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Pharmacognostical evaluation and phytochemical studies on Ayurvedic nutritional fruits of *Trapa natans* L.

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Abstract
Trapa natans L. is a small free-floating plant growing mainly in shallow water or swampy regions, native to Europe, Asia and Africa commonly called as the water chestnut or singhara in India. The plant consists of dried seeds which are enclosed in a thick and dark brown hard kernel that are delicious to eat possessing high protein, carbohydrates, starch, flavonoids and essential minerals. It favors nutrient rich water with pH range between 6.7 and 8.2 and the alkalinity between 12 and 128 mg/L of calcium carbonate. In Indian Ayurvedic System of Medicine, it's well known for medicinal properties and high nutritive value useful in the treatment of stomach, liver, kidney, spleen and genitourinary disorders. The kernels are used as appetizer, tonic, analgesic, anti-diarrheal, anti-inflammatory, anti-bacterial and anti-diabetic. The present work highlights the Phytochemical Evaluation, microscopic studies, nutritional aspects and Thin Layer Chromatography (TLC) of the fruits of *T. natans* L.

Keywords: Ayurvedic System, Phytochemical, Nutritive, Anti-diabetic, *Trapa natans*

1. Introduction
Trapa is a genus of aquatic herbs distributed in central and South-East Europe and temperate and tropical Asia belonging to the family Trapaceae. It is a monotypic genus represented by *T. natans* L., a polymeric species having a number of botanical varieties; of these varieties, var. *bispinosa* Makino is economically the most important in India. *T. natans* L. is commonly known as Sringhataka in Sanskrit and singhara in Kannada. It is a variable, aquatic herb occurring almost throughout the greater parts of India in lakes and ponds; also, extensively cultivated for the edible seeds. The stems are long, ascending in the water, submerged portions possessing pairs of green, spreading organs at intervals below the margins of leaf-scars; leaves floating crowded at the upper parts of stems, appearing as rosettes, rhomboidal, lower surface reddish purple to green, upper green and often variegated with long swollen petioles; flowers are white, solitary, opening above the surface of water in the afternoon; fruits bony, 4-angled, 2 opposite angles each with a scabrous spine, 1-seeded with starchy white seeds. The flowering starts during August-September and continuous, along with fruit-setting, for 60-120 days, depending upon the severity of the winter; the fruit-setting is adversely affected if the water is muddy. The fruit is ready for harvesting in c.21 days. The period of harvesting ranges from September to December but continuous up to February, depending upon the severity of cold [1].

The plant contains carbohydrates, minerals, calcium, phosphate, iron, copper, manganese, magnesium, sodium and potassium. The kernels contain some vitamins like thiamine, riboflavin, nicotinic acid, vitamin C, vitamin A, D-amylase and considerable amount of phosphorylases. The medicinal values of the fruits have been recognized in folklore medicine as a cure for various diseases. They also contain a great quantity of non-nutritional antioxidants, such as flavonoids, flavones and total phenolic contents. The seed contains carbohydrates, saponins, phytosterols, fixed oils and fat while the pericarp has tannins, flavonoids and glycosides [2].

The fruits of this plant are considered as antidiarrhoeal, refrigerant, nutritive and tonic and used in bilious affections. The nuts with milk are given in general debility, leucorrhoea and seminal weakness. The dried seeds are used as cooling and stomachic. Besides, the stem-juice is used beneficially in eye disease and as a poultice it acts as an agent for resolution of tumors [3]. The acrid juice of trapa is used for diarrhea and dysentery [4]. The whole herb has been reported for hepato-protective activity [5], Antibacterial activity [6, 7, 8,] Antifungal activity [9],

Anti-diabetic activity [10], Analgesic activity [11], Anti-inflammatory activity [12], Antioxidant activity and free radical scavenging activity [7, 13].

In Unani system of medicine it is being used in various diseases like sexual weakness, spermatorrhea, general debility, dysentery, dry cough, bleeding disorders, anal fissure, lumbago, dental caries, sore throat, bilious affections, bronchitis, tuberculosis, renal calculi and fatigue [14].

