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Research Article

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Phytochemical Characterization and Standardization of *Tamarindus indica* L. Leaves Using Advanced Analytical Techniques

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Abstract

Tamarindus indica L is tropical tree found throughout India, belongs to family Caesalpiniaceae. Tamarind fruit has been used to prepare many food products. Tender tamarind leaves are also edible and used for souring curries, sauces, and certain beverages in many parts of India. *T. indica* leaves are rich in nutrients and phytochemicals, which contribute towards their antioxidant, anti-inflammatory, and antimicrobial properties highlight their potential as functional foods and nutraceuticals. However, limited detailed investigations have been conducted to establish comprehensive quality control parameters for leaves of *T. indica* aimed at identification and detecting adulteration employing a combination of classical and modern chromatographic techniques. Therefore, the aim of present research is to develop standardization parameters. Pharmacognostic analysis of the leaves was accomplished by microscopy study, and physicochemical analysis. Macerated ethanol extract was employed for phytochemical screening. Chromatographic profiling was carried out through gas chromatography-mass spectrometry (GC-MS) chemo profiling and high-performance thin layer chromatography (HPTLC) fingerprinting. An optimized mobile phase system was developed based on preliminary thin layer chromatography (TLC) trials, subsequently employed to generate an HPTLC fingerprint using a chloroform:ethyl acetate (3:7) solvent system. Key diagnostic microscopic features observed included anomocytic stomata, a three-layered palisade parenchyma, and pericyclic fibers. Phytochemical screening revealed the presence of proteins, amino acids, alkaloids, carbohydrates, flavonoids, phenols, tannins, and saponin glycosides. HPTLC fingerprinting detected 17 and 15 peaks at 254 nm and at 366 nm, respectively. GC-MS chemo-profiling revealed the presence of a total of three phytochemicals, Present research will be useful as standardization parameter in order to identify, and to detect adulteration in *T. indica* leaf powder, extracts and its formulation.

Keywords

Tamarindus indica, Standardization, High-performance thin layer chromatography, chromatography, Gas chromatography-mass spectrometry

Introduction

T. indica L., commonly known as the tamarind tree, belongs to the Caesalpiniaceae (Fabaceae) family and is widely valued for its traditional medicinal properties. It is extensively distributed across tropical regions of Africa, North and South America, Brazil, as well as subtropical areas like China and India. The tamarind fruit is a key ingredient in the preparation of various food products, while the tender leaves are also consumed-often used as a souring agent in curries, sauces, and beverages throughout many parts of India [1]. The leaves are rich in bioactive compounds, including polyphenols, terpenoids, steroids, flavonoids, and saponins [2, 3]. Tamarind leaves are known for their strong antioxidant properties, largely due to their rich content of phenolic compounds and flavonoids. Research

has shown that these leaves effectively neutralize free radicals and help alleviate oxidative stress. Antioxidant potential has been validated through various *in vitro* assays, including 2,2-Diphenyl-1-picrylhydrazyl free radical scavenging and ferric reducing antioxidant power assays [4]. Tamarind leaves are rich in proteins, fats, fiber, and essential vitamins such as thiamine, riboflavin, niacin, ascorbic acid, and β-carotene, making them a nutritious addition to the diet. They also contain substantial amounts of flavonoids and other polyphenolic compounds, which are well known for their beneficial health effects [5]. In traditional medicine, tamarind leaves have been employed for their antimicrobial, analgesic, and anti-inflammatory effects [6]. These properties are attributed to the presence of lupeol, a triterpene known for its therapeutic benefits [7]. Thus, tamarind leaves are a rich source of nutrients and phytochemicals, highlighting their potential use as both food and nutraceuticals. Despite this, limited research has been conducted to establish quality control standards for *T. indica* leaves, whether in powdered form or as an extract, for use in formulations. HPTLC enables the simultaneous detection of multiple phytoconstituents, making it a preferred method for establishing identity, purity, and batch-to-batch consistency of polyherbal formulation. The integration of GC-MS in herbal standardization facilitates chemotaxonomic classification, detection of adulterants, and elucidation of bioactive markers, thereby supporting therapeutic efficacy and regulatory acceptance. Therefore, the aim of the present study is to develop standardization parameters. This includes pharmacognostic evaluation through microscopic examination, physicochemical analysis, phytochemical screening, HPTLC fingerprinting and GC-MS chemoprofiling of the leaves.

