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PHYTOCHEMICAL SCREENING AND NUTRACEUTICAL PROPERTIES OF *Ceiba pentandra* LEAVES, ROOT, AND STEM BARK

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Abstract
The phytochemical, proximate and vitamin, obtained from *Ceiba pentandra* leaves, root and stem bark was investigated. The qualitative phytochemical screening showed the presence of saponins, flavonoids, coumarins, anthroquinones, phenols, glycosides, quinines, proteins, tannins, terpenoids, triterpenoid and alkaloids. The quantitative phytochemical analysis results showed that the leaves, stem bark and root contain alkaloid (7.4±1.00%), flavonoid (10.60±0.025), saponins (4.80±1.00%). Tannins (9.00±0.11%), phenols (8.10±2.00%), alkaloid (4.6 ±0.2), flavonoids (4.40±1.10), saponins (8.00±0.20), tannins (5.50±0.10), phenols (9.20±1.10), alkaloid (5.7±1.01), flavonoids (5.80±0.05), saponins (8.8±0.11%), tannins (5.8±0.12%), phenols (12.5±0.11) respectively. The result of the proximate composition of the leaves, root and stem bark are: moisture (15.20±0.01%, 13.00±1.00%, 13.53±0.12%), Ash (6.30±1.02%, 2.30±0.02%, 9.40±0.11%), crude fibre (15.80±0.12%, 21.01±0.05%, 19.15±0.05%), protein (13.10±0.03%, 7.65±0.05%, 10.97±1.60%), lipids (2.85±0.11, 3%.02±0.12%, 1.35±0.60%), carbohydrates (47.75±0.13%, 52.02±1.10%, 45.06±1.00%) respectively. The result of the vitamins of *Ceiba pentandra* leaves as quantified with HPLC are Vitamin: C 0.7465736ug/ml, Vitamin B: 10.26573600ug/ml, Vitamin B2: 9.278400ug/ml, Vitamin A: 0.00562762ug/ml, Vitamin E: 0.0014129ug/ml. These results indicate that *Ceiba pentandra* is a rich source of phytochemicals and nutrients with significant nutraceutical potential, its leaves and roots, in particular as supplements to address protein and mineral deficiencies. Further research on these components could lead to the development of therapeutic or nutritional supplements.

Keywords: *Ceiba pentandra*, phytochemical, Nutraceutical properties, Carbohydrate, Vitamins.

1. Introduction
Plants classified as medicinal are those whose extracts from the roots, seeds, or leaves are used to treat illnesses or preserve health (Lawal et al., 2019). Plants' ability to treat human physiological conditions is mostly dependent on the phytonutrients they contain (Edeoga et al., 2005). According to Ameh and Eze (2010), the investigation of biomolecules from plants are essential for the evaluation of compounds with potential benefits. Since the beginning of time, people have utilized plants to heal various illnesses (Barnes et al., 2007). Labe et al. (2020) highlighted that plants' phytochemical characteristics provide information about their potential medical uses. Medicinal plants are rich in bioactive substances with pharmacological qualities; the phytochemical componets include saponin, coumarins, alkaloid, steroids, phenolic compound, tannins, saponins, flavonoids, anthocyanins,

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glycosides and terpenes (Ahmed et al., 2019). *Ceiba pentandra*, also referred to as the Kapok tree or Silk-Cotton tree, is mostly found in tropical, intertropical, and subtropical continents in the globe. *Ceiba pentandra* plant belongs to Malvaceae family, which was split off from the Bombacaceae family, and the order Malvales (Friday et al., 2011; Alvarado et al., 2002). The herb has antispasmodic, diuretic, and emetic properties in traditional medicine (Lim, 2012). According to Elumalai et al. (2012), it can be also used for the treatment of skin conditions, painful eye illnesses, bronchitis, insect bites, digestive disorders (diarrhea and dysentery), hormonal diseases (diabetes), bone diseases (arthritis), and persistent fever. The flower is used to cure constipation and gonorrhea (Friday et al., 2011). The aphrodisiac properties of both the bark and the root cause men to chew them to get a powerful erection (Joy, 1998). The plant exhibits hypoglycemic (Djomeni et al., 2006), hepatoprotective according to Bairwa et al., (2010), hypolipidimic (Aloke et al., 2010), anti-inflammatory (Shah and Alagawadi, 2011), and anti-ulcerogenic (Mohamed et al., 2018) properties, according to an evaluation of the pharmacological properties of solvent extracts from different plant parts. According to ethnic groupings, it is known in Nigeria by several names, including Rimi (Hausa), Bamtami (Fulani), Arabaogungun (Yoruba), and Akpu-ogwu (Igbo) (Anigo et al., 2013).

