

Decoding the Phytochemical Treasure and Physicochemical Properties of *Distimake quinquefolius* L. for Herbal Drug Development

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ABSTRACT

Distimake quinquefolius L. (Convolvulaceae) is a perennial climbing herbaceous vine reaching up to 5 m, characterized by palmately compound leaves with five elliptic to lanceolate leaflets and trumpet-shaped white flowers with faint off-white stripes. The species grows predominantly in disturbed habitats such as roadsides and agricultural fields. Physicochemical evaluation revealed total ash (8% w/w), acid-insoluble ash (0.5% w/w), water-soluble ash (4.5% w/w), and sulphated ash (9% w/w). Extractive values were found to be 19.2% w/w (water-soluble) and 9.6% w/w (alcohol-soluble), loss on drying (9.2% w/w), foaming index (<100), swelling index (3.5 cm), and heavy metal content. Phytochemical investigations confirmed the presence of alkaloids, phenols, tannins, flavonoids, triterpenoids, and steroids. GC-MS analysis revealed that presence of potential bioactive compounds of pharmacological relevance. Collectively, these findings indicate that *Distimake quinquefolius* L. possesses significant phytoconstituents and favorable physicochemical attributes, justifying further pharmacological and therapeutic studies.

Keywords: *Distimake quinquefolius* L., Physicochemical, Phytochemical, GC-MS Analysis.

INTRODUCTION

Distimake quinquefolius L. A.R. Simões & Staples, belonging to the Convolvulaceae family, is also known by synonyms such as *Ipomoea quinquefolia*, *Convolvulus quinquefolia*, and *Merremia quinquefolia*. Commonly referred to as Five-Fingered Morning Glory, Rock Rosemary, Snake Vine, Five-Leaf Distimake, and Five-Leaf Morning Glory, this plant is widely distributed in tropical and subtropical

regions including the Americas, the Bahamas, northern Brazil, India, and nearby areas. Phytochemically, it contains alkaloids, flavonoids, tannins, terpenoids, phytosterols, fatty acids, and cardiac glycosides. Traditionally, it has been used in ethnomedicine to treat fever, headache, skin ailments, joint pain, rheumatoid arthritis, scurvy, and digestive disorders [2]. This species typically thrives in disturbed or human-influenced habitats such as

roadsides, abandoned lands, and waste sites [1].

Distimake quinquefolius L. (Convolvulaceae) is a perennial, climbing herbaceous vine that may extend up to 5 meters in length and is sparsely covered with fine hairs. Its leaves are palmately compound, consisting of five sessile leaflets that are elliptic to lanceolate in shape with serrated edges. The plant bears solitary or small cymose clusters of flowers, featuring a white corolla accented with pale yellowish or off-white stripes. At maturity, it develops a capsule-type fruit [1]. The common name “Five-Fingered Morning Glory” refers to its characteristic palmately compound leaves with five distinct leaflets. These leaflets are oblong to lanceolate, typically 2.5–6 cm in length, borne on petioles measuring 2–5 cm, and occasionally extending up to 9 cm. Flowers arise from the leaf axils, appearing either singly or in small clusters. The pedicels are glandular toward the upper portion and may bear spreading bristly hairs. Sepals measure 4–8 mm, are narrowly ovate to oblong, blunt-tipped, and nearly uniform in size, though the outer pair may sometimes be slightly shorter. The corolla is pale yellow to whitish, funnel-shaped, and approximately 1.8–2.5 cm long. Fruits are rounded capsules about 9 mm in length, splitting into four valves, and contain blackish seeds around 4.5 mm long [2].

Phytochemical screening is a preliminary analytical approach employed to detect and identify different groups of bioactive compounds present in plants. This qualitative process serves as an important first step in natural product research, providing insights into potential pharmacologically active constituents that may be further investigated for their therapeutic applications and contribution to drug discovery [3]. Structured preliminary phytochemical screening, plays a crucial role in modern pharmacognosy and phytochemistry research [4]. Gas chromatography–mass spectrometry (GC–MS) is one of the most widely used and reliable techniques for the identification and

quantification of phytochemical constituents in plant extracts. It is highly efficient and well-suited for analyzing complex mixtures of bioactive compounds [5].

