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Madhulika Bhagat., Preeti Choudhary., Sonam
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RESEARCH ARTICLE

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INDIAN SPICES**

Madhulika Bhagat*, Preeti Choudhary., Sonam Chouhan., Rasleen Sudan and Sahil Gupta

School of Biotechnology, University of Jammu, Jammu 180006, India

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ABSTRACT

Spices are used widely in Indian culinary for flavour and aroma. They have also been known to impart several health benefits. In the current work, the essential oils of clove, carom, cumin, fennel and ginger were obtained by hydro distillation and analyzed by GC-MS. They were also investigated for their antimicrobial and antioxidant activities. Antibacterial assay showed that essential oil of carom seeds exhibited exceptional growth inhibition against all the bacterial strains with maximum inhibition against *Enterococcus faecalis* (40±0.17 mm) and its GC-MS analysis showed the presence of highest amount of thymol (49.6%). TLC-direct bio autography of carom essential oil confirmed that a single compound (Rf 0.325) was responsible for its remarkable antibacterial activity. Clove oil showed maximum inhibition against *Bacillus cereus* (24±0.28 mm) and ginger oil against *Micrococcus luteus* (35±0.39 mm). Clove and ginger essential oils showed the presence of high amounts of eugenol (82%) and - citral (18%). Antifungal assay demonstrated that all the five essential oils possessed remarkable antifungal activity against the three fungal strains. Antioxidant studies depicted that cumin, clove and ginger essential oil possessed maximum DPPH radical scavenging activity with lowest IC₅₀ (1.5µl/ml). Cumin also showed maximum chelation of ferrous ions (66%) in comparison to other spices. GC-MS analysis of cumin essential oil showed the presence of maximum amount of phenyl glycol (40%) and cuminaldehyde (30%). The results demonstrated that these oils possessed significant antibacterial, antifungal and antioxidant properties and may find their applications as food preservatives, medicine and herbal formulations.

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INTRODUCTION

Herbalists and folk practitioners have used plant remedies for centuries, but only recently scientists have begun to study the medicinal powers of common herbs and spices. As spices are known to be aromatic and pungent food ingredients, they have been used all over the world to intensify the flavour and food (Kivilompolo M et al., 2007). Because of having good medicinal value, they have been used in a large number of medicinal preparations for the treatment of several disorders, especially of the digestive system (Muthamma M K S and Hemang et al., 2008)

Spices are a rich source of essential oils. These essential oils have been long recognized for their antibacterial, antifungal, antiviral, insecticidal and antioxidant properties (Kordali and Kotan, 2005; Bakkali et al., 2006). As reviewed by many authors, the antimicrobial activity of plant essential oils has been extensively studied and they have been found to be effective against a number of microorganisms (Burt, 2004 and

Holley et al., 2005). Worldwide, bacterial and fungal infections are a major cause of diseases and deaths. The continuous use of synthetic drugs to fight infections has led to the generation of resistant microbial strains. However, antimicrobial agents from medicinal and dietary plants still provide a hope to this increasing problem. Similarly, antioxidants from dietary sources are the best option instead of taking synthetic drugs to curb diseases.

Free radicals are formed naturally in the body and play an important role in many normal cellular processes. At high concentrations, however, free radicals can be hazardous to the body and damage all major components of cells, including DNA, proteins and cell membranes and may play a role in the development of cancer and other health conditions. The body makes some of the antioxidants it uses to neutralize free radicals. These antioxidants are called endogenous antioxidants. However, the body relies on external (exogenous) sources, primarily the diet, to obtain the rest of the antioxidants it needs. The role of spices as sources of dietary antioxidants has been under extensive study. Because

*Corresponding author: **Madhulika Bhagat**
School of Biotechnology, University of Jammu, Jammu 180006, India

spices have very low calorie content and are a rich source of essential oils, they can prove to be reliable sources of antioxidant and antimicrobial agents in diet.

Keeping in view the nutraceutical value of spices, the present study was carried out to analyse the antimicrobial and antioxidant potential of five dietary spices which are used extensively in Indian food preparations viz. ginger, carom seeds, cumin, clove and fennel.

