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The Chemical Constituents and Biological Activities of Stem Bark Extract of *Theobroma Cacao*

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Abstract- The chemical composition of the extract of the stem bark of *Theobroma cacao* and its biological activity is hereby studied. Dried pulverised stem bark extract of *Theobroma cacao* was batch extracted with ethanol. This crude ethanol extract was screened for the presence of plant chemicals: the result showed the presence of alkaloid, tannin, saponin, glycoside, phenol, flavonoid and carboxylic acid. Four human pathogens; *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pnemoniae* and *Staphylococcus aureus* were used in the test for the biological activity and were discovered to be susceptible to the crude extract. The extract was acidified with concentrated hydrochloric acid (HCl) and extracted with chloroform ($CHCl_3$). The organic layer was basified with 1 M sodium hydroxide (NaOH) solution and the alkaline layer treated with HCl. The acidic component showed strong presence of tannin, saponin, phenol, alkaloid, mild presence of flavonoid and glycoside. The minimum inhibitory concentration (MIC) against the pathogens was at a concentration of 1 x 10^{-8} M for *Escherichia coli*, *Pseudomonas aeruginosa* and approximately 1 x 10^{-6} M for *Streptococcus pnemoniae* and *Staphylococcus aureus*. The inhibition zone diameter (IZD) was carried out at different concentrations of the plant extract. The concentration at 12.5 mg/mL was significant for all the microorganisms.

Keywords: activity, bark, chemicals, extract, pathogens, stem.

I. INTRODUCTION

The use of herbal medicines to cure/prevent illness and to lubricate the wheels of social interaction is a behaviour which antedates civilization and is present in every society irrespective of its level of sophistication (Sanjay and Yogeshwer, 2003). The drugs of today's modern society are products of research and development, whose raw materials are naturally occurring materials which are obtained from plants; either in the roots stems, leaves, fruits and seeds (Odugbemi, Akinsulire, 2006; Burkill, 1994).

Up till now, some of the widely used drugs of plant origin are still produced by extraction from plants though, for some, their chemical structures are known and the methods developed for their laboratory synthesis (Warren, 2002).

The cost of synthesis is high, therefore it is cheap and easier to access the plant chemical and in most cases, the natural product is better with minimal side effects compared to the synthetic ones (Xu and Zhao, 2004). As such despite the chemical structures currently available for the screening for the actions of therapeutic value, natural products of plants origin remain a most important source of new drugs. The majority of bioactive compounds are terpenoids, steroids, alkaloids, organic acids, polyketides, macrolides, pyranones, glycosides, phenolic compounds and derivatives. These compounds exhibit antibiotic, antitumour, antiviral, anti-inflammatory, immunomodulatory, enzyme inhibiting, cardiovascular, analgesic, antidiabetic, antioxidant, insecticidal, nematocidal e.t.c effects. Globally plants extracts are employed for their antibacterial, antifungal, antiviral, antihypertensive activities (Meyer et al., 1996; Xu and Zhao, 2004).

The continuous evolution of bacteria resistant to currently available antibiotics has been the main drive in the search for novel and more effective compounds that are bactericidal, and the focus is on plants because of their use historically and the fact that many people the world over rely on them for the treatment of infectious and non-infectious diseases (Martinez, et al., 1996). These plant chemicals were/are isolated from a wide array of plants and even those plants already known are being discovered of having new and interesting physiological properties, take *Theobroma cacao* (Wood and Lass, 1985) as an example.

The *Theobroma cacao* tree is a source of the world's most delicious and familiar products, chocolate. Chocolate which is gotten from the seeds of this plant contains so many valuable a compound amongst which is theobromine, a useful antioxidant.

In this study, the stem bark of *Theobroma cacao* was investigated; ethanol was used as the solvent for the extraction. The ethanol extract was screened for the presence and type of plant chemicals and whether *Escherichia coli*, *Pseudomonas aeruginosa, Streptococcus faecalis* and *Staphylococcus aureus* have intermediate or strong resistant/susceptibility to the extract.