This investigation focuses on assessing the physicochemical characteristics, conducting qualitative phytochemical analysis of the whole plant *Distimake quinquefolius* L. The study further aims to evaluate the ethanolic and aqueous extracts in order to identify and characterize their phytoconstituents, with detailed chemical profiling carried out through gas chromatography–mass spectrometry (GC–MS).

MATERIALS & METHODS

Collection and Authentication:

The whole plant of *Distimake quinquefolius* L. was collected from Veppampalayam village in Erode district, Tamil Nadu, India, and subsequently authenticated at the Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore. A voucher specimen (No. BSI/SRC/5/23/2024-25/Tech/365) has been deposited in the herbarium for future reference.

Chemicals:

All the reagents used were of analytical grade obtained from Modern Scientific Lab., Coimbatore.

Preparation of plant extracts:

The collected plant material of *Distimake quinquefolius* L. was thoroughly washed with water, shade-dried at room temperature, and subsequently ground into a fine powder. The powdered sample was stored in an airtight container for further analysis. A total of 75 g of the dried powder was subjected to Soxhlet extraction using ethanol and maceration using water as solvents. The resulting extracts were filtered through a vacuum filtration system and concentrated by evaporation to yield crude residues of 6.9 g (aqueous) and 8.6 g (ethanolic). The extracts were then preserved under refrigeration until further experimentation. The freshly prepared

extracts were chemically tested for the presence of different Phytoconstituents and Physicochemical analysis using standard methods [6-11].

Determination of Heavy metal analysis:

Procedure:

1. Weigh about 20 mg of the sample and place it in a Teflon digestion vessel.
2. Add 1 mL of ultrapure nitric acid to the vessel.
3. Digest the sample for approximately 45 minutes using a microwave digestion system.
4. Transfer the digested sample to a 50 mL volumetric flask and dilute to the mark with ultrapure water.
5. Prepare calibration standards ranging from 2 µg/mL to 10 µg/mL using ultrapure nitric acid, along with a blank solution.
6. Set the ICP-OES instrument with an RF power of 1.2 kW, plasma gas flow rate of 12 L/min, and nebulizer gas flow rate of 0.70 L/min.
7. Introduce the samples into the plasma via a nebulizer and spray chamber for analysis.

GC-MS Analysis:

Procedure:

The GCMS analysis was conducted by setting the autosampler to perform three rinses each with pre-solvent, solvent, and sample. Plunger speeds for suction and injection were set to high, with viscosity compensation enabled. The injection was

performed in normal mode with five pumps and no air gap. The syringe washing speed was high, with a wash volume of 6 µL.

The gas chromatograph was programmed to start the oven at 50 °C, increasing to 280 °C at 6 °C per minute, with the injector temperature at 250 °C using split injection mode. Flow control was set to linear velocity, with pressure at 68.1 kPa and a split ratio of 10.

The mass spectrometer was set with ion source and interface temperatures at 200 °C and 250 °C, respectively. A solvent cut time of 3.5 minutes was applied. Data acquisition was done in scan mode between 50 and 500 m/z, from 3 to 35 minutes.

RESULT

Physicochemical screening:

Ash Values:

The ash value determination is used to identify low-quality drugs, exhausted samples, and the presence of sandy or earthy contaminants. The recorded ash value results are presented in Table 1.

Extractive Values:

This method quantifies the number of active constituents extracted from a specific quantity of medicinal plant material using solvents. It is especially useful for materials lacking established chemical or biological assays. The accurately weighed, air-dried drug was extracted using aqueous and ethanolic solvents. The results are presented in Table 1.

Table 1: Physicochemical parameters of *Distimake quinquefolius L.*

Parameters	Values obtained (% w/w)
Ash value	
Total ash	8%w/w
Acid insoluble ash	0.5%w/w
Water soluble ash	4.5%w/w
Sulphated ash	9% w/w
Swelling index	3.5 cm
Loss on drying	9.2% w/w
Foaming index	Less than 100
Extractive value	
a) Ethanol	9.6% w/w
b) Aqueous	19.2% w/w

Determination of Heavy metal Analysis:

Table 2: Determination of heavy metal analysis

Elements	<i>Distimake Quinquefolius L.</i>
As	BDL
Cd	BDL
Hg	BDL
Pb	BDL

Note: BDL – Below Detection Limit

Standard Linearity:

Table 3: Values of Standard linearity of elements with wavelength & R² value

Element	Wavelength	R ² Value
Arsenic [As] (µg/ml)	188.980	0.9972
Cadmium [Cd] (µg/ml)	226.502	0.9964
Mercury [Hg] (µg/ml)	184.887	0.9985
Lead [Pb] (µg/ml)	220.353	0.9969

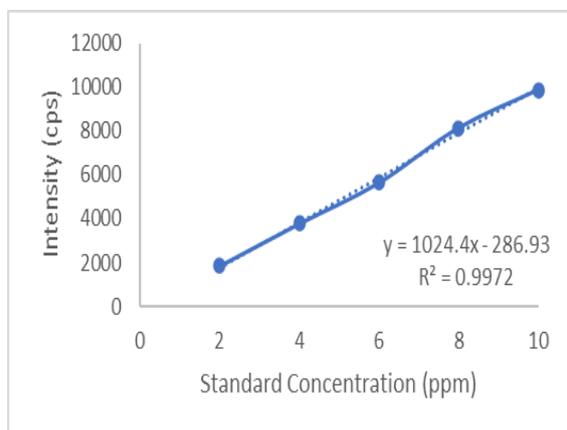


Fig 1: Linearity curve of element Arsenic

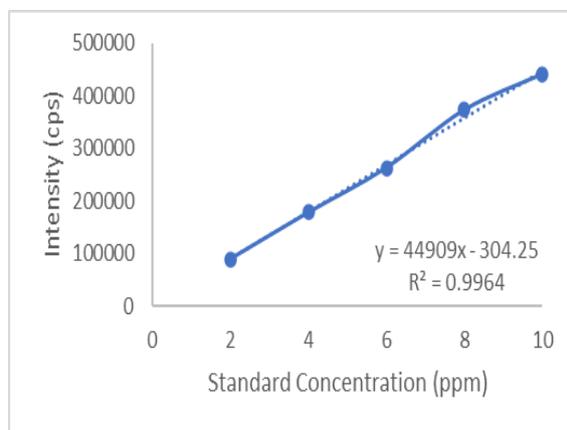


Fig 2: Linearity curve of element Cadmium

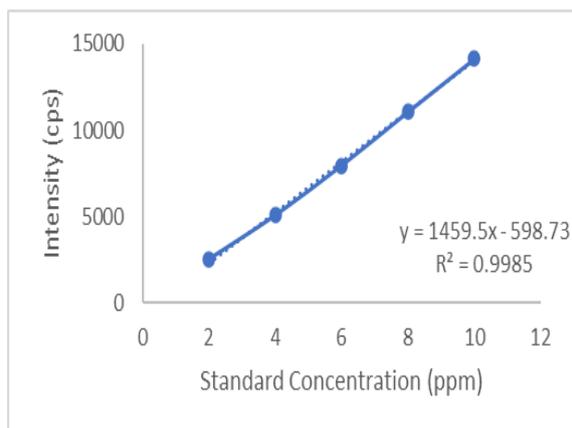


Fig 3: Linearity curve of element Mercury

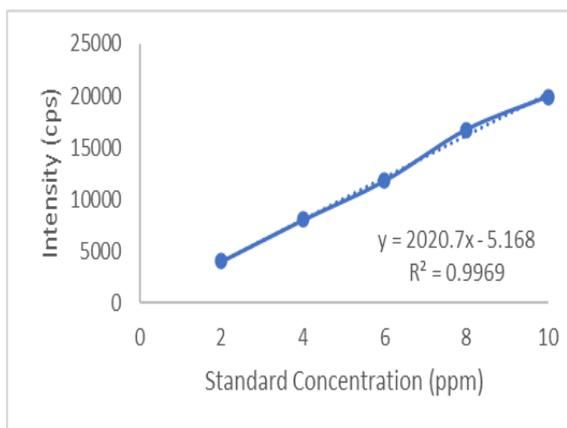


Fig 4: Linearity curve of element Lead

Distimake Quinquefolius Sample ICP-OES Graph:

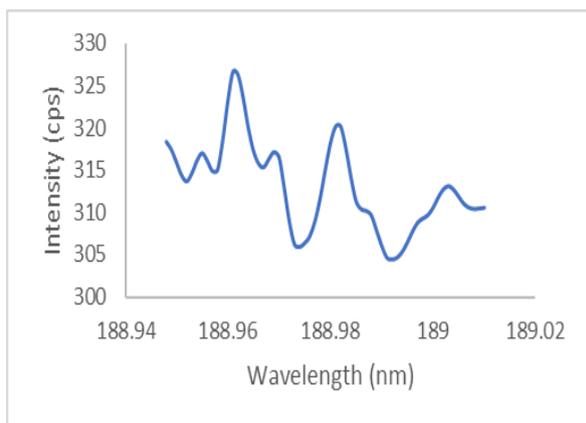


Fig 5: Linearity curve of element Arsenic

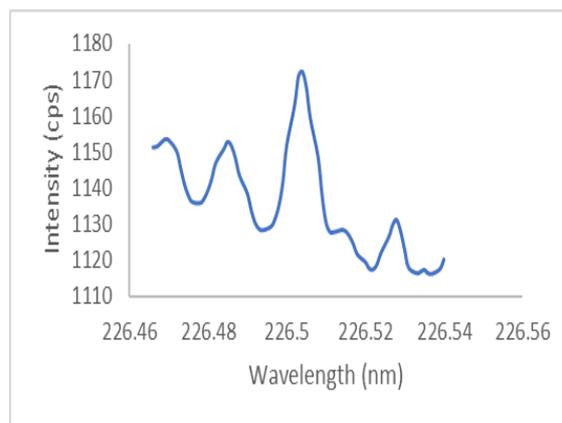


Fig 6: Linearity curve of element Cadmium

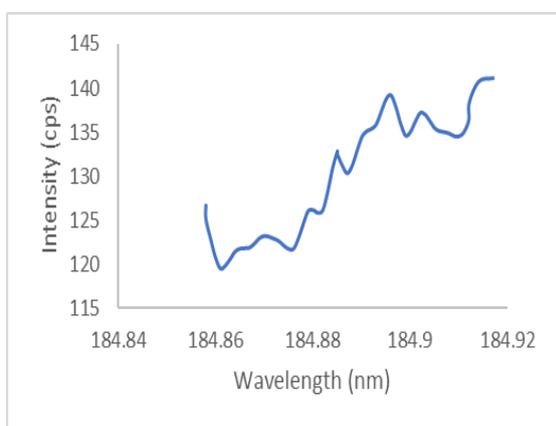


Fig 7: Linearity curve of element Mercury

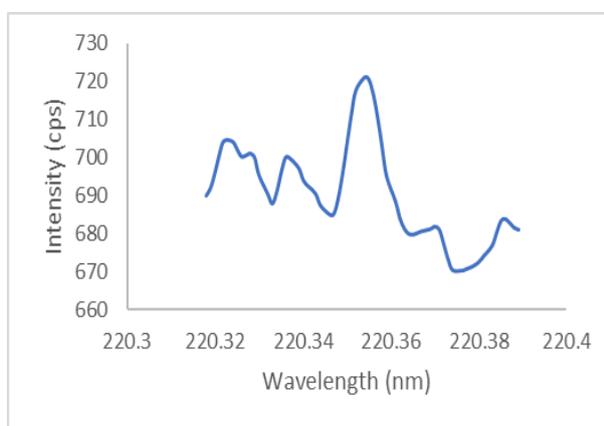


Fig 8: Linearity curve of element Lead

Phytochemical screening:

Table 4: Preliminary Phytochemical screening of *Distimake quinquefolius L.*

Phyto constituents	Ethanolic	Aqueous
Flavonoid	+	+
Tannin & Phenol	+	+
Reducing Sugar	+	+
Carbohydrate	+	+
Terpenoids	+	+
Alkaloids	+	+
Saponin	+	+
Volatile Oil	-	-
Cardiac glycoside	+	+
Steroids	+	-
Gums & Mucilage	-	-
Fixed Oils & Fats	-	-

+ Present, - Absent

GC-MS Analysis:

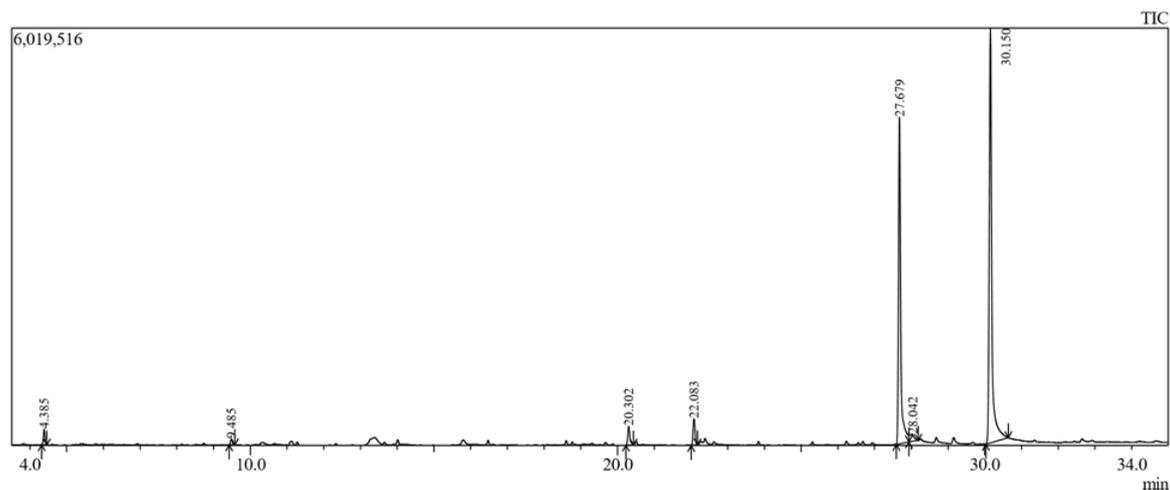


Fig 9: GC spectrum of ethanolic extract of *Distimake quinquefolius L.*

Table 5: Phytochemicals identified in ethanolic extract of *Distimake quinquefolius L.*

Peak	Retention Time	Start Time	End Time	Area	Height	A/H	Compound Name	Pharmacological Uses
1	4.385	4.317	4.458	684888	226817	3.02	Benzene, bromo-	Hypertriglyceridemia, cholesterol and treating respiratory infections
2	9.485	9.425	9.575	304710	85077	3.58	4-Vinylphenol	cytotoxic activity against MCF-7 cancer cell lines, antimicrobial effects against pathogens, insects, and fungi
3	20.302	20.225	20.433	1134721	274030	4.14	n-Hexadecenoic acid	antimicrobial, antioxidant, anti-inflammatory, and anticancer activities
4	22.083	22.008	22.175	1280663	381473	3.36	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	anticancer, anti-inflammatory, antioxidant, antiviral, and antimicrobial effects
5	27.679	27.592	27.933	20085118	4707588	4.27	14-Methylhexadecanoic acid, pyrrolidine	antifungal, antioxidant, anti-inflammatory, anticancer, antihyperglycemic activities
6	28.042	27.933	28.183	904216	93292	9.69	Cyclononasiloxane, octadecamethyl-	Synthetic compound
7	30.15	30.033	30.642	29887405	5980999	5	Nonadecanoic acid, pyrrolidine	inhibit cancer cell proliferation

DISCUSSION

The physicochemical evaluation of the crude drug indicated a total ash content of 8% w/w, reflecting a moderate level of inorganic matter. The acid-insoluble ash was 0.5% w/w, indicating minimal contamination by siliceous materials such as sand or soil. Water-soluble ash content was measured at 4.5% w/w, suggesting a significant presence of water-soluble inorganic salts important for physiological functions. The sulphated ash value slightly exceeded the total ash at 9% w/w, confirming the stability of mineral content when converted to sulphates. The swelling index was recorded as 3.5 cm, demonstrating the presence of mucilage and other hydrophilic compounds that contribute to demulcent or bulk-forming effects. Moisture content, as indicated by loss on drying, was 9.2% w/w, which is moderate and within acceptable limits, but proper storage is necessary to prevent microbial growth. The foaming index was below 100, indicating a low saponin content and minimal foaming ability. Collectively, these results suggest the crude drug has good quality with low extraneous matter, appreciable mucilage content, balanced inorganic components, and safe moisture levels, supporting its pharmacognostic suitability for medicinal applications.

The extractive value results showed that the aqueous extract had a higher yield (19.2% w/w) compared to the ethanolic extract (9.6% w/w). This suggests that water as a solvent is more effective at extracting a larger quantity of soluble compounds from the plant material, potentially including polar constituents. Conversely, ethanol, being a less polar solvent than water, may selectively extract fewer but possibly different phytochemicals. These findings highlight the influence of solvent polarity on the extraction efficiency and phytochemical profile of medicinal plants. The higher aqueous extractive value also indicates the presence of significant amounts of water-soluble constituents, which may contribute to the plant's therapeutic properties.

