IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

"EXTRACTION, ISOLATION, IDENTIFICATION & APPLICATION OF CARDIAC GLYCOSIDE FROM LEAF EXTRACT OF MURRAYA KOENIGII"

Patel Maitri, Dr Mittal Thakkar
Department of chemistry, Parul university, Vadodara, India

ABSTRCT

Murraya koenigii also distinguishes as a curry greenery trees and one of the most valuable medicinal plant in India and Srilanka. Curry leaves can be found in a variety of recipes. Curry leaves have a various health benefit and used for the treatment of various disease. The study extraction, isolation and identification of the cardiac glycoside in fresh curry leaves. Extraction of the curry leaves in alcoholic solvent and water. The study was done to find out the phytochemicalconstituents present in curry leaf [Murraya koenigii]. cardiac glycoside was extracted in alcohol. Cardiac glycosides are found in large quantities in alcoholic extraction of curry leaves. Identification tests and Isolations methods of the cardiac glycoside was done by chemical method. Quantification and identification of cardiac glycoside using various types of chromatography like Thin layer chromatography (TLC), liquid Chromatography-Mass spectrometry (LCMS). Antibactarial properties of Cardiac glycoside tested Escherichia coli (gram negative) by starch agar well diffusion method. Other Application of cardiac glycoside like antifungal properties of Cardiac glycoside tested candida by disc diffusion method, antioxidant ABTS method, antiviral properties.

Key words: phytochemicalconstituents, cardiac glycoside, Quantification, Antibacterial, Antifungal, Antioxidant, Antiviral

INTRODUCTION

Curry leaves, scientific name of Murraya Koenigii, Spices – M Koenigii at it belongs to the Rutaceae family. [1] This tree is inherent to India and Srilanka. Curry leaves also known as karieppilai, karipatta, sweet neem leaves and kadipatta etc [2]. Curry leaves, scientific name of Murraya Koenigii, Spices – M Koenigii at it belongs to the Rutaceae family. [1] This tree is inherent to India and Srilanka. Curry leaves also known as karieppilai, karipatta, sweet neem leaves and kadipatta etc [2].

The curry leaf tree is a tiny, pungent-smelling perennial plant that grows as undergrowth in forests [7]. The main benefit for medicinal plant therapeutic. Curry leaf is a popular leafy vegetable in India. Its leaves are commonly used to flavour meals in India cuisine. The leaves have changed colour [2]. Curry leaves has several properties that are advantages to human, hence this study aims to determine the phytochemical and proximate compositions, vitamin [4].

CLASSIFICATION OF MURRAYA KOENIGII: -

Native name: 'Curry patta'

Scientific name: Murraya koenigii (L.) Spreng

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Sapindaes

Family: Rutaceae

Genus: Murraya

Species: M. koenigii

Fruits

Roots

Flowers

Plant create secondary metabolites as an element of their defence system, which are characterized by a complex variety of composites from several classes, such as hydrocarbon, phenols, terpenes, alcohol, aldehyde, ketone, ester, and so on [10].

Murraya koenigii also contains protein, carbs, fiber, minerals, carotene, and vitamin C, all of which are essential nutrients [8]. It has extremely aromatic leaves that keep their colour and flavor even after drying. Beta-carotene is respectable source of fresh curry leaves. Curry leaves are amusing source of minor compounds, such as vitamin like C, A, B, E. Antibacterial, antifungal, and antiseptic properties are all present in it, as well as the capacity to improve hair skin quality. It has also aided in the management of blood sugar levels while also improving digestion [1].

