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Research Article

Identification of Flavonoids, Phenolics Profile by LC-MS/MS and Antioxidant Activity of Crude Extracts of Baobab (*Adansoniadigitata. L*) Fruit Pulp from Different Regions in Sudan

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Abstract

Flavonoids and Phenolics profiles of aqueous and ethanolic crude extracts of baobab fruit pulp from different geographical regions in Sudan were analyzed by LC-MS/MS. Eleven phenolic compounds were identified (Ferulic acid derivative, vanillic acid derivate, (4-6-Dimethyl-3(4'-hydroxyphenylcoumarins, (+)-(E)-caffeoyl-L-malic acid, Ferulic-caffeoyl glucose derivative, p-Hydroxybenzoic acid, Caffeoyl aspartic acid, caffeoyl glucose derivative, caffeoyl glucose, Galloylshikimic and p-Coumaroyl glycolic acid). Thirty-six of flavonoids, Stilbenes resveratrol derivate, octadecanoic and Citric acid were found moreover, antioxidant activity results were, DPPH assay for ethanolic and aqueous extracts ranged from 183.50 to 227.92 and 184.70 to 221.30 mg AEAC /g respectively, while for FRAP ethanolic and aqueous extracts ranged from 217.04 to 209.33 and from 205.80 to 191.64 mmol /g of Fe+2 respectively. Furthermore there were significant differences in total phenolic and flavonoids contents between the different extracts from different regions in Sudan. Total phenolics were ranged from 15.50 to 99.66 mg GA/g of Gallic Acid Equivalent (GAE) /g, while Flavonoids were ranged from 1.03 to 21.53 mg of CA/g. Ascorbic acid content was 372.52, 355.97, 354.13 and 345.82 mg/100g for Damazin, El Obeid, Umm Ruwaba and Nyala samples, respectively. This is the first report about phenolics and flavonoids profile of Sudanese baobab fruit and reveals new information about antioxidant activity of Sudanese baobab fruit indicating its potential as functional foods ingredient.

Keywords: Baobab Fruit; Flavonoids; Phenolics Profile; Antioxidant Activity

Introduction

Baobab (*Adansoniadigitata. L*) is a very large long-living tree widespread throughout the hot and drier regions of tropical Africa [1]. It belongs to the family, Bombacaceae, sub-family of Malvaceae [2]. In Sudan, it is known as Tabaldi or Gonglase, and is frequently found on sandy soils. It is found on seasonal streams in Kordofan, Darfur, and Blue Nile [3]. Its fruit has a woody pericarp and spongy pulp with uniform seeds [4]. (Several studies have indicated that baobab fruit pulp is rich in vitamins and minerals [5]. It contains a high amount of both soluble and insoluble dietary fiber [6]. This fruit is also known for its high content of vitamin C, which contributes to its overall antioxidant capacity [7]. It is also a good source of polyphones, including certain flavonoids [8].

Baobab (*Adansoniadigitata*) fruit is reported to be a good source of antioxidants and very significant in human nutrition [9]. Antioxidant is defined as a substance that when present in low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substance [10]. Moreover, Antioxidants are strongly correlated with the prevention of degenerative illness, such as cardiovascular, neurological diseases, cancer and oxidative stress dysfunction [11]. These facts are important considerations of the consumers who are more interested in safe and natural sources of antioxidants. Several studies were carried out to evaluate antioxidant potential of many botanicals and herbs, which could be useful as nutraceutical ingredients [12]. Ten aromatic compounds including isopropyl myristate and non anal were identified in the fruit pulp of Baobab using GC- MS [13].

Several compounds have been isolated from the pericarp using column chromatography and include: (–)-epicatechin, epicatechin-(4ß.8)-epicatechin (B2), epicatechin-(4ß.6)-epicatechin (B5), epicatechin- (2ß.O.7, 4ß.8)-epicatechin (A2), and epicatechin-(4ß.8)- epicatechin-(4ß.8)-epicatechin C1) [14]. Epicatechin is known to exhibit strong antioxidant activity and has positive health effect in diabetic mice [14]. However, to our knowledge, no study was conducted on flavonoids and phenolic acid profile of Sudanese baobab fruit pulp from different regions. Therefore the aim of this study is to investigate flavonoids, phenolics profile and antioxidant activity of crude extracts of Baobab (*Adansoniadigitata. L*) fruit pulp from different geographical regions in Sudan.

Material and Methods

Materials

Baobab fruits capsules were obtained from different geographical

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regions in Sudan; El Obeid (13° 11′ 0″ N, 30° 13′ 0″ E), Umm Ruwaba (12° 54′ 16.41″ N, 31° 12′ 28.32″ E), Nyala (12° 2′ 11″ N, 24° 52′ 37″ E) and Damazin (11° 46′ 0″ N, 34° 21′ 0″ E). The Fruit pulp was prepared by breaking the capsules manually, seeds were removed and pulp powder was sieved using appropriate mesh. The resulting fruit pulp was stored in dark polyethylene bags at -18°C until used.

