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Identification the phenolic compounds of *Adansonia digitata* different parts via LC-MS/QTOF and their antioxidant activity

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Abstract
Adansonia digitata L (Baobab) is a majestic, a deciduous and a large iconic tree revered in Africa for its nutritional and medicinal value. The plant parts are used to treat many diseases such as fever, diarrhea, dysentery, inflammation, asthma, malaria, anemia and microbial ailments. However, there is very little data on active ingredients in fruits, leaves, bark-stem and roots responsible for its biological properties. The aim of the present study was to quantify plant phenolic constituents and to evaluate antioxidant properties of different parts of *Adansonia digitata*. The LC-MS/QTOF experiment led to the characterization of 21 constituents including, rutin, fumaric acid, quercetin-3-*β*-*d*-glucoside, 4-hydroxybenzoic acid, protocatechuic acid, catechin, gentisic acid, vanillic acid and scutellarin. The flavanol catechin was found to be the most predominant phenolic constituent present in the four plant parts (0.0165-0.0233 mg/g of dry plant material). The highest amount of phenolic present was rutin (0.392 mg/g of dry plant material) in leaves extract. In DPPH radical scavenging assay, the effect on reducing free radicals increased in a dose dependent manner. On overall, among the four parts extracts, leaves extract showed highest antioxidant activity, with inhibition percentage ranging from 22.19% to 72.15%. In summary, findings reported that *Adansonia digitata* could be a natural source of phenolic constituents with antioxidant activity.

Keywords: *Adansonia digitata*, phenolics, LC-MS/QTOF, DPPH

1. Introduction
Medicinal plants extracts are main sources for a wide variety of active ingredients such as alkaloids, terpenoids, saponins, phenolics, fatty acids and tannins, which have been reported to have various biological activities [1]. The uses of plant-based products, for treatment and disease prevention become increasingly popular in most societies. According to the World Health Organization (WHO), about 85% of the populations in developing countries rely mainly on traditional medicine for their health care needs of which a major portion involves the use of plant extracts [2].
Adansonia digitata L. is known by many common names, the most common of which is Baobab [3]. The plant belongs to malvaceae family and is a deciduous tree, native to central Africa, where it is found in many countries such as Malawi, Benin, Zimbabwe, Mozambique, South Africa, Mali, Sudan, Senegal, Kenya, Cameroon, The Ivory Coast, Tanzania and Uganda [4, 5]. This tree is massive and grow up to 25 m high, deciduous in nature which may survive for hundreds of years and used for nutritional and medicinal purposes [6].
Various extracts from different parts (fruits, seeds, leaves, stem-bark, fruits pulp and roots) of *A. digitata* are employed in folk medicine in many Africa counties. For example, in South Africa, Cameroon and Central African Republic, its seeds and fruits mixed with water (decoction) to treat fever, diarrhea and dysentery [7]. In Sierra Leon leaves used to cure kidney bladder diseases, malaria, inflammation, asthma, fever, diarrhea, and blood clearing [8]. The Sudanese and Nigerian ethnic groups used aqueous extracts from the stem-bark to heal malaria and anemia [9], while the fruits have been used widely in Tanzania as ati-microbial diseases [10].
General phytochemicals screening of various parts of *A. digitata* has indicated the presence of phenolics, tannins, xanthones, terpenes, carbohydrates, saponins, cardiac glycosides, anthrax quinones, steroids and alkaloids [11, 12, 13]. Considering the nutritional and medicinal value of the *A. digitata* and previous investigations in our researches [14, 15, 16], an attempt done to quantify phenolic constituents and to evaluate antioxidant capacity of roots, stem-bark, leaves and fruits pulp extracts of the Sudanese medicinal plant *A. digitata.*

2. Materials and Methods
2.1. Collection of Plant Material
Fresh fruits pulp, leaves, bark-stem and roots of *A. digitata* were collected in July 2019 from AI-Fashir, North Darfur State, Sudan. The identification and taxonomy of the plant materials were done by the botanist in the Medicinal and Aromatic Plants and Traditional Medicinal Research Institute, National Center for Research (NCR), Khartoum, Sudan. A herbarium specimen (No 1579) was deposited.

