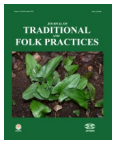
Volume 11 (01 & 02) December 2023, 01-09
ISSN 2278- 5906
http://jtfp.jntbgri.res.in



Journal of Traditional and Folk Practices

Pharmacognostic standardization, phytochemical profiling and *in vitro* antioxidant evaluation of *Tinospora crispa* (L.) Hook.f. & Thomson leaves

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Received: 10 July 2023 Accepted: 12 November 2023

Abstract

Tinospora crispa (L.) Hook.f. & Thomson (Menispermaceae) is a traditional medicinal plant renowned for its therapeutic properties in addressing various inflammatory disorders associated with the immune system. This study aims to provide a comprehensive evaluation of *T. crispa* leaves, encompassing macro-microscopic analysis and physiochemical profiling. The macro-microscopic assessment revealed distinctive features, including the presence of anomocytic stomata with a spacious air chamber in the lower epidermis of the lamina, the absence of spongy tissues, a collenchymatous hypodermis succeeded by a parenchymatous cortex in the midrib and a dome-shaped arrangement of sclerenchyma cells. Furthermore, chemical reactions of *T. crispa* leaf powder with diverse reagents led to characteristic colour changes visible under both normal light and UV light. The leaf extract displayed remarkable antioxidant activity, exhibiting inhibition percentages of 35%, 55%, 62%, 58% and 53% at concentrations of 20, 40, 60, 80 and 100 µg/ml respectively. The outcomes of this study contribute to the differentiation and identification of *T. crispa* leaves from closely related species, even when they are in crushed or powdered forms.

Keywords: Ash value, Foaming index, Physicochemical evaluation, Swelling index

1. Introduction

Traditional medicine, defined by World Health Organization (WHO, 2004) incorporates diverse cultural practices, notably India's ancient medical system. In India, healthcare is segmented into classical and oral systems. The former comprises Ayurveda, Yoga, Siddha, Homeopathy, Unani and Amchi, while the latter encompasses traditional knowledge, ethnomedicine, folk medicine and similar practices. These traditional medicines are economically accessible, especially benefiting rural communities in developing nations (Popovic *et al.,* 2016).

The evolution of medicinal plant usage has advanced from basic formulations to the isolation, identification and evaluation of bioactive compounds for drug discovery. Despite this progress, the absence of proper documentation and the implementation of rigorous quality control measures remain significant obstacles in the utilization of herbal medicines. Addressing this challenge, ensuring quality assurance through the standardization of drugs using diverse pharmacognostic parameters such as macro-microscopic, physicochemical and phytochemical assessments has become imperative (Wallis, 1985). These investigations play a pivotal role in authenticating plants and ensuring the uniform quality of herbal products, ultimately enhancing the safety and efficacy of natural remedies.

Tinospora crispa (L.) Hook.f. & Thomson, a traditional medicinal plant belonging to the Menispermaceae family, shares a close relationship with *Tinospora cordifolia* (Willd.) Hook.f. & Thomson, a plant widely used in Ayurveda to address a range of ailments. In various cultural traditions, including those of Thailand, Malaysia, Guyana, Bangladesh and the southern Indian province of Kerala, traditional healers incorporate *T. crispa* into their therapeutic practices to alleviate a variety of maladies (Thomas *et al*., 2016). Traditional uses of the plant span the treatment of conditions such as rheumatism, fever, backaches, muscle pain, abdominal discomfort, diabetes, internal inflammations, as well as its use as a tonic for enhancing overall well-being (Ahmad *et al*., 2016; Haque *et al*., 2017). Traditional healers in Kerala employ a leaf decoction of *T. crispa* for the treatment of diabetes mellitus. *T. crispa* possesses a broad range of pharmacological activities such as anti-inflammatory, antioxidant, immunomodulatory, cytotoxic, antimalarial, cardioprotective and anti-diabetic activities (Ahmad *et al*., 2016).

2. Materials and methods

2.1. Plant material

Fresh leaves of *T. crispa* were collected from its natural habitats of Nedumangad, Thiruvananthapuram, Kerala. Voucher specimens were deposited at Sree Narayana College Herbarium (SNCH 4734).

2.2. Methods

2.2.1. Macroscopic and organoleptic characterization: For the morphological analyses, fresh leaves of *T. crispa* were gathered and subjected to an examination of both qualitative and quantitative traits. The subsequent macroscopic and organoleptic characteristics of the fresh leaves were documented: size and shape, colour, surface properties, venation pattern, presence or absence of a petiole, characteristics of the apex, margin, base and lamina, as well as texture, odour and taste.

