

Microscopic Investigations and Pharmacognosy of *Striga orobanchioides* Benth.

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ABSTRACT

Objective: To study delineate Pharmacognosy of the stem of plant *Striga Orobanchoioides* Benth (Scrophulariaceae), prime plant in Indian system of medicine. A comprehensive account on standardization of herbal drug *Striga Orobanchoioides* Benth by using microscopic as well as Pharmacognostic parameters. In the field of herbal medicines, the main issues are quality, purity, and effectiveness, as in many cases herbal drugs are knowingly or unknowingly substituted or adulterated with similar species or varieties. **Methods:** The macroscopy, microscopy, physicochemical analysis, preliminary phytochemical testing of the powder of plant stem and other WHO recommended methods for standardization was done. **Results:** T S of young as well as old stem was studied. T.S. of the young stem confirmed the presence of thin epidermal layer, fairly wide cortex and thin vascular cylinder having the pith disintegrated or retained as homogeneous parenchymatous tissue. T.S. of the old stem confirmed the presence of cortical cells, parenchyma cells, sclerenchyma cells, vascular tissue. The lower conical part of the stem has epidermis cortical zone and vascular cylinder continued from its wide upper part. The cortical zone consists of parenchymatous ground tissue with sealed masses of sclerenchyma cells. The xylem tissue becomes gradually narrow and thin in the end of the stem. It includes compact radial lines of vessels, filies and xylem rays. **Conclusion:** The above parameters, being reported to the first time for the studied plant species, and are significant towards establishing the microscopic and Pharmacognostic standards for future identification and authentication of genuine herbal drug. It can be concluded that the Pharmacognostic outline of *Striga Orobanchoioides* plant is beneficial in developing standards for quality, purity and sample identification.

Key words: *Striga Orobanchoioides*, Pharmacognosy, Stem.

INTRODUCTION

Striga Orobanchoioides Benth (Family—Scrophulariaceae), is a leecher plant, lives on the roots of diverse plants principally on *Euphoria antiquorum*, *Dysophylla* and *Lepidagathis* and is dispensed up to 6000 ft in the hillocks, habitually on red and gravelly soils.¹ In Ayurveda, the plant is narrated as antidiabetic.² Primary learning have manifested noteworthy anti-implantation and estrogenic activity of ethanolic and distilled water extracts of the whole plant *S. orobanchoides*.³ The ethanolic extract has also manifested noteworthy antiandrogenic,⁴ antibacterial,⁵ antihistaminic and mast cell stabilising activities.⁶ Among the ethanolic extract, two known flavonoids, apigenin and luteolin which have also manifested antifertility properties have been isolated.^{7,8} Except for these studies, so far, no other chemical and biological investigations have been carried out on this plant.

Hence, the current study was done to delineate pharmacognosy of the stem of plant *Striga Orobanchoioides* Benth (Scrophulariaceae).

MATERIALS AND METHODS

Chemicals and instruments

Potassium hydroxide, hydrochloric acid, Phloroglucinol, glycerin and all other chemicals

used in the study were of analytical grade. Rotary microtome was used for taking sections.

Plant material

The plant specimen for the offered study was collected from Pimparne Mountain, Ahmednagar district, Maharashtra in August 2018 and was authenticated by Dr. Wabale Anil Sopanrao Head of Department of Botany PVP college of Arts, Science and Commerce, Pravaranagar, vide letter number PVPC/ 2018-19-HD-45 dated 28/08/2018, Specimen no. SRV 123.

Macroscopic and microscopic analysis

The paraffin embedded specimens of *Striga Orobanchoioides* were sectioned in with the help of rotary microtome. The thickness of the sections was 10 to 12 micrometer. The waxing of the sections was by customary procedure.⁹ The sections were stained with toluidine blue as per the method published by O'Brien *et al.*¹⁰ Since toluidine blue is a polychromatic stain.

The staining results were remarkably good and some site of chemical reactions were also obtained. For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid¹¹ were prepared. Glycerin

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mounted temporary preparations were made for macerated materials. Powdered materials of different parts were cleared with sodium hydroxide and mounted in glycerin medium after Figure 1.

