ORIGINAL ARTICLE

Estimation of Amarogentin content and standardization of different samples of Chirata collected from crude drug markets of Nepal

Bishun Dayal Prasad Patel1,2*, Shyambabu Yadav1, Shiv Mangal Prasad3, Kanti Shrestha4, Narendranath Tiwari5, Danik Lal BhARKher4,8

1Former Research Officer; 2Former Executive Director; 3Former Chairman; National Ayurveda Research and Training Center, Kathmandu, Nepal 1Head and Teaching Assistant, 1Professor and Former head; Department of Dravyaguna Vigyan, 1Head and Associate Professor; Department of Kaumrbrhiya, 1Former head and Professor; Department of Kayachikitsa, Ayurveda Campus, Institute of Medicine, Tribhuvan University, Kathmandu, Nepal, 6Senior Scientist; Natural Product Chemistry, Nepal Academy of Science and Technology, Khumaltar, Lalitpur, Nepal

ABSTRACT:

Background: Amarogentin is a major chemical constituent of bitter principle found in Swertia chirayita (Roxb.ex Fleming) Karsten; family Gentianaceae. Chirata is an endangered species because of it’s over exploitation for pharmaceutical industries. These species are mainly found in the Himalayas and used for gastritis, diabetes, jaundice, urinary disorders, liver disorders, etc. The bitter principle as a main indicator of the medical herb, Nowadays, other species of Swertia are substituted and traded by the name of Chirata. Therefore, the present study was undertaken to estimate Amarogentin and to standardize Chirata samples collected from different markets of Nepal in 2013 A.D.

Method and Materials: Different market samples of the species were analyzed for foreign matter, moisture content, extractive values; and bitter principles i.e. Amarogentin by using UFLC at Pacific Analytical Laboratory and Training Center, Lalitpur, Nepal. Result: The findings revealed that foreign matter, moisture content, extractive values and bitter principles i.e. Amarogentin content varied with different market samples. Methenolic extraction yielded higher percentage of extracts than that of chloroform extraction. Samples including more root and stem, in general, contained higher Amarogentin than samples containing more leaves. The samples containing higher moisture content were lesser in Amarogentin content. The finding of Amarogentin of sample collected from Charkot, Dolkha was 5mg/100gm. Conclusion: In this study, the findings suggest that sample collected from local market of Charkot, Dolkha district was Swertia chirayita and was the best quality.

Key words: Chirata, Swertia chirayita, Standardization, Amarogentin, TLC, UFLC.

INTRODUCTION

The plant KiratatiKta (Swertia chirayita (Roxb.ex Fleming) Karsten) belongs to family Gentianaceae; is a tropical family of small trees and herb which consists of 180 species worldwide. Among them, thirty species have been identified in Nepal and nine species are traded by the name of Chirata in nominal quantity. Swertia chirayita is endemic to the Nepal Himalayas; grows in the temperate zone of the mountain region between 1500 to 3000 masl. The most suitable growing altitude is 1800-2700 masl and is found in 55 hill and mountain districts out of total 77 districts of Nepal. The most valuable species of the genus is S. chirayita
(Roxb. ex Fleming) Karsten which is commonly known as *Chiraito* in Nepal and is indigenously distributed to northwest Himalayas at altitudes of 1100-3300 meter from Kashmir (India), Nepal to Bhutan. All members of this family are used as medicinal plants.

Chirata is known as Indian gentian which grows up to about 1.5 meters in height. The plant is erect with robust stem, profuse branching, normally 0.7-1.5 m tall, herbaceous, biennial, cylindrical or round stem with large pith at the middle portion, while the upper is four-angled, with a prominent decurrently line at each angle. The stems are orange brown or purplish in color and contain large continuous yellowish pith. The matured root is simple, tapering and stout, short; almost 7 cm long and usually half an inch thick. Some authors have described *Swertia chirayita* as an annual; and others as biennial or perennial.

Its leaves are broadly lanceolate, opposite pair about 10 cm long, without stalk, pointed at the tip. The plant has numerous flowers of greenish yellow or pale green in color, hermaphrodite in nature, tinged with purple, with long white or pink hairs, in large panicles, capsules egg-shaped, many light and tiny seeded and minutes or small shapped capsule fruits.

