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Original Research Article

Phytochemistry and Antibacterial Activity of *Prosopis juliflora* (SW.) DC

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Abstract

Ethnomedicine is currently practiced in Egypt, where has been documented by ethnobotanical surveys. The main objective of the current study is to evaluate phytochemistry and antibacterial potential of ethanolic and aqueous extracts of *P. juliflora* cultivated in Matruh region with a view to considering their contribution to human health. Qualitative and quantitative phytochemicals screening of pods and leaves was carried out. Five pathogenic bacteria were selected as recipients including four Gram-negative and one Gram-positive bacteria. Chocolate and blood agar solid media were applied for agar diffusion method. Extracellular proteins before and after treatment were measured and total protein assay was performed. Cytological effect of the ethanolic extracts was investigated via TEM. Phytochemical screening provides the occurrence of alkaloids, cardiac glycosides, coumarins, flavonoids, phenolics, steroids, tannins and terpenes in pods and leaves ethanolic and aqueous extract. In agar diffusion experiment, data showed that pod ethanolic extract (5%) was more active than the other three types of extracts. The extract elicited a decrease in the growth of Staphylococcus aureus in culture medium in comparison to the control. Both pods and leaves ethanolic extracts showed a decrease in the extracellular protein content of the tested S. aureus compared with control. TEM showed that pod ethanolic extract affected the growth of *S. aureus* cells. It caused severe damage in the cytoplasmic membrane and also the cell wall. The present study concluded that the pods and leaves extracts of *P. juliflora* possesses antibacterial potential against target bacterial type cultures.

Keywords: Prosopis juliflora, Pathogenic bacteria, Phytochemistry, Growth, extracellular proteins, Cytogenic effect.

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INTRODUCTION

Traditional medicine is widely practiced in Egypt, where has been documented by ethnobotanical surveys (El-Darier *et al*., 2001; El-Darier *et al*., 2002; El-Darier *et al*., 2014). The high cost related to conventional drugs has led to over reliance on traditional medicine since it is affordable and available to rural people. On the other hand, even when modern health facilities are available, traditional medicine is viewed as an efficient and an acceptable system from a cultural perspective (El-Darier and Fakhry, 2005; El-Darier *et al*., 2007; El-Darier and El-Mogaspi, 2009).

Microbial infections are major public health problems in the developed and developing countries. Due to unselective use of commercial antibiotics, the incidence of multiple antibiotic resistances in human pathogens is increasing (Jeyachandran and Mahesh, 2007). Millions of people were affected by infectious diseases caused by bacteria worldwide, representing a major cause of death and infirmity. Today infectious diseases account for one-third of all deaths in the world; the WHO estimates that nearly 50,000 people die each day throughout the world from infectious diseases. The discovery of antibiotics is considering an essential part in combating bacterial infections that once wasted humankind. Bacterial resistance to the currently available antibiotics is a worldwide concern, which is attributed to the indiscriminate and improper use of current antimicrobial drugs (Usha *et al*., 2010).

Application of plants and herbs extracts in the treatment of human ailments is a very ancient art, a practice that has been passed on for generations and scientists in Africa and other developing countries and other are conducting research into local plants abundant in the continent for their possible use in traditional medicine.

Prosopis juliflora (invasive Mesquite) is a shrub or small tree in the family Fabaceae. It is native to Mexico, South America and the Caribbean and has become established as an invasive weed in Africa, Asia,

Australia and elsewhere (CABI, 2017). This herb is well-known in the folk medicine because of its ethnobotanical importance (Hebbar *et al*., 2004). Badri *et al.,* (2017) reported that, the leaf extracts of *P. juliflora* showed the various degree of inhibitory activity against different bacterial species. The present study is a trial to explore phytochemistry and antibacterial potentiality of ethanolic and aqueous extracts of *P. juliflora* cultivated in Matruh region with a view to assessing their contribution to human health.

METHODS

Phytochemical screening

Preparation of plant extracts

Pods and leaves of *P. juliflora* were collected from El-Kasr region, Matruh governorate. Phytochemical screening was performed using standard phytochemical procedures. Thirty-gram dry powder was taken and ethanol or water was added so that the plant material got totally immersed in the solvent (Khan *et al*., 2010). The extraction period was prolonged for 72 hours. At the completion of the extraction, the extract was filtered through Whatman No. 1 filter paper and the solvent was evaporated. Later, each of the test samples was processed further to use to evaluate the presence of saponins, tannins, flavonoids, alkaloids, terpenes and sterols. Before doing so, each test sample was reconstituted in the respective solvents and divided into aliquots to perform the qualitative tests (Deepa *et al*., 2013).

