The Pharma Innovation Journal 2016; 5(9): 106-109

www.ThePharmaJournal.com

The Pharma Innovation

ISSN: 2277- 7695
TPI 2016; 5(9): 106-109

www.thepharmajournal.com
Received: 16-07-2016
Accepted: 17-08-2016

Sudha Joshi (Shrestha)
Department of Botany,
Patan Multiple Campus,
Tribhuvan University, Nepal

Correspondence
Sudha Joshi (Shrestha)
Department of Botany,
Patan Multiple Campus,
Tribhuvan University, Nepal

Pyrrolizidine alkaloids in some species of *Senecio* Linnaeus (Senecioneae: Asteraceae)

Sudha Joshi (Shrestha)

Abstract
Pyrrolizidine alkaloids were analyzed by Gas Chromatography Mass Spectrometry in five species of *Senecio* Linnaeus viz. *Senecio laetus* Edgew, *Senecio nudicaulis* Buch.-Ham. ex D. Don, *Senecio raphanifolius* Wall. ex DC., from series Erucifolii of section Jacobaea; *Senecio royleanus* DC., from series Nemorenses of section Jacobaea and *Senecio scandens* Buch.-Ham. ex D. Don. from section Flexicaulis, of tribe Senecioneae of family Asteraceae. Presence of five types of pyrrolizidine alkaloids viz. Senecionine, Seneciphylline, Integerrimine, Platyphylline and Jacozine have been confirmed in four different species of *Senecio* Linn. Presence of pyrrolizidine alkaloid is not confirmed in one species, *S. nudicaulis*. Different types of pyrrolizidine alkaloids are found to be taxonomically significant in infrageneric level. The section Jacobaea is characterized by the absence of pyrrolizidine alkaloid jacozine while section Flexicaulis is characterized by the presence jacozine. Within the section Jacobaea, the series Nemorenses is characterized by platyphylline and the series Erucifolii by Seneciphylline. Within the series Erucifolii, the *Senecio laetus* is characterized by senecionine and the *Senecio raphanifolius* by intergerrimine. As the presence of pyrrolizidine is not confirmed in the *Senecio nudicaulis* of series Erucifolii of section Jacobaea, revealing it as distantly related to its group members, *S. laetus* and *S. raphanifolius*; the fact is also revealed by the morphology based cluster analysis carried on the species of *Senecio* found in Nepal Himalaya. Thus it reflects that the pyrrolizidine alkaloids are in good correlation with the morphological data in genus *Senecio* Linn. and could be a good taxonomic marker at the infrageneric level.

Keywords: Correlation, infrageneric, pyrrolizidine alkaloids, morphological data. significant

1. Introduction
The genus *Senecio* L. is the largest and core genus of tribe Senecioneae of family Asteraceae Bercht. & J. Presl. (Compositae Giseke) and comprises at least 1200 species with worldwide distribution except in Antartica (Chen *et al.,* 2011) [1]. The species of the genus show high morphological variations with diversified habitats from tropical to alpine regions of the world. In Nepal Himalaya, the genus is represented by 14 species (Joshi & Bajracharya, 2014) [2].
Many plant species produce secondary metabolites to adopt in the harsh environment and also as a form of defense against other organisms. Members of the family Asteraceae are reported to produce a wide range of secondary metabolites such as monoterpenes, diterpenes, triterpenes, sequisterpenes and sesquiterpene lactones, polyacetylenes, flavonoides, phenolic acids, benzofurans, coumarins and pyrrolizidine alkaloids (Calabria, *et al*., 2009) [3]. Among them, one of the important secondary compound is the pyrrolizidine alkaloids (PAs) that is reported to have the hepatotoxic activity in mammals (Culvenor, *et al*., 1976; Mattocks, 1986) [4, 5]. Eupatorieae and Senecioneae are the only two tribes from family Asteraceae that were reported to produce pyrrolizidine alkaloids (Reimann, *e. al.,* 2004) [6]. Moreover, PAs containing plants are numerous and widespread and expected to be present in most of the environment (Smith & Culvenor, 1981) [7]. Presence of PAs as secondary compound had already been reported in many species of *Senecio* such as *S. brasiliensis* var. *brasiliensis* (Spreng.) Less., *S. conzyifolius* Baker, *S. heterotrichius* DC., *S. oleosus* Vell, *S. oxyphyllus* DC., *S. riograndensis* Matzenbacher, *S. bonariensis* Hook. et Arn., *S. grossidens* Dusen ex Malme, *S. icoglossus* DC., *S. juergensii* Mattfeld, *S. pulcher* Hook. et. Arn., *S. ceratophylloides* Griseb., *S. crassiflorus* var. *crassiflorus* (Poir.) DC., *S. crassiflorus* var. *maritimus* (Malme) Caberra (Trigo *et al*., 2002) [8]; *S. arbotanifolius* L., *S. adonidifolius* Loisel, *S. alpinus* (L.) Scop., *S. ambiguus* DC., *S. aquaticus* Hill, *S. boissieri* DC., *S. cannabifolius* Less., *S. carniolicus* Willd., *S. chrysanthemoides* DC., *S. cineraria* DC., *S. delphinifolius* Vahl, *S. erucifolius* L., *S. giganteus* Desf., *S. gnaphalodes* Sieber, *S. halleri* Dandy, *S. incanus* L., *S. jacobaea* L., *S. leucophyllus* DC., *S. minutes* DC., *S. othonnae* M. Bieb., *S. paludosus* L., *S. pancicii* Degen, *S. persoonii* De Not., *S. alpines* Koch (Pelser *et al*.,

