African Journal of Pharmaceutical Research & Development

Vol. 7 No.2; pp. 87-94 (2015)

A new antioxidant and antimicrobial compound isolated from *Alchornea cordifolia* leaves

Festus B. C. Okoye[1*], Damian C. Odimegwu[2], Chukwunonso Nnamdi Anyasor[1], Daniel L. Ajaghaku[3], Thaddeus H. Gugu[4], Patience O. Osadebe[5], Peter Proksch[6*]

[1]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, 420281 Awka, Anambra State, Nigeria
[2]Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, University of Nigeria, 410001 Nsukka, Enugu State, Nigeria
[3]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, 420281 Awka, Anambra State, Nigeria
[4]Department Pharmaceutical Microbiology & Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria
[5]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Nigeria, 410001 Nsukka, Enugu State, Nigeria
[6] Institut für Pharmazeutische Biologie und Biotechnologie, Universität Düsseldorf, Düsseldorf, Germany

ABSTRACT

A new luteoyl derivative, 1-O-galloyl-6-O-luteoyl-β-D-glucopyranoside, together with five known compounds were isolated from the methanol leaf extract of *Alchornea cordifolia*. The chemical structures of these compounds were confirmed by 1D and 2D NMR and HRMS analyses. The compounds exhibited significant antioxidant activity with IC_{50} values in the range of 1.56 to 3.11 µg/ml. Some of the activities were comparable to that of ascorbic acid (IC_{50} value of 1.56 µg/ml). The compounds also exhibited good antimicrobial activity against *Escherichia coli* with MIC values in the range of 105 to 180 µg/ml. The fraction from which these compounds were isolated, however, showed broad spectrum antimicrobial activity comparable to ciprofloxacin. This fraction also showed no toxicity against Hep 2 cell lines. In conclusion, extract of *A. cordifolia* leaves and the isolated compounds exhibited very strong anti-oxidant as well as antimicrobial activity. These activities further support the ethnomedicinal uses of this plant material.

KEYWORDS: A*ntioxidant, Antimicrobial, Alchornea cordifolia, Flavonoid glycosides*

INTRODUCTION

Compounds from plants have continued to be a source of inspiration for the development of novel therapeutic drug molecules. Over the years, the indigenous people have used herbal medicines for treatment of a variety of ailments. In some cases, these herbs used in their crude forms, exhibited therapeutic efficacies comparable to orthodox medicine. However, due to the need for standardization *vis á vis* dosage, the extracts are usually further purified up to the point of associating their therapeutic effects to defined chemical entities.
Alchornea cordifolia Schum-Thron (Euphorbiaceae) leaves have been used in ethnomedicine for a variety of ailments including inflammatory disease states and disorders associated with microbial infection. Extracts

*Authors of Correspondence: fb.okoye@unizik.edu.ng, +2348033607639;
proksch@uni-duesseldorf.de; +492118114163

of *A. cordifolia* leaves were shown to exhibit antibacterial activity comparable to ampicillin in the management of otitis media [1]. Several pharmacological investigations have been carried out to validate some of the claimed ethnomedicinal uses of the plant material. The anti-inflammatory [2,3], hepatoprotective [4], immunomodulatory [5], anti-bacterial [6,7] and anti-moluscidal activities [8] of the plant material are well documented. A few constituents responsible for the observed activities have also been described in the literature. The topical anti-inflammatory effect was attributed to the presence of eugenol, triterpenoids, daucosterol, acetyl aleuritolic acid and beta-sitosterol [9-11]. Smooth muscle relaxing flavonoids were also reported from the leaves of the plant [12]. Our literature survey, however, showed that till date, there is no study that has associated the antimicrobial and/or the antioxidant activities of this plant species to defined chemical compounds. This observation, together with our quest to further source novel therapeutic lead molecules from Nigerian medicinal plants, stimulated our interest to investigate this plant material for its antioxidant and antimicrobial potentials.

In the present study, we isolated and characterized some phenolic compounds from the plant material, which exhibited good antioxidant and antimicrobial potentials. Of particular interest is the isolation and structure elucidation of a new luteoly glucoside that has not been previously reported.

