ANTIBACTERIAL ACTIVITY AND GLC OF MEDICINAL SEED OILS

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ABSTRACT: *The work reported in this article is an extension of the project conducted by some researchers in Pakistan on the antibacterial activity of medicinal seeds and oils extracted from them using ethanol and acetone as solvents. Gas liquid chromatography (GLC) of oils from Brassica campestris (Sarson), Ricinus communis (Arund) and Eruca sativa (Taramira) was conducted to explore their lipid components for their assessment as active principles against bacteria. Extraction of oils of B. compestris, R. communis and E. sativa was carried out using ethanol and acetone as solvents. The extracted oils were quantitatively examined for antibacterial activity. The oil extracted from all three seeds with ethanol and acetone was active and inhibited the growth of S. aureus and E. coli.*

Keywords: Antibacterial, Activity, GLC, Medicinal, Seeds, Oils

1. INTRODUCTION

The control of infectious diseases has been one of the major problems faced by humanity throughout the history. Currently, it is worst hitting the developing countries due to lack of proper control of these diseases caused by the scarcity of medicines, low purchasing power, inadequate medical treatment, ignorance and indifferent attitude of public towards physical health.

The measures normally followed for the control are the chemotherapy, supply of balanced diet, maintenance of proper hygiene, etc. The antibiotics, the major tools of chemotherapy are very expensive and being widely misused via self-medication and substituted by unauthenticated alternative medical drugs due to their severe side effects. Even the medical practitioners are not making proper use of these drugs. Moreover, the misuse of antibiotics has widely caused the creation of resistant pathogens.

The use of medicinal seeds to treat various infectious diseases has been known in the Indo-Pak subcontinent and in some eastern countries such as China since ages. Drugs of plant origin have served as the means for the treatment of most human diseases and ailments. Their use has progressed with the passage of time and at present, these treatments are in practice as the major tool of eastern style medicine known as Unani-Hikmat (Greek Medicine). The natural plants and seeds carry significant antimicrobial activity and other characteristics, such as antipyretic, analgesic and divertic actions. That is why many scientists, particularly from the field of Biochemistry have become interested in their chemical analysis and the therapeutic action.

The systematic investigation of drugs on modern scientific lines was carried more about forty years ago and comparatively speaking much has been accomplished during this short span of time. A number of important medicinal plants prescribed by Kavirajes in China, Hakims in Pakistan and India have been carefully investigated in different contexts. Their chemical composition has been determined, pharmacological action of the active principles worked out by experimentation on animals, and finally suitable preparations made from the drugs have been tested on patients in the hospitals. It is only by such a thorough enquiry that the real merits of these drugs have been proved and their demand has been created for not only in Pakistan but in other parts of the world. This laborious work has brought into prominence the merits and qualities of certain drugs. These drugs may prove to be very valuable additions to the present armamentarium of the medicinal man to relieve the sufferings of humanity.

Lack of research in Oriental Medicine has compelled its scholars to collaborate with the scientists at large to rationalize their use as a treatment to cure various diseases. In Pakistan Hamdard Foundation is taking keen interest in the identification, classification, chemical analysis and the therapeutic action of medicinal plants and seeds.

Nature has provided Pakistan with a large number of plants and seeds which carry medicinal value. Some of these plants grow naturally and some are cultivated in the farms. Their chemical and medicinal investigation in the above context bears great importance. That is why some developing countries are conducting research on medicinal seeds and plants to develop technology for extraction and preparation of alternative drugs. The research workers in Pakistan are also actively involved in the exploitation of new sources of medicine.

Rashed determined the antibacterial activity of some medicinal plants and seeds against Escherichia coli and Staphylococcus aureus [1]. All water extracts of plants and seeds were found to be inactive except of Carum copticum (ajowain) which exhibited antibacterial activity against S. aureus. Homogenized seeds of Nigella sative (kalongi) and Carum copticum (ajowain) showed antibacterial activity against S. aureus. Extraction of active components of both ajowain and kalongi was carried out using ethanol and acetone as solvents. Alcohol extracted oil of kalonji and acetone extracted oil of ajowain were active and prevented the growth of S. aureus.

