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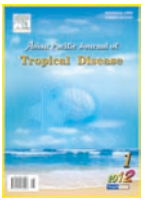

Document heading

Pharmacognostical and quality control parameters of *Stellaria media* Linn.

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ABSTRACT

Objective: The present study was attempted to evaluate the pharmacognostical and quality control parameters of *Stellaria media* Linn. **Method:** The plant was studied for taxonomic characters, standardization parameters viz. loss on drying, ash values, extractives values, foreign organic matter, crude fiber content, swelling index, haemolytic activity, foaming index, microbial contamination according to procedure mentioned in Indian Pharmacopoeia and the WHO Guidelines. The HPTLC studies were performed on pre-coated silica gel GF254 plates using Glycyrrizinic acid as a biomarker. **Result:** The present study provided taxonomic characters, pharmacognostical and physicochemical details, elemental analysis and microbial contamination of the plant which will help in laying down pharmacopoeial parameters. HPTLC profile developed for the plants will help in identification of the drug and also in isolating and identifying the biomarker compound responsible for the bioactivity. **Conclusions:** The pharmacognostical and quality control parameters presented in this paper may be helpful to establish the authenticity of *Stellaria media*. and can possibly differentiate the drug from its other species.

1. Introduction

Stellaria media Linn. (Caryophylaceae) commonly known as Chickweed, is found throughout the Himalayas upto an altitude of 4300 m [1]. The plant is indicated as anti-inflammatory, antipyretic, diuretic, emollient, emmenagogue and digestive in various traditional systems of medicines [2]. A poultice of plant can be applied to cuts, burns and bruises [3-4]. *Stellaria media* is reported to have antibacterial [5], anti-hepatoma [6], anti-oxidant [7], and anti-obesity effects [8-9]. Various phytoconstituents viz. lipids [10], penta-saccharide [11], and triterpenoid [12] have been reported from this plant. In spite of abundant uses, the pharmacopeial standards of *Stellaria media* is not been explored. In the present study pharmacognostic parameters of plant such as morphological and microscopical characters, physical standards like loss on drying, ash values, extractives values, foreign organic matter, crude fiber content, haemolytic activity, preliminary organic analysis, elemental analysis, microbial contamination and HPTLC profile of powdered drug was undertaken to standardize the plant of *Stellaria media* according to World Health Organization (WHO) guidelines [13].

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2. Materials and methods

2.1 Plant material

The fresh plant of *Stellaria media* Linn. was collected from the campus of Guru Jambheshwar University of Science and Technology, Hisar, Harayana. The plant was taxonomically identified and authenticated by Dr. H.B. Singh, Head, Raw Materials Herbarium and Museum Division of National Institute of Science Communication and Information Resources. The voucher specimen has been deposited in the herbarium section of the Pharmacognosy Division, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar. The fresh plant was taken for macroscopic and microscopic studies. The dried plants were ground to a coarse powder and subjected to physico-chemical parameters.

2.2 Pharmacognostical evaluation

Various morphological features viz. shape, size, colour, odour, taste and fracture were studied according to the method of Brain and Turner [14]. Microscopic studies were done using the method described by O Brien et al [15]. Photomicrographs were obtained by observing free-hand sections of drug under compound trinocular microscope (Zeiss Primostar). The powder analysis was done according to Trease & Evans [16] and Kokate [17]. Leaf constants such as stomatal index, vein islet number, veinlet termination

number and palisade ratio were determined according to method of Trease & Evans [16].

2.3 Standardization parameters

Various standardization parameters viz. loss on drying, ash values, extractives values, foreign organic matter, crude fiber content, swelling index, haemolytic activity, foaming index, microbial contamination were determined according to procedure mentioned in Indian Pharmacopoeia (1996) [18] and the WHO Guidelines (2005) [13]. Preliminary phytochemical analysis was carried out using standard conventional protocol [17]. The whole plant was analyzed for the presence of eleven elements by using Atomic Absorption Spectroscopy [13]. The HPTLC studies were performed for water extract on pre-coated silica gel GF254 plates using the suitable solvent system. Rf values, peak area, peak height, and spectrum of each peak were determined for the extract [19]. Glycyrrizinic acid (Sigma Aldrich) was used as a biomarker.