2.2.2. Microscopic characterization

(a) Anatomical studies of the leaf: Fresh leaf lamina and midrib sections were prepared by free-hand transverse cutting and staining with safranin. The stained sections were then mounted on glass slides using glycerine and examined under a light microscope equipped with a camera attachment and photomicrographs were captured (Trease and Evans, 2002).

(b) Quantitative leaf microscopy: The following quantitative microscopic studies of *T. crispa* leaf were carried out according to standard procedures.

(c) Determination of stomatal number and stomatal index: A leaf segment was subjected to a clearing process involving boiling with a sodium hypochlorite solution. The upper and lower epidermal layers were then individually peeled off. These peeled epidermal layers were set aside and subsequently mounted using glycerine water. The average stomatal count per square millimetre of the leaf's epidermis (stomata density) was calculated based on microphotographs taken using a microscope with an attached camera. This calculation was conducted separately for the upper and lower epidermis layers (Anonymous, 2011), employing the following equation:

Stomatal index (SI) = S×100/E + S (*Where, S= the number of stomata per unit area and E= the number of epidermal cells in the same unit area of the leaf*)

2.2.3. Powder analysis: Fresh leaves were isolated from the gathered plants, meticulously cleansed using fresh water and subsequently subjected to shade drying before being finely powdered. This leaf powder was then subjected to boiling with chloral hydrate for a duration of 5 to 10 minutes. Following this, staining was performed utilizing a solution composed of safranine, glycerine and iodine. This staining procedure aimed to ascertain the existence of lignified cells, calcium oxalate crystals and starch grains within the leaf sample (Khandelwal, 2002).

2.2.4. Fluorescence analysis: The fluorescence character of the leaf powder (40 mesh) was studied both in day light and UV light (254 and 366 nm) after treatment with reagents like sodium hydroxide, acetic acid, hydrochloric acid, nitric acid, iodine, ferric chloride, sulphuric acid, potassium dichromate, etc. The colour changes were noted.

2.2.5. Physicochemical analysis: Different physicochemical parameters of *T. crispa* leaf powder was determined according to the quality control methods for medicinal plant materials.

(a) Determination of pH: pH of 1% solution: Dissolved 1g of the leaf powder in 100 mL of distilled water, filtered and checked pH of the filtrate with a standardized glass electrode.

pH of 10% solution: Dissolved 10 g of the leaf powder in 100 mL of distilled water, filtered and checked pH of the filtrate with a standardized glass electrode.

(b) Determination of alcohol soluble extractive: Macerated 5 g of air-dried drug coarsely powdered with 100 mL of ethanol of specified strength in a closed flask for 24 h, shaking frequently during 6 h and allowed to stand for 18 h. Filtrate was evaporated to dryness in a tared flat bottom shallow dish, dried at 105°C and weighed. The percentage of alcohol- soluble extractive was calculated with reference to the air dried drug.

(c) Determination of water-soluble extractive: Followed as directed for the determination of alcohol soluble extractive using water instead of ethanol.

(d) Determination of petroleum ether soluble (40-60°C) extractive: Proceeded as directed for the determination of alcohol soluble extractive, using petroleum ether (40 – 60°C) instead of ethanol.

(e) Loss on drying: About 2-3 g of powder was accurately weighed in a China dish and kept in a hot air oven maintained at 105°C for 5 h. After cooling in a desiccator, the loss in weight was recorded. This procedure was repeated till constant weight was obtained.

Loss on Drying (LOD)% = Loss in weight/Weight of the drug (g) ×100

(f) Swelling Index: Drug powder (1g) was taken in a measuring cylinder (25 mL) and suspended in 25 mL distilled water for 1h by thorough mixing every 10 min. After 3 h, volume in ml occupied by the plant material including any sticky mucilage was measured. The experiment was repeated three times for accuracy and the swelling index was calculated.

(g) Foaming index: Finely divided crude drug (1g) was transferred into a 500 mL flask containing 100 mL of boiling water. The mixture was maintained at moderate boiling for 30 min. The mixture was cooled and filtered in to a 100 mL volumetric flask and added sufficient water to the filtrate to dilute the volume. The prepared decoction was poured in to 10 stoppered test tubes each 1 mL, 2 mL…….10 mL. The volume of the liquid in each tube was adjusted (10 mL) with water. The tubes were duly stoppered and shaken then in a lengthwise motion for 15 sec (two shakes per second) and allowed to stand for 15 min. The foam height in each tube was measured.

