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PHYTOCHEMISTRY AND ANTIMICOBIAL ACTIVITIES OF EXTRACTS OF *PARKIA CLAPPERTONIANA* STEM BARK

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ABSTRACT
The stem-bark of *Parkia clappertoniana* was subjected to photochemical screening using standard procedure. The method of cold solvent was used in the extraction. The stem-bark of *Parkia clappertoniana* as prepared by soaking 60g of it in 150ml of hexane for four days and filtered, concentrated by evaporation, dried and weighed. The procedure was repeated with chloroform, ethyl acetate, acetone and methanol sequentially in order of polarity. The stem-bark extracts of hexane, chloroform, ethyl acetate, acetone and methanol were screened for the presence of some phytochemical such as alkaloids, anthraquinones, saponins, terpenes, flavonoids and tannins. The result obtained revealed the presence of anthraquinones and terpenes in all the extracts. Flavoniods were found in the extracts except for hexane extracts. Saponin was only present in chloroform and acetone. Alkaloids and tannins were present in hexane, acetone and methanol extracts. The antimicrobial activities of the extracts were tested against some clinical isolates and the result of the sensitivity tests of the organisms to the extracts showed the extract had antibacterial activity against the test bacterial isolates.

Keyword: Parkia Clappertoniana, Stem Bark, Cold Solvent, Ethylacetate, Acetone, Methanol Hexane and Alkaloids.

INTRODUCTION
Medicinal plants constitute and important natural wealth of a country. They serve as important raw materials for the manufacture of traditional and modern medicine. Modern phytochemical investigation of plants thus becomes important. Plants have the ability to synthesize a wide variety of chemical compounds that

are used to perform important biological functions and to defend against attack from predators such as insects, fungi and herbivorous mammals (Gokulnath *et al.*; 2014). Many of these phytochemicals have beneficial effects on long-term health when consumed by humans and can be used to effectively treat human disease (Elumalai, and Eswariah (2012). A medicinal plant is any plant which in one or more of its or more of its organs, contains substance that can be used for therapeutic purpose of which is a precursor for synthesis of useful drugs (Sofowara 1982). Many of these plant has been used in traditional medicine for many years and they seem to work, such plants should be classify as medicinal plant even though some of their effectiveness has not been scientifically proven. Sofowora (1993) has stated that despite the aggressive activities in this field of chemistry in times, the task at its early stages. Sanherry and Brulum (1979) reported that only about 10% of all plants have been investigated in detail for bioactive agents. It has been noted that the leaves and stem-bark of *Parkia-clappertoniana* has been used for curative purposes.

The plant kingdom still holds many species of plants containing substances of medicinal value, which are yet to be discovered *P. clappertoniana* is one of the plants which have been used in traditional medicine many years. To the best of my knowledge little or no work has been done on the plant *P. clappertoniana* in this part of the world. The present work is designed to enrich the available scientific data of efficacy of *P. clappertoniana*. The phytochemical screening of the plant parts extracts revealed the presence of saponin, tannin, flavonoid, anthraquinones, glycosides, triterpenoids, steroids and alkaloids. All the extracts showed antibacterial activity against all the tested bacteria species. The stem bark extracts showed more antibacterial activity than the other tested parts of the plant (Adesina 2008). The roots and leaves of *Parkia clappertoniana* are pounded with water and used as an eye wash; the roots and the leaves were also reported to be active against dental caries, conjunctivitis (Millogo-Kone 2008).

It was also reported that an infusion of the stem bark was successfully used for the treatment of many infectious diseases such as diarrhoea, orchitis, dental caries, pneumonia, bronchitis, violent stomachaches, severe cough, infected wounds, otitis, dermatosis, amoebiasis, bilharziosis, leprosis, ankylosis, tracheitis, and conjunctivitis (Millogo-Kone 2008). The aims and objectives of the study were: to carry out the preliminary phytochemical screening of extracts from the stem bark of *P. clappertoniana* and to confirm or disprove the

efficacy of the plant *P. clappertoniana* by evaluating the antifungal and anti bacterial activities.

MATERIALS AND METHOD

Collection and Preparation of the Sample

The stem-bark of *parkia clappertoniana* was collected from their natural habitat in Bekwarra Local Government Area. The sample was air-dried for about 2 weeks and then milled into fine powder using a Thomas-willey milling machine. The method of cold solvent was used in the extraction. This was to prevent the escape of some volatile of the sample. The extract of the stem-bark was prepared by soaking 100g of the extract in 250 ml of hexane for four days. The resulting mixture was filtered by gravity filtration and the filtrate was concentrated by evaporation in a water bath dried and weighed. The procedure was repeated on the residue using the following solvents: chloroform, ethyl acetate, acetone and methanol sequentially in order of polarity. The extracts were store in a desiccator.

