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Research Article

Chemical Composition, Antimicrobial Activity and Chromosome Number of *Senecio giganteus* Desf. from Algeria

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ABSTRACT
The species *Senecio giganteus* is endemic to North Africa, especially in streams of mound. The essential oil isolated from aerial parts of *S. giganteus* from Ain Roua (Setif) region was submitted to the hydrodistillation; the yield obtained is very low 0.02% (v/w). The oil was analysed by GC and GC/MS. The chemical analysis has allowed identifying 40 compounds corresponding to 92.38 %. The oxygenated sesquiterpenes are dominant in the essential oil *of S. giganteus* with 21.22% and the hexadecanoic acid is the major component (17.80%), followed by isophytol (12.43%), pentanol 3 methyl (7.28%) and phytol (6.66%). The Essential oil of *S. giganteus* was tested for antimicrobial activity; it showed a modest effect against the strains tested. The karyological investigation of the population of *S. giganteus* has revealed a tetraploid chromosome number 2n = 4x = (20 + 2B), this number is reported for the first time in Algeria.

Keywords: *Senecio giganteus*, Essential oil, antibacterial activity, Chromosome, Algeria

INTRODUCTION

Senecioneae is the largest tribe of the *Asteraceae* family gathered about 150 genera and 3000 species, nearly a third of the species of the tribe are included in the genus *Senecio*[1,2]. In Algeria, the *Senecio* genus is represented by 18 species of which five species are endemic[3]. Several species of the tribe are used in folk medicine[4]. The species of genus *Senecio* are known to be toxic but also for their beneficial effects on cough, eczema, bronchitis, wound healing and facilitation of childbirth[5]. *S. vulgaris* is used to calm painful menstruation and *S. cineraria* to relieve eye problems[6]. The essential oils of the genus have been many studies[7-19]. The butanol extracts of the flowers of *S. giganteus* present an antioxidant power[20]. The major components of oil in genus *Senecio* are highly variable; 1,10-epoxy furano-eremophilane (55.30%) found in the essential oil of *S. aegyptius* in Egypt; the curcumene (42.8%) in the oil of *S. nemorensis* of Turkey and the α-pinene (33.97%) in oil of *S. graciliflorus* from India[7,10,15]. The genus *Senecio* includes several chemical families; hydrocarbon compounds, aliphatic and the oxygenated compounds[17]. The chemical composition of essential oils from species of the genus *Senecio* is influenced by soil type and characteristics of the plant[18]. The essential oils of the genus *Senecio* have shown several biological activities. The oils of *S. othonnae*, *S. nemorensis* and *S. racemosus*, showed antimicrobial and antifungal activity[10]. The essential oil of *S. aegyptius* possesses an antifungal and antibacterial activity[7]. The essential oil of *S. flammeus* is effective in the treatment of acute inflammations[16]; the essential oil of *S. nudicaulis* has an anti-antioxidant power[19], while the essential oil of *S. rufinervis* presents an analgesic activity[21]. The essential oil of the flowers of *S. graciliflorus* has antioxidant potential and high cytotoxicity against cell lines of lung cancer[15]. The essential oil of *S. graveolens* has antibacterial and antifungal effects[22], the oil of *S. amplexicaulis*, exhibits antifungal activity[23]. The genus *Senecio* is largely polyploid with a basic number (x = 5)[24-27]. The species of the genus show a great chromosomal diversity[26]. The species with 2n = 10 are located in Africa and probably this is the site of original genre[24,26,28]. The highest chromosome number is observed in *Senecio roberti-friesii* with 2n = 36x = 180 chromosomes[29]. It is noteworthy that several species of the genus *Senecio* are wearing chromosomes B, (*S. pogonias*, *S. sectilis*, *S. ragonesei*, *S. viridis*, *S. subulatus*, *S. uspallatensis*, *S. uspallatensis* and *S. uspallatensis*)[30]. The aim of this work is to study the chemical composition, the antimicrobial activity and determination of chromosomal number of *Senecio giganteus*, a species endemic to eastern Algeria.

