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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF *Eruca sativa* SEEDS AGAINST PATHOGENIC BACTERIA AND FUNGI

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ABSTRACT

The objective of the present study was to investigate the antimicrobial activity of chloroform, ethyl acetate, acetone, methanol and ethanol extracts of *Eruca sativa* seeds growing in Saudi Arabia. Major chemical constituents and functional groups of *E. sativa* extract were determined by Gas Chromatography -Mass Spectrometry and Fourier Transform-Infra Red Spectroscopy analysis. Seed extracts showed potent antimicrobial activity against pathogenic bacteria and fungi. Ethyl acetate extract was highly efficient in controlling the growth of *Staphylococcus aureus, Bacillus subtilus, Methicillin resistant Staphylococcus aureus, Streptococcus pyogenes* and *Escherichia coli*. Maximum zone of inhibition was exhibited by *S. aureus* (26mm). Similarly, methanol extract showed strong inhibitory effects on mycelial growth of all the fungi tested except for *Colletotrichum gleosporoides*. Maximum mycelial inhibition (82%) was recorded for *Colletotrichum musae*. The minimum inhibitory concentration and minimum bactericidal concentration results demonstrated the efficiency of the extracts screened. Furthermore, micrographs from scanning electron microcopy revealed the damaging effects of extracts on cell morphology as blebs, indentation and complete lysis at 0.8mg/ml. GC-MS analysis of ethyl acetate and methanol extracts has led to identification of important compounds which are known antifungals and antibacterials .

Key words: Antibacterial, Antifungal, GC-MS analysis, FT-IR, SEM, Cell lysis.

INTRODUCTION

Microbes are found everywhere. Some of them are pathogenic and infect humans, plants and animals. Since the mid-1970s, infectious diseases and its related health problems has become a major concern all over the world. Many of these diseases are stubborn and difficult to treat (Avery *et al*., 2006; Tekwu, 2012). Bacterial infections cause serious health problems in humans and can be life threatening (Khan *et al*., 2013), on the other hand post harvest fungal diseases of plants and its products cause huge crop and economic losses (Hassan, 2009). In general, antibiotics and antifungal drugs serve as important weapons in controlling and curing many infectious diseases (Bhalodia and Shukla, 2011). However, they are becoming less effective due to emergence of multiple drug resistance. The increasing prevalence in resistant strains of both bacteria and fungi could be attributed to misuse or overuse of standard antimicrobials (Waldvogel, 2004; Tenover, 2006; Aminov, 2010). Another problem is the toxicity and side effects related with the use of antimicrobials. Hence, to combat these stubborn microbes and toxicity related to antimicrobials, scientists are prompted to search for more effective antimicrobial drugs on horizon (Alviano and Alviano., 2009). Plant derived chemical substances serve as an excellent alternative source of antimicrobials which are nontoxic, safer and affordable (Upadhyay *et al*., 2014).Therefore, indigenous plants and its extracts need to be screened in search for potent antimicrobials.

Since time immemorial plants have served as huge reservoirs for numerous pharmaceutical products. Standard techniques and new approaches are being used to explore all parts of plants in search for wide array of chemical compounds that can be used as antimicrobials (Newman and Cragg, 2012).Traditionally, crude plant extracts are used as herbal medicine for the treatment of human infectious diseases (Zhang *et al*.,2006; Malini *et al*.,2013). Ethano-medicine still uses traditional herbal formulations, imbibed infusions and extracts all around the world. Many herbal extracts have shown to mediate important host responses, though their mechanism of action needs to be validated scientifically in most cases (Roberto *et al*., 2000; Cruz *et al*., 2007).Variety of phytochemicals are found in plants which includes terpenoids, flavanoids, tannins and alkaloids, all of these have shown in-vitro antimicrobial properties (Dorman *et al*., 2000; Talib and Mahasnneh., 2010). Recently several studies have shown the diverse biological and pharmacological properties of plant materials (Bussmann *et al*., 2010; Mouracosta *et al*., 2012; Ocheng *et al*., 2014; Vu *et al*., 2016).

Eruca sativa (Mill) is an annual herb and a member of Brassicaceae family. It is native to West Asia and Mediterranean region, but is widespread in Middle East. *E. sativa* is commonly referred to as Rocket or Argula. In Saudi Arabia, it is locally known as Jarjeer and

grows in northern Hejaz, Najd, and Eastern regions of Saudi Arabia (Migahid, 1978]. The leaves are used in salads and as a spice, while its seeds are used for the production of oil (Lamy *et al*, 2008). Traditionally, Jarjeer seeds and leaves are known for its medicinal properties. It is used as diuretic, laxative, stimulant in the treatment of stomach disorders, to improve digestion, to cure eye infection, kidney problems and is considered to be an excellent aphrodisiac (Yaniv *et al*., 1998; Alam *et al*., 2006). Ethanol extracts of *E. sativa* leaves and seeds have shown hepatoprotective and cytoprotective effect against liver cancer cells (Alqasoumi, 2010; Lamy *et al*., 2008). Recent studies have shown methanol extracts of *E. sativa* seeds to possess strong antibacterial activity against *Staphylococcus aureus* and *Bacillus cereus* (Salma 2015). Similarly Rani *et al*., reported methanol extracts of *E. sativa* seeds powder to have shown significant antimicrobial activity against *Enterobacter agglumerans* and *Penicillium funiculosum* (Rani *et al*., 2010). There are few reports available on antibacterial properties of seed extracts with different solvents (Khoobchandani *et al*., 2010; Gulfraz *et al*., 2011; Salma 2015), while reports related to antifungal activity of *E. sativa* seeds extracts on post harvest is scarcely available in literature. Similarly there are few reports on FT IR analysis of seed oil but FT IR analysis of ethyl acetate and methanol seed extracts has not been reported earlier to my knowledge. Hence this prompted us to screen the seed extracts, present work focuses on exploring different solvent extracts of *Eruca sativa seeds* against a panel of micro organisms and determine its chemical composition.

