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Investigation of in-vitro Antioxidant and Antibacterial Potential along with Phytochemical screening of Pterospermum acerifolium L. seed

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ABSTRACT

The aim of current study was to explore chemical composition and *in-vitro* antioxidant and antibacterial properties of *Pterospermum acerifolium*. Very few and sporadic works are there in the literature regarding the phytochemistry and biological activities of *P. acerifolium* (seed). Qualitative phytochemical analysis reflects the presence of tannins, steroids and flavanoids in the n-hexane extract and ethyl acetate extract of the seed was rich in alkaloids, glycosides, steriods and flavonoids. The n-hexane extract were assessed for antioxidant potential using DPPH free radical scavenging assay. The results showed promising anti-radical activity at 100µg/ml concentration. Evaluation of antibacterial effects was done against Gram-positive and Gram-negative bacteria. The antibacterial activity of *P. acerifolium* seed extract was measured through determining zone of inhibition by well diffusion method against *E. coli* and *S. aureus*. Ethyl acetate seed extract exhibited good antibacterial effects. However, that is comparatively lower as compared to standard (ofloxacin).

Keywords: *Pterospermum acerifolium*, Phytochemical analysis, Antioxidant activity, Antibacterial activity.

INTRODUCTION

Medicinal plants are local heritage with global importance. Among millions of plants and their different species, Muchakunda is having therapeutic predominance in certain disease conditions like skin disease, inflammation, bleeding disorder, gout, arthritis, psoriasis, bleeding piles, uterine bleeding. *Pterospermum acerifolium* (L) Willd belonging to the family Sterculiaceae is a tall tree having high medicinal value. The geographical distribution of the plant extends from North East India to Bangla Desh (Chittagong), Burma and Malayasia; cultivated in Pakistan and North America [1]. Reviewing the biological activity of the plant, It was reported that flowers are the organ used as tonic, anthelmintic, anti-inflammatory, laxative and to cure abdominal pain, ascites, ulcers, leprosy, urinary discharges and tumors. Also, flowers and

bark are charred, mixed with (kamala) and applied in suppurating small pox [2]. The aqueous and alcoholic extracts of this plant possesses various medicinal properties, including anti-inflammatory, analgesic, antipyretic and anti-hyperglycemic activities [3]. The current work deals with phytochemical screening test and biological profile (antibacterial effectiveness and antioxidant activity) of *P. acerifolium*.

MATERIALS AND METHODS

1.Collection and authentication of plant material

The seed samples were collected from wild with prior permission from various places within Bhopal. The sample was authenticated for its botanical identity from CSIR- NISCAIR (Delhi). A voucher specimen has been deposited in Raw Material Herbarium And Museum, Delhi (RHMD) Ref.no: NISCAIR/RHMD/Consult/2020/3775-76.

2. Extraction of plant material

The seed powder (300g) was firstly extracted with 700 ml of n-hexane in a soxhlet apparatus. The extraction was continued until the crude drug was completely exhausted. Then the extract was filtered and evaporated on a hot water bath to yield a soxhlet crude extract.

After that, same crude drug(300g) was extracted with 700 ml of ethyl acetate using same procedure.

3. Phytochemical Screening [4-6]

The extracts of *Pterospermum acerifolium* seed was further subjected to phytochemical tests for Alkaloids, Glycosides, Proteins and amino acids, Flavonoids, Steroids, Carbohydrates, Saponins and Tanins.

