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Chemical Composition And Antimicrobial Activity Of Ocimum Basilicum L Essential Oil

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Abstract: This study was designed to investigate the chemical constituents, antibacterial and antifungal activities of Ocimum basilicum. L essential oil extracted from the areal parts of the plant. Essential oil was extracted by hydro distillation for 3 hours using a Clevenger apparatus. Anhydrous sodium sulphate was used to remove the moisture from the extracted volatile oil. Essential oil was characterized using Gas Chromatography with Mass Spectrometry (GC-MS). The results showed 39 chemical constituents in the 99.92% of the total essential oil. Among them Linalool (41.28%), Geraniole (22.04%), Estragole (7.60%) and Neroleacetate (5.01%) are the major components and others are minor components. The essential oil showed potential antibacterial activity against gram positive bacteria than gram negative bacteria. S. aureus (32.0±0.9mm), B. cereus (32.0±0.9 mm), B. subtilis (29.1±1.2 mm), E.cloacae (25.2±1.0 mm), M.flavus (24.2±1.0 mm) showed strong antibacterial activity. Essential oil showed potent antifungal activity against yeast and fungal strains A.alternaria (32.1±0.1mm), A.fumigates (31.8±0.8mm), F.oxyporum (30.4±0.2mm), A.flavus (21.0±0.6mm), C.herbarum (20.2±0.1mm). Minimum inhibition concentration range between antibacterial and antifungal activity of the essential oils is 1.0±0.2 to 5.0±1.2µg/ml and 1.0±0.3 to 4.5±0.8 µg/ml respectively. The present study indicates that O.basilicum L. essential oil can be used for its antibacterial and antifungal activity.

Key Words: Ocimum bacilicum L.; Chemical constituents; Antibacterial; Antifungal; Essential oils

INTRODUCTION

Plants make many chemical compounds for biological functions, including defense against insects, fungi and herbivorous mammals. Over 12,000 active compounds are known to science. These chemicals work on the human body in exactly the same way as pharmaceutical drugs. According to the reports of the World Health Organization [1], at least 75-95% of the world's population of developing countries still uses plant extracts or their essential oils for treatment of illnesses. Essential oils and plant extracts are challenging the world now a days due to their potential as a source of biologically active compounds and natural antioxidants [2,3,4].These antioxidant activities of essential oils have formed the basis of many applications, including fresh and processed food preservation, pharmaceuticals, alternative medicine and natural therapies [2,5]. O. Bacilicum, L. popularly known as sweet basil in English, belongs to the family Lamiaceae, contains approximately 50-150 species and is found in tropical and sub tropical regions of Asia, Africa and Central and South America [6]. Basil is a condimental plant, used frequently in soups, desserts, pickles, pizza, spaghetti sauce, egg, cheese dishes, tomato juice, dressings, confectionery, salads, meat products etc. as a

flavoring agent. Traditionally, basil has been extensively utilized in food as a flavoring agent and in perfumery and medical industries [7]. The basil tea taken hot is good for treating Nausea, flatulence and dysentery [8]. The basil herb, especially its aromatic leaves possess excellent medicinal properties. That's why it has been used in traditional system of medicine as a tonic, vermifuge, diuretic, antispasmodic and for the treatment of upper respiratory tract infections [9]. The Arabian Peninsula [10] is the birth place of herbal drugs and the use of folk medicine has existed there since time immemorial. Traditional medicine is widely practiced still now in Saudi Arabia [11]. Based on the literature survey, the present study is the first to examine the chemical composition and Antibacterial and Antifungal activities of the essential oil from the areal parts of Ocimum basilicum L. in Jazan region, Saudi Arabia.

