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GC-MS profiling of bioactive components in the ethanolic extract of *Ophiorrhiza mungos Linn*

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Abstract
The study aimed to separate, isolate, and identify various phytochemical constituents present in the ethanolic extract of *Ophiorrhiza mungos Linn* leaves collected from the botanical garden near Himayatsagar Road, Moinabad, Hyderabad. Initial phytochemical screening was carried out to determine the nature of the compounds, followed by separation of individual components using HPTLC. Functional groups were identified through FTIR analysis, and final characterization of the constituents was performed using GC–MS. The screening revealed the presence of alkaloids, carbohydrates, volatile oils, flavonoids, glycosides, tannins, steroids, and triterpenoids. FTIR spectra indicated functional groups corresponding to alkane (C–H), aromatic compounds, sulfoxides, phosphor amides, esters, and disulfides, while GC–MS analysis identified compounds such as benzaldehyde, glycerine, n-hexadecanoic acid, 1,5,6,7-tetrahydro-4-indolone, and 9,12-octadecadienoic acid. These findings highlight the phytochemical potential of *Ophiorrhiza mungos Linn* leaves and provide a foundation for future research to explore their medicinal applications and therapeutic benefits.

Keywords: Phytochemical screening, FTIR, HPTLC, GCMS

Introduction

In the field of pharmacy, a drug is defined as any substance characterized by nutritive, curative, or preventive properties. When such properties are identified within botanical or plant-based preparations, the substance is technically classified as an herbal drug. This classification serves as the foundation for herbal medicine, a field that has evolved into a vital interdisciplinary nexus between traditional healing systems, such as Ayurveda and Unani medicine, and modern empirical science. The complexity of herbal medicine necessitates a multidimensional approach, as it integrates a vast array of scientific disciplines to validate the safety and efficacy of plant-derived therapeutics.

Ophiorrhiza mungos Linn., widely known as the Indian Snake Root or Mongoose Plant, is a perennial evergreen herb belonging to the Rubiaceae family. Geographically distributed across the Western Ghats of India, particularly in Kerala as well as the Himalayas, Southeast Asia, and China. This plant holds a significant position in both ethnomedicine and modern pharmacology. Morphologically, it is characterized by its erect, herbaceous structure and is recognized in various vernaculars, such as Avlipori (Malayalam) and Sarpakshi chettu (Telugu).

The pharmacological profile of *Ophiorrhiza mungos Linn.,* is primarily defined by its potent anti-cancer properties, largely attributed to the presence of the alkaloid Camptothecin. Research indicates high efficacy in treating diverse malignancies, including colon, lung, ovarian, and cervical cancers. Beyond oncology, a decoction of its leaves serves as a bitter tonic and stomachic to aid digestion. Traditionally, it is also utilized as a sedative, a laxative, and a critical remedy for snake bites. This combination of traditional use and modern cytotoxic potential makes it a vital subject for phytochemical investigation. Despite the potential of *Ophiorrhiza mungos Linn.,* need for the systematic isolation, characterization, and quantification of its bioactive constituents to facilitate pharmaceutical application.

Fig 1: Leaves of *Ophiorrhiza mungos linn*

Current literature lacks comprehensive data regarding these specific isolation and characterization, leaving a significant gap in research. To address these limitations and enhance analytical precision, advanced techniques such as High-Performance Thin-Layer Chromatography (HPTLC), Fourier Transform Infrared Spectroscopy (FTIR), and Gas Chromatography-Mass Spectrometry (GC-MS) are utilized. The findings from this study are expected to establish a framework for future pharmacological investigations and substantiate the therapeutic utility of *Ophiorrhiza mungos Linn.,* as a viable herbal alternative in modern medicine.

Materials and Methods

Sample Collection: Plant materials were collected from a herbal garden near Moinabad, Hyderabad, and authenticated by the Botanical Survey of India (Deccan Regional Centre). Prior to beginning the research, the sample were dried under shade for a duration of 15 to 20 days.

Preparation of extract with ethanol: The leaves of *Ophiorrhiza mungos linn* were collected. The collected leaves were cut into small pieces. Dried and pulverized into

coarse material. The coarse plant material of 200gm *Ophiorrhiza mungos linn* was taken. And the extraction process was done by using soxhlet apparatus with sufficient quantity of ethanol 99.99% for 3-4 hours at 71-75°C. The Ethanolic extract was collected and carried out for analysis after the completion of extraction.

