A Preliminary Study on the Nutraceutical Potentials of Aqueous Fruit Pulp Extracts of *Zizipus spina christii*, *Zizipus mauritiana* and *Chrysophyllum albidum*

Aliyu Daja¹*, Zainab Kassim Mohammed¹, Teddy Christopher Igein¹, Abubakar Danladi Yusuf¹

¹- Department of Biochemistry, Faculty of Science, University of Maiduguri, Nigeria  
*Correspondence: aliyudaja@unimaid.edu.ng

Abstract

Foods and/or food parts such as fruits are continually being investigated for their nutritional and medicinal benefits often because of their relative safety. This study was designed to investigate the aqueous fruit pulp extracts of *Zizipus spina christii*, *Zizipus mauritiana* and *Chrysophyllum albidum* for their phytochemical properties and probable antioxidant activities of the plants.

Established colorimetric methods were used to investigate the antioxidants and the quantitative phytochemicals of the extracts.

The study revealed the presence of alkaloids, flavonoids and phenolics in all the three extracts, tannins is lacking in *Chrysophyllum albidum* while all the extract were tested negative for saponins. *Z. mauritiania* had the highest flavonoids and total phenolic contents with values of 87.03 ± 0.02 mg/g QE and 65.76 ± 0.03 mg/g GAE respectively. *Z. spina christii* showed the highest vitamin C content 409.20 mg/100g, while, *Z. mauritiania* had the least 48.4 mg/100g of the three extracts. *C. Albidum* exhibited significantly (p<0.05) potent total antioxidant capacity compared to α-tocopherol (vitamin E) a known antioxidant. All the three extracts exhibited the ability of ferric reducing power compared to ascorbic acid (vitamin C).

This work has shown the fruits extracts of *C. albidum*, *Z. spina christii* and *Z. mauritiania* to be of promising nutraceutical potentials as likely new sources of raw materials for industries requiring antioxidants as medicinal and therapeutic agents.

Key words: Antioxidants, nutraceuticals, *C. albidum*, *Z. spina christii* and *Z. mauritiania*

Introduction

The term ‘nutraceuticals’ is often used to describe any food that confers extra health benefits in addition to the basic nutritional value found in them. Most of the nutraceuticals and natural products are obtained from plants and animals. Example, lycopene
extracted from plants, carnitine, creatine and carotenoids produced by fermentation (Dutta et al., 2017). They can be considered nonspecific biological therapies that are used to promote general wellbeing, control symptoms and prevent malignant processes (Meštrović, 2015). Foods and/or food parts such as fruits are continually being investigated for their nutritional and medicinal benefits, this is because they possess various chemicals such as antioxidants and polyphenols, that are beneficial to health particularly in preventing or retrogressing the disease conditions such as headache stress, anxiety, hypertension, Alzheimer disease, Parkinson disease and cancer (Keservani et al., 2016: Meštrović 2015).

Fruits are nutritionally dense food products that are in use from time immemorial by different cultures of the world to improve certain chronic health problems (Dutta et al., 2017). These fruits if properly harnessed can play a pivotal role in the therapeutic and nutraceutical industries. Zizipus spina christii (‘Kurna’ in Hausa), Zizipus mauritiana (‘Magariya’ in Hausa) and Chrysophyllum albidum (‘Agwaluba’ in Hausa) are a seasonal fruits that are commonly and mostly consumed by women and children. These set of fruits are reported to have different and several traditional medicinal benefits. C. Albidum for example improves breast milk formation in lactating mothers (Egunyomi and Oladunjoye, 2012). Both Z. spina christii and Z. mauritiana are widely reported to have folkloric medicinal benefit which include wound healing when applied to cuts and ulcer, treatment of pulmonary ailments, fever and gastrointestinal problems such as dysentery (Abalaka et al., 2010: Asgarpanah and Haghighat, 2012). There are scientific literatures that indicate the three plants to have antidiabetes, anti-inflammatory, antioxidant and antimicrobial potentials (Asgarpanah and Haghighat, 2012; Orijajogoun et al., 2013 and Bukar et al., 2015). However, most of these scientific reports are on the extracts of the stem, bark, root or leaves, there is very scanty report on the extracts of the edible fruit pulps especially of the ones grown in tropical Africa like Nigeria. Therefore, this preliminary study is aimed at evaluating the nutraceutical potentials of Zizipus spina christii, Zizipus mauritiana and Chrysophyllum albidum aqueous fruit pulp extracts.

