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Estimation of Total Phenolic, Flavonoid Content and *in-vitro* Evaluation of Anti Oxidant Activity of Different Extracts of *Pterospermum suberifolium* (L) Lam

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***Abstract*: Phenols and flavonoids are common in plants which act as antioxidants and free radical scavengers. The study aimed at estimation of total Phenolic, Flavonoid content and Antioxidant activity of *Pterosperum suberifolium* (L) Leaves. The various solvents like Hexane, Ethylacetate, Acetone, Methanol and Ethanol were used for extraction process by percolation using soxhlet apparatus. Determination of total phenol content for different extracts of leaves was done using Folin – Ciocalteau, total flavonoid content using Aluminium chloride by colorimetric method. Antioxidant nature of leaf was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DDPH) assay, Ferrous reducing antioxidant capacity assay and hydrogen peroxide scavenging activity and compared between the various extracts. Total phenol content was expressed in mg of Gallic Acid Equivalent (GAE) per g dry weight. The content of flavonoids was expressed in mg of Catechin equivalence (CE) per g of dry weight. Based on results, phenolic content is significantly high in Acetone extract (78.02 mg GAE/g. Extract). Similarly significant high concentration of flavonoids observed in methanol extract (85.25 mg CE/g Extract). For Antioxidant activity methanol extract showed highest activity and Hexane extract shown the least antioxidant property.**

***Index terms:* Catechin, Gallic Acid, Flavonoids, Phenols, *Pterosperum suberifolium* (L)**

I. INTRODUCTION

Plant derived medicinal components which are secondary metabolites are mostly stored in leaves and generally used in formulating many pharmaceutical compounds. Flavonoids and phenolic derivatives are important bioactive compounds (Kim *et al*). Phenolic compounds are key metabolites and classified as phenolic acids and polyphenols. These combine with mono to polysaccharides, linked to phenolic groups, or can occur as other derivatives (Harborne). Literature suggests good correlation between phenols, flavonoids ans antioxidant property (Prakit Chaithada *et al*). Out of several classes of phenols, phenolic acids, flavonoids and tannins are considered to be the key dietary compounds (Reddy CVK, *et al*). Numerous studies proved strong relation among the phenolics and the antioxidant potent nature of herbal products (Sarawong *et al*). This antioxidant nature helps in the decreasing lipid oxidation by conserving the food quality also by reducing risk of developing some diseases. The action of free radicals in disease state can involve in many health issues like atherosclerosis, diabetes, immunosuppression and neurodegeneration (Harman). Intake of natural antioxidants is inversely proportional to mortality from degenerative disorders (Gulcin). Polyphenols are proposed to prevent or treat of many diseases (Dimitrios Stagos). Phenolic compounds donate electrons as their hydroxyl ions directly contribute to antioxidant property (Bendary *et al*). Also some phenols help in producing endogenous antioxidant molecules (Cote J *et al*). As many reports in the literature suggests, phenolic compounds exhibit free radical inhibition, peroxide decomposition in biological systems and thus prevent oxidative stress (Oberoi H.S., *et al*).

II. LITERATURE SURVEY

Pterospermum genus is an angiosperm. Initially placed in Sterculiaceae, later it is included to Malvaceae in APG (The Angiosperm Phylogeny Group). Pterospermum is Greek word, in which "Pteron" and "Sperma," mean "winged seed."

Taxonomical classification: (India Biodiversity)

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Kingdom: Plantae
Class: Magnoliopsida
Order: Malvales
Family: Malvaceae
Genus: *Pterospermum*
Species: *Pterospermum suberifolium* Lam.
Synonyms:*Pentapetessuberifolia, Pterospermadendron suberifolium (L.) Kuntze, Pterospermum canescens Roxb.*

The vegetation of *Pterospermum suberifolium* (Ps) is a tropical dry evergreen forest type. (J.Jayapal et al). Ps of tropical dry evergreen forests of Coromandel Coast is important species from point of conservation (Muthulingam Udayakumar et al). Organoleptic study of *P. suberifolium* provided features of green, characteristic odor and bitter taste. Fluorescence analysis with visible & UV light by treating chemical reagents shows changes in color. The plant contains of flavonoids, steroids, saponins, tannins and glycosides (A.Jena *et al*). Petroleum ether, Chloroform and Ethanolic leaf extract has shown good activity against *Streptococcus, Bacillus Candida* spp etc (K. P. Jaiganesh *et al* 2011). GC-MS by methanol, chloroform and petroleum ether extracts of *Pterospermum canescens* Roxb resulted in 20, 6 and 15compounds respectively. Major constituents found are squalene, phytol and tocopherol (Jaiganesh *et al* 2012). Antioxidant activity for some solvent extracts showed highest in chloroform fraction. The total phenolic nature was calculated as high in Etyl acetate fraction when compared with other fractions. (Dibyajyoti Saha *et al***)**

