Alkaloids, Coumarin and Cinnamic Acid Derivative from *Murraya koenigii* (Linn.) Spreng.

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ABSTRACT: A total of seven compounds were isolated from the methanol extract of leaves of *Murraya koenigii* (Linn.) Spreng. The isolated compounds were characterized as arborinine (**1**), ferulic acid (**2**), umbelliferone (**3**), mahanimbine (**4**), koenimbine (**5**), koenidine (**6**) and *O*-demethyl murrayanine (**7**) by extensive spectroscopic studies, including high field NMR analysis as well as co-TLC with authentic samples, whenever possible. This is the first report of occurrence of arborinine (**1**) and ferulic acid (**2**) from *Murraya* species.

Key words: *Murraya koenigii*, Rutaceae, arborinine, ferulic acid, umbelliferone, mahanimbine, koenimbine, koenidine, *O*-demethyl murrayanine

INTRODUCTION

Murraya koenigii (Linn.) Spreng. (Bengali name- Chotokamini; Family- Rutaceae) is more or less a deciduous unarmed shrub or a small tree up to 6 meters in height, widely distributed throughout Bangladesh.[1] *M. koenigii* is a medicinal plant, various parts of which are used in diabetes, skin eruptions, poisonous bites, febrifuge and dysentery.[2] It was found to possess antimicrobial, anthelmintic, anti-inflammatory and hypoglecemic activities.[3] Previously isolated alkaloids from *M. koenigii* includes 3-methyl carbazole, murrayafoline A[4], mahanimbine, koenimbine, koenidine[5], mahanine, mahanimbicine[6], grinimbine, murrayanine[7], 2-methoxy-carloazole-3-carboxylate, 1-hydroxy-3-methyl carbazole[8], murrayanol, girinimbilol[9], bismurrayafoline E.[10] The isolation of flavonoid quercetin-D-glucoside[5], terpenoid like *β*-phellandrene, terpinen-4-ol[11], and coumarin like heraclenol, heraclenin, isoheraclenin, imperatorin, umbelliferone, osthol has also been reported.[12] Linalool, elemol, geranyl acetate, myrcene, allo-ocimene, *α*-Terpinene, (E)-*β*-Ocimene and neryl acetate had been isolated as essential oil from this plant.[13]

As a part of our continuing studies with medicinal plants of Bangladesh[14-18], we studied *M. koenigii* and we, herein, report the isolation of arborinine (**1**)[19], ferulic acid (**2**)[20], umbelliferone (**3**)[21], mahanimbine (**4**)[6], koenimbine (**5**)[5], koenidine (**6**)[5], and *O*-demethylmurrayanine (**7**)[22], where arborinine (**1**) and ferulic acid (**2**) are reported from *M. koenigii* for the first time.

MATERIALS AND METHODS

General experimental procedure. ^{1}H NMR and ^{13}C NMR spectra were acquired using Ultra Shield Bruker 400 and 100 NMR instrument, respectively using $CDCl_3$ and the chemical shifts are reported in ppm with respect to TMS or residual non deuterated

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solvent signals. RV10 Basic (IKA, Germany) was used for rotary evaporation. High performance liquid chromatographic system (Shimadzu-UFLC prominence), equipped with an auto sampler (Model-SIL 20AC HT) and UV-Visible detector (Model-SPD 20A) was used for the analysis. The data were recorded using LC-solution software. Analytical reversed phase C-18 (ODS column, 250×4.6 mm, 5 µm, Dynamix, Inc) was used for isolation.

Plant material. Leaves of *M. koenigii* were collected from University of Dhaka, Bangladesh, in April 2013. Voucher specimen for the plant has been maintained in Bangladesh National Herbarium, Dhaka, Bangladesh, for future reference. The leaves were first sun dried and then ground into a coarse powder using a grinding machine.

Extraction and isolation. The powdered leaf (1000 g) was soaked in 3.0 L methanol for 15 days and then filtered through a cotton plug followed by Whatman filter paper number 1. The extract was concentrated with a rotary evaporator. A portion (5 g) of the concentrated methanol extract was fractionated by the modified Kupchan partitioning protocol[23] into petroleum ether (0.65 gm), carbon tetrachloride (0.55 gm), chloroform (0.30 gm) and aqueous (2.5 gm) soluble materials.

