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Total Phenolic, Flavonoids and Ascorbic Acid contents in Baobab (*Adansonia digitata L.*) Fruit Pulp Extracts from Different locations in Sudan

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Abstract: *This study aimed to determine phenolics, flavonoids and ascorbic acid contents of Sudanese baobab fruit pulp. Baobab fruit (Adansonia digitata.L) samples were collected from four locations in Sudan (El Obeid, Um Ruwaba , Niyala and Damazin). Aqueous, aqueous ethanol and aqueous methanol were used for extracts. Dichlorophenol Indophenol reagent was using of Ascorbic acid content. There were significant differences in total phenolic and flavonoids contents between the different extracts from different locations in Sudan. Total phenolics were ranged from 15.50 to 99.66 mg GA/g of gallic acid equivalent (GAE) /g. Flavonoids were ranged from 1.03 to 21.53 mg of catechin (CA/g). Ascorbic acid content was 372.52, 355.97, 354.13and 345.82 mg/100g for Damazin, El Obeid, Umm Ruwaba and Niyala samples, respectively. These results indicated that baobab fruit is rich in natural antioxidants and could be used as functional foods ingredient and food supplementation.*

Keywords: Baobab fruit, Total Phenolic, Flavonoids, ascorbic acid, aqueous extracts.

1. Introduction

Baobab (*Adansoia digitata.L*) is a large deciduous tree originally found in Africa but it is also found in Asia and America to a lesser extent. In Africa, it extends from Senegal eastwards to Sudan, Kenya, then southern Africa and Madgashgar (Sidibe *et al*.,1998). It is found in belts in some regions of Sudan, particularly Kordofan, Blue Nile, and Darfur (Elamin, 1990), growing in sandy soils, short grass savanna areas and mountains. Its fruit has a woody pericarp a spongy pulp with uniform seeds (Sidibe & Williams, 2002). The fruit pulp has high contents of pectin, low protein, low fat, very little iron and is a relatively poor source of manganese, but contains exceptionally high calcium content (Osman, 2004) and high amounts of vitamin C (Sidibe & Williams, 2002) . Furthermore the Phytochemical screening of the baobab fruit pulp gave positive reaction for sterols, triterpenes, saponins, tannins and glycosides (Ramadan *et al.*, 1994).

Phenolic compounds are essential part of human diet and are provided antioxidant properties. These compounds have an aromatic ring bearing one or more hydroxyl groups and their structures may range from that of a simple phenolic molecule to that of a complex high-molecular weight polymer. The antioxidant activity of phenolic compounds depends on the structure, the number and positions of the hydroxyl groups and the nature of substitutions on the aromatic rings (Balasundram *et al*., 2006). Phenolic compound and flavonoids of fruits and vegetables used for human diet, may reduce the risk of cardiovascular disease (Cook & Samman, 1996).

Moreover vitamin C is defined as the generic term for all compounds exhibiting the biological activity of *L*-ascorbic acid. Vitamin C is required for the prevention of scurvy and maintenance of healthy skin, gums and blood vessels. It functions in collagen formation, absorption of inorganic iron, reduction of plasma cholesterol level, inhibition of nitrosamine formation, enhancement of the immune system, and reaction with singlet oxygen and other free radicals. As an antioxidant, it reportedly reduces the risk of arteriosclerosis, cardiovascular diseases and some forms of cancer (Harris, 1996).

Baobab (*Adansonia digitata*) is reported to be a powerful antioxidant and extremely important in human nutrition (Manfredini *et al.*, 2002). In Sudan there is no information about the varieties of *Adansonia*, however ecotypes from different areas are known with variations in fruits shape, size, weight, color and taste. Few studies are directed towards evaluation of nutritional and antioxidant potential of the fruit pulp of Sudanese baobab ecotypes established in different regions, therefore this study was conducted to determine the total phenolic, flavonoids contents and ascorbic acid of Sudanese Baobab (*Adansonia digitata.L*) fruit pulp from different regions.

2. Material and methods

2.1. Materials

Baobab fruits capsules were obtained from different locations in Sudan; El Obeid, Um Ruwaba, Niyala and Damazin. The Fruit pulp was obtained by breaking the capsules manually, seeds were removed and pulp powder was sieved using appropriate mesh. The resulting fruit pulp was stored in a dark polyethylene bag at -18ºC until used.