EXPERIMENTAL

Plant material and essential oil extraction: O. basilicum L. was collected as a whole plant from Greater Noida, during the flowering stage in October of 2020. The collected plant material was authenticated by a senior plant taxonomist,. As the aerial parts of the plant were used as flavoring agents and in traditional medicine, they were the focus of the investigation. Aerial parts were thus separated from the remaining plant material and were washed to remove soil particles, before being left to dry in shade at room temperature, after which they were ground by the grinder (IKA WERKE MF 10 basic). Following the procedure described by [12], the ground material (100 g of substance dissolved in 400 ml of double-distilled water in round-bottom flask of 1000 ml volume) was hydro distillated for 3 h using Clevenger apparatus (Klaus Hofmann Gmbh, Germany). Moisture was removed from the extracted oil using anhydrous sodium sulfate and the resultant oil was stored at 4 °C until required for further analysis.

GC-MS analysis conditions: The composition of the volatile oil was analyzed using gas chromatography (GC) and gas chromatography with mass spectrometry (GC-MS). The phyto-chemical analysis was carried out using the Agilent Technology 6890N instrument that allows for both GC and GC-MS. The chromatographic run was carried out at a column temperature in the 50−280 °C range, whereby 10 °C/min increments were used for temperatures below 140 °C, and 12 °C/min for 140° to 280 °C. Helium (99.99%) was used as carrier gas at a flow rate of 1.0 ml/min. Mass spectrometry analysis was performed to identify individual compounds, and the comparison of relative retention time with those of standard reference samples was performed. The mass spectra were interpreted using the reference library of the National Institute of Standards and Technology (NIST), US, along with Wiley 5 and mass finder, as well as data reported by [13]. The constituent percentages were measured based on the peak area.

Antimicrobial activity: Antimicrobial activity was assessed by using a wide range of gram-positive and gram-negative bacteria and fungi. The zone of inhibition against the microbes was determined using disc diffusion method, while the minimum inhibitory concentration (MIC) against each microorganism was determined using a micro broth dilution assay.

Microbial strains: The extracted oil was tested against a set of microorganisms. The bacteria and fungi strains were obtained from the laboratory of Microbiology, Sri Krishnadevaraya University,

India. The gram-positive micro-organisms used included Bacillus cereus (ATCC10876), Bacillus subtilis (ATCC 6633), Enterobacter cloacae (ATCC13047), Enterococcus faecalis (ATCC49452), Listeria monocytogenes (ATCC15313), Staphylococcus aureus (ATCC 25923), Micrococcus flavus (ATCC 9341), Staphylococcus epidrermis (A233) and Micrococcus luteus (ATCC9341).We also used the following gram-negative microorganisms: Acinetobacter baumannii (ATCC 19606), Escherichia coli (ATCC 25922), Klebsiella pneumonia (ATCC 27853), Proteus mirabilis (ATCC 35659), Pseudomonas aeruginosa (ATCC 27853), Salomella typhimurium (ATCC 13311), Citrobacter freundi (ATCC 13311), Enterobacteraerogenes (ATCC 13048).The fungi strains used comprised of Alternaria alternaria (MNHN 843390), Aspergillus flavus (MNHN 994294), Aspergillus fumigates (MNHN 566),Candida albicans (ATCC 26790), Cladosporiumherbarum (MNHN 3369), Fusarium oxyporum (MNHN 963917), Fusarium solani and Rhizoctomia saloni. All microorganisms were stored at 4 °C.

Determination of zone of inhibition by disc diffusion method: The antibacterial and antifungal activity of O. bacillicm L. volatile oil was investigated using the agar disc diffusion method. Microorganisms used were of referred Clinical and Laboratory Standards Institute, laboratory standards [14,15]. The culture suspensions were diluted so that 100.0 µL of the tested microorganisms contained 10^8cfu/mL of bacteria and 10^6cfu/mL of fungi strains. We utilized a Sabouraud Dextrose Agar (SDA) medium to spread the microorganisms. The discs were impregnated with 10.0 µL of the extracted essential oil before being placed on the agar medium containing the tested microorganisms. The plates were incubated at 37 °C for 24h for bacteria and at 30°C for 48h for fungal strains. Gentamicin and Amphotericin B served as reference compounds, allowing comparison of the antibacterial and antifungal activity exhibited by the essential oil. Each test was carried out in triplicate.

