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Physico-chemical analysis of some temperate Himalayan *Swertia species*

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Abstract

Swertia chirayita (Family Gentianaceae) source of Indian Ayurvedic drug “chirata”, is an important medicinal plant of Western Himalaya known for its potent activity against malaria, liver-disorder, fever, gastrointestinal infection and diabetes. Due to its high demand and scarcity, several other species of *Swertia viz. Swertia alata, Swertia angustifolia, Swertia cordata, Swertia purpurascens and Swertia paniculata* etc. are being used as substitutes /adulterants for *Swertia chirayita* in India, Japan, China, Pakistan and other Asian Countries. In order to check this adulteration and to assure genuineness of ‘true chirayita’, it is essential to know the detailed physico-chemical features of a drug plant. The present study was carried out on some *Swertia species* i.e., *S. chirayita, S. alata, S. cordata, S. angustifolia* and *S. purpurascens* for discriminating *Swertia chirayita* from its adulterants. Various ash and extractives values observed in this study may help in differentiating “True Chirayita” from other allied species.

Keywords: *Swertia chirayita*, adulterants, authentication, ash values, extractives

Introduction

Medicinal plants are playing very important role in traditional medicines for the treatment of various ailments. The world demand for raw materials of pharmaceutically important medicinal plants has increased manifolds during the recent past and most of this raw material is being unscientifically and indiscriminately collected from the natural habitats without any consideration for quality material (Handa, 2004; Mishra *et al.*, 2009) [10, 19]. The collection of medicinal plants from their natural habitats has reduced their population to such an extent that today pharmaceutical industries based on these plant materials are experiencing shortage of raw material. Authenticity, purity and quality control are important aspect to prevent the adulteration of target plant with other plant materials thereby ensuring the efficacy and potency of crude drugs. Ash and extractives analysis of crude drug are pharmacognostically important parameters for judging the purity and authenticity (Trease, 1949) [29]. In spite of use of herbal medicines as important mean for treatment of illness or as dietary supplements and health products, little attention has been given towards the standardization of medicinal plants. Adulterations and substitutions are frequent in raw material trade of medicinal plants. Whether these are being used in Indian pharmaceutical industries or are being supplied to international markets. Unintentional adulterations also exist in herbal trade as a result of 1) confusion in vernacular names between indigenous system of medicines and local dialects, sometimes not only the various species of particular genus but the entire different taxa are being sold under the same vernacular name (Rawat, 2005; Gupta, 2009) [24, 9], 2) lack of knowledge about authentic sample & non- availability of raw material due to unscientific harvesting from their natural habitats 3) near absent cultivation and similarity in morphology (Mitra and Khannan, 2010) [20], thus adversely effecting their bio-efficacy. This is more common in species that have persistent market demand and are in low supply especially endangered species having tremendous medicinal application. One of the important such medicinal plant found in Western Himalayas is *Swertia chirayita* Buch. Ham. a member of family Gentianaceae which is often adulterated with other less potent *Swertia species*. The plant is native of temperate Himalayas found at an altitude of 1200-3000m from Kashmir to Bhutan and in Khasi hills in Meghalaya at 1200-1500m (Blatter, 1984; Clarke, 1885) [7, 8]. *S. chirayita* is the source of important Indian Ayurvedic drug “chirata” and has been categorized as ‘critically endangered (Shrestha and Joshi, 1992; Samant *et al.*, 1998; Rai *et al.*, 2000; Badola and Pal, 2002) [28, 25, 21, 4]. Extensive habitat loss, low seed viability & germination as well as long gestation period contribute to its dwindling natural population (Samant *et al.*, 1998; Joshi and Dhawan, 2005) [25, 13]. High national and international demand for its raw material has prompted the National Medicinal Plant Board, New Delhi, India to prioritize this species amongst the 32 medicinal species for intensive cultivation (Kala and Sajwan, 2007) [14]. *Swertia chirayita*

