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Full Length Article

In vitro Antibacterial and Antifungal Activity of some Oils, Chemical Analysis and their FTIR Studies

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Abstract

In the present study, five oils (Clove, Arugula, Cactus, Almond and Sesame) were screened for their antimicrobial activity against a panel of pathogenic microbes. Poisoned food technique and Agar disc diffusion method were used to determine antifungal and antibacterial activity. Minimum inhibitory concentration**,** minimum bactericidal and fungicidal concentrations were determined by Broth dilution method. Oils showed varied antimicrobial activity against both bacteria and fungi. Clove oil showed strong antibacterial and antifungal activity followed by Arugula and Cactus, while Sesame oil was least inhibitory. Clove oil completely arrested the growth of *Colletotrichum gleosporoides*, while *S. aureus* and *E. coli* were inhibited with a large zone of inhibition (28.16 ± 0.28 mm; 24.00 ± 0.00 mm). The MIC of the oils against bacteria and fungi was in range of 0.25-32 µL/mL. The three potent oils were subjected to GC-MS and FTIR analyses to identify their chemical composition and functional groups. Major chemical components present in oils were Eugenol, Caryophyllene, Dibenzofuran, Cyclopentadecanol, di-t-Butylacetylene and 3,5-Cyclohexadiene-1,2-dione,3,5-bis(1,1-dimethylethyl)-; many of which are important antimicrobials in nature. Scanning electron microscopy of clove oil treated pathogens showed morphological changes, membrane disruption and cell leakage. In conclusion, essential oils of clove, arugula, and cactus would serve as promising candidates with broad application as natural product in controlling many pathogenic fungi and bacteria.

Keywords: Plant derived essential oils; GC-MS; FT-IR; Multidrug resistant; Post-harvest fungi; SEM; MIC

Introduction

Worldwide microbial infections are a major concern in primary health care and result in more deaths than any other single cause. Antimicrobial medications are considered as lifesaving drugs against all infectious diseases which are potential threats to life. They have played pivotal role in decreasing the mortality rates and increasing life expectancy. However, these miracle drugs which once revolutionized medical history had a short life due to emergence of multiple drug resistant strains and spread of resistance genes to pathogenic bacteria (Davies and Davies, 2010). Lack of new drugs, challenging regulatory requirement, misuse and overuse of drugs has further increased the crisis (Michael *et al.*, 2014). Another concern is the disturbance that arises in the gut micro flora due to antimicrobial therapy. Both antibiotics and antifungal drugs affect the commensal microbiota of the host besides targeting the pathogen. This imbalance can result in altered micro ecology**,** making the host vulnerable to various infections and may often cause detrimental effects on human immune system (Bailey *et al.*, 2010; Torey and Heather, 2012).

Another problem that needs immediate attention is control of post-harvest pathogens. In developing countries, plant pathogens cause considerable economic losses. Among them, fungal diseases play a major role accounting to 12% or more of these losses (Horbach *et al*., 2010; Shukla *et al*., 2012). Various fungal species of *Aspergillus, Fusarium, Alternaria and Penicillium* can produce mycotoxins and consumption of contaminated fruits and vegetables can be toxic to both humans and animals (Paster and Barkai-Golan, 2008). Currently, fungicide application is the most effective way to control fungal pathogens. However, repeated use of synthetic fungicides results in its accumulation in food chain**;** resistance by pathogens to fungicides, toxicity to non-target organisms and environmental problems (Lee *et al.*, 2009). Additionally, synthetic fungicides are more carcinogenic than herbicides and insecticides together (Wilson *et al*., 1997). Therefore, direct exposure of fungicides and its related side effects is a major threat to human and animal health (Marin *et al*., 2008).

Plants and their products have gathered renewed interest worldwide. Medications formulated with herbs, spices and oils have taken resurgence. Essential oils (EO) derived from medicinal and aromatic plants are a wide array of phytochemicals that are produced by plants to serve various protecting functions. They have been used since time immemorial both in traditional medicine and as home remedies. Essential oils are known to possess antibacterial, antifungal and anti-inflammatory properties (Gulluce *et al.*,

2007; Lang and Buchbauer, 2012). Recent studies have shown the potential of radical scavenging properties of essential oils and their role in controlling infectious diseases and food preservation (Nweze and Okafor, 2010; Saikat *et al*., 2010; Okoh *et al*., 2017). Additionally, essential oils are environmental friendly because they degrade quickly and do not leave any toxic residues; they have also shown low toxicity towards human beings (Isman, 2000).

Hence, keeping this in view**,** this study aimed at screening essential oils for their ability to combat some post-harvest fungi and human bacteria. To understand the role of chemical compounds and their functional groups in antimicrobial activity; oils were subjected to GCMS, FTIR analysis.

