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Characterisation and Identification of Taraxerol and Taraxer-14-en-3-one from *Jatropha tanjorensis* (Ellis and Saroja) Leaves

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Abstract. *Jatropha tanjorensis* leaves were collected, air dried and pulverised. The pulverised sample was extracted with solvents (*n*-hexane, ethylacetate and ethanol) of varying polarity to obtain the crude extracts. Repeated column and thin layer chromatographic separation of the crude extracts afforded two compounds which were characterised by their IR, MS, ^{1}H and ^{13}C-NMR spectral data. Comparison of the data with literature confirmed the compounds to be taraxerol and taraxer-14-en-3-one.

Keywords: *Jatropha tanjorensis*, taraxerol, taraxer-14-en-3-one, chromatography

Introduction

Jatropha tanjorensis is a plant in the Euphorbiaceae family. It is a perennial herb which is a hybrid specie with phenotypic character between *Jatropha curcas* and *Jatropha gossypifolia* (Prabakan and Sujatha, 1999). The plant is widely cultivated in Nigeria primarily for fencing, as a source of leafy vegetable and for medicinal purpose (Oboh and Masodje, 2009; O'Hara *et al*., 1998).

The leaf extract of the plant is employed traditionally in the treatment of anaemia, diabetes and cardiovascular diseases (Iwalewa *et al*., 2005; Olayiwola *et al*., 2004). Studies have been carried out to validate these claims of the traditional uses. The leaf extract has been shown to have hypoglycaemic properties (Olayiwola *et al*., 2004).

The antioxidant potential of the plant leaf has also been a subject of several studies (Atansuyi *et al*., 2012; Omobuwajo *et al*., 2011; Omoregie and Osagie, 2011) and all these studies confirmed the antioxidant properties of this plant. Toxicity studies have also been carried out on the leaf extract of *J. tanjorensis* with animal models, to ascertain its safety while, some of the studies suggest that it may not be safe (Oyewole *et al*., 2012; Ogoruvwe and Kori-Siakpere, 2012; Igbinaduwa *et al*., 2011; Akhigbe *et al*., 2009). Others suggested, it is safe for human consumption (Omobuwajo *et al*., 2011; Orhue *et al*., 2008).

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The antimicrobial potential of this plant has also been evaluated in several studies (Arun *et al*., 2012; Viswanathan *et al*., 2012; Sekaran, 1998) and the results showed excellent broad spectrum antimicrobial activity against the tested organisms.

Phytochemical analysis of the leaf extract revealed the presence of saponins, cardiac glycosides, flavonoids, terpenoids and tannins (Oyewole and Akingbala, 2011). However, except for the recent work of Viswanathan *et al*. (2012) who reported the isolation of friedelin, β-amyrin, stigmasterol and R(+)-4-hydroxyl-2-pyrrolidinone from the leaf extract of *J. tanjorensis*, there has been no other report in the literature on isolation and characterisation of the phytochemical constituents of this plant. Hence, this study was taken for isolation and characterisation of two terpenoid compounds, taraxerol and taraxer-14-en-3-one, from this plant.

Materials and Methods

Plant material. Fresh leaves of *J. tanjorensis* were collected in the month of April 2012 from Ladoke Akintola University of Technology (LAUTECH.), Ogbomoso, Nigeria. Identification was done in the Department of Pure and Applied Biology, LAUTECH. Harvesting was done with hands properly protected with glove to avoid contact with the milky sap of the plant which causes irritation and itching on contact with the skin.

Sample preparation. The leaves were air dried at room temperature for about two months. Thereafter, the dried leaves were pulverised.

Extraction and isolation. Pulverised sample 755 g was successively extracted with three solvents of varying polarity (*n*-hexane, ethylacetate and methanol), at room temperature (Taylor *et al*., 1983).

The crude *n*-hexane extract (13 g) was subjected to silica gel column chromatography and the column eluted with either one or a mixture of two of *n*-hexane, ethylacetate and methanol. Elution was done by gradually increasing the polarity of the solvent system starting with 100% *n*-hexane. The eluents were collected in fractions of 200 mL each. A total of 50 fractions were collected and analysed by thin layer chromatography, fractions with similar TLC profile were pooled together and concentrated to dryness *in vacuo*. Rechromatography of fraction 21 gave compound 1 (100 mg) as a white crystalline solid.

