Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(5): 2593-2598
Received: 25-07-2020
Accepted: 27-08-2020

Becerra V
Cátedra Toxicología, Facultad de Ciencias Médicas, Universidad Católica de Cuyo Sede San Luis. Felipe Velázquez 471 (5700) San Luis, Argentina

Gambaro R
Instituto de Genética Veterinaria "Ing. Fernando N. Dulout" (IGEVET-CONICET). Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata. 60 y 118 S/N (1900) La Plata, Buenos Aires, Argentina

Padula G
Instituto de Genética Veterinaria "Ing. Fernando N. Dulout" (IGEVET-CONICET). Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata. 60 y 118 S/N (1900) La Plata, Buenos Aires, Argentina

Seoane A
Instituto de Genética Veterinaria "Ing. Fernando N. Dulout" (IGEVET-CONICET). Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata. 60 y 118 S/N (1900) La Plata, Buenos Aires, Argentina

Palacios A
Cátedra Bioquímica, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata. 60 y 118 S/N (1900) La Plata, Buenos Aires, Argentina

Zeinsteger P
Cátedra Bioquímica, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata. 60 y 118 S/N (1900) La Plata, Buenos Aires, Argentina

Corresponding Author:
Zeinsteger P
Cátedra Bioquímica, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata. 60 y 118 S/N (1900) La Plata, Buenos Aires, Argentina

Effects of *Senecio selloi* (Spreng.) DC flower extract on mitochondrial membrane peroxidation and enzyme activities

Becerra V, Gambaro R, Padula G, Seoane A, Palacios A and Zeinsteger P

DOI: https://doi.org/10.22271/phyto.2020.v9.i5aj.12734

Abstract
Ethnobotanical use of plants may include poisonous species. *Senecio* (Asteraceae) is used as medicinal around the world. *Senecio selloi* (Spreng.) is widespread in Argentina. Purposes of this study were to investigate phytochemistry and *in vitro* activity of a methanolic extract of plant flowers on mitochondrial membrane peroxidation and enzyme activities. Chemiluminescence was used to test peroxidative effects, and thiazolyl blue tetrazolium bromide test (MTT) for cell viability. Results indicate presence of flavonoids and pyrrolizidine alkaloids. Regarding chemiluminescence, extract showed protective effect against peroxidation, while MTT demonstrated lower activities of mitochondrial dehydrogenases and cytosolic reductases, effects that were attributed to flavonoids and pyrrolizidine alkaloids, respectively. When ingested, pyrrolizidine alkaloids undergo hepatic biotransformation that cause damage to cells. On the contrary, toxicity does not take place when there is no metabolization. These situations may explain the ethnobotanical use of some poisonous senecios in human and veterinary medicine.

Keywords: Senecio, poisonous, chemiluminescence, MTT assay, ethnobotanical use

Introduction
Since ancient times, humans used natural resources such as minerals, animals, and plants. Considering the latter, they observed animals feeding on certain species and realized that some caused them to heal from diseases, while others caused their death. Consequently, plants were classified as medicinal or poisonous, knowledge that is still used nowadays.
Indigenous populations from many parts of the world have the knowledge that a same plant species has both beneficial and detrimental effects on health, considering the dose and the route of entry into the body, among other factors. Shamans from South America, for example, know that plants of the Solanaceae family have hallucinogen properties, such as *Brugmansia* spp. [1, 2], which neglected use may cause death [3]. In Europe, the use of such type of plants is known as well, and many species with these properties can be mentioned. As an example, *Conium maculatum* or "poison hemlock" is associated to both medicinal and poisonous uses through history, being the most famous and tragic case the so-called death of Socrates [4], a story which authenticity is still under review [5].
Humans may be exposed to poisonous plants when they voluntary intake species that are incorrectly considered as medicinal (as part of folk remedies), or when the consume dangerous plants that are mistaken with edible species [6]. Among them, some species of *Senecio* (Asteraceae) exert toxic effects on different organs in both humans and animals due to the presence of pyrrolizine alkaloids (PAs) [7]. PAs have a pyrrolizidine group in their structure and are toxic to the liver, lungs, kidneys; they are also genotoxic compounds. In particular, 1,2-dehydropyrrolizidine esters are metabolized by P450 enzymes to pyrrolic esters, compounds which are unstable electrophilic alkylating molecules [8] and some studies suggest that oxidative stress is involved in the hepatotoxic effects of them [9]. Oxidative stress is a condition caused by the excessive production of free radicals, in contrast to not enough activity of antioxidants by defense systems of the organism [10].
Interestingly, a few species of senecios are used as medicinal plants in folk medicine, some of them have demonstrated potential benefits under experimental conditions. For example, *S. brasiliensis* (Spreng.) Less, *S. sertularioides* DC and *S. chrysanthemoides* DC may be used to treat wounds [11, 12]. *S. serratuloides* extracts also exert antihypertensive effects [13] and *S. bifrae* Oliv. & Hiern has potential use to treat atherosclerosis, metabolic diseases such as hyperlipidemia and diabetes mellitus and neurodegenerative disorders [14]. *S. tenuifolius* DC has demonstrated antibacterial activity [15], while *S. smithioides* Cabrera possess antiplasmodial compounds [16].

