Scientific Research Journal,
Vol 20, No 2 (2023) 147-160
https://doi.org/10.24191/srj.v20i2.23459

Extraction of Phytochemical from Murraya Koenigii (L.) Spreng Leaves using Maceration Method

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Received: 06 August 2023
Accepted: 08 September 2023
Online First: 21 September 2023

ABSTRACT

Curry leaves, scientifically known as Murraya koenigii (L.) Spreng is employed as a flavoring agent in a wide range of dishes and possesses the potential for treatment due to its content of flavonoid, steroid, and phenolic compounds. These compounds exhibit antioxidant and antibacterial properties. In this work, the extraction of phytochemicals from M. koenigii leaves was satisfactorily performed. The extraction process was conducted using a maceration technique with three different solvents: 95 % ethanol, 99 % acetone, and 96 % methanol individually. The qualitative and quantitative of existing phytochemicals compounds in the M. koenigii leaves were analyzed through phytochemical screening and gas chromatography-mass spectrometry (GCMS). The percentage yield shows 21.42 % of methanolic extracts, 6.50 % of acetone extracts, and 11.66 % of ethanolic extracts. The major compounds present from screening phytochemicals are terpenoid, phenolic, glycoside, and saponin. GC-MS result showed the major compounds present in M. koenigii leaves are terpene, terpenoid, carotenoid and alkaloid.

Keywords: Murraya Koenigii; GC-MS; Maceration; Ethanol; Acetone; Methanol

INTRODUCTION

The curry plant (Murraya koenigii (L.) Spreng), which is native to India and Sri Lanka, is a tropical to subtropical plant of the family Rutaceae. In India, Sri Lanka, and other nearby nations, its leaves are utilized in a variety of recipes. The leaves, which are frequently used in curries, are commonly referred to as "curry leaves," but in most Indian languages, they are also known as "sweet neem leaves". Curry leaves have long been valued for their distinct flavor and culinary applications, but they also have a variety of health benefits that make them highly desirable [1]. M. koenigii is widely utilized in the Ayurvedic medicinal tradition as a significant herb of Indian origin with medicinal properties. From a previous study, the leaves of M. koenigii contain a variety of bioactive compounds and carbazole alkaloids, with total phenols, flavonoids, carotenoids, and acridine carbazole being the most noteworthy among them [2]. These plant secondary metabolites all have physiological effects on the body at various phases of development and keep the body disease-free [3]. Other metabolites, such as steroids and triterpene, contribute to antibacterial and antifungal properties. Thanks to the presence of these metabolites, curry leaves have antibacterial and antioxidant properties [4].

The maceration method was employed in this work in order to extract M. koenigii sample materials. This method is popular because it is straightforward, inexpensive, and may be used at room temperature. Previous study reports that maceration was an efficient and straightforward method for extracting bioactive compounds [5]. Three different solvents including ethanol, methanol, and acetone were employed in this study. Ethanol has been acknowledged as an effective solvent for extracting polyphenols and is safe for intake by humans [6]. While aqueous acetone is suitable for extracting higher molecular weight flavanols, methanol is generally found to be more successful in removing lower molecular weight polyphenols [7]. This research aims to extract and determine the phytochemical compound that contains in the M. koenigii leaves using phytochemical screening and gas chromatography–mass spectrometry (GC-MS).

EXPERIMENTAL DETAILS

M. koenigii leaves were bought from a local grocery in Bukit Gambir, Muar, Johor, Malaysia. The extraction method used was the maceration technique and the equipment used was a centrifuge (MPW MED. INSTRUMENTS, MPW-352R, Poland), GC-MS (AGILENT, Agilent 7000C Triple Quadrupole GC/MS, California).

Material Specifications

The materials that used in this experiment were 96 % methanol (HmbG), 95 % ethanol (HmbG), 99 % acetone (R&M Chemicals), 98 % sodium hydroxide (Emory), chloroform, concentrated sulphuric acid (H_2SO_4), potassium iodide (99.9 %) (R&M Chemicals), lead acetate solution, iron(III) chloride hexahydrate (Supelco), and distilled water.