MATERIALS AND METHODS

General experimental procedures

NMR spectra (^{1}H, ^{13}C, DEPT, HSQC amd HMBC) were recorded on AVANCE DMX 600 NMR spectrometers (Bruker, Rheinstetten, Germany). MS (ESI) and HRMS (ESI) were obtained with Finnigan LCQ Deca (Thermoquest, Bremen, Germany) and Maxis 4G 20213 mass spectrometers respectively. 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 (125 X 4 mm, length X internal diameter) was prefilled with Eurospher-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. Semipreparative HPLC was performed with Merck Hitachi L-7100 (Merck/Hitachi, Germany) coupled to a UV detector (L-7400). A linear gradient of HPLC grade methanol and nanopure water was used for separation. Vacuum Liquid Chromatography (VLC) was carried out on silica gel (230-400 mesh, Merck), Sephadex LH-20 was used for CC while TLC was performed on silica gel G_{254} coated plates (Merck) using the following solvent systems: DCM-MeOH (9:1 and 4:1).

Plant material

The leaves of *Alchornea cordifolia* were collected in February, 2012 from Nsukka, Enugu State, Nigeria. The plant material was collected and authenticated by Mr Alfred Ozioko of Bioresources Development and Conservation Centre. A voucher specimen has been deposited at the herbarium of Department of Pharmacognosy, University of Nigeria, Nsukka, Enugu State, Nigeria under the herbarium number 012/01. The leaves were air dried for 10 days and pulverized using a laboratory scale slow speed electric blender.

Extraction and fractionation

About 500 g of pulverized dried leaves of *Alchornea cordifolia* were extracted with 5 L of MeOH under continuous stirring with magnetic stirrer for 48 h at room temperature (25°C) and the extract concentrated *in vacuo* with a rotary evaporator to obtain a dark green semisolid. The dried extract (30 g, 6% w/w) was reconstituted in 20 mL of methanol and the dispersion made up to 200 mL with water, sonicated for about 10 min and subsequently partitioned successively with hexane (750 mL X 3), ethyl acetate (750 mL X 3) and n-butanol (500 mL X 2). All fractions were taken to dryness using rotary evaporator to obtain approximately 11.0 g (2.2% w/w) of HF, 9.6 g (1.9% w/w) of EF, 10.8 g (2.16 % w/w) of BF and 5.0 g (1% w/w) of WF respectively. About 5 g of ethyl acetate fraction (EF) was subjected to VLC (silica gel 500 g, sintered funnel 5 L) eluting with 500 mL each of hexane:ethyl acetate (100:0, 90:10, 80:20, 70:30, 60;40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100) and dichloromethane:MeOH (100:0, 90:10, 80:20, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100) resulting in 22 fractions EF1 to EF22. The quantities obtained from fractions EF1 to EF9 were very small compared to the other fractions and as such no further work was continued with these

fractions. HPLC analysis showed that fractions EF10 to EF16 contained the same constituents at varying quantities. Similarly, HPLC analysis showed that fractions EF17 to EF22 contained the same constituents at varying quantities. The latter fractions which showed good antioxidant and antimicrobial activities were separated on Sephadex LH-20 column (3 X 60 cm) eluted with 100% methanol and finally purified by semi-preparative reverse phase HPLC to obtain compounds **1** (15 mg), **2** (2 mg), **3** (3 mg), **4** (2.5) and **5** (6 mg)

Compound 1: Yield 15.0 mg: light brown powder; UV (PDA): λ_{max} 224 and 270 nm; ^{1}H NMR (600 MHz, DMSO-d_6): *δ*: 7.02 (2H, s, H-2'/6'), 6.50 (2H, s, H-5"/5'"), 6.21 (1H, d, *J*=7.3, H-1), 3.88 (1H, dd, *J*=7.4, H-2), 4.59 (1H, m, H-3), 4.22 (1H, m, H-4), 4.35 (1H, t, *J*=8.1, H-5), 4.25 (1H, dd, *J*=10.9, Ha-6), 3.94 (1H, dd, *J*=8.7, 10.9, Hb-6); 13C NMR (150 MHz, DMSO-d6): *δ*: 92,2 (d, C-1), 71.7 (d, C-2), 77.6 (d, C-3), 62.1 (d, C-4), 76.4 (d, C-5), 64.0 (t, C-6), 118.7 (s, C-1'), 108.7 (d, C-2'/6'), 145.6 (s, C-3'/5'), 139.0 (s, C-4'), 164.8 (s, C-7'), 115.5 (s, C-1"), 144.8 (s, C-2"/2'"), 135.4 (s, C-3"), 145.0 (s, C-4"), 106.1 (d, C-5"), 123.9 (C-6"), 167.1 (s, C-7"), 115.8 (s, C-1'"), 135.6 (s, C-3'"), 144.3 (s, C-4'"), 106.9 (d, C-5'"), 123.6 (s, C-6'"), 166.7 (C-7'"); MS (ESI+): *m/z* = 657 $[M+Na]^+$, 464.8 $[M\text{-galloy+H}]^+$; MS (ESI-): *m/z* =633.2 $[M-1]^-$, 463.2 $[M\text{-galloy-1}]^-$, 301 $[M\text{-galloy-glucosyl-1}]^-$; HRESIMS: calcd. for $C_{27}H_{22}O_{18}$ $[M+Na]^+$ 657.0698; found 657.0685 $[M+Na]^+$.

