British Journal of Pharmaceutical Research
12(1): 1-11, 2016, Article no.BJPR.25250
ISSN: 2231-2919, NLM ID: 101631759

SCIENCEDOMAIN *international*
www.sciencedomain.org

Senecio pterophorus DC. (Asteraceae) Essential Oils: Antibacterial, Antioxidant, Cytotoxic and Larvicidal Activities

Oladipupo A. Lawal[1,2*], Isiaka A. Ogunwande[2], H. M. M. Mzimela[3], Andy R. Opoku[4] and Adebola O. Oyedeji[1,5]

[1]Department of Chemistry, University of Zululand, Private Bag X1001, KwaDlangezwa 3886, South Africa.
[2]Department of Chemistry, Lagos State University, PMB 0001 LASU Post Office 001, Ojo Lagos, Nigeria.
[3]Department of Zoology, University of Zululand, Private Bag X1001, KwaDlangezwa 3886, South Africa.
[4]Department of Biochemistry and Microbiology, University of Zululand, Private Bag X1001, KwaDlangezwa 3886, South Africa.
[5]Department of Chemistry and Chemical Technology, Walter Sisulu University, Mthatha, 5099, South Africa.

Authors' contributions

This work was carried out in collaboration between all authors. Author OAL isolate the oils, assay the antibacterial, antioxidant and cytotoxic activities and wrote part of the manuscript. Author IAO managed the literature searches and wrote the final draft of the manuscript. Author HMMM assisted with the larvicidal activity. Authors ARO and AOO supervised the work. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2016/25250
Editor(s):
(1) Vasudevan Mani, Universiti Teknologi MARA (UiTM), Selangor, Malaysia.
(2) Ali Nokhodchi, Professor of Pharmaceutics and Drug Delivery, School of Life Sciences, University of Sussex, UK.
Reviewers:
(1) Francis W. Hombhanje, Divine Word University, Papua New Guinea.
(2) Luqman A. Khan, Central University, New Delhi, India.
(3) Anonymous, Université Abdelmalek Esaâdi, Morocco.
Complete Peer review History: http://sciencedomain.org/review-history/14903

Original Research Article

Received 23rd February 2016
Accepted 20th May 2016
Published 4th June 2016

**Corresponding author: E-mail: jumobi.lawal@lasu.edu.ng;*

ABSTRACT

The antibacterial, antioxidant, cytotoxic and larvicidal activities of essential oils obtained by hydrodistillation from the leaves of *Senecio pterophorus* DC were investigated in the present study. The oils were tested for the antibacterial effects against eleven reference microorganisms using agar-disc diffusion and microdilution-broth methods. The oils exhibited weak to moderate activity against the tested bacterial strains. The highest antibacterial activity was against *Staphylococcus aureus* (ATCC 6538 and ATCC 3983), *Streptococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 4983) and *Proteus vulgaris* (ATCC 0030). The zones of inhibition ranged from 14.0±2.0 to 21.0±2.6 mm while the minimum inhibitory concentrations were found between 0.63 and 10.0 mg/mL. In the antioxidant assay, the oils displayed poor ability to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and chelate iron (II) metal, but showed significant scavenging activity comparable to the standard antioxidants (Butyl hydroxyl anisole, butyl hydroxyl toluene, ascorbic acid and α-tocopherol) towards nitric oxide and hydroxyl scavenging procedures. The percentage mortalities of the oils at 250 mg/ml against *Artemia salina* nauplii and fourth-instar larvae of *Culex quinquefasciatus* was 100%. However, the lethal concentrations (LC_{50}) values ranged from 12.15 to 13.23 µg/mL while and LC_{90} was found between 30.50 and 39.45 µg/mL against *Artemia salina* nauplii. In addition, LC_{50} and LC_{90} values of 12.15-13.23 µg/mL and 30.50-39.45 µg/mL respectively were found against fourth-instar larvae of *Culex quinquefasciatus*.

Aims: The aim of this study was to isolate essential oils from the leaves of *Senecio pterophorus* DC (Asteraceae) and investigate the antibacterial, antioxidant, cytotoxic and larvicidal activities using standard procedures.

Study Design: The study involves the hydrodistillation of essential oils from the air-dried plant materials and investigation of the biological potentials of the oil.

Place and Duration of Study: Fresh plant materials of *Senecio pterophorus* were collected at full flowering stage in September, 2007 from Tongaat, Mtunzini and Gingindlovu in KwaZulu-Natal Province, South Africa.

Methodology: About 500 g of air-dried plant samples was shredded and their oils were obtained by separate hydrodistillation for 4 h at normal pressure, according to the British Pharmacopoeia. The antibacterial activity was determined by using agar-disc diffusion and microdilution-broth methods while the antioxidant assay was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and chelate iron (II) metal procedures as well by means of nitric oxide and hydroxyl scavenging assays. The cytotoxic and larvicidal activities were conducted by means of brine shrimps (*Artemisia salina*) and standard larvicidal procedures respectively.

Results: The essential oils displayed potents activities to the tested organisms.

Conclusion: The present study confirmed that the essential oils of *S. pterophorus* may serves as potential phytochemicals.

Keywords: Senecio pterophorus; Asteraceae; essential oils; antibacterial; antioxidant; cytotoxic and larvicidal activity.