Determination of MIC values: The minimal inhibition concentration (MIC) values were determined using the micro broth dilution assay method, as recommended by Clinical and Laboratory Standards Institute CLSI 2016). All tests were performed in Sabouraud Dextrose broth and Muller-Hilton broth, for bacteria and fungi, respectively. O. bacillus L. essential oil was dissolved in 10% Dimethyl sulfoxide. The suspensions were prepared to contain 5.0×10^5 and 2.0×10^3cfu/mL of bacteria and fungi, respectively. The standard strains of these suspensions were soaked onto the micro plates, which were subsequently incubated at 37 °C for 24h for bacteria and at 30°C for 48h for fungi. The MIC was defined as the lowest concentration of the compound required for inhibiting the microorganism growth, and the results obtained were used to compare the antibacterial and antifungal activity of the oil with the aforementioned reference standards.

Statistical analysis: All experiments performed as a part of the present study were conducted in triplicate, and the results were presented as mean ± standard deviation. The statistical analysis was conducted by ANOVA using the SPSS21 software package.

RESULTS AND DISCUSSION

The essential oil was extracted from the aerial parts of the O. basilicum L. by hydro distillation using Clevenger apparatus. The yield of the essential oil was 1.59% (w/w). The chemical constituents of the O. basilicum L. Essential oil are separated by Gas Chromatography (GC) and characterized by employing gas chromatography- mass spectrometry (GC-MS) .As a GC-MS analysis, 39 chemical

components are accounted for 99.92% of the essential oil. Linalool was identified as the major component (41.28%), followed by geraniol (22.04%), estragole (7.60%), neroleacetate (5.01%). Other components accounted for trace are minor percentages of the total chemical composition. The results are shown in Table1.

The present research results were compared with previous studies with same species from Brazil [16] linalool (46.97%), 1,8-cineole (14.97%), γ-cadinene (5.32%) and δ-cadinol (5.14%) were major constituents from (99.9%) of the total essential oil. Research from Northern California [17] linalool (39.8%); estragole (20.5%); methyl cinnamate (12.9%); eugenol (9.1%) main components. Essential oil extracted using hydro distillation and solvent free microwave extraction from Egypt [18] Linalool (48.4), methyl chavicol (14.3), methyl eugenol (3.7) and linalool (43.5), methyl chavicol (13.3), methyl eugenol (6.1) were major components from (99.0%) and (99.3%) of the total essential oil respectively. From Ethiopia [19,20] estragole (38.23%), 1-isopropyl-4- methylenecyclohex-1-ene (11.11%), p-mentha-1 (7.0%), 8-diene (6.01%) and copaene (25.5%), p-menth-2-en-1-ol (7.7%) were major components obtained from (94.67%) and (76.7%) of the total essential oil respectively. researchers from different places from India [21,22], linalool (69.87%), geraniol (9.75%), p-allylanisole (6.02%), 1,8–cineole (4.90%) and geraniol (34.89%), citral (23.51%), linalool (2.21%), and eugenol (1.33%) major components from total (99.87 %) and (61.91%) essential oil extracted from Karnataka and West Bengal respectively. Linalool (65.38%), eugenol (5.26%), τ-cadinol (8.18%) were major components from Romania [23] essential oil extracted from (99.91%) of total essential oil. Essential oil from South Benin [24] were represented estrogale (44.02), linalool (28.24) and 1,8-cineole (10.40) are major components from the total (97.12%) essential oil. Other researcher from Tunisia [25] linalool (42.1%), (E)-methyl cinnamate (16.9%) and 1, 8-cineole (7.6%) were major components (99.91%) of total essential oil.

