International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 22s, 2025
https://theaspd.com/index.php

Microbial Diversity and Antimicrobial Properties of Endophytes from Murraya Koenigii

Shahana Jabi[1], Preeti Juyal[2], Venus Dimri[3], Avnish Kumar[4]
[1]School of Basic & Applied Sciences, Shri Guru Ram Rai University, Dehradun
[2]School of Basic & Applied Sciences, Shri Guru Ram Rai University, Dehradun
[3]School of Basic & Applied Sciences, Shri Guru Ram Rai University, Dehradun
[4]SGRR (PG) College, Dehradun
Email of corresponding Author*: **juyalpreeti@gmail.com**

Abstract
Endophytic microorganisms, which form symbiotic associations by residing within plant tissues, are known of late for their bioactive compounds .This research aimed at harvesting the microbial diversity and antibacterial activity of endophytes associated with different parts of Murraya koenigii. A total of eight bacterial endophytic strains were isolated using standard isolation techniques and characterized morphologically and biochemically. Among them five were Gram positive, non-motile, non-spore forming bacilli and the remaining three were gram negative, motile, non-spore forming bacilli.
A total of eleven fungal endophytes were isolated from plant tissues, by colony and hyphal characters as stained by lactophenol they were identified as Aspergillus niger, Aspergillus flavus,Candida albicans, Phoma hedericola and Penicillium sublateritium.
Phytochemical screening exhibited Alkaloids, tannins and reducing sugars as the major constituents. Antimicrobial activity was analysed using agar well diffusion method against standard pathogens, several isolates demonstrated broad-spectrum antimicrobial activity, with notable inhibition zones and some showed limited or no activity.
These findings indicate that M. koenigii contains a broad spectrum of endophytes possessing useful antimicrobial activities with potential applications in pharmaceutical and agricultural biotechnology.
Keywords: *Murraya koenigii, endophytes, antimicrobial activity, microbial diversity, phytoconstituents.*

INTRODUCTION

The growing prevalence of multidrug-resistant pathogens is largely attributed to the indiscriminate use of antibiotics. Therefore, there is still a need to find novel antimicrobials from natural sources. In recent years, natural or herbal medicines have gained attention instead of antibiotics and chemically generated medications since they are harmless with no adverse effects. This belief is because these natural remedies are procured from plants. (Anita Joshi et al, 2009).

Medicinal plants have been in use since ancient times in treatment of various ailments. Many studies and clinical trials have demonstrated the therapeutic effect of herbal medicines on both mental and physical disorders. They reduce the dependency on synthetic drugs and treat the issue at deeper levels, causing less side-effects in comparison. These herbs contain bioactive phytochemicals which are isolated from different plant parts like leaves, stems, roots, flower and bark.

One of such medicinal plant is Murraya koenigii commonly known as curry leaf. The curry tree belongs to the family Rutaceae, which consists of over 150 genera and around 1,600 species. (Satyavati et al,1987). The curry leaves come in three varieties namely, Murraya koenigii (M. koenigii), Micromelum minutum (M. minutum), and Clausena indica (C. indica) (Abeysinghe et al., 2021). The detailed focus of this article is on Murraya koenigii**,** a type of shrub which can grow up to a height of 6 m. The diameter of its trunk is 15-40 cm. Each primary stems bear 11-25 leaflets. It is native to south Asia, majorly found in India, Bhutan, Nepal, Shri Lanka, Bangladesh and Pakistan. Murraya koeniggi is known by its different names depending upon the geographical locations such as Mitha neem in hindi; Pindosine in Burmese; Karrry bald in Danish; Feuilles de cari in French; Daun kari in Indonesian; Hoja in Spanish and more (Gahlawat et al., 2014). The curry leaves constitute of various phytochemicals or bioactive compounds like volatile oils, saponins, tannins, steroids, carbazole alkaloids, triterpene, quinones, phenols, and flavonoids (Tripathi et al.2018). These compounds carry antimicrobial, antifungal, nephroprotective, cardioprotective, anticancer, anti-inflammatory, and anti-oxidative activities. The plant is used for various purposes especially for medicinal uses such as treating piles, renal pain, dysentery, itching, vomiting, bruises, inflammation, fresh cuts, body aches, and treating snakebites, depending upon the plant part

used (Balakrishnan et al., 2020). The other traditional uses include weight loss, prevent hair loss, culinary, enhance digestion, curing vitamin deficiency and osteoporosis and anaemia in women, and more.

