ISSN 2664-4169 (Print) & ISSN 2664-7923 (Online)
South Asian Research Journal of Biology and Applied Biosciences
Abbreviated Key Title: South Asian Res J Bio Appl Biosci

| Volume-7 | Issue-3 | May-Jun -2025 | **DOI: https://doi.org/10.36346/sarjbab.2025.v07i03.001**

Original Research Article

Analysis of the Antibacterial and Antifungal Activity of Methanolic Seed Extract of Lepidium Sativum

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Article History
Received: 02.04.2025
Accepted: 07.05.2025
Published: 09.05.2025

Abstract: At a concentration of 200 mg/ml the methanol seed extract of L. sativum showed effects against tested bacteria. When tested against the diver's Staph. aureus bacterial strain, L. sativum methanol seed extract produced a 26 mm inhibition zone using a 1 ml solution but no inhibition zone formed when testing E. coli with E. faecelis because of the 0.12 ml extract solution. The research demonstrated that various seed extracts obtained from solvents produced distinct reactions toward gram-positive and gram-negative bacterial strains. A variation in antifungal potential against Aspergillus niger was observed along with extract quantity differences in both antioxidant strength and extract form during testing. A concentration of 1 mg provided the methanol extract of L. sativum seeds with its strongest anti-oxidation effect which produced 91.419 percent effectiveness while the lowest effect was noted at 0.12 resulting in 81.308. The content of active substance in solution steadily lowered while extract concentration decreased. The seed extract exhibited function as an antioxidant. The research evaluates the anti-oxidative effects and antibacterial properties exhibited by elements of the Gc seed.

Keywords: Lepidium Sativum, Antimicrobial, Antifungal, Seeds Extract.

INTRODUCTION

As a member of Cruciferae and reaching heights of 15–50 cm annually L. sativum grows as a yearly herb. Facing upwards the plant produces edible leaves that are upright and split and bald without hairs extending from 5 to 10 centimeters in length and measuring 2.5 to 3.5 centimeters across. Following the base of the plant the leaves are pinnatisect shaped and stalked before turning subsessile while the leaves at the stem base are linear with a sessile state and entire edge structure. The plant is infrequently pilose. A raceme is a complex flowering plant with many branches and bracteates (2021). The tiny, around 3 mm in diameter, white or pinkish flowers are a wonderful sight. Fruits range in size from 5 to 6.4 mm and are attached to a suberect to ascending pedicel; seeds are 3 lobed ovate-oblong, 3 mm long, and 1 mm broad; L. sativum grows quickly; it's suitable for all seasons, but winter is when it's at its best. Domesticated in Europe and southwestern Asia, the plant has since been disseminated as a salad green in many parts of the world, including the Americas, Europe, India, and Asia (2020). Since 1979 Sharma *et al*. (2018) has documented the traditional medical application of L. sativum seeds to treat asthma and other conditions. The medical literature reports that Bleeding piles, bronchitis and the cough flora of N.A. (2020) exist. In 1979, Angel and Chadha scurvy-related illnesses. According to Chopra *et al*. (2006) patients with liver disease develop various symptoms such as inflammation and rheumatic pain alongside spleen and liver chronic enlargement and flatulence and dysentery and indigestion and tenesmus and secondary syphilis and abortion and anaemia and weakness (Skaran *et al*., 2014). In 2013, Agarwal and Sharma published... Although all portions have commercial worth, the leaves and seeds are mostly harvested for their savoury salad flavour and the fact that they are ploughed everywhere. In 2018, Mohamad and colleagues published. The plant can also be used as a compress for wounds and contusions, according to Aqahtani *et al*. (2019). Antimicrobial properties of L. sativum are also utilised. In a 2019 study, Endrise *et al*. found that Cardioprotective, antioxidant, anti-inflammatory, Endrise *et al*. (2019), Tounsi *et al*. (2019), In a 2019 study, hypolipidemic Mohammed *et al*. (2019) found that diuretics Grover and Jain (2018) stomachic In 2016, Region *et al*. discovered a gastrointestinal stimulant, laxative, and gastro-protective... The antimicrobial and antioxidant properties