MATERIALS AND METHODS

Collection of plant material and extraction of essential oils -

Rhizomes of ginger (*Zingiber officinale*), carom seeds (*Trachyspermum ammi*), fennel (*Foeniculum vulgare*), cumin (*Cuminum cyminum*) and buds of clove (*Syzygium aromaticum*) were collected from the market and were subjected to hydro-distillation for 3 hours using a Clevenger-type apparatus for the extraction of essential oil and stored at 4 °C.

Phytochemical analysis of essential oils by GC-MS - Analysis of essential oil of different dietary spices viz. clove, cumin, carom, fennel and ginger was carried out at IIM, Canal road, Jammu, A GC-MS 4000 (VARIAN, usa) system with a Varian CP-SIL 8CB column (30 m × 0.32 mm i.d, 1 μm film thickness). Injector temperature was 230 °C. Oven temperature programme used was holding at 60 °C for 5 min, heating to 250 °C at 30 °C/min and keeping the temperature constant at 250 °C at for 10 min. helium was used as a carrier gas at a constant flow of 1.0 ml/min and an injection volume of 0.20 μl was employed. The MS scan parameters included electron impact ionization voltage of 70 eV, a mass range of 40-500 m/z. the identification of components of the essential oils was based on comparison of their mass spectra with those if NISTO5 (version 2.0) library.

Antibacterial activity - The antibacterial activity was performed using agar well diffusion technique⁷ as described by NCCLS, 1999. Briefly, 100 μl each of the bacterial suspensions (10⁸ CFU/ml) i.e. *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Alcaligenes denitrificans*, *Camphylobacter coli*, *Pseudomonas alcaligenis*, *Alcaligenes denitrificans*, *Micrococcus luteus* and *Klebsiella pneumonia* were spread on nutrient agar plate. The wells were created in the pre-seeded plates using borer. Essential oil or extract was added into each well. Finally the plates were kept for incubation at 37 °C for 24h. Chloramphenicol (10 μg/ml) was used as positive reference. Microbial growth was determined by measuring the zone of inhibition.

Antifungal activity - Antifungal activity of the essential oils was determined by poisoned food technique against three different plant pathogenic fungi viz. *Alternaria alternata*, *Curvularia lunata*, and *Bipolaris specifera* (Singh and Tripathi, 1999) The test component (essential oil) was added to the sterilized potato dextrose agar in 9 cm petri plates. After the preparation of plates containing different concentrations of essential oil, test fungus (5 mm) was inoculated in the centre of the agar plate (upside down). Plates were then incubated in dark at 26 °C. The extension diameter of hyphal growth was

measured at 24 hour interval, till growth of fungus in the plate without test component (control) reached the edge of the plate. The experiment was conducted in three replicates and results were expressed as average of the three. Fungal growth diameter in each plate containing different concentrations of test component was determined to calculate per cent growth inhibition. The antifungal indices were determined as: Antifungal index (%) = (1-Da/Db) × 100

Screening of antibacterial activity by TLC-Bioautography method-

To screen the antibacterial activity of the components of essential oil, direct bioautography was performed (Marston and Maillard, 1997). Carom essential oil was subjected to thin layer chromatography for the separation of its components. Silica gel 60 F₂₅₄ was used for the preparation of TLC plates. Different combinations of hexane:ethyl acetate solvent system were used as mobile phase and separation of analytes was checked by visualising under UV light (365 and 254 nm) or by spraying with vanillin/sulphuric acid spray reagent. Analytes of the essential oil separated on TLC plate were then dried to completely remove the solvent. *Bacillus subtilis* as a test organism was then allowed to grow on the TLC plate and incubated at 37 °C for 24 h in humid conditions. After incubation, the plate was sprayed with 2 mg/ml solution of INT. One of the replication of plate was developed with Godin reagent. Clear zones on chromatogram showed inhibition of growth.

Antioxidant studies

DPPH Radical Scavenging Assay - The DPPH radical capacity of spices was monitored at 517 nm by the method of Bozin (Bozin et al., 2006). To 1 ml of different concentrations of essential oils, 1 ml of DPPH was added (90 μM, in methanol). Volume was raised to 4 ml by adding methanol. Positive control was also set containing 1 ml of DPPH and 4 ml of methanol. The mixtures were kept at 25 °C in the dark for 1 hour. The absorbance was measured at 517 nm.

