Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2019; 11(3); 91-97
doi: 10.25258/phyto.11.3.1

ISSN: 0975-4873

Research Article

Phytochemical Screening and Anti-Multidrug Resistant *Pseudomonas aeruginosa* of some Fabaceae Medicinal Plants Growing in Aleppo-Syria

Abdel Aleem Bello[1], Zaher Samman Tahan[1], Adawia Kitaz[2], Byan Tiba[1]

[1]*Dept. of Plant Biology-Faculty of Science- University of Aleppo, Syria*
[2]*Department of pharmacognosy, Faculty of Pharmacy- University of Aleppo, Syria*

Received: 22nd Jan, 19; Revised 10th Mar, 19; Accepted 15th Apr, 19; Available Online:25th Jun, 19

ABSTRACT

Objective: The present study aimed to evaluate phytochemical screening and anti-multidrug resistant *Pseudomonas aeruginosa* for different extracts of leaves and fruits of *Glycyrrhiza glabra*, *Ceratonia siliqua*, and *Propsopis farcta* (Syrian medicinal plants from fabaceae). Methods: Phytochemical screening of active constituents was carried out for each of the leaves and fruits parts, using a number of color tests and qualitative reagents. The antimicrobial susceptibility to 15 antibiotics from 10 antibiotic groups (aminoglycosides, carbapenems, cephalosporins, fluoroquinolones, penicillins/ß-lactamase inhibitors, monobactams, Phosphonic acids, polymyxins, Phenicols, and Folate Pathway Inhibitors) were determined by disk diffusion method, according to recommendation of Clinical and Laboratory Standards Institute (CLSI). Characterization of *P. aeruginosa* isolates as /MDR/ multidrug resistant, /XDR/ extremely drug resistant and /PDR/ pandrug resistant was done according to standardized international terminology presented by European Centre for Disease Prevention (ECDP). Agar well diffusion method was used to study the antibacterial activity of extracts. Results: The results showed that Flavonoids presented in all the used plant extracts. The best antibacterial activity belonged to Chloroform extract of *Glycyrrhiza glabra* fruit, with zone of inhibition 20.33 mm on both PS64 and PS75 isolates. Whereas the least activity belonged to hexane fruit extracts of all plants which ranged between no zone of inhibition and 11 mm. Conclusion: The majority of plants extracts showed antibacterial activity against Multidrug Resistant *Pseudomonas aeruginosa*, therefore Syrian medicinal plants can provide protection against our natural enemies like bacterial pathogens, and can be used as new phyto-medicines against MDR bacterial pathogens.

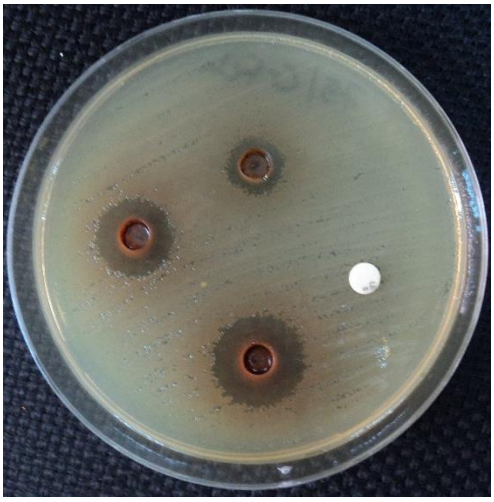
Keywords: *Glycyrrhiza glabra*, *Ceratonia siliqua*, *Propsopis farcta*, Phytochemical, multidrug resistant, extremely drug resistant, pandrug resistant.

