PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT PROPERTIES OF Hibiscus sabdariffa AND Moringa oleifera

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ABSTRACT

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This study evaluated some phytochemical constituents and antioxidant properties of two commonly consumed Nigerian vegetables, Hibiscus sabdariffa and Moringa oleifera. Both vegetables (dried) were screened for the non-phenolic phytochemicals: alkaloids, saponin, oxalate and phytate; the polyphenol phytochemical: polyphenol, anthocyanin, flavonoids, tannin; and the nutritive phytochemical, ascorbic acid. These vegetables were screened for antioxidant properties with respect to scavenging DPPH radical, in suppressing ferric thiocyanate and malonaldehyde formations in linoleic acid emulsions. Moringa oleifera had higher content of alkaloids (6.24%), flavonoids (29.42%), ascorbic acid (27.11%) and tannin (0.53%) than Hibiscus sabdariffa which had 3.11% alkaloid, 5.54% flavonoid, 16.76% ascorbic acid and 0.45% tannin contents. On the other hand, Hibiscus sabdariffa had higher polyphenols (1.10%), anthocyanin (1.15%), oxalate (0.53%) and saponin (1.19%) contents than Moringa oleifera which had 0.99% polyphenols, 1.10% anthocyanin, 0.50% oxalate and 1.13% saponin contents. Both vegetables exhibited antioxidant activities in scavenging DPPH radical, and in suppressing ferric thiocyanate and malonaldehyde formations in linoleic acid emulsions. It was not clear which of both vegetables that had higher antioxidant activity since Hibiscus sabdariffa was more antioxidative in suppressing malonaldehyde formation but less antioxidative in suppressing ferric thiocyanate formation than Moringa oleifera. Conclusively, combination of both vegetables will boost health-promoting and disease-preventing properties associated with the phytochemicals in these vegetables.

INTRODUCTION

Moringa oleifera and Hibiscus sabdariffa are used as medicinal and food ingredients in many parts of the world, including Nigeria in Africa. In northern Nigeria, both are highly sourced as food vegetables, particularly because of their health-promoting and disease-preventing properties strongly suspected to be due to the presence of many phytochemicals in them. Phytochemicals are a group of non-nutrient bioactive compounds found naturally in plant parts such as flowers, buds, leaves, fruits, roots, barks, spices and medicinal plants; and work in conjunction with other plant components as a defensive mechanism for the plants against diseases and many external attacks. Phytochemicals also provide characteristic colour, aroma and flavour in plants. They are plant metabolites (Sofowara, 1983). In humans, many phytochemicals have been found to be protective and preventive against many degenerative diseases and pathological processes such as in ageing (Burns et al., 2001), coronary heart disease, Alzheimer’s disease (Ames, 1983; Gey, 1990; Smith et al., 1996; Diaz et al., 1997; Rowland, 1999; Birt, 2006), neurodegenerative disorders, atherosclerosis cataracts, and inflammation (Aruoma, 1978). Both epidemiological and clinical studies provided evidence that most of these phytochemicals exhibit their protective and disease-preventing functions through their antioxidant activities (Shahidi, 1996). Typical phytochemical compounds that possess antioxidant activity include phenols, phenolic acids and their derivatives, ascorbic acid, flavonoids, phytic acid and many sterols. As antioxidants, these species are capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α-tocopherol radicals, and inhibit oxidases (Van-Acker et al., 1998; Oboh, 2006).

Moringa oleifera, commonly called the drum stick, is a tree native to India, but has been planted and domesticated in many other countries, including Nigeria. It is the most known and widely cultivated variety of the genus Moringa, family Moringaceae (Fugile, 2001). Moringa oleifera is also known by other common names such as Mallungay (Philippines), Benzolive tree (Haïti), Horse radish tree (Florida) and Nebeday (Senegal) (Price, 2000). In Nigeria, it is known as Zogale in Hausa, Okwe Oyibo in Igbo, Ewe Ile in Yoruba and Jeghel-adege in Triv. The leaves, seeds and flowers all have good nutritional and therapeutic value (Olushola, 2006). The seeds are eaten roasted. The flowers are eaten cooked in soups and resemble mushrooms while the leaves are eaten cooked as vegetables. The flowers and leaves are good sources of vitamins A, B group and (C when raw) and are among the best sources of minerals. The plant has been linked to the treatment or at least suppression of many degenerative diseases among many rural consumers.

