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QUANTIFICATION OF MAJOR PHYTOCHEMICALS OF *SWERTIA CHIRAYITA*, A MEDICINAL PLANT FROM NEPAL

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ABSTRACT

Swertia chirayita is an important medicinal plant from Nepal with anti-diabetic, anti-pyretic, anti-malarial and anti-inflammatory potential and used in therapeutic herbal preparations in parts of South Asia. The main phytochemicals in crude aqueous and ethanolic extracts of different plant parts of *Swertia chirayita* collected from nine different districts of Nepal representing West, East and Central Nepal were quantified using HPLC/DAD (High Performance Liquid Chromatography- Diode Array Detection). The quantities of these phytochemicals were also compared between wild and cultivated plant parts of *Swertia chirayita*. Amarogentin, mangiferin, swertiamarin were the main phytochemicals in all extracts. The highest quantity of all the three phytochemicals was found in IL (inflorescence and leaf mixture) of all the collected plants samples. There was no significant difference in the amounts of these three phytochemicals between extracts from wild and cultivated plants. The result from this study substantiates the validity of cultivated *Swertia chirayita* for medicinal purposes and trade.

Key words: *Swertia chirayita*, *HPLC/DAD*, wild, cultivated, phytochemicals, quantification, mangiferin, swertiamarin, amarogentin.

INTRODUCTION

Swertia chirayita (Roxb. ex Flem.) Karst. is an important medicinal plant endemic to the Himalayan regions. It is found growing in moist hillsides of temperate forests between the altitudes of 1200-3000 m and is indiscriminately collected from its wild habitat for trade and local medicinal use. *S. chirayita* is utilized extensively in Eastern traditional medicine such as Ayurveda, Unani, Siddha and also in traditional Chinese and Tibetan medicine as well as in local healing in Nepal. *S. chirayita* is used in traditional medicine for chronic fever, malaria, anemia, bronchial asthma, liver disorders, hepatitis, gastritis, constipation, dyspepsia, skin diseases, worms, epilepsy, ulcer, scanty urine, hypertension, melancholia and certain type of mental disorder, secretion of bile, blood purification and diabetes (Karan *et al.* 1999, Banerjee *et al.* 2000, Airi *et al.* 2002, Rai 2003, Gao-Feng *et al.* 2004 and Saha *et al.* 2004). In Ayurveda, *S. chirayita* is described as bitter (*tikta*) in taste and its thermal action defined as cooling (*shita*), easily digestible (*laghu*) and dry (*ruksha*) (Joshi and Dhawan 2005). It is an important

ingredient in many Ayurvedic health tonics, supplements, anti-diabetic and anti-cancer preparations, liver tonics, skin creams, soaps and even in hair oils. This species was first introduced in the Edinburgh Pharmacopoeia in 1839 and is reported in British and American Pharmacopeias to be used as an infusion or a tincture.

Swertia chirayita belongs to the Gentianaceae family and contains many of the compounds that are responsible for its therapeutic properties such as xanthones, flavonoids, terpenoids, iridoids and secoiridoid glycosides (Pant *et al.* 2000). This plant is used locally in Nepal as an infusion prepared by grinding the plant and steeping it in water overnight. In addition, *Swertia chirayita* has only recently been brought into cultivation with limited success. The objectives of the present study were: (i) to identify the main phytochemicals present in aqueous and ethanolic extracts of *Swertia chirayita*, (ii) to examine the possible differences of these phytochemicals in different plant parts (stem, root and inflorescence and leaf mixture (IL)) collected from different districts of Nepal, and (iii) to study possible differences in these phytochemicals present in aqueous and 12 % ethanolic extracts between wild and cultivated *Swertia chirayita*.

MATERIALS AND METHODS

Plant samples: *Swertia chirayita* plants were collected from nine districts of Nepal representing Western region: Dolpa, Bajura, Mugu, Central region: Sindhupalchok, Rasuwa and Dolakha and, Eastern region: Taplejung, Panchthar and Dhankuta. The wild and cultivated plants were collected from three districts in East Nepal: Dhankuta, Tehrathum and Sankhuwasabha. All plants were collected at the end of the flowering season in late August to October 2007 when the plants were in the seed dispersal phase.

