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PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF *ERUCA SATIVA* SEED

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Abstract

Antibacterial activity of various solvent extracts of *Eruca sativa* seed as well as seed oil was investigated against Gram+ve and Gram-ve bacterial strains. Maximum zone of inhibition was observed from seed oil followed by methanolic seed extracts from all bacterial strains compared with broad spectrum antibiotics gentamicine. MIC values of seed oil were within the ranges of 52-72 µg/ml as compared to 56-70 µg/ml standard antibiotic (Gentamicine). Proximate and Phytochemical analysis of seed of *E. sativa* showed presence of all essential phyto constituents required for promising traditional medicine. Analysis of seed oil by gas chromatography revealed that there was high concentration of Erucic acid (51.2%) followed by oleic acid (15.1%) and cis-11-eicosenoic acid (12.5%). In addition, minor quantities of other essential and non essential fatty acids were also present. Therefore the present study supports effectiveness of *E. sativa* seeds for its use in traditional medicine used in various human disorders.

Introduction

History of herbal remedies is very old; there are many medicinal herbs and spices, which find place in day-to-day uses. Many cooked foods including spices are providing remedies for cold, cough and stomach disorders of human system because of their medicinal properties. Herbal remedies can be taken in many forms ranging from infusions of herbs or spices to decoction of leaves and flowers from them. There is worldwide rrealization that any plant known for a particular bioefficacy should be explored. Moreover, authentic effects of any plant extracts on a particular human disorder prompt us to screen indigenous plants those also having potential for antioxidant and antimicrobial activity (Agrawal & Srivastava, 2008). There is considerable potential of raw plant material for a higher exposure to bioactive phytochemicals such as glucosinolates and their hydrolysis products like flavonoids, and vitamin C (Simoes *et al*., 2009).

Plants and herbal extracts have formed important position in modern medicine, due to their chemical and medicinal contents found in natural from. Their secondary metabolites represent a large reservoir of structural moieties which work together exhibiting a wide range of biological activities. Microorganisms have the genetic ability to transmit and acquire resistance to antibiotics and have become a major global health problem. This compelled the scientists to search out new drugs from plant origin (Khoobchandani *et al.*, 2010). Plant derived antimicrobial compounds might inhibit bacteria through different mechanisms and provide clinical values for the treatment of infections caused by resistant microbes (Stein *et al.*, 2005). Neman *et al*., (2003) reported that majority of antibacterial drugs in clinical uses are from natural origin. There is a need to evaluate the herbs

scientifically for their antimicrobial activity against the antibiotic-resistant microorganism in order to develop new drug from plant origin (Simoes *et al.,* 2009).

The vast majorities of antibiotics used today are produced by microorganisms, yeasts or fungi, which belong to the vegetable kingdom. Higher plants mainly produce antimicrobial compounds for their defense mechanism against infections constituting cellular metabolism. *Eruca sativa* locally known as *Taramira* belongs to the family Brassicaceae is grown in different parts of Indo-Pak subcontinent and Middle East. It is minor oil crop and used in traditional medicines as remedies for different diseases. There is sporadic information available about phytochemistry and bioactivity of this oily crop (Flanders & Abdulkarim, 1985). It is known as diuretic, anti-inflammatory and affects on blood circulation by various authors. Fresh plant material could provide more health benefits because cooking process destroy most of heat sensitive phytochemicals. *Eruca* seeds have high oil contents, protein glucosinolate and Erucic acid contents and commonly used as animal feed in Asia, particularly in India and Pakistan (Kim & Washii, 2006). The present study was conducted to assess the antibacterial activity as well as phyotochemical analysis of *Eruca* seeds and oil.

