



# Journal of Biological Sciences

ISSN 1727-3048

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## Research Article

# Comparative Antioxidant Capacity of Aqueous and Ethanol Fruit Extracts of *Tetrapleura tetraptera*

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## Abstract

**Background and Objective:** Most commonly used synthetic antioxidants have side effects and are carcinogenic according to research reports. Therefore the search for natural antioxidants from plants sources which are presumed to be safe, potent, nutritional and possessing therapeutic value is necessary. The objective of this study was to compare the antioxidant ability of aqueous and ethanol fruit extracts of *tetrapleura tetraptera*. **Materials and Methods:** The fruit of *Tetrapleura tetraptera* was procured, identified, aqueous and ethanol fruit extracts were prepared. The antioxidant activity was evaluated by various *in vitro* radical scavenging assays, namely, 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-2-picrylhydrazyl radicals (DPPH), hydroxyl radical, nitric oxide radical, ferric reducing antioxidant potential (FRAP), metal chelating and total antioxidant capacity (TAC). While antioxidant capacity was studied by assessing *in vitro* inhibition of lipid peroxidation, total phenolic content (TPC), total flavonoid content (TFC) and the reductive potential of the extracts. One-way ANOVA was used for statistical analysis. **Results:** The results indicated that both fruit extracts of *Tetrapleura tetraptera* showed strong antioxidant activity and capacity. Aqueous fruit extract, however, produced a higher effect than ethanol in eight assay models namely, Total phenolic content, reductive potential, ABTS, FRAP, DPPH, nitric oxide, anti-lipid peroxidation and metal chelating. The stronger and more potent free radical scavenging and antioxidant activities observed for aqueous extract over ethanol may be due to the high total phenolic content extractable by this solvent. The free radical scavenging and antioxidant activities of this plant suggest that it could be potential natural sources of antioxidants and could have greater importance as therapeutic agents in preventing or slowing oxidative stress-related degenerative diseases. **Conclusion:** The results of this study showed the potential of *Tetrapleura tetraptera* to scavenge free radicals. The more potent antioxidant capacity expressed by the aqueous extract implies that water is a better solvent in the extraction of phytonutrients present in this plant and it is therefore recommended for prevention and treatment of oxidative stress relative diseases.

**Key words:** Natural antioxidant, *Tetrapleura tetraptera*, reactive oxygen species, free radical scavengers, lipid peroxidation, oxidative stress

**Received:** January 12, 2017

**Accepted:** March 20, 2017

**Published:** April 15, 2017

**Citation:** Josiah Sunday Joel, Omoregie Ehi Sheena, Obuotor Efere Martins, Nwangwu Spencer Chukwumaobim Onyemauchekukwu and Adeyemi Adewumi Emmanuel, 2017. Comparative antioxidant capacity of aqueous and ethanol fruit extracts of *Tetrapleura tetraptera*. J. Biol. Sci., 17: 185-193.

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Reactive oxygen species (ROS), such as hydroxyl radicals, (OH), superoxide anion ( $O_2^-$ ), singlet oxygen, ( $^1O_2$ ) and hydrogen peroxides ( $H_2O_2$ ) are regularly synthesis as a result of exogenous factors or biological reactions<sup>1</sup>. Within the body, several of these ROS play a positive role such as cell growth regulation, energy production, intercellular signalling, phagocytosis, and/or production of biologically essential compounds<sup>2</sup>. Though, ROS may also be harmful, they can attack lipids in cell membranes and destroy DNA, facilitating oxidations that cause membrane damage such as membrane lipid peroxidation and a reduction in membrane fluidity and also cause DNA mutation leading to cancer. They also play a chief role in inducing oxidative stress which may lead to numerous ailments, such as anaemia, cancer, diabetes, degenerative diseases, inflammation and ischemia<sup>2</sup>.

