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**PHARMACOGNOSTIC, PHYTOCHEMICAL &  
CHROMATOGRAPHIC INVESTIGATION OF *STACHYTARPHETA  
JAMAICENSIS* (L.) VAHL. PLANT & TABLET**

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**ABSTRACT**

**Background:** *Stachytarpheta jamaicensis* (L.) Vahl. (Verbenaceae) is a common weed and ornamental plant, well-known as Brazilian tea, Aaron's rod. Ethnomedical survey reveals the use of this plant in diabetes and many other disease conditions. Establishing a preliminary analytical profile of the herb is a prerequisite to its use in clinical conditions. Hence the present study was undertaken with the objective to establish analytical standards of *Stachytarpheta jamaicensis* (L.) Vahl. plant & tablet.

**Material & methods:** The aerial part of the plant was collected, authenticated and subjected to macroscopic, microscopic, physico-chemical, preliminary phytochemical analysis using standard methodology. High performance thin layer chromatography was performed with Gallic acid as reference standard.

**Results:** The macroscopic & microscopic features show unique characters of the plant. The preliminary phytochemical analysis revealed the presence of carbohydrates, proteins, saponins, tannins in aqueous & alcoholic extracts, while alkaloids, glycosides, flavonoids were detected in alcoholic extract only. HPTLC analysis revealed 2 spots (Rf value 0.22,0.36) under UV short wave, 6 spots (0.14, 0.22,0.28, 0.36, 0.49, 0.70) under UV long wave in both powder & tablet.

**Discussion & conclusion:** The results obtained from the present study serves as a reference standard for *Stachytarpheta jamaicensis* (L.) Vahl. and its tablet. The product may further be evaluated for its medicinal uses.

**Keywords:** *Stachytarpheta jamaicensis* (L.) Vahl., Pharmacognosy, phytochemistry, Diabetes mellitus, Gallic acid

## INTRODUCTION

*Stachytarpheta jamaicensis* (L.) Vahl. (SJ) belongs to the family, Verbenaceae. It is commonly known as Brazilian tea, Blue porter weed, blue snake weed, Aaron's rod and is usually seen growing as a weed along road side, barren land fields & also grown in gardens for its beautiful flowers [1, 2, 3]. The plant is native to Mexico, Central America, tropical South America, south-eastern United States of America, the Carribean and and the Galapagos Islands [2].

*S. jamaicensis* (L.) Vahl. is an annual plant, reaching about 0.3- 0.9 m. high; stems erect, dichotomously branched & nearly glabrous with the young branches being quadrangular. Leaves 5-10 by 2.5-3.8 cm. elliptic, obtuse or acute, coarsely serrate, glabrous or nearly so, base much tapering and decurrent into the petioles which are consequently obscure. Flowers sessile, in long, slender glabrous spikes reaching 30cm long; corolla deep blue, tube 1 cm. long, slightly curved. Fruit is ribbed and splits into 2 pyrenes [4].

Various research surveys have documented ethnobotanical uses of the plant. The plant is used externally for purulent ulcers,

wound [4] and given internally for haemorrhoids [5], rheumatic inflammations [4], dysentery [4, 6]. Leaves are used in typhoid [7], malaria [8, 9], diabetes [10, 11], skin diseases [12], cough [13]. Previous phytochemical screening reveals the presence of carbohydrates, reducing sugars, alkaloids, glycosides, saponins, tannins, flavonoids, resins, proteins, oils, steroids, terpenoids from different extracts [3, 14]. Some of the isolated compounds are gallic acid [15], apigenin, ursolic acid, luteolin [16]. Earlier researches on *S. jamaicensis* have investigated multitude pharmacological activities like antimicrobial, antifungal, antioxidant, anti-inflammatory, antinociceptive, antidiarrheal, antihypertensive, antidyslipidaemia, hepatoprotective, anticancer, wound healing [17], anti-trypanosomal activity [18], antidiabetic [19, 20] & found effective in various experimental models.