II. General Experimental Procedures

Weighing was done on a weighing balance model 770Mak Kew and Mettler P1210; grinding was

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carried out using electric grinding machine model EC-101 Binatone. All reagents were of analytical grade; Mueller- Hinton agar was used for the biological activity of the sample. Authentic sample of the micro organisms were obtained from the Department of Applied Microbiology Ebonyi State University Abakaliki, Nigeria.

a) Plant Material

One kilogram (1kg) of fresh stem bark sample was obtained from Enugu, Enugu State Nigeria and authenticated in the Applied Biology Department, Ebonyi State University Abakaliki. The plant sample was washed to remove lichen, fungus and sand then oven dried at a temperature of 80° C for seven days.

b) Extraction

Five hundred grams (500 g) of the dried sample were ground into powder and soaked by soaking and percolation method in 2 x 1000 mL $CHCl_3$ (BDH, England) for 98 h. The chloroform extract was removed by filtration and the solution evaporated to dryness to reveal 123 g of a brown amorphous gel. This crude sample was screened for the presence and type of plant chemicals**.**

i. *Alkaloids*

Dragendorf's reagent, a solution of potassium bismuth iodide was used to determine the presence of alkaloids. About 0.85 g of bismuth nitrate (BDH, England), was dissolved in 10 mL of 0.5 M NaOH(Arondale, England), to this was added 10 mL of glacial acetic acid (BDH, England), and 40 mL of distilled water; this was labeled solution 1. Solution 2 was prepared by mixing 8 g of potassium iodide and 20 mL distilled water. Approximately 1 mL of each of solutions 1 and 2 were mixed with 2 mL of glacial acetic acid, 10 mL of water and 1 mL of 2 g plant sample (which was prepared by dissolving 2 g plant extract in 30 mL of distilled water). A brownish-yellow coloured mixture was obtained.

ii. *Flavonoids*

One grams (1 g) plant sample was dissolved in 2 mL methanol (BDH, England), to this was added 100 mg magnesium powder (Sigma-Aldrich, USA), and shaken. Three (3) drops of conc. HCl (BDH, England) was added, a red colouration developed within 2 min of the addition of the acid
(Xu, 2012; Ikan,1991).

iii. *Phenols*

a. Iron (III) Chloride Solution Test

About 50 mg of the plant sample was dissolved in 1 mL of water, to this was added 1 drop of neutral 1 % $FeCl_3$ (BDH, England) solution and shaken. After 2-3 sec, 0ne more drop of the ferric solution was added. A purple colour was observed (Furnis, et al., 2006).

b. Phthalein Test

Approximately 500 mg of the plant sample and 500 mg of phthalic anhydride (Sigma-Aldrich, USA) were mixed intimately in a test tube and 1 drop of conc. H_2SO_4 (BDH, England), added. The reaction test tube was allowed to stand for 5 min in a 50 mL beaker of hot paraffin oil. The test tube was removed and allowed to cool. Four (4) mL of 5 % NaOH was added and stirred until the fused mixture dissolved. This was diluted with 4 mL distilled water and filtered. A red colour was observed.

iv. *Tannins*

a. KOH Test

Ten (10) mg of the extract was added to 1 mL of freshly prepared 10 % KOH, a dirty precipitate was observed (Sofowora, 1984; Harborne, 1973; Nwokonkwo, 2009)

b. Iron (III) Chloride Solution Test

Three (3) drop of 5 % FeCl3 solution was added to a solution of 1 mL of plant extract prepared by dissolving 20 mg plant sample in 10 mL of water. Two (2) mL of water was added to the whole mixture, a greenish precipitate was obtained.

v. *Saponins*

Ten (10) mg of plant material was introduced into a 50 mL conical flask, to this was added 20 mL of distilled water and shaken vigorously, there was a lasting bubble effect after the agitation. Three (3) drop of arachis oil was added to the frothing mixture obtained from above; a stable emulsion developed.

vi. *Glycosides*

Five (5) mL of 50 % H_2SO_4 was added to 5 mL of the extract (100 mg extract in 10 mL water) and heated for 15 min and allowed to cool. To this was added 5 mL of Fehling's solution and boiled for 5 min, a brick red precipitate was observed.

vii. *Carboxylic Acid*

200 mg of sample was dissolved in 5 mL of ethanol and 1 mL conc. H_2SO_4 and warmed for 2 min. This was cooled and poured cautiously into 4 mL 0.5 M solution of sodium carbonate in an evaporating dish. A sweet fruity smell of an ester was perceived.