Qualitative Analysis

Phytochemical Screening

It refers to the process of identifying and detecting the presence of various phytocomponents in a plant extract or sample.

Fourier Transform Infrared Radiation (FTIR)

Fourier Transform Infrared (FTIR) spectroscopy was performed to identify the functional groups present in the samples using an Alpha-Bruker spectrometer operated via Opus 7.5 software. The measurements were conducted in Attenuated Total Reflectance (ATR) mode employing Zinc-Selenide (ZnSe) plates as the window material. Infrared radiation was generated by a silicon carbide (SiC) glower and modulated by an interferometer before interaction with the sample. The resulting signal was captured by a 1×1mm detector operating at an RMS voltage of 1.8×10^{-2} V. Data acquisition was optimized using a 16K Fourier transform size and a 5 kHz amplifier bandwidth. Spectra were recorded over a range of 4000–500 cm^{-1} by averaging 16 scans at a defined spectral resolution to ensure a high signal-to-noise ratio. The captured interferograms were subsequently processed to facilitate the qualitative analysis of the sample's chemical composition.

High-Performance Thin Layer Chromatography (HPTLC)

HPTLC was conducted using a Desaga Sarstedt system (Germany). Analytical chromatography was performed on pre-coated silica gel 60 F_{254} Aluminum-backed plates (200×100 mm, 0.2 mm thickness). Sample extracts (5µL) were applied as spots onto the stationary phase at an origin line 10 mm from the plate base using a precision micropipette. Development was carried out in a 20×10 cm, twin-trough chamber using a mobile phase of toluene: ethyl acetate: methanol in a 7:2:1 (v/v/v) ratio. Following development, the solvent front was marked and spots were visualized under UV radiation at 366 nm. Quantitative analysis was performed using Proquant software (version 1.6), and the retention factor (Rf) was determined by the ratio of the distance travelled by the solute to the distance travelled by the solvent front.

Gas Chromatography-Mass Spectrometry (GC-MS)

The chemical constituents of the sample were characterized using an Agilent 6890 Gas Chromatograph integrated with a 5973N Mass Selective Detector (MSD). Chromatographic separation was performed on an HP-5MS capillary (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness). Helium was utilized as the carrier gas at a constant flow rate of 1.0 mL/min, employing a 10:1 split ratio. The injector temperature followed a thermal gradient, initiated at 40 °C and increasing to a final temperature of 280 °C. For the oven program, the temperature was ramped from 150 °C to 300 °C at a linear rate of 10 °C/min, resulting in a total chromatographic runtime of 32 minutes. Regarding the detection parameters, the ionization source was maintained at 230°C, and mass spectra were acquired within a scan range of 29–600 Daltons at 0.5-second intervals. Data acquisition included the recording of relative average peak areas, retention times, molecular formulas, and molecular weights. For compound identification, the obtained mass spectra were compared against the NIST/WILEY (1999) spectral library database. This methodology ensured a high degree of precision in determining the qualitative and quantitative profile of the sample

Results

Phytochemical Screening

Table 1: Phytochemical Screening of *Ophiorrhiza mungos linn* Extract

S. NO	Phytochemical Test	Results
1	Alkaloids	+
2	Amino Acids	-
3	Carbohydrates	+
4	Volatile oils	+
5	Flavonoids	+
6	Glycosides	+
7	Tannins	+
8	Steroids	+
9	Triterpenoids	+
10	Proteins	-

FTIR analysis

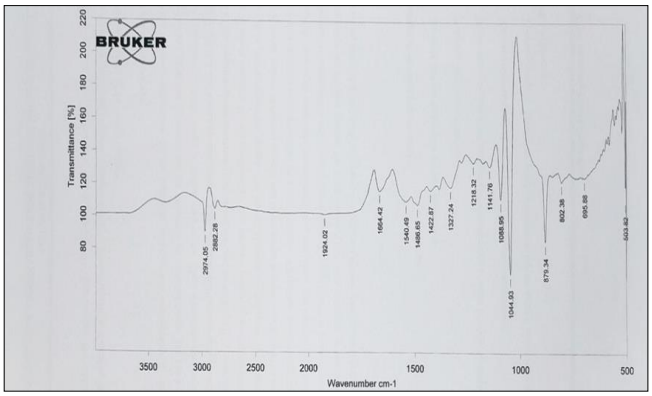

Fig 2: Interferogram of *Ophiorrhiza mungos linn* extract

HPTLC Analysis

Table 2: Peak list of Ethanolic extract of *Ophiorrhiza mungos linn* at UV 366nm

Peak number	Y-pos	Area	Area %	Height	Rf value
1	9.9	826.94	52.14	611.04	0.01
2	21.3	16.75	1.06	8.25	0.17
3	27.6	57.09	3.60	22.14	0.26
4	52.5	98.57	6.21	47.91	0.60
5	62.2	530.90	33.47	196.98	0.74
6	76.7	39.14	2.47	18.68	0.94
7	78.0	16.68	1.05	12.91	0.96

