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Altitudinal Variation of Bitter Principle of *Swertia Chirayita* and Its Standardization

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Abstract

Swertia chirayita was obtained at an altitude of (1750-3250m) and three strata were distinguished on the basis of altitude. The bitterness values at strata 1 (1750-2250m), strata 2 (2251 – 2750m) and Strata 3(2751 – 3250m) was 1.33 %, 1.43% and 1.52% respectively; which shows slightly increasing trend with altitude and the average bitterness principle of the plant samples was 1.42 ± 0.06% . The parameters studied for ash standardization for total ash value, acid insoluble ash, and water soluble ash were 5.05± 0.08 %, 0.72± 0.06% and 0.84± 0.05%, respectively. Extract values; methanol extract 16.06 ± 0.41 % and water extract was 17.11± 2.33%. The thin layer chromatography used for the extraction of powder drug and the presence of bitter compounds was confirmed. This study provide the first data in standardization of Chirayita specifically in western Himalayas of Nepal and revealed the quality and purity of chirayita drugs setting down pharmacopoeia standards for future reference.

Keywords: S. chirayita Linn. Strata, Thin layer chromatography.

Introduction

Swertia belongs to *Gentianaceae* family consisting of more than 135 species is widely distributed in temperate areas of Asian, African, Europen, and American continent. *S. Chirayita* has been listed as one of the major medicinal plants for domestication and research in Nepal. In Nepal there are 31 species of *S. Chirayita* available from eastern to western region and from tropical to alpine zones, ranging from 600 m to 5600 m altitude[1,2]. Its trade is more than 45% of the world's total volume in which only 1% is being used locally in Nepal and the rest is being exported to Asian and western country to fulfill the demand of various medicinal plant based industries[3]. *S. chirayita* is a versatile medicinal plant having bioactive chemical compounds and properties such as amaroswerin, amarogentin, chiratinin, gentianine, flavonoids, xanthones, terpenoids, iridoids, and secoiridoid glycosides[4]. The bioactive compounds of *S. chirayita* and their derivatives are reported to have anti-inflammatory, anti-diabetic, anti-hepatotoxic, anti-leishmanial, anti-carcinogenic, anti-viral and anti-helmithic properties. It possesses the property of bitter tonic but, unlike most others bitters it doesn't constipate bowels. Instead it tends to produce a regular action and a free discharge of biles. Like the other members of the family *Gentinaceae*, *chiraita* may also nauseate and oppress the stomach in overdoses[5]. Traditionally *S. chirayita* is used for fever, malaria, anemia, bronchial asthma, hepatitis, constipation, skin diseases. Herbal medicines viz. Ayush-64, Diabecon, Mensturyl syrup, Melicon V ointment and *Mahasudarshan* churn which contains *chirayita* powder and its extract in varied quantities are used for its antipyretic, hypoglycemic, antifungal and antibacterial properties[6].

The chemical screening of the *chirayita* revealed a great deal of variation with respect to bitter content percent relatively higher bitter content of 1.14% was recorded in Kinnaur at 2300m and the lowest bitter content of 1.08% in Solan at 2350m altitude of India [7]. Hence this study is completely based in the variation of bitter principle with respect to altitude. The efficacy of any drug depends upon its properties; therefore, their standardization is a tool to check the authenticity of a drug and it also helps to characterize the constituents that lead to establish the structure, activity, and mode of action of drug. Hence, this study was done with an aim to provide a fruitful data of standardization of *S. chirayita* using pharmacopoeia guidelines. The parameters studied were total ash, water soluble ash, acid insoluble ash, alcohol soluble extractive, and water soluble extractive.

Methodology

Study Area: The study area is Tamu and Kamre forest of Bhoje VDC which belongs to Bhujung Unit Conservation Office of Annapurna Conservation area, Nepal. The geographical location of the sample plots in the study areas are shown in Figure-1. The study area was divided in to three strata starting from 1750m to 3350m with a difference of 500m. Strata I, II and III includes an altitude of 1750 to 2250m, 2250m to 2750m and 2750m to 3250m, respectively.

