Phytochemical Constituents and Comparative Antioxidative Effects of Some Medicinal Plants

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Authors’ contributions
This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study was carried out to compare the in-vitro antioxidant potentials, antidiabetic and phytochemical constituents of methanolic leaf extracts of Anthocleista djalonensis, Chrysophyllum albidium, Bauhinia thonningii, Daniellia oliveri, and Cola nitida. The results of this study show that all the plant extracts have strong antioxidant potentials against various radicals. The extracts scavenged DPPH and ABTS radicals, in a concentration-dependent manner and scavenged nitric oxide radicals with IC₅₀ values of 152.39, 186.36, 213.40, 303.58 and 355.53 µg/ml for C. albidium, D. oliveri, C. nitida, A. djalonensis and B. thonningii, respectively. All the extracts also inhibited the induction of lipid peroxidation and α-amylase activity in a concentration-dependent manner, while the degree of ferric reducing power by the extracts was of the order C. albidium > D. oliveri > B. thonningii > C. nitida > A. djalonensis. Phytochemical and gas chromatography analyses carried out on the extracts revealed the presence of known chemical constituents. The amounts of total

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phenolics in \textit{A. djalonensis}, \textit{C. albidium}, \textit{B. thonningii}, \textit{D. oliveri}, and \textit{C. nitida} were 68.39 mg/g, 95.11 mg/g, 61.03 mg/g, 103.74 mg/g, and 63.31 mg/g, respectively, in gallic acid equivalents. In all cell-free assays, \textit{C. albidium} and \textit{D. oliveri}, the two plants with higher amounts of phenolic compounds, were found to be more effective as antioxidants than other plant extracts with lower phenolic contents under the same experimental conditions. Therefore, the effectiveness of the antioxidant and antidiabetic activities of these plant extracts may be related to their phenolic content. The presence of phenolics and various antioxidant compounds in the plants may explain the strong pharmacological potentials of these plants.

\textit{Keywords: Antioxidant; lipid peroxidation; phytochemical constituents; gas-chromatography.}

1. INTRODUCTION

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are very important because of their vital roles in the host defence system and several biological processes [1, 2]. However, excessive production of these reactive species has been reported in the pathogenesis of a vast variety of conditions, such as diabetes mellitus, inflammatory diseases, ageing, atherosclerosis, hypertension, cancer and AIDS [1, 2]. The side effects of reactive species (ROS) can, however, be neutralized by the antioxidant defence system, which helps to remove reactive species, thereby protecting biological sites [3].

One major source of natural antioxidants is plants, and interest in phytochemicals of plant origin that serve as natural antioxidants has been increasing because they are viewed as potential therapeutic drugs for the treatment of reactive species-mediated diseases [4]. The risk of developing reactive species-mediated diseases is significantly reduced when the diet is plant-based with a high level of vegetables and fruits [5, 1]. Therefore, this study aimed to investigate the antioxidant effects of five selected plants, \textit{A. djalonensis}, \textit{C. albidium}, \textit{B. thonningii}, \textit{D. oliveri}, and \textit{C. nitida}, which are known to prevent some diseases in Africa.

\textit{Anthocleista djalonensis} (Longaniaceae) is a tall tree that can reach 15 metres in height. Its roots, leaves, and stem bark are used locally in southwestern Nigeria for treating different diseases, such as malaria, fungal skin infections and male infertility [6-8]. The roots of the plant are also used for treating intestinal problems, constipation and regulating menstruation [9]. \textit{Chrysophyllum albidum} Linn. (Sapotaceae), known locally in Nigeria as “Udara”, is used in folklore medicine for the treatment of diarrhoea and stomach ache [10]. Its leaf and stem bark have been demonstrated in many studies to have antiplatelet, antimicrobial, hypoglycaemic, antiplasmodial and antioxidant properties [11-14].

