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ANTIBACTERIAL ACTIVITY OF LEPIDIUM SATIVUM AND ALLIUM PORRUM EXTRACTS AND JUICES AGAINST SOME GRAM POSITIVE AND GRAM NEGATIVE BACTERIA

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SUMMARY: The antibacterial effect of ethanolic and aqueous extracts of medicinal plants Lepidium sativum (cress garden) and Allium porrum (leek), in addition to their juices, was investigated on Gram negative and Gram positive bacteria (Klebsiella pneumoniae, Proteus, Pseudomonas aeruginosa, Staphylococcus aureus, and Streptococcus mutans). All bacteria under this study were obtained from human infections from Hawlery Ferkary Hospital in Erbil City – Iraq, by using the well diffusion technique. It was observed that the extracts of both plants had an inhibitory effect on all the bacteria under study, except Klebsiella pneumoniae, whereas the juices of both plants did not have any effect on these bacteria. The minimum inhibitory concentration (MIC) of L. sativum extracts was determined and it was 3% for Klebsiella pneumoniae and Proteus, whereas other bacterial species were sensitive to all concentrations of the extracts. The MIC of ethanolic extract of A. porrum was 8% for S. aureus and 9% for P. aeruginosa, whereas K. pneumoniae and Proteus were insensitive to all concentrations in contrast to S. mutans that was sensitive to all concentrations. The MIC of aqueous extract of Alllium porrum did not affect K. pneumoniae and Proteus in contrast to other bacteria.

Key words: Lepidium sativum, Allium porrum, antibacterial agent, Gram positive, Gram negative.

INTRODUCTION

The medicinal plants are being used for treatment of infections is an age-old practice especially in developing countries (1). Plants still remain a major source for drug discovery in spite of the great development of synthetic molecules. Consequently, the uses of traditional plant extract in the treatment of various diseases have been flourished (2). According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety, and efficiency. In recent years, pharmaceutical companies have spent considerable time and money in developing therapeutics based on natural products extracted from plants. The rising incidence of multidrug resistance among pathogenic microbes has further necessitated the need to search for newer antibiotic sources. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, phenolic compounds, which are

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part of essential oils, Tannin, terpenoids, alkaloids, and flavonoids. These metabolites have been found in vitro to have antimicrobial properties. Many plants have been evaluated not only for their inherent antimicrobial activity but also for their action as a resistance-modifying agent. The enhancement of antibiotic activity or the reversal of antibiotic resistance by natural or synthetic nonconventional antibiotics has lead to the classification of these compounds as modifiers of antibiotic activity (3 and 4). Besides small molecules from medicinal chemistry, natural products are still major sources of innovative therapeutic agents for various conditions, including infectious diseases. Current research on natural molecules and products primarily focuses on plants since they can be sourced more easily and selected on the basis of their ethnomedicinal use. The antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms. Several reports are available in literature regarding the antimicrobial activity of plant crude extracts and the procedure for the bioassay-guided fractionation to yield active principles from these plants (5). In recent years, human pathogens have developed resistance in response to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. Undesirable side effects of certain antibiotics and the emergence of previously uncommon infections led the scientists to look for new antimicrobial substance from various sources, especially from medicinal plants. The screening of plant extracts and products presents potential sources of new antimicrobial agents (1).

Lepidium sativum L., (LS) cress, locally known as Rashad, is a fast-growing annual tall glabrous herb with an erect stem belonging to the Brassicaceae family. This herb is widely distributed in many countries across the globe (2, 6 and 7) and is a native herb. LS seeds contain volatile essential aromatic oils, active principle, and fatty oils; carbohydrate, protein, and fatty acid; β-carotene, riboflavin, and niacin; and ascorbic acid, flavonoids, and isothiocynate glycosides (8 and 9).

Allium genus has over 500 members, each differing in maturing time, color, and taste, but having similar biochemical, phytochemical, and neutraceutical contents. *Alliums* are revered to possess antibacterial and antifungal activities and include powerful antioxidants, sulfur, and other numerous phenolic compounds, which arouse significant interests in researchers (10).

MATERIALS AND METHODS

Bacteria tested

The bacteria under study (*Klebsiella pneumoniae, Proteus, Pseudomonas aeruginosa, Staphylococcus aureus, and Streptococcus mutans*) were obtained from human infections from Hawlery Ferkary Hospital in Erbil City–Iraq. The isolates were inoculated on agar to obtain a single colony, which were subcultured on the same medium to check for the purity of the isolated bacteria. Purified isolates were identified using morphological, cultural, and some biochemical tests, as a more accurate method for identification.

Plant Extraction

Collection and preparation of plant samples

Both plants L. sativum (cress garden) and Allium porrum (leek) were obtained from market in Erbil City. The plants were washed with tap water using soap powder, and then were washed with distilled water. They were then air dried, powdered, and stored in polyethylene bags in refrigerator at 4°C for further processes.

