



Received on 16 March 2014; received in revised form, 02 June 2014; accepted, 09 July 2014; published 01 October 2014

## PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITIES OF CRUDE EXTRACT OF *LEPIDIUM SATIVIUM* SEEDS GROWN IN ETHIOPIA

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### Keywords:

*Lepidium sativium*,  
Phytochemical screening, Crude  
extract, Antimicrobial activities

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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<sup>1</sup>.

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<sup>2</sup>. *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.

<p><b>QUICK RESPONSE CODE</b></p> 	<p><b>DOI:</b> 10.13040/IJPSR.0975-8232.5(10).4182-87</p> <hr/> <p>This article can be accessed online on <a href="http://www.ijpsr.com">www.ijpsr.com</a></p> <hr/> <p>DOI link: <a href="http://dx.doi.org/10.13040/IJPSR.0975-8232.5(10).4182-87">http://dx.doi.org/10.13040/IJPSR.0975-8232.5(10).4182-87</a></p>
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The seeds are rich in minerals and vitamins; especially vitamins C, A, B, and E<sup>3</sup>. A paste of seed flour mixed with water is used on chapped lips, against sunburn and other skin disorders. Seeds are chewed to cure throat disease, asthma, and headache. It is an important spice and medicinal plant which is scattered into and grown with other crops, particularly teff in Ethiopia. More uses of *L. sativum* in Ethiopia: for colic, abdominal pain, dysentery, swellings, and aphrodisiac<sup>4</sup>. The seeds are used to treat a variety of skin complaints as well as colds, stomach upsets, and swollen glands. In many parts of Ethiopia, a special dish called "Feto Fitfit" is prepared from the seeds<sup>5</sup>.

Several recent studies pointed out the traditional uses of *L. sativum* seeds extract in controlling many clinical problems such as anti-asthmatic, anti-scorbutic, aperients, diuretic, poultice, galactagogue, aperient, alterative, tonic, demulcent, carminative, emmenagogue, and stimulant. The leaves of this plant also used as anti-scorbutic, diuretic, renal diseases, hypertension, and stimulant<sup>6,7</sup>.

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<sup>8</sup>. 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<sup>9</sup>.

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<sup>10</sup>.

Compounds isolated from this plant were reported to have free radical-scavenging and antioxidant properties<sup>11,12</sup>. The work is done on the biological activity and medicinal application of these compounds are still insufficient in many of

previous reports. To the best of our knowledge, Phytochemical screening seeds extract of *L. sativum* and its antimicrobial activities on a large variety of microbial strains to have a clear picture of the spectrum of antimicrobial activities of this herb is not worked up to date in Ethiopia.

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 (*Aspargillus 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 was investigated. The antimicrobial activities of plant seeds extract were compared with that of Chloramphenicol and Bavistin as reference antibiotics.

## MATERIALS AND METHODS:

### Collection and Identification of the Plant

**Material:** Dry seeds of *L. sativum* were collected from Eastern Ethiopia in December 2013. The Botanical specimens of the plant were identified by Mr. Abeduruzak Abdulhahi, and the voucher specimen was deposited at the Herbarium of the Department of Plant Science, Haramaya University. After collection, the seeds were washed repetitively and air-dried in the shade to make it easily grindable

**Extraction of Seeds of *L. sativum*:** Air dried seeds of *L. sativum* were ground by blander and packed in polyethylene bags to avoid entrance of air and any other mixing of the surrounding material. A 150 g air dried and a powdered seed of *L. sativum* was extracted with n-hexane for 6 hrs in Soxhlet apparatus. The defatted powdered seeds of the plant were removed from Soxhlet apparatus and air dried at room temperature.

Then after, the marc was further extracted with chloroform/methanol (1:1) for 7 hrs and filtered using Whatman no. 1 filter paper and concentrated by rotary evaporator at 40 °C to yield yellowish gray crude extract (17.45% w/w) and the crude extract was kept in refrigerator at 4 °C until analysis.

**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, cholesterols, flavonoids, saponins, terpenoid, glycosides, steroids, tannins, and phenols, respectively, according to the standard procedure<sup>13-17</sup>.

**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*)<sup>18</sup>. The bacterial cultures were inoculated into the Muller 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 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<sup>19</sup>.

## 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 phytosterols, flavonoids, saponins, alkaloids, tannins, carbohydrates, steroids, phenols, terpenes, cholesterols, 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<sup>20</sup>. 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**

S. no.	Chemical constituents	Chloroform/methanol crude extract
1	Phenols	+
2	Alkaloids	+
3	Phytosterols	+
4	Steroids	+
5	Saponins	+
6	Carbohydrates	+
7	Terpenoids	+
8	Glycosides	+
9	Tannins	+
10	Proteins	+
11	Cholesterol	+
12	Flavonoid	+

+ = 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. typhi* and *E. coli* at 10 and 20  $\mu$ L 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  $\mu$ L and 20  $\mu$ L) 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<sup>21</sup>. 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<sup>20</sup>.

