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Phytochemical profile and biological activities of Sudanese baobab (*Adansonia digitata* L.) fruit pulp extract

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Abstract

The present work aimed to investigate the phytochemical profile and biological activities of Sudanese baobab (*Adansonia digitata* L.) fruit pulp extract. Baobab fruit pulp serves as food, and has been used in traditional medicine in Africa for the treatment of several diseases, and believed to possess many biological activities. The sample of baobab fruit was collected from Blue Nile State in Sudan. The fruit pulp was obtained from baobab pods, sieved, extracted with methanol (80%), and analysed for its antioxidant activity, and alpha-glucosidase and nitric oxide (NO) inhibitory activities. The phytochemical constituents of the pulp were determined by LC-MS and ^{1}H-NMR techniques. The results for antioxidant activity evaluated by DPPH and FRAP for methanolic extract were 232.70 mg Ascorbic Acid Equivalent Antioxidant Capacity (AEAC)/g and 222.28 mmol/g of Fe^{2+}, respectively. Baobab pulp extract showed greater capability in inhibiting the generation of NO from the stimulated RAW264.7 cells at 98.45% inhibition and IC_{50} of 36.55 µg/mL; and α-glucosidase enzyme activity at 97.94% inhibition and IC_{50} of 58.59 µg/mL. The LC-MS/MS analysis of the baobab extract showed that 52 compounds were found in the baobab pulp, including 19 flavonoids, ten phenolic acids, four lipids (glycero-3-phosphocholine derivative, 1,2-didodecanoyl-sn-glycero-3-phospho-(1'-sn-glycerol), 1-pentadecanoyl-2-(13Z,16Z-docosadienoyl)-glycero-3-phosphate, and glycero-3-hosphocholine derivative), sugars, organic acid, iridoids, adenosine, scopoletin, and taraxerone. The NMR detected 21 metabolites from baobab pulp extract including carbohydrates, organic acids, vitamin, amino acids, phenolic compounds, alkaloids, and fatty acids. The present work is the first of its kind in comprehensively analysing the Sudanese baobab fruit pulp, combined with qualitative characterisation of chemical components using NMR, LC-MS, α-glucosidase, and NO inhibitors. Baobab fruit pulp contains several beneficial phytochemicals and biological activities, and it has potential positive effect on general human's health.

Introduction

Baobab (*Adansonia digitata* L.) is a huge deciduous tree originally found in Africa, and has spread to Asia and America to a lesser extent. In Africa, it extends from Senegal eastwards to Sudan, Kenya, and South Africa (Sidibe and Williams, 2002).. In Sudan, it is found in belts across Kordofan, Blue Nile, and Darfur (Elamin, 1990). The fruit pulp has excessive contents of pectin with low protein and fat. It has very small amount of iron and manganese, but exceedingly high content of calcium and vitamin C (Sibibe and Williams, 2002). Moreover, the phytochemical screening of the baobab fruit pulp showed sterols, triterpenes, saponins, and tannins (Ramadan *et al.,* 1994) which have effects on scavenging free radicals that have been embroiled in numerous sickness conditions in people including joint inflammation, haemorrhagic stun, atherosclerosis, ischemia, reperfusion damage of numerous organs, Alzheimer's and Parkinson's diseases, gastrointestinal dysfunctions, tumour advancement, and carcinogenesis (Bagchi *et al.,* 2000). Numerous sorts of free radicals exist; however, the most worrying in biological system is from oxygen, and referred as reactive oxygen species (ROS) such as nitric oxide (NO), which is a free radical delivered from L-arginine by the enzyme nitric oxide synthase (NOS). NO, a pro-inflammatory, plays a crucial part within the regulation of immune functions, neurotransmission, and vasodilatation. Nevertheless,

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NO overproduction happens in irregular physiological conditions, and results in different inflammatory and neurodegenerative illnesses. Therefore, the inhibition of NO is an essential factor for the prevention and additional treatment of numerous sicknesses (Sharma *et al.,* 2007). With the exception of reactive oxygen species (ROS), the overrun of reactive nitrogen species (RNS) has conjointly been related to oxidative stress and with chronic inflammation that are involved in the pathophysiology of assorted diseases like inflammation, diabetes, atherosclerosis, and carcinogenesis (Moncada and Higgs, 1993).

Broadly speaking, metabolomics is a holistic study aimed at identifying metabolites in biological system (Trimigno *et al.,* 2015). Plant foods contain a complex mixture of metabolites in terms of structure, chemical properties, and concentrations specific to species. Many components are already present in the crude products, whereas others can arise during storage and processing. Understanding the biochemical response to postharvest treatments is of great importance for the selection of better processing and storage conditions (Oms-Oliu *et al.,* 2013). There are several analytical platforms for carrying out metabolic studies including nuclear magnetic resonance (NMR), gas chromatography mass spectrometry (GC-MS), and liquid chromatography mass spectrometry (LC-MS) (Sotelo and Slupsky, 2013). In metabolomics, ^{1}H-NMR spectroscopy has been broadly used as an analytical tool because of its robustness, speed, and high-throughput as well as the exceptionally easy technique of sample preparation (Kim *et al.,* 2010). Also, MS-based techniques are also widely used because they offer extra velocity, sensitivity, and selective qualitative and quantitative analyses. Furthermore, efficient identification of each constituent is enabled by way of application of tandem mass (MS/MS) (Seeram *et al.,* 2006).

