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Original Article

Investigation on the Phytochemistry, Antioxidant, and Hepatoprotective Properties of the Ethanolic Extract of *Gymnostachyum febrifugum* Benth

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ABSTRACT
Background: The phytochemical evaluation of the ethanolic extract of the plant, *Gymnostachyum febrifugum* Benth. (Acanthaceae family) was carried out to evaluate its therapeutic potential. Despite being part of traditional medicine, not much research has been done on the plant. **Materials and Methods:** The study aimed to understand the plant's phytochemistry and evaluate its therapeutic potential. For this purpose, DPPH, ABTS, FRAP, Total Antioxidant Assay, MTT Assay, and HR-LCMS techniques were employed. **Results:** Major secondary metabolites in the ethanolic extract were flavonoids, tannins, phenolics, and steroids. The free radical scavenging capacity of the ethanolic extract of the plant was evaluated using four different methods, proving the plant to possess high antioxidant properties. Hence, the hepatoprotective potential of ethanolic extract was evaluated using an MTT assay on Hep G2 cells induced with hepatotoxicity using Acetaminophen. When treated with ethanolic extract, the cell viability was found to be 85.32% at 12.5 μg/mL concentration. IC_{50} for ethanolic extract was attained at 75.77 μg/mL. Also, chemical characterization of the extract was performed using the HR-LCMS technique. Around 64 compounds were obtained in chromatogram using positive ESI mode, of which 24 are unknown and 40 are known compounds. Out of 37 compounds obtained in the negative ESI mode, 27 were known, and 10 were unknown.

Keywords: *Gymnostachyum febrifugum* Benth. , Acanthaceae, Antioxidant assay, HR-LC MS, MTT assay, hepatoprotective.

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INTRODUCTION

Gymnostachyum febrifugum Benth. belonging to the Acanthaceae family is an elegant, rare, endemic, and ornamentally important plant, indigenous to India and distributed along the Southern Western Ghats of India. The plant usually grows near the banks of small running water bodies. It is a small scrapigerous, stemless attractive herb with large ovate green leaves, beautiful pink flowers with yellow lips, and a woody rootstock.[1,2] Locally known as, '*Nelamuchchala*' or '*Navuneeti*' the root of this plant is traditionally used along various areas of south western ghats of India for the management of fever, indigestion, headache, Metrorrhagia, Purpureal fever[2], ulcers, cough, and stomatitis.[3] In folklore medicine, root decoction of the plant at a dose of 10-15 mL thrice daily is used for the management of fever in children.[4]

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This plant has febrifugal properties and is also used as an antidote for viper bites.[5] The plant and its chemical constituents have not been studied extensively. Based on the literature review, the methanolic extract of the roots of *G. febrifugum* Benth. possessed very good antioxidant and antimicrobial potential.[6] The current study focuses on the phytochemical evaluation of the ethanolic extract of the whole plant, its antioxidant activity, hepatoprotective potential, and phytochemical fingerprinting using the HR-LCMS technique.

MATERIALS AND METHODS

All chemicals and reagents used were of analytical or HPLC grade. DPPH (1,1-diphenyl-2-picrylhydrazyl), and ABTS (2,20 -azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) were procured from Merck. MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and HIMEDIA (Dulbecco's modified eagle's media) was obtained from Sigma Aldrich. Human hepatic cell lines of HEP G2 were procured from NCCS Pune.

Plant collection and authentication

Fresh plants were collected from their natural habitat at Kakkayam, Kozhikode district of Kerala, India in March 2021. The plant was identified and authenticated by Dr. V.B. Sreekumar. A specimen voucher (authentication no-18126) was deposited in the herbarium, Dept. of Botany, KFRI (Kerala Research Forest Institute), Peechi, Thrissur.

