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FATTY ACIDS OF *HIPPOCRATEA AFRICANA* ROOT: ANTIOXIDANT AND ANTI-MICROBIAL ACTIVITY

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ABSTRACT: In this study, the fatty acid content, antioxidant activities and antimicrobial potential of oil obtained from the root of *Hippocratea africana* was investigated. Results showed that the oil was characterized by oleic acid (65.73%), palmitic acid (20.34%) and linoleic acid (6.90%) as the most abundant fatty acids with a oleic acid/linoleic acid ratio of 9.52. The oil possessed potent antioxidant activity (IC_{50} = 75.00µg/ml) and exhibited significant metal chelating potentials (EC_{50}= 7.4mg/ml). It also showed notable antimicrobial activity against the tested human pathogens. MIC values indicated bactericidal activity against *S. aureus* (MIC =0.05mg/ml), *S. pyogenes* (MIC =0.17mg/ml) and fungicidal activity against *C. albican* (MIC =0.06mg/ml). These results support to some extent, the medicinal potential of *Hippocratea africana* root.

INTRODUCTION: *Hippocratea africana* (Willd.) Loes is a plant of the *Hippocrateaceae* family. It is a perennial climber without hairs and reproduces from seeds. The plant is widely distributed in tropical Africa. The root of the plant is used traditionally by the Ibibio's of the Niger Delta region of Nigeria in the treatment of various ailments such as fever, malaria, body pains, diabetes and diarrhoea[1]. Literature survey has revealed little work on this plant species. The plant root extract has also been reported to possess *in vivo* antiplasmodial activity, analgesic, anti-inflammatory, antidiarrhoel, antiulcer, hypolipidemic, as well as antidiabetic activity [2-4].

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In previous studies, isolated compounds from genus *Hippocratea* include lupeol acceate, hippocrateine I-III, mayteine, pentacyclic tritepenoids, β-sitosterol, epicatechin, friedelin, canophyllal, sesquiterpene evinonate alkaloids, as well as benzyl isothiocyanates. These compounds exhibited varying biological activities including anticancer activity, gastro protective properties, antimicrobial and anti-inflammatory activities, antiguardial and antifeedant activities [5-10]. The fatty acid and antioxidant activity of tropical species of *Hippocratea africana* root has not been reported.

In this study, we report the content of fatty acids as well as the potential antioxidant and antimicrobial activity of *Hippocratea africana* root oil

MATERIALS AND METHODS:

Collection and Extraction of Oil from Test Plant

Roots of *Hippocratea africana* were collected from the wild in Uyo, Akwa Ibom State, in November, 2012, and authenticated by a taxonomist in the

Department of Botany and Ecological Studies, University of Uyo, then dried at 40^{o}C for two weeks. The roots were carefully derived and oil was extracted from powdered plant organ by maceration in petroleum ether (40-60^{o}C) for 48 hours. Filtration and evaporation *in vacuo* gave yellow oil.

Determination of fatty acid profile of plant root: The fatty acid content of the root oil was determined by gas chromatography. Fifty microlitre of oil was solubilised in 950µl of hexane and esterified using sodium ethoxide [12]. The fatty acid composition was analysed by GC (Hewlett-Packard 6890) equipped with a flame ionization detector (FID), a split injector, and an HP Innowax column (30m x 0.25mm, d_f 0.25µm). The oven temperature programming was initially held at 60oC for two minutes, heated at 8^{o}C/min up to 240^{o}C and maintained isotherm for 15 min. The temperatures used in the injector and detector were 250 and320^{o}C respectively. Samples of 1µL were injected adopting a split ratio of 1:20. Hydrogen was used as the carrier gas at a linear speed of30ml/L Fatty acids were identified by comparison of retention times of FAME with the standard component FAME mixture.

Determination of Antioxidant potential of root oil: The antioxidant potential of the root oil was determined by evaluating its DPPH radical scavenging activity and by measuring its metal chelating potentials.

Evaluation of DPPH Activity: Precisely 1ml of *H. africana* root oil at varying concentrations was mixed with1ml of 0.004% methanol solution of DPPH. The mixture was shaken vigorously and allowed to stand for 30 min at room temperature in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 517nm. The procedure was repeated for the blank and control. The radical scavenging activity was calculated using the equation:

DPPH scavenging effect (%)

$$= [(A_{blank} - A_{sample})/A_{blank}] \times 100.$$

Sample concentration providing EC_{50} was calculated from the graph plotting inhibition percentage against extract concentration [12]. BHA and Vitamin E were used as positive controls.

