



## ***In-Vitro* Antioxidant Activity and Determination of Total Phenolic, Flavonoid Contents of Peperomia Pellucida Linn**

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### **ABSTRACT**

*Peperomia Pellucida Linn* (Piperaceae) has been traditionally used for the treatment of various ailments such as abdominal pain, acne, boils, colic, fatigue, gout, headache, renal disorders, and rheumatic joint pain. Preliminary phytochemical screening showed the presence of phenols and flavonoids in *P. Pellucida*. Thus the present study, aims to determine the *in vitro* antioxidant activity and to estimate the phenolic and flavonoid content in the methanolic extract of the whole plant of *Peperomia Pellucida Linn*. Total phenolic content (TPC) was estimated using Folin-Ciocalteu's reagent and total flavonoid (TF) content by Aluminium chloride colorimetric method. DPPH (2, 2-diphenyl-1-picryl hydrazyl), ABTS (2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) and nitric oxide scavenging method were carried out to evaluate the *in vitro* antioxidant activity. Total phenolic and flavonoid contents were found to be 3.91g gallic acid equivalent per 100 g and 5.80g quercetin equivalent per 100 g of methanolic extract respectively by spectrophotometric method. IC<sub>50</sub> value of methanolic extract of *Peperomia Pellucida Linn* were found to be 351.56±0.21µg/ml, 76.51±0.19µg/ml and 282.30±0.42µg/ml respectively in DPPH, ABTS and nitric oxide scavenging assays. These primary findings suggest that the methanol extract of *P. Pellucida Linn* possess phenolic and flavonoid constituents that are responsible for antioxidant activity.

**Keywords:** *P. Pellucida*, antioxidant activity, flavonoids, ABTS, DPPH

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## INTRODUCTION

Free radical is a chemical species which contains an unpaired electron spinning on the peripheral layer around the nucleus. Free radicals generated from the oxygen are called Reactive oxygen species (ROS) which cause damage to other molecules by extracting electrons from them in order to attain stability<sup>1</sup>. Living cells may generate free radicals and other reactive oxygen species by-products as a results of physiological and biochemical processes. Free radicals can cause oxidative damage to lipids, proteins and DNA, eventually leading to many chronic diseases, such as cancer, diabetes, aging, and other degenerative diseases in humans<sup>2</sup>.

Antioxidants are substances, when present at low concentration (compared with those of an oxidizable substrate), significantly delays or prevents oxidation of that substrate<sup>3</sup>. In recent years, there has been an increasing interest in finding natural antioxidants, which can protect the human body from free radicals and retard the progress of many chronic diseases. Natural antioxidants such as  $\alpha$  tocopherol and ascorbic acid are widely used because they are regarded as safer and causing fewer adverse reactions but their antioxidant activities are lower than the synthetic antioxidants, which have been restricted by legislative rules because they are suspected to have some toxic effects and are possible carcinogens<sup>4</sup>. Therefore, there is considerable interest in finding safer antioxidants from natural sources to replace the synthetic ones.

Plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity<sup>5</sup>. Studies have shown that many of these antioxidant compounds possess anti-inflammatory, anti-atherosclerotic, anti-tumor, anti-mutagenic, anti-carcinogenic, anti-bacterial, and anti-viral activities<sup>6</sup>. The ingestion of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing<sup>7</sup>.

*Peperomia Pellucida* Linn, widely used in traditional medicine belongs to the family Piperaceae. It has been used for treating abdominal pain, acne, boils, colic, fatigue, gout, headache, renal disorders, and rheumatic joint pain. In some parts of Asia such as Bangladesh, the aerial parts including leaves of the plant have been used by local traditional healers to treat the excited mental disorders. Several scientific studies reported the anti-bacterial and analgesic activities of *P. pellucida* and some other studies reported the isolation of bioactive phytoconstituents with strong anti-fungal and anti-cancer properties from *P. Pellucida*<sup>8</sup>. The present study was to analyze the phenolic, flavanoid content and the in-vitro antioxidant activity of the *Peperomia*

*pellucida*.