Extraction

The plant material collected was thoroughly washed under running water and dried under the shade for 10 days. The dried plants were powdered and sieved through 45 mesh sieves. Successive Soxhlet extraction was carried out on 400g crude powder using 1000 mL of solvents: Petroleum Ether followed by Ethyl acetate and Ethanol.

$$\% \text{ Yield}=(W1X100)/W2$$

Where W1 is the amount of extract (grams) after drying and W2 is the amount of the dry plant material (grams) taken for extraction. The % yield of extract was 0.70 (Petroleum Ether), 4.95 (Ethyl acetate), and 16.07 (Ethanol) respectively. The extracts were dried and stored at 2-8°C.

Preliminary Phytochemical Screening

Preliminary phytochemical screening was carried out on the ethanolic extract of the plant and was positive for tests for Phenolics, Steroids, Flavonoids, Tannins, and Saponins. The quantitative analysis of these secondary metabolites was also carried out.[7-11]

Antioxidant activity

DPPH Radical–Scavenging Activity

The ethanolic extract's 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity was evaluated and the % activity was calculated using the following Formula.[12]

$$\% \text{ DPPH scavenging activity}=(a0-a1)/a0\times100$$

Where, a0 is the absorbance of the control and a1 is the sample absorbance. The extract exhibited a linear increase in activity with an increase in concentration.

ABTS Radical Scavenging Activity Assay

The ethanolic extract was also subjected to evaluate the ABTS radical (2,20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) scavenging capacity and was compared with Ascorbic acid standard solution. The % activity of the extract was measured by evaluating the scavenging capacity of the ethanolic extract against the free radical, ABTS.[12]

Determination of Ferric Reducing Antioxidant Power (FRAP)

The antioxidant ability of the ethanolic extract to reduce Fe^{3+} to Fe^{2+} ions was evaluated by the FRAP method of analysis and is expressed in AAE (Ascorbic Acid Equivalents)/mL units. The absorption maximum of the blue colour formed by the reduced ferrous ion with TPTZ reagent was measured at 593 nm. An increase in the absorbance indicates the antioxidant capacity of the extract or Ascorbic acid to reduce ferric ions to ferrous ions.[12]

Determination Of Total Antioxidant Capacity Assay

The ethanolic extract's total antioxidant activity was evaluated using the phosphomolybdenum method. The absorbance was measured at 695 nm using a UV-visible spectrophotometer and higher absorbance exhibited higher antioxidant activity. The calibration curve was plotted for various concentrations of Ascorbic acid and the unknown concentration was extrapolated from the graph.[13] All the reagents used were of analytical grade and procured from Merck. The standard, Ascorbic acid was obtained from Sigma Aldrich.

In vitro Evaluation of Hepatoprotective Activity

The hepatoprotective ability of the ethanolic extract was evaluated using an MTT assay on HEP G2 cells. Hepatotoxicity was induced by exposing the cells to Acetaminophen and the regenerative capacity of the cells was evaluated after treatment with the plant extract.

Evaluation of hepatic cell regenerative capacity

The healthy, human hepatic cell lines of HEP G2 were seeded and incubated (37°C) in a 96-well plate. After attaining sufficient cell growth, cell toxicity was induced by adding 20 mM Acetaminophen solution followed by incubation for one hour. 1 mg of the ethanolic extract was thoroughly mixed in 1 mL of 0.1% DMSO and filtered. Various concentrations of the extract (100 μg, 50 μg, 25 μg, 12.5 μg, and 6.25 μg in 500 μL) were prepared in 5% DMEM. 100 μL of each concentration was added to the wells in triplicate and incubated in a 5% CO_2 incubator.

The plates were viewed in an inverted phase contrast tissue culture microscope at intervals of 24 hr up to 72 hr and the images were recorded. Toxicity was evaluated based on the changes in the morphology of the cells. Cytotoxicity and the cell regenerative capacity of the ethanolic extract were also assessed using an MTT assay. 30 μL of sterile MTT solution prepared in Phosphate-Buffered Saline (PBS) was added to all test, standard (Silymarin), and control wells. After 4 hr of incubation at 37°C, the supernatant solution was removed and 100 μL of DMSO was added to solubilize the Formazan crystals formed. Absorbance was measured at 540 nm using a microplate reader and percentage growth inhibition, and cell viability was calculated.