Materials and Methods

Sample collection

Fresh samples of the C.albidum, Z. spina christii, and Z. mauritiana were purchased from Gomboru market of Maiduguri, Borno state, Nigeria. After collection of the fresh samples, the rotten and spoiled ones were separated from the good
ones and were rinsed with tap water to remove dirt. The fleshy pulps of *C. Albidum* were separated from the whole fruits by hand while that of *Z. spinacristii, and Z. mauritiana* was done with pestle and mortar. All samples were shade dried at room temperature.

**Sample Extraction**

Cold maceration method of extraction was carried out at the research laboratory, Faculty of Science, Department of Biochemistry, University of Maiduguri, Borno state, Nigeria. Powdered samples were measured in quantities of 10 g and dissolved into 1L (in ratio of 1:10) of distilled water and stirred at an interval of 5 minutes for about 3 hours. It was then allowed to settle for 24 hours. The solution was filtered using fine cloth and the solute was discarded. The solvent was evaporated using a hotplate at 40°C until the water content evaporated leaving the sample extract. The extract was then collected into a brown container and labelled.

**Qualitative phytochemical analysis**

Phytochemical analysis of the sample was performed according to the methods described below:

**Test for tannins**

The plant extract (200 mg) was added to 10 ml of distilled water. The process was immediately followed by filtration; to the 2 ml of the filtrate, 2 ml of 10% ferric chloride (FeCl₃) was added. Positive test was indicated by a blue-black precipitate (Trease and Evans 1996).

**Test for alkaloids**

Exactly 200 mg of extract was added to 10ml methanol before subsequent shake and filtration. To 2 ml of the filtrate, 1 ml of 1% HCl was added before the mixture was then boiled for 5 minutes, filtered and 6 drops of Mayer’s reagent were added to 1 ml of the filtrate. A positive test was indicated by a creamy precipitate (Trease and Evans 1996).

**Test for saponins**

The extract was subjected to frothing test. The filtrate (0.5ml) and 5ml distilled water was taken and shaken in a test tube. When frothing or foaming persist for 5 minutes, it indicated the presence of saponins (Awe and Sodipo 2001).

**Test for flavonoids**

The presence of flavonoids was determined by adding 200 mg of plant extract to 10 ml ethanol and filtered. Two millilitre of filtrate and 5 drops of concentrated HCl and magnesium chips (13 pieces) were taken into a test tube and mixed. Presence of flavonoids was indicated by a pink tomato colour (Trease and Evans 1996).
Quantitative Phytochemical Analysis

Phyto-constituents of the sample were quantitatively estimated according to the methods described below:

**Determination of Total Phenolic Contents (TPC)**

Total phenolic content of plant extract was determined by Folin-Ciocalteau reagent according to the method of Antolovich *et al.* (2002), with minor modifications as reported by Massoumeh *et al.* (2013). In brief, 200 µl of extract was mixed with 1ml of 1:10 Folin-Ciocalteau reagent followed by addition of Na$_2$CO$_3$ (800 µl, 7.5%). The assay was carried out in a set of test tubes. After the incubation at room temperature for 2 hours in dark, the absorbance at 600 nm was recorded. Gallic acid served as standard reference. TCP was expressed as mg Gallic acid equivalents per gram of dried extract (mg GAE g$^{-1}$) (Gallic acid equivalent).