The petroleum ether soluble partitionate was subjected to gel permeation chromatography over lipophilic Sephadex LH-20 and a total of 15 fractions were collected. On the basis of their TLC behavior, fraction 8 and 9 were subjected to preparative thin layer chromatography (PTLC) using 1% ethyl acetate in toluene to yield ferulic acid (**2**, R_f = 0.78), mahanimbine (**4**, R_f = 0.64), koenimbine (**5**, R_f = 0.32), and koenidine (**6**, R_f = 0.45). Fraction 13 and 15 of the carbon tetrachloride soluble partitionate obtained from gel permeation chromatography was subjected to high performance liquid chromatography separation using 40% water in acetonitrile as mobile phase with the flow rate of 3 ml/min and pressure of 57 kgf/cm^2 yielded arborinine (**1**, R_t = 7.228 min) and *O*-demethyl murrayanine (**7**, R_t = 10.828 min) (Figure 1).

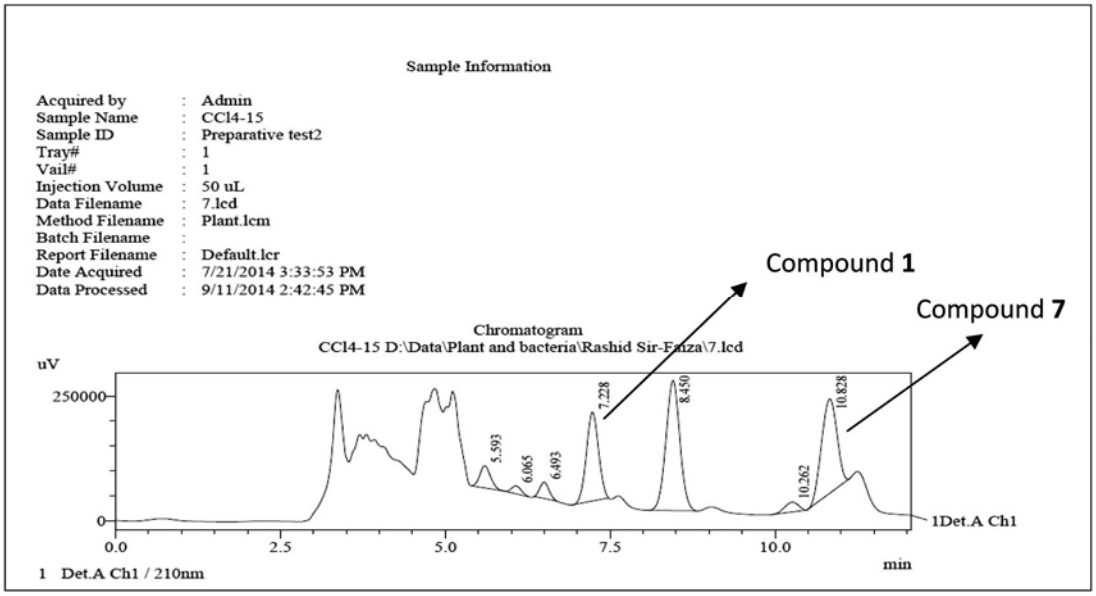


Figure 1. Separation of compound **1** and **7** using High Performance Liquid Chromatography (HPLC).

The carbon tetrachloride soluble partitionate was subjected to gel permeation chromatography over lipophilic Sephadex LH-20 and a total of 15 fractions were collected. On the basis of their TLC behavior, fraction 15 was subjected to preparative thin layer

chromatography (PTLC) using 30% ethyl acetate in toluene to yield umbelliferone (**3**, R_f = 0.56).

Properties of isolated compounds.

Arborinine (1): colourless mass; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.84 (3H, s, N-10), 3.92 (3H, s, 2-OCH_3), 4.01 (3H, s, 3-OCH_3), 6.27 (1H, s, H-4), 7.25 (1H, d, *J* = 8.5 Hz, H-7), 7.50 (1H, d, *J* = 8.5 Hz, H-5), 7.72 (1H, d, *J* = 8.5 Hz, H-6), 8.42 (1H, dd, *J* = 8.5, 2.0 Hz, H-8); ^{13}C NMR (100 MHz, $CDCl_3$): δ 34.2 (N-Me), 56.0 (3-OCH_3), 60.8 (2-OCH_3), 86.7 (C-4), 105.7 (C-12), 114.6 (C-5), 120.7 (C-13), 121.5 (C-7), 126.7 (C-8), 130.2 (C-2), 133.9 (C-6), 140.4 (C-11), 141.9 (C-14), 156.3 (C-1), 159.4 (C-3), 180.8 (C-9).

Ferulic acid (2): white crystalline mass; ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.91(3H, s, 2-OMe), 5.82 (1H, s, OH-1), 6.29 (1H, d, *J* = 15.9 Hz, H-8), 6.95 (1H, d, *J* = 8.2 Hz, H-6), 7.04 (1H, s, H-3), 7.08 (1H, br d, H-5), 7.60 (1H, d, *J* = 16.0 Hz, H-7).

