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Isolation and Anti-Bacterial Activity of the Active Components from the Stem- -Back of *Enantial chlorantha*

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Authors' contributions

This work was carried out in collaboration between both authors. Authors AMP and AS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AMP and AS managed the analyses of the study and managed the literature searches. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

A known quantity of coarsely blended stem-back of *Enantia chlorantha* was progressively extracted with hexane, ethyl acetate and ethanol respectively. The resulting crude extracts were separated into various components using thin layer chromatographic and preparative thin layer chromatographic techniques. The antibacterial activity of every isolated component was carried out using five test organisms which are *Klebsiella pneumonia, Salmonel typhi, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli.*
The result of the antibacterial assay conducted indicated that some of the isolated components had significant activities against the test organisms employed.

Keywords: Enantia chlorantha; crude extracts; chromatographic; antibacterial; assay.

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1. INTRODUCTION

Antibiotic resistance is a problem that continues to challenge the health care sector in a large part of the world in both developing and developed countries. The spread of multidrug resistant (MDR) bacteria in hospital and community settings remains a widely unresolved problem and a heavy burden to health services [1]. Despite advances in antibiotic therapy, infectious complications remain an important cause of mortality and morbidity among hospitalized patients. Although medical practitioners can resort to second or third choice drugs for treating these patients, the use of these synthetic drugs may subject the patient to a higher risk, due to the possibility of the drugs producing more harmful side effects. To address this challenge, actions must be taken to reduce this problem, such as controlling the use of antibiotics, understanding the genetic mechanisms of resistance and developing new antibiotics and new therapeutic strategies. Advances in identifying new sources of natural products with antimicrobial activities and expanding antibiotic chemical diversity are providing chemical leads for new drugs [2]. The vast majority of modern medications were derived originally from ancient herbal traditions. The practices of plant based traditional medicine are founded on hundreds of years of belief and observations, which predate the development of modern medicine. Medicinal plants have been used for centuries as remedies for human diseases as they contain components of therapeutic value. There are numerous plant's natural products which have antifungal, antibacterial and antiprotozoal activities that could be used either systemically or locally [3]. Several plants containing volatile oils, polyphenols and alkaloids as active constituents are utilized as popular folk medicines, while others gained popularity in the form of finished products collectively named phytomedicines [4]. During the second half of the 20th century, the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the classical antibiotics led researchers to investigate the antimicrobial activities of medicinal plants. Antimicrobials of plant origin have enormous therapeutic potential [3]. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties. One of the vital activities possessed by these medicinal plants is antimicrobial, the scarcity of infective diseases in plants is in itself an indication of the successful defense mechanisms developed by them [5]. The substances that can either inhibit the growth of bacteria or kill them, with no toxicity or minimum toxicity to host cells are considered candidates for developing new antimicrobial drugs [6]. Some of the bioactive compounds could hinder the life processes of disease-causing bacteria, either by itself or in combination with other therapeutic agents [7]. In recent years, antimicrobial properties of medicinal plants are been increasingly reported from different parts of the world [8–10]. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However, very little information is available on such activity of medicinal plants [6]. There have been numerous documentations found in the medical literature concerning the significance of traditional medicinal plants as alternatives to synthetic antibacterial and antifungal medications [11–17]. Most of these published works come from many countries that are still practicing the use of herbal medicine for the treatment of various diseases for practical and economic reasons. These studies are valuable resources for local medical scientists who seek to explore and substantiate the antibacterial and antifungal activities of Philippine medicinal herbs, particularly against MDR bacteria knowledge on the different antimicrobial assays and the plants' bioactive compounds are vital for the design of future studies [2].

Enantia chlorantha (*Annonaceae*) is an ornamental tree of up to 30 metre high with dense foliage and spreading crown. The stem is fluted; the outer bark is thin, dark brown while the inner bark is light brown. Due to the relative abundance of this plant in nature and its antibacterial activities of medicinal plants, it is important to study the antibacterial potency of *Enantia chlorantha.* This research work is therefore aimed at isolating the active components from the-stem bark of *Enantia chlorantha,* subject these isolated components to biological tests and to compare their potency with an available antibiotic.

2 MATERIALS AND METHODS

2.1 Collection of Plants Materials

The plant material in this research work is the stem- back of *Enantia chlorantha* (African yellow

wood) which was purchased from a market in Ikare- Akoko Ondo State.

