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Research Article

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Isolation of two pentacyclic triterpenoid from methanol root extract of tacazzea apiculata (periplocaceae) oliv.

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Abstract Tacazzea apiculata is a large perennial climbing plant that belongs to the family Periplocaceae. It is widely distributed throughout the tropics and sub-tropical part of Africa. It is commonly used traditionally for the management of pain related ailments. The root part of Taccazea apiculata was subjected to isolation and identification of chemical constituents. The extract was purified and isolated by column chromatography and thin layer chromatography (TLC). The isolated compounds was then subjected to 1H NMR and 13C-NMR for identification of protons and carbon atoms. From the interpretation of the spectral data, the isolated compounds was found to be 3-acetyloxy-olean–12-en-28–oic acid and Lup-20(29)-en-3-ol.

Keywords Tacazzea apiculata, Extraction, Chromatography, Structural Elucidation.

1. Introduction

Tacazzea apiculata belongs to the family Periplocaceae, it is a large perennial climbing but sometimes apparently sub-erect with flexuous reddish brown stems, Corona-lobes intertwined above the stigma, the leaves are glabrous or more or less densely pubescent below, apex rounded or emarginate and apiculate, base rounded or cordate and flowers are green inside, more or less red outside it climbs up to 20 m long and 0.15 mm in diameter [10]. It is widely distributed throughout the tropics and sub-tropical part of Africa. It is commonly used traditionally for the management of hemorrhoids, wound healing, cancer, inflammation, epilepsy, access phlegm, venereal disease, abdominal pain and worm expulsion [1]. The family are confined to the old World and are found in Africa and Asia. The genera are monotypic or composed of a small number of species, with the exception of Raphionacme haeneliae which consists of 36 species [11;7]. The family comprises of 50 genera and 175 species. Approximately 20 of these genera and about 85 species occur in Africa. In Madagascar 13 genera occur of which only Coptolepis also occurs in both Africa and Asia, while Baseoizenm occurs only in Africa and Bneolepis Decrie ex Moq also in Asia [11]. The majority of the African genera of the Periplocaceae are climbers, some of these enormous lianas such as Tacazzea decner and Mondia skeels. A number are shrubs, especially those from arid regions, e.g. E. ectadium and M. curroria Planch. Raphionacme is the only herbaceous genus, although a number of its species are climbers [11].

2. Experimental

2.1. Collection, Identification and Preparation of Plant Material

The fresh leaf of Tacazzea apiculata was collected in May, 2019 at Samaru, Zaria Local Government Area of Kaduna State. It was identified by Mr. Namadi Sunusi at the Herbarium unit of the Department of Botany, Faculty of life Sciences, Ahmadu Bello University Zaria by comparing with Herbarium reference voucher specimen (No. 066633). The root bark was shade dried, pounded to powder, labelled and stored at room temperature for use.

2.2. Extraction, Partitioning

2kg of the pulverized root of the plant was macerated using methanol with occasional shaking for 72 hours and the extract was concentrated by evaporating the solvent using rotary vacuum evaporator to afford 140 g extract, subsequently referred to as methanol root extract (MRE). The MRE (130 g) was partitioned using n-hexane, dichloromethane, ethyl acetate and n-butanol to obtain fractions coded HF (hexane fraction), DCMF (dichloromethane fraction), EF (ethylacetate fraction) and BF (butanol fraction) respectively. Dichloromethane fraction was found to have a best TLC profile and was subjected to column chromatography over a silica gel (60-120 µm).

2.3. Isolation and Purification of Compounds

The column was packed using wet slurry method with hexane. Slurry of the silica gel (60-120 mesh) was poured into column and allowed to settle. The sample (dichloromethane fraction) was adsorbed on silica gel by dissolving it in 5ml of hexane and adding small quantity of silica gel to form a paste. The paste was dried, triturated and loaded on to the previously packed column. The column was eluted using gradient elution technique; starting with hexane 100% (800 mL) followed by 95:5 hexane/ethyl acetate, 90:10 hexane/ethyl acetate, 85:15 hexane/ethyl acetate, 80:20 hexane/ethyl acetate, 75:25 hexane/ethyl acetate, 70:30 hexane/ethyl acetate, 65:35 hexane/ethyl acetate, 60:40 hexane/ethyl acetate down to 100% ethyl acetate (400 mL) and the column was finally eluted with 100% methanol. A total of 94 collections of 100 mL aliquot each were made. The collections were pooled together based on similarities in their TLC profiles to obtain 8 major bulk fractions coded A-H.