MATERIALS AND METHODS

Plant materials

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Senecio giganteus is an endemic plant of North Africa. Stems are fluted, with 2 cm in diameter. The leaves are whitish below; the flowers are yellow, grouped in corymbs. The akenes are very small, hairless[3] (Figure 1). *Senecio giganteus* is collected from eastern Algeria, Ain roua (Setif) (Figure 2). Aerial parts were collected during the flowering stage in June 2014. The air dried materials were subjected to hydro-distillation for 3h using a Clevenger apparatus type. Voucher specimens were deposited in the herbarium of the Department of Biology and Ecology, Setif University, Algeria. The oil obtained was collected and dried over anhydrous sodium sulphate and stored in screw capped glass vials in a refrigerator at 4-5°C prior to analysis. Yield based on dried weight of the samples was calculated.

Essential oil analysis

The essential oils were analyzed on a Hewlett-Packard gas chromatograph Model 5890, coupled to a Hewlett-Packard model 5971, equipped with a DB5 MS column (30 m X 0.25 mm; 0.25 µm), programming from 50°C (5 min) to 300°C at 5°C/min, with a 5 min hold. Helium was used as the carrier gas (1.0 mL/min); injection in split mode (1:30); injector and detector temperatures, 250 and 280°C, respectively. The mass spectrometer worked in EI mode at 70 eV; electron multiplier, 2500 V; ion source temperature, 180°C; MS data were acquired in the scan mode in the m/z range 33-450. The identification of the components was based on comparison of their mass spectra with those of NIST mass spectral library[31-33] and those described by Adams, as well as on comparison of their retention indices either with those of authentic compounds or with literature values[34].

Antimicrobial activity

The antimicrobial activities of the essential oil of *S. giganteus* were evaluated against One Gram positive bacteria (*Staphylococcus aureus* ATCC2592), three Gram negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Klebsella pneumonia* ATCC 70060, *Escherichia coli* ATCC 25922 and *Shigella sp*) and the yeast *Candidat* albicans ATCC 10231. The bacterial inoculums were prepared from overnight broth culture in physiological saline (0.8 % of NaCl) in order to obtain an optical density ranging from 0.08-01 at 625 nm. Muller-Hinton agar (MH agar) and MH agar supplemented with 5 % sheep blood for fastidious bacteria were poured in Petri dishes, solidified and surface dried before inoculation. Sterile discs (6 mm Φ) were placed on inoculated agars, by test bacteria, filled with 10 µl of mother solution and diluted essential oil (1:1, 1:2, 1:4, and 1:8 v: v of DMSO). DMSO was used as negative control. Bacterial growth inhibition was determined as the diameter of the inhibition zones around the discs. All tests were performed in triplicate. Then, Petri dishes were incubated at 37°C during 18 to 24h aerobically (bacteria). After incubation, inhibition zone diameters were measured and documented. The bactericidal and bacteriostatic tests on the five bacterial strains using pure oil of S. giganteus are performed in the present study.

Caryology

For karyotypic analysis, the squashing method is used. The root-tip meristems of from germinating seeds were usually used for chromosome preparations. A pre-treatment at room temperature for 1.5 hours was usually applied before fixation of the root-tips, in a 0.05% water solution of colchicine. After fixation in a cold mixture of ethanol acetic acid (3:1), the root-tips were stored in cold 70° ethanol until used. The following procedure involved the maceration in 45% acetic acid for 15 min. staining of chromosomes is made of emerging root-tips in acetic orcein with heating for one minute. Cutting off the meristems and squashing them in a drop of orcein.

