Preliminary Phytochemical Screening, Pharmacognostical Analysis, and Identification of Bio-Active Compounds of Commelina Benghalensis Using Gc-Ms Technique

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ABSTRACT- ***OBJECTIVE****: The study deals with the preliminary phytochemical screening, pharmacological evaluation, and identification of bioactive compounds present in Commelina benghalensis using the GC-MS technique. The main objective of the work was to analyze the phytochemical constituent, conduct pharmacognostical studies, and identify biologically active compounds from the selected plant.* ***METHOD****: Fresh leaves of Commelina benghalensis were collected, shade-dried, and powdered into a coarse form. The powdered plant material was subjected to solvent extraction using ethanol and ethyl acetate. The obtained extract was then used for preliminary phytochemical screening to detect various secondary metabolites. Further analysis was carried out using GC-MS to identify the active phytochemical constituent present in the plant.* ***RESULT: The*** *results of the study emphasize the phytochemical and pharmacognostical importance of Commelina benghalensis and confirm the presence of several bioactive compounds identified through GC–MS analysis.*

INTRODUCTION:

Traditional medicine plays a vital role in healthcare systems worldwide. Human survival would be challenging without the widespread use of plants and plant-derived products. Since ancient times, people from diverse civilizations have relied on plants for the treatment of numerous ailments. The World Health Organization (WHO) defines a medicinal plant as a herbaceous species that possesses phytochemical compounds responsible for therapeutic activities. In recent years, there has been growing interest among scientists and healthcare professionals in medicinal plants following the validation of their therapeutic benefits.

Comedian beng halensis is a medicinal herb that belongs to the family Commelinaceae. It is a perennial species indigenous to the tropical regions of Asia and Africa and is commonly referred to as Bengal dayflower or dew flower. The plant is a robust, spreading herb that grows annually and reaches a height of approximately 40 cm. Its roots emerge from the basal nodes. The species is readily recognized by its small, attractive bluish-violet flowers. The leaves are ovate to elliptical in shape, occasionally appearing slightly triangular, and are deep bright green in colour, measuring about 4–7 cm in length. The fruit capsules are broadly ovoid to oblong, approximately 4–5 mm long, while the seeds are ovoid in form.

Traditional uses: In India, *Commelina benghalensis* is traditionally used to treat ailments such as headache, constipation, leprosy, fever, snakebite, jaundice, mouth thrush, insanity, epilepsy, and psychosis. It is also known for its anti-inflammatory, demulcent, emollient, and depressant properties. In Lesotho, the plant is used to manage infertility in women. In China, it is used as a diuretic. In Pakistan, it is employed in the treatment of leprosy and is also used as a laxative and anti-inflammatory agent. In Bangladesh, the plant is used to treat insomnia, cataracts, night blindness, and other eye disorders, as well as suppurative sores, snakebites, inflammation, burns, conjunctivitis, headaches, toothaches, and eczema.

Fig. 1: Commelina benghalensis

Taxonomical classification

Kingdom: Plantae
Subkingdom: Tracheobionta (Vascular plants)
Superdivision: Spermatophyta (Seed plants)
Division: Magnoliophyta (Angiosperms)
Class: Liliopsida (Monocotyledons)
Subclass: Commelinidae Order: Commelinales
Family: Commelinaceae
Genus: *Commelina*
Species: Commelina *benghalensis* L.

Botanical Name: Commelina benghalensis Linn.

Common Name: Bengal Dayflower

Vernacular Name:

Tamil: Kana *Vazhai*, Aduthinna *Palai*

Telugu: Konda *Gogu*, *Bodda Gaddi*

Malayalam: Kakkapoo, *Vazha Poo*

Kannada: Kadu *Kanagale*, *Bodda Soppu*

Hindi: Kankaua, *Benghal Dayflower*

Bengali: Kanshira, *Kana Shak*

Marathi: Kena

English: Bengal *Dayflower*, *Tropical Spiderwort*

QUALITATIVE PHYTOCHEMICAL ANALYSIS

Phytochemicals are natural chemical substances found in plants that are beneficial. Chloroform solvent extract obtained from the Synedrella nodiflora was subjected to various qualitative tests for the identification of various plant constituents present in the species.