However, botanical information, identification and chemical studies have been reported in many literature reviews, the comprehensive studies involving phytochemical evaluation, pharmacognosy and TLC fingerprint studies have become sparsely dense. Hence, the present work is an attempt to develop and report wide-spreading parameters including powder microscopy, macroscopical, microscopical, phytochemical, TLC profile of the fruits of *Trapa natans* L.

2. Regional names in India [15]

English- Singhara nut, Water chestnut; Hindi- Sinhaada, Singhara; Kannada - Mullu kombu balli, Mullu kombu beeja, Neeru acrotu, Singhara, Singaade; Malayalam- Karimpolam, Ponaver tsjeraua; Marathi- Shingada, Singhaada; Sanskrit- Shrungataka, Jalakantaka, Jalaphala, Jalashaya, Jalavalli; Tamil - Mullikaai, Pannimonthan kizhangu, Sinvaara; Telugu- Kubjakam, Kubyakam, Pandi gadda.

3. Traditional Uses

Trapa natans L. has been identified as the Ayurvedic drug **Shrungataka** [16].

Shrungataka is an important drug mentioned by a majority of the ayurvedic lexicons, texts, explaining about its different properties and actions. Some of the synonyms given for Shrungataka clearly states the anatomical characters and assist in its identification. Some of the common synonyms are Jalakanda – referring to its aquatic nature, Trikona kanda- referring to the shape of its triangular shape. Shrungataka is known to possess Madhura Kashaya rasa (sweet and astringent taste), is Ruksha Guru (dry and heavy for digestion), it is Sheeta veerya (cold in potency, anabolic in nature). The drug is known to reduce the vitiated Pitta dosha (One of the three bio forces responsible for the metabolism). Shrungataka, hence can be used in treating a number of diseases caused due to Pitta dosha [17].

On the other hand, Sushrutha mentions Shrungataka among the drugs used for increasing the quantity of Kapha dosha [18]. In Dysuria caused due to vitiated pitta, a decoction made of *T. natans* is administered. The drug is known to be a diuretic and also pittahara, hence acts both against the aetiology and symptoms [19]. In diseases of Vata, Sushrutha advises the use of Shrungataka along with Shunti (*Zingiber officinale* Roscoe) and Kasheruka (*Scirpus grossus* L.) [20]. Sushruta also advocates the use of Shrungataka as a Galactogogue [21]. Shrungataka is also used as a haemocoagulant, particularly in Post partum haemorrhages [22]. One of the most important medicines used in Ayurveda in fractures and to promote bone strength is Gandha taila and Shrungataka is one of its ingredients [23]. Other formulations of Ayurveda with Shrungataka as an ingredient area: Mahamayura ghruta given in Shiroroga (Diseases of Head), Eladi taila prescribed in fractures, Vrushya ghruta and Apathyakarasvarasa used as aphrodisiacs, Amruta prasha ghruta and Sarpiguda used as rejuvenators, etc [24]. In Visarpa (Erysipelas), a paste of shrungataka with other pittahara dravyas can be applied with ghee [25].

In general, the drug Shrungataka is Prajasthapana that helps in stabilisation of foetus during pregnancy, it is Dahaprashamana (allays burning sensation), Vrushya Shukrajanana (Aphrodisiac), and Balya (tonic). It is also Mutrala (diuretic), Trushna nigrahana (allaying thirst), and Sthambana (Coagulant) [17].

4. Utilization and Nutritional Aspects

In many parts of India, the fruit is an important source of food, especially during the times of scarcity. The fresh, tender kernels are sweet, delicious and farinaceous, and the flavor resembles that of chestnuts and they are nutritious. The nuts are eaten raw when tender and fresh or after cooking or boiling and roasting. The meal prepared by grinding the dried kernels is used as a substitute for cereal flour and is also sometimes used as an adulterant of butter [1].

The kernels are good source of minerals and are reported different constituents like proteins, fat, fiber, other carbohydrates and mineral matter: calcium, phosphorus, and iron, copper, manganese, magnesium, sodium, potassium, Iodine; vitamin contents: thiamine, riboflavin, nicotinic acid, vitamin C, vitamin A etc. The presence of β- amylase and a considerable amount of phosphorylases and tannins has been reported in the kernels [1].