RESULTS

The essential oil, of *Senecio giganteus*, isolated by hydro-distillation from the aerial parts, was obtained in very low yield 0.02% (v/w). The analysis by gas chromatography/mass spectrometry (GC-MS) (Figure 4 and 5) of the chemical composition of essential oils, we allowed the identification of 40 compounds in oil representing 92.38% of the total oil. The compounds, identified in this oil and their relative abundance, are presented in their order of appearance (Table 1). The major compounds of the essential oil of *S. giganteus* are hexadecanoic acid (17.80%), followed by isophytol (12.43%), 3-methyl pentanol (7.28%), phytol (6, 66%) and the spathulenol (4.47%). Oxygenated sesquiterpenes are dominant in the essential oil of *S. giganteus* with 21.22%, followed by diterpenes (19.09%) and fatty acids with 17.80%. One Notices the poverty of monoterpenes compared to the sesquiterpenes and diterpenes. The anti-bacterial activity of the essential oil was determined by the disc diffusion method. Five strains bacteria and yeast and three controls (antibiotics) are used in this study (Table 2). The diameters of inhibition zones generated by the essential oil are well below those produced by antibiotics. The essential oil of *S. giganteus* shows moderate activity against *Escherichia coli* ATCC 25922 and *Shigella* sp. with a diameter of inhibition of (12-14mm). This oil is weakly active against *Staphylococcus aureus* ATCC 25923, *Klepsela pneumonia* ATCC 70060 and *Pseudomonas aeruginosa* ATCC 27853, with inhibition diameter of (7.5-11 mm). The yeast, *Candidate albicans*, is resistant to the essential oil of *S. giganteus*. The bactericidal and bacteriostatic tests on the five bacterial strains using the pure oil of *S. giganteus* show a bacteriostatic effect. To our knowledge *S. giganteus*, Algerian endemic species, has never been the subject of karyological study. The microscopic observation of the metaphase plates of root meristems, allowed to observe a tetraploid chromosome number, $2n = 4x = 20 + 2B$, with the presence of two B chromosomes (Figure 5). This chromosome number is identified for the first time. The basic chromosome number of this species is $x = 5$.

DISCUSSION

The essential oil yield of *S. giganteus* in the region of Ain Roua (Setif) is considered very low (0.02%) compared with the performance of the population of Constantine (0.7%)[14]. While our result is inserted into the genus of