of Ullah L. sativum Gc. *et al*. (2019) and numerous others due to its diverse pharmacological effects. According to another source, the antioxidant activity of L. sativum is used to boost melanin formation in response to exposure to UV-c rays. As stated by Arabia *et al*. (2010). In plant physiological studies, as a signal organism to determine the toxicity levels of environmental contaminants, and for the experimental evaluation of several disease-causing microorganisms, L. sativum is extensively utilised. The antibacterial characteristics and anti-oxidant activity of the components of Gc seeds are the focus of the current investigation (Saeid *et al*., 2022). Staphylococcus aureus is a hardy bacterium that holds on to fomites for extended periods of time before releasing them into the environment, making it resistant to drainage and heat. The transmission of staphylococcal aliens can be reduced by simply washing hands before and after handling food or people who may be affected. In addition to causing necrotising lobar pneumonia in alcoholics, diabetes, chronic obstructive pulmonary disease, UTIs, and bacteremia, especially in hospitalised patients, Klebsiella are big, non-motile bacilli. Despite being a natural component of animal and human gut flora, Escherichia coli (E. coli) can cause illness in settings outside of the gastrointestinal tract. A dysentery-like illness, characterised by fever and bloody faeces, can be caused by E. coli. It can also induce traveler's diarrhoea and persistent diarrhoea, which can affect young children. A variety of human infections can be caused by enterococci, the most common of which is Enterococcus faecalis. Chronic apical periodontitis and endodontic infections, such as obturator root canals, have led to the eradication of Enterococcus faecalis. Extreme stress does not kill the creature. The endodontic handling failures that have been linked to this creature can be better understood if more is known about it. Published by Richard *et al*. in 2007. The fungus Asparagillus niger is a member of the class Euascomycetes; it lives in both terrestrial and aquatic habitats around the globe and reproduces all year round through spores. The fungus finds application in numerous sectors, including those dealing with food and drugs. In humans, this strain has the potential to cause respiratory discomfort, particularly in people with impaired immune systems. BIO_BARS Aspergillus spp.

MATERIALS AND METHODS

Preparing Plant Extracts

Lepidium sativum seeds were sourced from a nearby market for this investigation (figure 1). To get crude methanol extract, 250g of plant powder (seed) was soaked in 500 ml of solvent (methanol) at room temperature with shaking for one week in a water bath. After filtering, the plant powder was extracted again, this time with an extraction concentration of 40C°. This oven-based process removed organic solvents. Now that the crude extracts had been collected, El-Sayed *et al*. (2012) could finally conduct the bioassays. Making a single concentration (200 mg/ml) required dissolving 1 gramme of crude extract in 1 millilitre of dimethyl sulfoxide (DMSO) and adding 5 millilitres of distilled water.

Methods for Collecting and Identifying Fungi and Bacteria

This study examined the response of Klebsiella pneumonia and Staphylococcus aureus as well as E. coli and Enterococcus faecalis among gram-negative bacteria and Aspergillus niger as the gram-positive fungus. This study obtained its microorganisms from the Al-Amin Research Centre and cutting-edge biotechnology which operates in Najaf city.

Methods for Making a Bacterial Dialysis Solution

Once the bacterial suspension was generated, it was incubated at 37°C for 24 hours to culture the bacteria on nutrient agar. After 3–4 weeks, the colonies were harvested and incubated for another 4–5 hours in a test tube filled with nutrient broth at 37°C. To achieve the desired turbidity, it was compared to a McFarland tube.

Assessing the activity of plant extracts

In order to determine the plant extract's susceptibility, we used the well agar diffusion method. We spread different volumes of bacterial suspension on the media surface, and then we made five equal wells in a Muller Hinton plate with a diameter of 6mm using a crok borer. We added 0.1 ml of extract to each well, and then we incubated the plate overnight at 37 C°. To measure the inhibitory zone's diameter, we used a ruler. As a control, we used DMSO (Dimethysufoxide) as per Agrove (1985).