Foaming index=1000/a (*Where, 'a' is the volume of the plant decoction for forming foam of height 1 cm)*

(h) Determination of total ash: About 2-3 g of crude drug powder were weighed and placed in a tared silica dish. The powder was then scattered on the bottom of the dish and incinerated by gradually increasing the heat, ensuring it did not exceed a dull red heat, until it was free from carbon. After cooling, the dish with the incinerated powder was weighed. The percentage weight -to-weight (% w/w) of total ash was calculated with reference to the air-dried drug.

(i) Determination of acid insoluble ash: The ash was boiled with 25 ml of diluted hydrochloric acid for 5-10 min; collect the insoluble matter in a Gooch crucible, washed it with hot water, ignited and weighed it. The percentage of acid-insoluble ash with reference to the air -dried drug was conducted. Finally, determine the % w/w of acid-insoluble ash with reference to the air-dried drug was determined.

(j) Determination of water-soluble ash: To the crucible containing the total ash, 25 mL of water was added and boiled for 5-10 minutes. Collected insoluble matter in a Gooch crucible was added it with hot water and then ignited it in a crucible for 15 minutes at a temperature not exceeding 450°C. Next, subtracted the weight of the insoluble matter from the weight of the ash. The difference in weight represents the water-soluble ash. Calculated the percentage of water-soluble ash with reference to the air-dried drug. Finally, determine the % w/w of water-soluble ash with reference to the air-dried drug was determined.

2.2.6. Preparation of methanolic extract: The leaves of the plant were carefully collected and thoroughly washed with water to remove any impurities. Subsequently, the collected plant material was subjected to shade drying, after which it was finely powdered using a blender. The leaf powder was then separated and 60 g of the plant powder was placed into a Soxhlet extractor. The extraction process was carried out using 200 mL of methanol (with a boiling point of 64 to 65°C) for a duration of ten hours. Following the extraction, the resulting extract was transferred to a conical flask and concentrated. To remove the solvent, the extract was evaporated to dryness using a rotary evaporator under vacuum conditions, following the method described by Harborne *et al.* (1998).

2.2.7. Determination of antioxidant potential: The methanolic extract of *T. crispa* leaves was used for the analyses of antioxidant activity

(a) DPPH radical scavenging assay: Various concentrations of methanolic extract were prepared, ranging from 100 to 1000 µg/mL. A solution of DPPH was freshly prepared by dissolving 0.0039 g of DPPH in 100 mL of methanol. Subsequently,1 mL of the methanolic plant extract at different concentrations was combined with 2 mL of the DPPH solution. This reaction mixture was then incubated in darkness for 20 minutes. The dilution of DPPH with methanol devoid of the extract served as the control, while pure methanol was employed as the blank. The absorbance was measured at 517 nm against the blank reference (Katalinic *et al*., 2006). The assay was executed in triplicate.

$$\% \text{ of Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of the sample})}{\text{Absorbance of control}} \times 100$$

2.2.8. Statistical analysis

All the data were expressed as mean ± standard deviation (SD).

3. Results and discussion

3.1. Macroscopic and organoleptic characterization

The leaf exhibited a dark green colour with cordate shape and reticulate venation, featured a distinctive pulvinate base. It possesses a bitter taste and upon drying, a characteristic tea-like aroma is noticeable from the powder.

The leaf of *T. crispa* exhibited a distinctive dark green colour, cordate shape, an alternate arrangement and a pronounced bitter taste. Upon drying, the powdered form emits a characteristic tea-like aroma, serving as a distinctive identifier. The petiole boasts a pulvinous base, measured approximately 12 ± 3 cm. The leaf surface of *T. sinensis* (Lour.) Merr., are densely tomentose while smooth in *T. crispa* and length of petiole in *T. cordifolia* is 4-6 cm while 12 ± 3 in *T. crispa* (Parveen *et al*., 2020). Cordate leaf shape is present in *T. crispa* but the leaf shape in *T. baenzigeri* Forman is cordate or reniform and there is a two node present at leaf base, it is previously reported in *Tiliacora funifera* Oliv. and *Tinospora* in family Menispermaceae (De *et al*., 2016).