Uses: *Pterospermum suberifolium* leaves mixed with the other plants can treat fractured bones (R. Kotaimuthu). Leaf paste is applied to treat fracture and inflammation (VP Silja *et al*)

III. MATERIALS AND METHODS

A. Sample Preparation

A shade dried and powdered material was extracted with hexane, ethyl acetate, acetone, methanol and ethanol in soxhlet apparatus. Each extract of 0.5g weighed, phenols and flavonoids were extracted with 50ml methanol on ultrasonic bath for 20min. An aliquot(2ml) of extracts ultra centrifugated 5min at 14000rpm.

B. Determination of total phenolic content:

An aliquot(1ml) of extract or standard Gallic acid (50, 100, 150, 200, 250 µg/ml) added to 25ml of volumetric flask, holding 9ml distilled water. Areagent blank of water also made. 1ml of Folin-Ciocalteau phenol reagent added to mixture and agitated. 5min later 10ml of 7%Na_2CO_3 solution added to above. It was diluted to 25ml with water. Incubated for 90 min at RT, absorbance against reagent blank was determined at 750 nm using UV-Visible spectrometer (Y. Anusha *et al*). This data was expressed in mg. GAE/g. Extract)

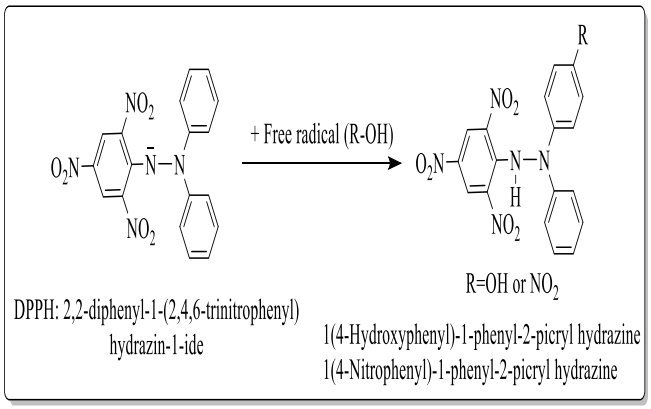
C. Determination of total flavonoid assay

It was performed by $AlCl_3$ colorimetric assay. An aliquot(2.5ml) extracts or standard (+)-catechin (100, 200, 400, 600, 800 and 1000 µg /ml) was introduced to a 25ml flask containing 10ml of deionized water. To flask 0.75 ml 5 % $NaNO_2$ added. 5min later 0.75ml 10%$AlCl_3$ was added. At 6th min, 5ml 1M NaOH was added and volume made upto 25ml with H_2O. Mixed well and absorbance measured against blank at 510nm with UV-VIS Spectrophotometer (R. Prathibha *et al*). The data was expressed as mg. of CE/g extract.

D. In-vitro anti oxidant activity

This was performed by various methods as follows;

1) DPPH radical scavenging assay



DPPH: 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazin-1-ide

1(4-Hydroxyphenyl)-1-phenyl-2-picryl hydrazine
1(4-Nitrophenyl)-1-phenyl-2-picryl hydrazine

Antioxidant activity of different extracts of *P. suberifolium* determined by DPPH radical scavenging method. DPPH radical have maximum absorption at 517nm that disappears on reduction by antioxidant component. DPPH solution in methanol(0.1mM) prepared and 1ml solution mixed with 2.5mL of test compound/standard methanolic solution (different concentrations range in from 5 to 25µg/mL). The samples were incubated at room temperature for 30min in dark chamber and then, decrease in absorbance at 517nm was measured (AS). Ablank sample containing 1ml DPPH and 4ml methanol was prepared and absorbance measured(AB). Radical scavenging was calculated by following formula (Y. Anusha *et al*).