The most popular claim is that consumption of baobab fruit pulp could prevent one from sicknesses. This could be related to the enhancement of immune function of the body against attacks of foreign agents. Anti-inflammatory property can therefore become one of the factors that can be associated with protection from potential illnesses. It is believed that the management of too much production of NO by macrophages should significantly help in the treatment of inflammatory illnesses (Terra *et al.,* 2007). Thus, the present work aimed to evaluate the antioxidant property, and NO and α-glucosidase inhibitory activities; and to identify the phytochemical profiles of baobab fruit pulp.

Materials and methods

Plant materials

Baobab fruits capsules were collected in 2013 from Damazin (Blue Nile State, 11.2 N and 34.1 E), Sudan. The fruit pulp was prepared by manually breaking the capsules, followed by seed removal, and the pulp powder was sieved using appropriate mesh. The resulting fruit pulp was stored in a dark polyethylene bag at -18°C until used.

Chemicals

Ascorbic acid, 2,2-diphenly-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), $FeC1_36H_2O$, KH_2PO_4, Griess reagent, curcumin, lipopolysaccharide (LPS), phosphate buffered saline (PBS), recombinant murine IFN-γ, dimethylsulfoxide (DMSO), phosphate buffer, α-glucosidase enzyme, *p*-nitrophenyl-α-D-glucopyranose (PNPG), glycine, and quercetin were purchased from Sigma-Aldrich (Hamburg, Germany). Cell culture medium, Dulbecco's Modified Eagle's Medium (DMEM) containing HEPES and L-glutamine for both, with and without phenol red, penicillin-streptomycin antibiotic solutions, foetal bovine serum (FBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and TrypLE Express enzyme were purchased from Gibco/BRL Life Technologies Inc. (Eggenstein, Germany). For NMR analysis, deuterated methanol-d4 (CH_3OH-d_4) and trimethylsilyl propionic (TSP) acid-d4 sodium salt were purchased from Merck (Darmstadt, Germany). For LC-MS analysis, Optima™ LCMS grade solvents including water, acetonitrile (ACN), methanol, formic acid, and ammonium formate were purchased from Fisher Scientific (Geel, Belgium).

Preparation of fruit pulp extracts

Baobab fruit pulp methanolic extract was prepared by the method of Kim and Lee (2002) with some modification. Twenty grams of the baobab fruit pulp was used for extraction with 80 mL of solvent and methanol/water (4:1) (v/v) at room temperature for 5 h, using an orbital shaker (Unimax 1010DT D-91128, Schwabach, Germany; serial No. 071006060) at 200 rpm and 30°C. Then, the homogenate was centrifuged for 10 min at 3,600 rpm (Eppendorf Centrifuge 5804; Hamburg, Germany), and the supernatant was removed. The residue was extracted once again at the similar 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 methanol), and were concentrated in a rotary evaporator (Büchí 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 extract which was kept in dark glass bottle at -18°C until used.

Ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) analysis

The sample for analysis was prepared by dissolving 2 mg of methanolic extract in 1 mL of MeOH (LC-MS grade), and then filtered with 0.22 µ m membrane filter. The UHPLC-MS/MS analysis was carried out using a Thermo Scientific Q Exactive™ Focus Quadrupole-Orbitrap mass spectrometer with heated electro spray ionisation (HESI-II) source, coupled to a Surveyor UHPLC binary pump and auto-sampler (Thermo Fisher Scientific, Bremen, Germany). Liquid chromatography was performed using a C18 column (ACQUITY UPLC HSS-T3, 1.8 µm, 2.1 × 100 mm; Waters, Milford, MA, USA). A gradient mobile phase consisting of water (containing 0.1% formic acid and 10 nM of ammonium format; solvent A) and acetonitrile (containing 0.1% formic acid; solvent B) was used for the separation of the analyte. The gradient program started with 5 to 100% solvent B from 0 to 20 min. The injection volume was 5 µL, and the flow rate was 0.25 mL/min. The MS parameters were adjusted as follows: spray volt-pressure, -3.5 kV; capillary temperature, 320°C; sheath and auxiliary nitrogen gas flow, 50 and 10 arbitrary units. The mass resolution was set at 70,000 FWHM with collision-induced dissociation (CID) energy was adjusted to 30%. The UHPLC-MS/MS analysis was performed in negative ion mode, and the total ion chromatograms (TIC) were recorded for 100 - 1500 *m/z*. The spectrum preprocessing was carried out using Thermo Xcalibur 2.2 (Thermo Fisher Scientific, Bremen, Germany), and the identification of the compounds was supported by MS/MS data and comparison with online database (http://metabolomicsworkbench.org).

NMR measurement

About 30 mg of the methanolic extract was dissolved in 700 µL of CH_3OH-d_4 containing 0.1% TSP. The sample solution was then subjected to sonication for 30 min, and centrifuged at 13,000 rpm for 10 min to separate the supernatant from the residue. Following this, 600 µL of the supernatant was transferred to an NMR tube for ^{1}H-NMR analysis. The ^{1}H-NMR measurement was carried out as previously described (Kim *et al.,* 2010). The ^{1}H-NMR data of the extract were obtained from a 500 MHz Varian INOVA NMR spectrometer (Varian Inc., California, USA), functioning at a frequency of 499.887 MHz at room temperature (25°C). The acquisition time for ^{1}H-NMR spectrum was 3.54 min, and 64 scans were performed. A PRESAT sequence was once used to suppress residual water signal with low energy selective irradiation. The acquired NMR spectrum was manually phased, baseline corrected, and referenced to TSP as an internal standard using MestReNova software version 6.0.2 (Mestrelab Research S.L., Coruña, Spain).