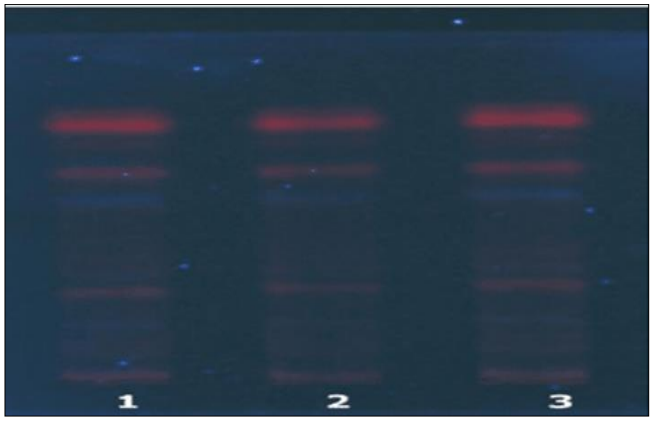

Fig 3: Visual detection of HPTLC plates at UV 366nm

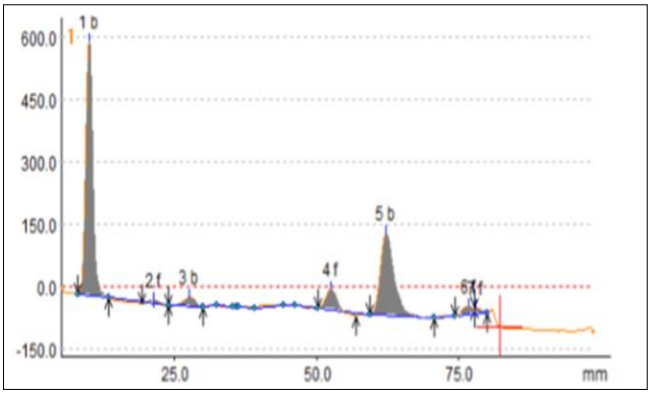

Fig 4: Densitogram of ethanolic leaves extract of of *ophiorrhiza mungos linn*

GC-MS analysis

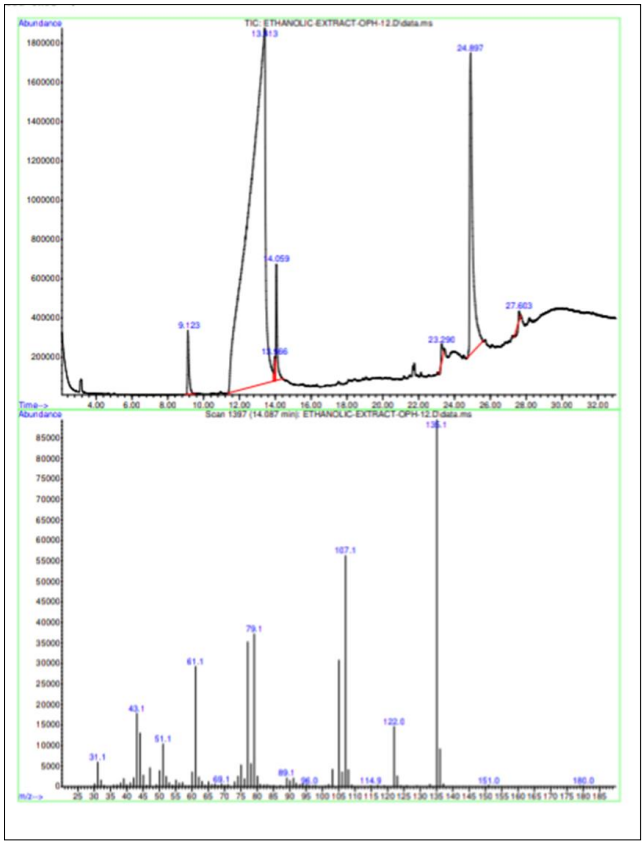

Fig 5: GC-MS chromatogram of *Ophiorrhiza mungos linn* extract

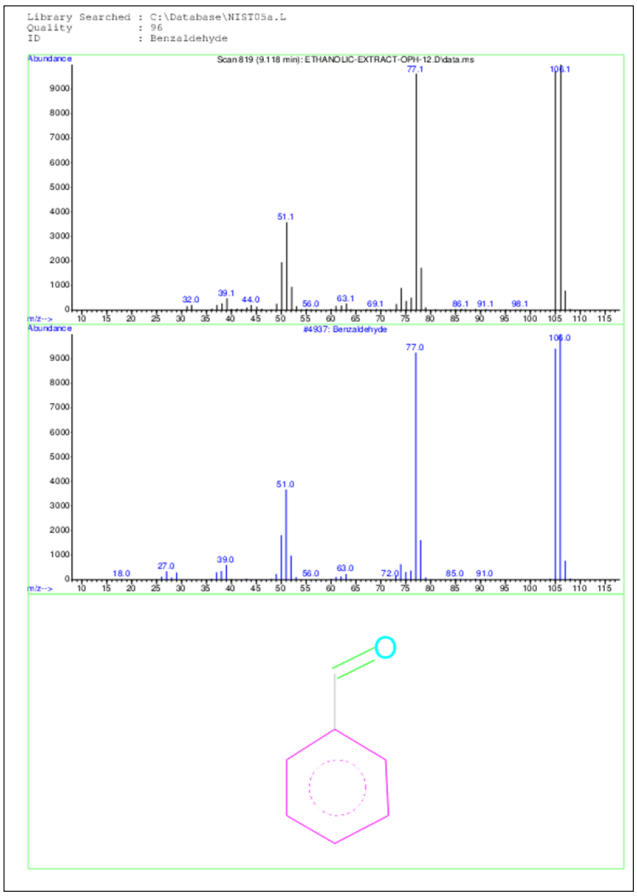

Fig 5a: Benzaldehyde

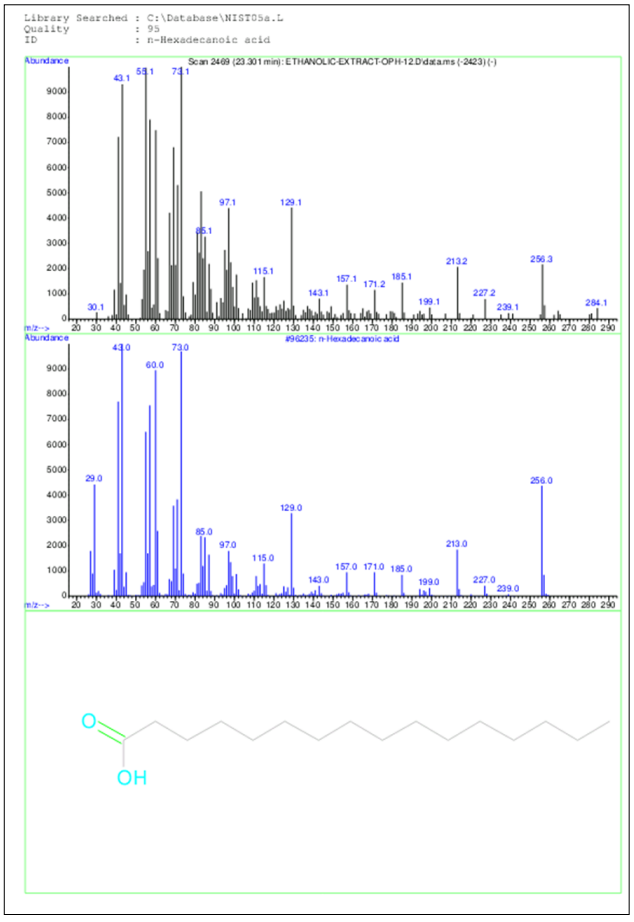

Fig 5b: n-Heaxadecanoic acid

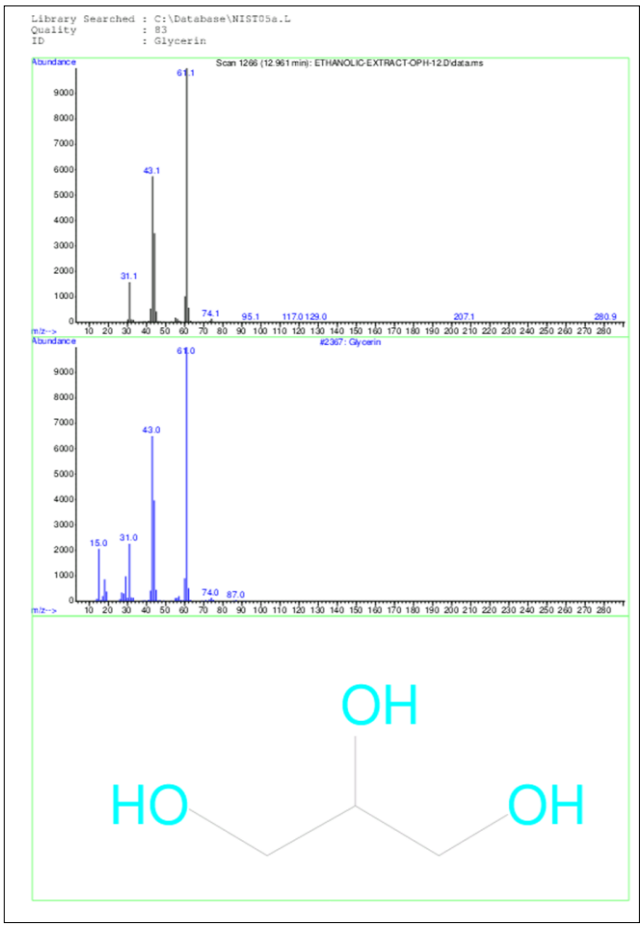

Fig 5c: Glycerin

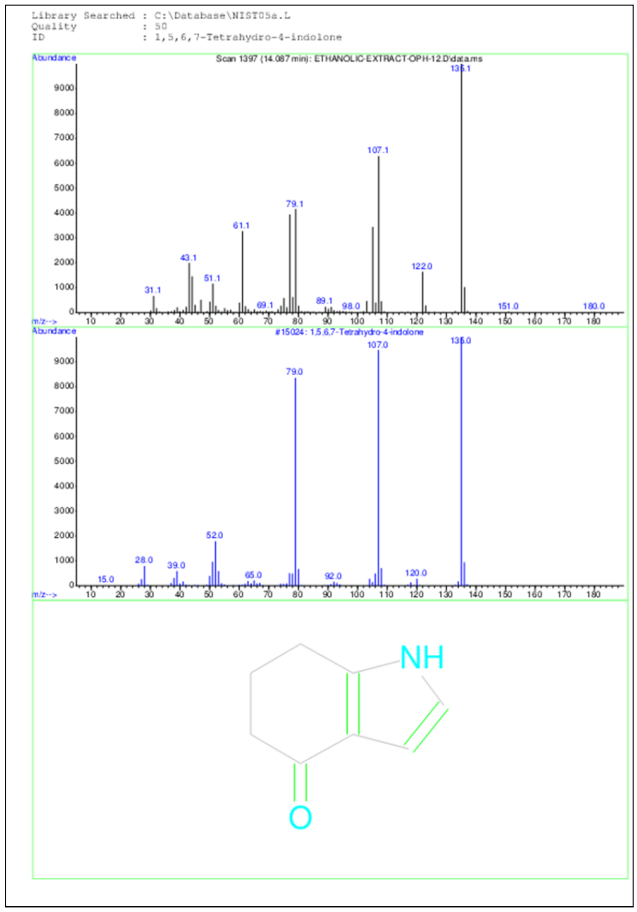

Fig 5d: 1,5,6,7 tetrahydro-4- Indolone

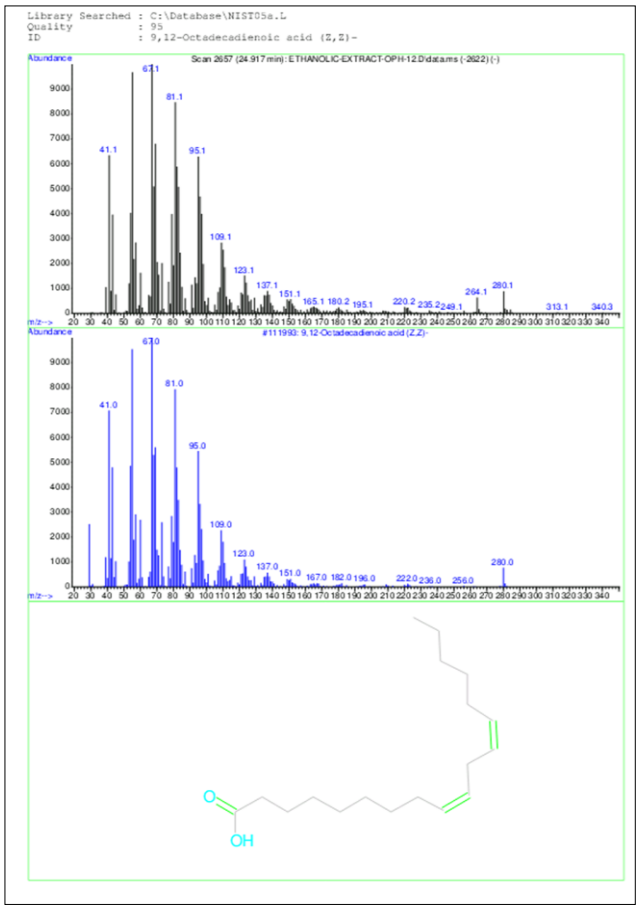

Fig 5e: 9,12 Octadecadienoic acid

Table 3: List of Chemical Constituents

S.NO	Compound Name	RT	Molecular formula	Molecular Weight(g/mole)
1	Benzaldehyde	9.11	C_7H_6O	106.12
2	Glycerine	12.96	$C_3H_8O_3$	92.09
3	n- hexadecenoic acid	23.30	$C_{16}H_{32}O_2$	256.43
