Original Article

LC/MS characterization of secondary metabolites of Sphagneticola trilobata J. F Pruski Leaves

Sonali Labhade[1,2], Smita Jain[1], Sohan Chitlange[2], Swapnil Sharma[1]
[1]Department of Pharmacy, Banasthali Vidyapith, Rajasthan, India
[2]Department of Chemistry, Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research Pimpri, Pune
Email: sonalilabhade16@gmail.com
DOI: 10.47750/pnr.2023.14.03.171

Abstract

Spectroscopic and chromatographic methods were derived to identify the secondary metabolites from Sphagneticola trilobata, an essential medicinal plant. Invitro DPPH radical scavenging activity of different fractions was carried out by DPPH assay. The LC/MS analysis was performed so as to determine characteristic active fractions. Several phytoconstituents such as Rhamnetin 3- laminaribiosideMethyl 7-epi-12-hydroxyjasmonate glucoside8-Epiiridodial glucoside tetraacetateMethyl 7-epi-12- hydroxyjasmonate glucosideCamellianin A Gibberellin A110, 3-Hydroxychavicol 1- [rhamnosyl-(1->6)- glucoside]4'-O-methyl-(-)- epicatechin-3'-O-betaglucuronide6-Ketoestriol Rhamnetin 3-laminaribioside 3,4-Dicaffeoyl-1,5-quinolactone Methyl 7-epi-12- hydroxyjasmonate glucosideIridodial glucoside tetraacetate Eugenol O-[a-L-Arabinofuranosyl-(1- >6)-b-D-glucopyranoside]4'-O-methyl-(-)-epicatechin-3'- O-beta-glucuronide2',7-Dihydroxy-4'-methoxy-8-prenylflavan 2',7-diglucosidexi-3-Hydroxy-5-phenylpentanoic acid O-beta-D-Glucopyranoside were identified for first time, invitro and invivo studies are to be reported in further studies. The characterization of S.trilobata proved to be helpful for the potential use of this plant in novel medicinal products.

Keywords: Flavonoids, LCMS, Phenols, sphagneticola trilobata.

1. Introduction

Sphagneticola trilobata being perrinial herb is present at tropical temperature (Vivi Mardina et al.). The plant belonsg to family Astereceae. It exhibit severel biological activities like antibacterial, antioxidant, antifungal, analgesic, antidiabetic (1,2). The presence of phenolic chemicals, which are crucial for plant growth and play a significant role in their defensive systems, is linked to several features of plant products [3,4,5]. Strong biological activity of plant phenolics and flavonoids highlights the need for their characterisation. With more than 8000 different phenolic structures now known, including anything from straightforward compounds like phenolic acids to highly polymerized substances like tannins, phenolics are the most prevalent secondary metabolites of plants. Several investigation have decscibed the chemcial constituents of essential oils presnet in the different parts of S.trilobata [6]. Due to lack of evidence the current study aimed to characterise secondary metabolites from S.trilobata J F. pruski leaves [7].

2. Result and Discussions

2.1 Preliminary characterization and separation of Compounds

The hydromethanolic extract was partitioned for Water, butanol and chloroform fractions. Preliminary analysis was done using HPTLC. Based on the evaluation at 275 nm butanol fraction showed major Rf 0.08, 0.14, 0.26, 0.36, 0.63, 0.71 and 0.81, while major bands were observed at 0.14, 0.19, 0.32 and 0.72 for chloroform fraction and major bands for Rf 0.03, 0.18, 0.51, 0.57 were observed for water fraction. Fractions Fr A1–A3 were screened for radical scavenging activity using DPPH. The characterization of the same will be reported later.