The nutritive values of flour, prepared from dried kernels are as follows: moisture-10.6, protein-8.0, fat-0.6 and minerals-2.6%; calcium-69, phosphorous-343, iron-2.8 and thiamine-0.44mg/100g. The partial substitution of rice, ragi or jowar in the diet of rats to an extent of 25% by flour of water chestnut is reported to have shown significantly larger gains in the body-weight of rats as compared with the corresponding un-substituted diets. The biological value of the proteins of water chestnut was found to be higher than that of proteins in wheat [1].

The starch isolated from the flour, consists of 15% amylose and rest amylopectin, coating penetrating qualities, and gelatinizes quickly at low temperature and also suitable for textile-sizing as a good substitute for corn-starch in ice cream manufacture [1].

5. Materials and Methods

5.1. Plant Material *Trapa natans* L. fruits were collected from Anand District, Gujarat, India and it was identified and authenticated by Survey of Medicinal Plants Unit (Ref no AP-3274), National Ayurvedic Dietetics Research Institute (NADRI), Ashoka pillar, Jayanagar, Bangalore. The fruits were washed thoroughly and shade dried, pulverized by mechanical grinder, passed through 40 mesh sieves and stored in a closed vessel. The powdered fruit material was used to carry out pharmacognostical evaluation and chemical analysis for different parameters according to standard methods. Photomicrographs were captured with Catcam Image Analyzer and Nikon digital camera.

5.2. Macroscopic and Microscopic Analysis The macroscopical characters of the fruits of *T. natans* L. were observed and the seeds were soaked in 70% alcohol for 24 hours to take freehand sections cleared with chloral hydrate solution and water, stained with saffranin according to standard prescribed methods [26- 28].

5.3. Powder Microscopy The powdered fruit material was stained with phloroglucinol and concentrated HCl to study the lignified cells, trichomes, fibres, xylem vessels, etc., obtaining observations through image analyzer [26-28].

5.4. Physico-chemical analysis The physico chemical parameters like moisture content, loss on drying, total ash,

acid-insoluble ash, alcohol and water-soluble extractive values were carried out as per the standard procedures of Indian Pharmacopeia [28].

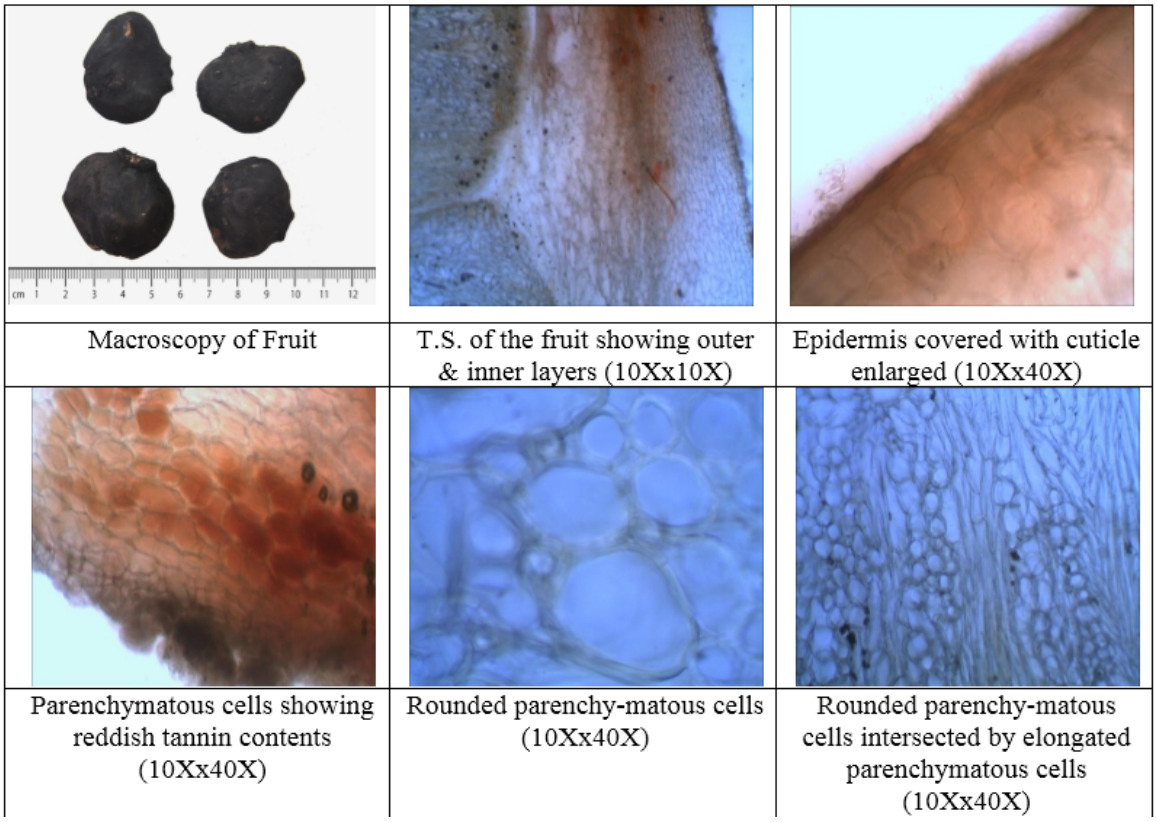
5.5. Phytochemical analysis the crude powder or crude drugs extracted in different solvents are tested for the presence of various phytoconstituents such as proteins, carbohydrates, saponin, starch, phenols, flavonoids present in them by standard procedures [29].