Photographs of different magnifications were taken with Nikon lab photo 2 microscopic unit. For normal observations bright field was used. For the study of crystals starch grains and lignified cells polarized light was employed. 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. Descriptive terms of the anatomical features are as given in the standard anatomy books.¹²

Determination of leaf constants

Following leaf constants were determined (Evans, (1997); Kokate, (2001); Kokate *et al.*, (2003).

a) Stomatal number: Stomatal number is the average amount of stomata per square millimeter of epidermis.

b) Stomatal index: Stomatal index is the ultimate divisions of the epidermis of a leaf percentage which have been changed into stomata.

$$I = \frac{S}{E+S} \times 100$$

S = quantity of stomata per unit area and

E = amount of ordinary epidermal cells in the same unit area.

c) Vein-islet number: Vein-islets per sq. mm calculated from four contiguous squares in the central portion of the lamina, midway in the middle of the midrib and the margin.

d) Veinlet termination number: The quantity of veinlet terminations was determined per sq. mm of the leaf surface.

e) Palisade ratio: The average number of palisade cells under each higher epidermal cell is called the palisade ratio.

Physiochemical analysis

Different extractive values like alcohol soluble extractive, water soluble extractive values and Ash values were carried out by standard methods of IP.¹³

Foreign organic matter was ascertained from the weight of the drug taken and moisture content was ascertained by loss on drying method in terms of percent w/w as per standard procedure raised in Indian Pharmacopoeia.¹⁴

Preliminary phytochemical screening

Preliminary phytochemical screening was accomplished using standard procedure ascertained by Kokate (1994).¹⁵

RESULTS

Macroscopic characteristics

A pubescent herb 2–24 in. high; stems and branches angular, leaves scale-like, opposite, alternate and scattered.

Microscopic characteristics

a) Young stem: the young stem is conical in sectional view with one end being thick and the opposite end narrow (Figure 1.1)

The stem consist of thin epidermal layer, fairly wide cortex and thin vascular cylinder. The pith is disintegrated or retained as homogeneous parenchymatous tissue (Figures 1.1 and 1.2)

The vascular cylinder is thin comprising xylem cells and phloem elements (Figure 1.2). The xylem elements consist of narrow angular

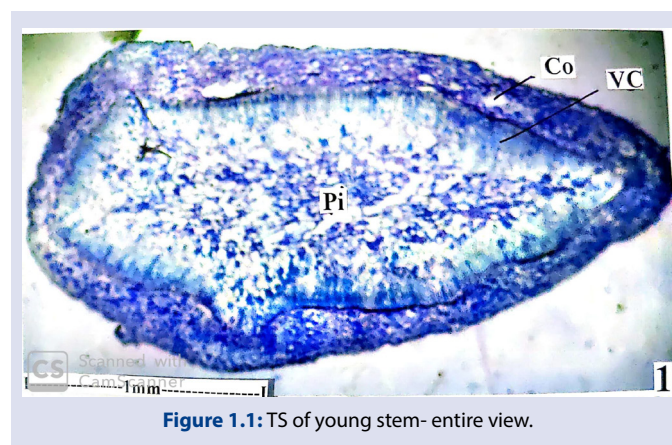


Figure 1.1: TS of young stem- entire view.

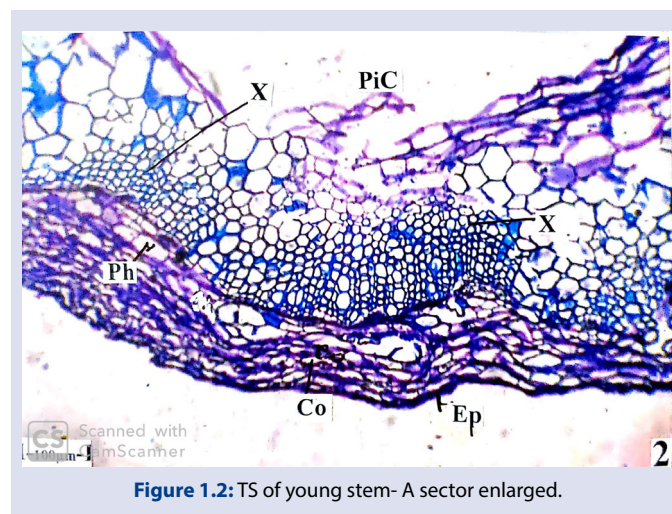


Figure 1.2: TS of young stem- A sector enlarged.

