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Lupeol Acetate Isolated from n-Hexane Extract of *Tapinanthus globiferus* Leaf

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ABSTRACT
A study was carried out to isolate and characterise Lupeol Acetate from the leaves of *Tapinanthus globiferus*. The dried pulverized plant material was extracted for 24 hours with n-hexane, dichloromethane, ethyl acetate and methanol solvents. The /n-hexane fraction obtained was subjected to column chromatography followed by preparative thin layer chromatography, which resulted in isolation of a colourless crystalline compound. The spectral data (^{1}H NMR, ^{13}C NMR and FTIR) and literature comparison infer that the isolated compound is a pentacyclic triterpenoid namely Lupeol Acetate.

Keywords: *Tapinanthus globiferus*, Lupeol Acetate, Structure elucidation

INTRODUCTION

Tapinanthus globiferus is a plant commonly used in folkloric medicine and highly consumed in Nigeria. *T. globiferus* known as mistletoe (in English) belongs to the family Loranthaceae. It is a woody, spreading shrub with blackish, smooth stems made rough by the presence of lenticels. It is popularly called "afomo" in South Western Nigeria. *T. globiferus* is commonly consumed for the treatment of hypertension, ulcers, diabetics, weakness of vision, and for promoting muscular relaxation before delivery (Obatomi *et al*., 1994; Deeni *et al*., 2002; Ademiluyi and Oboh, 2008).

Latex from several medicinal plants is rich in triterpenes. These are molecules formed by thirty carbon atoms and six isoprenoid units (with five carbon atoms each) (Patočka, 2003). The triterpenes are divided into several families with different base structures. Lupeol, betulin, betulinic acid and calenduladiol are triterpenes belonging to the lupine family. As far as their biological activities are concerned, the pentacyclic triterpenes including lupeol are a group of promising secondary plant metabolites (Laszczyk, 2009).

Lupeol is an important constituent of *Tapinanthus globiferus* and may be closely related to its anti-inflammatory action. Several researchers have isolated lupeol acetate from medicinal plants because of its known antitumor, antifungal and anti-inflammatory actions (Larrosa and Duarte, 2005; Sanusi *et al*., 2013; Kipkemei, 2017; Daniel *et al*., 2010).

The present study aim at isolating and characterizing the compound(s) from *T. globiferus* grown on *Piliostigma thonningii* tree.

MATERIALS AND METHODS

Plant Materials Collection, Preparation and Identification

Tapinanthus globiferus leaves were collected from the plant farm of the Faculty of Pharmaceutical Science Usmanu Danfodiyo University, Sokoto, in the month of November, 2015. It was identified and authenticated by U.S. Gallah, a consultant taxonomist at the Department of Pharmacognosy, UDUS where a herbarium specimen was deposited and a voucher number PCG/UDUS/FABA/0117 issued. It was air dried for three weeks, powdered with the aid of a clean mechanical grinder and stored in an air tight glass container until use (Ogbiko *et al*., 2018).

Extraction and fractionation

Three hundred grams (300 g) of *T. globiferus* powdered leaves was separately extracted by soaking the pulverized plant material separately with solvents of increasing polarity starting with n-hexane, dichloromethane, ethyl acetate and methanol for 24 hours. The solvents were decanted and filtered with Whatman filter paper. The filtrates were concentrated under reduced pressure at 45°C in a rotary evaporator (Stuart RE 300) and dried at room temperature to constant weight to obtain the n-hexane fraction (NHF), dichloromethane (DCF), ethyl acetate fraction (EAF) and methanol fraction (MF) respectively. The percentage yields were

determined for all the concentrated fractions before they were stored at 4ºC till use (Cyril *et al*., 2017).

