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Evaluation of Antioxidant and Antibacterial activity of leaf extracts of *Prosopis cineraria* (L.) Druce

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ABSTRACT:

The present research, antibacterial and antioxidant activities of leaf extracts of *Prosopis cineraria* were evaluated. Free radical scavenging activity was screened by using 1, 1, diphenyl-2-picrylhydrazyl (DPPH) and Reducing power assay. Total phenol and flavonoid content was also quantitatively estimated in both the extracts. Total phenoilc content in the leaf was found to be 548 and 654 mg GAE/g extract, while the total flavonoid content in the leaf were found to be 184 mg/100g to 231 mg QE/g extract respectively. For antibacterial activity, aqueous and methanol extracts were tested for its antibacterial activity against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Proteus vulgaris*, *Sheigella flexneri* and *Klebsillae pneumonia*. Out of two extracts, methanol extract showed significant activity against *S.aureus* followed by *S.pneuminae* and *E.coli*. Aqueous extract exhibited no activity.

Keyword: DPPH, DMSO, Disc diffusion, Phytocompounds, Medicinal plant

INTRODUCTION

Free radicals are class of highly reactive molecules derived from the metabolism of oxygen. The fast production of free radicals may lead to oxidative damage to biomolecules and results in disorders such as degenerative disorders, cancer, diabetes, neutral disorder and aging (Hyun *et al*., 2006; Sas *et al*., 2007). Synthetic antioxidants and antimicrobials in use have been produces severe side effects (Gao *et al*., 1999; Williams *et al*., 1999); therefore, there is a need for more effective, less toxic and cost antioxidant and antimicrobials from natural sources. Medicinal plants, as the source of medicine, have been playing an important role in the health amenities around the world. Majority of the world's population depends on plant and their extracts for primary health care (Kunwar and Bussmann, 2008). A good number of our population particularly those living in rural areas rest on largely herbal remedies for the treatment of different types of infectious diseases. Nevertheless, little scientific research has been done to investigate the plants of this region used in herbal medicine and various problems associated with them. Therefore, during the present study, the background information of *Prosopis cineraria* was collected from in and around Salem District, Tamilnadu. *Prosopis cineraria* (L.) Druce (Syn. *Prosopis spicigera* L.) (Family Fabaceae (Leguminosae), sub. family Mimosaceae) is a small to moderate sized tree found in the regions of Arabia and various parts of India such as Rajasthan, Gujarat, Haryana, Uttar Pradesh and Tamilnadu. *Prosopis* spices have also been used in indigenous system of medicine as a folk medicine for various ailments. Reported to be astringent, demulcent, and pectoral, ghaf is a folk remedy for various ailments. In India, the flowers are mixed with sugar and administered to prevent miscarriage (Anshika Gupta *et al*., 2014).

MATERIALS AND METHODS

Plant materials

Fresh plant leaves were collected randomly from the gardens and villages of Salem district, Tamilnadu from the natural stands. The botanical identity of these plants was confirmed by Dr.V.Nandagopalan, Associate Professor, National College, Tiruchirappalli, Tamilnadu. The voucher specimens are deposited at the Department of Botany, National College (Autonomous), Tiruchirapalli-620 001, Tamilnadu, India.

Preparation of Extracts

Aqueous extract

Samples of 10g were immersed in 100 ml of distilled water, mixed and allowed to soak for 24hrs. Then the mixer was filtered through Whatmann No.1 filter paper to get pure extract.

Methanol extract

Air dried powder of 10 g was placed in a conical flask containing 100 ml of methanol plugged with cotton and then kept on a rotary shaker at 200 rpm for 24 hrs. Later, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 rpm for 15 min. The supernatant was collected and the solvent was evaporated to make volume one fourth of its original volume.

Antimicrobial activity

Disc diffusion method

Antimicrobial activity was carried out by the disc diffusion method. The antimicrobial assays of aqueous and methanol extracts were performed by Bauer *et al*. (1966). Each plant extract was tested at two different concentrations (100 and 200 µg/ml) to see their inhibitory effects against microbial pathogens. Sterile paper discs (6 mm in diameter) prepared from Whatman No. 1 filter paper was impregnated with drug, containing solution placed on the inoculated agar. The inoculated plates were incubated at 37°C for 24 h. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone for

the test microorganisms.