Chemical substances has a lot of side effect on the users and many people have died while some have sustained many injuries in their bodies as a result of its usage. At present, greater fraction of the world populace today focuses on products from plant as well as those isolated from animal for treatment of diseases. Some individuals uses plant products in curing diseases (Sandhya *et al*., 2006). In the United Nations, most of the drugs used for treatment of various types of ailments are isolated from plant active compound originating from plants. Between 1983 and 1994, considering the drugs in use today for treatment majority are biosynthesized, while some are modified through chemical reactions from their origin (Yoder, 2005).

This has prompted the research work on the evaluation of medicinal potential of the leaves, root and stem bark of *Ceiba pentandra* plant which are known to be a common vegetable in various part of African countries. The aim of the research is based on isolating, characterizing, and assessing the phytochemical and nutraceutical properties of the leaves, stem bark, and roots of *Ceiba pentandra*

Materials and Method

Sample collection and preparation

The fresh and young parts of the plant such as the roots ,stembark and leaves were collected from agricultural farm in Uhuagu village Ezere, Awgu Local Government Area Enugu state in the month of September 2021.The specimen were identified at Center for Ethnomedicine and Drug Development (CEDD) in Nsukka by Dr. Alfred ozioko with voucher number intercede1064. The harvested plant parts were washed thoroughly with clean tap water, cut into small size and air dried at room temperature for three weeks (21days) and pulverized to fine powder using mechanical grinding machine, wrapped with newspaper, stored in air -tight container and kept in refrigerator until the time of extraction.

Procedure for the extraction

200g of the collected parts of the plant was extracted using soxhlet extractor fitted with 1000mL round botton flask, successive extraction was carried out using n-hexane, ethyl acetate and methanol based on their order of polarity the extraction process took 12h to get to completion. The extracts was collected in a separate container and concentrated in rotatory evaporators.

Phytochemical Screening

Test for Alkaloid: A few drops of Meyars reagent were added to 2 mL of the extract, followed by acidification with dilute hydrochloric acid (HCl). The appearance of a brown or yellow precipitate indicates the presence of alkaloids (Perekh and Chands, 2008).

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Test for Glycosides: To 1 mL of each extract, 0.5 mL of glacial acetic acid and 3 drops of 1% aqueous ferric chloride solution were added. The formation of a brown ring at the interface suggests the presence of glycosides (Perekh and Chands, 2008).
Test for Tannins: Adding a few drops of 5% w/v $FeCl_3$ solution to 2 mL of the ethanolic extract resulted in a green tint, indicating gallo tannins, or a brown tint, indicating faux tannins (Trease and Evans, 1989).
Test for Terpenoids: A reddish-brown precipitate formed when 0.5 mL of chloroform and a few drops of concentrated sulfuric acid were added to 1 mL of each extract, indicating the presence of terpenoids (Perekh and Chands, 2008).
Test for Saponins: The addition of 6 mL of distilled water to 2 mL of each extract, followed by vigorous shaking, produced bubbles or persistent foam, indicating saponins (Trease and Evans, 1989).
Test for Flavonoids: When 2 mL of each extract was mixed with a few drops of 20% sodium hydroxide, a bright yellow color appeared, which vanished after adding a few drops of 70% diluted hydrochloric acid. This color change indicates the presence of flavonoids (Trease and Evans, 1989).
Test for Coumarins: Adding 1 mL of a 10% NaOH solution to each extract produced a yellow color, indicating the presence of coumarins (Harborne, 1973).
Test for Anthraquinones: The addition of 2% HCl to 1 mL of each extract resulted in a red precipitate, indicating anthraquinones (Sofowara, 1993).
Test for Quinones: The presence of quinones was confirmed by the appearance of a red color upon adding 1 mL of saturated H_2SO_4 to 1 mL of each extract (Sofowara, 1993).
Test for Protein (xanthoproteic test): Nitric acid solution is applied dropwise to 1 mL of each extract. Protein was present as evidenced by the formation of yellow color (Sofowara, 1993).
Triterpenoid: Each extract was subjected to 1.5 mL of the Lieberman-Buchard reagent, which is a composition of acetic anhydride and hydrogen peroxide. Triterpenoid content is indicated by the formation of bluish-green color (Hashmi et al., 2021).