Materials and Methods

Bacterial and Fungal Strains

The bacterial strains used in this study were *Pseudomonas aeroginosa* ATCC *27853, Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC 25966, Methicillin resistant *Staphylococcus aureus* (MRSA) ATCC 3345, *Streptococcus pyogenes* ATCC19615, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *S. aureus* ATCC 25923. All the test strains were procured from King Khalid hospital, Riyadh, Saudi Arabia. Phytopathogens, *Penicillium citrinum*, *Alternaria alternata*, *Fusarium oxysporum*, *F. solani*, *Aspergillus niger*, *Aspergillus fumigatus* and *Colletotrichum gloeosporioides* were acquired from Botany and Microbiology Department (King Saud University). Stock cultures of fungi and bacteria were maintained on slants of Potato dextrose agar and Nutrient agar. Antibiotic discs were purchased from Sigma-Aldrich (USA).

Essential Oils (EO) and Media

All the essential oils were purchased, and the same lot of oils were used throughout our study. Following oils were used in this study: *Opuntia ficus-indica* (Cactus oil) and *Eruca sativa* (Arugula oil) were purchased from Wadi Al Nahil for essential oils and oud, Saudi Arabia. *Prunus dulcis* (almond oil), *Sesamum indicum* (Sessame oil), *Syzygium aromaticum* (Clove oil) were purchased from Gopaldas Visran Pharmaceuticals, Tegraj and company Pvt Ltd, India. Based on literature survey oils were purchased from known commercial suppliers with ascertained purity. Potato dextrose agar, Muller Hinton agar, Muller Hinton broth and Potato dextrose broth were purchased from Himedia, Riyadh, Saudi Arabia.

Antibacterial Activity

Disc diffusion assay: Antibacterial activity of oils was determined by disc-diffusion method (Gulluce *et al.,* 2007; CLSI, 2014). Mueller–Hinton Agar plates (9 cm) were used to test the sensitivity of bacterial strains towards essential oils. Each plate was seeded with 24 h old bacterial suspension (10^6 CFU/mL) and spread evenly with a sterilized cotton swab. After drying, 6 mm sterile filter paper discs were impregnated with 10 μL of essential oils separately and placed on the surface of the Muller Hinton agar plates individually. All the plates were sealed with parafilm and incubated for 24 h at 37°C. Diameter of the inhibition zones was measured (mm) after 24 h. Following antibiotic discs were used as positive control keeping in mind the antibiotic resistance of pathogens: Vancomycin (30 μg), Ampicillin (10 μg**)**, Tetracycline (30 μg), Gentamycin (40 μg) and Optichin (10 μg). All the tests were carried out in triplicates and mean inhibition was calculated by Standard deviation.

Poisoned Food Technique

Antifungal activity of essential oils was carried out by poison food technique with slight modification (Sharma and Tripathi, 2006). A 9-cm sterile petri plate was poisoned with measured amount of essential oil. To ensure thorough mixing of essential oils, they were dispersed as an emulsion in sterile agar suspension (0.2%) and immediately added to PDA prior to being emptied into Petri dishes. After solidification, mycelia plug of 6mm from 7 days old culture plate of the test fungi was placed in the centre of the plate in an upside-down manner and sealed with parafilm. Inoculated plates were incubated at 25 ± 2°C for one week and all the experiments were run in triplicates. PDA plates without oil served as Control plates. After seven days, the diameter of the fungal colony in test plates and control was measured (mm) and percentage mycelium inhibition was calculated. Calculation for percentage mycelia inhibition was done as follows:

$$PI = (Co\text{-}TM)/Co \times 100$$

Where, PI (Percentage inhibition), Co-Control and TM-Treatment.

Minimum Inhibitory Concentration

Minimum inhibitory concentration of essential oils was determined by broth micro-dilution method for fungi and bacteria, with slight modification (CLSI, 2008, 2012). A two-fold dilution series of oil was prepared in the range of 0.03 – 64 μL/mL by using Muller Hinton broth for bacteria and Potato dextrose broth for fungi. Each tube containing the dilution was inoculated with bacterial and fungal suspension according to the guidelines. Tubes were incubated at 37°C (24 h) for bacteria and 28 ± 2°C for 72 h for fungi. Positive control was Muller Hinton broth and Potato dextrose broth without oil. The lowest concentration showing no visible growth (turbidity) was regarded as minimum inhibitory concentration.

Minimum Microbicidal Concentration (MMC)

Minimum bactericidal (MBC) and fungicidal concentration (MFC) was determined by following the method of Espinel-Ingroff *et al*. (2002) with slight modification. Briefly 10 µL of inoculum was withdrawn from the culture tubes which had no visual turbidity and inoculated on MHA or PDA plate for

bacteria and fungi respectively. Respectively these sub-cultured plates were then incubated at 37°C for a period of 24 h for bacteria and 28°C for 96 h for fungi. The concentrations which did not show growth on subculture plates were taken as MBC and MFC. All assays were carried out in duplicate.

GC MS

GC-MS analysis of essential oils was carried out on Agilent technologies model 7890GC coupled with a mass detector Agilent 5975 GC/MSD. The Analytic column was Agilent J&W HP-5MS (phenyl methyl siloxane, 30 m × 0.25 mm, 0.25 µm). Carrier gas Helium (1 mL/min) was used to separate components. The different instrument conditions were standardized as follow, injector temperature was set at 250°C (mass analyzer). During GC extraction the program of oven temperature was 2 min at 45°C, increased to a temperature of 200°C at a rate of 3°C/min. delay time for solvent was 5 min. Transfer line temperature was 280°C, Mass spectra were taken at an ionization mode with an electron impact at 70 eV; Ion source temperature was 230°C, mass scan range was 50–400 m/z. The GC run time was 90 min. Essential oils (10 μL) were diluted in n hexane (1 mL). NIST libraries were used for identification and interpretation of mass spectrum.

