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EVALUATION OF ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF *Jasminum auriculatum*

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ABSTRACT

The present study aimed at evaluating the *in-vitro* study of antibacterial and antioxidant activities of crude extracts (methanol, Acetone and Ethyl acetate) of medicinal plant Jasminum auriculatum stem. The antioxidant activity of these extracts was determined by using DPPH (1, 1-Diphenyl -2-picryl hydroxyl) assay. It was found that the crude extract of methanol solvent of *Jasminum auriculatum* stem was significantly highest extract. Reducing power assay (RPA) significant results were observed in methanol extract of *Jasminum auriculatum* has estimated parameters. All the extracts showed lower antioxidant activity compared to ascorbic acid which was used as reference. It was found that various pathogenic microorganisms studied (*S. aureus, E. coli and P. aerugenosa*) were largely inhibited by extracts (methanol, acetone and ethyl acetate) of dried stem of *Jasminum auriculatum* using reference antibacterial drug ampycilin, by agar well diffusion method. The present study thus suggests the use of this medicinal plant may be exploited for health supplements.

Key words : *DPPH (1, 1-Diphenyl -2-picryl hydroxyl) assay, Reducing power asaay, antioxidant activity, agar well diffusion, antibacterial activity.*

Nature has been a source of medicinal agents since times immemorial. The importance of herbs in the management of human ailments cannot be overemphasized. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of many intractable diseases (1). The plants that posses therapeutic properties or exert beneficial pharmacological effects on the animal body generally designated as “medicinal plants”. Plant materials remain an important resource to combat serious diseases in the world (2). Approximately 62-80% of the world’s population still relies on traditional medicines for treatment of common illness (3). Over 50% of all modern clinical drugs are of natural origin. And natural products play important role in drug development in the pharmaceutical industry .

Jasmine is one of the oldest fragrant flowers and is especially appreciated in India. The term jasmine is probably derived from the Persian word ‘Yasmin’ meaning ‘fragrance’. Jasmines are widely grown in warm parts of southern Asia, Europe, Africa and the Pacific regions. Jasmine belongs to the family Oleaceae. Although more than 2,000 species are known, 40 species have been identified in India and 20 are cultivated in South India (5).

Five crude extracts were made from leaves and stems of *Jasminum subtriplinerve Blume* (Oleaceae) and investigated for antimicrobial, antioxidant and cytotoxic activities (6).

MATERIALS AND METHODS

Plant material : Plant has been collected from Fragrance and Flavour development centre (F.F.D.C.), Ministry of Micro, Small and Medium Enterprises, Government of India, Kannauj (U.P).

Extraction : The methodology which was used for the extraction as follows :

- The plant material was dried at room temperature under shade and ground to make it powder.
- Plant material was then soaked into solvents viz, ethyl acetate, acetone and methanol separately for 24 hours.
- The extracts were concentrated under reduced pressure.

The same method was repeated for 3 times.

Chemicals and reagents : DPPH, methanol, ethyl acetate, acetone, ascorbic acid, distilled water Phosphate buffer (0.2M, pH 6.6), Potassium ferricyanide (1%), Trichloroacetic acid (10%), Ferric chloride (0.1%).

Microorganisms : The antibacterial activity was tested

against the following 3 selected strains: *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. The selected bacterial strains were obtained from Microbiology and Fermentation technology lab.

I. Evaluation Antibacterial activity

Agar well diffusion method : Agar well diffusion method elucidated by (7) was followed. The antibacterial activity of crude extracts of *Jasminum auriculatum* was evaluated by using agar well diffusion method. The Nutrient Agar plates were prepared by pouring 15 ml of molten media into sterile Petriplates. About (10^8 - 10^9) colony-forming units per ml was used. Wells or cups of 10 mm size were made with sterile borer into agar plates containing the bacterial inoculums. 10 µl of microbial broth culture was spread on the surface of nutrient agar plates, 100 µl volume of the plant extract of concentration (0.5,1.0,1.5,2.0 mg/ml) was poured into a well of inoculated plates. Ampicillin (10 mg/ml) was used as a positive control which was introduced into a well instead .Solvents methanol, acetone, ethyl acetate, hexane and distilled water was used as a negative control, which was introduced into a well instead of plant extract.

