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Nutritional value and antioxidant properties of four wild fruits commonly consumed in Sudan

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Abstract

Fruit pulps of doum (*Hyphaene thebaica* L. Mart.), baobab (*Adansonia digitata* L.), tamarind (*Tamarindus indica* L.) and jujube (*Ziziphus spina-christi* L. Willd.) sampled from Nuba Mountains, Sudan were characterized for their proximate composition, mineral contents, total soluble phenols, total carotenoids and total antioxidant capacity. Mineral contents were high, total soluble phenols ranged 14 − 45 mg GAE/g DW and total carotenoids were between 7 and 16 mg/kg DW. Total antioxidant capacity reached 120 – 425 µmoles TE/g DW when measured in hydrophilic extract using DPPH assay. The richness of these fruits in minerals and antioxidant compounds makes them considerable sources of nutrition and of potential impact on human health.

Introduction

Collection of wild foods is a tradition that rural communities in the developing countries practice to secure their food supply especially during times of food shortage (Loghrust, 1986; Lockett and Grivetti, 2000; Msuya *et al*., 2010). The physical and economic accessibility in addition to high diversity of wild foods are crucial factors for survival of communities suffering, periodically or seasonally, from food scarcity and poverty (Falconer and Arnold, 1989). Also the long accumulating knowledge and experience that local population develops over generations, concerning uses, gathering, processing and storage of wild foods, make these foods of considerable importance (Somnasang and Moreno-Black, 2000). Furthermore, wild foods play a major role in diet of rural children and have a nutritional value in some cases superior to the quality of domesticated foods (Ogle and Grivetti, 1985; Legwaila *et al*., 2011). In addition, sale of wild foods of high marketability makes an indirect contribution to enhancing household's food security by generating alternative income opportunities. The socioeconomic potential of wild food in developing world was discussed (Agea, 2010; Lulekal *et al.*, 2011).

In rural areas of the Sudan, where a wide genetic diversity of plants exist, edible wild fruits play a vital role in securing food and enhancing household's economy (Gebauer *et al*., 2002; El Tahir and Gebauer, 2004; Goenster *et al*., 2011). Most of these fruits are consumed in different forms and have a wide use in folk medicine in everyday life of local population (El Gazali *et al*., 1987; Abdelmuti, 1991). Despite their significant role, wild edible fruits are under-estimated and only a little attention has been paid to explore their various potentials. In this study, we aimed at reporting the nutritional value and antioxidant properties of fruits pulps of doum (*Hyphaene thebaica* L. Mart.), baobab (*Adansonia digitata* L.), tamarind (*Tamarindus indica* L.) and jujube (*Ziziphus spina-christi* L. Willd.), belonging to the families Arecaceae, Bombacaceae, Fabaceae - Caesalpinioideae and Rhamnaceae, respectively. In addition to nutritional value and antioxidant properties, this paper intends to draw attention to the potential of these wild edibles and value their genetic resources for ex- and in-situ conservation.

Materials and Methods

Chemicals

Standards were purchased from Sigma Aldrich (St. Louis, MO). All the chemicals were High Performance Liquid Chromatography (HPLC) – grade obtained from J.T. Baker (Baker Mallinckrodt, Mexico). HPLC–grade water was prepared using a Milli–Qplus purification system (Millipore

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Table 1. Color parameters L, a, b, c and hº, Proximate composition and minerals concentrations of the analyzed pulps on dry base*