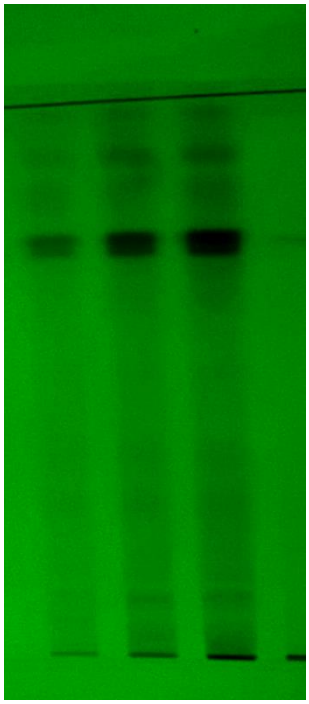

Fig 1: TLC profiling of butanol, chloroform and water fractions of S. trilobata

2.2 DPPH Radical Scavenging activity

As a direct and reliable method for determining radical scavenging activity [11], the DPPH radical is one of the free radicals that is frequently used for testing the preliminary radical scavenging activity of plant extracts [10]. Using DPPH, the radical scavenging activity of the subfractions Fr A1, Fr A2, and Fr A3 was investigated in vitro. The significant DPPH radical scavenging capacity of fractions Fr A1 and Fr A3 was demonstrated by their respective EC50 values of 4.59 and 4.16. (Table 1). Joshi et al. (2011) [12] reported that the DPPH radical scavenging activity of the Hydroalcoholic extract of S. trilobata had an EC 50 value of 6.46 0.371 g/ml. In the current investigation, the fraction A3 displayed a higher level of DPPH radical-fighting activity.

Table 1: DPPH radical scavenging assay of several fractions of S. trilobata

Fractions/Standard	EC50 (µg/ml)
Fr A1	4.59 ± 0.19
Fr A2	6.32±0.21
Fr A3	4.16 ± 0.15
Catechin	3.41± 0.19

2.3 LC/MS characterization of active fractions:

Fraction F3 was found to be more active fraction against DPPH assay. LC/MS characterization was performed for same in positive mode. Varying collision cell voltage was used to perform mass fragmentation. F3 fraction showed several peaks Table 2. The peak corresponding to [M + H] + 321.04, 494.1402, 476.2128, 517.1308, 665.2806, 374.1815, 460.1815, 598.2493, 538.2637, 226.1802, 612.1698 were identified as Methyl 7-epi-12-hydroxyjasmonate glucoside, an glycoside, 4'-O-methyl-(-)-epicatechin-3'-Obeta-glucuronide3-Hydroxychavicol 1-[rhamnosyl-(1->6)-glucoside] eugenol, O-[a-L-Arabinofuranosyl-(1->6)-b-D-glucopyranoside], 4'-O-methyl-(-)-epicatechin-3'-O-betaglucuronide, xi-3-Hydroxy-5-phenylpentanoic acid O-beta-D-Glucopyranoside, 1-O-E-Cinnamoyl-(6-arabinosylglucose), (+)-7-epi-Syringaresinol 4'-glucoside, Myricanol 5-glucoside, Eremopetasinorol, Epicatechin-(4beta->8)-gallocatechin already reported (Rui Jun Cai et al., 2017) respectively.

Table 2: LC/MS analysis of FrA3

Sr. No.	Rf value	Common name /IUPAC name	Molecular formula	Molecular weight [M+H]+ (experimental)	m/z ratio [M + H]+ (calculated)
1	12.109	6-Ketoestriol	C18 H22 O4	302.1518	302.1508
2	13.025	Rhamnetin 3-laminaribioside	C28 H32 O17	640.163	641.1702
3	13.025	Rhamnetin 3-laminaribioside	C28 H32 O17	640.163	641.17
4	14.539	3,4-Dicaffeoyl-1,5-quinolactone	C25 H22 O11	498.1152	499.1223

