



## Phytochemical Screening, Antimicrobial and Antioxidant Activity of *Lepidium sativum* Seeds Extract

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### Authors' contributions

This work was carried out in collaboration among all authors. Author AHN administrated the study. Author ABO conducted the experiments and wrote the first draft of manuscript. Author MMA helped supervise the study and revised the manuscript. Author OAOI managed the literature and revised the manuscripts. Authors IYE and MAA read and revised the manuscript. All authors read and approved the final manuscript.

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### ABSTRACT

Plants play a significant role in drug discovery and development of pharmaceuticals process. In many countries, medicinal plants consider the main source of primary health care.

**Aims:** This study aimed to investigate the phytochemical screening, antimicrobial and antioxidant activities of *Lepidium sativum* seeds extracts and fractions.

**Methodology:** Seeds of *L. sativum* were collected and extracted by maceration in methanol and the extract was fractionated using hexane, chloroform, ethyl acetate and an aqueous solvent. Phytochemical screening of crude extract was performed using standard methods. The crude extracts and the fractions were tested against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus niger* and *Candida albicans*. The radical scavenging (DPPH) was determined according to the standard methods.

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**Results:** The phytochemical screening result of the crude extract showed the presence of flavonoids, alkaloids, tannins, saponins, phenols, glycosides and terpenoids. Antimicrobial activities results were showed variation in the inhibition zones from 8-20 mm; the best activity was in *A. niger* 20 mm at concentration 100 mg/mL of crude extract, however in *S. Aureus* 16 mm at the same concentration of ethyl acetate fraction. The results of free radical scavenging activity results obtained were higher at ethyl acetate fraction (76±0.02%), chloroform fraction (74±0.01%) and aqueous fraction (70±0.09%), while moderate at the crude extracts (46±0.07%) and low at hexane fraction (21±0.04%).

**Conclusion:** It could be concluded that the crude extracts and fractions of *L. sativum* had high potential as an antibacterial and antioxidant agent, which can be used for medicinal purposes.

**Keywords:** *Lepidium sativum*; seeds extract; phytochemical screening; antimicrobial activity; antioxidant activity.

## 1. INTRODUCTION

The world is lush with naturally grown medicinal plants that abound in health-enhancing phytochemicals and nutrients. Medicinal plants are considered the richest bio-resource of drugs for traditional systems of medicine and modern medicines as well as for nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities of synthetic drugs [1,2,3]. Plants play a significant role in drug discovery and development of pharmaceuticals process. In many countries, medicinal plants consider the main source of primary health care. Nearly 80% of world populations depend on traditional medicine to treat various illnesses. The bioactive secondary metabolites isolated from plants constitute drug discovery. Recently, there is a significant increase in the discovery of molecular targets that may be applied to the discovery of novel materials for the diagnosis and treatment of human diseases [4]. *Lepidium sativum* seeds (family: *Cruciferae* & *Brassicaceae*) in Sudan is known as 'Hab ALrashad'. It is widely available in the market and has very low cost [5]. 'Hab ALrashad' has names i.e. garden cress, garden pepper cress, peppergrass, pepperwort, elrashad and town cress due to its Townes or enclosure [6] which is natively distributed throughout tropical regions of India, North America and parts of Europe [7], Egypt and West Asia, although it is now cultivated in the entire world and its seeds are used fresh or dried, as a seasoning with a peppery flavour. Boiled seeds are consumed in drinks, either ground in honey or as an infusion in hot milk [8]. *L. sativum* can grow in any type of climate and soil condition and can tolerate slight soil acidity [6]. Also, *L. sativum* is an annual herb grows up to 50 cm height and its seeds contain volatile oils [9]. Furthermore, *L. sativum* seeds are small, oval-shaped, pointed and triangular at

one end, smooth, reddish-brown in colour, a furrow present on both surfaces extending up to two thirds downward, a slight wing-like extension present on both the edges of seed. On soaking in water, seed coat swells and gets covered with transparent, colourless, mucilage with a mucilaginous taste. Also, the seeds are a rich source of proteins, dietary fibre, iron and omega-3 fatty acids [10]. Moreover, *L. sativum* seeds claimed to possess varied medicinal properties like galactagogue, aperient, diuretic, alterative, tonic, demulcent, aphrodisiac, carminative and emmenagogue. Mucilage of the seeds allays the irritation of the mucous coat of intestines [7]. *L. sativum* is being used as a folk medicine to treat allergies and wounds in and around Chickballapur, Karnataka, India. This study aimed to investigate the important characteristics of crude extracts and fractions of *L. sativum* seeds to present the phytochemical screening, antimicrobial and antioxidant activities that use in traditional medicines and pharmaceuticals process.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

The seeds of *L. sativum* used in this study were collected from the local market in Omdurman, Sudan in May 2019. The authentication or identification of the plant seeds was done by a botanist from the National Center of Research (NCR), Khartoum, Sudan.

