

In Vitro Effect of Plant Parts Extract of *Senecio glaucus* L. on Pathogenic Bacteria

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Abstract: *Senecio glaucus* L. is an annual herb that grows in several Egyptian desert habitats. The diversity of habitats inhabited by this species, as well as its distribution, chemical composition, and biological activity, are all unknown. This research aimed to examine the chemical composition of *S. glaucus* from various environments in Egypt, as well as the antioxidant and antimicrobial activities. The general assessment of the analytical results for different parts of *S. glaucus* showed that the capitula and leaves in both inland and coastal samples were rich in bioactive constituents than the other parts as following (capitula > leaf > root > stem). Based on the results of IC₅₀, the antioxidant properties of the eight parts of two samples follows the sequence capitula > root > leaf > stem for the coastal sample, and capitula > leaf > stem > root for the inland sample. The IC₅₀ values ranged from 25.94 to 41.20 mg/ml in coastal sample, where the IC₅₀ values ranged from 28.02 to 42.83 mg/ml in desert sample, compared to ascorbic acid (IC₅₀ = 13.30 mg/ml). The antimicrobial potential of MeOH extracts of *S. glaucus* parts collected from different habitats exhibited different inhibitory spectrum behavior with varying degrees of inhibition against six Gram-positive bacteria and four Gram-negative bacteria. In both coastal and inland samples, the *E. coli* inhibition zone was the most susceptible bacterium. Whereas, in the case of the coastal sample, the inhibition zone of *B. subtilis* was the most sensitive bacterium. The results of the antibacterial test were compared with 3 standard antibiotics.

Keywords: *Senecio*; Asteraceae; phytochemical; antioxidant; antimicrobial.

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1. Introduction

Millions of people worldwide depend on natural habitats and the wild plants that thrive there, which often act as a buffer against drought, scarcity, and hunger and play critical roles in maintaining livelihoods and well-being. This is especially true for those who live in the rural areas of many developing countries [1,2]. Hippocrates, who taught the art of healing by using plant-based medicines, used plants and their extracts to treat diseases around 460-370 BC. [3]. Medicinal plants are highly beneficial to people's and communities' well-being [4].

Although most research focuses on sustainable development and the need for advanced clean technology, returning to nature's potential for discovering and creating new pharmacological agents from renewable resources may be viable [5]. The main reason for this is that these natural derivatives have specific properties, including continuous immunomodulatory activity with high selectivity and efficacy. Generally, medicines contain just one active substance, synthetically, whereas medicinal plants, on the other hand, are simply a synergistic combination of dozens, if not hundreds, of natural chemicals [6,7]. Natural chemicals (secondary metabolites) are essential because they are used in various sectors,

including pharmaceuticals, cosmetics, agricultural and industrial products, and dietary supplements [8-10]. Additionally, medicinal plants contain a high concentration of vitamins and minerals easily absorbed by the human body [6-11].

Asteraceae family (Compositae) is an extremely large and common Flowering plant family of 23,600 currently known species, divided into 1,620 genera and 13 subfamilies, found in temperate and tropical climates, with a strong presence in the Mediterranean and Western Asia. Several herbs were once used to treat microbial infections in traditional medicine [12]. In the flora of Egypt, Asteraceae is represented by about 228 species in 98 genera [13]. Many family members have commercial importance for human usage as; food, ornamentals, oil production, insecticides, medicinal practices, and industrial applications [14].

In Egypt, *Senecio flavus*, *S. glaucus*, *S. vulgaris*, *S. aegyptius*, *S. belbeysius*, and *S. hoggariensis* are found among the nearly 1250 *Senecio* species found worldwide [15]. This genus's botanical, pharmacological, and toxicological properties are significant [16-18]. In Egypt, *Senecio* species found in desert wadies and sandy plains are used as a central nervous system sedative, emetic, and diuretic [19]. *Senecio glaucus* L. (vernacular name, Morrar) is an annual herb with two subspecies (subsp.) that grow in Egypt. The first subsp. is *S. glaucus* subsp. *glaucus* and the second is *S. glaucus* subsp. *coronopifloius* (Maire) C. Alexander. Subsp. *coronopifloius* grow in dry, acidic soils along the coast, desert wadis, and the edges of agriculture and it is much more widespread throughout Egypt than subsp. *glaucus* [15].