Evaluation of Metal Chelating Activity: Metal chelating activity was determined according to the method of Decker and Welch[13], with some modifications. Briefly, 0.5ml of oil was mixed with 0.05ml of 2mM$FeCl_2$ and 0.1ml of 5mM ferrozine. The total volume was diluted with 2ml methanol. Then, the mixture was shaken vigorously and left standing at room temperature for 10mins. After the mixture had reached equilibrium, the absorbance of the solution was measured spectrophotometrically at 562nm. The percentage inhibition rate of ferrozine – Fe^{2+} complex formation was calculated using the formula:

Scavenging activity (%)

$$= [(A_{control} - A_{sample})/A_{control}] \times 100$$

Where $A_{control}$ = absorbance of ferrozine – Fe^{2+} complex, and A_{sample} = absorbance of sample. EDTA was used as a positive control.

Antimicrobial Activity: Antimicrobial activity of *H. africana* root oil was assayed by the disc diffusion method [15]. Two gram positive (*Staphylococcus aureus*, *Streptococcus pyogenes*), three gram-negative bacteria (*Salmonella typhi*, *Pseudomonas aeruginosa* and *Escherichia coli*) and a yeast (*Candida albican*) were assayed. Innocula of test isolates were prepared with fresh cultures of microbial strains and cultivated on tryptic soy agar for 24 hours at 37^{o}C. Yeast culture was incubated on Sabouraud agar at 28^{o}C for 48 hours.

The density of the innocula was set to 0.5McFarlands unit. Empty sterilized disks of 6mm diameter were impregnated with 1ml of oil each. 1 ml of the innocula was mixed with 17ml of Muller-Hinton agar and Sabouraud agar respectively and poured into sterile petri dishes. The disc impregnated with sample was then placed on top of the inoculated agar and pressed slightly. Plates were incubated at 37^{o}C for 24hours (for bacterial strains) and at 28^{o}C for 48hours (for fungal strain). Inhibition zones formed on the medium were evaluated in millimeters. Gentamicin, ciprofloxacin, penicillin and fluconazole were used as positive controls [15].

Minimum inhibitory concentration (MIC) of the oil was determined using the micro-dilution broth susceptibility assay in accordance with the Clinical and Laboratory Standards Institute (NCCLS) [16]. MIC is the lowest concentration of oil that inhibits the growth of microorganism.

RESULTS AND DISCUSSION: The fatty acid content of *Hippocratea africana* root oil was determined by gas chromatography. Obtained results (**Table 1**) indicate that the oil is especially characterized by oleic acid (65.73%) palmitic acid (20.34%) and linoleic acid (6.90%).

The total saturated fatty acid percentage was 25.11%, while the total amount of unsaturated fatty acid was 74.87%, of which 65.73% was monounsaturated. The oil also had an oleic acid/linoleic acid ratio of 9.52, indicating stable oil capable of preventing the formation of bad cholesterol (LDL). The high content of monounsaturated fatty acid in the plant root confers on it certain benefits. Monounsaturated fats are associated with decreased low density lipoprotein (LDL) cholesterol and possibly increased high density lipoprotein (HDL) cholesterol [16]. Also, oleic acid has been reported to control lipid oxidation through signalling/ transcriptional pathway, and may provide possibilities to treat metabolic diseases associated with lipid dysfunction [17].

It has also been reported that oleic acid decreases the content of saturated long chain fatty acids in cultured skin fibroblast from patients with adrenoleukodystrophy (ALD) [18]; hence the oil may be seen as a good source of unsaturated fatty acids. Keskin and Kacar [19] reported lower values (5.78-25.7%) of oleic acid in the roots of some *Astragalus* species. *Hippocratea africana* root also contained 9.13% of EFA's (linoleic and linolenic acid). Linoleic and linolenic acids have been reported to nourish skin, hair and nails. They also help eliminate eczema, psoriasis and dandruff and help prevent hair loss [20].

Our values are lower than reports for *Nyctanthes arbortristis* root [21] but higher than values recorded for *Vallaris solanace* (Roth) *Kuntze* root bark [22]. These variations may reflect influence of factors such as vegetation, degree of ripeness, climate, soil cultivation and test methodologies.