MATERIALS AND METHODS

Plant Material: Seeds of *E. sativa* were procured from Department of Botany, King Saud University, Saudi Arabia. Seeds were surface sterilized using 1% mercuric chloride, washed thoroughly with sterilized distil water and shade dried completely. The dried seeds were ground well into powder by using an electric grinder (Philips, Brazil) and stored in air sealed plastic bags for further analysis.

Preparation of Seed extract: Preparation of crude seed extract was carried out with different organic solvents following the method of Kaur and Arora (2009) with slight modification. Different organic extracts with increasing polarities were used for extraction purpose (chloroform, ethyl acetate, acetone, ethanol and methanol). Finely powdered seeds of *E. sativa* were dissolved in respective solvents separately and placed on rotary shaker (200 rpm) for 72h. All extracts were then passed through Whatman No.1 filter paper and the filtrates were concentrated using rotary evaporator. Individual extracts were reconstituted with their respective solvents to give a stock solution of 200mg/ml (Ashafa, 2013)

Test organisms: Methicillin resistant *Staphylococcus aureus* (MRSA) ATCC 12498, *Bacillus subtilis* ATCC 6633, *Streptococcus pyogenes* ATCC 19615, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 700603 were used as indicator microbes. All the bacterial strains used in this study for the evaluation of antibacterial activity were obtained from the Department of Microbiology, King Khaled Hospital, Riyadh, Saudi Arabia. *Colletotrichum musae, Colletotrichum gleosporoides, Fusarium oxysporum, Fusarium solani* and *Alternaria alternata* were obtained from Department of Botany and Microbiology, King Saud University, Saudi Arabia

Antibacterial activity

Well diffusion assay: Agar Well diffusion method (Valgas *et al*., 2007) was used to determine the antibacterial activity of seed extracts. Bacterial suspension (0.5 MacFarland) for each bacterial species was prepared in sterile nutrient broth and 200µl of this suspension was inoculated on the surface of solidified Muller Hinton agar plates and spread evenly. Inoculated plates were allowed to dry. After drying, the inoculated medium of each plate was punched with 6 mm diameter wells. Each well was filled with 100µl of the seed extract and incubated at 37 °C for 24 h. Antibacterial activity was measured as the diameter of the zone of inhibition (mm) around each well and all tests were carried out in triplicates. Different antibiotics discs were used to carry out antibiotic susceptibility test and the best zone with largest diameter was chosen and tabulated. The antibiotics screened were, Impenem (Im 10 µg, Oxoid), Tetracycline (30 µg), Sulphamethoxazole trimetoprine(25 µg), Vancomycin (30 µg), Optichin 10 µg. Antibiotic discs served as positive control while negative control was the extracting solvent.

Determination of Minimum inhibitory (MIC) and Minimum bactericidal concentration (MBC) of plant extract against bacteria: Minimum inhibitory concentration was carried out by tube macro dilution assay with slight modification (CLSI, 2008, 2012). Muller Hinton broth (MHB) was used for all the tests. A double fold dilution of the extracts was made using MHB to obtain various concentrations (0.2mg/ml to 100 mg/ml). Equal volumes of broth and extract were added to sterile test tubes containing 5 x10^5 CFU/ml of bacterial inoculums and incubated for 24 hr at 37°C. The highest dilution (lowest concentration) showing no visible growth was regarded as MIC. The MBC was determined as an adjunct to MIC test (Espinel-Ingroff *et al*., 2002 and Olorundare *et al*., 1992) .Briefly contents from the tubes

showing no visible growth were sub cultured on Muller Hinton agar plates and incubated at 37 °C for 72h , the concentration of the extract that did not show any growth was taken as MBC. All experiments were performed in triplicate. Control tube contained only broth and inoculum without extract

Antifungal activity: Potato dextrose agar was used as a culture medium. Extracts were screened for their antifungal ability by the poisoned food technique (Balouiri *et al*, 2016; Dhiman *et al.,* 2016). To a 9 cm sterile petri dish, 1 ml of extract followed by 19 ml of PDA was added, thoroughly mixed by gentle swirling and allowed to solidify. A mycelia plug of 6mm diameter was removed from the periphery of seven days old actively growing colony and inoculated in the center of the plate aseptically. Culture plates were incubated at 25 ± 2°C and diameter of the fungal colonies was measured after seven days. Control treatment was without the extract. Each sample was assayed in triplicate and the mean values were observed.

Percentage inhibition of mycelial growth was evaluated by measuring the relative growth of fungus in treatment and control and calculated by using the following formula.

$I = (C-T)/C \times 100$

Where I stands for percentage inhibition, T is the treatment and C denotes the mean growth rate of control.

Determination of Minimum inhibitory and Minimum fungicidal concentration (MIC, MFC) of plant extract against fungal pathogens: Minimum inhibitory concentration was carried out by tube macro dilution assay with slight modification (Valgas *et al*., 2007; CLSI, 2008, 2012). A double fold dilution of the extracts was made using Potato dextrose broth and concentrations ranging from 0.2mg/ml to 100 mg/ml were used. To sterile test tubes containing $2X10^6$ spore/ml suspensions, equal volumes of extract and broth were added. The tubes were incubated for 28 ± 20^0C for 72h for fungi. Highest dilution (lowest concentration) showing no visible growth was regarded as MIC. The contents from the tubes showing no visible growth were sub cultured on agar plates and incubated at 27^0C for 96h .The concentration which did not show any growth on fresh agar medium was regarded as MFC (Espinel-Ingroff *et al*., 2002 and Olorundare *et al*., 1992). Control tube contained only broth and inoculum without extract treatment. All experiments were performed in triplicate.

GC -MS analysis: Methanol and Ethyl acetate extracts were directly used for the Gas Chromatography-Mass Spectrometry analysis. Analysis was carried out on Clarus 500 Mass spectrometer and Gas chromatography. The different parameters involved in the operation of the Clarus 500 MS were standardized as follows:(Inlet line temperature 200°C; Ion Source temperature230°C. Mass spectra were taken at 70 eV; solvent delay time 5min, acquisition mode- scan 40-550 amu. Gas Chromatography used in the analysis employed a fused silica column packed with Elite-1 [100% dimethyl poly siloxane, 30 nm × 0.25 nm ID × 1µm df] and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The 2µl sample extract injected into the instrument was detected by the Turbo gold mass detector (Perkin Elmer) with the aid of the Turbo mass 5.1 software. The oven temperature program was 2 min at 45°C, 1.5°C/min to 100°C, 2 °C /min to 200 °C during the GC extraction process; split ratio 25:1. The injector temperature was set at 250°C (mass analyzer). The GC run time was 90 minutes. The identification of the phytocompounds and interpretation of Mass spectrum was done with the aid of standards database of NIST libraries.