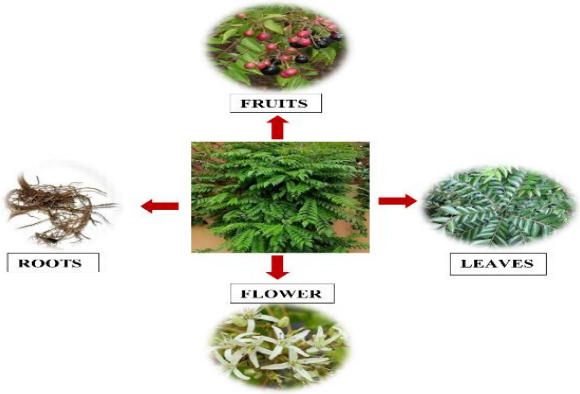


Fig 2: Different parts of Murraya koenigii

These plants also provide a habitat for numerous microorganisms including bacteria, fungus, and yeast which are native to a living plant, residing in internal tissues of living plant such as leaves, stem, roots, bark, seeds and flowers. These microorganisms are harmless to the plant and are referred as endophytes. They usually complete their life cycle within the living plant tissue and exhibit various bioactive compounds including alkaloids, steroids, phenols, quinones, xanthones, lignans, terpenoids, saponins, tannins, lactones etc. These bio active compounds have been researched and have many properties like anticancer, antimicrobial, antioxidant, antidiabetic, anti-inflammatory, antiviral, etc. and more activities (Hashem et al., 2023). Endophytes are also beneficial to the plant as they help in increasing nutrient absorption rate, plant growth, hormone production and stress tolerance. Because of the genetic exchange between the host plant and the native endophytes, they produce similar secondary metabolites or phytochemicals. (Mahlangu et al., 2024).

Murraya koenigii is known for its culinary and medicinal importance, but the microbial endophytes residing this plant remain relatively underexplored. Recent studies have shown that these endophytes, particularly bacterial strains isolated from curry leaves, exhibit promising probiotic and antimicrobial properties, suggesting their potential for use in therapeutic applications (Bandara et al., 2023).

MATERIAL AND METHODS

Sample Collection

Fresh Murraya koenigii specimens were collected from the Medicinal Garden of SGRR (PG) College, Dehradun, India. Samples were transported in sterile containers and processed within 24 h to maintain microbial viability.

Sterilization Protocol and Endophyte Isolation Procedures

For bacterial isolation, plant tissues (leaves, stems, roots) were washed under running water to remove debris, followed by immersion in 70% ethanol for a few seconds and 0.5–3.5% sodium hypochlorite for 1–2 min. Tissues were rinsed thoroughly in sterile double-distilled water and aseptically transferred to LB agar plates (Kharwar et al., 2009).

For fungal isolation, tissues were immersed in 70% ethanol for 1–3 min, treated with 4% sodium hypochlorite for 3–5 min, briefly dipped in 70% ethanol, and rinsed with sterile water. After air-drying in a laminar flow cabinet, internal segments were placed on PDA plates and incubated at 28 °C for 5–6 days (Maheshwari, 2006). Uninoculated control plates were maintained to check for contamination.

To remove the sample's exterior tissues and excise its inner tissues, a sterile knife blade was necessary. For approximately five to six days, the PDA plates were maintained to track the growth of any fungal endophytes. All the plates were incubated at 28°C to encourage the growth of fungal endophytes and was checked routinely for any microbial growth. (Maheshwari, 2006). Sub-culturing was carried out after the microbial growth was observed. After being examined for purity, each endophytic culture was moved on a newly made PDA plate. Additionally, appropriate controls that did not include the inoculation of plant tissues were maintained. The isolated endophytes of bacteria and fungi were identified.

