Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
Impact Factor (RJIF): 5.94
www.plantsjournal.com
JMPS 2025; 13(5): 142-152

Received: 07-06-2025
Accepted: 10-07-2025

Praveen T
Department of Studies in Botany, Davangere University, Shivagangotri, Davangere, Karnataka, India

Siddappa B Kakkalameli
Department of Studies in Botany, Davangere University, Shivagangotri, Davangere, Karnataka, India

Corresponding Author:
Siddappa B Kakkalameli
Department of Studies in Botany, Davangere University, Shivagangotri, Davangere, Karnataka, India

Studies on phytochemical, GC-MS analysis *in vitro* cytotoxic assay and their biological Activities of *Cynarospermum asperrimum* (Nees) Vollesen. (Acanthaceae)

Praveen T and Siddappa B Kakkalameli

Abstract
The present investigation deliberates the chemical profiling and biological activities of *Cynarospermum asperrimum* (Nees) Vollesen. Using three different polar and non-polar solvents. The proximate analysis reveals the presence of various components, including total carbohydrates, total protein, and total lipids. The qualitative and quantitative phytochemical analysis reveals that it is rich in Phytoconstituents, showing the presence of polyphenols, alkaloids, flavonoids, and tannins, among others. The GC-MS analysis shows significant bioactive compounds linked to multiple health benefits, The FTIR analysis shows functional groups based on peak values, the peaks are detected from 4000cm^{-1} to 600cm^{-1}. The antibacterial activity (*Escherichia coli* and *Staphylococcus aureus*) and Antifungal activity (*Aspergillus flavus and Pichia anomala)* show significant zones of inhibition. Antioxidant assay by the DPPH method reveals that the IC50 value is 57.24±3.83 µg/ml in the methanol extract, due to the presence of various antioxidant phytochemicals, indicating the potential for scavenging free radicals. Distilled water, and chloroform show moderate results. The *in vitro* Cytotoxicity by MTT assay against MCF-7 and HepG2 cell lines both show significant results with IC_{50} values of MCF-7 58.38±3.13 µg/ml, and HepG2 67.57±4.1 µg/ml, in the methanol extract. These findings suggest *Cynarospermum asperrimum* (Nees) Vollesen. Leaf has promising functional pharmaceutical applications.

Keywords: Antimicrobial, antioxidant, *Cynarospermum asperrimum* anti-cancer (MCF-7, HepG2), FTIR, GC-MS, phytochemical, proximate analysis

Introduction
Cynarospermum asperrimum (Nees) Vollesen is a flowering plant in the family Acanthaceae. The native range of this species is western India, where it grows primarily in the seasonally dry tropical biomes. Acanthaceae comprises 346 genera and 4300 species, most of which are herbs, shrubs, and vines, cosmopolitan and distributed in the Old and New World. Mainly present in Africa, Central America, Malaysia, and Indonesia, with a few species extending to South Europe, Japan, the Southern coast of New Holland, and Southern to the Cape of Good Hopes, In India, and all over the world, a large number of plants are used to treat several ailments. Acanthaceae genera are peculiar to the Southern parts, the Indian Archipelago, and Malayan Peninsula, but have spread from the Sutlej to the Sylhet and the lower ranges of the Himalayas [1]. Approximately eighty percent (80%) of the globe's population relies on conventional medicine to provide primary healthcare [2]. Herbal medicines are the basis of treating and curing diseases and physical conditions through traditional methods such as Unani, Ayurveda, and Siddha. Grassy medicines have various remedial uses such as healing wounds, and treating swellings due to infection, scabies, leprosy, skin lesions, diarrhea, snake bites, ulcers, etc [3]. The Acanthaceae members are the richest source of drugs for conventional systems of medicine, nutraceuticals, folk medicines, and pharmaceutical intermediates, as well as synthetic drugs [4]. Various species of Blepharis have been traditionally claimed to possess medicinal potential, including anti-inflammatory, anti-nociceptive, anti-fungal, anti-bacterial, antispasmodic, antioxidant, aphrodisiac, and cytotoxic activities. The members of Acanthaceae are conventionally used to treat bone fractures, urinary discharges, skin diseases, and allergies [5]. Present research was performed to study FTIR, GC-MS analysis, phytochemical (Qualitative and Quantitative), Antimicrobial, Antioxidant, and Anticancer activities.

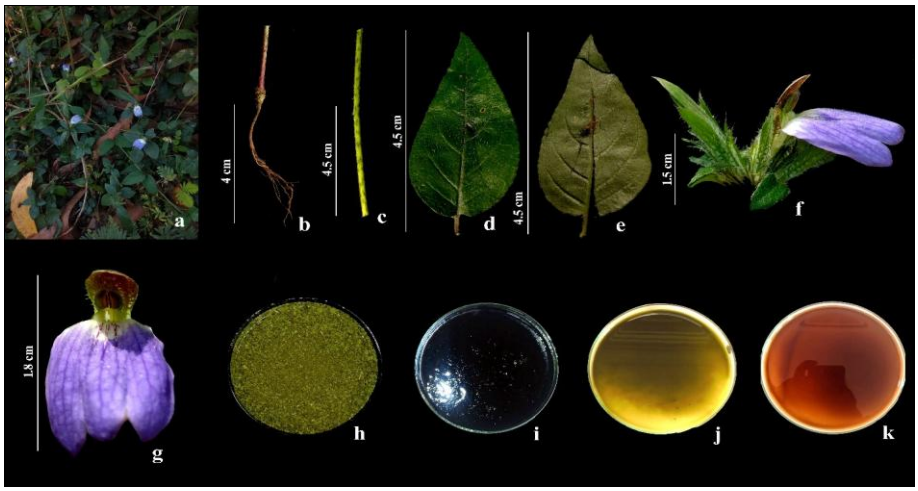

Fig 1: A. Habit, B. Root, C. Stem, D. Adaxial leaf, E. Abaxial leaf, F. Inflorescence, G. Flower, H. Dry powder, I. Methanol extract, J. Aqueous extract, K. Chloroform extract.

Materials and Methods

Collection and Identification of Plant Material

The collection of *Cynarospermum asperrimum (Nees) Vollesen* collected from Chigule village, Belgaum District, Karnataka, India, during February and the plant was authenticated by referring to the standard flora and the herbarium was identified by A N Sringeswara, curator, Mahatma Gandhi Botanical Garden, University of Agricultural Sciences, GKVK Bangalore, collection Number 008, Accession number UASB 5633.

Preparation of Plant Extract

The leaves were washed to remove the dirt and extraneous matter. Leaves were shade-dry, the leaf was powdered with the help of a grinder machine, and employed for Soxhlet extraction. The sample is weighed about 25 grams, packed with the help of No. 1 Whatman filter paper, with 250 mL of Distilled Water. Methanol and Chloroform solvents were used to extract, respectively and the crude extract is collected and used for further analysis.

Proximate compositions

The plant-dried powder was determined by (AOAC, 1995). Moisture content was analyzed by drying the biomass for 10 hours at 100 °C, using the Soxhlet method. Lipid content was estimated by the phenol-sulfuric acid method. Carbohydrates were determined by the Kjeldahl method, protein was measured in a muffle furnace at 550 °C the ash content was determined. Nutritive Value.

Total phenolic content estimation

The TPC using FC reagent, the Gallic acid is used as a standard antioxidant. The results are expressed in (GAE)/mg extract. 100 µg of appropriately diluted extracts, 0.5 mL of FCR was added to it, incubation at room temperature (10 min), and then the 7% Na^2CO^3s solution (2 mL) solution was added. The content was boiled for 1 min, and absorbance and color were recorded at 750nm using a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) [6].

Total flavonoid estimation

The flavonoid was estimated according to the method[7,] 200 µg of sample, chromogen reagent 5 mL (methanol and 25 mL concentrated HCl and 0.1% cinnamaldehyde solution in a cooled mixture of 75 mL) was added. After 10 min incubation, the absorbance was recorded at 640nm. The flavonoid was express in µg catechin equivalents (CE)/mg of extracts.

