken as reference standards. Experiments were performed in triplicate and averaged. IC_{50} value was determined from percent inhibition versus concentration graph.

ABTS radical scavenging assay : For ABTS assay, the method of Adedapo *et al.* (2008) was adopted. Stock solution contained 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing two stock solutions in equal quantities and allowing them to react for 12 hr at room temperature in dark. The solution was then diluted by mixing 1 ml ABTS solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm by spectrophotometer. Fresh ABTS solution was prepared for each assay. 1 ml of different concentrations of oil (10, 20, 30, 40, 50 and 60 µg ml^{-1}) dissolved in methanol was allowed to react with 1 ml of ABTS solution and the absorbance was taken at 734 nm after 7 min using the spectrophotometer. ABTS scavenging capacity of oil was compared with that of BHT and BHA and percentage inhibition was calculated as ABTS radical scavenging activity.

Determination of total phenolic contents : Total phenolic content of four essential oils was determined according to the method of Taga *et al.* (1984) with slight modifications. 100 µl of each pure essential oil was dissolved in 10 ml of methanol. Now, 2 ml of this solution was made up with 0.3% HCl to 5 ml. A 100 µl aliquot of this resulting solution was added to 2 ml of 7.5% Na_2CO_3 and after 2 min 100 µl of Folin Ciocalteau (Hi Media) reagent (diluted tenfold with distilled water) was added and mixed well. After 30 min incubation, absorbance of mixtures was recorded spectrophotometrically at 750 nm. Total phenolic content was calculated as gallic acid equivalent (GAE) from a calibration curve of gallic acid standard solutions and expressed as mg of gallic acid per 100 µl of essential oil sample.

Statistical analysis : Experimental results were expressed as mean ± SD of three parallel measurements. Analysis of antioxidant activity and total phenolic content were carried out using Microsoft office excel programme. IC_{50} value of antioxidant activity was calculated by Sigma plot. Antibacterial activity, MIC

Table 1 : Antibacterial activity of different essential oils against the bacterial strains tested based on disc diffusion assay

Essential oils from aromatic plants	Local name	Family	Zone of inhibition (mm)			
			B. subtilis	*S. aureus*	*S. flexneri*	*E. coli*
Aegle marmelos (L.) Correa	Bel	Rutaceae	24±0.81	19.66±0.47	21±0.81	17.33±0.47
Azeratum conyzoides L.	Ajagandha	Asteraceae	9.66±1.28	-	-	-
Callistemon lanceolatus (Sm.) DC.	Bottlebrush	Myrtaceae	13.33±0.46	14±1.41	11.33±1.24	-
Chenopodium ambrosioides L.	Ban bhathuwa	Amranthaceae	23.66±0.94	14.33±1.69	13.33±0.47	12.66±0.47
Citrus aurantifolia (Christm.) Swingle	Kaghzi nimbu	Rutaceae	23.66±0.45	-	11.66±1.24	11.33±0.46
Citrus limone (L.) Burm. f.	Bara nimbu	Rutaceae	18.66±0.45	10±1.61	-	-
Citrus sinensis (L.) Osbeck	Mausami	Rutaceae	14.66±0.45	10±0.81	-	-
Curcuma domestica Valeton	Haldi	Zingiberaceae	16.67±0.94	16.66±0.45	15±0.81	14±0.81
Cymbopogon citratus (DC.) Stapf	Lemmongrass	Poaceae	#	40.33±1.24	32.33±0.46	35.67±0.47
Eucalytus citridora Hook	Eucalyptus	Myrtaceae	20.33±0.46	21.66±0.45	18±0.81	21±0.81
Hyptis suaveolens (Linn.) Poit	Wilayati tulsi	Lamiacece	18±1.61	-	-	-
Murraya koenigii Spreng.	Kurry pattha	Rutaceae	17.33±1.24	10±0.81	10.67±0.47	12.66±0.47
Ocimum basilicum Linn.	Kali tulsi	Lamiaceae	30±1.61	29.33±1.38	27±0.81	31±0.81
Ocimum canum Linn.	Bantulsi	Lamiaceae	16.33±0.45	10±0.81	-	10.33±0.47
Ocimum gratissimum Linn.	Ramtulsi	Lamiaceae	-	11.33±1.24	13.67±0.47	-
Ocimum sanctum Linn.	Krishna tulsi	Lamiaceae	20.33±0.45	-	10±0.81	11.33±0.47

complete zone of inhibition

and MBC were obtained by calculating average of three experiments.