The absorbance and percentage viability obtained are listed in Table 1. A graph was plotted using a Graph pad prism, and the IC_{50} value was calculated and is provided in Figure 1. The direct microscopic observation is provided in Figure 2.

Phytochemical evaluation by HR-LCMS

The ethanolic extract of *G. febrifugum* Benth. was subjected to HR-LCMS analysis and chemical fingerprints were obtained using high-resolution liquid chromatography and mass spectrometry (MS Q-TOF, model-G6550A, Agilent technologies). The Hypersil GOLD C18 column was used for the separation with a dimension of 100x2.1 mm-3 µ (G1316C) at a column temperature of 40°C. A gradient mobile phase system was used with varying ratios of 0.1% Formic acid in water and Acetonitrile, with a constant flow rate of 0.3 mL.

RESULTS AND DISCUSSION

The freshly collected, dried, and pulverized whole plants of *G. febrifugum* were subjected to successive soxhlet extraction with solvents of increasing polarity: Petroleum ether, followed by Ethyl acetate, and finally Ethanol. The phytochemical evaluation of the ethanolic extract tested positive for phenolics, steroids, flavonoids, glycosides, saponins, and tannins. The quantitative analysis of the ethanolic extract of *G. febrifugum* specifies that the plant is rich in flavonoids-5.81%±0.354,[7] phenolics-3.88%±0.057,[8] steroids-2.36%±0.156,[9] saponins-6.65%[10] and tannins-0.77%.[11]

Antioxidant activity Evaluation

The antioxidant potential of the ethanolic extract of *G. febrifugum* Benth. was evaluated using 4 different methods. The extract showed strong DPPH radical inhibition and the IC_{50} was found to be 57 µg/mL. The capacity to reduce ferric to ferrous ions (FRAP) was calculated as 260 µg of Ascorbic acid units/g. IC_{50} value obtained from the ABTS scavenging activity method was 59.5 µg/mL and the Total Antioxidant activity was calculated as 340 µg/g Ascorbic acid equivalents.

In vitro Hepatoprotective Assessment using MTT Assay

The ethanolic extract of *G. febrifugum* was evaluated for its hepatoprotective effects by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay on healthy HepG2 cells. Hepatotoxicity was induced in healthy HepG2 cells by Acetaminophen (20 mM).[14] The percentage viability of acetaminophen (20 mM) exposed Hep G2 cells when treated with 12.5 µg/mL of ethanolic extract of *G. febrifugum* Benth. was 85.32%. This was done in comparison with standard Silymarin whose percentage viability was found to be 63.54 at the same concentration, (Figure 3). However, the percentage of cell viability was found to decrease with the increasing concentration of the extract. The IC_{50} value was plotted using the GraphPad Prism and was found to be 75.77 µg/mL.

HR LC-MS Phyto chemical Profiling

The chromatogram obtained with HR-LCMS analysis[15-17] of ethanolic extract indicated the presence of various phytochemical constituents. Both positive and negative modes of ionization studies were performed. The positive ESI profile showed 24 unknown and 40 known compounds and the negative ESI profile showed 27 known and 10 unknown compounds. The fingerprint obtained was interpreted by comparing their retention time, molecular formula, and mass against the database at SAIF library, IIT, Mumbai. Copies of the original spectra are obtainable from the corresponding author.

