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Research Article

Pharmacognostic and Physico-Chemical Studies on Leaves of *Syzygium zeylanicum* (L.) DC

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ABSTRACT

Syzygium zeylanicum syn. *Syzygium lineare* (Family – Myrtaceae), is also known as Poochapazham or Kaatuvazhana (Malayalam). It is a widespread evergreen large shrub. The present investigation deals with the qualitative and quantitative microscopic evaluation of the leaf material and establishment of its quality parameters, including physicochemical and phytochemical evaluation. In the microscopic studies, the leaf was found to be dorsiventral and the chief characters of transverse section includes single plano convex and collateral vascular bundle, which consists of several short 3 celled xylem rows and a thin layer of phloem on the lower end and mesophyll consists of 2 layer of thin, vertically oblong, compact palisade cells and lower part of 5 or 6 much lobed spongy parenchyma. . Chief characters of powder include thick, wavy epidermal cells, the cells being much lobed; stomata appear in deep pits and calcium oxalate druces seen scattered in surface view of the lamina. Leaf constants were analysed. Physicochemical parameters such as moisture content, chlorophyll estimation, ash values and extractive values were evaluated. Phytochemical screening revealed the presence of many therapeutically important classes of phytoconstituents such as alkaloids, flavonoids, phenolics, glycosides, sterols, terpenoids, saponins and carbohydrates. Such a study would serve as a useful tool in standardization of the leaf material, isolation of medicinally important phytoconstituents, performing pharmacological investigations and ensuring quality formulations in the future. It would also help in distinguishing the plant material of *Syzygium zeylanicum.*

INTRODUCTION

Herbal medicines are promising choice over modern synthetic drugs. They show minimum or no side effects and are considered to be safe. Generally herbal formulations involve use of fresh or dried plant parts. Correct knowledge of such crude drugs is very important aspect in preparation, safety and efficacy of the herbal product. Pharmacognosy is a simple and reliable tool, by which complete information of the crude drug can be obtained[1]. The therapeutic use of herbal medicine is gaining considerable momentum in the world during the past decade. Hence, quality control standards for various medicinal plant used in indigenous system of medicine are becoming more relevant, an important factor, which contributes the consistent quality of herbal products, is to have adequate control on the quality of medicinal plants[2]. The main aim of the present study is to investigate the pharmacognostical and phytochemical properties of leaves of *Syzygium zeylanicum*, an ethnomedicinally important plant. This investigation will be a useful marker for identification of the crude drugs obtained from the investigated taxa. *Syzygium zeylanicum* syn. *Syzygium lineare* (Family – Myrtaceae), is also known as Poochapazham or Kaatuvazhana (Malayalam).It is a widespread evergreen large shrub, attaining a height up to 1.5-2m, with soft wooded stem and widespread branches. Dark green lanceolate leaves, flowers yellow colored and with white berries. Leaves are applied externally in joint pains. Oil obtained from the leaves is used in rheumatism. The plant is reported to be stimulant, antimicrobial and anti-rheumatic, vermifuge[3]. The present research work is concerned with the leaves of the above mentioned Indian medicinal plant *Syzygium zeylanicum*, which has reported folklore uses but yet not thoroughly explored so far for their exploitation in medicinal use. The first and foremost step is the qualitative and quantitative microscopic evaluation of the leaf material of *S. zeylanicum* and establishment of its quality parameters, including physicochemical and phytochemical evaluation. This thorough evaluation would be useful in standardization of the leaf material.

MATERIALS AND METHODS

Plant material collection and authentication: The leaves of plant *Syzygium zeylanicum* (L.) DC. were collected from Mahatma Gandhi University campus, Athirampuzha, Kottayam, India, in the month of February and were positively identified and confirmed by the botanist, Mr. Joby Paul, School of Environmental science, M.G University, Athirampuzha, Kottayam and the voucher specimen, numbered 1439, has been submitted to the Department of Pharmacognosy, University College of Pharmacy, and School of Environmental science, M.G University for future references. The fresh mature leaves were used for the study of macroscopic and microscopic characters, whereas the dried uniform leaf powder was

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Table 1 : Morphology

Properties	Observation
Color	dark green
Odour	aromatic
Taste	Bitter

Fig: 1 *Syzygium zeylanicum* leaves

Table 2: Determination of leaf constants

Vein islet number	8.75
Vein termination number	8.5
Stomatal number	17.5
Number of epidermal cells	49.5
Stomatal index	26

used for the extraction of active constituents of the plant, physicochemical and phytochemical investigation.

