**In vitro Antioxidant Activities of Aqueous and Ethanol Extracts of Mangifera indica Leaf, Stem-Bark and Root-Bark**

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**ABSTRACT**

Introduction: *Mangifera indica* is a rampant Nigerian plant with history of application in traditional medicine for the treatment of degenerative diseases by indigenous Nigerians. Methods: The leaf, stem-bark and root-bark of *M. indica* were screened for phytoconstituents. The dried powdered plant parts were extracted using aqueous and ethanol solvents. The antioxidant activity of the concentrated extracts was determined using the ferric reducing anti-oxidant power (FRAP), 2, 2′-diphenyl-1-picrylhydrazyl (DPPH) and thiobarbituric acid reactive substance (TBARS) assay methods. Result: The results obtained were analysed statistically by one – way analysis of variance with level of statistical significance taken as p<0.05. In FRAP assay, leaf and stem-bark aqueous extracts showed the highest antioxidant activity with values 92.75 ± 0.48% and 94.49 ± 0.48% respectively while the root-bark ethanolic extract (88.67 ± 1.49%) showed the highest activity. In DPPH assay, the leaf, stem-bark and root-bark ethanolic extracts showed the best percentage DPPH scavenging activity with values 79.09 ± 0.42%, 59.86 ± 0.32% and 80.70 ± 0.42% respectively. In TBARS assay, the leaf and root-bark aqueous extracts showed the best activities with values 97.75 ± 0.56% and 99.48 ± 0.33% and the stem-bark ethanolic extract (99.62 ± 0.26%). Conclusion: The study shows that both the aqueous and ethanol extracts of *M. indica* could be useful to combat free radical mediated diseases.

Key words: *Mangifera indica* leaf, 2,2′-diphenyl-1-picrylhydrazyl (DPPH), Thiobarbituric acid reactive substance (TBARS), Stem-Bark, Root-Bark, Antioxidant activity.

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**INTRODUCTION**

Recently, antioxidant activity (AOA) has been used as a parameter to characterize various products’ health-enhancing properties. This is connected with a common opinion on antioxidants’ crucial role in the prevention of oxidative stress\(^1\) related diseases and on the reduction of total mortality;\(^2-3\) associated with diets rich in plant foods, particularly fruits and vegetables.\(^4\)

In most areas of food manufacturing, precise knowledge of antioxidant capacity (AOC) is becoming of great importance; thus many analytical methods and measuring systems have been developed. Certain antioxidants or reactions are method-specific, so researchers don’t just depend on a particular method only, but employ several others for determining the AOC of fruits and vegetables.\(^5\)

Cancer prevention with the aid of bioactive fractions as well as with specific phytochemical obtained from plants with proof of cancer-inhibiting properties pose promising alternatives to the present cancer drugs.\(^6-7\)

Antioxidant compounds have been widely used to reduce clinical toxicity\(^8\) induced by cancer drugs in patients; there is yet to be any observed reduction in these drugs’ efficacy.\(^9\)

From time immemorial, plants have been used as a source for drug compounds, and plant derived medicines have made large contributions to human health and wellbeing.\(^10\) Many plants known to possess antioxidant properties have been proposed in the treatment\(^11\) and prevention of different pathologies induced by oxidative stress.\(^1,12\) The mango plant belongs to the family Anacardiaceae. It is classified as *Mangifera indica*.\(^13\)

Two main phenolic compounds were isolated from *M. indica* leaves aqueous decoction, specifically mangiferin and C-glucosylibenzophene.\(^14\) Six different extracts from the leaves of *Mangifera indica* was extracted. In that study, a hexane-ethyl acetate extract was characterized by gas chromatography- mass spectroscopy. Ten constituents from 10 peaks were identified. Terpinyl acetate (5.80%) and phytol isomer (5.12%) are as the major constituents, with the minor constituents being oxirane (3.57%), sabine (3.24%), beta-pinen (3.34%), beta-myrccene (3.23%) , cymene (3.68%), alpha-limonene (2.82%), eucalyptol (1,8-cineol (4.71%), 1,3-benzodioxole, 5-(2-, (3.68%) were identified.\(^15\)

The resin has been found to be useful in the treatment of cracked foot, syphilis, infection of ringworm and fungi.\(^19\) The ethno-medicinal survey done by Ene,\(^20\) showed that one of the plants commonly used amongst the three major ethnic groups (*Igbo, Hausa and Yoruba*) in Nigeria for malaria therapy is the mango. The leaves of MI are also used as an antidiabetic agent in Nigerian people medicine.\(^22\)

This present study seeks to evaluate the *in vitro* antioxidant activities of aqueous and ethanolic extracts of *Mangifera indica* leaf, stem-bark and root-bark.