Fourier Transform Infrared (FTIR) Fingerprint Analysis: To identify the characteristic functional groups present in the seed extract of *E. sativa* Fourier transform infrared (FTIR) spectrophotometer was used. After centrifuging the extract at 3000 rpm for 10 min, it was filtered through Whatmann No. 1 filter paper by using high pressure vacuum pump. The sample was diluted to 1:10 with the same solvent. Perkin Elmer 2000 spectrophotometer system with a scan range from 400 to 4000 cm−1 was used to obtain FT-IR spectrum.The spectrum was analyzed with the help of Bruker OPUS software.

Scanning electron microscopy: *Staphylococcus aureus* was chosen for Scanning electron microscopy studies. Bacterial suspension of cells treated with ethyl acetate extract at an MIC of 0.4mg/ml and 0.8mg/ml and without extract (control) was taken and centrifuged at 8000 × g for 10min; the supernatant was discarded and the bacterial suspension was spread on a glass slide and fixed by immersing it in 2.5% (4°C) glutaraldehyde overnight followed by rinsing it thrice in phosphate buffer(pH 7.2).It was then post fixed in Osmium tetroxide(1%) for an hour. Series of ethanol gradient (60%, 70%, 80%, 90%, and 100%) was used to dehydrate the bacterial cells and dried with CO_2. Dried samples were coated with gold and were examined under LTD JSM-6060LV (JEOL-Japan) scanning electron microscope

RESULTS

Antibacterial activity of *Eruca* seed extracts: The antibacterial activity of five different organic extracts of *Eruca sativa* (chloroform, ethyl acetate, acetone, ethanol and methanol) were screened for both Gram positive and Gram negative bacteria. It was found that all the extracts screened had variable effects of inhibition on test organisms (Table 1). Maximum zone of inhibition for S. *aureus* was shown by both ethyl acetate and chloroform

extracts (25.66±0.57, 23.16±0.76) followed by methanol and ethanol (16.00±1.00, 14.33±2.08). Methicillin resistant *Staphylococcus aureus* showed maximum zone of inhibition with Ethyl acetate (19.33 ±0.28) while it was resistant to both ethanol and methanol extracts. *B. subtilis* showed excellent inhibition by ethyl acetate followed by acetone and methanol extracts (25.16±0.76, 19.50±0.50, 16.50±1.50). *S. pyogenes* and *E. faecalis* exhibited poor inhibition by all the extracts used. Among the Gram negative bacteria screened in this study, *E.coli* was strongly inhibited by chloroform extracts (22.50±0.86) and *K. pneumoniae* by acetone extracts (24.83±0.28), *Pseudomonas aeroginosa* was least inhibited with chloroform and ethyl acetate extracts while was resistant to ethanol, methanol and acetone extracts .

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC): Minimum inhibitory concentration (MIC) was carried out for ethyl acetate and methanol. Both the extracts showed varied levels of inhibitory effects on test organisms. Amongst the Gram positive bacteria screened, *S. aureus* and *B. subtilis* were inhibited at a lower concentration of 0.4mg/ml with ethyl acetate extracts while the bactericidal concentrations was 0.8 mg/ml for *S. aureus* and 1.6 mg/ml for *B. subtilis. E. faecalis* was inhibited at 6.25mg/ml and the bactericidal concentration was 12.5 mg/ml with ethyl acetate extracts. However, the gram negative bacteria *E. coli* was sensitive to ethyl acetate extract and was inhibited at 0.4mg/ml but *K. pneumoniae* and *P. aeroginosa* responded at higher concentrations. In comparison to ethyl acetate extracts, methanol extracts were less effective against all the bacteria screened. Both MRSA and *P. aeroginosa* were not inhibited by methanol extracts (Table 3).

Antifungal activity of *E.sativa* seed extracts: All the fungi screened showed antifungal activity towards the eruca extracts except for *C. gleosporoides*. Amongst all the extracts screened, methanol followed by ethanol extracts were found to be effective in controlling the mycelial growth of test fungi. All the solvent extracts effectively controlled the mycelial growth of *C. musae* with methanol showing 81.44% inhibition, ethyl acetate 75.55%, ethanol 73.58% and acetone 71.84%. *A. alternata* and *F. solani* showed mycelial inhibition in the range of 39.62-57.40% and 34.07-50.00% with various seed extracts. Methanol, ethanol and ethyl acetate extracts were able to inhibit the mycelial growth of *F. oxysporum* very effectively. However, chloroform and acetone extracts were unable to cause any inhibition (Table 2).

Minimum Inhibitory concentration (MIC) and Minimum fungicidal concentration (MFC): All the fungi screened were inhibited by both ethyl acetate and methanol extracts except for *C. gleopsoroides* which did not show mycelial growth inhibition, hence was not tested. Both the extracts showed strong fungicidal effect on *C. muase* at a very low concentration of 0.2mg/ml, while the MIC and MFC values varied between 0.2mg/ml-12.5mg/m for *A. alternata* , *F. oxysporum* and *F. solani* (Table 3)