\textit{Bauhinia thonningii} Schum. (Cesalpiniaceae) is a shrub that can reach a height of 6 meters. Its leaves are used in traditional medicine to treat dysentery, malaria, gastric ulcers, sore throat and cough [15, 16]. Wound healing, as well as the antiviral and antifungal potentials of the plant, has been demonstrated in previous studies [17, 18]. \textit{Daniellia oliveri} (Caesalpiniaaceae) is a wood plant, and the root, bark and leaf extracts of the plant are used in folklore medicine in Nigeria to treat diabetes, gastrointestinal disturbances, yellow fever, and as a diuretic [19, 20]. The antioxidant and antimicrobial potentials of the leaf extract have been demonstrated in many in vitro and in vivo studies [21, 22].

\textit{Cola. nitida} (Sterculiaceae) is native to West Africa, where people chew nuts as a stimulant because of their caffeine content [23]. Cola nuts are known to prevent thirst, sleep, and hunger and act as antidepressants [24]. The antioxidant potential of cola nuts has been demonstrated in a previous study, and many phytochemicals, such as kolatin, \textit{d}-catechin, L-epicatechin, and theobromine, have been identified in nuts [25]. Based on the widely reported health benefits of the five selected plants, the present study aims to explore the antioxidant properties and phytochemical constituents of the leaf extracts of the selected medicinal plants.

2. MATERIALS AND METHODS

2.1 Reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, thiobarbituric acid, sodium carbonate, sodium nitroprusside, naphthylethylenediamine dichloride, \textit{\alpha}-amylase and potassium hexacyanoferrate, and trichloroacetic acid were obtained from Sigma–Aldrich Chemical Co. Ltd. (England). All other reagents and chemicals used were of analytical grade.
2.1 Plant materials and extract preparation

The leaves of the plants were collected from local farms at Ogbomoso, Oyo States, Nigeria. The plants are Anthocleista djalonensis, Chrysophyllum albidiun, Bauhinia thonningii, Daniellia oliveri, and Cola nitida. The plants were properly authenticated by Prof. Ogunkunle J.A. The sample of each plant was deposited in the herbarium of the Biology Department of Ladoke Akintola University of Technology, Ogbomosho, Nigeria with voucher numbers LHO402, LHO403, LHO405, LHO411 and LHO407 for each plant. The dried plant materials were ground and then extracted in absolute methanol (50 g in 250 mL). The mixtures were allowed to stand at room temperature for three days with occasional agitation, the mixture was filtered out using Whatman No 1 filter paper, and the filtrates were concentrated using a rotary evaporator.

2.1.2 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The assay was performed as previously described by Schlesier et al., [26]. In the DPPH assay, the radical solution was prepared by dissolving 2.4 mg DPPH in 100 mL of ethanol. Antioxidants reduce the free radical 2,2-diphenyl-1-picrylhydrazyl, which has an absorption maximum at 517 nm. DPPH (1.95 ml) was measured as a blank, and for the photometric assay, 1.95 ml DPPH solution and 50 μl antioxidant solution (plant extract) were mixed. The reaction was measured after 30 minutes until ΔA=0.003 min-1. The antioxidantive activity was calculated using the following equation:

\[
\% \text{ Inhibition activity} = \frac{A \text{ (DPPH)} - A \text{ (Extract)}}{A \text{ (DPPH)}} \times 100
\]

2.2 Trolox Equivalent Antioxidant Capacity Assay (ABTS)

The assay was performed essentially as described by Re et al., [27]. 1ml of freshly prepared ABTS solution and various concentrations of extract ranging from 20-100μL were mixed for 45 seconds and measured immediately after 1 minute at 734nm. The extract antioxidant activity was determined using the following equations:

\[
\% \text{ Inhibition activity} = \frac{A \text{ (ABTS)} - A \text{ (Extract)}}{A \text{ (ABTS)}} \times 100
\]

2.3 Ferric Reducing Antioxidant property (FRAP) Assay

The Fe³⁺ reducing power of the extract was determined by the method of Oyaizu [28]. Various concentrations of extract (0.75 mL) were mixed with 0.75 mL of potassium hexacyanoferrate [K₃Fe(CN)₆] (1%, w/v) and 0.75 mL of phosphate buffer (0.2 M, pH 6.6). The mixture was incubated for 20 min at 50 °C in a water bath. Then, 0.75 mL of 10% trichloroacetic acid (TCA) solution was added to stop the reaction, which was centrifuged for 10 min at 3000 r/min. The supernatant (1.5 mL) was mixed with 0.1 mL of ferric chloride (FeC₁₅) solution (0.1%, w/v) and 1.5 mL of distilled water for 10 min. The absorbance at 700 nm was measured as the reducing power, and the higher the absorbance was, the greater the reducing power.

