

## Novel carbazole alkaloid from *Murraya koenigii* (L.) Spreng

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**Abstract:** Background: The plant *Murraya koenigii* (L.) Spreng, native to Pakistan and India and a part of the Rutaceae family, plays a vital role in the Indian Ayurvedic medicine system. Studies have shown that this plant's bark, roots, and leaves contain many carbazole alkaloids. These alkaloids are known to have substantial therapeutic properties.

**Methods:**

The crushed and powdered organs of *M. koenigii* were extracted with several solvents (acetone, chloroform, and methanol). Then, using column chromatography, a dull brown oily substance, MK- 1 (1.05 g), was obtained with chloroform and methanol (7:3).

**Result**

We have successfully isolated a new carbazole alkaloid, 3-geranyl 8-hydroxy 6, 7-di methoxy 3', 3'-dimethyl 1, 2-pyranocarbazole, from the seeds of *M. koenigii*. The structure was further elucidated by cross-referencing our NMR, UV, IR, and MS data with that found in the published literature.

**Conclusion:**

Comparing the previously published literature data clearly explained that this alkaloid is new to *M. koenigii*.

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## 1. INTRODUCTION

A significant breakthrough of the current time will be identifying various rejuvenating molecules that may halt or minimize the pathology of multiple illnesses. The potential for adverse effects and health concerns associated with synthetic chemicals has prompted the quest for natural compounds to replace them. Several phytoconstituents have offered superior therapeutic benefits to standard medical therapy.

Faisalabad is geographically situated amidst the Chenab River and the Ravi River, two prominent water bodies in Pakistan. The Chenab River exhibits a westward flow, whereas the Ravi River is situated towards the southeast. According to data on medicinal plants, this region

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has many plant species. Various medicinally important "Rutaceae" species have a wide distribution. Some of the plants in this family, which have great medicinal value, also are found in Faisalabad. Phytochemical investigations revealed that medicinal plants in the Rutaceae family produce many active chemicals responsible for disease-curing activities. As a result of some highly active compounds, medicinal plants of the Rutaceae family have proven to be more helpful. *Murraya koenigii* (L.) Spreng. (Family Rutaceae) is indigenous to India, Pakistan, Azerbaijan, and numerous Asian nations (Chopra *et al.*, 2002; Satyavati *et al.*, 1987). In India and Pakistan, *Murraya koenigii* is known as *kathnim*, *mitha neem*, *curry patta*, *gandhela*, *barsanga*. People have used the organs of *Murraya koenigii* for a long time to make herbal remedies like the leaves to help ease morning sickness; they can also treat diarrhoea and sniffles. Moreover, *Murraya koenigii* branches are often used to clean teeth and build teeth and gums (Chopra *et al.*, 2002; Satyavati *et al.*, 1987; Gautam *et al.*, 2020; Aniq *et al.*, 2021; Batool *et al.*, 2020).

The essential oils extracted from this plant have anti-inflammatory, analgesic, and anti-amoebic properties and are used to treat various ailments. The astringent and stimulating properties of the leaves and other organs are employed to treat cuts, joint discomfort, and other bodily aches, as well as anticancer. This plant has identified many phytochemicals, mainly carbazole alkaloids (Abeysinghe *et al.*, 2021; Balakrishnan *et al.*, 2020). The biologically active carbazole alkaloids in *Murraya koenigii* make this plant a go-to for medicinal purposes, such as cytotoxic, anti-microbial, anti-bacterial, anti-tumour, antioxidant, hypoglycaemic, anti-inflammatory and analgesic also used to treat leprosy (Abeysinghe *et al.*, 2021; Balakrishnan *et al.*, 2020; Mandal, 2016; Tripathi *et al.*, 2018; Knölker & Reddy, 2008). Therefore, we have chosen *Murraya koenigii*, for our research in light of the above context, abundantly found in Faisalabad, Pakistan.