1. INTRODUCTION

Senecio pterophorus DC. (syn: *S. pterophorus* DC. var. *apterus* Hary) popularly known as African daisy (English) or inkwandlakwandlan (Zulu) is an aggressive weed commonly found growing on moist forest thickets, open sites and along roadsides with grey-green stems usually covered with white hairs [1,2]. The leaves (*ca*. 50-120 mm long, 3-25 mm wide with 6-8 forward pointing teeth) are leathery and rough with margins very curled and short wings at the base. The flowers are numerous and form corymbiform panicles with yellow corollas [1-3]. It is native to Lesotho, South Africa (Cape and KwaZulu-Natal Provinces) and Swaziland [2,3], but now found in many countries [4,5]. *Senecio pterophorus* have been shown to contain alkaloids of the pyrrolizidine group, which are known to be hepiltotoxins, hepatocarcinogens, genotoxic and teratogenic [6-9]. But, different organs of this plant have been reportedly used as medicine and vegetable [1,10]. Previous studies on the phytochemical analysis of *S. pterophorus* afforded acetylseneciphylline, spartioidine, retrorsine, senecionine, seneciphylline, pterophorin, rosmarinine and isorosmarinine [7,10,11].

The chemical compositions of essential oils of *S. pterophorus* collected from three different locations of South Africa have been reported

Senecio pterophorus [12]. The major components of the oils were limonene (10.3-32.3%), myrcene (14.4-19.7%), sabinene (13.0-18.0%), α-phellandrene (3.4-16.9%), *p*-cymene (15.6-16.7%), (*E*)-β-ocimene (7.3-10.9%), (*Z*)-ocimene (3.0-9.4%) and α-pinene (2.6-6.2%). To the best of our knowledge, literature survey shows that there are no information on the biological activity of essential oil of *S. pterophorus*. However, there are reports on the biological activities of essential oils of some species of the genus *Senecio* [13-27].

In continuation of our growing interests on the chemical composition and biological activities of essential oils from poorly studied species of South African flora [28-30], the present study is reporting for the first time, the antibacterial, antioxidant, cytotoxic and larvicidal activities of essential oils of *Senecio pterophorus* growing wild in KwaZulu-Natal Province, South Africa.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2-dexyribose, sodium nitroprusside, trichloroacetic acid (TCA), thiobarbituric acid (TBA), naphthylethylenediamine dihydrochloride, 4,4- [3-(2-pyridinyl)-1,2,4-triazine-5,6-dryl] bisbenzene sulphonic acid (ferrozine), Folin-Ciocalteau reagent, 2-dexyribose, sodium nitroprusside, Ethylenediaminetetraacetic acid (EDTA), Dimethylsulfoxide (DMSO), Ferrous ammonium sulfate, Quercetin, Gallic, Ammonium molybdate and Hydrogen peroxide were obtained from Sigma-Aldrich Co., Ltd (Steinheim, Germany). All other chemicals and solvents are of analytical grade.

2.2 Plant Materials

Fresh leaves of *Senecio pterophorus* were collected at full flowering stage in September, 2007, from Tongaat, Mtunzini and Gingindlovu in KwaZulu-Natal Province, South Africa. The botanical identification of the plant materials was carried out by Dr. R. N. Ntuli, Department of Botany, University of Zululand, KwaDlangezwa. Voucher specimens [OAL 35, 36 and 38 (ZULU)] were deposited at the University Herbarium.

2.3 Hydrodistillation of Essential Oils

Air-dried and pulverized leaves (300 g each) were separately subjected to separate hydrodistillation in a Clevenger-type glass apparatus for 3 h in accordance with the British Pharmacopoeia specification [31]. The distilled oils were preserved in different sealed sample tubes and stored under refrigeration until analysis.

2.4 Antibacterial Activity

2.4.1 Microbial strains

The essential oils of *S. pterophorus* were tested against 11 reference bacterial strains viz., *Bacillus cereus* (ATCC 10702), *Bacillus pumilus* (ATCC 14884), *Staphylococcus aureus* (ATCC 3983), *Staphylococcus aureus* (ATCC 6538), *Streptococcus faecalis* (ATCC 29212), *Enterobacter cloacae* (ATCC 13047), *Escherichia coli* (ATCC 4983), *Klebsiella pneumoniae* (ATCC 2983), *Proteus vulgaris* (ATCC 0030), *Pseudomonas aeruginosa* (ATCC 19582) and *Serratia marcescena* (ATCC 9986). The stock cultures were obtained from Applied and Environmental Microbiology Research Group (AEMREG), Department of Biochemistry and Microbiology, University of Fort Hare, Alice 5011, South Africa and were maintained at 4℃ in Mueller-Hinton agar (MHA) (Oxoid, Germany).

2.4.2 Disc diffusion assay

The antibacterial activity of *S. pterophorus* leaf essential oils was tested against the above Gram-positive and Gram-negative bacteria strains by the agar disk diffusion method [32]. The microorganisms were grown overnight at 37℃ in 20 ml of Mueller-Hinton broth (Oxoid). The cultures were adjusted with sterile saline solution to obtain turbidity comparable to that of McFarland no. 5 standard (1.0×10^8) CFU/mL. Petri dishes, 90 mm (Merck, South Africa) containing 12 mL of sterilized Mueller-Hinton agar (Oxoid) were inoculated with these microbial suspensions. Sterile Whatman No.1 (6 mm) discs papers were individually placed on the surface of the seeded agar plates and 10 µL of essential oils in Hexane/DMSO mixture (1:1, 5 mg/mL) were applied to the filter paper disk. The plates were incubated at 37℃ for 24 H and the diameter of the resulting zones of inhibition (mm) of growth was measured. All tests were performed in triplicates. Chloramphenicol (25 µg), gentamycin (5 µg) and tetracycline (30 µg) were used as positive controls, while, hexane and 1% DMSO solution served as negative controls.