Hibiscus sabdariffa (Rosselle) also known as guinea sorrel or Indian sorrel is a medicinal herb cultivated for its seeds, calyx and leaf; and is grown in the tropics, subtropics and other parts of the world (Dalziel, 1973) but is utilized beyond these areas of cultivation globally. Rosselle is used in preparation of local, non-alcoholic...
beverage, tea, jam, industrial wine, and marmalade (Mounigan et al., 2007). In Northern Nigeria, the production of a non-alcoholic drink called Zobo that is prepared from the red calyces is popular. The leaves and the white calyces are eaten as vegetable after cooking. In Western Indies, the calyces are freshly used in making jelly, syrup, gelatin, puddings and cakes while the dried calyces are used in ice cream, butter, pies and sauces (Adegunloye et al., 1996; Bolade et al., 2009). The flowers and fruits are used to produce drugs for cough and bronchitis (Chewonarin et al., 1999). Rosselle is reported to be diuretic, digestive, antiseptic, sedative, purgative, emollient, demulcent and astringent (Olateye, 2007). The calyces have many medicinal applications to cure kidney stone, pyrexia, liver damage, hypertension and leukemia (Abu-Tarboush et al., 1997; Estrella et al., 2000). This study is poised to evaluate the phytochemical composition and antioxidant properties of Moringa oleifera and Hibiscus sabdariffa. This will generate more knowledgeable information about their potential for more utilisation.

MATERIALS AND METHODS

Sample collection and preparation: Moringa oleifera (leaves) and Hibiscus sabdariffa. (Flowers) were purchased from rural farmers in the local market in Keffi, Nasarawa state, Nigeria. The vegetables were separately sorted to get fine grades which were cleaned, washed, drained and oven-dried at 55°C for 12 hours. They were packed in polythene bags and stored in air-tight containers for laboratory analysis. Determination of oxalate content: Oxalate content of samples was determined using the method of Dye (1956) as modified by Oke (1966). A blend of each ground plant sample (1.0 g), 190 ml of distilled water and 10 ml of 6M HCl in 250 ml volumetric flask was digested in a water bath at 90°C for 4 hours, and then centrifuged at 2000 rpm for 5 min. The supernatant was diluted to 250 ml with distilled water; and then titrated with concentrated ammonium hydroxide solution in drop wise manner, using methyl orange as an indicator which changed from pink coloration to faint yellow at the endpoint of titration. The resulting solution was heated at 90°C for about 20 minutes on a water bath and 10ml of 5% Calcium Chloride (CaCl2) solution to precipitate oxalate as Calcium oxalate. The resulting solution was allowed to stand overnight, centrifuged and the residue dried at 60°C for 48 hours. The dry precipitate was weighed and triplicate weights expressed as percentage oxalate content. Each determination was done in triplicates and the mean values taken.

Determination of total alkaloid content: The alkaloid content of samples was determined as described by Harborne (1973). Ground samples (5.0 g) of test material was mixed with 50 ml of 10 % acetic acid in absolute ethanol and allowed to stand for 4 hours. The mixture was filtered through Whatman no 1 filter paper and the filtrate concentrated to ¼ of its original volume on a water bath maintained at 90°C. Alkaloid was precipitated from each sample, using a concentrated ammonium hydroxide solution (NH4OH) and then allowed to sediment. Precipitates were collected, washed with concentrated NH4OH and then dried in a hot air oven. The residue is alkaloid and is calculated thus: %Alkaloid = W2-W1 / W x 100.

Where, W1 = Initial weight before drying, W2 = Final weight after drying, W = weight of sample.

Determination of anthocyanin content: Total anthocyanin content was determined using the method of Fuleki and Francis (1969). A portion of the sample extract, 10 ml (1 mg/ ml H2O) was diluted to 50 ml with distilled water and divided into two equal parts. These were grouped into two batches, with one batch adjusted to pH of 1.0 while the second batch was adjusted to pH of 4.5. The absorbance of samples was read at 535 nm, and the difference in absorbance readings reading at the two pH calculated.