Materials and Method

Collection and authentication

The leaves of *T. indica* were collected from the herbal garden at school of pharmacy, RK University, Gujarat, India, during the month of December 2024. Herbarium was prepared and authentified by botanist at school of science, RK University and further a voucher specimen was deposited at school of pharmacy, RK University with herbarium Number SOP/COG/12/2025 (Figure 1).

Pharmacognostic study

Pharmacognostic study of samples of *T. indica* leaves were performed by taking transverse sections and determining leaf constants. Essential leaf constants, such as the stomatal index, stomatal number, vein islet count, and vein islet termination number and palisade ratio, were evaluated through camera lucida (mirror type)-assisted microscopic examination [8].

Drying and extraction

The fresh leaves were dried by placing them in a hot air oven set at 40 °C for a duration of 24 h. 40 gm of dry powder from *T. indica* leaves were ultra-sonicated for 1 h with methanol and further macerated for 23 h at room temperature. The resulting extract was then evaporated using a water bath maintained at temperatures below 50 °C. This dried extract was utilized for phytochemical screening, TLC, HPTLC, and GC-MS analysis.



Figure 1: Leaves of *T. indica.*

Fluorescence analysis

Subsequently, the consistency, percentage yield, and fluorescence characteristics of methanol extract of *T. indica* leaves were examined under daylight and at 366 nm UV light. These fluorescence evaluations may aid in confirming the purity of the drug [9].

Physico-chemical investigations

The dried leaves *T. indica* in powdered form were utilized to assess various parameters, including moisture content, ash values, foaming index, hot water extractive value, cold water extractive value, alcohol extractive value, and swelling index [10].

Moisture content

The moisture content of *T. indica* leaf powder was assessed using a standard gravimetric approach [10]. Accurately weighed 5 g of the powdered material was dried in a hot air oven maintained at 100 - 105 °C until a constant weight was achieved. All measurements were conducted in triplicate, and results are reported as mean percentage (% w/w) ± standard error of the mean (SEM).

Ash values

Physicochemical ash parameters, including total ash, acid-insoluble ash, and water-soluble ash, were evaluated following the guidelines of the World Health Organization (WHO)

[10]. For total ash determination, 2 g of powdered leaves were incinerated in a pre-weighed silica crucible at 500 - 600 °C using a muffle furnace until a uniform, carbon-free ash was obtained. Acid-insoluble ash was calculated by boiling the ash in 25 mL of diluted hydrochloric acid for 5 min, filtering the mixture, and drying the residue retained on the filter paper. For water-soluble ash, the ash was boiled with 25 mL of distilled water, followed by filtration. The weight of the insoluble matter was subtracted from the total ash to compute the water-soluble fraction. Ash values were expressed as mg/g of air-dried material and reported as mean % w/w ± SEM based on triplicate analysis.

Extractive values

Hot water, cold water, and alcohol-soluble extractive values were determined in accordance with WHO protocols [10]. In the hot water extractive test, 4 g of leaf powder was refluxed with 100 mL of distilled water for 1 h. After cooling, the volume was readjusted to 100 mL, filtered, and 25 mL of the filtrate was evaporated to dryness on a water bath. The residue was weighed to estimate the extractive yield. For the cold-water method, 4 g of sample was macerated with 100 mL of water for 24 h - shaken intermittently during the first 6 h and left undisturbed for the remaining 18 h. The filtrate (25 mL) was evaporated, and the residue was weighed. Alcohol-soluble extractives were determined using the same procedure, with methanol replacing water. All values were obtained in triplicate and are presented as mean % w/w ± SEM.

Swelling index

One gram of powdered *T. indica* leaves was introduced into a 25 mL graduated cylinder. The initial volume was recorded, followed by the addition of 25 mL of distilled water. The mixture was shaken every 10 min for 1 h and then left undisturbed for 3 h at room temperature. The final volume was noted, and the swelling index was calculated by subtracting the initial volume from the final volume. Each test was performed in triplicate, and results are presented as mean ± SEM.