The crude extract is known to be active against a wide variety of microorganisms, including Gram-negative and Gram-positive bacteria. Therefore, *L. sativum* 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<sup>22</sup>.

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%<sup>23</sup>. 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 for the fungal species such as *Aspergilla fumigates*, *F. solani*, *A. niger*, and *Aspergilla flavus*<sup>24</sup>.

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.



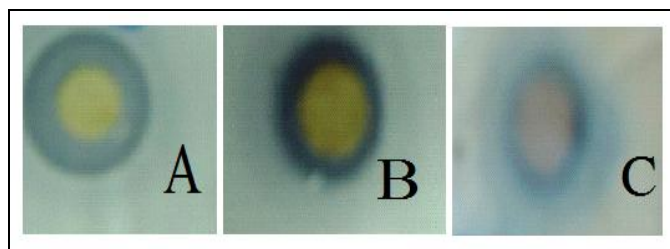


FIG. 1: INHIBITION ZONE OF CHLOROFORM/METHANOL CRUDE EXTRACT SEEDS OF *L. SATIVUM* AGAINST; (A = *S. AUREUS*), (B = *B. SUBTILLIS*) AND (C = *E. COLI*)

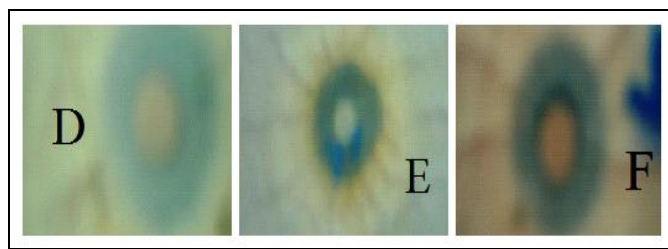


FIG. 2: INHIBITION ZONE OF CHLOROFORM/METHANOL CRUDE EXTRACT SEEDS OF *L. SATIVUM* AGAINST; (D = *F. SOLANI*), (E = *A. NIGER*) AND (F = *F. OXYSPORUM*)

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

Cpds	Average inhibition (I) (mm) of microorganisms													
	Gram (-) Bacteria				Gram (+) Bacteria				Fungi					
	<i>E. coli</i>		<i>S. typhi</i>		<i>S. aureus</i>		<i>B. subtilis</i>		<i>A. niger</i>		<i>F. solani</i>		<i>F. oxysporum</i>	
	10 μL	20 μL	10 μL	20 μL	10 μL	20 μL	10 μL	20 μL	10 μL	20 μL	10 μL	20 μL	10 μL	20 μL
CE	10.50 ± 0.52	12.13 ± 0.60	10.30 ± 0.62	11.35 ± 0.25	15.90 ± 0.56	17.43 ± 0.65	13.45 ± 0.55	16.17 ± 0.27	13.73 ± 0.35	15.00 ± 0.45	12.50 ± 0.4	14.83 ± 0.35	12.23 ± 0.16	13.38 ± 0.15
CAL	19.40 ± 0.80	21.30 ± 0.75	20.18 ± 0.34	22.73 ± 0.24	24.23 ± 0.70	26.40 ± 0.72	25.98 ± 0.63	27.53 ± 0.47	-	-	-	-	-	-
Bav	-	-	-	-	-	-	-	-	22.47 ± 0.65	24.37 ± 0.55	23.06 ± 0.5	25.03 ± 0.35	20.47 ± 0.40	24.39 ± 0.32
DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-	-

CAL = Chloramphenicol, DMSO = dimethylsulfoxide, Cpds = compounds, CE = crude extract, Bav = bavistin, - = No inhibition was observed, Gram (-) = Gram negative, Gram (+) = Gram positive, and the inhibition zoon 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. sativium* 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. sativium* 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. sativium* sources should be scientifically evaluated and then disseminated properly and the knowledge about the botanical preparation of traditional sources of medicinal application of *L. sativium* 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.

**ACKNOWLEDGEMENT:** The authors would like to thanks, Mr. Kesatebrhan Haile (Assistant Professors Organic Chemistry) and family members for their constructive advice, fruitful discussions, friendly treatment, and sustained moral support which invariably helped us a lot during this research work.

**CONFLICT OF INTEREST:** Nil

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**How to cite this article:**

Berehe SG and Boru AD: Phytochemical screening and antimicrobial activities of crude extract of *Lepidium sativum* seeds grown in Ethiopia. Int J Pharm Sci & Res 2014; 5(10): 4182-87. doi: 10.13040/IJPSR.0975-8232.5(10).4182-87.

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