Total alkaloid estimation

The 200 µg of sample is dissolved in 1 ml of HCl 2N, then filtered and washed with 10 ml of chloroform. Similarly, the different aliquots (20, 40, 60, 80, and 100 µg/ml) of atropine standard (Sigma Chemical, USA) were used. Then add Bromocresol green (BCG), 5 ml solution (prepared by heating 69.8 mg bromocresol green with NaOH 2N3 ml) and PB solution (pH 4.7), then add the water dissolved and make up to 10 ml. Absorbance were measured at 470nm blank prepared as above, but without atropine or sample. The alkaloid content is expressed in µg of AE/mg of extract [7].

Total Tannins estimations

The tannin was estimated in samples by the method [8]. an aliquot of a sample (0.05 ml), taken in a TT, and the volume was made up to 0.5 ml with water. Later, 0.25 ml of the Folin-Ciocalteu reagent (1N) added &then mixed with 1.25 ml of sodium carbonate solution (20%). Incubated for 40 min, vortexed, the absorbance were recorded at 725 nm. The results were expressed as µg tannic acid equivalents (TAE)/mg of extract.

Qualitative Phytochemical Analysis

Qualitative phytochemical analysis was done by using specific chemical reagents for different secondary metabolites of phytochemicals (Phenols, Alkaloids, Terpenoids, Saponins, Steroids, Glycosides, Flavonoids, Tannins, Proteins, and Carbohydrates) [9].

Antimicrobial activity

The antimicrobial activity by the Agar Diffusion Method. Samples were tested against the bacterial strains, gram-positive *Staphylococcus aureus* MTCC-7443 and gram-negative *Escherichia coli* MTCC-7410. Incubation at 37 ℃ for bacteria. The inoculum was adjusted to approximately 5 × 105 CFU/ml with sterile saline solution. Samples were dissolved in 20 mg/mL in DMS as a stock solution and loaded in different concentrations of 200 µg to 800 µg for different wells. The Medium Muller Hinton agar for Bacteria and Czapek 's-Dox agar media. The two fungal species, *Aspergillus flavus* MTCC-9606 and *Pichia anomala* MTCC-237, 72 h of incubation at 28℃ for fungi After incubation, the diameter of the inhibition zone (mm) was measured [10].

GC-MS Analysis

gas chromatography-mass spectrometry (GC-MS) to determine its phytochemical constituents. The systematic procedure was carried out through a Shimadzu QP2010S system operating in electrospray ionization (ESI) mode, equipped with an ELITE-5MS capillary column (film thickness: 0.25 µm, length: 30 m, internal diameter: 0.25 mm). The GC oven temperature was initially programmed at 80 °C and ramped to 450 °C at a rate of 20 °C per minute to enable effective separation of analytes. Sample injection was carried out using a 2 mm direct injection technique. Compound identification was performed by correlating relative retention times and mass spectral data with authenticated reference spectra from the National Institute of Standards and Technology (NIST) library. The analytical approach adhered to established methodologies to ensure precise compound characterization, aligning with the protocol [11].

FTIR Analysis

Fourier Transform Infrared Spectroscopy (FTIR) was employed to analyze the bioactive constituents. A spectrophotometer (Waltham, MA, USA), Thermofisher Scientifics USA was utilized for the assessment. The dried powder of biosynthesized nanoparticles was blended with potassium bromide (KBr) to form a compressed pellet. The KBr pellets were evaluated within the spectral range of 400 to 4000 cm¹, enabling the identification of infrared absorption bands. The presence of functional biomolecules in the sample was documented through spectral data analysis within the 400-4000 cm¹ range, facilitating a comprehensive characterization of the nanoparticles' chemical composition [12].

DPPH Assay

The radical-scavenging activities of the samples studied using the stable DPPH radical, D/t concentration (0 to 100 µg/mL) of samples, 2 mL of DPPH (100 µM), methanol 3 mL of methanol, and the mixture was incubated in a dark room at room temperature at 45 min. At the end of the incubation, absorbance was recorded using a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) at 517 nm against the blank (Without sample/Standard). The free radical capacity of the samples is calculated and expressed in IC_{50} Vit-C as a standard [13].

Cytotoxicity

Cytotoxicity Evaluation: The IC_{50} value was evaluated using MTT assay which the cultured cells are about $1\times10^{5,}$ were seeded in a 96-well plate and incubated at 48 h with 37 °C in a 5% CO_2 incubator. After 48 hrs., the mono layer was washed with medium, and 100 µL of different test concentrations of samples were added onto the mono layer, and the cells were further incubated under the same conditions. The cultured medium was removed, 100 µL of the MTT solution was added to each well, and incubated at 37 °C for about 4 hrs. After removal of the supernatant, 100 µL of DMSO was added to each of the wells and incubated for 10 minutes to solubilize the formazan crystals. The optical density was measured at 590 nm. The percentage growth inhibition was calculated, and the results were expressed in terms of IC_{50} values using a dose-response curve [14].

Results and Discussion

Proximate composition

The proximate analysis of *Cynarospermum asperiimum* leaf extract shows that the total protein is 31.34±2.21%, total carbohydrates 12.64±1.06%, total lipids 8.6±0.25%, total ash 17.50±0.59%, moisture content 1.97±0.52%, and nutritional value 210.32 Kcal. Shown in (Table 1). *Astercantha longifolia* leaf extract evaluates the proximate composition of the protein 18.41 0.26%, ash 14.210.38%, and crude fiber 22.770.37%.[15] *Blepharis repens* reported the presence of protein 6.52mg/g, carbohydrates 6.42mg/g, lipids 7.14mg/g, and total phenolics 38.86mg/g [16].

Table 1: Proximate composition of *Cynarospermum asperiimum* leaf extract.

Sl No	Samples	Total protein (%)	Total carbohydrates (%)	Total lipids (%)	Total ash content (%)	Moisture (%)	Nutritive value/100g
01	CALF	31.34±2.21	12.64±1.06	8.6±0.25	17.50±0.59	1.97±0.52	210.32Kcal

Qualitative phytochemical analysis

The qualitative phytochemical analysis of *Cynarospermum asperiimum* leaf extract shows that the presence of phenols, tannins, alkaloids, terpenoids, flavonoids, saponins, glycosides, steroids, proteins, and carbohydrates in methanol, terpenoids, and steroids were absent in distilled water extract, alkaloids, tannins, saponins, and glycosides were absent in chloroform extract, as shown in Table 2. The *Blepharis repens* reported the presence of carbohydrates, tannins, alkaloids, flavonoids, steroids, and tri-terpenoids [17]. *Blepharis maderaspatensis* reported the presence of various phytochemicals such as alkaloids, flavonoids, phenol, steroids, saponin, tannin, terpenoids, glycoside, xanthoprotein, and fixed oil [18]. *Blepharis sindica* reported the presence of carbohydrates, protein, tannin, saponin, glycoside, alkaloid, steroid, and reducing sugar [19].

Table 2: Qualitative phytochemical analysis of *Cynarospermum asperiimum* leaf extract.

Sl No	Phytochemical tests	CALF M	CALF DW	CALF CHL
1	Phenols	+	+	+
2	Alkaloids	+	+	-
3	Flavonoids	+	+	+
4	Tannins	+	+	-
5	Terpenoids	+	-	+
6	Saponins	+	+	-
7	Glycosides	+	+	-
8	Steroids	+	-	+
9	Proteins	+	+	+
10	Carbohydrates	+	+	+

Note: "+" = Present, "-'' = Absent.

