Assessment of in vitro activities and chemical profiling of Senecio hoggariensis growing in Algerian Sahara

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Abstract. Arab Y, Sahin B, Ceylan O, Zellaguai A, Olmez OT, Kucukaydin S, Tamfu AN, Ozturk M, Gherraf N. 2022. Assessment of in vitro activities and chemical profiling of Senecio hoggariensis growing in Algerian Sahara. Biodiversitas 23: 3498–3506. The in vitro antioxidant, anticholinesterase, tyrosinase inhibitory, antibiofilm, and anti-quorum sensing activities of the ethyl acetate extract of Senecio hoggariensis, growing in Algerian Sahara, were studied along with its chemical constituents using HPLC-DAD. The chromatographic analysis unveiled seven phenolic compounds, including p-coumaric acid as a major component. Additionally, the extract showed moderate DPPH radical scavenging activity, compared to known standards. At 200 µg/mL, the extract disclosed significant acetylcholinesterase (AChE), butyryl-cholinesterase (BChE) and tyrosinase inhibition rates with respective values of 37.01±1.48%, 18.87±4.18% and 45.99±1.81%. Likewise, the extract exhibited a good antioxidant activity against Candida albicans ATCC 10239 biofilm production with an inhibition ratio of 59.56±0.40%, at 50µg/mL. The anti-quorum sensing by QS-regulated violacein pigment production inhibition test was determined using Chromobacterium violaceum CV026 and CV12472. The swarming motility inhibition assay was determined using Pseudomonas aeruginosa PA01. It is evident from the findings that Senecio hoggariensis could be considered potential antioxidant, anti-QS, and antibiofilm compounds. However, the origin of anti-biofilm and anti-quorum sensing activities of the ethyl acetate extract could be revealed by further studies on the mechanism of action of active compounds that can be isolated via activity-guided fractionation.

Keywords: Antibiofilm, anticholinesterase, antioxidant, anti-quorum sensing, anti-tyrosinase, Senecio hoggariensis

INTRODUCTION

The genus Senecio belonging to the Asteraceae family is widely distributed in tropical and subtropical regions of Africa, America, Asia and Europe, containing more than 1300 species (Tidjani et al. 2013). Most of them are common annual weeds, while some are cactiform and succulent perennials. Their flowers are arranged in clusters at the top of the plants with different colors like white, yellow, red, or purple (Albayarak et al. 2016). Senecio spp. is traditionally used for ornamental purposes and in folkloric medicine to treat wounds. They are reported to have antimicrobial, anti-inflammatory, antiemetic and vasodilator activities (Yang et al. 2011). Besides their importance, certain Senecio species have adverse effects against livestock due to their high toxicity attributed to the presence of pyrrolizidine alkaloids and some furano-eremophilanes. These are the most important characteristic constituents of Senecio plants responsible for more livestock deaths than any other poisonous plants. Besides pyrrolizidine alkaloids (PAs), a comprehensive review of Senecio plants showed that they possess flavonoids, coumarins, phenolic acids, alkalines, terpenoids, and steroids. Moreover, they exhibited potent antimicrobial, antitubercular, anti-inflammatory, antitaster, cytotoxic, antimimetic, antifeedant, and insecticidal properties (Yang et al. 2011). Apart from eremophilanolide derivatives, PAs and sesquiterpenes seem to be one of the most abundant constituents. Regarding bioactivities, antioxidant and antimicrobial activities seem to be the most predominant (Milad 2014).

The limited chemical and biological studies on S. hoggariensis incited us to explore their phenolic compositions and various bioactivities thereof. This species grow in the Saharan mountains in Hoggar, Algeria, Niger (Air), Chad (Tibesti), and Egypt (Gebel Elba and Sinai) (Lebrun 1981). Herein, we report the phenolic constituents of ethyl acetate extract of S. hoggariensis for the first time using HPLC-DAD, followed by antibiofilm, anti-quorum sensing, antioxidant, anti-tyrosinase and anticholinesterase activities.
MATERIALS AND METHODS

Chemicals and instrumentation

The optical densities for bioassays were measured by using SpectraMax340PC® (Microplate reader by Molecular Devices, Silicon Valley, USA). The phenolic profiling of the sample was done using Shimadzu 20AT series (HPLC-DAD (Shimadzu Corporation, Japan). Quercetin, ethylenediaminetetraacetic acid (EDTA), sodium chloride, ferrous chloride, and copper (II) chloride dihydrate (CuCl2·2H2O), were acquired from Merck (Darmstadt, Germany). DPPH (1,1-diphenyl-2-picrylhydrazyl), butylated hydroxytoluene (BHT), β-carotene, α-tocopherol, neocuproine, polyoxyethylene sorbitan mono palmitate (Tween-40), ferrene, ABTS (2,2′-azino-bis (3-ethylbenzothiazoline- 6-sulfonic acid) diammonium salt), linoleic acid, kojic acid, BChE (butyrylcholinesterase) from horse serum (EC 3.1.1.8, 11.4 U/mg) and AChE (acetylcholinesterase) from electric eel (Type-VI-S, EC 3.1.1.7, 425.84 U/mg), mushroom tyrosinase (EC 232-653-4, 250 KU, ≥1,000 U/mg), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), galantamine, butyrylthiocholine chloride, acetylthiocholine iodide, L-DOPA (3,4-dihydroxy- D-phenylalanine), the certificated reference compounds used to screen the chemical constituents of sample were purchased from Sigma-Aldrich GmbH (Steinheim, Germany). Solvents and chemicals were of analytical grade.