Antioxidant activity assays

DPPH assay

A spectrophotometric analysis using DPPH, and a comparison with the ascorbic acid calibration curve was performed (Tuberoso *et al.,* 2010). This assay was based on the ability of the antioxidant to scavenge the radical cation 1,1-dipheny1-2-picrylhydrazyl radical (DPPH). Fifty microliters of diluted sample (5 mg/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, mg/g). Spectrophotometric readings were carried out at 517 nm, using a 10 mm plastic cuvette after an incubation period of 60 min in the dark. Samples were analysed in triplicates, and the mean values were calculated.

FRAP assay

The FRAP assay evaluates antioxidants as reductants of Fe^{3+} to Fe^{2+}, which was 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 (Tuberoso *et al.,* 2010). The FRAP assay was performed by preparing a ferric complex TPTZ and Fe^{3+} (0.3123 g TPTZ and 0.5406 g $FeC1_3 6H_2O$ in 100 mL acetate buffer, pH 3.6). Fifty microliters of diluted sample (5 mg/mL, with solvent) were dissolved in 2 mL of ferric complex, and after 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 mmol/L) correlating the absorbance with the concentration, and results were expressed as mmol/L of Fe^{2+}. Samples were analysed in triplicates, and the mean values were calculated.

In vitro α-glucosidase inhibitor activity assay

The α-glucosidase inhibition activity was carried out according to the protocols described by Pramai *et al.* (2018). The *p*-nitrophenyl-α-D-glucopyranose (PNPG), which acted as a substrate, was dissolved in 50 mM phosphate buffer (pH 6.5). The prepared extracts were pipetted into the wells of 96-well micro plate, and incubated at room temperature for 5 min. Following this, 75 µL of the PNPG was loaded to every well containing the sample, blank substrate, negative control, and positive control; whereby the rest of the wells were added with 75 µL KH_2PO_4 buffer (30 mM). The incubation time of 15 min was then commenced. The reactions for the sample, blank, substrate, and negative control were inhibited by the addition of 50 µL of 2 M glycine (pH 10). In the meantime, 50 µL of deionised water was pipetted into the remaining wells of 96-well micro plate. The absorbance was read at 405 nm using a micro-plate reader (SPECTRA max PLUS; Molecular devices, LLC, Sunnyvale, CA, USA). Quercetin served as the positive control. The α-glucosidase inhibition activity of the baobab extract was calculated as percentage inhibition using the following formula = $[(a_n - a_s) / a_n] \times 100$, where, a_n refers to the absorbance difference between the negative control and the blank substrate, and as refers to the variation between the absorbance of the sample and the blank sample. IC_{50} value denotes the concentration of sample required to inhibit 50% α-glucosidase activity.

In vitro nitric oxide inhibitory activity

The NO inhibitory activity test and cell viability were assessed following the protocols reported by Abas *et al.* (2006) with some modifications. RAW264.7 cells, the murine macrophage cell line was acquired from American Type Culture Collection (ATCC). Briefly, the RAW 264.7 cells were seeded into the wells of 96-well tissue culture plates, and stimulated with the inducer consisting of 200 U/mL of recombinant murine IFN-γ and 10 µg/mL polysaccharide. A volume of 50 µL of the prepared extracts were pipetted into every well of cell plate with a final concentration of dimethyl sulfoxide (DMSO) at 0.4% per well. The cells were incubated for 17 h at 37°C, with 5% CO_2 in humidified incubator. The amount of nitrite (NO_{2-}) was determined with the freshly prepared Griess reagent (1% sulphanilamide, 0.1% N-(1-naphthyl)-ethylene diamine dihydrochloride, and 2.5% H_3PO_4) at room temperature. Curcumin served as the positive control. The absorbance was read at 550 nm using Spectramax Plus (Molecular Devices) UV/Vis micro-plate reader.

Statistical analysis

Results are reported as the mean ± standard deviation of triplicates ($n = 3$).

Results and discussion

LC-MS analysis of baobab fruit pulp extract

Total ion chromatogram (TIC) profiles indicated that most of the major peaks detected in methanolic baobab fruit pulp extract was attributable to the presence of phenolic acids, flavonoids, sugars, organic acid, lipids, iridoid glycosides, and others (Figure 1). The retention times (RT), *m/z*, and MS/MS data of the detected metabolites are summarised in Table (1). Few researchers have reported the presence of polyphenols including procyanidins (Coe *et al.,* 2013), procyanidin B2, (-)-epicatechin, (-)-epigallocatechin-3-*O*-gallate, and gallic acid (Tembo *et al.,* 2017) in baobab fruit pulp. This investigation is the first report of phenolics and

