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Phytochemical Assessment of The Extracts of Stem (Bark) and Leaves of Theobroma Cocoa Materials: Experimental Procedure and Its Comparison to Literature

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ABSTRACTS

Plants had already long been utilized as medicines to treat illnesses. Theobroma cocoa is a plant with antifungal, antibacterial, antioxidant, antiemetic, larvicidal, hepatoprotective, anti-diarrheal, anti-inflammatory, antimalarial, anthelmintic, antiarthritic, wound healing, and anticarcinogenic potential. The purpose of this study was to look into the phytochemical compositions of crude extracts of Theobroma cocoa leaves and stem (bark) materials. Carbohydrates, saponins, and phlobatannins were found in higher concentrations in both the stem (bark) and the leaves, whereas tannins, glycosides, resins, and alkaloids were found in lower concentrations in both samples. Depending on the solvent used for extraction, different phytochemical compositions are obtained in each part of the tree. However, for the majority of the phytochemicals, water extraction yielded higher concentrations.

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1. INTRODUCTION

Cocoa, botanically known as Theobroma cocoa, is a tropical and subtropical plant that belongs to the genus Theobroma in the Sterculiaceae family. There are over 20 recognized Theobroma species. Some of these species are found in Africa, most notably in Nigeria, Cameroon, Sierra Leone, Ghana, and Equatorial Guinea, while others are found in Asia, including Brazil, Central America, and the Caribbean Islands. Cocoa originated in Mexico and was introduced into Africa in the sixteenth century, eventually making its way to Nigeria. Since 1887, when cocoa seedlings from the old botanic Garden at Ebute Metta (Lagos) were sent to Ibadan for trial, the Nigerian government has been interested in cultivating cocoa. This explains why cultivation was first recognized in Ibadan, Oyo State, Nigeria, and later spread to other Western Nigerian states such as Ondo, Ekiti, and Ogun.

Phytochemicals are a large number of plant-derived substances that are believed to be caused much of the disease protection provided by diets high in fruits, vegetables, beans, cereals, and plant-based beverages such as tea and wine. Phytochemicals are classified into tannins, flavonoids, glycosides, saponins, alkaloids, triterpenoids, and sterols based on their chemical structure (Tiwari *et al*., 2011; Yang *et al*., 2021). The leaves, stem bark, and roots are the most commonly used plant parts. The roots are dried, dissolved in water, and then taken orally to treat malaria and venereal diseases, as well as stomachache and premenstrual syndrome symptoms. The stem bark is also crushed, soaked in water, and taken orally to treat diarrhea, as well as applied to help treat abscesses and a variety of other skin lesions (Kabuka *et al*., 2022).

The leaves' extracts have been reported to have a variety of ethnomedical applications. Some authors have reported on the properties of Alkaloids as well as the anti-inflammatory and anti-bacterial properties of tannins (Akinmoladun *et al*., 2007). Flavonoids have also been shown to have diuretic, antibacterial, and antifungal properties (Mallikharjuna *et al*., 2007). It has been proposed that the phytochemical compounds extracted from the crude extracts of these plants could be useful drug sources. Another study on the phytochemical screening of Strychnos potatorum L., a medicinal plant - conducted by (Mallikharjuna *et al*., 2007) revealed that the root, stem (bark), and the seed of this plant contain alkaloids, flavonoids, glycosides, saponins, phenols, and sterols.

Several authors (Akinmoladun *et al*., 2007, Mallikharjuna *et al*., 2007, Ayeni and Yahaya, 2010, Abdulgafar *et al*., 2011) have worked on various plant species to detect the phytochemical compound that they contain. None have been tested on Theobroma cocoa. As a result, it is necessary to work on it. Despite the widespread use of cacao, there is little information on the nutrients and phytochemistry composition of the stem and leaves; thus, the current study focuses on the phytochemical study of the cocoa leaves and stem (bark). The findings are expected to add to our understanding of phytochemistry.

2. METHODS

Fresh cocoa (cacao) leaves and stem (bark) were collected in Ipinsa, Ondo State, Nigeria (see **Figure 1**). Both were rinsed with clean water and sun-dried separately for three weeks before being chopped with a mortar and pestle and ground into a fine powder with an electric blender and stored in airtight sample containers pending laboratory analyses. C1 (leaves) and C2 stem labels were applied to the samples (bark).