A literature review on the phytochemical investigation of the aerial parts of *Senecio* species indicates a large variety of sesquiterpenoids [20], diterpenoids [21], triterpenoids [22], volatile oils [23], and alkaloids [20,24]. The different extracts isolated from *Senecio* showed antioxidant, antimicrobial, insecticidal antiviral properties, anti-inflammatory *in vitro* [25-28]. Therefore, this work aimed to examine the chemical composition of *Senecio glaucus* subsp. *coronopifloius* from different Egyptian habitats and investigate their antioxidant and antimicrobial properties for better exploitation of these natural products.

2. Materials and Methods

2.1. Plant material collection and extract preparation.

The entire plant of *Senecio glaucus* subsp. *coronopifloius* in the flowering stage was collected in March 2020 from Wadi Araba (Eastern Desert) and Gamesa City at Mediterranean coastal. Dr. Yasser A. El-Ameir, Lecturer of Plant Ecology, Botany Department, Faculty of Science, Mansoura University, Egypt, characterized the plant, according to Bolous [15]. A voucher specimen was deposited in the faculty of Sciences, Mansoura University.

The plant sample was handily cleaned, washed several times with distilled water, divided into four portions root, stem, leaf, and flower, dried for 24 hours at 55-60 °C in an air-forced oven to reduce moisture content before grinding. 200 grams of each dried plant part was soaked separately in 85% methanol and shake periodically. The dried residue was dissolved in dimethyl sulfoxide (DMSO) for further use after the extracts were filtered and evaporated.

2.2. Antibacterial Bioassay.

2.2.1. Tested bacteria.

The different extracts of *Senecio glaucus* were tested against four gram-negative bacteria, including *Klebsiella pneumoniae* (ATCC10031), *Escherichia coli* (ATCC10536),

Salmonella typhi (ATCC25566), and *Pseudomonas aeruginosa* (ATCC9027) as well as six gram-positive bacteria, including *Streptococcus epidermidis* (EMCC1353t), *Staphylococcus aureus* (ATCC6538), *Bacillus subtilis* (DMS1088), *Listeria monocytogenes* (ATCC19116), *Bacillus cereus* (DMS30054), and *Streptococcus pneumoniae* (LAB3672).

2.2.2. Antibacterial activity.

Filter paper discs with a diameter of five milliliters were sterilized and immersed in the prepared plant extracts overnight before being loaded onto the nutrient agar medium seeded with the pathogenic microorganisms under investigation for the antibacterial assay [29]. After the agar plates had been incubated for 24 hours at 37°C, the inhibition zone diameter (mm) was measured.

2.3. Qualitative phytochemical screening.

The phytochemical components were identified using standard procedures as defined by Sofowora [30], Trease and Evans [31], and Harborne [32].

2.4. Quantitative determination of the chemical constituents.

Total phenolics, flavonoids, and alkaloids were determined using assays developed by Chlopicka *et al.* [33], Stankovic [34], and Jasuja *et al.* [35]. Obadoni and Ochuko [36] identified a method for evaluating saponins content, while tannins were defined by Van Buren and Robinson [37].

2.5. Antioxidant activity.

The free radical DPPH (1,1-diphenyl-2-picrylhydrazyl) was used to calculate antioxidant activity [38]. One milliliter of 0.15×10^{-3} M DPPH was applied to one milliliter of various concentrations of prepared extracts (50, 40, 30, 20, 10, and 5 ppm). One milliliter of DPPH was mixed with one milliliter of the solvent to make a control. The absorbance was estimated at 517 nm after thirty minutes of incubation at room temperature in the absence of light. IC₅₀ values were graphically determined, and the antioxidant activity was expressed as:

$$\% \text{ Radical scavenging activity} = \left[1 - \frac{A_{\text{sample}}}{A_{\text{control}}} \right] * 100$$

2.6. Statistical analysis.

The experiments were done in triplicates, and mean \pm standard deviation (M \pm SD) was measured. CoStat (CoHort Software, www.cohort.com, USA, version 6.311) was used to analyze the antimicrobial and antioxidant results, which was done using one-way ANOVA and Duncan's test at a probability level of 0.05.