Pharmacognostic studies

Macroscopic studies: Morphological studies were done using simple microscope. The shape, apex, base, margin, taste and odour of leaves were determined.

Microscopic studies

T.S of the Leaf: The leaf samples were cut and fixed in FAA (formalin-5 ml + acetic acid-5 ml + 70% ethyl alcohol-90 ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary butyl alcohol[4]. Infiltration of the specimen was carried out by gradual addition of paraffin wax (melting point-58-60ºC) until tertiary butyl alcohol solution attained super saturation. The specimens were cast into paraffin blocks.

The paraffin embedded specimens were sectioned with the help of rotary microtome. The thickness of the sections was 10-12 µm, dewaxing of the sections was by customary procedure[5]. The sections were stained with toluidine blue[6]. The staining results were remarkably good; and some cytochemical reactions were also obtained. The dye imparted pink color to the cellulose walls, blue to the lignified cells, dark green to the suberin, violet to the mucilage, blue to the protein bodies etc.

Powder microscopy: Powdered materials of different parts were cleared with 5% sodium hydroxide and mounted in glycerin medium after staining. Different cell components were studied and measured. Photographs of different magnifications were taken with Nikon labphoto 2 microscopic units. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was used. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale bars.

Determination of leaf constants: Few leaves were boiled with chloral hydrate in a test-tube placed on a boiling water bath, mounted the preparation in glycerin water. The camera lucida was set and Traced the stomata, epidermal cells, vein-islets and veinlet termination by looking through the microscope when a superimposed image of the leaf portion and the paper is seen at the same time[7].Stomatal index was calculated[8] using the formula: SI = S/(E+S) X 100. S- Number of stomata per unit area, E- Number of epidermal cells in the same

Physiochemical studies: The moisture content, total ash, water-soluble ash, acid-insoluble ash, alcohol and water-soluble extractive values were determined as a part of its physiochemical parameters[9]. The chlorophyll estimation was also carried out on fresh leaves[10].

Phytochemical studies: Fresh leaves were collected and shade dried at room temperature to remove moisture, and size reduced. Successive solvent extraction were carried out with solvents of increasing polarity i.e. petroleum ether, chloroform, ethyl acetate and alcohol. The extract obtained was collected and concentrated. The concentrated extract was then weighed and stored for further studies. The percentage yield of the extracts were calculated and tabulated. Qualitative chemical tests were carried out in various extracts[11].

RESULT

Pharmacognostic studies

Macroscopic studies*:* The leaves of *Syzygium zeylanicum* were observed to be dark green, ovate elliptic to linear lanceolate, coriaceous, opposite, petiolate (upto 7 mm long), pinnate veination, acuminated, shining on upper side, entire margin. Branchlets are yellowish brown when dry, round, old branches greyish brown. Flowers shortly pedicellate, forming axillary or terminal cymes, calyx funnel shaped. Berries are white, thick and fleshy, leathery, ellipsoid to sub-globose, 1-seeded. (Fig: 1)The leaves of *Syzygium zeylanicum* were found to have aromatic odour and bitter taste (Table: 1).

Microscopic studies: In the microscopic studies, the leaf was found to be dorsiventral, and shows all the typical characteristics of leaf,

T.S of leaf through Midrib: The leaf has smooth and even surface and midrib of the leaf is not much thicker than the lamina (Fig: 2). The midrib is slightly depressed on the adaxial side and slightly convex on abaxial side. It is 300 µm thick. The midrib vascular bundle is single Plano convex and collateral. It is prominent and occupies the entire space of the midrib. It consists of several short 3 celled xylem rows and a thin layer of phloem on the lower end. A thick layer of fibers occurs on the abaxial end as well as on the adaxial part (Fig: 2).

