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ISOLATION, IDENTIFICATION, CHEMICAL CHARACTERIZATION AND ANTIMICROBIAL ACTIVITIES OF THE ESSENTIAL OIL OF CAPPARIS CARTILAGENIA DECNE.

[1]Mylabathula Mary Moses, [2]Desam Nagarjuna Reddy, [3]J. Naveena Lavanya Latha

[1]Research Scholar, [2]Assistant professor, [3]Associate professor

[a]Department of Biotechnology, Krishna University, Machilipatnam, AP, India

Abstract

This study was designed to investigate the phytochemicals and antimicrobial activity of the volatile oil of the plant extract of *Capparis cartilaginea.* GC and GC-MS analysis of the essential oil resulted in the identification of eighteen chemical constituents representing 98.88 % of the oil. Isopropyl isothiocyanate (32.03 %), butyl isothiocyanate (24.95 %), 2-nitrilebutane (6.23 %), and isobutyl isothiocyanate (5.61 %) were the major components. The essential oil shows significant antibacterial and antifungal activity than principle components.

The antibacterial activity of the essential oil was significant against *Enterococcus faecalis, Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermis,* and *Escherichia. coli,* with zones of inhibition of diameters 35.12 ± 0.12 mm, 32.06 ± 0.02 mm, 30.24 ± 0.12 mm, 30.14 ± 0.04 mm, and 28.08 ± 0.10 mm, respectively. Significant antifungal activity was also noted, based on zones of inhibition, for *Candida albicans* (35.14 ± 0.22 mm), *Aspergillus fumigates* (33.08 ± 0.04 mm), *Aspergillus flavus* (33.02 ± 0.04 mm), and *Alternaria alternata* (32.16 ± 0.14 mm). The minimum inhibition concentration for essential oils ranged between 0.5 ± 0.01 and 2.0 ± 0.19 µg/ml. Our findings indicate that *C. cartilaginea* essential oil could be useful as a natural antibacterial and antifungal treatment.

Key words: *Capparis cartilaginea* L.; Essential oil; Antibacterial activity; Antifungal activity; GC; GC-MS; Isopropyl isothiocyanate; Butyl isothiocyanate; 2-nitrilebutane.

1. Introduction

Plants are the great source for producing new drugs of benefit to mankind. Every part of the Plants like roots, stems, leaves, buds, flowers, fruits and seeds synthesize hundreds of chemical compounds for their functions such as defense against insects, fungi, diseases and herbivorous animals. These chemical compounds are rich in antimicrobial properties equal to the pharmaceutical drugs that are being used now a days. Most of the medicinal and aromatic plants are rich sources with Antibacterial properties which can be used to fight against bacterial infections.

C. cartilaginea is a perennial plant. These are xerophytes and growing in a broad range of climatic conditions. *C.cartilagenia* belongs to the Family Capparaceae or Capparidacece, comprises 39 genera and 650 species. These are all distributed through warm regions (Boulos, 1999; Trombetta et al.,2005). These are shrubs, trees or creepers. These stems are long, abnormal and hairless. Bark is waxy coated and it appears in whitish grey and yellowish green. Leaves are elliptical, large and attractive flowers are produced during flowering period. Fruits are berry and globes having many seeds. *C. cartilagenia* is grown in Northern and Eastern side of Africa, and the most of the places in Asia, including India, and also found in the UA (Rivera et al.,2002) and Saudi Arabia. *Capparis* species which are grown in harsh areas are not preferred food choice of goats because of the high concentrated secondary metabolites (Garcia et al., 2008).

Plant parts of *C.cartilagenia* being used widely in medicinal field (Galib et al., 2016; Ingrid et al., 2012). Various biochemical compounds, alkaloids, phenols, sterols or glycosides present in *Capparis* sp. might be medicinally important and /or nutritionally valuable. (Tlili et al., 2011; Romeo et al., 2007; Abreu et al., 2012; Affronti et al., 2015; Carty et al., 2003; Cragg et al., 2013; Hussam et al., 2016; Newman et al., 2012; Orel et al., 2014; Ruikar et al., 2009). Extracts of different parts of *Capparis* show biological activity against large number of pathogens (Aghili Khorasani, 2008). Ethanol extract of *C.cartilagenia* causes reduction in blood pressure and heart rate. Aqeous extract of *C. cartilagenia* causes inhibition of non-epinephrine paralysis and tooth ache. Bark pout of *C.cartilagenia* found effective as antihelmintec, constipative and purgative. Anti-tubercular property of *C.cartilagenia* ethanol extract could be considered as a very specific property (Mishra et al.,2007). *Capparis cartilagenia* is used to treat itching, shortness of breath, head cold and for tumors. (Mothana et al., 2009; Rivera et al., 2003; Youssef, 2013; Miller et al., 2004; Al-Dubai et al., 1996). Literature reveals that this is the first study on *Capparis cartilagenia* in Jazan region, Saudi Arabia

