Phytochemical Screening and Antioxidant Activities of Leaf and Root Bark Extracts of *Combretum paniculatum*.

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**Abstract**

*Combretum paniculatum* (combretaceae), has been used widely in ethnomedicine in the treatment of chronic diarrhea and dysentery, flatulence, vomiting, colic, and enlarge spleen and liver. The leaf and root of *C. paniculatum* were pulverized and extracted by serial exhaustive method with solvents i.e chloroform, ethyl acetate and ethanol in order of increasing polarity, the extracts were investigated for their phytochemical composition and antioxidant activities. The results of the qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, steroids, phlobatannins and cardiac glycosides in both leaf and root extracts. The results obtained from quantitative phytochemical analysis shows that flavonoids has the highest yield of 21.00%, saponin 20.40%, alkaloids have the lowest percentage yield of 13.80%. The results of the Total Antioxidant Potential (TAP) revealed that ethanol leaf extract has the highest total antioxidant activity (TAP) followed by ethanol root extract. After 90 mins incubation, 80.2% of DPPH radicals were quenched by ethanol leaf extract.

**Keywords:** Phytochemical, antioxidant, qualitative, quantitative and *Combretum paniculatum*

**Introduction**

The use of plants as medicinal sources is as old as the history of man. It has been recognized for some time that people consuming diets rich in plant foods (e.g. fruits and vegetables and whole grain cereals) are at reduced risk of developing chronic diseases. These include cardiovascular disease (Dauchet et al., 2006), cancer (WHO, 2002) and other chronic conditions such as age-related eye conditions (Hogg and Chakravarthy 2004) and lung disorders (e.g. obstructive pulmonary disease). The relationship between plant food intake and health has been the focal point of much scientific investigation in recent years in trying to identify the specific plant components that convey health benefits. Recently, the search for specific plant components that convey health benefits has widened to encompass the vast range of 'non-nutritive' compounds present in plant foods, and their potential to improve health. Evidence is growing that most plant constituents, belonging to the group termed “bioactive compounds”, which may help to promote optimal health and to reduce the risk of chronic diseases such as cancer, coronary heart disease, stroke and perhaps Alzheimer’s disease, (Edeoga et al., 2005). Medicinal plants over the years have been known for their medicinal and nutritional value in relation to
their bioactive and antioxidant components, which can substitute most conventional drugs in their medicinal importance, however little research have been carried out on the phytochemical constituents of different most African-based plants parts, (Molyneux et al., 2007).

Many synthetic medicinal drugs has fail in their medicinal value as most diseases today turn to develop resistance to these synthetic drugs, however there is need to search for an alternative method to will-stand these diseases. *C. paniculatum* (called forest frame in English, Alo/Gusa in Tiv) is a shrub with leaves 3cm, deep root system with vivid scarlet flowers attaining 15 m length widely spread in tropical Africa along Benue valley. *C. paniculatum* (combretaceae), has been used widely in ethno medicine in the treatment of chronic diarrhea and dysentery, flatulence, vomiting, colic, and enlarge spleen and liver (Cheng et al; 2003).

**Materials and Methods**

**Sample Collection, Preparation and Extraction**
The leaves and the root of *C. paniculatum* plant were collected in the forest of Asogo, Usher, Shiakpev, of Yooyo ward in Katsina-ala Local Government Area of Benue state, Nigeria, and were properly identified in the Department of Biological Sciences, Federal University Wukari. The plant materials were air dried and pulverized using electric blender. The extracts were obtained by 250 g of the samples (leaves and stem each and separately) was soaked in 1250ml of ethyl acetate and 200 g in 750ml of ethanol for the root for 72 hours. The extracts was first filtered and the filtrate was evaporated and concentrated into solid extracts using rotatory evaporator, kept under room temperature overnight to remove all solvent. This process was repeated for ethanol. The extracts were kept refrigerated until required for analysis.

**Qualitative Phytocemical Screening**
The extract of each solvent was used to examine the presence of different phytochemical constituents.

**Test for Flavonoids**
The extract was treated with a few drops of FeCl₃ solution. Formation of a blackish red colour indicates the presence of flavonoids.

**Test for Tannins**
To 1 ml of the solvent extract, few drops of 1% FeCl₃ solution were added. The appearance of a blue, black, green precipitate indicated the presence of tannins.

**Test for Saponins**

**Froth test**
Extract were dilute with distilled water to 20 ml and this was shaken in graduated cylinder for 15 minutes. The formation of 1cm of foam indicates the presence of saponins.

**Foam test**
0.5ml of extract was shaken with 2ml of water. The foam produced persist for 10 minuntes indicates the presence of saponins.

**Test for steroids**
1ml of extract was treated with acetic acid follow by the addition of H₂SO₄(aq). A red colouration indicates the presence of steroids.

