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Pharmacognostic and preliminary phytochemical screening of *Trachyspermum khasianum* H. Wolff.

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Traditional systems of medicine are one of the widely practised systems of medicine in the northeastern part of India. Since ancient times plants are believed to have miraculous healing properties and playing a vital role in the management of different disease conditions. This study aimed to determine the pharmacognostical and phytochemicals properties of a rare ethnomedicinal plant *Trachyspermum khasianum* H. Wolff mainly found in the north-eastern part of India (Assam and Meghalaya). *T. khasianum* is a plant used as traditional medicine for the treatment of throat-pain, toothache and stomach ache by traditional healers of Meghalaya, India. The pharmacognostical evaluations of *T. khasianum* H. Wolff i.e., leaves, stems and roots like macroscopy and microscopy were carried out separately. The physicochemical parameters include moisture contents, water extractive, ethanol extractive, chloroform extractive, total ash values such as acid insoluble ash and water-soluble ash were evaluated. The different prepared extracts were submitted for various phytochemicals screening such as tests for alkaloids, glycosides, phenolic compounds, flavonoids, tannins, carbohydrates, proteins, and amino acids, fixed oils and fats, terpenoids, and diterpenes. Further study required to be carried out to determine the specific components responsible for the reported therapeutic activities.

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Introduction

Since ancient times, plants are believed to have a prevalent role in health care management[1]. Natural products including plants, animals, and minerals have been the basis of the treatment of diseases from time immemorial[2,3]. According to the World Health Organisation (WHO), traditional medicines are used by approximately 80% of the world population[4]. In recent surveys, revealed that almost 50% of the prescription drugs are available made from natural products and raw materials[5]. The traditional system of medicine became more popular in developed as well as in developing countries due to the general belief that drugs occurring from natural sources are safe, effective, time tested and having fewer side effects[6,7].

The northeastern part of India considered as a heritage of herbal remedies and blessed with splendid diversity of ethnomedicinal plants. Approximately 1500 medicinally active plant species are reported from this region, which having miracles healing properties against several diseases. Yet, there may be several plants that are yet to be revealed and identify scientifically. The authentic identification, as well as preservation of the traditional knowledge of medicinal plants, are equally important. WHO has given a clear guideline about the importance, processes of characterization and identification of ethnomedicinal plants[8,9]. The assessment of quality, standard, and purity of the plant-derived material may vary depends on several parameters, hence, for better and safe use of medicinal plants, proper identification and characterization like morphological, microscopical, macroscopical and pharmacognostical, and phytochemical are required to be performed[10,11].

In this present research work, the plant *Trachyspermum khasianum* H. Wolff, belonging to family Apiaceae or Umbelliferae (Flock-flowered plants) was selected. *Trachyspermum* comes from Greek words as “Trachy” means rough & “spermum” mean seeded, and the synonym is *Carum khasianum*, C.B. Clarke[12,13]. Traditional healers of Meghalaya, use the aerial part of the plant and crunched along with rhizome of *Zingiber officianalis* (Zingiberaceae) then, used together for the treatment of toothache, throat pain, and stomach-ache as traditional medicine. The study was carried out to determine the

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pharmacognostical and phytochemicals parameters using the leaves, stem, and roots part of the plant[14,15].

T. khasianum is rarely grown in the terrestrial region with sandy soil. It is a biennial or perhaps perennial herb with 30-60 cm tall. This plant is a wild-plant and mostly found in some parts of Bangladesh and India (Assam and Meghalaya)[16]. Due to the evolution of agricultural modern technology used by the endogenous people for their daily need and commercial use of all parts of the agricultural land, the species is poorly regenerating in their natural habitat and pose a threat for extinction[17].

Materials and Methods

Materials

All Chemicals, reagents and solvents used in the study were obtained from Hi-Media laboratory, Mumbai and solvents were procured from SD Fine Chemical, Mumbai

Collection of plant

Around 2 kg of fresh *T. khasianum* plants were collected from Mairang village, Meghalaya, India in September 2018. After the collection, the fresh plants were washed in running water and dried at room temperature for 2-3 weeks[18,19].