## MATERIALS AND METHODS

### Plant Materials

The fresh plants of *Peperomia pellucida* were collected from kollam district of kerala and it was authenticated by Dr V.J. Dominic , Sacred heart college, Thevara. . A voucher specimen was deposited in the department for future reference. The whole plant were washed thoroughly with sufficient water. It was dried in shade and powdered using mechanical grinder and sieved to get the powder of uniform size.

### Preparation of extract

The powdered material was subjected to soxhlet extraction with various solvents ranging from non-polar to polar. The solvents used were petroleum ether, chloroform, methanol and water. Each time before extraction with next solvents the marc was air-dried. All the extracts were concentrated by distilling the solvent at low temperature. They were then weighed and percentages of different extractive values were calculated with respect to air-dried substance. The methanolic extract was selected for phenolic, flavonoid content and antioxidant activity on the basis of phytochemical screening and literature survey.

### Determination of total phenolic content

The total phenolic content was determined using Folin–Ciocalteu reagent with analytical grade gallic acid as the standard<sup>9</sup>.Methanolic solution of the extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of methanolic solution of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% NaHCO<sub>3</sub>. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of NaHCO<sub>3</sub>.

The samples were thereafter incubated in a thermostat at 45°C for 45 min. The absorbance was recorded at 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. Percentage of total phenolic content was calculated from calibration curve of gallic acid plotted using the same procedure. Total phenolics were expressed in terms of gallic acid equivalent (mg/g of GA/g of extract). All the tests were performed in triplicate.

### Determination of Total Flavonoid Content

Total flavonoid content was determined according to the procedure by Aluminium chloride colorimetric method. To a 10 ml volumetric flask 4 ml of water and 1 ml of plant extract (0.1

mg/ml) were added followed by 0.3 ml of 5 % sodium nitrite and 0.6 ml of 10 % aluminum chloride after 5 minutes and 2 ml of 1 M Sodium hydroxide after 6 minutes. The volume was made up with distilled water. The solution was mixed thoroughly and absorbance was measured at 510 nm. Total flavonoid contents were expressed as milligrams of quercetin equivalent per milligram of dry weight. All the tests were performed in triplicate<sup>10</sup>.

### Antioxidant activity assays

#### DPPH radical scavenging assay<sup>11</sup>

The effect of extracts on DPPH(1,1-diphenyl-2-picryl-hydrazyl) radical was estimated using the method of Liyana-Pathiranan and Shahidi. A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this was mixed with 1.0 ml of different concentrations (0.02 – 0.1 mg) of the extract in methanol. The reaction mixture was vortexed thoroughly and left in the dark at room temperature 27°C for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as reference. The ability to scavenge DPPH radical was calculated by the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \frac{[(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})]}{(\text{Abs}_{\text{control}})} \times 100,$$

where  $\text{Abs}_{\text{control}}$  is the absorbance of DPPH radical + methanol;  $\text{Abs}_{\text{sample}}$  is the absorbance of DPPH radical + sample extract /standard. The  $\text{IC}_{50}$  value was defined as the concentration (in  $\mu\text{g/ml}$ ) of extract that inhibits the formation of DPPH radical by 50%<sup>12</sup>.

#### ABTS radical scavenging assay<sup>13</sup>

The method of Re et al. (1999), was adopted for this experiment. The stock solutions included 7 mM ABTS. + solution and 2.4 mM potassium persulfate solution. Varying concentrations of the methanolic extract of *Peperomia pellucida* (1.0 ml) was allowed to react with 1 ml of the ABTS.+ solution and the absorbance taken at 734 nm after 7 min using the spectrophotometer . The ABTS.+ scavenging capacity of the extract was compared with that of standard butylated hydroxytoluene (BHT) and percentage inhibition capacity of  $\text{ABTS}^+$  of the extract was calculated from the following equation:

$$\% \text{ scavenging activity} = \frac{[(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})]}{(\text{Abs}_{\text{control}})} \times 100$$

