Pak. J. Bot., 42(4): 2949-2953, 2010.

ANTIMICROBIAL POTENTIAL OF SEED EXTRACT OF ERUCA SATIVA

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Abstract

The antimicrobial activity of crude water extract, Aqueous extract as well as Methanolic extract of *Eruca sativa* were investigated *In vitro* by using Agar well diffusion method. All extracts were tested against 2 Gram negative bacteria and four fungal species. Plant extracts exhibited displayed highest antibacterial activity while fungal species viz. *Penicillium lilacinum, Paecilomyces variotii, Spadicoides stoveri, Penicillium funiculosum* showed variable degrees of inhibition even at lower concentration.

Introduction

Scientific experiments on the antimicrobial properties of the plants compounds were first documented in the late 19th century (Zaika, 1975). Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoides, alkaloids and flavonoid, which have been found to have antimicrobial properties *In vitro* (Cowan, 1999). Extracts of many plants are now known to exhibit antimicrobial activity. Different plant extract have been evaluated for their antimicrobial properties by Mahmoud (1999); Digrak *et al*., (1999); Bowers & Locke (2000); Eksteen *et al*., (2001); Hol & Van-veen (2002); Magama *et al*., (2003); Gulluce *et al*., (2003) and Afolayan (2003). Pretorius *et al*., (2002) tested crude extracts from 39 plant species for their antifungal potential against 7 economically important plant pathogenic fungi. Muhsin *et al*., (2001) observed remarkable reduction in growth of 18 fungal species due to crude garlic bulb extract.

Eruca sativa Miller (Brassicaceae, synonym *Eruca vesicaria* Rocket), commonly known as “Tarmira”, “Rocket salad” or “Garden salad” is a diploid annual herbaceous plant growing up to 80cm. It is in leaf all year, in flower from May to August and the seeds ripen from July to September. (Hedrick, 1972; Larkcom, 1980).

Young leaves - raw or cooked have distinct strongly spicy flavour (Vilmorin *et al*., 1960; Komarov, 1968; Hedrick, 1972; Harrison *et al*., 1975). A few leaves added to a salad are acceptable though the flavour is too strong for many tastes (Facciola & *Cornucopia*, 1990).

Flowers - raw has a similar taste to the leaves, they make a nice garnish on the salad bowl (Larkcom, 1980).

The seed yields semi-drying oil (Chittendon, 1956; Hedrick, 1972; Komarov, 1968) which is edible if stored 6 months (Usher, 1974) It contains 32% fat, 27% protein. It is known as 'jamba oil’. (Facciola, 1990).

Literature survey reveals that Rocket was at one time used medicinally, though it is now used only as salad herb (Bown & Kindersley, 1995). The leaves are antiscorbutic, diuretic, stimulant and stomachic (Uphof, 1959; Grieve, 1984). The seed is rubefacient and stimulant. The powdered seed possesses antibacterial activity, but no alkaloids have been isolated (Chopra *et al*., 1986).

The oil from the seed is said to have aphrodisiac properties (Phillips & Rix, 1995). The oil of the seed contains erucic acid. A number of plant species have been reported to possess natural substances, which inhibit the growth of many fungi. In the present study the different extract of plant were used to inhibit the growth of microorganism's. The crude juice *Eruca sativa* were active only on *Escherichia coli, Salmonella typhi* and *Bacillus subtilis* (Abdou *et al*., 1972). This is the first attempt to study the antifungal activity of *Eruca sativa* seed powder.

Material and Method

Preparation of seed powder: The seed of *Eruca sativa* were collected from the local market of Hyderabad and Sanghar, Pakistan. These seeds were washed in sterile water. Extract of seeds were obtained according to following methodology.

Solvent extraction: The extraction was done according to Parekh *et al*., (2005) with slight modification. Ten grams of seeds were dried material was crushed in an electrical grinder (ANEX AG-694). Crushed seeds were extracted with 100 ml of methanol kept on rotatory shaker for 24 h thereafter, it was filtered through eight layered Muslin cloth then through Whatman No. 1 filter paper and centrifuged at 5000 X g for 15 min. the supernatant was collected and the solvent was evaporated at 40°C by rotary evaporator (Eyela N- 1000) to make the final volume one fifth of the original volume. It was stored at 4^oC in air tight bottles.