Evaluation of antifungal efficacy

The analysis of antifungal properties in the plant extract utilized petri plates filled with sterilized potato dextrose agar at the concentrations of 1, 0.5, and 0.12 mg for Aspergillus niger. The scientists measured mycelial radial growth from cultures kept at 27 °C for seven days to gather results for comparison with negative control tests. The percentage of fungal inhibition in treatment required use of the below mathematical formula:

L = [(C – T)/C] x 100

"L" stands for "percent inhibition." The radial expansion of the pathogen in the presence of plant extracts is denoted by T, while C represents the colony radius in the control plate. Authors: Shivapratap and colleagues (2004). In order to determine the antioxidant properties of methanol seed extract, the plant extract was diluted with methanol at four different concentrations (1, 0.5, 0.25, and 0.12) and then tested using the DPPH assay, a commonly used stable radical

compound (Amarowicz *et al.*, 2004). When it is reduced with an antioxidant, it forms the non-radical form DPPH-H, which causes its absorption to drop. In contrast, the DPPH radical, which is purple because of its unpaired electron, has a significant absorbance at 517 nm. (Gursoy *et al*., 2010). A solution concentration of 80 µl/ml was achieved by performing the DPPH radical cation technique using DPPH (8 mg) diluted in (methanol) MeOH (100 ml). Pellegrini and colleagues (1999). An ELISA reader was used to measure the absorbance at 514 nm after incubating a mixture of 100 ml of DPPH reagent and 100 µl of sample in a 96-well microplate at room temperature for 30 minutes. A control group was given 100% methanol.

One way to quantify the DPPH scavenging effect is using the following formula Ishimaru *et al*., (1995):

$$\text{Radical scavenging (\%)} = [(A)\ \text{control} - (A)\ \text{sample} \setminus (A)\ \text{control}] \times 100$$

You can find the IC50 DPPH values by extrapolating from a regression study; they indicate the sample concentrations needed to block 50% of DPPH radicals. Based on this IC50 value, the antioxidant was evaluated. The following concentrations of plant extract were prepared: 91.419, 89.125, 88.105, and 81.308 micrograms/ml; a methanol control was also included.

Evaluation of Antioxidant and Antibacterial Activity (LSD)

The data was analyzed with the SPSS program version 23, specifically with the Least Significant Difference (LSD) test set at $P \leq 0.05$.

RESULTS AND DISCUSSION

Investigating the Antimicrobial Effects

Table 1 shows the effects of a 200 mg/ml concentration of methanol seed extract on various bacteria; the antibacterial activity of different species of L. sativum varied; the results showed that the largest inhibition zone value was 26 mm for Staph. aureus, a strain of diver's bacteria, at 1 ml of L. sativum methanol seed extract, while E. coli and E. faecelis showed no inhibition zone at 0.12 ml. Figures 2, 3, 4, and 5 further supported these findings. A number of studies have shown that gram-negative bacteria are more resistant than gram-positive bacteria, and that there was variance in the irresponsible to different solvent seed extract among gram-positive and gram-negative bacteria. This variation may be due to differences in the bioactive chemicals. Kluytmans and colleagues (2013) concluded. According to Wu *et al*. (2020), essential oils have a wide range of antimicrobial activity due to their complex chemical composition. The reason behind this is that essential oils can penetrate microbial cells, altering their structure and function. The variation in susceptibility between gram-negative and gram-positive bacteria is mainly related to the different structures of their cell walls.