A potent scavenger of these reactive oxygen species may serve as a possible preventive intervention for free radical-mediated diseases<sup>3</sup>. The cell has several protective (antioxidant) mechanisms to detoxify or prevent the formation of ROS in healthy individuals; equilibrium exists between free radical production and the antioxidative defence system<sup>2</sup>. Oxidative stress resulting from the imbalance between oxidants generation and destruction by antioxidant may lead to damage in the structure and function of the cell membrane<sup>4</sup>. Defence mechanism against oxidative stress in the human body includes antioxidant enzymes like peroxidases, catalases, superoxide dismutases, proteins e.g albumin or transferrin as well as small molecules like ascorbic acid, vitamin E or secondary metabolic plant products. Many synthetic antioxidant mediators have been established to remediate oxidative stress. Yet, factors like lack of availability, expensive and side effects remained as the main impediment in fighting oxidative stress. Due to these side effects, interest has increased considerably in the search for naturally occurring antioxidants for use as foods, dietary supplements or drugs. Some secondary metabolic plant products, particularly phenolic compounds<sup>5</sup>, found in fruits and vegetables<sup>6</sup> and medicinal plants<sup>7</sup> play a potential role in the prevention of human diseases, due to their antioxidant activities.

*Tetrapleura tetraptera* belongs to the family of *Mimosaceae*. It is called Aridan and Oshosho by Yorubas and Ibo people of Nigeria respectively. It is generally found in the coastal tropical forest of Africa. The plant is a strong, perennial tree with a single stem, dark green leaves, dispersion branches and dense woody base. The tree reaches 20-25 m in height

and may attain a girth of 1.5 m and has sharp buttress<sup>8</sup>. It is normally used for building poles, carvings, firewood, pestle and tool handles.

The *Tetrapleura tetraptera* fruits and seeds have abundant macro-elements and nutrients such as iron, magnesium, phosphorus, potassium, sodium, sucrose, fructose and glucose<sup>9</sup>. Josiah *et al*<sup>9</sup> also reported that *T. tetraptera* fruits contains minerals such as  $Ca^{2+}$ ,  $Fe^{2+}$ ,  $PO_{4-2}$ ,  $Na^+$ ,  $k^+$ ,  $Mg^{2+}$ ,  $Mn^{2+}$ ,  $Se^{2+}$ ,  $Zn^{2+}$ ,  $Cu^{2+}$  and  $Cl^-$  as well as vitamins like ascorbic acid,  $\beta$ -carotene, thiamine, riboflavin and niacin, with phytochemicals which include saponin, anthraquinones, steroids, flavonoids, terpenoids, alkaloids, tannins. The dry fruit has a pleasant aroma and popularly used as a spice in Southern and Eastern Nigeria. The fruits are usually used for the management of convulsions, fevers, flatulence, inflammation, jaundice, leprosy, post-partum contraction and rheumatism. Also, its leaves are essential for the treatment of epilepsy and present strong molluscicidal activity<sup>10</sup>.

Therapeutic plants and herbs were the initial sources of substances used for the treatment and management of different ailments. Herbal medicine has been found to have impressive credentials which have no equivalent in modern medicine<sup>11</sup>.

Interest in the role of antioxidants in human health has prompted research in the field of food science and medicinal herbs to assess the role of herbs as antioxidants. Hence, the present investigation was designed to evaluate the comparative antioxidant potential and free radical scavenging capability of aqueous and ethanol extracts of *T. tetraptera*.

## MATERIALS AND METHODS

**Collection and extraction of plant materials:** Two fresh fruits of *Tetrapleura tetraptera* (50 g) were purchased from Onitsha market in Anambra State. They were authenticated by Prof Eigbonnaire a Botanist of the Department of Biological Sciences, Igbinedion University, Okada. This study was conducted between April and July, 2012. The fruits were shade dried and ground into uniform powder using a Thomas-Willey milling machine, the powdered sample was used for the analysis. Weighed portions (20 g) of the dried plant material were macerated with five volumes (1:5w/v) of petroleum ether (60-80°C), followed by 95% ethanol for 24 h. The resulting supernatant obtained was filtered with a Whatman (No.1) filter paper and concentrated in a rotary evaporator under reduced pressure at 40°C to give the crude ethanol extract. Similarly, the macerated sample from the petroleum ether extraction was first air-dried and then macerated with 10 volume of

distilled water for 24 h. The mixture was then filtered using a Whatman (No. 1) filter paper and thereafter concentrated using a rotary evaporator and lyophilized to give the crude aqueous extract.

**Determination of total phenolic content:** Modified Folin-Ciocalteu method was used for the estimation of total phenolic content in the aqueous and ethanol fruit *Tetrapleura tetraptera* extracts as described by Roura *et al.*<sup>12</sup>.

