Isolation of Steroids from Acetone Extract of *Ficus iteophylla*

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**Abstract:** Two steroids were isolated from the leaves of *Ficus iteophylla* (Family: moraceae) a plant popularly used in African traditional medicine to treat variety of illnesses. The leaf part of the plant was investigated phytochemically using a standard procedure. Schematic fractionation of its ethanol extract by acetone and methanol and subsequent column chromatography of the acetone fraction over silica gel G (60-120) mesh size led to the isolation of 3β-cholest-5-ene-3, 23-diol (1) and 24 ethyl cholest-5-ene- 3β-ol (2). The structure of these two compounds was elucidated using 1H-NMR, 13C- NMR and DEPT analysis. To the best of our search this is the first report on the isolation of these compounds from the leaves of *Ficus iteophylla*.

**Key words:** *Ficus iteophylla*; 1H- NMR; 13C- NMR, DEPT; 3β-cholest-5-ene-3; 23-diol; 24 ethyl cholest-5-ene-3β-ol

**INTRODUCTION**

*Ficus iteophylla* belongs to family moraceae, The bark is used to treat dysentery and rheumatic pain (Burkill, 1997). The root has a wide usage for treating paralysis, tuberculosis, epilepsies, convulsion, spasm and pulmonary troubles (Burkill, 1997). The leaf part is reported to have analgesic, anti-inflammatory activity (Abdulmalik et al., 2011) and antibacterial activity (Ahmadu et al., 2006). It is also reported to contain two furanocoumarines (Ahmadu et al., 2004) and two flavonoid glycosides (Ahmadu et al., 2006). In continuation of investigation of bioactive metabolites from *Ficus iteophylla*, we report herein the isolation and identification of steroids from the leaf part of the plant.

**MATERIALS AND METHODS**

**Plant material:** The plant samples were collected from Ahmadu Bello University, Zaria Nigeria in the month of March, 2006. It was authenticated by comparing with the existing one by Mallam Musa Muhammad of the Herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.

**Extraction procedure:** The powdered leaves (850 g) of the plant was exhaustively extracted with petroleum ether(60-80°C) using Soxhlet apparatus, the marc was dried and extracted with ethanol (96%) in same way. The solvent in both cases were removed at reduced pressure to give 15 and 50 g of Petroleum Ether (PE) and ethanol (EE), respectively. The Ethanol Extract (EE) was successively partitioned with acetone followed by methanol to give acetone extract coded EEAc (8 g) and methanol extract coded EEM (16 g).

**Column chromatography of acetone extract from ethanolic extract:** The acetone extract from ethanol extract (EEAc) was dissolved in small quantity of acetone and was adsorbed on silica gel. It was allowed to dry and ground into a fine powder. The fine powder was applied over a well-packed silica gel G (60-120 mesh size) column. The column was then eluted gradually with Hexane: Ethyl acetate mixture, with polarity increased gradually. Eluents were collected as 30 mL fraction and the progress of the separation was monitored by thin layer chromatography, similar fractions were pooled together.

**Instruments:** The melting point was determined using Gallemkemp capillary and melting point apparatus and they were uncorrected. The 600 MHz H-NMR spectra were recorded in CDCl3 with Tertamethyl Silane (TMS) as internal standard. The 13C NMR and DEPT were recorded at 400MHz. The DEPT experiments were used to determine the multiplicities of carbon atoms. Thin layer chromatography (TLC) was performed on TLC Silica gel 60 F254 pre-coated (Merck). The spots were visualized by spraying with 10% H2SO4 followed by heating at 100°C for 5 min.

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RESULTS AND DISCUSSION

Thin layer Chromatography of Compound 1 and Compound 2 (Fig. 1). Chromatographic separation of the acetone fraction over a silica gel G (60-120) mesh size led to isolation of two compounds that gave positive Salkowski and Liebermann-Burchard test specific for steroids.

Compound 1 was obtained as white crystal, its melting point is 228-230°C, Rf value is 0.515 (Hexane/Ethylacetate). The structure was established by 1H-NMR and 13C-NMR at 600MHz in CDCl3. The 1H-NMR spectrum of the isolated compound showed a series of proton signal (δH1,0 - δH1,8) attributed to resonance of overlapping of methylenes and methines a characteristic frame work of steroid (Yun-Song et al., 2006). It showed a multiplet at δH 3.59 and 3.15. The signal at δH 3.59 and 3.15 revealed the presence of two hydroxyl group, these signal were for proton on carbon a  adjacent to alcohol. The signal at δH 3.59 was ascribed to C-3 (XU and Zeng, 2000) and that at δH 3.15 was ascribed to C-23. The C-6 oleifinic proton appeared at δH 5.48. The 1H-NMR showed vicinal coupling between C-3 methine proton and C-2 methylene at δH 1.98 and δH 1.85 (López et al., 2008), the spectrum further displaced signal at δH 0.85 (C-18) and δH 1.20 (C-26 and C-27). The 13C-NMR showed peaks at δC 11.8, δC 11.7, δC 19.0 corresponding to C-18, C-19 and C-21, respectively. C- 5 resonated at δC 158.1 while C-6 resonated at δC 116.8. Some of the signals are shown in (Table 1). Based on above evidence the structure of compound 1 was identified as 3β-cholest-5-ene-3,23-diol.

Compound 2 was obtained as white crystal, its melting point is 106-108°C, Rf value is 0.350 (Hexane/Ethylacetate). The structure was established by DEPT, 1H-NMR and 13C-NMR at 400MHz in CDCl3. Its
'HNMR revealed the presence of hydroxyl group at $\delta_{\text{H}}$ 3.52 while the oleifinic proton appear at $\delta_{\text{H}}$ 5.35 which shows that there is double bond between C-5 and C-6. The spectra further revealed the presence of six methyl groups $\delta_{\text{H}}$ 0.69, 1.03, 0.92, 0.82, 0.82, and 0.85 corresponding to $\delta_{\text{C}}$ C-18, C-19, C-21 C-26, C-27, and C-29, repectively. The 13C NMR spectrum showed the presence of 29 carbon signals in the molecules. The DEPT spectrum exhibited six methyl, eleven methylene and nine methine, while the remaining three signals in the broad band spectrum were due to the quaternary carbon atom. All the signals are shown in (Table 1). Base on the evidence above compound 2 was identified as 24-ethyl cholest-5ene-3β-ol. These two compounds probably could be responsible for the analgesic and anti-inflammatory activity already reported.

REFERENCES