2. Materials and Methods

2.1.Plant collection and extraction of essential oil

Capparis cartilaginea L. was collected near the Jazan local market, during the flowering stage (September 2016). Senior taxonomist Ramesh Moochikkal, Faculty of Botany, acknowledged the plant specimen (JAZUH 1148) and preserved at the Department of Botany, of Jazan University. The aerial parts were separated, frozen by refrigerator at -10°C and dried with freeze drier (ZIRBUS Va Co 10-11, German Technology) -80°C for 24hrs at different stage, and different pressure, and then ground to a powder with a mixer (IKA WERKE MF 10 basic) to pass through a 50 mm sieve.

A 100 g sample of the powder was subjected to hydro distillation (Viuda-Martos et al., 2011) for 3 h in 500ml of distilled water in a 1000 ml round bottom flask using a Clevenger apparatus (Klaus Hofmann Gmbh, Germany). The resulting yellow oily liquid was collected, dried over anhydrous Na_2SO_4, and preserved under 4°C for forthcoming analysis.

2.2.Gas Chromatography and Mass Spectrometry Analysis

The oils were subjected to qualitative and quantitative analysis by using gas chromatography and mass spectrometry method with an Agilent Technology 6890N instrument that allows both GC and GC-MS. The Gas chromatographer was equipped with a FID detector, HP-5MS 5% capillary column with a film of 30m x 0.25mm x 0.25 µm dimensions, a split-splitless injector, and helium (99.99 %) at 1.0 ml/min was used as carrier gas. The sample solution (0.1µL) was injected in the split mode (1:10) at an injection temperature of 280 °C and a detector temperature of 300 °C. The following temperature gradient program was used: In the beginning the temperature of the column was 40 °C for five minutes, temperature linearly raised from 40 - 180 °C at 5 °C/min and then 8 °C/min from 180 to 280 °C. when the sample was injected manually. The peak area percentage was obtained from the FID signal.

GC-MS was conducted on the same instrument fitted with an HP-5MS capillary column of dimensions-30 m × 0.25 mm × 0.25 µm, and a column temperature gradient- 40 to 280 °C at 5 °C/min. The injection was performed at 240 °C, the carrier gas was helium (99.99 %) at 1ml/min. Gas chromatography with Mass spectrometry (Agilent technology 6890N) was done for essential oil Qualitative and quantitative analysis using the electron impact ionization(70ev) mode. and mass spectra were recorded at 70eV (in a mass range from 25 to 500 m/z). A 0.1µl volume of the sample was injected in split mode.

2.3. Identification of chemical constituents

Essential oil components were identified by comparison with standard reference substances and their mass spectral data were assembled via NIST and Wiley 5 online mass spectrum libraries or authentic compounds. Data were confirmed by comparison of retention indices or authentic compounds or the data published in the literature (Adams,2007; Davis,1990). With similar operating conditions indices of retention are set in comparison to a homologous series of alkanes (C_5-C_{25}). Based on the concentration levels chemical components were received from the FID highest areas and heights.

2.4.Antibacterial and Antifungal Activity

Disc diffusion method was used to investigate antibacterial and antifungal activity on broad spectrum of fungi and gram positive and gram-negative bacteria. Minimum inhibition concentrations (MIC) were determined using a microdilution method for microorganisms.