**Determination of total flavonoids content:** The aluminium chloride colorimetric method was used for total flavonoid content assay according to Gomez-Caravaca *et al.*<sup>13</sup>.

**Determination of total antioxidant capacity:** The assay was carried according to the method of Umamaheswari and Chatterjee<sup>14</sup>. The method is based on the reduction of molybdenum (IV) to molybdenum (V) by the extract and the subsequent formation of a green phosphate/molybdenum (V) complex at acid pH.

**Estimation of lipid peroxidation:** The anti-lipid peroxidative property of the extract was determined using a modified thiobarbituric acid reactive species (TBARS) assay described by Dasgupta and De<sup>15</sup>. In this assay, the end product of lipid peroxidation using egg yolk homogenate as lipid-rich media was quantified by determining the formed malondialdehyde (MDA) which react with the thiobarbituric acid under an acidic condition to form an MBA-TBA adduct. This pink coloured product is then measured spectrophotometrically at 532 nm.

**Estimation of nitric oxide radical inhibition activity:** The nitric oxide radical scavenging activity was measured by Griess reaction. The reductive potential was determined according to the method described by Alam *et al.*<sup>16</sup>.

**Determination of the reductive potential:** The reductive potential of the extract was determined according to the method described by Alam *et al.*<sup>16</sup>.

**Estimation of ferric reducing antioxidant power (FRAP):** Ferric reducing antioxidant power assay is based on the ability of antioxidants to reduce  $Fe^{3+}$  to  $Fe^{2+}$  in the presence of 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ), forming an intense blue  $Fe^{2+}$ -TPTZ complex with an absorption maximum at 593 nm. This reaction is pH-dependent (optimum to 3.6). The assay is according to Benzie and Strain method described by Eghdami *et al.*<sup>17</sup>.

**Determination of 2,2-azinobis 3-ethyl-benzothiazoline-6-sulfonate (ABTS) free radical scavenging activity:** The radical scavenging activities (RSC) of the extracts were also measured by ABTS radical cation assay<sup>18</sup>.

**Determination of DPPH radical scavenging activity:** The hydrogen donating or radical scavenging properties of the extracts were determined using the stable radical DPPH (2, 2-diphenyl-2-picrylhydrazyl hydrate) according to the method of Blois described by Alam *et al.*<sup>16</sup>.

**Determination of the hydroxyl radical scavenging activity:** The Hydroxyl radical (Deoxyribose method) the assay was performed as reported by Halliwell with minor modification<sup>19</sup>, the extent of deoxyribose degradation was measured by the TBA reaction.

**Determination of the metal chelating activity:** The Metal Chelating activity was determined as described by Alan *et al.*<sup>16</sup>.

**Statistical analysis:** All analyses were determined in triplicates, One-way ANOVA and Tukey's pair-wise test were carried out for the determination of significant differences ( $p < 0.05$ ) between the means. Data were analysed using Graph Pad Prism Version 6<sup>20</sup>.

## RESULTS

The results of the antioxidant capacity of fruit extracts of *Tetrapleura tetraptera* investigated by eleven *in vitro* methods are shown in Table 1-6.

To adequately assess oxidative stress in living systems a single measurement of antioxidant status will not be sufficient but a 'battery' of determinations will be required. The results include investigation of eleven *in vitro* assays of antioxidant activities of aqueous and ethanol fruit extracts of *Tetrapleura tetraptera* (Tt).

**Total phenol content:** The results of total phenol content of aqueous and ethanol fruit extracts of *Tetrapleura tetraptera* (Tt) are shown in Table 1, different solvent extracts showed different levels of total phenolic content. The total phenolic content was expressed as  $\mu g$  of gallic acid equivalent (GAE), Tt aqueous fruit extract revealed a significantly higher ( $p < 0.05$ ) total phenolic content than ethanol fruit extract.

**Total flavonoid content determination:** The results of total flavonoid content are shown in Table 1, the results are solvent dependent and expressed as  $\mu g$  of quercetin equivalent (QE).

