

Characterization, Phytochemical Screening and Antimicrobial Properties of stem bark extract of *Theobroma cacao* (Cocoa)

Timilehin Francis Olaleye¹, Oluwagbenga John Ogunbiyi^{2*}

¹Dept. of Industrial Chemistry, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria & Dept. of Chemistry, Faculty of Science, Air Force Institute of Technology, Kaduna, Nigeria

²Biology Unit, Faculty of Science, Air Force Institute of Technology, Kaduna, Nigeria

*Corresponding Author: 2rayacoa@gmail.com, Tel: +2347030076292

Available online at: www.isroset.org

Received: 05/Aug/2022, Accepted: 10/Oct/2022, Online: 31/Dec/2022

Abstract–*Theobroma cacao* (cocoa) is one of the medicinal plants that most of its properties is yet to be fully unravel as it is underutilized in most part of the countries. Several reports have focused on few of its uses in the area of ethno-medicine and folk medicine. The aim of this study is to characterize the phytochemical screening and antimicrobial properties of *Theobroma cacao* stem bark extract. The phytochemicals were extracted from dried milled stem bark of cocoa (*Theobroma cacao*) using solvent extraction method. The results showed the presence of alkaloids, tannins, saponins, glycosides, phenols, carboxylic acids and flavonoids. Antimicrobial acid metabolite indicates the presence of micro-organisms such as *Escherichia coli*, *Streptococcus pneumonia*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, while the neutral and basic metabolites activities indicates nil-potential towards same micro-organisms. The proximate analysis of cocoa stem bark extract was also evaluated and the antimicrobial activities of the extract were compared with standard antibiotics.

Keywords–Cocoa, Stem bark, Phytochemical, Antimicrobial, Metabolites, Characterization.

I. INTRODUCTION

Theobroma cacao, commonly referred to as cocoa is a tropical plant usually grown in some part of Africa, most importantly in South-Western part of Nigeria including the states such as Ondo, Ogun, Oyo, Osun and Ekiti. The broad-leaved, evergreen crop grows between 20^oC North and South of the equator and reaches a height of around 17 meters. Its fruit are fibrous pods that range in length from 15 to 20 cm, have a diameter of 7.5 to 10 cm, and contain 20 to 40 seeds, or "beans," that are each approximately 2.5 cm long and embedded in a sweet-tart pulp [1],[2]. If the proper cocoa is grown, raised in a seed bed that is planted in the proper kind of soil, and shaded sufficiently, cocoa farming can be financially successful. Procyanidins, theobromine, caffeine, catechins, and (-)-epicatechin are all abundant in cocoa [3],[4],[5],[6]. It is properly pruned, weeded, mulched, fertilized, and protected from disease and pest attacks as it grows. It is also properly collected and processed. Although it is consumed and processed in temperate nations, cocoa is a cash crop that is mostly grown in tropical regions [7].

Phytochemicals are biologically active chemical compounds that occur naturally in plants [8]. Several plants have been reported to contain phytochemical constituents which corroborate their usefulness in phytomedicine [9],[10],[11],[12]. In recent time, some underutilized plants used as chewing sticks in Nigeria has been reported to contain some phytochemical constituents as well as antimicrobial properties which support its

usefulness [13]. The phytochemical screening of cocoa stem bark has shown that the stem bark of this plant is composed of the major ingredients of the alkaloids, tannins, flavonoids, saponins, polyphenols, glycosides, carboxylic acids [14],[15],[16],[17],[18].

Cocoa seed is used for treatment of infections such as asthma, bronchitis, diarrhea, intestinal diseases as well as an expectorant for lung congestion. The seed coat is used in the treatment of bladder, kidney and liver ailments. More so, it is employed in the diabetic treatment and as a general remedy [19],[20],[21],[22]. Cocoa butter is a good source of cholesterol and supplement that is used as a component of body cream and lotion to take care of the skin. Report has shown that about 80 percent of the world's population rely mainly on the use of plant extract [23]. However, despite the fact that cocoa is widely used, little is known about the nutrients and phytochemistry of the stem bark. For this reason, the current study focuses on the phytochemical analysis of the cocoa stem bark and assess the antibacterial potency of some identified metabolites. We anticipate that the discoveries will deepen our understanding of phytochemistry while the identified antibacterial properties will elucidate the importance of the stem bark of cocoa in ethno-medicine and its usage in folklore medicine.

II. RELATED WORK

Recently, phytochemical assessment of the extracts of stem (bark) and leaves of *Theobroma cacao* materials was

evaluated using certain experimental procedures and comparison were done with literatures [24]. Result of the phytochemical screening obtained from this work revealed that the extracts from the two samples contain alkaloids, carbohydrates, saponins, phlobatannins, tannins, glycosides, and resins. It was suggested that these extracts may be employed in the pharmaceutical industry to produce medications [24].

