AENSI Journals

Australian Journal of Basic and Applied Sciences

ISSN:1991-8178

Journal home page: www.ajbasweb.com

Fatty Acid Composition, Antioxidant and Antimicrobial Activity Of *Homalium Letestui* Stem

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ARTICLE INFO

Article history:
Received 25 January 2014
Received in revised form
8 April 2014
Accepted 20 April 2014
Available online 10 May 2014

Keywords:
Homalium letestui, antioxidant activity, fatty acid profile, antimicrobial activity

ABSTRACT

Background*: Homalium letestui* is a medicinal plant in the Niger Delta region of Nigeria used for the treatment of various ailments. **Objective**: The fatty acid composition, *in–vitro* antioxidant and antimicrobial activity of *Homalium letestui* stem (HLS) oil was studied using standard methods. **Results**: Oleic, linoleic and palmitic acids were the most abundant fatty acids (65.35, 7.32 and 19.95% respectively). Also, the oil showed significant DPPH radical scavenging activity (EC_{50} = 13.02mg/ml), moderate metal chelating activity (EC_{50} = 19.58mg/ml) and notable ferric reducing potential (EC_{50} = 10.80mg/ml), with a total phenolic content of 56.36mgGAE/g. Antimicrobial analysis using the disc diffusion method revealed promising antimicrobial activity against *S. pyogenes* (21.1mm), *C. albicans* (18.6mm), *S. aureus* (18.2mm), and *E. coli* (16.4mm). Based on the broth dilution method, the oil showed minimum inhibitory concentration (MIC) values against *S. pyogenes* (0.41mg/ml) and *E. coli* (0.65mg/ml). **Conclusion**: The present results suggest that *Homalium letestui* stem oil possess notable antioxidant and antimicrobial properties and supports to some extent, its use in traditional medicine.

To Cite This Article: Basil Ita, Raphael Ngochindo., Fatty Acid Composition, Antioxidant and Antimicrobial Activity Of *Homalium Letestui* Stem. ***Aust. J. Basic & Appl. Sci.,*** *8(6): 416-422, 2014*

INTRODUCTION

Homalium letestui Pellegr (Flacourtiaceae) is a forest tree growing up to 80 – 100 ft and found in the rain forest of West Africa (Hutchison and Daziel, 1963). The plant parts, particularly the stem bark and root are used in various decoctions traditionally by the Ibibio's of the Niger Delta to treat stomach ulcer and malaria, as well as an aphrodisiac. Work done on this particular plant species is scanty. The antiplasmodial and antidiabetic activities of the ethanolic root extract have been reported (Okokon *et al.*, 2006; Okokon *et al*., 2007).

The stem extract has been reported to possess cytotoxic, immunomodulatory, and antileishmanial activities (Okokon *et al*., 2013a) as well as anti-inflammatory and antinociceptive activities (Okokon *et al*., 2013b). From the family Flacourtiaceae and other *Homalium* species, the anticancer (Ismail *et al*., 2012), antioxidant (Madan *et al*., 2009; Tyagi *et al*.,2010), antileishmanial, antitrypanosomal and antitrichomonal (Desrivot *et al*.,2007), antibacterial (Chung *et al*., 2004), antiviral (Ishikawa *et al*., 2004), cytotoxic (Silva *et al*., 2008; Salvador *et al*., 2011), antifilaricidal (Cho-Ngwa *et al*., 2010) and anthelmintic activity (Gnananath *et al*.,2012)of extracts have also been reported. In the present work, we report the *in-vitro* antioxidant potential and antimicrobial activity of fatty acids from *Homalium letestui* stem using various models.

MATERIALS AND METHODS

Collection and Extraction of Oil from Test Plant:

Stems of *Homalium letestui* were collected from the wild in Uyo, Akwa Ibom State, in December, 2010, and authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, then sun-dried for two weeks. The stems were carefully derived, ground and oil was extracted by maceration in petroleum ether (40-60^{o}C) for 48 hours at room temperature. Filtration and evaporation *in vacuo* gave a light yellow oil.

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Determination of fatty acid profile:

The fatty acid content of oil from the stems of *Homalium letestui* (HLS) was determined by gas chromatography. Fifty microlitre of oil was solubilised in 950µl of hexane and esterified using sodium ethoxide (Christie, 1993). 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 60^{o}C 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 of 30ml/L. Fatty acids was identified by comparison of retention times of FAME with the standard component FAME mixture.

