PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITIES OF CRUDE EXTRACT OF LEPIDIUM SATIVUM SEEDS GROWN IN ETHIOPIA

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ABSTRACT: Lepidium sativum Linn. locally known as ‘fetto’ is a fast-growing edible herb which belongs to the family Brassicaceae is traditionally used for the treatment of various human ailments including cold, headache, colic, abdominal pain, dysentery, swellings and aphrodisiac in Ethiopia. Phytochemical screening and antimicrobial activities were conducted on seeds of Lepidium sativum grown in Ethiopia. Qualitative phytochemical screening test of chloroform/methanol crude extract showed the presence of phytochemicals: flavonoid, cholesterol, terpenoids, steroids, carbohydrates, glycosides, tannins, alkaloids, phenols, phytosterols, proteins, and saponins. Antimicrobial activities of crude extract were tested against four bacteria: two Gram-negative bacteria (Escherichia coli and Salmonella typhi) and two Gram-positive bacteria (Staphylococcus aureus and Bacillus subtilis) and three fungi (Aspergillus niger, Fusarium oxysporum, and Fusarium solani). The crude extracts of the seeds of the plant were active against the tested bacteria and fungi. The antimicrobial activities of plant seeds crude extract were compared with that of chloramphenicol against bacteria and bavistin against fungi as reference antibiotics. The ethnopharmacological knowledge of Lepidium sativum was documented. It was concluded that Lepidium sativum seeds crude extract used for the treatment of various diseases possess antibacterial and antifungal and this also justify its use in the traditional medicine.

INTRODUCTION: Medicinal plants are now more focused than ever because they have the capability of producing many benefits to society indeed to mankind, especially in the line of medicine and pharmacological. The medicinal power of these plants lies in phytochemical constituents that cause definite pharmacological actions on the human body. The search for new pharmacologically active agents obtained by screening natural resources such as plant extracts has led to the discovery of many clinically useful drugs for the treatment of human diseases. Based on ethnobotanical information and results obtained in the literature, L. sativum (family-Brassicaceae) was selected for this study. Several plants of this family are used as antidiabetic, antibacterial, antifungal, anticancer, antirheumatic and show potent insecticidal effects. L. sativum (family-Brassicaceae) Fetto (Amharic), Garden cress, (English) is an erect annual herb of up to 60 cm in height. Garden cress is indigenous to western Asia. It is used as an ornamental crop and its seedlings as salad source.
The seeds are rich in minerals and vitamins; especially vitamins C, A, B, and E.

Antioxidant and antimicrobial substances like saponins, glycosides, flavonoids, and alkaloids are found to be distributed in this plant, yet these compounds were not well established.

Several recent studies pointed out the traditional uses of *L. sativum* seeds extract in controlling many clinical problems such as anti-ulcerative, anti-inflammatory, diuretic, poultice, galactagogue, aperient, alterative, tonic, demulcent, carminative, emmenagogue, and stimulant. The leaves of this plant also used as anti-scrobutic, diuretic, renal diseases, hypertension, and stimulant.

Antibiotics or antimicrobial substances like saponins, glycosides, flavonoids, and alkaloids are found to be distributed in this plant, yet these compounds were not well established.

The phytoconstituents such as phenols, anthraquinones, alkaloids, glycosides, flavonoids, and saponins were previously reported to have bioactive principles. From these phytoconstituents, saponins have been reported to exhibit hemolytic and foaming activity, antifungal, anti-inflammatory, and molluscicidal.

Chemical constituents seeds of the plant mainly contains; alkaloids such as lepidine, glucotropaeolin, N, N’-dibenzyl urea, N, N’-dibenzyl thiourea, sinapic acid and its choline ester (sinapin); The bioactive flavonoids such as 5-4-dihydroxy-7, 8, 3, 5 tetramethoxyflavone, and 5-3-dihydroxy-6, 7, 4’ trimethoxyflavone have been isolated.

Compounds isolated from this plant were reported to have free radical-scavenging and antioxidant properties.

The objective of the present study on *L. sativum* seeds was to estimate the possible antimicrobial activities of seed extract (chloroform/methanol) at dose of (10 and 20 µL) against four pathogenic bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Bacillus subtilis*) and three fungi (*Aspergillus niger*, *Fusarium oxysporum* and *Fusarium solani*) and phytochemical screening of both primary and secondary metabolite present in the crude extract of ‘fetto’ (*L. sativum*) grown in Ethiopia.
Preliminary Phytochemical Screening of Crude Solvent Extracts: The crude extract of the plant was used for screening of phytochemicals for the presence or absence of primary and secondary metabolites such as carbohydrates, proteins, and phytosterols, alkaloids, cholesterol, flavonoids, saponins, terpenoids, glycosides, steroids, tannins, and phenols, respectively, according to the standard procedure 13-17.