III. MATERIALS AND METHODS

Plant material

Fresh dried cocoa (*Theobroma cacao*) stems barks were obtained from Ogbuete market, Enugu, Enugu State while a taxonomist at the Department of Applied Biology, Ebonyi State University (EBSU), Abakaliki authenticated the sample. These dry cocoa stem barks were further solar-dried under favorable conditions and pulverized to powder using an electric grinding machine (milled into fine particles). The milled sample was then packaged in a moisture proof polythene bag in a cool and dry environment.

Extract preparation

The powdered stem bark of cocoa (about 200 g) was soaked in 1000 ml of Chloroform (CHCl₃) for 72 hours. The chloroform was allowed to evaporate completely under the influence of increased temperature (heat) to give a brownish gel like solid which was dissolved in 30 ml of chloroform and 10 ml of distilled water. The filtrate was used for the antimicrobial experiment and preparation of basic, acidic and neutral metabolites. The brownish crude that was formed was used for acidic, basic and neutral medium for further antimicrobial analysis.

Phytochemical screening of this filtrate revealed the presence of flavonoids, alkaloids, saponins, tannins, glycosides etc.

Preparation of basic metabolites

The previously produced chloroform filtrate was combined with 20 ml of diluted HCl, 30 ml of chloroform, and a separating funnel. The mixture was shaken with periodic pressure releases before being allowed to settle for roughly an hour. In order to prepare neutral metabolites, the bottom chloroform layer was removed. 1 M sodium hydroxide (NaOH) solution was applied to the hydrochloric acid (HCl) layer until the mixture turned basic. To create a gel that was dissolved in 95% chloroform and filtered, the resulting solution was allowed to totally evaporate at ambient temperature. Without additional purification, this filtrate was employed for the antimicrobial analysis. Alkaloids, glycosides, saponins, carboxylic acid, and tannins were all present in this filtrate after preliminary phytochemical screening.

Preparation of neutral metabolites

The previously acquired layer of chloroform was put into a separate funnel, treated with 30 ml of 1M NaOH solution, vigorously shaken with periodic pressure releases, and left in a lab environment for around three hours until

equilibrium was reached. From this, two layers were obtained. The bottom chloroform layer was then taken out and allowed to completely evaporate at room temperature, forming a brownish gel that was then dissolved in 95% chloroform and filtered. The filtrate was used for antimicrobial analysis without further purification. Carboxylic acids, flavonoids, and phenols were found in this filtrate after a preliminary phytochemical screening was conducted on it.

Preparation of acidic metabolites

In order to turn the previously produced aqueous alkaline layer acidic, concentrated HCl was added moderately. At room temperature, the mixture was allowed to completely evaporate, yielding a brownish gel that was then dissolved in 95% chloroform and filtered for antimicrobial screening. This filtrate had saponins, phenols, flavonoids, and tannins, according to preliminary phytochemical testing.

Qualitative phytochemical screening

The different active metabolites (basic, neutral and acidic metabolites) were screened by phytochemical screening through qualitative test analysis reported by [25] with slight modification.

Test for saponins

Saponins produce froth when shaken with water or oil.

Method: 2 ml of the sample (taken from the extract previously prepared) was added into 2 ml of olive oil and was vigorously shaken. Frothing formation was observed.

Test for flavonoids

Flavonoids are prone to forming precipitate in acidic solutions.

Method: 1 ml of the sample was dissolved in 5 ml of dilute sodium hydroxide (NaOH) and filtered. Then concentrated tetraoxosulphate (VI) acid (H₂SO₄) was added moderately until the solution turned acidic (examined using litmus paper). Coloured precipitate was observed.

Test for carboxylic acids

Method: A strip of blue paper turned red when dipped into the sample, this affirms the presence of carboxylic acid.

Test for phenols

Method: A mixture containing 0.5 ml of the sample and 4 ml of distilled water was heated after which 1 ml solution of FeCl₃ was added to the filtrate. The presence of free phenols was confirmed following a dark brown precipitate formed.

Test for tannins

This test is based on the observable colour change as tannin reacts with iron (II) salts.

Method: The sample (2 ml) was diluted to 5 ml with distilled water, followed by the addition of two drops of ferric chloride. The presence of tannins was confirmed by the formation of brown coloured precipitate.

Test for alkaloids

Method: 1 ml of 5% H₂SO₄ was added to 2 ml of the sample in a Meyer's reagent treated test tube. The presence of alkaloids was indicated with a creamy white precipitate formed.

Test for cardiac glycosides

Method: 5 ml of 50% H₂SO₄ was heated in water bath for 5 minutes. Then, 5 ml of Fehling solution was added and boiled. The presence of glycosides was signaled with the formation of a brick red precipitate.

