Botanica Orientalis – Journal of Plant Science (2016) **10**: 12–18 ISSN 1726-6858

Research

Volatile organic metabolites and their importance in *Senecio* L. (Senecioneae: Asteraceae)

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

Abstract

The present work aims to characterize volatile secondary metabolites in *Senecio* L. for delimiting taxa at the infrageneric level. Analysis of *n*-hexane fraction of ethanolic extracts of five species of *Senecio* L. (*S. laetus* Edgew., *S. nudicaulis* Buch.-Ham. ex D. Don, *S. raphanifolius* Wall. ex DC., *S. royleanus* DC. and *S. scandens* Buch.-Ham. ex D. Don) from Nepal Himalaya revealed the presence of a number of volatile secondary metabolites. Among them, 17 metabolites, *viz*., acorenol, bergamotene, cadin, cadinene, calarene, caryophyllene, cumialdehyde, cycloprop(e)azulene, elemol, farnesene, hexanol, intermedeol, muurolene, naphthalene, naphthalenone, propenoic acid and tridecane were considered for their potential use in chemotaxonomy of the genus. All species were characterized by the presence of cadin, elemol, farnesene, muurolene, naphthalenone and propenoic acid and absence of acorenol, cycloprop(e)azulene, hexanol and intermedeol. Cumialdehyde is present in all species except *S. royleanus*; cadinene is present in all tested species except *S. nudicaulis* and *S. royleanus*. Moreover, *S. nudicaulis* can be delimited from other species by the presence of bergamotene; *S. royleanus* can be delimited from other species by the presence of tridecane and caralene. Caryophyllene is present in *S. raphanifolius* and *S. scandens* and absent in *S. laetus.* Naphthalene is present in *S. nudicaulis, S. royleanus* and *S. scandens,* and absent in *S. laetus* and *S. raphanifolius*. Presence and absence of a single metabolite or a group of volatile organic metabolites indicates its significance as the taxonomic marker for delimiting taxa at infrageneric level. An artificial dichotomous key is prepared to delimit the taxa.

Key-words: chemotaxonomy, hexane fraction, secondary metabolites, taxonomic marker.

Introduction

The genus *Senecio* L. is the largest and the core genus of the tribe Senecioneae of the family Asteraceae. It comprises at least 1200 species with worldwide distribution, except in Antartica (Chen *et al*. 2011). The species of the genus show high morphological variations possibly due to very diverse habitats ranging from tropical to alpine regions of the world. Nepal houses 14 species of *Senecio* (Joshi and Bajracharya 2014).

Secondary metabolites are the organic compounds produced by plants to adapt to the harsh environmental conditions, and also as a sort of defense mechanism against pathogenic micro-organisms and herbivores. Members of the family Asteraceae are reported to produce a wide range of secondary metabolites, including monoterpenes, diterpenes, triterpenes, sesquiterpene lactones, polyacetylenes, flavonoides, phenolic acids, benzofurans, coumarins and pyrrolizidine alkaloids (Calabria *et al*. 2009).

Senecioneae is the species-rich tribe among the eleven tribes of the family Asteraceae (Bremer 1994). This tribe is characterized by the complete absence of or weak presence of polyacetylene compounds which are commonly found in most of the other tribes (Robins 1977). This tribe is also characterized by the complete absence of coumarins (Zdero and Bohlmann 1990).

Among secondary metabolites present in plants, alkaloids have been used as chemosystematic markers at the family and subfamily level (Hartmann and Witte 1995). The production of pyrrolizidine alkaloids in Asteraceae is confined to only two tribes, Eupatorineae and Senecioneae (Reimann *et al.* 2004). The pyrrolizidine alkaloids are reported to be a suitable taxonomic marker in *Senecio* as they have good correlation with the morphological data in various taxa of the genus (Trigo *et al.* 2003; Joshi 2016).

Volatile organic metabolites produced by plants are well known for their pharmaceutical effects from the very long time. Besides, there are some reports of use of these compounds in delimiting the taxa at specific and infraspecific level. Essential oil types are found to be useful in the infraspecific classification of the genus *Ocimum* (Grayer *et al.* 1996). Similarly, essential oil types are also reported to be useful in the classification of *Artemisia* species (Maggio *et al.* 2012). However, the volatile organic metabolites have never been considered for the purpose of delimiting the species and to find interrelationship within and among the species of *Senecio* so far. In this paper, an effort is made to find the importance of volatile organic metabolites in delimiting taxa within *Senecio*.