Table 1: Phenolic content and antioxidant activities of fruit extracts of *Tetrapleura tetraptera* (Tt)

| Plant extract | Total phenolic<br>( $\mu\text{g mL}^{-1}$ GAE) | Total flavonoid<br>content ( $\mu\text{g mL}^{-1}$ QE) | Total antioxidant<br>capacity ( $\mu\text{g mL}^{-1}$ AAE) | Reductive potential<br>( $\mu\text{g mL}^{-1}$ AAE) | ABTS<br>( $\mu\text{g mL}^{-1}$ TEAC) | FRAP<br>( $\mu\text{g mL}^{-1}$ AAE) |
|---------------|--|--|--|---|---------------------------------------|--------------------------------------|
| Tt aqueous    | 432.1 $\pm$ 11.97                              | 133.3 $\pm$ 12.93                                      | 667.9 $\pm$ 11.30  | 9.7 $\pm$ 0.09                                      | 6,835.5 $\pm$ 1.17                    | 2,566.6 $\pm$ 21.14                  |
| Tt ethanol    | 165.5 $\pm$ 2.57                               | 232.1 $\pm$ 49.38                                      | 1,388.4 $\pm$ 0.00   | 3.4 $\pm$ 0.04                                      | 38,415.6 $\pm$ 0.43                   | 460.8 $\pm$ 25.37                    |

Data presented as the Mean  $\pm$  SEM of three determinations

The total flavonoid content was significantly higher ( $p < 0.05$ ) in Tt ethanol fruit extract compared to Tt aqueous fruit extract.

**Total antioxidant capacity determination:** The results of total antioxidant capacity as shown in Table 1 are solvent dependent and expressed as  $\mu\text{g}$  ascorbic acid equivalent (AAE). Total antioxidant capacity of Tt ethanol fruit extract was significantly higher ( $p < 0.05$ ) than Tt aqueous fruit extract.

**Reductive potential assay:** Table 1 showed the results of reductive potential (RP), which was significantly higher ( $p < 0.05$ ) in Tt aqueous fruit extract as compared with Tt ethanol fruit extract. Reducing powers provide effectiveness in the conversion of free radicals to more stable products and thus brings about the termination of free radical initiated chain reaction.

**Ferric reducing antioxidant power (FRAP) assay:** The results of ferric reducing antioxidant power (FRAP) showed in Table 1, the results are expressed as ascorbic acid equivalent, the results are solvent dependent and showed that ferric reducing antioxidant power of Tt aqueous fruit extract was significantly higher ( $p < 0.05$ ) than Tt ethanol fruit extract. The assay is based on the ability of antioxidants to reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  in the presence of 2, 4, 6-Tri 2-pyridyl-s-triazine (TPTZ).

**ABTS free radical scavenging assay:** The results of 2, 2-Azinobis 3-ethyl- benzothiazoline-6-sulfonate (ABTS) free radical scavenging assay showed in Table 1 are solvent dependent and is expressed as Trolox equivalent antioxidant capacity (TEAC). The results revealed that ABTS free radical scavenging was significantly higher ( $p < 0.05$ ) in Tt ethanol extract than Tt aqueous.

**DPPH free radical scavenging assay:** The results of the DPPH free radical scavenging capacity showed in Table 2 revealed an increase in percentage inhibition of the free radical formation. The DPPH  $\text{IC}_{50}$  value for Tt aqueous was significantly lower ( $p < 0.05$ ) than Tt ethanol and is comparative with the ascorbic acid used as the positive control. The lower the  $\text{IC}_{50}$  the more effective the DPPH activity, the results, therefore, indicate that Tt aqueous was a more potent free radical scavenger of the DPPH radical than Tt ethanol extract.

**Nitric oxide radical scavenging activity:** Table 3 revealed the effect of different concentrations of both aqueous and ethanol fruit extracts of Tt and ascorbic acid (positive control) on nitric oxide radical scavenging activity. The two extracts