2005) [9]; *S. madagascariensis* Poir. (Gardner, *et al*., 2006) [10]; *S. nemorensis, S. aquaticus* Hill, *S. vernalis* Waldst, *S. jacobaea* L**.** (Kostova, *et al.*, 2006) [11]; *S. scandens, S. chrysanthemoides* DC. (Roeder, 2000) [12]; *S. brasiliensis* var. *S. stabianus* Lacaita (Tundis, *et al.,* 2009) [13].

Secondary compounds, have long been known for their pharmacological effects. However, there are some reports of the use of these secondary compounds in identifying the species and delimiting the allied species. Essential oil types from *Ocimum basilicum* were found to be useful in the classification of the species at the infraspecific level (Grayer, *et. ai.,* 1996) [14]. Similarly, essential oil types were reported to be useful in the classification of *Artemisia* species, one of the members of family Asteraceae (Maggio, *et al.,* 2012) [15]. In some *Senecio* species also, many PAs patterns were reported to have in good agreement with the morphological data and are reported suitable to be used as chemo-taxonomical markers at the infra-generic level (Trigo, *et al*., 2003) [8]. In this paper, PAs in five species of *Senecio* was analyzed by GC-MS and their suitability as taxonomic marker is evaluated.

2. Material and Methods

2.1 Plant materials

The composite samples of aerial parts of five species of *Senecio* viz. *Senecio laetus* Edgew., *Senecio nudicaulis* Buch.-Ham. ex D. Don, *Senecio raphanifolius* Wall. ex DC., from series Erucifolius of section Jacobaea; *Senecio royleanus* DC., from series Nemorenses of section Jacobaea and *Senecio scandens* Buch.-Ham. ex D. Don. from section Flexicaulis, of tribe Senecioneae of family Asteraceae, were collected from various parts of Nepal Himalaya at full flowering stage. The collected specimens were identified and authenticated by consulting the protologue texts and literatures (D. Don, 1825; De Candollea, 1837; Edgeworth, 1846; Hooker, 1882; Jeffrey & Chen, 1984) [16, 17, 18, 19, 20] and by matching with type specimens housed at various herbaria. The voucher specimens were deposited at Tribhuvan University Central Herbarium (TUCH), Central Department of Botany, Tribhuvan University, Nepal and National Herbarium and Plant Laboratories (KATH), Department of Plant Resources, Government of Nepal.

2.2 Alkaloid Extraction

The crude alkaloid was extracted from the air dried plant material by the standard method as described by Tundis *et al.*, (2009) [13] with some modification. The collected plant specimens were cleaned, air dried under the shade at normal room temperature and after completely drying, the material was pulverized to fine powder by mixture grinder. 20 gm of powered material was extracted with 95% ethanol at 60°C for about 6 hours by soxhlet method. The extract was concentrated at reduced pressure by using the rotary evaporator. The obtained extract was suspended in distilled water to get the slurry and then fractionated with n-hexane and chloroform successively. The remaining aqueous fraction was acidified with 2.5% sulphuric acid (H_2SO_4) to pH 2.5 and stirred overnight with about 5.0 g Zn powder to reduce the native or artificial N-oxides. The aqueous acidic solution was filtered and treated with 25% ammonia (NH_4OH) until the samples were alkaline (pH 11). The combined aqueous alkaline solution was fractionated thrice with 20 ml. of chloroform ($CHCl_3$). The combined chloroform layer was filtered through anhydrous sodium sulphate. The obtained chloroform extracts were evaporated to dryness that contains the crude alkaloids and were stored under the refrigeration untill analysis.

