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Extraction, Phytochemical, Antibacterial Screening and Spectroscopic Analysis of the Crude Samples of Stem Bark Extract of *Lonchocarpus cyanescens*

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ABSTRACT

Powdered stem bark of Lonchocarpus cyanescens was batch extracted with chloroform, methanol and water to yield varying proportions of the extracts: Stem Bark Aqueous Extract (SAE), Stem Methanol Extract (SME) and Stem Chloroform Extract (SCE) respectively. The thin-layer chromatography revealed 5-6 constituents for each extract. The extracts were each washed in a column packed with alumina using n-butanol-glacial acetic acid-water (5:1:4) upper layer and screened for the presence of phytochemicals. The extracts indicated the presence of alkaloids, saponins, tannins, cardiac glycosides, flavonoids and phenols. The ultraviolet-visible (UV-VIS) spectroscopy of SCE showed peaks between 650-776.0 nm, SME between 497.0-795.5 nm and SAE showed bands between 208.5-760.1 nm. The Infrared Spectroscopy (IR) of SCE showed significant broad stretches at 3444.03 cm^{-1} for the presence of hydrogen bonds (OH), 2932.50 cm^{-1} C=C of aromatic compounds, 1645 cm^{-1} for aromaticity and overtoned at 1186 cm^{-1}. The IR of SME showed identical peaks at 3442.09 cm^{-1}. 2931.90 cm^{-1}, 1644.37 cm^{-1} and 1063.78 cm^{-1}. The IR peaks or bands of SAE appeared at 3431.48 cm^{-1}, 2944.44 cm^{-1}, 1634.73 cm^{-1} and 1096.57 cm^{-1}. The Gas Chromatography-Mass Spectrometry (GC-MS) of SCE showed a molecular peak (m/z) at 282, SME at 206 and SAE at 256. The antimicrobial activity of the extracts showed sensitivity at a concentration as low as 1.0×10^{-4} mg/mL against four human pathogens; Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi and Staphylococcus aureus. The extracts did not inhibit the growth of Klebsiella pneumonia and Streptococcus pneumonia.

Keywords: Bark, Batch, Constituents, Sensitivity, Spectroscopy

INTRODUCTION

Medicinal plants are used for the treatment of illnesses and are of great importance in folk and orthodox health care. The use of these plants in folk or herbal medicine is common in rural areas of many developing countries [1,2]. The medicinal and pharmaceutical properties of plants are as a result of the type of chemical substances which they produce and store. These substances are utilized as food by man and animals and also because they exert certain physiological effects in them they are referred to as secondary metabolites or active constituents, they impart certain therapeutic properties in these plants [3]. *Lonchocarpus cyanescens* is a medicinal plant that belongs to the family *Leguminosae papilionoideae* and is known as *Philenoptera cyanescens*. It is called African indigo; the Igbos of South East Nigeria call it 'anunu' while the Hausas name it 'talaki'. It is known as 'elu' by the Yorubas and 'ebelu' by the Edo People of Nigeria. It is a deciduous scan dent shrub with alternate leaves with flat fruits which are 1-5 seeded. The aerial parts yield indigo which is a useful colourant for dyeing cloths in West Africa in ancient times [4-6]. The plant is utilized in traditional medicine; the leaves are used as food in sauces, spices, condiments, flavouring [7,8]. The leaves are also used as medicines as laxative, in the treatment of leprosy, parasitic infection and veneral diseases. The leaves and roots are applied as poultice to treat skin disease and ulcers, arthritic conditions, sores, boils and anthelmintic [9,10]. Bioactivity effects of the root of *L. cyanescens* have been demonstrated in its anti-inflammatory, anti-arthritic, anti-diarrhoea, anti-dysentery eye treatment, as abortifacient, in veneral disease and as genital stimulant/depressant. It has some additional pharmacological properties including antiviral, antifungal, anti-protozoa and antibacterial activities. Onwuliri and Wonang averred that traditional medicine is the one of the first means of medical treatment known to many in the developing world. In the same vein, it is another phase of medical treatment that is becoming very prevalent in both emerging and advanced countries of the world. Pei, 2001 concluded that "traditional medical knowledge of medicinal plants and their uses by indigenous culture are not only important for preservation of cultural and traditional biodiversity but also for community healthcare and drug development now and in the future" [11-17].