2.1.1. Preparation of fruit pulp extracts

Baobab fruit pulp aqueous, methanolic and ethanolic extracts were prepared by the method of Kim and Lee (2002) with slight modification. Twenty gram of the sample from each was extracted separately with 60 ml of three different solvents: methanol/water (4:1) v/v, ethanol/water (4:1) v/v and water at room temperature for five hour using an orbital shaker (Germany, serial No.071006060) at 200 rpm and temperature at 30 °C. Then, the homogenate was centrifuged for 10 min at 3600 rpm (Eppendorf Centrifuge 5804, Hamburg, Germany) and the supernatant was removed. The residue was extracted once again at the same conditions. Then, both supernatants were filtered through Whatman filter paper (Whatman International Limited, Kent, England) using a chilled Buchner funnel .The filtrate was transferred into evaporating flask with an additional of 50 ml 80% (aqueous ethanol) and were concentrated in a rotary evaporator (BüchíRotavapor, Switzerland) at 45 °C. The resulting concentrates were then mixed with 15 ml of deionised (DI) water to obtain a NANO pure water system (Barnstead, Dubuque, Iowa, USA). The concentrates were then frozen and freeze-dried (Great Britain. Serial No. K12173-5) to obtain crude extracts powder and kept in dark glass bottles at -18°C until used for analysis.
The residue samples extract was calculated using the following equation.

$$\textbf{Yields extract \%:} \quad \frac{w2}{w1} * 100$$

W1=weight of sample taken
W2=weight of residue extract

2.2. Methods

2.2.1.Total phenolics content

Total phenolics content (TPC) was determined by spectrophotometric
determination using Folin–Ciocalteu reagent according to the method of Tuberoso *et al*. (2010) with some modifications. One hundred µl of the sample (5mg/ml) were diluted with deferent solvents and added to 0.5 ml of 10% Folin–Ciocalteau's phenol reagent. After 5 min, 3 ml of 10% Na_2CO_3 (w/v) were added, the mixture was shaken, and then diluted with water to a final volume of 10 ml. After a 90 min incubation period at room temperature, the absorbance was read at 725 nm on a 10 mm quartz cuvette using a spectrophotometer (UV -1800, serial No.A11454805048CD, SHIMADZU, Japan), against a blank. The total polyphenol content results, of the samples were expressed as the gallic acid as mg/g of gallic acid equivalent (GAE), using a calibration curve of a freshly prepared gallic acid standard solution (10-100 mg/ml). All the samples were analyzed in four triplicates and the mean values were calculated.

2.2.2. Total flavonoids content

Total flavonoids content was determined spectrophotometer using aluminum chloride by the method of Kim *et al*. (2003). Four ml of distilled water were added to 1 ml of the fruit pulp extracts (5mg/ml). Then, 5% sodium nitrite solution (0.3 ml) was added, followed by addition of 10% aluminum chloride solution (0.3 ml). Test tubes were incubated at ambient temperature for 5 min, and added 2 ml of 1M sodium hydroxide was added to the mixture and completed the volume of reaction mixture were made up to 10 ml with distilled water. The mixture was thoroughly vortexed and the absorbance of the pink colour developed was read at 510 nm. A calibration curve was prepared by catechin and the results were expressed as mg catechin equivalents (CEQ) /g. All the samples were analyzed in four triplicates and the mean values were calculated.

2.2.3 Determination of ascorbic acid

Determination of ascorbic acid was done using 2-6- Dichlorophenol Indophenol reagent according to AOAC (1990). This reagent is reduced by ascorbic acid to become colorless. It is prepared as follows: 0.2 g of 2-6- Dichlorophenol Indophenol dye was dissolved in 200 ml distilled water, and then filtered through Whatman filter paper (No. 2) Into 500 ml Volumetric Flask and made up to volume with distilled water. The dye was standardized as follows: 50 g of standard ascorbic acid was weighed and made up to volume–by distilled water in 250. Volumetric flask and 5ml aliquot were diluted with 5ml oxalic acid 10 % and titrated with the dye solution to a pink end point. One kg of ascorbic acid is equivalent to one ml of the dye used. Thus: strength of the dye =1/titre.

Procedure:

Thirty grams of the sample were blended with about 100 ml of 04% oxalic acid for two minutes in a blender. The blended mixture was made up to 500ml in a volumetric flask with 04% oxalic acid and filtered. The ascorbic acid in the filtrate was titrated against standard 2-6 Dichlorophenol Indophenol .The ascorbic acid was calculated as follows:

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{Titre} \times \text{dye strength} \times 100}{\text{Factor}}$$

$$\text{Factor} = \frac{\text{Sample wt.} \times \text{Sample volume for titration}}{\text{Total volumeof sample}}$$

2.3. Statistical analysis

Data were analyzed using one-way ANOVA using MINITAB16 Statistical Software for Windows (State College, PA. USA). Significant level of less than 0.05($p<0.05$) considered significant.

3. Results and Discussion

3.1. Extraction yield

The yield of extracts obtained from 20 g of different Sudanese baobab fruit pulp using different solvents were shown in Table (1). The highest yield percentages of solid residue was obtained using aqueous as extraction solvents for baobab fruit pulp from Niyala (72.85 %) followed by Umm Ruwaba(71.50%) and then Damazin (63.80%) but the lowest yield was that from El Obeid (47.55%) . Our results show that the extract yields vary depending on the origin region of baobab fruit and solvent used for the extraction.