**MATERIALS AND METHODS**

**Plant material**

Fresh leaf, stem-bark and root-bark of mature *Mangifera indica*, were collected from Gerei, Adamawa State, Nigeria. They were identified and authenticated in the department of Plant Science, Modibbo Adama University of Technology, Yola, Nigeria in the month of August 2013. The samples were carefully washed under running tap water followed by...
distilled water. They were shed dried for fourteen days at room temperature to constant weight and pulverized to a fine powder using a sterilized mixer grinder KenwoodBL237.

**Preparation of Ethanol and Aqueous Extracts**

Two hundred grams of pulverized sample were mixed with ethanol, 1000 ml and placed on a magnetic stirrer with stirring for 24 h. The extracts were filtered using a sterilized Whatman® filter paper No. 1 to obtain a particle free extract. The samples were re-extracted with the solvent three times, filtered and the extract pooled. The extracts were concentrated using rotary evaporator (Cole-Parmer®) at less than 50°C. Extracts were made solvent free by final drying in oven at (40°C - 50°C) to obtain a powder or paste material.23

For the aqueous one, same Procedure was followed as used in ethanol the only difference was in solvent used which was water. The filtrate was evaporated to dryness in hot air oven and stored in refrigerator. The concentrated extracts were used for preliminary screening of phytochemicals as well as antioxidant activity evaluations.

**Phytochemical Screening**

The individual extracts were subjected to the qualitative phytochemical screening for the presence of alkaloids, saponins, tannins, steroids, flavonoids, phenols, terpenoids and cardiac glycosides. They were identified by characteristics colour change using standard procedures.24-26

**Quantitative determination of phenols and flavonoids**

**Preparation of fat free sample**

Two grams of the sample were defatted with 100 ml of diethyl ether (≥98.0%, Merck®) using a soxlet apparatus for two hours.

**Determination of total phenols by spectrophotometric method**

The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. Five ml of the extract was pipette into a 50 ml flask, then 10 ml of distilled water was added. Two ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol (≥99.0%, Merck®) was also added. The sample was made up to 50ml and reacted for 30 min for colour development. The absorbance was measured on the GC at 505 nm.25

**Determination of Extraction Yield**

Ten grams of the plant extract were extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed to a constant weight.

**Antioxidant activity assays**

**FRAP (Ferric Reducing Antioxidant Power) ASSAY**

The antioxidant activity of the plant extracts was estimated using FRAP27 with minor modifications. A stock solution of the extracts and standard ascorbic acid (≥99.0%, Merck®) were prepared in the concentration of 10 mg/ml. In the ferric reducing antioxidant power assay, 1 ml of test sample of both extract in different concentration was mixed with 1 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide in separate test tubes. The reaction mixtures were incubated in a temperature-controlled water bath at 50°C for 20 min followed by addition of 1 ml of 10% trichloroacetic acid (≥99.0%, Merck®). The mixtures were then centrifuged for 10 min at room temperature. The supernatant obtained (1 ml) was added to 1 ml of distilled water in a test tube and 0.2 ml of 0.1% FeCl3 (≥99.99%, Merck®) also added. The blank was prepared in the same manner as the samples except that the extract was replaced by distilled water. The absorbance of the reaction mixture was measured at 700 nm. The reducing power was expressed as an increase in A700 after blank subtraction.

**DPPH (2, 2'-diphenyl-1-picrylhydrazyl) assay**

The antioxidant activity of the plant extracts was also estimated using the DPPH radical scavenging27 with little modifications. DPPH solution (0.04% w/v) was prepared in 95% ethanol (≥99.8%, Merck®). A stock solution of the extracts and standard ascorbic acid were prepared in the concentration of 10 mg/ml. From stock solution 2ml, 4ml, 6ml, 8ml and 10ml of this solution was taken in five test tubes respectively. The final volume of each test tube was made up to 10 ml to give concentrations of 20 mg/ml, 40 mg/ml, 60 mg/ml, 80 mg/ml and 100 mg/ml respectively. Then, 2 ml of freshly prepared DPPH solution (0.04% w/v) was added to each of these test tubes. The reaction mixture was incubated in the dark for 15 min and thereafter the optical density was recorded against the blank at 523 nm. For the blank, 2 ml of DPPH solution in ethanol was mixed with 10 ml of ethanol and the optical density of the solution recorded after 30 min. The decrease in optical density of DPPH on addition of test samples in relation to the control was used to calculate the antioxidant activity, as percentage inhibition (% IP) of DPPH radical.

