

***In vitro* Antimicrobial Assessment of *Lepidium sativum* L. Seeds Extracts**

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Abstract: The antimicrobial activity of the petroleum ether, methanol and water extracts of *Lepidium sativum* seed extracts against six opportunistic pathogens namely *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and one fungus *Candida albicans* was assessed using the concentrations of 2.5, 5 and 10%. The antimicrobial activity of plant seeds extracts were compared with that of Gentamicin or Ketoconazol, as reference antibiotics. The petroleum ether extract of *Lepidium sativum* seeds in different concentrations (2.5-5-10%) were found to be active antimicrobials against all the test microorganisms with a strong antifungal activity at the concentration 2.5 and 10%. At the concentration of 5%, the methanolic extract of this plant had no activity against *Candida albicans*. *Staphylococcus aureus* and *Candida albicans* were resistant to 2.5 and 5% water extracts, whereas the latter was also resistant to 5% methanolic extract. The antimicrobial activity of Gentamicin and Ketoconazol against the same test microorganisms was compared with that of the extracts of *L. sativum* seeds.

Key words: Antimicrobial activity, *C. albicans*, *E. coli*, *K. pneumoniae*, *Lepidium sativum*, *P. vulgaris*, *P. aeruginosa*, *S. aureus*

INTRODUCTION

The accumulated information on World's plants goes far beyond more identification and taxonomic classification. Our knowledge of plant physiology biochemistry, genetics, adaptation to new environments and breeding has steadily grown and several scientific disciplines have contributed to the development of new tools for effective utilization of plants by man.

Medicinal plant products were proved useful in minimizing the adverse effects of various chemotherapeutic agents as well as in prolonging longevity and attaining positive general health (Kaushik and Dhiman, 2002). The increasing global interest in the medicinal potential of plants during the last few decades is therefore quite logical.

Lepidium sativum, known as pepper cress or Elrashad, belongs to the family Brassicaceae (cruciferae) and it is an erect, annual herb grows up to 50 cm height. The leaves are variously lobed and entire, flowers are white small and found in racemes and Fruits are obovate pods, about 5 mm long, with two seeds per pods. The seeds and leaves of the plant contain volatile oils (Watt and Breyer Brandwijk, 1962). The plant is eaten and seed oils are used in treating dysentery and diarrhea (Broun and Massey, 1929) and migraine (Merzouki *et al.*, 2000). The plant was found to contain glucosinolate and glucotropaeolin (Songsak and Lockwood, 2002).

Lepidium sativum plant and seeds are considered one of the popular medicinal herbs used in the community of Saudi Arabia, Sudan and some other Arabic countries as a good mediator for bone fracture healing in the human skeleton. A number of recent studies pointed out the traditional uses of *Lepidium sativum* seeds extract in controlling many clinical problems. They were used as anti-asthmatic antiscorbutic, aperient, diuretic, galactogogue, poultice and stimulant. The leaves are antiscorbutic, diuretic and stimulant (Eddouks *et al.*, 2002). It was found that oral administration of the aqueous *Lepidium sativum* extract exhibited a significant decrease in blood pressure (Maghrani *et al.*, 2005).

Lepidium sativum L. seeds increase weight gain as they are found to contain 18-24% of fat. Thirty four percent of the total fatty acids are alpha linolenic acid; and the oil has alpha linoleic acid which could give it nutritional advantages (Diwakara *et al.*, 2008). The primary fatty acids in *Lepidium sativum* oil were oleic (30.6 wt %) and linolenic acids (29.3 wt%) and was found to contain high concentrations of tocopherols. It contains good amount of lignans and antioxidants, which can stabilize the n-3 polyunsaturated fatty acids in its seed oil. The primary phytosterols in *Lepidium sativum* were sitosterol and campesterol, with avenasterol (Bryan *et al.*, 2009).

The objective of the present study on *Lepidium sativum* seeds was to estimate the possible antibacterial

activity of several seed extracts (Petroleum ether, methanolic and water) at concentrations of (2.5, 5 and 10%) against six pathogenic bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and one fungus *Candida albicans*). The antimicrobial activity of plant seeds extracts were compared with that of Gentamicin or Ketoconazol, as reference antibiotics.

MATERIALS AND METHODS

Materials:

Plant materials: *Lepidium sativum* L. seeds (Fig. 1) were obtained from a local market in Khartoum (March, 2011), authenticated by the scientists of the Aromatic and Medicinal Plants Research Institute and brought to the research laboratory, University of El Neelain, Khartoum, Sudan The plant tissues were cleaned, shade dried and ground by a mechanical grinder.