5	14.656	Methyl 7-epi-12-hydroxyjasmonate glucoside	C19 H30 O9	402.1892	300.1
6	14.656	Methyl 7-epi-12-hydroxyjasmonate glucoside	C19 H30 O9	402.1892	420.223
7	15.37	8-Epiiridodial glucoside tetraacetate	C24 H34 O11	498.21	318.02
8	15.37	Iridodial glucoside tetraacetate	C24 H34 O11	498.21	516.2439
9	16.996	Methyl 7-epi-12-hydroxyjasmonate glucoside	C19 H30 O9	402.1885	321.04
10	17.445	Camellianin A	C29 H32 O15	620.1759	327.9
11	17.526	4'-O-methyl-(-)-epicatechin-3'-Obeta-glucuronide	C23 H26 O12	494.1442	494.1402
12	18.294	Gibberellin A110	C20 H28 O5	348.1944	248.14
13	18.584	3-Hydroxychavicol 1-[rhamnosyl-(1->6)-glucoside]	C21 H30 O11	458.1788	476.2128
14	18.584	Eugenol O-[a-L-Arabinofuranosyl-(1->6)-b-D-glucopyranoside]	C21 H30 O11	458.1788	476.2128
15	18.832	4'-O-methyl-(-)-epicatechin-3'-O-betaglucuronide	C23 H26 O12	494.142	517.1308
16	19.781	2',7-Dihydroxy-4'-methoxy-8-prenylflavan 2',7-diglucoside	C33 H44 O14	664.273	665.2806
17	19.876	xi-3-Hydroxy-5-phenylpentanoic acid O-beta-D-Glucopyranoside	C17 H24 O8	356.1472	374.1815
18	21.314	1-O-E-Cinnamoyl-(6-arabinosylglucose)	C20 H26 O11	442.1474	460.1815
19	22.399	(+)-7-epi-Syringaresinol 4'-glucoside	C28 H36 O13	580.2154	598.2493
20	23.706	Myricanol 5-glucoside	C27 H36 O10	520.2297	538.2637
21	26.183	Eremopetasinorol	C13 H20 O2	208.1461	226.1802
22	33.28	Epicatechin-(4beta->8)-gallocatechin	C30 H26 O13	594.1359	612.1698

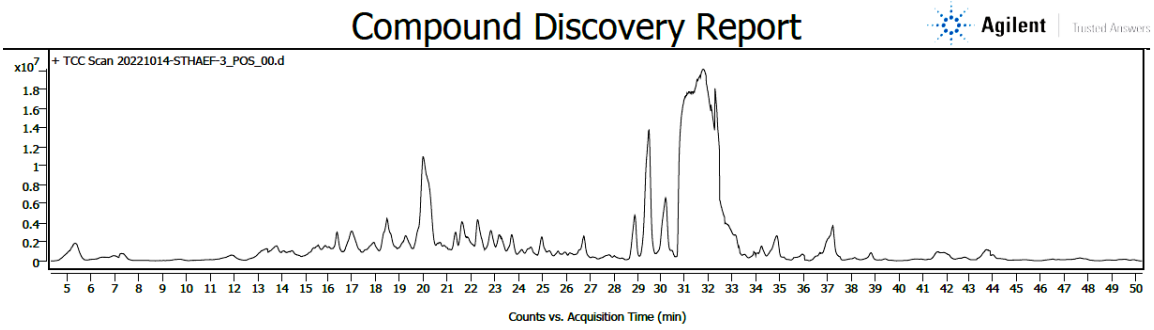

Fig 2: LC/ESI/MS Chromatogram for S. trilobata Fraction

Conclusions:

In the present study, a total of 22 compounds were reported from the Hydroalcoholic extract fraction of S. trilobata. Most of the metabolites identified on the basis of LC-MS data were Diterpenes, glycosides in nature. Based on the mass spectrum a diterpenes, andrographolide and a flavones were there in peak quantity in negative ion modes. The range of the phytoconstituents identified presents us novel category of molecules that should be screened for their pharmacological activities. It can also be inferred that the mixture of diterpenoids and flavonoids might be displaying synergetic effect against S. trilobata in the in vitro assays. The existing study led to identification of diterpenoids flavonoids and glycoside using LC MS characterization and can be used a great quality control methods. S. trilobata is widely used in the traditional medicine for malaria treatment by the tribal healers of Amarkantak region and therefore, based on current results, bioactivity guided fractionation, purification, and characterization of the active extract is urgently required for further development of potentially active pharmacological moiety.