3. Result

3.1 Macroscopic characters

(a) Roots : Shallow, fibrous, fragile, slender tap root; Stems : Weak, trailing, 5-40 cm in length.
(b) Leaves : Opposite, smooth, oval, entire, yellowish green, lower leaves are stalked and vary in size from 3-20 mm in length, while the upper new leaves lack stalks and upto 25 mm in length.
(c) Flowers: White starry, about a quarter inch in diameter, leafy cymes or solitary in leaf axils, hermaphrodite, petals-5 and 2-parted, shorter than sepals, sepals-5, stamens have reddish-violet anthers.
(d) Fruits : Capsule like fruit contain many tiny seeds.
(e) Seeds : Reddish brown (Figure 1).

3.2 Microscopic characters

(a) Stem: The transverse section of stem shows a single row of flattened epidermal cells, fitted closely along with radial walls with well defined cuticle extended over it, having uniserrate, glandular, multi-cellular hair and a few stomata. The cortex consists of 4-5 layers of large rounded parenchymatous cells without intercellular spaces. The endodermis, inner most layer of cortex consists of closely fitted barrel shaped cells without intercellular space. The vascular bundles have xylem and phloem. The phloem lies externally and is composed of thin walled cells. The xylem lies internally and shows the presence of thick walled xylem vessels. The central portion i.e pith is composed of rounded or polygonal thin walled parenchymatous cells without intercellular spaces (Figure 1a).
(b)Leaf: The transverse section of leaf shows a single layered upper and lower epidermis with cuticle and stomata. The mesophyll lies between two epidermal layers and differentiated between palisade and spongy parenchyma. The palisade parenchyma is one layered of elongated cylindrical cells, right angle to upper epidermis. It is followed by spongy parenchyma consisting of 3-4 layers of rounded irregular loosely arranged cells. Vascular bundle consists of xylem lying towards the upper epidermis and phloem towards lower epidermis. Surrounding the vascular bundles there is compact layer of thin walled parenchymatous cells (Figure 1b).

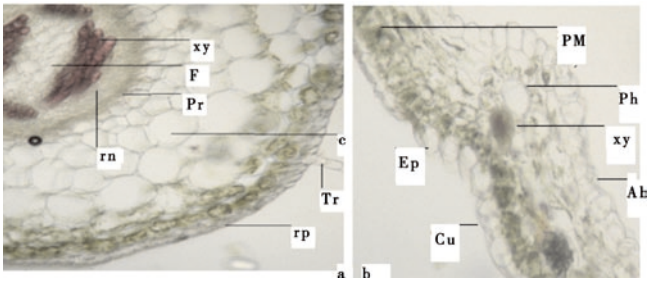

Figure 1. T.S of stem (a) T.S of leaf (b) Ep-Epidermis, Co-Cortex, Xy-Xylem, Ph-Pholem, Pr-Pericycle, Tr-Trichome, P- Pith, PM-Palisade mesophyll, AbE- Abaxial epidermis, Cu- Cuticle

3.3 Powder microscopy

On microscopic examination, powder showed presence of reticulate xylem, leaf fragment with epidermis, mesophyll cells and stomata, and multi-cellular, uniserrate glandular trichomes (Figure 2 a-c).

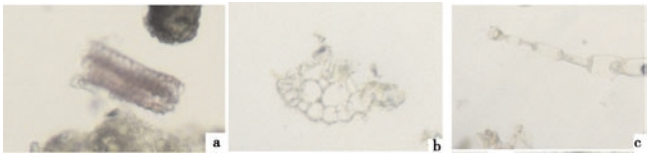

Figure 2. Powder microscopy (a) Reticulate xylem vessel (b) Leaf fragment (c) Trichome

3.4 Leaf constants

The various leaf constants were determined. Stomatal index was 5% (adaxial); 9% (abaxial), palisade ratio was 6-11, vein islet number was 7-12 /sq.mm, and veinlet termination number 15-29 /sq.mm for plant leaf.