Thin layer chromatography

Pre-coated silica TLC plates (6 × 8 cm) were used and each fraction was applied as small droplet. n-hexane: ethyl acetate (8:2), dichloromethane: methanol (9: 1), ethyl acetate: n-hexane (8:2) and ethyl acetate: methanol: water (8:1:1) were used as solvent compositions for the NHF, DCF, EAF and MF respectively. Spots were visualized under day light, ultraviolet light (254 nm and 365 nm) and then following spraying with 10% tetraoxosulphate (vi) acid followed by heating in an oven for 5 minutes at 105 ºC. The extract with the best separation was further purified using the column chromatography technique (Hassan *et al*., 2018).

Purification using column chromatography

One hundred and twenty grams (120 g) of silica gel (60-120 mesh) was made into slurry with 100% n-hexane and was packed into a 2.5 × 63 cm glass column and allowed to stand for about one hour to attain stability. 3 g of n-hexane extract was pre-adsorbed on 3 g of silica gel and loaded onto the column. The loaded sample was eluted gradiently starting with n-hexane (100 %), n-hexane: ethyl acetate (99: 1), n-hexane: ethyl acetate (98: 2), n-hexane: ethyl acetate (97: 3) and n-hexane: ethyl acetate (96: 4). A total of 60 fractions were collected and regrouped based on their TLC profiles and R_f values, fractions 1 to 20 were combined together. The fractions consist of one major spot and some impurities. The fractions were then subjected to preparative TLC for further purification. A colourless crystalline non oily compound was isolated, and was coded MB (Hassan *et al*., 2018).

Spectroscopic characterization

Spectroscopic analyses using ^{1}H-NMR and ^{13}C-NMR were carried out. A mixture of 0.5 cm^3 of deuterated chloroform with 20 mg of the pure compound was used for identification. The assay was developed at 22°C using tetramethylsilane (TMS) as an internal reference. ^{13}C signals were acquired under standard conditions, and the NMR spectrum was recorded at high resolution on a 200 MHz Varian Mercury spectrometer (Palo Alto, CA, USA). 4 mg of the isolated compound was also subjected to FTIR analysis.

RESULTS AND DISCUSSION

Extraction

The percentage yield after the gradient extraction of the plant material is summarized in Table 1.

Table 1: Percentage yield of the fractions of *T. globiferus.*

Fraction	Percentage Yield
N-hexane	3.80
Dichloromethane	3.50
Ethyl acetate	9.60
Methanol	35.00

Methanol gave the highest yield because it is a highly polar solvent and has been reported to be a better solvent for the consistent extraction of plant materials when compared with other solvents due to its high polarity (Eloff, 1998).