Cytotoxicity assay

The human larynx carcinoma cell line HEp-2 (Invitrogen, Germany) was maintained in Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal calf serum (FCS) and penicillin/streptomycin (100 U/mL) as previously described [13]. Effect of the active fraction (ACL-Active) on the viability of this cell line was determined by a MTT cell viability assay. HEp-2 cells (Human Larynx carcinoma cell line) seeded in a 96-multiwell plate and cultured in DMEM containing increasing concentrations of test sample were incubated at 37^{o}C in 5% CO_2 for 48 h. The culture medium was replaced with fresh medium containing 50 µL of MTT (5 mg/mL) and incubated further for 1 h to allow the formazan production. After that the MTT containing medium was aspirated and 200 µL of DMSO was added to lyse the cells and solubilize the water insoluble forma zone [14]. The optical density of lysates were determined at 550 nm using a multiwellmicroplate reader and the concentration of 90% cellular toxicity (TC_{90}) determined by simple regression analysis.

Antimicrobial assay

Antimicrobial activity of the compounds was screened on laboratory strains of bacteria and fungi using previously reported protocols [15]

Antioxidant assay

The anti-oxidant activity of the compounds were screened as previously reported [16]

RESULTS & DISCUSSION

The crude methanol extract of *A. cordifolia* leaves was fractionated into hexane (HF), ethyl acetate (EF), butanol (BF) and water (WF) fractions by liquid-liquid fractionation. The ethyl acetate fraction, which exhibited very strong antioxidant and antimicrobial activities, was further separated on VLC column and the active fractions were subjected to Sephadex LH-20 gel chromatography and semi-preparative reverse phase HPLC to obtain the new compound (**1**) and five known compounds (**2a/b -5**).

Compound **1** was obtained as light brown powder. It showed UV maxima at 224 and 270 nm. LC-ESIMS of **1** exhibited strong peaks at *m/z* 657.0 $[M+Na]^+$ in the positive mode and at *m/z* 633.2 $[M-H]^-$ in the negative mode, which is consistent with the molar mass of 634 g/mol. The molecular formula was established as $C_{27}H_{22}O_{18}$ based on the HR-ESIMS pseudomolecular ion peak at *m/z* 657.0685 $[M+Na]^+$. ESI-MS/MS showed fragments from loss of gallic acid *m/z* 464.8 $[M-170+H]^+$ and subsequent loss of a hexose sugar *m/z* 303 $[M-170-162+H]^+$.

