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Research Article

Chemical composition of crude extract and essential oils isolated from baobab plant *Adansonia digitata*

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Abstract

Antioxidant activities of the crude ethanolic and essential oils extracts of baobab plant, *Adansonia digitata* was determined using standard methods. Extracts of the fresh leaves were obtained using modified steam distillation. The antioxidant activities of the essential oils was screened using DPPH method in comparison to ascorbic acid as standard and the IC_{50} values for the plant's crude ethanolic extracts was found to be 0.5590 in *Adansonia digitata*, and 0.4281 for ascorbic acid; while the IC_{50} values obtained for essential oil was 0.4280 for ascorbic acid and absent in *Adansonia digitata. Adansonia digitata* was analysed by GC-MS, and 5 compounds from it were obtained and identified. They were 1,5-diamino-2-ethyl-1,2-dihydro-3H-pyrole-3,3,4-tricarbonitrile (37.14%), Phenylaniline (54.95%), Isopropenyl-4,5-dimethyloctahydroindene-4-carboxylic acid (3.26%), Octadecanoic acid (2.91%) and Phenyl-4-(1H-tetrazol-4,5-ylcarbamoyl)-butyric acid (1.75%). The results, thus support the traditional use of the plants to treat chronic diarrhoea, fever, diabetes, kidney stone, asthma, general fatigue, digestive disorder, respiratory infection and insect bites. Hence, suggesting its usage in the formulation of new antioxidant and antibacterial drugs and its credence as food.

Keywords: *Adansonia digitata*; DPPH; Antioxidant; GC-MS Analysis; Essential oil.

Introduction

The use of natural products with therapeutic properties is as ancient as human civilization and for a long time, minerals, plants and animal products were the main sources of drugs [1]. Traditional medicine has been an important source of potentially useful new compounds for the development of chemotherapeutic agents, as about 25% of the drugs prescribed worldwide come from plants and about 121 of such active compounds are in current use [2].

Nigeria is one of the biggest baobab producers, making powders that are commonly used as an ingredient for making famous Hausa cuisine known as "Miyar kuka". It is reported that its fresh leaves contain high amount of vitamin C as well as other nutritional elements such as alpha and beta carotene, rhamnose, uronic acid, tannins, potassium, calcium, glutamic acid, mucilage and other sugars. Therapeutically, the baobab leaves have several benefits as they can perform certain forms of allergies with their anti-histamine and hyposensitive properties. It have been reported that they can also treat asthma, fatigue, inflammation, insect bites, kidney and gall bladder diseases. Also, powdered baobab leaf poultices are also used to treat sores [3].

More so, they contain essential oils which are known to be volatile secondary metabolites formed by aromatic plants and can generally be recognized by their characteristic odour. These oils are projected to protect the plant by acting as antifungal, antibacterial, insecticidal or antiviral components and are also thought to promote the dispersion of seeds and pollens by attracting insects [4-6].

However, despite, the large number of the baobab trees in Nigeria and Africa at large, it has not been fully utilized, as it is commonly used only in making soup for feeding [7]. This work explores the chemical composition of

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Adansonia digitata (*A. digitata*) plant specifically to determine the antioxidant property of the crude extracts and essential oils isolated from the leaves of the plant.

Material and methods

Materials used

Incubator, rotatory evaporator, steam distillation apparatus, gas chromatograph coupled to a mass spectrometer, weighing balance, ethanol, n-hexane, ethyl acetate, Molish reagent, Mayer's reagent, HCl, NaOH, conc. H_2SO_4, Fehling's solution A & B, $FeCl_3$, conc. nitric acid, chloroform, benzene, ammonium solution, Wagner's reagent, iodine solution, diethyl ether, ammonium hydroxide

Collection of plant materials

The plant leaves were collected from Tunfure Area Council of Akko Local Government, Gombe State, Nigeria, and identified at the Department of Biological Science, Gombe State University. The leaves were allowed to dry properly under shade at room temperature. It was then ground into fine powder using pestle and mortar.

Preparation of aqueous extracts of the leaves

The powdered sample was extracted using cold maceration at room temperature with ethanol for 72 hr. The extracts obtained was filtered and concentrated under reduced pressure with a rotary evaporator. The extraction procedures were repeated to obtain aqueous, n-Hexane, and ethyl acetate crude extracts of the leave samples [8,9].

Extraction of essentials oil

500 g of pulverised form of the fresh leaf sample was placed inside a steam distiller, according to the British Pharmacopoeia (BP) method. The distillation process was carried out for a period of 2-3 hr and the oils obtained settled on top of water. The water was removed with the aid of separating funnel [10].

