INDIAN JOURNAL OF SCIENCE AND TECHNOLOGY

RESEARCH ARTICLE

Phytochemical Study, Antioxidant Activity and GC-MS Analysis of *Soymida febrifuga* A. Juss

Smita Sinalkar[1], **Seema Kokitkar**[1]*

1 Department of Biotechnology, Changu Kana Thakur College of Arts, Commerce and Science, (Autonomus), New Panvel, Maharashtra, India

OPEN ACCESS

Received: 20-04-2024

Accepted: 03-05-2024

Published: 28-06-2024

Citation: Sinalkar S, Kokitkar S (2024) Phytochemical Study, Antioxidant Activity and GC-MS Analysis of *Soymida febrifuga* A. Juss. Indian Journal of Science and Technology 17(26): 2691-2697. https://doi.org/10.17485/IJST/v17i26.1280

* **Corresponding author**.

seemaskk11@gmail.com

Funding: None

Competing Interests: None

Published By Indian Society for Education and Environment (iSee)

ISSN
Print: 0974-6846
Electronic: 0974-5645

Abstract

Objectives: To determine antioxidant activity and identify bioactive compounds present in n-Hexane & Ethyl acetate extracts of stem bark of *Soymida febrifuga* A. Juss. **Methods:** In the present study stem bark of *Soymida febrifuga* A. Juss was powdered and subjected to Soxhlet extraction using n-Hexane and Ethyl acetate as solvents. Extracts were analysed for the presence of phytochemicals and antioxidant activity. The Bio-active compounds were identified by Gas chromatography-mass spectrometry. **Findings:** The qualitative analysis revealed presence of various phytochemicals. GC-MS analysis confirmed the presence of total 28 Medicinally active compounds which included Tetradecane, Hentriacontane, Octacosane, Docosane and Eicosane in n-Hexane extract. 15 Medicinally active compounds were found in ethyl acetate extract which included Purolan, 1,2-Dipropenyl cyclobutane, Hentricontane, 2,6,7-Trimethyl decane, Tetrahydrofurfuryl propionate and Decyl propanoate. **Novelty:** Though the phytochemical analysis of *Soymida febrifuga* have been performed by some research groups but there are no reports on identification of Bio-active compounds by GC-MS. The results obtained from present study confirms presence of many compounds of biological significance that warrants further biological and pharmacological study of *Soymida febrifuga* A. Juss as a potential herbal drug.

Keywords: *Soymida febrifuga*; GC-MS analysis; DPPH assay; n-Hexane; Ethyl acetate

1 Introduction

The contribution of medicinal plants in improving human health and well-being is significant throughout the history. Use of plant based antioxidants and herbal medicine has become popular as preventive medicine due to side effects of synthetic drugs[(1)]. Worldwide modern medicines are replaced by phytomedicines in the form of key staring materials, intermediates, active ingredients and finished products as their effectiveness has been proven scientifically with either minimal or no adverse effects.

Global research is continuously focusing on finding out active components from these herbal plants that will be useful in new drug development. These active ingredients

should be assessed for their chemical composition, structures, biosynthetic pathway, or solubility before the usage in drug formulation. This information is also helpful to establish efficacy, quality and safety of the medicinal plant.

Soymida febrifuga (Roxb) A. Juss is plant which is popularly known for its medicinal values. It is found in tropical deciduous dry forest and widely distributed in the hilly area of South, North Western and Central India [(2)].It belongs to lofty deciduous family Meliaceae and referred as Mamsarohini in Sanskrit. The name of the plant itself suggests its curative properties as *Soymida* is synonym to God and *febrifuga* means expulsion of fever [(3)].

Stem bark of *Soymida febrifuga* contains bitter resin which cures sore throat, cough, tridosha fevers, asthma and remove vata [(4)].It consists of secondary metabolites such as lupeol, 2 tetranortriterpenoids with modiefied furan ring, methyl angloensate, deoxyandirobin and sitosterol which are responsible for cytotoxic activity [(5)].

The plant also exhibited cardiotonic, hypoglycemic, anti-inflammatory and analgesic activity when tested using tail flick method [(6)]. Stem bark extract also revealed the presence of 5-lipoxygenase as an inhibitory compound responsible for anticancer activities. A new flavone 5, 7 dihydroxy 3,4 dimethoxy flavones has been isolated from stem bark [(7)].