Determination of Antioxidant potential:

Evaluation of DPPH Activity:

Precisely 1ml of oil sample 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 50% effective concentration (EC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration (Kivrak *et al*., 2009). 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 (1990), 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.

Evaluation of Ferric Reducing Antioxidant Power (FRAP):

The reducing power of the oil was determined according to the method of Oyiazu (1986). The oil (0.1 - 20mg/ml) in ethanol (2.5ml) was mixed with 2.5ml of 200mM sodium phosphate buffer (pH 6.60 and 2.5ml of 1% potassium ferricyanide and the mixture was incubated at 50^{o}C for 20 minutes. After 2.5 ml of 10% trichloroacetic acid (w/v) was added, the mixture eas centrifuged at 200g for 19 minutes. The upper layer(5ml) was mixed with 5ml of deionised water and 1ml of 0.1% ferric chloride and the absorbance was measured at 700nm against a blank. A higher absorbance indicated a higher reducing power. EC_{50} value (mg/ml) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation. Ascorbic acid and BHA were used as positive controls.

Determination of Total Phenolics:

The concentration of total phenolics in the oil was determined with the Folin- Ciocalteu reagent using the method of Lister and Wilson (2001). 0.1ml of oil was dissolved in 0.5ml (1/10 dilution) of the Folin-Ciocalteu reagent and 1ml of water/methanol (1:2) was added. The solutions were mixed and incubated at room temperature for 1 min. After 1 min, 1.5ml of 20% Na_2CO_3 solution was added. The final mixture was shaken and incubated for 2 h in the dark at room temperature. The absorbance of the oil was measured at 760nm using a UV-Vis spectrophotometer. Gallic acid was employed as the standard and the results expressed in mg of gallic acid per gram (mgGAE/g) of oil.

Antimicrobial Activity:

Antimicrobial activity of *H. letestui* stem oil was assayed by the disc diffusion method (Kivrak *et al*., 2009). Two Gram positive (*Staphylococcus aureus*, *Streptococcus pyogenes*), three Gram-negative bacteria (*Salmonella typhi*, *Pseudomonas aeruginosa* and *Escherichia coli*) and a yeast (*Candida albicans*) 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 millimetres. Gentamicin, ciprofloxacin, penicillin and fluconazole were used as positive controls (Kosalec *et al*., 2013).
The activity index (AI) of the oil against the human pathogens was calculated using the formula:
Activity Index (AI) = zone of inhibition of oil /zone of inhibition for the standard antibiotic drug.

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, 1999). MIC is the lowest concentration of oil that inhibits the growth of microorganism.

Results:

Fatty acid profile:

The light yellow oil obtained by maceration of the stems of *H. letestui* in petroleum ether was subjected to gas chromatographic analysis to determine its content of fatty acids and compared with a standard mixture of fatty acid methyl ester (FAME). The result of the fatty acid analysis is shown in Table 1.

Table 1: Fatty acid profile of *Homalium letestui* stems oil.

Fatty acid	Structure	%	R_t
Myristic	(C14:0)	0.31	14.70
Palmitic	(C16:0)	19.95	16.04
Stearic	(C18:0)	4.54	18.16
Behenic	(C22:0)	0.06	23.97
Lignoceric	(C24:0)	0.13	25.37
Σ SFA		**24.99**	
Palmitoleic	(C16:1)	0.01	16.46
Oleic	(C18:1)	65.36	19.52
Σ MUFA		**65.37**	
Linoleic acid	(C18:2)	7.32	21.03
Linolenic acid	(C18:3)	2.28	21.82
Σ PUFA		**9.60**	

Results revealed oleic and linoleic acids (65.36 and 7.32% respectively) as the most abundant unsaturated fatty acid with palmitic acid (19.95%) being the most abundant saturated fatty acid.

Antioxidant Potential:

The antioxidant activity of *Homalium letestui* stem oil was analysed using different models:- DPPH radical scavenging activity, metal chelating activity and ferric reducing activity.

DPPH radical scavenging activity increased in a dose-dependent manner. At 1mg/ml, the oil exhibited a radical scavenging activity of 28.41%; this increased to 68.81% at 20mg/ml (Fig.1). EC_{50} values for the oil and controls are given in Table 2.

Table 2: EC_{50} values of *Homalium letestui* stem oil (HLS) and reference compounds.

