Garla Venkateswarlu/ Afr.J.Bio.Sc. 6(5) (2024). 5871-5887 ISSN: 2663-2187

https://doi.org/ 10.33472/AFJBS.6.5.2024. 5871-5887

AFJBS

African Journal of Biological Sciences

PHYTOCHEMICAL EVALUATION AND GC-MS ANALYSIS OF ETHANOLIC LEAVES EXTRACT OF *STRYCHNOS COLUBRINA*.

Garla Venkateswarlu[1], Ramesh Bodla[1*]

[1]Dept. of Pharmaceutical Chemistry, Delhi Institute of Pharmaceutical Sciences and Research, Delhi Pharmaceutical Sciences and Research University, New Delhi-110017, India.

[1]*Address for correspondence: Dr. Ramesh Bodla

Associate Professor, Dept. of Pharmaceutical Chemistry,

Delhi Institute of Pharmaceutical Sciences and Research,

Delhi Pharmaceutical Sciences and Research University, New Delhi-110017.

E-mail id- rameshbodla@gmail.com

Article History
Volume 6, Issue 5, 2024
Received: 09 May 2024
Accepted: 17 May 2024
doi: *10.33472/AFJBS.6.5.2024. 5871-5887*

Abstract:

Medicinal plants play a foremost role in the basic healthcare systems of countries. The therapeutic properties of the plant are determined by the biological substances present in the plant components that are used to produce medicinal remedies.

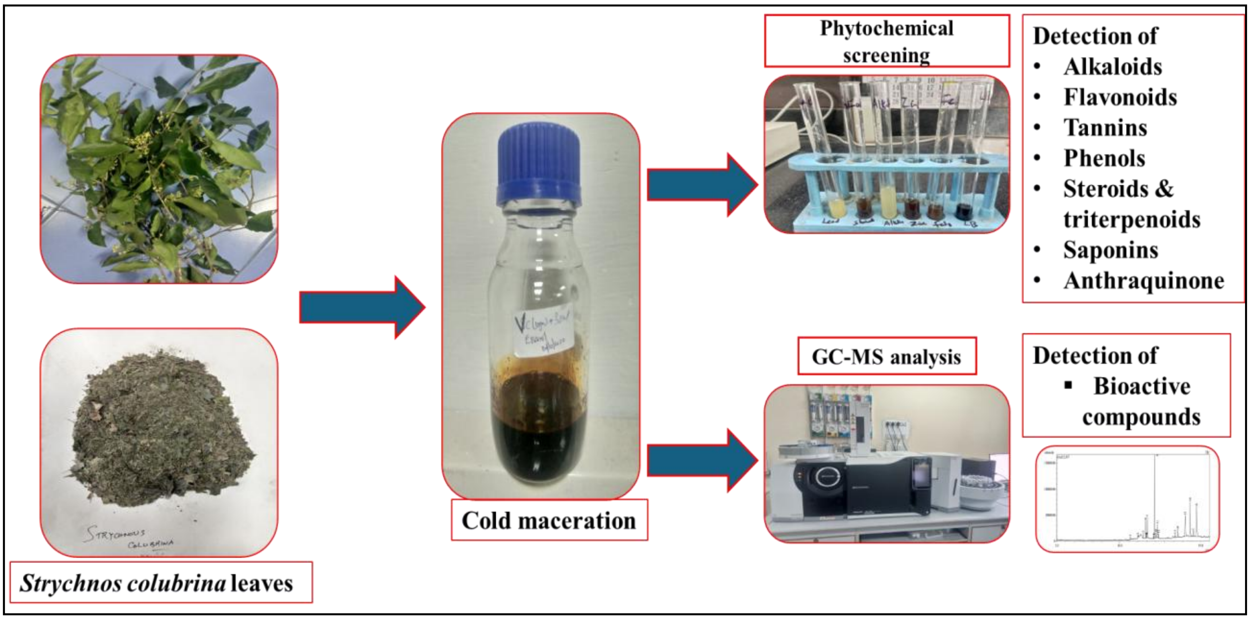
Strychnos colubrina belongs to the Loganiaceace family. It is generally spread in the Chittoor District exactly at Kambakkam hills, Ambakkam, Sadhumalamma kotta, Bramhadevudigundem (Mamandur). Traditionally, plant have been significantly used to treat fever, rheumatism, anthelmintic, cutaneous disorders, and mania. Objective: The current research aimed to explore the phytochemical constituents of the leaves of Strychnos colubrina through phytochemical evaluation, and gas chromatography-mass spectrometry (GC-MS) analysis. Methods: The shade-dried leaves were powdered and extracted with ethanol using the cold maceration method; we conducted a phytochemical examination to evaluate the characteristics of secondary metabolites and used gas chromatography-mass spectrometry (GC-MS) to find the individual phytocompounds in the ethanolic leaf extract. Phytochemicals were determined using molecular weights (m/z) obtained from GC-MS chromatograms. Phytocompounds were identified using the NIST library and spectral peak interpretation.

Results: The phytochemical evaluation detected the presence of alkaloids, flavonoids, tannins, phenols, steroids, glycosides, and anthraquinone. GC-MS analysis, eighteen various phytochemical compounds were found in Strychnos colubrina leaf extract. The percentage of main bioactive compounds were n-Hexadecanoic acid (5.84%), Hexadecanoic acid, ethyl ester (5.00%), Phytol (13.867%), Ethyl. Alpha-linolenate (5.37%), gamma.

-Sitosterol (13.22%), squalene (11.58%), Lupeol (20.06%) was observed as the 7 major constituents in the ethanol extract, the six minor constituents such as 1, 2-O-Isopropylidene-D-xylofuranose, TBDMS derivative (1.47%), Hexadecanoic acid, methyl ester (1.22%), alpha. - Linolenic acid (2.26%), Glycerol. Beta. - palmitate (1.16%), Stigmasterol (4.40%), beta. -Amyrone (2.76%). The detection of bioactive compounds is based on the retention time, peak area, molecular formula, and probability. Conclusion: After the results, it could be determined that Strychnos colubrina may have anti-cancer, anti-diabetic, antioxidant, anti-microbial, and hepatoprotective and hypocholesterolemic due to the presence of secondary metabolites in the ethanol extract.