Senecio formosus Kunth, present in some countries of South America, has been used as a folk remedy to heal wounds and ingested as infusions [17]. Regarding the ethnoveterinary use of *Senecio* plants, *S. scandens* Buch-Ham ex D. Don is used to treat constipation, diarrhea, and to heal skin diseases in domestic animals by veterinarians from some provinces of China [18].

Senecio selloi (Spreng.) DC (SS) is a subshrub of 1-meter height, bushy in the base, with striated stems which are leafy in the lower half and with less leaves in the upper half, glandulous pubescence. Leaves are oblong lanceolate-spatulate, obtuse in the apex, margins are serrated-crenate, heavily glandulous-pubescent in both sides, 40-120 mm long and 12-35 mm wide. Capitulum are long pedicellate, with many daisy-like yellow flowers (Fig. 1). The plant is widespread from south Brazil to northeastern Argentina. It is classified as poisonous to cattle and other species and have caused deaths in ruminants in Argentina [19]. There are no data regarding its ethnobotanical use in the country.

Fig 1: *Senecio selloi* (Spreng.) DC from Buenos Aires, Argentina

Considering this background, we wanted to determine if *Senecio selloi* could have similar properties to those senecios mentioned above. Purpose of this study were to investigate the phytochemistry of SS and the *in vitro* activity of different concentrations of a methanolic extract on mitochondrial membrane peroxidation and enzyme activities, to determine whether if they affect these cell structures and processes.

Material and Methods

Chemicals

All solvents and chemicals used in this study were of analytical grade. Rutin and retrorsine standards were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Plant material

Plants were collected during November 2018 from Brandsen Department, Buenos Aires, Argentina. Botanical identification was performed by Ing. Agr. Néstor Bayón, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata. Boucher samples were deposit at the Laboratorio de Bioquímica, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata.

Extract preparation

Fifteen grams of air-dried SS flowers were milled until a cotton-like material was obtained, methanol was used as the extraction solvent (200 ml), and extraction was performed by means of a magnetic stirrer at room temperature during 24 hours under dim light. Later, the extract was filtered using vacuum filtration and then rotaevaporated until dryness using a Senko® equipment. A residue of 4.0607 g was obtained, which was re-suspended in 100 ml 1% HCl and then put in a separation funnel. Chloroform was added to extract the lipidic compounds (chlorophyll and waxes), which were discarded. The remaining acidic solution was alkalinized using 12 ml 20% NH_3 (pH 10-12), a color change of the solution was observed at this point. Final separation consisted in the addition of 250 ml chloroform: methanol (4:1). The chloroform-methanol extract was filtered on anhydrous sodium sulphate and then rotaevaporated until dryness. A residue of 42 mg was obtained.

Phytochemical determinations

For the phytochemical analysis of the extract, the following qualitative determinations were performed: flavonoids (zinc hydrochloride reduction test, Shinoda's test), tannins (ferric chloride test), lipids (iodine reaction), carbohydrates (phenol 5% + concentrated H_2SO_4), steroids (acetic anhydride + concentrated H_2SO_4, Liebermann-Burchard's reaction), anthraquinones (sodium hydroxide test, Borntrager's test), pyrrolizidine alkaloids (Ehrlich's reagent), cardenolides (Legal's reagent), and leucoanthocyanins (concentrated HCl + amyl alcohol, Rosenheim's reaction) [20, 21].