To our best knowledge yet there are no reports available on GC-MS analysis of *Soymida febrifuga* stem bark to confirm the presence of these bioactive compounds.

Many herbal medicines and their products are mostly prepared from crude extracts, which consists of a complex mixture of various plant secondary metabolites responsible for therapeutic activity. To identify and evaluate these phytoconstituents with their structures, Gas Chromatography coupled with mass spectrometry is a powerful technique as it has great separation potency which produces data of chemical fingerprint with high accuracy and precision [(8)].

In the present study the GC-MS analysis of stem bark has been attempted for the very first time to identify active compounds by GC-MS analysis.

2 Methodology

2.1 Collection and authentication of plant material

Plant material *Soymida febrifuga* A. Juss was collected from Nanded, India. The plant material was identified and authenticated from Botanical Survey of India, Pune. The authentication number assigned was Certificate number – No. BSI/WRC/IDEN.CER/2021/H1.

2.2 Extraction of plant material

Stem bark of *Soymida febrifuga* A. Juss was dried under shade for 5 days and milled to obtain a coarse powder. Soxhlet method was used for extraction. A portion of (30 gm) coarse powder was placed in Soxhlet apparatus followed by the extraction with the 300 ml of n-Hexane and Ethyl acetate as solvents for 48 hours at 60-65°C. The solvents were evaporated by rotary vacuum evaporator and dried n-Hexane and Ethyl acetate crude extracts were stored in refrigerator for further use.

2.3 Phytochemical analysis

Preliminary Phytochemical analysis of *Soymida febrifuga* A. Juss stem bark for secondary metabolites such as alkaloids, tannins, phenols, flavonoids, sterols, terpenoids, proteins, saponins, quinones and cardiac glycosides was carried out as per earlier reported methods [(9)].

2.4 DPPH assay

The free radical scavenging potential of extracts of *Soymida febrifuga* A. Juss stem bark against DPPH (1,1-diphenyl-2-picrylhydrazyl) was determined using UV Spectrophotometer at 517 nm [(10)]. Aliquots of (10, 20, 30 and 40 μg/ml) were dissolved in test tubes containing 3 ml of methanol and 0.5 ml of DPPH. Ascorbic acid was used as the standard and blank was prepared with methanolic dilution of DPPH. This solution mixture was incubated in dark at room temperature for 30 min.

The percentage of radical scavenging activity of tested extract and ascorbic acid was calculated by below formula:

Free radical scavenging activity (%) = [Ac- As] /Ac X 100

Where Ac=Absorbance of control at 517 nm and As=Absorbance of sample. The concentration of sample required to scavenge 50% of DPPH free radical (IC_{50}) was determined from the linear regression analysis of percentage inhibitions plotted against the respective concentration.

2.5 GC-MS analysis of Medicinally active compounds

The extracts of *Soymida febrifuga* A. Juss stem bark were analyzed by the GC-MS device consisted of Perkin Elmer Clarus 600C GCMS system with single quadrupole mass analyzer. The column used was GsBP-5 MS column (30M x 0.25 mm with 0.25 microns thickness). The temperature of injector was set at 250°C. The carrier gas was helium with flow rate of mobile phase set at 1.2 ml/mins. The temperature of the oven was raised from 45^0 C to 290°C at a rate of 10°C/min which was held for 5 minutes, the sample volume injected was 1.0 μl and the analysis was done in split less mode. The transfer line temperature was 280°C and source temperature was 230°C. The mass range was selected from 40 to 450.

2.6 Identification of Medicinally active compounds

Identification of mass spectrum of GC-MS was done using database of National Institute Standard and Technology (NIST) library and Flavors and Fragrances of Natural and Synthetic Compounds (FFNSC 3) library.

3 Results and Discussion

3.1 Phytochemical Analysis

The phytochemical analysis of n-Hexane and Ethyl acetate extract of *Soymida febrifuga* A. Juss stem bark has been analysed and results are presented in Table 1.

