JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

CHEMICAL COMPOSITION ANALYSIS AND EVALUATION OF ANTIOXIDANT, ANTIACETYLCHOLINESTERASE AND CYTOTOXICITY OF *MURRAYA KOENIGII* (L.) SPRENG FRUIT OIL

*Gogul Ramnath Madhusudhanan**[1], *Shathya Jayaraman*[2], *Ramaraj Thirugnanasampandan*[3], *Bhuvaneswari Gunasekaran*[2], *Akila Gopalakrishnan*[2]

Address(es): Gogul Ramnath Madhusudhanan
[1] Department of Biotechnology, Sri Ramakrishna College of Arts & Science, Nava India, Avinashi Road, Coimbatore-641006, Tamil Nadu, India.
[2] Plant Tissue Culture and Bioprospection Laboratory, PG and Research Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore-641029, Tamil Nadu, India.
[3] PG and Research Department of Botany, Kongunadu Arts and Science College, Coimbatore-641029, Tamil Nadu, India.

*Corresponding author: gogul@srcas.ac.in

https://doi.org/10.55251/jmbfs.6135

ARTICLE INFO

Received 8. 5. 2022
Revised 1. 8. 2023
Accepted 2. 8. 2023
Published 1. 12. 2023

Regular article

OPEN ACCESS

ABSTRACT

Plant derived essential oils are easily available source for healthcare purpose and it creates increased attention due to undesirable effects of chemically synthesized drugs. *Murraya koenigii* (L.) Spreng. raw fruit oil was extracted, and its chemical constituents was analyzed. Antioxidant and acetylcholinesterase inhibitory effect was tested under *in vitro*. MTT assay for evaluating cytotoxic effect on HeLa cells was done. β-phellandrene was identified as major compound in the extracted fruit oil. Scavenging of DPPH radical, inhibition of lipid peroxidation and protection of deoxyribose were found to be excellent. A dose dependent antiacetylcholinesterase activity of oil was observed with IC_{50} value as 448.12±0.02µg/mL. A significant growth inhibitory effect of oil on cervical cancer cells (HeLa) was measured with an IC_{50} value of 194.1±0.03µg/mL. Overall, the essential oil extracted from raw fruit of *Murraya koenigii* (L.) Spreng exhibited several biological properties, and it could be considered for plant-based medicine preparations.

Keywords: *Murraya koenigii*, Essential oil, β-phellandrene, Cytotoxicity, Acetylcholinesterase

INTRODUCTION

Murraya koenigii, belongs to Rutaceae is most widely distributed in tropical and subtropical countries. It is commonly represented as curry leaves in India **(Akanksha Rani *et al*., 2017).** It has been used as a traditional medicine for treating rheumatism, traumatic injury and snake bites due to the presence of important bioactive compounds **(Ashokkumar *et al*., 2013)**.
Leaf essential oil of *M. koenigii* contains monoterpenes as predominant constituent which has antioxidant, antibacterial property and also plays an important role in cosmetic industry **(Lal *et al*., 2005; Mini Priya *et al*., 2014)**. Several studies have reported the chemical constituents of essential oil extracted from various parts of *M. koenigii* such as fruit, seed cotyledon, leaf and flower. Very few reports were dealt with the volatile analysis of *M. koenigii* raw fruit but not concerned with its medicinal values **(Mallavarappu *et al*., 2000; Walde *et al*., 2005, 2006)**. According to our knowledge the present work is considered as the first attempt which focused on volatile constituent analysis and biological properties of essential oil extracted from *M. koenigii* fruit.

MATERIALS AND METHODS

Collection of fruit and oil extraction

Raw fruit of *Murraya koenigii* (Linn.) Spreng was collected in the months of June and July from Coimbatore, Tamil Nadu, India. Essential oil extraction was carried out using Clevenger apparatus through hydrodistillation process for 2 hours. Sodium sulphate anhydrous was used for separating oil from water and the purified oil was tightly sealed and stored at 4 ºC for future studies.

