Antibacterial activity of *Zygophllumfabago*L. Leaves extracts

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Abstract

The present research consist of qualitative chemical analysis for both aqueous and alcoholic extracts of *Zygophyllumfabago*leaves which showed that the glycosides, alkaloids, flavonoids, terpens and steroids were found in both extracts, while saponines and phenolic compounds were found in alcoholic extract only, and both extracts contain no tannins. The other part of research was the determination of the antimicrobial activity of these extracts against eight species of pathogenic bacteria (*Esherichia coli, Klebsilla Pneumonia, Proteus volgaris, Proteus mirabilis, Salmonella Sp., Pseudomonas aeruginosa, Streptococcus Pyogens*, *Staphyllococcusaureus*) with six different concentration began with 40 mg/ml to 100 mg/ml by using agar well diffusion method which showed that alcoholic extracts more effective than aqueous ones and that both *Staphyllococcousaureus*and *Escherishia coli* were the most affected species.

Keywords: *Zygophllufabago*L., Chemical analysis, Antmicrobial activity, Active compounds.

Introduction

Many plant leaves extracts were investigated for their antimicrobial properties, the increasing interest of using natural products instead of the costly synthetic drugs is mainly related to synthetic drug side effects represented by hyper sensitivity toxicity to human tissues and organs and passive reactions which may lead to death [1].

The most critical purpose of using natural alternatives is the bacterial resistance to antibiotics and the highlighted prevalence of multi drug resistance strains which can be seriously threatening to human health and communities [2].

Recently, many researchers have countered this problem in many bacterial e.g *Pseudomonasaeruginosa, Staphyllococussaureus*, *Esherishiacoli*, and other seriously human pathogens [3].

Zygophillumfabago member of the family Zygophillaceae commonly called Bean – Coped or Syrian bean–caper, a sub-shrub is widely distributed in Iraq and neighboring countries. *Z. fabago* anti fungal activities are well studied [4], in contrast very few researches discussed their antibacterial activities thus research aims to investigate the chemical characteristics and antibacterial properties of this plant extract against some G(+) ve and G(-)ve bacterial strains.

Materials and Methods

Bacterial strains

Clinical bacterial strains used in this study were provided from strain bank laboratory, Biology dept., Baghdad University, Bacterial strains were isolated from clinical specimens and identified according to [5] (Table (1)).

All bacterial strains were cultured on nutrient agar slants (Himedia, India) and kept at 4ºC and subcultured when ever used.

Table (1)
Bacterial Species Used in the study and their isolation source.

Bacterial Strains	***Source of isolation***
Escherichia coli	Urinary tract infection
Klebsilla Pneumonia	Respiratory tract infection
Proteus volgaris	Urinary tract infection
Proteus mirabilis	Gastro intestinal tract infection
Salmonella Sp.	Gastro intestinal tract infection
Pseudomonas aeruginosa	Skin burns
Streptococcus Pyogens	Sour through
Staphyllococcusaureus	Skin burns

Preparation of aqueous and alcoholic extracts

Fresh leaves were collected from Baghdad and dried then grounded. Both aqueous and alcoholic extracts were done according to [6]. Briefly 100 g of grounded sample was weighted and placed in 1 L conical flask then soaked in 500 ml of distilled water, sealed with foil and incubated in shaker – incubator at room temperature for 24 hrs then allowed to stand for 24 hrs at room temperature.

For ethanolic extracts the same procedure was done but using 70% ethanol alcohol instead of distilled water in ratio of 1:5 (W/V). Both extracts were filtered by 4 layers of gauze and centrifuged at 2000 rpm for 10 min., and then supernatants were filtered by Whatman No.4 filter–paper, filtrate mixture was concentrated by oven for 72 hrs to obtain crude extracts. Finally all extracts were stored in dark sterile screw bottles at 4°C until use.

Chemical characterization of the active compounds in plant extracts

Detection of glycosides

It was achieved according to [7] by adding 2ml of the reagent to 1 ml plant extract, boiled in water bath for 5 min. The appearance of red precipitate indicates a positive result.

Detection of alkaloids

It was achieved according to [7] (prepared by mixing 1 ml of 40% formaldehyde with 10 ml con. H_2SO_4); White precipitate refers to the presence of alkaloid.

Detection of tannins

It was achieved according to [7] by using 1% acetate the appearance of gelatinous white precipitate indicates positive result of tannins.

Detection of Flavonoids

It was achieved according to [8]. By adding few drops of con.$H_2SO_4$to 1 ml of plant extract the appearance of red or brown color indicate positive result.

Detection of saponins

It was achieved according to [7] by using 5% aqueous mercury chloride.

Detection of phenolic compound

It was achieved according to [7) by adding 2ml of 1% ferric chlorid to 3ml of plant extracts, the formation of blue –green color indicate positive result.

Detection of steroid and andterpens

According to [8] by Mixing 1 ml of plant extract with 2ml of chloroform then 1drop of acetic acid and 1 drop of con. H_2SO_4 were added to the mixture. The appearance of brownish color refers to terpens presence. Then the tubes were remain for 10 min. the formation of blue color indicate positive result for steroids.

Antimicrobial activities of plant extracts

Aqueous and alcoholic extracts weretested for it antimicrobial activities byWell–diffusion method seven serial concentrations of plant extract were prepared (40, 50, 60, 70, 80 and 100 mg/ml)by Sterile Mulher – Hinton broth [2].All bacterial strains used in the test were adjusted to 10^{-8} cfu./ ml by macferland tube No. 0.5 and cultured on Muller–Hinton agar plate.

