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SCREENING *IN-VITRO* ANTIFUNGAL ACTIVITY OF *RAPHANUS SATIVUS* L. VAR. *CAUDATUS*.

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ABSTRACT

The aim of present investigation was to evaluate antifungal activity of ethanolic extract of *Raphanus sativus* L. var. *caudatus*. The antifungal activity using agar disk diffusion method was determined against six fungal strains; *Aspergillus niger*, *Trichphyton rubrum*, *Microsporum canis*, *Fusarium lini*, *Candida glabrata* and *Candida albicans*. Four different concentrations (50, 100, 200 and 400µg/ml) of *Raphanus caudatus* extract and standard drug (Miconazole) were tested against fungi and zone of growth inhibition were measured. *Raphanus caudatus* exhibited remarkable antifungal activity ($p < 0.05$) against all fungi at all tested concentrations except *Fusarium lini* which was inhibited only at higher concentrations (400 and 800µg/ml). It is noted that with increase in concentration, the zones of growth inhibition were also increased. Thus *Raphanus caudatus* could be a lead for development of antifungal agents in future.

KEYWORDS: *Raphanus caudatus*, ethanolic extract, antifungal activity, Miconazole.

INTRODUCTION

Medicinal plants are used for various diseases around the globe. In fact, almost all the cultures of world depend upon herbs for primary health care because they are economical, easily available with fewer side effects (Mukhergi et al. 2007). It was evaluated that about 25% of various type of herbal constituents have been extracted from plants (Mukhtar et al. 2008).

Raphanus sativus L. Var. caudatus belongs to the family Brassicaceae, is a plant of the radish genus Raphanus. These are basically Radish pods purple or green in color, are

consumed for properties attributed to Raphanus. These are known as Mungraa or Sungraa in Pakistan & India (Khare, 2007).

Radish pods are rich in ascorbic acid, folic acid and potassium. They are a good source of vitamin B6, riboflavin, magnesium, copper and calcium. *Raphanus caudatus* (RC) was suggested to be the functional food since it was reported to have several kinds of minerals, vitamins and some active pharmaceutical metabolites such as sulforaphene and sulforaphane (Songsak and Lockwood, 2002, Pokasap, et al. 2013). Both isothiocyanate compounds have proven role against prostate, breast, colon and ovarian cancers by virtue of its cancer-cell growth inhibition and cytotoxic effects on cancer cells (Holst and Williamson, 2004). RC contained phenolic compounds which showed antioxidant activity (Charoonratana, et al. 2014). Radish is found to be antimicrobial (Abdou, et al. 1972), anti-fungal (Terres et al. 1992), antiurolithiatic (Vargas, et al. 1999), anti-inflammatory (Moon and Kim, 2012) and antioxidant (Takaya, et al. 2003). The leaf, seed and root of *Raphanus sativus* are claimed to have various medicinal uses (Gutiérrezand and Perez, 2004). Fatty acids are the major nutritional composition of interests in seed. Other nutritional components include minerals, vitamin, proteins and polysaccharides (Sham, et al. 2013).

Among plants, Brassicaceae have been reported having significant antifungal activity. Moreover it has known for long time that radish possesses strong antifungal properties, its seeds are also reported for antifungal activities with different antifungal components (Duke and Ayensu, 1985, Bown 1995). In contrast RC, one of the varieties of radish, has not been reported against fungal infections so far. The present study is designed to explore antifungal potential of RC.

MATERIAL AND METHODS

Collection of plant materials

Fresh and healthy pods of Raphanus sativus L. were collected from District Karachi (Pakistan) and were identified from herbarium, University of Karachi, Pakistan. The plant pods were washed thoroughly with double distilled water to avoid contamination.

The plant pods were dried in air under shade at room temperature and stored in properly labeled tightly well closed containers.

Extraction of plants material

The dried pods were grinded with the help of mechanical grinder. Extraction of plant pods were carried out with ethanol using soxhlet apparatus (Davey et al. 2010). 100 grams of powdered pods of plant were placed in thimble of soxhlet apparatus and extracted by 500 ml of ethanol. All extracts were filtered using autoclaved Whatmann's filter paper and centrifuged at 2400 × *g* for 15 minutes **(**Wittschier et al. 2009**).** All the extracts were dried in rotary evaporator at 45° C until semi- solid extract obtained. Percentage yield of extract was calculated. The extract was stored at 4^{o}C in the refrigerator until further use. Four different concentrations of crude extract were subjected to antifungal studies.

***IN-VITRO* ANTIFUNGAL ACTIVITY**

Following six different fungi were selected for antifungal evaluation:

Aspergillus niger (ATCC 1015), *Trichphyton rubrum* (ATCC MYA 4438), *Microsporum canis* (ATCC 10214), *Fusarium lini* (NRRL 2204), *Candida glabrata* (ATCC 90030) and *Candida albicans* (ATCC 36082). The yeasts and molds were grown in Sabouraud dextrose agar and PDA media, respectively, at 28°C. The stock cultures were maintained at 4°C.

