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Antimicrobial Profiling of *Lepidium sativum* Seed - A Comparative Study with Different Solvent Extracts

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Abstract:*Lepidium sativum (Family: Brassicaceae) seeds obtained from local medicinal plant vendors of Thiruvananthapuram, Kerala was taken up for the study.Soxhlet extraction with hexane, butanol, ethyl acetate, methanol and water were done successively to obtain the crude extracts. The extracts were used for phytochemical screening and antimicrobial studies. Antibacterial and antifungal activities were performed by Agar Well Diffusion Method and Tube Method respectively. Amount of phytoconstituents present was greater in methanol extracts showing steroids, terpenoids, alkaloids, phenolic compounds, flavonoids, carbohydrates, saponins, fixed oils and fats. Antibacterial activity revealed that the ethyl acetate extract had the greatest inhibition with maximum zone for Micrococcus luteus and Staphylococcus aureus. Antifungal activity was highest for methanol extract. The results of the experiments were promising for broad-spectrum microbicidal action and the evidence can be used for further pharmacological studies and possible drug exploration.*

Key Word*:Lepidium sativum, Soxhlet, phytochemical screening, antimicrobial.*

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I. INTRODUCTION

Medicinal plants and herbal remedies havebeen in use from time immemorial by man for protection fromvarious ailments, disorders and diseases. Production of synthetic drugs decreased the priority for natural remedies. But the health hazards due to the continuous usage of chemical drugs and multidrug resistivity of microbes urge the scientific community to focus more on natural sources. Therapeutic efficacies of newer compounds from underutilized plants and other natural sources will help the pharmaceutical companies to formulate new drugs that are safer to use, cost effective and will be available for common populations.

Lepidium sativum a member of the Brassicaceae family is a well-known medicinal plant widely grown in Asia and Europe. The plant particularly the seeds and seedlings are used in fortification of cuisines and salads popularly by the Arabs. The seeds are used in various Ayurvedic preparations and home remedies in many parts of the world especially in India since Vedic ages (Chandra and Vinod, 2017).

L.sativum (Garden cress) is an erect herbaceous annual plant of 15-45 cm, with small white flowers in long racemes and the pods are obovate, elliptic, rotundate, irregular at apex and winged. The brownish red coloured seeds are oval with one end pointed and conical at the other with smooth surface (Baregama and Goyal, 2019). The seeds contain 20-25% oil with linolenic acid (32-35%) as the main fatty acid, protein 27%, 35-54 % carbohydrates (90% non-starch polysaccharides and 10% starch), 14-26% fat and 8% crude fiber. The seeds also possess natural antioxidants eugenol, carotenoids, tocopherols, bioactive phytoconstituents like imidazole alkaloids, lepidine, sinapic acid, sinapine which makes it a vital therapeutic drug with antimicrobial, antioxidant, anticancer, and anti-inflammatory properties (Khalid *et al*., 2020).

The plant also called as Chandrasur / Asalio in India is cultivated mostly in the northern part of the country and transported to other states. Kerala, the store house of herbal medicine also uses Asali seeds in various traditional medicinal preparations for postnatal care and lactating mothers for improving their health and milk production (Jazir Haneef *et al*., 2015). The seeds are said to be used in various porridge preparations during medicinal diet regimen and fasting. So through the current work the much underutilized seeds of *L.sativum* available in Kerala premises are taken and its phytochemical and antimicrobial profiling with various solvents are studied to know the reliability of the seed for various pharmacological studies in future.

II. MATERIALS AND METHODS

Preparation of Seed Extracts

Lepidium sativum seeds were obtained from local medicinal plant vendors of Thiruvananthapuram. The seeds were identified and authenticated (voucher specimen TBGI, TSB.10.94.1.1.14) by the taxonomist of Jawaharlal Nehru Tropical Botanical Garden and Research Institute (JNTBGRI), Kerala.

Dry seeds were cleaned manually, crushed using a grinder and subjected to hot extraction using Soxhlet apparatus. 100 g of crushed *L.sativum* seed**s** were extracted successively with 500 mL of n-Hexane, n-Butanol, Ethyl Acetate, Methanol and water. Except aqueous extract all other extracts were removed from the respective solvents using a rotary vacuum evaporator (Ika, Germany). The extracts were stored in sterile pre-weighed screw capped containers at 4°C until used.