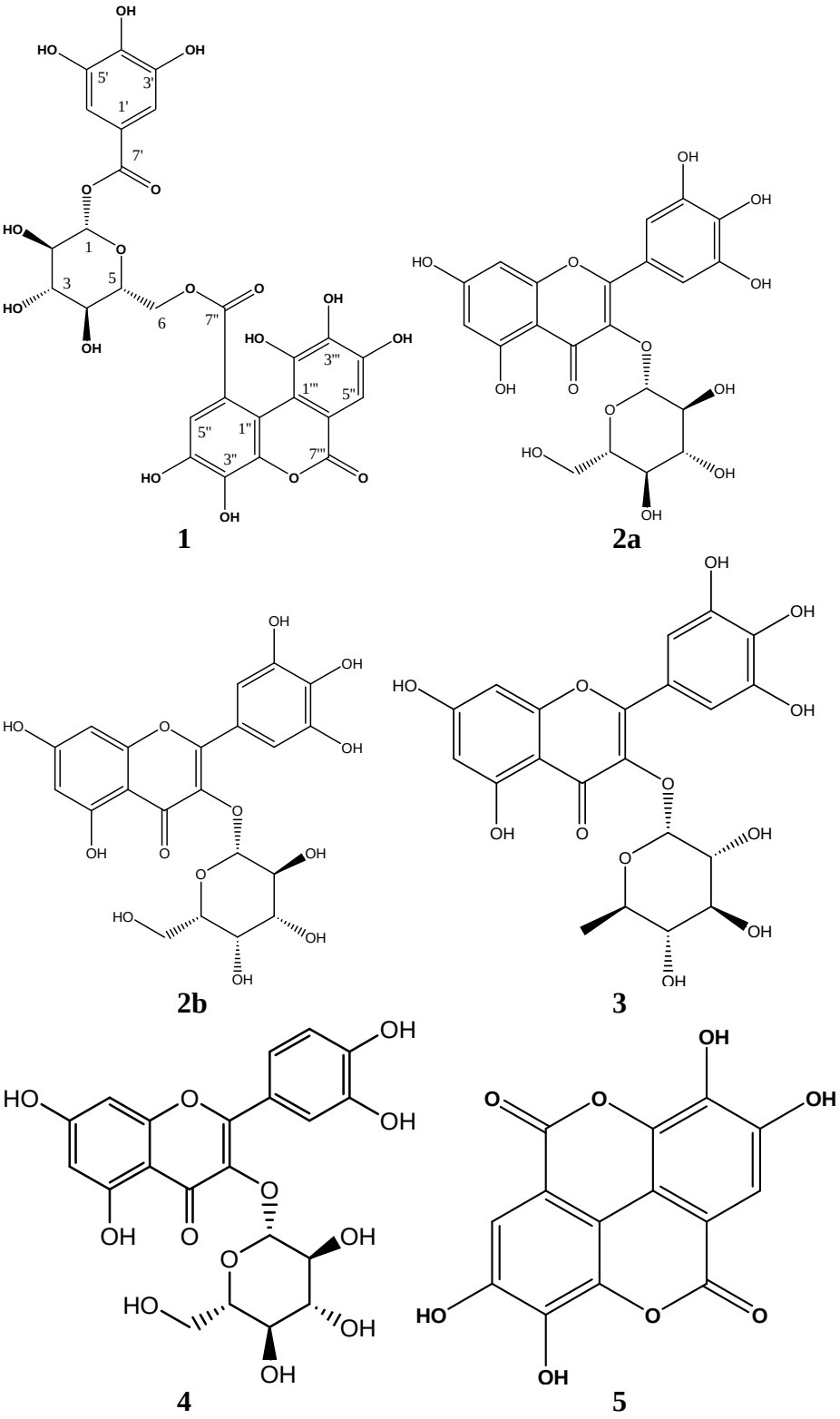

Figure 1: The chemical structures of the isolated compounds

The galloyl moiety was further confirmed by the proton NMR signal at δ_H 7.02 (s, 2H) assigned to H 2'/6' of gallic acid residue. The presence of the hexose unit was also confirmed by the signals at δ_H 6.21 (d, *J*=7.3, 1H) assignable to the anomeric proton (H-1), which showed set of correlations with the other sugar protons in the COSY spectrum. Comparison of the proton and C-13 NMR of the sugar moiety with previous NMR data [17-19] suggested that the sugar was glucose and the coupling constant of the anomeric proton (*J*=7.3) supported a *β*D glucopyranoside configuration. The linkage of the glucose and the galloyl moiety was determined to be between the C-1 of the glucose moiety and the C-7' of the galloyl moiety based on the cross peaks observed in the HMBC spectra. The signals at δ_H 6.50 s (1H) and 6.56 s (1H) assignable to H-5" and H-5"' respectively and together with the observed correlations in the HMBC spectrum suggested the presence of luteoyl moiety (partial open ring form of ellagic acid). The linkage of the luteoyl moiety was similarly determined to be between the C-6 of the glucose moiety and the C-7" of the luteoyl moiety based on the observed HMBC cross peaks. This was also supported by the deshielded position of the C-6 signal at δ_C 64.0 ppm. All the proton and carbon signals of the compound were assigned by the analysis of the DEPT, HSQC and HMBC (Table 1). Compound **1** was thus deduced as 1-O-galloyl-6-O-luteoyl-*β*-D-glucopyranoside (Figure 1). To the best of our knowledge, this is the first report of this molecule in literature. A similar compound, 1-O-galloyl-6-O-luteoyl-α-D-glucoside was previously reported [20]. Our comparison of the NMR data of the two compounds (Table 1) showed great similarity, but a marked difference in the observed values of the coupling constants of the anomeric protons of the two hexose sugar units. The coupling constant value of 7.3 Hz observed for compound **1** as against 3.7 Hz recorded for the reported compound [20] suggested a trans diaxial orientation of the anomeric proton of compound **1** relative to its H-2, which is consistent with *β*-D-glucopyranoside substructure.

