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Secondary Metabolite variation in some species of Senecio L. from Nepal Himalaya.

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Secondary metabolites in different species of *Senecio* L. collected from different localities of Nepal Himalaya were analysed. Secondary metabolites like volatile oil, fatty acid, coumarin, alkaloid, sterols and triterpene, emodins, polyphenol, reducing sugar, anthocyanosides, anthracenosides, flavon aglycones, tannin, cardiac glycosides, flavonoids, saponin, terpenoid, steroides were screened in the aerial parts of *Senecio alatus* Wall. ex DC., *Senecio chrysanthemoides* DC., *Senecio densiflorus* Wall.ex DC., *Senecio diversifolius* Wall. ex DC., *Senecio graciliflorus* DC., *Senecio rufinervis* DC., *Senecio scandens* Buch.-Ham.ex D.Don., *Senecio triligulatus* Buch.-Ham. ex D.Don., *Senesio vagans* Wall. ex DC. and *Senecio wallichii* DC..The air dried plant material (powder) was extracted with 95% ethanol by soxhlet method. The aqueous slurry of the extract was subsequently fractioned with hexane, chloroform and butanol. Qualitative phytochemical analysis was carried out by using standard methods by their reactions with specific reagents. Out of the 18 secondary metabolites tested, 14 were detected from ten species of *Senecio* L. Alkaloid was detected from all the species. Among the species, *S. diversifolius, S. graciliflorus* and *S. scandens* were found to be rich in secondary metabolites while the *S. wallichii* was found to have less secondary metabolites. The result indicates the richness of secondary metabolites in *Senecio* species and thus could be potential source of useful compounds.

Keyword: Secondary metabolite, *Senecio*, Nepal Himalaya, Phytochemical, Detected.

1. Introduction

The genus *Senecio* L. of the species rich tribe Senecioneae of Asteraceae is one of the largest genera (Kostova *et al.*, 2006)[1]of flowering plants comprising about 1250 species (Mabberley, 1998)[2] and are worldwide in distribution with remarkable amount of morphological variations . Nepal Himalaya harbours 24 species of *Senecio* L. (Press *et al*., 2000; Hara *et al*., 1982) [3,4] mostly being confined to the temperate and sub- alpine zones of the region.

The different species of *Senecio* L. is herb or shrub, sometimes scandent ; leaves alternate; inflorescence capitulum; capitula radiate or discoid, usually yellow; involucral bracts usually one seriate, often subtended by calyculate bract; ray florets in one seriate, pistilate, fertile, pappus copious or sparse or sometimes absent; disc florets few to numerous, tubular, bisexual, fertile, five dentate, usually yellow, pappus copious .

The secondary metabolites, also known as the natural products are important for ecological fitness and survival of plants as a means to

defend themselves against herbivores, bacteria , viruses as well as other competing plants (Wink, 2010)[5]. Members of the family Asteraceae are reported to produce the wide ranges of secondary metabolites including monoterpenes, diterpenes, triterpenes, sequisterpenes and sesquiterpene lactones, polyacetylenes, flavonoides, phenolic acids, benzofurans, coumarins and pyrrolizidine alkaloids, the alkaloids being confined to Senecioneae and Eupatoriaceae with few exceptions (Calabria *et al.,* 2009)[6]. Cronquist in 1981 had suggested that the prosperity of the family was mainly due to their secondary compound chemistry which provided the effective defence (Bremer, 1994)[7]. But polyacetylene common in other tribes of Asteraceae are rare or absent in Senecioneae (Robins, 1977)[8] and coumarin common in Asteroid tribe are also reported to be absent from Senecioneae (Zdero and Bohlmann, 1990)[9]. Out of the varied numbers of the secondary metabolites, pyrrolidizine alkaloids (PAs) is one of the important compound. PAs are frequently found in some genera of tribe Senecioneae and Eupatorineae and some distantly related families of Angiosperms (Pelser *et al*., 2005)[10]. Nearly all species of *Senecio* were reported to have PAs as the main secondary metabolites (Bicchi, 1989)[11]. PAs have been reported from different *Senecio* species like *S. arbotanifolius* L., *S. adonifolius* Loisel , *S. alpinus* (L.) Scop., *S. chrysanthemoides* DC. etc. (Pelser *et al.*, 2005)[10]; *S. madagascariensis* Poir. (Gardner *et al*., 2006)[12]; *S. nemorensis* L., *S. aquaticus* Hill., *S. vernalis* Waldst, *S. jacobaea* L. (Kostova *et al*., 2006) [1]; *S. scandens* and *S. chrysanthemoides* DC. (Fu *et al*., 2002)[13]; *S. stabianus* Lacaita (Tundis *et al*., 2009)[14]. Different species of *Senecio* have been used in traditional medicine as anthelmintic, diaphoretic, diuretic, for the treatment of scurvy, as poultice, said to be useful in treatment of sickness of stomach (Sahu *et al*., 2011)[15], as antipyretic and calmative, to treat against cholera and lung diseases (Qureshi *et al*., 2007)[16]. In Rasuwa district of Nepal, the *S. graciliflorus*, *S. scandens* and *S. wallichii* were used to treat fever, increase appetite, for indigestion, to care chest pain and headache. The hexane fraction of *S. stabianus* was also reported to inhibit the viability of cancer cell lines (Tundis *et al.*, 2009)[14].

