

Isolation and Characterization of Kaempferol-7-*O*-methyl ether from *Solenostemon monostachys* (P.Beauv.) Briq. (Lamiaceae)

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ABSTRACT

Solenostemon monostachys (Lamiaceae) is a common herb found in the southern part of Nigeria where it is used in the treatment of diabetes, tuberculosis, threatened abortion and other common ailments. Preliminary phytochemical screening has shown the presence of glycosides, flavonoids, tannins, saponins and anthraquinones. Phytochemical investigation of the dichloromethane extract of the leave, involving the use of flash chromatography and gel filtration on sephadex LH-20 was carried out and this has led to the isolation of kaempferol-7-*O*-methylether.

KEYWORDS

Solenostemon monostachys, Kaempferol-7-*O*-methylether, dichloromethane extract.

INTRODUCTION

Medicinal plant species are so regarded because they are sources of well known and medically useful secondary products as wide-ranging as pain killers like morphine, stimulants like nicotine, caffeine, cocaine and depressants with high potency in the management of ailments in humans. Generally, medicinal plants are unique in containing compounds that are end-products of long biosynthetic pathways and are usually not needed in such plants' metabolic processes. Davis and Heywood (1963) reported that these compounds called secondary metabolites include: alkaloids,

flavonoids, terpenes, glycosides, essential oils and other organic constituents. These constituents are usually produced and stored in different parts of the plants like the roots, leaves, fruits and seeds (Kochhar, 1981). Knowledge about these medicinally active constituents makes their application in therapy possible as contained in the various pharmacopoeias.

Solenostemon is a genus of perennial plants, native to tropical Africa, Asia, Australia, the East Indies, the Malay Archipelago, and the Philippines (Idu and Ndukwu, 2006 and Folu. *et al.*, 2009). They are commonly known as Coleus, a name which

derives from an earlier classification under the genus name *Coleus*, which is currently treated as two: with species included in either the genus *Solenostemon* or in another genus, *Plectranthus* (Griffiths, 1994). *Solenostemon* includes about 150 species; they grow in moist soil so in cultivation require warmth and frequent watering, but thrive better in a shady position where leaf colours are often enhanced. Flower spikes are greenish-purple, small and insignificant (GRIN. 2007).

Solenostemon monostachys is commonly found in the southern part of Nigeria where it is used in the treatment of diabetes (Erah *et al.* 1996). Other uses of the plant includes: antiemetic, antidotes (venomous stings, bites, etc.), arthritis, rheumatism, eye treatment, febrifuges, diuretics, laxatives, menstrual cycle disorders, paralysis, epilepsy, convulsions, spasm and pulmonary disease. Miyase and his colleagues (1977) reported the isolation of six coleons (Diterpenoids) from the plant leaves. These are coleons C and H, 2 alpha-formoxy coleon C, 12 beta-o-acetyl coleon Z, 12 beta-o-acetyl-7-o-fonnyl-7-o-desacetyl coleon Z. 17 -acetoxoy -12 beta-o-acety I coleon Z, and 12 beta-o-acety 1-17 -formoxy coleon Z. Further phytochemical investigation was carried out in order to isolate more compounds which are responsible for the various biological activities of this plant.

MATERIALS AND METHODS

Equipment and reagents

IR spectrum was recorded on a Buck Scientific IR M500 instrument (Buck Scientific Inc, Norwalk, Connecticut, USA). ¹H NMR spectrum was recorded on a Varian

Gemini 200 (250 MHz) (Varian Inc, Palo Alto, California, USA). Chemical shifts are reported as δ in ppm relative to tetramethylsilane (TMS). ¹³C NMR spectrum was recorded on a Varian Gemini 200 (63 MHz) (Varian Inc, Palo Alto, California, USA). Chemical shifts were reported as δ ppm relative to TMS. Mass spectrum was acquired on a Finnigan MAT 44S mass spectrometer (Thermo Finnigan San Jose, California, USA) operating at 70eV under electron impact mode. All the analytical grade solvents used, the silica gel for column chromatography and sephadex LH-20 were obtained from Sigma Aldrich (Germany). The TLC plates were obtained from Meck (Germany).