The leaves and the whole plant have been used against diabetes and experimental study on animals also reveals its antidiabetic activity [19, 20]. But not clinically investigated as per the knowledge of authors and hence the plant was chosen

to clinically evaluate for its antidiabetic effect. To make it palatable & easy to administer, tablets was prepared from the aerial parts of the plant. Prior to clinical trial commencement, the present study was undertaken to pharmacognostically evaluate the raw material and establish quality standards of the pharmaceutical product, tablet of *S. jamaicensis* (L.) Vahl. (SJ tablet)

## MATERIAL & METHODS

### Plant collection & authentication

The whole plant of *Stachytarpheta jamaicensis* (L.) Vahl. was collected from local fields in Hassan district. The authentication of the sample was carried out by at Department of Dravyaguna, Sri Dharmasthala Manjunatheshwara College of Ayurveda & hospital, Hassan, Karnataka, India and a voucher herbarium specimen (SDMHSN-DG-20/11) maintained in the same laboratory for future reference. A part of the whole plant including stem, leaves, and flowers was kept separately for macroscopic, microscopic & sensory evaluation and the rest was cut into smaller pieces, dried under shade. A portion of the dried sample was kept in an air tight container for macroscopic and sensory evaluation while another portion was pulverized into coarse powder. It was stored in a well closed container, free from environmental climatic changes, any other contamination till usage for further studies.

### Macroscopy

The external features of whole plant of *S. jamaicensis* (L.) Vahl. were documented using digital camera. The macroscopic features of the leaves, including type of margin, venation, size, shape, base, apex, mid-rib, surface character and texture were observed and noted. Similarly, the features of the stem were also documented. The organoleptic properties such as colour, odour and taste were also observed and noted as per standard procedures [21]. The macroscopic features were compared to previous published literature.

### Microscopy

The microscopic sections of different parts and powder microscopy were carried out as per standard procedures [21]. The different parts like stem, leaf of fresh plant of SJ were cut into thin transverse section using a sharp blade and the sections were stained with safranin and observed under a compound microscope. The sections were photographed using camera under bright field light.

### Powder microscopy

A pinch of *Stachytarpheta jamaicensis* (L.) Vahl. powder was put on the slide, treated with various reagents, mounted in glycerine and powder characters observed under a compound microscope. The features were photographed using camera under bright field light.

### Physicochemical standards

The physicochemical evaluation includes tests as per Ayurvedic Pharmacopeia of India for herbs. This included foreign matter (%w/w), total ash (%w/w), acid insoluble ash (%w/w), alcohol soluble extractive (%w/w), and water-soluble extractive (% w/w). Analysis of the sample as per API norms [22] was conducted at Department of Dravyaguna, Sri Dharmasthala Manjunatheshwara College of Ayurveda & hospital, Hassan, Karnataka, India. All the tests were performed in triplicates and data presented as Mean  $\pm$  SD.

#### **Preliminary phytochemical evaluation**

The aqueous and alcoholic extracts of whole plant of *Stachytarpheta jamaicensis* (L.) Vahl. were screened for phytoconstituents using standard reagents and methodology [21].

#### ***Stachytarpheta jamaicensis* (L.) Vahl.**

##### **Tablet (SJ tablet) preparation**

SJ tablets were prepared as per the standard procedures delineated [23]. Authenticated aerial parts of SJ was collected, chopped into small pieces and dried under shade. After ensuring complete drying, the raw drug was pulverized to coarse powder. A portion of coarse powder was stored separately in an air tight container. The rest of the coarse powder was further pulverized to obtain fine powder. Decoction was prepared using coarse powder. The prepared decoction was used to triturate the

fine powder of SJ and kept for drying. The dried powder was punched to obtain 500mg tablets. The prepared tablets were stored in air tight container for further analytical study.

##### **Quality standards for *Stachytarpheta jamaicensis* (L.) Vahl. Tablet (SJ tablet)**

The quality parameters for SJ tablet were carried out as per standard guidelines [24]. This included the general description, organoleptic evaluation, microscopic characters, physicochemical parameters like loss on drying, total ash (%w/w), acid insoluble ash (%w/w), alcohol soluble extractive (%w/w), and water-soluble extractive (% w/w) and other tests like Friability, hardness, pH (5% aqueous extract), uniformity of weight and disintegration time of the tablet. Analysis of the sample as per norms was conducted at Departments of Dravyaguna & Rasashastra-Bhaishajya kalpana, Sri Dharmasthala Manjunatheshwara College of Ayurveda & Hospital, Hassan, Karnataka, India.