Aqueous extraction: Ten grams of dried seed and crushed plant material was extracted in 100 ml of distilled water for 6h at slow heat. Every two hour it was filtered through 8 layered muslin cloth and centrifuged at 5000X g for 15 min., than the supernatant was collected. The procedure was repeated twice and after 6 hour the supernatant was concentrated to make the final volume. The extract was then stored at 4^oC in air tight bottles.

Crude water extract: The plant extract were prepared using the modified method of Alade & Irobi (1993). Briefly 100g of dried powdered seeds were soaked in 500 ml of distilled water for 72 h. then mixture was kept on rotary shaker for 24 h. It was filtered through 8 layered muslin clothe. Then it was refluxed followed by agitation at 200 rpm for 1h. The filtrate obtained was concentrated under vacuum at 40°C (Eyela N-1000) to obtain the dry extract. The extract was then stored at 4^oC in pre sterilized air tight flasks. To avoid contamination and prospective chemical alteration, the extract was ensured to be used within 3-4 days.

Determination of antimicrobial activity

Microorganisms: Cultures of fungi were collected from the Mycology Laboratory University of Sindh Jamshoro viz. *Paecilomyces variotii*, *Spadicoides stoveri, Penicillium lilacinum, Penicillium funiculosum*. Cultures of Bacteria viz. *Enterobacter agglomerans* and *Hafnia alvei* were collected from the Mycology & Plant Pathology Department, University of the Punjab, Lahore.

Well diffusion technique: Screening of antimicrobial activity was performed by well diffusion technique (Kivanc & kunduhoglu, 1997). The Mueller Hinton Agar (MHA) plates were seeded with 0.1 ml of the standarized inoculum of each test organism. The inoculum was spread evenly over plates with glass spreader. The seeded plates were allowed to dry in the incubator at 25-35°C for 20 minutes. A standard cork borer of 8 mm was used to cut uniform wells on the surface of MHA and 100 µl of each extract was introduced in the wells. The inoculated plates were incubated at 35 ± 2°C for 24 hours and zone of inhibition was measured to the nearest millimeter.

Antifungal activity

Diffusion plate method: Antifungal activity was tested against *Paecilomyces variotii, Spadicoides stoveri, Penicillium lilacinum, Penicillium funiculosum.* The diffusion plate method was used to test *Eruca sativa* with slight modification as reported by Terras *et al*., (1995). In this technique 0.1 ml of the fungal spore suspension (grown for 3 days in 10ml of Potato dextrose agar) was thoroughly mixed with 20 ml of melted PDA and poured into sterilized Petri plates. When the agar was set 3 holes of 8 m.m diameter were made on each of the seeded plate. These holes were filled with 100 µl of the testing sample. Experiments were performed in triplicate. The Petri plates were incubated at 35°C for 7 days. All culture plates were examined after 24-96 hours. The zone of inhibition produced by the plant extract was compared with control.

Minimum inhibitory concentration (MIC) evaluation: The MIC was evaluated on plant extracts that showed antimicrobial activity. This test was performed at four concentrations of each extract. (6.3, 12.5, 25, 50 v/v) employing the same modified agar well diffusion method. Calculations of MIC were determined by Standard deviation and mean of replicates.

Results and Discussion

Different plants and their parts (flowers, buds, leaves, stem, seed, skin, pulp) have been used for thousands of years to enhance the flavor and aroma of food. In addition, plants are rich in wide variety of secondary metabolites. The seed is rubefacient and stimulant. The powdered seed possesses antibacterial and antifungal activity, but no alkaloids have been isolated (Chopra *et al*., 1986). In this connection, the present study was conducted to evaluate the antimicrobial activity of *Eruca sativa* seeds.

The seed extract having variable degree of inhibition. The crude water extract of seed showed moderate antifungal activity while it showed highest antibacterial activity (39.5 mm ± 1.734 SD) against *Hafnia alvei* and *Enterobacter agglumerans* exhibited (33.5 mm ± 1.36 SD). It has been observed that crude water extract posses highest antibacterial activity. Crude water extract showed significant inhibition against some fungal strain like *Spadicoides stoveri* (17.2mm ± 1.429 SD) and *Paecilomyces variotii* (19.2 mm ± 0.960 SD) while some fungal strain having insignificant inhibition.