**Test for phlobatanins**
About 2ml of aqueous extract was added to 2ml of 1% HCl and the mixture was boiled. Deposition of a red precipitate was an evidence for the presence of phlobatanins.

**Test for glycosides**
Extract was dissolved in water, follow by addition of sodium hydroxide and the formation of yellow colour indicates the presence of glycosides.

**Test for alkaloid**

**Meyer's test**
Filterate were treated with Mayer’s reagent (potassium mercuric iodide). The formation of yellow colour indicates the presence of alkaloids.

**Wagner’s reagent**
Filterate were treated with Wagner’s reagent (iodine in potassium iodide). Formation of brown/redish precipitate indicate the presence of alkaloids.

**Dragendoff test**
Filteres were treated with Dragendroff’s reagent (solution of potassium bismuth iodide). Formation of red precipitate indicates the presence of alkaloids.

**Test for terpenoids**

**Salkowski test**
To 1ml of the solvent extract, 2ml of chloroform was added. Then 3ml of conc. H₂SO₄ was added carefully to form a layer. A reddish brown colouration of the interface indicated the presence of terpenoids.

**Determination of Total Antioxidant Activities**
Radical scavenging activity and the presence of hydrogen donors in the extracts of *C. paniculatum* were examined using the DPPH method by reduction of methanol according to Ramadan et al., (2003). A methanolic solution of DPPH radicals was freshly
prepared at a concentration of $10^4 \text{M}$. The radical in the absence of antioxidant compound, was stable for more than 3 hours of normal kinetic assay. For evaluation, 100 µl of each sample was mixed with 500 µl methanolic solution of DPPH radicals ($10^4 \text{M}$) and the mixtures were vortexed for 10 s at ambient temperature. Against a blank of methanol without DPPH, the decrease in absorption at 517 nm was measured in 1 cm quartz cells after 30, 60 and 90 minutes using a UV-260 visible recording spectrophotometer (Shimadzu, Kyoto, Japan). Antiradical action toward DPPH radical was estimated from the difference in absorbance with or without the sample (control) and the percentage of inhibition was calculated from the following equation:

$$\%\text{ inhibition} = \frac{\text{absorbance of control} - \text{absorbance of the test sample}}{\text{absorbance of control}} \times 100.$$ 

The variation in the DPPH values for replicates was always between 3 to 10% relative standard deviation (RSD). When the RSD was higher than 10%, the analyses were repeated to confirm the value. The samples of each item were analysed and the main values as well as the SD were given.

**Quantitative Analysis of detected Phytochemicals.**

**Determination of Flavonoid**

Five grams of crude leaves and one gram of crude root powder was taken in a 250 ml conical flask and 50 ml and 10 ml of 70% ethanol was added to it. Magnetic stirrer was used to mix the solution for 3 h and filtration of the solution was done using Whatman number 1 filter paper. The remaining powdered material was re-extracted once again with 70% ethanol and filtered in a similar way. Both the filtrate were mixed and transferred into a crucible and evaporated to dryness in a hot water bath of 40°C and weighted.

**Determination of Saponin**

Five grams of 9.33% yield of crude leaves and one gram of 6.87% yield of crude root powder was taken in a 250 ml conical flask and 50 ml and 10 ml of 20% ethyl acetate was added to it. The mixture was heated in a hot water bath of 55°C for 5 h with continuous stirring. The mixture was filtered using Whatman number 1 filter paper and the supernatant liquid was separated. The solid residue was mixed with 20% ethyl acetate and heated in similar way for about 5 h, the solution was filtered and mixed with previously filtered solution. The combined filtered solution was placed on a hot water bath of 40°C and heated still the volume was reduced to 20% of its initial volume. The concentrated sample was transferred into a 250 ml separating funnel and 10 ml of diethyl ether was added to it and shaken vigorously. The aqueous layer was separated carefully after settling down the solution. The purification process repeated again. Sixty milliliters of ethanol extracts were washed twice with 10 ml of 5% aqueous NaCl solution. The remaining solution was heated in a water bath at 40°C until the solvent evaporates and then solution turns into semi dried form. The sample was then dried in an oven. The saponin content was calculated by the following equations:

$$\text{Percentage of saponin} = \frac{\text{WEP}}{\text{WS}} \times 100$$

where, WEP= Weight of oven dried end product; WS= Weight of powdered sample taken for test.

**Determination of Alkaloid**

Five grams of 10.15% yield of crude leaves and one gram of 7.05% yield of crude root powder was taken in a 250 ml conical flask and 125 ml and 25 ml of 20% CH$_3$COOH in ethanol was added to it. Magnetic stirrer was used to mix the solution for 10 h at room temperature. The solution was done using Whatman number 1 filter paper and the resultant was placed on a hot water bath (40°C) until the extract volume turns 1/4th of its initial volume. Concentrated NH$_4$OH was added drop wise which forms thick precipitate. NH$_4$OH was added till the formation of the precipitate was complete. The whole solution was allowed to settle down. The precipitate was collected by filtration, dried in an oven and weighted.

