



# A report on the quality control parameters of aerial parts of *Pluchea lanceolata* (DC.) Oliv. & Hiern, Asteraceae

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**RESUMO:** “Relatório sobre os parâmetros de controle de qualidade das partes aéreas de *Pluchea lanceolata* (DC.) Oliv. & Hiern, Asteraceae.” *Pluchea lanceolata* (DC.) Oliv. & Hiern, Asteraceae, é uma planta que tem sido usada como medicinal na Índia como um antipirético, analgésico anti-reumático e calmante. Este trabalho tem como objetivo estabelecer os padrões morfológicos e anatômicos e caracterizar o extrato(s) de *P. lanceolata* pela triagem fitoquímica preliminar como parâmetro de controle de qualidade para a matéria-prima.

**Unitermos:** *Pluchea lanceolata*, controle de qualidade, físico-química, microscopia, fluorescência.

**ABSTRACT:** *Pluchea lanceolata* (DC.) Oliv. & Hiern, Asteraceae, is a weed that has been used as an indigenous medicine in India as an antipyretic, analgesic, anti-rheumatic and nervine tonic. This paper aims at setting the morphological and anatomical standards and to characterize the extract(s) of *P. lanceolata* by preliminary phytochemical screening as quality control parameter for the raw material.

**Keywords:** *Pluchea lanceolata*, quality control, physico-chemical, microscopy, fluorescence.

## INTRODUCTION

*Pluchea lanceolata* (DC.) Oliv. & Hiern, Asteraceae, commonly known as Rasna in Hindi; Yukta or Elaparnee in Sanskrit, is a perennial weed grown in warm climatic regions of India. Controversies exist about the identity of Rasna, however *Pluchea lanceolata* is most widely accepted under shrub (Singh et al., 1972). It is a succulent, erect plant, traditionally used for dyspepsia, bronchitis (Dwivedi et al., 1949) and rheumatoid arthritis (Anonymous, 1969). It is also used as antipyretic, analgesic, bitter, laxative, and nervine tonic (Chopra et al., 1958) in Ayurveda. The drug has been scientifically validated for certain pharmacological effects namely anti-inflammatory (Prasad et al., 1966; Srivastava et al., 1990), uterine relaxant (Jadhav & Bhutani, 2005) and antioxidant effects. A number of phytoconstituents,  $\alpha$ -amyirin,  $\beta$ -amyirin caproate, stigmaterol (Bhatnagar et al., 1972) few triterpenes morentenol acetate, morentenol, neolupenol (Chawla et al., 1991; Kaith, 1995),  $\Psi$  taraxasterol acetate (Ames et al 1954; Srivastava et al., 1990), quercetin and isorhamnetin (Bahl et al., 1968) have been isolated from the plant.

In spite of its numerous medicinal attributes, we did not find a systematic report on its quality control parameters. The present investigation was therefore,

undertaken to set standards and to characterize the extract of *Pluchea lanceolata* by preliminary phytochemical screening.

## MATERIAL AND METHODS

### Plant material

The shade dried, aerial parts of the plant *Pluchea lanceolata* (DC.) Oliv. & Hiern, Asteraceae, was collected from National Botanical Research Institute (NBRI) Lucknow (India), in the month of October 2008 and authenticated by Dr. AKS Rawat, Senior scientist, NBRI, Lucknow India. A voucher specimen no. pp-569 was deposited in the Department of Pharmacognosy, Manipal, College of Pharmaceutical Sciences, Manipal. The plant material was further size reduced and stored until further use in an air tight container. Fresh plant material was obtained for the macroscopical and microscopical evaluation.

### Chemicals

All the chemicals used were of analytical grade from NICE chemicals Ltd, Kochi, Kerala, India.

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## Macroscopic and microscopic analysis

The macroscopy and microscopy of the plant was studied according to the methods of Brain & Turner (1975a), the cross sections were prepared and stained. The microscopic analysis of powder was done according to the method of Brain & Turner (1975b) and Kokate et al. (1986). Leaf constants viz. vein termination and stomatal index were studied according to the method of Evans (2003).

## Physico-chemical analysis

Air dried plant material was used for the quantitative determination of ash and extractive values (WHO/QCMMPM guidelines, 1992). Fluorescence analysis of the extract(s) was carried out by the method of Chase & Pratt, (1949) and Kokoski et al., (1958).

## Preliminary phytochemical screening

Preliminary phytochemical screening was carried out by using standard procedure described by Kokate et al. (1986) and Harbone (1998). Total flavonoid content was determined as described by Singleton & Rossi (1965).