Authentication of the plant

The herbarium sheet was prepared and authenticated by Dr N. Devi., Professor and Head, Department of Botany, Guwahati University with Reference No. Herb./GUBH/2018/97 dt. 11/10/2018.

Morphology evaluation

Plant morphology study was carried out by standard methods to determine the different external parameters of stems, roots, and leaves that were observed[20,21]. The macroscopy or morphology characteristic of this plant include size, colour, odour, taste, etc. were noted[22].

Microscopic evaluation

Transverse section microscopy

This method is used for the identification of crude drugs on cellular level[23]. The fresh parts of the plant were cut into thin sections using a sharp blade and observed under a microscope after suitable staining[24].

Powder microscopy

The dried parts of the plant were crushed to form a powder and then passed separately through the sieve no. 40 to get uniform powder mass[25]. The powder sample was then placed inside the Petri plates and submerged using different chemicals. After that, the treated samples were placed under a microscope and examined[26,27].

Physico-chemical parameter

Moisture content (Loss on drying)

The study was performed by taking 1 g of the powdered leaves, stems, and roots separately and transferred into a petri dish. The petri dish was put inside a hot air oven at a temperature of 105-110 ºC and the weighed was taken till it gets constant[28].

Determination of total ash value

About 2.5 g of accurately weighed powdered drug was transferred into a two silica crucible separately and kept inside muffle furnace at a temperature of 450-500 ºC. The powdered drug was heated till white ash is obtained. The weight was taken in an electronic balance and the percentage for total ash value was calculated[29]. The obtained ash further used for the determination of water-insoluble and acid-insoluble ash.

Determination for water-insoluble ash

The total ash obtained was boiled with 25 mL of distilled water for 5-6 minutes. The content was filtered, and then it was washed with hot water. The insoluble matter was screened using Whatman filter paper. The residue was dried in a hot air oven at temperature 110 ºC for 15 minutes. The final weight was measured in an electronic balance and the percentage was calculated[30].

Determination for acid-insoluble ash

The total ash obtained was boiled with 25 mL of 2N hydrochloric acids for 5 minutes. This was filtered, and then it was washed with hot water. The insoluble matter was washed with hot water and separated by the filtration process using Whatman filter paper. The weight was taken and calculated[31].

Extractive values

The dried powdered plant of the leaves, stem, and roots was extracted with water, ethanol, petroleum ether and chloroform using the maceration process. The powdered plant material was weighed for about 5 g and then transferred into a 250 mL conical flask. The conical flask was filled with different solvents for 100 mL separately. The flask was kept aside for 24 hours at room temperature with continuous agitation for the first 6 hours, then allowed to stand for 18 hours. The mixtures were then filtered through

Whatman filter paper. After that, only 25 mL of the filtrate was taken and kept above the heating mantle for drying, at a constant temperature. The final extractive values were calculated in percentage[32].

Fluorescence analysis

The dried parts of the leaves, stems, and roots were powdered separately. The obtained powders of the plant were treated following standard methods examined under visible light and ultraviolet light at two different wavelengths at 254 and 365 nm, respectively under the ultraviolet chamber[33]. The observed colour was noted.

Preliminary phytochemicals screening

The dried coarsely powdered of different plant parts (i.e., leaves, stems, and roots) were sieved through sieve no. 30. About 100 g of each powdered were extracted with 96% ethanol and water using Maceration process, for 72 hours at room temperature. After that, the whole content was filtered using Whatman No.1 filter paper and the filtrate was collected in a conical flask. Both the extracts obtained were concentrated using a rotary vacuum evaporator at temperature (40-45 °C) and then, transferred into a closed container for further use[34].

Phytochemical tests were performed using both ethanol and aqueous extracts to determine the presence of different phytochemicals following established standard protocol[33,34]. The plant extracts were subjected for the test of alkaloid-like substances, carbohydrates, fixed oils and fats, glycosides (Cardiac, Anthraquinone, Saponin, Coumarin), phenolic compounds and tannins, proteins and amino acids, flavonoids, lignin, terpenoids, and diterpenes. The preliminary phytochemical screening was carried out on the powdered samples and observed for sharp changes in colours were noted[35,36].

