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Investigation of *in-vitro* antioxidant activity of *Pterospermum acerifolium* Linn.

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Abstract

In traditional system of medicine *Pterospermum acerifolium* Linn. is widely used for the treatment of various diseases. It is a flowering plant belongs to the family Sterculiaceae. The present investigation was undertaken to evaluate the active phytoconstituents present in the leaves of the plant and to access the *in-vitro* antioxidant activity of the plant.

Key- words: *Pterospermum acerifolium, In-vitro* antioxidant activity, Traditional claims

Introduction

Pterospermum is a flowering plant genus included in the family *Sterculiaceae* and most subsequent systematics. *Pterospermum* is based on two Greek words, "Pteron" and "Sperma," meaning "winged seed". It has a wide application in traditional system of Indian medicine for example, in ayurvedic anticancer treatment flowers are mixed with sugars and applied locally. Flowers and bark, charred and mixed with kamala applied for the treatment of smallpox. Flowers made into paste with rice water used as application for hemicranias. Stem bark of the plant was found to have antimicrobial activity. Isolation of boscialin glucosides from leaves of *P. acerifolium* has been reported. Hepatoprotective effect of ethanolic extract of leaves of *P. acerifolium* was also reported. Chronic effects of *P. acerifolium* on glycemic and lipidemic status of type 2 model diabetic rats was found beneficial. The barks are reported to be used as anti-ulcer, anti-inflammatory, analgesic and anti-oxidant activity[1]. The present work was undertaken to evaluate the *in-vitro* antioxidant activity of the plant.

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Fig. 1: ***Pterospermum acerifolium***

Material and Methods

Selection of Plant

Pterospermum acerifolium leaves were selected for present study.

Collection, Authentication and Drying of plant material

The leaves of *Pterospermum acerifolium* were identified, and collected in the month of december from the Bhopal, Delhi, Banglore, Orissa. The plants were authenticated by a Botanist. The plants were washed, shade dried, pulverized into moderately coarse powder and stored in air tight container for further use.

Plant Authentication

Plant was authenticated by Dr. Zia Ul Hasan H.O.D. of Botany Safia Science College Bhopal. Authentication Voucher Specimen No. 304/Bot/Safia/11.

Extraction

The plant were cleaned properly and washed with distilled water to remove any kind of dust particles. Cleaned plants were coarsely powdered in hand grinder. Powdered plants were weighed (250g). Extraction will be performed by maceration using hydro alcohol as solvent in the ratio 3:7.after 7 days it was filtered and the filterate was kept on water bath 37^0c for 5 days.[2-4]

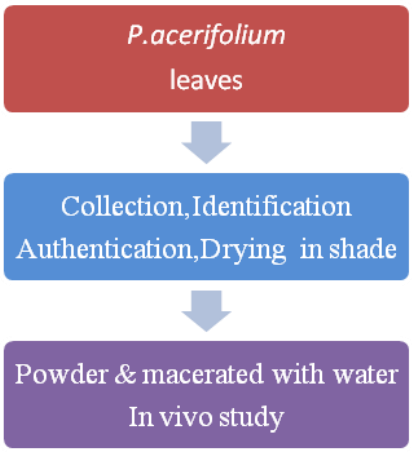


Fig. 2: Extraction (Cold Macertion)

Phytochemical investigation[2-4]

Qualitative Test Analysis

In order to detect the various constituents present in the different extracts of *Pterospermum acerifolium*, these were subjected to the tests as per methods described by Ansari, and Khandelwal. Phytochemical screening was revealed for the presence of Alkaloids, Glycosides, Carbohydrates, Tannins, Resins, Flavonoids, Steroids, Proteins and Amino acids.

Determination of Alkaloids

Table 1: Identification test of Alkaloids

S.No.	Identification test	Procedure	Observation
1	Mayer's Test	Test solution + Mayer reagent (Potassium mercuric iodide solution)	White or yellow precipitate
2	Dragendorff's Test	Test solution + Dragendorff's reagent (Potassium iodide + bismuth nitrate)	Showed orange red precipitate
3	Wagner's Test	Test solution + Wagner's reagent (iodine solution)	Brown or reddish brown precipitate
4	Hager's Test	Test solution + Hager's reagent (saturated solution of picric acid)	Gives characteristic crystalline ppt.