Preservation of Endophytic isolates for Preliminary Characterization and future use

The purified endophytic isolates were transferred separately to Luria Bertani agar and Potato Dextrose agar slants and broths depending on the case for bacterial and endophytes respectively. Finally, all the

purified endophytes were maintained at 4°C till further used. Different biochemical tests were done for identification of bacterial and lactophenol staining for fungal endophytes. The bacterial isolates were tested for their morphological and biochemical characteristics. Gram staining was performed to determine the characteristics of the cell wall, cell shape and the arrangement of cells. The morphology of the endophytic bacterial strains was observed under a microscope. 15 µL of a bacterial culture were heat-fixed onto a slide and then stained. Lactophenol was used to stain fungal strains . The structures were observed using a microscope. The samples were then compared to other samples reported in the literature (Schilmiller et al., 2008; Sutton, 1980; Carmichael et al., 1980).

Secondary Metabolite Profiling of Endophytic Isolates

To produce secondary metabolites LB broth and Potato Dextrose broth were prepared and autoclaved and the endophytic bacterial and fungal cultures were inoculated in the prepared medium separately within the flasks. Flasks were incubated at 28°C for 10-14 days in shaker after inoculation. Extraction of secondary metabolite was done with different solvents (Chloroform, Ethyl acetate) after incubation and later the organic phase was collected and kept for drying at 37°C. The extracts dry weight was determined.

Antimicrobial Assay

Agar well diffusion method was used to analyse antimicrobial activity. Test organisms included E.coli NCIM 2065,S.abony NCIM 2257, M.luteus ATCC 9341, L.plantarum NCIM 2083 ,MRSA 35 ,MRSA 8 ,A.niger NCIM 1196 ,C.albicans NCIM 3471.Zones of inhibition were measured and compared with standard antibiotics.

RESULTS

In the present investigation, after the surface sterilization of plant parts different bacterial and fungal endophytes from Murraya koiengii were isolated on Luria-Bertani (LB) and Potato dextrose agar (PDA) medium respectively. (fig 2 and 3)

Diversity of Endophytes

A total of eight Bacterial endophytes were isolated from plant tissues (Table1 and Graph 1) and were characterized based on morphological and biochemical tests, out of which five were Gram positive, non-motile, non-spore forming bacilli and the remaining three were categorised as gram negative, motile, non-spore forming bacilli as mentioned in Tables 2a.&2b.

A total of eleven fungal endophytes were isolated from plant tissues, by colony and hyphal characters as stained by lactophenol at National Centre of Fungal Taxonomy, New Delhi five were found to be Aspergillus niger, two were Aspergillus flavus, two were Candida albicans, one was Phoma hedericola and one was Penicillium sublateritium. (Table 3)

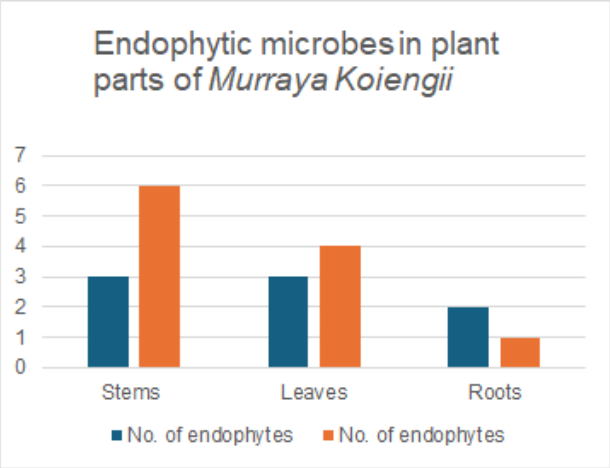
The findings indicate the presence of diverse bacterial and fungal endophytes associated with the plant samples, highlighting their potential role in plant health and ecological interactions.

Fig 2: Initial inoculation of sterilized plant parts of M.koiengii

Fig 3: Endophytes on LB and PDA Media

Table 1: Percent endophytic cultures isolated from aerial and non aerial parts of Murraya koiengii

Parts of Murraya koiengii	Percentage Endophytes Isolated	
	Bacterial	Fungal
Leaves	3	4
Stem	3	6
Roots	2	1



Graph 1: **Graphical representation in percentage of endophytic cultures isolated from aerial and non-aerial parts of Murraya koiengii**

Table 2a. Gram Positive Bacterial Endophytes and their Biochemical Characterization