In the various communities of Nepal, different species of Chirata are categorized into three types. The local dwellers locally recognize them by the name of *Pate* (growing at marshy land), *Bhale* (male) and *Pothi* (female). *Pate* grows in moist areas so it is called Simsar Chiraito (Chirata grows at marshy land). *Bhale chiraito* grows at open field in Central Region of Nepal and is also known as Sirlinge that is graded as number two *Chiraito*. *Pothi Chiraito* is also known as Danthe that is graded as number one or Kali Chiraito (Black chirata) in Central Region of Nepal. *Pate, Bhale and Pothi* are botanically identified as *Swertia ciliata* (D. Don ex G. Don) B.L. Burtt, *Swertia angustifolia* Buch.-Ham ex D. Don. and *Swertia chirayita* respectively. *Swertia chirayita* is authenticated with *Kiratatikta* and is official drug in Ayurveda. The later one has high economic and medicinal values because of containing higher percentage of bitter principle known as chiratin (Amarogentin).

It is listed under medicinal and aromatic plants (MAPs) in Nepal which is cash generating non-timber forest Product species (NTFPS) for the people of high hills areas in the mid and eastern region since decades. Its harvesting time is after monsoon (October-November). The plants are usually harvested just when the seeds begin to set in and dried in the sun for use afterwards.

*Swertia chirayita*, a Gentian species can be evaluated through the medicinal properties as a nontoxic and safe ethno-medicinal herb mainly utilized for its bitterness in Ayurveda and bitter bioactive compounds in other medical science. Namely iridoids, xanthones, mangiferin and C-glucoflavones, has been recorded. The properties, biosynthesis and distribution of each group of compounds are described. The iridoids (mainly secoiridoid glucosides) The chemical constituents of *Swertia chirayita* include secoiridoid bitters, alkaloids, xanthones and triterpenoids. UV spectra, on-line electrospray ionization mass (ESI-MS) and SWARTIANIN (C26H48O15), amarosvetin, gentiopicroside and swertiamarin are the reported bitter secoiridoid glycosides of the plant.

Chirata is a popular plant for its medicinal, ethnovotanical, economic, environmental and historical values in Nepal. It is used as an ingredient for manufacture of different Ayurveda and allopathic medicines. The whole plant is bitter in taste; and used for local remedies and manufacturing several Ayurvedic medicines since time immemorial. It is used for more than two dozens of diseases, disorders and ailments. It is a bitter tonic, appetizer, febrifuge, anthelmintic, stomachic, laxative, blood purifier and effective against microbial. It is also effective against malarial fever, jaundice, gastritis, influenza, diabetes, headache, obesity, leucoderma, scabies, wounds, hysteria, convulsion, urinary disorder and Impetigo contagiousa etc. It is effective tonic in case of general weakness and during convalescence. It can also be used in uric acid deposits. In fact, this bitter herb promotes digestion of fats and regulates blood sugar levels. Its effectiveness is also observed for medication of leishmaniasis; a parasitic disease usually found in tropical regions. This plant is also recommended for treating intestinal worms, burning of the body, bronchial asthma and regulating the bowels etc.

It is widely used in India to treat fever, malaria and liver diseases. Decoction of *Swertia chirayita* with cardamom (*Elettaria cardamomum* Linn.), Turmeric (*Curcuma longa* Linn.) and Kutaki (*Picrorhiza kurrooa* Royle ex. Bent.) is given for gastrointestinal infections and along with Ginger (*Zingiber officinale* Linn.). It is considered good for fever. When given along with Neem (*Azadirachta indica* Linn.),
Manjishta (*Rubia cordifolia* Linn.) and Gotu kola (*Centella asiatica* Linn.), it serves as a cure for various skin problems. It is used in combination with other drugs in case of scorpion bite.\(^{20}\)

The *Swertia chirayita* is a therapeutic plant and its medicinal usage has been recognized in the Indian pharmaceutical codex as well as the British and American Pharmacopoeias. In addition, the curative value of the herb has also been recorded by Ayurveda and other conventional medical systems, such as Siddha and Unani.\(^{21}\)

