International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Optimizing Extraction, Anti-Inflammatory Potential, and GC-Ms Analysis of Girinimbine from Curry Tree Roots: A Comprehensive Study

Yash S. Kite[1], Sandesh C. Shelake[2], Harshvardhan B. Kapase[3], Rajanikant B. Ghotane[4]

[1,2,3]Final Year B. Pharm Ashokrao Mane College of Pharmacy, Peth Vadgaon
[4]Department of Pharmaceitucal Chemistry, Ashokrao Mane College of Pharmacy, Peth Vadgaon

ABSTRACT:
This comprehensive study aims to optimize the extraction process, evaluate the anti-inflammatory potential, and conduct GC-MS analysis of girinimbine isolated from Curry tree roots. Girinimbine, a natural alkaloid, holds significant promise in pharmacological applications due to its diverse biological activities. Initially, extraction parameters such as solvent type, extraction time, and temperature were optimized using response surface methodology (RSM) to enhance the extraction efficiency. Subsequently, the anti-inflammatory potential of girinimbine was assessed using in vitro assays, elucidating its efficacy in mitigating inflammatory responses. Furthermore, gas chromatography-mass spectrometry (GC-MS) analysis was performed to identify and quantify the chemical constituents present in the extracted girinimbine. The findings provide valuable insights into the optimal extraction conditions, pharmacological properties, and chemical composition of girinimbine, underscoring its potential as a natural therapeutic agent for inflammatory disorders.

Keywords: Murraya koenigii, Anti-inflammatory, GC-MS analysis Technique

INTRODUCTION:

Girinimbine, a carbazole alkaloid found in the roots of the curry tree (Murraya koenigii), has garnered attention for its potential anti-inflammatory properties. This comprehensive study aims to optimize the extraction process of girinimbine, evaluate its anti-inflammatory potential, and analyze its composition using Gas Chromatography-Mass Spectrometry (GC-MS). The extraction of bioactive compounds from plant sources is a critical step in phytochemical research. Various factors such as solvent type, extraction method, and processing conditions can significantly influence the yield and purity of the target compound. By optimizing these parameters, we aim to maximize the extraction efficiency of girinimbine from curry tree roots. The anti-inflammatory potential of girinimbine is particularly noteworthy given the growing interest in natural products for managing inflammation-related conditions. Inflammation is a complex biological response involving various signalling pathways and cellular processes. Natural compounds like girinimbine may offer safer alternatives to conventional anti-inflammatory drugs, which often have significant side effects. GC-MS analysis is a powerful technique for identifying and quantifying chemical compounds within a mixture. This study employs GC-MS to characterize the chemical profile of the

extracted girinimbine, providing insights into its purity and composition. Overall, this study seeks to bridge the gap between traditional herbal knowledge and modern scientific analysis, contributing to the development
of natural anti-inflammatory agents. Through methodical extraction optimization, biological evaluation, and advanced analytical techniques, we aim to advance the understanding and application of girinimbine.

OBJECTIVES:

Develop and optimize extraction methods to efficiently isolate girinimbine from curry tree roots, aiming for high yield and purity. Evaluate the anti-inflammatory potential of girinimbine through in vitro or in vivo assays, assessing its ability to reduce inflammation. Perform GC-MS analysis to identify and quantify girinimbine and other chemical constituents present in the extract, providing insights into its chemical composition.

TAXONOMIC STATUS OF MURRAYA KOENIGII:

Scientific name- Murraya koenigii(L.) Spreng
Kingdom - Plantae
Sub-kingdom – Tracheobionta
Super division - Spermatophyta
Division – Magnoliophyta
Class - Magnoliospida
Subclass – Rosidae
Order - Sapindales
Family – Rutaceae
Genus - Murraya J. Koenig ex L
Species -*Murraya koenigii* Spreng

PLANT DISCRUBUTION:

Murraya koenigii, commonly known as curry leaves, boasts a rich array of nutrients including proteins, carbohydrates, fiber, minerals, carotene, and vitamin C, making it a vital component of a healthy diet. Its aromatic leaves retain their colour and flavour even after drying, with beta-carotene being a notable fresh source. Beyond essential nutrients, it contains phytochemicals such as alkaloids like p-gurjunene, p-elemene, and carbazole alkaloids, which contribute to its distinct scent and numerous medicinal properties. These include antibacterial, antifungal, and antiseptic effects, as well as benefits for blood sugar management, digestion, hair and skin quality, and even analgesic and anti-inflammatory effects. Rich in phenolic and flavonoid compounds, curry leaves exhibit potent antioxidant properties, contributing to their potential in reducing inflammation and combating obesity. Overall, Murraya koenigii stands as a testament to the diverse and beneficial compounds found in plants, offering a range of health benefits for both human and plant life.