Quantitative phytochemical analysis

The quantitative estimation of Polyphenols was in distilled water, methanol, and chloroform. 182.58±4.38, 377.16±10.40, and 244.66±4.38 µg/mg. Flavonoids estimation in distilled water, methanol, and chloroform extract. 124.58±4.79, 356.40±7.16 and 147.03±2.70 µg/mg. Tannins estimation in distilled water, methanol, and chloroform 48.81±3.69, 143.44±4.14, 11.26±2.29 µg/mg. Alkaloids estimation in distilled water, methanol, and chloroform extract. 132.43±2.42, 243.01±4.76 and 71.58±6.29 µg/mg, respectively. (Table 3). The *Blepharis repens* (Vahl) Roth reported the presence of carbohydrates, alkaloids, tannins, and flavonoids were reported [20].

Table 3: Quantitative phytochemical analysis of *Cynarospermum asperiimum* leaf extract.

Sl. No.	Samples	Polyphenols (µg/mg GAE)	Flavonoids (µg/mg QE)	Tannins (µg/mg TAE)	Alkaloids (µg/mg AE)
1	CALF DW	182.58± 4.38	124.58± 4.79	48.81± 3.69	132.43± 2.42
2	CALF M	377.16± 10.40	356.40± 7.16	143.44± 4.14	243.01± 4.76
3	CALF Chl	244.66± 4.38	147.03± 2.70	11.26± 2.29	71.58± 6.29

LF-Leaf, DW-Distilled Water, M-Methanol, Chl-Chloroform.

GC-MS (Gas chromatography-mass spectroscopy)

Chromatogram CALF D:\KUD\Plant Extract\CALF_031_30-12-2024_31.qgd

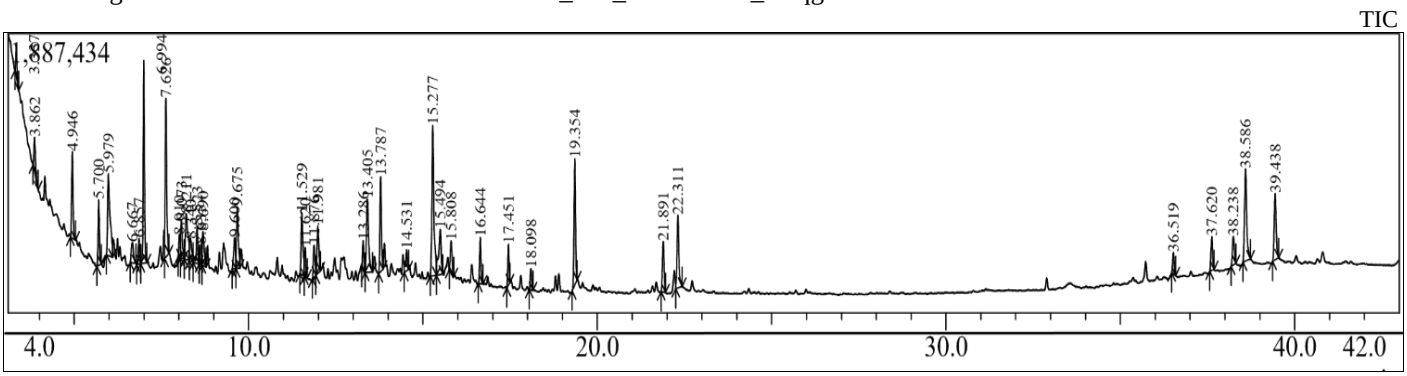

Fig 2: Chromatogram of *Cynarospermum asperiimum* methanol leaf extract.

The GC-MS analysis of methanol extract shows a total of 40 bioactive compounds with different areas of % and Retention times shown in (table 4), (Figure 2 & 3). Furfural RT 3.862, Butyrolactone<gamma->RT 4.946, Furfural <5-methyl->RT 5.700, Phenyl alcohol RT 5.979, Pseudocumene RT 6.667, Pineapple ketone RT 7.626, Maltol RT 8.010, Phenethyl alcohol RT 8.073, Laciniata furanone G RT 8.635, Verdoracine RT 11.981, Hexanoic acid RT 13.405, Tetradecanol <n->RT 14.531, Neophytadiene RT 17.451, Linolenate<methyl->RT 22.311, Larixol RT 38.586, Torulosol RT 39.438, (table 4). The Blepharis maderaspatensis (L) leaf ethanolic shows the presence of total 17 bioactive compounds major compounds are n-Hexadecanoic acid, phytol, tocopherol, squalene, cholestane-3-ol, 3,7,11,15-tetrameth-2-hexadecen-1-ol, 1-Hepatriacotanol, 2-tridecen-1-ol, (E), 3-Hexadecyloxycarbony 1-5-(2-hydroxyethyl)-4- methylimidazolium ion these compounds are antioxidant, anticancer, anti-inflammatory, anti-infertility, antitumor, anti-carcinogenic, chemopreventive, Flavors, fragrance agent, diuretic anti-asthma activity were reported [21]. The *Blepharis repens* leaves and fruit extract revealed the presence of 16 compounds major linolenic acid, n-hexadecanoic acid, vitamin E, squalene, stigmasterol, hexadecanoic acid, etc [22]. *Blepharis boerhavifolia* reported that azulene, 1H-cyclopenta, tetramethylbicyclo, caryophyllene, trimethyl-11-methylene, gamma-neoclovene, beta-humulene, methanoazululene, Tetradecanoic acid, 13-tetradecenal, which show significant anti-inflammatory, anti-oxidant, antibacterial, anti-tumor, anti-epileptic, and local anesthetic activity [23].

Table 4: GC-MS Analysis of Bioactive Compounds from *Cynarospermum asperrimum (Nees) Vollesen*

Peak#	Retention Time	Area	Area%	Similarity	Base m/z	Compound Name
1	3.337	436498	0.85	82	43.00	trans-2,3-Dimethyloxirane
2	3.862	608641	1.18	97	96.00	Furfural
3	4.946	1660262	3.22	96	42.05	Butyrolactone <gamma->
4	5.700	1189428	2.31	98	110.05	Furfural <5-methyl->
5	5.979	2627737	5.10	86	94.05	Phenyl alcohol
6	6.667	628542	1.22	78	105.10	Pseudocumene
7	6.857	514958	1.00	95	79.05	Benzyl alcohol
8	6.994	3709955	7.20	76	128.05	Thiofuran <2-methyl-, 3methyl>
9	7.626	3142166	6.10	85	43.00	Pineapple ketone
10	8.010	436832	0.85	90	126.05	Maltol
11	8.073	813625	1.58	96	91.05	Phenethyl alcohol
12	8.211	1613897	3.13	85	85.00	Valeric anhydride
13	8.340	659203	1.28	75	43.00	Pentanal <2-methyl->
14	8.523	730350	1.42	73	43.00	Acetate <2,4-dimethyl-, ethyl-, 1,3-dioxolane>
15	8.635	484094	0.94	71	111.05	Laciniata furanone G
16	8.690	786851	1.53	73	56.00	Pentalactone <gamma->
17	9.600	744067	1.44	83	120.05	Tolualdehyde <meta->

18	9.675	1459168	2.83	95	97.05	Hydroxy methyl furfural
19	11.529	1535057	2.98	72	74.05	Isopentanoate <methyl->
20	11.620	532270	1.03	83	157.15	Calacorene <alpha->
21	11.876	681177	1.32	81	123.05	Benzene <para-dimethoxy->
22	11.981	1117232	2.17	72	159.15	Verdoracine
23	13.286	488649	0.95	69	125.05	Thiophene <2-formyl-, 5-methyl->
24	13.405	2215973	4.30	85	60.00	Hexanoic acid
25	13.787	1965047	3.81	73	135.05	Salicylaldehyde <4-methyl->
26	14.531	484387	0.94	88	43.05	Tetradecanol <n->
27	15.277	4075569	7.91	70	123.05	Undecene <1-oxaspiro [5.5]-, 4-methyl->
28	15.494	1562271	3.03	66	169.05	Undec-2-en-1-yl acetate
29	15.808	644260	1.25	63	169.05	Cyclopentan-1,2-dione <3,5-dimethyl->
30	16.644	901110	1.75	73	43.00	Vinylcyclohexane <1-acetoxy-, 2-sec-butyl->
31	17.451	688769	1.34	95	68.05	Neophytadiene
32	18.098	409862	0.80	90	82.10	Neophytadiene
33	19.354	2633195	5.11	95	73.05	Hexadecanoic acid <n->
34	21.891	1153016	2.24	92	95.10	Neophytadiene
35	22.311	1818272	3.53	91	79.05	Linolenate <methyl->
36	36.519	452830	0.88	67	147.10	Spathulenol
37	37.620	742410	1.44	44	430.40	Ionone <dimethyl->
38	38.238	742474	1.44	64	145.10	Torulosol
39	38.586	2653694	5.15	66	83.10	Larixol
40	39.438	1804528	3.50	65	145.15	Torulosol
		5154836	100.0			

