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Isolation of Phytoconstituents from the leaves of *Murraya koenigii* Linn

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ABSTRACT

Fractionation of petroleum ether partitioned ethanol extract and crude petroleum ether extract of the leaves of *Murraya koenigii* Linn (Rutaceae) led to the isolation of 5,8-dimethyl furanocoumarin (1) and 1-al, 3[6', 6' dimethyl 5-hexene] carbazole (2) and β-sitosterol (3). Their structures were elucidated by spectroscopic methods such as UV, IR, NMR and LCMS. All the compounds were isolated for the first time from this plant.

Keywords: *Murraya koenigii* Linn; Isolation; 5,8-dimethyl furanocoumarin; 1-al, 3[6', 6' dimethyl 5-hexene] carbazole; β-sitosterol; spectroscopic method.

INTRODUCTION

Murraya koenigii Linn (Rutaceae) commonly known as Meethi neem, is an aromatic more or less deciduous shrub or a small tree up to 6 m in height found throughout India up to an altitude of 1500 m and are cultivated for its aromatic leaves (Wealth of India, 1998). In Traditional System of Medicine, it is used as antiemetic, antidiarrhoeal, dysentery, febrifuge, blood purifier, tonic, stomachic, flavoring agent in curries and chetneys. The oil is used externally for bruises, eruption, in soap and perfume industry (Prajapati et al., 2003). The phytoconstituents isolated so far from the leaves are alkaloids viz., mahanine (Narasimhan et al., 1970), koenine, koenigine, koenidine (Narasimhan et al., 1975), girinimbiol, girinimibine (Adebajo et al., 2006), koenimbine, O-methyl murrayamine A, O-methyl mahanine, isomahanine, bismahanine, bispyrayafoline (Tachibana et al., 2003) and other phytoconstituents such as coumarinic glucoside, scopotin, murrayanine (Adebajo et al., 2000), calcium, phosphorus, iron, thiamine, riboflavin, niacin, vitamin C, carotene and oxalic acid (Wealth of India, 1998). The essential oil from leaves yielded di-α-phellandrene, D-sabinene, D-α-pinene, dipentene, D-α-terpinol and caryophyllene (Gopalan et al., 1984). It is reported to possess antioxidant, antibacterial, antifungal, larvicidal, anticarcinogenic, hypoglycemic, hypolipidemic, anti-lipid peroxidative and anti-hypertensive activity (Iyer and Uma, 2008).

In the present work, we have isolated 5,8-dimethyl furanocoumarin (1), 1-al, 3[6', 6' dimethyl 5-hexene] carbazole (2) and β-sitosterol (3) from the petroleum ether partitioned ethanol extract and crude petroleum ether extract of dried leaves of *Murraya koenigii* respectively. All the compounds were isolated for the first time from this plant.

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MATERIAL and METHODS

Plant material

Murraya koenigii Linn leaves were collected and authenticated by Central Council for Research in Ayurveda and Siddha, Bangalore. A voucher specimen (RRI/BNG/SMP/Drug Authentication/2008-09/267) has been preserved in our Department of Pharmacognosy, The Oxford College of Pharmacy, Bangalore.

General instrument details

UV: Shimadzu UV VIS-1700; IR: JASCO FTIR 5300; LCMS: Agilent 1100 LC-MSD APCI; ^{1}H-NMR (500 MHz): Bruker Avance 500.

Extraction and isolation procedure

Coarsely powdered leaves (750 gm) were extracted with petroleum ether followed by chloroform and ethanol by the process of continuous extraction (soxhlation). The crude extract was evaporated to dryness in a rotary film evaporator, with the percentage yield being 3.20 %, 0.46 % and 3.50 % w/w in term of dry plant material. Alcohol extract was partitioned with petroleum ether, chloroform and acetone. All the partitioned extracts were dried. Petroleum ether partitioned fraction of the alcohol extract was subjected to column chromatography over silica gel (60-120 mesh) using petroleum ether, taking 250 ml fraction each time. From petroleum ether 100%, fractions 24-28 (MK-1); petroleum ether 100%, fractions 135-294 (MK-2) and crude petroleum ether extract on standing gave precipitate (MK-3) on further purification by fractional crystallization yielded compound 1(10 mg), compound 2 (15 mg) and compound 3 (06 mg) respectively.

RESULTS and DISCUSSION

The structures of compound isolated were elucidated on the basis of spectral data.

Compound 1 was isolated as colorless powder. Its molecular formula was determined as $C_{13}H_{20}O_3$ on the basis of mass spectrum by exhibiting a quassi molecular ion at m/z 230 (M +) and molecular weight was established as 230. Its IR spectrum exhibited characteristic broad bands at 3336 cm^{-1} for the presence of hydroxy group, peak at 2920 and 1622 cm^{-1} for aromatic ring in the structure and band at 1729 cm^{-1}

for carbonyl group. The ^{1}H-NMR spectrum showed a singlet signal at δ 7.27 confirming the aromatic ring; two-singlet signal at δ 4.08 and δ 4.11 shows the presence of oxygen with in the heterocyclic ring. With the above data and mass spectrum, the compound 1 is identified as 5,8-dimethyl furanocoumarin.

Compound 2 was isolated as colorless powder. Its molecular formula was determined as $C_{21}H_{23}NO$ on the basis of mass spectrum by exhibiting a quassi molecular ion at m/z 305 (M +) and molecular weight was established as 305. Its IR spectrum exhibited characteristic broad bands at 3526 cm^{-1} for the presence of –NH group, peak at 2920 cm^{-1} for aromatic ring in the structure which confirms by the ring stretch peak at 1917 cm^{-1}. The ^{1}H-NMR spectrum showed a singlet signal at δ 7 confirming the aromatic ring and a singlet signal at δ 5.8 shows the presence of nitro in the heterocyclic ring. The triplet signal at δ 0.8 is due to the methyl group, singlet at δ 1.2 and triplet at δ 2.30 are due to double bonded carbon. With the above data and mass spectrum, the compound 2 is identified as 1-al, 3[6', 6' dimethyl 5-hexene] carbazole.

Compound 3 was isolated as white amorphous powder, m.p.: 139-142^{0}C. Positive test for Liebermann Burchardt test indicated the presence of tetracyclic triterpenoid compound. Its IR spectrum exhibited characteristic bands at 3288 cm^{-1} for hydroxyl group. The ^{1}H-NMR as well as ^{13}C-NMR data were found to be identical with the spectrum of those already reported earlier for β-sitosterol (Sethi et al., 1978). It was further confirmed by TLC and CO-TLC method with the reference standard of β-sitosterol.

All the compounds were isolated for the first time from this plant.

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