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ANALYSIS OF PHYTOCONSTITUENTS AND *IN VITRO* ANTIFUNGAL EVALUATION OF METHANOLIC EXTRACT OF *LEPIDIUM SATIVUM* LINN. SEEDS

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ABSTRACT

Lepidium sativum Linn. is a small annual herb placed in the family Cruciferae. It is used in various Ayurvedic drugs in the management of hiccup, diarrhoea, asthma, bronchitis and cough. Seeds are rich source of omega-3 fatty acids, proteins, dietary fiber, iron, other essential nutrients and phytochemicals. In the present paper, methanolic extract of *Lepidium sativum* seed obtained by Soxhlet extraction was examined for phytochemical screening to detect the phytoconstituents. Pharmacological relevance of the extract for antifungal activity was evaluated at different concentrations of 10, 30, 60 and 90 mg/ml against human pathogenic and opportunistic fungi such as *Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Fusarium* sp*, Penicillium* sp *and Penicillium marneffi*. Imidazole was used as the reference antibiotic. It was observed that methanolic extract showed alkaloids, steroids, terpenoids, flavonoids, phenolic compounds, fixed oils, fats and *Aspergillus flavus* was the most sensitive fungi, inhibited at 30 mg/ml.

KEYWORDS: ***Lepidium sativum*, methanolic extract, phytoconstituents, antifungal.**

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INTRODUCTION

The world is lush with naturally grown medicinal plants that abound in health enhancing phytochemicals and nutrients. Contribution of medicinal plants to human healthiness against different ailments began since time immemorial. Resistance of pathogenic microorganism to antimicrobial agents and reappearance of diseases which were thought to be under control made the treatment of infectious diseases more problematic. The folklore use of medicinal plants and their experimental validation suggests that the natural products play a key role in plant-pathogen interactions [1, 2]. *Lepidium sativum* Linn. (Cruciferae) commonly known as 'Garden cress' is well recognized plant in European communities as Herba Lepidii Sativi as a source of vitamins, diuresis effect, a bile stimulant and cough reliever. It is a popular medicinal herb among the Arab and Asian communities for multiple ailments and therapeutic applications [3]. The plant is cultivated as a culinary vegetable all over Asia [4]. Glucosinolates are the major secondary metabolites of *L.sativum* and the extracts have chemopreventive effects in inhibiting carcinogenesis [5]. The seeds of *Lepidium sativum* are small, oval-shaped, smooth surfaced, about 2-3mm long and 1-1.5mm wide with reddish brown colour. When soaked in water the seed coat swells and gets covered with transparent, colourless, mucilage [6,7]. It is texted as 'Chandrashoor' in Sanskrit and the plant has been considered as an important nutritional and medicinal plant in India since the Vedic era (between 500-1700 B.C). The seed is described as hot, bitter, galactogogue [8] and is incorporated in the Chaturbeeja for the management of backache, tympanitis pain and nervous disorders [9]. The seeds contain edible oil which is rich in alpha linolenic acid with ideal ratio of omega 3&6– fatty acids [10,11] as well as imidazole alkaloids [12]. Chemical constituents like lepidine, lepidine B, C, D, E and F, semilepidinoside A and B [13], glucotropaeolin, N,N'-dibenzyl urea, N,N'-dibenzylthiourea, sinapic acid and its choline ester (sinapin), flavonoids such as 5-4'-dihydroxy-7,8,3',5-tetramethoxyflavone and 5-3'-dihydroxy-6,7,4'-trimethoxyflavone have been isolated from the seeds [14]. Seeds possess antioxidant, anti-inflammatory, anti tumor activity and are useful as poultices for sprains and in leprosy, ophthalopathy, leucorrhoea, scurvy, seminal weakness, bronchial asthma, cough, hemorrhoids etc[15,16,5]. The present study reports detection of the phytochemical constituents in the crude methanolic extract of *Lepidium sativum* seeds and antifungal activity of the methanolic extract at 10, 30, 60 and 90mg/ml concentrations against selected fungal strains.

