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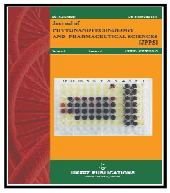

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In vitro antibacterial activity of extracts and alkaloid fraction of *Prosopis juliflora* (Sw.) DC. leaves

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Abstract

In the present work, phytochemical screening and *in vitro* antibacterial activity of extracts of *Prosopis juliflora* (Sw.) DC. leaves were carried out against various bacteria like *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella typhimurium*, *Streptococcus agalactiae* and *Staphylococcus aureus*. Chloroform, methanol and aqueous extracts were prepared using the Soxhlet extraction method. Per cent yield was calculated for each extract. Phytochemical screening was carried out for all the extracts. Antibacterial activity of all three extracts and crude alkaloid fraction of *P. juliflora* leaves were carried out using disc diffusion assay. Standard antibacterial discs were used to compare the antibacterial activity of the extracts. Additionally, the antibacterial activity of extract after removal of chlorophyll was also evaluated. Aqueous extract showed the highest extractability. All the extracts showed the presence of alkaloids. Aqueous extract and crude alkaloid fraction of *P. juliflora* leaves showed the highest *in vitro* antibacterial activity against all the bacteria as compared to chloroform, methanol and aqueous extracts. Methanol extract of *P. juliflora* leaves showed significantly higher antibacterial activity against all the bacteria as compared to extract without removal of chlorophyll. Results showed that alkaloids present in the *P. juliflora* leaves had shown antibacterial activity. Thus, *P. juliflora* leaves can be a good source of antibacterial phytochemicals. Further study related to structural identification may be helpful to explore the potential of the active phytochemicals present in the *P. juliflora* leaves.

1. Introduction

The emergence of multidrug-resistance (MDR) bacteria is the most emerging and challenging problem in the field of antibacterial chemotherapy for humans and animals. The MDR leads to the use of higher-classed antibiotics and increases the side effects in the body (Nikaido 2009). Several bacteria including *Staphylococcus aureus* (SA), *Klebsiella pneumoniae* (KP), *Escherichia coli* (EC), *etc.*, are considered notorious bacteria and requires higher antibiotics and a combination of the same to treat the infection. This may lead to an increase in resistance and also significant side effects (Giamarellou, 2010). Therefore, it is very necessary to explore newer antibacterial agents which can be useful to eradicate harmful pathogens.

A large number of medicinal plants and their phytochemicals are reported to have antibacterial activity. Not only in Ayurveda, but almost all systems of medicine like the Unani system, Traditional Chinese medicine, *etc.*, claimed to treat many infections with the use of medicinal plants. Saurashtra region of Gujarat (India) is considered a biodiversity-rich area for medicinal plants. People from this region are using many medicinal plants to treat infections.

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It is well-documented that stockmen from this area also treat the infection on their animals with medicinal plants (Bhatt *et al.*, 2019).

Different extracts of various medicinal plants like *Pueraria tuberosa* tuber, *Psoralea corylifolia* seed, *etc.*, had shown antibacterial action against *S. aureus*, while *Ficus racemosa bark* and *Moringa oleifera* leaf against *E. coli*. Phytochemicals present in medicinal plants like alkaloids, saponin, terpenoids, flavonoids, *etc.*, might be responsible for the antibacterial action (Pandya *et al.*, 2019).

Prosopis juliflora (Sw.) DC. belongs to the family Leguminosae (Fabaceae) and it is commonly growing in the roadsides and wastelands throughout India. Traditionally, pods and leaves are used as feed for lambs. Leaves are also used to prepare cosmetics in folklore practices. Leaves are used to cure the local infection. *P. juliflora* contains majorly alkaloids in all the parts of the plant. These alkaloids have antifungal, plant growth inhibiting and antioxidant activities (Bhatt *et al.*, 2019; Ukande *et al.*, 2019).