GC/GCMS analysis

The extracted essential oil was analyzed by Gas Chromatography. The Perkin Elmer Clarus 680 GC (Waltham, Massachusetts, United States) employed had Elite-5 MS capillary column (5% biphenyl, 95% dimethylpolysiloxane, 30 m × 0.25 mm, 0.25 µm film thickness) and a flame ionization detector. The temperature was set at 260 °C during injection of 1 µL of oil. 10:1 split ratio was maintained. Initial oven temperature was set at 60 °C for 2 min. At the rate of 10 °C min^{-1}, the oven temperature was increased upto 290 °C and maintained for 6 min. Separation of compounds was done using helium gas at a flow rate of 1 mL/min. The percentage of oil constituents were measured by chromatographic peaks.
Gas Chromatography/ Mass Spectrometry analysis of essential oil was done in a Perkin Elmer Clarus 680 GC (Waltham, Massachusetts, United States) with Elite-5 MS (5% biphenyl, 95% dimethylpolysiloxane, 30 m × 0.25 mm, 0.25 µm film thickness) interfaced with Clarus 600 Mass Spectrometer. The conditions used for GC were same as for MS analysis. For mass detector, the temperature for transfer line and ion source temperature was set at 230 °C. The ionization mode electron impact was set at 70 eV. Based on retention indices and mass spectra, the chromatographic peaks were identified and analysed using NIST spectral library **(NIST 2008)**.

Antioxidant assay

Different concentrations of *M. koenigii* fruit oil was prepared using 10% dimethyl sulfoxide (DMSO) and used for antioxidant studies. Butylated hydroxytoluene (BHT) was used as standard.
In vitro antioxidant activity of *M. koenigii* fruit oil was evaluated by scavenging of DPPH **(Mensor *et al*., 2001)** and hydroxyl radicals **(Zhao *et al*., 2006)**, chelation of metal ions **(Singh and Rajini 2004)**, inhibition of linoleic acid peroxidation **(Choi *et al*., 2002)** and prevention of deoxyribose degradation **(Halliwell *et al*., 1987)**. The percentage of inhibition was calculated as follows: % Inhibition = $[(A_B - A_A)/A_B] \times 100$, where A_B, blank absorption, A_A, sample absorption.

Antiacetylcholinesterase activity

According to **Ingkaninan *et al*. (2002)**, the enzymatic activity was measured. The reaction mixture contained 500 µL of 3 mM DTNB, 100 µL of 15 mM AChI, 275 µL of 50mM tris-Hcl buffer (pH 8) and various concentrations of essential oil. 25 µL of buffer was added in the blank and it was replaced by 25 µL of solution containing 0.28 UmL^{-1} of enzyme in the sample added wells. The reaction was observed, and absorption was measured at 405 nm for 5 mins at different time interval. Physostigmine was used as standard.

Cytotoxicity

Cell culture

HeLa cell was purchased from National Centre for Cell Science, Pune. For cell line maintanance, DMEM containing fetal bovine serum (10%), L-glutamine (1%), streptomycin (1%) and penicillin (1%) was used and incubated at 37 ºC in a moistened incubator (Nectarnova, Canada) containing 5% CO_2.

MTT assay

To each well of 96 well plate, $1x10^4$ cells/100 µL medium was added and was incubated for 24 hours. The cells were treated with 25-200 µg/mL of essential oil for 48 hours. After adding 20 µl of MTT (5 mg/mL) in phosphate buffered saline, the plate was kept for incubation at 37 °C for 4hours. Medium was removed and 100 µL of DMSO was added. The plate was kept for incubation at 37 °C for 10 min and absorption was measured at 570 nm **(Lau *et al*., 2004)**. Doxorubicin was used as standard. % of cell viability = ([AB − AA]/AB) x100, where AB, blank absorption, AA, test absorption.

Statistical study

In vitro antioxidant, antiacetylcholinesterase and cytotoxicity data were analyzed using the statistical package for social sciences (16.00, SPSS Inc. Chicago, IL) for IC_{50} calculation.

RESULTS AND DISCUSSION

Essential oil extraction and chemical composition analysis

Hydrodistillation of 100 g of *Murraya koenigii* Spreng raw fruit yielded 250 µL transparent pale yellow oil with strong aromatic smell. A total of three compounds constituting 100% were identified. β-phellandrene, a monoterpene was identified as the major constituent which accounts 92.83%. The oil was mainly comprised of monoterpenes (Table 1).