## 2. MATERIAL and METHODS

### 2.1. Instrumental

UV spectrometer Perkin-Elmer Lambda Bio 20 (Perkin Elmer, USA) was used to record the ultraviolet absorption spectrum. On Perkin-Elmer 1710 Fourier transform spectrometer (Perkin Elmer, USA), IR spectroscopy was carried out utilising the KBr disc. As an internal standard, tetramethylsilane (TMS) is used to calculate  $\delta$  values (ppm). The FEBMS were recorded using the JEOL SX 1021/DA-6000 mass spectrometer (JEOL Ltd. Japan). The Bruker AVANCE DRX-400 (German) was used to record NMR spectra (400, 100 MHz). Silica gel (60–120 mesh) was used for column chromatography. The chemicals and reagents used in this experiment were AR quality from E-Merck (Pakistan).

### 2.2 Plant material

*Murraya koenigii* seeds were gathered in June 2022 throughout remote rural areas of Faisalabad District, Punjab province. Faisalabad is a city situated between 31°25'0"N latitude and 73°5'28"E longitude. The collected plant specimens were shown to be authentic by the botany department of Government College University in Faisalabad, Pakistan.

### 2.3 Extraction

It is best to utilise it as soon as it is collected and dried because dried material that has been stored for an extended period might alter dramatically. *Murraya koenigii* air-dried seeds (1.2 kg), were crushed into a fine powder and defatted with petrol-ether (3 Lx5 times). Subsequently, the sample was subjected to Soxhlet extraction using acetone, chloroform, and methanol. We only considered the chloroform extract for further investigation. A rotator was used to evaporate the chloroform extract below 50 °C temperature to produce a reddish-brown substance (28.6 g). The chloroform extract was reddish-brown (28.6 g) after being evaporated in a rotator at

temperatures below 50 °C. Next, column chromatography was performed on this brownish-red material. After being well mixed, a silica gel solution, 135 g in pet.-ether, was put into a 150 mm long column with a circular area crosssection 50 mm in diameter.

After the absorbent had finished settling, the column was opened so the extra pet.-ether could flow through it. The column was finally settled after the slurry was digested to produce a methanolic extract using silica gel in a pet.-ether. Increasingly polar solvents and solvent combinations were used to elute the column without interruption. A dull brown oily substance **MK-1** (1.05 g) was obtained with chloroform and methanol (7:3). Likewise, the alcoholic extract was eluted in the column with increasing polarity solvents and solvent mixtures. Elution with acetone: methanol (7:1) afforded **MK-2** (0.57 g). Similarly, after being eluted with a mixture of chloroform and ethyl acetate (6:4), the ethyl acetate extract produced the chemical **MK-3** (0.35 g).

