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Research Article

Antimicrobial Activity of Lepidium Sativum against multi-drug resistant and sensitive Pseudomonas aeruginosa: A microbiological study from Khartoum State, Sudan

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ABSTRACT

Background: L. sativum, are traditionally used for the treatment of various diseases and thought to have medicinal value. Isolates from many part of the world is now multidrug resistant. Therefore, there is an urgent need to look for and test an alternative herbal drug.
Objective: The present study aimed to evaluate the antibacterial activity of L. Sativum seed extract against multi drug resistant (MDR) and sensitive Pseudomonas aeruginosa clinical isolates.
Subjects and Methods: An ethanolic and aqueous stock extracts were prepared from L. sativum seed plant then serial dilutions were prepared and the obtained concentrations (50, 25, 12.5 and 6.2 mg/ml) were tested against 30 multidrug-resistant and 35 sensitive clinical isolates of Pseudomonas aeruginosa using wells diffusion method.
Results: It was found that L. sativum seed extracts had antimicrobial activity against MDR and sensitive isolates at different concentrations of 100, 50 and 25 according to the mean ± SD (standard deviation) of the maximum zones of inhibition. The total number of isolates that were sensitive to both extracts were 49/130 (37%) which represented 17/60 (28.3%) MDR and 32/70 (45.7%) sensitive isolates. The aqueous extract exhibited more inhibitory effect than ethanolic extract 43 (66%) vs. 6 (9%) against the examined isolates (n=65).
Conclusion: The study concluded that the L. sativum extracts had an antibacterial activity against the susceptible and MDR isolates which may enable it to be used an alternative treatment for medicinal purposes.

Introduction

Medicinal plants have been widely used in traditional medicine for the intention of health a long time ago for the deterrent and healing of many health-related ailments (1). 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 (2-3). According to WHO (World Health Organization) about more than 80% of the world's total population, regularly, depends on traditional medicine and products for its healthcare needs especially in third world countries (4). Rural people adopt traditional treatment due to their proximity to

traditional healers and their firm belief that they know the nature of their diseases and often do not reach primary care centers to receive treatment (5-6-7). Therefore, there is a real need to investigate plants to better understand their properties, safety, and efficiency (8). Therefor. Thus Pharmaceutical companies have spent considerable time and money developing medication from plant extracts. The use of plant extracts and phytochemicals, both have antimicrobial properties, which give a greater significance treatment. In the last years, many studies have been conducted in different countries to prove such efficiency (9).

L. sativum famous as garden cress belongs to the family Brassicaceae (cruciferae). In Sudan it is known as 'Hab Alrashad (10-11-12). It is annual herb grows up to 50 cm height and its seeds contain volatile oils (13). The plant is native to Egypt and South west Asia. It's cultivated in India, North America and parts of Europe (14-15). L. sativum seeds possess varied medicinal properties like aperient, diuretic, expectorant, aphrodisiac, antibacterial, gastrointestinal stimulant, gastro protective, laxative (16-17-18). Cress seed is reported to exhibit anti-rheumatic bronchodilator potential. The paste of L. sativum seed is applied in rheumatic joints to relive the pain and swelling. It is also used for hiccup, diarrhea, dysentery, and disease of the skin caused by impurities of blood. Ethanolic extracts of cress seed were effective in treating inflammatory bowel disease (19).

Multi-drug resistant bacterial strains in hospitals and community consider a serious communal pathological state since the time of the invention of antimicrobial drugs, especially infections caused by Pseudomonas species and Ps. aeruginosa particularly (20). So, this study was undertaken to determine the antimicrobial activity of L. sativum against multi-drug resistant and Sensitive Ps. aeruginosa in Khartoum Sudan since very few studies were published regarding this works in our country.

Subjects and Methods

Plant Material:

The seeds of L. sativum used in this study were collected from the local market in Omdurman, Sudan in February 2021. The authentication or identification of the plant seeds was done by a botanist from the National Center of Research (NCR), Khartoum, Sudan.