Plant collection and extraction

For collection of plant samples, Senecio hoggariensis Batt. & Trab. Plants were collected in April during their flowering time from El-Hoggar mountains (South-West Algeria). Dr. Youcef Halice, Technical Research Centre of Touggourt, identified confirmed the plant identity up to species level. It was deposited under voucher specimen number ZA67 at the laboratory of Biomolecules and Plant Breeding, Larbi Ben M’Hidi University, Oum El Bouaghi, Algeria.

The aerial parts (100 g) of the plant were air-dried, grinded, and macerated with 80% aqueous methanol at room temperature. After filtration, the liquid phase was evaporated under reduced pressure using a rotary evaporator to obtain a solid residue. The obtained material was further dissolved in water and re-extracted using n-hexane, chloroform and ethyl acetate and successively evaporated to dryness under reduced pressure.

Total phenolics and flavonoids contents

The total phenolic contents (TPC) of the extract were calculated using the Folin-Ciocalteau Reagent (FCR) as well as external calibration with Gallic acid. In brief, 2.5 mL of FCR (diluted 1/10 with distilled water) was mixed with 0.5 mL of each extract diluted solution in methanol. After 5 minutes, 2 mL of sodium carbonate aqueous solution Na2CO3 (75 g/L) was added to the mixture and incubated for 30 minutes at 40°C then the absorbance was measured at 760 nm. Using the Gallic acid calibration curve, the results are presented as mg of Gallic acid equivalent (GAE)/g of dry extract. All experiments were carried out in triplicates with averaged results (Suleria et al. 2020). The extract was also analyzed spectroscopically to determine flavonoid content using quercetin (5-20 μg/mL) as standard. One (1 mL) of the extract (1 mg/mL) was added to 1mL of AlCl3 (2%), incubated for 10 min at room temperature. Then, the absorbance was measured at 430 nm, and the results were expressed as (μg QE/mg extract) (Durak and Uçak 2015).

Quantitative analysis of phenolic compounds by HPLC-DAD

The chemical constituents of sample extract were determined by the reverse-phase HPLC-DAD system using a validated method against 27 standards (Tokul-Ölmez et al. 2020). The ODS-3 column (Inertsil, 150 mm × 4.0 mm i.d, 4 μm film thickness) was used to separate the compounds. The column oven temperature was set to 40 °C. Each extract's stock solution (8 mg/mL) was prepared in methanol/water (80/20,v/v). The stock solutions were pre-filtered using a disposable LC disk filter (Agilent 0.45 μm). The mobile phases used are 0.5% acetic acid in water (A) and methanol (B). The gradient elution program was of 40 minutes, as 0-0.01 min (0-20% B); 0.01-2 min (20-60% B); 2-15 min(60-80% B); 15-30 min(100% B); 35-35 min (100-10% B); and 35-40 min (10-0% B). The flow was 1.5 mL/min, and a 20 μL sample was introduced. The inherent compounds were detected using a photodiode array detector (PDA) in the range of 230-350 nm with reference to the UV data and retention time of each commercial standard. Each analysis was performed in triplicate.

Antioxidant activity

Free radical-scavenging activity (DPPH assay)

The antiradical activity of ethyl acetate extract was tested by the DPPH free radical assay (Kozłowska et al. 2016). DPPH is a colored radical that has a maximum absorbance at 517 nm, and upon reduction, its absorbance decreased. Briefly, 0.1 mM DPPH (160 μL) was mixed with 40 μL of the sample solution of various concentrations and incubated for 30 minutes in the dark and the absorbance was measured at the same wavelength. The antioxidant activity of tested extract was compared with the known standards. The DPPH radical scavenging was calculated using the equation given below:

\[
\text{DPPH Free radical scavenging activity (mg/ml)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100
\]

From the inhibitory activity versus concentration graph, the IC50 (μg/mL) values were calculated.

Lipid peroxidation inhibitory activity

The lipid peroxidation inhibition activity of the plant extract was estimated using the β-carotene-linoleic acid test (Şahin 2013). β-carotene (0.5 mg) was dissolved in chloroform (1 mL), 25 μL of linoleic acid, and 200 mg of Tween-40 emulsifier were mixed in a flask. The mixture was evaporated under a vacuum to remove the chloroform, and 100 mL of oxygen-saturated water was added by vigorous stirring. The prepared reagent (160 μL) was added
separately into wells containing 40 μL of extracts. The absorbance at zero time of each reaction was recorded at 470 nm using a 96-well microplate reader. Each reaction was incubated at 50°C for 2 hours. α-tocopherol, BHT, BHA and quercetin were used as standards. The equation below was used to estimate the bleaching rate (R) of β-carotene.

\[ R = \frac{\ln a - \ln b}{t} \]

Where ln: natural logarithm, a is the absorbance at 0 time while b is absorbance after two hours. t is the total time in minutes.