The ability to scavenge DPPH radical in percent was calculated as

DPPH radical scavenging effect (%) = $\frac{A_C - A_T}{A_C} \times 100$

Chelation power on ferrous (Fe²⁺) ions- The chelating effect on ferrous ions of the essential oils was estimated by the method of Dinis with slight modifications (Dinis et al., 1994) Briefly, 20 μl (10 times diluted with DMSO) of each essential oil was raised with methanol to make final volume to 3ml. Then, 60 μl of 2 mM FeCl₂ was added. The reaction was initiated by the addition of 120 μl of 5 mM ferrozine into the mixture, which was then left at room temperature for 2 min before determining the absorbance of the mixture at 562 nm. The ratio of inhibition of ferrozine-Fe²⁺ complex formation was calculated using the equation: % inhibition = ([absorbance of control - absorbance of test sample]/absorbance of control) × 100.

RESULTS

GC-MS analysis- In the current study, rhizomes of ginger, seeds of carom, fennel, cumin and buds of clove were subjected

to hydro-distillation for isolation of essential oils. The main chemical constituents of the essential oils were determined by using gas chromatography and mass spectrometry as depicted in Table 1.

Table 1 Major compounds present in the essential oils of spices analysed by GC-MS

S.No.	Major Compounds	Carom	Fennel	Ginger	Clove	Cumin
1.	- thujene	0.145	-	-	-	0.120
2.	- pinene	0.677	-	1.205	-	0.153
3.	L - pinene	0.116	0.246	-	-	0.252
4.	- terpinene	0.136	-	-	-	-
5.	p- cymene	26.832	0.106	1.749	-	8.883
6.	Limonene	0.059	2.876	1.322	-	0.124
7.	- phellandrene	0.155	-	9.126	-	-
8.	-terpinene	22.185	-	1.112	-	5.242
9.	Thujol	0.095	-	-	-	-
10.	Thymol	49.600	-	1.917	-	-
11.	- pinene	-	0.544	1.368	-	-
12.	Camphene	-	0.142	6.016	-	-
13.	6-Methyl-5-heptene-2-one	-	-	0.232	-	-
14.	Eucalyptol	-	-	8.406	-	0.079
15.	Terpinolene	-	-	0.344	-	-
16.	Linalool	-	-	1.376	-	-
17.	- citronellal	-	-	0.563	-	-
18.	Borneol	-	-	6.940	-	-
19.	- terpineol	-	-	0.894	-	-
20.	- citral	-	-	8.065	-	-
21.	- citral	-	-	18.031	-	-
22.	L-Bornyl Acetate	-	-	0.305	-	-
23.	- elemene	-	-	0.213	-	-
24.	Curcumene	-	-	4.447	-	-
25.	Germacrene	-	-	0.560	-	-
26.	Zingiberene	-	-	14.044	-	-
27.	-Farnesene	-	-	3.030	-	-
28.	Tau-Maurolene	-	-	0.922	-	-
29.	-Bisbolene	-	-	1.452	-	-
30.	-Funebrene	-	-	4.009	-	-
31.	Elemol	-	-	0.410	-	-
32.	Nerolidol	-	-	0.547	-	-
33.	Germacrene B	-	-	0.147	-	-
34.	(+)-Aromadendrene	-	-	0.21288	-	-
35.	Eugenol	-	-	-	82.414	-
36.	- caryophyllene	-	-	-	1.919	-
37.	- caryophyllene	-	-	-	0.472	-
38.	Eugenol Acetate	-	-	-	15.196	-
39.	Cis-Ocimene	-	0.185	-	-	-
40.	Fenchone	-	17.120	-	-	-
41.	Camphor	-	0.126	-	-	-
42.	Methyl Chavicol	-	2.254	-	-	-
43.	Anethole	-	76.389	-	-	-
44.	Sabinene	-	-	-	-	0.274
45.	4-(1-methylethylidene) cyclohexane	-	-	-	-	0.201
46.	4-terpineol	-	-	-	-	0.082
47.	4-isopropyl-1,3-cyclohexadien-1-yl methanol	-	-	-	-	1.025
48.	2-tert-butyl-4-methylfuran	-	-	-	-	0.327
49.	Cuminaldehyde	-	-	-	-	30.852
50.	2-carene-10-al	-	-	-	-	7.926
51.	phenyl glycol	-	-	-	-	40.508