Ps. aeruginosa clinical isolates:

The Clinical isolates of Ps. aeruginosa (n=30 MDR) that represented Multi drug resistant to different antibiotics (Gentamycin (10µg), Tobramycin (10µg), Amikacin (30 µg), Meropenem (10 µg), Imipenem (10 µg), Ceftazidime (30 µg), Cefpime (30 µg), Ciprofloxacin (5 µg) and Levofloxacin (5 µg), and sensitive isolates (n=35) that were sensitive to these mentioned antibiotics were included in this study. They were obtained from Fedail hospital during the period from February 2021 to August 2021 from different clinical specimens and from patients with different age and gender. The isolates were purified by sub culturing in sterile Nutrient agar media and incubated at 37 $\overset{o}{C}$ over night.

Preparation of the crude extract:

Ethanolic and aqueous extracts of L. sativum were prepared by using overnight maceration techniques according to the method described by Harbone (21) . A total of 50 ground material were macerated in 500 ml of each solvent (ethanol 70% and sterilized distilled water) for 3 days at room temperature. Occasional shaking for 24 h at room temperature was performed and, the supernatant was decanted. Thereafter, the supernatant was filtered under reduced pressure by rotary evaporation at 40°C. Each residue was weighed and the yields percentage were calculated and then stored at 4°C in tightly sealed glass vial ready for use. The remaining extract which not soluble was successively extracted using ethanol and sterilized distilled water with the described technique. The extract was kept in deep freezer for 48 hours until they were completely dried. The extract was kept and stored at 4 °C until required.

Determination of antimicrobial activity of plant extracts:

At the time of testing, 0.1 gram of ethanolic and aqueous extract L. sativum were measured using electronic balance and dissolved in 1 ml of dimethyl sulphoxide (DMSO 10%) and distal water respectively, to prepare stock solution concentration at 100 mg/mL, then (1:1) serial dilution with distal water was done to obtain concentrations at 50, 25, 12.5 and 6.2 mg/ml. Antibacterial activity was assessed by Agar well diffusion technique. Sterile dry surface Mueller Hinton agar was used to exam antimicrobial activity of L. sativum extract. For each isolate two to three of freshly 24 hours colonies were emulsified in sterile normal saline then they were adjusted to 0.5 McFarland standard turbidity with approximately 1 – 5 x 106 CFU/ml. Cotton swab was immersed in the suspension and inoculated on a plate of Mueller-Hinton agar. The inoculation was eventually distributed all over the plate surface. Then, holes with a diameter of 6 to 8 mm were punched aseptically with a sterile blue tip, and a volume (100 µL) of the extract solution at desired concentration were introduced into the wells with standard automatic pipette. Then the agar plates were incubated aerobically at 37 $\overset{o}{C}$ aerobically for 24 h. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested; the zone diameter around each well was manually measured by ruler and recorded (22).

Interpretation of results

The formation of clear inhibition zone around the wells of about >8mm diameters were taken as significant susceptibility measurement. The mean value and standard deviation value were used for analysis (23).

Statistical analysis

The collected data with the laboratory results were analyzed by the statistical package of social science (SPSS) soft program version 20, with reference p-value (0.05), P-value $\leq$.05 conceders as significant result. Frequencies and percent obtained in frequency tables, chi-square test for goodness of fit used to test these frequencies. The relations between variables tested using cross tables and chi-square (Fisher exact) test for independence.

Results

The collected clinical isolates (n=65) of Pseudomonas aeruginosa (35 Multi drug resistant MDR and 30 sensitive) had been collected from different clinical specimens, 17 (26%) from urine, 15 (23%) wound swabs, 11 (17%) sputum, 9 (14%) ear swabs, 2(3%)

body fluid, 3 (5%), tissue biopsy, 2(3%) blood culture and 6 (9%) from cerebrospinal fluid (CSF).

The antibacterial activity of ethanolic and aqueous seed extracts of L. sativum were tested against the clinical isolates at different concentrations (100, 50, 25, 12.5 and 6.2 mg/ml). It was found that L. sativum seed extracts have a potential antibacterial activity against the sensitive and M multi drug resistant isolates.