Antioxidant activity assay

The 2, 2- diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was carried out for the evaluation of the antioxidants activities. The essential oil was dissolved in methanol, and various concentrations (5, 10, 25, 50 and 100 µL/ml or µg/ml, as the case may be) were used. The assay mixture contained in a total volume of 1ml were; 500 µL of the oil, 125 µL prepared DPPH (1ml in methanol), and 375 µL solvent (methanol). After about 30 minutes of incubation at 25^0C, the decrease in absorbance was measured at λ = 517nm. The radical scavenging activity was then calculated from the equation:

Percentage (%) of radical scavenging activity = (Absorbance of control – Absorbance of sample)/ Absorbance of control X 100

The concentration of sample required to scavenge 50% of DPPH free radical (IC_{50}) was determined from the curve of percent inhibition plotted against their respective concentration [2].

Preparation of essential oil samples for GC-MS analysis

Essential oil was diluted to 7% by chloroform. An inert gas (helium) was introduced from a large gas cylinder through the injection part, the column and the detector. The flow rate of the carrier gas was adjusted to ensure reproducible retention time and to minimize detector dirt. The sample was then injected by a micro syringe through a heated injection part when it was vaporized and carried into the column [11].

Gas Chromatography - Mass Spectrometry (GC-MS) Analysis

Essential oil composition was determined by using a gas chromatography-mass spectrometry (GC-MS) analysis [11]. Trace GC ULTRA gas chromatograph was coupled to a Polaris Q MS ion trap mass spectrometer. The column was VB-5 (Methylpolysiloxane, 5% phenyl), 30 m x 0.25 mm x 0.25 µm film thickness with helium as carrier gas. Injection was performed at 220°C in the split mode, and 1µL of sample was injected. GC oven temperature was kept at 40°C for 2 min and programmed to 180˚C at a rate of 4˚C/min. This was increased to 300°C at a rate of 20°C/min, and kept constant at 300˚C for 2 min. The MS operating parameters were as follows: ionization potential, 70 eV; ionization current, 2 A; ion source temperature, 200°C; and resolution, 1000. Mass units were monitored from 30 to 450 m/z.

he components of the oil were identified by comparison of the mass spectra fragmentation patterns with those found in data bases or libraries. The final temperature was increased to 220°C with 5°C/min heating ramp for 20 min; injector temperature, 250°C; detector (FID)

temperature, 275°C; carrier gas, helium; inlet pressure, 40-60 psi; linear gas velocity, 39 cm/s; column flow rate, 1.5 ml/min; split ratio, 40:1; injected volume, 1 L. The retention indices were calculated for all volatile constituents using a homologous series of n-alkanes C_8 - C_{16}. Chemical constituents were identified by comparing their mass spectra peaks [12].

Result and discussion

Inhibition in Percentage of DPPH free radical by the crude ethanolic extract and essential oils of A. digitata leaf using ascorbic acid at 517 nm

Both the essential oils and the ethanolic extract from the leaves of *A. digitata* showed a reasonable zone of inhibition as shown in tables 1 to 4 and figure 1 and 2. Based on previous findings, there is a high correlation between antioxidant activity and phenolic compounds [13]. This implies that compounds that have tannins in nature are expected to exhibit antioxidant activities even though other phenolic compounds like flavonoids also possess antioxidant activities and they are known to be in a synergistic relationship with tannins in plants [14]. From the information obtained, the plant's ethanolic, n-Hexane, ethylacetate as well as aqueous crude extracts contained phenolic compounds as well as tannins in high quantity. Therefore, *A.digitata* plants are very good antimicrobial agents [15].

The crude ethanolic extracts and essential oil extracts have proved the scavenging effects of the leaves of *A. digitata* on DPPH. The results obtained from the spectrophotometer were expressed as inhibition in percentage as they were compared with the standard antioxidant (ascorbic acid). The lowest concentration of the essential oils (5 µl/ml) and crude ethanolic extract (5 µg/ml) showed the lowest inhibition value for the leaves of *A. digitata* at 4.75% and 33.87% respectively which is very far away from the standard (ascorbic acid) with a value of 68.18%. On the other hand, the highest concentration of the essential oil extracts, showed appreciably higher inhibition at 37.85%. The highest concentration for the crude extract showed the highest inhibition at 82.93%, which is very close to the standard (ascorbic acid) at 85.30%. Therefore, there was a distinguishing increase in inhibition as the essential oils and crude extract concentration increases [16].

Table 1. Antioxidant activity of *A.digitata* readings from spectrophotometer of crude ethanolic extract using ascorbic acid as standard blank solution 1.810

Concentration (µL/mL)	*A. digitata* (%)	Ascorbic acid (%)
5	1.197	0.576
10	1.166	0.457
25	0.956	0.415
50	0.398	0.385
100	0.309	0.266

Table 2. Antioxidant activity inhibition of *A. digitata* expressed in percentage (%) of crude ethanolic extract using ascorbic acid as standard

Concentration (µL/mL)	*A. digitata* (%)	Ascorbic acid (%)
5	33.870	68.180
10	35.580	74.750
25	47.200	77.100
50	78.100	78.730
100	62.400	85.300

Table 3. Antioxidant activity of *A. digitata* readings from spectrophotometer of essential oil extract using ascorbic acid as standard blank solution 1.810