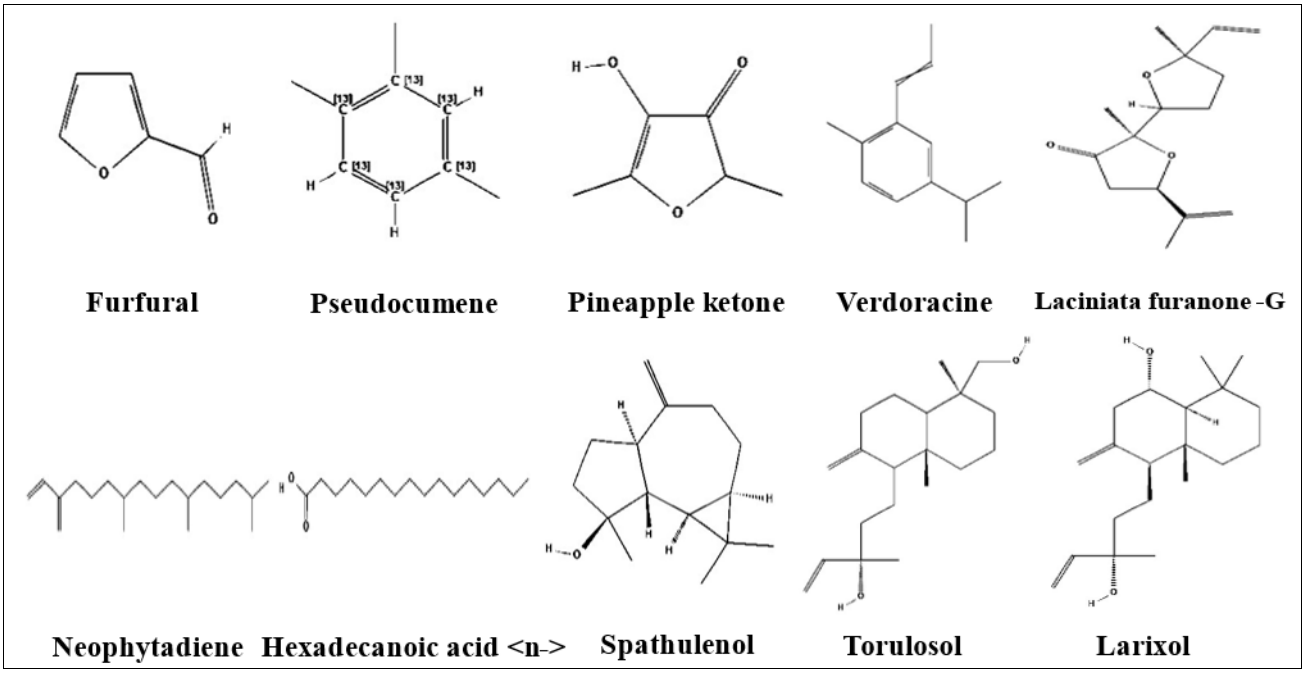

Fig 3: Compound structures of GC-MS analysis of *Cynarospermum asperrimum (Nees) Vollesen* using a methanol solvent.

FTIR Analysis

The FTIR analysis to know the functional group of bioactive components based on peak values to know the compound class was shown in (Graph 1), (Tables 5,6, and 7). The significant peaks are detected from 4000 cm-1 to 600^{-1}. The methanol extract revealed that the C-H stretching, O-H stretching, N-H stretching, C-H stretching, O=C=O stretching, C≡C stretching, C=C stretching, N-O stretching, C-H stretching, O-H bending, O-H bending, C-O stretching, C-O stretching, and S=O stretching. Corresponding to the Aldehyde, alcohol, aliphatic primary amine, alkane, carbon dioxide, alkyne, conjugated alkene, nitro compound, alkane, carboxylic acid, alcohol, aromatic ester, tertiary alcohol, sulfoxide. The distilled water revealed that the C-H stretching, O-H stretching, N-H stretching, C=C stretching, O=C=O stretching, C≡C stretching, C=C stretching, corresponding to the alcohol, alcohol, secondary amine, carbon dioxide, carbodiimide, α, β-unsaturated ketone, alkane, fluoro compound, aromatic ester, and anhydride. The chloroform extract revealed that the O-H stretching, O-H stretching, N-H stretching, O=C=O stretching, N=C=N stretching, C=C stretching, C-H stretching, C-F stretching, C-O stretching, and CO-O-CO stretching corresponding to the alcohol**,** alcohol**,** secondary amine**,** carbon dioxide**,** carbodiimide**,** α, β-unsaturated ketone**,** alkane**,** fluoro compound**,** aromatic ester**,** and anhydride. *Blepharis linariifolia* reported that C-X for organic halogen, C-O for esters, aliphatic NO_2 for nitro compound, C-C for aromatics, N-O for nitro compound, N-H for 1^{o} amine, C=O carbonyl, C-H for organic compound, C-H aldehydes, C-H for alkenes, O-H for phenol, O-H for alcohol, O-H for free hydroxy alcohol, phenol, Si-OH for silicon compound [24].

