

# PHYTOCHEMICAL AND *IN VITRO* ANTIOXIDANT ACTIVITY OF *Psilotrichum elliotti* Baker – A PROMISING MEDICINAL PLANT

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## Abstract

The present study was performed to explore the biochemical estimation and antioxidant activities of *Psilotrichum elliotti* Baker (whole plant) belonging to *Amaranthaceae* using different solvents such as petroleum ether, chloroform, ethyl acetate, methanol and aqueous extract. The estimation of total carbohydrate, protein, phenols, tannin and flavonoids were quantified using the standard biochemical procedures. *in vitro* antioxidant activity of DPPH assay, Hydroxyl scavenging, Hydrogen peroxide radicals, Superoxide scavenging, antioxidant capacity were determined using standard procedures. Methanolic extract has shown the highest antioxidant activity when compared to other solvents and the wide range of phenols ( $28.89 \pm 3.6$  GAE mg/g) and flavonoid ( $27.33 \pm 3.4$  QE mg/g) component were present in the methanolic extract. The results of this analysis found out that *Psilotrichum elliottii* can offer a treasured source of natural antioxidant properties and nutritive biochemical compounds that could assist pharmaceutical sectors discover novel drugs.

**Keywords:** Antioxidant activity, Biochemical analysis, Phenols, *Psilotrichum elliottii*.

## 1.Introduction

Plant vegetation was the inspiration of many conventional medicine systems around the world for lots of years and held to offer people with new treatments and plant extracts are used to deal with roughly one among the world's population [1]. Plant-derived components, particularly phenolics, tannins and flavonoids, have been found to have anti-aging, antioxidant and anticancer properties [2,3]. Reactive oxygen species simply react with loose radicals to form radicals. ROS are distinct varieties of activated oxygen, which include loose radicals like superoxide anion radicals ( $O_2^-$ ) and hydroxyl radicals (OH), in addition to non-loose radical species like  $H_2O_2$  and singlet oxygen ( $O_2$ ) Antioxidants

defend dwelling organisms from damage because of unregulated ROS synthesis and consequent lipid peroxidation, protein degradation, and DNA breakage [4]. Synthetic antioxidants such as butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinone, and gallic acid are available, but they have been linked to harmful health consequences in the past. As a result, their use has been severely restricted, and there is a growing movement to replace them with naturally occurring antioxidants [5]. The Amaranthaceae family contains 174 genera and around 2,500 species in various regions of the world, some species of the genus are utilized as leaf vegetables, while others are considered weeds. The plants contain phytonutrients that suppress free radicals, as per previous research [6]. Several species of this genus have been evaluated for their antioxidant properties, including *Amaranthus viridis*, [7] *Amaranthus spinosus*, [8]. Flavonoids, alkaloids, tannins, phenolics, and glycosides were found in *Psilotrichum nudum* identified by phytochemical screening [9]. In addition to their antioxidant activity, they have been reported to have antimicrobial, anti-inflammatory, anti-malaria, anti - cancerous and anti-diabetic activity [10,11,12]. *Psilotrichum elliotii* is a perennial herbaceous plant that grows in dry grassland and hillsides. Amaranthaceae family plants contain bioactive substance like Phytosterols, polysaccharides, specifically flavonoids and phenolic compounds [1,2]. Due to its high quality and rich pharmacological background, *Psilotrichum elliotii* has been chosen for further study and treatment of numerous medical objectives such as eye sight correction, diarrhoea, asthma treatment, snake bite treatment, and blood purification [13]. A research study has showed up scientific evidence of *Psilotrichum elliotii* for its therapeutic benefits. The present study focused on the biochemical and antioxidant activities of the entire plant of *P. elliotii*.

## 2. Materials and Methods

### 2.1. Plant material

*Psilotrichum elliotii* Baker. is a plant species collected from the Sivanmalai Hills in the Tiruppur District. This plant was later identified specimen number (BSI/SRC/5/23/2021/Tech 246) and authenticated by “Scientist E” Head of Botanical Survey of India, Southern Regional Centre in Coimbatore. The herbarium was stored at the Department of Botany at Nirmala College for Women in Coimbatore, Tamil Nadu, for future reference. The entire plant was shade dried and ground into a coarse powder using an electric blender.

### 2.2. Hot Water Extraction

In a beaker, (150) g of finely powdered dry plant material was combined with 500 mL of distilled water. The mixture was heated on a hot plate for 20 minutes at 30°-40°C with constant stirring minutes. The water extract was then filtered using filter paper and utilized for qualitative and quantitative analysis.

### 2.3. Soxhlet Extraction

A Soxhlet apparatus was used to extract the entire plant of *P. elliotii* powdered material (200 g) in a series of solvent extractions. The solvents were chosen in accordance

with their polarity. Petroleum ether (PET), chloroform (CET), ethyl acetate (EET), aqueous (AET) and methanol (MET) solvents were used in a Soxhlet's apparatus for extraction. The solvent was extracted from the residue under vacuum, providing extracts of petroleum ether, chloroform, ethyl acetate, methanol, and aqueous extracts were dried weight of extract each for a solvent had been used to calculate the percentage of yield. Preserved in a desiccator for further analysis.

