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Isolation and characterization of 2, 4-dihydroxycinnamic acid from the stem bark of *Adina microcephala* Delile

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Adina microcephala **which belongs to the family Rubiaceae is widely distributed in Africa. It is used in African traditional medicine for the treatment of diarrhoea and other stomach or digestive tract problems.** ***A microcephala*** **stem bark was collected, identified, dried and pulverized. The pulverized plant material was subjected to Soxhlet extraction using petroleum ether (60-80°C), chloroform, ethyl acetate and methanol. The ethyl acetate extract was subjected to further purification by chromatographic techniques to yield a compound labeled R_1 which was then fully characterized as 2, 4-dihydroxycinnamic acid using IR, 1D and 2D NMR spectroscopic techniques.**

Key words: 2,4-Dihydroxycinnamic acid, *Adina microcephala*, Rubiaceae, stem bark.

INTRODUCTION

Adina microcephala Delile is an evergreen plant and a small to large tree of up to 40 m tall, with slender branches near thebase. It is synonymous to: *Breonadia salicina*, *Breonadia microcephala* and *Nauclea microcephala* (Hyde et al., 2016). The root decoctions of *A. microcephala* are drunk as a purgative in Tanzania and against tachycardia in South Africa. An infusion of the powdered stem is given to breast-fed children in Ethiopia for the treatment of diarrhoea and vomiting. In Nigeria, the plant is locally called *hakoren babauniya* or *káïányàr kaúrmìì* or *káïányàr ràfíí* (Hausa) and *emido* in Yoruba. It is commonly known as Africa teak or water shea-nut (Burkill, 1985). The bark of *A. microcephala* is rich in tannins (Neuwinger, 1994). Leaf extracts of *B. salicina* have also been found to reduce activity of both Gram-negative and Gram-positive bacteria (Mahlo, 2010).

It is believed that these same anti-bacterial properties of *B. salicina* can be used for the preservation of foods (Al-Qurainy et al., 2013). Antifungal compounds isolated and characterised from the leaves of *B. salicina* protect oranges from infection (Mahlo, 2010). Cinnamic acids are used as precursor for the synthesis of commercially important cinnamic *Adina microcephala* Delile is an evergreen plant and a small to large tree of up to 40 m tall, with slender branches near the esters, which are obtained from various plant sources and find application in flavourings, synthetic indigo, perfumery, cosmetic industries and in pharmaceutics (Sharma, 2011). A

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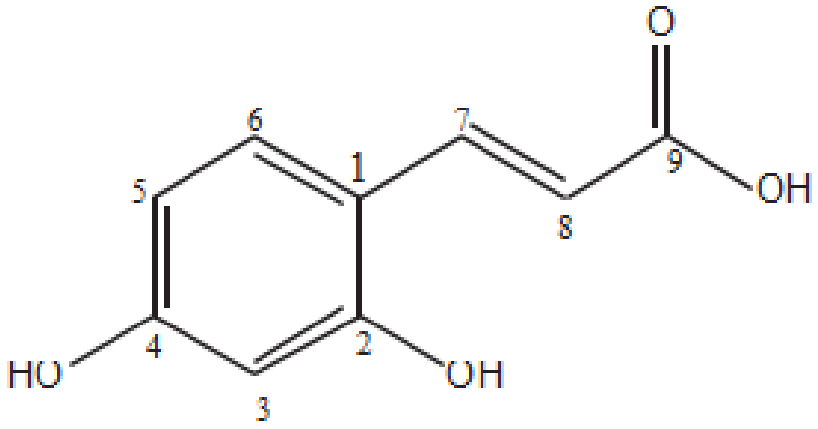

Figure 1. 2,4-dihydroxycinnamic acid.

major use of cinnamic acids is in the manufacturing of the methyl, ethyl, and benzyl for the perfume industry (Budavari, 1996). Cinnamic acid is also a precursor to the sweetener, aspartame via enzyme-catalyzed amination to phenylalanine (Garbe, 2002). Cinnamic acid derivatives are also naturally occurring substances found in fruits, vegetables, and flowers and are consumed as dietary phenolic compounds, which play a vital role in the formation of commercially important intermediate molecules used for the production of different pharmaceutical ingredients (Sharma, 2011).

The aim of the study was to isolate and characterize chemical constituent(s) present in the ethyl acetate extract of the stem bark of *A. microcephala.*

MATERIALS AND METHODS

Plant sample

The stem bark of *A. microcephala* was collected from Kundingi area, Samaru-Zaria (11° 04/N, 07° 42/ E), located in the Northern Guinea Savannah zone of Nigeria in May, 2015. The plant was identified and authenticated by the curator of the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria, and with a Voucher Specimen Number 345. The stem bark was air dried, powdered, and stored in air tight container before use.