MATERIALS AND METHODS

1. Preparation of seed extract

Lepidium sativum seeds were commercially purchased identified and voucher specimen (TSB.10.94.1.1.14) was deposited at Jawaharlal Nehru Tropical Botanical Garden and Research Institute (JNTBGRI), Kerala. 100g of seeds were powdered and extracted in a soxhlet apparatus with different organic solvents (100g in 500ml of respective solvents) for 12 hours [17]. The extracts collected were concentrated under reduced pressure using a rotary vacuum evaporator (Ika, Germany) and stored in sterile containers at 4˚C until used. Of the different extracts, methanolic extract was chosen for the study since it was found to be the most potent against the selected fungi during primary antifungal screening.

2. Phytochemical analysis of seed extracts

Preliminary phytochemical screening was done for detecting the phytoconstituents in the methanolic extract [18,19].

2.1 Liebermann Burchard test

To the extract dissolved in chloroform a few drops of acetic anhydride, followed by con. H_2SO_4 were added along the sides of the test tube. Presence of red coloured ring indicated terpenoids and the change in red colour to green marked the presence of steroids.

2.2 Shinoda test

Magnesium turnings and dil.HCl were added to the alcoholic extract of drug, formation of deep-red to magenta colour indicated the presence of flavonoids.

2.3 Mayer's test
Few drops of Mayer's reagent (K_2HgI_4) were added to the extract, formation of creamy-white precipitant indicated alkaloids.

2.4 Dragendorff's test (Spot test)
A spot of the extract (dissolved in chloroform) was applied onto a filter paper using capillary tube. Dragendorff's reagent was added onto the dried spot, allowed to dry and washed the spot with water. No change in spot colour showed presence of alkaloid.

2.5 Molisch's test
The extract was mixed with a few drops of Molisch reagent (alpha naphthol). Conc. H_2SO_4 was added along the sides of the test tube. Formation of purple coloured ring at the junction indicated the presence of carbohydrates.

2.6 Biuret test
Few drops of Biuret reagent (KOH, $CuSO_4$ and sodium potassium tartarate) were added to the extract. The blue colour turned to violet, indicating the presence of protein.

2.7 Ferric Chloride test
A few drops of 5 % ferric chloride were added along the sides of the test tube to a mixture of 200µl of the extract and 2ml of distilled water. A dark green colour showed the presence of phenolic compounds.

2.8 Coumarin test
The extract was dissolved in methanol. Appearance of yellow colour on the addition of NaOH and decolouration with the addition of Con. HCl were detected pointing to the presence of coumarin

2.9 Foam test
10ml of distilled water was added to the extract and shaken well for few minutes, formation of frothing persisted for 60-120 seconds in the presence of saponins.

2.10 Test for fixed oils and fats (spot test)
A small quantity of extract was pressed between two filter papers. Oil stain on the paper indicated the presence of fixed oils and fats.

3. Antifungal activity screening
3.1 Standard microorganism
The fungi used in the study were obtained from the collections maintained at the Department of Biotechnology, University of Kerala, Thiruvananthapuram and Centre for Health Sciences, University of Calicut, Kozhikode, Kerala. Fungal strains included human pathogenic strains like *Candida albicans, Fusarium* sp*, Microsporum* sp*, Penicillium marneffi and laboratory contaminants / opportunistic fungi like Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Penicillium* sp*, Rhizopus* sp.

3.2 Antifungal activity by tube method
The antifungal activity of seed methanolic extract was tested against the listed fungi by test tube method [20]. Different concentrations of the extract -10mg/ml, 30mg/ml, 60mg/ml and 90mg/ml- were tested against the fungi. Sabouraud Dextrose Agar (SDA) was taken as the culture medium. Different concentrations of the extract (initially dissolved in DMSO) were added directly to the media to obtain extract-SDA slants of 1ml volume. Mixed well and after solidification of slant media, the test fungi were inoculated. The tubes inoculated with *Candida albicans* was incubated at 37˚C for 24 – 72 hours. Other tubes were incubated at room temperature and results were recorded after 7-10 days. Imidazole (100µg/ml of SDA) and DMSO were kept as the drug and solvent controls respectively.