Keywords: Strychnos colubrina, phytochemical, GC-MS analysis, ethanol extract.

Graphical abstract:



1. INTRODUCTION:

Plants have discovered a rich reservoir of natural resources in the form of herbal remedies. Several medicinal plants have long been utilised in traditional medicine to cure a number of illnesses. Plant-based therapies have demonstrated efficacy in treating and controlling illnesses and are widely utilised in ethnomedicinal traditions [1-3]. The exorbitant expense of conventional medications and their restricted accessibility, particularly in rural populations worldwide and specifically in developing nations, have led to a persistent reliance on traditional therapies. Approximately 75-90% of the global population continues to depend on plants and plant extracts as their major source of healthcare. The extensive use of plant-based medications for treating diseases has generated a global interest in evaluating the bioactive phytochemical components and pharmacological effectiveness of medicinal plants. The majority of medicinal plant components are utilised as unprocessed pharmaceuticals and have been documented to exhibit diverse therapeutic qualities [4, 5]. Medicinal plants, adaptable to various climates, provide both treatments and livelihoods for many rural areas. Their chemical compounds confirm their therapeutic potency. The plant resources are providing us essential raw material for food, shelter, climatic balance and medicine. The existence of life on earth cannot be imagined without plants. The medicinal value of plant kingdom is only partially exploited. The various parts of the plants such as leaves, fruits, barks, roots, rhizomes or even flowers are used as medicines. All these plant parts are packed with active biomolecules like flavonoids, sterols, terpenes, nucleic acids, saponins, glycosides, alkaloids etc.

In traditional medicine, medicinal plants are considered an essential component of drugs for treatment. The kingdom of plants is an ideal spot for finding new potential medications, and the importance of medicinal plants has come to prominence in recent years. Medicinal plants generate a diversity of bioactive molecules and are significant providers of several medicinal compounds. Herbal plant extracts are immensely helpful as well as one of the main forms of medicine. These serve as vital to fostering production and preventing an array of illnesses. These are the lower-cost sources for effective therapies and treatments for multiple infections. Because they hold an extensive number of secondary metabolites, like flavonoids, alkaloids, phenolics, and tannins, which enhance innate immune response, growth, and resistance to disease toward pathogenic microorganisms in humans and other organisms, extracts from medicinal plants have recently gained attention as a viable substitute. Approximately 80% of people in affluent nations utilize a variety of medicinal plants as traditional medicines, such as antifungals, anticancer medications, and antibacterial pharmaceuticals, in a variety of ways. Secondary metabolites, which are incredibly varied chemically and taxonomically with unknown roles, are abundant in medicinal plants. Numerous phytochemicals are employed extensively in scientific study, veterinary medicine, agriculture, and human therapy [6-9].

Strychnos colubrina, an endemic tree in peninsular India, is a large, climbing woody shrub with bifid tendrils. Its ovate-elliptic leaves are undulate or entire, acute or acuminate, rounded at the base, 3-nerved, coriaceous, and shiny. Flowers grow in cymes with corolla tube and lobes of equal length, and a woolly throat. The hirsute ovary and style measure 2.5-3 mm across. Creamy white flowers are 2-3 cm long. Axillary and old wood cymes produce globose orange-yellow berries, 1-1.8 cm across, with crustaceous pericarp. Seeds are 1-3, circular, flat, and 0.6-1.2 cm in diameter. Flowering and fruiting occur from September to November. The roots used in dyspepsia, intermittent fevers, malaria, swellings in chicken pox, joint discomfort, and diarrhoea, snake bite. The fresh leaves used in tumor and the bark is used as a febrifuge, dyspepsia, intermittent fever, and malarial cachexia. Fruits used in the treatment of mania. The stem decoction is used as a therapy for whooping cough, the dried leaf powder is smoked to alleviate asthma, the leaf paste is applied to cleanse scars from pox wounds, the root bark is employed to lower fever, fresh twigs serve as insecticides, and the dry leaf powder is utilised to preserve food grains.

Native healers also suggest using leaf extract to prevent the rise in blood sugar levels. It is employed as a traditional remedy for curing headaches and catarrh. The pharmacological studies exposed anti- depressant, analgesic, anti-inflammatory, anti- parkinsonian, and anti- microbial activities. The anti-inflammatory and wound healing activities of aqueous and ethanolic extracts of *Strychnos colubrina* leaves have been examined using an acute inflammation paradigm. This research presents phytochemical evaluation and GC-MS analysis of bioactive compounds in the ethanolic leaf extract of *Strychnos colubrina* [10-17].

2. MATERIALS AND METHODS

2.1. Plant material.

Collection and identification of plant material

Strychnos colubrina used for the research was collected from Tirupati, Andhra Pradesh, India. The plant was authenticated by Dr. Sankararao Mudadla, Botanical Survey of India, Hyderabad. The voucher number is BSI/DRC/2021-22/Tech./Identification/522. The botanical specimen of *Strychnos colubrina* was cleansed using tap water and dried by exposure to air.

Fig: 1. *Strychnos colubrina*

2.2. Preparation of extract

The plant material was dried and ground to powder in a mechanical grinder. The leaf powder of *Strychnos colubrina* was weighed, immersed in ethanol, incubated for 72 hours, and filtered through Whatman No.41 paper. The extracts were concentrated by evaporating under reduced pressure with a rotary evaporator. Prior to conducting phytochemical and GC/MS analysis, the concentrated extracts were stored at 4°C in an airtight container [18-20].

2.3. Phytochemical evaluation:

The ethanolic leaf extract of *Strychnos colubrina* was qualitatively analyzed for secondary metabolites using standard methods [21-25].

2.4. GC/MS Experimental System and Measurements

GC/MS analysis was conducted using a Shimadzu TQ8040 NX GS-MS instrument coupled with a silica capillary column TG-5-MS (30.0 m×0.25 mm, film thickness 0.25µm). For GC/MS detection, an electron impact ionization system with an ionizing energy of 70 eV was used, with a scanning mass range set at 29–400 (m/z). Helium carrier gas with a flow rate of linear velocity (41.4 cm/s) was utilized. Prior to initiating the phytochemical analysis, the oven temperature was set at 60°C for a duration of 1 minute. After this phase, the temperature was increased to 300°C at a rate of 3°C/min and maintained isothermally for 15 minutes. The temperature specifications for the injector port, ion source, and detector were 280°C, 220°C, and 280°C, respectively. The total GC run time was 20 minutes. The NIST Library database was employed to retrieve the components' names, molecular weights, and structures [26-28].