2.4 Isolation of SKY 1

Fraction (7-11) coded C from the major bulk fractions showed a significant proportion of the compound of interest which was further subjected to repeated column chromatography over silica gel (60-120 mesh) and was eluted with n-hexane 100% followed by hexane: ethyl acetate 95:5 was used. 50 Collections of (2 mL each) were made; collections which had similar TLC profiles were combined together, collections 36-44 gave a homogeneous single spot on TLC with two different solvents system hexane and ethyl acetate (9:1; 1:1). This compound was coded SKY 1.

2.5 Isolation of SKY 2

The fraction (17-27) coded E from the major bulk fraction also indicated a significant proportion of the compound of interest and was also subjected to repeated column chromatography over silica gel (60-120 µm). The column was eluted using gradient elution technique; starting with hexane: ethyl acetate 95:5 through 90:10. 35 collections (2 ml each) were made; collections observed to have same TLC profile were combined together, collections 20-24 showed a homogeneous single spot on TLC with two different solvents system hexane and ethyl acetate (8:2; 1:1). This compound was coded SKY 2.

3. Results and Discussion

The Compound SKY 1 was isolated as a white powder and was observed to be soluble in dichloromethane and Ethylacetate

. The 1H NMR spectra of compound SKY 1 (Figure 1) showed the presence of several overlapping protons at δH 0.75, 0.78, 0.84, 0.88, 0.91, 0.92 and 1.11 assigned to C-23, C-24, C-25, C-26, C-27, C-29 and C-30 respectively due to overlapping of methyl and methylene protons typical of steroid and triterpenoid nucleus [8]. A prominent proton signal which resonate at δH 2.04 is an acetate methyl proton at C-31, also a proton signal at C-3 which resonate at δH 4.48 (1H, t, J =8. Hz, H-3) is a deshielded proton due to the presence of ester functional group that is attached to it and olefenic proton at C-12 shows a resonance peak at δH 5.33 (1H, t, J = 3.12).

The APT spectrum of SKY 1 (Figure 2) showed a total of thirty two (32) carbon signals which include eight methyl carbon signals at δC 28.0 (C-23), 16.6 (C-24), 15.4 (C-25), 17.1 (C-26), 26.0 (C-27), 33.0 (C-29), 23.5 (C-30) and 21.3 (C-31'), ten methylenes carbon signals at δC 38.0 (C-1), 23.5 (C-2), 18.1 (C-6), 32.4 (C-7), 22.8 (C-11), 27.6 (C-15), 23.3 (C-16), 45.8 (C-19), 33.7 (C-21) and 32.4 (C-22), five methine carbon signals including olefinic carbon signal at δC 80.9 (C-3), 55.2 (C-5), 47.5 (C-9), 122.5 (C-12), 40.8 (C-18) and nine quaternary carbon signals at δC 37.6 (C-4), 39.2 (C-8), 36.9 (C-10), 143.6 (C-13), 41.6 (C-14), 46.5 (C-17), 30.6 (C-20), 184.3 (C-28) and 171.1 (C-32'). A carboxylic and ester functional groups appeared at δc 184.03ppm and 171.04ppm, respectively. These spectroscopic data were found to be in agreement with those previously reported by [12] for Oleanolic acid acetate, thus confirming the identity of compound SKY 1 as 3-acetyloxy- olean – 12- en- 28 –oic acid (Figure 3). 3β-acetyl-oleanolic acid is a derivative of oleanolic acid and it is relatively non –toxic, hepatoprotective and exhibits antitumor and antiviral Properties (Liu, 1995).

