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ELLAGIC ACID DERIVATIVE AND OTHER COMPOUNDS FROM *ALCHORNEA CORDIFOLIA* ROOT BARK EXHIBITED STRONG ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES

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ABSTRACT

Chemical investigation of the ethyl acetate fraction of the methanol root bark extract of *Alchornea cordifolia* led to the isolation of an ellagic acid derivative, 3, 3'-di-O-methylellagic acid, and the detection of other phenolic compounds. The ethyl acetate sub-fractions were screened for their ability to scavenge 2,2-diphenyl-1-picrylhydrazyl radical, while the isolated compound was screened for its inhibitory effect against bacteria and fungi isolates. The chemical structure of the isolated compound was confirmed by 1D and 2D NMR, while those of the detected compounds were deduced from HPLC-DAD analysis. The fractions exhibited dose dependent antioxidant activity with IC_{50} values in the range of 82.8 to 80.0 µg/ml. The compound also exhibited good antimicrobial activity against *Staphylococcus aureu*, *Bacillus subtilis* and *Escherichia coli* with MIC values in the range of 1.5 -0.375 mg/ml. It can be concluded that the root bark extract of *A. cordifolia* and its phenolic compound exhibited strong antioxidant as well as antimicrobial activities. These findings further support the ethnomedicinal use of the root back extracts of this plant material in the management of bacterial infections and offer great prospects for the isolated compound as lead candidate in drug discovery.

KEYWORDS: Antioxidant, Antimicrobial, *Alchornea cordifolia*, Ellagic acid, Phenolic compounds

INTRODUCTION

Natural products and their derivatives have continually played an important role in drug development [1]. Many therapeutic drugs were either derived from natural products, their analogues or were designed based on the pharmacophore from natural products [2]. Apart from drug discovery, therapeutic use of natural products especially those derived from plants has been a growing area in alternative therapies [3]. Based on the therapeutic potentials of natural products, continual efforts are geared towards identification, purification and isolation of their active principles as a potential leads in drug discovery.

Alchornea cordifolia Schum-Thron (Euphorbiaceae) is a widely distributed plant in Africa. All its parts are used for therapeutic purposes in ethnomedicine. Most of these claims have been validated through several scientific studies. The anti-inflammatory activities of the

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leaves and root bark extracts with their associated compounds have been reported [4-6]. The immunomodulatory activity [7,8], hepatoprotective [9], antibacterial [10], and anxiolytic activities [11] of the plant material are well documented. Recently, our research group reported a new antimicrobial and antioxidant luteoyl derivative together with several known phenolics from the leaves [12]. In the present study we investigated the root extracts, which hitherto is poorly investigated, for its antimicrobial and/or the antioxidant activities as well as isolated specific chemical entities responsible for these activities.

MATERIALS AND METHODS

Plant

Root barks of *A. cordifolia* were collected in June, 2013 from Nsukka, Enugu State, Nigeria and were authenticated by Mr. Alfred Ozioko, a taxonomist with the Bioresource Development and Conservative Centre, Nsukka. The root barks were air-dried at room temperature for two weeks and then reduced to powder using a mechanical grinder.

Extraction and fractionation

The pulverized root bark (500 g) of *A.cordifolia* was extracted with 2.5 L of methanol under continuous stirring with magnetic stirrer for 48 h at room temperature (25^{0}C) and the extract concentrated *in vacuo* with a rotary evaporator at 50^{o}C. The dried extract (25 g) was reconstituted in 20 mL with water, the dispersion made up to 200 mL with water, sonicated for about 10 min and subsequently partitioned successively with hexane (300 mL X 3), ethyl acetate (300 mL X 3) and n- butanol (300 mL X 2). All fractions were taken to dryness using rotary evaporator to obtain hexane (HF), ethyl acetate (EF), butanol (BF) and water (WF) fractions respectively.

Chromatographic separations and purification

About 5 g of ethyl acetate fraction was subjected to VLC (silica gel 500 g, 230-400 mesh; Merck; sintered funnel 5 L) eluting with 500 mL each of hexane:ethylacetate (100:0, 80:20, 60:40, 40:60, 20:80, 0:100) and dichloromethane: methanol (90:10, 70:30, 50:50, 0:100) resulting in 10 fractions EF1 to EF10. Fractions EF5-EF7 which showed very similar chromatogram and also good antioxidant activity were combined and further separated on Sephadex LH-20 column eluting with 100% methanol and finally purified by semi-preparative reverse phase HPLC to obtain compound **1**.

General experimental procedures for structure elucidation

The NMR spectrum (1H) was recorded on AVANCE DMX 600 NMR spectrometers (Bruker, Rheinstetten, Germany). MS (ESI) were obtained with Finnigan LCQ Deca (Thermoquest, Bremen, Germany). Analytical HPLC was carried out with a Dionex P580 HPLC system (Dionex Softron, Germering, Germany) coupled to a photodiode array detector (UVD340S). Routine detection was at 235, 254, 280 and 354 nm. The separation column was (125 X 4mm, length X internal diameter) was prefilled with Eurosphere-10 C18 (Knauer, Berlin, Germany), and a linear gradient of nanopure water (adjusted to pH 2 by addition of formic acid) and methanol was used as eluent.