Ten compounds were identified in the essential oil of carom with thymol as the major compound (49.6%) followed by p-cymene (26.8%) and - terpinene (22%) (Fig 1, Table 1). The essential oil of ginger exhibited the presence of thirty volatile compounds out of which - citral (18%) followed by zingiberene (14%) were the major ones. - phellandrene (9%), eucalyptol (8.4%) and - citral (8%) were found in moderate amounts (Fig 2, Table 1). Clove essential oil demonstrated the presence of four compounds in which eugenol (82%) and eugenol acetate (15%) were found to be in maximum quantity (Fig 3, Table 1). Out of the fifteen compounds identified in the

essential oil of cumin, phenyl glycol (40%) and cuminaldehyde (30%) were the major compounds (Fig 4, Table 1). The essential oil of fennel showed the presence of anethol (76%) as the major compound followed by fenchol (17%) out of ten identified compounds (Fig 5, Table 2).

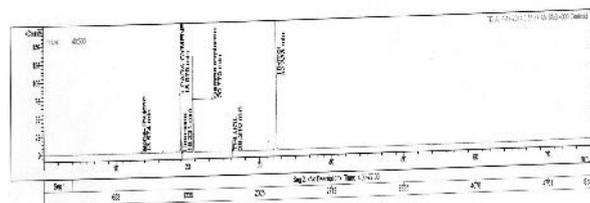


Fig 1 Chromatogram showing the chemical profile of Carom essential oil

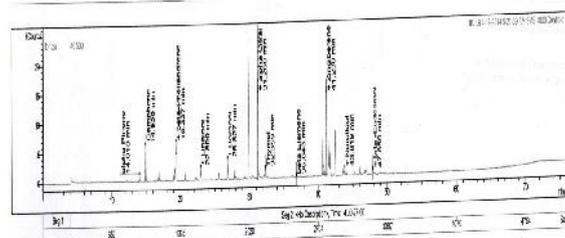


Fig 2 Chromatogram showing the chemical profile of Ginger essential oil

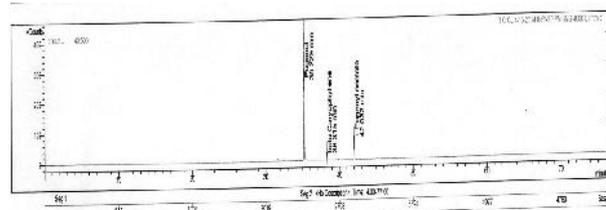


Fig 3 Chromatogram showing the chemical profile of Clove essential oil.

Table 2Antibacterial activity of essential oils as determined by agar well diffusion assay Values are represented as a result of triplicate experiments (Mean ± S.D)

S.No.	Bacterial strains	Zones of inhibition (mm)					
		Positive control	Carom	Clove	Cumin	Fennel	Ginger
1	<i>Bacillus subtilis</i>	21±0.21	34±0.23	6±0.03	-	-	11±1.13
2	<i>Bacillus cereus</i>	25±0.11	23±0.57	24±0.28	-	-	10±0.27
3	<i>Alcaligenes denitrificans</i>	19±0.23	26±0.78	13±0.54	7±0.11	-	7±0.75
4	<i>Micrococcus luteus</i>	25±0.31	28±1.10	15±0.73	-	-	35±0.39
5	<i>Enterococcus faecalis</i>	20±0.45	40±0.17	12±0.62	-	-	20±0.71
6	<i>Pseudomonas alcaligenes</i>	23±0.52	24±0.53	9±0.44	8±0.18	-	10±0.92
7	<i>Camphylobacter coli</i>	20±0.12	32±0.86	-	-	-	-
8	<i>Klebsiella pneumoniae</i>	24±0.22	32±0.91	-	-	-	-

Table 3 IC50 (%) values of antifungal activities of the Essential oils against the three fungal strains

Fungal strains	Carom oil	Clove oil	Cumin oil	Fennel oil	Ginger oil
<i>A.alternata</i>	0.012	0.014	0.045	0.006	0.073
<i>C.lunata</i>	0.021	0.016	0.038	0.016	-
<i>B.specifera</i>	0.016	0.014	0.036	0.016	0.023