Minimum inhibitory concentration (MIC)

For determination of MIC, 1 ml of broth medium was taken into 10 test tubes for each bacterium. Different concentrations of plant extract ranging from 0.125 to 8 µg/ml^{-1} concentration were incorporated into the broth and the tubes were then inoculated with 0.1 ml of inoculums of respective bacteria (10^5CFU ml^{-1}) and kept at 37°C for 24 h. The test tube containing the lowest concentration of extract which showed reduction in turbidity when compared with control was regarded as MIC of that extract (Muhamed *et al*., 2011).

Antioxidant activity

DPPH radical scavenging activity

DPPH scavenging activity was carried out by the method of Blois (1958). Different concentrations (1000, 500, 250, 125, 62.5 and 31.2 mg/ml) of crude extracts were dissolved in DMSO (dimethyl sulfoxide) and taken in test tubes in triplicates. Then 5 ml of 0.1mM ethanol solution of DPPH (1, 1, Diphenyl-2-Picrylhydrazyl) was added to each of the test tubes and were shaken vigorously. They were then allowed to stand at 37ºC for 20 minutes. The control was prepared without any extracts. Methanol was used for base line corrections in absorbance (OD) of sample measured at 517nm. A radical scavenging activity was expressed as 1% scavenging activity and was calculated by the formula:

$$\text{OD\% radical scavenging activity} = \frac{\text{Control OD} - \text{Sample}}{\text{Control O.D}}$$

Determination of reducing power assay

Reducing activity was carried out by using the method of Oyaizu (1986). Different concentration (1000, 500, 250,125, 62.5 and 31.2 mg/ml) of crude extracts were prepared with DMSO and taken in test tube as triplicates. To test tubes 2.5 ml of sodium phosphate buffer and 2.5 ml of 1% Potassium ferric cyanide solution was added. These contents were mixed well and were incubated at 50ºC for 20 minutes. After incubation 2.5ml of 10% Trichloroacetic acid (TCA) was added and were kept for centrifugation at 3000rpm for 10 minutes. After centrifugation 5ml of supernatant were taken and to this 5ml of distilled water was added. To this about 1ml of 1% ferric chlorite was added and was incubated at 35ºC for 20 minutes. The OD (absorbance) was taken at 700nm and the blank was prepared by adding every other solution but without extract and ferric chloride (0.1%) and the control was prepared by adding every other solution but without extract. The reducing power of the extract is linearly proportional to the concentration of the sample.

Total phenolic content

Total phenolic contents were determined by Folin Ciocalteu reagent (McDonald *et al*., 2001). A dilute extract of each crude extracts (0.5 ml of 1:10g ml^{-1}) or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5ml, 1:10 diluted with distilled water) and aqueous sodium carbonate (4ml, 1 M). The mixtures were allowed to stand for 15 min and the total phenols were determined by colorimetry at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg/ml solutions of gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg g^{-1} of dry mass), which is a common reference compound.

Determination of total flavonoids

Aluminum chloride colorimetric method was used for flavonoids determination (Chang *et al*., 2002). Each crude fruit extracts (0.5ml of 1:10 g/ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1ml of 10% aluminum chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). The calibration curve was prepared by preparing quercetin solution at concentrations 12.5 to 100g ml^{-1}in methanol.

RESULTS AND DISCUSSION

The antibacterial activity of crude extracts was compared according to their zone of inhibition against the several microbial pathogens. The plant extracts showed their activity against both gram positive and gram negative organisms. Table 1 shows the microbial growth inhibition of methanol and aqueous extracts of the plant species and their MIC. The plant extracts showed their activity against both gram positive and gram negative organisms. Methanol extract showed more or less antibacterial activity against all six selected microbial pathogens. Out of the two extracts, methanol extract was found to be more potent against all the microbes showing 11 mm and 10 mm zone of inhibition against *S.aureus*, *S. pneumoniae* and *E.coli* respectively whereas aqueous extract showed comparatively moderate effect (Table 1). Aqueous extract of leaves exhibited low antibacterial activity compared to methanol extract, which certainly indicates that methanol extract contain higher concentration of bioactive compounds such as alkaloids, glycosides, volatile oils, which are all found in more abundant amount in *Prosopis cineraria*.