Preparation of fruit pulp extracts: Baobab fruit pulp aqueous and ethanolic extracts were prepared by the method of [15] with some modification. Twenty grams of the baobab fruit pulp were used for extraction with 60 ml of two different solvents: 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 under the same conditions. Then, both supernatants were filtered through Whatman filter paper (Whatman International Limited, Kent, England) using a chilled Buchner funnel. The filtrates were transferred into evaporating flask with an additional 50 ml of 80% aqueous ethanol and were concentrated in a rotary evaporator (Buchi Rotavapor, Switzerland) at 45 °C. The resulting concentrate was then mixed with 15 ml of deionised (DI) water (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 powders which were kept in dark glass bottles at -18 °C until used for analysis.

Methods

Identification of phenolic compounds by LC-MS/MS: The samples were analyzed according to the method described by [16] with some modifications. Electro-spray ionization mass spectrometry (LC-EPI-MS/MS) was used to identify phenolic acids and flavonoids profile. UHPLC software Application Flexar FX-15 Pump, equipped with a binary gradient solvent pump, a degasser, and an auto sampler, column oven connected to 3200 QTRAP Mass spectrometer (AB Sciex, USA) was used for this purpose. Chromatographic separations were carried out at 25 °C, on column Phenomenex Synergi Fusion 100 mm X 2.1 mm, 5 μM, with a mobile phase consisting of water containing 5 mM ammonium and 0.1% formic acid (solvent A) and acetonitrile containing 0.1% formic acid and 5mM ammonium (solvent B), the flow rate was 0.2 mL/min and 250 μL of samples were injected. The QTRAP-MS system was equipped with Enhanced Product Ion (EPI) operated in the negative-ion mode. EPI worked at the following setting: capillary temperature 500 °C, curtain gas at 10 psi, and negative ionization mode for a mass range from m/z 100 to 1500. The data was acquired and processed using Analyst Software Version 1.5.2. Triplicate injections were made for each sample. The analytes were identified by comparing retention time and m/z values obtained by MS and MS2 with the mass spectra identified according to the corresponding spectral characteristics: mass spectra, accurate mass, characteristic fragmentation and characteristic retention time, the Internet database of accurate mass spectrometry data (www.chemspider.com), mass spectra, mass bank and database: *http://www.phenol-explorer.eu* were used for their identification.

Antioxidant activity assays: Antioxidant activity of the extracts was evaluated using DPPH (1, 1-dipheny1-2-picrylhydrazyl radical) and FRAP (ferric reducing antioxidant power) according to the method described by [17] with some modifications.

DPPH assay: DPPH test was determined spectrophotometrically. This assay depends on the limit of the capacity of the antioxidant to scavenge the radical cation 1, 1-dipheny1-2-picrylhydrazyl radical (DPPH). Fifty microlitres of diluted sample (5mg /ml, with solvent) were dissolved in 2 mL of 0.04 mmol/L DPPH in methanol. A calibration curve in the range 0.05-1.0 mmol/L was used for the Ascorbic acid, and data were expressed as Ascorbic acid equivalent antioxidant capacity (ASAC, mmol/g). Spectrophotometric readings were carried out at 517 nm, using plastic cuvette (10 mm) after an incubation period of 60 min in the dark. Samples were analyzed in four replicates and the mean values were calculated.

FRAP assay: Ferric reducing antioxidant power evaluates antioxidants as reductants of Fe^{3+} to Fe^{2+}, which is chelated by 2,4,6-tris(pyridin-2-y1)-1,3,5-triazine (TPTZ) to form a Fe^{2+}– TPTZ complex absorbing at 593 nm. The FRAP test was done by preparing a ferric complex TPTZ and Fe^{3+} (0.3123 g TPTZ, 0.5406 g $FeC1_36H_2O$ in 100 ml acetate buffer pH 3.6). Fifty microlitres of diluted sample (5 mg/ml, with solvent), was dissolved in 2 ml of ferric compound and, followed by an incubation period of 4 min in the dark, absorbance at 593 nm was measured with a spectrophotometer. Quantitative analysis was performed according to the external standard method ($FeSO_4$, 0.1-2.00 mmol/L). Correlation of the absorbance with the concentration was expressed as mmol/g of Fe^{2+}. Samples were analyzed in four replicates and the mean values were calculated.

Total phenolics content: Total Phenolics Content (TPC) was determined by spectrophotometric determination using Folin-Ciocalteu reagent according to the method of [17] 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.

Total flavonoids content: Total flavonoids content was determined spectrophotometer using aluminum chloride by the method of [18]. Four ml of distilled water were added to 1 ml of the fruit pulp extracts (5 mg/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.