Table 1. Phytochemical Analysis of n-Hexane and Ethyl acetate extracts of *Soymida febrifuga* A. Juss stem bark

Sr. No.	Constituents	n-hexane extract of *Soymida febrifuga*	Ethyl acetate extract of *Soymida febrifuga*
1	Alkaloids	-	+
2	Tannins	+	+
3	Phenols	+	-
4	Flavonoids	+	+
5	Steroids	-	-
6	Terpenoids	+	+
7	Saponins	-	-
8	Proteins	-	-
9	Quinones	+	+
10	Cardiac glycosides	-	-

(+ = Present; - = Absent)

The n-Hexane extract of *Soymida febrifuga* A. Juss stem bark revealed the presence of Tannis, Phenols, Flavonoids, Terpenoids and Quinones.

The Ethyl acetate extract of *Soymida febrifuga* A. Juss stem bark revealed the presence of Alkaloids, Tannis, Flavonoids, Terpenoids and Quinones. While Steroids, Saponins, Proteins and Cardiac glycosides were found to be absent in both the extracts.

Alkaloids from medicinal plant have proven to have mechanism of action in antidiabetic activity. They also play a role in the management of neurodegenerative disorders, inhibition of cancer growth, microbial infections, viral diseases [11].

The presence of flavonoids in both the extracts indicates the role of plant against microbes, allergies, tumours, scavenging of free radicals, platelet aggregation and ulcers [12].

As described in previous studies Syringetin which is an o-menthlylated flavonol belonging to the flavonoids group has been isolated from the chloroform extract of root heartwood of *Soymida febrifuga* plant [13].

Phenols were present in n-Hexane extract which confirms the antimicrobial capability of the plant. Literature study confirms the structure-activity relationship of phenol for p-hydroxy benzoic acid and different functional groups with easter side chains corroborate antibacterial activity [14].Earlier study on hydroalcoholic extract of stem bark authenticated to have significant antibacterial and antifungal activities with reference to standard [15].

Terpenoids presence in both the extracts validated ability of plant in management and prevention of lymphoma. As per prior studies a tetranortriterpenoid Methyl anglolensate is extracted from the root callus of the plant [16].

Plant tannins plays variety of biological functions they are responsible for anti-haemorrhagic and anti-diarrhoeal activity [17].Confirmation of tannins in both the extracts validate the use of stem bark powder in wound healing.

Quinones were present in both the extracts which are the most diverse group of plant metabolites. Recent findings on plant based quinones presented their role in Alzheimer's disease[18].

Previous reports on phytochemical study of stem bark revealed the presence of flavonoids, glycosides, steroids, carbohydrates, tannins, phenols and diterpenes[19].

3.2 DPPH assay

Antioxidant activity of the extracts of *Soymida febrifuga* A. Juss was assessed by DPPH assay, in which scavenging of free radical (DPPH) was measured. The IC_{50} value which is concentration of substrate that brings about 50% inhibition of the DPPH, was used to express the antioxidant potential and to compare antioxidant potential of various samples. In the present study the extract bleached DPPH indicating the free radical scavenging activity and IC_{50} values of n-hexane extract was found to be 29.27 μg/ml and for Ethyl acetate extract it was 13.56 μg/ml (Figures 2 and 3). IC_{50} value of Ascorbic acid used as a standard was found to be 13.81 μg/ml (Figure 1). The activity was found to be dose dependent.

Prior report on hydroalcoholic extract also emphasized that it produced a dose dependent inhibition of free radicals generation of DPPH, hydroxyl radical and superoxide anion which is in agreement with the results obtained in current study[20].

Plant phytochemicals such as flavonoids, phenols, tannins and terpenoids have been reported to possess free radical scavenging activities. These chemicals block various hormonal actions and metabolic pathways which are responsible for development of cancer. Phenolic compounds such as caffeic acid, ferulic acids and vanillin have been reported for antioxidant and anticarcinogenic activities. Thus, the antioxidant action may be attributed due to the presence of these compounds.

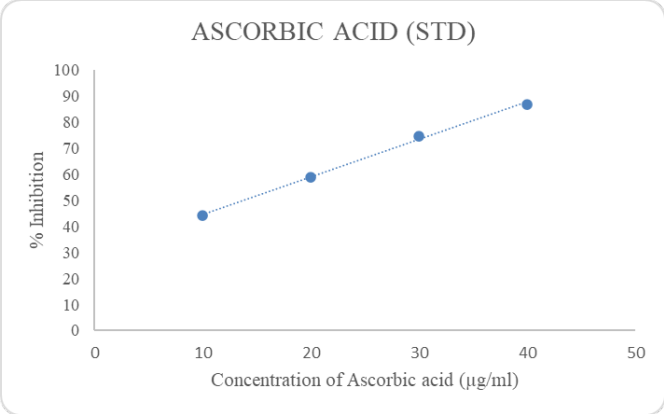

Fig 1. Graph of Concentration of Ascorbic acid VS Percentage inhibition of DPPH radical