Preliminary Phytochemical Screening

This is a preliminary qualitative test for the presence of the secondary metabolites Qualitative analysis of the crude extracts were carried on the extracts as described by Brain and Turner (1975), Sofowora (1993) and Trease and Evan (2000) and Ushie *et al.*; (2013) to identify the presence of the classes of secondary metabolites (Alkaloid, anthreagumones, flavoniods tannins, saponins, steroids and phenol).

Bioassay

This is the study of antimicrobial activity of these crude extracts of the stem-bark of *Parkia clappertoniana*. Against micro-organism. The antimicrobial activity on the clinical isolates was carried out with hexane, chloroform, ethyl acetate, acetone and methanol extracts from the stem-bark of *Parkia clappertoniana*. The test bacteria were *Esherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeuroginosa* While the test fungi were *Candida albicans*, *Rhizopus spp*. The test organisms were collected from University of Calabar Teaching Hospital Calabar. The bacterial assay procedures of WaterWorth (*197*8) and Perez *et al.,* (1990) were employed with small modification. The methods involved the preparation of the culture medium and inoculation. Aseptic technique was used to avoid contamination.

Preparation of the Media

Two media were employed for this research: Muller Hinton Agar (MHA) for bacteria culture and Sabourated Destrose Agar (SDA) with chloramphenicol for fungi culture. The media was prepared by dissolving 38g of Muller Hinton Agar (MHA) in 1litre of distilled water, while 65g of Sabourated Destrose Agar (SDA) was dissolved in 1litre of distilled water. They were sterilized at 121^0C for 15 minute in an autoclave and subsequently allowed to cool to agent 45^0C (temperature at which the agar remain molten) and pour in plate (Petric dishes) allow to get or solidified.

Standardization of Inoculums

The five test organisms were sub-cultured with nutrient broth using a wire loop (done aseptically) and incubated for 24hours at 35^0C for bacteria and 48hours at 25^0C for fungi. The growth of the micro organism in the broth by the turbidity produced was adjusted to match 0.5 mcfarland standards (10^9 cfu/ml) which was further adjusted to 10^3 cfu/ml and 10^3 for bacteria and fungi respectively.

Inoculation of the Plates and Application of the Extract

The agar plates Muller Hinton Agar (MHA) and Sabourated Destrose Agar (SDA) was inoculated by spreading a small volume (0.05ml to 0.10ml) of the liquid inoculums (sub-cultured molten broth) by means of wire rod (on a spreader) in such a way that the surface of the agar in the plates were covered with microbes. One microbe was inoculated to one plate making a total of five plates for five microbes. Five wells for hexane, chloroform, ethyl acetate, acetone and methanol extracts and two for the control (tetracycline, fulcin) were made. The plant extracts is diluted using dilution method and in each of the appropriately labeled well (holes) dilute plant extract were introduced. Tetracycline and fulcin were also introduced in the other two wells (holes) as control. The inoculated plates were left on the bench for about an hour to allow the extracts diffuse in the agar. The Muller Hinton Agar (MHA) and Sabourated Destrose Agar (SDA) were aerobically incubated at 37^0C for 23hours for the bacteria and 48hours for the fungi. The diameter of zones of inhibition was measured by means of linear instrument in millimeter (ruler) and recorded.

RESULTS AND DISCUSSION

Results

Nature and Yield of Extract from the Stem Bark of *Parkia clappertoniana*

The results obtained from the cold extraction of secondly metabolites from the stem-bark of *P. clappertoniana* using hexane, chloroform, ethyl acetate, acetone and methanol in order of increasing polarity gave yields of 1.7 (2.8%), 1.6 (2.6%), 1.1 (1.8%), 11.9 (8.5%) and 6.7 (11.1%) respectively. The summary of the results are given in table 4.1.

Table 1: Nature and Yield of Extract from the Stem-Bark of *Parkia clappertoniana*

Solvents	Extract Colour	Extract Texture	Extract Recovery	Percentage Recovery (%)
Hexane	Brown	powder	1.7	2.8
Chloroform	Brown	Sticky solid	1.6	2.6
Ethytacetate	Brown	Sticky	1.1	1.8
Acetore	Dark brown	Hard solid	11.9	18.5
Methanol	Dark brown	Hard solid	6.7	11.1

Result of the Preliminary Phytochemical Screening of Stem-Bark Extracts of *Parkia clappertoniana*

The phytochemical analysis of the hexane, chloroform, ethyl acetate, acetone and methanol plant extract revealed the presence of anthraqunones, terpenes, saponmins, tannins, flavonoid and alkaloids. The result obtained reveals the presence of anthraquinones and terpense in all the extracts. Flavonoids was present in all the extracts except in hexane. Saponins was present only in chloroform and Acetore. Tannins and alkaloids were present in methanol, acetone and hexane. These are presented in table 4.2 below