calcium, phosphorous, Protein, carbohydrate, carotene, nicotinic acid, vitamin C are the primary nutrients contained in Murraya koenigii [5]. The greatest and important chemical ingredients answerable for its instance alkaloids p-gurjunene, p-elemene, and carbazole alkaloids have a distinct scent [9]. The major advantages of medicinal plants for therapeutic purposes in various diseases are their safety, as well as their cost, efficacy, and simplicity of access [11]. Carbazole alkaloids are plentiful existing in its twig and root preparation of this plant [13]. Curry leaves can help with indigestion, stomach aches from excessive acid production, diarrhea, and other digestive problems [12]. Murraya koenigii leaves ethanol extract demonstrated analgesic and anti-inflammatory effects [18]. When ingested in significant numbers, curry leaves have been shown to be beneficial in strengthening hair roots and avoiding more hair loss fresh curry leaves are soaked in boiling water on the Indian subcontinent. Coconut oil as a moisturiser for the skin [6]. Those learning the activities of wide number of secondary metabolic chemicals found in plants, as well as their functions in human and plant life [19]. Plants generated phytochemicals for the variety of reasons, including inset defence and diseases prevention. Phytochemicals create in food active in human biology and provide health advantage in a variety of situation [15]. Various research has been shown that many plants are amusing source of antioxidant [16]. Curry leaves contains considerable amounts of phenolic and flavonoids compound that are responsible for liquid reduction and anti- obesity actions due to rich antioxidant capacity [17].

STUDY OF CARDIAC GLYCOSIDE: -

Cardiac glycosides are a class of drug-like compound that have been studies extensively and found to be useful in the development of future drugs [20]. Congaing cardiac glycosides were initially utilized medicinally by the ancient Egyptians and Romans as emetics and heat remedies. Ancient [22]. Cardiac glycoside containing Plants have also caused human poisoning that can be lethal, the main cardiac glycosides involved in the deaths caused by skewers made from oleander twigs for meat are oleander and nerine [27]. Digitalis is the most well-known cardiac glycoside generated from plants. Which is used therapeutically in hearts illness. It was initially derived from foxglove leaves and has been utilized for therapeutic purposes [21]. Cardiac glycoside is a diverse family if naturally resulting compound that bind to and inhibit Na^+ / K^+ ATPase [26] Cardiac glycoside contains two forms of aglycones: cardenolides and bufadienolides. Cardenolides have an unsaturated 5 – membered ring at C 17, Bufadienolides, on the other hand, feature a six-membered lactone ring that is doubly unsaturated [27].

TYPES OF CARDIAC GLYCOSIDE: -

- Ouabin
- Digoxigenin
- Bufalin
- Digoxin
- Digitoxin
- Oleandrin

EXPERIMENTAL

The extraction method of fresh leaves and dry leaves of curry leaves in methanol and aqueous.

Collection of plant source: - [Curry leaves]

Curry leaves were gathered from an Indian family ‘s home. To eliminate the filth and dust, these were thoroughly washed.

Then dry in the shade to keep the therapeutic properties. With the use of a mixer grinder, the dried leaves were crushed. The strength of the curry leaves that were used in the extraction.

EXTRACTION OF FRESH AND DRY CURRY LEVAES IN METHANOL AND AQUEOUS: -

Preparation of plant extract: -

The experiment was carried out in water and methanol. Take 1 gm of plant source was dissolves in 25 ml of water and methanol, The prepared solution were then maintained for 24 hours. In closed tubes at room temperature [methanol extract Boling in 5 min] Filtered the solution with Whatman filter paper. The filtered solution is used in further identification tests and isolation method [1].

IDENTIFICATION TESTS OF CARDIAC GLYCOSIDE: -

Cardiac glycosides [Keller killiani’s test]

One drop of ferric chloride solution was added to 1 mL acetic acid (glacial), and one ml of strong sulphuric acid was added. The presence of cardiac glycosides was revealed by a brown colour ring at the interface.

ISOLATION METHOD OF CARDIAC GLYCOSIDE: -

The curry leaves extracted with 50% methanol at low temperature, observed via way of means of the adding of Lead(II) acetate way to centrifugation is used to remove contaminants and precipitates from the mixture, the cardiac glycoside present in the supernatant is extracted with chloroform extract, that is evaporated underneath a vacuum, and the residue [cardiac glycoside] is further purified via chromatography.

CHROMETOGRAPHIC TECHNIQUE: -

TLC [Thin layer chromatography]: -

Extracted cardiac glycoside sample using changed solvent, and several forms of cardiac glycoside were studied in all fraction by TLC.