3. Results and Discussion

3.1. Inhibitory effect of medicinal plant.

The development of multidrug-resistant pathogenic bacteria in humans and animals, as well as the negative side effects of certain antibiotics, has sparked interest in developing and finding new plant-based antibiotics [39]. In this experiment, the antimicrobial potential of

methanolic extracts of *Senecio glaucus* parts (root, stem, leaf, and capitula) collected from different habitats (coastal and inland desert) exhibited a wide variety of inhibitory spectrum behaviors against six Gram-positive bacteria and four Gram-negative bacteria, as measured by the diameters of inhibition zones formed by the extracts (Tables 1 & 2).

In the case of Gram-negative bacteria, the inhibition zone of *Escherichia coli* was found to be the most susceptible bacterium in the current study's coastal and inland desert samples. The most potent inhibitor of MeOH extract was *Escherichia coli* (root: 20 and 25mm, stem: 15 and 10mm, leaf: 15mm each, capitula: 21 and 10 mm) for coastal and desert samples, respectively. In comparison, *Pseudomonas aeruginosa* had negative results in the case of MeOH extracts of root and leaf for coastal sample, Although the findings for *Klebsiella pneumoniae* were negative in the case of MeOH extracts of stem for desert sample (Table 1). The results were compared to three standard antibiotics; *Escherichia coli* was shown to be the most active inhibitor of ampicillin, cefotaxime, and tetracycline antibiotics (20, 30, and 20mm, respectively), followed by *Klebsiella pneumoniae* (5, 20, and 20mm, respectively). *Pseudomonas aeruginosa* was found to be ampicillin and tetracycline antibiotic-resistant, while *Salmonella typhi* was found to be ampicillin-resistant (Table 1).

Table 1. The antibacterial activities are represented by the inhibition zone diameter(mm*) of the extracted MeOH from *Senecio glaucus* and standard antibiotics.

Plant sample	Plant parts	Gram-negative bacteria			
		<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	<i>Salmonella typhi</i>
Coastal sample	Root	20	10	NA	19
	Stem	15	10	6	15
	Leaf	15	8	NA	1
	Capitula	21	10	6	11
Desert sample	Root	25	12	8	7
	Stem	10	NA	7	1
	Leaf	15	13	6	5
	Capitula	10	10	7	7
Standard antibiotic (10 mg L ⁻¹)					
Ampicillin		20	5	NA	NA
Cefotaxime		30	20	10	10
Tetracycline		20	20	NA	10

*Values are average (n = 3), NA: Not active.

Table 2. The antimicrobial activities are represented by the inhibition zone diameter (mm*) of the extracted MeOH from *Senecio glaucus* and standard antibiotics.

Plant sample	Plant parts	Gram-positive bacteria					
		<i>Bacillus cereus</i>	<i>Bacillus subtilis</i>	<i>Listeria monocytogenes</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>	<i>Streptococcus pneumoniae</i>
Coastal sample	Root	NA	21	NA	NA	7	8
	Stem	NA	10	7	NA	6	6
	Leaf	NA	8	NA	NA	11	10
	Capitula	NA	8	6	NA	7	6
Desert sample	Root	NA	15	NA	NA	7	6
	Stem	NA	12	NA	NA	7	6
	Leaf	NA	19	NA	NA	7	7
	Capitula	NA	8	NA	NA	7	7
Standard antibiotic (10 mg L ⁻¹)							
Chloramphenicol		5	20	25	30	10	10
Gentamicin		10	5	20	22	20	11
Penicillin		10	23	20	20	20	15