##### **High performance thin layer chromatography (HPTLC) of the plant and tablet of *Stachytarpheta jamaicensis* (L.) Vahl**

1gm of sample of *Stachytarpheta jamaicensis* (L.) Vahl powder and SJ tablet was dissolved in 20.0ml of methanol, kept overnight, filtered, removed solvent under reduced pressure and dissolved in

10.0ml of methanol. 4 $\mu$ l of each of the above extract, gallic acid (marker compound) was applied on a pre-coated silica gel F<sub>254</sub> on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. Sample was developed in Toluene: Ethyl Acetate: Formic acid (5: 2.5: 0.1). The developed plates were visualized in short UV, long UV and then derivatised with Vanillin sulphuric acid (VSA) reagent subsequently scanned under UV 254nm, 366nm and 620nm (after derivatisation). R<sub>f</sub>, colour of the spots, densitometric scan were recorded.

## OBSERVATION & RESULTS

### Macroscopic features

The macroscopic features of the leaf & stem are tabulated in the **Table 1 & 2**, respectively. (**Figure 1**) The leaves and stem had characteristic odour and was bitter to taste.

### Microscopic features

Transverse section of the stem is circular in outline, slightly compressed. The outer most layer is a single layer of epidermis covered with cuticle. The cortex is composed of outer 3-4 layers of collenchymatous cells, followed by closely packed 6-7 layers of chlorenchymatous cells. The cortex is followed by the vascular bundles, with the phloem outside and xylem within. At the centre is the pith made up of parenchymatous cells. Cells

with purple pigment are present in the cortex (**Figure 2**).

Transverse section of the leaf through the midrib, shows a single layer of upper epidermis and a single layer of lower epidermis. At the midrib, below the upper epidermis are 3-4 layers of collenchyma cells, while above the lower epidermis, are 2-3 layers of collenchyma cells. At the centre the vascular bundles are arranged as an arc. The Mesophyll is composed of 2 layers of palisade parenchyma and spongy parenchyma (**Figure 3**).

### Powder microscopy

The powder of the plant revealed the presence of starch grains, epidermal cells with stomata, vessels, fibres. The leaf epidermal cells show anticlinal sinuous walls with diacytic stomata (**Figure 4**).

### Physico-chemical standards

The physico-chemical standards of the *Stachytarpheta jamaicensis* (L.) Vahl. are tabulated in **Table 3**. Similarly, the physical standards of SJ tablet are tabulated in **Table 4**.

### Phytochemical evaluation

The preliminary phytochemical analysis revealed the presence of carbohydrates, proteins, saponins, tannins in aqueous & alcoholic extracts, while alkaloids, glycosides, flavonoids were detected in alcoholic extract only.

**High performance thin layer chromatography of the plant and tablet of *Stachytarpheta jamaicensis* (L.) Vahl**  
HPTLC photo documentation of methanolic fraction of *Stachytarpheta jamaicensis* (L.) Vahl powder and tablet revealed 2 spots (Rf values- 0.22, 0.36) at UV short wave; 6 spots (0.14, 0.22, 0.28,

0.36, 0.49, 0.70) at UV long wave; while post derivatisation, powder & tablet showed common spots at Rf value 0.49 & 0.78. There was an extra spot at Rf value 0.58 in powder and at 0.53 in tablet (**Figure 5 & Table 5**). Gallic acid, which was used as a marker compound (Rf 0.08) was not detected in either powder or tablet.

**Table 1: Macroscopic features of *Stachytarpheta jamaicensis* (L.) Vahl leaf**

Parameters	Observations
Leaf type	simple
Leaf phyllotaxy	Opposite, decussate
Leaf shape	Ovate or obovate
Leaf size	4-14 cm long and 2-6 cm wide
Leaf base	Symmetrical, tapering into the petiole
Leaf apex	Acute to obtuse
Midrib	Raised on the underneath
Leaf margin	Serrate to dentate
Leaf surface	Hairy along the veins on the under surface of the leaf
Leaf texture	Herbaceous
Leaf colour	Green on the upper surface, slightly dull green on the lower surface. Purple on the midrib till $\frac{3}{4}$ of its length