The common cardiac glycosides are separated and identified using a fast thin-layer chromatographic method. A 10 ml chloroform solvent is used for the TLC of cardiac glycoside.

TLC [Using different solvent]

For the better separation, if we use other solvent and perform TLC. And compered the both TLC plats and show the sports of plates. A 9.5 ml chloroform + 0.5 ml methanol + 1 drop of n- hexane. Perform TLC and visualized in uv chamber for the separation of the sample [25].

LCMS(LIQUIDCHROMETOGRAPHY-MASS SPECTROSOCPY): -

The samples were analyzed using an ACQUITY UPLCTM system for water (Waters; Milford, MA, USA), that was coupled to a triple quadrupole-ESI hybrid source. The chromatographic separation was done using a SUNFIRE C18 column (250 mm 4.6 mm, 5m) and positive mode LC-ESI-MS/MS. The stage of mobility was (A) ethyl nitrile and (B) 5mm ammonium ethanoate in 1.5 % methanol, with a gradient system of 95% B in 0–1 minute, 70% B in 1–10 minute, 40 % B in 10–14 minute, 40 % B in 14–16 minute, 20 % B in 16–24 minute, 20 % B in 24–32 minute, 95 % B in 32–35 minute, 95 % B in 35–40 minute, 95 as a nebulizing and drying gas, nitrogen gas was employed. For each investigation, the narrow voltage of the ESI source potential was 3.5 kV, and the cone potential was 30 V. Temperatures of 125°C for the supply and 350°C for the desolation were used into a positive ionization mode, electro spray mass spectra from m/z one hundred to m/z one thousand were noninheritable. The information was assembled and processed using the Mass wildcat V4.1 SCN 714 software package.

Detector name: - thermo ION trap

Method: - Approx. concentration

Sheat gas flow: - 15 Arb

Spray volume: - 5kv

APPLICATION OF CARDIAC GLYCOSIDE: -

Anti- Microbial [Microbiological assay]

Diffusion of agar wells methodology was wont to verify antibacterial properties of isolated steroid present in our Curry leaves extraction. The numerous microorganisms found throughout this investigation were thoroughly cultivated on starch agar. A swab was used to transfer microbe colonies to agar plats, and the muddiness was visually adjusted with the broth to match that of a vortexed 0.5 McFarland turbidity typical. among fifteen min od altering the inoculant turbidity standard of McFarland 0.5, to remove excess inoculant, a sterile cotton swab was swayed back into the inoculum and alternated against the tube wall on top of the liquid. The whole surface of the agar plate was swabbed 3 times with a cotton swab to transfer the inoculum, and the plates were rotated by almost 60° between streaks to ensure uniform dispersion. For all microorganisms, the inoculum preparation and culture medium vaccination procedures remained the same. Every microbe was infected with a steroid extracted from curry leaves. Consequently, an entire set of plates was infected and left to square for at least three minutes but no more than 15 minutes before producing wells for several permutations to be formed. On the apex of the infected agar plates, a hollow cylinder with a 5 mm period was warmed and ironed. It was immediately eliminated by means of making a well inside the plate; also, one well was formed on each plate, one for beneficial control, poor control, and extraction of curry leaves [figure]. Every properly acquired 5 µl of a specific substance. After 15 minutes of chemical

utility, plates had been incubated at 37°C in an apparatus. For each antibacterial antibiotic [E-coil], microbe, incubation was modified to 24 h in hot water. Plates were incubated within the dessert apple and Filde's antibacterial jar for antibacterial organisms, while microorganisms were transformed into genteel inside the apparatus at 37°C for 48 hours. Plates were browsed once the incubation period was ended and the boom sector had become convergent or almost confluent. A Vernier caliper was used to measure the diameter of the inhibitory zone to the next full metric linear unit. The effects have been in comparison to the [Streptomycin] reference values [26].

Anti-fungal properties: -

natural cultures of every of the selected fungal species had been grown individually on potato dextrose agar (PDA). For the antifungal test, these pure cultures were employed.

