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Short Communication

Arjunolic Acid from the Root Bark of *Terminalia catappa* Linn

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ABSTRACT

Terminalia catappa (Linn.) is used in traditional medicine to treat dysmenorrhea and typhoid fever in the Tiv speaking parts of Benue State, Nigeria. The aim of this study was to investigate the plant for its chemical contents that could be responsible for the reported medicinal activity. Extraction of root bark of the plant was done with n-hexane, ethyl acetate and methanol. The ethyl acetate extract was subjected to column chromatography on silica gel 60H, 200-400 mesh, eluted gradient wise with hexane:ethyl acetate. Fractions (ND95-97) obtained from hexane:ethyl acetate (60:40) were combined based on similar TLC R_f values. On standing, the combined fractions gave a white solid with melting point 230 – 231 ºC which gave a positive result to the Liebermann-Burchard test for pentacyclic triterpenes. Its mass spectrum showed a molecular ion peak at *m/z* 506.3879 $[M + H_2O]^+$. On the basis of ^1H, ^{13}C, 2D (HMBC, HSQC, COSY) NMR spectral data, mass spectrometry, and by comparison of spectra data with literature, ND95-97 was characterized as Arjunolic acid (2,3,23-trihydroxyolean-12-en-28-oic acid). To the best of our knowledge, this is the first report of the isolation of Arjunolic acid from the root bark of *Terminalia catappa*.

Keywords: Arjunolic acid, *Terminalia catappa*, Triterpene, Root.

Introduction

Plants contain several chemical components including lipids, flavours, fragrances, pigments, etc. which serve man's daily needs. Prominent among these is their use as medicines.[1] Records from ancient civilizations like Mesopotamia dating about 2600 BC describe approximately 1000 plant-derived products used for the treatment of ailments ranging from coughs and colds to parasitic infections and inflammation.[2] The Chinese *Materia Medica* and the Egyptian *Ebers Papyrus* are other records of ancient use of natural products in human and animal medicine.[2] Crude aqueous extracts of various plants are still used for the treatment of diseases like malaria.[3] The dependence on plants as source of medication has continued to increase, especially among rural dwelling low income earners because plants are affordable and readily available. The World Health Organization (WHO) has estimated that about 80% of the population in African countries depends on herbal medicine for their primary health care needs.[4] For rural dwellers, herbal medicine provides a relatively convenient alternative to orthodox medicine which in some cases is either not affordable or not readily available.

Terminalia catappa Linn. (Combretaceae) is a herbal medicinal plant commonly called Indian almond in English and *fruutu in* Tiv language. It is a tropical tree that can grow up to 35 m high and grows mainly in the tropical regions of Asia, Africa, and Australia.[5] The tree is deep rooted in the sand but can have shallow lateral root system where the water table is high.[6] In Indian traditional medicine, its leaves are used to treat leprosy, scabies, bleeding, cough and asthma.[6] The stem bark has been reported to treat bilious fever, diarrhea, thrush, sore throat and abscess.[7]

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In Nigeria, its use in traditional medicine varies with tribe. Among the Tiv speaking people of Benue state, its root bark is used to treat dysmenorrhea and typhoid fever, however, these claims have not been validated scientifically. In addition, its fruits are used for food. Its Seeds were found to contain Oleic acid, Linoleic acid, Phosphorus, Sodium, Potassium, Iron, Magnesium, Calcium and the amino acids - tryptophan and Lysine.[8] Methanol extracts of its stem bark administered (500 mg/kg body weight) to induced diabetic rats for 7 days significantly ($p < 0.05$) reduced their fasting blood glucose level comparable to that obtained with Metformin (vended drug), indicating that the stem bark extract possesses anti-diabetic activity.[9] Tannins, flavonoids, triterpenes and glycosides have been isolated from different parts of the plant.[10-16] The present study investigated the root bark of the plant for its chemical contents that could be responsible for the reported medicinal activity.

Materials and Methods

General experimental procedure

NMR spectra (^1H and ^{13}C) were performed on a Bruker DRX 400 spectrophotometer (400 MHz for proton and 125 MHz for ^{13}C) in DMSO using Tetramethylsilane (TMS) as internal standard, while mass spectrometry was done with a Bruker Compass DataAnalysis 4.4 mass spectrometer. Column chromatography was performed on silica gel 60H, 200-400 mesh. Thin layer chromatography (TLC) was performed on pre-coated aluminum backed (Silicycle) TLC plate silica gel (0.2 mm) and visualized by spraying with conc. Sulfuric acid and then heated to 105ºC.