2.2. Preparation of *Adansonia digitata* Different Parts Extracts
The dried roots, stem-bark, leaves and fruits of *A. digitata* were ground into powder. About 20 g of each powder part was macerated with ethanol at room temperature (27 ± 2 °C) overnight with slight stirring and shaking. The ethanolic extracts were then filtered with filter papers (Whatman

no.1). The filtrate was concentrated via rotary evaporator and kept for further analysis [17]. For LC-MS/QTOF analysis, stock solution of each crude extract (fruits pulp, leaves, bark-stem and roots) 200 ppm was filtered through a 0.22 µm membrane filter (PES).

2.3. LC-MS/QTOF Analysis of Extracts

Characterization of phenolic compounds in the fruits pulp, leaves, stem-bark and roots extracts of *A. digitata* was done via LC-MS/QTOF technic. The analysis was carried out on a CTO-20A HPLC, 2LC-10ADvp pumps, Auto sampler Sil-10ADvp (Shimadzu, Japan). Sample isolation was done on an Agilent Shim-pack VP-ODS column (150 mm × 4.6 mm i.d.,1.8 µm) using gradient elution. The analysis was carried out by gradient systems with a flow rate of 1 mL/min at 25 °C. The mobile phase consisted of solvent A (water 70%) and solvent B (acetonitrile 30%) gradually changed to 90% solvent B within 25 min and held for another 5 min. Total running time was 30 min. The sample injection volume was 10 µL and the wavelength was 230 nm. LC system was coupled with 6210 Time of Flight (TOF) mass spectrometer equipped with an electro spray ionization source was used to achieve the MS analysis. The characterization of the constituents was based on the comparison of their mass spectra with those in the system's spectral library.

2.4. Antioxidant Activity

The antioxidant capacity of the four different parts extracts of *A. digitata* (fruits pulp, leaves, stem-bark and roots) was done by the method described by [18]. Briefly, stock solution of each extract (1000 µg/mL) was prepared and tested in series concentrations of 50, 100, 150, 200 and 250 µg/mL. Then, 750 µL of stock solution of sample and 1500 µL of a 2% solution of DPPH in methanol were introduced into sterile tubes. For each test sample a negative control and a blank solution are prepared. The reaction mixture was incubated in dark at room temperature for 40 min. The absorbance of each mixture was measured at 517 nm. The radical scavenging activity of DPPH was calculated as follows:

$$\%\text{Scavenging} = \left(\frac{Absorbance_{control} - Absorbance_{sample}}{Absorbance_{control}} \right) \text{X100}$$

3. Results and Discussion

3.1. LC-MS/QTOF Characterization of Phenolic Constituents in Extracts

The LC-MS/QTOF analysis of *A. digtata* different parts extracts led to identify 21 constituents which belong to wide classes of phytochemicals such as organic acids, phenolic acids, flavonoids and their derivatives (Table 1, Fig 1-4). The major phenolic constituents identified in plant parts were, rutin, quercetin-3-*β*-D-glucoside, 4-hydroxybenzoic acid, gentisic acid, catechin and diosmin. The phenolic catechin which is flavanol was found to be the most abundant constituent present in the four parts extracts of *A. digtata* plant (0.0165-0.0233 mg/g of dry plant material). The highest content of phenolic constituent present was rutin (0.3192 mg/g of dry plant material) in the leaves extract. The compound quercetin-3-*β*-D-glucoside which is flavonol was fund only in the leaves Extract with amounts of 0.0886 (mg/g of dry plant material).

Table 1: Phenolic constituents (mg/g of dry plant material) in different parts of *A. digitata* as identified by LC-MS/QTOF

