

## Standardization of *Tulsi* (*Ocimum sanctum* Linn.)

Singh J.<sup>1\*</sup> Gupta S. J.<sup>2</sup> and Singh A. K.<sup>3</sup>

<sup>1</sup>Dept. of Dravyaguna, I.M.S. B.H.U. Varanasi, U.P., India

<sup>2</sup>Dept. of Shalya Tantra, I.M.S. B.H.U. Varanasi, U.P., India

<sup>3</sup>Dept. of Dravyaguna, I.M.S. B.H.U. Varanasi, U.P., India

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### Abstract

India has a rich heritage of plants as medicines; Indian systems of medicines utilize 80 percent of the material derived from the plants. Large numbers of plants are utilized in various systems of medicine practiced in India and local health traditions for the treatment of human diseases since time immemorial. The use of Tulsi as source of medicine in human has been in vogue since antiquity. Tulsi (*Ocimum sanctum* Linn. Gurke.) has been used for thousands of years in Ayurveda due to its diverse healing properties. Tulsi is legendary and the 'incomparable one' from India. It is one of the holiest and most cherished of the many healing and healthy giving herbs of the orient. Morphological and anatomical characters play a vital role in crude drug standardization. However, a proper documentation of medicinal plants of *Ocimum* spp. is lacking and many times adulterants are passed as genuine drugs. In present investigation, the *Ocimum sanctum* has been selected for the standardization due to its medicinal importance.

### Keywords

*Tulsi, Morphological, Standardization, Pharmacognostical, TLC*



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## INTRODUCTION

Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases. Medicinal plants have played a key role in the world health<sup>1-2</sup>. In view of this, WHO recognized the need of standardization and quality control for herbal medicines.<sup>3</sup> WHO has published guidelines in order to define basic criteria for evaluating the quality, safety and efficacy of herbal medicines aimed at assisting national regulatory authorities, scientific organizations and manufacturers in this particular area<sup>4</sup>.

There are over 2500 plant species in India having documented medicinal value, WHO has listed about 20,000 plant species in the world which are used in manufacturing different medicines<sup>5</sup>. Standardization of herbal drugs is the most desirable at this time when worldwide interest on herbal medicine has gained momentum. Morphological and anatomical characters play a vital role in crude drug standardization. Morphological characters involve size, arrangement, venation, texture, surface characters, markings and hardness of the plant materials. The *Ocimum* (Basil) comprises some of the most popular herbs in the world. It belongs to the family

*Lamiaceae*, subfamily *ocimoideae* and includes more than 150 different species and varieties distributed in the tropical regions of Asia, Africa, Central and South Africa considered as one of the largest genera of the *Lamiaceae* family.

The name Tulsi is a Sanskrit word which means “matchless one.”<sup>6,7</sup> Among the plants known for medicinal value, the plants of genus *Ocimum* are very important for their therapeutic potentials. Because of its popularity basil is often referred to as King of herbs, being widely utilized due to its economic, nutritional, industrial and medicinal properties.<sup>8-10</sup> In traditional systems of medicine, different parts (leaves, stem, flower, root, seeds and even whole plant) of *O. sanctum* have been recommended for the treatment of bronchitis, bronchial asthma, malaria, diarrhoea, dysentery, skin diseases, arthritis, chronic fever and insect bite (Prakash and Gupta, 2005)<sup>11</sup>.

Scientifically, the *Ocimum* species have proved to possess various biological activities such as antibacterial (Obinna et al., 2009<sup>12</sup>; Parag et al., 2010<sup>13</sup>) antifungal (Bansod and Rai 2005<sup>14</sup>; Singh, 2010) antioxidant (Ramesh and Satakopan 2010<sup>15</sup>;

Hanif et al. 2011<sup>16</sup>), and anti-diabetic (Chandra et al., 2008)<sup>17</sup>.

Tulsi consists of dried whole plant of *Ocimum sanctum* Linn. (Fam. Lamiaceae); an erect, annual herb 30 - 60 cm height, much branched, found throughout the country. In the present investigation *Ocimum tenuiflorum* (*O.santum*) have been selected for the standardization studies due to their medicinal importance.

### Synonyms:

Sanskrit	:	Surasa, Apetrakshasi, Bhutghni, Bahumanjari, Sulabha,
Assamese	:	Tulasi
Bengali	:	Tulasi
English	:	Holy Basil
Gujrati	:	Tulasi, Tulsi
Hindi	:	Tulasi
Kannada	:	Tulasi, Shree Tulasi, Vishnu Tulasi
Malayalam	:	Tulasi, Tulasa
Marathi	:	Tulas
Punjabi	:	Tulasi
Tamil	:	Tulasi, Thulasi, Thiru Theezai
Telugu	:	Tulasi
Urdu	:	Raihan, Tulsi

### Plant Description:

#### a) Macroscopic

**Root** - Thin, wiry, branched, hairy, soft,

blackish-brown externally and pale, violet internally.

