

Effects of aqueous and ethanol extracts of Akaka plants *Allium akaka* Gmel on some standard pathogenic bacteria

Abdulghany Omer Ismaeel Sarmamy

Department of Biology / College of Science / University of Salahaddin / Kurdistan
Region / Republic of Iraq

Email : abdulghani.ismaeel@su.edu.krd

Abstract:

The present study was conducted, to determine the bactericidal effects of aqueous and ethanol extracts of vegetative parts of Akaka plants *Allium Akaka* Gmel. On some standard pathogenic bacteria such as *Staphylococcus aureus*(ATCC 25923),*Escherichia coli* (ATCC 35218),*Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella Pneumoniae* (ATCC 1031). Plant vegetative parts were extracted (crude extraction) using distilled water or ethanol (80%). Concentrations of 250, 500, 1000, 2000, 4000, 8000, 10000 and 20000 $\mu\text{g ml}^{-1}$ or $\mu\text{g disc}^{-1}$ using Disk Saturation Technic (DST) and Disk Loading Technique (DLT) respectively and 20000 $\mu\text{g ml}^{-1}$ delusions using Enzyme Linked Immunosorbent Assay (ELISA) were applied .Sterilized water and Streptomycin were used as control. Data was analyzed statistically using SPSS and treatment means were compared using Duncan Multiple Range Test at probability range 0.01. Results showed that Akaka plant extracts contains antibacterial chemical compounds that affect bacterial growth. Minimum Inhibition Concentrations (MIC) of aqueous extract was 4000 $\mu\text{g disc}^{-1}$ for *Staphylococcus aureus* and 8000 $\mu\text{g disc}^{-1}$ for other three bacteria using DLT. MIC of ethanol extract was 2.48 $\mu\text{g ml}^{-1}$ for *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and 4.95 $\mu\text{g} : \text{ml}^{-1}$ for *E. coli*. Minimal Bactericidal Concentration (MBC) of ethanol extracts using ELISA Technique was 10000 $\mu\text{g ml}^{-1}$ for

Staphylococcus aureus and *Klebsiella pneumoniae*, 20000 $\mu\text{g ml}^{-1}$ for *E. coli* and 625 μgml^{-1} for *Klebsiella pneumoniae*.

Keywords: Akaka plant *Allium akaka* Gmel., Extraction, Bacteria.

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Introduction

Many higher plants accumulate extractable organic chemical compounds in quantities sufficient to be economically useful as pharmaceuticals / antibiotics. Higher plants species are less surveyed for antibacterial and antifungal activities in our country. In many developing countries, traditional medicine is still the pole of health-care, and most of the drugs and cures come from natural sources, such as, plants. Even in developed countries, the raw materials for manufacturing essential drugs are extracted from medical plants using their natural properties of healing. A lot of people are turning to herbal medications, especially for treating minor illness. Akaka is one of the medicinal plants belongs to Liliaceae family naturally grown in the central sector of alpine region of Iraq on the mountains of Helgurd, Kodo and Qendil. Akaka reproduced by bulbs mainly and by seeds, distributed throughout the Kwestan lawns of the northern parts of Erbil governorate in

Kurdistan Region-Iraq. Plant parts are used in food making, folk medicine for treating cases of high blood pressure and for regulating blood cholesterol. The plant contains sulphur compounds with an onion flavor. It also acts as a tonic to the digestive system and circulatory system. Plant bulbs - raw or cooked were used as an onion substitute in food making. The un-mature plants are a great delicacy and they were used as additives to rice in pilaw (traditional Kurdish cooked rice). Akaka leaves (raw or cooked) and flowers (raw) were used as adornment on salads. Published literatures in this field had clearly showed the effects of such a plant extracts on fungal and bacterial growth (14, 15 and 16).

This study was aimed to determine the main groups of chemical constituents and the antibacterial effects of aqueous and ethanol extracts of akaka against some standard pathogenic bacteria using different Techniques (methods).