Sundar studied the fatty acid composition of six seed oils of Alyogine hakeifolia, Alyogine, huegelii, Gossypium austral, Hibiseus coastessi, Lawrencia viridigrisea and Radyera farragei. They contained 13.5-18.6% oil. Linoleic acid dominated (60.0 – 68.2%) in the component fatty acids followed by palmitic acid and oleic acid. Cyclopropene fatty acids, Sterculic acid and malalic were present in small concentration (1.0-4.4%, 0.1-1.5% respectively). Dihydrosterculate acid was present in small quantities [2].

Javed and Khan tested water extracts and homogenized powders of six medicinal plant seeds against Staphylococcus

aureu and Escherichia coli. All water extracts were inactive except of Brassica compastris (Sarson) which exhibited antibacterial activity against both the organisms. Homogenized seeds of Eruca sativa (Taramira), Ricinis communis (Arund) and Brassica compastris (Sarson) showed antibacterial activity against S. aureus as well as against E. coli. The results indicated that the active components of B. compastris were soluble and that of R. communis and E. sativa were completely insoluble in water. Extraction of active components of E. sativa, B. compastris and R. communis was carried out using ethanol and acetone as solvents. The extracted oils of E. sativa, R. communis and B. compastris with ethanol and acetone were active and inhibited the growth of both S. aureus and E. coli [3].

Ahmad, et al (1998) screened 82 Indian medicinal plants traditionally used in medicine for their antibacterial activity against several pathogenic and opportunistic microorganisms using agar well diffusion method. The results indicated that out of 82, 56 exhibited antibacterial activity against one or more test pathogens. Extracts of five plants showed strong and broad spectrum activity as compared to rest of 51 plant extracts which demonstrated moderate activity. On the whole the alcoholic extracts showed greater activity than their corresponding aqueous and hexane extracts [4].

Leonardo determined in vitro antimicrobial activity of a castor oil-based irrigant against Gram-positive cocci (*Micrococcus luteus, Staphylococcus aureus, Enterococcus faecalis, Staphylococcus epidermidis, Streptococcus mutans*, and *Streptococcus sobrinus*), Gram-negative rods (*Escherichia coli* and *Pseudomonas aeruginosa*), and the yeast *Candida albicans* applying two-layer agar diffusion technique. All bacterial strains were inhibited by 2.0% chlorhexidine gluconate. Endoquil was effective against Gram positive microorganisms, and 0.5% NaOCI was effective only against *S. aureus* [5].

The oil of *Cassia absus* was extracted with petroleum ether and acetone by Saad, et al.. The oil was subsequently investigated for its fundamental properties and antibacterial activity and the residue of *Cassia absus*, left after the extraction was checked for its antibacterial activity. It was observed that the oil extracted with petroleum ether was inactive against *Escherichia coli* and *Staphylococcus aureus* while that extracted with acetone was active against both pathogens. The residue left after extraction with petroleum ether was active and that left after extraction with acetone was inactive. The properties of oils such as color, melting point, specific gravity, refractive index, viscosity, iodine value, acid value, saponification value, ester value, peroxide value, Richert-Meisal value and unsaponifiable matter differed slightly. The major acid components of oil found by TLC were caproic acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, oleic acid, etc. The TLC also indicated the presence of some unknown components [6].

