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Original Article

GC-MS Profiling, Standardization and *in vitro* Evaluation of Antibacterial Activity in *Hemidesmus indicus* L. (R.Br.) Roots Extracts

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ABSTRACT

Aim: The study was aimed to evaluate the pharmacognostic features of *Hemidesmus indicus*, analyze the antibacterial properties of ethanolic and aqueous root extracts, and identify bioactive compounds using GC-MS analysis. **Materials and Methods:** We conducted pharmacognostic analyses, including organoleptic, macroscopic, microscopic, and physio-chemical evaluations, phytochemical screening, and fluorescence analysis. We also used standard protocols for TLC fingerprinting. Antibacterial activity against gram-negative bacteria was assessed using the agar well diffusion method. Additionally, we conducted GC-MS analysis to identify major compounds in the plant extracts. **Results:** In our *H. indicus* root examination, we identified three regions: wood, inner cortex, and outer phellem. Key findings: moisture content at 9.0% w/w, ethanol and water-soluble extractives at 11.21% and 16.89% w/w, total ash at 4.21% w/w, acid-insoluble ash at 1.14% w/w, and water-soluble ash at 3.12% w/w. Phytochemical screening detected alkaloids, flavonoids, tannins, polyphenols, triterpenoids, carbohydrates, and proteins. TLC analysis of the ethanolic extract showed varying R_f values. The aqueous extract exhibited stronger antibacterial activity against *E. coli* than the ethanolic extract. GC-MS analysis identified over 30 prominent compounds in the ethanolic extract. **Conclusion:** In conclusion, this study provides valuable insights for the standardization of *H. indicus* root extracts. The aqueous root extract displayed notable antibacterial properties. Furthermore, our GC-MS analysis identified the presence of phenolic, Fatty acids, and terpenoids compounds in the root extract of *H. indicus*, which hold potential therapeutic applications.

Keywords: *Hemidesmus indicus*, Pharmacognostic, Fluorescence analysis, Standardization, GC-MS.

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INTRODUCTION

The plant species *H. indicus*, sometimes known as Indian sarsaparilla, is a member of the Asclepiadaceae family. The use of plants as antibacterial, anti-inflammatory, and antitumor medicines has been demonstrated by numerous pharmacological research. The plant's roots are used in herbal remedies to treat various diseases and are available in the market. It thrives in mesophilic to semi-arid plains and can be found at altitudes of up to 600 m across the entire region of India. Iran, Bangladesh, Pakistan, Sri Lanka, and other countries also have it. It is a thin, lactiferous, twining shrub that is found over most of India.[1,2] The leaves are arranged in opposite pairs and have short petioles. They are elliptically oblong to linear-lanceolate in shape, with a dark green upper surface and a lighter underside that may occasionally have pubescence. Flowers have a deep five-lobed calyx and are greenish on the outside but purple on the inside. Black seeds have a flattened, silvery-white coma around them.[3,4] The anticlockwise-twined stem and branches are thick, elongated, and narrow and they have a purplish-brown tint. The roots are fragrant, woody, and thin. The fruit consists of two straight, slender, narrowly cylindrical follicles that spread widely apart.[5,6] The plant yields numerous flat, oblong seeds adorned with a tall tuft of silky white hairs. It boasts a rich medicinal heritage in South India and is highly coveted in Ayurveda, recognized by its local name, "Nannari". *H. indicus* serves as a substitute demulcent, tonic, and diaphoretic. Traditionally, it has been employed to address venereal diseases, skin ailments, urinary infections, emotional distress, and impotence. Furthermore, it is believed to help prevent abdominal distention, arthritis, gout, rheumatism, and epilepsy. A chemical that kills bacteria or prevents their development is an antimicrobial agent. Antibiotics, antifungals, and antivirals are used to treat microbial infections all over the world.[7-12]

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MATERIALS AND METHODS

Material, Chemicals and Reagents

Conical flask, Beakers, Spatula, Glass funnels, Measuring cylinders (various sizes), Autoclave, Top-loading Mettler balance, Mortar and pestle, Test tube holder, Analytical balance, Capillary tubes and Filter paper. Reagents used for the analysis of ethyl acetate, chloroform, n-hexane, methanol, ferric chloride, Mayer-Wagner reagent, HCl, NaOH, sulfuric acid, Dimethyl Sulfoxide (DMSO), Potassium Ferricyanide [$K_2Fe(CN_6)$], (Mettler-Toledo India Pvt. Ltd.,), Ciprofloxacin (Glenmark Pharmaceuticals Ltd.,).

Procurement of plant materials

Fresh root of *Hemidesmus indicus* were purchased from Thiruvananthapuram district of Kerala in October 2022. The plant's authenticity was verified at the National Botanical Research Institute (NBRI) in Lucknow, Uttar Pradesh, and a complete plant specimen has been archived in the repository, bearing the CDR No. The mature roots were collected and subsequently washed for 5 min under a stream of tap water, followed by three additional rinses with sterile distilled water. The remaining half of the drug samples underwent a grinding process to achieve a particle size of 60 meshes. Afterward, they were subjected to a seven-day shade drying period and then stored in an airtight container to prevent any potential contamination from moisture or other factors. Some of these drug samples were also air-dried without any alteration for the purpose of conducting morphological examinations.

Organoleptic and macroscopy studies of root of *H. indicus*

Macroscopic and organoleptic assessments of the raw drug involved examining its characteristics such as shape, color, size, taste odour, and other relevant attributes. The samples were put through a macroscopic examination that included visual, tactile, and other sensory inspections. For a better assessment of surface features, a dissecting microscope was employed with a magnifying lens.[12-15]

Qualitative microscopy

Under the microscope, we observed the surface and transverse sections of Indian sarsaparilla root. To enhance the visibility of microscopical structures, we stained the sections using a phloroglucinol and HCl solution in a 1:1 ratio, following the established standard procedure. Various microscopical features were identified and captured in photomicrographs.[16]

Powder microscopy

We conducted microscopic examinations on powdered dried roots to identify various components. For the detection of lignified elements, we employed a staining reagent consisting of phloroglucinol and HCl in a 1:1 ratio. Additionally, a small quantity of finely powdered leaves was also observed under the microscope. To prepare the leaf sample for examination, we mounted it in glycerol, adding 1-2 drops of a 0.1%w/v phloroglucinol solution and concentrated HCl. A coverslip was placed over the powder before the microscopic examination. During this process, we were able to identify the periderm, the presence of phellem along with epidermal cells, xylem vessels, fibers, phloem, and other relevant structures within the cells. These cell structures were meticulously observed and subsequently captured in photomicrographs.[17-19]