**Results and Discussion**

**Table 1: Percentage Yield of the Plant Combretum Paniculatum.**

<table>
<thead>
<tr>
<th>S/N</th>
<th>Sample</th>
<th>Weight of Plant (g)</th>
<th>Weight of Extract (g)</th>
<th>Percentage Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethanol leaf</td>
<td>250</td>
<td>25.38</td>
<td>10.15%</td>
</tr>
<tr>
<td>2</td>
<td>Ethanol root</td>
<td>200</td>
<td>14.09</td>
<td>7.05%</td>
</tr>
<tr>
<td>3</td>
<td>Ethylacetate leaf</td>
<td>250</td>
<td>23.33</td>
<td>9.33%</td>
</tr>
<tr>
<td>4</td>
<td>Ethylacetate root</td>
<td>200</td>
<td>13.74</td>
<td>6.87%</td>
</tr>
</tbody>
</table>
Table 2: Results of Quantitative Phytochemical Result of *Combretum paniculatum* Crude Extract, (Leaf)

<table>
<thead>
<tr>
<th>S/N</th>
<th>Test(sample)</th>
<th>Weight of Crude(g)</th>
<th>Weight of dried filtrate</th>
<th>Concentration mol/dm³</th>
<th>% crude calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flavanoids</td>
<td>5.00</td>
<td>1.05</td>
<td>0.210</td>
<td>21.00</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloid</td>
<td>5.00</td>
<td>0.69</td>
<td>0.138</td>
<td>13.80</td>
</tr>
<tr>
<td>3</td>
<td>Saponins</td>
<td>5.00</td>
<td>1.02</td>
<td>0.204</td>
<td>20.40</td>
</tr>
</tbody>
</table>

Table 3: Result obtained from qualitative phytochemical of *Combretum paniculatum*

<table>
<thead>
<tr>
<th>S/N</th>
<th>Phytochemicals</th>
<th>Tests</th>
<th>ELE</th>
<th>ERE</th>
<th>EALE</th>
<th>EARE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flavanoids</td>
<td>Ferric test</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Tannins</td>
<td>Extract+few drops of 1% FeCl₃</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Saponins</td>
<td>Front test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Steroids</td>
<td>Extract+acetic acid +H₂SO₄</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Phlobotannins</td>
<td>Extract+1% HCl in Boiling water</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Glycoceids</td>
<td>Extract+H₂O+NaOH</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Alkaloids</td>
<td>Mayer's</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wagner</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dragendroff's</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Terpenoids</td>
<td>Salkowski Test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

**Keys:** + = presence of phytochemical, - = absence of phytochemical. ELE = Ethanol leaf extract, ERE = Ethanol root extract, EALE = Ethyl acetate leaf extract, EARE = Ethyl acetate root extract.

Table 4: Results of Quantitative Phytochemical Result of *Combretum paniculatum* Crude Extract, (Root).

<table>
<thead>
<tr>
<th>S/N</th>
<th>Test(sample)</th>
<th>Weight of Crude(g)</th>
<th>Weight of dried filtrate</th>
<th>Concentration mol/dm³</th>
<th>% crude calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flavanoids</td>
<td>1.00</td>
<td>0.02</td>
<td>0.020</td>
<td>2.00</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloid</td>
<td>2.00</td>
<td>0.17</td>
<td>0.085</td>
<td>8.50</td>
</tr>
<tr>
<td>3</td>
<td>Saponins</td>
<td>1.00</td>
<td>0.04</td>
<td>0.040</td>
<td>4.00</td>
</tr>
</tbody>
</table>

Table 5: result of Total Antioxidant Concentration of *Combretum paniculatum* of crude extract.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>30 mins</th>
<th>60 mins</th>
<th>90 mins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate leaf</td>
<td>38.42 ± 0.05</td>
<td>24.50 ± 0.03</td>
<td>19.80 ± 0.03</td>
</tr>
<tr>
<td>Ethanol leaf</td>
<td>20.60 ± 0.03</td>
<td>16.50 ± 0.02</td>
<td>10.30 ± 0.02</td>
</tr>
<tr>
<td>Ethanol root</td>
<td>37.20 ± 0.04</td>
<td>30.60 ± 0.02</td>
<td>23.40 ± 0.03</td>
</tr>
<tr>
<td>Ethyl acetate root</td>
<td>62.64 ± 0.04</td>
<td>48.22 ± 0.02</td>
<td>36.90 ± 0.02</td>
</tr>
</tbody>
</table>

**Key:** TAC = Total Antioxidant Concentration

The results for the preliminary qualitative phytochemical screening of the *C. paniculatum* extract is presented in table 2. The results showed the presence of tannins, steroids and alkaloids in all the extracts (ethanol leaf and root extract and ethyl acetate leaf and root extracts). Flavanoids were present in ethanol leaf extract, ethyl acetate leaf extract and ethyl acetate root extract but was absent in ethanol root extract. Saponins were present in ethanol leaf and root extract but were absent in both ethyl acetate leaf and root extract. Phlobotannins were...
present in ethanol leaf and roots extracts but were absent ethanol leaf and ethyl acetate extracts. Glycosides were found in both ethanol leaf and ethyl acetate root extract. These are indications that *C. paniculatum* is very rich in secondary metabolites, which are probable medicinal active constituents.