Analysis	Doum	Jujube	Tamarind	Baobab
Color parameters				
L	70.01±0.22	75.85±0.69	58.40±0.94	84.28±0.46
a	7.50±0.06	5.43±0.15	7.02±0.93	2.28±0.03
b	17.44±0.23	16.97±0.17	13.05±0.45	10.59±0.72
C	18.99±0.26	17.84±0.28	13.58±0.59	10.90±0.12
hº	66.74±0.32	72.28±0.29	58.41±0.25	77.92±0.09
Proximate composition				
Moisture (%)	7.50±0.01	10.53±1.02	12.82±1.05	4.23±0.01
Fat (%)	0.90±0.00	2.55±0.02	2.71±0.14	0.21±0.00
Protein (%)	2.62±0.03	4.34±0.12	4.75±0.02	2.39±0.07
Ash (%)	7.04±0.02	5.16±0.05	4.23±0.12	4.33±0.02
Carbohydrates (%)	64.46±2.11	74.31±2.52	69.48±1.28	81.35±2.24
Fiber (%)	17.48±1.21	3.11±0.01	6.01±0.25	7.49±0.08
Mineral concentrations				
Ca (mg / g DW)	0.48 ± 0.02	1.73 ±0.12	1.01 ±0.00	3.14 ±0.05
K (mg / g DW)	8.02 ±0.53	8.40 ±0.27	7.78 ±0.51	18.78 ±0.1
Mg (mg / g DW)	0.54 ±0.01	0.73 ±0.10	1.32 ±0.00	2.08 ±0.15
Na (mg / kg DW)	133.58±3.45	95.4 ±2.61	46.7 ±0.17	110.4 ±2.10
Fe (mg / kg DW)	13.4 ±0.91	11.0 ±0.09	6.1 ±0.23	61.7 ±3.21
Cu (mg / kg DW)	1.5 ±0.03	2.5 ±0.08	2.3 ±0.11	6.1 ±0.11
Zn (mg / kg DW)	2.1 ±0.01	3.3 ±0.03	1.9 ±0.02	2.9 ±0.03
Mn (mg / kg DW)	1.5 ±0.01	3.6 ±0.21	1.4 ±0.00	2.2 ±0.10
Al (mg / kg DW)	20.8 ±0.25	21.8 ±0.09	7.5 ±0.21	21.5 ±0.21

* All the given values are means of three determinations ± standard deviation

Corporation, Bedford, MA).

Fruit materials

Mature and air dried fruits of doum, baobab, tamarind and jujube were purchased from three different sellers in local market in El Rashad (lat. 11° 40` – 11° 55 N` and long. 30° 45` – 31° 25` E), Eastern Nuba Mountains, South Kordofan State, Sudan. A composite pulp sample from each fruit type was milled (using M20 universal grinding mill, IKA work) to pass a sieve of 0.2 mm. The color parameters L (lightness/darkness), a (red/green) and b (yellow/blue) were measured on composite milled samples using a Minolta spectrophotometer (Minolta, Co. Ltd., Osaka, Japan) and the metric chroma (C) and hue-angle (h°) were calculated to characterize each of the investigated samples for its chromaticity (Table 1).

Proximate composition

Crude fat, crude fiber, crude protein, ash, moisture and carbohydrates were determined according to the methodology of AOAC (AOAC, 2000). Oil was extracted with petroleum ether (boiling point 40–60°C) using a Soxhlet fat extraction unit (B–810 Soxhlet, Büchi Labortechnik AG, Flawil, Switzerland). Crude protein was determined according to Kjeldahl method by a digestion system (Büchi Kjeldahl line K–437) and a distillation unit (Büchi Auto Kjeldahl unit K-370, Büchi Labortechnik AG, Flawil, Switzerland) with a titrator connection and using a conversion factor of 6.25. Crude fiber was measured by filter bag technique that subjects the sample to an acid (0.255N H_2SO_4) and a base (0.313N NaOH) digestions. The filter bag technique includes one blank bag of known weight in the run to determine the ash correction factor. A fiber analyzer vessel (ANKOM 200/220 Fiber Analyzer, ANKOM Technology, NY, USA) was used. For ash determination a muffle furnace controlled to 550ºC was used, moisture content was determined by oven drying method and carbohydrates were calculated by difference.

Mineral contents

Minerals were extracted and analyzed as described by Gul and Safdar (2009) with some modifications. Cold digestion was allowed for 16 h with concentrated HNO_3 (16 N) and a hot digestion, at temp 50–60°C, was allowed until the appearance of

white fumes. A dilution of 1:10, 1:100 or 1:1000 using HNO_3 (1 N) was used. The analysis was performed by an atomic absorption spectrophotometer (AAnalyst 700 atomic absorption spectrometer, Perkin Elmer, Massachussets, USA).