Isolate	Gram Staining/Shape/Motility/Spore formation	Sugar Fermentation Test	Amylase test	Indole test	Methyl red test	VP test	H_2S production	Citrate test
S1	Gram positive, Bacilli, Non-motile, spore forming	+	+	_	+	-	_	-
S2	Gram positive, Bacilli, Non motile, Non spore forming	+	+	_	+	-	_	-
L1	Gram positive, Bacilli, Non motile, Non spore forming	+	+	_	+	-	_	-
L2	Gram positive, Bacilli, Non motile, Non spore forming	+	+	_	+	-	-	-
R2	Gram positive, Bacilli, Non motile, Non spore forming	+	+	_	+	-	-	-

Table 2b: Gram Negative Bacterial Endophytes and their Biochemical Characterization

Isolate	Gram Staining/Shape/Motility /Spore formation	Sugar Fermentation Test	Amylase test	Indole test	Methyl red test	VP test	H_2S production	Citrate test
S3	Gram negative, Bacilli, motile, Non spore forming	+	+	+	_	+	+	+
L3	Gram negative, Bacilli, motile, Non spore forming	+	+	+	_	+	+	+
R1	Gram negative, Bacilli, motile, Non spore forming	+	+	+	_	+	+	+

Table 3: Fungal Endophytes

Isolate	Fungal Endophyte
S1	Aspergillus niger
S2	Aspergillus niger
S3	Aspergillus niger
S4	Aspergillus flavus
S5	Aspergillus flavus
S6	Aspergillus niger
L1	Aspergillus niger
L2	Candida albicans
L3	Candida albicans
L4	Penicillium sublateritium
R1	Phoma hedericola

Phytochemical Screening

Crude extract of plant parts of Murraya Koenigii was subjected to phytochemical screening. Alkaloids, tannins and reducing sugars are the predominant phytochemicals which are present and anthraquinones presence was absent in extracts of all plant parts as depicted in Table 4.

Table 4: Phytochemical Screening of Crude extract of Plant parts

	Phytoconstituents							
Solvent extracts	**Alkaloids**	**Tannins**	**Flavanoids**	**Saponin**	**Steroids**	**Cardiac glycosides**	**Reducing sugars**	**Anthraquinones**
Leaves	+	+	+	+	+	+	+	_
Stems	+	+	_	_	_	_	+	_
Roots	+	+	+	_	_	_	+	_

Activity of Bacterial and Fungal Isolates

The antimicrobial activity of bacterial and fungal isolates from different plant parts was analysed for pathogens such as E.coli NCIM 2065, S.abony NCI2257, M.luteus ATCC 9341, L.plantarum NCIM 2083,MRSA 35,MRSA 8,A.niger NCIM 1196,C.albicans NCIM 3471.

Erythromycin and fucanazole were used as positive control.

Bacterial endophytic isolates from stems showed the best activity compared to leaves and roots. The results are depicted in table 5 and Graph 2 and fig 4.

Fungal endophytes showed no relevant activity against the above-mentioned pathogens.

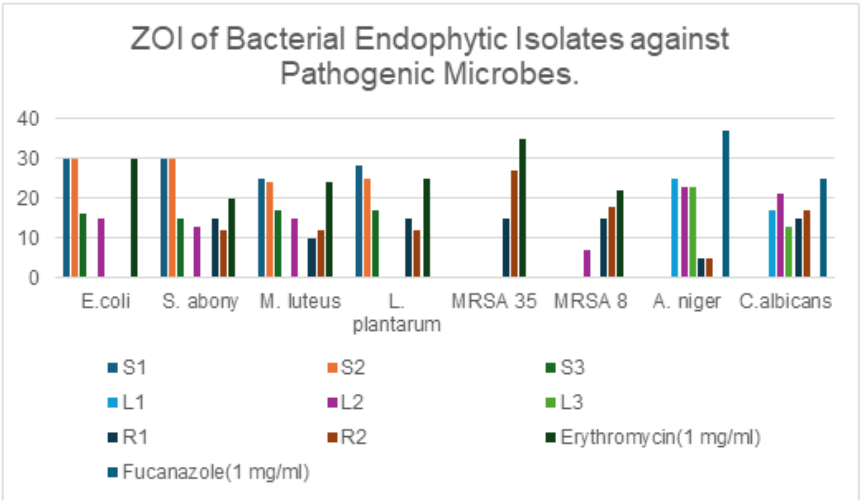
Endophytic fraction identified as C.albicans from leaf showed significant activity against E.coli , L.plantarum , S.abony,and MRSA 35 as shown in table 6and Graph 3.