In vitro antifungal activity of ethanolic extract of *Raphanus caudatus* against six pathogenic fungi were evaluated by the agar disk diffusion test (Bauer et al 1966; Rios et al 1988). The extract was dissolved in DMSO (dimethyl sulfoxide), sterilized by filtration using sintered glass filter and stored at 4°C. For the determination of zone of inhibition, fungal strains were taken as a standard antibiotic for comparison of the results. Four concentrations (50, 100, 200 and 400µg/ml) of *Raphanus caudatus* extract and standard drug (Miconazole) were prepared in double-distilled water using nutrient agar tubes. The zones of inhibition of fungal growth were determined by measuring sizes of inhibitory zones (including the diameter of disk) after 7 days at 28°C. All experiments were carried out in triplicate, the solvent ethanol was also evaluated alone for its inhibitory effect and it has shown no inhibitory effect at tested concentrations.

RESULTS

In the present study, four different concentrations of RC ethanolic extract against six fungal strains were evaluated. The results were measured as zone of growth inhibition. The extract has shown significant antifungal activity against all fungi at all tested concentrations except *Fusarium lini* which was inhibited only at higher concentrations (400 and 800µg/ml) of RC extract. It is noted that with increase in concentration, the zones of growth inhibition were

also increased. Antifungal effects of standard drug Miconazole and RC extract against different fungal strains are presented in table 1 and 2 respectively. The zones of inhibition for different fungal strains were found in the range of 17 -28 mm and 14 – 20mm for Miconazole and RC ethanolic extract respectively (Figure 1-2).

Table 1: Antifungal activity of Miconazole (Standard drug) against different fungal strains.

Standard drug Miconazole	**Zone of inhibitions (mm)**			
	100	**200**	**400**	**800**
Aspergillus niger	17	19	22	26
Trichphyton rubrum	19	20	23	25
Microsporum canis	17	18	19	22
Fusarium lini	18	19	20	25
Candida glabrata	18	20	23	26
Candida albicans	17	21	25	28

Values are Mean ± SD of three experiments.

Table 2: Antifungal activity of RC ethanolic extract against different fungal strains.

S.No	**Name of Fungus**	**Concentrations of RC ethanolic extract (µg/ml)**			
		100	**200**	**400**	**800**
		Zone of inhibitions (mm)			
1	*Aspergillus niger*	14	15	17	20
2	*Trichphyton rubrum*	14	15	16	18
3	*Microsporum canis*	13	13	14	15
4	*Fusarium lini*	-	-	14	16
5	*Candida glabrata*	15	16	17	19
6	*Candida albicans*	16	18	20	23

Values are Mean ± SD of three experiments; - indicates no zone of inhibition.

STATISTICAL ANALYSIS

Statistical differences of results for antifungal assay were carried out by ANOVA (Analysis of variance) test. Results with p value < 0.05 were taken as significant. All data manipulation and statistical analysis were carried out by using Statistical Package for Social Sciences (SPSS for Windows version 20, SPSS inc., Chicago, IL, USA).

DISCUSSION

Fungal infections are very common in individuals with compromised immune systems and especially in the diabetic patients. Additionally very old and very young individuals are also at risk.

Plants have enriched profile of antimicrobial constituents for example flavonoids, alkaloids, polysaccharides, coumarins, glycosides, lignans, saponins, polyines, thiophenes, proteins and polyphenolics (Jassim and Naji, 2003). It has been estimated that approximately 250000 to 500000 species of plants exist but few of them have been evaluated for their antifungal potential. The plants could be a lead for the development of antifungal compounds as they have already been inhibiting phytopathogenic fungi to which they are exposed in their environment (De Lucca et al 2005).

Due to its high yield and excellent nutritional value Radish has been grown all over the world (Sham et al 2013). Radish and its different varieties have been reported for their antifungal potential. Earlier, the ethanolic extract of root juice of radish exhibited antifungal activity against *Candida albicans* (Caceres, 1987). In another study, Takasugi et al 1987 isolated spirobrassinin, one of the phytoalexin from Raphanus sativus L. var. hortensin which showed substantial antifungal potential. Two peptides rich in cystein designated as RsAFP1 and RsAFP2 were isolated from radish showed notable antifungal activity against different fungi (Terras et al 1992). Moreover antifungal activity of these peptides have been suggested via receptor mediated mechanism (Thevissen et al 1996).