Table 1 Chemical composition analysis of *Murraya koenigii* fruit essential oil

S.No	RT[a]	RI[b]	Compounds[c]	%[d]
1	4.874	928	α-pinene	5.747
2	6.830	1029	β-phellandrene	92.832
3	13.363	1188	Terpineol	1.421
Total				100

[a]Retention time of compound
[b]Retention indices in reference to n-alkanes series on the Elite-5MS column
[c]Identification of compounds based on MS NIST (2008) library data
[d]Percentage of compound in total oil

α-pinene and β-phellandrene were found to be dominant constituents in leaf essential oil of *M. koenigi* **(Rajeswara Rao *et al*., 2011)**. Similarly, the present work regarding essential oil composition analysis of *M. koenigii* fruit revealed the presence of two compounds. **Mallavarapu *et al.* (2000)** reported the presence of β-phellandrene in *M. koenigii* fruit oil which accounts 23% but it was found to be increased to 92.8% in present analysis report. The percentage of α-pinene found in present study was comparatively less when compared with previous findings which was reported to be 46.32% and 48.1% in *M. koenigii* raw fruit oils respectively **(Mallavarapu *et al*., 2000; Walde *et al*., 2005)**. The variations identified in essential oil constituents of *M. koenigii* reported earlier and in present study may be due to the difference in geographical location, climate and soil. The similar statement has been concluded by **Padmakumari (2009)**.

***In vitro* antioxidant activity**

Antioxidant potential of *M. koenigii* fruit oil was determined by various *in vitro* tests and their IC_{50} values were calculated (Table 2). Oil was found to be more efficient in scavenging DPPH, inhibiting linoleic acid peroxidation, preventing deoxyribose degradation when compared with standard antioxidant butylated hydroxytoluene (BHT) whereas it was less effective in chelating metal ions and scavenging hydroxyl radicals.

Table 2 *In vitro* antioxidant activity of *M. koenigii* fruit oil

Assays	*IC_{50} (µg/mL)	
	Oil	BHT
DPPH assay	95.858±0.03	140±0.02
Hydroxyl radical scavenging	289.25±0.04	140±0.02
Metal chelation	550.66±0.05	120±0.02
Inhibition of linoleic acid peroxidation	27.83±0.06	145±0.02
Prevention of deoxyribose degradation assay	27.05±0.02	75±0.02

*IC_{50}-inhibitory concentration 50% value expressed as the mean ±standard deviation of three replicates

The leaf essential oil of *M. koenigii* possesses good antioxidant potential as they contain monoterpenes as predominant constituents **(Mini Priya *et al*., 2014)**. From the evidence of the literature, monoterpenes identified in this study may play a major role in scavenging free radicals. **Gunathilake *et al*. (2016)** supports the antioxidant results of current work by reporting *M. koenigii* as a good antioxidant supplement among other green leafy vegetables.

Antiacetylcholinesterase activity

According to cholinergic hypothesis, the therapeutic approach for treating Alzheimer's disease should overcome the deficiency of acetylcholine by inhibiting acetylcholinesterase **(Hasselmo and Giocomo 2006)**. The essential oil showed a dose dependent inhibition of acetylcholinesterase. A minimum inhibitory concentration required for inhibiting acetylcholinesterase was calculated to be 448.12±0.02 µg/mL and it was found to be less effective when compared with positive control physostigmine which showed IC_{50} value as 143±0.01 µg/mL.
The important effective compounds namely β-Phellandrene and α-pinene were reported as strong AchE inhibitors by **Marco *et al.* (2010)** and **Miyazawa and Yamafuji (2005)** respectively. The combined antagonistic action of β-Phellandrene and α-pinene might be responsible for the AchE inhibitory effect of *M. koenigii* oil in the present study.

