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A Study of Methanol Seed Extract of *Lepidium Sativum* Effect on Some Entero Bacteria and Fungi *(Asparagillus Niger)*

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ABSTRACT

The results of the current study indicated the effect of methanol seed extract at a concentration of 200mg/ml there was a variation effect of *L. sativum* methanol seed extract against the studied bacteria, in the diameter of the inhibition zone with diver's bacterial strain*Staph. aureus* gave the highest inhibition zone value was 26mm at 1ml of *L. sativum* methanolseed extract while *E. coli* and *E. faecelis* did not give any inhibition zone at 0.12 ml of *L. sativum*methanol seed extract, there was a variation of effect among gram-negative and positive bacteria in their response to different solvent seed extracts.

There was a variation effect in the antifungal activity of the methanol seed extract against *Aspergillus niger* the results established the methanol seed extract appeared the differences in their antioxidant activity and diverse variety. It was variation in the activity in relation to concentration (activity decrease with decrease extract concentration), Seed extract showed antioxidant activity in this study, the anti-oxidation effect of the *L. sativum* seeds extract by methanol, which give the highest percentages at concentration 1mg, reaching 91.419 and the lowest value at concentration 0.12, which amounted to 81.308. Thepresent study deals with, antimicrobial properties and Anti-oxidant activity constituents of Gcseeds.

KEYWORDS: L. sativum, antioxidant activity, antibacterial activity,Gc.

ARTICLE DETAILS

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INTRODUCTION

L. *sativum* is an annual (Family *Cruciferae*), upright, split, glabrous edible herb 15–50 cm in height, erect, rarely pilose with pinnatisect basal leaves that are 5-10 cm long, 2.5-3.5 cm broad, stalked to subsessile, while cauline leaves are linear and sessile with entire margin. Racemes are much-branched, consist of 20-40 flowered, bracteates Shah, et al (2021). Flowers white or pinkish are tiny, its size about 3 mm. Fruit is inform to rachis that ison sub erect to ascending pedicel and 5 to 6.4 mm in size, seeds are brownish-red in color, 3lobed ovate- oblong 3 mm long 1 mm broad and fast-growing, L. *sativum* can be grown all over around the year at all ground but the most favorable season is winter. The plant domesticis Europe and southwestern Asia, introduced and plowed throughout United States, France, England, India, Asia, etc. as a salad plant flora of N.A. (2020). Certified registration on phytochemical side imply that fresh seeds in specific are fertile in minerals, carbohydrates, vitamins A, C, and K, protein, fat and dietary fibers Jangra and Madan (2018).

L. *sativum* was utilize traditionally to treat numerous diseases like asthma treated by use seeds [Sharma et al. 2018] bronchitis and cough flora of N.A. (2020), bleeding piles Angel and Chadha (1979) scorbutic diseases Chopra et al (2006) liver complaints Sakran et al (2014),spleen and liver chronic enlargement, flatulence, diarrhea, dysentery, indigestion, rheumaticpain, inflammation, viscous humors, tenesmus, secondary syphilis, abortion, anemia and weakness Agarwal and Sharma (2013).

It is plowed everywhere principally for its leaves and seeds of which the later are used as salad for sever flavor in spite of all parts have commercial value Mohamad et al (2018). In compress for wounds and contusion the plant can also be used Aqahtani et al (2019).

L. *sativum* is also used for anti-bacterial Endrise et al (2019) anticarcinogenic Tounsi et al (2019) anti-inflammatory Endrise et al (2019), cardioprotective, antioxidant Chen et al (2019) hypolipidemic Mohamed et al (2019) diuretic Jain and Grover (2018) stomachic Region et al (2016) gastrointestinal stimulant, laxative, gastro-protective Ullah

et al(2019) and many more because it's varied pharmacological actions. 7, 10- hexadecatrienoic acid, 11-octadecenoic acid, 7, 10, 13- hexadecatrienoic acid, and behenic acid phytoconstituents found in *L. sativum* are shown to hold free radical scavengingactivity (DPPH) Endrise et al (2019).