Antimicrobial assay

Antimicrobial activity of the compounds was screened on laboratory strains of bacteria and fungi using the method as previously described [12].

Antioxidant assay

The antioxidant activity of the fractions were screened using the method described previously [13] with modification Obiagwu *et al*., (2014). Freshly prepared DPPH solution (0.5 ml, 0.6 mmol) was added to 0.5 ml of different concentrations of the fractions (7.8125, 15.625, 31.25, 62.5, 125, 250, 500 and 1000 µg/mL). The volume of the solution was adjusted with methanol to a final volume 5 ml. The control tube contains 4.5 ml methanol and 0.5 ml of DPPH. After incubation in the dark for 30 min at room temperature, absorbance was taken at 517 nm. Ascorbic acid was used as standard. The DPPH radical percentage scavenging potentials were calculated from the equation below.

$$\% \text{ Inhibition of free radical} = \left[\frac{A_0 - A_t}{A_0}\right] \times 100$$

Where A_0 is the absorbance of the control, and A_t is the absorbance of the test/standard.

Statistical analysis

The results were presented as mean values. The antioxidant 50% inhibitory concentration (IC_{50}) was determined from a plot of percentage scavenging potentials against concentration using Microsoft Excel 2010.

RESULT AND DISCUSSION

Compound **1** was isolated from the combined fractions EF5-7 as a brown solid. The UV spectrum of compound **1** exhibited two absorption maxima at 248 and 374 nm characteristic of ellagic acid derivatives. LC-ESIMS of compound **1** showed strong peaks at m/z 331.0 $[M+1]^+$ and 682.6 $[2M+23]^+$ in the positive mode and m/z 329.9 [M-1] in the negative mode which is consistent with a molar mass of 330 g/mol and a molecular formula of $C_{16}H_{10}O_8$. The mass difference of 28 amu when compared with ellagic acid suggested that compound **1** is a di-O-methyl derivative of ellagic acid. The 1H-NMR spectrum of compound **1** is similar to that previously reported for ellagic acid, showing a prominent 2 proton singlet peak at δ_H 7.58 ppm assigned to H-5/5'. The major difference, however, is the presence of 6 proton singlet peak at δ_H 4.18 assignable to two equivalent methoxy groups, Me-3/3'. No further information was obtained from the H-COSY due to lack of neighbouring protons on the adjacent carbons. Based on these data and comparison with NMR data from previously reported studies [12,14], compound **1** was elucidated as 3,3'-di-O-methylellagic acid.

Table 1: 1HNMR Spectrum of Compound 1 and reported 3,3'-di-O-methylellagic acid

Position	δ_H (Compound 1)*	δ_H [14])#
1	-	-
2	-	-
3	-	-
4	-	-
5	7.58 s	7.54 s
6	-	-
7	-	-
1'	-	-
2'	-	-
3'	-	-
4'	-	-
5'	7.58 s	7.54 s
6'	-	-
7'	-	-
Me-3	4.18 s	4.05 s
Me-3'	4.18 s	4.05 s

*Spectrum measured at 600 MHz in CD_3OD
#Spectrum measured at 500 MHz in DMSO-d_6

Table 2: IC_{50} Values of the DPPH free radical scavenging activity of the crude extract and fractions

Text Fractions	IC_{50} (µg/ml)
Crude Extract	77.9
HF	236.9
EF	92.8
BF	98.2
EF4	194.5
EF5	82.8
EF6	88.9
EF7	80.0
EF8	81.6
EF9	286.6
AA	9.4

HF = Hexane fraction, EF = Ethyl acetate fraction, BF = Butanol fraction, EF4 to EF9 = the ethyl acetate VLC fraction, AA = ascorbic acid

The HPLC-DAD analysis of the two other active ethyl acetate sub-fractions (EF4 and EF8) showed that protocatechuic acid (**2**) and ellagic acid (**3**) were the major compounds present in EF4 and EF8 respectively.

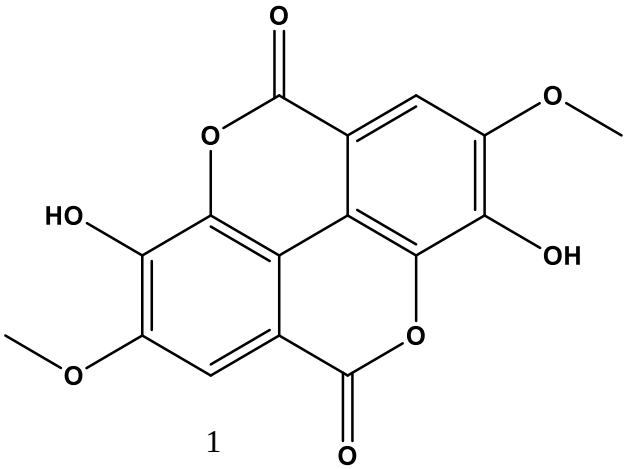
The crude extract and fractions exhibited good antioxidant activity with ethyl acetate fraction showing comparably better activity (Table 2). The ethyl acetate sub-fractions also exhibited good anti-

oxidant activity using the DPPH free radical scavenging assay (Table 2). HPLC-DAD analysis showed that these fractions contain mainly phenolic compounds. Phenolic compounds are best known for their ability to scavenge free radicals. Their radical scavenging potentials are however dependent on the number and position of their free hydroxyl groups. Hydrogen donating potentials of the isolated phenolic compounds were demonstrated by their ability to inhibit DPPH radical. As hydrogen donors, these compounds are not only limited to direct scavenging activity but are also able to form chelation complexes with metal ions that mediate the processes of free radical production. Phenolic compounds can as well through hydrogen-bonding interactions inhibit enzymes involved in radical generation processes.

