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Research Article – Phytochemistry

Isolation and partial characterization of alkylferulate from *Entada africana* (Guill. & Perr.) stem bark extract

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Abstract

Investigation of the bioactive constituents of *Entada africana* crude extract afforded the isolation of alkylferulate. The hexane soluble portion of acetone/methanol (1:1v/v) crude stem bark extract of *Entada africana* was subjected to column chromatography on silica gel 60 (60-200) mesh size. Gradient column elution yielded an isolate coded AC4 with Rf value of 0.65 in hexane/diethyl ether (3:2). The isolate was characterized using IR, NMR and in comparison with literature data. The analysis of spectroscopic data and literature comparison strongly suggests that AC4 is an alkylferulate; a known hypolipidemic agent in addition to other biological uses. The isolation of alkylferulate partly lays credence to the use of *Entada africana* in traditional medicine practice.

Keywords: *Entada africana,* Isolation, Alkylferulate, bioactive, Characterization

Introduction

Alkyl ferulates are esters of ferulic acid and aliphatic alcohols. It is known to occur in several plant families such as Pinaceae, Rubiaceae, Podocarpaceae, Euphorbiaceae, Aristolochiaceae and the Leguminosae among others (Katagiri *et al.,* 1997). Despite widespread occurrence, it is believed that the main source of alkyl ferulates is *Commiphora wightii* (Dev, 1989). Previous report had shown that alkylferulates had been isolated from *Tecomella undulata* (Joshi *et al.,* 1986) and *Dendrobium clavatum* (Chang *et al.,* 2001). *Entada africana* is of the family Leguminosae and the isolation of alkyl ferulate from its stem bark in this present study justifies earlier literature report about its occurrence in this plant family. Consequently, we report for the first time the isolation and partial characterization of alkylferulate from *Entada africana* stem crude extract.

Materials and methods

Instruments/Equipments

Glass column (75 x 3.5) cm, TLC, plate (silica gel 60 [illegible] Rule, , Chromatogram & Iodine tanks. Water Coolant (Stuart-SRC4), Water Bath (Stuart RE300DB), Vacuum Pump (CAT. RE3022C), Rotavapor (Stuart RE300/MS), Digital balance ae-*ADAM* (PW254). IR-PerkinElmer Universal ATR (100 FT-IR Spectrometer); ^{1}H &^{13}C -NMR – 400 MHz BruckerAvance, Chloroform ($CDCl_3$).

Solvents

(n-Hexane, Dichloromethane, Diethyl ether Ethyl acetate, Acetone & methanol) all are Analytical grade Reagents from LobaChemie Company.

Sample Collection, Identification and Preparation

The plant sample was collected by a herbalist and subsequently identified by a botanist at Department of Biological Sciences Gombe State University, Nigeria. The plant stem bark sample were cut sufficiently enough to small pieces to allow for quick drying at room temperature. The shade dried sample was ground to powder and kept until use. About 2.70 Kg of the plant sample was initially defatted using hexane. The defatted sample was allowed to dry and then extracted with ten (10) Liters of acetone/methanol (1:1v/v) using maceration technique (Tiwari *et al*., 2011) to give 134 grams of crude extract after concentration of filtrate on rotavapor at 45°C. Furthermore the crude extract was thoroughly washed with hexane. Concentration of the hexane soluble portions afforded about seven (7.0) grams of crude extract. This was subjected to column chromatography.

Isolation of Alkylferulate from *Entada africana* (Guill.&Perr.)

The hexane soluble portion (≈7.0 g) of the acetone/methanol (1:1v/v) crude extract was subjected to open column chromatography. Purification was carried out using gradient elution with hexane/ethyl acetate in the ratio [illegible] 1000 mL per ratio of combined solvents volume. A total of 156 fractions of 100 mL each were obtained. These were concentrated on a rotary evaporator and combined on the basis of their TLC profiles. Fraction No. 56 was found to be pure after repeated recrystallization in methanol and gave a single spot with Rf value of 0.65 in hexane/diethyl ether (3:2). It was coded AC4 and submitted for IR and NMR spectroscopic analysis.

Results

Table 1.IR Data of AC4

IR (cm^{-1})	3332.60 (O-H vib), 2915.27 (C-H vib), 2848.40 (C-H vib), 1705.52(C=O vib), 1632.27 (C=C, conjugated vinyl), 1514.74 (C=C, aromatic), 1472.59

Table 1 Conted.....

	(-CH_2- deformation), 1430.18 & 1377.32 (-CH_3-deformation), 1269.77 (-C-O--stretch); 1157.35 (C-O) Esters; 979.76 (C=C) vinyl vibrations; 844.14-814.81 (*p-disubstituted benzene)*.