Foaming index

To determine the foaming index, 1 g of leaf powder was boiled with 100 mL of distilled water in a 500 mL conical flask for 30 min. After cooling and filtering, the volume was adjusted to 100 mL. Serial volumes ranging from 1 to 10 mL of the decoction were transferred into separate stoppered test tubes and diluted to 10 mL with distilled water. Each tube was shaken vigorously for 15 s and left to stand for 15 min. The foam height was measured, and the foaming index was established based on the height of the stable foam formed.

Preliminary phytochemical screening

Preliminary phytochemical screening of *T. indica* leaves was conducted using methanol extract, and the presence of various phytochemical classes were determined through standard qualitative chemical tests as outlined in established protocols [11, 12].

TLC analysis

TLC was conducted in accordance with the procedure established by Harborne [13]. The mobile phase was developed using a methanol extract. Approximately 50 pilot TLC plates were prepared with the methanol extract, utilizing various solvent combinations in differing proportions, such as chloroform, methanol, acetic acid, ammonia and ethyl acetate. The pre-coated aluminum plates with silica gel F_{254} served as the stationary phase. A working solution of the extract was prepared at a concentration of 2 mg/mL and applied onto TLC plates. After chromatographic development using the selected mobile phase, the plates were air-dried under ambient conditions. Detection was performed under UV light at 366 nm, and the migration of phytoconstituents was evaluated by calculating their retention factor (R_f) using the following equation:

$$R_f = \frac{Distance\ travelled\ by\ solute}{Distance\ travelled\ by\ solvent}$$

HPTLC fingerprinting

An HPTLC fingerprint profile was established for the methanolic leaf extract of *T. indica*. The sample was prepared by reconstructing the dried methanol extract in methanol to achieve a final concentration of 2 mg/mL. A volume of 10 µL of the prepared solution was applied onto a 10 × 10 cm aluminum-backed HPTLC plate pre-coated with silica gel 60 F_{254} (0.2 mm thickness; E. Merck KGaA Pvt. Ltd., India). Chromatographic application was performed using the CAMAG LINOMAT 5 automated sample applicator. Plate development was carried out in a mobile phase system comprising chloroform and ethyl acetate in a 3:7 ratio. Post-development, the plates were air-dried and subsequently scanned at 254 nm and 366 nm using the CAMAG TLC Scanner 3. R_f values were recorded for the resolved phytochemicals [14].

Metabolite-profiling by GC-MS analysis

GC-MS was employed to chemically profile the methanol extract of *T. indica* leaves. The analysis was carried out at the Bioresearch and Characterization Centre, RK University, Gujarat, India. The GC-MS system comprised an agilent 7820A gas chromatograph coupled with an Agilent 5977B mass selective detector (MSD), utilizing an HP-5 nonpolar capillary column (30 m × 0.32 mm i.e., 0.25 µm film thickness) composed of 5% phenyl methyl polysiloxane. Helium was used as the carrier gas at a constant flow rate of 1.2 mL/min. A 1 µL aliquot of the sample was introduced in split mode at a 10:1 ratio. The mass spectrometer operated in scan mode, covering a mass range of 50 - 650 m/z. Compound identification was performed by comparing the acquired spectra with those in the National Institute of Standards and Technology database. Furthermore, a detailed literature review was conducted to investigate the pharmacological relevance of the identified phytochemicals.

Results and Discussions

Pharmacognostic study

Morphology description of *T. indica* leaves

The leaves observed were alternate and compound, comprising 10 - 18 pairs of opposite leaflets. Each leaflet displayed an oblong shape with an obtuse base and apex, and smooth, entire margins. The venation pattern was pinnate, and the leaves exhibited green coloration.

Microscopic study of *T. indica* leaves

The transverse section and surface preparation studies are illustrated in figure 2 and figure 3, respectively. Analysis of the surface of *T. indica* leaves indicated the presence of anomocytic stomata, epidermal cells with straight walls, and unicellular trichomes that provide coverage. The transverse section of *T. indica* leaves reveals a lamina that displays a dorsiventral structure, characterized by an upper epidermis made up of a single layer of rectangular cells. Below this layer, the palisade parenchyma is organized into three tightly packed layers, extending over the midrib area. The vascular bundle consists of lignified xylem paired with non-lignified phloem. Sclerenchyma is intermittently located between the vascular bundle and the parenchyma. The parenchyma itself is loosely arranged in three to four layers. The lower epidermis has a structure that closely mirrors that of the upper epidermis.