1. Test for Alkaloids Dragen dorff's test

To 1 ml of extract, add 1 ml of Dragendorff's reagent (potassium bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

Mayer's test

To 1 ml of extract, add 1 ml of Mayer's reagent (potassium mercuric iodide solution). A whitish yellow or cream-colored precipitate indicates the presence of alkaloids.

2. Test for Glycosides

Test solution: It was prepared by dissolving the sample in alcohol.

Killer Killani Test

To test the solution, a few drops of ferric chloride solution and concentrated sulphuric acid were added; the formation of two layers occurred, a lower layer of reddish-brown color and an upper layer of bluish-green color simultaneously.

3. Test for Triterpenoids

Test solution: It was prepared by dissolving the extract in chloroform.

Salkowski test

A few drops of conc. sulphuric acid were added to the test solution and allowed to stand for some time. The lack of formation of yellow color in the lower color indicates the absence of triterpenoids.

4. Test for carbohydrate Molisch test

To 2-3 ml of the test solution, add a few drops of Molisch reagent solution and shake. Concentrated sulfuric acid was added from the side of the test tube. A violet ring was formed at the junction of two lipids.

5. Test for Proteins

Test solution: It was prepared by dissolving extract in water and making aqueous extract.

Biuret test

To about 3 ml of the extract, 40% sodium hydroxide solution and a few drops of 1% copper sulfate solution were added. White produces blue color.

6. Test for Anthraquinone

Borntrager's Test

Add 10 ml of 10% ammonia solution with few ml of Aq., shake with ml of benzene and filter (shake vigorously for 30 mins) appears as a pink, violet, or red color solution, it shows the presence of anthraquinones.

7. Test For Flavonoids

Test solution:

To a small amount of extract, an equal amount of 2M HCl was added and heated for about 30-40 mins. The extract was cooled down and again extracted with ethyl acetate, which was further concentrated to dryness and ready to be used as the test sample.

Lead acetate test

To a small quantity of extract, lead acetate was added. Formation of yellow-colored precipitate shows the presence of flavonoids.

8. Test for Quinone

Conc. HCL test

Plant extract with conc. HCl appears a green color; it shows the presence of quinones.

8. Test for Tannins

Test solution: The test solution is prepared by dissolving the extract in water and alcohol.

Ferric chloride solution test

To 1 ml of the extract, ferric chloride was added. Formation of a dark blue or greenish-black color shown in the presence indicates the tannins.

Lead acetate test:

A few ml of 10% lead acetate was added to the test solution. The presence of the white precipitate indicates the tannins.

9. Test for Cholesterol:

Add 2 ml of chloroform with 10 drops of acetic anhydride and add 2-3 drops of conc. H_2SO_4. . Appears a red-rose color shows the absence of cholesterol.

10. Test for Diterpenes

Plant extract is dissolved in distilled water, and adding 3-4 drops of copper acetate solution appears emerald green, which shows the presence of diterpenes.

Test for Terpenoids : Add 2 ml of chloroform with 5 ml of plant extract and add 3 ml of conc. H_2SO_4 (boiled in a water bath). Do not appear in a grey-colored solution in the absence of terpenoids.

11. Test for Saponins

Test solutions: It was prepared by dissolving extract in water and making an aqueous solution extract.

Foam Test

The drug extract was vigorously shaken with water. Persistent foam formation indicates the absence of saponins.

12. Test for Fat Solubility test

Fats are insoluble in water and ethanol. But soluble in chloroform, benzene, and ether. Presence of Fat.

13. Test for Sterols Libermann-Burchard Test

1 gm of the test substance was dissolved in a few drops of chloroform, 3 ml of acetic anhydride and 3 ml of glacial acetic acid were added, it was warmed and cooled under the tap, and drops of conc. H_2SO_4 were added along the side of the test tube. The appearance of a bluish-green color shows the absence of sterols.