**TBARS (Thiobarbituric Reactive Substance) ASSAY**

TBA method used for evaluating the extent of lipid peroxidation. At low pH (2–3), and high temperature (100°C), melondialdehyde (MDA) binds TBA to form a red complex that can be measured spectrometrically at 532 nm. A volume of 2 ml of 20% trichloroacetic acid (TCA) and 2 ml of 0.67% TBA solutions were added to 2 ml of the mixtures containing 4 mg of the sample in 4 ml of 99.5% ethanol (final concentration 0.02%). This mixture was kept at 100°C for 10 min and after cooling to room temperature. It was centrifuged at 3000 rpm for 20 min. The antioxidant activity was based on the absorbance of the supernatant at 532 nm on the final day of the assay.27-28 The percentage of antioxidant activity was calculated by the following formulae for all the methods.

\[
\text{Percentage of antioxidant activity} = 100 \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}
\]

**Statistical Analysis**

Results were expressed as mean ± SEM. The statistical analysis of data were done using one – way analysis of variance (one – way ANOVA) with level of significance taken as p<0.05, with the aid of GraphPad Prism version 5.01 (GraphPad Software, San Diego, CA, USA).

**RESULTS**

**Phytochemical analysis**

The preliminary phytochemical analysis of the leaf extract revealed the presence of alkaloids, tannin, saponins, terpenoids, flavonoids, phenols and cardiac glycoside (Table 1). The stem-bark and root-bark revealed the presence of tannin, saponins, terpenoids, flavonoids, phenols and cardiac glycoside. The quantitative determination of both phenols and flavonoid is shown in Table 2. Matt. indica root-bark with 34.50 ± 0.42% showed highest amount of phenols and the leaf (4.8 ± 0.55%) recorded the highest flavonoids.

**Antioxidant activities**

The results of various antioxidant activities studied are shown in Tables 3 - 7. The values of the various antioxidant activities ranged from 20mg/ml - 100mg/ml. Mangifera indica ethanol root-bark extract has 68.67 ± 0.05 mg/ml as the highest activity and the lowest activity was recorded at 20 mg/ml (5.27 ± 0.33%) for the leaf (Table 3). Mangifera indica aqueous leaf extract has 94.49 ± 0.48% at 100 mg/ml as the highest activity and the lowest activity was recorded at 20 mg/ml (24.95 ± 0.50%)
Table 1: Qualitative Phytochemical Analysis of Different Parts of *Mangifera indica*.

<table>
<thead>
<tr>
<th>Plant (extracts)</th>
<th>Alkaloids</th>
<th>Tannin</th>
<th>Saponin</th>
<th>Terpenoids</th>
<th>Steroids</th>
<th>Flavonoids</th>
<th>Phenols</th>
<th>Cardiac glycoside</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. indica</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>leaf</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Stem bark</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Root bark</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

Key: + = Present, - = Absent

Table 2: Total Phenol and Flavoniod Contents of Different Parts of *Mangifera indica*.

<table>
<thead>
<tr>
<th>Plant (extracts)</th>
<th>Total phenol contents (%)</th>
<th>Flavonoid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. indica</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>leaf</td>
<td>33.75 ± 1.79</td>
<td>4.8 ± 0.55</td>
</tr>
<tr>
<td>Stem bark</td>
<td>34.00 ± 0.51</td>
<td>3.1 ± 0.06*</td>
</tr>
<tr>
<td>Root bark</td>
<td>34.50 ± 0.42</td>
<td>2.8 ± 0.15*</td>
</tr>
</tbody>
</table>

Results are mean ± SEM for three determinations

* Significantly (p<0.05) different when compared to leaf in the same column

Table 3: Antioxidant Activity (% Inhibitions) of the Ethanol Extracts in FRAP Assay.

<table>
<thead>
<tr>
<th>Plant (extracts)</th>
<th>20 mg/ml</th>
<th>40mg/ml</th>
<th>60mg/ml</th>
<th>80mg/ml</th>
<th>100mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. indica</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>leaf</td>
<td>5.27 ± 0.33</td>
<td>14.04 ± 0.27*</td>
<td>35.87 ± 0.59ab</td>
<td>41.90 ± .47a</td>
<td>45.07 ± 0.60abc</td>
</tr>
<tr>
<td>Stem bark</td>
<td>14.57 ± 0.64*</td>
<td>24.72 ± 0.37*</td>
<td>49.52 ± 0.51a</td>
<td>54.86 ± 1.56b</td>
<td>60.27 ± 0.59abc</td>
</tr>
<tr>
<td>Root bark</td>
<td>19.44 ± 0.36 ab</td>
<td>30.02 ± 0.39a</td>
<td>58.94 ± 0.51abk</td>
<td>61.81 ± 4.42abcd</td>
<td>68.67 ± 1.49abcd</td>
</tr>
</tbody>
</table>

Results are mean ± SEM for three determinations

* Significantly (p<0.05) higher when compared to 20 mg/ml in the same row

Table 4: Antioxidant Activity (% Inhibitions) of the Aqueous Extracts in FRAP Assay.