Quantitative microscopy of *T. indica* leaves

The constants associated with the leaves of *T. indica* are summarized in table 1. The stomatal counts were determined to be between 204 and 208, and the stomatal indices were observed to range from 20.76 - 21.89. Furthermore, the counts for vein islets and vein terminations were recorded as 64 - 92 and 12 - 56, respectively, while the palisade ratio exhibited a variation from 4.66 - 6.

Phytochemical screening of leaves of *T. indica*

Preliminary phytochemical analysis provides insights into the various classes of phytoconstituents found in the leaves of *T. indica*, which contribute to its therapeutic properties. The findings from this analysis are summarized in table 2. The screening identified the presence of alkaloids, flavonoids, phenolics, carbohydrates, coumarin glycosides, tannins and saponins. The occurrence of these phytochemical classes in the leaf matrix may contribute to the observed pharmacological activities. Several flavonoid compounds have been extensively associated with diverse therapeutic effects such as antioxidants, hypotension, bleeding piles, hepatoprotective, and diabetes are well documented [15]. Certain polyphenolic compounds are known to suppress pathogenic bacterial growth, while others support the proliferation of beneficial gut microbiota. Therefore, the presence of these phytochemical classes, along with traditional ethnomedicinal claims associated with *T. indica* leaves, provides a meaningful correlation between traditional usage and potential therapeutic efficacy.

Fluorescence analysis of *T. indica* leaves

The methanol extract of *T. indica* leaves was evaluated for its physical consistency, extraction yield, and fluorescence characteristics. Fluorescence behavior of the powdered leaf methanol extracts was observed under visible light and ultraviolet illumination at 366 nm. The extraction process yielded 12% w/w of methanol-soluble constituents. Consistency of *T. indica* leaves was found sticky. The color of the *T. indica* leaf methanol extract was light green in daylight and reddish brown at 366 nm UV. To identify and authenticate an extract made from *T. indica* leaves, fluorescence analysis might be utilized.

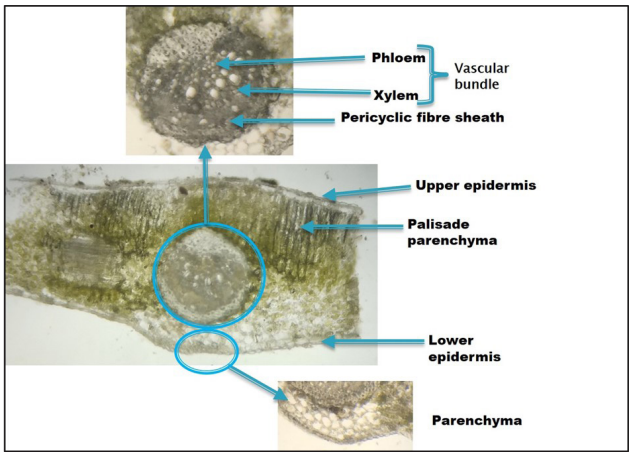


Figure 2: Transverse section of *T. indica* leaves.

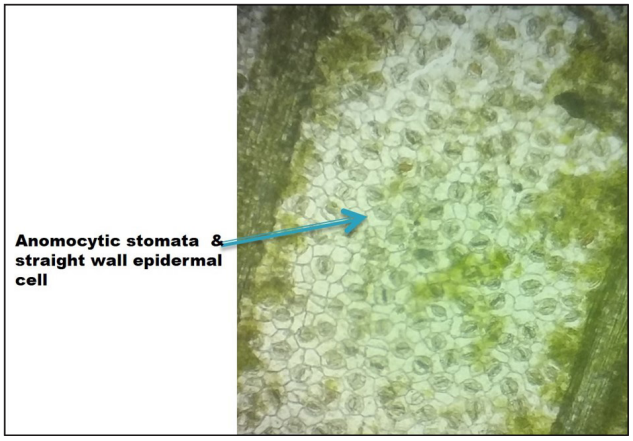


Figure 3: Surface preparation of *T. indica* leaves.