Table 2: DPPH free radical scavenging activity of fruit extracts of *Tetrapleura tetraptera* (Tt)

| Concentration<br>( $\mu\text{g mL}^{-1}$ ) | Inhibition (%)   |                  | Concentration<br>( $\mu\text{g mL}^{-1}$ ) | Standard<br>(ascorbic acid) |
|--|------------------|------------------|--|-----------------------------|
|  | Tt aqueous       | Tt ethanol       |  |                             |
| 1000                                       | 50.57 $\pm$ 1.90 | 89.71 $\pm$ 1.64 | 20.0                                       | 89.64 $\pm$ 2.10            |
| 500  | 27.03 $\pm$ 1.18 | 82.54 $\pm$ 0.77 | 10.0                                       | 80.73 $\pm$ 1.33            |
| 250  | 17.25 $\pm$ 2.01 | 50.22 $\pm$ 1.59 | 5.0  | 34.93 $\pm$ 1.03            |
| 125  | 7.27 $\pm$ 0.86  | 29.06 $\pm$ 5.18 | 2.5  | 14.22 $\pm$ 0.59            |
| 62.5                                       | 3.74 $\pm$ 1.001 | 13.19 $\pm$ 0.72 | 1.25                                       | 5.15 $\pm$ 0.87             |
| 31.25                                      | -                | 6.09 $\pm$ 0.21  | 0.625                                      | 8.04 $\pm$ 2.00             |
| $r^2$                                      | 0.8506           | 0.9755           |  | 0.9887                      |
| IC <sub>50</sub> ( $\mu\text{g mL}^{-1}$ ) | 14.12 $\pm$ 1.76 | 24.25 $\pm$ 8.28 |  | 8.94 $\pm$ 115.30           |

Data presented as the Mean  $\pm$  SEM of three measurements

Table 3: Nitric oxide radical scavenging activity of fruit extracts of *Tetrapleura tetraptera* (Tt)

| Concentration<br>( $\mu\text{g mL}^{-1}$ ) | Inhibition (%)     |                      | Concentration<br>( $\mu\text{g mL}^{-1}$ ) | Standard<br>(ascorbic acid) |
|--|--------------------|----------------------|--|-----------------------------|
|  | Tt aqueous         | Tt ethanol           |  |                             |
| 1000                                       | 76.31 $\pm$ 1.510  | 29.54 $\pm$ 4.883    | 250.0                                      | 98.41 $\pm$ 0.127           |
| 800  | 48.90 $\pm$ 4.363  | 21.25 $\pm$ 2.517    | 125.0                                      | 87.78 $\pm$ 2.323           |
| 600  | 33.25 $\pm$ 3.944  | 13.90 $\pm$ 0.286    | 62.5                                       | 53.79 $\pm$ 4.094           |
| 400  | 16.88 $\pm$ 5.017  | 4.14 $\pm$ 0.679     | 31.25                                      | 32.04 $\pm$ 6.325           |
| 200  | 4.82 $\pm$ 1.60    | 1.54 $\pm$ 0.197     | 15.625                                     | 11.93 $\pm$ 0.322           |
| $r^2$                                      | 0.9613             | 0.9724               |  | 0.9941                      |
| IC <sub>50</sub> ( $\mu\text{g mL}^{-1}$ ) | 760.10 $\pm$ 11.65 | 1669.45 $\pm$ 234.87 |  | 64.02 $\pm$ 6.91            |

Data presented as the Mean  $\pm$  SEM of three measurements

Table 4: Hydroxyl radical scavenging activity of fruit extracts of *Tetrapleura tetraptera* (Tt)

| Concentration<br>( $\mu\text{g mL}^{-1}$ ) | Inhibition (%)    |                   | Concentration<br>( $\mu\text{g mL}^{-1}$ ) | Standard mannitol |
|--|-------------------|-------------------|--|-------------------|
|  | Tt aqueous        | Tt ethanol        |  |                   |
| 100  | 35.52 $\pm$ 16.33 | 58.53 $\pm$ 1.63  | 100  | 42.36 $\pm$ 0.88  |
| 80   | 24.25 $\pm$ 0.38  | 47.34 $\pm$ 2.64  | 80   | 35.52 $\pm$ 9.29  |
| 60   | 13.50 $\pm$ 0.75  | 45.65 $\pm$ 1.51  | 60   | 23.36 $\pm$ 0.63  |
| 40   | 9.33 $\pm$ 1.13   | 42.27 $\pm$ 0.50  | 40   | 10.92 $\pm$ 1.88  |
| 20   | 7.10 $\pm$ 1.26   | 39.17 $\pm$ 0.88  | 20   | 4.44 $\pm$ 1.51   |
| $r^2$                                      | 0.8807            | 0.919             |  | 0.9875            |
| IC <sub>50</sub> ( $\mu\text{g mL}^{-1}$ ) | 162.73 $\pm$ 0.70 | 75.582 $\pm$ 1.51 |  | 114.21 $\pm$ 1.00 |

