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In vitro antioxidant activity and total phenolic, flavonoid contents of the crude extracts of *Pterospermum acerifolium* wild leaves (Sterculiaceae)

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ABSTRACT

Different extracts of Pterospermum acerifolium Willd leaves were investigated for its Free radical scavenging property of Acetone, ethanol, water extracts of 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.
Present paper has also evaluated for total Phenolic and flavonoids contain in Acetone, ethanol, water extracts.

Keywords: *Pterospermum acerifolium*, DPPH, Reducing power, Nitric oxide, Folin – Ciocalteu Method, Aluminium Chloride Colorimetric Method.

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, is widely distributed in India particularly in sub-Himalayan tract and outer Himalayan valleys.

In Phytochemical review it was found that the plant was rich in various types of chemical constituents such as amino acids, sugars, flavonoids and glycosides. The seeds and bark of Pterospermum acerifolium plant contains amino acids and sugars where as flower contains 3, 7-diethyl-7methyl-1, 5-pentacosanolide, n-hexacosan-1, 26-diol and kaempferol, kaempferide, β-

sitosterol, β-amyrin, friedelin which is useful in leucorrhoea, inflammation, ulcer, and leprosy. The leaves is haemostatic, rich in Luteolin and kaempferol, 3-O-B-D galactoside and quercitin glycosides.[3] In some areas flowers and bark are charred and mixed with Kamala and applied in suppurating small pox.[1]

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 different antioxidant methods with determination of total Phenolic and flavonoids contain**.** [4]

EXPERIMENTAL SECTION

Plant material

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

Extraction

Hot continuous extraction, Soxhletion process was used for the extraction of the plant material with solvent Solvents were choose according to increasing order of its polarity like petroleum ether, benzene, chloroform, acetone and ethanol. In Cold maceration procedure water is use as a solvent.

In vitro –antioxidant study

Acetone, Ethanol, water extract of leaves of *Pterospermum acerifolium* were tested for its free radical scavenging property using 3 different in vitro models .All experiment were performed thrice and the result averaged.

DPPH radical scavenging activity:

Methanolic solution of each extract i.e. acetone, ethanol, hydro alcoholic, 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.

Scavenging Effect (%) = [Abs. of control -Abs. of Sample/Abs. of Control] x 100

Results are shown in Table no.1. Figure 1 illustrate decrease in the concentration of DPPH radical due to the scavenging of different extracts

Nitric oxide radical scavenging activity:

1 ml Sodium nitroprusside (10mM) in phosphate buffered saline was mixed with different concentration of1 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 1ml 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 no.2. Figure 2 illustrates the percentage inhibition of nitric oxide radicals by different extracts.

Reductive activity:

The reducing power of the extracts was determined according to the method of Oyaizu (1986). Various concentrations of the extracts (100- 1000 ug/ ml) in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml, 0.2M, pH 6.6) and 1% potassium ferricynide (2.5 ml). The mixture was incubated at 50^0C 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. [6, 7] Results are summarized in Table no.3

Total flavonoids contain by Aluminium Chloride Colorimetric Method:

Aluminium chloride colorimetric method was used for flavonoids determination above mentioned plant extract samples were mixed with 1.5 ml of methanol, 0.1 ml 10% AlCl3, 0.1 ml of 1M. Potassium Acetate & 2.8 ml distilled water.

It remained at room temperature for 30 minutes the absorbance of the reaction mixture was measured at 415 nm with double beam U.V spectrometer. The calibration curve was prepared by preparing quercitin solution at concentration 20 to 100ug/ml in methanol. Results are summarized in table no.4

$$TFC\ (\%) = \frac{\text{Absorbance x Dilution Factor}}{E^{1\%}, 1^{cm} \text{ x Weight of extract (gm)}} \text{ x } 100$$

$E^{1\%}, 1^{cm}$ = Specific absorption of the Quercetin $AlCl_3$ Complex (500)

Total Phenolic contain by folin – ciocalteu method: Standard calibration curve

Weigh accurately 10 mg of standard Gallic acid & dissolve in 100ml distilled water in a volumetric flask (100ug/ml of stock solution). From the above stock solution pipette out aliquots of (2 to10 ml) in 25 ml volumetric flask. Add 10 ml of distilled water & 1.5 ml of folin ciocalteu reagent, diluted according to the label specification to each above volumetric flask after 5 minute add 4ml of 20% sodium carbonate solution make up the volume to 25ml with distilled water and incubate for 30 minutes at room temperature. Record the absorbance at 765nm.