INTRODUCTION

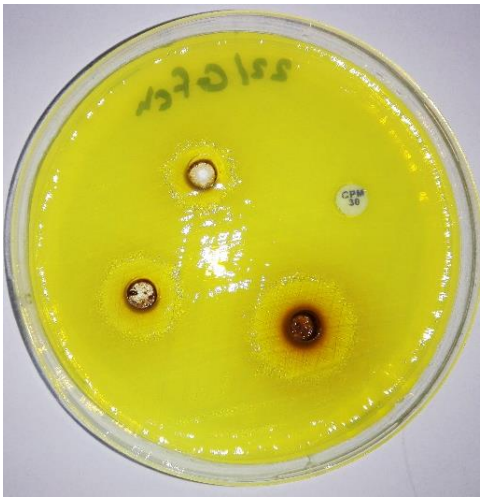
Herbal and medicinal plants are from the oldest and the most using in treating various diseases, they used in all cultures and civilizations, and still used by 80% of world populations. Fabaceae Lindley (=Leguminosae A. L. de Jussieu) Bean or Pea family. Comprised 630 genera, and 18,000 species (Third largest family after Asteraceae and Orchidaceae). Cosmopolitan in distribution. The family Fabaceae was traditionally subdivided into three subfamilies, Mimosoideae, Caesalpinioideae and Papilionoideae (Faboideae)[1,2]. *Glycyrrhiza glabra* is one of the useful medicinal plants from fabaceae, belonged to subfamily Papilionoideae (Faboideae). Glycyrrhiza is derived from the ancient Greek term glykos, meaning sweet, and rhiza, meaning root[3]. *Ceratonia siliqua* is the scientific name of carob tree from fabaceae, belonged to subfamily Caesalpinioideae, derives from Greek keras, horn, and Latin siliqua, alluding to the hardness and shape of the pod. The common name originates from the Hebrew kharuv, from which are derived the Arabic kharrub[4]. Prosopis the name used by Dioscorides for butterbur, *Propsopis farcta* is the scientific name of Dwarf Mesquite, from fabaceae, belonged to subfamily Mimosoideae.

Plant produces a wide variety of secondary metabolites which are used either directly as precursors or as lead compounds in the pharmaceutical industry. It has been expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However, very little information is available on such activity of medicinal plants and out of the 4,00,000 plant species on earth, only a small number has been systematically investigated for their antimicrobial activities[5]. *Pseudomonas aeruginosa* is an opportunistic pathogen in human. The most worrisome characteristic of this bacterium is its low antibiotic susceptibility, which is attributable to low permeability of the bacterial cellular membranes and action of multidrug efflux pumps[6]. This study aimed to: Evaluate phytochemical screening for *Glycyrrhiza glabra*, *Ceratonia siliqua*, and *Propsopis farcta*. Evaluate anti-multidrug resistant *Pseudomonas aeruginosa* for Methanolic 70%, chloroform, and Hexane leaves and fruits

**Author for Correspondence: dr.adakitaz@alpuniv.edu.sy*



a



b

Figure 1: a) chloroform extract of *Glycyrrhiza glabra* fruit on PS75. b) chloroform extract of *Glycyrrhiza glabra* fruit on PS22.

Table 1: Antibiotic groups and agents proposed for characterization of MDR, XDR and PDR in *P. aeruginosa.*

Antibiotic groups	Antimicrobial agents
Aminoglycosides	Gentamicin Tobramycin Amikacin
Carbapenems	Imipenem Meropenem
Cephalosporins	Ceftazidime Cefepime
Fluoroquinolones	Ciprofloxacin Levofloxacin
Penicillins/ß-lactamase inhibitors	Piperacillin-Tazobactam
Monobactams	Aztreonam
Phosphonic acids	Fosfomycin
Polymyxins	Colistin
Phenicols	Chloramphenicol
sulfonamides	Cotrimoxazole

extracts of *Glycyrrhiza glabra*, *Ceratonia siliqua*, and *Propsopis farcta.*

MATERIALS AND METHODS

Plant Material

Leaves and fruits of *Glycyrrhiza glabra, Ceratonia siliqua and Propsopis farcta* were collected in July 2017 from Aleppo University Campus, the plant materials were dried under shade at room temperature, then powdered using mechanical grinder and kept in airtight glass container until use.

Phytochemical Screening

Phytochemical examinations were carried out according to a standard methods[7,8].

Detection of alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered. Then filtrates used for the next two tests.

Wagner's Test

Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids[8].

Dragendroff's Test

Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids[8].

Detection of saponins (Froth Test)

Extracts were diluted with distilled water to 20 ml and were shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins[8].

Detection of phenols (Ferric Chloride Test)

Extracts were treated with 3-4 drops of ferric chloride solution 1%. Formation of bluish black color indicates the presence of phenols[7].

Detection of tannins (Gelatin Test)

1% gelatin solution containing sodium chloride was added to the extract. Formation of white precipitate indicates the presence of tannins[8].

Detection of flavonoids

Alkaline Reagent Test

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids[8].