Pre-processing of M. Koenigii Leaves

About 100 g of M. koenigii leaves were weighed. It was washed under tap water to eliminate the adhered dust particles and dried at a temperature of 60 °C for 4 h. After drying, it was ground and then blended to be the powder for extraction [8].

Preparation of M. Koenigii Leaves Extract

The preparation of M. koenigii extract was adopted from Sablania *et al.* [8] with slight modification. In brief, 5 g of M. koenigii leaves powder was added to 100 mL of methanol, and the sample was kept for 56 h away from sunlight or a dark place. This helps in maintaining a stable temperature and prevents the degradation of the extracted compounds. Next, the sample was centrifuged at a speed of 10000 rpm and 24 ℃ for 10 min to obtain the aqueous extract of M. koenigii leaves and it was filtered using filter paper to separate the fine solid particles from the liquid and the filtrates were completely dried off. The steps were repeated using ethanol and acetone. The extracts were analysed for their phytochemical properties.

Percentage Yield

The percentage yield was calculated as shown in Equation (1) [9]:

$$\% \text{ Yield} = \left(\frac{\text{weight of } M.koenigii \text{ leaves extract}}{\text{weight of fresh } M.koenigii \text{ leaves}}\right) \text{x } 100\% \quad (1)$$

Phytochemical Screening

The phytochemical screening was conducted to detect the specific species-based compounds and characteristics that are present in M. koenigii leaves. The conducted test was specific to the terpenoid, phytosterol, alkaloid, tannin, phenolic, and glycoside [10]. Each test's method is described in the following.

Terpenoid (Salkowski's Test)

Five mL of extracted M. koenigii leaves were mixed in two mL of chloroform. Three mL of concentrated sulphuric acid was added to the solution carefully. The reddish-brown color of the interface indicated the positive result of terpenoids.

Phytosterol (Salkowski's Test)

Two mL of extracted M. koenigii leaves was dissolved in five mL of chloroform, the solution was mixed until it dispersed well. A few drops of concentrated sulphuric acid were added to the solution. The formation of a brown ring indicated the positive result of phytosterols.

Alkaloid (Dragendroff Test)

Two mL of potassium bismuth iodide solution (Dragendroff reagent) was added to five mL of extracted M. koenigii leaves. The formation of an orange-red precipitate indicates the presence of alkaloids.

Tannin (Lead acetate test)

In a test tube containing about five mL of aqueous extract, a few drops of 1 % solution of lead acetate were added. The formation of a yellow or red precipitate indicated the presence of tannins.

Phenolic (Ferric Chloride Test)

One mL of extracted solution of the sample, two mL of distilled water followed by a few drops of 10 % aqueous ferric chloride solution were added. The formation of bluish-black color indicated the presence of phenols.

Glycoside

A small amount of extracted solution of samples was dissolved in one mL of water and then aqueous sodium hydroxide was added. The formation of a yellow colour indicated the presence of glycosides.

Saponin (Froth Test)

The extract was diluted with 20 mL of distilled water and was shaken in a test tube for 15 min. The formation of foam indicated the presence of saponin.

Flavonoid (Ferric Chloride Test)

Two mL of an extracted solution of the sample, followed by a few drops of 10 % aqueous ferric chloride solution were added. The formation of intense green color indicated the presence of flavonoid.

Gas Chromatography-Mass Spectrometry Analysis

Gas Chromatography-Mass Spectrometry (GC-MS, GC Agilent® 7890B, MS Agilent® 5977B, Agilent Technologies, Santa Clara, CA, USA) was utilised to confirm the chemical constituents of methanolic extract leaves of M. koenigii. Head column pressures for the HP-5MS (30 m x 0.25 mm x 0.25 μm film thickness) panel were 70 kPa. GC-MS has acquired on the

following conditions: Carrier gas He; flow rate 1.0 mL/min; split 1:100; injection volume 1.0 μL; injection temperature 280 °C; oven temperature progress included an initial hold at 70 °C for 2 min, then increased 7 °C/min to 320 °C hold for 1 min. The temperature of ion sources was maintained at 250 °C. The mass spectrum was obtained by electron ionization at 70 eV, and the detector operated in scan mode 30 to 500 Da atomic units. Chemical constituents of methanolic extract leaves of M. koenigii were identified by comparing retention indices with mass spectral database libraries National Institute of Standards and Technology (NIST). The chromatograms from GC-MS captured existing sample constituents in terms of peak area count.