**Compound MK-1:** brown oily substance, C<sub>40</sub>H<sub>40</sub>N<sub>2</sub>O<sub>6</sub>; UV (MeOH)  $\lambda_{\max}$  (log $\epsilon$ ) nm: 211.0 (4.60), 240.6 (4.66), 285.4 (4.40 sh), 295.2 (4.56), 325.2 (3.90), 342.2 (3.92), and 356.6 (3.86); IR (KBr)  $\nu_{\max}$ : 3390, 2934, 2875, 1671, 1468, 1459 (*gem* dimethyl), 1102, 1076, 999 and 907 cm<sup>-1</sup>; <sup>1</sup>H NMR(400 MHz, CDCl<sub>3</sub>)  $\delta$ : 10.69 (1H, brs, -OH), 9.30(1H, brs, -NH), 7.91(1H, brs, H-5), 7.66(1H, brs, H-4), 5.68 (1H, d, *J* = 9.5 Hz, H-1'), 5.59 (1H, d, *J* = 9.5 Hz, H-2'), 5.16 (1H, d, *J* = 7.1 Hz, H-6''), 5.09 (2H, d, *J* = 7.1 Hz, H-2''), 3.35 (2H, d, *J* = 7.2 Hz, H-1''), 2.09 (2H, d, *J* = 7.5 Hz, H-5''), 1.98 (2H, d, *J* = 7.5 Hz, H-4''), 1.47 (3H, s, 10'' -CH<sub>3</sub>), 1.54 (3H, s, 8'' -CH<sub>3</sub>), 1.45 (3H, s, 9'' -CH<sub>3</sub>), 1.37(3H, s, 3' -Me), 1.29 (3H, s, 4' -Me), 3.96 and 4.02 (6H, s, 8, 9 -OCH<sub>3</sub>); <sup>13</sup>C NMR(100 MHz, CDCl<sub>3</sub>)  $\delta$ : 153.4(C-2), 151.4(C-6), 136.2 (C-3''), 134.9 (C-9a), 132.9(C-7''), 128.7 (C-8a), 106.2 (C-1), 138.1 (C-8), 127.8 (C-2'), 136.2(C-7), 125.3 (C-6''), 123.1 (C-2''), 123.9 (C-4), 121.2 (C-4a), 117.4 (C-1'), 125.3 (C-3), 122.6(C-4b), 98.7(C-5), 77.1(C-3'), 59.1(-OCH<sub>3</sub>), 57.2 (-OCH<sub>3</sub>), 38.6 (C-4''), 29.8 (C-1''), 28.7 (5' -CH<sub>3</sub>), 28.4(4' -CH<sub>3</sub>), 27.1 (C-5''), 23.7(9'' -CH<sub>3</sub>), 19.1(8'' -CH<sub>3</sub>), 17.5(10'' -CH<sub>3</sub>); Mass spectra *m/z*: 461[M]<sup>+</sup>, 324[M-C<sub>10</sub>H<sub>17</sub>]<sup>+</sup>, 137

### 3. RESULTS

The compound was obtained as a brown oily substance. According to the EIMS study of this compound, a quasimolecular ion *m/z* 462 [M+H]<sup>+</sup> was found, compatible with a molecular weight of 461 and molecular formula C<sub>29</sub>H<sub>35</sub>NO<sub>4</sub>. This compound also produces blue-violet colour spots with H<sub>2</sub>SO<sub>4</sub> (conc.), characteristic of the carbazole alkaloids (Chakraborty & Roy, 2003; Chakraborty & Roy, 1991). Ultraviolet spectrum of this compound exhibited typical absorptions  $\lambda_{\max}$  at 211.0 (4.60), 240.6 (4.66), 285.4 (4.40 sh), 295.2 (4.56), 325.2 (3.90), 342.2 (3.92), 356.6 (3.86) characteristic of a carbazole skeleton (Chakraborty & Roy, 2003; Chakraborty & Roy, 1991). Infrared spectra of this chemical revealed absorption bands at 3390, 2934, 2875, 1671, 1468, 1459, 1102, 1076, 999, and 907 cm<sup>-1</sup>, which revealed a pyrano carbazole system. According to the spectroscopic evidence, the compound was carbazole and had a pyran ring, attached to the carbazole at C-1 and C-2 (Chakraborty & Roy, 2003; Chakraborty & Roy, 1991).

A broad singlet observed at  $\delta$  9.30 in the <sup>1</sup>H NMR spectrum corresponds to the -NH proton, while an additional singlet observed at  $\delta$  10.69 is assignable to the -OH proton. Other two singlets have been observed at  $\delta$  7.66 and 7.91 for carbazole ring, H-4 and H-5 protons. The singlets nature of H-4, and H-5 confirmed that the remaining positions of carbazole were substituted. Signals were observed at  $\delta$  3.35 (2H, d, *J* = 7.1 Hz, H-1''), 1.98 (2H, d, *J* = 7.5 Hz, H-4''), and 2.09 (2H, d, *J* = 7.5 Hz, H-5''), allocated for methylene protons. Furthermore, signals detected at  $\delta_{\text{H}}$  5.09 (1H, d, *J* = 7.1 Hz, H-2''), and 5.16 (1H, d, *J* = 7.1 MHz, H-6''), were attributed to two methine protons (Kapil, 1971; Chakraborty, 1977; Bhattacharyya & Chakraborty, 1987).

