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Phytochemical Screening and Antioxidant Potential of Chloroform Extract of *Adansonia digitata* Leaves

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Abstract
In this study, the presence of steroids, flavonoids, cardiac glycosides, anthraquinone, saponin and alkaloids in the chloroform extract of Adansonia digitata leaves were investigated using standard methods. Our result shows the presence of the afore-mentioned metabolites except Anthraquinone and saponins. Subsequently, Antioxidant activity in 0.2, 0.4, 0.6, 0.8 and 1 µg/ml of the extract was determined using FRAP and DPPH radical scavenging activity methods respectively. Antioxidant activity was observed at all concentrations used in FRAP assay, with 0.2, 0.4 and 1.0 µg/ml of the extract having the highest percentage of FRAP inhibition of 76.92, 83.14 and 80.58% respectively, whereas only 0.2 and 1.0 µg/ml of the sample in DPPH assay shows DPPH percentage inhibition of 11.16 and 23.21% respectively. Putting these together, it was observed that, chloroform extract of A.digitata leaves displays antioxidant activities even at low concentration owing to its abundance phytochemicals composition.

Keywords: A. digitata, antioxidants, inhibition, phytochemicals, metabolites.

INTRODUCTION
The human body is in constant production of damaging agents due to exposure to both biotic and abiotic stresses. These agents are harmful and capable of causing anomalies and

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interference to the normal physiological processes, they are termed free radicals (oxidants). In order to curtail the effect of oxidants, complex systems of natural enzymatic and non-enzymatic antioxidants defence mechanisms are involved, that counteract their harmful effects. Various diseases arise due to exposure to Free radicals such as cardiovascular disease, cancer, Alzheimer's disease, Parkinson's disease, neural disorders, atherosclerosis; alcohol induced liver disease and ulcerative colitis (Alam et al., 2012). Dietary intake of antioxidants has been found to complement body defensive system against free radicals. Several reports have indicated that antioxidants in foods particularly antioxidant nutrients contribute significantly in diseases prevention (Alam et al., 2012). *A. digitata* is one of the important trees with great potential owing to its various nutrients content. It is commonly called baobab tree (*Adansonia digitata L.*) and dead rat tree. It is an important multipurpose food tree of the semi-arid and sub-humid zones of sub-Saharan Africa, including countries in Western Africa (e.g. Senegal, Mali, Niger, Benin and Nigeria), Southern Africa (e.g. Namibia, South Africa, Mozambique, Zambia, Malawi) and Eastern Africa (e.g. Sudan, Ethiopia, Kenya, Tanzania) (Wickens, 2008). The genus *Adansonia* belongs to the family *Bombacaceae*. A recent publication suggests that there are two different species of Adansonia in mainland Africa, namely the tetraploid *A. digitata* ('lowland baobab) and the diploid *A. kilima* ('hill baobab'), which is believed to occur on higher altitudes and has a slightly different flower morphology from *A. digitata*, among other minor differences (Pettigrew *et al*. 2012). The remarkable, long-lived baobab tree has a short, swollen trunk with a girth of up to about 28 m, ending in thick, wide-spreading branches that carry a large, round canopy reaching a height of up to 25 m (Sibibe and Williams 2002). It has large palmate leaves and showy whitish flowers that open at night and are pollinated by fruit bats and nocturnal moths (Wickens, 2008). The fruits are capsulated with a hard, woody shell and many seeds embedded in a whitish powdery fruit pulp. Almost all parts of baobab are useful for human beings (Wickens, 2008), with fruits and leaves being the most important for food and nutrition security of local communities (Sibibe and Williams 2002; Buchmann *et al*, 2010). Baobab leaves are superior in nutritional quality to fruit pulp, and contain significant levels of vitamin A. The leaves are a staple for many populations in Africa, and are eaten fresh or dried (De Caluwe *et al*, 2010). The naturally dry, whitish fruit pulp is high in sugars, vitamin C and minerals such as calcium, magnesium and iron (Stadlmayr *et al*. 2013). It can be eaten fresh or processed into porridge, juice, jam, ice cream and sweets (Sibibe and Williams 2002). The seeds are rich in protein and fat and can be roasted and eaten as snack or pressed into oil for consumption and industrial use, particularly for cosmetic products (Gebauer et al. 2002). The leaves are known to have high protein, beta carotene and iron content and are used fresh as leafy vegetable or dried and powdered as a soup ingredient (Sibibe and Williams 2002; Buchmann *et al*. 2010 and Chadare *et al*., 2008).

