

Phytochemical and Antimicrobial Screening of native plant *Swertia chirayita* (Roxb. ex Fleming) karst from Rasuwa district of Nepal

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ABSTRACT

Swertia chirayita is one of the highly traded medicinal plants of Nepal. This plant was chosen for investigation as folk medicines and then collected from the diverse habitats of Rasuwa district of Nepal and specimens have been checked and identified at National Herbarium and Plant Laboratories (NHPL), Godavari, Lalitpur. The identified plant was subjected to study physiochemical and biological activity. The stem, root and leaves of the plant were dried, made powder and mixed at the ratio of 1 : 1 : 1 and then extracted using methanol solvent by cold and warm method. The percentage yield from the plant was highest in warm methanol with 3.73%, followed by cold methanol with 2.28%. Plant extract showed the presence of phytochemicals like basic alkaloids, coumarin, glycosides, steroids, quinones, flavonoid and terpenoids. The antibacterial activity of the extract showed significant bioactive by inhibiting the growth of selected pathogenic microbial species for the test. The zone of inhibition (ZOI) shown by the extracts was comparable to the standard antibiotics. Similarly, proximate composition was also carried out. The antioxidant activity of the sample was found to be 62.54% at 500 µg/mL and 15.32% at 100 µg/mL by radical scavenging method and showed significant antioxidants potential.

Keywords: Anti-inflammatory, cultivation, phytochemical, proliferations, antioxidant

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].

S. chirayita (Roxb. ex Fleming) karst can reach the height of one to one and half meter. It can be found growing in broadleaf forests and on open slopes along the Himalayan Mountains, from 1200 to 3000 meters [4]. Several different species of chiraito (about 16 different species are recorded) are found in Nepal. Chiraito

belongs to the family Gentianaceae and can be propagated in the nursery by seed. Many farmers in Nepal have cultivated chiraito on their private land because of good market price and high demand. The Department of Plant Resources (DoPR), Ministry of Forest, Nepal Government, has prioritized chiraito as herbs and also has chosen it for agricultural research technology development [5]. Since many decades, herbal medicine has had more relevance in health care with positive impact for both global health and trade. Not less than 25 % of the drugs prescribed worldwide have come from plants and 121 such compounds are in current use [6]. A large proportion of the world's population, around 80% use traditional medicines for their health care needs [7]. The plant *S. chirayita* has been prized in mountainous country like Nepal and China as a tonic medicine and has been reported to possess anticarcinogenic [8]; hypoglycemic [9]; antihepatotoxic [10]; antihelmintic [11]; anti-inflam-

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imatory [12], antipyretic [13] and antiviral [14] properties. Due to its medicinal importance and trade value the plant *S. chirayita* already has been vulnerable and endangered in Nepal [15] as people harvest indiscriminately from its wild habitat. It seems to be very urgent to control such malpractices, calling for immediate efforts for its conservation. Without delay, it is necessary to conduct good cultivation and sustainable harvesting as suitable options for phytochemicals retention. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. The term is generally used to refer to those chemicals that may affect health but are not yet established as essential nutrients. There are currently many phytochemicals possibly having medicinal properties in clinical trials for a variety of diseases. Plant sterols, flavonoids, and sulphur containing compounds are three classes of micronutrients found in fruits and vegetables. Some well-known phytochemicals are lycopene in tomatoes, isoflavones in soy and flavonoids in fruits. To assess health promotive, protective and therapeutic effects of this plant, phytochemical screening is necessary to determine its potential importance in modern therapeutic and preventive medicines and demands a greater focus on this plant.

MATERIALS AND METHOD

Materials

The bitter herb named *S. chirayita* was collected and sent to National Herbarium and Plant Laboratories, Godavari (Kathmandu) for its validation. Then it was analyzed, in the laboratory of Department of Biotechnology at Kathmandu University (KU), Department of Food Technology and Quality Control (DFTQC) under the Ministry of Agriculture Development Nepal (MOAD/N) and Department of Plant Resources (DoPR), to study its physiochemical and biological activity such as yield, antimicrobial activity, proximate composition, phytochemical properties and antioxidant activity. Materials used for extraction were Soxhlet extraction apparatus, methanol, hexane, beakers, grinders, filter papers, funnel, measuring cylinder, water bath, magnetic stirrer, and separating funnel.