Another author described that the melanin production increase on insinuation to UV-c radiation by utilized the L. *sativum* antioxidant activity Arabia et al (2010).*L. sativum* is widely used as a test organisms in plant physiologically study, as a signal organism to check toxicity levels of circumferential pollutants, and in experimentally assessing various microorganism that can cause disease Saeid et al (2022),the present study deals with antimicrobial properties and Anti-oxidant activity constituents of Gc seeds.

2.2. Bacteria and Fungi

1- Staphylococcus: are hardly, being reluctant to drainage and heat, and can hold for long periods on fomites which can then avail origin of goal. Ordinary washing before and after approach with food or potentially infected individuals decreases the transport of staphylococcal aliens.

2- *Klebsiella*: are large, non-motile bacilli, cause necrotizing lobar pneumonia individuals compromised by alcoholism because it contain a luxurious capsule ,it also cause diabetes or chronic obstructive pulmonary disease, urinary tract infections and bacteremia particularly in hospitalized patients.

3- *E. coli*: in spite of it is part of normal flora in humans and animals, but can be pathogenic both within and outside of Gl tract. *E. coli* cause fever and bloody stools that could a dysentery

– like syndrome. It also effect on young children by cause traveler diarrhea and persistentdiarrhea.

4- *Enterococcus faecalis*: Enterococci are a persistent cause of broad diversity of infections inhumans. *Enterococcus faecalis* has been removed from endodontic infections like obturator root canals with chronic apical periodontitis. The organism can remain alive excessive challenge Learning about the creature may aid to hold endodontic handling failures attributed to this creature Richard et al (2007).

5. *Asparagillus niger*: is a fungus belonging to the class Euascomycetes they reproduce through spores that are present year long, this fungus is used in many industries including food and drug, the fungus resides throughout the world in both terrestrial and aquatic environment. This strain can be pathogenic in humans creating respiratory distress especially in immunocompromised individuals BIO_BARS_Aspergillus _spp.

MATERIALS AND METHODS

Plant used in experiment

In this study we used seed of *Lepidium sativum* were provided from local market figure 1.

Plant Extracts Preparation

The plant powder (250g of seed) was soakedin at room temperature with shaking with 500 ml of the solvent (methanol) for one week using water bath day by day followed by filtering and once more extraction, the extraction concentration at 40C° was used to remove organicsolvents using an oven and getting crude methanolextract. Collected crude extracts were, finally, ready for bioassay examination El-Sayed et al (2012).

One concentration was prepared (200mg/ml) by dissolving 1gm of crud extract 1ml ofDimethysufoxide (DMSO) and completing volume to 5ml using distilled water.

Collection and Diagnosis of Bacteria and fungi

There were four samples of gram negative of bacteria (*Klebsiella pneumonia, Staphylococcus aureus, and E.coli* and *Enterococcus faecalis*) and one sample of fungi (*Asparagillus niger*) diagnosed bacteria and fungi are obtained from Al-Amin Research Center and advanced biotechnology in the holy city of Najaf, used in current study.

Preparation of Bacterial Suspension

To culture bacteria on nutrient agar and incubation for 24 h at 37 C° the bacterial suspension was prepared , colony weretaken after 3-4 weeks and incubation for 4-5 h in 37C° by test tube contenting on nutrient broth, after that, to obtained suitable turbidity it was compared withMcFarland tube.

Plant extract activity test

Method of well agar diffusion was used to study the susceptibility test for plant extract by spread (1, 0.5, 0.25 and 0.12) ml from bacterial suspension on surfaceof media, then, makingfive equals well in Muller Hinton plat, diameter of 6mm by crok borer 0.1 ml from extract were added to each well, after then incubation the plate overnight in 37 C° and then measured the diameter of inhibition zone to detect the activity of test plantextract on growthof bacteria used ruler DMSO (Dimethysufoxide) was used as control Agrove (1985).