**Stem** - Erect, herbaceous, woody, branched; hairy, sub quadrangular, externally purplish-brown to black, internally cream, coloured; fracture, fibrous in bark and short in xylem; odour faintly aromatic.

**Leaf** - 2.5-5 cm long 1.6 - 3.2 cm wide, elliptic oblong, obtuse or acute, entire or serrate, pubescent on both sides; petiole thin, about 1.5-3 cm long hairy; odour, aromatic; taste, characteristic.

**Flower** - Purplish or crimson coloured, small in close whorls; bracts about 3 mm long and broad, pedicels longer than calyx, slender, pubescent; calyx ovoid or campanulate 3-4 mm bilipped, upper lip broadly obovate or suborbicular, shortly apiculate, lower lip longer than upper having four mucronate teeth, lateral two short and central two largest; corolla about 4 mm long, pubescent; odour, aromatic; taste, pungent.

**Fruit** - A group of 4 nutlets, each with one seed, enclosed in an enlarged, membranous, veined calyx, nutlets sub-globose or broadly elliptic, slightly compressed, nearly smooth; pale brown or reddish with small black marking at the place of attachment to the thalamus; odour, aromatic; taste, pungent.

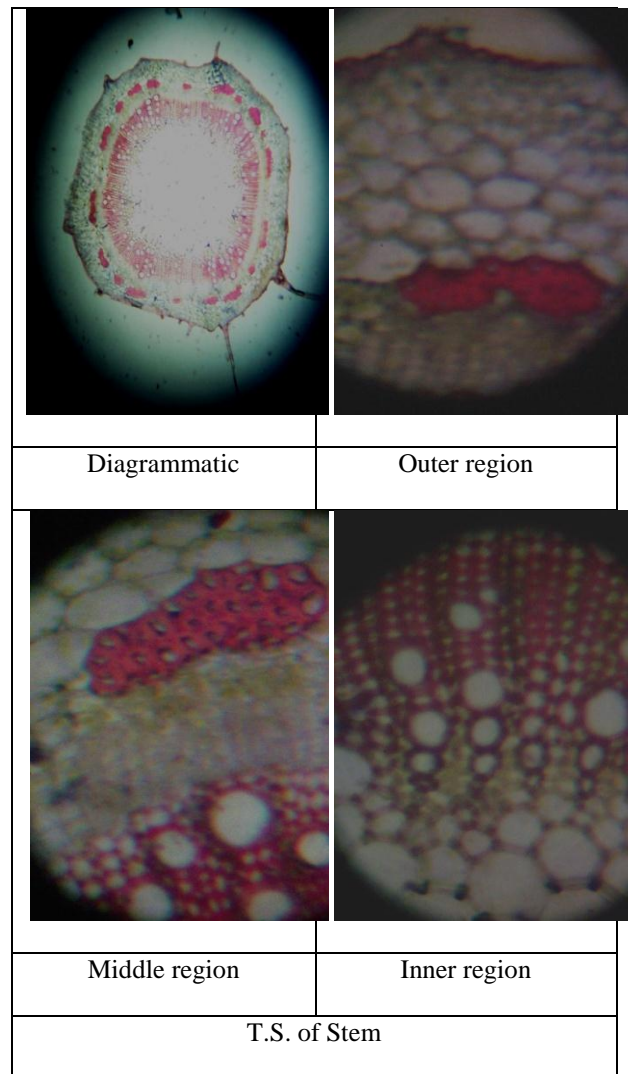
**Seed** - Rounded to oval; brown,

mucilaginous when soaked in water, 0.1 cm long, slightly notched at the base; no odour; taste, pungent, slightly mucilaginous.



### b) Microscopic

**Stem** - Shows a single layered epidermis with uniseriate, multicellular covering trichomes having 5-6 cells, occasionally a few cells collapsed; cortex consists of 10 or more layers of thin-walled, rectangular, parenchymatous cells; phloem consists of sieve elements, thin-walled, rectangular parenchyma cells and fibres; fibres found scattered mostly throughout phloem, in groups and rarely in singles; xylem occupies major portion of stem consisting of vessels, tracheids fibres and parenchyma; vessels pitted; fibres with pointed ends; centre occupied by narrow pith consisting of round to oval, thin-walled, parenchymatous cells.