RESULT AND DISCUSSION

Maceration Extraction

The extracted leaves of M. koenigii undergo identification to determine the phytochemical compounds. Figure 1 shows the extraction of M. koenigii leaves from three different solvent which is methanol, ethanol, and acetone respectively. Table 1 shows the colour observation of the extraction of M. koenigii leaves. Based on the results (Table 1), the extraction of M. koenigii with methanol shows the highest percentage yield of crude extract which is 21.42 %, therefore leading to identify in-depth of the phytochemical compounds in this herbal plant. A similar result was found in the previous study [11], which methanol was used for the extraction of Severinia buxifolia and resulted in the highest extraction yield followed by ethanol and acetone. Methanolic extract and ethanolic extract had greater extraction yields than acetone extracts, showing that highly polar solvents had better extraction efficiency [11]. It proved that the solvent and sample composition has been determined to be the most significant variables under the same extraction time and temperature conditions. This study reveals that the yield percentage of methanol extracts (21.42 %) exceeds the percentages for ethanol extracts (11.66 %) and acetone extracts (6.50 %), as indicated in Table 1. Due to the higher percentage yield, methanolic extract of leaves of M. koenigii was selected for the phytochemical screening for identifying the types of phytochemicals present.

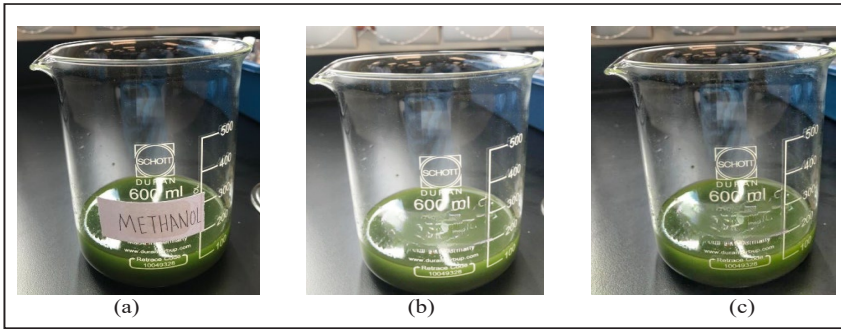

Figure 1: The extraction of M. koenigii leaves from (a) Methanol, (b) Ethanol and (c) Acetone

Table 1: Solvent extraction of M. koenigii leaves

Type of solvent	Color	Percentage of yield (%)
Methanol	Dark green residue	21.42
Ethanol	Dark green residue	11.66
Acetone	Dark green residue	6.50

Phytochemical Screening

Figure 2 and Table 2 show the results of phytochemical screening for terpenoid, phytosterol, alkaloid, tannin, phenolic, and glycoside. Terpenoid, phytosterol, phenolic, glycoside and saponin are the most critical found in this plant. Terpenoid shows a positive result with the formation of a radish-brown colour [12], and phytosterol also shows a positive result with the formation of a brown ring [13]. The same goes well for phenolic, glycoside, and saponin, which showed positive results in the formation of bluish-black colour [10], yellow colour [10], and foam [12], respectively. However, alkaloids, tannins, and flavonoids did not show any preferable results in the formation of orange-red precipitate [14], yellow or red precipitate [15] and intense green colour [15] respectively. In short, M. koenigii contains terpenoid, phytosterol, phenolic, glycoside and saponin.