Phytochemicals literally mean "plant chemicals." Scientists have identified thousands of different phytochemicals, found in vegetables, fruits, beans, whole grains, nuts and seeds. Eating lots of plant foods rich in phytochemicals may help to prevent at least one in every five cases of cancer, as well as other serious ailments such as heart disease. Plant phytochemicals comprises of Primary metabolites, which include amino acids, simple sugars, nucleic acids, and lipids, and secondary plant metabolites. (Buchmann *et al.*,2010). Compounds contributing to fundamental metabolism are termed primary metabolites. In contrast secondary metabolites are limited in their distribution; both throughout the plant and between different species. Plant phytochemicals can be categorized into three main groups, namely: alkaloids, phenolics and terpenoids (Buchmann *et al.* 2010).

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. The name derives from the word alkaline and was used to describe any nitrogen - containing base. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and

animals and are part of the group of natural products (also called secondary metabolites) (Ng, *et al*.2015). Many alkaloids can be purified from crude extracts by acid - base extraction. Many alkaloids are toxic to other organisms. They often have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals. Examples are the local anesthetic and stimulant cocaine, the stimulant caffeine, nicotine, the analgesic morphine, or the antimalarial drug quinine. Some alkaloids have a bitter taste (Ng, *et al*.2015).
Phenolic are aromatic benzene ring compounds with one or more hydroxyl groups produced by plants mainly for protection against stress (Yoshida K, *et al* 2009). Example of phenols include: flavonoids, isoflavonoids, neoflavonoids, triflavonoids, lignans, neolignans,condense tannin (Lattanzio, 2013)

Terpenoid are also group of polyunsaturated organic compound produce by plant as their secondary metabolites, that serves different physiological functions, terpenoid are refers to a terpene that has been modified, such as by the addition of oxygen. Isoprenoids are, therefore, the building blocks of other metabolites such as plant hormones, sterols, carotenoids, rubber, and phytol tail of chlorophyll (Zwenger and Basu, *2008*).

An antioxidant is "any substance that delays, prevents or removes oxidative damage to a target molecule" (Halliwell and Gutteridge 2015). The oxidised substrate could be e.g. proteins, lipids or DNA. Many substances are shown to have antioxidative properties in vitro but do not necessarily work in the same way in vivo. The substance could be metabolised and lose its antioxidative properties when applied in vivo. The antioxidative ability is influenced by the qualities of each individual substance, such as the antioxidative mechanism, the target biomolecule, the place of action (extra- or intra-cellular) and the concentration needed for antioxidative effect (Halliwell and Gutteridge 2015).