Table 1: Compounds identified by LC-EPI-MS/MS in extracts of baobab fruit pulp samples.

Aqueous Extracts					Ethanolic Extracts			
Sample	RT	Negative ions [M_H]_ (m/z)	Fragment MS-MS ions (m/z)	Compound name	RT	Negative ions [M_H]_ (m/z)	Fragment MS-MS ions (m/z)	Compound name
El Obeid	1.32	897	351.03 ,527.04,175.01 ,545.11,721.07	Cyaniding-3-glycoside	1.43	191	110.97, 86.97	Citric acid
	1.92	515.12	110, 86 ,172	Citric acid derivative	1.63	571	228.95, 166.97, 86.99	Resveratrol derivative
	3.37	952	892.22 , 611.19, 674.16 , 820.25	Granatin B, dehydrohexahydroxydiphenoyl	4.1	689	397.07, 289.07, 245.05, 535.13	Procyanidintrimer T
	4.21	407	255.03 ,407.03 , 227.01 ,211.01, 243.03 ,283.03	Kaempferol-3-Glucoside	5.27	220	166.85, 184.87, 201.80	vanillic acid derivative
	4.45	687	289.06 ,397.04,245.06,235.04	Procyanidintrimer	10.69	749	255.22, 283.27	Kaempferol glycoside derivative
	7.11	293	221.15, 205.14, 148.08, 177.12 192.14, 236.09	Ferulic acid derivative	10.93	735	698.57, 374.28	Malvidin 3-glucoside-
	8.07	727.42	653.4	Cyanidin 3-O-xylosyl-rutinoside	-	-	-	-
	11.85	901	842	Delphinidin 3-O-[6-O-(p-coumaroyl)-β-D-glucopyranoside]-5-O-[4-O-acetyl-6-O-malonyl-β-D-glucopyranoside	-		-	-
	12.67	887	826.68 , 854.64	Malvidin 3-O-(6-O-(4-O-malonyl-α-rhamnopyranosyl)-β-glucopyranoside)-5-O-β-glucopyranoside	-			
	13.62	886	825.68,853.62	Kaempferol 3-isorhamninoside-7-rhamnoside	-	-	-	-
Umm Ruwaba	0.6	295	295.06, 176.97,234.97,178.96	(+)-(E)-caffeoyl-L-malic acid	1.67	571	228.95, 166.97, 86.99	Resveratrol derivative
	1.21	1042	527.11, 175.00 ,351.01		4.19	245	159.02, 173.03, 187.03	(epi)Catechin
	1.44	583.03	193, 341 .449	Ferulic- caffeoyl glucose derivative	7.32	265	96.93,79,95	Adenosine
	4.09	865	289.06 , 407.04, 245.05, 577.11	Procyanidintimer	8.52	452	255.22	Pinocembrin derivative
	5.42	277	116.99 ,145.06	Caffeoyl aspartic acid	9.73	473	392.31,452.29	Unknown
	9.63	729	729	Cyanidin 3-O-xylosyl-rutinoside	10.57	454	454	7,8,3',4',5'-Pentamethoxy-6",6"-dimethylpyrano [2",3":5,6] flavones
	9.99	815	410.35, 279.23,764.48	Ipriflavone derivative	11.3	749	255.24, 283.26,227	Kaempferol glycoside derivative
					12.02	752	255.24 , 283.26,227.00	Isomer Kaempferol glycoside derivate
	10.722	851	814.57, 410.35	Unknown	12.63	719	682.61, 383.38, 337.37,426.39	Unknown
	11.08	642	625.53, 387	Quercetin 3,4'-di-O-β-glucopyranoside	13.24	683	337.48 ,383.45, 426.45	Petunidin 3-glucoside-5-(6"-acetylglucoside)
	11.68	817	493.22,449.25,279	Methyl-myricetin-trihexoside (B)	-	-	-	
	12.41	681	337.36 ,383.36,426.39,253.23, 223.22 ,365.36 ,408.38 644.59 , 662.60	Petunidin 3-glucoside-5-(6"-acetylglucoside)	-	-	-	-
	12.41	681	337.36 ,383.36,426.39, 253.23, 223.22 ,365.36 ,408.38 644.59 , 662.60	Petunidin 3-glucoside-5-(6"-acetylglucoside)	-	-	-	-
	12.78	869	519.20, 504.24	Theaflavin 3,3'-O-digallate	-	-	-	-
Nyala	1.31	387	341	caffeoyl glucose derivative	1.43	517	110.97, 86.99	Citric Acid derivative
	1.44	897	351.03 ,527.04,175.01 ,545.11, 721.07	Unknown	1.67	571	228.95, 166.97, 86.99	Resveratrol derivative
	3.12	1097	1079.36,615	Cyanidin 3-glucoside-5,3'-di-(caffeoylglucoside)	7.07	293	221.14, 205.11,2 36.09	Ferulic acid derivative
	7.81	297	183.00,197.02	Oxooctadecanoic acid	8.88	485	485	Unknown
	8.44	325	183,169.99	Galloylshikimic acid	9.72	683	664.00,337.36, 383.32, 664	Petunidin 3-glucoside-5-(6"-acetylglucoside)
	9.91	680	337.35 ,383.34, 426.37	Petunidin 3-glucoside-5-(6"-acetylglucoside)	11.05	682	664.00, 337.44 ,383.41, 426.43	Isomer Petunidin 3-glucoside-5-(6"-acetylglucoside)

