

Phytochemical Profiling of Methanolic Leaf Extracts of Vietnamese *Murraya koenigii*, a Highly Valued Traditional Medicine, using UHPLC-Q-TOF-MS/MS

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ABSTRACT

Murraya koenigii (L.) Spreng (curry tree) is a tropical to subtropical tree originally from South Asia. The leaves of curry trees are an essential ingredient of Indian cuisine and are used as a flavoring in many dishes. The leaves are also widely used in Indian traditional medicine and possess the therapeutic potential due to the high content of biologically active compounds, including but not limited to flavonoids, steroids, alkaloids, phenolic compounds, etc. These compounds exhibit antioxidant, hypoglycemic, anticancer, antibacterial, and some other curative properties. It is well known that phytochemical composition is dependent on plant habitat. *M. koenigii* grows in Vietnam, where it is called “cà ri,” and is used in its cuisine. However, there is no data about the phytochemical profile of this plant from Vietnam. In this study, the extract of curry tree leaves was studied. For long-term methanol extraction, a maceration technique for 7 days was used. The extract was analyzed by UV-spectroscopy and ultra-high-performance liquid chromatography-quadrupole time-of-flight tandem mass spectrometry (UHPLC-Q-TOF-MS/MS). In the end, about two hundred matches with known substances including flavonoids, alkaloids, steroids, terpenoids, phenolic, and other compounds were identified in *M. koenigii* extract. In general, the phytochemical composition of Vietnamese *M. koenigii* resembles those of plants from other countries. However, several new compounds of unknown structure were found. Therefore, a furthermore detailed study of *M. koenigii* and other *Murraya* species native to Vietnam is needed.

Keywords: Curry tree, Phytochemical composition, Maceration, Methanol extraction, Mass-spectrometry

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INTRODUCTION

Murraya koenigii (syn. *Bergera koenigii*) (L.) Spreng (curry tree) (Fig. 1) is a tropical to subtropical plant from the family Rutaceae [1]. In India, where curry tree leaves are widely used, it has many local names, for example in Hindi - karī patī and in Bengal - kari gachha. This plant is known as cà ri, xan tróc, or chùm hôi trắng in Vietnam [2]. In some European countries, this curry tree is referred to as currybaum (German), kerrieboom (Dutch), arbre à curry (French), albero del curry (Italian), and árbol de curry (Spanish).

M. koenigii is one of the plants that people have been using for different purposes. People in India, Indonesia, and Bangladesh have used curry tree leaves as a flavoring spice since ancient times. Curry leaves are most widely used in cooking along the southern and western coasts of India and are an essential component of Indian cuisine. Various parts of the curry tree have medicinal properties and have found use in Indian traditional medicine. Recently, the therapeutic properties of *M. koenigii* have been confirmed by several studies. Thus, the *M. koenigii* root components heal kidney disease and protect the liver [3,4], the bark has shown anti-diabetic effect in rats [5], while the leaves are taken as a stomachache remedy [6]. Different parts of the curry tree, especially its leaves, have been the object of extensive

research to identify active substances and study their biological activity. *M. koenigii* leaves have been found to contain compounds with various biological properties, including antioxidants [7,8], antihyperglycemic [9], anticancer [10], and antimicrobial [11,12]. The found compounds include steroids, terpenoids, saponins, carbazoles, and flavonoids [13-15]. Thus, the study of n-hexane extract of curry tree leaves from Indonesia [16] revealed that these leaves contained active terpenoid and steroid compounds. *M. koenigii* leaves have also been found to be cytotoxic to the HeLa cell line [16]. A study on a methanolic extract of curry tree leaves demonstrated that it had anti-inflammatory activity both in *in vitro* and *in vivo* models [17]. Methanol extract of *M. koenigii* leaves from India was studied by Gas Chromatography–Mass Spectrometry (GC-MS) [18]. This extract was found to contain 9,12-octadecadienoic acid (0.6%), 1,2-benzene dicarboxylic acid diisooctyl ester (2.55%), 1,2-ethanediol monoacetate (2.79%), c-himachalene (2.88%), isolongifolene (3.86%), ethyl α -D-glucopyranoside (13.36%) and 1-methyl-pyrrolidine-2-carboxylic acid (69.00%) [18]. The 9,12-octadecadienoic acid demonstrated potent antimicrobial, strong antioxidant, and anti-inflammatory activities [18]. Recently, new carbazole alkaloids mumunin and girinimbin were identified in methanol extract of curry tree stem bark from West Bengal (India) [14].