Umbelliferone (3): white needle like mass; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.18 (1H, d, *J* = 9.6 Hz, H-3), 6.74 (1H, dd, *J* = 8.0, 2.1 Hz, H-6), 6.77 (1H, d, *J* = 2.0 Hz, H-8), 7.29 (1H, d, *J* = 8.8 Hz, H-5), 7.61 (1H, d, *J* = 9.2 Hz, H-4).

Mahanimbine (4): deep yellow gum; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.44 (3H, s, 3-CH_3), 1.56 (3H, s, 4`-$CH_3$), 1.64 (3H, s, 4`-CH_3), 1.76 (2H, t, *J* = 8.0 Hz, 1`-$CH_2$), 2.15 (2H, m, 2`-CH_2), 2.32 (3H, s, 5-CH_3), 5.10 (1H, t, *J* = 7.0 Hz, 3`-CH_2), 5.61 (1H, d, *J* = 10.0 Hz, H-2), 6.64 (1H, d, *J* =10.0 Hz, H-1), 7.16 (1H, t, *J* = 8.0 Hz, H-8), 7.29 (1H, t, *J* = 8.0 Hz, H-9), 7.36 (1H, br d, *J* = 8.0 Hz, H-10), 7.65, (1H, s, H-6), 7.85 (1H, s, 11-NH), 7.89 (1H, d, *J* = 8.0 Hz, H-7).

Koenimbine (5): white gummy residue; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.48 (3H, s, H-5`), 1.56 (3H, s, H-4`), 2.32 (3H, s, 3-CH_3), 3.90 (3H, s, 6-OCH_3), 5.69 (1H, d, *J* = 10.0 Hz, H-2`), 6.61 (1H, d, *J* = 10.0 Hz, H-1`) , 6.94 (1H, dd, *J* = 2.4, 8.5 Hz, H-7), 7.28 (1H, d, *J* = 8.5 Hz, H-8), 7.41 (1H, d, *J* = 2.4 Hz, H-5), 7.62 (1H, s, H-4).

Koenidine (6): yellow gum; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.45 (3H, s, H-5`), 1.49 (3H, s, H-4`), 2.32 (3H, s, 3-CH_3), 3.95 (3H, s, 7-OCH_3), 3.98 (3H, s, 6-OCH_3), 5.69 (1H, d, *J* = 10.0, H-2`), 6.60 (1H, d, *J* = 10.0 Hz, H-1`), 6.93 (1H, s, H-8), 7.38 (1H, s, H-4), 7.55 (1H, s, H-5).

***O*-demethyl murrayanine (7):** colourless mass; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.95 (1H, br t, H-6), 6.98 (1H, dd, 1-OH), 7.47 (1H, br d, *J* = 8.0 Hz, H-7), 7.50 (1H, d, *J* = 8.4 Hz, H-2), 7.88 (1H, d, *J* = 3.6 Hz, H-8), 7.99 (1H, d, *J* = 8.5 Hz, H-5), 8.30 (1H, s, NH), 8.49 (1H, s, H-4), 10.07 (1H, s, 3-CHO).

RESULTS AND DISCUSSION

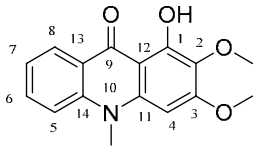
A total of seven compounds (**1**–**7**) were isolated from the methanol extract and its carbon tetrachloride and pet ether soluble fraction of leaves of *M. koenigii* by gel permeation chromatography over lipophilic Sephadex LH-20, preparative thin layer chromatography (PTLC) and high performance liquid chromatography (HPLC) on C_{18} bonded silica gel HPLC and repeated chromatographic separation by gel permeation chromatography. The structures of the isolated compounds were primarily solved by high field NMR data analysis.

The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **1** revealed signals characteristic of a polycyclic acridone-type alkaloid, where two of the three hydroxyl groups were methylated (OMe). The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum displayed a total of 16 carbon resonances, including an N-CH_3 signal at δ 34.2 and a signal for carbonyl carbon at 180.8 ppm. The DEPT spectrum indicated that out of the 16 carbons, 8 had attached protons. It also demonstrated the presence of three methyls, five methines and eight quaternary carbon resonances.