2.5.Source of microbial strains

The essential oil was investigated against the following microorganisms. Gram-positive bacterial strains included *Bacillus cereus* (ATCC10876), *Bacillus macerans (M58), Bacillus megaterium (M3), Bacillus subtilis (ATCC6633), Bacilla abortus (A77), Bukholdria cepacia (A255), Enterobecter cloacae (ATCC13047), Enterococcus faecalis (ATCC49452), Listeria monocytogenes* (ATCC15313), *Staphylococuss aureus* (ATCC25923), Micrococus flavus (ATCC 9341), *Staphylococcus epidermis* (A 233), *Clavibacter michiganense* (A 277), and *Streptococcus pyogenes* (ATCC 176). Gram-negative microorganisms included Acinetobacter baumannii (ATCC 19606), Escherichia coli (ATCC 25922), Klebsiella pneumonia (ATCC 27853), *Proteus mirabilis* (ATCC 35659), *Salmonella typhimurium* (ATCC13311), *Citrobacter freundi* (ATCC13311), *Enterobacter aerogenes* (ATCC13048), *Salmonella enteritides* (I K27), *Proteus vulgaris* (A 161), *Pseudomonas syringae* (A 35), and *Xanthomonas campestris* (A 235). The fungal strains were *Alternaria alternaria* (MNHN 843390), *Aspergillus flavus* (MNHN 994294), *Aspergillus fumigates* (MNHN 566), *Candida albicans* (ATCC 26790), *Cladosporium herbarum* (MNHN 3369), *Fusarium oxyporum* (MNHN 963917), *Aspergillus versicolor, Fusarium acuminatum, Fusarium solani, Fusarium tabacinum, Moliniana fructicola, Penicillum spp., Rhizoctonia solani, Sclerotinia minor, Sclerotinia sclerotiorum*, *Trichophyton mentagrophytes,* and *Trichophyton rubrum*. The ATCC, NRRL, and clinical isolate type microorganisms received from Microbiology department, Faculty of Pharmacy, Jazan University, Jazan, Saudi Arabia. Bacteria and *C. albicans* were inoculated on SDA (Sabouraud Dextrose Agar) and cultured overnight at 37°C to check purity. Microorganisms were transferred to Mueller Hinton Broth (MBH) for further incubation for another 24 h at 37 °C.

2.6. Disc Diffusion Method

Disc diffusion method was used to evaluate the antibacterial activity of C. cartilaginea essential oil as per the protocol of NCCLS (NCCLS, 2001) (Pfaller et al.,1998). Essential oils and microorganisms were dissolved in 2 % dimethyl sulfoxide (DMSO), which was used for further dilutions. 96- well microtiter plates were used to keep the diluted culture suspensions in 100µl. The solutions of tested microorganisms contained 10^8CFU/mL of bacteria and 10^6 cfu/ml of fungi strains spreding Sabouraud Dextrose Agar (SDA) respectively. 10.0 µl suspension of essential oil and microorganism was pipetted into each well containing agar medium. The inoculated plates were incubated at 37°C for 24h for clinical bacterial strains and at 30°C for 48 h for fungal strains. Gentamicin and amphotericin B served as reference compounds, allowing comparison of the antimicrobial activity of the volatile oil. Tests were done in triplicate.

Micro dilution method

The minimal inhibition concentration (MIC) values were evaluated in microorganisms using a micro broth dilution assay as recommended by CLSI,2016, all the tests were performed in Sabouraud Dextrose broth and Muller-Hilton broth, respectively. Essential oils and microorganisms were dissolved in 2 % dimethyl sulfoxide (DMSO), which was used for further dilutions. Suspensions were diluted to 5.0×10^5 and 2.0×10^6 cfu/ml for bacteria and fungi, respectively. Standard strains of these suspensions were soaked onto micro plates, and incubated for bacteria at 37 °C for 24 hours and for fungi at 30°C for 48 hours. The minimum inhibition concentration (MIC) was marked as the least concentration of the compound required to inhibit microorganism growth, and the results were used to estimate the antimicrobial activity of the oil to the reference standards.

2.7. Statistical analysis

All experiments were conducted in triplicate, and the results were presented as mean ± standard deviation. Statistical analysis was conducted by ANOVA using the SPSS21 software package.

3. Results and discussion

3.1.Chemical constituents of the C. cartilaginea L.

C. cartilaginea L. essential oil was extracted by hydrodistillation using a Clevenger apparatus, expressed as a percentage (1.9 % v/w). Eighteen chemical constituents were found in the GC and GC-MS analysis of the essential oil. They are accounted for 98.88 % of the total oil. The principal components were isopropyl isothiocyanate (36.43 %), butyl isothiocyanate (24.95 %), methyl isothiocyanate (16.04 %), 2-cynobutane (6.23 %), isobutyl isothiocyanate (5.61 %), and ethyl isothiocyanate (4.44 %); other components accounted for trace or minor percentages of the total chemical composition. The results are shown in Table 1.