Concentration (µL/mL)	*A. digitata* (%)	Ascorbic acid (%)
5	1.720	0.580
10	1.670	0.460
25	1.670	0.420
50	1.460	0.390
100	1.130	0.270

Table 4. Antioxidant activity inhibition of *A. digitata* expressed in percentage (%) of essential oil extract using ascorbic acid as standard

Concentration (µL/mL)	*A. digitata* (%)	Ascorbic acid (%)
5	4.750	68.180
10	7.620	74.750
25	7.960	77.100
50	19.340	78.730
100	37.850	85.300

Furthermore, it is important to state that generally the antioxidant activities of the crude ethanolic extracts tend to be higher in all cases than the antioxidant activities of the essential oil extracts. In comparison with the standard (ascorbic acid), *A. digitata* has a lower IC_{50}

value as seen in table 5. Hence it has a lower antioxidant activity than ascorbic acid.

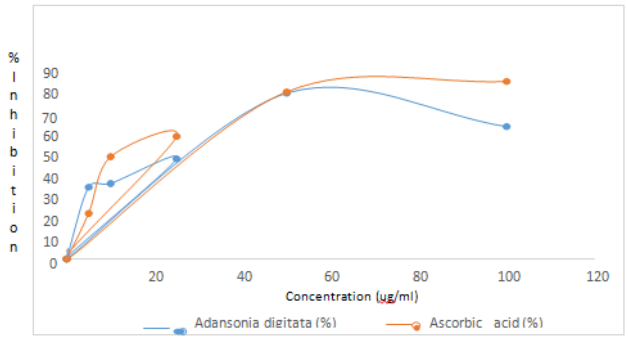

Figure 1. Antioxidant activity of crude ethanolic extract of *A. digitata* using ascorbic acid as standard

Nonetheless, the variation in the yield of the essential oils compared to other research may be due to factors like site of collection, time of collection, part and form of the plant used and the extraction methods employed [17].

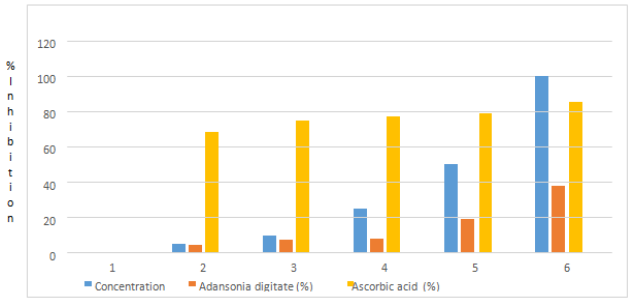

Figure 2. Antioxidant activity inhibition of *A. digitata* expressed in percentage of essential oil extract using ascorbic acid as standard

Table 5. IC_{50} values obtained for the ethanolic extract and essential oil extract of *A. digitata.*

	IC_{50} value ethanolic extract	IC_{50} value essential oil
A. digitata	0.559	Nil
Ascorbic acid	0.428	0.279

Table 6. Percentage yield of the essential oils of *A. digitata*

Plant	Plant part	Weight (g)	Volume of essential oils (ml)	Appearance	% Yield
A. digitata	Fresh leaves	1000	3.670	Colourless	0.367

GC-MS analysis

The essential oil was analysed by GC-MS and 5 components were identified alongside their respective retention time and area percentage as showed in table 7. The major components in the essential oil are 1,5-diamino-2-ethyl-1,2-dihydro-3H-pyrole-3,3,4-tricarbonitre (37.14%); [1,1Biphenyl]-2-amine, phenylaniline (54.95%); Isopropenyl-4,5-dimethyloctahydroindene-4-carboxylic acid (3.26%); Octadecanoic acid (2.91%); and Phenyl-4- (1H-tetrazol-4,5-ylcarbamoyl)-butyric acid (1.75%).

Essential oil yield of A. digitata

1000 g of the fresh plant leave was subjected to steam distillation for the extraction of essential oils. The results obtained as seen in table 6, showed that *A. digitata* has percentage yield of 0.37%.

Conclusions

The antioxidant activities of the essential oil and ethanolic extract of *A. digitata* against standard ascorbic acid indicated the potential and potency of the plant as an antioxidant as shown in this work. This supports the traditional use of the plant in curing various ailments in Niheria and for research, discovery and development of new antioxidant compounds globally. Hence, there is a new for more active research on this plant as it can be used as possible raw materials for the formulation of new drugs.

Table 7. GC-MS analysis of essential oil of *A. digitata*

Constituents	Retention time (min)	Area (%)
1,5-diamino-2-ethyl-1,2-dihydro-3H-pyrole-3,3,4-tricarbonitrile	13.525	37.140
[1,1-Biphenyl]-2-amine, phenylaniline	14.179	54.950
Isopropenyl-4,5-dimethyloctahydroindene-4-carboxylic acid	14.606	3.260
Octadecanoic acid	17.698	2.910
Phenyl-4-(1H-tetrazol-4,5-ylcarbamoyl)-butyric acid	22.405	1.750

Conflict of interest

The Authors of this work declare no conflict of interests.

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