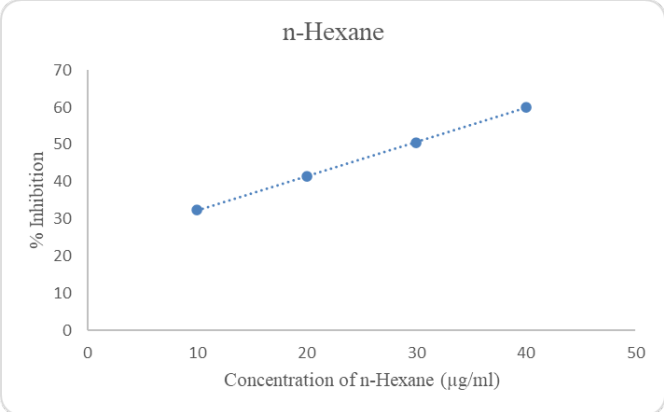

Fig 2. Graph of Concentration of n-hexane extract of *Soymida febrifuga* A. Juss VS Percentage inhibition of DPPH radical

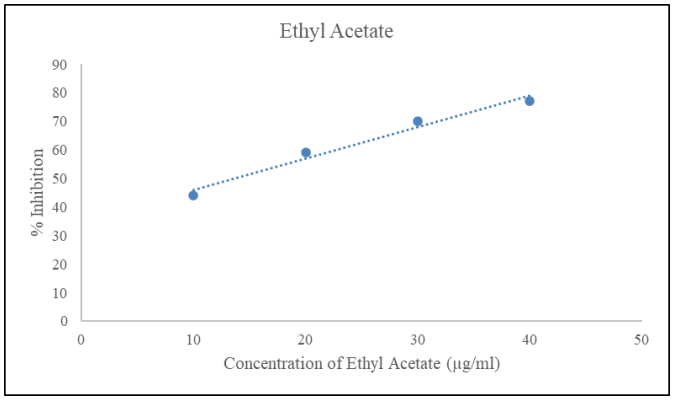

Fig 3. Graph of Concentration of Ethyl acetate extract of *Soymida febrifuga* A. Juss VS Percentage inhibition of DPPH radical

3.3 Gas Chromatography-Mass Spectrometry analysis of Medicinally active compounds present in *Soymida febrifuga* A. Juss stem bark

Gas Chromatography – Mass Spectrometry analysis of *Soymida febrifuga* A. Juss stem bark revealed the presence of many bioactive compounds.

The GC-MS chromatogram of n-Hexane extract is presented in Figure 4 and retention time (RT), name of the compound, molecular formula, molecular weight (MW), peak area (%) and structure of identified compounds is presented in **Supplementary table A**.

Total 28 medicinally active constituents were found to be present in n-Hexane extract of *Soymida febrifuga* A. Juss stem bark. The highest percentage of Tetradecane was found with retention time 10.52 minutes and percentage peak area of 8.75 percentage, followed by Octatriacontane, 1,38-Dibromo- with retention time 29.27 minutes and percentage peak area of 8.57 percentage. Least percentage were observed for Methane, Dichloronitro- with retention time 5.84 minutes and percentage peak area 0.46 followed by Heptacosane with retention time 17.16 minutes and percentage peak area 0.84 percentage.

Figure 5 and **Supplpementary table B** represent the GC-MS chromatogram and structures of identified compounds from Ethyl acetate extract.

In this extract the highest percentage of 1-Decanethiol with retention time 12.54 minutes and percentage peak area 10.697 percentage followed by 3,7,11-Trimethyl 3-docdecanol with retention time 21.33 minutes and percentage peak area 9.163 percentage was observed. The least percentage was for Purolan with retention time 5.37 minutes and percentage peak area 1.468 percentage followed by Tetrahydrofurfuryl propionate with retention time 9.68 minutes and percentage peak area 1.872 percentage.

Seeds of *Soymida febrifuga* has been investigated earlier via GC-MS analysis and it showed the highest percentage of Desulphosinigrin and fatty acids observed were 9,12-Octadecadienoic acid (Z,Z)-, methyl ester.