Sample collection

Plant samples of *S. chirayita* were collected from Rasuwa district of Nepal which is located in the 2,360 masl (28°7'N 85°17'E), at the end of flowering season in late August of 2015 to August 2016 as shown below and then subjected to process at biochemical laboratory.

Sample preparation

The plants were divided in to 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) and mixed thoroughly at the rate of 1 : 1 : 1. The powdered samples were analyzed to determine proximate composition whereas the extract of plant materials so obtained after warm and cold extraction were used to study yield, phytochemical properties, antimicrobial assay and antioxidant activity.

Extraction

The extraction was done using Sox let extractor which is a versatile tool that can be used to separate a single gram to hundreds of grams with near 100% recovery [16]. Ten grams of the dried powdered sample (root, shoot and leaves) at the ratio of 1 : 1 : 1 was mixed and packed into thimble of the Sox let Apparatus. 200 mL HPLC grade Methanol (Qualigens, Fisher Scientific, CAS No 67, 56-1) was added to the thimble (to make 10%, w/v extract). Then the apparatus was operated and continued for 48 hours regularly monitoring the circulation of water in the condenser. For the cold extraction, 10 g of the same powdered sample was taken in a beaker and 200 mL methanol was added and the mixture was stirred for 30 minutes. The mixture was kept in room temperature for 48 hours. After 48 hours, the solution was filtered and fresh methanol was added. The filtrate was kept in water bath for drying. The mixture of the collection flask is recovered and taken for further purification process. The Soxhlet extraction methanol was then treated for Hexane in order to remove Chlorophyll pigment. It was carried out in separating funnel. After warm extraction the solvent was removed yielding the extracted compound. The non-soluble portion of the extracted solid remained in the thimble was discarded. The process was repeated until the chlorophyll pigment was completely extracted in hexane (ratio of hexane and methanol extract is 1 : 2). Drying of the treated methanol was done in water bath.

Proximate and phytochemical analysis

Proximate analysis included moisture, carbohydrate, protein, lipid, crude fiber, and ash which were tested to determine nutritional value as macro and micro nutrient [17]. The bioactive components present in the plant extracts were also tested to review as alkaloids, sterols, terpenes, tannins, coumarin, saponin, glycosides,

and quinones respectively [18].

Antimicrobial screening

Antimicrobial activity of the extracts was assessed to confirm its anti-proliferation effect [19]. The process includes sterilization to all equipment used such as media, Petri plates, and cotton swab before performing the experiments. Similarly, proper labeling of the Petri plates were also done before performing lab work. All the determination of antibacterial activities of plant extracts were done in triplicates for accurate result. The cultures of bacteria were made revive by inoculating in broth media. It was then grown up after incubation for 18 hours at 37°C. On the other hands, agar plates with Hilton media were prepared. The plates were inoculated by swabbing with bacterial suspension or with 18 h fresh culture ($10^5 - 10^6$ colony forming unit “CFU”/mL). After 20 minutes, wells of 6 mm diameter were made in solid agar medium with the help of gel puncher and filled with 50 µL of test samples of *S. chirayita*. The positive control wells were filled with antibiotics (chloramphenicol) as standard drug. Each plate was incubated for 24 hours at 37°C. The diameter of the zone of inhibition (ZOI) was measured in millimeter as antimicrobial effect.

The test microorganisms as shown in Table 1 were taken from Kathmandu University Teaching Hospital (KUTH, Dhulikhel), National Institute of Science and Technology (NIST), Khusibu, Kathmandu and National Public Health Laboratory (NPHL, Department of Health Services (DoHS), Teku, Kathmandu under the Ministry of Health (MoH).

