

## Comparison of Phytochemicals of Native Plant, *Swertia Chirayita* (Roxb. ex Fleming) Karst from Rasuwa District in Nepal

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### Abstract

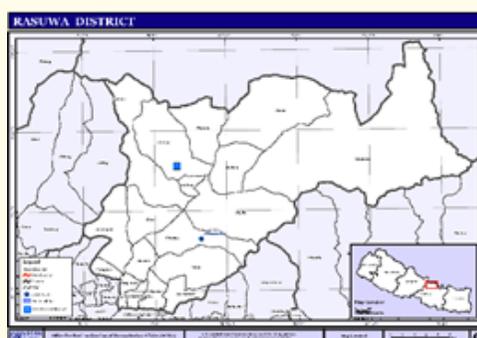
*Chiraito* (*Swertia chirayita*) as medicinal and aromatic plants (MAPs) is considered as a major source of rural household income and also contribute to the local economics in Nepal. Great majority of species are collected from wild and very few are practiced for cultivation. The rapid loss of traditional medical knowledge and practices due to their dependency on verbal transformation has been a great challenge to modern technology; as a result its application to design therapeutic food has been in shadow. Phytochemicals namely Amarogentin, Mangiferin and Swertiamarin were determined from the root, shoot and leaves of nine samples collected from the 9 different Village Development Committee (VDCs) of Rasuwa district to compare the difference of phytochemicals using High Performance Liquid Chromatography-DA Detector. The plant extracts so prepared in warm distilled water and ethanol respectively were subjected to measure phytochemicals where no significant difference observed between the extracts of these samples.

**Keywords:** Aqueous Solution; Extraction; HPLC; Methanol; Phytochemicals; *Swertia*

### Introduction

*Swertia chirayita* (Roxb. ex Fleming) karst. Commonly known as *Chiraito* in Nepal and its entire plant is used as a source of active phytochemicals [1]. This species was first introduced in the Edinburgh Pharmacopoeia in 1839 and is reported in British and American Pharmacopoeias to be used as an infusion or a tincture. The plant has been used as a beneficial remedy for lung, liver, stomach and kidney ailments and anticancer preparation [2]. It is a rich source of the bitterest compound amarogentin, known in nature and this phytochemical is present in this plant [3].

The chemical constituents of this plant have been analyzed, characterized and reviewed by several groups [4-5]. The compound isolated from *Swertia* include compound like chiratin, ophelic acid, palmitic acid, oleic acid, stearic acid, alkaloids, glycosides and a large number of xanthones. The first isolated dimeric xanthone was chiratinin. Other important phytochemicals include swertiamarin, mangiferin, amarogentin, gentiopecrin, swerchirin, swertanone, and chiratol. A detail list of compounds isolated from *Swertia Chirayita* is available in literature [8,9]. A review of naturally occurring xanthone including gentisin, mangiferin, swerchirin is available in literatures [10] but still *Swertia Chirayita* has received inadequate attention till date to estimate their medicinal properties. Problem often arise due to adulterants as a substitute of *Swertia Chirayita* for unhealthy trade and unavailability of the major target compounds in their pure form commercially. In general, each and every part of this plant is dried, grinded and used locally in Nepal as an infusion prepared by steeping in tap water overnight. In addition, *Swertia chirayita* has only recently been brought into cultivation with limited success. The significant of the present study was to identify the main phytochemicals present in polar solvents like aqueous and ethanol extracts for the assessment of possible differences.



**Figure 1:** Map of the VDCs in Rasuwa District, Nepal from where the plant samples were collected 1) Bridhim 2) Chilime 3) Gallang 4) Goljung 5) Haku 6) Shertung 7) Syafru 8) Tipling and 9) Thuman.

## Materials and Methods

### Plant samples

*Swertia chirayita* plants were collected from the nine Village Development Committee (VDC) of Rasuwa district, Nepal representing Central region. All plants were collected at the end of the flowering season in late August to October 2017 when the plants were in the seed dispersal phase. The standards of mangiferin, swertiamarin and amarogentin were supported from the Department of Plant Resources, Government of Nepal (GON).

### Sample Collection

The plants *Swertia Chirayita* were collected from the nine different location of Village development committee (VDC) Rasuwa for study. The VDC were as below.

