

## IN VITRO ANTIOXIDANT ACTIVITY, MINERAL CONTENT AND HPLC ANALYSIS OF *TALINUM PORTULACIFOLIUM* (FORSSK.) ASCH. EX SCHWEINF. LEAF AND STEM

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### ABSTRACT

The present study was carried out to determine the phenolic content, antioxidant activity, mineral content and HPLC analysis of methanol extract of *Talinum portulacifolium* leaf and stem extracts. Antioxidant activities were performed in terms of DPPH• (1, 1-diphenyl-2-picrylhydrazyl) stable free radical scavenging, hydroxyl radical (•OH) scavenging, nitric oxide (NO) scavenging, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) scavenging activities, reducing power, chelating capacity (ICC) and ferric reducing antioxidant power (FRAP). The methanol extract of *T. portulacifolium* leaf and stem showed stronger activity patterns towards all the tested assays. The leaf extract possessed higher antioxidant activity and mineral content than the stem extract. The HPLC analysis enabled the qualitative and quantitative identification of the flavonoid quercetin. The results of the study clearly indicate that the ethanol extract of *T. portulacifolium* can be used as a potential source of antioxidant agents.

**Keywords:** *T. portulacifolium*, Antioxidant activity, Reducing capacity, FRAP, HPLC.

### INTRODUCTION

Free radicals in the form of reactive oxygen species (ROS) and reactive nitrogen species are implicated in numerous diseases such as inflammation, metabolic disorders, repulsion damage, atherosclerosis and carcinogenesis<sup>1, 2</sup>. The consumption of plant foods, such as antioxidant supplements or antioxidant-containing foods may be used to protect against various diseases, including cancer, cardio and cerebrovascular diseases. They also help the human body to reduce oxidative damage or protect oxidative deterioration<sup>3</sup>. Natural antioxidants can be phenolic compounds ( $\alpha$ -tocopherols, flavonoids, and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, and amines), or carotenoids as well as ascorbic acid, whereas synthetic antioxidants are compounds with phenolic structures of various degrees of alkyl substitution<sup>4</sup>. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used as antioxidants since the beginning of this century. Restrictions on the use of these compounds, however, are being imposed because of their carcinogenicity and cause an increased interest towards natural antioxidant substances<sup>5, 6</sup>. Antioxidant compounds add to food products, especially to lipids and lipid-containing foods and can increase shelf life by retarding the process of lipid peroxidation, which is one of the major reasons for deterioration of food products during processing. An alternative natural and safe source of food antioxidant is found<sup>7</sup>. Thus, the search for natural antioxidants, especially extracted from plant origin, has notably increased in recent years<sup>8</sup>.

Fruits and vegetables contain different antioxidant compounds, such as vitamin C, vitamin E and carotenoids, whose activities have been established in recent years. Flavonoids, tannins and other phenolic constituents present in food of plant origin are also potential antioxidants<sup>9, 10</sup>.

The genus *Talinum* consists of approximately 500 species across the world. It is said to be used as an aphrodisiac<sup>11</sup>. The leaf powder of this plant mixed with boiled milk is used to treat diabetes<sup>12, 13</sup>. Ethanol extract of *Talinum triangulare* exhibited free radical and lipid peroxidation inhibitory or scavenging activity<sup>14</sup>. It was reported that the methanol extract of *Talinum portulacifolium* leaves contain alpha-glucosidase inhibitory activity<sup>15</sup>. Hexane, ethanol and aqueous extracts of *Talinum portulacifolium* leaf possess antihyperglycemic and antioxidant effects<sup>16</sup>. But there are no systematic scientific studies on the comparative mineral content and *in vitro* antioxidant activities of methanol extract of leaf and stem of this plant. The present study was undertaken to scientifically

investigate the polyphenolic composition, mineral content and antioxidant activity of leaf and stem extracts of *T. portulacifolium*.

### MATERIALS AND METHODS

#### Chemicals

Folin-Ciocalteu reagent, potassium ferricyanide, butylated hydroxytoluene (BHT), L-ascorbic acid, quercetin, 1, 1-diphenyl-2-picrylhydrazyl (DPPH•), 2, 4, 6-tripyridyl-s-triazine (TPTZ), N-1-naphthyl ethylene diamine dihydrochloride, ethylenediaminetetraacetic acid disodium salt (EDTA-Na<sub>2</sub>) and trichloroacetic acid (TCA) were purchased from Merck Co., India. Gallic acid was purchased from Riedel-de-Hahn, Germany. All other chemicals and solvents used were of analytical grade and were made in India.

#### Collection of plant material

Plant parts of *T. portulacifolium* were collected from Trichur district of Kerala and was taxonomically identified by Dr. R. Gopalan, Taxonomist, Karpagam University and was authenticated in Botanical Survey of India (southern circle), Coimbatore, India. The leaves and stem were separated and shade dried and powdered. The powder was used for the preparation of the extract.