Materials and Methods

Plant samples collection and preparation: Seeds of *E. sativa* were purchased from the local herbal store and specimen was identified in Taxonomy Lab. in the Department of Botany, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan. The identified voucher specimen (No. 20) was deposited in the Department of Botany for record. Seeds were surface sterilized using 1% mercuric chloride ($HgCl_2$) and ground into powder form by using electrical grinder and preserved in plastic bags at 4ºC for further analysis. Organic extracts of seeds were prepared using three different solvents with increasing polarity (Kaur & Arora, 2009). Dried seed powder was weighed accurately and subjected to extraction in a soxhlet apparatus at 60˚C using different solvents.

Before extraction with the next solvent, the powder was air dried to remove the adhering solvent. The extract obtained was filtered, concentrated in rotary evaporator and dried in oven at 600ºC (Roopashree *et al*., 2008). Percentage yield of each extract was calculated and dried extract was stored in air tight containers for further study.

Proximate analysis: Proximate analysis of seed samples consists of moisture, total oil, crude protein, crude fiber and ash content (Duke & Atchley, 1984). Nitrogen content was estimated by the Kjeldhal method (Anon., 1984) and amount of crude protein was estimated by using factor (Nx6.25). Ash contents and crude fiber contents were determined by using methods of AOAC (Anon., 1990). Carbohydrate content was estimated by difference (Khalifa, 1996). Total oil was extracted by using solvent extraction in which 150 g of powdered sample was placed into cellulose paper cone and extracted by using light petroleum ether (b.p 40ºC -60ºC) in a 5 liter Soxhlet extractor for 8 h (Anon., 1984). Total oil was then recovered by evaporating solvent using rotary evaporator and residual solvent was removed by drying in an oven at 60ºC and preserved at 4ºC for further uses.

Fatty acid analysis with gas chromatographic: Fatty acid methyl ester (FAME) was prepared by treating 10 mg of lipid with two mL hexane followed by the addition of 0.2

mL of 2 M methanolic KOH. The tube was vortexed for two minutes at room temperature and after a light centrifugation aliquot of the hexane layer that was collected for GC analysis. All gas chromatography analysis was performed on a Perkin Elmer, Clarus 500 series under the following condition: Column, HP Innowax capillary: 60.0m x 0.25mm x 0.25 um, oven temperature programme. The column held initially at 60˚C for 5 minutes after injection, then increased to 140˚C with 10˚C/min. heating ramp for 20 minutes and increased to 200˚C with 5˚C/min heating ramp for 20 minutes. Then temperature was increased to 220˚C with 5˚C/min heating ramp for 20 minites. Injector temperature 250˚C, detector (FID) temperature 275˚C, carrier gas $H_{2:}$ inlet pressure 45 psi linear, gas velocity 39 cm/sec, column flow rate 2.4 mL/min; split ratio, 40:1 and injector volume 1µL. The chromatogram was collected for identification of different fatty acid compounds present in *E. sativa* seed oil and was compared with Nist library.

Analysis of plant samples for phytochemicals: Samples of *Eruca* seeds were analyzed for phytochemicals to get information for their active ingredients. Total alkaloids, flavonoids and cardiac glycosides were determined by using method reported by Kaur & Arora (2009). Saponin and tannin contents were determined by routine analytical procedures. Whereas phenolic acids were analyzed by using Folin-Ciocalteu reagent method and results were expressed as milligrams gallic acid equivalent per gram of dry weight (mg GAE/g dry weight) as described by Chahardehi *et al*. (2009)**.** Ascorbic acid in samples was determined by using method reported by Okwu & Josiah (2006).

Determination of antibacterial activity: Total six clinical isolates were obtained from outdoor patients visiting National Institute of Health, Islamabad and other local hospitals. Gram+ve bacteria were *Staphylococcus aureus, Staphylococcus epidermidis* and Gram– ve were *Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumonia* and *Escherichia coli* selected for this study. Bacterial strains were identified by using methods of Heggers *et al*., (1996). Gentamicine, a broad spectrum antibiotic was obtained from pharmaceutical company, Islamabad and used as standard in the study. The above mentioned culture were maintained in agar nutrient media and stored in a refrigerator at low temperature and recultured after 15 days before experiment.