The following equation was used to calculate the percent lipid peroxidation inhibitory activity (AA):

\[ AA(\text{inhibition } \%) = \left( \frac{R_{\text{control}} - R_{\text{sample}}}{R_{\text{control}}} \right) \times 100 \]

The graph presenting inhibitory activity against concentration was used to calculate the IC50 (µg/mL) value.

**Cupric reducing antioxidant capacity (CUPRAC)**

The standard CUPRAC method with slight modifications was adopted (Maryam et al. 2016) and the absorbance was recorded using an ELISA reader. The aqueous solution including 50 μL of CuCl2·2H2O (10 mM), neocuproine (7.5 mM in absolute ethanol), and NH4Ac buffer (100 mM, pH 7.0) was added to 50 μL of sample extract at various concentrations to make 200 μL of final volume then incubated for one hour at room temperature. The absorbance was recorded at 450 nm. The blank contains the same reactants except for the plant extract. The antioxidant standards were used for comparison. The results were expressed as A0.5.

**ABTS cation radical scavenging activity**

ABTS⁺ scavenging activity assay presents some advantages over DPPH scavenging test which is not convenient with water insoluble or bulky structures compounds. Therefore, the ABTS⁺ scavenging activity of the extracts was also verified (Gupta et al. 2016). Briefly, 7 mM of ABTS and 2.45 mM of potassium persulfate were dissolved in water, kept for 16 hours in the dark to provide ABTS⁺ solution. The tested ABTS⁺ solution was prepared by diluting it with ethanol to get an absorbance of 0.700±0.025 at 734 nm in a one cm pathway. To each well containing 40 μL of the extract in methanol of various concentrations, 160 μL of diluted ABTS⁺ solution was added and incubated for ten minutes, then the absorbance was measured at 734 nm. For comparison, quercetin, BHA, BHT, and α-tocopherol were used and each assay was performed in triplicate. The sample’s capability to scavenge ABTS⁺ was calculated using the formula given for the DPPH assay. The results of ABTS⁺ scavenging activity were presented as IC50.

**Metal chelating activity assay**

The ferrous ion chelating potential of extract was measured using the following standard procedure with slight modifications (Kozłowska et al. 2016). To each well containing 40 μL of the extract in methanol at various concentrations, 40 μL of FeCl₂ (0.2 mM) and 40 μL of ethanol were added. Then 80 μL of ferrone (0.5 mM) was added to initiate the reaction. After incubation at room temperature for 10 minutes, the absorbance was measured at 593 nm. The EDTA was used as a chelating standard. The results are presented as inhibition (%) at 100 µg/mL concentrations.

**Anticholinesterase activities**

The inhibition of acetylcholinesterase (AChE; 5.32 × 10-3U) and butyrylcholinesterase (BChE; 6.85 × 10-3U) of the extract was tested using Ellman’s method (Öztürk et al. 2014). In a 96 well plate, each concentration (25-200 µg/mL) of the sample in ethanol (10 µL) was incubated at 25°C for 15 min with 20 µL of enzyme solution and 150 µL of sodium phosphate buffer (100 mM, pH8). After incubation, Ellman’s reagent, DTNB (0.5 mM, 10 µL), and substrates (10 µL) were added to each well to make 200 µL final volume. Then measurement was performed at 412 nm for 10 minutes and galantamine was used as a standard. The percent of both enzymes inhibition was calculated using the following formula.

\[ A\text{ChE/BChE inhibition activity (}\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \]

Where Acontrol is the enzyme inhibitory activity of blank and A sample is the enzyme inhibitory activity of the sample. Each test was conducted in triplicate. The results are presented as inhibition (%) at an extract concentration of 200 µg/mL.

**Determination of tyrosinase inhibitory activity**

In vitro tyrosinase inhibitory potential of plant extract was assessed using mushroom tyrosinase by following the Hearing method (Benso et al. 2018). The L-Dopa was employed as a tyrosinase substrate. Kojic acid was used as a standard to compare the activity. The tyrosinase inhibition (%) at each sample concentration (µg/mL) was calculated as that used in AChE and BChE assays.