1) Bridhim 2) Chilime 3) Gallang 4) Goljung 5) Haku 6) Shertung 7) Syafru 8) Tipling and 9) Thuman

**Sample Preparation:** The plants were divided into root, shoot and leaf and shade dried at room temperature of 24 - 30°C. These different parts of the plants were grounded in the spice grinder (Spark machines, manufactured under quality system, ISO-9001:2000, India). The powdered samples were subjected to extraction in warm polar solution (Water; DW and ethanol).

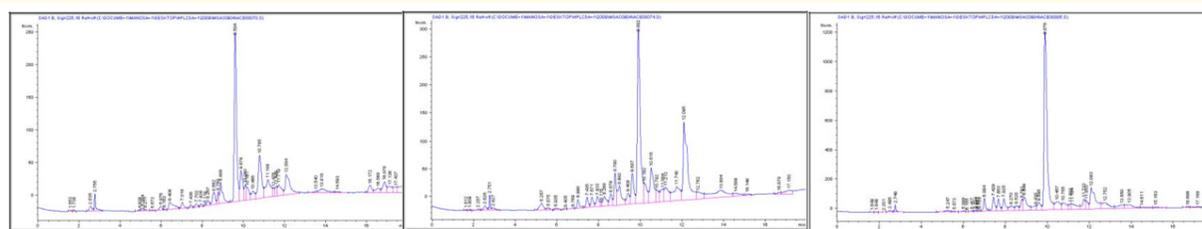
**Extraction of the samples:** The warm extraction was done which is a versatile tool that can be used to separate a single gram to hundreds of gram with near 100% recovery [11]. Five grams of the dried powdered plant sample was mixed with 500 mL distilled water and kept in water bath at 70°C for 48 hours. Similarly 500 mL ethanol Qualigens, Fisher Scientific was added into 5 g individual samples packed into thistle funnel and extraction was done 48 hours at 70°C to make 1% w/v extract. The mixture was stirred for 30 minutes prior to heat treatment at room temperature. After 48 hours, the solution was filtered using No. 1 filter paper the following day.

### Chromatographic Analysis

According to Khanal S., *et al.* [12], Crude extracts of two mL was taken and then filtered through a 0.2 µm filter and 5 µL were injected in a High Performance Liquid Chromatography Agilent 1100 series equipped with auto-sampler and DA-Detector 1100 diode array detector (Agilent Technologies, Palo Alto, CA). The solvents used for gradient elution were 10 mM phosphoric acid (pH 2.5) and 100% methanol [13]. The methanol concentration was increased to 60% for the first 8 min and to 100% over the next 7 minutes, then decreased to 0% for the next 3 min and was maintained for the next 7 minutes (total run time, 25 minutes). The analytical column used was Agilent Zorbax SB-C18, 250 mm × 4.6 mm i.d. with packing material of 5 µm particle size at a flow rate of 1 mL/min at ambient temperature. During each run the absorbance was recorded at 225 nm and 306 nm and the chromatogram integrated using Agilent Chemstation enhanced integrator. Standard solution of Amarogentin, Mangiferin and Swertiamarin were used to perform Calibration by injecting at different concentrations. Peak identification was performed by comparison of retention times and diode array spectral characteristics with the standards so as to determine the level of phytochemicals using the standard curve and expressed in mg/g dry weight of the sample.

## Results and Discussion

The phytochemicals namely mangiferin, amarogentin and swertiamarin were observed in aqueous and ethanol extracts of all plant parts (Figure 2) that shows the variation in quantity of mangiferin, a C-glucosylxanthone (1, 3, 6, 7-tetrahydroxy-2-[(2S, 3R, 4R, 5S, 6R)-3, 4, 5-trihydroxy-6-(hydroxymethyl) oxan-2-yl] xanthen-9-one) present in aqueous and ethanol extracts of different plant parts of *Swertia chirayita* collected from nine different VDC of Rasuwa district of Nepal.