The leaves of *L. cyanescens* is well known, the fermentation of which yields the dye indigo which is still being used as local homemade dye, biology stains, inks, tattoos and mordants for materials in Nigeria [16]. Indigo *L. cyanescens* is a well-known fast dye since the 19th century and its derivatives had/ are being synthetically produced by new methods [17].

The focus of this paper was on the investigation of the phytochemicals present in the stem bark extract from three different solvents, partial characterization by the UV-VIS, IR, GC-MS spectroscopic analysis and the antibacterial sensitivity of these extracts. The focus on the stem bark extract of the plant species would probably be one of the new reports concerning this part of the plant.

MATERIALS AND METHODS

Sample collection

The stem bark of the plant was collected on 25th of March, 2015, at Unwana, Afikpo North, L.G.A of Ebonyi State. It was identified and authenticated by a Taxonomist, Professor Onyekwelu S.S.C of Applied Biology Department, Ebonyi State University, Abakaliki.

Preparation of sample

The stem bark of *Lonchocarpus cyanescens* weighing 1.0 kg was collected, cut into small pieces, washed with pipe borne water and sterilized with distilled water. This was allowed to dry in an airy room temperature for 7 days. The sample was ground into powder and stored in sterile cellophane until needed. The ground powder weighing 600 g was soaked in 2000 ml of distilled water, methanol and chloroform for 7 days sequentially.

The aqueous extract was collected after decantation, the water removed by gentle heating using a sand bath. The aqueous filtrate was 3.250 g and labeled as Stem aqueous Extract (SAE). The methanol extract after evaporation of the solvent weighed 2.634 g and was labeled Stem methanol Extract (SME). The chloroform extract was recovered using the same method; the yield was 4.699 g and labelled Stem chloroform Extract (SCE).

Antibacterial test

The antibacterial tests were carried out in the Department of Applied Microbiology, Ebonyi State University, Abakaliki. The test organisms/pathogens used were *Streptococcus pneumonia*, *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Salmonella typhi and pseudomonas aeruginosa.* The medium used for the identification was Mueller-Hinton nutrient agar.

Phytochemical screening

The aqueous extract weighing 0.5 g was re-dissolved in 50 mL of the mother solvent and used for the phytochemical screening of the active constituents present. They were based on stain-test observed with specific reagents. The screening of the sample was determined according to the standard method [18].

Test for phenols

The extract 0.5 g was introduced into a 50 ml beaker, to this was added 4 mL of distilled water and heated. 1.0 mL of 1 M $FeCl_3$ solution was added to the solution. The reaction gave bluish colour thereby confirming the presence of phenol.

Test for alkaloids

One gram of the extract (1.0 g) was added to 2mL of Wagner's reagent (2 g of iodine and 6 g of KI in 100 ml of distilled water). The formation of a reddish brown coloration and turbidity indicated the presence of alkaloids.

Test for tannins

The extract weighing 0.5 g was diluted with 5 mL of distilled water; 2 drops of 1 M solution of $FeCl_3$ were added. A blue-black colored precipitate that occurred confirmed the presence of tannins.

Tests for flavonoids

The extract weighing 0.5 g was dissolved in 5 mL of dilute NaOH and filtered. Then 2 mL of 50% solution of HCl was added until the solution became acidic (tested with litmus paper). The formation of precipitate which changed to red colour confirmed the presence of flavanoid [19].

Tests for steroids

The extract (0.5 g) was introduced into a 10 mL test tube; to this was added 2 mL of chloroform. Then, 5 ml of concentrated H_2SO_4 was added carefully down the side of the tube to form a layer. On cooling, a pink coloration appeared at the ring interphase confirming the presence of steroids.

Column chromatography

A cylindrical column of 55 cm × 2.5 cm was packed with 187 g of neutral alumina after being solvated with the upper layer n-BuOH-AA-H2O (5:1:4) solvent system. Exactly 1.00 g of SAE, SME and SCE were dissolved in 20 ml of corresponding solvents used in the extraction respectively, introduced into the column, and washed severally with distilled water, methanol and chloroform respectively. Portions of 20 mL of the eluents were collected and identical portions monitored by TLC were pooled together, and the solvent was allowed to evaporate. This was set aside for instrumental analysis [20].