2.4.3 Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of the oils was determined by microtitre plate dilution method as described by Eloff [33]. The bacterial cultures were incubated in Müller-Hinton (MH) broth overnight at 37℃ and a 1:1 dilution of each culture in fresh Müller-Hinton broth was prepared prior to use in the micro dilution assay. Stock solution concentrations of the essential oils (40 mg/mL) were obtained by adding 4 mg of essential oils to 500 µL DMSO and 500 µL Hexane. Sterile water (100 µL) was pipetted into all wells of the microtitre plate, before transferring 100 µL of stock solutions of essential oils to the wells of row A. Serial dilutions were made from the first well vertically in the plate and excess volume of 100 µL was discarded from the wells in row H to obtain concentrations ranging from 10 to 0.078 mg/mL. Bacterial culture (100 µL) of approximate inoculums size of 1.0×10^8 CFU/mL was added to all wells and incubated at 37℃ for 24 H. After incubation, 40 µL of 0.2 mg/mL p-iodo-nitotetrazolium violet (INT) solution was added to each well and incubated at 37℃. Wells were examined after about 20-30 min. of incubation. Microbial growth was indicated by the presence of a reddish colour, which is produced when *p*-iodo-nitrotetrazolium violet (INT), a dehydrogenase activity detecting reagent, is reduced by metabolically active microorganisms to the corresponding intensely coloured formazan. The minimum inhibitory concentration (MIC) is defined as the lowest concentration that produced an almost complete inhibition of visible microorganism growth in liquid medium.

2.5 Antioxidant Activity

2.5.1 Scavenging effect on 1,1-diphenyl-2-picrylhydrazyl

The DPPH radical scavenging activity of *S. pterophorus* essential oils was determined by modifying the method of Han et al. [34]. Two milliliter of various concentrations (1-5 mg/100 ml) of the extract in methanol was added to 2 ml of DPPH radical solution in methanol (final concentration of DPPH was 0.2 mM). The mixture was shaken vigorously and allowed standing for 30-60 min; the absorbance of the resulting solution was measured at 517 nm using UV-Visible Genesys 20 spectrophotometer; after the solution had been allowed to stand in the dark for 60 min. The absorbance of the samples, the control and the blank were measured in comparison with methanol. Lower absorbance of the reaction mixture indicates higher DPPH scavenging activity.

DPPH scavenging activity was calculated using the following formula:

DPPH scavenging activity (%) = {1- (S-SB)/(C-CB)} X 100%

where S, SB, C and CB were the absorbances of the sample, the blank sample (2.0 mL of methanol plus 0.2 mL of sample at different concentrations), the control (2.0 mL of DPPH solution plus 0.2 mL of methanol), and the blank control (methanol) respectively. The concentration providing 50% inhibition (IC_{50}) was calculated from the graph of percentage inhibition against oil concentrations.

2.5.2 Hydroxyl radical scavenging activity

The ability of the different concentrations (10-250 µg/mL) of *S. pterophorus* essential oils to scavenge the hydroxyl radical generated by Fenton reaction was measured according to the modified method of Nagai et al. [35]. The Fenton reaction mixture containing 200 µL of 10 mM 2-deoxyribose was mixed with 1.2 mL of 0.1 M phosphate buffer (pH 7.4) containing 200 uL of the extract. Thereafter, 200 µL of 10 mM H_2O_2 was added to the mixture before incubation for 4 h at 37℃. Later, 1 mL of 2.8% trichloroacetic acid (TCA) and 1 mL of 1% thiobarbituric acid (TBA) were added and placed in a boiling water bath for 10 min. The resultant mixture was allowed to cool to room temperature and centrifuged at 300 X g for 5 min. Absorbance was recorded at 532 nm in a UV-VIS spectrophotometer. The percentage inhibition was calculated by the formula:

$$\% \text{ Inhibition} = \{(A_0 - A_1)/A_0 \times 100\}$$

where A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the essential oils. The IC_{50} value represented the concentration that caused 50% inhibition of radical formation.

2.5.3 Nitric oxide radical ($NO^{\cdot}$) scavenging

Scavengers of nitric oxide compete with oxygen and reduction the production of nitric oxide was measured according to the modified method of Badami et al. [36]. The reaction mixture (3 mL) containing 2 mL of 10 mM sodium nitroprusside,

0.5 mL of phosphate buffer saline (pH 7.4, 0.01 M) and 0.5 mL of different concentrations (10-250 µg/mL) of *S. pterophorus* essential oils were incubated at 25℃ for 150 min. Thereafter, 0.5 mL of the reaction mixture containing nitrite was pipette and mixed with 1 mL of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotisation. Then, 1 mL of naphthylethylenediamine dihydrochloride (0.1%) was added, and allowed to stand for 30 min in diffused light. The absorbance of the pink coloured chromophore was measured using UV-Visible Genesys 20 spectrophotometer at 540 nm against the corresponding blank solution.

$$\% \text{ Inhibition} = \{(A_0 - A_1)/A_0 \times 100\}$$

Where A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the essential oils. The concentration providing 50% inhibition (IC_{50}) was calculated from the graph of percentage inhibition against oil concentrations.

2.5.4 Metal chelating activity

The Fe^{2+} chelating effect of essential oils of *S. pterophorus* was measured according to the method of Senevirathne et al. [37]. To 0.5 mL of various concentrations (5-250 µg/ml) of each oil in methanol, 1.6 mL of deionized water and 0.05 mL of $FeCl_2$ (2 mM) were added. After 30 s, the reaction was initiated by the addition of 5 Mm ferrozine (0.1 mL). Then, the mixture was shaken and left at room temperature for 10 min. Absorbance of the mixture was measured spectrophotometrically at 562 nm. Citric acid and ethylenediaminetetraacetic acid were used as standard.

The inhibitory effect of the oils was calculated as:

$$\% \text{ Inhibition} = \{(A_0 - A_1)/A_0 \times 100\}$$

Where A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the essential oils. The concentration providing 50% inhibition (IC_{50}) was calculated from the graph of percentage inhibition against oil concentrations.