Bhattacharjee and Ghosh determined antimicrobial activity of the essential oil of Cestrum diurnum a single or multi-stemmed shrub also known as Day Jasmine. The essential oil of the mature leaves of C. diurnum was analyzed by Gas liquid chromatography (GLC) and Gas liquid chromatography-mass spectrometry (GLC-MS) and 14 components were detected. The main constituents were palmitic acid (27.62%), stearic acid (4.62%) and oleic acid (3.06%). The essential oil of mature leaves of C. diurnum was tested for antimicrobial activity against pathogenic strains of Gram positive (Staphylococcus aureus, Bacillus subtilis) and Gram negative (Escherichia coli, Pseudomonas aeruginosa) bacteria. The oil showed strong in vitro activity against P. aeruginosa and S. aureus [7].

Mahesh, and Satish tested the antimicrobial activity of some important medicinal plants against plant and human pathogens. The methanol leaf extracts of Acacia nilotica, Sida cordifolia, Tinospora cordifolia, Withania somnifer and Ziziphus mauritiana showed significant antibacterial activity against Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus and Xanthomonas axonopodis, Puccinia malvacearum antifungal activity against Aspergillus flavus [8].

Turgis, *et al* studied antimicrobial activity of mustard essential oil against Escherichia coli $O157:H_7$ and Salmonella typhi with an objective to know how mustard essential oil affected the cell membrane of these organisms. The researchers claimed that the treatment with mustard essential oil affects the concentration of intracellular component, such as ATP in both bacteria and also the pH suggesting that cytoplasmic membrane is involved in the antimicrobial action of mustard essential oil resulting in a loss of cell homeostasis and thus Mustard essential oil can be used as an effective antimicrobial agent [9].

Khoobchandani, et al determined the antimicrobial activity of various solvent extracts of *Eruca sativa* (aerial and root) and seed oil against-antibiotic resistant Gram-negative (Escherichia coli, Pseudomoms aeruginosa and Shigella flexneri) and Gram-positive (Staphylococcus aureus and Bacillus subtilis) bacteria and compared it with analytical profile of traditional Eruca sativa seed oil. *Eruca* sativa seed oil was found to be the most active, exhibiting a maximum zone inhibition of 97% for Gram-positive bacteria and of 74–97% for Gram-negative bacteria. Analytical investigation on main volatile and non-volatile components revealed that the seed oil of E. sativa exhibits promising pharmacological effects ensuring the presence of bioactive components responsible for the observed benefits [10].

Shoaib et. al. conducted chemical analysis of some edible oils of Syzigium aromaticum (Clove), Nigella sativa (Kalonji) and Eruca sativa Miller (Taramira) and determined antimicrobial activity against bacteria and fungi by agar well diffusion assay. Klebsiella pneumonia, Aspergillus lavus and Cunninghamella were found to be more sensitive organisms showing large zones of inhibition. Eruca sativa showed highest antifungal potential as compared to other tested oils. Thus researchers concluded that these oils can be used as bio control agents to treat bacterial and fungal infections [11].

The review suggests that nature is enriched extensively by medicinal plants and thus there is no end to enquiry for exploration of the presence of active principles in the herbs, plants and seeds for preparing cures for the ailing humanity from this source. Here the work partly done by our predecessors on Brassica campestris (Sarson), Ricinus communis (Arund) and Eruca sativa (Taramira) was extended

further to determine the components of these oils responsible for antibacterial activity.

2. MATERIALS AND METHODS

It was planned to carry out the work in the following stages: Extraction of oil from medicinal seeds and quantitative determination, identification of fatty acid composition of oil by GLC and determination of activity of GLC separated components.

2.1. Seed Samples

Seeds investigated for the antibacterial activity were Brassica campestris (Sarson), Ricinus communis (Arund) and Eruca sativa (Taramira). These were purchased from the local market of Lahore.

2.2. Extraction of Oil

The extraction of oil from 250g seed powder was accomplished with 300 ml ethanol as solvent using Soxhelt apparatus for 20 hours. The solvent was recovered by distillation and yield of oily residue was calculated. The extracted oil was stored in a refrigerator. The seed material left after extraction with solvent was subjected to another extraction for 20 hours using acetone as solvent. Acetone was distilled off and the oily residue was also stored.