Root Microscopy

Transverse slices of the root were examined under a photomicrograph for this investigation. Safranin and Fast Green staining chemicals were used in accordance with accepted procedures. If there was staining or not, many distinguishing characteristics were noted. With or without staining, the numerous distinguishing characteristics were examined and noted.[20]

Physiochemical evaluation of root of *H. indicus*

Determination of extractive value

Five grammes of the air-dried root powder from the *H. indicus* root were taken in six sealed flasks individually. Three flasks received 100 millilitres (mL) of 95% v/v ethanol, while the remaining three flasks received 100 mL each of distilled water. The flasks were placed for 18 hr without stirring after being placed for 6 hr with periodic shaking. The material was filtered. In tared flat china dishes, 25 mL of both aqueous and ethanol filtrates were individually evaporated. The evaporated residues were dried until they reached a constant weight at 105ºC.The results for the ethanol and water-soluble extractives of *H. indicus* root are presented below in Table 1.[21]

Determination of Ash value

Ash value is a crucial standardization measure for assessing the quality and purity of unprocessed plant material. After incineration, total ash is made up of leftover particles that are composed of inorganic salts such as silicates, phosphates, and carbonates of Calcium (Ca), Sodium (Na), Potassium (K), and Magnesium (Mg). To determine the total ash content of air-dried *H. indicus* root, 2 g of the root material were individually incinerated at 450ºC until a constant weight was attained. This process was carried out in six pre-weighed silica crucibles. Next, in a separate procedure, the ash from the first three crucibles was subjected to further treatment. It was heated in 25 mL of water, and the water-insoluble ash was collected using ashless filter paper. Afterward, the collected water-insoluble ash was washed in hot water until reaching a consistent weight, and then it was incinerated again at 450ºC. Separately, we utilized 25 mL of 2M HCl to boil the ash in the remaining three (3) silica crucibles. Similarly, as in the previous step, we collected the acid-insoluble

ash by washing it in warm water until reaching a constant weight, using ashless filter paper for this purpose. The resulting values for the total ash, water-soluble ash, and acid-insoluble ash of *H. indicus* are presented as their average in Table 1.[22,23]

Moisture content by loss on drying method

Utilizing the thermogravimetric concept, the loss on drying approach was used to calculate the moisture content. 2 g of fine root powder were placed on a tinny China dish and dried in an oven set to 100ºC to 105ºC. Afterward, I let the dish cool in a desiccator. There were recorded three successive weighing with a maximum 0.5 mg difference between them. Moisture loss was used to describe the weight loss.[24]

Determination of swelling index

The WHO states that the swelling index is a measurement of the swelling of 1 g of crude medication in volume (mL). Put 1 g of root powder in a twenty five (25) mL measuring container with a cork, then mix in 25 mL of water for 24 hr, gently stirred the mixture every now and then. Without tampering, this combination was left at ambient temperature. The amount of space the powdered medication and gooey mucilage took up in millilitres (mL).[25]

Determination of foaming index

The WHO specifies that the capacity of a herbal drug's aqueous decoction to generate foam is used to calculate the foaming index. A 100 mL conical flask was filled with precisely weighed 1 g of root powder, boiling water were added, and the mixture was then allowed to cool. After filtering, the volume is increased to 100 mL in a volumetric flask. One to ten millilitres of the decoction were placed in each of the ten test tubes in turn. Water was added to the remaining capacity to bring it up to 10 mL, and the tubes were then corked shut. The test tubes were vigorously shaken and then allowed to stand undisturbed for duration of 15 min. Each test tube's foam was measured for height. After measuring the froth, the foaming index was calculated.[26]

Phytochemical screening

Pharmacological activities in crude drugs are typically attributed to secondary metabolites. To assess the presence or absence of various compounds, including phytosterols/triterpenoids, flavonoids, polyphenols, tannins, alkaloids, proteins, carbohydrates, and more, both ethanolic and aqueous extracts of the powdered drug were screened.[22]

Fluorescence Study

The whole plant powder underwent fluorescence analysis using a conventional procedure. The plant powder was subjected to several solvent treatments, including both acidic and basic, to conduct the analysis. Following treatment, they were subjected to both short- and long-wavelength UV radiation and daytime observation. An essential tool for screening substances with the ability to display various colours under UV light is fluorescence analysis. Some substances can be transformed into fluorescent derivatives even if they are not fluorescent in and of themselves when they are exposed to solvents. This analysis noticed the shift in colours.[27,28]

Heavy metal analysis

To perform the heavy metal analysis of root extracts from *H. indicus*, an atomic absorption spectrophotometer was employed. This analysis involved the preparation of standards for Nickel (Ni), Arsenic (As), Lead (Pb), and Cadmium (Cd), and the subsequent development of a calibration plot. The drug samples were then analyzed using this calibration plot as a reference.[29]

Thin Layer Chromatography (TLC)

Thin-layer chromatography, also known as fingerprint profiling, is a technique employed for the separation of non-volatile compounds. In this method, samples were applied to TLC-precoated plates made of Silica gel 60-F254 (Merck KGaA, Darmstadt, Germany) in the form of bands using capillaries. The chromatographic plates were then placed in a TLC chamber that had been pre-saturated with a solvent system. After the compounds had undergone separation between the mobile and stationary phases, the TLC plates were carefully removed. The solvent was allowed to evaporate at room temperature. Subsequently, the TLC plates were examined both in daylight and in a chamber illuminated with UV light at wavelengths of 254 nm and 365 nm. The spots on the plates were quantified by determining the retention factor (R_f). It's important to note that the R_f value varies and depends on the polarity of both the mobile and stationary phases.[30-32]

GC-MS analysis (Gas Chromatography-Mass Spectrometry)

GC-MS is a highly effective tool for obtaining both qualitative and quantitative information about compounds, including their molecular weight. The ethanolic extract ofH. *indicus* was subjected to GC-MS analysis. GC analysis was carried out using the GC Clarus 500 Perkin Elmer system under the specified conditions. The injector temperature was maintained at 260ºC, and a capillary column known as Elite 5 (composed of 100% Dimethyl poly siloxane) was used. The oven temperature followed a program from 110ºC to 280ºC, with a gradient of 5ºC per min, and an initial hold time of 2 min. The ionization voltage was set at 70V, and the detector voltage at 2100 V. The detector used was the mass detector TurboMass Gold by Perkin Elmer, and the software employed was Turbo Mass. For the analysis, a 1 mL ethanol extract was injected into the GC-MS system. The spectral data of the unidentified component were matched against spectra of known components archived in the NIST and MS libraries. This matching process enabled the identification of the component's name, molecular weight, and structure within the test materials.