	EC_{50} value* (mg/ml).		
	Radical scavenging activity	Metal chelating ability	Ferric reducing antioxidant power
HLS	13.02	19.58	10.80
BHA	0.38	-	0.60
Vit. E	0.50	-	-
Vit.C	-	-	0.05
EDTA	-	0.01	-
Total phenolic content[a]	= 56.36		

*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. [a] = mgGAE/g oil

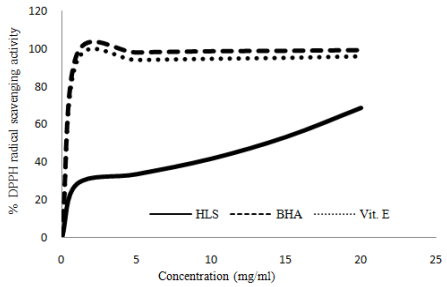

Fig. 1: DPPH radical scavenging activity of HLS oil.

The ability to chelate metals was interrupted by the oil in a dose dependent manner (Fig. 2). At 1mg/ml, metal chelating ability was 30.1%; this increased to 37.8% at 10mg/ml. The oil showed a maximum chelating potential of 45.6% at 20mg/ml with an EC_{50} of 19.58mg/ml.

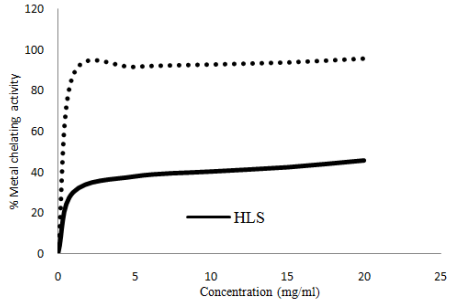

Fig.2: Metal chelating activity of HLS oil.

Like metal chelating activity, the ferric reducing antioxidant power of the oil increased with increasing concentrations of oil (Fig. 3) and was potent at concentrations of 10mg/ml and above. Reducing power was of the order Vit. C (EC_{50}= 0.05mg/ml) > BHA (EC_{50} = 0.06mg/ml) > HLS oil (EC_{50} = 10.80mg/ml).

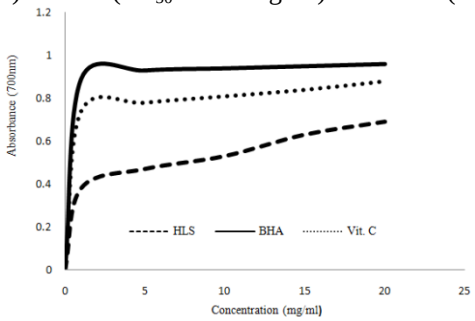

Fig. 3: Ferric reducing antioxidant power of HLS oil.

Antimicrobial activity:

The antimicrobial activities of oil from *Homalium letestui* stem at varying concentrations was determined by the disc diffusion method and the mean value of inhibition zone recorded in millimetre diameter. The activity index was also determined and the minimum inhibitory concentration (MIC) assayed by the broth dilution method. These results are given in Table 3.

Table 3: Antimicrobial activity of HLS oil and reference antibiotics on selected human pathogens.

	HLS oil			RA		
Microorganism	IZ	AI	MIC[a]	IZ	AI	MIC[b]
Staphylococcus aureus	18.2	0.39	1.38	46.3	1	11.00
Streptococcus pyogenes	27.1	0.67	0.41	39.1	1	10.00
Salmonella typhi	11.2	0.26	NT	43.4	1	8.50
Escherichia coli	16.4	0.40	0.65	40.4	1	11.20
Proteus vulgaris	12.5	0.26	NT	45.3	1	8.60
Candida albican	18.6	0.47	1.42	38.2	1	9.10

IZ = inhibition zone in mm; AI= activity index; MIC[a] = minimum inhibitory concentration in mg/ml; MIC [b] = minimum inhibitory concentration in µg/ml. RA = reference antibiotics; *Staphylococcus aureus*- Penicillin; *Streptococcus pyogenes* – Gentamicin; *Salmonella typhi*- Ciprofloxacin; *Escherichia coli*- Penicillin; *Proteus vulgaris* – Ciprofloxacin; *Candida albicans* - Fluconazole

Discussion:

Fatty acid profile:

The oil was particularly rich in the monounsaturated fatty acid oleic acid constituting about 65.36% of the total fatty acid content. Saturated fatty acids constituted about 24.99 % of the total fatty acid content, with palmitic and stearic acids (19.95% and 4.54% respectively) being the most abundant. The essential fatty acids linoleic and linolenic acids were also present at levels of 7.32 and 2.28 % respectively, accounting for approximately 9.60 % of the total fatty acid content. The significant amount of oleic acid in the stem may confer on it certain health benefits. Consumption of oleic and linoleic acids has been associated with decreased low density lipoprotein cholesterol, hypotensive effects as well as hindering the progression of adrenoleukodystrophy (Rizzo *et al*., 1986), thus the stem oil may serve as a good source of these acids.