Quantification of total flavonoids

A modification of Maksimovic *et al*. (2005) technique was performed for the determination of total flavonoids [22]. For this, 0.1 ml of the extract with 1.4 ml deionized water and 0.5 ml flavonoid reactive (133 mg ferric trichloride and 400 mg sodium acetate diluted in 100 ml of a solvent prepared with 140 ml methanol, 50 ml distilled water, and 10 ml acetic acid) were incubated at room temperature for 5 min. Absorbance was determined at 430 nm. Total flavonoid content was calculated as mg rutin equivalent per g dry weight. Prior to this, a calibration curve was prepared with decreasing concentrations of rutin diluted in methanol (1, 0.500, 0.250, 0.125, and 0.0625 mg of rutin).

Quantification of total pyrrolizidine alkaloids

One milliliter of the methanolic extract of SS was put in a 20 ml test tube and evaporated until dryness at 75 ºC on a hot plate. Then, 1 ml of 5% ascorbic acid was added to the residue, with the addition of 150 µl 5% sodium nitroprusside, and heated during 1 min at 75 ºC. Ehrlich´s reagent (150 µl) was added and heated again a 75 ºC for 1 min; a magenta color was observed. The same procedure was performed with a standard solution of retrorsine (500 µg/1 ml methanol, Sigma-Aldrich), and for a negative control (prepared with ascorbic acid, sodium nitroprusside and Ehrlich´s reagent, without SS extract). As little as 5 µg of most unsaturated PAs can be measured by this method [23]. After all the tubes reached room temperature, 4 ml acetone were added to each one. Absorbance was determined at 565 nm. Total concentration of pyrrolizidine alkaloids was obtained considering the concentration of the standard solution of retrorsine and its absorbance, and the absorbance of the SS extract, according to the following equation:

$$\text{Total concentration of PA} = \frac{\text{ABS SS Extract x Concentration of retrorsine standard (mg/ml)}}{\text{ABS of retrorsine standard}}$$

Where:
ABS: Absorbance
SS: *Senecio selloi*

Thin layer chromatography (TLC)
For TLC, aliquots of SS methanolic extract were applied on silica gel 60 plates. A retrorsine standard (Sigma-Aldrich) was also applied to each plate. For the development, three mobile phase systems were used as follows: S1 dichloromethane: methanol: ammonia 25% (85:14:1), S2 chloroform: acetone: diethylamine (50:40:10), S3 toluene: ethyl acetate: diethylamine (70:20:1) [24]. After development, plates were sprayed with concentrated H_2O_2, heated at 80 °C for 15 min, then sprayed with Ehrlich's reagent, and heated again for another 15 min at 80 °C.

Animals and membrane preparation
Female Wistar AH/HOK rats, 7 weeks-old, weighing 120-137 g, from the Laboratory Animal Facility, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, were used. All rats were fed commercial rat chow and water ad-libitum. The rats were sacrificed by cervical dislocation and the liver was rapidly removed, cut into small pieces and extensively washed with 0.15 M NaCl. A 30% (w/v) homogenate was prepared in a 0.25 M sucrose solution, 10 mM Tris-HCl pH 7.4 using a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 3,000 G for 5 min; pellets were discarded, and the supernatant was centrifuged at 10,000 G for 10 min to obtain mitochondrial pellets. The pellets were suspended in solution a (0.25 M sucrose solution, 10 mM Tris-HCl pH 7.4, 0.01 M PMFS). All operations were performed at 4 °C and under dim light.

Peroxidation of rat liver mitochondria
Chemiluminescence and peroxidation were initiated by adding ascorbate to mitochondria [25]. Mitochondria (0.5 mg mitochondrial protein) with addition of SS extract (0.025, 0.1, 0.4 and 0.8 µg) were incubated at 37 °C with 0.01 M phosphate buffer pH 7.4, 0.4 mM ascorbate, final volume 2 ml. Phosphate buffer is contaminated with sufficient iron to provide the necessary ferrous or ferric iron (final concentration in the incubation mixture was 2.15 µM) for peroxidation [26]. Mitochondria preparations, which lacked ascorbate, were carried out simultaneously. Membrane light emission was determined over a 180 min period, chemiluminescence was recorded as counts per minute (cpm) every 10 min, and the sum of the total chemiluminescence was used to calculate cpm/mg protein. Chemiluminescence was measured as counts per min in a liquid scintillation analyzer Packard 1900 TR equipment with a program for chemiluminescence.

Protein determination
Proteins were determined by the method of Lowry *et al.* (1951) using BSA as standard [27].