Methanolic extract of seed exhibited antibacterial activity against *Enterobacter agglumerans* and *Hafnia alvei*. Methanolic extract also found to be affected against *Penicillium funiculosum* (17.8mm ± 0.888 SD) and *Paecilomyces variotii* (37.9mm± 0.458 SD).

As the work for the development of herbal medicines is in progress worldwide, the present report will help in isolation of new products. Besides, the same may also be used for the treatment of plant pathogenic fungi as conventional method.

Table 1. Antimicrobial activity of crude water extract of *Eruca sativa*.

S. No.	Name of organism	Mean Zone of inhibition in mm and standard deviation(SD			
		6.2 %	12.5%	25%	50%
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
1.	*Spadicoides stoveri*	5.1 ± 2.206	8.9 ± 0.503	12.4 ± 0.862	17.2 ± 1.429
2.	*Paecilomyces variotii*	7.5 ± 0.650	11.9 ± 1.115	15.9 ± 0.907	19.2 ± 0.960
3.	*Penicillium funiculosum*	5.8 ± 0.305	12.5 ± 0.602	14.2 ± 1.101514	17.8 ± 0.888
4.	*Penicillium lilacinum*	0.13 ± 0.20	9.3 ± 0.802	17.2 ± 0.854	20.9 ± 1.401
5.	*Enterobacter agglomerans*	12.8 ± 0.793	17.5 ± 0.702	22.3 ± 1.059	33.5 ± 1.365
6.	*Hafnia alvei*	15.6 ± 0.832	20.8 ± 0.650	26.2 ± 0.702	39.5 ± 1.734

Table 2. Antimicrobial activity of methanolic extract of *Eruca sativa.*

S. No.	Name of organism	Mean Zone of inhibition in mm and standard deviation(SD			
		6.2 %	12.5%	25%	50%
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
1.	*Spadicoides stoveri*	9.9 ± 0.503	22.10 ± 0.20	30.1 ± 0.503	35.0 ± 0.850
2.	*Paecilomyces variotii*	12.5 ± 0.624	24.8 ± 0.360	32.0 ± 0.3214	37.9 ± 0.458
3.	*Penicillium funiculosum*	14.0 ± 0.208	28.9 ± 0.264	34.9 ± 0.152	39.5 ± 20.675
4.	*Penicillium lilacinum*	12.0 ± 0.152	19.9 ± 0.305	26.9 ± 0.264	29.5 ± 0.680
5.	*Enterobacter agglomerans*	15.0 ± 0.20	18.5 ± 0.264	27.8 ± 0.251	32.2 ± 0.650
6.	*Hafnia alvei*	18.10 ± 0.20	22.0 ± 0.50	28.8 ± 0.513	34.9 ± 0.490

Table 3. Antimicrobial activity of Supernatant of Aqueous extract of *Eruca sativa*.

S. No.	Name of organism	Mean Zone of inhibition in mm and standard deviation(SD			
		6.2 %	12.5%	25%	50%
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
1.	*Spadicoides stoveri*	13.0 ± 0.321	19.2 ± 0.556	20.9 ± 0.754	25.3 ± 0.360
2.	*Paecilomyces variotii*	14.0 ± 0.152	18.5 ± 0.450	23.1 ± 0.305	28.5 ± 0.556
3.	*Penicillium funiculosum*	10.0 ± 0.152	11.9 ± 0.152	13.7 ± 0.20	17.2 ± 0.251
4.	*Penicillium lilacinum*	10.9 ± 0.264	13.0 ± 0.50	16.9 ± 0.10	21.2 ± 0.351
5.	*Enterobacter agglomerans*	16.0 ± 0.20	23.9 ± 0.264	28.5 ± 0.404	36.0 ± 0.655
6.	*Hafnia alvei*	17.8 ± 0.251	24.9 ± 0.10	31.9 ± 0.10	39.9 ± 0.503

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(Received for publication 8 April 2008)