Sample collection and preparation

The root bark of *T. catappa* was collected from the wild around Federal Housing Estate in North Bank, Makurdi. The plant material was identified at the Department of Forest Products and Production, Federal University of Agriculture, Makurdi. The specimen was assigned the voucher number UAM/FH323 and deposited in their herbarium. The root bark was air-dried under shade to a constant weight and then pulverized into coarse powder.

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Extraction
The pulverized *Terminalia catappa* root bark (1 kg) was extracted by maceration in n-hexane for 72 h at room temperature with intermittent manual agitation. After 72 h, the solvent was decanted and filtered using Whatman No. 1 filter paper. The extraction process was repeated using ethyl acetate and methanol. The filtrates were concentrated using a rotary evaporator at 40ºC.

Phytochemical screening
Phytochemical screening tests were carried out on the crude n-hexane, ethyl acetate and methanol extracts following standard procedures.[17, 18]

Isolation and characterization
The crude ethyl acetate extract (6 g) was dissolved in ethyl acetate (20 mL) and adsorbed onto silica gel (6 g) then the solvent was allowed to evaporate completely to form a slurry. The slurry was applied as a concentrated band onto a gravity column and eluted gradient-wise, starting with hexane (200 mL), followed by mixtures of hexane:ethyl acetate 95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45, 50:50 (200 mL each).[19,20] Fractions were collected in 20 mL vials and allowed to stand until the solvents evaporated to dryness at room temperature. Similar column fractions were combined after TLC analysis. [21] Fractions 95, 96 and 97 eluted with hexane:ethyl acetate 60:40 gave similar TLC profile (single brown spots when charred with concentrated sulphuric acid, R_f values 0.40 (EtOAc:MeOH 9:1). The fractions 95, 96 and 97 also were subjected to the Liebermann-Burchard test for terpenoids. The combined fractions 95-97 were recrystallized in ethyl acetate to yield compound 1 labeled ND95-97. The compound was subjected to spectroscopic analysis (NMR spectroscopy and mass spectrometry).

Results and Discussion

Phytochemical Screening
Results of phytochemical screening (Table 1) showed that all the extracts contained alkaloids and tannins. Flavonoids were not detected in the hexane extract; however, they were present in the ethyl acetate and methanol extracts. Steroids and terpenes were detected only in the ethyl acetate extract. Cardiac glycosides and saponins were not detected in any of the extracts.

Extraction and Isolation
The ethyl acetate extract (6 g, whitish yellow solid) subjected to column chromatography, eluted gradient-wise with n-hexane:ethyl acetate yielded 119 fractions. TLC profile showed that fractions 95, 96 and 97 eluted with hexane:ethyl acetate 60:40 are similar: single brown spots when charred with concentrated sulphuric acid on silica gel TLC plates; R_f values (0.40; EtOAc: MeOH, 9:1). In addition, they had similar physical properties: appearance (white solid), and melting point (230 – 231ºC). They also tested positive to the Liebermann-Burchard test for terpenoids.

Table 1: Phytochemical Analysis of the Root Bark Extracts *of Terminalia catappa* Linn.

Constituent	Inference		
	Hexane extract	EtOAc extract	Methanol extract
Alkaloids	+	+	+
Flavonoids	-	+	+
Saponins	-	-	-
Tannins	+	+	+
Steroids and triterpenes	-	+	-
Cardiac glycosides	-	-	-

Key: + = present, - = not detected, EtOAc = Ethyl acetate.