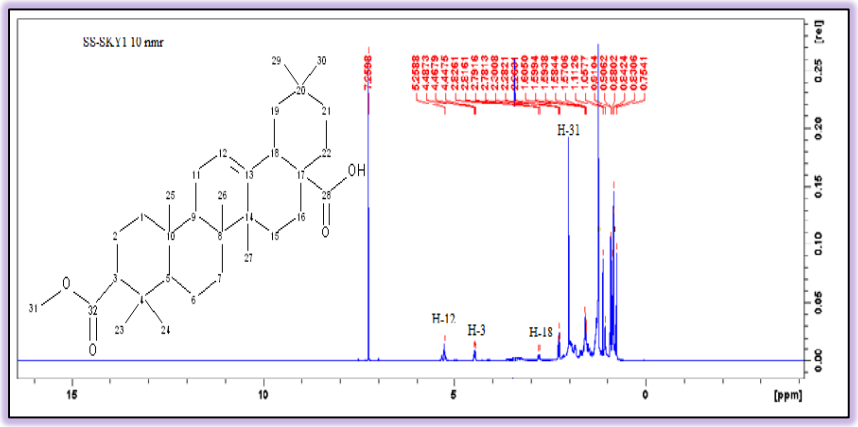

Figure:1 1H NMR spectrum of Isolated compound SKY 1

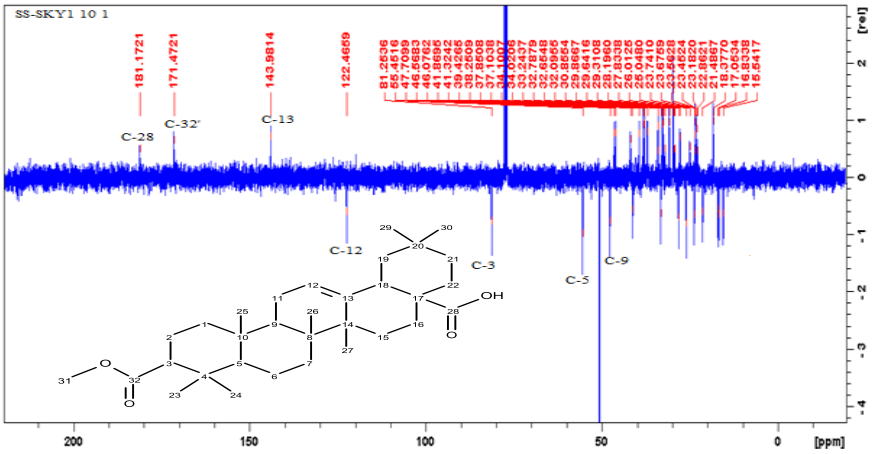

Figure: 2 APT Spectrum of Isolated compound SKY 1

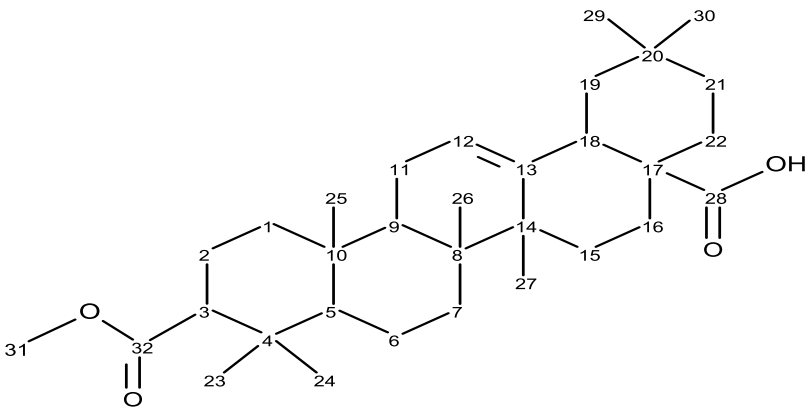

Figure: 3 Structure of Isolated compound SKY 1

Spectroscopic Characterization of Isolated Compound SKY 2

The 1H NMR spectrum of SKY 2 (Figure 4) showed overlapping proton signals due to methyl and methylene protons typical of steroid and triterpenoid nucleus [8]. It also revealed a signal at δH 3.17 (1H, t) due to carbinol proton at position C-3. This deshielded proton is as a result of OH group typical of steroids and triterpenoids. The spectrum also displayed two broad singlets at δH 4.55 (1H, s) and δH 4.66 (1H, s) an important characteristic of olefinic protons (H-29a and 29b) of lupeol nucleus. The spectrum also showed seven methyl proton signals typical of tertiary methyl groups at (δH 0.77, 0.80, 0.82, 0.92, 0.93, 1.03 and 1.66 3H each) which are the characteristic of triterpenoids. The APT spectrum of SKY 2 (Figure 5) showed a total of thirty (30) carbon signals which seven methyl carbon at δC 28.1 (C-23), 15.5 (C-24), 16.1 (C-25), 16.3 (C-26), 14.7 (C-27), 17.6 (C-28) and 18.5 (C-30) , eleven methylene carbon signals at δC 38.9 (C-1), 27.5 (C-2), 18.2 (C6), 34.4 (C-7), 21.1 (C-11), 25.3 (C-12), 27.3 (C-15), 35.7 (C-16), 29.5 (C-21), 39.8 (C-22), 109.5 (C-29), six methane at δC 77.5 (C-3), 55.3 (C-5), 50.6 (C-9), 38.2 (C-13), 48.2 (C-18), 47.7 (C-19) and six quaternary carbon signals at δC 38.9 (C-4), 40.2 (C-8), 37.3 (C-10), 41.7 (C-14), 47.6 (C-17) and a highly deshielded carbon signal at δC C-20. These spectroscopic data were found to be in agreement with those previously report in the literature by [5] for Lupeol, thus confirming the identity of compound SKY 2 as lupeol (Figure 6). Lupeol has been reported to exhibit a spectrum of pharmacological activities against various disease conditions these include inflammation, arthritis, diabetes, cardiovascular ailments, renal disorder, hepatic toxicity, microbial infections and cancer [2; 4; 3; 8].