<table>
<thead>
<tr>
<th>Plant (extracts)</th>
<th>20 mg/ml</th>
<th>40mg/ml</th>
<th>60mg/ml</th>
<th>80mg/ml</th>
<th>100mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. indica</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>leaf</td>
<td>58.16 ± 0.48</td>
<td>58.86 ± 0.73</td>
<td>81.37 ± 0.51ab</td>
<td>86.55 ± 0.34ab</td>
<td>92.75 ± 0.48abcab</td>
</tr>
<tr>
<td>Stem bark</td>
<td>48.56 ± 0.62*</td>
<td>62.26 ± 0.67a</td>
<td>68.09 ± 0.58a</td>
<td>74.26 ± 0.52a</td>
<td>94.49 ± 0.48abcd</td>
</tr>
<tr>
<td>Root bark</td>
<td>24.95 ± 0.50ab</td>
<td>27.09 ± 0.53abk</td>
<td>34.70 ± 0.60abk</td>
<td>42.96 ± 0.56abk</td>
<td>48.04 ± 0.68abcdab</td>
</tr>
</tbody>
</table>

Results are mean ± SEM for three determinations

* Significantly (p<0.05) higher when compared to 20 mg/ml in the same row

Table 5: Antioxidant Activity (% Inhibitions) of the Ethanol Extracts in DPPH Assay.

<table>
<thead>
<tr>
<th>Plant (extracts)</th>
<th>20 mg/ml</th>
<th>40mg/ml</th>
<th>60mg/ml</th>
<th>80mg/ml</th>
<th>100mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. indica</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>leaf</td>
<td>5.27 ± 0.33</td>
<td>14.04 ± 0.27*</td>
<td>35.87 ± 0.59ab</td>
<td>41.90 ± .47a</td>
<td>45.07 ± 0.60abc</td>
</tr>
<tr>
<td>Stem bark</td>
<td>14.57 ± 0.64*</td>
<td>24.72 ± 0.37*</td>
<td>49.52 ± 0.51a</td>
<td>54.86 ± 1.56b</td>
<td>60.27 ± 0.59abc</td>
</tr>
<tr>
<td>Root bark</td>
<td>19.44 ± 0.36 ab</td>
<td>30.02 ± 0.39a</td>
<td>58.94 ± 0.51abk</td>
<td>61.81 ± 4.42abcd</td>
<td>68.67 ± 1.49abcd</td>
</tr>
</tbody>
</table>

Results are mean ± SEM for three determinations

* Significantly (p<0.05) higher when compared to 20 mg/ml in the same row

Table 6: Antioxidant Activity (% Inhibitions) of the Aqueous Extracts in DPPH Assay.

<table>
<thead>
<tr>
<th>Plant (extracts)</th>
<th>20 mg/ml</th>
<th>40mg/ml</th>
<th>60mg/ml</th>
<th>80mg/ml</th>
<th>100mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. indica</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>leaf</td>
<td>58.16 ± 0.48</td>
<td>58.86 ± 0.73</td>
<td>81.37 ± 0.51ab</td>
<td>86.55 ± 0.34ab</td>
<td>92.75 ± 0.48abcab</td>
</tr>
<tr>
<td>Stem bark</td>
<td>48.56 ± 0.62*</td>
<td>62.26 ± 0.67a</td>
<td>68.09 ± 0.58a</td>
<td>74.26 ± 0.52a</td>
<td>94.49 ± 0.48abcd</td>
</tr>
<tr>
<td>Root bark</td>
<td>24.95 ± 0.50ab</td>
<td>27.09 ± 0.53abk</td>
<td>34.70 ± 0.60abk</td>
<td>42.96 ± 0.56abk</td>
<td>48.04 ± 0.68abcdab</td>
</tr>
</tbody>
</table>

Results are mean ± SEM for three determinations

* Significantly (p<0.05) higher when compared to 20 mg/ml in the same row

Table 7: Antioxidant Activity (% Inhibitions) of the Ethanol Extracts in TBARS Assay.