Antimicrobial Activities of Crude Extract of the Seeds of L. sativium: Chloroform/methanol crude extract seeds of L. sativium was evaluated in vitro for antimicrobial assay by using the paper disc diffusion method against Gram-positive bacteria (Staphylococcus aureus and Bacillus subtilis) and Gram-negative bacteria (Escherichia coli and Salmonella typhi) and three fungi (Aspergillus niger, Fusarium oxysporum and Fusarium solani 18. The bacterial cultures were inoculated into the Mueller Hinton Agar (MHA) and incubated at 37 °C. Fungal cultures were inoculated into Potato Dextrose Agar (PDA) and incubated at 27 °C. All the microbial cultures were obtained from Plant Pathology laboratory of the School of Plant Science, Haramaya University. Chloramphenicol was used as a standard drug against bacteria whereas bavistin was used against fungi. Dimethyl sulfoxide (DMSO) was also used as a negative control.

Preparation of Inoculums: The bacterial test strains were transferred from the stock cultures and streaked on MHA plates and incubated for 24 h at the 30 °C oven. Well, separated bacterial colonies were then used as inoculums. Then spores of the test fungi were harvested by washing the surface of the colony using 10 mL sterile distilled water. The mycelial plugs of fungi from stock cultures were transferred to PDA plates and incubated for 7 days at the 27 °C oven. The MHA and PDA medias were autoclaved at 121 °C and 1.03 bars for 15 min to sterilize and cooled to about 45 °C in a water bath. The microorganisms were then transferred to their media using a sterile loop and mixed by gentle swirling the flasks and then poured to sterile Petri plates, allowed to solidify and used for the bioassay test.

Testing for Antimicrobial Activities: Filter paper discs of 6 mm diameter placed in a beaker were sterilized in an oven at 180 °C for 1 h. Then 10 and 20 μL of the solutions of the crude extract was pipetted to the discs in three replications. The paper discs impregnated with the sample were then transferred with sterile forceps to medias seeded with a spore suspension of test fungi and bacterial strains as described above. The crude extract was evaluated by measuring the zone of inhibition against the test bacteria and fungi after an incubation period of 24 h and compared to that of commercial standard drugs 19.

RESULTS AND DISCUSSION:
Percentage Yield of the Crude Extract: The air-dried powdered seeds of L. sativium (150 g) was crushed and defatted with n-hexane by soxhlet extractor for 6 h. Then, the defatted air-dried powdered seed of the plant was extracted with chloroform/methanol (1:1) by soxhlet extractor for 7 h to yield yellowish gray crude extract (17.45% w/w).

Phytochemical Screening: Phytochemical research is closely related to the needs of finding new and effective pharmaceuticals. As an initial step of the phytochemical screening research allows to determining qualitatively the main groups of chemical constituents present in the plant. Phytochemical screening was done using color forming and precipitating chemical reagents on the dried seed of L. sativium to generate preliminary data on the constituents of the plant extract.

The phytochemical screening test results of chloroform/methanol crude extract of L. sativium seeds are summarized in Table 1. The results of this study indicated the presences of phytoconstituents like flavonoids, saponins, alkaloids, tannins, carbohyrdates, steroids, phenols, terpenes, cholesterol, proteins, and glycosides in L. sativium seeds.

According to the previous study, a qualitative phytochemical screening test of ethanol extract of L. sativium seed from India showed the presence of a different class of compounds such as cardiac glycosides, anthraquinone glycosides, flavonoids, alkaloids, tannins, proteins, steroids and saponins in the plant 20. The presences of phytoconstituents like flavonoids, saponins, tannin, and phenols in the crude extract are likely to be responsible for the antimicrobial activities.
TABLE 1: PHYTOCHEMICAL SCREENINGS OF CHLOROFORM/METHANOL CRUDE EXTRACT OF L. SATIVUM SEEDS

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Chemical constituents</th>
<th>Chloroform/methanol crude extract</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Phytoesters</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Proteins</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>Cholesterol</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>Flavonoid</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = the presence of phytochemical constituents

Antimicrobial Activities of L. sativum Seed Crude Extract: The antimicrobial activities of the crude extract of L. sativum seeds were tested by the disc diffusion method and are shown in Table 2. The inhibition of each microorganism by the crude extract was measured as the average of two cross diameters after 24 h of inoculation of the microorganism. The crude extract of L. sativum showed moderate antifungal activities against A. niger, F. solani and F. oxysporum and antibacterial activities against S. aureus, B. bacillus, S. typi and E. coli at 10 and 20 μL concentrations Table 2.

The antibacterial and antifungal inhibition effect of the crude extract was increasing with increasing concentrations. Table 2 also reveals that the standard commercial drug (chloramphenicol and bavistin) showed the greatest inhibition effect against both tested bacteria and fungi in both doses (10 μL and 20 μL) compared with the crude extract. DMSO was used as negative control and did not show any antimicrobial activities against the tested microorganism.

