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Isolation and Characterization of Lupeol Acetate from the Root Bark of *Tacazzea apiculata* (Oliv.)

*Yakubu, M. B., Mohammad, F. S. and Musa, B.
Department of Applied Chemistry, Kaduna Polytechnic, Kaduna, Nigeria
Corresponding Author:bidam.shinku@gmail.com

ABSTRACT

The aim of the present study was to isolate and characterize lupeol acetate from the ethyl acetate fraction of the root bark of *Tacazzea apiculata*. *Tacazzea apiculata* is a plant that is variously used in ethno-medicine to treat inflammation, hemorrhoid, cancer, wound healing, worm expulsion, skin disease and abdominal pain. To isolate this compound, the ethyl acetate fraction was subjected to a combination of thin layer chromatography (TLC), column chromatography and preparative thin layer chromatography. The structure of the isolated compound was determined by analysis of its FTIR and ^{1}HNMR spectral data, as well as comparison with reported data. Lupeol acetate was obtained as a white needle - like compound with melting point of 214-216 ^{o}C. This is the first report of isolation of lupeol acetate from the root bark of *Tacazzea apiculata* Oliv.

Key words*:* Column chromatography, Lupeol acetate, Isolation, *Tacazzea apiculata and* triterpenoid.

INTRODUCTION

Tacazzea apiculata Oliv (Family: Apocynaceae) is a woody climber, indigenous to tropical Africa. It is popularly called Craw-craw vine. The plant is known by the Hausa people of northern Nigeria as "*Yadiyar kada"*. The flowers are edible [1]. In South Africa, the twig is powdered and taken with milk or water as "tonic" to improve the general health condition of the body. The leaves are used to treat skin diseases [2]. In northern Nigeria the powdered root mixed with milk or honey is taken orally to relief pains in pile. The plant is also reported to be used in traditional medicine for the treatment of some forms of cancers and inflammations [3]. The powdered leaves are used to treat snake bite stings by venomous animals. The decoction of the root is used for convulsion and epilepsy by the Hausa people of North-western Nigeria [4].

Triterpenoids are widespread in nature, and can be found in fruits, vegetables, cereals, fungi, ferns, monocotyledonous and dicotyledonous plants, animals and marine organisms [5].

Lupeol acetate, a pentacyclic triterpenoid, is an important constituent of *T. apiculata*, and may be closely related to its anti-inflammatory activity. Several researchers have isolated lupeol acetate from medicinal plants because of its known antimicrobial, antifungal, antivenom and anti-inflammatory actions [6-9]. The aim of this study is to isolate and characterized lupeol acetate from the ethyl acetate fraction of the root bark extract of *T. apiculata.*

MATERIALS AND METHODS

Collection, Identification and preparation of plant materials

Samples of *T. apiculata* were collected from Kangimi Village in Zaria and transported to the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria, for authentication. A voucher No. 6975 was deposited in the Herbarium for reference purposes. The leaves, twigs and the root were separated and air-dried under room temperature and crushed into powder using pestle and mortar. The root materials were stored in plastic container and properly kept in the lab until needed for use [10]. The powdered root bark material (500 g) was extracted successively using 2 L of methanol. The solution was filtered and concentrated *in-vacuo* using rotary evaporator. The methanol extract was reconstituted in warm water and partitioned with n-hexane and ethyl acetate to obtain the n-hexane and ethyl acetate fractions respectively [11].

Thin layer chromatography (TLC) of the ethyl acetate fraction.

TLC was performed on the ethyl acetate fraction obtained. Pre-coated plates, gF254 (Silica gel on Aluminum support) 3 cm by 7 cm were used. After drying, the spots were visualized using UV light (254 nm and 366 nm) and by spraying with 10% H_2SO_4, followed by heating in an oven. R_f values were calculated as quotient of the distance moved by the spots divided by the solvent front.

Isolation and purification using column chromatography

Hexane slurry of silica-gel powder (200 g) was packed in a glass column (70 x 30 cm). The extract (5 g) in a fine powdered form was loaded onto the column and allowed to stabilize for onehour before elution started. The column was eluted using gradient profile. The elution began with 100% n- hexane and ethyl acetate was added gradiently from 0 to 100%. Eighty fractions of 50 ml each were collected and monitored by TLC and sprayed with 10 % sulfuric acid.