2.3 GC-MS analysis

The crude alkaloid fraction was run and analyzed by Gas Chromatography Mass Spectrometry (GCMS-QP 2010 Plus, Shimadzu Co.). Identification of the individual alkaloid was based on the comparison of the mass spectral data with computer matching against NIST library 05 and was confirmed by the determination of retention time and mass spectra and mass fragmentation patterns.

3. Result and Discussion

The GC-MS analysis of crude alkaloid extracted from aerial parts of 5 species of *Senecio* confirmed the presence of alkaloids in 4 species of *Senecio* viz. *S. laetus, S. raphanifolius, S. royleanus* and *S. scandens* (Table 18). All together presence of five types of PAs viz. Senecionine, Seneciphylline, Integerrimine, Platyphylline and Jacozine (Fig.1) are confirmed in four species. All the PAs present in them are of 12 membered and close chain type.

Among the three species of series Erucifolii of section Jacobaea, *S. nudicaulis* lacks the PAs while *S. raphanifolius,* has two types of PAs, seneciphylline and integerrimine and *S. laetus* with senecionine and seneciphylline. The species, *S. royleanus* of series Nemorenses has the PA, platyphylline, which was not been found in other species. In *S. scandens* of section Flexicaulis, the PA, Jacozine, is present and absent in other species studied.

Looking at the picture of PAs pattern of *Senecio* spp. from Nepal Himalaya, PAs are found to be different in sections and series as well as in species level. The species *S. laetus* and *S. raphanifolius* of series Erucifolii of section Jacobaea is characterized by seneciphylline while *S. royleanus* of series Nemorenses of section Jacobaea is characterized by the the platyphylline. Moreover, the *S. scandens* from section Flexicaulis has the different PA, the jacozine. Thus, it seemed that PAs could be the good taxonomic marker in infrageneric level in delimiting the section, series and species within the studied group.

Absence of PAs in *S. nudicaulis*, a member of series Erucifolii of section. Jacobaea, in present study reveals that though presently it was kept under the series Erucifolii of section Jacobaea along with *S. laetus* and *S. raphanifolius*, but found to be distantly related to those species, the fact that was also revealed by the morphology based cluster analysis of *Senecio* species from Nepal Himalaya (Joshi, 2016: Unpublished data).

Table 1: Pyrrolizidine alkaloids analyzed on crude alkaloid extract of *Senecio* spp.

SN	Name of plant	Alkaloid	Molecular weight	Molecular formula
1.	*S. laetus*	Senecionine	335	$C_{18}H_{25}NO_5$
		Seneciphylline	333	$C_{18}H_{23}NO_5$
2.	*S. nudicaulis*	-	-	-
3.	*S. raphanifolius*	Seneciphylline	333	$C_{18}H_{23}NO_5$
		Integerrimine	335	$C_{18}H_{22}NO_5$
4.	*S. royleanus*	Platyphylline	337	$C_{18}H_{27}NO_5$
5.	*S. scandens*	Jacozine	349	$C_{18}H_{23}NO_6$

