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ANTIMICROBIAL POTENTIAL OF SEED EXTRACT OF *RAPHANUS SATIVUS*

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Abstract

The antimicrobial activity of crude water extract, Supernatant as well as Methanolic extract of *Raphanus sativus* were investigated in vitro using Agar well diffusion method. All Extracts were tested against two gram negative, two gram positive bacteria and four fungal cultures. Plant extracts exhibited concentration dependent antimicrobial properties. The extracts displayed highest antibacterial activity against *Hafnia alvei, Enterobacter agglomerans, Lactobacillus and Bacillus thuringiensis* 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 in vitro to have antimicrobial properties. (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). In addition, 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.

Raphanus sativus L. belongs to family Brassicaceae and its common name are Radish, Japanese Radish, Leafy Daikon, Daikon and Fodder Radish. It flowers from June to August, and the seeds ripen from July to September. Radishes have long been grown as a food crop, but they also have various medicinal actions. The roots stimulate the appetite and digestion, having a tonic and laxative effect upon the intestines and indirectly stimulating the flow of bile (Chevallier, 1996). Consuming radish generally results in improved digestion, but some people are sensitive to its acridity and robust action (Chevallier, 1996). The leaves, seeds and old roots are used in the treatment of asthma and other chest complaints (Duke & Ayensu, 1985). The juice of the fresh leaves is diuretic and laxative (Chopra *et al.*, 1986). The seed is carminative, diuretic, expectorant, laxative and stomachic (Yeung, 1985, Duke & Ayensu, 1985, Chopra *et al.*, 1986). It is taken internally in the treatment of indigestion, abdominal bloating, wind, acid regurgitation, diarrhoea and bronchitis **(**Bown, 1995). The root is antiscorbutic, antispasmodic, astringent, cholagogue, digestive and diuretic (Lust, 1983; Duke & Ayensu, 1985). It is crushed and used as a poultice for burns, bruises and smelly feet (Duke & Ayensu 1985). Radishes are also an excellent food remedy for stone, gravel and

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scorbutic conditions (Grieve, 1984). The root is best harvested before the plant flowers (Lust, 1983). Its use is not recommended if the stomach or intestines are inflamed (Lust. 1983). The plant contains raphanin, which is antibacterial and antifungal (Duke and Ayensu, 1985; Bown, 1995). It also has been found to be strongly active on *Escherichia coli, Pseudomonas pyocyaneus, Salmonella typhi* and *Bacillus subtilis*, etc **(**Abdou *et al.* 1972). It inhibits the growth of Staphylococcus aureus, streptococci, Pneumococci. (Yeung, 1985) It is also active against many food born pathogenic and food spoilage bacteria such as *Listeria, Micrococcus, Enterococcus, Lactobacillus and Pedicoccus* spps. (Yildium & Johnson, 1998). The plant also shows anti-tumour activity (Duke & Ayensu, 1985). Seeds contain different antifungal and antibacterial protein. The seed contain fatty oil 30 percent, ash 3.5 percent, volatile oil, sulphuric acid, erucic and C9H15NS2. *Raphanus sativus* seeds also contain two highly potent antifungal proteins (AFPs) called Rs- AFP1 and Rs- AFP2, homolgous to 2 pea pod proteins specifically induced upon fungal infection, and to γ – thionin and sorghum α- amylase inhibitor.

Materials and Method

Preparation of seed powder: The seed of *Raphanus sativus* 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 dried and crushed in 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 5000g 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° C in air tight bottles.

Aqueous Extraction: Ten grams of dried 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 5000g for 15 min. 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° C 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 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° C 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: Cultures of Fungi were collected from the Mycology Laboratory University of Sindh Jamshoro e.g. *Paecilomyces variotii,* (Gilman

J.C and E.V. Abbott (1927) *(Spadocoides stoveri, Penicillium lilacinum (Thom 1910) Penicillium funiculosum.*

Bacterial culture: Cultures of Bacteria 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 37° 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 30-37° C for 24 hours and zone of inhibition was measured to the nearest millimeter. (mm).