	10.48	655	309.33, 355.32	Malvidin 3,5-O-diglucoside	11.65	719	682.64, 383.40, 337.39	Unknown
	11.66	642	625.56,387.29	Quercetin 3,4'-di-O-β-glucopyranoside	12.5	879	842.61, 438.36, 680.68	Procyanidin dimer digallate(A-type)
	12.86	832	796.59 , 634.6, 616.6,	Quercetin 3-(6'''-sinapylglucosyl) (1->2)-galactoside	13.11	879	842.64, 438.36, 680.76	Isomer Procyanidin dimer digallate (A-type
Damazin	1.19	866	175.00, , 527.09,351.02	Robinetinidol-(4α->8)-catechin-(6->4α)-robinetinidol	0.59	295	277.06, 177.01 , 235.02	Caffeoyl aspartic acid
	1.55	431	179.06,59.02,112.99	caffeoyl glucose	1.43	517	110.96, 173.01 ,86.98	Citric Acid derivative
	3.24	1097	1079, 1037.28	Delphinidin 3-glucoside-5-(6-caffeoylglucoside	5.87	593	284.01, 255.02, 227.05, 145.06	Kaempferol-3-rutinoside
	4.09	407	255.02, 283.01, 227.05	Kaempferol glycoside	7.19	293	221.15, 205.11, 148.04,177.08	Ferulic acid derivative
	4.45	687	669.03, 407.07, 397.07, 289.07, 245.05	Procyanidin C1	7.92	593	277.21, 152.98, 241.00, 315.03	Epicatechin 3,5-di-O-gallate
	4.81	577	289.07, 407.05, 245.05, 161.02	Procyanidin B1	9.72	433	152.99, 170.97 ,279.27	Quercetin-3-Arabinoside
	7.29	222	168.84, 184.82,	p-Coumaroyl glycolic acid	8.88	452	255.22, 196.042, 78.96	3-Hydroxyphloretin 2'-O-glucoside
	9.39	540	255.22, 480.26	Pinocembrinderivative	10.81	616	162.83, 581.23	Cyanidin 3-O-sambubioside
	10.11	639	620.63, 602.54,572.51, ,382.35, 394.34, 364.33, 338.34	Malvidin 3-O-(6''-p-coumaroyl-glucoside)	11.65	861	824.60, 644.59, 662.65	Pelargonidin 3-(6''-malonylglucoside)-5-glucoside
	10.84	639	620.63, 602.54,572.51, ,382.35, 394.34, 364.33, 338.34	Isomer Malvidin 3-O-(6''-p-coumaroyl-glucoside)	11.9	713	666.59, 410.38, 267.25, 367.37,255.00	Delphinidin 3-(6-malonylglucoside)-5-glucoside
	10.59	681	337.32, 383.34	Pelargonidin 3-(6''-malonylglucoside)-5-glucoside	12.99	713	666.61, 410.44, 422.42, 267.32,255.00	Isomer Delphinidin3-(6-malonylglucoside)-5-glucoside
	11.69	750	255.23, 283.27	Kaempferol glycoside derivative	-		-	-
	13.38	669	650.00, 323.38 369.37	Delphinidin 3-glucoside-5-(6-acetylglucoside)	-	-	-	-

Determination of ascorbic acid: Determination of ascorbic acid was done using 2-6- Dichlorophenol Indophenol reagent according to [19]. 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 5 ml aliquot were diluted with 5 ml 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 500 ml 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:

Ascorbic acid (mg/100g) = (Titre × dye strength × 100)/Factor

Factor = (Sample wt. × Sample volume for titration)/(Total volume of sample)

Statistical Analysis

Data were analyzed using one-way ANOVA using MINITAB16 [20] Statistical Software for Windows (State College, PA. USA) with a significant level of $p<0.05$.