2.5.5 Reducing power

The reducing power of essential oils of *S. pterophorus* was evaluated according to the procedure described by Oyaizu [38]. An aliquot (2.5 mL) of different concentrations of each oil (5-250 µg/mL) in methanol was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was incubated at 50℃ for 20 min and 2.5 mL of 10% TCA was added to the mixture, prior to centrifuging at 1000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and (0.5 mL, 0.1%) $FeCl_3$. The absorbance was measured spectrophotometrically at 700 nm. BHA and ascorbic acid were used as standards. Higher absorbance of the reaction mixture indicated greater reducing power.

2.6 Cytotoxicity Activity

2.6.1 Brine shrimp test

The brine shrimps (*Artemisia salina*) eggs were obtained from Fish designs (Distributor of live ornamental fish and products) in Mtunzini, South Africa.

2.6.2 Preparation of artificial sea water

The pH of natural seawater was taken and found to be 7.8 at a temperature of 28-30℃. About 5.0 g of non-iodized coarse salt was added to 1.0 L of warm water to produce a pH of 7.8. This was the artificial seawater used.

2.6.3 Hatching of the brine shrimp eggs

The brine shrimps eggs three teaspoons were mixed with artificial seawater (500 mL) and left to stand for about 15-20 minutes. The mixture was put into an inverted 2 L glass bottle containing artificial seawater (1.0 L) with the base cut off., and aeration supplied through an open airline, with a weighed down air-stone. The glass bottle was wrapped with black sheet. The hatching water was kept at a temperature of 27 to 30℃, using a 100 w incandescent light bulb hanged over the hatching glass bottle, and left for 18 to 24 h for hatching of the eggs. The hatched nauplii were observed under the microscope for mobility and were transferred to a bowl in order to secure a large surface for oxygenation of the water and the bioassay performed. A drop of dry yeast suspension (3 mg in 5 mL artificial seawater) was added as food source.

2.6.4 The brine shrimp assay

The brine shrimp test was carried out using the method of Meyer et al. [39]. Ten shrimps (counted on a slide) were introduced into the beakers (30 mL) containing 50 µL of different

concentrations of essential oils (10, 20, 50, 100 and 250) µg/mL in 1% DMSO and made up to 5 mL volume with the artificial sea water. Each beaker was shaken lightly to ensure a homogeneous test solution and was left at room temperature. The control was prepared with 24.95 mL of degassed distilled water and 50 µL of DMSO solution without essential oils to which shrimp larvae were added. The tubes were maintained under illumination and each test was performed in duplicate. Survivors were counted after 24 H, and a criterion for death was loss of locomotive action of nauplii. The percentage mortality at each concentration and control was calculated using Abbots formula [40] and lethal concentration (LC_{50}) values were determined by probit analysis program, version 1.5. Cytotoxic activity was reported as LC_{50} with 95% confidence intervals, representing the concentrations in µg/mL with 50% larvae mortality rate in 24 h.

2.7 Larvicidal Activity

2.7.1 Mosquito

Larvae of *Culex quinquefascitus* were collected from the storage water tanks in the Hatchery unit of the Department of Zoology, University of Zululand, KwaDlangezwa campus. The mosquito species was identified by Mzimela, H.M.M and were maintained at ambient rearing conditions in the environmental room. All bioassays were conducted at 28±1℃, 60.0±5℃ R.H, and 12 h light and 12 h dark photoperiod. A 5% yeast suspension was used as food source.

2.7.2 Mosquito larvicidal test

Test for mosquito larvicidal activity was conducted according to the method of Rafikali and Nair [41] with some modifications. Five fourth-instar mosquito larvae were collected with a Pasteur pipette, placed on filter paper to remove excess water and transferred to the beakers (100 mL) each containing 29.0 mL of degassed distilled water and 1000 µL of different concentrations of essential oils (10 - 250 µg/mL) in 1% DMSO solution. Each beaker was shaken lightly to ensure a homogeneous test solution and was left at room temperature. Each test was performed in duplicate. The control was prepared with 29.0 mL of degassed distilled water and 1000 µL of DMSO solution without essential oils to which larvae were added. Observation on larval mortality was recorded after 24 h exposure, during which no food was given to the larvae. Larvae were considered dead, when they did not react to touching with a needle. The percentage of mortality using Abbots formula [40] and lethal concentrations (LC_{50}) values were determined by probit analysis program, version 1.5. Larvicidal activity was reported as LC_{50} with 95% confidence intervals, representing the concentrations in µg/mL with 50% larvae mortality rate in 24 H.

2.8 Statistical Analysis

The mean and standard deviation of three experiments were determined. Statistical analysis of the differences between mean values obtained for experimental groups were calculated as means ± standard deviation (SD) of three independent measurements using Microsoft excel program, 2003 and Origin 6.0 for IC_{50}. Data were subjected to one way analysis of variance (ANOVA). *P* values ≤ 0.05 were regarded as significant and *P* values ≤ 0.01 as very significant.

3. RESULTS AND DISCUSSION

3.1 Antibacterial Activity

The zones of inhibition (ZI, mm) and minimum inhibitory concentrations (MIC, mg/mL) of *S. pterophorus* essential oils against the microorganisms are summarized in Tables 1 and 2 respectively. All the essential oils were active against *S. aureus* (ATCC 3983; ZI, 14.0±2.0 mm – 17.7±0.6 mm), *S. aureus* (ATCC 6538; ZI, 17.3±2.5 mm – 20.7±1.2 mm), *E. coli* (ATCC 4983; ZI, 15.3±2.5 mm – 21.0±2.6 mm), *P. vulgaris* (ATCC 0030; ZI, 16.7±2.1 mm – 17.7±1.5 mm) and *P. aeruginosa* (ATCC 19582; ZI, 11.0±1.0 mm – 15.3±1.5 mm).

