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Proximate Analysis, Phytochemical Screening and Antimicrobial Activity of Baobab (*Adansonia digitata)* Leaves.

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Abstract: *Baobab (Adansonia digitata L.) is known in Africa as the tree of life because there are many traditional uses for every part of it, from the leaves to the roots. This study carried out the proximate analysis of baobab leaves, screened the phytochemicals present and determined the leaves' aqueous extract antimicrobial activity to suit the traditional way of preparation and use. Phytochemicals screening was carried out on the ethanolic, methanolic and aqueous extracts of the leaves. The phytochemicals screened were reducing sugar, flavonoids, terpenoids, saponins, tannins, alkaloids, anthraquinones, steroids, resins, phenols, cardiac-active glycosides. The aqueous extract of baobab leaves was tested for its antimicrobial activity against the following organisms; Staphylococcus aureus, Esherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Saimonelle typhi, Klebsiellae pneumonae, Candida albicans, Aspergillus niger, Rhizopus stolomifer, Penicillum rotatum*at at different concentrations of 200, 100, 50, 25, 12.5, 6.25 mg/ml, with water, gentamycin 10 µg/ml(Bacterial), tioconazole70% (fungi) as negative and positive controls respectively. The proximate analysis revealed baobab leaves contain protein (13.6%), fat (2.71`%), sugar(0.01%), ash(4.08%), crude fibre (2.45%), Moisture content(78.2%),Vitamin C (14.98mg/100g). *All phytochemicals screened were found to be present.* The result showed that the aqueous extract of the baobab leaves was active against the tested organisms except *klebsiellae pneumonae. Staphylococcus aureus* is more sensitive to the antimicrobial activity of the extract. The aqueous extract displayed broad spectrum effect because it has activities on Grams positive and Grams negative bacteria and fungi.

Keywords: *Adansonia digitata, Staphylococcus aureus, Esherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Saimonelle typhi, Klebsiellae pneumonae, Candida albicans, Aspergillus niger, Rhizopus stolomifer, Penicillum rotatum.*

I. Introduction

Baobab (A. digitata), a tree plant belonging to the Malvaceae family, is widespread throughout the hot, drier regions of tropical Africa (De Caluwé et al., 2010; Bremer et al., 2003;FAO, 1988). It is a deciduous, massive and majestic tree up to 25 m high, which may live for hundreds of years (Gebauer et al., 2002). The trunk is swollen and stout, up to 10 m in diameter, often buttressed; usually tapering or cylindrical and abruptly bottle-shaped. Branches are large and distributed irregularly. The bark is smooth, reddish brown to grey, soft and fibrous (Gebauer et al., 2002).Leaves are alternate and foliate. Overall mature leaf size may reach a diameter of 20 cm. Flowers are pendulous, solitary or paired in leaf axils, large and showy. Flower bud is globose, sometimes ovoid (Sidibe and Williams, 2002). The fruit of the baobab tree hangs singly on long stalks with an ovoid, woody and indehiscent shell 20 to 30 cm long and up to 10 cm in diameter with numerous hard, brownish seeds, round or ovoid, up to 15 mm long, which are embedded in a yellowish-white, floury acidic pulp (Nnam and Obiakor,2003). The ripe fruit pulp appears as naturally dehydrated, powdery, whitish colored and with a slightly acidulous taste (Vertuani et al., 2002).

Baobab leaves, bark and fruit are used as food and for medicinal purposes in many parts of Africa. In the Sahel, for example, baobab leaf is a staple the Hausas used to make "miyan kuka" soup. Powdered leaves are used as anti-asthmatic agent and known to have antihistamine and anti-tension properties. The leaves are also used traditionally to treat a wide variety of conditions including fatigue, as a tonic, and for insect bites Guinea worm and internal pains, dysentery, diseases of the urinary tract, opthalmia and otitis in some parts of Africa (Dan and Dan, 1986; Sidibe and Williams, 2002). According to Nordeide et al, 1996, baobab leaves are potential protein source to be used to complement the amino acid profile to improve the overall protein quality of the local diet. Baobab leaves are also significant sources of minerals (Nordeide et al, 1996, Barminas et al., 1998; Lockett and Grivetti,2000; Boukari et al., 2001) .Some studies reported that baobab leaves is an important source of iron (Yazzie et al,1994; Sidibe and Williams,2002), and have a higher content of iron compared to numerous other wild – gathered foods, and are a rich source of calcium(Glew et al, 1997; Barminas et al, 1998; Lockett and Grivetti, 2000;). Vertuani et al, 2002, also show that baobab leaves possess anti-oxidant properties.