Plant materials

The Plants were collected in Ugbowo area of Benin City, South- South Nigeria between May and June 2009. Documented folklore use and oral information from the residents formed the basis for the selection of the parts of the plants collected. The authentication of the plant material was done by Dr. B.A. Ayinde of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, where a voucher specimen was deposited with herbarium number 1023.

Preparation of the plant material and extraction

The leaves of the plant were carefully picked and washed to remove sand. They were dried in the sun to constant weight and pulverized using mortar and pestle. The powdered leaf material (500 g) was exhaustively extracted with dichloromethane by cold maceration; and then subsequently extracted with methanol.

Purification of the Dichloromethane extract

The dichloromethane fraction (11.02 g) was eluted on flash column chromatography using hexane and ethyl acetate gradient wise (i.e. hexane : ethyl acetate, 90:10, 80:20, 70:30, 60:40, and 50:50). The fractions collected were pooled into five groups (A-E) based on their thin layer chromatography (TLC) profile; (solvent system, hexane: ethyl acetate, 9:1) and were concentrated at low temperature. Repeated gel filtration of fraction A, on sephadex LH-20 (a slurry of 10 g of the sephadex was prepared using methanol) furnished a collection of eluate (5 mLs of the eluate was collected in twenty tubes) with a single spot on TLC; the solvent was removed at 40°C to leave behind yellow crystals which was characterized with the aid of infra red spectroscopy, ¹H and ¹³C nuclear magnetic resonance (NMR) and mass spectrometry (MS). The data obtained were compared with literature information (Markham, 1972) to arrive at the structure.

RESULTS AND DISCUSSION

The compound was isolated as yellow crystals (20 mg); Melting Point (225-227°C); IR (KBr) $V_{\max} \text{cm}^{-1}$: 3480 (-OH, sharp band), 3260 (-OH, broad band), 2463, 2364, 1654 (C=O), 1607, 1503, 1420, 1378, 1180, 1101, 985, 820. ¹H-NMR (DMSO-d₆) δ ppm: 3.9 (3H, s, -OCH₃), 6.2 (1H, d, J = 2 Hz, C₆-H), 6.5 (1H, d, J = 2 Hz, C₈-H), 6.9 (2H, d, J = 8.8 Hz, H-3', H-5'), 7.9 (2H, d, J = 8.8 Hz, H-2', H-6'), 10.3 (1H, s, OH), 10.8 (1H, s, OH), 12.9 (1H, s, OH). ¹³C-NMR (DMSO-d₆) δ ppm: 53.0, 94.4, 99.3, 103.3, 104.1, 116.4, 121.6, 128.9, 130.5, 137.4, 157.8, 161.6, 161.9, 164.2, 164.6, 182.2. EI-MS m/z (%): 300

(M⁺ 19), 270 (100), 242 (37), 164 (93), 153 (52), 121 (76).

The compound was found to have molecular weight of 300 g as indicated by the presence of molecular ion peak at m/z 300 in EI-positive mass spectrum. The IR spectrum of the compound displayed two absorption band at 3480 and 3260 cm⁻¹ for chelated and non-chelated OH groups respectively. The IR spectrum also showed absorption bands at 1654 and 1607 cm⁻¹ indicating the presence of α , β -unsaturated C=O and at 1503, 1420 cm⁻¹ for stretching of ether function. The ¹H-NMR spectrum displayed two meta-coupled doublet (J=2.0 Hz) each for 1H at δ 6.2 (H-6) and 6.5 (H-8) and two ortho-coupled A₂B₂-type doublet (J=8.8 Hz) at δ 6.9 (H-3', 5') and 7.9 (H-2', 6') in aromatic region. These proton signals suggested the presence of a tetra-substituted and a 1,4-di-substituted phenyl ring. The latter ring was further confirmed to be *p*-hydroxyphenyl system from the ¹³C-chemical shift of the carbon signals at δ 130.5 (C-2', 6') and δ 116.4 (C-13,15) which fairly corresponded with those of hydrogen bearing carbons of *p*-cresol (δ 115.3, 130.2) (Pouchart, and Campbell, 1974). In aliphatic region the ¹H-NMR spectrum displayed an integrated singlet for 3H at δ 3.9 which was assigned for -OCH₃ group attached at C-7 position, which is confirmed by downfield ¹³C-chemical shift of C-7 at δ 164.6. The ¹³C-NMR spectrum also showed the presence of C=O group at δ 182.2, a benzylic carbon (C-2) at δ 157.8 and oxygen bonded ethylenic carbon (C-3) at δ 137.4. On the basis of these spectral data the compound was identified as rhamnocitrin (kaempferol-7-O-methyl ether). It was further confirmed by comparison of spectral data with reported values (Markham, 1972).