The essential oils from Gingindlovu and Tongaat exhibited good activity towards *S. faecalis* (ATCC 29212; ZI, 15.7±2.1 mm and 19.3±2.3 mm respectively). Only the essential oil from Tongaat exhibited moderate activity against *K. pneumoniae* (ATCC 2983; ZI, 11.3±1.5 mm). Also, the oils from Mtunzini and Gngindlovu displayed some activity towards *S. marcescena* (ATCC 9986; ZI, 16.0±2.2 mm and 13.0±2.0 mm, respectively). All the studied oil samples showed no activity towards the growth of *B. cereus, B. pumilus* and *E. cloacae* (Table 1).

The minimum inhibitory concentration (MIC) values ranging from 0.63 to 10.0 mg/mL (Table 2), revealed a wide range of activity for the

Table 1. Antibacterial activity of essential oils of *S. pterophorus*

Microorganisms	Inhibition zones (IZ)[a]					
	Mtunzini	Gingindlovu	Tongaat	Gent[b]	Chl[c]	Tetra[d]
B. cereus	6.6±0.5	9.7±1.5	6.6±0.5	14.0±2.0	23.7±1.3	13.3±1.3
B. pumilus	6.0±0.0	6.3±0.6	9.7±2.5	13.3±1.7	16.3±1.3	14.0±1.3
S. aureus[e]	17.3±1.5	17.7±0.6	14.0±2.0	17.3±0.9	16.7±1.3	18.7±0 .9
S. aureus[f]	17.3±2.5	18.3±1.2	20.7±1.2	14.3±1.3	13.7±1.3	ND
S. faecalis	ND	15.7±2.1	19.3±2.3	16.0±1.6	20.3±1.3	ND
E. cloacae	ND	6.0±0.0	6.0±0.0	17.7±0.5	13.3±1.3	13.0±1.4
E. coli	15.3±2.5	21.0±2.6	19.0±2.0	21.3±1.3	23.7±1.3	23.0±1 .4
P. vulgaris	16.7±3.1	16.7±2.1	17.7±1.5	23.7±1.3	20.0±1.4	17.6±1 .3
K. pneumoniae	7.3±1.2	8.7±1.2	11.3±1.5	21.3±0.9	21.0±2.0	6.0±0.0
P. aeruginosa	11.0±1.0	12.7±2.1	15.3±1.5	20.7±0.9	22.7±1.7	14.7±0 .5
S. marcescena	16.0±2.2	13.0±2.0	6.0±0.0	14.3±0.5	6.0 ±0.0	15.7±1. 3

[a]*IZ: Inhibition zones diameter (mm) including diameter of sterile disc (6 mm); Values are given as mean ± SD (3 replicates); Essential oil tested 10 µl/disc and 25 µg/mL methanolic solution of Chloramphenicol.* [b]*Gent - Gentamycin;* [c]*Chl - Chloramphenicol;* [d]*Tetra –Tetracycline.* [e]*ATCC 3983;* [f]*ATCC 6538; ATCC = American Type Culture Collection; CSIR = Council for scientific and industrial research*

Table 2. Minimum inhibitory concentrations of essential oils of *S. pterophorus*[a]

Microorganisms	Mtunzini	Gingindlovu	Tongaat	Gent[b]	Chl[c]	Tetra[d]
B. cereus	ND	10	5	0.63	0.08	1.25
B. pumilus	10	10	5	1.25	0.63	1.25
S. aureus[e]	2.5	2.5	2.5	0.31	0.31	0.31
S. aureus[f]	2.5	1.25	0.63	0.63	0.31	0.31
S. faecalis	ND	1.25	0.63	1.25	0.16	ND
E. cloacae	ND	ND	10	2.5	5	2.5
E. coli	5	1.25	1.25	0.16	0.08	0.31
P. vulgaris	5	1.25	10	0.08	0.63	0.63
K. pneumoniae	10	10	5	0.63	0.63	-
P. aeruginosa	10	5	ND	0.63	0.31	0.63
S. marcescena	1.25	5	1.25	1.25	ND	0.63

[a]*MIC values are given as (mg/mL);* [b, c & d]*Methanolic solutions of Gentamycine, Chloramphenicol and Tetracycline - 5µg/mL;* [e]*ATCC 3983;* [f]*ATCC 6538; ND = Not Determined*

essential oils studied. The oil samples displayed weak to strong antibacterial activity against the tested bacteria strains. Moderate activity (MIC ≤ 2.5 mg/mL) was observed against *S. aureus* strains by all the oil samples. While, little or no activity was seen against *B. cereus*, *B. pumilus*, *E. cloacae*, *K. pneumoniae*, *P. vulgaris* strains and *P. aeruginosa*. The most active oils against *S. aureus* strains, *E. coli* and *S. faecalis* were Gingindlovu and Tongaat oil samples, both having MIC values between 0.63 to 2.5 mg/mL. On the other hand, Mtunzini and Tongaat oil samples displayed the most excellent activity against *S. marcescena* (MIC = 1.25 mg/mL). Generally, the oil of *S. pterophorus* from Tongaat showed greater activity than the Gingindlovu and Tongaat oil samples against most of the tested microorganisms. When compared with standard antibiotics (gentamycin, chloramphenicol and tetracycline.), the oils of *S. pterophorus* showed MIC values from weak to moderate range, with both Gram-positive and Gram-negative bacteria been responsive, although, susceptibility was found more with Gram-positive bacteria strains [42,43].