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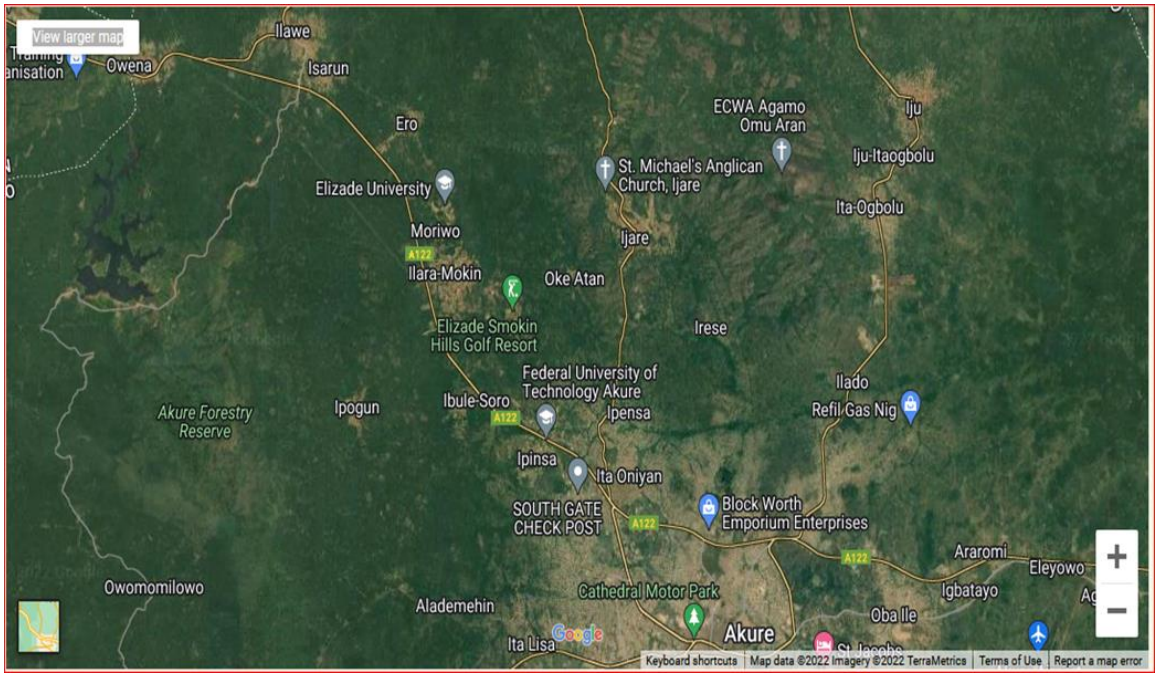

Figure 1. The sampling location in Ipinsa, Nigeria.

For the experimental procedure, we did the following protocols. Water, chloroform, and ethanol were used to extract the ground cocoa leaves and stem (bark). Extracts of leaves and stem (bark) samples were prepared by soaking 10g of each powdered sample in 750cm3 of each solvent (ethanol, distilled water, and chloroform) and shaking for 3 hours with a mechanical shaker. Filter papers were then used to filter the extracts (Abulude, 2001). Before analysis, the filtrates were stored in various plastic containers at room temperature. Different images of the samples are shown in **Figure 2**.

Seeds wet, dried, and powder (chocolate)

Pod, stem and leaves

Fresh leaves

Figure 2. The different parts of the Cocoa (Theobroma cacao L).

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Phytochemical analyses were done in the following:

(i) Test for Carbohydrates using Fehling's Test. In a test tube, 5 mL of Fehling A and B mixture was added to 2 mL of each extract. The mixture was then boiled for 2 minutes. Copper oxide precipitation in the form of bricks was observed.

(ii) Test for Tannins. To 1 ml of extract, two drops of FeCl3 (5%) were added. Each extract produced a dirty-green precipitate.

(iii) Test for Glycosides. In a test tube, 10 mL of H2SO4 (50%) was added to 1 mL of each extract. For 5 minutes, the mixture was immersed in boiling water. Fehling solutions A and B (10 mL) were added and boiled. A brick-red precipitate was observed, indicating the presence of glycosides.