Spectral analysis of the MB isolate

The spectra of the isolated compound MB are represented in Figures 1 - 3

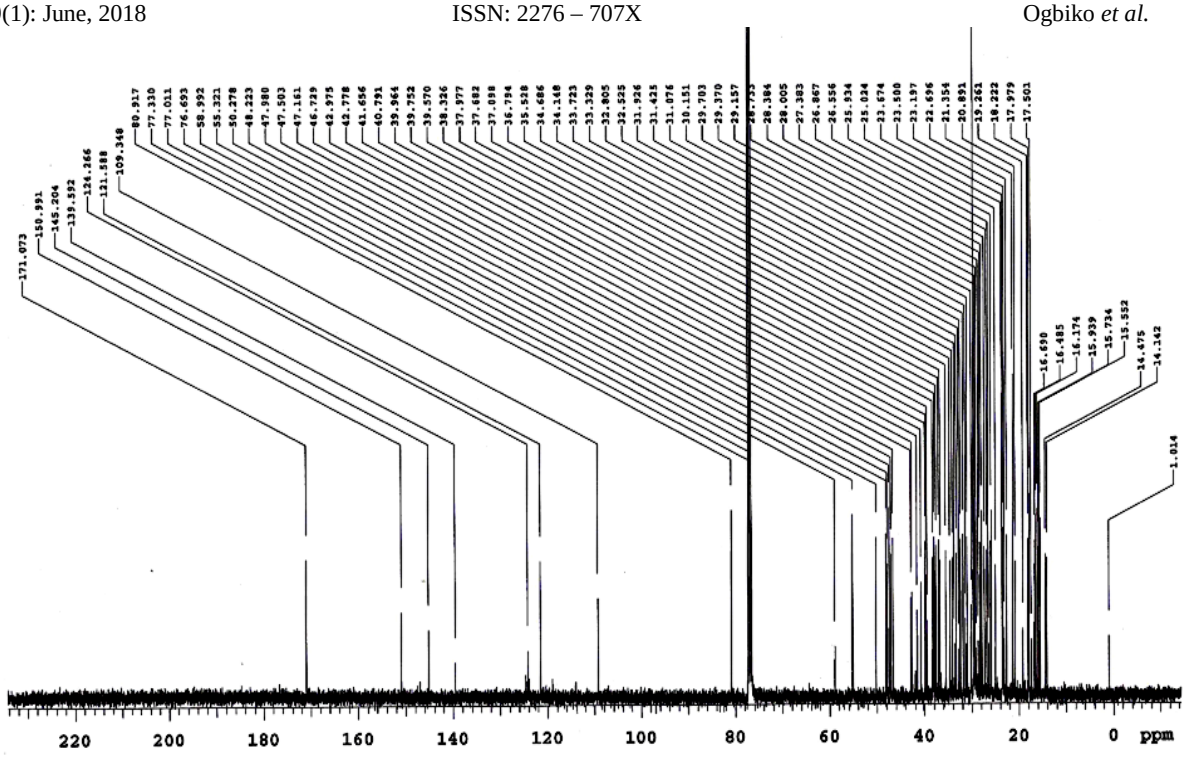

Figure 1: Carbon 13 NMR spectrum of compound MB

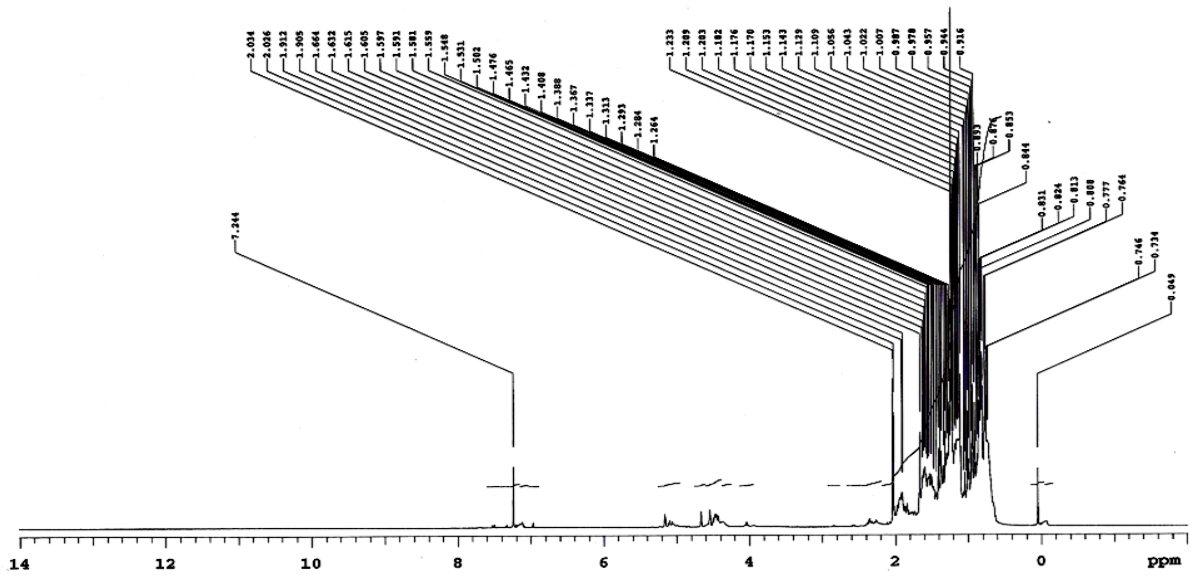

Figure 2: Proton NMR spectrum of compound MB

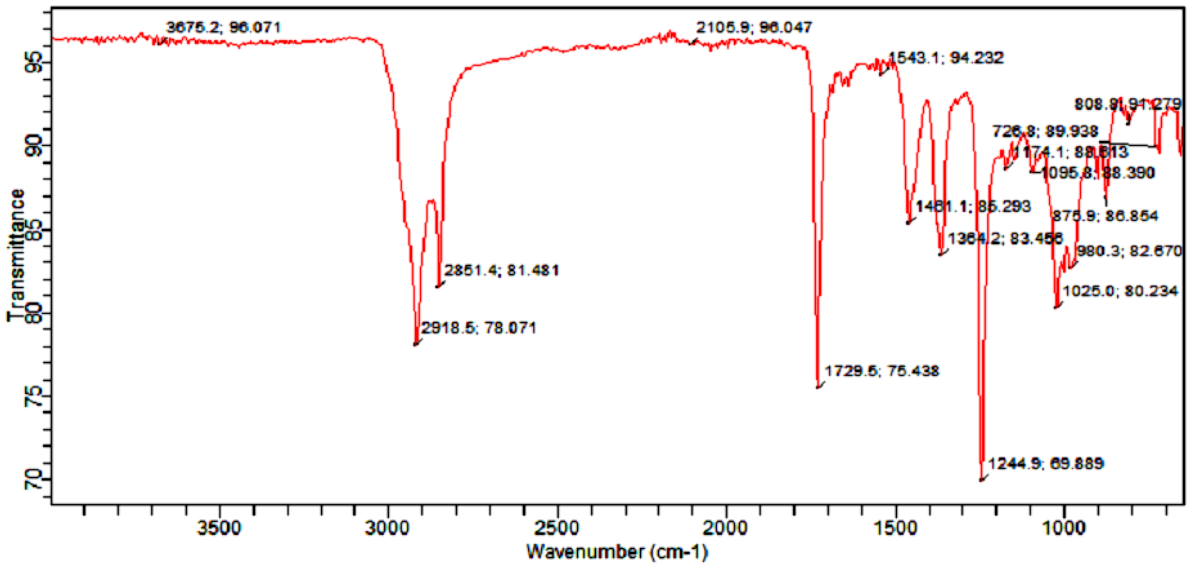

Figure 3: IR spectrum of compound MB

Table 1: Spectra data for Lupoel acetate (Jamal *et al.*, 2008) and compound MB ($DCCl_3$)

Position	Lupoel acetate*		Compound MB	
	$^{\delta}$H (Coupling) in Hertz	$^{\delta}$C (ppm)	$^{\delta}$C (ppm)	$^{\delta}$H (Coupling) in Hertz
1	-	38.6	38.57	-
2	-	21.7	27.53	-
3	4.47 (1H, dd, 7.4, 12.0)	81.2	81.19	4.42