2.3. Quantitative Determination of the Antibacterial Activity of Oils Extracted from Seeds

Media

The antibacterial activity of oils was determined against S. aureus and E. coli. The cultivation medium used for both was Oxoid's antibiotic medium number 3 (liquid broth); most widely used medium for cultivation of bacteria.

Its composition was as under in table 2.1:

Table 2.1: Composition of medium for cultivation of bacteria

Constituents	Gram/liter
Peptone (Oxoid L. 34)	5.0
Yeast extract (Oxoid L. 21)	1.5
Lab-lemico powder (Oxoid L. 29)	1.5
Dextrose	1.0
NaCl	3.5
Dipotassium hydrogen phosphate	3.68
Potassium dihydrogen phosphate	1.32
pH = 7.0	

Preparation of Medium

500 ml of the prepared medium was taken in a one-liter flask and tween-20 was added up to the concentration of 20% as an emulsifier. The resultant medium was sterilized and cooled to room temperature. Three sets each consisting of seven plugged and sterilized tubes which were numbered from zero to six were taken. One of the sets was used as reference set.

In each of the tubes of reference set, 10 ml of sterilized medium was poured and the tubes were carefully replugged. The rest of the medium in the flask was then inoculated by a loop full of one of the standard cultural suspension and mixed. 10 ml of the incubated medium was poured in each tube of the two sets. The oil extracted with ethanol was added to each tube of the three sets with the help of a micro-syringe as given below in table 2.2.

Table 2.2: Amount of oil extracted in each tube.

Tube No.	0	1	2	3	4	5	6
Amount of Oil	0	10	20	30	40	50	60
Amount of Oil in ppm	0	1000	2000	3000	4000	5000	6000

The tubes were incubated at 35^{o}C for 20 hours. The absorbance of each set of tubes after having been shaken was read at 530 nm using Spectronic-21.

2.4. Identification of Fatty Acid Composition by GLC

Etherification of fatty acids of different fractions:

To carry out GLC, the acids being non-volatile, were converted into volatile methyl esters which were subsequently subjected to GLC. The Details of the technique are described below.

Methyl esters of fatty acid, 1.3 diglyceride, 1.2 diglyceride, 1-monoglyceride, 2- monoglyceride and the whole oil were prepared with boron triflouride methanol reagent according to the method described by Morrison and Smith [12].

Each fraction collected above was placed in different Tef lined screw cap tube and boron-trifluoride-methanol reagent was added under nitrogen in appropriate quantity (one ml reagent for 4 mg of lipid). The tubes were closed with the screw caps and were then heated on a boiling water bath for the requisite time as shown below in table 2.3.

Table 2.3: Requisite time of tubes

Lipid class	Percentage of volume	Time at 100^{o}C
Triglyceride	25% BF_3-methanol 20% Benzene 55% methanol	30 min
Free fatty acid	100% BF_3-methanol	2 min
Diglyceride	100% BF_3-methanol	10 min
Monoglyceride	100% BF_3-methanol	10 min

After heating, the tubes were cooled and then opened. The esters were extracted with n-pentane. The extracts were washed with saturated solution of sodium chloride. After washing, the extracts were dried by adding anhydrous sodium sulphate and then filtered. Evaporation of the solvent from the filtrate resulted in 97-99% extraction of esters. The esters thus prepared were further purified by preparative TLC on silica gel-G with hexane, diethylether (9:1) as developing solvent.

Identification of fatty acids by GLC

A pye Unicam series 204 gas liquid chromatograph was used for the identification of fatty acids. First of all a suitable column was prepared which separated the fatty acids according to their boiling points. A 1.5m x 4nm glass column was filled with diethylene glycol succinate (stationary phase) on the support of diatomite C 100-120 mesh.