Results

Morphology

T. khasianum is rarely grown in a terrestrial region with sandy soil. It is a branched biennial or perhaps perennial herb with 30-60 cm tall. Leaves were 3-6 cm long, 1-2 cm wide, sessile, green in colour. The stem was striated, inflorescence compound umbel with 16 umbellets, each containing around 16 flowers; flowers actinomorphic, white, male and bisexual; corolla 5, petals bilobed; stamens 5, alternating with the petals; ovary inferior; stigma knob-like; fruit aromatic, ovoid, cordate, cremocarp with a persistent stylopodium. Roots are cylindrical or slightly tapering, 1-5 cm long, 0.5-1.5 cm in diameter at the ground frequently branching. Morphological characterization of the plant shown in Table 1. The plant is shown in Fig. 1.

Microscopy

Transverse section of leaf

The fresh leave was transversely sectioned through the midrib region and was mounted with phloroglucinol, dil. HCl and stained with safranin. Upper and lower epidermis, palisade cell along with collenchyma, xylem and phloem are shown in Plate 1a.

Transverse section of stem

The fresh stem was transversely sectioned and mounted with phloroglucinol, dil. HCl, stained with safranin and was observed which showed cork cells, cortex, collenchyma, parenchyma cells, xylem, and phloem were seen in Plate 1b and 2.

Transverse section of root

The transversely sectioned root part of the plant was mounted on the slide after treatment phloroglucinol, dil. HCl and stained with safranin. Microscopic observation was carried out, that show the presence of stone cells, parenchyma cells, vascular bundles, cork cells, and phloem. Observations are shown in Plate 1c.

Powder microscopy

When the powder of *T. khasianum* H. Wolff was mounted with phloroglucinol, dil. HCl and stained with safranin following cells component were observed –Stomatal cells, Parenchymatous cells with starch grains, Stone cells, Trichomes, Vessels, Flatted

Table 1 — Morphological characterization of plant *Trachyspermum khasianum* H. Wolff.

Characters	Stems	Roots	Leaves	Flowers
Colour	Green/Reddish green	Light brown/ Dark brown	Green	White
Odour	Aromatic	Aromatic	Aromatic	Aromatic
Taste	Pungent/spicy	Pungent/spicy	Pungent/spicy	-
Size	20-30 cm long and 0.5-1 cm in diameter	1-5 cm long and 0.5-1.5 cm in diameter	3-6 cm long and 1-2 cm width.	2-3 mm in diameter
Shape	Striated	Cylindrical or slightly tapering	Sessile	Actinomorphic