Preparation of inoculums: Suspensions of organisms were prepared as per McFarland´s standard. A 24 hours old culture was used for the preparation of bacterial suspension using sterile isotonic solution of Sodium chloride (90.9% w/v) containing approximately $1.5x10^8$ cells/ml. The turbidity was adjusted at the optical density of the bacterial suspension to that of 0.05 ml of 1.17% of barium chloride and 9.95ml of 1% sulphuric acid (Roopashree *et al*., 2008).

Agar well diffusion method: The media was prepared by dissolving all the ingredients in distilled water and subjected to sterilization in an autoclave at 121ºC for 15 minutes. The Petri plates were washed thoroughly and sterilized in oven. A total of 30 ml sterile molten agar medium was seeded by organisms and allowed to solidify at room temperature. After 10 minutes, wells were prepared with a sterile cork borer at a distance of 15 mm from each other. The open wells were filled with 0.05 ml extract and incubated at 37ºC for 24 hours. The diameter of the zone of inhibition was measured in millimeter and compared with a broad spectrum antibiotic (Gentamicine) as positive control (Roopashree *et al*., 2008).

Minimum inhibitory concentration (MIC): Minimum inhibitory concentration (MIC) of the *Eruca* oil was determined by using tube dilution method in Mueller –Hinton broth medium. Duplicate tubes of each dilution (10-100 µg/mL) were inoculated with 5 x 10^5 of the test tube of bacterial strain cultured in incubator at 37ºC for 24 h. The antimicrobial agent (Gentamicine) was inoculated in the assays as positive control at equal concentration. The absorbance of broth suspension was measured at 420 nm against the blank with spectrophotometer (U 2001-Hitachi). The minimum inhibitory concentration for bacteria was determined as lowest concentration of seed oil inhibiting the test cultures showing no detectable growth (Ettebong & Nwafor, 2009).

Statistical analysis: Statistical analysis was carried out using the student´s *t*-test, for the estimation of results as mean ± SD (standard deviation) and percentage values of different Phytochemicals.

Results and Discussion

Composition analysis of *Eruca* seeds: The seed extract was analyzed for screening chemical composition and revealed that it contained crude protein with the percentage of 29.83±0.8, followed by oil (27.67±1.8%), moisture (6.02±0.5), carbohydrates (3.09±0.4%), ash (2.60±0.5%) and fiber (1.60±0.7%) (Table 1). The presence of these nutrients indicates that seeds can be used as food or feed purposes.

Phytochemical analysis of *Eruca* seeds indicates that alkaloids, cardiac glycosides, flavonoids, phenolics, ascorbic acid, saponins and tannins are present in the seed samples (Table 2). All phyto constituents found in seed samples responsible for different bioactivities including antimicrobial activity against various pathogenic microorganism (Ettebong & Nwafor, 2009).

Alkaloids and their synthetic derivatives are being used as basic therapeutic agents for their analgesic, antispasmodic and bactericidal effects. Natural ascorbic acid is vital for the body performance (Okwu & Josiah, 2006; Aiyelaagbe & Osamudiamen, 2009). The presence of phenolic compound in the seed indicates its antimicrobial properties against pathogenic bacteria (Khoobchandani *et al.,* 2010). Tannins are reported to exhibit antiviral, antibacterial and antitumor activity and also used as diuretic (Aiyelaagbe & Osamudiamen, 2009). Cardiac glycosides are helpful to overcome various human diseases. Saponin has the property of precipitating and coagulating red blood cells (Okwu & Josiah, 2006).