#### Preparation of methanol extract

Methanol (80%) extract was prepared by solvent extraction in a soxhlet extractor with 5 times its volume of methanol (1:5 v/v). The extract was concentrated *in vacuo* at 40 ± 1<sup>o</sup> C by rotary flash evaporator (Buchi type rotavapor, Switzerland) under reduced pressure to obtain the dry extract. The dry extract was re-dissolved in methanol and the stock solution was kept at -4<sup>o</sup> C to protect from light until further use. The stock solution was used to determine total phenol, total flavonoid, total flavonol and antioxidant capacity.

#### Total Phenolic Content (TP)

The total phenolic content (TP) was determined colorimetrically using Folin-Ciocalteu phenol reagent method<sup>17</sup>. Briefly, diluted extract (1 mL) was added with diluted Folin-Ciocalteu reagent (1 N, 1 mL). After 3 min of reaction, sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) (35%, 2 mL) was added and the mixture was incubated for 30 min at room temperature. The absorbance was read at 765 nm using UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan). The analyses were performed in triplicate. The TPC was expressed as mg gallic acid equivalents from a gallic acid standard curve (mg GAE/100 g fresh mass, R<sup>2</sup> = 0.9968).

### Total Flavonoid Content (TF)

The determination of total flavonoid content (TF) in the *T. portulacifolium* extract was based on the method reported previously<sup>18</sup>. The absorbance of TF was measured at 510 nm using UV-Vis spectrophotometer with reference standard prepared with quercetin concentrations. The analyses were performed in triplicate. The TF was estimated from a quercetin standard curve and the results were expressed as mg quercetin equivalents (mg QE/100 g fresh material,  $R^2 = 0.9665$ ).

### Total flavonol Content (TFI)

Total flavonol in the plant extracts were estimated using the method reported previously<sup>19</sup>. To 2.0 mL of sample (standard), 2.0 mL of 2%  $AlCl_3$  ethanol and 3.0 mL (50 g/L) sodium acetate solutions were added. The absorption at 440 nm was read after 2.5 h at 20°C. Extract samples were evaluated at a final concentration of 0.1 mg/ml. Total flavonoid content was calculated as quercetin (mg/g) using the following equation based on the calibration curve:  $y = 0.0255x$ ,  $R^2 = 0.9812$ , where x was the absorbance and was the quercetin equivalent (mg/g).

### Antioxidant Activity

#### Reducing Capacity (RC)

The reducing capacity of the *T. portulacifolium* extract was measured using the potassium ferricyanide reduction method<sup>20</sup>. Various concentrations of the extract and standards (25, 50, 100, 250, 500  $\mu g mL^{-1}$ ) were added to 2.5 ml of (0.2 M) sodium phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide [ $K_3Fe_3(CN)_6$ ] (1%) solution and vortexed. After incubation at 50°C for 20 min, 2.5 ml of TCA (10%, w/v) was added to all the tubes and centrifuged (Remi, India) at 3000 x g for 10 min. Afterwards, upper layer of the solution (5 mL) was mixed with deionized water (5 mL). To this, one milliliter of  $FeCl_3$  (1 %) was added to each test tube and incubated at 35°C for 10 min. The formation of Perl's Prussian color was measured at 700 nm in a UV-Vis spectrophotometer. Increased absorbance of the reaction mixture indicated increasing reducing power. Here, the  $EC_{50}$  value is the effective concentration at which absorbance was 0.5 % for the reducing capacity. BHT and BHA were used for comparison.

#### Scavenging Capacity towards DPPH•

The determination of DPPH• stable radical scavenging activity of the *T. portulacifolium* extract was based on the method as described previously<sup>21</sup>. Briefly, one milliliter of aliquots of the extract and standards (20, 40, 60, 80, 100  $\mu g mL^{-1}$ ) was added to MeOH solution of DPPH• (5 mL, 0.1 mM) and vortexed. After 20 min reaction at 25°C, the absorbance was measured at 517 nm against a blank in a UV-Vis spectrophotometer. BHT was used for comparison. The percentage quenching of DPPH• was calculated as follows: Inhibition of DPPH• (%) =  $1 - \text{Sample}_{517nm} / \text{Control}_{517nm} \times 100$ , where,  $\text{Sample}_{517nm}$  was absorbance of the sample and  $\text{Control}_{517nm}$  was absorbance of control. The results were expressed as  $EC_{50}$ , which means the concentration at which DPPH• radicals were quenched by 50%.