Table 1: Quantitative microscopy of *T. indica* leaves.

S no.	Leaf constant	Values
1	Stomatal no.	204 - 248
2	Stomatal index	20.76 - 21.89
3	Vein islet number	64 - 92
4	Vein termination no	12 - 56
5	Palisade ratio	4.66 - 6

Table 2: Phytochemical screening of *T. indica* leaves methanol extract.

S no	Phytochemical class	Result
1	Carbohydrates	+
2	Alkaloids	+
3	Phytosterols	-
4	Cardiac glycosides	-
5	Proteins and free amino acids test	+
6	Flavonoids	+
7	Terpenoids	-
8	Phenolic compounds	+
9	Tannins	+
10	Saponin glycosides	+
11	Fixed oil	-
12	Cyanogenic glycoside	-
13	Anthraquinones	-
14	Coumarin glycoside	-

Note: + indicates positive test and - indicates negative test.

Physico-chemical investigation of *T. indica*

In accordance with WHO guidelines, physicochemical analysis of powdered *T. indica* leaves was conducted [13]. The outcomes of physicochemical evaluations, including moisture content, total ash, acid-insoluble ash, water soluble ash, foaming index, water and alcohol extractive values, and swelling index, are summarized in table 3.

Table 3: Physicochemical analysis of leaves of *T. indica*.

S no.	Physicochemical parameters	Value % w/w
1	Loss of drying	8.55 ± 0.26
2	Total ash	15 ± 0.5
3	Acid insoluble ash	4.6 ± 0.15
4	Water soluble ash	2.7 ± 0.02
5	Alcohol extractive value	14.40 ± 0.42
6	Hot water extractive Cold water extractive	28.47 ± 0.32 32.23 ± 0.47
7	Swelling index	3 ± 0.30
8	Foaming index	< 100

Note: Values are mean % w/w of air-dried drugs ± SEM, where n = 3.

Mobile phase development by TLC of leaves of *T. indica*

Methanol extracts of *T. indica* leaves were obtained through maceration and subsequently subjected to TLC for the optimization of an appropriate mobile phase, intended for subsequent HPTLC fingerprinting. Various solvent system compositions were systematically evaluated to achieve optimal resolution, reproducibility, and minimal spot tailing. Approximately 50 TLC trials were conducted to identify a mobile phase capable of providing maximal compound separation. The optimized solvent system for the methanol extract of *T. indica* leaves was determined to be chloroform:ethyl acetate in a 3:7 ratio. TLC analysis of the methanol extract of *T. indica* leaves revealed four distinct spots with R_f values of 0.32, 0.47, 0.87, and 0.94 under UV light at 366nm, as illustrated in figure 4. This mobile phase was selected for further application in HPTLC fingerprint development.

