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The Antimicrobial activity of *Allium porrum* Water Extract against some pathogenic bacteria

الفعالية ضد الحيوية للمستخلص المائي لنبات الكراث ضد بعض البكتريا المرضية

Rana K.Naem[1]*, Noora A. Hadi[1]
[1]Dept. Of Biotechnology /College of Science / Univ. of Al-Nahrain

ABSTRACT

This study was designed to evaluate antimicrobial activity of *Allium porrum* aqueous extract against some pathogenic bacteria. So that, the aqueous extract of *A. porrum* was screened for its anti-microbial activity using the plate agar diffusion method. It was tested against six bacterial species; three Gram-positive bacteria (*Bacillus subtilis, Streptococcus pneumonia* and *Staphylococcus aureus*) and three Gram-negative bacteria (*Escherichia coli, Proteus vulgaris* and *Pseudomonas aeruginosa*). The susceptibility of these different bacterial species toward the extracts of this plant was compared with each other and with selected antibiotics (gentamicin and tetracycline) used as positive control. Results showed that aqueous extract of *A.porrum* had antimicrobial activity against all bacterial species.Then the active components which exist in aqueous extract were detected. Result indicated that amino acid with sulphate, glycosides, saponin and phenol were present in the aqueous extract.
Keywords: *Allium porrum,* aqeous extract, antimicrobial activity, sulphar compound

الخلاصة

صممت الدراسه لتقييم التاثير ضد البكتيري للمستخلص المائي لنبات الكراث على بعض البكتيريا المرضيه التي تم عزلها من بغداد.تم تحضير المستخلص المائي لنبات الكراث ودراسة تأثيره في نمو ست عزلات بكتيرية مرضيه مختلفة ثلاثة منها موجبه لصبغه كرام (*Bacillus subtilis, Streptococcus pneumonia* and *Staphylococcus aureus*) وثلاثة سالبه لصبغه كرام.(*Escherichia coli, Proteus vulgaris* and *Pseudomonas aeruginosa*) باستخدام طريقه الانتشار عبر الوسط ألزرعي. قورنت النتائج بين الأنواع الستة وبين تأثير نوعين من المضادات الحيوية الواسعة الانتشار (جنتاميسين وتيتراسايكلين) على هذه الأنواع من البكتيرية المرضية.اظهرت النتائج بان المستخلص المائي لنبات الكراث يمتلك فعاليه ضد مايكروبيه ضد البكتريا الموجبه والسالبه لصبغه كرام. وأخيرا اجريت فحوصات كيميائيه مختلفة لدراسة التحليل الكيميائي للمستخلص ومعرفه مكوناته الكيميائية. اظهرت النتائج وجود الكلايكوسيدات ,الفينول ,الصبونين,احماض امينيه حاويه على الكبريت في المستخلص المائي لنبات الكراث.

Introduction

Several plants were widespread for their many therapeutic and pharmaceutical virtues, especially antioxidant, anti-tumor, and anti-infectious activities. A big part of the world's population still relies on the benefits of food for the treatment of common illnesses [21]. These benefits provide from their big content on bioactive compounds [8].

A. porrum , Common Name: leek plant, Family: Alliaceae. The leek (*Allium porrum*) is an annual herbaceous biennial plant. It is closely related to onion. The leaves and long white bunched stem are eaten cooked or added to salads [20].

Leeks contain excellent amounts of vitamin C, as well as folate and amounts of some of the B vitamins, vitamin E, copper, potassium and iron. [17].

The extract of *A. porrum* contains high levels of sulfur compounds that work on the inhibition of microbial growth through prevent of one of basic needs for microbial cell growth and reproduction, for

example, the paramenobenzoic acid is a metabolic compound in which the microbial cell needs to form folic acid and this acid is the key material for the continued growth and multiplication of microbial cell and the sulfur compounds contained within the *A. porrum* extract prevents the formation of this baramenobenzoic acid [13]. *A. porrum* used in treatment of many disease example stomach ulcer, , sores, wounds, Tuberculosis, reduced blood pressure and Anti-helminthic [4, 9]. Allicin has antibacterial properties against a wide range of bacteria. Allicin even showed effectiveness against multidrug resistant strains of *E. coli*. *E. coli* is mainly known for causing life-threatening causes of food poisoning. Additional studies have reported that consuming allicin may kill bacteria that cause stomach and intestine ulcers [1].

Materials and Methods

Plant Material

A. porrum was used throughout this study collected from locall markets. The collected leeks cleaned with water and then air-dried, coarsely powdered and then extracted.

Microbial Organisms

Three Gram-positive bacteria (*Bacillus subtilis, Streptococcus pneumonia* and *Staphylococcus aureus*) and three Gram-negative bacteria (*Escherichia coli, Proteus vulgaris* and *Pseudomonas aeruginosa*) was used throughout this study and kindly obtained from Al-Nahrain University / Department of Biotechnology. Gram-negative strains were cultured using nutrient broth and brain-heart infusion broth for Gram-positive strains.

Crud Extract Preparation

Water extract was prepared by adding 10ml of boiled distilled water to 5g of coarsely powdered plant leaves of *A. porrum* in a beaker placed on warm water bath at 45°C with occasional stirring for 4 hrs. The aqueous extract was then filtered and rewashed with small volume of boiled distilled water and added to the filtrate. Finally the filtrate was concentrated by evaporation at 45°C using a rotary evaporator in which the final volume of crude extracts was 5ml which was used immediately **[**16**].**

Preparation of Tested Microorganisms

The average number of viable *Bacillus subtilis,Streptococcus pneumonia*, *Staphylococcus aureus, Escherichia coli, Proteus vulgaris* and *Pseudomonas saeruginosa* microorganisms per ml of the stock suspensions was determined by means of the viable counting technique [15]. About (10^8-10^9) colony-forming units(CFU) per ml was used. Each time, a fresh stock suspension was prepared; the experimental conditions were maintained constant.