A xanthone rich extract of this plant has shown significant anti-inflammatory\(^{22}\) activity in acute, subacute, chronic and immunological models and swerchirin, a xanthone from *Swertia chirayita* is a potent hypoglycaemic agent.\(^{23,24}\) Methanol extracts of this plant having antidiabetic activity contain mangiferin, Amarogentin, amaroswerin, sweroside and swertiamarin as active constituents.\(^{25}\) Xanthone derivatives like mangostin, isomangostin and mangostin triacetate are known to possess significant anti-inflammatory activities. Reports also suggest that several varieties of xanthones show potent anti-platelet, anti-cancer, CNS stimulant, anti-fungal and antimalarial effects.\(^{19}\) Extract of *Swertia chirayita* is used as an anthelmintic and hepatoprotective agents whereas antimalarial and hypoglycemic activities of this medicinal plant are also known.\(^{11}\) It is also reputed for its antidiarrhoeal properties.\(^{26}\) Trimethoxyxanthone (TMX) treatment successfully reduced the metastatic potential of Ehrlich ascites carcinoma (EAC) induced solid tumor, with in vitro validation TMX on the MCF-7 cell line.\(^{27}\)

Since 2002, demand of Chirata is increasing in the national and international market although producers/farmers of the plant is not getting appropriate price in the market. One of the reasons of that is the lack of certification and quality standard. In order to getting reasonable price, quality of the product must be known for promoting in the markets. Quality of Chirata cultivated or collected from Bhojpur district is considered one of the best among others.

Nepal exports about 50% of the world’s total traded volume of dry product amounting 300 to 450 metric tons per year. Major buyer is India (about 70%), China (about 20%) and other two dozen of Asian, European, American and African countries. Local use is very low (about 5%). Related species, which are traded from Nepal in small quantities that are used as substitute or adulterants with *S. chirayita* are; *Swerita angustifolia*, *S. kingi*, *S. paniculata*, *S. bimaculata*, *S. multicaulis*, *S. pedicellata*, *S. ciliata*, *S. nervosa* and *S. racemosa*. The synonyms are; *Swertia chirayita* (Wall.) C. B. Clarke; *Gentianna chirayita* Roxb. Ex. Fleming. This species is known by different names locally and abroad.

The bitter principle of *Swertia* species characterizes its quality in the testing sample. Indian pharmacopoeia also fixed its limit in percent (1.3 - 2.5%). This information to traders, cultivators, consumers and manufacturers is beneficial in all aspects.

Therefore, the present study was undertaken to estimate Amarogentin and to standardize Chirata samples collected from different markets of Nepal.

**MATERIAL AND METHODS:**

**Market Selection:** *Swertia chirayita* is commonly known as Chiraito in Nepal. It has different name in different communities and ethnic groups of hilly and mountain regions of central and eastern Nepal. It was rarely reported in the districts of mid-western region of Nepal. Gorkha, Kaski and Lamjung districts are known for wild collection of Chirata. Panchthar, Dhankuta, Bhojpur, Sakhuwasabha and Ilam districts belong to eastern region of Nepal and are well known for cultivation of Chirata. The raw materials are usually exported to India by route of Kakarvitta and Biratnagar custom. Hence, these districts were selected to represent all types of market samples of Chirata.

**Raw Materials Collection and Coding:** A total eleven samples of raw materials of the plant sold by the name of Chiraito; and often taken as *S. chirayita* was collected from different markets of eleven districts representing packet area of natural habitat in the districts of Chirata from all over the country and coded as Kakarbhitta; Jhapa (KJ1), Hetauda; Makawanpur (HM2), Butwal; Rupandehi (BR3), Narayanghat; Chitwan (NC4), Dharan; Sunsari (DS5), Birgunj; Parsa (BP6), Pokhara; Kaski (PK7), Raxaul; India (RB8), Charikot; Dolkha (CD9) Dhankuta Bazaar; Dhankuta (DD10) and Ilam Bazaar; Ilam (II11) in March 2013 A.D. The dried samples of raw materials were sold by the name of Chirata in the local market of Nepal. The samples were collected in amount of one kilogram and kept at room temperature (25-30°C); and were deposited at herbarium museum of National Ayurveda Research and Training Center, Kathmandu, Nepal before carrying out laboratory testing.
Testing Laboratory: The samples were carried out investigating foreign matter, moisture content, extractive values and thin layer chromatography (TLC) for analysis of the samples. The qualitative investigation of Amarogentin was carried out by TLC and quantification of Amarogentin using UFLC at Pacific Analytical Laboratory and Training Center, Khumaltar, Lalitpur, Nepal in April 2013 A.D.