2.2 Extraction

Extraction is the crucial first step in the analysis of medicinal plants, because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization. The basic operational include steps, such as;

- Pre- washing
- Drying of plant materials or freeze drying
- Grinding to obtain a homogeneous sample and often improving the kinetics of analytic extraction and also increasing the contact of sample surface with the solvent system.

Proper action must be taken to assure that potential active constituents are not lost, distorted or destroyed during the preparation of the extract from plant samples. If the plant was selected on the basis of traditional uses [18], then it is needed to prepare the extract as described by the traditional healer in order to mimic as closely as possible the traditional 'herbal' drug. The selection of solvent system largely depends on the specific nature of the bioactive compound being targeted. Different solvent systems are available to extract the bioactive compound from natural products. The extraction of hydrophilic compound uses polar solvent such as methanol. Ethanol since they are highly polar. Ethyl acetate removes moderately polar compound. In some instances, extraction with hexane is used to remove Chlorophyll [19] which is a non- polar solvent. As the target compound may be non- polar to polar and thermally labile, the suitability of the methods of extraction must be considered. Various methods, such as Sonification, heating under reflux, Soxhlet extraction and other are commonly used.

2.3 Extraction with Hexane (Defating Process)

259.50 *g* powdered Stem- bark of *Enantia chloranta* was put into *an* extractor bottle and 750 *ml* of hexane was added and the content was allowed to extract for five days with occasional shaking. After the extraction process, the content of mixture was filtered under reduced pressure. The filtrate obtained was poured into a round bottomed and was distilled in order to recover the solvent used for defatting process. The resulting crude filtrate left was poured into a beaker and allowed to stand. The crude extract was exposed to the air for any trace of solvent left in crude to evaporate. It was then labeled EC/ SB/HEX/ CRUDE. The remaining residue was returned into the same extractor bottle for further extraction.

2.4 Extraction with Ethyl Acetate

205.5 *g* of the residue of the plant material after the extraction with hexane solvent was extracted with ethyl acetate which is a moderately polar solvent in the extractor bottle. The same procedure for the previous extraction was carried out. The resulting crude extract was exposed into the air and the crude in the beaker was labeled EC/SB/EtoAC/CRUDE.

2.5 Extraction with Ethanol

162.5 *g* of the remnant obtained from the ethyl acetate extract was extracted with 750 *ml* ethanol. The resulting mixture was distilled off. The crude filtrate was weighed and labeled EC/ SB/ Etoh/CRUDE.

2.6 Preparative Thin Layer Chromatography Plates

30 *g* of silica gel powder was weighed and poured into a reagent bottle and 60 *ml* of distilled water was added to it. The content in the reagent bottle was vigorously sharked for two minutes to form a thick slurry. The slurry was poured on a 20 *cm* x 20 *cm* glass plate placed on a wooden support. The solution was evenly spread on the glass plate and allowed to dry, and then activated in the oven at 90$^{\circ}C$ for 1 hour.

2.7 Chromatography Separation of the Hexane Crude Extract

The TLC of hexane crude extracts was carried out by trying several solvent systems until a satisfactory solvent system was obtained for the crude extract. 1.50 *g* of the crude extract was dissolved in 8 *ml* of chloroform; 1.3 *ml* of the solution was spread on the origin of a PTLC plate with the aid of a Pasteur pipette. After complete evaporation, the PTLC plate was put into a chromatography tank containing 200 *ml* mixture of diethyl ether and hexane in ratio 1:1. At the end of the separation, three distinct bands were obtained. These were separately scrapped into three beakers and the content of each beaker

Table 1. The percentage yields of each extract using hexane, ethyl acetate and ethanol extraction media

Wt of starting material (g)	Solvent	Wt of crude extracts (g)	Percentage yield (%) $W2 = \frac{W2-W1}{W1} X100$
259.50	Hexane	10.70	4.12
205.50	Ethyl acetate	19.30	9.39
162.50	Ethanol	23.50	14.5

Wt= weight, W_1= weight of container alone, W_2= weight of extract and container, W_0= weight of the initial dry sample

was eluted with 80 *ml* ethanol. Each isolated component was filtered, evaporated on a water-bath and coded accordingly.