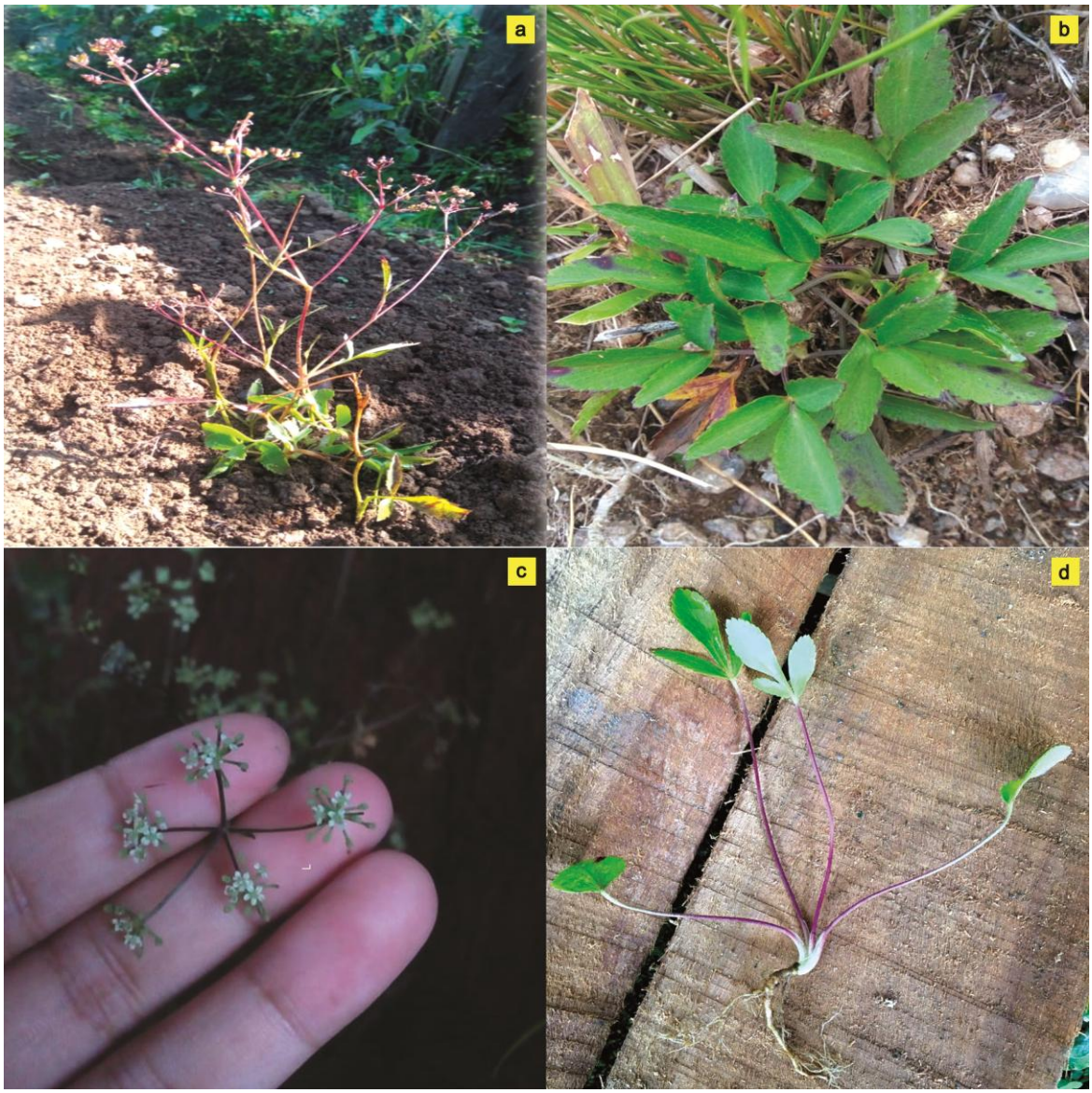

Fig. 1 — a) Whole plants of *T. khasianum* H. Wolff, b) Leaves of *T. khasianum* H. Wolff, c) Flowers of *T. khasianum* H. Wolff, d) Stem and roots of *T. khasianum* H. Wolff.

Starch grains, Fragment of vessels, Fibres, Xylem, Phloem, Stomata are shown in Plate 3.

Physico-chemical parameter

Moisture content and ash value

The result of moisture content for leaves, roots, and stems was recorded as 12±0.58, 9±0.44%, and 8±0.65% respectively. The total ash value for leaves, roots, and stems were recorded as 10.6±0.66, 8.4±0.55, and 11±0.48%, respectively, acid insoluble ash value for leaves, roots and stems as 0.8±0.78, 1.2±0.52, and 0.8±0.35% respectively, and water-insoluble ash value for leaves, roots and stems recorded as 5.6±0.45, 4.8±0.34, and 6.8±0.23%, respectively as shown in Table 2.