Materials and Methods

Preparation of Plant Extracts: Plant samples were collected from Qendil Mountain, immediately brought to the laboratory and dried out as soon as possible. Plant parts were milled by electrical grinder passed through 2mm mesh, and extracted by macerating 100gm of the powder in 200ml of ethanol 80% or distilled water in volumetric flask renounced for 24 hours on an electric shaker. Plant extracts were filtered twice first by passing it through folded layers of gauze and by Buchner apparatus, then concentrated using Rotary Vacuum Evaporator (RVE) at 40-45° C. (8 and 9) and sterilized by passing the extract solutions through bacterial filter (Seitz). Five grams of concentrated raw extracts were poured into a flask, 5 ml of dimethyl sulphoxide (DMSO) and 45 ml of sterilized distilled water were added, to prepare a solution of $100000\mu\text{g ml}^{-1}$ as a stock solution. Different plant extract concentrations were then prepared from the stock solution.

Determination of MIC and MBC (Minimum Bactericidal Concentration) Disc Diffusion Technique:

Paper discs of 7mm in diameter, were prepared, from thick filter paper and sterilized by keeping them in an incubator at 70 °C for 48 hours. Paper disks were placed in beakers, containing extract solutions of different concentrations, for 10 hours for saturation {Disk Saturation Technique (DST)} and then, placed in an incubator for 6 hours. at 40°C until drying. While in the second method, the requested amounts of different concentrations for each treatment were placed on the paper discs {Disk Loading Technique (DLT)} using micropipettes of different volumes (13). Both nutrient broth and nutrient agar, were prepared according to Harigan and McCane,(10).

Enzyme Linked Immunosorbent Assay (ELISA):

Inoculums was prepared, according to (6 and 5). 100µl of Mueller-Hinton Broth (MHB) was

dispensed into the wells of a micro titer plate 100µl of different extraction concentrations, from working solution was transferred into the well number 1 (far left of plate). The extract was mixed with MHB in the well number 1 by sucking up and down 10 times to make the well number 1 twofold dilution of the stock. 100 µl was withdrawn, from well number 1 and added to the well number 2, in the same row. This procedure was repeated down, until well number 12 and the 100 µl from well number 12 was discarded, then 10µl of bacterial suspension was added to wells No.1-12. This process was repeated for all treatments with triple replications and three of the reminder wells were used as control. Plates were scanned with an ELISA reader at absorbance 630nm before and after incubation at 37C° for 24 hrs. The MIC (minimum inhibition concentration) was detected by difference between before and after incubation. MBC (minimum bactericidal concentration) was taken by sub culturing the wells of

all plates after reading by ELISA (6 and 5).

Preparation of Standard Pathogenic Bacteria:

Staphylococcus aureus (ATCC 25923), *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC 1031) were from the laboratories of Rizgary Teaching Hospital-Erbil and College of Science, University of Salahaddin-Erbil.

Chemical Studies:

The preliminary detection of some active compounds of Akaka plant extract:

Alkaloids:

Ten ml of theaqueous and ethanol extracts were stirred with 36% HCl and then 1-2 drops of the picric acid reagent was added. The appearance of a yellow precipitation indicates the presence of alkaloids (12).

Saponins:

Three ml of AgCl₂ was added to 5 ml of aqueous and ethanol

plant extracts, the appearance of a white precipitate, indicated the presence of Saponins (20). For assurance, 5 ml of aqueous and ethanol extracts, were mixed, with twenty ml of distilled water and then agitated in a test tube for 15 minutes, formation of froth, indicates the presence of Saponins(11).

Flavonoids:

Solution A: One gram, of dry powdered of aqueous and ethanol extracts, were dissolved, in 10ml of 95% ethanol, leaved in a boiling water bath for 2 min.

Solution B: Ten ml of 50% of sodium hydroxide (NaOH) was added to 10 ml of 50% ethanol. Equal volumes, from both solutions A and B were mixed; a yellow color was developed, indicating the presence of Flavonoids (17).