Cytotoxicity

The cytotoxic effect of *M. koenigii* fruit oil in HeLa cells was determined by MTT assay. The oil showed considerable growth inhibitory action with an IC_{50} value of 194.1±0.03 µg/mL. The positive control doxorubicin showed IC_{50} value of 10.5±0.01.
Hakkim *et al*. (2015) has reported two important terpenes such as α-pinene and β-Phellandrene in frankincense oil which showed a significant cytotoxicity against breast cancer cell (MDA-MB-231). In agreement with the literature, the cytotoxicity of *M. koenigii* fruit oil may be due to the synergistic action of β-Phellandrene and α-pinene. Since, α-pinene has been reported to be a good anticancer agent against different cancer cells it might have contributed to the observed cytotoxicity even though at less concentration in this study **(Aydin *et al*., 2013; Wang *et al*., 2012)**.

CONCLUSION

The present study revealed a detailed report for the first time on medicinal importance of essential oil extracted from raw fruit of *Murraya koenigii* (L.) Spreng. The present study results suggest that oil could be used as effective candidate for preparing herbal based medicine for treating various diseases.

Acknowledgements: The authors wish to thank SIF-VIT University, Vellore for providing instrumentation facilities to carry out chemical composition analysis

REFERENCES

Akanksha Rani, Mamta Bisht, Chitra Pande, Geeta Tewari, Sunita Bhatt & Monika Matiyani. (2017). Effect of Drying on the Volatiles of Leaves of *Murraya koenigii* (L.) Spreng. *Journal of Essential Oil Bearing Plants*, 20, 552-558. https://doi.org/10.1080/0972060X.2017.1317606

Ashokkumar, K., Selvaraj, K. & Saradha Devi, K.M. (2013). Reverse phase-high performance liquid chromatography-diode array detector (RP-HPLC-DAD) analysis of flavonoids profile from curry leaf (*Murraya koenigii*). *Journal of Medicinal Plants Research*, 7, 3393-3399. https://doi.org/10.5897/JMPR2013.5150

Aydin, E., Turkez, H. & Geyikoglu, F. (2013). Antioxidative, anticancer and genotoxic properties of a-pinene on N2a neuroblastoma cells. *Biologia*, 68, 1004-1009. https://doi.org/10.2478/s11756-013-0230-2

Choi, B., Kang, S., Ha, Y., Park, G. & Ackman, R.G. (2002). Conjugated linoleic acid as a supplemental nutrient for common carp (*Cyprinus carpio*). *Food Science and Biotechnology*, 11, 457-461

Gunathilake, K.D.P.P. & Ranaweera, K.K.D.S. (2016). Antioxidative properties of 34 green leafy vegetables. *Journal of Functional Foods*, 26, 176-186. https://doi.org/10.1016/j.jff.2016.07.015

Hakkim, F.L., Al-Buloshi, M. & Al-Sabahi, J. (2015). Frankincense derived heavy terpene cocktail boosting breast cancer cell (MDA-MB-231) death in vitro. *Asian Pacific Journal of Tropical Biomedicine*, 5, 824–828. https://doi.org/10.1016/j.apjtb.2015.06.008

Halliwell, B., Gutteridge, J.M.C. & Aruoma, O.I. (1987). The deoxyribose method: a simple test-tube assay for determination of rate constants for reactions hydroxyl radical. *Analytical Biochemistry*, 165, 215-219. https://doi.org/10.1016/0003-2697(87)90222-3

Hasselmo, M.E. & Giocomo, L.M. (2006). Cholinergic modulation of cortical function. *Journal of Molecular Neuroscience*, 30, 133–135. https://doi.org/10.1385/JMN:30:1:133

Ingkaninan, K., Temkitthawon, P., Chuenchon, K. & Yuyaem, T. (2003). Screening for acetylcholinesterase inhibitory activity in plants used in Thai