QUALITATIVE PHYTOCHEMICAL ANALYSIS

S.NO	PHYTOCHEMICAL	ETHYL ACETATE	ETHANOL	WATER
1.	Alkaloid	+	-	+
2.	Glycosides	+	+	+
3.	Triterpenoids	+	-	-
4.	Carbohydrates	-	-	-
5.	Proteins	+	+	+
6.	Anthraquinones	-	-	-
7.	Flavanoids	-	-	-
8.	Quinone	+	+	-
9.	Tannins	+	-	+
10.	Cholesterol	+	+	+
11.	Diterpenes	+	+	+
12.	Terpenoids	-	-	+
13.	Saponins	+	-	-
14.	Fat	+	-	-
15.	Sterols	+	+	+

PHARMACOGNOSTICAL STUDIES

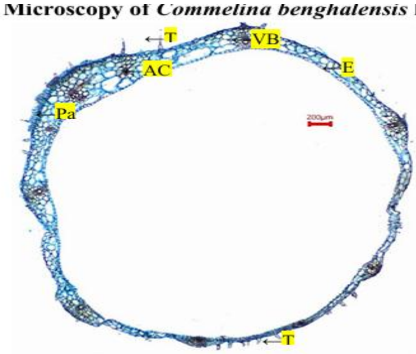
METHODOLOGY

1. **Microscopy (SOP No. PCOG-005):** The sample was preserved in fixative FAA for more than 48 h. The preserved specimens were cut into thin transverse sections using a sharp blade, and the sections were stained with 0.8% safranin and 0.5% Astra blue. Transverse sections were photographed using an Axiolab 5 trinocular microscope attached to a Zeiss Axiocam 208 color digital camera under bright field light. Magnifications were indicated by a scale bar.

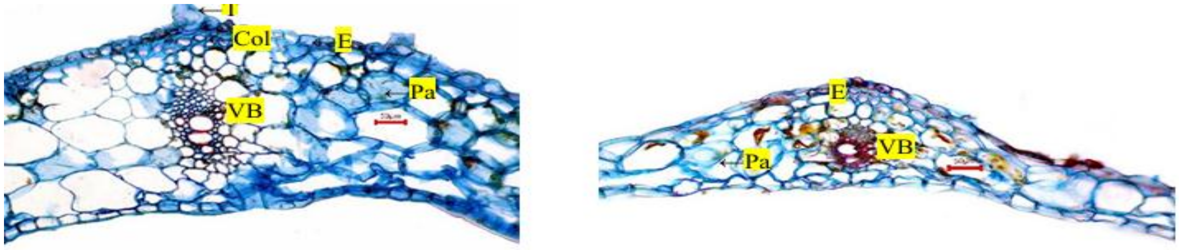
   RESULT Microscopy Leaf sheath

   The TS of the leaf sheath is nearly circular-shaped in outline with an outer single-layered epidermis covered by cuticle and bears simple covering trichomes; a hypodermal layer made up of aerenchyma cells can be seen beneath the epidermis, followed by parenchymatous ground tissue; vascular bundles are arranged in a ring from one end to the other; bundles are collateral and covered by parenchymatous bundles; xylem and phloem are formed of normal vascular elements; some of the parenchyma cells contain micro sphenoidal, acicular, rod-shaped, and prismatic crystals; and few cells contain any cell contents (Fig. 1).

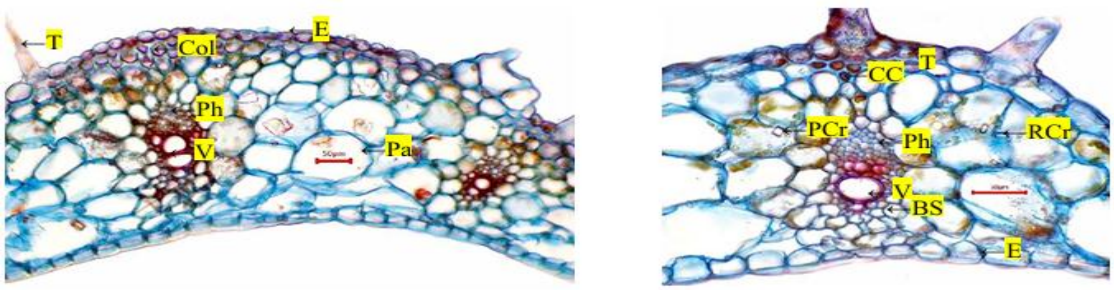
Figure 1. Microscopy of *Commelina benghalensis* leaf sheath