#### Scavenging Capacity towards Hydroxyl ion ( $\cdot OH$ )

Hydroxyl radicals ( $\cdot OH$ ) were generated by a Fenton reaction model system, and the scavenging capacity towards the  $\cdot OH$  radical was measured using deoxyribose method with minor modifications<sup>22</sup>. To 1 mL of the extract (concentrations- 50, 100, 150, 200, 250  $\mu g mL^{-1}$ ), 1 mL of phosphate buffer (50 mM; pH 7), 0.2 mL of EDTA (1.04 mM), 0.2 mL of  $FeCl_3 \cdot 6H_2O$  (1.0 mM) and 0.2 mL of 2-deoxy-D-ribose (60 mM) were added. Following incubation in a water bath at 37°C for 60 min, 2 mL of cold TBA (in 50 mM NaOH) and 2 mL of TCA (25% w/v aqueous solution) were added to the reaction mixture. The mixture was then incubated at 100°C for 15 min. After cooling, the absorbance of the pink chromogen developed was recorded at 532 nm in a spectrophotometer. BHT and catechin were used for comparison. The percentage scavenging of  $\cdot OH$  was calculated as follows: Inhibition of  $\cdot OH$  (%) =  $1 - \text{Sample}_{532nm} / \text{Control}_{532nm} \times 100$ , where,  $\text{Sample}_{532nm}$  was absorbance of the sample and  $\text{Control}_{532nm}$  was absorbance of control.

### Scavenging Capacity towards Nitric Oxide (NO)

Nitric oxide (NO) generated from sodium nitroprusside (SNP) in aqueous solution at physiological pH was estimated by the use of Griess reaction with minor changes<sup>23</sup>. The reaction mixture (3 mL) containing SNP (10 mM, 2 mL), phosphate buffer saline (0.5 mL) and the methanol extract of *T. portulacifolium* at different concentrations and standards (50, 100, 150, 200, 250  $\mu g mL^{-1}$ ) were incubated at 25°C for 150 min. After incubation, 0.5 mL of the incubated solution containing nitrite was pipetted and mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 mL of N-1-naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min at 25°C. The absorbance of pink coloured chromophore formed during diazotization was immediately measured at 540 nm in a UV-Vis spectrophotometer. BHT and catechin were used for comparison. The percentage scavenging of NO was calculated as follows: Inhibition of NO (%) =  $1 - \text{Sample}_{540nm} / \text{Control}_{540nm} \times 100$ , where,  $\text{Sample}_{540nm}$  was absorbance of the sample and  $\text{Control}_{540nm}$  was absorbance of control.

### Scavenging Capacity towards Hydrogen Peroxide ( $H_2O_2$ )

The  $H_2O_2$  scavenging activity of extract was determined by the method as described previously<sup>24</sup>. The extracts (100, 200, 300, 400, 500  $\mu g mL^{-1}$ ) were dissolved in 3.4 ml of 0.1 M phosphate buffer pH 7.4) and mixed with 600  $\mu L$  of 43 mM solution of hydrogen peroxide. The absorbance value of the reaction mixture was recorded at 230 nm. Percentage of scavenging activity was calculated with the formula  $\text{Control OD} - \text{Sample OD} / \text{Control OD} \times 100$ .

### Ferric Reducing Antioxidant Power (FRAP)

The reducing capacity of the extract was measured using FRAP assay<sup>25</sup>. FRAP reagent (1.8 ml) was mixed with 0.2 mL of test sample, then incubated at 37°C for 10 min in a water bath. The FRAP reagent contained acetate buffer (300 mmol/L pH 3.6), 2, 4, 6- tripyridyl-s-triazine (TPTZ) solution (10 mmol/L HCl) and  $FeCl_3 \cdot 6H_2O$  solution (20 mmol/L distilled water). After incubation the absorbance were measured immediately at 593 nm. The calibration curve was plotted with OD vs concentration of  $FeSO_4$  in the range of 0.1 mmol and the total antioxidant activity was expressed as  $\mu mol Fe(II)/100g$ .

### Iron Chelating Capacity (ICC)

The Iron chelating capacity (ICC) was investigated using the methods as described previously<sup>26</sup>. Briefly, different concentrations of both the sample extracts and standards (50 - 250  $\mu g mL^{-1}$ ) were mixed  $FeCl_2$  (2 mmol) and ferrozine (5 mmol). The mixture was made into 0.8 mL with deionized water. After 10 min incubation at room temperature, the absorbance of ferrous ion-ferrozine complex was measured at 562 nm in UV-Vis spectrophotometer. EDTA was used as controls for iron chelating assay. The percentage of Iron chelation was calculated as follows:

$$\text{Ferrous ion chelating capacity (\%)} = 1 - \text{Sample}_{562nm} / \text{Control}_{562nm} \times 100,$$

Where,  $\text{Sample}_{562nm}$  was absorbance of the sample and  $\text{Control}_{562nm}$  was absorbance of control.