Wilson-Tauböck-Test

1 ml of Extracts were heated until dryness, and then treated with Wilson-Tauböck reagent, and were dried again, The occurrence of an intense fluorescence under UV light (λ 365 nm) is a positive test for the presence of flavonoids.

zirconium chloride test

3 ml of Extracts were treated with few drops of zirconium chloride solution. Formation of intense yellow color, indicates the presence of flavonoids.

Detection of Coumarins (UV Test)

Extracts were treated with 0.5 ml of 10 % ammonia. The occurrence of an intense fluorescence under UV light (λ 365 nm) is a positive test for the presence of coumarins and derivatives[9].

Table 2: Phytochemical analysis of the leaves and fruit of *Glycyrrhiza glabra*, *Ceratonia siliqua* and *Propsopis farcta*

	test	*G.g* GL	GF	*C.s* CL	CF	*P. f* PL	PF
Alkaloids	Wagner's Test	+	-	-	-	-	-
	Dragendroff's Test	+	-	-	-	-	-
Saponins	Froth Test	+	+	+	+	-	+
Phenols	Ferric Chloride Test	+	+	+	+	+	+
Tannins	Gelatin Test	+	+	+	+	+	+
	Alkaline Reagent Test	+	+	+	+	+	+
Flavonoids	Wilson-Tauböck-Test	+	+	+	+	+	+
	zirconium chloride test	+	+	+	-	+	+
Coumarins	UV Test	+	+	+	-	+	+
Cyanogenic Glycosides	Guignard Test	-	+	+	+	+	+
Diterpenes	Copper acetate Test	+	+	-	-	-	-
Anthraquinones	Borntrager test	-	-	-	-	-	-
Terpens	Salkowski test	+	+	-	-	+	-

G.g: *Glycyrrhiza glabra*, C.s: *Ceratonia siliqua*,P. f: *Propsopis farcta*, G.L: *Glycyrrhiza glabra* leaves, G.F: *Glycyrrhiza glabra* fruits, C.L: *Ceratonia siliqua* leaves, C.F: *Ceratonia siliqua* fruits, P.L: *Propsopis farcta* leaves, P.F: *Propsopis farcta* fruits.

Table 3: Antimicrobial susceptibility of 9 *P. aeruginosa* isolates (mm).

	PS9	PS21	PS22	PS40	PS51	PS56	PS60	PS64	PS75
GEN	S	R	R	S	R	R	R	S	R
Tob	S	R	R	S	R	R	R	S	R
Ak	I	R	R	I	R	R	R	S	R
IMP	S	I	R	S	R	R	R	S	R
MER	S	R	R	S	I	R	I	S	R
CAZ	S	I	I	I	R	R	R	S	R
FEP	S	R	R	S	R	R	R	S	R
CIP	S	R	R	S	R	R	R	S	R
LEV	S	R	R	S	R	R	R	S	R
PIT	S	R	R	I	R	R	R	S	R
ATM	S	S	I	S	R	R	R	S	R
FOS	R	R	I	R	R	S	R	R	S
CT	R	R	R	R	R	S	S	S	S
C	S	R	R	R	R	R	R	R	R
COT	R	R	R	R	R	R	R	R	R

GEN: Gentamicin, Tob: Tobramycin, Ak: Amikacin, IMP: Imipenem, MER: Meropenem, CAZ: Ceftazidime, FEP: Cefepime,CIP: Ciprofloxacin, LEV: Levofloxacin, PIT: Piperacillin-Tazobactam, ATM: Aztreonam, FOS: Fosfomycin, CT: Colistin, C: Chloramphenicol, COT: Cotrimoxazole.

Table 4: Phenotypes of 9 *P. aeruginosa* isolates according to antibiotic resistance

	Amino	Carba	cepha	fluo	peni	Mono	Phospho	Poly	phe	sulf	Number of antibiotic groups	
PS9	S	S	S	S	S	S	R	R	S	R	3	
PS64	R	S	S	S	S	S	R	S	R	R		
PS40	S	S	S	S	S	S	R	R	R	R	4	
PS22	R	R	R	R	R	S	S	R	R	R		
PS56	R	R	R	R	R	R	S	S	R	R		8
PS75	R	R	R	R	R	R	S	S	R	R		
PS21	R	R	R	R	R	S	R	R	R	R	9	
PS60	R	R	R	R	R	R	R	S	R	R		
PS51	R	R	R	R	R	R	R	R	R	R		10

Amino: Aminoglycosides, Carba: Carbapenems, Cepha: Cephalosporins, Fluo: Fluoroquinolones, Peni: Penicillins/ß-lactamase inhibitors, Monoba: Monobactams, Phospho: Phosphonic acids, Poly: Polymyxins, phe: Phenicols, sulf: sulfonamides.

