World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

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Chemical composition, antimicrobial and antioxidant activities of the essential oils from *Senecio longiscapus* Bojer leaves (Asteraceae)

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World Journal of Biology Pharmacy and Health Sciences, 2021, 07(02), 009–018

Publication history: Received on 28 June 2021; revised on 02 August 2021; accepted on 04 August 2021

Article DOI: https://doi.org/10.30574/wjbphs.2021.7.2.0079

Abstract

The present work aims to study the chemical composition and the antimicrobial and antioxidant properties of the essential oil of *Senecio longiscapus* (SLEO) leaves. SLEO was extracted from fresh leaves by hydrodistillation with a yield of 3%. It is clear, yellow, with a relative density of 0.7466 at 20°C, a refractive index of 1.4959, an optical rotation of +3°47, an acid number of 1.53 and an ester number of 12.49. Gas chromatography/mass spectrometry (GC/MS) analysis of the SLEO identified 17 components, representing more than 99.09% of the overall composition. The main component of SLEO was sabinene (53.28%) and elemicin (15%), β-pinene (9.85%), methyleugenol (5.58%), α-pinene (4.84%) and mircene (2.37%) were the major components. At 7.48 mg/disk, SLEO inhibited the growth of all germs tested including four GRAM (+) and five GRAM (-) bacteria and one yeast. The zones of inhibition (ZI) ranged from 12 mm (*Yersinia enterolitica*) to 40 mm (*Bacillus subtilis*). The antioxidant activity of SLEO by the DPPH method was IC50 = 4.601 µg/ml. When administered orally at doses as high as 5 g/kg body weight, SLEO was not toxic to mice. Its non-toxicity, antimicrobial and antioxidant activities could make SLEO an alternative in the treatment of infectious diseases.

Keywords: *Senecio longiscapus*; Essential oils; Chemical composition; Antimicrobial activity; Antioxidant activity; Toxicity

1. Introduction

The growing interest in essential oils is reflected in the extensive research being carried out around the world on aromatic plants. Aromatic plants produce essential oils (EO) that have great therapeutic power and interesting biological activities such as antibacterial, antifungal, larvicidal, insecticidal and antioxidant properties [1].

Asteraceae (Compositeae) is one of the plant families that are good producers of essential oils. In Madagascar, this family is represented by 550 species of which 500 are endemic [2].

Senecio is the largest and most complex genus in the family of the Asteraceae having more than 1500 species distributed widely throughout the world [3, 4]. In Madagascar, the *Senecio* genus is represented by 85 species and 78 of which are endemic [5]. Present in various formations in the Central Madagascar region, the *Senecio* genus is mainly found in open areas such as natural clearings and forest edges. The mountain environment with rocky soil is particularly favourable to its development with a rather remarkable microendemism [6].

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The essential oils of the genus *Senecio* have been the subject of a large number of phytochemical studies and several biological activities have been demonstrated [7] but the studied species for essential oils constituents are still few and not more than 10 % of the species belonging to this genus [8]. A number of studies have shown that several *Senecio* species have antimicrobial, antifungal and cytotoxic activities [3].

Many species from *Senecio* genus have been used in traditional medicine [8, 9] and their pharmacological activities have been demonstrated: anti-inflammatory [9], antimicrobial [10] [3, 8, 10], antioxidant activities [3, 4, 8, 11], cytotoxic [8, 11] and so on.

However, nearly thirty species of *Senecio*, because of their high pyrrolizidine alkaloids content, are very dangerous for both humans and animals [12, 13]. For examples, *S. jacobaea, S. douglasii, S. riddellii* caused the majority in the cattle losses in western US [12] and *Senecio inaequidens* was implicated in a livestock poisoning epidemic in South Africa [14].

We are interested in *Senecio longiscapus* which is an aromatic plant endemic to central Madagascar. According to our surveys of healers and traditional practitioners, *Senecio longiscapus* is used to treat sexually transmitted infections, stomach aches and intestinal parasites (vermifuge). In addition, the available literature on this plant has so far only reported botanical studies.

The main objectives of this study were to determine the composition and physicochemical characteristics of *Senecio longiscapus* leaf essential oil (SLEO) and to explore its potential antibacterial and antioxidant activities and toxicity.

2. Material and methods

2.1. Plant materials

Senecio longiscapus leaves constituted our study material (figure 1). The leaves were collected in February 2021 in the Amoron' i Mania region, Ambositra district, Ivony commune (20°34'42.39''S; 47°09'53.01''E, altitude 1776 m) when the plant was not bearing flowers or fruits.