#### 2.1.1 Preparation of the crude extract

About 100 g of dried ground *L. sativum* seeds were accurately weighed and extracted by the soxhlet (for defatting), then the residue was macerated using methanol for five days, filtered and the solvent was evaporated; the resulted

crude extract was kept in a glass bottle. A 10 g of the crude extract was dissolved in ethanol 80%, and then fractionated using different solvents with different polarity namely; hexane (F1), chloroform (F2), ethyl acetate (F3) and aqueous (F4). The crude extract and fractionations were prepared according to the method described by Nour et al. [11] and Alebiosu and Yusuf [12].

### 2.1.2 Preparations of crude extract concentrations

A stock solution of crude extract (200 mg/mL) was prepared by dissolving one g of crude extract in 5 mL DMSO (10%); then a series of concentrations (25, 50 and 100 mg/mL) were prepared by serial dilution. Also, 100 mg/mL of each fraction was prepared.

## 2.2 Phytochemical Screening of *L. sativum*

Qualitative phytochemical screening of crude extract was performed using the methods describes by Harborne [13] and Ishag et al. [14]; for screening of alkaloids with dragendoff's reagent; tannin and phenolic compounds with (0.1% FeCl<sub>3</sub>); saponins and glycosides with (foaming test); flavonoids using 10% FeCl<sub>3</sub>, potassium hydroxide, terpenoids (Salkowski's test).

## 2.3 Antimicrobial Activity Test

The microorganisms used in this study were included two strains of gram-positive bacteria (*S. aureus* and *B. subtilis*) and gram-negative bacteria (*E. coli*, and *P. aeruginosa*); in addition to two fungi (*A. niger* and *C. albicans*). The strains of microorganisms were delivered from microbiology laboratory of Faculty of Pure and Applied Science, International University of Africa, Khartoum-Sudan.

### 2.3.1 Antibacterial activity test

The cup-plate agar diffusion method was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. One mL of bacterial stock suspension 10<sup>8</sup> - 10<sup>9</sup> CFU/mL was mixed with 100 mL of molten sterile Mueller Hinton Agar which was maintained at 40°C. About 20 ml of the inoculated Mueller Hinton Agar were distributed into sterile Petri-

dishes. The agar was left to set were cut by a sterile cork borer (No.4). The cups (8 mm in diameter) were filled with 0.1 mL of each extract (25, 50, 100, and 200 mg/mL) and fractions at the concentration (100 mg/mL) using automatic microliter pipette, and allowed to diffuse at room temperature for 2 h. The plates were then incubated in the upright position at 37°C for 24 h. The plates were observed for the presence of inhibition of bacterial growth that was indicated by a clear zone around the wells. The size of inhibition zones was measured and the antibacterial activity was expressed in terms of the average diameter of the zone of inhibition in millimetres, gentamicin was used as a reference antibiotic [15].

### 2.3.2 Antifungal activity test

Fresh cultures of the fungus (*A. niger* and *C. albicans*) prepared by sub-culturing on Sabouraud dextrose agar (SDA) at 28°C for 24 h were used throughout the study. The inoculum was evenly spread on the surface of 10 cm Petri dishes containing SDA medium and exposed to dry. Then, the paper discs were impregnated with 20 µL of crude extracts. After 5 min, antifungal discs were dispensed onto the surface of the inoculated agar plates, after the plates were incubated at 25°C for 24 h. After the colonies grew, the zones of inhibition around the disks were measured and recorded; nystatin was used as a reference antibiotic [16].

## 2.4 Antioxidant Activity of the Crude Extract and Fractions DPPH Radical Scavenging Assay

The DPPH radical scavenging was determined according to the method described by Shimada et al. [17] with some modification. In 96-wells plate, the test samples were allowed to react with 1, 1-diphenyl-2-picrylhydrazyl stable free radical (DPPH) for half an hour at 37°C. The working concentration of DPPH was (300 µM). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, the decrease in absorbance was measured at 517 nm using multiple reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group and Propyl Gallate. All tests and analysis were run in triplicate.

### 3. RESULTS AND DISCUSSION

The collected seeds of *L. sativum* were extracted with methanol and the crude extract yield obtained was 18.7%; beside the resulted crude extract was fractionated with different solvent and the yield for each fraction was mentioned in Table 1.

**Table 1. The fractions yield of *L. sativum* seeds**

No	Fractions	Yield%
1	F1	9.30
2	F2	16.70
3	F3	1.96
4	F4	70.00

Key: F1: hexane fraction; F2: chloroform fraction; F3: ethyl acetate fraction and F4: aqueous fraction

The crude extract of *L. sativum* was subjected to phytochemicals screening tests and the obtained results shown in Table 2.

