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Phytochemical Screening and Antibacterial Activities of the Root Bark Extracts of *Hippocratea africana* (Willd.) Loes. ex Engl.

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Authors' contributions

This is a collaborative research between all authors. Author ADF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ANM and AOF managed the analyses of the study. Author FVAD managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The root of *Hippocratea africana* (Celastraceae) is used in ethnomedicine in South-Western Nigeria to treat infectious and parasitic diseases. This study aimed at identifying the compound(s) that are responsible for the antimicrobial activities of the roots and also to contribute to the chemistry of the plant species. Investigation of the phytochemical constituents and antimicrobial activity of the n-hexane and methanol root bark extracts of *Hippocratea africana* was carried out. These extracts were subjected to screening of preliminary phytochemical test, which showed the presence of alkaloids, flavonoids, glycosides, phytosterols, phlobatanins and diterpenes. The crude methanol extract exhibited the largest zone of inhibition (16 mm in diameter with 700 mg/mL extract) against

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Morganella morganii and the largest zone of inhibition of the n-hexane crude extract (8 mm in diameter with 700 mg/mL) against *E. coli*. The methanol extract was subjected to column chromatography. Four isolates (A, B C & D) were obtained. Isolates C exhibited largest zone of inhibition (21 mm in diameter at 15 mg/mL) against *Escherichia coli* while isolate D exhibited the lowest zone of inhibition (3.7 mm in diameter at 15 mg/mL) against *Klebsiella neumoniae.*

Keywords: Hippocratea africana extract; antibacterial activity; chemical studies.

1. INTRODUCTION

Plants act as the potent biochemicals and have been the components of phytomedicine from ancient times. In rural areas of developing countries, herbal materials continue to be used as the primary source of medicine [1]. According to the World Health Organization, an estimated 3.5 billion people in the developing world depend on medicinal plants as part of their primary health care [2]. At least 122 compounds, i.e. 80% of which were used for the same or related ethnomedical purposes, have been derived from 94 species occurring worldwide [3], but only 1% has been phytochemically investigated. Of recent, the screening of plant extracts and plant products has demonstrated that higher plants represent a potential source of novel drug prototypes [4].

The roots are used in the treatment of diverse ailments such as fever, body pains, malaria, diarrhea and diabetes [5]. It was reported to exhibit in-*vivo anti*-plasmodial activity with lethal dosage (LD_{50}) of 2.45 mg/kg body weight in mice. [5] also reported that *H. africana* possesses anti-inflammatory, analgesic, anti-pyretic properties and anti-malaria activity. It was also reported that the plant contains important quantities of phytochemicals such as alkaloids, cardiac glycosides and flavonoids [6]. Other studies reported ethanolic root bark extracts of *H. africana* possess promising blood schizontocidal activity. This group reported that chemo-suppressive effect of *H. africana* at 400 and 600 mg/kg were 81.8 and 90.9%, respectively while that of chloroquine at 5 mg/kg was 92.5%. Other species of *Hippocratea* possess anti-inflammatory activities are *H. excelsa* [7] and *H. indica* [8]. [9] reported that there was no significant change in urea concentration but there was significant increase in glucose concentration in tested female group. There was also a slight increase in total protein and albumin concentration which was not significant [10].

H. africana, (Yoruba: *ponju-owiwi,* Hausa: *godyi*, Tiv: *ipungwa*) is a woody wiry stem, with green twigs and bright green leaves; flowers fragrant, petals green, anthers orange; a very variable species; mainly in fringing forest in the savannah regions, savannah woodland, riverine fringes and wide spread in tropical Africa, S. Africa, Madagascar, India, China and Philippines [11]. They are not attacked by termites and are favoured for their durability as binding materials after splitting. The root is used in Nigeria for treatment of skin infections. In Sierra Leone, the leaves of *H. africana,* are made into tisane for cold and occasionally a decoction of the liane for treatment of oedema [12]. This paper reports the potentials of local medicinal plants for their antimicrobial properties. The choice of this plant was based on ethno botanical information from literature on their uses for the treatment of various [11]. This study aimed to determine the qualitative phytochemistry of *H. africana* root bark extract, with view to determine chromatographic fraction and the antibacterial effects of the of the root extract against some selected bacterial species.

2. MATERIALS AND METHODS

2.1 Collection of Plant Samples

The root bark of the plant *H. africana* was collected from Mosunse village, Osiele, Ogun State, Nigeria in the month of March, 2015. The taxonomic identity of the plant was confirmed at the Department of Botany, with voucher number CRUH 042016 University of Lagos.