However, our values are higher than reports for fatty acids from the stem oil of *Maytenus royleanus* (Uddin *et al*., 2012).

Antioxidant potential:

The ability to scavenge radicals is of utmost importance due to the deleterious effects of free radicals in biological systems. Overproduction of these free radicals may lead to oxidative damage to biomolecules such as lipids, DNA and protein. Primary antioxidants react with $DPPH^{\cdot}$ and convert it to 1, 1- diphenyl-2-picryl hydrazine, resulting in the bleaching of the purple colour of DPPH solution at 517nm. This is usually thought to be due to the their hydrogen donating abilities. DPPH radical scavenging activity of the stem oil increased in a dose-dependent manner. At 1mg/ml, the oil exhibited a radical scavenging activity of 28.41%; this increased to 68.81% at 20mg/ml (Fig.1). However, these were lower than values obtained for BHA and Vit. E used as reference radical scavengers in this study. EC_{50} values indicated BHA (0.38mg/ml) > Vit. E (0.50mg/ml) > HLS oil (13.02mg/ml). Lower EC_{50} values indicate higher radical scavenging activity. Results indicate that the oil under study had moderate proton donating ability and could serve as free radical inhibitors. This may be attributed to the synergistic effects of lipophylic phytochemicals such as carotenoids, pigments, tocopherols and polyphenolic compounds present in the oil which are known to be effective free radical scavengers (Luzia and Jorge, 2010). Secondary antioxidant activity was measured using the metal chelating activity and the ferric reducing antioxidant power. Metal ions have been reported to cause lipid peroxidation which could lead to the production of free radicals; hence metal chelating activity indicates antiradical and antioxidant properties. In this study, the oil exhibited promising metal chelating ability, which increased with increasing concentration of the oil. The observed activity was however lower than the control EDTA which is known to be an excellent metal chelator. EC_{50} values indicated that the contents of the stem oil (EC_{50}= 19.58mg/ml) were less effective in chelating metal ions than *Flacourtia sepiaria* Roxb (Sreejith *et al*., 2013).

Like metal chelating ability, ferric reducing antioxidant power (FRAP) increased with increasing concentration of the oil. Higher FRAP values give higher antioxidant capacity because FRAP is based on reducing ferric ion, where antioxidants are the reducing agent. Results indicated that the oil had notable ferric ion (Fe^{3+}) reducing antioxidant power, but which was however less efficient than the controls Vit. C and BHA.

Antimicrobial activity:

The oil was bactericidal against *S. pyogenes* (27mm, AI-0.67, MIC = 0.41mg/ml) and *E. coli* (16mm, AI-0.40, MIC=0.65mg/ml); bacteristatic against *S. aureus* ((18mm, AI-0.39, MIC=1.38mg/ml) and *C. albicans* ((18mm, AI- 0.47, MIC 1.42mg/ml) and resistant to the rest. However the oil was less potent than the reference antibiotics used. The most abundant compounds in the oil are oleic acid (65.7%), palmitic acid (20.34%) and linoleic acid (6.89%) and these fatty acids have been shown to exhibit antimicrobial properties, particularly against gram positive bacteria and yeast (Kabara *et al*., 1972). Lauric acid has been shown to possess antibacterial activity (Ouattara *et al*., 1997). 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* (Farrington *et al*., 1992);*H. pylori* (Hazell and Graham, 1990); *C. albican* (Bergsson *et al*., 2001) and *E. coli*. Also, the antimicrobial activity of phenolic compounds have been documented (Yoshikawa *et al*., 2008), thus, it could be suggested that the observed antimicrobial activity of HLS oil could be associated with its content of fatty acids and phenolic compounds.

In conclusion, studies on *H. letestui* stem oil indicated potent antioxidant and antimicrobial properties, which could lead to the establishment of some valuable compound that can be used to formulate new and more potent drugs. Further studies are needed to identify the biologically active compounds and determine their efficacy.

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