Antifungal Activity assay: -

The disc diffusion technique was used to test antifungal activity. The medium was initially made by liquifying In distilled water, autoclave potato dextrose agar for 15 minutes at 121℃. For the antifungal experiment, 20 ml of sterile PDA medium was put into sterilized Petri dishes and allowed to harden. Spore interruption was made in 0.9 % salty water and familiar to a last concentration of 1.5 10^5 cfu/ml. The solution of cardiac glycosides was diluted. On the disinfected plates containing PDA, a 1–week-old fungal culture plug (candida) was inserted in the center. Approximately 10 liters of material were injected onto sterile disc sheets. The discs were then put on top of the fermentation medium. After 4-5 days of incubation at 300 C, the colony diameter was measured and reported. A fungal strain's growth inhibition was determined. A Vernier caliper [diameter 18 mm] was used to measure the inhibitory zone's diameter to the closest entire millimeter [10].

Anti-Oxidant properties: -

Assay for radical scavenging using ABTS
Decolorization of the radical cation ABTS test [5] was used to assess the ability of plant materials to scavenge free radicals. The ABTS + cationic radical was created by combining 7 mg of ABTS in water with 2.45 milligrams of atomic number 19 persulfate (1:1) and keeping it for 1216 hours before use. The ABTS+ solution changed into then diluted with methanol to provide a 734 nm absorbance of 700 nm.
The absorbance was measured 30 minutes after the initial mixing of 5 liters of plant extract with 3.995 liters of diluted ABTS+ solution. Each experiment was carried out with a solvent blank. At least three times, all of the measurements were completed. ABTS+ scavenging effect (%) = ((AB–AA)/ AB)100 (2), in which AB is the absorbance of the ABTS radical + methanol, and AA is the absorbance of the ABTS radical + pattern extract/well known. As a control drug, Trolox was employed [28].

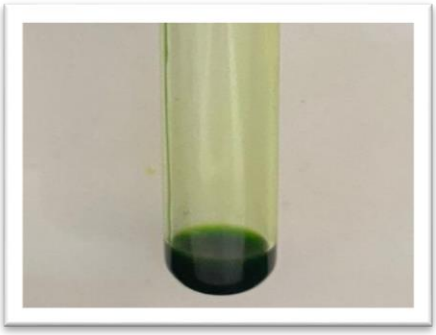
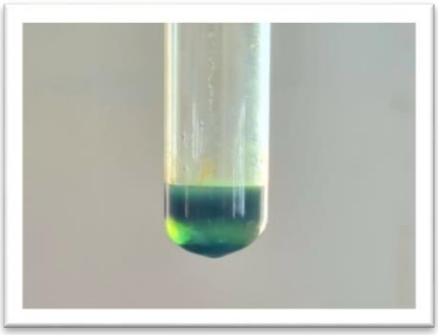
RESULT AND DISCUSSION: -

Identification test of Cardiac glycoside: -

Cardiac glycosides [Keller killiani’s test]

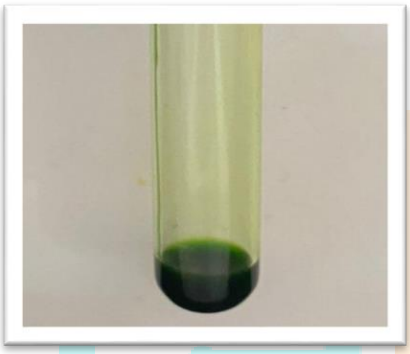
One drop of ferric chloride solution was added to 1 mL acetic acid (glacial), and 1 ml of strong sulphuric acid was added. The presence of cardiac glycosides was revealed by a brown colour ring at the interface.

Identification test in methanol extract [Dry Leaves]

Take 2-3 ml extract in one test tube and add one ml glacial acetic acid contain 1 drop of ferric chloride and then added one drop of concentrated sulfuric acid. If cardiac glycoside present brown ring obtains. In this methanolic extract brown ring is obtain. So cardiac glycoside is present.