Drugs and Chemicals: The Laboratory works were used HPLC graded reagents namely Methanol (Molychem Ltd., India), Chloroform (CDH Pvt. Ltd, India) and Amarogentin (Sigma-Aldrich, India Ltd.).

Organoleptic Parameters: Organoleptic characters of collected samples were evaluated observing characteristics of the samples by Panchagyanendriya Pariksha (Five sense organ examination), a method of sample examination in Ayurveda. The characters like colour, odour, taste and consistency of all eleven samples are presented in figure 1 and findings shown in the table 1.

Foreign Matter: The parts of the organs or organs other than the required are called foreign organic matter. These may include remains of same plant, part/s other than part’s used, molds, earthy material, animal excreta; and each crude drug has its own limits for presence of foreign matter.²⁸

Procedure: 100 gm (W) of each dried samples of Chirata were taken. Foreign matter viz. earth, sand, soil, gravels, weeds, vegetative parts were separated; and the samples were again weighed (W₁). The percent of foreign matters was calculated by the formula; % of Foreign Matter is equal to (W-W₁/W x100) and result is presented in table 2.

Moisture Content: Estimation of the amount of water and volatile contents in a crude drug when the drug is dried under specified conditions is called moisture content of the crude drug. The moisture content of a crude drug is responsible for decomposition of crude drug due to chemical change or microbial attack.²⁸

Procedure: The dried each samples were cut into smaller pieces; and were accurately weighed in amount of 10 gm (W). The eleven dried and cleaned conical flasks of capacity 250 ml were weighed (W₂). The cut pieces were kept in the conical flasks and were measured for each (W₃). All the weights of raw materials, conical flasks and conical flasks with raw materials were recorded. The flasks were heated in hot air oven at 105 °C; taking weight of flasks after each hour interval for 6 hours. The flasks were taken out from oven and stoppered immediately. Then the stoppered flasks were cooled in desiccator for 30 minutes before weighing for every reading. It was heated until two consecutive reading were found similar, or might differ by 0.25% or constant (W₄). The percent of moisture content was calculated by the formula; % of moisture content is equal to (W₂-W₃/W x 100) and result is presented in table 3.

Extraction (Percolation Method): This is most frequently used procedure to extract active ingredients in the preparation of tinctures and fluid extracts. The dried and ground each sample of 100gm of the raw material (W) were moistened with an appropriate amount of the hydro-alcoholic (4:1) menstruum and allowed to stand for approximately 4 hours in a well closed container, after which the mass was packed with 400 ml solvent of hydro alcohol; ethyl alcohol (EtOH) and water (H₂O) in the proportion of four is to one i.e. four times of the quantity of raw crude powder for 72 hours.²⁸ Then the extract was filtered and concentrated with a rotary evaporator and was subsequently defatted to get the dried extract yield. Then the mixture was stirred with a cleaned stick in an interval of 1 hour for 6 hours every day and left for 48 hours. The mixture was poured in a large container, filtered using wetman filter paper and dried on Rota vapor (Weight of Conical flask was taken; W₅). The whole process was repeatedly done for three times to obtain maximum solutes (active compounds) in extracts. Finally, the extracts were lyophilized to remove moisture, weighed (Weight of Conical flask + extract; W₆) and calculated the extractive value using the following formula; Extractive value Percolation method) is equal to (W₂ - W₁/W x 100 and result is presented in table 4.

TLC Profile of Swertia Plants: The above extracts was carried out thin layer chromatography (TLC) on Silica gel 60 F254 pre-coated aluminum plates of Merck Brand.
Solvent System for Chromatography: Lower layer of Chloroform: Methanol : Water (65:25:10) was used for carrying out TLC of different extracts of eleven samples.
Visualization of Spots in TLC: The spots on TLC plates were viewed by keeping the developed plates in UV light chamber at wavelength 254 nm.