Determination of phenolic total soluble contents and compounds

Total soluble phenols (TSP) were determined following Folin-Ciocalteu method by Singleton and Rossi (1965) as described by Yahia and Mondragon-Jacobo (2011). One g of sample was homogenized with 20 mL of 80% acetone and 2% formic acid (in a ratio of 80% acetone: 20% formic acid) using an Ultra Turrax model T25 (IKA Works, Willmington, NC), and this was followed by sonication in a Bransonic 2510 sonicator (Bransonic Ultrasonic Co., Danbury, CT) for 5 min. The homogenates were then centrifuged in a high speed centrifuge HERMLE Z 323 K (LaborTechnik, Wehingen, Germany) for 15 min at 2°C and 19 000 g. The aliquot of the extract was filtered through a Whatman no. 1 filter paper (Whatman Inc., Clifton, NJ) and the supernatants were collected. Re-extraction of the residues was done repeating the above steps. The extracts were concentrated using a Büchi rotary evaporator R – 200 (Büchi Labortechnik, Postfach, Switzerland) at 40°C for 45 min. The concentrated sample was diluted with 25 mL of methanol and HPLC- grade water was added to complete the volume to 50 mL.

Determination of TSP was done using Folin-Ciocalteu reagent assay. The prepared sample extract was diluted by adding HPLC - grade water, in a ratio of 1:10. A 30 µL of the diluted sample extract was placed in a micro-plate and 150 µL of Folin–Ciocalteu reagent diluted 1:10 followed by 120 mL of Na_2SO_4 (7.5 %) were added. The contents were dark incubated for 3 h at room temperature. The absorbance was measured at 630 nm using Dynex MRX micro-plate reader (Dynex Technology Chantilly, VA). TSP content was expressed as mg GAE/g DW (milligrams of Gallic Acid Equivalents per g Dry Weight).

Phenolic compounds were identified and quantified as described by Yahia *et al.* (2010 a; 2010 b) using high perfomance liquid chromatoraphy HPLC (Hewlett–Packard GmbH HP 1100 series, Waldbronn; Germany) equipped with a diode–array detector DAD. A 250 × 4.6 mm i.d., 3.5 µm, Symmetry RP18 column (Waters Co, Milford, CT) was used. Formic acid (1%) and acetonitrile in a ratio 98: 2 at a flow rate of 0.5 mL per min was used as a mobile phase. The phenolic compounds of interest in this study were gallic acid, p-hydroxybenzoic acid, protocatechuic acid and vanillic acids (hydroxybenzoic acids); caffeic acid, chlorogenic acid, cinnamic acid, p-coumaric acid, ferulic acid, 2-hydroxycinnamic acid and sinapic acids (hydroxycinnamic acids); kaempferol, myricetin and quercetin (flavonols) and catechin and epicatechin (flavan-3-ols) and were measured at 280 and 320 nm. Standards calibration curves were prepared for quantification.

Total carotenoids content (TC)

Extraction of TC was done using AOAC method (AOAC, 2000) and following the modifications reported by Soto-Zamora *et al*. (2005). A 10 mL of extraction solution (10 hexane: 6 ethanol: 7 acetone: 7 toluene) was added to 0.5 g of sample and saponification was done with 3 mL of 40% KOH in 80% methanol. The mixture was heated in a water path at 56°C for 20 min. After cooling, 10 mL of hexane followed by 10 mL of Na_2SO_4 (10%) were added. Vortex was done each time a solvent was added. The extract was allowed to stand for 1 h at room temperature for phase separation. The organic upper phase was collected and the volume was registered. Total carotenoids were determined by measuring absorbance at 470 nm in a Beckman DU 65 spectrophotometer (Beckman Instruments, Fullerton, CA). The Beckman spectrophotometer was calibrated with hexane as blank. The test tubes were kept in dark all the time during the process.