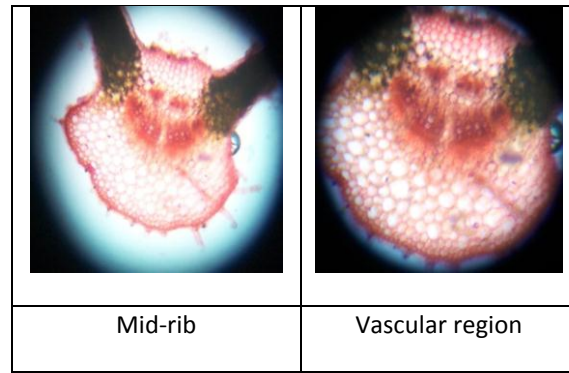
### Leaf-

**Petiole** - shows somewhat cordate outline, consisting of single layered epidermis composed of thin-walled, oval cells having a number of covering and glandular trichomes; covering trichomes multicellular 1-8 celled long, rarely slightly reflexed at tip; glandular trichomes short, sessile with 1-2 celled stalk and 2-8 celled balloon-shaped

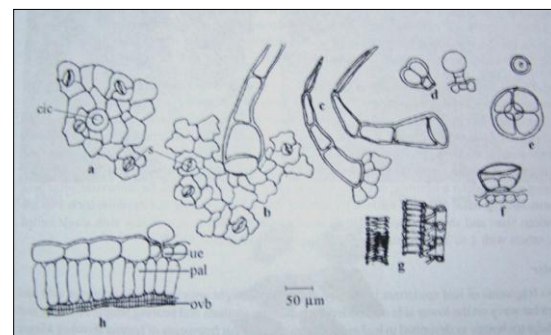
head, measuring 22-27 in dia; epidermis followed by 1 or 2 layers and 2 or 3 layers of thin-walled, elongated, parenchyma cells towards upper and lower surfaces respectively; three vascular bundles situated centrally, middle one larger than other two; xylem surrounded by phloem.

**Midrib** - epidermis, trichomes and vascular bundles similar to those of petiole except cortical layers reduced towards apical region.

**Lamina** - epidermis and trichomes similar to those of petiole; palisade single layered followed by 4-6 layers of closely packed spongy parenchyma with chloroplast and oleo-resin; stomatal index 10-12-15 on upper surface and 14 - 15 - 16 on lower surface; palisade ratio 3.8; vein islet number 31 - 35.



T.S. of leaf

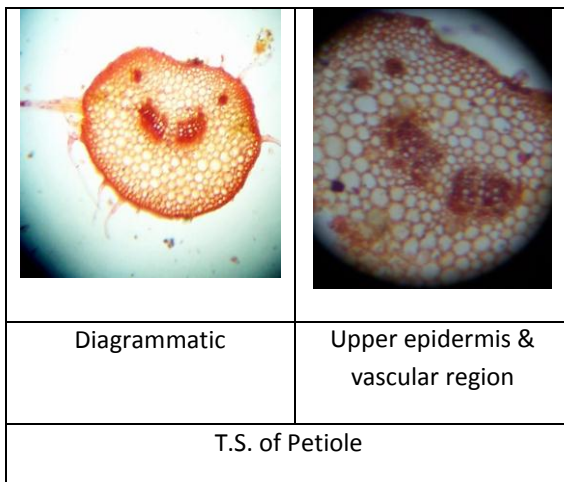


Powder Microscopy of leaf

- a. Upper epidermis surface view
- b. Lower epidermis surface view
- c. Covering trichomes
- d. Glandular trichomes
- e. Sessile glandular trichomes
- f. Cupshaped trichomes
- g. Fragments of vessels
- h. Lamina in sectional view

Cic=cicatrix; s=stomata, ovb=obliquely cut vascular bundles; pal=palisade; ue=upper epidermis

**Powder** - Greenish: shows thin-walled, parenchymatous cells, a few containing reddish brown contents, unicellular and multicellular-trichomes either entire or in pieces; thin walled fibres, xylem vessels





with pitted thickenings, fragments of epidermal cells in surface view having irregular shape, oil globules, rounded to oval, simple as well as compound starch grains having 2-5 components, measuring 3-17  $\mu$  in diameter; both anomocytic and diacytic type of stomata present on both surfaces, slightly raised above the level of epidermis.