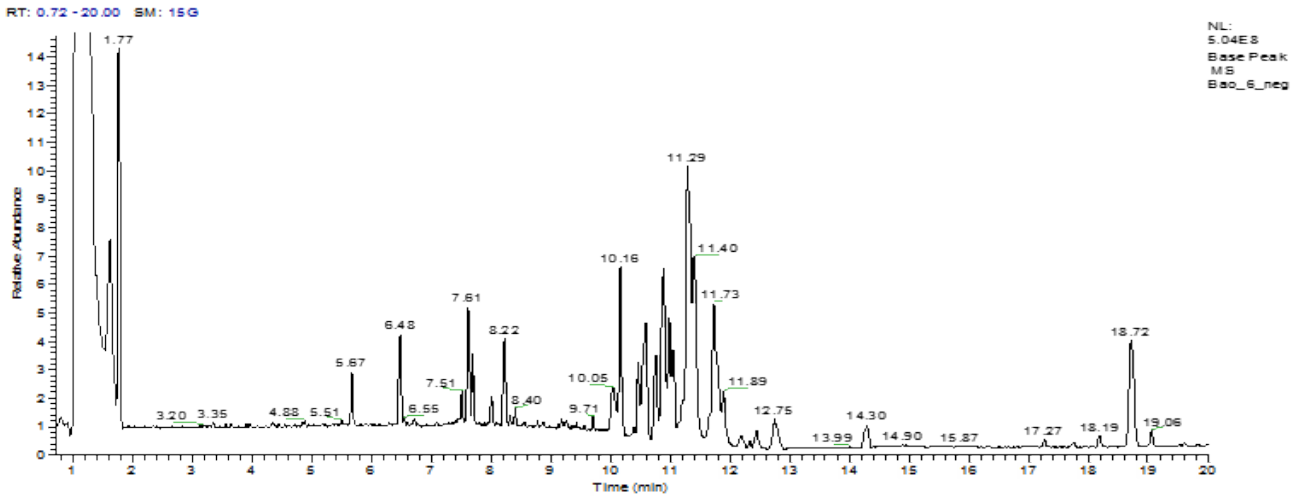

Figure 1. Total ion chromatogram of baobab extract in negative ion mode; for peak assignments, see Table 1.

flavonoids of methanolic extract of Sudanese baobab fruit pulp.

Identification of phenolic acids

Baobab fruit pulp revealed the presence of several phenolic acids which were identified based on its *m/z* in the negative ion and their MS/MS data (Table 1). Peak (5) at *m/z* 405.0285 and the retention time (RT) of 1.76 min with fragment ions at *m/z* 191 showed the characteristic of quinic acid moiety, suggesting a molecular weight of caffeoyl quinic derivative. Other phenolic acids such as coumaric acid 2-glucoside (*m/z* 325, peak 11, RT 5.43), coumaric acid (*m/z* 163, peak 28, RT 8.03), feruloyl-β-D-glucose (*m/z* 355, peak 16, RT 6.33) and homovanillic acid (*m/z* 180, peak 29, RT 8.21) were also identified.

Peaks 7, 8, 9, and 10 showed similar molecular ion peaks at *m/z* 341 indicating that they are probably isomers, tentatively identified as 1-*O*-caffeoyl-hexose, 1-*O*-caffeoyl-β-D-glucose, 6-*O*-caffeoyl-β-D-glucose, and 6-*O*-caffeoyl-α-D-glucose based on comparison with previous reports (Schütz *et al.,* 2004; Li *et al.,* 2017). Sudanese baobab fruit appeared to contain several components of phenolics. However, few researchers have reported the presence of polyphenols including procyanidins and gallic acid (Coe *et al.,* 2013; Tembo *et al.,* 2017).

Identification of flavonoids

Methanolic baobab fruit pulp extract contained several flavonoid glycosidesas as probably chemical indicators was presented in Table 1 and Figure 1. Epicatechin and its derivatives were the most abundant flavonoids detected in the extract. (-)-Epicatechin 3-*O*-gallate (peak 14) was identified by the deprotonated molecular ion at *m/z* 440.8891, and the fragment ions at *m/z* 305 and 190. Peaks 15, 19, 20, 22, 32, and 37 (Figure 1) which showed characteristic ions at *m/z* 289, 865, 289, 304, 440, and 593 (Table 1) attributable to (+)-epicatechin isomer, procyanidin C1, (+)-epicatechin, (-)-gallocatechin, catechin 3-*O*-gallate, and gallocatechin-(4alpha->8)-epicatechin. Peak 18 at *m/z* 403.0648 and RT = 6.2 min was identified as Osajin. Peak 21, 30, and 33 (RT = 7.3, 8.24, and 8.44 min, respectively) were identified as kaempferol-3-*O*-glucoside, kaempferide 3-rhamnoside-7-(6"-succinyl glucoside), and kaempferol-3-*O*-rutinoside based on molecular ion at *m/z* 447.0935, 707.1233, and 593.1307, respectively. Peak 41 (RT = 10.46 min) was identified as pinobanksin 5-galactosyl-(1->4)-glucoside based on the deprotonated molecular ion at *m/z* 595, and fragment ions at m/z 433 and 279. The assignment of fustin 7-rhamnoside was based on deprotonated molecule at *m/z* 433, and its fragment ions at *m/z* 289 and 279. For peaks 34 and 35, *m/z* 372.90 (RT = 8.47 and 8.78 min) were identified as chrysosplenetin and its isomer. Peak 44 (RT = 11.75 min) with a deprotonated molecular ion peak at *m/z* 316 was tentatively assigned as myricetin. Peak 46 at *m/z* 407.18 (RT = 12.21 min) as paratocarpin D. Peak 48 (RT = 12.31 min) was identified as quercetin based on the deprotonated molecular ion at *m/z* 301, which is consistent with the fragmentation patterns reported by Braca *et al.* (2018) and Ismail *et al.* (2019). Moreover, LC-MS/MS results showed similarity to some flavonoids profile such as quercetin, catechin, epicatechin, and procyanidin dimer 1 compared to those stated by Braca *et al.* (2018).