Figure 1: *Senecio giganteus*

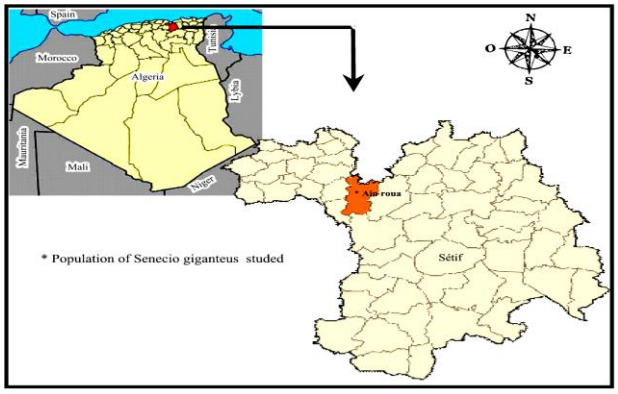

Figure 2: Population of *Senecio giganteus* studed

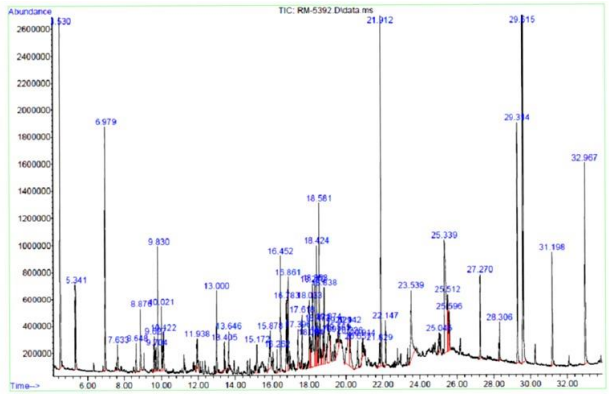

Figure 3: GC/FID profiles of *Senecio giganteus*

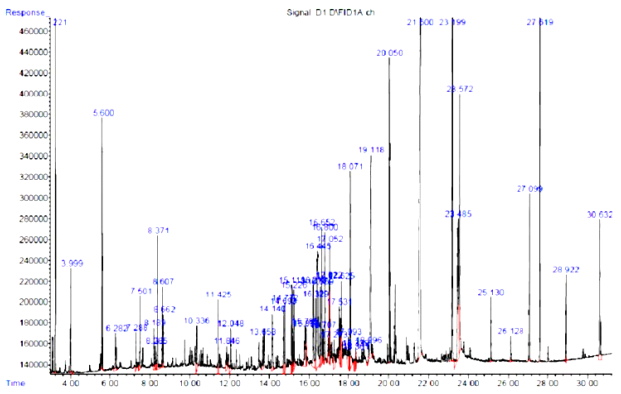

Figure 4: GC/MS profiles of *Senecio giganteus*

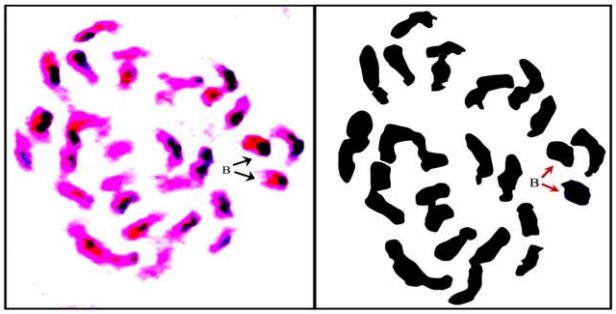

Figure 5: Caryotype of *Senecio giganteus* (2n= 4x= 20 + 2B). (Magnification = HI 100X).

literature data, *S. aegyptius* from Egypt (0.05%)[7], *S. polyanthemoides* of South Africa (0.07 %)[9], *S. perralderianus* of Algeria (0.1%)[13] and *S. graveolens* of Argentina (0.5%)[22]. The essential oil chemical profile of *S. giganteus* differs from other species of the genus. The chemical composition of *S. giganteus* of Constantine region (Algeria) shows similarity with our results, with the presence of five terpene compounds (eicosane, nerolidol<Z>, pentacosane, pentyl furan<2> and tridecene<1>)[14]. To our knowledge this is the only study that was done on the essential oil of this species. The chemical composition of *S. giganteus* is integrated to the overall context of the genus *Senecio*, by the presence of hexadecanoic acid, major compound in the genre, as in *S. flammeus*[16]. *S. giganteus* is characterized by the prevalence of oxygenated sesquiterpenes, moving closer to the chemical composition of species (*S. nudicaulis* and *S. adenotrichius*)[35], *S. rowleyanus* of Egypt[36], *S. royelanus* of India[37], *S. belgaumensis* of India[12], *S. vulgaris* and *S. angulatus* of France[17]. While the chemical composition