4. *In-vitro* antioxidant assay [7,8]

Assay : DPPH free radical scavenging assay

4.1 Material used

1. Regents and Chemical

DPPH, Methanol, Ascorbic Acid

2. Glassware

Graduated pipette, volumetric flask (5 ml/10 ml) or, test tubes, Beaker (25/50 ml)

3. Instrument

Digital weighing balance, Spectrophotometer, Incubator

4.2 Procedure

Different concentrations (20, 40, 60, 80, 100µg/mL) of test sample and 0.1mM DPPH solution was prepared in methanol. Ascorbic acid was used as standard. 2mL of DPPH solution and 1 mL of methanol was used as control. 1 ml of plant extract of various concentrations was mixed with 2 mL of DPPH

solution and control separately. The mixture was incubated for 10 minutes in the dark and absorbance was measured at 515 nm by spectrophotometer using methanol as blank. The percentage inhibition of DPPH radical was calculated by following formula:

% Inhibition= [A(control) – A(sample)]/A(control) x 100

where A(control) is the absorbance of the control reaction (containing all reagents except the test extract), and A(sample) is the absorbance of the test extract. IC50 was calculated by plotting % inhibition as a function of sample concentration. IC50 is defined as extract concentration necessary to inhibit 50% of DPPH solution.

5. *In vitro* antibacterial study [9]

Assay : Well diffusion assay

Cultures used : *E. coli* (MTCC-119), *S. aureus* (MTCC-96)

5.1 Material used

1. Nutrient Agar Media (1L)

One liter of nutrient agar media was prepared by dissolving 35.0 g of commercially available nutrient agar medium (Hi Media) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

2. Standard Drug Used: Ofloxacin

5.2 Preparation of suspension of bacteria

Approximately 4 to 5 well isolated colonies of the bacterial strain are inoculated into 5 ml of nutrient broth and incubated at 37°C.

5.3 Procedure

Petri plates containing 20ml Nutrient Agar medium were seeded with 24hr culture of bacterial strains. Wells were cut and 15µl of the given sample (of different concentrations) were added. The plates were then incubated at 37°C for 24 hours. The antimicrobial activity was assayed by measuring the diameter of the inhibition zone formed around the well.

The plates were examined for the presence and the size of inhibitory zones. The diameter of the inhibitory zone (including the diameter of the well) was measured by using a millimetre scale up to the nearest millimetre. Zone of inhibition were measured with digital vernier calliper.

RESULTS

The extracts of *Pterospermum acerifolium* were subjected to phytochemical screening for the detection of various phytoconstituents. The results are mentioned in table 1:

S.No.	Plant constitutes	Test/reagent	N-hexane extract	Ethyl acetate extract
1.	Alkaloids	Dragendroff's reagent	-	+
		Mayer's reagent	-	+

		Wagner's reagent	-	+
		Hager's reagent	-	+
2.	Anthraquinone glycosides	Brontrager test	+	+
		Modified borntrager test	-	+
3.	Cardiac glycosides	Killer-killani test	-	+
		Legal test	-	-
		Baljet test	-	-
4.	Proteins & amino acids	Biuret test	+	-
		Xanthoprotic test	-	-
		Millon test	-	-
		Ninhydrin test	-	-
5.	Steroids	Salkowski reaction	+	+
		Libermann-burchad test	+	+
6.	Tanins	Ferric chloride test	+	+
		Gelatin test	+	-
7.	Flavonoids	Shinoda test	+	+
8.	Carbohydrates	Molish test	-	-
		Barfoed test	-	-
9.	Saponins	Foam test	-	-

Table 1: Phytochemical screening of successive Seed extracts of *Pterospermum acerifolium*

***In-vitro* Antioxidant Activity**

S.No	Concentration (µg/ml)	Absorbance	% Inhibition	IC_{50} Value
1.	10	0.605	47.8	
2.	15	0.408	64.8	
3.	20	0.306	73.6	9.329
4.	25	0.218	81.2	
5.	30	0.116	90	

