Isolation of Carbazole Alkaloids from *Murraya koenigii* Leaves and their Cytotoxic Activities

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Murraya koenigii, from the family Rutaceae, is commonly known as the curry leaf plant. It is widely grown across Southeast Asia, mainly in India and Sri Lanka. *M. koenigii* is known for its carbazole alkaloids which interact intricately to produce pharmacodynamic effects. In this study, carbazole alkaloids were isolated and characterised. The leaves of *M. koenigii* were macerated with hexane, dichloromethane and methanol to produce three crude extracts. From the hexane crude, eight fractions were eluted via column chromatography. Two compounds were successfully isolated by preparative TLC with a solvent system of hexane:DCM (3:2). Both compounds were characterised by NMR (1D and 2D spectroscopy) and FTIR, and successfully identified as mahanimbine (**1**) and murrayazolinol (**2**). When tested individually by TLC, mahanimbine had an R_f value of 0.69 with a solvent system of hexane:DCM (2:3), while murrayazolinol had an R_f value of 0.62 with a solvent system of hexane:DCM (1:1). Compounds **1** and **2** were tested for cytotoxic activity using MTT assays. Mahanimbine (**1**) showed moderate cytotoxicity against HeLa (CD_{50} = 21.4 ± 3.0 µg/mL) and weak activity against HL-60 (38.2 ± 6.2 µg/mL), whereas murrayazolinol (**2**) exhibited weak cytotoxicity against HeLa (46.0 ± 4.4 µg/mL) and HL-60 (42.2 ± 3.6 µg/mL), with low activity against normal NIH/3T3 cells (>60 µg/mL). Assessment of their cytotoxic activity provides valuable insights into their potential as lead compounds for anticancer drugs. These results confirm the pharmacological value of *Murraya koenigii* and its potential in natural therapeutic product research.

Keywords: Carbazole alkaloids, *Murraya koenigii*, Rutaceae, mahanimbine, murrayazolinol

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Murraya koenigii L. Spreng (*M. koenigii*), also known as the curry leaf tree, comes from the Rutaceae family and is native to India, Sri Lanka, and Bangladesh. All parts of the plant including the leaves, fruits, bark and roots are used in traditional Indian (Ayurvedic) medicine to treat various illnesses. This plant has been proven to have an abundance of carbazole alkaloids with various potent biological activities such as antimicrobial, metabolic, anti-inflammatory and anticancer effects [1-7]. Several recent reviews have also highlighted that *M. koenigii* contains an abundance of pyranocarbazoles such as mahanimbine, girinimbine and koenimbine which were reported to have broad bioactivities, reinforcing the plant's medicinal relevance [8].

Mahanimbine has become the leading marker compound in *M. koenigii*. There are many reports of its biological activities. In 2011, Nagappan found that mahanimbine inhibited various antibiotic-resistant bacteria and had cytotoxic effects [9]. In 2016, Dahiya found that mahanimbine had potent antianxiety activity at 3 mg/kg, po, compared to diazepam at 2 mg/kg, po [10]. Also in 2016, Jagtap concluded that mahanimbine could prevent HFD-induced hyperlipidemia and fat accumulation in adipose and lung tissues in mice, as well as restricting progression of systemic inflammation and oxidative stress [11]. In 2022, Hobani tested the therapeutic properties of mahanimbine against breast cancer cells. This contemporary in-vitro study demonstrated that the mitochondrial apoptosis induced by mahanimbine exhibited anti-invasive and anti-angiogenic effects in breast cancer cells. This expands on earlier findings about the antibacterial and metabolic effects of this compound [12]. The decision to isolate mahanimbine was made because it was one of the most pharmacologically active carbazoles in the curry leaf plant, as indicated by previous work.