(iv) Test for Saponins using a frothing test. In a test tube, 2 mL of each extract was vigorously shaken for 2 minutes. There was frothing, indicating the presence of saponins.

(v) Test for Resins. Each extract received a 5 mL of copper addition. The resulting solution was vigorously shaken and allowed to separate. A green precipitate was observed, indicating the presence of resin.

(vi) Test for Phlobatannins. 5 mL of distilled water was added to 5cm3 of each extract and boiled for 2 minutes with 5 mL of HCl (1%). There was no visible reaction, indicating the absence of phlobatannins.

(vii) Test for Flavonoids. 2 mL of extract was heated in a water bath with 10cm3 ethyl acetate and cooled. The layers were separated and the color of the NH3 layer was noted (red coloration formed).

(viii) Test for Alkaloids. In each test tube, 1 mL of HCl (1%) was added to 3 mL of extract. A few drops of Mayer's reagent were added to each extract. The presence of alkaloids was indicated by the presence of a creamy white precipitate.

3. RESULTS AND DISCUSSION

Table 1 shows the phytochemical results of the stem (bark) and leaves of cocoa (Theobroma cacao). It shows that tannins, carbohydrates, glycosides, saponins, resins, phlobatannins, and alkaloids were present in the two samples. From **Table 1** and **Table 2**, it could be observed that both stem (bark) and leaves have a higher concentration, while glycosides are slightly present in leaves than in stem (bark). The test also revealed that both leaves and stem (bark) have equal concentrations of saponins, while resins are in higher concentration in stem (bark) than in leaves. Finally, the test for phlobatannins shows that the concentration is very high in both leaves and stem (bark) while the test for alkaloids showed higher concentration in leaves than stem (bark).

The results were similar to those elsewhere (Madziga *et al*., 2010; Abulude *et al*., 2014; Adi-Dako *et al*., 2016; Nas and Ali, 2017; Sahrawat *et al*., 2018; Isah and Aminu, 2019; Kabuka *et al*., 2022). A detailed comparison is shown in **Table 3**. Saponins occur constitutively in a great many plant species, in both wild plants and cultivated crops. In cultivated crops, the tripterperiod saponins are generally predominant, while steroid saponins are common in plants used as herbs or for their health-promoting properties. Saponins may be considered a part of plants' defense systems, and as such have been included in a large group of protective molecules found in plants (Morrissey and Osbourn, 1999). Some saponins have also been found to have anti-oxidative or reductive activity. A group of saponins produced in legumes, namely, group B soya saponins, contain an antioxidant moiety attached (Yoshiki *et al*., 1998). Many saponins are known to be antimicrobial to inhibit molds and protect plants from insect attacks. Saponins have high toxicity against fungi (Delmas *et al*., 2000).

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Table 1. Phytochemical results of the Cocoa extracts.

Sample	Carbohydrate	Tannins	Glycoside	Saponins
C_1 (Ethanol)	+	+ +	-	-
C_2 (Ethanol)	+ +	+ + +	-	-
C_1 (H_2 O)	+	+	+ +	+ + +
C_2 (H_2 O)	+ + +	+ +	+	+ + +
C_1 (Chloroform)	+ + +	-	-	+
C_2 (Chloroform)	+ + +	+ +	-	+

Note: C1 is the Leaves, C2 is the stem (bark), + is the low value, + + is the moderate value, + + + is the high value, and - is the absent.

Table 2. Phytochemical results of the Cocoa extracts.

Sample	Resins	Phlobatannins	Flavonoids	Alkaloids
C_1 (Ethanol)	+	+	-	+
C_2 (Ethanol)	+ +	+	-	+ +
C_1 (H_2 O)	-	+	-	+ + +
C_2 (H_2 O)	-	+	-	+
C_1 (Chloroform)	+ +	+ + +	-	-
C_2 (Chloroform)	+ + +	+ + +	-	-

Note: C1 is the Leaves, C2 is the stem (bark), + is the low value, + + is the moderate value, + + + is the high value, and - is the absent.

Table 3. Comparisons of this study's results with other studies.