Helium gas served as the mobile phase, and the obtained results were subsequently analyzed and interpreted.[33-36]

Antibacterial activity in *H. indicus* roots extracts

Gram-negative microorganisms, including *Escherichia coli* (MTCC687), were employed to evaluate the antibacterial activity. The agar well diffusion assay was employed to assess the antibacterial effectiveness of root extracts derived from *H. indicus*. The petri plates were filled with solidified nutrient agar, and these plates were then inoculated by applying an inoculum with sterilized cotton swabs that had been previously soaked in the bacterial culture. The inoculum was evenly spread across the solid agar medium. Subsequently, varying concentrations (100, 150, and 200 µg/mL) of both aqueous and ethanolic root extracts were added to individual wells in the agar plates. All plates containing the extract-loaded wells were sited in an incubator set at 37°C for 24 hr. The assessment of antibacterial activity entailed the measurement of the diameter of the inhibition zone surrounding each well. In this study, a concentration of 50µg of ciprofloxacin was utilized as the positive control, while distilled water was employed as the negative control. To assess the antibacterial properties of the plant extracts, the zone of inhibition (expressed in mL) around the wells was calculated and compared with the standard antibiotic, Ciprofloxacin.[37-39]

Statistical Analysis

For separately treatment, triplicate plates were organized, and the average zone of inhibition, excluding the well, was recorded. All the collected data were presented as mean values with accompanying standard deviations.

RESULTS

Macroscopic evaluations

In Figure 1, the roots exhibit the following characteristics: they are typically fragmented, slender, woody, brown, and sparsely branched. They are thick, rigid, hard, elongated, and cylindrical in shape, with a wavy and rough outer surface. Externally, they appear dark, tortuous, with transverse cracks and longitudinal fissured bark portions, while internally, they display a yellowish-brown hue. The rootlets are wiry and, thin with a cork that is thin, easily separable, and peels off in flakes. The root fragment has a diameter of approximately 3.0 cm, with a solid central core measuring about 0.4±0.8 cm in diameter. The outer surrounding cylindrical portion is approximately 0.3±0.7 cm in breadth, and the length of the root fragment varies within the range of 1.6±3.4 cm. When fractured, the roots tend to break with a short, splintery pattern. In terms of taste, they are characterized as sweetish, acrid or astringent, while their odour is described as agreeable, very aromatic, and slightly acidic.

Evaluations of transverse sections of *H. indicus* root

Transverse slices of the *H. indicus* roots revealed three unique regions: the wood regions, the inner cortex, and the outer phellem. Phellem cells were brownish-pink in color, whereas cork cells were rectangular, radially flattened, and filled with brownish-coloured granules. Each cortical cell is completely filled with large-sized starch granules. The brain is covered in a variety of fantastic ducts. Variable-sized xylem vessels alternate with phloem and uniseriate medullary rays. The cambium is composed of 1-2 layered compressed cells with many, frequently uniseriate, sporadically biseriate medullary rays. It was very normal to find secondary cortical cells in numerous levels. The secondary phloem is composed of ray cells, parenchyma, sieve components, and a number of lactiferous ducts (Figures 2 and 3). The centre is composed of woody tissues and thin medullary rays that intersect the xylem.

Evaluations of Powder microscopy of *H. indicus* roots

The fine root powder, exhibiting a yellowish-brown hue, displayed the following characteristics: There is a plentiful presence of sturdy stone cells with lignified walls, featuring prominent pit canals that come in a range of sizes and shapes, including triangular, oval, rectangular, semi-circular, and elongated tracheid-like

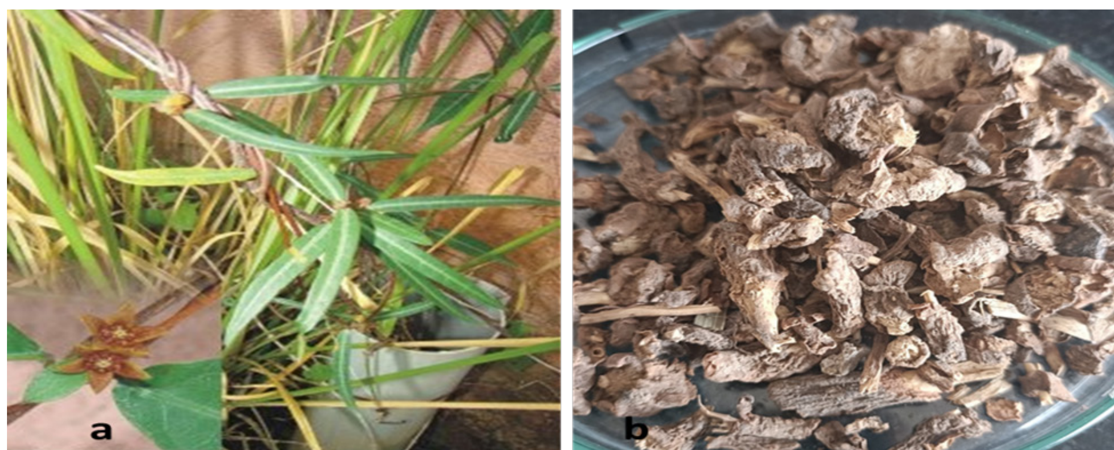

Figure 1: *Hemidesmus indicus* plants and dry roots.