Quantitative Phytochemical Analysis

Alkaloid determination

In a 250 mL beaker, 5 g of the ground plant sample was mixed with 200 mL of a 10% ethoxyl ethane solution in ethyl alcohol. The mixture was covered and allowed to stand for 4 hours. After filtration, the filtrate was concentrated to a quarter of its original volume using a water bath. Concentrated NH4OH was then added drop by drop to precipitate the alkaloids until the precipitation process was complete. After centrifuging the whole mixture, the supernatant was removed, cleaned with diluted NH4OH, and filtered. Total alkaloids were determined by drying the residue to a consistent weight (Harborne, 1973).

Determination of Flavonoids

For one hour, 100 mL of 80% aqueous methanol was refluxed with 10 g of plant material. The mixture was filtered through Whatman No. 1 filter paper into a pre-weighed 200 mL evaporating dish. The filtrate was then evaporated to a constant weight and weighed (Bohm and Kocipai-Abyazan, 1974).

Estimation of Total Phenols

To extract the phenolic compounds, 5 g of the defatted sample was heated with 50 mL of ether for 15 minutes. Then, 5 mL of the extract, 2 mL of NH_4OH solution, and 5 mL of concentrated amyl alcohol were combined with 10 mL of distilled water. The mixture was topped up to 50 mL and allowed to develop color for 30 minutes. After which a UV-VIS Spectrophotometer set to 505 nm was used to determine the optical density of the combination (Edeoga et al., 2005).

Determination of Tannins

A 0.5 g sample was shaken with 50 mL of distilled water in a mechanical shaker for one hour. The mixture was then filtered into a 50 mL volumetric flask, and the volume was adjusted accordingly. To 5 mL of the filtrate, 2 mL of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide were added. The mixture was left to stand for 10 minutes to allow for color development, after which the absorbance was measured at 120 nm (Van-Burden and Robinson, 1981; Prasad et al., 2008).

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Saponin Determination

Hot water bath was used to heat 20 g of powdered material and 100 mL of 20% aqueous ethanol for four hours, with continuous stirring at around 55°C. After filtering the mixture, an additional 200 mL of 20% ethanol was used to extract the residue. The combined extracts were then concentrated to 40 mL by evaporation over a water bath at approximately 90°C. The concentrate was partitioned with 20 mL of diethyl ether, discarding the ether layer and collecting the aqueous layer. The partitioning was repeated twice with 60 mL of n-butanol. The n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The saponin content was determined as a percentage after the residual solution was evaporated to a constant weight (Obdoni and Ochuku 2001).

Proximate Analysis

The AOAC (2005) approach was utilized for detailed examination of *Ceiba pentandra* leaves, roots, and stembark.