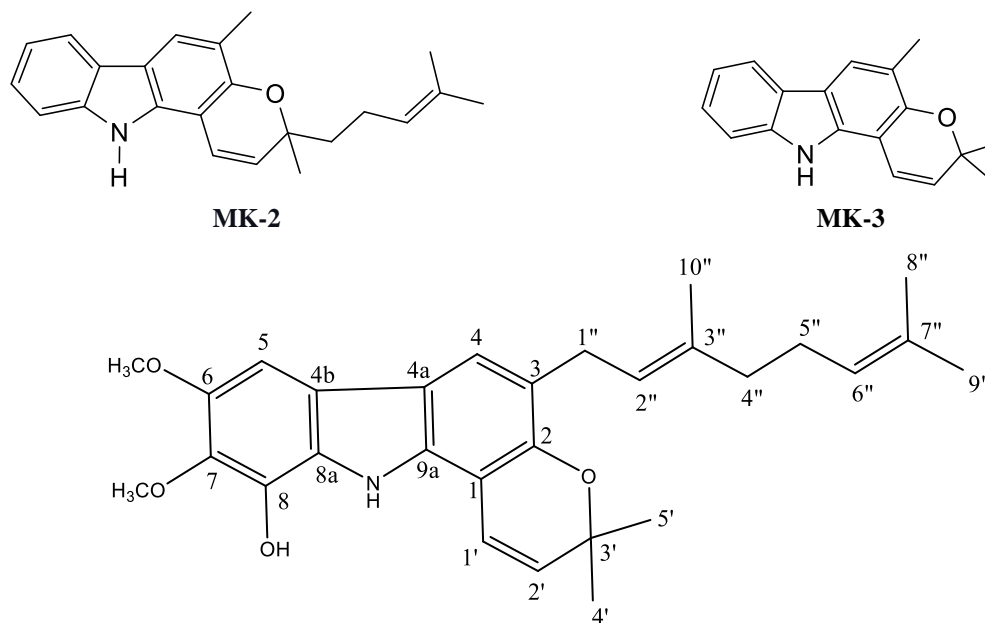
Additionally, singlets have been found in  $^1\text{H}$  NMR at  $\delta$  1.47 (3H, s, 8'' -CH<sub>3</sub>), 1.45 (3H, s, 9'' -CH<sub>3</sub>), and 1.54 (3H, s, 10'' -CH<sub>3</sub>) were unambiguously assigned to three methyl groups. Based on these findings, the molecule has a geranyl-substituted group. The  $^{13}\text{C}$  chemical shift of  $\delta$  115.3 for the geranyl-substituted carbon strongly suggests the presence of the geranyl group in C-3. In HMBC correlations of H-1'' at  $\delta$  3.35 with C-3 ( $\delta$  115.3), C-2 ( $\delta$  153.4), and C-4 ( $\delta$  122.1), the geranyl moiety was confirmed to be located at C-3 (Kapil, 1971; Chakraborty, 1977; Bhattacharyya & Chakraborty, 1987). Furthermore, doublets are at  $\delta$  5.68 (1H, d, 9.5 Hz, H-1'), and 5.59 (1H, d, 9.5 Hz, H-2') are allocated to double bonds protons H-1' and H-2' of the pyran ring. Additionally, spectra revealed two peaks at  $\delta_{\text{H}}$  1.37 and 1.29 associated with gem-dimethyl groups on pyran rings (Kapil, 1971; Chakraborty, 1977; Bhattacharyya & Chakraborty, 1987).