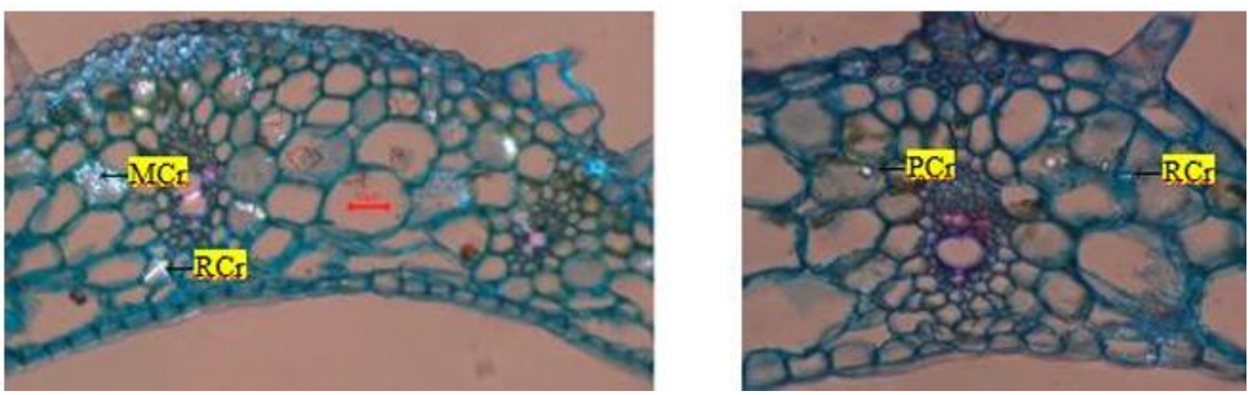
TS of leaf sheath



Enlarged view of leaf sheath



Enlarged view



TS of leaf shealth under polarized field

AC-aerenchyma**; BS-**bundle sheath**; E-epidermis; Mcr-**micro sphenoidal crystals**; pa-**parenchyma; **pcr-**prismatic crystals; **Ph-**phloem; **Rcr-**rod-shaped crystals; **T-**trichome**; V-**vessel; **VB-**vascular bundle

LEAF

TS of leaf shows lower convex and upper depressed midrib surface with lateral laminar extensions. 9fig:2)

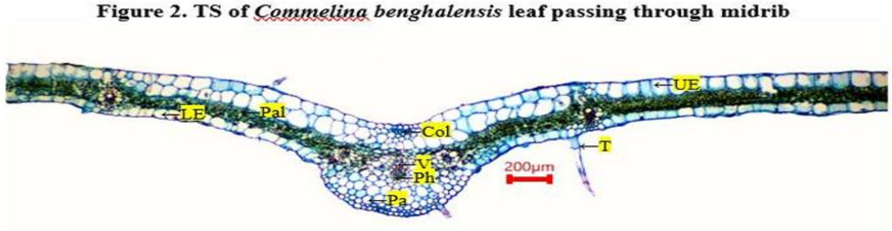
MIDRIB

TS of the midrib shows upper and lower epidermis formed of oval-shaped cells by cuticle and bears simple covering trichomes; a patch of collenchymatous hypodermis is followed by a few layers of parenchyma cells; ground tissue is aeren chymatous, embedded with 3 vascular bundles arranged in a half ring in which the center bundle is comparatively larger than the lateral bundle; the vascular bundle is collateral and covered by parenchymatous bundle sheath cells; xylem is arranged towards the upper side and phloem towards the lower; xylem and phloem are formed of normal vascular elements. (fig. 2)

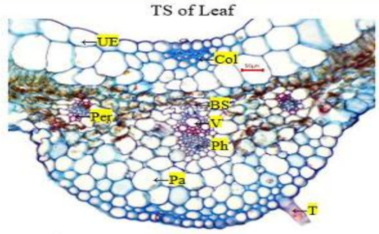
LAMINA

TS of lamina shows the upper and lower epidermis consists of oval to barrel-shaped cells covered by cuticle and bears simple covering trichomes; mesophyll tissue is differentiated into upper single-layered long, columnar palisade cells followed by 3 to 4 layers of loosely arranged spongy parenchyma cells with intercellular spaces; vascular bundles are collateral and covered by parenchymatous bundle sheath and some patches of pericyclic fibers at the lower side; xylem and phloem are formed of normal vascular elements; rod-shaped crystals are found distributed in some of the epidermal cells and mesophyll tissues (Fig. 2).