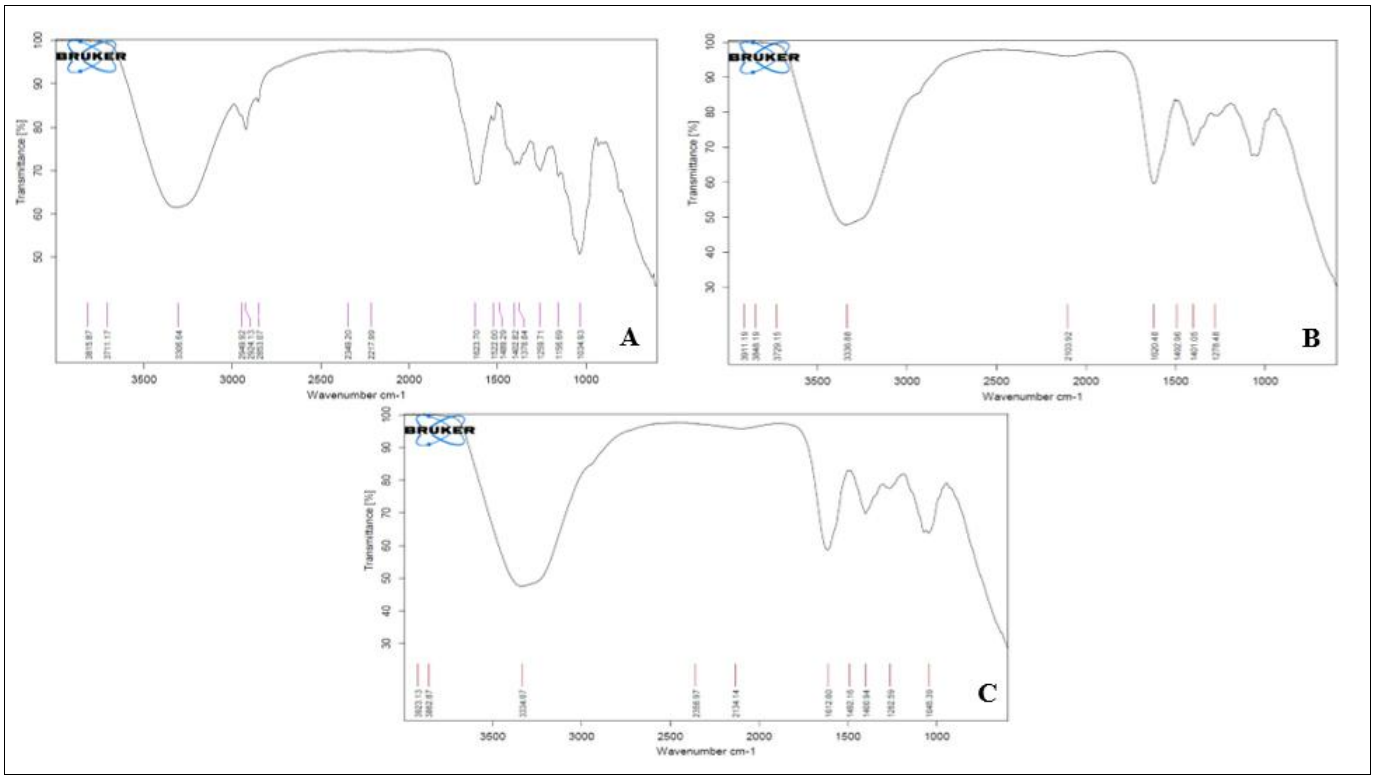

Fig 4: FTIR Chromatogram showing peak values for bioactive functional groups of *Cynarospermum asperrimum (Nees) Vollesen*, A. Methanol, B. Chloroform, C. Distilled water

Table 5: FTIR Peak Values interpretation for bioactive functional groups of *Cynarospermum asperrimum (Nees) Vollesen.* Methanol leaf extract.

Absorption(cm-1)	Intensities of functional group	Functional Group	Compound Class
3815.87	Weak, medium	C-H stretching	Aldehyde
3711.17	Medium sharp	O-H stretching	alcohol
3306.64	medium	N-H stretching	Aliphatic primary amine
2949.92	medium	C-H stretching	alkane
2924.13	medium	C-H stretching	alkane
2853.07	medium	C-H stretching	alkane
2349.20	strong	O=C=O stretching	carbon dioxide
2217.99	weak	C≡C stretching	alkyne
1623.70	medium	C=C stretching	conjugated alkene
1522.00	strong	N-O stretching	nitro compound
1489.29	medium	C-H stretching	alkane
1402.82	medium	O-H bending	carboxylic acid
1376.84	medium	O-H bending	alcohol
1259.71	strong	C-O stretching	aromatic ester
1156.69	strong	C-O stretching	tertiary alcohol
1034.93	strong	S=O stretching	sulfoxide

Table 6: FTIR Peak Values interpretation for bioactive functional groups of *Cynarospermum asperrimum (Nees) Vollesen.* Distilled water leaf extract.

Absorption(cm-1)	Peak details	Functional Group	Compound Class
3923.13	Medium sharp	O-H stretching	alcohol
3862.87	Medium sharp	O-H stretching	alcohol
3334.67	medium	N-H stretching	secondary amine
2356.97	strong	O=C=O stretching	carbon dioxide
2134.14	strong	N=C=N stretching	carbodiimide
1612.60	strong	C=C stretching	α,β-unsaturated ketone
1492.16	medium	C-H stretching	alkane
1400.94	strong	C-F stretching	fluoro compound
1262.59	strong	C-O stretching	aromatic ester
1045.39	strong, broad	CO-O-CO stretching	anhydride

Table 7: FTIR Peak Values interpretation for bioactive functional groups of *Cynarospermum asperrimum (Nees) Vollesen.* Chloroform leaf extract.

Absorption(cm-1)	Peak details	Functional Group	Compound Class
3923.13	Medium sharp	O-H stretching	alcohol
3862.87	Medium sharp	O-H stretching	alcohol
3334.67	medium	N-H stretching	secondary amine
2356.97	strong	O=C=O stretching	carbon dioxide
2134.14	strong	N=C=N stretching	carbodiimide
1612.60	strong	C=C stretching	α,β-unsaturated ketone
1492.16	medium	C-H stretching	alkane
1400.94	strong	C-F stretching	fluoro compound
1262.59	strong	C-O stretching	aromatic ester
1045.39	strong, broad	CO-O-CO stretching	anhydride

Antimicrobial activity

It shows moderate antimicrobial activity exhibiting inhibition zones in the tested concentration in all three extracts. The maximum activity was found in *Staphylococcus aureus* with an inhibitory zone of 15.66±1.15 mm *and Escherichia coli* with an inhibitory zone of 16.66±1.1 mm in methanol. 16.33±0.57 mm and 17.33±0.57 mm in chloroform. 15.33±0.57 mm and 16.33±0.57 mm in distilled water. No antifungal activity was found at the tested concentrations. Kanamycin is used as a Standard. Shown in (Table 8), (Figure 4). The *cynarospermum asperiimum* reveals that the inhibition zones against S. *aureus are* 6.1mm. *E. coli* 5.3 mm. *P. aeruginosa* 5.3mm. *P. vulgaris* 4.9 mm [25]. *Blepharis ciliaris* revealed that the inhibition zone against *S. aureus* was 25.0±2.0 mm, *B. subtilis* was 23.0±2.5 mm, and *E. coli* was 12.0±1.5 mm. *S. marcescens* was 10.00±2.00 mm.[26] *Blepharis persica* reported that zone of inhibition against *Staphylococcus aureus* 9.4±0.5 mm, *Escherichia coli* 8.3±0.5 mmin ethanolic extract, *Staphylococcus aureus* 6.5±0.5 mm, *Escherichia coli* 6.2±0.5 mm in Aqueous extract [27].

Table 8: Antimicrobial Activity of *Cynarospermum asperiimum* leaf extract against gram-positive and gram-negative bacteria and two fungal strains.

Sl.	Sample Name	Conc. (mg)	*S. aureus*	*E. coli*	*A. flavus*	*P. anomala*
1	CALF M	1	ND	13.66±1.15	ND	ND
		2	13.66±1.15	15.66±1.15	ND	ND
		3	15.66±1.15	16.66±1.15	ND	ND
		Std. Kanamycin	20.66±1.15	21.33±1.15	ND	ND
2	CALF Chl	1	ND	ND	ND	ND
		2	13.33±0.57	13.66±1.15	ND	ND
		3	16.33±0.57	17.33±0.57	ND	ND
		Std. Kanamycin	20.66±0.57	21.33±0.57	ND	ND
3	CALF DW	1	ND	ND	ND	ND
		2	12.33±0.57	13.66±1.15	ND	ND
		3	15.33±0.57	16.33±0.57	ND	ND
		Std. Kanamycin	20.33±0.57	21.66±0.57	ND	ND