Fractions 24 to 29 were combined and rechromatographed using the solvent system as stated above and fractions collected at 15 mL interval, compound 2 (65 mg) crystallised out of fraction 10 of this column.

Melting points were determined on a Kofler apparatus and are uncorrected. IR spectra were recorded by using a Thermo Nicolet 5700 FT-IR spectrometer, in $CHCl_3$. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on an Agilent DD2 400 NMR spectrometer at 400 MHz and 100 MHz, respectively. The chemical shifts as δ-values are reported in parts per million (ppm) relative to teteramethylsilane (TMS, δ=0) as internal standard.

The positive and negative ion high resolution ESI mass spectra were obtained from a Bruker Apex III Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (Bruker Daltonics, Billerica, USA) equipped with an infinity cell, a 7.0 Tesla superconducting magnet (Bruker, Karlsruhe, Germany), an RF-only hexapole ion guide and an external electrospray ion source (Agilent, off axis spray). Nitrogen was used as drying gas at 150 °C. The sample solutions were introduced continuously via a syringe pump with a flow rate of 120 μL/h. The data were acquired with 512 k data points; zero filled to 2048 k by averaging 16 scans and evaluated using the Bruker XMASS software (Version 7.0.8).

The electrospray (ESI) mass spectra were performed on a SCIEX API-3200 instrument (Applied Biosystems, Concord, Ontario, Canada) combined with a HTC-XT autosampler (CTC Analytics, Zwingen, Switzerland). The samples were introduced via auto sampler and loop injection. All solvents used for extraction and column chromatography were General Purpose Reagent (GPR), redistilled before use. Column chromatography was carried out on Merck Si gel 60, while thin layer chromatography (TLC) were done with aluminium sheet pre coated with normal phase silica gel 60 F254 (Merck, 0.20 mm thickness). The TLC was run using suitable solvent systems. Spots were located on the developed TLC plates by visualisation under ultraviolet light at 254 and 366 nm.

Results and Discussion

There are few reports on the isolation and characterisation of the phytochemical constituents of *J. tanjorensis*, however, a recent study reported the isolation of friedelin, B-amyrin, stigmasterol and R-(+)-4-hydroxyl-2-pyrrolidinone from the leaf extract of the plant (Viswanathan *et al*., 2012). These phytoconstituents present in the methanol extract were suggested to be responsible for the broad spectrum antimicrobial activity of the plant. Now, two compounds are isolated and reported in this work.

The infra red spectrum of compound 1 (Fig. 1) (m.p 238-240 °C) revealed absorptions at 3048.7 cm^{-1} and 3007.7 cm^{-1} due to =C–H of alkene, 2956.7, 2913.8 and 2847.9 cm^{-1} due to C–H stretch of alkane; 1706 cm^{-1} due C=O strectching vibration and at 1471, 1461 and 1447 cm^{-1} as a result of the bending vibrations of =C–H. The ESI MS of the compound gave its molecular mass as 424 corresponding to the molecular formula $C_{30}H_{48}O$. The mass spectrum of the compound showed intense peaks at m/z 300, 285 and 204. Table 1 showing the ^1H

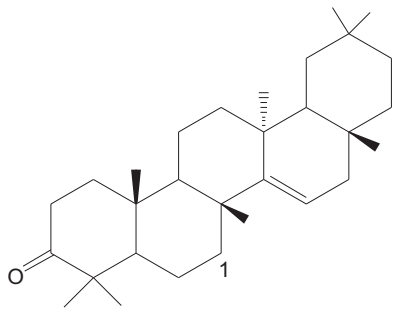

Fig. 1. Structure of Taraxer-14-en-3-one.