Extraction and isolation

The dried pulverized stem bark (148 g) of *A. microcephala* was extracted exhaustively and successively with petroleum ether (60-80°C), chloroform, ethyl acetate and methanol, respectively to afford the four extracts upon evaporation of the solvent at reduced pressure using rotary evaporator. The ethyl acetate extract (7 g) was chromatographed on a silica gel column and eluted with gradient elution technique using a solvent systems of petroleum ether:ethyl acetate mixtures 95:05, 90:10, 85:15 and 80:20, respectively to yield 25 fractions. Compound R_1 (5 mg) was obtained from fraction Fr-2 (eluate of petroleum ether:ethyl acetate = 80:20), purified further using preparative thin layer chromatography and a solvent mixture of petroleum ether:ethyl acetate 75:25.

Equipment and chemicals

Thin layer chromatography analysis, silica gel 60 F_{254} (Merck) was used, column chromatography was performed using Merck silica gel (60-120) mesh, while spots on thin layer chromatography plates were visualized by spraying with 10% H_2SO_4, followed by heating at 100°C for 5 min. The IR spectrum was measured on a Shimadzu FT-IR8 400S Fourier transform infrared spectrophotometer. Nuclear magnetic resonance (NMR)-spectra were recorded on a Bruker Avance spectrometer (400 MHz) for ^{1}H- and (100 MHz) for ^{13}C-NMR. Internal standard was residual solvent signal with methanol as a solvent (Figure 1).

RESULTS AND DISCUSSION

The IR spectrum indicated the presence of carbonyl (1735 cm^{-1}), hydroxyl (3361 cm^{-1}) and aromatic groups (1550-1480 cm^{-1}). The aromatic region of the proton NMR spectrum of compound R revealed the presence of 5 proton signals at $ä_H$ 6.20(H, d, J = 9.44 Hz), 6.72(H, d J =2.12 Hz), 6.82 (H,dd, J = 2.24, 8.52 Hz), 7.47 (H, d, J =8.52 Hz) and 7.86 (H, d, J = 9.48 Hz). The ^{13}C spectrum of R revealed the presence of 9 carbon atoms, of which one carbon atom was assigned to a carboxylic acid carbonyl at $ä_C$ 163.76; six unsaturated carbons at $ä_C$ 103.44, 113.17, 114.56, 130.69, 157.27 and 160.17 (Table 1). Three of the six unsaturated carbons are methine carbons (103.44, 114.56, and 130.69) and two oxymethine carbons $ä_C$ 157.25 and 163.17; and two olefinic carbons at $ä_C$ 146 and considering that the proton NMR spectrum revealed five aromatic protons. The six unsaturated (olefinic) carbons could be assigned to a mono-substituted benzene ring, joined to a three carbon straight olefinic carboxylic acid chain, with two significantly deshielded carbons being oxygenated, while three of the remaining up-field aromatic carbon atoms bore single aromatic proton (Ponce et al., 2009).

Table 1. NMR spectral data for compound 1 (CD_3 OD, 400 MHZ).

No.	ä$_C$	ä$_H$	DEPT
1	113.17	-	C
2	163.17	-	C
3	103.44	6.72 (H, d, *J*=2.12 Hz)	CH
4	157.27	-	C
5	114.5	6.82 (H, dd, *J*=2.24, 852 Hz)	CH
6	130.69	7.47 (H, d, *J*=8.52 Hz)	CH
7	146.10	7.86 (H, d, *J*=9.48 Hz)	CH
8	112.33	6.82 (H, dd, *J*=2.24, 8.52 Hz)	CH
9	163.76	-	C

The ^{1}H and ^{13}C assignments were further supported by the two-dimensional (2D) NMR data including ^{1}H-^{1}H COSY, HSQC and heteronuclear multiple-bond correlation (HMBC) experiments. In the five carbon polyhydroxy carbon chain, ^{1}H-^{1}H COSY experiment indicated the following connectivity: on the aromatic ring H-5'/H-6', suggesting that the two aromatic protons are adjacent to each other and ortho-couple to each other. The H-^{1}H COSY experiment showed connectivity between H-3'/H-5', H-3'/H-4' suggesting that the aromatic protons are meta couple to each other. Furthermore, H-7 on the carboxylic side chain showed ^{1}H-^{1}H COSY with H-8' of the olefinic carbon. The connectivity of the carboxylic side chain was supported by the HMBC correlations of H- 7'/C-3, H-7'/C-1', H-7'/C-6 H-7'/C-4, H-7'/C-2 and H-7'/C-9.

From the aforementioned 1D and 2D NMR spectra analyses, the compound isolated from the ethyl acetate extract of the stem bark of *A. microcephala* is 2, 4-dihydroxycinnamic acid (Figure 1). Dihydroxycinnamics acids are found to possess significant pharmacological activity and are used as a precursor of pharmaceutical industries (Sharma, 2011).

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