The ethanolic extract against the M multi drug resistant isolates showed maximum zone of inhibition (20 mm) at 100 mg/ml with a Mean±SD 2.4±6.3, followed by (16 mm) at 50mg/ml (Mean±SD 1.3±4.2) in comparison the aqueous extract at the same concentration showed the maximum zone of inhibition (20 mm) with a Mean±SD 5.1±7.5 followed by (15mm) (Mean±SD 4.4±6.4) as shown in Table (1).

Regarding the sensitive clinical isolates the maximum zone of inhibition with a Mean±SD which represented by methanolic extract at 100 and 50 mg/ml were (18 mm) Mean±SD 1.4±4.9, (15mm) Mean±SD 1.1±3.7 respectively while the aqueous extract represented (14 mm) Mean±SD 10.1±3.8, (12mm) Mean±SD 6.1±4.6 respectively at these concentrations as shown in Table (2).

The inhibitory action of each extact (ethanolic and aqueous) against the collective number of isolates (n=65) was calculated as in Table (3)

The frequency of antimicrobial effect of aqueous and ethanolic extract was compared against the Mmulti drug resistant Ps. aeruginosa (n=30) P. value =0.03 and the sensitive isolates (n=35) P. value= 0.7 as expressed in Table (4)

Table 1: Means of inhibition growth diameter of E ethanolic and Aaqueous extract of L. sativum against Mmulti drug resistant Ps. Aaeruginosa.

Ethanolic extract					
Concentration (mg/ml)	100	50	25	12.5	6.2
Minimum	0.00	0.00	0.00	0.00	0.00
Maximum	20.00	16.0	15.00	13.00	0.00
Mean±SD	2.4±6.3	1.3±4.2	0.9±2.3	0.43±2.3	0.00±0.00
Aqueous extract					
Minimum	0.00	0.00	0.00	0.00	0.00
Maximum	20.00	15.00	13.00	8.00	0.00
Mean±SD	5.1±7.5	4.4±6.4	2.4±4.5	0.26±1.46	0.00±0.00

Table 2: Means of inhibition growth diameter of Ethanolic and Aqueous extract of L. sativum against Ssensitive Ps. Aaeruginosa

Ethanolic extract					
Concentration (mg/ml)	100	50	25	12.5	6.2
Minimum	0.00	0.00	0.00	0.00	0.00
Maximum	18.00	15.00	10.00	0.00	0.00
Mean±SD	1.4±4.9	1.1±3.7	0.28±1.6	0.00±.00	0.00±.00
Aqueous extract					
Minimum	0.00	0.00	0.00	0.00	0.00
Maximum	14.00	12.00	11.00	0.00	0.00
Mean±SD	10.1±3.8	6.1±4.6	1.0±3.0	0.00±.00	0.00±.00

Table 3: Antimicrobial effect of L. Sativum against the total number of isolates (n=65)

	Aqueous	**Ethanolic**	**Total**	**P. value**
Sensitive	43 (66%)	6 (9%)	49 (37.7%)	
Resistant	22 (34%)	59 (91%)	81 (62.3%)	0.06
Total	65 (100%)	65 (100%)	130 (100%)	

The formation of clear inhibition zone around the wells of about >8 mm diameters were taken as significant susceptibility measurement (23).

Table 4: Frequency of Aantimicrobial effect of L. Sativum extracts against the Mmulti drug resistant and sensitive Ps. Aaeruginosa

	L. Sativum extracts against the M multi drug resistant Ps. aeruginosa (n=30)			
	Aqueous	**Ethanolic**	**Total**	**P. value**
Sensitive	12 (40%)	5 (17%)	17 (28.3%)	
Resistant	18 (60%)	25 (83%)	43 (71.7%)	0.03
Total	30 (100%)	30 (100%)	60 (100%)	
	L. Sativum extracts against the sensitive Ps. aeruginosa (n=35)			
	Aqueous	Ethanolic	Total	P. value
Sensitive	31 (88%)	1 (3%)	32 (45.7%)	
Resistant	4 (12%)	34 (97%)	38(54.3%)	0.7
Total	35(100%)	35 (100%)	70 (100%)	

Discussion

Antibiotic resistance toward commonly used medicinal drugs could also be a dangerously growing threat to our existence. Plants come with a variety of biomolecules and metabolites that have vital biological functions. In the fight against multidrug-resistant bacteria, these natural chemicals are a gold mine. Green synthesis could lead to the creation of plant-based antimicrobials as an alternative to commonly used pharmaceuticals. L. sativum, is a fast-growing herb found all over the world. The antimicrobial, antioxidant and anti-inflammatory properties of L. sativum seed are intriguing (24).