Saponin content determination: Ground sample (20 g) of the test material was extracted for 3 hours with 100 ml of 20% ace tone using a Tecator soxhlet unit, applying extraction method of AOAC (2000). Each determination was done in triplicate and the mean values taken.

Phytate content determination: Phytate content was determined by the photometric method of Latta and Eskin (1980). Each test sample (2 g) was extracted with 100ml of 2.4 % HCl by shaking vigorously in a vortex mixer for 1h at room temperature (26 ± 2°C) and then filtered through Whatman no 5 filter paper. The filtrate (5ml) was mixed with 1 ml of 0.1M Na-EDTA, 0.75M NaOH solution and then made up to 25 ml with distilled water before being placed on an ion-exchange (AG1X4, 100-200 mesh) column. The column was washed with 15ml of distilled water and then 15ml of 0.1M NaCl before being eluted with 15ml of 0.7 M NaCl. The eluate was collected and wet digested in a Kjeldhal apparatus with a mixture of concentrated H2SO4 (0.5 ml) and HCl (3 ml). The digest was cooled to room temperature, 10 ml of distilled water added, and the mixture heated again on a water bath at 80°C for 10 minutes. The resulting solution was mixed with 2 ml of 2.5% ammonium molybdate solution in 1N H2SO4, 1 ml concentrated sulphuric acid; and then made up to 50 ml in a 50 ml volumetric flask. Each solution was allowed to stand for 15 min before reading absorbance at 640 nm against a blank without the plant material. Each determination was done in triplicate.

Total polyphenol content determination: Total polyphenol content was determined by mixing 0.5 ml aliquot of the sample extract with equal volume of distilled water, 0.5 ml of Folin-Ciocalteu’s reagent and 2.5ml of
saturated sodium carbonate; and the absorbance measured after 40 min at 720 nm (Singleton et al., 1990). Each determination was done in triplicates and the mean values reported.

Ascorbic acid content determination: The ascorbic acid (vitamin C) contents of the samples were determined using AOAC (2000) method. About 5 g of each sample was extracted by mixing with 100 ml of distilled water, and 10 ml of the extract mixed with 25 ml of 20% of glacial acetic acid and titrated against standardized 2.6-dichloroindophenol (0.05 g / 100 ml) solution. Ascorbic acid was used as a standard, and the result expressed as mg/100 g of the test samples. Each determination was done in triplicates and the mean values reported.

Tannin content determination: Tannin content was determined using the Vanillin-HCl method as described by Price and Butler (1977). Ground test material (0.5 g) was extracted at room temperature (26 ± 2°C) with 3 ml of methanol for 60 seconds. The extracts were each reacted with 3 ml of 0.1 M FeCl₃ in 0.1 N HCl and 3 ml of 0.008 M K₂Fe(CN)_6. Absorbances of samples were read after 2 minutes at 720 nm, Tannic acid was used as a standard and values expressed as mg/100 g of test materials. Each determination was done in triplicates and the mean values reported.

Total flavonoid content determination: The AlCl₃ method of Lamaison and Carnet, (1999) was used to determine total flavonoid contents of the sample extracts. The plant material (10 g) was extracted with 100 ml of 20% aqueous solution at room temperature, and filtered through Whatman no1 filter paper. Aliquots of 1.5 ml of extracts were added to equal volumes of a solution of 2% AlCl₃, 6 H₂O in methanol solvent. The mixture was vigorously shaken and absorbance taken after 10 minutes of incubation at room temperature. Catechin was used as a standard for the calibration curve but values were converted to mg/100 g of samples. Each determination was done in triplicates and the mean values reported.

Thiobarbituric acid (TBA) method of determining antioxidant activity: TBA values of linoleic acid emulsion (Benzie, 2003; Gulcin, 2004) as affected by addition of the plant extracts was evaluated (Buege and Aust, 1978). A mixture of 0 to 4.0 mg of plant material in 4 ml absolute ethanol, 4.1 ml of 2.5 % linoleic acid in absolute ethanol, 8.0 ml of 0.05M phosphate buffer (pH 7) and 3.9 ml of di absolute water (3.9 ml) and kept in screw capped container for 40 minutes in the dark. To 0.1 ml of the resulting solution was added 9.7 ml of 75% (v/v) ethanol and 0.1 ml of 30 % (v/v) ammonium thiocyanate. Lastly, after adding 0.1 ml of 20 mM ferrous chloride in 3.5% (v/v) HCl to the reacting mixture, the absorbance was measured at 500 nm after 3 minutes and again after every 24 hours until when the absorbance reached the maximum value. Triplicate determinations were done for each sample and mean values reported.