<table>
<thead>
<tr>
<th>Plant (extracts)</th>
<th>20 mg/ml</th>
<th>40mg/ml</th>
<th>60mg/ml</th>
<th>80mg/ml</th>
<th>100mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. indica</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>leaf</td>
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<td>Root bark</td>
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<td>58.94 ± 0.51abk</td>
<td>61.81 ± 4.42abcd</td>
<td>68.67 ± 1.49abcd</td>
</tr>
</tbody>
</table>

Results are mean ± SEM for three determinations

* Significantly (p<0.05) higher when compared to 20 mg/ml in the same row

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*Mangifera indica* ethanol root-bark extract at 100 mg/ml showed best percentage DPPH scavenging activity with 80.70 ± 0.42% and the lowest activity was recorded at 20 mg/ml (41.64 ± 0.37%) for the stem-bark (Table 5). *Mangifera indica* aqueous leaf at 100 mg/ml showed the best percentage DPPH scavenging activity with value 71.45 ± 0.36% and the lowest activity was recorded at 20 mg/ml (0.70 ± 0.06%) for the stem-bark in DPPH assay (Table 6). The percentage inhibition of malondialdehyde (MDA) by the extract showed the following percentage inhibition compared to other parts of the plant in TBARS, at 100 mg/ml the ethanol extract of *M. indica* stem-bark has the highest activity with 99.62 ± 0.26% and in the aqueous extract, the root-bark showed the best activity with value 99.48 ± 0.33% (Table 7). Among the various antioxidant activities tested, TBARS assay
Bioactive compounds are increasingly being introduced in the treatment of cancer because of their accessibility, antioxidant activity with less adverse effects when compared with any traditional chemotherapeutic agents for prostate cancer prevention. Bioactive compounds also serve as health promoting compounds as a result of their anion activities at 100mg/ml were more effective compared with other activities.

**DISCUSSION**

Recently, due to the fear of after effects, people will often opt for natural plant products for cancer therapy, either as a complementary or an alternative therapy. Bioactive compounds are increasingly being introduced in the treatment of cancer because of their accessibility, antioxidant activity with less adverse effects when compared with any traditional chemotherapeutic agents for prostate cancer prevention. Bioactive compounds also serve as health promoting compounds as a result of their anion activities at 100mg/ml were more effective compared with other activities.

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to anticancer agents could enhance their pharma kinetic behavior as well as target specificity.

Thus, the presence these compounds in *Mangifera indica*, leaf, stem-bark and root-bark, may serve as a potential source of effective bioactive compounds for cancer prognosis and treatment. The reported pharmacological activities of major phytochemicals from *Mangifera indica* stem-bark, leaves, heartwood, roots and fruits include antioxidant,20,47 radioprotective,49 antitumor,51 immunomodulatory, anti-allergic, anti-inflammatory,49 antidiabetic,49 lipolytic , antitumor,51 antiviral, antifungal, antibacterial56 and antiparasitic properties,56 which may support the numerous traditional uses of the plant.

In the present study, all the plant parts investigated showed significant level of antioxidant activity, at the lowest concentration (i.e. 20 mg/ml). The activity increased as the concentration increased in all the assays, viz: FRAP, DPPH and TBARS, a finding in agreement with those of several other studies.46,47 Looking at the values of both the ethanol and aqueous extracts from FRAP, DPPH and TBARS assays at 100mg/ml, there are no significant differences and as such the finding disagrees which found that the antioxidant activity of plant extracts decline along with an increase in solvent polarity This could be due to the differences in solvent system ratios to water. The two solvents used in our study are 99% pure. The results of this work when comparing Tables 3 and 4 are in line with findings of Litescu et al.17 showed that FRAP assay provides fast and reliable results for plasma, single antioxidants in pure solution and for mixtures of antioxidants in aqueous solutions. However, the results obtained from this study may not compare well with the limited information in the previous study. This may be attributed to difference in solvent used to extract, pH as well as mechanisms of the redox potentials as well as assay type.

CONCLUSION

Data from this study show that aqueous and ethanol extracts of *Mangifera indica* leaf, stem-bark and root-bark in FRAP assay show high anti-oxidant activity. Also, ethanol extracts in DPPH assay is more effective. The data obtained from this study may not compare well with the limited information in the previous study. This may be attributed to difference in solvent used to extract, pH as well as mechanisms of the redox potentials as well as assay type.

ACKNOWLEDGEMENT

We are grateful to Dr. S. Sarkiyya, the head department of Biochemistry, School of Life Science Modibbo Adama University of Technology Yola for the kind permission to use his laboratory for this study. Also to the laboratory technician Mallam Raji Lawan Murtalan for his assistance in the laboratory during the experiment and the analysis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

2,2’-diphenyl-1-picrylhydrazyl (DPPH) and Thiobarbituric acid reactive substance (TBARS).

REFERENCES