Identification of lipids

As shown in Table 1, peak 23 and RT = 7.51 was identified by the deprotonated molecular ion at *m/z* 713.47, and the fragment ions at *m/z* 678, 418, 394, and 207. Peak 24 (RT = 7.51 min) was identified as glycero-3-phosphocholine derivative based on the deprotonated molecular ion at *m/z* 836.58. Peaks 51 (RT = 12.75 min) was identified as 1,2-didodecanoyl-sn-glycero-3-phospho-(1'-sn-glycerol). These results could be considered the first report for the present of these components in baobab fruit pulp.

Identification of sugars and organic acids

Some sugars and organic acids including (R)-3,3-dimethylmalic, citric, D-ascorbic, dehydroascorbic, bassic, cinnamic, and 2-oxopimelic acids were found in methanolic baobab pulp extract (Table 1 and Figure 1). Peak 1 at *m/z* 503.16 (RT = 1.15 min), peak 2 at *m/z* 683.22 (RT = 1.28 min), and peak 3 at *m/z* 683.22 (RT = 1.61 min) were identified as sugars compound such as maltotriose, maltose, and sucrose, respectively. These findings differed from those reported by Braca *et al.* (2018) who found that the sugar moieties are represented in all cases by a hexose unit (glucose).

Identification of iridoids and other components

Iridoids exhibit a wide range of bioactivity namely antitumor, anti-inflammatory, neuroprotective, antioxidant, cardiovascular, hypoglycaemic, hypolipidemic, antispasmodic, antiviral, antimicrobial, immunomodulator, and antiallergic effects (Bas *et al.,* 2007). Iridoids were detected in baobab fruit pulp extract (Figure 1). Peak 43 at *m/z* 699.38 (RT = 11.26), peak 47 at *m/z* 537.32 (RT = 12.22), and peak

Table 1. Compounds identified by LC-MS2 in methanolic extract of baobab fruit pulp.

No	RT (min)	*m/z* [M-H$^-$]	*m/z* MS2	Compound
1	1.15	503.1619	341, 161, 143	Maltotriose
2	1.28	683.2252 [2M-H]$^-$	341, 161, 143	Maltose
3	1.61	683.2252 [2M-H]$^-$	341, 161, 143	Sucrose
4	1.62	161.0445	118, 105, 73, 71, 68, 59	(R)-3,3-dimethylmalic acid
5	1.76	405.0285	354, 191, 173, 155, 147, 129, 111, 87	Caffeoylquinic derivative
6	2.33	191.0190	147, 103, 87	citric acid
7	4.8	341.1091	204, 179, 161	1-*O*-caffeoyl-hexose
8	4.96	341.1090	179, 161, 133	1-*O*-caffeoyl-β-D-glucose
9	5.11	341.1090	179, 161, 133	6-*O*-caffeoyl-β-D-glucose
10	5.21	341.0879	221, 203, 179, 161, 133	6-*O*-caffeoyl-α-D-glucose
11	5.43	325.0930	187, 163, 145	Coumaric acid 2-glucoside
12	5.51	174.9552	156, 119, 106, 91	D-ascorbic acid
13	5.67	481.1538	407, 345, 362, 190, 170, 113, 69	Decyl beta-D-maltopyranoside
14	5.92	440.8891	305, 190, 175, 169, 147	(-)-Epicatechin 3-*O*-gallate
15	6.08	289.0719	274, 203, 188, 165, 161, 151, 147, 137, 123, 109, 97	(+)-Epicatechin isomer
16	6.33	355.1036	217, 193, 175, 161	Feruloyl-β-D-glucose
17	6.49	172.957	155, 137, 114, 95	Dehydroascorbic acid
18	6.5	403.0648	318, 290, 245, 179, 137, 125, 113	Osajin
19	6.54	865.199	739, 713, 695, 577, 289, 273, 245, 137	Procyanidin C1
20	6.56	289.0718	274, 203, 188, 165, 161, 151, 147, 137, 123, 109, 97	(+)-Epicatechin