5.6. Thin Layer Chromatography (TLC) Dried fruit powder was extracted with petroleum ether (60-80 ^{0}C), chloroform and ethanol by using soxhlet extraction apparatus and water bath. The dried extractives were obtained after evaporation of solvent under reduced pressure by rotary evaporator. TLC studies of these extracts were carried out by using, commercially available precoated plates with standardized adsorption layers, i.e. Silica gel 60 F_{254}, (Merck, Germany) at room temperature as per the standard procedures [30].

6. Results

6.1. Macroscopy Fruit blackish 2-4 cms long, obovoid, 4 angled with 2 spines on opposite angles, 2 other spines usually obsolete, indehiscent, 1 seeded. Seeds are 2-3cm long and 2.5-3.5cm wide with slightly creamish or white in colour. Seeds taste slightly sweetish with agreeable odour.

6.2. Microscopy T.S. of the outer kernel shows a single layered epidermis covered by thin brown cuticle, followed by epidermis i.e., many layered thin-walled parenchymatous cells arranged closely, filled with brown content of tannin and some of the cells shows black/brown calcium oxalate crystals. In the parenchymatous cells, many helical to spiral xylem vessels are found scattered. Followed by this, the innermost layer/middle region of kernel shows thin walled elongated parenchymatous cells, intersected by closely arranged rounded parenchymatous cells showing reddish brown tannin content. This is followed by cotyledon region where the outer most layer testa is made up of single layer of thin-walled epidermal layer made up of reddish coloured cells, followed by many layered closely arranged, thin-walled polygonal parenchymatous cells filled with abundant rounded to oval simple to compound starch grains with prominent hilum. Some of the cotyledonary cells show helical to spiral xylem vessels (Plate I).



Macroscopy of Fruit	T.S. of the fruit showing outer & inner layers (10Xx10X)	Epidermis covered with cuticle enlarged (10Xx40X)
Parenchymatous cells showing reddish tannin contents (10Xx40X)	Rounded parenchy-matous cells (10Xx40X)	Rounded parenchy-matous cells intersected by elongated parenchymatous cells (10Xx40X)