**Corresponding author*: e-mail - sudhashresthajoshi@gmail.com; Tel – 977-9841466330.

Materials and Methods

PLANT MATERIALS

The composite samples of aerial parts of five species of *Senecio* (*S. laetus* Edgew., *S. nudicaulis* Buch.-Ham. ex D. Don, *S. raphanifolius* Wall. ex DC., *S. royleanus* DC. and *S. scandens* Buch.-Ham. ex D. Don) were collected from different localities in Nepal Himalaya at full flowering stage. The collected specimens were identified and authenticated by comparing the character states of specimens with protologue texts and other literature (Don 1825; De Candolle 1838; Edgeworth 1846; Hooker 1882; Jeffrey and Chen 1984) and verifying the specimen with the type specimens. The voucher specimens were deposited at Tribhuvan University Central Herbarium (TUCH) and National Herbarium and Plant Laboratories (KATH).

EXTRACTION METHOD

The collected plant specimens were cleaned, air dried under the shade at normal room temperature. The material was pulverized to fine powder by mixture grinder upon complete drying. Twenty grams of powered materials were 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 concentrated extract was treated with sterile water to make the slurry. The aqueous slurry was fractionated with *n*-hexane, petroleum ether, for three times. Obtained *n*-hexane fraction was concentrated at reduced pressure in a rotary evaporator. The fractions were stored at low temperature in the refrigerator until further analysis.

GC-MS ANALYSIS

The volatile organic compounds present on *n*-hexane fraction of ethanol extract were analyzed by gas chromatography mass spectrometry (GCMS-QP 2010 Plus, Schimadzu, Japan) by head space method. Identification of the compound 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 fragmentation patterns.

Results

GC-MS analysis of *n*-hexane fraction of ethanolic extracts in different species of *Senecio* revealed the presence of a number of volatile organic metabolites. Among them, some of the metabolites, viz., farnesene, cadinene, bergamotene, elemol, cadin, naphthalenone, cumialdehyde, caryophyllene, tridecane, acorenol, intermedeol, murrolene, nephthalene, cycloprop(e) azulene, hexanol, calarene and propenoic acid showed species specific pattern of distribution. The compounds, such as cadin, elemol, farnesene, muurolene, naphthalenone and propenoic acid were present in all the species of *Senecio*, while compounds, such as acorenol, cycloprop(e)azulene, hexanol and intermedeol were present in none.

The compounds considered in the study along with their retention time are presented in Table 1 while the classification and occurrence of these compounds in different species is given in Table 2. The chromatograms revealed by GC-MS analysis for different species are presented in Figures 1-5. The metabolite cumialdehyde, ten carbon compound, is present in all species except *S. royleanus*. Similarly, cadinine, a 15-carbon bicyclic sesquiterpene, is present in species except in *S. nudicaulis* and *S. royleanus*; while naphthalene, 10 carbon polcyclic hydrocarbon, is present in all species except in *S. laetus* and *S. raphanifolius.*

The analysis revealed that *S. royleanus* is characterized by the presence of calarene (15 carbon sesquiterpene) and tridecane (13 carbon alkane hydrocarbon), and absence of cumialdehyde. The species *S. nudicaulis* can be delimited from other species by the presence of bergemotene (15 carbon terpenoid), and absence of cadinine (15 carbon bicyclic sesquiterpene).

Three species, viz., *S. scandens, S. laetus* and *S. raphanifolius* are characterized by the presence of the metabolites farnesene, cadinine, elemol, cadin, naphthalenone, cumialdehyde, murrolene, and propenoic acid and absence of bergamotene, tridecane and calarene. However, the secondary compound naphthalene is present only in *S. scandens* and absent in *S. laetus* and *S. raphanifolius.*

Two species, *S. laetus* and *S. raphanifolius* are characterized by the presence of farnesene, cadinine, elemol, cadin, naphthalenone, cumialdehyde, murrolene and propenoic acid and absence of bergamotene, tridecane, naphthalene and calarene. However, within these two species, *S. raphanifolius* has caryophyllene, while *S. laetus* lacks this compound. The metabolite caryophyllene is also present in *S. scandens.*

Discussion

The species, *S. royleanus* is found to be quite distinct from other species due the presence of calarene and tridecane, and absence of cumialdehyde, indicating distant relationship of the species with its group members. Similarly, *S. nudicaulis* which lies in series Erucifolii of section Jacobaea (Mill.) Dumort along with *S. laetus* and *S. raphanifolius,* is found to be different from them in having the secondary metabolite bergemotene. Likewise, *S. scandens* which lies in section Flexicaulis C. Jeffrey & Y.L. Chen, shows the close relationship with *S. laetus* and *S. raphanifolius* of section Jacobaea, in having the metabolites like farnesene, cadinine, elemol, cadin, naphthalenone, cumialdehyde, murrolene, and propenoic acid, and in the absence of bergamotene, tridecane and calarene. However, *S. scandens* can be delimited from *S. laetus* and *S. raphanifolius* by the presence of naphathalene.