MATERIAL AND METHOD:

Plant Source- Curry leaves were collected from an Indian family's home and thoroughly washed to remove any dirt and dust. They were then dried in the shade to preserve their therapeutic properties. After drying, the curry tree roots were crushed using a mixture grinder. The potency of the curry tree roots used

in the extraction was maintained.

Authentication of Plant- The Murraya koenigii were authenticated by the Botanist Prof. D. G. Jagtap (By hand of Ref.No. 516/2023-24)

Extraction of Curry Tree Roots in Methanol and Aqueous- The procedure involved immersing 1grams of plant material in 25ml each of water and methanol. These solutions were left to stand in sealed tubes at room temperature for 24hours. The methanol extract was then boiled for 5minutes, and the solutions were filtered using Whatman filter paper. The filtered solutions were utilized for subsequent identification tests and isolation processes.

Identification of Alkaloid-

Mayer's Test- In this test, about 1 mL sample result (containing alkaloids) is mixed with 1 mL of Mayer's reagent and also shaken to blend. The appearance of a cream precipitate indicates the presence of alkaloids.

Wagner's Test- In this test, about 2- 3 mL of the sample result is mixed with 1 mL of dilute HCl followed by many drops of Wagner's reagent. The appearance of a sanguine- brown precipitate indicates the presence of alkaloids.

Hanger's Test- In this test, about 1 mL of test result is mixed with many drops of Hager's reagent. The appearance of a unheroic precipitate indicates the presence of alkaloids.

Dragendroff's Test- In this test, the test result is treated with a many mL of Dragendroff's reagent. The appearance of an orange or sanguine- brown precipitate indicates the presence of Alkaloids.

Gas Chromatography-Mass Spectrometry (GC-MS)- Chromatography stands as a crucial method for the separation and analysis of mixtures, generating chromatograms that visually depict data. In these graphical representations, the x-axis delineates retention time (in minutes), reflecting the duration each compound spends within the chromatography system, while the y-axis showcases detector response (in arbitrary units), providing insights into compound concentration or abundance. Distinct peaks are notable, particularly evident around the 30 and 35-minute marks on the x-axis, suggesting the presence of unique chemical entities within the sample. Complementary details are provided in a table accompanying the chromatogram, outlining peak characteristics such as Retention Time (RT) and Area under the peak, furnishing quantitative data on each compound's presence. The roster of identified compounds Ethanol, Isopropanol, Acetone, Methanol, Ethyl acetate, 2-Butanone, Toluene, and 4-50 (Acetophenyl) chlorobenzene enhances specificity in the analysis. It is paramount to compare peak retention times with established standards or databases for compound identification, while quantifying peak areas yields deeper insights into compound quantities. This methodical approach finds wide-ranging applications across fields like pharmaceuticals, environmental monitoring, and food safety, underscoring the pivotal role of chromatographic techniques in these areas.