Table 1. Macroscopic and organoleptic characters of *T. crispa* leaf

Sl. No.	Macroscopic parameters		Observations*
1	Phyllotaxy		Alternate
2	Type		Simple
3	Lamina	Size	11600 mm^2 -12900 mm^2
		Length	15 cm -17.5 cm
		Width	13 cm -14.8 cm
4	Blade class		Mesophyll
5	Laminar shape		Cordiform
6	Apex		Acuminate
7	Apex angle		Acute
8	Base		Lobate
9	Base angle		Wide obtuse
10	Surface		Glabrous
11	Margin		Entire
12	Venation		Reticulate
13	Vein category	Primary vein	Basal actinodromous
		Secondary vein	Brochidodromous
		Teritiary vein	Alternate percurrent
14	Petiole		12 ± 3
15	Petiole base		Pulvinate
16	Colour upper		Dark green
17	Colour lower		Light green
18	Odour	Before drying	No characteristic odour
		After drying	A characteristic odour same as that of tea powder
19	Lobation		Unilobed
20	Laminar symmetry		Symmetrical
21	Taste		Bitter

**Mean ± SD of 25 observations*

3.2. Microscopic characterization of *T. crispa* leaf

3.2.1. Anatomical studies of petiole: The cross-section (a circular outline) consists of a single-layered parenchymatous epidermis followed by a collenchymatous hypodermis that comprises 2-3 cell layer, succeeded by a parenchymatous cortex. The endodermis consists of a single cell layer, while the fibrous pericycle is arranged in a wavy pattern spanning 2-5 cell layers. Within this configuration, 7-9 vascular bundles are organized in a circular arrangement. Xylem, displaying an endarch arrangement and phloem are present. The cortex is predominantly parenchymatous and large (Plate 1).

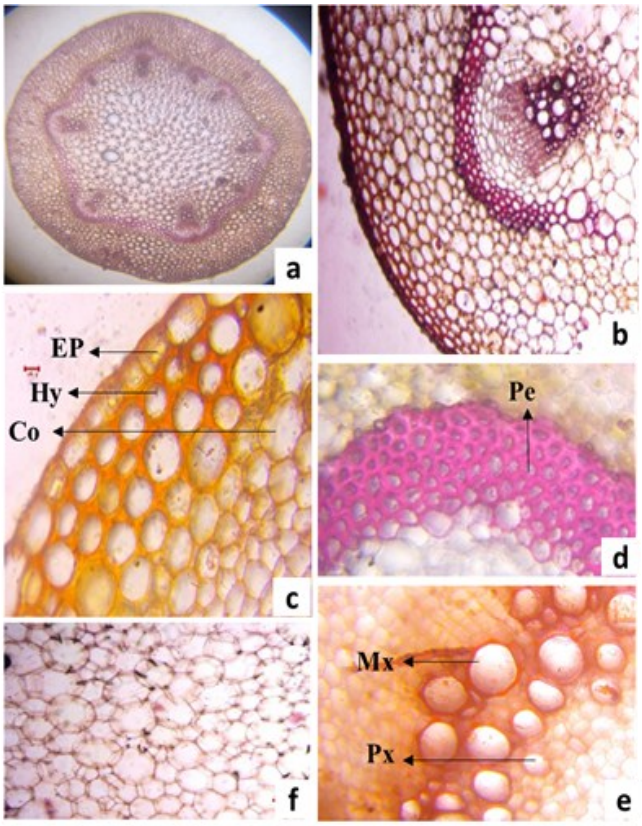

Plate 1. Microscopic studies in petiole. a. Cross-section of petiole; b. A single vascular bundle; c. Epidermis and hypodermis; d. sclerenchyma; e. Metaxylem and Protoxylem; f. Cortex (EP-Epidermis, Hy-Hypodermis, Co-Cortex, Pe-Pericycle, Mx-Metaxylem, Px-Protoxylem)

3.2.2. Anatomical studies of midrib: The outermost layer consists of the epidermis, succeeded by a collenchymatous hypodermis. The cortex is primarily parenchymatous and within it, there is a single, sizable collateral vascular bundle. Notably, a distinctive dome-shaped arrangement of phloem has also been observed (Plate 2a).

3.2.3. Anatomical studies of lamina: The cross-section (C.S) of the leaf lamina reveals a single-layered upper and lower epidermis. Notably, the lower epidermis features anomocytic stomata, accompanied by a distinctive large sub-stomatal chamber. The spongy tissues are entirely absent within this structure (Plate 2b).