Phenols:

Samples of the extracts were treated with 5% FeCl₃ reagent formation of a deep blue black

color, revealed the presence of phenols (19).

Resins:

Ten ml of dry aqueous and ethanol extracts, were added to 20 ml of 4% HCl, appearance of turbidity indicated the presence of resins (2).

Glycosides:

From each of the aqueous and ethanol extracts one ml was filtrated and then the filtrates placed in test tubes separately, and treated with 2 ml of Fehling reagent. The appearance of red – brown precipitate, indicated the presence of saccharides then retreated by adding 1 ml of the plant extracts to 5 ml Benedict's reagent, placed in boiling water bath for 5 minutes then cooled. The appearance of red precipitate confirmed the presence of saccharides (4).

Tannins:

From each of the aqueous and ethanol extracts, 0.5gm were stirred with 10ml of distilled water and then filtered. Few drops of 1%

ferric chloride solution were added to 2ml of the filtrate. Occurrence of a blue-black precipitate indicates the presence of tannins (21).

Carbohydrates:

From each of the aqueous and ethanol extracts, 0.5 ml was filtrated then the plant extracts were mixed with 3 drops of α – naphthol solution separately shaken vigorously then 1 ml of concentrated H_2SO_4 was added. The purple ring at the separation surface confirmed the presence of carbohydrates (3).

Terpenoids:

From each of the aqueous and ethanol extracts 5 ml was mixed, with 2 ml of chloroform and then 3ml of concentrated H_2SO_4 was carefully added, formation of a layer of red to brown color at the interface indicated the presence of terpenoids(19).

Results and Discussion

Chemical Studies:

Chemical studies revealed that, *Allium akaka* plant extracts contain many chemical compound groups such as alkaloids, Saponins, flavonoids, Phenolic compounds, resins, Glycosides, Tannins and carbohydrates (Figures 1,2,3,4,5& 6), which are physiologically active chemical compounds and can be of valuable advantages for using in different purposes, especially as antibacterial agents.

Bacterial Growth Inhibition (BGI) Effects, of Akaka Extracts on Standard Human Pathogenic Bacteria (SHPB):

BGI Effects of Akaka Aqueous Extracts (AAE) on SHPB using Disk Loading Technique (DLT):

Table 1 showed, that Akaka aqueous extracts affected the growth of SHPB and inhibited their growth highly significantly. The minimum concentration (MC) that inhibited the growth of *Staphylococcus aureus* was $4000 \mu\text{g disc}^{-1}$ and $10000 \mu\text{g disc}^{-1}$ for *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and