2.2 Extraction

Fresh root barks of *H. africana* were collected and washed thoroughly in running tap water to remove debris and dust particles (root bark was removed with cutlass) and then rinsed in sterile distilled water, shade dried at room temperature, weighed (465 g) and ground in a mechanical grinder. The powdered material was then kept in airtight containers until use. For extraction of bioactive components, the powdered samples were soaked into 1000 mL of n-hexane and methanol exhaustively and serially each for 72 h

with stirring at 24 h interval respectively. The extracts obtained were filtered, first through muslin cloth then through filter paper and subsequently concentrated to a gummy material under reduced pressure at 50°C by rotary vacuum evaporator. The extracts were then transferred to small vials and stored in the refrigerator.

2.3 Thin Layer Chromatography

The thin layer chromatography technique adopted was that due to Buncak, Paul; (US Patent 4388193) of 1983 [13]. A large number of solvent systems were used to achieve a good resolution. Finally, the solvents hexane: ethyl acetate and ethanol were used [14]. About 0.5 g of the crude extract was dissolved in 2 mL of methanol in a beaker. In each case, 1 cm was measured from the base of the thin layer chromatography plate (TLC Silica gel 60 F_{254}), marked with a pencil and labeled. Capillary tube was used to spot the plates with the crude extract. Small quantity of the concentrated solution was collected with capillary tube by dipping it in the solution. This was then used to spot the plates. The solvents system used for the development of the plates was n-hexane, ethyl acetate and ethanol (24:4:2 volumes). After pre-saturation for 30 mins, the mobile phase was used for the development of the plate. Later the developed plates were dried and observed under UV lamp (264 or 366 nm) to detect the bands on the TLC plates. The movement of the active compounds was expressed by its retention factor values (R_f) which were calculated for the sample. (R_f = distance move by the solute front/ distance moved by the solvent front).

2.4 Preliminary Phytochemical Analysis

The methanol root bark extracts were subjected to standard analysis for the presence of phytoconstituents as described by Harborne [15].

2.5 Test Microorganisms

Four bacterial species were employed as test organisms (isolated from healthy individuals). These included *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae* and *Morganella morganii* which were obtained as fresh pure cultures and identified by the Department of Microbiology, Crawford University, Igbesa, Nigeria. The bacterial strains were maintained on Mueller-Hinton agar slants in the refrigerator at 4°C prior to use.

2.6 Antibacterial Assay

Antibacterial activity was determined against four pathogens by agar well method [16]. Different concentrations of n-hexane and methanol crude extracts of *H. africana* was tested against pathogenic bacteria. The test microorganisms were seeded into respective medium by spread plate method 100 µl (10^6 cells/mL suspensions) with the 24 hours cultures of bacteria grown on nutrient broth medium. Petri dishes (measuring 90 mm diameter) containing 15-20 mL of nutrient agar medium were used. Sterile cork borer was used aseptically to bore five 6 mm holes. A volume of 0.2 mL of various concentrations of crude n-hexane and methanol extracts (150, 600 and 700 mg/mL) were poured into the holes punched on test organism-seeded plates. Gentamicin (15 mg/mL) was used as the reference antibacterial agent. Reconstitution of the extract was done by 20% of extraction solvents (n-hexane and methanol) as negative control. The antibacterial assay plates were incubated at 37°C for 24 hours. After the incubation period, the diameter of inhibition zone was measured in millimeter. All the experiments were performed in duplicates and average diameter zone of inhibition was recorded.

2.7 Column Chromatography of Root Bark Extracts *H. africana*

The powdered, dried root bark (456 g) was extracted serially and exhaustively in turn with *n*-hexane and methanol. On removal of the solvents under reduced pressure, it gave residues of 4.3717 g dark-yellow semi-liquid (0.940% yield) and 123.9099 g brick-red semi-liquid (27.2329% yield) for n-hexane and methanol respectively. The mass 3 g of methanol root extract of *H. africana* was dissolved in methanol. Silica gel (60-200 mesh) was added to the solution of the extract. The mixture was thoroughly stirred in an evaporating dish and air-dried. Cotton wool was introduced into the glass column with the use of a long glass rod, followed by the sea sand which acted as support. The slurry silica gel (60-200 mesh) was introduced into the column and packed smoothly. Process for the isolation and purification of taxols and taxanes from *Taxus* spp US 5279949 A, was adopted for the column chromatographic separation of the sample of *H. africana* [17]. The glass column and its contents were packed uniformly to avoid cracking. The root bark methanol extract of *Hippocratea africana* was introduced gently on the top of the

silica gel, while sea sand was used as support on the top. Thereafter, the eluting solvent (n-hexane) was added while the tap of the column was opened. The elution started with 100% n-hexane and 15 mL fraction was collected each time. The polarity of the eluting solvent was increased gradually, starting with 100% n-hexane then 5% ethyl acetate in n-hexane up to 100% ethyl acetate and eventually using ethanol. Each fraction was concentrated and the concentrated fraction was spotted to identify the number of components present. Fractions with similar TLC properties were combined together. The crystals obtained (A to D) were washed severally with 100% isopropyl alcohol.