Standard microorganisms: The organisms used in the present study were kindly provided by the scientists at Khartoum National Health Laboratory and designated as follows: *Staphylococcus aureus* ATCC/25923, *Escherichia coli* ATCC/27853, *Klebsiella pneumoniae* ATCC/3565, *proteus vulgaris* ATCC/27853, *Pseudomonas aeruginosa* 27853/ATCC and *Candida albicans* ATCC/7596.

(NCTC = National Collection of Type Culture, Colindale, England, ATCC = American Type Culture Collection, Rockville, Maryland, USA).

Antibiotics: Gentamicin and Ketoconazol (Shanghai pharmaceutical Co. Ltd., China).

Culture media: The Nutrient broth (Oxoid Ltd., London) formed the basis of most media used in microbiological studies. Nutrient agar (Oxoid Ltd., London) was used to prepare enriched culture media.

Mueller-Hinton agar (Oxoid Ltd., London) was used for all antibiotic sensitivity tests for standard drug as well as for plant extracts evaluation. Sabouraud's dextrose agar (Difco, USA) was used as enriched culture media for fungi.

Preparation of plants extracts: The powdered sample (50 g, of each seeds plant) was accurately weighed, and separately extracted with petroleum ether (90% at 60-80°C for 2 h) in a Soxhlet apparatus. The petroleum ether extract was evaporated by a Buchi Rotaevaporator under reduced pressure. The extracted plant material was air-dried and repacked in the Soxhlet apparatus and then extracted with methanol (99.8% for 2 h). The extract was similarly evaporated exhaustively; air dried for about 18 h and the yield was preserved in a covered flask. The



Fig. 1: *Lepidium sativum* seeds

plant residue was further extracted over night at room temperature (25-30°C) with distilled water, filtered and freeze dried.

Preparation of the standard bacterial suspensions: One ml aliquots of a 24 h broth culture of the test organisms was aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 h. The bacterial growth was harvested and washed off with 100 mL sterile normal saline, to produce a suspension containing about 10^8 - 10^9 colony forming units per mL. The suspension was stored in a refrigerator at 4°C till used. The average number of viable organisms per mL of the stock suspension was determined by means of the surface viable counting technique (Collee *et al.*, 1996). Serial dilution of the stock suspensions were made in sterile normal saline solution and 0.02 mL volumes of the appropriate dilution were transferred by micropipette to the surface of dried nutrient agar plates. The plates were allowed to stand for 2 h at room temperature for the drops to dry, and then incubated at 37°C for 24 h. After incubation, the number of developed colonies in each drop was counted. The average number of the colonies per drop (0.02 mL) will be multiplied by 50 and by the dilution factor to give the viable count of stock suspensions, expressed as the number of colony forming units per mL of suspension. Each time a fresh stock suspension will be prepared, all the above experimental condition will be maintained constant so that suspensions with very close viable count would be obtained.

Preparation of standard fungal organisms: The fungal standard cultures from the Medicinal and Aromatic Plant Research Institute were maintained on Peptone water, incubated at 25°C for 4 days. The fungal growth mats were harvested and washed with sterile normal saline and finally suspended in (100 mL) of sterile normal saline and stored in refrigerator till used.

Determination of antimicrobial activity of plant extracts: At the time of testing, the extracts were reconstituted to concentrations of 2.5, 5 and 10% in

dimethyl sulphoxide (DMSO). Antimicrobial activity was assessed by the agar-well diffusion method (Kingsbury and Wagner, 1990). The inoculum size of each tested bacterium was adjusted to a suspension of 10^6 cells. The inoculum suspension was spread over a Mueller Hinton Agar (MHA) plate, to achieve confluent growth, and allowed to dry. 10 mm- diameter wells were bored in the agar using a sterile cork borer (NO. 4) and the agar discs were removed. A 100 μ L aliquot of the reconstituted extract was placed into a well with standard Pasteur pipette and the plate was held for 1 h at room temperature for diffusion of extract into the agar. Subsequently, the plate was incubated for 18 h at 37°C. After incubation, the diameters of the inhibition zones were measured to the nearest mm. Three replicates were made from each concentration and comparative activity was recorded. The antimicrobial activity of the plant extract against the standard microorganisms was evaluated and compared with that of Gentamicin (bacteria) and Ketoconzol (yeast).