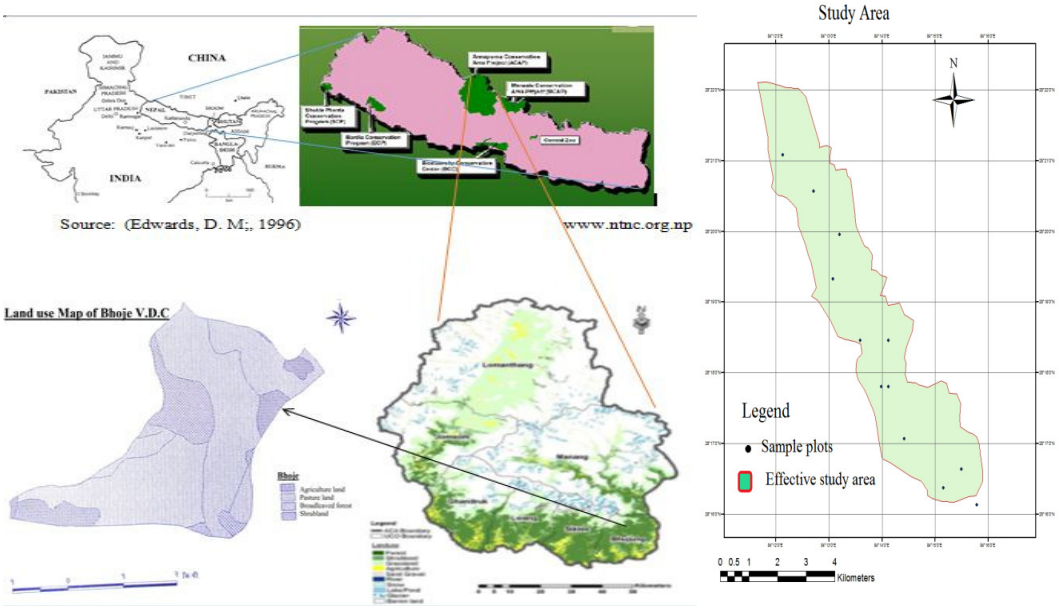

Figure-1
Location of the study area[8-10]

Plant Material: The samples of S. *chirayita* were collected from 12 plots, 4 plots in a transect line of each of 3 altitudinal range. The *chirayita* is collected within the plots leaving 25% of the area for regeneration [5, 6] by taking permission from Bhujung Conservation Area Management Committee. The Taxonomic evaluation was established by Head, Herbarium in-charge, Systematic Botany Discipline, Forest research Institute, Dehradun.

Determination of Bitter Principle: The bitterness principle is determined for all the three samples of three strata's separately following the procedures as mentioned in Ayurvedic pharmacopeia of India, volume 1 part 1;1986. The 2 gram of powder (No. 60 sieve) of *S. chirayita* is mixed with boiling water containing 0.5gram of calcium carbonate till the last portion of the extract is devoid of bitterness. Then it was concentrated in vacuum (Life lysed) and the residue was dissolved in hot alcohol. This was filtered at hot, and then residue was washed thrice on the filter with 10ml hot alcohol. The alcohol solution was removed from the filtrate and the residue was repeatedly taken with 25, 15, 15, 15, and 15 ml of hot water. The aqueous solution was shake and extracted repeatedly with 25, 20, 15, 15 and 10 ml of Ethyl Acetate, extracts was collected, evaporated, dried and weighed[11]. The powder of three samples from three different strata is taken repeatedly and replicated thrice for the consistency in the result.

Determination of Ash Values and Extractive Values: The ash was determined by three different methods which measures, total ash, acid insoluble ash and water soluble ash. Acid-insoluble ash residue was obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. Water-soluble ash was obtained by calculating the difference in weight between the total ash and the residue after treatment of the total ash with water. Water soluble extractive and alcohol soluble extractive values were calculated by standard methods. The different Ash values and extractive values were determined following the standard procedures as mentioned in Quality control of herbal plants, WHO; 2011 and the experiment was replicated thrice for the consistency in the results[8].

Thin Layer Chromatography (TLC) Experiment: For TLC test, air dried and coarsely powdered material (4 G) was accurately weighed and taken in a glass stopper conical flask, and 100ml of 90% ethanol was added and shake for 6 hours continuously. Then the solution was allowed to stand for 18 hrs and filtered rapidly taking care without loss of any solvent. The solvent was removed and dissolved in 100 mg of extract in 5 ml of 90 % ethanol. Then this solution was used for TLC profile[12]. The methanolic extracts of *S. chirayita* were applied on TLC aluminum plate pre-coated with Silica gel 60 GF254 and developed using a solvent system containing Ethyl acetate : Methanol : Water (7.7 : 1.3 : 0.8, v/v/v) as a mobile phase. The detection was done with 10% $FeCl_3$ solution. The TLC plate was observed immediately after spraying[13]. The bitter principles of *S. chirayita* turn the colour of TLC plate yellow brown to yellow green (Figure-3). This confirmed the presence of bitter compound in the samples[13].

Results and Discussion

The bitterness test of the material revealed some variation with respect to bitter content in different strata. Strata 1 have a bitter

content of 1.33 % while the samples from strata 2 and 3 have bitter content of 1.43 % and 1.52 % respectively. The average value of bitterness principle of this region is 1.42 ± 0.06 % the result is supported by the "The wealth of India- 1976" which reveals that *chirayita* contains not less than 1.3 % of bitterness value. The percentage values of bitterness principle in different strata show a slight variation between the samples of different altitudinal range. The difference is 0.11% between strata 1 and strata 2 and only 0.09 % between strata 2 and 3. The variables that affect the bitterness values was altitudinal range and others factor might be soil pH and other soil parameters. The literature revealing the bitterness principle from the Nepalese sample is lacking. The bitter principle value of samples of different strata is tabulated in Table-1.

