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# EVALUATION OF ANTIOXIDANT ACTIVITIES OF LEAVES, STEM AND WHOLE PLANT OF PEPEROMIA PELLUCIDA

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## ABSTRACT

*Peperomia pellucida (L.)* HBK (Piperaceae) has been used as a drug (Rasayan) in the Ayurvedic system of medicines. Recently, leaves and stem of this herb are used as food items, especially in salads. In the present study, leaves, stem and the whole plant of phenotypes grown in West Bengal were tested for their antioxidant properties. Among the five antioxidant assays (viz. ABTS radical decolorization assay, DPPH radical decolorization assay, assay for total phenolics content, FRAP assay and hydroxyl radical scavenging assay) used in the present study, the whole plant gave the best result, in terms of gallic acid equivalents. Leaves, however, showed greater antioxidant potential in comparison to stem in both ABTS and DPPH assays, indicating higher amount of polar and non-polar biomolecules in the leaves. All the parts as well as the whole plant showed comparable hydroxyl radical scavenging abilities, clearly indicating its potential use in benefits of human.

**KEYWORDS:** Antioxidant, Leaves, Stem, Plant, *Peperomia pellucida*

## INTRODUCTION

*Peperomia pellucida* (L.) HBK, also known as shiny bush or silver bush, belongs to Piperaceae family. It is an herbaceous plant found in many South American and Asian countries. It grows to a height of about 15 to 45 cm and is characterized by succulent stems, shiny, heart-shaped leaves and tiny, dot-like seeds attached to several fruiting spikes[1] The species develops during rainy periods (often in the spring) and thrives in loose, humid soils under the shade of trees[2] Ethno-medicinal uses of this plant has been ascribed in treating abdominal pain, abscesses, acne, boils, colic, fatigue, gout, headache, renal disorders, and rheumatic joint pain[3,4] Geographically, this plant has multi-functional attributes, including mental disorder treatment in Bangladesh; haemorrhages treatment in Bolivia, cholesterol reduction in Brazil, and renal problem and uric acid reduction in Guyana and Philipines[5]. Chemical bioactives present included flavonoids, phytosterols, apiols and substituted styrenes, whereas the biological studies of crude extracts showed anti-inflammatory, antioxidant, bactericidal and analgesic activities.[6]

*Peperomia pellucida* leaves and stems may also be eaten as vegetable.[7] In salads, the fresh plant has the crispness of carrot sticks and celery[8] The leaves and the stem are used to prepare traditional dishes during *Bihu* festival in Assam.[9] Despite its widespread range of ethnopharmacological uses in India sub-continent, there is very little scientific records available on its pharmacological and biological activities as well as its chemical constituents.[8] The plant is now copious in distribution and is abundantly available in South-East Asia, especially in Malaysia, Indonesia and Thailand. A body of literature is now available about the nutritional and pharmacognostic activities of this plant. However, the plant is now abundantly growing in the Indian subcontinent, especially in Bengal, of which very little activities have been reported. The present study delineate antioxidant activities of the species grown in West Bengal, India, probably for the first time. Individual parts of the plant, viz. leaves, stem and the whole plant are also analyzed for their antioxidative potentials, if any.

## MATERIALS AND METHODS

2, 2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), ABTS, were obtained from Sigma, USA. 2,2′-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Himedia, India. Analytical grades of Gallic acid, Folin-Ciocalteau's solution, 2-Thiobarbituric acid, sodium hydroxide and sodium carbonate, were obtained from Merck, India. 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) and 2-Deoxy-D-ribose was procured from SRL, India. All other reagents and chemicals used were of analytical grade procured from local sources. Demonized distilled water was used in the entire study.

**Preparation of extracts**
The samples were collected from various pond banks of Barasat, Kolkata. The samples were checked for any dirt or visible damages prior to the study. Such damaged samples were discarded. 5 gms of whole plant or parts thereof were taken in 50 ml of aqueous 70% (V/V) methanolic solution. The mixture was then heated at 80$^{o}$C for 5 minutes, cooled to room temperature and centrifuged at 8000 rpm for 5 mins. The clear supernatants were stored at 4$^{o}$C for further *in vitro* antioxidant assays.

**ABTS radical decolorization assay**
The assay was performed using a previously described procedure.[10] The oxidant was generated by persulfate oxidation of 2,2′-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid. The oxidant solution was mixed with the sample/standard solutions in such a way that total volume of the solution reached 1 ml. The absorbance at 734 nm in a Systronics spectrophotometer (model – 2202) was read at room temperature, 4 minutes after mixing. The results were expressed as Gallic acid equivalents.

**DPPH radical decolorization assay**
The assay was performed using a previously described procedure.[10] DPPH solution (0.1 Mm) was mixed with sample/standard solution and the decrease in absorbance of the mixture after 20 minutes of incubation in the dark was monitored at 517 nm in a Systronics spectrophotometer (model – 2202). The results were expressed as Gallic acid equivalents.