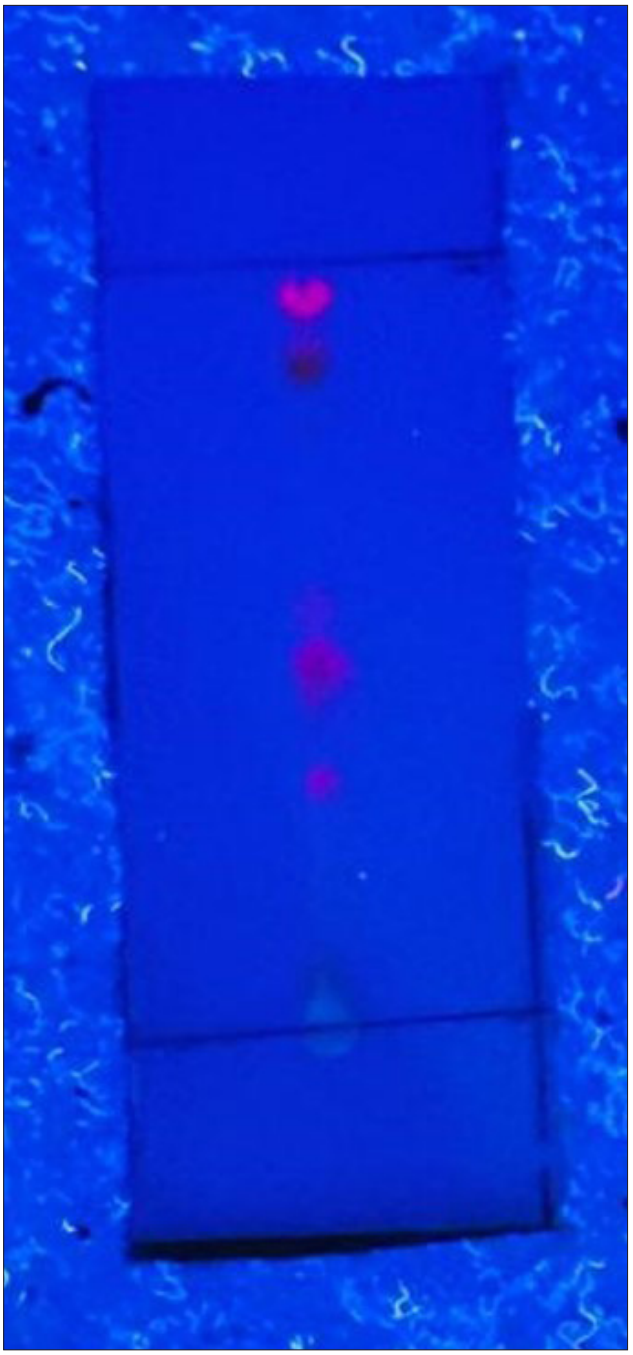


Figure 4: TLC of methanol extract of leaves of *T. indica*; chloroform: ethyl acetate (3:7).

HPTLC fingerprinting profile

HPTLC serves as a reliable, sensitive, and precise analytical approach for the development of chromatographic fingerprints and marker-based standardization of herbal drugs. It is widely employed not only for the identification and quantification of phytoconstituents, and assessment of purity, but also for evaluating parameters such as stability, dissolution behavior, and content uniformity in both raw botanical materials and formulated herbal products. Since 1991, the WHO has recognized chromatographic fingerprinting as a valid method for the identification and quality control of medicinal plants [16, 17]. HPTLC also plays a critical role in the detection of adulteration in plant materials, alcoholic extracts, herbal formulations, and crude drugs [18-20]. In the present study, HPTLC fingerprinting was carried out for the methanol leaf extract of *T. indica* utilizing a mobile phase composed of chloroform and ethyl acetate (3:7). At a detection wavelength of 254 nm, seventeen distinct peaks were observed (Figures 5 and figure 6, table 4), while fifteen peaks were detected at 366 nm under UV illumination (Figures 5 and figure 7, table 5). The resulting HPTLC profile using this solvent system demonstrates its potential utility for the authentication and quality assessment of *T. indica* leaves, their methanol extracts, and related alcoholic preparations. HPTLC fingerprinting of *T. indica* can serve as an indispensable tool to detect adulteration and identify methanol extract of *T. indica* leaves.

Metabolite-profiling by GC-MS Analysis

GC-MS is a widely employed analytical technique for the qualitative and quantitative analysis of volatile and semi-volatile organic constituents within complex matrices of plant materials [21-24]. In the present investigation, GC-MS was utilized to conduct comprehensive chemo-profiling of the methanol extract obtained from the leaves of *T. indica*. The

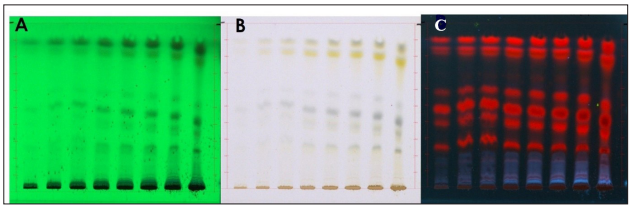


Figure 5: HPTLC fingerprinting of methanol extract of *T. indica* leaves at **(A)** 254 nm, **(B)** in daylight, and **(C)** at 366 nm.