Results and Discussion

Phenolic Compounds Identified in Extracts of Baobab Fruit Pulp from Different Geographical Regions in Sudan

The results of phenolic compounds in baobab fruit pulp extracts are shown in Table 1. The molecular weights of these compounds are between 137 and 1097 Da, suggesting that they belong to the class of simple and complex phenolic and flavonoids compounds. These compounds were fragmented to full scan spectra from 100 to 1500 Da by MS/MS on negative mode. The results of these analyses were compared to the fragments of mass spectra, mass bank and database: http://www. Phenol- explorer.eu was used for their identification. In Aqueous extract the phenolics and flavonoids profiles of baobab fruit pulp from different geographical regions in Sudan were found to be composed of different components. The results of an aqueous extracts of baobab fruit pulp from El Obeid are shown in Figure 1 (A). As shown in the figure, the extract contains one phenolic acid namely Ferulic acid derivative and 12 flavonoids component namely Cyanidin-3-glucoside, Kaempferol-3-Glucoside, Procyanidintrimer, Cyanidin 3-O-xylosyl- rutinoside, Ipriflavone derivate, Pinocembrin derivate, Kaempferol glycoside derivate, Kaempferol glycoside derivate isomer, Delphinidin 3-[6-O-(p-coumaroyl)-β-D-glucopyranoside]-5-O-[4-O-acetyl-6-Omalonyl-β-D-glucopyranoside,Malvidin3-O-(6-O-(4-O-malonyl-α-rhamnopyranosyl)-β-glucopyranoside)-5-O-β glucopyranoside and Kaempferol3-isorhamninoside-7-rhamnoside.

Figure 1(B) shows the results of an aqueous extracts of baobab fruit pulp from Umm Ruwaba. It contains four phenolic acids namely,(+)-(E)-caffeoyl-L-malic acid , Ferulic- caffeoyl glucose derivative, p-Hydroxybenzoic acid and Caffeoyl aspartic acid and

Table 2: Antioxidant activity expressed as mmol/g equivalents of Ascorbic acid and $Feso_4.7H_2O$.

	Ethanolic Extracts		Aqueous Extracts	
Baobab fruit Geographical Regions	**DPPH mmol/g**	**FR AP mmol/g**	**DPPH mmol/g**	**FR AP mmol/g**
El Obeid	201.1±0.00^c	211.03±0.02^b	193.67±0.04^c	209.50±0.00^a
Umm Ruwaba	183.50±0.00^d	209.33±0.02^c	221.30±0.00^a	205.80±0.00^a
Nyala	205.87±0.01^b	210.99±0.03^b	184.70±0.00^d	206.83±0.01^a
Damazin	227.92±0.00^a	217.04±0.01^a	200.17±0.03^b	191.64±0.70^b

Values are means ± SD (n=4); means with different superscripts in the same column are significantly different (p≤ 0.05).

eight flavonoids component namely, Procyanidin C1, Cyanidin 3-O-xylosyl- rutinoside, Ipriflavonederivative, Ipriflavonederivative, Quercetin 3,4'-di-O-*β*-glucopyranoside, Methyl-myricetin-trihexoside (B), Petunidin 3-glucoside-5-(6''-acetylglucoside) and Theaflavin 3,3'-O-igallate.

Figure 1(C) shows the results of an aqueous extracts of baobab fruit pulp from Nyala, it has two phenolic acids (caffeoyl glucose derivative and Galloyl shikimic acid) and five flavonoids components namely v Cyanidin 3-glucoside-5,3'-di-(caffeoylglucoside), petunidin3-glucoside-5-(6''-acetylglucoside), Malvidin3,5-O-diglucoside, Quercetin3,4'-di-O-β-glucopyranoside and Quercetin 3-(6'''-sinapylglucosyl)(1->2)-galactoside.

The results of an aqueous extract of baobab fruit pulp from Damazin are shown in Table 1 Figure (D). It has two phenolic acids, namely caffeoyl glucose and p-Coumaroyl glycolic acid and 11 flavonoids component, namely Robinetinidol-(4*α*->8)-catechin-(6->4*α*)-robinetinidol, Delphinidin 3-glucoside-5-(6- caffeoylglucoside, Kaempferol glycoside, Procyanidin C1, Procyanidin B1, Pinocembrin derivative, Malvidin 3-O-(6''-p-coumaroyl-glucoside), Pelargonidin 3-(6''-malonylglucoside)-5-glucoside, Kaempferol glycoside derivative and Delphinidin3-glucoside-5-(6-acetylglucoside) .

The aqueous extracts of baobab fruit pulp from different geographical regions in Sudan contain various components of phenolic acids and flavonoids. Phenolic acid (Ferulic acid) was found in baobab fruit pulp from El Obeid and Umm Ruwaba. While caffeoyl glucose derivative was found in Nyala and Damazin baobab fruit. On the other hand the flavonoids component Procyanidin C1existsin all of baobab fruit pulp samples except the sample from Nyala, Cyanidin 3-O-xylosyl- rutinoside and Ipriflavone derivative were found in the samples from El Obeid and Umm Ruwaba. Kaempferol glycoside was found in baobab fruit from El Obeid and Damazin, while Quercetin 3-(6'''-inapylglucosyl) was found in baobab fruit from Umm Ruwabaand Nyala.