Antifungal activity test

Plant extract examined at three concentrations for *Aspergillus niger* at (1, 0.5, 0.12) mg to assess antifungal activity in petri plates with 15 ml of sterilized potato dextrose agar when seven days of incubation at 27 °C is gown the *Aspergillus niger* radial growth of mycelium wasmeasured the actively growing mycelium 5 mm diameter of the disc of the pathogen with negative control the results were compared the following formula used for calculate percentinhibition of the fungus in treatment:

$$L = [(C - T)/C] \times 100$$

The L is refer to percent inhibition C is represent the colony radius in control plate and T is the radial growth of the pathogen in the presence of plant extracts Shivapratap et al (2004).

Antioxidant Activity Study

Plant extract examined at four concentrations by dilution with methanol (1, 0.5, 0.25, 0.12) using DPPH (2, 2diphenyl-1-picrylhydrazyl) free radical scavenging assay was used to assess the ability of methanol seed extract as antioxidant agent, to assay freeradical scavenging activity of natural compounds, DPPH is stable radical compound often utilized Amarowicz et al (2004).

Upon reduction with an antioxidant its absorption decreases due to the formation of its non- radical form DPPH-H while the DPPH radical has a strong absorbance at 517 nm due to its unpaired electron and giving the radical a purple color Gursoy et al (2010).

The DPPH radical cation method was performed according to the DPPH reagent was DPPH (8 mg) dissolved in (methanol) MeOH (100 ml) for a solution concentration of 80 µl/mlPellegrini et al (1999).

To determine the scavenging activity, in a 96- well microplate 100 ml DPPH reagent was mixed w ith100 µl of sample and incubated at room temperature for 30min afterincubation the absorbance was measured 514 nm using an ELISA reader, and 100% methanolwas used as a control.

The following formula used to measure the DPPH scavenging effect:

Radical scavenging (%) = [(*A*) control− (*A*) sample\ (*A*) control]×100 Ishimaru et al(1995).

The concentrations of samples required for inhibition of 50% of DPPH radicals representthe IC50 DPPH values can obtained by extrapolation of regression investigation. The antioxidant was evaluated based on this IC50 value.

Plant extract prepared in following concentrations (91.419, 89.125, 88.105, 81.308 microgram /ml) in addition to methanol control treatment.

Data Analysis for Antibacterial and Antioxidant Activity (LSD)

Data were analyses using Least Significant Difference (LSD) at P≤0.05using SPSS programversion 23.

RESULTS AND DISCUSSION

Antibacterial Activity Study

The results in table 1 indicated the effect of methanol seed extract at concentration of (200mg/ml) there was a variation among *L. sativum* in the antibacterial activity of the methanol seed extract against the studied bacteria, in the diameter of inhibition zone withdiver's bacterial strain Staph. aureus gave the highest inhibition zone value was (26mm) at1ml of *L. sativum* methanol seed extract while *E. coli* and *E. faecelis* not gave any inhibitionzone at 0.12 ml of L. sativum methanol seed extract results also indicated in figures 2, 3, 4 and 5.

There was variation among gram negative and positive bacteria in theirresponse todifferent solvent seed extract, this might be attributed to the difference in the bioactive compounds generally, gram negatives are more resistant than gram-positive bacteria as reported in many studies Kluytmans et al (2013).

Wu et al (2020) reported that the wide range of antimicrobial activity of essential oils because of its complex chemical composition variation in susceptibility among gram-negative and gram-positive related mainly to differing structures of cell walls of bacteria the main cause of alterations in cell's structure and functional is hydrophobic nature of the essential oils which allows them to penetrate microbial cells.

The results in figure 6 indicated the effect of methanol seed extract at concentrationof (200mg/ml) there was a variation among L. *sativum* in the antifungal activity of the methanol seed extract against the studied fungi (*Aspergillus niger*) AL-Saadi (2018) examineda group of medicinal plants essential oil including *E. sativa* against group of *Aspergillus* its results indicate oil ability to inhibit fungi growth in addition to their ability to produce aflatoxin his results agreed with many authors that the major antimicrobial agent is eurcic acid Gemede et al (2019) reported the ability of medicinal plants essential oils against *A. niger* growth Noshirvani and Fasihi (2018) examined 75 medicinal plant essential oil result in theirpossess antifungal activity against *A. niger*.