GC-MS: GC-MS chromatogram analysis of the ethyl acetate extract and methanolic extract (Figure 2 and 3) of *Eruca sativa* showed a total of 18 peaks and 11 main peaks of which 5 and 7 peaks were not identified in both the extracts. The Mass spectra of the phytocompounds were compared with NIST library and identified. A list of major compounds identified by their retention time, molecular formula and molecular weight are listed in the table (Table 4). Compounds mainly comprised of hydrocarbons, esters, alcohols, aromatic compounds, phenols and ketones. Acetyl acid , butyl ester; O-Xylene ; Ethanol , 2-butoxy; Butane ,1,1,oxy bis(2,1-etanediyloxy) bis; Ethanol ,2-(2-butoxyethoxy); Thiocyanic acid , methylene ester; 1,2,3,4,5,6-Hexahydro-1, 1,5,5-tetramethyl-2,4a-mathanonaph-7(4aH)-one; Vitamin A aldehyde; Testosterone propionate; Phenyl cyclohexyl ketone; Fenipentol; Benzyl alcohol; Benzenemethanol, 3-phenoxy;2-Cyclohexene-1-one, 3-phenyl-; 2,5-Cyclohexadiene-1, 4-dione, 2,6-bis (1,1-dimethylethyl) were identified in ethyl acetate extracts whereas the methanol extracts revealed fewer compounds which includes Cyclohexene ,1-methyl-4-(1-methyl ethylidene), Silane, phenyl, Teripinolene, 1,2,3,4,5,6-Hexahydro -1,1,5,5-tetramethyl-2, 4a-mathanonaph-7 (4aH)-one, Testosterone propionate, Benzene acetic acid, alpha-hydroxy-,ethyl ester, Benzene methanol ,alpha-(ethoxymethyl) (Table 5).

FT-IR: The FT-IR analysis of the ethyl acetate seed extracts of *E. sativa* revealed the presence of characteristic absorption spectrum which is presented (figure 4), while the frequency range and the functional group obtained from absorption spectrum are presented in Table 6.The spectrum shows the presence of biological active functional groups such as the CH_2 asymmetric and symmetric stretching at 2923.34 cm-1 and 2853.68 cm-1 .The absorption at 1738.56 cm-1 is due to C=O stretching. The peak at 1463.98 cm-1 represents C=C stretching, while 1372.35 cm -1is due to CH, CH2 bending. Peaks obtained at 1160.99 cm-1and 1097.76 cm-1 and the prominent peak at 1044.71 is due the C-O stretching region (resulting from stretching vibrations of C-O and C-O-C).The peak at 607.40 cm-1 is a distinctive finger print region.

Scanning electron microscopy: Scanning electron microscopy images of *S. aureus* showed structural changes when compared to control. It was observed that the cells in control treatment which was without any treatment were smooth cocci with clear intact and regular

margins, whereas the extract (0.4mg/ml) induced changes in cell morphology of *S. aureus* (Figure 1A). As seen in the micrographs (Figure 1B, C) cells show prominent blebs, clustering and corrugated cell wall. At a concentration of 0.8mg/ml, cell aggregation and complete cell lysis occurred which is clearly seen in the Figure (Figure 1D, E).

Table 1. Antibacterial activity of crude organic extracts of *Eruca sativa* seeds.

Microorganisms	Zone of Inhibition(mm)					
	Extracts used					
	Chloroform	Ethyl acetate	Acetone	Ethanol	Methanol	Antiibiotics
Staphylococcus aureus	23.16±0.76	25.66±0.57	13.83±1.60	14.33±2.08	16.00 ± 1.00	(Amp)27.67±1.16
MRSA	8.00±0.00	19.33±0.28	16.16±1.04	0.00±0.00	0.00±0.00	(V)26.00±0.00
Bacillus subtilis	0.00±0.00	25.16±0.76	19.50±0.50	12.00±0.00	16.50±1.50	(T)27.33±0.58
Streptococcus pyogenes	9.00±0.00	16.50±0.50	8.83±0.57	10.16±0.28	13.33±1.04	(ST)22.60±0.20
Enterococcus faecalis	8.50±0.50	15.00±0.00	11.50±0.50	12.00±1.00	18.33±0.50	(O)25.83±0.57
Escherichia coli	25.66±0.57	22.50±.0.86	8.00±0.00	8.50±1.00	9.00±1.00	(T)36.40±0.17
Klebsiella pneumonia	9.00±1.00	12.16±0.76	24.83±0.28	9.66±0.28	18.66±0.50	(ST)29.0±0.00
Pseudomonsa aeruginosa	8.16±0.28	8.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	(IM)9.83±0.28

0.00-Indicates no inhibition. MRSA-Methicillin resistant Staphylococus aureus.Values are mean inhibition zone (mm) ± SD of three replicates. Antibiotics used -Ampicillin (10µg) Tetracycline (T, 30 µg), Sulphamethoxazole trimetoprine, (ST, 25 µg), and Impenem (Im, 10 µg) Vancomycin (V,30µg),Optichin(O,10µg)

Table 2. Inhibitory activity of organic extracts on the radial growth of fungi tested

Fungi used	Percent(%) inhibition of the mycelial growth						
	Extracts used						
	Chloroform	Ethyl acetate	Acetone	Ethanol	Methanol	Standard	Control
C. musae	58.88±1.92	75.55±2.93	71.84±2.79	73.58±3.69	81.84±2.79	(C)100	0.00±0.00
C. gleosporoides	0.00±0.00	0.00±0.00	6.07±0.06	0.00±0.00	0.00±0.00	(C)100	0.00±0.00
A. alternate	47.03±2.79	51.10±2.22	55.18±1.69	39.62±1.28	57.40±3.51	(M)100	0.00±0.00
F. oxysporum	0.00±0.00	71.03±1.12	0.00±0.00	60.36±1.69	64.22±2.22	(M)98.66±0.06	0.00±0.00
F.solani	38.06±1.22	34.07±1.28	50.00±0.00	35.55±2.22	49.99±1.11	(M)90.16±0.28	0.00±0.00

0.00-Indicates no inhibition. (C) - Carbendazim 0.2%, (M)- Mancozeb. 0.00-Indicates no inhibition. (C)-Carbendazim 0.2%,(M)-Mancozeb 0.2%. Each values represented in tables are means ± SD (N=3).