Test for terpenoids (Salkowski test)

Method: 2 ml of the sample was dissolved in 5 ml of chloroform then addition of concentrated H₂SO₄ was followed. The presence of terpenoids was indicated by the formation of a reddish brown colouration of the interface.

Chromatographic analysis of sample

The crude extract of the sample was purified and diluted with chloroform and set on a marked TLC (chromatographic paper) on three different points and dipped inside a mixture solution of 10 ml of butanol, 8 ml

of distilled water and 2 ml of acetic acid to determine the chromatographic flow rate of the sample through the solvent phase of the chromatographic layer and to determine the distance travelled through the solvent phase medium and the flow rate. ($Rf_1 = 0.4898$, $Rf_2 = 0.4082$ and $Rf_3 = 0.3878$). The major use of this technique was to identify compounds based on their flow rate values and to monitor organic reactions.

Antimicrobial screening of the active metabolites

These were carried out in line with standard protocols of microbiological procedures in Microbiology Department at Ebonyi State University, Abakaliki. Test micro-organisms used were *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus pneumoniae*.

IV. RESULTS AND DISCUSSION

The result of phytochemical screening conducted on various active metabolites of cocoa stem bark was presented in Table 1 while the chromatographic analysis of the sample was presented in Table 2.

Table 1: Phytochemical screening on various active metabolites

<i>Phytochemical</i>	<i>Acid metabolites</i>	<i>Basic metabolites</i>	<i>Neutral metabolites</i>
<i>Tannins</i>	++	+	-
<i>Saponins</i>	+++	++	-
<i>Flavonoids</i>	+	-	++
<i>Steroids</i>	-	-	-
<i>Glycosides</i>	+	-	+++
<i>Phenols</i>	+++	++	++
<i>Carboxylic</i>	-	+++	++
<i>Alkaloids</i>	+++	+	+

KEYS:

+++ = Strongly positive

++ = Positive

+ = Fair

- = Not detected

Table 2: Chromatographic analysis of sample (TLC)

<i>Sample drops</i>	<i>Distance travelled by compound (cm)</i>	<i>Distance travelled by solvent (cm)</i>
<i>1st drop</i>	2.4	4.9
<i>2nd drop</i>	2.0	4.9
<i>3rd drop</i>	1.9	4.9

$$\text{Flow rate} = (Rf) = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent}}$$

$$Rf_1 = 0.4898$$

$$Rf_2 = 0.4082$$

$$Rf_3 = 0.3878$$

$$\text{Average flow rate (Rf)} = \frac{0.4898 + 0.4082 + 0.3878}{3}$$

$$\text{Therefore: Rf} = 0.4286$$

The result of the antimicrobial properties on various active metabolites of the sample and their correlation were shown in Table 3 and 4 respectively.

Table 3: Antimicrobial result of extract and various active metabolites

Test organism	Crude	Acidic	Basic	Neutral	Ampiclox
<i>Streptococcus Pneumonia</i>	28	30	0	0	31
<i>Pseudomonas Aeruginosa</i>	25	30	0	0	28
<i>Escherichia coli</i>	27	31	0	0	25
<i>Staphylococcus Aureus</i>	21	27	0	0	35
<i>Candida albicaus</i>	-	-	-	-	-
<i>Coliform bacilli</i>	-	-	-	-	-

Table 4: Correlation of antimicrobial analysis of extract and active metabolites

100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	Augmentin	Amoxicillin	Organisms
10	05	02	02	01	25	12	<i>Straphylococcus Aureus</i>
08	06	03	μ	μ	30	30	<i>Pseudomonas Aeruginosa</i>
17	12	09	02	μ	24	24	<i>Escherichia Coli</i>
20	05	04	04	μ	33	22	<i>Streptococcus pneumoniae</i>

μ= no effect

100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	Control (ampiclox)	Organisms
10	05	02	04	02	25	<i>Escherichia coli</i>
07	04	01	03	02	22	<i>Pseudomonas aeruginosa</i>
08	05	01	03	03	20	<i>Streptococcus pneumonia</i>
08	05	02	01	02	16	<i>Staphylococcus aureus</i>

The phytochemical screening of the cocoa stem bark shows the presence of alkaloids, tannins, saponins, glycosides, carboxylic acids, flavonoids, and phenols. The phytochemical screening result of the extract and metabolites are presented in Table 1, which showed that the extract contained alkaloid, tannins, saponins, glycosides, carboxylic, acids flavonoids, and phenols.

These results were similar to the study earlier conducted by [25]. On the contrary, alkaloids, glycosides and tannins were found in the chloroform extract of the stem back which were absent in the earlier report of [24]. The presence of the identified phytochemicals suggests that the extract or its various active metabolites may possess antibacterial potential against several human pathogens when compared with standard antibiotics.