Research from Pakistan [26] represents the chemical composition of essential oils in summer, autumn, winter and spring. For summer linalool (56.7%), α-bergamotene (9.2%), epi α-cadinol (11.4%), linalool (60.5%), α-bergamotene (7.4%), α-cadinol (12.4%) , for autumn linalool (60.6%), α-bergamotene (7.8%), α-cadinol (8.6%) for winter and linalool (58.6%) α-bergamotene (7.6%), α-cadinol (10.4%), for spring season of the total essential oil (99.86%), (99.0%), (98.0%) and (99.7%) of total essential oil from different regions respectively. For asummer and winter the percentage of linalool composition was similar and also closely same in autumn and winter seasons. Essential oil was extracted from different places (Lema and Utengule) from Tanzania [6] 1,8-cineole (54.3%), α-terpineol (6.6%), β-pinene (8.15%) and E-myroxide (19.6%), rosefuran epoxide (6.03%), α-copaene (7.5%), α-humulene (6.28%), caryophyllene oxide (11.4%), humulene epoxide II (11.0%) were reported from total (91.99%) and (82.11%) essential oil respectively. Other researcher [27] from France, estragole (52.60%), limonene (13.64%), fenchone (5.70%), exo-fenchyle acetate (10.99%) were major components (95.04%) of the total essential oil. Research from different places [28] (Yatta, Sagana and Kariti) of Kenya, linalool (28.2%), camphor (32.6%), terpinen-4-ol (12.0%), linalool (95.7%), geranial (49.6%), neral (30.9%), citronellol (6.5%) were major components from the (99.7%, 95.7% and 94.6%) of the total essential oil respectively. essential oil extracted from Sagana was found only one component that is linalool. Essential oil extracted from Austria [29], linalool (28.6%), estragole (21.7%), (E)-methyl cinnamate (14.3%) total (97.0%) of the oil. Research from Algeria [30] linalool (32.83%), linalyl acetate (16.0%), elemol (7.44%), geranyl acetate (6.18%), myrcene (6.12%) and allo-ocimene (5.02%) were major components from the (97.56%) of the total essential oil. Essential oil extracted from Oman [31] shows linalool (69.86%), p-allylanisole (6.02%) and 1,8-ciniole

(4.90%) were major components from (99.87%) of the total essential oil. Compared to the present study the chemical composition and percentage of major composition is totally different, due to the geographical conditions and seasons. Researches (West Bengal and Karnataka) from India [22,21], from Ethiopia [20,19], Tanzania [6], France [27], representing major components are totally different from the present study.

The antibacterial activity of the extracted essential oil, tested with the disc diffusion method for zone of inhibition (mm) and minimum inhibition concentration (µg/mL) are shown in Table 2. Based on the zone of inhibition diameter, the essential oil shows the highest antibacterial activity against the bacteria S. aureus, B. cereus, B. subtilis, E. cloacae, M. flavus, L. monocytogenes, E. coli and E. faecalis with respect to zone of inhibition 32.0±0.9, 32.0±0.9, 29.1±1.2, 25.2±1.0, 24.2±1.0, 22.2±1.2, 22.4±0.3 and 20.4±0.6 mm (ρ≤0.01) respectively. Moderate activity was observed against M. luteus, C. freundi, E. aerogenes, S. epidrermis, S. typhimurium and P.mirabilis 15.4±0.9, 14.1±0.6, 12.3±0.4, 10.0±0.7, 10.0±0.1 and 10.5±0.9 mm (ρ≤0.01) respectively. Minimal activity was observed against A. baumannii, K. pneumonia and P. aeruginosa 9.3±0.3, 5.1±0.2 and 4.2±0.4 mm (ρ≤0.01) respectively. These results indicated that the essential oil was obtained from potential antibacterial activity. The MIC values for the essential oils were in the range between (1.0±0.2to 5.0±1.2 µg/mL). The results are shown in Table 2.