holds a valuable position in Indian systems of medicines, and forms a household remedy primarily as a blood purifier, carminative, digestive, expectorant, febrifuge, anthelmintic, antidiarrhoeic, antiperiodic and laxative, and is also useful in flatulence, indigestion, skin diseases, malarial fever, gout, bronchial asthma (Anonymous, 1976; Joshi and Dhawan, 2005) [1,13]. Ruthless and unscientific exploitation by pharmaceutical houses coupled with ever increasing popularity of this herb owes largely for the present status of "True Chirata" having become a rare item in commerce (Karan *et al.*, 1997) [15]. As a result, other related species are substituted in trade for "chirata". The morphological similarity among different species of *Swertia*, especially in dry form simplifies the act of substitution (Bhatia *et al.*, 2003) [5]. Some of the common adulterants/substitutes of *Swertia chirayita* are *S. angustifolia*, *S. alata*, *S. bimaculata*, *S. ciliata*, *S. densifolia*, *S. minor* and *S. lawii* (Anonymous, 1976; Joshi and Dhawan, 2005) [1, 13]. Besides species belonging to genus *Swertia*, *Swertia chirayita* is also substituted by the species belonging to other genera like *Andrographis paniculata* (green chirayita), *Exacum tetragonum*, *E. bicolor*, *E. pedunculatum* and *Slevolgia orientalis* (Joshi and Dhawan, 2005) [13]. With the increasing use of herbal medicines worldwide and the rapid extension of the global market for its products, the safety and quality of medicinal plant materials and finished products have become a major concern. Correct characterization and quality assurance of starting material is an essential step to ensure reproducible quality of herbal medicine which will help us to justify its safety and efficacy. To overcome this problem, it is almost inevitable to standardize the drug for their rational therapeutic use. The present paper aims to develop physico-chemical parameters to identify the genuine 'chirayita' to strengthen the efficacy of the formulations.

Material and Methods

Determination of physico-chemical characteristics

Physico-chemical constants such as percentage of total ash, acid-insoluble ash, acid soluble ash, sulphated ash as well as water and alcohol soluble extractives determined as per the methods of Indian Pharmacopoeia (Anonymous, 1955; Anonymous, 1998) [3, 2].

Total Ash

Accurately weighed air dried and powdered plant material (3g) was taken in each of five previously ignited and weighed silica crucibles. The material was then evenly spread and crucibles were kept in the muffle furnace. The temperature was gradually increased to 500-600°C until white ash was obtained indicating absence of carbon. After complete burning of organic matter, the muffle furnace was switched off and allowed to cool. Crucibles were then taken out and weighed again. The percentage of total ash was calculated as per the following formula.

$$\text{Total ash \%} = \frac{(\text{weight of crucible} + \text{Ash}) - \text{weight of crucible}}{\text{weight of sample}} \times 100$$

Acid Insoluble Ash

The total ash obtained above was gently boiled over water bath for 5 minutes with 25ml of dilute hydrochloric acid. The solution was filtered through an ashless filter paper and thoroughly washed with hot distilled water. The residue along with filter paper was ignited in preweighed silica crucibles in

the muffle furnace at temperature of 500-600°C for 5-6 hours. After cooling the crucibles were again weighed and percentage acid insoluble ash was calculated as below.

$$\text{Acid insoluble ash content} = \frac{(\text{wt of crucible} + \text{residue}) - \text{wt of crucible}}{\text{weight of sample}} \times 100$$

Acid Soluble Ash

The weight of acid insoluble ash was subtracted from the weight of total ash and acid soluble ash was calculated as below:

$$\text{Acid soluble ash} = \frac{\text{Total ash} - \text{acid insoluble ash}}{\text{weight of sample}} \times 100$$

Sulphated Ash

Accurately weighed air dried and powdered plant material (3g) was taken in pre-weighed silica crucibles and moistened with few drops of concentrated sulphuric acid. The contents were then gently ignited at temperature of 500°C for two hours. The contents in the crucibles were again moistened with sulphuric acid and reignited for 5-6 hours. Percentage of sulphated ash was calculated as follows

$$\text{Sulphated ash (\%)} = \frac{(\text{wt. of ash} + \text{crucible}) - \text{wt. of crucible}}{\text{weight of sample}} \times 100$$

Alcohol soluble extractives

Accurately weighed (5g) of air dried and powdered plant material was macerated with 100ml of ethanol (90%) in a glass-stoppered conical flask for 24 hours. The contents were shaken during the first 6 hours and then allowed to stand for next 18 hours. The contents were then rapidly filtered to avoid loss of ethanol. The final volume of filtrate was noted and 25ml of the filtrate was evaporated in a tared china dish. The residue so obtained was dried at 105°C to a constant weight. On the basis of total volume of the filtrate and the total weight of air dried plant material, percentage of alcohol soluble extractives was calculated.

