Isolation and Characterization of Lupeol from the Whole Plant of Phaulopsis bateri

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ABSTRACT: This work was aimed at isolating and characterizing the bioactive constituents from the crude methanol extract of the whole plant of Phaulopsis bateri. The crude extract which was subjected to a silica gel column chromatography separation using petroleum ether/ethyl acetate blend, eluted a white crystalline substance with a melting point of 215-216°C. PBA shows recognizable peak at 79.02ppm which is characteristic of a carbon carrying an oxygen and the ¹HNMR spectrum which shows an olefinic proton at δ 4.71 and 4.56 respectively was identified as lupeol which is a C₃₀H₅₀O moiety. Identification of the compound was purely by spectral analysis.

DOI: https://dx.doi.org/10.4314/jasem.v23i2.5

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Dates: Received: 17 December 2018; Revised: 26 January 2019; Accepted 31 January 2019

Keywords: Phaulopsis barteri, Lupeol, C₃₀H₅₀O, Chromatography.

Africa is endowed with a wide array of medicinal plants which play important roles in the treatment of various ailments. Various members of the Acanthaceae family are widely distributed all over the world. Acanthus ilicifolius Linn is distributed in South Africa, Seacoast of India, Philippines and Australia (Okoli, et al., 2008). Acanthus montanus (Nees) T. Anders. is a small herb with sparse branches and soft stems, widespread in Africa, the Balkans, Romania, Greece and the Eastern Mediterranean (Okoli, et al., 2008). Plants belonging to Acanthaceae family are medicinally important. In the Cameroons, Acanthus montanus is used traditionally to treat various ailments namely; cough, carious teeth, pharyngitis, dysmenorrhea, gastritis, false labour, epilepsy and intestinal helminthiasis. In other regions of Africa it is used to alleviate urethral discharge, chest pain, constipation, rheumatic pains, and syphilis (Adjanohoun, et al 1996. H. M. Burkill, 1985). Leaf extract of Hygrophila auriculata exhibited a good antibacterial activity on Escherchia coli, Staphylococcus aureus, Bacillus subtilis and Pseudomonas aeruginosa (Patra et al., 2009). The ground leaves and stem of Odontonema callistachyum are applied on open wounds in order to heal in Sierra Mazateca (Heinreich, et al., 2008). Adhatoda vasicais used in Ayurvedic medicine for the treatment of various ailments of respiratory systems like bronchitis, asthma and it is also used in the treatment of malaria, dysentery and diarrhea (Jain, 1984). Phaulopsis barteri is another member of this family with ethno medicinal usage in northern Nigeria although no form of scientific report is available on it.

The objective of this paper, is to isolate and characterize the bioactive compound(s) from the crude methanolic extract of the whole Phaulopsis barteri plant.

MATERIALS AND METHODS
Phaulopsis barteri was collected from Zaria, Kaduna state, Nigeria in the month of August, 2016 and identified by Mallam Namadi S. of the Herbarium, Department of Biological Sciences, Faculty of life Science, Ahmadu Bello University, Zaria, Nigeria. The voucher specimen, number -01130 was kept in the Herbarium. The sample was air-dried, pulverized using wooden pestle and mortar and stored in paper bags and kept away from moisture until they were ready for use. Below is a picture of the plant.

Fig 1. The plant, Phaulopsis barteri
The plant, *Phaulopsis barteri*, is a herbaceous plant which is about 100cm high. The leaves are dark green, acuminate at both ends, ovate or oblong. It has a vertical spike of flowers about 6.45 to 2.54cm high. The plant is known as Eran-koje in Yoruba, Apaogbe in Igbo and Danyas in Hausa.

**Extraction procedure:** The organic constituents were extracted from the dried plant material using cold maceration method. The coarse powdered whole plant material was packed into a container and extracted with methanol until exhaustive extraction was achieved. The solvent was removed using the rotary evaporator at 40°C and was later air dried.

The solvent system used for the column was obtained via TLC process. The residue was chromatographed on a silica gel column with gradient elution using petroleum ether and ethyl acetate (Fulgentis *et al.*, 1990). Among the eluents was a white crystalline substance which was further purified using silica gel preparative thin layer chromatography to obtain a compound called PBA. Chemical characterization of the substance obtained was achieved using NMR spectral studies.

**Spectral measurements:** The $^1$Hnmr and $^{13}$Cnmr spectra of this compound was obtained using Bruker AMX -400 instrument using DMSO as solvent. The results are as obtained in Figures 1 and 2.

**RESULTS AND DISCUSSION**

$^{13}$C-nmr spectrum of PBA had signals at; (ppm) 38.77, 27.26, 79.02, 38.87, 55.31, 18.33, 34.42, 40.84, 50.45, 37.18, 20.94, 25.15, 38.07, 42.84, 27.46, 35.59, 48.72, 53.49, 50.08, 150.99, 29.86, 40.01, 27.99, 15.37, 16.12, 15.98, 14.55, 18.01, 110.03, 19.31. The $^1$Hnmr had peaks recorded as; (ppm) 3.15 (triplet of a double doublet), 4.71 (multiplet), 4.56 (multiplet), 1.28 (singlet), 1.04 (singlet), 1.07 (singlet), 1.01 (singlet), 0.98 (singlet), 0.97 (singlet) and 0.91 (singlet). The $^{13}$C-NMR (400Hz) of PBA: $^{13}$C-NMR spectrum of PBA, the chemical shift observed at 79.02 ppm is typical of a carbon attached to an oxygen. The signals observed to peak at 37.3, 29.9, 18.1, 34.4, 50.5, 21.0, 25.2, 27.5, 35.7, 28.1 and 38.1 ppm are all characteristic of methylene groups. The peaks observed at 38.8, 40.1, 42.9, 43.1, 48.4 and 150 ppm are quaternary carbons. The signal at 29.8, 14.6, 16.2, 16.1, 15.5, 18.4, 109.5 and 19.3 ppm are typical of methyl groups. All these were observed comparatively to be typical of the spectrum of lupeol (Iman *et al.*, 2007). In H-NMR spectrum of lupeol, H-3 proton appeared as a triplet of a doublet (tdd) at chemical shift 3.15 (J=4.5 and 1.1 MHZ) and H-29 olefinic proton showed a multiplet at chemical shift 4.71 and 4.56 respectively. Seven methyl protons also appeared at chemical shift 1.28, 1.04, 1.07, 1.01, 0.98, 0.97 and 0.91 (3H each, s, CH3). All these were observed comparatively to be typical of the spectrum of lupeol (Iman *et al.*, 2007).

**Conclusion:** Compound PBA, isolated from the methanol extract of the whole plant of *Phaulopsis barteri* was identified as lupeol. The structure of the isolated compound was identified purely by spectroscopic methods and by comparing their physical properties reported in the literature. This is the first time lupeol is isolated from the plant of *Phaulopsis barteri*.
Acknowledgement: The authors are very grateful to the entire staff and management of the Department of Chemistry, Ahmadu Bello University, Zaria for the laboratory that was used for this work.

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