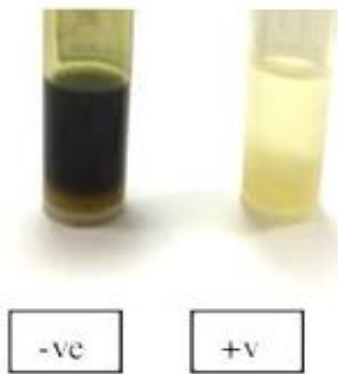


Fig.1: Saponins

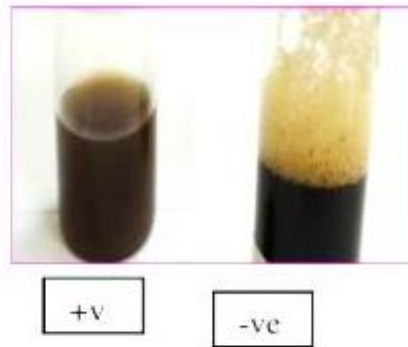


Fig. 2: Flavonoids



Fig. 3: Phenolic

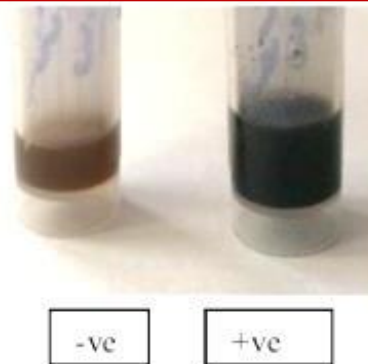


Fig. 4: Tannins

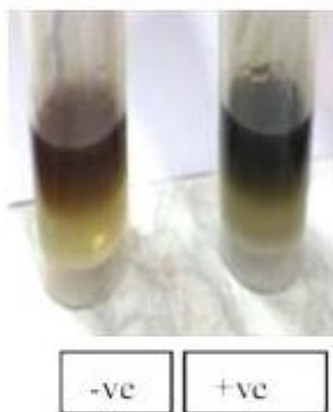


Fig.5: Carbohydrates

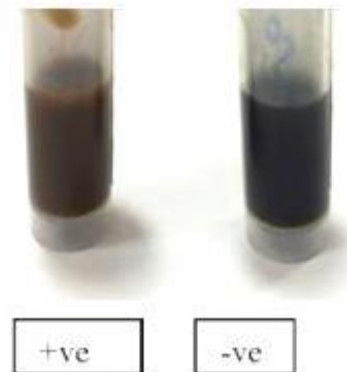


Fig.6: Terpenoids

Table 1: Effects of Akaka aqueous extracts on control of some standard humanpathogenic bacteria using disc loading technique.

Plant Extract Concentrations $\mu\text{g disc}^{-1}$	<i>Staphylococcus aureus</i>	<i>E. coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella Pneumoniae</i>
	Inhibitional Growth Circle Diameter (mm)			
Control	1.00±.0000 a*	1.00±.0000 a	1.00±.0000 a	1.00±.0000 a
250	1.00±.0000 a	1.00±.0000 a	1.00±.0000 a	1.00±.0000 a
500	1.00±.0000 a	1.00±.0000 a	1.00±.0000 a	1.00±.0000 a
1000	1.00±.0000a	1.00±.0000 a	1.00±.0000 a	1.00±.0000 a
2000	1.00±.0000 a	1.00±.0000 a	1.00±.0000 a	1.00±.0000 a
4000	7.00±.2887 b	1.00±.0000 a	1.00±.0000 a	1.00±.0000 a
8000	7.33±.1667 b	1.00±.0000 a	1.00±.0000 a	1.00±.0000 a
10000	10.00±.2887 c	7.67±.2887 b	7.67±.1667 b	7.67±.1667 b
20000	11.00±.5774 c	8.33±.5774 c	8.33± .3333c	8.33±.3333 c
Streptomycin	15.00±.2887 d	10.33±.5774 d	10.33± .3333d	10.33±.3333 d

*Means with the same letters are not different significantly depending on Duncan Multiple Rang Test at 0.01 of significance.

E.coli. The highest inhibitions were registered at concentration of 20000 $\mu\text{g disc}^{-1}$, which were 11.0, 8.3, 10.3 and 11.7mm of inhibition growth circle diameter (IGCD), for *Staphylococcus aureus*, *E.coli*,

Pseudomonas aeruginosa and *Klebsiella pneumoniae* respectively. The BGI effects of AAE on the growth of SPB may be attributed, to its physiologically active chemical constituents, such as carbohydrates which are well known, for their ability in penetration the barriers, and effects as antibacterial agents (22), and act as moisture holding compound that delay water and solute absorption and binding toxins (16). Or due to tannins, which effect on protein synthesis and bind to adhesions, (16) or both *Klebsiella pneumoniae* was sensitive, against AAE more than the three other bacterial genera. These results compatible with the findings of Abdullah, (1), but disagreed with El-Safey and Ali (7).