thin walled vessels and narrow thick walled fibers. Phloem elements are in thin compressed layers.

b) Old Stem: the old stem is conical with broad end measuring 2 mm thick and narrow end measuring 1 mm thick (Figure 2).

The wider part of the stem has thin less prominent epidermal layer followed internally followed by some 4 layers of tangentially elongated thin walled cortical cells.

Further inner part has circular thin walled parenchyma cells. Thick are of sclerenchyma cells is located adjacent to the vascular tissue figure 3.1. The vascular strand of the wider stem part has thin layer of phloem elements and fairly thick cylinder of xylem. The xylem elements are in compact parallel lines of fibers vessels and parenchyma cells figures 3.1 and 4.1. All these cells are thick walled and lignified.

Lower narrow part of the stem: the lower conical part of the stem has epidermis cortical zone and vascular cylinder continued from its wide upper part. The cortical zone consists of parenchymatous ground tissue with sealed masses of sclerenchyma cells. The xylem tissue becomes gradually narrow and thin in the end of the stem. It includes compact radial lines of vessels, fibres and xylem rays figure 3.2. The vessels are narrow angular and thin walled. The fibers are small thick walled and lignified. They occur in large irregular masses in the cortical zone figure 3.2.

Powder microscopy

Powder preparation of the stem shows the following elements

a) Parenchyma cells: long cylindrical wide parenchyma cells are common in the powder figure 5.1. The cells have dense cell contents.

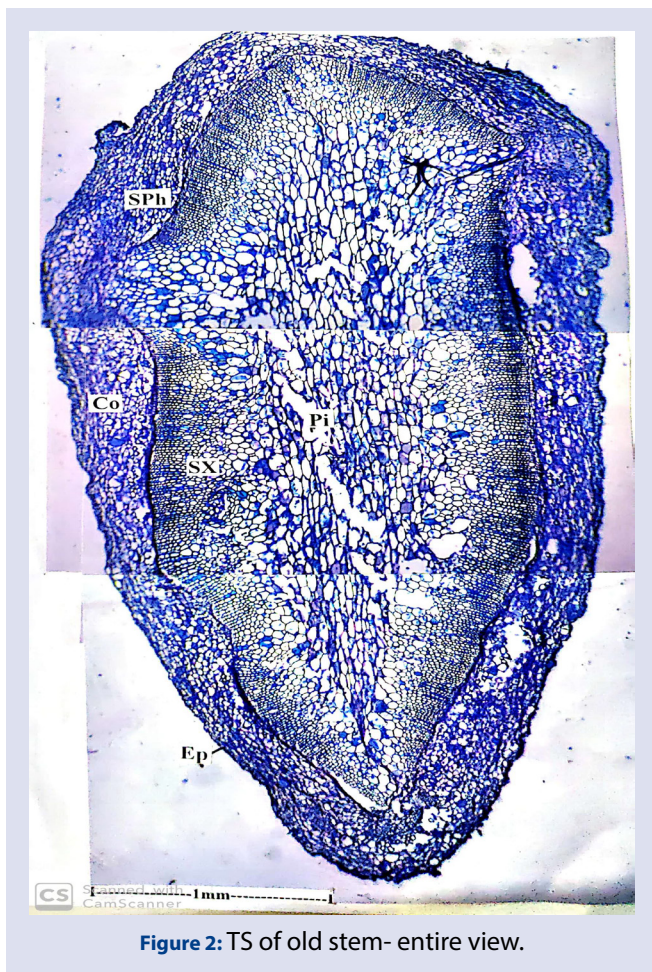


Figure 2: TS of old stem- entire view.