Table 1. Volatile organic metabolites and their retention time in *n*-hexane fraction of ethanolic extracts of selected species of *Senecio* L.

Compound	*Senecio laetus*	*Senecio nudicaulis*	*Senecio raphanifolius*	*Senecio royleanus*	*Senecio scandens*
alpha-Acorenol	-	-	-	-	-
beta-trans-Bergamotene	-	12.028	-	-	-
Cadin-4-en-10-ol	13.660	13.654	13.661	13.311	13.653
delta-Cadinene	12.423	-	12.958	12.463	-
Calarene	-	-	-	10.731	-
Caryophyllene	-	-	11.837	-	13.733
Cumialdehyde	9.501	9.494	9.501	-	9.494
1H-Cycloprop[e]azulene,decahydro-1,1,7-trimethyl-1-4-methylene[1]	-	-	-	-	-
alpha-Elemol	13.250	13.245	13.251	12.862	13.244
(E)-beta-Farnesene	12.032	12.622	12.033	12.191	12.026
2-ethylhexanol	-	-	-	-	-
Intermedeol	-	-	-	-	-
gamma-Muurolene	12.698	12.693	12.700	12.101	12.690
Naphthalene,1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)	-	12.954	-	12.596	12.952
1(2H)-Naphthalenone,octahydro-4a,8-dimethyl-7-(1-methylethyl)-1	14.721	14.715	14.723	14.532	14.715
2-Propenoic acid, 3-phenyl, methyl ester	11.277	11.274	11.278	9.698	11.275
n-Tridecane	-	-	-	10.065	-

Table 2. Classification of volatile organic metabolites of *Senecio* spp.

Compound name	Class	Type	Mol. Formula	Occurrence in spp.
Bergamotene	Terpenoid	Close chain	$C_{15}H_{24}$	*S. nudiaculis*
Cadin	Sesquisterpenoid	Close chain	$C_{15}H_{26}O$	All species
Cadinene	Bicyclic sesquisterpenes	Close chain	$C_{15}H_{24}$	*S. laetus, S. raphanifolius, S. scandens*
Calarene	Sesquisterpene	Close chain	$C_{15}H_{24}O$	*S. royleanus*
Caryophyllene	Bicyclic sesquisterpene	Close chain	$C_{10}H_{24}$	*S. raphanifolius, S. scandens*
Cumialdehyde	Isopropylbenzaldehyde	Close chain	$C_{10}H_{12}O$	*S. laetus, S. nudicaulis, S. raphanifolius, S. scandens*
Elemol	Sesquisterpene	Close chain	$C_{15}H_{26}O$	All species
Farnesene	Sesquisterpene	Open chain	$C_{15}H_{24}$	All species
Muurolene	Enzyme	Close chain	$C_{15}H_{24}$	All species
Naphthalene	Polycyclic hydrocarbon	Close chain	$C_{10}H_8$	All species
Naphthalenone	Bicyclic aromatic derivative	Close chain	$C_{10}H_{10}O_3$	All species
Propenoic acid	Organic acid	Open chain	$C_3H_4O_2$	All species
Tridecane	Alkane hydrocarbon	Open chain	$C_{13}H_{28}$	*S. royleanus*