**Determination of minimum inhibitory concentrations (MIC)**

The lowest concentration of extract showing no visible bacterial growth is called MIC. It was determined by a microtiter broth dilution assay (CLSI 2006) by using four bacterial strains including *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *E. faecalis* ATCC 29212 and one yeast *C. albicans* ATCC 10239. The Mueller-Hinton Broth (MHB) was used as a test medium, whereas, inoculum density was 5x105 CFU/mL. Before being read, the cell suspensions (100 µL) were incubated separately under aseptic conditions with extract at various concentrations (6.25, 12.5, 25, 50 and 100 µg/mL) at 37°C for one day.
Effect of extract on bacterial biofilm formation

The biofilm-forming ability of tested microorganisms was tested using a microplate biofilm assay with extract at 1/1, 1/2, 1/4 and 1/8 minimum inhibitory concentration (MIC) concentrations (Wang et al. 2017). Briefly, the tested microbes as mentioned for MIC were incubated at static in glucose (0.25%) containing sterile Tryptose Soy Broth (200 µL) at 37°C for 48 hours. After incubation, the wells were drained and washed with water. The crystal violet solution (0.1%) was used to stain the remaining bacteria and washed after 10 minutes with water to eliminate the crystal violet solution. The biofilm formed in each well was suspended with 33% glacial acetic acid (200 µL) and was shaken for 5 minutes. Toa sterile tube, the solution (125 µL) was transferred separately from each well. The volume was completed to 1 mL using sterile distilled water. The absorbance was recorded at 550 nm, and % biofilm inhibition was calculated using the equation below:

\[
\text{biofilm inhibition (96) = } \left( \frac{O D_{550 \text{ sample}} - O D_{550 \text{ control}}} {O D_{550 \text{ control}}} \right) \times 100
\]

Bioassay for quorum-sensing inhibition (QSI) activity using CV026

Method of Koh and Tham (2011) was used to perform quorum sensing inhibition (QSI) activity extract. The bacterial CV026 culture (100 µL) was transferred to the warm molten Soft Top Agar (5 mL). Then, the exogenous AHL source 20 µL of C6-HSL (100 µg/mL) was gently mixed and overlayed onto the solidified Luria Bertani Agar (LBA) plate. After solidification, the 50 µL extracts (sub-MIC concentrations) were transferred to each well and incubated for three days at 30°C. The QSI was visualized by monitoring a cream or white-colored halo around each well against tested CV026 bacteria. Thus, the inhibition zones could be measured to exhibit antimicrobial activity.

Violacein pigment inhibition assay

QSI potential of Senecio plant extract was qualitatively determined against Chromobacterium violaceum ATCC 12472 (Packiavathy et al. 2012). The overnight culture (10 µL) of C. violaceum with 0.400 optical density at 600 nm was transferred to plates that contains LB media (200 µL). The prepared samples with and without sub-MICs of ethyl acetate extract were incubated for twenty-four hours at 30°C. The absorbance was recorded at 585 nm to assess the reduction in the violacein pigments. The formula presented below was used to determine the violacein percent inhibitory activity.

\[
\text{Violacein pigment inhibition (96) = } \left( \frac{O D_{585 \text{ control}} - O D_{585 \text{ sample}}} {O D_{585 \text{ control}}} \right) \times 100
\]

Swarming motility assay

The effect of the extract on the inhibition of Pseudomonas aeruginosa was assessed by following the described procedure (Merritt et al. 2011) with few modifications. The swarming plates were prepared by using D-glucose (0.5%), agar (0.5%), NaCl (0.5%), and peptone (1%). The plates were treated with Senecio extract (50, 75, and 100 µg/mL) followed by inoculation with a fresh culture of P. aeruginosa PA01. A control medium without extract was also prepared for comparison. All the plates were incubated at 37°C in an upright position for eighteen hours. The plate without the extract was maintained as a control. The bacterial growth and extension were measured as swarm motility.

Statistical analysis

Results were calculated and presented as means ± Standard Deviation (n = 3). Data were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test using GraphPad Prism software (version 8.0.1); p values < 0.05 were regarded as significant.

RESULTS AND DISCUSSION

Total phenolics and flavonoids contents

According to the results shown in Table 1, the amounts of total phenolics and flavonoids of ethyl acetate extract of S. hoggariensis were found to be 100.27±0.74 µg GAE/mg extract and 80.06±1.14 µg QE/mg extract, respectively. The extraction yield was 0.28% with respect to dry weight.

High-Performance Liquid Chromatography (HPLC) analysis

The investigation of the phenolic compounds was carried out using a HPLC equipped with PDA detector. The compounds were identified by comparing their spectroscopic characteristics and retention times (RT) with reference compounds. Seven compounds were detected among which p-coumaric is the predominant (10.85 mg/g extracts) followed by caffeic acid (3.38 mg/g). Other constituents were quercitin (0.93 mg/g), 3,4-dihydroxybenzoic acid (0.87 mg/g), 4-hydroxy benzoic acid (0.68 mg/g), vanillic acid (0.38 mg/g) and 6,7-dihydroxy coumarin (0.32 mg/g) (Table 2).

Antioxidant activity

The antioxidant activity was evaluated using five complimentary tests, namely, β-carotene- linoleic acid, ABTS, DPPH, metal chelating, and CUPRAC assays. As shown in Table 3, the results of β-carotene bleaching test are compared with those of BHT and BHA, α-Tocopherol and quercetin. The analysis of variance revealed significant difference between tested samples and the standards. The inhibition of lipid peroxidation was recorded with IC₅₀ values of 18.98±1.50 µg/mL. Since IC₅₀ of extract is lower than 50 µg/mL, the extract could be considered with good potential for lipid peroxidation.