Fungicide Efficacy

Figure 6 shows that at a concentration of 200 mg/ml, the methanol seed extract had an effect on the Aspergillus niger fungus, however this effect varied across the different varieties of L. sativum. The results show that essential oils of medicinal plants, including E. sativa, can inhibit the growth of fungi and also produce aflatoxin (AL-Saadi, 2018). His findings also corroborate those of many other authors who have found that eurcic acid is the primary antimicrobial agent. The capacity of essential oils from medicinal plants to inhibit the growth of Acinetobacter niger was documented by Gemede *et al*. (2019). Noshirvani and Fasihi (2018) tested 75 essential oils from medicinal plants and found that some of them have antifungal effects against Aspergillus niger. In this study, the results were shown in table 2 and figures 7 and 8. It was found that the methanol seed extract had varying levels of antioxidant activity, which varied with concentration. The activity decreased as the concentration of the extract decreased, and the seed extract showed antioxidant activity. The results of the anti-oxidation effect of the methanol extract of L. sativum seeds are shown in table 2 and figure 7. The highest percentages were obtained at a concentration of 1 mg, reaching 91.419, while the lowest percentages were obtained at a concentration of 0.12, amounting to 81.308. These variations in antioxidant activity can be explained by the different solvents that were used. Thaker and Pawar (2006). Not to mention that there is fluctuation when the concentration increases According to Maltas and Yildiz (2012), different cultivars' extracts may have different flavonoid and phenolic contents, which in turn might affect the scavenging capabilities of free DPPH radicals. This was previously observed by Mltas *et al*. (2011). Several authors have shown a correlation between the amount of phenolic compounds in plant extracts and their antioxidant activities; for example, Owusu-Ansah *et al*. (2010) and Maltas and Yildiz (2012) found a correlation between the scavenging activity of plant extracts and the solvent employed for extraction.

Figure 1. Seeds of *Lepidium sativum*

Table 1. Antibacterial effect of methanol L. sativum seed extract

Bacteria \	*Staph. aureus*	*Kleb. Pneumonia*	*E. coli*	*E. facelis*
Seed 1ml	26mm	22mm	20mm	19mm
Seed 0.5 ml	24mm	20mm	18mm	17mm
Seed 0.25	18mm	16mm	15mm	15 mm
Seed 0.12	15mm	11mm	-	-

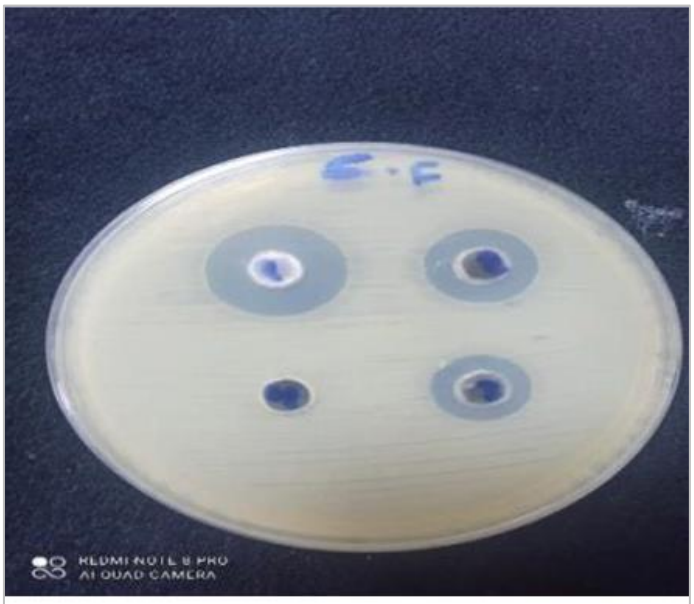

Figure 2. Antibacterial effect (E. faecelis) on the L. sativum methanol seed extract.