The plates thus prepared was left at room temperature for ten minutes allowing the diffusion of the extract into the agar (8). After incubation for 24 hrs at 37, the plates was observed .If antibacterial activity will be present on the plates, it was indicated by an inhibition zone surrounding the well containing the plant extract. The zone of inhibition was measured and expressed in millimeters. Antibacterial activity was recorded if the radius of zone of inhibition was greater than 4 mm (9). The antibacterial activity results was considered as inactive if ? 4.5 mm ; 4.5-6 mm as partially active; while 6.5-9mm as active and greater than 9 mm as very active (10).

II. Evaluation of antioxidant activity

Scavenging activity on DPPH radical : The DPPH radical scavenging assay elucidated by was followed.

Procedure : Different dilutions of extract (200, 400, 600, 800 µg/ml) were prepared. DPPH solution was also prepared by dissolving 6.0 mg of DPPH in 100 ml methanol. Then, 1 ml of extract from each dilution was added into the test tube containing 2 ml of DPPH -solution. Control was prepared by adding 1 ml of methanol to 2 ml of DPPH solution. Ascorbic Acid was used as standards. The mixture was shaken vigorously and was left to stand in the dark for 30 min. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The scavenging activity of each extract on DPPH radical was calculated using the following equation:

Table-1: Antibacterial activity of *Jasminum auriculatum* methanol extract by agar well diffusion method.

Concentration mg/ml	Mean radius of inhibtion zones of bacteria (mm)		
	S. aureus	*E. coli*	*P. aeruginosa*
0.5	11.00 ± 1.73	13.00 ± 1.00	13.00 ± 2.23
1.0	13.00 ± 1.00	14.00 ± 3.46	13.00 ± 1.85
1.5	14.00 ± 3.46	16.00 ± 1.73	16.00 ± 2.22
2.0	16.00 ± 2.65	18.00 ± 1.73	18.00 ± 1.79
-ve control	11.00 ± 3.61	12.00 ± 2.65	12.00 ± 1.34
+ve control	14.00 ± 1.32	16.00 ± 1.73	16.00 ± 1.53

Values were expressed as Mean + S.D. (n = 3)

SE = 0.865 CD = 1.834

Table-2 : Antibacterial activity of *Jasminum auriculatum* acetone extract by agar well diffusion.

Concentration mg/ml	Mean radius of inhibtion zones of bacteria (mm)		
	S. aureus	*E. coli*	*P. aeruginosa*
0.5	11.00 ± 0.87	12.00 ± 1.73	13.00 ± 1.85
1.0	11.00 ± 1.48	13.00 ± 2.65	14.00 ± 2.50
1.5	12.00 ± 2.65	16.00 ± 2.65	14.00 ± 1.76
2.0	13.00 ± 3.46	16.00 ± 1.73	16.00 ± 1.75
-ve control	11.00 ± 1.37	11.00 ± 2.65	11.00 ± 2.00
+ve control	14.00 ± 1.32	16.00 ± 2.00	16.00 ± 1.00

Values were expressed as Mean + S.D. (n=3)

SE = 1.092 CD = 2.315

Table-3: Antibacterial activity of *Jasminum auriculatum* ethyl acetate by agar well diffusion.