RESULTS

1. Phytochemical Screening
The qualitative phytochemical screening of methanolic extract of *Lepidium sativum* seeds is summarized in Table 1. Screening showed the presence of many important secondary metabolites like alkaloids, steroids, terpenoids and flavonoids which are of great value in health care.

Table 1
Phytochemical screening of methanolic extract of Lepidium sativum seeds

Chemical test	Phytoconstituent	Observation	Result
Liebermann Burchard	steroids, terpenoids	red-green colour	+
Shinoda	flavonoids	red colour	+
Mayer's Test	alkaloids	creamy precipitate	+
Dragendorff's spot test	alkaloids	red spot	+
Molisch's	carbohydrates	purple ring	+
Biuret test	protein	violet colour	+
5% $FeCl_3$	phenols	blue green colour	+
Coumarin	HCl decolouration	decolouration of yellow	+
Foam test	saponins	stable foam	+
Spot test	fixed oils, fats	translucent oil spot	+

2. Antifungal activity

The antifungal potential of the methanolic extract of *Lepidium sativum* seed against the tested fungi at different concentrations of 10mg/ml, 30mg/ml, 60mg/ml and 90mg/ml is given in Table 2. Maximum activity was observed at a concentration of 90mg/ml.

Table 2
Antifungal activity of Lepidium sativum methanolic seed extract

Fungus	10mg/ml	30mg/ml	60mg/ml	90mg/ml
Aspergillus flavus	G	NG	NG	NG
Aspergillus fumigatus	G	G	G	NG
Aspergillus niger	G	G	SG	SG
Candida albicans	G	G	SG	NG
Fusarium sp	G	G	G	NG
Microsporum sp	G	G	SG	NG
Penicillium sp	G	G	G	NG
Penicillium marneffi	G	G	G	NG
Rhizopus sp	G	SG	SG	NG

G:growth NG: no growth (inhibition) SG: slow growth of the fungus towards the end of the week

It is revealed that, the methanolic extract at a concentration of 30mg/ml completely inhibited the growth of *Aspergillus flavus*. Towards the end of the incubation period *Rhizopus* sp. showed slow and weak growth on 30 mg/ml and 60 mg/ml slant and was completely inhibited at 90 mg/ml. At a concentration of 90 mg/ml the fungi *Aspergillus fumigatus*, *Candida albicans*, *Fusarium* sp, *Microsporum* sp, *Penicillium sp*, *Penicillium marneffi* were completely inhibited.

DISCUSSION

Increased consumption of Cruciferous vegetables has been associated with a decreased risk of infectious diseases [21], cancer [22] and cardiovascular disease [23]. *Lepidium sativum* seeds are rich source of proteins (22.47$\pm$0.78), dietary fiber (30$\pm$0.47), minerals and essential amino acids (28.53%) [24]. The methanolic extract of seed evaluated by Indumathy and Aruna [25] was found to contain total phenols (8.651mg GAE/gm) and flavonoids (4.023 mg CAE/gm). The presence of phenolic and flavonoid compounds in the seeds might be responsible for its strong antioxidant capacity. Toxicology studies of seeds revealed that they can be considered as non-toxic and safe[26]. Imidazole; an important organic member of Cruciferae family, is used in various medications has an extensive spectrum of biological activities such as anti cancer, anti-inflammatory, analgesic, antiviral and antidepressant [27]. There exist reported studies on antibacterial and antifungal activity of imidazole [28,29,30]. Ulrich et al [13] has isolated imidazole alkaloid lepidin and its derivatives from the seeds of *Lepidium sativum*. Various reported studies with *Lepidium sativum* indicated that they have a strong antimicrobial activity. Rahul et al

