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In vitro Antimicrobial and Antioxidant Activity of the Crude Extracts of *Pterospermum acerifolium* Willd Leaves (Sterculiaceae)

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Abstract

Different extracts of *Pterospermum acerifolium* Willd leaves were investigated for its antimicrobial activity on various microorganisms, i.e., 5 bacterial species and 3 fungal species. Among all extracts, water extract showed maximum antibacterial activity against all species. Present paper has also evaluated free radical scavenging property of acetone, ethanol, water extracts by different *in vitro* models, i.e., 1,1-diphenyl-2-picryl hydrazyl (DPPH), nitric oxide and reducing power. The ethanol extract found to have more free radical scavenging activity among all extracts.

Keywords: *Pterospermum acerifolium, Antimicrobial, Minimum inhibition concentration, Antioxidant, DPPH, Reducing power, Nitric oxide*

1. Introduction

Pterospermum acerifolium Willd (Sterculiaceae) having Muchkund as local Indian name, was evaluated for preliminary antimicrobial and antioxidant activity. *Pterospermum acerifolium* Willd., a large tree belonging to the Sterculiaceae, was widely distributed in India particularly in sub-Himalayan tract and outer Himalaya valleys. In the Konkan the flowers and bark are charred and mixed with Kamala and applied in suppurating small pox (Kirtikar and Basu, 1993).The flowers are sharply bitter, acrid, tonic, laxative anthelmentic removes cough (in Ayurveda). Also useful in leucorrhoea, inflammation, ulcer, leprosy. Leaves are used as haemostatic. Phytochemical review found that flavonoids like Kampferol, Kampferide, luteolin and steroids and triterpenoids like β-sitosterol, taraxerol, friedelin, sugars, fatty acids are reported in this plant (Asima and Satyesh, 1991; The Wealth of India, 2003).

Plants are potent biochemists and have been components of phytomedicine since times immemorial. Many plant species have been utilized as traditional medicines but it is necessary to establish the scientific basis for the therapeutic actions of traditional plant medicines as these may serve as the source for the development of more effective drugs. In view of that the different extracts of *Pterospermum acerifolium* Willd were screened for potential antibacterial activity againstsome medically important bacterial strain and for different antioxidant methods (Varshney *et al.*, 1972).

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2. Materials and Methods

2.1. Collection of Plant Material

Fresh leaves of *Pterospermum acerifolium* Willd collected in the month of August to September from Amravati district, Maharashtra. The fresh leaves of *Pterospermum acerifolium* Willd were dried under shade and powder in a mixture grinder. The powder leaves packed in a paper bags and stored in air tight container until use.

2.2. Extraction

The Soxhlet process was used for the extraction of plant material with particular solvent, choose accoding to increasing its polarity like petroleum ether, benzene, chloroform, acetone, ethanol. Cold maceration technique used for water solvent. Qualitative phytochemical analysis of all extracts was performed to know chemical constituents of extracts.

2.3. Antimicrobial Study

Microorganisms – All the strains of microorganism were obtained from National Chemical Laboratory, Pune. Table 1 is showing gram-positive, gram-negative and fungal microorganisms used for the study.

Table 1: Gram Positive, Gram Negative and Fungal Microorganisms

S. No.	Name of Microorganism	Code
1.	*Staphylococcus aureus*	NCIM 2079
2.	*Proteus vulgaris*	NCIM 2813
3.	*Bacillus subtilis*	NCIM 2063
4.	*E.coli*	NCIM 2065
5.	*Pseudomonas aerugenosa*	NCIM 2036
6.	*Aspergillus niger*	NCIM 545
7.	*Aspergillus flavon*	NCIM 610
8.	*Candida albicans*	NCIM 3100