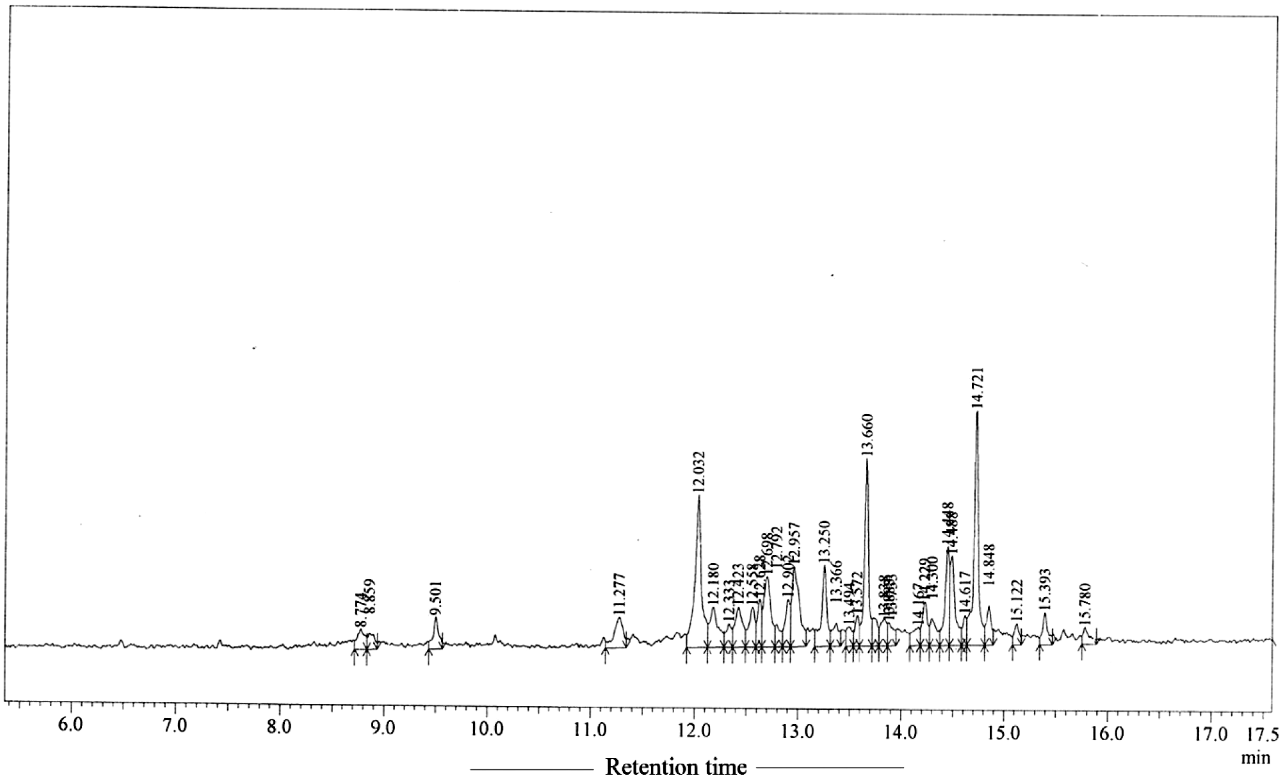

Figure 1. Chromatogram of *Senecio laetus* Edgew.