Note: - 'ND' indicates no activity shown at the tested concentrations

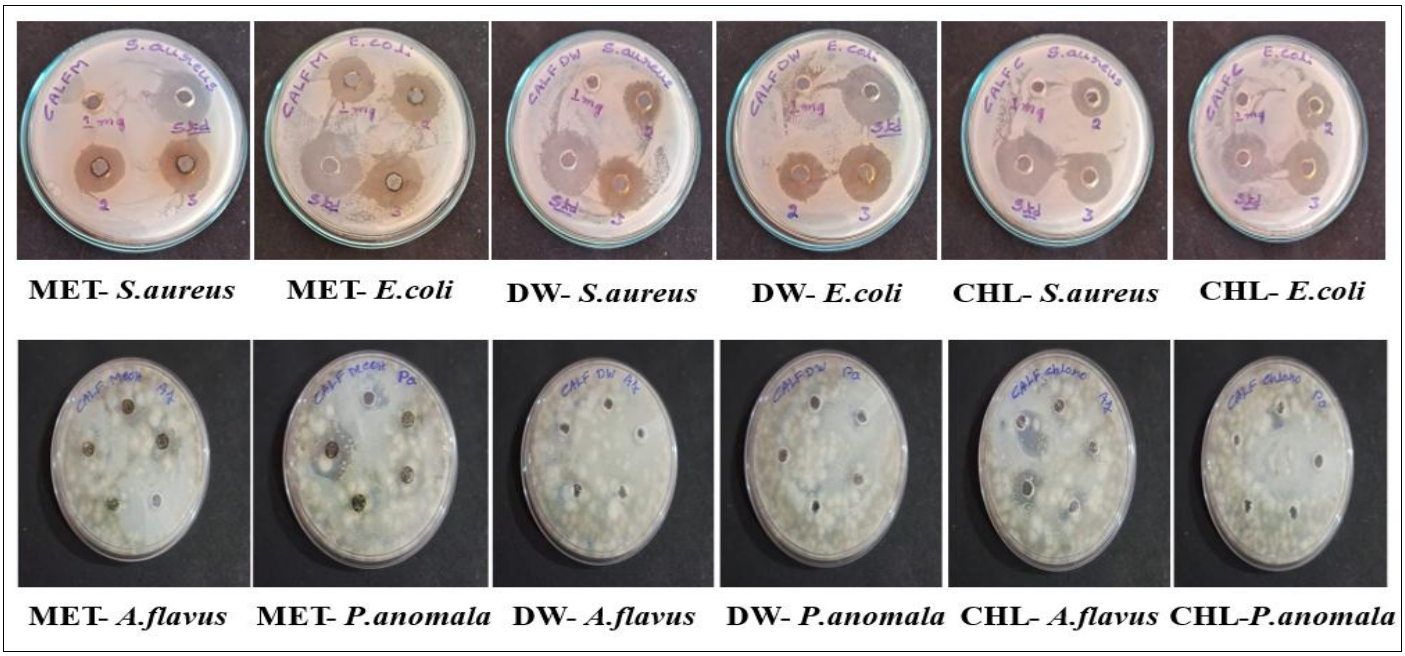

Fig 5: Antimicrobial activity of methanolic leaves extract/ distilled water/ Chloroform of *Cynarospermum asperiimum*, showing zone of inhibition at different concentrations.

Antioxidant activity

The antioxidant activity shows that the IC_{50} value is 57.24±3.83 µg/ml in methanol extract, in distilled water 155.38±17.81 µg/ml, and 164±7.79 µg/ml in Chloroform extract. Vit-C is taken as a standard shown in. (Table 9). (Graph 2). The *Blepharis edulis* revealed that the IC_{50} values were 218.4 µg/ml, 73.7 µg/ml, 71.2 µg/ml, and 81.4 µg/ml in water, 70% MeOH, and MeOH. To conclude, free radicals were detected by the DPPH assay [28]. The Blepharis maderaspatensis revealed that the IC_{50} values were 910 µg/ml, 498 µg/ml, and 210 µg/ml, in pet ether, ethyl acetate, and methanolic extracts [29]. *Blepharis edulis* reported that significant IC_{50} values were 218.4 µg/ml, 73.7 µg/ml, 71.2 µg/ml, and 81.4 µg/ml [30].

Table 9: Antioxidant activity of *Cynarospermum asperiimum* leaf extract.

Sl. No.	Samples	DPPH (IC_{50} µg/ml)
1	CALF M	57.24±3.83
2	CALF DW	155.38±17.81
3	CALF CHL	164.73±7.79
4	Std (Vit-C)	26.21±2.84

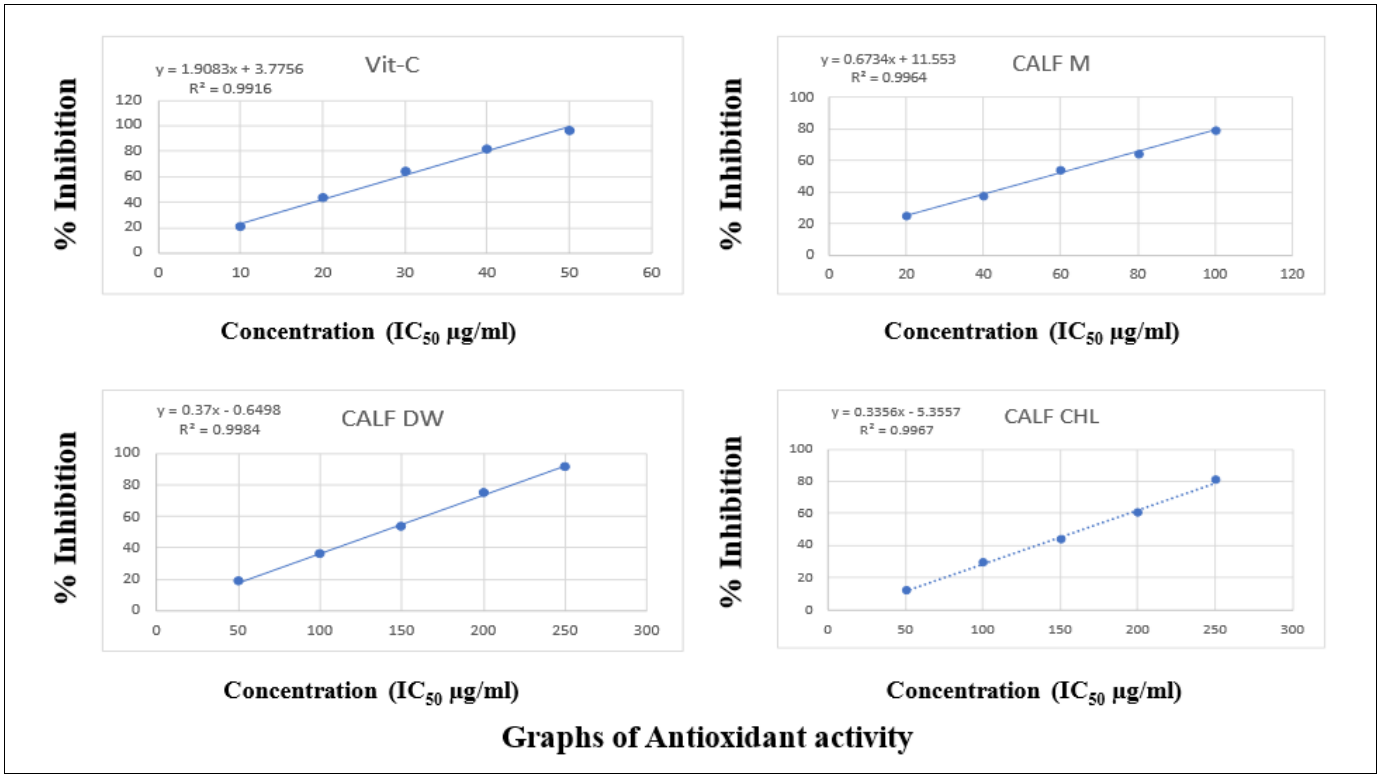

Fig 6: The Graph shows the percentage of inhibition of the free radical scavenging assay

Cytotoxicity by MTT Assay

The percentage of cytotoxicity against MCF-7 (Breast cancer) and HepG2(Liver cancer) here the chloroform showed a good % of cytotoxicity 66.76±3.37 µg/ml in the MCF7 cell line, in other solvents showed a moderate % cytotoxicity in methanol extract shows 58.38±3.13 µg/ml in the MCF7 cell line, 67.57±4.10 µg/ml in the HepG2 cell line, in Distilled water shows moderate cytoxicity against both the cell lines at the tested concentration here the Doxorubicin taken as a standard was shown in (table 10) (figure 3) (graph 3). The *Blepharis maderaspatensis* revealed that the % cytotoxicity with an IC_{50} value of 31.59 µg/ml, against A375 (skin melanoma cell), 55.74 µg/ml against HT29 (colon cancer cell), and HepG2 cell line shows better response at 100 µg/ml against the tested concentration [31]. *Blepharis edulis reported* that promising activity against MCF-7, 25.24± 2.3 µg/ml, HepG2, 18.27±1.9 µg/ml, and HCT-116, 20.35±2.1 µg/ml.[32] The lower IC_{50} values are more potent it has a stronger interaction with cellular targets. The higher IC_{50} values indicate less potency, it has a weaker interaction with cellular targets [33].