Similar fractions were pooled together and concentrated *in-vacuo*. The fractions were combined into five fractions B1 (9 mg), B2 (18 mg), B3 (10 mg) and B4 (20 mg) and B5 (12 mg) based on their TLC spot profile. Fraction B2 had two spots which were separated using preparative thin layer chromatography. Fifteen (15 mg) of the major compound was obtained and coded as **TAB2**.

Structural determination of the bioactive compound TAB2

The structure of the compound TAB2 was determined using ^{1}HNMR spectroscopy and Infrared spectroscopy (IR). The Infrared (IR) spectra of the isolated compound which revealed the functional groups present in the compound was recorded as KBr pellets on a FT-IR spectrometer (Perkin-Elmer RX1, USA). The KBr pellet (13 mm diameter) was prepared by mixing the finely ground solid sample (1 mg) and powdered KBr (1:100, w/w) and pressed under high pressure, 4000 pound per square inch (psi) by a hydraulic pellet press (ICL, USA). IR spectra were recorded from an accumulation of 40 scans at wave numbers from 4000 to 600 cm^{-1} with a resolution of 4 cm^{-1}. The^{1}H NMR (400 MHz), spectra of the isolated compound were recorded on an NMR spectrometer (JEOL- JMN-ECX 400, Japan). Fifteen milligram of sample was dissolved in deuterated solvent, filtered and transferred into NMR tube (Kimble Chase-400 MHz, Vineland) until it achieved a height of 4 cm. All chemical shifts (δ) were in parts per million (ppm) and coupling constants (J) were expressed in Hz. All the spectra were analysed and the results compared with published literatures to determine the structure of the isolated compound.

Melting Point Determination/ Sample Preparation

About 0.5 mg of the solid sample was loaded into separate capillary tube and the melting points determined on an electronic melting point apparatus. The melting point was taken as a range of the beginning and total melting temperatures.

Triterpenoid test for isolated compound TAB2

The isolated compound TAB2 (1 mg) was placed in a test tube; a few mg of Liebermann-Buchard reagent (glacial acetic acid+Conc.H_2SO_4) was added. The formation of green colour indicates that the compound TAB2is suspected to be a triterpenoid [15].

RESULTS AND DISCUSSION

The results of percentage yield of the extracts are presented in Table 1. Methanol extract had a mass of 22.5 g which is about 4.5% of the total weight of the plant, while n-hexane had the

lowest weight of 12.3 g representing 2.5% of the total weight of plant. The ethyl acetate gave 15.6 g which is about 3.1% of the total weight of the plant. The observed higher yield of the methanol extract is due of it high polarity. Dhawan and Gupta reported that highly polar solvents are better solvents for extraction of plant material than other solvents [12].

Table 1: Percentage yield of extract obtained from 500 g of powdered plant.

S/No	Solvents used	mass of extracts / fractions (g)	percentage yield (%)
1	Methanol	22.5	4.5
2	Ethyl acetate	15.6	3.1
3	n-Hexane	12.3	2.5

Thin layer chromatography analyses of the ethyl acetate fraction indicated five (5) phyto-constituents, their R_f values and the various colours in 10% H_2SO_4. The result is as presented in Table 2. The results of fractions obtained from the column chromatography (Table 3) showed five fractions labeled B1 to B5 at various solvent systems. Fraction B1 and B5 had three (3) spots each, fraction B3 and B4 revealed five (4) spots each while fraction B2 had two (2) spots.

Table 2: Thin layer chromatography of the ethyl acetate extract

Compound spots	R_f Values	Colour in 10% H_2SO_4
1.	0.57	Purple
2.	0.54	Orange
3.	0.64	Brown
4.	0.32	Purple
5.	0.16	Brown

Key: R_f = Retention factor

Preparative thin layer chromatography was used to separate fraction B2 to obtain a white-needle like compound labeled as **TAB2.** The compound gave a purple TLC single spot in 10% H_2SO_4. The physical attributes of the compound **TAB2** is as shown in Table 4. The crystals had melting point of 214-216 ^{O}C and was in agreement with those cited in literature [13, 14]. The name, Lupeol acetate, was assigned to this compound by comparing its spectroscopic data from IR and

^{1}HNMR analysis which compares very well with those of authenticated samples reported by Jamal *et al.*, [15] for lupeol acetate.