From the literature reviews on baobab, information was provided on its phytochemistry and pharmacology, the species taxonomy, distribution, utilization, agronomy, agro-ecology (Sidibe and Williams, 2002; Diop et al., 2005; De Caluwé et al., 2010). Gebauer et al., 2002, brought out information on baobab

botany, ecology, origin, propagation, main uses, genetic improvement and especially its importance for nutrition and poverty alleviation. However, the information on the nutritional components of baobab leaves is not much .This prompted the proximate analysis of this study. Also, reports on the antimicrobial activity of baobab leaves aqueous extract as being prepared locally for various medicinal purposes is scanty. Thus, this research work carried out the antimicrobial activity of baobab leaves and screened the phytochemicals present.

II. Materials And Methods

Sample Collection And Preparation

Adansonia digitata(baobab) leaves were collected at Botany Department, University of Ibadan, Oyo state, Nigeria. The leaves were air dried at room temperature, and reduced to fine powder using a blender. Twenty grammes (20g) each of the samples was accurately weighed into three different 500ml beaker and 400ml each of water, methanol, and ethanol was added to the flasks respectively. These were left for 72 hours. The crude extracts were then decanted and kept for the various analyses.

Proximate Analysis

The proximate analysis was done according to AOAC methods 1990

Phytochemicals Screening

Tannins

Five ml of the extracts was measured and boiled with 10ml of water in a test tube, followed by filtration. Few drops of 0.1% ferric chloride were added. Brownish green precipitate indicates the presence of tannins.

Terpeniods

Five ml of the extracts was measured and was added to 2ml of chloroform. 3ml of concentrated tetraoxosulphate (VI) acid was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

Flavonoids

Five ml of ammonia was added to the extracts and 1ml of concentrated tetraoxosulphate (VI) acid was added. A yellow colouration that disappears on standing indicates the presences of flavonoids.

Saponins

Five ml of the extracts was added to 5ml of distilled water in a test tube , the solution was shaken vigorously and observed formation of stable persistent froth indicates the presence of saponins.

Reducing sugar

Ten ml of the extracts was added to 5ml of water was added to boiling Fehling's solution A and B in a test tube. The solution was observed for a colour change.

Alkaloids

Five ml 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.

Anthraquinones

Five ml of the extracts was shaken with 4ml benzene, it was filtered when hot, the filtrate was shaken with 2ml of 10% ammonia solution The absence of violet colour in the ammoniacal (lower phase) indicates the presence of free anthraquinones.

Steroids

Two ml of the extracts was mixed with 2ml of chloroform. Concentrated sulphuric acid was carefully added to form a lower layer. The formation of a reddish-brown colour at the interface indicates the presence of steroids

Cardiac-active glycoside

Two ml of the extracts was mixed with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was followed by the addition of 1ml of concentrated sulphuric acid. A brown ring which was formed at the interface confirmed the presence of cardiac glycosides.

Resins
Five ml of copper acetate solution was added to 5ml of the extracts. The resulting solution was shaken vigorously and allowed to stand. Formation of a green coloured solution is an evidence for the presence of resins

Phenols
Two ml of the extracts was mixed with ferric chloride solution. A green or dirty green precipitate indicates the presence of phenolic compound.

Antimicrobial Screening
Microorganisms

Pure cultures of Staphylococcus aureus, Esherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Saimonelle typhi, Klebsiellae pneumonae, Candida albicans, Aspergillus niger, Rhizopus stolomifer, Penicillum rotatum were obtained from the Medical Microbiology department of the University College Hospital (UCH) Ibadan, Nigeria. Bacterial cultures were maintained on nutrient agar slant and the fungi on potato dextrose agar slant, both at 7^{o}C.