Table 1. Total phenolic content (TPC) and total flavonoid content (TFC) of S. hoggariensis

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>Yield %</th>
<th>Polyphenols a</th>
<th>Flavonoids b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate extract</td>
<td>0.28</td>
<td>100.27±0.74</td>
<td>80.06±1.14</td>
</tr>
</tbody>
</table>

Note: aTotal phenolic content (µg GAE/mg extract); bTotal flavonoids content (µg QE/mg extract); results are expressed as means ± SD (n=3).
Table 2. Retention time, calibration curves, regression coefficient ($R^2$), linearity ranges, LODs and recoveries of phenolic standards at 254 nm

<table>
<thead>
<tr>
<th>Compound</th>
<th>RT $^a$ (min)</th>
<th>Calibration equation</th>
<th>$R^2$ $^b$</th>
<th>Linear range (µg/mL)</th>
<th>LOD $^c$ (µg/mL)</th>
<th>LOQ $^c$ (µg/mL)</th>
<th>Recovery (%)</th>
<th>RSD $^d$ within day (n=7)</th>
<th>RSD between days (n=7)</th>
<th>S. hoggariensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,4-Dihydroxybenzoic acid</td>
<td>14.10</td>
<td>y=76181x-88801</td>
<td>0.9995</td>
<td>3.13-100</td>
<td>3.42</td>
<td>10.35</td>
<td>102.35±4.21</td>
<td>3.19</td>
<td>1.22</td>
<td>0.78±0.08</td>
</tr>
<tr>
<td>4-Hydroxybenzoic acid</td>
<td>19.50</td>
<td>y=111102x+21691</td>
<td>0.9993</td>
<td>1.56-50.0</td>
<td>1.58</td>
<td>4.79</td>
<td>100.82±3.89</td>
<td>4.00</td>
<td>2.41</td>
<td>0.68±0.10</td>
</tr>
<tr>
<td>6,7-Dihydroxycoumarin</td>
<td>21.99</td>
<td>y=34377x-32740</td>
<td>0.9940</td>
<td>5.00-50</td>
<td>3.98</td>
<td>12.07</td>
<td>104.11±5.06</td>
<td>4.94</td>
<td>3.72</td>
<td>0.32±0.06</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>22.37</td>
<td>y=74653x-9634.1</td>
<td>0.9998</td>
<td>1.56-100</td>
<td>1.56</td>
<td>4.68</td>
<td>103.58±4.43</td>
<td>5.06</td>
<td>3.88</td>
<td>0.38±0.02</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>22.94</td>
<td>y=67972x-32965</td>
<td>0.9880</td>
<td>3.00-30.0</td>
<td>4.54</td>
<td>13.75</td>
<td>102.67±4.92</td>
<td>4.01</td>
<td>5.87</td>
<td>3.38±0.15</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>28.43</td>
<td>y=18300x+6153.3</td>
<td>0.9998</td>
<td>6.25-400</td>
<td>5.46</td>
<td>16.56</td>
<td>101.60±2.36</td>
<td>3.14</td>
<td>0.44</td>
<td>10.85±0.31</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>29.93</td>
<td>y=35737x+12977</td>
<td>0.9999</td>
<td>2.34-300</td>
<td>3.96</td>
<td>11.99</td>
<td>100.99±3.54</td>
<td>3.20</td>
<td>0.51</td>
<td>0.93±0.02</td>
</tr>
</tbody>
</table>

Note: $^a$ RT: Retention time of the compound in minutes, $^b$ R2: linearity of the calibration graph, $^c$ LOD: Limit of Detection in mg/mL and LOQ: Limit of Quantification in mg/mL, $^d$ RSD: Percentage. The values expressed herein were the mean ± S.E.M. of three parallel measurements.
Table 3. Antioxidant activity of ethyl acetate extracts of *S. hoggariensis* by β-Carotene-linoleic acid, DPPH*, ABTS**, CUPRAC and metal chelating assays*

<table>
<thead>
<tr>
<th>Extract</th>
<th>β-Carotene linoleic acid assay IC₅₀ (µg/mL)**</th>
<th>DPPH assay IC₅₀ (µg/mL)**</th>
<th>ABTS assay IC₅₀ (µg/mL)**</th>
<th>CUPRAC assay A₅₀ (µg/mL)**</th>
<th>Metal chelating assay Inhibition (%) (at 100 µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. hoggariensis</em></td>
<td>18.98±1.50⁹</td>
<td>46.40±3.95⁹</td>
<td>26.19±0.96⁹</td>
<td>53.10±3.79⁸</td>
<td>28.66±2.70⁸</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>2.10±0.09⁹</td>
<td>12.26±0.07⁹</td>
<td>4.31±0.10⁹</td>
<td>10.20±0.011⁸</td>
<td>NT</td>
</tr>
<tr>
<td>BHT</td>
<td>1.34±0.04⁹</td>
<td>45.37±0.47⁹</td>
<td>4.10±0.06⁹</td>
<td>3.80±0.00³</td>
<td>NT</td>
</tr>
<tr>
<td>Quercetin</td>
<td>1.81±0.11⁹</td>
<td>2.07±0.10⁹</td>
<td>1.18±0.03⁹</td>
<td>NT</td>
<td>44.09±0.87⁷</td>
</tr>
<tr>
<td>EDTA</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>96.50±0.07⁷</td>
</tr>
</tbody>
</table>