Results and Discussion

Results of antibacterial disc diffusion assay are summarized in Table 1. Zones of inhibition ranged from 10-40 mm. Oils of *Cymbopogon citratus*, *Ocimum basilicum*, *Aegle marmelos* and *Chenopodium ambrosioides* showed potential antibacterial activity against specific pathogens. *C. citratus* oil formed the highest zone of inhibition against all the four bacterial pathogens followed by *O. basilicum* oil. No other oil was found effective against all the pathogens collectively irrespective of being effective against single bacterial pathogen (Table 1).

C. citratus oil showed complete inhibition of *B. subtilis* while 40.33, 32.33 and 35.67 mm inhibition zone was recorded against *S. aureus*, *S. flexneri* and *E. coli*. *C. citratus* oil exhibited strong antibacterial activity against *B. subtilis, S. aureus, S. flexneri* and *E. coli* with MIC values of 2, 8, 8 and 16 µl respectively. *O. basilicum* oil inhibited the growth of *B. subtilis*, *S. aureus*, *S. flexneri* and *E. coli* forming zones of 30, 29.33, 27 and 31 mm respectively. MIC value for *B. subtilis* was found to be 8 µl and 16 µl for rest of the three pathogens. MIC and MBC values are given in Table 2.

Out of 16 oils tested in the present study, 2 oils exhibited strong antibacterial action against *B. subtilis*, *S. aureus*, *S. flexneri* and *E. coli,* while 3 oils showed moderate activity. *C. citratus* oil showed highest inhibitory activity against all the four bacterial pathogens. The present study is in confirmation with the reports of Naik *et al*. (2010); Arputha *et al*. (2012) and Singh *et al*. (2011). In the present study, Gram positive strains were found to be more susceptible to *C. citratus* oil than the Gram negative strains. Similar results were reported by Barbosa *et al*. (2009). *O. basilicum* oil proved good in inhibiting *S. aureus* after *C. citratus* oil as reported by Stefan *et al*. (2013).

The MIC results of *C. citratus* and *O. basilicum* oil against *S. aureus* and *E.coli* are in fair correlation with the studies of Singh *et al*. (2011) and Mohaddam *et al*. (2011) respectively. An important characteristic of essential oils and their components is their hydrophobicity which enables them to partition lipids of the bacterial cell membrane, disturbing the cell structures and rendering them more permeable. Extensive leakage from bacterial cells or exit of critical molecules and ions will lead to death (Prabuseenivasan *et al*., 2006). The mode by which microorganisms are inhibited by essential oils and their chemical compounds, seem to involve different mechanisms. It has been hypothesized that inhibition involves phenolic compounds because these compounds sensitize phospholipid bilayer of the

Table 2 : MIC and MBC data of effective oils, *C.citratus* oil and *O.basilicum* oil against Gram positive and Gram negative bacteria

Bacterial Strains	*C. citratus* oil		*O. basilicum* oil	
	MIC	MBC	MIC	MBC
B. subtilis	2µl	4µl	8 µl	8 µl
S. aureus	8 µl	≤16 µl	16 µl	≤32µl
S. flexneri	8 µl	≤16 µl	16 µl	32 µl
E. coli	16	32	16	16

Table 3 : Effect of antibiotics against tested Gram positive and Gram negative pathogenic bacteria