### Mineral Analysis

The levels of Na and K were determined using flame photometry. The standard solutions of 100 mg/ml of Na and K were prepared from NaCl and KCl salt. Working standard of 0, 2, 4, 6, 8, 10 mg/l were prepared from the standard solution by serial dilution. Each standard was aspirated in to the flame photometer (Jenway FP9) and its emission recorded to prepare a standard curve. The prepared sample solutions for each extract were also aspirated in to the flame photometer and its emission recorded. The Na and K concentrations were calculated from the standard curve. The levels of Ca and Mg were determined by titrimetry. Five drops of 2% KCN followed by 5 drops of hydroxyl ammonium chloride were added to 10 ml of sample solution. Ammonium buffer was then added to raise the pH to 11.3 followed by the addition of 3 drops of Eriochrome Black T indicator. The solution obtained was titrated against 0.01 M EDTA until a blue color was obtained. Phosphorous determination was carried out using vanadomolybdate method<sup>27</sup>.

### HPLC Analysis

Identification of the compounds was achieved by comparing the retention time of the compounds with corresponding standards. The analytical HPLC system (P-40000, Thermo separation products, USA) employed consisted of a quaternary HPLC pump, photodiode array detector (UV 6000 LP) and a recorder. HPLC analysis was performed using a water prevail C18 analytical column (15 cm × 4.6 mm id, 5µm particle size; ALTech, IL, USA) was used. The auto-injection system (spectra system-AS 3000) consisted of a 20 µl sample loop. The mobile phase consisted of 20% acetonitrile in aqueous formic acid (v/v). The Peak responses were detected at 280 nm using UV detector. In both cases, a flow rate of 1.0 mL/min was maintained. Identification and peak assignment of the isolated compound was based on comparison of its retention time with corresponding standard and by spiking of sample with the standard. Quantification of the isolated compounds was done using total peak area and each peak with external standard.

### Statistical Analysis

The experimental data were reported as mean ± standard error of three parallel measurements. Linear regression analysis was performed quoting the correlation coefficient.

## RESULTS AND DISCUSSION

### Total phenol, flavonoid and flavonol contents

In our work, we examined *T. portulacifolium* for its total phenolic content (TP) using folin-ciocalteu reagent method. The total phenolic, flavonoid and flavonol content of methanolic extracts of leaf and stem, expressed as gallic acid equivalents (GAE) and quercetin equivalents (QE), was dependent upon the solvent and the method used in the extraction (Table 1). The results indicated that leaf extract features a substantial content of total phenol as compared with stem extract. The content of total phenol of leaf was found to be 9000±1.040 mg GAE/100 g of FM and that of stem was equal to 2880±0.644 mg GAE/100 g of FM. The content of total

phenolics in *T. portulacifolium* was found higher than *T. triangulare*<sup>28</sup>, but it was found to be lower than *Amaranthus gangeticus* and *A. paniculatus*<sup>29</sup>. Hence, leaf and stem of *T. portulacifolium* can be considered as good source of phenolics. They have been reported to possess antioxidant activity which allows them to scavenge both active oxygen species and electrophiles to inhibit nitrosation and to chelate metal ions, to have the potential for autooxidation and the capability to modulate certain cellular activities<sup>30</sup>. Flavonoids are considered to be the strong scavengers of reactive oxygen species (ROS)<sup>31, 32</sup>. In this study, TF content was measured by aluminium colorimetric method. The TF content of leaf was 8800 ± 0.946 mg QE/100 g of FM and that of stem was 960±0.917 mg QE/100 g of FW. Total flavonol content of leaf extract was found to be 1840±0.720 mg QE/100g of FW and that of stem was 360±0.544 mg QE/100 g of FW. From the results, it was found that leaf extract of *T. portulacifolium* possessed higher content of total phenolic, flavonoid and flavonols.

### Antioxidant Activity

#### Reducing Capacity

The reducing capacity was determined as the reduction of Fe<sup>3+</sup> to Fe<sup>2+</sup> and is increased with increasing concentration of the extracts. The presence of antioxidants in the extracts results in the reduction of the Fe<sup>3+</sup>/ferricyanide complex to its ferrous form which is measured by the intensity of the resultant Prussian blue color complex. The higher absorbance at high concentration indicates the strong reducing capacity. Higher reducing capacity was found in leaf (0.59 ± 0.009) than in stem (0.48 ± 0.0076) whilst that of BHT was 0.688 ± 0.0087 at a concentration of 100 µg/mL (Figure 1). The reducing power of *T. portulacifolium* was found to be higher than some other leafy vegetable like *Amaranthus lividus*<sup>33</sup>. Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the total antioxidant activity of many fruits and vegetables<sup>34</sup>. From the study, it was found that the reducing power of *T. portulacifolium* might be due to their hydrogen donating ability<sup>35</sup>.

Table 1: Total phenol, flavonoid and flavonol content of *T. portulacifolium*

Plant part	Total phenol (mg GAE/100g)	Total flavonoid (mg QE/100g)	Total flavonol (mg QE/100g)
Leaf	9000 ± 1.040	8800 ± 0.946	1840 ± 0.720
Stem	2880 ± 0.644	960 ± 0.917	360 ± 0.544

Each value in the table was obtained by calculating the average of three experiments (n=3) ± standard deviation.