#### Nitric oxide Scavenging Activity<sup>14</sup>

Nitric oxide was estimated spectrophotometrically by the method at 546 nm. Sodium nitroprusside (5mM) in phosphate buffered saline was mixed with different concentrations of methanolic extract of *peperomia pellucida* (100-500  $\mu\text{g/ml}$ ) dissolved in methanol and incubated at 25°C for 30 min. Then 1.5 ml of the incubated solution was removed and diluted with 1.5 ml of Griess reagent (1% Sulfanilamide, 2% phosphoric acid, and 0.1% naphthyl ethylene diamine

dihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with naphthylethylene diamine was measured at 546 nm. Percentage scavenging activity (antioxidant activity) can be calculated by the following formula

$$\% \text{ scavenging activity} = [(Abs_{\text{control}} - Abs_{\text{sample}})/(Abs_{\text{control}})] \times 100$$

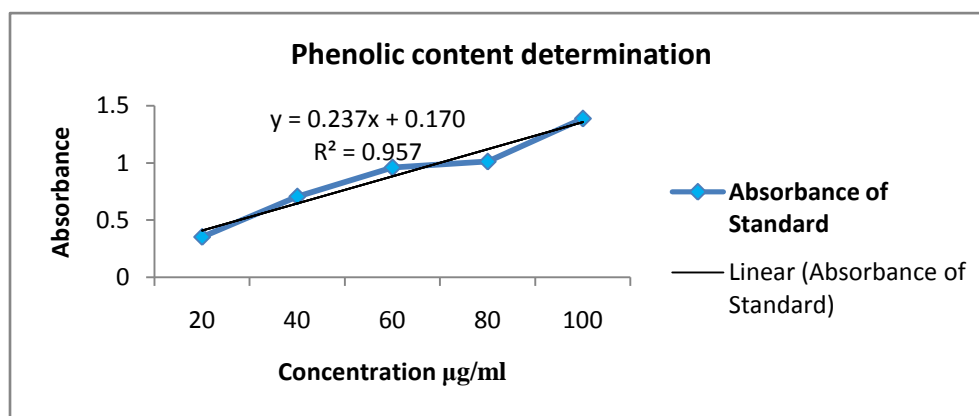
## RESULTS AND DISCUSSION

### Total Phenolic content:

Total phenolic content was estimated by Folin-Ciocalteu method. Total content of phenolic compound was calculated as Gallic acid equivalent. The obtained observations are mentioned in table 1 and graph plotted showing absorbance vs concentration (in figure. 1). Total Phenolic content was found to be 3.91gm gallic acid equivalent per 100 g methanolic extract by using the equation ( $y = 0.237x + 0.170$ )

**Table 1: Phenolic Content of Methanolic Extract of *Peperomia pellucida* linn**

Concentration( $\mu\text{g/ml}$ ) (Gallic Acid)	Absorbance
20	0.3526
40	0.7052
60	0.9589
80	1.0106
100	1.3863
Methanolic extract <i>Peperomia pellucida</i> (1mg/ml)	0.1789



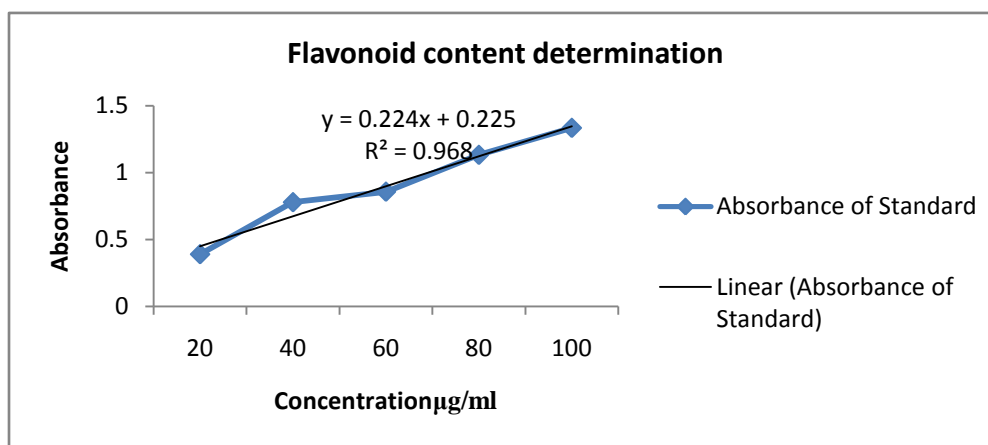
**Figure. 1: graph of absorbance against concentration for total phenolic content Total Flavonoid content**

The total flavonoid content was estimated by Aluminium chloride colorimetric method. In this method flavonoid content was determined as quercetin equivalent. The obtained observations are mentioned in table 2 and graph plotted showing absorbance Vs concentration (figure. 2). Total

flavonoid content was found to be 5.80 g quercetin equivalent per 100 g methanolic extract by using the equation ( $y = 0.224x + 0.225$ ).