Qualitative and quantitative phytochemicals screening of pods and leaves

Qualitative test for saponins, tannins and terpenes were performed according to Odhiambo *et al*., (2014) while for steroids, alkaloids and flavonoids were according to Kavishankar *et al*., (2011). Furthermore, coumarins and phenol were identified according to Harborne, (1998). On the other hand, quantitative evaluation of flavonoids, alkaloids, saponins, phenol and tannins were according to Okwu and Ukanwa (2007), Poornima and Ravishankar (2009), Aliyu *et al.,* (2008), Hussain *et al.,* (2011) and Price and Butler (1977) respectively.

Bacterial species

Five pathogenic bacterial species (*Acinetobacter baumannii complex, Klebsiella pneumonia SSP pneumonia, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*) were selected as recipients to carry out the present study including only *Staphylococcus aureus* as Gram-positive bacteria. All of these bacterial species were obtained from Microbiology lab, High Institute of Public Health, Alexandria University and were maintained at 4°C on both chocolate agar slants and blood agar slants as well as Luria-Bertani (LB) slants.

Culture media

Chocolate agar and blood agar solid media were applied for agar diffusion method while Luria-Bertani (LB) broth medium was used for growth measurement of bacteria, extracellular protein and total protein assay.

Agar well-diffusion method

The agar diffusion method was used to test the antagonistic activity of the plant extracts against the five recipient pathogenic bacteria. The resulting inhibition zones were measured in centimeters (Bauer *et al.,* 1966 and Balouiri *et al.,* 2016).

Measurement of extracellular protein

Extracellular proteins of the five different pathogenic bacteria before and after treatment were measured according to (Lowry *et al.,* 1951).

Total protein assay

Preparation of cell lysate

The method assay was conducted according to the method described by Bradford (1976).

Transmission Electron Microscopy (TEM)

The freshly samples of cells not exposed to the plant extract (control) and others subjected to ethanolic and aqueous extracts were fixed using a universal electron microscope fixative. Series of dehydration steps were followed using ethyl alcohol and propylene oxide. The samples were then embedded in labeled beam capsules and polymerized. This section of cells was cut using LKB 2209-180 ultra-microtome and stained with a saturated solution of uranyl acetate for 30 min and lead acetate for 2 min (Mc Dowell and Trump, 1976). Electron micrographs were taken using a transmission electron microscope (JEM-100 CX JOel), at the Electron Microscope Unit, Faculty of Science, Alexandria University.

Statistical Analysis

Data collected on different parameters were analyzed statistically by using the COSTAT 2.00 statistical analysis software manufactured by Co-Hort Software Company. For analysis of variance and means data were separated using Fisher's protected least significant difference (LSD) test at 5% probability level (Steel *et al*., 1997).

RESULTS

Phytochemical Screening

Data in Table 1 provides the occurrence of some phytochemicals (alkaloids, cardiac glycosides, coumarins, flavonoids, phenolics, steroids, tannins and terpenes) in pods and leaves ethanolic and aqueous extract of *P. Juliflora.* It was recorded that alkaloids attained moderate concentration in both pods and leaves of the study species either in ethanolic or aqueous extract. Cardiac glycosides were absent in leaves but

reached low concentration in pods with both extracts. Coumarins attained low concentration in all cases except in pods with aqueous extract, it attained low moderate concentration. In the same context, flavonoids achieved low concentration in all cases except in pods with ethanolic extract it attained moderate concentration. It was clear that phenolics attained high and moderate concentration in pods and leaves, respectively in both extracts. Proteins attained moderate and low concentration in pods and leaves, respectively in the two extracts. Steroids were detected in leaves only either in ethanolic or aqueous extracts with high concentration. Clearly, tannins and terpenes attained low concentration in both pods and leaves with the two types of extracts.