Results

Chemical characterization of *Z.fabago*Plants extracts

Results of the chemical detection of the active components of the crude aqueous and alcoholic extracts lasted in the Table (2).

Antibacterial activities of *Z.fabago*aqueous and alcoholic

This study showed the activity of *Z. fabago*extracts by the cap plant method and detecting the inhibition zones of different Gram +ve and –ve Bacterial isolates. Results showed that activities were varied according to the type of extract, its concentration and bacterial species (Table (3)).

Results showed that alcoholic extract were generally more effective than the aqueous one and *Z. fabago* had abroad antimicrobial spectrum against G(–) ve and G(+) ve and that *S. aureus*and *E.coli* were the most susceptible strains respectively.

Table (2)
Detection of active components in crude aqueous and ethanolic extract of Z. fabago.

Active compound	Reagent	Positive result	Result	
			Aqueous extract	Ethanolic extract
glycoside	Benedict reagent	Red color	+	+
alkaloid	Mayers reagent	White precipitate	+	+
Tannine	1 % lead acetate	Gelatinous white precipitate	-	-
Flavonoids	H_2So_4	Red or brown color	+	+
Saponines	5 % mercury chloride	White precipitate	-	+
Phenolic Compund	1% $Fecl_3$	Blue – green color	-	+
Terpens and Steroids	Chloroform + aceticacid	Browncterpens Convert to blue (steroids)	+	+
(+)ve: positive results (-)ve: negative results				

Discussion

The result of this study proved that *Z. fabago* extract had abroad range antimicrobial activity in both alcoholic and aqueous extracts, particularly against fungi. Few researches discussed its affectivity against bacteria [4, 9] specially in Iraq. Results showed that the effectiveness of extracts were varied according to type of extract and bacterial strains, and in concentration depending manner, Extracts were more effective against G(+)ve strains (in most cases) specially against *Staph. aureus* and followed by *E. coli* while the less affectivity occurred due to the differences of cell wall structure as G(–)ve bacteria cell is more complicated cell wall consist of two layers (outer and inner layer) separated by periplasmic membrane that means the membrane of G(–)ve cell contains high level of lipids (90 – 95%) which does not provide suitable medium for entrance of the antimicrobial agents [10].

These results came corresponded with previous study in Pakistan which showed that *E. coli* was the most susceptible strain [9].

Table (3)
Antibacterial activity of different concentrations of Z.fabago extracts against different bacteria.

Organism		Concentration (µg/ml)						
	Extract	40	50	60	70	80	90	100
Escherichia coli	Alcoholic	5	7	7	9	13	16	18
	aqueous	0	1	1	3	4	5	6
Klebsiella pneumonia	Alcoholic	1	2	2	3	4	6	8
	Aqueous	0	0	1	2	2	3	4
Salmonella typhi	Alcoholic	4	4	5	6	8	8	12
	Aqueous	0	1	2	2	3	4	5
Pseudomonas aeruginosa	Alcoholic	1	1	2	3	4	4	5
	Aqueous	0	0	0	1	1	2	2
Proteus volegaris	Alcoholic	2	3	3	6	8	9	10
	Aqueous	0	1	2	2	3	4	5
Staphyllococcusaureus	Alcoholic	4	6	6	8	13	17	20
	Aqueous	0	6	3	3	4	5	6
Enterococcus faccalis	alcoholic	1	2	3	4	5	6	7
	Aqueous	0	0	1	2	2	3	3
Streptococcus pyogens	alcoholic	1	2	3	4	4	5	7
	Aqueous	0	0	1	2	3	4	4

Conclusion

-Plant leaves contained the major active compounds: glycosides, alkaloids, flavonids,terpens, steroids, saponines, and phenols. While tannins were not detected.

-*Zygophyllumfabago* extracts were more effective against *S. aureus*from G(+)ve and *E. coli* from G(-)ve.

الخلاصة

تضمن البحث الحالي تحليل المحتوى الكيميائي النوعي لكل من المستخلصين المائي والكحولي لاوراق نبات *Zygophyllumfabago* والذي اظهرت نتائجه بأن كلا المستخلصين يحتويان على المركبات التالية (الكلايكوسيدية والقلويدية والفلافونية والتربينية والستيرودية) بينما وجدت المركبات الصابونية والفينولية في المستخلص الكحولي فقط، كما وتبين بأن كلا المستخلصين لا يحتويان على المركبات التانينية. كما وتضمن البحث دراسة الفعالية ضد الميكروبية لكلا المستخلصين على ثمانية انواع من البكتيريا المرضية (*Escherichia coli* ، *Klebsilla Pneumonia*، *Proteus volgaris* ، *Proteus mirabilis* ، *Salmonella Sp.* ، *Pseudomonas aeruginosa* ، *Streptococcus Pyogen*، *Staphyllococcusaureus*) وبستة تراكيز مختلفة تبدا من التركيز 40 mg/ml وصولا الى 100 mg/ml، باستخدام الأطباق متعددة الحفر وأظهرت نتائج الدراسة بأن المستخلص الكحولي هو أكثر فعالية من المائي وأن بكتيريا *Staph. aureus* و *E. coli* هي أكثر الأنواع تأثرا ولكلا المستخلصين

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