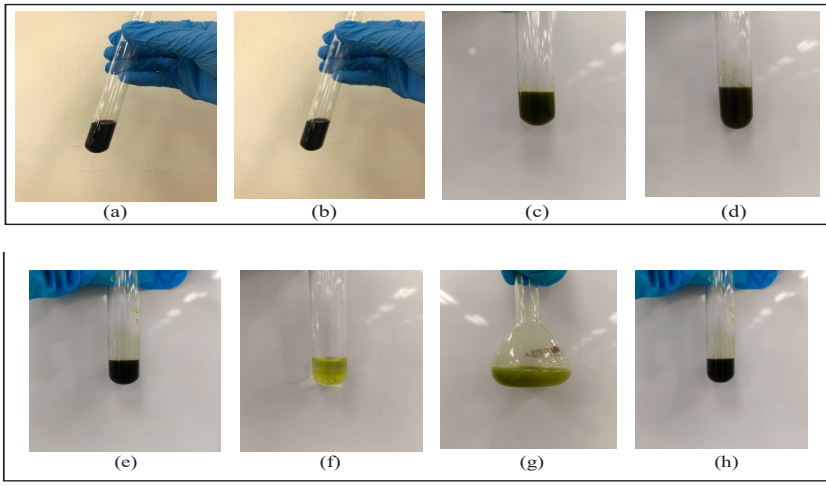

Figure 2: Detection of Phytochemicals (a) Terpenoid, (b) Phytosterol, (c) Alkaloid, (d) Tannin, (e) Phenolic, (f) Glycoside, (g) Saponin, (h) Flavonoid.

Table 2: The result of phytochemical screening of leaves of Murraya koenigii (L.) Spreng

Phytochemical constituents	Result
Terpenoid	+
Phytosterol	+
Alkaloid	-
Tannin	-
Phenolic	+
Glycoside	+
Saponin	+
Flavonoid	-

Footnotes: "+" = presence and "-" = absence

Gas Chromatography-Mass Spectrometry (GC-MS)

Table 3 tabulates the list of compounds found in the Methanolic extract of M. koenigii leaves from the GC-MS determination. There are 18 chemicals that are successfully identified in the present study. Table 3 shows the list of compounds based on their retention time, molecular weight, and chemical formula. A similar result was found from the

previous study [16], which are Caryophyllene, Phytol, 9-Octadecenoic acid (Z)-,2,3-dihydroxy propyl ester, and Mahanimbine. The retention time of the targeted compound is (7.715, 16.619, 28.382 , and 30.260) min respectively. The other existing compound such 9,12-Octadecadienoyl chloride, (Z, Z)-,1-Methyl-pyrrolidine-2-carboxylic acid, Humulene, 1,4-Dimethyl-7-(prop-1-en02-yl) decahydroazulen-4-ol, 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester, Oleic Acid, Glycidyl (Z)-9-Heptadecanoate, i-Propyl 11,12-methylene-ocatadecanoate, 5,16-Heptadecadiene-1,2,4-triol, triacetate, Butyl 9-tetradecenoate and 9-Octadecenoic acid (Z)-oxiranylmethyl ester are the compounds that not related with any phytochemical activities in M. koenigii leaves.

The relation between the test of phytochemical (Figure 2 and Table 2) can be related to the GC-MS result (Table 3). Some of the phytochemical compounds are Caryophyllene, Phytol, 9-Octadecenoic acid (Z)-, 2,3-dihydroxy propyl ester, and Mahanimbine. Caryophyllene is bicyclic sesquiterpenes. It is the terpenoid phytochemical that was present during the phytochemical screening test [17]. The compounds of Phytol, 9-Octadecenoic acid (Z)-, 2,3-dihydroxy propyl ester, and Mahanimbine are confirmed detected by utilising GC-MS analysis, instead of the experimental phytochemical screening. Phytol is diterpene alcohol is the terpene phytochemical [18], 9-Octadecenoic acid (Z)-, 2,3-dihydroxy propyl ester is the zeaxanthin of the carotenoid phytochemical [19]. Additionally, Mahanimbine is the carbazole alkaloid which is the main compound in M. koenigii leaves successfully determined its presence based on GC-MS analysis. This similar observation was supported by a previous study [20], in which Mahanimbine successfully determined its presence in these species which showed its alkaloid compounds.