Table 5: Zone of Inhibition of Bacterial Endophytic Isolates against Pathogenic Microbes.

Bacterial Endophytic fractions	**Diameter of zone of inhibition (mm)**							
	Pathogens studied							
	E.coli	**S. abony**	**M. luteus**	**L. plantarum**	**MRSA 35**	**MRSA 8**	**A. Niger**	**C.albicans**
S1	30.0	30.0	25.0	28.0	NA	NA	NA	NA
S2	30.0	30.0	24.0	25.0	NA	NA	NA	NA
S3	16.0	15.0	17.0	17.0	NA	NA	NA	NA
L1	NA	NA	NA	NA	NA	NA	25.0	17.0
L2	15.0	13.0	15.0	NA	NA	07.0	23.0	21.0

L3	NA	NA	NA	NA	NA	NA	23.0	13.0
R1	NA	15.0	10.0	15.0	15.0	15.0	05.0	15.0
R2	NA	12.0	12.0	12.0	27.0	18.0	05.0	17.0
Erythromycin(1 mg/ml)	30.0	20.0	24.0	25.0	35.0	22.0	NT	NT
Fucanazole(1 mg/ml)	NT	NT	NT	NT	NT	NT	37.0	25.0

*NA No activity *NT Not tested



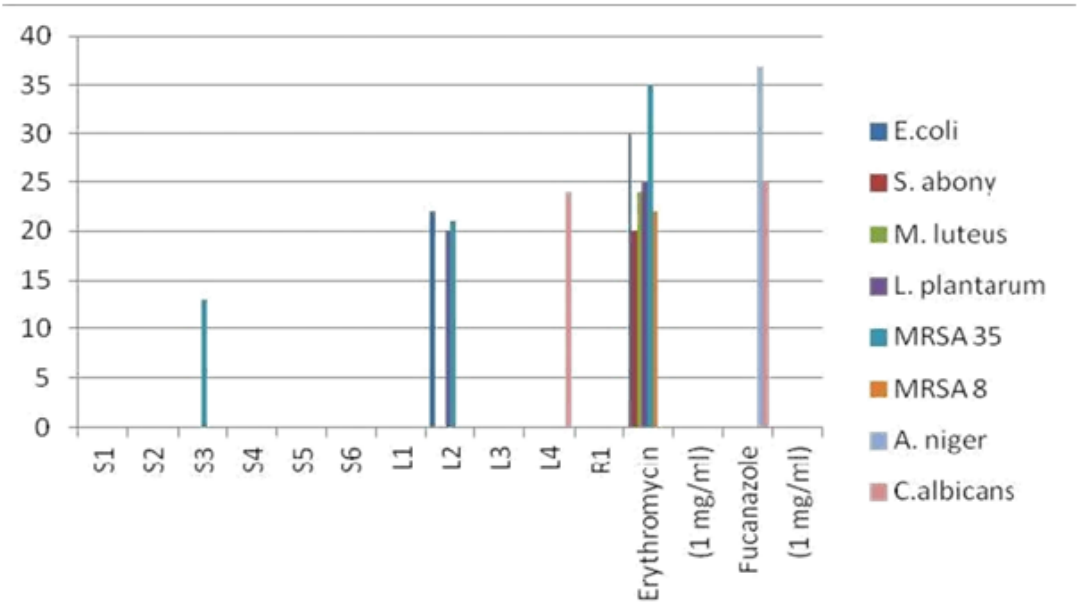
Graph 2: Depicts Antimicrobial activity of Bacteria endophytic isolates from plant parts

Fig 4: Antimicrobial activity of Bacteria endophytic isolates from plant parts

Table 6: Zone of Inhibition of Fungal Endophytic Isolates against Pathogenic Microbes.