Detection of Cyanogenic Glycosides (Guignard Test)

Picrate papers were prepared by dipping a filter paper in saturated aqueous picric acid neutralized with sodium

Table 5: zone of inhibition of *Glycyrrhiza glabra* extracts.

	GLM	GLCH	GLH	GFM	GFCH	GFH
PS9	10.33	6	6	6	12.67	6
PS21	10.67	9.33	6	6	15.33	6
PS22	12.67	8.67	12.33	12.67	20	6
PS40	7	6	6	8.3	12.33	6
PS51	10.67	6	6	9.33	14.33	6
PS56	10.67	6	6	11.33	17.67	6
PS60	9	10	9.67	7	13	11
PS64	10.33	8	12	11.33	20.33	10
PS75	11	9.33	9.67	12.67	20.33	6

GLM: methanolic extract of *Glycyrrhiza glabra* leaves, GLCH: chloroform extract of *Glycyrrhiza glabra* leaves, GLH: hexane extract of *Glycyrrhiza glabra* leaves, GFM: methanolic extract of *Glycyrrhiza glabra* fruits, GFCH: chloroform extract of *Glycyrrhiza glabra* fruits, GFH: hexane extract of *Glycyrrhiza glabra* fruits.

Table 6: zone of inhibition of *Ceratonia siliqua* extracts (mm)**.**

	CLM	CLCH	CLH	CFM	CFCH	CFH
PS9	14.33	6	6	6	6	6
PS21	13.67	6	6	12	10.33	6
PS22	17.33	12	10.33	12.33	12.67	6
PS40	15.33	8	10	10	9.33	6
PS51	14	6	6	9.33	6	6
PS56	16	6	6	11.67	11.33	6
PS60	17.67	6	9	10	8	6
PS64	15.33	7.33	11	10.33	9	6
PS75	14.67	10.67	11.67	9.67	10.33	8

CLM: methanolic extract of *Ceratonia siliqua* leaves, CLCH: chloroform extract of *Ceratonia siliqua* leaves, CLH: hexane extract of *Ceratonia siliqua* leaves, CFM: methanolic extract of *Ceratonia siliqua* fruits, CFCH: chloroform extract of *Ceratonia siliqua* fruits, CFH: hexane extract of *Ceratonia siliqua* fruits.

bicarbonate NaHCO3. Extract in the test tube were treated with few drops of water and two drops of toluene, The tube was then firmly corked, with a moistened picrate paper suspended inside from the cork and left to incubate at 40°C for 2h. A color of picrate papers change from yellow to reddish-brown indicates the enzymic release of HCN[7].

Detection of diterpenes (Copper acetate Test)

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes[8].

Detection of Anthraquinones (Borntrager test)

0.5g of the plant extract was shaken with 10 ml of aqueous sulphuric acid and then filtered while hot, the filtrate was then shaken with 5 ml of benzene, the benzene layer separates and half its own volume of 10% ammonia solution was added. A violet or red coloration in the ammonical (lower) phase indicates the presence of combined Anthraquinones[10].

Detection of Terpenoid (Salkowski test)

About 0.2g extract was mixed with 2ml Chloroform and 3ml of concentrated sulphuric acid was added carefully to form a layer. A reddish brown coloration of the interface formed indicates the presence of terpenoids[10].

Antibacterial activity

Plant Extraction

The leaves and fruit of *Glycyrrhiza glabra*, *Ceratonia siliqua* and *Propsopis farcta*, were collected from Aleppo university campus. The plant materials shade dried at room temperature, then pulverized into fine powder using electric grinder. each of the powdered materials were extracted by maceration with methanol 70%, chloroform, and hexane for 24 hours. The extracts were filtered and then re-extracted by sonication for 60 minutes. Then concentrated under vacuum to obtain a crude extract using rotary evaporator[11] with minor modifications.

The crude extracts were weighed and kept at 4 C° prior to test. The final dried extracts were dissolved in dimethyl sulphoxide (DMSO), to get the final concentration 20% for each extract.