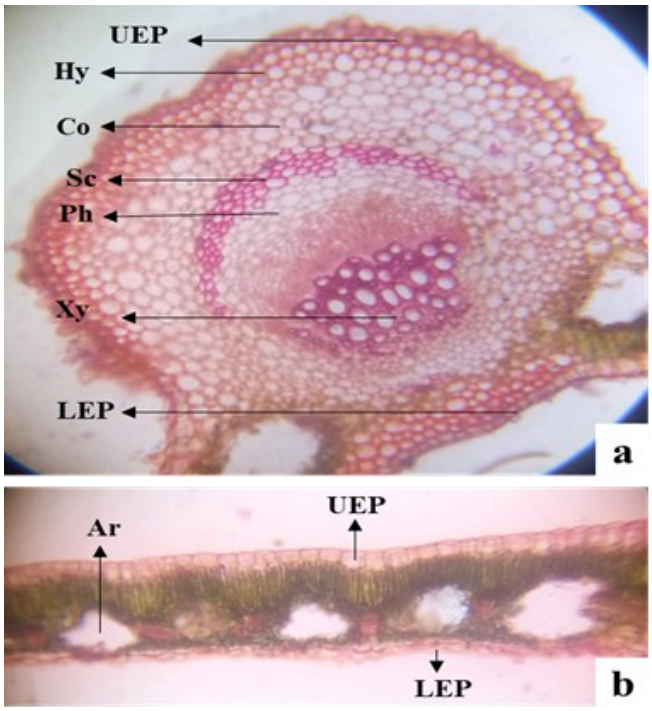

Plate 2. Microscopic studies in midrib and lamina of *T. crispa.* a. Cross-section of midrib; b. L.S of lamina (UEP-Upper epidermis, Hy-Hypodermis, Co-Cortex, Sc-Sclerenchyma, Ph-Phloem, Xy-Xylem, LEP-Lower epidermis, Ar-Air cavity)

Microscopic examination of *T. crispa* unveiled several notable features. The transverse section (T.S) of the lamina showcased anomocytic stomata on the lower epidermis, characterized by a spacious air chamber. Notably, the absence of spongy cells is observed. Shifting to the cross-section (C.S) of the midrib, a single large collateral vascular bundle becomes apparent, accompanied by the characteristic dome-shaped arrangement of sclerenchyma cells. The C.S of the petiole revealed a fibrous pericycle in a wavy pattern comprising 2-5 layers, alongside 7-9 vascular bundles forming a circular arrangement. Xylem with endarch characteristics, phloem and a substantial parenchymatous cortex complete this structure.

In *T. cordifolia,* midrib in the adaxial side is slightly convex, but prominently convex in *T. crispa*. In *T. sinensis* midrib is irregular wavy with numerous trichomes and the vascular bundles is 5-6 in *T. cordifolia* and 10–12 in the other two species. Sclerenchyma cells in the petiole are more prominent in *T. crispa* than the other two species (Parveen *et. al.,* 2020). Midrib vascular bundles in *T. crispa* is well-developed with collateral vascular bundles and sclerenchyma cells are absent whereas in *T. sinensis* single median open vascular bundles with cup shaped xylem and phloem is present with cap like structure of sclerenchyma cells (Noorunnisa *et al*., 2019).

3.2.4. Quantitative leaf microscopy: The leaf exhibited a densely reticulate venation pattern, prominently featuring primary, secondary and tertiary veins. Anomocytic stomata are exclusively present on the lower leaf surface, conspicuously absent on the upper surface. The stomatal count is recorded at 190.40 ± 21.65 mm² and the stomatal index measures 12.13 ± 0.97 stomata/mm² (Plate 3).

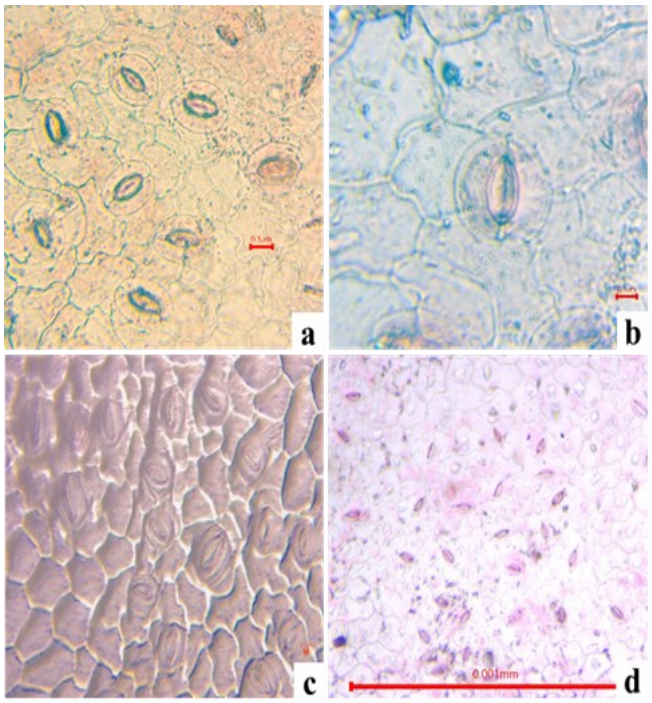

Plate 3. Stomatal details of *T. crispa.* a. Leaf peeling of lower surface showing anomocytic stomata; b. A stoma; c. Stomatal impression of lower surface; d. Numerous stomata

Anomocytic stomata are present in both *T. crispa* and *T. baenzigeri* which corroborated with the characteristic of family Menispermaceae. In *T. crispa* stomata are present only at the lower (abaxial) epidermis while *T. baenzigeri* stomata are present on both lower and upper (adaxial) epidermis (Ortiz *et al*., 2007). Quantitative leaf characteristics were observed and the result is shown in Table 2.