**Table 2. Qualitative phytochemical screening of crude extracts of *L. sativum* seeds**

No	Compounds	Results
1	Flavonoids	+
2	Alkaloids	+
3	Tannins	+
4	Saponins	+
5	Phenols	+
6	Glycosides	+
7	Terpenoids	+

Key: (+) presence of compounds; Methanol used for extract; crude extracts dissolved in distilled water

Phytochemical screening results of the crude extract of *L. sativum* seeds shown in Table 2 above; confirmed the presence of flavonoids, alkaloids, tannins, Phenols, glycosides, terpenoids and saponins in the methanolic extract. These results revealed that the crude extract of *L. sativum* has quite a number of chemical constituents, which may be responsible for the different pharmacological actions related to the existing secondary metabolites that promote characteristic antimicrobial activities. These results are in agreement with those confirmed by Solomon and Aman, [18] that performed the phytochemical analysis of chloroform/methanol extract of *L. sativum* seeds; where their results indicated the presence of flavonoids, saponins, alkaloids, tannins, phenols, terpenes and glycosides in the extract.

The results are in the same trend of those obtained by Yohannes et al. [19] that investigated the ethanolic and methanolic extracts of *L. sativum* seeds and found the occurrence of alkaloids, saponins, tannins, flavonoids, terpenoids in the methanolic extracts. Also, the results are agreeing with those reported earlier by Chatoui et al. [20].

Based on the above discussion the phytochemicals present in the crude extracts of *L. sativum* seeds can serve as a valuable source of information and provide appropriate standards to establish a base for identification and elucidation of the different types of bioactive chemicals.

The crude extracts of *L. sativum* seeds were subjected to antimicrobial tests and the obtained results shown in Table 3.

The crude extract of *L. sativum* seeds was screened against two Gram-negative bacteria (*E. coli*, *P. aeruginosa*), two Gram-positive bacteria (*B. subtilis*, *S. aureus*) and two fungi (*A. niger* and *C. albicans*); using the cup plate agar diffusion method. The inhibition zones of tested microorganisms varied in the range from 8 to 20 mm. The extracts showed good inhibition activity against *A. niger* which were 18, 20 and 19 mm in concentration 50, 100 and 200 mg/mL respectively. While the concentration 100 mg/mL showed high inhibition activity against tested microorganisms compared to other concentrations where the results were 14 mm in *E. coli*, 14 mm in *C. albicans*, 13 mm in *S. aureus*, 13 mm in *B. subtilis*; 20 mm in *A. niger*. The results obtained for *E. coli* in different concentration agreed with Shama et al. [9] results for 100 mg/mL and their result for 25 mg/mL was 17 mm; while the concentration 25 mg/mL showed no inhibition in our study. Also, Pragma et al. [21] found the antimicrobial activity of *L. sativum* against bacteria using methanol extract 12.5 mm and this result is similar to our result. The *S. aureus* were found 10, 13, 11 and 9 mm in different concentration as mentioned in Table 3 which is higher compared to the result obtained by Yohannes et al. [19]; found 10.2 mm in 250 mg/mL and it lower than Shama et al. [9] that found 14 mm in concentration of 100 mg/mL. Moreover, the obtained results are the same as Iqbal et al. [22] results which were 8 mm. The difference in the results may be due to the preparation of the sample. The inhibition zones of *B. subtilis* were 13, 10 mm in the concentration

**Table 3. Antimicrobial activity of crude extracts of *L. sativum* seeds**

No.	Conc mg/mL	Zone inhibition in mm					
		<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>	<i>C. albicans</i>	<i>A. niger</i>
1	200	13	14	11	-	-	19
2	100	14	13	-	13	14	20
3	50	10	11	-	10	10	18
4	25	-	9	-	-	-	-
DMSO (10%)	-	-	-	-	-	-	-
Gentamicin	50	32	35	23	29	-	-
Nystatin	50	-	-	-	-	17	20

Key: *E. coli*: *Escherichia coli*; *S. aureus*: *Staphylococcus aureus*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *B. subtilis*: *Bacillus subtilis*; *C. albicans*: *Candida albicans*; *A. niger*: *Aspergillus niger*; Solvent used is DMSO (10%)

of 100, 50 mg/mL, respectively which is lower compared to the result obtained by Solomon and Aman, [18] since, they found 13.4, 16.1 mm in 50, 100 mg/mL. The inhibition zones result obtained of *A. niger* were higher compared to the result reported by Solomon and Aman, [18] found 15 mm.