Note: *The values (IC₅₀ and A₅₀ and Inhibition%) presented represent means ± SD, followed by the different script letters within the same column indicates significant difference statistically using Tukey test at p<0.05. **IC₅₀ values correspond to the µg/mL concentration of 50% inhibition while ***A₅₀ values correspond to the µg/mL concentration of 0.500 absorbance. NT: not tested

Furthermore, the extract displayed relatively good DPPH radical scavenging activity with IC₅₀ values of IC₅₀: 46.40±3.95 µg/mL. Nonetheless, its scavenging activity was higher than those of α-tocopherol (IC₅₀: 12.26±0.07 µg/mL) and BHT (IC₅₀: 45.37±0.47 µg/mL). In ABTS assay, the studied extract exhibited an IC₅₀ of 26.19±0.96 µg/mL, higher than those of the standards BHT and α-tocopherol with IC₅₀: 4.10±0.06 µg/mL and IC₅₀: 4.31±0.10 µg/mL, respectively.

In CUPRAC assay the antioxidant activity increased with increasing absorbance. The potential of activity was expressed as A₅₀ corresponding to an Absorbance of 0.5 which was calculated through the absorbance versus concentration graph. As seen in Table 3, *S. hoggariensis* extract exhibited moderate cupric reducing antioxidant capacity (A₅₀: 53.10±3.79 µg/mL) than the standard α-tocopherol (A₅₀: 10.20±0.01 µg/mL).

Regarding metal chelating activity, the extract demonstrated an IC₅₀ much higher than 100 µg/mL. *S. hoggariensis* extract exhibited an appreciable metal chelating activity where The percentage inhibition was 28.66±2.70% at 100 µg/mL compared with quercetin (44.09±0.87 µg/mL) and EDTA (96.50±0.07 µg/mL) (Table 3).

Anticholinesterase activity

Ethyl acetate extract of *S. hoggariensis* was subjected to acetylcholinesterase (AChE) and butyryl-cholinesterase (BChE) inhibitory activity tests. The percentage inhibition increased with the increasing concentration. Various concentrations of extract (25-200 µg/mL) were used revealing relatively moderate activity against both enzymes. The results of the highest concentration at 200 µg/mL were reported in Table 4. The extract showed slight AChE and BChE inhibitory activities with respective values of 37.01±1.48% and 18.87±4.18%. Under the same conditions, galantamine highlighted 81.41±1.03% and 75.54±1.05% values against AChE and BChE, respectively.

Tyrosinase inhibition

*S. hoggariensis* extract had moderate inhibitory activity (45.99±1.81%) against tyrosinase enzyme at 200 µg/mL. Under the same conditions, kojic acid displayed 83.6±0.2% (Table 4).

Antimicrobial and antibiofilm activity

The antimicrobial activity of the extract was determined by the broth microdilution method using 96 well plates. The results obtained after evaluating the antimicrobial activity corresponding to the MIC of the extract are shown in Table 5. In this study, two Gram-positive bacteria, two Gram-negative bacteria, and one yeast were used. The MIC values were found to be in the range of 50 to 100 µg/mL against all microorganisms. *S. aureus* showed the highest susceptibility to tested extract, with a MIC of 50 µg/mL. The extract at the MIC and sub-MIC concentrations inhibited biofilm formation by the test microorganisms in various percentages. The highest antibiofilm activity was observed in *C. albicans* ATCC 10239 with 59.56±0.40%, followed by 49.55±1.00 against *S. aureus* ATCC 25923 biofilm production at the 50 µg/mL concentration (Table 5).

Anti-QS potential

Anti-QS activity assay using *Chromobacterium violaceum* (CV026) and Violacein inhibition assay by *C. violaceum* (CV12472). QS bioassay of extract of the plants using CV026 revealed strong anti-QS activities. The ethyl acetate extract of *S. hoggariensis* showed a QS-inhibition zone diameter of 11.00±0.42 mm at 25 µg/mL, as shown in Table 6.

Concerning violacein inhibition assay on CV12472 it is worthy to note that before carrying out the test, MIC values should be determined and the values alongside violacein inhibition percentages are reported in Table 6. In this qualitative analysis, the extract showed inhibition of QS-mediated violacein production in *C. violaceum* ATCC 12472 in a dose-dependent manner. At MIC concentrations, there was 100% inhibition of violacein pigment by the extract. The inhibition decreased for the extract at MIC/16 concentration.