The results of ethanolic extracts are shown in Figure 2. As shown in Figure 2 (A) the ethanolic extract of baobab fruit pulp from El Obeid contains one phenolic acid (Vanillic acid derivative) and six flavonoids components (Procyanidintrimmer, Kaempferol glycoside derivative, Malvidin 3-glucoside, Pelargonidin 3-O-[2-O-(*β*-D-xylopyranosyl)-6-O-(methyl-malonyl)-bata-D-alactopyranoside,Pelargonidin3-(6''-malonylglucoside)-7-(6'''caffeylglucoside) and Cyanidin 3-(6''-(E)-p-coumarylsambubioside)-5-glucoside.

Figure 2(B) shows the results of ethanolic extract of baobab fruit pulp from Umm Ruwaba which contains one phenolic acid (4,6-Dimethyl-3(4'-hydroxyphenyl) coumarins) and seven flavonoids components (epi-Catechin, Adenosine, Pinocembrin derivative,

Table 3: 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 extract**	**Aqu eth OH**
El Obeid	21.38±0.20^a	42.29±0.24^b	1.03±0.08^a	10.77±0.23^b
Umm Ruwaba	15.50±0.30^d	58.46±0.22 ^{a}	2.00±0.10^a	15.76±0.67^b
Nyala	17.16±0.05^c	35.36±0.34^c	1.62±0.02^b	5.77±0.07^c
Damazin	22.62±0.07^a	58.93±0.22^a	1.46±0.02^b	16.06±0.15^a

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

7,8,3',4',5'-Pentamethoxy-6'',6''-dimethylpyrano[2'',3'':5,6] flavones, Kaempferol glycoside derivate, Isomer Kaempferol glycoside derivative and Petunidin 3-glucoside-5-(6''-acetylglucoside).

Figure 2(C) shows the results of ethanolic extract of baobab fruit pulp from Nyala, it has one phenolic acid (Ferulic acid derivative)andfour flavonoids component (Petunidin 3-glucoside-5-(6''-acetylglucoside), Isomer Petunidin 3-glucoside-5-(6''-acetylglucoside), Procyanidin dimer digallate (A-type) and Isomer Procyanidin dimer digallate (A-type).

Figure 2(D) shows the results of ethanolic extract of baobab fruit pulp from Damazin, it has two phenolic acids (Caffeoyl aspartic acid and Ferulic acid derivative) and eight flavonoids components (Kaempferol-3-rutinoside, Epicatechin 3,5-di-O-gallate, Quercetin-3-Arabinoside, 3-Hydroxyphloretin 2'-O-glucoside, Cyanidin 3-O-sambubioside, Pelargonidin 3-(6''-malonylglucoside)-5-glucoside, Delphinidin3-(6-malonylglucoside)-5-glucoside and Isomer Delphinidin 3-(6-malonylglucoside)-5-glucoside.

In ethanolic extracts of baobab fruit pulp form different geographical regions in Sudan various component of phenolics and flavonoids were found, The same phenolic acid (Ferulic acid derivative) was in baobab fruit pulp from Nyala and Damazin, while Kaempferol glycoside was found in baobab fruit pulp from El Obeid and Umm Ruwaba, Pelargonidin 3-(6''-malonylglucoside) was found in baobab fruit pulp from Obeid and Damazin. Petunidin 3-glucoside-5-(6''-acetylglucoside) was found in baobab fruit pulp from Umm Ruwaba and Nyala. The variation in phenolic acids and flavonoids profiles may be due to the differences in soil of the regions in Sudan and could be attributed to the climatic and geographical characteristics of the regions studied. The effects of these variables on the phenolic composition have also been mentioned by other authors [21] and [22]. Few researchers have reported the presence of polyphenols including procyanidins [23] and [24], procyanidin B2,

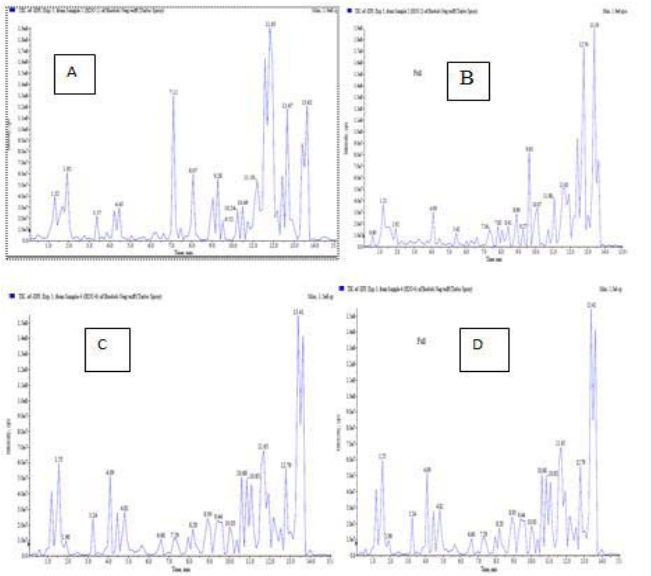

Figure 1: Full spectrum LC- MS/ MS using aqueous extract of baobab fruit pulp from El Obeid (A), Umm Ruwaba (B), Nyala (C) and Damazin (D).