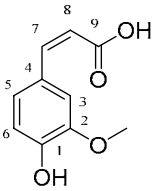
The ^{1}H NMR spectrum exhibited signals for a highly characteristic ABCD spin system with four aromatic proton resonances at δ 7.50 (1H, d, *J* = 8.5 Hz), 7.72 (1H, t, *J* = 8.5 Hz), 7.25 (1H, t, *J* = 8.5 Hz) and 8.42 (1H, br d, *J* = 8.5 Hz), which could be assigned to four adjacent protons H-5, H-6, H-7 and H-8, respectively on ring A. A sharp singlet at δ 6.27 that integrated for one proton was attributable to the aromatic proton at C-4 of ring C. Two sharp singlets, each of three proton intensity, at δ 3.92 and 4.01 were ascribed to two methoxyl groups. The ^{13}C NMR

spectrum also showed two methoxyl carbons resonating at δ 56.0 and 60.8. The high field value at δ 56.0 was assigned to the sterically hindered methoxyl group at C-2, while that at 60.8 could be attributed to C-3 methoxyl carbon. The signal at δ 180.8 clearly showed that the C-9 position had a ketonic functionality. On the other hand, the three proton singlet at δ 3.84 could be assigned to the methyl on a nitrogen atom (N-CH_3). This was substantiated by the ^{13}C resonance at δ 34.2.

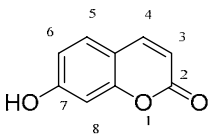
The above spectral features are in close agreement to those observed for arborinine (**1**), the identity of which was confirmed by co-TLC with authentic sample.[19] On this basis, the identity of compound **1** was established as arborinine (**1**). Although arborinine (**1**) has previously been isolated from many plants belonging to the family Rutaceae[19], this is the first report of its occurrence from *Murraya* species.



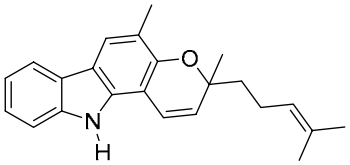
Arborinine (**1**)



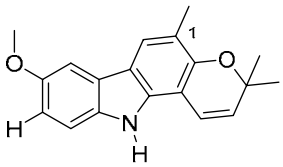
Ferulic acid (**2**)



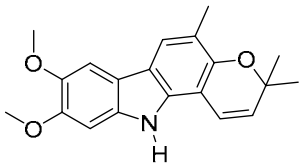
Umbelliferone (**3**)



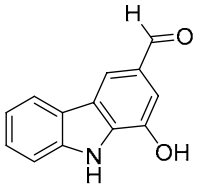
Mahanimbine (**4**)



Koenimbine (**5**)



Koenidine (**6**)



O-demethyl murrayanine (**7**)

The ^{13}C NMR spectrum (150 MHz, $CDCl_3$) of compound **2** showed a total of 11 carbon resonances, including a carboxylic acid group at δ 167.4 and a methoxyl group at 55.0. The ^{1}H NMR spectrum ($CDCl_3$, 600 MHz) displayed a singlet of three proton intensity at δ 3.91 suggesting the presence of a methoxyl gruop. It also exhibited two doublets centred at δ 7.60 (1H, J = 1.5 Hz) and 6.95 (1H, J = 8.4 Hz) and a double doublet (J = 8.4, 1.5 Hz) at δ 7.08 typical for a 1,3,4-trisubstituted aromatic moiety. The doublets (J = 16.9 Hz) centred at δ 7.60 and 6.29 could be assigned to the *trans* coupled protons H-7 and H-8, respectively. The relatively low field resonance of H-7 could be explained by its beta position to the carbonyl group, probably in the form of a carboxylic acid. Thus the structure of compound **2** was deduced as ferulic acid (**2**). The ^{1}H and ^{13}C NMR resonances in compound **2** were assigned by 2D NMR data notably HSQC and HMBC (Table 1) which allowed to revise the previous assignments made by Sajjadi *et al.*(2012).[20] This is the first report of its isolation from *M. koenigii.*

Table 1. HMBC and HSQC correlations observed for Ferulic acid.

Proton	HMBC correlations		HSQC
	2J	3J	
H-3	146.8 (C-2)	144.6 (C-7), 147.9(C-1)	109.3
H-5		109.3 (C-3), 144.6 (C-7)	123.1
H-6		127.1 (C-4), 146.8 (C-2), 147.9 (C-1)	114.7
H-7		109.3 (C-3), 115.8 (C-8), 123 (C-5), 127.1 (C-4), 167.4 (C-9)	144.6
H-8	127.1 (C-6)	167.4 (C-9)	115.8
1-OH		114.6 (C-6), 146.8 (C-2)	-
2-OMe		146.8 (C-2)	55.9

The compound **3** - **7** were identified as umbelliferone[21], mahanimbine[6], koenimbine[5], koenidine[5] and *O*-demethyl murrayanine[22], respectively through co-TLC with authentic samples, whenever possible and their structures were elucidated by comparison their spectral data with the published values reported previously.

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