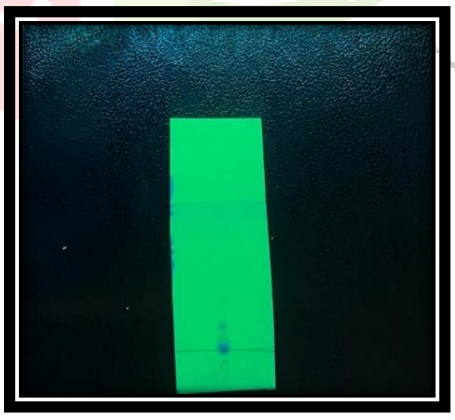
Identification test in methanol extract [Fresh Leaves]




Take 2-3 ml extract in one test tube and add one ml glacial acetic acid contain 1 drop of ferric chloride and then added one drop of concentrated sulfuric acid. If cardiac glycoside present brown ring obtains. In this methanolic extract brown ring is obtain.
So cardiac glycoside is present.

RESULTS FOR THE CHROMETOGRAPHY: - [TLC]

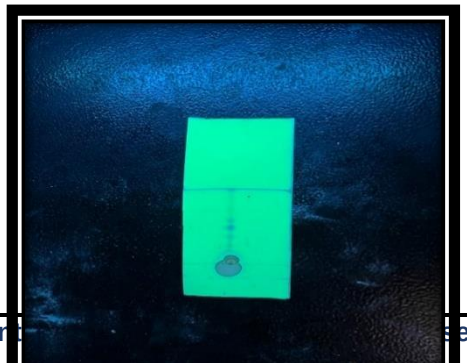
[1].



SEPARATION OF CARDIAC GLYCOSIDE

[MOBILE PHASE METHANOL]

[2]