Percentage of inhibition= [(AB-AS)/AB] x 100

2) *Ferrous reducing antioxidant power assay*

Fe^{2+} can be assessed by measuring production of Perl's Prussian blue at 700 nm. PS extracts 0.25ml and standard solution of different concentrations (12.5–150 µg/ml), 0.625 ml (0.2M) potassium buffer and 0.625 ml of 1%[K_3Fe $(CN)_6$] solution were added to test tubes. An incubation period of 20 min at 50°C was provided to complete the reaction. To it 0.625 ml of 10% trichloro acetic acid (TCA) solution was pipetted into test tubes. The whole mixture was subjected to centrifugation for 10 min at 3000rpm; 1.8 ml supernatant was

withdrawn from test tubes and mixed with 1.8ml of water and 0.36 ml of 0.1% ferric chloride solution. A blank solution was prepared and incubation was done similarly, and its absorbance was measured at 700 nm. This was performed in triplicate for each concentration (Md. Mahbubur Rahman *et al*).

3) *Hydrogen peroxide scavenging assay*

The ability of scavenging H_2O_2 was performed as per Ruch *et al*(1989) method. H_2O_2 (40mM) solution was prepared in phosphate buffer (pH 7.4). Extracts (100 µg/ml) were added to it (0.6ml, 40mM) and absorbance was determined at 230nm 10 minutes later against blank solution with phosphate buffer without H_2O_2 (Serhat Keser *et al*). Percentage of H_2O_2 scavenging of *P. suberifolium* extracts and ascorbic acid were calculated by formula.

$$\% H_2O_2 \text{ Scavenged} = [(Ac - As)/Ac] \times 100$$

Where Ac is absorbance of the control and As is absorbance of *P. suberifolium* extracts or ascorbic acid

IV. RESULTS AND DISSCUSION

A. Determination of total phenolic content (TPC)

In the current study TPC of Hexane extract (HE), Ethyl acetate extract(EAE), Acetone extract(AE), Methanol extract(ME) and Ethanol extract(EA) of Ps leaves of 1mg equivalent to 24, 58, 69,78 and 66 mg Gallic acid equivalents/gram of extract respectively of Gallic acid(GA) was detected and depicted in Table-1. Standard graph of GA depicted in Fig-1 and graphical representation of TPC in Fig-2.

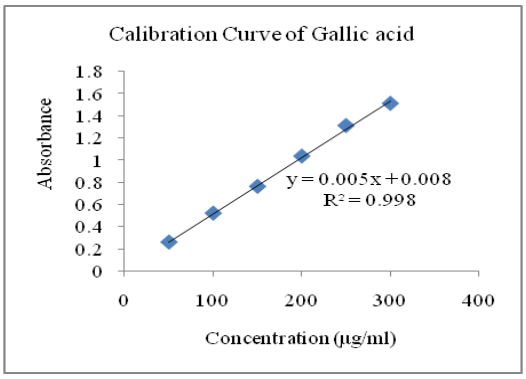


Fig. 1: Calibration curve of GA

Table 1: Total Phenolic content of *Pterosperum suberifolium* leaves Extracts

Sample	**Unknown Concentration (µg/ml)**	**Phenolic Content mg GAE/g.Extract**
Hexane extract	240.00	24.00
Ethyl acetate ext	582.35	58.25
Acetone extract	780.20	78.02
Methanol extract	704.25	70.42
Ethanol extract	660.79	66.07

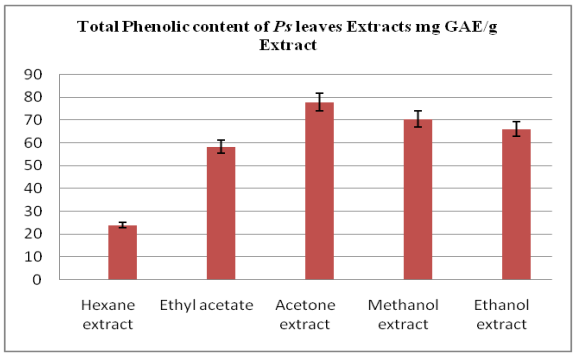


Fig. 2: Bar diagram of TPC of P.s leaf Extracts

From the result it was observed that Acetone leaf extract has more Phenolic content (78.02 mg GAE/gr. Extract) than other extracts. Order of phenolic nature was observed as AE>ME>EE>EAE>HE

B. Determination of total flavonoid content (TFC)

TFC of Hexane, Ethyl acetate, Acetone, Methanol and Ethanol extracts of Ps leaves of 1mg equivalent to 53, 68, 79, 85 and 82 /100 gram equivalents respectively of Catechin was detected and depicted in Table-2. Standard graph of Catechin depicted in Fig-3 and graphical representation of TFC in Fig-4.