The plant was identified by comparison of an herbarium made from the collected material with the voucher specimen n°3553 of the Botanical and Zoological Park of Tsimbazaza (Antananarivo) made by Hildebrandt in 1933.

Figure 1 *Senecio longiscapus* Bojer. **Source**: The authors

2.2. Microbial strains

The microbial strains used were pathogens commonly sought in medical and food microbiological analysis and/or control. They include 4 Gram (-), 4 Gram (+) bacteria and one yeast (Table 1).

Table 1 List of bacterial strains used

Germ-tests	Gram	Reference
Listeria monocytogenes	+	ATCC 19114
Staphylococcus aureus	+	ATCC 6538
Clostridium perfringens	+	ATCC 13124
Bacillus cereus	+	ATCC 14579
Escherichia coli	-	ATCC 10145
Vibrio fischeri	-	ATCC 49387
Enterobacter aerogenes	-	ATCC 13048
Shigella flexneri	-	ATCC 12022
Yersinia enterocolitica	-	ATCC 23715
Candida albicans		ATCC 10321

2.3. Animals

OF-1 strain Albino mice (*Mus musculus*), weighing 25 ± 2 g, were provided by the Pasteur Institute of Madagascar (IPM) breeding farm.

2.4. Extraction of the essential oil

The extraction of the essential oils from the fresh leaves of *Senecio longiscapus* was carried out by hydrodistillation using a Clevenger type apparatus [15].

2.5. Physico-chemical characterization

The physico-chemical parameters to be determined and the references used are presented in Table 2.

Table 2 Parameters to determine and the standards used

Parameters	Standards used
Relative density	AFNOR, NF-T 75-111
Refraction index	AFNOR, NF-T 75-112
Optical rotation	AFNOR, NF-T 75-13
Acid index	AFNOR, NF-T 75-103
Ester index	AFNOR, NF-T75-104

2.6. Essential oil analysis

The chemical composition of the essential oil was determined by gas chromatography/mass spectrometry (GC/MS) [16]. The sample was analyzed using an AGILENT 5973 chromatograph (Network mass selective detector). It is equipped with a DBWAX column (0.25 mm x 30 m x 0.25 µm). The temperature of the column was programmed from 40°C to 250°C. The injector and detector temperatures are set at 280°C. Helium is the carrier gas used, with a flow rate of 1 ml/minute, the volume of sample injected being 1 µl. The peaks obtained were identified using AMDIS software Version 2.69 (Automated Mass Spectral Deconvolution and Identification System).

2.7. Assessment of antimicrobial activity

All the methods used for antimicrobial assay were detailed in our previous papers [17, 18].

2.8. Assessment of antimicrobial activity

The sensitivity of microorganisms to the essential oil was determined by the agar diffusion method or aromatogram. Sterile paper disks (6 mm in diameter BioMérieux, REF 549916) were soaked with pure essential oil and placed on the surface of the inoculated Mueller-Hinton Agar (Scharlau®). The Petri dishes were incubated at 37°C for 24 h and the zones of inhibition were measured. The sensitivity to the essential oil was classified according to the diameter of the zones of inhibition as: not sensitive (-) for diameters less than 8 mm; sensitive (+) for diameters 9–14 mm; very sensitive (++) for diameters 15–19 mm and extremely sensitive (+++) for diameters larger than 20 mm [19].

Antibiotic and antifungal used as references in this study were respectively Neomycin 30 µg/disk and Miconazole 500 µg/disk.

2.9. Antioxidant activity determination

The antioxidant capacity was evaluated by the method using free radical scavenging against DPPH (2, 2-Diphenyl-1-Pycryl Hydrazyl). The method was detailed in previous paper [20].

2.10. Toxicity determination

A volume of 0.3 ml of EO per 25 ± 2 g of body weight was administered to mice by oral route by means of an intubation cannula with a curved distal. Four batches of 5 male mice were used. Another one receiving physiological serum (NaCl 0.9%) was used as control. The mice were observed for 24 h.

3. Results

3.1. SLEO physico-chemical parameters

The extraction yield and the physico-chemical parameters of SLEO are shown in Table 3.