[31] reported the presence of alkaloids, amino acids, essential oils, phenolic compounds and glycosides in the ethanolic extract of *Lepidium sativum* seeds. They also reported antifungal zone against *Fusarium equisetum* at 6 mg/ml and *Aspergillus flavus*, *Alternaria alternate* at 8 mg/ml concentrations in Potato Dextrose Agar (PDA). Iqbal et al [32] detected the presence of alkaloid, flavonoid, saponins, tannin and phenol in *L.sativum* and also found the antimicrobial activity against *Bacillus subtilis*, *Proteus vulgaris*, *E.coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Aspergillus niger* and *Candida albicans*. Of the different fungal strains tested, reports [33,34,35] pointed that *Candida albicans* was inhibited by various extracts of *Lepidium sativum*. The antimicrobial activity of the petroleum ether, methanol and water extracts of *L.sativum* seeds against six pathogenic microorganisms viz. *S.aureus*, *E.coli*, *Klebsiella pneumonae*, *Proteus vulgaris*, *P.aeruginosa* and fungus *C.albicans* was studied by Shama et al [35]. Of these, different concentrations of petroleum ether (2.5,5 and 10%) were found to be more active against all the six pathogens. Members of the genus *Lepidium*, such as *Lepidium meyenii*, *Lepidium apetalum* as well as cabbage, broccoli etc. of Cruciferae family exhibit strong antimicrobial activity. The oil of *L.meyenii* has phytotoxic, cynobactericidal and antitermite activity[36]. *L.virginicum* showed activity against *S.aureus* and *C.albicans* with inhibition zone of 10 mm and 12 mm respectively[37]. Goralska et al[38] stated the fungistatic properties of glucosinolates from seeds of Cruciferous plants such as *Brassica oleracea* (broccoli), *Raphanus sativus* (small Radish), *Brassica oleracea* (white cabbage) *Sinapsis alba* (white mustard) against *Candida albicans*, obtained from different sections of the gastrointestinal and respiratory tracts. In this the seed extracts of broccoli exhibited highest antifungal activity against *C.albicans* with an inhibition zone of 38 mm. Of the various solvent extracts of *Brassica oleracea* (broccoli) the acetone and methanolic extract showed Minimum Inhibitory Concentration (MIC) values of 10 - 320 µg/ml were recorded against most of the pathogens tested (*B.cereus*, *B.subtilis*, *S.aureus*, *E.coli*, *S.typhimurium* and *Shigella flexneri*). *B.subtilis* ATCC 6633 (15.4 mm) and *B.cereus* ATCC 10876 (16.3 mm) were found to be the most sensitive organisms to the broccoli extract [39].

CONCLUSION

The awareness of the chemical constituents in a plant helps in understanding the value of folkloric remedies and discloses the wrapped areas of therapeutics. In this study, the presence of phytochemicals such as alkaloids, steroids, terpenoids, flavonoids, saponins, phenolic compounds, fixed oils and fats were detected in the crude methanolic extract of *Lepidium sativum* seeds. The strong antifungal action of the plant may be due to the presence of these secondary metabolites. Detailed research with purified fractions helps in sourcing the antifungal component of the plant for the development of newer antifungal agents.

REFERENCES

1. Riazullah.,IqbalHussain and Badrullah. Phytochemical and anti-microbial activity of *Lepidium sativum* L. J. Medicinal plant research, Vol 6(26): 4358-4361, (2012)
2. Deepa M and Padmaja C K. A study on the antibacterial effects of the various extracts of *Excoecariaagallocha* L. Int J Pharm Bio Sci,5(4): (B) 169 – 178,(2014)
3. Abdullah bin Habeeballah bin Abdullah Juma. The effects of *Lepidium sativum* seeds on fracture-induced healing in rabbits. Medscape general medicine,9(2):23,(2007)
4. Najeeb-ur-Rehman., Arif-ullahKhan., Khalid M Alkharfy and Anwarul Hassan Gilani. Pharmacological basis for the medicinal use of *Lepidium sativum* in airways disorders. Evidence-based