Extract preparation

The ethanolic and aqueous extracts of both plants were prepared by maceration method according to the procedure discussed in Reference 11 with slight modification. A total of 10 gm of the plant powder was steeped in 100 ml of each solvent (ethanol and sterilized distilled water) for 3 days, and then filtered through eight-layered muslin cloth. They were further filtered using filter paper (Whatman No.1) and centrifuged at 3000×g for 10 minutes. The supernatants were collected separately and stored in sterile bottles at 4°C.

Preparation of plant juic

Concentrated juices of cress garden and leek were obtained by crushing and squeezing them separately. Both juices were filtered and stored at 4°C until use (12).

Well diffusion technique

Screening of antibacterial activity was performed by well diffusion technique (13). The Nutrient agar (NA) plates were seeded with 0.1 ml of the inoculums of each tested organism. The inoculums were spread evenly over the plates with a loop. A standard cork borer of 8-mm diameter was used to cut uniform wells on the surface of the NA, and 100 µl of each concentration of plant extracts or juices was introduced in the well. The plates were incubated for 24 hours at 37°C, and the zones of inhibition was measured to the nearest millimeter (mm).

Preparation of inoculum

Two to three colonies from the pure growth of each tested organism were transferred each to 5 ml nutrient broth. Broths were incubated overnight at 37°C (14).

Agar dilution method

The NCCLS-approved agar dilution method with slight modification was followed. A series dilution of each extract ranging

from 10% (v/v) to 1% (v/v) was prepared in NA. After solidification of media, the plates were inoculated with bacterial suspensions using the streak method.

Inoculated plates were incubated at 37°C for 24 h. Minimum inhibitory concentrations (MICs) were determined after 24 h, as the lowest concentration of the extract inhibiting the visible growth of each organism on the agar plate. The presence of one or two colonies was disregarded (15). All experiments were applied in triplicates.

Statistical analysis

The data were analyzed using the Complete Random Design (CRD), with each treatment performed in triplicates. Their means were compared using the LSD Least Significant Difference Test (LSD). No statistical analysis was performed for the effect of juices, as the juices were ineffective on all the bacterial species under study.

RESULTS AND DISCUSSION

All bacterial species included in the test (Tables 1, 2, 3, and 4) showed susceptibility toward ethanolic and aqueous extracts of both plants under study except *K. pneumoniae*. It was also observed that the ethanolic extracts of both plants acted as better antibacterial agents than the aqueous extracts. This may be attributed to the difference in the activity of the active compounds when extracted with different solvents (16); the results of statistical analysis also showed a significant difference ($p < 0.05$). *K. pneumoniae* showed resistance against all extracts. This is observed in other studies as well(17) in which *K. pneumoniae* was found resistant to all extracts, including A. porrum, used in their studies. This may be attributed to the presence of a capsule in *K. pneumoniae* structure that protects it from the effect of plant extract or prevents the entrance of these extracts to inner of the cell.

The antibacterial activity of *L. sativum* is stronger than that of A. porrum; published reports also suggest that the antimicrobial activity of *A. porrum* is very limited (10 and 13). It is noted from the present result that the extracts of *L. sativum*, especially the ethanolic extract, had maximum antibacterial activity, which is identical with results obtained from other researchers (4, 5 and 18).

The juices of both plants did not show any antibacterial activity against bacteria tested in the study (Table 5).

The minimal inhibitory concentration (MIC) was determined by agar dilution method (Tables 6, 7, 8, and 9). The MIC of both extracts of *L. sativum* was found to be 3% against *K. pneumoniae* and *Proteus*, whereas other bacterial species were sensitive to all concentrations of these extracts. The MIC of ethanolic extract of *A. porrum* was 8% against *S. aureus* and 9% against *P. aeruginosa*, whereas *K. pneumoniae* and *Proteus* were insensitive to all concentrations of this extract in contrast to *S. mutans* that was affected by all concentrations. The MIC of aqueous extract of *A. porrum* did not affect *K. pneumoniae* and *Proteus* in contrast to other bacterial species.

However, negative results do not indicate that the bioactive constituents are absent or that the plant is inactive. Active compounds may be present in insufficient quantities in the crude extracts; therefore, the dose levels employed would not be sufficient enough to exhibit the inhibitory activity. The lack of inhibitory activity can thus only be proven by using large doses. Alternatively, even if the active principle is present in high enough quantities, there could be other constituents exerting antagonistic effects on the positive effects of the bioactive agents, thus zeroing the antibacterial activity of the principle. It is also possible that the extracts may be active against other bacterial species that were not tested (5).

It is concluded that both plant extracts have the antibacterial activity against G+ and G- bacteria; it requires further investigations in this field.

Table 1: Antibacterial activity of ethanolic extract of Lepidium sativum.