The antioxidants primarily act by three different mechanisms; either by preventing the formation of radicals, by scavenging radicals or by repairing the damages that oxidative stress has caused. The first step in the antioxidative defence involves preventive compounds that suppress the generation of reactive oxygen species (ROS), such as the metal binding proteins transferrin and albumin. The second step includes the radical-scavenging antioxidants. These antioxidants inhibit initiation of the oxidation chain, prevent chain propagation and terminate chain reaction by forming a stable by-product. Vitamin C (ascorbic acid) and vitamin E (mainly -tocopherol) are important scavenging antioxidants. The third step of antioxidant defence repairs damages caused by free radicals by means of e.g. DNA repair enzymes and lipases. The antioxidant defence includes both antioxidants produced in the body (endogenous) and antioxidants derived from the diet (exogenous) (Willcox *et al.,* 2004). The endogenous antioxidants are found both intracellularly and extracellularly. Uric acid, bilirubin, ubiquinol, glutathione and the metal binding proteins albumin, transferrin and ferritin are examples of non-enzymatic endogenous antioxidants. Superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px) are examples of enzymatic endogenous antioxidants. Exogenous antioxidants are provided by the diet and include vitamins and other antioxidative plant compounds but also essential minerals required in the active site of antioxidative enzymes such as selenium in GSH-Px (Alam *et al*., 2013). In this research we assessed some phytochemicals in the chloroform extract of *Adansonia digitata* leaves and subsequently determined the antioxidant potential of the same.

METHODOLOGY

Plant material:

Adansonia digitata leaves were collected from Andaza, Kiyawa, Local Government, Jigawa state, Nigeria, and identified by Baha'udeen Sa'id Adam of herbarium unit in the plant

biology Department, Bayero University Kano, in the month of August 2016. The sample was given a herbarium accession number as BUKHAN 0036, and a voucher of the sample was deposited in the department.

Extract preparation:
The *Adansonia digitata* leaves were dried under shed and grinded into fine powder, using pestle and mortar. Then a 100g of the grinded powder was dissolved in 500ml of chloroform, and incubated for 48hours at room temperature. The extract was then filtered using maceration Method, and the supernatant was then boiled to evaporation. A portion of the dried extract was used against 20ml of chloroform to make a solution for the phytochemical screening.

Phytochemical screening
We carried out phytochemical analysis of the plant sample extract based on the method adopted by Ekwueme *et al,* 2015. Simple chemical test was used to qualitatively analyzed the presence of phytochemicals namely; Steroids, Flavonoids, Cardiac glycosides, Anthraquinone, Saponin and Alkaloids in chloroform extract of *Adansonia digitata* leaves

Test for Steroids
A known quantity of the test sample was extracted in the chloroform and filtered. The filtrate was mixed with 2 ml of conc. H_2SO4 carefully so that the sulphuric acid formed a lower layer. A reddish-brown colour at the interphase indicated the presence of steroidal ring.

Test for flavonoids
Portion of the extract was added with few drops of 20% sodium hydroxide, formation of intense yellow colour was observed. To this, few drops of 70% dilute hydrochloric acid were added and yellow colour was disappeared. Formation and disappearance of yellow colour indicates the presence of flavonoids in the sample extract.

Test for Glycosides
Dilute sulphuric acid (5 ml) was added to the portion of the extract in a test tube and boiled For 15 min in a water bath, then cooled and neutralized with 20% potassium hydroxide solution. 10 ml of a mixture of equal parts of Fehling's solution A and B was added and boiled for 5 min. A more dense brick red precipitate indicated the presence of glycoside.

Test for Anthraquinones
Portion of the extracts was added to 4ml of benzene and shaken, it was filtered when hot, the filtrate was shaken with 2ml of 10% ammonia solution. The disappearnace of violet colour in the ammoniacal phase (lower phase) indicates the presence of free anthraquinones..

Test for Saponins
Aliquot of the extract was diluted with 20ml of deionized water, shaken vigorously and observed. Persistent foaming indicated the presence of saponins.

Test for Alkaloids
Portion of the extracts was diluted with 10ml alcohol, boiled and filtered. 5ml of filtrate was added to 2ml of ammonia. 5ml of chloroform was also added and shaken gently; 10ml of acetic acid was added. Then Wagner's reagent was also added. Reddish brown precipitate was positive for the presence of alkaloids (Abiona *et al.*, 2015)

Antioxidant activity

FRAP (Ferric reducing/antioxidant power) assay

The total reducing power of the extracts were measured using the FRAP method described by (Benzie and Strain, 1999) with slight modification. To perform this assay, 1 mL of each extract (0.2–1 µg/mL) was incubated with 1 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide at 50 oC for 30 minutes. Thereafter, 1 mL of 10% trichloroacetic acid was used to acidify the reaction mixtures. After the acidification, 1 mL of the sample was mixed with 1 mL of distilled water and 200 µL of 0.1% $FeCl_3$. The absorbance of the resulting solution was read at 700 nm in a spectrophotometer. The absorbance of the samples is proportional to the reduction capability of the extracts. The results were expressed as a percentage of the absorbance of the sample to the absorbance of ascorbic acid.