Swarming inhibition assay

The extract was tested for anti-QS potential on swarming motility due to pyocyanin levels against *P. aeruginosa* PA01. The results presented in Figure 1 revealed that the extract positively intervened in the swarming of PA01. Accordingly, a substantial inhibition in the migration of PA01 was achieved. At the highest tested concentration (100 µg/mL), the swarming inhibition with pyocyanin was 26.02±0.21%.
Table 4. Acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and tyrosinase inhibitory activities of the *S. hoggariensis* extract at 200 µg/mL.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>AChE assay (µmol/min/mg)</th>
<th>Enzyme inhibitory activities (inhibition%)</th>
<th>BChE assay (µmol/min/mg)</th>
<th>Tyrosinase assay (µmol/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. hoggariensis</em></td>
<td>37.01±1.48</td>
<td>18.87±1.18b</td>
<td>45.99±1.81b</td>
<td></td>
</tr>
<tr>
<td>Galantamine”</td>
<td>81.41±1.03a</td>
<td>75.54±1.05a</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>Kojic acid”</td>
<td>NT</td>
<td>NT</td>
<td>83.6±0.2</td>
<td></td>
</tr>
</tbody>
</table>

Note: *The values (Inhibition%) represent means ± SD. Followed by the different scripts within the same column indicates significant difference statistically using Tukey test at p<0.05. **Standards used in the studies. NT: not tested.

Table 5. MIC and antibiofilm activity of *S. hoggariensis* extract

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Schwarfg motility (%)</th>
<th>% inhibition on biofilm formation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aeruginosa</em> ATCC 27853</td>
<td>100</td>
<td>59.04±1.9</td>
</tr>
<tr>
<td><em>E. coli</em> ATCC 25922</td>
<td>100</td>
<td>59.02±4.70</td>
</tr>
<tr>
<td><em>S. aureus</em> ATCC 25923</td>
<td>50</td>
<td>49.55±1.00</td>
</tr>
<tr>
<td><em>E. faecalis</em> ATCC 29212</td>
<td>100</td>
<td>58.13±1.00</td>
</tr>
<tr>
<td><em>C. albicans</em> ATCC 10239</td>
<td>50</td>
<td>59.56±0.40</td>
</tr>
</tbody>
</table>

Note: =: No inhibition

Table 6. Screening of ethyl acetate of *S. hoggariensis* for anti-quorum sensing activity against *C. violaceum* CV026, inhibition of violaein production against *C. violaceum* CV12472

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>MIC against CV026</th>
<th>Anti-quorum sensing activity</th>
<th>MIC against CV12472</th>
<th>Inhibition of violaein production</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>11.00±0.42b</td>
<td></td>
<td>50</td>
<td>100±0.00</td>
</tr>
<tr>
<td></td>
<td>7.00±0.49b</td>
<td>MIC/2</td>
<td>61.73±1.00</td>
<td></td>
</tr>
<tr>
<td><em>S. hoggariensis</em></td>
<td>MIC/4</td>
<td>-</td>
<td>28.4±0.59</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MIC/8</td>
<td>-</td>
<td>10.42±1.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MIC/16</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Note: =: No inhibition the values (MIC) represent means ± SD. (QS inhibition zone diameters in mm)*

The Folin–Ciocalteu method and the aluminum chloride colorimetric method for determining total content of phenols and flavonoids, respectively, are quick and accurate assays, giving crucial information regarding phytochemical quantities. However, these approaches do not provide a complete picture of phenolic compound quantification in complicated samples. Therefore, the phenolic profile of *S. hoggariensis* ethyl acetate extract was investigated further using HPLC-DAD and the results were presented in Table 4. Our results revealed the presence of mixture of bioactive phytochemicals from phenolic acids, coumarins to flavonoids. The phenolic composition of *S. hoggariensis* is still limited (Lahlou 2014), which allowed to identify the presence of certain flavonoids like Q 3-glucoside, I 3-rutinoside and I 3-mono sulphate in traces.

The ethyl acetate fraction demonstrated a significant potent antioxidant capacity. This is the first contribution of the antioxidant activity of *S. hoggariensis*, therefore, it is important to compare data obtained with other plants under the same genus. Significant antioxidant activities were recorded in ethyl acetate fractions for *S. inaequidens*, *S. vulgaris*, (inhibition of DPPH equal to 61.60% and 44.57% of inhibition, respectively, at concentration of 0.31 mg/mL
A study on the activity of Senecio hoggariensis

(Conforti et al. 2006a), S. gibbosus (IC50 of 0.01 mg/mL on DPPH) (Conforti et al. 2006b) and in S. angulatus (IC50 of 21.85 µg/mL on DPPH) (Bousetta et al. 2021). These findings showed higher inhibition than that obtained in the present study. The diverse procedures utilized to obtain the ethyl acetate fraction can be used to explain these dissimilarities. Conforti et al. (2006b) extracted the plant materials with methanol; then the methanolic extract was acidified with 2.50% H2SO4 and partitioned with n-hexane, dichloromethane and ethyl acetate. Conversely, in our study the fractionation steps were carried out without acidification. The antioxidant activity of the extract can be attributed to the content of p-coumaric acid and other phenolics such as rutin elicagic acid and quercetin. P-coumaric acid is a phenolic acid of the hydroxyxinnamic acid family, synthesized from phenylalanine and tyrosine. It has antifungal, antiviral, anti-melanogenic, antioxidant and anti-inflammatory effects and various biological activities. It is also a well-documented antioxidant, known to reduce oxidative stress and have appreciable radical scavenging activity (Yue et al. 2018).