Total antioxidant capacity (TAC)

TAC was measured using two extracts; lipophilic extract (LPE) and hydrophilic extract (HPE) and two assays; 2, 2–diphenyl–1–picrythydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) as described by Yahia and Mondragon– Jacobo (2011). One gram of sample was homogenized with 10 mL of hexane/ dichloromethane (1:1, v:v) in an Ultra Turrax model T25 basic homogenizer (IKA Works, Willmington, NC). The homogenate was sonicated for 5 min in a Bransonic 2510 sonicator (Bransonic Ultrasonic Co., Danbury, CT) and centrifuged in a HERMLE Z 323 K (Labortechnik, Wehingen, Germany) at 15000 g and 4ºC for 10 min. The supernatant was collected and the residue was subjected to re-extraction repeating the same steps. The supernatants collected from the first and second extractions were mixed and evaporated to dryness at 40ºC and low pressure using R-200 Büchi rotary evaporator (Büchi Labortechnik, Postfach, Switzerland). After evaporation 10 mL of HPLC – grade acetone was added and the extract was filtered through Whatman filter paper no. 2 and designated as LPE. The residue after the second extraction was homogenized in 20 mL of acetone/ water/acetic acid (70: 29.5: 0.5, v: v: v) and sonicated

Table 2. Composition of different phenolic compounds in mg/kg DW, analyzed by HPLC, TSP in mg GAE/g DW and TC in mg/kg DW of the analyzed pulps*

Phenolic compound	Doum	Jujube	Tamarind	Baobab
Hydroxybenzoic acids				
Gallic acid	ND	ND	504.9±21.54	ND
Vanillic acid	336±7.20	416±10.21	75±2.31	3120±22.08
p-hydroxybenzoic acid	408±7.21	331±14.23	42±1.24	ND
Protocatechuic acid	ND	1.1±0.00	ND	ND
Hydroxycinnamic acids				
Caffeic acid	293.3±3.53	243.4±3.21	ND	42.8±0.14
Sinapic acid	1367.6±18.35	337.9±13.34	ND	179.2±5.21
p-coumaric acid	ND	46.3±2.25	161.8±2.35	ND
Cinnamic acid	69.4±2.11	95.7±2.28	104.0±4.10	330.4±6.87
Metoxicinnamic acid	2219.4±20.12	88.4±2.14	289.2±2.08	ND
2-hydroxicinnamic acid	217.9±8.24	ND	ND	ND
3-4 dihydrocinnamic acid	330.7±3.25	273.5±2.21	41.5±0.19	ND
Chlorogenic acid	584.6±2.55	663.9±4.87	36.8±0.11	362.8±11.45
Flavanols				
Myricetin	243.2±2.56	ND	ND	ND
Quercetin	58.2±1.43	ND	ND	ND
Flavan-3-ols				
Epicatechin	133.6±1.02	129.0±2.21	20.0±0.02	39.3±0.97
Catechin	572.5±0.99	1308.4±17.26	363.41±3.74	ND
TSP	45.08±1.02	32.08±1.09	18.17±0.58	13.92±0.73
TC	7.01±0.6	12.29±0.63	14.92±1.81	16.13±1.25

* All the given values are means of six determinations ± standard deviation. ND= not detected.

and centrifuged as described above. The supernatant was collected and the residue was subjected to re-extraction. The collected supernatants from the first and second extraction were mixed, filtered through Whatman filter paper no. 2 and designated as HPE.

DPPH and FRAP assays were performed using a microplate reader. An amount of 280 µL of DPPH/ methanol solution or FRAP reagent per hole was placed first in a microplate followed by 20 µL of extract per hole. Methanol was included as a blank. The microplate was allowed for 30 min incubation in dark. DPPH was prepared with 100 mM DPPH/ methanol. FRAP reagent was prepared with 50 mL of 300 mM acetate buffer (pH 3.6), 5 mL of 10 mM 2,4,6-tripyridyl-2-triazine (TPTZ) in 40 mM of HCl, and 5 mL of 20 mM of $FeCl_3$. TAC determination was taken at 490 nm for DPPH and at 360 nm for FRAP in a MRX microplate reader (Dynex Technology, Chantilly, VA). TAC was expressed as Trolox equivalents (TE) in µmoles/g DW.