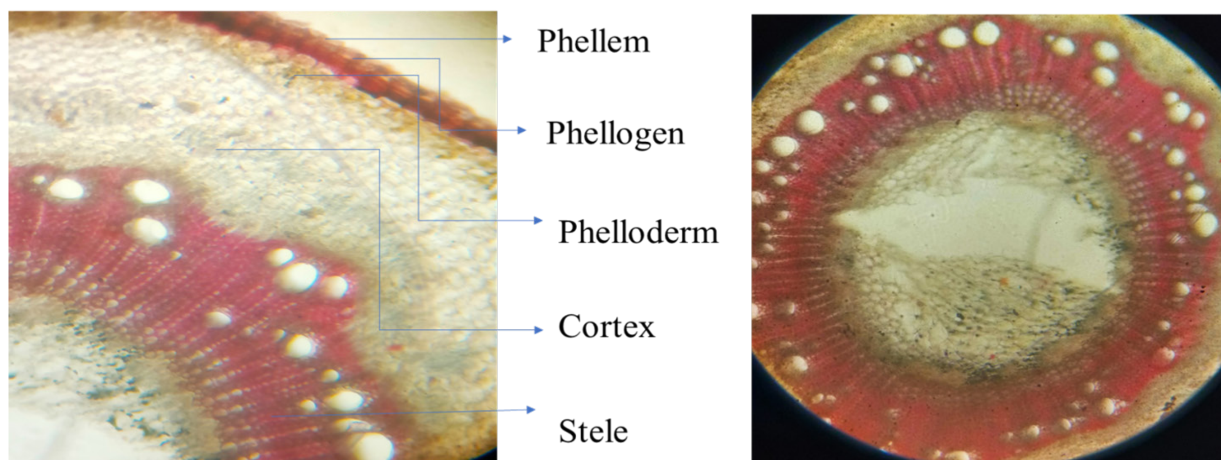

Figure 2: T.S. of root of *H. indicus*-4x and 40X.

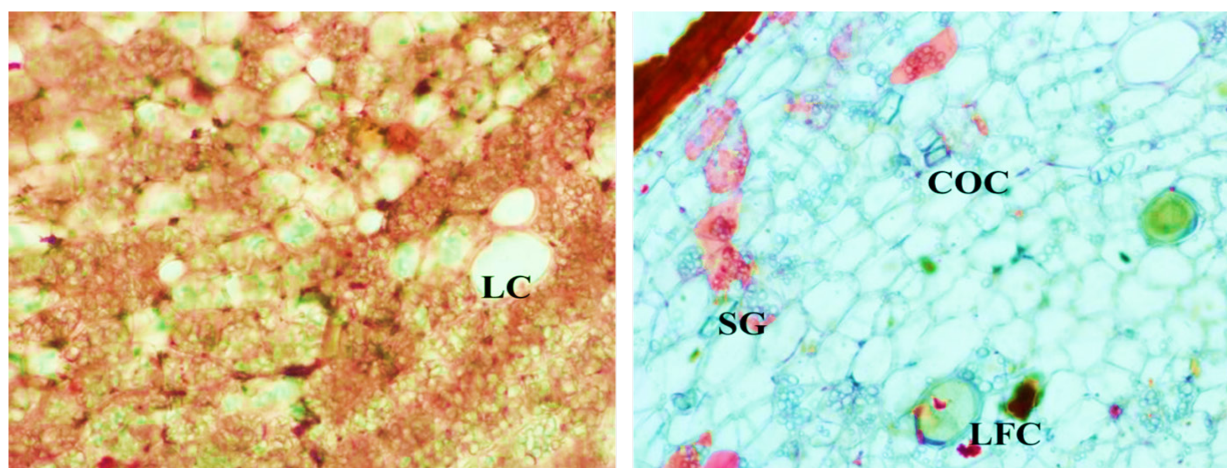

Figure 3: T.S. of *H. indicus* roots at 10x Cortex and starch grain (LC: Lactiferous Canal, SG: Starch Grain, COC: Calcium Oxalate Crystal and LFC: Latex Filled Cells).

forms. Angular or semi angular, flattened, lignified-walled cork cells. Clusters of xylem vessels displaying both pitted and spiral characteristics, sometimes accompanied by fibers. There are also single-celled, aseptate, long fibers with an undulating inner wall. Additionally, there are numerous Ca-oxalate prismatic crystals with varying shapes, including rectangular, triangular, and irregular forms. There is an abundance of starch grains, comprising both simple and compound grains with three to four components and a central hilum. Squarish and Spacious, types of parenchymatous cells are presents. Additionally, there are clusters of light brownish polygonal cells with angular and opaque characteristics. In contrast, there is a limited presence of long, lignified tracheid fibers with a serrated inner wall. Furthermore, a small number of groups of xylem parenchyma cells containing cellular contents can also be observed in Figure 4.

Physio-chemical parameters

Typically, physicochemical criteria were employed to determine the identity, potency, and purity of the drug source. These characteristics were also utilized to identify any potential adulterants. To assess the purity of *H. indicus* roots, several parameters were examined, including moisture content, extractive values for both ethanol and water solubility, total ash content, acid insoluble ash, water-soluble ash, swelling index, and foaming index as shown in Table 1.

Phytochemical screenings

The outcomes of the initial phytochemical screening of ethanolic and aqueous extracts revealed the existence of proteins, flavonoids, phytosterols/triterpenoids, alkaloids, tannins, carbohydrates, and polyphenols. These findings are presented in Table 2.