#### **2.4. Percentage of recovered extract**

After weighing the amount of crude extract recovered after each extraction, the percentage of yield was calculated by using the formula,

$$\text{Extract recovery percentage} = \frac{\text{Amount of extract recovered (g)}}{\text{Amount of plant sample (g)}} \times 100$$

#### **2.5. Extraction and Estimation of total Carbohydrate content**

Total soluble carbohydrate was determined using the Anthrone method. Different extracts of *P. elliotii* were hydrolyzed for three hours in a boiling water bath with 5 ml of 2.5N HCl, then cooled to room temperature. To halt the effervescence, use sodium carbonate to neutralize it. Fill to a capacity of 15 mL, centrifuge, and collect the supernatant. The carbohydrate content of each extract was measured in triplicate. Divide the material into 1ml aliquots for analysis. 4mL ice-cold anthrone reagent, heated for 10 minutes in a boiling water bath. After quick cooling with running tap water, read green to dark colour using a Visible Spectrophotometer set to 630nm. The total soluble carbohydrate in each sample was calculated and represented in (Standard deviation) mg/g yield weight using glucose as a reference.

#### **2.6. Extraction and Estimation of total Protein content**

The total soluble protein content of different extracts of *P.elliotii* determined using the [14] method. Different solvents extracts of *P.elliotti* with (1 mL) samples in 5 mL of Tris-HCl buffer (pH 7.0). The homogenates were centrifuged for 20 minutes at 5000 rpm. Now, 5 mL of 10% trichloroacetic acid (TCA) was added to the supernatant and heated for 3 minutes in a water bath. The treated samples were centrifuged for 20 minutes at 5000rpm after cooling. In 5 mL of 0.1N NaOH, the pellets were solubilized. After that, 5ml of alkaline reagent was well mixed into the test tube containing 0.1ml of protein extract. After allowing the samples to cool for 10 minutes at room temperature, 0.5ml of folin reagent was added. In a temperature-controlled water bath, the reaction mixture was incubated for 30 minutes (37° C). The color intensity increased as it cooled. In a visible spectrophotometer, the sample was measured at 650nm. Protein measurement was done in triplicate, and total soluble proteins in each sample were evaluated using bovine serum albumin Standard curve as a reference and expressed in mg/gm yield weight.

#### **2.7. Extraction and Estimation of total Flavonoid contents**

The flavonoid content of different extracts was determined using the aluminium chloride colorimetric technique [15]. Each extracts solution (1 mg/ml) was mixed with

1.25 mL distilled water. The combination was then treated with a 0.075mL (5%) sodium nitrite solution, which was incubated for 5 minutes before being treated with 0.15mL (10%) aluminium chloride. The mixture was allowed to remain at room temperature for 6 minutes before adding 0.5 mL of 1 M sodium hydroxide and diluting with 0.275 mL distilled water. A visible spectrophotometer was used to measure the absorbance of the reaction mixture at 510 nm straight away. For the standard curves, quercetin was utilized as the standard. The quercetin equivalent in mg/gm yield weight was used to calculate the flavonoid concentration.

## 2.8. Extraction and Estimation of total Phenol contents

Total phenol content was determined using a Folin-Ciocalteu reagent-based assay with slight modifications [16] 1 ml of each extract, 5 ml of Folin-Ciocalteu reagent (diluted tenfold) and 4 ml (75 g/l) Na<sub>2</sub>CO<sub>3</sub> were added. A Visible Spectrophotometer was used to measure the absorbance of the created colour at 765 nm after it was kept at 20°C for 30 minutes. 1 ml aliquots of various concentration of gallic acid solutions were used as standards for the calibration curve. The tests were all replicated 3 times. The gallic acid equivalent in mg/gm yield weight was used to calculate the phenol concentration.

## 2.9. Extraction and estimation of total tannin content

The tannin content of different extract of *P.elliottii* was determined using a slightly modified Folin and Ciocalteu procedure. Each extract contains 0.5 mL plant material, 3.75 mL distilled water, 0.25 mL Folin Phenol reagent, and 0.5 mL 35 percent sodium carbonate solution. The absorbance was measured at 725 nm. Tannic acid dilutions of varied concentrations were employed as calibration curve standards. The concentration of tannic acid was measured in mg/gm of yield weight.

## 2.10. DPPH (2,2-diphenyl-1-picrylhydrazyl) Scavenging Activity

The DPPH radical scavenging activity of crude extracts of *P.elliotti* plant in various solvents (petroleum-ether, chloroform, ethyl acetate, water, and methanol) was tested. [17] methodology was used to measure DPPH radical scavenging activity. 0.05g of dry extract was dissolved in 50mL of methanol to make extract solutions. An aliquot of 2mL of 0.004 percent DPPH solution in methanol and 1mL of plant extract in methanol at various concentrations (50, 100, 200, 500, and 1000 g/ml) were mixed and incubated at 25°C for 30 minutes, with absorbance measured at 517nm using a spectrophotometer against a DPPH control containing only 1 ml of methanol in place of the extract. Before the absorbance measurements, a DPPH solution in methanol was produced on a regular basis. The stable free radical DPPH is purple in colour. When diphenyl picryl hydrazine is reduced, it produces yellow Diphenyl picryl hydrazine. All of the trials were repeated three times, with the findings being averaged. Ascorbic acid was employed as a reference point. The percentage inhibition was estimated using the formula below.  $(A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}) \times 100 = \text{percent inhibition}$  Where  $A_{\text{control}}$  and  $A_{\text{sample}}$  denote blank sample absorption and tested extract solution absorption, respectively.