No	Compound	Fruits pulp	Leaves	Stem-barks	Roots
1	Fumaric acid	0.0263	0.0722	0.2059	0.2166
2	Gentisic acid	0.0021	0.0260	0.0052	0.0021
3	Chlorogenic acid	0.0009	0.0000	0.0008	0.0000
4	Catechin	0.0201	0.0181	0.0165	0.0233
5	4-hydroxybenzoic acid	0.0034	0.0455	0.0196	0.0096
6	Protocatechuic acid	0.0000	0.0108	0.0028	0.0024
7	Caffeic acid	0.0005	0.0000	0.0000	0.0000
8	Vanillic acid	0.0022	0.0061	0.0136	0.0015
9	Syringic acid	0.0035	0.0109	0.0050	0.0027
10	Rutin	0.0000	0.3192	0.0013	0.0000
11	Polydatine	0.0000	0.0000	0.0039	0.0000
12	Scutellarin	0.0000	0.0000	0.0035	0.0132
13	Quercetin-3-*β*-d-glucoside	0.0000	0.0886	0.0000	0.0000
14	Sinapic acid	0.0000	0.0000	0.0044	0.0000
15	Naringin	0.0000	0.0054	0.0009	0.0002
16	Diosmin	0.0000	0.0203	0.0059	0.0047
17	Apigetrin	0.0000	0.0116	0.0000	0.0000
18	Neohesperidin	0.0000	0.0000	0.0003	0.0000
19	Morin	0.0021	0.0104	0.0019	0.0016
20	Cinnamic acid	0.0018	0.0056	0.0015	0.0013
21	Apigenin	0.0000	0.0075	0.0000	0.0000

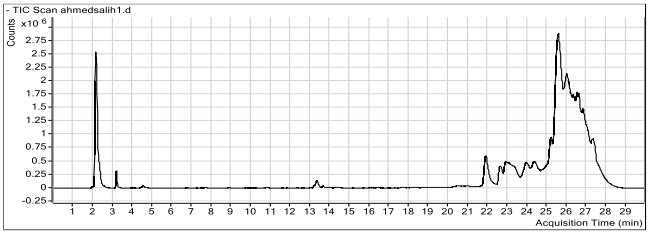

Fig 1: LC-MS/QTOF chromatogram of *A. digitata* fruits pulp extract.

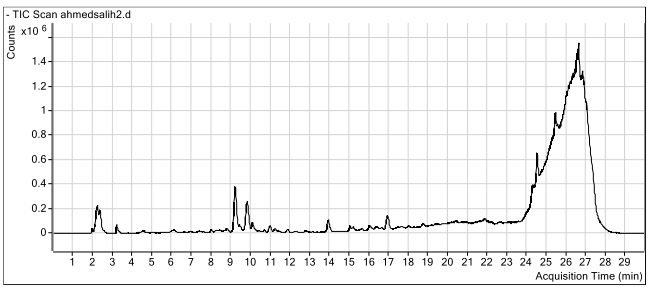

Fig 2: LC-MS/QTOF chromatogram of *A. digitata* leaves extract.

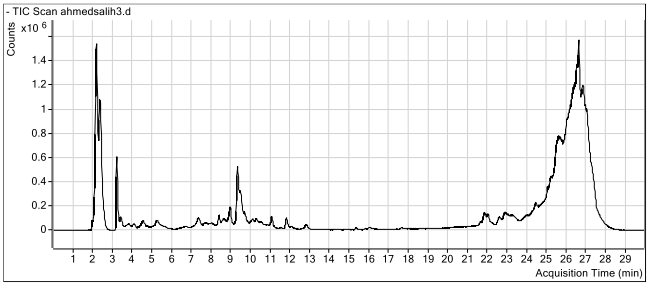

Fig 3: LC-MS/QTOF chromatogram of *A. digitata* stem-bark extract.

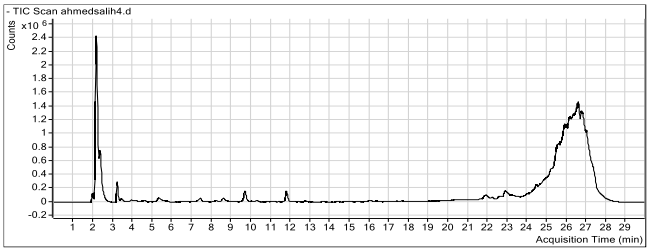

Fig 4: LC-MS/QTOF chromatogram of *A. digitata* roots extract.

3.2. Antioxidant Activity of *A. digitata* Different Parts

As shown in Table 2 and Fig 5, all extracts revealed DPPH radical scavenging capacity and was in the following order: Leaves > steam-bark > roots > fruits pulp. Leaves extract have the highest DPPH radical scavenging with 72%. Stem-bark and roots extracts demonstrated percentages inhibition of 70% and 67%, respectively. While the fruits pulp extract possessed weak activity, the percentage of inhibition was found to be 44%. The scavenging capacity of all extracts on the DPPH radical was found to be strongly dosage dependent manner (Fig 5).