of the species *S. perralderianus*[13], *S. leucanthemifolius*[38] and *S. atacamensis*[39] is significantly different from the chemical composition of *S. giganteus*. The results of bacteriological analyzes of *S. giganteus* are generally similar to those in the literature. Essential oils of our species show moderate activity against *Escherichia coli* and *Staphylococcus aureus*. The same results are cited for species *S. graveolens* and *S. pedunculatus*[40,41] and *S. othonnae* and *S. nemorensis*[10]. The essential oils of *S. pogonias* and *S. oreophyton* show antibacterial activity against *Escherichia coli* and *Klebsiella pneumoniae*[42]. While, the bacteria *Staphylococcus aureus, E. coli, K. pneumoniae* and *Pseudomonas aeruginosa* are resistant to the oil of *S. glaucus* of Egypt[43]. The yeast *Candida albicans* is resistant to the essential oil of *S. giganteus* by against it is sensitive to the essential oil of *S. pedunculatus*[40]. The oil of *S. glaucus* from Egypt shows moderate activity against *C. albicans*[43]. The karyological study of *S. giganteus* showed the presence of a tetraploid karyotype with 2n = 4x = 20 + 2B. The presence of chromosomes B in this genre is confirmed in several species[30,44-47]. This result is consistent with those found in other species of the genus *Senecio*. The species *S. madagascariens* Argentina[28], *S. leucanthemifolius, S. glaucus* and *S. squalidus* of Morocco[48-49]; *S. aethnensis, S. squalidus* and *S. chrysanthemifolius* of Sicily have a basic chromosome number (x = 5)[50]. This basic number is