Extractive values

The extractive value of different parts of *T. khasianum* were reported as aqueous extractive (leaves: 5.6±0.15% w/w, stems: 4.8±0.28% w/w, roots: 5.4±0.44% w/w), ethanol extractive (leaves: 2.5±0.27 % w/w, stems: 1.5±0.24% w/w, roots: 3±0.78% w/w), chloroform extractive (leaves: 0.5±0.28% w/w, stems: 0.5±0.12% w/w, roots: 1±0.49% w/w) and petroleum ether (leaves: 0.5±0.12% w/w, stems: 0.25±0.18% w/w, roots:

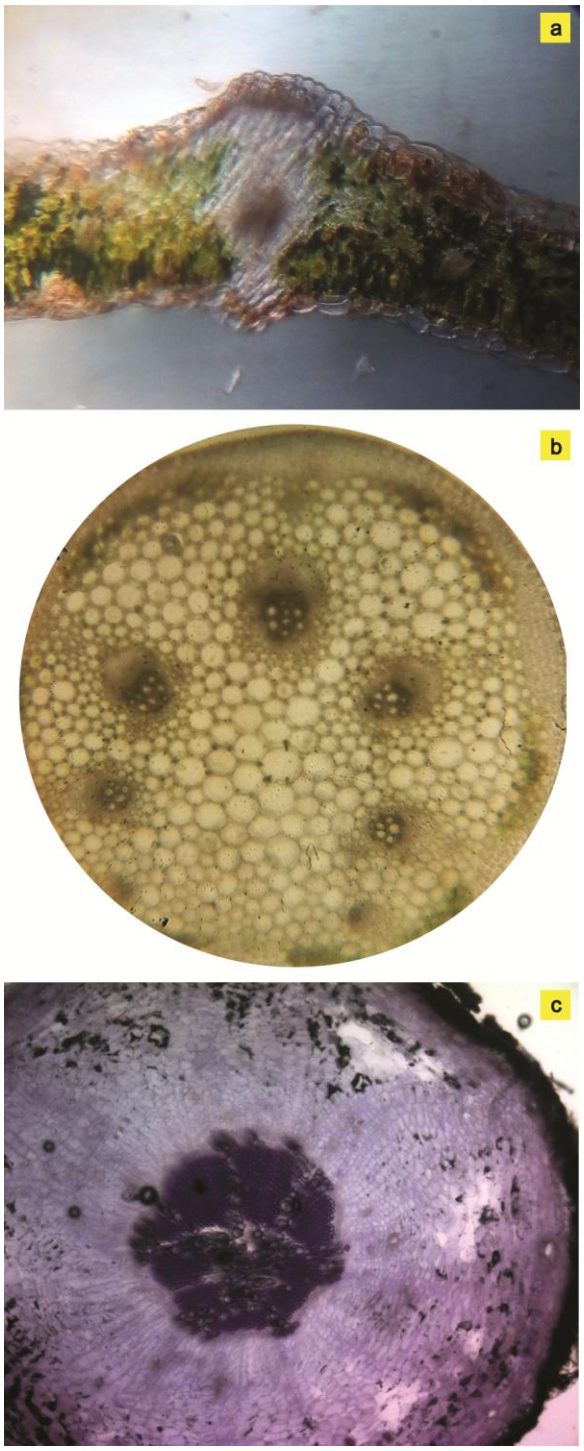

Plate 1 — a) Transverse Section of leaves, b) Transverse Section of stems, c) Transverse Section of roots.

0.25±0.31% w/w) as shown in Table 2. The maximum extractive value was found in the water solvent minimum was in chloroform. In all studied, parts soluble extractive values found in rank are aqueous>ethanol>chloroform>petroleum ether.