Effects of AAE on Control of SHPB using DST:

Table 2 showed that AAE affected the growth of SHPB and inhibited their growth highly significantly. The MC inhibited the growth, of *Staphylococcus aureus* was $4000\mu\text{g ml}^{-1}$ and $8000\mu\text{g ml}^{-1}$

¹for *E. coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The highest inhibitions were registered at concentration of $20000\mu\text{g ml}^{-1}$ which was 9, 7.2, 7.5 and 7.3mm Inhibition Growth Circle Diameter (IGCD) for *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* respectively. The effects of AAE concentrations were more effective, and accurate when used as DLT compared with DST, which may be because the disks that containing a distinct amount of the chemical compounds more accurately by using DLT.

Effects of Akaka Ethanol Extracts (AEE), on Control of SHPB using DLT:

Table 3 showed that AEE affected on the growth of SHPB and significantly inhibited their growth. The MC that inhibited the growth of *Staphylococcus aureus* was $2000\mu\text{g disc}^{-1}$, $8000\mu\text{g disc}^{-1}$ for *Klebsiella pneumoniae* and $10000\mu\text{g disc}^{-1}$ for *E. coli* and *Pseudomonas aeruginosa*. The

highest IGCD, were registered at mm for *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* concentration of 20000 $\mu\text{g disc}^{-1}$ which were 13.7, 8.3, 15.3 and 13

Table 2: Effects of Akaka aqueous extracts on control of some standard human pathogenic bacteria using disc saturation technique.

Plant Extract Concentrations $\mu\text{g ml}^{-1}$	<i>Staphylococcus aureus</i>	<i>E. coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumoniae</i>
	Inhibitional Growth Circle Diameter (mm)			
Control	1.00±.0000 a*	1.00±.0000 a	1.00±.0000 a	1.00±.0000 a
250	1.00±.0000 a	1.00±.0000 a	1.00±.0000 a	1.00±.0000 a
500	1.00±.0000 a	1.00±.0000 a	1.00±.0000 a	1.00±.0000 a
1000	1.00±.0000 a	1.00±.0000 a	1.00±.0000 a	1.00±.0000 a
2000	1.00±.0000 a	1.00±.0000 a	1.00±.0000 a	1.00±.0000 a
4000	1.00±.0000 a	1.00±.0000 a	1.00±.0000 a	1.00±.0000 a
8000	7.17±.1667 b	1.00±.0000 a	1.00±.0000 a	1.00±.0000 a
10000	7.33±.3333 b	7.1667±.1667b	7.33±.5774 b	7.67±.1667 b
20000	9.00±.5774 c	7.1667±.1667b	7.50±.5000 b	7.33±.3333 b
Streptomycin	13.33±.1667 d	10.50±.2887 c	14.00±.5000 c	10.67±.3333 c

*Means with the same letters are not different significantly depending on Duncan Multiple Rang Test at 0.01 of significance.

Table 3: Effects of Akaka ethanol extractson control of some standard

humanpathogenic bacteria using disc loading technique.

Plant Extract Concentrations $\mu\text{g disc}^{-1}$	<i>Staphylococcus aureus</i>	<i>E. coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumoniae</i>
	Inhibitional Growth Circle Diameter (mm)			
Control	1.00 ±.0000a*	1.00 ±.0000 a	1.00±.0000 a	1.00 ±.0000 a
250	1.00 ±.0000a	1.00±.0000 a	1.00 ±.0000a	1.00 ±.0000 a
500	1.00±.0000 a	1.00±.0000 a	1.00 ±.0000a	1.00 ±.0000 a
1000	1.00±.0000 a	1.00 ±.0000 a	1.00 ±.0000a	1.00 ±.0000 a
2000	5.00 ±.2887a	1.00 ±.0000 a	1.00 ±.0000a	1.00 ±.0000 a
4000	6.00 ±.2887b	1.00 ±.0000 a	1.00 ±.0000a	1.00 ±.0000 a
8000	7.67 ±.0000a b	1.00 ±.0000 a	1.00 ±.0000a	9.33 ±.3333 b
10000	6.60 ±3.3005 b	7.70 ±.3606 b	7.33±.3604 b	9.83 ±.1667 b
20000	13.67±.3333 c	8.33±.5774 b	7.33 ±.3604b	13.00 ±.5774 c
Streptomycin	18.50±.2867d	12.67±5774 c	11.33 ±. 5765c	14.00 ±.5774 c

*Means with the same letters are not different significantly depending on Duncan Multiple Rang Test at 0.01 of significance.

respectively, compared with 18.5, 12.7, 19.8 and 14 mm for *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*

respectively when treated with streptomycin.