**Figure 2:** Chromatogram of Ethanol extract of root, stem and leaves respectively (left to right).

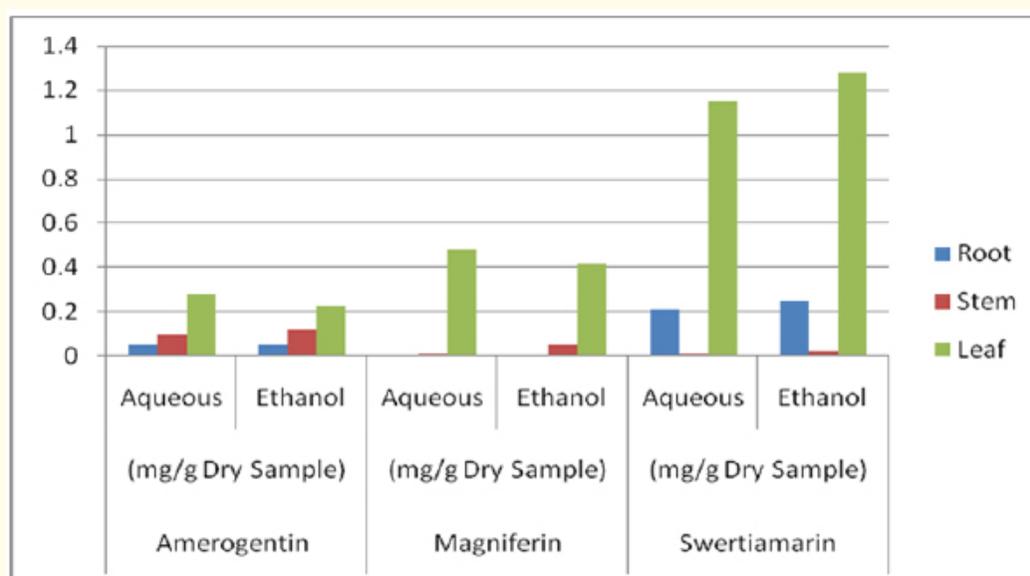
The quantity of mangiferin was found highest in both aqueous and ethanol extract of leaves for all *S. chirayita* plant samples. In general, both aqueous and ethanol extracts of stem had higher quantity of mangiferin than both extracts of root for all collected samples.

The quantity of mangiferin was highest in aqueous and ethanol extracts (0.48 and 0.42 mg/g DW) of leaves.

Parts	Amerogentin (mg/g Dry Sample)		Mangiferin (mg/g Dry Sample)		Swertiamarin (mg/g Dry Sample)	
	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol
Root	0.05	0.05	0.004	0.002	0.21	0.25
Stem	0.10	0.12	0.01	0.05	0.01	0.02
Leaf	0.28	0.23	0.48	0.42	1.15	1.28

**Table 1:** Variation in overall quantity of amarogentin, mangiferin and swertiamarin.

The quantities of mangiferin and its derivatives were lowest in aqueous and ethanolic extracts (0.004 and 0.002 mg/g DW) of roots. The name Mangiferin was found in many medicinal plants after *Mangifera indica* (Mango), the leaves of which is reported to possess considerable hypoglycemic property due to this phytochemical [14].

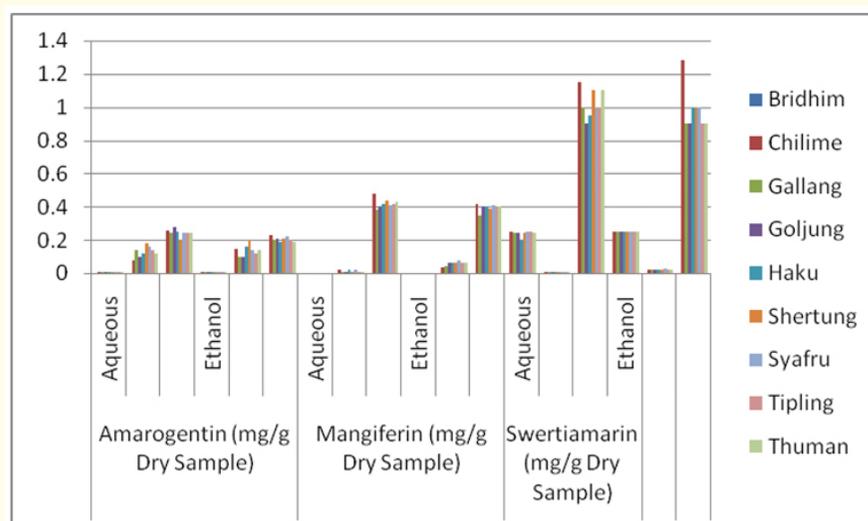


**Figure 3:** Variation in overall quantity of amarogentin, mangiferin and swertiamarin present in aqueous and ethanol extracts of stem, root and leaf of *Swertia chirayita* collected from nine VDCs of Rasuwa district of Nepal.