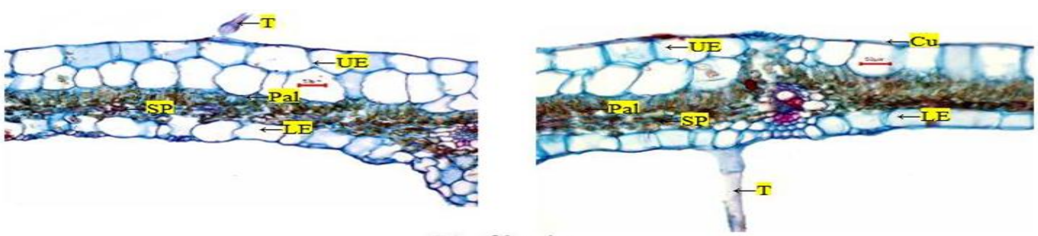
Figure 2. TS of *Commelina benghalensis* leaf passing through midrib



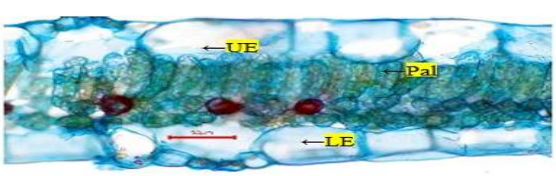
TS of Leaf



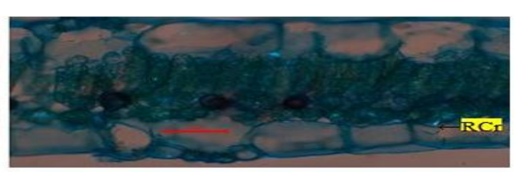
TS of Midrib



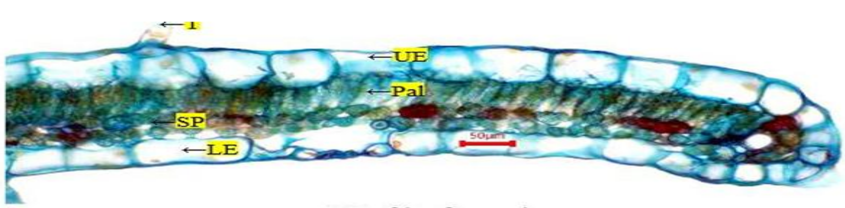
TS of lamina



Enlarged lamina portion



Lamina under polarizer field



TS of leaf margin

GAS CHROMATOGRAPHY-MASS SPECTROMETRY

The present study focuses on the analysis of phytochemical constituents performed using gas chromatography-mass spectrometry. In the ethyl acetate and ethanolic extract of *Commelina benghalensis,* bioactive phytochemicals were detected by the screening method of GC-MS. These extracts show relative concentrations of the bioactive phytoconstituents at different peak heights. The list of phytoconstituents with their percentage of peak area and retention time.

PROCEDURE

Sample preparation:

Weigh 1 g of homogenized sample (chloroform and ethanol) into a 15 ml plastic tube. Add 1 ml of ethyl acetate and vortex for 5 minutes.

Then transfer to a 1 ml vial for injection of µl of the sample into GC-MS

INSTRUMENT CONDITIONS:

GC–MS analysis was conducted using a Shimadzu QP2020 gas chromatograph–mass spectrometer fitted with a split/splitless injector and an electron impact (EI^+) ionization source. Chromatographic separation was performed on an HP-5MS capillary column (30 m × 0.32 mm internal diameter, 0.25 µm film thickness). A sample volume of 1.0 µL was injected, and the injector temperature was maintained at 285°C.

High-purity helium (99.999%) served as the carrier gas at a constant flow rate of 1.0 mL/min. The oven temperature was initially set at 50°C and programmed to increase gradually to 290°C, where it was held for 10 minutes to ensure complete elution of analytes. The split valve was opened 3 minutes after injection to purge the injector. All injections were performed using a 10 µL microsyringe.