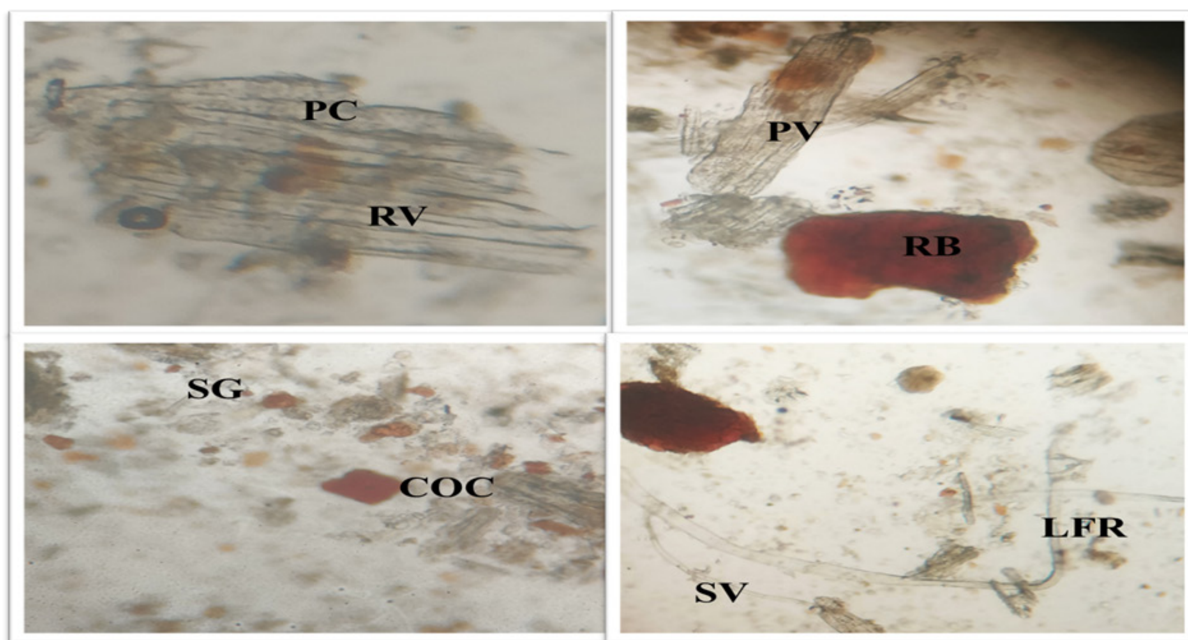

Figure 4: Powder characteristics of *H. indicus* root. (RV: Reticulated vessel, PC: Parenchyma cell PV: Pitted vessel, RB: Resinous Block, COC: Calcium oxalate crystal, SG: Strach grain, LFR: Long fibres, SV: Spiral vessel.

Table 1: Physiochemical parameters of *H. indicus* root.

Parameters	Result
Ethanol soluble Extractive value	11.21%
Water soluble extractive value	16.89%
Water soluble Ash	3.12%
Acid Insoluble Ash	1.14%
Total Ash	4.21%
Moisture content	9.0%
Swelling index	1.9%
Foaming index	>100

n=3: dry weight basis.

Fluorescence analysis

Fluorescence is a phenomenon that is displayed by many medications and may be used to identify the different chromophores of chemical elements in plant material. A few components fluoresced in the visible spectrum of daylight. Many natural chemicals, such as alkaloids like berberine, which do not glow noticeably in daytime, fluoresce under the influence of UV radiation. If the material itself is not fluorescent, it is frequently possible to use various reagents to transform it into fluorescent derivatives or breakdown products. As a result, this approach is frequently used to evaluate some crude drugs qualitatively, and it also serves as a crucial component of pharmacognostic evaluation. The information from the fluorescence characteristics was given in Table 3 and indicated different tones of brown and yellow.

Table 2: Phytochemical analysis of different extract of *H. indicus* root.

Secondary metabolites	Extracts	
	Ethanol Extract	Aqueous Extract
Alkaloids	+	-
Steroids	+	+
Tannins	+	+
Flavonoids	+	+
Phenolic compounds	+	+
Saponins	+	+
Lignin	+	+
Saponins	+	+
Triterpenoids	+	+
Proteins	+	+
Carbohydrates	+	+
Amino acids	+	+

Thin layer chromatography

A standard solution was prepared using ethanolic root extract of *H. indicus*, and it was quantitatively applied to pre-coated silica gel TLC plates. These plates were subsequently developed using a mobile phase consisting of Toluene: Ethyl acetate: Formic acid: Methanol (in a ratio of 6:4:1:0.5) and visualized using 0.5% anisaldehyde-H2SO4 and Ultraviolet (UV) lamps with wavelengths of 254 nm (short wave) and 365 nm (long wave).

Figure 5 illustrates the presence of 5 distinct spots, and the corresponding R_f values for these bands in the solvent system can be found in Table 4.

Heavy metal assessment

The levels of heavy metals such as Arsenic (As), as well as metals like Cadmium (Cd), Nickel (Ni), and Lead (Pb), in the roots of *H. indicus* were determined to be within the acceptable or permissible limits, as indicated in Table 5.

Antibacterial activity

The study examined the antibacterial properties of *H. indicus* root extracts, both in ethanol and aqueous solutions, against Gram-negative bacteria, specifically *E. coli*. To quantitatively assess their antibacterial effectiveness, the researchers measured the diameter of the clear zones in Petri dishes containing bacterial cultures. The observed antibacterial action of these extracts seems to be attributed to the existence of secondary metabolites such as alkaloids, terpenoids, phenolic compounds, tannins, and flavonoids. These compounds have the potential to interfere with microbial growth and metabolic processes. This microbiological assay aimed to assess the antibacterial potential of the aqueous and ethanolic extracts. Results show that the aqueous extract demonstrated significant efficacy at 100 µg/mL, while the ethanolic extract exhibited similar effectiveness at 150 µg/mL. Notably, the aqueous extract's efficacy significantly increased at 200 µg/mL, approaching the standard value. Table 6 compares the inhibition zones produced by the aqueous extract of *H. indicus* (100, 150, and 200 µg/mL), the ethanolic extract (100, 150, and 200 µg/mL), and the standard Ciprofloxacin against *E. coli*.

Gas Chromatography-Mass Spectrometry (GC-MS)

Exploring and extracting plant materials play a crucial role in advancing, modernizing, and maintaining the quality control of herbal preparations. Additionally, the study of medicinal/therapeutics plants contributes to a better understanding of plant toxicity, thereby safeguarding both humans and animals from natural toxins. Consequently, our current research aimed

Table 3: **Fluorescence Analysis of *H. indicus* root powder.**

Treatment	Under Ordinary Light	Under short wavelength 254 nm	Under long wavelength 365 nm
Powder as such	Dark brown	Pale brown	Blackish brown
Powder+Aqueous I N NaOH	Dark brown	Dark brown	Dark brown
Powder+50% HCl	Light brown	Pale brown	Brown
Powder+50% HNO_3	Light brown	Brown	Dark brown
Powder+H_2SO_4	Light brown	Pale brown	Greenish brown
Powder+Methanol	Light brown	Blackish brown	Dark brown
Powder+$FeCl_3$	Blackish brown	Dark brown	Dark brown
Powder+Picric acid	Yellowish brown	Brown	Pale brown
Powder +Iodine	Dull brown	Dull brown	Blackish green
Powder + Alcoholic I N NaOH	Brown	Pale brown	Blackish brown

Table 4: **Thin layer chromatograms of ethanolic extract of *H. indicus* roots.**

Extract	Solvent system	Spots	R_f value
Ethanolic extract	Toluene: ethyl acetate : formic acid: methanol (6:4:1:0.5)	5	0.45, 0.49, 0.58, 0.61 and 0.72

Table 5: **Heavy metals analysis of *H. indicus root*.**

Metal	Concentration (ppm)	Limit (ppm)
Cadmium	0.168±0.02	0.3
Arsenic	Not detected	3.0
Lead	0.61±0.01	10.0
Nickel	0.33±0.017	0.05-5 ppm

n= 3.