Calculations:

$$\text{Ferric reducing antioxidant power \%} = \frac{\text{Absorbance of sample}}{\text{Absorbance of ascorbic acid}} \times 100$$

DPPH (2, 2 -diphenyl-1-pycryl-hydrazyl) free radical scavenging action:

The assay is based on the measurement of the scavenging capacity of antioxidants towards 2,2 -diphenyl-2-picrylhydrazyl (α,α-diphenylpicrylhydrazyl; DPPH). The odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine. The molecule 2,2 -diphenyl-2-picrylhydrazyl (α,α-diphenylpicrylhydrazyl; DPPH) is a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecule does not dimerize. The delocalization of electron also gives rise to the deep violet color, characterized by an absorption band centered at about 520 nm. When a solution of DPPH is mixed with that of a substrate (AH) that can donate a hydrogen atom, then this gives rise to the reduced form and the color changes from violet to colorless or pale yellow (kedare and singh, 2011). We adopted the method of Shimamura *et al*, 2014, with some slight modifications. The free radical scavenging activity of the fraction was measured in vitro by 1,1 diphenyl- 2 picrylhydrazyl (DPPH) assay. About 0.3mM solution of DPPH in 95% ethanol was prepared and 2 ml of this solution was added to 1ml of the fraction dissolved in ethanol at different concentration. The mixture was shaken and allow to stand at room temperature for 30 minutes and the absorbance was measured at 517nm using a spectrophotometer. The percentage of the free radical scavenging activity at different concentration was determined. The antioxidant activity was expressed as

Calculations:

$$\text{\% Inhibition} = \frac{[Abr - Aar]}{Abr} \times 100$$

Where Abr = Absorbance before reaction

Aar = Absorbance after reaction (Alam *et al*, 2013)

Statistical Analysis

The data were also analyzed statistically using one way analysis of variance (ANOVA) and Differences among the means were determined for significance at $P < 0.05$.

RESULT

Phytochemicals screening:

A preliminary study on the composition of important bioactive metabolites in *A. digitata* leaves was performed. Qualitatively, the presence of secondary metabolites; namely steroids, flavonoids, cardiac glycosides, anthraquinone, saponin and alkaloids were tested using

chloroform extract of *A. digitata* leaves. Only anthraquinone and saponin were found to be absent in the leaf extract while the remaining metabolites were present (Table 1)

Phytochemicals	Inference
Steroids	+
Flavonoids	+
Cardiac glycosides	+
Anthraquinone	–
Saponin	–
Alkaloids	+

(+) indicate a positive result and (-) indicate a negative result

Table 1: Qualitative analysis of some phytochemicals in the chloroform extract of *A. digitata* leaves.

Ferric Reducing Antioxidant Power (FRAP)

The antioxidant power which is defined as the ability of a compound to inhibit oxidation was determined using ferric reducing antioxidant power (FRAP) method. As evident from Figure 1, *A. digitata* leaf extract displayed antioxidant activity expressed as percentage FRAP inhibition at all concentrations (0.2, 0.4, 0.6, 0.8 and 1 µg/ml) respectively. However, the most pronounced activity was obtained at 0.2, 0.4 and 1 µg/ml concentration representing 76.92, 83.14 and 80.58% respectively.