The standards of mangiferin, swertiamarin and amarogentin were purchased from Chromadex™ (Irvine, CA). Phosphoric acid and HPLC grade methanol were purchased from Fisher Scientific (Fairlawn, NJ). This study was undertaken in Department of Food Science, University of Massachusetts, Amherst, USA.

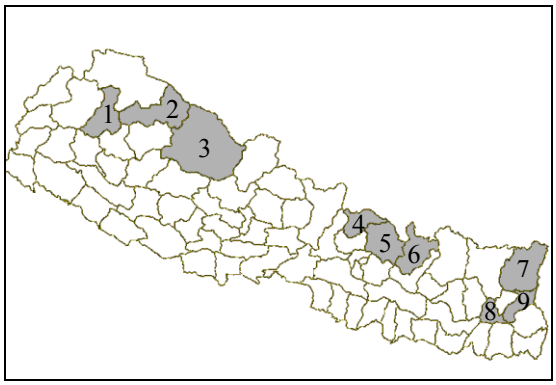


Fig. 1. Map of Nepal showing districts from where the plant samples were collected. (1: Bajura, 2: Mugu, 3: Dolpa, 4: Rasuwa, 5: Sindhupalchok, 6: Dolakha, 7: Taplejung, 8: Dhankuta 9: Panchthar)

Sample Extraction: Individual plant samples were divided into root, shoot and mixture of inflorescence with leaf (IL) and dried at room temperature of 20-25ºC. The separated plant parts were ground using coffee grinder (Mr. Coffee, Sunbeam Products Inc., Boca Raton, FL). One gram of each of the dried plant sample was mixed with 100 ml of either water or 12% ethanol (to make 1%, w/v extract). Ethanolic extracts at 12% were chosen as lowest concentration for alcoholic beverages for effectively extracting bound phenolic compounds which would have physiological relevance in the medicinal use of *S. chirayita*. This mixture was left at room temperature (25ºC) for 12 h (overnight). The sample was then filtered the next day using Whatman No 1 filter paper.

HPLC Analysis: Two ml of the extracts were filtered through a 0.2 µm filter and 5 µL were injected in a HPLC Agilent 1100 series equipped with autosampler and DAD 1100 diode array

detector (Agilent Technologies, Palo Alto, CA). The solvents used for gradient elution were (A) 10 mM phosphoric acid (pH 2.5) and (B) 100% methanol. The methanol concentration was increased to 60% for the first 8 min and to 100% over the next 7 min, then decreased to 0% for the next 3 min and was maintained for the next 7 min (total run time, 25 min). 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. Calibration was performed by injecting the standards of amarogentin, mangiferin and swertiamarin at different concentrations. Peak identification was performed by comparison of retention times and diode array spectral characteristics with the standards. The quantity of the phytochemicals was calculated using the standard curve and expressed in mg/g DW (dry weight of the sample).

Statistical Analysis: Statistical analysis was completed using one-way analysis of the variance (ANOVA). Post-hoc comparisons were carried out using LSD test or planned comparison done in Statistic software package version 5.0 (StatSoft, Inc., Tulsa, OK) ($p < 0.05$).

RESULTS AND DISCUSSION

Three main phytochemicals: mangiferin, amarogentin and swertiamarin were identified in aqueous and 12 % ethanolic extracts of all plant parts (Figs. 2, 3, 4 and 5).

Mangiferin and its derivatives: Fig. 6 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 12% ethanolic extracts of different plant parts of *Swertia chirayita* collected from nine different districts of Nepal.

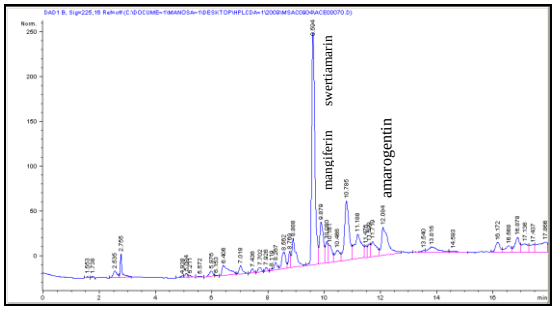


Fig. 2. HPLC chromatogram of 12% ethanolic extract of root of wild *S. chirayita* showing three main phenolic compounds: swertiamarin, mangiferin, amarogentin.