Antibacterial activity- The results of antibacterial activity of the essential oils are depicted in Table 2. The essential oils were examined by agar well diffusion assay against various bacterial strains and inhibitory activity was determined by the zone of inhibition. Essential oil of carom showed remarkable inhibitory activity against all the bacterial strains in comparison to the positive control (chloramphenicol): *E. faecalis* (40±0.17 mm), *B. subtilis* (34±0.23 mm), *C. coli* (32±0.86 mm), *K.*

pneumoniae (32±0.91 mm), *M. luteus* (28±1.10 mm), *A. denitrificans* (26±0.78 mm), *P. alcaligenes* (24±0.53 mm), and *B. cereus* (23±0.57 mm). Essential oil of clove also exhibited significant antibacterial activity most of the bacterial strains with maximum inhibition against *B. cereus* (24±0.28 mm) followed by *M. luteus* (15±0.73 mm) and *A. denitrificans* (13±0.3 mm). Essential oil of cumin showed low activity against *P. alcaligenes* (8±0.18) and *A. denitrificans* (7±0.11 mm). However, other bacterial strains were found to be resistant to the cumin essential oil. Essential oil of fennel exhibited no antibacterial activity against any bacterial strain whereas essential oil of ginger showed maximum inhibition against *M. luteus* (35±0.39 mm) followed by *E. fecalis* (20±0.71mm), moderate inhibitory activity against *B. subtilis* (11±1.13 mm), *B. cereus* (10±0.27 mm) and *P. alcaligenes* (10±0.29mm) and negligible activity against *A. denitrificans*, *C. coli* and *K. pneumonia*.

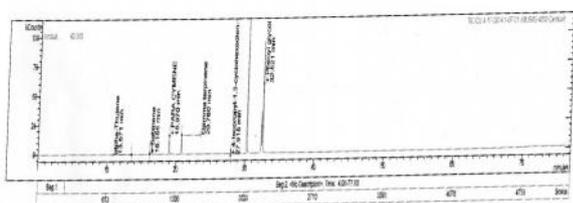


Fig 4 Chromatogram showing the chemical profile of Cumin essential oil

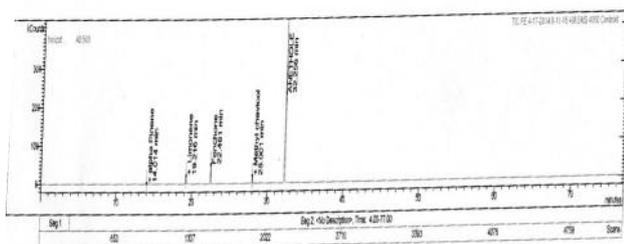


Fig 5 Chromatogram showing the chemical profile of Fennel essential oil

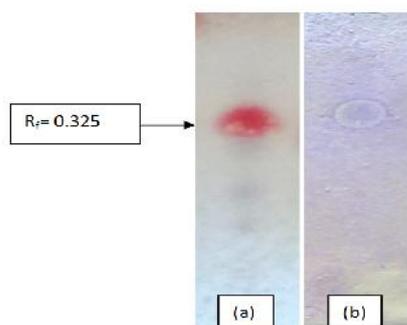


Fig 6 Visualization of antibacterial compounds in Carom essential oil: (a) represents TLC separation of compounds in Carom oil, (b) bioautography representing antibacterial compounds present in the Carom oil. The compound with R_f 0.325 in plate (a) was showing antibacterial activity in the plate

Antifungal activity- Antifungal activities of the essential oils were evaluated by poisoned food technique against three fungal plant pathogens viz. *Alternaria alternata*, *Curvularia lunata*, *Bipolaris specifera*. The results as depicted in Table 3 indicated that all the five essential oils showed noteworthy antifungal activities against all the three fungal strains. However, fennel oil showed remarkable antifungal activity against *A. alternate*

with IC_{50} values 0.006%, followed by carom oil and clove oil. *C. lunata* was found to be significantly inhibited by clove oil and fennel oil with IC_{50} values 0.016% followed by carom oil (IC_{50} 0.021%). *B. specifera* was considerably inhibited by clove oil (IC_{50} 0.014%) followed by carom oil (IC_{50} 0.016%) and fennel oil (IC_{50} 0.016%). Essential oil of cumin demonstrated significant antifungal activity against *B. specifera*, *C. lunata* and *A. alternata* with IC_{50} values 0.036%, 0.038% and 0.045% (v/v) respectively. Essential oil of ginger demonstrated significant antifungal activity against *B. specifera* and *A. alternata* with IC_{50} values 0.023% and 0.073% (v/v) respectively.