Preparation Of Graded Concentration Of The Leaves Extract

One gram of the aqueous leaves extract was weighed and dissolved into 5ml of the solvent of extraction (water) for proper dissolution which was 200mg/ml, 2.5ml from 200mg/ml was shaken and put into another 2.5ml to give 100mg/ml, this was done till the 6^{th} test tube that gives 6.25mg/ml. The 7^{th} and 8^{th} tubes were negative and positive controls (water and gentamycin) respectively.

Pour Plate Method (Bacteria) Agar Diffusion

From overnight culture, 0.1ml of each organism was taken into 9.9ml of sterile distilled water(SDW) to give 10-2 (1:100) of the dilution of the organism. 0.2ml of each of the diluted organism was put into the prepared sterile nutrient agar. It was then poured aseptically into the sterile plates and allowed to set for about 45 minutes. Using a sterile cork borer of 8mm diameter, the wells were made on the nutrient agar plate. Into each well the graded concentration of the extract were put into the wells including the controls. The duplicates were made to ascertain the results obtained. The plates were allowed on the bench for about 2hrs to allow the extract to diffuse properly into the nutrient agar i.e. pre-diffusion. The bacterial plates were incubated uprightly at 37°C and the fungal plates at 28°C for 24hrs. The zone of inhibition was measured in mm.

III. Results And Discussion

The results of the study are presented in the tables below:

Table1. Proximate Composition of Baobab Leaves.

Content	**Value**
Protein	13.6%
Fat	2.71%
Sugar	0.01%
Ash	4.08%
Crude Fibre	2.45%
Moisture content	78.20%
Vitamin C	14.98mg/100g

Table2. Phytochemicals Composition Of Baobab Leaves Result

Phytochemical screened	Methanol extract	Ethanol extract	Aqueous extract
Reducing sugar	+	+	+
Flavonoids	+	+	+
Terpenoids	+	+	+
Saponnins	+	+	+
Tannins	+	+	+
Alkaloids	+	+	+
Anthraquinones	+	+	+
Steroids	+	+	+
Resins	+	+	+
Phenols	+	+	+
Cardiac-active glycosides	+	+	+

Keys:
+: present, - : not present

Table 3. Result Of Antibacterial Activity Of The Aqueous Extract

Concentration in extract mg/mi	Staphylococcus aureus	Esherichia coli	Bacillus subtilis	Pseudomonas aeruginosa,	Saimonelle typhi
200	20	14	16	12	12
100	18	12	14	10	10
50	14	10	12	-	-
25	12	-	10	-	-
12.5	10	-	-	-	-
6.25	-	-	-	-	-
-ve	-	-	-	-	-
+ve	38	36	38	40	36

- : No inhibition, -ve : Negative control (water), +ve : Positive control(gentamycin 10 µg/ml) .

Table 4. Result Of Antifungal Activity Of The Aqueous Extract

Concentration in extract mg/mi	Klebsiellae pneumonae	Candida albicans	Aspergillus niger	Rhizopus stolomifer	Penicillum rotatum
200	-	16	14	17	14
100	-	14	12	10	12
50	-	10	10	-	10
25	-	-	-	-	-
12.5	-	-	-	-	-
6.25	-	-	-	-	-
-ve	-	-	-	-	-
+ve	38	28	28	26	28

Key:
- : No inhibition, -ve : Negative control (water), +ve : Positive control(Tioconazole (70%)) .

IV. Discussion

The result of phytochemicals screening shows that all the phytochemicals screened were present. The antibacterial result as shown in table 3, above shows that the sample aqueous extract was active against the test organisms, which confirms its use traditionally . The table shows that as the concentration increases the zone of inhibition increases and vice versa. The sample's inhibitory effect was more pronounced on Staphylococcus aureus and less in Pseudomonas aeruginosa and Saimonelle typhi. The antifungal activity shown on table 4, revealed that the sample has no inhibitory effect on Klebsiellae pneumonae, but inhibited Candida albicans , Aspergillus niger, Penicillum rotatum and Rhizopus stolomifer growth at concentration of 200,100 and 50 mg/ml except Candida albicans which was not affected by the sample at 50 mg/ml concentration. The sample is