Table 1: Chemical composition of *Senecio giganteus* essential oil

Yield (%)	KI	0.02	Yield (%)	KI	0.02
Number of compound		40	Number of compound		40
Total		92.38	Total		92.38
Pentanol 3-methyl	833	7.28	Muurola-4(14),5-diene-trans	1493	1.17
Heptenal (2E)	947	3.40	Δ-amorphene	1511	0.65
Pentylfuran-2	984	0.64	Kessane	1529	0.54
Decene-1	986	1.42	Nerolidol (Z)	1531	2.19
β-ocimene (Z)	1032	0.45	Spathulenol	1577	4.47
β-ocimene (E)	1044	1.16	Caryophyllene oxide	1582	3.09
Octen-1-ol (3Z)	1047	0.52	Salvial 4(14) en 1 one	1594	2.06
n-octanol	1063	0.38	Humulene epoxide II	1608	2.75
Octenol (5Z)	1065	1.73	Himachalol	1652	0.67
Linalool	1095	1.09	α-cadinol	1652	0.03
n-nonanal	1100	0.68	Amorpha-4,9-dien-2-ol	1700	4.24
α-terpineol	1186	0.53	Mint sulfide	1740	0.20
Dodecene-1	1187	0.95	Phytol	1942	6.66
Tetradecatriene (3Z,6Z,9Z)	1288	0.39	Isophytol	1946	12.43
Tridecene-1	1290	0.54	Hexadecanoic acid	1959	17.80
β-damascenone (E)	1383	0.26	Eicosane (C20)	2000	0.87
Caryophyllene (Z)	1408	1.14	Tetracosane	2400	0.48
Neryl acetone	1434	1.03	Pentacosane	2500	2.65
α-humulene	1452	0.90	Hexacosane	2600	1.29
β-ionone (E)	1483	1.22	Nonacosane	2900	2.43
Chemical Class					
Monoterpene hydrocarbons	2.15				
Oxygenated monoterpenes	2.92				
Sesquiterpene hydrocarbons	2.69				
Oxygenated sesquiterpene	21.22				
Diterpenes	19.09				
Hydrocabons	11.54				
Fatty acid	17.80				
Alcool	10.06				
Aldehyde	4.08				
Others	0.83				

Table 2: Inhibition diameter of essential oil of *Senecio giganteus*

Microbial strains	Controls			Dilution			
	CN	CTX	CS	1	1/2	1/5	1/10
Staphylococcus aureus ATCC 25923	0	18	18	8	10	12	11
Echerichia coli ATCC 25922	25	33,5	15	12	11	9	11
Klebsiella pneumonia ATCC 70060	17	19	12	11	10	9	9,5
Shigella sp.	30	14	15	14	13	11,5	11
Pseudomona aeruginosa ATCC 27853	26	18	15	9	8	7,5	9
Candida albicans ATCC 10231	0	0	0	0	0	0	0

CN = gentamicine, CTX = cefotaxime, CS = colistin sulfate

controversial between x = 5 and (x = 10)[24-25,27], while x = 5 is confirmed for the genus *Senecio*[28].

CONCLUSION

The chemical analysis of the essential oil of *Senecio giganteus* show that the oil contains 40 compounds when the Hexadecanoic acid is the major constituent (17.80%), and reported that the oil is rich of oxygenated sesquiterpenes. This result is differing from the other species of genus *Senecio*. The testing of the antimicrobial activity of essential oils of *S. giganteus* shows that the oil has a moderate antibacterial activity and it was inactivated against yeast. The karyological study of *S. giganteus* based on chromosome counting, allows us to determine a tetraploid with 2n = 4x = 20 + 2B, with a basic chromosome number x = 5 and this result are reported for the first time in Algeria. This study thus reflects that *Senecio giganteus* could be considered as a potential natural source of oxygenated sesquiterpenes and the karyological results can help in the classification of the species and the genus *Senecio*.