Table 2: Preliminary Screening of Stem-bark Extracts from *Parkia clappertoniana*

He = Hexane extract CE = Chloroform extract

	Phyto-chemicals	Reagents	HE	CE	EAE	AE	ME
1	Alkaloids	(a) Wagers	+	_	_	+	+
		(b) Mayer	+	_	_	+	+
2	Tannins	Ferric chloride	+	_	_	+	+
3	Saponnin	Forth test	_	+	_	+	_
4	Flavonoids	(a) Lead acetate test	_	+	+	+	+
		(b) Iron(iii) chloride	_	+	+	+	+
5	Anthraquini-nes	Extract in benzene + ammonia solution	+	+	+	+	+
6	Terpenes	Extract + chloroform and conc H_2SO_4	+	+	+	+	+

EAE= ethyl acetate extract AE = Acetone extract

ME = Methanol extract

Antimicrobial activity test results of the stem bark extracts.

Activity of the hexane, chloroform, ethyl acetate, acetone and methanol from the stem-bark of *P. clappertoniana* was tested on five clinical isolates. The measured zone of inhibition of the pathogens by the extracts are summarized in the table 4 below:

Table 4: Zone of Inhibition of Antimicrobial Activity of the Extract in MM from the Stem-Bark of *P. Clappertoniana*.

Test organisms	HE	CE	EAE	AE	ME	TCN	FUL 1g/5ml
Pseudomonas aeruginosa	-	10	13	12	17	30	NA
Esherichia coli	-	5	6	10	18	30	NA
Staphylococcus aerus	27	6	11	15	11	36	NA
Candida albican	-	-	-	-	5	-	5
Rhizopus spp	6	-	-	-	5	-	6

Note:
He = Hexane extract CE = Chloroform extract EAE= ethyl acetate extract
AE = Acetone extract
ME = Methanol extract TCN = Tetracycline FUL = Fulcin - = No zone of clearance
NA = Not applicable

DISCUSSION

The Phytochemical screening of the hexane, chloroform, ethyl-acetate, acetone and methanol extract reveals the presence of saponin, tannin, alkaloids, flavoniod, terpene and anthraquinones. Anthraquinnose and terpenes were detected in all the extracts. The flavonionds were found in all the extracts except in one. Alkoniods and tannins were not present in all the extracts. Saponins were not also present in all the extract. The absence of these secondary metabolites in some of the extract does not necessary confirm the absence of such constituents since the presence of active components is usually influenced by climates, drying and storage procedures, sensitivity colour reaction due to the presence of pigments, method of analysis and the plant saponins were detected in only chloroform and acetone.

Saponins are precursors of important therapeutic drugs such as cortisone and contraceptive estrogens. The pharmacological activities associated with saponins include anti-tumor, anti-mutagenic, anti-inflammatory, anti-viral. Flavoniods were detected in all the extract except in hexane extract.

Flavoniods are widely distributed in plant fulfilling many functions. Flavoniods have been shown to have a wide range of biological activities in in-vitro studies. Example include anti-allergic, anti-inflammatory (Yamamoto *et al,* 2001), anticancer and anti-diarrheal activities (Schuler *et al* 2005). Alkaloids were present in hexane, acetone and methanol. Many alkaloids are used in medicine usually in the form of salt (Hesse, 2005). Many synthetic and semisynthetic drugs are structural medications of the alkaloids which were designed to enhance or change the primary effect of the drugs and reduce unwanted side effect (Hesse, 2005). Anthraquinones was present in all the extracts. Anthraquinone has a derivative used as drugs. They include laxatives, antimalaria and antineroplastic used in the treatment of plastics. Tannins were present in hexane, acetone and Tannins are administered internally to check diarrhea and intestinal bleeding and as an antidote for metallic, alkaloididal and glycosidic poisons. Terpenes were present in all the extracts. Terpenes are shown to be present for pharmaceutical application for example as anti-malaria and anti-cancers (Mo H and Elson C. E. (1999).

The result of the susceptibility test of the organisms to the extracts showed the extract had antibacterial activity against the test bacterial isolates. All the extracts of the stem-bark inhibited antibacterial activity against *S. aureus* and also the same for *E. Coli* and *P. aeuroginosa* which was negative in the hexane extracts. Methanol extract exhibited very significant antibacterial activity against *S. aureus*, *P. aeuroginosa E. Coli* and antifungal activity against *C. albican* and *R. spp*. *Rhizopus spp* was negatively inhibited by the stem-bark extracts of chloroform, Ethyl acetate an acetone while *C. albican* was negatively inhibited by all the stem-bark extracts except methanol extracts. The conventional controlled tetracycline and fulcin consistently showed the superior antimicrobial than the extracts.

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