Mitochondrial enzyme activities
Thiazolyl blue tetrazolium bromide assay (MTT) is used to determine the respiratory activity of mitochondrial succinate-tetrazolium reductase system, which converts the yellow tetrazolium salt into a blue formazan dye, assessing cell viability [28, 29]. Bovine lymphocytes were stimulated with 100 µg/ml phytohemagglutinin and cultured in Ham F12 supplemented with fetal bovine serum and antibiotics at 37 °C during 48 h. Treatments were performed on the last 24 h. Measurement of absorbance was performed at 550 nm with a microplate spectrophotometer (Multiskan™ Go, Thermo Fisher Scientific). Results were expressed as the mean ± SEM of absorbance (arbitrary units, AU) from three independent experiments. Treatments were: 1) negative control 2) positive control (ethanol 10%) 3) methanol controls (3.3 µl/ml, 6.6 µl/ml, 10 µl/ml, 13.3 µl/ml and 26.6 µl/ml); and SS extracts 4) T1: 25 µg/ml 5) T2: 50 µg/ml 6) T3: 100 µg/ml 7) T4: 200 µg/ml and 8) T5: 400 µg/ml.

Results
Phytochemical analysis of SS extract
Table 1 shows the results of the phytochemical analysis of SS flower extract. Qualitative chemical determinations were performed to assess the presence of those constituents that exert anti-oxidative or toxic properties.

Table 1: Qualitative analysis of SS methanolic extract. Chemical reactions were used as follows:

Shinoda	+
$FeCl_3$	+
Iodine	+
Phenol 5% + concentrated H_2SO_4	+
Liebermann-Burchard	+
Bornträger	-
Ehrlich	+
Legal	-
Rosenheim	-

Table 1. Qualitative analysis of SS methanolic extract. Chemical reactions were used as follows: Shinoda for flavonoids, $FeCl_3$ for tannins and phenolic OH, iodine for lipids, phenol 5% + concentrated H_2SO_4 for carbohydrates, Liebermann-Burchard for steroids, Bornträger for anthraquinones, Ehrlich for pyrrolizidine alkaloids, Legal for cardenolides, and Rosenheim for leucoanthocyanins.

Thin layer chromatography
Figures 2a, 2b and 2c show the chromatograms of SS extract. For S1 mobile phase system, a purple spot was observed for the extract, which Rf was similar to that of the retrorsine standard; other spots were observed as well. In the case of S2, only retrorsine spots were observable, as well as for S3. Rf values are shown in Table 2.

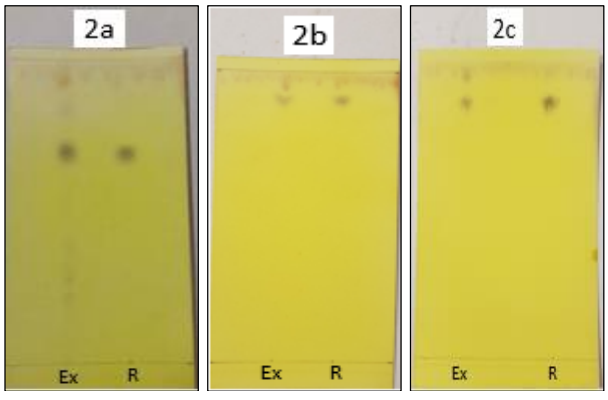

Fig 2a: S1 chromatogram, 2b: S2 chromatogram, 2c: S3 chromatogram. Ex: SS extract, R: retrorsine standard

Table 2: Rf values of SS extract and retrorsine standard for the three mobile phase systems

Compound	S1	S2	S3
SS extract	0.69	0.88	0.86
Retrorsine standard	0.68	0.89	0.87

Total flavonoid concentration in the extract
A total of 125 µg was determined for the extract.

Total pyrrolizidine alkaloid concentration in the extract
A total of 641 µg was determined for the extract. Considering the SS extract total residue weight (42 mg) and the weight of flowers used in the preparation of the extract (15 g), total PAs concentration represents 1.5% and 0,004%, respectively.

Light emission of rat liver mitochondria during lipid peroxidation. Chemiluminescence
The incubation of rat liver mitochondria in the presence of ascorbate-Fe^{++} resulted in the peroxidation of membranes, as evidenced by emission of light (chemiluminescence). After incubation of mitochondria in an ascorbate-Fe^{++} system at 37 ºC for 180 min, the cpm originated from light emission was lower in the SS extract group than in the control group and was concentration dependent. Figure 3 shows the total light emission obtained from SS extract group and control group. The values were from 977±206 (total chemiluminescence cpm x 10^{-3}) in the control group to 474±79 (total chemiluminescence cpm x 10^{-3}) with the addition of 0.8 µg SS extract/mg of protein and statistically significant $p < 0,007$.