RESULTS AND DISCUSSION

Phenolic and Nutritive (ascorbic acid) phytochemical components

Table 1 shows the composition of Phenolic and Nutritive (ascorbic acid) phytochemicals in methanolic extracts of Hibiscus sabdariffa (flowers) and Moringa oleifera (leaves). The polyphenol content ranged from 0.99 mg/g in Moringa oleifera to 1.10 mg/g in Hibiscus sabdariffa. Phenols are one of the major groups of non-nutritive dietary components that have been associated with the inhibition of cancer, atherosclerosis, as well as for age-related degenerative brain disorder (Chang et al., 2006; Wang et al., 2009). The high polyphenol content in Hibiscus sabdariffa is of health benefit to consumers as a potential source of natural antioxidant. Anthocyanin content was 1.15 mg/g in Hibiscus sabdariffa and 1.10 mg/g in Moringa oleifera. Anthocyanins are known to inhibit low density lipoprotein (LDL) oxidation and LDL-mediated macrophage apoptosis, serving as a chemo-preventive agent (Tseng et al., 1998). Hibiscus sabdariffa which has higher content of anthocyanin (Table 1) are also sourced as a good food colorant in wine and related product.

Flavonoid content ranged from 5.54 mg/g in the methanolic extracts of Hibiscus sabdariffa to 29.42 mg/g in Moringa oleifera. Thus, Moringa oleifera had about at least 5 times flavonoid content as in Hibiscus sabdariffa. Moringa oleifera is better as a good source of flavonoid than Hibiscus sabdariffa. Flavonoids in human diet may reduce the risk of various cancers as well as prevent menopausal symptoms (Ross and Kasum, 2002; Padayatty et al., 2003). Epidemiological studies suggest that the consumption of flavonoid is effective in lowering the risk of...
coronary heart diseases (Rice-Evans et al., 1997). Thus Moringa oleifera could be useful in treating coronary heart disease.

Tannin content as obtained in this study ranged from 0.35 mg/g in *moringa oleifera* to 0.45 mg/g in *Hibiscus sabdariffa*. Tannin content in these two vegetables is much lower than those obtained by other workers (Tsaknis et al., 1999). Edible plant materials containing tannins are known to be astringent, and are used for treating intestinal disorders such as diarrhea and dysentery (Smirnoff, 2001). The presence of tannins in *Moringa oleifera* and *Hibiscus sabdariffa* supports their use in traditional curing of many different diseases. Li et al. (2003; Cho et al., 2004) reviewed the biological activities of tannins and observed that tannin have remarkable activity in cancer prevention and anticancer activities. The result also supports the use of *Moringa oleifera* and *Hibiscus sabdariffa* in many herbal remedies. The leaves of moringa oleifera are rich in tannins (Table 1), and this could be the main reason why it is useful in curing urinary tract infections (Fahey, 2005). *Hibiscus sabdariffa* had ascorbic acid content of 16.7 mg/g while *Moringa oleifera* had ascorbic acid content of 27.11 mg/g. The ascorbic acid content in *Hibiscus sabdariffa* was higher than the value (14.0 mg/g) reported by Duke (1983). Both vegetables are very high in ascorbic acid content, and could serve as good food supplement for ascorbic acid, a nutritive phytochemical.

Table 2: Composition of some non-nutritive phytochemicals in *Hibiscus sabdariffa* and *Moringa oleifera*

<table>
<thead>
<tr>
<th>Parameters</th>
<th><em>Hibiscus sabdariffa</em></th>
<th><em>Moringa oleifera</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid (mg/g)</td>
<td>3.11 ± 0.02</td>
<td>6.24 ± 0.11</td>
</tr>
<tr>
<td>Saponin (mg/g)</td>
<td>1.19 ± 0.11</td>
<td>1.13 ± 0.03</td>
</tr>
<tr>
<td>Phyate (mg/g)</td>
<td>1.61 ± 0.04</td>
<td>0.75 ± 0.01</td>
</tr>
<tr>
<td>Oxalate (mg/g)</td>
<td>0.53 ± 0.44</td>
<td>0.50 ± 0.42</td>
</tr>
</tbody>
</table>

Values represent mean of three determinations ± standard deviation.