Table 1: Qualitative phytochemical screening of pods and leaves of *Prosopis juliflora* in ethanolic and aqueous extracts

No.	Constituent	Ethanolic extract		Aqueous extract	
		Pods	Leaves	Pods	Leaves
1	Alkaloids	++	++	++	++
2	Cardiac glycosides	+	-	+	-
3	Coumarins	+	+	++	+
4	Flavonoids	++	+	+	+
5	Phenolics	+++	++	+++	++
6	Steroids	-	+++	-	+++
7	Tannins	+	+	+	+
8	Terpenes	+	+	+	+

+; low concentration, ++; moderate concentration, +++; high concentration, -; Absent

Data in Table 2 provides the concentration (%) of some secondary metabolites such as alkaloids, flavonoids, phenols, saponins and tannins in pods and leaves of *P. juliflora*. It was recorded that flavonoids attained the highest concentration values in pods (22%) and leaves (16 %). In contrast, tannins achieved the lowest values (0.66 and 0.33 %) in pods and leaves, respectively.

Table 2: Quantitative phytochemical screening of leaves and pods of *Prosopis juliflora*

Constituent	Concentration (%)	
	Pods	Leaves
Alkaloids	5.2 ± 0.09	3.6 ± 0.06
Flavonoids	22 ± 0.58	16 ± 0.39
Phenols	0.87 ± 0.21	0.66 ± 0.11
Saponins	3.92 ± 0.33	2.2 ± 0.23
Tannins	0.66 ± 0.08	0.33 ± 0.07

3.1. Antagonistic activity of plant extracts

The concentration of the different plant extracts applied in the present study was 5%. In the agar diffusion experiment, data showed that pod ethanolic extract (5%) was more active than the other three types of extracts. The maximum effect of both ethanolic and aqueous extracts was demonstrated against *Staphylococcus aureus* (Figure 1) while the minimum was established on *Acinetobacter baumannii*, *Klebsiella pneumonia*, *E. coli*, and *Pseudomonas aeruginosa* (Figure 2).

Figure 1: Inhibition zone for the effect of pod ethanolic extract against *Staphylococcus aureus* in chocolate (A) and blood (B) agar media

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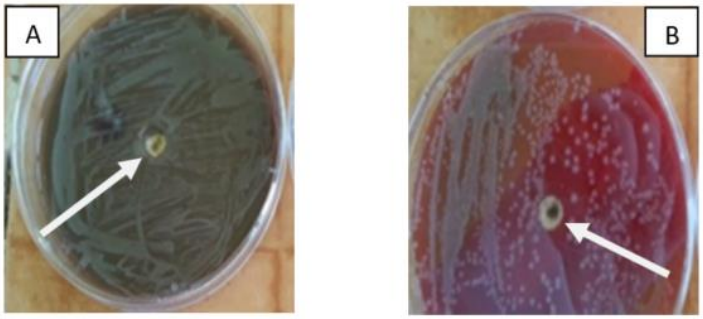

Figure 2: Inhibition zone for the effect of leaf ethanolic extract against *Acinetobacter baumannii complex*, *Klebsiella pneumonia SSP pneumonia*, *Escherichia coli*, and *Pseudomonas aeruginosa* in chocolate (A) and blood (B) agar media

Effect of plant extracts on bacterial growth in culture media

Pod ethanolic extract elicited a decrease in the growth of *Staphylococcus aureus* in culture medium in comparison to the control (Figure 3).

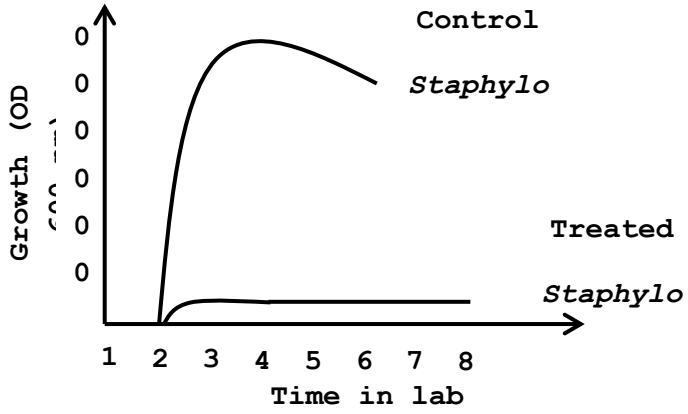

Figure 3: Effect of pod ethanolic extract on the growth of *Staphylococcus aureus*

Effect of ethanolic plant extract on *Staphylococcus aureus* extracellular protein

Both pods and leaves ethanolic extracts showed a decrease in the extracellular protein content of the tested *S. aureus* compared with control (Figure 4).