The relative concentrations of various compounds eluted as a function of retention time were calculated from the area under the curve from the chromatogram, Figures 3a and 3b. The list of known compounds is provided in Tables 2 and 3. The most abundant phytochemicals identified from the extract include

Table 1: ***In vitro*** **analysis of hepatoprotective properties of Ethanolic extract on toxicity-induced HEP G2 cells.**

Sl. No.	Sample	Concentration (µg/mL)	%Viability
1	Control	-	100
2	Acetaminophen	20 mM	47.63
3	Silymarin	6.25	55.92
4		12.5	63.54
5		25	74.66
6		50	80.69
7		100	94.21
8	Ethanolic extract	6.25	79.5
9		12.5	85.32
10		25	64.96
11		50	54.54
12		100	35.22

Extract 3 – IC50 75.77 µg/ml (R^2 = 0.9901)

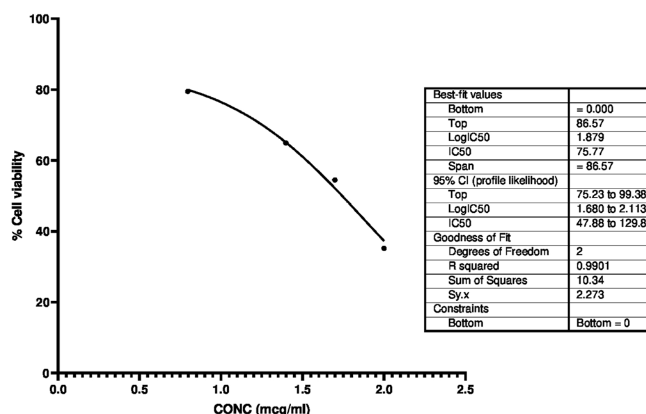

Figure 1: IC_{50} value of Ethanolic extract from MTT assay.