Table 6: Antibacterial activity of *H. indicus* in ethanolic and Aqueous extract.

Microbial strain	Diameter of Zone of inhibitions (mm)						
E. coli	Ethanolic extract			Aqueous Extract			Ciprofloxacin
	100 µg/mL	150 µg/mL	200 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL	50 µg
	16.1±0.2	22.5±0.7	24.1±0.3	23.1±0.3	26.1±0.1	26.4±0.2	29.8±0.2

N=3.

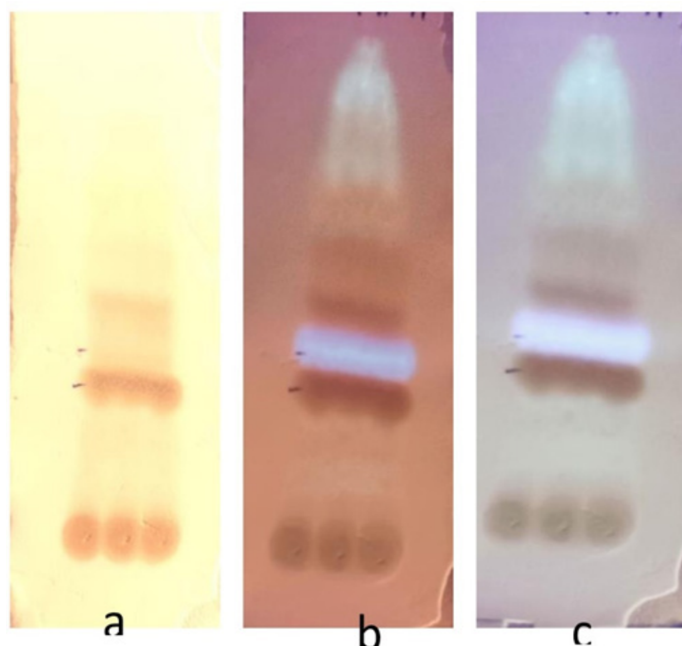

Figure 5: (a) Thin-Layer Chromatography (TLC) of the root ethanolic extract from *H. indicus* under visible light after being treated with the anisaldehyde-sulfuric acid reagent. (b) TLC of the ethanolic extract from the leaves under UV light at a short wavelength of 254 nm. (c) TLC of the ethanolic extract under UV light at a long wavelength of 365 nm.

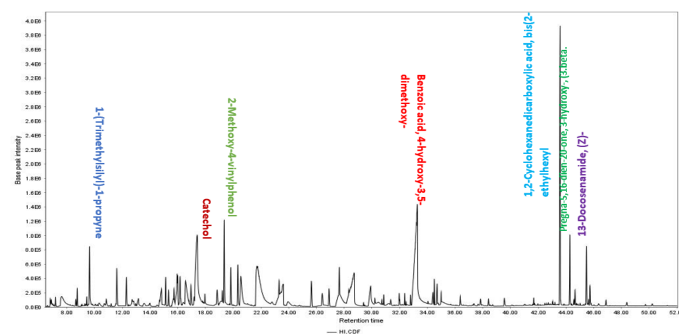

Figure 6: Gas chromatogram of ethanolic extract of *H. indicus root.*

to identify the bioactive compounds within the roots ethanolic extracts of *H. indicus*, employing gas Chromatography - mass Spectroscopy. In Table 7 and Figures 6 and 7, you can find detailed information about the active components, including their concentration (peak area %), Retention Time (RT), Molecular Weight (MW), and molecular formula.

DISCUSSION

The growing popularity of herbal remedies necessitates robust quality control measures to ensure their safe and effective use. Bioassays, guided by WHO guidelines, are pivotal for standardizing herbal medicines. Initial steps involve macroscopic and microscopic examinations to authenticate and purify herbal drugs. Morphological studies, a cost-effective method, encompass sensory observations and microscopic examinations, aiding drug authentication. This study provides anatomical and morphological descriptions of *H. indicus* roots, essential for quality control. Standardization is vital across medicinal systems to assure herbal medicine quality, enabling plant identification and differentiation from substitutes. Ensuring identity, quality, purity, and safety precedes human consumption. Microscopic, macroscopic, and fluorescence analyses authenticate raw materials and aid in *H. indicus* identification. These cost-effective standards benefit users and help assess raw material purity. Moisture content and loss on drying indicate drug quality. High moisture levels reduce drug efficiency, as they can lead to active compound hydrolysis. Moisture content in this case: 9.0% w/w. Minimal moisture is desirable to protect the drug from degradation. Ash content assesses foreign contaminants in crude drugs. Total ash includes physiological and non-physiological ash. Acid insoluble ash measures silica content. Water-soluble ash analyzes water-soluble components. The total ash value here is 4.21% w/w, well below the 14% limit, indicating a pure and effective crude drug.[23]

The extractive values were assessed for both ethanol and water, resulting in 11.21% and 16.89% respectively. The greater water-soluble extractive value suggests that the root's constituents have a higher solubility in water. These values are crucial for approximating the phytochemical composition of the herbal plant, but they can fluctuate if impurities or depleted materials are introduced into the original substance.[24]

Crude drugs are occasionally polluted with toxic metallic element such as Cadmium, Arsenic, Nickel, and Lead, even in minute quantities. These metals can be harmful to humans, particularly during gestational exposure, leading to ovotoxicity, hepatotoxicity, and renal disorders. Thus, it is essential for these metals to remain within acceptable limits in crude drugs to ensure their safety and efficacy for consumption.[28] Further observations revealed that the foaming index surpassed 100, suggesting the presence

Table 7: Chemical composition of ethanolic root extract of *H. Indicus*.