21	7.3	447.0935	285, 162, 151	Kaempferol-3-*O*-glucoside
22	7.4	304.9141	175, 147, 119	(-)-gallocatechin
23	7.51	713.4742	678, 418, 394, 207	1-pentadecanoyl-2-(13Z,16Z-docosadienoyl)-glycero-3-phosphate
24	7.51	836.5875	791, 585, 524, 389, 218, 207	glycero-3-phosphocholine derivative
25	7.61	826.5587	791, 325, 178	glycero-3-phosphocholine derivative
26	7.69	485.3344	439, 421, 370, 337, 242, 224, 195, 161	Bassic acid
27	7.7	475.3056	339, 289, 190, 175, 137	(-)-Asbestinine 2
28	8.03	163.0391	119, 93	coumaric acid
29	8.21	180.9724	168, 153, 141, 93	Homovanillic acid
30	8.24	707.1233	593 (loss of succinate 114), 447, 285	Kaempferide 3-rhamnoside-7-(6"-succinylglucoside)
31	8.37	146.9602	104, 91	Cinnamic acid
32	8.4	440.889	463, 305, 277, 249, 195, 175, 147	Catechin 3-*O*-gallate
33	8.44	593.1307	447, 285, 151, 145	Kaempferol-3-*O*-rutinoside
34	8.47	372.9015	267, 249, 229, 209, 191, 189, 175, 147, 119, 94	Chrysosplenetin
35	8.78	372.9017	267, 249, 229, 217, 199, 189, 175, 147, 119, 97	Chrysosplenetin isomer
36	9.72	310.9312	205, 183, 175, 147, 119	3(3',4'-Dimethoxyphenyl)-7-methoxycoumarin
37	10.05	593.2734	315, 294, 241, 209, 165, 153	Gallocatechin-(4alpha->8)-epicatechin
38	10.17	423.134	367, 337, 323	Taraxerone
39	10.27	191.1071	175, 124, 118, 93	Scopoletin
40	10.45	265.1479	150, 97, 80	Adenosine
41	10.46	595.2891	433, 279, 153	Pinobanksin 5-galactosyl-(1->4)-glucoside
42	11.06	433.2361	289, 279, 171, 153	Fustin 7-rhamnoside
43	11.26	699.3816	415, 397, 287, 263, 255, 217, 179, 161	Welloside

44	11.75	316.9479	289, 271, 141, 113	Myricetin
45	12.17	172.9571	155, 137	2-oxopimelic acid
46	12.21	407.1875	339, 308, 289, 255, 183	Paratocarpin D or 3'-Prenyl-3-(2-hydroxy-3-methylbutyl-3-enyl)-4,2',4'-trihydroxychalcone
47	12.22	537.3284	339, 255, 179, 161	Feruloylcatalpol
48	12.31	301.1662	285, 217, 151, 135	Quercetin
49	12.34	339.2000	310,279, 216, 183	3'-(2-Hydroxy-3-methylbut-3-enyl)-4,2',4'-trihydroxychalcone
50	12.45	480.3098	461, 435, 413, 345, 283, 196	1-octadecanoyl-sn-glycero-3-phosphoethanolamine
51	12.75	609.2584	552, 549, 515, 473, 405, 181	1,2-didodecanoyl-sn-glycero-3-phospho-(1'-sn-glycerol)
52	14.23	671.2682	489, 421, 331, 179	8-*O*-caffeoyl-6'-*O*-caffeoylajugol

52 at *m/z* 671.26 (RT = 14.23) were identified as 8-*O*-caffeoyl-6'-*O*-caffeoylajugol, feruloylcatalpol, and 8-*O*-caffeoyl-6'-*O*-caffeoylajugol, respectively. Similar fragmentations pattern was reported by Li *et al.* (2017). Other compounds such as scopoletin and taraxerone were identified in baobab stem bark and leaves (Mishra *et al.,* 2019). However, adenosine and (-)-asbestinine 2 were not detected in another research related to baobab.

Metabolite identification of baobab fruit pulp extract based on ^{1}H-NMR

Several beneficial health and medicinal effects of baobab fruit have been previously reported (Lamien-Meda *et al.,* 2008). Nevertheless, there has not been any scientific identification that focused on the relationship of metabolite content with the evaluations of biological activities of baobab fruit. In the present work, the methanolic extract of baobab fruit was subjected to NMR analysis. The representative ^{1}H-NMR spectrum and identified metabolites of baobab fruit extract are shown in Figure 2 and Table 2. Carbohydrates including maltotriose, sucrose, maltose, arabinose, glucose, and xylose were among the characterised metabolites. Besides the carbohydrates, organic acid and vitamin such as citric acid and ascorbic acid were detected; as well as amino acids such as glycine, aspartic acid, glutamic acid, and GABA. Several components of phenolics were identified in methanolic extract comprising protocatechuic acid, caffeoyl-α-D-glucose, feruloylcatalpol, caffeoylajugol, and methylgallic acid. Other metabolites like 3-methylxanthine, 1-methylnicotinamide, β-sitosterol, and fatty acids were also identified. These results were coordinated with previous ones in which metabolites, such as specific methylgallic acid, sucrose, glucose, ascorbic acid, and citric acid were readily identified from baobab juice extracted by aqueous (Tembo *et al.,* 2017) using NMR spectroscopy.

Biological activities in baobab fruit methanolic extract

DPPH and FRAP assays

The antioxidant activity of the baobab fruits was analysed using the free radical scavenging capacity (DPPH) and ferric reducing antioxidant power (FRAP), and shown in Table 3.