Complementary and Alternative Medicine, Article ID 596524:1-8,(2012)
5. Sawsan Hassan Mahassni and Roaa Mahdi-Al-Reemi. Cytotoxic effect of an aqueous extract of *Lepidium sativum* L. seeds on human breast cancer cells. Indian Journal of Traditional Knowledge, 12(4):605-614,(2013)
6. Anonymous. The Ayurvedic Pharmacopoeia of India. Part-1,1st Edn, Vol-1. Ministry of health and family welfare,India,26(2001)
7. Anil Kumar D. Ayurvedic Drug Plants, Daya Books Publisher, New Delhi, India,97(2006)
8. NidhiAgarwal and Sheel Sharma. Appraisal of Garden Cress (*Lepidium sativum* L.) and product development as an all pervasive and nutrition worthy food stuff. Annals, Food Science and Technology,14(1):77-84,(2013)
9. KshitijChauhan.,Nishteswar K and Chauhan M. G.Pharmacognosticalevaluation of seeds of *Lepidium sativum* Linn. International Journal of Pharmaceutical & Biological Archives, 3(3):627-631,(2012)
10. Kassie F., Laky B., Gminski R., Mersch-SundermannV., ScharfG., Lhoste E and Kansmuller S. Effects of garden and water cress juices and their constituents, benzyl and phenethylisothiocyanates, towards benzo (a) pyrene-induced DNA damage: a model study with the single cell gel electrophoresis/Hep G2 assay. Chemico-Biological Interactions,142:285–296,(2002)
11. Kassie F., Pool-Zobel B., Parzefall W and Knasmuller S. Genotoxic effects of benzyl isothiocyanate, a natural chemopreventive agent. Mutagenesis,14:595–604,(1999)
12. Shehzad M., Tanveer A., Ayub M., Mubeen K., Sarwar N., Ibrahim M and Qadir I. Effect of weed-crop competition on growth and yield of garden cress (*Lepidium sativum* L.). J. Med. Plants Res, 5(26):6169-6172,(2011)
13. Ulrich H Maier., HeidrunGundlach and Meinhart H Zenk. Seven imidazole alkaloids from *Lepidium sativum*. Phytochemistry, 49(6):1791-1795,(1998)
14. DivanjiManohar., Viswanatha G.L., Nagesh S., Vishal Jain and Shivaprasad H.N. Ethnopharmacology of *Lepidium sativum* Linn (Brassicaceae): A Review. International Journal of Phytothearpy Research, 2(1):1-7, (2012)
15. Gokavi S.S., Malleshi N.G and Guo M. Chemical composition of garden cress (*Lepidium sativum*) seeds and its fractions and use of bran as a functional ingredient. Plant Foods for Human Nutrition (Formerly QualitasPlantarum), 59:105–111, (2005)
16. Gu¨venc , Okada , AkkolE.K., Duman H and Okuyama T. Investigations of anti-inflammatory, antinociceptive, antioxidant and aldose reductase inhibitory activities of phenolic compounds from *Sideritisbrevibracteata*. Journal of Ethnopharmacology, 61:161–166, (1998)
17. Gupta C Prakash., Joshi P and Lohar D R. Evaluation of antibacterial activity of *Lepidium sativum* Linn. seeds against food borne pathogens. Int.J.Chemical and Analytical Science, 1(4):74-75, (2010)
18. Harbone J B. Phytochemical Methods. A Guide to Modern Techniques to Plant Analysis. 3rdEdn. Chapman and Hall, New York: 56-79, (1998)
19. Thankamani V., Joel James and Lipin Dev. Phytochemical screening and antimicrobial activity of *Alstoniascholaris* Flower. I.J.PRD, 3(4):172-178, (2011)
20. Pandey B P. A text book of Botany-Fungi. Chand S and CompanyLtd., New Delhi, India, 416 (1994)