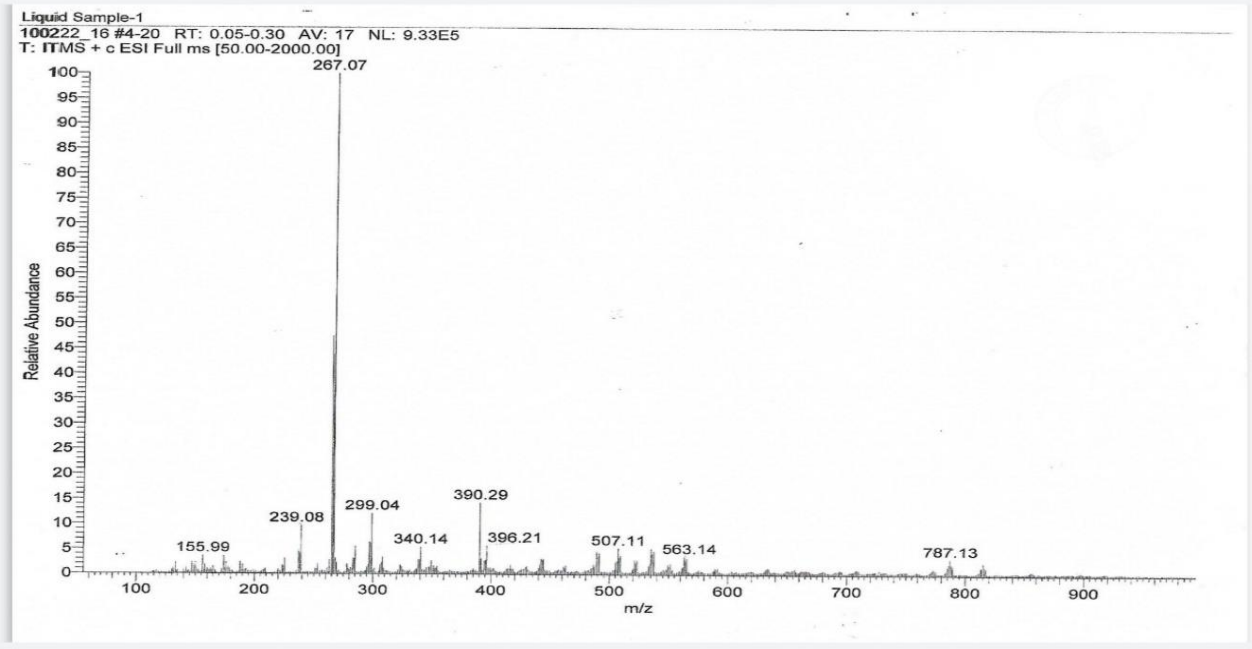


BETTER SEPRECTION OF CATDIAC GLYCOSIDE [MOBILE PHASE 9.5 ML CHLOROFORM+0.5 MLMETHANOL + 1 DROP OF N-HEXANE]

RESULT OF LCMS (LIQUID CHROMETOGRAPHY-MASS SPECTROSCOPHY): -

Sr No.	Parameters	Units	Specification	Result
1	Identification	-	-	Liquid extract in methanol from Murraya koenigii
2	Colour	-	-	Colour less
3	Solubility	-	-	Miscible in water

Sr No.	Name	Chemical Formula	M.W (g/mol)	Relative abundance
1	Bufalin	$C_{24}H_{34}O_4$	386.532	390.29
2	Digoxin	$C_{41}H_{64}O_{45}$	780.949	787.13
3	Oleandrin	$C_{32}H_{48}O_9$	576.727	507.11
4	Ouabin	$C_{29}H_{44}O_{12}$	584.659	563.14



LCMS ANALYSIS

Result of LCMS: -

Liquid Chromatography Mass Spectrometry (LC-MS/MS) is a fantastically touchy and specific analytical method for determining the identities and concentrations of chemicals in your pattern. Observe the chromatograms show additives as a characteristic of retention time and mass to rate ratio through mass relative abundance, as a consequence that the general output from a complete LC-MS is a graph with two horizontal axes. According on the researcher's aim, the graph's focus may be adjusted. In LCMS graph at a relative abundance of cardiac glycoside 390.29 – Bufalin, 787.13 – Digoxin, 507.11 – Oleandrin, 563.14 - Ouabin shown in chromatogram.

RESUTLS FOR THE APPLICATION OF CARDIAC GLYCOSIDE: -

Anti – Microbial: -

SAMPLE REFERENCE

Results of antibacterial activity: -

According to the antibacterial activity test results of the cardiac glycoside by assay method, the cardiac glycoside were effective against E. coli. The antibacterial activity of cardiac glycoside methanolic extraction was shown to be most effective against bacteria with a zone of inhibition of 10mm. The presence of a bioactive component in the extract of medicinal plants was suggested by the antibacterial activity. The greatest activity against E. coli was found in the cardiac glycoside extracted from methanolic curry leaves extraction, as shown in Figure.

Anti-fungal properties: -

Result of anti-fungal activity: -

According to the antifungal activity test results of the cardiac glycoside by assay method, the cardiac glycoside were effective against candida. The antibacterial activity of Cardiac glycoside methanolic extraction was shown to be most effective against bacteria with a zone of inhibition of 18mm. The presence of a bioactive component in the extract of medicinal plants was suggested by the antibacterial activity. The

greatest activity against candida was found in the cardiac glycoside extracted from methanolic curry leaves extraction, as shown in Figure.

Result of anti-oxidant activity: -

According to the antioxidant activity test results of the cardiac glycoside by ABTS assay method the curry leaves extract was effective. The antioxidant activity of curry leaf methanol extract was shown to be also most effective and 80% Antioxidant property contain.