3. MATERIALS AND METHOD

3.1 Plant Material:

The fresh leaves of S. trilobata (1000 g) was collected from the garden of Star City Apt, Pune India, during August-September 2022 and was authenticated by the Plant Systematics and Genetetic Resources Division of the Botonical Survey of India western regional centre, Koregaon Park, Pune, India. And voucher specimen was deposited Research (CMPR), Arya Vaidya Sala, Kottakkal, Kerala, India, and voucher specimen was deposited in the 'SDLST-1' herbarium.

3.2 Instruments and chemicals:

A UV-visible spectrophotometer (Shimadzu, 1800) was used to perform the DPPH radical scavenging experiment. Using a precoated silica plate, HPTLC was performed (Camag). Agilent 6520 accurate mass Q-TOF LC/MS and Agilent LC 1200 equipped with Extend-C18 column of 1.8 _m, 2.1 50 mm were used for the LC-ESI-MS study. The Folin-Ciocalteu reagent was purchased from Mumbai, India's Merck Specialities Pvt. Ltd. From Hi Media Laboratories Pvt. Ltd., DPPH was purchased. Quercetin, gallic acid, and catechin were purchased from Mumbai's Research Labs of Fine Chem Industries. All other chemicals used were of the normal analytical quality and came from the Mumbai-based Research Labs of Fine Chem Industries.

3.3 Extraction:

The fresh leaves were kept for shade dry almost about 8 days. The cold maceration method was used to extract the phytoconstituents form the dry material. Hydromethanolic solvent was used [13]. The extract was dried by using rotary evaporator [14].

3.4 Separation of phytoconstituents:

The extract was further processed for fractionation in 3 fractions chloroform, n-butanol, water. The preliminary chemical analysis of the fractions was done by using bioguided fractionation method [15-17]. The sample was dissolved in a mixture of Methanol and spotted (10 µL) with a 100 µL sample syringe (Hamilton, Bonaduz, Switzerland) in the shape of a band that was 6 mm wide on a precoated silica gel aluminium plate 60 F254 (5 cm 10 cm) with 250 m thickness (E. MERCK, Darmstadt, Germany) (Switzerland). Slit dimensions of 5 mm 0.45 mm and a scanning speed of 20 mm/sec were used. The linear development was conducted using toluene, ethyl acetate (EA), methanol, and formic acid (5: 4: 2: 0.5) as the mobile phase in a 10 cm x 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland). 10 minutes was the ideal chamber saturation period for the mobile phase. Chromatogram development took 8 cm of run length. The characterization of this compound is on progress which will be reported later. Until LC/MS characterization, the remaining fractions (Fr A3) were kept in separate vials based on invitro cytotoxicity assay.

3.5 DPPH radiacal scavenging assay:

Using DPPH, which is based on the idea of scavenging the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical, several subfractions (Fr A1–A3) were evaluated for their radical scavenging activity. After stirring the solutions for which various fractions and standard catechin had already been made, DPPH was added to them. Following keeping each mixture in the dark for thirty minutes, the absorbance was determined at 517 nm using a blank as a reference [18-20].

3.6 Invitro cytotoxicity assay:

Hep G2 cells were purchased from NCCS, Pune so as to perform the cell viability and cytotoxicity assay. Cells were cultured for 72 hrs before use. MTT assay was performed to measure the invitro cytotoxicity of the cell lines [21-23].

3.7 LC/MS characterization of active fractions

HPLC determination was done using 1 mg/ml of DHF2, on Agilent 1260 infinity II with Agilent 6540 UHP Accurate –Mass Q-TOF LCMS using Agilent eclipse XDB-C18, 3.0X150 mm, 3.5 micro column. The gradient elution was carried out with 0.1 % formic acid in acetonitrile (B). A- 0.1 % formic acid in H2O. The flow rate was 0.3 ml/min. The fraction was dissolved in H20/MeOH followed by direct injection into the M-QTOF LCMS. Capillary temp was 350 0C, voltage -3500V, Nozzle voltage 1000 V, fragmentor voltage -175V. However, full scan mode with mass range of m/z 100-1700 with the positive

polarity was applied.