Ultra-Flow Liquid Chromatography: The UFLC analysis was performed on a Shimadzu chromatographic system consisting of a quaternary pump, manual injector and dual λ UV absorbance diode array detector. The built-in LC-
Solution software system was used for data processing. Chromatographic separation was achieved on a Supelco, (2.7 μm) column (10 cm- 4.6 mm). Separation of Amarogentin was achieved by injecting 20 μL samples with a mobile phase consisting of acetonitrile and 0.1 % trifluoro acetic acid (25:75). The analysis time was 10 min for both the standard and samples with a flow rate of 0.7 ml/min and the 230 nm as detection wave length. UFLC was carried out to quantify Amarogentin in the eleven crude drug market samples of Chirata. The laboratory work was done at Pacific Analytical Laboratory and Training Center, Lalitpur, Nepal was and shown in table 3.

RESULTS:

Organoleptic evaluation: Samples of medicinal plants collected from the different markets of Nepal was examined by organoleptic method at NARTC, Kirtipur. The sample collected from Charikot (CD9), Panchthar Bazar (PP1), Hetauda (HM2) and Dharan (DS5) had strong bitterness and recognized with all parts of plants viz. root, stem, flower and fruits shown in figure 1. The sample collected from Kakarbhitta was deteriorated and old that might have had less phyto-constituents.

Figure 1: Chirata samples collected from Kakarbhitta; Jhapa (KJ1), Hetauda; Makawanpur (HM2), Butwal; Rupandehi (BR3), Narayanghat; Chitwan (NC4), Dharan; Sunsari (DS5), Birgunj; Parsa (BP6), Pokhara; Kaski (PK7), Raxaul; Bihar (RB8), Charikot; Dolkha (CD9), Dhanakuta Bazar; Dhankuta (DD10) and Ilam Bazar; Ilam (II11).

Characteristics of touch, odor, taste, appearance and macroscopic of individual samples collected during study period are listed in the table 1 below.

Table 1: Organoleptic characteristics of different market samples

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Taste</th>
<th>Visual</th>
<th>Odour</th>
<th>Touch</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>KJ1</td>
<td>Strong bitter</td>
<td>Greenish brown</td>
<td>ND</td>
<td>Rough</td>
<td>Whole plant including flowers, deteriorated</td>
</tr>
<tr>
<td>HM2</td>
<td>Strong bitter</td>
<td>Greenish brown</td>
<td>ND</td>
<td>Rough</td>
<td>Fresh, matured stem; maximum, root; few, flowers; more</td>
</tr>
<tr>
<td>BR3</td>
<td>No bitter</td>
<td>Brown leaves</td>
<td>ND</td>
<td>Rough</td>
<td>Brown and old leaves only</td>
</tr>
<tr>
<td>Sample Code</td>
<td>Taste</td>
<td>Visual</td>
<td>Odour</td>
<td>Touch</td>
<td>Remarks</td>
</tr>
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<td>---------------</td>
<td>-------</td>
<td>-------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>NC4</td>
<td>No bitter</td>
<td>Dried green</td>
<td>ND</td>
<td>Rough</td>
<td>Fresh, immature dried shoot, Leaves; maximum</td>
</tr>
<tr>
<td>DS5</td>
<td>Strong bitter</td>
<td>Greenish brown</td>
<td>ND</td>
<td>Rough</td>
<td>Fresh, whole plant excluding flowers</td>
</tr>
<tr>
<td>BP6</td>
<td>No bitter</td>
<td>Dried green</td>
<td>ND</td>
<td>Rough</td>
<td>Fresh, immature dried shoot, Leaves; maximum, with foreign matters</td>
</tr>
<tr>
<td>PK7</td>
<td>Mild bitter</td>
<td>Brown leaves</td>
<td>ND</td>
<td>Rough</td>
<td>Brown and old leaves only</td>
</tr>
<tr>
<td>RB8</td>
<td>No bitter</td>
<td>Dried green</td>
<td>ND</td>
<td>Rough</td>
<td>Fresh, immature whole plant, foreign matters, root, flowers &amp; fruits; absent.</td>
</tr>
<tr>
<td>CD9</td>
<td>Strong bitter</td>
<td>Brown</td>
<td>ND</td>
<td>Rough</td>
<td>Fresh, whole plant with flowers</td>
</tr>
<tr>
<td>DD10</td>
<td>Mild bitter</td>
<td>Brown</td>
<td>ND</td>
<td>Rough</td>
<td>Fresh, matured whole plant with root, stem, flowers, leaves; absent</td>
</tr>
<tr>
<td>II11</td>
<td>Mild bitter</td>
<td>Pale brown</td>
<td>ND</td>
<td>Rough</td>
<td>Fresh, stems; more, root, leaves &amp; flowers; absent</td>
</tr>
</tbody>
</table>