traditional rejuvenating and neurotonic remedies. *Journal of Ethnopharmacology*, 89, 261-264. https://doi.org/10.1016/j.jep.2003.08.008
Lal, R.K., Khanuja, S.P.S., Misra, H.O., Bansal, K. & Naqvi, A.A. (2005). Genetic diversity in the secondary metabolite traits of curry leaf (*Murraya koenigii* (Linn). Spreng.). *Indian Perfumer*, 49, 519-524
Lau, C.B., Ho, C.Y., Kim, C.F., Leung, K.N., Fung, K.P., Tse, T.F., Chan, H.H. & Chow, M.S. (2004). Cytotoxic activities of Coriolus versicolor (Yunzhi) extract on human leukemia and lymphoma cells by induction of apoptosis. *Life Sciences*, 75, 797-808. https://doi.org/10.1016/j.lfs.2004.04.001
Mallavarapu, G.R. & Laxmi Rao, Ramesh, S. (2000). Volatile constituents of the leaf and fruit oils of *Murraya koenigii* Spreng. *Journal of Essential Oil Research*, 12, 766-768. https://doi.org/10.1080/10412905.2000.9712211
Marco, B., Federica, M., Rosa, T., Monica, R.L,, Filomena, C., Nicodemo, G.P., Giancarlo, A.S. & Francesco, M. (2010). Acetylcholinesterase and butyrylcholinesterase inhibitory activity of *Pinus* species essential oils and their constituents. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 25, 622-628. https://doi.org/10.3109/14756360903389856
Mensor, L.I., Menezes, F.S., Leitao, G.G., Reis, A.S., Dos Santos, T., Coube, C.S. & Leitao, S.G. (2001). Screening of Brazilian plants extracts for antioxidants activity by the use of DPPH free radical method. *Phytotheraphy Research*, 15, 127-130. https://doi.org/10.1002/ptr.687.
Mini Priya, R., Blessed Beautlin, P. & Nija, S. (2014). Chemical composition, antibacterial and antioxidant profile of essential oil from *Murraya koenigii* (L.) leaves. *Avicenna Journal of Phytomedicine*, 4, 200-214. PMID: 25050318
Miyazawa, M. & Yamafuji, C. (2005). Inhibition of acetylcholinesterase activity by bicyclic monoterpenoids. *Journal of Agricultural and Food Chemistry*, 53, 1765-1768. https://doi.org/10.1021/jf040019b
NIST/NIH/EPA. 2008. Mass spectral library with search program data version: NIST v08. Standard reference data program, National Institute of Standards and Technology, Gaithersburg
Padmakumari, K.D. (2009). Volatile constituents from the leaves and flowers of *Murraya koenigii* (Linn.) Spreng. *Journal of Essential Oil Bearing Plants*, 12, 722-727. https://doi.org/10.1080/0972060X.2009.10643781
Rajeswara Rao, B.R., Rajput, D.K. & Mallavarapu, G.R. (2011). Chemical diversity in curry leaf (*Murraya koenigii*) essential oils. *Food Chemistry*, 126, 989-994. https://doi.org/10.1016/j.foodchem.2010.11.106
Singh, N. & Rajini, P.S., 2004. Free radical scavenging activity of an aqueous extract of potato peel. *Food Chemistry*, 85, 611-616. https://doi.org/10.1016/j.foodchem.2003.07.003
Walde, S.G., Jyothirmayi, T., Prabhakara Rao, P.G., Shivaswamy, R. & Srinivas, P. (2005). Flavour volatiles of leaves, fruits and seed cotyledons of *Murraya koenigii* L. *Flavour and Fragrance Journal*, 20, 169-172. https://doi.org/10.1002/ffj.1436
Walde, S.G., Jyothirmayi, T., Prabhakara Rao, P.G. and Srinivas, P. (2006). Flavour volatiles of flowers and stalks of *Murraya koenigii* L. *Flavour and Fragrance Journal*, 21, 581-584. https://doi.org/10.1002/ffj.1737
Wang, W., Li, N., Luo, M., Zu, Y. & Efferth, T. (2012). Antibacterial activity and anticancer activity of *Rosmarinus officinalis* L. essential oil compared to that of its main components. *Molecules*, 17, 2704-2713. https://doi.org/10.3390/molecules17032704
Zhao, G.R., Xiang, Z.J., Ye, T.X., Yuan, Y.J. & Guo, Z.X. (2006). Antioxidant activities of *Salvia miltiorrhiza* and *Panax notoginseng*. *Food Chemistry*, 99, 767-774. https://doi.org/10.1016/j.foodchem.2005.09.002