**Table 2: Flavonoid Content of Methanolic Extract of *Peperomia pellucida linn***

Concentration( $\mu\text{g/ml}$ ) (Quercetin)	Absorbance
20	0.3897
40	0.7785
60	0.8571
80	1.1325
100	1.3345
Methanolic extract <i>Peperomia pellucida</i> (1mg/ml)	0.2380



**Figure. 2: Graph of absorbance against concentration for total flavonoid content**

**Table 3. DPPH radical scavenging activities of methanolic extract of *P.pellucida* and Ascorbic acid as standard at different concentrations**

Concentration $\mu\text{g/ml}$ )	% Inhibition	
	Ascorbic acid	<i>P.pellucida</i>
100	28.23 $\pm$ 0.24	13.86 $\pm$ 0.30
200	49.42 $\pm$ 0.82	28.57 $\pm$ 0.23
300	69.40 $\pm$ 0.32	39.44 $\pm$ 0.41
400	81.2 3 $\pm$ 0.34	55.39 $\pm$ 0.46
500	98.40 $\pm$ 0.38	74.56 $\pm$ 0.35

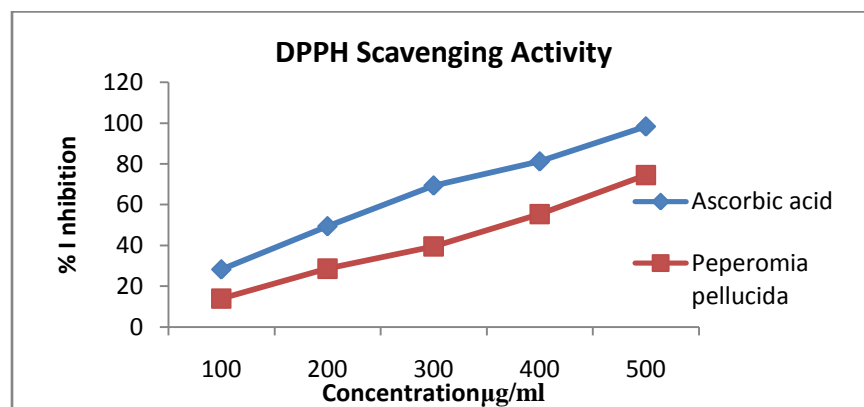
(Values are expressed as SEM of 3 readings)

#### Antioxidant activity:

##### DPPH method:

In order to determine the extent of scavenging effect, methanolic extract of the *Peperomia pellucida linn* was tested for antioxidant activity using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical. Methanolic extract showed significant antioxidant activity [ $\text{IC}_{50} = 351.56 \pm 0.21 \mu\text{g/ml}$ ] and it was compared to standard ascorbic acid [ $\text{IC}_{50} = 210.98 \pm 0.34 \mu\text{g/ml}$ ]. (Table 3,

Figure. 3).