viii. *Preparation of Acidic Component*

Approximately 2 g of plant sample was dissolved in 20 mL of HCl and extracted with 2 x 30 mL $CHCl_3$ using a separatory funnel. The $CHCl_3$ layer was treated with with 30 mL of 1 M NaOH solution. The aqueous alkaline layer was treated with 30 mL of 0.5 M HCl and the resulting solution evaporated to dryness to reveal a light brown gel which was used for the biological test. The preliminary phytochemical tests of the residue showed the presence of saponin, phenol, flavonoid, tannin and carboxylic acid (Ejele and Alinor, 2010; Ejele and Nwokonkwo, 2013).

c) *Biological Activity Test*

Biological activity tests were done by Applied Microbiology Department Ebonyi State University Abakaliki. Four human pathogens; *Escherichia coli*, *Pseudomonas aeruginosa, Streptococcus pnemoniae* and *Staphylococcus aureus* were used for the susceptibility tests.

i. *Broth Dilution Assay*

Ten sterile capped tubes were used; 2.0 mL of 100 mg/mL of the plant solution was introduced into the first test tube. About 1.0 mL of sterile broth was added to all the test tubes. One mL (1.0) was transferred to the second test tube, the content was mixed and 1.0 mL of it transferred to the third test tube. The process was repeated till the eighth tube, the ninth tube was used as the control.

A suspension of the microorganisms *Escherichia coli*, *Pseudomonas aeruginosa, Streptococcus pnemoniae* and *Staphylococcus aureus* were made to appropriate turbidity in 5.0 mL of Mueller-Hinton broth to give a slight turbid suspension. This suspension was diluted aseptically by introducing 0.2 mL of the suspension into 40 mL of Mueller-Hinton broth. One (1.0) mL of the diluted suspension was added to each of the test tubes and incubated at 35^0C overnight. Signs of visible microbial growth were examined (Woods and Washington, 1999; Nwokonkwo, 2010).

ii. *Agar Well Diffusion Assay*

A suspension of *Escherichia coli*, *Pseudomonas aeruginosa, Streptococcus pnemoniae* and *Staphylococcus aureus* were made in Mueller-Hinton broth at an appropriate turbidity. A sterile cotton swab was streaked over Mueller-Hinton broth using swab sticks. A cork borer was used to bore 7 cm hole on the inoculated media using sterile hole-borer. The plant extract was prepared in different concentrations of 100 mg/ mL, 50 mg/ mL, 25 mg/ mL, 12.5 mg/ mL and 6.25 mg/ mL.

1 mL of each concentration of the plant extract was inoculated into each borer, and the plates incubated at 35^0C for 24 h; after which the diameter of the zone of growth inhibition around each hole was measured to the nearest mm.

III. Results

The stem bark extract of *Theobroma cacao* showed the presence of plant chemical indicated inTable 1. The minimum inhibitory concentration (MIC) and the inhibition zone diameter (IZD) results are shown in Tables 2 and 3.

Table 1 : Result of the Phytochemical Screening of the Stem Bark Extract of *Theobroma cacao*

Phytochemical	Result
Alkaloid	+++
Tannin	++
Saponin	+++
Glycoside	+
Phenol	+++
Flavonoid	+
Carboxylic acid	+

+++ Significant presence, ++ appreciable present, + moderate presence

Table 2 : Minimum Inhibitory Concentration of the Stem Bark Extract of *Theobroma cacao*

Clinical Organism	Concentration (M)	Activity
Escherichia coli	1×10^{-8}	+
	1×10^{-7}	+
	1×10^{-6}	+
	1×10^{-5}	+
	1×10^{-4}	+
	1×10^{-3}	+
	1×10^{-2}	+
	1×10^{-1}	+
Pseudomonas aeruginosa	1×10^{-8}	+