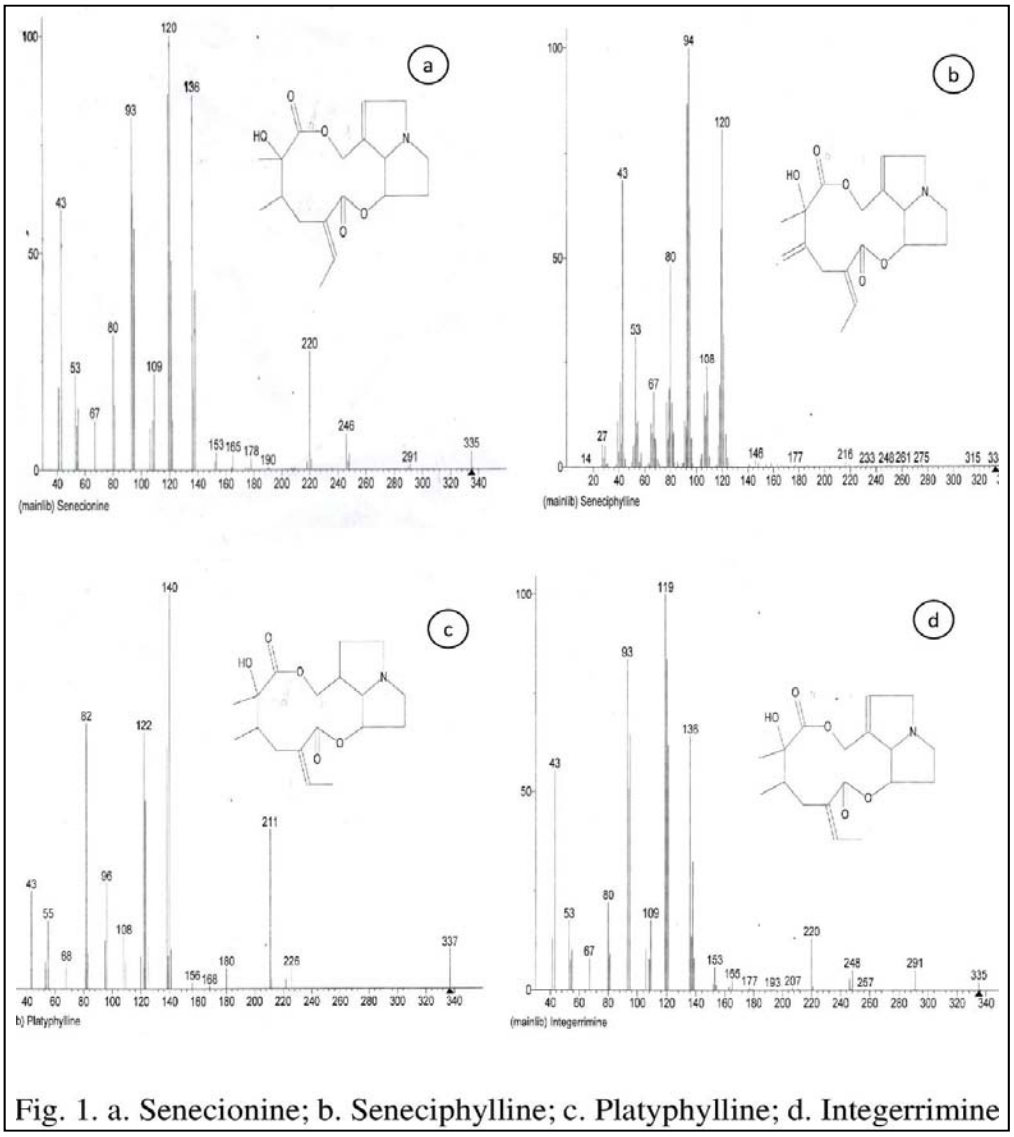

Fig. 1. a. Senecionine; b. Seneciphylline; c. Platyphylline; d. Integerrimine

4. Conclusion

From the analysis, it can be concluded that the PAs in *Senecio* species are significant in infrageneric level in delimiting the sections, series and the species within the studied group. The section Flexicaulis is characterized by the presence of jacozine; series Nemorenses of section Jacobaea by the platyphylline; series Erucifolii of section Jacobaea, by the seneciphylline. Within the species of series Erucifolii of section Jacobaea, *S. laetus* is characterized by the presence of senecionine and *S. raphanifolius* by the integerrimine. The species, *S. nudicaulis* though presently under the section Erucifolii of section Jacobaea along with *S. laetus* and *S. raphanifolius*, is found to be distantly related with its group members as it lacks the PAs. The analysis also revealed that the PAs are in correlation with the morphological data within then species studied from the genus *Senecio* Linn. of family Asteraceae.

5. Acknowledgements

Central Department of Botany, Tribhuvan University; Patan Multiple Campus, Tribhuvan University; Nepal Academy of Science and Technology, Khumaltar and Pacific Analytical Laboratory and Training Center, Dhapakhel, Lalitpur are gratefully acknowledged for their assistance and cooperation.

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