In addition to *M. koenigii*, structurally related carbazole alkaloids have also been reported in other Rutaceae species, most commonly *Clausena*, *Glycosmis*, and *Micromelum*. This indicates that the carbazole scaffold is relatively widespread within this family; as such the presence of these compounds in

M. koenigii alone does not automatically establish its therapeutic distinctiveness [13]. What differentiates *M. koenigii* is its comparatively high abundance of mahanimbine and similar carbazoles in its leaves. This makes it a practical and renewable source for compound isolation [8]. This study aims to determine whether the carbazole alkaloids from *M. koenigii* demonstrate unique or superior biological activities relative to those found in other Rutaceae species. Their pharmacological effects were evaluated at the isolated compound level rather than relying on results obtained from crude extracts [14].

As part of our ongoing phytochemical studies on *M. koenigii* [15-18], we isolated carbazole alkaloids from the hexane extract of *M. koenigii* leaf and evaluated their cytotoxic activity. The carbazole structures were characterized by extensive spectroscopic techniques such as IR, UV, NMR, and MS. Their cytotoxic activities towards HL-60 and HeLa cells are reported here for the first time in the hope of providing new and beneficial insights into their potential therapeutic application.

EXPERIMENTAL

Plant Materials

A total of 5 kg of of *M. koenigii* leaves were harvested from a plantation in Sibu, Sarawak in January 2023. A voucher specimen (TM1008) of the plant was deposited in the herbarium and identified by a botanist in Universiti Pendidikan Sultan Idris.

Chemicals and Materials

Hexane (HXE-0723-00-4E-131023, HmbG Chemicals), dichloromethane (241022.1, Fisher Scientific), and methanol (I1230409 229, Merck) were used for maceration and as solvents for column chromatography which used Silica Gel $60F_{254}$, 230-400 mesh ASTM as the stationary phase (TA5016985 845, Merck). Thin layer chromatography was performed on precoated aluminium-supported silica gel $60F_{254}$ thin layer chromatography (TLC) sheets (HX98205654, Merck). Preparative thin layer chromatography was performed using Silica Gel $60F_{254}$ containing gypsum as the stationary phase. Deuterated chloroform (A0399402, Acros Organics BV) was used as the solvent to prepare samples for NMR analysis.

For the cytotoxic assay, three cell lines obtained from the American Type Culture Collection (ATCC) were the human promyelocytic leukaemia cell line (HL-60), cervical cancer cell line (HeLa) as well as normal mouse embryonic fibroblasts (NIH/3T3) cell line. RPMI-1640 (Sigma-Aldrich), a culture medium with 10 % v/v foetal calf serum 10270 (Gibco) and a Penicillin-Streptomycin Mixed Solution (5000 units/mL of penicillin and 5 mg/mL of streptomycin in 0.85 % sodium chloride, Kyota, Japan) was used as a complete growth medium (CGM). Phosphate Buffer Solution (PBS) was used as the buffer solution. Trypsin/EDTA (TrypLE™ Express, Gibco) was used to suspend adherent and semi-adherent cells, while 0.4 % Trypan Blue Solution (Sigma Aldrich) was used as a cell stain for cell counting. MTT Dye (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), a yellow tetrazole, was added to the cell cultures which were then incubated up to 4 hours. Dimethyl sulfoxide (DMSO) was used as a solvent.

Characterisation Methods

A UVP UGL-58 Handheld Ultraviolet (UV) lamp was used to observe the developed TLC sheets. 1D (^{1}H, ^{13}C, and DEPT) and 2D (COSY, HMQC, HMBC) Nuclear Magnetic Resonance (NMR) spectra were recorded with a JEOL at 500 MHz. The chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz. LC-MS was used to determine the mass spectra (MS) of the compounds while a Hitachi UH5300 Ultraviolet-Visible (UV-Vis) Double Beam Spectrophotometer was used to obtain the UV spectrum with a wavelength range of 200-1000 nm. A PerkinElmer Spectrum 100 Fourier Transform Infrared Spectrometer (FT-IR) was used to obtain the IR spectra of both compounds.