Moisture Content Determination

Clean beaker was dried in an oven at 105°C for one hour, and then allowed to cool in a desiccator. The initial weight of the empty beaker was recorded (Wa), followed by its weight after adding 2 g of the sample (Wb). The beaker was then dried in the oven at 105°C until it reached a constant weight. After cooling in a desiccator, the final weight was measured (W3). The % moisture content in the sample was determined using the equation

Percentage (%) moisture content = $\frac{(W_b - Wa)}{(W_c - W_a)} \times 100\%$

W_a = mass of empty aluminium dish, W_b = mass of dish + sample before drying

W_c = mass of beaker + sample after drying.

Ash Content determination

2g of the ground sample were placed into the crucible, which was then weighed (W1). The crucible was cleaned thoroughly and weighed again (W1). The sample was then allowed to cool for ten hours, during which time it turned into a whitish-grey ash, and the ash was weighed again (W3). The ash content percentage of the samples was determined using the equation.

Percentage (%) Ash content $= \frac{W_c - Wa}{W_b - W_a} \times 100\%$

Whence: W_a= mass of empty crucible W_b = mass of empty crucible + sample before ashing, W_c = mass of crucible ash in grams.

Determination of Crude Fiber

A 50 mL solution of trichloroacetic acid (TCA) was added to a round-bottom flask containing two grams of the sample to determine its crude fiber content. The mixture was then heated to boiling and refluxed for forty minutes. After cooling to room temperature (27^{o}C), filter paper was used to separate the residue. The residue underwent four hot water washes and one petroleum ether wash. The filter paper holding the residue was then dried in an oven at 50°C for 24 hours. The sample was weighed, then heated to 650°C to measure the ash content and determine the crude fiber proportion.

Percentatge crude fiber = $\frac{W_b - W_c}{W_a} \times 100\%$

W_a = mass of sample

W_b = mass of ash + crucible

W_c = mass of empty crucible

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Determination of Crude Protein

The crude protein concentration of the samples was determined using a Kjeldahl tablet. Two grams of the sample were placed in a digestion tube, and 20 mL of concentrated sulfuric acid (H_2SO_4), 2 Kjeldahl tablets, and the sample were added. The mixture was digested for 5 hours at 420°C. After cooling, 90 mL of distilled water was added to the digested solution. Approximately 50 mL of 40% caustic soda (NaOH) was then added, and the mixture was placed on a heating mantle in a distillation chamber. To capture ammonia, a conical flask containing 30 mL of 4% boric acid with bromocresol green and methyl red indicators was positioned under the distillation chamber, resulting in a color change from orange to green. The solution was titrated with 0.1 M hydrochloric acid (HCl) until the color turned pink, and the burette reading was recorded. The (Cp) was then determined using the appropriate equation:

$$\% \, Cp = [(A-B) \times M \times F \times 6.250 / \text{ mg of samples}] \times 100 \%$$

Whence A = volume (milliliter) of acid used for titrating the sample, B =volume (mL) of acid used for titrating blank sample (O); M = Molarity of the acid used for the titrimetric analysis.

RESULTS

Table 1; Qualitative phytochemical screening of *Ceiba pentandra* leaves

Phytochemicals	n-hexane extract	Ethly acetate extract	Methanol extract	Aqueous extract
Saponins	+	++	++	+++
Flavonoids	+	+++	++	+
Coumarins	-	+++	++	+
Anthroquinones	+	++	-	++
Phenols	++	++	+	+
Glycosides	-	++	+++	+++
Quinines	-	+	++	+++
Proteins	-	++	-	-
Tannins	+++	+++	++	-
Triterpenoids	+++	++	++	-
Alkaloids	++	+++	+	++

Key: - = Not detected + = scarcely present++ = moderately present +++ = abundantly present

Table 2: Results of qualitative phytochemical screening of *Ceiba pentandra* stem bark

Phytochemicals	n-hexane extract	Ethly acetate extract	Methanol extract	Aqueous extract
Saponins	-	+	++	+++
Flavonoids	++	-	+	+
Coumarins	++	-	+	++
Anthroquinones	+	+++	++	+
Phenols	-	+	+++	-
Glycosides	+	+++	+++	+
Quinines	+	++	++	+
Proteins	++	+	-	-
Tannins	+	++	+	-
Triterpenoids	++	-	-	-
Alkaloids	+++	++	+++	+++