3. RESULTS

3.1 High Abundance 27.23% was Obtained from the Methanol Extract, with Least Abundance 0.94% from the N-hexane *H. africana* Extract

The yield from the extraction is shown in Table 1.

The TLC analysis of *H. africana* root bark extracts revealed three components and their measured R_f values as shown in Table 2.

The qualitative phytochemical analysis of root bark extracts of *H. africana* revealed the presence of various phytochemicals, like alkaloids and so on, and absence of phytochemicals like Saponins etc. as shown in Table 3.

Crude Root bark Extract of *H. africana* showed inhibition as high as 15 mm in *M. morganii* and lowest at 8 mm for *E. coli* whereas gentamicin showed highest inhibition of 16 mm in *M. morganii* as presented in Table 4.

The isolated compounds from *H. africana* denoted; A-D. Isolates C showed highest inhibition of 21 mm in *E. coli* and lowest inhibition of 3.7 mm by isolate D in *K. pneumoniae* as presented in Table 5.

Four eluents were obtained from column chromatographic analysis of *H. africana* root bark, after combining fractions with similar TLC profile as presented in Table 6.

Isolated compound D from *H. africana* root bark gave 35.14% yield while the lowest yield was compound A which was 0.006% as presented in Table 7.

Table 1. Percentage yield and colour of *H. africana* root bark extracts

Extract	Weight of plant materials (g)	Weight of extract (g)	Percentage (%) yield	Colour of extract
n-hexane extract	465	4.3717	0.9402	Dark yellow liquid
Methanol	465	123.9099	26.6473	Brick red semi-solid

Table 2. R_f values and TLC analysis of methanol root bark extract of *H. africana*

Plant extract	Developing solvents	Adsorbent	Visualizing agent	No of components	R_f values
Methanol Extract	n-hexane: Ethylacetate: Ethanol (24:4:2)	Silica gel	UV lamp	3	0.20, 0.30, 0.45

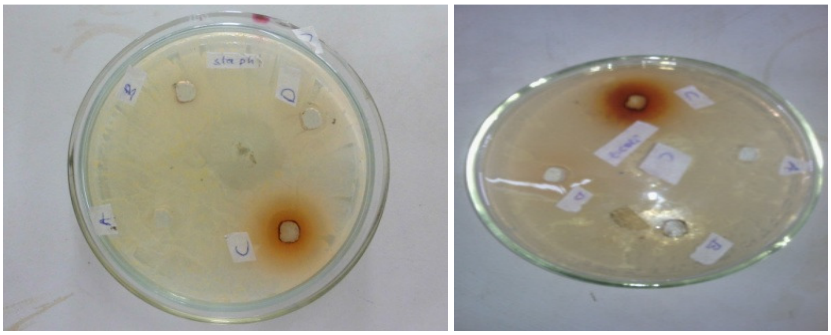

Plate 1. The antibacterial activity test of pure isolates against *S. aureus* and *E. coli*

Table 3. Phytochemical constituents of the root bark extracts of *H. africana*

Phytoconstituents	Methanol extract
Alkaloids	+ve
Glycosides	+ve
Saponins	-ve
Phenolic compounds	-ve
Tannins	-ve
Phytosterols	+ve
Reducing sugars	+ve
Amino acids	-ve
Flavonoids	+ve
Diterpenoids	+ve
Phlobatanins	+ve

"+ve = Present; –ve = Absent"

4. DISCUSSION

Many plants produce secondary metabolites such as alkaloids, flavonoids, phenols, terpenoids, steroids and quinines that are used in pharmaceuticals, cosmetics and pesticide industries. The present study confirms the traditional medical practice and previous pharmacological observations and supplement treatment for other health problems such as fever, malaria, body pains, diabetes and diarrhea [5]. In the present study, phytochemical screening of methanol root extracts showed important indication about the presence of metabolites. The methanol extraction of root barks of *H. africana* gave the highest yield of 27.23%, while the n-hexane extraction gave the least yield of 0.94% (Table 1). Also, the preliminary TLC analysis of *H. africana* root bark extracts revealed the presence of three components and their measured R_f values are shown in Table 2. Alkaloids, glycosides, phlobatannins, flavonoids, reducing sugars, steroids, phytosterols, flavonoids and terpenoids were found to be present in the methanol extracts of *H. africana* root bark (Table 3).

The antibacterial test carried out on the crude root bark extract of *H. africana* revealed zone of inhibition as high as 15 mm in *M. morganii* and lowest at 8 mm for *E. coli* whereas gentamicin (control) showed highest inhibition of 16 mm in *M. morganii* as shown in Table 4. Column chromatographic analysis of *H. africana* results in the isolation of compounds A to D. Compound D, from *H. africana* root bark gave 35.14% yield, while the lowest yield was compound A, which was 0.006%. The antibacterial test carried out on the isolated compounds revealed that compound C showed the highest inhibition of 21 mm in *E. coli* and lowest inhibition of 3.7 mm by Compound D in *K. pneumoniae*. Compound A had no antibacterial activity, while compound B showed various levels of activity in different organism as shown in Table 5.