4	-	38.0	40.19	-
5	-	55.6	55.57	-
6	-	18.4	18.39	-
7	-	34.4	34.39	-
8	-	41.0	41.04	-
9	-	50.5	50.53	-
10	-	37.3	37.27	-
11	-	21.1	21.13	-
12	-	24.0	25.28	-
13	-	36.2	38.23	-
14	-	43.0	43.02	-
15	-	25.3	27.53	-
16	-	35.8	35.76	-
17	-	43.2	43.19	-
18	-	48.5	48.47	-
19	-	48.2	48.20	-
20	-	40.2	40.19	-
21	-	151.2	151.21	-
22	-	30.0	29.98	-
23	1.03 (3H, s)	16.4	16.69	1.01
24	0.85 (3H, s)	27.6	28.14	0.95
25	0.85 (3H, s)	16.2	16.38	1.43
26	0.79 (3H, s)	14.7	16.69	0.87
27	0.94 (3H, s)	18.2	18.19	0.81
28	4.69 (1H, s), 4.57 (1H, s)	109.6	109.54	4.62 and 4.57
29	0.87 (3H, s)	16.7	16.69	1.23
30	1.69 (3H, s)	19.5	19.54	1.66
a	-	171.3	171.26	-
b	2.05 (3H, s)	28.2	28.18	1.59