GC-MS analysis of leaves of *Soymida febrifuga* in past studies has showed presence of total 15 compounds such as 1-Heneicosyl formate, Cis-Vaccenic acid, Cis-Vaccenic acid however presence of same compounds was not observed in stem bark[21].

Earlier finding has reported that Heptacosane, Octadecane and Hexacosane has significant antiviral effect against Coxsackie virus B4[22].

Presence of Dodecane which is an aliphatic compound responsible for antibacterial, antifungal and antioxidant activity justifies the use of stem bark decoction in vaginal infections, dental diseases and sore throats.

Tetradecane have antimicrobial activity, wound healing activity, anti-viral activity and antitumor activities.

Eicosane has inhibitory effect on food borne pathogens and it has antimicrobial activity against clinical pathogens and has sugar phosphatise inhibitor activity.

Tetratricontane act as an antifungal agent against fungal spores, germination and also has antioxidant and antitumor activity.

Alkanes, glycol, ketones, esters and phenolic compounds are the major volatile compounds which have antidiabetic, hypolipidemic, anti-HIV, alpha reductase inhibitory activity and antoarthritic activities.

Presence of all these bioactive compounds makes it suitable candidate for anticancer and antitumor studies.

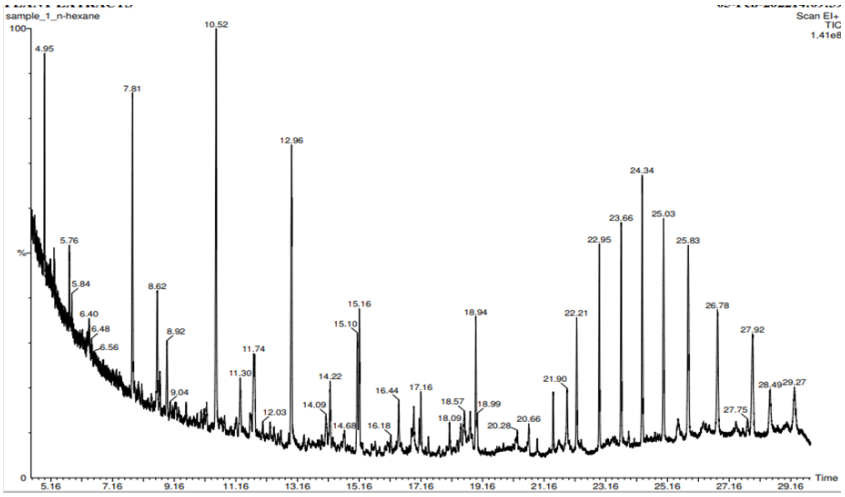

Fig 4. GS-MS chromatogram of n-Hexane extract of *Soymida febrifuga* A. Juss stem bark

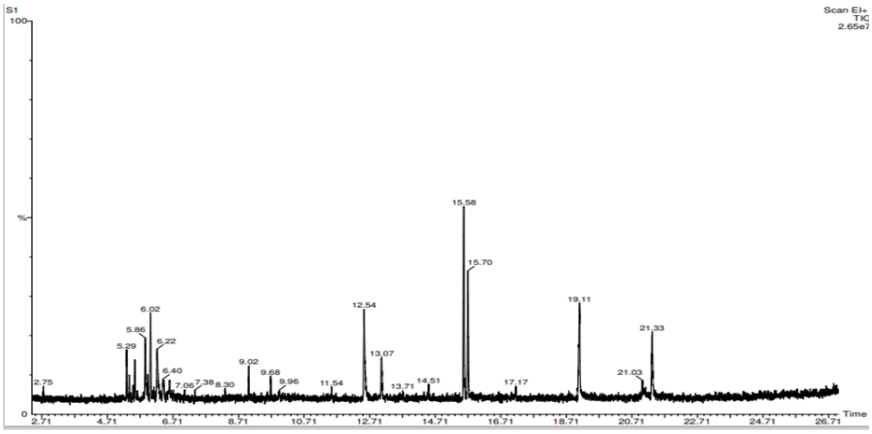

Fig 5. GS-MS chromatogram of Ethyl acetate extract of *Soymida febrifuga* A. Juss stem bark