The use of cholinesterase inhibitors is a suitable and effective way to treat neurological illnesses cognitive symptoms (Tamfu et al. 2019). The ability of the plants to inhibit cholinesterase enzymes indicated its potential use in remediying Alzheimer disease. Our results showed that the ethyl acetate extract presented a moderate inhibitory activity against AChE, meanwhile, it was found to be low against BChE enzymes. Tyrosinase is an essential enzyme responsible for the initiation of browning of freshly harvested fruits. It is a copper-containing multifunctional enzyme belonging to the oxidase group that catalyzes the initial two steps of mammalian melanogenesis. Similarly, the hyperpigmentation of human skin is undesirable and is considered comparable to the enzymatic browning of fruits. This has forced researchers to seek novel and more compelling tyrosinase inhibitors for their safe use in functional foods and harmless cosmetics (Chang 2012). The present extract displayed moderate anti-tyrosinase activity in comparison with Kojik acid standard. There is no research on antityrosinase activities of Senecio spp. so far in the literature, to our knowledge, this is the first review on tyrosinase inhibitors.

The antimicrobial activity of extract could be related to their flavonoids and phenolic acid contents. In particular p-coumaric acid was quantified as a major compound in the Senecio sp., p-coumaric acid is known to have a good antimicrobial activity not only against E. coli but also against other Gram-negative bacteria such as Salmonella typhimurium and Shigella dysenteriae. It can alter the cell membrane permeability and bind DNA, causing increased cell membrane permeability, consequently damaging barrier function due to loss of cytoplasmic contents. The p-coumaric acid binds with anionic phosphate of a double helix of bacterial DNA and creates a groove in the helix. This adversely affects the genetic process, including replication, transcription and expression of a bacterium. Due to these facts, the p-coumaric acid exhibited a dual mechanism towards bactericidal activity by disrupting bacterial cell membranes and binding the genomic DNA of bacteria to hinder cellular functions, ultimately causing cell death (Lou et al. 2012).

The indiscriminative use of antibiotics to treat bacterial infections is a serious issue causing the development of drug-tolerant strains with persistent and more severe infections, this is due to the formation of highly resistant biofilms by multi-drug resistant strains ( Husain and Ahmad 2013). Therefore, QS inhibition can be considered an advanced strategy to control microbial pathogenesis. Thus, the quorum sensing inhibition (QS) protocols could play a vital role to biomonitor organisms with easily detectable QS responses (Saurav et al. 2017). Herein, the inhibition of QS was detected by the presence of colorless zone (s). Similarly, viable cells around the wells could be differentiated from the growth inhibition zone (antibacterial activity). The swarming migrations of bacterial strains have a significant role in the formation of QS-mediated drugs. The extracts inhibited swarming motility in P. aeruginosa PA01. To date, no studies were reported on the effect of Senecio spp, on the production of violacein or any other QS-mediated phenomenon. However, certain phenolic compounds amongst other metabolites in these plants could be responsible for the observed anti-QS activity. Naturally occurring phenolic compound p-coumaric acid has already been shown to interact with bacterial quorum sensing, sometimes by triggering or by inhibiting the QS-process system (Othman et al. 2019).

In conclusion, the phytochemical analysis and antioxidant, anticholinesterase, tyrosinase inhibitory, anti-QS potential properties of ethyl acetate of S. hoggariensis growing in Algeria desert were reported for the first time. Using HPLC-DAD analysis, seven compounds were identified including p-coumaric acid as the major constituent. Considering the results obtained, it can be concluded that the plant possessed relatively potent radical scavenging capacity while showing moderate enzyme inhibitory activity in tyrosinase and acetylcholinesterase inhibitory activities. It can be concluded that p-coumaric acid is mainly responsible for critical antioxidant activity. It also possessed high antimicrobial activity and potential inhibition of biofilm formation. The present study also demonstrated the anti-QS properties of S. hoggariensis extract against C. violaceum CV 026 grown in the presence of C6-HSL. The tested plant extracts inhibited the Violacein synthesis in C. violaceum CV 12472 successfully. The said bioactivity of extracts can be mainly attributed to p-coumaric acid, which demonstrated anti-QS activity against C. violaceum. Senecio hoggariensis could be considered for potential antioxidant, anti-QS, and biofilm compound. However, the origin of anti-biofilm and anti-quorum sensing activities of the ethyl acetate extract could be revealed by further studies on the mechanism of action of active compounds that can be isolated via activity-guided fractionation.

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