Fungal endophytic fraction	Diameter of zone of inhibition (mm)							
	Pathogens studied							
	E.coli	**S. abony**	**M. luteus**	**L. plantarum**	**MRSA 35**	**MRSA 8**	**A. Niger**	**C.albicans**
S1	NA	NA	NA	NA	NA	NA	NA	NA
S2	NA	NA	NA	NA	NA	NA	NA	NA
S3	NA	NA	NA	NA	13.0	NA	NA	NA
S4	NA	NA	NA	NA	NA	NA	NA	NA
S5	NA	NA	NA	NA	NA	NA	NA	NA
S6	NA	NA	NA	NA	NA	NA	NA	NA
L1	NA	NA	NA	NA	NA	NA	NA	NA
L2	22.0	NA	NA	20.0	21.0	NA	NA	NA
L3	NA	NA	NA	NA	NA	NA	NA	NA
L4	NA	NA	NA	NA	NA	NA	NA	24.0
R1	NA	NA	NA	NA	NA	NA	NA	NA
Erythromycin 1mg/ml	30.0	20.0	24.0	25.0	35.0	22.0	NT	NT
Fucanazole (1 mg/ml)	NT	NT	NT	NT	NT	NT	37.0	25.0

*NT Not tested *NA Not activity



Graph 3: Depicts Antimicrobial activity of Fungal endophytic isolates from plant parts

DISCUSSION

Studies have shown that the curry leaf plant, Murraya koenigii, is home to a wide variety of endophytic microbes, comprising fungi and bacteria. Due to their antibacterial properties,these endophytes play a vital role in the plant's natural defense systems.

Strong antimicrobial activity against both Gram-positive and Gram-negative infections has been demonstrated by Bacillus species, particularly Bacillus subtilis and Bacillus cereus. They have an inhibitory effect on human and phytopathogens such Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa because of their capacity to create lipopeptides and other secondary metabolites. (Lincy & Saranraj, 2014).By fixing atmospheric nitrogen, solubilizing phosphate, and generating indole-3-acetic acid (IAA), bacterial endophytes also aid in the growth of plants. This dual function in defense and growth promotion raises the possibility that M. koenigii's endophytic microbiome plays a crucial part in the plants ability to adapt to a variety of environmental circumstances. (Nalini & Sunita, 2018).

Recent investigations have revealed that endophytes from M. koenigii produce a broad spectrum of secondary metabolites with bioactive properties. For instance, bacterial isolates such as species of Bacillus, Pseudomonas, and Streptomyces have shown strong antibacterial and antifungal activities, likely due to the production of lipopeptides, polyketides, and other antimicrobial compounds (Kumari et al., 2023)
It has been linked to a variety of fungal endophytes, according to Rangari et al. (2017). Mucor spp. and Aspergillus flavus were discovered to be related with leaf tissues, while Fusarium oxysporum, Cladosporium spp., Rhizopus spp., and Candida spp. were primarily identified from stem tissues, according to their analysis. This distribution points to a potential tissue-specific colonization pattern among endophytic fungi, suggesting that various plant sections may offer unique ecological niches that impact the richness and prevalence of endophytes.

CONCLUSION

The disquisition of endophytic microorganisms from Murraya koenigii has revealed a different and functionally rich microbiome comprising both bacterial and fungal species. These endophytes are known to contribute significantly to the host factory's medicinal parcels by producing a wide range of bioactive secondary metabolites, similar as alkaloids, flavonoids, and phenolics(Patel & Thakkar, 2022).

The endophytic microbiome of Murraya koenigii holds immense potential in the quest for new antimicrobial agents. By leveraging the natural chemical arsenal produced by these microorganisms, innovative solutions to tackle antibiotic resistance can emerge. Future research must prioritize uncovering the intricate symbiotic relationships within M. koenigii to advance this promising avenue of drug discovery.

The current study emphasizes Murraya koenigii's importance as an important reservoir of endophytic microbes by highlighting the extensive microbial diversity found within its tissues. Important antibacterial activity against a variety of harmful pathogens was shown by isolated endophytes from various plant sections. According to these results, M. koenigii may include endophytes that could be used in biotechnological processes, including the creation of new antimicrobial drugs. The identification of bioactive substances with potential medicinal applications may result from additional molecular and biochemical analysis of these endophytes. All things considered, this study highlights the value of medicinal plants as reservoirs of endophytic microorganisms with intriguing therapeutic applications.