Statistical analysis

It was done using Statview statistical program. Results were presented as mean ± standard deviation of at least three replicates.

Results and Discussion

Proximate composition

The proximate composition and carbohydrates of the analyzed pulps are presented in Table 1. Results for baobab pulp were comparable to the results of Osman (2004) and of lower values in comparison to the study of Compaoré *et al.* (2011) that investigated Burkina Faso's samples. Tamarind pulp's values were in agreement with the values reported by Rao and Mathew (2001). Jujube pulp showed values comparable to the values published by Amoo and Atasie (2012).

Mineral contents

As shown in Table 1 the most abundant Ca, K, Mg, Fe and Cu were measured in baobab pulp. Wild fruits investigated for their chemical composition in various publications (Nour *et al.*, 1980; Saka and Msonthi, 1994; Osman, 2004; Compaoré *et al.*, 2011) had a wide range of mineral contents. The reported level of mineral contents in this and other studies

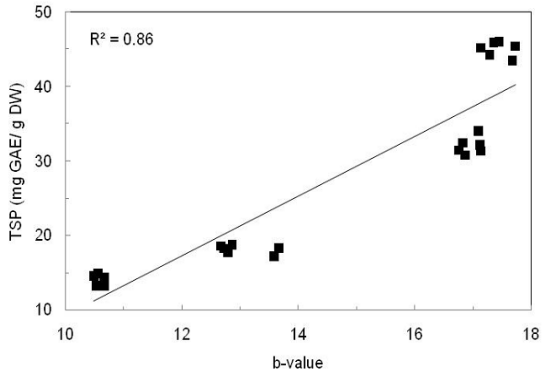

Figure 1. Correlation between total soluble phenols (TSP) and color b-value of the analyzed pulps

make a considerable contribution to the recommended daily intake which is 1000 mg Ca, 3500 mg K, 350 mg Mg, 15 mg Fe (IOM 1997).

Determination of phenolic total soluble contents and compounds

TSP values ranged from 13.92 mg GAE/g DW in baobab to 45.08 mg GAE/g DW in doum (Table 2). Different TSP values were published elsewhere; e.g. Mohamed *et al*. (2009) reported 64.90 mg GAE/g DW for doum pulp, Lamien–Meda *et al.* (2008) reported 40.58 for baobab pulp and 8.89 mg GAE/g DW for tamarind pulp. The work of Lamien–Meda *et al*. (2008) showed high polyphenols for the fourteen investigated Burkina Faso's wild fruits. TSP in wild fruits investigated in this and other studies were superior to that detected in domestic fruits. TSP values found in apple, plum, apricot, mulberry and strawberry were in the range 4.10 – 31.20 mg GAE/g DW (Sultana *et al*., 2012). The commonly consumed dry fruits in India (almond, apricot, brown raisins, cashew nuts, dry dates, fresh dates, figs, ground nut, piyal seeds, walnuts) showed TSP in the range 0.99 – 9.59 mg GAE/g DW (Reddy *et al.*, 2010). Consumption of fruits and vegetables was reported to prevent a number of chronic diseases (Lock *et al*., 2005). Asami *et al*. (2003) attributed the bioactive potential of fruits and vegetables to their high content of polyphenols. Our TSP and b-color values were highly correlated, showed an R^2= 0.86 (Figure 1). The relation between color and phenolic content was reported in the study of Xu *et al.* (2007) where colored beans and black soybeans exhibited higher TSP and TAC values than those of yellow peas, green peas, and chickpeas.