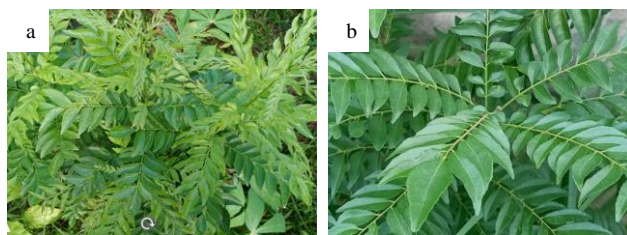


Fig. 1 Vietnamese *M. koenigii* (L.) Spreng. a) young plant. b) Leaves.

Differences in phytochemical composition are well documented for the same plant species from different geographical regions [19,20]. Although curry leaves are used in Vietnamese cuisine [21,22], there is no data about the phytochemical composition of *M. koenigii* from this region. It should be mentioned that several methods are applied for phytochemical analysis of plant extracts including traditional chemical methods [16] and GC-MS [18]. Recently, ultra-high-performance liquid chromatography-quadrupole time-of-flight tandem mass spectrometry (UHPLC-Q-TOF-MS/MS) has been introduced for this purpose. Compared with analysis by GC-MS, UHPLC-Q-TOF-MS/MS is more efficient and highly selective in obtaining ion fragments of analytes for determining their chemical structures. It is also very sensitive and requires a small quantity of samples [23]. In this work, we used UV spectroscopy and UHPLC-Q-TOF-MS/MS for the investigation of the long-term methanol extract of curry tree leaves harvested in Vietnam. As a result, about two hundred matches with known substances including flavonoids, alkaloids, steroids, terpenoids, phenolic, and other compounds were identified in *M. koenigii* extract. Several new compounds of unknown structure were found as well. Further study is needed for their detailed characterization. This is the first so comprehensive phytochemical analysis of *M. koenigii* native to Asia in general and Vietnam in particular.

MATERIALS AND METHODS

Materials

Analytical grade formic acid ($\geq 98\%$), HPLC grade methanol, HPLC grade acetonitrile, and deionized water for HPLC were obtained from Scharlau (Barcelona, Spain). The powder of *M. koenigii* dried leaves was purchased from DORI LLC (Ly Son, Quang Ngai province, Vietnam). The leaves were collected from plants growing on Ly Son Island, Quang Ngai province, Vietnam.

Sample Preparation

100 mL of methanol was added to 10.0 g of *M. koenigii* leaves powder, and the mixture was allowed to stay for 7 days protected from sunlight or in a dark place to maintain a stable temperature and prevent the extracted compounds from degradation. After that, to obtain the methanolic extract of *M. koenigii* leaves, the mixture was centrifuged at 10000 rpm for 10 min (24 °C). Before UHPLC-Q-TOF-MS/MS analysis, the obtained extract was filtrated through a 0.45- μ m membrane filter.

Spectral Analysis

UV spectra of methanol extract were registered using Evolution 60S UV-Visible Spectrophotometer (Thermo Electron Scientific Instruments LLC, Madison, WI, USA) at College of Natural Sciences, Can Tho University, Can Tho City, Viet Nam. The spectra were recorded using a scan interval of 1 nm in the range from 200 to 400 nm.

Analysis of the Chemical Composition of *M. koenigii* by UHPLC-Q-TOF/MS

ExionLC™ UHPLC system (AB SCIEX, USA) was used for the UHPLC analysis. The chromatographic separation of the extract was carried out at room temperature on a Hypersil GOLD C18 column (150×2.1 mm, 3 μ m) (Thermo Fisher Scientific, USA). The mobile phase consisted of water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B); the elution was done with a linear gradient: 0–4 min, 2–20% B; 4–30 min, 20–68% B; 30–32 min, 68–98% B; 32–40 min, 98% B at a flow rate of 0.4 mL/min. The sample was injected in a volume of 5.0 μ L [24]. AB SCIEX X500R QTOF mass spectrometer (AB SCIEX, USA) coupled to the UPLC via an electrospray ionization (ESI) interface was used to acquire high-resolution MS and MS/MS spectra in both negative and positive ion modes. The operating parameters were optimized as follows: the heater gas (GS 2), 45 psi nebulizer gas (GS 1), 45 psi; curtain gas, 30 psi; ion source temperature, 500 C. The mass ranges for the TOF MS/MS and TOF MS were set at m/z 50–1500 and 70–2000, respectively. For the positive ion mode, the ion spray voltage was set at 5.5 kV, the declustering potential (DP) was 80 V, the collision energy (CE) was 20 eV, and the collision energy spread (CES) was 10 eV [24]. For the negative ion mode, the ion spray voltage was set at –4.5 kV, the DP was –70 V, the CE was performed at –20 eV, and the CES was 10 eV. To record and process the raw data, SCIEX OS software version 1.2.0.4122 (AB SCIEX, USA) was used.