4 Conclusion

This study makes a qualitative screening and antioxidant potential of phytochemicals present in stem bark of *Soymida febrifuga*. Further identification of the compounds has been done by GC-MS analysis. Qualitative screening for phytochemicals from the stem bark of *Soymida febrifuga* revealed the presence of wide range of phytochemicals which establishes a relationship between the active constituents of the plant extracts and its biological potential. *In vitro* antioxidant study showed the inhibition of free radicals by *Soymida febrifuga* stem bark in a dose dependant manner. This makes it a suitable candidate for cytotoxicity analysis, supplemental studies on Cancer cell lines (*In vitro*) are recommended to support the present findings. The present article showed GC-MS analysis of stem bark of *Soymida febrifuga* as the first report. The GC-MS analysis revealed the presence of total 28 compounds in n-Hexane and 15 compounds in Ethyl acetate extracts which contribute to activities like anti-inflammatory, chemoprotective, antitumor and alpha reductase inhibitor activities. The results and findings of present study suggests that stem bark of *Soymida febrifuga* can be used as a valuable source for plant-based drug discovery. The presence of bioactive compounds justifies the traditional use of this plant for the treatment of various health ailments. Scientific studies have mentioned that the plant with alternative potential when homogenized with other drug or plants exhibits a prominent result in contrast to individual plant and also the sum total of their individual effect. Thus, investigation reports from this study also make *Soymida febrifuga* a prospective plant for polyherbal formulation or combination therapy to attain desirable therapeutic results. However, further analysis is required to study its bioactivity and exact mechanism of action.

Acknowledgement

The authors are thankful to the Principal, Changu Kana Thakur College of Arts, Commerce and Science, New Panvel, Navi Mumbai for continuous support for the successful completion of research work.