Table 3. Minimum Inhibitory concentration and Minimum microbicidal activity. (MBC,MFC -mg/ml)of *Eruca sativa* seed extract for bacteria and fungi

Microorganism	MIC		MBC	
Bacteria	**Ethyl acetate**	**Methanol**	**Ethyl acetate**	**Methanol**
Staphylococcus aureus	0.4	6.25	0.8	12.5
MRSA	0.8	NI	6.25	NI
Bacillus subtilis	0.4	12.5	1.6	25
Streptococcus pyogenes	1.6	6.25	1.6	6.25
Enterococcus faecalis	6.25	12.5	12.5	12.5
Escherichia coli	0.4	25	3.1	25
Klebsiella pneumonia	25	25	50	>100
Pseudomonsa aeruginosa	>50	NI	>100	NT
Fungi				
C.musae	0.2	0.2	0.2	0.2
C.gleopsoroides	NI	NI	NT	NT
A.alternata.	6.25	6.25	6.25	12.5
F.oxysporum	0.2	0.2	0.2	0.8
F.solani	12.5	0.2	12.5	0.2

NI-Not Inhibited; NT-Not Tested

Table 4. Phytoconstituents found in the ethyl acetate seed extracts of *Eruca sativa*

S. No	Name of the Compound	Retention Time (min)	Molecular formula	Molecular weight
1	Acetyl acid, butyl ester	5.42	$C_6H_{12}O_2$	116
2	O-Xylene	7.49	C_8H_{10}	106
3	Ethanol, 2-butoxy	9.16	$C_6H_{14}O_2$	118
4	Butane, 1,1,[oxy bis(2,1-etanediyloxy)]bis	9.16	$C_{12}H_{26}O_3$	218
5	Ethanol, 2-(2-butoxyethoxy)-	9.16	$C_8H_{18}O_3$	162
6	Thiocyanic acid, methylene ester	13.751	$C_3H_2N_2S_2$	130
7	1,2,3,4,5,6-Hexahydro-1,1,5,5-tetramethyl-2,4a-mathanonaphthalen-(4aH)-one	55.16	$C_{15}H_{22}O$	218
8	Vitamin A aldehyde	55.16	$C_{20}H_{28}O$	284
9	Testosterone propionate	55.68	$C_{22}H_{32}O_3$	334
10	Phenyl cyclohexyl ketone	65.31	$C_{13}H_{16}O$	188
11	Fenipentol	68.93	$C_{11}H_{16}O$	164
12	Benzyl alcohol	68.93	C_7H_8O	108
13	Benzenemethanol,3-phenoxy	77.68	$C_{13}H_{12}O_2$	200
14	2-Cyclohexen-1-one,3-phenyl-	78.51	$C_{12}H_{12}O$	172
15	2,5-Cyclohexadiene-1,4-dione,2,6-bis(1,1-dimethylethyl)	79.03	$C_{14}H_{20}O_2$	220

Table 5. Phytoconstituents found in the methanol seed extracts of *Eruca sativa.*

S.No	Name of the Compound	Retention Time (min)	Molecular formula	Molecular weight
1	Cyclohexene, 1-methyl-4-(1-methylethylidene)	31.61	$C_{10}H_{16}$	136
2	Silane, phenyl-	31.61	C_6H_8Si	108
3	Teripinolene	51.02	$C_{10}H_{16}$	136
4	1,2,3,4,5,6-Hexahydro-1,1,5,5-tetramethyl-2,4a-mathanonaph-7(4aH)-one	55.16	$C_{15}H_{22}O$	218
5	Testosterone propionate	55.16	$C_{22}H_{32}O_3$	334
6	Benzene acetic acid, alpha-hydroxy-,ethyl ester	68.86	$C_{10}H_{12}O_3$	180
7	Benzene methanol, alpha-(ethoxymethyl)-	68.86	$C_{10}H_{14}O_2$	166

Table 5. FT-IR spectral peak values and the major functional groups present in *Eruca sativa* seed extracts (ethyl acetate extracts).

Peak values (frequency, cm-1)	Functional group
2923	CH_2 asymmetry stretching
2853	CH_2 symmetry stretching
1738	C=O stretch
1463	C=C stretching
1372	CH bending
1235	C=O stretch
1160	C-O stretching
1097	C-O stretching
1044	C-O stretching