## 2.11. Hydroxyl scavenging activity

The amount of hydroxyl radical scavenging activity of different extracts of *P.elliottii* was determined using the method, [18] which involved analyzing the reaction between Deoxyribose and various plant extracts for hydroxyl radical production using the Fe<sub>3</sub><sup>+</sup>/Ascorbate/EDTA/H<sub>2</sub>O<sub>2</sub> system. The 2-deoxyribose degradation product is quantified by condensation with TBA.

$$\% \text{ inhibition of NO} = \text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}} / \text{Abs}_{\text{sample}} \times 100$$

Where, Abs<sub>control</sub> was the absorbance of the control and Abs<sub>sample</sub> was the absorbance presence in the samples.

## 2.12. Hydrogen Peroxide Scavenging activity

The hydrogen peroxide scavenging activity of various extracts of *P.elliottii* was tested using the previously established method. [19] At 230nm, hydrogen peroxide's UV light absorption may be easily tested. When plant extracts scavenge hydrogen peroxide, their absorbance reduces at this wavelength, a feature that can be used to quantify their H<sub>2</sub>O<sub>2</sub> scavenging ability. The technique is based on the condensation of 2-deoxy ribose with TBA to quantify the breakdown product. The Fe<sub>3</sub><sup>+</sup> - Ascorbate - EDTA - H<sub>2</sub>O<sub>2</sub> system produced a hydroxyl radical (Fenton reaction). TBARS were used to detect deoxyribose degradation. and the % inhibition was calculated. The amount of hydrogen radical was calculated following the equation % inhibition of NO = Abs<sub>control</sub> - Abs<sub>sample</sub> / Abs<sub>sample</sub> × 100.

## 2.13. Superoxide Scavenging Activity

The Nitro Blue Tetrazolium dye (NBT) reduction method was used to remove the superoxide radical (O<sub>2</sub><sup>-</sup>) created by the photo reduction of riboflavin. The scavenging activity of various extracts of *P. elliotti* was measured using the [19] method, which has previously been used. In a spectrophotometer, the absorbance was measured at 560 nm against the control samples. The reference substance was ascorbic acid. All of the tests were done three times and the results were analyzed in means± SE. By comparing the results of the control and test samples, the % inhibition was calculated.

$$\% \text{ inhibition of NO} = \text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}} / \text{Abs}_{\text{sample}} \times 100$$

## 2.14. Statistical analysis

To demonstrate variances in the various experimental data, the results were expressed as means± SE. When (*P* > 0.05), differences are considered significant. where <sup>a</sup>><sup>b</sup>><sup>c</sup>><sup>d</sup> in each column.

## 3. Result and Discussion

The yield percentage (Table -1) provides whole plant extracts of *P. elliotti* using various solvents. Methanol solvent yielded the highest percentage of extract (17.14%), followed by ethyl acetate solvent (13.21%), and chloroform (10.66%) and petroleum ether yielded the lowest percentage (9.08 %). The yielding of poorest percentage was obtained

from aqueous extract (6.12 %). These findings demonstrated that high polar solvents might dissolve more chemical components in *P. elliotii* plant extracts.

**Table 1: Extract recovery percentage of *Psilotrichum elliotii***

S. No.	Solvents	Yielding Percentage
		<i>Psilotrichum elliotii</i>
1	Petroleum ether	9.08%
2	Chloroform	10.66%
3	Ethyl acetate	13.21%
4	Methanol	17.14%
5	Aqueous	6.12%

Natural antioxidants presence in herbs are essential for reducing or preventing the harmful effects of oxidative stress, the sources are significantly safer to consume because they are less toxic and have fewer negative impacts. Ascorbic acid, vitamin E, carotenes, and phenolic acids are all found in plants that has been shown to reduce disease rates. Anti-inflammatory, antioxidant, anti-diabetic, antibacterial, and anti-cancer effects have been discovered in alkaloids, flavonoids, and phenolic compound [20].