Determination of Glycosides and Carbohydrate

Table 2: Identification Tests of Glycosides

S.No.	Identification test	Procedure	Observation
1	Raymond's Test	Test solution + 1 ml of 50% ethanol + 0.1% solution of dinitrobenzene in ethanol + 2-3 drops of 20% sodium hydroxide solution	Appearance of violet colour, which changes into violet.
2	KillerKillani Test	2 ml of extract + glacial acetic acid + one drop of 5% $FeCl_3$ + conc. H_2SO_4.	Reddish brown colour appeared at the junction of the two liquid layers and upper layer appeared bluish green.
3	Legal Test	Test solution dissolved in few drops of pyridine + a drop of 2% sodium nitroprusside + a drop of 20% sodium hydroxide	Deep red color produced.

		solution	

S.No.	Identification test	Procedure	Observation
1	Molisch's Test	2-3 ml. extract +few drops of α-naphthol solution (20% in ethyl alcohol) + 1 ml. conc. H_2SO_4 added along the side of the test tubes.	Violet ring was formed at the junction of two liquids.
2	Fehling's Test	Extract heated with dil. HCL +NaOH + Fehling's solution A & B	Brick red precipitate was formed
3	Benedict's Test	Extract +equal volume of Benedict's reagent. Heat for 5 min.	Solution appears Green, Yellow or Red

Determination of Tannins, Flavonoids, and Resins

Table 3: Identification Tests of Tannins

S.No.	Identification test	Procedure	Observation
1	Vanillin- HCl Test	Extract+ vanillin-HCl reagent (1 g vanillin + 10 ml. alcohol + 10 ml. conc. HCl)	Formation of pink or red color.
2	Gelatin Test	Extract solution + aqueous solution of gelatine	White buff color precipitate was formed.

Table 4: Identification Tests of Flavonoids

S.No	Identification test	Procedure	Observation
1	Lead acetate test	Filter paper strip was dipped in the alcoholic solution of extract. Ammoniated with ammonia solution	Color changed from white to orange.
2	Shinoda Test	Extract + 5 ml. 95% alcohol + few drops of conc. HCl + 0.5 g magnesium turning.	Pink color observed

Table 5: Identification Tests of Resins

S.No.	Identification test	Procedure	Observation
1	Color detection with ferric chloride	Extract + alcohol + few drops of $FeCl_3$ solution.	Green color appears
2	Turbidity Test	Extract solution (2 g of drug in methanol) +5 ml distilled water.	Turbidity appears

Table 6: Identification Test of Steroids

S.No.	Identification test	Procedure	Observation
1	Libermann-Burchard Test	2 ml. extract + Chloroform + 1- 2ml. acetic acid + 2 drops H_2SO_4 from the side of the test tube	First red, then blue and finally green color appeared.

2	Salkowski Reaction	2 ml. of extract +2 ml. chloroform + 2 ml. conc. H_2SO_4. Shake well.	Chloroform layer appeared red color and acid layer shows greenish fluorescence.

***In-vitro* antioxidant assay**[5-7]

Broadly defined, an antioxidant is a compound that inhibits or delays the oxidation of substrates even if the compound is present in a significantly lower concentration than the oxidized substrate. The scavenging of reactive oxygen species (ROS) is one of possible mechanism of action. Others include the prevention of ROS formation by metal binding or enzyme inhibition. Chain breaking antioxidants prevent damage by interfering with the free radical propagation cascades. Several methods are used to measure the antioxidant activity of a biological material. The most commonly used ones are those involving chromogen compounds of radical nature that stimulate the reductive oxygen species. These methods are popular due to their ease, speed and sensitivity.

Total Flavonoid Content

Flavanones, flavones and flavonols are three types of flavonoids which occur in natural compounds. They posses biological properities antioxidant properties, but as anti-carcinogenic and anti-inflammatory agents,lipid anti-peroxidation effects.[46]

Procedure:

For TFC different concentration of plant extract (1mg/ml) and different concentration of rutin as a standard were taken. 0.5ml of sample solution was taken and 2.5ml of distilled Water and 0.150ml of 5% $NaNO_2$ was added. After 6 min, 75 µL of 10% $AlCl_3$ was added. After another 5min, 0.5 mL of 1 M NaOH was added to the mixture. Immediately, the absorbance of the mixture was determined at 510 nm *versus* prepared water blank.

Total Phenolic Content

Phenolic compounds are secondary metabolites which synthesize in plants. They showed biological prosperities such as: antioxidant, antiapoptosis, anti-aging, anticarcinogen, anti-inflammation, anti-artherosclerosis, and cardiovascular protection.