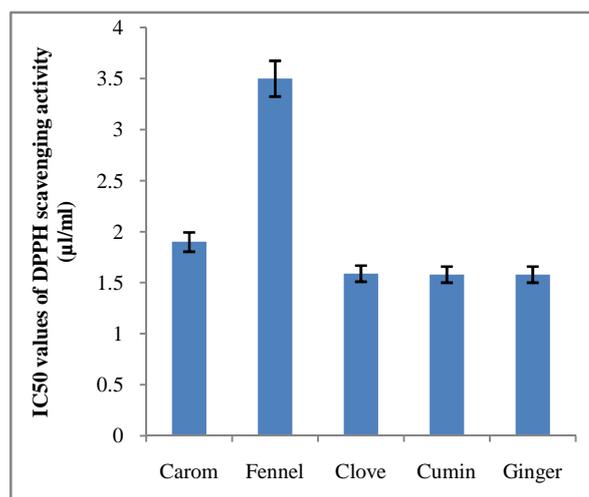


Fig 7 Graph showing the DPPH radical scavenging activity of essential oils of spices

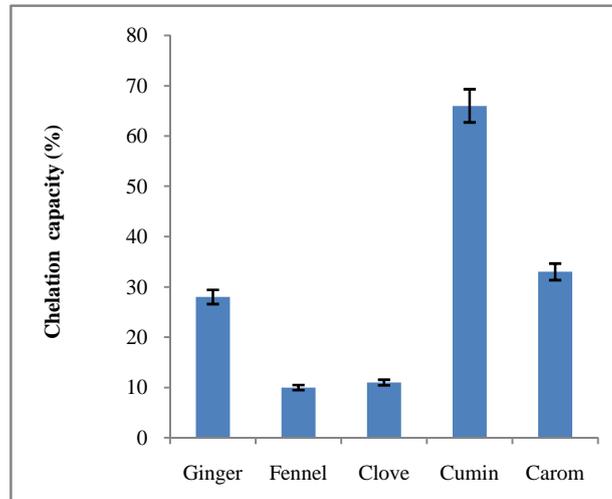


Fig 8 Graph representing Chelation capacity on ferrous ions of essential oils of spices

Screening of antibacterial activity by direct Bio-autography- Antibacterial potential of the essential oil of carom was also determined by TLC- bio-autography technique. Analytes of the essential oil were separated on TLC plate and *Bacillus subtilis* was used as test organism. Application of the essential oil of carom in direct bio-autography for observing antibacterial activity showed one inhibition zone at R_f value 0.325 as shown in Fig 6. One zone of inhibition indicated the presence of one active antibacterial compound present in essential oil separated on TLC plate.

Antioxidant activity

DPPH radical scavenging activity- The essential oils when analysed for antioxidant capacity exhibited variable results. Cumin oil, ginger oil and clove oil possessed the highest antioxidant capacity followed by carom oil and fennel oil (Fig 7). Order of antioxidant capacity of the five essential oils in terms of IC₅₀ values: Cumin oil (1.581 µl/ml) = Ginger oil (1.581 µl/ml) = Clove oil (1.590 µl/ml) > Carom oil (1.982 µl/ml) > Fennel oil (3.521 µl/ml).

Chelation capacity on ferrous ions- As depicted in Fig 8, cumin oil showed the highest chelation capacity with 66% chelation. Carom oil and ginger oil showed moderate chelating effect of 33% and 28% on ferrous ions. Fennel oil and clove oil showed negligible chelation. Therefore, decreasing order of chelation capacity of the five essential oils: cumin oil (66%) > carom oil (33%) > ginger oil (28%) > clove oil (11%) > fennel oil (10%).

