Comparative determination of antioxidant activities and phytochemicals from fractions of ethanol extract of *Senna occidentalis* using GC-MS

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Abstract

Medicinal plants have been the mainstay of traditional herbal medicine. Their medicinal properties are due to the presence of phytochemicals which exert pharmacological effects on the body. In this study, phytochemicals from partially purified fractions of ethanolic extract of *Senna occidentalis* leaf were comparatively determined using GC-MS. Fractionation was carried out using solvents of different polarities, namely n-Hexane, Chloroform, Ethyl acetate, Ethanol, Methanol and water. The total phenolic content (TPC), Total flavonoids content, metal chelating activity of the fractions were determined. The result for Total Anti-Oxidant Capacity (TAC) showed that fractions 2a and 6a have the highest capacity (128.8 µg/ml), followed by 6b (112 µg/ml), 4a (86.8 µg/ml), 7a (84 µg/ml) and lastly 9a&2b (81.2 µg/ml). The decrease in the TAC of the fractions was in the order: 2a&6a>6b>4a>7a>9a&2b>3a&10a, with 3a and 10a having the lowest TAC. The result for Total Phenolic Content (TPC) showed that fraction 1b had the highest concentration of total phenol content (97.5 mg/ml), followed by F9b (78 mg/ml). F6b has the lowest concentration (1.95 mg/ml). The result for Total Flavonoids Content (TFC) shows that fraction 1a had the highest concentration (2373 mg/ml), followed by F3a (2226 mg/ml). Fractions F8a, 8b, F9a, F9b, and F10b had the lowest concentration (21.0 mg/ml). The fractions with higher TAC, TPC and TFC were subjected to GC-MS analysis, revealing compounds of diverse pharmacological and industrial applications. The fraction eluted with ethyl acetate + chloroform contained fatty acid esters with % Area ≥ 1 (Heptanoic acid octyl ester 55.42%), the fraction eluted with ethyl acetate + methanol contains both fatty acid esters and glycosides presented as % Area ≥1 (Cyclononene 23.70 %). The fraction eluted with Methanol showed high concentrations of essential oils presented as % Area ≥1 (Oleic Acid 59.57%). These properties of *Senna occidentalis* revealed that the fractions contain the active ingredients that are the major targets for drug development. The presence of high concentrations of flavonoids, phenolic compounds, fatty acids and essential oils in this plant does not only predispose it to a very good antioxidant property but can also can be used as a natural source of antioxidants towards the prevention of disease progression.
1. Introduction

Plants are important sources of drugs; especially in traditional medicine [1]. It is a common practice in Nigeria and other parts of the world to use plants in the form of crude extracts, decoction, infusion or tincture to treat common infections and chronic conditions [3]. According to the world health organization (WHO), over 70% of the world’s population rely on medicinal plants for primary health care and there are reports from various researchers about biologically active on natural substances of plant origin with desirable antimicrobial and antioxidant properties [2].

Despite tremendous progress in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health. The impact is particularly large in developing countries due to the relative unavailability of medicines and the emergence of widespread drug resistance [3]. The active principle of many drugs found in plants is phytochemicals [4]. The medicinal value of these phytochemicals is due to the presence of chemical substances that produce definite physiological action on the human body [4]. Some chemical substances of biological importance include; alkaloids, tannins, saponins, glycosides, flavonoids, phosphorus and calcium for cell growth, replacement, and body building [4]. During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics has led to the search for new antimicrobial agents mainly from plant extracts with the goal to discovering new chemical structures, which overcome the above disadvantages [6, 7]. Current research on natural compounds and products primarily focuses on plants since they can be sourced more easily and be selected based on their ethno-medicinal uses [8]. Interest has also increased recently in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants which are being restricted due to their carcinogenicity [9].