Table 3: Fraction from column chromatography of Ethyl acetate extract

Fractions	Eluting solvents	Number of major spots
B1	Hexane; Ethyl acetate (95**:**5)	3
B2	Hexane; Ethyl acetate (90**:**10)	2
B3	Hexane; Ethyl acetate (85**:**5)	4
B4	Hexane; Ethyl acetate (80**:**20)	4
B5	Hexane; Ethyl acetate (70**:**30)	3

Table 4: TLC profile of the isolated compounds TAB2

Compound code	solvent system	Observed colour of Spot	R_f Value	Melting point (^{o}C)
TAB2	Hexane ethyl acetate (9:1)	1	0.56	214-216

The compound TAB2 was isolated as a white needle –like crystals (15 mg) with melting point of 214 – 216 °C. Retention factor (R_f) value of 0.56 was recorded on TLC plate using n-hexane: ethyl acetate (9:1) as the solvent system. The isolated compound TAB2 was characterized using ^{1}H NMR and the results are shown in Table 7 and Figure 1. Based on the spectrum, the signal showed eight methyl signals at δH 2.04 (3H, H-2), 0.82 (3H, H- 23), 0.83 (3H, H-24), 0.85 (3H, H-25), 1.02 (3H, H-26), 0.94 (3H, H-27), 0.76 (3H, H-28) and 1.69 (3H, H-30) ppm. A doublet at 4.56 and 4.68 ppm shows C-29 atomic shifts (2H, dd, H-29a, 29b), along with that methyl signal at δH 1.69 ppm for C-30 suggested that the isolated compound TAB2 was a lupane-type triterpenoid [16]. A multiplet at δH 4.03 ppm is characteristic of α-oriented proton at C-3, and a characteristic singlet of methyl α-oriented proton at δH 2.37 ppm for C-32 suggested that TAB2 is an ester derivative of lupeol-type triterpenoid [17]. Based on the above findings a comparison of the chemical shift of TAB2 isolated from the ethyl acetate fraction of the root bark of *Tacazzea apiculata* compared very well with the compound lupeol acetate, $\mathbf{C_{32}H_{52}O_2}$, also known as (3β)-Lup-20(29)-en-3-yl acetate.

Table 5: Characteristic FTIR intensities (Vcm^{-1}) of compound TAB2

Compound	C=O Ketone	C-H Methyl	C-O Acetate	CH Methylene	C=C Alkene	CH (Ben.vib.) Methyl	CH (Ben.vib) Ethylene
TAB2	1718.3	2926.0	1259.8	2851.1	1640	1457.4	1384.1

KEY: V= Wave number; alip = aliphatic; ben.vib =bending vibration

Table 6: Liebermann – Buchard Test for Isolated compound TAB2

Isolated Compound	Test	Result	Inference
TAB2	Liebermann's test	+	Terpenoids/steroids

Table 7: Comparison of δ_H(Hz) HNMR of TAB2 with lupeol acetate of standards in $CDCl_3$ [15].

H- Position	Isolated TAB2	Literature value	Number of Hydrogen
1	0.96	0.99	2H
2	1.60	1.62	2H
3	4.03	4.47	H
4	-	-	-
5	0.78	0.79	H
6	1.51	1.50	2H
7	1.46	1.49	2H
8	-	-	-
9	1.28	1.28	H
10	-	-	-
11	1.38	1.39	2H
12	1.68	1.66	2H
13	1.65	1.65	H
14	-	-	-
15	1.49	1.67	2H
16	1.42	1.47	2H
17	-	-	-
18	1.35	1.35	H
19	2.41	2.40	H
20	-	-	-
21	1.91	1.91	2H
22	1.36	1.38	2H
23	0.82	0.84	3H
24	0.83	0.83	3H
25	0.85	0.85	3H
26	1.02	1.03	3H
27	0.94	0.94	3H

