Pharmacognostic Study and Quantifiction of Active Component (Amarogentin) Of *Swertia Chirayita* by HPLC

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Abstract: ***The present study deals with comparative pharmacognostic parameters involving moisture content, ash values, extractive values and quantification of active component (Amarogentin) in whole plant of two different sites of Garhwal [Chakrata (CK) and Kaddukhal(KK)] in Uttarakhand of Swertia chirayita have been carried out. The quantification of amarogentin evaluated by HPLC, results indicate that Chakrata site has higher percentage of amarogentin than Kaddukhal site. Adulteration and substitution of medicinal plants can be prohibited by standardization and authentication.***

Key words: Pharmacogenestic study, Amarogentin, HPLC, Swertia chirayita

I. INTRODUCTION

Plants have been used from ancient time for the curing of a variety of diseases and the efficiency of these drugs mainly depends upon the appropriate use and sustained availability of the valid raw materials [1].Those plants that contains active constituents which could be used for remedial purposes or for the synthesis of useful drugs are called medicinal plants [2].

The genuineness of the plant material plays a significant role for detection of standard plant material and comparison of adulterants. The Pharmacognostic study deals the structural, physical, chemical and sensory characters of the plant-drug which includes the history, botanical identification, collection, preparation and standardized of the plant to estimate the chief active constituent.

The total ash value reflects the Carbonate, Phosphate, Oxides, Silicate, and Silica,Which represents the purity index of medicinal plants. The acid-insoluble ash value represents the amount of silica and the water soluble ash is the water soluble portion of the total ash in the plant [3].

The *Swertia chirayita* an important species of Gentianaceae family. Floristic *Swertia chirayita* is a robust plant 60 to 180 centimeters in length, branching, teret except near top, stout, branching towards top, stem round ; quadrangular towards top, green. Leaves 70-90 X 35-40 mm broadly lanceolate, sessile, elliptic acute, 5- 7- nerved. Flowers tetramerous, calyx smaller than corolla, oblong, tip acute, green, 1- nerved, 5-6 mm in size. Corolla is gamopetalous, greenish petals which are violet in centre. Bear two glands on each petal- each gland is a depression, which is bright green inside. Margin of the depression is covered by long purple hair which converges to form a summit. Its Androecium has 4- versatile filaments and anthers purple in colour, filament base slightly dilated. The tap root has yellowish pith with no smell. Fruit is a capsule bearing numerous minute seeds. The plant mostly grows in open, moist places and forest openings [4].

The ethno botanical utilization of this species is well known, it is used in its crude form for treatment against fever, treatment against scorpion sting, as a tonic for liver and heart. Its utility as an antidiabetic agent has also been recognized. In Ayurveda an infusion of the *Swertia chirayita* is generally employed for stomachic, febrifuge and anthelmintic treatment.

The wide spread use of *Swertia chirayita* in traditional medicine reflects its pharmacological importance for ages. Its antithelmintic, hypoglycemic and antipyretic properties are attributed due to amarogentin, swerchirin, swertiamerin and other active principles of the herb. The plant has been classed in critical endangered because existing populations of *Swertia chirayita* are diminishing due to various biotic factors[4].

During the preliminary surveys of the *Swertia chirayita* from the known site, it was found that most of the sites had very poor or non significant population to become representative site of *Swertia chirayita* expect for two sites, where the population of *Swertia chirayita* were significant and possessed good vigor.

II. MATERIALS AND METHOD

2.1 Collection Of Plant Material:

The plant specimen of *Swertia chirayita* were collected in the end of September from two different locations, the first being from the Kauntalani Nursery, Chakrata Forest Division, District Dehra Dun, Uttarakhand and the second site for the collection of plant material is Kaddukhal of Saklana Forest range in Narendranagar Forest Division.

2.2. Identification Of The Plant Material

The plant species has been confirmed and identified as *Swertia chirayita* by the plant taxonomist of Department of Botany, Forest Research Institute, Dehradun. and accession number **16430** has been assigned.

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2.3. Chemicals REquired

Hydrochloric acid (HCl), Silver Nitrate ($AgNO_3$) and Methanol (CH_3OH) (Renkem Fine Chemicals Limited, Okhla, Delhi).

2.4.1. Moisture content (%)

Take 2g accurately weighed coarse dried powdered sample in a pre weighed petri dish and drying it in oven at 105°C till constant weight was achieved and was calculated as follows [5].

$$Moisture\ content\ \left(\%\frac{w}{w}\right) = \frac{weight\ of\ oven\ dried\ drug\ sample}{weight\ of\ dry\ drug\ sample} \times 100$$

2.4.2. Total ash (%)

2g accurately weighed drug sample (coarse powder) was heated upto 450°C in an electrical muffle furnace until the burnt ash become free from carbon, then cooled and weighed[5].

$$Total\ Ash\ \left(\%\frac{w}{w}\right) = \frac{weight\ of\ total\ ash}{weight\ of\ \ dry\ drug\ sample} \times 100$$

2.4.3. Acid Insoluble ash (%)

The ash obtained after incineration was treated with 6 Normal HCl and the solution was boiled for 5 minutes and filtered. The residue was washed intermittently with distilled water. The filtrate was tested with Silver Nitrate ($AgNO_3$) to check Chloride ion for absence or presence. The residue left in the filter paper (ash less) was again incinerated and was completely converted to ash. After cooling, the weight of ash was noted and the acid-insoluble ash obtained was calculated by the formula given below [5].

$$Acid\ insoluble\ Ash\ \left(\%\frac{w}{w}\right) = \frac{weight\ of\ Acid\ insoluble\ ash}{weight\ of\ total\ ash} \times 100$$