The inhibitional effects of AEE may be due to the effectiveness of phenolic compounds terpenes and tannins which effects on substrate

deprivation (22) and inhibits micro-organisms, as well as, they bind to proteins and enzyme inhibitors respectively (18).

Phenolic compounds and tannins are well-known to effect on degradation of microbial phytotoxins (23).

Staphylococcus aureus was sensitive, against the different concentrations of AEE, more than three other bacterial genera.

Effects of AEE, on control of Standard Human Pathogenic Bacteria (SHPB) using DST:

Table 4 showed that AEE affected the growth of SHPB and significantly inhibited their growth. The MC that inhibited the growth, of *Staphylococcus aureus* and *Klebsiella pneumoniae* was 8000 $\mu\text{g ml}^{-1}$ and 10000 $\mu\text{g ml}^{-1}$ for *E. coli*, and *Pseudomonas aeruginosa*. The largest IGCD were registered at the concentration of 20000 $\mu\text{g ml}^{-1}$ which were 8.3, 7.3, 7.3 and 8.3 mm for *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*

respectively, compared with 15.3, 11.0, 11.3 and 12 mm for *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* respectively, when treated with streptomycin.

Effects of AEE on SHPB using Enzyme Linked Immunosorbent Assay {(ELISA (Biotech))}:

Table 5 showed that the different concentrations of AEE used in this study significantly affected the growth of SHPB, compared with control. There were highly significant differences between all concentrations in their effects on the growth of SHPB. Concentrations of 20000 and 10000 $\mu\text{g ml}^{-1}$ of Akaka were superior in their effects on the growth of *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, and registered the lowest values of light absorbance (0.9700 and 0.9950), (0.9320 and 0.9550), (0.9400 and 0.9650) and (0.9500 and 0.9560) respectively, compared with control (1.6000,

Table 4: Effects of Akaka ethanol extractson control of some standard humanpathogenic bacteria using disc saturation technique.

Plant Extract Concentrations $\mu\text{g ml}^{-1}$	<i>Staphylococcus aureus</i>	<i>E. coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella Pneumoniae</i>
	Inhibitional Growth Circle Diameter (mm)			
Control	1.00±.0000 a*	1.00 ±.0000a	1.00±.0000 a	1.00 ±.0000a
250	1.00±.0000 a	1.00±.0000 a	1.00 ±.0000a	1.00±.0000 a
500	1.00±.0000 a	1.00±.0000 a	1.00 ±.0000a	1.00 ±.0000a
1000	1.00±.0000 a	1.00±.0000 a	1.00±.0000 a	1.00±.0000 a
2000	1.00±.0000 a	1.00±.0000 a	1.00±.0000a	1.00 ±.0000a
4000	1.00±.0000 a	1.00±.0000 a	1.00±.0000 a	1.00±.0000 a
8000	7.00 ±.5774 b	1.00 ±.0000a	1.00±.0000 a	7.33 ±.3333 b
10000	7.33±.3333 bc	7.00±.5774 b	7.33±.3333 b	8.00 ±.5774 b
20000	8.33 ±.3333 c	7.50±.2887 b	7.33 ±.3333 b	8.33±.3333 b
Streptomycin	15.33±.3333 d	11.00±.5774 c	11.33±.3333 c	12.00±.5774 c

*Means with the same letters are not different significantly depending on Duncan Multiple Rang Test at 0.01 of significance.

1.8200, 1.8500 and 1.3400) for thefour SPB respectively, which means the lowest bacterial growth.

The inhibitional effects of AEE were decreased as the concentrations decreased (table 5).