Sl. No.	Retention time (RT)	Compound Name	Molecular formula	Molecular weight	Peak area %	Nature of the compound
1	7.85	Dodecane	$C_{12}H_{26}$	170.34	4.34	Alkane
2	8.678	3-Aminopyrazine 1-oxide	$C_5H_6N_2O$	111	0.38	Organic compound
3	37.983	Octadecenoic acid	$C_{18}H_{36}O_2$	284	6.30	Stearic acid
4	37.738	Ethyl Oleate	$C_{20}H_{38}O_2$	310	1.67	Fatty acid ester
5	34.920	Hexadecenoic acid, Ethyl ester	$C_{18}H_{36}O_2$	284	2.28	Fatty acids
6	34.807	Hexadecenoic acid	$C_{16}H_{32}O_2$	256	13.8	Fatty acids
7	37.540	Oleic acid	$C_{18}H3_4O_2$	282	10.94	Fatty acids
8	17.101	m-Guaiacol	$C_7 H_8O_2$	124.1	1.03	Phenolic compound
8	44.540	Dotriacontane	$C_{32}H_{66}$	450.8664	0.45	Alkane
9	45.368	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$	337	1.00	The amide of docosenoic acid
10	44.540	Pregna-5,16-dien-20-one, 3-hydroxy-, (3.beta.	$C_{21}H_{30}O_2$	314.4617	1.34	Steroids
11	23.454	Vanillin	$C_8H_8O_3$	152.15	0.94	Phenolic aldehyde
12	27.369	Benzoic acid, 2-hydroxy-6-methoxy-	$C_8H_8O_4$	168	0.99	Benzoic acid
13	23.160	Eugenol	$C_{10}H_{12}O_2$	164.2	0.25	Phenolic compound
15	31.912	Tetradecanoic acid(Myristic acid)	$C_{14}H_{28}O_2$	228.3709	0.90	Fatty acids
16	17.488	5-Hydroxymethylfurfural	$C_6H_6O_3$	126.1100	3.56	Aldehyde
17	41.285	Eicosanoic acid	$C_{20}H_{40}O_2$	312.5	0.69	Fatty acids
18	40.745	Methyl eicos-11-en-14-ynoate	$C_{21}H_{38}O_2$	320.5	0.90	Fatty acids esters
19	36.012	Heptadecanoic acid /margaric acid	$C_{17}H_{34}O_2$	270.4	1.23	Fatty acids
20		2-[2-[2-[2-(2-Butoxyethoxy) ethoxy]ethoxy]et	$C_{16}H_{32}O_7$	336.4	0.49	Glycol ethers
21	38.286	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312.5	1.41	Fatty acid Ester
22	39.807	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652.9	0.35	Ester of ascorbic acid
23	29.938	Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	$C_9H_{10}O_4$	182.17	0.20	Aldehyde
24	27.510	Phenol, 4-ethenyl-2,6-dimethoxy-	$C_{10}H_{14}O_3$	182.2	0.34	Phenols
25	36.601	2-Pentatriacontanone	C35H70O	506.9	0.31	Ketone
26	21.631	Decane, 1-bromo-2-methyl-	$C_{11}H_{23}Br$	235.2	0.48	Haloalkanes
27	43.111	2-Eicosen-5-olide	$C_{22}H_{40}O_2$	430.4	0.23	Cycle esters
28	26.763	Ethyl 2,4-dihydroxy-6-methylbenzoate	$C_{11}H_{12}O_4$	196.2	0.22	Organic compound
29	31.074	1-Isobutyl-7,7-dimethyl-octahydro-isobe nzofu	$C_{14}H_{26}O_2$	226.3	0.23	Organic compound
30	49.594	Cholesta-4,6-dien-3-ol, (3.beta.)-	$C_{27}H_{44}O$	384.6	2.04	Sterols
31	45.703	Squalene	$C_{30}H_{50}$	410.7	0.16	Triterpenoid

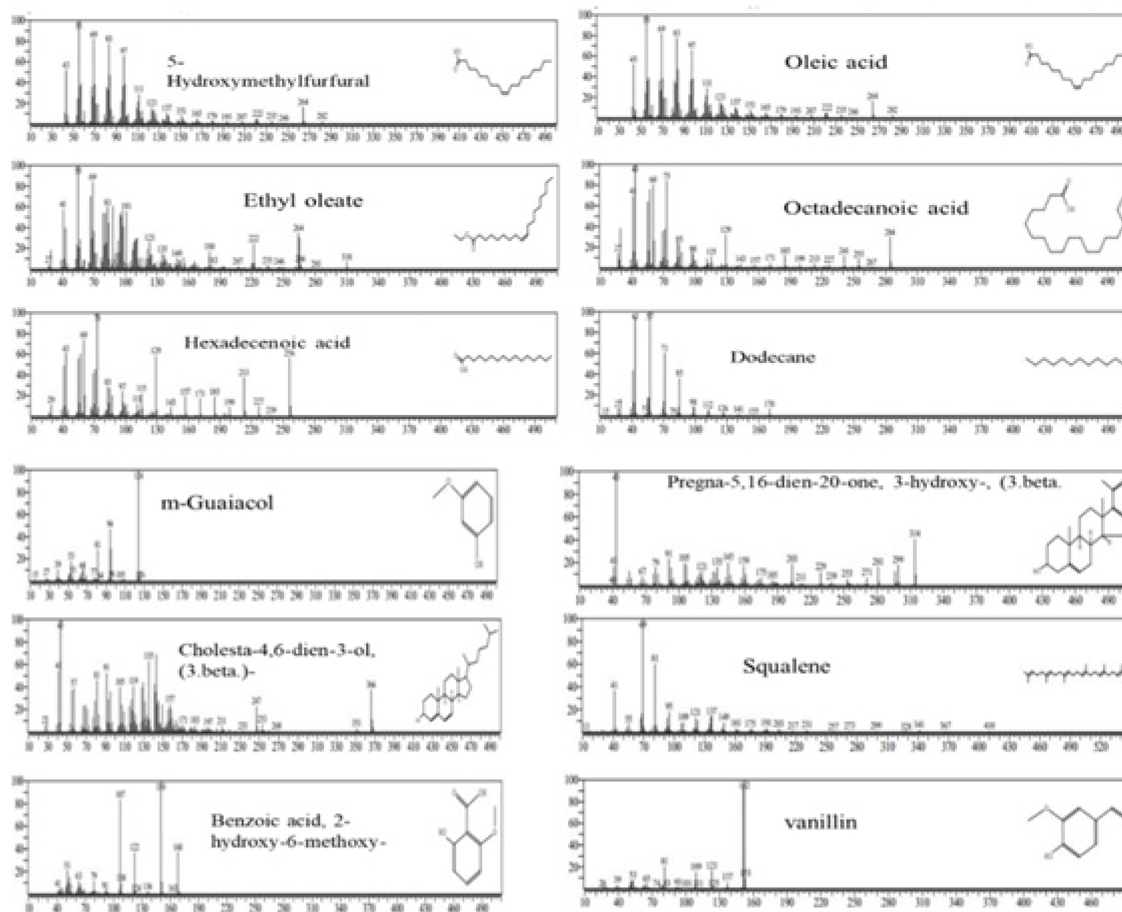

Figure 7: Mass spectra of chief compounds from root (ethanolic extract) of *H. indicus*.