*Note: ND = Not detected*

The samples collected from Birgunj (BP6), Butwal (BR3), Narayangadh (NC4) and Raxaul (RB8) had no bitterness and had more leaves with foreign matters. These samples seemed similar in all respect. It can be concluded that the origin of these samples might be same. The rest of the samples collected from Pokhara (PK7), Dhankuta (DD10) and Ilam (II11) had mild bitterness and had fresh and matured whole plant without flowers.

**Foreign Matter and Moisture Content:** Samples were graded to separate foreign matters and substitute plant materials. The collected sample of Chirata plant materials were evaluated for foreign matters and moisture content and result are presented in the table 2 below. The sample collected from Charikot, Dolka (CD9) had the least (1.733±0.208) percent of foreign matter among all the samples of Chirata. The samples collected from Hetauda, Makawanpur (HM2), Dhankuta Bazar, Dhankuta (DD10) and Ilam Bazar, Ilam (II11) were found 2.300±0.265, 2.033±0.153 and 2.000±0.200 percent of moisture content respectively, which was higher percentage than the sample CD9. In rest of the samples were found more than 3 percent of moisture content.

### Table 2 : Foreign Matter and Moisture Content in the samples of Chirata

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Foreign matter Mean ± SD in %</th>
<th>Moisture content Mean ± SD in %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KJ1</td>
<td>3.167±0.764</td>
<td>10.600±0.529</td>
</tr>
<tr>
<td>III2</td>
<td>2.300±0.265</td>
<td>7.500±0.500</td>
</tr>
<tr>
<td>BR3</td>
<td>3.867±0.153</td>
<td>4.633±0.551</td>
</tr>
<tr>
<td>NC4</td>
<td>3.933±0.058</td>
<td>6.700±0.608</td>
</tr>
<tr>
<td>DS5</td>
<td>3.933±0.058</td>
<td>8.967±0.058</td>
</tr>
<tr>
<td>BP6</td>
<td>3.867±0.153</td>
<td>10.633±0.321</td>
</tr>
<tr>
<td>PK7</td>
<td>3.933±0.153</td>
<td>11.200±0.721</td>
</tr>
<tr>
<td>RB8</td>
<td>3.900±0.100</td>
<td>8.967±1.002</td>
</tr>
<tr>
<td>CD9</td>
<td>1.733±0.208</td>
<td>7.467±0.503</td>
</tr>
<tr>
<td>DD10</td>
<td>2.033±0.153</td>
<td>10.500±0.500</td>
</tr>
<tr>
<td>II11</td>
<td>2.000±0.200</td>
<td>10.000±1.000</td>
</tr>
</tbody>
</table>

Out of eleven samples included in this study, the highest percentage of moisture content (11.200±0.721) was observed in the samples collected from Pokhara, Kaski (PK7) district followed by Birgunj, Parsa (BP6) which was 10.633±0.321 percent. In contrast, the sample collected from Butwal, Rupandehi (BR3) had the least percentage of moisture content (4.633±0.551) followed by Narayangadh, Chitwan (NC4) and Charikot, Dolka (CD9) which were 6.700±0.608 and 7.467±0.503 percent respectively. Other samples were observed percent of moisture content in between 4.633±0.551 and 11.200±0.721.
**Thin Layer Chromatography:** The dried methanolic extract was used for developing TLC profile. The TLC plates were developed in solvent system Chloroform:Methanol:Water (65:25:10).