Table 3: GC-MS chromatogram of methanolic extract of M. koenigii leaves

No.	Name of Compound	Formula	Molecular Weight (g/mol)	Retention Time (Min)
1.	9,12-Octadecadienoyl chloride, (Z, Z)-	$C_{18}H_{31}C_{lO}$	298	3.129
2.	1-Methyl-pyrrolidine-2-carboxylic acid	$C_6H_{11}NO_2$	129	4.649
3.	1-Methyl-pyrrolidine-2-carboxylic acid	$C_6H_{11}NO_2$	129	4.727
4.	Caryophyllene	$C_{15}H_{24}$	204	7.715
5.	Humulene	$C_{15}H_{24}$	204	8.151
6.	1,4-Dimethyl-7-(prop-1-en02-yl) decahydroazulen-4-ol	$C_{15}H_{26}O$	222	8.561
7.	1,4-Dimethyl-7-(prop-1-en02-yl) decahydroazulen-4-ol	$C_{15}H_{26}O$	222	8.660
8.	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{40}O_4$	356	13.143
9.	Phytol	$C_{20}H_{40}O$	296	16.619
10.	Oleic Acid	$C_{18}H_{134}O_2$	261	19.182
11.	Glycidyl (Z)-9-Heptadecanoate	$C_{20}H_{36}O_3$	324	24.236
12.	i-Propyl 11,12-methylene-ocatadecanoate	$C_{22}H_{42}O_2$	338	27.043
13.	9-Octadecenoic acid (Z)-,2,3-dihydroxypropyl ester	$C_{21}H_{40}O_4$	356	28.382
14.	Mahanimbine	$C_{23}H_{25}NO$	331	30.260
15.	5,16-Heptadecadiene-1,2,4-triol, triacetate	$C_{23}H_{38}O_6$	395	31.308
16.	Butyl 9-tetradecenoate	$C_{18}H_{34}O_2$	344	32.242
17.	9-Octadecenoic acid (Z)-oxiranylmethyl ester	$C_{21}H_{38}O_3$	338	34.232
18.	9-Octadecenoic acid (Z)-oxiranylmethyl ester	$C_{21}H_{38}O_3$	338	36.710

CONCLUSION

Methanolic extract of M. koenigii leaves has the highest percentage of yield which is 21.40 % compared to ethanolic (11.66 %) and acetone (6.50 %). The methanolic extract works best in the extraction of M. koenigii leaves because it shows the highest polarity, compared to ethanol and acetone. The methanolic extract was used to determine the phytochemical activities by having the phytochemical screening test and GC-MS analysis. Terpenoids, phytosterols, phenolic, glycoside, and saponin were presented in phytochemical screening tests. The GC-MS results confirmed the presence of phytochemical compounds including Caryophyllene, Phytol, 9-Octadecenoic acid (Z)-, 2,3-dihydroxy propyl ester, and Mahanimbine. The previously mentioned secondary metabolites found in leaves are abundant in phenolic compounds, which have been reported to exhibit significant antibacterial properties. The M. koenigii contains a variety of phytochemical mixtures that allow traditional medical experts to use the entire plant for a variety of diseases.

ACKNOWLEDGMENT

This research was supported by Universiti Tun Hussein Onn Malaysia (UTHM) for the TIER 1 grant (Q513) as financial research support. The authors are also grateful to the management for providing adequate laboratory facilities in the execution of this study.

REFERENCES

[1] R. N. F. Nazurah, M. A. Noranizan, M. A. R. Nor-Khaizura, and Z. A. Nur Hanani, 2022. The potential of chitosan-based film with curry leaf essential oil as natural insect-repellent food packaging, *Food Packaging and Shelf Life*, 34, p. 100993.

[2] S. Batool, R.A. Khera, M. Asif Hanif, M. Adnan Ayub, and S. Memon, 2020. Curry Leaf, *Elsevier eBooks*, pp. 179–190, Jan. 2020.

[3] J. Mittal, 2017. Curry Leaf (Murraya koenigii): A Spice with Medicinal Property, *MOJ Biology and Medicine*, 2(3).

[4] M. Maruthupandi, P. Varatharajan, S. Banu, M. H. Mamat, and N. Vasimalai, 2022. White light emitting diode and anti-counterfeiting applications of microwave assisted synthesized green fluorescent carbon dots derived from waste curry leaves, *Results in Optics*, 8, 100249–100249.

[5] N. Ćujić, K. Šavikin, T. Janković, D. Pljevljakušić, G. Zdunić, and S. Ibrić, 2016. Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique, *Food Chemistry,* 194, 135–142.