Procedure:

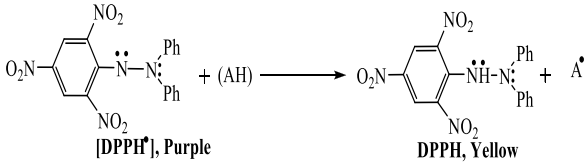
The amount of total phenolic in extracts was determined with the folin ciocalteu reagent. Galic acid was used as a standard and the total phenolic were expressed as mg/g gallic acid equivalent (GAE) .Concentration of 0.01 to 0.1, mg/ml of gallic acid were prepared in methanol.Concentration 1mg/ml of plant extract were also prepared in methanol and 0.5ml of each sample were introduced in to test and mixed with 2.5ml of a 10 fold dilute folin-Ciocalteu reagent and 2ml of 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature before the absorbance was at read at 760 nm spectrometrically. All determination was performed in triplicate. The folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols, there by producing a blue colour upon reaction. This blue colour is measured spectrophotometrically. Thus total phenolic content can be determined.

DPPH Radical Scavenging Assay:DPPH method

Chemicals 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) and L-ascorbic acid were purchased from Sigma Chemical Co. other chemicals and solvents used in this experiment were of the highest quality available.

Principle

DPPH is nitrogen centred free radical that show strong absorbance at 517 nm. DPPH assay is based on the measurement of the scavenging ability of antioxidants towards the stable DPPH radical. The free stable radical is reduced to the corresponding hydrazine when it reacts with hydrogen donors; this ability was evaluated by more frequently used decolouration assay, which evaluates the absorbance decrease at 517 nm produced by the addition of the antioxidant to a DPPH solution in methanol. This method is widely used to check the free radical scavenging antioxidants. To evaluate the antioxidant activity of specific compounds or extracts (antioxidant) were allowed to react with a stable radical $DPPH^{\bullet}$ in a methanol solution. Extent of DPPH radical scavenged was determined by the decrease in intensity of violet colour in the form of IC_{50} values.



Preparation of extract dilution

100 mg of the methanol extract was weighed and dissolved in 100 ml of methanol to get 1000 µg/ml stock solution. Lower concentrations (10-100µg/ml) were prepared by diluting serially with methanol.

Preparation of standard dilution

Ascorbic acid (10 mg) was weighed and dissolved in 10 ml of methanol to get 1000 µg/ml stock solutions.

Lower concentrations (10-100µg/ml) were prepared by diluting serially with methanol.

Method

The stable 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extract. The 0.1 mM solution of DPPH in methanol (22.2 mg in 1000 ml) was freshly prepared. Different concentrations of the extract were added with an equal volume to methanol solution of DPPH. After 30 min at room temperature, the absorbance was recorded at 517 nm. Ascorbic acid was used as standard. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals. Radical scavenging activity was calculated by the following formula:

$$\% \text{ inhibition of DPPH radical} = \frac{\text{Control - (sample with DPPH- sample without DPPH)}}{\text{Control}} \times 100$$

IC_{50} value was determined from the plotted graph of scavenging activity against the different concentrations of methanol extract, which is defined as the total antioxidant necessary to decrease the initial DPPH radical concentration by 50%. The measurements were carried out three times and their scavenging effect was calculated based on the percentage of DPPH scavenged.

Results and Discussion

The leaves of *Pterospermum acerifolium* were selected, identified, collected and authenticated. The leaves of *Pterospermum acerifolium* were selected, identified, collected and authenticated.

Table 7: % yield of Extract

S.No.	Solvent	Time of extraction	Colors of extract	Yield	% Yield
1.	Hydroalcoholic	One week	Greenish black	0.12 g	12 %
2.	Hydroalcoholic	One week	Greenish black	0.098g	9.8 %
3.	Hydroalcoholic	One week	Greenish black	0.06 g	6%
4.	Hydroalcoholic	One week	Greenish black	0.07 g	7%
Note: 250 g of crude drug was taken for the extraction.					

Extraction of plant sample which are collected from different regions has been performed by cold maceration using hydro alcohol as solvent in the ratio 3:7. Preliminary phytochemical screening was performed for different sample extract.It was noted that hydroalcoholic extract of different samples contains Alkaloids,Glycosides, Tannins,Carbohydrates, Flavonoids, and Steroids.