*Values are average (n = 3) , NA: Not active

In this study, the MeOH extracts from two samples (coastal and desert) showed promising inhibitory activity against Gram-positive bacteria *B. subtilis*. The most potent

inhibitor of MeOH extract was *B. subtilis* (root: 21 and 15mm, stem: 10 and 12mm, leaf: 19mm, capitula: 8 mm each) for coastal and desert samples, respectively. Whereas *S. epidermidis* is the most active inhibitor of leaf MeOH extract (11mm) for the coastal sample. Moreover, in all MeOH extract from two samples of *S. glaucus*, the pathogens *S. pneumoniae* and *S. epidermidis* were the most susceptible bacteria. In contrast, *B. cereus*, *L. monocytogenes*, and *S. aureus* tested negative in root and leaf extracts for the coastal sample and all extracts from all sections of the desert sample. Furthermore, *B. cereus* and *S. aureus* were found to be immune to MeOH extracts from the stem and capitula for coastal samples (Table 2). The results were compared with three standard antibiotics; *S. aureus* was the most active inhibitor (30 and 22 mm) of chloramphenicol and gentamicin, respectively, and *B. subtilis* was the most active inhibitor of penicillin (23 mm), followed by *L. monocytogenes* (Table 2).

The overall susceptibility of *E. coli* (G-ve) and *B. subtilis* (G+ve) pathogens confirmed the findings of a previous preliminary investigation against *E. coli* and *B. subtilis*, respectively [40-42]. Also, Elsayed *et al.* [43] reported that *Calotropis procera* extract was found to have a major inhibitory effect against two Gram-positive (*Bacillus subtilis* and *S. aureus*) and two Gram-negative (*E. coli* and *P. aeruginosa*) bacteria. Based on the findings, gram-negative bacteria seem to be more susceptible to *Senecio glaucus* extracts than Gram-positive bacteria. Our previous studies on compounds isolated from wild species are in agreement with these findings [10,28,44]. The difference in sensitivity between Gram-negative and Gram-positive bacteria may be due to differences in cell wall structure [45,46].

Since most plants have an amazing ability to produce a large range of secondary metabolites, such as alkaloids, glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinones, and coumarins, many wild plants exhibited inhibition zones of various diameters toward pathogens [47]. Plant-derived antimicrobial compounds are derived from these biomolecules [48]. In the treatment of bacterial infections, some natural products are beneficial. *S. glaucus* reportedly exhibits significant cytotoxic and antioxidant activities [49,50]. *S. glaucus* also produces bioactive compounds such as phenolics, saponins, flavonoids, and tannins, as well as certain volatile essential oils [28,51,52].

3.2. Phytochemical constituents.

Phytochemical screening and qualitative of the powder and crude extract of *S. glaucus* parts (root, stem, leaves, and capitula) were performed. The results were recorded in Table 1. In this study, the use of different plant parts revealed different responses to the presence of phytoconstituents, and the terms of scores are used as -,+,++,+, according to the intensity of color or precipitates generated. As a result, the qualitative estimation of the bioactive compounds in the parts of *S. glaucus* was observed. Within the same sample and between the coastal and the inland (desert) samples, *S. glaucus* displayed substantial variance. The two samples were rich in varying amounts of alkaloids, flavonoids, phenols, saponins, and tannins (Table 1). However, two samples have reported traces or absence of phytoconstituents. Glycosides and steroids were found in trace amounts (+) in both samples. Anthraquinones are absent (-) from the all-plant parts, either coastal or inland samples, whereas terpenoids are present in the stem and capitula parts and are absent from the root and leaf parts in both plants samples (Table 1). Several factors that influence the extraction of bioactive compounds from natural flora, including extraction method, raw materials, extraction solvent, plant species and age, and soil type, have to affect the phytochemical composition [53,54].

Table 3. Qualitative phytochemical analysis of four parts of *Senecio glaucus* collected from the Egyptian desert.

Screening test	<i>S. glaucus</i>							
	Costal sample				Desert sample			
	Root	Stem	Leaf	Capitula	Root	Stem	Leaf	Capitula
Alkaloids	+	+	+	++	+	+	+	++
Flavonoids	+	+	++	++	+	+	++	+++
Phenols	++	++	++	+++	++	+	++	++
Saponins	+++	++	+	+++	++	+	++	++
Tannins	++	+	+	++	++	+	+	+
Steroids	+	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+	+	+
Anthraquinones	-	-	-	-	-	-	-	-
Terpenes	-	+	-	+	-	+	-	+

A positive mark (+) indicates the presence of the phytochemical. A negative mark (-) indicates the absence of the phytochemical.