Antioxidant activity

It measures the reduction of chemical reaction when free radical scavenging compound are added [20]. For this 1,1-diphenyl-2-picrylhydrazyl radical also known as DPP (Sigma-Aldrich) was used as an indicator of antioxidant properties. Crude extract of 25 mg was taken and dissolved in methanol (HPLC grade) and diluted up to 50 mL. Then, 100 and 500 µg/mL test samples were prepared as stock solution. Vitamin C was taken as standard reagents (antioxidants). Five mL of each solution was taken in a test tube and 1 mL of 0.001 M of DPPH solution was mixed to it and kept at dark for 30 min. Similarly, 5 mL methanol was taken and 1 mL of DPPH solution was added, for control (A₀) solution. At the end, the mixtures were triplicate examined for the antioxidant property using Optima UV Visible spectrophotometer having wavelength of 517 nm. Percentage

Table 1. Test organisms

No.	Microorganism	Sources	Institute
1.	<i>Klebsiella pneumonia</i>	Pus sample	KUTH
2.	<i>Citrobacter freundii</i>	Blood sample	NIST
3.	<i>Escherichia coli</i>	Urine sample	KUTH
4.	<i>Salmonella typhi</i>	Blood sample	KUTH
5.	<i>Staphylococcus aureus</i>	Pus sample	KUTH
6.	<i>Shigella dysenteriae</i>	Food handler	NPHL

Table 2. Yield of extraction

No.	Solvent used			
	Methanol (Warm)	Methanol (Cold)	Cold: Warm	%
1.	3.96	2.56	0.646	64.65
2.	3.84	2.31	0.601	60.01
3.	3.58	2.15	0.6	60.0
4.	3.72	2.22	0.596	59.6
5.	3.57	2.14	0.599	59.9

Table 3. Phytochemical screening

No.	Parameter	Test
1.	Basic alkaloids	+
2.	Coumarin	+
3.	Saponin	-
4.	Glycosides	+
5.	Steroids	+
6.	Quinones	+
7.	Reducing Sugar	-
8.	Flavonoid	+
9.	Terpenoids	+

Table 4. Proximate analysis

No.	Parameters	Test (%)
1.	Moisture	10.26 ± 0.29
2.	Crude Fat	1.65 ± 0.03
3.	Crude Protein	4.95 ± 0.31
4.	Crude Fiber	1.65 ± 0.09
5.	Ash Content	1.54 ± 0.12
6.	Total Sugar	0.01665 ± 0.001

DPPH inhibition was determined using formula below:

$$\text{Antioxidant activity \%} = \frac{A_0}{A_1} \times 100$$

where A₀ is absorbance of control and A₁ is absorbance of extract.

RESULTS AND DISCUSSION

After the completion of extraction, the yield was

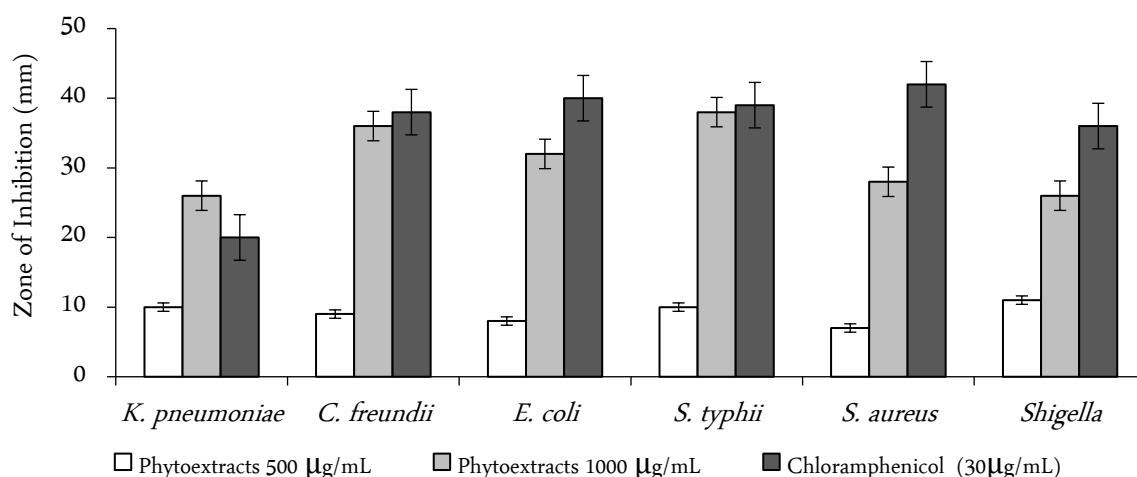


Figure 1. Zone of Inhibition (ZOI)

calculated as shown in Table 2 and it shows that the extraction is uniform and best in warm method. The yield was calculated using the following formula. The ratio of cold to warm extraction was maximum 0.646 and minimal 0.596 respectively. It means the yield was less in cold method as compared to warm methanol.