**Determination of Total Flavonoid**

Total flavonoid content of the extract was determined by the aluminium chloride colorimetric assay as described by Chatatikun and Chiabchalard, (2013) modified. Briefly, the extract 500 µl (1mg/ml) or standard solution of quercetin in 80% ethanol was added to 100 µl of 10% the aluminium chloride solution followed by 1.5 ml of 95% ethanol. Ethanol 80% was used as reagent blank. Then 100 µl of 1M sodium acetate was added to the mixture into a test tube. All reagents were mixed and incubated for 40 minutes at room temperature protected from light. The absorbance was measured at 415 nm with micro plate reader. Total flavonoid content of the extract was expressed as mg Quercetin Equivalents (QE) per gram of dry plant material.

**Alkaloids Determination**

Alkaloid was determined using Harborne (1973) method: Five grams of the sample was weighed into 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. The mixture was filtered and the extract concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until there was complete precipitation. The precipitation was washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid and it was dried and weighed.

**Determination of Ascorbic Acid**

Ascorbic acid was determined using the method of Association of Vitamin Chemists as described by Kirk and Sawyer (1998). Briefly, 5 g of sample was dispersed in 50 ml
of EDTA/TCA solution and homogenized. The homogenate was filtered using a filter paper and more of the extractant was used to wash the residue in the filter paper until 100 ml filtrate was obtained. A 20 ml portion of the filtrate was measured into a conical flask and 10 ml of 30% potassium iodide solution was added to it. It was mixed well and then followed by four drops of 1% starch solution. The mixture was titrated against 0.01M CuSO₄ solution until a blue-black colour appeared. A reagent blank was also titrated using 20 ml of distilled water. The vitamin C content was calculated based on the relationship that 1 ml CuSO₄ = 0.88 mg Vitamin C. Therefore,

\[
\text{Vitamin C mg/100g} = \frac{100}{W} \times 0.88 \times (T-B) \times \frac{VT}{VA}
\]

Where,

\( W \) = Weight of sample, \( T \) = Titre value of sample, \( B \) = Titre value of Blank, \( VT \) = Total extract volume, \( VA \) = Volume of extract titrated

**Determination of Total Antioxidant Activity**

Total antioxidant activity of the extract was evaluated spectrophotometrically according to Prieto *et al.*, (1999) using a phosphomolybdenum method based on the reduction of Mo(VI) to Mo(V) by antioxidants and the subsequent formation of specific green phosphate/Mo(V) compounds. A 0.3 ml aliquot of 100 µgml⁻¹ sample solutions was combined with 2.7 ml of reagent solution (0.6 molL⁻¹ H₂SO₄, 28 mmolL⁻¹ sodium phosphate, 4 mmolL⁻¹ ammonium molybdate) in a test tube. The tube was capped and incubated in a boiling water bath at 95°C for 90 min. After cooling to room temperature, the absorbance of the reaction mixture at 695 nm was measured. For the blank, 0.3 ml of ethanol was mixed with 2.7 ml of reagent solution. A typical blank solution containing 2.7 ml of reagent solution and the appropriate volume of methanol used to dissolve the sample was incubated under the same conditions as the rest of the samples. Stock solution of α-tocopherol in ethanol was prepared. The antioxidant activity of each extract was determined as equivalents of α-tocopherol using an extinction coefficient of \( 4 \times 10³ \) L mol⁻¹cm⁻¹ and expressed as µmol α-tocopherol equivalent/g dried plant material.

**Reducing Power**

Reducing power of the extracts was determined according to the method of Oyaizu, (1986). Extracts and standard antioxidants at 50-500 µgml⁻¹ in 1ml of distilled water were mixed separately with 2.5 ml of 0.2 molL⁻¹ phosphate buffer (pH 6.6) and 2.5 ml of 10 gL⁻¹ K₃[Fe(CN)₆] and incubated at 50°C for 20 min. Then 2.5 ml of 100 mlL⁻¹ of TCA was added to
each mixture and the mixtures were centrifuged at 5000 rpm for 20 min. Finally, 2.5 ml aliquots of the supernatants were mixed with 2.5 ml of distilled water and 0.5 ml of 1gL⁻¹ FeCl₃. The absorbance of each solution at 700 nm was measured. Higher absorbance indicates better reducing power under the reaction conditions.