Data presented as the Mean  $\pm$  SEM of three determinations

expressed a percentage increase in inhibition of nitric oxide radical formation in an increasing order in respect to the concentrations of the plant extracts. A percentage increase in nitric oxide radical scavenging ability was noticed with increasing concentration of plant extracts. Tt aqueous fruit extracts expressed a higher percentage inhibition compared with Tt ethanol extract. Tt aqueous fruit extract showed a significant reduction ( $p < 0.05$ ) in IC<sub>50</sub> when compared with Tt ethanol fruit extract. This indicated that the plant extracts possess a weak nitric oxide radical scavenging ability when compared to the control.

**Anti-lipid peroxidation assay:** The results obtained Table 5 showed that Tt aqueous and ethanol fruit extracts exhibited a percentage increase in the inhibition of the formation of the thiobarbituric acid reactive substances (TBARS) with increasing concentration of the extracts. The results obtained showed

that the plant extract produced an inhibition of the formation of the thiobarbituric acid reactive substances (TBARS). Tt aqueous extract showed a significantly lower ( $p < 0.05$ ) value of an IC<sub>50</sub> as compared to Tt ethanol and standard BHT.

**Hydroxyl radical scavenging capacity assay:** In the results of the study showed in Table 5, revealed percentage increase in the inhibition of the hydroxyl radical formation with increasing concentration of aqueous and ethanol fruit extracts of Tt. The IC<sub>50</sub> hydroxyl radical scavenging capacity was significantly reduced in Tt ethanol compared to Tt aqueous fruit extract. The results indicated that Tt ethanol fruit extract possesses an effective hydroxyl radical scavenging capacity.

**Metal chelating activity assay:** Table 6 revealed the effect of different concentrations of both aqueous and ethanol fruit extracts of *Tetrapleura tetraptera* (Tt) and EDTA on metal

Table 5: Lipid peroxide formation inhibition activity of fruit extracts of *Tetrapleura tetraptera* (Tt)

| Concentration<br>( $\mu\text{g mL}^{-1}$ ) | Inhibition (%)      |                      | Concentration<br>( $\mu\text{g mL}^{-1}$ ) | BHT standard      |
|--|---------------------|----------------------|--|-------------------|
|  | Tt aqueous          | Tt ethanol           |  |                   |
| 10   | 61.79 $\pm$ 1.89    | 69.40 $\pm$ 1.75     | 500.0                                      | 72.50 $\pm$ 3.20  |
| 5  | 53.60 $\pm$ 0.37    | 53.43 $\pm$ 0.71     | 250.0                                      | 53.00 $\pm$ 0.88  |
| 2.5  | 47.04 $\pm$ 1.20    | 44.42 $\pm$ 0.00     | 125.0                                      | 36.37 $\pm$ 1.18  |
| 1.25                                       | 28.33 $\pm$ 1.42    | 36.22 $\pm$ 0.33     | 62.5                                       | 32.70 $\pm$ 2.68  |
| 0.625                                      | 18.82 $\pm$ 0.75    | 31.84 $\pm$ 0.00     | 31.25                                      | 19.50 $\pm$ 3.01  |
| 0.3125                                     | 12.69 $\pm$ 1.80    |                      | 15.625                                     | 13.97 $\pm$ 1.66  |
| r <sup>2</sup>                             | 0.9531              | 0.9720               |  | 0.9727            |
| IC <sub>50</sub> ( $\mu\text{g mL}^{-1}$ ) | 3.108.36 $\pm$ 1.14 | 4.639.10 $\pm$ 140.8 |  | 219.54 $\pm$ 2.50 |