2.4 Nitric Oxide Scavenging Activity

This was carried out using the method described by Garrat [29]. Two millititres of 10 mM sodium nitroprusside was dissolved in 0.5 ml phosphate buffer at pH 7.4. The dissolved mixture was mixed with 0.5 ml of S. siamea extract at ranging concentrations. The mixture was incubated at room temperature for two and a half hours. Then, 0.5 ml of incubated sample plus 0.5 ml of Griess reagent was re-incubated for thirty minutes, and the absorbance was measured at 546 nm. Percentage inhibition was calculated as:

\[
\text{Inhibition of NO radical} = \frac{A_o - A_s}{A_o} \times 100
\]

2.5 Lipid Peroxidation Inhibition Assay

This is a modified thiobarbituric acid reactive substance (TBARS) assay as described by Janero [30]. The lipid source was egg yolk homogenate, and Fenton Reagent (FeSO₄/ H₂O₂) was the source of free radicals. The reaction mixture containing 0.5 ml egg yolk homogenate (10% in distilled water V/V), 0.05 ml FeSO₄ (0.07 M) and 0.1 ml of the extract was incubated for 30 min. The absorbance was read at 532 nm, and the percentage induction of lipid peroxidation was 100% in the control, which was compared to the reduction in the plant extract samples.

2.6 Inhibition of Alpha-amylase

The determination of α-amylase inhibition was carried out using a modified dinitrosalicylic acid
(DNS) method previously described by Bernfeld [31]. One millilitre of methanolic extracts of plant extract was preincubated with 1 U/mL α-amylase for 30 min. One millilitre of starch solution (1% w/v) was added to the mixture and incubated for 10 min at 37 °C. Then, 1 mL DNS reagent (12.0 g of sodium potassium tartrate tetrahydrate in 8 mL of 2 M NaOH and 96 mM 3,5-dinitrosalicylic acid solution) was added to the reaction to stop it. This was followed by heating in a boiling water bath for 5 min. A control was prepared by using buffer (20 mM sodium phosphate buffer with 6.7 mM sodium chloride, pH 6.9 at 20 °C) instead of plant extracts. The absorbance was read at 540 nm.

\[
\text{% inhibition} = \frac{A_o - A_i}{A_o} \times 100
\]

Where \(A_o\) is the absorbance of the control and \(A_i\) is the absorbance of the sample.

2.7 Phytochemical Composition Screening

Qualitative analysis of the plant material methanolic extract was carried out by testing for the presence of flavonoids, terpenoids, tannins, phenols, saponins, phytosterol, alkaloids, phlobotannins and cardiac glycoside. Each assay was carried out following the method of Sofowora et al. [32] for flavonoids, Ejikeme et al. [33] for terpenoids and tannins, Santhi et al. [34] for phenol, Harbone et al. [35] for phytosterol and alkaloids, and Ajiboye et al. [36] for phlobotannins.

2.7.1 Determination of total phenolic compound in methanolic extract of plant materials

The total phenolics of the plant materials were determined by the Folin-Ciocalteu method as described by Miliauskas et al. [37]. For the preparation of the calibration curve, 1 ml Folin-Ciocalteu reagent (diluted tenfold) and 4 ml (75 g/L) sodium carbonate were mixed with 1 ml aliquots of 0.24, 0.075, 0.0105 and 0.3 mg/ml ethanol gallic acid solutions. The absorbance was read at 765 nm after 30 min, and a calibration curve was drawn. One millilitre Folin-Ciocalteu reagent and 4 ml (75 g/L) sodium carbonate were mixed with 1 ml of plant material, and after 30 minutes, the absorbance was read for the determination of plant phenolics. The total content of phenolic compounds in plant materials (gallic acid equivalent) was calculated using the following formula:

\[
C = \frac{c \cdot v}{m}
\]

Where \(C\) is the total content of phenolic compound (mg/g plant extract, in GAE), \(c\) is the concentration of Gallic acid established from the calibration curve in mg/ml, \(v\) is the volume of extract in ml, and \(m\) is the weight of pure plant extract.