FTIR

Oil samples were subjected to Fourier transform infra-red analysis using a Nicolet 6700 Spectrometer (Thermo Scientific, USA) equipped with a beam splitter and DTGS detector. The IR spectrum in the scan range of 400–4000 cm^{-1} was obtained and analyzed by using a OMNIC software.

Scanning Electron Microscopy

To determine the effect of clove and arugula oil on morphology of the *S. aureus* and *E. coli*, the overnight grown cultures were treated with their MIC concentration of 0.25 μL/mL (clove oil) and 2 μL/mL (arugula oil) respectively (Li *et al*., 2014). This suspension was incubated at 37°C in a water bath shaker at 150 rpm for 4 h. After which it was centrifuged at 9000 rpm for 10 min and the supernatant was discarded. Remaining pellets was subjected to three washes with Phosphate buffer (pH 7.4). Glutaraldehyde (2.5%) was used for fixing, followed by treatment with osmium tetroxide (1%), and finally dehydrated in a series of graded alcohol gradient between 60 100%. After drying with carbon dioxide, the cells were mounted onto stubs coated with gold (thin layer) and examined scanning electron microscope [LTD JSM-6060LV (JEOL-Japan)].

Results

Antibacterial Activity of Essential Oils by Disc Diffusion Method

The antibacterial activity exhibited by essential oils against pathogenic bacteria is presented in Table 1. Clove and arugula oil inhibited all the pathogens tested with significant inhibition zones. Clove oil inhibited *S. aureus* with a largest zone of inhibition (28.16 ± 0.28 mm) followed by *E. coli* (24.33 ± 0.57 mm) and *B. subtilus* (22.00 ± 0.00 mm). Arugula and cactus oil also proved to be highly potent in inhibiting *S. aureus and B. subtilus* (23.00 ± 0.00, 20.16 ± 0.28 mm). Almond oil had moderate inhibitory effect on all the bacterial strains while sesame oil was least active.

Both Gram positive and negative organisms screened showed sensitivity to clove and arugula oil; however, sesame oil inhibited Gram-positive bacteria weakly but was unable to inhibit gram negative isolates. *S. aureus* and *B. subtilus* were inhibited by all the oils tested.

Minimum Inhibitory and Minimum Bactericidal Activity of Oils (MIC and MBC)

Minimum inhibitory concentrations were tested for all the bacterial isolates used in the study, irrespective of their poor zones of inhibition (Table 2). The MIC of the oils was in the range of 0.25–32 μL/mL, while the MBC was 0.25–64 μL/mL. Clove oil showed inhibitory activity at a lowest concentration of 0.25 μL/mL. MBC ranged between 0.25 to 8 μL/mL, except *E. faecalis* it was 16 μL/mL. *S. aureus* and *B. subtilus* showed bactericidal activity with clove oil at a very low concentration of 0.25 μL/mL and 1 μL/mL respectively. Sesame oil failed to inhibit MRSA at the highest concentration of 64 μL/mL. Since most of the organisms were not inhibited by the highest concentration of sesame, cactus and almond oil, they were not tested for bactericidal activity.

Antifungal Activity by Poisoned Food Technique

Poisoned food technique was used to determine the effect of oils on mycelial growth of post-harvest pathogens. Amongst all the oils screened sesame and cactus oil had poor inhibitory effect on fungi tested while all the test pathogens were highly sensitive to clove, arugula, and almond oil (Table 3).

C. gloeosporioides showed complete inhibition (100%) with clove oil followed by *P. citrinum* (92.07 ± 2.78) and *A. alternata* (90.00 ± 1.00). However, both almond and cactus oil had no effect on the growth of *C. gloeosporioides*. All the oils screened had significant inhibitory effect on *A. niger* and *A. fumigatus* except cactus oil. Similarly, all the oils inhibited *P. citrinum* strongly except sesame oil.

Minimum Inhibitory and Minimum Fungicidal Activity of Oils (MIC and MFC)

Essential oils inhibited mycelial growth of test fungi with an MIC in the range of 0.03–64 μL/mL. Most fungi were inhibited by oils in the range of 0.03–32 μL/mL. However, the resistant strains of fungi required a higher concentration of 64 μL/mL. Clove oil inhibited the growth of *C.*

Table 1: Antibacterial activity of essential oils against human pathogenic bacteria