Bacterial Isolation

The bacterial isolates were collected from patients visiting Aleppo University Hospital, during the period January – March 2018. The isolates were identified according to the standard microbiological methods, include: Gram stain (negative rods), Oxidase test (positive), Culture on MacConkey agar (gives pale yellow colonies), Culture on selective media: Cetermide (gives pigments are more obvious), and Pseudomonas agar for fluorescein (gives fluorescent diffusible yellow fluorescein pigment)[12].

Preparation of bacterial inoculums

A 24 h old culture of bacterial isolates were emulsified in sterile normal saline and adjusted to 0.5 McFarland (equal to 1.5×10^8 colony forming units (CFU)/ml)[13].

Antimicrobial susceptibility test

Disk diffusion method was used for detection of antimicrobial susceptibility in clinical isolates of *P. aeruginosa* according to the Clinical and Laboratory Standards Institute (CLSI) guidelines[14].

Defining of MDR, XDR and PDR in *P. aeruginosa* isolates were done according to standardized international

Table 7: zone of inhibition of *Propsopis farcta* extracts (mm).

	PLM	PLCH	PLH	PFM	PFCH	PFH
PS9	9.67	7.33	6	6	10.67	6
PS21	12.33	6	6	10.33	6	6
PS22	15.33	14.67	9.67	16.67	11.67	6
PS40	11.67	8	6	8.67	7.67	8
PS51	10	6	6	11.67	9.33	6
PS56	13.33	6	6	13.67	6	11
PS60	13	6	10	12	11.33	6
PS64	12.33	15	12	11.33	14	6
PS75	12.67	12.67	9.33	12	10	9.67

PLM: methanolic extract of *Propsopis farcta* leaves, PLCH: chloroform extract of *Propsopis farcta* leaves, PLH: hexane extract of *Propsopis farcta* leaves, PFM: methanolic extract of *Propsopis farcta* fruits, PFCH: chloroform extract of *Propsopis farcta* fruits, PFH: hexane extract of *Propsopis farcta* fruits.

document[15], by the results of antimicrobial susceptibility of *P. aeruginosa* to all antimicrobial agents listed in Table 1. Therefore, isolates of *P. aeruginosa*, which have shown non-susceptibility to at least one agent of ≥3 antibiotic groups considered MDR, and isolates exhibit non-susceptibility to at least one agent of ≥6 antibiotic groups known as XDR and PDR was defined as "non-susceptibility to all agents in all antibiotic groups"[6].

Determination of Antibacterial activity

Antibacterial assay in vitro was performed by using agar well diffusion method. The medium (Muller-Hinton agar medium) was sterilized by autoclaving. About 25 ml of the medium infused into each sterilized Petri plate. The plates were left at room temperature until solidification. Then each plate was separately inoculated with different isolates by using seed layer aseptically on the whole surface of the agar with cotton swab. Four wells of 6 mm in diameter was made in each plate using a sterile mineral borer. 25, 50, and 75 µl from each extract placed into wells. The fourth well was supplemented with dimethyl sulphoxide serving as negative control. Antibacterial activity of the extract was determined by measuring the diameter of clear zone around the well (zone of inhibition)[13].

RESULTS

The study on the presence of Secondary metabolites showed that Flavonoids and phenols presented in all the extracts of studied plant species. However, Anthraquinones are completely absent in all the plants. Diterpenes was detected in *Glycyrrhiza glabra* extracts. The presence and absence of Alkaloids, saponins, Phenols, Tannins, flavonoids, Coumarins, Cyanogenic Glycosides, Diterpenes, Anthraquinones, and Terpens of individual plant species are presented in Table 2.

Antimicrobial susceptibility test

Disk Diffusion Assay

Multidrug resistance pattern of isolates was determined by measuring the diameters of zones of inhibition in millimeter (mm) around the discs by following CLSI guidelines[14]. Out of nine isolates, three isolates of them (33.3%) ps9, ps40 and ps64 were considered as MDR, and six isolates (66.7%) ps22, ps56, ps75,ps21, ps60, and ps51 were considered as XDR. The results obtained from disk diffusion test are illustrated in Table 3.