TABLE 1: FATTY ACID PROFILE OF *HIPPOCRATEA AFRICANA* ROOT OIL

Fatty acid	Structure	%
Myristic	(C14:0)	0.23 ± 0.02
Palmitic	(C16:0)	20.34 ± 1.09
Stearic	(C18:0)	4.40 ± 0.21
Behenic	(C22:0)	0.03 ± 0.01
Lignoceric	(C24:0)	0.07 ± 0.01
Σ SFA		**25.11 ± 1.34**
Palmitoleic	(C16:1)	0.01 ± 0.01
Oleic	(C18:1)	65.73 + 2.14
Σ MUFA		**65.74 + 2.15**
Linoleic acid	(C18:2)	6.90 ± 0.62
Linolenic acid	(C18:3)	2.23 ± 0.02
Σ PUFA		**9.13 ± 0.64**

Antioxidant activity: DPPH assay is one of the most widely used method for evaluating the antioxidant activity of plant extracts. In this assay, the antioxidants ability to scavenge a stable radical DPPH is measured. The radical (violet colour in MeOH solution) react with suitable reducing agent, during which the electrons become paired off and the solution is progressively reduced (stoichiometrically) to a yellow coloured product, diphenylpicrylhydrazine, with the addition of extracts in a concentration dependent manner at 517nm.

In this study, oil from the root of *Hippocratea africana* demonstrated notable antiradical activity, and this increased with increasing concentration of the oil **(Figure 1)**.

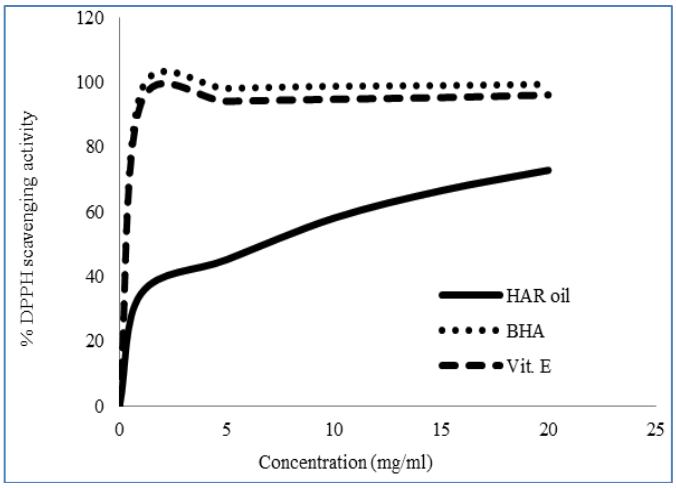

FIGURE 1: DPPH SCAVENGING ACTIVITY OF HIPPOCRATEA AFRICANA ROOT OIL

The concentration at which the DPPH radical was scavenged by 50% (EC_{50}) was extrapolated and found to be 9.80mg/ml for the oil (**Table 2).** The obtained EC_{50} value for the oil was however lower than the activity of the positive controls BHA and vitamin E with EC_{50} values of 0.38 and 0.50 mg/ml respectively. The results obtained in the present work, have shown that the root oil of *Hippocratea africana* is a more effective antioxidant than the ethanolic root bark extract of *Salacia campestris* (Hippocrateaceae) [23]. The observed antioxidant activity may be related to its high levels of unsaturated fatty acids and other lipophilic phytochemicals such as tocopherols, carotenoids, pigments, etc. not evaluated in this study.

Metal chelating ability: Ferrous ion is known to be good pro-oxidant and phenolic compounds have the ability to chelate pro-oxidant metal ions such as iron, thus preventing free- radical formation [29]. The chelating ability of *H. africana* root oil increased in a dose-dependent manner (**Figure 2**). At 5mg/ml, metal chelating ability of the oil was 35.7 %; this increased to 55.6% at 20mg/ml while that of the standard tended to remain constant at higher concentration likely due to the limitation of Beer - Lamberts law. EC_{50} values (**Table 2**) indicated that the activity of the oil was lower than that of EDTA which is known to be an excellent metal chelator.

TABLE 2: EC_{50} VALUES OF *HIPPOCRATEA AFRICANA* ROOT OIL.

	EC_{50} value* (mg/ml)	
	DPPH radical scavenging activity	**Metal chelating ability**
Hippocratea africana root oil	9.80	14.22
BHA	0.38	-
Vit. E	0.50	-
EDTA	-	0.01

*EC_{50} value, the effective concentration at which 1,1-diphenyl, 2, picrylhydrazyl (DPPH) radical were scavenged by 50%, ferrous ions were chelated by 50%, the absorbance was 0.5 for ferric reducing antioxidant power. EC_{50} value was obtained by interpolation from linear regression analysis.