Antifungal activity - diffusion plate method: Antifungal activity was tested against *Paecilomyces variotii, Spadocoides stoveri, Penicillium lilacinum, Penicillium funiculosum.* The diffusion plate method was used to test *Raphanus sativ*us 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 10 ml 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 bore were made on each of the seeded plate. These holes filled with 100 µl of the testing sample. Experiments were performed in triplicate. The Petri plates were incubated at 30-35 ° 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.

Statistical analysis: Calculations of MIC were determined by Standard deviation and mean of replicates.

Results and Discussion

Antimicrobial proteins and peptides in plants have most commonly been discovered in seeds where they accumulate to high level and may also function as storage proteins. The seed extract having variable degree of inhibition. The crude water extract of seed inhibited moderate antifungal activity while showed highest antibacterial activity (47 mm± 1.00 SD) against *Hafnia alvei* and *Enterobacter agglomerans* exhibited (27 mm ± 2.08 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* (42mm ± 1.00 SD) and *Paecilomyces variotii* (18 mm ± 0.52 SD) while some fungal strain having insignificant inhibition.

Methanolic extract of seed exhibited strong antibacterial activity against *Bacillus thuringiensis (*51mm ± 2.00 SD), *Enterobacter agglomerans* and *Hafnia alvei* (16 mm ±1.00 SD) Methanolic extract also found to be affected against *Penicillium funiculosum* (21 mm ±3.20 SD) and *Paecilomyces variotii* (18mm± 0.57 SD).

Aqueous supernatant extract of Raphanus sativus were effective against *Penicillium funiculosum* (30mm±1.52SD) *Hafnia alvei* showed *(*17mm±3.21SD) at very low concentration *Bacillus thuringiensis* (14mm±2.51SD*) Lactobacillus* (16mm±2.08SD).

As the work for the development of herbal medicines 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 *Raphanus sativus.*

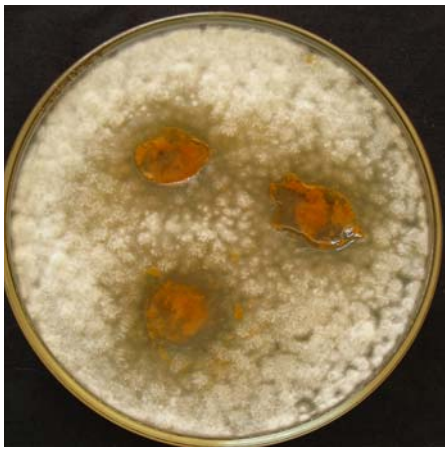
Name of Organism	Mean Zone of inhibition in mm and standard deviation(SD			
	6.3 % Mean±SD	12.5% Mean±SD	25% Mean±SD	50% Mean±SD
Spadicoides stoveri	13± 2.08	20± 3.00	29± 2.51	42±1.00
Paecilomyces variotti	13± 1.00	18± 0.57	27±1.52	44± 1.52
Penicillium funiculosum	8 ±0.57	13±1.15	24±1.00	31± 1.52
Penicillium lilacinum	8± 0.57	16± 2.08	23±1.52	33 ±1.00
Enterobacter agglomerans	13±2.08	18± 2.00	27±2.08	36±1.52
Hafnia alvei	14±1.00	23± 2.64	29±1.52	47±1.00
Bacillus thuringiensis	15±1.52	22±1.00	30±2.51	40±2.00
Lactobacillus	14±2.08	23±2.00	32±2.08	39±3.05