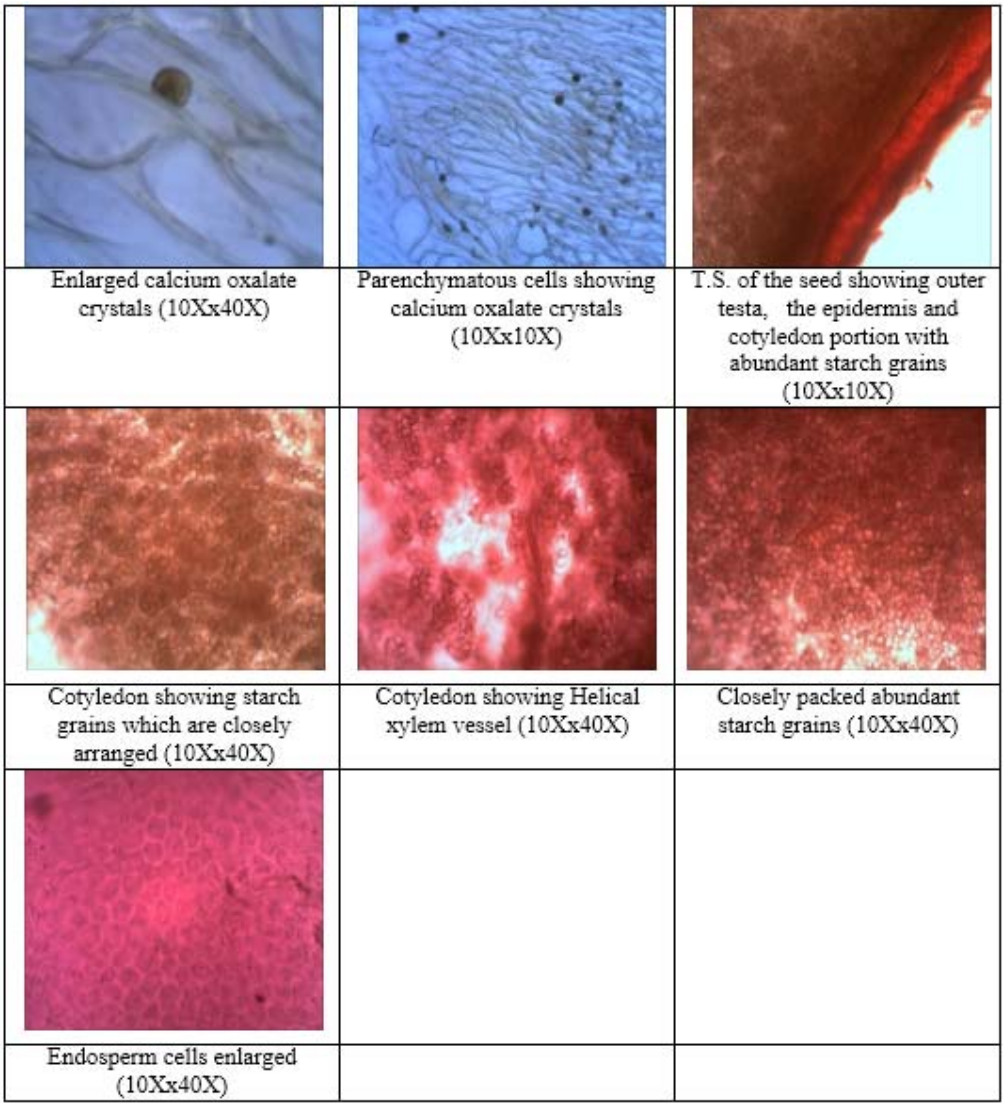


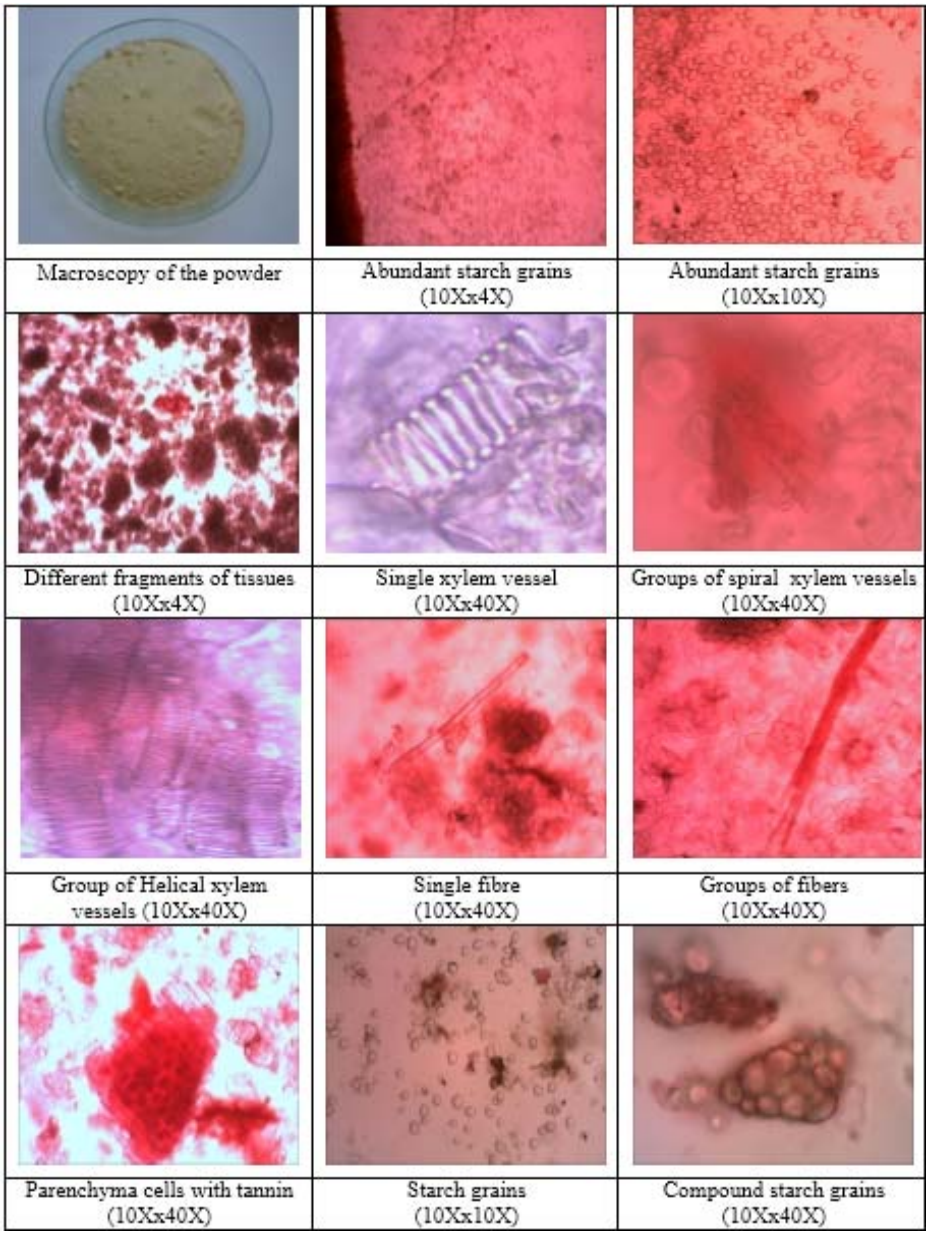
Plate 1: Microscopic studies of the Fruit of *Trapa natans* L

6.3. Powder Microscopy: (Plate II) Powder is creamish white, smooth to touch with agreeable odour, taste slightly sweetish. When powder treated with chloral hydrate and water, the following different fragments of tissues were observed and noted under the microscope,

- Presence of abundant mat like thick starch grains, which are arranged compactly /closely in the cotyledanary region of seed.
- Presence of plenty of simple to compound starch grains, which are rounded to oval with prominent hilum.
- Abundant fragments of helical to spiral xylem vessels.
- Elongated xylem fibres in groups and single.
- Elongated thin walled and thin walled rounded parenchymatous cells.
- Parenchymatous cells with reddish tannin contents.
- Abundant rounded starch grains in groups.