Data presented as the Mean  $\pm$  SEM of three determinations

Table 6: Metal chelating ability of fruit extracts of *Tetrapleura tetraptera* (Tt)

| Concentration<br>( $\mu\text{g mL}^{-1}$ ) | Inhibition (%)   |                   | Concentration<br>( $\mu\text{g mL}^{-1}$ ) | Standard (EDTA)  |
|--|------------------|-------------------|--|------------------|
|  | Tt aqueous       | Tt ethanol        |  |                  |
| 0.10                                       | 44.69 $\pm$ 3.76 | 6.640 $\pm$ 0.770 | 0.10                                       | 39.45 $\pm$ 1.66 |
| 0.08                                       | 21.02 $\pm$ 1.88 | 4.297 $\pm$ 0.330 | 0.08                                       | 33.91 $\pm$ 3.09 |
| 0.06                                       | 13.59 $\pm$ 0.00 | 3.047 $\pm$ 0.110 | 0.06                                       | 27.19 $\pm$ 9.06 |
| 0.04                                       | 7.81 $\pm$ 1.33  | 3.047 $\pm$ 0.110 | 0.04                                       | 22.50 $\pm$ 3.09 |
| 0.02                                       | 4.61 $\pm$ 2.32  | 1.328 $\pm$ 0.330 | 0.02                                       | 8.36 $\pm$ 1.66  |
| r <sup>2</sup>                             | 0.9518           | 0.9110            |  | 0.9536           |
| IC <sub>50</sub> (mg mL <sup>-1</sup> )    | 0.1602           | 0.8403            |  | 0.1245           |

Data presented as the Mean  $\pm$  SEM of three determinations

chelating ability. The two extracts expressed increased in metal chelating ability in dose-depended manner. The metal chelating ability of Tt aqueous showed significant low ( $p < 0.05$ ) IC<sub>50</sub> compared to Tt ethanol. The results obtained were comparable to the positive control EDTA used.

## DISCUSSION

The antioxidant activities of ethanol and aqueous fruit extracts of *Tetrapleura tetraptera* were investigated and compared in the present study. The results of the study revealed that the fruit extracts of *Tetrapleura tetraptera* possess antioxidant activities. The potent scavenging of free radicals exhibited by this plant based on the results obtained from ABTS, DPPH, lipid peroxide formation, hydroxyl radical scavenging and metal chelating ability could be attributed to the synergistic effect and the key contributions of many phytochemicals that are present in the plant. Based on DPPH radical scavenging assay it was evident that aqueous fruit extract of *Tetrapleura tetraptera* exhibited strong antioxidant potential than extract. This expression of a stronger antioxidant potential displayed by aqueous fruit extract is consistent with the results showed by nitric oxide, metal chelating, FRAP, reductive potential and lipid peroxidation. However, the ethanol fruit extract of *Tetrapleura tetraptera* showed a much more potent antioxidant potential as revealed

by obtained results of hydroxyl radical scavenging and ABTS. These results showed that different solvent extracts have a noticeable effect on scavenging free radicals.

The phenolics constitute of plants majorly act as primary antioxidants<sup>21</sup>. They have high redox potentials which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers<sup>22</sup>. The results from this study showed that Tt aqueous fruit extract contained a higher phenolic content than Tt ethanol extract. The high phenolic content of Tt aqueous indicate high antioxidant potentials, however, the results did not suggest a correlation between total phenolic content and total antioxidant capacity considering the high value of total antioxidant capacity observed with the Tt ethanol extract. This lack of correlation is in agreement with literature report<sup>23</sup>. Estimation of phenolic content is important because several studies reported the relationships between phenolic contents and antioxidant property, some authors reported the high relationship between phenolic content and the antioxidant activity<sup>24,25</sup>. The observed high DPPH radical scavenging activity and phenol content in Tt aqueous extract agreeing with the direct correlation between phenolic content and antioxidant activity previously reported<sup>26</sup>. On the other hand, Tt ethanol extract had a low amount of phenols but had good DPPH radical scavenging activity. These results are in agreement with the previous report<sup>27</sup> that there is no correlation between phenolic content and antioxidant activity.

Flavonoids are the major family of phenolic compounds that are generally seen in human diets. In this study, Tt ethanol fruit extract expressed a higher amount of total flavonoid content (TFC) than aqueous. In accordance, Tt ethanol fruit extract expressed higher amount of total antioxidant capacity (TAC) which suggested a correlation between TFC and TAC of the extracts. The antioxidant activities of flavonoids are as a result of numerous different mechanisms, such as chelation of metal ions such as iron and copper, scavenging of free radicals and inhibition of enzymes responsible for a free radical generation<sup>28</sup>.