Bacterial isolates	Essential oils (Zone of inhibition in mm)					
	Arugula	Almond	Cactus	Clove	Sesame	Antibiotic discs
MRSA	18.66 ± 0.57	10.83 ± 0.28	NI	20.33 ± 0.57	10.00 ± 0.00	24.00 ± 0.00(A)
S. aureus	23.00 ± 0.00	19.00 ± 1.00	20.16 ± 0.28	28.16 ± 0.28	11.33 ± 0.57	27.67 ± 1.16(A)
S. pyogenes	10.33 ± 0.57	NI	18.00 ± 0.00	21.66 ± 1.57	15.00 ± 0.00	23.00 ± 0.58(A)
B. subtilis	19.50 ± 1.00	17.50 ± 0.00	20.83 ± 0.28	22.00 ± 0.00	13.66 ± 0.50	27.33 ± 0.58(T)
E. faecalis	15.50 ± 0.50	NI	NI	16.16 ± 0.57	NI	25.83 ± 0.57(O)
E. coli	20.00 ± 1.00	11.00 ± 0.00	18.66 ± 0.57	24.33 ± 0.57	NI	20.00 ± 0.00(G)
P. aeroginosa	16.66 ± 1.15	14.33 ± 0.57	NI	18.83 ± 0.28	9.00 ± 1.00	0.00(G)
K. pneumoniae	17.83 ± 0.57	15.00 ± 1.00	14.00 ± 0.00	20.83 ± 0.76	NI	14.33 ± 0.58(O)

MRSA-Methicillin resistant *S. aureus*, Inhibition zone (mm) ± SD of three replicates
NI-Indicates no inhibition, Antibiotics: **A**-Ampicillin; **V**- Vancomycin; **T**- Tetracycline; **G**- Gentamycin; **O**-Optichin

Table 2: Minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC) of essential oils (µL/mL)

Bacterial isolates	Essential oil source									
	Arugula		Almond		Cactus		Clove		Sesame	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
MRSA	8	16	NI	NT	NI	NT	4	4	32	>64
S. aureus	2	4	16	32	16	16	0.25	0.25	16	32
S. pyogenes	NI	NT	NI	NT	32	64	4	4	8	16
B. subtilus	4	8	8	16	8	8	0.5	1	NI	NT
E. facecalis	16	32	NI	NT	NI	NT	8	16	NI	NT
E. coli	2	4	NI	NT	8	16	2	2	NI	NT
P. aeroginosa	8	16	32	>64	NI	NT	4	4	NI	NT
K. pneumoniae	4	8	32	32	32	64	2	4	NI	NT

Table 3: Antifungal activity of essential oils and percentage inhibition of mycelia growth

Fungal isolates	Essential oils (Percentage mycelia inhibition)					
	Arugula	Almond	Cactus	Clove	Sesame	Control
C. gloeosporioides	62.59 ± 1.28	NI	NI	100	47.33	C-100.00
F. monoliforme	44.88 ± 0.19	21.94 ± 0.48	NI	78.33 ± 1.67	NI	M-100.00
F. oxysporum	56.14 ± 0.06	30.00 ± 1.00	42.00 ± 1.00	67.40 ± 1.28	32.77 ± 0.69	M-98.66
F. solani	63.77 ± 1.07	40.18 ± 0.31	36.44 ± 0.76	84.07 ± 0.64	29.00 ± 1.00	M-90.16 ± 0.28
P. citrinum	70.37 ± 0.64	86.10 ± 0.95	40.00 ± 1.00	92.07 ± 2.78	27.03 ± 1.28	M-90.16 ± 0.28
A. alternata	59.00 ± 1.00	72.14 ± 0.12	50.00 ± 0.00	90.00 ± 1.00	31.33 ± 0.57	M-83.33 ± 0.58
A. niger	78.00 ± 1.00	49.33 ± 0.57	NI	76.66 ± 1.11	54.77 ± 0.38	C-72.00 ± 0.00
A. fumigatus	82.00 ± 0.00	57.58 ± 1.15	30.00 ± 0.00	88.51 ± 0.64	64.66 ± 1.15	C-80.00 ± 1.00

C- Carbendazem , M-Mancozeb - (0.2%)**.** All the values are ± SD of three replicates

Table 4: Minimum inhibitory and minimum fungicidal concentrations (MFC) of essential oils

Fungal isolates	Essential oil source									
	Arugula		Almond		Cactus		Clove		Sesame	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
C. gloeosporioides	4	8	NI	NT	NI	NI	0.03	0.03	32	32
F. monoliforme	32	64	32	> 64	NI	NI	4	4	NI	NI
F. oxysporum	2	4	32	32	16	32	2	4	>64	NT
F. solnai	8	8	16	32	> 64	NT	2	2	>64	NT
P. citrinum	2	4	2	4	32	32	0.06	0.125	NI	NT
A. alternata	4	8	8	8	8	16	0.5	1	>64	NT
A. niger	4	4	8	32	NI	NT	4	4	16	32
A. fumigatus	2	4	8	16	>64	NT	1	2	32	32

NI-indicates no inhibition and NT-indicates not tested

gloeosporioides at a very low concentration of 0.03 μL/mL followed by *P. citrinum* at 0.06 μL/mL. Almond inhibited *P. citrinum* at 2 μL/mL. Highest MIC was shown by sesame oil > 64 μL/mL. Minimum bactericidal concentrations ranged between 0.03–8 μL/mL for clove followed by 4–64 μL/mL for arugula and almond and 16–> 64 μL/mL for cacti and sesame oil (Table 4).

