

Pharmacognostic and Phytochemical Studies of *Stellaria media* Linn.

Disha Arora and Anupam Sharma* Pharmacognosy Division,

University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh- 160 014, India

Abstract

Aim: Stellaria media Linn. (Caryophyllaceae) has been traditionally used for a variety of ailments such as inflammation, asthma, blood diseases and in the treatment of mental tension. In the present investigation, various pharmacognostic standards of the plant have been generated so that authentic *S. media* could be explored for its traditional claims.

Materials and Methods: Various parameters like macroscopic and microscopic characters of the leaves and stem of *S. media* were studied along with physico-chemical and phytochemical analysis.

Results: The macroscopic studies revealed that the stem is weak, branched, pale green and slightly swollen at the joints; leaves are oval shaped, smooth and are arranged on the stem in pairs. Microscopically, leaf showed the presence of epidermis, palisade cells, trichomes and vascular bundles; stem revealed the presence of epidermis, hypodermis, cortex, vascular bundles and pith. Total ash, acid insoluble ash, ethanol soluble extractive and water soluble extractive values were 11.24, 2.04, 6.3, 37.7 % w/w respectively.

Conclusion: Since standardization of herbal medicines is essential for ensuring reproducible therapeutic action, data evolved in this investigation could be used in laying down pharmacopoeial standards for the drug studied.

Keywords: Pharmacognostic studies, Phytochemical analysis, Standardization, Stellaria media

INTRODUCTION

Pharmacognostic study is the preliminary step in the standardization of crude drugs. Detailed pharmacognostic evaluation gives valuable information regarding the morphological and histological characteristics of the crude drugs [1]. There are a number of crude drugs where the plant source has not yet been scientifically identified. Hence pharmacognostic study gives the scientific information regarding the authenticity, quality and purity of the plant drugs [2]. Stellaria media Linn. (Caryophyllaceae) is well known as an invasive weed in gardens, fields and grounds in the world [3]. The plant is reported to be useful in inflammations of the digestive, renal, respiratory and reproductive tracts. The plant is employed in plasters used for broken bones and swellings [4]. It also possesses diuretic, expectorant and anti-asthmatic properties [5]. Some phenolic acids, flavones [6], fatty esters [5] and gypsogenin [7] have been reported from this species. The objective of the present study was to establish various pharmacognostic standards and to evaluate preliminary phytochemical and physico-chemical parameters that can facilitate identification and assist in the preparation of monograph of the plant.

MATERIALS AND METHODS Collection and authentication of plant material

S. media was collected from Panjab University, Chandigarh. Identity of the plant was confirmed through Head, Raw Materials, Herbarium & Museum at National Institute of Science Communication and Information Resources (NISCAIR), New Delhi 110 067. The fresh as well as shade dried powdered plant material was subjected to macroscopic, microscopic, phytochemical and physico-chemical studies.

Pharmacognostic Studies

Macroscopic characters

For macroscopic evaluation, appropriate parameters like taste, odour, size, shape, colour and texture of *S. media* were studied [8].

Microscopic studies

Microscopic examination was carried out by taking transverse hand sections of the fresh leaves and stems of *S. media*. Description of the tissues was supplemented with photomicrographs. Photographs were taken with Nikon Labphoto 2 microscopic units. For normal observations, bright field was used [10]. Powder characteristics of whole herb powder were also studied and measured [10, 11].

Determination of vein-islet number and veinlet termination number

Fresh leaves of *S. media* were cleared by boiling (5 min) in chloral hydrate solution. The cleared leaves were mounted in glycerin. Camera lucida was set up on a compound microscope and was divided into 4 squares each of one mm² by using stage micrometer. The stage micrometer was replaced with the cleared leaf preparation, and the vein-islets and vein terminations were traced in the four continuous squares [11]. Number of vein-islets and veinlet terminations within the squares were counted. Total number of vein-islets and veinlet terminations in four adjoining squares was divided by 4 to get the value for one mm². Ten sets of such counts were recorded and the mean value was calculated.

Determination of stomatal number and stomatal index

Leaf fragments of the plant were cleared from middle of the lamina by boiling (5 min) in chloral hydrate solution. By using forceps, the upper and lower epidermis were peeled and mounted in glycerin. Camera lucida was set up on a compound microscope and was divided into 4 squares each of one mm² by using stage micrometer. The stage micrometer was replaced with a cleared leaf preparation, and the epidermal cells and stomata were traced [11]. The number of epidermal cells and stomata within the squares were counted. About 400 cells were counted and the number of stomata per mm² of leaf preparation and the stomatal index were calculated by using the formula: SI=100S/E+S. The values for each surface of the leaf were calculated.