*Senna occidentalis* is a small shrub, about 3 feet high, belongs to the *Leguminosae* family. It is native to the tropical regions of America and naturalized in Australia, eastern Africa, southern and eastern USA [10]. It is part of continued efforts and mandate to investigate Nigeria’s medicinal floras, since the little studies carried out on this plant showed that the nature and amount of the phytochemicals vary according to the season and geographical location [11]. Despite the immense technology advancement in modern medicine, 75% of African populations still rely on traditional medicinal plants for their daily healthcare needs [11]. Medicinal plants are used in the treatment of diseases either alone or in combination with other plants. In recent years, some of the widely used African medicinal plants have been selected for investigation of their chemical constituents in an attempt to establish a scientific basis for their ethno medicinal uses [12]. *Senna occidentalis* is used as a remedy for typhoid fever. There is little or no knowledge of its toxicity in the study area therefore, it is paramount to analyse and determine the phytochemical contents and its antioxidant effects with the aim of providing a base line data for the plant medicinal properties in and around the study area.

2. Materials and Methods

2.1. Materials

The materials include mortar and pestle, Beakers, Conical flasks, Retort stand with clamps, Filter paper (Whatman No.1), Masking tape, Spatula, Rotary evaporator, Water Bath, Analytical Weighing balance, Measuring Cylinder, Mass spectrophotometer, volumetric flask, glass column.
2.2. Reagents
All reagents used were of analytical grade and include ethanol, methanol, ethyl acetate, chloroform, n-Hexane, silica gel, aluminium chloride (AlCl₃), trichloroacetic acid, tributyric acid, potassium phosphate, DPPH, ferrous chloride (FeCl₂), folin-ciocalteau, sodium carbonate.

2.3. Sample collection
Healthy looking and fresh leaves of *Senna occidentalis* were obtained from Wukari LGA, Taraba State.

2.4 Sample preparation
Only healthy leaves were used, as they were examined to be free from diseases. The leaves were air dried at room temperature for two weeks, to reduce the moisture content and to prevent enzyme action. The dried leaves were pulverized using mortar and pestle.

2.5 Ethanolic extraction
The air-dried, pulverized leaves were soaked in sufficient volume of ethanol for 24 hours at room temperature, at a ratio of 1:4 g/mL and continually stirred after every 5 hours. After 24 hours, the extracts were first filtered out using clean white sieving mesh and then with Whatman No 1. Filter paper. The ethanol used for extraction was recovered from the extract using Rotary evaporator. This helped to concentrate the extract which was dried using water bath.

2.6. Fractionation of Ethanolic extract
The dried ethanol extract was subjected to Column chromatography to separate the extract into its component fractions. Silica gel was used in packing the column while varying solvent combinations of increasing polarity were used as the mobile phase.

2.7. Packing of Column
This was done according to the method of Yakubu et al. [13]. The lower part of the glass column was stocked with glass wool with the aid of glass rod. 235g of silica gel of mesh size 60-200 was dissolved in 255mL of absolute n-Hexane to make the slurry (activation of silica gel). The chromatographic column (30mm diameter by 40mm height) was packed with silica gel and the solvent was allowed to freely flow into a conical flask. The column was held in a retort stand. At the end of the packing process, all taps were locked and the set up was allowed to stand for 24 hours to stabilize.

2.8. Elution
The method of Yakubu et al. [13] was adopted for the elution. 2g of the ethanol extract was dissolved in 15mL absolute ethanol and the solution was applied into the chromatographic column (30 mm in diameter and 400mm in height). Elution of the extract was done with solvent system of gradually increasing polarity, beginning with n-hexane, chloroform, ethlyacetate, methanol, ethanol and finally water. The following solvent ratio (V/V) in mL were sequentially used in the elution process:

- n-Hexane: chloroform 100:00, 50:50
- chloroform: ethylacetate 100:00, 50:50
- ethylacetate: methanol 100:00, 50:50
- methanol: ethanol 100:00, 50:50
- ethanol: distilled water 100:00, 50:50
- distilled water 100

A measured volume (300mL) of each solvent combination was poured into the column using a separatory funnel. The eluted fractions were collected in aliquots of 150mL using conical flasks.
2.9. Determination of total Antioxidant capacity (TAC)
DPPH radical scavenging activity was measured as per the procedure of Shimada et al. [14]. The absorbance was measured in triplicate for each fraction. Total antioxidant capacity (TAC) was calculated as mg/ml Trolox equivalent (TE) using the regression equation from the calibration curve.

\[
\% \text{ DPPH radical scavenging} = \left(\frac{A_C - A_t}{A_C}\right) \times 100
\]

- \(A_C\) is the absorbance of the control
- \(A_t\) is the absorbance of the test sample

2.10. Determination of total Phenolic content
Total phenolic content (TPC) of the extract was estimated following the phosphomolybdic/phosphotungstic acid complex procedure of Velioglu et al. [9]. Gallic acid was used as standard. Data for each concentration was recorded in triplicates.