GC/MS Analysis

Essentials oils (Clove, Arugula and Cacti) exhibiting significant antimicrobial activity was subjected to analyses by GCMS technique. The major compounds identified in clove oil were Eugenol and Caryophyllene along with 2-Heptanone; 3-Allyl-6-Methoxyphenol; Acetyl eugenol (Table 5).

The major bioactive chemical compounds identified from arugula oil were Dibenzofuran; 6-Methyl-6-hepten-4-yn-2-ol; Acetic acid, mercapto-, methyl ester; Dimethyl Sulphur; Cyclopentadecanol; 9-Hexadecen-1-ol, (Z)-; Isocyanic Acid n-Octadecyl Ester; Oxalocyclododecan-2-one; Brassylic acid (Tridecanedioic acid, dimethyl ester) and Cis-9-octadecen-1-ol (Table 6).

The chemical compound found in cactus oil were di- t-Butylacetylene, 9-Tetradecan -1-ol, Bicyclo [2.2.1] hept-5-en-2-ol; 2,4 Hexadiene; 3,5,Cyclohexadiene-1-2-dione,3,5-bis (1,1-dimethylethyl); and 1,2,3,4,5,6-Hexahydro-1,5,5-tetramethyl-2,4a-methanonaph-7(4Ah)-one (Table 7).

Table 5: Chemical composition of clove oil

Name of the compound	Molecular weight	Formula
2-Heptanone	114	$C_7H_{14}O$
Acetyl eugenol	222	$C_{15}H_{26}O$
3-Allyl-6-methoxy phenyl acetate	206	$C_{12}H_{14}O_3$
Eugenol	164	$C_{10}H_{12}O_2$
Caryophyllene	204	$C_{15}H_2O_3$

Table 6: Chemical composition of Arugula oil

Name of the compound	Molecular weight	Formula
Acetic acid, mercapto-, methyl ester	106	$C_3H_6O_2S$
Dimethyl sulphur	62	C_2H_6S
6-Methyl-6-hepten-4-yn-2-ol	124	$C_8H_{12}O$
Dibenzofuran	168	$C_{12}H_8O$
Cyclopentadecanol	226	$C_{15}H_{30}O$
9-Hexadecen-1-ol, (Z)-	240	$C_{16}H_{32}O$
Isocyanic Acid n-Octadecyl Ester	295	$C_{19}H_{37}NO$
Oxalocyclododecan-2-one	184	$C_{11}H_{20}O_2$
Brassylic acid (Tridecanedioic acid, dimethyl ester)	272	$C_{15}H_{28}O_4$
Cis-9-octadecen-1-ol	268	$C_{18}H_{36}O_4$

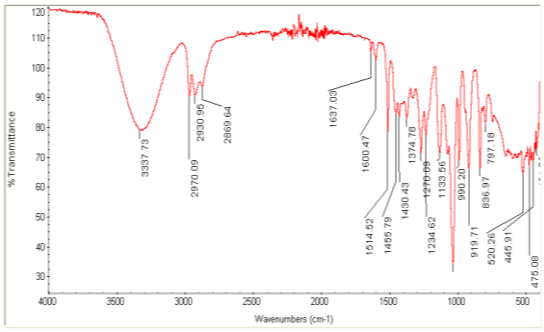

Fig. 1: IR spectrum of Clove oil

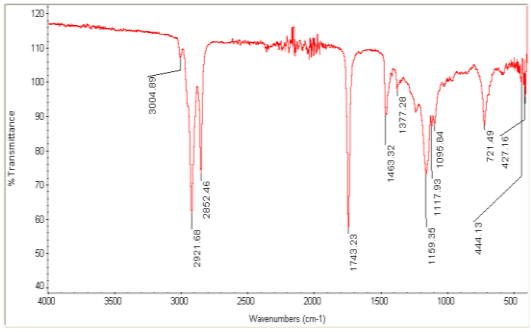

Fig. 2: IR spectrum of Arugula oil

FTIR Analysis

FTIR analysis was carried out for clove and arugula oil to identify the functional group. The characteristic IR spectrum of clove oil obtained is presented in Fig. 1. A broad and clear band at 3337 cm^{-1} represents the region of OH stretching of hydroxyl group which is associated with phenols. Series of peaks at 2970, 2930 and 2869 cm^{-1} represents C-H stretch vibrations for alkanes (both symmetric and asymmetric). The band 1637 cm^{-1} represents the C=C stretch of alkenes. Peak at 1600 cm^{-1} is due to C=C ring stretch of aromatic compounds. Alcohols and phenols represent the C-O-H bending at 1514 cm^{-1}. Peaks at 1455, 1430 and 1374 cm^{-1} represent the CH_2 and CH_3 deformation. Peaks between 1035, 1133 and 1234 cm^{-1} represent the C-O stretching. Peaks at 990 and 919 cm^{-1} are due to CH and CH_2 bending vibrations. The peaks at 797 and 836 cm^{-1} is due to CH bending of aromatic compounds.