Table 10: Anticancer activity of *Cynarospermum asperiimum* leaf extract.

Sl. No.	Samples	MCF7 (IC_{50} µg/ml)	HepG2 (IC_{50} µg/ml)
1	CALF M	58.38±3.13	67.57±4.10
2	CALF DW	233.26±8.90	166.82±81.73
3	CALF CHL	66.76±3.37	119.64±5.05
4	Std (Doxorubicin)	23.51±1.95	15.85±1.43

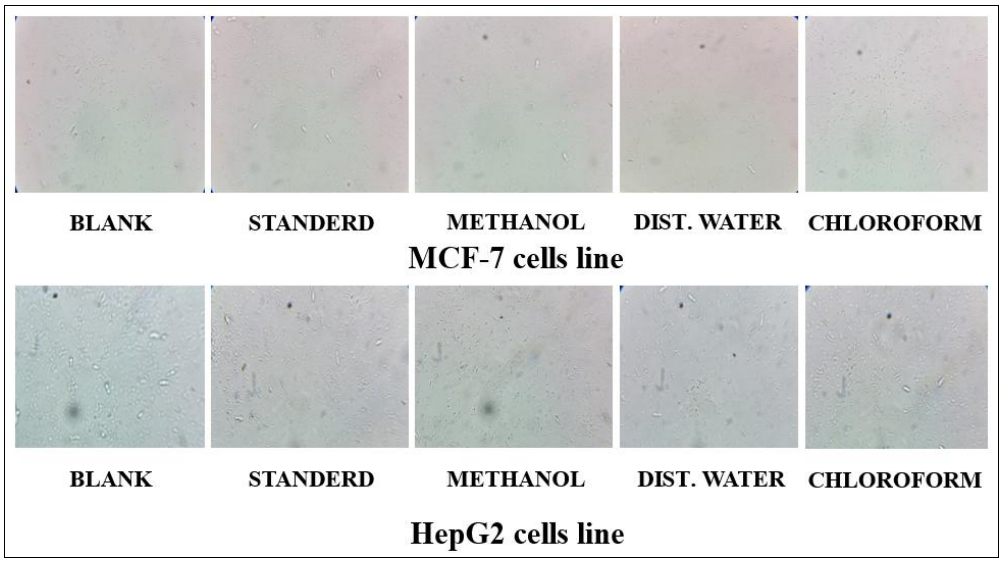

Fig 7: Microscopic images show the percentage of cytotoxicity of *Cynarospermum asperiimum* leaf extract at a higher concentration of 160 µg/ml

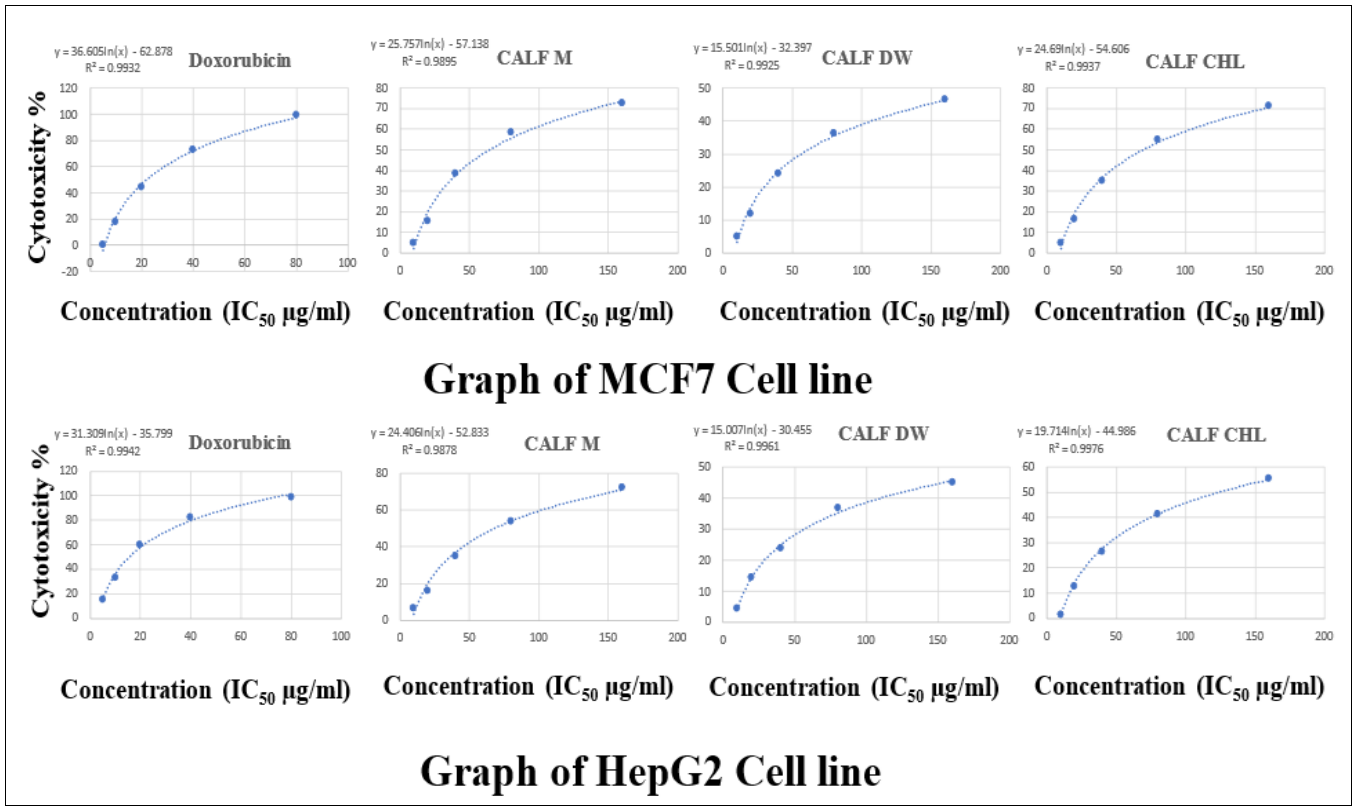

Fig 8: Cytotoxicity graph of Methanol, Distilled water & Chloroform leaf extract at a higher concentration.

Statistical analysis

All data are reported as mean±SD of three replicates, and all the statistical analyses were computed with IBM SPSS Statistics version 23.

Conclusion

The present study reveals that the various phytochemicals are present in *Cynarospermum asperiimum*. These are linked to various pharmacological and biological activities. The various compounds identified might be a good source of novel drugs as the GC-MS analysis shows a good anti-cancer potential from MCF-7 and HepG2, which show good therapeutic values in crude extracts against various ailments. From the present research, the Torulosol bioactive compound shows potent activity. It is concluded that the methanol crude extract shows a promising tool in the pharmaceutical and therapeutic applications. Further study is needed to isolate the Pure compound and determine the pharmacological activities of compounds reported in our study.

Acknowledgment

The Authors gratefully acknowledge the P.G. Department of Studies in Botany, Davangere University, Shivagangotri, Davangere, Karnataka. India. Dr. Sudharshan B L Cheliyan Biotech Solutions, Mysore. And the Sophisticated Analytical Instrumentation Facility (SIAF), Karnatak University, Dharwad, for providing lab facilities to conduct experiments.

Conflict of interest

The authors declare there is no conflict of interest.