28	0.76	0.78	3H
29	4.68	4.69	2H
30	1.69	1.68	3H
31	-	-	-
32	2.04	2.05	3H

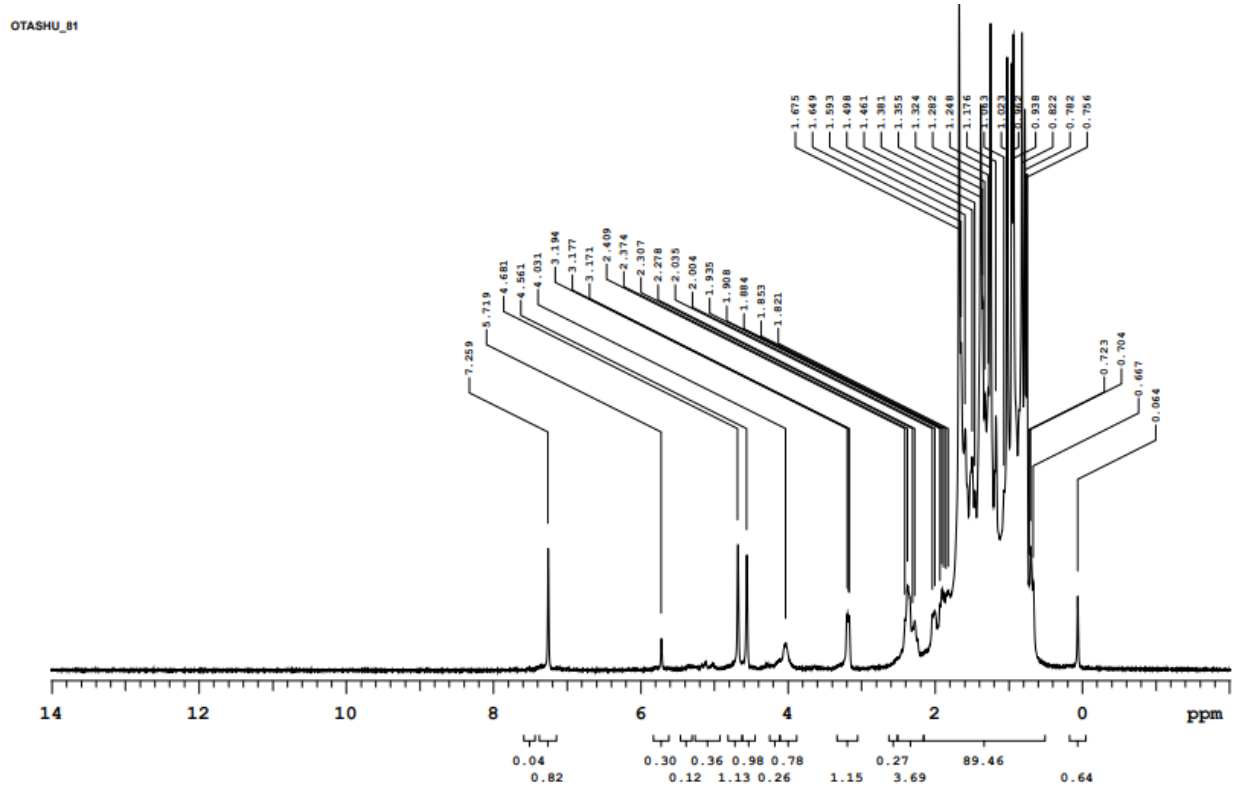

Figure 1:[1]HNMR spectrum of lupeol acetate

The presence of acetate group in the lupeol compound was amplified by the presence of carbonyl group. (C = O) at the wave number at 1733 cm-¹ and the absorption of C-O at wave number of 1259 cm-¹ on theFTIR spectra (Figure 3). The compound isolated has a band at 1673 cm^{-1} indicating C = C vibrations, (C29) [17]. The IR absorbance values are in concordance with Silverstein *et al*., [18]. Therefore, the isolated compound TAB2 was identified as lupeol acetate (Figure 2)**.** The compound isolated as lupeol acetate is known for its biological activity such as anti-inflammatory, antioxidants, anti-cancer antivenom and antimicrobial activities [19, 20].