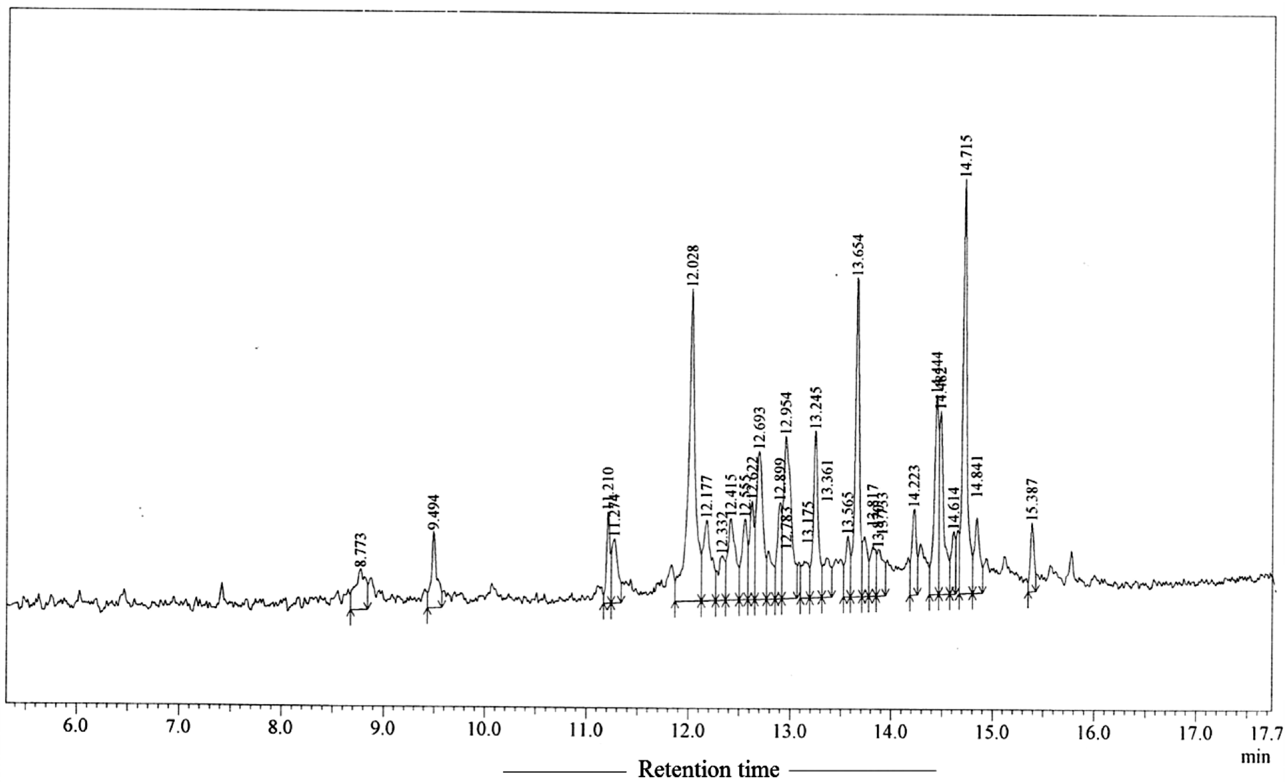

Figure 2. Chromatogram of *Senecio nudicaulis* Buch.-Ham. ex D. Don

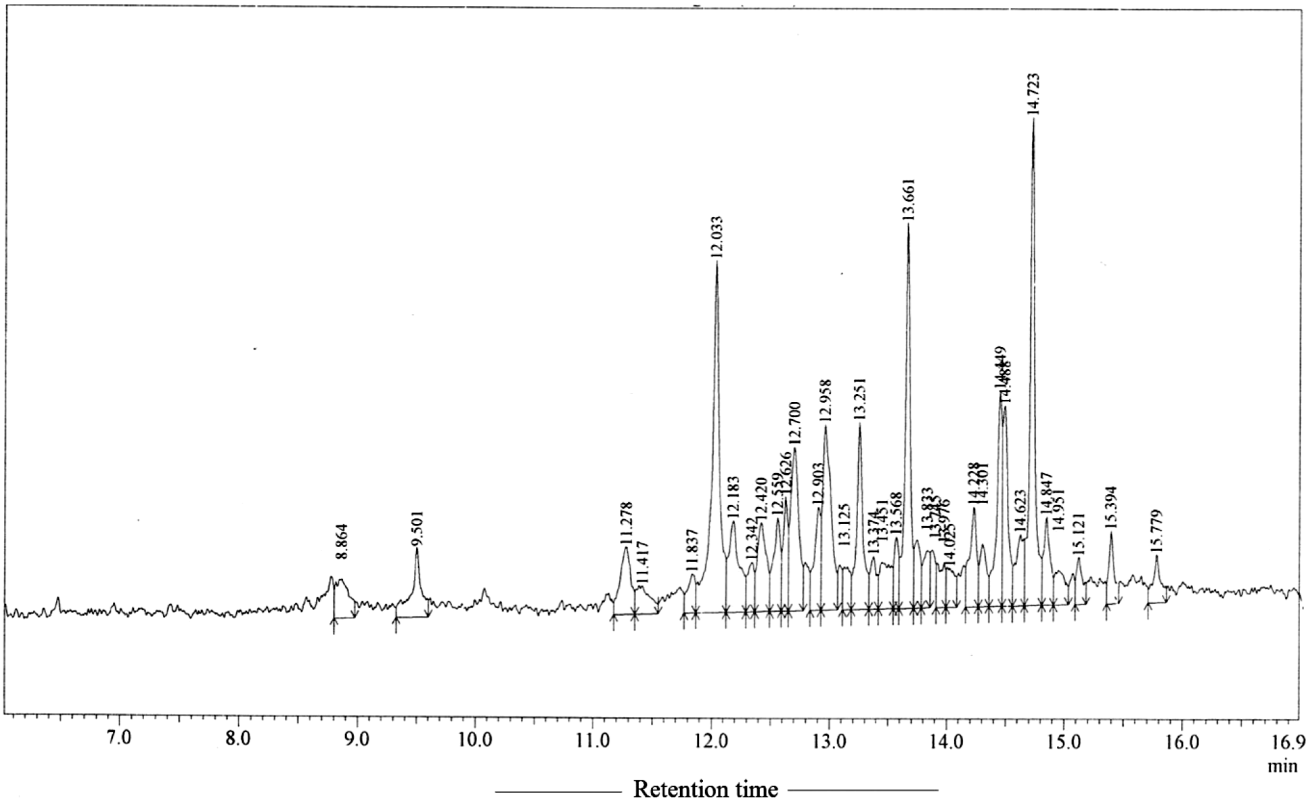

Figure 3. Chromatogram of *Senecio raphanifolius* Wall. ex DC.