Concentration mg/ml	Mean radius of inhibtion zones of bacteria (mm)		
	S. aureus	*E. coli*	*P. aeruginosa*
0.5	11.00 ± 1.46	13.00 ± 2.65	12.00 ± 0.87
1.0	12.00 ± 1.00	14.00 ± 3.46	12.00 ± 0.88
1.5	12.00 ± 1.82	15.00 ± 2.65	14.00 ± 0.92
2.0	13.00 ± 1.85	16.00 ± 1.73	17.00 ± 0.87
-ve control	12.00 ± 1.30	11.00 ± 1.73	11.00 ± 0.60
+ve control	16.00 ± 1.00	16.00 ± 3.61	16.00 ± 0.30

Calculation : % scavenging activity = [Absorbance of control – Absorbance of sample / Absorbance of control] × 100.

Reducing power assay : Antioxidant activity by reducing power assay (Yen and Duh. 1993) was as followed.

Procedure : The reducing power of the test sample was determined by taking different concentration of the leaf extract (200, 400, 600, 800 µg/ml) in 1 ml methanol. They were mixed with 2.5ml of phosphate buffer and 2.5ml of potassium ferric cyanide [$K_3Fe(CN)_6$] in test tubes . The mixtures were incubated for 20 min .at 50 C°. At the end of the incubation 2.5ml of tri-chloroacetic acid was added to the mixtures followed by centrifuging at 500 rmp for 10 min. The upper layer (2.5ml) was mixed in 2.5ml distilled water and 0.5ml of ferric chloride and the absorbance was measured at 700nm. The reducing power test was run in triplicates.

Statistical analysis : Results were reported as Mean ± S.D. and data were tested by two-way analysis of variance.

RESULTS AND DISCUSSION

The results of antibacterial activity are given in Table-1, 2 and 3. From the tables, it is clear that all the extract at various concentrations have shown antibacterial activity against the three bacterial strains which are used. Methanol and ethyl acetate extracts have shown better activity than acetone extract. The results of antioxidant activity by DPPH method, 200 µg/ml of methanol extract exhibited free radical scavenging potential (53.57%), which was higher than acetone (22.44) and ethyl acetate extract (48.46). Likewise in 400 µg/ml methanol extract showed the highest free radical scavenging activity (60.20%) as compared to acetone extract (28.57) and ethyl acetate extract (50.51%). Methanol extract of 600 µg/ml had higher free radical scavenging activity (67.34%) than acetone extract (34.18%) and ethyl acetate (62.75%) whereas 800 µg/ml of methanol extract had highest free radical scavenging activity (69.89%) as compared to acetone extract (47.44%) and ethyl acetate extract (68.87%). It was observed that all the three extract had lower antioxidant activity compared to the ascorbic acid (78.06%) as shown in Table-4 and fig-1. The results of antioxidant activity by reducing power assays as shown in Table 5 and fig 2 are the maximum reducing power was observed *i.e.*0.327 with 50µg/ml of methanol extract of *Jasminum auriculatum* as compared to acetone extract 0.241 and ethyl acetate 0.307. In case of methanol extract of sample 100µg/ml, highest reducing power was 0.355 than acetone extract 0.264 and ethyl acetate extract 0.320. Methanol extract of test sample 200 µg/ml showed highest reducing power of 0.371 as compared to acetone extract 0.280 and ethyl acetate extract 0.331. Maximum antioxidant activity of *Jasminum auriculatum* extracts by reducing power assay at 400µg/ml concentration was observed that is 0.389 in methanol, the next higher reducing power was

Table-4: DPPH free radical scavenging assay (%) of extracts of Jasnimum auriculatum.

Methanol OD 517 nm	%	Ethyl acetate OD 517 nm	%	Acetone OD 517 nm	%	Ascorbic acid OD 517 nm	%	Conc. (µg/ml)
0.091 ± 0.0020	53.57	0.101 ± 0.0030	48.46	0.152 ± 0.0783	22.44	0.061 ± 0.0021	68.87	200
0.082 ± 0.0070	60.20	0.097 ± 0.0020	50.51	0.146 ± 0.0025	28.57	0.060 ± 0.0015	69.89	400
0.064 ± 0.0015	67.34	0.074 ± 0.0025	62.75	0.129 ± 0.0020	34.18	0.056 ± 0.0025	71.42	600
0.059 ± 0.0025	69.89	0.062 ± 0.0021	68.87	0.103 ± 0.0015	47.44	0.043 ± 0.0015	78.06	800

Values were expressed as MEAN ± S.D. (n=3) SE = 0.008 CD = 0.017

Table-5: Reducing power activity of Jasminum auriculatum extract.

