PROXIMATE CONTENT AND ANTIOXIDANTS ACTIVITIES OF *BOMBAX COSTATUM* LEAF EXTRACTS

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Abstract

Medicinal plants are a rich source of bioactive constituents for traditional systems of medicine, modern medicines, neutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. One of which is the wild edible plant-*Bombax costatum* plant, of the subfamily Bombacoideae and family Malvaceae; it has reported to have high nutritional value and possess phytochemicals, which could be attributed to its medicinal properties. The plant's leaves were extracted and, the yield showed that Ethyl acetate had the highest yield of 5.07 %, followed by methanol 2.70 %, hexane 2.23 % and the least was acetone with 1.61 %. The proximate analysis were carried out following standard methods of analysis by AOAC, showed that the plant sample had an average moisture content of 5.92 g/100 g; ash content, 14.42 g/100 g; average crude protein 26.16 g/100 g; crude lipid 9.50 g/100 g, and crude fibre of 9.10g/100g. The antioxidant activity using the DPPH (1,1-Diphenyl-2-Picrylhydrazyl) radical scavenging assay, showed that the extracts exhibited various degrees of antioxidant activities when compared with the Standard (Vitamin C). The result of the minimum inhibitory concentration (IC_{50}) shows that Vitamin C had the highest scavenging ability (22.52 mg/mL), while the crude extracts were in the order: methanol, highest (57.56 mg/mL), acetone (71.22 mg/mL), ethyl acetate (76.08 mg/mL), and hexane (87.94 mg /mL). These solvents extracts gave a wide difference and varying percentage inhibitions from that of the Standard. This could be as a result of the bioactive constituents present in each extract. Hence, there is the need to explore the potentials of this plant's leaves as a source of natural supplement and antioxidant as well as a precursor for antioxidant drugs development especially in the pharmaceutical industries. Also, the toxicity and anti-nutritional contents be properly investigated.

Keyword: *Bombax costatum*, Proximate, Antioxidant, Nutritional, Medicinal

Introduction

Medicinal plants a rich bio-resource of drugs for traditional systems of medicine, modern medicines, neutralceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ushie *et al*., 2019). Some of these plants are cultivated through thorough efforts by man while others are naturally grown without any input from man, such as the wild edible plants.

Wild edible plants known to grow and reproduce naturally in their natural habitat without being cultivated (Motti, 2022) and plant parts such as fruits, roots, flowers and leaves from native and naturalized species are widely consumed across Tropical Africa. In West Africa, a large part of the recorded useful plants is potentially consumed by local populations and are a source of intervention during food crisis (Shomkegh *et al.*, 2013; Boedecker et al., 2014). This includes wild edible plants, which also plays an important role in food safety, both as a component in daily diets and as resource food (Boedecker *et al.*, 2014).

Most species used as vegetables are the leaves of wild edible plants with the dried leaves widely used as condiments and flavourings for sauces and cooked cereals; this may be boiled or pounded and added directly, while fresh leaves are mostly used as pot-herbs in soups and sauces (Catarino et al., 2019). One of the examples of WEP is the *Bombax costatum* plant, it is widely distributed with the region of Africa (Oraebosi *et al*., 2020) and used widely for its ethno-medical purposes (Sharifi-Rad *et al*., 2020) in treating infectious diseases including sexually transmitted diseases (Muhammad *et al.*, 2017), skin diseases, yellow fever, headache, promote lactation, tonic for fatigue, epilepsy (Oyen, 2011), menstrual cramps (Inngjerdingen et al., 2004). *B. costatum* is of the subfamily Bombacoideae and family Malvaceae (Das et al., 2021), commonly called Red-flowered silk cotton tree in English, Kapok and simply Bombax (Inngjerdingen *et al*., 2004; Das *et al*., 2021). It is locally called *Kurya* or *Gujjiya* (Hausa), and *Kutupkaci* (Nupe) *Genger* (Tiv) (Shomkegh et al., 2013; (Muhammad *et al.*, 2017). It has been reported that the leaves, fruits, flowers and immature pods of *B. costatum* are edible in many countries; it is used as a vegetable with highly nutritive value (Shomkegh *et al*., 2013); it's reported to possesses phytochemicals, which may be responsible for its antimicrobial and antioxidant activities, (Eugene *et al*., 2018). A major setback in the commercial utilization of African herbs is the lack of adequate data, most of the published data on the nutritional content of *B. costatum* leaves are at variant from each other. This has led to the evaluation of the nutritional content (proximate) and antioxidants properties of the plant especially the leaves to

ascertain the nutrients taken and how it can contribute to food security at large.