3.2 Antioxidant Activity

The results of the antioxidant activity of *S. pterophorus* essential oils (expressed in terms of IC_{50} value) are showed in Table 3. In DPPH and metal chelating methods, the oils displayed weak ability to scavenged 1,1-diphenyl-2-picrylhydrazyl radical and form Fe^{2+} complex for peroxidation protector. The essential oil samples possessed lower inhibitory concentration values on DPPH and metal chelating (250 µg/mL) than BHA (IC_{50}: 10.72 µg/mL), citric acid (7.42 µg/ml), EDTA (7.42 µg/ml) and BHA, vitamin C and α-tocopherol (IC_{50}: < 10.0 µg/ml). On the other hand, a stronger free radical scavenging activity on hydroxyl radical and nitric oxide was observed with inhibitory concentration values (IC_{50}) of 13.90 µg/mL (Mtunzini) and < 14.07 µg/mL

(Tongaat) when compared with vitamin C (IC_{50}: < 15.19 µg/ml) in nitric oxide scavenging activity and almost the same in hydroxyl radical scavenging activity (IC_{50}: < 10.0 µg/ml).

The reducing ability of the essential oils to prevent peroxide formation was evaluated and the result displayed in Fig. 1. The essential oils and standard antioxidants showed a dose dependent concentration for Fe (III) reduction. However, the tested oil samples displayed poor ferric reducing power in comparison with the standard antioxidants. At 250 µg/mL, the reducing power of the essential oil samples and standard antioxidants decreases as follows: α-tocopherol > BHA > ascorbic acid > BHT > Gingindlovu > Mtunzini >Tongaat.

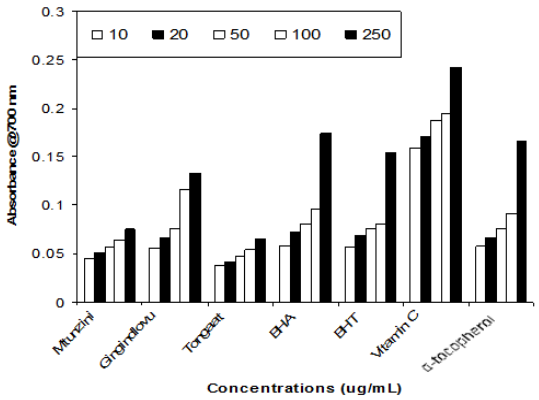

Fig. 1. Reducing power of *Senecio pterophorus* essential oils and synthetic antioxidants

Each value is mean ± S.D (n = 3)

3.3 Cytotoxic Activity

The cytotoxic activity of the oils was determined using the brine shrimp lethality assay. The bioassay represents a rapid, inexpensive and simple method of testing plant extracts lethality, which in most cases correlates well with cytotoxicity and anti-tumuor activity. Table 4 showed the results of cytotoxic activity of *S. pterophorus* essential oils against *A. salina* nauplii after 24 H exposure to different concentrations of the oils, gallic acid and 1% DMSO and water. The degree of lethality was found to be directly proportional to the different concentrations of the oils ranging from the lowest concentration (10 µg/mL) to the highest concentration (250 µg/mL). Maximum mortalities were recorded at the concentration of 250 µg/mL, whereas least mortalities were seen at 10 µg/mL. The essential oils of *S. pterophorus* showed significant cytotoxicity against *A. salina* nauplii with 24 H at LC_{50} values ranging from 12.15-13.23 µg/mL.

3.4 Larvicidal Activity

The results of the percentage mortality and lethal concentrations (LC_{50} and LC_{90} values with 95% conference limits) of the essential oils of *S. pterophorus* against the fourth-instar larvae of *C. quinquefascitus* are summarized in Table 4. The larvicidal activity of *S. pterophorus* essential oils against the fourth-in-star larvae of *Culex quinquefascitus* shows a positive correlation between the different concentrations, with the mortality rate being directly proportional to the concentrations. At lower concentrations (10-20 µg/mL) less than 40% mortality was observed after 24 h exposure. However, higher mortality rate (80-100%) was produced for the oils at concentrations between (100-250 µg/mL), where larvae displayed some restless movement and then died. No larvae emergence into pupae or adult between these concentrations within 24 h exposure. But, the larvae developed into pupae and then adult within 48 h in the control experiments. Fig. 2 summarized the lethal concentrations (LC_{50} values with the corresponding 95% confidence intervals) of essential oils of *S. pterophorus* against fourth-in-star larvae of *C. quinquefascitus*. The bioassay also showed that the essential oils of *S. pterophorus* were toxic against fourth-in-star larvae *Culex quinquefascitus* with LC_{50} values ranging from 24.04-85.00 µg/mL.

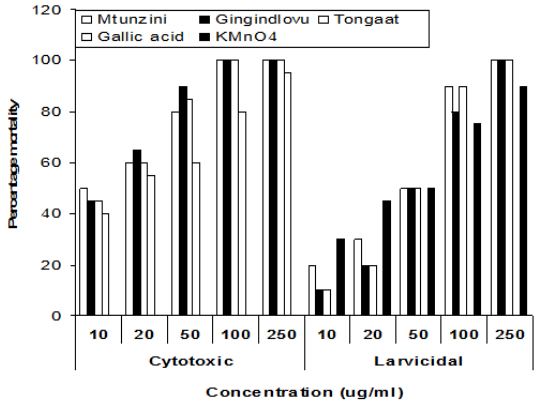

Fig. 2. Percentage of mortality of essential oils of *S. pterophorus* against the nauplii of *Artemisia salina* and fourth-in-star larvae of *Culex quinquefascitus*

Table 3. Antioxidant activity of *S. pterophorus* essential oils[a]

Essential oil/Standard antioxidant	Free radical scavenging activities (IC_{50})[d]			Metal chelating[e]
	DPPH	($^{\cdot}OH$)	($NO^{\cdot}$)	
Mtunzini	> 250	< 10	13.90	> 250
Gingindlovu	> 250	< 10	28.87	> 250
Tongaat	> 250	< 10	14.07	> 250
BHA	10.70	< 10	< 10	-
BHT	< 10	< 10	< 10	-
Citric acid	-	-	-	7.42
EDTA	-	-	-	14.81
Vitamin C	< 10	< 10	15.19	-
α-tocopherol	< 10	< 10	11.96	-