The general assessment of the analytical results for different parts of *S. glaucus* showed individual specificity of each studied part and the rich, diverse spectrum of secondary metabolites differing from one another in both coastal and inland (desert) plant samples; and revealed that the capitula and leaves parts of both the inland and coastal samples were a rich source of saponins, tannins, phenols, flavonoids, and alkaloids than the other parts as following (capitula > leaf > root > stem); On the other hand, the inland plant sample produces comparable findings to the coastal sample, but with lower values (Figure 1). The highest total content of saponins, tannins, phenols, flavonoids, and alkaloids was found in plant costal sample was found in capitula (22.10, 14.75, 13.37, 7.08, and 6.60 mg/g) followed by leaf (19.75, 12.40, 11.02, 6.73 and 4.25 mg/g); Similar results were reported for inland sample capitula (20.15, 12.80, 10.42, 5.13 and 3.62 mg/g) followed by leaf (17.80, 10.45, 9.07, 4.78 and 2.30 mg/g) as shown in Figur 1. In this study, the phytochemical analysis of *S. glaucus* was significantly higher than that of El-Amier *et al.* [28] on the same species from the coastal desert. Furthermore, the total phenolic contents of *Senecio* plants grown in Egypt and Turkey were comparable [49,55]. These differences in bioactive constituents may be attributed to environmental variance, whereas the phytochemical composition has been shown to be influenced by geographical variability, temperature, and soil type [53,54,56].

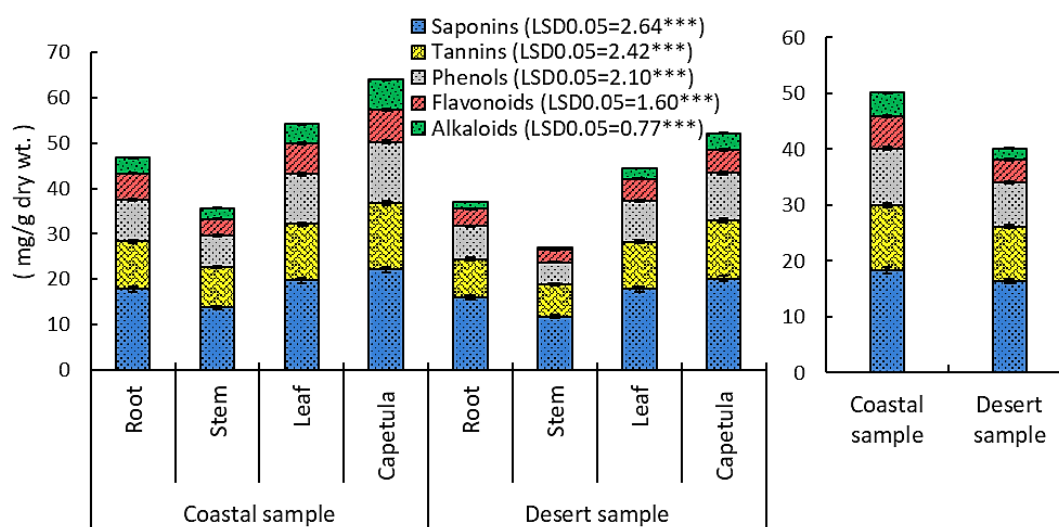


Figure 1. Secondary compounds (mg g⁻¹ dry wt) of *Senecio glaucus* collected from the Egyptian desert.

3.3. Antioxidant activity.

The antioxidant activity of methanolic extracts from *Senecio glaucus* (root, stem, leaf, and capitula) was calculated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals, with the concentration of an antioxidant required to reduce the initial levels of DPPH by 50% (IC₅₀). The IC₅₀ inverted the antioxidant force, whereas the lower the IC₅₀, the higher the antioxidant activity [57]. In this experiment, ascorbic acid was used as a standard compound.