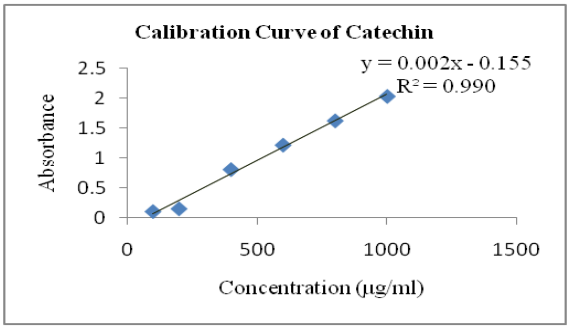


Fig. 3: Calibration curve of Standard Catechin

Table 2: Total Flavonoid content of *Pterosperum suberifolium* leaves extracts.

Sample	**Concentration (µg/ml, established from calibration curve**	**Total Flavonoid Content mg CA/g. Extract**
Hexane extract	538.28	53.82
Ethyl acetate extract	687.13	68.71
Acetone extract	797.13	79.71
Methanol extract	852.50	85.25
Ethanol extract	824.39	82.43

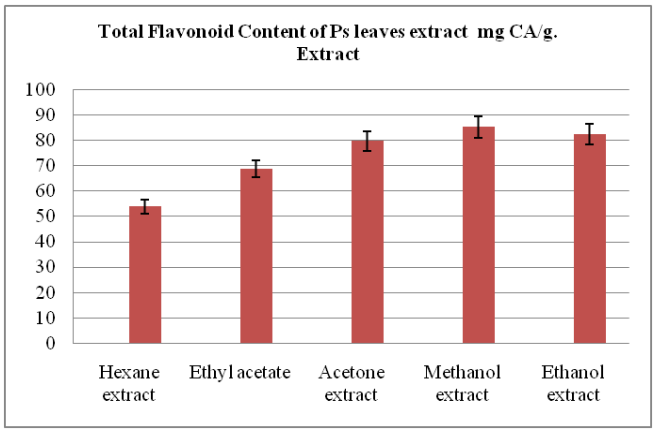


Fig. 4: Bar diagram of TFC of Ps leaf extracts

Based on the result it was observed that Methanol leaf extract has more Flavonoid content (78.02 mg CA/g Extract). Order of Flavonoids nature was observed as ME>EE>AE>EAE>HE.

C. In-vitro anti oxidant activity

1) DPPH radical scavenging assay

It determines capability of Ps extracts to reduce DPPH radical to respective hydrazine by changing unpaired electrons to pair once. In the present study inhibition of DPPH radical indicates that extracts cause reduction in DPPH radical. Percentage scavenging of DPPH examined at various concentrations was shown in table-3, fig-5 using Ascorbic Acid as standard and illustrates the effect of Hexane, Ethylacetae, Acetone, Methanol and Ethanol extracts on DPPH radicals.

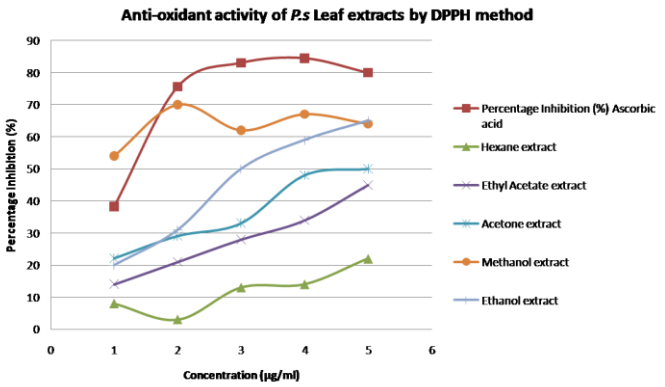


Fig. 5: DPPH radical scavenging assay of different solvent extracts of P.s leaves

Table 3: Effect of various extracts of P.s leaves on DPPH radicals

Conc (µg/ml)	Percentage Inhibition (%)					
	Ascorbic acid	HE	EAE	AE	ME	EE
5	38.23	8	14	22	54	20
10	75.63	3	21	29	70	31
15	83.11	13	28	33	62	50
20	84.55	14	34	48	67	59
25	80	22	45	50	64	65

The Anti-oxidant activity of Ps using DPPH method was observed more in methanolic extract compared to other solvent extracts. Order of Anti-oxidant activity of plant extracts by DPPH method-ME> EE > AE > EAE> HE.