The results in both table 2 and figures 7 and 8 were established the methanol seed extract appeared the differences in their antioxidant activity and diverse variety, it was variation in the activity in relation to concentration activity decrease with decrease extract concentration and seed extract showed antioxidant activity in this study.

As shown in table 2 and figure 7 for the anti-oxidation effect of the L. *sativum* seeds extract by methanol which give the highest percentages at concentration 1mg reaching 91.419 and the lowest value at concentration 0.12 which amounted to 81.308 the differencesin the antioxidant activity can be attributed to the differences in the effectiveness of the solvents was used Pawar and Thaker, (2006).

In addition to variation with increase concentration Maltas and Yildiz (2012) the variable flavonoid and phenolic contents in the extracts of the cultivars can be cause to the variable scavenging activities of the free DPPH radicals Mltas et al (2011).

A strong correlation between antioxidant activities of plant extracts and their contents of phenolic compounds a number of authors have reported Owusu-Ansah et al (2010) and relation between solvent used in extraction and scavenging activity Maltas and Yildiz (2012).

CONCLUSION

Methanol seed extract was effectively bearing antibacterial and antioxidant activity it's necessary to conduct screening study for phytochemicals isolation and examining them to determine the most effective phytochemical potency for pharmaceutical purpose. More searches are needed for isolation and identification of others microorganism like anaerobic bacteria, fungi and virus.

Figure 1. Seeds of Lepidium sativum

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

Extract Bacteria	Seed 1ml	Seed 0.5	Seed 0.25	Seed 0.12
Staph. aureus	26 mm	24mm	18mm	15mm
Kleb. pneumonia	22mm	20 mm	16mm	11mm
E. coli	20mm	18 mm	15mm	-
E.faecelis	19mm	17mm	15mm	-

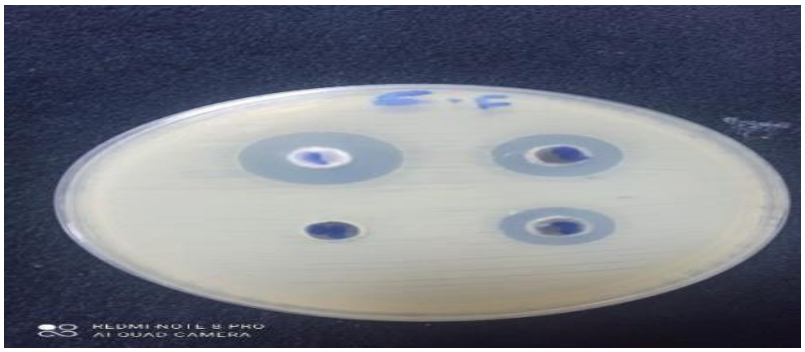

Figure 2. Antibacterial effect (E.faecelis) on the L. *sativum* methanol seed extract