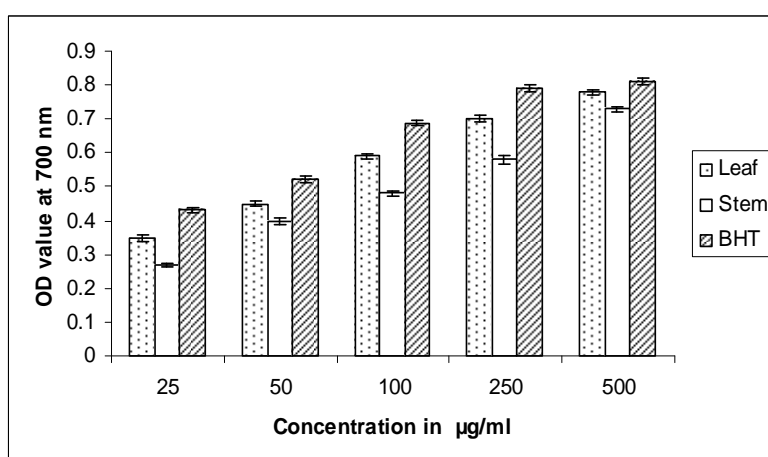


Fig. 1: Reducing capacity of *T. portulacifolium* leaf and stem

### DPPH Scavenging Activity

The proton free-radical scavenging ability is a known mechanism for antioxidation. The DPPH• assay constitutes a quick and low cost method, which has frequently been used for the evaluation of the antioxidative potential of various natural products<sup>36</sup>. When DPPH•

proton free radical, encounters proton radical scavengers, its purple color fades rapidly as a measurement of absorption at 517 nm<sup>37</sup>.

The extracts exhibited impressive DPPH• scavenging with inhibition of 84.75 %, 73 % and 98.46 % at 100 µg mL<sup>-1</sup> for leaf, stem and BHT respectively. Based upon the measured EC<sub>50</sub> values,

the DPPH scavenging activity of leaf extract ( $16 \pm 1.34 \mu\text{g mL}^{-1}$ ) of *T. portulacifolium* was higher when compared to stem extract ( $26 \pm 1.10 \mu\text{g mL}^{-1}$ ) where as that of BHT was  $26.12 \pm 0.04 \mu\text{g mL}^{-1}$  (Table 2). From the results it was found that DPPH scavenging activity of *T. portulacifolium* was higher than some other leafy vegetables like *Chenopodium album*, *Moringa oleifera* and *Centella asiatica*<sup>38</sup> but it was lower than *Mukia maderaspatana* and *Solanaum trilobatum*<sup>39</sup>.

#### OH Scavenging Capacity

Hydroxyl radical ( $\cdot\text{OH}$ ) which is the most reactive free radical, has the capacity to conjugate with nucleotides in DNA, cause strand breakage, and lead to carcinogenesis, mutagenesis and cytotoxicity<sup>40</sup>. The extracts of *T. portulacifolium* were evaluated for their ability to scavenge  $\cdot\text{OH}$  radicals using 2-deoxyribose degradation assay. The extracts were capable of inhibiting  $\cdot\text{OH}$  radical formation in concentration dependent manner. The  $\cdot\text{OH}$  scavenging activity of leaf was 54.36 % and that of stem was 50.33 % at the concentration of  $100 \mu\text{g mL}^{-1}$ . The  $\text{EC}_{50}$  value of methanol extract of leaf ( $80 \pm 0.89 \mu\text{g mL}^{-1}$ ) and stem ( $100 \pm 1.40 \mu\text{g mL}^{-1}$ ) were significantly higher than that of BHT ( $16.44 \pm 0.04$ ) (Table 2). The  $\cdot\text{OH}$  scavenging activity of *T. portulacifolium* was found to be higher than *C. album*, *M. oleifera* and *C. asiatica*<sup>38</sup>.

#### NO Scavenging Capacity

In addition to reactive oxygen species, NO is also implicated in chronic inflammation, cancer and other pathological conditions. The NO generated from SNP at physiological pH reacts with oxygen ( $\text{O}_2$ ) to form nitrite ions. The methanol extract of *T. portulacifolium* competed with  $\text{O}_2$  to react with nitrite ions and thus inhibits the NO generation. The NO scavenging capacity was depended on concentration of the extract. The leaf extract was potent in scavenging NO by 51 % and stem 45 % at the concentration of  $100 \mu\text{g/mL}$ , while BHT showed scavenging activity of 53.6%. As compared with the  $\text{EC}_{50}$  values, the scavenging capacity of leaf extract ( $95 \pm 1.21 \mu\text{g mL}^{-1}$ ) was significantly higher than that of stem extract ( $120 \pm 1.15 \mu\text{g mL}^{-1}$ ). The  $\text{EC}_{50}$  value of BHT was found to be  $46.34 \pm 0.08$  (Table 2).