Antimicrobial screening of active metabolite of aqueous extract

The extracts were screened for antimicrobial sensitivity. The agar-diffusion method was used. The microorganisms were maintained on agar slants and sub cultures were freshly prepared before use. The inocular were made in 5 mL nutrient broth and given for 24 h at 37.5°C. The final inocular was prepared with nutrient agar medium 5 mL, at 48°C sealed with test organism. Plates were prepared by pouring 20 mL of freshly prepared agar into 20 mm ×100 mm Petri dishes and adjusted to 45°C. About 5 mL inoculums was poured to the surface of the prepared plates and allowed to solidify for 5 min. Stainless steel cylinders were used to bore the surface; different concentrations of the samples were inoculated and incubated for 24 h at 37°C. After the inoculation period, inhibition zones were recorded as the diameter of the growth free zones. Ampiclox 500 mg/ml was used as the positive control [21,22].

UV-Visible analysis

The UV-Visible analysis was done on with Shimadzu Spectrophotometer with model UV 2500 PC series V.2.30 to record the absorption maxima of the SAE, SME and SCE [23].

Infrared spectroscopic analysis

Infrared spectroscopy was done on Perkin-Elmer 337 Infracord spectrophotometer. Absorption spectra were recorded in the range between 4000-700 cm^{-1}. The samples were mixed with 10 mg KBr and ground intimately; the powdered mixture was compressed under pressure to produce KBr discs which were analyzed by the equipment [23].

GC-MS spectroscopic analysis

GC-MS spectroscopy was carried out in GC-MS QP2010 plus instrument on the samples SAE, SCE and SME. The detection was on the principle of electron ionization system at 70 eV. Using helium gas at a flow of 1 mL/min and the extracts volume of 2 ul, the molecular weights and pattern of fragmentations were given and compared with National Institute of Standard and Technology, NIST 05-S Library [23].

RESULTS

Results of the various analyses

The result of the phytochemical screening showing the different plant chemicals found in the various solvents used was as presented in Table 1. UV-VIS scan of the presence of conjugation and the absorption in the visible and ultraviolet regions was contained in Table 2. Infrared analysis of the significant functional groups present in these extracts appeared in Table 3. The GC-MS result that showed the molecular weight of each extract and their fragments was depicted in Table 4, while the antimicrobial screening result was shown in Table 5.

Table 1: Phytochemical analysis of SAE, SME and SCE extracts

Phytochemicals	SAE	SME	SCE
Saponin	+	+	+
Alkaloid	+	+	+
Tannin	+	+	_
Flavonoid	+	+	+
Steroid	+	+	_
Phenol	+	+	+

+ Present; - Absent

Table 2: UV-visible spectral scan analysis of SAE, SME and SCE in nanometers (nm)

UV-Vis bands	SAE	SME	SCE
1	750.00	799.50	776.00
2	738.00	731.00	705.00
3	320.00	718.50	659.50
4	311.50	663.50	650.50
5	208.50	604.50	NS
6	NS	497.00	NS

NS = Not significant (weak)

Table 3: FTIR analysis result of SAE, SME, and SCE extract in per centimetres (cm^{-1})

Infrared result	Extracts			
Bands	SAE	SME	SCE	Characteristic group
1	3431.48	3442.09	3444.03	OH
2	2944.44	2931.90	2932.50	C=C-H
3	2344.55	2349.38	2361.91	C=C-H
4	2122.73	2123.70	1641.48	Aromatic stretch
5	1634.73 1403.26	1644.37 1405.19	1387.83 1259.56	Aromatic overtone
6	1096.57	1259.56	1186	
7	414.71 406.03 402.1	1063.78 409.89 (single bond)	549.73	three band sharp Solvent used.

Table 4: GC-MS molecular fragment peaks of SAE, SME and SCE

Sample	m/z molecular/fragment peaks
SAE	41, 57, 60,70, 256 (M^+)
SME	41, 57, 74, 91, 191, 206, (M^+)
SCE	41, 69, 83, 97, 282 (M^+)

M^+ molecular ion peak

Table 5: The inhibition zone diameter (IZD) and concentration of the different plant samples

Microorganism	Water extract				Methanol extract				Chloroform extract				Control (Amoxicillin)			
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{--1}	10^{-2}	10^{-3}	10^{-4}
Klebseilla pneumonia	-	-	-		-	-	-	-	-	-	-		5	2	5	5
Streptococcus pneumonia	-	-	-	-	-	-	-	-	-	-	-	-	10	12	10	10
Staphylococcus aureus	2	-	-	-	-	-	-	-	-	2	-	8	-	-	-	1
Samonella typhi	2	7	7	-	2	-	-	2	-	-	-	-	-	10	12	14
Escherichia coli	2	-	-	-	-	-	-	6	4	1	-	-	-	-	2	2
Pseudomas aureginosa	-	-	-	-	-	-	-	-	8	10	-	4	-	6	2	4