Table 1. Antimicrobial activity *Prosopis cineraria*

Name of the Strains	Zone of Inhibition (mm)				
	Methanol (mg/ml)		Aqueous (mg /ml)		Synthetic drug (Chloramphenicol)
	100	200	100	200	
Staphylococcus aureus	9	11		10	15
Streptococcus pneumoniae	-	10	-	-	18
Bacillus cereus	-	9	-	8	17
Escherichia coli	-	10	-	-	16
Pseudomonas aeruginosa	-	8	-	-	15
Klbseillae pneumoniae	-	-	-	-	17
Salmonella typhi	-	9	-	-	20
Proteus vulgaris	-	-	-	-	15
Shigella flexneri	-	-	-	-	16

Table 2. Determination of MIC and MBC of crude extracts *Prosopis cineraria*

S.No.	Name of the Strains	Methanol		Aqueous	
		MIC (mg /ml)	MBC (mg /ml)	MIC (mg /ml)	MBC (mg /ml)
1.	*Staphylococcus aureus*	1.0	0.125	0.250	-
2.	*Streptococcus pneumoniae*	-	0.250	-	-
3.	*Bacillus cereus*	-	0.500	0.250	4
4.	*Escherichia coli*	-	0.250	-	-
5.	*Pseudomonas aeruginosa*	-	2	-	-
6.	*Klbseillae pneumoniae*	-	-	-	-
7.	*Salmonella typhi*	-	4	-	-
8.	*Proteus vulgaris*	-	-	-	-
9.	*Sheigella flexneri*	-	-	-	-

Table 3. Antioxidant activity (DPPH Assay) *Prosopis cineraria*

Concentrations (µg/ml)	Methanol	Aqueous	Standard (Ascorbic acid)
1000	89.36 ± 0.30	80.86 ± 0.61	84.13 ± 1.45
500	76.5 ± 0.5	71.56 ± 0.51	
250	54.2 ± 1.11	52.33 ± 2.51	
125	29.33 ± 2.08	20.5 ± 0.95	
62.5	15.26 ± 0.94	14.83 ± 0.76	
31.25	10.43 ± 0.01	9.54 ± 0.43	

Each Value is SEM ± 5 individual observations

Table 4. Reducing power assay of *Prosopis cineraria*

Concentrations (µg/ml)	Methanol	Aqueous	Standard (Ascorbic acid)
1000	0.828 ± 0.17	0.730 ± 0.01	0.742 ± 0.45
500	0.546 ± 0.19	0.538 ± 0.12	
250	0.419 ± 0.25	0.386 ± 0.15	
125	0.236 ± 0.18	0.228 ± 0.16	
62.5	0.118 ± 0.09	0.101 ± 0.09	
31.25	0.063 ± 0.01	0.543 ± 0.05	

Each Value is SEM ± 5 individual observations

Table 4. Total phenol and Flavonoids contents in the crude extracts of *Prosopis cineraria* (mg/100g of crude extracts)*

Extracts	Total Phenols content*	Flavonoids content*
Methanol	654 ± 2.54	231 ± 0.43
Aqueous	548 ± 1.45	184.03 ± 1.87

*Each value in the table was obtained by calculating the average of three analyses ± standard deviation.

Since the activities were quite dynamic, MIC and MBC were determined in extracts optimal activity conditions. Table 3 provides MBC and MIC values. Results showed different values of MBCs and MICs, suggesting a selective activity of the extracts. In order to elucidate whether the observed antimicrobial effects were microbicide or microbiostatic, MBC/MIC ratios were calculated. Extracts with ratios greater than 1 were considered as microbiostatic, while the other extracts are microbicide. Results on antibacterial activities of the extracts are illustrated in Table 2. From these data, it can concluded that some extracts have either microbicide or microbiostatic activities on specific strains (Table 2).

The scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples, including plant extracts. The measured DPPH radical scavenging activity is shown in table 3. The *P.cinearia* leaves extract scavenging antioxidant activity was significantly increased with increasing concentration of the extract. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability. Lack of hydrogen donor bioactive constituents in the extract, slow rate of the reaction between DPPH and the substrate molecules resulting in low readings for antioxidant activity probably might explain the low DPPH antioxidant activity of the *P.cinearia* leaves extract (Stepek *et al*., 2005; Gilani *et al*., 2008).

The reducing power of crude extracts and the standard drug (Ascorbic acid) is shown in Table 4. The methanol extract of leaf had shown significantly higher reducing power than the aqueous extract *P.cinearia* leaf in a dose-dependent manner. Absorbance of solution was increased with concentration of plant extract, indicating the concentration of hydrogen donating compounds present in the extracts was increased or reducing power of extracts was increased. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The total flavonoid content varied widely among the extracts and ranged from 184 mg/100g to 231 mg/100 g of fresh

weight. Total phenol content different among the crude extracts ranged from 548 to 654 mg/100g of fresh weight (Table 5). The present study revealed the inhibitory effect of leaf extracts of selected plant against microbial pathogens. The extracts also exhibited marked antioxidant activity which may be attributed to the phenolic content of extracts. The plants can be used as potential source for the development of antimicrobial and antioxidant agents.

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