Analysis of *Eruca* oil by GC (Fig. 1) and estimation of relevant fatty acids by comparing peaks, retention time and amount of oil (Table 3) indicates that both essential and non essential fatty acids are present in the oil. However, Erucic acid (51.2%) was the main fraction found in the seed oil. Many workers believed that antimicrobial activity of *Eruca* oil is mainly due to higher concentration of Erucic acid, which was present in both free and triglyceride form (Khoobchandani *et al*., 2010). Mono and polyunsaturated fatty acids like Oleic acids (15%) *cis*-11- eicosenoic acid methyl ester (12.5%) and linoleic acid methyl ester (6.9%) were abundant. Presence of these fatty acids support edible uses of *Eruca* seeds indicating that oil contained valuable fatty acids required for edible purposes (Flanders & Abdulkarim, 1985).

Table 1. Composition (%) analysis of *Eruca* seeds.

Sample	Dry matter (%)	Moisture (%)	Crude protein (%)	Crude oil (%)	Crude fiber (%)	Ash (%)	Carbohydrate (%)
1	94.0±2.2	6.0±0.3	29.58±0.7	27.0±1.8	1.6±0.4	2.5±0.1	3.05±0.45
2	92.1±1.5	6.5±0.6	30.1±0.6	28.1±2.1	1.5±0.6	2.7±0.8	3.12±0.5
3	93.4±2.6	6.1±0.7	29.8±1.2	27.9±1.5	1.7±1.2	2.6±0.7	3.09±0.5
Average	93.17±2.1	6.02±0.5	29.83±0.8	27.67±1.80	1.60±0.7	2.60±0.5	3.09±0.4

Table 2. Estimation of (%) of photochemical from seeds of *Eruca sativa*.

Sample	Alkaloids	Flavonoids	Phenols	Saponins	Tannins	Cardiac glycosides	Ascorbic acid
1	11.46± 0.12	25.63±2.1	26.66±0.4	6.17±1.2	3.12 ±0.7	2.08±0.17	21.42±0.56
2	10.23 ± 1.5	23.41 ±1.5	28.14 ±1.6	5.28 ± 1.6	4.15 ±0.6	2.19 ±1.5	22.11 ±0.5
3	12.14 ±2.1	24.25 ± 2.6	26.13 ± 2.4	7.14 ±2.4	5.18 ±1.4	3.11 ±1.6	23.14± 3.5
Average	11.27±1.24	24.43±2.06	26.97±1.47	6.20±1.73	4.15±0.90	2.76±1.09	22.22±1.52

Values are in terms of Mean ±SD after triplicate analysis (n = 3)

Table 3. Free fatty acid content identified by using GC from seed oil of *Eruca sativa*.

No of Peak	Retention time	Compound name	Corresponding acid	Fatty acids µmol/ml [a]	% w/w
1	34.2	Caporic acid methyl ester	C10: 0	0.282 ± 0.1	0.008
2	35.2	Lauric acid methyl ester	C12:0	0.567 ± 0.4	0.017
3	37.5	Palmitoleic acid methyl ester	C16:1	3.358 ± 0.2	0.136
4	38.3	Palmitic acid methyl ester	16:0	64.128 ± 1.4	2.378
5	39.1	Linoleic acid methyl ester	C18:2	173.16 ± 2.8	6.938
6	41.0	Oleic acid methyl ester	C18:1	389.21 ± 3.5	15.1
7	41.4	Linolenic acid methyl ester	C18:3	0.112 ± 2.6	0.005
8	46.2	*cis*-11-Eicosenoic acid methyl ester	C20:1	274.18 ± 3.7	12.514
9	52	Erucic acid methyl ester	C22: 1	1031.5 ± 3.8	51.212
10	53.2	Behenic acid methyl ester	C22:0	23.4 ± 2.4	1.261
11	57.2	Nervonic acid methyl ester	C24:1	18.2 ± 1.6	0.218
12	63.1	Lignoceric acid methyl ester	C24:0	4.12 ± 0.3	0.148

Fatty acids analysis for fraction of *Eruca* seed oil*:* a. Calculation of whole oil; b. Amount (%) calculated on the total fatty acid fraction