[6] N. Q. Nguyen, 2020. The effects of different extraction conditions on the polyphenol, flavonoids components and antioxidant activity of Polyscias fruticosa roots, IOP Conference Series: *Materials Science and Engineering*, 736, p. 022067.

[7] E. Brglez Mojzer, M. Knez Hrnčič, M. Škerget, Ž. Knez, and U. Bren, 2016. Polyphenols: Extraction methods, antioxidative action, bioavailability and anticarcinogenic Effects, *Molecules*, 21(7), p. 901.

[8] V. Sablania, S. J. D. Bosco, and M. Bashir, 2019. Extraction process optimization of Murraya koenigii leaf extracts and antioxidant properties, J*ournal of Food Science and Technology,* 56(12), 5500–5508.

[9] J. Klinger, (21 Apr 2021) "Top 5 Factors That Can Influence Botanical Extraction Yield," Retrieved from www.customprocessingservices.com.

[10] R. Rajvanshi, K. Mittal, 2019. Phytochemical analysis of curry leaves. *International Journal of Science and Research (IJSR)*, 8(9), 366-367.

[11] D.H. Truong, D. H. Nguyen, N. T. A. Ta, A. V. Bui, T. H. Do, and H. C. Nguyen, 2019. Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-

inflammatory activities of severinia buxifolia, *Journal of Food Quality,* 2019, pp. 1–9, 8178294.

[12] S. A. Saidi, T. M. Al-Shaikh, O. A. Alghamdi, and K. Hamden, 2022. Ephedra alata subsp. alenda (Ephedraceae) leaf extracts: phytochemical screening, anti-diabetic, anti-obesity and anti-toxic activities on diabetic-induced liver-kidney-testes toxicities and inhibition of α-amylase and lipase enzymes, *Heliyon*, 8(12), p. e11954.

[13] N. Singh, A. Chatterjee, W. K. Chanu, P. M. Vaishalli, C. B. Singh, and V. A. Nagaraj, 2023. Antimalarial activity of Toona ciliata MJ Roem aqueous methanolic leaf extract and its antioxidant and phytochemical properties, *Journal of Traditional and Complementary Medicine*, in press.

[14] N. Kancherla, A. Dhakshinamoothi, K. Chitra, and R. B. Komaram, 2019. Preliminary analysis of phytoconstituents and evaluation of anthelminthic property of cayratia auriculata (In Vitro), *Maedica,* 14(4), 350–356.

[15] J. M. Dahanayake, P. K. Perera, P. Galappatty, H. D. S. Melshandi Perera, and L. D. A. M. Arawwawala, 2019. Comparative phytochemical analysis and antioxidant activities of tamalakyadi decoction with its modified dosage forms, *Evidence-Based Complementary and Alternative Medicine*, 2019, 1–9.

[16] S. Azhagu Madhavan, S. Vijayakumar, R. Sripriya, S. Rajalakshmi, 2021. Phytochemical screening and GC–MS analysis of bioactive compounds present in ethanolic leaf extract murraya koenigii, *Bulletin of Environment, Pharmacology and Life Sciences,* 10(4), 158-164.

[17] Jelita, B. Wirjosentono, Tamrin, and L. Marpaung, 2019. Phytochemical screening and chemical analysis of ethanol extract of kari leaves (murayya koeginii) using GC-MS method, *Journal of Physics: Conference Series*, 1232, p. 012012.

[18] T. Taj, R. Sultana, H. Shahin, M. Chakraborthy, M. G. Ahmed. 2021. Phytol a phytoconstituent, its chemistry and pharmacological actions,

Gis Science Journal, 8, 395-406.

[19] Y. L. Powder-George and F. K. Mohammed, 2018. GC-MS analysis of the bioactive phytoconstituents of various organic crude extracts from the seed kernels of Manilkara bidentata (balata) collected in Trinidad, W.I., *Natural Product Research*, 32(3).

[20] P. Mondal, J. Natesh, A. Ajees, and S. M. Meeran, 2022. Mahanimbine isolated from murraya koenigii inhibits P-glycoprotein involved in lung cancer chemoresistance, *Bioorganic Chemistry,* 129, p. 106170.