The cell walls are thin. The cells are 200 to 310mm long and 20 mm wide.

b) Xylem elements: xylem elements with double helical thickenings and closed spiral thickenings are found in loose bundles figure 5.2. These elements are primary xylem elements and they have thick lignified walls. The xylem elements are 100 to 270 mm long and 20 mm wide.

c) Fibers: liberiform fibers are common with the powder.

There are two types of fibers

- i) Thin long highly thick walled fibers with pointed ends are narrow fibers figure 5.2 and 6.2 these fibers are 500 mm long and 10 mm thick
- ii) Wide fibers also common in the powder. These fibers are 250 to 580 mm long and 20 mm wide. The cell himen is wide and the cell walls are thin (figures 6.1 and 6.2).

d) Vessel elements: two types of vessel element are seen in the powder.

i) Long narrow cylindrical vessel elements with thick cell walls these cells are called narrow vessels figure 7.1 some of the vessel elements have narrow tails at us ends figure 7.1. Others are tail less. The narrow vessel elements have oblique and wall perforation.

The narrow vessel elements are 410mm long and 30 mm wide.

ii) Wide vessel elements figure 7.2:

The wide vessel elements are unusually wide and dilated thick and elliptical in shape. They are 250 to 370 mm long and 72 to 120 mm wide. The cells are thin walled with wide or narrow elliptical lateral wall pits. The end walls have wide horizontal perforations.

Leaf constants

The parameters evaluated are presented in Table 1.

Preliminary phytochemical screening

Preliminary phytochemical screening mainly divulged the presence of alkaloid, tannins, glycosides, carbohydrates, proteins and flavonoids in ethanol extract (Table 2).

Fluorescence analysis

The result of fluorescence analysis is shown in Table 3. The leaf powder fluoresced green under daylight with short UV light (254 nm), dark green under long UV light (365 nm). The *Striga orobanchioides* leaf showed the characteristic fluorescent green treated with 50% H₂SO₄, concentrated HNO₃, 50% HNO₃, NaOH, ethanol under UV light.

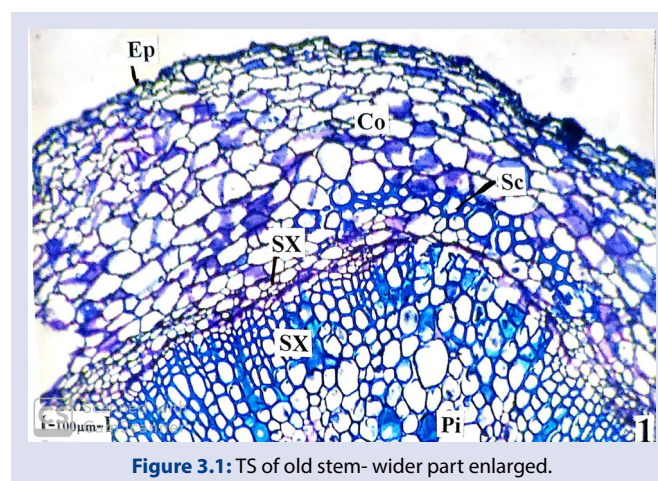


Figure 3.1: TS of old stem- wider part enlarged.

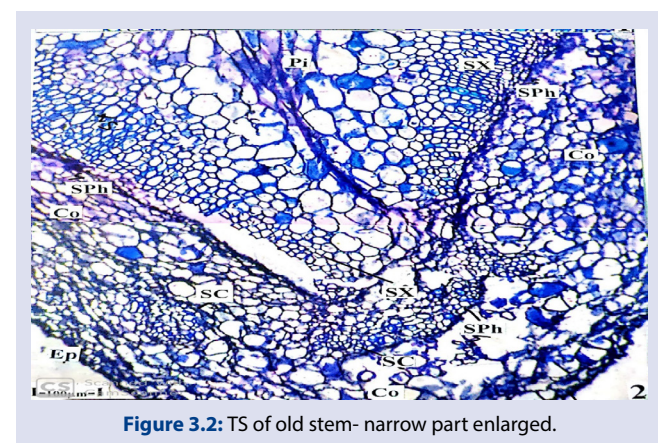


Figure 3.2: TS of old stem- narrow part enlarged.

