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PHYTHOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF ETHANOL LEAF EXTRACT OF BAOBAB (ADANSONIA DIGITATA)

Biotechnology

Hussaini U. Bulakarima Department of Biological Sciences, Faculty of Science Borno State University, Maiduguri, Nigeria

Fatima Muhammad Bahir Department of Biotechnology, Faculty of Life Sciences University of Maiduguri, Maiduguri, Nigeria

Ibrahim M. Bala Department of Chemistry, Faculty of Physical Sciences University of Maiduguri, Nigeria

ABSTRACT

The tree species of Adansonia digitata possesses wide range of medicinal properties effective against various infectious diseases. The present work has aim to study the antimicrobial activity and phytochemical screening of leaf extracts of A. digitata against pathogenic spp. Antimicrobial activity of ethanolic extracts of leaf extracts of A. digitata has been studied to find out their activity against pathogenic bacteria viz., Escherichia coli, Staphylococcus aureus, Streptococcus pyeogene and Salmonella. The activity of this extracts leaf part against both gram positive as well as gram negative pathogenic bacterial strains was screened through well diffusion technique by using minimum inhibitory concentration (MIC) method. According to the findings, the leaf extracts of A. digitata exhibited antibacterial activity against Staphylococcus aureaus and phytochemical test constituents on carbohydrate, flavonoids, terpenoids, tannis, glycoside, cardiacglycoside, saponinglycosides and alkanoid. Thus, results provided evidence that the species A. digitata can be used as a potential source of antimicrobial agent in the treatment of various infectious diseases.

KEYWORDS

Phytochemical, Ethanol, Baobab, MIC, Microbes

INTRODUCTION

Baobab (A. digitata), a Malvaceae family tree is widely spread throughout the dry, warmer parts of tropical Africa (FAO, 1988). The deciduous big and majestic tree is capable of reaching heights of 25 meters and has a lifespan of a few centuries (Gebauer et al., 2002). Trunk has a thick and swollen morphology with diameters of up to 10 meters commonly found with a cylindrical or tapering shape that abruptly is bottle-like frequently being buttressing. The branches show irregular distribution with significant size. The bark has been described as smooth, red-brown or grey in colour and it has a fibrous and soft texture (Gebauer et al., 2002). The plant forms a large lateral root system which develops in the form of tuberous structures. The leaves are alternate in nature and foliate in structure. The juvenile foliage is often simple in nature. Mature leaf width can be up to 20 centimetres. Pendulous flowers either singly or two together in the axil of the leaves are characteristic of the plant. They are large and highly conspicuous in their appearance. The flower bud is usually globose, though sometimes ovoid in form (Sidibe and Williams, 2002). The baobab tree fruit grows singly on long stalks with an ovoid, woody, and indehiscent exocarp 20 to 30 centimetres long and 10 centimetres in diameter (Nnam and Obiakor, 2003). Inside the shell there are many hard, brown seeds that are either spherical or ovoid, 15 millimetres long, lodged in a yellowish-white, floury and acidic pulp (Nnam and Obiakor, 2003). The mature fruit pulp appears as naturally dehydrated, powdery and whitish in colour with a faintly acidulous taste (Vertuani et al., 2002).

Economic Importance

The baobab tree has numerous uses, with each part of the plant found to be useful (Igboeli et al., 1997; Gebauer et al., 2002). The leaves for instance are used in preparing soups. Seeds are used as a thickening agent in cooking but can also be fermented to provide flavour or roasted for eating as snacks (Addy and Eteshola, 1984). The pulp can be consumed directly or converted into a drink while the bark is utilized in the manufacture of ropes (Igboeli et al., 1997). Different parts of the plant provide food, shelter, clothing and medicinal resources as well as materials appropriate for hunting and fishing activities (Venter and Venter, 1996; Sidibe and Williams, 2002). The baobab tree makes a significant contribution to the economic livelihood and employment for both rural and urban families. For example, in 1990, in Burkina Faso, about 92,445 tons of baobab leaves were collected, equivalent to an economic valuation of US$18.1 million (Coulibaly, 1993). Earlier biochemical studies have revealed that the leaves, seeds and pulp of baobab are rich in nutrients (Becker, 1983; Glew et al., 1997; Diop et al, 2005; Nkafamiya et al).