The structures of the five known compounds were identified based on their ^{1}H NMR, ^{13}C NMR, and mass spectrometric data as well as comparison with published data as the isomeric mixture of myricentin-3-O-*β*-D-glucopyranoside (**2a**) and myricentin-3-O-*β*-D-galactopyranoside (**2b**), myricentin-3-O-α-L-rhamnopyranoside (**3**), quercetin-3-O- *β*-D-glucopyranoside (**4**) and ellagic acid (**5**) [21-23]. (Figure 1).

All isolated compounds were screened for antioxidant activity using the DPPH free radical scavenging assay and antimicrobial activity against strains of Gram positive and Gram negative bacteria and fungi. The compounds exhibited strong DPPH free radical scavenging activity comparable to the standard drug, ascorbic acid (Figure 2, Table 2). The isolated compounds (**1-5**) showed antimicrobial activity against *Escherichia coli* while only **5** was active against *Staphylococcus aureus* (Table 3).

The antibacterial activity of the VLC fraction from which the compounds were isolated was, however, comparable to that of ciprofloxacin in terms of potency and spectrum (Table 3).

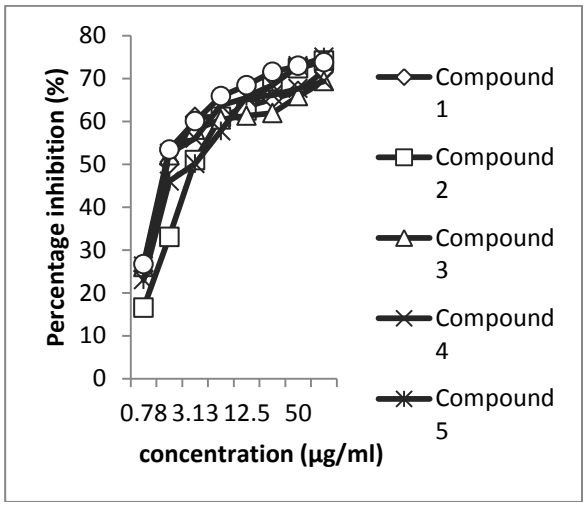

Figure 2: DPPH free radical scavenging assay of the isolated compounds

Table 1: NMR data of compound 1 and reported 1-O-galloyl-6-O-luteoyl-α-D-glucoside

	Compound 1*			Reported 1-O-galloyl-6-O-luteoyl-α-D-glucoside# (Matsuura et al., 2005)	
Position	**δ_H**	**δ_C**	**HMBC**	**δ_H**	**δ_C**
1	6.21 d (7.3)	92.2	7'	6.37 d (3.7)	94.9
2	3.88 d (7.4)	71.7		3.99 m	69.3
3	4.59	77.6		4.81 m	71.5
4	4.22	62.1		4.47	62.4
5	4.35 t (8.1)	76.4		4.53 ddd (2.1, 5.0, 11.4)	76.1
6	4.25 3.94 dd (8.7, 10.9)	64.0	7''	4.99 dd (2.1, 11.1) 4.15 dd (5.0, 11.1)	64.9
1'	-	118.7		-	120.5
2'	7.02 s	108.7	7', 4', 5'	7.06 s	110.9
3'	-	145.6		-	146.3
4'	-	139.0		-	140.4
5'	-	145.6		-	146.3
6'	7.02 s	108.7	7'. 3', 4'	7.06 s	110.9
7'	-	164.8		-	166.7
1''	-	115.5		-	116.7
2''	-	144.8		-	145.3
3''	-	135.4		-	137.6
4''	-	145.0		-	145.9
5''	6.50 s	106.1	7'', 3'', 4'', 6''	6.66 s	108.3
6''	-	123.9		-	125.4
7''	-	167.1		-	170.1
1'''	-	115.8		-	117.2
2'''	-	144.8		-	145.4
3'''	-	135.6		-	138.2
4'''	-	144.3		-	145.5
5'''	6.56 s	106.9	7''', 3''' 4''', 6'''	6.69	110.9
6'''	-	123.6		-	125.3
7'''	-	166.7		-	168.5

*Spectra Measured at 600 (^{1}H) and 150 (^{13}C) MHz in DMSO-d6

#Spectra measured at 500 (^{1}H) and 125 (^{13}C) MHz in CD_3OD

Table 2: IC_{50} values of the DPPH free radical scavenging activity of the isolated compounds.