Water soluble extractives

The same procedure as described for alcohol soluble extractives was used except replacing ethanol (90%) with chloroform water, which was prepared by adding 2.5ml of chloroform to 1000ml of distilled water. Percentage of water soluble extractives was calculated in the same manner as the alcohol soluble extractives.

Results and Discussion

Physico-chemical parameters

Physico-chemical evaluation of plant material helps in ascertaining the quality of the raw herbs. Out of the various quality related tests, determination of ash content is one of the most facile means to ascertain authenticity and purity of medicinal plant materials (Trease, 1949) [29]. The amount and composition of ash obtained after combustion of plant material varies considerably according to the plant part, age and place of collection (Vermani *et al.*, 2010) [30]. The total ash content accounts for the total amount of residual material remaining after ignition of the plant material. This includes both 'physiological ash' which is derived from the plant tissue itself and 'non physiological ash' comprising extraneous matter such as sand, stones and soil which are sometimes

deliberately added to the raw material. For herbal medicines with considerable level of physiological ash, the value of the total ash content alone is not sufficient to reflect the quality of herbal medicines. Upon treatment with dilute acid and further ignition, the residues left behind are mostly silica materials such as sand and siliceous earth. Therefore, the acid-insoluble ash content serves as another supplementary piece of evidence to illustrate the quality of the plant materials. Besides acid insoluble ash, sulphated ash is another ash parameter which also helps in ascertaining the quality of plant materials. In the present study, the plants of five *Swertia* species were analyzed for their physicochemical values and the results are given in Table 1. Total ash content values for all the five *Swertia* species were found significant which lied between the minimum value in *S. purpurascens* (4.00%) and the maximum in *S. cordata* (5.81%). Out of the five species total ash content for *S. chirayita* (5.69%) and *S. alata* (4.59%) were found close to the value for *S. cordata* (5.81%). However, all the values were significantly different. Acid insoluble ash contents for all the five *Swertia* species lied between the minimum value in *S. alata* (0.51%) and the maximum in *S. cordata* (0.94%). *S. chirayita* and *S. purpurascens* showed the same value (0.83%) for acid insoluble ash contents. Acid soluble ash content was found minimum in *S. purpurascens* (3.18%) and maximum in *S. cordata* (4.85%) which was however, at par with the value for *S. chirayita* (4.81%). *S. alata* and *S. angustifolia* showed intermediate values of acid soluble ash. Maximum content of sulphated ash was found in *S. chirayita* (7.78%) and minimum in *S. purpurascens* (5.24%). In case of *S. chirayita* (the actual *Swertia* species used in the pharmaceutical industries), the total ash content has been reported to be less than 6.00 percent and acid insoluble ash less than 1.00 percent (Anonymous, 1955; Anonymous, 1998) [3,2]. Results are also supported by reports of Latif and Rehman (2014) [17] and Sayadd *et al.* (2013) [26] who have recorded total ash of 2.40%, 4.89% and acid insoluble ash of 0.49%, 0.96% respectively, in *S. chirayita*. Ash contents have also been recommended for quality evaluation of plant based drugs like *Saraca asoca* (Bisht *et al.*, 2017) [6], *Aconitum heterophyllum* (Rajakrishnan, 2016) [22], *Picrorhiza kurroa* (Sharma and Kumar, 2012) [27], *Nardostachys jatamansi* (Jha *et al.*, 2012) [12] and also for Ayurvedic polyherbal formulation 'palasabijadicurna' (Rastogi *et al.*, 2008) [23]. Ash value determination furnishes the basis for judging the identity and cleanliness of any drug and gives information relative to its adulteration/contamination with inorganic matter, thus ash