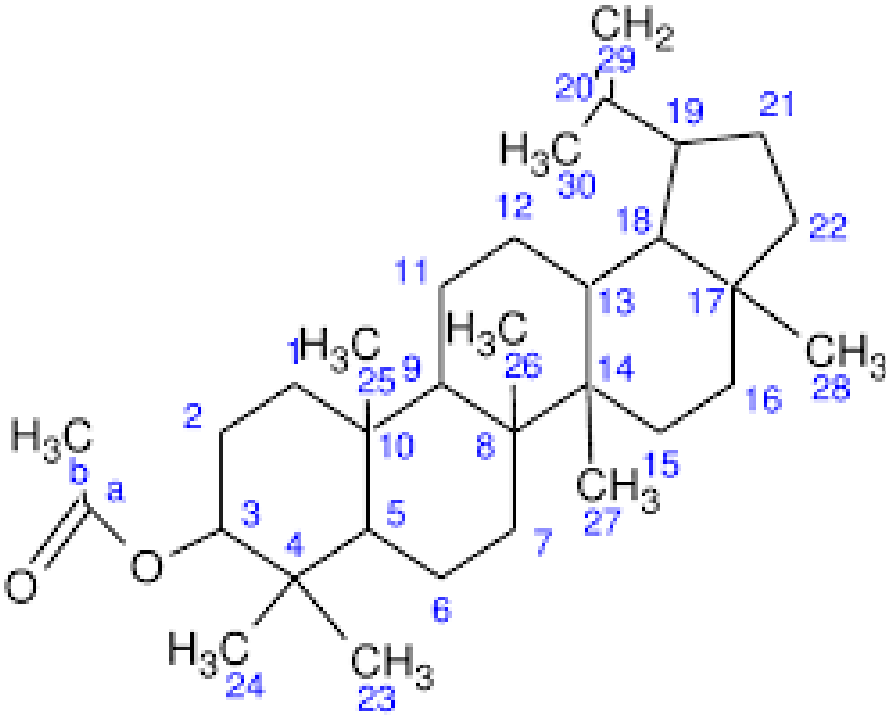

Figure 2: Complete structure of lupeol acetate

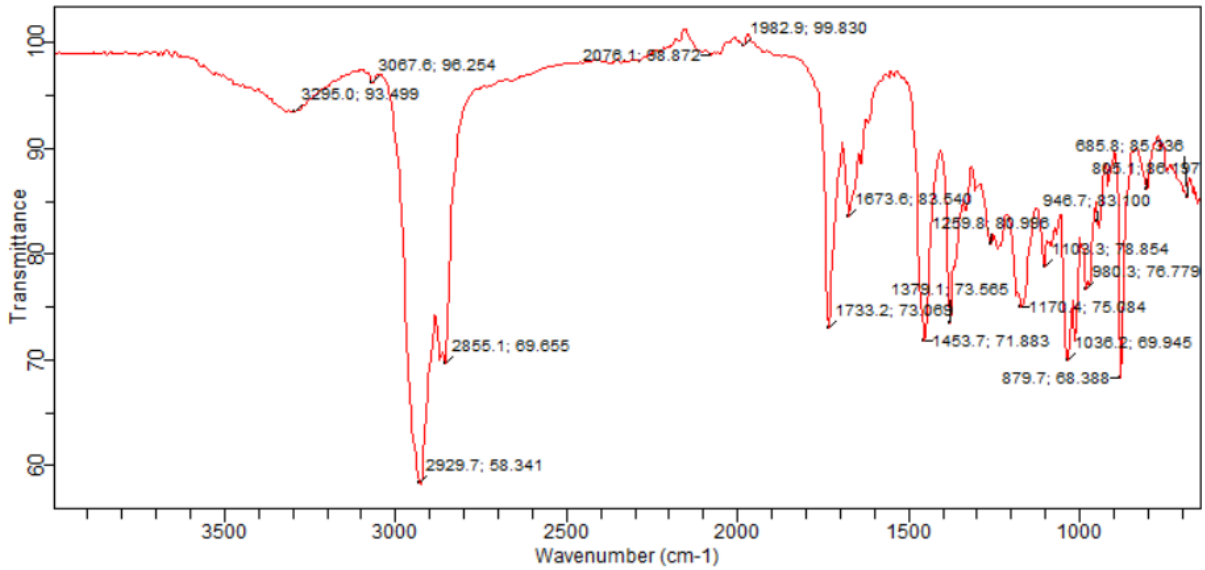

Figure 3: FTIR Spectrum of TAB2

CONCLUSIONS

Based on column chromatography and spectroscopic analysis of FTIR and ^{1}HNMR data, lupeol acetate (TAB2) have been isolated and characterized. This compound is reported for the first time as constituents of *Tacazzae apiculata*. The plant extracts of *Tacazzae apiculata* could therefore be seen as a potential source of useful drugs and this justifies the claim by the traditional healers that *Tacazzae apiculata* is used to cure illnesses. The continued use of this plant by traditional medicine users is therefore encouraged.

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