2.5. Statistical Analysis:

All qualitative tests/analyses were done in triplicate.

3. RESULTS AND DISCUSSION

3.1. Qualitative phytochemical screening

The qualitative analysis *Strychnos colubrina* ethanolic extract showed the presence of phytochemical constituents which is shown in Table 3.

Table 1: Qualitative phytochemical analysis of *Strychnos colubrina* leaf extract

S. No	Phytochemicals	Tests/Reagents	Results
1	Alkaloids	Mayer’s test, Wagner’s test, Hager’s test, Dragendorff’s test, Tannic acid test	+
2	Flavonoids	Lead acetate test, Shinoda test, Alkaline test, Zinc Hcl test	+
3	Tannins & Phenols	$Fecl_3$ test, Lead acetate test	+
4	Steroids	Libermann-Buchard test, Salkowski test	+
5	Anthocyanins	Anthocyanins test	**-**
6	Glycosides	Glycoside test	+
7	Saponins	Honeycomb test, Foam test	+

Garla Venkateswarlu / Afr.J.Bio.Sc. 6(5) (2024). 5871-5887

8	Anthraquinone	Bontrager's test	+

Note: + indicates the presence of constituents and – indicates the absence of constituents.

It is evident from the table.1 that the ethanol extract recorded the maximum number of chemical constituents including alkaloids, flavonoids, tannins, phenols, glycosides and anthraquinone. Presence of alkaloids compounds is of importance in pharmaceutical application as these compounds are responsible for several biological functions like antimalarial, antiasthma, anticancer, cholinomimetic, vasodilatory, antiarrhythmic, analgesic, and antibacterial in the human body. The presence of flavonoids, known to be effective free radical scavengers, indicates that this plant may have antioxidant qualities. Tannins and phenols are linked to anticancer, virucides, antioxidant, antimicrobial and anti-inflammatory, antidiabetic, wound healing, cardiovascular protection and antidiarrhoeics. Saponins have been associated in antiinflammatory. Glycosides have been shown to be linked to the reduction of blood pressure, saponins are responsible for antifungal, antiparasitic and antimicrobial and anthraquinones act as chemotherapeutic agent used for the treatment of secondary progressive, progressive relapsing, or worsening relapsing-remitting multiple sclerosis.

GC-MS analysis of *Strychnos colubrina*

Eighteen compounds have been detected by GC-MS analysis in the studies on the active constituents in the plant *Strychnos colubrina* leaf ethanolic extract. The active compounds with their retention time (RT), molecular formula, molecular weight (MW), and concentration (peak area%) are presented in Table-1. The GC-MS chromatogram of the eighteen peaks of the compounds detected is shown in Figure-1. The compounds which the mass spectroscopy identified were presented. The GC-MS revealed a total number of components in the ethanol extract. The results revealed that Phytol (19.72 %), Lupeol (20.06%), Squalene (11.58%) and gamma.-Sitosterol (13.22%) was found as the 4 major components in the ethanol extract, the seven minor compounds such as n-Hexadecanoic acid (5.84%) Hexadecanoic acid, ethyl ester (5.00%), Ethyl .alpha.-linolenate (5.37%), alpha.-Linolenic acid (2.26%), Stigmasterol (4.40%), and beta.-Amyrone (2.76%).

Garla Venkateswarlu / Afr.J.Bio.Sc. 6(5) (2024). 5871-5887

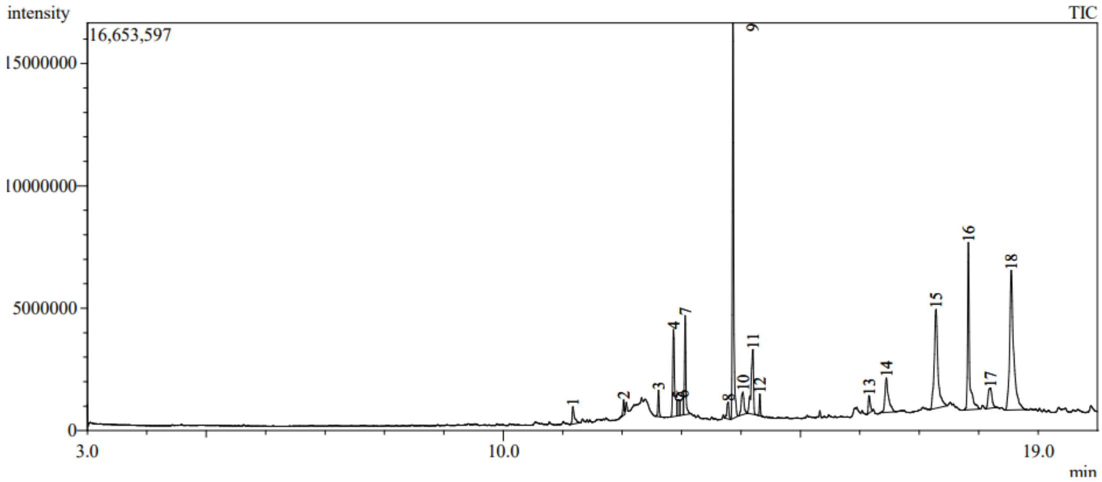


Fig:1 GC-MS spectral analysis of leaf ethanolic extract of *Strychnos colubrina*

Table:2 Bioactive compounds identified in the ethanolic extract of *Strychnos colubrina*