values are helpful in determining the quality and purity of drug. In addition to ash content, extractive values too make a valuable test to check the quality of drug. The compounds present in the plants dissolve in different solvents according to their polarities. The non-polar compounds dissolve in non-polar solvents such as petroleum ether; mediumly polar compounds dissolve in solvents such as chloroform, acetone, ether; whereas polar compounds dissolve in polar solvents such as ethanol/water. In the present studies, alcohol soluble extractives were recorded minimum (15.20%) in *S. purpurascens* and maximum (22.03%) in *S. angustifolia* which was statistically at par to 21.57% in *S. cordata*. However, water soluble extractives lied between the minimum value of 18.20% (*S. purpurascens*) and maximum of 24.40% (*S. angustifolia*). In case of *S. chirayita* alcohol soluble and water soluble extractives were 18.57 and 21.53 percent respectively. All the five *Swertia* species showed significant higher values for water soluble extractives than their alcohol. For 'true chirayita' i.e. *S. chirayita* each of the alcohol and water soluble extractives have been recommended to be not less than 10.00 percent (Anonymous, 1955; Anonymous, 1998) [3, 2]. Sayadd *et al.* 2013 [26] have reported 12.39 % alcohol soluble and 13.17% water soluble extractive in *S. chirayita*. Extractives values have also been recommended for standardization of sudharshanachurna-polyherbal formulation (Karthi *et al.*, 2012) [16], fruits of *Terminalia bellerica* (Meena *et al.*, 2010) [18], *Butea monosperma* (Iqbal *et al.*, 2010) [11].

Conclusion

Swertia Chirayita is highly demanded nationally as well as internationally and is difficult to obtain in crude drug market of India as other of *Swertia* are being sold under the name chirayita; therefore it is essential to establish internationally recognized guidelines for assessing their quality. Among the various quality test, physico-chemical parameters are also helpful for the identification and authentication of drug used in herbal industries. Thus, the physico-chemical standards, such as total ash, acid soluble & insoluble ash, sulphated ash and extractive values analysis will be certainly helpful to identify the chirayita drug for its adulterant species even in the crushed or powdered forms. This type of study may lead to the authentication of chirayita samples procured from markets. Further, the study will help in setting down Pharmacopoeial standards for future reference in determining the quality and purity of *Swertia chirayita*.

Table 1: Ash and extractives values of different *Swertia* species

| Species Parameters | Total ash (%) | Acid insoluble ash (%) | Acid soluble ash (%) | Sulphated ash (%) | Alcohol soluble extractives (%) | Water soluble extractives (%) |
|--------------------------|---------------|------------------------|----------------------|-------------------|---------------------------------|-------------------------------|
| <i>Swertia chirayita</i> | 5.69(2.39) | 0.83(0.91) | 4.81(2.19) | 7.78(2.79) | 18.57(4.31) | 21.53(4.64) |
| <i>S. alata</i> | 4.59(2.14) | 0.51(0.71) | 4.09(2.02) | 6.39(2.53) | 18.50(4.30) | 20.93(4.58) |
| <i>S. cordata</i> | 5.81(2.41) | 0.94(0.97) | 4.85(2.20) | 7.65(2.77) | 21.57(4.64) | 23.17(4.81) |
| <i>S. angustifolia</i> | 4.43(2.11) | 0.73(0.85) | 3.74(1.94) | 5.83(2.42) | 22.03(4.69) | 24.40(4.94) |
| <i>S. purpurascens</i> | 4.00(2.00) | 0.83(0.91) | 3.18(1.78) | 5.24(2.29) | 15.20(3.90) | 18.20(4.27) |
| SE _d (±) | 0.006 | 0.013 | 0.008 | 0.019 | 0.025 | 0.014 |
| Cd _{0.05} | 0.012 | 0.029 | 0.019 | 0.042 | 0.057 | 0.031 |

Values in parentheses are square root transformation values

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