Procedure for filling of column

In a 250 ml round bottom flask, 1.5g of diethylene glycol succinate was dissolved in 40ml of dichloromethane, 13.5 g of diatomite C of 100-120 mesh was slowly added into the flask. The solvent was removed on a rotary evaporator very gently to avoid the breakage of particles. After the removal of solvent, the remaining material was heated on a water bath at 100^{o}C for half an hour under moderate vacuum.

A high vacuum pump was connected to the column outlet and it was filled progressively using small quantities of packing material with gentle tapping. When the column was full, its inlet was connected to a gas line along with the pressure meter. Gas supply was adjusted to give a pressure of about 0.7 kg/cm^2. The column was filled with more material and the above process was repeated till the column was packed to

2cm from top. Quartz wool was placed above the material to protect the column from any mechanical damage.

Conditioning of the column

The column packed as above was mounted in the oven of the chromatograph in such a way that its inlet was connected with the injection pot while its outlet was not connected with detector initially. A carrier gas with flow rate 20 ml/min was flown through the column at 200°C for 48 hours. In this manner the volatile material of the packed column was removed after conditioning and the outlet of the column was connected to a flame ionization detector and thus the column was ready for analysis.

2.5 Fatty Acid Composition of Different Lipid Classes by GLC

1-2 ml of lipid sample was injected into the column each time. The temperature of the injector, column and detector were maintained at 220°C, 200°C, and 250°C respectively. The carrier gas (nitrogen) whose flow rate through the column was adjusted to 20 ml/min.

The peaks were recorded by comparison of their retention times with those of the standard methyl esters analyzed under the same conditions (chromatogram attached). The area of each peak was calculated by the geometrical formula (1/2 x base x perpendicular). Percent peak area are quoted as acid composition.

3. RESULTS

Antibacterial activity of oils extracted from B. campestris (Sarson) using ethanol and acetone as solvents against S. aureus and E. coli are reported in Table 3.1 to Table 3.4.

Table 3.1: Antibacterial activity of oils from B. campestris extracted with ethanol as solvent against S. aureus.

Oil in PPM	Optical Density	%age growth	%age inhibition
0	0.42	100.0	0.0
1000	0.39	92. 8	7.0
2000	0.34	80.9	19.1
3000	0.32	76.2	23.8
4000	0.28	66.8	33.3
5000	0.25	59.5	40.5
6000	0.22	52.3	47.6

Table 3.2: Antibacterial activity of oils from B. campestris, extracted with ethanol as solvent against E. coli.

Oil in PPM	Optical Density	%age growth	%age inhibition
0	0.39	100.0	0
1000	0.37	94.0	5.1
2000	0.35	89.7	10.3
3000	0.32	82.0	18.0
4000	0.31	79.5	20.5
5000	0.27	69.2	30.9
6000	0.20	51.3	48.7

Table 3.3: Antibacterial activity of oils from B. campestris extracted with acetone as solvent against S. aureus.

Oil in PPM	Optical Density	%age growth	%age inhibition
0	0.42	100.0	0.0
1000	0.42	100.0	0.0
2000	0.42	100.0	0.0
3000	0.40	95.2	4.8
4000	0.40	95.2	4.8
5000	0.39	92.8	7.2
6000	0.38	90.5	9.5

Table 3.4: Antibacterial activity of oils from B. campestris extracted with acetone as solvent against E. coli.

Oil in PPM	Optical Density	%age growth	%age inhibition
0	0.39	100.0	0.0
1000	0.39	100.0	0.0
2000	0.38	97.4	2.6
3000	0.37	94.9	5.1
4000	0.37	94.9	5.1
5000	0.36	92.3	7.7
6000	0.35	89.7	10.3

The details for the results of determination of antibacterial activity of oil from B. campestris using ethanol and acetone as solvents are reported as models. As the same procedure was followed to determine the antibacterial activity of oils of R. communis and E. sativa, the details are not given. Here only antibacterial activities of the investigated medicinal seed oils have been reported and comparison made (Table 3.5). The readers may refer to Kausar and Khan [3] for consultation if needed.