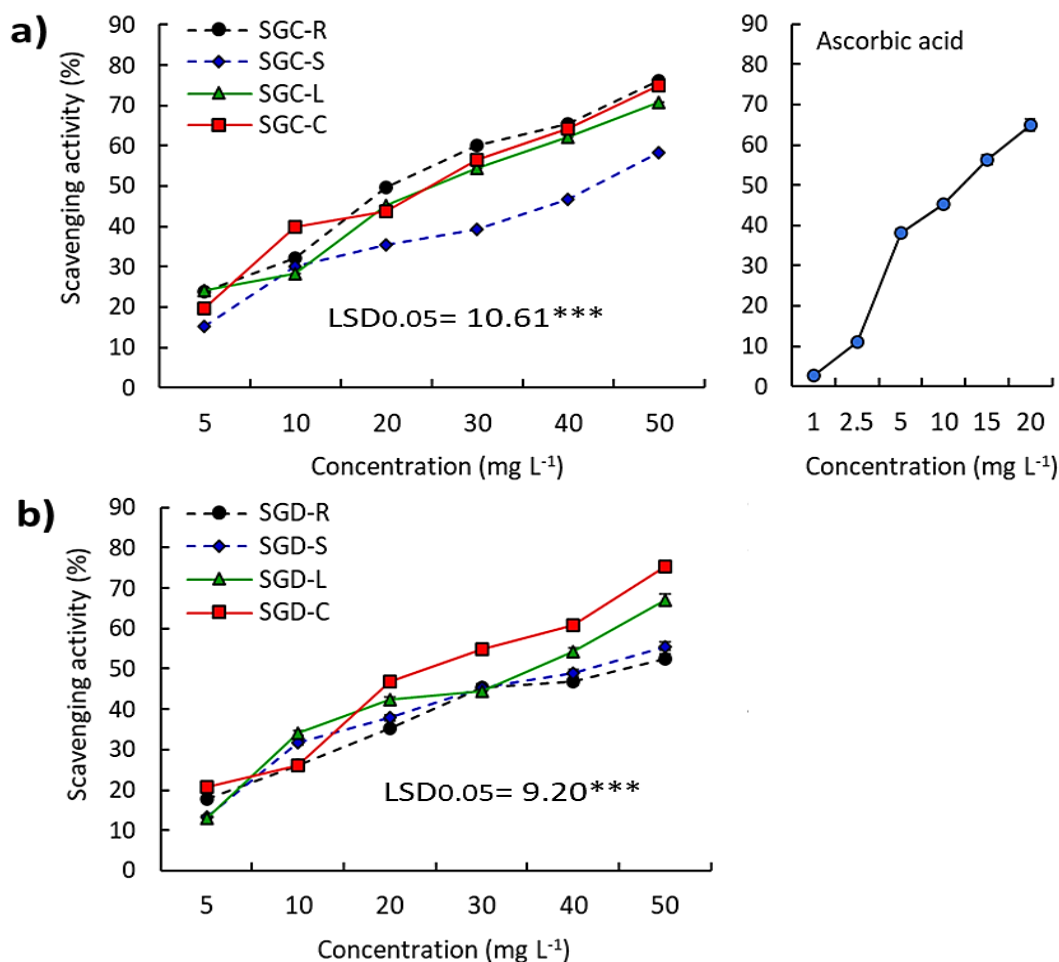


Figure 2. Scavenging activity percentage of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) by MeOH extract of *Senecio glaucus* collected from a) the coastal area and b) the inland desert as well as ascorbic acid as standard. (SGC-R: *S. glaucus* coastal root, SGC-S: - stem, SGC-L: -leaf, SGC-C: -capitula, SGD-R: *S. glaucus* desert root, SGD-S: -stem, SGD-L: -leaf, SGD-C: -capitula).

According to the findings, methanolic extracts of *Senecio glaucus* had an antioxidant activity that was dose-based ($P \leq 0.05$) and comparable to ascorbic acid as a reference norm (Figure 2). At 50 mg/ml, the extracts of root, stem, leaf and capitula showed scavenging activities of 76.17%, 58.23%, 70.68% and 74.97% for coastal sample, and 52.61%, 55.56%, 67.20% and 75.23% for inland sample, respectively. However, the lowest concentration (5 mg/ml) shows the lowest antioxidant activity in the two samples (Figure 2).