Fibres

Fibres obtained from the inner bark are strong and are widely used in the production of ropes, basketry, snares and fishing lines and are also used in weaving. Other fibres may be obtained from rotten wood and have been used for packaging. Other fibres that can be used for rope making are obtained from the bark of roots (Sidibé and Williams, 2002).

Dye

The roots are used in the East African region in the manufacture of a soluble red dye. The green bark is also used not just as a dye but also for ornamentation (Sidibé and Williams, 2002).

Seed Shell

The strong fruit exocarp is used in the making of vessels to be used in food and drink containment (Sidibé and Williams, 2002).

Fuel

The wood itself is considered a poor source of fuel; however, the shells of fruit are utilized as a burning material in Tanzania. In addition, fruit shells are also used as tools for the harvesting of water (Sidibé and Williams, 2002).

Medicinal Properties of Baobab

Antipyretic Activity

People suffering from malaria in areas like Africa, India, Sri Lanka and the West Indies are known to eat a mash made of dried baobab bark as a febrifuge to reduce the fever of the disease (Wickens and Lowe, 2008; Brady, 2011). The fruit pulp and seeds are also widely used for their anti-pyretic properties (Ramadan et al., 1993; Wickens and Lowe, 2008). In addition, baobab fruit pulp has been shown to significantly lower raised body temperature without affecting normal body temperature (Ramadan et al., 1993). In a study by Ramadan et al. (1993), Adansonia digitata underwent in vivo testing for the purpose of determining the biological anti-pyretic activity of the fruit pulp using rodent models. The results indicated a drastic temperature decrease of 1.94°C among the subjects given an aqueous baobab extract at a concentration of 800 mg/ml, compared to a paltry 0.42°C decrease in the control group.

Analgesic Property

The analgesic activity of baobab pulp was also investigated by Ramadan et al. (1993). The results showed that an aqueous baobab pulp extract given at 800 mg/kg yields activity that is comparable and long-lasting when compared to traditional analgesics equivalent to the analgesic action of 50 mg/kg acetylsalicylic acid. Such activity could be due to the presence of phytochemical constituents such as sterols, saponins and triterpenes in the aqueous extract (Ramadan et al., 1993). The analgesic activities were also supported by Masola et al. (2009) possibly as a result of the above phytochemicals found in the fruit pulp.

Anti-microbial Activity

Addition of baobab pulp powder to an acidic environment, e.g., during

tempe fermentation of fermented soybeans using the fungus Rhizopus oligosporus, can potentially inhibit the growth of pathogenic microorganisms like Salmonella sp., Bacillus sp., and Streptococcus sp. (Afolabi and Popoola, 2005). Besides that increasing baobab pulp powder concentrations were also found to have a parallelism with elevated levels of the number of lactic acid bacteria, which would benefit consumers in the sense that most of the lactic acid bacteria species would be nontoxic and been known to exhibit enzymatic hydrolysis capability to break oligosaccharides in soybeans into disaccharides and monosaccharide constituents. As reported by Yagoub (2008), the baobab extracts acquired by petroleum ether, ethanol and aqueous techniques demonstrated antimicrobial activity on Escherichia coli.