Table (1): The yields %of solid residue after extraction and evaporation from 20 g dried baobab fruit pulp

Sample	Aqu extract	Aqu eth OH	Aqu meth OH
El Obeid	47.55 ± 0.05^{c}	22.95 ± 0.04^{a}	15.15 ± 0.06^{b}
Umm Ruwaba	71.50 ± 0.27^{a}	18.95 ± 0.04^{d}	11.85 ± 0.08^{c}
Niyala	72.85 ± 0.05^{a}	21.85 ± 0.04^{b}	17.25 ± 0.05^{a}
Damazin	63.80 ± 0.28^{b}	20.00 ± 0.10^{c}	17.45 ± 0.07^{a}

Aqu**:** Aqueous extract, Aqu eth OH : Aqueous ethanol, Aqu meth OH : Aqueous methanol
Each value is mean ± standard deviation of four replicates expressed on dry matter basis. Values that bear different superscript letter in the same Column are significantly different at $p<0.05$.

3.1. Total Phenols and Flavonoids Contents

The total phenols and Flavonoids contents of Baobab fruit pulp are shown in Table (2) and expressed as mg of gallic acid (GA)/g) and catechin equivalent (CA) mg /g of extracts, respectively. There are significant differences ($p\leq 0.05$), in total phenolic contents in aqueous, aqueous ethanol and aqueous methanol extracts; which ranged from 15.50 to 99.66 mg GA/g. Those values were higher when compared to result by Lamien–Meda *et al*. (2008) .They reported value of 35.18mg GAE /g while Salih, and Yahia phenolic content were 13.92 mg GAE /g for baobab pulp . The highest concentration of phenols was measured in aqueous methanol , aqueous ethanol and aqueous solvents extracts in all samples. That may be due to effect of polarity. High solubility of phenols in polar solvents provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction (Mohsen and Ammar, 2008). No significant difference between Niyala and Damazin samples in phenolic contents of the aqueous ethanol extracts.

The concentration of flavonoids in extracts ranged from 1.03 to 21.53 mg of CA/g showing significant difference ($p\leq 0.05$).These values were higher compared to those reported by Lamien–Meda *et al*. (2008).The highest concentration of flavonoids was in samples from Damazin and Niyala . The concentration of flavonoids in fruit pulp extracts depends on the polarity of solvents used in the extract preparation (Min and Chun-Zhao, 2005).The variations in phenolic, and Flavonoids content could probably be due to genetic variation, and soil chemical composition.

Table (2): Total Phenols and Flavonoids contents of baobab fruit pulp (mg/g) equivalents of Gallic acid and chatechin

sample	TPC			TFC		
	Aqu extract	Aqu eth OH	Aqu meth OH	Aqu extract	Aqu eth OH	Aqu meth OH
El Obeid	21.38±0.20^{b}	42.29± 0.24^{b}	84.49 ± 0.34 ^{b}	1.03 ± 0.08^{c}	10.77 ± 0.23^{b}	11.66 ± 0.10^{b}
Umm Ruwaba	15.50 ±0.30^{d}	58.46 ± 0.22 ^{a}	99.43 ± 0.80^{a}	2.00 ± 0.10^{a}	15.76 ± 0.67^{a}	4.07 ± 0.11^{d}
Niyala	17.16 ± 0.05^{c}	35.36 ± 0.34^{c}	41.37 ± 0.13^{c}	1.62 ± 0.02^{b}	5.77 ± 0.07^{c}	4.62 ± 0.07^{c}
Damazin	22.62 ± 0.07^{a}	58.93 ± 0.22^{a}	99.66 ± 0.18^{a}	1.46 ± 0.02^{b}	16.06 ± 0.15^{a}	21.53 ± 0.12^{a}

Values are means ±SD (n=4); means with different superscripts in the same column are significantly different ($p \leq 0.05$).
Aqu**:** Aqueous extract, Aqu eth OH : Aqueous ethanol, Aqu meth OH : Aqueous methanol
TPC: Total phenols content, TFC: Total flavonoids content

3.2 **Ascorbic acid (vitamin C)**

As presented in Table (3), the results showed significant difference ($P \leq 0.05$) in ascorbic acid content of baobab fruit pulp. The sample obtained from Damazin had the highest ascorbic acid content (372.52 mg/100g) followed by El Obeid and Umm Ruwaba samples (355.97 mg/100g and 354.13), while that from Niyala showed the lowest vitamin level (345.82 mg/100g). These values were within range reported by Besco et al. (2007) from 150-499 mg/100 and lower than the result found by Nnam and Obiakor, (2003) which was 373 mg/100 g.

Table (3): Ascorbic acid content (mg/100 g) of baobab fruit pulp

Sample	Ascorbic acid
El Obeid	355.97 ± 4.17^{b}
Umm Ruwaba	354.13 ± 2.59^{b}
Damazin	372.52 ± 2.00^{a}
Niyala	345.82 ± 1.15^{c}

Each value is mean ± standard deviation of three replicates expressed on dry matter basis. Values that bear different superscript letter in the same Colum are significantly different at $p<0.05$.

4. Conclusion

The results presented here constitute the first information on the total phenols and flavonoids contents of baobab fruit pulp by different solvent extracts of four different locations in Sudan. Damazin variety showed the highest amount on total polyphenol, total flavonoids and ascorbic acid contents. Sudanese baobab fruit pulp could be a good source of natural antioxidant compound.

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