Materials and Methods

Sample collection and preparation

The leaves of *B. costatum* plant were collected from the environment of the Federal University, Wukari, in wukari Local Government Area, Taraba State, Nigeria. The plant was identified by the Department of Forestry, Federal University, Wukari, Taraba state, Nigeria. The plucked leaves were properly air-dried at room temperature for three weeks, pounded into fine powder using pestle and mortar and stored in a well closed container for further use.

200 g of the powdered sample was weighed and transferred into extraction container for serial exhaustive extraction using cold maceration method for 96 hours (4 days) using 300 mL each of hexane, ethyl acetate, acetone, and methanol. At the end of each extraction, the extract was filtered using a fine clothe and evaporated to dryness using rotary evaporator. The percentage yields were calculated and noted.

Proximate Analysis

The method adopted for the study was the gravimetric method of the Association of Official Analytical Chemist (AOAC. 2005).

Moisture content determination

About 2.0 g each of the fresh seed of the sample was weighed into empty pre-weighed crucibles and dried in a ventilated electronically heated oven at 60º for 24 hours, this was then removed and cooled in a desiccator containing magnesium sulphate as drying agent then reweighed. This was done in triplicate. The moisture content was determined using the equation below:

Moisture content (%) = (Loss of weight on drying ÷ Initial sample weight) × 100

Ash content determination

Three crucibles were pre-weighed and about 2.0 g each of the sample was weighed into the crucibles and put in a muffle furnace and ashed at 600ºC for 3 hours. The ashed samples were removed and cooled in a desiccator and reweighed. The content was then calculated using the equation:

Ash content (%) = (Weight of fresh sample ÷ Initial sample weight) × 100

Fiber content determination

This was done using the digestion method which involves:

Acid digestion: The fat free sample was weighed and transferred into 400 mL beakers. 50 mL of 1.25 % H_2SO_4 was added and the mixture was made up to 200 mL with distilled water. These were filtered through a Buchner funnel and the residue was washed with warm distilled water until it was acid free.

Base Digestion: The residues left after acid digestion were transferred into 400 mL beakers, 50 mL of 1.25 % NaOH was added and made up to 200 mL mark with distilled water. These were heated for 30 minutes with constant stirring. The contents of the beaker were filtered using Buchner funnel and washed several times with hot water until free from sodium hydroxide. Finally, the residue was washed twice with 95 % methanol. These were transferred into crucibles, dried at 100 ºC in an oven, weighed and ignited in a furnace at 550 ºC. The weight of ash noted. The fibre content was calculated using the formula below:

% Fibre content = (Weight of ash ÷ Initial sample weight) × 100

Nitrogen content determination

About 5.0 g of the powdered sample was weighed into 250 mL standard Kjeldahl flask containing standard Kjeldahl catalyst and anti-bombing chip. 30 mL of concentrated H_2SO_4 was introduced into the flask and gently heated for an hour, then, vigorously heated for 8 hours to obtain a clear solution. This solution was cooled and transferred into 100 mL standard volumetric flask and made up with distilled water. 10 mL of this was then measured into a semi micro Kjeldahl Mackham distillation apparatus and treated with 30 ml of 40 % NaOH, 10 ml of 2 % boric acid, few drops of double indicator (0.1 % methyl reagent and 0.1 % methyl blue in 100 mL of ethanol) and distilled to about triple the original volume was obtained. The distillate was then titrated with 0.1 M HCl solution until a purple-pink end point was reached. The percentage nitrogen content in the sample was calculated using the formula:

$$\% \text{ Nitrogen} = \frac{(\text{Titre value} - \text{Blank}) \times 0.01 \times DF \times CF \times 250}{1000 \times \text{Weight of sample}} \times 100$$

Fat content determination

About 5.0 g of the sample was weighed and extracted using petroleum ether as solvent in a Soxhlet extractor for 10 hours, the flask and its content were removed, dried and weighed accurately. The amount of lipid extracted was obtained as the difference between the weight of the flask before and after, using the formula below:

% Fat content = Weight of fat ÷ Weight of sample × 100

Total carbohydrate content determination

This was obtained using the differential method as:

Total Carbohydrate = 100 – (% Moisture □ % Ash □ % Fat □ % Protein □ % Fibre)

Antioxidant Assay

The antioxidant activity was evaluated by DPPH (1,1-Diphenyl-2-Picrylhydrazyl) radical scavenging method as described by Ushie *et al*., (2019) and Kendeson *et al*., (2021) with some modifications. Different concentrations of the standard (Vitamin C) and extract were prepared and tested (0.0313, 0.0625, 0.125, 0.25, and 0.50 mg/mL) and 1.5 mL of 1 mM of DPPH in ethanol was added to each of the standard/sample solutions. The mixture was shaken vigorously using a vortex mixer (SA7) and allowed to stand at room temperature for half an hour then the absorbance was measured at 517 nm using UV/Vis spectrophotometer. A control solution prepared with the same amount of methanol and DPPH only. The percent DPPH scavenging effect was calculated by using equation:

% inhibition = [(A – B) ÷ A] × 100.

Where A = the Absorbance of control and B = the Absorbance of standard/sample. All measurements of free radical scavenging activity were performed in duplicates. The concentrations of samples resulting in 50 % inhibition on DPPH (IC_{50} value) were calculated from linear regression equations.

Results and Discussion

Extracts of *B. costatum* leaves by various solvents in order of polarity with yields and percentage recovery is shown in Table 1 below: The result of the extraction of the leaf of *B. costatum* has shown that the percentage yield of the crude extracts as shown in Table 1 indicates that methanol had the highest yield and hexane the lowest yield, implying that all the solvents extracted some components of the plant's material at varying quantity. This variation could be as a result of the differences in the solvent's polarity and the nature of the constituents present (Ajanal et al., 2012; Truong *et al*., 2019).

Table 1: Table 1: Solvent, Extracts' Nature and Percentage yield

Solvents	Extracts colour	Extracts texture	% yield
Hexane	Green	Soft	2.23
Ethyl acetate	Dark green	Soft	5.07
Acetone	Dark green	Soft	1.61
Methanol	Reddish brown	Soft	2.70

Table 2: Result for the Proximate Analysis for *B. costatum* leaves

S/No	Parameter	% Average
1	Moisture Content	5.91
2	Ash Content	14.42
3	Crude Protein Content	16.16
4	Crude Lipid Content	9.50
5	Crude Fibre Content	9.10
6	Carbohydrate Content	44.91

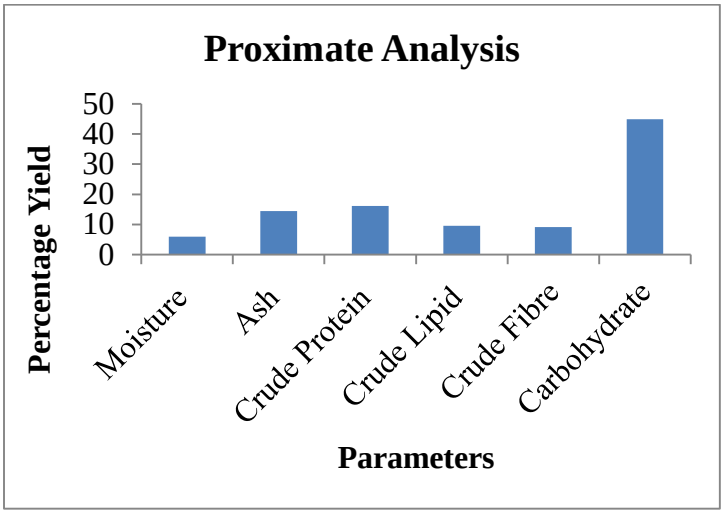

Fig 1: Chat showing the proximate analysis of *B. costatum* leaves.

Table 3a: Absorbance of *B. costatum* extracts and Standard at 517 nm UV-Vis Spectrophotometer

Concentration (mg/mL × 10^{-2})	Absorbance				
	Methanol	Acetone	Ethyl acetate	Hexane	Vitamin C
3.13	0.169	0.176	0.178	0.182	0.147
6.25	0.148	0.164	0.170	0.177	0.134
12.50	0.137	0.156	0.162	0.168	0.127
25.00	0.125	0.138	0.155	0.151	0.081
50.00	0.109	0.123	0.140	0.138	0.025