The MIC of AEE has begun from $2.48\mu\text{g ml}^{-1}$ against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* and from $4.95\mu\text{g ml}^{-1}$ against *E. coli*.

The inhibitional effects of Akaka may be due to the effectiveness of phenolic compounds, flavonoids and tannins that are well known to effect on the substrate deprivation (22), and capable of modulating the activity of enzymes and bind to proteins respectively (18).

Bactericidal Effects of Akaka Extracts on SHPB:

The minimal bactericidal concentration (MBC) of AAE using DLT was $20000\mu\text{g disc}^{-1}$ for *Staphylococcus aureus*. MBC of AEE using DLT was $20000\mu\text{g disc}^{-1}$ for *Staphylococcus aureus* and *Klebsiella aeruginosa*. MBC of AEE using ELISA Technique was $10000\mu\text{g disc}^{-1}$ against *Staphylococcus aureus*, *E. coli* and *Klebsiella pneumoniae*, and $5000\mu\text{g disc}^{-1}$ against *Pseudomonas aeruginosa*.

Conclusions

Based on the results obtained, from the present study, it can be concluded that, the ELISA technique is the accurate method for determining the inhibitional concentrations and minimal bactericidal concentrations (MBC) than DLT and DST, and DLT is better than DST.

Alcohol solvents showed to be suitable for preparing plant extracts used for controlling bacterial growth because many of the physiologically active chemicals present in the plant tissues are soluble in alcohol more than in water. Akaka extracts has been shown to be effective as anti-bacterial agent due to its containing of physiologically active chemical compounds.

Recommendations

I recommend, forming team groups of scientific researchers in different fields of specializations for conducting detail studies, on the Medicinal plants grown in Kurdistan Region to establish a good information data bank about

their chemical consisting benefits from this national wealth
biological effects and taking for pest control and medication.

**Table 5: Effects of Akaka ethanol extractson control of some standard
humanpathogenic bacteriausing ELISA technique**

Plant Extract Concentrations	<i>Staphylococcus aureus</i>	<i>E.coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumonia</i>
$\mu\text{g ml}^{-1}$	Light Absorbance (nm) $\bar{X} \pm \text{Std. Error}$			
20000	0.9750 $\pm .0058\text{a}^*$	0.9320 $\pm .0116 \text{ a}$	0.9400 $\pm .0029$ a	0.9500 $\pm .0058\text{a}$
10000	0.9800 $\pm .0017\text{a}$	0.9550 $\pm .0087\text{ab}$	0.9650 $\pm .0058$ a	0.9560 $\pm .0058$ a
12500	0.9980 $\pm .0058\text{a}$	0.9570 $\pm .0000 \text{ b}$	0.9730 $\pm .0029$ a	0.9750 $\pm .0017$ a
625	1.0445 $\pm .0024\text{b}$	1.4600 $\pm .0058\text{c}$	0.9950 $\pm .0017$ a	1.0190 $\pm .0058$ b
312.5	1.0570 $\pm .0058\text{b}$	1.4900 $\pm .0087\text{cd}$	1.1630 $\pm .0075$ b	1.0320 $\pm .0029$ bc
156.25	1.0260 $\pm .0058\text{b}$	1.5200 $\pm .0058 \text{ d}$	1.1950 $\pm .0577$ b	1.0520 $\pm .0058\text{c}$
78.13	1.1620 $\pm .0127\text{c}$	1.5270 $\pm .0098 \text{ d}$	1.4500 $\pm .0116$ c	1.0890 $\pm .0058\text{d}$
39.06	1.2300 $\pm .0058\text{d}$	1.5500 $\pm .0116 \text{ d}$	1.5400 $\pm .0029$ d	1.1600 $\pm .0058$ e
19.8	1.2670 $\pm .0116\text{e}$	1.6260 $\pm .0116 \text{ e}$	1.6033 $\pm .0073$ de	1.1760 $\pm .0058$ ef