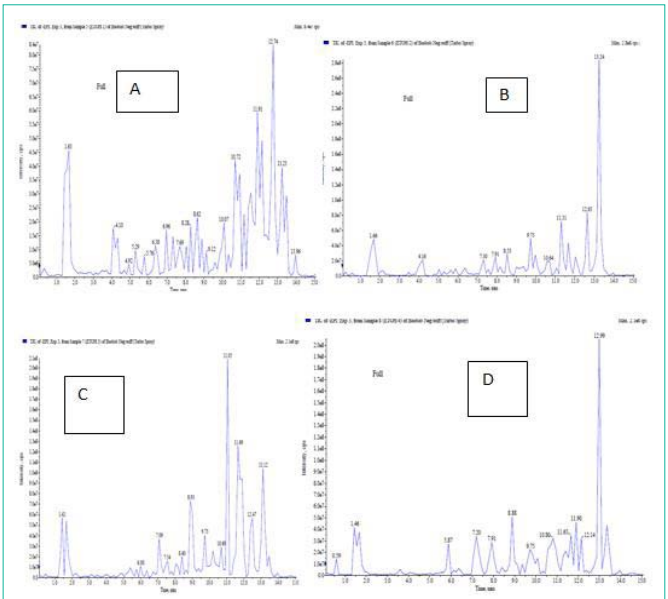

Figure 2: Full spectrum LC- MS/ MS using Ethanolic extract of baobab fruit pulp from El Obeid (A), Umm Ruwaba (B), Nyala (C) and Damazin (D).

(-)-epicatechin, (-)-epigallocatechin-3-O-gallate and gallic acid [25] in baobab fruit pulp. Moreover baobab fruit pulps were found to be rich in procyanidins and flavonol glycosides [26] and [27].

This investigation is the first report of phenolics and flavonoids profile of Sudanese baobab fruit pulp. These results suggest that Sudanese baobab fruit pulp is a promising functional food ingredient.

Other Phenolics and Unidentified Compounds

Several compounds are found in the negative ion mode. As shown in Table 1, the molecular ion m/z (571) base fragment 228 indicates that Stilbenes resveratrol derivative is found in all baobab fruit pulp ethanolic extracts except baobab fruit from Damazin. M/z (517). Citric acid derivative was found in baobab fruit from Nyala and Damazin in ethanolic extracts, however in aqueous extracts it was found only in the sample from El Obeid. m/z191 Citric acid was found in ethanolic extract of the baobab fruit from El Obeid. m/z 311(15, 16-dihydroxy-9Z,12Z-octadecadienoic acid) was found in baobab fruit from Umm Ruwaba in ethanolic extracts, m/z 297 (Oxooctadecanoicacid) was found in baobab fruit from Nyala in an aqueous extract. Unidentified compounds in pulp extracts were indicated by molecular ion [M-H] m/z 1042,473,851,897,485,719, and fragment ions of compounds are shown in Table 1.

Antioxidant Activity Assays

The DPPH and FRAP antioxidant capacity results are shown in Table 2. DPPH assay and FRAP results of the antioxidant capacities showed significant difference ($p \leq 0.05$) between the baobabs fruit pulp extracts. Moreover, FRAP antioxidant activity of the aqueous extracts revealed that baobab fruit from Damazin was significantly different ($p \leq 0.05$) from that of the other three geographical regions. These differences could be due to the fact that Damazin area in which Baobab trees grow in mountainous with heavy rains, resulting in different characteristics of baobab fruits as compared to that from other geographical regions in Sudan. The environmental conditions may have also affected tupess of phenolic compound, as the environment could contribute in the selection of phenolic biosynthetic pathways. It is also probable that different baobab ecotypes in these regions are partially responsible for these variations.

Table 4: 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}
Nyala	345.82±1.15^{c}

The DPPH test is the oldest indirect method for determining the antioxidant activity which is based on the ability of the stable free radical 2,2-diphenyl-1-picrylhydrazyl to react with hydrogen donors including phenols [2]. The bleaching of DPPH solution increases regularly with increasing amount of fruit in a given volume. The bleaching action is mainly attributed to the presence of antioxidant compounds like polyphenols in the solution. The antioxidant capacity was evaluated by DPPH assay in aqueous and ethanolic extract compounds of the baobab fruit pulp from different Geographical Regions in Sudan (El Obeid, Umm Ruwaba, Nyala and Damazin).