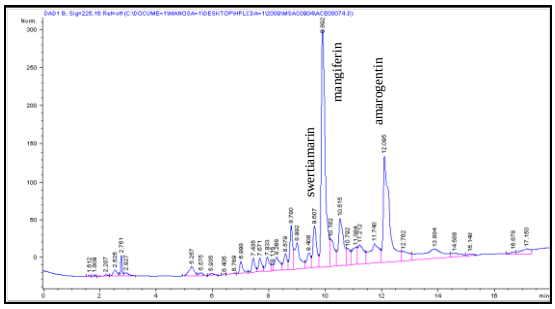


Fig. 3. HPLC chromatogram of 12% ethanolic extract of stem of wild *S. chirayita* showing three main phenolic compounds: swertiamarin, mangiferin, amarogentin.

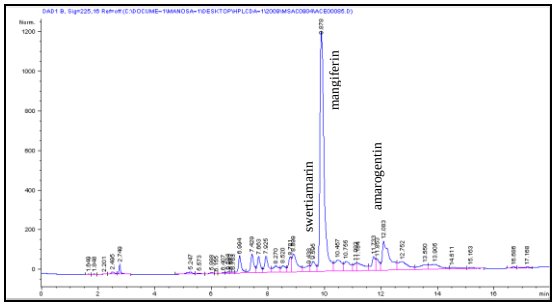


Fig. 4. HPLC chromatogram of 12% ethanolic extract of IL (mixture of inflorescence and leaf) of wild *S. chirayita* showing three main phenolic compounds: swertiamarin, mangiferin, amarogentin.

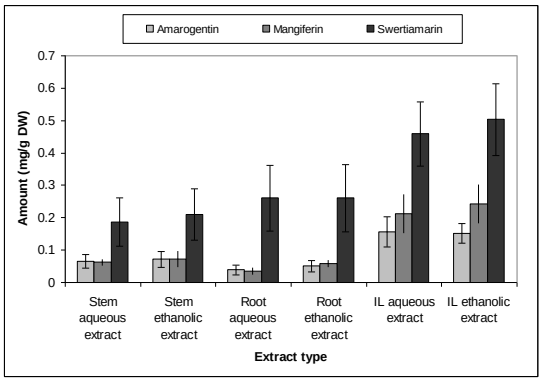
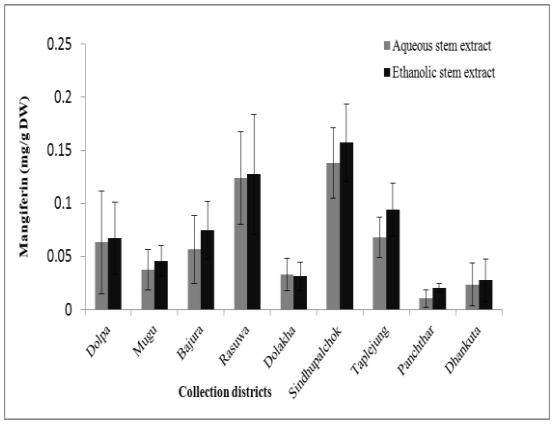


Fig. 5. Variation in overall quantity of amarogentin, mangiferin and swertiamarin (expressed in mg/g DW) present in aqueous and 12% ethanolic extracts of stem, root and IL (mixture of inflorescence and leaf) of *Swertia chirayita* collected from different districts of Nepal.

The quantity of mangiferin was found to be highest in both aqueous and 12% ethanolic extract of IL (mixture of inflorescence and leaf) for all *S. chirayita* plant samples. In general, both aqueous and 12% ethanolic 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 ethanolic extracts (0.46 and 0.4 mg/g DW) of IL from Rasuwa. The quantities of mangiferin and its derivatives were lowest in aqueous and ethanolic extracts (0.003 and 0.001 mg/g DW) of roots from Dolakha. There was no significant difference in the quantity of mangiferin present in different plant parts in aqueous and ethanolic extracts of *S. chirayita* collected from East, West and Central regions of Nepal ($p > 0.05$). However, the amount of mangiferin was slightly higher in aqueous and ethanolic extracts of wild plant samples in comparison to the extracts from cultivated plant samples (Fig. 7). The quantity of mangiferin was lowest in both aqueous and ethanolic extracts of wild and cultivated plant samples; and highest in both aqueous and ethanolic extracts of IL of wild and cultivated plant samples.