Annex 1: List of the specimens studied along with their localities.

SN	Taxon	Locality
1.	*S.alatus* Wall. ex DC.	Kalingchowk, Dolkha (SJ2123); Langtang, Rasuwa (SJ1970)
2.	*S.chrysanthemoides* DC.	Godawari, Lalitpur (SJ2116)
3.	*S.densiflorus* Wall. ex DC.	Simbhanjhang, Makawanpur, (SJ2185); Langtang Rasuwa (SJ1923)
4.	*S.diversifolius* Wall.ex DC.	Kalingchowk, Dolkha (SJ2122)
5.	*S,graciliflorus* DC.	Kalingchowk, Dolkha (SJ2124); Langtang, Rasuwa (SJ1960)
6.	*S.rufinervis* DC.	Langtang, Rasuwa (SJ1990)
7.	*S.scandens* Buch.-Ham. ex D.Don.	Phulchowki, Lalitpur (SJ2109)
8.	*S.triligulatus* Buch.-Ham. ex D.Don.	Near mude bazzar, Dolkha (SJ2112)
9.	*S.vagans* Wall.	Lakure Bhajhang, Lalitpur (SJ8103)
10.	*S.wallichii* DC.	Langtang, Rasuwa (SJ1965)

The literature review showed that a number of works on chemical part of *Senecio* had been carried out, the most being specially confined on the PAs profile and the researches on secondary metabolites of *Senecio* species are still insignificant. More over the Himalayan species

still remain unexplored. Hence we feel it worth to do some preliminary research on secondary metabolites of some species of *Senecio* found in Himalayan region.

2. Materials and Methods:

2.1 Plant materials

The composite samples of aerial parts of different species of *Senecio* were collected from different localities of Nepal Himalaya at full flowering season. The specimens were authenticated by comparing the collected specimens with the type specimen and protologue texts (Don, 1825; De Candolllea, 1837)[17,18]. List of the specimens studied along with their localities are given in Annex 1.

2.2 Extraction of plant material

The collected materials were cleaned, air dried under shade at room temperature and pulverized to fine powder using electric grinder. The powered material was weighed in the electronic balance. About 20gms of material was extracted with 95% ethanol by soxhlet method at about 60^0C. The extract was concentrated using the rotary evaporator. The aqueous slurry was made from the obtained extract and it was fractioned with the solvents with different polarities viz; hexane, chloroform and n-butanol so that four fractions were obtained. The solvent of each fraction was also evaporated.

2.3 Phytochemical screening

Phytochemical screening of different secondary chemicals were performed on different fractions and extract using the standard procedures as mentioned in literature (Sasidharan *et al*., 2011; Savithramma *et al.,* 2011; Benmehdi, 2012; Raaman, 2006; Amar *et al.,* 2012; Tiwari *et al.,* 2011; Kumar *et al.,* 2012; Ayoola *et al*., 2008; Todkar *et al*., 2012; Siddiqui, 2009)[19-28] with some modifications. The screening was performed in replicate samples.

2.4 Test for volatile oil

About 2ml of ethanolic extract was mixed with 0.1ml of NaOH and a small quantity of dilute HCl and the solution was shaken and allowed to stand. The formation of white precipitations indicates the presence of volatile oil.

2.5 Test for fatty acid (Saponification method)

The hexane fraction was saponified with 50% potassium hydroxide solution. The alkaline solution was acidified with conc. HCL and extracted with ether (5ml x 3times). The ether fraction obtained was concentrated. A drop of concentrated fraction of ether was spotted on the filter paper. The persistence of spot even after evaporation of solvent indicates the presence of fatty acid.