![TLC plates](image)

**Figure 2:** TLC plates viewed under UV light (254 nm), TLC plates developed in Chloroform:Methanol:Water (65:25:10), (RS - Reference Standard, Ag - Amarogentin (1-11 Samples number)

The developed plates were also viewed under UV light at wavelength 254 nm (Figure 2). The plates immediately after spotting on TLC plate was run in the same solvent, light orange coloured spots appeared at Rf 0.59 of the standard compound Amarogentin (Ag) presented in Figure 1. The prominent light orange coloured spot at Rf 0.59 was of Amarogentin in the reference standard (RS). The presence or absence and intensity of colour was critically observed in the spots of all the treatments in the TLC plate. Critical observation of the TLC plates showed that the light or dark orange coloured spots in the same horizontal line belongs to Amarogentin. In some treatments very light coloured spots corresponding to the spots of Amarogentin were observed which indicates very low concentration of these compounds in the sample.

**Ultra-Flow Liquid Chromatography:** This technique was applied for the quantification of Amarogentin in eleven study samples of Chirata.

**Extractive Values and Quantification of Amarogentin:**
There were variation of extractive values among the samples of different locations collected during the study. The investigation was found that there were lesser extractive values in Chloroform than in Methanol solvent.

Furthermore, extractive value in Chloroform was the highest (4.597±0.015) percent extracted from the sample of Birgunj, Parsa (BP6) whereas was the least (0.563±0.055) from the sample of Butwal, Rupandehi (BR3). The samples collected from Kakarbhitta, Jhapa (KJ1), Hetauda, Makawanpur (HM2) and Narayangadhada, Chitwan (NC4) had comparatively higher yield in between 3 to 4 percent. The samples collected from Pokhara, Kaksi (PK7), Raxaul, Bihar (RB8), Charikot, Dolkha (CD9), Dhankuta Bazar, Dhankuta (DD10) and Ilam Bazar, Ilam (II11) were extracted below 2 percent in Chloroform solvent.

Similarly, extractive value in Methanol was the highest (11.433±0.208) percentage extracted from the sample of Pokhara, Kaksi (PK7) whereas was the least (0.700±0.100) from the sample of Hetauda, Makawanpur (HM2) and followed by the percentage of the samples from Dhankuta Bazar, Dhankuta (DD10), Kakarbhitta, Jhapa (KJ1) and Dharan, Sunsari (DS5) that had 2.200±0.100, 3.600±0.100 and 3.733±0.058 respectively.

The samples collected from Raxaul, Bihar (RB8), Narayangadhada, Chitwan (NC4), Butwan, Rupandehi (BR3), Ilam Bazar, Ilam (II11) had around 4 percent yield in methanolic extract.

**Table 3 : Percent extractive values in the samples of Chirata**

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Extractive Value</th>
<th>Amarogentin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chloroform</td>
<td>Methanol</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD in %</td>
<td>Mean ± SD in %</td>
</tr>
<tr>
<td>KJ1</td>
<td>3.833±0.058</td>
<td>3.600±0.100</td>
</tr>
<tr>
<td>HM2</td>
<td>3.800±0.100</td>
<td>0.700±0.100</td>
</tr>
<tr>
<td>BR3</td>
<td>0.563±0.055</td>
<td>4.333±0.153</td>
</tr>
<tr>
<td>NC4</td>
<td>3.100±0.100</td>
<td>4.100±0.100</td>
</tr>
<tr>
<td>DS5</td>
<td>0.200±0.010</td>
<td>3.733±0.058</td>
</tr>
</tbody>
</table>
The samples collected from Charikot, Dolakha (CD9) and Birgunj, Parsa (BP6) had around 10 percent yield in methanolic extract. Hence, maximum yield of extract in particular solvent was considered higher quality of crude drugs for commercial or therapeutic uses.

Amarogentin is the biomarker for quality evaluation of *Swertia chirayita* which was quantified by Ultra-Flow Liquid Chromatography (UFLC) technique. The solvent system was Chloroform, Methanol and Water used and run in UFLC. The Amarogentin content was the highest (3.000±0.100 mg/100gm) in the sample of Charikot, Dolakha (CD9) followed by Pokhara, Kaski (PK7) and Ilam Bazar, Ilam (II11) that was found 1.480±0.026 and 0.803±0.015 mg/100gm respectively. The Amarogentin content was found around 0.2 mg/100gm in the sample of Kakarbhitta, Jhapa (KJ1), Hetauda, Makawanpur (HM2), Raxaul, Bihar (RB8), Narayangadh, Chitwan (NC4) and Dharan, Sunsari (DS5). The rest of the samples had Amarogentin content in between 0.3 to 0.4 mg/100gm.