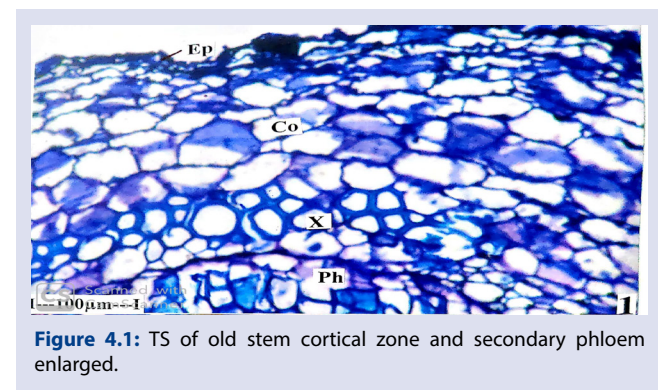


Figure 4.1: TS of old stem cortical zone and secondary phloem enlarged.

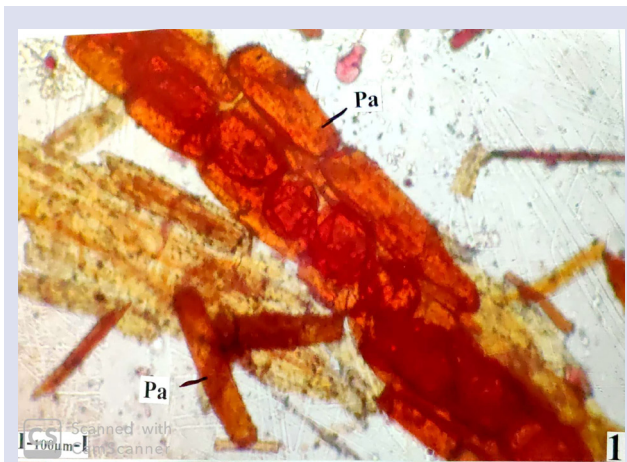


Figure 5.1: Isolated thick parenchyma cells [Powder microscopy].

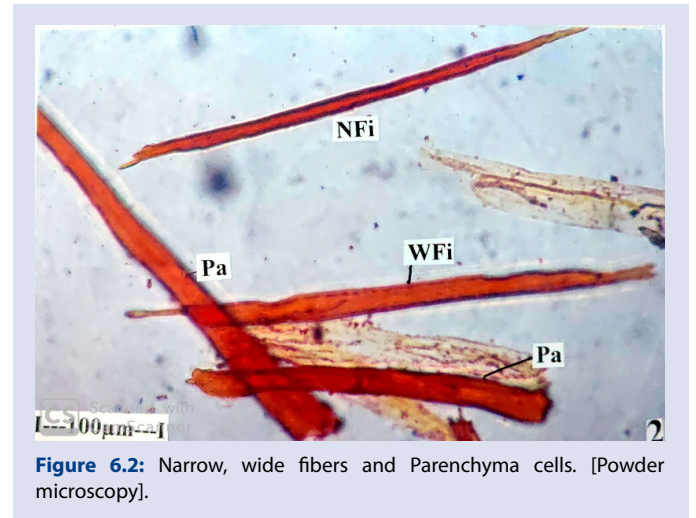


Figure 6.2: Narrow, wide fibers and Parenchyma cells. [Powder microscopy].