**Table 2: Total carbohydrate, protein, flavonoid, phenolic & tannin content *P. elliotii* Present in various extracts.**

Extracts	Carbohydrate (mg GLC/g yield of extract)	Protein (mg BSI/g yield of extract)	Flavonoid (mg QE/g yield of extract)	Phenol (mg GAE/g yield of extract)	Tannin (mg TE/g yield of extract)
Petroleum ether	13.15±1.2 <sup>c</sup>	2.42 ± 0.3 <sup>d</sup>	5.16 ± 0.9 <sup>b</sup>	9.94± 2.1 <sup>c</sup>	4.42± 1.7 <sup>a</sup>
Chloroform	18.65 ±0.5 <sup>b</sup>	6.71± 0.2 <sup>c</sup>	9 ± 2.1 <sup>a</sup>	13.10±0.3 <sup>d</sup>	8.42 ± 1.4 <sup>c</sup>
Ethyl acetate	20.4 ± 0.2 <sup>a</sup>	10.28± 0.8 <sup>d</sup>	18.16±0.6 <sup>c</sup>	21.52±0.1 <sup>a</sup>	14.42 ± 0.9 <sup>b</sup>
Methanol	23.15 ± 0.2 <sup>c</sup>	13.14 ± 0.4 <sup>a</sup>	27.33±2.4 <sup>d</sup>	28.89 ±0.6 <sup>b</sup>	19.85± 0.1 <sup>c</sup>
Aqueous	4.65 ±1.3 <sup>a</sup>	1.14 ±2.3 <sup>b</sup>	10.61 ±1.0 <sup>a</sup>	6.26 ±0.4 <sup>c</sup>	2.71± 0.6 <sup>d</sup>

GLC- Glucose, BSA- Bovine serum albumin, QE- Quercetin Equivalents, GAE- Gallic Acid Equivalents, TE- Tannic acid Equivalents.

Values are mean of triplicate determination (n=3)  $\pm$  SD. Statistically significant at  $p < 0.05$  where  $a > b > c > d$  in each column.

Total Carbohydrate, Protein, Flavonoid, Phenols, and Tannin content have been shown in (Table -2). Methanolic extract of *Psilotrichum elliotii* species plant indicated higher quantities of Phenol ( $28.9 \pm 3.6 \text{ mg/g}$ ) and Flavonoid ( $27.33 \pm 3.4 \text{ mg/g}$ ) and lesser content of protein was observed in petroleum ether extract ( $2.42 \pm 1.03 \text{ mg/g}$ ) and Aqueous extract showed less amount of Tannin content ( $2.71 \pm 4.13 \text{ mg/g}$ ) [21] reported that Methanol, Chloroform and Aqueous extracts have the highest total phenolic content, whereas lower concentrations of Tannin levels were observed in *Amaranthus tricolor* plant. Natural antioxidants found in herbs and spices are responsible for reducing or avoiding oxidative stress's negative effects. Natural sources are far less hazardous and have less adverse effects, making them far healthier to use. Polyphenols, flavonoids, and phenolic chemicals, found herbs, are free radical scavengers [22].

### 3.1. DPPH radical scavenging activity

The ability of medicinal plants to scavenge DPPH radicals is often cited as a measure of their antioxidant activity. The efficiency of radical scavenging at varied concentrations (50-1000 g/ml) of *P. elliotii* entire plant extract are analyzed for *in vitro* antioxidant studies, with BHT as the standard. The effect of radical scavenging was observed to grow with increasing concentrations in general. As a result, the DPPH radical scavenging capability of extract gradually increased in a dose-dependent manner. The ethyl acetate (16.3, 29.2, 41.4, 65.8, 75.1  $\mu\text{g/ml}$ ) and methanol (24.5, 47.3, 58.4, 73.1, 96.6  $\mu\text{g/ml}$ ) plant extracts indicated the greatest radical scavenging activity show that (Table -3) compared with Standard synthetic antioxidant BHT. Antioxidant activity and determination of total phenolic and flavonoid contents of *Amaranthus spinosus* root extract DPPH radical scavenging activity assay analyses the ability of the extract to donating hydrogen or to scavenge free radicals, [5] stated that ethyl acetate extract of the plant had the maximum radical scavenging activity, with  $61.47 \pm 4.8\%$ , followed by the petroleum ether extract with  $53.05 \pm 25.2\%$ , and the methanol extract with  $46.57 \pm 1.9\%$ , of the findings extracts. Free radical scavenging activity was shown to rise as plant concentration increased, leading to an improvement in % inhibition. The results are shown in (Figure – 1) as a percentage inhibition of DPPH.

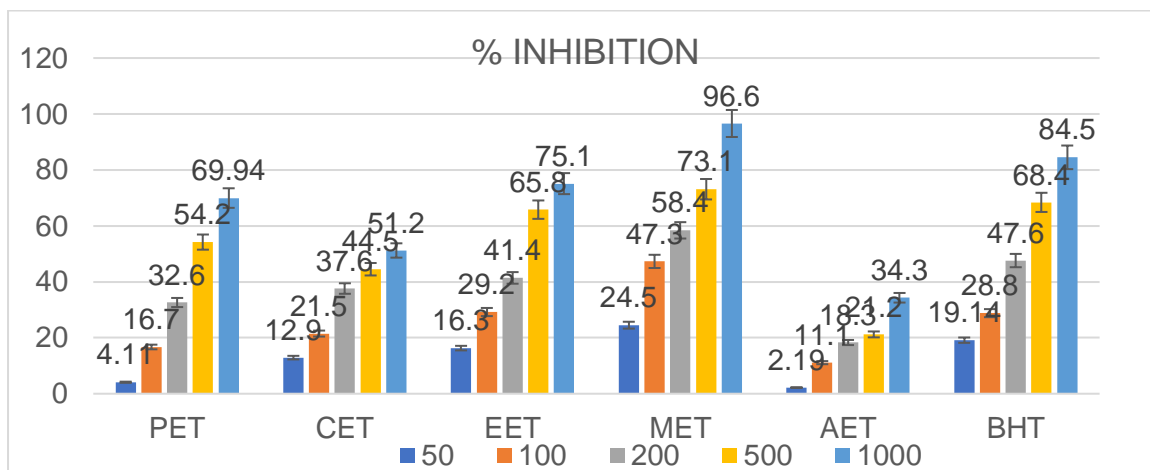
**Table 3: DPPH free radical scavenging activity of various solvent extracts of *P. elliotii*.**