2.11. Determination of total Flavonoid content
Flavonoids were determined using the aluminium chloride colometric method of Chang et al. [15]. Quercetin standard was used for derivation of the calibration curve. Total flavonoids were expressed as mg/ml quercetin equivalent (QE).

The concentration of flavonoid in the sample was estimated from the calibration curve.

2.12. Metal chelating Activity
The metal chelating activity of the extract fraction with ferrous ions were measured in triplicates following the method of Dinis et al. [16].

Measurements were carried out for all fractions, the chelating activity of the extract at different concentrations was calculated as follows:

\[
\% \text{ chelating activity} = \left(\frac{A_1 - A_2}{A_0}\right) \times 100
\]

Where \(A_0\) = Absorbance of the control (without extract);
\(A_1\) = Absorbance of reaction mixture, \(A_2\) = Absorbance without FeCl₂

3. Results
The Total flavonoids, Total phenols, Total Antioxidant Capacity (TAC), Anti-Lipid Peroxidation Inhibition Assay (TBARS) of \textit{Senna occidentalis} are stated below:

3.1. TAC (µg/ml)
The Total Anti-Oxidant Capacity (TAC) shows that fraction 2a and 6a have the highest capacity (128.8µg/ml), the next being 6b (112µg/ml), 4a (86.8 µg/ml), 7a (84 µg/ml) and 9a&2b (81.2µg/ml). The decrease in the TAC of the fractions is in order; 8a≥4a>2b≥5a>9a> with 3b and 10a having the lowest TAC. This is presented in fig 1 below:
Figure 1: Total Antioxidant Capacity

n-hexane:(100:00), 2=n-hexane/Chloroform (50:50), 3=Chloroform (100:00), 4=chloroform /ethyl acetate (50:50), 5=ethyl acetate (100:00), 6=ethyl acetate/ methanol (50:50), 7=Methanol (100:00), 8=methanol/ethanol (50:50), 9=ethanol (100:00), ethanol/water (50:50), 10=water (100:00).

3.2. Total Flavonoids Content
The result for Total Flavonoids Content (TFC) shows that fraction 1a has the highest concentration of total flavonoids content (2373mg/ml), followed by F3a (2226mg/ml). F8a, 8b, F9a, F9b, and F10b have the lowest concentration (21.0mg/ml). This is presented in fig 2 below:
Figure 2: Total Flavonoids Content

Fraction 1 = n-hexane (100:00), 2 = n-hexane/Chloroform (50:50), 3 = Chloroform (100:00), 4 = chloroform /ethyl acetate (50:50), 5 = ethyl acetate (100:00), 6 = ethyl acetate/ methanol (50:50), 7 = Methanol (100:00), 8 = methanol/ethanol (50:50), 9 = ethanol (100:00), ethanol/water (50:50), 10 = water (100:00).

3.3. Total phenol Content

The result for Total Phenol Content (TPC) shows that fraction 1b has the highest concentration of total phenol (97.5mg/ml), followed by F9b (78mg/ml). F6b has the lowest concentration (1.95mg/ml). This is presented in fig 3 below:
Fig 3: Total phenol Content

Fraction 1 = n-hexane (100:00), 2 = n-hexane/Chloroform (50:50), 3 = Chloroform (100:00), 4 = chloroform/ethyl acetate (50:50), 5 = ethyl acetate (100:00), 6 = ethyl acetate/methanol (50:50), 7 = Methanol (100:00), 8 = methanol/ethanol (50:50), 9 = ethanol (100:00), 10 = water (100:00).
3.4. Anti-lipid peroxidation inhibition Assay (TBARS)

The result of anti-lipid peroxidation inhibition assay (TBARS), after test on the liver homogenate of Wistar rat revealed that fractions 1a, 1b, 2b, 3a, 3b, 6a, and 6b have the highest anti-lipid peroxidation assay (97.1-97.3), followed by fractions 7a and 7b. The lowest anti-lipid peroxidation was observed in fraction 5a as shown below:

![Figure 4: Anti- Lipid Peroxidation Inhibition Assay](image)

\[\text{Fraction 1 = n-hexane:(100:00), 2 = n-hexane/Chloroform (50:50), 3 = Chloroform (100:00), 4 = chloroform/ethyl acetate (50:50), 5 = ethyl acetate (100:00), 6 = ethyl acetate/methanol (50:50), 7 = Methanol (100:00), 8 = methanol/ethanol (50:50), 9 = ethanol (100:00), ethanol/water (50:50), 10 = water (100:00).}\]
3.5. GC-MS Result of fractions of Ethanolic extract of *Senna occidentalis*

The fractions with high Total Antioxidant Activity were subjected to GC-MS. The compounds identified from the fraction eluted with were presented in the order of decreasing % Area, with Heptanoic acid octyl ester having the highest % Area of 55.42. These compounds are basically fatty acid esters.

Table 1: GC-MS scan for fraction 2 (n-Hexane + chloroform fraction)

<table>
<thead>
<tr>
<th>S/n</th>
<th>Rt</th>
<th>Compound</th>
<th>Chemical structure, formula and molecular weight</th>
<th>% Area</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>66.264</td>
<td>Heptanoic acid octyl ester</td>
<td>C7H14O2, 130.187</td>
<td>55.42</td>
<td>This compound serves as Membrane stabilizer, energy storage, nutrient.</td>
</tr>
<tr>
<td>2</td>
<td>66.768</td>
<td>15-Hydroxypentadecanoic acid</td>
<td>C15H30O3, 258.4</td>
<td>12.53</td>
<td>15-Hydroxypentadecanoic acid is suitable reagent used in the following studies: As an internal standard in the quantification of formation of 1-1 hydroxylauric acid by gas chromatography.</td>
</tr>
<tr>
<td>3</td>
<td>64.907</td>
<td>E-11-Tetradecenoic acid</td>
<td>C16H30O2, 254.41</td>
<td>7.16</td>
<td>Cytotoxic, apoptosis inducing effect, anti-microbial activity</td>
</tr>
<tr>
<td>4</td>
<td>56.068</td>
<td>3-Octen-1-ol</td>
<td>C8H16O, 128.21</td>
<td>6.37</td>
<td>Anti-microbial effect.</td>
</tr>
<tr>
<td>5</td>
<td>56.495</td>
<td>Hexanoic acid</td>
<td>C6H12O2, 116.1583</td>
<td>2.98</td>
<td>The primary use of hexanoic acid is in the manufacture of its esters for artificial flavors, and in the manufacture of hexyl derivatives, such as hexylphenols. It also has Antibacterial effect</td>
</tr>
<tr>
<td></td>
<td>Molecular Weight</td>
<td>Structure</td>
<td>Activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>------------------</td>
<td>-----------</td>
<td>---------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>64.365</td>
<td>Cyclopentaneundecanoic acid</td>
<td>Anti-fungal activity, Antioxidant activity.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C_{16}H_{30}O_{2}</td>
<td>268.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>62.543</td>
<td>Benzoxazol-3-carboxylic acid</td>
<td>Antimicrobial activity; anti-inflammatory activity; anti-diabetic activity; anti-cancer activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C_{8}H_{4}BrNO_{3}</td>
<td>163.13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>63.822</td>
<td>Tetraacetyl-d-xylonic nitrile</td>
<td>Antibacterial effect. This nitrile increases water solubility or decreases susceptibility to oxidative metabolism in the liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C_{14}H_{17}NO_{9}</td>
<td>343.28608</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>51.028</td>
<td>Cyclohexanobutanoic acid</td>
<td>Antimicrobial effect The acid is of considerable commercial importance as a raw material in the manufacture of esters of lower alcohols for use as flavouring agents; its anhydride is used to make cellulose butyrate, a useful plastic</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C_{10}H_{18}O_{2}</td>
<td>170.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>63.124</td>
<td>trans-Traumatic acid</td>
<td>This compound helps induce cell division, heal damaged tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C_{12}H_{20}O_{4}</td>
<td>228.285</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2: GC-MS scan for fraction 6 (Ethyl acetate + Methanol Fraction)