The analysis of the *Distimake Quinquefolius L.* sample using ICP-OES showed that toxic heavy metals such as arsenic, cadmium, mercury, and lead were present at levels below the detection capabilities of the method. This confirms the sample complies with safety guidelines regarding heavy metal contamination. The technique employed, Inductively Coupled Plasma–Optical Emission Spectroscopy, enables precise and sensitive detection of trace elements in plant extracts following sample digestion. Calibration curves and linearity data validated the method's accuracy and reliability for measuring these elements within regulatory limits. Overall, the findings indicate that the plant material is free from harmful heavy metal contamination, making it safe for use in pharmaceutical and medicinal research.

Phytochemical screening of *Distimake quinquefolius L.* highlighted a diverse array of bioactive compounds present in both ethanol and aqueous extracts. Key constituents such as flavonoids, tannins, phenols, reducing sugars, carbohydrates, terpenoids, alkaloids, saponins, cardiac glycosides, and steroids were identified in varying amounts, indicating a complex phytochemical composition that likely underpins the plant's therapeutic properties. Both extracts contained flavonoids and tannins, known for their powerful antioxidant, anti-inflammatory, and antimicrobial effects. The presence of reducing sugars and carbohydrates denotes nutritional and energy-related components within the plant. Therapeutically significant terpenoids and alkaloids were detected, which are linked to anticancer, analgesic, and antimicrobial activities. Saponins may contribute to immune enhancement and expectorant functions, while cardiac glycosides suggest possible benefits in managing heart conditions. Steroids were exclusively found in the ethanolic extract, reflecting the influence of solvent polarity on phytoconstituent extraction. Tests for volatile oils, gums, mucilage, fixed oils, and fats yielded negative results, indicating

either their absence or minimal levels in these extracts. Overall, this broad phytochemical spectrum not only supports the traditional medicinal use of *Distimake quinquefolius* L. but also informs appropriate solvent choices for future extraction and formulation efforts.

The GC-MS analysis of the ethanolic extract of *Distimake quinquefolius* L. revealed seven major phytochemical compounds, each represented by distinct peaks in the chromatogram. Among these, Benzene, bromo- was identified as an aromatic hydrocarbon known for its role in managing hypertriglyceridemia and cholesterol, although it also possesses carcinogenic potential and is used in treating respiratory infections. 4-Vinylphenol, a phenolic compound detected, exhibited strong cytotoxic activity against MCF-7 cancer cell lines and is used as a flavoring agent as well as for its antimicrobial effects against pathogens, insects, and fungi. The fatty acid n-Hexadecenoic acid (palmitic acid) was found, which is widely used in food, cosmetics, and industry and has demonstrated antimicrobial, antioxidant, anti-inflammatory, and anticancer activities. 3,7,11,15-Tetramethyl-2-hexadecen-1-ol showed diverse biological activities including anticancer, anti-inflammatory, antioxidant, antiviral, and antimicrobial effects. 14-Methylhexadecanoic acid, another fatty acid, is known for its antimicrobial and antifungal properties. Pyrrolidine, an alkaloid identified, exhibits a range of pharmacological effects such as antifungal, antioxidant, anti-inflammatory, anticancer, antihyperglycemic activities, and is utilized in neuropharmacology. Lastly, Nonadecanoic acid, also a fatty acid, is reported to inhibit cancer cell proliferation. These identified compounds underscore the therapeutic potential of *Distimake quinquefolius* L., reflecting its rich phytochemical composition with multiple bioactive properties.

CONCLUSION

The physicochemical and phytochemical analyses of *Distimake quinquefolius* L. confirm its high-quality and safe medicinal profile with balanced inorganic content and low toxic metals. The aqueous extract's higher yield suggests solvent polarity significantly affects phytochemical extraction. Diverse bioactive compounds identified, including flavonoids, alkaloids, and fatty acids, underpin the plant's broad therapeutic potential. GC-MS results highlight multiple compounds with notable anti-inflammatory, anti-cancer, and anti-microbial activities. Overall, these findings validate the plant's pharmacognostic suitability and support its traditional medicinal use and future pharmaceutical development.

Declaration by Authors

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Conflict of Interest: The authors declare no conflict of interest.

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