Table 3b: % Inhibition for Standard and *B. costatum* extracts

Concentration (mg/mL × 10^{-2})	% Inhibition				
	Methanol	Acetone	Ethyl acetate	Hexane	Vitamin C
3.13	15.08	11.56	10.55	08.54	26.13
6.25	25.63	17.59	14.57	11.05	32.66
12.50	31.16	21.60	18.59	15.58	36.18
25.00	37.19	30.65	22.61	23.61	59.30
50.00	45.23	38.19	29.64	30.65	87.44
IC_{50}	**57.56**	**71.22**	**76.08**	**87.94**	**22.52**

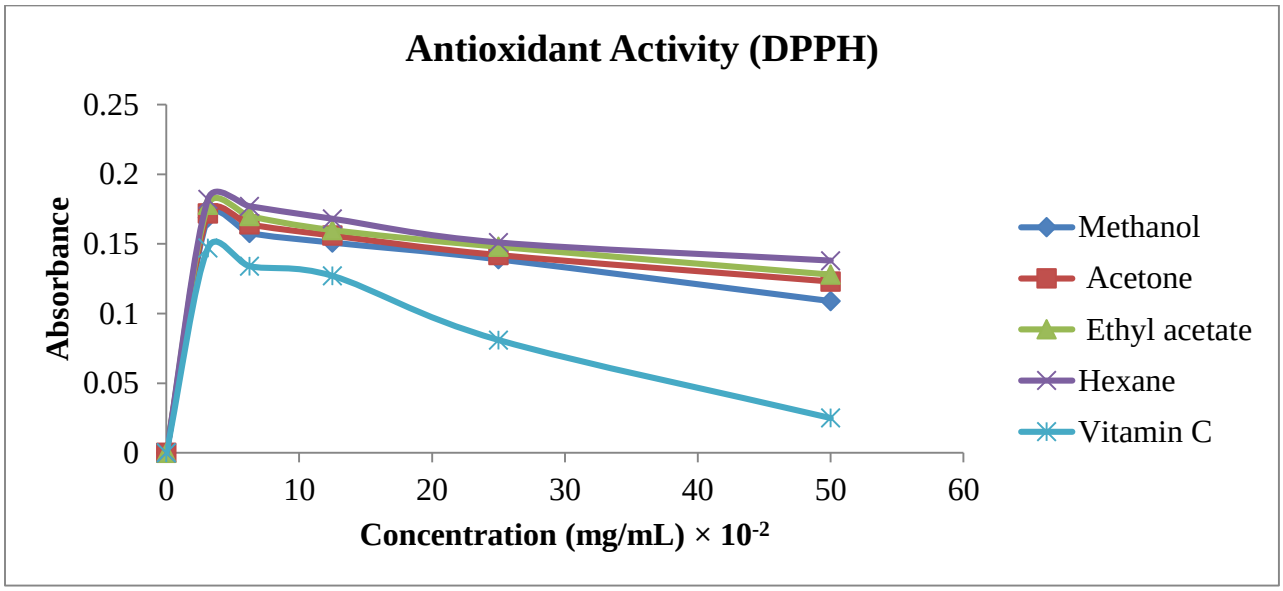

Fig 2: Chat showing the Absorbencies of Standard and *B. costatum* leaf Crude extracts.