The compounds identified from the fraction eluted with Ethyl acetate + Methanol were presented in order of decreasing % Area, Cyclononene having the highest % Area.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Rt</th>
<th>Compound</th>
<th>% Area</th>
<th>Chemical structure, formula and molecular weight</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55.952</td>
<td>Cyclononene</td>
<td>23.70</td>
<td><img src="image" alt="Cyclohexane" /></td>
<td>Cytotoxicity, Antimicrobial effect.</td>
</tr>
<tr>
<td>2</td>
<td>56.378</td>
<td>alpha.-D-Glucopyranoside.</td>
<td>10.39</td>
<td><img src="image" alt="Alpha-D-Glucopyranoside" /></td>
<td>Anticancer effect.</td>
</tr>
<tr>
<td>3</td>
<td>64.442</td>
<td>Tetraacetyl-d-xylonic nitrile</td>
<td>8.23</td>
<td><img src="image" alt="Tetraacetyl-d-xylonic nitrile" /></td>
<td>Antibacterial activity.</td>
</tr>
<tr>
<td>4</td>
<td>64.907</td>
<td>8-Nonenoic acid</td>
<td>6.88</td>
<td><img src="image" alt="8-Nonenoic acid" /></td>
<td>Antifungal effect.</td>
</tr>
<tr>
<td>5</td>
<td>50.912</td>
<td>beta.-D-Glucopyranose</td>
<td>5.51</td>
<td><img src="image" alt="beta.-D-Glucopyranose" /></td>
<td>Anti-diabetic, antihypertensive antihyperlipidemia.</td>
</tr>
<tr>
<td>6</td>
<td>35.327</td>
<td>(Aminomethyl)cyclopropane</td>
<td>4.02</td>
<td><img src="image" alt="Aminomethyl)cyclopropane" /></td>
<td>Antidepressant effect.</td>
</tr>
<tr>
<td>7</td>
<td>63.783</td>
<td>Isobutyl nonyl carbonate</td>
<td>3.54</td>
<td><img src="image" alt="Isobutyl nonyl carbonate" /></td>
<td>this ester is mainly used in herbal composition</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>Value</td>
<td>Compound</td>
<td>Molecular Structure</td>
<td>Properties</td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>--------</td>
<td>---------------------------</td>
<td>---------------------</td>
<td>--------------------------</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>63.046</td>
<td>4-Tetradecene</td>
<td><img src="image" alt="Structure" /></td>
<td>258.4 s for cosmetic purposes.</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>62.504</td>
<td>Dichloroacetic acid,</td>
<td><img src="image" alt="Structure" /></td>
<td>Antimicrobial activity.</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>61.418</td>
<td>9-Oxabicyclo[6.1.0]nonane</td>
<td><img src="image" alt="Structure" /></td>
<td>Antioxidant</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>17.300</td>
<td>n-Heptyl acrylate</td>
<td><img src="image" alt="Structure" /></td>
<td>Antiinflammatory, antibacterial</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>12.570</td>
<td>Methanamine, N-methoxy-</td>
<td><img src="image" alt="Structure" /></td>
<td>This compound is used in the illicit production of methamphetamine.</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>15.206</td>
<td>3-Hexyn-1-ol</td>
<td><img src="image" alt="Structure" /></td>
<td>Antiproliferative</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>17.067</td>
<td>Cyclopropane, 1,1-dimethyl-</td>
<td><img src="image" alt="Structure" /></td>
<td>This compound is used as Intermediate for montelukast</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>61.883</td>
<td>4-Nonene, 5-nitro-</td>
<td><img src="image" alt="Structure" /></td>
<td>Antimicrobial</td>
<td></td>
</tr>
</tbody>
</table>
Table 3: GC-MS scan for fraction 7 (Methanol fraction)