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Table 3: Results of qualitative phytochemical screening of *Ceiba pentandra* roots

Phytochemicals	n-hexane extract	Ethly acetate extract	Methanol extract	Aqueous extract
Saponins	-	+	++	+++
Flavonoids	+++	++	+	+
Coumarins	++	+	++	+
Anthroquinones	+++	++	+	-
Phenols	+	+++	-	-
Glycosides	+	+++	++	+
Quinones	+	+++	++	+
Proteins	++	+	+++	+
Tannins	+++	+	-	-
Triterpenoids	-	-	+	-
Alkaloids	++	++	+++	+++

Key: - = Not detected + = scarcely present++ = moderately present +++ = abundantly present

Table 4: Result of quantitative phytochemical analysis of leaves, stem barks and roots *Ceiba pentandra*

Phytochemicals	Leaves	Stem barks	Roots
Alkaloid (%)	7.40 ± 1.00	4.60 ±0.20	5.70 ±1.01
Flavonoids(%)	10.60 ±0.02	4.40 ±1.10	5.80 ±0,05
Saponin (%)	4.80 ±1.00	8.00 ±0,20	8.80 ±0.11
Tannin (%)	9.00 ±0.11	5.50 ±0,10	5.80 ±0.12
Phenol (%)	8.10 ±2.00	9.20 ±1.10	12.50 ±0.11

Analysis was done in triplicate and result presented as Mean ± SD

Table 5: Result of proximate analysis of leaves, Stem barks and roots *Ceiba pentandra.*

Proximate composition (%)	Leaves	Stem barks	Roots
Moisture	15.20 ±0.01	13.00 ±1.00	13.53±0.12
Ash	6.30 ±1.02	2.30±0.02	9.40±0.11
Crude fibre	15.80 ±0.12	21.01±0.05	19.15±0.05
Protein	13.10 ±0.03	7.65±0.05	10.97 ±1.60
Lipids	2.85 ±0.11	3.02±0.12	1.35± 0.60
Carbohydrates	47.75 ±0.13	52.02±1.10	45.06 ±1.00

Analysis was done in triplicate and result presented as Mean ± SD

Table 6: Results of Vitamins in *Ceiba pentandra* leaves quantified with HPLC

Vitamins	Concentration (ug/ml)	Retention time (min)	Peak area (mAU*s)	Conc./Area
C	0.7465736	1.39	3.91002	0.190952
B1	2.6573600	1.07	15.71666	0.169079
B2	9.2787400	1.78	4.82521	1.92297
B3	ND	1.32	ND	ND
A	0.00562762	1.30	0.817189	0.00688656
E	0.0014129	3.34	0.0812922	0.0173806

ND = not detected

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Discussion

Plants, as natural products, are a significant source of potentially valuable therapeutic agents. (Tona et al., 1998). Chemical substances derived from Plant have gained substantial interest recently due to their diverse applications. Medicinal plants are a valuable source of bioactive compounds used in traditional medicine, modern pharmaceuticals, nutraceuticals, dietary supplements, folk remedies, pharmaceutical intermediates, and as chemical building blocks for synthetic drugs (Ncube et al. 2008). The biological and pharmacological potential of numerous plants remains unexplored. Globally, scientists are investigating the potential of pharmacologically active compounds from medicinal plants (Karmegam et al., 2012). Herbal medicinal plants have been in use in various parts of the globe this is because they have low level of side effect, highly effective, affordable and they do not act as narcotic substances (Ahmad and Beg, 2001)