Anti-diarrhoea Activity

In a research study by Tal-Dia et al. (1997), the curative effectiveness of a traditional local solution made from dried baobab fruit, water and sugar was comparatively evaluated in a systematic manner with the standard solution recommended by the World Health Organisation (WHO) for the cure of acute diarrhoea among children. The WHO standard solution is described as an oral rehydration salt (ORS) solution containing glucose, sodium, potassium, and citrate dissolved in drinkable water. The results were evaluated according to the pattern of diarrhoeal attacks and the simultaneous weight gain of the subjects. Although the results showed that the WHO solution had higher efficacy compared to the baobab mixture, comparative analysis showed that there was no statistically significant difference between the two solutions regarding diarrhoea duration and weight gain. In addition, it was noted that the local traditional solution had some benefits, including being a very good source of nutrients, being cheaper than the WHO solution and having better access within African cultures than the WHO option. The antidiarrheal action of baobab is mainly due to two important factors: the astringent action of tannins that suppress osmotic secretions and the anti-inflammatory activity of baobab mucilage on the intestinal mucosal membrane (Wickens and Lowe, 2008). Moreover, the content of tannins, mucilage, cellulose and citric acid in the baobab fruit could be responsible for its anti-pathogenic action against diarrhoea (Gruenwald and Galizia, 2005).

Phytochemicals of Antimicrobial Aroperty

Earlier studies on the ethanolic extract of A. digitata have shown the occurrence of different bioactive constituents, such as tannins, phlobatannins, terpenoids, cardiac glycosides and saponins in the leaf which are responsible for the high antimicrobial activity shown by the crude extracts of the plant (Masola et al., 2009).

MATERIALS AND METHODS

Materials

Weighing Balance	Petri Dishes
Filter paper	Foil Paper
Beaker (250ml)	Funnel
Fume chamber	
Conical flask (250ml)	
Solvent extraction	

Chemicals and Reagents

Ethanol	Ferric chloride	Barfoed's reagent
Ether	Distilled water	Molis's reagent
Acetone	Fehling's solution A and B	Acetic anhydride
Chloroform	Dragendroff's reagent	Potassium hydroxide

Methods

Sample Collection and Identification

The fresh plant leaves of Adansonia digitata were collected from Mairi Kuwait Maiduguri, Borno State around 5:00pm in the month of December 2021. The leaves sample was identified by taxonomist from the department of Biological Science University of Maiduguri.

Sample Preparation

The fresh leaves sample was cleaned air-dried under room temperature for several days. The air dried leaves of the plant material was pulverized using mortar and pestle then subjected to the following analysis.

Soxhlet Extraction

1.5 L of ethanol was poured into round flask; 300 g of the sample was placed in the thimble and was inserted in the centre of extractor. The soxhlet was heated at 60 degree Celsius, when solvent was boiling. The vapour rises through vertical tube into condenser at the top. The liquid condensate drips into the filter paper thimble in the centres, which contain solid sample to be extracted. The extract sleeps through the pore of the thimble and the siphon tube when it flows back down into the round bottom flask. This was allowed to continue for 6 hours. It was then removed from the tube, dried in the oven, coded in the desiccators and weighted again to determine out at 6 hours interval unit of the sample weight becomes equal. The experiment was repeated by placing 300 g of the sample into the thimble again. The weight of oil extracted was determined for each 30minutes interval, at the end of the extraction, the resulting immature containing the oil was heated to recover solvent from the oil.

Phytochemical Screening

The extract of the leave were screened qualitatively for phytochemical constituents using standard procedures (Brain and Turner, 1975; Sotowora, 2008; Evans, 2009).

Test for Tannins

Each extract (0.5g) to be tested was stirred with 10ml of distilled water and filtered. The filtrate was used for the following tests as described by Evans (2009).

I. Ferric chloride test.

To 2 ml of the filtrate, a few drop of 1% ferric chloride solution were added; occurrence of blue-black precipitate showed the presence of Tannins.

II. Lead acetate method;

A mixture of equal volume of 10% ethanoate was added to 2ml of the filtrate. The formation of white precipitate was an indication for the presences of tannis Evans (2009).

Test for Glycosides

I. Test for Anthroaquinoanes (Borntrager's Test)

Each extract (0.5 g) was shaken with 10ml of benzene and filtered. Then 5 ml 10% ammonia solution was added to the filtrate and the mixture was shaken. The appearance of violet colour in the ammonical (lower) phase was taken as the presence of tree Anthraquinones Evans (2009).