2.4. Antibacterial Assay

2.4.1. Agar-Well Diffusion Method

The agar diffusion method was used to screen the antibacterial activity of various extract of leaves of *Pterospermum acerifolium* Willd. 0.2 mL of each of the seeded broth containing 10^{-7} test organisms was inoculated on the plates of solidified agar and spreaded uniformly. Then 8 wells were cut in the agar layer of each plate with an aluminum bore of 6 mm diameter. In every plate 6 different extracts of concentration 20 mg/mL dissolved in DMSO were added while in 7th and 8th wellstandard tetracycline and control DMSO was added. Then all plates were incubated at 37 ^{o}C ± 1 for 18 h. After the incubation period the mean diameter of the zone of inhibition in mm obtained around the well was measured which has been shown in Table 2.

2.5. Antifungal Assay

Anti-fungal study was carried out through same procedure as used in antibacterial study only difference was media used for antifungal study was Sabouraud dextrose agar media (SDA MEDIUM) (Dugler and Gonuz, 2004; Lino and Apak, 2006). Results are shown in Table 2.

3. Determination of Minimum Inhibitory Concentration (MIC)

3.1. Two-Fold Serial Dilution Method

The test was carried out individually for different microorganisms with respect to the benzene, water solvent extract which shows comparative more effective result than other solvent on bacteria. This testing was done in the seeded broth by two-fold serial dilution technique. The solutions of different extracts were prepared of concentration 20 mg/mL in DMSO. For both gram positive and gram negative organism a series of 7 assay tubes for concentration, i.e., 20, 10, 5, 2.5, 1.25, 0.625, 0.3125 mg/mL for each extract were used. While standard tetracycline concentration was taken as 5, 2.5, 0.625, 0.3125, 0.15625, 0.078072 mg/mL. One positive control for each microorganism was prepared by adding nutrient

broth with respective microorganism inoculums. To each concentration test tube required volume of sterile nutrient broth and inoculums was added and kept for 24 h incubation at 37 °C. After incubation period the growth of microorganism by considering turbidity was measured by using turbidometer (Parekh *et al.*, 2006). Results are shown in Table 3.

Table 2: Zone of Inhibition in mm (Including Bore Diameter 6 mm)

Microbial Extract	PT	BE	CH	AC	ET	WT	STD
Strain 20 mg/mL							
Gram +ve							
Staphylococcus aureus	8±1.0	19.33±0.58	14.331±1.53	16.33±1.53	15±1.0000	18.66±0.5773	22±1
Bacillus subtilis	10±1.0	17.33± 2.08	16 ±1	16.33±0.58	17.33±1.1547	19±1.0	24.33±0.5
Gram –ve							
E. coli	9.33± 0.58	18.33±0.58	13±1.0000	13±1.0	13.66±0.5773	19±1.0	23±1.7
P. aerugenosa	9.33±0.5773	17±1.0	12.33±2.0816	16.33±1.1547	15±1.00	18.33±0.5773	24±1
Proteus vulgaris	10±1.0	17 ± 1.00	14±1.0000	17±1.0000	16 ± 1.00	17.66±0.5773	24.33±0.5
Fungi							
Aspergillus niger	15 ± 1.15	19.33± 0.57	14.66 ± 0.57	14.66 ± 1.5	19.33 ± 0.57	18.33 ± 0.57	28.33 ± 0.57
Candida albicans	12 ± 1	20.33 ± 0.57	16.33 ± 1.5	16 ± 1	17.66 ± 0.57	17.33 ± 0.57	27 ± 1
Aspergillus falvon	13 ± 1	19.33 ± 0.1	15.66 ± 0.5	15 ± 2	19.33 ± 1.52	18.33 ± 0.57	29 ± 1

Note: Where PE: Pet ether, BE:Benzene extract, CH: Chloroform extract, AC: Acetone extract, ET: Ethanol extract, WT: Water extract, STD: Standard drug.