Test Compounds	IC_{50} (µg/ml)
ACL-Active	1.56
1	1.56
2	3.11
3	1.56
4	1.48
5	2.34
Ascorbic acid	1.46

ACL-Active = the VLC fraction from which the compounds were isolated

Similarly, the antifungal activity this fraction was comparable to that of ketoconazole. The pronounced broad spectrum antimicrobial activity of this fraction, which was not replicated for the individual compounds, suggested that the compounds possibly act in synergism. This fraction also exhibited very low cytotoxic activity against Hep 2 cell line (TC_{90} = 563.30 µg/ml), also suggesting that the observed antimicrobial activity was not due to direct cytotoxic effect of the constituents. This active fraction may found useful application as both stabilizer and preservative in creams or oral liquid pharmaceutical preparations.

Table 3: Antimicrobial activity of the isolated compounds

Test Comps	*Bacillus subtilis*	*Staph aureus*	*Salmonella typhi*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albican*	*Aspergillus niger*
ACL-active	38	25	50	39	100	100	100
1	NA	NA	NA	182	NA	NA	NA
2	NA	NA	NA	120	NA	NA	NA
3	NA	NA	NA	105	NA	NA	NA
4	NA	NA	NA	105	NA	NA	NA
5	NA	91	NA	120	NA	NA	NA
CPFX	50	72	25	114	100	ND	ND
KETX	ND	ND	ND	ND	ND	100	120

NA = Not active (i.e. MIC values greater than 250 µg/ml); ND = Not determined; ACL-active = the VLC fraction from which the compounds were isolated; CPFX = ciprofloxacin, KETZ = ketoconazole.

CONCLUSION

In conclusion, our present investigation of the extracts of *A. cordifolia* leaves resulted in the isolation of six phenolic compounds including the new compound, 1-O-galloyl-6-O-luteoyl-β-D-glucopyranoside. These compounds exhibited strong antioxidant activity but were found to show possible synergistic antimicrobial activity. The presence of these compounds may be contributing to the observed efficacy of the plant material in ethnomedicine.

ACKNOWLEDGMENT

Dr. F.B.C. Okoye acknowledges the Return Fellowship Grant awarded to him by Alexander von Humboldt Foundation.

CONFLICT OF INTEREST

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

REFERENCES

1. Anonymous. 2008. Quoted from *Treatments - Treatment of Otitis media with Alchornea cordifolia leaves extracts. - Mamaherb – What actually works in natural health* found at http://www.mamaherb.com/Treatments/View.aspx?id=9902 [Published: Dynamic Content on Wed, 29 Oct 2008 22:31:28 GMT] [565 characters, 85 words]

2. Osadebe PO, Okoye FBC. 2003. Anti-inflammatory effects of crude methanolic extrats and fractions of *Alchornea cordifolia* leaves. J Ethnopharmacol. 89: 19 – 24.

3. Mavar-Manga H, Brkic D, Marie DEP, Quetin-Leclercq J. 2004. In vivo anti-inflammatory activity of *Alchornea cordifolia* (Schumach. & Thonn.) Müll. Arg. (Euphorbiaceae). J Ethnopharmacol. 92: 209 – 214.

4. Osadebe PO, Okoye FBC, Uzor PF, Nnamani NR, Adiele IE, Obiano NC. 2012. Phytochemical analysis, hepatoprotective and antioxidant activity of *Alchornea cordifolia* methanol leaf extract on carbon tetrachloride-induced hepatic damage in rats. Asian Pac J Trop Med. 5: 289-293.