Reports on the metal chelating abilities of Hippocrateaceae is rare, therefore it becomes difficult to compare our result with previous studies from the same family. However, lower chelating ability was reported for *Tabernaemontana corymosa*[24], while *Ferula gummosa* Boiss roots exhibited higher chelating properties [25].

Antimicrobial activity: The antimicrobial activity of oil from *Hippocratea africana* root against human pathogens is shown in **Table 3**. The oil exhibited notable antimicrobial activity, and was potent against *S. aureus* (31mm, 67% inhibition),*C. albicans* (22mm, 58%inhibition) and *S. pyogenes* (16mm, 41% inhibition).

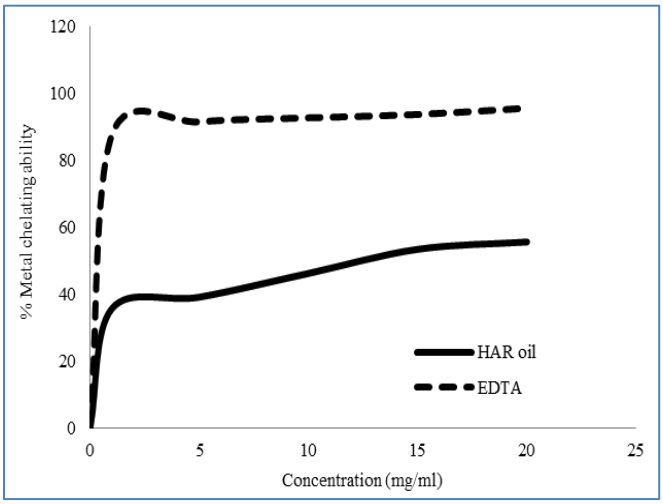

FIGURE 2: METAL CHELATING ABILITY OF HIPPOCRATEA AFRICANA ROOT OIL

An equal and more than 15mm mean zone of inhibition in the disc diffusion method were documented and the minimum inhibitory concentration (MIC) determined by the broth dilution method. MIC values indicated bactericidal activity (MIC < 0.8mg/ml) against *S. aureus, S. pyogenes* and *C. albicans*, but bacteriostatic (MIC = 0.8-2mg/ml) against *P. vulgaris.* Other bacterial isolates tested were resistant to the plant root. The values recorded were however less potent than the control antibiotics used. Generally, the plant exhibited better antimicrobial activity against Gram positive bacteria than Gram negative bacteria. This is in agreement with reports from Kivrak [15] who observed higher activity towards gram positive bacteria from the oils of *S. potentillifolia*. Also, Miller [26] reported that long chain fatty acids have higher antimicrobial activity against gram positive than gram negative bacteria. The antimicrobial activity of the plant root may in part, be attributed to its content of saturated/unsaturated fatty acids.

Lauric acid has been shown to possess antibacterial activity [27]. Long chain unsaturated fatty acids such as linoleic and oleic acids have been reported to be bactericidal to important pathogenic microorganisms such as *S. aureus, H. pylori, C. albicans* and *E. coli* [28].

TABLE 3: ANTIMICROBIAL ACTIVITY OF H. *AFRICANA* ROOT OIL

	Oil*			Reference antibiotics*	
	Disc diffusion (mm) (%)	MIC (mg/ml)		Disc diffusion (mm) (%)	MIC (mg/ml)
Staphylococcus aureus	31 (67)	0.05	Penicillin	46 (100)	11
Streptococcus pyogenes	16 (41)	0.17	Gentamicin	39 (100)	10
Salmonella typhi	11 (26)	nt	Ciprofloxacin	43 (100)	8.5
Escherichia coli	9 (23)	nt	Penicillin	40 (100)	11.2
Proteus vulgaris	15 (33)	1.9	Ciprofloxacin	45 (100)	8.6
Candida albicans	22 (58)	0.06	Fluconazole	38 (100)	9.1

*Values are zones of inhibition in mm; (%) =percentage efficacy relative to the standard drug. nt= not tested

CONCLUSION: In the present study, the fatty acid profile, antioxidant activity and antimicrobial potential of oil obtained from the root of *Hippocratea africana* were investigated. The oil was most abundant in the unsaturated fatty acids-oleic and linoleic acid and the saturated fatty acid-palmitic acid. The oil exhibited antimicrobial activities against the tested microorganisms, but was particularly active against *Staphylococcus aureus, Streptococcus pyogenes* and *Candida albicans*. The plant root possessed notable antioxidant activity, which was supported by its good metal chelating abilities. Further work will focus on the fractionation and isolation of the specific compounds from the oil responsible for the observed activity.

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