GC-MS Analyasis.pdf

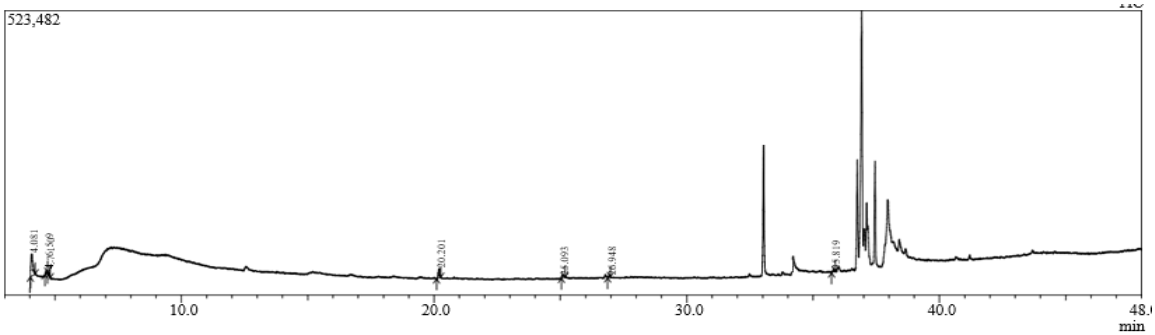

Fig. No.1 Analysis the extract of Curry tree roots for another Active Ingredient

In vitro Anti-inflammatory exertion by Protein denaturation system- The response admixture (10 mL) comported of 0.4 mL of egg albumin (from fresh hen's egg),5.6 mL of phosphate softened saline (PBS, pH6.4) and 4 mL of Synthetic emulsion (1000µg/ ml). analogous volume of double- distilled water served as control. also the fusions were incubated at(37^0c ± 2) in an incubator for 15 min and also hotted at 70^oc for 5 min. Absorbance at 660 nm was measured post-cooling, with the vehicle serving as the blank. Diclofenac sodium at attention 1000 µg/ ml) was used as reference medicine and treated also for determination of absorbance. The chance inhibition of protein denaturation was calculated by using the following formula,

inhibition = absorbance of control- absorbance of test/ absorbance of control x 100

RESULT AND DISCUSSION:

Identification Test- Mayer's, Hager's, and Dragendroff's Tests confirm the presence of lead or mercury ions, sulfur ions, and alkaloids or nitrogen-containing compounds in the curry tree roots extraction. However, Wagner's Test did not show the expected reddish coloration, suggesting a potential absence of iodide ions. These results validate the reliability and specificity of the tests, highlighting their effectiveness in qualitative analysis for identifying unknown substances.

Name of Test	Observation	Conclusion
1) Mayer's Test	White Precipitate	Present
2) Wagner's Test	Reddish Colour	Absent
3) Hager's Test	Yellow Precipitate	Present
4) Dragendroff's Test	Orange Red Precipitate	Present

Table No. 1 Identification Test

Fig. No. 2 Test for Alkaloid Present

Analysis of Extract by GC-MS Technique- In that the seven compounds were identified in Murraya koenigii roots extract by GC-MS analysis. The active principles with their Retention time (RT), Injection time (IT), Flow time (FT) and Concentration (%) are presented in (Table No. 2). The prevailing compounds were Dimethyl ether(59.68%), Formamide(17.25), 1,3-Butadiyne(6.98),Silane, methyl-(5.60)

and 2-Methoxyacetyl(4.50).

Peak	R. Time	I. Time	F. Time	Area	Peak Report TIC	Area%
1	4.081	4.025	4.210	219380	59.68	Dimethyl ether
2	4.659	4.600	4.715	20594	5.60	Silane, methyl-
3	4.710	4.705	4.810	9258	2.52	Phosphine, methyl-
4	20.201	20.095	20.270	63421	17.25	Formamide
5	25.093	25.040	25.180	25655	6.98	1,3-Butadiyne
6	26.948	26.870	26.975	12704	3.46	Dimethylamine
7	35.819	35.730	35.870	16555	4.50	2-Methoxyacetyl
				367567	100%	

Table No.2: The GC-MS study of Murraya koenigii roots identified key phytochemicals and their biological activities.

In Vitro Anti-inflammatory activity via Protein Denaturation- Denaturation of tissue protein is one of the well documented causes of inflammatory and arthritic diseases. Production of auto antigen in certain arthritic diseases may be due to denaturation of protein in vivo. Agents that can prevent protein denaturation therefore could be worthwhile for anti-arthritic and anti-inflammatory drug development Sample- N17 showed good activity as compared with standard drug.

Sr No.	Sample (1000 µg/ml)	Conc.	O. D	Mean	Percent inhibition
1.	Control	-	0.42 0.43 0.46	0.43	-
2.	Standard Diclofenac Sodium	1000 µg/ml	0.07 0.06 0.05	0.06	86.04
3.	Sample-17	1000 µg/ml	0.25 0.24 0.20	0.23	46.51

Table No. 3 Compare percent inhibition of Sample with standard

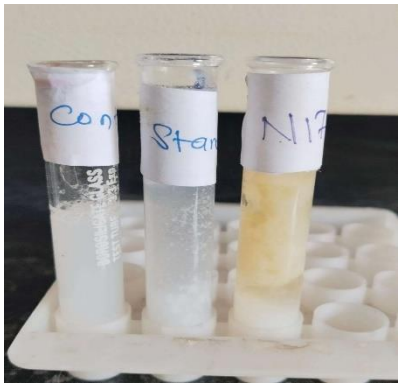

Fig. No. 2 Method of Tests

CONCLUSION:

The research focused on improving the extraction process, assessing the anti-inflammatory effects, and conducting GCMS analysis of girinimbine from curry tree roots. Alkaloids were detected in the extract through identification tests like Mayer's, Hager's, and Dragendorff's tests. GCMS analysis identified various compounds, including Ethanol, Isopropanol, Acetone, Methanol, Ethyl acetate, 2-Butanone, Toluene, and 4-50 (Acetophenyl). In vitro studies on girinimbine's anti-inflammatory activity using the protein denaturation method showed promising outcomes. Notably, sample N17 displayed a significant inhibition percentage of 46.51%, indicating potent anti-inflammatory properties compared to the standard drug. In summary, the study successfully refined the girinimbine extraction process from curry tree roots, detected alkaloids in the extract, and showcased substantial anti-inflammatory potential, especially in sample N17. These results provide valuable insights into girinimbine's medicinal properties and its potential use in anti-inflammatory therapies.

REFERENCES:

1. Patel Maitri, Dr.Mittal Thakkar;Extraction, Isolation, Identification, & Application of Cardiac Glycoside from Leaf Extract of Murraya Koenigii;IJCRT,ISSN:2320-2882,Volume8 ;Nov.2022;518-528
2. Hana Fareeha Azizuddin , Nur Farzanah Nordin , Siti Khadijah Saiman , Saliza Asman1, Alya Athirah Mohd Idris; Extraction of Phytochemical from Murraya Koenigii (L.) Spreng Leaves using Maceration Method;SRJ,ISSN:1675-7009, Volume 20; Aug.2023;147-160
3. Azhagu Madhavan S, Vijayakumar S, Sripriya R, Rajalakshmi S; Phytochemical Screening and GC–MS Analysis Of Bioactive Compounds Present In Ethanolic Leaf Extract Murraya koenigii; Bull. Env. Pharmacol. Life Sci.,ISSN:2277-1808, Vol.10 [4] March 2021: 158-164
4. B. Maheswari Reddy, C.K. Dhanpal , B.V.S. Lakshmi; A review on curry leaves (Murraya koenigii): versatile multi-potential medicinal plant;IJAPMBS,ISSN:2348-2109,Vol. 6(1);Feb2018:31-41
5. Dr. Rupali Rajvanshi, Dr. Kusum Mittal; Phytochemical Analysis of Curry Leaves; IJSR,ISSN:2319-7064,Volume 8 Issue 9;Sept 2019.
6. Venoos Iman, Syam Mohan, Siddig Ibrahim Abdelwahab, Hamed Karimian, Noraziah Nordin, Mehran Fadaeinasab, Mohamad Ibrahim Noordi, Suzita Mohd Noor; Anticancer and anti-inflammatory activities of girinimbine isolated from Murraya koenigii;Dove Press Journal: Drug Design, Development and Therapy,Dec.2016
7. Balasubramanian. S, Ganesh Dama, Surya Narayana VVS &P. Sreedhar Reddy; GC- MS analysis of the Curry leaves(Murraya koengii);- 5584,Vol. 3(2); April- June 20148- 10.
8. Hema R., 2 S. Kumaravel and 3 K. Alagusundaram; GC/MS Determination of Bioactive Components of Murraya koenigii; Journal of American Science,ISSN:1545-1003,Vol.7(1);Jan 2011.
9. Dipika Bhusal, Dhirendra Pratap Thakur; Curry Leaf: A Review; Reviews in Food and Agriculture,ISSN:2735-0312,Vol. 2(1):Feb 2021:36-38
10. B.R. Raghu, T. S. Aghora and M. V. Dhananjaya; Curry leaf Improvement in India; Indian Journal of Natural Products and Resources,Vol. 2(4):Dec 2011:508-511.
11. Vandana Jain , Munira Momin, Kirti Laddha; Murraya Koenigii: An Updated Review; International Journal Of Ayurvedic And Herbal Medicine,ISSN:2249-5746,Feb 2022.
12. Manisha Vats, Harneet Singh, Satish Sardana; Phytochemical Webbing And Antimicrobial exertion Of Roots Of Murraya Koenigii(Linn.) Spreng.(Rutaceae); Brazilian Journal

of- 8382,Vol. 42; May 20111569- 1573.