Extraction and Fractionation

The leaves of *M. koenigii* (600 g) were air-dried to a constant weight and pulverized. The dry plant materials were macerated with 1 L of hexane (Hex) (3 times × 24 hrs) and filtered. The filtrates were combined and concentrated to dryness to give a dried Hex extract (17.18 g) [19]. The dried plant residues were then subsequently extracted with 1 L of dichloromethane (DCM) (5 times × 24 hrs) to yield 54.21 g of DCM extract. Lastly, the plant residues were extracted with methanol (MeOH) (1 L × 3 times × 24 hrs) to give 35.84 g of MeOH extract. The Hex and DCM extracts were then subjected to silica gel column chromatography using a gradient solvent system of Hex, DCM and MeOH. A gradient solvent system of DCM and MeOH was used for the MeOH extract. The combined fractions of the extracts were dried using a rotary evaporator; a total of eight fractions (H1 – H8) were eluted from the Hex extract, and five fractions each from the DCM (D1 – D5) and MeOH extracts (M1 – M5).

Isolation and Characterisation of Bioactive Compounds

The subfractions were further isolated and purified. Preliminary fractionation was performed by column chromatography and eluted with a solvent gradient of Hex, Hex-DCM, DCM and DCM-MeOH. Each fraction was monitored by TLC. The TLC sheets were developed in a closed tank saturated with eluent

vapour. The purified compounds were identified by a single spot on the TLC visualized under UV light at 254 nm. Fraction 4 of the Hex extract was subjected to preparative TLC which yielded two compounds: mahanimbine [**1**, R_f value = 0.69 in Hex:DCM (2:3)] and murrayazolinol [**2**, R_f value = 0.62 in Hex:DCM (1:1)]. The structural elucidation and identification of the isolated compounds were performed using a combination of 1D (^{1}H, ^{13}C, and DEPT) and 2D (COSY, HMQC, HMBC) NMR, LC-MS, UV, and IR analyses.

Spectral Data

Mahanimbine (1): brown solid (19.5 mg); m.p: 93-95 °C; IR: 3428 (NH), 2923 (C-H stretching), 1469 (aromatic C-C), 1157 (C-O), 738 (C-H bending); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.46 (3H, s, 11-Me), 1.59 (3H, s, 4'-Me'), 1.67 (3H, s, 4'-Me"), 1.77 (2H, t, J = 10.0 Hz, H-1'), 2.18 (2H, m, H-2'), 2.34 (3H, s, 3-Me), 5.12 (1H, t, J = 9.0, 8.5 Hz, H-3'), 5.65 (1H, d, J = 9.7 Hz, H-10), 6.65 (1H, d, J = 9.7 Hz, H-9), 7.18 (1H, td, J = 9.0, 1.2 Hz, H-6), 7.31 (1H, td, J = 9.0, 1.2 Hz, H-7), 7.37 (1H, d, J = 10.0 Hz, H-8), 7.67 (1H, s, H-4), 7.75 (br s, NH), 7.91 (1H, d, J = 9.8 Hz, H-5); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 16.2 (3-Me), 17.7 (4'-Me'), 22.8 (C-2'), 25.7 (4'Me"), 25.8 (11-Me), 40.8 (C-1'), 78.2 (C-11), 104.2 (C-1), 110.4 (C-8), 116.7 (C-4a), 117.6 (C-9), 118.5 (C-5a, C-3'), 119.4 (C-5), 119.5 (C-6), 121.3 (C-4), 124.0 (C-3), 124.3 (C-7), 128.6 (C-10), 131.8 (C-4'), 134.9 (C-8a), 139.5 (C-1a), 150.0 (C-2); HRESIMS *m/z*: found 332.2008 $[M+H]^+$ (Calcd. for $C_{23}H_{25}NO$: 331.4507 $[M]^+$).