Table 2. Quantitative leaf microscopy of *T. crispa* leaf

Sl. No.	Parameters	Mean ± SD*
1	Stomatal number – upper surface	0
2	Stomatal number – lower surface	190.40 ± 21.65
3	Stomatal index – upper surface	0
4	Stomatal index – lower surface	12.13 ± 0.97

**Mean ± SD of 25 observations*

3.3. Powder analysis

The powder as such is green in colour with a characteristic smell like that of a tea powder and it is bitter in taste, it helped to check the authenticity of the plant material. Analysis of *T. crispa* leaf powder revealed the presence of vessels, tracheids, fibres, etc. In the powder study (Plate 4), distinctive characteristics such as the presence of fibers, tracheids and spiral vessels facilitate the identification of *T. crispa* leaf, even when it's in crushed or powdered form.

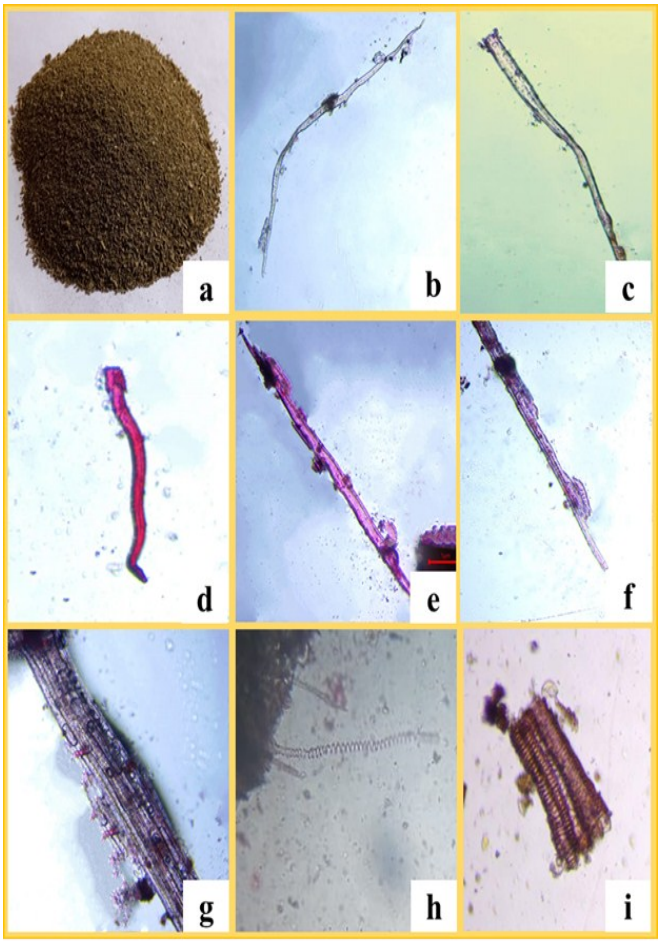

Plate 4. Powder analysis a. Leaf powder of *T. crispa*; b, c & d. Xylem fibres; e & f. Xylem tracheids; g. Vessels; h & i - Spiral xylem vessel

3.4. Fluorescence analysis

Chemical tests of *T. crispa* leaf powder with different reagents were done and observed under visible and UV light. Under UV light, the six samples (2, 3, 4, 5, 8 and 10) showed the presence of fluorescence (Plate 5). The results were compared with their respective observations in visible light and they were represented in Table 3.

The vivid colours and fluorescence exhibited when the plant powder is treated with specific reagents and observed under both UV and visible light serve as pharmacognostical benchmarks for identifying the *T. crispa* leaf in powdered form. The fluorescence observed under UV light strongly implies the presence of fluorescent compounds within the plant. Leaf and stem show fluorescence when treated with HCl, HNO_3, ethanol, etc, but when treated with H_2SO_4 leaf show fluorescence and stem doesn't show any fluorescence (Asmi and Vilash, 2020).