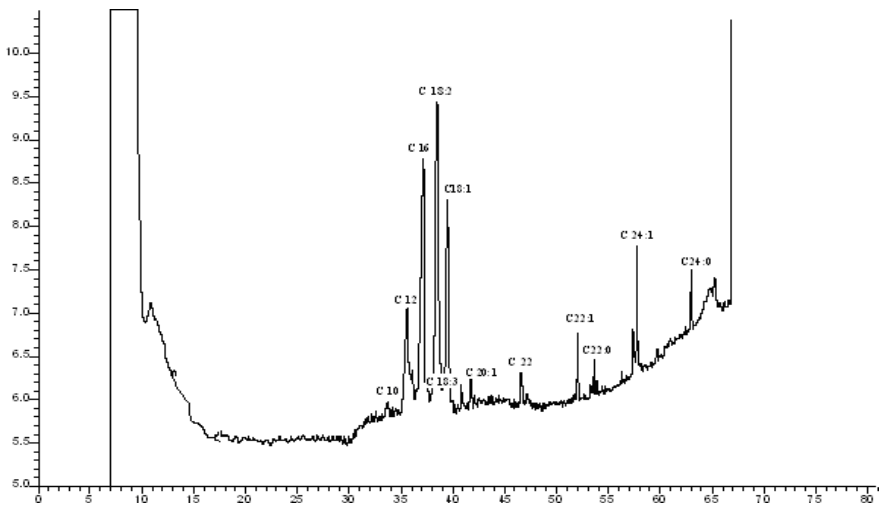

Fig. 1. GC Chromatogramm obtained by the injection of 5 µL of fatty acid (methyl esters) from *Eruca* seeds.

Antimicrobial activity of *Eruca* seed extracts in three different organic solvents as well as seed oil has been tested against Gram+ve and Gram–ve bacterial strains. The results indicated that the different extracts of *Eruca* seeds exhibit antibacterial activity. Seed oil and methanolic extracts have shown higher activity than the other extracts and standard antibiotic gentamicine (Table 4). Bacterial strains were tested in 25, 50 and 100 µg/ml n-hexane, ethanolic and methanolic extracts for 24 h by well diffusion assays. It was observed that all extracts showed growth inhibition of various clinical isolates. Zone inhibition of all tested samples for Gram+ve and Gram–ve bacterial strains were compared to gentamicine which is broad spectrum antibiotic. *Klebsiella pneumoniae Staphyllococcus epidermidis* were found to be less susceptible as compared to other clinical isolates (Table 4).

Minimum inhibitory concentration: The minimum inhibitory concentration (MIC) for Gram-ve and Gram+ve bacteria was determined (Table 5). The seed oil showed antimicrobial activity in the concentration ranges of 50-72 µg/ml for Gram+ve and Gram-ve bacteria. Whereas, MIC value of reference compound (Gentamicine) was found in the ranges of 56- 70 µg/ml for both Gram+ve and Gram-ve bacteria (Table 5).

Discussion

The chemical composition of *Eruca* seeds and Phyto chemical analysis revealed that seeds contained various nutrients which are required for food or feed purposes (Khalifa, 1996). The average oil content of *Eruca* was 27.67%. However, contents of oil depend on many factors including maturity of the seed and the degree of plant irrigation (Flanders & Abdulkarim, 1985). The presence of crude protein (29.58%) shows that it is a good source of feed supplement. Whereas, *Eruca* seed extract contains important secondary metabolite such as flavonoids, alkaloids, tannins, phenols, saponins, ascorbic acid and those are used as remedies of many diseases and frequently required in traditional medicines. Essential oil especially Erucic acids was present in high concentration those are responsible for antibacterial activity, that could be used for the preparation of drugs required for human and animal health (Alam *et al*., 2007).