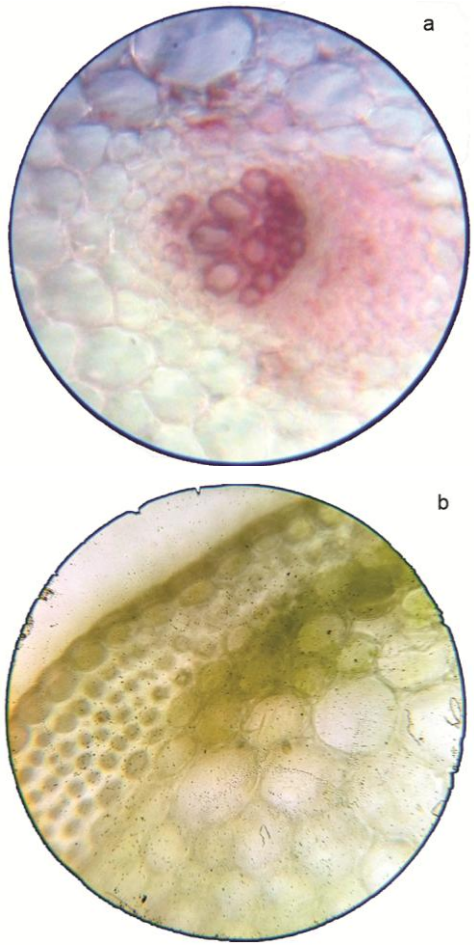

Plate 2 — a), b) Transverse section of stems.

Hence, the extractive value in the aqueous medium was found maximum as compared to other. Further, majority of active component present in the extract are expected to be hydrophilic.

Fluorescence analysis

The fluorescence analysis of dried powdered parts of the *T. khasianum* such as leaves, stems, and roots was treated separately with various chemical reagents and exposed to visible, ultraviolet light (Short UV and long UV). The changes in appearance and colour were observed and recorded (Table 3). The herbal formulation showed characteristic colouration upon treatment with different chemical reagents. The observation of different powdered plant parts under UV and visible light may be considered as a primary diagnostic tool for the identification of the pure crude drug.

Preliminary phytochemicals screening

The phytochemicals screening of both the extracts indicates the possibility of the presence of various

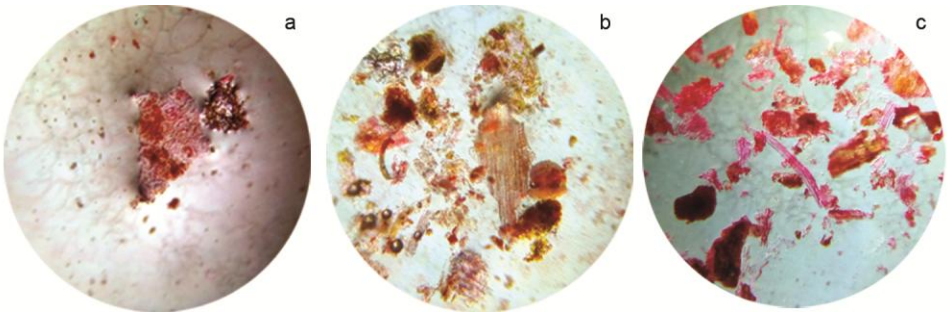

Plate 3 — a), b),c) Powder microscopy of plant.

Table 2 — Physico-chemical parameter on plant *Trachyspermum khasianum* H. Wolff

S. No.	Physico-chemical parameter	Parts		
		Leaves (% w/w)	Roots (% w/w)	Stems (% w/w)
1	Loss on drying	12±0.58	9±0.44	8±0.65
2	Total ash value	10.6±0.66	8.4±0.55	11±0.48
3	Acid-insoluble ash value	0.8±0.78	1.2±0.52	0.8±0.35
4	Water-insoluble ash value	5.6±0.45	4.8±0.34	6.8±0.23
5	Water extractive value	5.6±0.15	5.4±0.28	4.8±0.44
6	Ethanol extractive value	2.5±0.27	3±0.78	1.5±0.24
7	Chloroform extractive value	0.5±0.28	1±0.49	0.5±0.12
8	Petroleum ether extractive value	0.5±0.12	0.25±0.31	0.25±0.18

chemical constituents such as alkaloids, glycosides, fixed oils and fats, phenolic compounds and tannins, protein and amino acids, flavonoids, lignins, terpenoids and diterpenes as shown in Table 4.