Mangiferin, found in many medicinal plants is named after *Mangifera indica* (Mango), the leaves of which is reported to possess considerable hypoglycemic property due to this phytochemical (Muruganandan *et al.* 2005). In India, the leaf and bark extract of *Mangifera indica* is used for the treatment of immuno-deficiency diseases such as arthritis, diabetes, hepatitis, cardiac and mental disorders (Sanchez *et al.* 2000). Besides possessing antioxidant (Sanchez *et al.* 2000), anti-tumor (Guha *et al.* 1996), antiviral (Zheng and Lu 1990), antiatherogenic (Murugananadan *et al.* 2005), immunodialtory (Guha *et al.* 1996), anti-proliferative, immunodilatory, cardiotonic and diuretic properties (Andreu *et al.* 2005), it is also reported to have significant anti-diabetic activity similar to the clinical drug glibenclamide and acts by stimulation of insulin production from the pancreas, extra-pancreatic action and enhancement of glycolytic enzymes (Sellamuthu *et al.* 2009). Mangiferin significantly increased heart tissue phospholipids in isoproterenol induced cardio-toxic rats suggesting cardioprotective and hypolipidemic effects (Nair and Devi 2006). Mangiferin has also been reported to show suppressive effects on blood lipids in diabetes (Leiro *et al.* 2003).

The infusion of *Swertia chirayita* is locally used in Nepal as an anti-diabetic. A review of literature indicates that mangiferin seem to have several modes of actions for counteracting diabetes and its complications; directly by stimulating insulin production, enhancing glycolytic enzymes, inhibiting α-glucosidase and other enzymes such as maltase, sucrase, isomaltase and aldose reductase (Yoshikawa *et al.* 2001) and indirectly by its antioxidant capacity and analgesic (Dar *et al.* 2005), anti-inflammatory (Garrido *et al.* 2003), antiatherogenic (Muruganandan *et al.* 2005), cardioprotective and antihyperlipidemic properties (Nair and Devi 2006).



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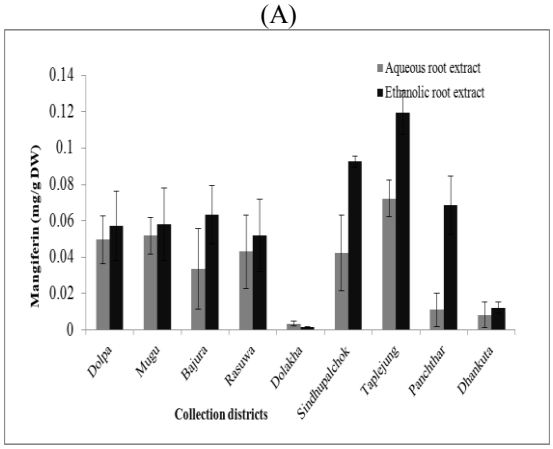

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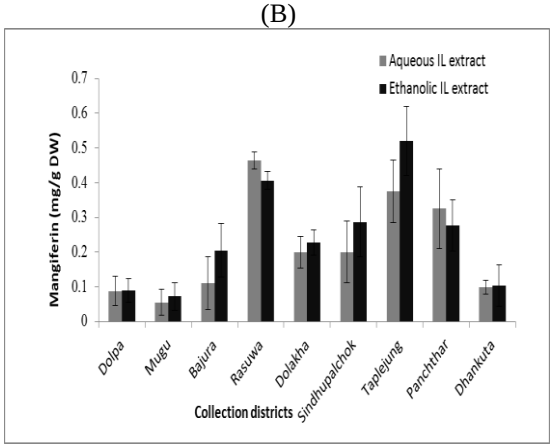


(C)

Fig. 6. Variations in mangiferin (mg/g DW) present in aqueous and 12% ethanolic stem (A), root (B) and IL (mixture of inflorescence and leaf) (C) extracts of *Swertia chirayita* collected from different districts of Nepal.