Table 2: DPPH radical scavenging potential of *A. digitata* different parts

Con µg/mL	Leaves	Stem-barks	Roots	Fruits pulp
50	22	18	16	10
100	41	35	32	19
150	52	46	42	30
200	69	62	59	40
250	72	70	67	44

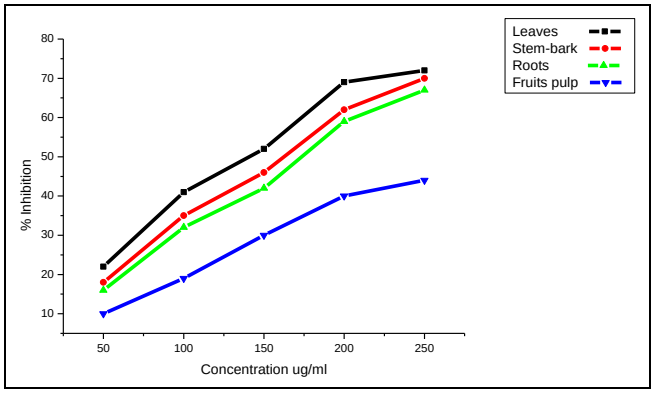

Fig 5: DPPH radical scavenging potential of *A. digitata* different parts

Similarly [19], summarized the anti-oxidant capacity of various fruits and vegetables. He was found that, the anti-oxidant capacity of *A. digitat*a extracts varies according to the plant part used. The antioxidant activity of leaves was 48.1 mmol/100 g which was almost five times higher than that of fruit pulp. This was in contrast to a study done by [20] who determined the antioxidant capacity of *A. digitata* leaves and fruits pulp extracts in comparison with *A. digitat* fruits seeds extract. The results clearly indicated that the antioxidant activity of *A. digitata* fruits seeds (9.35 mmol/100 g) was found to be significantly higher than that of the fruits pulp (6.96 mmol/100 g) and leaves (6.39 mmol/100 g).

The phenolic constituents, a major class of phytochemicals have been reported to have a wide array of biological activities due to its numerous properties [21]. For instance, the flavonol rutin (quercetin-3-O-*β*-rutinoside), has been revealed to have antidiabetic, anti-inflammatory, antioxidant and anticancer activities [22, 23]. Various mechanisms of reactions have been found to be responsible for its antioxidant properties in both in vivo and in vitro models. Firstly, it was reported that its chemical structure can directly scavenge reactive oxygen species (ROS) [24]. Secondly, it increases the production of glutathione (GSH) and cellular oxidative defence systems are believed to be upregulated by an increased expression of numerous antioxidant enzymes such as superoxide dismutase (SOD) [25, 26]. Finally, rutin inhibits xanthine oxidase which is involved in generating ROS [27]. In this study, the presence of high amounts of rutin is mainly found in leaves part and could be attributable to the observed high antioxidant property of this extract.

4. Conclusion

This study emphasizes the significant difference in phenolic constituents between different parts of *A. digitata* (fruits pulp, leaves, stem-bark, and roots) and their significant influence on antioxidant capacity. Twenty-one constituents were characterized via LC-MS/QTOF, representing different classes of phytochemicals such as flavonoids, flavanols, phenolic compounds and organic acids. The major phenolic constituents were rutin, quercetin-3-*β*-D-glucoside, 4-hydroxybenzoic acid, gentisic acid, catechin and diosmin. The flavanol catechin was found to be the most predominant phenolic constituent present in the four plant extracts (0.0165-0.0233 mg/g of dry plant material). The highest amount of phenolic constituent present was rutin (0.3192 mg/g of dry plant material) in the leaves. All the parts showed various degrees of oxidant activity on the tested DPPH radical scavenging, and were in the following order: Leaves > steam-bark > roots > fruits pulp. In summary, findings reported that *A. digitata* could be a natural source of phenolic constituents with antioxidant activity.

5. Acknowledgement

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