Table 2: Antimicrobial activity of Methanolic extract of *Raphanus sativus.*

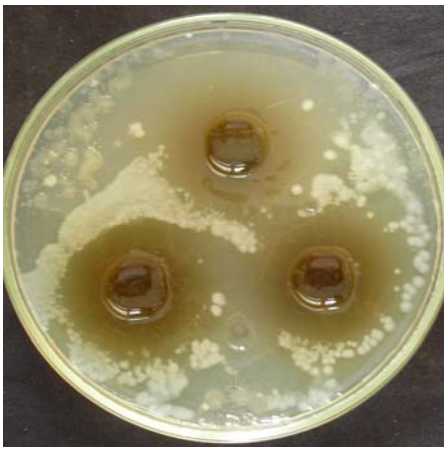
Name of Organism	Mean Zone of inhibition in mm and standard deviation(SD			
	6.3 % Mean±SD	12.5% Mean±SD	25% Mean±SD	50% Mean±SD
Spadicoides stoveri	14± 2.51	23± 2.64	30± 2.08	45± 3.05
Paecilomyces variotti	15± 2.64	21± 2.00	32±2.08	48± 1.52
Penicillium funiculosum	11±1.52	14±2.51	21±3.20	29± 2.64
Penicillium lilacinum	12± 1.52	16± 2.00	24±2.08	30 ±2.60
Enterobacter agglomerans	14±1.52	23± 2.50	26±3.00	43±2.51
Hafnia alvei	16±1.00	22± 3.05	32±3.52	52±3.05
Bacillus thuringiensis	19±2.50	24± 2.08	35±2.51	51±2.00
Lactobacillus	20±2.50	25±2.08	38±3.60	45±3.05

Table 3: Antimicrobial activity of Supernatant of Aqueous extract of *Raphanus sativus*

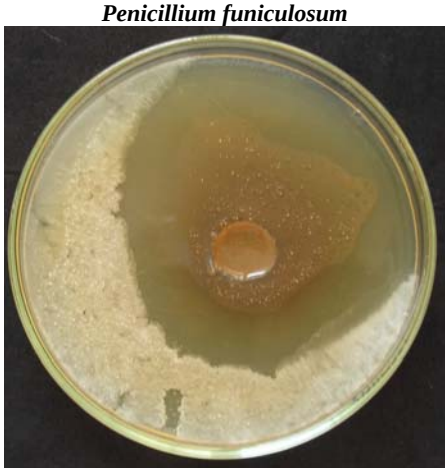
Name of Organism	Mean Zone of inhibition in mm and standard deviation(SD			
	6.3 % Mean±SD	12.5% Mean±SD	25% Mean±SD	50% Mean±SD
Spadicoides stoveri	12± 1.52	19± 2.08	23± 2.51	32±2.08
Paecilomyces variotti	13± 2.80	19±2.51	26±1.527	38± 3.51
Penicillium funiculosum	9 ±1.00	12±1.157	20±2.00	30± 1.52
Penicillium lilacinum	10±1.527	15± 1.154	21±2.08	27 ±2.00
Enterobacter agglomerans	12±2.08	24± 0.57	28±1.527	34±2.08
Hafnia alvei	17±3.21	26± 2.51	28±2.08	52±3.05
Bacillus thuringiensis	14±2.51	27±2.64	37±2.08	43±3.21
Lactobacillus	16±2.08	24±2.51	37±3.60	53±2.08



Penicillium funiculosum



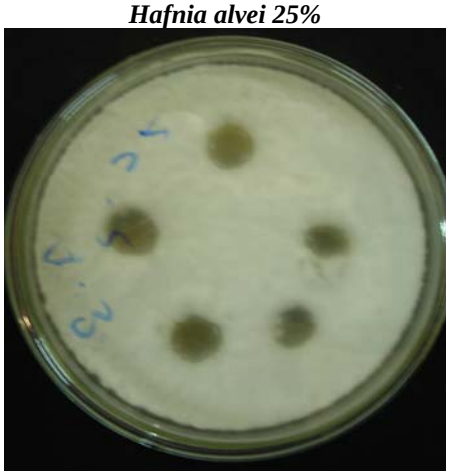
Enterobacter agglomerans



Hafnia alvei 50%

Hafnia alvei 25%

Paecilomyces variotii



Penicillium lilacinum (inverted view)

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