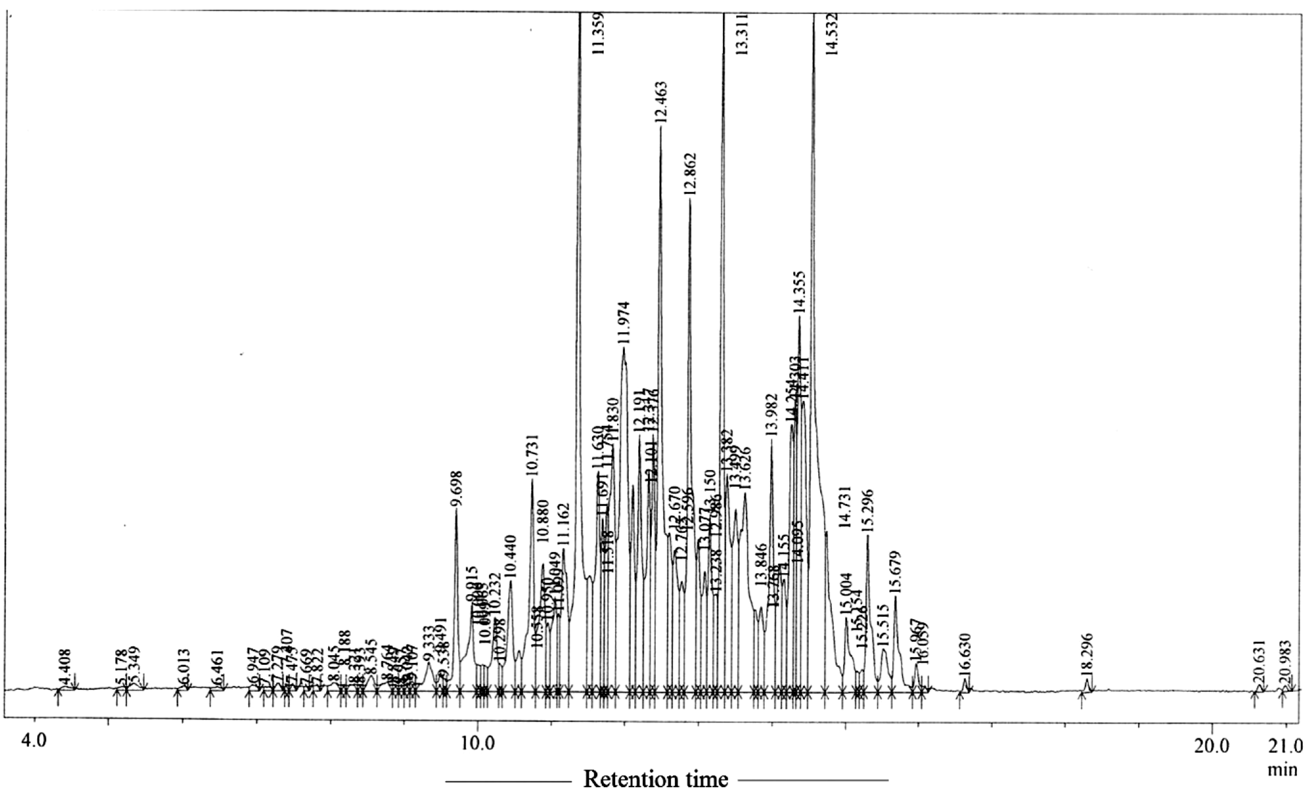

Figure 4. Chromatogram of *Senecio royleanus* DC.