**Table 2: Macroscopic features of *Stachytarpheta jamaicensis* (L.) Vahl stem**

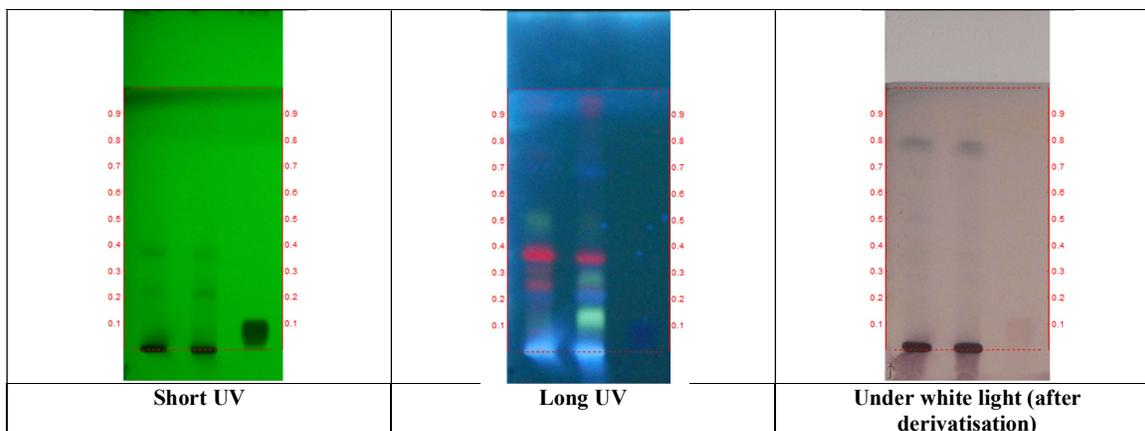
Parameters	Observations
Shape	Oval, slightly flattened
colour	Green, purple tinged on the inner side
Surface	Hairy at angles, strigose

**Table 3: Physico chemical standards of *Stachytarpheta jamaicensis* (L.) Vahl plant**

<i>Stachytarpheta jamaicensis</i> (L.) Vahl plant (n= 3)	Mean± SD
Foreign matter	0.07±0.12
Loss on drying at 105°C	17.3±0.17
Total ash	7.67±0.58
Acid insoluble ash	1.67±0.58
Water soluble extractive	24.33±1.15
Alcohol soluble extractive	15.67±0.58

**Table 4: Quality standards for *Stachytarpheta jamaicensis* (L.) Vahl tablet**

<i>Stachytarpheta jamaicensis</i> (L.) Vahl tablet	Mean± SD (n=3)
Loss on drying at 105°C	12.2
Total ash	8.67±0.58
Acid insoluble ash	2.00±0.00
Water soluble extractive	42.33±1.53
Alcohol soluble extractive	16.00±0.00
Friability	1.68%
Hardness	3 kg
Uniformity of Weight	0.56-0.50
Disintegration time	18min 26 sec
pH	3.96



Track 1- *Stachytarpheta jamaicensis* powder – 4µl

Track 2- *Stachytarpheta jamaicensis* tablet – 4µl

Track 3- *Gallic acid* (Marker compound- MC) – 2µl

Solvent system – Toluene: Ethyl Acetate: Formic acid (5.0: 2.5: 0.1)

Figure 5: HPTLC photo documentation of methanolic fraction of *Stachytarpheta jamaicensis* (L.) Vahl powder and tablet

Table 5: Rf value of *Stachytarpheta jamaicensis* (L.) Vahl powder and tablet

Short UV			Long UV			Under white light (after derivatisation)		
SJ powder	SJ tablet	Gallic acid	SJ powder	SJ tablet	Gallic acid	SJ powder	SJ tablet	Gallic acid
-	-	0.08 (Green)	-	-	0.08 (F. blue)	-	-	0.08 (Purple)
-	-	-	0.14 (F. green)	0.14 (F. green)	-	-	-	-
0.22 (Green)	0.22 (Green)	-	0.22 (F. green)	0.22 (F. green)	-	-	-	-
-	-	-	0.28 (F. green)	0.28 (F. green)	-	-	-	-
0.36 (Green)	0.36 (Green)	-	0.36 (F. red)	0.36 (F. red)	-	-	-	-
-	-	-	0.49 (F. green)	0.49 (F. green)	-	0.49 (Purple)	0.49 (Purple)	-
-	-	-	-	-	-	-	0.53 (Purple)	-
-	-	-	-	-	-	0.58 (Purple)	-	-
-	-	-	0.70 (F. blue)	0.70 (F. blue)	-	-	-	-
-	-	-	-	-	-	0.78 (Purple)	0.78 (Purple)	-