6.4. Diagnostic Characters

- Presence of abundant simple to compound starch grains in cotyledon region.
- Presence of abundant spiral to helical xylem vessels.
- Presence of reddish tannin content in the parenchymatous region of fruit kernel region.
- Presence of black calcium oxalate crystal in the parenchymatous region of inner portion of fruit kernel.
- Presence of hard and thick fruit kernel, which is not easily breakable by hand, in dry condition.
- Presence of abundant mat like thick starch grains, which are arranged compactly/closely in the cotyledanary region of seed.



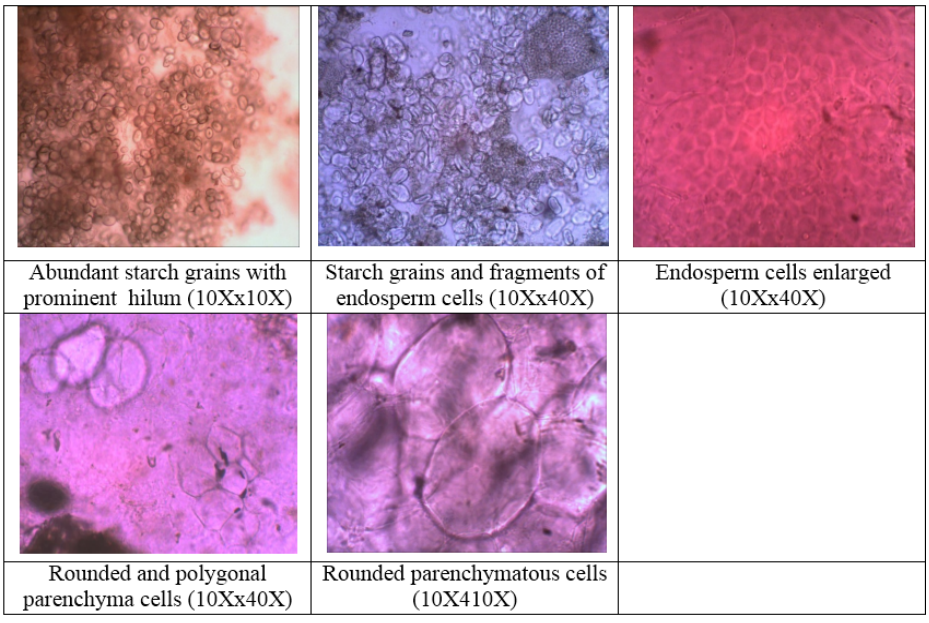


Plate 2: Powder Microscopic studies of the Fruit of *Trapa natans* L

6.5. Physico-chemical Analysis The physico chemical parameters like moisture content, loss on drying, total ash, acid-insoluble ash, alcohol and water-soluble extractive values were carried and recorded the values in Table 1.

6.6. Phytochemical analysis the phytochemical parameters in different solvents were tested for the presence of various phytoconstituents such as proteins, carbohydrates, saponin, starch, phenols, flavonoids present in them by standard procedures and recorded the values in the Table 2.

6.7. Thin Layer Chromatography the TLC was carried out for three different solvent extracts i.e. Hexane: Ethyl acetate (8:2) for Petroleum ether (PE) extract; Hexane: Ethyl acetate (8:2) for Chloroform extract and Hexane: Ethyl acetate (8:2) for Ethanol extract of fruit powder. After developing, the plates were dried under room temperature for 5-10 minutes and observed under UV-254 & UV-366. Photographs were taken and the R_f values were recorded in Table 3 (Plate III).

6.8. R_f values Petroleum Ether extract - under 254nm: 0.77, 0.95 and under 366: 0.52; after derivitazation: 0.13, 0.25, 0.35, 0.50, and 0.93; Chloroform extract - under 254nm: 0.47, 0.65, 0.75, 0.93 and under 366nm: 0.52; after derivatization: 050, 0.61, 0.93, and 0.96; Ethanolic extract - under 254nm: 0.40, 0.75, 0.83, 0.93 and under 366 nm: 0.52, 0.87.
After derivitazation: 0.08, 0.13, 0.18, 0.25, 0.56 and 0.96.