The crude extract has moderate inhibition effect in comparison with the standard drug against the tested bacteria and fungi due to naturally occurring combinations of phytochemical constituents might have synergistic effects. Presences of constituents like flavonoids and tannins in the extract are likely to be responsible for the antimicrobial activity. So the antifungal and antibacterial activity of crude extract might be due to the presence of some active secondary metabolite in the plant seeds.

The crude extract is known to be active against a wide variety of microorganisms, including Gram-negative and Gram-positive bacteria. Therefore, L. sativium seed extract is valuable not only for increasing the shelf life of foodstuffs but also it could be a future target for replacing synthetic antibacterial agents. As revealed from the results presented in Table 2, the antibacterial activities of the tested seed extract of the plant were more pronounced on the Gram-positive bacteria (S. aureus and B. subtilis) than the Gram-negative bacteria (E. coli and S. typhi). This may be because Gram-negative bacteria have an outer phospholipidic membrane carrying the structural lipopolysaccharide components, which makes their cell wall impermeable to antibacterial chemical substances.

The Gram-positive bacteria, on the other hand, are more susceptible to having an outer peptidoglycan layer which is not an effective permeability barrier. Therefore, the cell walls of Gram-negative organisms are more complex than the Gram-positive ones and act as a diffusional barrier. Thus, making them less susceptible to the antibacterial agents when compared with Gram-positive bacteria.

Comparisons of the current finding with literature were showed a similar result. In vitro disc diffusion method depicted that the methanol and water extracts of seeds of L. sativum obtained from Sudan have potent antibacterial activities against S. aureus, E. coli, Klebsiella pneumonia, Proteus vulgaris and Pseudomonas aeruginosa which was assessed at the concentrations of 2.5, 5 and 10%.

Furthermore, the antifungal activities of the methanol and its fractions of chloroform, ethyl acetate and water extracts of the roots, stems and leaves of L. sativum were investigated against the fungal species such as Aspergilla fumigates, F. solani, A. niger, and Aspergilla flavus.

The spectrum presented in Fig. 1 and Fig. 2 revealed that the crude extract of seeds of L. sativum has a good inhibition zoon against the tested bacteria (S. aureus, B. subtilis, and E. coli) and fungi (F. solani, A. niger and F. oxysporum), respectively.
**FIG. 1: INHIBITION ZONE OF CHLOROFORM/METHANOL CRUDE EXTRACT SEEDS OF L. SATIVUM AGAINST; (A = S. AUREUS), (B = B. SUBTILLIS) AND (C = E. COLI)**

**FIG. 2: INHIBITION ZONE OF CHLOROFORM/METHANOL CRUDE EXTRACT SEEDS OF L. SATIVUM AGAINST; (D = F. SOLANI), (E = A. NIGER) AND (F = F. OXYSPORUM)**

**TABLE 2: ANTIMICROBIAL ACTIVITIES OF L. SATIVUM SEEDS CRUDE EXTRACT**

<table>
<thead>
<tr>
<th>Cpd</th>
<th>Average inhibition (I) (mm) of microorganisms</th>
<th>Fungi</th>
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<tbody>
<tr>
<td></td>
<td>E. coli</td>
<td>S. typhi</td>
</tr>
<tr>
<td></td>
<td>µL</td>
<td>µL</td>
</tr>
<tr>
<td>CE</td>
<td>10</td>
<td>12.13</td>
</tr>
<tr>
<td></td>
<td>± 0.52</td>
<td>± 0.60</td>
</tr>
<tr>
<td></td>
<td>± 0.80</td>
<td>± 0.75</td>
</tr>
<tr>
<td>Bav</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

CAL = Chloramphenicol, DMSO = dimethylsulfoxide, Cpd = compounds, CE = crude extract, Bav = bavistin, - = No inhibition was observed, Gram (-) = Gram negative, Gram (+) = Gram positive, and the inhibition zone were reported in mean (n=3) ± standard deviation.

**CONCLUSION:** From the above study, it is concluded that the presence of phytochemical constituents revealed in the chloroform/methanol crude extract of seeds of *L. sativum* could contribute to their antimicrobial activities. The crude extract was active against the tested bacteria (*E. coli, S. typhi, B. subtilis*, and *S. aureus*) and fungi (*A. niger, F. oxysporum* and *F. solani*). The antifungal and antibacterial characteristics of this herb can be further investigated so as to be used in the treatment of fungal and bacterial infections, respectively. Thus, *L. sativum* crude extract can be used against the selected pathogenic and some microorganisms and may provide better alternatives or supplements to the conventional antibacterial and antifungal additives in foods.

These local ethnomedical preparations and prescriptions of *L. sativum* sources should be scientifically evaluated and then disseminated properly and the knowledge about the botanical preparation of traditional sources of medicinal application of *L. sativum* can be extended for future investigation into the field of pharmacology, phytochemistry, ethnobotany and other biological actions for modern drug discovery. Extensive research in the area of isolation and characterization of the bioactive components of this plant is required so that better, safer and cost-effective drugs for treating bacteria and fungi infections can be developed.

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**CONFLICT OF INTEREST:** Nil

**REFERENCE:**