For each test, negative control DMSO 10% showed no inhibition against all bacterial strains and fungi tested. The inhibition zones of tested Gentamicin as antibiotic varied in the range from 23-35 mm. The obtained results of the negative control (DMSO 10%) showed no activity against all strains of Bacteria and fungi compared to *L. sativum* crud results. Also, the activity results of gentamicin varied in the range from 23 to 35 mm. However, the result of nystatin varied also in the range from 17 to 20 mm. Our obtained results indicate that *L. sativum* can be used as antibiotic.

The Fractionate of *L. sativum* seeds were subjected to antimicrobial tests and the obtained results shown in Table 4.

The antimicrobial activity of fractions of *L. sativum* shown in Table 4 confirmed that hexane fraction (F1) inhibited only *S. aureus* (15 mm) and *B. subtilis* (16 mm), but it is very low in other bacteria and fungi strains. The chloroform fraction (F2) displayed high inhibition on *E. coli* 14 mm, while there was low inhibition at *S. aureus* and *P. aeruginosa*. On the other hand, the fungi were not affected. Moreover, the ethyl acetate fraction (F3) inhibited the growth of all tested microorganisms. *B. subtilis* is only microorganisms not affected by ethyl acetate

fraction. Also, *E. coli* showed a low inhibition zone 11 mm. On the other side, the high inhibition zone recorded in all fractions appeared in *S. aureus* 16 mm and then *P. aeruginosa* and *C. albicans* 15 mm. Aqueous fraction (F4) showed no inhibition for all tested microorganisms except *S. aureus* zone which was 13 mm. Hexane fraction revealed a less inhibition effect on the tested microorganisms compared to the chloroform and ethyl acetate fractions used in this study and higher than an aqueous fraction. The ethyl acetate is the most active fraction that had high inhibition effect than the other fractions.

The crude extract and fractions of *L. sativum* were subjected to antioxidant activity test and the obtained results shown in Table 5.

As shown in Table 5; the percentage screening of extract and fractions inhibition of DPPH scavenging activity ranged from 21%  $\pm$  0.04 to 76%  $\pm$  0.02. The results of antioxidant activity of *L. sativum* seeds showed high antioxidant activity against the DPPH free radical in ethyl acetate fraction (76  $\pm$  0.02 RSA%), chloroform fraction (74  $\pm$  0.01 RSA%), and aqueous fraction (70  $\pm$  0.09 RSA%) and showed moderate antioxidant activity in crude extracts (46  $\pm$  0.07 RSA%), and low hexane fraction (21  $\pm$  0.04 RSA%) compare to the control of propyl gallate levels (91  $\pm$  0.01 RSA%) that used as standard drug level. The results of crude extract were high compared with the study reported by Hiba [16] and Chairman et al. [23] who found that methanol extracts of *L. sativum* exhibited low antioxidant activity (57%, 11.63%, respectively).

**Table 4. Antimicrobial activity of fractions at the concentration of 100 mg/mL of *L. sativum***

No.	Zone of inhibition in mm				
	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>	<i>C. albicans</i>
F1	-	15	10	16	-
F2	14	12	12	9	-
F3	11	16	15	-	15
F4	10	13	-	-	10

Solvent: DMSO (10%)

**Table 5. The antioxidant activity results of *L. sativum***

No.	Fraction	%RSA* $\pm$ SD (DPPH)
1	F1	21 $\pm$ 0.04
2	F2	74 $\pm$ 0.01
3	F3	76 $\pm$ 0.02
4	F4	70 $\pm$ 0.09
5	Crude extract	46 $\pm$ 0.07
Standard	Propyl gallate	91 $\pm$ 0.01

Key: RSA\* = Radicals scavenging activity

#### 4. CONCLUSION

The aim of the study is screening phytochemical and in vitro antimicrobial and antioxidant activity of *L. sativum* seeds extract. The phytochemical analysis showed the presence of alkaloids, flavonoids, tannins, saponins, phenols, glycosides and terpenoids of the extracts of the seeds and this indicates that the extracts contain some prominent secondary metabolites. The cognisance of the chemical constituents of the extracts helps in understanding the value of folkloric medications and discloses the wrapped areas of therapeutics. The different crude extracts and fractions exhibited a wide range of antimicrobial and antioxidant activities. Based on the observed results, the methanol extracts and fractions are extractive for antimicrobial, antioxidant activities of the seeds of *L. sativum*. Furthermore, antimicrobial studies with additional organisms might be relevant to give a better picture of the activity of the extracts. Therefore, the results recommended the use of *L. sativum* in traditional medicines, especially for people living in remote areas as an alternative treatment for eye diseases. Also, the results confirmed that crude extracts and fractions of *L. sativum* had high potential in vitro antimicrobial and antioxidant activity; where the fraction of ethyl acetate had the highest antimicrobial and antioxidant activity compared to other fractions.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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