II. Test for combined Anthraquinones

The extract (0.5 g) was shaken with 10ml aqueous sulphuric acid, and then filtered, while hot, filtrate was shaken with 5ml of benzene; the benzene layer separated and half its own volume of 10% ammonia solution were added. The presence of red colour in the ammonical (lower) phase was taking as the presence of combined anthraquinones (Evans, 2009).

Test for Cardiac Glycosides

I. Salkowski's test

The extract (0.5 g) was dissolved in 2 ml of chloroform. Sulphuric acid was carefully added to the mixture by the side of the test tube to form a lower layer. The appearance of reddish-brown colour at the interphase was an indication of the presence of steroidal ring (i.e.) a glycon portion of cardiac glycosides (Sotowora, 2008).

Test for Terpernoids

Each extract (0.5 g) was dissolved in ethanol and 1ml of acetic anhydride was added, followed by the addition of concentrated sulphuric acid. A colour changed from pink to violet showed the presence of terpenoids (sotowora 2008).

Test for Flavonoids

I. Shinoda's Test

Each extract (0.5 g) was dissolved in ethanol then warmed and filtered. Three pieces of magnesium chips were added to the filtrate, followed by a few drops of concentrated hydrochloric acid. A pink, orange to purple colouration was an indication of the presence of flavonoids (Evans 2009).

Test for Alkaloids

Each (0.5g) of the extract were stirred with 5 ml of 1% aqueous hydrochloric acid on water bath and then filtered. The filtrate (3 ml) was divided into 3 portions in a test tube and was used for the test below.

I. Dragendroff's Test

Two drops of Dragendroff's reagent were added to the first portion of filtrate; the occurrences of an orange-red precipitate indicate the presence of alkaloids.

II. Mayer's Test

Ten (10 ml) of methanol was added to the second filtrate, 1%

hydrochloric acid was added; the mixture was then boiled and filtered. Mayer's reagent (6 drops) was added to 1ml of the filtrate. A creamy, reddish-brown precipitate was indication of Alkaloids (Evans, 2009).

Test for Carbohydrates

The extract (0.5g) was dissolved in distilled water and filtered; 1 ml of the filtrate was mixed with 1 ml of Barfoed's reagent in a test tube and then heated in a water bath for a period of 2 minutes.

A red precipitate of cuprous oxide is considered as a positive test (Trease and Evans, 2002).

Test for Free Reducing Sugars (Fehling test)

The extract of (0.5 g) was dissolve in distilled water and filtered. The filtrate was heated with 5ml of equal volume of Fehling's solution A and B. Formation of a red precipitate of cuprous oxide (Cu_2O) indicate the presence of reducing sugar (Trease and Evans, 2002).

I. General test (Molish's Test)

Few drops of Molish's reagent were added to the extract dissolved in water. This was followed by addition of 1ml of concentrated Tetraoxosulphate (vi) acid (H_2S0_4) by the side of the test tube, so that the acid will form a layer beneath and was diluted with 5 ml of distilled water, the formation of a red or dull violet at the interphase of the layers indicates a positive test (Trease and Evans, 2002).

Disc Diffusion Sensitivity Test

Disc diffusion techniques are used by most laboratories to test routinely for antimicrobial sensitivity. A disc of blotting paper is impregnated with a known volume and appropriate concentration of an antimicrobial and this is placed on a plate of sensitivity testing agar uniformly inoculated with test organism. The antimicrobial diffuses from disc into the medium and the growth of the test organism is inhibited at a distance from the disc that is related (among other factors) to the sensitivity of the organism. Strains sensitive to the antimicrobial are inhibited at a distance from the disc whereas resistant strains have smaller zones of inhibition or growth up to edge of the disc.