Based on the results of IC₅₀, the antioxidant activity of the eight parts of two samples follows the sequence capitula > root > leaf > stem for coastal sample, and capitula > leaf > stem > root for inland sample (Figure 3). In the present study, the IC₅₀ values ranged from 25.94 to 41.20 mg/ml in the coastal sample, where the capitula extract showed the highest antioxidant activity by IC₅₀ = 25.94 mg/ml, while the stem extract showed the lowest antioxidant activity by IC₅₀ = 41.20 mg/ml. On the other side, the IC₅₀ values ranged from 28.02

to 42.83 mg/ml in the desert sample, where the capitula extract showed the highest antioxidant activity by $IC_{50} = 28.02$ mg/ml, while the root extract showed the lowest antioxidant activity by $IC_{50} = 42.83$ mg/ml (Figure 3). All the tested extracts have shown antioxidant scavenging activities but with values higher than that of ascorbic acid ($IC_{50} = 13.30$ mg/ml).

S. glaucus MeOH extract could be attributed to the antioxidant activity of major compounds, such as volatiles [51,52], phenolics, saponins, flavonoids, and tannins [28,52], antimicrobial [28], cytotoxic [49], antioxidant and anticancer properties [50]. Besides, the MeOH extract from *S. glaucus*, which grew in Egypt, has been found to have antioxidant activity using the DPPH method, $IC_{50} = 79.57 \pm 0.74$ μ g/mL. Species of the genus of *Senecio* are known to be rich in alkaloid and phenolic composites [24,49,55]. Recent studies have demonstrated the significant contribution of many flavonoids, phenolic, and alkaloids to the full antioxidant activity of many medicinal plants [10,58].

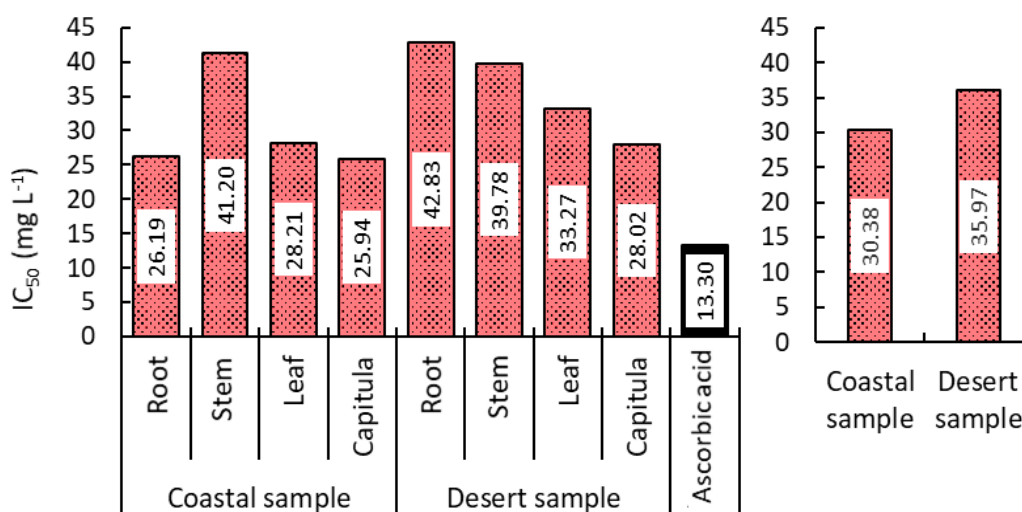


Figure 3. IC_{50} values of the different parts of *Senecio glaucus* collected from the Egyptian desert and ascorbic acid (standard).

4. Conclusions

Finally, *Senecio glaucus* is major wildlife used in traditional medicine for the treatment of many diseases. This study demonstrated that *S. glaucus* can become a sustainable source of antimicrobials because of the good inhibitory action against *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Bacillus subtilis*, and *Streptococcus pneumoniae*. *S. glaucus* could also serve as an advantageous source for antioxidants and green materials with antioxidant scaping (30.38-35.97 mg/ml) but higher than ascorbic acid levels ($IC_{50}=13.30$ mg/ml). Besides, the phytochemicals of *S. glaucus* can be used as a raw material to produce cheaper pharmaceuticals and many more important commercialized products of public use.

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Conflicts of Interest

The authors declare no conflict of interest.

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