2.6 Test for coumarin (Borntiager's test)

The ether fraction obtained after saponification of hexane fraction or chloroform/n-butanol fraction was dissolved in hot water, cooled and the solution was divided into two parts. The first one was taken as the standard and the second one was made alkaline by adding 10% NH_4OH. These two solutions were observed under the UV (254nm) light. The occurence of intense florescence in second solution in comparison to first standard indicates the presence of coumarin.

2.7 Test for alkaloid (Dragendroff's test)

About 30 mg of each fraction was treated with 3 ml of dilute acid (2% HCl/ H_2SO_4) and then filtered. The filtrate was treated with 4/5 drops of dragendroff's reagent. The appearance of orange precipitate indicates the presence of alkaloid.

2.8 Test for sterols and triterpene (Libermann-Burchard test)

About 20 mg of each fraction was dissolved in 1ml of acetic acid. To this solution 2ml of concentrated H_2SO_4 was added from the side of test tube without disturbing the solution. The occurrence of violet ring at the junction of two layers indicates the presence of triterpene and the green upper layer indicates the presence of sterol.

2.9 Test for emodins (Borntiager test)

The ether fraction of hexane after saponification was treated with 25% (v/v) ammonium hydroxide solution and shaken vigorously and the test tube was allowed to stand for few minutes to separate

two layers. The decolorization of upper layer and change of lower alkaline layer into red indicates the presence of emodin.

2.10 Test for polyphenol (Ferric chloride test)
The residue obtained from 1ml of chloroform/n-butanol fraction was mixed with sterile water. To this solution 3/4 drops of 1% ferric chloride solution was added. Development of bluish black colour indicates the presence of polyphenol.

2.11 Test for reducing sugar (Fehling's test)
The fractions (2ml) were treated with Fehling's reagent (1:1 mixture of Fehling's A and B). The mixture was warmed over the water bath for about 15-30 minutes. The development of brick red colour indicates the presence of reducing compounds.

2.12 Test for anthocyanosides
The chloform/n-butanol fraction was hydrolyzed by refluxing with equal volume of 10% HCl for 30 minutes. The hydrolyzed extract was re-extracted thrice with hexane (10ml) so that acid layer and hexane layers were obtained. The acidic solution was basified with sodium carbonate using the litmus paper as indicator. The appearance of green or blue colour but not the gray colour indicates the presence of anthocyanosides.

2.13 Test for anthracenosides
About 15 ml of chloform/n-butanol fraction was hydrolyzed by refluxing with equal volume of 10% HCl for 30 minutes. After cooling, the hydrolyzed extract was re-extracted thrice with hexane (10ml) so that acid layer and hexane layers were obtained. About 2ml of hexane solution was treated with 1ml of 25% ammonium hydroxide solution and was shaken vigorously. The test tube was allowed to stand for few minutes to separate the two layers. The appearance of red colour in the lower alkaline layer indicates the presence of anthracenosides.

2.14 Test for flavon aglycones (Shibata,s reaction)
The hexane fraction was saponified with 50% KOH. The ether fraction obtained after saponification was dissolved in 2ml of methanol. A small piece of metallic magnesium and 5 drops of conc. HCl was added. Appearance of red or orange colour indicates the presence of flavons.

2.15 Test for tannin (Ferric chloride test)
About 2ml of aqueous fraction was treated with few drops of 1% ferric chloride solution. The appearance of dark green colour indicates the presence of tannin.

2.16 Test for cardiac glycosides (Keller-Killiani test)
About 0.5gm of ethanolic extract was diluted with 5ml of sterile water. To this solution 2ml of glacial acetic acid containing few drops of ferric chloride solution was added. The mixture was again treated with 0.5ml of concentrated Sulphuric acid. The appearance of brown ring at the interface indicates the presence of cardiac glycosides.

2.17 Test for flavonoids (Ammonium test)
About 3ml of aqueous filtrate of the material was treated with the 1ml of dilute ammonia (10%) and then with 1 ml of concentrated sulphuric acid. The appearance of yellow colour after adding the ammonia and disappearance of yellow colour after adding concentrated sulphuric acid indicates the presence of flavonoids.

2.18 Test for saponin (Froth test)
The chloroform/n-butanol/aqueous fraction was mixed with hot sterile water and shaken vigorously for about 15 minutes. The appearance of persistent froth for about 10 minutes indicates the presence of saponin.