In the present investigation, the antimicrobial activity of various test samples in comparison with Gentamicine was determined and found to proceed in a dose-dependent manner for different bacterial strains. Those are becoming a clinical problem in hospital patients. Gram-positive bacteria, *S. aureus* is known to cause serious diseases such as pneumonia, meningitis etc., in hospital patients (Curran & Al-Salihi, 1980). *E. coli* and *P. aeruginosa* cause the urinary tract infections (UTI), pulmonary tract infections, burns, wounds, dysentery-like diarrhoea and other blood infections and similar also true for *K. pneumonia*, *S. typhii* and *S. epidermidis* (Ryan & Ray, 2004). Seed oil showed maximum inhibition of growth against all the antibiotic-resistant bacteria. MIC values for the *Eruca* seed oil showed that this oil had almost equal activity with respect of the broad-spectrum antibiotic Gentamicine (Table 5).

Table 4. Antibacterial activity of different Extracts of *E.sativa* seeds.

Eruca seed extracts	Extract concentration (µg/ml)	Bacterial strains Diameter of zone of inhibition in mm					
		S. aureus	*P. aeruginosa*	*E. coli*	*S. typhi*	*K. pneumonia*	*S.epidermidis*
n- hexane extract	25	1.0 ± 1.2	-	10.0 ± 0.1	-	6.1 ± 1.5	-
	50	17.0 ± 0.22	-	12.0 ± 0.45	-	8.4 ± 0.4	-
	100	18.0 ± 0.44	-	19.5 ± 1.3	15.9 ± 2	8.8 ± 1.2	-
Methanolic extract	25	8.0 ± 0.23	-	8.1 ± 0.4	0.6 ± 1.2	8.1 ± 1.8	-
	50	21.0 ± 0.11	-	1.6 ± 1.5	0.7 ± 0.8	9.2 ± 4.6	-
	100	24.0± 0.98	18.0 ± 0.54	18.0 ± 1.53	11.0 ± 1.56	9.8 ± 1.5	2.5 ± 1.21
Ethanolic extract	25	-	12.0 ± 0.11	10.0 ± 0.66	-	6.5 ± 1.2	-
	50	-	15.0 ± 0.22	15.0 ± 0.71	-	8.4 ± 0.55	0.7 ± 0.5
	100	11.0 ± 1.16	17.7 ± 0.47	17.0 ± 0.71	12.0 ± 4.48	9.1 ± 0.6	-
E. Sativa seed oil	25	13.0 ± 0.99	-	17.1 ± 2.1	8.1 ± 1.5	4.5 ± 1.2	4.8 ± 0.2
	50	23.0 ± 0.34	-	22.0 ± 0.6	14.2 ± 1.4	8.1 ± 1.6	6.7 ± 1.2
	100	28.4 ± 0.4	20.3 ± 1.1	24.0 ± 0.34	21.6 ± 2.1	11.1 ± 1.1	9.1 ± 1.1
Gentamicine	25	22.0 ± 0.06	21.0 ± 0.1	20.5 ± 0.78	18.5 ± 0.1	9.9 ± 0.01	13.2 ± 0.64
	50	24.0 ± 0.8	25.0 ± 0.55	32.0 ± 0.66	21.0 ± 0.2	11.0 ± 0.08	14.1 ± 0.12
	100	24.0 ± 1.2	31.0 ± 0.11	33.0 ± 0.1	22.0 ± 0.4	14.0 ± 0.1	16.2 ± 0.67

DMSO, dimethyl sulfoxide- no inhibition. Values are in terms of Mean ±SD after triplicate analysis.

Table 5. Minimum inhibitory concentration (MIC) of eight extracts of *Eruca* seeds.

Bacterial strains	*E. Sativa* seed oil	Gentamicine
Staphyllococcus aureus	50	60
Pseudomonas aeruginosa	58	56
Escherichia coli	65	60
Salmonella typhi	70	64
Klebsiella pneumoniae	68	70
Staphyllococcus epidermidis	72	69
Pasturella	68	70

Comparison of *Eruca* seed oil and Gentamicine for minimum inhibitory concentration

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