Discussion

In this research work all the important Morphological, microscopic, pharmacognostic and phytochemical properties of different parts of *T. khasianum* was characterised following standard guideline. The preliminary morphological study observation states that the whole plant is around 25-30 cm long and 3-4 cm in diameter with and characteristic aromatic flavour and pungent or spicy taste. The leaves of the plant are sessile, the stem is striated and the root part is slightly cylindrical. The microscopic study of different parts of plant shows the presence of collenchyma, parenchyma cells, xylem, and phloem that may be considered and identical parameters for future reference. Powder analysis plays a significant role in the identification of crude drugs and to measure its purity. The total ash value of the evaluated samples was found to be 10.6±0.66, 8.4±0.55, and 11±0.48% w/w followed by acid-insoluble (0.8±0.78, 1.2±0.52, 0.8±0.35% w/w) and water-soluble ash (5.6±0.45, 4.8±0.34, and 6.8±0.23% w/w) are reported for leaves, root, and stems respectively. The ash value analysis reflects the amount of organic and inorganic matters present in the sample and considered as a qualitative standard in determining authenticity and purity of the sample. Moisture content analysis was also carried out and the observation is tabulated in Table 2. It is an important parameter as the presence of excess water, at ambient temperature may lead to the activation of enzymes that play a role in the proliferation of living organism. Fluorescence analysis is an important phenomenon displayed by different phytoconstituents present in plant materials. The presence of such content may be confirmed through the observation of fluorescence property under visible light and ultraviolet light. Any substances which are not fluorescent by themselves, they may often be decomposing into fluorescent derivatives or decompose products by using different multifarious chemical reagents. Hence, fluorescent analysis is an important parameter for the pharmacognostic standardization of crude drugs. An extractives value of different solvent indicates the quantity and nature of active phytoconstituents present in the plant and extracted with a particular solvent. The result of the study indicates that the majority of active Phytoconstituent show comparatively better solubility in water, hence it may

Table 3 — Fluorescence analysis study of different parts of plant *Trachyspermum khasianum* H. Wolff.

Experiment particulars	Leaves			Roots			Stems		
	Visible light	UV Fluorescence		Visible light	UV Fluorescence		Visible light	UV Fluorescence	
		SW (254 nm)	LW (365 nm)		SW (254 nm)	LW (365 nm)		SW (254 nm)	LW (365 nm)
Powder as such	Brown	Dark green	Dark brown	Light brown	Brown	Dark brown	Brown	Yellowish-brown	Dark brown
Powder+1N Aqueous NaOH	Yellowish-brown	Light green	Dark brown	Light brown	Light green	Dark brown	Yellowish-brown	Light green	Light green
Powder+1N Alcoholic NaOH	Light green	Dark green	Dark brown	Light brown	Greenish brown	Dark brown	Greenish brown	Light green	Dark brown
Powder+1N HCl	Light brown	Yellowish-brown	Dark brown	Light brown	Yellowish-brown	Dark brown	Light brown	Yellowish-brown	Dark brown
Powder+conc. H_2SO_4	Brown	Greenish brown	Dark brown	Brown	Greenish brown	Dark brown	Brown	Greenish brown	Dark brown
Powder+50% H_2SO_4	Light brown	Greenish brown	Dark brown	Light brown	Yellow-brown	Dark brown	Light brown	Greenish brown	Dark brown
Powder+conc. HCl	Light green	Dark green	Dark brown	Light brown	Light green	Dark brown	Light brown	Greenish brown	Dark brown
Powder+conc. HNO_3	Yellowish-brown	Greenish brown	Dark brown	Yellow-brown	Greenish brown	Dark brown	Light brown	Greenish brown	Dark brown
Powder+50% HNO_3	Yellowish-brown	Greenish brown	Dark brown	Light brown	Greenish brown	Dark brown	Light brown	Greenish brown	Dark brown
Powder+Ferric chloride	Yellowish green	Black	Greenish brown	Yellowish-brown	Dark brown	Greenish brown	Yellowish-brown	Dark brown	Greenish brown
Powder+NH_3	Greenish brown	Dark brown	Brownish green	Yellowish-brown	Dark brown	Greenish brown	Yellowish-brown	Dark brown	Greenish brown
Powder+Petroleum ether	Brown	Dark brown	Light brown	Brown	Dark brown	Light brown	Brown	Dark brown	Light brown
Powder+Chloroform	Greenish brown	Dark brown	Light brown	Light brown	Dark brown	Brown	Brown	Dark brown	Light brown
Powder+Acetone	Brown	Dark brown	Light brown	Light brown	Dark brown	Slight brown	Brown	Dark brown	Slight brown
Powder+Ethyl acetate	Brown	Dark brown	Light brown	Light brown	Dark brown	Slight brown	Light brown	Dark brown	Slight brown
Powder+Acetonitrile	Brown	Dark brown	Light brown	Light brown	Dark brown	Brown	Brown	Dark brown	Slight brown
Powder+Picric acid	Yellowish-brown	Reddish-brown	Greenish brown	Yellowish-brown	Reddish-brown	Greenish brown	Yellowish-brown	Reddish-brown	Greenish brown
Powder+2 Propanol	Brown	Light brown	Dark brown	Brown	Dark brown	Light brown	Brown	Dark brown	Light brown
Powder+Ethanol	Dark brown	Black	Light brown	Light brown	Dark brown	Yellowish-brown	Brown	Dark brown	Light brown
Powder+Water	Brown	Dark brown	Light brown	Light brown	Brown	Yellowish-brown	Brown	Dark brown	Light brown