The most abundant phenolic compounds recorded in our doum, jujube, tamarind and baobab

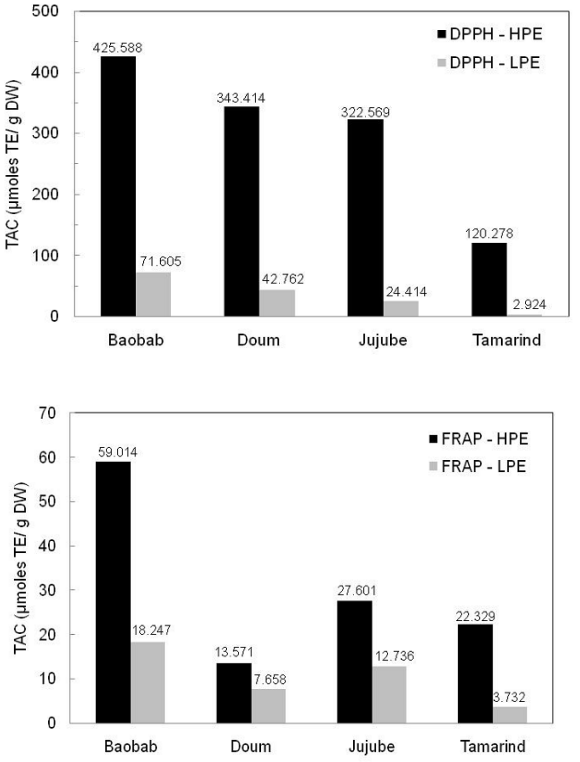

Figure 2. Total antioxidant capacity (TAC) of hydrophilic (HPE) and lipophilic (LPE) extracts measured by DPPH and FRAP assays for the analyzed pulps

pulps were metoxicinnamic acid, catechin, gallic acid and vanillic acid, respectively (Table 2). None of the investigated pulps showed ferulic acid and kaempferol. Jujube and tamarind showed higher p–coumaric cid and jujube and doum pulps exhibited higher caffeic acid contents in comparison to the domestic fruits investigated by Sultana *et al*. (2012).

Total carotenoids content

We measured TC amounting to about 16 mg/kg DW in baobab, 14 mg/kg DW in tamarind, 12 mg/kg DW in jujube and 7 mg/kg DW in doum pulps (Table 2). The low carotenoids levels recorded in these pulps might be attributed to the dryness of the samples. Smith *et al*. (1996) found that the fresh samples contained twice the carotenoids content as dry samples.

Total antioxidant capacity (TAC)

TAC (Figure 2) measured from HPE was in the range 120.278 – 425.588 µmoles TE/g DW when using DPPH assay and in the range 22.329 to 59.014 µmoles TE/g DW when using FRAP assay. The DPPH values were higher than FRAP values and HPE values were higher than those of LPE. Baobab pulp had the highest TAC values when measured from the two extracts and assays and tamarind came last except when measured from HPE using FRAP assay. DPPH

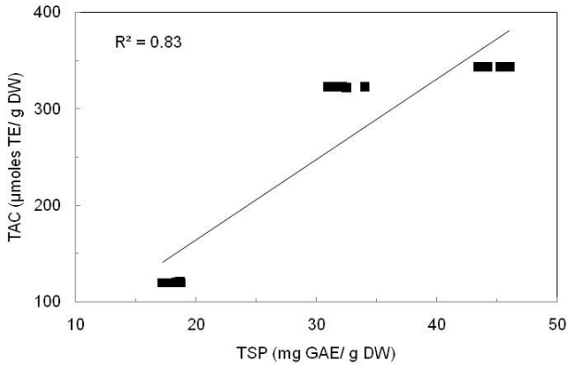

Figure 3. Correlation between total antioxidant capacity (TAC), measured from HPE using DPPH assay, and total soluble phenols (TSP) for the analyzed pulps, excluding baobab

measured in HPE was highly correlated to TSP (R^2 = 0.83) when excluding baobab pulp (Figure 3). Strong correlation between total phenolics content and TAC reported in this and other studies (Deighton *et al*., 2000; Dykes *et al*., 2005; Lamien-Meda *et al.*, 2008) concluded the significant role that total phenols can play in antioxidant activity.

Conclusion

The investigated wild fruit pulps possess high phenolic compounds and antioxidant capacity and good amount of minerals but low carotenoids. Total soluble phenolic contents were positively correlated to the color b–value and antioxidant capacity. Baobab pulps, which had the highest TAC but lowest TSP, behaved differently. We concluded that our analyzed pulps seem to have a high nutritional value and bioactive potential. Biological studies to explore the advantageous effect of these wild fruits for human health are needed.

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