Extracts	Concentration of extracts ( $\mu\text{g/mL}$ )					
	50	100	200	500	1000	IC <sub>50</sub>
PET	$4.11 \pm 0.7^b$	$16.7 \pm 0.2^c$	$32.6 \pm 0.4^a$	$54.2 \pm 0.1^d$	$69.4 \pm 0.5^b$	2.05
CET	$12.9 \pm 0.4^a$	$21.5 \pm 0.7^d$	$37.6 \pm 0.5^c$	$44.5 \pm 0.3^b$	$51.2 \pm 0.8^d$	4.65
EET	$16.3 \pm 0.3^c$	$29.2 \pm 0.5^a$	$41.4 \pm 0.2^d$	$65.8 \pm 0.6^b$	$75.1 \pm 0.1^d$	3.19
MET	$24.5 \pm 0.2^d$	$47.3 \pm 0.1^b$	$58.4 \pm 0.7^a$	$73.1 \pm 0.5^c$	$96.6 \pm 0.3^b$	2.41

<b>AET</b>	2.19±0.4 <sup>d</sup>	11.1± 0.2 <sup>b</sup>	18.3 ±0.07 <sup>c</sup>	21.2± 0.9 <sup>a</sup>	34.3± 0.6 <sup>c</sup>	6.07
<b>BHT</b>	19.14± 0.2 <sup>a</sup>	28.8± 1.3 <sup>c</sup>	47.6± 0.1 <sup>b</sup>	68.4 ± 0.5 <sup>d</sup>	84.5± 0.7 <sup>a</sup>	2.85

PET- Petroleum ether extract, CET- Chloroform extract, EET-Ethyl acetate extract, MET- Methanol extract, AET- Aqueous extract, BHT- Butylated hydroxy toluene.

Values are mean of triplicate determination (n=3) ± SD. Statistically significant at  $p < 0.05$  where  $a > b > c > d$  in each column.



**Figure - 1 The antioxidant activity of the *P. elliotii* entire plant extracts as measured by DPPH Assay. Statistically significant at  $p < 0.05$**

### 3.2. Hydroxyl scavenging activity

In a  $FeCl_3$ -EDTA-ascorbic acid and  $H_2O_2$  reaction combination, the extract and standard ascorbic acid both inhibit hydroxyl radical-mediated deoxyribose degradation. Although being lower than the standard antioxidant (ascorbic acid), the results observed for all extracts of the plant indicated that it is a relatively strong source of antioxidant activity. Hydroxyl radical species are one of the fastest initiators of lipid oxidation, extracting hydrogen atoms from unsaturated fatty acids. As a result, one of a live body's most efficient defenses against numerous diseases is the elimination of hydroxyl radicals [23]. The methanol extract has the highest antioxidant inhibition, and (Table-4) demonstrates that the  $IC_{50}$  value of various extracts (3.36, 2.37, 2.3, 2.62, 3.94 and 3.26  $\mu g/ml$ ) respectively. In this study, the Ascorbic acid concentration in 1000  $\mu g/ml$  was found to have a 77.3 % inhibition hydroxyl radical scavenging effect, while the same concentration of methanolic extract *P. elliotii* had a 92.39 % inhibition result, indicating that plant extract can be considered a good hydroxyl radical scavenger. The value was the plant's scavenging capability by the constant degradation of  $H_2O_2$  to the efficacy of all solvent extracts of the entire plant *P. elliotii* compared to with ascorbic acid. Increased concentration of extract have greater ability of hydroxyl scavenging activity. This indicated extract much higher than that of the synthetic antioxidant ascorbic acid.