2.8 Gas Chromatography Analysis

The gas chromatography (GC) study was performed using Shimadzu GC-17A gas chromatography fitted with a flame ionization detector (FID) and an autosampler. The GC column used was a fused silica capillary column OV-1, DB-1 (30 m x 0.53 mm, 0.5 μm film thickness), at 75 °C and programmed to 75 °C at 240 °C/min with a 5 min hold. The injector and detector were at 240 and 250 °C, respectively. Approximately 1 μL of each sample was injected, and the relative quantity of the chemical compounds present in the extract of plant materials was expressed as a percentage based on the peak area produced in the chromatogram. The identification of plant material constituents was carried out by comparison of GC retention times of plant materials with GC retention times of desired standard compounds.

3. RESULTS

3.1 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Activity

All the plant extracts demonstrated concentration- and time-dependent scavenging activity by quenching DPPH radicals (Figure 1). The scavenging potentials of the plant extracts were compared with gallic acid as a positive control. The IC50 values (defined as the concentration of test compound required to produce 50% inhibition) for DPPH scavenging of gallic acid and plant extracts were in decreasing order of gallic acid > C. albidium > D. oliveri > C. nitida > A. djalonensis > B. thonningii (Figure 1).

3.2 Ferric Reducing Antioxidant Power (FRAP) Assay

The reducing abilities of plant extracts were evaluated and compared with standard ascorbic acid. The reductive capabilities were found to increase with increasing concentrations of the plant extracts and standard ascorbic acid.
Comparatively, the reducing abilities of plant extracts were lowered compared with standard ascorbic acid in decreasing order of *C. albidium* > *D. oliveri* > *B. thonningii* > *C. nitida* > *A. djalonensis* at the same concentration and experimental conditions (Figure 2).

**Fig. 1.** The effects of different concentrations of plant extracts and Gallic on the inhibition of the DPPH radical.

**Fig. 2.** Ferric reducing power of plant extracts and ascorbic acid at different concentrations.
3.3 Lipid Peroxidation Inhibition Assay (TBARS)

The ability of different concentrations of plant extracts to reduce the induction of lipid peroxidation was compared with the control sample, where lipid peroxidation induction was 100%. The decrease in the induction of lipid peroxidation was observed with an increased concentration of plant extracts. The plant extracts inhibited the induction of lipid peroxidation at μg/ml concentrations in decreasing order of C. nitida > D. oliveri > C. albidium > A. djalonensis > B. thonningii (Fig. 3).

3.3.1 Nitric oxide scavenging activity

The nitric oxide scavenging abilities of plant extracts were compared with the standard ascorbic acid. All plant extracts demonstrated concentration-dependent scavenging abilities, with IC50 values (defined as the concentration of test compound required to produce 50% inhibition) for nitric oxide scavenging by ascorbic acid. C. albidium, D. oliveri, C. nitida, A. djalonensis and B. thonningii were 152.39 μg/ml, 186.36 μg/ml, 213.40 μg/ml, 303.58 μg/ml, 355.62 μg/ml and 1057.08 μg/ml, respectively (Fig. 4).

3.4 Inhibition of α-Amylase

All the plant extracts significantly inhibited α–amylase activity in this study. The level of inhibition was found to be concentration-dependent, and the maximum percentage inhibition of α–amylase activity obtained at 250 μg/dL for A. djalonensis, C. albidium, B. thonningii, D. oliveri, and C. nitida was 71.93%, 92.98%, 77.19%, 82.46% and 70.18%, respectively (Figure 5).