Murrayazolinol (2): brown solid (8.4 mg); m.p: 289-290 °C; IR: 3412 (OH), 2923 (C-H stretching), 1459 (aromatic C-C), 747 (C-H bending); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.25 (2H, s, H-10), 1.27 (1H, s, 4'-Me'), 1.45 (1H, s, 11-Me), 1.61 (1H, s, H-1'), 1.91 (1H, s, 4'-Me"), 1.98 (1H, m, H-3'), 2.32 (1H, s, 3-Me), 3.30 (2H, d, J = 6.4 Hz, H-9), 3.66 (1H, s, H-2'), 7.14 (3H, td, J = 8.9, 1.1 Hz, H-6), 7.22 (1H, td, J = 9.0, 1.2 Hz, H-7), 7.46 (3H, s, H-8), 7.48 (1H, s, H-4), 7.88 (2H, d, J = 9.45 Hz, H-5); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 15.5 (3-Me), 21.9 (C-10), 23.1 (4'-Me'), 25.1 (11-Me), 30.2 (4'-Me"), 36.2 (C-1'), 36.9 (C-9), 48.7 (C-3'), 60.5 (C-4'), 72.0 (C-2'), 79.5 (C-11), 107.5 (C-1), 113.6 (C-8), 114.0 (C-4a), 118.5 (C-3), 119.2 (C-6), 119.4 (C-4), 119.9 (C-5), 122.7 (C-7), 127.5 (C-5a), 140.7 (C-8a), 142.5 (C-1a), 155.1 (C-2); HRESIMS *m/z*: found 348.1957 $[M+H]^+$ (Calcd. for $C_{23}H_{25}NO_2$: 347.4501 $[M]^+$).

Cytotoxic Bioassay

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to assess the cytotoxicity of the compounds isolated from the leaves of *M. koenigii* in accordance with the revised methods of Bahuguna (2017) [20] and Kumar (2018) [21], as well as the modified method of Mosmann (1983) [22].

Different concentrations (0.5–60.0 µg/mL) of test substances were obtained by serial dilution in RPMI-1640 or DMEM media for adherent and suspension cell lines, depending on the requirements of the cell line. A 100 µL of a cell suspension (1.0×10^5 cells/mL) in CGM was added to each well of a sterile 96-well microplate that contained the test compounds to create suspension cells. For adherent cells, 1.0×10^4 cells were seeded in each well, and left to adhere for 24 hours. After adhesion and aspiration of media, 100 µL of compound-containing medium was added. The controls included were a negative control, which contained only cells and medium, a positive control using the anticancer chemotherapy drug, vincristine (VCR), at 60 µg/mL each, and lastly a blank control which only contained the medium.

The cells were incubated in a humidified environment with 5 % CO_2 for 72 hours at 37 °C. Then each well received 20 µL of MTT solution (5 mg/mL in PBS), and the plates were incubated for a further 4 hours to enable the production of formazan crystals. Following incubation, 100 µL of DMSO was added to each well to dissolve the formazan crystals, and the supernatants were carefully aspirated. To ensure total dissolution, the plates were gently shaken for ten to twenty minutes in the dark.

An ELISA microplate reader was used to record the absorbance of each well at 570 nm with 630 nm as the reference wavelength. The following formula was used to calculate the cytotoxicity percentage:

$$\% \text{ Cytotoxicity} = \left[\frac{(\text{Abs of untreated cells}) - \text{Abs of treated cells})}{\text{Abs of untreated cells}}\right] \times 100$$

The percentage of cell viability against the log concentration of test compounds produced dose-response curves. Non-linear regression analysis in GraphPad Prism was used to calculate the half-maximal cytotoxic dosage (CD_{50}), which is the concentration at which 50 % of cells were inhibited in comparison to untreated controls. Their potency was categorized as potent (<5 µg/mL), moderate (5–25 µg/mL), and weak (>25 µg/mL) [23]. Data was expressed as mean ± SD (n = 3), and statistical comparisons between compounds were performed using one-way ANOVA followed by Tukey's post-hoc test, with significance set at $p < 0.05$.