RESULTS

To extract phytochemicals from dried *M. koenigii* leaves collected in the Vietnamese province Quang Ngai, methanol was used as a solvent. This solvent dissolves both polar (alkaloids etc.) and non-polar (terpenoids, fatty acids, etc.) plant metabolites well. A maceration technique, a very soft extraction method, was applied to preserve maximally natural compounds. To increase the yield of extracted substances, the process continued for 7 days. The extract obtained was analyzed by UV spectroscopy (Fig. 2). The spectrum displays three strong adsorption maxima at 205, 240, and 290 nm. Less pronounced maxima can be seen at 330 and 360 nm. A quite intense absorption near 205 nm and a less intensive absorption in the 255 to 275 nm range is typical for aromatic compounds. The presence of electron-donating substituents in the benzene ring or conjugation of rings results in the bathochromic shift in the adsorption maximum. So, the adsorption maxima at 205 and 290 nm suggest the presence of substituted and/or condensed aromatic compounds in the extract.

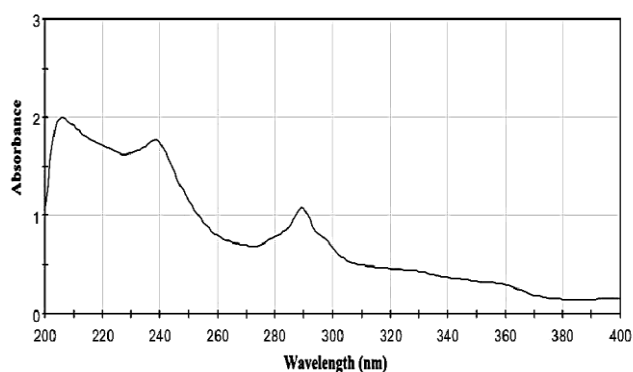


Fig. 2 UV spectrum of the methanol extract of the *M. koenigii* leaves.

The maximum at 240 nm may correspond to conjugated double bonds of different natures. The maxima at 330 and 360 nm may

evidence the presence of compounds with more than two conjugated aromatic rings. A non-targeted analysis was done by UHPLC-Q-TOF-MS/MS to annotate the chemical compounds in the methanolic extract of curry tree leaves. For the analysis, both negative and positive ionization modes were used. The obtained chromatographic profiles are shown in Figure 3. Interestingly, the positive ionization mode (Fig. 3A) in which only a few intensive signals were observed in the area corresponding to the elution of hydrophobic compounds at the end of the acetonitrile gradient (from 18 to 21 minutes) revealed fewer intensive signals than the negative mode (Fig. 3B). The processing of raw data revealed several hundreds of compounds with molecular masses in the range from 70 to above 1000 Da (Table S1). In positive mode, for the compounds that give the most intense signals, 13 matches (Table 1) with known compounds were found and in negative mode 19 matches (Table 2), i.e., a total of 32 matches with known secondary metabolites were tentatively identified. 296.1657; 330.1877 and 627.3245), respectively, could not be identified (Table 3). These may be new compounds that have not been identified in plants so far. Their characteristics represent a task for further research. It should be noted that, along with many identified substances, six compounds with the precursor mass ions $[M - H]^-$ at m/z (265.1062; 220.1483; 358.1823; 294.1508; 328.1118 and 625.3057) and $[M + H]^+$ at m/z (267.1220; 222.0934; 360.1975;

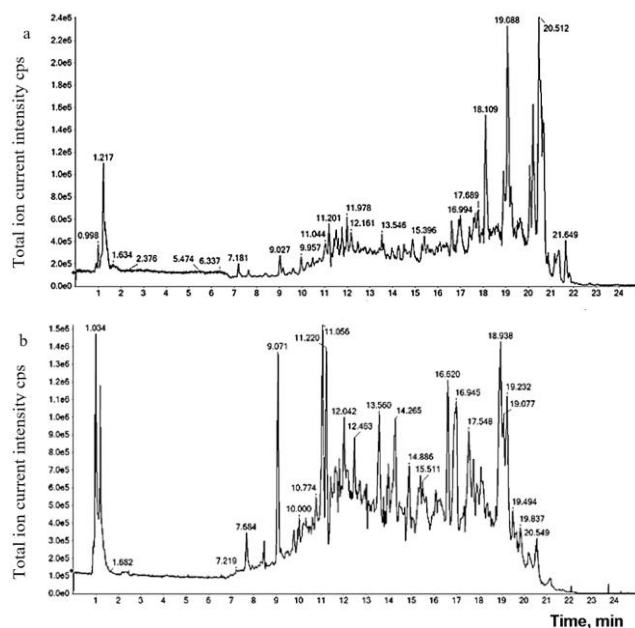


Fig. 3 Chromatographic profiles of the methanol extract of *M. koenigii* leaves were obtained using UHPLC-Q-TOF-MS/MS in both positive (a) and negative (b) ionization modes. The ordinates show the total ion current (TIC). A cps is a count per second, that is the number of ions that hit the detector per second.