3.8 Statistical analysis

Data were presented as mean ± standard deviation (SD) of three determinations. Results were calculated by employing statistical software (Graph Pad Software, La Jolla California USA).

4. CONCLUSION:

The in vitro metabolites of flavonoids, glycosides, Phenols have been successfully identified based on LC/ESI/MS analysis. Based on the 22 metabolites identified and their MS data. . This is the first report on the secondary metabolites in leaves of S. Trilobata and is considered a useful guide for understanding its metabolism in vivo. Therefore, further exploration on the isolation and detection of antioxidant component are obligatory to discover phytoconstituents of a probable use.

Declaration of interest

The authors declare that there is no conflict of interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

The author declared that they have no conflict of interest.

REFERENCES

1. Husain N, Kumar A. Comparative study of phytochemical constituents in flower of Wedelia trilobata, Achyranthes aspera and Chrysanthemum from Durg District of Chhattisgarh, India. Int. J. Curr. Microbiol. App. Sci. 2015;4(4):150-6. [Google Scholar], [Publisher], [Crossref]
2. Govindappa M, Bharath N, Shruthi HB, Sadananda TS, Sharanappa P. Antimicrobial, antioxidant and in vitro anti-inflammatory activity and phytochemical screening of Crotalaria pallida Aiton. African Journal of Pharmacy and Pharmacology. 2011 Dec 8;5(21):2359-71. [Google Scholar], [Publisher], [Crossref]
3.. Cai RJ, Yin XL, Liu J, Zhao GZ. Characterization and identification of in vitro metabolites of (-)- epicatechin using ultra-high performance liquid chromatography-mass spectrometry. Tropical Journal of Pharmaceutical Research. 2017;16(12):2985-90. DOI: 10.4314/tjpr.v16i12.24
4. Adedapo A A, Mogbojuri O M, Emikpe B O. Safety evaluations of the aqueous extract of the leaves of Moringa oleifera in rats. Journal of medicinal plants Research. 2009 Aug 31;3(8):586-91. [Google Scholar], [Publisher], [Crossref]
5. Lai H Y, Lim YY, Tan S P. Antioxidative, tyrosinase inhibiting and antibacterial activities of leaf extracts from medicinal ferns. Bioscience, biotechnology and biochemistry. 2009 Jun 23; 73(6):1362-6. [Google Scholar], [Publisher], [Crossref]
6 Fawole OA, Amoo SO, Ndhlala AR, Light ME, Finnie JF, Van Staden J. Anti-inflammatory, anticholinesterase, antioxidant and phytochemical properties of medicinal plants used for pain-related ailments in South Africa. Journal of Ethnopharmacology. 2010 Feb 3; 127(2):235-41. [Google Scholar], [Publisher]
7. Yoshida T, Konishi M, Horinaka M, Yasuda T, Goda AE, Taniguchi H, Yano K, Wakada M, Sakai T. Kaempferol sensitizes colon cancer cells to TRAIL-induced apoptosis. Biochemical and Biophysical Research Communications. 2008 Oct 10;375(1):129-33. [Google Scholar], [Publisher], [Crossref]
8. Taddei A, Rosas-Romero AJ. Antimicrobial activity of Wedelia trilobata crude extracts. Phytomedicine. 1999 May 1;6(2):133-4. [Google Scholar], [Publisher]
9 Turker AU, Usta C. Biological screening of some Turkish medicinal plant extracts for antimicrobial and toxicity activities. Natural Product Research. 2008 Jan 20;22(2):136-46. [Google Scholar], [Publisher], [Crossref]
10. Devare SM, Patil JA, Gaikwad SA, Torne RC, Deshpande NR, Salvekar JP. Antioxidant potential of Artemisia pallens roots. Int. J. PharmTech Res. 2013;5:1360-3.
11. Aksoy L, Kolay E, Ağılönü Y, Aslan Z, Kargıoğlu M. Free radical scavenging activity, total phenolic content, total antioxidant status, and total oxidant status of endemic Thermopsis turcica. Saudi journal of biological sciences. 2013 Jul 1;20(3):235-9.
12. Agrawal H, Joshi R, Gupta M. Isolation, purification and characterization of antioxidative peptide of pearl millet (Pennisetum glaucum) protein hydrolysate. Food Chemistry. 2016 Aug 1;204:365-72.
13. Brito S, Crescente O, Fernández A, Coronado A, Rodriguez N. Eficacia de un ácido kaurénico extraído de la planta venezolana Wedelia trilobata (Asterácea) contra Leishmania (Viannia) braziliensis. Biomedica. 2006 Oct;26:180-7. [Google Scholar], [Publisher], [Crossref]
14. Lin FM, Chen LR, Lin EH, Ke FC, Chen HY, Tsai MJ, Hsiao PW. Compounds from Wedelia chinensis synergistically suppress androgen activity and growth in prostate cancer cells. Carcinogenesis. 2007 Dec 1;28(12):2521-9. [Google Scholar], [Publisher], [Crossref]
15. Ma BJ, Wen CN, Gao Y, Ren FC, Wang F, Liu JK. ent-Kaurane diterpenoids from the plant Wedelia trilobata. Natural Products and Bioprospecting. 2013 Jun;3:107-11. [Google Scholar], [Publisher]
16. Balekar N, Nakpheng T, Katkam NG, Srichana T. Wound healing activity of ent-kaura-9 (11), 16-dien-19-oic acid isolated from Wedelia trilobata (L.) leaves. Phytomedicine. 2012 Oct 15;19(13):1178-84. [Google Scholar], [Publisher], [Crossref]
17. Mizokami SS, Arakawa NS, Ambrosio SR, Zarpelon AC, Casagrande R, Cunha TM, Ferreira SH, Cunha FQ, Verri Jr WA. Kaurenoic acid from Sphagneticola trilobata inhibits inflammatory pain: effect on cytokine production and activation of the NO–cyclic GMP–protein kinase G–ATP-sensitive potassium channel signaling pathway. Journal of Natural Products. 2012 May 25;75(5):896-904. [Google Scholar], [Publisher], [Crossref]
18. Li Y, Hao X, Li S, He H, Yan X, Chen Y, Dong J, Zhang Z, Li S. Eudesmanolides from Wedelia trilobata (L.) Hitchc. as potential inducers of plant systemic acquired resistance. Journal of agricultural and food chemistry. 2013 Apr 24; 61(16):3884-90. [Google Scholar], [Publisher], [Crossref]
19. Taddei A, Rosas-Romero AJ. Antimicrobial activity of Wedelia trilobata crude extracts. Phytomedicine. 1999 May 1;6 (2):133-4. [Google Scholar], [Publisher]