Table 1: Physicochemical parameters

S. No.	Name of the parameter	Values (%) w/w
1	Description	Creamish or white in color
2	Foreign matter	Less than 1.0%
3	pH (5% w/v aq. solution)	6.8
4	Loss on drying at 105 ^{0}C	9.80
5	Total ash	2.19
6	Acid-insoluble ash	0.03
7	Water-soluble extractive	2.54
8	Alcohol-soluble extractive	0.98

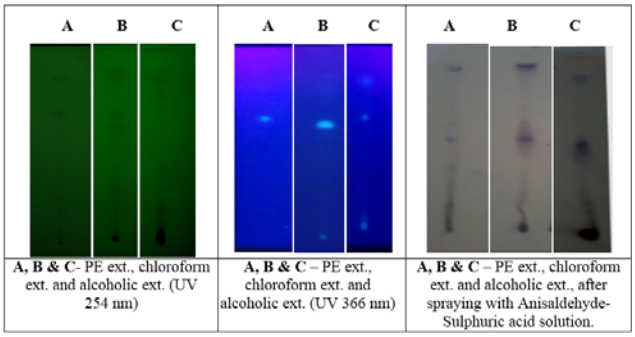
Table 2: Preliminary Phytochemical Tests

S. No.	Natural product group	Test for natural products	Extract used for the test	Presence(+)/Absence(-)
1	Alkaloids	Dragendorff's test	Aqueous	-
		Hager's test	Aqueous	-
		Mayers's test	Aqueous	-
		Wagner's test	Alcoholic	-
2	Carbohydrates	Benedict's test	Aqueous	+
		Fehling's test	Aqueous	+
		Molisch's test	Aqueous	+
3	Flavonoids	Shinoda test	Alcoholic	+
4	Phenols	Ferric chloride test	Aqueous	+
5	Proteins	Biuret's test	Aqueous	+
		Ninhydrin	Aqueous	+
6	Saponins	Foam test	Aqueous	+
7	Steroids	Salkowski reaction	Petroleum ether	-
8	Tannins	Ferric chloride test	Aqueous	+
		Lead acetate test	Aqueous	+
9	Fixed oils	-	Petroleum ether	+
10	Glycosides	-	Alcoholic	+
11	Starch	Iodine test	Aqueous	+
12	Resin	-	Chloroform	-
13	Amino acids	Ninhydrin test	Aqueous	+

Table 3: Extractive values by Soxhlet extraction

S. No.	Solvent	Values (%) w/w
1	Petroleum ether (40-60 ^{0}C)	0.94
2	Chloroform	0.77
3	Ethanol	2.37

Plate 3: TLC Fingerprint of *Trapa natans* L



A, B & C- PE ext., chloroform ext. and alcoholic ext. (UV 254 nm)

A, B & C – PE ext., chloroform ext. and alcoholic ext. (UV 366 nm)

A, B & C – PE ext., chloroform ext. and alcoholic ext., after spraying with Anisaldehyde-Sulphuric acid solution.

7. Conclusion

The global interest towards traditional medicines is growing day by day due to its safety and lesser side effects. Traditional herbal medicines are naturally occurring plant derived substances with minimal or no industrial processing that have been used to treat illness within local or regional healing practices. *Trapa natans* L. is one among them commonly called as shrungataka, is a good dietary source of nutrition and the whole plant has various pharmacological applications mainly as analgesic, antibiotic, anti-diabetic and immuno-modulatory activities. From the above study, it could be concluded that the preliminary phytochemical screening showed the presence of carbohydrates, phenols, flavonoids, proteins, saponins, tannins, starch and amino acids. The aqueous extract was the richest in the presence of phytoconstituents. The pharmacognostical evaluation revealed the presence of abundant helical to spiral xylem vessels, reddish brown tannin contents in the parenchymatous cells and also the presence of black calcium oxalate crystals in the innermost cells of fruit kernel. These results may be useful criteria for identification and standardization of the genuine drug, comparison with other *Trapa* species and also the isolation of compounds could be carried out for further studies to elucidate the molecular mechanism of interactions of its various components with human body in different diseases.

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