9.9	1.2800 ±.0058e	1.6600 ±.0058 f	1.6400 ±.0058 e	1.1900 ±.0029 f
4.95	1.3500 ±.0029f	1.7100 ±.0058 g	1.7133 ±.0088 f	1.2440 ±.0058 g
2.48	1.5500 ±.0087g	1.8020 ±.0046h	1.7900 ±.0029 g	1.3150 ±.0087 h
0	1.6000 ±.0116h	1.8200±.0028 h	1.8500 ±.0058 i	1.3400±.0058 i

*Means with the same letters are not different significantly depending on Duncan Multiple Rang Test at 0.01 of significance.

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**تأثير المستخلصات المائية والكحولية لنبات الاكاكا *Allium akaka* Gmel. على نمو
بعض انواع البكتريا**

القياسية

عبد الغني عمر اسماعيل سارمي

قسم علوم الحياة / كلية العلوم / جامعة صلاح الدين / إقليم كردستان/ جمهورية العراق

E-mail: abdulghani.ismaeel@su.edu.krd

المستخلص

أجريت الدراسة الحالية لمعرفة تأثير مستخلص الأجزاء الخضرية لنبات الاكاكا في السيطرة على نمو بعض انواع البكتريا القياسية الممرضة للإنسان (*Staphylococcus aureus* (ATCC 25923) و *Pseudomonas aeruginosa* (ATCC 27853) و *Escherichia coli* (ATCC 35218) و *Klebsiella Pneumoniae* (ATCC 1301) . استخلصت الأجزاء الخضرية للنبات بالماء المقطر (80%) وتم تحضير المستخلص 250، 500، 1000، 2000، 4000، 8000، 10000 و 20000 مايكروغرام. مل⁻¹ او مايكروغرام. قرص⁻¹ استعملت تكتيكي اشباع الاقراص بالمستخلص Disk Saturation Technique (DST) وتحميل الاقراص بالكميات المطلوبة من المستخلص و Disk Loading Technique (DLT) وعلى التوالي، و20000 مايكروغرام. مل⁻¹ أذيتت باستعمال تكتيك تخفيفات ما يسمى Enzyme Linked Immunosorbent Assay (ELISA). الماء المقطر والمضاد الحيوي Streptomycin استعملت كعامل مقارنة (control). حلت البيانات احصائيا باستعمال نظام SPSS وقورنت المتوسطات بحسب اختبار دنكن متعدد الحدود وعل مستوى احتمال 0.01.

دلت النتائج ان مستخلص نبات الاكاكا يحتوي على مركبات كيميائية ضد البكتريا تؤثر على نمو البكتريا. و اقل تركيز من المستخلص المائي مثبط لنمو البكتريا (MIC) Minimum Inhibitional Concentrations كان 4000 مايكروغرام. قرص⁻¹ لـ *Staphylococcus aureus* و 8000 مايكروغرام. قرص⁻¹ للبكتريا الثلاث الاخرى باستعمال DST و DTT. اقل تركيز من المستخلص الكحولي كان 2.48 مايكروغرام. مل⁻¹ لبكتريا *Staphylococcus aureus* ، و 4.95 مايكروغرام. مل⁻¹ و *Pseudomonas aeruginosa* و *Klebsiella Pneumoniae* و اقل تركيز قاتل للبكتريا *Escherichia coli* Minimal Bactericidal

Kufa Journal For Agricultural Sciences 2018 100 – 120 :10 (3)

Concentration (MBC) من المستخلص الكحولي باستعمال تكنيك (ELISA) كان
10000 مايكروغرام. مل⁻¹ لبكتريا *Staphylococcus aureus* و *Klebsiella Pneumoniae*
20000 مايكروغرام. مل⁻¹ لبكتريا *Escherichia coli* و 625 مايكروغرام. مل⁻¹ لبكتريا
Klebsiella Pneumoniae .

كلمات مفتاحية: نبات الاكاكا *Allium akaka* Gmel. ، المستخلص، البكتريا

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