References

1) Jabbar AA, Abdullah FO, Abdulrahman KK, Galali Y, Abdullah AS. GC-MS Analysis of Bioactive Compounds in Methanolic Extracts of Papaver decaisnei and Determination of Its Antioxidants and Anticancer Activities. *Journal of Food Quality*. 2022;2022:1–12. Available from: https://doi.org/10.1155/2022/1405157.
2) Pandey S, Vajpai K, Patel P, Tiwari M. Phytochemical And Fatty Acids Profile of Soymida febrifuga (Roxb.). *NeuroQuantology*. 2022;20(12):2483–2489. Available from: https://www.neuroquantology.com/media/article_pdfs/2483-2489.pdf.
3) Danapur V, Seetharam YN. Pharmacognostic and preliminary phytochemical studies on Soymida febrifuga A.Juss. *International Journal of Pharmacognosy*. 2019;6(2):80–84. Available from: https://ijpjournal.com/bft-article/pharmacognostic-and-preliminary-phytochemical-studies-on-soymida-febrifuga-a-juss/.
4) Rajput AP, Kachhava BC. Phytochemical analysis and biological activities of soymida febrifuga (roxb.) juss (meliaceae): an overview. *International Journal of Research and Analytical Reviews*. 2019;6(2):826–834. Available from: http://ijrar.com/upload_issue/ijrar_issue_20543632.pdf.
5) Nandeshwar VS, Kalkar SA, Agrawal PS. Green synthesis of silver nanoparticles using soymida febrifuga: optimization, characterization and for dye degradation in photocatalytic activity via green way. *International Journal For Innovative Research In Multidisciplinary Field*. 2022;8(5):9–16. Available from: https://www.ijirmf.com/wp-content/uploads/IJIRMF202205002.pdf.
6) Danapur V, Seetharam YN. Pharmacological Studies On Soymida Febrifuga (Roxb.) A. Juss. A Less Known Medicinal Plant. *World Journal Of Pharmacy And Pharmaceutical Sciences*. 2022;11(6):1711–1719. Available from: https://www.wjpps.com/Wjpps_controller/abstract_id/16752.
7) Singh AK, Chandra KK, Kumar R, Bhardwaj AK. Phytochemical Analysis and Medicinal Properties of Soymida febrifuga (Roxb.) A. Juss: a Review. *Advances in Bioresearch*. 2022;13(6):37–43. Available from: https://soeagra.com/abr/abr_nov2022/6.pdf.
8) Konappa N, Udayashankar AC, Krishnamurthy S, Pradeep CK, Chowdappa S, Jogaiah S. GC–MS analysis of phytoconstituents from Amomum nilgiricum and molecular docking interactions of bioactive serverogenin acetate with target proteins. *Scientific reports*. 2020;10:1–23. Available from: https://doi.org/10.1038/s41598-020-73442-0.
9) Shaikh JR, Patil MK. Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*. 2020;8(2):603–608. Available from: https://doi.org/10.22271/chemi.2020.v8.i2i.8834.
10) Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, et al. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of Ficus religiosa. *Molecules*. 2022;27(4):1–19. Available from: https://doi.org/10.3390/molecules27041326.
11) Adhikari B. Roles of alkaloids from medicinal plants in the management of diabetes mellitus. *Journal of Chemistry*. 2021;2021:1–10. Available from: https://doi.org/10.1155/2021/2691525.
12) Seck I, Hosu A, Cimpoiu C, Ndoye SF, Ba LA, Sall C, et al. Phytochemicals content, screening and antioxidant/pro-oxidant activities of Carapa procera (barks) (Meliaceae). *South African Journal of Botany*. 2021;137:369–376. Available from: https://doi.org/10.1016/j.sajb.2020.11.019.
13) Chmiel M, Stompor-Gorący M. The spectrum of pharmacological actions of syringetin and its natural derivatives-a summary review. *Nutrients*. 2022;14(23):1–12. Available from: https://doi.org/10.3390/nu14235157.
14) Dubale S, Kebebe D, Zeynudin A, Abdissa N, Suleman S. Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in Ethiopia. *Journal of experimental pharmacology*. 2023;15:51–62. Available from: https://doi.org/10.2147/JEP.S379805.
15) Yadav R, Kashaw V, Dudhe R. Antibacterial and antifungal screening of Amoora rohituka, Melia Azedarach and Soymida febrifuga stem barks. *Journal of Advanced Scientific Research*. 2018;9(02):34–40. Available from: https://www.sciensage.info/index.php/JASR/article/view/288.
16) Cho Y, Park MN, Noh S, Kang SY, Kim B. Review of Natural Compounds for the Management and Prevention of Lymphoma. *Processes* . 2020;8(9):1–25. Available from: https://doi.org/10.3390/pr8091164.
17) Sawant SV, Kokitkar SS. Phytochemical Profiling and GC-MS analysis of n-Hexane extract of Lavandula bipinnata (Roth) Kuntze leaves. *The Journal of Plant Science Research*. 2022;38(1):357–363. Available from: https://doi.org/10.32381/JPSR.2022.38.01.38.
18) Chen DB, Gao HW, Peng C, Pei SQ, Dai AR, Yu XT, et al. Quinones as preventive agents in Alzheimer's diseases: focus on NLRP3 inflammasomes. *Journal of Pharmacy and Pharmacology*. 2020;72(11):1481–1490. Available from: https://doi.org/10.1111/jphp.13332.
19) Bharadwa PV, Gogari PK, Chaudhari VS, Goyal YP. In vitro Antioxidant, Antimicrobial and Phytochemical studies on Soymida febrifuga bark extract. *International Journal of Advanced Science and Technology*. 2020;9(5s):818–824. Available from: http://sersc.org/journals/index.php/IJAST/article/view/7759.
20) Unadkat K, Jani D, Parmar N. Rasayana Effects of Herbs Mentioned in Dhavantari Nighantu along with Conventional Interpretations. *International Journal of Ayurveda and Pharmaceutical Chemistry*. 2021;14(2):196–201. Available from: http://ijapc.com/upload/MNAPC-14-I2-(v14-i1-27)-P-196-201.pdf.
21) Kachhava BC, Rajput AP. Phytochemical, fourier transform infrared (FTIR) spectroscopy and gas chromatography: Mass spectroscopy (GCMS) studies on Soymida febrifuga a juss. *International journal of Botany Studies*. 2021;6(6):544–549. Available from: https://www.botanyjournals.com/assets/archives/2021/vol6issue6/6-6-45-382.pdf.
22) Joshi S, Singh S, Sharma R, Vats S, Alam A. Gas chromatography-mass spectrometry (GC–MS) profiling of aqueous methanol fraction of Plagiochasma appendiculatum Lehm. & Lindenb. and Sphagnum fimbriatum Wilson for probable antiviral potential. *Vegetos*. 2023;36(1):87–92. Available from: https://dx.doi.org/10.1007/s42535-022-00458-4.