The compounds identified from the methanol fraction were presented in order of decreasing % Area, with oleic acid having the highest % Area.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Rt</th>
<th>Compound</th>
<th>%Area</th>
<th>Chemical structure, formula and molecular weight</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>66.536</td>
<td>Oleic Acid</td>
<td>59.57</td>
<td>C_{18}H_{34}O_{2} 282.47</td>
<td>It is important for proper membrane fluidity, hormone responsiveness, infectivity of pathogens, mineral transport and immune competence. Anti-inflammatory effect.</td>
</tr>
<tr>
<td>2</td>
<td>56.029</td>
<td>7,11-Hexadecadienal</td>
<td>13.37</td>
<td>C_{16}H_{28}O 236.39</td>
<td>It serves as Pollinator, it is used in assessing insect population</td>
</tr>
<tr>
<td>3</td>
<td>56.456</td>
<td>D-Allose</td>
<td>6.57</td>
<td>C_{6}H_{12}O_{6} 180.1559</td>
<td>Serves as an immunosuppressant, could improve allograft survival and reduce tissue injury Antioxidant effect</td>
</tr>
<tr>
<td>4</td>
<td>64.403</td>
<td>Tetraacetyl-d-xylonic nitrile</td>
<td>2.98</td>
<td>C_{14}H_{17}NO_{9} 343.29</td>
<td>Antimicrobial activity the nitrile increases water solubility or decreases susceptibility to oxidative metabolism in the liver</td>
</tr>
<tr>
<td>5</td>
<td>64.985</td>
<td>2-Tetradecanol</td>
<td>2.82</td>
<td>C_{14}H_{30}O 214.393</td>
<td>used as an ingredient in cosmetics such as cold creams for its emollient properties</td>
</tr>
<tr>
<td>6</td>
<td>16.912</td>
<td>1,6-Dideoxy-l-mannitol</td>
<td>1.38</td>
<td>C_{6}H_{14}O_{4} 150.17</td>
<td>Antiglaucoma, osmotic diuretic effect It is used to reduce acutely raised intracranial pressure The use of mannitol, when inhaled, as a bronchial irritant as an alternative method of diagnosis of exercise-induced asthma has been proposed</td>
</tr>
</tbody>
</table>
4. Discussion

4.1. Total Antioxidant Capacity

In this study, the total antioxidant capacity of ethanol extract of *Senna occidentalis* leaf ranges from 56µg/ml-128.8µg/ml, as shown in (fig. 3.1). This shows that the ethanol extract of *Senna occidentalis* contains appreciable amounts of antioxidants. The result presented above has shown that some fractions of the ethanol extract of *Senna occidentalis* possess appreciable potency than others. The antioxidant activities of the fractions with DPPH free radical scavenging assay showed valuable results. DPPH, a stable free radical with characteristic absorption at 517nm, was used to study the radical scavenging effects of the extract. Iwueke and Nwodo, [17] stated that DPPH free radical scavenging by antioxidants is due to their hydrogen donating ability. Antioxidants scavenge or quench reactive oxygen species (ROS) and reactive nitrogen species (RNS) products of respiration, including free radicals. The terms “antioxidant activity” and “antioxidant capacity” have different meanings: antioxidant activity deals with the kinetics of a reaction between an antioxidant and the prooxidant or radical it reduces or scavenges, whereas antioxidant capacity measures the thermodynamic conversion efficiency of an oxidant probe upon reaction with an antioxidant. Measuring the antioxidant activity/capacity levels of food and biological fluids (e.g., human serum) is carried out for the meaningful comparison of the antioxidant content of foodstuffs and for the diagnosis and treatment of oxidative stress-associated diseases in clinical biochemistry [18].

4.2. Total Flavonoids

The total flavonoid content of the ethanol extract of *Senna occidentalis* ranged from 21mg/ml-2373mg/ml. The highest concentration was found in fraction 1a (2373 mg/ml) and the lowest concentration was found in fractions 8a, 8b, 9a, 9b, and 10a.(21mg/ml). The variation in the concentration of flavonoids in fractions of ethanol extract of *Senna occidentalis* might be due to the difference in polarity of the eluting solvents. The results of this study suggest that *Senna occidentalis* is rich in flavonoids and has a good antioxidant activity. It can be used as a natural source of antioxidants to prevent the progression of many diseases.
4.3. Total Phenolic Content

The total phenolic content (TPC) of *Senna occidentalis* ethanol extract range from 1.95mg/ml-97.5mg/ml. The highest amount of phenolic content was detected in fraction 1b. Ethanol is found to be a very good solvent for extraction of phenols and other antioxidants. The results of this study suggest that *Senna occidentalis* is rich in phenolic compounds and have a good antioxidant activity. It can be used as a natural source of antioxidants to prevent the progression of many diseases.