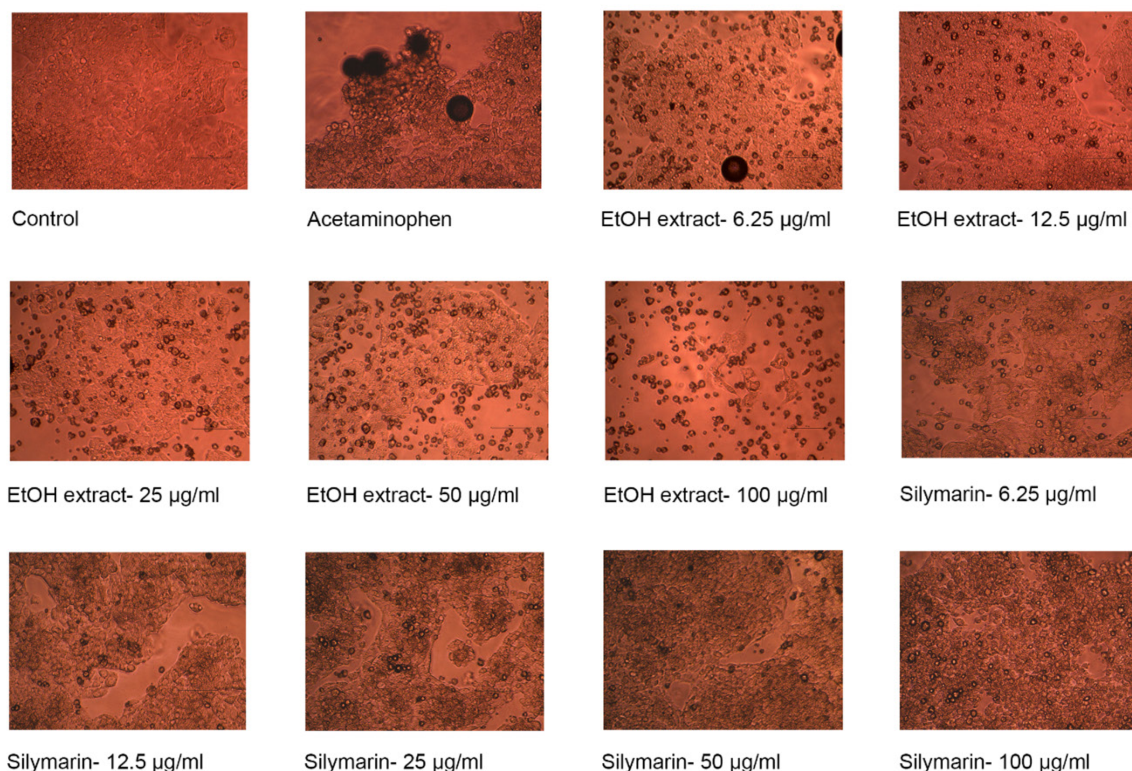

Figure 2: Cytotoxicity Assay by Direct Microscopic Observation.

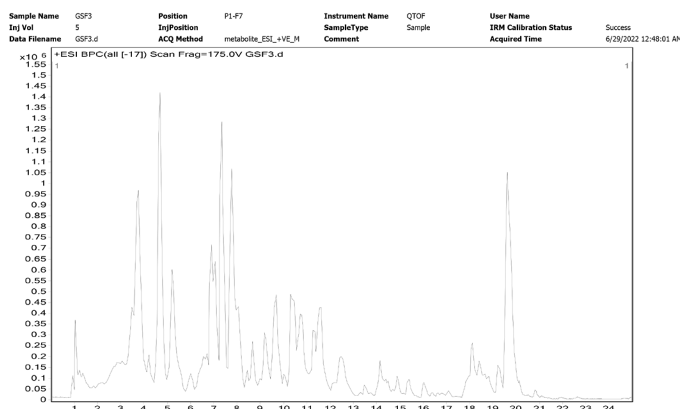

Figure 3a: HR-LCMS Chromatogram (Positive ESI) of Ethanolic extract.

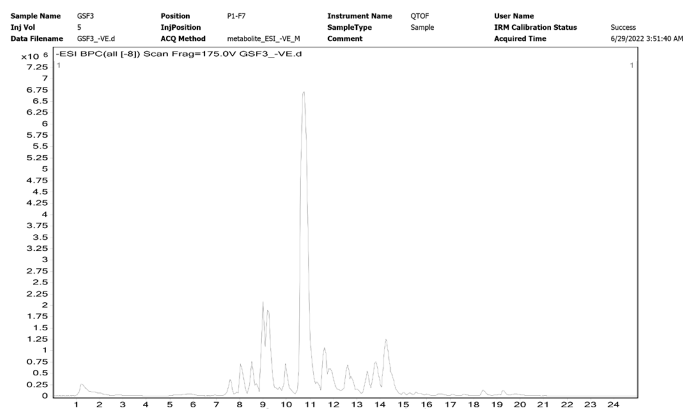

Figure 3b: HR-LCMS Chromatogram (Negative ESI) of Ethanolic extract.

2-(4-Methyl-5-thiazolyl) ethyl decanoate, Pinacidil,[18] Fosinopril,[19] Licoricesaponin A3,[20] Avocadynofuran. Based on the literature review, Pinacidil belongs to the class of potassium channel openers and acts as an antihypertensive drug. Fosinopril is an ACE inhibitor and is beneficial for patients with hypertension and left ventricular hypertrophy. Licoricesaponin A3, a natural triterpenoid has been proven to possess potent inhibitory activity against SARS CoV 2 virus and is also cytotoxic against the human cancer cell lines MGC-803, SW620, and SMMC-7721. Some of the most abundant secondary metabolites available from the negative ionization spectrum were Saccharocin,[21] an aminoglycoside antibiotic, chlorogenic acid a polyphenol, and Caryoptin a diterpene.

The phytochemical and pharmacological analysis of *G. febrifugum* demonstrates that the plant has good antioxidant properties and has high phenolic and terpenoid content. The plant shows good hepatoprotective properties at lower doses and further investigations on this plant can lead to safe and potential usage of the plant for several therapeutic needs.