DISCUSSION

Spices have been used all over the world for their aroma and to enhance flavour of food preparations. They are also a rich source of essential oils which can be utilised for health benefits. The essential oils in plants are secondary metabolites which have been proven to harbour various biological activities like antimicrobial, antivirals, anti-inflammatory, antioxidant, insecticides etc (Bakkali *et al.*, 2008). In view of increasing risk factors of humans to various deadly diseases, there has been a global trend towards the use of natural substances present in medicinal plants and dietary plant foods as therapeutics and as promising alternative for synthetic compounds in respect of low cost, highly compatible with dietary intake and no harmful effects inside the human body.

Owing to the growing trend of using plant foods to impart health benefits to humans, the present study was made to assess the antimicrobial and antioxidant potential of essential oils of five extensively used Indian spices *viz.* carom, clove, cumin, fennel and ginger. Essential oils are natural, complex, multi-component systems composed mainly of terpenes in addition to some other non-terpene components. At present, approximately 3000 essential oils are known, 300 of which are commercially important especially for the pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries. The essential oils extracted by hydro-distillation when subjected to antibacterial study showed that carom essential oil maximally inhibited the growth of all the bacterial strains in comparison to other essential oils and positive control (Table 2). Also, GC-MS analysis of carom essential oil showed the presence of rich amounts of thymol, p-cymene and - terpenine. Thymol has been scientifically proven to be a potent antibacterial and fungicide. It has also been known to reduce bacterial resistance to antibiotics (Palaniappan and Richards, 2010).

Also, direct bioautography of carom essential oil showed single zone of inhibition against *B. subtilis* which helps in concluding that a single compound is responsible for its antibacterial activity which needs to be identified. Clove and ginger essential oils also showed moderate inhibition against a few

bacterial strains. GC-MS analysis of clove and ginger essential oils showed eugenol (82%) and - citral (18%) as the major compounds respectively (Table 1). Therefore, the presence of these active compounds may be responsible for their bacterial growth inhibition. Strong *in vitro* evidence indicates that essential oils can act as antibacterial agents against a wide spectrum of pathogenic bacterial strains including *Listeria monocytogenes*, *L. innocua*, *Salmonella typhimurium*, *Escherichia coli*, *Shigella dysenteria*, *Bacillus cereus*, *Staphylococcus aureus* and *Salmonella typhimurium* (Schmidt *et al.*, 2005; Jirovetz *et al.*, 2005; Dadlioglu *et al.*, 2004; Nguefack and Jakobsen, 2004; Hulin *et al.*, 1998). Antifungal activity analysis showed that all the essential oils were active against the three fungal pathogens with carom essential oil again showing the best antifungal activity. This may be again due to the presence of high amount of thymol.

Antioxidant studies showed that cumin, clove and ginger essential oils had almost similar DPPH radical scavenging activity followed by that of carom and fennel essential oils. The presence of phenyl glycol and cuminaldehyde in cumin essential oils might be responsible for its antioxidant property (Table 1). Ginger essential oil also had a wide range of compounds, some of which are potent antioxidants. The synergistic effect of the major compounds present in these essential oils might also be responsible for their antimicrobial and antioxidant potential. Chelation of ferrous ions is also a measure of antioxidant capacity because ferrous ions are indirectly involved in the generation of free radicals in the body.

Among the five essential oils, cumin again exhibited exceptional chelation capacity followed by carom and ginger. Excess of metal ions can lead to various anomalies in the body. The iron (II) chelating activity of plant extracts is of great significance because it has been proposed that the transition metal ions contribute to the oxidative damage in neurodegenerative disorders like Alzheimer's and Parkinson's diseases (Aparadh *et al.*, 2012). Also, chelation therapy is a common practice of neutralising iron overload in the body especially in cases of treatment of Thalessemia and other anemias (Ebrahimzadeh *et al.*, 2008). Therefore, cumin essential oil can be used in therapeutics for chelation of excess ferrous ions in the body.

CONCLUSION

The present study highlights the biological effects of some commonly used Indian spices. In addition to imparting flavour, they can be used as food preservatives against bacterial and fungal pathogens. Also, few of them like cumin, clove and ginger can also be promoted as source of natural antioxidants. However, it is important to develop a better understanding of their mode of biological action for new applications in human health, agriculture and the environment.

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