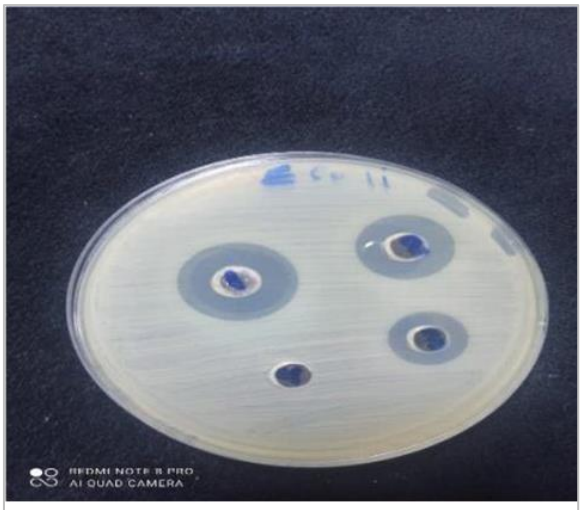

Figure 3. Antibacterial effect (E. coli) on the L. sativum methanol seed extract.

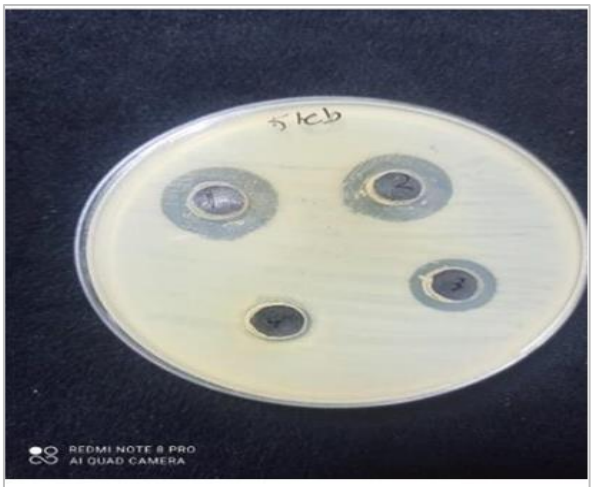

Figure 4. Antibacterial effect (klebsiella) on the L.sativum methanol seed extract.

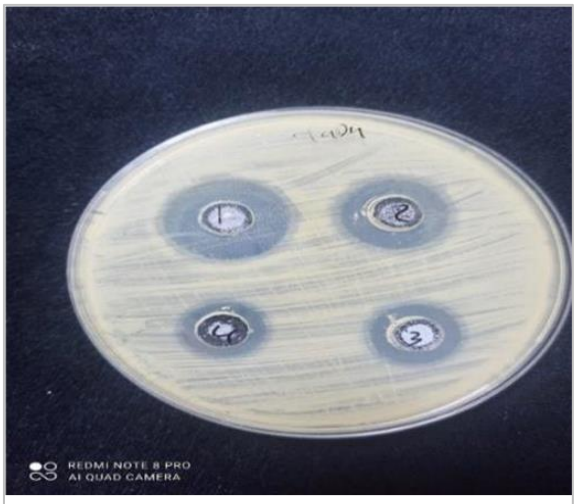

Figure 5. Antibacterial effect (staphylococcus) on the L. sativum methanol seed extract.