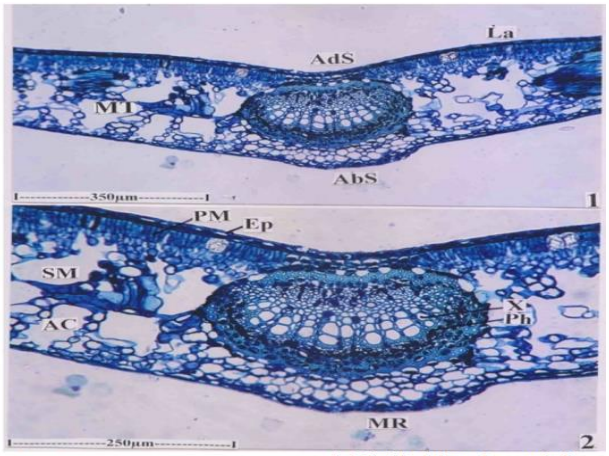

Fig : 2 1. T.S of leaf through mesophyll
2 . Midrib enlarged

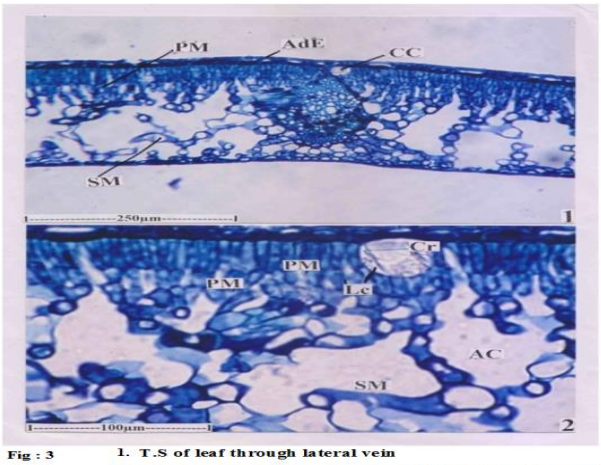

Fig : 3 1. T.S of leaf through lateral vein
2. T.S of lamina showing hypodermal lithocyst with crystal

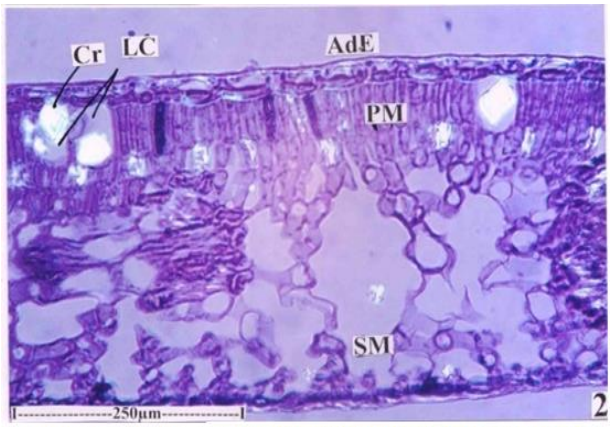

Fig : 4 T.S of lamina showing sub marginal crystal bearing lithocyst (under polarized light)