Non-phenolic phytochemical components

Table 2 shows the composition of some non-phenolic phytochemical in the two vegetables, *Moringa oleifera* and *Hibiscus sabdariffa*. *Moringa oleifera* had 6.24 mg/g alkaloid, 1.13 mg/g saponin, 0.75 mg/g phyate and 0.50 mg/g oxalate while *Hibiscus sabdariffa* had 3.11 mg/g alkaloid, 1.19 mg/g saponin, 1.61 mg/g phyate and 0.53 mg/g oxalate. The oxalate content was relatively low, exactly 0.53 mg/g in both vegetables. The alkaloid content was high in both vegetables, but the alkaloid content (6.24 mg/g) of *Moringa oleifera* was about 2 times that (3.11 mg/g) of *Hibiscus sabdariffa*. The high alkaloid content could account for their popular use in the traditional treatment of hypertension. These secondary metabolites have been associated with numerous physiological activities in mammalian cells in various studies (Sofowora, 1993; Abo et al., 1999; Nweze et al., 2004; Mishra, 2009). Many plants containing alkaloids and flavonoids have diuretic, antispasmodic, anti-inflammatory and analgesic effect (Owoyele et al., 2002; Ujowundu et al., 2010). Saponin reduces the uptake of glucose and cholesterol at the gut through intra-lumenal physicochemical interaction. This could confer a chemoprotection against heart diseases. The presence of phyate in foods has been associated with reduced mineral absorption from the food due to its formation of complexes with most mineral. However, presence of phyate in high fibre foods may reduce the incidence of breast cancer and cardiovascular diseases. Oxalic acid content in food would be an indication of toxicity level of the food. However, oxalate at low level advantageously confers antioxidant activity in both food and humans.

Antioxidant activity

The antioxidant activity was evaluated using the Ferric thiocyanate (Osawa and Namiki, 1981) and thiobarbituric acid (Buege and Aust, 1979) methods. These methods tested the levels of inhibition of lipid peroxidation in linoleic acid emulsion by the ethanol extracts of these two vegetables. α-tocopherol and BHT (butylated hydroxytoluene) were used as positive artificial antioxidants. The ferric thiocyanate method determined the amount of peroxides formed at the initial stage while the thiobarbituric acid method determined the amount of decomposed...
carbonyl compounds formed from the peroxides at later stage of the lipid peroxidation (Mackeen et al., 2000). The vegetable extracts exhibited different capacity in inhibiting lipid peroxidation. At initial stage, *Moringa oleifera* exhibited higher antioxidant activity than the *Hibiscus sabdariffa*, as indicated by the ferric thiocyanate assay (Figure 1) but the reverse was the case at a later stage as indicated by the thiobarbituric assay. Antioxidant activity increased with increasing extract concentrations for all the samples.

![Figure 1: Antioxidant activities of the extracts of *Hibiscus sabdariffa* and *Moringa oleifera* by ferric thiocyanate method](image1.png)

![Figure 2: Thiobarbituric acid (TBA) value of Linoleic acid as affected by ethanolic extracts plants after lain days of storage](image2.png)
CONCLUSION

The result of this study shows that both Hibiscus sabdariffa and Moringa oleifera possess a number of phytochemicals in high quantity and exhibit high antioxidant activities. These vegetables contain alkaloid, tannin, flavonoids, ascorbic acid, saponin, anthocyanin, polyphenols, oxalate and phytate in detectable amounts. Both vegetables also exhibited high antioxidant properties in suppressing ferric thiocyanate and malonaldehyde formations in linoleic acid emulsions. However it is not very clear which of them possess more antioxidant properties since Moringa oleifera exhibited higher antioxidant activity in suppressing ferric thiocyanate formation but lower antioxidant activity in suppressing malonaldehyde formation. Further study is required on this account.

REFERENCES


Rice-Evans, C. A., Miller, N. J. and Panganga, , G. (Antioxidant properties of phenolic compounds. Trends in Plant Science, 2: 152-159