In Vitro Testing of Anti-Microbial Activity of the crude extract

The plate agar diffusion method was adopted according to the method described elsewhere [14] in order to assess the antibacterial activity of the prepared extracts. An amount of 0.6ml of standardized bacterial stock suspensions (10^8-10^9) CFU per ml was aliquot of mixed with 60ml of sterile nutrient agar. About 20µl of the inoculated nutrient broth were distributed into sterile Petri dishes of nutrient agar. The agar was left to adsorbed in each of these plates, 1 well (10 mm in diameter) were cut using a sterile cork borer No. 4. The agar discs were removed and filled with 0.1ml of the crude extract and allowed to

diffuse at room temperature for two hrs. The plates were then incubated in the upright position at 37ºC for 18 hrs. Two replicates were carried out for the extract against each of the tested organism. Simultaneous addition of the respective solvents (Gentamicine and Tetracycline) instead of extracts was carried out as controls. After incubation, the diameters of growth inhibition zones were measured.

Active component detection

The most important chemical ingredients in the crude extract of *A. porrum* was detected using different reagent according to [12],(chemical ingredients in *A. porrum* aqueous extract was summarized in Table (2).

Results and Discussion

The aqueous extract of leaves of *A. porrm* was subjected to preliminary screening for antimicrobial activity against six bacterial species: *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Streptococcus pneumonia*, *Bacillus subtilis* and *Staphylococcus aureus*. According to Table (2), the crude extract of *A. porrum* showed high activity against Gram positive bacteria rather than Gram negative bacteria, the inhibition zone reached 31 mm in diameter against *Bacillus subtilis*, *Staphylococcus aureus* and Streptococcus *pneumonia*. On the other hand the zone of inhibition reached to 26, 25, 24 mm in diameter against to *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Escherichia coli* respectively.

The *A. porrum* contains numerous organic sulfur compounds, including trans-S-(l- propenyl) cysteine sulfoxide, S-methyl-cysteine sulfoxide, S-propylcysteine sulfoxide in addition to cycloalliin; flavonoids, phenolic acids, sterols including: (cholesterol, stigma sterol, b-sitosterol), saponins sugars and a trace of volatile oil composed mainly of sulfur compounds. The presence of these compounds may explain the antimicrobial activity of *Allium porrum*. These results were similar to those obtained by Merih and Buket, 1997; which were reported that the leek inhibited the growth of *Escherichia coli* and *staphylococcus aureus* [10, 3].

Table (2): Antibacterial activity of crude aqueous extract of *A.porrum* against some pathogenic bacterial species as compared with Gentamicin and Tetracycline.

Bacterial strain	Mean Diameter of growth Inhibition Zone in (mm)		
	Crude aquoes extract of A. porrum	**Gentamicine**	**Tetracycline**
B. subtilis	**31**	**30**	**27**
S. aureus	**30**	**20**	**31**
S. pneumonia	**30**	**24**	**28**
E.coli	**24**	**25**	**24**
P. aeruginosa	**26**	**25**	**-**
P. vulgaris	**25**	**22**	**-**

On the other hand Breu and Dorsh in 1994 noted that the aqueous and alcoholic extracts of leaves have a powerful antibacterial activity as they inhibit the growth of the bacteria *E. coli, Streptococcus sp., Serratia marcesceus, Pseudomonas aeruginosa, Staphylococcus aureus* and *Salmonella typhi* [2].

The cell wall structural nature of Gram-negative enteric bacteria may be responsible for the observed susceptibility. For instance, the cell wall of Gram-negative bacteria contains 15-20% polysaccharides and 10-20% lipid, whereas that of Gram-positive bacteria contain 35-60% polysaccharides and only 0-2% lipid [6]. The cell membrane of *E. coli* has been reported to contain 20% lipid [19]. The polysaccharides and the lipid contents of the cell wall affect the permeability of allicin and other porrum constituents, and thus the observed susceptibility to porrum by the diarrheagenic organisms [7, 18].

The antimicrobial activity of crude extract of *A. porrum* was compared with that of the antibiotics (gentamicine and tetracycline) which considered to popular in treatment of diseases against those six types of standard bacterial species, the effect of crude extract reached to that of antibiotics at concentration 100 µg/ml.

Chemical analysis (Table, 2) showed that the aqueous extract of *Allium porrum* has many biological active compound example free amino acids, amino acids that contain sulphar, glycosides and saponins. It is known that Alliaceae family contains many sulphate compounds in addition to other chemical compound which considered being pharmaceutically valuable [5, 11].

Table (2): Different Chemical contents of *Allium porrium*.

Reagent	Aqueous Extract	Notes
Ninhydrin	Blue color	detect Free amino acids
NaOH + Lead Acetate	White Precipitation	detect amino acid with sulphate
Benedict	Brown Precipitation	detect Glycosides
Dragendroff Wagner Mayer	Negative Reaction	No Alkaloids
Molish	Purple Ring	detect Carbohydrate
$HgCl_2$ 5%	White Precipitation	detect Saponine
$FeCl_3$ 1%	Negative Reaction	detect Phenol
Alcoholic KOH	Negative Reaction	No Flavonids
Lead Acetate 1%	Negative Reaction	No Tannins

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