**Results and Discussions**

In this study, the presence of tannins, alkaloids, flavonoids and phenols were detected in the aqueous extracts of the fruit pulp of *Z. spina-christii* and *Z. mauritiana* while saponins were absent (Table 1) however, Okala *et al.*, (2014) indicate the presence of saponins in a similar work on metanolic fruit extract of *Z. mauritiana*. The aqueous fruits extract of *C. albidum* was devoid of tannins in addition to saponins. Phytochemicals such as alkaloids, saponins, terpenoids, phenols, polyketides, tannins, flavonoids e.t.c, in plants are used as ingredients in many nutraceutical and pharmaceutical products (Abdukarim and Azlan, 2012). These compounds are found in different parts of all plants (leaves, stem, bark, roots, fruits and seeds) in varying concentrations and are known to exert biological activities against many physiological conditions.

Table 1: Qualitative phytoconstituent of the aqueous extract of *C. albidum, Z. spinacristii,* and *Z. mauritiana*

<table>
<thead>
<tr>
<th>S/n</th>
<th>Phytochemical</th>
<th><em>C. albidum</em></th>
<th><em>Z. spina-christii</em></th>
<th><em>Z. mauritiana</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Tannins</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Phenolics</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Present = Absent

Quantitative estimation of these phytochemicals in the aqueous fruits extracts (Table 2) showed presence of high amount of flavonoids and total phenolics in *Z. mauritiana* with values of 87.03 ± 0.02 mg/g QE and 65.76 ± 0.03 mg/g GAE respectively. Memon *et
aliyu et al., 2012 reported Z. mauritiana fruits from Sindh, India to contain 12.8 mg/g GAE phenolic contents with 0.5µmol/g QE of antioxidant activity. This shows that the fruits of Z. mauritiania in Maiduguri, Nigeria may be of good antioxidant potential.

Table 2: Quantitative Phyto-constituent of aqueous fruit Extracts of C. Albidum, Z. spina-christii and Z. mauritiana

<table>
<thead>
<tr>
<th>Sno.</th>
<th>Phytochemical</th>
<th>C. albidum</th>
<th>Z. spinacristii</th>
<th>Z. mauritiania</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Tannins (mg/g tannic acid)</td>
<td>ND</td>
<td>36.20±0.00</td>
<td>63.72±0.00</td>
</tr>
<tr>
<td>2.</td>
<td>Alkaloid (g)</td>
<td>0.43±0.01</td>
<td>0.28±0.01</td>
<td>0.19±0.01</td>
</tr>
<tr>
<td>3.</td>
<td>Flavonoid (mg/g QE)</td>
<td>1.34±0.00</td>
<td>14.00±0.03</td>
<td>87.03±0.02</td>
</tr>
<tr>
<td>4.</td>
<td>Total phenolic (mg/g GAE)</td>
<td>21.46±0.01</td>
<td>26.99±0.02</td>
<td>65.76±0.03</td>
</tr>
<tr>
<td>5.</td>
<td>Ascorbic acid mg/100g</td>
<td>162.8±0.12</td>
<td>409.20±0.13</td>
<td>48.4±0.11</td>
</tr>
</tbody>
</table>

Values are mean ± SD of three replications. ND not detected.