Physico-chemical parameters

For physico-chemical evaluation, ash values viz., total ash, acid insoluble ash, and extractive values viz., alcohol soluble extractive value, water soluble extractive values were determined in triplicate [12, 13].

Determination of total ash value:

Powdered plant material (2 g) was taken in a tared silica crucible and was incinerated at a temperature not exceeding 450 °C until free from carbon. The resultant ash was cooled and weighed. The percentage of ash was calculated with reference to the air-dried drug.

Acid insoluble ash value:

The total ash obtained from 2 g of the powdered plant material was boiled (5 min) with 25 ml of dilute hydrochloric acid, and the insoluble matter was collected on an ashless filter paper. It was then washed with hot water, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

Determination of alcohol soluble extractive value:

Accurately weighed powder (5 g) of the plant material was taken and macerated with 100 ml of 95% alcohol for 24 h. The contents were frequently shaken during the first 6 h and allowed to remain for 18 h. After 24 h, the extract was filtered and 25 ml of the filtrate was evaporated. The extract was dried at 105 °C to a constant weight.

Determination of water soluble extractive value:

Water soluble extractive value was determined using the procedure described for alcohol soluble extractive, except that chloroform-water was used for maceration.

Phytochemical screening

Dried, coarsely powdered *S. media* (200 g) was successively Soxhlet extracted with petroleum ether, chloroform and methanol. The marc was air dried and water extract was obtained by boiling with distilled water for 2 h, filtered, concentrated and dried in an oven at 40-50 °C. All the four extracts were dissolved in respective solvents and were screened for different classes of phytoconstituents [14, 15, 16].

RESULTS AND DISCUSSION

Macroscopic characters

The stem was procumbent, weak and much branched. It was juicy, pale green and slightly swollen at the joints. A line of hairs ran up the stem on one side only, which upon reaching a pair of leaves is continued on the opposite side. The leaves were succulent, pale green, oval shaped and smooth. The leaves were arranged on the stem in pairs, the lower leaves being larger (up to 25 mm) than the upper leaves.

Microscopic studies Transverse section of leaf:



Figure 1: Transverse section of *Stellaria media* leaf Representative photomicrograph (× 100) of transverse section of *S. media* leaf

T. S. passing through midrib showed upper epidermis, palisade cells, crescent shaped vascular bundles and collenchymatous cells.

Stellaria media leaf was dorsiventral (Figure 1). Transverse section passing through midrib showed upper epidermis followed by single layered palisade cells in lamina portion. Mesophyll consisted of 4-6 layers of spongy parenchymatous cells with intercellular spaces. Midrib showed arc shaped vascular bundles and collenchymatous cells.

Transverse section of stem:

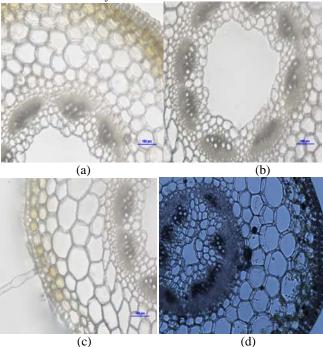


Figure 2: Transverse section of *Stellaria media* stem (a, b, c, d): Representative photomicrographs (× 100) of transverse section of *S. media* stem

T. S. showing (a) epidermis followed by pigment cells; (b) vascular bundles in a ring with central pith region of mature stem; (c) a trichome; (d) central pith region of young stem T.S. of stem showed epidermis, comprising thick walled quadrangular cells (Figure 2). Epidermis bore trichomes, usually 3-5 celled. Collenchyma and epidermis were followed by 2-3 layers of loosely packed pigmented cells. Next was a layer of thin walled, tightly packed cells of endodermis which surrounded the underlying phloem, xylem and the central pith. The vascular bundles were situated in a ring. Pith consisted of circular to oval, thin walled parenchymatous cells in young stem while in mature stem, it comprised of hollow space.

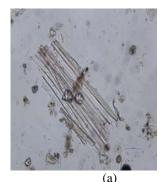
Quantitative microscopic analysis:

Various leaf constants viz. vein-islet number, veinlet termination number, stomatal number, stomatal index were determined as per standard methods and recorded (Table 1).

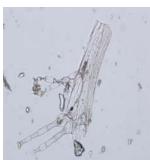
| Range | |
|----------------------------|--|
| 10.20- 12.24 -14.28 | |
| 2.04- 4.08 -6.12 | |
| 24.48- 27.54 -30.61 | |
| 11.00- 12.02 -13.04 | |
| | |

Powder characteristics:

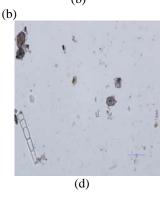
The powdered plant material showed abundant rosette and few prismatic calcium oxalate crystals, epidermal fragments with fragments of lamina (Figure 3). Thick walled fibers, diacytic stomata, trichomes (collapsed, covering and glandular), annular and spiral vessels were also observed in the powdered plant material.