## RESULTS AND DISCUSSION

### Macroscopic characters

The plant is an erect allelopathic, perennial under shrub growing up to 30-100 cm high, with a cylindrical stem of 2-3 mm in diameter. Stem is herbaceous and cylindrical with smooth outer surface is hairy, branched and the branches are terrete, ashy and pubescent. Leaves are simple (0.6-1.6 x 2.5-2.7 cm), alternate coracious, sessile, oblong or lanceolate, obtuse apiculate narrowed at the base, margin is entire and apex is round. The inflorescence is compound corymbs usually with purple tinged flowers.

### Microscopic characters of leaf

#### Leaf

Transverse section passing through midrib of leaf (Figure 1A) reveals its isobilateral nature that has upper and lower epidermis with thick cuticle, traversed with stomata. The leaf has both covering and glandular trichomes; the covering trichomes were uniseriate, multicellular (2-5 cells of about 90 µm in size) and lignified while the glandular trichomes were sessile as well as stocked. Although collenchymatous tissues lie under both upper and lower epidermis it is strongly developed towards the upper side. Vascular bundles are colletral, centrally located; meristele is incircled by a parenchymatous bundle sheath.

Transverse section of the leaf passing through

lamina (Figure 1C) reveals a row of small sized palisade under both upper and lower epidermis in continuation within midrib. The remaining mesophyll comprises of spongy parenchymatous cells partially filled with oil globules, small sized cluster and rosette (Figure 1D) calcium oxalate crystals; vessels traversing mesophyll was clearly seen in the section.

#### Stem

The transverse section of the stem (Figure 2A) is almost circular in outline covered with thick cuticle. Epidermis consists of single layer of thick walled cells along with covering and glandular trichomes. Covering trichomes are uniseriate, multicellular with two to many thick walled cells while glandular ones are sessile as well as stalked. Collenchymatous hypodermis lies underneath the epidermis, followed by 5-7 layered parenchymatous cortex. A ring of open collateral vascular bundles is seen in the outer cortex region. Each vascular bundle consists of well developed phloem and xylem. Phloem is made up of sieve tube, companion cells and parenchyma. Cambium is distinct 2-3 layered while the centre portion is occupied by collenchymatous pith.

### Leaf constants

Vein islet and vein termination numbers in 1 mm<sup>2</sup> leaf surface was determined and recorded (Table 4). Stomatal indices of both upper and lower epidermis were also determined and recorded in Table 4.

### Powder microscopy

Powder microscopy showed patches of upper (Figure 3A) and lower epidermis (Figure 3B) in surface view along with ranunculaceous type of stomata, plenty of uniseriate unicellular as well as multicellular covering trichomes (Figure 3C) were also seen along with glandular trichomes with or without stalks. The preparation also showed transversely cut fragments of lamina (Figure 3D) with a row of palisade underneath upper epidermis, fragments of fibres (Figure 3E), vessels (Figure 3F), with spiral and reticulated thickening. Rosette shaped crystals (Figure 3G) of calcium oxalate were seen in unstained slide preparation.

### Physicochemical analysis

Air dried material was used for quantitative determination of physiochemical values. Total, acid insoluble and water soluble ash (Table 1) was determined for five times and its mean+SE was recorded. Similarly, hexane, alcohol and water soluble extractives were determined for five times and it mean+SE was recorded (Table 2). Alcohol and water extractive was determined

as per WHO recommendations while hexane soluble extractive was determined due to the medicinal attributes of the extract. Water soluble extractive was found to be very high when compared to other extractable matter in the drug.

### Preliminary phytochemical screening

The phytochemical profiling of the plant revealed the presence of protein starch, fixed oil, steroid, glycosides and triterpenoid and flavonoids. This serves as an important tool for the quality assurance of plant for future studies. Total flavonoid content of the plant was determined in five different samples and was found to be 67.24±1.4 µg/mL. The fluorescence analysis is a tool for the determination of constituents in the plant that gives a definite idea of the chemical nature. Thus the fluorescence analysis of the drug powder was carried out and data is presented in the Table 4.

### CONCLUSION

The plant *Pluchea lanceolata* (DC.) Oliv. & Hiern, Asteraceae, a weed, is a perennial undershrub commonly known as rasna. Rasna, however is a controversial name and hence a well established quality control and identification parameters are highly essential for the plant. It is popular for its medicinal properties in the indigenous system of medicine and some of its traditional claims have been scientifically validated. In the present paper, the macroscopical and microscopical findings will lay down the standards which will be useful for the detection of its identity and authenticity. The other parameters viz. ash value, extractive value, leaf constants and microscopy add to its quality control and quality assurance. Thus the above finding will serve the purpose of quality control and assurance for the future studies.