*C. paniculatum* has been reported to have anti-inflammatory, antispasmodic, anti-cancer, anti-HIV (1&2), and analgesic agent. This attributed to the presence of flavanoids, alkaloids, glycosides, steroids and saponins in their compositions (Savithramma et al., 2010). Saponins are precursors of important therapeutic drugs such as cortisomes and contraceptive estrogens (Ramadan et al., 1993). Saponins are mild detergents used in intracellular histochemistry staining to allow antibody access to intracellular proteins. The saponins are antioxidant, anticancer, anti-inflammatory activities and weight loss (Masola et al., 2009).

*C. paniculatum* can be used as an analgesic, anaesthetics and as social drugs in view of the fact that it contains alkaloids. Alkaloids content in plants are used in medicine as anaesthetics agents (Herourat et al., 1988). Harbone in (1988) also reported on the analgesic properties of alkaloids. Alkaloid has contributed to the majority of the poisons neurotoxins and traditional psychedelics and social drugs (e.g. Nicotine, caffeine, methamphetamine (ephedrine), cocaine, and opiates consumed by humans).

Flavanoids were detected in the extract, according to Okoli and Okere (2010), flavanoids are potent water soluble super antioxidants and free radical scavengers which present oxidative cells damage, have strong anti-cancer activities and inhibit tumor growth. *C. paniculatum* is important in pharmacy because it contains steroidal compounds. Okwu (2001) reported that steroidal compounds are of important and interest in pharmacy due to their relationship with sex hormones known to effect the development and control of the reproductive tracts in humans and molt insects.

Pharmacologically, glycosides have been found to be useful in the treatment of several illnesses for instant cardiac glycoside have long been employed as important ingredients for arrow poisons and drugs (Trease and Evans, 1989). The presence of terpenoids that have carboxylic acids groups could also be responsible for the active of the organic extract of *C. paniculatum* (Njoku and Obi, 2009). Phlobatanins have been reported for it wound healing properties. They also have anti-inflammatory and analgesic and antioxidant properties (Samdumu, 2007).

The results of the quantitative phytochemical analysis are presented in table 3 and 4 and analysis were carried out on three detected phytochemicals namely; alkaloids, flavonoids and saponins. The quantitative estimation reveals that, these secondary metabolites are present in different amount in the leaves of the plant. The quantitative results obtained from quantitative phytochemical analysis shows that flavonoids has the highest yield of 21.00%, saponin 20.40%, alkaloids have the lowest percentage yield of 13.80%. Saponins are very important as they are shown to also have hypolipidemic and anti-cancer activity. The natural anti-cancer agent saponin reacts with cholesterol rich plasma membrane of various cancer cells and arrests their proliferation (Rao et al., 1995). The high level of this saponins and flavonoids in the leaves and roots of *C. paniculatum* indicates that the plant can be use to produce anti-cancer and other drugs. Flavonoids and alkaloids contribute various medicinal properties such as analgesic, anti-oxidant and astringent activity (Cheng et al., 1998).

The antioxidant activities of the crude extracts of *C. paniculatum* is presented in table 3. The antioxidant activity of different plant extracts from *C. Paniculatum* was determined using a methanol solution of DPPH reagent. DPPH is a stable radical. A freshly prepared DPPH solution exhibits a deep purple colour with an absorbance maximum at 517 nm. This purple colour generally fades when antioxidant molecules quench DPPH free radicals resulting in decrease in absorbance at 517nm (Amarowicz et al., 2003). The results revealed that ethanol leaf extract has the highest total antioxidant activity (TAP) followed by ethanol root extract. After 90 minutes incubation, 80.2% of DPPH radicals were quenched by ethanol leaf extract. The extracts that perform the highest antioxidant activity have the highest concentration of phenols due to their hydroxyl groups (Tosun et al., 2009).

**Conclusion**

This present study showed that *C. paniculatum* is very rich in secondary metabolites, which are probable medicinal active constituents which have antioxidant potentials and can be employed in the treatment of various ailments claimed by ethno-medicinal practice, hence the study is justified the use *C. paniculatum* by traditional medicine.

**Conflict of interest**
The authors have declared no conflict of interest in the course of the research.

References