**Estimation of total phenolics content**
Total phenolics compound contents were determined by the Folin-Ciocalteau method.[11] The samples/standards were mixed with Folin-Ciocalteau reagent (1:10 diluted with distilled water) for 5 min and aqueous sodium carbonate (1 M) was then added. The absorbance of the reaction mixture was then measured at 765 nm in a UV-Vis spectrophotometer (model – Systronics 2202). Gallic acid was used as standard. The results were expressed in terms of gallic acid equivalent/gm sample.

**Ferric reducing antioxidant power: FRAP**
Ferric reducing antioxidant power of the samples was estimated with a previously described procedure.[12] Briefly, a maximum of 100 µl of extract solution or standard was mixed with 1.9 ml of FRAP reagent and incubated at 37$^{o}$C for 30 mins. FRAP reagent was prepared by mixing 0.1 M aqueous acetate buffer (pH 3.6), TPTZ solution and ferric chloride solution. After the stipulated time period, absorbance was measured at 593 nm in a UV-Vis spectrophotometer (model – Systronics 2202). Gallic acid is used as standard. Results were expressed as Gallic acid equivalents (GAE).

**Hydroxyl radical scavenging assay**
Hydroxyl radical scavenging potentials of the samples were estimated with a previously described procedure.[13] Briefly, 10 mM each of $FeSO_4.7H_2O$, EDTA, 2-deoxy-D-ribose and $H_2O_2$ solutions were prepared in water. Each solution of above four with sample/standard solution was mixed in a test tube to get a final volume of 1 ml and incubated at 37$^{o}$C for 90 mins. $H_2O_2$ solution was added last. After the incubation, 2.8% (w/v) aqueous TCA solution and 1% (w/v) aqueous TBA solution were added to the reaction mixture and kept at boiling water bath for 20 mins. Development of the pink chromophore was measured at 532 nm in a UV-Vis spectrophotometer (model – Systronics 2202). Results were expressed as Gallic acid equivalents (GAE).

**Statistical analysis**
Experimental results are expressed as mean ± SD of three individual samples. The statistical analysis was done by using the software 'SPSS Statistics 17.0' (IBM Corporation, USA).

## RESULTS AND DISCUSSION

ABTS radical decolorization activity of the different parts of *Peperomia pellucida* indicated that total plant possessed maximum radical scavenging capacity (Fig.1). Between leaves and stems, higher activity was shown by the leaves. This indicated that contribution of leaves in the radical scavenging potential of the whole plant extract was greater.

DPPH assay is used to determine the scavenging potential of the different parts of *Peperomia pellucida* indicated that total plant possessed maximum radical scavenging capacity (Fig. 2). Between leaves and stems, higher activity was shown by leaves. This indicated that contribution of leaves in the radical scavenging potential of the whole plant extract was greater.

DPPH assay was used to determine the scavenging potential of antioxidant extracts based on their capabilities as hydrogen donor in tandem with electron transfer.[14] ABTS assay, on the other hand, indicates radical neutralization by electron transfer mechanism only. The two assays in this study indicated possible involvement of the two mechanisms in the radical scavenging abilities of the subject herb. Moreover, using the two assays, it was ascertained that the herb contains both polar and non-polar bioactives in substantial amounts.[15]

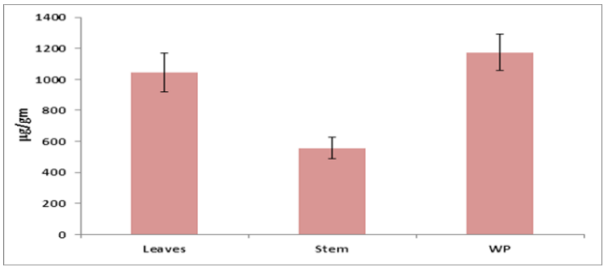


**Fig. 1: ABTS radical scavenging activities of whole plant (WP) and different parts. Results are expressed as Mean±SD in gallic acid equivalent (GAE).**

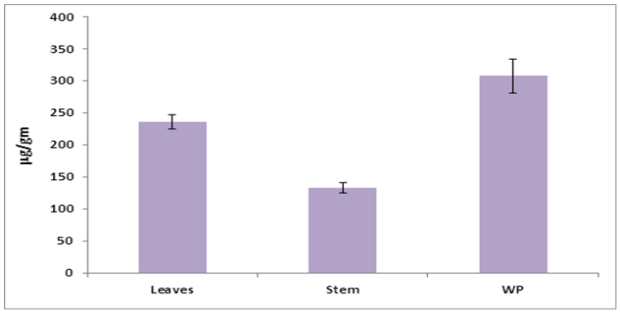


**Fig. 2: DPPH radical scavenging activities of whole plant (WP) and different parts. Results are expressed as Mean±SD in gallic acid equivalent (GAE).**

Total phenolic contents of the whole plant and different parts of *Peperomia pellucida* indicated that the whole plant possessed maximum radical scavenging capacity (Fig. 3). Between leaves and stems, higher activity was shown by leaves. This indicated that contribution of leaves in the radical scavenging potential of the whole plant extract was greater.

