



Isolation and Characterization of Di-(2-ethylhexyl) Phthalate from the Leaves of *Combretum micranthum* (Altum) Combretaceae

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Abstract

Combretum micranthum is a medicinal plant used in traditional medicine for treatment of wounds, sores, diarrhoea, pain, fever and skin infections. This study was aimed at isolation and characterization of compounds from the leaves of *Combretum micranthum*. The pulverized leaves of the plant were gradiently extracted with hexane, ethyl acetate and methanol using soxhlet apparatus. The isolation of the compound was done using silica gel gravity column chromatography and preparative thin layer chromatography which yielded yellow compound 'A'. The characterization of the isolated compound was carried out using ¹³C-NMR, ¹H-NMR and GC-MS. Data obtained from the spectral analyses, suggested the compound to be di-(2-ethylhexyl) phthalate.

Key words: *Combretum micranthum*; Di-(2-ethylhexyl) phthalate; preparative TLC; Extraction; Analysis.

1.0 INTRODUCTION

The *Combretaceae* is a large family of trees, shrubs, herbs, comprising of about 20 genera and 600 species widely spread in the tropical and subtropical regions of the world. The largest genera are *Combretum* and *Terminalia* with about 370 and 200 species respectively. *Combretum micranthum* is locally called *Geza* (Hausa language). It is widely distributed all over the continent of Africa but appears to be dominant in the Savannah Belt. It is found from Sudan to Nigeria and from Gambia to Congo (Iwu, 1993). It is a spreading shrub, creeper or small tree usually 2-5 m tall, but can reach up to 15 m height while the branches may reach a length of 20 m. The leaves are opposite, oval and acuminate. It has white flowers and small fruits that are scaly and four winged (Roy *et al.*, 2014).

C. micranthum is used in traditional medicine for the treatment of wounds, sores, fever (especially malaria fever), cough and bronchitis (Roy *et al.*, 2014). The leaves are used as diuretic, anti-emetic, anti-malarial and anti-diarrheal agents. Other uses include; treatment of skin infections, hypertension, dysentery and abdominal pain in nursing mothers (Burkill, 1985; Sani and Aliyu, 2011; Agboke *et al.*, 2012). An ointment prepared from the pulverized dry fruits is used in suppurating wounds and abscesses. The roots and leaves

2.0 MATERIALS AND METHODS

2.1 Collection and Identification of Plant material

The leaves of *C. micranthum* were collected from Ruggar Shauda town, in Dogondaji District, Tambuwal Local Government Area of Sokoto State, Nigeria. It was identified at the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto where a voucher specimen (UDUSH/ANS/0064) was deposited for future reference.

2.2 Sample Preparation

The leaves of the plant were shade dried and pulverized using a wooden pestle and mortar where fine powder was obtained. The powdered plant material was stored in an air tight polythene bag until required for use.

2.3 Extraction of the plant sample

The powdered plant sample (300 g) was packed into a thimble and gradiently extracted with n-

infusion are used as a febrifuge. Decoction of the roots is used in the treatment of guinea worm infestation in West Africa (Roy *et al.*, 2014).

A number of pharmacological studies have been conducted on different parts of the plant. Chika and Bello (2010) reported a significant hypoglycemic and antidiabetic activity of aqueous leaf extract of *Combretum micranthum*. Ferrea *et al.* (1993) reported the antiviral activity of *C. micranthum* against herpes simplex viruses 1 and 2. The methanol leaves extract of the plant exhibited a significant anti-inflammatory activity (Olajide *et al.*, 2003). The roots of *C. micranthum* have been reported to possess anticonvulsant (Danmalam *et al.*, 2011) and antimicrobial activities (Taura *et al.*, 2012; Udoh *et al.*, 2012). The leaves of the plant demonstrated good antioxidant activity (Karon *et al.*, 2005). β -sitosterol has been isolated and characterized from the hexane extract of the root of *C. micranthum*. Epicatechin, catechin epigallocatechin, galocatechin, bartogenic acid-28- β -D-glucoside have been isolated and characterized from the acetylated methanol extract of the roots (Lima *et al.*, 2012). Extensive literature search revealed that very few compounds have been isolated from the leaves of the plant. This research is aimed at isolation and characterization of compound(s) from the leaves of *C. micranthum*.

hexane, ethyl acetate and methanol using a soxhlet apparatus for 3 hours each. The extracts were freed from the solvent using a rotary evaporator.

2.4 Chromatographic Separation

Silica gel (120 g) was made into slurry with 100% n-hexane and packed in a glass column (2.5×63 cm) and allowed to stand for an hour for the column to stabilize. The ethyl-acetate extract (3.0 g) was pre-absorbed on silica gel (1 g) and loaded onto the column. The sample was gradiently eluted using hexane (100%); hexane: ethyl acetate (9:1) up to hexane: ethyl acetate (6:4). A total of 450 fractions were collected and combined based on their TLC profiles to give 13 major fractions coded A1-A13. Fraction A10 consisting of one major spot and little impurities was subjected to further purification using preparative thin layer chromatography (PTLC). The purification was done on pre-coated TLC

glass plates (20×20 cm) and 0.25 mm thickness. Hexane: ethyl acetate (7:3) was used as solvent system. The region containing the band of interest was circled and scrapped off. The scrapped sorbent was transferred into 250 cm³ beaker and extracted using a mixture of ethyl acetate and methanol (9:1) and filtered using Whatman filter paper No. 20. The filtrate was concentrated by exposure to air and afforded a yellow oily compound **A**. TLC analysis of compound **A** using two solvent systems, hexane: ethyl acetate (7:3) and (9:1) and sprayed with