These 9 isolates characteristic in 5 phenotypes, two isolates (PS9 and PS64) were resistant to at least one agent belonged to 3 antibiotic groups, one isolate (PS 40) was resistant to at least one agent belonged to 4 antibiotic groups. three isolates (PS 22, PSS56, and PS75) were resistant to at least one agent belonged to 8 antibiotic groups, two isolates (PS21 and PS60) were resistant to at least one agent belonged to 9 antibiotic groups, and one isolate (PS51) was resistant to at least one agent belonged to 10 antibiotic groups.

Antibacterial activity

Activity of Glycyrrhiza glabra extracts

The results showed that the majority of *Glycyrrhiza glabra* extracts had activity against all tested *P. aeruginosa* isolates Table 5, specially chloroform extract of *Glycyrrhiza glabra* fruit had the best antibacterial activity, with zone of inhibition 20.33 and 20 mm on PS64, PS 75, and PS22 isolates figure 1.

Activity of Ceratonia siliqua extracts

The results showed that almost extracts of *Ceratonia siliqua* had activity against all tested *P. aeruginosa* isolates Table 6, methanolic extract of *Ceratonia siliqua* leaves had the best antibacterial activity, with zone of inhibition 17.67 mm on PS60 isolate.

Activity of Propsopis farcta extracts

The results showed that almost extracts of *Propsopis farcta* had activity against all tested *P. aeruginosa* isolates Table 6, methanolic extract of *Propsopis farcta* fruit had the best antibacterial activity, with zone of inhibition 16.67 mm on PS22 isolate.

DISCUSSION

The study on the presence of Secondary metabolites showed that Flavonoids and phenols presented in all the extracts of studied plants. Flavonoids have two main activity, which are antibacterial and Antidiarrhoeal, the mechanisms of action of their antibacterial activity which are related to Complex with cell wall and binds to adhesins[8]. Flavonoids are one of the major groups of active plant compounds, they act through inhibiting both cytoplasmic membranes function and DNA synthesis. Protein and RNA syntheses are also affected, but in low level[16]. Phenols also have antibacterial activity and their activity return to: Binds to adhesins, enzyme inhibition, substrate deprivation, complex with cell wall, membranes

disruption, and metal ion complexation[8]. Many studies showed that antibiotic efflux is a major mechanism of antibiotic resistance in *Pseudomonas aeruginosa* due to mix efflux proteins. The majority of bacteria efflux systems are non-drug specific proteins that can recognize and export a broad range of chemically and structurally unrelated compounds, from bacteria without drug alteration or degradation[17]. Recently[18] indicated that extracts of different plants, used as herbal medicinal products, contain inhibitors of efflux in Gram-negative bacteria (the polyacetylene falcarindiol, for example). This is a very important finding, as it gives hope that Plant secondary metabolites (PSMs) could be truly useful in fighting multidrug resistant strains. It is not surprising that there are differences in the antimicrobial effects of plant species, due to the phytochemical properties and differences among species. It is quite possible that some of the plants that were ineffective in this study do not possess antibiotic properties, or the plant extracts may have contained antibacterial constituents, quite in sufficient concentrations so as to be effective[19]. However, plant extracts in this study showed activity against *pseudomonas aeruginosa* with values higher than many antibiotics, that may related to its content of many kinds of secondary metabolites, which have antibacterial activity and have a mechanism of action difference from that in antibiotics.

CONCLUSION

Glycyrrhiza glabra, *Ceratonia siliqua*, and *Propsopis farcta* which is important Syrian medicinal plants from fabaceae rich in an important secondary metabolites. and maybe that is why that plants had good antibacterial activity against Multidrug Resistant *Pseudomonas aeruginosa*, therefore Syrian medicinal plants can provide protection against our natural enemies like bacterial pathogens and can be used as new phyto-medicines against MDR bacterial pathogens.

ACKNOWLEDGEMENTS

The authors are grateful to Plant Biology Department-Faculty of Science, and Aleppo University for providing necessary technical and financial support for this research.