The IR spectrum for Arugula oil is shown in Fig. 2. It showed a peak at 3004 cm^{-1} which is due to the C-H stretch. The two peaks at 2921 and 2852 cm^{-1} are due to C-H stretching i.e., symmetric CH_2 stretching and the asymmetric CH_3 and CH_2 stretching respectively. Spectrum showed a strong and prominent peak at 1743 cm^{-1} corresponds to vibrations of carbonyl group (C=O). At 1463 cm^{-1} the band represents the deformation vibration of =C-H; the peak at 1377 cm^{-1} is attributed to the bending vibrations of CH_2 groups. The bands at 1159, 1117 and 1095 cm^{-1} are due to C-O ester groups and CH_2 group vibrations. The band at 721 cm^{-1} is assigned to CH_2 rocking vibrations.

Scanning Electron Microscopy

The scanning electron micrographs of both treated and untreated cells of *S. aureus* and *E. coli* are shown in Fig. 3 (A–D) and 4 (A–C). The micrographs of control (not treated) sample show cells with smooth, spherical (3A) or Rod (4A) shape. Cells which were not treated had regular, intact and well-defined margins (3 and 4A), while the microphotograph shown in Fig. 3 B–D and 4 B–C are a clear illustration of cells treated with clove oil. Treated cells show pitting, protrusions, blebs and clumping leading to distortion in shape. Cell membrane disruption was quite evident which caused the cellular contents to leak out and eventually lead to cell lysis.

Discussion

Present study screened some essential oils against a panel of pathogenic bacteria and post-harvest fungi. Our results show that both Gram positive and negative bacteria were sensitive to clove and arugula oil as they showed large zones of inhibition and low MIC values. Cactus and almond oils inhibited *S. aureus* and *B. subtilus* strongly but could not inhibit other bacterial strains effectively. Similar observations were reported from earlier studies where clove oil had exhibited strong inhibitory effect on Gram positive and negative bacterial strains (Babu *et al*., 2011; Seenivasan *et al*., 2011). In separate studies clove oil inhibited *S. aureus, B. subtilus, K. pneumoniae* and *E. coli* with large zones and low MIC values 0.5–4 μL/mL and 0.5–4 µL/mL, which is in agreement with our findings (Matan *et al*., 2006; Upadhyay *et al*., 2010; Sharma *et al*., 2014a). Promising antibacterial activity was shown by arugula oils as all the bacterial pathogens screened were inhibited with effective zones.

Table 7: Chemical composition of cactus oil

Name of the compound	Molecular weight	Formula
di-t-Butylacetylene	138	$C_{10}H_{18}$
9-Tetradecen-1-ol	212	$C_{14}H_{28}O$
Bicyclo[2.2.1]hept-5-en-2-ol	110	$C_7H_{10}O$
3,5-Cyclohexadiene-1,2-dione,3,5-bis(1,1-dimethylethyl)-	220	$C_{14}H_{20}O_2$
1,2,3,4,5,6-Hexahydro-1,5,5,5-tetramethyl-2,4a-methanonaph-7(4aH)-one	218	$C_{15}H_{22}O$

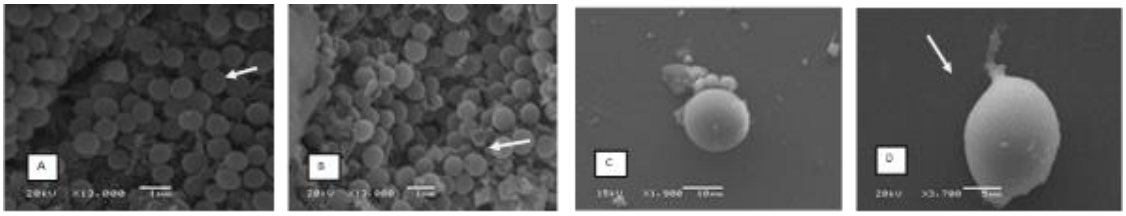

Fig. 3: Scanning electron micrograph of *S. aureus* treated with clove oil
B, C and D: Cells treated with clove oil exhibit dentations, clumping, blebbing, membrane disruption and cell leakage

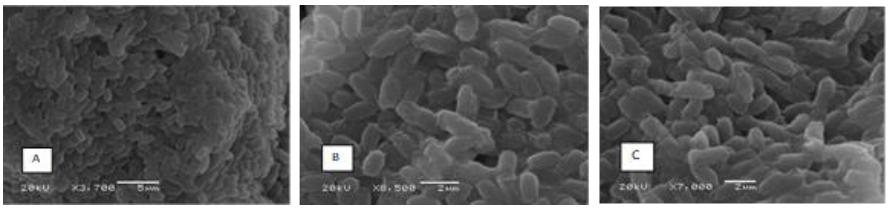

Fig. 4: Microphotographs of *E. coli* cells (treated and untreated)
B and C: Cells treated with clove oil show distorted shape, membrane disintegration and clumping

