International Journal of Science and Research Archive
eISSN: 2582-8185
Cross Ref DOI: 10.30574/ijsra
Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

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Antibacterial activity of *Gardenia jasminoides* Ellis leaves extracts on bacteria isolated from burns infected

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International Journal of Science and Research Archive, 2023, 09(02), 935–940

Publication history: Received on 28 June 2023; revised on 24 August 2023; accepted on 26 August 2023

Article DOI: https://doi.org/10.30574/ijsra.2023.9.2.0641

Abstract

This study provides scientific information about the aqueous and methanol extracts of *Gardenia jasminoides* Ellis leaves based on its antimicrobial potential against gram positive and gram negative bacteria isolated From burns infection using the broth dilution and disc diffusion method. Results of this study indicate the presence of many phytochemicals which have antimicrobial activity against broad spectrum of bacteria.The methanol extract of *G. jasminoides* showed highest activity than aqueous ones . The minimum inhibitory concentration (MIC)of the aqueous extract on the tested organisms was25_100mg/ml while in the methanol extract ranged between 25_50mg/ml on the tested organisms and the minimum bacterial concentration (MBC) of the aqueous extract was 25-200 mg/ml while the methanol extract ranged between 25-100 mg/ml. Essential element (Pb, Na, K, Ca, Fe, Zn, P, Mn, Co and Cu) at different concentration. The result of this study demonstrate that *G.jasminoides* constituent revealed that this plant have antimicrobial activity against test organism and this may be suggest the use at this extract in treatment of infectious disease

Keywords: Extracts of *Gardenia Jasminoides*; Antimicrobial Activity; Aqueous and Methanol Extracts; Infectious Disease

1. Introduction

It is well known that infectious diseases are responsible for a high proportion of health problems, especially in developing countries. The situation has created immense clinical problems for infectious disease treatment. More scientists are in search for new antimicrobial substances derived from plants. Historically, plants provide us with a good source of anti-infective agents [1]. However, an emerging problem associated with misuse of antibiotic therapy is the worldwide emergence of higher level tolerance of target organisms against available broad spectrum antibiotics .As a result, and in the light of the rapid spread of multidrug resistance, the development of new antimicrobial or anti pathogenic agents that act upon new microbial targets has become a very pressing priority [2]. In the traditional systems of medicine, plants are used in the form of crude extracts, infusions and powders to treat common infections without scientific evidence of efficacy [3]. Plants are rich in a variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides and volatile oils [4]. *Gardenia jasminoides Ellis* is a plant from Rubiaceae family that historically originated from Ghafghaz and Near East. Gardenia extract uses for medicine properties to treat many aliments such haemorhage in human ,skin diseases ,stomach pain ,ephemeral fever ,and many phytochemical compounds which have a potential effect as anti-oxidants ,anti-microbial , anti-diabetic ,and anti-inflammatory [5]&[6]. Extracts of *G. jasminoides* have a various pharmacological and benefical effects on nerves ,cardiovascular ,and digestive systems also anti-depressant and anti-inflammatory activities [7]. Some researches pointed to an importance of *G. jasminoides* Ellis leaves extracts for antibacterial and anti-fungi , these extracts have an initial phytochemical compounds such (Iridoid-glycosides , flavonoids , tannins , alkaloids) [8]&[9] .

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2. Material and methods

2.1. Collection of plant samples

The medicinal plant in for the experiment was identified according to various literatures, Collected the leaves of plants from herbous house/ College of Science/ Baghdad university were washed thoroughly and chopped into small pieces shade dried and grinded into powdered form.

2.2. Test microorganisms

Bacterial *species Shigella dysenteriae; Aeromonas hydrophila; Escherichia coli; Klebsiella spp; Serratia marcescens and Staphylococcus aureus* were all obtained from Medicine-city in Baghdad.

2.3. Culture medium and inoculum

The stock cultures of microorganisms used in this study were maintained on Plate Count Agar slants at +4°C. Cell suspansions were prepared by inoculation of each bacteria into 10 ml of Nutrient broth. Incubation was performed at 37°C for 24 h. On the next day Mueller-Hinton Agar (MHA) was prepared and cooled to -5°C. Bacterial suspension was added into MHA to give a final concentration of 10^7 bacteria/ml and plated out.

2.4. Phytochemical screening

The plant leaves extracts were screened for phytochemical constituents using standard procedures of analysis .These were done at College of Science/ Dept.Biology/ Al-Mustansiryah University [11].