REFERENCES

1. Abeysinghe, D. T., Alwis, D. D. D. H., Kumara, K. A. H., & Chandrika, U. G. (2021). Nutritive importance and therapeutic uses of three different varieties (Murraya koenigii, Micromelum minutum, and Clausena indica) of curry leaves: An updated review. Evidence-Based Complementary and Alternative Medicine, 2021, 5523252.
2. Gahlawat, D. K., Jakhar, S., & Dahiya, P. (2014). Murraya koenigii (L.) Spreng: An ethnobotanical, phytochemical and pharmacological review. Journal of Pharmacognosy and Phytochemistry, 3(3), 109–119.
3. Tripathi, Y. C., Anjum, N., & Rana, A. (2018). Chemical composition and in vitro antifungal and antioxidant activity of essential oil from Murraya koenigii (L.) Sprengel leaves. Asian Journal of Biomedical and Pharmaceutical Sciences, 8(65), 1–6.
4. Balakrishnan, R., Vijayraja, D., Jo, S. H., Ganesan, P., Su-Kim, I., & Choi, D. K. (2020). Medicinal profile, phytochemistry, and pharmacological activities of Murraya koenigii and its primary bioactive compounds. Antioxidants, 9(2), 101. https://doi.org/10.3390/antiox9020101
5. Hashem, A. H., Attia, M. S., Kandil, E. K., & El-Gazzar, N. (2023). Bioactive compounds and biomedical applications of endophytic fungi: A recent review. Microbial Cell Factories, 22, 107. https://doi.org/10.1186/s12934-023-02118-x
6. Mahlangu, S. G., Zulu, N., Serepa-Dlamini, M. H., & Tai, S. L. (2024). Isolation, identification, and biological characterization of bacterial endophytes isolated from Gunnera perpensa L. FEMS Microbiology Letters, 371, fnae056. https://doi.org/10.1093/femsle/fnae056
7. Bandara, A. C., Abayasekara, C. L., Karunaratne, A. M., & Panagoda, G. J. (2023). Probiotic potential of bacterial endophytes isolated from leaves of Murraya koenigii L. Journal of the National Science Foundation of Sri Lanka, 51(3), 279–286. https://doi.org/10.4038/jnsfsr.v51i3.11385
8. Joshi, A., Shahane, V. D., Gore, V., & Bharadwaj, R. (2009). Antibacterial potential of endophytic bacteria isolated from medicinal plants. Hindustan Antibiotics Bulletin, 47–48(1–4), 7–12.
9. Lincy, S., & Saranraj, P. (2014). Antibacterial activity of endophytic bacteria isolated from the leaves of Murraya koenigii (Linn.) Spreng. International Journal of Research in Applied, Natural and Social Sciences, 2(7), 33–40.
10. Nalini, M. S., & Sunita, M. S. (2018). Endophytic fungi: A potential source of bioactive compounds. Journal of Applied Pharmaceutical Science, 8(5), 124–131. https://doi.org/10.7324/JAPS.2018.8547
11. Patel, A. R., & Thakkar, V. R. (2022). Bioactive potential of fungal endophytes from medicinal plants including Murraya koenigii: A review. Biocatalysis and Agricultural Biotechnology, 41, 102329
12. Kumari, R., Kaur, R., & Singh, S. (2023). Diversity and bioactivity of endophytic bacteria isolated from Murraya koenigii (L.) Spreng. Microbial Research, 266, 127232.
13. Maheshwari, R. (2006). What is an endophytic fungus? Current Science, 90(10), 1309–1310.

14. Kharwar, R. N., Verma, V. C., Kumar, A., Gond, S. K., Harper, J. K., & Hess, W. M. (2009). Javanicin, an antibacterial naphthaquinone from an endophytic fungus of Neem, Chloridium sp. Current Microbiology, 58(3), 233–238.
15. Schilmiller, A. L., Last, R. L., & Pichersky, E. (2008). Harnessing plant trichome biochemistry for the production of useful compounds. The Plant Journal, 54(4), 702–711.
16. Carmichael, J. W., Kendrick, B., Conners, I. L., & Sigler, L. (1980). Genera of Hyphomycetes. University of Alberta Press.
17. Sutton, B. C. (1980). Coelomycetes: Fungi Imperfecti with pycnidia, acervuli and stromata. Commonwealth Mycological Institute.
18. Rangari, S. N. H., & Rangari, S. K. (2017). Isolation of fungal endophytes from Murraya koenigii and Costus pictus—Valuable medicinal plants. Current Agriculture, 1(1), 1–6.