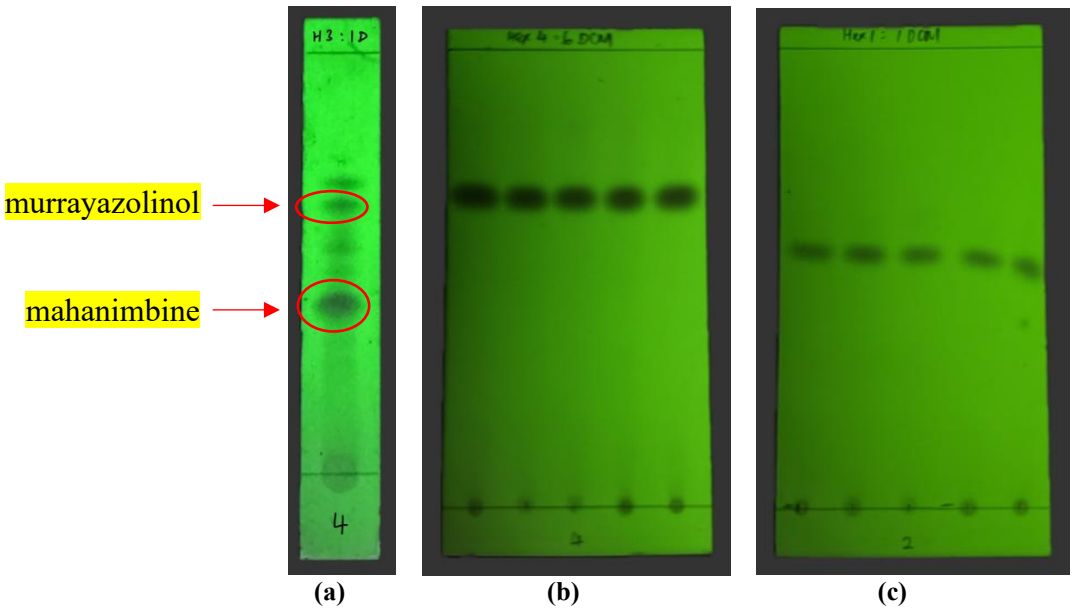

Figure 1. TLC of (a) fraction 4, (b) mahanimbine (1), and (c) murrayazolinol (2).

RESULTS AND DISCUSSION

Hexane Crude Fractionation

A total of 17.18 g of crude hexane was successfully extracted from 600 g of *M. koenigii* Leaves. Eight fractions were obtained from this extract, and two compounds were successfully isolated from the fourth fraction, mahanimbine (**1**) and murrayazolinol (**2**).

The hexane yield (≈ 2.7% w/w) and the isolation of the lipophilic carbazoles, mahanimbine (**1**) and murrayazolinol (**2**) from fraction four is relevant as recent reports demonstrated that these pyranocarbazoles in *M. koenigii* were low polarity extracts, reflecting that the recovery rate was due to solvent polarity dependency [8, 24]. Unlike many previous studies which only tested the crude extracts or individual compounds, we purified the compounds isolated from this plant and compared their cytotoxic activities directly, in order to obtain a clearer understanding of their true therapeutic potential.

Thin Layer Chromatography

Five distinct spots can be seen in Figure 1(a), which used a solvent system of Hex:DCM (3:1). Mahanimbine (**1**) was the first spot from the bottom with an R_f value of 0.4, while murrayazolinol (**2**) was the fourth spot from the bottom with an R_f value of 0.65. Figure 1(b) and (c) show the TLC results of mahanimbine (**1**) and murrayazolinol (**2**) after a purification process by PTLC. Mahanimbine (**1**) had an R_f value of 0.69 with a Hex:DCM (2:3) solvent system, while murrayazolinol (**2**) had an R_f value of 0.62 with a Hex:DCM (1:1) solvent system.