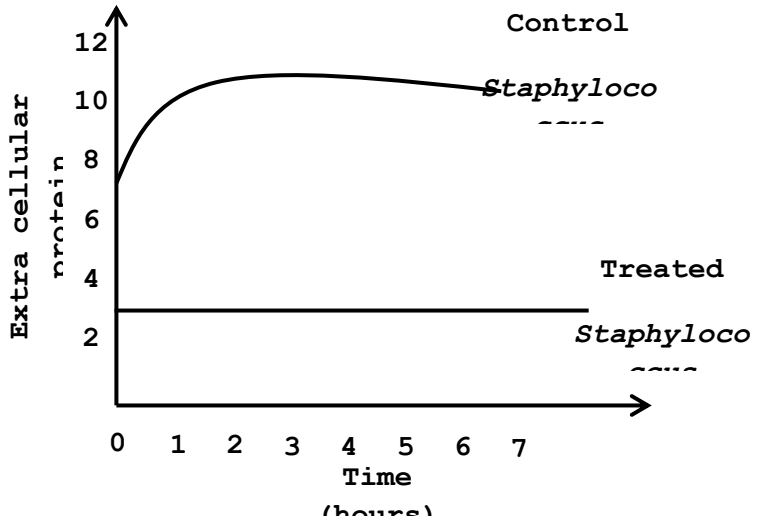

Figure 4: Effect of pod ethanolic extract on extracellular protein content of *Staphylococcus aureus*

Effect of plant extract on *Staphylococcus aureus* total proteins

Data showed that total protein decreased by about 82% compared to control in case of pod ethanolic extract and approximately 50% in case of leaf ethanolic extract. With respect to leaf aqueous extract, total proteins were reduced by about 27% compared to control (Table 3).

Table 1: Effect of pods and leaves ethanolic and aqueous extracts on *Staphylococcus aureus* total proteins

Sample	Absorbance (1)	Absorbance (2)	Absorbance (3)	Average	Cells weight (1ml)	Protein concentration (mg/ml)
Control	0.531	0.534	0.536	0.533	20.1	1.021
Ethanolic extract						
Pods	0.118	0.116	0.118	0.117	0.521	0.218
Leaves	0.254	0.251	0.254	0.253	0.813	0.480
Aqueous extract						
Pods	0.641	0.642	0.643	0.642	2.14	1.022
Leaves	0.388	0.387	0.389	0.388	1.18	0.741

Cytological effect of pod ethanolic extracts on *Staphylococcus aureus* cells

TEM showed that pod ethanolic extract affected the growth of *S. aureus* cells. It caused severe damage in the cytoplasmic membrane and also the cell wall (Figure 5).

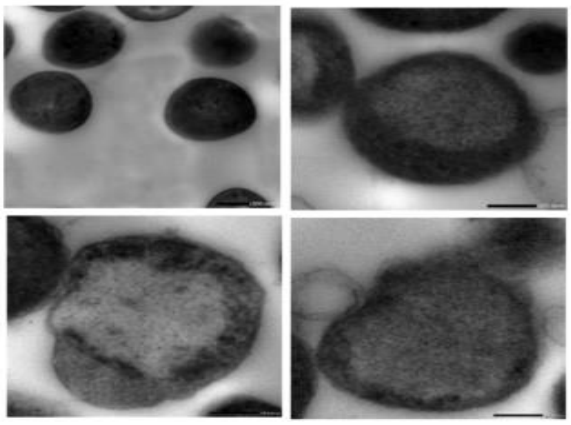

Figure 5: TEM graphs showing effect of pod ethanolic extract on *Staphylococcus aureus* cells

Furthermore, it also initiated abnormalities in morphology and a decrease or increase in cell volume as shown in Figure 6.

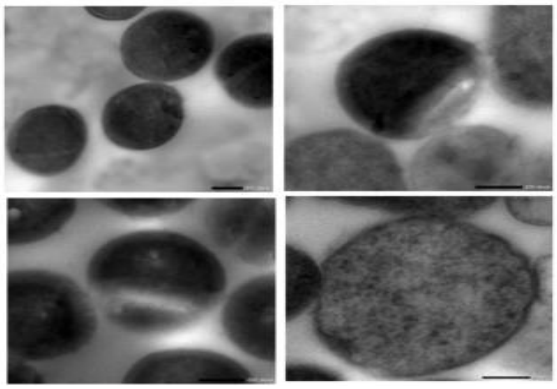

Figure 6: TEM graphs showing effect of pod ethanolic plant extract on *Staphylococcus aureus* cells