The essential oil shows potent antifungal activity against yeast and fungal strains. Essential oils against A. alternaria, A. fumigates and Fusarium oxyporum 32.1±0.1, 31.8±0.8 and 30.4±0.2 mm ((p ≤ 0.01) shows strong antifungal activity respectively. In addition A. flavus and Cladosporium herbarum 21.0±0.6 and 20.2±0.1 mm (ρ≤0.01) show moderate antifungal activity respectively. The minimal antifungal activity was shown by F. solani, C. albicans, R. solani 16.2±1.0, 15.4±0.6 and 13.0±0.7 (ρ≤0.01) respectively. The minimal inhibition concentration values are in the range between 1.0±0.3 to 4.5±0.8 µg/ml for yeast and fungi respectively. The results were shown in Table 3.

In the present study O. basilicum essential oil shows potential antibacterial activity for gram positive bacteria than gram negative bacteria. Research from [32,23,22,6,26] O. basilicum essential oil shows potential antibacterial activity against a wide range of gram positive microorganisms. A research [4] reported O. basilicum essential oils showed intermediate antibacterial activity for Gram positive bacteria. Whereas O. basilicum essential oils [2, 33] against to gram positive bacteria more sensitive than those of their counterpart and also antifungal activity of O. basilicum essential oil was [2, 34] reported and its main component was linalool. The antimicrobial activity of the essential oils from O. basilicum may be due to the presence of high percentage of linalool [34-37].

Chemical constituents of the essential oils of the plant extracts and their inhibition capacity upon different types of microbes are varying from the regions. Geographical conditions, seasonal variations, the age of the plants, the parts of the plants, genetic differences and experimental conditions are also responsible for the changes in the types of chemical compounds as well as the inhibition processes towards the different microbes. The present study declared that because of the Linalool (41.28%) and Geraniole (22.04%), Estragole (7.60%) and Neroleacetate (5.01%) major components the essential oil showed strong antibacterial activity.

The essential oil from Ethiopia [19] by GC-MS analysis and identifying 15 chemical constituents with Estragole (38.22%), 1-isopropyl-4-methlenecyclohex-1-ene (11.104%) being the dominant ones. Estragole holds the highest composition of the essential oil. The essential oil exhibits strong antifungal activity against two tested fungal strains which are A.niger and R. bataticola. The strong

inhibition activity of sweet basil oil was observed against R. bataticola (8.20 mm and 12.75mm).The essential oil shows the strong antibacterial activity against two tested bacteria E. coli and S. aureus . The oil has a stronger antibacterial activity toward E. coli than S. aureus. The essential oil which is hydro distillated from aerial parts of the Omani basil [31] contains 36 chemical constituents and in these the major components were Linalool (69.87%) followed by geraniol (9.75%), p-allylanisole (6.02%), 1,8-cineole (4.90%), trans-bergamotene (2.36%) and neryle acetate (1.24%). The essential oil exhibits strong antibacterial activity. The basil oil inhibited the growth of two Gram- positive (S. aureus and B. cereus) and two Gram - negative bacteria. B. cereus showed the highest susceptibility to the extracted oil followed by E. coli, S. epidermis, K. pneumonia and P. aeruginosa were found to be highly resistant to O. basilicum L essential oil.