Table 2. NMR Data of AC4

^{1}H NMR δ ppm ($CDCl_3$, 400 MHz)	7.61 (1H, d, J = 15.89 Hz); 7.08 (2H, m, J = 4.18 Hz); 6.92 (1H, d, J = 8.12 Hz); 6.30 (1H, d); 5.87(1H, s); 4.19 (2H, m, J = 6.7 Hz); 3.93(3H, s); 3.65 (1H, t, J = 6.62 Hz); 3.49 (1H, s); 1.66 (2H, m, J = 7.03 Hz); 1.27 (br, s), 0.88 (3H,t, J = 6.72 Hz)
^{13}C NMR δ ppm ($CDCl_3$, 400 MHz)	167.40 (C-1), 147.89 (C-7), 146.75 (C-6), 144.63 (C-3), 127.07 (C-4), 123.04 (C-9), 115.69 (C-8), 114.5 (C-2), 109.29 (C-5), 76.70 (C-3'), 64.63 (C-4'), 63.12 (C-2'), 55.94 (-OCH_3 aromatic); 22.70 –32.82 $(CH_2)_n$; 14.10 (-CH_3- terminal).

Discussions

Characterization of Entada africana Isolate AC4

The IR spectrum of AC4 (Table 1) revealed an O-H stretching vibrational frequency of 3332.60 cm^{-1}, while the frequencies at 2915.27 cm^{-1} and 2848.40 cm^{-1} represent the C-H stretching vibrations. The frequency at 1705.52 cm^{-1} stands for the stretching vibrations of the carbonyl (C=O) group of a conjugated ester. The frequency at 1632.27 cm^{-1} represents the vinyl carbon-carbon double (C=C) of alkenes stretching vibrations while the frequency at 1514.74 cm^{-1} represent the aromatic conjugated C=C stretching vibrations of an ester. From ^{1}H NMR (Table 2), the following chemical shifts indicates the presence of feruloyl moiety [δ 7.61 (1H, d), δ 7.08 (2H, m), δ 6.92 (1H, d), δ 6.30 (1H, d), and δ 3.93 (3H, s –OCH_3)] (Lo *et al*., 2001). The chemical shifts at δ 0.88 (3H, m) (Bernards and Lewis, 1992) represents that of a terminal methyl group which is further confirmed by the ^{13}C chemical shift of δ 14.10 ppm while that at δ 1.27 (78 H, br, s) represents those of methylene $(CH_2)_{39}$ groups which accounts for the 78 H of a long aliphatic hydrocarbon chain (*see* appendix for H-NMR spectrum).

A carbonyl methylene at δ 4.19 (2H, m) can be assigned to that of an oxygenated methylene group (-OCH_2-) directly attached to the carbonyl carbon of an ester functional group (Singh *et al*., 2008). Oxygenated methine protons at δ 3.49 ppm, δ 3.65 ppm and δ 5.87 ppm indicates the presence of highly oxygenated ester which is further substantiated by the following ^{13}C chemical shifts at δ 76.70 ppm (C-3'), δ 64.63 ppm (C-4') and δ 63.12 ppm (C-2') as in fig.1 below. Literature comparison with reports of Dobhal *et al.* (1999) and Sarup *et al*. (2015) suggests that AC4 is an alkyl ferulate (Fig. 1). The above data in Tables 1 & 2 are quite consistent with literature and the structure below as reported by Dobhal *et al*. (1999) and Zhu *et al*. (2001). Consequently AC4 proposed molecular formula is $C_{54}H_{98}O_7$ which is in full agreement with the fig.1 structure. Literature reports clearly indicate the existence of alkyl ferulates as mixtures and are difficult to separate (Katagiri *et al.*, 1997; Sob *et al.*, 2011). Consequently the spectroscopic data above sufficiently identified AC4 as an alkylferulate that had been isolated for the first time from *Entada africana.*

In comparison to gallic acid and catechin found in tea essential for their antioxidant activity, the alkyl ferulates reportedly demonstrates higher antioxidant activity than catechin (a flavonoid) with an IC_{50} value of 16 µg/mL (28 µM). It is also reported to demonstrate significant Cytotoxicity against MCF-7 (breast tumor cells) and PC-3 (prostate tumor cells) with an IC_{50} value of 14.3 µg/mL (25 µM) for both cells (Zhu *et al*., 2001).

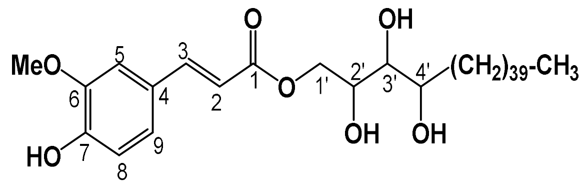

Fig.1: 2,3,4-trihydroxy alkylferulate

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