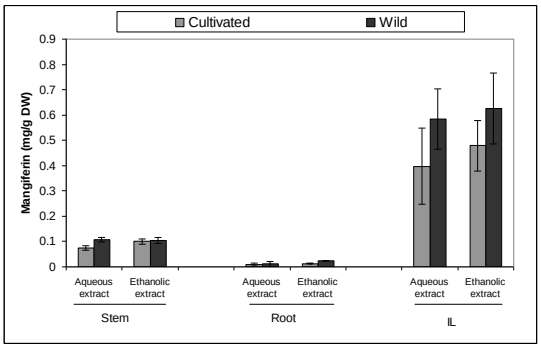
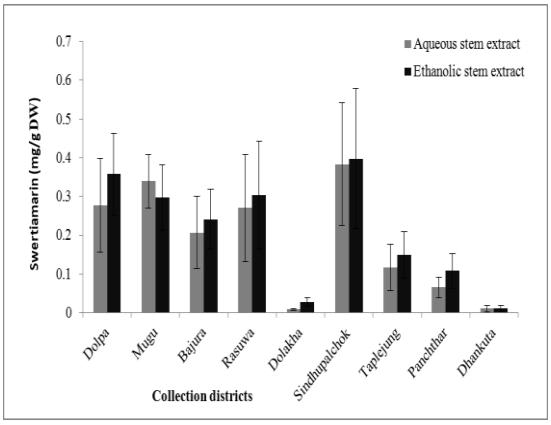
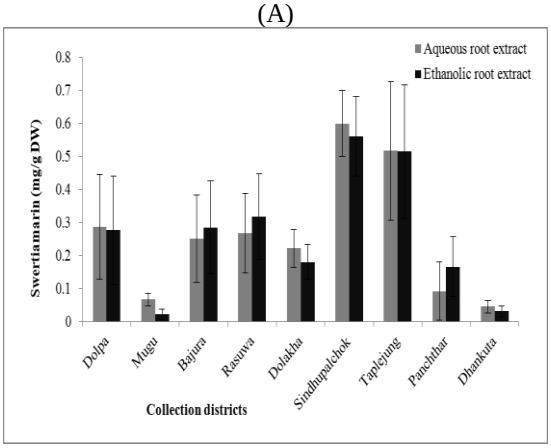


Fig. 7. Variations in mangiferin (mg/g DW) present in aqueous and 12% ethanolic root extracts of cultivated and wild *Swertia chirayita*.

Swertiamarin and its derivatives: In general, higher quantities of swertiamarin, a secoirirdoid glycoside ((5R, 6S)-5-ethenyl-4a-hydroxy-6-[(2S, 3R, 4S, 5S, 6R)-3, 4, 5-trihydroxy-6-(hydroxymethyl)oxan-2-yl] oxy-3, 4, 5, 6-tetrahydropyrano [3,4-c]pyran-1-one) were found in aqueous and ethanolic extracts of roots than in aqueous and ethanolic extracts of stem for all collected plant samples. Quantities of swertiamarin were highest in aqueous and 12% ethanolic extracts of IL of all collected *Swertia chirayita* plant samples (Fig. 8). In addition, higher quantities of swertiamarin were found in 12% ethanolic 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 ethanolic extracts of *S. chirayita* collected from East, West and Central regions of Nepal ($p > 0.05$). While the highest quantity of swertiamarin were found in aqueous and 12 % ethanolic IL extracts (1.15 and 1.28 mg/g DW) from Sindhupalchok, the lowest quantity of swertiamarin were found in aqueous and ethanolic stem extracts (0.01 mg/g DW each) from Dhankuta. Although there was no significant difference in quantities of swertiamarin between aqueous and ethanolic extracts of wild and cultivated plant parts ($p > 0.05$), in general, higher



(A)



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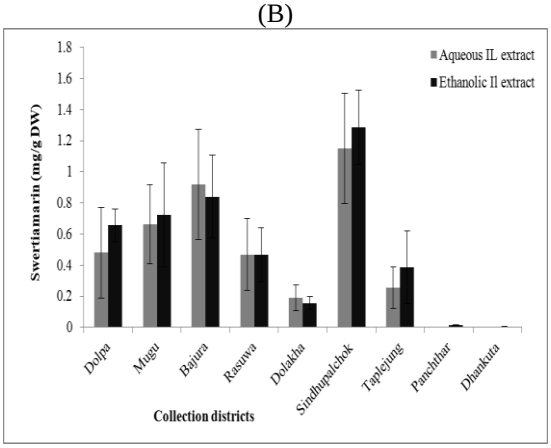


(C)

Fig. 8. Variations in swertiamarin (mg/g DW) present in aqueous and 12% ethanolic stem (A), root (B) and IL (mixture of inflorescence and leaf) (C) extracts of *Swertia chirayita* collected from different districts of Nepal.

quantities of swertiamarin were found in aqueous and ethanolic extracts of wild *Swertia chirayita* plants in comparison to the cultivated *Swertia chirayita* plants (Fig. 9). The quantities of swertiamarin were highest in aqueous and ethanolic root extracts (0.25 and 0.29 mg/g DW) from wild plants and lowest in aqueous and ethanolic stem extracts (0.04 and 0.07 mg/g DW) from cultivated plants (Fig. 9).