Khoobchandani *et al*. (2010) reported, oil from arugula seeds were highly effective in inhibiting Gram-positive bacteria when compared to Gram negative strains. Gulfraz *et al*. (2011), found arugula oil to be potent in inhibiting all the test isolates with low MIC values 50–72 µg/mL except *P. aeroginosa*. In another study by Shoaib *et al.* (2014), arugula oil was found to be more effective than clove oil in inhibiting pathogenic bacteria which is contrary to our findings, where we report lowest MIC with clove oil 0.25–8 μL/mL followed by arugula oil 8–16 μL/mL. Almond oil showed variable inhibitory effect on test pathogens, *S. aureus* and *B. subtilus* exhibited relevant zones of inhibition while *S. pyogenes* and *E. faecalis* were not inhibited at all. Other studies show sweet almond oil to possess strong inhibitory property against *S. aureus, E. coli* and *K. pneumoniae*; they were inhibited with low MIC and MBC values of 0.25–1 μL/mL and 2–4 μL/mL (Upadhyay *et al*., 2010). Contrary to all the aforementioned studies, Gupta *et al*. (2008a) reported all the bacterial strains tested to be resistant to almond oil (Gupta *et al*., 2008a). In our study cactus oil showed significant antibacterial activity with test pathogens except *MRSA, E. faecalis* and *P. aeroginosa* which were highly resistant. There is very little data available on antibacterial and antifungal activity of almond, cactus oil and sesame oil. With the meager information available it shows that cactus oil has strong to moderate antibacterial activity. Recent studies on *O. stricta* essential oils show pathogenic bacteria to be sensitive at 20 mg/mL concentration and the MIC values were between 1.25–20 mg/mL for *B. cereus, E. coli, B. licheniformis and P. aeroginosa* (Moosazadeh *et al.,* 2014). Multiple drug resistant bacteria and nosocomial microbes were screened recently in two separate studies and both the studies show methanol and ethanol extracts of *O. ficus-indica* to possess significant antimicrobial activity with an MIC ranging between 12.5–50 mg/mL (Wasnik and Tumane, 2016). Sanchez *et al*. (2016) screened methanol extracts of *O. ficus-indica* against nosocomial microorganisms and found the MBC to be in the range of 1 mg/mL to >15 mg/mL. In present study sesame oil was moderately active in inhibiting gram-positive bacteria while gram negative bacteria were resistant. According toZaki, sesame oil possessed significant antibacterial activity against *S. aureus, P. aeroginosa, E. coli* and *Streptococcus* spp. while *K. pneumoniae, Enterobacter* spp. and *Micrococcus* spp. were not inhibited (Zaki *et al*., 2015). Methanol extracts of sesame leaves, fruits and seeds had significant inhibitory activity against pathogenic bacteria (Sharma *et al*., 2014b).

All the Essential oils tested caused variable degree of mycelia growth inhibition. Clove oil and arugula inhibited all the fungal isolates. *C. gloeosporioides* showed 100% inhibition with clove oil, their MIC and MBC values were very low as well (0.03 μL/mL). Similarly, Enzo *et al*. (2012) reported complete inhibition of *C. gloeosporioides* at 1% v/v concentration of clove oil. In another study**,** clove oil caused inhibition of *Alternaria spp., Aspergillus niger, Rhizopus* spp. and *Penicillium* spp. with an MIC ranging between 2.5–10%, which is in agreement with our findings (Gupta *et al*., 2008b; Rana *et al*., 2011). Earlier a study conducted on

phytopathogens found arugula oil to be potent in inhibiting all the isolates screened (Amna *et al*., 2014). However, almond and cactus oil could not inhibit *C. gloeosporioides* and *F. monoliforme*, *F. solani*, *P. citrinum* effectively, while sesame oil needed a high MIC of > 64 µL/mL to inhibit most of the fungi tested. Reports concerning the antifungal activity of almond, cacti and sesame are scarce. A recent study screened panel of fungi with oil of *Armeniaca sibirica* (bitter almond) and found it to inhibit fungi at a very low EC50 (Huiling *et al*., 2016). Various parts of three different species of cacti *O. macrorhiza, O. microdasys* and *O. lindheimeri var linguiformis* were tested for their antifungal property against plant pathogens and it revealed that all the volatile extracts were ineffective in controlling the growth of *F. solani, F. oxysporum, Botrytis cinera* and *Pythium ultimum* except *A. alternata* (Bergaoui *et al.,* 2007). However, our findings show that except for *F. monoliforme, A. niger* and *C. gloeosporioides* other fungal isolates exhibited poor mycelia inhibition. Uniyal *et al.* (2012), studied the antifungal ability of sesame oils against two species of *Aspergillus* which cause Aspergilloma. They found sesame oil to be potent in controlling their growth with large zones of inhibition. Our study reports similar findings with *A. niger* and *A. fumigatus.*

Broadly all the essential oils displayed variable effectiveness against different test pathogens**,** Clove and Arugula being the most effective. Generally, antifungal activity was remarkable in comparison to antibacterial. Fungi were found to be more sensitive to essential oils than bacteria (Shelef, 1983; Ceylan and Fung, 2004).