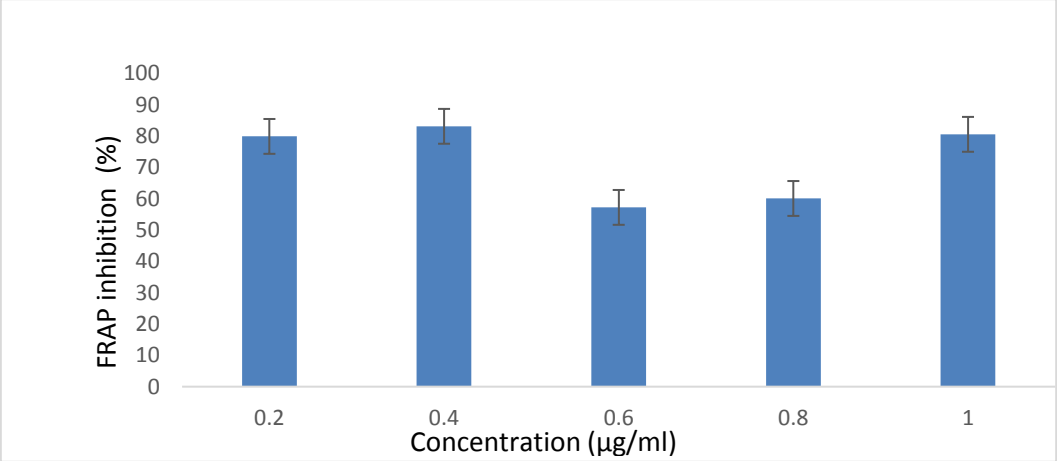

Fig 1: FRAP inhibition (%) against sample concentration (µg/ml)

DPPH Radical scavenging activity

To further asses the antioxidant potential result of *A. digitata* leaf extract obtained using FRAP assay, we used DPPH radical scavenging activity assay with ascorbic acid serving as a standard. 0.2, 0.4 and 0.6 µg/ml of the sample extract does not reveals positive inhibition of DPPH while 0.8 and 1.0 µg/ml displays DPPH inhibition of 11.16 and 23.21% respectively (Figure 2).

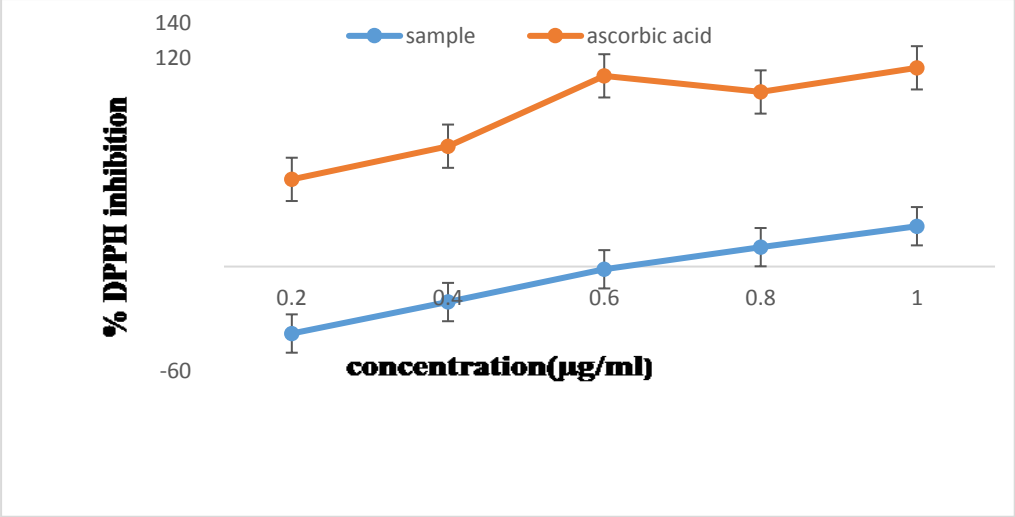

Fig.2: Comparison between Antioxidant Activity of Ascorbic Acid and that of *A. digitata* chloroform leaves extract measured as % DPPH inhibition, each value is presented as mean of triplet treatments.