Table 3: Antimicrobial activity of the isolated compound

Test Compd	Conc. (mg/ml)	*S.aureus*	*E.coli*	*B.subtilis*	*P.aerugionsa*	*C. albicans*	*A. niger*
		Inhibition zone diameter (mm)					
Compd 1	6	6	5	7	-	-	-
	3	4	4	6	-	-	-
	1.5	2	3	5	-	-	-
	0.75	-	2	3	-	-	-
	0.375	-	-	2	-	-	-
	0.1875	-	-	-	-	-	-
Cipro	0.05	8	6	10	3	ND	ND
	0.025	6	4	8	1	ND	ND
Keto	0.002	ND	ND	ND	ND	6	4
	0.001	ND	ND	ND	ND	4	3

ND = Not determine, (-) = No inhibition, Cipro = Ciprofloxacin, Keto = Ketoconazole



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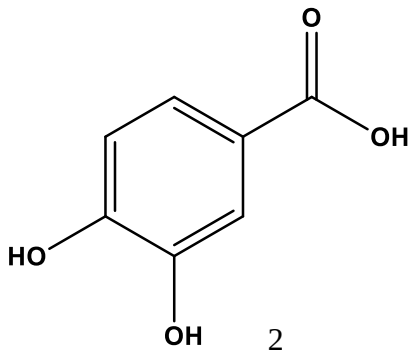

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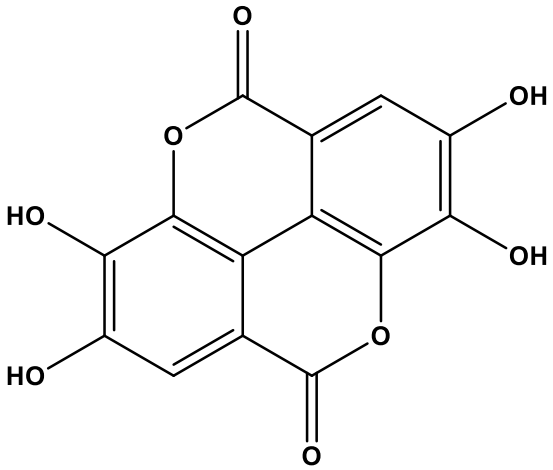

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Figure 1: The Chemical Structures of the Isolated and Detected Compounds

Pharmacological activities of phenolic compounds like protocatechuic acid have been suggested to be closely correlated with its antioxidant activities [15]. Its *in-vitro* antioxidant activity has been shown to be more effective than reference standard – Trolox in h lipid and aqueous medium [16]. Ellagic acid

has also been reported to demonstrate comparable activity with known antioxidant reference standards [17]. Poor correlation between *in vitro* and *in vivo* antioxidant activity of phenolic compounds have been traced to many factors that include poor absorption and high metabolic degradation. Enhancing the lipophilicity of phenolic compounds has been shown to improve their antioxidant activities in biological systems. One of the proven techniques for enhancing lipid solubility of phenolic compounds is through methylation of some of the hydroxyl groups. The dimethyl derivative isolated from the active fractions may, thus, have contributed to the slightly better antioxidant activity of these fractions compared to that containing only ellagic acid.

The isolated compound was subjected to antimicrobial activity assay. The result as shown in Table 3 indicated potent activity against *Staphylococcus aureus*, *Bacillus subtillis* and *Escherichia* coli.

Antimicrobial activities of phenolic compounds are well known. Their activities in some studies have been shown to be more effective than reference standards [18]. Ellagic acid has demonstrated profound antimicrobial activity in many previous studies that have qualified it a lead antimicrobial compound for drug development [19,20]. Structural activity relationship analysis and molecular docking studies have also shown that methoxyl functional group is important for anti-methicillin-resistance *Staphylococcus aureus* activity of phenolic compounds [21]. Methoxy substitutions have also been found to play an important role in controlling inflammation diseases [22], antidiabetic [23] and antifungal activities [24] of phenolic compounds. The presence of the dimethyl moiety in the isolated compound may have contributed to its antimicrobial activity.

CONCLUSION

The root bark extract of *A. cordifolia* and its phenolic fraction exhibited strong antimicrobial as well as antioxidant activities. These activities may be as a result of the 3, 3' dimethyl ellagic acid isolated from the most active fractions. These findings offer great prospects for this compound as lead candidate in drug discovery.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The authors alone are responsible for the content and writing of this article.

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