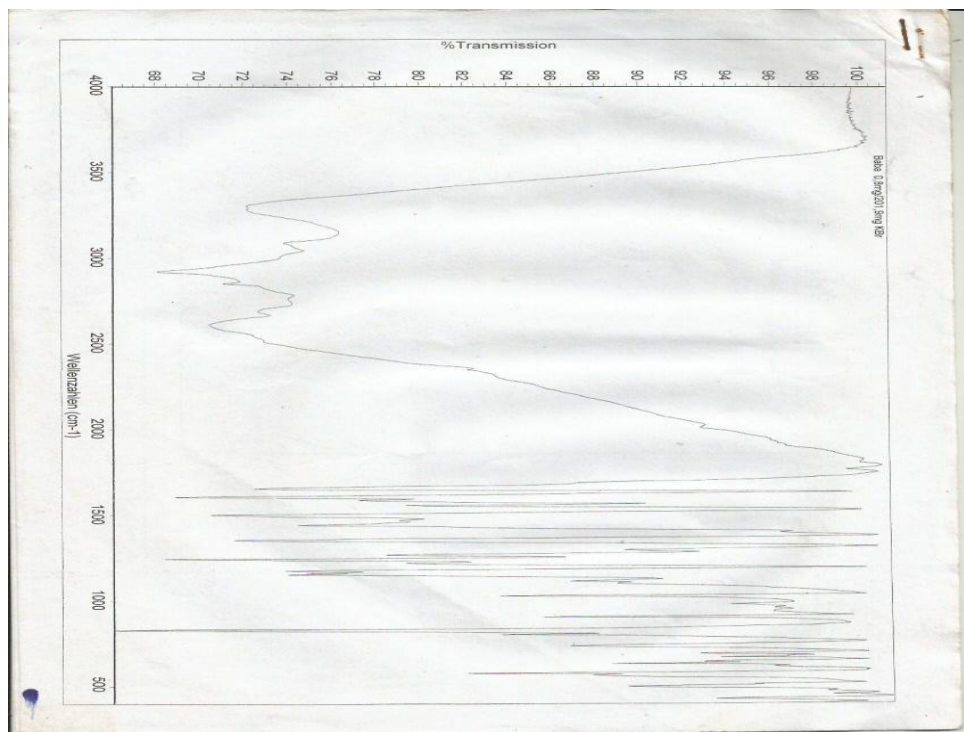


Figure 1: IR spectrum of the isolated kaemferol 7-O-methyl ether

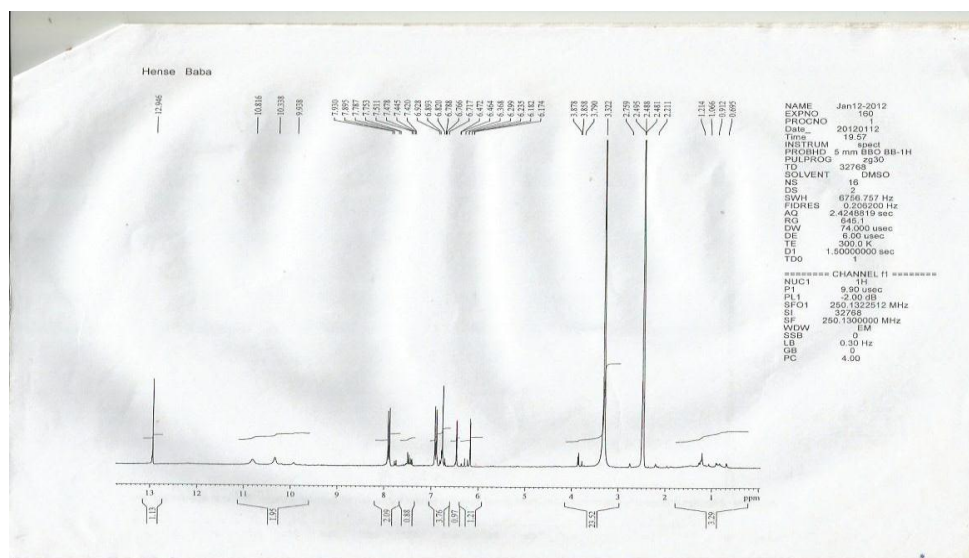


Figure 2 : ¹H NMR spectrum of the isolated kaemferol 7-O-methyl ether

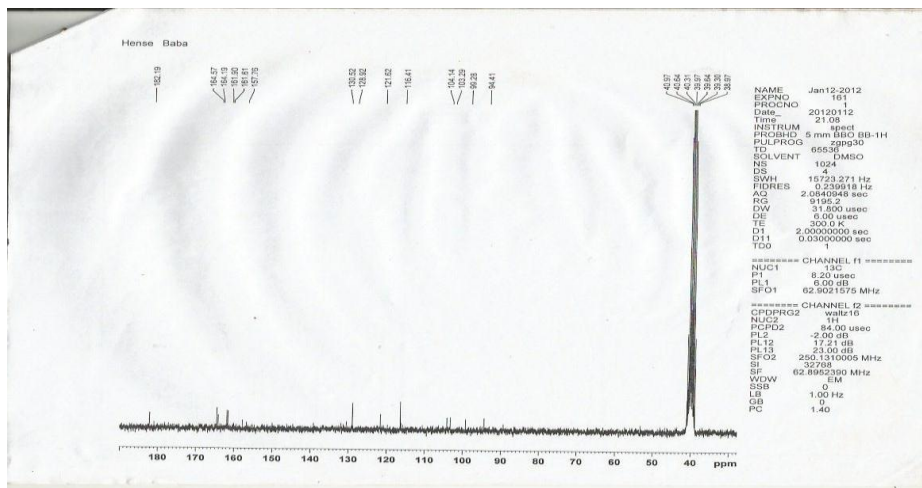


Figure 3: ¹³C NMR spectrum of the isolated kaempferol 7-O-methyl ether

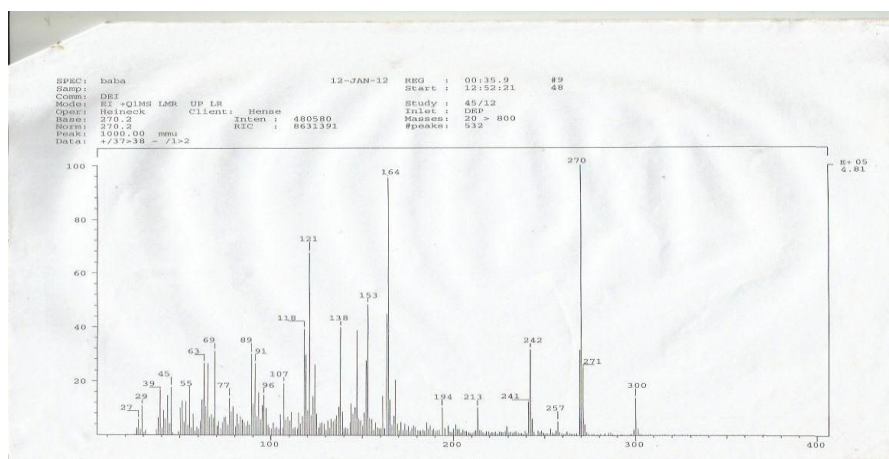
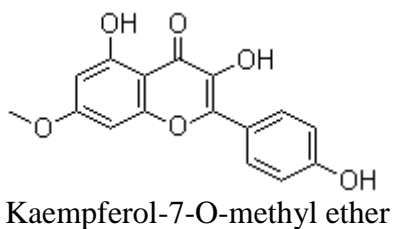


Figure 4: MS of the isolated kaempferol 7-O-methyl ether



CONCLUSION

Kaempferol-7-O- methyl ether is being reported here for the first time from this plant. Further work is on-going to purify the other fractions of this plant extract in order to isolate other compounds which may have biological activity.

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