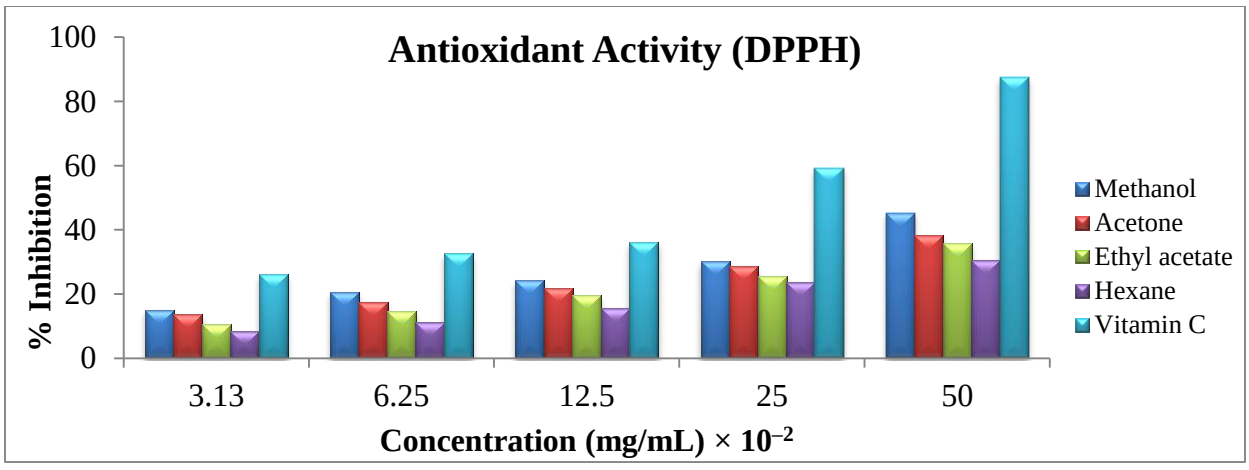

Fig 3: Chat showing the Percentage Inhibition of Standard and *B. costatum* leaf Crude extracts.

The result of extracts (Table 1) showed that all the four solvents used were able to extract some components of the plant leaves though at varying quantity. Ethyl acetate had the highest yield of 5.07 %, followed by methanol 2.70 %, hexane 2.23 % and the least was acetone with 1.61 %. The difference in the percentage yield could be as a result of the difference in polarity of the solvents, the nature of the constituents involved, variety of bioactive compounds and their differing solubility properties in different solvents (Ajanal et al., 2012; Truong et al., 2019). Methanol being a higher polar solvent was expected to have higher yield, rather, ethyl acetate had the highest in yield. This could be that the optimal solvent for extraction depends on the particular plant materials and the compounds to be isolated (Mahdi–Pour *et al*., 2012).

The result of the proximate composition of *B. costatum* leaves as shown in Table 2 & Fig 1 revealed that the sample had an average moisture content of 5.92 g/100 g. The low moisture content could give a longer shelf life and also ease transportation of the plant leaves (Aletan, 2019). The Ash content of 14.42 g/100 g indicates high mineral content and improved the nutritional quality (Fagbolun, 2012). The average crude protein content of 26.16 g/100 g indicates that the plant leaves are proteineous and could serves as a main source of food nutrient for the less privileged population in developing countries including Nigeria. Therefore, *B. costatum* leaves are plant protein supplement (Emebu, 2011). Crude lipid of 9.50 g/100 g is not too low as supplement for fats and oil in the body. The crude fibre of 9.10g/100g indicates that the sample is a good source of roughage in the diet. The available carbohydrate content could serve as fuel and energy that is required by the body for daily activities and exercise (Aletan, 2019). Adequate carbohydrate is also required for optimum function of the brain, heart, nervous system, digestive system and immune system while carbohydrate deficiency causes depletion of the body tissues (Offor et al., 2014). The health benefits of medicinal plants are attributed to the free radical scavenging potential of the active constituents (phytochemicals) present in these plants and can be used in health management for conditions which involves free radicals, such as cardiovascular and neurodegenerative diseases (Ushie et al., 2019; Kendeson et al., 2021).

The antioxidant activity was assayed using DPPH (1,1-Diphenyl-2-Picrylhydrazyl) radical scavenging method, which is an *in vitro* activity that involves quantification of free radical scavenging ability based on hydrogen or electron reduction (Kendeson et al., 2021). *B. constatum* leaf extracts of different solvents as presented in Table 3 a & b shows that the plant extracts exhibited various degrees of antioxidant activities as compared with Vitamin C - the Standard - as indicated by the IC_{50} values (Table 3 b). The result of the minimum inhibitory concentration (IC_{50}) shows that Vitamin C had the highest scavenging ability (22.52 mg/mL), while the crude extracts were in the order: methanol, highest (57.56 mg/mL), acetone (71.22 mg/mL), ethyl acetate (76.08 mg/mL), and hexane (87.94 mg /mL). These solvents extracts gave a wide difference and varying percentage of inhibition from that of the Standard. This could be as a result of the bioactive constituents present in each extract, factors relating to the experimental environment which may have caused changes in quantity and quality of action (Zahra *et al.*, 2019).

Conclusion

It was observed from the proximate analysis that the leaves extracts of *B. costatum* possess a rich source of nutritional value and could be a good source of diet and a as source of nutritional supplements in combating micronutrients deficiency both in human and other animals. Hence, there is the need to explore the potentials of this plant's leaves as a source of natural supplement and antioxidant as well as a precursor for antioxidant drugs development especially in the pharmaceutical industries. Also, the toxicity and anti-nutritional contents be properly investigated.

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