**Table 1.** Ash values of *Pluchea lanceolata* (DC.) Oliv. & Hiern, Asteraceae.

Sl. N <sup>o</sup>	Parameters	Values % (w/w)
1	Total ash	21.1±0.63
2	Acid insoluble ash	3.125±0.53
3	Water soluble ash	2.48±0.48

**Table 2.** Extractive values of *Pluchea lanceolata* (DC.) Oliv. & Hiern, Asteraceae in different solvents.

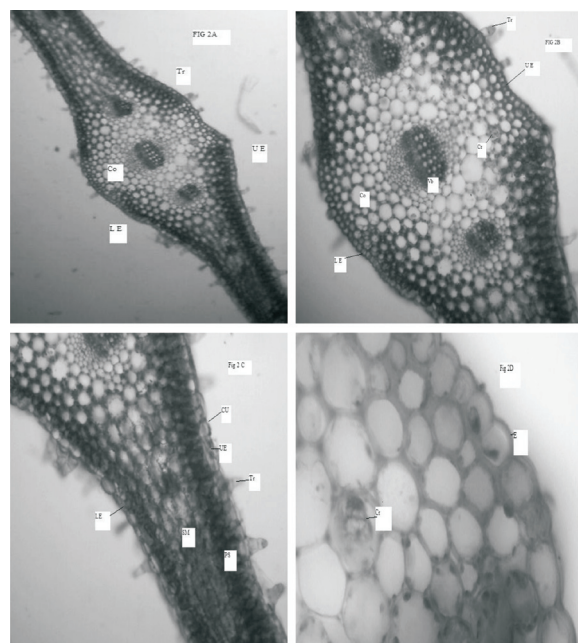
Sl. N <sup>o</sup>	Solvent	Values
1	Alcohol (absolute)	4.91±0.56
2	Water	27.78±0.2
3	n-Hexane	2.07±0.43

**Table 3.** Fluorescence analysis of leaf and stem powder of *Pluchea lanceolata* (DC.) Oliv. & Hiern, Asteraceae.

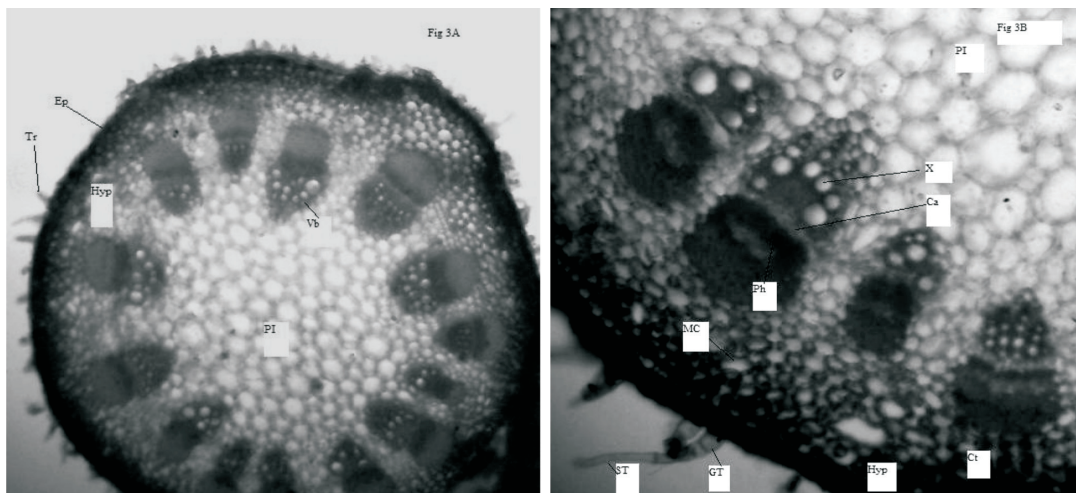
Treatment	Short UV light	Long UV light	Day light
Powder as such	Green	Light green	Dark green
Powder + 1N HCl	Greenish brown	Black	Green
Powder + 1N H <sub>2</sub> SO <sub>4</sub>	Brown	Dark violet	Backish brown
Powder + 1N HNO <sub>3</sub>	Green	Dark brown	Yellowish orange
Powder + Picric acid	Greenish yellow	Blackish green	Bright yellow
Powder + 5% FeCl <sub>3</sub>	Greenish yellow	Blackish green	Greenish yellow
Powder + iodine	Brownish	Black	Brownish orange
Powder + glacial acetic acid	Green	Dark violet	Green