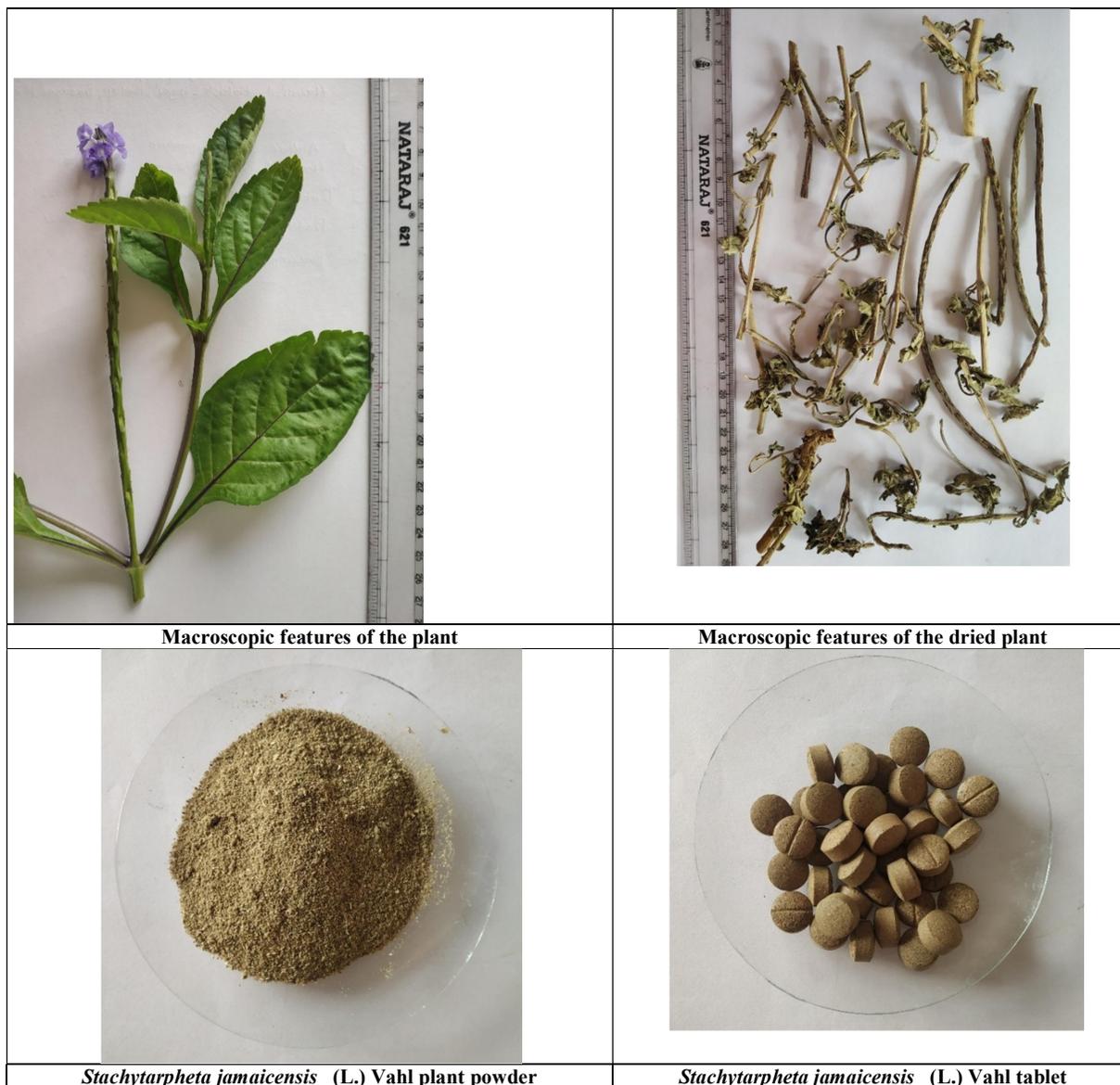