Nuclear Magnetic Resonance (NMR) and Infrared (IR) Spectra

Compound **1** was obtained as a brown solid. The IR spectrum of compound **1** indicated the presence of an N-H bond at 3428 cm^{-1}, C-H stretching at 2923 cm^{-1}, C-O stretching at 1157 cm^{-1}, and C-H bending at 738 cm^{-1}. The molecular weight was deduced by LC-MS and identified as $C_{23}H_{25}NO$ at m/z 332.2002 $[M+H]^+$ while the UV spectrum showed absorbance peaks at λ_{max} 238 and 288 nm due to the pyranocarbazole group. Based on the ^1H NMR spectrum from Table 1, compound **1** had a broad downfield signal at $^{\delta}7.75$ which represented the N-H bond in the carbazole nucleus. Signals $^{\delta}7.91$ (H-5), $^{\delta}7.67$ (H-4), $^{\delta}7.37$ (H-8), $^{\delta}7.31$ (H-7) and $^{\delta}7.18$ (H-6) were all concluded to be unsubstituted carbazole ring carbons. The presence of pyran rings attached to the carbazole ring were denoted by to two olefinic protons at $^{\delta}6.65$ (H-9) and $^{\delta}5.65$ (H-10). Lastly, the ^1H NMR showed signals for an aromatic methyl attached to C-3 at $^{\delta}2.34$, a methyl group attached to C-11 at $^{\delta}1.46$ and a prenyl group, 2-methylpent-2-enyl which was attached adjacent to the oxygen in the pyran ring of the pyranocarbazole at C-11 with signals at $^{\delta}5.12$ (H-3'), $^{\delta}2.18$ (H-2'), $^{\delta}1.77$ (H-1'), $^{\delta}1.66$ (4'-Me") and $^{\delta}1.59$ (4'-Me'). The ^{13}C NMR data for compound **1** presented in Table 1 confirmed the presence of a total of 23 carbon atoms. The carbon signals were assigned to their locations in compound **1** using DEPT, ^1H-^1H COSY, HMQC and HMBC spectra. Compound **1** was identified as mahanimbine [25].

Table 1. ^{1}H & ^{13}C NMR [500 MHz, δ_H] data for compounds 1 and 2.

Position	1		2	
	δH	δC	δH	δC
NH	7.75 (*br s*)			
1		104.2		107.5
1a		139.5		142.5
2		150		155.1
3		124		118.5
4	7.67 (1H, *s*)	121.3	7.48 (1H, *s*)	119.4
4a		116.7		114
5	7.91 (1H, *d*, 9.8 Hz)	119.4	7.88 (2H, *d*, 9.45 Hz)	119.9
5a		118.5		127.5
6	7.18 (1H, *td*, 9.0, 1.2 Hz)	119.5	7.14 (3H, *td*, 8.9, 1.1 Hz)	119.2
7	7.31 (1H, *td*, 9.0, 1.2 Hz)	124.3	7.22 (1H, *td*, 9.0, 1.2 Hz)	122.7
8	7.37 (1H, *d*, 10.0 Hz)	110.4	7.46 (3H, *s*)	113.6
8a		134.9		140.7
9	6.65 (1H, *d*, 9.7 Hz)	117.6	3.3 (2H, *d*, 6.4 Hz)	36.9
10	5.65 (1H, *d*, 9.7 Hz)	128.6	1.25 (2H, *s*)	21.9
11		78.2		79.5
3-Me	2.34 (3H, *s*)	16.2	2.32 (1H, *s*)	15.5
11-Me	1.46 (3H, *s*)	25.8	1.45 (1H, *s*)	25.1
1'	1.77 (2H, *t*, 10.0 Hz)	40.8	1.61 (1H, *s*)	36.2
2'	2.18 (2H, *m*)	22.8	3.66 (1H, *s*)	72
3'	5.12 (1H, t, 9.0, 8.5 Hz)	118.5	1.98 (1H, m)	48.7
4'		131.8		60.5
4'-Me'	1.59 (3H, *s*)	17.7	1.27 (1H, *s*)	23.1
4'-Me"	1.67 (3H, *s*)	25.7	1.91 (1H, *s*)	30.2

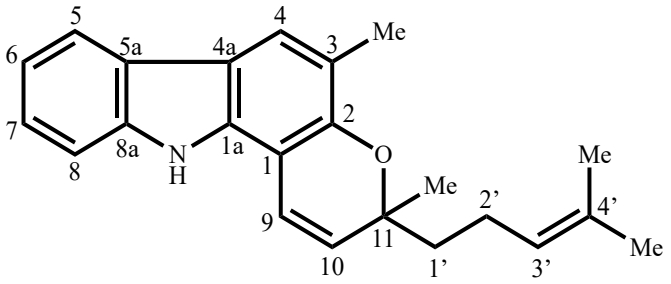

Figure 2(a). Compound 1, mahanimbine.