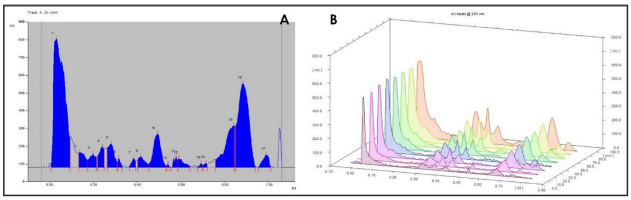


Figure 6: HPTLC **(A)** 2D, and **(B)** 3D chromatogram of *T. indica* leaves methanol extract at 254 nm.

Table 4: R_f value and area under curve (AUC) of methanol extract of *T. indica* leaves at 254 nm.

Peak no.	R_f	AUC	Area %
1	0.03	34084	37.64
2	0.14	2366.5	2.61
3	0.2	2034.4	2.25
4	0.24	2407	2.66
5	0.28	3753.2	4.14
6	0.32	602.5	0.67
7	0.39	797.9	0.88
8	0.42	1751.8	1.93
9	0.49	6211.1	6.86
10	0.52	157.8	0.17
11	0.58	857.7	0.95
12	0.59	1301.1	1.44
13	0.69	255.6	0.28
14	0.71	317.7	0.35
15	0.84	9601.2	10.6
16	0.88	22154.3	24.47
17	0.89	1899.1	2.1

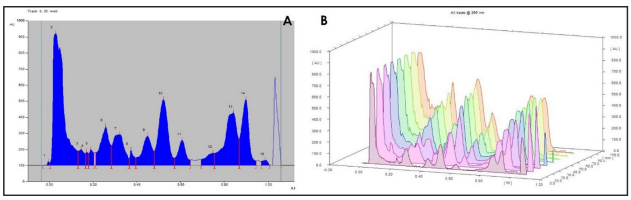


Figure 7: HPTLC **(A)** 2D, and **(B)** 3D chromatogram of *T. indica* leaves methanol extract at 366 nm.

outcomes comprising peaks, names of phytoconstituents, area percentages, retention time (RT), and individual fragmentation patterns-are presented in table 6. A total of three phytoconstituents were identified in the methanol extract of *T. indica* leaves. All of them, Butanedioic acid, 2,2-dihydroxy, dimethyl ester; Hexadecanoic acid, methyl ester; and 9,12,15-octadecatrienoic acid, methyl ester has been reported to possess notable pharmacological and therapeutic activities. Butanedioic acid, 2,2-dihydroxy, dimethyl ester accounted for

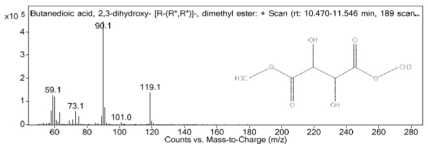
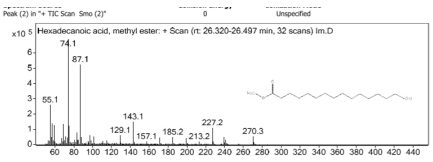
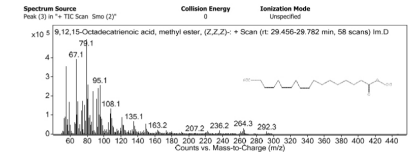
Table 5: R_f value and AUC of methanol extract of *T. indica* leaves at 366 nm.

Peak no.	R_f	AUC	Area %
1	-0.00	203.6	0.16
2	0.03	38174.5	29.87
3	0.15	2441.5	1.91
4	0.17	863.7	0.68
5	0.19	1710.7	1.34
6	0.26	9246.5	7.23
7	0.32	9335.7	7.30
8	0.37	1537.2	1.20
9	0.45	7734.2	6.05
10	0.52	16638.4	13.02
11	0.61	5328.9	4.17
12	0.75	2885.4	2.26
13	0.84	17872.9	13.98
14	0.90	13054.3	10.21
15	0.99	788.8	0.62