## OBSERVATIONS

**Table 1** Identity, Purity and Strength

Parameter	Results
Foreign matter	Not more than 2 %
Moisture content	3.6 %
Total ash	15.6 %
Acid – insoluble ash	2.5%
Sulphated ash	16.7 %
Alcohol – soluble extractive	9.6 %
Water – soluble extractive	20.4 %

**Table 2** Phyto-Chemical Test

Chemical constituents	Result
Alkaloids -	+ve
Glycosides -	+ve
Flavonoids -	+ve
Steroids -	+ve
Saponins -	+ve
Tannins -	+ve

**FLORESCENCE STUDY:** [Chase & Pratt, 1949.,Kokaski, et al.,1958) with some modification]

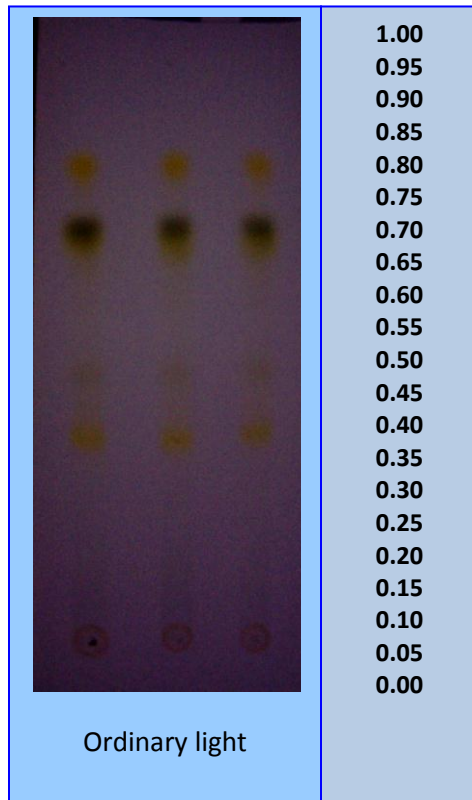
**Table 3** UV Spectrography

S.NO.	Treatment	Under ordinary light	Under UV - Long (366nm)
1.	Drug as such	Grey	Light brown
2.	Drug + Nitrocellulose	Dark brown	Green
3.	Drug + Picric acid	Yellow	Green
4.	Drug + HCl <sub>conc.</sub>	Grey	Green
5.	Drug + H <sub>2</sub> SO <sub>4 conc.</sub>	Black	Brown
6.	Drug + HNO <sub>3 (50%)</sub>	Yellow	Greenish brown
7.	Drug + 1 N Na OH in Me OH	Dark brown	Brown
8.	Drug + 1 N Na OH in Water	Greenish yellow	Reddish brown
9.	Drug + NH <sub>4</sub> OH	Blackish brown	Green
10.	Drug + FeCl <sub>3</sub>	Greenish brown	Dark green
11.	Drug + Acetic acid Glacial	Grey	Dark brown
12.	Drug + Sudan-III	Grey	Brown

### \*Visualized in ordinary light

<b>Stationary phase</b>	TLC Aluminium sheet silica gel 60 F 254 plate
<b>Mobile phase</b>	Toluene: Ethyl Acetate: Formic acid (7: 2.7: 0.3)
<b>Rf value of spots Visualized in ordinary light</b>	0.35 (Yellow) , 0.45 (Light yellow) , 0.68 (Green), & 0.78 (Yellow)