Methanol	Ethyl acetate	Acetone	Ascorbic acid	Concentration (µg/ml)
0.327 ± 0.0012	0.307 ± 0.0023	0.241 ± 0.0015	0.422 ± 0.0026	50
0.355 ± 0.0031	0.320 ± 0.0010	0.264 ± 0.0012	0.443 ± 0.0015	100
0.371 ± 0.0015	0.331 ± 0.0015	0.280 ± 0.0015	0.465 ± 0.0042	200
0.389 ± 0.0020	0.362 ± 0.0156	0.293 ± 0.0031	0.482 ± 0.0017	400

Values were expressed as MEAN ± S.D. (n=3) SE = 0.003 CD = 0.007

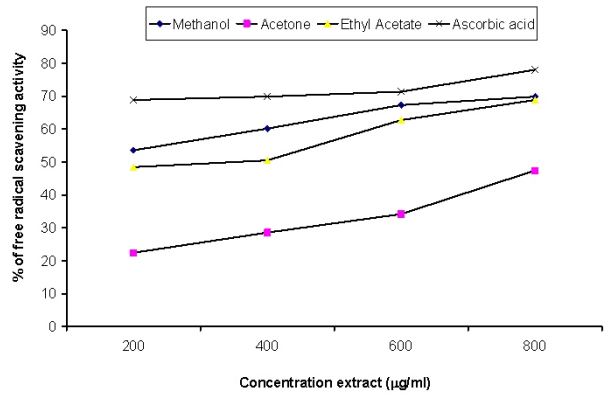

Fig.1: DPPH free radical scavenging assay (%) of stem extracts of *Jasnimum auriculatum.*

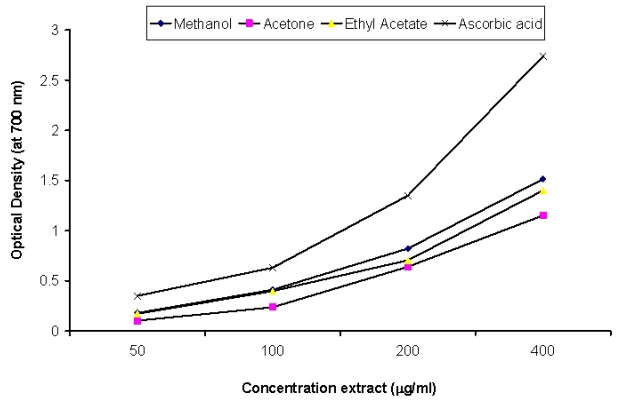

Fig. 2: Reducing power activity of *Jasminum auriculatum* extract.

observed in ethyl acetate extract that is 0.362. But acetone extract was 0.293 showing low reducing power compared to others.

The data showed that the entire sample increased their reducing ability when the concentration of extract was increased. There was slight difference in reducing ability of all the three extracts. Hence methanol, acetone and ethyl acetate of *Jasminum auriculatum* had antioxidant activity. All samples of extract showed lowest reducing power as compare to ascorbic acid (0.482)**.**

CONCLUSION

The results of the study suggest that the extracts of *Jasminum auriculatum* stem are effective against the tested pathogens and possess antioxidant activity. The present study supports the view that several ethno plants might be useful as antibacterial agents. The encouraging results of *J. auriculatum* stem with the various antioxidant tests proved the plant as a reducing agent and effectiveness as scavengers of free radicals. Hence, it is worthwhile to isolate and elucidate the bioactive principles that are responsible for the antioxidant activity that is underway.

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