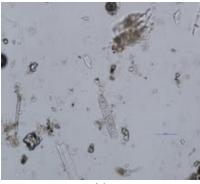






(c)





(e)

Figure 3: Powder microscopy of Stellaria media (a) to (e): Representative photomicrographs (× 100) of powder microscopy of S. media powder

(a) fibres with calcium oxalate crystals; (b) covering trichomes and prismatic calcium oxalate crystals; (c) epidermis with 4-5 celled glandular trichomes; (d) rosettes of calcium oxalate crystals; (e) vessel

Physico-chemical standards

Ash values viz., total ash, acid insoluble ash, and extractive values viz., alcohol soluble and water soluble extractive values were calculated (Table 2).

| Parameter | Mean value*(% w/w) | |
|----------------------------|--------------------|--|
| Total ash | 11.24 | |
| Acid insoluble ash | 2.04 | |
| Ethanol soluble extractive | 6.30 | |
| Water soluble extractive | 37.70 | |
| *n_2 | | |

n=3

Phytochemical screening

The results of all the four extracts subjected to qualitative chemical tests were recorded (Table 3).

Table 3: Phytochemical analysis of extracts of Stellaria media

| Class of phytoconstituents | Petroleum ether extract | Chloroform extract | Methanol extract | Water extract |
|-------------------------------|-------------------------------|-----------------------|---------------------|------------------|
| Alkaloids | - | - | - | - |
| Anthracene glycosides | - | - | - | - |
| Cardiac glycosides | - | - | - | - |
| Steroids | + | + | - | - |
| Saponins | - | - | - | + |
| Flavonoids | _ | + | + | - |
| Tannins | - | + | + | - |
| Carbohydrates | _ | - | + | + |
| Proteins | _ | - | + | + |
| Fixed oils/Fats | + | - | - | - |

CONCLUSION

The information generated regarding various pharmacognostic parameters and phytoconstituents of *Stellaria media* shall be very useful to ascertain the identity and to determine the quality and purity of the plant material in future studies.

ACKNOWLEDGEMENTS

The authors duly acknowledge University Grants Commission, New Delhi, for providing financial assistance.

REFERENCES

- [1]. Sharma, S. K., *Recent approach to herbal formulation development and standardization*, http://pharmainfo.net, 2004.
- [2]. Dhanabal, S. P., Suresh, B., Sheeja, E., Edwin, E., Indian J. Nat. Prod. 2005, 21(1), 9–11.
- [3]. Shan, Y., Zhou, J., Zhao, H. G., Feng, X., Dong, Y., Xia, B., Chem. Nat. Compd. 2010, 46(4), 667–668.
- [4]. Anonymous, *The Wealth of India*, Raw Materials, Publications and Information Directorate, Vol X, CSIR, New Delhi 1976.
- [5]. Pande, A., Shukla, Y. N., Tripathi, A. K. Phytochemistry, 1995, 39(3), 709–711.

- [6]. Kitanov, G., Pharmazie, 1992, 47(6), 470-471.
- [7]. Hodisan, V., Sancraian, A., Farmacia, 1989, 37(2), 105–109.
- [8]. Kirtikar, K. R., Basu, B. D., Indian Medicinal Plants, Vol II, International Book Distributors, Dehradun 2006, 939.
- [9]. Anonymous, *Indian Pharmacopoeia*, 3rd Edition, Vol II, Ministry of Health and Family Welfare, Government of India, The Controller of Publication, New Delhi 1985.
- [10]. Wallis, T. E., *Text Book of Pharmacognosy*, 15th edition, T.A. Churchill, London 1985, 575– 582.
- [11] Evans, W. C., *Trease and Evans Pharmacognosy*, 15th edition, W. B. Saunders, Baillere Tindall, London 1983, 538–547.
- [12]. Kokate, C. K., Practical Pharmacognosy, 4th edition, Vallabh Prakashan, New Delhi 2003, 122–126.
- [13]. Anonymous, *Indian Pharmacopoeia*, Vol. II, 4th edition, Ministry of Health and Family Welfare, Government of India, The Controller of Publications, New Delhi 1996.
- [14]. Farnsworth, N. R., J. Pharm. Sci., 1966, 55, 225-286.
- [15]. Harborne, J.B., Methods of extraction and isolation, Chapman & Hall, London 1998, 60–66.
- [16]. Brain, K. R., Turner, T. D., Practical evaluation of phytopharmaceuticals, 1stedition, Wright-Scientechnica, Bristol 1975.