Funding statement
Not applicable

Ethics statement
Not applicable

References

1. Rattan R. Bioactive phytochemicals from *Acanthaceae* - Mini review. J Emerg Techn Innov Res. 2023;10(5):580-585.
2. Ranjitha MC, Akarsh S, TR PK, Darshini SM, Vidya P. Antibacterial activity of some plants of Karnataka, India. Journal of Pharmacognosy and Phytochemistry. 2016;5(4):95-99.
3. Sene M, Mandal A, Dey SK. Antimicrobial activity of ethanolic extracts of eight medicinal plants used in traditional medicine in India. 2015.
4. Neelambika HS, Sampath KKK, Vindhya K, Leelavathi S. Preliminary phytochemical investigations of *Blepharis maderaspatensis* (L.) Heyne ex Roth and *Blepharis molluginifolia* Pers. whole plant. 2014.
5. Vijayalakshmi S, Kripa KG. Therapeutic uses of plants of genus *Blepharis*—A systematic review. Int J Pharma Bio Sci. 2016;7:B236-B243.
6. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants using the Folin-Ciocalteu reagent. In: Methods in Enzymology. 1999;299:152-178.
7. Delcour JA, Varebeke DJD. A new colorimetric assay for flavonoids in pilsner beers. Journal of the Institute of Brewing. 1985;91(1):37-40.
8. Makkar HPS. Measurement of total phenolics and tannins using the Folin-Ciocalteu method. In: Quantification of Tannins in Tree and Shrub Foliage. Dordrecht: Springer; 2003. p. 49-52. https://doi.org/10.1007/978-94-017-0273-7_3
9. Pant DR, Pant ND, Yadav UN, Khanal DP. Phytochemical screening and study of antioxidant, antimicrobial, antidiabetic, anti-inflammatory, and analgesic activities of extracts from the stem wood of *Pterocarpus marsupium* Roxburgh. Journal of Intercultural Ethnopharmacology. 2017;6(2):170-178.
10. Ranjitha N, Krishnamurthy G, Manjunatha MN, Naik HB, Pari M, Vasantakumarnaik NK, *et al*. Electrochemical determination of glucose and H_2O_2 using Co(II), Ni(II), Cu(II) complexes of novel 2-(1,3-benzothiazol-2-ylamino)-N-(5-chloro-2-hydroxyphenyl)acetamide: synthesis, structural characterization, antimicrobial, anticancer activity, and docking studies. Journal of Molecular Structure. 2023;1274:134483.
11. Chakraborty B, Raju SK, Abdulrahman IA, Karthikeyan P, Sreenivasa N, Kathirvel B. *Streptomyces* filamentous strain KS17, isolated from microbiologically unexplored marine ecosystems, exhibited a broad spectrum of antimicrobial activity against human pathogens. Process Biochemistry. 2022;117:42-52. https://doi.org/10.1016/j.procbio.2022.03.010
12. R C, Darshan, Siddappa BK. Phytochemical screening, proximate analysis and phyto-synthesis of zinc oxide metallic nanoparticles from the leaf of *Abutilon persicum* (Burm. f.) Merr. and their biological applications. Asian Journal of Applied Chemistry Research. 2025;16(2):173-200. https://doi.org/10.9734/ajacr/2025/v16i2337
13. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181(4617):1199-1200. https://doi.org/10.1038/1811199a0
14. Tolosa L, Donato MT, Gómez-Lechón MJ. General cytotoxicity assessment using the MTT assay. In: Vinken M, Rogiers V, editors. Protocols *in vitro* Hepatocyte Research. Methods in Molecular Biology. Vol. 1250. New York: Humana Press; 2015. p. 333-348. https://doi.org/10.1007/978-1-4939-2074-7_26
15. Dash AK, Dutta GK, Sahoo G, Mishra SK, Sardar KK, Bisoi PC. Phytochemical screening, mineral, and proximate composition of *Asteracantha longifolia* leaf extracts as a quality livestock feed. Journal of Medicinal Plants Research. 2012;6(21):3786-3799.
16. Choudhary D, Prasad R, Meena KL, Jatoliya S. Phytochemical analysis and genetic characterization of genus *Blepharis* in Rajasthan, India. South Asian Journal of Experimental Biology. 2023;13(3):1-8.
17. Rathod SK, Thakare VM, Kanhere HS. Pharmacognostic and phytochemical evaluation of *Blepharis repens*. Journal of Pharmacognosy and Phytochemistry. 2020;9(2):805-809.
18. Mohan VR, Amish AD, Kalidass C, Maruthupandian A. Pharmacognostical and phytochemical investigation of whole plant of *Blepharis maderaspatensis* (L.) Heyne ex Roth. Pharmacognosy Journal. 2010;2(14):1-5.
19. Priyadarshi A, Kumari R, Sharma AK, Jaiswal ML. Preliminary pharmacognostic and phytochemical investigation of *Blepharis sindica* T. Anders seeds. Ancient Science of Life. 2016;36(2):78-83.
20. Deore SL, Bhansali PB, Baviskar BA. Pharmacognostic, phytochemical and *in vitro* biological evaluation of *Blepharis repens* (Vahl) Roth. Pharmacognosy Journal. 2020;12(5):1021-1030.
21. Suriyavathana M, Indupriya S. GC-MS analysis of phytoconstituents and concurrent determination of flavonoids by HPLC in ethanolic leaf extract of *Blepharis maderaspatensis* (L.) B. Heyne ex Roth. World Journal of Pharmaceutical Research. 2014;3(9):405-414.
22. Dhawale PG, Ghyare BP. Determination of phytoconstituents of *Blepharis repens* (Vahl.) Roth leaves and fruit through GC-MS. World Journal of Pharmaceutical Research. 2016;3:883-889.
23. Sulieman AME, Idriss H, Alshammari M, Almuzaini NA, Ibrahim NA, Dahab M, *et al*. Comprehensive *in vitro* evaluation of antibacterial, antioxidant, and computational insights into *Blepharis ciliaris* (L.) B.L. Burtt from Hail Mountains, Saudi Arabia. Plants. 2024;13(24):3491-3505.
24. Na S. Phytochemical composition and evaluation of antimicrobial activity of *Blepharis linariifolia* (Pers.) seeds. Bioscience Journal. 2017;32(2):101-108.
25. Sene M, Mandal A, Dey SK. Antimicrobial activity of ethanolic extracts of eight medicinal plants used in traditional medicine in India. 2015.
26. Sulieman AME, Idriss H, Alshammari M, Almuzaini NA, Ibrahim NA, Dahab M, *et al*. Comprehensive *in vitro* evaluation of antibacterial, antioxidant, and computational insights into *Blepharis ciliaris* (L.) B.L. Burtt from Hail Mountains, Saudi Arabia. Plants. 2024;13(24):3491-3505.
27. Kaushik V, Chaudhary G, Ahmad S, Saini V. Antimicrobial potential of *Blepharis persica* (Burm. f.) O. Kuntze (seeds). International Journal of Phytopharmacology. 2016;7(3):92-95.
28. Mahboubi M, Haghi G, Kazempour N, Hatemi AR. Total

phenolic content, antioxidant, and antimicrobial activities of *Blepharis edulis* extracts. Songklanakarin Journal of Science and Technology. 2013;35(1):23-30.
29. Mythri M, Dev KS, Muthu AK. Estimation of flavonoids and screening of *in vitro* antioxidant activities of various extracts of aerial parts of *Cassia absus* (Linn.). Journal of Evolution of Medical and Dental Sciences. 2020;9(32):2266-2270.
30. Mahboubi M, Haghi G, Kazempour N, Hatemi AR. Total phenolic content, antioxidant, and antimicrobial activities of *Blepharis edulis* extracts. Songklanakarin Journal of Science and Technology. 2013;35(1):23-30.
31. Milind BP, Suganthi V, Vishnu BM. Dose and time-dependent cytotoxicity of *Blepharis maderaspatensis* (L.) against various human cancer cell lines. 2025.
32. Abdel Maboud T. Cytotoxic potentials and phytoconstituent profiling of *Blepharis edulis* (Forssk.) Pers. using UHPLC/Q-TOF-MS-MS. Al-Azhar Journal of Pharmaceutical Sciences. 2021;63(1):37-56.
33. Gordani N, Cheong BE, Teoh PL. Antiproliferative effect of *Strobilanthes crispus* on MCF-7 cell line. Transactions on Science and Technology. 2017;4(3-3):414-419.