The antioxidant capacity evaluated by DPPH radical scavenging using ascorbic acid as standard (R^2 = 0.9866) was 232.70 mg AEAC/g for methanolic baobab fruit pulp extract. The FRAP assay was conducted to measure the ability of antioxidant in reducing Fe^{3+} to Fe^{2+}. The result of the FRAP obtained was slightly lower than DPPH method. The FRAP recorded value of 222.28 mmol/g of Fe^{+2} for methanolic extracts. This result is slightly lower than that reported by Besco *et al.* (2007) of 240.5 µmol/g. The total antioxidant capacity value of baobab pulp was nine times higher than that of orange pulp, with values of 240.5 µmol/g for baobab pulp and 24.3 of µmol/g for orange pulp (Besco *et al.,* 2007).

Alpha-glucosidase inhibitor activity assay

Diabetes mellitus is a chronic disorder caused by changed metabolism of carbohydrate, thus creating the hyperglycaemic state (Zheng *et al.,*

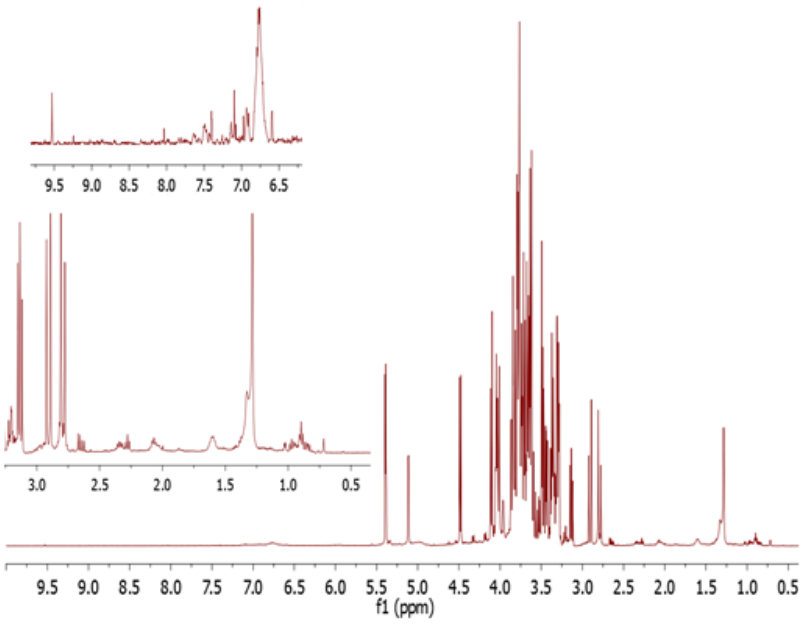

Figure 2. Representative ^{1}H-NMR spectrum identified metabolites in methanolic extract of baobab fruit pulp.

Table 2. ^{1}H-NMR (in CD_3OD + 0.1% TSP) characteristics of the identified metabolites in baobab fruit methanolic extract.

No.	Tentative compound	δH (ppm)
1.	1-Methylnicotinamide	9.24 (s), 8.92 (d, J = 6.0 Hz), 8.86 (d, J = 8.0 Hz), 8.19 (m), 4.47 (br s, overlapped)
2.	3-Methylxanthine	8.03 (s)
3.	Protocatechuic acid	7.41 (d, overlapped), 7.36 (dd, overlapped), 6.92 (d, overlapped)
4.	Caffeoyl-α-D-glucose	7.10 (br s), 6.80 (d, overlapped), 6.97 (m), 5.56 (d, J = 3.5 Hz), 4.46 (dd, overlapped), 4.32 (m)
5.	Feruloylcatalpol	5.18 (d, J = 8.0 Hz), 6.33 (d, J = 6.5 Hz), 5.096 (d, J = 8.0 Hz), 6.83 (d, overlapped), 7.16 (dd, J = 7.5, 1.8 Hz), 3.85 (s)
6.	Caffeoylajugol	5.56 (d, J = 3.5 Hz), 7.10 (br s), 6.78 (d, J = 8.0 Hz), 6.95 (m), 7.57 (d, J = 15.5 Hz), 6.30 (d, J = 15.5 Hz), 1.33 (s), 3.39 (t, J = 9.0 Hz), 4.65 (J = 7.5 Hz), 2.36 (dd, J = 13.0, 6.5 Hz), 1.99 (m)
7.	Methylgallic acid	6.97 (s), 3.76 (s)
8.	Maltotriose	5.40 (d, J = 4.0 Hz), 5.11 (d, J = 3.5 Hz), 4.48 (4.18 (d, J = 8.0 Hz)), 4.04 (m), 3.28 - 3.83 (m, overlapped), 3.13 (t, J = 9.0 Hz)
9.	Sucrose	5.40 (d, overlapped), 4.18 (d, J = 7.5 Hz), 4.06 (m, overlapped), 3.42 - 3.87 (m, overlapped)
10.	Maltose	5.40 (d, overlapped), 5.38 (d, overlapped), 4.67 (d, overlapped), 3.96 (m, overlapped), 3.42 - 3.87 (m, overlapped), 3.22 (t, overlapped)
11.	β-Sitosterol	5.35 (t, J = 5.0 Hz), 3.58 (m, overlapped), 0.72 (s), 1.02 (s), 0.97 (d, J = 7.0 Hz), 0.86 (d, J = 7.0 Hz), 0.84 (d, J = 7.0 Hz), 0.87 (t, J = 7.0 Hz, overlapped)
12.	Arabinose	5.29 (d, overlapped), 5.28 (d, J = 4.0 Hz), 4.52 (d, overlapped), 3.50 - 4.11 (m, overlapped)
13.	Glucose	5.22 (d, J = 4.0 Hz), 4.64 (d, J = 8.0 Hz), 3.68 - 3.86 (m), 3.34 - 3.45 (m), 3.18 (t, overlapped)
14.	Xylose	5.20 (d, J = 4.0 Hz), 4.57 (d, overlapped)
15.	Ascorbic acid	4.53 (d), 4.01 (m, overlapped), 3.75 (m, overlapped)
16.	Glycine	3.57 (s)
17.	Citric acid	2.90 (d, J = 15.0 Hz), 2.79 (d, J = 15.0 Hz)
18.	Aspartic acid	3.88 (m, overlapped), 2.82 (dd, overlapped), 2.65 (dd, J = 16.0, 8.0 Hz)
19.	GABA	3.00 (t, J = 7.0 Hz), 2.28 (t, J = 7.5 Hz), 1.87 (m)
20.	Glutamic acid	2.35 (m), 2.14 (m), 2.07 (m)
21.	Fatty acids	1.28 - 1.32 (m)