Table 3: Results of Minimum Inhibitory Concentration

Microbial Extract	BE (mg/mL)	ET (mg/mL)	WT (mg/mL)
Strain			
Staphylococcus aureus	2.5	5	2.5
Bacillus subtilis	2.5	5	5
E. coli	1.25	2.5	2.5
P. aerugenosa	5	5	2.5
Proteus vulgaris	5	10	2.5
Aspergillus niger	5	2.5	5
Candida albicans	10	10	10
Aspergillus flavon	10	5	5

3.2. In vitro Antioxidant Study

Acetone, ethanol, water extract of leaves of *Pterospermum acerifolium* were tested for its free radical scavenging property using three different *in vitro* models. All experiment was performed thrice and the result averaged.

3.3. DPPH Radical Scavenging Activity

Methanolic solution of each extract, i.e., acetone, ethanol, hydroalcoholic, water (10, 20, 40, 60, 80, 100 µg/mL) was mixed with 400 mM DPPH (Sigma Aldrich) methanol solution at a ratio 1:3. The mixture was left in dark at room temperature for 90 min. The absorbance of the resulting solution was measured by spectrophotometer (Shimandzu 1700) at 517 nm. The capability of scavenging DPPH radical was then calculated by using following equation. Results are shown in Table 4.

Scavenging Effect (%) = [(Abs. of control – Abs. of sample) / Abs. of control] × 100

Table 4: DPPH Radical Scavenging Activity of Each Extracts

S. No.	Extract	Concentration (µg/mL) and % Inhibition						IC50(µg/mL)
		10	20	40	60	80	100	
1.	WT	20.76±0.06	40.80±0.11	67.10±0	74.05±0.06	83.39±0.06	92.26 ± 0.02	34.00
2.	ET	28.23±0.1	42.52±0.10	70.90±0.06	79.32±0.06	86.21±0.06	94.37±0.06	27.88
3.	AC	23.99±0.06	39.80±0.06	66.27±0.01	79.47±0.06	81.47±0.06	93.27±0.02	32.45
4.	Vit. C	24.92±0.06	54.68±0.06	78.04±0.06	85.34±0.06	91.56±0.01	95.58±0.06	20.58

Note: WT-Water, ET-Ethanol, AC-Acetone, Std.Ascorbic acid.

3.4. Nitric Oxide Radical Scavenging Activity

1 mL sodium nitroprusside (10 mM) in phosphate buffered saline was mixed with different concentration of 1 mL aqueous, hydro alcoholic, ethanol, and acetone extract dissolved in methanol and incubated at room temperature for 180 min. The same reaction mixture without the extract of the sample but with equivalent amount of phosphate buffer served as the control. After incubation period 1 mL Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% N-(naphthyl) ethylenediamine hydrochloride NEDA) was added to equivalent amount of sample. The absorbance of chromophore formed during diazotization of nitrite with sulphanilamide and subsequent coupling with NEDA was measured at 546 nm for the determination. Results are shown in Table 5.

Table 5: Nitric Oxide Scavenging Activity of Extracts

S. No.	Extract	Concentration (µg/mL) and % Inhibition						IC50(µg/mL)
		10	20	40	60	80	100	
1.	WT	25.81±0.146	32.74±0.030	41.25±0.214	60.91±0.005	66.98±0.005	79.98±0.01	52
2.	ET	28.48±0	39.29±0.005	57.86±0.02	67.96±0.005	74.38±0.011	82.74±0.02	44
3.	AC	23.10±0.185	32.75±0	45.16±0.01	57.70±0.005	67.01±0	80.24±0	52
4.	HA	22.61±0.036	32.15±0.005	42.59±0.005	60.13±0.005	66.76±0.005	77.37±0.17	53
5.	Vit.C	34.00±0.006	48.51±0.017	58.39±0.06	67.71±0	80.12±0	87.18±0	39.99

Note: WT-Water, ET-Ethanol, AC-Acetone, Std.Ascorbic acid.