**Figure1** T.L.C. Tulsi (Whole plant)\* Visualized in ordinary light



**Figure 2** T.L.C. Tulsi (Whole plant) Visualized after Spray of Methanolic Sulphuric acid reagent

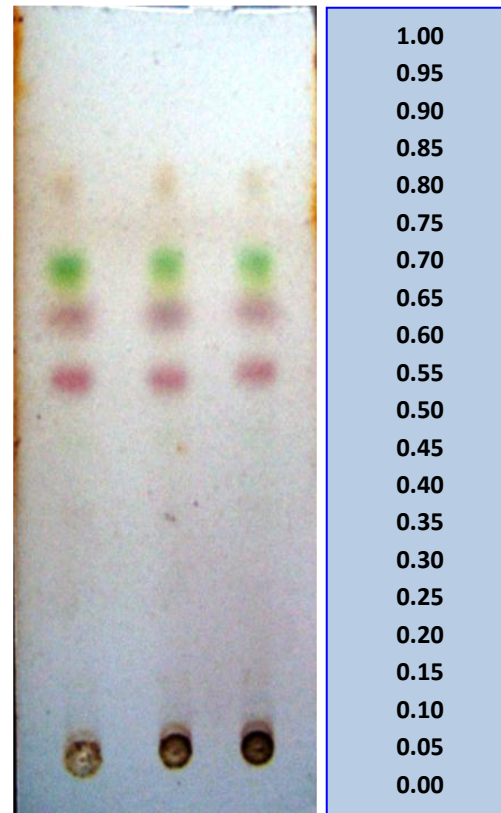


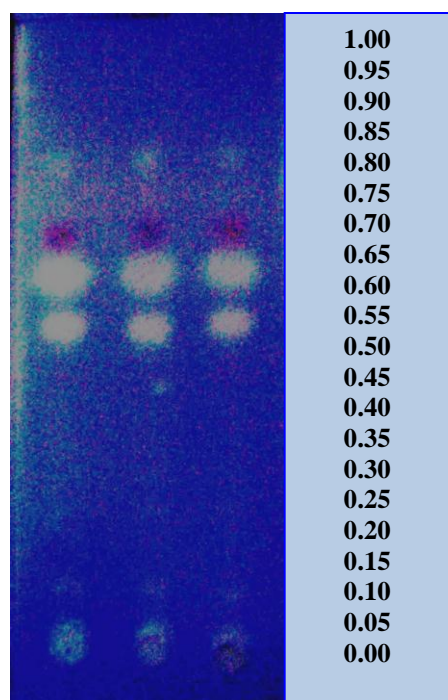
Figure 2 Visualized after Spray of Methanolic Sulphuric acid reagent and heated 105<sup>0</sup>C

<b>Stationary phase</b>	<b>TLC Aluminium sheet silica gel 60 F 254 plate</b>
<b>Mobile phase</b>	Toluene : Ethyl Acetate : Formic acid (7: 2.7: 0.3)
<b>Rf value of spots Visualized after Spray of Methanolic Sulphuric acid reagent &amp; 5 minuts at 110<sup>0</sup>C</b>	0.53 (Pink), 0.60 (Pink), 0.68 (Green), & 0.80 (Pink)

Figure 3 Visualized in UV Long Wave (366nm) after Spray of Methanolic Sulphuric acid reagent and heated 105<sup>0</sup>C

<b>Stationary phase</b>	<b>TLC Aluminium sheet silica gel 60 F 254 plate</b>
<b>Mobile phase</b>	Toluene : Ethyl Acetate : Formic acid (7: 2.7: 0.3)
<b>Rf value of spots Visualized after Spray of Methanolic Sulphuric acid reagent &amp; 5 minuts at 110<sup>0</sup>C</b>	0.53, 0.60, 0.68 (Pink), & 0.80

**Figure 3** T.L.C.Tulsi (Whole plant) Visualized in UV Long Wave (366nm) after Spray of Methanolic Sulphuric acid



### T.L.C. Methodology

T.L.C. of Tulasi oil obtained by stem distillation has been carried out on Silica gel 'G' plate using Toluene : Ethyl acetate : Formic acid (7:2.7:0.3) used as a mobile phase. Tulsi oil has been diluted in chloroform-toluene (1: 10). Eugenol has to be applied as standard also diluted in (1 : 30) ratio in same solvent and 10 µl of each to be applied in band form on the plate. After running distance of 10 cm the plate, plate has been air dried for 15 minutes and then it has been kept in the oven for 2 to 5 minutes. After cooling of the plate vanillin – Methanolic Sulphuric acid reagent spray has

been done in thoroughly and heated the plate at 110° C for 1-5 minutes. Under observation Rf. values of eugenol and caryophyllence has been recorded. Eugenol (orange brown) approx Rf. value 0.7 caryophyllence (reddish violet) runs to solvent front.

### PROPERTIES AND ACTIONS ACCORDING TO AYURVEDIC TEXT

**Rasa** : Katu, Tikta,  
**Guna** : Laghu, Ruksha, Tikshna,  
**Virya** : Ushna  
**Vipaka** : Katu  
**Karma** : Kapha-vatahara, Pittavardhini, Hridya, Dipana, Rucya, Durgandhinashak

### THERAPEUTIC USES

It is used for Swasa, Kasa, Hikka, Chardi, Krimiroga, Kustha, Asmari, and Netraroga etc.

### DOSE

Drug juice: 1-3 ml.  
 Drug powder: 1-2 gm.

### IMPORTANT FORMULATIONS



Market available preparations are Tribhuvanakirti Rasa, Muktapancamrta Rasa, Muktadi Mahanjana, Manasamitra Vataka and Tulsi Arka.

### CONSTITUENTS

Tulsi contains Essential oil containing eugenol, apigenin, apigenin-7-O-glucuronide, vicenin, gallic acid & urosolic acid etc.

### CONCLUSION

The results of the present study have established the specifications of the quality profile of the drug *Ocimum sanctum* Linn. plant. The drug should be standardized before any research and the results should be within specifications.

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