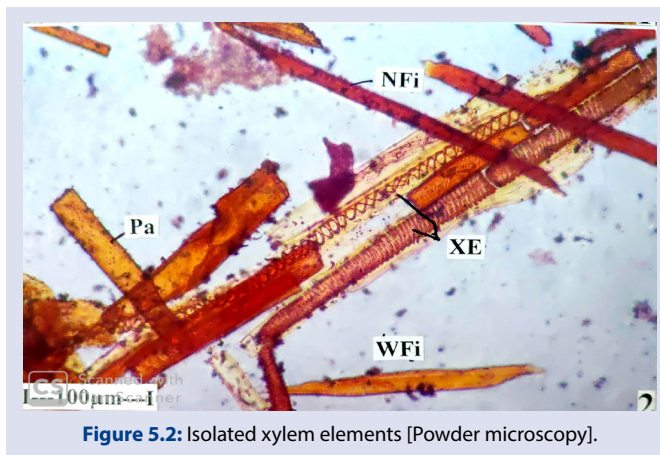


Figure 5.2: Isolated xylem elements [Powder microscopy].

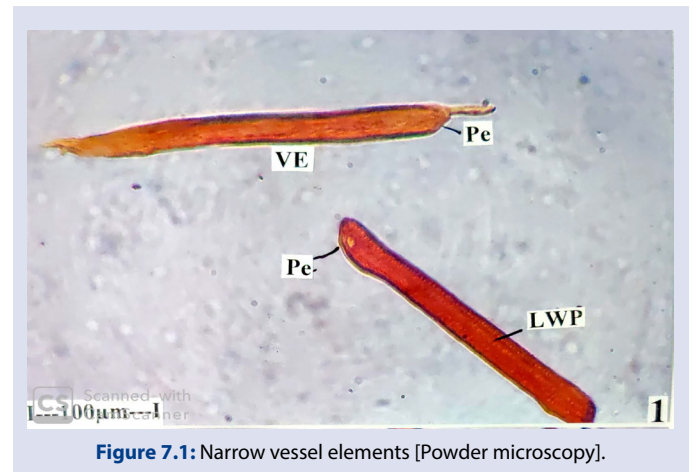


Figure 7.1: Narrow vessel elements [Powder microscopy].

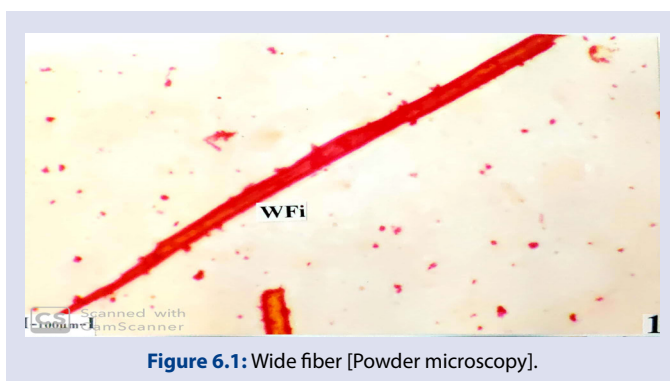


Figure 6.1: Wide fiber [Powder microscopy].

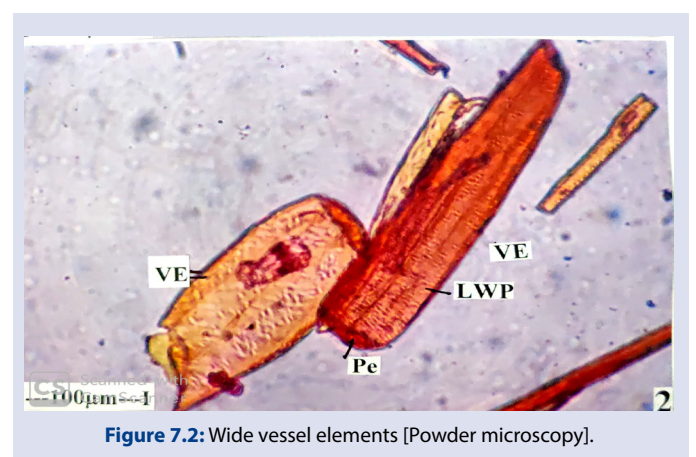


Figure 7.2: Wide vessel elements [Powder microscopy].

Table 1: Leaf constants of *Striga orobanchioides*.