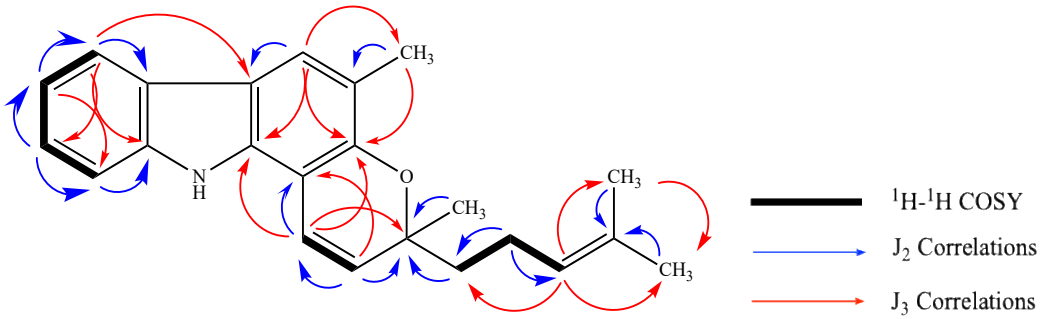

Figure 2(b). COSY and HMBC correlations for compound 1.

Compound **2** was a brown solid. The IR spectrum of compound **2** confirmed the presence of an additional OH group due to the absorption peak at 3412 cm^{-1}. Compounds **1** and **2** showed similarities due to an aromatic methyl group attached to C-3 at $^{\delta}2.32$ which confirmed a similar substitution pattern in both compounds. Differences which helped identify compound **2** included the absence of the N-H signal, as well as the additional substitution of a monoterpenoid moiety instead of a prenyl group. A carbinyl hydrogen of a secondary alcohol confirmed the presence of the monoterpenoid moiety, which showed a multiplet at $^{\delta}3.66$. The ^{13}C NMR spectrum of compound **2** in Table 1 confirmed the presence of 23 carbon atoms. The carbon signals were assigned to their locations in compound **2** with data from the DEPT, ^{1}H-^{1}H COSY, HMQC and HMBC spectra. Compound **2** was characterized as murrayazolinol [26].

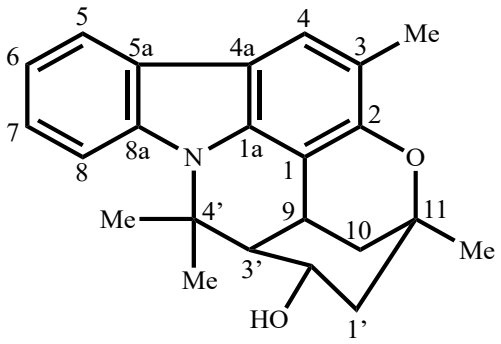

Figure 3(a). Compound 2, murrayazolinol.

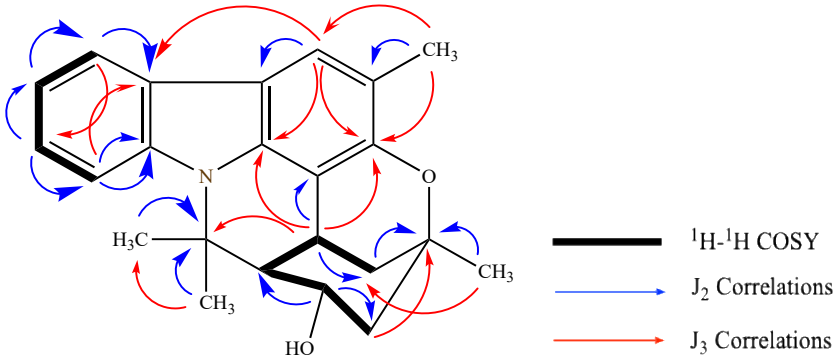

Figure 3(b). COSY and HMBC correlations for compound 2.

Table 2. Cytotoxic Activity of Isolated Compounds from *M. koenigii* in CD_{50} values (µg/mL).