For sample preparation, 1 g of the homogenized plant material was weighed into a 15 mL plastic centrifuge tube, followed by the addition of 5 mL of ethyl acetate. The mixture was vortexed for 5 minutes, and the resulting extract was transferred into a 1 mL vial. An aliquot of 1 µL of the extract was then injected into the GC–MS system for analysis.

TABLE 1: Bioactive phytoconstituents of ethanolic extract of *Commelina benghalensis*

PEAK	COMPOUND NAME	R.TIME	AREA	AREA%
1.	Acrylic acid	11.116	39903983	24.55
2.	Pyrrolidinone	12.310	21989425	13.53
3.	Dimethyl phthalate	13.29	21596909	13.29
4.	2-Pyrrolidinone	10.317	8186691	5.04
5.	Pyridine	2.881	7383074	4.54
6.	N,N-Dimethylaminoethanol	2.695	5668502	3.49
7.	Dianhydromannitol	12.732	4876211	3.00
8.	Cyclohexanone	14.658	5698287	3.51
9.	Acetaldoxime	3.266	4557534	2.80
10	Mequinol	10.651	3245318	2.00
11.	p-Toluidine	12.890	3631492	2.23
12.	Indole	14.039	3573328	2.20
13.	2-Cyclopenten-1-one	9.437	2309840	1.42
14.	Cyclohexane	10.780	2039156	1.25
15.	1H-Pyrrole-2,5-dione	13.080	2396679	1.47
16.	2-Methoxy-4-vinylphenol	14.299	2413486	1.48
17.	N-succinimide	15.157	2221349	1.37
18.	Dimethyl phthalate	16.211	2646745	1.63
19.	1,2-Benzenedicarboxylic acid	17.304	2143545	1.32
20.	Cytidine	16.792	2109429	1.30
21.	Lidocaine	26.728	1457042	0.90
22.	Niacinamide	15.472	1732390	1.07
23.	2,4[1H,3H]-Pyridinamide	15.805	1559498	0.96
24.	1,2-Cyclopentanedione	7.052	1491434	0.92

TABLE 2: Bioactive phytoconstituents of Ethyl acetate extract of *Commelina benghalensis*

PEAK	COMPOUND NAME	R.TIME	AREA	AREA%
1.	Phytol	35.993	465342474	13.58
2.	Stigmasterol	46.604	419377320	12.24
3.	Gamma-Sitosterol	47.441	253985663	7.41
4.	1-Hexacosanol	44.321	183697534	5.36
5.	Phytyl sterate	51.763	17147567	5.00
6.	Campesterol	46.134	157471046	4.59
7.	3,7,11,15-Tetramethylhexadec-2-en-1-yl-acetate	37.919	123187817	3.59
8.	Bicyclo [2.2.1]heptan-2-ol	16.694	106944225	3.12
9.	Hydrocinnamic acid	14.759	95413664	2.78
10.	Caffeine	25.167	80034095	2.34
11.	Phytyl	41.712	72341914	2.11
12.	Delta-Tocopherol	43.051	93725993	2.73
13.	Dotriacontanol	41.263	54901311	1.60
14.	Pentatriaontane	42.544	62556343	1.83
15.	1-Hexacosanol	45.546	45811047	1.34
16.	2-Hexacosanol	46.836	46896990	1.37
17.	Cis-1-chloro-9-octadecene	48.623	65101542	1.90
18.	Beta-Amyrin	48.810	14054876	0.41
19.	Alpha-Amyrin	48.809	51406793	1.50
20.	Phytyl stearate	47.670	47802905	1.39

Conclusion:

Commelina benghalensis was extracted using different solvents, and the percentage yield obtained from each solvent was determined. Phytochemical screening was carried out to identify the presence of bioactive constituents in the selected extracts. The detection of these phytochemicals through preliminary analysis indicates the potential medicinal value of the plant.

The plant extract was further analyzed using gas chromatography–mass spectrometry (GC–MS) under standard operating conditions. Compounds were separated based on their retention times and detected using a flame ionization detector (FID). The resulting chromatogram displayed multiple peaks, each representing distinct bioactive compounds. Gas chromatography confirmed the chemical diversity of the extract by effectively separating volatile constituents. The identified compounds are likely contributors to the therapeutic properties attributed to *Commelina benghalensis*.

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