Preliminary Phytochemical Screening

Phytochemical screening of the seed extracts to detect the presence of bioactive phytoconstituents was done using the standard phytochemical methods of Harborne (1984).

In vitro Antimicrobial Activity

Strains used for antimicrobial studies

The bacterial cultures were obtained from the standard culture collections maintained at the Department of Biotechnology, University of Kerala, Thiruvananthapuram. They included *Micrococcus luteus* (MTCC 106), *Klebsiella pneumoniae* (MTCC 3384), *Pseudomonas aeruginosa* (MTCC 424), *Escherichia coli* (MTCC 40) and *Proteus vulgaris* (MTCC 426). *Staphylococcus aureus, Salmonella typhi*, *Salmonella paratyphi* and *Shigella* sp. were clinical strains. Single colony grown on aNutrient Agar plate was transferred to 5mL of Nutrient Broth and incubated for 2-3 hours at 37˚C to obtain 0.5 McFarland turbidity standards (approximately 1-2 x 10^8 colony forming units per mL) and were used for testing antibacterial activity.

Antifungal studies were done on human pathogenic strains like *Candida albicans*, *Fusarium* sp., *Microsporum* sp., *Penicillium marneffei* and laboratory contaminants/ opportunistic fungi like *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Penicillium* sp., *Rhizopus* sp., *Cryptococcus* sp., *Curvularia* sp., *Epidermophyton* sp. obtained from the collections maintained at the Department of Biotechnology, University of Kerala, Thiruvananthapuram and also from Centre for Health Sciences, University of Calicut, Kozhikode, Kerala.

Media used

Nutrient Agar was used for the regular maintenance of cultures, nutrient broth for inoculum preparations, Mueller-Hinton Agar (MHA) and Sabouraud's Dextrose Agar (SDA)were chosen as the medium for antibacterial and antifungal testing respectively. The required amount of media was dissolved in corresponding amount of sterile double distilled water and sterilized by autoclaving at 15 lbs pressure at 121˚C for 15 min.

Preparation of test samples

A single concentration of 100 mg/ml of crude extract was used for the antimicrobial studies. Streptomycin (0.125 mg/mL) and Imidazole (0.100 mg/mL**)** were taken as the positive control (standard drugs) for antibacterial and antifungal respectively. DMSO was used as the solvent control.

Antibacterial Activity by Agar Well Diffusion Method

The antibacterial activity of the *L.sativum* seed extracts was assessed using Agar-Well Diffusion method as described in European Pharmacopeia with slight modification **(**Thankamani *et al*., 2011)**.** MHA plates were prepared and wells of 6 mm diameter were cut using a sterile borer. 100 µL of each of the 2 hour culture of test bacteria was loaded onto the agar plate. The inoculum was swabbed uniformly over the entire agar surface using a sterile swab and allowed to dry for 5 minutes. 100 µL of various extracts were added into the wells. Incubation of plates was done at 37˚C for 24 hours. The diameter of the inhibition zones representing the antibacterial effect of the extracts was measured in millimeters at the end of the incubation period.

Antifungal Activity by Tube Method

The extracts were added directly to the media to obtain 1ml in each tube. The content were mixed well and kept in slanting position to solidify. After sterility checking, the test fungi were inoculated into respective test tubes (Pandey, 1994). The tubes inoculated with *Candida albicans* were incubated at 37˚C for 24-72 hours. Other tubes were incubated at room temperature and results were recorded over 7-10 days.

III. RESULTS AND DISCUSSION

Phytochemical screening showed the presence of pharmacologically significant secondary metabolites in various extracts (Table 1). Methanol extract of seeds confirmed the maximum number of tested phytoconstituents. Steroids, terpenoids, fixed oils and fat were present in Hexane, Butanol, Ethyl Acetate and Methanol extracts, with greater display in Hexane extract. Butanol, Ethyl Acetate and Methanol extracts had alkaloid content and the amount was greater in Methanol. Methanol extract also showed strong presence of

phenolic and flavonoid compounds and weak indication of carbohydrates and proteins. Aqueous extract exhibited the presence of carbohydrates and proteins.