	1×10^{-7}	+
	1×10^{-6}	+
	1×10^{-5}	+
	1×10^{-4}	+
	1×10^{-3}	+
	1×10^{-2}	+
	1×10^{-1}	+
Streptococcus pnemoniae	1×10^{-8}	-
	1×10^{-7}	-
	1×10^{-6}	-
	1×10^{-5}	+
	1×10^{-4}	+
	1×10^{-3}	+
	1×10^{-2}	+
	1×10^{-1}	+
Staphylococcus aureus	1×10^{-8}	-
	1×10^{-7}	-
	1×10^{-6}	+
	1×10^{-5}	+
	1×10^{-4}	+
	1×10^{-3}	+
	1×10^{-2}	+
	1×10^{-1}	+

+ effective, - ineffective

Table 3 : Inhibition zone Diameter (IZD) mm of the Stem Bark Extract of *Theobroma cacao*

Test organism	Inhibition zone diameter(mm) Concentration (mg/mL) 100	50	25	12.5	6.25
Escherichia coli	30±1	28±1	25±2	23±1	-
Pseudomonas aeruginosa	30±2	25±1	23±1	22±1	-
Streptococcus pnemoniae	31±1	27±2	24±2	22±2	-
Staphylococcus aureus	27±1	24±2	20±2	22±2	-

-No activity, 20- 25 Appreciable activity, > 25 High activity

IV. Discussions

Table 1 showed the presence of seven different phytochemicals; alkaloid, saponin and phenol were present in significant amount; tannin was also present in substantial amount while glycoside, flavonoid and carboxylic acid were also indicated. Alkaloids have biological activity and are often the active constituents of various medicinal plants (Manske, 2007; Pelletier, 2001) and comprise the largest family of natural organic products. Saponins are able to destroy cell membranes of micro organisms and show heamolytic, spermicidal and cytotoxic activity. Phenols are natural products that have strong antibacterial and antifungal effects, flavonoids are compounds that offer disease and bacterial defense. Glycoside and flavonoid have also been discovered to exhibit biological and physiological effects. The plant sample showed Minimum Inhibitory Concentration (MIC) at a concentration of 1×10^{-8} M for *Escherichia coli* and *Pseudomonas aeruginosa,* while the MIC for *Streptococcus pnemoniae* was at a concentration of 1×10^{-5}M and 1×10^{-6} M concentration for *Staphylococcus aureus*. That is to say that even at the highest dilution of 1×10^{-8} the plant sample was still

active against two of microorganisms; *the Escherichia coli* and *Pseudomonas aeruginosa.* The inhibition zone diameters (IZD) for the organisms at a concentration of 100 mg/mL were 30±1, 30±2, 31±1 and 27±1; at a concentration of 50 mg/mL, the IZD values were 28±1, 25±1, 27±2 and 24±2; at 25 mg/mL, 25±2, 23±1, 24±2 and 20±2 while at 12.5 mg/mL, the IZD values were 23±1, 22±1, 22±2 and 22±2 for *Escherichia coli*, *Pseudomonas aeruginosa, Streptococcus pnemoniae and Staphylococcus aureus* respectively. At a concentration of 6.25 mg/mL the growth of *Escherichia coli*, *Pseudomonas aeruginosa, Streptococcus pnemoniae and Staphylococcus aureus* were not inhibited. Since the IZD values of the plant extract at 12.5 mg/mL were appreciable, the implication was that this plant extract could serve as a source for the treatment/cure for some bacterial infections especially those posed by the four pathogens investigated. The use of ethanol as a choice solvent was because an infusion or decoction of a glass of the stem bark of *Threobroma cacao* could be made with vodka, brandy or even champagne on "rocks" and taken. The IZD values were high values when compared to the fact that at IZD of 15-20 mm the growth of a microorganism could be inhibited. *Theobroma cacao* is a very useful plant in that while the fruiting body of *Theobroma cacao* produces an antioxidant the stem bark is a good antimicrobial agent: hence this plant part may be looked at as a potential source of antimicrobials.

The quest for natural products from plants having significant physiological properties is growing everyday and cuts across the globe; plants' extracts can be given singly or as concoctions for various ailments. People are relying on herbs to meet their various health needs; these are safe, may be consumed with little or no side effect, easily accessible and cheap.

With the improvement of process formulation and production technology, various formulation can be made available using plant extracts in form of tablets, capsules, granules, oral liquid, injections which can be used to treat bacterial infections, cardio-cerebrovascular diseases, liver, kidney, lung disorders with satisfactory effects (Guo et al., 2001; Xueli et al., 2001).

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