Table 2: HR-LCMS constituent analysis of Ethanolic extract (Negative ESI).

Sl. No.	Name	RT	Formula	Mass	Abundance
1	Syringic acid	9.709	$C_9H_{10}O_5$	198.0519	726
2	Kaempferol	9.092	$C_{15}H_{10}O_6$	286.0477	5441
3	10-Oxo-11-octadecen-13-olide	14.619	$C_{18}H_{30}O_3$	294.22	14712
4	Chlorogenic acid	7.54	$C_{16}H_{18}O_9$	354.0952	74841
5	Linalyl caprylate	18.406	$C_{18}H_{32}O_2$	280.2404	68688
6	Verbasoside	7.553	$C_{20}H_{30}O_{12}$	462.1747	
7	Quinic acid	8.027	$C_7H_{12}O_6$	192.0625	
8	Plantamajoside	8.501	$C_{29}H_{36}O_{16}$	640.2035	
9	(+)-Gallocatechin	8.651	$C_{15}H_{14}O_7$	306.0771	
10	Suspensaside	8.853	$C_{29}H_{36}O_{16}$	640.2028	101367
11	Isoacteoside	8.962	$C_{29}H_{36}O_{15}$	624.2087	
12	Clocortolone pivalate	9.727	$C_{27}H_{36}C_1FO_5$	494.2236	115902
13	Tetrahydrofolyl-[Glu](2)	9.981	$C_{24}H_{30}N_8O_9$	574.2148	52278
14	NPC$_\$$	10.058	$C_{28}H_{30}N_4O_6$	518.2215	446109
15	Cinnamoside	10.324	$C_{24}H_{38}O_{12}$	518.2371	32328
16	Ptelatoside A	10.582	$C_{19}H_{26}O_{10}$	414.1548	
17	Myricanol 5-laminaribioside	10.827	$C_{33}H_{46}O_{15}$	682.2707	
18	(2S)-2-Butanol O-[b-D-Apiofuranosyl-(1->6)-b-D-glucopyranoside]	11.241	$C_{15}H_{28}O_{10}$	368.1682	310592
19	b-D-Xylopyranosyl-(1->4)-a-L-rhamnopyranosyl-(1->2)-L-arabinose	11.331	$C_{16}H_{28}O_{13}$	428.1528	
20	Androsterone sulfate	11.707	$C_{19}H_{30}O_5S$	370.1834	
21	Saccharocin	11.864	$C_{21}H_{40}N_4O_{12}$	540.2609	511961
22	Caryoptin	12.252	$C_{26}H_{36}O_9$	492.2436	134862
23	Daphnoline	12.408	$C_{35}H_{36}N_2O_6$	580.2562	119653
24	Tigecycline	12.621	$C_{29}H_{39}N_5O_8$	584.2666	126328
25	Hematoporphyrin	12.623	$C_{34}H_{38}N_4O_6$	598.2823	256878
26	L-365260$_\#$	13.353	$C_{24}H_{22}N_4O_2$	398.1784	
27	Tetrahydrofolyl-[Glu](2)	13.928	$C_{24}H_{30}N_8O_9$	574.2128	

$ -4-Hydroxy-3-Nitrophenylacetyl-Epsilon-Aminocaproic Acid Anion.# -1-[(3R)-1-methyl-2-oxo-5-phenyl-3H-1,4-benzodiazepin-3-yl]-3-(3-methylphenyl) urea.

Table 3: HR-LCMS constituent analysis of ethanolic extract (positive ESI).