The results of the antimicrobial activity against tested bacteria and fungi clearly exhibited that the strains were susceptible to various extracts of *L.sativum* seeds at 100mg/ml concentration. This reveals the broad-spectrum antimicrobial potential of the seed against Gram positive and negative bacteria and to clinical and opportunistic fungi. With respect to the standard drug Streptomycin, ethyl acetate extract was the most effective extract followed by methanol and butanol extracts.The highest zone of inhibition was exhibited by G+ve strains of *Micrococcus luteus* (20mm) and *Staphylococcus aureus* (15mm). *Klebsiella pneumoniae* and *Proteus vulgaris* were resistant to the tested concentrations of extracts. The tested concentration of aqueous extract was not effective to create any inhibition on tested bacteria.

Antifungal activity of the extracts was assessed by the absence of fungal growth on the medium slant. Preliminary antifungal activity of the seed extracts revealed that methanol extract was the most potent extract followed by butanol and ethyl acetate extracts. Out of the twelve fungal strains methanol extract completely inhibited the growth of ten fungi and two fungi (*Curvularia* sp. and *A.niger*) showed very weak / slow growth towards the end of the incubation period. Hexane extract exhibited restricted growth in *C.albicans*, *Curvularia* and *Fusarium* species, but did not show any inhibitory action towards other strains. In aqueous extract *Penicillium* sp., showed slow and weak growth towards the end of incubation period but other fungal strains were not inhibited. The inhibitory action of different seed extracts on tested microbial strains is given in the Table 2,3 and Figure 1,2.

The secondary metabolites produced by the plants for its survival and protection against various stress conditions often have immense pharmaceutical properties (Adema *et al*., 2020). Reports confirmed the presence of bioactive phytocompounds such as triterpenes, alkaloids, cardiotonic glycosides, flavonoids, glucosinolates, tannins and sterols in *L.sativum* seeds (Ghante *et al*., 2011; Brotonegoro and Wiharti, 2001). Glucosinolates and its derivatives are the major secondary compounds of *L.sativum* and are reported to have antioxidant and chemoprotective effects (Behrouzian *et al*., 2014 ; Mahassni and Al-Reemi 2013). Benzyl isothiocyanate in the seeds are stated to possess antibacterial activity against severe infections and food borne diseases (Prakash *et al*., 2010). The seeds also contain rare imidazole alkaloids, lepidine and semilepidine that increase the antimicrobial efficacy of the seed (Shukla *et al*., 2012).The results of the analysis go in accordance with the reports of Rahul *et al*., 2012 ; Hussain *et al*., 2011 ; Akrayl and Tawfeeq, 2012. Most of the antimicrobial phytocompounds referred were extracted in medium polar solvents and this could be one of the reasons for the enhanced antimicrobial property in ethyl acetate and methanol extracts. Resistance of certain microbes to the tested concentration does not mean that the seed extract do not have any effect. It only describes that the bioactive constituents is insufficient in quantity to display a positive effect or it may be due to the antagonistic effect of certain phytocompounds on bioactive constituents lowering the potency of the extract (Parekh and Chanda, 2008).Toxicological studies demanded the seeds to be safe, non-toxic and their consumption in diet can reduce the risk of cardiovascular diseases, cancer and other infectious diseases (George *et al*., 2015).Even though antimicrobial activities of *L.sativum* seeds have been done by various researchers such an extended list of strains might not be found. Thus the present work proved that the *L.sativum* seeds available in the region possess equivalent efficacy for antimicrobial activity and in phytoconstituent gradation as in other reports published. The work is of high relevance in the field of pharmacology and with further experimentations *L.sativum* seed preparations or the purified fractions can be utilized against infectious diseases and multidrug resistance of microbes.

Table 1: Qualitative analysis of phytochemicals present in *L.sativum* seed extracts

Chemical tests	Compounds	Hexane	Butanol	Ethyl Acetate	Methanol	Aqueous
Liebermann Burchard	Steroids, Terpenoids	+	+	+	+	-
Shinoda	Flavonoids	-	-	±	+	-
Mayer's test	Alkaloids	-	+	+	+	-
Molisch's test	Carbohydrates	-	-	±	±	+
Biuret test	Proteins	-	-	-	±	+
5% $FeCl_3$	Phenolic compounds	-	±	+	+	-
Foam test	Saponins	-	±	±	±	-
Spot test	Fixed oils and fat	+	+	+	+	-