In addition, two singlets were seen at  $\delta$  3.96 and 4.02 (6H, s, 2-OCH<sub>3</sub>) associated with two methoxy groups in the carbazole structure at C-6 and C-7. Furthermore, signals observed at  $\delta_{\text{H}}$  4.02 /  $\delta_{\text{C}}$  56.3 and  $\delta_{\text{H}}$  3.96 /  $\delta_{\text{C}}$  55.8 were assignable for methoxy protons positioned at C-6 and C-7. In the mass spectra, the existence of a fragment with a mass of  $m/z$  137 indicated the existence of a geranyl group in the structure (Scheme 1). Accordingly, based on these spectral shreds of evidence and literature comparisons, the isolated compound should be 3-geranyl 8-hydroxy 6, 7-di methoxy 3', 3'-di methyl 1, 2-pyranocarbazole (**MK-1**). This compound is isolated for the first time from the seeds of *Murraya koenigii*.

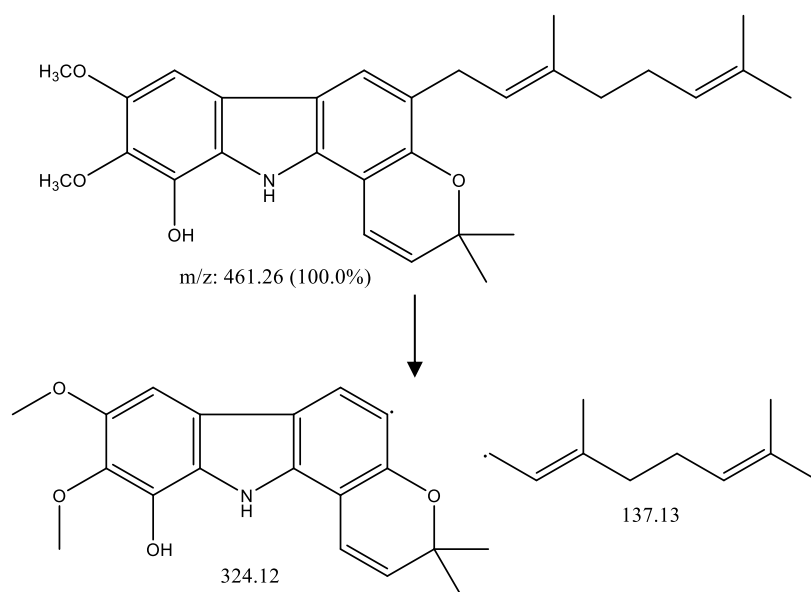
The two known compounds were also isolated and identified as 3,5-dimethyl-3-(4-methylpent-3-enyl)-11*H*-pyrano[3,2-*a*]carbazole (**MK-2**, mahanimbine), 3,3,5-trimethyl-11*H*-pyrano[3,2-*a*]carbazole (**MK-3**, Girinimbine) through matching their NMR values to one previously published (Kapil, 1971; Chakraborty, 1977; Bhattacharyya & Chakraborty, 1987).

Mahanimbine lower blood glucose, also prevents obesity, mitigates metabolic abnormalities caused by excessive fat consumption, and has anti-aging and anti-anxiety effects. It fights against leukemia also bladder, and pancreatic cancer (Hobani, 2022). Whereas girinimbine has been shown to have anticancer action in vitro, and it also has potent antioxidant and gastro-protective properties (Singh, et. al., 2023).

**Scheme 1. (MK-1)**



3-geranyl 8-hydroxy 6, 7-di methoxy 3', 3'-di methyl 1, 2-pyranocarbazole (MK-1)



**4. DISCUSSION and CONCLUSION**

Accordingly, based on these spectral shreds of evidence and literature comparisons, the isolated compound should be called as 3-geranyl 8-hydroxy 6, 7-di methoxy 3', 3'-dimethyl 1, 2-pyranocarbazole. This compound is isolated for the first time from the seeds of *Murraya koenigii*.

**Declaration of Conflicting Interests and Ethics**

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

### Authorship Contribution Statement

**Arifa Mehreen, Shagufta Kamal:** Methodology, Supervision, and Validation. **Sevinj Musayeva Vagif:** Resources, Visualization. **Muhammad Qaisar:** Software, Formal Analysis. **Sumia Urainab:** Writing original draft. **Asad Ullah:** Investigation.

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