The antioxidant capacity evaluated by DPPH radical scavenging using ascorbic acid as standard (R2 = 0.9866) was found to be 227.92, 205.87, 201.10 and 183.50 mg AEAC/g for ethanolic extract from Damazin, Nyala, El Obeid and Umm Ruwaba respectively. Moreover, for aqueous extracts antioxidant capacity was found to be 221.30 mg AEAC/g for Umm Ruwaba, 200.17mg AEAC/g, Damazin 193.67mg AEAC/g El Obeid and 184.70mg AEAC /g for Nyala.

DPPH assays free radical scavengers to a certain extent, however, the free radical scavenging efficiency of each species differs depending on the solvents used and the varieties studied as suggested previously by [29]. Polar solvents are commonly used for extraction

of antioxidants, therefore generally higher antioxidant activity could be observed in samples that were extracted with ethanol than in aqueous extracts. The highest antioxidant activity was observed in the ethanolic extracts of baobab fruit pulp from Damazin than those of the other geographical regions in Sudan.

In the aqueous extracts the highest antioxidant activity was observed in baobab fruit pulp from Umm Ruwaba. This might suggest that most of the antioxidants present were less polar and could be extracted by less polar solvent like water. Also, the variation of antioxidant activity obtained from baobab fruit pulp from different geographical regions in Sudan with different solvents systems in baobab fruit pulp might be affected by types and structures of phenolic compound. It had been reported that antioxidant activity is greatly influenced by number and configuration of hydroxyl group, as well as gylcosylation [30], for instance, flavonoids without hydroxyl group (e.g. isoflavone, flavanone) tend to have reduced antioxidant activity.

The FRAP assay measured the ability of antioxidant to reduce Fe (3+) to Fe (2+). The results of the FRAP obtained were slightly lower than these of DPPH method. The FRAP values ranged from 217.04 to 209.33 mmol /g of Fe^{+2} for ethanolic extracts and 205.80 to 191.64 for aqueous extracts (Table 2), using $FeSO_4.7H_2O$ as standard (R2=0.999). In this assay, significant difference was observed between FRAP values of the two types of extracts from different baobab fruit pulp samples. Moreover, FRAP antioxidant activity of the aqueous extracts revealed that of Damazin baobab fruit pulp was significantly different ($p \leq 0.05$) from that of samples from other geographical regions in Sudan. These differences could be due to the fact that Baobab trees grow in mountainous area with heavy rain fall in Damazin as compared to that from other geographical regions in Sudan and contained different components of phenolic acids and flavonoids profile.

A study by [31] of baobabs from several locations in the Nuba Mountains, Sudan, reported a substantial genetic variation in which two distinct gene pools appeared. The variations in phenolics, flavonoids profile and antioxidant activity of baobab samples in this study, could probably be due to genetic, environmental factors or both, as well as soil chemical composition.

Total Phenols and Flavonoids Contents

The total phenols and Flavonoids contents of Baobab fruit pulp are shown in Table 3 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 and ethanolic extracts; which ranged from 15.50 to 99.66 mg GA/g. Those values were higher when compared to result by [8]. They reported value of 35.18 mg GAE /g while [32] phenolic content were 13.92 mg GAE /g for baobab pulp. The highest concentration of phenols was measured in ethanolic 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 [33]. No significant difference between Nyala and Damazin samples in phenolic contents of the aqueous ethanol extracts.

The concentration of flavonoids in extracts ranged from 1.03 to 16.06 mg of CA/g showing significant difference ($p \leq 0.05$). These values were higher compared to those reported by [8]. The highest concentration of flavonoids was in samples from Damazin and Umm Ruwaba. The concentration of flavonoids in fruit pulp extracts depends on the polarity of solvents used in the extract preparation [34]. The variations in phenolic, and Flavonoids content could probably be due to genetic variation, and soil chemical composition.

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 $p<0.05$.

Conclusion

The results of this study are the first report on phenolics and flavonoids profile in Sudanese baobab fruit pulp. Eleven phenolic compounds and thirty-six flavonoids were identified in baobab fruit pulp for the first time. The baobab fruit pulp samples showed characteristic qualitative profile of flavonoids and phenolic compounds, and high antioxidant capacity. Based on the finding of this study, we conclude that Sudanese baobab fruit pulp is rich in phenolics, flavonoids, and a good source of natural antioxidant. The identified compounds may contribute to the health-promoting properties of baobab fruit which could be a potential functional foods, and pharmaceutical ingredient, with potential economic value for the local people in these regions.

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