4	1,5,6,7 tetrahydro-4- Indolone	14.08	C_8H_9NO	135.16
5	9,12 octadecadienoic acid	24.91	$C_{18}H_{32}O_2$	280.44

Discussion

The leaves of the herbal plant i.e., *Ophiorrhiza mungos linn* are erect, herbaceous, perennial plant found from the forest of Telangana, Kerala, Andaman and Nicobar Islands etc.

The extraction of *ophiorrhiza mungos linn* leaves was done by using ethanol as a solvent through Soxhlet apparatus.

The phytochemical screening of the extract was done in which chemical constituents are recognized such as alkaloids carbohydrates, volatile oils, flavonoids, glycosides, tannins, steroids, and triterpenoids as listed in table no.1.

In HPTLC technique, ethanolic extract was spotted on silica gel G plate developed with Toluene: Ethyl Acetate: Methanol (7:2:1) as mobile phase which shows seven major spots under UV 366nm.

The HPTLC technique was used for the separation of chemical constituents, utilizing a DESAGA Sarstedt Gruppe system HPTLC instrument (Germany) was used along with automatic TLC applicator and UV visible cabinet as an

imaging system, paroquet 1.6 version software as listed in table no.2.

Peaks obtained on FTIR spectrum by using Alpha- Bruker instruments which leads to the determination of the functional group as follows 2973.88(alkane (C-H),1649.81(aromatic alkane), 1380.61(S=O),1274.00 (p=o Phosphor amides) 581.89 (S-OR Esters),514.70 (S-S disulfide) as shown in figure no.2.

GC-MS analysis of ethanolic extract was done by using Agilent 6890GC with 5973 N MSD for the identification and characterization of five active constituents such as Benzaldehyde, Glycerine, n- hexadecenoic acid,1,5,6,7 tetrahydro-4- Indolone, and 9,12 octadecadienoic acid as listed in table no.3.

Conclusion

In this study, the leaves of *Ophiorrhiza mungos Linn.* were utilized for extraction using a Soxhlet apparatus, with ethanol selected as the solvent of choice. Preliminary phytochemical screening was conducted to determine the nature of the chemical constituents present in the ethanolic extract. Further separation, characterization of functional groups, and identification of phytoconstituents were performed using analytical techniques such as Fourier Transform Infrared Spectroscopy (FTIR), High-Performance Thin-Layer Chromatography (HPTLC), and Gas Chromatography–Mass Spectrometry (GC–MS). The spectral data obtained were compared with the NIST library of IICT for compound identification. This comprehensive investigation highlights the potential medicinal relevance of *Ophiorrhiza mungos Linn.*in herbal therapeutics and provides a foundation for further traditional uses, and pharmacological properties.

Acknowledgement

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Conflict Of Interest Statement

The authors declare no conflict of interest.

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