Table 1 Compounds identified in the methanolic extract of *M. koenigii* leaves by UHPLC-Q-TOF-MS/MS in positive ionization mode

No.	RT ¹ (min)	Experimental mass (m/z) [M+H] ⁺	Compound name	Molecular formula	Monoisotopic mass	Ref.
1	1.26	130.0853	N-Methyl proline	C ₆ H ₁₁ NO ₂	129.0789	[27]
2	7.18	195.1142	Ferulic acid	C ₁₀ H ₁₀ O ₄	194.0579	[27]
3	9.91	163.0406	Umbelliferone	C ₉ H ₆ O ₃	162.0316	[26]
4	11.05	163.1496	1,7-Dimethyl-1,3,7-cyclodecatriene	C ₁₂ H ₁₈	162.1408	[26]
5	11.22	303.0515	Quercetin	C ₁₅ H ₁₀ O ₇	302.0426	[25-27]
6	12.17	531.8691	D-α-Tocopherol succinate	C ₃₃ H ₅₄ O ₅	530.3971	[26]
7	13.54	275.2025	Toddalenone	C ₁₅ H ₁₄ O ₅	274.0841	[27]
8	15.40	278.1562	Murrastinine C	C ₁₈ H ₁₅ NO ₂	277.1102	[27]
9	16.98	277.2170	Stearidonic acid	C ₁₈ H ₂₈ O ₂	276.2089	[27]
10	17.62	431.2347	α-Tocopherol	C ₂₉ H ₅₀ O ₂	430.3810	[27, 28]
11	18.17	264.1398	Girinimbine	C ₁₈ H ₁₇ NO	263.1310	[25, 27, 28]
12	19.09	348.1971	Cohumulone	C ₂₃ H ₂₅ NO ₂	347.1885	[26]
13	20.55	332.2024	Mahanimbidine	C ₂₃ H ₂₅ NO	331.1936	[27]

¹Retention time

Table 2 Compounds identified in the methanolic extract of *M. koenigii* leaves by UHPLC-Q-TOF-MS/MS in negative ionization mode

No	RT ¹ (min)	Experimental mass (m/z) [M-H] ⁻	Compound name	Molecular formula	Monoisotopic mass [39]	Ref.
1	1.07	179.0583	Caffeic acid	C ₉ H ₈ O ₄	180.0422	[29]
2	7.69	387.1193	Secologanin	C ₁₇ H ₂₄ O ₁₀	388.1369	[26]
3	9.06	325.1276	p-Coumaric acid hexoside	C ₁₅ H ₁₈ O ₈	326.1001	[29]
4	10.01	179.0571	5-Methylthioribose	C ₆ H ₁₂ O ₄ S	180.0456	[26]
5	10.76	595.1300	Quercetin 3-O-sambubioside	C ₂₆ H ₂₈ O ₁₆	596.1377	[26]
6	11.11	327.0885	(-)-Rhododendrin	C ₁₆ H ₂₄ O ₇	328.1522	[26]
7	11.23	463.0878	Isoquercetin	C ₂₁ H ₂₀ O ₁₂	464.0954	[26]
8	12.18	405.1201	Morroneiside	C ₁₇ H ₂₆ O ₁₁	406.1475	[26]
9	12.47	226.0517	Mukoline	C ₁₄ H ₁₃ NO ₂	227.0946	[25]
10	13.55	327.2176	(-)-Rhododendrin	C ₁₆ H ₂₄ O ₇	328.1522	[26]
11	14.37	210.0574	2-Methoxy-3-methylcarbazole	C ₁₄ H ₁₃ NO	211.0997	[26]
12	14.86	307.1919	Inulicin	C ₁₇ H ₂₄ O ₅	308.1623	[26]
13	15.50	221.1557	Selin-11-en-4-ol	C ₁₅ H ₂₆ O	222.1983	[30]
14	16.56	311.2239	Octadecenedioic acid	C ₁₈ H ₃₂ O ₄	312.2300	[26]
15	16.95	315.2552	Geranyl glucoside	C ₁₆ H ₂₈ O ₆	316.1885	[26]
16	17.55	277.2184	Longistylin C	C ₂₀ H ₂₂ O	278.1670	[26]
17	19.25	362.2134	Murrayanol	C ₂₄ H ₂₉ NO ₂	363.2198	[25, 26]
18	19.85	339.2156	Behenic acid	C ₂₂ H ₄₄ O ₂	340.3341	[26]
19	20.55	330.1869	Mahanimbine	C ₂₃ H ₂₅ NO	331.1936	[25, 26]

¹Retention time

Table 3 Unidentified compounds detected by UHPLC-Q-TOF-MS/MS in positive and negative ionization modes in the methanol extract of *M. koenigii* leaves.