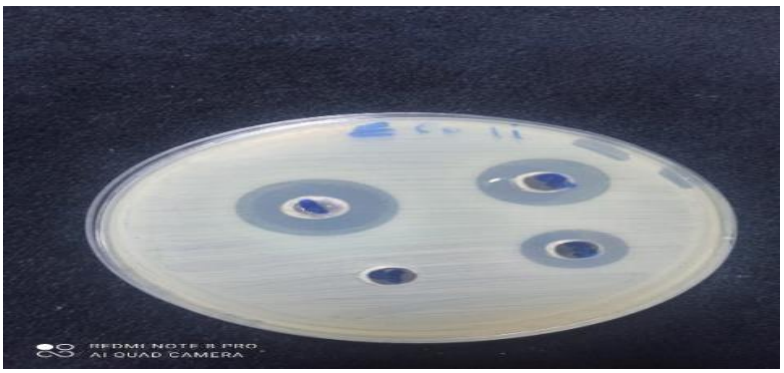

Figure 3. Antibacterial effect (E.coli) on the L.*sativum* methanol seed extract

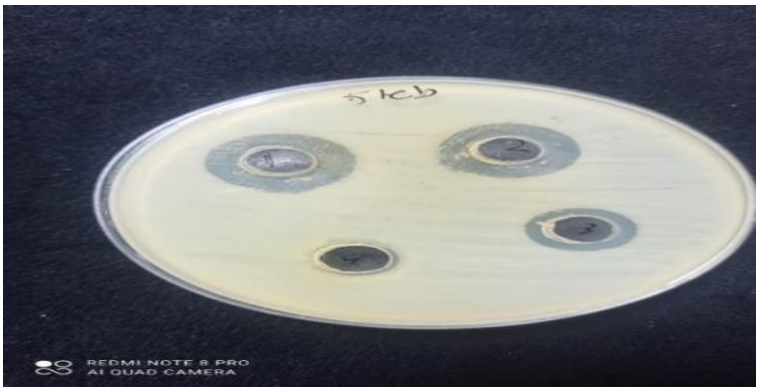

Figure 4. Antibacterial effect (klebsiala) on the L.*sativum* methanol seed extract

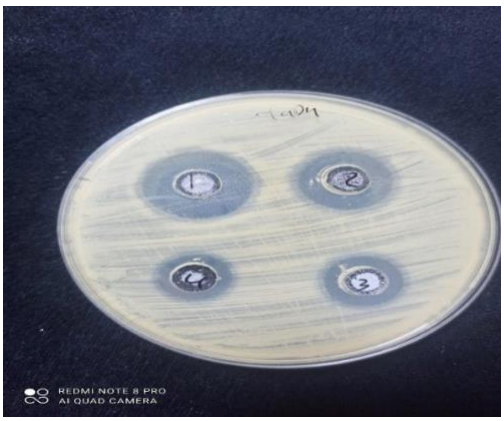

Figure 5. Antibacterial effect (staphylococcus) on the L. *sativum* methanol seed extract

Figure 6. Anti fungi (*Aspergillus niger*) effect of L. *sativum* methanol seed extractAntioxidant Activity Using DPPH Assay

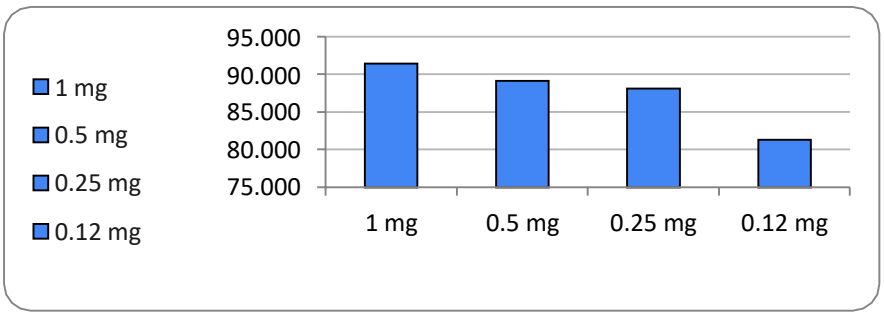

Figure 7. Antioxidant activity (Scavenging activity %) of L. *sativum* genotype seed methanol extract using DPPHassay

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

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

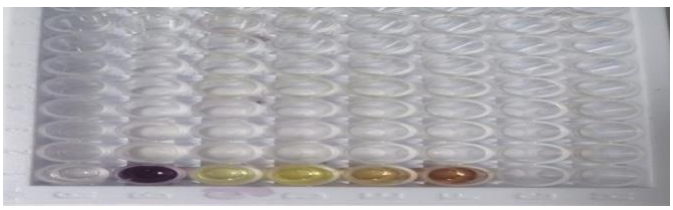

Figure 8.Antioxidant activity (Scavenging activity %) of L. *sativum* genotype seed methanol extract using DPPH assay

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