Gram positive Antibiotics	*B. subtilis*	*S. aureus*	Gram negative antibiotics	*E. coli*	*S. flexneri*
Azithromycin (15µg)	29±0.81	25.66±0.47	Amphicillin (30µg)	-	-
Chloremphenicol (30µg)	25.66±0.81	10±1.25	Cefotaxime (30µg)	-	27.66±0.81
Ciproflaxacin (30µg)	26±0.81	16.66±0.47	Cefpodoxime (10 µg)	-	-
Clindamycin (2µg)	-	-	Ceftriaxone (30 µg)	21±0.81	28.66±0.47
Erythromycin (15µg)	23±0.812	26.33±0.45	Ceftizoxime (30 µg)	29±0.81	-
Gatifloxin (5µg)	29±0.91	20.66±0.88	Ciprofloxacin (5µg)	28±0.81	29.33±0.45
Lincomycin (15µg)	14.33±0.47	-	Gatifloxacin (5µg)	23.66±0.47	15.66±0.47
Lomefloxacin (10µg)	28.66±0.47	17.33±0.61	Gentamycin (10µg)	-	10±1.25
Moxifloxacin (5µg)	25.33±0.45	21.66±0.88	Levofloxacin (5µg)	22±0.61	17.33±0.61
Penicillin (10µg)	-	-	Nalidixic acid (30 µg)	19.33±0.45	30.33±0.45
Roxithromycin (30µg)	31±0.81	28±0.61	Nitrofurantoin (30 µg)	15±1.20	14.33±0.47
Telcoplanin (30µg)	24.66±0.48	-	Norfloxacin (10µg)	20.33±1.38	22.66±0.88
Tetracycline (30µg)	24.66. ±0.47	13.66±0.48	Ofloxacin (5µg)	26.66±0.45	24.66±0.47
Vancomycin (30µg)	31.33±0.88	14.66±0.81	Sparfloxacin (5µg)	19.33±0.45	23.33±0.91

- No sensitivity

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microbial cytoplasmic membrane causing increased permeability, unavailability of vital intracellular constituents and impairment of bacterial enzymes system (El-Mougy *et al*., 2012). In the present study, lemon grass and basil oils were found to be effective against both Gram positive and Gram negative bacterial strains but Gram positive strains were found more susceptible.

Various antibiotics showed varying degree of susceptibility pattern in the present study (Table 3). *B. subtilis* was sensitive to all antibiotics except penicillin, and with clindamycin and lincomycin intermediate resistance was recorded. *S. aureus* was found resistant to clindamycin, lincomycin, penicillin and telcoplanin and sensitive to azithromycin, moxifloxacin, roxithromycin, whereas with other antibiotics intermediate resistance was recorded (Table.3). Antibiotic susceptibility pattern of test organisms in the present study was in correlation with the studies of Jeyakumar *et al.* (2011) where a difference in the sensitivity pattern of *B. subtilis* was reported. *B. subtilis* showed resistance against penicillin which is in correlation with the study of Hashemi *et al*. (2008). However, the findings of Adewumi *et al.* (2009) demonstrated that *B. subtilis* exhibited resistance to erythromycin which is in contrast with the findings of present study. *S. aureus* was found sensitive to chloremphenicol and tetracycline which is also comparable to the study of Onwubiko and Sadiq (2011). *S. aureus* showed resistance to clindamycin while for same antibiotic *S. aureus* was found to be sensitive in the study of INSAR group, India (INSAR, 2013).

It was observed that indiscriminate use of antibiotics without prescriptions in the developing countries where there are no regulatory policies in this respect, has rendered the commonly used antibiotics completely ineffective in treatment of *S. aureus* infections (Onwubiko and Sadiq, 2011). Ciprofloxacin, nitrofurantoin, ceftizoxime exhibited highest sensitivity against

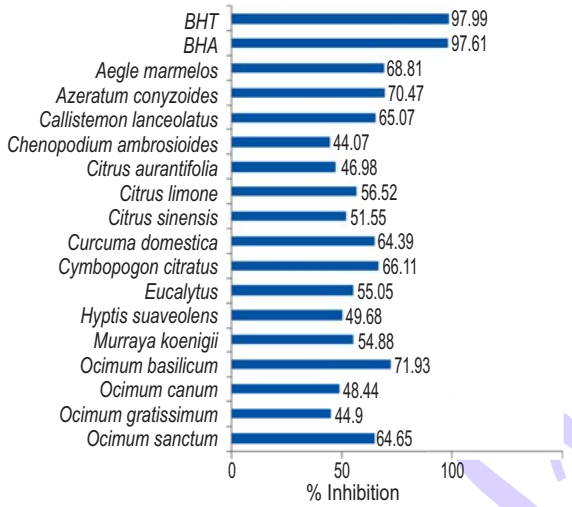

Fig. 1 : Free radical-scavenging activity of 16 essential oil evaluated by DPPH assay and comparison with that of reference BHA and BHT