2.4.4. Water soluble ash (%)

The ash obtained after incineration was boiled in 25 ml of water for 5 minutes and the insoluble matter was collected on an ashless filter paper. It was washed with hot water and ignited in a crucible for 15 minutes. After cooling, the weight of insoluble matter was subtracted from the weight of total ash and the percentage of water soluble ash obtained was considered as under[5]:

$$Water\ soluble\ ash\ \left(\%\frac{w}{w}\right) = \frac{weight\ of\ water\ soluble\ ash}{weight\ of\ \ total\ ash} \times 100$$

2.4.5. Water soluble extractive (%)

Stirred 5 g accurately weighed powder in a 100 ml distilled water in a conical flask and kept overnight. It was then filtered and the filtrate was evaporated on a water bath in a pre weighed, dried, evaporating dish. The evaporated filtrate was then dried to a constant weight in an oven. The water soluble extractive percentage was calculated using following formula[5].

$$Water\ soluble\ extractive\ \left(\%\frac{w}{w}\right) = \frac{weight\ of\ water\ soluble\ extract}{weight\ of\ \ dry\ drug\ sample} \times 100$$

2.4.6. Alcohol soluble extractive (%)

Stirred 5g accurately weighed sample powder and 100ml methanol in a conical flask, and kept overnight and after filtration the filtrate was evaporated on water bath in, a dried, pre-weighed a clean and porcelain evaporating dish. After evaporation of the solvent, the evaporating dish was transferred to an oven for drying to obtain a constant weight of a residue, which was determined by using following formula [5].

$$Alcohol\ soluble\ extractive\ \left(\%\frac{w}{w}\right) = \frac{weight\ of\ alcohol\ soluble\ extract}{weight\ of\ \ dry\ drug\ sample} \times 100$$

2.4.7. Determination of pH

5g accurately weighed drug sample dissolved in 50 ml distilled water was kept for about 30 minutes. It was filtered and pH was noted by digital pH meter[5] (Systronics Inc.).

2.5. Method for quantification of amarogentin

2.5.1. Standard Solution

In methanol a stock solution of A*marogentin* (1.6 mg/10 ml) was prepared. The aliquots of 0.5, 1.0, 1.5, 2.0 and 2.5 ml of stock solution were transferred to 10 ml volumetric flasks and the volume in each flask was adjusted to 10 ml with mobile phase to obtain working standard solutions containing 0.5, 1.0, 1.5, 2.0, 2.5 mg of *amarogentin* per ml, respectively[6]

2.5.2. Test Solution

Refluxed 5 g of dried powdered of each sample with 50 ml methanol for 4 hours. It was filtered and concentrated to dryness using Rota vapor and transferred to a separating funnel by adding 50 ml of distilled water to this solution. After this, it was partitioned with 3X50 ml of Chloroform and then Chloroform layer was discarded.

Similarly it was partitioned with overnight saturated Butanol (3X50 ml). The Butanol washings were pooled and concentrated. It was reconstituted with 10 ml methanol and 10 µl of this solution was injected to the HPLC system for analysis[6].

2.5.3. Mobile Phase

Methanol containing 5 % water, filtered through 0.45 µm membrane filter, degassed

By sonication at a flow rate of 1 ml/min was used in the present analysis.

2.5.4. Detector

All the samples were run for 20 minutes and components of the mixture were detected at 235 nm using Photodiode Array Detector (PAD) 2996 was used in the analysis.

III. RESULTS AND DISCUSSION

The outcomes of the proximate analysis of *Swertia chirayita* are shown in **Table 1**. The general moisture level in site KK was more than the site CK. For storage of drugs the moisture content needs be minimized to control the growth of bacteria, yeast or fungi [7]. Our findings indicated that, moisture content of the specimen was found to be 9% and10% for the two samples of CK and KK respectively.

Table 1: Physico-Chemical Analysis Of *Swertia Chirayita*

Parameter	CK	KK
Moisture content (%)	9%	10%
Total ash (%)	4.75%	4.70%
Acid Insoluble ash(%)	0.895%	0.811%
Water soluble ash (%)	2.165%	2.132
Water soluble extractive(%)	12.15%	11.61%
Alcohol soluble extractive(%)	11.05%	11.23%
pH	7.1	6.85

The results of comparative quantitative analysis of amarogentin in *Swertia chirayita* of two different sites are depicted in **Table 2**

Result indicates that CK site has higher percentage of amarogentin than KK site, which may be due to genetic differences in different populations coupled with edaphic, climatic factors.

Table 2: Comparative Analysis Of Amarogentin (%) In Swertia Chirayita

Population	Amarogentin%		
	Wild	Cultivated	Percent difference
CK	0.094±0.003	0.093 ±0.001	2.46
KK	0.122±0.002	0.119±0.001	1.06

The amarogentin quantity at both the site CK and KK was found to be .094 and 0.122 respectively. Though the amarogentin content is less than the expected level, it may be attributed to the environmental, edaphic factors, change in the use pattern of land, forest management practices. The ash content reported is not more than 6% [8] as the results of the present investigation indicated the ash content 4.75% and 4.70% respectively in the samples under investigation, which is well within the limit and the results are upheld by the previous investigations. So the material can be recommended for use as medicine and generally the whole plant is used as medicinal component. The percentage yield of all extractive are well within the limit as mentioned in Ayurveda Pharmacopoeia [8].

IV. CONCULSION

The information regarding the chemical constituents present in the raw plant obtained from extractive values and also inform the solubility of specific constituents in particular solvents. The demand for genuine *Swertia chirayita* drug material is increasing with its potential use against diseases like Dengue, Chicken guinea, but the major natural quantities are insignificant as compared to the demand.

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