No.	RT ¹ (min)	Experimental mass (m/z) [M-H] ⁻	RT (min)	Experimental mass (m/z) [M+H] ⁺
1	12.47	265.1062	12.47	267.122
2	15.5	220.1483	15.4	222.0934
3	16.96	358.1823	16.96	360.1975
4	17.66	294.1508	17.67	296.1657
5	18.1	328.1118	17.95	330.1877
6	20.32	625.3057	19.95	627.3245

¹Retention time

DISCUSSION

Earlier studies have shown that the most represented compounds in *M. koenigii* extracts are flavonoids, terpenoids, alkaloids, and polyphenols [25-30]. The compounds of all these types were detected in the methanol extract of Vietnamese *M. koenigii*. They were tentatively identified based on the analysis of the m/z value of the molecular ion (precursor ion) of each molecule and its fragments obtained by Q-TOF-MS/MS. The PubChem, Mass bank, and ChemSpider databases, as well as literature data, were used as reference information.

From a therapeutic point of view, the most significant compounds in *M. koenigii* extracts are carbazole alkaloids, which exhibit several medicinal properties [31]. They are the key bioactive constituents of this plant. In this work, several carbazole alkaloids (mahanine, mahanimbine, murrayacine, mukoline, girinimbine, murrayanol and many others) were found. Similar compounds were found in several previous studies, for example, in the hydroalcoholic extract of the curry tree leaves [32]. It was shown that some *M. koenigii* carbazole alkaloids were cytotoxic to cancer cell lines. Thus, mahanimbine manifested cytotoxicity in the human breast cancer MCF-7 cell line [33], and girinimbine effectively induced programmed cell death in the human hepatocellular carcinoma cell line HepG2 [34]. Interestingly, in our work, various carbazole alkaloids were observed in both acquisition modes in several places of chromatographic profile. This suggests that they form non-covalent complexes with other substances, and these complexes are partially stable under the HPLC conditions used.

Another important component of *M. koenigii* extracts is flavonoids. The antioxidant activity of flavonoids is their main biological activity [35]. The antioxidant activity of flavonoids may prevent damage by free radicals in several ways, by scavenging reactive oxygen species (ROS). Among the major flavonoids found in our work were quercetin, isoquercetin, and some others as well as their glycoside derivatives like quercetin 3-O-sambubioside. Characterization of flavonoids in *M. koenigii* was reported earlier in several studies, for example, several flavonoids were discovered in the ultrasound-assisted extract of curry tree leaves [36].

The high content of terpenoids is usually found in extracts of *M. koenigii* green leaves [37]. Nevertheless, several representatives of this class of compounds, including juniper camphor, inulicin, geranyl glucoside, and some others, were found in our study of the powder of dry *M. koenigii* leaves.

In addition to these widely represented classes of phytochemicals, our work revealed several less common ones. Thus, several coumarins, including 2-hydroxychromone, and umbelliferone were found. Similar compounds were reported for extracts of *M. paniculata* leaves [38]. Furthermore, long-chain fatty acids, including

behenic, and octadecenoic acids were observed. Some amino acids such as N-methyl proline were tentatively identified as well. So, some of the above compounds were earlier identified in *M. koenigii* extracts. Because *M. koenigii* contains a wide range of healing phytochemicals, traditional medicine practitioners use the whole plant or parts of it to treat a variety of conditions.

CONCLUSION

In conclusion, using the method of UHPLC-Q-TOF-MS/MS, the methanol extract of leaves of the curry tree *M. koenigii* grown in Vietnam was analyzed. The compounds identified tentatively included flavonoids, terpenoids, carbazoles, coumarins, alkaloids, and fatty acids, among others. In particular, 19 matches were detected using the negative ionization mode and in 13 the positive ionization mode. Moreover, the structures of six compounds could not be identified, which deserves further detailed investigation. However, this is the first such comprehensive phytochemical analysis of *M. koenigii* growing in Asia in general and Vietnam in particular.

Data Availability Statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Conflict of Interests

The authors declare that they have no competing interests.

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