Following the same procedure, the fatty acid percentage composition of oils from B. campestris, R. communis and E. sativa extracted with ethanol and acetone as solvents is reported in Table 3.6.

Table 3.5: Comparison of antibacterial activities of oils from B. campestris, R. communis and E. sativa extracted with ethanol and acetone as solvents against S. aureus and E. coli.

Oil n PPM	Percentage Inhibition %											
	Brassica compesteris				Eruca sativa				Rananculus muricatus			
	Ethanol		Acetone		Ethanol		Acetone		Ethanol		Acetone	
	S. aureus	E. coli	S. aureus	E. coli	S. aureus	E. coli	S. aureus	E. coli	S. aureus	E. coli	S. aureus	E. coli
0	0.0	0	0.0	0.0	00.0	00.0	0.0	0.0	0.0	0.0	0.0	0.0
1000	7.0	5.1	0.0	0.0	57.2	69.2	0.0	2.6	2.4	2.6	0.0	0.0
2000	19.1	10.3	0.0	2.6	76.2	89.2	2.4	2.6	2.4	5.1	0.0	0.0
3000	23.8	18.0	4.8	5.1	78.6	71.8	2.4	5.1	7.2	7.7	0.0	0.0
4000	33.3	20.5	4.8	5.1	85.7	79.5	2.4	7.7	14.3	10.3	0.0	2.6
5000	40.5	30.9	7.2	7.7	92.9	82.1	2.4	10.3	16.7	20.5	0.0	2.6
6000	47.6	48.7	9.5	10.3	97.6	89.8	7.2	12.8	28.6	33.4	2.4	2.6

Table 3.6: Comparison of fatty acid percentage composition of oils from B. campestris, R. communis and E. sativa extracted with ethanol and acetone as solvents

Fatty Acids	Fatty Acid Percentage %					
	Brassica compesteris		*Eruca sativa*		*Rananculus muricatus*	
	Ethanol	Acetone	Ethanol	Acetone	Ethanol	Acetone
Myrestic	-	-	-	-	-	00.49
Palmitic	05.26	03.86	08.82	11.15	04.93	10.90
Palmitoleic	-	-	-	-	00.12	-
Stearic	01.50	01.21	01.54	-	03.55	08.59
Oleic	11.09	09.35	17.64	26.19	15.71	33.38
Linoleic	12.77	07.67	03.64	14.39	20.20	36.71
Linolenic	10.43	06.62	24.26	18.79	02.64	03.07
Eicosenoic	07.94	08.01	30.05	28.90	05.44	02.42
Ricinoleic	-	-	-	-	43.74	03.71
Erucic	50.60	58.34	-	-	-	-
Unidentified	11.41	04.93	00.00	-	03.64	0.073

4. DISCUSSION

The central theme of the work reported here was the study of antibacterial activity of ethanol and acetone extracted oils of medicinal seeds i.e.: Brassica compesteris, Eruca sativa and Rananculus muricatus to check the results of the previous workers and their subsequent analysis by GLC.

The results reported here on antibacterial activity agree with the results of [1], [3], [4], [6], [10], [11] and others; thus this study joins hands in claiming that nature is very rich in antimicrobial agents and thus this source may be exploited for preparing herbal drugs for effective treatment of different infectious diseases. For example, our results show that highest activity of E. sativa ethanol extracted oil against E. coli was 97.8% and of acetone extract 89.6% (Table 5) strongly agree with [10] who reported that *E.* sativa seed oil was found to be the most active, exhibiting a maximum zone inhibition of 97% for Gram-positive bacteria and of 74–97% for Gram-negative bacteria, Our results (Table 5)also agree strongly with the conclusion of [4] that on the whole the alcoholic extracts showed greater activity than hexane extracts. Here other solvent was acetone.