Compound	CD_{50} values (µg/mL)		
	HL-60	HeLa	NIH/3T3
Mahanimbine (**1**)	38.2 ± 6.2	21.4 ± 3.0	NA
Murrayazolinol (**2**)	42.2 ± 3.6	46.0 ± 4.4	> 60
Vincristine (VCR)	< 1.0	< 1.0	> 60

*Each value is expressed as the mean ± relative standard deviation of biological triplicates (n = 3). For the last column, values followed by different letters are significantly different at $p \leq 0.05$ as measured by the one-way ANOVA and Tukey's post-hoc test.

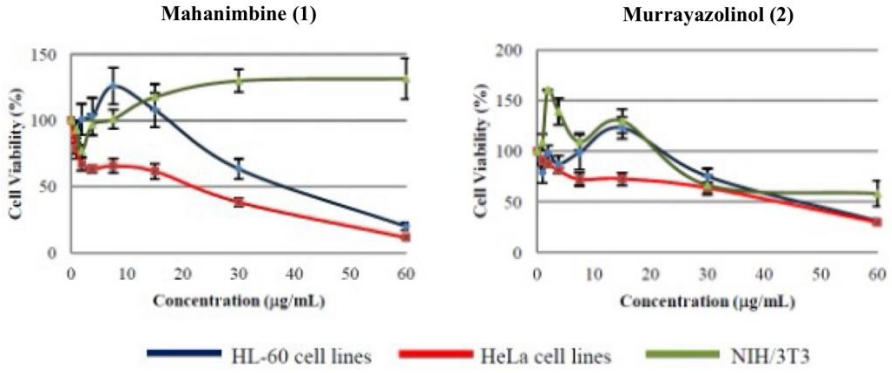

Figure 4: Dose-response curves showing the cytotoxic activities of isolated compounds from *M. koenigii*.

Cytotoxic Activity

Further tests were conducted on compounds **1** and **2** to examine their cytotoxic potential using MTT assays against three targeted cancer lines, HL-60, HeLa and NIH/3T3.

The results show that the compounds were not active against normal cells (NIH/3T3), due to the linear pattern shown for the percentage of viability of the NIH/3T3 cell line with concentrations of 10 to 60 µg/mL for both compounds and the positive control.

Both compounds inhibited the growth of HL-60 and HeLa cell lines when compared to the positive control (VCR, $CD_{50} < 1.0$ µg/mL), but only at moderate to weak activity, whereby mahanimbine (**1**) had moderate activity towards HeLa cell lines. The weak activities of both compounds may be due to **1**, a pyranocarbazole with an electron-donating group at position 3 of the carbazole nucleus, while **2** was a

cyclic monoterpenoid pyranocarbazole with a cyclic ring system.

The cytotoxic activity assessment of these two compounds provides valuable insights into their potential as lead compounds for anticancer drug development. Although the observed activity was moderate, it establishes a clear foundation for further optimization through structural modification or combination studies. In conclusion, the results confirmed the pharmacological value of *M. koenigii* and promote its relevance to natural product therapeutic research. It also highlights the importance of the continued exploration of other compounds or carbazole alkaloids present in this plant.

CONCLUSION

This study aimed to isolate and evaluate cytotoxic carbazole alkaloids from *M. koenigii*, addressing the limited data available based on isolated compounds from this medicinal plant. Through hexane fractionation, mahanimbine (**1**) and murrayazolinol (**2**) were successfully isolated. Both compounds demonstrated weak to moderate cytotoxic activities towards the HL-60 and HeLa cell lines, indicating that they possessed some baseline cytotoxic potential but were not as intrinsically potent in their native form. These findings provide a clear foundation for further mechanistic studies and structure-activity relationship (SAR) development, since carbazole alkaloids are known to show their enhanced biological activity following targeted substitution or semi-synthetic modifications. Overall, this study clarified the contribution of individual isolated compounds to its cytotoxic effects and serves as a chemical starting point rather than identifying finalized therapeutic agents.

Future work will be focused on isolating more carbazole alkaloids from DCM and MeOH extracts. Cytotoxic activity will also have to be evaluated for newly isolated compounds to continue the exploration of the cytotoxic effects of *M. koenigii*.

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