Leaf constant	Value per sq. mm
Stomatal Number	112
Stomatal Index	18
Palisade Ratio	5
Vein Islet Number	4.3
Vein Termination Number	6.7

Table 2: Preliminary phytochemical screening of *Striga orobanchioides*.

Chemical constituent	Chemical test	<i>Striga orobanchioides</i> plant extract (ethanol extract)
Alkaloid	Dragendorff's test	+
	Mayer's test	-
Steroids	Salkowaski test	-
	Liebermann-burchard test	-
Triterpene	Vanillin-sulphuric acid test	+
	Ferric chloride test	+
Tannin	Dilute nitric acid test	+
	Keller-killani test	+
Glycoside	Molish test	+
	Fehling's test	+
Carbohydrate	Shinoda test	-
	Lead acetate test	+
Saponins	Foam formation test	-
	Biuret test	+
Proteins	Millon's test	+
	Ninhydrin test	-

Table 3: Fluorescence analysis of *Striga orobanchioides* leaf and stem.

S. no.	Experiments	Visible/daylight		UV Low (254 nm)		UV High (365nm)	
		Leaf	Stem	Leaf	Stem	Leaf	Stem
1	Powder	Green	Buff	Green	Dark green	Dark green	Herbage green
2	Powder+1N Aqueous NaOH	Yellowish green	Rust	Greenish yellow	Jelly green	Dark blue	Greyish green
3	Powder+ 1 N Alcoholic NaOH	Yellowish green	Buff	Dark green	Herbage green	Dark brown	Fluorescent green
4	Powder+50% HNO ₃	Greenish yellow	Green	Fluorescent green	Dark green	Violet	Dark green
5	Powder+ Conc. H ₂ SO ₄	Light brown	Green	Light green	Dark green	Brown	Dark green
6	Powder+ 50% H ₂ SO ₄	Brown	Green	Fluorescent green	Greyish green	Dark green	Fluorescent green
7	Powder+ Conc. HNO ₃	Light green	Green	Fluorescent green	Herbage green	Dark green	Fluorescent green
8	Powder+ Conc. HCl	Green	Brown	Light green	Dark green	Dark green	Herbage green
9	Powder+50% HNO ₃	Greenish yellow	Green	Fluorescent green	Herbage green	Violet	Herbage green

The stem powder fluoresced buff under daylight, Dark green with short UV light (254 nm), herbage green under long UV light (365 nm). The *Striga orobanchioides* stem showed the characteristic fluorescent green treated with 50% H₂SO₄, concentrated HNO₃, 50% HNO₃, NaOH, ethanol under UV light

Physicochemical constants

Ash value is accustomed to determine quality and purity of undistinguished drug. Ash value holds inorganic radicals like phosphates carbonates and silicates of sodium, potassium, magnesium, calcium etc. Diverse physicochemical parameters such as total ash, water soluble ash and acid insoluble ash of whole plant of *Striga orobanchioides* was estimated to be 7.67, 0.08 and 0.09 % w/w, respectively. Moisture content in the plant was estimated to be 3.87 % w/w. foreign organic matter in the plant was estimated to be 0.72 % w/w. The extractive values are primarily useful for the determination of the exhausted or adulterated drug. Extractive values such as ethanol soluble extract and water soluble extract of whole plant of *Striga orobanchioides* was estimated to be 18.76 and 14.5% w/w respectively.

DISCUSSION

Standardization is a crucial estimate of quality, purity and originality. Microscopic technique is unembellished and economical routine to begin with proving the veracious recognition of the starting stuff. As no any Pharmacognostic work is noted on this medicinally formidable plant, the current study was tackled to recline the standards which could be useful for proving its originality. Macro and micro standards are utilitarian recognizing variables estimation of the drug. The particulars secured from the preliminary phytochemical screening will divulge the utilitarian recognizing about chemical nature of the drugs. Total ash values and extractive values are utilitarian in identification and authentication of the plant material.^{16,17} Extractive values are utilitarian to assess the chemical constituents of starting stuff.¹⁸ Preliminary phytochemical screening mainly divulged the presence of alkaloid, tannins, glycosides, carbohydrates, proteins and flavonoids in ethanol extract. T.S. of the young stem committed the existence of thin epidermal layer, fairly wide cortex and thin vascular cylinder having the pith disintegrated or retained as homogeneous parenchymatous

tissue. T.S. of the old stem committed the existence of cortical cells, parenchyma cells, sclerenchyma cells, vascular tissue.