Antifungal and Antioxidant table: -

Sr no	Parameters	Unit	Specification	Result
1	Identification	_	_	Liquid extract in methanol from Murraya koenigii
2	Colour	_		Colour less
3	Antifungal Activity	Mm	Zone	18
4	Antioxidant activity	%	ABTS assay, At ambient temperature	86%

CONCLUSION: -

Murraya koenigii was one of our medicinal herbs. This paper summarizes the therapeutic applications, several health benefit, phytochemistry, and pharmacological characteristics of M. koenigii. Among additional bioactive substances found in M. koenigii include alkaloids, polyphenols, terpenoids, and flavonoids, cardiac glycoside. Antibacterial, antifungal, and antioxidant activities have been found in M. koenigii and its derivatives. Cardiac glycoside was variety of medicinal properties and therapeutic application. Isolation of cardiac glycoside and their biological activity. Quantification and identification of cardiac glycoside was done by LCMS and TLC (chromatographic technique). I conclude that cardiac glycoside separation and qualities such as antibacterial, antifungal, and antioxidant activity were conducted in this study. As a result, the price of those useful vegetation ought to be underscored and the bioactive components of Murraya Koenigii need to be further researched and used against illnesses that broaden resistance, to which resistance has developed, as well as synergistic research.

ACKNOWLEDGEMENT: -

I would like to express my gratitude to my supervisor Dr. Mittal Thakkar, Assistant Professor, Department of chemistry. I would like to express my gratitude and sincere appreciation for her guidance, valuable care, supervision, encouragement, and kindness throughout this study. I convey my special acknowledgment to Parul University, for providing all essential facilities required for the dissertation work as well as to complete the M.Sc. course. I offer sincere thanks to Dr. Trilok Akhani, Principal of Parul institute of applied science for providing me necessary infrastructure. I also take this opportunity to give my thank note to Dr. Kushant Parikh [H.O.D] for their nice timely support. I would like to thank my friend Ms. Vishmita Raulji for her timely co-operation in my dissertation work as well as my M.Sc. tenure. I am also thankful to my lab mates Ankush Patel, Shreya Kachhiya, and Vishal Patel for their cheerful cooperation during their entire tenure.

REFRENCES: -

[1] Dr. Rajvanshi, Dr. Kusum mittal "Phytochemical Analysis of Curry leaves," International Journal of science and research, 2018

[2] Dr suman Singh, P. K. more and Sandhya Madan Mohan "CURRY LEAVES (Murraya koenigii linn. spengal)- A MIRICAL PLANT," Indian J. Sci Rec. 4, vol. 1, pp. 46-52, 2014

[3] Human AL herbi, Dr. Uma M. Irfan and Dr.Sarah Ali "THE ANTIBACTERIAL EFFRECT OF CURRY LEAVRES (Murraya koenigii)" EUROPEN JOURNAL OF PHARMACEUTICAL, vol 3, no10 ,pp, 382 – 387, 2016

[4] CE Igara , DA Omoboyowa, A A Ahuchaoug, NU Orji and MK ndukwe "Phytochemical and nutritional profile of Murraya koenigii (linn) Spreng leaf" Journal of Pharmacognosy and phytochemistry, vol 1, no 2, pp. 07 – 09, 2016

[5] Prasan. R. Bhandari, "Curry leaf (Murraya Koenigii) or Cure leaf: Review of its curative Properties," Review of its curative properties," vol. 1, no 2, pp. 92 – 97, 2012

[6] A. B. Sharangi, S. Guha "Wonders of leafy spices: Medicinal proper ties Ensuring Human Health" Science International 1 (9), pp. 312-317, 2013

[7] Mini Priya Rajendran, Blessed Beautlin Pallaiyan, Nija Selvaraj "Chemical composition, Antibacterial and antioxidant profile of essential oil from Murraya koenigii (L.) leaves" Avicenna Journal of Phytomedicine (AJP), vol. 4, No.3, pp. 200-214, 2014

[8] Muthulinggam Nishan, Partiban Subramanian "Murraya Koenigii (Curry leave)- A review on its potential." International journal of Pharma Tech Research, vol.7, No. 4, pp. 566-572

[9] Satish Chand Saini, Dr Gopu Bala Show Reddy "A Review on Curry Leaves (Murraya Koenigii): Versatile Multi-Potential Medicinal Plant" American Journal of Phytomedicine and Clinical Therapeutics (AJPCT), vol.3, No. 04, pp. 363-368