The total ash content, total acid insoluble ash content and total water soluble ash content of the *chirayita* in the study area was obtained as 5.05%, 0.72% and 0.84% likely with this result Sayyed et al.[8] also confirmed the values as 4.89% , 0.96% and 0.843% w/w simultaneously. According to the Ayurvedic pharmacopeia of India vol. 1 part 1 the values is less than 6 % for total ash content and not more than 1 % for total acid insoluble ash content and total water soluble ash content respectively which shows the consistency of the results.

On the other hand the methanol extract, and the water extract of the *chirayita* in the study area was obtained as 16.05% and 17.11% similarly, Sayyed et al. also confirmed the values as 12.39% and 13.17% w/w simultaneously[8]. According to the Ayurvedic pharmacopeia of India vol. 1 part 1 the values of total methanol extract and total water extract is not less than 10 % which shows the consistency of the results.

The bitter principles of *S. chirayita* turn the colour of TLC plate yellow brown to yellow green after spraying $FeCl_3$ solution to the TLC plate (Figure-3). This confirmed the presence of bitter compound in the samples[13].

Table-1
Bitter Principle values of samples of principles with respect to altitude

Strata	% Bitterness
1750-2250	1.33
2251 – 2750	1.43
2751 – 3250	1.52
Standard Dev.	0.10
Standard Error	0.06
Average	1.42 ± 0.06

Table-2
Standardized values of *S. Chirayita* obtained from the research as compared to API (1976)

Standards	Values (%)	API(1976)
Total Ash content	5.05 ± 0.88	less than 6%
Total Acid insoluble Ash content	0.72 ± 0.13	not more than 1%
Total Water solubleAsh content	0.84 ± 0.05	not more than 1 %
Total Methanol extract	16.05 ± 0.41	not less than 10%
Total Water extract	17.11 ± 2.33	not less than 10%

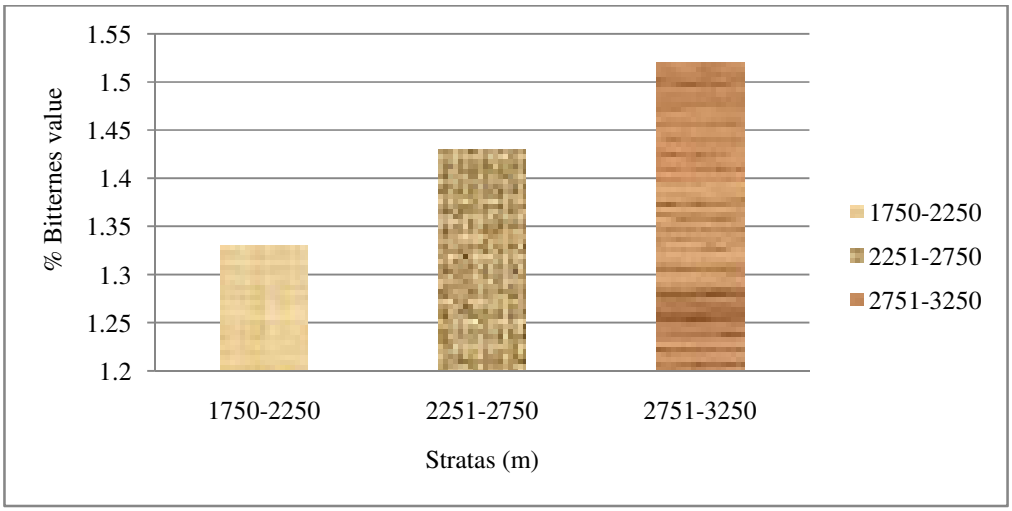

Figure-2
Diagram showing the trend of bitterness values at different strata

Figure-3
Thin layer chromatography observations a) Before spray of $FeCl_3$ and b) after spraying

Conclusion

This study concluded that the average bitter principle of *S. chirayita* is 1.42 ± 0.06 % and the bitter principle shows slightly increasing trend with respect to altitude. Collections from higher altitude gave higher bitter content and can be selected for cultivations and further improvement by adopting appropriate agro technology.

Standardization is an integral part for any study and is necessary when we are exploring any biological activity of a drug, and to make drug authentic. It is therefore necessary to work out physicochemical standards of drugs. As many parameters in this study provide one of the first data in standardization of *chirayita* specifically in western Himalayas of Nepal. This study will help in setting down pharmacopoeial standards for future reference in determining the quality, purity and authenticity of *S. chirayita Linn.*

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