The Egyptian basil [18] consists of 42 chemical constituents. The most predominant compounds were Linalool (33.9%), Eugenol (8.31%) and 2, 6- Dimethyl-6- (4-methyl-3- pentenyl)-bicycle[3.1.1]hept-2-ene (8.04%) of the oil constituents. The bacterial strains which are used for the antibacterial activity of the essential oil are Escherichia coli, Staphylococcus aureus, Bacillus cereus and the fungal strains which are used in the antifungal activity are Saccaromyces cerevisial, Aspergillus niger, Candida albicans. Shows Strong antimicrobial activity of the Egyptian O. basilicum L essential oil was shown in E.coli and S. cerevisial. The qualitative and quantitative analysis of the Republic of Srpska [38] basil essential oil was done by GC-MS and GC-FID spectrometry and 65 chemical constituents were identified. Among these Linalool (31.6%) and Methyle chavicol (23.8%) are dominant. All tested microbial strains were shown strong antibacterial activity towards the commercial antimicrobial agents Ciproflaxacin and Gentamicin. S.enterica, P.stuartii. coagulase positive Staphylococus and Streptococcus group D were shown stronger antimicrobial activity in comparison to commercial antimicrobial agent Ciprofloxacin. E.coli, S.enterica, P. stuartii, coagulase-positive Staphylococcus, Streptococcus group D and Salmonella spp. were exhibited stronger antimicrobial activity on compared to commercial antimicrobial agent Gentamicin. In the same time, the oil showed stronger antifungal activity on C.albicans than commercial antimicrobial agent Metronidazol. The essential oil of the O.basilicum from Benin [24] consists of estragol (or methichavicol) (44.60%), Linalool (24.60%) and 1,8-cineole (8.22%) major elements among 25 chemical constituents. Qualitative and quantitative analysis of the essential oil was done by GC-FID and GC-MS analysis. The antimicrobial effect of the essential oil is varied with the different microbial strains because of the major elements. Antibacterial activity of this essential oil showed very week in the inhibition of the tested microorganisms L.monocytogenesis and S.typhimurium.

The relation between the percentage of the chemical constituents, the most naturally abundant compound and the interaction between them and the antibacterial activity was reported previous studies [39]. Presence of more number of free heteroatoms such as oxygen and nitrogen containing components has exhibited contribution to maximum antibacterial activity [40]. Oxygenated terpenes show potential antibacterial activity compared to normal terpene hydrocarbons. The present study has further extended knowledge on the contribution of the chemical constituents of essential oils to anti-agent properties. The present study shows more antibacterial activity of the essential oil toward gram-positive than gram negative bacteria, due to the natural abundance of ketones and alcohols in the essential oils. Mentha pulegium essential oil shows highest antimicrobial activity against penicillium and mucor, because of a ketone major chemical constituent [40].

3. Conclusion

Essential oils and their components generally displayed strong antibacterial and antifungal properties which are useful in daily life in foods and as preventive agents from various diseases. O.basilicum essential oil also shows strong antibacterial and antifungal activities because of the chemical constituents like Linalool and Geraniole. This comprehensive review revealed that although the essential oil from different geographical regions exist various chemical constituents, they are being used successfully in a traditional way as well as modern methods such as antimicrobial and antifungal activities.

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Table 1: Chemical composition of Ocimum bacilicum L essential oil

[a]Reported K. I	[b]K. I of present study	Name of the compound	% of the compound
953	959	Camphene	0.28
980	983	β- pinene	1.99
1031	1028	Limonene	0.60
1033	1059	Eucalyptol	2.00
1040	1027	β-ocimene	1.53
1061	1182	6-methyl-2- (oxiran-2-yl)hept-5-en-2-ol	0.49
1080	1164	Linalool oxide	0.95
1088	1096	Terpinolene	0.31
1098	1109	Linalool	41.28
1143	1121	Camphor	0.42
1149	1148	β-sesquiphellandrene	0.10
1165	1156	Borneol	1.36
1195	1195	Estragole	7.60
1228	1228	Geraniol	22.04
1237	1233	Pulegone	0.09
1270	1252	Geranial	0.29
1288	1285	P-menth-1-ene-8-ol	0.31
1356	1392	Eugenol	3.38
1361	1361	Eugenol methyl ether	0.22
1365	1364	Nerol acetate	5.01
1375	1372	β-elemene	0.37
1390	1384	β-cubebene	0.16
1436	1432	α-bergamotene	3.21
1438	1446	Caryophyllene	0.49
1439	1440	δ-guaiene	0.36
1458	1440	β-farnesene	0.39
1477	1477	γ-muurolene	0.60
1519	1517	Calamenene	0.13
1547	1522	Elemol	0.08
1564	1564	Nerolidol	0.09
1576	1573	Spathulenol	0.11
1581	1581	Caryophyllene oxide	0.11
1640	1640	τ-cadinol	2.52

1642	1642	Cubenol	0.48
1647	1647	methyl cis-jasmonate	0.06
1652	1652	α-cadinol	0.13
1673	1672	Bisabolol	0.09
1730	1732	Geranic acid	0.14
1949	1945	Phytol	0.06

[a]Reported K.I: Kovats Index reported in the Literature;

[b]K.I: Kovats index of present study

Table: 2. Antibacterial activity of Ocimum basilicum L essential oil (5.0 µg/ml) in disc diffusion method.