#### H<sub>2</sub>O<sub>2</sub> Scavenging Capacity

Hydrogen peroxide is a highly important reactive oxygen species due to its ability to penetrate biological membranes. However, it may be toxic if converted to  $\cdot\text{OH}$  in the cell if it reacts with  $\text{Fe}^{2+}$  and possibly  $\text{Cu}^{2+}$  ions<sup>41</sup>. In the study, the extracts were capable of scavenging  $\text{H}_2\text{O}_2$  dose dependently. The leaf extract was potent in

scavenging  $\text{H}_2\text{O}_2$  by 53.45 % and stem 38.78 % at a concentration of  $100 \mu\text{g/mL}$ , while BHT showed scavenging activity of 54.5 %. As compared with the  $\text{EC}_{50}$  values, the scavenging capacity of leaf and stem extracts were  $70 \pm 1.25 \mu\text{g mL}^{-1}$  and  $285 \pm 1.03 \mu\text{g mL}^{-1}$  respectively which has was significantly lesser scavenging capacity than that of BHT ( $65 \pm 0.254 \mu\text{g mL}^{-1}$ ) (Table 2). From the study, it was found that  $\text{H}_2\text{O}_2$  scavenging capacity of the extracts were higher than some other Nigerian green leafy vegetables like *Talinum triangulare*, *Amaranthus hybridus*, *A. caudatus*, *Solanum macrocarpon*, *Celocia argentia* etc.<sup>14</sup>. Earlier it was proven that dietary phenols protect mammalian and bacterial cells from cytotoxicity induced by hydrogen peroxide<sup>42</sup> indicating that the observed  $\text{H}_2\text{O}_2$  scavenging activity of *T. portulacifolium* could be due to the presence of phenols.

#### Iron Chelating Capacity

Iron has the most important lipid pro-oxidant. It is known that  $\text{Fe}^{+2}$  accelerates lipid peroxidation by breaking down hydrogen and lipid peroxides forms by Fenton free radicalic reaction; ( $\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}\cdot$ )<sup>43</sup>.  $\text{Fe}^{+2}$  ion can form complexes with ferrozine. In the presence of chelating agents, the complex formation is prevented, resulting in a decrease in the red color of the complex. In this study, the methanol extracts of *T. portulacifolium* and the reference antioxidant (EDTA) interfered with the formation of ferrous-ferrozine complex, suggesting that they possessed chelating activity and captures ferrous ion before ferrozine. Figure 6 shows dose dependent chelating effect of the methanolic extracts of *T. portulacifolium* leaf and stem. At a concentration of  $100 \mu\text{g/mL}$ , chelating activity of the extracts and reference antioxidant were of the following order: EDTA > leaf extract > stem extract with  $\text{EC}_{50}$  values of  $120 \pm 1.34$ ,  $170 \pm 1.78$  and  $60 \pm 1.40$  respectively (Table 2). From the results it was found that *T. portulacifolium* possessed higher ICC than *A. lividus*<sup>33</sup>.

#### Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay treats the antioxidants contained in the sample as reductant in a redox and links it to a colorimetric reaction. The value reflects the reducing power of the antioxidants. The leaf extract showed comparatively higher FRAP values than the stem extract. At a concentration of  $100 \mu\text{g/mL}$ , the FRAP value of leaf extract was  $80 \mu\text{M Fe}^{2+}/100\text{g}$  fresh material and that of stem was  $75 \mu\text{M Fe}^{2+}/100\text{g}$  material (Table 2). The FRAP values of leaf and stem extracts was found to be lower than some other leafy vegetables like *Mukia maderaspatana*, *Solanum trilobatum*<sup>39</sup> and *Melothria maderaspatana*<sup>44</sup>.

Table 2: Antioxidant capacities of *T. portulacifolium*

Assays	Leaf	Stem	BHT	EDTA
DPPH <sup>a</sup>	$16 \pm 1.34$	$26 \pm 1.10$	$26.12 \pm 0.04$	-
OH <sup>a</sup>	$80 \pm 0.89$	$100 \pm 1.40$	$16.44 \pm 0.04$	-
NO <sup>a</sup>	$95 \pm 1.21$	$120 \pm 1.15$	$46.34 \pm 0.08$	-
H <sub>2</sub> O <sub>2</sub> <sup>a</sup>	$70 \pm 1.25$	$285 \pm 1.03$	$65 \pm 0.254$	$60 \pm 1.40$
ICC <sup>a</sup>	$120 \pm 1.34$	$170 \pm 1.78$	-	-
FRAP <sup>b</sup>	80	75	-	-

<sup>a</sup>Expressed as  $\text{EC}_{50}$  value: the effective concentration at which the antioxidant capacity was 50%.  $\text{EC}_{50}$  was obtained by interpolation from linear regression analysis. Data are mean  $\pm$  standard deviation ( $n = 3$ ). <sup>b</sup>Expressed as  $\mu\text{M Fe}^{2+}/100\text{g}$  sample