Sl. No.	Name	RT	Molecular Formula	Avg Mass	Abundance
1	Esprocarb	0.955	$C_{15}H_{23}NOS$	265.1497	110850
2	D-1-Aminopropan-2-ol O-phosphate	0.978	$C_3H_{10}NO_4P$	155.0327	86027
3	Lentiginosine	3.408	$C_8H_{15}NO_2$	157.1089	210830
4	2-(4-Methyl-5-thiazolyl)ethyl decanoate	3.723	$C_{16}H_{27}NO_2S$	297.1779	486355
5	(±)-Currayangine	4.213	$C_{23}H_{25}NO$	331.1955	76599
6	n-decanohydroxamic acid	4.521	$C_{10}H_{21}NO_2$	187.1551	81746
7	Pinacidil	4.572	$C_{13}H_{19}N_5$	245.1624	822813
8	S-(Phenylacetothiohydroximoyl)-L-cysteine	5.072	$C_{11}H_{14}N_2O_3S$	254.0743	106389

Sl. No.	Name	RT	Molecular Formula	Avg Mass	Abundance
9	para-Trifluoromethylphenol	5.149	$C_7H_5F_3O$	162.0298	269337
10	Clavamycin D	5.426	$C_{13}H_{21}N_3O_6$	315.1437	137228
11	Biotripyrrin-b	6.116	$C_{25}H_{27}N_3O_6$	465.1945	73240
12	Pseudoaconitine	6.348	$C_{36}H_{51}NO_{12}$	689.3435	83278
13	(Z)-3-(1-Formyl-1-propenyl)pentanedioic acid	6.437	$C_9H_{12}O_5$	200.0685	152789
14	2-Aminoethylphosphocholate	6.582	$C_{26}H_{46}NO_7P$	515.3061	74206
15	Gentisyl alcohol	6.647	$C_7H_8O_3$	140.048	167520
16	3,5-Dihydroxyphenyl 1-O-(6-O-galloyl-beta-D-glucopyranoside)	6.837	$C_{19}H_{20}O_{12}$	440.0921	97182
17	Fosinopril	6.857	$C_{30}H_{46}NO_7P$	563.3035	296719
18	6''-(4-Carboxy-3-hydroxy-3-methylbutanoyl)hyperin	6.863	$C_{27}H_{28}O_{16}$	608.1313	86250
19	Z-Arg-Arg-NHMec	7.147	$C_{30}H_{39}N_9O_6$	621.3082	77575
20	Candletoxin A	7.53	$C_{35}H_{44}O_9$	608.2993	78070
21	(3a,5b)-24-oxo-24-[(2-sulfoethyl)amino]cholan-3-yl-b-D-Glucopyranosiduronic acid	9.142	$C_{32}H_{53}NO_{11}S$	659.3254	241089
22	Furaneol 4-glucoside	10.037	$C_{12}H_{18}O_8$	290.0989	87186
23	124-1	10.038	$C_{37}H_{44}O_{14}$	712.2635	88279
24	Licoricesaponin A3	10.361	$C_{48}H_{72}O_{21}$	984.463	225563
25	Avocadynofuran	10.598	$C_{17}H_{26}O$	246.1975	185249
26	AFN911@	10.655	$C_{29}H_{33}N_7O_2$	511.2725	242719
27	Flumethasone pivalate	10.658	$C_{27}H_{36}F_2O_6$	494.2461	267765
28	1alpha,3beta,22R-Trihydroxyergosta-5,24E-dien-26-oic acid 3-O-b-D-glucoside 26-O-[b-D-glucosyl-(1->2)-6-acetyl-b-D-glucosyl] ester	10.699	$C_{48}H_{76}O_{21}$	988.4932	242672
29	28-Glucosyl-30-methyl-3b,23-dihydroxy-12-oleanene-28,30-dioate 3-[arabinosyl-(1->3)-glucuronide]	10.702	$C_{48}H_{74}O_{21}$	984.4704	251335
30	5-Methyltetrahydropteroyltri-L-glutamate	11.285	$C_{25}H_{36}N_8O_{12}$	640.244	75730
31	(7'R)-(+)-Lyoniresinol 9'-glucoside	12.368	$C_{28}H_{38}O_{13}$	582.2384	112997
32	Triphenyl phosphate	14.143	$C_{18}H_{15}O_4P$	326.0672	125929
33	Flavidulol C	14.551	$C_{34}H_{42}O_4$	514.3088	83219
34	Ganoderic acid F	18.125	$C_{32}H_{42}O_9$	570.2803	168459
35	Cepharanthine	18.47	$C_{37}H_{38}N_2O_6$	606.2762	77627
36	Myricanene B 5-[arabinosyl-(1->6)-glucoside]	18.765	$C_{32}H_{42}O_{13}$	634.2721	89007
37	Gnididin	18.783	$C_{37}H_{44}O_{10}$	648.2861	111002
38	Hypaconitine	19.255	$C_{33}H_{45}NO_{10}$	614.3062	185766
39	Delphinine	19.507	$C_{33}H_{45}NO_9$	599.3141	197807
40	Goshonoside F3	19.526	$C_{32}H_{52}O_{13}$	644.3525	77084
41	Lanceotoxin A	19.758	$C_{32}H_{44}O_{12}$	620.2935	616363