3.5 Phytochemical Analysis

Phytochemical analysis of the methanolic extract of A. djalonensis and D. oliveri indicated the presence of flavonoids, terpenoids tannins, phenol, phytosterol, saponins, flavonol and alkaloid. While C. albidium indicated the absence of flavonol and C. nitida only showed the presence of flavonoids, tannins, phenol, saponin and alkaloid (Table 1).
Fig. 4. The effects of different concentrations of plant extracts on the inhibition of Nitric oxide radical formation

Fig. 5. The effects of different concentrations of plant extracts on inhibition of α-amylase activity
Table 1. The result of phytochemical analysis on powder sample of plant extracts

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Test</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A. djalonensis</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>Alkaline reagent</td>
<td>Yellowish colouration</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Chloroform test</td>
<td>Reddish brown colouration</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric Chloride</td>
<td>Blue black colouration</td>
<td>+</td>
</tr>
<tr>
<td>Phenol</td>
<td>Ferric Chloride</td>
<td>Blue black colouration</td>
<td>+</td>
</tr>
<tr>
<td>Phytosterol</td>
<td>Chloroform</td>
<td>Formation of brown ring</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>Foam test</td>
<td>Formation of foam</td>
<td>+</td>
</tr>
<tr>
<td>Flavonol</td>
<td>Lead Acetate test</td>
<td>Yellowish precipitate</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>Mayer’s test</td>
<td>Cream colour precipitate</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 2. The total phenolic content of plant extracts

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>Total phenolic compounds mg/g plant extract (in GAE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. djalonensis</td>
<td>68.39 ± 2.17</td>
</tr>
<tr>
<td>C. albidium</td>
<td>95.11 ± 3.36</td>
</tr>
<tr>
<td>B. thonningii</td>
<td>61.03 ± 1.84</td>
</tr>
<tr>
<td>D. oliveri</td>
<td>103.74 ± 3.72</td>
</tr>
<tr>
<td>C. nitida</td>
<td>63.31 ± 2.33</td>
</tr>
</tbody>
</table>

3.5.1 Quantitative determination of total phenolic compound in plant methanolic extracts

The phenolic content of methanolic plant extracts was determined using a colorimetric assay (Folin-Ciocalteu) and by constructing a standard curve using gallic acid as the standard. The total amounts of phenolic compounds present in the methanolic extracts of A. djalonensis, C. albidium, B. thonningii, D. oliveri, and C. nitida were 68.39 mg/g, 95.11 mg/g, 61.03 mg/g, 103.74 mg/g, and 63.31 mg/g, respectively, in gallic acid equivalents (Table 2).

3.6 GC Analysis

The compounds present in methanolic extracts of A. djalonensis, C. albidium, B. thonningii, D. oliveri, and C. nitida are shown in Table 3. Their identification and characterization were based on their elution order in a GC column. The elution time and the names of compounds present in each of the plant extracts are presented in Table 3.

4. DISCUSSION

This work set out to evaluate the antioxidant activity of five medicinal plants using several in-vitro assays: DPPH radical scavenging activity, ferric reducing power activity, nitric oxide scavenging activity, lipid peroxidation inhibition activity and α-amylase inhibition activity. In the DPPH assay, the stable radical, which is violet in colour, changes to yellow after reduction owing to either hydrogen or electron transfer. Substances that possess this activity can be termed antioxidants and, therefore, free radical scavengers [38]. In the present study, the extracts of A. djalonensis, C. albidium, B. thonningii, D. oliveri, and C. nitida showed antioxidant potential and were able to scavenge DPPH radicals in a concentration- and time-dependent manner. The degree of DPPH radical scavenging was in the order C. albidium > D. oliveri > C. nitida > A. djalonensis > B. thonningii. Therefore, the extracts of the plant used in this study may serve as an important source of free radical scavengers that support its efficacy in managing different ailments.
Table 3. Biologically active chemical compounds present in plant extracts