S. No	Bioactive compound name	Retention time (min)	Peak area %	Molecular formula	Similarity	Biological activity reported	Reference
1	1,2-O-Isopropylidene-D-xylofuranose, TBDMS derivative	11.170	1.47	$C_{14}H_{28}O_5Si$	72	Not reported	
2	Neophytadiene	12.023	0.77	$C_{20}H_{38}$	95	antioxidant compound, antipyretic, analgesic, anti-inflammatory, antimicrobial.	29
3	Hexadecanoic acid, methyl ester	12.612	1.22	$C_{17}H_{34}O_2$	96	Antimicrobial, Antioxidant, Antiandrogenic 5-Alphareductase inhibitor activity, anti-hypercholesterolemic	30

						property.	
4	n-Hexadecanoic acid	12.865	5.84	$C_{16}H_{32}O_2$	94	5-Alpha reductase inhibitor activity, Anti-inflammatory property. Antioxidant, Hypocholesterolemic,	31
5	Benzoic acid, 4-methyl-2-trimethylsilyloxy-, trimethylsilyl ester	12.944	1.29	$C_{14}H_{24}O_3Si_2$	55	Anti inflammatory	32
6	trans-2-undecenoic acid	13.020	1.73	$C_{11}H_{20}O_2$	76	Antifungal, antibacterial	33
7	Hexadecanoic acid, ethyl ester	13.061	5.00	$C_{18}H_{36}O_2$	94	Anti-bacterial, Antitumour, Antifungal,	34
8	Linolenic acid, methyl ester	13.784	1.24	$C_{19}H_{32}O_2$	92	antifungal, cytotoxic, antioxidant, antibacterial.	35
9	Phytol	13.867	19.72	$C_{20}H_{40}O$	97	Anticancer, Anticonvulsant activity Anti-diabetic, Antimicrobial, Anti-diuretic properties, Neuroprotective, Antioxidant, Antiinflammatory Antidepressant,	36

10	alpha. - Linolenic acid	14.034	2.26	$C_{18}H_{30}O_2$	91	Anticancer	37
11	Ethyl. alpha. - linolenate	14.198	5.37	$C_{20}H_{34}O_2$	96	Antimicrobial	38.
12	Octadecanoic acid, ethyl ester	14.317	0.90	$C_{20}H_{40}O_2$	94	Antibacterial	39
13	Glycerol. beta. -palmitate	16.155	1.16	$C_{19}H_{38}O_4$	93	Anti inflammatory	40
14	Stigmasterol	16.447	4.40	$C_{29}H_{48}O$	85	Neuroprotective, anti-osteoarthritis, anticancer, anti-diabetic, anti-inflammatory, antiparasitic, immunomodulatory, antifungal, antioxidant antibacterial,	41
15	gamma. - Sitosterol	17.280	13.22	$C_{29}H_{50}O$	80	Antifungal, antibacterial	42
16	Squalene	17.826	11.58	$C_{30}H_{50}$	95	Antitumor, antioxidant	43
17	beta. - Amyrone	18.194	2.76	$C_{30}H_{48}O$	84	Antifungal	44
18	Lupeol	18.547	20.06	$C_{30}H_{50}O$	91	Antiprotozoal, anticancer, anti-inflammatory.	45