In vitro antimicrobial activity of alkaloids (Juliflorine, julifloricine) and alcoholic extract of the plant have also been studied previously (Ahmad *et al.*, 1986, 1989; Sathiya and Muthuchelian, 2008). However, the data of *in vitro* antibacterial activity of different types of extracts of *P. juliflora* are lacking. Thus, in the present study, *in vitro* antibacterial action of chloroform, methanolic and aqueous extracts of *P. juliflora* leaves and alkaloid isolated from *P. juliflora* was evaluated against various types of bacteria.

2. Materials and Methods

2.1 Collection of the plant material

Leaves of *Prosopis juliflora* (Sw.) DC. were collected from the premises of Junagadh Agricultural University, Junagadh (Gujarat) India. Leaves were shade dried and then were subjected to grinding to make powder.

2.2 Preparation of extract and phytochemical screening

Twenty-five grams of leaves were extracted with solvent extraction. Leaf material was subjected to de-fat with n-hexane in Soxhlet apparatus then extracted with chloroform, methanol and aqueous. Solvents were evaporated under reduced pressure in the rotary evaporator below 60°C. All the extracts were dried and weighed properly. All the extracts were labelled and stored at –20°C. All the solvents and chemicals of analytical grades (Merck Pvt. Ltd. or SD Fine Pvt. Ltd., India) were used for qualitative analysis for the presence of different phytochemical constituents in different extracts of plants as per the method described by Sarker *et al*. (2006).

Per cent extractability was calculated by the following formula:

$$\frac{\text{Total amount of extract obtained}}{\text{Total amount of powder used for extraction}} \times 100$$

2.3 Isolation of crude alkaloid fraction from *Prosopis juliflora* leaves

Isolation of crude alkaloid mixture from *P. juliflora* leaves powder was done by acid-base method **(**Manske, 1965). Fifty grams of powder was defatted with n-hexane in the Soxhlet apparatus for 72 h. Hexane was recovered and methanol was added to the dry defatted extract and allowed standing for 48 h. Methanol was recovered and methanol extract was dissolved in water and acidified up to pH 1-2. The acidified extract was shaken with diethyl ether to remove polar debris. Ether was removed and the aqueous phase was basified with 20% NaOH solution which precipitated out the crude alkaloid mixture. The basic extract was shaken with two quantities of chloroform which pulled the crude alkaloid. The chloroform layer was separated and evaporated under a vacuum. The 100 mg crude alkaloid mixture was dissolved in a diluted dimethyl sulfoxide (DMSO) solution and used for further evaluation.

2.4 Removal of chlorophyll from *P. juliflora* methanol extract

In separate set of experiment, for removal of chlorophyll, powder of *P. juliflora* was defatted with petroleum ether for 72 h and dried by removing petroleum ether in a rotary evaporator below 60°C (Sarkar *et al*., 2006). Methanol was added to extract the powder and allowed to stand for 24 h. After 24 h, methanol was evaporated under reduced pressure and extract was dried and used to evaluate antibacterial activity against tested bacterial strains as mentioned below.

2.5 Bacterial culture

Bacterial cultures of *Bacillus cereus* (ATCC 11778), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (NCIM 5082), *Salmonella typhimurium* (ATCC 23564), *Streptococcus agalactiae* (NCIM 2401) and *Staphylococcus aureus* (ATCC 9144) were procured from National Chemical Laboratory (NCL), Pune.

2.6 Chemicals and reagents

Nutrient broth, nutrient agar, MRS agar, MRS broth, Mueller Hinton agar, sterile blank disc and antibacterial discs were procured from HiMedia Lab, India and media were prepared for sub-culturing and to test the antimicrobial activity of plant extracts and standard antibacterial drugs as per specifications of the manufacturer.