**DISCUSSION:**

The samples collected from different crude drug markets of Nepal having whole plant with flowers exhibited strong bitterness that indicates higher concentration of bitter principle (Amarogentin). The sample of Charikot (Dolkha) was fresh and possessed complete parts of plants that had also strong bitterness. Sharma et al. (2018) reported similar findings in their previous study. The Ayurvedic Pharmacopoeia has standardized the limit of bitter principle for *Swertia chirayata* as 1.3 percent. The findings of foreign matter, moisture content, Chloroform and Methanol extractive values in the sample collected from Charikot; Dolakha (CD9) were 1.733±0.208 percent, 10.500±0.500 percent, 1.800±0.100 percent and 10.800±0.100 on the basis dry weight respectively. The Ayurvedic Pharmacopoeia of India has set the limit for foreign matter, alcohol and water soluble extractive values; not more than 2 percent, not less than 10 percent and not less than 10 percent on the weight by weight basis respectively. The finding for moisture content of the sample collected from Butwal, Rupandehi was the least (4.633±0.551) in percentage among other study samples. Similarly, the finding of Methanolic extract in the sample collected from Pokhara (Kaski) was the highest (11.433±0.208) in percentage compared to other samples of the study.

Qualitative examination on Thin Layer Chromatography (TLC) plates showed that the light or dark orange coloured spots in the same horizontal line with reference standard (Amarogentin) of RF value 0.59 belongs to Amarogentin. In some treatments very light coloured spots corresponding to the spots of Amarogentin were observed which indicates very low concentration of these compounds in the sample whereas the dark coloured spots indicates high concentration in the sample. Similar previous study was carried out by Wagner et al., (1984) who developed thin layer chromatography profiles of some medicinal and aromatic plants so that the genuine raw drugs can be distinguished from substitutes/adulterants. *Swertia chirayita* had been reported to contain Amarogentin which is a bitter secoiridoid using thin layer chromatography. Gupta et al., (2009) has also used HPTLC along with preliminary phytochemical and UV analysis for the authentication of *Hibiscus rosa sinensis* Linn. Meena et al., (2010) had used thin layer chromatography in authentication of the fruits of *Terminalia bellirica* and identified the adulterants. The findings of UFLC was quantified the highest yield (3mg/100gm in Methanolic crude extract) of Amarogentin in...
the sample collected from Charikot, Dolkha district. Similar previous studies support the finding done in *Swertia chirata* by Keil et al. (2000), Parthraj et al. (2019), Sharma et al. (2019), Kaur et al. (2019) and Sharma et al. (2020)\textsuperscript{,3,8,39,40,32}

The findings of physico-chemical parameters and bioactive compound (Amarogentin) of Charata crude drug market samples from Charikot, Dolkha meets the standard of *Swertia chirayita* recommended by The Ayurvedic Pharmacopoeia of India and other studies on the same species. Hence, the sample collected from Charikot, Dolkha is standard crude drug for quality production.

**CONCLUSION :**

*Swertia chirayita* Buch. - Hams. ex Wall. (chirata) which is under high demand by the various Ayurvedic and other pharmaceutical industries causes its extreme exploitation and leads to categorize the herb as an endangered species. In the scarcity of authentic drug, traders substitute other species and sell out under the name of Chirata. A standard set for extractive values, qualitative analysis by Thin layer chromatography and quantitative analysis by Ultra-Flow Liquid Chromatography are the major parameters for evaluation of standard of crude drug sample collected from different markets in Nepal. In this study, different crude drug market samples of Chirata was evaluated for their Amarogentin content and extractive values which suggests their quality and authentication. The sample collected from Charikot, Dolkha (CD9) was evaluated as the standard quality raw materials for therapeutic uses.

**CONFLICT OF INTEREST :** There is no known conflict of interest associated with this publication.

**RECOMMENDATION :** This study is helpful in setting down biochemical standards for future reference in determining the purity, quality and authenticity of *Swertia chirayita*.

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