(* Jamal *et al*., 2008)

The compound MB was colourless crystalline solid which is soluble in n-hexane, ethyl acetate and chloroform. The ^{13}C NMR of the isolated compound (Figure 2) and presented in Table 1 shows that the isolated compound contains 30 carbon signals. Prominent among these signal are ∂ = 109.54 ppm (C-28) and 151.21 ppm (C-21) account for the lupane skeleton (Rosas *et al*., 2007). Comparing the spectra of lupeol acetate isolated by Jamal *et al*., (2008) and that of the isolated compound (Table 1), the chemical shift on C-3 of the isolate MB is 81.19 ppm which corresponds to Lupeol acetate C-3 signal of 81.2 ppm isolated by Jamal *et al*., (2008). A chemical shift of 171.26 on ''a" is a signal of the acetate group attached to C-3. This corresponds to the signal of 171.3 as reported by Jamal *et al*., (2008) and 171.2 ppm (Lucetti *et al*., 2010). The ^{1}H NMR spectrum of MB (Figure 1) also shows proton signals at 4.62 ppm and 4.57 ppm (H-3) representing the exocyclic double bond proton of H-29 and is in close agreement with lupeol acetate isolated by Jamal *et al*., (2015). The IR spectrum of isolate MB (Figure 3) indicates a strong absorption band at 1729 cm^{-1} which is a characteristic of a carbonyl carbon of aliphatic ester (C=O) without a conjugation and having an overtone at 3676 cm^{-1}. A strong absorption band at 1244 cm^{-1} which is a carbon to oxygen single bond (C-O) was also observed. Absorption band of 2918 cm^{-1} and 2851 cm^{-1} is an aliphatic C-H stretching vibration. The interpretations of these spectra lead us to unequivocally report for the first time the isolation of Lupeol acetate from the leaves of *Tapinanthus globiferus.* Based on the spectral data (^{1}H NMR, ^{13}C NMR and FTIR), the proposed structure of MB is presented in Figure 4.

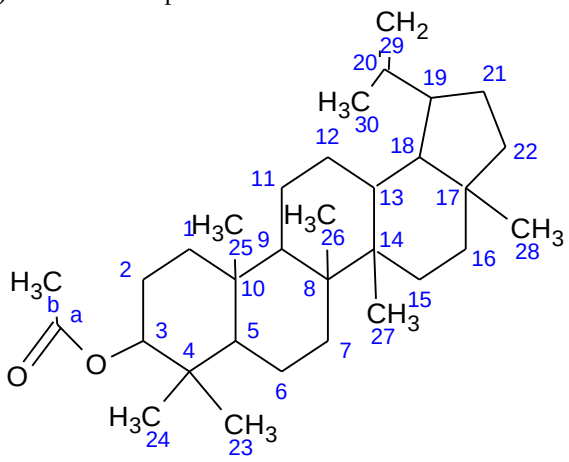

Figure 4: Proposed structure of lupeol acetate.

CONCLUSION

Lupeol acetate a known pentacyclic triterpenoid has been isolated and characterised from the n-hexane extract of *Tapinanthus globiferus* leaves. The presence of lupeol acetate and potentially other phytochemicals may explain the use of the leaves of the plant traditionally to treat inflammation.

Declaration of Conflicts of Interest

The authors declared that no competing interest exits.

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