- No activity; >2-12 inhibitory activity

DISCUSSION

The phytochemical analysis shown in Table 1 indicated the presence of saponins, alkaloids, tannins, flavonoid, steroid and phenol for the three samples used. This showed that the stem bark of *L. cyanescens* contained phytochemicals that would be useful pharmacologically and physiologically [24,25].

The UV-visible showed the different absorption maxima for the various samples. About six bands were indicated in each sample. The SAE extract had strong bands at 320.00 nm, 311.50 nm and 208.50 nm in the UV region and weak and bands at 776.50 nm and 738.50 nm [23]. The SME result showed a strong absorption at 663.50 nm, a moderate band at 604.50 nm and weak bands at 795.50 nm, 731.00 nm, 718.50 nm and 497.00 nm in the visible region of the spectrum. SCE showed four weak absorption bands in the visible region of the electromagnetic spectrum. The bands at 718-795 nm indicated the presence of indigo compound in these three samples; this is because indigo dye exhibited absorption maxima from 620-720 nm [26].

The infrared absorption scan indicated that SCE, SME and SAE had strong broad band at 3444.0 cm^{-1}, 3442.09 cm^{-1} and 3431.48 cm^{-1}. Bands at this region of the infrared are associated with O-H stretch of hydrogen bond of alcohol aryl alcohol. The weak band one at 2932.86 cm^{-1} for SAE and strong bands at 2931.90 cm^{-1} and 2944 cm^{-1} for the SCE and SME extracts equally indicated the C-H stretch of aromatic compounds. Again, the pronounced strong bands at 1634.73 cm^{-1} for SAE, 1641.48 cm^{-1} for SCE and 1644.37 cm^{-1} for SME were characteristic stretches for aromatic group. The medium bands at 1403.26 cm^{-1}, 1387.83 cm^{-1}, 1405.19 cm^{-1} for SAE, SCE and SME respectively were overtones for aryl stretch. The rest of the bands were characteristic bands in the fingerprint region of the infrared spectrum [27-29]. SAE compound gave a molecular peak of m/z 256 and a base peak at 43 and other peaks at 41, 57, 60, 70 and 83. SME had a molecular ion at m/z 206, a base peak at m/z 57 and fragments at m/z 41, 74, 91 and 191 while SCE compound was a compound of molecular weight 282, with the base peak at m/z 55 and other fragments at m/z 41, 69, 83 and 97. The antibacterial inhibitory analysis of the three samples showed that *K. pneumonia* and *S. faecalis* were not inhibited.

The samples inhibited *S. aureus* in varying proportions: For SAE at 1×10^{-1} mg/mL with IZD values of 2 mm. *S. typhi* at concentration of 1×10^{-1}, 1×10^{-3} mg/mL with IZD values of 2 mm, and 7 mm respectively. *E. coli* at with 1×10^{-1} mg/mL having with IZD 2 mm. No inhibitory activity was shown by SAE against *P. aeruginosa* at all the concentrations. SME inhibited *E. coli* at a concentration of 1×10^{-3} mg/mL with IZD 6 mm but was inactive against *P. aeruginosa, S. aureus, S. pneumonia* and *K. pneumonia*. SCE inhibited *S. aureus* at concentrations of 1×10^{-2} mg/mL and 1×10^{-4} mg/mL with IZD values of 2 mm and 8 mm respectively; inhibited *E. coli* at 1×10^{-1} mg/mL and 1×10^{-2} mg/mL with IZD 4 mm and 1 mm respectively and also inhibited *P. aeruginosa* significantly giving IZD values of 8 mm, 10 mm and 4 mm at 1×10^{-1} mg/mL, 1×10^{-2} mg/mL and 1×10^{-4} mg/mL respectively. SCE was more potent against *E. coli* and *P. areuginosa* compared to the control at the corresponding concentrations used. This particular extract could serve as a potential antibacterial agent against *E. coli* and *P. areuginosa* the SAE extract could be harnessed against *S. typhi*. The isolation of these extracts into single pure isolates vis a vis their antibacterial potency is target of further research.

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