No previous research has investigated the chemical composition and antibacterial and antifungal activity of *C. cartilaginea* essential oil. One paper (Ahmed et al., 2007) from Egypt reported the chemical composition

of butyl isothiocyanate (65.03 %), 6-methylsulfonylhexyl isothiocyanate (29.86 %), 7-methylsulfonylheptyl isothiocyanate (0.066 %), and 5-benzylsulfonyl-4-bentenyl isothiocyanate (0.91 %) from an ethanol extract and showed antibacterial activity similar to that described in the present study. The ethanol extract had butyl isothiocyanate as a similar constituent to that found in the essential oil, but the percentage of the butyl isothiocyanate differed, as did the other components. (Galib et al., 2016) reported only phytochemical and antibacterial activity studies, (Alasbahi et al., 1999) from Yemen investigated the antibacterial activity of various solvent extractions, while other researchers from Iran (Rahimifard et al., 2015) reported antibacterial activity and flavonoid content. A report from Saudi Arabia (Abutaha et al.,2014) reported larvicidal activities.

3.2. Antibacterial and antifungal activities of C. cartilaginea L. essential oil

Disc diffusion method is used for zone of inhibition(mm) of C. cartilaginea essential oil to find the results of antimicrobial activity as shown in Table 2. The minimum inhibition concentrations (µg/ml), determined with the microdilution method, are shown in Table 3.

The essential oil showed potent antibacterial activity in the range of 3.12 ± 0.02 to 35.12 ± 0.12 mm. according to the disc diffusion method. Strong antibacterial activity was observed against *E. faecalis* (35.12 ± 0.12 mm), *B. cereus* (32.06 ± 0.02 mm), *S. aureus* (30.24 ± 0.12 mm), *S. epidermis* (30.14 ± 0.04 mm), *E. coli* (28.08 ± 0.10 mm), and *B. macerans* (27.05 ± 0.12 mm) ($\rho \leq 0.05$), while only moderate or minimal antibacterial activity was found for the other bacteria, including *B. abortus* (23.06 ± 0.01 mm), *C. michiganense* (23.04 ± 0.04 mm), *S. pyogenes* (22.02 ± 0.06mm), *L. monocytogenes* (17.11 ± 0.02mm), *M. flavus* (15.01 ± 0.10mm), *K. pneumonia* (15.04 ± 0.08mm), *P. mirabilis* (15.12 ± 0.14), *B. subtilis* (14.12 ± 0.12mm), *A. baumannii* (13.08 ± 0.12mm), *E. cloacae* (12.18 ± 0.14mm), *B. megaterium* (12.03 ± 0.04mm), *S. enteritides* (11.12 ± 0.11mm), *S. typhimurium* (9.06 ± 0.06mm), *E. aerogenes* (8.02 ± 0.07mm), *B. cepacia* (8.06 ± 0.12mm), *P. vulgaris* (4.09 ± 0.10mm), *P. syringae* (3.16 ± 0.02mm), and *X. campestris* (3.12 ± 0.02mm) ($\rho \leq 0.05$). The essential oil showed good antibacterial activity when compared with antibiotics and its major components, isopropyl isothiocyanate, butyl isothiocyanate, and methyl isothiocyanate, which showed potent antibacterial activity against *B. cereus, E. faecalis, S. epidrermis,* and *E. coli*. Antibacterial activity, in general, was better against gram-positive than gram-negative bacteria. Minimum inhibition concentrations for the essential oil ranked from 0.5 ±0.01to 2.0 ± 0.19 µg/ml

The essential oil showed potent antifungal activity against *C. albicans*, *A. fumigates A. flavus, A. alternate,* and *Penicillium spp.,* with zones of inhibition of diameters 35.14 ± 0.22 mm, 33.08 ± 0.04 mm, 33.02 ± 0.04 mm, 32.16 ± 0.14 mm, and 30.24 ± 0.02 mm ($\rho \leq 0.05$) respectively. Moderate inhibition was observed for *F. acuminatum*, *F. solani,* and *R. solani* (22.50 ± 0.02 mm, 22.42 ± 0.04 mm, and 20.22 ± 0.14 mm, respectively). Minimal activity was observed against *A. versicolor*, *F. oxyporum*, *C. herbarum*, *M. fructicola, T. rubrum, S. sclerotiorum, S. minor,* and *T. mentagrophytes* (16.33 ± 0.04 mm, 16.44 ± 0.06 mm, 15.24 ± 0.14 mm, 15.12 ± 0.03 mm, 11.14 ± 0.02 mm, 10.28 ± 0.04 mm, 9.14 ± 0.12 mm, and 9.16 ± 0.04mm, respectively) ($\rho \leq 0.05$). Antifungal activity was compared with the antifungal activity of the synthetic drug