DISCUSSION

Prosopis juliflora yields several compounds including alkaloids, tannins, phenolics, steroids, terpenes, flavonoid, proteins, sugars, and fatty acids (Singh, 2012). Some of these compounds may exhibit therapeutic activities such as antibacterial activity. For example, juliprosinene and juliflorinine isolated from *P. juliflora* exhibit antibacterial effect on bacteria such as *E. coli, Staphylococcus. aureus, Klebsiella pneumoniae,* and *Shigella sonnei* (Prabha *et al*., 2014). Tajbakhsh (2015) stated that the extract of *P. juliflora* seed pods was effective against *Staphylococcus aureus, Staphylococcus epidermidis, E. coli,* and *Pseudomonas aeruginosa* but the Minimum Inhibitory Concentration (MIC) for gram-positive species was lower than that of for gram-negative species. In present study, five different pathogenic bacterial species including Gram-negative and Gram-positive bacteria were used. The effect of the four plant extracts (pods and leaves; aqueous and ethanolic) were tested by the agar diffusion method. Ethanolic extract was more active than aqueous one. The maximum effect of the ethanolic extracts was shown against *S. aureus* but has a minimum effect on the other types of bacteria. The aqueous extract had no effect on all types of the recipient bacteria. Contrariwise, Thakur *et al* (2014) reported that cold and hot extracts of *P. juliflora* leaves at 100 mg/ml concentration significantly inhibited the growth of all test bacteria viz., *Bacillus subtilis, Escherichia coli, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Salmonella typhi* and *Salmonella typhimurium.*

Both ethanolic extracts (pods and leaves) showed a decrease in the extracellular protein content of the tested *S. aureus* compared with control and the decrease was prominent in case of pod ethanolic >leaf ethanolic > leaf aqueous. It produced severe damage in the cytoplasmic membrane and cell wall and elicited abnormalities in morphology and cell volume. The leaf methanolic extract of *P. juliflora* were screened for

antibacterial activity against seven Gram negative bacteria (*Escherichia coli, Escherichia coli ESBL, Shigella flexneri, Salmonella typhi, Klebsiella pneumoniae, Pseudomonas aeruginosa and Proteus mirabiliss*), and three Gram positive bacteria (*Enterococcus faecalis, Listeria monocytogenes and Bacillus cereus*) using the cup-plate agar diffusion method. The extract employed pronounced activity against the tested bacteria as indicated by diameter of growth inhibition zones that varied from 12 to 41 mm except *S. typhi* has no inhibition zones (Badri *et al.*, 2017). *P. juliflora* pod methanolic crude extract tested on two tested Gram- negative bacteria (*E. coli and Klebsiella* spp.) and three Gram-positive bacteria (*Staphylococcus aureus, Bacillus* spp. *and Streptococcus* spp.) indicated an inhibition of all tested bacteria except *Klebsiella* spp. The tests on Gram-positive bacteria (*S. aureus, Streptococcus* spp. *and Bacillus* spp.) showed higher sensitivity than for Gram-negative bacteria (*E. coli and Klebsiella* spp.). The findings generally indicated that Gram-positive organisms were more susceptible to the extract of *P. juliflora* pods than Gram-negative organisms (Tajbakhsh*et al*., 2008, and 2011). The less susceptibility of Gram-negative bacteria to antibacterial substances in such studies may be associated with their outer membrane and lipopolysaccharide molecules which provide the barrier against penetration of antimicrobial molecules. Gram-positive bacteria do not have this type of outer membrane and cell wall construction (Willey *et al*., 2008). Moreover, Singh and Verma, (2011) investigated the antibacterial effect of alkaloid rich fractions of *P. juliflora* taken from different parts of plant including leaf, pods and flower. The leaf extract showed the highest antibacterial properties compared to the other organs. Thakur *et al*. (2014) stated that cold and hot extracts at 100 mg/ml concentration significantly inhibited the growth of all test bacteria viz., *Bacillus subtilis, E. coli, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Salmonella typhi and Salmonella typhimurium*. Overall, 100% bacteria produced the zone of inhibition during the screening process showing appreciable inhibitory effect.

The present study concluded that the pods and leaves extracts of *P. juliflora* possesses antibacterial potential against target bacterial type cultures. Therefore, further investigations regarding the mode of action and other related pharmacological studies such as in vivo investigation, drug formulation and clinical trials are highly recommended. Isolation and characterization of the bioactive components can be further done by systematic screening of the most active solvent fraction which could lead to the possible source of new antibacterial agents.

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