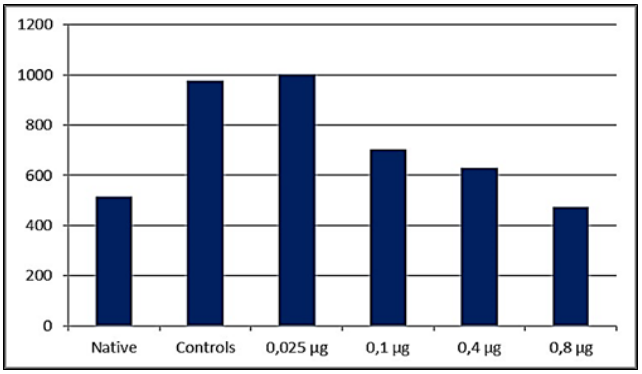

Fig 3: Peroxidation (total chemiluminescence) of rat liver mitochondria with different amount of SS extract. Data are given as the mean ±SD of six experiments. Native means system without ascorbic-Fe^{++}

Mitochondrial enzyme activities
For all the cases, viability of cells (MTT assay) treated with SS methanolic extract is lower than their controls, except for T4, which are almost equal (Fig. 4). Treatments T1, T3 and T5 showed significant differences with their respective methanol controls and with negative control. Although methanol decreases cell viability, SS methanolic extract increments this effect in a statistically significant manner. SS flower extract significantly decreases cell viability ($p<0.001$) with concentrations starting from 100 µg/ml, as shown by lower activities of mitochondrial dehydrogenases and cytosolic reductases (100 µg/ml 18.6%; 200 µg/ml 36.9%).

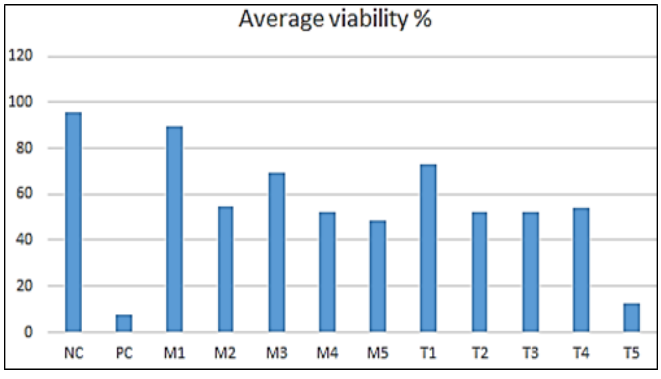

Fig 4: MTT assay of SS methanolic extract

NC: negative control; PC: positive control; M1: methanol 3.3 µl/ml, M2: methanol 6.6 µl/ml, M3: methanol 10 µl/ml, M4: methanol 13.3 µl/ml, M5: methanol 26.6 µl/ml; T1: SS extract 25 µg/ml, T2: SS extract 50 µg/ml T3: SS extract 100 µg/ml, T4: SS extract 200 µg/ml, T5: SS extract 400 µg/ml.

Discussion
There is a consensus that toxicity of a certain toxicant depends on the dose and the route of entry. On the other hand, considering organisms, toxic effects also depend on age, sex, as well as idiosyncrasy [30]. In plants, the variety of compounds with pharmacological and toxicological potential in just one species is considerable. It has been demonstrated that some *Senecio* species contain considerable amounts of flavonoids and phenolic compounds, such as *S. brasiliensis* [10], which are associated to its benefits for treating skin wounds [11], hypertension [13], atherosclerosis and hyperlipidemia [31]. *Senecio chrysanthemoides* DC flowers are crushed and applied on wounds as antiseptic, whereas root powder is used against rheumatic pain [12]. Our results show that *Senecio selloi* has *in vitro* antioxidant effects, as demonstrated by the protective effect on peroxidation of mitochondria membranes. *In vitro* peroxidation studies are useful for the elucidation of possible mechanism of peroxide formation *in vivo* [32]. The antioxidant property of our methanolic extract could be associated to the presence of flavonoids. It is well-known that polyphenolic compounds, like flavonoids, act as scavengers of reactive oxygen species, which are implicated in skin inflammation and infection [33, 34]. These antioxidant mechanisms could be attributable to:

1) Direct scavenging of free radicals and chelating free Fe^{2+} and Cu^{2+}
2) Preventing free radical formation by inhibiting specific ROS-producing enzymes, or improving the integrity of the mitochondria membrane in stress conditions
3) Maintaining an optimal redox balance in cells by activating a range of antioxidant enzymes and non-enzymatic antioxidants [35]

Although there are no references of ethnobotanical use of SS in Argentina, the utilization of other species of the genus in folk medicine is well known [36].
Senecios mentioned above are also poisonous. *S. chrysanthemoides* DC possess Pas [37], such as integerrimine, senecionine and retrorsine, among others [38]. According to Mendoça Soares *et al*. (2019), *S. brasiliensis* contains the pyrrolizidine alkaloid integerrimine [39]. For *Senecio scandens* Buch.-Ham. ex D. Don, an herb used in skin diseases in Chinese traditional medicine [40], PAs were also identified [41]. Our findings also demonstrate the presence of two PAs in our extract, one of them being retrorsine, and the other probably senecionine. PAs do not exert toxicity until they are bioactivated by the liver, and active metabolites may cause alterations to this organ [42], as well as lungs [43] and kidneys [44]. They may also have effect against anti-oxidative processes, such as dehydromonocrotaline, the primary metabolite of monocrotaline (present in *Crotalaria* sp.), a PA that after P450 activation acts as an inhibitor of the respiratory chain complex I NADH oxidase [45]. From the results of MTT, we can state that mitochondrial enzymes such as dehydrogenases and cytosolic reductases are negatively affected by PAs in a concentration dependent manner, as demonstrated by the decrease in cell viability. These effects may contribute in turn with the toxicological properties of PAs present in SS.

Conclusion

PAs need a metabolic pathway that includes hepatic metabolization to cause damage to cells. On the contrary, when applied to the skin, such toxicity does not take place due to lack of metabolization. Considering the content of flavonoids and other polyphenolic compounds, this may explain the ethnobotanical use of some poisonous *Senecio* species in both human and veterinary medicine in some parts of the world.

Acknowledgements

This work was supported by Secretaría de Ciencia y Técnica, National University of La Plata. We would like to thank Ing. Néstor Bayón, Facultad de Ciencias Agrarias y Forestales, National University of La Plata, for the botanical identification of plant material.