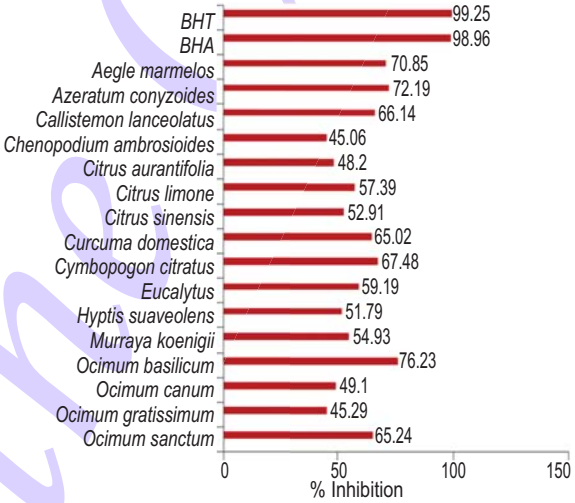

Fig. 2 : Free radical-scavenging activity of 16 essential oils evaluated by the ABTS assay and comparison with that of reference BHA and BHT.

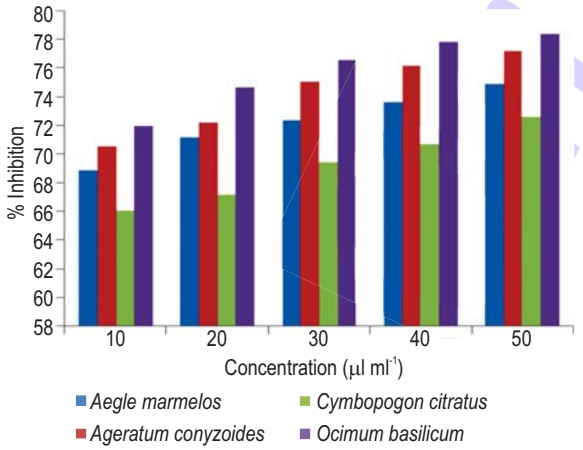

Fig. 3 : DPPH free radical scavenging activity of essential oils at different concentration

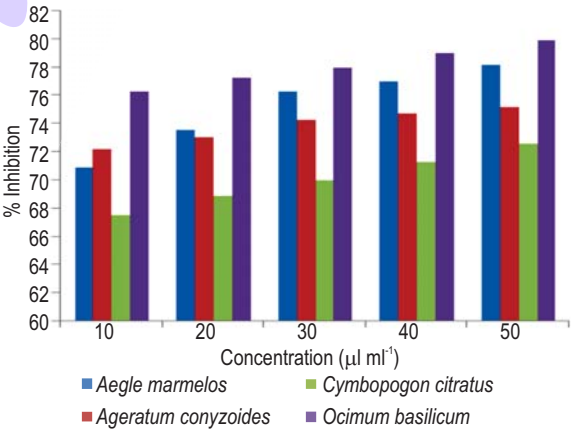

Fig. 4 : ABTS radical cation decolourization assay of essential oils at different concentrations

Table 4 : Radical scavenging activity and total phenolic contents of tested essential oils

Essential Oils	IC_{50} value in µl ml^{-1} (DPPH assay)	IC_{50} value in µl ml^{-1} (ABTS assay)	Phenolic content (mg 100 $µl^{-1}$ EO as GAE)
Aegle marmelos	15.92	14.28	0.238
Azeratum conyzoides	14.81	14.81	0.322
Cymbopogon citratus	17.92	17.21	0.231
Ocimum basilicum	14.10	12.12	0.406