The phytochemical screening of *Ceiba* pentandra leaf, root, and stem bark extracts using n-hexane, ethyl acetate, and methanol revealed the presence of flavonoids, coumarins, triterpenoids, proteins, saponins, anthraquinones, quinones, phenols, alkaloids, tannins, and glycoside. These compounds are known for their therapeutic activity against disease-causing pathogens, suggesting their potential pharmacological use in developing new health-beneficial compounds (Muthukumaran et al., 2017**).** Phytochemicals that are secondary metabolites from plant serves as defense mechanism against several microorganisms (Paliwal et al., 2011). The quantitative phytochemical analysis of *Ceiba pentandra* is presented in Table 1-4. The findings from this study showed that the leaves contain more alkaloid compared to the stem bark and root, this contradicts the results of Labe *et al.,*2020 who reported that alkaloid is absent in both leaves ,root and stem bark of *Ceiba pentandra* but agrees with the findings of Iroka et al.(2014) who reported that the alkaloid content of the leaves ,stem bark and roots of *Ceiba pentandra* are 6.32%,3.79% and 0.76% respectively. Alkaloids serve as essential medicinal agents with analgesic, antispasmodic, and antibacterial properties (Stray, 1998). Plants containing alkaloids are commonly used in medicine to alleviate headaches and fever (Labe et al., 2020). Alkaloid have anti-inflammatory properties (Kuras et al., 2009). Flavoinoids are reported to be predominant (10.60%) in the leaves of *Ceiba pentandra*. This supports the result of findings of Labe *et al*., (2020) Flavonoid are natural occurring substances from plant. They have been found to possess therapeutic properties, including anticancer, antioxidant, antibacterial, cardioprotective, anti-inflammatory, immune-boosting, and skin-protective effects against UV radiation (Kumar, 2013). Flavonoid lowers the risk of heart diseases (Okwu, 2004). The amount of saponin in the root is higher than the leaves and stem bark. Saponin is used in the production of shampoo,insecticides, synthesis of steroidal hormone such as cortisone and estrogenic contraceptive (Dubrovsky,2005; Okeke and Nwachukwu 2009), phenols are present in both leaves ,stem bark and root of the plant the root has more phenol than other parts of the plant phenols has antimicrobial activity and are used in frormulating germicides, disinfectants and antiseptics (Hogan,2008).

The proximate analysis of *Ceiba pentandra* (Table 5) shows the result of the basic nutritional components of the leaves, stembark and roots of the plant. Carbohydrate has the highest proximate content in the leaves, stem bark and root. Carbohydrates are metabolized in the body to generate glucose, which can either be utilized right away or stored as glycogen in the muscles and liver for later energy use. The protein content is highest in the leaves, with the stem bark containing the lowest amount of protein. Proteins are essential for body repair, tissue replacement, immune support, and aiding cell growth and division (Okeke and Elekwa, 2006). Among the proximate contents, the root of *Ceiba pentandra* has the lowest lipid level. Fats are vital for energy production, and they also help regulate blood pressure and support cellular function (Dutta, 1981). The leaves have the highest moisture content, followed by the root. Moisture acts as a universal solvent, carrying nutrients throughout the body and enabling organs to function properly. Fiber contributes to lowering blood cholesterol and helps prevent colon cancer and cardiovascular diseases (Boutwell, 1998).

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Vitamins

The vitamin content in the leaves was quantified using high-performance liquid chromatography (HPLC) and shown in Table 6. The result showed that water-soluble vitamins are largely present in the leaves than fat soluble vitamins, the most abundant vitamin is vit.B2 with a concentration of 9.2787400ug/ml followed by Vit.B1 (2.657360ug/ml). The least vitamin found in the leave is vitamin E at a concentration of 0.0014129ug/ml while vitamin B3 was not detected in the leaves of *Ceiba pentandra*.

Conclusion

The medicinal properties of plants are largely due to their secondary metabolites. Phytochemical analysis of the leaves, root, and stem bark of *Ceiba pentandra* showed the presence of alkaloids, saponins, flavonoids, tannins, and phenols, each in different concentrations. The plant is also rich in proximate and vitamin content, with its leaves and roots showing strong potential to supplement protein and mineral deficiencies commonly found in developing countries. It should be incorporated into our diet in order to improve its quality and there by improve the overall health and general wellbeing of people.

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