3.0 RESULTS AND DISCUSSION

The preparative thin layer chromatography carried out on column fractions A10 led to the isolation of a yellow compound (**A**). The mass spectrum of the compound revealed molecular ion (M+1) peak at m/z 391. Other characteristic fragment peaks observed were at m/z 104, 149, 221 and 279. The mass spectrum revealed the molecular formula of compound **A** to be C₂₄H₃₈O₄. The proton NMR (Table 1) revealed the presence of aromatic protons (doublets) at 7.69 and 7.52, indicating 2 protons (H's at positions 12 and 11) are in the same chemical environment (substituted benzene). The peak at 4.19 is likely to be a CH₂ group (position 6) attached to electronegative oxygen while a peak at 2.15 indicates a CH (position 5) bonded to two methylene groups. A complex multiplet at 1.28 to 1.41 and another peak at 1.69 indicates several CH₂ groups (position 2, 3, 4 and 8) resonating in the same chemical environment, hence the overlapping signals. Peaks at 0.89 to 0.97 indicates the presence of a terminal CH₃ attached to CH₂ (positions 1 and 9). The peaks obtained from the spectra of the compound are typical of a dialkyl phthalate (Habib and Karim, 2009; Asker *et al.*, 2015).

The ¹³Carbon-NMR (Table 1) revealed peaks at 131.11, 129.05 and 129.01 indicative of aromatic carbon atoms in which C-12, C-11 and

10% sulphuric acid revealed a single homogenous spot.

2.5 Spectroscopic Studies

Compound **A** was subjected to ¹H-NMR and ¹³C-NMR analyses using NMR Spectrophotometer-Bruker 400 MHz. The GC-MS analysis was carried out using GC-MS Agilent Technologies model 7890A. The ionization of the compound was obtained by electron impact mass spectroscopy (EIMS).

C-10 resonated in the same chemical environment respectively. The peak at 68.36 is as a result of the down field (-O-CH₂) group attached to an electronegative oxygen atom (C-6), while the peak occurring up field at 38.91 indicates the presence of a CH group neighboring the (-CH₂-) benefitting from the effect of anisotropy (C-5). Other peaks at 23.93, 22.91, 29.91 and 30.77 are of (-CH₂-) groups (position 2, 3, 4 and 8). The peaks at 14.35 and 19.36 represent (-CH₃) groups at positions 1 and 9 (Habib and Karim, 2009; Fadipe *et al.*, 2014; Asker *et al.*, 2015).

By comparing the data obtained from the spectral analyses with those published in literature (Habib and Karim, 2009; Fadipe *et al.*, 2014; Asker *et al.*, 2015), the compound was characterized and the structure proposed as di-(2-ethylhexyl) phthalate (Figure 1). Di-(2-ethylhexyl) phthalate had previously been isolated from the ripe fruits of *Nauclea latifolia* (Fadipe *et al.*, 2014) and the flowers of *Calotropis gigantea* (Habib and Karim, 2009). Di-(ethylhexyl) phthalate has been reported to possess moderate antibacterial activity against Gram positive *B. subtilis* and *S. aureus*. This suggests the use of di-(2-ethylhexyl) phthalate in the treatment of infections caused by these organisms (Habib and Karim, 2009; Fadipe *et al.*, 2014).

Table 1: ^1H & ^{13}C -NMR data of compound a di-(2-ethylhexyl) phthalate compared with literature values.

Position	^{13}C - NMR	^1H -NMR	^{13}C - NMR	^1H -NMR
			(Habib and Karim, 2009; Asker <i>et al.</i> , 2015)	
1	14.35	0.89	14.11	0.84
2	23.93	1.28-1.41	24.80	1.28-1.41
3	22.91	”	22.68	”
4	29.91	”	29.50	”
5	38.91	2.60	40.76	2.15
6	68.36	4.15	65.21	4.17
7	-	-	171.10	-
8	30.77	2.30	29.69	1.69
9	19.36	0.97	20.79	0.93
10	129.01	-	124.75	-
11	129.05	7.52	118.95	6.96
12	131.11	7.69	132.65	7.11

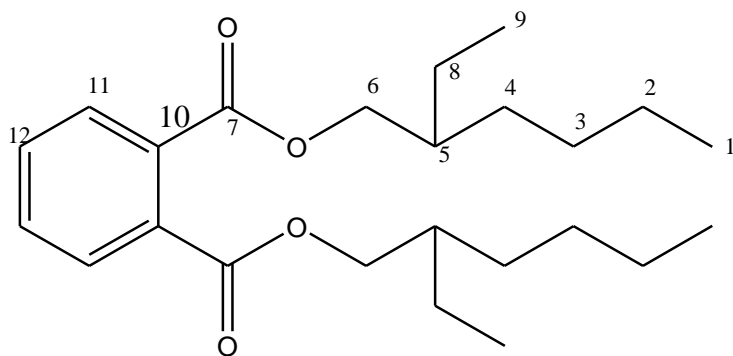


Figure 1: Di-(2-ethylhexyl) phthalate or Bis-(2-ethylhexyl) phthalate.

4.0 CONCLUSION

Di-(2-ethylhexyl) phthalate have been isolated and characterized from the leaves of *Combretum micranthum*. Base on available literature on this plant, this is the first report of isolation of this compound in *Combretum micranthum*.

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