The ethanol extract of *Senna occidentalis* produced in-vitro anti-inflammatory activity that justifies its use in traditional system of medicine in Nigeria and some parts of Africa (West Africa). However, further detailed investigations are needed to ascertain the mechanisms and constituents behind its anti-inflammatory actions. Chakrabarty and Chawla, [19] reported that it exhibits stronger antioxidant activity as compared to α-tocopherol, this could be due to its high phenol concentration.

4.4. Metal chelating Activity

The metal chelating activity was determined according to the method of Dinis et al. [18]. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, complex formation is disrupted with the result that the red color of the complex is decreased. Measurement of colour reduction, allows the estimation of the chelating activity of the coexisting chelator. The transitional metal ion, Fe^{2+} possess the ability to move single electron by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals [20].

The main strategy to prevent ROS generation that is associated with redox active metal catalysis involves chelating of the metal ions. Fractions of the ethanol extract of the plant actively interfered with the formation of ferrous and ferrozine complex, suggesting that it has an appreciable level of chelating activity by capturing ferrous ion before ferrozine.

4.5. Anti-lipid peroxidation inhibition

The result of anti-lipid peroxidation inhibition assay (TBARS), after test on the liver homogenate of Wistar rat revealed that fraction 1a,1b, 2b,3a, 3b, 6a, and 6b have the highest anti-lipid peroxidation assay (97.1-97.3), followed by fraction(s) 7a and 7b. The lowest anti-lipid peroxidation was observed in fraction 5a. Thiobarbituric acid reactive substances (TBARS) are formed as a byproduct of lipid peroxidation (i.e. as degradation products of fats) which can be detected by the TBARS assay using thiobarbituric acid as a reagent. TBARS can be upregulated, for example, by heart attack [21]. Because reactive oxygen species (ROS) have extremely short half-lives, they are difficult to measure directly. Instead, what can be measured are several products of the damage produced by oxidative stress, such as TBARS [22]. Assay of TBARS measures malondialdehyde (MDA) present in the sample, as well as malondialdehyde generated from lipid hydroperoxides by the hydrolytic conditions of the reaction. MDA is one of several low-molecular-weight end products formed via the decomposition of certain primary and secondary lipid peroxidation products. However, only certain lipid peroxidation products generate MDA, which is neither the sole end product of fatty peroxide formation and decomposition, nor a substance generated exclusively through lipid peroxidation [23].

4.6. Compounds identified from GC-MS Result of Analysis

The compounds identified from the GC-MS analysis were presented in the order of decreasing % Area. The fraction eluted with ethyl acetate + chloroform contains fatty acid esters with %Area ≥ 1(Heptanoic
acid octyl ester 55.42%), the fraction eluted with ethyl acetate + methanol contains both fatty acid esters and glycosides presented as % Area ≥1(Cyclononene 23.70 %). The fraction eluted with Methanol showed high concentrations of essential oils presented as % Area ≥1 (Oleic Acid 59.57% which has anti-inflammatory effect). The anti-cancer activity of this plant is due to the presence of compounds such as 1,6-Dideoxy-1-mannitol, alpha.-D-Glucopyranoside, E-11-Tetradecenoic acid, etc.

Trans-traumatic acid is responsible for the wound healing effect of this plant. Beta.-D-Glucopyranose accounts for its anti-diabetic, antihypertensive (coma) antihyperlipidemia effects. The presence of (Aminomethyl)cyclopropane makes this plant a good antidepressant. D-Allose was identified which gives the plant a good antioxidant effect. Also, 2-Tetradecanol is used as an ingredient in cosmetics such as cold creams due its emollient properties. 7,11-Hexadecadienal serves as a pollinator, and in assessing insect population.

5. Conclusion
The different methods employed in the determination of phytochemicals from fractions of ethanol extract of Senna occidentalis, showed the presence of high content of flavonoids and phenolic compounds with attendant antioxidant properties for free radical scavenging, metal chelating, and anti-lipid peroxidation. The activities of these phytochemicals prevent the progression of myriad of diseases. There is also the presence of its protective bioactive compounds such as Oleic Acid, Heptanoic acid octyl ester, 8-Nonenoic acid etc. in appreciable amounts that enables it to exert pharmacological effects, one of which is effect against lipid peroxidation of biological membranes.

Data Availability
The data used to support the findings of this study are included within the article.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

References