Estimation of Total Phenolic contained in extracts

1 ml of each extract (1mg / ml) was mixed with the same reagent as performed above. The absorbance was read after 30 min. at 765 nm for determination of phenolic. All determination was performed in triplicate. Total content (%) of phenolic compound in plant different extracts was calculated as Gallic acid equivalent (GAE) [8]

$$\mathbf{GAE = [(C \times V) / M] \times 100}$$

C = the conc. of Gallic acid established from calibration curve mg/ml
V = Volume of extract (ml)
M = the weight of dried plant extract (mg)

Results are summarized in table no.5

Table No.1: DPPH radical scavenging activity of each extracts

Sr. No	Extract	Concentration (µg/ ml) and % inhibition						IC 50 (µg/ml)
		10	20	40	60	80	100	
1)	WT	20.76±0.06	40.80±0.11	67.10±05	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

WT-Water, ET-Ethanol, AC- Acetone, Std.-ascorbic acid

Table No.2: Nitric oxide scavenging activity of extracts

Sr. No	Extract	Concentration (µg/ ml) and % inhibition						IC 50 (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)	Vit. C	34.00±0.06	48.51±0.017	58.39±0.06	67.71±0	80.12±0	87.18±0	39.99

WT-Water, ET-Ethanol, AC- Acetone, Std.-ascorbic acid

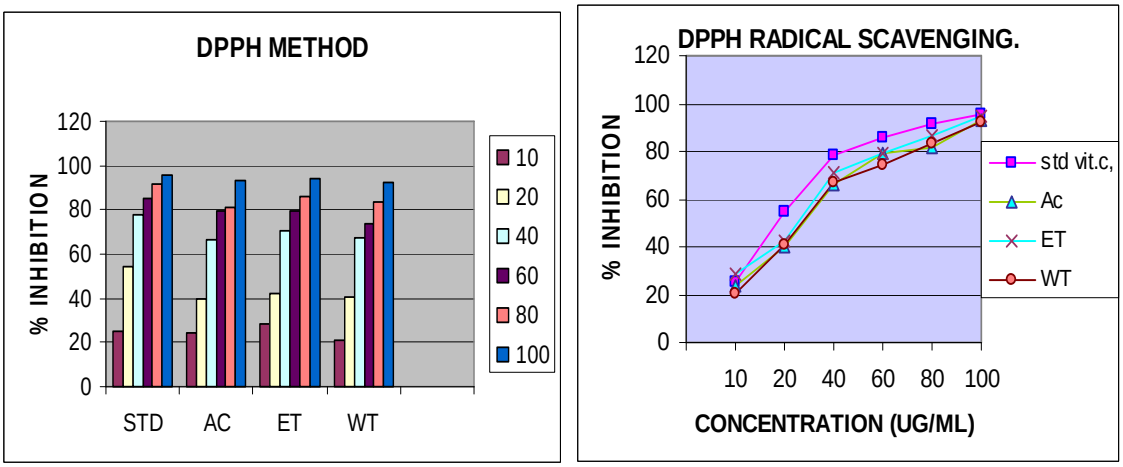


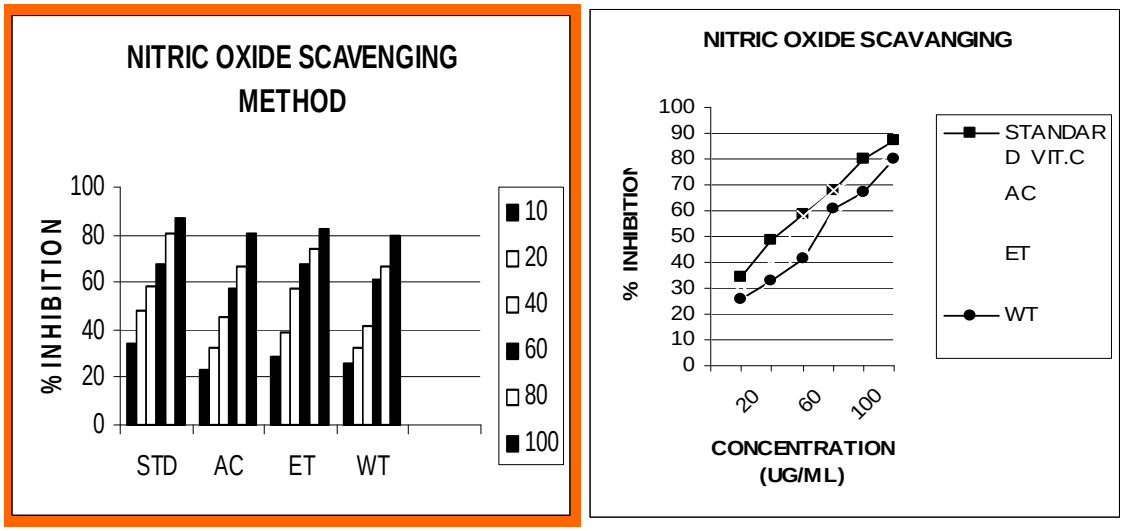
Fig no. 1: In vitro antioxidant activity of different extracts by DPPH method