**Table 4.** Leaf constants of *Pluchea lanceolata* (DC.) Oliv. & Hiern, Asteraceae.

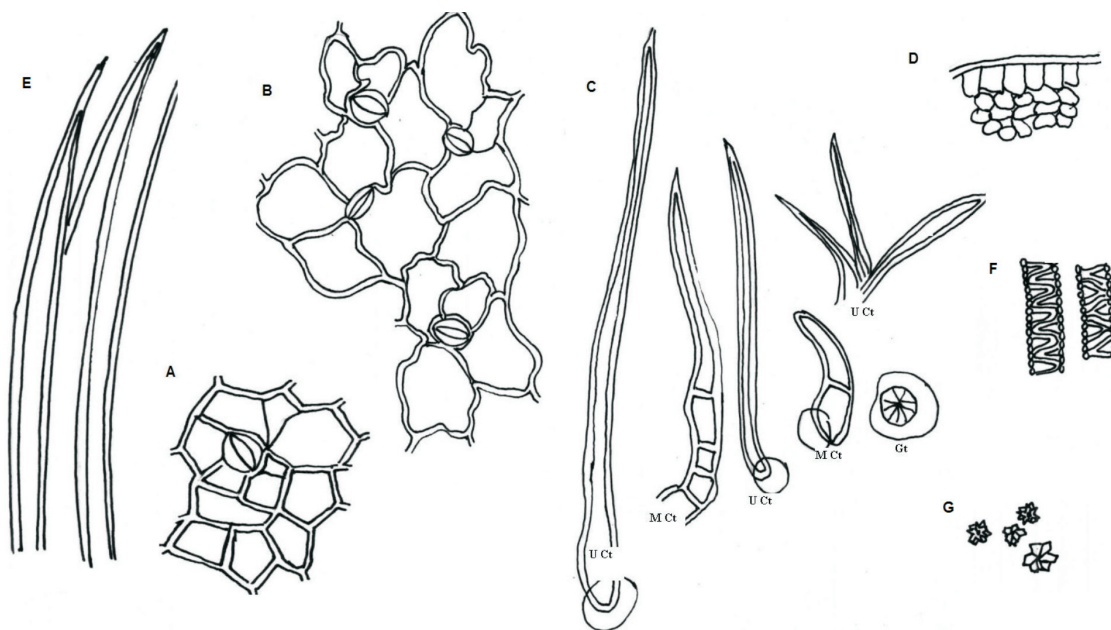
Sl. N <sup>o</sup>	Parameters	Value (1 mm <sup>2</sup> )
1	Vein islet number (1 mm <sup>2</sup> leaf surface)	25
2	Vein termination number	32
3	Stomatal index of upper epidermis	18.33
4	Stomatal index of lower epidermis	20



**Figure 1.** Microscopy of the leaf of *Pluchea lanceolata* (DC.) Oliv. & Hiern, Asteraceae. A. transverse section of leaf passing through the midrib. B. enlarged view of the transverse section of leaf through the midrib. C. enlarged view of the transverse section of leaf through lamina. D. portion of leaf with calcium oxalate crystals. E. epidermis; Cu. cuticle; SM. spongy mesophyll; PS. palisade; UE. upper epidermis; LE. lower epidermis; Tr. trichomes; Co. collenchyma; Cr. crystals; Vb. vascular bundles.



**Figure 2.** Microscopy of stem of *Pluchea lanceolata* (DC.) Oliv. & Hiern, Asteraceae. A. transverse section of the stem. B. enlarged view of transverse section of stem. Ep. epidermis; Hyp. hypodermis; Tr. trichomes; Vb. vascular bundle; PI. pith; ST. simple trichomes; GT. glandular trichomes; X. xylem; Ph. phloem; Ca. cambium; MC. mucilage canal; Ct. cortex.



**Figure 3.** Powder microscopy of *Pluchea lanceolata* (DC.) Oliv. & Hiern, Asteraceae. A. Upper epidermis in surface view with anomocytic stomata; B. Lower epidermis on surface view with anomocytic stomata; C. Trichomes in the powder microscopy. U Ct. unicellular covering trichomes; M Ct. multicellular covering trichomes; Gt. glandular trichomes. D. Transversely cut fragments of lamina. E. Fibers with pointed ends; F. Reticulate vessels. G. Rossette type of calcium oxalate crystals.

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