According to Nair and Devi [15], Mangiferin significantly increases heart tissue phospholipids in isoproterenol induced cardio-toxic rats suggesting cardio protective and hypolipidemic effects. It has also been reported to show suppressive effects on blood lipids in diabetes [16].

In general, higher quantities of swertiamarin, a secoiridoid glycoside ((5R, 6S)-5-ethenyl-4a-hydroxy-6-[(2S, 3R, 4S, 5S, 6R)-3, 4, 5-tri-hydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-3, 4, 5, 6-tetrahydropyrano [3,4-c]pyran-1-one) were found in aqueous and ethanol extracts of roots than in aqueous and ethanol extracts of stem for all collected plant samples. Swertiamarin was highest in aqueous and ethanol extracts of leaves of all collected *Swertia chirayita* plant samples. In addition, higher quantities of swertiamarin were found in ethanol extracts than in aqueous extracts of all collected plant samples. There was no significant difference in the quantities of swertiamarin present in different plant parts in aqueous and ethanol extracts of *S. chirayita* collected from different locations of Rasuwa district of Nepal. While the highest quantity of swertiamarin were found in aqueous and ethanol leaves extracts (1.15 and 1.28 mg/g DW) at Chilime whereas the lowest quantity of swertiamarin were found in aqueous and ethanol stem extracts (0.01 mg/g DW each) at Syafru. Although there was no significant difference in quantities of swertiamarin between aqueous and ethanol extracts of other plant parts ( $p > 0.05$ ). Swertiamarin, has been studied to have a number of pharmacological properties such as hepato protective and anti-inflammatory, antioxidant and anti-

spastic [17]. Swertiamarin has also been reported as a potent lipid lowering agent comparable to the clinical drug atorvastatin which may also contribute to its cardio-protective and anti-atherosclerotic role [18]. The result from this study indicates that swertiamarin and its derivatives are present in all plant parts of *Swertia chirayita* but are mostly concentrated in the root and in leaf.



**Figure 4:** Variation in overall quantity of amarogentin, mangiferin and swertiamarin present in aqueous and ethanol extracts of samples collected from nine VDCs of Rasuwa district of Nepal.

Quantities of amarogentin, a secoiridoid glycoside ([[(2S, 3R, 4S, 5S, 6R)-4, 5-dihydroxy-6-(hydroxymethyl)-2 [[[(5S)-5-hydroxy-1-oxo-4, 4a, 5, 6-tetrahydro-3H-pyrano [3, 4-c] pyran-6-yl]oxy] oxan-3-yl] 2, 4-dihydroxy-6-(3-hydroxyphenyl) benzoate) were found to be highest in aqueous and ethanol leaves extracts (0.28 and 0.23 mg/g DW) from Goljung and lowest in aqueous and ethanol root extracts (0.007 and 0.002 mg/g DW) from Gallang. In general, aqueous extracts had higher quantities of amarogentin than ethanolic extracts of all plant parts for all collected plant samples. Quantities of amarogentin were highest in aqueous and ethanolic extracts of leaves and lowest in aqueous and ethanolic extracts of root for all collected plant samples. It is reported that Amarogentin is also known as one of the bitterest compound known to mankind and its bitterness has been tasted even at a dilution of 1: 58,000,000 [19]. It is a known topoisomerase inhibitor [20], chemo preventive and is reported to have anti-leishmanial [21] with gastro protective properties [22].

## Conclusion

The phytochemicals i.e., amarogentin, mangiferin and swertiamarin were present in highest quantities in leaves aqueous and ethanol extracts. So, it can be concluded that this plant's part could be used as medicinal preparations. Root extracts showed the lowest amounts of mangiferin and amarogentin whereas amount of swertiamarin were found to be the lowest in aqueous and ethanol stem extracts. This indicates that plants cultivated at nine VDC were found effective. *Swertia chirayita* is used for therapeutic purpose after infusion in local healing. The presence of these main phytochemicals may suggest their relation to the therapeutic properties especially the high quantity of mangiferin and its derivatives which is reported to be a potent anti-diabetic.

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