2.5. Antibacterial activity

The plate-hole diffusion assay as described by [12] was used to determine the growth inhibition of bacteria by the plant extract .The isolated bacteria from burn infection were obtained .The tests were carried out by using a stock concentration of 500mg/ml prepared by dissolving 1g of the methanol extract (MTE) and aquatic extract into 2ml of distilled water. Nutrient agar was prepared and 25ml each was poured into sterile petri dish. This was allowed to solidify and dry. Using a sterile cock-borer of 9mm diameter three equi-distant holes per plate were made in the set agar and were inoculated with 0.5ml over night suspension of the bacteria. Thereafter, the wells (holes) were filled with the extract solution at varying concentrations of 500mg/ml, 400mg/ml and 300mg/ml respectively. This was done in triplicate and the plates were incubated at 37ºC for 18hours. The antibacterial activities were observed and measured using a transparent meter rule and recorded if the zone of inhibition was ‹10mm [13].

2.6. Minimum Inhibitory Concentration (MIC)

MIC is defined as the lowest concentration where no visible turbidity is observed in the test tube (bacteriostatic concentration). Reuben et al.[14] was employed. In this method, the broth dilution technique was utilized where the plant extract was prepared to the highest concentration of 500mg/ml (stock concentration) in sterile distilled water and serially diluted (two-fold) to a working concentration ranging from 0.780 mg/ml to 200mg/ml using nutrient broth and later inoculated with 0.2ml suspension of the test organisms. After 18 hours of incubation at 37ºC, the test tubes were observed for turbidity. The least concentration where no turbidity was observed was determined and noted as the minimum inhibitory concentration (MIC) value.

2.7. Minimum Bacterial Concentration (MBC)

The MBC is defined as the lowest concentration where no bacterial growth is observed (bacteriocidal concentration). This was determined from the broth dilution resulting from the MIC tubes by sub culturing to antimicrobial free agar as described by Usman et al., (2007) In this technique, the contents of the test tubes resulting from MIC was streaked using a sterile wire loop on agar plate free of bacteria and incubated at 37ºC for 18 hours. The lowest concentration of the extract which showed no bacterial growth was noted and recorded as the MBC[15].

2.8. Determination of Essential elements

The work was carried out in the centeral laboratory, College of Science/ Dept.Biology/Baghdad university .Three gram of dried plant were taken and mixed with 8ml of concentrated H_2So_4 (98%) and 2ml of $HClO_3$ (60%) in conical flask for 24 hours which covered by watch class. Then left this mixture for 6 hours on the sand bath at 80C° , until the digestion material converted to white powder. Then add 8ml of deionized water to this powder and the trace elements were determined by flame atomic absorption spectrophotometer [18].

3. Results and discussion

The results of phytochemical screening for *G.jasminoides Ellis* leaves are shown in Table1 which reveals the presence of Alkaloids, Phenol, Cardiac glycosides, Flavonoids, Terpenes, Tanins, Ratenges, Coumarines,and Essensial oil . These results agree with other study that found a phytochemical compounds in leaves extracts of *G.jasminoides* [9] , also [16] showed a similar compounds to our study but in *G.jasminoides* flowers .

Table 1 Phytochemical screening of Methanol**,** Hot and Cold water extract of *G. jasminoides* Eills leaves

Number	Constituents	Methanol extract	Hot water extract	Cold water extract
1	Alkaloids			
	i.Dragendorff's test ii.Meyer's test	+	+	+
2	Phenol			
		+	+	+
3	Cardiac glycosides			
	Killer-killanis test	+	+	+
4	Flavonoids			
	i.Shinoda's test ii.FeCl3 test	+	+	+
5	Saponins			
	Frothing test	-	-	-
6	Terpenes			
	Salkowski test	+	+	+
7	Steroids			
	Libarman-Burchard's test	-	-	-
8	Tanins			
	i.FeCl3 test			
	ii.Lead acetate test	+	+	+
9	Ratenges			
		+		
10	Coumarines			
		+		
11	Essensial oil			
		+		

The result of antibacterial activity of plant extract against test organism list in Table2. In this study was correlation between concentration of test plant extract and the inhibition zone of pathogenic isolates. As is shown, the methanol extract of *G. jasminoides* was more effective than two aqueous extract (hot and cold) for the same plant ,and the hot aqueous extract of plant was more effective than cold extract. *S.aureus* shwoed zone of inhibition for aqueous and methanol extract. while all gram negative bacteria, *K.spp, A..hydrophila ,S.marcesence, S.dysenteriae* and *E.coli* exhibit difference in zone of inhibition respectively.The result of this study in agreement with other research which showed that plant extracts with well documented antimicrobial activities could possess antipathogenic as well as antivirulent activities, which may not be linked to the growth and inhibition of the microorganism [5]& [17]. [18] studied *In vitro* antimicrobial activity of crude dichloromethane, methanol and aqueous extracts from medicinal plants in Yemeni ethnomedicine and showed good activity against gram positive and negative.