broad spectrum because it has activities on Grams positive and Grams negative bacteria and fungi. The positive (+ve) controls are purified drugs, therefore, Their activities on the test organisms were high. The proximate analysis reveals that baobab leaf is rich in protein (13.6%), fat (2.71%) and vitamin C (14.98%). It is also rich in mineral content since its ash content is 4.08%. It is very low in sugar content (0.01%), which makes it suitable for diabetic patients consumption. It also contain crude fibre (2.45%) which is now known to reduce risk of some of the world's most prevalent disease like obesity, diabetes, high blood cholesterol, cardiovascular disease, and numerous gastrointestinal disorders (Tungland and Meyer,2002; Venn and Mann,2004; Theuwissen and Mensink, 2008).The percentage moisture content is 78.2. Report by other researchers revealed that baobab leaf is rich in protein(11-17%) and Fibre(10 to 19 %), (Belewu et al., 2007; Heuze et al.,, 2013). Leung et al., 1968, reported 77g water, 3.8g protein, 0.3gfat, 2.8gfibre, 16.1 g carbohydrate, 52mg ascorbic acid in fresh baobab leaves per 100 g edible portion. Ibrahim et al., 2014 reported genetic diversity in nutritional traits in baobab leaves , in which he reported percentage moisture, fat, ash, protein, fibre and carbohydrate contents in the range, 76.14-80%, 0.24-1.10%, 0.65-1.44% , 3.88-5.64%, 1.60-2.60% , 12.82-17.92% respectively. Also Sidibe & Williams (2002), based on the data of Becker (1983), Yazzie et al. (1994) and Nordeide et al. (1996), the leaves contain (expressed on dry weight basis):13–15% protein, 4–10% fat (Sidibe & Williams, 2002). Chadare et al. (2009) calculated that, without considering the conversion factor or the effect of processing, the consumption of 20 g of dry leaf material would cover 10 to 16% of the protein recommended daily intake for children.

Vertuani et al., 2002; Besco et al., 2007; Lamien-Meda et al.,2008; Blomhoff et al., 2010; Brady, 2011 who worked on baobab fruit pulp reveals that it is high in natural vitamin C, which acts as antioxidant, and thus could help prevent oxidative stress related diseases such as cancer, ageing, inflammation and cardiovascular diseases as they may eliminate free radicals which contribute to these chronic diseases (Kaur and Kapoor, 2001; Blomhoff et al., 2010). Chadare et al., 2009 asserted that pulp and leaves of baobab exhibit antioxidant activity. It was reported that the daily recommended dose of vitamin C can be obtained from 23 g of baobab powder (Chadare et al., 2009). A variety of chemicals have been isolated and characterised from A. digitata. They belong to the classes of terpenoids, flavonoids, steroids, vitamins, amino acids, carbohydrates and lipids (Shukla et al., 2001). This analgesic activity of aqueous baobab fruit pulp extract was attributed to probably the presence of sterols, saponins and triterpenes in the aqueous extract by Ramadan et al., 1993. Masola et al., 2009, also mention that the analgesic activities were probably due to the presence of sterols, saponins and triterpenes in the fruit pulp (Donatien Kaboré et al., 2011).Yagoub (2008), showed that the petroleum ether, ethanol and aqueous extracts of baobab showed antimicrobial activity against Escherichia coli. Afolabi and Popoola, 2005,created an acid medium for fermentation of tempe (fermented soybeans using the fungus Rhizopus oligosporus),by the addition of baobab pulp powder reveals that it could prevent the growth of pathogenic bacteria such as Salmonella sp., Bacillus sp. and Streptococcus sp. (Donatien Kaboré et al., 2011).

V. Conclusion

The result of the analysis revealed that baobab leaves contain significant nutritional components that are good for human health and maintenance. They also contain active ingredients against Staphylococcus aureus, Esherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Saimonelle typhi, Candida albicans, Aspergillus niger, Rhizopus stolomifer, and Penicillum rotatumat.

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