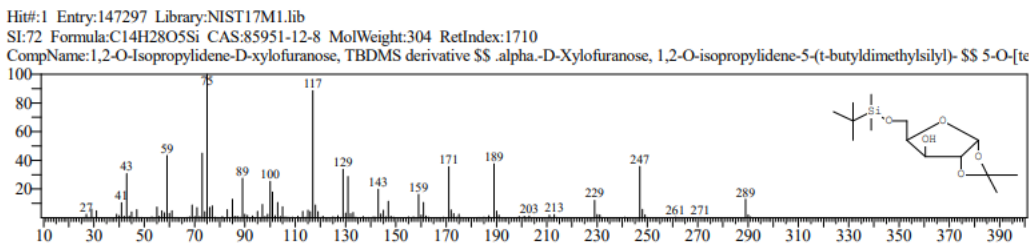


Fig: 2 GC-MS spectra of 1,2-O-Isopropylidene-D-xylofuranose, TBDMS derivative

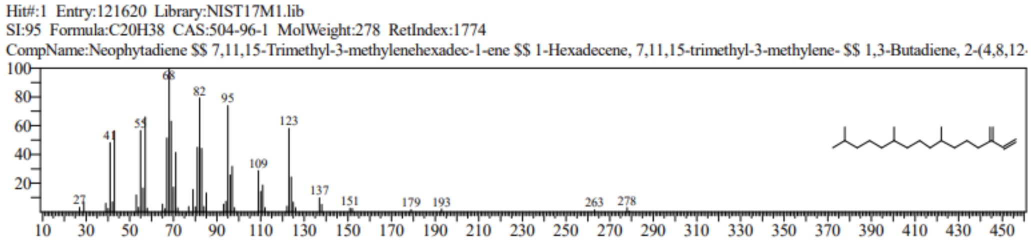


Fig: 3 GC-MS spectra of Neophytadiene

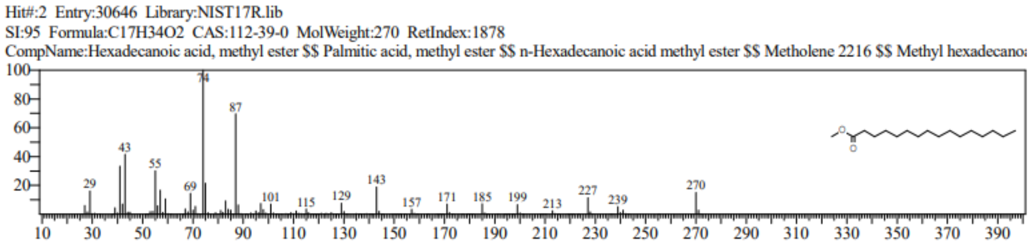


Fig: 4 GC-MS spectra of Hexadecanoic acid, methyl ester

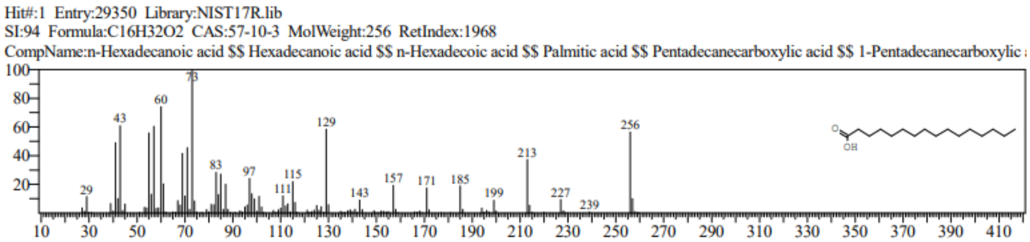


Fig: 5 GC-MS spectra of n-Hexadecanoic acid

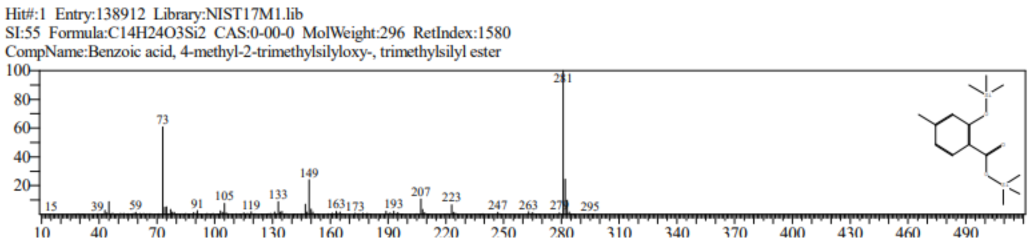


Fig: 6 GC-MS spectra of Benzoic acid, 4-methyl-2-trimethylsilyloxy-, trimethylsilyl ester