Table 3 Extraction yield and physico-chemical parameters of SLEO

Yield	Density	Refractive	Optical rotation	Acid index	Ester index
3%	0.7466	1.4959	+ 3°47	1.53	12.49

3.2. SLEO chemical composition

The GC-MS analysis of the SLEO identified 17 constituents representing approximately 99.09% of the overall composition. (Figure 2 and Table 4). Sabinene (53.28%) is the main constituent and the major compounds were elemicin (15%), β-pinene (9.85%), methyleugenol (5.58%), α- pinene (4.84) and mircene (2.37%).

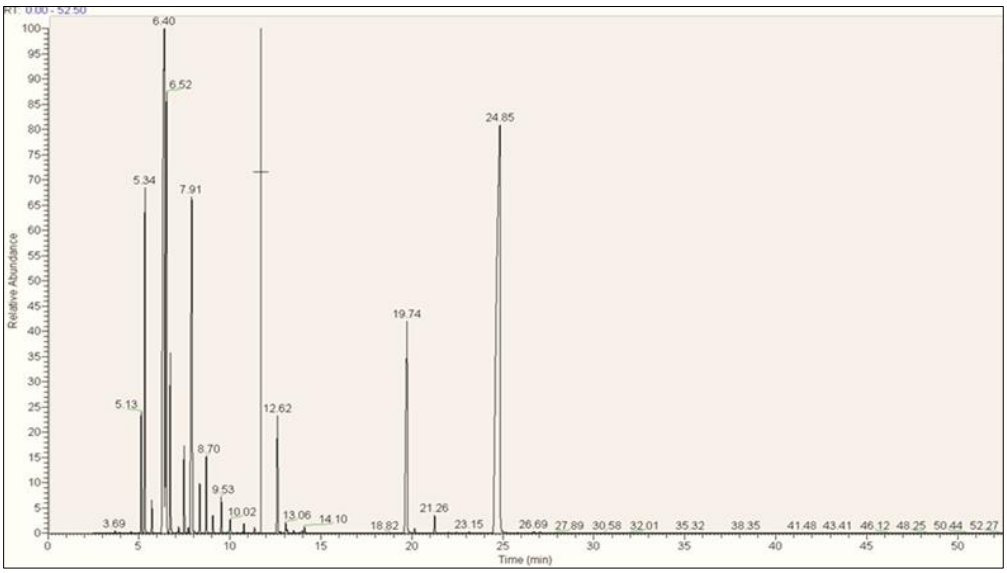

Figure 2 Chromatographic profile of SLEO

Table 4 SLEO major components

Peak number	Retention time (min)	Component	Relative rate (%)
1	5.1267	α-tujene	1.32
2	5.3370	α-pinene	4.84
3	5.7066	camphene	0.35
4	6.4024	sabinene	53.28
5	6.5222	β-pinene	9.85
6	6.7294	mircene	2.37
7	7.4816	terpinolene	0.97
8	8.3441	trans ocimene	0.63
9	8.7020	γ-terpinene	0.97
10	9.0658	trans sabinene hydrate	0.33
11	9.5273	terpinolene	0.46
12	10.0223	Linalol	0.13
13	12.6253	terpinene-4-ol	1.97
14	19.7439	methyleugenol	5.58
15	20.1727	β-caryophyllene	0.24
16	21.2563	α-humulene	0.80
17	24.8396	elemicin	15
Total			99.09

3.3. SLEO antimicrobial activity

SLEO antimicrobial activity was tested at 745.2 mg/ml against nine strains of bacteria and one yeast. The IZs obtained are presented in the Table 5.

Table 5 Antimicrobial activity of SLEO

Strains	Inhibition zone diameters (mm)			
	SLEO (7.45 mg/disk)	Strain sensitivity	Neomycin (30 µg/disk)	Miconazole (500 µg/disk)
Listeria monocytogenes	15	++	18	
Staphylococcus aureus	20	+++	19	
Clostridium perfringens	18	++	24	
Bacillus cereus	40	+++	22	
Escherichia coli	30	+++	21	
Vibrio fischeri	20	+++	18	
Enterobacter aerogenes	20	+++	27	
Shigella flexneri	12	+	26	
Yersinia enterocolitica	12	+	27	
Candida albicans	35	+++		25

+++ : Extremely sensitive; ++ : Very sensitive; + : Sensitive

Based on the standard of Ponce *et al*, [19], SLEO was active on all microbial strains tested with IZs ranging from 12 mm (*Shigella flexneri*) to 40 mm (*Bacillus cereus*).

3.4. SLEO antioxidant activity

The antioxidant activities of SLEO and ascorbic acid are presented in Table 7 and figure 3.

