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EVALUATION OF ANTIFUNGAL EFFECT ON ETHANOLIC EXTRACT OF *LEPIDIUM SATIVUML.*SEED

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ABSTRACT

Lepidium sativum is one of the medicinal plants having great potency in treating certain common diseases traditionally. This research aim at testing the antifungal activity of ethanolic extract of *Lepidium sativum* seeds against *Fusarium equiseta, Aspergillus flavus, Alternaria* alternate in Potato Dextrose Agar (PDA). Qualitative phytochemical screening showed presences of essential oil, phenolic compounds, alkaloids, Glycosides, tannins, amino acid, steroids. The antifungal activity of ethanolic extract of *Lepidium sativum* was evaluated by employing various concentration (2-8mg).All the concentration of seed extract inhibited the fungal growth. Among different doses, the diameter of inhibition zone ranged from 4 to 22 mm in various fungal species. Hence, the results of the present investigations indicate the *Lepidium sativum* extracts possess antifungal activity that can be exploited as an ideal treatment for future fungal disease.

Keywords: *Lepidium sativum*, Antifungal activity, Potato Dextrose Agar (PDA), Inhibition zone, Phytochemical screening.

INTRODUCTION

Infectious diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide (Laredo JV *et al*., 1995). Fungi are the fifth most common pathogens after *Entero bacteriaceac*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and Coagulase-negative staphylococci. During the past several years, there has been an increasing incidence of fungal infections due to a growth in immunocompromised population such as organ transplant recipients, cancer and HIV/AIDS patients. The number of multi-drugs resistant microbial strains with reduced susceptibility to antibiotics are continuously increasing. The small number of drugs available for their treatment, most of them fungi static and emerging resistance permanently encourage the search for alternatives and led us to find them among low cost and low toxicity traditional therapies and natural products (Knobloch K *et al*., 1989).

Lepidium sativum Linn. (Cruciferae) is commonly known as garden cress. Garden cress is a fast-growing, edible herb that is botanically related to watercress and mustard, sharing their peppery, tangy flavor and aroma. In some regions, garden cress is known as garden pepper cress, pepper grass, pepperwort or poor man's pepper This annual plant can reach a height of 60 cm (~24 inches), with many branches on the upper part. The white to pinkish flowers are only 2 mm (1/12 of an inch) across, clustered in branched racemes. *Lepidium sativum* have been widely used to treat a number of ailments in traditional system of medicine throughout India. Preliminary phytochemical study of *Lepidium sativum* following standard procedures showed presence of flavonoids, coumarins, sulphur glycosides, triterpenes, sterols and various imidazole alkaloids. The major secondary compounds of this plant are glucosinolates.

The alkaloids of *Lepidium sativum* are member of the rare imidazole alkaloids that is known as lepidin. Despite the widespread traditional/edible uses of *Lepidium sativum*, there is very few pharmacological works done. Phytopharmacological screening of alkaloid and glucosinolates are untouched so far.

Lepidium sativum seeds have been used in traditional medicine since ancient times in India. The

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seeds are aperients, diuretic, tonic, demulcent, aphrodisiac, rubefacient, carminative, galactagogue and emmenagogue. It is supplemented in the diet of lactating women to increase the milk secretion during post natal period and recommended for diarrhea and dysentery. The roots are bitter, acrid and are useful in treatment of secondary syphillis and tenesmus and used as a condiment. The aqueous extract of *Lepidium sativum* L. seeds exhibits hypoglycaemic activity in normal and diabetic rats without affecting insulin secretion. *L. sativum* seeds are shown to reduce the symptoms of asthma and improve lung function in asthmatics (AL-Yahya MA *et al*., 1994; Shinde N *et al*., 2010; Yadav YC *et al*., 2010; Yadav YC *et al*., 2011; Al Hamedan WA, 2010).

MATERIALS AND METHODS

Plant material

Lepidium sativum seeds were collected from Mandsur (M P), India. This was identified by Department of Botany, University of Rajasthan, Jaipur. A voucher specimen (RUBL 21063) of same plant material is preserved with herbarium head of Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India.

Preparation of crude extract

120 gm of powdered drug was defatted with petroleum ether (60-80°c) for 72hrs using Soxhlet apparatus. Defatted drug was removed from Soxhlet apparatus and dried at room temperature. Dried defatted drug was further extracted with ethanol for 72hrs. Ethanol extract of seeds of *L. sativum* was collected through distilling off ethanol and thick and concentrated *L. sativum* ethanol extract was procured (yield value 9%).