3.5. Reductive Activity

The reducing power of the extracts was determined according to the method of Oyaizu (1986). Various concentrations of the extracts (100-1000 µg/mL) in 1 mL of deionized water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1% potassium ferricynide (2.5 mL). The mixture was incubated at 50 °C for 20 min. aliquots of trichloroacetic acid (2.5 mL, 10%) were added to the mixture, which was then centrifuged at 1036 x g for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared $FeCl_3$ solution (0.5 mL, 1%). The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. These reducing powers of extract compare with standard ascorbic acid (Rajeshwar *et al.*, 2005; Mondal *et al.*, 2006). Results are summarized in Table 6.

Table 6: Reducing Power Assay

S. No.	Extracts	Conc. µg/mL	Absorbance at 700 nm
1.	Water	100	0.3751±0.005
		200	0.5050±0.0001
		400	0.6952±0.005
		600	0.8287±0.0009
		800	1.0246±0.0003
		1000	1.2702±0.01
2.	Ethanol	100	0.3981±0.00001
		200	0.6168±0.004
		400	0.8933±0.0007
		600	1.0117±0.004
		800	1.1987±0.005
		1000	1.3615±0.017
3.	Acetone	100	0.2914±0.003
		200	0.4771±0.003
		400	0.7015±0.0005
		600	0.9418±0.0006
		800	1.1011±0.006
		1000	1.2064±0.0002
4.	L-Ascorbic acid	100	0.4681±0.0008
		200	0.6126±0.001
		400	0.9311±0.0005
		600	1.1972±0.001
		800	1.3157±0.06
		1000	1.5784±0.015

4. Results and Discussion

Phytochemical investigations clears that *Pterospermum acerifolium* Willd leaves contain tannin, saponins, triterpenoids, cardiac glycosides, flavonoids.

Antimicrobial studies by agar well diffusion method has shown that all extracts of leaves of *Pterospermum acerifolium* has considerable antibacterial and antifungal activity against all microbial strains. Though all extracts were found effective against bacteria and fungi, benzene and water showed maximum inhibition against Gram positive and Gram negative bacteria. Petroleum ether extract showed less inhibition. MIC of water extract in average on both Gram positive and Gram negative was found to be 2.5 mg/mL while MIC of benzene and ethanol extract was found to be in a range 2.5 to 5 mg/mL.

In antifungal study ethanol and water extract showed good antifungal activity. MIC of ethanol on *A. niger, A. flavons, Candida albicans* was found to be 2.5, 5, 10 mg/mL respectively and MIC of water extract was found to be 2.5, 5, 10 mg/mL respectively.

In vitro antioxidant study results of DPPH, nitric oxide and reducing power method has cleared that all extracts possesses significant antioxidant properties and antimicrobial effect may be correlated to this property. In DPPH method, acetone, ethanol, water extracts and ascorbic acid (100 µg/mL) exhibited 93.27%, 94.37%, 92.26%, and 95.58% of percentage inhibition with IC50 values 32.45, 27.88, 34.00, 20.58 µg/mL respectively.

Nitric oxide radical generated from sodium nitroprusside at physiological pH was found to be inhibited by acetone, ethanol and water extracts. The concentration of acetone, ethanol, water extract and ascorbic acid needed for 50% inhibition was found to be 52, 44, 53, 39.99 µg/mL respectively.

For measurements of the reductive ability, we investigated the Fe^{3+} to Fe^{2+} transformation in presence of different extracts of *Pterospermum acerifolium*. The reducing capacity of compound may serve as significant indicator of its potential antioxidant. Reducing power of selected diluted extract found to be significant and as good as L-Ascorbic acid.

5. Conclusion

All extracts of leaves of *Pterospermum acerifolium* showed considerable antibacterial and antifungal activity. Acetone, ethanol, water extracts shows significant antioxidant activity and so further studies are required to isolate and characterize the active phytochemical constituents responsible for activity to obtain effective antimicrobial lead molecules of plant origin.

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