[a](n = 3, X ± SEM), [b]IC_{50} -inhibitory concentration, (.OH) - Hydroxyl radical scavenging; (NO.)- Nitric oxide radical scavenging; - Not observed

Table 4. Cytotoxic and larvicidal activities of essential oils of *S. pterophorus*[a]

Activity	Essential oil	LC_{50} (95% CI)	Slope ± SE	Chi square ($\chi 2$)
Cytotoxic	Mtunzini	12.15 (5.93-17.95)	2.02+0.47	1.93
	Gingindlovu	12.27 (7.74-16.19)	1.89+0.42	3.33
	Tongaat	13.23 (7.16-19.00)	2.15+0.48	1.51
	Gallic acid	11.45 (6.63-38.15)	1.63+0.14	2.62
Larvicidal	Mtunzini	30.50 (17.56-48.60)	2.23+0.54	0.97
	Gingindlovu	39.45 (23.88-63.49)	2.26+0.53	0.87
	Tongaat	39.29 (25.21-60.47)	2.66+0.61	1.17
	$KMnO_4$	40.73 (34.47-58.82)	2.79+0.53	1.62

LC_{50} values in µg/mL with the corresponding 95% confidence intervals (95% CI)

Although, there is no literature report concerning the biological activities of essential oils of *S. pterophorus*, however, the results of this study on antibacterial, antioxidant, cytotoxic activities were in agreement with some previous investigation on the essential oils of several species of the genus *Senecio* [17-19,22,23,44-46]. But, the cytotoxic activity of *S. pterophorus* essential oils which showed significant lethality to *A. salina* nauplii may be an indication of the presence of potent cytotoxic constituents.

4. CONCLUSION

The present study indicated that the essential oils of *S. pterophorus* possessed some biological activities of importance, which needs further investigation to determine the active compounds.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Nordenstam B. Senecioneae and liabeae-systematic review. In the biology and chemistry of the compositae, Heywood VH, Harborne JB, Turner BL, Eds. Academic Press: London, U.K. 1977;2:799-830.
2. Hilliard OM. Compositae in Natal. University of Natal Press. Pietermaritzburg. 1997;385-502.
3. Hutchings A, Scott AH, Lewis G, Cunningham AB. Zulu medicinal plants: An Inventory. University of Natal Press, Scottsville. 1966;328-30.
4. Chamorro L, Caballero B, Blanco-Moreno JM, Cano L, Garcia-Serrano H, Masalles RM, et al. Ecología y distribución de *Senecio pterophorus* (Composite) en la Península Ibérica. Anales del Jardín Botánicode alien species from the European mainland. Anales del Jardín Botán Madrid. 2006;63(1):55-62.
5. Pino J, Afan I, Sans FX, Guierrez C. *Senecio pterophorus* DC. A new alien species from the European mainland. Anales del Jardín Botanic Madrid. 2000; 58(1):188-9.
6. Suau R, Cabezudo B, Rico R, Najera F, Lopez-Romaro JM, Garcia AI. Pyrrolizidine

alkaloids from three Spanish *Senecio* species. Biochem System Ecol. 2002; 30:981-84.
7. Perez-castorena A, Martinez F, Villasenor JL, de Vivai RA. Pyrrolizidine alkaloids from four *Senecio* species. Biochem System Ecol. 2000;28:279-82.
8. Silva CM, Bolzan AA, Heinzamann BM. Pyrrolizidine alkaloids from *Senecio* species. Quim Nova. 2006;29(5):1047-53.
9. Castells E, Mulder PPJ, Pérez-Trujillo M. Diversity of pyrrolizidine alkaloids in native and invasive *Senecio pterophorus* (Asteraceae): Implications for toxicity. Phytochem. 2014;108:137-46.
10. Caño L, Escarre´ J, Vrieling K, Sans FX. Palatability to a generalist herbivore, defence and growth of invasive and native *Senecio* species: Testing the evolution of increased competitive ability hypothesis. Oecologia. 2009;159(1):95-106.
11. Liddell JR, Logie CG. A Re-investigation of the alkaloids of *Senecio pterophorus*. Phytochem. 1993;34(6):1629-31.
12. Lawal OA, Oyedeji AO. Volatile Constituents of African daisy *Senecio pterophorus* DC. from South Africa. Nat Prod Commun. 2010;5(11):1811-4.
13. Cimanga KK, Kambu TL, Apers S, De Bruyne T, Hermans N, Totte J, et al. Correlation between chemical compositions and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. J Ethnopharm. 2002;79(2):213-20.
14. Conforti F, Loizzo MR, Statti G, Houghton P, Menichini F. Biological properties of different extracts of two *Senecio* species. Int J Food Sci Nutr. 2006;57(1):1-8.
15. Dorman HJD, Deans SG. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. J Appl Microb. 2006; 88(2):308-6.
16. Silva GNS, Spader TB, Alves SH, Mallmann CA, Heinzmann BM. Composition and evaluation of the antimicrobial activity of the essential oil of *Senecio selloi* Spreng DC. Rev. Bras. Pl. Med., Campinas. 2013;15(4):503-507.
17. El-Hawary SS, Galal AE, Yousif MF, Kiraollos FN. GC-MS and bioactivity of the essential oil of *Senecio rowleyanus* Jacobs. Phcog Mag. 2008;4(16):273-7.
18. El-Shazly A, Doral G, Wink M. Chemical composition and biological activity of the essential oils of *Senecio aegyptius* var. *discoideus* Boiss. Z Natur. 2002;57c(5-6): 434-5.
19. El-Tantawy ME. Chemical composition and biological activity of the essential oil of *Senecio micanioides* otto cultivated in Egypt. Azhar J Pharm Sci. 2000;26:294-306.
20. Grace MH, Khattab AM. Chemical constituents and molluscicidal activity of *Senecio cineraria.* D. C. Egyptian J Pharm Sci. 1998;39(1/3):253-66.
21. Loizzo MR, Statti R, Tundis GA, Conforti R, Bonesi F, Autelitano M, et al. Antibacterial and antifungal activity of *Senecio inaequidens* DC. and *Senecio vulgaris* L. Phytother Res. 2004;18(9):777-9.
22. Perez C, Agnese AM, Cabrera JL. The essential oil of *Senecio graveolens* (Compositae): Chemical composition and antimicrobial activity test. J Ethnopharm. 1999;66(1):91-6.
23. Rondón M, Araque M, Morales A, Gualtieri M, Rojas J, Veres K, et al. Chemical composition and antibacterial activity of the essential oil of *Lasiocephalus longipenicillatus* (*Senecio longipenicillatus*). Nat Prod Commun. 2006;1(2):113-5.
24. Ugur A, Sarac N, Duru ME. Antimicrobial activity and chemical composition of *Senecio sandrasicus* on antibiotic resistant Staphylococci. Nat Prod Commun. 2009;4(4):579-84.
25. Sharma P, 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):883-6.
26. Albayrak S, Aksoy A, Yurtseven L, Yaşar A. A comparative study on antioxidant and antimicrobial activities of four *Senecio* L. species from Turkey. Int J Sec Metab. 2015;2(1):26-36.
27. Ugur A. Ertem H, Beyatli, Y. Antibacterial properties of *Senecio sandrasicus* on multidrug-resistant *Stenotrophomonas maltophilia.* Pharm Biol. 2006;44(4):253-7.
28. Oyedeji OO, Lawal OA, Shode FO, Oyedeji AO. Chemical composition and antibacterial activity of the essential oils of *Callistemon citrinus* and *Callistemon viminalis* from South Africa. Molecules. 2009;14(6):1990-8.
29. Lawal OA, Ogunwande IA, Opoku AR, Kasali AA, Oyedeji AO. Chemical composition and antibacterial activity of