Bacterial strains	Zone inhibition (mm[a])		MIC[a] (µg/mL)	
	Essential oil	RA[b]	Essential oil	RA[b]
B. cereus	30.2±1.3	28.2±1.1	1.0±0.2	0.5±0.2
B. subtilis	29.1±1.2	29.3±1.0	1.5±0.4	0.5±0.2
E. cloacae	25.2±1.0	31.1±1.2	1.5±0.4	0.5±0.2
E. faecalis	20.4±0.6	21.4±0.8	2.0±0.2	0.5±0.2
L. monocytogenes	22.2±1.2	26.1±0.9	2.0±0.2	0.5±0.2
S. aureus	32.0±0.9	34.2±1.1	1.5±0.4	0.5±0.2
M. flavus	24.2±1.0	28.2±0.7	1.5±0.4	0.5±0.2
S. epidrermis	10.0±0.7	25.4±1.2	4.5±0.8	0.5±0.2
M. luteus	15.4±0.9	22.3±0.8	4.0±0.4	0.5±0.2
A. baumannii	9.3±0.3	20.2±1.1	4.5±0.8	1.0±0.6
E. coli	22.4±0.3	26.1±1.0	2.5±0.2	0.5±0.2
K. pneumonia	5.1±0.2	20.2±1.2	5.0±1.2	0.5±0.2
P. mirabilis	10.5±0.9	22.2±0.8	4.0±0.8	1.0±0.8
P. aeruginosa	4.2±0.4	29.4±0.8	5.0±1.2	0.5±0.2
S. typhimurium	10.0±0.1	24.6±0.7	4.5±0.8	0.5±0.2
C. freundi	14.1±0.6	20.3±1.1	4.5±0.8	0.5±0.2
E. aerogenes	12.3±0.4	19.3±0.8	4.0±0.4	1.0±0.6

[a]values represent means ± standard deviations for triplicate experiments

[b]RA: Reference of antibiotics Gentamicin for bacteria used was 10 µg/disc

Table-3: In vitro antifungal activity of essential oil

Fungal strains	Essential oil[a]		Amphotericin[b]	
	DD[c]	MIC[d]	DD[c]	MIC[d]
Alternaria alternaria (MNHN 843390)	32.1±0.1	1.5±0.6	27.2±0.4	1.5±0.4
Aspergillus flavus (MNHN 994294)	21.0±0.6	1.5±0.6	25.7±0.8	2.5±0.6
Aspergillus fumigates (MNHN 566)	31.8±0.8	1.0±0.3	20.6±0.5	1.0±0.1
Candida albicans (ATCC 26790)	15.4±0.6	2.0 ±0.1	22.8±0.3	2.0±0.6
Cladosporium herbarum (MNHN 3369)	20.2±0.1	1.5±0.6	16.1±0.6	2.5±0.7
Fusarium oxyporum (MNHN 963917)	30.4±0.2	1.5±0.2	28.4±0.1	1.5±0.2
F. solani	16.2±1.0	3.5±0.6	20.2±1.0	1.0±0.6

R. solani	13.0±0.7	4.5±0.8	19.3±1.1	1.0±0.6

[a]essential oil impregnated with 1.0 µL/disc.

[b]Amphotericin B impregnated with (10 µg/disc).

[c]disc diameter (mm) included, [d] minimal inhibition concentrations in (µg/mL)