WT-Water, ET-Ethanol, AC- Acetone, STD.-standard ascorbic acid

Table No.3: Results of reducing power method

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

Fig no. 2: In vitro antioxidant activity of different extracts by Nitric Oxide method



WT-Water, ET-Ethanol, AC- Acetone, STD. - standard ascorbic acid

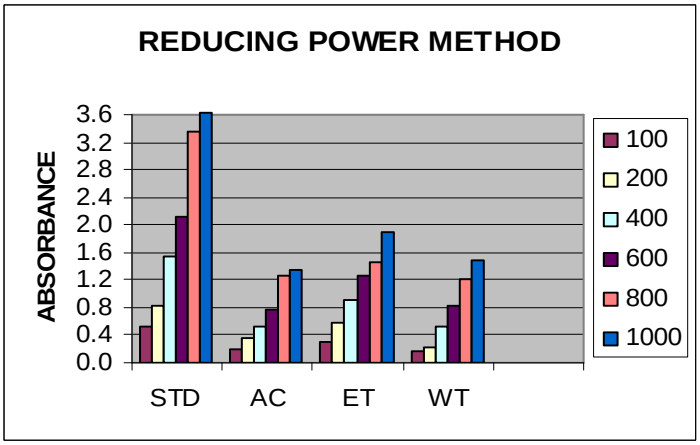


Fig no. 3: In vitro antioxidant activity of different extracts by reducing power method
WT-Water, ET-Ethanol, AC- Acetone, STD-standard ascorbic acid

Table No.4: Results of % flavonoids contain in sample

Sr.No.	Extract	Abs. at 415 nm	% Content	Mean (%) ± SD
1	Water	0.2511	4.988	4.98± 0.01
		0.2487	5 .00	
		0.2500	4.974	
2	Ethanol	1.2196	24.396	24.40±0.01
		1.2203	24.424	
		1.2212	24.424	
3	Acetone	0.6383	12.76	12.76 ± 0
		0.6383	12.76	
		0.6384	12.76	

Table No.5: Result of Total Phenolic contained in sample

Sr.No.	Extract	Abs. at 765 nm	% Content(GAE)	Mean (%) ± SD
1	Water	2.1150	55.00	55.58±0.6
		2.1610	56.32	
		2.1300	55.43	
2	Ethanol	2.1560	49.98	46.82±3.1
		2.0101	43.59	
		2.0115	46.90	

RESULT

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

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 (100µg/ml) exhibited 93.27%, 94.37%, 92.26%, and 95.58% of percentage inhibition with IC50 values 32.45, 27.88, 34.00, 20.58ug/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, 52, 53, 39.99 ug/ml. respectively.

For measurements of the reductive ability, we investigated the Fe^{3+} to $Fe2^{+}$ 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.

DISCUSSION

Aluminum chloride colorimetric method shows, ethanol extract of leaves contain maximum amount of flavonoid i.e 24.40% and folin – ciocalteu method for total phenolic contain clears that plant contain large amount of phenolic compound i.e. Water extract contain 55.58% and Ethanol extract contain 46.82%

CONCLUSION

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.

REFERENCES

[1] KR Kirtikar and BD Basu. Indian Medicinal Plants, 2nd edition edited by K S Mhaskar & J F Cains (Sri Satguru Publications, Delhi) **2001**, Vol. (I): 374.
[2] Asima Chatterjee, Satyesh Chandra Pakrashi The Treaties On Indian medicinal plants, 2nd edition, vol. (III), 16.
[3] The Wealth of India; a dictionary of Indian raw materials & industrial products; 2nd edition, CSIR, New Delhi, **2003**, vol. (IV), J-Q, 423-424.
[4] SC Varshney, AI Rizvi and PC Gupta, *Phytochemistry*, **1972**, 2 (2), 856 – 858.

[5] JB Harborne, **1998**: 56, 115-120, 81-83, 92-96.
[6] Mondal Susanta Kumar, Chakraborty Goutam, M Gupta & UK Mazumder, *Indian Journal of Experimental Biology*, **2006**, 44(1): 39-44.
[7] Rajeshwar Yerra , GP Senthil Kumar, Gupta Malaya, Mazumder Upal Kanti, *European Bulletin of Drug Research*. **2005**, 13(1): 31-35
[8] Quality standards of Indian medicinal plants. Volume (I) Indian Council of Medicinal Research, New Delhi **2003**, 208-211, 42-46.