Table 2 Antibacterial Activity of *G. jasminoides Ellis* leaves Extracts against Test Organisms

Extract/concentration Mg/ml	**Zone of inhibition (mm)**						
	Cone.	K.spp	S.marcescens	A.hydrophila.	S.dysenteriae	E.coli	S.aureus
Methanol Extract	500	26	18	23	11	14	42
	400	23	16	21	10	12	36
	300	19	15	18	8	11	33
Hot aqueous Extract	500	20	16	21	11	12	39
	400	17	15	20	9	11	36
	300	16	12	18	7	9	31
Cold aqueous Extrac	500	17	15	15	9	10	36
	400	16	14	13	8	8	29
	300	14	13	11	7	6	21
Control (water)	-	-	-	-	-	-	-
Control (Methanol)	-	-	-	-	-	-	-

The minimum inhibition concentration MIC and minimum bacterial concentration MBC results are shown in (Table 3, 4) respectively. The highest MIC and MBC values is an indication that either the plant extracts are less effective on some bacteria or that the organism has the potential of developing antibiotic resistance, while the low MIC and MBC values for other bacteria is an indication of the efficacy of the plant extract. The result of this study was agreement with other research which showed antimicrobial activity of ethanol, methanol, ethyl acetate and water extract of *Gardenia jasminoides Ellis* by disc diffusion method, from this study it was found that *G. jasminoides* revealed antimicrobial activity against some gram positive and gram negative bacteria, filamentous fungi [8]& [16] . These MIC values for the different bacteria though relatively high, are definitely demonstrative of the potential clinical use [19]. The microorganisms were least sensitive to the aqueous crude extracts due to negligible secondary metabolites in it [20]. [21] Talk about the use of this extract in the treatment of wounds and Injuries in the traditional medicine of India.

Table3 Minimum Inhibitory Concentration (MIC) values for Bacterial Isolates Against *G. jasminoides* Ellis extracts

Bacterial Isolates	**Extract concentration (mg/ml)**											
	25			**50**			**100**			**200**		
	M	**H**	**C**	**M**	**H**	**C**	**M**	**H**	**C**	**M**	**H**	**C**
K.spp	_	_	_	_	_	_	I	I	+	I	I	+
S.marcescens	_	_	_	_	_	_	_	_	_	I	I	+
A.hydrophila	_	_	_	_	_	_	_	_	_	I	I	+
S.dysenteriae	_	_	_	_	_	_	_	_	_	I	I	_
E.coli	_	_	_	_	_	_	_	_	_	I	_	_
S.aureus	I	_	_	+	I	I	I	+	+	+	+	+

- = Resistance (growth of bacteria); + = Concentrations show no turbidity (inhibition of bacterial growth); I = least concentration showing no turbidity (MIC); M=Methanol extract; H= Hot aqueous extract ; C= Cold aqueous extract

Table 4 Minimum BacteriaConcentration (MBC) values for Bacterial Isolates Against *G. jasminoides* Ellis extracts

Bacteria Isolates	Extract concentration (mg/ml)											
	25			50			100			200		
	M	H	C	M	H	C	M	H	C	M	H	C
K.spp	_	_	_	B	B	B	+	+	+	+	+	+
S.marcescens	_	_	_	_	_	_	B	B	B	+	+	+
A.hydrophila	_	_	_	B	+	+	B	B	+	+	+	+
S.dysenteriae	_	_	_	_	_	_	_	_	_	_	_	_
E.coli	_	_	_	_	_	_	_	_	_	_	_	_
S.aureus	_	B	B	B	+	+	+	+	+	+	+	+

- = Resistance (growth of bacteria); + = Concentrations show no turbidity (inhibition of bacterial growth); B= = Minimum Bactericidal (MBC); M=Methanol extract; H= Hot aqueous extract; C= Cold aqueous extract

The results of determined essential elements (Pb, Na, K, Ca, Fe, Zn, P, Mn, Co,and Cu) in *Gardenia jasminoides Ellis* (Table5) releavel the prescence of this elements at different concentration ,this results agrrement The results of [7].

Table 5 Essential elements concentration of *G. jasminoides* Ellis leaves

Elements	Concentration	*Gardenia jasminoides* Ellis
Pb	ppm	0.3
Na	Ppm	613
K	%	1.8
Ca	%	0.85
Fe	Ppm	620
Zn	Ppm	94.2
P	%	0.21
Mn	Ppm	6.4
Co	Ppm	1.9
Cu	Ppm	5.2

4. Conclusion

Results of this study demonstrate that G.jasminoides Ellis constituent revealed that this plant has antimicrobial activity against test orgainsms and this may be suggest the use at this extract in treatment of infectious disease.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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