RESULTS AND DISCUSSION

Antimicrobial activity of lepidium sativum seeds extracts against tested organisms: The Agar well diffusion method was used in this study to assess the antimicrobial activity of *Lepidium sativum*. According to Omenka and Osuoha (2000), this method allows better diffusion of the extracts into the medium thus enhancing contact with the organisms.

The antimicrobial activity in terms of zone of inhibition (in mm diameter) of petroleum ether, methanol and water extracts of *Lepidium sativum* seeds at the different concentrations of 2.5, 5 and 10% against six pathogenic organisms, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Candida albicans* was presented in Table 2. The results indicated that the concentrations 2.5, 5, and 10% of the three types of *Lepidium sativum* extracts were active against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. The results also showed that the petroleum ether was the best solvent for extracting antimicrobial substances from this plant compared to methanol and water. This was shown by its high inhibitory effect against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus vulgaris* at 2.5% (Table 1 and Fig. 2, 3 and 4). *Proteus vulgaris* was well inhibited by 2.5% petroleum ether and 10% of both methanolic and aqueous extracts (Fig. 5). The most susceptible microorganism to petroleum ether extract was *Candida albicans* at the concentration of 2.5 (32 mm) and 10% (33 mm) (Table 1 and Fig. 6). Methanol and aqueous extract at different concentrations showed moderate inhibitory action to the test organisms.

Table 1: Evaluation of antimicrobial activity of petroleum ether, methanol and aqueous extracts of *Lepidium sativum* seeds

Organisms	Inhibition zone (mm)*			
	Extract conc.%	Petroleum ether extract	Methanol extract	Aqueous extract
<i>Staphylococcus aureus</i>	2.5	25	15	(-)
	5	13	11	(-)
	10	13	14	18
<i>Escherichia coli</i>	2.5	25	17	10
	5	14	14	12
	10	24	13	19
<i>Klebsiella pneumoniae</i>	2.5	26	16	11
	5	12	15	12
	10	19	11	17
<i>Proteus vulgaris</i>	2.5	21	18	10
	5	13	10	15
	10	17	20	19
<i>Pseudomonas aeruginosa</i>	2.5	18	17	9
	5	15	12	10
	10	14	10	16
<i>Candida albicans</i>	2.5	32	9	(-)
	5	11	(-)	(-)
	10	33	19	21

(-): No inhibition was observed; Conc.: concentration; *: mean of three replicates

Table 2: Evaluation of antimicrobial activity of gentamicin and ketoconzol

Organisms	Gentamicin conc. (%)	Inhibition zone (mm)
<i>Staphylococcus aureus</i>	10	32
<i>Escherichia coli</i>	10	32
<i>Klebsiella pneumoniae</i>	10	35
<i>Proteus vulgaris</i>	10	34
<i>Pseudomonas aeruginosa</i>	10	32
	Ketoconzol conc.%	
<i>Candida albicans</i>	10	33

*: mean of three replicates; Conc.: concentration

It can be suggested that *Staphylococcus aureus* and *Candida albicans* were the most resistant organisms to the concentrations of 2.5 and 5% of the aqueous and the latter was found also resistant to methanolic extract at the concentration of 5%. Maximum activity of aqueous extract was seen against *Candida albicans* at concentration of 10% (Fig. 7). Poor inhibitory effect was detected against.

Pseudomonas aeruginosa and *Candida albicans* at 2.5% methanolic extract and both 2.5 and 5% water extract. The plant extracts compared favourably with the standard antibiotic Gentamicin and Ketoconzol (Table 2).

Phytochemical screening of *Lepidium sativum* seeds revealed the presence of flavonoids, Alkaloids, sterols and/or triterpenes, tannins and glucosinolates (Brotonegoro and Wiharti, 2001). These compounds are known to be biologically active. Tannins have been found to form irreversible complexes with proline-rich proteins (Hagerman and Butler, 1981) resulting in the inhibition of the cell protein synthesis. This activity was exhibited

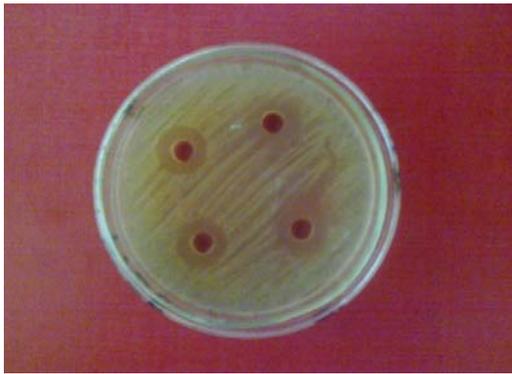


Fig:2: Inhibition zone of *Lsativum seeds* petroleum ether and methanol extracts (2.5%) against *S. aureus*