3.5 Physicochemical parameters

The physicochemical parameters total ash, acid-insoluble ash, water soluble ash and sulpahted ash values were found to be 13.3, 9.25, 6.45 and 15.6 % w/w respectively. The alcoholic and water soluble extractive values by hot and cold methods were 4.75 and 8.5 % w/w; 7.15 and 11.75 % w/w respectively. The foreign organic matter was 1.45 %. The percentage moisture content was found to be 3.5 % w/w. The crude fiber content 6.0 % w/w, swelling index was 7.5, and foaming index was found to be less than 100. The haemolytic activity was found to be 0.66 units/ g.

3.6 Preliminary phytochemical screening

The preliminary phytochemical screening of the alcoholic extract indicated the presence of mainly carbohydrates, saponins, phenols, tannins, phytosterols and flavonoids.

3.7 Elemental analysis

The Atomic Absorption Spectroscopy study showed the presence of cadmium, lead, zinc, copper, nickel, sodium,

magnesium, iron, cobalt, manganese and mercury in aqueous and alcoholic extracts of plant but below the WHO permissible limits and therefore safe to use.

3.8 Microbial contamination

Determination of microbial contamination of alcoholic and aqueous extracts of plant showed complete absence of *Escherichia coli, Salmonella typhi, Psuedomonas aerginosa, Staphylococcus aureus, Clostridia* and *Shigella*.

3.9 HPTLC studies

A qualitative densitometric HPTLC profile was developed for water extract of whole plant as a preliminary fingerprinting of the extract. Most of the compounds have shown maximum absorbance at 254 nm. Chloroform : methanol (90:10) was found to be a suitable solvent system for the separation of constituents of whole plant extract. The bands in the sample were obtained at Rf 0.08, 0.13, 0.14, 0.16, 0.19, 0.80, 0.86, 0.95, 1.00. The band at Rf 0.80 is corresponding to standard i.e. Glycyrrhizinic acid (Rf 0.81) indicative of presence of triterpene oleanolic acid saponin. The above Rf values and number of spots are invariable for the extract at particular solvent system studied (Figure 3).

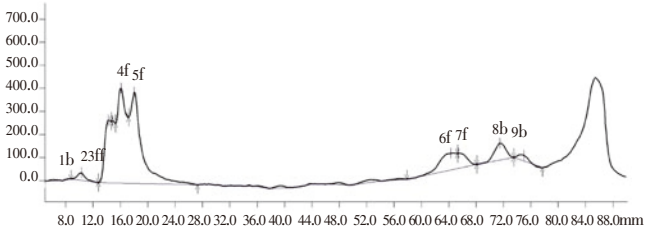

Figure 3. HPTLC profile of water extract of *Stellaria media* powder

4. Discussion

The quality control of crude drugs and herbal formulations is of paramount importance in justifying their acceptability in modern system of medicine. But one of the major problems faced by the herbal drug industry is non availability of rigid quality control profile for herbal material and their formulations. The microscopic characters, leaf constants, quantitative analysis, and physicochemical parameters studied here can be used for judging the adulteration and purity of this drug. Since these parameters studied are constant and any change in these values are indicative of substitution and adulteration with the plant *Stellaria media*. The preliminary phytochemical analysis revealed the presence of carbohydrates, saponins, glycosides, phenols, tannins, phytosterols and flavonoids. These constituents may be possibly responsible for the biological activities of *Stellaria media*. HPTLC profile helps in standardization and also for undertaking work on isolating and identifying the bioactive compounds.

5. Conclusion

As there is no detailed pharmacognostic anatomical work on *Stellaria media* Linn. is reported. Therefore present work is taken up in the view to completely standardize the herb in accordance to parameters of WHO Guidelines. The profile presented in this paper may be proposed as parameters to establish the authenticity of *Stellaria media* and can possibly help to differentiate the drug from its other species. The information gathered from above studies could be of value in the preparation of the herbal monograph for its proper identification and evaluation.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgment

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