Total Antioxidant Capacity means the ability of free radical scavenging by the radical scavenging antioxidants present in the test sample<sup>29</sup>. In this study the TAC was remarkably high for the ethanol extracts of Tt than the aqueous counterpart, indicating ethanol to be a better solvent for extraction of this compound and that ethanol extracts of Tt having higher relative antioxidant efficiencies. Also, the antioxidant activities of these extracts varied markedly probably due to differences in structures of phenolic compounds extracted by the different solvents and primarily related to their hydroxylation and methylation patterns.

The results of the reductive potential revealed that the aqueous extracts expressed a relatively higher reductive potential than the ethanol extracts. In this study, the ferric reducing antioxidant power (FRAP) was found to be remarkably high in Tt aqueous extract than Tt ethanol. The ferric-reducing ability measured for a biological sample may indirectly reflect the total antioxidant power of sample<sup>30</sup>.

The results from this study showed that nitric oxide radical generated from sodium nitroprusside at physiological pH was inhibited by both aqueous and ethanol Tt extracts. Tt aqueous expressed a much better inhibition than Tt ethanol. Generally, the nitric oxide scavenging activity of the two extracts showed a weak nitric oxide scavenging activity relative to the standard ascorbic acid.

It has been reported that lipid peroxidation induces disruption and changes in biological membranes which may lead to potentially toxic products<sup>31</sup>. Therefore, it is an essential task for radical scavenging antioxidants to suppress lipid peroxidation. The results obtained from this study showed that Tt extracts inhibited the formation of the thiobarbituric acid reactive substances (TBARS) with Tt aqueous expressing a higher percentage inhibition than Tt ethanol. The hydroxyl radical is the utmost reactive among the reactive oxygen species which induce severe damage to biomolecules by extracting hydrogen atoms from membrane lipids and brings about peroxidation of lipid<sup>32</sup>. *Tetrapleura tetraptera* ethanol showed a higher scavenging ability of the hydroxyl radical than Tt aqueous extracts.

From the results of the metal chelating ability of the two extracts, it was evident that the extracts possessed Fe<sup>2+</sup> chelating activity and this might play a protective role against oxidative damage induced by metal catalysed decomposition reactions. *Tetrapleura tetraptera* aqueous extract expressed a higher percentage metal chelating activity which compared closely with the standard EDTA. The high metal chelating power might be due to the high concentration of phenolic compounds that can chelate metal ions.

This study discovered the antioxidant potentials and free radical scavenging capabilities of *Tetrapleura tetraptera* plant. This study helped the researchers to uncover the synergy action between the phytonutrients and the endogenous antioxidant enzymes activities of *Tetrapleura tetraptera*, there has been a dearth of knowledge in this aspect. Therefore, *Tetrapleura tetraptera* especially its aqueous extract is recommended as a natural antioxidant, to reduce oxidative stress which has been implicated in the development of chronic and degenerative diseases. The limitations of the current study are the inability to analysis chemical constituents in *Tetrapleura tetraptera*, its toxicity and its endogenous antioxidant activity.

## CONCLUSION

The present study compared the free radical scavenging and antioxidant activities of aqueous and ethanol fruit extracts of *Tetrapleura tetraptera*. The results revealed that both fruit extracts of *Tetrapleura tetraptera* possess free radical scavenging and antioxidant activities, however, aqueous fruit extract has comparatively more potent *in vitro* free radical scavenging activity and antioxidant capacity than ethanol fruit extract. This capacity may be correlated with the total phenolic contents in the plant. The free radical scavenging and antioxidant activities of this plant suggest that it could be potential natural sources of antioxidants which may possess better importance as medicinal agents in averting or reducing oxidative stress-related degenerative diseases.

## SIGNIFICANCE STATEMENT

This study discovered the antioxidant potentials and free radical scavenging capabilities of *Tetrapleura tetraptera* plant, which makes the plant beneficial and can be recommended as natural antioxidant in diet, to reduce free radicals especially, reactive oxygen species, which at high concentration, results into oxidative stress which has been implicated in the development of chronic and degenerative diseases. This study helped the researchers to



uncover the synergy action between the phytonutrients and *in vitro* antioxidant enzymes activities of *Tetrapleura tetraptera*, there has been a dearth of knowledge in this aspect.

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