References

1. Shepard GH Jr. Psychoactive botanicals in ritual, religion, and shamanism. In: Ethnopharmacology, Elisabetsky E, Etkin N. (Eds.). Encyclopedia of Life Support Systems (EOLSS); Theme 6.79. Oxford, UK: UNESCO/Eolss Publishers, 2005.
2. Torres CM. The use of psychoactive plants by ancient indigenous populations of North Andes. J Psychedelic Stud. 2019; 3(2):198-211. Doi: 10.1556/2054.2018.015.
3. Kerchner A, Farkas A. Worldwide poisoning potential of *Brugmansia* and *Datura*. Forensic Toxicol. 2020; 38:30-41. Doi: 10.1007/s11419-019-00500-2.
4. Reynolds T. Hemlock alkaloids from Socrates to poison aloes. Phytochemistry 2005; 66:1399-406.
5. Dayan A. What killed Socrates? Toxicological considerations and questions. Postgrad Med J. 2009; 85(999):34-37. Doi: 10.1136/pgmj.2008.074922.
6. Diaz GJ. Toxicosis by plant alkaloids in humans and animals in Colombia. Toxins. 2015; 7:5408-5416. Doi: 10.3390/toxins7124892.
7. Tokarnia CH, Farias M, Barbosa JD, Vargas P, Döbereiner J. Plantas/micotoxinas que afetam o fígado. In: Plantas tóxicas do Brasil, para animais de producción, 2º ed., Heliantus, Rio de Janeiro, 2012.
8. Chen T, Mei N, Fu PP. Genotoxicity of pyrrolizidine alkaloids. J Appl Toxicol. 2010; 30:183-196.
9. Yan X, Kang H, Feng J, Yang Y, Tang K, Zhu R *et al*. Identification of toxic pyrrolizidine alkaloids and their common hepatotoxicity mechanism. Int. J Mol. Sci. 2016; 17(3):318. Doi: 10.3390/ijms17030318.
10. Macedo GE, Gomes KK, Rodrigues NR, Martins IK, Wallau GDL, Carvalho NL *et al*. *Senecio brasiliensis* impairs eclosion rate and induces apoptotic cell death in larvae of *Drosophila melanogaster*. Comp Biochem Physiol C Toxicol. Pharmacol. 2017; 198:45-57. Doi: 10.1016/j.cbpc.2017.05.004.
11. Gould AN, Penny CB, Patel CC, Candy GP. Enhanced cutaneous wound healing by *Senecio serratuloides* (Asteraceae/Compositae) in a pig model. S Afr. J Bot 2015; 100:63-68. Doi: 10.1016/j.sajb.2015.05.006.
12. Kumar M, Paul Y, Anand VK. An ethnobotanical study of medicinal plants used by the locals in kishtwar, Jammu and Kashmir, India. Ethnobotanical Leaflets. 2009; 13:1240-1256.
13. Tata CM, Sewani-Rusike CR, Oyedeji OO, Gwebu ET, Mahlakata F, Nkeh-Chungag BN. Antihypertensive effects of the hydro-ethanol extract of *Senecio serratuloides* DC in rats. BMC Complement Altern Med. 2019; 19(1):52. Doi: 10.1186/s12906-019-2463-2.
14. Ajiboye BO, Ojo OA, Okesola MA, Akinyemi AJ, Talabi JY, Idowu OT *et al*. In vitro antioxidant activities and inhibitory effects of phenolic extract of *Senecio biafrae* (Oliv and Hiern) against key enzymes linked with type II diabetes mellitus and Alzheimer´s disease. Food Sci. Nutr. 2018; 6(7):1803-1810. Doi: 10.1002/fsn3.749.
15. Manubolu M, Goodla L, Ravilla S, Obulum VR. Activity-guided isolation and identification of anti-staphylococcal components from *Senecio tenuifolius* Burm. F. leaf extracts. Asian Pac J Trop Biomed. 2013; 3:191-195.
16. Mollinedo P, Vila JL, Arando H, Sauvain M, Deharo E, Bravo JA. Anti-infective assessment of *Senecio smithioides* (Asteraceae) and isolation of 9-oxoeuryopsin, a furanoeremophilane-type sesquiterpene with antiplasmodial activity. Nat Prod Res. 2016; 30(22):2594-2597. Doi: 10.1080/14786419.2015.1115994.
17. Toro González G, Rojas Villamil E, Arango Uribe G. Seneciosis. Enfermedad veno-oclusiva del hígado (EVOH) en Colombia. 1964-1996. Identificación, manejo y solución de un problema. Rev. Acad. Colomb. Cienc. Exactas Fis Nat. 1997; 21:35-56.
18. Shen S, Qian J, Ren J. Ethnoveterinary plant remedies used by Nu people in NW Yunnan of China. J Ethnobiol and Ethnomed. 2010; 6:24.
19. Odriozola E. Poisoning by plants, mycotoxins and algae in Argentinian livestock. In: Poisoning by plants, mycotoxins and related toxins (Riet-Correa F, Pfister J, Schild AL, Wierenga TL, eds.), Edit. CABI, Oxfordshire, 2011.
20. Bruneton J. Farmacognosia. Fitoquímica. Plantas medicinales. 2ª. Ed. Editorial Acribia. Zaragoza, 2001, 1100.
21. Harborne JB. Phytochemical methods. A guide to modern techniques of plant analysis. Chapman & Hall, 3th Ed., 1998, 302.
22. Maksimovic Z, Malencic D, Kovacevic N. Polyphenol contents and antioxidant activity of *Maydis stigma* extracts. Bioresour Technol. 2005; 96:873-877.
23. International Programme on Chemical Safety. Pyrrolizidine Alkaloids. Environmental Health Criteria 80. WHO, Geneva, 1988.
24. Hussein KH, Enas JK. Extraction, isolation and characterization of pyrrolizidine alkaloids present in *Senecio vulgaris* Linn grown in Iraq. J Pharmacog Phytochem. 2016; 5(6):28-37.
25. Wright JR, Rumbaugh RC, Colby HD. The relationship between chemiluminescence and lipid peroxidation in rat hepatic microsomes. Arch Biochem Biophys. 1979; 192:344-351.
26. Tadolini B, Hakim G. The Mechanism of Iron (III) Stimulation of Lipid Peroxidation. Free Radic Res. 1996; 25:221-227.
27. Lowry OH, Rosebrough NJ, Farr AL. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951; 193:265-75.
28. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assay. J Inmunol Methods. 1983; 65:55-63.
29. Robb J, Norval M, Neill WA. The use of tissue culture for the detection of mycotoxins. Lett Appl Microbiol. 1990; 10:161-165.
30. Robinson L. Factors That Modify Toxicity. In: A Practical Guide to Toxicology and Human Health Risk Assessment, L. Robinson (Ed.), 2020.