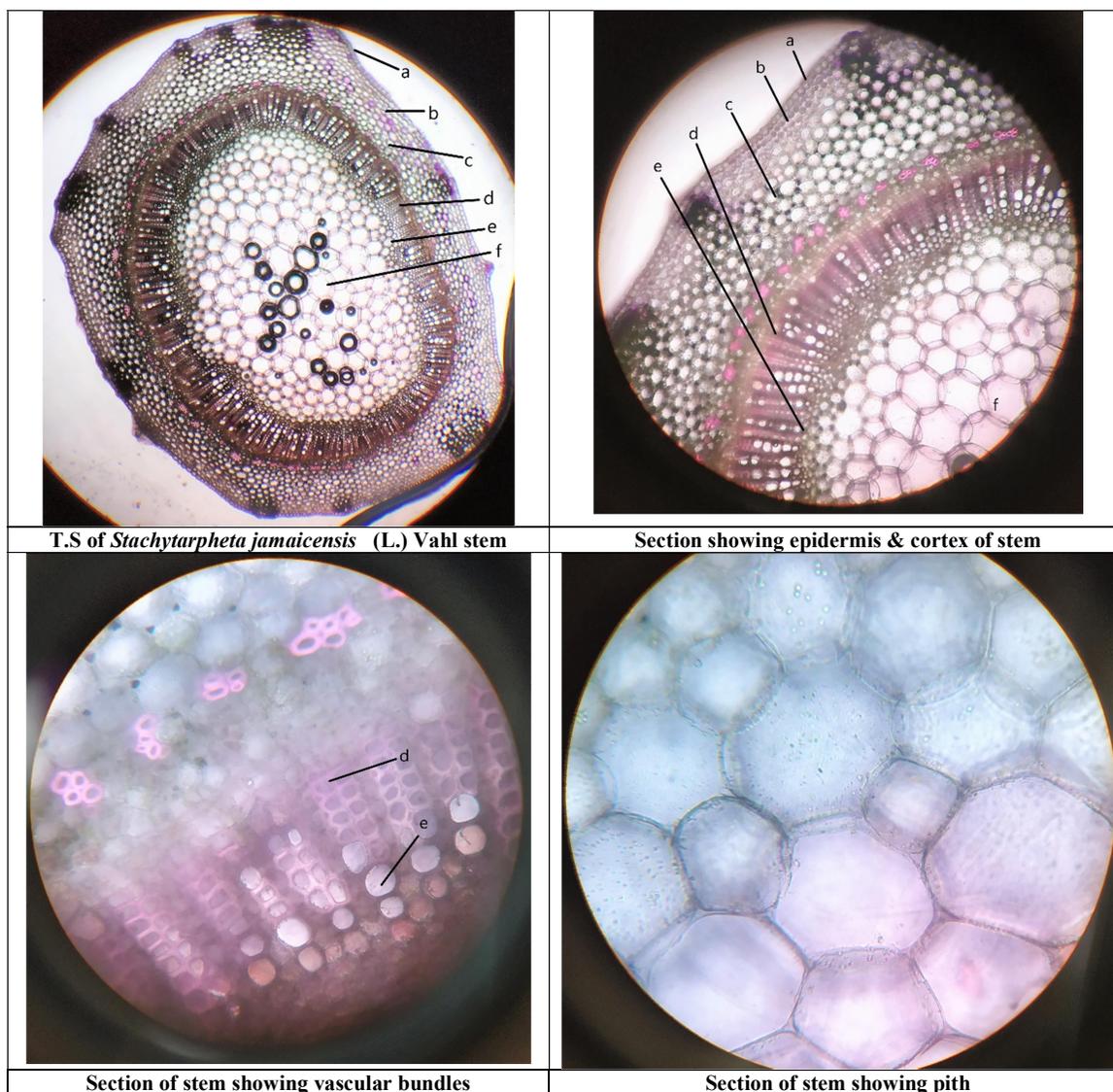