Table 3. Biological activities in baobab fruit methanolic extract.

α-Glucosidase		Nitric oxide		Antioxidant activity (mmol/g)	
%inhibition (at 1000 µg/mL)	IC_{50} (µg/mL)	%inhibition (at 500 µg/mL)	IC_{50} (µg/mL)	DPPH	FRAP
97.94 ± 0.30	58.59 ± 4.56	98.45 ± 0.59	36.55 ± 1.79	232.70 ± 0.00	222.28 ± 0.04

*Each value is an average of three replicates (mean) ± standard deviation.

2007). One therapeutic approach in the early stage of diabetes is to decrease post-prandial hyperglycaemia. It is well known that complex polysaccharides are hydrolysed by amylases to dextrins, which are further hydrolysed to glucose by intestinal α-glucosidase before entering blood circulation through intestinal epithelium absorption. For this reason, inhibition of these enzymes could reduce the high post-prandial blood glucose levels in diabetics (Tundis *et al.,* 2007). The enzyme reaction was performed *in vitro* using *p*-nitrophenyl-α-D-glucopyranoside (*p*-NPG) as the substrate, which was hydrolysed by a-glucosidase to release *p*-nitrophenol, a colour agent that can be monitored at 405 nm (Babu *et al.,* 2004). *In vitro* studies had shown that baobab fruit extracts interfere with starch degradation and reduce sugar release from starch-rich foods such as bread (Coe *et al.,* 2013). However, there is no study that established inhibition of these enzymes by Sudanese baobab fruit extracts on the *in vitro* α-glucosidase inhibitory activity. Table 3 shows the α-glucosidase inhibition activity of methanolic extract of baobab fruit pulp. Methanolic extract yielded high α-glucosidase inhibition activity of more than 90%, with IC_{50} of 58.59 µg/mL. Recently it has been confirmed that proanthocyanidins are potential natural α-glucosidase inhibitors (Hsu *et al.,* 2018). Furthermore, flavonoid derivatives have also been established to be active as α-glucosidase inhibitors (Silva *et al.,* 2016). Numerous identified compounds (see Table 1) have been previously established to be able to inhibit α-glucosidase, thus explaining the extract activities.

Nitric oxide inhibitory activity

Nitric oxide is a free radical product in mammalian cells, involved in the regulation of various physiological processes similar to regulation of vascular tone, inhibition of platelet, leukocyte aggregation and adhesion, and inhibition of cell proliferation (Bredt and Snyder, 1990). However, excess production of NO is associated with numerous diseases. Table 3 shows the NO inhibitory activity of methanolic extract of baobab fruit pulp of more than 98.45, with IC_{50} of 36.55 µg/mL. Ayele *et al.* (2013) recorded a high IC_{50} value compared with inhibition of nitric oxide by methanolic baobab leave extract (IC_{50} = 28.6 mg/mL). This information is interrelated to several phenolic components in the methanolic baobab pulp extract (see Tables 1 and 2). The phenolic compounds have long been believed to have the potential to inhibit NO and peroxynitrite production (Conforti and Menichini, 2011). Also, flavonoids such as apigenin, wogonin, luteolin, tectorigenin, and quercetin inhibited NO production the most among 26 flavonoids derivatives tested by Kim *et al.* (1999), thus indicating that the presence of antioxidant molecules in all the methanolic baobab pulp extract is responsible for their inhibitory effect. Previous epidemiological studies have shown an inverse relationship between the consumption of antioxidant-rich food and the decrease of risk factors for some human diseases (Udenigwe *et al.,* 2009). In addition, the secondary metabolites of plants have also been reported to act as excellent anti-inflammatory agents in oxidative stress and inflammation (Ghasemian *et al.,* 2016).

Conclusion

The methanolic extract of baobab fruit pulp contains a variety of phytochemical compounds, has strong antioxidant activity, and strong inhibitory effects against α-glcosidase and nitric oxide. The inhibitory effects of baobab fruit pulp extract on NO generation and α-glucosidase may suggest beneficial health effects, and could be effective in management of type 2 diabetes. Moreover, the present work might be useful in providing fundamental information for the development of pharmacological products or functional foods from baobab fruit pulp.

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