Table 1: Physical appearance and per cent extractability of the different extracts of *P. juliflora* leaves

Name of plant	Name of extract	Physical appearance	Extractability (%)
Prosopis juliflora	Chloroform	Green lumpy	7.2
leaves	Methanol	Dark green dried mass	11.76
	Aqueous	Dark brown dry mass	14.84

Table 2: Phytochemical screening of various extracts of *P. juliflora* leaves

***P. juliflora* leaves**			
Phytochemicals	**Chloroform extract**	**Methanol extract**	**Aqueous extract**
Alkaloids	+	+	+
Glycosides	-	-	-
Saponins	-	-	-
CHO	-	+	-
Proteins	-	-	-
Flavonoids	-	-	+
Tannins	-	+	-
Steroids	-	-	-
Triterpenes	-	+	-

CHO: Carbohydrate; +/- = Presence/absence of phytochemical constituents

Table 3: Zone of inhibition in mm (Mean ± SE) of antibacterial against various bacteria

Antibacterial	*B. cereus*	*E. coli*	*K. pneumoniae*	*S. typhi*	*S. agalactiae*	*S. aureus*
Ceftriaxone	24.01 ± 0.17^{a}	29.81 ± 0.46^{a}	28.06 ± 0.69^{c}	27.83 ± 0.28^{b}	22.93 ± 0.17^{a}	24.01 ± 0.17^{a}
Tetracycline	25.96 ± 0.46^{a}	30.16 ± 0.11b^{a}	28.47 ± 0.52^{c}	22.42 ± 0.52^{a}	33.53 ± 0.52^{b}	25.96 ± 0.46^{a}
Levofloxacin	24.39 ± 0.52^{a}	41.48 ± 0.46^{b}	21.36 ± 0.40^{b}	31.69 ± 0.34^{b}	33.78 ± 0.34^{b}	24.39 ± 0.52^{a}
Gentamicin	24.97 ± 0.16^{a}	29.13 ± 0.13^{a}	14.54 ± 0.46^{a}	17.47 ± 0.69^{a}	24.51 ± 0.46^{a}	24.97 ± 0.16^{a}

Mean values with a different superscript in a column significantly differ at $p<0.05$

Table 4: Zone of inhibition in mm (Mean ± SE) of different extracts and crude alkaloid fraction from *P. juliflora* leaves against various bacteria

Plant extract	*B. cereus*	*E. coli*	*K. pneumoniae*	*S. typhi*	*S. agalactiae*	*S. aureus*
P. juliflora (C)	10.92 ± 0.17^{a}	11.19 ± 0.15^{a}	12.04 ± 0.10^{a}	12.62 ± 0.26^{a}	11.17 ± 0.19^{a}	10.58 ± 0.27 ^{a}
P. juliflora (M)	13.41 ± 0.23^{a}	12.91 ± 0.17^{a}	16.52 ± 0.25^{ab}	17.76 ± 0.26 ^{ab}	17.15 ± 0.17^{ab}	16.73 ± 0.31^{b}
P. juliflora (A)	13.21 ±0.16^{a}	13.82 ± 0.24^{ab}	14.33 ± 0.21^{a}	16.36 ± 0.26^{a}	14.14 ± 0.12^{a}	10.92 ± 0.16^{a}
P. juliflora alk	20.77 ± 0.11^{b}	22.03 ± 0.50^{b}	20.90 ± 0.29^{b}	19.39 ± 0.31^{b}	21.18 ± 0.34^{b}	18.39 ± 0.70^{b}

C = Chloroform extract, M= Methanol extract, A= Aqueous extract. Mean values with different superscript in a column are significantly differ at $p<0.05$

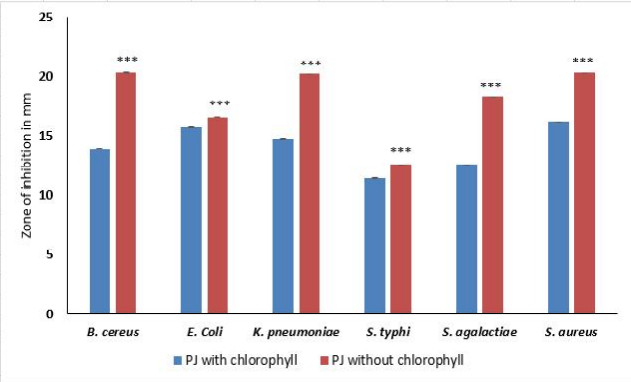

Figure 1: Zone of inhibition in mm (Mean ± SE) of *Prosopis juliflora* methanol extract (with and after removal of chlorophyll) against various bacteria.* Significant at $p<0.01$.**