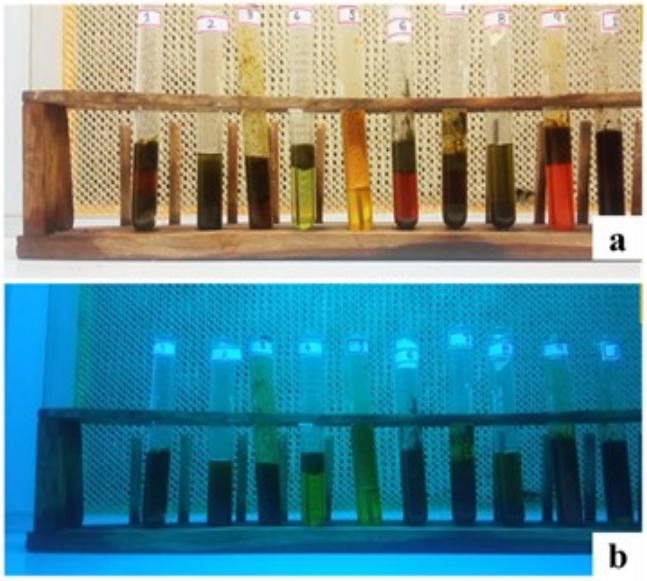

Plate 5. Fluorescence analysis a. Observation under day light; b. Observation under UV light

3.5. Physicochemical analysis

Physicochemical parameters of *T. crispa* leaf powder were evaluated and observations are presented in Table 4.The assessment of physicochemical parameters contributed to the establishment of pharmacopoeial standards. The nearly neutral pH (7.94 ± 0.23%) of the various water solutions of the drug powder suggests that the crude drug is unlikely to cause any acidic-related issues during internal or external applications. The extractive values, obtained using different solvents such as alcohol, petroleum ether and water (Table 4), help to identify the presence of various types of adulteration and exhaustion materials.

Table 3. Observations of *T. crispa* leaf powder under visible light and UV (254 nm and 366 nm) light

Sl. No.	Treatment	Observation (Colour developed)	
		Visible light	**UV light**
1	Powder + 5 ml NaOH (1N)	Red PMS 484	Black 4 2X
2	Powder + 5 ml Methanol (80%)	Green PMS 584	Green PMS 584 *
3	Powder + 1 M NaOH + Water	Yellow PMS 1225	Green PMS 375 *
4	Powder + 1 M HCl	Green PMS 372	Green PMS 367 *
5	Powder + dil HNO_3	Yellow PMS 142	Green PMS 584 *
6	Powder + 5% Iodine	Red PMS 1815	Black 3
7	Powder + dil Ammonia	Red PMS 505	Black 3 2X
8	Powder + ethanol	Green PMS 374	Green PMS 382 2X *
9	Powder + $K_2Cr_2O_7$	Orange PMS 1655	Black 5 2X
10	Powder + 1M H_2SO_4	Yellow PMS 458	Yellow PMS 601 *

*Pantone matching system *Presence of fluorescence*

Table 4. Physicochemical parameters of *T. crispa* leaf powder

Sl. No.	Physicochemical parameters		Mean ± SD*
1	pH of Water solution	1% w/w	7.94 ± 0.23 %
		10% w/w	7.64 ± 0.32 %
2	Alcohol soluble extractive		12.45 ± 0.51 %
3	Water- soluble extractive		15.04 ± 0.12 %
4	Petroleum ether soluble extractive		9.34 ± 0.42 %
5	Loss on drying (LOD)		11 ± 0.23 %
6	Swelling index		3 ml
7	Foaming index		>100
8	Total ash		12 ± 0.25 %
9	Water- soluble ash		4 ± 0.02 %
10	Acid insoluble ash		6 ± 0.01 %

**Mean of 9 observations ± SD*

The relatively low swelling index (3 ml) observed in this study suggests the absence of mucilaginous substances in the sample under investigation, but in stem the swelling index is 0.1 which is too small as compared with leaf (Asmi and Vilash, 2020). The leaf powder of *T. crispa* exhibited minimal loss on drying compared to other plant materials. The foaming index (>100) indicated a low saponin content in the leaf. Ash values play a pivotal role in assessing the identity and purity of crude drugs, particularly in powdered form. The total ash value constitutes % w/w in the plant. The water-soluble ash constitutes 4 ± 0.02 %w/w, while acid-insoluble ash amounts to 6 ± 0.01 % w/w, signifying that more than half of the ash is soluble in acid. Sayyada *et al*., (2014) studied that the acid insoluble ash in stem is 0.61% -1.01 %, compared to the leaf it is too small.