Plants	Plant Parts	Extraction Types	Results (Phytochemicals)	References
Cocoa (*Theobroma cacao* L)	Leaves and stem	80% Ethanol, Chloroform, Distilled Water	Absence of flavonoids. Tannins, glycosides, paleobotanies, and alkaloids were absent in chloroform extractions. Resins were absent in water extractions. Carbohydrates, tannins, flavonoids. glycosides, paleobotanies, and alkaloids were present in all the extractions	This study
Cassia abbreviata	Roots, leaves, and stem bark	Ethanol (96% v/v) and distilled water	High concentrations of tannins, reducing sugars, and sterols are found in the stem bark and roots. Flavonoids, phenolics, and proteins were abundant in the stem bark, whereas anthraquinone, glycosides, and alkaloids were found in the leaves and roots, respectively.	Kabuka *et al*. (2022)
Theobroma cacao L	Cocoa pod husk	Hot water soluble and hot aqueous citric acid-soluble extracts	There was a good presence of tannins, alkaloids, and saponins.	Adi-Dako *et al*. (2016)

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Table 3 (continue). Comparisons of this study's results with other studies.

Plants	Plant Parts	Extraction Types	Results (Phytochemicals)	References
Azadirachta indica and *Dodonea viscosa*	Leaves	Methanol, petroleum ether extract, and distilled water	Saponins and phenols were found in all of the extracts. Tannin was present in all of the extracts except the *A. indica* leaves extract (methanolic and petroleum ether). Only *A. Indica* methanolic and *D. viscosa* (methanolic and petroleum ether) leaves extracts contained flavonoids. Except for the *A. indica* methanolic extract, all of the extracts contained volatile oil. Glycoside was found only in *A. Indica* aqueous extract and *D. viscosa* methanolic extract. Steroids were found in the extracts of *A. indica* and *D. viscosa* (methanolic and petroleum ether) (aqueous and petroleum ether extract). Terpenoids were present in all extracts except *A. indica* (methanolic and petroleum ether) and *D. viscosa* (methanolic and petroleum ether) (petroleum ether extract)	Isah and Aminu (2019)
Flamboyant tree (*Delonix regia*)	Roots, bark, and leaves	Water, ethyl acetate, and ethanol extracts.	A simple quantitative method was used to identify tannins, saponins, carbohydrates, glycosides, resins, paleobotanies, alkaloids, and flavonoids in the samples.	Abulude *et al*. (2014)
Azadirachta Indica	Stem bark and leaves	Methanolic and aqueous	Both stem bark and leaf extracts contained alkaloid, tannin, anthraquinone, flavonoid, phenols, and terpenoid, according to preliminary phytochemical analysis.	Nas and Ali (2017)
Azadirachta indica	Leaves	95% EtOH and distilled water	The ethanolic and aqueous extracts of *A. Indica* contained phenolic compounds, tannins, and steroids, while the aqueous extract contained flavonoids, phenolic compounds, tannins, saponins, and alkaloids.	Hikaambo *et al*. (2022)
Acalypha wilkesiana	Leaves	Aqueous	Carbohydrates, Tannins, and flavonoids were found in high concentrations, while Paleobotanies and Saponins were found in low concentrations. Alkaloids, cardiac glycosides, and trace amounts of terpenes and steroids There were no anthraquinone derivatives present.	Madziga *et al*. (2010)
Azadirachta Indica	Leaves	Ethyl acetate, acetone, butyl ethanol, benzene, and toluene	Plant extract phytochemical analysis revealed positive results for saponins, tannins, phenols, proteins, glycosides, terpenoids, carbohydrates, flavonoids, and alkaloids.	Sahrawat *et al*. (2018)

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4. CONCLUSION

This research work has revealed the potential of the stem (bark) and leaves of cacao in the area of pharmacology as sources of useful drugs. Therefore, this study has provided some biological ground for ethnopharmacological uses of the leaves and stem (bark) of cacao in the treatment and prevention of some diseases in humans including plants and animals. Since the result of the phytochemical screening has shown that the extracts of the two samples contain carbohydrates, saponins, phlobatannins, tannins, glycosides, resins, and alkaloids, it is recommended that these extracts should be used in the production of drugs in the area of pharmaceutical.

5. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. Authors confirmed that the paper was free of plagiarism.

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