2) Hydrogen peroxide scavenging capacity

In this study it was identified that various extracts of Ps shown H_2O_2 scavenging activity in dose dependent manner. Ascorbic acid was standard and the percentage scavenging activity was compared with Hexane, Ethyl acetate, Acetone, Methanol and Ethanol extracts. The results were tabulated in table-4 and figure-6.

Table 4: Hydrogen peroxide scavenging capacity of Ps leaf extracts

Conc (µg/ml)	Percentage Inhibition (%)					
	Ascorbic acid	HE	EAE	AE	ME	EE
5	1.83	0.08	0.19	0.91	1.12	1.04
10	3.63	0.19	0.44	1.02	2.48	2.12
15	4.56	0.31	0.61	1.69	3.51	2.98
20	5.95	0.68	0.94	1.98	4.82	3.25
25	6.56	0.82	1.23	2.65	5.61	4.52

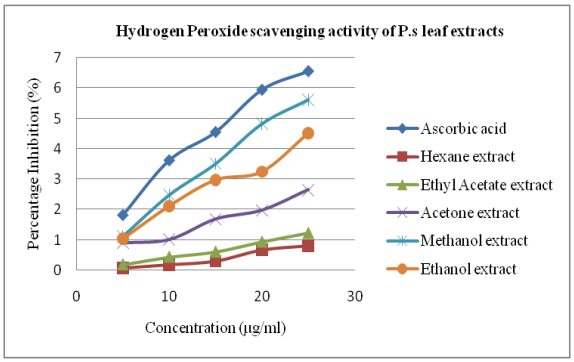


Fig. 6: Hydrogen peroxide scavenging assay of P.s leaves

3) Ferrous reducing antioxidant capacity assay

Here reducing power of extracts (Fe^{3+} to Fe^{2+}) was compared with Ascorbic acid. In this assay the reductive capability of the extracts increased in increasing concentrations. The absorbance values at 700nm were tabulated (Tab-5) and the graphical representation was given the Fig-7.

Table 5: Ferrous reducing antioxidant capacity assay of P.s leaf extracts

Conc (µg/ml)	Absorbance					
	Ascorbic acid	HE	EAE	AE	ME	EE
5	0.19	0.06	0.10	0.13	0.16	0.14
10	0.36	0.11	0.25	0.26	0.31	0.29
15	0.59	0.16	0.34	0.39	0.52	0.48
20	0.78	0.19	0.49	0.54	0.68	0.61
25	0.95	0.24	0.56	0.66	0.86	0.82

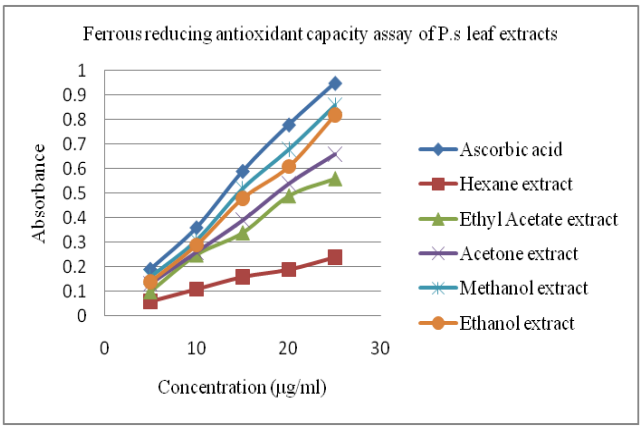


Fig. 7: Ferrous reducing antioxidant capacity of P.s leaf extracts

From the result it was observed that methanolic extract shown higher absorbance which means more reducing power than other extracts. While compared to ascorbic acid the antioxidant activity was moderate.

CONCLUSION

Based on present study it was concluded that methanolic extract of *Pterosperum suberifolium* leaves has considerable amounts of flavonoids and phenols and has moderate antioxidant activity which can act against free radicals to reduce their negative effects on various organs in the human body. We can notice the acetone extract got more phenols and methanol extract with high flavoniods, and finally methanol extract showed better antioxidant activity, this can be explained by saying that besides phenolics there may be other photochemical compounds that contribute to the antioxidant activity (Giovanna Piluzza *et al*). However as the flavonoid content was high in methanolic extract this can also lead to high antioxidant activity for methanolic extract (Josiane Alhage *et al*). The yield of the constituents varies by varying the process of extraction (Souad Senhaji *et al.*,), so this idea must be applied for better results. Further investigation of various pharmacological activities, isolation and characterization of potent phytochemicals is necessary to give a detailed profile of the plant.

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