Table 2: Antibacterial activity of *L.sativum* seed extracts by Agar Well Method

Bacterial Strains	Zone of Inhibition (mm in diameter)						
	Hexane	n-Butanol	Ethyl Acetate	Methanol	Aqueous	DMSO	Streptomycin
E.coli	R	R	8	8	R	R	16
Klebsiella pneumoniae	R	R	R	R	R	R	14
Micrococcus luteus	R	17	20	17	R	R	26
Proteus vulgaris	R	R	R	R	R	R	19
Pseudomonas aeruginosa	R	8	12	8	7	R	15
Salmonella paratyphi	10	9	13	9	R	R	15
Salmonella typhi	11	9	13	11	R	R	16
Shigella sp.	R	10	12	R	R	R	15
Staphylococcus aureus	R	14	15	8	R	R	16

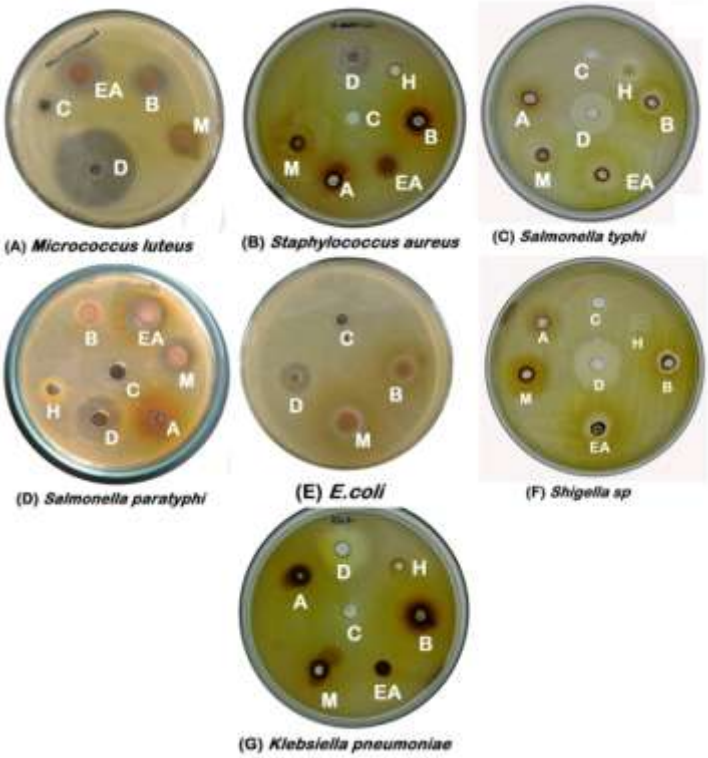
R: resistant to the extract

Table 3 : Antifungal activity of *L.sativum* seed extracts by Tube Method

Strain No	Fungal Strains	Hexane	n-Butanol	Ethyl Acetate	Methanol	Aqueous
1F	*Penicillium marneffei*	+	-	±	-	+
2F	*Cryptococcus* sp.	+	±	-	-	+
3F	*Candida albicans*	±	-	-	-	+
4F	*Curvularia* sp.	±	+	+	±	+
5F	*Penicillium* sp.	+	±	±	-	±
6F	*Epidermophyton*	+	-	±	-	+
7F	*Microsporum* sp.	+	-	±	-	+
8F	*Fusarium* sp.	±	-	±	-	+
9F	*Aspergillus flavus*	+	±	±	-	+
10F	*Aspergillus niger*	+	±	±	±	+
11F	*Rhizopus* sp.	+	-	±	-	+
12F	*Aspergillus fumigatus*	+	-	±	-	+

+ : fungal growth on slant, ± : slow and weak growth of fungus on slant, - : no fungal growth on slant

Figure 1 : Antibacterial activity of *L.sativum* seed extracts



C:DMSO (solvent control), H: Hexane extract, B: n-Butanol extract, EA: Ethyl acetate extract, M: Methanol extract, A: Aqueous extract, D: Drug (Streptomycin)

Figure 2 : Antifungal activity of *L.sativum* seed methanol extract

1F 2F 3F 5F 6F 7F 8F 9F 11F 12F

1F:*Penicillium marneffei*, 2F: *Cryptococcus* sp., 3F: *Candida albicans*, 5F: *Penicillium* sp., 6F:*Epidermophyton*, 7F:*Microsporum* sp., 8F:*Fusarium* sp., 9F:*Aspergillus flavus*, 11F*Rhizopus* sp., 12F:*Aspergillus fumigatus*

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