Antioxidants when present in foods offer nutraceutical properties because they provide therapeutic activity in addition to the nutritional benefits. Phenolic, flavonoids and terpenoids-containing compounds have strong antioxidant activities and are now used as alternative sources of food preservatives because of their antimicrobial activities to confer two benefits (Babbar et al., 2015). There is a high degree of correlation between the total phenolic content and antioxidant activity in many plants parts, fruits and vegetables studied (Kaur and Kapoor, 2000; Klimczak et al., 2007; Jayaprakasha et al., 2008 and Maier et al., 2009). Antioxidants activities of plants is due to the ability of the antioxidant molecules present as phyto compounds in the plants to chelate metal ions, scavenge free radicals or donate protons or electrons. In this way they help fight oxidative damages that are associated to many age-related degenerative diseases such as Parkinson’s, Alzheimer’s and coronary heart diseases, diabetes, hypertension and cancers. The total antioxidant capacities of the fruit extracts studied (Table 3) showed C. albidum to possess a significantly same antioxidant activity to standard alpha tocopherol. This
finding suggests that the aqueous fruits extract of *C. albidum* may be rich in the tocopherol-like antioxidant activity and this could be the reason for the folkloric use of the fruits and other parts of the plant in improving breast milk formation of lactating mothers, wound healing, fever and gastrointestinal disorders among many other ailments.

Ascorbic acid is another antioxidant compound that is required for many metabolic functions in maintaining homeostatic balance. The aqueous fruits extracts of *Z. spina-christii* had a highest concentration of ascorbic acid $409.20 \pm 0.13$ mg/100g compared to *C. albidum* $162.8 \pm 0.12$ mg/100g and *Z. mauritiania* $48.4 \pm 0.11$ mg/100g respectively (Table 2) this also showed that these extracts have the tendency of radical scavenging activity. Vitamin C helps in fighting diseases, improving fertility and is required by many metabolic pathways in maintenance of general well-being.

**Table 3:** Total antioxidant capacity of *C. albidum, Z. spina-christii* and *Z. mauritiania*

<table>
<thead>
<tr>
<th>Sno.</th>
<th>Plant extracts</th>
<th>TAC μmolα-tocopherol equivalent /g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>C. albidum</em></td>
<td>$125 \times 10^{-6} \pm 0.00$</td>
</tr>
<tr>
<td>2.</td>
<td><em>z. spina-christii</em></td>
<td>$101 \times 10^{-6} \pm 0.03$</td>
</tr>
<tr>
<td>3.</td>
<td><em>z. mauritiania</em></td>
<td>$343 \times 10^{-7} \pm 0.01$</td>
</tr>
<tr>
<td>4.</td>
<td>α-tocopherol (std)</td>
<td>$149x 10^{-6} \pm 0.19$</td>
</tr>
</tbody>
</table>

Values are mean ± SD of three replications. Std = standard.

*P < 0.05. Value with asterisk is significantly potent in activity compared to the standard tocopherol
Figure 1: Ferric reducing power activities of the three plants samples

The extracts of these plants also display a tremendous ferric reducing capability (Figure 1) which indicates that it could prevent fenton/harber-waise reaction that can elicit an avalanche of reaction resulting in destruction of cellular integrity (Mohan et al., 2012). This finding is similar to that of Dandare et al., (2017) and Okala et al., (2014) which showed that both C. Albidum, and Z. mauritiania respectively have potent antioxidant properties. This is corroborating the general assertion that many plants including C. albidum, Z. spinachristii and Z. mauritiania have been documented to possess health benefits such as anti-inflammatory, antimicrobial, antihypertensive and anti-diabetic activities due to the presence of phytochemicals (Asgarpanah and Haghighat, 2012; Orijajogoun et al., 2013 and Bukar et al., 2015).

The three fruits studied all possessed some degree of antioxidant activities. It is no wonder why the people in our community constantly consume them whenever they are in season so that they can benefit from the acclaimed potentials.

Conclusion

This work has shown the fruits extracts of C. albidum, Z. spinachristiiand Z. mauritiania to be of promising nutraceutical potentials and as a likely new sources of raw materials for industries requiring antioxidants as medicinal and therapeutic agents.

References

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