2.8 Chromatography Separation of the Ethylacetate Crude Extract

1.5 *g* of the crude extract was dissolved in 8 *ml* of ethyl acetate 1.3 *ml* of the solution was technically spread on the origin of a PTLC plate with the aid of a Pasteur pipette. After complete evaporation (air- dry for 20 *mins*) the PTLC plate was put into a chromatography tank containing 200 *ml* mixture of chloroform and ethyl acetate in ratio 1:1. At the end of the separation, six distinct bands were obtained. These were separately scrapped into six beakers and the content of each beaker was eluted with 80 *ml* chloroform. Each isolated component was filtered evaporated on a water bath and coded accordingly.

2.9 Chromatography Separation of the Ethanolic Crude Extract

1.5 *g* of the ethanolic crude extract was dissolved in 8ml of ethanol; 1.3 *ml* of the solution was technically spread on the origin of a PTLC plate with the aid of Pasteur pipette. After complete evaporation (about 20 *mins*); the PTLC plate was put into a chromatography tank containing 200 *ml* mixture of ethyl acetate and methanol in ratio 1:1. At the end of the separation three distinct bands were obtained. These were separately scrapped into three beakers and the content of each beaker was eluted with 80 *ml* of diethyl ether. Each isolated component was filtered, evaporated on a water- bath and coded accordingly.

2.10 The Bioassay of the Extracts

The bioassay of the extract was done by using Agar diffusion techniques according to Bett et al. 2007. Muller Hinton agar was prepared by dispensing 8.4 *g* of agar powder into 300 *ml* of distilled water. Hot plate with stirrer was used to homogenize the agar on an autoclave at 121°*C* for 15 minutes. The agar was allowed to cool to temperature of 45°*C* and poured into sterile plates and allowed to solidify. The test organisms (*Klebsiella pneumonia*, *Salmaonel typhi*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E. coli*) were used to seed the plates; a plate per organism. Wells were made in the agar using cork borer, and the extracts were dispensed into the wells and allowed to diffuse into the agar. Tetracycline, an orthodox antibiotic was used as a control. The setup was incubated at 37°*C* for 24 hours. At the end of the incubation period, the zones of inhibitions displayed on the plates were measured in millimeters with the aid of a ruler and were recorded accordingly.

3. RESULTS

3.1 Percentage Yields

The percentage yield of the extracts using the three different extraction media in relation to the weight of the starting materials and weight of the crude extracts is summarized in Table 1.

3.2 Colour of Isolated Fractions

The colour of the different isolated components while using the different extraction media is summarized in Table 2.

3.3 Zone of Inhibition of the Tested Organisms

The zone of inhibitions for each of the tested microorganisms in correlation to the isolated components while using different extraction media is summarized in Table 3.

4. DISCUSSION

The percentage yields of the crude extracts of *Enantia chloratha* using hexane, ethyl acetate and ethanol as extracting media were relatively low compared to the starting material. The percentage yields were 4.12%, 9.39% and 44.49% of hexane, ethyl acetate and ethanolic extracts respectively as shown in Table 1. Only the ethanolic extracts of the Stem-bark appeared to be appreciable high and this is probably because ethanol is highly polar and capable to extract both polar and non-polar components of the plant materials. The two isolated fractions of the hexane extracts of the stem-bark of *Enantia chlorantha* showed positive activities against all the test organisms employed which are EC/SB/HEX/01 and EC/SB/HEX/01 which displayed various zones of inhibition against *Klebsiella pneumonia, Salnnonel typhi, E. coli Staphylococcus aureus* and *pseudomonas aerugmosa* respectively. This is a satisfactory result when compared with the zone of inhibition of the control used which is tetracycline. The remaining fractions of hexane, ethyl acetate and ethanol that are left indicated appreciated activities against the test organisms with the few exception: EC/SB/EtoAC/PTLC/01 which indicated a negative action against *Klebsiella pneumonia, Salnnonel typhi, E.coli* and *Pseudomonas aerugmose* while that of EC/SB/EtoAC/PTLC/03 indicated negative action against *Klebsiella pneumonia* and *Salnnonel typhi.* Finally, EC/SB/EtoAC/PTLC/06 revealed a negative activity against *Klebsiella pneumonia, Pseudomonas aeruginosa.* By using the aid of pharmaceutical improvement and modifications, these isolated fractions can be developed into powerful antibiotics.