GCMS analysis of clove, arugula and catus was carried out to get an insight into its chemical composition. It is known that FTIR in combination with GCMS is valuable in providing molecular structure and its important functional groups. The major components observed in clove oil were eugenol followed by caryophyllene. Similarly, earlier studies reported Eugenol as dominant compound in clove oil (Matta, 2010; Marya *et al*., 2012). Recently, two separate studies show predominant compound in clove oil to be eugenol, while caryophyllene, Chavibetol and Acetyl eugenol acetate were found in lesser amounts (Kasai *et al*., 2016; Najla and Perveen, 2017). Previous studies report the strong antimicrobial activity by clove oil, eugenol alone and combination of both (Combrincka *et al*., 2011; Rosello *et al*., 2015). Analysis of essential oil from arugula showed the presence of major compounds like, Dibenzofuran, Cyclopentadecanol, 9-Hexadecen-1-ol, (Z). Previous studies report compounds including isothiocyanates, aldehydes, furans and nitriles (Miyazawa *et al*., 2002; Khoobchandani *et al*., 2010). However, our studies show few compounds without isothiocyantes.

GCMS analysis of cactus shows the presence of various compounds amongst which some of them such as Bicyclo [2.2.1] Hept-5-en-2-ol; di-t-Butylacetylene; 9-Tetradecen-1-ol, 3,5-Cyclohexadiene-1,2-dione,3,5-bis(1,1-dimethylethyl)-;1,2,3,4,5,6-Hexahydro-1,5,5,5-tetramethyl -2,4a-methanonaph-7 (4aH)-one. Similarly, in two separate studies, different Cactus species growing in Arizona and California were reported to possess major components belonging to alcohols, alkanes, furanoid forms of oxides and fatty acids (Wright and Cetzer, 2013, 2014). Compounds like 9-Tetradecen-1-ol, sesquiterpenes and ketones have been reported earlier from different species of *Opuntia* (Arena *et al*., 2001; Osuna-Martinez *et al*., 2014). Earlier reports state fruits and cladodes of *O. ficus-indica* as rich source of phytochemicals such as unsaturated fatty alcohols and flavonoids (Ginestra *et al*., 2009). In a study conducted on flower extracts of *O. ficus indica*, 26 compounds were identified which were dominated by alcohols and carboxylic acids followed by esters, ketones and sesquiterpenes (Ennouri *et al*., 2014).

As mentioned above, GCMS analysis of clove, arugula and cacti essential oils revealed fewer compounds in comparison to earlier reports (Miyazawa *et al*., 2002; Bergaoui *et al*., 2007; Rasheeha *et al*., 2013). This can be attributed to many factors like method of drying and extraction, time of harvest, geographical location, and age of plant (Singh *et al*., 2010; Calo *et al*., 2015).

Present study shows various functional groups to be present in essential oils which can be correlated with the GCMS studies. FTIR analysis of clove and arugula oils show presence of important bioactive functional groups such as phenols, sesquiterpenes, triglycerides, ester groups, aldehydes and ketones. Besides, it shows the characteristic bands related to oils and fats. Our findings agree with other studies where similar spectrums have been reported with clove oil and arugula oil (Sharma *et al*., 2014a, c).

Plant phenols and phenolic hydroxyl groups in general have been reported to be excellent in controlling bacterial and fungal infections (Taguri *et al*., 2004). Phenolic compounds in oils are known to alter and interfere with the cell permeability and cellular energy generation system (ATP) thereby damaging and disrupting the cytoplasmic membrane (Bajpai *et al*., 2009). In our study we identified Eugenol, (GCMS) which is known to effectively control both bacteria and fungi (Devi *et al*., 2010). Earlier studies report inactivation of essential enzymes due to eugenol, as it disturbs the genetic material functionally by reacting with cell membrane (Cox and Markham, 2007; Devi *et al*., 2010). This explains the strong antibacterial activity exhibited by clove oil in our study. A very low MIC of 0.25 µL/mL caused *S. aureus* cells to bleb and cell membrane disruption which eventually lead to leakage of cellular content and lysis. The SEM micrograph depicts the damaging effect of clove oils which could be attributed to eugenol activity.

Similarly, various studies have shown damaging effects of essential oils and its components like eugenol, thymol and carvacrol on pathogenic bacteria and fungi (Gill and Holly, 2006; Jian *et al*., 2016). Lipophilic nature of essential oils aids in enhancing the permeability of plasma membrane. This further explains the significant antifungal and antibacterial results of essential oils. Caryophyllene, a

sesquiterpene has also shown antimicrobial activity in earlier studies but is not as potent as eugenol (Dorman and Deans, 2000). However, some reports state monoterpenes to possess higher antimicrobial activity (Sokovic and Griensven, 2006).

Essential oils are a mixture of various compounds and their antimicrobial activity depends on its functional groups. Though the major compounds are known to play a key role in antimicrobial activity, the role of less abundant constituents that act synergistically and through additive interactions cannot be ignored (Burt, 2004). According to Kalemba *et al*. (2012) the antimicrobial activity of essential oils are due to the following components, ranked as follows phenols > alcohols > aldehydes = ketones > ethers hydrocarbons.

Conclusion

This study demonstrates the strong antimicrobial activity of some essential oils like clove, arugula and cactus. Their effectiveness could be attributed to the presence of phenols, terpenes and terpenoids. Since clove and arugula oil has shown bactericidal and fungicidal activities, these oils can serve as an excellent alternative to antimicrobials. Moreover**,** the Food and Drug Administration has declared clove, eugenol, and some other essential oils as safe substances. Further research to understand the molecular mode of action of different constituents of essential oils is needed as they have different targets.

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