2.7 *In vitro* antibacterial activity of various extracts of *P. juliflora* leaves

Aqueous and methanol extracts were reconstituted in sterile distilled water and chloroform extract was dissolved in sterile distilled water and dimethyl sulphoxide (DMSO) at a concentration of 200 mg/ml. Solution of extracts (50 µl) was dispensed on blank sterile discs and sterilised under exposure to UV light for 30 min. Standard antibacterial discs (ceftriaxone 30 mcg, gentamicin 10 mcg, levofloxacin 5 mcg, tetracycline 30 mcg) were used to observe the sensitivity of bacteria. The antibacterial activity of different extracts and antibacterial drugs by disc diffusion assay (Murray *et al.*, 1999) was evaluated in terms of measuring the zone of inhibition (ZOI) in mm.

2.8 Statistical analysis

All data are presented as mean ± SE. Data were analysed statistically using the Duncan Multiple Range Test (DMRT) test to compare the means of different groups. $p < 0.05$ was considered for statistical significance.

3. Results and Discussion

The physical appearance and per cent extractability of the different extracts of *P. juliflora* leaves is depicted in Table 1. The highest extractability has been observed with an aqueous extract of *P. juliflora*. Phytochemicals pre sent in various extracts of *P. juliflora* leaves are presented in Table 2. Alkaloid has been detected in methanolic and chloroform extracts of *P. juliflora.* Zones of inhibition in mm (Mean ± SE) of different extracts against tested bacteria are given in Table 3. All antibacterial drugs have shown good activity against tested bacteria. Mean zones of inhibition in mm of different extracts of *P. juliflora* and alkaloid against tested bacteria are given in Table 4.

Aqueous, chloroform and methanol extracts of *P. juliflora* leaves have shown moderate activity against *Bacillus cereus* (ATCC 11778), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (NCIM 5082), *Salmonella typhimurium* (ATCC 23564), *Streptococcus agalactiae* (NCIM 2401) and *Staphylococcus aureus* (ATCC 9144). *P. juliflora* crude alkaloid rich fraction was found to have very comparatively higher activity against all bacteria (ZOI ranging from 19.39 to 22.03 mm).

Antibacterial activity of *P. juliflora* leaf powder after removal of chlorophyll was also evaluated. Zones of inhibition of *P. juliflora* methanol extract (with and after removal of chlorophyll) against various bacterial isolates are given in Figure 1. Zones of inhibition against all the bacteria were significantly ($p<0.01$) increased with extract after removal of chlorophyll.

In vitro antimicrobial activity of alkaloids (juliflorine, julifloricine) and alcoholic extract of the plant were also evaluated by a few researchers (Ahmad *et al.*, 1986; 1989; Sathiya and Muthuchelian, 2008). Antimicrobial activity alkaloid fraction has been found more as compared to chloroform extract of *P. juliflora*. Shachi Singh *et al.* (2011) also observed good activity of alkaloids isolated from ethanolic extract of the plant. In the pre sent study, different types of bacteria were used to evaluate *in vitro* antibacterial effect of various types of extracts of *P. juliflora* leaves which is different from the previous reports.

4. Conclusion

It has been concluded that alkaloids are present in the leaves of *P. juliflora* which might be responsible for having the antibacterial effect of various extracts of *P. juliflora* leaves. The extract of *P. juliflora* leaves after removal of chlorophyll had shown comparable higher *in vitro* antibacterial activity. The plant would be an excellent source of bioactive natural products for therapeutic purposes. However, it is needed to test antibacterial potential of each alkaloid fraction present in *P. juliflora*.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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