Microorganisms Used

- Staphyloccus aureus
- Streptococcus pyogene
- Escherichia coli
- Salmonella typhi
- Candida albicans

RESULTS AND DISCUSSION

Table 4.1 Phytochemical Constituents Extracts of Adansonia Digitata Leaves

S/N	Constituents	Test	Inference
1.	Carbohydrate	i. Molish test ii. Test for combined reducing sugar iii. Test for monosaccharide iv. Test for ketoses v. Test for free reducing sugar	+ - + + +
2.	Flavonoids	i. Shinoda`s test ii. Ferric chloride test iii. Lead acetate test iv. Sodium hydroxide test	+ - + +
3.	Terpenoids	i. Terpenoid test	+
4.	Tannins	i. Ferric chloride ii. Lead acetate	+
5.	Glycoside	i. Salkwoski`s test ii. Lieberman burchard`s test	+ -
6.	Cardiacglycosides	i. kELLER KILlian`s test	-
7.	Saponinglycosides	i. Saponin glycoside test	+
8.	Alkaloid	i. Dragendroff's reagent ii. Meyer`s reagent	- -

Key: Positive indicates (+) presence, and Negative (-) absence of Secondary Metabolites

Table 4.2 The Results of Antimicrobial Activity

S/N	Microorganisms used			Concentration of Extract Used	
		800 mg/ml	600 mg/ml	400 mg/ml	200 mg/ml
1.	Staphyloccus aureus	7.1 mm	7.1 mm	7.1 mm	7.1 mm
2.	Streptococus pyogene	R	R	R	R
4.	Escherichia coli	R	R	R	R
5.	Salmonella typhi	R	R	R	R
8.	Candida albricans	R	R	R	R

Key: R indicates Resistance of microorganims

DISCUSSION

Plants are basic biological structures used in the formation of chemical compounds, most of which are used to increase health and fight diseases. Phytochemical examination of Adansonia digitata, crude ethanolic leaf extract as shown in Table 1, revealed an array of bioactive secondary metabolites. The examination proved the existence of carbohydrates, saponins, and flavonoids with cardiac glycosides and alkaloids being significantly absent. These types of compounds are known to be therapeutically effective against a wide range of pathogens, thus explaining their traditional use in the treatment of a wide variety of diseases (Usman et al., 2005). The presence of flavonoids in this plant supports previous studies cited in the literature review that this species could be a good source of antioxidant, carcinogenic and cytotoxic bioactive compounds (Evans et al., 2009). Flavonoids have been proved to have anti-inflammatory, anti-allergic, antibacterial and antifungal effects (Usman et al., 2005). The presence of saponins is important owing to their conventional use in medicine, specifically the treatment of malaria. Antimicrobial testing was carried out against four strains of bacteria (two Gram-positive and two Gram-negative) and a single isolate, i.e., Candida albicans, Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, and Salmonella typhi. At concentrations of 800 mg/ml, 600 mg/ml, 400 mg/ml, and 200 mg/ml, the greatest zone of inhibition was 7.1 mm against Staphylococcus aureus, showing that Adansonia digitata's efficacy is concentration-independent while resistance was noted against the other pathogens at all the above concentrations.

CONCLUSION

Baobab tree is a versatile species with a wide range of uses in food, non-food products and medicine. Every part of the baobab tree is said to have use. Baobab's edible parts (pulp, seeds, and leaves) are distinguished by their density in nutrients. Nevertheless, acceptability and effective utilization of baobab parts as a nutritional food are limited by the existence of anti-nutritional elements like protease inhibitors, tannins, and phytates; however, suitable processing methods may suppress or remove these anti-nutrients. Baobab leaves, bark, roots, pulp and seeds have been utilized for several medicinal purposes throughout different parts of Africa and exhibited significant pharmacological activities, including antioxidant activity, prebiotic-like action, anti-inflammatory, analgesic, antipyretic, anti-diarrheal, and anti-dysentery activities, in addition to excipient properties. Further studies regarding the bioavailability and pharmacokinetics of the plant are of utmost importance in order to verify safety. This review is expected to be a major stimulus for research and development projects towards expanding knowledge and utilization of the baobab tree.

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