58.66% of the area and is recognized for its pharmacological effects, including pesticide and anticancer activities and as a food flavoring agent [25]. Hexadecanoic acid, methyl ester, constituted 33.08% of the total area and is known for its antioxidant, insecticidal, and hemolytic activities, along with its role in cholesterol regulation [26]. The phytochemical 9,12,15-octadecatrienoic acid, methyl ester showed the highest area percentage at 100% and is valued for its anti-inflammatory, hepatoprotective, and anticancer potential [25]. Based on the GC-MS findings and supporting literature, it is evident that the identified phytoconstituents, which appear in significant concentrations, exhibit antioxidant, anticancer, anti-inflammatory, and hemolytic properties. Preliminary phytochemical analysis confirmed the presence of flavonoids, tannins, and polyphenolic phytochemical class, all of which are well-documented for their antioxidant potential [27-31], while as per our review of literature for pharmacological studies on *T. indica* [6], it was revealed that the plant possesses significant antioxidant potential. Consequently, GC-MS analysis facilitated the identification of bioactive constituents, thereby substantiating the antioxidant potential attributed to *T. indica*. The GC-MS chromatographic profile of the methanol leaf extract is presented in figure 8. Overall, present GC-MS chemo-profiling can serve as an essential step toward understanding the phytochemical profile of *T. indica* leaves. Moreover, present investigation of GC-MS analysis can be used as a valuable tool for identification of alcoholic extract of *T. indica* leaves.

HPTLC and GC-MS are critical tools in the standardization and quality evaluation of herbal raw materials and formulations. HPTLC enables the development of characteristic chromatographic fingerprints that facilitate the identification, authentication, and detection of adulteration in plant-derived substances. It supports qualitative and semi-quantitative estimation of phytoconstituents and aligns with WHO guidelines recommending fingerprinting as a reliable approach for herbal drug standardization [11, 32]. In contrast, GC-MS provides molecular-level chemoprofiling by identifying volatile and semi-volatile phytochemicals based on their RT and mass fragmentation patterns. This technique is particularly valuable

Table 6: Phytoconstituents identified in *T. indica* by GC-MS analysis.

Peak no.	Phytochemical	RT	Area	Area %	Counts vs./z of peak
1	Butanedioic acid,2,2-dihyroxy, dimethyl ester	11.483	70578010.42	58.66	
2	Hexadecanoic acid, methyl ester	26.165	39802485.89	33.08	
3	9,12,15-octadecatrienoic acid, methyl ester	29.393	120314045.3	100	

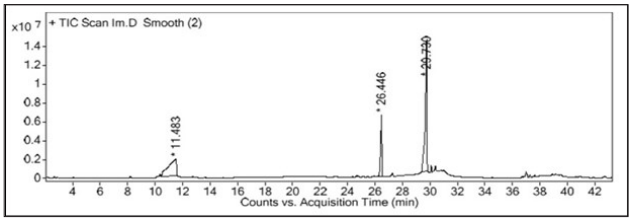


Figure 8: GC-MS Chromatogram of *T. indica* leaves methanol extract.

for detecting terpenoids, fatty acids, esters, phenolics, and other bioactive compounds in trace concentrations, contributing to marker-based standardization and pharmacological correlation [33-35]. When used together, HPTLC and GC-MS offer a synergistic and comprehensive phytochemical assessment framework, essential for ensuring the reproducibility, efficacy, and safety of herbal products.

Conclusion

The present study establishes comprehensive standardization parameters for *T. indica* leaves, offering scientifically validated identification criteria critical for maintaining the quality, safety, and authenticity of herbal materials. The successful development of a preliminary monograph encompassing both raw leaves and their alcoholic extracts positions this botanical as a viable candidate for regulated inclusion in pharmacopoeial standards. By integrating macroscopic, microscopic, physicochemical, and phytochemical evaluations with advanced techniques such as HPTLC fingerprinting and GC-MS chemoprofiling, this investigation lays a robust foundation for future pharmacognostic and phytopharmaceutical studies. These chromatographic profiles not only facilitate the identification and quantification of bioactive constituents but also serve as essential tools for detecting adulteration and ensuring batch-to-batch consistency in herbal formulations. Ultimately, the findings contribute significantly to the standardization and quality assurance frameworks required by the herbal industry and regulatory authorities, thereby enhancing the therapeutic reliability of *T. indica*-based products and promoting public health protection.

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None.

Conflict of Interest

None.

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