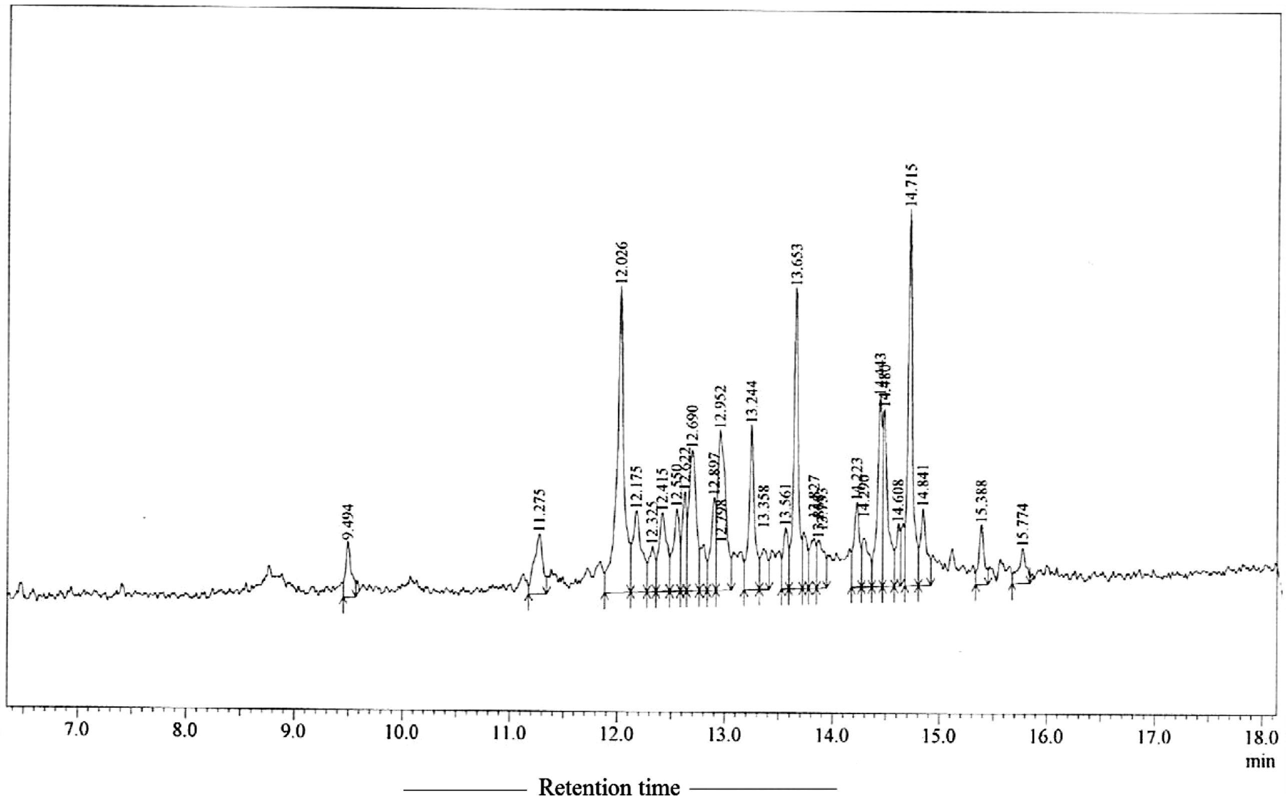

Figure 5. Chromatogram of *Senecio scandens* Buch.-Ham. ex D. Don

The cluster analysis of Nepalese species of *Senecio* based on gross and micro- morphological characters shows *S. royleanus* along with other species, viz., *S. graciliflorus, S. biligulatus* and *S. topkegolensis* forming a distinct sister cluster with the cluster formed by *S. nudicaulis, S. scandens, S. laetus* and *S. raphanifolius*. Within the cluster formed by *S. nudicaulis, S. scandens, S. laetus* and *S. raphanifolius*, the species *S. nudiaculis* was found to from the sister cluster with *S. scandens, S. laetus* and *S. raphanifolius* (Joshi 2016: unpublished data). In the same analysis, it was also found that *S. laetus* and *S. raphanifolius* are sisters to each other. The species *S. laetus* differs from *S. raphanifolius* in lacking the caryophyllene in it. The metabolite caryophyllene that was reported to have antibacterial and antifungal effects with strong antioxidant activity and inhibitory effects against colon cancer (Dahham *et al*. 2015) is thus found to be significant in delimiting the closely related species. The result thus indicates that the distribution of volatile secondary metabolites is in good correlation with the morphological data and could be the taxonomic marker in delimiting infrageneric taxa in *Senecio*.

Previous reports have shown only the pyrrolizidine alkaloids to have good agreement with the morphological data and are suitable as the taxonomic markers (Trigo *et al.* 2003; Joshi 2016) in *Senecio*. The importance of volatile secondary metabolites in taxonomy of *Senecio* has never been studied. The secondary metabolites, which are well known for their pharmaceutical effects with significant industrial importance, are thus also revealed significant in delimiting a single species or group of species in *Senecio* and are suitable to be used as good taxonomic markers at infrageneric level. Moreover, it is also envisaged that revelation of presence of different types of volatile organic metabolites will help in bio-prospecting of species of *Senecio* in future.

Based on presence and absence of particular volatile organic metabolite, an artificial dichotomous key has been prepared to delimit the selected species in genus:

1a. Presence of tridecane and calarene; absence of cumialdehyde .... *S. royleanus*
1b. Absence of tridecane and calarene; presence of cumialdehyde 2
2a. Presence of bergamotene; absence of cadinene ....*S. nudicaulis*
2b. Absence of bergomotene; presence of cadinene 3
3a. Presence of naphthalene ....*S. scandens*
3b. Absence of naphthalene 4
4a. Presence of caryophyllene ....*S. raphanifolius*
4b. Absence of caryophyllene ....*S. laetus*

Acknowledgements

I would like to gratefully acknowledge the Central Department of Botany, Tribhuvan University; Patan Multiple Campus, Tribhuvan University; Nepal Academy of Science and Technology, Khumaltar; and Department of Plant Resources, Thapathali for their cooperation in the completion of present work. Comments and suggestions of two anonymous reviewers on the earlier version of the manuscript greatly helped to improve the quality of the paper.