Doi: 10.1002/9781118881880.ch5.
31. Ajiboye BO, Edobor G, Ojo AO, Onikanni SA, Olaranwaju OI, Muhammad NO. Effect of aqueous leof extract of *Senecio biafrae* on the hyperglycaemic and serum lipid profile of alloxan-induced diabetic rats. Int. J of Dis Disorders. 2014; 2(1):59-64.
32. Rauchová H, Kalous M, Drahota Z, Koudelová J, Mourek J. Lipid peroxidation in isolated membranes of cerebral cortex, heart and kidney. Physiol Res. 1993; 42(5):323-327.
33. Spooner R, Yilmaz O. The role of oxygen reactive species in microbial persistence and inflammation. Int. J Mol. Sci. 2011; 12(1):334-352. Doi: 10.3390/ijms12010334.
34. Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive oxygen species in inflammation and tissue injury. Antioxid Redox Signal. 2014; 20(7):1126-1167. doi:10.1089/ars.2012.5149.
35. Surai PF. Silymarin as a Natural Antioxidant: An Overview of the Current Evidence and Perspectives. Antioxidants (Basel). 2015; 4(1):204-47.
36. Scarpa GF, Rosso CN, Anconatani L. Etnobotánica médica de grupos criollos de Argentina: reconocimiento, análisis y puesta en valor de los datos presentados por el gobierno argentino en la exposición universal de París en 1889. Darwiniana 2016; 4(2):291-315. Doi: 10.14522/darwiniana.2016.42.714.
37. Pelser PB, De Vos H, Theuring C, Beuerle T, Vrieling K, Hartmann T. Frequent gain and loss of pyrrolizidine alkaloids in the evolution of *Senecio* section *Jacobea* (Asteraceae). Phytochemistry. 2005; 66(11):1285-1295.
38. Roeder E, Wiedenfeld H. Pyrrolizidine alkaloids in medicinal plants of Mongolia, Nepal and Tibet. Pharmazie 2009; 64:699-716. Doi: 0.1691/ph.2009.9653.
39. Mendoça Soares S, Domigues R, Baldo Gaspar E, Azevedo Dos Santos P, Marques Canuto K, Pelegrine Minho A *et al. In vitro* ovicidal effect of a *Senecio brasiliensis* extract and its fractions on *Haemonchus contortus*. BCM Vet Res. 2019; 15:99. Doi: 10.1186/s12917-019-1843-7.
40. Jin B, Liu Y, Xie J, Luo B, Long C. Ethnobotanical survey of plant species for herbal tea in a Yao autonomous county (Jianghua, China): results of a 2-year study of traditional medicinal markets on the Dragon Boat Festival. J Ethnobiol Ethnomed. 2018; 14:58. Doi: 10.1186/s13002-018-0257-0.
41. Xiong A, Yang L, Ji L, Wang Z, Yang X, Chen Y *et al*. UPLC-MS based metabolomics study on *Senecio scandens* and *S. vulgaris*: an approach for the differentiation of two *Senecio* herbs with similar morphology but different toxicity. Metabolomics. 2012; 8(4):614-623. Doi: 10.1007/s11306-011-0354-8.
42. Xu J, Wang W, Yang X, Xiong A, Yang L, Wang Z. Pyrrolizidine alkaloids: and update on their metabolism and hepatotoxicity mechanism. Liver Res. 2019; 3(3-4):176-184. Doi: 10.1016/j.livres.2019.11.004.
43. Dumitrascu R, Koebrich S, Dony E, Weissmann N, Savai R, Pullamsetti SS *et al*. BCM Pulmonary Med 2008; 8:25. Doi: 10.1186/1471-2466-8-25.
44. Hayashi Y, Lalich JJ. Renal and pulmonary alterations induced in rats by a single injection of monocrotaline. Proc. Soc. Exptl. Biol. Med. 1967; 124:392-396.
45. Mingatto FE, Dorta DJ, Dos Santos AB, Carvalho I, Da Silva CH, Da Silva VB *et al.* Dehydromonocrotaline inhibits mitochondrial complex I. A potential mechanism for hepatotoxicity of monocrotaline. Toxicon. 2007; 50(5):724-730.