The chromatographic analysis carried out on the extract are shown in Table 2, which depicts and illustrates the flow rate of the crude extract in a thin layer chromatography (TLC) by evaluating and deducing the distance travelled by the compound with respect to the distance travelled by the solvent on the chromatographic plate.

The antimicrobial drugs; Ampiclox, Augmentin and Amoxicillin were used to test for the inhibitory action of these microbes; *Streptococcus pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*. The acidic metabolites showed more activeness and effectiveness towards antibacterial activity. The greatest antimicrobial activity was against *Staphylococcus aureus* with 35 mm zone of inhibition. It was observed that the basic, crude extract and neutral metabolites were ineffective towards the antimicrobial activity. The results in Table 4, showed that there was a correlation in antimicrobial activities of the active metabolites when compared to the standard used in the test by preparing test solutions in 100, 50, 25, 12.5 and 6.25 mg/ml used in the microbial tests against 100 and 30 mg/ml standard solutions.

V. CONCLUSION AND FUTURE SCOPE

Findings from this study on phytochemical screening, suggest that the identified phytochemicals may be the reason why cocoa stem bark extract is pharmacologically active. Their antimicrobial activities may also be responsible for their usefulness in the management and treatment of various diseases in folk medicine. Data obtained from this research suggest that cocoa stem bark

hold much promise as antibiotics against certain bacteria identified in this work. However, the need to find out the existence of these biologically active principles responsible for its pharmacological roles using other parts of this plant is necessary in the future research.

VI. RECOMMENDATION

This study however, has shown that the extract of *Theobroma cacao* stem bark and its secondary metabolites contained potential antibacterial activities against some human pathogens to a reasonable extent. This was explained in terms of the presence of certain phytochemicals contained therein like alkaloids, flavonoids, tannins, saponins, glycoside and phenols, attention should be drawn to harnessing cocoa stem bark medicinal properties in orthodox and herbal medicine. Conservation and plantation of *Theobroma cacao* should be one of the major area of concern for researchers, farmers and government in order to unravel some of its unknown potential thereby providing information on its underutilization.

ACKNOWLEDGEMENT

The authors are grateful to the Department of Industrial Chemistry, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria for their support during this research.

AUTHOR CONTRIBUTIONS

Olaleye TF conceived the idea and designed this research work while Ogunbiyi OJ gathered all the data and prepared the manuscript for publication.

FUNDING STATEMENT

No funding available for this research.

CONFLICT OF INTEREST

Authors declare no competing interest in the publication of this article.

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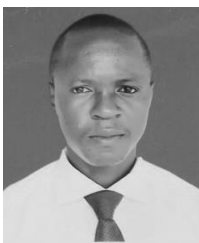
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AUTHOR'S PROFILE

Mr. Timilehin Francis Olaleye pursued B.Sc. and M.Sc. Industrial Chemistry from Ebonyi State University, Abakaliki, Ebonyi State, Nigeria and University of Ilorin, Ilorin, Nigeria in 2013 & 2019 respectively. He is currently working as a Lecturer in Department of Chemistry, Faculty of Science, Air Force Institute of Technology, Kaduna, Nigeria since 2020. He is a member of Chemical Society of Nigeria since 2009, Life member of CSN since 2014. He has published more than 5 research papers in reputed international journals and has attended several conferences including CSN. His main research work focuses on Analytical and Phytochemical Screening of underutilized plants. He has over 2 years of teaching and 5 years of research experience.



Mr. O J Ogunbiyi pursued B.Sc. (Hon) Biochemistry from Adekunle Ajasin University, Akungba-Akoko, Ondo State Nigeria and M.Sc., M.Phil. Biochemistry from University of Benin, Benin-City, Nigeria in 2009, 2015 & 2019 respectively. He is a PhD candidate in Department of Biochemistry, University of Benin,



Nigeria. He is currently working as a Lecturer in Department of Biology, Faculty of Science, Air Force Institute of Technology, Kaduna, Nigeria since 2020. He is a member of Society for Experimental Biology of Nigeria also known as Nigerian Society of Experimental Biology (NISEB) since 2013, a member of Nigerian Society of Biochemistry and Molecular Biology (NSBMB) since 2012. He is also a member of Nigerian Institution of Professional Engineers and Scientists (NIPES) since 2020. He has published more than 15 research papers in reputed international journals including *Biokemistri* (a journal of NISEB) most of which is also available online. He has attended conferences both local and international (through webinar). In addition, he is a reviewer for some academic journals, such as Science Publishing Group Reviewer for American Journal of Bioscience. His main research work focuses on Environmental Toxicology: Biochemical and Molecular evaluation of the effects of certain heavy metals associations on reproductive organs. He has over 2 years of teaching in tertiary institution and 6 years of research experience.