Fig. 5: Inhibition zone of *L. sativum seeds* water extract (10%) against *Candida albicans*

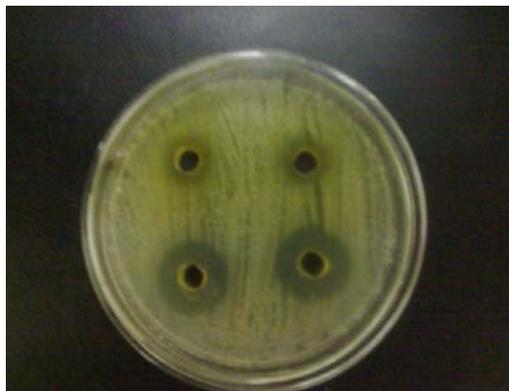


Fig. 3: Inhibition zone of *L. sativum seed* petroleum ether extract (2.5%) against *E. coli*



Fig. 6: Inhibition zone of *L. sativum seeds* petroleum ether and methanol extracts against (10%) *Candida albicans*

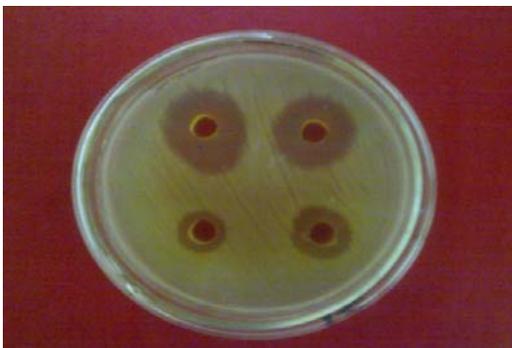


Fig. 4: Inhibition zone of *L. sativum seeds* petroleum ether (2.5%) against *Klebsiella pneumoniae*



Fig. 7: Inhibition zone of *L. sativum seeds* water extract (10%) against *Candida albicans*

against the test organisms with different concentration with the plant extracts in this study. Apart from antimicrobial activity exhibited by tannins, they also react with proteins to provide the typical tanning effect. Medicinally, this is important for the treatment of inflamed or ulcerated tissues (Mota *et al.*, 1985). Tannins have important roles such as stable and potent

antioxidants (Trease and Evans, 1983). Herbs that have tannins as their main component are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery (Dharmananda, 2003), thus exhibiting antimicrobial activity. One of the largest groups of chemical produced by plants are the alkaloids and their amazing effect on humans has led to the

development of powerful pain killer medications (Raffauf, 1996).

In classifying the antibacterial activity as gram-positive or gram-negative, it would generally be expected that a much greater number of extracts would be active against gram-positive than gram-negative bacteria (Mc Cutcheon *et al.*, 1992). In the present findings, 7/9 samples (77.78%) extract showed activity against gram-positive bacteria *S. aureus* (Fig. 7), not compatible with above view. *Staphylococcus aureus* was found susceptible to various plant extracts evaluated in different studies such as methanolic extract of *Psidium guajava* (Akinpelu and Onakoya, 2006), *Verbascum* sp. (Dulger and Hacıoglu, 2002), *Solanum tomentosum* (Aliero and Afolayan, 2006), *Curcuma longa* (turmeric) (Kaushik and Singh, 2000; Thongson *et al.*, 2004). In another study, *Psidium guajava* (Goiaba) leaf extract found to have significant antimicrobial activity against *Staphylococcus aureus* (Gnan and Demello, 1999). However, the numbers of bacteria screened in this study have been restricted to five: four gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*) and one gram-positive (*S. aureus*) due to limitation of resources.

Many medicinal plants have been found effective in the cure of bacterial diseases. Due to increasing antibiotic resistance of microorganisms and side effects of synthetic antibiotics medicinal plants are now gaining popularity in the treatment of bacterial infections. Medicinal plants are considered as clinically effective and safer alternatives to the synthetic antibiotics. Extensive research in the area of isolation and characterization of the active principles of these plants are required so that better, safer and cost effective drugs for treating bacterial infections can be developed.

CONCLUSION

From the above study, it is concluded that the traditional plants may represent new sources of antimicrobials with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine. These local ethnomedical preparations and prescriptions of plant sources should be scientifically evaluated and then disseminated properly and the knowledge about the botanical preparation of traditional sources of medicinal plants can be extended for future investigation into the field of pharmacology, phytochemistry, ethnobotany and other biological actions for drug discovery.

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