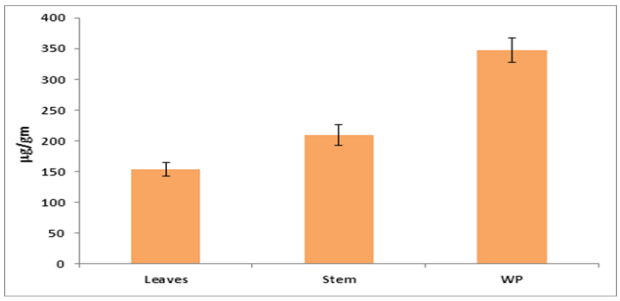


**Fig. 3: Total phenolics contents of whole plant (WP) and different parts. Results are expressed as Mean±SD in gallic acid equivalent (GAE).**

The reducing power of the different parts of *Peperomia pellucida* indicated that total plant possessed maximum reducing (Fig. 4). This commensurate with the results of ABTS and DPPH assays, as it was proved by the two assays that the plant extract possess different types of bioactives, which might have wide ranges of polarity. Between leaves and stems, higher activity was shown by leaves. This indicated that contribution of leaves in the radical scavenging potential of the whole plant extract was greater.

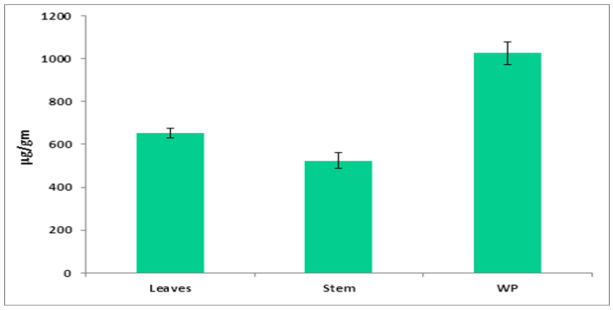


**Fig. 4: Ferric reducing antioxidant potential (FRAP) of whole plant (WP) and different parts. Results are expressed as Mean±SD in gallic acid equivalent (GAE).**

Hydroxyl radical scavenging potentials of the different parts of *Peperomia pellucida* indicated that total plant possessed maximum radical scavenging capacity (Fig.5). Between leaves and stems, higher activity was shown by leaves. However, the differences in the activities of the different extracts were non-significant.

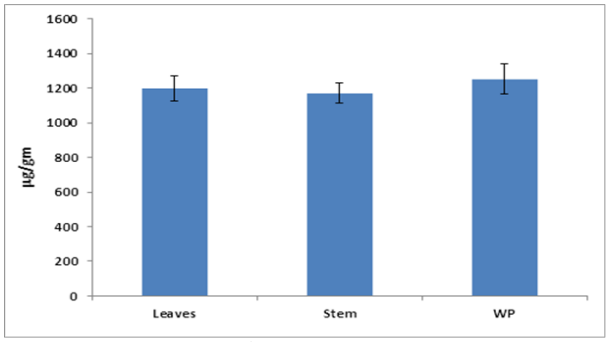


**Fig. 5: Hydroxyl ($OH^{\bullet}$) radical scavenging abilities of whole plant (WP) and different parts. Results are expressed as Mean±SD in gallic acid equivalent (GAE).**

Ability to remove hydroxyl radical, albeit *in vitro*, has immense importance in the application of the herb as food as well as nutraceuticals. The extracts removed significant levels of free radicals, which indicated that they might play a potential role systemically to eliminate the most harmful free radicals present in human body.

## CONCLUSION

*Peperomia pellucida* leaves and stems are nowadays being eaten as vegetable and in salads as the fresh plant has the crispness of carrot sticks and celery. The leaves and the stem are also used to prepare traditional dishes during *Bihu* festival in Assam. However, very little antioxidant activities have been reported for the plant growing in the eastern part of India. The present study indicated that the herb contained both polar and non-polar antioxidant bioactives abundantly. Its' ability to scavenge hydroxyl free radicals *in vitro* indicated that it might play an impending role systemically to eradicate this most harmful free radical present in human body. Thus, it can be concluded that whole plant or parts thereof *Peperomia pellucid*a can be considered as a potential source of different antioxidant components, which need to be exploited at the moment where degenerative disease like cardiovascular disease, cancer etc has become very prevalent. So it can be utilized in household food preparations as a great source of potential antioxidant.

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