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Competing interests

The authors declare that they have no competing interests

REFERENCES

1. Jeanmonod D, Schlüssel A and Gamisans J. *Asteraceae* -II. In Compléments au Prodrome de la Flore Corse. Ed. Conservatoire et Jardin Botaniques. Genève, 2004, 256 p.
2. Pelser PB, Bertil N, Joachim WK and Linda EW. An ITS phylogeny of tribe *Senecioneae* (*Asteraceae*) and a new delimitation of *Senecio* L. *Taxon* 2007; 56(4): 1077-1104.
3. 3.Quézel P et Santa S. Nouvelle Flore de l'Algérie et des régions désertiques méridionales, Tome II, CNRS, Paris, 1963, 963p.
4. Nordenstam B, Pelser PB, Kadereit JW and Linda E. Watson : Chapter 34, Senecioneae, S. 503-525, In : Victoria Ann Funk, A. Susanna, T. F. Stuessy & R. J. Bayer (Hrsg.) : Systematics, Evolution, and Biogeography of *Compositae* ; *International Association for Plant Taxonomy* : Vienna, 2009.
5. Hammond GB, Fernandez ID, Villegas LF and Vaisberg AJ. A survey of traditional medicinal plants from the Callejo´n de Huaylas, Department of Ancash, Peru. *Journal of Ethnopharmacology* 1998; 61(1): 17-30.
6. Paume Marie Claude. (2009). Sauvages et Toxiques : Plantes des bois, des prés et des jardins. *Edisud*, Aix en Provence, 2009, 255p.
7. El-Shazly A, Doral G and Wink M. Chemical composition and biological activity of essential oils of *Senecio aegyptitus* var. discoideus Boiss. *Verlag der Z. Naturforsc C* 2002; 57: 434-439.
8. Lawal AO and Oyedeji AO. Chemical composition of the essential oils of the flowers, leaves and stems of two *Senecio polyanthemoides* Sch. Bip. samples from South Africa. *Molecules* 2009; 14: 2077-2086.
9. Lawal AO and Oyedeji AO. Volatile constituents of *Senecio pterophorus* (African Daisy) DC. from South Africa. *Natural Product Communications* 2010; 5: 1811-1814.
10. Üçüncü O, Kahriman N, Terzioğlu S, Karaoğlue SA and Yayli N. Composition and antimicrobial activity of the essential oils from flowers of *Senecio othonnae*, *S. racemosus*, and *S. nemorensis*. *Nattural Product Communications* 2010; 5(5): 831-834.
11. Arrabal C, Garcia FM, Arraiza MP and Garcia SG. Chemical composition of essential oil of *Senecio coincyi*an endemic species of the central Iberian Peninsula. *Nattural Product Communications* 2011; 6: 123-126.
12. Joshi RK. GC /MS analysis of the essential oil of *Senecio belgaumensis* flowers. *Natural product communications* 2011; 6(8): 1145-1146.
13. Lograda T, Ramdani M, Chalard P, Gharzouli R, Feguiredo G, Chalchat JC. (2012). Essential oil and antimicrobial activity of *Senecio perralderianus*. *International Journal of Medicinal and Aromatic Plants* 2012; 2: 632-637.
14. Chibani S, Gherboudj W, Kabouche A, Touzani R, Aburjai T and Kabouche Z. GC-MS Analysis of *Senecio giganteus* Desf. from Algeria *Journal of Essential Oil-Bearing Plants* 2013; 16(1): 123-125.
15. Lone SH, Bhat KA, Bhat HM, Majeed R, Anand R, Hamid A and Khuroo MA. Essential oil composition of *Senecio graciliflorus* DC: comparative analysis of different parts and evaluation of antioxidant and cytotoxic activities. *Phytomedicine* 2014; 21(6): 919-925.
16. Xiao KJ, Wang WX, Dai JL, Zhu L. Anti-inflammatory activity and chemical composition of the essential oils from *Senecio flammeus*. *EXCLI Journal* 2014; 13: 782-791.
17. Andreani S. (2014). Valorisation d'espèces envahissantes des genres *Xanthium* et *Senecio* : Caractérisation, variabilité chimique et activités des huiles essentielles. Thèse de Doctorat, *Université de Corse-Pascal Paoli*, 2014, 264p.
18. Andreani S, Paolini J, Costa J and Muselli A. Essential-oil composition and chemical variability of *Senecio vulgaris* L. from Corsica. *ChemBiodivers* 2015; 12(5):752-66.
19. Sharma P and Shah GC. Composition and antioxidant activity of *Senecio nudicaulis* Wall.ex DC. (*Asteraceae*): a medicinal plant growing wild in Himachal Pradesh, India. *Nat Prod Res* 2015; 29(9): 883-886.
20. Mezache N, Derbre S, Akkal S, Laouer H and Seraphin D. Richomme, Pascal Fast counter current chromatography of n-butanolic fraction from *Senecio giganteus* (*Asteraceae*). *Natural Product Communications* 2009; 4(10): 1357-1362.
21. Mishra D, Bisht G, Mazumdar PM and Sah SP. Chemical composition and analgesic activity of *Senecio rufinervis* essential oil. *Pharm Biol* 2010; 48(11): 1297-301.
22. Perez C, Agnese AM and Cabrera JL. The essential oil of *Senecio graveolens* (*Compositae*): chemical composition and antimicrobial activity tests. *Journal of Ethnopharmacology* 1999; 91-96.
23. Singh R, Ahluwalia V, Singh P, Kumar N, Prakash Sati O and Sati N. Antifungal and phytotoxic activity of essential oil from root of *Senecio amplexicaulis* Kunth. (*Asteraceae*) growing wild in High Altitude Himalayan Region. *Natural Product Research* 2015; 30(16): 1875-1879.
24. Ornduff R, Raven PH, Kyhos DW and Kruckeberg AR. Chromosome numbers in *Compositae*. III. *Senecioneae. American Journal of Botany* 1963; 50: 131-139.
25. Turner BL and Lewis WH. Chromosome numbers in the *Compositae*. IX. African species. *Journal of South Africa Botany* 1965; 31: 207-217.
26. Lawrence ME. *Senecio* L. (*Asteraceae*) in Australia: chromosome numbers and the occurrence of polyploidy. *Aust J Bot* 1980; 28:151-165.