In conclusion, the present work was tackled with a perspective to reline standards which could be utilitarian to detect the authenticity of the medicinally utilitarian plant. Pharmacognostic evaluation can be utilitarian to substantiate and authenticate the drug.

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CONFLICTS OF INTEREST

We declare that we have no conflicts of interest.

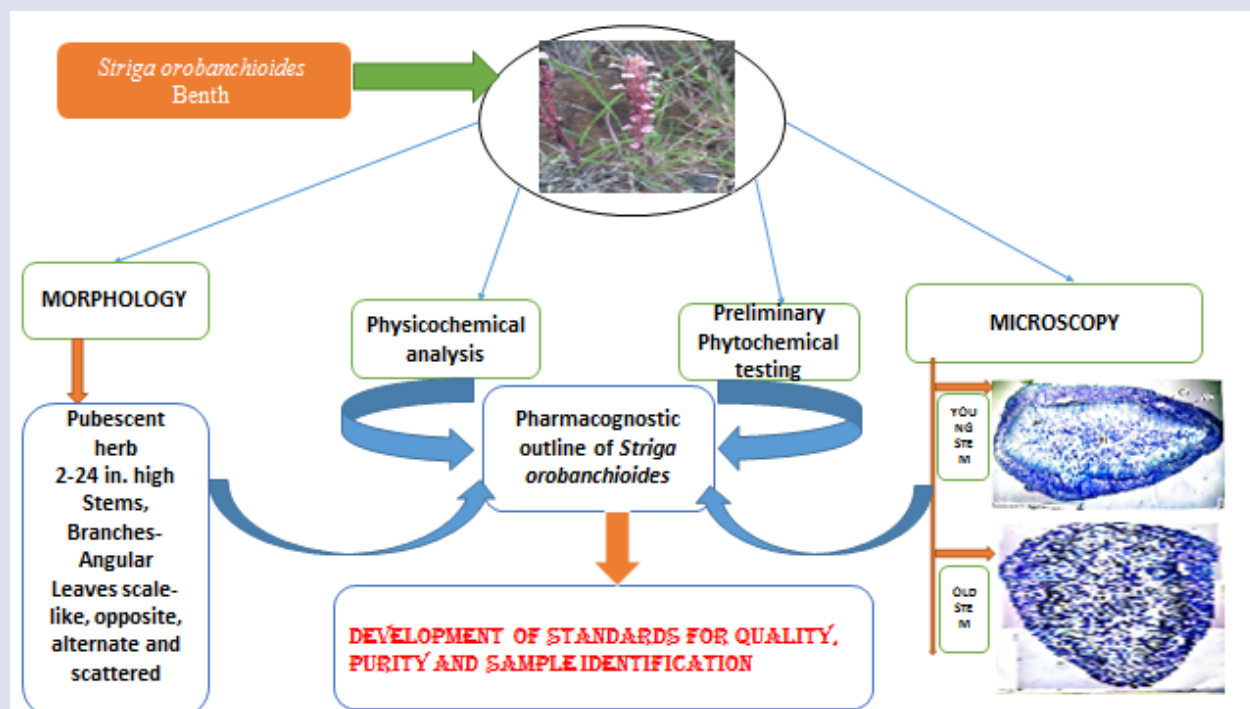
LIMITATIONS OF THE STUDY

The limitations are cost, size, maintenance, researcher training and image artifacts resulting from specimen preparation. This type of microscopic study is a large, cumbersome, expensive piece of equipment, extremely sensitive to vibration and external magnetic fields.

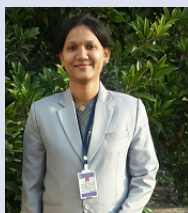
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GRAPHICAL ABSTRACT



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