Concentration of plant extract	*Zone of inhibition in mm				
	Klebsiella pneumoniae	*Proteus spp.*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Streptococcus mutans*
100%	-	16	17	19	18
75%	-	15	15.5	15	15
50%	-	13	15	13	12
25%	-	-	13	11	9
12.5%	-	-	-	-	8
LSD	Non significant	3.52	4.21	3.47	6.57

*Values calculated as mean of triplicates. -: No inhibition zone or less than 8 mm.

Table 2: Antibacterial activity of aqueous extract of *Lepidium sativum.*

Concentration of plant extract	*Zone of inhibition in mm				
	Klebsiella pneumoniae	*Proteus spp.*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Streptococcus mutans*
100%	-	13	15	16	16
75%	-	-	11	11	9
50%	-	-	-	-	8
25%	-	-	-	-	-
12.5%	-	-	-	-	-
LSD	Non significant	1.15	7.84	4.62	3.27

*Values calculated as mean of triplicates. -: No inhibition zone or less than 8 mm.

Table 3: Antibacterial activity of ethanolic extract of *Allium porrum.*

Concentration of plant extract	*Zone of inhibition in mm				
	Klebsiella pneumoniae	*Proteus spp.*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Streptococcus mutans*
100%	-	-	15.5	17	-
75%	-	-	12	13	-
50%	-	-	10	-	-
25%	-	-	-	-	-
12.5%	-	-	-	-	-
LSD	Non significant	Non significant	3.88	8.10	Non-significant

*Values calculated as mean of triplicates. -: No inhibition zone or less than 8 mm.

Table 4: Antibacterial activity of aqueous extract of *Allium porrum.*

Concentration of plant extract	*Zone of inhibition in mm				
	Klebsiella pneumoniae	*Proteus spp.*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Streptococcus mutans*
100%	-	-	15	17	17
75%	-	-	13	15	14
50%	-	-	-	-	-
25%	-	-	-	-	-
12.5%	-	-	-	-	-
LSD	Non significant	Non significant	3.65	3.65	2.31

*Values calculated as mean of triplicates. -: No inhibition zone or less than 8 mm.

Table 5: Antibacterial activity of *Lepidium sativum* juice.

Concentration of L. sativum	*Zone of inhibition in mm				
	Klebsiella pneumoniae	*Proteus spp.*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Streptococcus mutans*
100%	-	-	-	-	-
75%	-	-	-	-	-
50%	-	-	-	-	-
25%	-	-	-	-	-
12.5%	-	-	-	-	-
Concentration of A. porrum	*Zone of inhibition in mm				
	Klebsiella pneumoniae	*Proteus spp.*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Streptococcus mutans*
100%	-	-	-	-	-
75%	-	-	-	-	-
50%	-	-	-	-	-
25%	-	-	-	-	-
12.5%	-	-	-	-	-

*Values calculated as mean of triplicates. -: No inhibition zone or less than 8 mm.

Table 6: MIC of ethanolic extract of *Lepidium sativum* (%v/v).

Concentration of plant extract	*Klebsiella pneumoniae*	*Proteus spp.*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Streptococcus mutans*
1%	R	R	S	S	S
2%	R	R	S	S	S
3%	S	S	S	S	S
4%	S	S	S	S	S
5%	S	S	S	S	S
6%	S	S	S	S	S
7%	S	S	S	S	S
8%	S	S	S	S	S
9%	S	S	S	S	S
10%	S	S	S	S	S

Table 7: MIC of aqueous extract of *Lepidium sativum* (%v/v).

Concentration of plant extract	*Klebsiella pneumoniae*	*Proteus spp.*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Streptococcus mutans*
1%	R	R	S	S	S
2%	R	R	S	S	S
3%	S	S	S	S	S
4%	S	S	S	S	S
5%	S	S	S	S	S
6%	S	S	S	S	S
7%	S	S	S	S	S
8%	S	S	S	S	S
9%	S	S	S	S	S
10%	S	S	S	S	S

Table 8: MIC of ethanolic extract of *Allium porrum* (%v/v).

Concentration of plant extract	*Klebsiella pneumoniae*	*Proteus spp.*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Streptococcus mutans*
1%	R	R	R	R	S
2%	R	R	R	R	S
3%	R	R	R	R	S
4%	R	R	R	R	S
5%	R	R	R	R	S
6%	R	R	R	R	S
7%	R	R	R	R	S
8%	R	R	R	S	S
9%	R	R	S	S	S
10%	R	R	S	S	S

Table 9: MIC of aqueous extract of *Allium porrum* (%v/v).

Concentration of plant extract	*Klebsiella pneumoniae*	*Proteus spp.*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Streptococcus mutans*
1%	R	R	S	S	S
2%	R	R	S	S	S
3%	R	R	S	S	S
4%	R	R	S	S	S
5%	R	R	S	S	S
6%	R	R	S	S	S
7%	R	R	S	S	S
8%	R	R	S	S	S
9%	R	R	S	S	S
10%	R	R	S	S	S

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