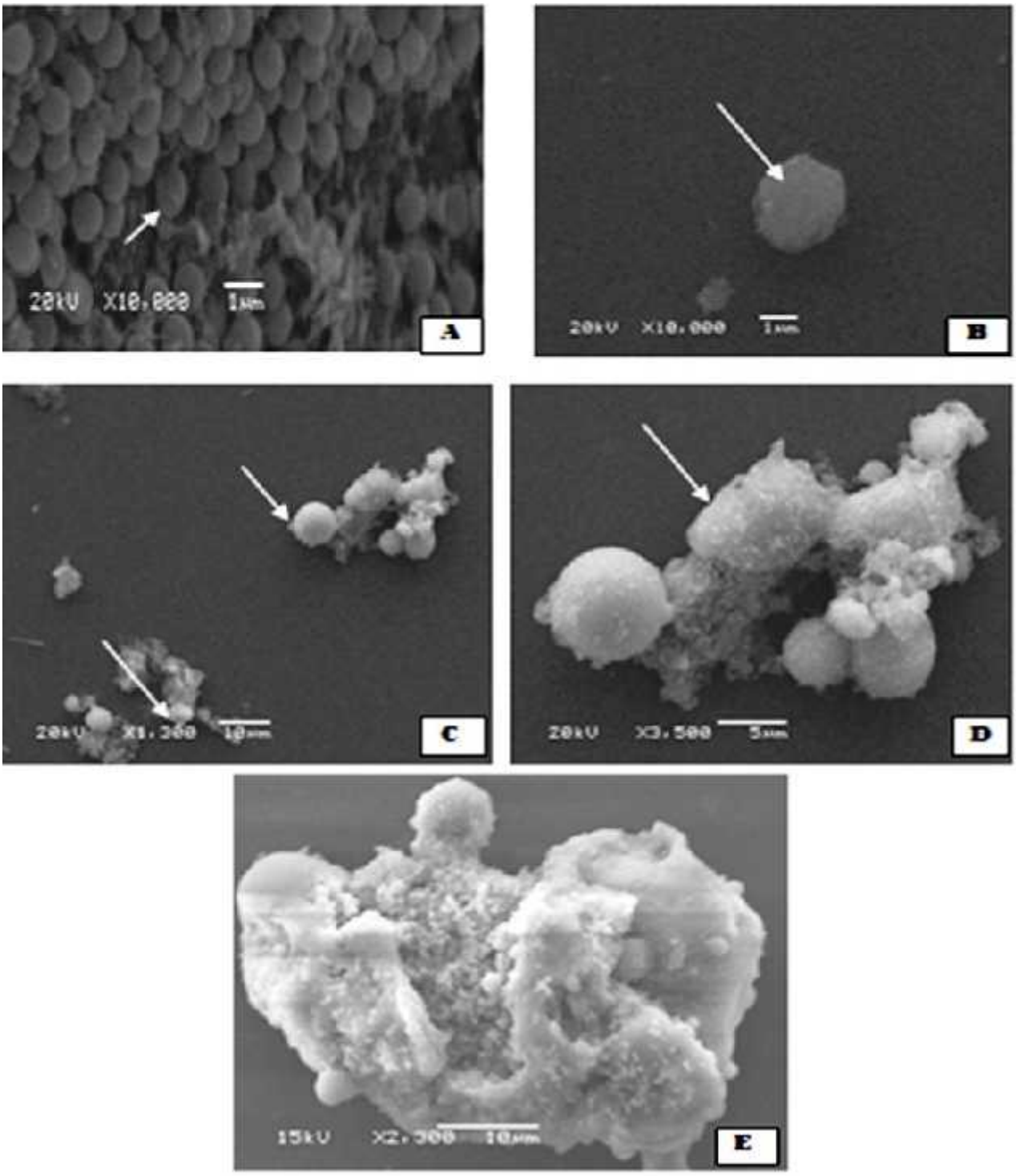

Figure 1.SEM micrograph showing the *S.aureus* cells with various levels of degeneration when treated with Eruca seed extract (ethyl acetate extract). Figure 1A –shows intact, regular and smooth cocci .B -shows irregular margin, C and D-Cells showing blebs, rupture and aggregation.1E- Clustering and complete destruction of cells