Figure 1: Macroscopic features of *Stachytarpheta jamaicensis* (L.) Vahl



T.S of *Stachytarpheta jamaicensis* (L.) Vahl stem

Section showing epidermis & cortex of stem

Section of stem showing vascular bundles

Section of stem showing pith

Figure 2: T.S of *Stachytarpheta jamaicensis* (L.) Vahl stem (a- epidermis, b- collenchyma below epidermis, c- Chlorenchyma, d- phloem, e- xylem, f- pith)

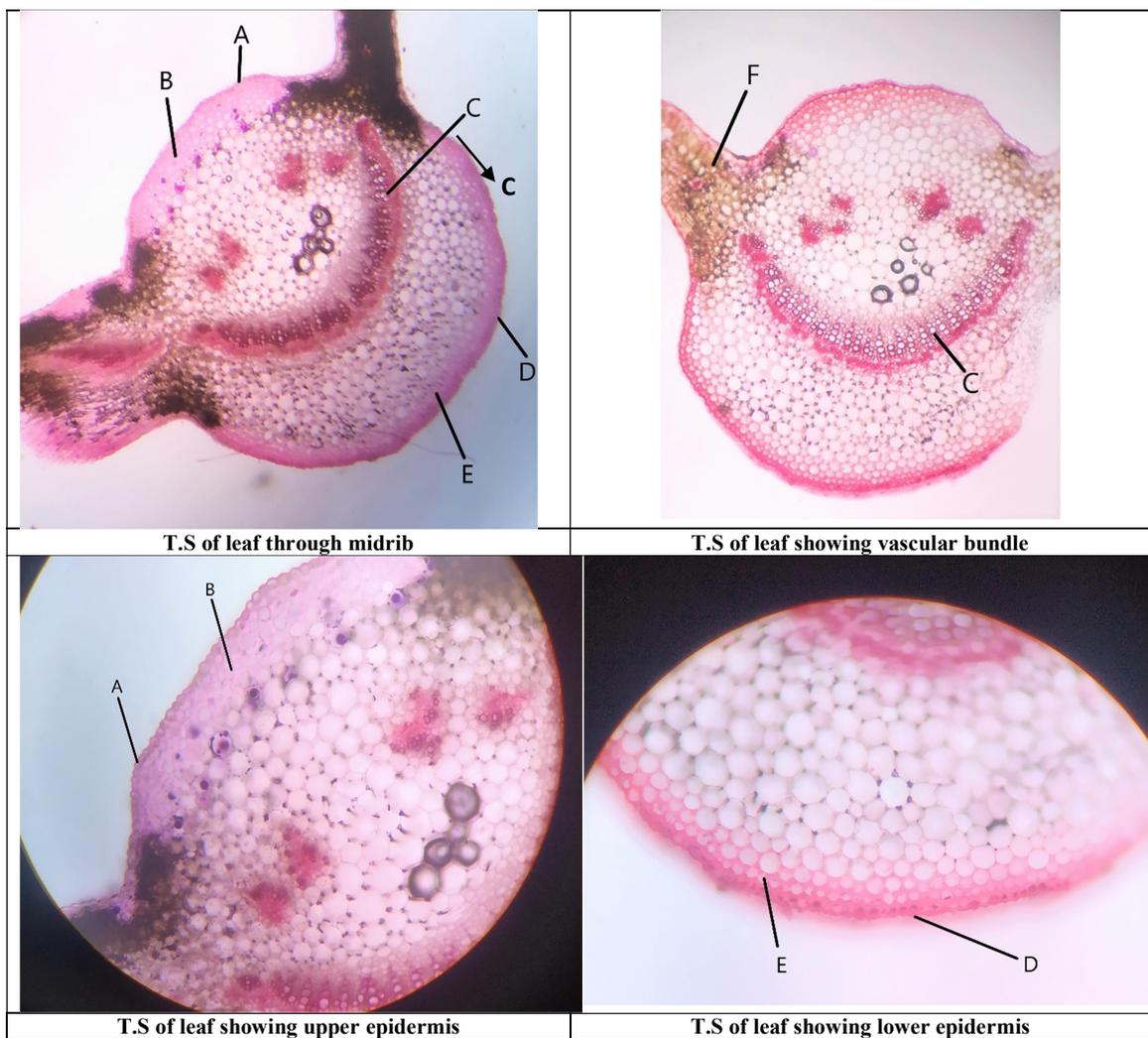


Figure 3: T.S of *Stachytarpheta jamaicensis* (L.) Vahl leaf through the midrib (A- upper epidermis, B- collenchyma under epidermis, C- vascular bundle, D- lower epidermis, E- collenchyma above lower epidermis, F- Palisade parenchyma)