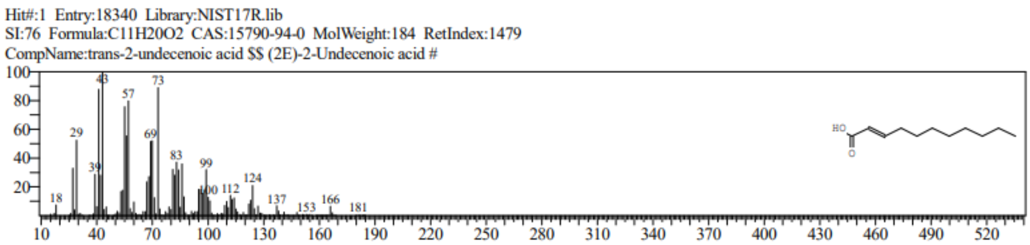


Fig: 7 GC-MS spectra of trans-2-undecenoic acid

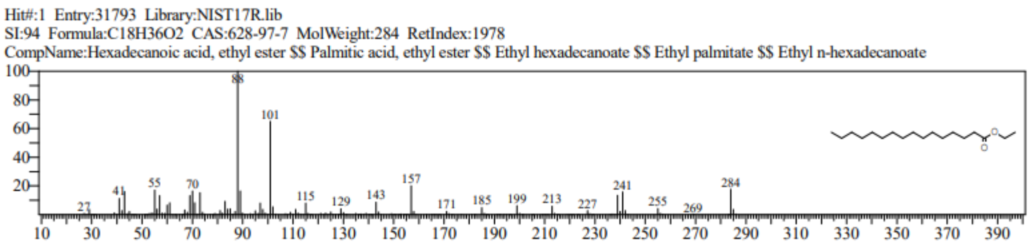


Fig: 8 GC-MS spectra of Hexadecanoic acid, ethyl ester

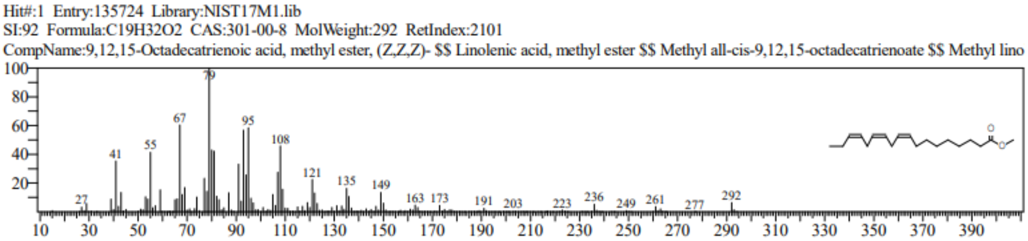


Fig: 9 GC-MS spectra of Linolenic acid, methyl ester

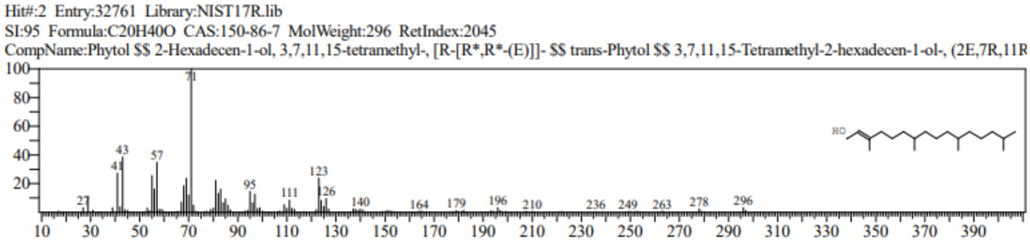


Fig: 10 GC-MS spectra of Phytol

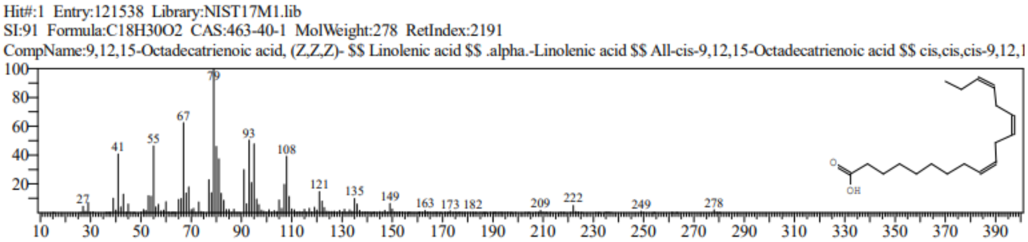


Fig: 11 GC-MS spectra of alpha. -Linolenic acid

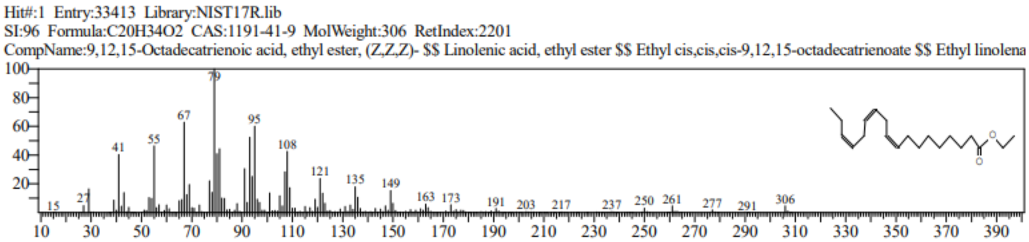


Fig: 12 GC-MS spectra of Ethyl. alpha. -linolenate

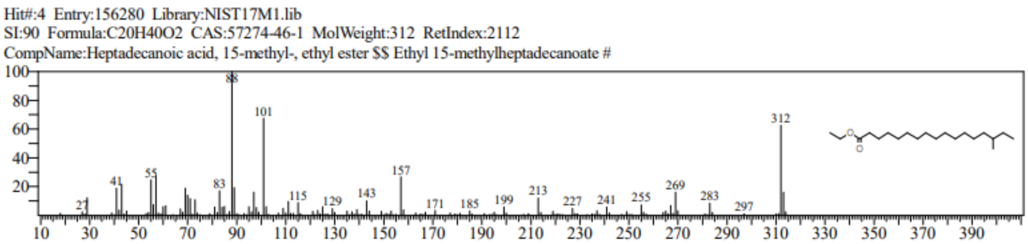


Fig: 13 GC-MS spectra of Octadecanoic acid, ethyl ester

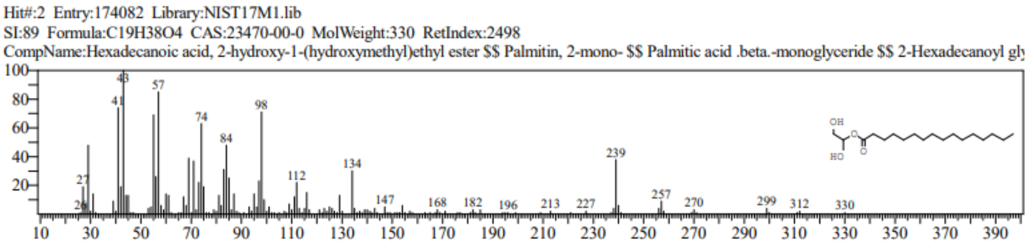


Fig: 14 GC-MS spectra of Glycerol. beta. -palmitate

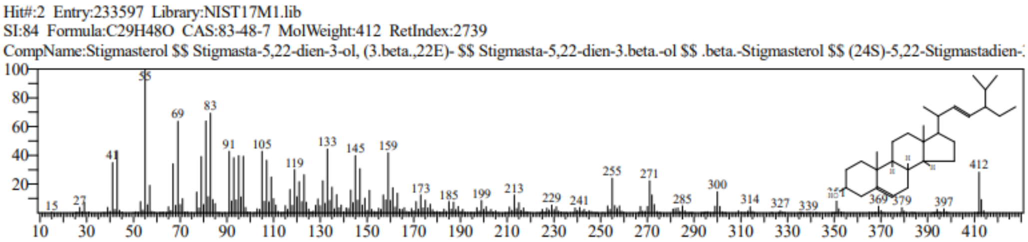


Fig: 15 GC-MS spectra of Stigmasterol

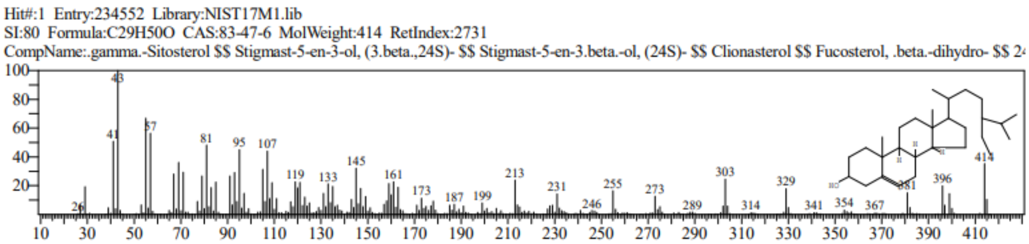


Fig: 16 GC-MS spectra of gamma. -Sitosterol

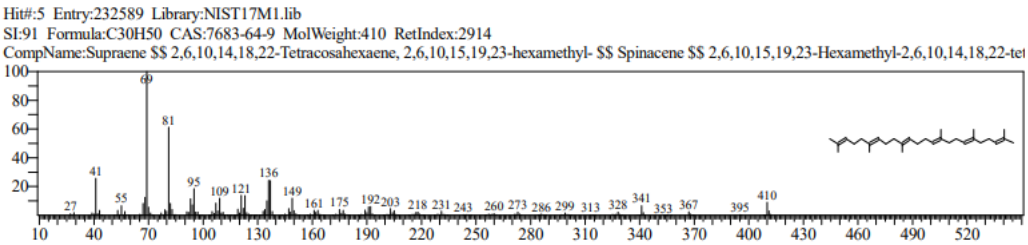


Fig: 17 GC-MS spectra of Squalene

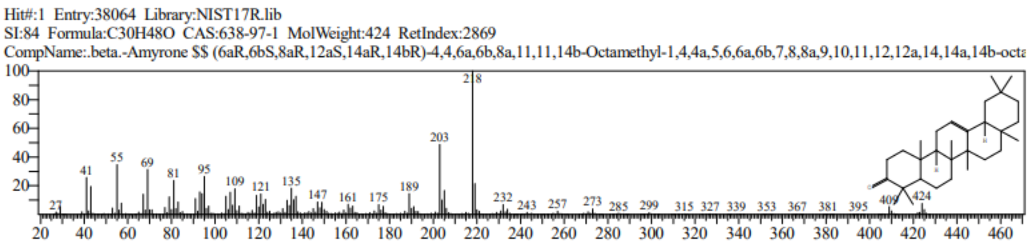


Fig: 18 GC-MS spectra of beta. -Amyrone

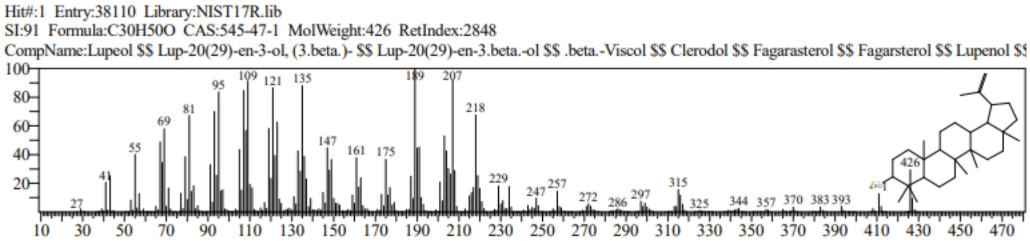


Fig: 19 GC-MS spectra of Lupeol

4. CONCLUSION:

In this work, phytochemical screening indicated the existence of various phytochemicals like alkaloids, flavonoids, tannins, phenols, glycosides, and anthocyanins. Gas Chromatography-Mass Spectrometry (GC-MS) analysis was used to recognize eighteen chemical components from an ethanolic extract of *Strychnos colubrina* leaves. Traditional healers utilize plant leaves for a variety of illnesses, which is justified by the existence of several bioactive chemicals.

Based on the results found in the present investigation, it may be concluded that the biological activities of the associated phytocomponents used for antioxidant, anti-microbial, antiinflammatory, hepatoprotective, and anti-cancer activities. Therefore, *Strychnos colubrina* is recommended as a source of phytopharmaceutical value.

Acknowledgement:

None

Conflict of an interest:

None

5. REFERENCES

1. Sudhira L, Rao SV, Kamakshamma J. Phytochemical Screening, antioxidant and antibacterial activity of Strychnos colubrina L. as an important endangered medicinal species in eastern ghats. Journal of pharmaceutical sciences and research. 2015 May 1;7(5):242.

2. Preethimol F, Suseem SR. A Review on an Endemic Indian Species: Strychnos colubrina Linn. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2016;7(1):2021-5.

3. Francis P, Suseem SR. A review on an endemic Indian species: Strychnos colubrina Linn. RESEARCH JOURNAL OF PHARMACEUTICAL BIOLOGICAL AND CHEMICAL SCIENCES. 2016 Jan 1;7(1):2021-5.

4. Bisset NG, Philcox D. Identification and clarification of Strychnos colubrina L.(Loganiaceae). Taxon. 1971 Aug;20(4):537-43.

5. Karthikeyan R, Koushik OS, Babu PS. Anti Pyretic Effect of Methanolic Extract of Strychnos colubrina L. Bark by Brewer's Yeast Induced Pyrexia in Albino Rats. Journal of Pharma and Biosciences. 2011;2(2):501-8.