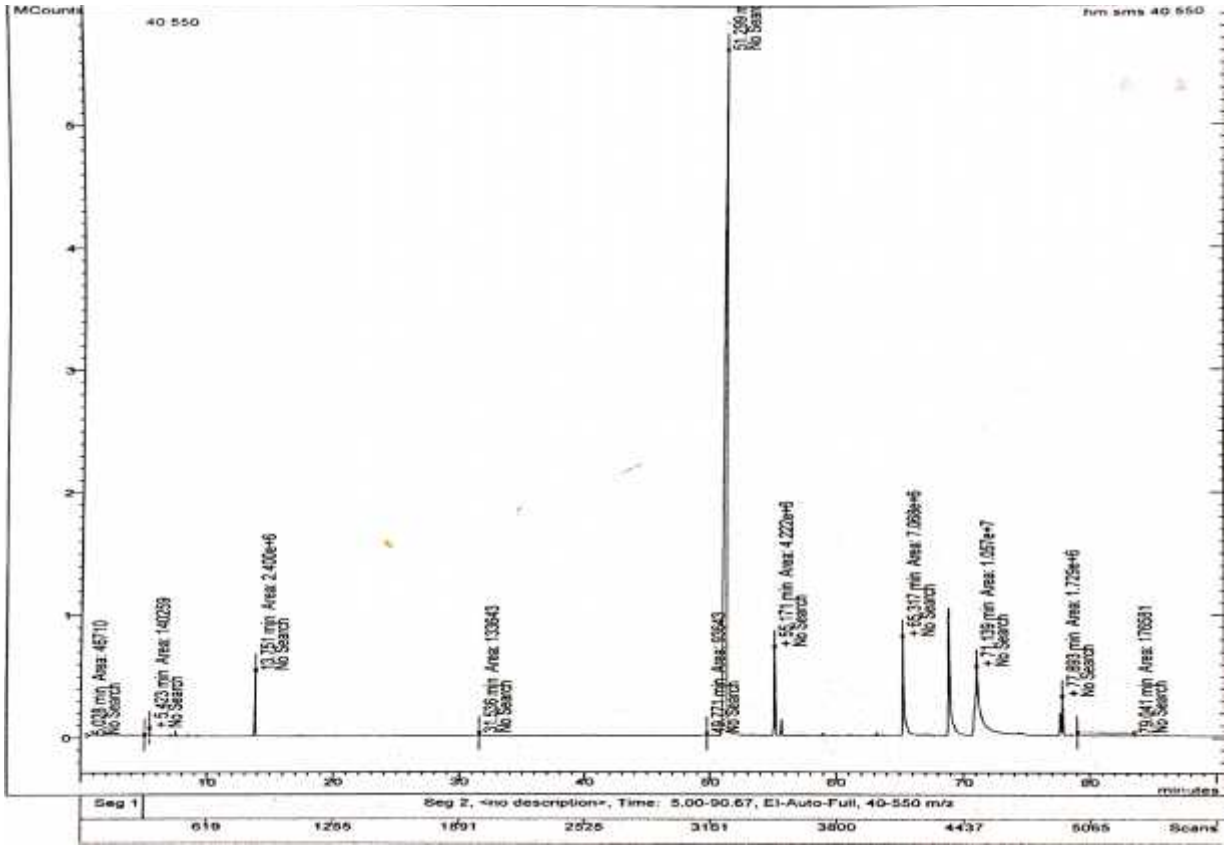

Figure 2. GC-MS Chromatogram of ethyl acetate extracts of *Eruca seeds*

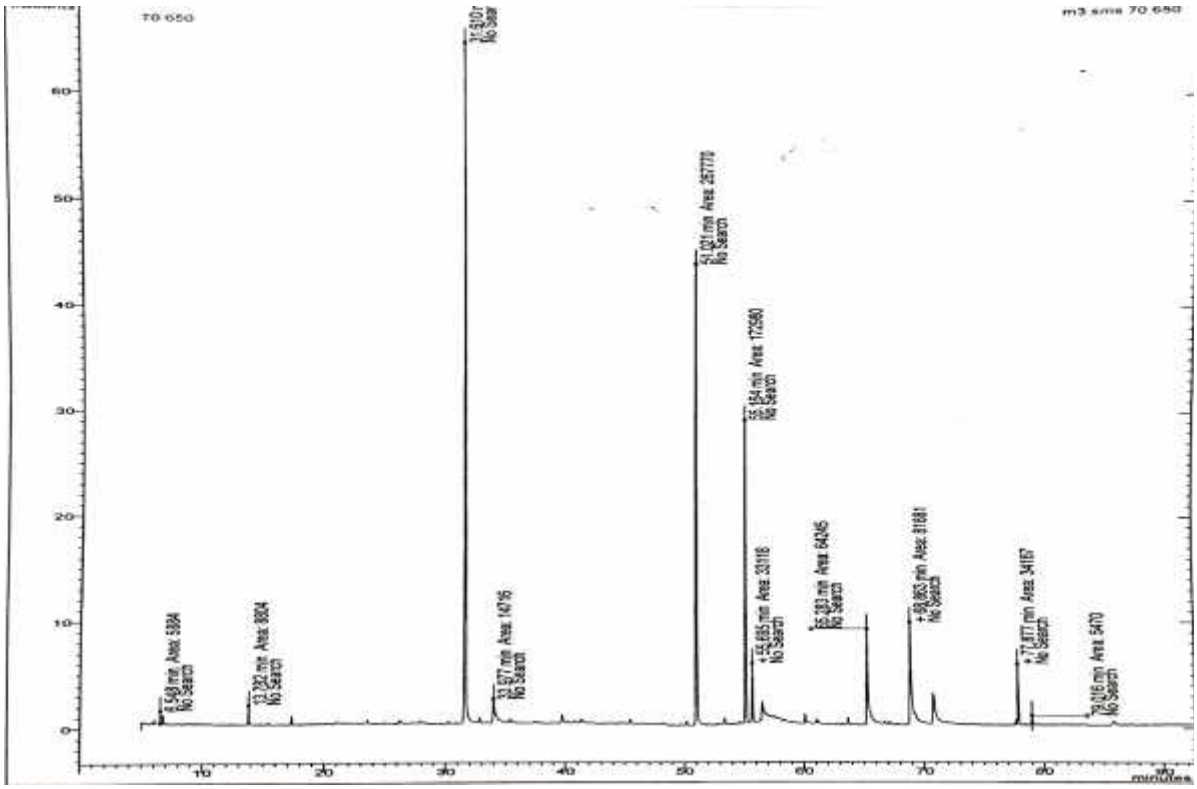

Figure 3. GC-MS chromatogram of methanolic seed extracts of *Eruca sativa*

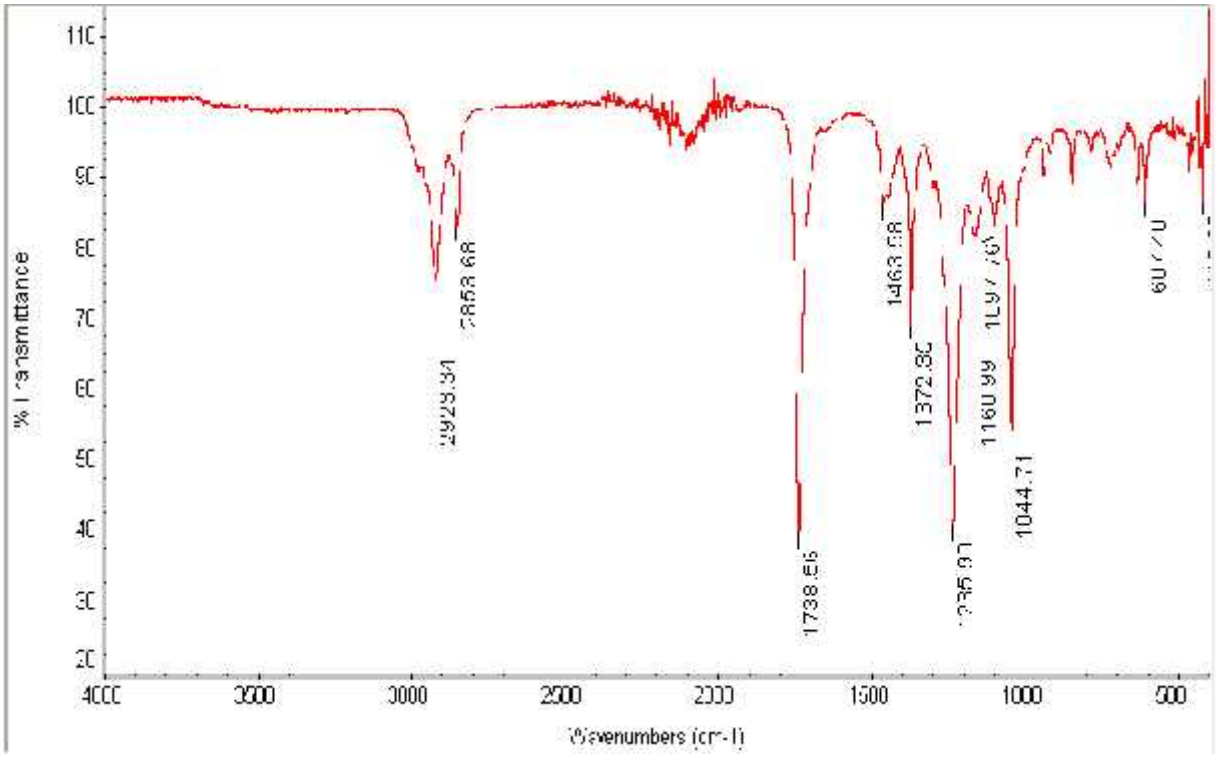

Figure 4. FT-IR spectrum of *Eruca sativa* seed (ethyl acetate extracts).