The study by [9] on antimicrobial activity of mustard essential oil against Escherichia coli O157:H_7 and Salmonella typhi adds further value to the statement on richness of nature in antimicrobial agents by highlighting the mode action of Brassica campestris on cytoplasmic membrane involvement in the antimicrobial action of mustard essential oil resulting in a loss of cell homeostasis and recommending that the mustard essential oil can be used as an effective antimicrobial agent.

The oils found active here are commonly used in Indo-Pak subsequent as local agents for protecting wounds from infections and cure some infectious diseases. The oil of Eruca sativa for example is locally applied to cure scalp and itching. Our results confirm that their common traditional use as curative agents are quite rational. The antibacterial activity may be due to the active factors found in the oil extracted. Thus a rational approach was to identify the active principles for which GLC was carried out.

The comparison of composition of the ethanol and acetone extracts of Eruca sativa (Taramira) and Rananculus muricatus (Castor) reveals that the solvent influences the percentage extraction of different acids. In ethanol extract for example the amount of eieosenoic acid and ricinoleic acid is greater than that of these acids in the acetone extract while the percentage yields of palmitic, palmitoleic, stearic, oleic, linoleic, and linolenic acids are greater in acetone extracts than in the ethanol extracts (Table 6). On the other hand, in case of B. compastris (Sarson) greater percentage of palmitic, stearic, oleic, linoleic and linolenic acids is extracted by ethanol as a solvent, while eicosenoic and erucic acids are greater in acetone extract.

In R. muricatus and E. sativa oils fatty acids with more than 18-C atoms seem to be more soluble in ethanol but in B. compastris reverse is the case. Results were compared with the work of Wood, et al [13]. The fatty acids distribution of castor oil reported by Wood, et al: Ricinoleic acid (87 - 95%), linoleic (4.5 - 5.0%), oleic (traces), saturated acids (1%). An optically active isomer of 9, 10 dihydoxy steric acid was also present in small amounts. While our yield was palmitic (4.92 & 10.9), stearic (3.55 & 8.6%), oleic (15.7 & 33.4%), linoleic 20.2 & 36.7%), linolenic (2.64 & 3.06%), eieosenoic (5.4 & 2.4%) and ricinoleic (43.74 & 3.7%) in ethanol and acetone extracts respectively.

The results were also compared with the work of Ahmad et al [14]. These researchers reported the %age variation of different acids present in E. sativa oil as palmitic (3 - 7%), stearic (1 - 3%), oleic (11 - 27%), linoleic (9 - 19%), linolenic (16 - 22%), while our yields were palmitic (8.8 & 11.15%), stearic (1.5 & 0.0%), oleic (17.65 & 26.19%), linoleic (3.6 & 14.39%), linolenic (24.25 & I8.78%) and eicosenoic (30.0 & 28.9%) in ethanol and acetone extract respectively. Same workers reported palmitic (up to 15%), stearic (up to 8%), oleic (0.2 - 60%), linoleic (6 - 27%), linolenic (9 - 22%), eicosenoic (3 - 58%) and unidentified (0.0 - 6.0%) in B. c oil, while our yield was palmitic (5.26 & 3.86%), stearic (l.5 &1.2%), oleic (11 & 9.36%), linoleic (12.77 & 7.67%), linolenic (10.43 & 6.6%), eicosenoic (7.9 & 8%) erucic (50.6 & 58.34%) and unidentified (11.4 & 4.9%) in ethanol and

acetone extracts respectively. Our results table No. 6. fall in the ranges reported by them. The difference in the yield reported by the above workers and here may be due to different solvents used as the extractants.

The antibacterial activity of the individual identified acids could not be determined because these as well as their glycerides were not readily available in the market and student worker had time constraint due to in-time submission of her research performance for the award of the degree. So the antibacterial activity of the oils could not be associated to any specific fatty acid. The future research work may be carried in this direction.

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