2.19 Test for terpenoid (Salkowski test)
About 2ml of chloroform fraction was taken. To this 3 ml of concentrated sulphuric acid was carefully added to form a layer. A reddish brown coloration at the interface indicates the presence of terpenoid.

2.20 Test for steroides (Liebermann Burchard reaction)

The chloroform/n-butanol (1ml) fraction was mixed with acetic acid (1ml), to which equal volume of concentrated sulphuric acid was added from the side. The development of blue, green ring indicates the presence of steroid.

3. Result and Discussion:

The preliminary phytochemical tests for secondary metabolites in ten species of *Senecio* studied showed the variations in the presence and absence of the compounds tested. Volatile oil, fatty acids, coumarin, alkaloids, sterols, triterpenes, emodin, polyohenols, reducing compounds, tannins, flavonoids, saponins, terpenoids and steroids were detected among ten species of *Senecio* (Table1.). All the species are found to contain the alkaloid while the emodin is of rare occurrence and found only in *S. scandens.* Flavon aglycones Anthocyanosides, Anthracenosides and Cardiac glycosides were not detected in any of the species (Table1). *S. diversifolius*, *S. graciliflorus* and *S. scandens* were found to be rich in secondary metabolites while *S. wallichii* was poor in secondary metabolites (Table1).

Detection of alkaloid in all species agrees with the previous literature that most species of *Senecio* contain the alkaloid. Though the coumarin reported to be absent in tribe Senecineae, was detected from most of the species of *Senecio* studied. Flavonoids, reported to have diverse functions in plants with ecological significanc , attracting the pollinators, seed and fruit dispersal, providing protection against UV light and function in plant–plant interaction and plant–microbe signaling interactions (Calabril et al 2009)[6] was also detected in many species.

Table 1: Result of phytochemical screening in *Senecio* species:

Group of compound	Name of Taxon									
	S.ala	***S.chr***	***S.den***	***S.div***	***S.gra***	***S.ruf***	***S.sca***	***S.tri***	***S.vag***	***S.wal***
Volatile oil	+	+	+	+	+	+	+	+	-	-
Fatty acids	+	-	-	+	+	-	-	+	+	-
Coumarin	-	+	+	-	+	+	+	-	-	-
Alkaloids	+	+	+	+	+	+	+	+	+	+
Sterols	+	-	+	+	+	+	+	+	+	+
Triterpenes	+	-	+	-	-	+	-	-	-	+
Emodin	-	-	-	-	-	-	+	-	-	-
Polyphenols	-	+	+	+	+	-	+	+	+	+
Reducing compounds	+	+	+	+	+	+	+	+	+	-
Anthocyanosides	-	-	-	-	-	-	-	-	-	-
Anthracenosides	-	-	-	-	-	-	-	-	-	-
Flavon aglycones	-	-	-	-	-	-	-	-	-	-
Tannins	-	+	+	+	+	-	+	+	+	+
Cardiac glycosides	-	-	-	-	-		-	-	-	-
Flavonoids	-	+	+	+	+	-	+	-	+	-
Saponins	+	+	-	+	-	+	+	+	-	+
Terpenoides	+	-	-	+	+	+	+	-	-	+
Steroids	-	-	-	+	+	-	-	+	+	-

Note: + ve indicates the presence and – ve indicates the absence of the compound tested.

Abbreviation of the *Senecio* species: *S. ala* = *Senecio alatus*, *S. chr* = *Senecio chrysanthemoides*, *S. den* = *Senecio densiflorus*, *S. div* = *Senecio diversifolius*, *S. gra* = *Senecio graciliflorus*, *S. ruf* = *Senecio rufinervis*, *S. sca* = *Senecio scandens*, *S. tri* = *Senecio triligulatus*, *S. vag* = *Senecio vagans* and *S. wal* = *Senecio wallichii*.

4. Conclusion:

This work has for the first time reported the phytochemical constituents of ten species of *Senecio* from Nepal Himalaya. The secondary metabolites like volatile oil, fatty acid, coumarins, alkaloids, sterols, triterpene, emodin, polyphenol, reducing compounds, flavonic glycosides, tannins, flavonoides, steroids and saponin were present in some amount in those species of *Senecio* found naturally in Nepal. Phytochemical screening of secondary metabolites are essential in order to find out the pharmacological effects of the species. Different types of secondary metabolites have already been reported to have the pharmaceutical effect and thus they should be the potential source of useful drugs, insecticides and possibly the herbicides. However further researches are required to identify the bioactive compound and to know the true potentials of different species.

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