Table 2 : DPPH radical scavenging assay of Ascorbic Acid

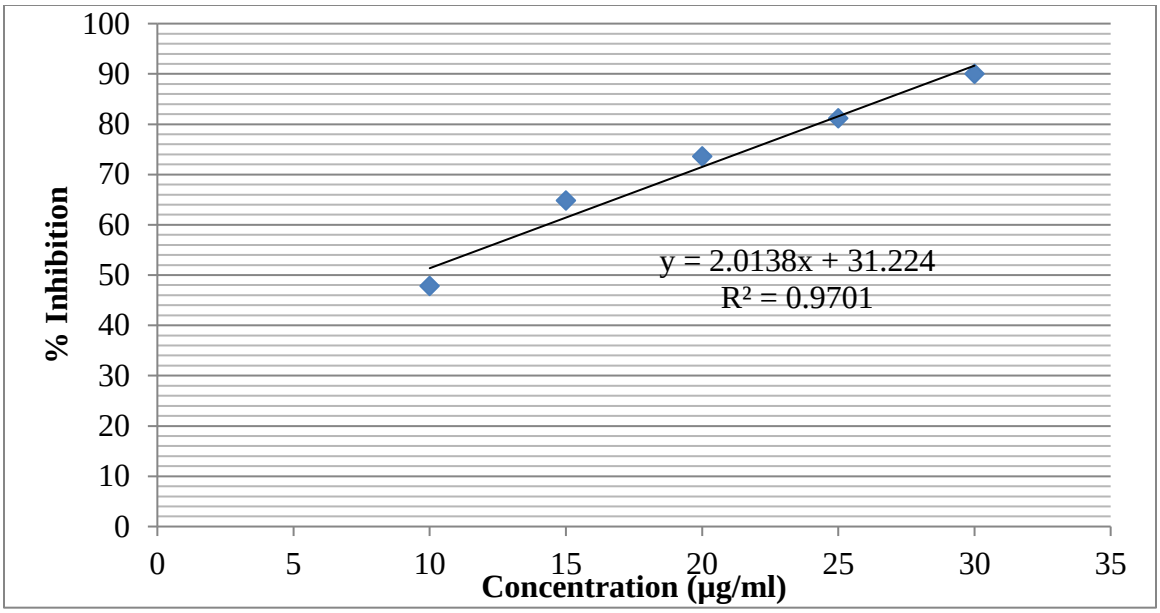


Fig. 1: DPPH radical scavenging activity of Ascorbic acid

S.No	Concentration (µg/ml)	Absorbance	% Inhibition	IC_{50} Value
1.	20	0.786	32.24	55.56
2.	40	0.687	40.77	
3.	60	0.537	53.70	
4.	80	0.448	61.37	
5.	100	0.306	73.6	