REFERENCES

1. Singh G., 2006- Diversity of Seed Plants and their Systematics (Diversity of Flowering Plants), SBD Khalsa College, New Delhi, 50 pages.
2. Takhtajan A., 2009- Flowering Plants. Springer Science and Business Media B.V., Second Edition, 871 pages.
3. Sharma V., Katiyar A., and Agrawal R. C., 2016- *Glycyrrhiza Glabra*: Chemistry and Pharmacological Activity. *Springer International Publishing Switzerland*, 14 pages.
4. Batlle, I. and J. Tous. 1997-Carob tree. *Ceratonia siliqua* L. Promoting the conservation and use of underutilized and neglected crops. 17. Institute of Plant Genetics and Crop Plant Research, Gatersleben/*International Plant Genetic Resources Institute*, Rome, Italy.
5. Selvamohan T., Ramadas V., and Kishore S. S., 2012- Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria, *Advances in Applied Science Research*, , 3 (5):3374-3381
6. Saderi H and Parviz Owlia P., 2015-Detection of Multidrug Resistant (MDR) and Extremely Drug Resistant (XDR) Pseudomonas aeruginosa Isolated from Patients in Tehran, Iran. *Iranian Journal of Pathology*. 10 (4), p:265-271..
7. Harborne J. B., 1998- Phytochemical Methods, A guide to modern techniques of plant analysis, Third edition, Published by Chapman and Hall, 302 pages
8. Tiwari P., Kumar B., Kaur M., Kaur G., and Kaur H., 2011- Phytochemical screening and Extraction: A Review. *Internationale Pharmaceutica Sciencia*. (1)1, 98-106.
9. Kumar R. S., Venkateshwar C., Samuel G., and Rao S. G., 2013- Phytochemical Screening of some compounds from plant leaf extracts of Holoptelea integrifolia (Planch.) and Celestrus emarginata (Grah.) used by Gondu tribes at Adilabad District, Andhrapradesh, India, *International Journal of Engineering Science Invention*, Volume 2, Issue 8, PP.65-70.
10. A. S. Aska.,Kubmarawa, D., 2016- Preliminary Phytochemical Screening of Some Indigenous Medicinal Plants Used In the Treatment of Tuberculosis in Bauchi State, Nigeria., *IOSR Journal of Applied Chemistry (IOSR-JAC)*,e-ISSN: 2278-5736.Volume 9, Issue 4 Ver. I, PP 48-5248.
11. Sharifi-Rad J., Hoseini-Alfatemi S. M., Sharifi-Rad M., and Miri A., 2014- Phytochemical screening and antibacterial activity of different parts of the *Prosopis farcta* extracts against methicillin-resistant *Staphylococcus aureus* (MRSA). *Minerva Biotecnologica*, 26(4), 287-193.
12. Brenner D J.; Krieg N R.; Staley J T., 2005- Bergey's Manual- of Systematic Bacteriology. Second Edition, *Springer*, USA, P: 325.
13. Jahan M., Abuhena Md., Azad A. K., and Karim M.M., 2018- In vitro antibacterial and antibiofilm activity of selected medicinal plants and spices extracts against multidrug resistant *Pseudomonas aeruginosa*
14. CLSI. 2017- Performance Standards for Antimicrobial Susceptibility Testing., 27th ed. CLSI supplement M100.Wayne, PA: Clinical and Laboratory Standards Institute.
15. Magiorakos A.-P., Srinivasan A., Carey R. B., Carmeli Y., Falagas M. E., *et al.*, 2012- Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance, European Society of Clinical Microbiology and Infectious Diseases No claim to original US government works, Clinical Microbiology and Infection, Volume 18 Number 3, pp 268–281.
16. Radulovic N.S., Blagojevic P.D., Stojanovic -Radic Z.Z. and Stojanovic N.M., 2013-Antimicrobial Plant Metabolites: Structural Diversity and Mechanism of

Action.Current Medicinal Chemistry. 20 (7933), 932-952.

17. Adwan Gh, Abu-Shanab B, Adwan K., 2010- Antibacterial Activities of some Plant Extracts Alone and in Combination with Different Antimicrobials against Multidrug-Resistant *Pseudomonas aeruginosa* Strains, *Asian Pacific Journal of Tropical Medicine*, p: 266-269.
18. Garvey, M.I.; Rahman, M.M.; Gibbons, S.; Piddock, L.J.V. 2010- Medicinal plant extracts with efflux inhibitory activity against Gram-negative bacteria. *Intern.J. Antimicrob. Agents*, 37(2), 145-151.
19. Chaudhary S., Negi A., and Dahiya V., 2010- The Study of in vitro Antimicrobial Activity and Phytochemical Analysis of Some Medicinal Plants in Chamoli Garhwal Region, *PHCOG J*, Vol 2, Issue 12, PP 481–485.

.