<table>
<thead>
<tr>
<th>A. djalonensis constituents</th>
<th>Retention time</th>
<th>C. albidium constituents</th>
<th>Retention time</th>
<th>B. thonningii constituents</th>
<th>Retention time</th>
<th>D. oliveri constituents</th>
<th>Retention time</th>
<th>C. nitida constituents</th>
<th>Retention time</th>
<th>1-butanol</th>
<th>Retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinine</td>
<td>1.43</td>
<td>Thiamine</td>
<td>1.43</td>
<td>Vitamin E</td>
<td>5.06</td>
<td>Ascorbic acid</td>
<td>2.43</td>
<td>1-butanol</td>
<td>2.63</td>
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<tr>
<td>Amplexine</td>
<td>1.66</td>
<td>Beta-carotene</td>
<td>1.66</td>
<td>Ascorbic acid</td>
<td>5.80</td>
<td>Delphinidin</td>
<td>6.91</td>
<td>Caffeine</td>
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<tr>
<td>Brucine</td>
<td>1.86</td>
<td>Ascorbic acid</td>
<td>1.86</td>
<td>Beta carotene</td>
<td>6.48</td>
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<td>8.63</td>
<td>Quinine</td>
<td>4.20</td>
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<td>Djalonolenol</td>
<td>2.15</td>
<td>Pectin</td>
<td>2.15</td>
<td>C-methylflavonol</td>
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<td>Quercetin</td>
<td>10.21</td>
<td>Quinidine</td>
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<tr>
<td>Strychnine</td>
<td>5.06</td>
<td>Linoleic acid</td>
<td>5.06</td>
<td>I-tartaric acid</td>
<td>7.45</td>
<td>Quercitrin</td>
<td>11.68</td>
<td>Tannic acid</td>
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<td>Djalonesone</td>
<td>5.81</td>
<td>Anacadic acid</td>
<td>5.81</td>
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<td>Germacrene d</td>
<td>12.58</td>
<td>Quinoline</td>
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<td>Loganine</td>
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<td>Nicotinic acid</td>
<td>6.21</td>
<td>2-phenoxychromo</td>
<td>7.86</td>
<td>Catechin</td>
<td>13.05</td>
<td>Linoleic acid</td>
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<td>Loganoside</td>
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<td>Oleic acid</td>
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<td>D-cadinene</td>
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<td>9-octadecanoic acid</td>
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<tr>
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<td>B-3-o-methylchiroiol</td>
<td>13.11</td>
<td>Chlorogenic acid</td>
<td>17.83</td>
<td>Ephedrine</td>
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<td></td>
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<td>Teraxerol</td>
<td>13.31</td>
<td>Quercetin dehy</td>
<td>18.88</td>
<td>Nicotine</td>
<td>9.13</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Coumarin</td>
<td>19.88</td>
<td>Strychnine</td>
<td>9.41</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Alpha copaene</td>
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Owoade et al.; JOCAMR, 16(4): 121-133, 2021; Article no.JOCAMR.74793
The ability of selected plant extracts to reduce Fe$^{2+}$/Fe$^{3+}$ was determined by FRAP assay. The reducing ability of a compound is commonly linked to its electron transfer activity, thus highlighting its potential antioxidant power [39, 40]. All the plant extracts used in this study were found to have strong ferric reducing power, and this ability was found to be concentration-dependent. The degree of ferric reducing power was in the order C. albidium > D. oliveri > B. thonningii > C. nitida > A. djalonensis. Since the FRAP assay is easily reproducible and linearly related to the molar concentration of the antioxidants present, it can be reported that the selected plant extracts for this study were capable of donating electrons that reacted with free radicals, converting them into stable products.

Nitric oxide (NO) has been implicated in chronic inflammation, cancer and other pathological conditions [41]. NO inhibitors have been shown to have beneficial effects on some aspects of tissue inflammation and damage observed in models of inflammatory bowel disease. Plant and plant products may have the ability to counteract the effect of NO formation and, in turn, may be of considerable interest in preventing the effects of excessive NO generation in humans. The extracts of A. djalonensis, C. albidium, B. thonningii, D. oliveri, and C. nitida used in this study scavenge NO in a concentration-dependent manner. The nitric oxide scavenging abilities of selected plant extracts were compared with standard ascorbic acid, and the results showed that the selected plants have good antioxidant potential against NO formation.