essential oil of *Warbugia salutaris* (Bertol f.). Chiov from South Africa. J Biol Active Prod from Nat. 2014;4(4):272-7.

30. Lawal OA, Ogunwande IA, Opoku AR, Kasali AA, Oyedeji AO. Chemical composition, antibacterial and cytotoxicity activities of essential oil from the leaves of *Helichrysum odoratissimum* from South Africa. J Essent Oil Bearing Plants. 2015;18(1):236-41.
31. Nevas M, Korhonen A, Lindstrom M, Turkki P, Korkeala H. Antibacterial efficiency of Finnish spice essential oils against pathogenic and spoilage bacteria. J Food Prot. 2004;67(1):199-202.
32. Sonboli A, Babakhani B, Mehrabian AR. Antimicrobial activity of six constituents of essential oil from *Salvia*. *Zeitschrift fur Naturforschung C:* J Biosci. 2006;61(3-4):160-4.
33. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med. 1998;64(8):711-13.
34. Han J, Weng X, Bi K. Antioxidants from a Chinese medicinal herb – *Lithospermum erythrorhizon*. Food Chem. 2008;106(1):2-10.
35. Nagai T, Myoda T, Nagashima T. Antioxidative activities of water extract and ethanol extract from field horsetail (*tsukushi) Equisetum arvense* L. Food Chem. 2005;91(3):389-94.
36. Badami S, Rai SR, Suresh B. Antioxidant activity of *Aporosa lindleyana* root. J Ethnopharmacol. 2005;101(1-3):180-4.
37. Senevirathne M, Kin SH, Siriwardhana N, Ha JH, Lel KW, Jeon YJ. Antioxidant potential of *Ecklonia cava* on reactive oxygen species, scavenging, metal chelating, reducing power and lipid peroxidation inhibition. Food Sci Tech Int. 2006;12(1):27-38.
38. Oyaizu M. Studies on products of browning reactions: Antioxidant activities of products of browning reaction prepared from glucose amine. Japan J Nutr. 1986;44: 307-15.
39. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med. 1982;45(5):31-4.
40. Rafikali AM, Nair MG. Mosquitocidal, nematicidal and antifungal compounds from *Apium graveolens* L. seeds. J. Agric Food Chem. 2001;49:142-145.
41. Abbott WS. A method for computing the effectiveness of an insecticide. J Econ Entom. 1925;18:265-67.
42. Lahlou M. Methods to study the phytochemistry and bioactivity of essential oils. Phytother Res. 2004;18(6):435-48.
43. Vijoen AM, van Vuuren SF, Gwebu T, Demirci B, Baser KHC. The geographical variation and antimicrobial activity of African wormwood (*Artemisia afra* Jacq.) essential oil. J Essent Oil Res. 2006;18(1):19-25.
44. Sharma P, Shah GC. Composition and antioxidant activity of *Senecio nudicaulis* Wall. x DC. (Asteraceae): A medicinal plant growing wild in Himachal Pradesh, India. Natural Product Research. 2015;29(9):883-886.
45. Lone SH, Bhat RA, Haroon M. Bhat HM, Majeed R, Anand R, Hamid A, Khuro MA. Essential oil composition of *Senecio graciliflorus* DC: Comparative nalysis of different parts and evaluation of antioxidant and cytotoxic activities. Phytomedicine. 2014;21:919-925.
46. Singh R, Ahluwalia V, Singh P, Kuma N, Sati OP, 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.
DOI: 10.1080/14786419.2015.1079910

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/14903