References

Bremer K. 1994. *Asteraceae: Cladistics and Classification*. Timber Press, Portland, Oregon, USA.

Calabria L.M., Emerenciano V.P., Scott M.T. and Mabry T.J. 2009. Secondary chemistry of Compositae. In: *Systematics, Evolution, and Bibliography of Compositae* (V.A. Funk, A. Sussana, T.F. Stussey and R.J., Bayer, eds.), pp. 73–88. International Association of Plant Taxonomy (IAPT), Vienna, Austria.

Chen Y., Nordenstam B., Jeffrey C. and Vincent L. 2011. *Senecio* Linnaeus. In: *Flora of China*: *Asteraceae*, V*ol* 20–21 (W. Zhengyi, P.H. Raven and H. Deyuan, eds.), pp. 508. Science Press, Beijing, China and MBG Press, St. Louis, USA.

Dahham S.S., Tabana Y.M., Iqbal M.A, Ahamed M.B., Ezzat M.O., Majid A.S. and Majid A.M. 2015. The anticancer, antioxidant and antimicrobial properties of the sesquiterpene β-caryophyllene from the essential oil of *Aquilaria crassana*. *Molecules,* 20: 1108–1129.

De Candolle A.P. 1838. *Prodromus Systematis Naturalis Regni Vegetabilis* Parts VI, 1585–1597. Parisii: Sumptibus Sociorum Treuttelet Wurtz, Paris.

Don D. 1825. *Prodromus Florae Nepalensis*. Londini, J. Gale.

Edgeworth M.P. 1846. Descriptions of some unpublished species of plants from north-western India. *Transactions of the Linnean Society of London,* 20: 74.

Grayer R.J., Kite G.C., Goldstone F.J., Bryan S.E., Paton A. and Putievsky E. 1996. Intraspecific taxonomy and essential oil chemotypes in sweet basil, *Ocimum basilicum*. *Phytochemistry,* 43: 1033–1039.

Hartmann T. and Witte L. 1995. Chemistry biology and chemoecology of pyrrolizidine alkaloids. In: *Alkaoids: Chemical and Biological Perspectives* (S.W. Pelletier, ed.)*,* pp. 155–233. Pentagon Press, Oxford.

Hooker J.D. 1882. *Flora of British India.* L. Reeve & Co., London.

Jeffrey C. and Chen Y.L. 1984. Taxonomic studies on the tribe Senecioneae (Compositae) of Eastern Asia. *Kew Bulletin*, 39: 205–446.

Joshi S. 2016. Pyrrolizidine alkaloids in some species of *Senecio* Linnaeus (Senecioneae: Asteraceae). *The Pharma Innovation,* 5: 106–109.

Joshi S. and Bajrachraya D.M. 2014. *Senecio echaetus* Y.L. Chen & K.Y. Pan (Senecioneae: Asteraceae) – an addition to the flora of Nepal. *Pleione,* 8: 508–512.

Maggio A., Rosselli S., Bruno M., Spadaro V., Raimondo F.M. and Senatore F. 2012. Chemical composition of essential oil from Italian populations of *Artemisia alba* Turra (Asteraceae). *Molecules,* 17: 10232–10241.

Reimann R., Nurhayati N., Backenkohler A. and Ober D. 2004. Repeated evolution of the pyrrolizidine alkaloid mediated defense system in separate angiosperm lineages. *The Plant Cell*, 16: 2772–2784.

Robins D.J. 1977. Senecioneae–chemical review. In: *The Biology and Chemistry of the Compositae*, 2 (V.H. Heywood, J.B. Harborne and B.L. Turner. eds.), pp. 831–850. Academic Press, London.

Trigo J.R., Leal I.R., Matzenbacher N.I. and Lewinsohn T.M. 2003. Chemotaxonomic value of pyrrolizidine alkaloids in southern Brazil *Senecio* (Senecioneae: Asteraceae). *Biochemical Systematics and Ecology,* 31: 1011–1022.

Zdero C. and Bohlmann F. 1990. Systematics and evolution within the Compositae, seen with the eyes of a chemist. *Plant Systematics and Evolution,* 171: 1–14.