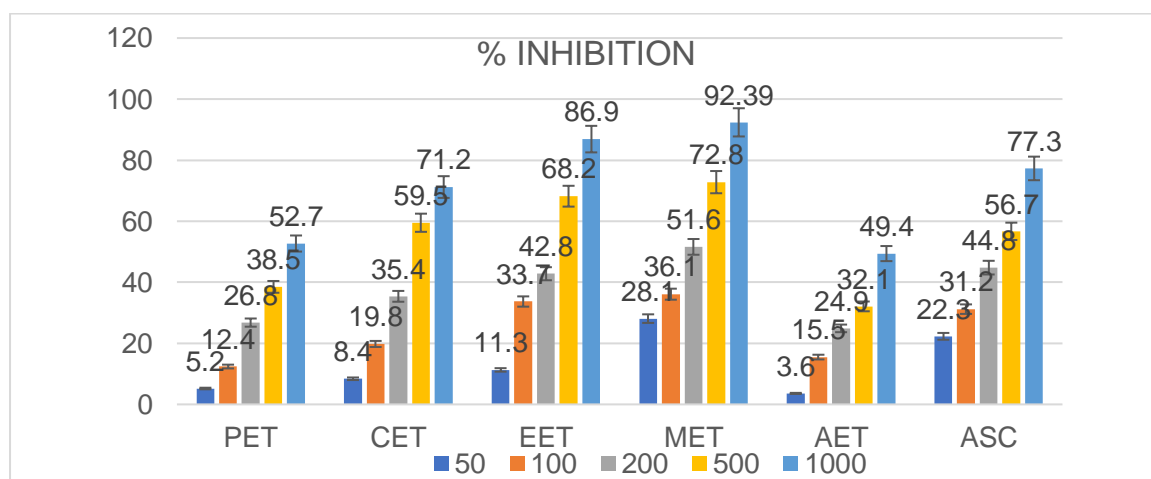


**Table 4: Hydroxyl scavenging activity of various solvent extracts of *P. elliotii*.**

Extracts	Concentration of extracts (µg/mL)					
	50	100	200	500	1000	IC <sub>50</sub>
PET	5.2±2.6 <sup>a</sup>	12.4±2.4 <sup>d</sup>	26.8 ±0.2 <sup>b</sup>	38.5±0.3 <sup>a</sup>	52.7±0.2 <sup>c</sup>	3.36
CET	8.4± 1.54 <sup>b</sup>	19.8± 1.3 <sup>a</sup>	35.4± 0.8 <sup>d</sup>	59.5±0.6 <sup>c</sup>	71.2±0.4 <sup>a</sup>	2.37
EET	11.3± 0.2 <sup>a</sup>	33.7± 2.3 <sup>b</sup>	42.8±1.3 <sup>d</sup>	68.2±0.2 <sup>a</sup>	86.9± 2.1 <sup>c</sup>	2.3
MET	28.1± 0.3 <sup>c</sup>	36.1± 1.4 <sup>a</sup>	51.6± 2.7 <sup>b</sup>	72.8±1.6 <sup>a</sup>	92.39±0.5 <sup>d</sup>	2.62
AET	3.6±1.3 <sup>d</sup>	15.5±0.4 <sup>c</sup>	24.9 ± 1.4 <sup>b</sup>	32.1±0.3 <sup>a</sup>	49.4± 1.6 <sup>d</sup>	3.94
ASC	22.3±2.7 <sup>d</sup>	31.2±0.7 <sup>c</sup>	44.8±1.8 <sup>a</sup>	56.7± 1.4 <sup>c</sup>	77.3±0.2 <sup>b</sup>	3.26

PET- Petroleum ether extract, CET- Chloroform extract, EET-Ethyl acetate extract, MET- Methanol extract, AET- Aqueous extract, ASC- Ascorbic acid.

Values are mean of triplicate determination (n=3) ± SD. Statistically significant at  $p < 0.05$  where  $a > b > c > d$  in each column.



**Figure - 2 The antioxidant activity of the *P. elliotii* entire plant extracts as measured by Hydroxyl scavenging activity. Statistically significant at  $p < 0.05$  where  $a > b > c > d$  in each column.**

### 3.3. Hydrogen peroxide scavenging activity

Different extract of *P. elliotii* analyzed for hydrogen peroxide scavenging activity. Methanol extract showed significant scavenging ability IC<sub>50</sub> (1.6 µg/ml) than the standard BHT (1.52 µg/ml) other extracts also have appreciable hydrogen peroxide scavenging activity (Table - 5). The capacity of extracts to scavenge H<sub>2</sub>O<sub>2</sub> is related to phenolics, which can transfer electrons to H<sub>2</sub>O<sub>2</sub>, neutralized it and converting it to water. The effectiveness of the extracts to scavenge Hydrogen peroxide was concentration dependent. The electron-donating abilities of the crude methanolic extracts are higher from BHT. The hydrogen peroxide scavenging activity of the ethanolic seed extract of *Morinda citrifolia*