6. Francis P, Suseem SR. Phytochemical Analysis and Anti-inflammatory Screening of Strychnos colubrina Linn. Research Journal of Pharmacy and Technology. 2016;9(2):165-9.

7. Mohan A, Deepa MS. A review on ethno botanical importance of an endemic species of Western Ghats: Strychnos colubrina Linn. Sp.(Vallikanjiram). International Journal of Herbal Medicine. 2021;9(1):19-22.

8. Karthikeyan R, Suryabhavana A, Srinivasa P. Anthelmintic Activity of Methanol Extract of Strychos Colubrina L. Bark. J Pharm Res. 2016;1(1):1-3.

9. Kishore IV, Ragalatha R, Krishna N, Vijayalakshmi M, Mallikarjuna K. ANTIMICROBIAL SCREENING OF DIFFERENT SOLVENT EXTRACTS OF AN ENDANGERED MEDICINAL PLANT, STRYCHNOS WALLICHIANA STEUD EX DC.

10. Priyadarsini AI, Chakrapani IS, Shamshad S, Swamy NT. Pharmacological evaluation of Strychnos colubrina L., an endangered medicinal plant.

11. Mors WB, do Nascimento MC, Pereira BM, Pereira NA. Plant natural products active against snake bite—the molecular approach. Phytochemistry. 2000 Nov 1;55(6):627-42.

12. Bhogireddy N, Naga A, Ramesh B, Pradeep M, Reddy OV, Gaddaguti V. Anti-inflammatory and anti-diabetic activities with their other ethnomedicinal properties of the plants. J Med Plants Stud. 2013;1(5):87-96.

13. Bukke, S.P.N., Gali, A.K., Igbinoba, S.I., Garla, V., Hussaini, B., Goruntla, N. and Onohuean, H., 2024. Anti-Apoptotic and Anti-Inflammatory Protective Mechanisms of Gmelina Arborea Stem Bark Extract on Ischemic Reperfusion Injury in Albino Wistar Rats. *RPS Pharmacy and Pharmacology Reports*, p.rqae015.

14. Francis P, Suseem SR. Isolation, structural characterization and anti-inflammatory screening of three compounds from Strychnos colubrina linn extract. Research Journal of Pharmacy and Technology. 2022;15(3):1235-40.

15. Francis P, Suseem SR. Phytochemical Analysis and Anti-inflammatory Screening of Strychnos colubrina Linn. Research Journal of Pharmacy and Technology. 2016;9(2):165-9.

16. Rajeswari G, Murugan M, Mohan VR. GC-MS analysis of bioactive components of Hugonia mystax L.(Linaceae).

17. Gherman C, Culea M, Cozar O. Comparative analysis of some active principles of herb plants by GC/MS. Talanta. 2000 Oct 2;53(1):253-62.

18. Houghton PJ, Osibogun IM. Flowering plants used against snakebite. Journal of Ethnopharmacology. 1993 May 1;39(1):1-29.

19. Weir W. Cases of Cholera Asphyxia, in Which the Strychnine Was Employed. Glasgow Medical Journal. 1832 May;5(18):140.

20. Farnsworth NR. Biological and phytochemical screening of plants. Journal of pharmaceutical sciences. 1966 Mar 1;55(3):225-76.

21. Mojab F, KAMALNEZHAD M, Ghaderi N, VAHIDI PH. Phytochemical screening of some species of Iranian plants.

22. Odebiyi OO, Sofowora EA. Phytochemical screening of Nigerian medicinal plants II. Lloydia. 1978 May 1;41(3):234-46.

23. Sharma GN, Dubey SK, Sati N, Sanadya J. Phytochemical screening and estimation of total phenolic content in Aegle marmelos seeds. International Journal of Pharmaceutical and Clinical Research. 2011;2(3):27-9.