Swertiamarin, has been reported to possess a number of pharmacological properties such as hepatoprotective and antiedematogenic/anti-inflammatory, free radical scavenging and antispastic (Vaijanathappa and Badami2009). 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 (Vaidya *et al.* 2009). Swertiamarin has been reported to have very low toxicity and is anti-bacterial (Kumarasamay *et al.* 2003), anticholinergic. (Suparna *et al.* 1998) and antinociceptive (Jaishree *et al.* 2009). 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 inflorescence and leaf mixture.

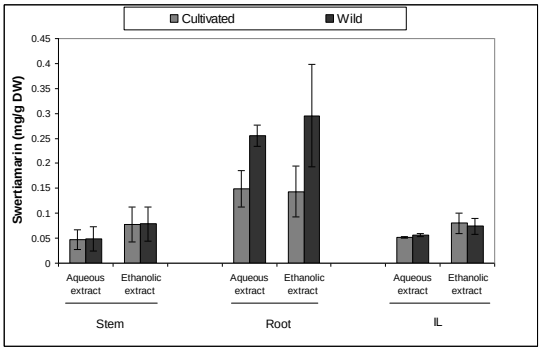
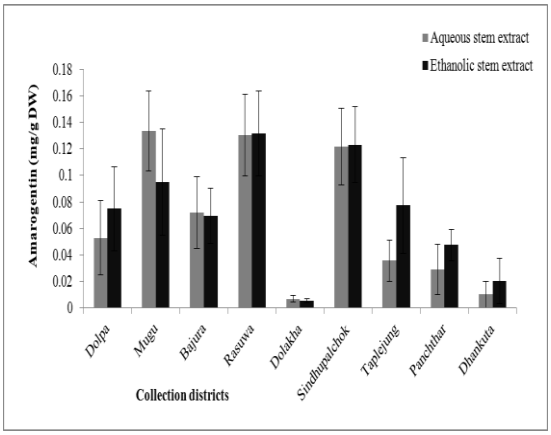


Fig. 9. Variations in swertiamarin (mg/g DW) present in aqueous and 12% ethanolic root extracts of cultivated and wild *Swertia chirayita*.



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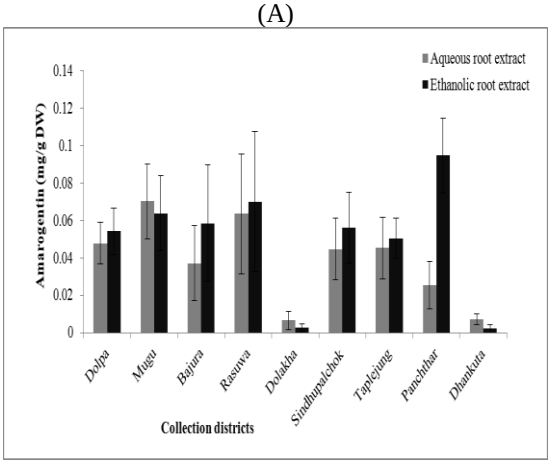

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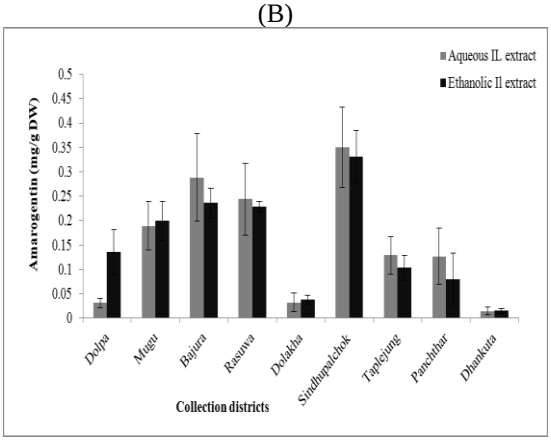

(C)

Fig. 10. Variations in amarogentin (mg/g DW) present in aqueous and 12% ethanolic stem (A), root (B) and IL (mixture of inflorescence and leaf) (C) extracts of *Swertia chirayita* collected from different districts of Nepal.