of saponins within the roots. Additionally, the swelling index reached 9.0%, indicative of the mucilaginous nature of the root's content.[23,25] In the identification of raw herbal materials, the presence of specific phytoconstituents is of utmost importance, as they can exert distinct physiological effects on humans. A preliminary qualitative test has validated the existence of bioactive compounds in the roots, encompassing flavonoids, phytosterols/triterpenoids, polyphenols, alkaloids, tannins, carbohydrates, and proteins, highlighting the diverse array of phytoconstituents present.[22]

A fluorescence analysis is employed as a qualitative method to authenticate plant materials. This analysis reveals distinct colours associated with specific compounds. In cases where non-fluorescent compounds are present, certain reagents are added to induce their conversion into luminous derivatives compounds. The distinctive fluorescent characteristics and colours observed in the powdered roots of *H. indicus*, as detailed in Table 3, can be valuable tools for analyzing purposes.[27,28] Additionally, Thin-Layer Chromatography (TLC) is a crucial method employed for the initial profiling of numerous phytoconstituents. In the case of the ethanolic extract, TLC was performed, resulting in the identification of 5 distinct R_f values, indicating the presence of 5 phytochemicals within the extract, as outlined in Table 4 and Figure 5.[29] For a more comprehensive qualitative analysis, a GC-MS analysis of the roots of ethanolic extract of *H. indicus* was conducted. The results revealed the presence of flavonoids, alkaloids, steroids, glycosides, saponins, terpenoids, and phenolic compounds. The concentration (expressed as peak area %), Retention Time (RT), Molecular Weight (MW), and molecular formula of these active components are comprehensively outlined in Table 7, Figures 6 and 7.

Identified phytocompounds, including tetradecanoic acid, squalene, 13-Docosenamide, (Z)-, cholesta-4,6-dien-3-ol, (3.beta.)-, m-Guaiacol, Oleic acid and n-hexadecenoic acid, exhibit diverse properties such as antioxidant, antifibrinolytic, 5-alpha-reductase inhibition, antimicrobial, and haemolytic activities. Octadecadienoic acid (Z, Z) have been reported to possess hypocholesterolemic, anti-inflammatory, and antiarthritic activity. Squalene, categorized as a triterpene, is a phenolic compound found in the latex and resins of certain plants. It is believed that these compounds function as chemical defences against pathogens causing diseases in both humans and animals. In addition to its antimicrobial activity, squalene has been noted for its antioxidant, anti-tumor, anticancer,

chemo preventive, hepatoprotective, gastroprotective, pesticide, and sunscreen properties. Esters, with a growing number of commercial applications, find extensive use in cosmetics, flavors, fragrances, detergents, and pharmaceuticals industries. Ethyl oleates, a type of ester, serve as lubricants, plasticizers, hydraulic fluids, and biological additives. Furthermore, the analysis of extracts using GC-MS has exposed the presence of various anticancer constituents, such as 2-hydroxy, 4-methoxy benzoic acid, which exhibit anticarcinogenic activity.[33-36]

The observed antibacterial action of these extracts appears to be associated with the existence of secondary metabolites like terpenoids, alkaloids, flavonoids, tannins, and phenolic compounds which have the potential to interfere with microbial growth and metabolism. This microbiological assay aimed to determine the antibacterial potential of the ethanolic and aqueous extracts. The results indicate that the aqueous extract exhibited significant efficacy at 100 µg/mL, while the ethanolic extract demonstrated similar effectiveness at 150 µg/mL. Notably, the aqueous extract's efficacy increased significantly at 200 µg/mL compared to the ethanolic extract. At 200 µg/mL concentration, the aqueous extract's efficiency approached that of the standard value. [37-39]

CONCLUSION

A pharmacognostic analysis of the root will establish crucial identification standards for future research. Antibacterial investigation has confirmed potent antimicrobial properties in the aqueous root extract against the *E. coli* strain. GC-MS analysis identified key bioactive compounds: hexadecenoic acid methyl ester, ethyl oleate, oleic acid, tetradecanoic acid, and squalene, belonging to fatty acids, flavonoids, terpenes, and steroids. These compounds offer promise for developing antimicrobial drugs. Additionally, the analysis revealed diverse anticancer compounds like 2-hydroxy, 4-methoxy benzoic acid, showcasing anticarcinogenic properties. Exploring this plant's potential for various biological activities may lead to therapeutic formulations in pharmaceuticals, cosmetics, and culinary applications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

GC-MS: Gas chromatography and mass spectrophotometry; **TLC:** Thin layer Chromatography; **HCl:** Hydrochloric acid; **NaOH:** Sodium hydroxide; **R_f:** Retardation factor.

SUMMARY

Hemidesmus indicus is a vital herbal plant in Ayurvedic and traditional folk medicine. The current study focused on the pharmacognostic assessment of *Hemidesmus indicus* root, aiming to contribute to the accurate identification and authentication of this species for future research endeavors. The study involved a comprehensive examination of the freshly dried roots at a macroscopic level. In addition, both the transverse section and the powdered form of the roots were thoroughly investigated. The physico-chemical evaluation encompassed the determination of various parameters, including moisture content, ash values, foaming index, and extractive values. Furthermore, the root powder was subjected to identification through fluorescence analysis using different reagents. The study also delved into the phytochemical analysis of both aqueous and ethanolic extracts derived from the plant leaves, and their antibacterial activities against *Escherichia coli* were thoroughly explored. The phytochemical analysis revealed the presence of flavonoids, polyphenols, phytosterols/triterpenoids, alkaloids, carbohydrates, tannins, and proteins. TLC fingerprinting of ethanolic extract was done for the separation of phytoconstituents for qualitative analysis. Through GC-MS analysis, several potential bioactive compounds have been identified, including hexadecenoic acid methyl ester, ethyl oleate, oleic acid, tetradecanoic acid, and squalene. Based on the antimicrobial activity findings mentioned above, it was noted that the aqueous extract demonstrated significant antibacterial efficacy at a concentration of 200 µg/mL.

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