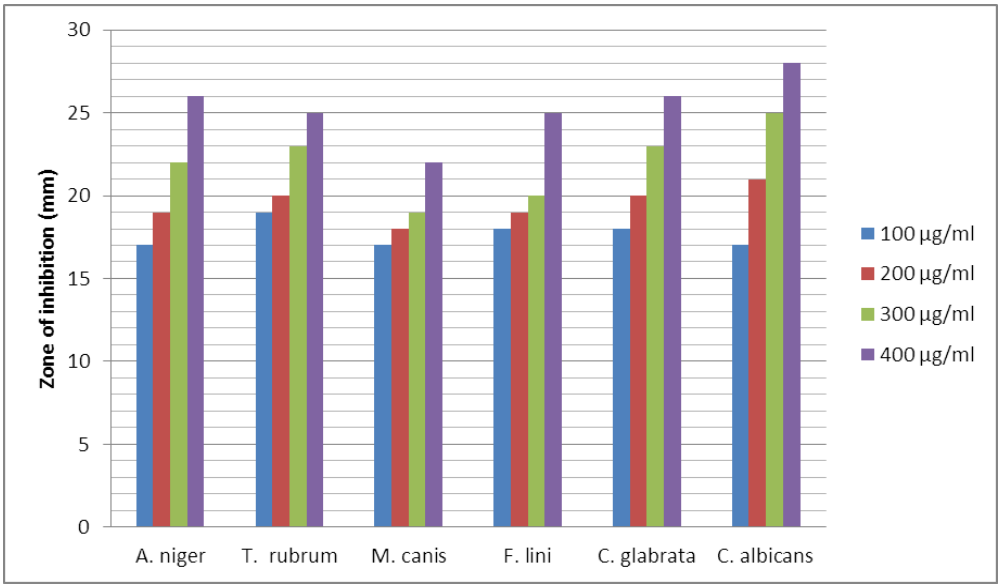

Figure1: Antifungal activity of Miconazole against different fungal strains.

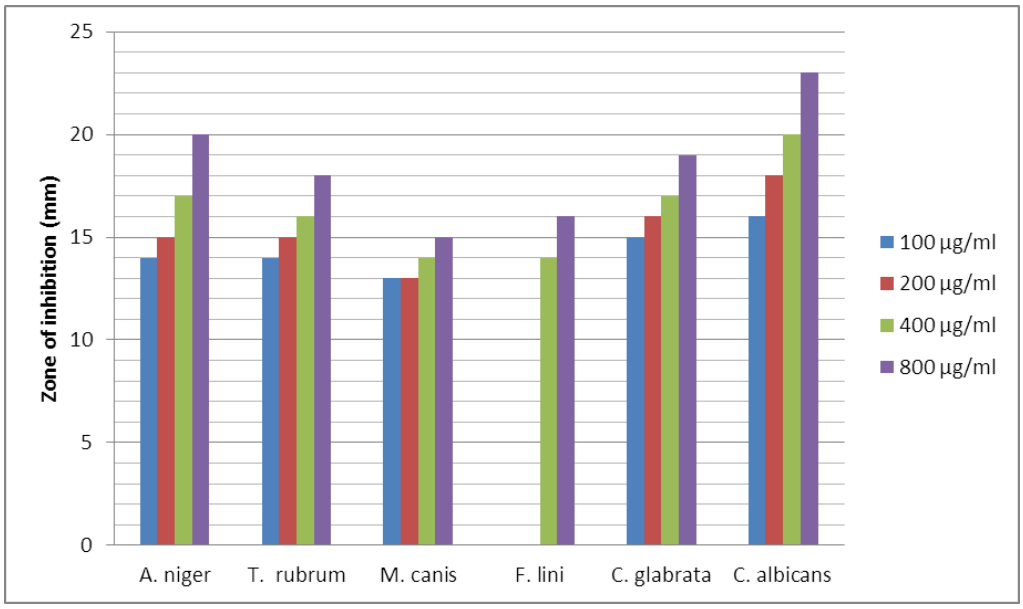

Figure2: Antifungal activity of RC ethanolic extract against different fungal strains.

Shin and Hwang, 2001 isolated two antifungal substances named as RAP1 (Raphanus Antifungal Peptide-1) and RAP2 (Raphanus Antifungal Peptide-2) from Korean radish seeds. Their structure and molecular masses were determined using different chromatographic and non-chromatographic techniques. Both substances were effective fungicidal agents especially against *Candida albicans* and *Saccharomyces cerevisiae*. The antifungal activity of RC in the present investigation could be related to the presence of peptides.

Lee et al 2013 showed that ethyl acetate extract of Korean radish leaves contain significant amount of polyphenols and flavonoids which are responsible for considerable antimicrobial activities against different gram positive bacteria's.

It has been reported that isothiocyanate compounds in Raphanus and other members of Brassicass are responsible for antifungal activities (Smolinska and Horbowicz 1999). The presence of sulphoraphane, sulphoraphene and different other isothiocyanates have been also reported in the pods of Raphanus (Songsak and Lockwood 2002). In addition Ferulic acid and caffeic acid in Raphanus also exhibited antifungal activities. Thus the occurence of these compounds in RC could be linked to its antifungal activity in the present study.

Radish and its seeds are reported to have 2S albumin showed antifungal activity by enhancing the permeability of plasma membranes of fungi (Terras et al 1995).

CONCLUSION

In the present investigation, *Raphanus sativus* L. var. *caudatus* has shown remarkable invitro antifungal potential. In vitro study gives role model for screening of drugs and so helps in further evaluations of activities of drugs. Further research including in vivo studies is required to elucidate the antifungal phytochemicals of the plant with their target of action.

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