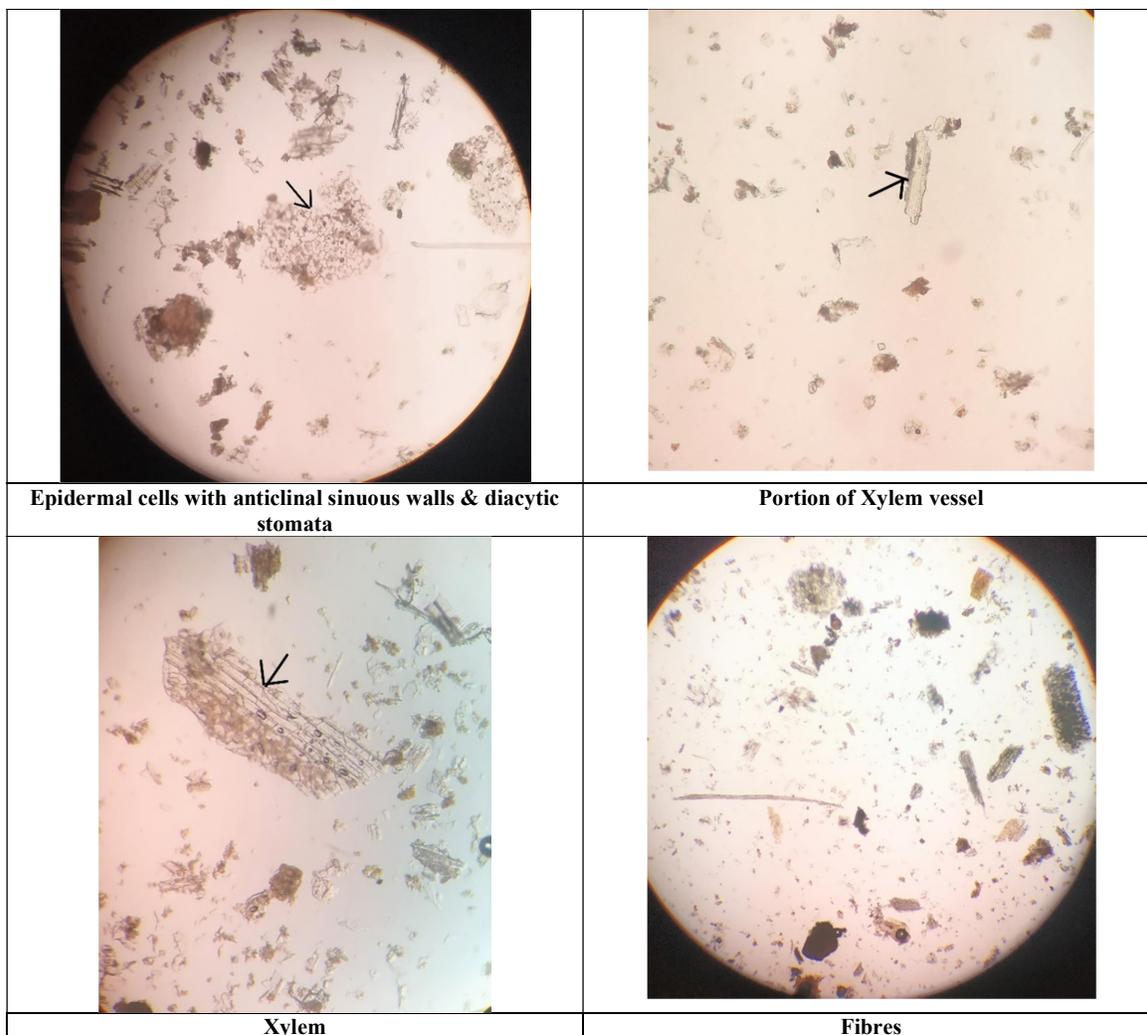


Figure 4: Powder microscopy of *Stachytarpheta jamaicensis* (L.) Vahl plant

## DISCUSSION

The present study was undertaken to develop analytical standards of *S. jamaicensis* (L.) Vahl plant and its tablet. The macroscopic features of the plant parts correlate to published literature [2, 17]. The microscopic section of the stem and leaf, powder microscopy revealed structures similar to previous findings [14, 25, 26, 27]. Physico-chemical standards of SJ plant was carried out and the values of Total ash, Acid insoluble ash & extractive values

agree with previous studies [14, 25]. The analytical standards obtained for the tablet may be used as reference standards for the product. Preliminary phytochemical evaluation reveals the presence of secondary metabolites in the plant and the results of the present study agree with previous studies. In an earlier study, the powdered leaf extracts were screened for its phytochemicals and found to contain carbohydrates, alkaloids, saponins, flavonoids, phenolic compounds [19, 25,

28]. These bioactive components present in the plant are responsible for the varied pharmacological action as evidenced in the antioxidant activity [29], hypoglycaemic potential [19], antiarthritic, anti-inflammatory activities [30] of SJ. Gallic acid is one of the active biocomponents of SJ and hence was chosen as a marker compound. But in the present study it was not detected in both the samples, probably due to its quantity, difference in solvent used in the analysis. In a previous study, gallic acid was identified through HPLC analysis of ethanolic extract & it was detected in the concentration of 4.43mg/L from SJ extract obtained from supercritical CO<sub>2</sub> extraction, 3.92mg/L from soxhlet extraction [15]. Thus, the other phytoconstituents present may also be used as reference for identification.

### CONCLUSION

The macroscopy, microscopy, physico-chemical evaluation, phytochemical analysis & chromatographic profile of the present study serves as reference standards for *S. jamaicensis* (L.) Vahl plant powder and its tablet. The herb & product may further be evaluated for various pharmacological activities on human population.

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