[10] YC Tripath, Nishat Anjum, Ashish Rana "Chemical Composition and In vitro Antifungal and antioxidant Activities of Essential Oil from Murray koenigii (L.) Spreng. Leaves" Asian Journal of Biomedical and Pharmaceutical Sciences. Vol. 8 pp. 7-11

[11] Sinha Parul, Akhtar Javed, Batra Neha, Jain Honey, Bhardwaj Anuj "Curry leaves–A medicinal herb" Asian Journal of Pharmaceutical Research 2 (2), 51-53, 2012

[12] PCD Perera, N Dahanayake "Current Status and Future Prospect of Curry (Murraya koenigii) Leaves in South Asia" Journal of AgriSearch 2 (3), 212-217, 2015

[13] Salve RV, Syed HM, More SG and Shinde EM " Effect of different drying treatment on composition, nutritional and phytochemical content of curry leaves" The Pharma Innovation Journal 2020; 9(7): 584-589

[14] Shah Rajesh Kumar, Das Loveleena, Sangma Godwin " Medicinal property of Murraya koenigii-a review" International Research Journal of Biological Sciences 2 (9), 80-83, 2013

[15] Syed Muhammad Nouman, Aamir Shehzad, Masood Sadiq Butt, Muhammad Issa Khan, Mahwish Tanveer "Phytochemical profiling of curry (Murraya koenigii) leaves and its health benefits" Pak. J. Food Sci 25 (4), 204-215, 2015

[16] Ammar Altemimi 1, Naoufal Lakhssassi, Azam Baharlouei, Dennis G. Watson 2 and David A. Lightfoot "Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts" Plants 2017, 6, 42

[17] Salve RV, Syed HM, More SG and Shinde EM "Effect of different drying treatment on composition, nutritional and phytochemical content of curry leaves" The Pharma Innovation Journal 2020; 9(7): 584-589

[18] Ajay Kumar, Shipra Nitin, Narottam Singh "Pharmacognostical, Phytochemical and Pharmacological Evaluation of Murraya koenigii (Curry Tree)" JUL 2021, IRE Journals, Volume 5 (1)

[19] Radheshyam Sharma, Umesh Kumar **"**Exploration and Phytochemical Estimation of *Murraya koenigii* Leaves for Pharmaceutical Applications" Asian J. Pharm. Res. 2019; 9(3):159-168.

[20] https://www.intechopen.com/chapters/53014

[21] Dj Radford, K Cheung, R Urech, Ir Gollogly and Duffy "Immunological detection of cardiac glycoside in plant" Australian Veterinary Journal. Vol.71 (8) August 1994

[22] J. Radenkova-Saeva1 and P. Atanasov "Cardiac glycoside plants self-poisoning" Acta Medica Bulgarica, Vol. XLI, 2014, № 1

[23] Charusheela Ramteke, Tapan Chakrabarti, Berjaya Ketan Sarangi, and Ram-Avatar Pandey "Synthesis of Silver Nanoparticles from the Aqueous Extract of Leaves of Ocimum sanctum for Enhanced Antibacterial Activity" Journal of Chemistry Volume 2013, 7pages

[24] "Cardiac Glycosides in Medicinal Plants" Aromatic and Medicinal Plants - Back to Nature

[25] B. J. WHITE1 AND DENNIS 0ETH "Separation of Cardiac Glycosides by Thin-Layer Chromatography Proceedings of the Iowa Academy of Science", Vol. 73 [1966], No. 1,

[26] Loannis Prassas and Eleftheriose p. Diamandis "Novel therapeutic application of cardiac glycoside" Nature Reviews Drug Discovery, vol.7 pp, 926-935, 2008

[27] A. McVANN, I. HAVLIK, P. H. JOUBERT, F. S. E. MONTEAGUDO "Cardiac glycoside poisoning involved in deaths from traditional medicines" SAfrMedJ 1992; 81: 139-141

[28] Dimitrina Zheleva-Dimitrova, Paraskev Nedialkov, Gerassim Kitanov "Radical scavenging and antioxidant activities of methanolic extracts from hypericum species growing in Bulgaria" Pharmacognosy Magazine, vol.6(22), April 2010