Values expressed are mean of three replicates

E. coli. Similar results were demonstrated by Khoshbakht *et al*. (2013). *E. coli* showed poor degree of sensitivity towards gentamycin which is contrary with the findings of Khalili *et al*. (2012) and in correlation with the study of Khoshbakht *et al*. (2013). Gram negative *S. flexneri* was found to be highly sensitive to all antibiotics with highest degree of sensitivity towards nalidixic acid and ciprofloxacin and these results are in confirmation with the study of Mandomando *et al.* (2009). *S. flexneri* showed resistance to amphicillin, likewise Reda *et al.* (2011). Susceptibility of gentamycin was low in the present study while other studies mentioned above showed effectiveness of this drug, which can be attributed to its uncontrolled use. Bacteria showed resistance to amphicillin and were found susceptible to ciprofloxacin and nitrofurantoin. It may be endorsed to its rare availability and expensiveness as compared to other antibiotics.

DPPH radicals are widely used to investigate the scavenging activity of natural compounds. *A. marmelos, A. conyzoides, C. citratus* and *O. basilicum* essential oils notably reduced the concentration of DPPH free radical (Fig. 1) with an efficacy lower than that of reference BHA and BHT. Highest antioxidant activity was observed in *O. basilicum* (71.93%), *A.conyzoides* (70.47%) *A. marmelos* (68.81%) oils and *C. citratus* (66.11%). IC_{50} value of these oils was found to be 14.10, 14.81, 15.92 and 17.92 µl ml^{-1} (Table 4). Higher concentration of oils were more effective in quenching free radicals (Fig. 3).

In ABTS radical cation decolourization assay, highest antioxidant activity was observed in *O.basilicum* (76.23%) followed by *A. conyzoides* (72.19%) *A. marmelos* (70.85%) *and C. citratus* (67.48%) oils, with IC_{50} value of 12.12, 14.81, 14328 and 17.21 µl ml^{-1} respectively (Table 4). These oils were found to be good scavengers of ABTS free radicals (Fig. 2). Rise in oil concentration was found to enhance the radical scavenging abilty. At 50 µl ml^{-1} concentration, highest activity of oil was observed (Fig. 4). Results were in correlation with the data found in DPPH assay or it can be said that per cent inhibition was better than those provided by radical scavenging activity. Proton radical scavenging is an important attribute of antioxidants. ABTS, a protonated radical, has characteristic absorbance maximum at 734 nm which decreases with scavenging of proton radicals (Mathew and Abraham, 2006).

Many studies have demonstrated correlation between phenolic content and antioxidant activity (Yang *et al.,* 2002). On the other hand, Bajpai *et al.* (2005) reported no correlation between total phenolic content and antioxidant potential of several medicinal plants. Phenolic compounds may contribute directly to the antioxidative action (Lu *et al*., 2011). Phenolic compounds are secondary metabolites of plants and also good hydrogen donors, which makes them good antioxidants (Dudonne *et al*., 2009; Sim *et al*., 2010) and they can act as antioxidants by many potential pathways such as freeradical scavenging, oxygen radical absorbance and chelation of metal ions (Gupta and Gupta, 2011).

Total phenolic equivalent ranged from 0.231 to 0.406 mg 100$µl^{-1}$ EO as GAE (Table 4). Highest total phenolic content was observed in *O. basilicum* (0.406mg 100$µl^{-1}$ EO as GAE) and lowest in *C. citratus* (0.231 mg 100$µl^{-1}$ EO as GAE). Total phenolic equivalents among four essential oils were as follows: *O. basilicum*> *A. conyzoides*, > *A. marmelos* > *C. citratus* (Table 4).

To a certain level a correlation can be establish between total phenolic content (TPC) and antioxidant activity in the present study conducted. Highest antioxidant activity with lowest IC_{50} value was observed in *O. basilicum* oil followed by *A. conyzoides, A. marmelos* and *C. citratus* oils. Total phenolic content showed the same order of results with highest TPC in *O. basilicum* and *A. conyzoides* oils except the oils of *C. citratus* and *A. marmelos* showing contradictory values. All the four oils were found to have good antioxidant activity as well as high total phenolic content along with promising antibacterial activity, except for *A. conyzoides* which showed poor antibacterial activity against both bacterial strains. DPPH and ABTS assay proved that all the four oils were found to have natural antioxidant activity, especially source of *O. basilicum* and can be used as potential natural source of antioxidants and antibacterials.

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