5. Nworu CS, Vladimir T, Okoye FBC, Akah PA, Esimone CO, Überla K. 2010. Activation of murine lymphocytes and modulation of macrophage functions by fractions of *Alchornea cordifolia* (Euphorbiaceae) leaf extract. Immunopharmacol Immunotoxicol. 32: 28-36

6. Okeke IN, Ogundaini AO, Ogungbamila FO, Lamikanra A. 1999. Antimicrobial Spectrum of *Alchornea cordifolia* leaf extract. Phytother Res. 13:67-69

7. Ebi GC. 2001. Antimicrobial activities of *Alchornea cordifolia.* Fitoterapia 72: 69 – 72.

8. Adewunmi CO, Agbedahunsi JM, Adebajo AJ, Aladesanmi AJ, Murphy N, Wando J. 2001. Ethno-veterinary Medicine: Screening of Nigerian Medicinal Plants for Trypanocidal Properties. J Ethnopharmacol. 77: 19-24.
9. Mavar-Manga H, Haddad M, Pieters L, Baccelli C, Penge A, Quetin-Leclercq J. 2008. Anti-inflammatory compounds from leaves and root bark of *Alchornea cordifolia* (Schumach. & Thonn.) Müll. Arg. J Ethnopharmacol. 115: 25 – 29.
10. Osadebe PO, Ebi GC, Okoye FBC. 2008. Anti-inflammatory effects of triterpenoids from *Alchornea ordifolia* leaves. Rec Progr Med Plants 22: 571 – 577.
11. Okoye FBC, Osadebe PO, Nworu CS, Omeje EO, Okoye NN, Esimone CO. 2011. Topical anti-inflammatory constituents of lipophilic leaf fractions of *Alchornea floribunda* and *Alchornea cordifolia*. Nat Prod Res. 25: 1941-1949.
12. Ogungbamila FO, Samuelson G. 1990. Smooth muscle relaxing flavonoids from *Alchornea cordifolia*. Acta Pharm Nord. 2: 421-422.
13. Corse E, Machamer CE. 2002. The cytoplasmic tail of infectious bronchitis virus E protein directs golgi Targeting. J Virol. 76: 1273-1284.
14. Shruti S, Nikhil K, Roy P. 2013. Effects of low dose treatment of tributyltin on the regulation of estrogen receptor functions in MCF-7 cells. Toxicol Appl Pharmacol. 269: 176-186
15. Ukwueze SE, Osadebe PO, Okoye FBC. 2015. A new antibacterial benzoquinone glycoside from *Psidium guajava* (Linn). *Natural Product Research*. Epub Ahead of print DOI: 10.1080/14786419.2014.1003188.
16. Agbo MO, Lai D, Okoye FBC, Osadebe PO, Proksch P. 2013. Antioxidative Polyphenols from Nigerian Mistletoe *Loranthus micranthus* (Linn.) Parasitizing on *Hevea brasiliensis*. Fitoterapia 86: 78-83.
17. Gohar AA, Maatooq GT, Niwa M. 2000. Two flavonoid glycosides from *Chenopodium murale*. Phytochemistry 53: 399-403.
18. Gudej J. 2003. Kaempferol and quercetin glycosides from *Rubus idaeus* L. leaves. Acta Poloniae Pharmaceutica - Drug Res. 60: 313-316.
19. Okoye FBC, Sawadogo WR, Sendker J, Aly A, Quandt B, Wray V, Hensel A, Esimone CO, Debbab A, Diederich M, Proksch P. 2015. Flavonoid glycosides from *Olax mannii*: Structure elucidation and effect on nuclear factor kappa B pathway. J. Ethnopharmacol. 176: 27-34
20. Subeki, Matsuura H, Takahashi K, Yamasaki M, Yamato O, Maede Y, Katakura K, Kobayashi S, Trimurningsih, Chairul, Yoshihara T. 2005. Anti-babesial and Anti-plasmodial Compounds from *Phyllanthus niruri.* J Nat Prod. 68: 537-539
21. Markham KR, Ternai B, Stanley R, Geiger H, Mabry T J. 1978. Carbon-13 NMR studies of flavonoids-lavonoids NMR studies of flavonoid glycosides and their acylated derivatives. Tetrahedron 34:1389-1397.
22. Arot Manguro LO, Ugi I, Lemen P. 2004. Further flavonol glycosides of *embelia schimperi* leaves. Bull Chem Soc Ethiop. 18: 51-57.
23. Islam M, Al-Amin M, Ali Siddiqi MM, Akter S, Haque MM, Sultana N, Chowdhury AMS. 2012. Isolation of Quercetin-3-O-beta-D-glucopyranoside from the Leaves of *Azadirachta indica* and Antimicrobial and Cytotoxic screening of the Crude Extracts. Dhaka Univ. J. Sci. 60: 11-14.