Table 7 SLEO and ascorbic acid antioxidant activities

Concentration (µg/ml)	Percentage* of inhibition (%)	
3.515	28.76	66.8
7.03	57.19	71.97
14.06	72.30	82.14
28.125	73.52	86.48
56.25	73.93	95.04
112.5	79.52	99.03
225	79.41	99.48
450	79.62	99.41
900	79.90	99.47
1800	79.41	99.47

*: Each value was the average of the values of 3 tests

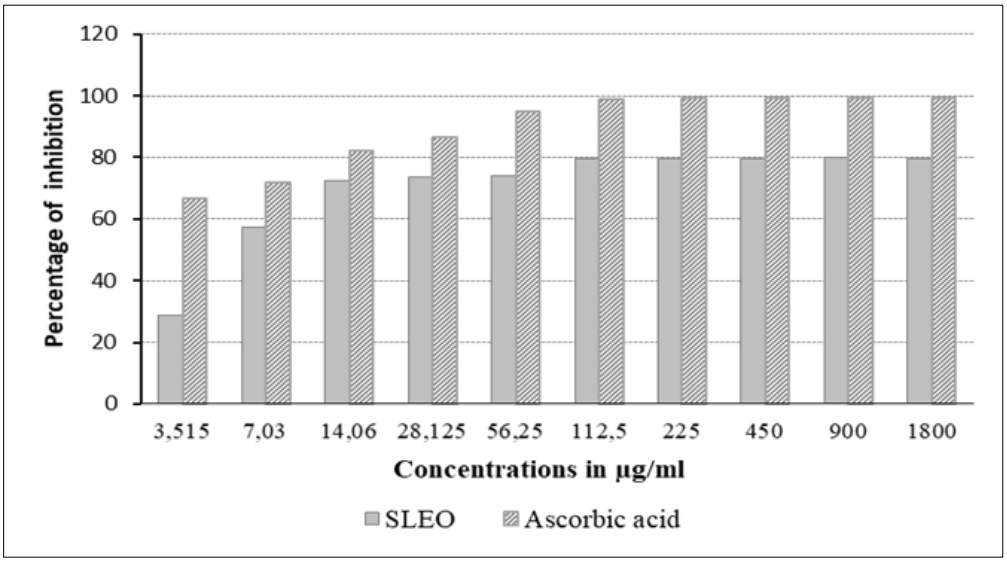

Figure 3 Effects of concentrations on the scavenging activity of SLEO and ascorbic acid against DPPH

For both SLEO and ascorbic acid, scavenging activity against DPPH increased with concentrations and the maximum of activity was reached at 112, 25 µg/ml. The IC50s for SLEO and ascorbic acid were 4.601 µg/ml and 3.245 µg/ml respectively**.**

3.5. SLEO toxicity

Four increasing doses of SLEO (1117.7, 2235.5, 4471.0 and 8942 mg/kg) were administered orally to 4 homogeneous batches of 5 mice of 25 g±2. Another batch of 5 mice serving as a control received physiological serum (NaCl 0.9%). No symptoms of intoxication were observed with the 4 doses tested during 24 h.

4. Discussion

The yields of essential oils are extremely variable depending on the plants considered, but they are generally very low, below 1% [21]. The extraction yield of SLEO was much higher (3%).

Seventeen (17) components representing 99.09% of the overall composition were detected in SLEO. This quantity was close to that observed for example in *S. giganteus* (18) [22], *S. argophylloides* (16) and *S. viridis* (15) [23]. As shown in Table 8, the main components and their proportion (%) in relation to the overall oil vary according to the species of *Senecio*. Sabinene, with a percentage of 53.28%, was the main component of SLEO.

Table 8 Number of components detected and major components in different *Senecio* species

***Senecio* species**	**Plant part**	**Components**		**Major components**
		Number	**%**	
S. longiscapus	leaf	17	99.09	Sabinene (53.28%)
S. giganteus [22]	aerial part	18	82.8	α-pinene (19.4%) and 6,10,14-trimethyl-2-pentadecanone (19.1%)
S. vulgaris [24]	aerial part	54	95.2	α-humulene (57.3%)
S. nemorensis [25]	flower	76	86.8	γ-curcumene (42.8%)
S. graciliflorus [11]	flower	20	95.50	α-pinene (33.97%)
S. nudicaulis [4]	aerial part	30	95.3	caryophyllene oxide (24.99%)
S. flammeus [9]	aerial part	48	98.41	α-farnesene (11.26 %)
S. glaucus [8]	flower	33	97.1	m-mentha-4,8-diene (31.4%)
	shoot	33	96	m-mentha-1(7), 8-diene (25.6%)
S. subulatus [23]	aerial part	20	95.5	p-cymene (33.3%) and β-pinene (31.2%)
S. argophylloides [23]	aerial part	16	99.8	camphene (52.7%)
S. viridis [23]	aerial part	15	94.8	α-thujene (31.7)