20. Balekar N, Katkam NG, Nakpheng T, Jehtae K, Srichana T. Evaluation of the wound healing potential of Wedelia trilobata (L.) leaves. Journal of Ethnopharmacology. 2012 Jun 14;141(3):817-24. [Google Scholar], [Publisher], [Crossref]
21. Patil PS, Venkatanarayanan R, Argade PD, Shinde PR. Assessment of pharmacognostic and phytochemical standards of Thespesia populnea (L.) root. Asian Pacific Journal of Tropical Biomedicine. 2012 Jan 1;2 (3):S1212-6. [Google Scholar], [Publisher]
22. Fucina G, Rocha LW, da Silva GF, Hoepers SM, Ferreira FP, Guaratini T, Cechinel Filho V, Lucinda-Silva RM, Quintão NL, Bresolin TM. Topical anti-inflammatory phytomedicine based on Sphagneticola trilobata dried extracts. Pharmaceutical biology. 2016 Nov 1;54(11):2465-74. [Google Scholar], [Publisher]
23. Balekar N, Nakpheng T, Srichana T. Wedelia trilobata L.: A phytochemical and pharmacological review. Chiang Mai Journal of Science. 2014 Jul 1;41(3):590-605. [Google Scholar], [Publisher], [Crossref]
24. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. springer science & business media; 1998 Apr 30.