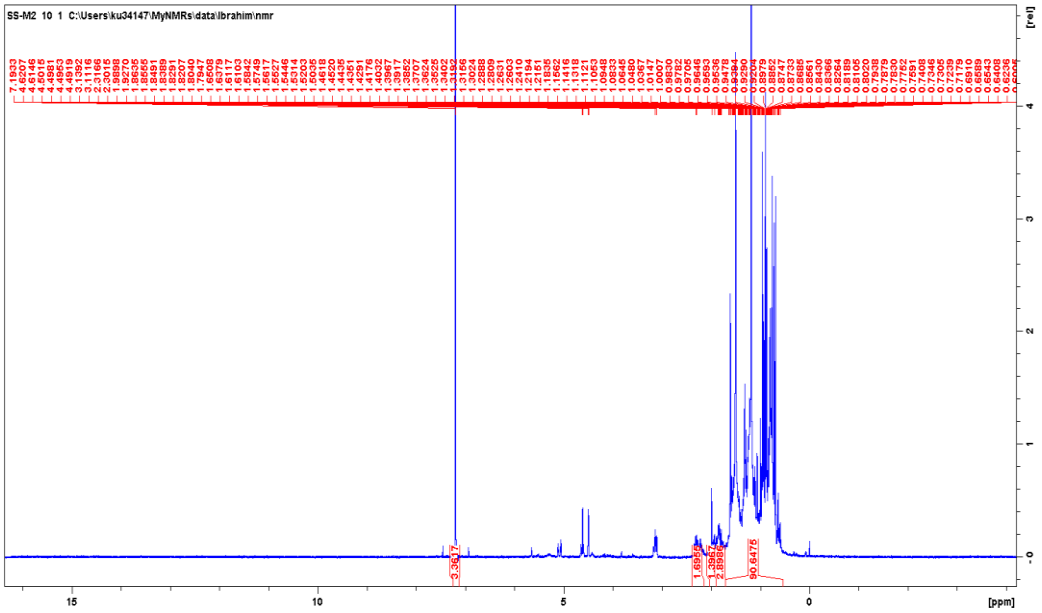

Figure: 4 1HNMR spectrum of Isolated compound SKY 2

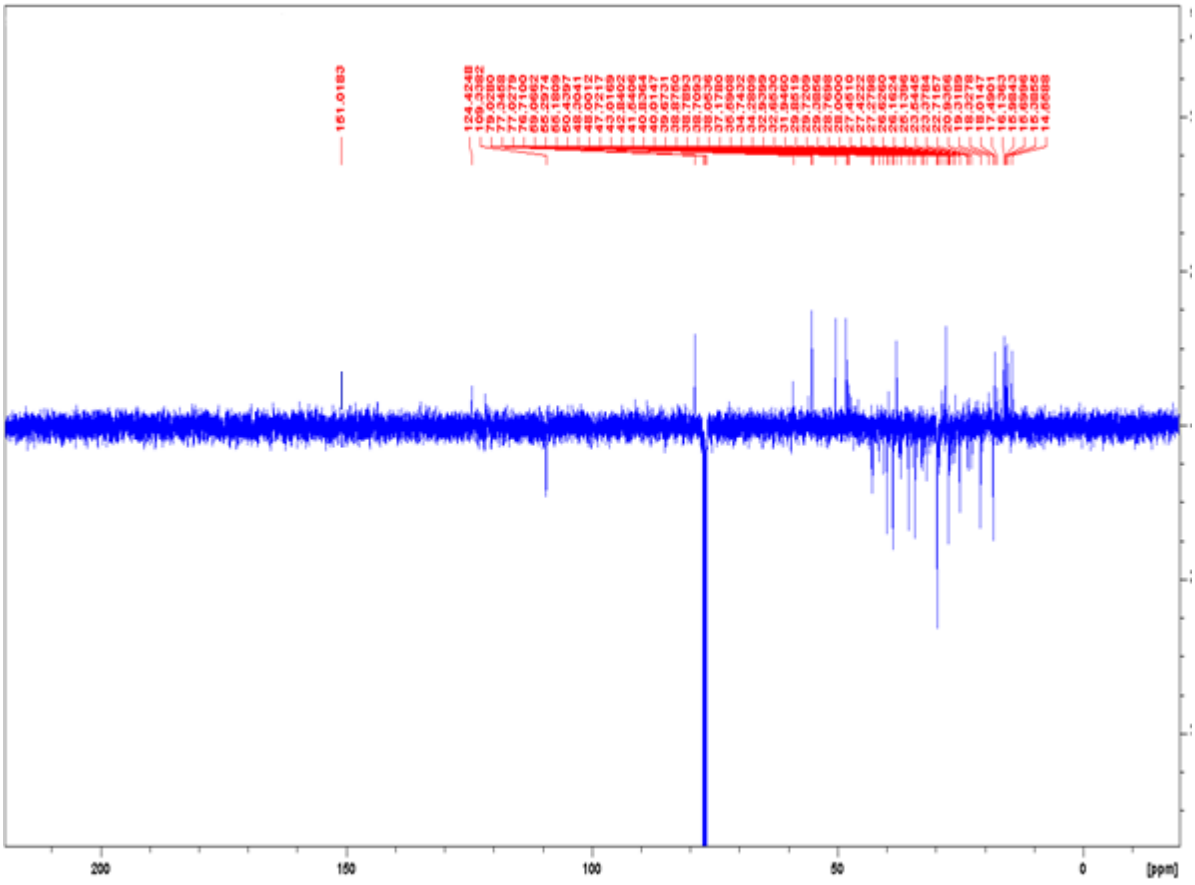

Figure: 5 APT Spectrum of Isolated compound SKY 2

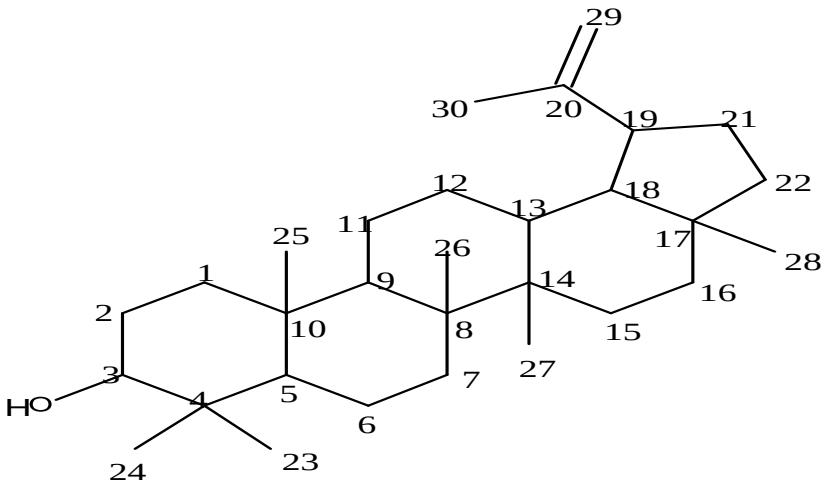

Figure: 6 Structure of Isolated compound SKY 2

4. Conclusions

Based on the spectral data and references, the structure of the two compounds isolated from methanol root extract of the plant T. apiculata are 3-acetyloxy- olean – 12- en- 28 –oic acid a derivative of oleanolic acid and SKY 2 was found to be Lup-20(29)-en-3-ol.

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