Phytochemical Analysis of Lepidium sativum seeds Extracts

The extract was subjected for phytochemical investigations by qualitative chemical tests. Standard phytochemical methods were used to test for the presence of saponins, alkaloids, tannins, anthraquinones, cardiac glycosides, glycosides, amino acid & protein and flavonoids.

In-vitro antifungal susceptibility

The procedure for the Potato dextrose agar (PDA) method. PDA powder was dissolved in distilled water to a final concentration of 39 g/liter and then sterilized at 121°C for 15 min. The sterilized PDA solution was placed in a water bath, and the temperature was cooled to and maintained at 55 to 60°C. The antifungal agent stock solutions were mixed with the PDA solution to produce a series of different final concentrations as 2%, 6% and 8%. Drug-free agar containing only 1% DMSO was used as a positive control and without inoculation one plate used as negative control. The mixtures of antifungal agent and PDA solutions were poured directly into the plates. After the plates were cooled to room temperature, freshly made fungal suspension (5 ×103 to 2 ×104/ml) was inoculated onto the agar plate. The plates were incubated aerobically at 35°C for 7 days and then measure the growth of the fungi on the plate. Diameter of zone of inhibition was measured using zone reader and given table 3.

RESULT AND DISCUSSION

The result of the present study indicate that ethanolic extract of *L. sativum* showed antifungal activity against *Fusarium equiseta,Aspergillus flavus, Alternaria alternate* in different concentration. *Fusarium equiseta, Aspergillus flavus, Alternaria alternate* were inhibited by Ethanolic extract. It shows maximum inhibition zone 12mm, 13mm and 16 mm in different dose respectively (Table-3). The extract shows highest antifungal activity against Alternaria alternate and less antifungal activity against *Aspergillus flavus, Fusarium equiseta* (Adam K *et al*., 1998; Kaufman HK, 1997).

Table 1. Phytochemical screening of ethanolic extract of *Lepidium sativum* seeds

Nameof components	Name of chemical tests	Observation
Test of glycosides		
Cardiac glycosides	Legal test	Pink colour
Anthroquinone glycoside	Brontrager test	Ammonia layer turn pink to red Colour
Flavonoids	Shinoga test	Pink Colour
Test of alkaloids	Dragendraff's test	Orange-brown Ppt
	Mayer	Ppt
Tannins and Phenolic Compounds	5% FeCl3 Solution	Deep blue-black ppt**.**
	Dil. Iodine Solution	Transient red colour
protein and amino acid Compounds	Biuret test	Violet colour
Test of Steroid	Salkowshi reaction	Chloroform layer appear, red and blue

Table 2. Phytochemical screening of ethanolic extract of *Lepidium sativum* seeds

S.No	Name of Tests	Results
1	**Glycosides**	+ve
	Cardiac glycosides	+ve
	Anthroquinone glycoside	+ve
	Flavonoids	+ve
2	**Alkaloids**	+ve
	Dragendraff's test	+ve
	Mayer test	+ve
3	**Tannins and Phenolic Compound**	+ve
4	**Proteins**	+ve
5	**Steroid**	+ve

Fungal strains: Human pathogen *Aspergillus flavus* and two plant pathogens (*Fusarium equiseti*, *Altenaria alternate*) were used

Drug used: Cotrimazole was used as reference standard for antifungal studies.

Table 3. Zone of inhibition of seed extract of *Lepidium sativum* for antifungal activity

Fungus	Zone of growth (In mm)			
	LSE 2mg/ml	**LSE 6mg/ml**	**LSE 8mg/ml**	**Clotrimazole 1mg/ml**
Fusarium equiseta	4	12	10	20
Aspergillusflavus	9	11	13	18
Alternaria alternate	12	8	16	22

*LSE- *Lepidium sativum* extract

Qualitative phytochemical screening showed presence of essential oil, phenolic compound, glycosides, amino acids alkaloids. Presence of constituents like flavonoids, tannin in the extract are likely to be responsible for the antimicrobial activity.so the antifungal activity of seed extract might be due to presence of some active secondary metabolite in the plant. Our result indicate the potential usefulness of *L. sativum* in the treatment of various pathogenic diseases as it may help in the discovery of new chemical classes of antibiotics that could serve as selective agent for the maintence of human health and may provide biochemical tools for the study of fungal infection diseases. The discovery of a potent remedy from plant origin will be a great advancement in fungal infection therapies.

CONCLUSION

Ethanolic extract of *L. sativum* possess significant antifungal activity against selective pathogens. Further studies aim at isolation and purification of active phytoconstituents. There is a need to test the in-vivo activity of the extract apart from the effect on many other fungi. This plant is an ideal candidate in the research for new bioactive phytocompound suggesting that a more extensive biological and chemical bioassay guided fractionation is required in order to isolate and characterize such bioactive compound.

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