Table 3: DPPH radical scavenging assay of n-haxane extract

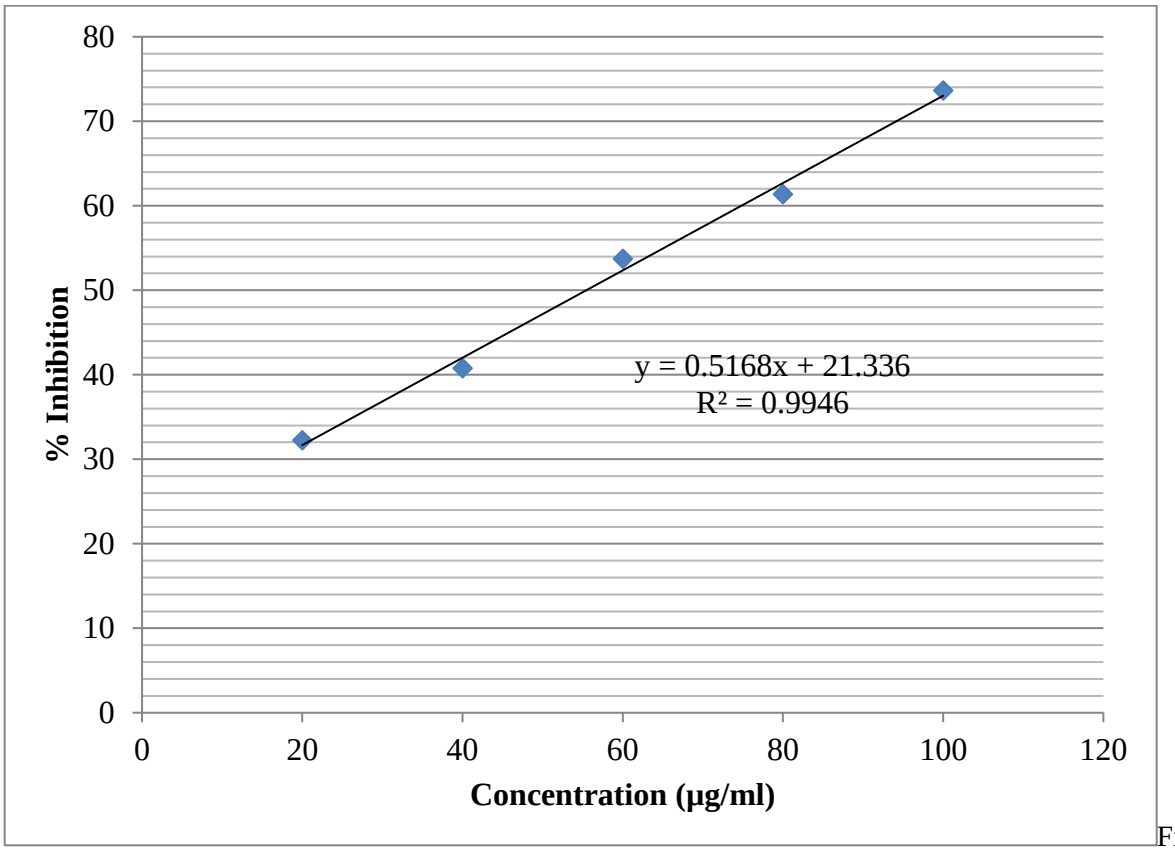


Fig. 2: DPPH radical scavenging activity of n-hexane extract

***In-vitro* Anti Bacterial Activity**

Standard (Ofloxacin)	
Concentration	**Zone of inhibition (mm)**
10 µg/ml	33

Table 4: Antibacterial activity of standard

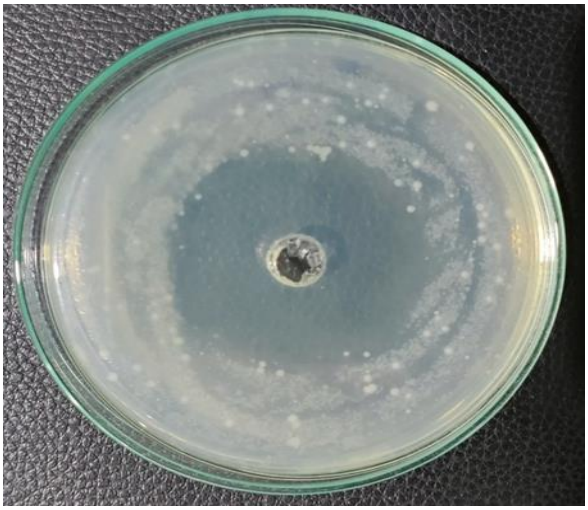
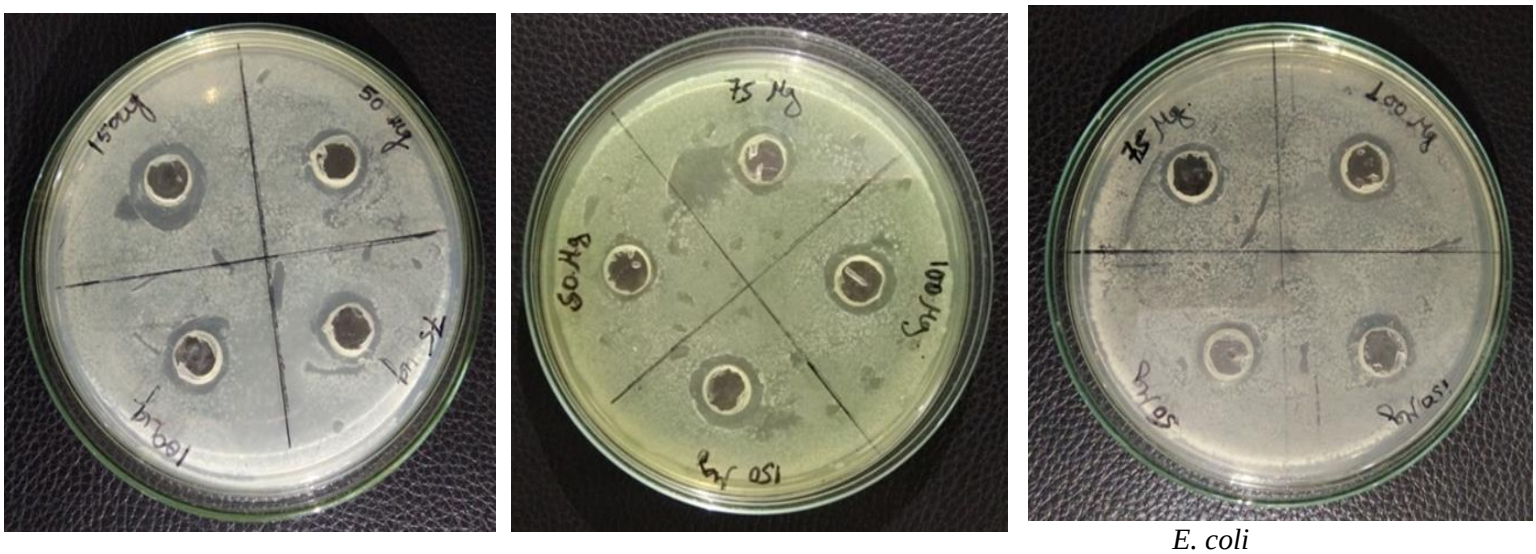


Fig. 3: Plate: Standard Oflaxacin (10 µg/ml)

Name of the Organism	Zone of inhibition (mm)				
	Plates	**50 µg/ml**	**75 µg/ml**	**100 µg/ml**	**150 µg/ml**
E. coli	**1**	**7.6**	**8.5**	**9.2**	**13.6**
	2	**7.2**	**8.3**	**9.1**	**11.5**
	3	**7.5**	**8.4**	**9.3**	**11.4**
	Mean±SD	7.43±0.169	8.4±0.816	9.2±0.081	12.16±1.014

Table 5: *In-vitro* Anti Bacterial Activity of Ethyl acetate extract against



E. coli

Fig. 4: Plates of Ethyl acetate extract against *E. coli*

Name of the Organism	Zone of inhibition (mm)				
	Plates	50 µg/ml	75 µg/ml	100 µg/ml	150 µg/ml
Staphylococcus aureus	**1**	**8.5**	**8.8**	**9.8**	**10.9**
	2	**9.0**	**9.4**	**9.3**	**11.0**
	3	**8.8**	**9.0**	**9.7**	**10.7**
	Mean±SD	7.43±0.169	8.4±0.816	9.2±0.081	12.16±1.014

Table 6: *In-vitro* Anti Bacterial Activity of Ethyl acetate extract against *Staphylococcus aureus*