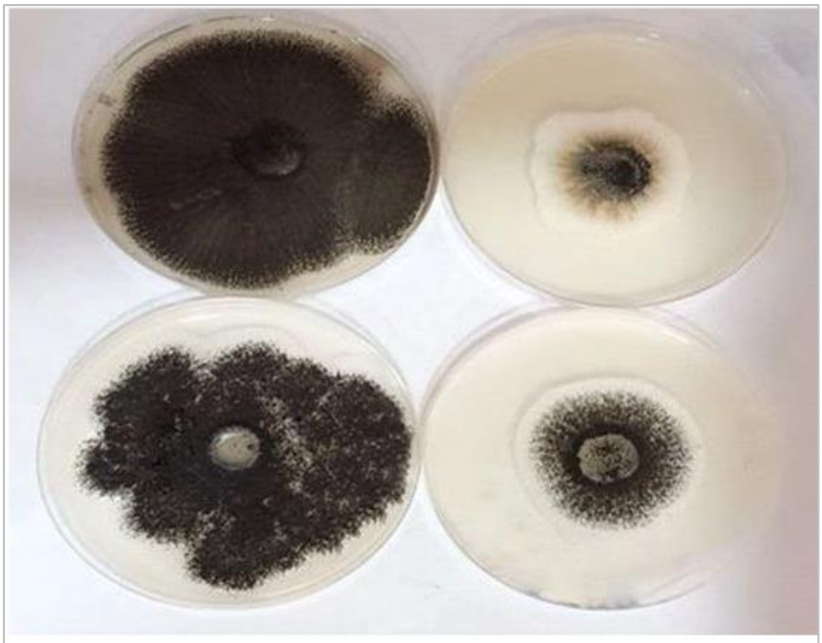

Figure 6. Anti-fungi (Aspergillus niger) effect of L. sativum methanol seed extract Antioxidant Activity Using DPPH Assay

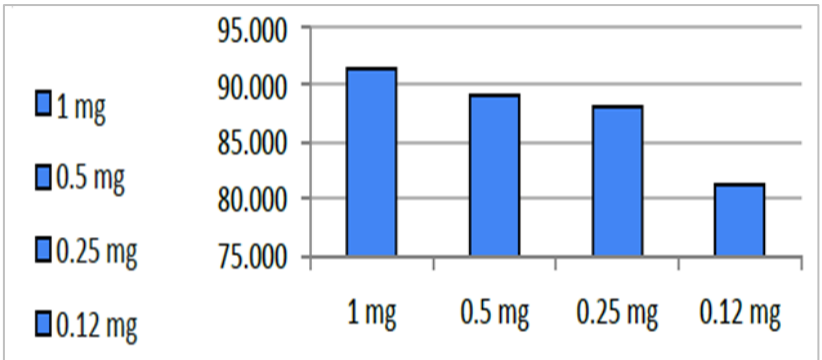

Figure 7. Antioxidant activity (Scavenging activity %) of L. sativum genotype seed methanol extract using DPPH assay

Table 2. Antioxidant activity (Scavenging activity %) of L. sativum genotype seed methanol extract using DPPH assay

Anti-oxidant%	Test result	concentration	Sample name
91.419	0.101	1mg	C1
89.125	0.128	0.5mg	D1
88.105	0.14	0.25mg	E1
81.308	0.22	0.12mg	F1
	1.177	control	

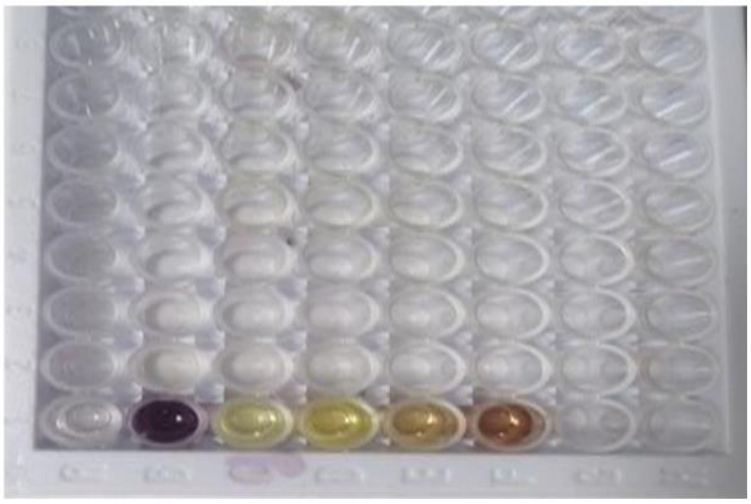

Figure 8. Antioxidant activity (Scavenging activity %) of L. sativum genotype seed methanol extract using DPPH assay

CONCLUSION

Screening studies should be conducted to isolate phytochemicals and evaluate their efficacy for pharmaceutical purposes; as methanol seed extract exhibited effective antibacterial and antioxidant activities, this is crucial. Additional research is required to isolate and identify other microorganisms, such as viruses, fungi, and anaerobic bacteria.

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