Characterization of ND95-97
ND95-97 displayed a molecular ion peak at m/z 506.3879 $[M + H_2O]^+$ in the HRESIMS (High Resolution Electrospray Ionization Mass Spectrometry), consistent with the molecular formula $C_{30}H_{48}O_5$. The ^{1}H-NMR spectrum of ND95-97 recorded in DMSO-d_6 showed signals at 1.74 ppm and 1.36 ppm. These signals are characteristic signals for non-equivalent methylene protons attached to the same carbon.[22] Other proton signals were observed at δ_H 3.48 ppm (1H, m), 3.17 ppm (1H, d, J = 9.4 Hz), 3.03 ppm (1H, d, J = 10.6 Hz) and 3.30 ppm (1H, d, J = 10.6 Hz). The signals are similar to signals reported for protons bonded to carbons that are attached to hydroxyl groups;[23] they indicate the presence of three hydroxyl groups in the compound. This is consistent with reported data on Arjunolic acid[22]. Other signals were for an olefinic proton at δ_H 5.17 ppm (1H, t, J = 3.6 Hz) and six methyl protons at δ_H 0.54 ppm (3H, s), δ_H 0.91 ppm (3H, s), δ_H 0.71 ppm (3H, s), δ_H 1.10 ppm (3H, s) and δ_H 0.87 ppm (6H, s). The ^{13}C-NMR spectrum showed signals for one carboxylic acid carbon at δ_C 178.7 ppm, two olefinic carbons at 121.4 ppm and 144.0 ppm. These chemical shifts are characteristic of Olean-12-ene triterpenes.[24] Other signals were for six methyl carbons, ten methylene carbons including a hydroxyl methylene at δ_C 63.9 ppm, six methine carbons including an olefinic carbon at δ_C 121.4 ppm, two oxygen-bearing carbons at δ_C 67.4 ppm and δ_C 75.5 ppm, and six other quaternary carbons. A total of 30 carbon signals were observed indicating a triterpene. The ^{1}H- and ^{13}C-NMR spectra of compound ND95-97 are consistent with NMR data published for compounds that have an oleanane pentacyclic moiety.[24-26] The ^{1}H- and ^{13}C-NMR data for ND95-97 agrees with NMR data published for Arjunolic acid.[22,28] Compound ND95-97 was thus identified as Arjunolic acid (2,3,23-trihydroxyolean-12-en-28-oic acid) (Figure 1) by comparison of the spectral data with previous reports.[22-24, 28] Table 2 showed the NMR data for ND95-97.

Conclusion

This study resulted in the isolation and characterization of a pentacyclic, olean-12-ene triterpeniod, Arjunolic acid from ethyl acetate extract of the root bark of *Terminalia catappa* Linn. Arjunolic acid had earlier been reported from ethyl acetate and methanol extracts of the stem bark of *Terminalia arjuna*,[16] hexane root bark extract of *Terminalia avicennioides* and *Terminalia chebula.*[22,23] The isolation of Arjunolic acid from *Terminalia catappa* suggests that Arjunolic acid is of chemotaxonomic significance in the genus *Terminalia*.

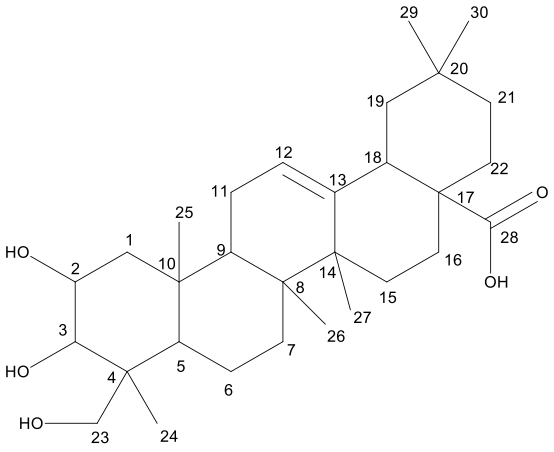

Figure 1: Chemical Structure of compound ND95-97 (Arjunolic acid).

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Table 2: ^{1}H- and ^{13}C-NMR Data for ND95-97.

ND95-97			
Position	**δ_H**	**δ_C**	**^{13}C-type**
1	1.74, 1.36	47.1	CH_2
2	3.48 (m)	67.4	CH
3	3.17 (*J* = 9.4 Hz)	75.5	CH
4	-	42.5	C
5	2.01	45.7	CH
6	1.73, 1.33	17.5	CH_2
7	1.56, 1.10	32.8	CH_2
8	-	40.8	C
9	1.54	37.4	CH
10	-	38.3	C
11	1.83, 1.45	23.4	CH_2
12	5.17 (1H, t, *J* = 3.57 Hz)	121.4	CH
13	-	144.0	C
14	-	41.4	C
15	1.72	27.2	CH_2
16	1.70, 3.33	31.9	CH_2
17	-	46.0	C
18	2.74 (dd, *J* = 13.7, 4.3 Hz)	40.2	CH
19	1.67, 1.22	46.7	CH_2
20	-	30.4	C
21	1.87, 1.77	32.1	CH_2
22	3.27, 1.20	45.4	CH_2
23	3.03 (d, *J* = 10.6Hz), 3.30 (d, *J* = 10.6)	63.9	CH_2
24	0.54 (3H)	13.7	CH_3
25	0.91 (3H)	16.9	CH_3
26	0.71 (3H)	16.8	CH_3
27	1.10 (3H)	25.7	CH_3
28	-	178.7	C
29	0.87 (3H)	23.0	CH_3
30	0.87 (3H)	22.6	CH_3

Conflict of interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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