Sabinene does not seem to be common in the genus *Senecio* EOs. It is also the main component of the EO of *S. graveolens* aerial part (23.8%) [10], but is present at much lower percentages in the EOs of *S. polyanthemoides* leaves (3.2%) [26], *S. flammeus* (2.6%) [9], *S. mustersii* aerial part (1.6%) [10] and *S. graveolens* leaves (0.8%) [27].

Although sabinene (53.28%) was quantitatively by far the main component, other components such as elemicin (15%), β-pinene (9.85%), methyleugenol (5.58%), α-pinene (4.84) and mircene (2.37%) were not negligible and might have important roles in the biological properties of SLEO.

According to the values of its physico-chemical parameters, SLEO was a light oil (density <1), clear and yellow, with a low refraction index (1.4959), dextrogyre (+3°47), a low acid number (1.53) and an acid ester number of 12.49.

SLEO, at 7.45 mg/disk, exhibited high activity on 60% of the microorganisms tested, in particular on *Bacillus cereus (*IZ = 40 mm), *Candida albicans* (IZ = 35 mm) and *Escherchia coli* (IZ = 30 mm). On several germs, SLEO was as active (on *Staphylococcus aureus, Vibrio fischeri*) or even much more active (on *Bacillus subtilis, Escherchia coli, Candida albicans*) than the reference antibiotics (Neomycin 30 µg/disk or miconazole 500 µg/ml/disk).

SLEO showed an antioxidant activity that increased with concentration and was maximal at 112.5 µg/ml. Its efficacy was about 70% of that of ascorbic acid. For comparison, the IC50s of the EOs from different parts of some *Senecio* species determined by the DPPH method assay and using ascorbic acid as standard reference are presented in Table 9. Based on the size of the difference between the EO and ascorbic acid IC50 values, SLEO has an antioxidant activity comparable to that of *S. nudicaulis*, higher than that of *S. graciliflorus* but significantly lower than that of *S. glaucus*.

As mentioned in Table 9, several *Senecio* species are known to be dangerous to humans and animals. Therefore, before considering the exploitation of the interesting biological properties already identified, it was necessary to evaluate the toxicity of SLEO. Acute toxicity tests on mice showed that at a dose as high as 5 g/kg body weight by the oral route, SLEO did not cause any symptoms of intoxication for 24 hours. This result is in agreement with our field surveys that no adverse effects due to the use of *S. longiscapus* have been reported. This is an important advantage as it does not limit the uses of SLEO, especially for therapeutic purposes. However, this result should be complemented by further toxicity tests.

Table 9 Comparison of the IC50s of EOs of some *Senecio* species and ascorbic acid determined by the DPPH scavenging method

Senecio		**IC50 (µg/ml)**	
Species	**Plant part**	**EOs**	**Ascorbic acid**
S. longiscapus	leaf	4.601	3.245
S. graciliflorus [11]	flower	21.6 (flower)	14.3
	leaf	24.8	
S. glaucus [8]	flower	1.6	16
	shoot	1.9	
S. nudicaulis [4]	aerial part	10.61	12.08

Other known biological properties of components that are among the main components in SLEO should be explored. Its non-toxicity, good antimicrobial and antioxidant activities could make SLEO an alternative in the treatment of infectious diseases.

5. Conclusion

The chemical composition and physicochemical characteristics of the essential oil of *Senecio longiscapus* leaves are well established. Two important pharmacological properties, antibacterial and antioxidant, and the absence of toxicity of the essential oil have been demonstrated. The results obtained on the antimicrobial activity of SLEO provided scientific basis of the use of *Senecio longiscapus* in traditional medecine.

Compliance with ethical standards

Acknowledgments

We sincerely acknowledged the Pasteur Institute of Madagascar (IPM) for providing us with the experimental animals.

Disclosure of conflict of interest

The authors declare no conflict of interests.

Statement of ethical approval

All the tests on animals were approved and in line with the standard established by Ethics Committee of Pasteur Institute of Madagascar**.**

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