#### Mineral Content

Mineral content of *T. portulacifolium* leaves in decreasing order in mg/kg is 51424, 22101, 10578, 367.12 and 365 while that of stem is 41511, 547, ND, 262.33 and 156 for K, Mg, Ca, P and Na respectively (Table 3). Calcium and phosphorous are associated with each other for growth and maintenance of bones, teeth and muscles<sup>45, 46</sup>. The calcium level in the leaves studied was high when compared with some green leafy vegetables like *Amaranthus hybridus*<sup>47</sup>, but was low compared to the values reported in some leafy vegetables commonly consumed in Lagos, Nigeria<sup>48</sup>. The phosphorous content compares favorably with that of *A. hybridus*<sup>47</sup> and *Ipomea batatas*<sup>49</sup>. Magnesium content of the leaves was high compared to the values reported in some green vegetables<sup>47, 49</sup>. This is a component of chlorophyll<sup>50</sup>. It is an important mineral element in connection with ischemic heart

disease and calcium metabolism in bones<sup>51</sup>. Potassium and sodium content of the leaves was high compared to the values reported in some edible vegetables and fruits of Iran and India<sup>52</sup>. From the obtained results, it is clear that the leaf extract possessed more content of minerals than the stem extract.

Table 3: Mineral composition of *T. portulacifolium*

Minerals	Leaf	Stem
Calcium	10578	ND
Magnesium	22101	547
Sodium	365	156
Potassium	51424	41511
Phosphorus	367.12	262.33

<sup>1</sup> Expressed as mg/kg; ND – Not Detected upto 2 ppm

### HPLC Analysis

HPLC method enabled qualitative and quantitative analysis of Quercetin in *T. portulacifolium*. From the calibration curve results, the amount of Quercetin, in the sample injected was calculated. Figure 2 depicts the chromatogram of Quercetin. *T. portulacifolium* leaves contain higher amount of quercetin (0.15 mg/kg) (Figure 3) than *T. portulacifolium* stem (0.08 mg/kg) (Figure 4). Quercetin (3, 5, 7, 3', 4'-pentahydroxyflavone), a bioflavonoid, frequently found in

consumed foods including apples, berries, onions, tea and vegetables<sup>53</sup>. Many natural antioxidants derived from plants play very important role because of their safe use with no side effects.

Quercetin is one such multidimensional antioxidant present in plants especially in green vegetables. Indeed, quercetin has many beneficial effects on human health, including cardiovascular protection, anticancer activity, cataract prevention, antiviral activity and anti-inflammatory effects<sup>54</sup>.