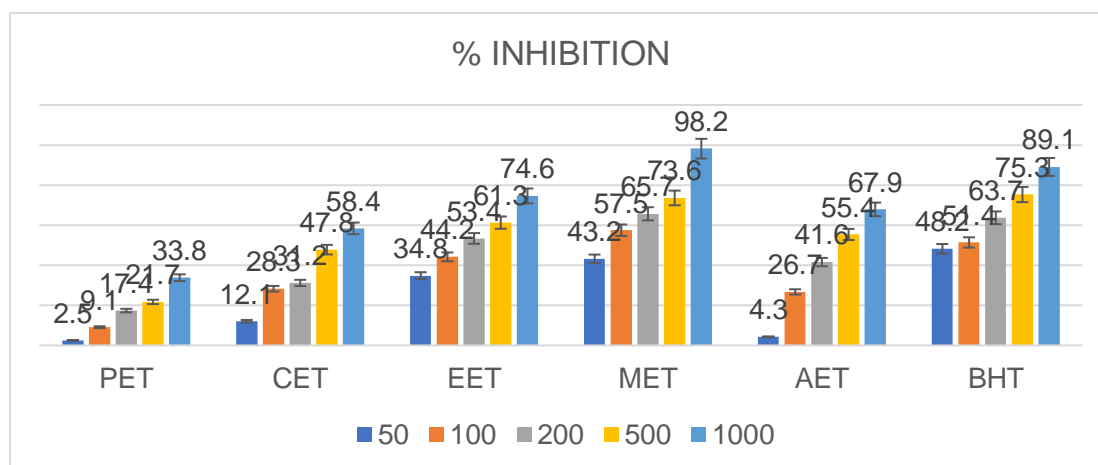
and the standard reagent Butylated hydroxyl toluene was found to be 82 % and 92 %, respectively [24].

**Table 5: Hydrogen peroxide scavenging activity of various solvent extracts of *P. elliotii*.**

Extracts	Concentration of extracts (µg/mL)					
	50	100	200	500	1000	IC <sub>50</sub>
PET	2.5±0.2 <sup>d</sup>	9.1±0.4 <sup>a</sup>	17.4± 2.1 <sup>b</sup>	21.7±0.5 <sup>c</sup>	33.8±0.75 <sup>b</sup>	5.89
CET	12.1 ± 0.8 <sup>b</sup>	28.3± 2.4 <sup>d</sup>	31.2± 0.4 <sup>c</sup>	47.8±1.4 <sup>d</sup>	58.4±0.1 <sup>a</sup>	4.28
EET	34.8± 0.3 <sup>a</sup>	44.2± 0.6 <sup>d</sup>	53.4±0.9 <sup>a</sup>	61.3 ±0.2 <sup>c</sup>	74.6 ±0.3 <sup>b</sup>	2.62
MET	43.2 ±2.0 <sup>c</sup>	57.5 ±0.3 <sup>b</sup>	65.7±0.8 <sup>a</sup>	73.6±1.6 <sup>d</sup>	98.2± 0.07 <sup>a</sup>	1.6
AET	14.3 ±0.1 <sup>c</sup>	26.7 ±1.8 <sup>a</sup>	41.6 ±0.7 <sup>d</sup>	55.4±0.1 <sup>c</sup>	67.9±0.2 <sup>b</sup>	2.72
BHT	48.2±0.7 <sup>a</sup>	51.4±0.1 <sup>c</sup>	63.7±0.10 <sup>d</sup>	75.3±0.2 <sup>a</sup>	89.1±2.1 <sup>b</sup>	1.52

PET- Petroleum ether extract, CET- Chloroform extract, EET-Ethyl acetate extract, MET- Methanol extract, AET- Aqueous extract, BHT- Butylated hydroxy toluene.

Values are mean of triplicate determination (n=3) ± SD. Statistically significant at *p*< 0.05 where <sup>a>b>c>d</sup> in each column.



**Figure - 3 The antioxidant activity of the *P. elliotii* entire plant extracts as measured by Hydrogen peroxide scavenging activity. Statistically significant at *p*< 0.05**

### 3.4. Super oxide radical scavenging activity

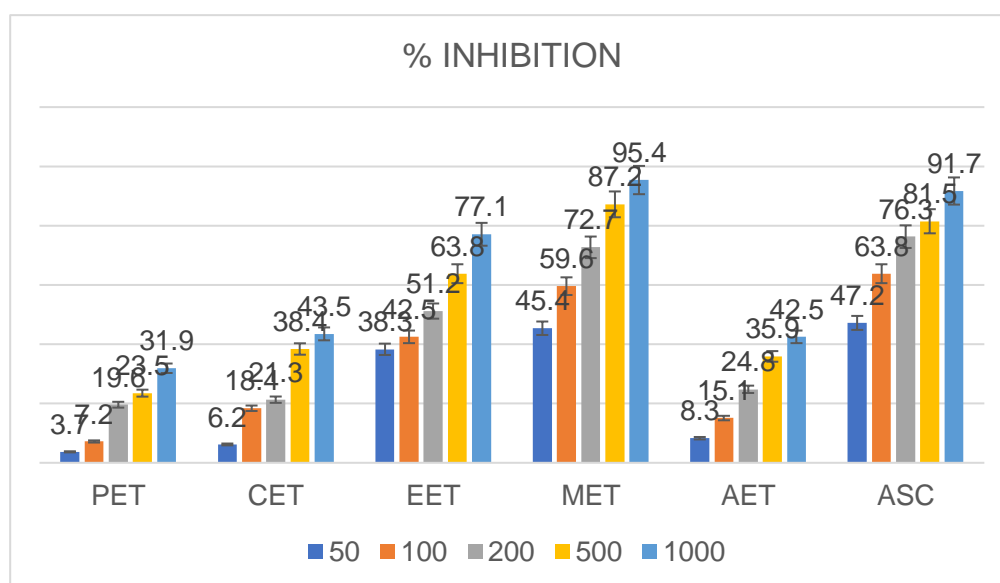
The ability to decrease Nitro Blue Tetrazolium (NBT) utilizing PMS-NADH coupling can be used to assess superoxide radicals produced by dissolved oxygen. The ability of the *P. elliotii* and the reference chemical copper sulphate to quench superoxide radicals in the reaction mixture is indicated by their decrease in absorbance at 560 nm. Superoxide scavenging activity of ethyl acetate extract (38.3, 42.5, 51.2, 63.8, 77.1  $\mu\text{g/ml}$ ) and methanol extract (45.4, 59.6, 72.7, 87.2, 95.4  $\mu\text{g/ml}$ ) showed appreciable free radical scavenging activity compared to other extracts of *P. elliotii*. The  $\text{IC}_{50}$  value of methanolic extract (1.27  $\mu\text{g/ml}$ ) was found to be higher than that of standard ascorbic acid (0.92  $\mu\text{g/ml}$ ). [25] reported that superoxide anion scavenging activity of *Aerva lanata* to that of standard quercetin, it was discovered that has equivalent free radical scavenging activity against super oxide anions. Several destructive intermediates generated during the inflammatory process, superoxide anions, have been proven to increase the risk of inflammation-related illnesses such as arthritis and atherosclerosis [26].