amphotericin B. The results are shown in Table 3. Minimum inhibition concentration (MIC) values for essential oil ranged from 1.0 ± 0.02 to 5.0 ± 0.12 µg/ml.

The anti-microbial activity of the oil and the interaction between its chemical constituents and the most naturally abundant compounds were shown in earlier studies (Delaquis et al., 2002; Dorman et al., 2000; Abutaha et al., 2014. The results of the present study indicate a greater antibacterial activity against gram-positive bacteria than against gram-negative bacteria.

4. Conclusion:

Essential oils and their chemical constituents often show potent antimicrobial, antifungal and antioxidant activities. This study indicated *C. cartilaginea* oil and its chemical constituents have potent antibacterial and antifungal activity, making this oil very useful in daily life as a food preservative and as a protective agent against various diseases. However, further research is required to investigate other parts of the plant as natural sources of this essential oil and to confirm its biological activities.

Conflict of interest

The authors take the responsibility for the content and writing of the paper. The authors declare no conflict of interest either financial or non-financial.

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Table-1: Chemical constituents of the essential of *Capparis cartilaginea*.

Lit. R.I[a]	R.I	Name of the Compound	% Composition
689	689	Isobutyl cyanide	2.69
708	709	Methyl isothiocyanate	16.04
744	743	2-cynobutane	6.23
786	785	Ethyl isothiocyanate	4.44
837	837	Isopropyl isothiocyanate	36.43
919	919	Isobutyl isothiocyanate	5.61
959	959	Butyl isothiocyanate	24.95
1101	1109	Linalool	1.03
1150	1148	Menthone	0.31
1237	1232	Pulegone	0.20
1253	1228	3,7-dimethyl-2,6-octadien-1-ol	0.14
1342	1342	3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-2H-chromene	0.12
1356	1352	Eugenol	0.16
1315	1317	Benzyl isothiocyanate	0.06[t]
1389	1384	β-cubebene	0.05[t]
1498	1494	Caryophyllene	0.25
1680	1680	Nerolidyl acetate	0.04[t]
-	-	O-tolyl isocyanide	0.13
		Total	98.88

RI: Retention index, HP-5ms capillary column (30 m × 0.25 mm × 0.25 µm), He carrier gas, FID detector for n-alkane series C_5-C_{24}. [a]literaruere retention index (Admas 2005) DB-5, capillary column (30. M x 0.26 mm x 0.25 µm), He carrier gas, FID detector. [t]% of composition is trace amount

Table: 2. Antibacterial activity of *Capparis cartilaginea* L (10.0µg/ml) of essential oil