Table 8: Qualitative analysis of hydroalcoholic extracts of *Pterospermum acerifolium*

S. N	Test	Sample (Hydroalcoholic extract)			
		Bhopal	Delhi	Bangalore	Orissa
1.	**Alkaloids**				
(a)	Mayer test	+ve	+ve	+ve	+ve
(b)	Wagner test	+ve	+ve	+ve	+ve
2.	**Glycosides**				
(a)	Legal test (Cardiac glycoside)	+ve	+ve	+ve	+ve
(b)	Libberman burchard test	+ve	+ve	+ve	+ve
(c)	Foam test (Saponinglycoside)	+ve	+ve	+ve	+ve
3.	Tannins				
a.	Vanillin-HCL test	+ve	+ve	+ve	+ve
b.	Gelatin test	+ve	+ve	+ve	+ve
4.	Flavonoids				
a.	Vanillin-HCL test	+ve	+ve	+ve	+ve
b.	Ferric- Cl test	+ve	+ve	+ve	+ve
5.	Steroid(Triterpenoids)				
a.	Salkowaski test	+ve	+ve	+ve	+ve
b.	Libermann	+ve	+ve	+ve	+ve
6.	Amino-acids				
a.	Ninhydrin	-ve	-ve	-ve	-ve

	test				
b.	Cysteine test	-ve	-ve	-ve	-ve
7.	Proteins				
a.	Precipitate test	-ve	-ve	-ve	-ve
b.	Biuret Test	-ve	-ve	-ve	-ve
8.	Carbohydrate				
a.	Molish test	+ve	+ve	+ve	+ve
b.	Benedict test	+ve	+ve	+ve	+ve
9.	Fats & Fixed oil				
a.	Saponification test	-ve	-ve	-ve	-ve
10.	Volatile oil				
a.	Sudan III	-ve	-ve	-ve	-ve
11.	Resins				
a.	Turbidity test	-ve	-ve	-ve	-ve
b.	Ferric- Cl test	-ve	-ve	-ve	-ve

(+) Positive, (-) Negative

The results showed that the total Phenolic content was (243.33, 237,366.6,200mcg/g) in hydroalcolic fractions and was expressed as mcg/g equivalent to gallic acid (w/w). The total Flavonoid content was (30.15,73.33,35,15mcg/g) in hydroalcolic fractions and was expressed as mcg/g equivalent to Rutin (w/w). In present study antioxidant potential of *Pterospermum acerifolium* was observed on the basis of % inhibition by test sample when compared with DPPH. It was observed that sample showed good line of fit for % inhibition in concentration range of 10-50mcg/ml with R^2=0.991 line of regression for % inhibition at above said conc. (y) which revealed that test samples posses IC-50 for ascorbic acid (used as standard oxidant) was 200.84,202,199.8,192mcg/ml respectively on biostatical interpretation.

Table 9: Total Phenolic Content

Extract	Absorbance	TPC (mcg/g) Gallic Acid equivalent
Hydro Alcoholic Extract(S_1)	0.229	243.33
Hydro Alcoholic Extract(S_2)	0.224	237
Hydro Alcoholic Extract(S_3)	0.34	366.6
Hydro Alcoholic Extract(S_4)	0.19	200

Table 10: Total Flavanoid Content

Extract	Absorbance	TFC(mcg/g) Rutin equivalent
Hydro Alcoholic Extract(S_1)	2.97	30.15
Hydro Alcoholic Extract(S_2)	3.22	73.33
Hydro Alcoholic Extract(S_3)	2.99	35
Hydro Alcoholic Extract(S_3)	2.87	15

Table 11: IC-50 of different extract for DPPH

S.No.	Sample	IC-50
1	Ascorbic acid	160.16 µg/ml
2	Leaf extract (Sample1)	200.84 µg/ml
3	(Sample2)	202 µg/ml
4	(Sample3)	199.8 µg/ml
5	(Sample4)	192 µg/ml

Conclusion

P.acerifolium leaves were collected and extracted with hydroalcoholic solvent. Leaves extract (LCP) were investigated for their antioxidant potential using DPPH assay.As phenolic compounds and flavonoids present in plants are having most significant antioxidant potential, Total Phenolic Contents (TPC) and Total Flavonoid Contents (TFC) was also estimated using Gallic acid and Rutin as standard. DPPH radical scavenging assay is widely used method to assess antioxidant activities in a relatively short time. DPPH is a stable free radical and accepts an electron or hydrogen radical to turn into a stable diamagnetic molecule. Conversion of colour from purple to yellow showed the decline in absorbance of DPPH radical at 517nm caused by reaction between antioxidants present in extract and free radical. In present investigation all four sample extracts revealed significant antioxidant activity. IC-50 for LCP was found to be 200.84,202,199.8,192mcg/ml. Thus extract was found to possess best antioxidant activity in DPPH assay.

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