**Figure. 3: DPPH Scavenging activity of methanolic extract of *Peperomia pellucida* and ascorbic acid**

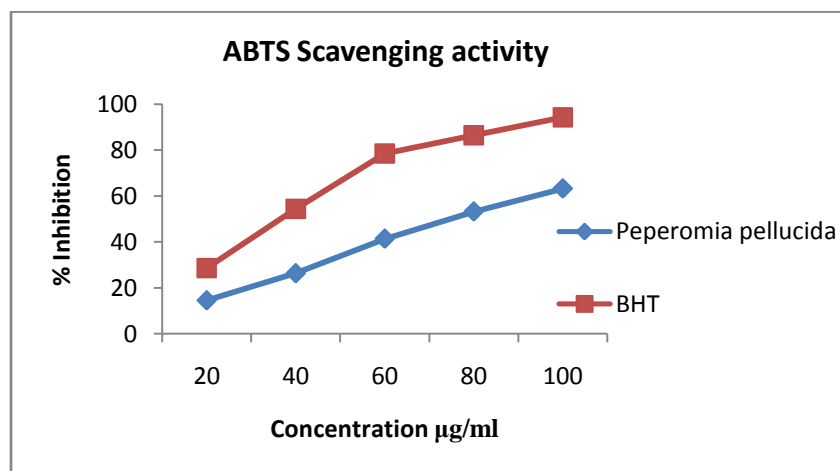
#### ABTS RADICAL SCAVENGING METHOD

The methanolic extract of *P. Pellucida* showed potent antioxidant activity ( $IC_{50} = 76.51 \pm 0.19 \mu\text{g/ml}$ ) in ABTS method which is comparable to the standard (BHT) used ( $IC_{50} = 37.47 \pm 0.19 \mu\text{g/ml}$ ). The results are presented in Table 4. Figure.4

**Table 4. ABTS radical scavenging activities of methanolic extract of *P.pellucida* and BHT as standard at different concentrations**

Concentration (µg/ml)	% Inhibition	
	BHT	<i>P.Pellucida</i>
20	28.59±0.72	14.56±0.52
40	54.32 ±0.62	26.35±0.43
60	78.49 ±0.56	41.38±0.32
80	86.45±0.81	53.23 ±0.12
100	94.21 ±0.21	63.23±0.25

(Values are expressed as SEM of 3 readings)



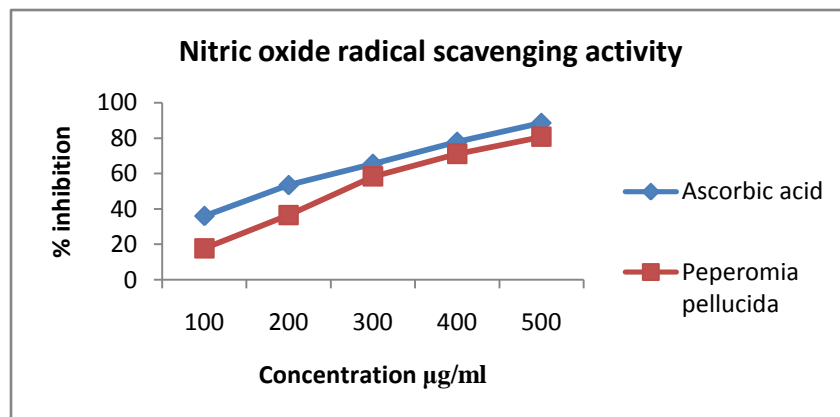
**Figure. 4: ABTS Scavenging activity of methanolic extract of *Peperomia pellucid* & BHT**

### Nitric oxide Scavenging Activity

In nitric oxide scavenging activity study, *P.Pellucida* proved to be a good nitric oxide radical fighter by its showing IC<sub>50</sub> value of 282.30±0.42 µg/ml. The results were compared with standard antioxidant ascorbic acid whose IC<sub>50</sub> value was calculated as 190.28±0.38µg/ml. The results are presented in Table 5.Figure.5

**Table 5. Nitric oxide radical scavenging activities of methanolic extract of *P.pellucida* and Ascorbic acid as standard at different concentrations**

Concentration (µg/ml)	% Inhibition	
	Ascorbic Acid	<i>P.Pellucida</i>
100	35.94 ±0.23	17.74 ±0.54
200	53.41 ±0.35	36.53±0.21
300	65.44 ±0.43	58.32 ±0.33
400	77.89 ±0.61	71.05 ±0.12
500	88.57±0.44	80.67 ±0.41



**Figure. 5: Nitric oxide radical Scavenging activity of methanolic extract of *Peperomia pellucida* and Ascorbic acid**

### CONCLUSION

The methanolic extract of *Peperomia pellucida linn* indicated strong antioxidant activity which might be helpful in preventing or slowing the progress of various oxidative stress induce diseases such as diabetes, liver diseases, cancer, ageing etc. This may be related to the high amount of phenolic and flavonoid compounds present in this plant's methanolic extract. Further experiments are required to explore the mechanism of action.

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