DISCUSSIONS

Many phytochemicals were found to be present in various plants in varying amounts. These phytochemicals are regarded as biologically active due to their significant influence on various physiological processes. More than 25% of the prescribed drugs were found to be originated from plants. Despite the discovery and isolation of many vital bioactive compounds from various plants, only few were successfully developed and utilized for clinical use (Ekwueme *et al*, 2015). Detection or otherwise of a particular phytochemicals depends on the type of solvent and plant parts (Dai and Mumper, 2010). Cost of production of chemically synthetized drugs, safety concern and low bioactivities are the driving forces that render bioactive compounds from natural sources to be an alternative of the synthetic ones. In our studies, the qualitative phytochemical screening using chloroform extract of *A. digitata* leaves shows the presence of alkaloids, sterols, flavonoids, and cardiac glycoside while saponins and anthraquinone were absent in the sample extract (table 1). This result is in agreement with that of Ogbaga *et al*., 2017 who shows that water extract of *A. digitata leaves* contains glycosides, steroids, and flavonoids. Ebaid *et al*, 2019 also analyzed phytochemicals of methanolic extract of *A.digitata* leaves and found that flavonoids, saponins, steroids and alkaloids were present. This result indicates that, *A. digitata* leaves as used by many Africans especially in the preparation of some dishes, could be of great benefits to human well-being. The results also implied that the baobab leaves could be of great potential in nutraceutical, pharmaceuticals and food industries for making drugs and supplements. The subsequent antioxidant activity tests shows capability of the extract to prevent the oxidative effect of free radicals. Ferric reducing antioxidants power experiment (FRAP) shows a detectable oxidants scavenging activities of the extract at all concentrations due to the ability of the antioxidants presence in the sample extract at various concentrations to reduce free radical (Figure 1). Although FRAP inhibition increases with sincrease in extract concentration from 0.2 to 0.4 µg/ml, there was decrease in the inhibition at 0.4 and 0.8 µg/ml concentrations, this could be due to the incomplete FRAP reduction by the antioxidants presence in the extract. This might happen possibly as a result of low incubation time in the course of the experiment. Furthermore, the DPPH inhibition experiment confirms the antioxidant power of the extract at 0.8 and 1.0 µg/ml of the extract while there was no detectable level of antioxidant activity (negative value) at 0.2, 0.4 and 0.6 µg/ml concentration compared to the standard (Figures 2).

This could be attributed to the low extract concentration and relatively higher solvent volume leading to the higher sample dilution and highly undetectable inhibition.

Flavonoids have been shown to exhibit various functions such as antioxidant (Brunetti *et al*, 2013), regulators of cell proliferation, anticancer (Ren *et al*, 2003), antimicrobial (Cushnie *et al*, 2005), and anti-inflammatory (Guardia et al, 2001). The phytochemicals present in the extract are likely responsible for the antioxidant activity of chloroform extract of *A. digitata* leaves. Furthermore, antioxidant activity of the *A. digitata* leaves could be due to some antioxidant not been tested for. Vertuani *et al*, 2002, have shown that A. *digitata* leaves contain provitamin A which can be converted to vitamin A (antioxidant). Structurally, some functional groups such as hydroxyl group present in aromatic ring of these compounds could account for their antioxidant power.

CONCLUSION

The result of this study has shown that *A. digitata* contain steroids, flavonoids, cardiac glycosides and alkaloids while Anthraquinone and saponins were absent. These chemicals were found to exhibit numerous bioactivities such as antioxidant, anticancer, anti-inflammatory and antimicrobial activities. Subsequent oxidants scavenging activity (antioxidant activity) of *A. digitata* leaves extract as determined by FRAP and DPPH assay methods has revealed that, A. *digitata l*eaves extract could have health-promoting effect via its ability to scavenge free radicals.

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