DISCUSSION

Alternative medicine has given us enormous number of therapeutic agents in the past and the search is still ongoing (Olajuyigbe *et al*., 2012; Khan *et al*., 2012). Recent reports show the alarming rise in the antimicrobial resistant strains leading to less effective drugs on the horizon, coupled with few new discoveries of antimicrobials (Khan *et al*., 2012).Researchers in the recent years have reported Brassicaceae members to be a rich source of phytochemicals (Beevi *et al*., 2009), many of the chemical compounds found in Brassicaceae are potential antioxidants ,cancer preventive ,antibacterial, antifungal, antimutagenic and antitumor agents (Ghazanfar and Al Sabahi, 1993 ; Fimognari *et al*., 2004 ; Barillari *et al*., 2005 ; Yahuda *et al*.,2009; Hanafi *et al*., 2010). *E. sativa* is a known member of the same family, its extracts and oils contain important secondary metabolites such as alkaloids, flavanoids, tannins and phenols which are known to possess antimicrobial and antioxidant properties(Gulfraz *et al*.,2011;Salma 2015). The presence of phenolic compound, glucosinolates, isothiocyanates, and erucin in the seed indicates its antimicrobial properties against pathogenic bacteria (Khoobchandani *et al.*, 2010, Aires *et al*., 2009).

Present study was conducted to analyze the antibacterial and antifungal activities of *E. sativa* seed extracts. Our results clearly demonstrate that amongst the five different solvents extracts screened for their antimicrobial activity, ethyl acetate extracts exhibited maximum antibacterial activity while methanol was highly potent in controlling the growth of fungi tested. Similarly, Khoochandani *et al*. (2010) also reported ethyl acetate extracts of aerial and root parts to possess good antibacterial activity against *B. subtilus* and *S. aureus.* In another study ethyl acetate fraction of *C. intybus* seeds was found to be the most effective against *E. coli* and *S. aureus* (Mehmood *et al*., 2012). However Gulfaraz *et al.* (2011) reported *Eruca* seed oil followed by ethyl acetate extracts to be potent in controlling all bacteria used in his study.

Mycelia growth of fungi screened were effectively controlled methanol extracts followed by ethanol extracts. Rani *et al*. (2010) reported varying degrees of inhibition of *Penicillium lilacinum, Paecilomyces variotii, Spadicoides stoveri, Penicillium funiculosum by* both methanolic and crude water extracts of *E. sativa*.

MIC results and diameter of inhibition zones clearly indicates that the gram positive bacteria were more sensitive to the extracts than Gram negative bacteria. This kind of response by gram positive bacteria could be attributed to the peptidoglycan layer which is mesh like and more accessible to the permeation of the extracts (Qadan *et al*., 2005; Ramesh *et al*., 2007) , while the outer lipopolysaccharide membrane of the Gram negative bacteria serves as an effective permeability barrier restricting the entry of the plant extracts. The

proteins, in particular porins constitute a selective barrier to hydrophilic solutes with the exclusion limit of 600Da (Gurinder Kaur *et al*., 2009). It has been reported earlier that Gram-negative bacteria are usually more resistant to the plant-origin antimicrobials and even show no effect, compared to Gram-positive bacteria (Ramesh *et al*., 2007; Stefanello *et al*., 2008; Taj Karimi *et al*., 2010). According to Sahgal, the morphological structure of the bacterial cells and their composition could be responsible for the differences in the MIC values (Sahgal *et al*., 2011).

Eruca seed extract induced morphological effects like blebing, aggregation, distortion in shape and rupture of cells, which is clear in the micrographs of *S. aureus*. Our results are in agreement with the findings of other studies where similar structural deformities were observed in MRSA and *S. aureus* (Darah and Lim, 2015; Su *et al*., 2015; Jiamboonsri *et al*., 2011). According to Darah *et al*. (2013) antimicrobial compounds in extracts interact with cell membrane and cause ultra-structural changes which are clear expressions of profound biochemical changes. Braga and Ricci (2002) reported that the integrity of cell wall and bacterial structure are imperative in maintaining the vitality and the virulence of bacteria

Plant extracts contain wide array of phytoconstituents and many are known to be bioactive compounds which exhibit various diverse pharmacological activities (Gu R *et al*., 2014).Their secondary metabolites serve in plant defence mechanism, against predation by insects, microorganisms and herbivores. Extracts exhibiting highest antimicrobial activity were subjected to GCMS analysis to identify the molecular structure by interpreting its mass spectrum. Further, extracts were subjected to FTIR as it is an important and valuable tool for identifying and characterizing the functional group (chemical bonds) present in these compounds (Elberhardt *et al*., 2007; Hazra *et al*., 2007). Additionally, the spectrum obtained from FTIR analysis is considered like a molecular fingerprint because of its uniqueness (Sasidharan *et al*., 2011). GCMS analysis elucidated the presence of important phytochemicals like ,O-Xylene , benzoic acids and its derivatives which are known to possess antibacterial, antifungal and defensive functions (llondu and Bosah., 2015; Friedman *et al*., 2003).Role of esters as antifungal compound was also reported earlier (Ahmad *et al*., 2012; Hema *et al*., 2011).In a recent study 2 ,5-cyclohexadiene-1,4-dione-2,6-bis (1,1- dimethylethyl), was isolated , identified and purified from *Trichoderma* strain (t-33) and was reported as an active antifungal compound (Li *et al*., 2015).

The Major regions of the peaks obtained in the IR spectrum in our study; 2923; 2853; 1738; 1463 and 1160 were also reported in earlier studies conducted with *Eruca seed* oil (Mumtaz *et al*., 2012; Sharma *et al*., 2014). However, the peak 1235, 1044 and 607 were not reported in oils. In a bio-organic molecule, functional groups influence the biological activity as they contribute significantly to their inherent acid -base properties, partition coefficient, stereochemistry and solubility etc (Zavod and Knittel, 2008). Our results indicate that the *E. sativa* seed extracts contain bioactive functional groups like hydrocarbon, esters, fatty acids, aldehydes and ketones. All of these compounds are known to possess antimicrobial, anti-secretory, anti-ulcer, cytoprotective, neuroprotective, anti-inflammatory and potent antioxidant properties as mentioned in previous reports (Alqasoumi *et al*., 2009; Miranda *et al*., 2014; Samia *et al*., 2014).

Conclusion: The present study demonstrates that *E.sativa* seed extracts possess strong antimicrobial activity. Presence of important bioactive compounds with antimicrobial properties further authenticates it as a promising candidate for pharmaceutical industry. Hence, further research is needed to isolate bioactive compounds which may be recommended as potent antimicrobials for therapeutic purposes as an alternative medicine.

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