The Agar well diffusion method was used in this study to assess the antimicrobial activity of ethanolic and aqueous seed extracts of L. sativum against Ps. aeruginosa (MDR and sensitive isolates). According to Omenka and Osuoha (2000) (25), this method allows better diffusion of the extracts into the medium thus enhancing contact with the organisms.

The present study showed that L. sativum seed extracts had a potential antimicrobial activity against MDR and sensitive Ps. aeruginosa clinical isolates at different concentration 100, 50 and 25. Whereas the aqueous extract exhibited more inhibitory effect than ethanolic extract against Ps. aeruginosa (MDR and sensitive isolates) when comparing the mean values that it gave with that of ethanolic extract (Table 1 and 2) also when comparing the inhibited number among the total isolates (n=65), 43 (66%) Vs. 6 (9%) (Table 3). MDR isolates (40% vs. 17%) and sensitive isolates (88% vs. 3%) (Table 4). In contrast other study that carried out by Shama et al. (2011) (13) in Sudan to test the antimicrobial activity of the petroleum ether, methanol and water extracts of L. sativum seed extracts against six opportunistic pathogens including one Ps. aeruginosa. They found the petroleum ether extract in different concentrations (2.5-5-10%) were most active antimicrobials against all the tested microorganisms. Whereas Hero and Jwan, (2012) (23) found ethanolic extract was more inhibitory to Ps. aeruginosa than aqueous extract, other study done by Nafyad Ibrahim and Ameha kebede (2020) (26) in Ethiopia that found methanol extract of L.

sativum showed highest activity as compared to aqueous extract. These variations may be due to differences in geographical area, strains and sample size as these studies were worked on only one ATCC Ps. aeruginosa compared with Pseudomonas strains that in our this study.

All isolates (n= 65) were subjected to aqueous and ethanolic extracts. It was found the total number of isolates that were sensitive to both extracts were 49/130 (37%) as shown in (Table 3) and according to MDR and sensitive isolates, 17/60 (28.3%) and 32/70 (45.7%) were sensitive to both extracts respectively (Table 4). This finding demonstrated the potent effect of L. sativum seed extracts against Ps. aeruginosa clinical isolates used in the present study specially MDR which they were resistant to nine commonly used antibiotics (aminoglycosides, 3rd generation cephalosporine and carbapenem) that may lead this extracts to be an alternative treatment to infectious diseases caused by this organism. Similarly, different many studies were reported antimicrobial activity of L. sativum seed extracts against different microorganisms including Ps. aeruginosa, of these two studies that were done in Sudan by Shama et al. (2011) (13) and, Awdalla et al.(2020) (24) and other study conducted in Iraq by Hero and Jwan, (2012) (23) Therefore, L. sativum seed extract antibacterial activity may be related to their ability to inactivate cell envelope transport proteins, enzymes, microbial adhesions. and may be complex with polysaccharides (27).

Conclusion

The study concluded that L. sativum seed extracts revealed antibacterial activity against Ps. aeruginosa clinical isolated used in this study. Accordingly, the traditional plants may represent a new source of antimicrobials that can establish a scientific base for the use of plants in modern medicine. The ethanol and aqueous seed extracts showed variation in the degree of their efficiency against the studied bacteria; with increased affections of aqueous extract than ethanolic extract which it may reflects the differences in chemical constituents of L. sativum extract or the differences in the examined strains.

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Conflicts of interests

The authors declare that there are no conflicts of interest.

Ethical permission

Approval was taken from the Research Ethical Committee of Al-Neelain University and informed consent was obtained from the mentioned hospital.

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