Lipid peroxidation is a primary toxicological event because various reactive aldehydes generated from oxidized lipids react with cellular nucleophiles such as proteins, nucleic acids and glutathione (GSH) to form adducts that are related to various pathologies [42]. Lipid peroxidation is caused by the generation of free radicals from a variety of sources, including organic hydroperoxides, redox cycling compounds and iron-containing compounds [43]. Using the TBARS assay, extracts of A. djalonensis, C. albidium, B. thonningii, D. oliveri, and C. nitida were found to be capable of preventing the formation of malondialdehyde (MDA) in a concentration-dependent manner in this study by reducing the percentage induction of MDA when compared to the maximum induction of 100% observed in the control. The rate at which induction of MDA was inhibited by the extracts was in the order C. nitida > D. oliveri > C. albidium > A. djalonensis > B. thonningii.

Management of the blood glucose level is an essential approach in the control of diabetes complications. Inhibitors of carbohydrate hydrolysing enzymes (α-amylase) have been helpful as oral hypoglycemic medicines for the control of hyperglycemia exclusively in patients with type-2 diabetes mellitus [44]. In this study, all the plant extracts showed strong α-amylase inhibitory activity, which could be attributed to the presence of phenolic compounds in the plant extracts [45].

The antioxidant activities of plant extracts are usually linked to their phenolic content, although it is well accepted that nonphenolic antioxidants such as ascorbic acid, beta carotene, and vitamin E might also contribute to the antioxidant activity of plant extracts [46, 47]. Phenolic compounds are commonly found in the plant kingdom, and they have been reported to have multiple biological effects, including antioxidant activity [45]. In the present study, the amounts of phenolic compounds in the plant extracts were determined. All the plant extracts were found to contain a high amount of different kinds of phenolic compounds that were distributed differently. The quantity of phenolic compounds in the plants was in the decreasing order of D. oliveri > C. albidium > A. djalonensis > C. nitida > B. thonningii. Therefore, it was considered that the high antioxidant potential of all the plant extracts used in this study could be attributed to the high amount of phenolic compounds present in them.

The nature of phenolic compounds present in the plant extracts was analysed qualitatively. The study revealed the presence of flavonoids, tannins, phenols, saponins and alkaloids in all the plant extracts. Further screening of the extracts by gas chromatography revealed the presence of various phenolic compounds, ascorbic acid, beta carotene, and vitamin E in the plant extracts. In addition, the presence of various fatty acids, such as oleic acid and linolenic acid, was also identified in the extracts. The phenolic compounds identified in selected plant extracts as well as ascorbic acid and beta carotene are well known for their antioxidant properties; for example, ascorbic acid and vitamin E are well-known standard antioxidant compounds, and their radical scavenging potentials have been demonstrated in several studies [45-47], while beta carotene has strong
antioxidant potential due to its chemical structure and interaction with biological membranes [48, 49]. The fatty acids identified in some of the plant extracts have been reported to be effective against cardiovascular diseases [50].

A detailed look at all the cell-free antioxidant assays used in this study showed that *D. oliveri* and *C. albidium*, which have higher amounts of phenolic compounds than the remaining plant extracts, responded to have stronger antioxidants than other plant extracts in all the assays used in this study, suggesting that the antioxidant activity of the plant extracts may be associated with their phenolic content [47]. Therefore, it is proposed that the phenolic compounds of the plant extracts may play an important role in the observed antioxidant activities of the extracts.

Although the antioxidant potential of selected plant extracts has been ascribed to the polyphenol content of all the extracts in this study, other antioxidant compounds, such as ascorbic acid, vitamin E, and beta carotene, which are not subject to this study, may also contribute. Therefore, the antioxidant activity of all the plant extracts observed in this study can be traced to various antioxidant compounds and phytochemicals present in the plant extracts.

5. CONCLUSION

The evaluation of the antioxidant potentials of *A. djalonensis*, *C. albidium*, *B. thonningii*, *D. oliveri*, and *C. nitida* methanolic extracts proved that all the extracts have a strong antioxidant effect. There is substantial evidence that the induction of oxidative stress is a key process in the onset of many diseases. Therefore, the antioxidant activity of *A. djalonensis*, *C. albidium*, *B. thonningii*, *D. oliveri*, and *C. nitida* observed in this study may be due to the presence of various antioxidant compounds, such as beta carotene, vitamin E, ascorbic acid and phenolic compounds, which scavenge oxidant species.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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