Microbial Strains	**Zone of inhibition (mm[a])**					**MIC[d](µg/mL)**			
	E.O[c]	RA[b]	Iso.i[e]	But.i[f]	Meth.i[g]	E.O[c]	Iso.i[e]	But.i[f]	Meth.i[g]
B. cereus	32.06±0.02	28.44±0.10	30.0±0.04	25.2±0.04	15.2±0.02	0.5±0.01	0.5±0.02	0.5±0.02	4.0±0.02
B. macerans	27.05±0.12	22.28±0.04	24.0±0.02	20.0±0.02	10.2±0.02	0.5±0.04	0.5±0.02	0.5±0.02	5.0±0.05
B. megaterium	12.03±0.04	20.02±0.12	10.2±0.02	10.0±0.02	6.2±0.04	1.0±0.08	2.0±0.04	2.5±0.04	5.0±0.05
B. subtilis	14.12±0.12	14.10±0.06	10.0±0.04	10.0±0.02	8.0±0.02	1.0±0.14	2.0±0.04	2.5±0.04	5.0±0.05
B. abortus	23.06±0.01	8.02±0.04	15.0±0.06	15.2±0.02	10.0±0.06	0.5±0.02	1.5±0.02	1.5±0.02	5.0±0.05
B. cepacia	8.06±0.12	14.45±0.02	6.4±0.04	5.0±0.02	3.0±0.02	1.5±0.06	2.5±0.06	3.0±0.05	5.0±0.05
E. cloacae	12.18±0.14	16.28±0.04	8.2±0.04	10.0±0.02	6.2±0.04	1.5±0.14	2.5±0.06	2.5±0.05	5.0±0.05
E. faecalis	35.12±0.12	18.32±0.12	30.2±0.02	25.4±0.02	15.4±0.04	0.5±0.18	0.5±0.02	1.5±0.02	3.0±0.04
L. monocytogenes	17.11±0.02	14.23±0.12	13.2±0.04	15.2±0.02	10.0±0.04	1.0±0.06	1.5±0.04	2.0±0.02	4.0±0.02
S. aureus	30.24±0.12	10.42±0.14	18.0±0.02	15.0±0.02	12.2±0.02	0.5±0.03	1.0±0.02	2.5±0.04	3.0±0.04
M. flavus	15.01±0.10	10.28±0.08	14.2±0.02	13.2±0.02	8.2±0.04	1.0±0.07	1.5±0.04	3.0±0.02	5.0±0.04
S. epidrermis	30.14±0.04	16.32±0.04	25.4±0.06	22.0±0.02	20.0±0.02	1.5±0.07	0.5±0.02	0.5±0.02	1.0±0.02
C. mmichiganense	23.04±0.04	15.03±0.06	20.0±0.06	23.0±0.02	14.0±0.02	0.5±0.12	0.5±0.02	0.5±0.02	4.0±0.02
S. pyogenes	22.02±0.06	10.04±0.10	20.0±0.02	18.2±0.02	15.0±0.06	0.5±0.12	0.5±0.02	1.5±0.02	0.3±0.02
A. baumannii	13.08±0.12	15.12±0.10	10.3±0.02	9.4±0.02	5.2±0.02	1.5±0.03	2.0±0.04	3.0±0.04	5.0±0.05
E. coli	28.08±0.10	16.24±0.12	22.2±0.04	20.0±0.02	18.2±0.02	0.5±0.09	0.5±0.02	1.0±0.02	3.0±0.04

K. pneumonia	15.04±0.08	14.22±0.04	10.2±0.02	10.0±0.02	5.0±0.02	1.0±0.14	2.0±0.04	3.0±0.04	5.0±0.05
P. mirabilis	15.12±0.14	13.36±0.04	10.2±0.02	10.0±0.02	5.0±0.04	1.0±0.17	2.0±0.04	3.0±0.04	5.0±0.05
S. typhimurium	9.06±0.06	10.03±0.04	7.0±0.04	7.2±0.02	4.2±0.02	2.0±0.15	2.5±0.02	3.0±0.05	5.0±0.05
C. freundi	6.04±0.04	10.04±0.02	5.0±0.02	5.0±0.02	3.2±0.06	2.0±0.19	2.5±0.02	3.0±0.05	5.0±0.05
E. aerogenes	8.02±0.07	14.24±0.02	5.0±0.04	5.0±0.02	3.2±0.02	2.0±0.12	2.5±0.02	3.0±0.05	5.0±0.05
S. enteritides	11.12±0.11	14.36±0.14	10.4±0.04	8.0±0.02	4.0±0.06	1.5±0.02	2.5±0.02	3.0±0.05	5.0±0.05
P. vulgaris	4.09±0.10	13.02±0.04	3.0±0.02	2.0±0.02	2.0±0.02	2.0±0.02	2.5±0.02	3.0±0.05	5.0±0.05
P. syringae	3.16±0.02	15.09±0.04	3.0±0.02	2.0±0.02	2.0±0.02	2.0±0.16	2.5±0.02	3.0±0.05	5.0±0.05
X. campestris	3.12±0.02	10.04±0.04	3.0±0.02	2.0±0.02	2.0±0.02	2.0±0.12	2.5±0.02	3.0±0.05	5.0±0.05

[a]values represent means ± standard deviations for triplicate experiments; [b]RA: Reference of antibiotics Gentamicin for gram positive and amikacin for gram negative bacteria used was 20 µg/disc; E.O[c]: Essential oil; MIC[d] Minimum inhibition concentration; Iso.i[e]: isopropyl isothiocyanate; But.i[f]: butyl isothiocyanate; Meth.i[g]: methyl isothiocyanate.