The Phytochemical screening of *S. chirayita* showed the presence of sterols, terpenoids, alkaloids, flavonoids, quinones, coumarins, and glycosides in methanol extracts have been shown in Table 3.

The zone of inhibition (ZOI) shown by the plant extract was significant to the level of bioactive but likely comparable to that of antibiotics like chloramphenicol as shown in Figure 1.

The Figure 1, reveals significant antibacterial activities of the plant *S. chirayita*. The ZOI in the extract at 500 µg/mL is less, where as it was higher at 1000 µg/mL. It shows higher the extract, higher the inhibition effect.

The proximate analysis of the plants is shown in Table 4. The dried samples have highest amount of moisture while the total reducing sugar is the lowest. The results obtained from the proximate composition showed very poor in macronutrient content, as is evident from low ash content.

Alkaloids have anti-cholinergic, anti-inflammatory, anti-spasmodic, hyperglycemic, sedative, tranquilizer and vasodilator [21]. Terpin hydrate is a derivative of terpine, an expectorant and humectants, it is commonly used in the treatment of acute or chronic bronchitis and related condition. Flavonoids have antiallergic, anti-cancer, antioxidant, anti-inflammatory and anti-viral activities. The flavonoid quercetin is known for its ability to relieve hay fever, eczema, sinusitis and asthma. Red wines contain high level of flavonoids and reduce heart

disease [22]. Tea flavonoids reduce the oxidation of low density lipoprotein, lowers the blood levels of cholesterol and triglycerides [20]. Soy flavonoids (isoflavonoids) can also reduce blood cholesterol and can help to prevent Osteoporosis. Tanins are used as antidote to poisoning by alkaloids depending on their capacity to form insoluble tannates, applied on the skin to pull out poisons from bee stings or poison oak bringing in instant relief. Coumarin has blood thinning, anti-fungicidal and anti-tumor activities. It increases the blood flow in the veins and decrease capillary permeability. Coumarin has been used in the treatment of lymph edema. Saponins are used on injection and results in the lysis of blood cells, haemolysis. Saponin stomatolytic solutions are being used for treating malaria. People suffering from allergic and those who are suffering from dermatitis will be benefited if they use the liquid soap solutions prepared from saponin.

The antimicrobial screening of Methanol extracts of the plates showed encouraging results by inhibiting most of the microbial species selected for test which proved that the plant extract has anti-microbial activity. The zone of inhibition (ZOI) shown by the plant extract was significant to the level to be bioactive and comparable to that of antibiotics like ciprofloxacin (Cf30), gentamycin (G10), tetracyclin (T10). Therefore, it can be concluded that the extract is biologically active and more research needs to be carried out for its confirmatory.

The antioxidant activity of the sample was found to be 62.54% at 500 µg/mL and 15.32% at 100 µg/mL by radical scavenging method and showed significant antioxidants potential. The results obtained also showed that it can be used as medicinal point of view locally. The chemical constituents of this plant have been analyzed,

characterized and reviewed by several groups [23]. The compound isolated from *Swertia* include compound like chiratin, ophelic acid, palmitic acid, oleic acid, stearic acid, alkaloids, glycosides and a large number of xanthenes. The first isolated dimeric xanthone was chiratinin. Other important phytochemicals include swertiamarin, mangiferin, amarogentin, gentiopeccrin, swerchirin, swertanone, and chiratol. A detail list of compounds isolated from *S. chirayita* is available in literature [24]. A review of naturally occurring xanthone including gentisin, mangiferin, swerchirin is available in literatures [25] but still *S. chirayita* has received inadequate attention till date to estimate their medicinal properties. Problem often arise due to adulterants as a substitute of *S. 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, *S. chirayita* has only recently been brought into cultivation with limited success. The present study will help the researchers as basic data for future research in exploiting the hidden potential of this important plant which has not been explored so far.

CONCLUSION

It can be concluded that *S. chirayita* and its part can be used as medicinal preparations. The phytochemicals were present in highest quantities in leaves, stem and root. This indicates that plants cultivated in Rasuwa district of Nepal are found effective against pathogenic microorganism with antioxidant property. Local people who are using this plant for therapeutic purpose after infusion in local healing can be expressed as science based medicinal practice.

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