3.6. Determination of antioxidant potential of the plant

For the evaluation of antioxidant activity, the methanolic extract of *T. crispa* leaves was employed.

3.6.1. Determination of antioxidant activity using DPPH radical scavenging assay: The leaf extract of *T. crispa* showed a noticeable shift in colour when observed within the DPPH solution, transitioning from a deep violet to yellow across all five concentrations. The reduction in absorbance of the DPPH solution indicated an elevation in DPPH scavenging activity. The antioxidant capacity displayed a progressive enhancement with increasing sample concentration up to a certain threshold, beyond which it exhibited a diminishing trend (Fig. 1).

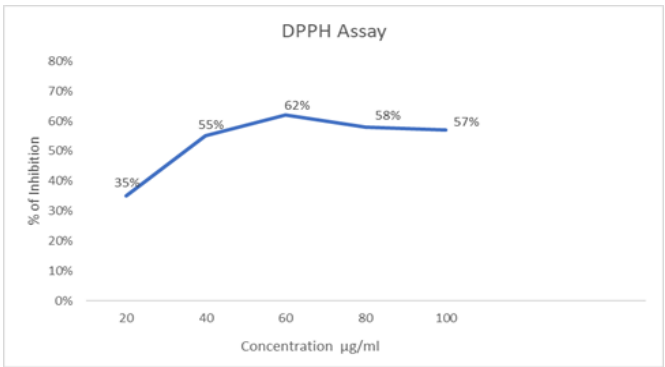

Fig. 1. DPPH radical scavenging activity of methanolic extract of *T. crispa* (leaf)

Concerning the leaf extract, the percentage of inhibition was determined to be 35%, 55%, 62%, 58% and 53% at concentrations of 20, 40, 60, 80 and 100 µg/ml, respectively.

In the DPPH assay conducted in this study, antioxidant activity exhibited an incremental pattern with increasing concentration up to a certain threshold, beyond which a decline was observed. The outcomes highlight *T. crispa* as a promising source of antioxidants, with phenolic compounds, including flavonoids, serving as potent free radical scavengers. Froemming (2011) also reported notable antioxidant activity in the methanol extract of *T. crispa* leaves, as evidenced by the total flavonoid content, total phenolic content and DPPH free radical scavenging activity. The presence of phenolic compounds in *T. crispa* substantially contributes to its antioxidant attributes. Heida *et al*., 2013 reported that the stem extract of *T. crispa* exhibits high antioxidant activity, it is due to the presence of apigenin and magnoflorine because they contain hydroxyl groups which donate the electron to reduce the DPPH radicals. Mohammad *et al*., (2011) also proved the high antioxidant activity of *T. crispa* in their study.

4. Conclusion

In conclusion, the macroscopic and organoleptic characterization of *T. crispa* leaf reveals distinctive features such as a dark green colour, pulvinous based petiole, smooth surface, cordate shape, pulvinous based petiole and bitter taste. The characteristic tea-like aroma after drying is a significant identifier of the plant in its powdered form. Microscopic studies indicate unique anatomical structures in the petiole, midrib and lamina. The prominent sclerenchyma cells in the midrib in *T. crispa* make this plant easily distinguishable from other species. Stomata present only in the lower surface make a distinction from some other species. Fluorescence analysis demonstrates the presence of fluorescent compounds under UV light. Physicochemical analysis provides important data on pH, extractive values, ash content and other parameters. The DPPH radical scavenging assay shows antioxidant potential with the percentage of inhibition varying with concentration. These findings contribute to the identification and differentiation of *T. crispa* leaf from closely related species, even in crushed or powdered forms.

This study has provided valuable insights into its botanical identity, microscopic structure and physicochemical attributes of *T. crispa*. These findings are pivotal for establishing the authenticity, quality and standardization of this medicinal plant, a crucial aspect in traditional medicine and herbal product development. The distinctive macroscopic and microscopic features identified in both its leaf and stem, along with the unique characteristics of the plant powder, offer reliable markers for its identification and differentiation from other botanical species. The observed antioxidant activity enhances the potential pharmacological significance, aligning with its traditional use for various health concerns. This detailed pharmacognostic understanding lays a solid foundation for further research, facilitating informed decisions in the utilization, formulation and regulation of *T. crispa*-derived products in the realm of natural medicine.

Acknowledgements

The authors express their gratitude to Dr. S V Manoj, Principal and Dr. A P Nisha, Head of the Department, as well as all the faculty members of the Department of Botany and Management of Sree Narayana College, Kollam, for their invaluable support and provision of necessary facilities. Special thanks are extended to the DBT Star Scheme for providing instrumentation facilities.

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