Table 4. Mean inhibition antibacterial activity of crude root bark extract of *H. africana* against pathogenic bacteria

Pathogenic bacteria	Gentamicin 10 mg/mL	Extract (Zone of inhibition)					
		Methanol extract (mg/ mL)			n-hexane extract (mg/mL)		
		150	600	700	150	600	700
M. morganii	16.0	0.0	14.0	16.0	0.0	0.0	0.0
E. coli	14.0	0.0	12.0	13.0	0.0	0.0	8.0
S. aureus	6.4	0.0	0.0	0.0	0.0	0.0	0.0
K. pneumoniae	5.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 5. Mean inhibition antibacterial activity of pure isolate of root extract of *H. africana* against pathogenic bacteria

Pathogenic bacteria	Gentamicin 10 mg/mL	Extract (Zone of inhibition in mm) methanol extract 15 mg/mL			
		A	B	C	D
M. morganii	17.0	0.0	13.0	14.3	15.1
E. coli	25.0	0.0	10.0	21.0	12.0
S. aureus	24.0	0.0	16.0	15.0	9.0
K. pneumoniae	6.0	0.0	4.0	5.1	3.7

Table 6. Result of the fractionation after combining the eluted fraction

Fraction	Colour and nature	Designation
1-10	Colourless	A
11-25	Light amber	B
26-32	Dark red	C
33-35	Amber	D

Table 7. Result of the fractionation colour, yield and percentage yield

Isolates	Colour	Weight (g)	Percentage yield (%)
A	Colourless	0.0019	0.0060
B	Light amber	0.1669	5.5600
C	Dark red	1.0442	34.8100
D	Amber	1.0543	35.1400

According to WHO report, 80% of the world populations are taking interest in indigenous herbal medicines usually in form of fruits, vegetables, roots, bark or their extracts for the treatment of diseases and for maintenance of health [18]. Phytochemical screening of the plant material under research revealed the presence of alkaloids, flavonoids, glycosides, phytosterols, phlobatanins and diterpenes. The crude methanol extract exhibited the largest zone of inhibition (16 mm in diameter with 700 mg/mL extract) against *Morganella morganii* and the largest zone of inhibition of the n-hexane crude extract (8 mm in diameter with 700 mg/mL) against *E. coli*. The methanol extract was subjected to column chromatography. Three isolates (A, B & C) were obtained. Isolates B exhibited largest zone of inhibition (16 mm in diameter at 150 mg/mL) against *Staphylococcus aureus* while isolate C exhibited zone of inhibition (21 mm in diameter at 150 mg/mL) against *Escherichia coli.* It appeared that *H. africana* could be a potential natural source of new antimicrobial agent. According to Heikens et al. [19], the presence of alkaloids in both extract could partly be responsible for the observed antimicrobial properties as seen from the analysis. Flavonoids are also known to help in alleviating tissue and organ inflammation, free radicals, ulcers, viruses and tumor hence the extracts obtained from this plant could be of great assistance to people with inflammatory disorders. Anowi et al. [20], mentioned in their report that the presence of flavonoids in an extract may be the reason for its use in the treatment of intestinal disorders. Antibacterial activity may also be attributed to the presence glycosides as observed by Ren-Bo An et al. [21] and Kaur et al. [22]. The results of the present study also complement the traditional usage of the studied plants which possess several known and unknown bioactive compounds with bio-activity. Identification of these isolated bioactive compounds showed that new drugs can be formulated to treat various diseases and disorders. TLC profiling of root bark extracts gave a striking separation, a pointer to the presence of a number of phytochemicals. Various phytochemicals gave different R_f values in different solvent systems. Different R_f values of the compound also reflected their level of polarity. This information will help in the selection of appropriate solvent systems for further separation of compounds from the plant extracts. It appeared that *Hippocratea africana* could be a potential natural source of new antimicrobial agent.

5. CONCLUSION

The plant screened for phytochemical constituents was seen to contain a lot of organic compounds and hence showed a high potential for use as a source of drugs for treating ailments and also for health improvement. The possible application of this plant in medical treatment could be as a result of the presence of various compounds that are vital for good health. These findings suggest that *H. africana* root bark could be a potential source of natural antibiotics. The root bark of *H. africana* can provide lead molecules which could be a useful substrate for the synthesis of new broad spectrum antibiotics for the treatment of infections caused by microorganisms. Further identification and characterization of the active compounds will be our priority in future studies.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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