Amarogentin and its derivatives: 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 12% ethanolic IL extracts (0.28 and 0.23 mg/g DW) from Bajura and lowest in aqueous and 12% ethanolic root extracts (0.007 and 0.002 mg/g DW) from Dhankuta and Dolakha (0.006 and 0.002 mg/g DW) (Fig. 10). In general, aqueous extracts had higher quantities of amarogentin than 12% ethanolic extracts of all plant parts for all collected plant samples. Quantities of amarogentin were highest in aqueous and 12% ethanolic extracts of IL and lowest in aqueous and 12% ethanolic extracts of root for all collected plant samples. Quantities of amarogentin were slightly higher in aqueous and ethanolic extracts of wild plants in comparison to extracts of cultivated plants for all plant parts although there was no significant difference ($p > 0.05$) (Fig. 11).

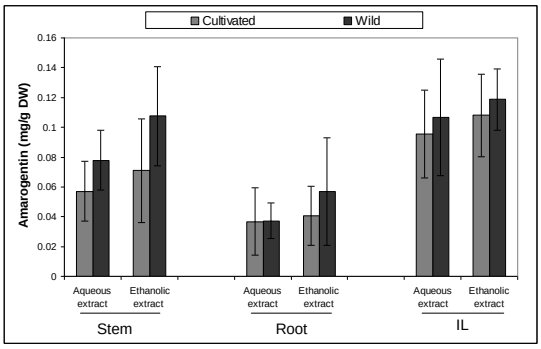

Fig. 11. Variations in amarogentin (mg/g DW) present in aqueous and 12% ethanolic root extracts of cultivated and wild *Swertia chirayita*.

Amarogentin is also known as one of the bitterest compound known to mankind. Its bitterness can be tasted even at a dilution of 1: 58,000,000 (Singh 2008). It is a known topoismerase inhibitor (Ray *et al.* 1996), chemopreventive and is reported to have anti-leishmanial (Medda *et al.* 1999) and gastroprotective properties (Niiho *et al.* 2006).

CONCLUSION

All three phytochemicals i.e., mangiferin, swertiamarin and amarogentin were present in highest quantities in IL aqueous and ethanolic extracts. Therefore, this plant part could be used for medicinal preparations. Aqueous and ethanolic root extracts had the lowest amounts of mangiferin and amarogentin whereas amount of swertiamarin were found to be lowest in aqueous and ethanolic stem extracts. In general, the plants collected from central Nepal had slightly higher quantity of all three phytochemicals compared to East and West Nepal but there was no difference between both the extracts collected from all three regions. This indicates that plants from all parts of Nepal are equally as effective; contrary to the local belief that plants from East Nepal are more biologically active.

These three phytochemicals mangiferin, amarogentin and swertiamarin have been reported to possess properties such as antihypoglycemic, antilipidemic, antiatherogenic, cardioprotective, immunodilatory, cardiotonic, anti-inflammatory, analgesic, antioxidant, gastroprotective, hepatoprotective, anticholerogenic which may be responsible for its therapeutic action against diabetes, hypertension, chronic fever and their complications. In Nepal, locally the infusion of *Swertia chirayita* is used for diabetes, hypertension and chronic fever. The presence of these main phytochemicals may suggest their relation to the therapeutic properties of this infusion used in local healing in Nepal especially the high quantity of mangiferin and its derivatives which is reported to be a potent anti-diabetic.

Since this species is greatly exploited, conservation is highly recommended. The highest concentration of all three phytochemicals were found in its leaf and inflorescence mixture, the optimum time for harvesting this plant could be during seed set when the inflorescence are fully formed. Collecting the plant after seed set ensures seed dispersal on its wild habitat which is the sustainable method for harvesting this plant from the wild. Since there was no difference in the amounts of all the three phytochemicals between extracts from wild and cultivated plants, cultivation of *Swertia chirayita* could be a sustainable strategy for its conservation and trade.

ACKNOWLEDGEMENTS

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