27. Lawrence ME. *Senecio* L. (*Asteraceae*) in Australia: nuclear DNA amounts. *Aust J Bot* 1985; 33: 221-232.
28. Lopez Mariana G, Wulff AF, Poggio L and Xifred CC. South African fireweed *Senecio madagascariensis* (*Asteraceae*) in Argentina: relevance of chromosome studies to its systematics. *Botanical Journal of the Linnean Society* 2008; 158: 613-620.
29. Lopez Mariana G, Xifred CC, Poggio L and Wulff AF. Deep cytogenetics analysis reveals meiotic recombination depletion in species of *Senecio* (*Asteraceae*). *Botanical Studies* 2013; 54(1): 1-11.
30. Lopez Maruiana G, Wulff AF and Xifreda CC. Chromosome contribution to Andean Polyploid Species of *Senecio* (*Asteraceae*), from Argentina, Caryologia 2002; 55(1): 27-35.
31. Masada Y. Analysis of Essential Oils by Gas Chromatography and Mass spectrometry.: Wiley / Halsted, New York, 1976, 334 p.
32. NIST (2002). Automated Mass Spectral Deconvolution and Identification System, Ver. 2.1, DTRA, 2002.
33. McLafferty FW, Stauffer DB (2004). Wiley Registry of Mass Spectral Data. *6th* electronic Ed with NIST02, John Wiley & Sons, New York, NY, USA, 2004.
34. Adams RP (2007). Identification of essential oil components by gas chromatography/mass spectroscopy. *(4th Ed.)* Carol Stream. IL, Allured Publishing Corporation. USA, 2007, 804p.
35. Niemeyer HM. Composition of the essential oils from five aromatic species of *Asteraceae*. *Journal of Essential Oil Research* 2007; 21: 350-352.
36. El Hawary SS, Galal AE, Yousif MF and Kirollos FN. GC-MS and bioactivity of the essential oil of *Senecio rowleyanus* Jacobs. *Pharmacognosy Magazine* 2008; 4(16): 273-277.
37. Bisht CMS, Melkani AB, Dev V and Beauchamp PS. 1,10-β-epoxy-6-oxofuranoeremophilane and other terpenoids from the essential oil of *Senecio roylanus* DC. *Journal of Essential Oil Research* 2011; 23: 102-104.
38. Ouchbani T, Ouchbani S, Boufhid R, Merghoub N, Guessous AR, El Mzibri M and Essassi E M. (2011). Chemical composition and antiprolative activity of *Senecio leucanthemifolius* Poiret. Essential oil. *Journal of Essential Oil Bearing Plant* 2011; 14(6): 815-819.
39. Benites J, Bravo F, Rojas M, Fuentes R, Moiteiro C and Venâncio F. Composition and antimicrobial screening of the essential oil from the leaves and stems of *Senecio atacamensis*. Phil. from Chile. *Journal of the Chilean Chemical Society* 2001; 56(2): 712-714.
40. Navadha J, Sah GC and Devendra M. Gc-Ms Analysis and Antimicrobial Activity of Essential Oil of *Senecio Pedunculatus*. *Journal of Applied Chemistry* 2013; 6(3): 49-51.
41. Pumaylle KO, Paredes L, Dagnith LBL, Silva Paz RJ. Extraction, characterization and evaluation of antibacterial activity of essential oil of *Senecio graveolens* Wedd (Wiskataya). *Scientia Agropecuaria* 2007; 3: 291-302.
42. Lopez S, Beatriz L, Aguero MB, Lopez ML, Hadad M, Zygadlo J, Caballero D, Stariolo R, Suero E, Feresin GE, Tapia A. Chemical composition, antibacterial and repellent activities of *Azorella trifurcata, Senecio pogonias*, and *Senecio oreophyton* essential oils. *Arabian Journal of Chemistry* 2014; doi:10. 1016/j.arabjc. 2014.11.022.
43. El-Amier YA, Abdelghany AM, Abed Zaid A. Green Synthesis and Antimicrobial Activity of *Senecio glaucus* -Mediated Silver Nanoparticles. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 2014; 5(5): 631-642.
44. Lopez MG, Wulfe AF, Poggio L and Xifreda CC. Chromosome numbers and meiotic studies in species of *Senecio* (*Asteraceae*) from Argentina. *Botanical Journal of the Linnean Society* 2005; 148(4): 465-474.
45. Watanabe, K. 2002: Index to chromosome numbers in the *Asteraceae*. http://www.asteraceae.cla.kobe-u.ac.jp/index.html.
46. Oberprieler C and Vogt R. Chromosome numbers of North African phanerogams. II. *Willdenowia* 2002; 23: 211-238.
47. Humphries CJ, Murray BG, Bocquet G and Vasudevan KN. Chromosome numbers of phanerogams from Morocco and Algeria. *Bot. Not* 1978; 131: 391-406.
48. Alexander JCM. The mediterranean species of *Senecio* sections *Senecio* and *Delphinitolius*. *Notes Roy. Bol. Gard. Edinburgh* 1979; 37: 387-428.
49. Vogt R, Oberprieler C. Chromosome numbers of North African phanerogams. VIII. More counts in *Compositae*. *Willdenowia* 2008; 38: 497-519.
50. Abbott RJ, James JK, Forbes DG and Comes HP. Hybrid origin of the Oxford Ragwort, senecio squalidus L: morphological and allozyme differences between *S. squalidus* and *S. rupestries* Waldst. and Kit. *Watsonia* 2002; 24: 17-29.