Table 2. The result of the isolated fractions from preparative thin layer chromatographic separation and their respective colours

Fraction	Colour
EC/SB/HEX/PTLC/01	Brown
EC/SB/HEX/PTLC/02	Pink
EC/SB/HEX/PTLC/03	Orange
EC/SB/EtoAC/PTLC/01	Brown
EC/SB/ EtoAC/PTLC/02	Pink
EC/SB/ EtoAC/PTLC/03	Grey
EC/SB/ EtoAC/PTLC/04	Light yellow
EC/SB/ EtoAC/PTLC/05	Light brown
EC/SB/ EtoAC/PTLC/06	Blue
EC/SB/ Etoh/PTLC/01	Brown
EC/SB/ Etoh/PTLC/02	Lemon
EC/SB/ Etoh/PTLC/03	Ash

Table 3. Diameter of zone of inhibition of the tested organisms from the three extracts

Isolated components	Diameter of zone inhibition in mm				
	Klebsiella pneumonia	**Salmonell typhi**	***E. coli***	***Staphylococcu aureus***	***Pseudomona aeruginosa***
Control (tetracydin)	30	22	25	18	20
EC/SB/HEX/PTLC/01	24	20	15	25	19
EC/SB/HEX/PTLC/02	20	20	15	10	30
EC/SB/HEX/PTLC/03	25	20	20	25	20
EC/SB/EtoAC/PTLC/01	-	-	-	11	-
EC/SB/ EtoAC /PTLC/02	22	-	10	20	-
EC/SB/ EtoAC /PTLC/03	-	-	15	15	10
EC/SB/ EtoAC /PTLC/04	15	-	12	10	15
EC/SB/ EtoAC /PTLC/05	10	20	8	9	10
EC/SB/ EtoAC /PTLC/06	-	25	13	-	-
EC/SB/ Etoh/PTLC/01	12	20	14	20	-
EC/SB/ Etoh/PTLC/02	15	12	15	30	10
EC/SB/ Etoh /PTLC/03	10	13	30	30	10
Hexane crude extract	25	24	25	20	25
EtoAC crude extract	15	20	20	25	30
Ethanol crude extract	11	15	13	15	30

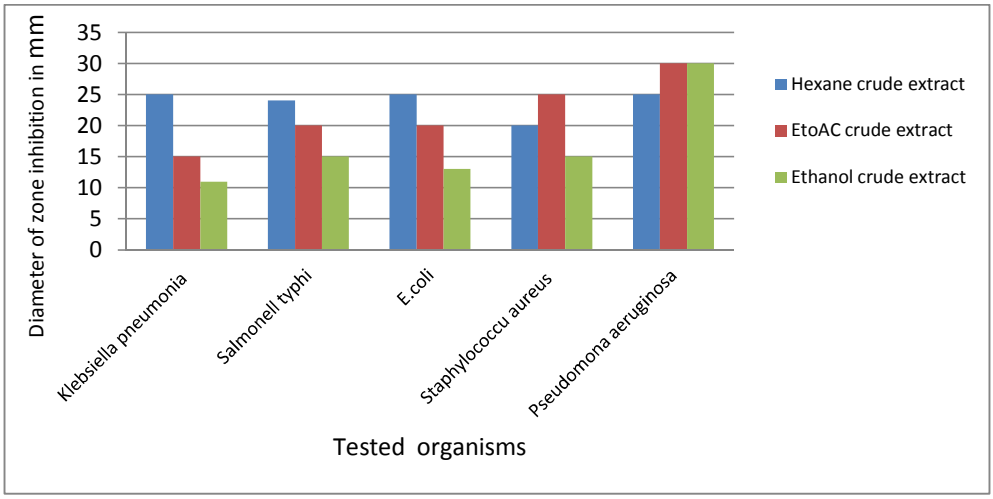

Fig. 1. Comparison of the zone of inhibition of tested organisms from three crude extracts

5. CONCLUSION

The extraction of the stem-back of *Enantia chlorantha* with solvents of increasing polarities hexane, ethyl acetate and ethanol was successful. The chromatographic fractions of hexane, ethyl acetate and ethanol crude products gave satisfactory antibacterial activity against all the test organisms employed. Further research work is hereby recommended for the phytochemical screening, infrared and H.N.M.R. analysis of the isolated compounds. This would probably lead to the structural elucidation of some of the active isolated components.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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