24. Khaled-Khodja N, Boulekbache-Makhlouf L, Madani K. Phytochemical screening of antioxidant and antibacterial activities of methanolic extracts of some Lamiaceae. Industrial crops and products. 2014 Nov 1;61:41-8.

25**.** Swamy MK, Arumugam G, Kaur R, Ghasemzadeh A, Yusoff MM, Sinniah UR. GC-MS based metabolite profiling, antioxidant and antimicrobial properties of different solvent extracts of Malaysian Plectranthus amboinicus leaves. Evidence-Based Complementary and Alternative Medicine. 2017 Oct;2017.

26. Mujeeb F, Bajpai P, Pathak N. Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of Aegle marmelos. BioMed research international. 2014 Oct;2014.

27. Elaiyaraja A, Chandramohan G. Comparative phytochemical profile of Indoneesiella echioides (L.) Nees leaves using GC-MS. Journal of pharmacognosy and phytochemistry. 2016;5(6):158-71.

28. Ponnamma SU, Manjunath K. GC- MS analysis of phytocomponents in the methanolic extract of Justicia wynaadensis (Nees) T. anders. International Journal of pharma and bio sciences. 2012;3(3):570-6.

29. Amrati FE, Bourhia M, Saghrouchni H, Slighoua M, Grafov A, Ullah R, Ezzeldin E, Mostafa GA, Bari A, Ibenmoussa S, Bousta D. Caralluma europaea (Guss.) NE Br.: Anti-inflammatory, antifungal, and antibacterial activities against nosocomial antibiotic-resistant microbes of chemically characterized fractions. Molecules. 2021 Jan 26;26(3):636.

30. Ashmawy NA, Al Farraj DA, Salem MZ, Elshikh MS, Al-Kufaidy R, Alshammari MK, Salem AZ. Potential impacts of Pinus halepensis Miller trees as a source of phytochemical compounds: Antibacterial activity of the cones essential oil and n-butanol extract. Aroforestry Systems. 2020 Aug;94:1403-13.

31. Tyagi T, Agarwal M. Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of Pistia stratiotes L. and Eichhornia crassipes (Mart.) solms. Journal of Pharmacognosy and phytochemistry. 2017;6(1):195-206.

32. Jalalvand AR, Zhaleh M, Goorani S, Zangeneh MM, Seydi N, Zangeneh A, Moradi R. Chemical characterization and antioxidant, cytotoxic, antibacterial, and antifungal properties of ethanolic extract of Allium Saralicum RM Fritsch leaves rich in linolenic acid, methyl ester. Journal of Photochemistry and Photobiology B: Biology. 2019 Mar 1;192:103-12.

33. P Costa J, Islam T, S Santos P, B Ferreira P, LS Oliveira G, VOB Alencar M, FCJ Paz M, Ferreira LF, M Feitosa C, MGL Citó A, P Sousa D. Evaluation of antioxidant activity of phytol using non-and pre-clinical models. Current Pharmaceutical Biotechnology. 2016 Nov 1;17(14):1278-84.

34. Lee W, Woo ER, Lee DG. Phytol has antibacterial property by inducing oxidative stress response in Pseudomonas aeruginosa. Free radical research. 2016 Dec 1;50(12):1309-18.

35. Syad AN, Rajamohamed BS, Shunmugaiah KP, Kasi PD. Neuroprotective effect of the marine macroalga Gelidiella acerosa: Identification of active compounds through bioactivity-guided fractionation. Pharmaceutical biology. 2016 Oct 2;54(10):2073-81.

36. Phukan H, Bora CR, Mitra PK. Phytochemical screening and GC-MS analysis of methanolic leaf extract of an endemic plant kayea assamica. IOSR J. Pharm. Biol. Sci. 2017;15:7-16.

37. Stark AH, Crawford MA, Reifen R. Update on alpha-linolenic acid. Nutrition reviews. 2008 Jun 1;66(6):326-32.

38. Mbabazi I, Wangila P, K'Owino IO. Antimicrobial activity of Euclea divinorum hern (ebenaceae) leaves, tender stems, root bark and an herbal toothpaste formulated from its ethanolic root bark extract.

39. Pu ZH, Zhang YQ, Yin ZQ, Jiao XU, Jia RY, Yang LU, Fan YA. Antibacterial activity of 9-octadecanoic acid-hexadecanoic acid-tetrahydrofuran-3, 4-diyl ester from neem oil. Agricultural Sciences in China. 2010 Aug 1;9(8):1236-40.

40. Lu P, Bar-Yoseph F, Levi L, Lifshitz Y, Witte-Bouma J, de Bruijn AC, Korteland-van Male AM, van Goudoever JB, Renes IB. High beta-palmitate fat controls the intestinal inflammatory response and limits intestinal damage in mucin Muc2 deficient mice. PLoS One. 2013 Jun 12;8(6):e65878.

41. Bakrim S, Benkhaira N, Bourais I, Benali T, Lee LH, El Omari N, Sheikh RA, Goh KW, Ming LC, Bouyahya A. Health benefits and pharmacological properties of stigmasterol. Antioxidants. 2022 Sep 27;11(10):1912.

42. Burčová Z, Kreps F, Greifová M, Jablonský M, Ház A, Schmidt Š, Šurina I. Antibacterial and antifungal activity of phytosterols and methyl dehydroabietate of Norway spruce bark extracts. Journal of biotechnology. 2018 Sep 20;282:18-24.
43. Spanova M, Daum G. Squalene–biochemistry, molecular biology, process biotechnology, and applications. European journal of lipid science and technology. 2011 Nov;113(11):1299-320.
44. Elfadil H, Fahal A, Kloezen W, Ahmed EM, van de Sande W. The in vitro antifungal activity of Sudanese medicinal plants against Madurella mycetomatis, the eumycetoma major causative agent. PLoS neglected tropical diseases. 2015 Mar 13;9(3):e0003488.
45. Gallo MB, Sarachine MJ. Biological activities of lupeol. Int. J. Biomed. Pharm. Sci. 2009 Oct;3(1):46-66.