**Table 6: Super oxide radical scavenging activity of various solvent extracts of *P. elliotii***

Extracts	Concentration of extracts ( $\mu\text{g/mL}$ )					
	50	100	200	500	1000	$\text{IC}_{50}$
PET	3.7 $\pm$ 0.8 <sup>c</sup>	7.2 $\pm$ 1.7 <sup>a</sup>	19.6 $\pm$ 2.1 <sup>c</sup>	23.5 $\pm$ 0.4 <sup>b</sup>	31.9 $\pm$ 1.9 <sup>d</sup>	6.24
CET	6.2 $\pm$ 0.6 <sup>a</sup>	18.4 $\pm$ 2.4 <sup>b</sup>	21.3 $\pm$ 0.5 <sup>d</sup>	38.4 $\pm$ 0.2 <sup>c</sup>	43.5 $\pm$ 0.1 <sup>a</sup>	4.98
EET	38. 3 $\pm$ 0.1 <sup>b</sup>	42.5 $\pm$ 0.7 <sup>a</sup>	51.2 $\pm$ 0.9 <sup>c</sup>	63.8 $\pm$ 1.4 <sup>d</sup>	77.1 $\pm$ 1.2 <sup>c</sup>	2.53
MET	45.4 $\pm$ 0.2 <sup>a</sup>	59.6 $\pm$ 0.4 <sup>c</sup>	72.7 $\pm$ 1.7 <sup>b</sup>	87.2 $\pm$ 0.2 <sup>a</sup>	95.4 $\pm$ 0.5 <sup>d</sup>	1.27
AET	8.3 $\pm$ 0.6 <sup>c</sup>	15.1 $\pm$ 2.1 <sup>d</sup>	24.8 $\pm$ 0.2 <sup>b</sup>	35.9 $\pm$ 1.9 <sup>a</sup>	42.5 $\pm$ 0.8 <sup>d</sup>	5.44
ASC	47.2 $\pm$ 0.2 <sup>c</sup>	63.8 $\pm$ 1.3 <sup>a</sup>	76.3 $\pm$ 0.8 <sup>b</sup>	81.5 $\pm$ 0.3 <sup>c</sup>	91.7 $\pm$ 1.4 <sup>d</sup>	0.92

PET- Petroleum ether extract, CET- Chloroform extract, EET-Ethyl acetate extract, MET- Methanol extract, AET- Aqueous extract, ASC- Ascorbic acid.

Values are mean of triplicate determination (n=3)  $\pm$  SD. Statistically significant at  $p < 0.05$  where  $a > b > c > d$  in each column.



**Figure- 4 The antioxidant activity of the *P. ellioti* entire plant extracts as measured by Super oxide scavenging Activity. Statistically significant at  $p < 0.05$**

#### 4. Conclusion

The findings of the study proved that the variety of phytochemical substances that can effectively defend from oxidative damage through free radical scavenging activities. Various extracts of entire plant of *P. ellioti* exhibited more amount of Carbohydrate, Protein, Phenol, Flavonoid and Tannin content. Among the solvents studied methanol showed the highest amount of phenols and flavonoids, and the Pharmacological data confirms the methanolic extract's harmless effect on human RBCs (Red blood cells). Furthermore, the methanol extract of the entire plant of *P.ellioti* registered a strong antioxidant activity by DPPH, Hydroxyl Scavenging activity, Hydrogen peroxide Scavenging activity and Super oxide Scavenging activity. Hence, the entire plant extracts of *P. ellioti* was found to contain a wide variety of free radical scavengers that may be beneficial for human health.

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#### 6. References

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