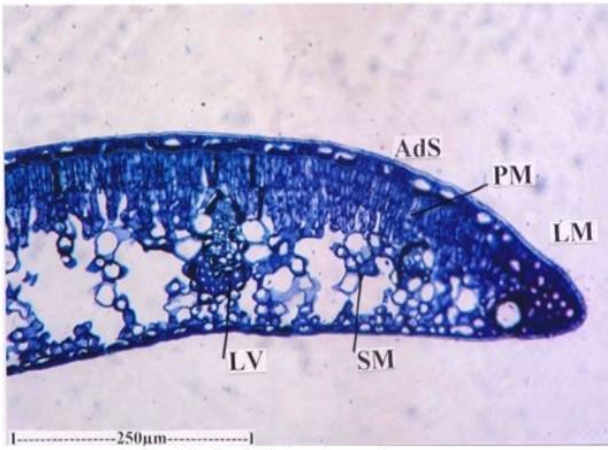

Fig: 5 T.S of leaf margin

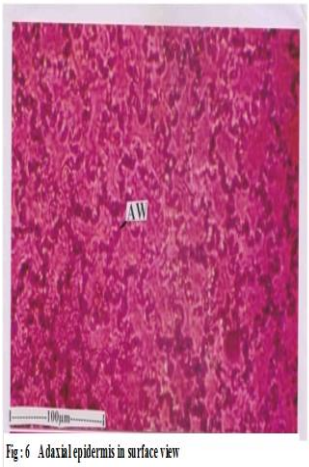

Fig : 6 Adaxial epidermis in surface view

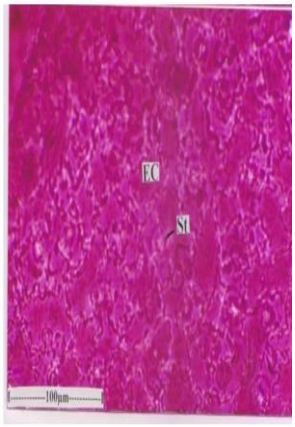

Fig : 7 Abaxial epidermis in surface view

Fig : 8 Sub epidermal crystals viewed in surface view under polarized light

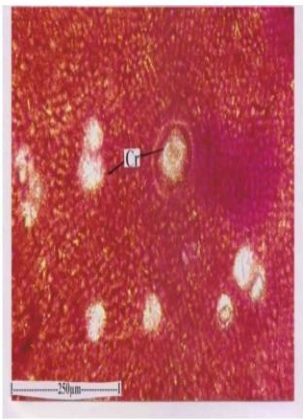

Fig : 9 Sub epidermal crystals viewed in surface view under bright field

Lamina: The lamina is 250 µm thick. The adaxial epidermal layer is thick, narrowly rectangular with thick walls. The abaxial epidermis is further thin and cells are small and spindle shaped. The mesophyll tissue consists of adaxial part of 2 layer of thin, vertically oblong, compact palisade cells and lower part of 5 or 6 much lobed spongy parenchyma linked with each other forming wide air spaces. Some of the palisade cells are modified into wide, circular lithocysts containing prominent calcium oxalate crystals of druces (Fig: 3 and Fig: 4)

Leaf margin: The leaf margin is narrowly conical with blunt end. It is 170 µm thick. The epidermal layer of the leaf margin is slightly enlarged with thicker cuticle. The extreme margin of the lamina consists of compact thick walled cells. The sub marginal part has normal palisade spongy differentiation of the mesophyll (Fig: 5)

Powder microscopy: The powder microscopy of the leaf shows the following inclusions; Adaxial epidermal cells:

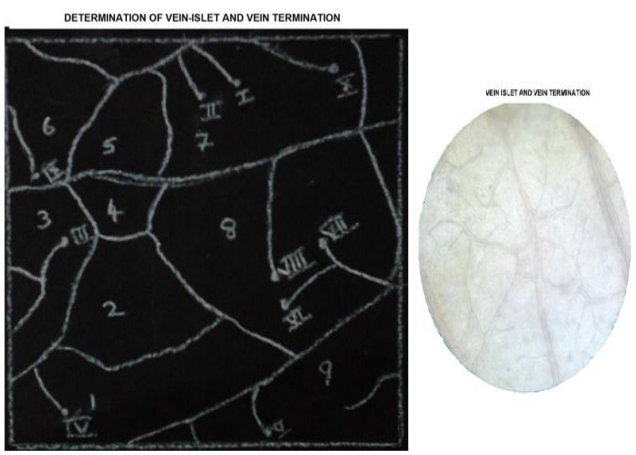

Fig : 10 Determination of vein islet and vein termination

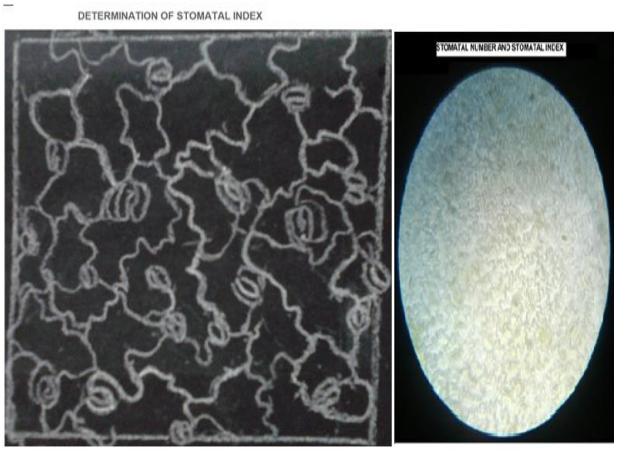

Fig : 10 Determination of stomatal number and stomatal index

seen in surface view of the peeling. The cells are thick walled, highly wavy, making the epidermal cells amoeboid in outline (Fig: 6). Abaxial epidermal cells: the abaxial epidermal peeling consists of thick, wavy epidermal cells, the cells being much lobed. Stomata appear in deep pits (Fig: 7). Crystals: calcium oxalate druces are seen scattered in surface view of the lamina. Druces occur within modified circular lithocysts. The druces are 60 µm in diameter (Fig: 8 and Fig: 9)

Determination of leaf constants: Vein islet, vein termination and stomatal index of *S. zeylanicum* fresh leaves were shown in Fig: 10, Fig: 11 and tabulated in table: 2.

Table 3: Physicochemical screening

Parameters	Results
Total ash	3.19±0.01 %w/w
Acid insoluble ash	0.17±0.02 %w/w
Water soluble ash	1.23±0.04 %w/w
Water soluble extractive value	23.8±1.35 %w/w
Alcohol soluble extractive value	9.73±0.23 %w/w
Chlorophyll a	9.13 mcg/ml
Chlorophyll b	4.87 mcg/ml
Total chlorophyll	13.10 mcg/ml
Total carotenoids	2.41 mcg/ml

Physicochemical Evaluation

Moisture content (Loss on drying): Moisture content of fresh leaves *S. zeylanicum* was found to be 41.0±0.07%w/w. Ash values, extractive values and chlorophyll content of the drug were studied and tabulated in table: 3. Colour, consistency and weight of various extracts were shown in table: 4.

Preliminary phytochemical evaluation:

Qualitative chemical tests were carried out in various extracts (Petroleum ether (PEE), Chloroform (CHE), Ethyl acetate (EAE), Alcoholic (ALE) and Aqueous (AQE) extracts. The results of the chemical tests for each extract are tabulated in the following table: 5. (++) indicate active constituents in high amount, (+) indicate active constituents in low amount, (-) indicates the absence of active constituents.

DISCUSION

Plants serve as vast source for varied phytoconstituents exhibiting varied pharmacological property. Identifying such potential plants is of significance in medicine. So it becomes necessary to study the pharmacognostic characteristic of the plant before its use in the field of research and also in pharmaceutical formulation. Moreover it also helps in distinction from other allied species and adulterants. In this connection, in the present study the pharmacognostical characteristics of the stem and leaf of the plant *S. zeylanicum* (L.) DC leaves were examined.

The present studies revealed that pharmacognostic screening can serve as a basis for preparation of the herbal monograph for proper identification, authentification and standardization of drugs. The present study on the leaf of *Syzygium zeylanicum* will help to identify the correct species of the plant, since no such scientific data are available[11].

The qualitative and quantitative analysis of various extracts of *S. zeylanicum* were carried out and extracts showed the presence of various chemical constituents such

Table 4: Character of various extracts

Extracts	Colour and consistency	Extractive value (%w/w) on dry weight basis
Total ethanolic extract (TEE)	Dark green sticky mass	18.00
Petroleum ether extract (PEE)	Green powder	5.56
Chloroform extract (CHE)	Light green powder	4.2
Ethyl acetate extract (EAE)	Brownish green powder	2.64
Alcoholic extract (ALE)	Brownish green oily mass	6.1
Aqueous extract (AQE)	Brown powder	10.6

as alkaloids, glycosides, phenolics, flavonoids, tannins, saponins, carbohydrates and steroids. This shows high level of its possible medicinal value. Ethyl acetate and aqueous extracts showed the presence of most of these phytochemicals, possessing antioxidant related activities.

Table 5: Qualitative chemical tests of extracts

Phytoconstituents	PEE	CHE	EAE	ALE	AQE
Alkaloids	-	+	-	-	-
Glycosides	-	-	-	+	+
Phenolics	-	-	++	-	+
Flavones and Flavonoids	-	-	++	+	++
Carbohydrates	-	-	-	-	+
Terpenoids	+	-	++		-
Sterols	+	-	++	-	-
Proteins and aminoacids	-	-	-	-	-
Saponins	_	_	_	_	+

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