Fig. 5: Plates of Ethyl acetate extract against *Staphylococcus aureus*

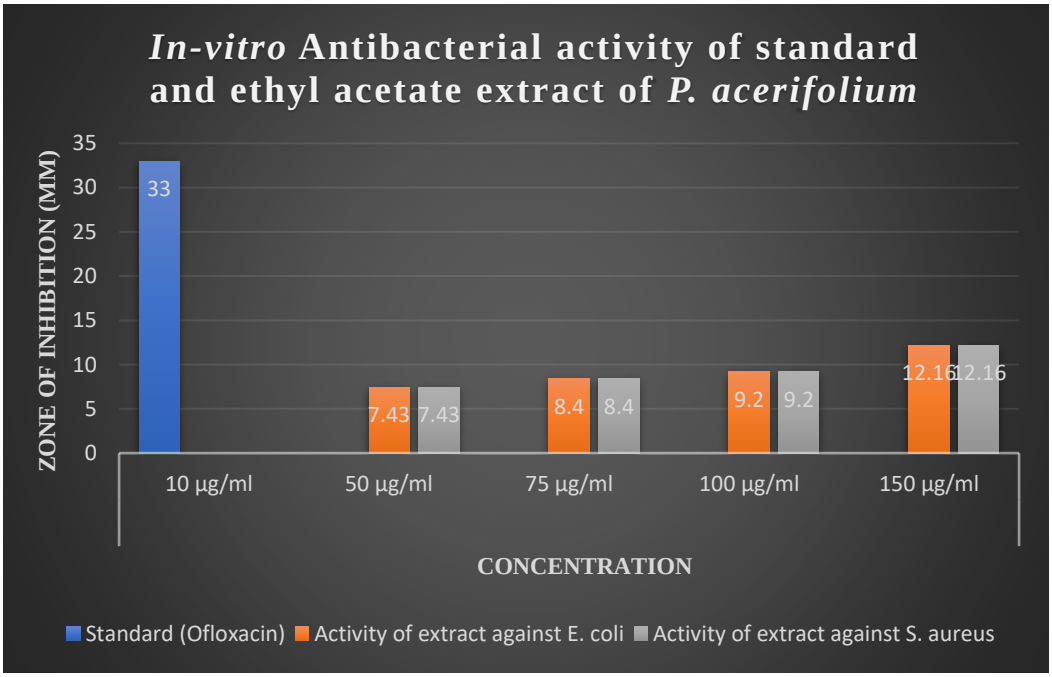


Fig. 6: Antibacterial effect of ethyl acetate extract of *P. acerifolium* seed shows higher zone of inhibition 12mm approximately at concentration 150µg/ml against both bacterial strains. However, that is comparatively lower than standard drug (Ofloxacin) which shows 33mm at conc. 10µg/ml.

DISCUSSION

Phytochemical screening test is important for the isolation of new, rare and novel compounds before a bulk extraction. The phytochemical screening on qualitative level showed that ethyl acetate extract was rich in Alkaloids, Glycosides, Steriods and flavonoids and n-hexane extract have Flavonoids, Tanins and Steroids (Table 1).

As per literature review, plants having alkaloids and flavonoids were reported for their antimicrobial properties [10]. In previous work it has been observed that alkaloids and phenolic compounds have strong interaction with microbial cells through enzymes and proteins [11,12]. Flavonoids and tanins are responsible for plant's antioxidant property.

The crude n-hexane extract of title plant was evaluated at accumulative concentrations i.e. 20, 40, 60, 80, 100µg/mL for anti-radical effect. Ascorbic acid was used as standard. The crude extract showed significant antioxidant activity against DPPH. The activity increased as concentration was increased (Figure 2).

The crude ethyl acetate extract of seed of *Pterospermum acerifolium* was evaluated for antibacterial study. The antibacterial activity of crude extract of seed of P. acerifolium was investigated by well diffusion assay against two selected bacterial strains. The antibacterial activity showed significant reduction in bacterial growth in the term of zone of inhibition that indicated that the plant possesses antibacterial activity against *E. coli* and *Staphylococcus aureus* (Table 5 and 6; Figure 6).

CONCLUSION

The aim of current study was to explore *Pterospermum acerifolium* seed for the presence of phytoconstituents, which indicated the presence of various classes of compounds. Pterospermum acerifolium seed also possess good antibacterial and antioxidant activity which may be due to the presence of identified secondary metabolites which need further study.

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