SW- Short wavelength, LW- Long wavelength

be stated the Phytoconstituent are hydrophilic on nature. The Phytochemical analysis study shows possibilities of the presence of different phytochemical group present in the tested sample includes tannins, glycosides, alkaloids, proteins, terpenoids, diterpenes, lignin and flavonoids. It is assumed that the alkaloid, tannins, flavonoids and/or glycosides present in the plant shall be responsible for the claimed therapeutic effects, either individually is in combination.

Table 4 — Phytochemicals screening of plant *Trachyspernum khasianum* H. Wolff

Compounds	Ethanolic extracts			Aqueous extracts			Inference
	Leaves	Stems	Roots	Leaves	Stems	Roots	
Alkaloids like substances test							May be present
Mayer's test	-	-	-	-	-	-	
Wagner's test	+	+	+	+	+	+	
Hager's test	+	+	+	-	-	+	
Dragendroff's test	+	+	+	+	+	+	
Carbohydrates test							Absent
Molish's test	-	-	-	-	-	-	
Benedict's test	-	-	-	-	-	-	
Fehling's test	-	-	-	-	-	-	
Test for fixed oils and fats							May be present
Saponification test	+	+	+	-	-	+	
Test for glycosides							May be present
Keller killiani test (Cardiac glycoside)	+	+	+	-	-	-	
Bontrager's test	-	-	-	-	-	-	
Foam test	-	-	-	+	+	+	
Coumarin glycosides	+	+	-	+	+	+	
Test for phenolic compounds and Tannins							May be present
Ferric chloride test	+	+	+	+	+	+	
Gelatin test	-	+	+	-	-	-	
Lead acetate test	+	+	+	+	+	+	
Test for proteins and amino acids							May be present
Millon's test	-	-	-	+	+	+	
Biuret test	-	-	-	-	-	-	
Ninhydrin test	-	-	-	-	-	-	
Xanthoproteic test	+	+	+	+	+	+	
Test for flavonoids							May be present
NaOH test	+	+	+	+	+	+	
H_2SO_4 test	+	+	+	+	+	+	
Test for lignin							May be present
	+	+	+	+	+	+	
Test for terpenoids							May be present
	+	+	+	+	+	+	
Detection of diterpenes							May be present
Copper acetate test	+	+	+	+	+	+	

(-) negative; (+) positive;

Conclusion

The preliminary physico-chemical, phytochemical and pharmacognostic study the different parts of *T. khasianum* H. Wolff was successfully performed following the standard protocol. As of now, no scientific record is available about this plant. Therefore, the present study outcome specified the salient microscopic, morphological, pharmacognostic and phytochemicals parameters that are imperative for identification and significant quantification of future reference. From the above study, it can be concluded that active phytochemicals predicted to be present in the plant are hydrophilic and shall be responsible for the reported medicinal activity. However, further research studies are required to find out the specifically responsible phytoconstituents for the establishment of the therapeutic activities of the *T. khasianum.*

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