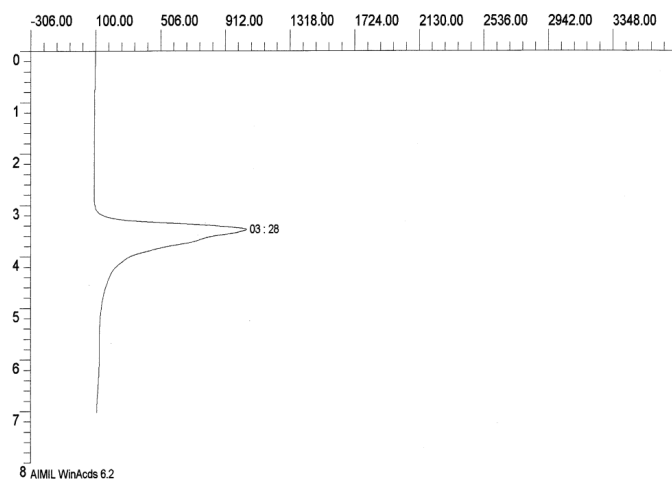


Fig. 2: HPLC Analysis of Quercetin

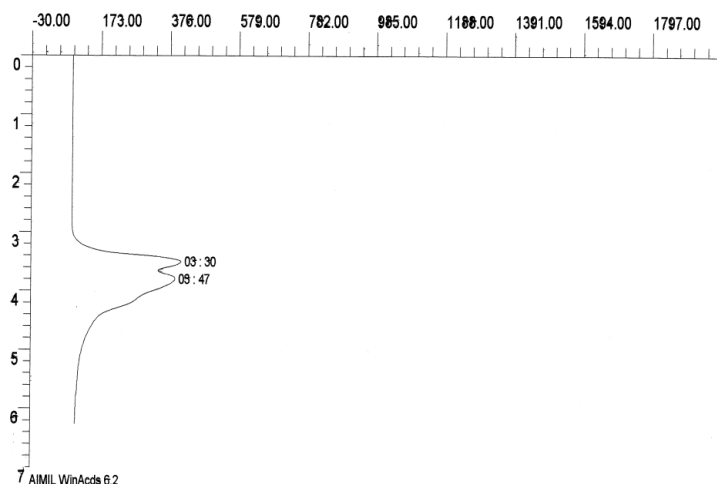


Fig. 3: HPLC Analysis of *T. portulacifolium* Leaf extract

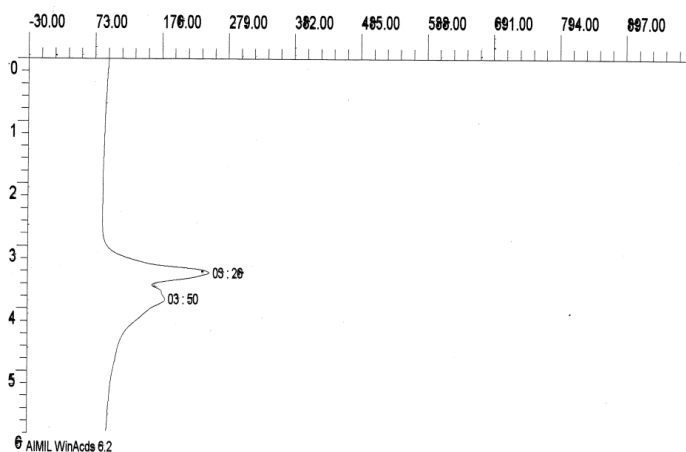


Fig. 4: HPLC Analysis of *T. portulacifolium* Stem extract

Parameters	Leaf extract	Stem extract	Standard
RT (min/sec)	03:30	03:26	03:28
Content (mg/kg)	0.15	0.08	98.00

### The Relationship between Antioxidant Activity and Total Phenolics and Flavonoids Content

Phenolic compounds are believed to account for a major portion of the antioxidant activity in many plants. The total phenolic content of leaf extract exhibited an apparent linear relationship with nitric oxide radical scavenging activity ( $r^2 = 0.985$ ), hydrogen peroxide scavenging activity ( $r^2 = 0.944$ ), reducing capacity ( $r^2 = 0.975$ ), DPPH radical scavenging activity ( $r^2 = 0.988$ ) and hydroxyl scavenging activity ( $r^2 = 0.960$ ). The total phenolic content of stem extract also exhibited linear relationship with nitric oxide radical scavenging

activity ( $r^2 = 0.990$ ), hydrogen peroxide scavenging activity ( $r^2 = 0.981$ ), reducing capacity ( $r^2 = 0.963$ ), DPPH radical scavenging activity ( $r^2 = 0.955$ ) and hydroxyl scavenging activity ( $r^2 = 0.974$ ) (Table 4). The total flavonoid content and flavonol content was also well correlated with the leaf and stem extracts in all the tested assays. Many works have shown that there has been a positive correlation between polyphenol content and antioxidant activity<sup>55,56</sup>.

Therefore, it was considered that the high antioxidant capacity of the leaf and stem extract of *T. portulacifolium* could be attributable to its high amount of polyphenol content.

**Table 4: Correlation coefficient between polyphenol content and antioxidant activity**

Plantpart	Polyphenol	DPPH	OH	NO	H <sub>2</sub> O <sub>2</sub>	RC	ICC
Leaf	TPC	0.988	0.96	0.985	0.944	0.975	0.980
	TFC	0.978	0.953	0.975	0.934	0.965	0.970
	TFIC	0.961	0.940	0.965	0.916	0.942	0.954
Stem	TPC	0.955	0.974	0.990	0.981	0.963	0.909
	TFC	0.943	0.956	0.983	0.967	0.951	0.914
	TFIC	0.932	0.947	0.976	0.943	0.935	0.891

TPC- Total phenol content, TFC- Total flavonoid content, TFIC- Total flavonol content

The leaf and stem extract of *T. portulacifolium* exhibited appreciably high antioxidant activity as manifested through different antioxidant assays like reducing capacity, DPPH radical scavenging assay, hydroxyl scavenging assay, nitric oxide scavenging assay, hydrogen peroxide scavenging assay, metal chelating assay and FRAP assay. Comparing the activities of two sets of extracts, leaf extract had superior activity in terms of antioxidant activity than the stem extract. This may be due to the presence of more polyphenol compounds and minerals in the leaf extract. The observed variation in the antioxidant activity was further justified by HPTLC chemical profile. It revealed that number of polyphenol compounds in leaf and stem varied slightly and that can be responsible for the observed slight disparity in their antioxidant activity<sup>57</sup>.

### CONCLUSION

In this study we have focused on antioxidant properties of leaf and stem of *T. portulacifolium* which has been rarely reviewed. The high reducing capacity of leaf and stem extracts efficiently scavenged DPPH, NO, OH<sup>•</sup>, H<sub>2</sub>O<sub>2</sub> free radicals and hydrogen peroxide *in vitro*. These activities of the plant were strongly correlated with its phenolic and flavonoid contents. However, the leaf extract possessed more antioxidant capacity and mineral content than the stem extract and this may be due to the presence of more amounts of phenolic compounds in the leaf extract. HPTLC polyphenolic profile of the leaf and stem also support the fact that leaf extract possess more antioxidant activity. As a result, leaf and stem extracts of *T. portulacifolium* seem to be good sources of natural antioxidants.

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