Table 1. ^{1}H and ^{13}C NMR data for compound 1

Position	δC (ppm)	C type	δH(ppm)
1	38.3	CH_2	1.90; 1.60
2	34.1	CH_2	2.60 ; 2.36
3	217.5	C=O	-
4	47.6	C	-
5	55.8	CH	1.90
6	19.9	CH_2	1.75 ; 1.60
7	35.1	CH_2	1.70 ; 1.60
8	38.9	C	-
9	48.7	CH	1.70
10	35.8	C	-
11	17.4	CH_2	1.70 ; 1.60
12	37.68	CH_2	1.70; 1.60
13	37.72	C	-
14	157.6	C=	-
15	117.2	HC=	5.57
16	36.6	CH_2	2.10 ; 1.85
17	37.5	C	-
18	48.8	CH	1.70
19	40.6	CH_2	1.70; 1.50
20	28.8	C	-
21	33.6	CH_2	1.75; 1.60
22	33.1	CH_2	1.75 ; 1.60
23	26.1	CH_3	0.99
24	21.5	CH_3	0.99
25	14.8	CH_3	1.03
26	29.9	CH_3	1.14
27	25.6	CH_3	1.14
28	29.8	CH_3	1.03
29	33.3	CH_3	0.83
30	21.3	CH_3	0.83

and ^{13}C-NMR spectra data for compound 1, the ^{1}H NMR spectrum shows a double doublet of an olefinic proton at δ5.57 ppm among other signals confirming the olefinic double bond while the ^{13}C-NMR confirms a total of thirty carbon atoms, eight of which are methyl carbons, ten methylene carbons, four methine carbons and eight quartenary carbons including carbonyl at δ217.5 ppm and the unsaturated carbon atoms at δ157.6 and 117.2 ppm.

The Infra red spectra of compound 2 (Fig. 2) (M.pt 277–280 °C) revealed, among others, a broad absorption band centred at 3843.0 cm^{-1} due to O-H stretching vibration, absorptions at 3052.6 cm^{-1} due to =C-H stretching vibration, absorptions at 2914.0 and 2848.0 cm^{-1} due to C-H stretching vibration of alkanes and at 1641 cm^{-1} due to C=C stretching vibrations. The ESI MS of the compound gave a molecular mass of 426 corresponding

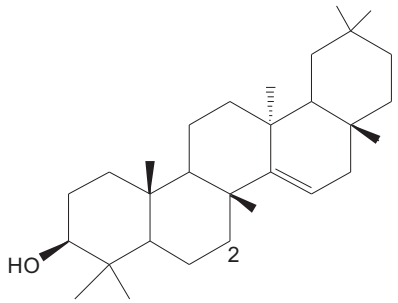

Fig. 2. Structure of Taraxerol.

Table 2. ^{1}H and ^{13}C NMR data for compound 2

Position	δC (ppm)	C type	δH (ppm)
1	37.7	CH_2	1.60; 1.15
2	27.2	CH_2	1.60; 1.40
3	79.1	C—OH	3.2, 3.6
4	39.0s	C	-
5	55.5	CH	1.40
6	18.8	CH_2	1.60; 1.15
7	35.1	CH_2	1.95; 1.60
8	38.8	C	-
9	48.8	CH	1.40
10	35.8	C	-
11	17.5	CH_2	1.15
12	37.7	CH_2	1.15
13	37.6	C	-
14	158.1	C=	-
15	116.9	HC=	5.5
16	36.7	CH_2	2.01; 1.95
17	38.0	C	-
18	49.3	CH	1.40
19	41.3	CH_2	1.15
20	28.8	C	-
21	33.7	CH_2	1.15
22	33.1	CH_2	1.15
23	28.0	CH_3	0.82
24	15.44	CH_3	0.82
25	15.4	CH_3	0.99
26	29.9	CH_3	1.10
27	25.9	CH_3	1.10
28	29.8	CH_3	0.99
29	33.3	CH_3	0.82
30	21.3	CH_3	0.82

to a molecular formula of $C_{30}H_{50}O$. The mass spectrum of the compound also showed intense peaks at m/z 302 and 204. The ^{1}H NMR of compound 2 confirmed the presence of OH group in the compound through the double doublet centred at δ3.20 ppm, the methylene group is also confirmed through the double doublet at δ5.50 ppm. The ^{13}C NMR spectrum revealed a total of thirty carbon atoms distributed as follows: 8 methyl, 10 methylene, 5 methine and 7 quartenary carbons as presented in Table 2. The spectra characteristics of these compounds confirm compound 1 to be taraxer-14-en-3-one while compound 2 as taraxerol. These interpretations are in excellent agreement with the literature data for these compounds isolated and characterised from *Myrica rubra* and *Euphorbia pubescens* (Valente *et al*., 2004; Sakurai *et al*., 1987).

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