21. Sofrata A., Santangelo E M., Azeem M., Borg-KarlsonA K., Gustafsson A and PütsepK.Benzylisothiocyanate, a major component from the roots of *Salvadorapersica* is highly active against gram-negative bacteria. PLoS One 6(8): e23045, (2011).
22. Navarro S L., Li F., Lampe J W. Mechanisms of action of isothiocyanates in cancer chemoprevention: an update. Food Funct, 2: 579-587, (2011)
23. Yuan G., Sun B., Yuan J and Wang Q. Effects of different cooking methods on health-promoting compounds of broccoli. J Zhejiang UnivSci B 10(8): 580-588, (2009)
24. SnehalDoke and ManishaGuha. Garden cress (*Lepidium sativum* L.) Seed - An Important Medicinal Source: A Review J. Nat. Prod. Plant Resour, 4(1):69-80,(2014)
25. Indumathy R and Aruna A. Free radical scavenging activities, total phenolic and flavonoid content of *Lepidium sativum* (Linn.).International Journal of Pharmacy and Pharmaceutical Sciences, 5(4):634-637, (2013)
26. Datta P K., Diwakar B T., Viswanatha S., Murthy K N and Naidu K A. Safety evaluation studies on Garden cress (*Lepidium sativum* L.) seeds in Wistar rats. International Journal of Applied Research in Natural Products, 4 (1):37-43,(2011)
27. Vijayta Gupta and Vinay Kant. A review on biological activity of imidazole and thiazole moieties and their derivatives. Science International,1 (7):253-260,(2013)
28. RajatGhosh and Biplab De. Review on: synthesis, chemistry and therapeutic approaches of imidazole derivatives. Int.J.Pharm.Sci.Rev.Res.,23(2):237-46,(2013)
29. KumariShalini., Pramod Kumar Sharma and Nitin Kumar. Imidazole and its biological activities: A review. Pelagia Research Library Der ChemicaSinica, 1(3): 36-47,(2010)
30. AmitaVerma., Sunil Joshi and Deepika Singh. Imidazole: Having Versatile Biological Activities. Hindawi Publishing Corporation Journal of Chemistry Volume 2013, Article ID 329412, 12 pages
31. Rahul Kumar Sharma., KapilVyas and HansrajManda. Evaluation of antifungal effect on ethanolic extract of *Lepidium sativum* seed. Int.J.of Phytopharmacology,3(2):117 120,(2012)
32. IqbalHussain., Moneeb Ur RehmanKhattak., Riazullah.,Zia Muhammad., Naeem Khan., Farhat Ali Khan., ZahoorUllah and SajjadHaider. Phytochemicals screening and antimicrobial activities of selected medicinal plants of Khyberpakhtunkhwa. Pakistan African Journal of Pharmacy and Pharmacology, 5(6):746-750,(2011)
33. BhasinPragya., Dinesh Bansal., Anita Punia and Anita R Sehrawat. Antimicrobial activities of *Lepidium sativum*: Medicinal plant used in folklore remedies in India. J Pharmacy Research, 5(3):1643-1645,(2012)
34. Darwish R M and Aburjai T A. Effect of ethanomedicinal plants used in folklore medicine in Jordan as antibiotic resistant inhibitors on *Escherichia coli*. Complement. Altern.Med,10(9):1-8,(2010)
35. Shama I Y Adam., Shayma A M Salih and Warda S Abdelgadir.*In vitro* antimicrobial assessment of *Lepidium sativum* L.seed extract. Asian J Medical Sciences 3(6):261-266,(2011)
36. Tellez M., Khan I., Kobaisy M., Schrader K, Dayan F and Osbrink W. Composition of the essential oil of *Lepidium meyenii* (Walp.). Journal of Phytochemistry,61:149-155,(2002)

37. Joy R. Borchardt., Donald L Wyse., Craig C Sheaffer., Kendra L Kauppi., Gary Fulcher R., Nancy J Ehlke., David D Biesboer and Russell F Bey. Antioxidant and antimicrobial activity of seed from plants of the Mississippi river basin. Journal of Medicinal Plants Research., 2(4):81-93,(2008)
38. Góralska K., Dynowska M and Ciska E. Fungistatic Properties of Glucosinolates –a Reconnaissance Study. Polish J. of Environ. Stud, 18(3):377-382,(2009)
39. Sibi G., AbhilashaShukla., Dhananjaya K., Ravikumar K.R. and Mallesha H. *In vitro* antibacterial activities of Broccoli (*Brassica oleracea*L.varitalica) against food borne bacteria. Journal of Applied Pharmaceutical Science, 3(05):100-103,(2013).