@ - a metabolite of imatinib (benzylic hydroxylation).

CONCLUSION

This study is focused on identifying the phytoconstituents and therapeutic potential of the ethanolic extract of *Gymnostachyum febrifugum* Benth. In folklore medicine, the root of the plants has been used for its antipyretic, anti-viral, and anti-ulcer properties. Despite this, very little work has been reported on this plant. This study included the qualitative and quantitative evaluation of phytoconstituents and the pharmacological potential of the ethanolic extract. The ethanolic extract of the whole plant was found to possess good antioxidant and hepatoprotective

properties. Based on the study, the regenerative potential of the toxicity-induced hepatocytes, after treatment with the ethanolic extract was found to be dose-dependent. The cell viability increased to 85.32% at a concentration of 12.5 μg/mL which then decreased substantially at higher concentrations. Hence, it may be concluded that dose adjustments and phytoconstituent isolation are important before the use of the whole plant for any treatment. Based on the phytoconstituent analysis by HR-LCMS, several compounds were identified in comparison with the library. Most of the compounds identified possess potential therapeutic benefits and hence shall be used beneficially if processed properly.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DPPH: 1,1-diphenyl-2-picrylhydrazyl; **ABTS:** 2,20 -azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); **FRAP:** Ferric Reducing Antioxidant Power Assay; **MTT:** 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide); **HR-LCMS:** High-resolution Liquid Chromatography Mass Spectrometer; **IC_{50}:** Half maximal Inhibitory concentration; **ESI:** Electrospray Ionization; **HepG2:** Hepatoblastoma cell line; **NCCS:** National Centre for Cell Science; **TPTZ:** 2,4,6-Tripyridyl-s-triazine reagent; **DMSO:** Dimethyl sulfoxide; **DMEM:** Dulbecco's Modified Eagle Medium; **MS Q TOF:** Mass Spectrometry Quadrupole Time-of-Flight; **SAIF:** Sophisticated Analytical Instrumentation Facility; **ACE inhibitor:** Angiotensin-converting enzyme.

SUMMARY

Gymnostachyum febrifugum Benth. of the Acanthaceae family, despite being part of traditional medicine has not been studied extensively. From the study conducted it was evident that the ethanolic plant extract exhibited potential antioxidant properties in comparison with the standard, Ascorbic acid. Based on these results the *in vitro* evaluation of hepatoprotective activities was carried out. It was observed that the extract possesses good regenerative capacities for hepatocytes subjected to acetaminophen toxicity compared to Silymarin. However, the hepatocyte regenerative capacity was subject to vary with concentration. The extract exhibited reduced cell viability with increasing concentration, which might indicate the potential anticancer properties of the extract. Hence, even though the product exhibits several therapeutic benefits proper characterization, isolation, and evaluation of the phytoconstituents must be performed before its therapeutic usage.

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