Phytochemical and Biological Studies of *Senecio glaucus* subsp. *coronopifolius*

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ABSTRACT

Compositae family is not just an extensive family, but, as expected, a different family. *Senecio* genus is the biggest genus in the family. In the present study, a phytochemical screening of main secondary metabolites present in *S. glaucus* extracts has been done. In addition to, the antimicrobial, antioxidant and reducing power activity have been measured.

Keywords: Antimicrobial, antioxidant, phytochemical screening, Reducing power, *Senecio glaucus*.

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I. INTRODUCTION

Plants are serving the human beings in number of ways, therefore they are considered as gift from the nature for them. Their uses as medicine have been discussed from a long time. These plants based natural chemicals can be extracted from different parts of the plant, which reveals that every part of the plant contains these biologically active compounds (Tiwari *et al.*, 2011). The medicinal plants got considerable importance according their antibacterial, antimalarial, antipyretic, anti-inflammatory, antiviral, anticancer, and antifungal activities (Piero *et al.*, 2011). *Senecio glaucus* subsp. *coronopifolius* [Asteraceae] is significantly distributed and have been used in folk medicine for the treatment of different ailments particularly treatment of cancer, cough suppressant, eczema inflammation, and muscular pain. The Plant has been checked for its secondary metabolites; alkaloids (Singh *et al.*, 2017); sesquiterpenoids, monoterpeneoids (Bohlmann *et al.*, 1985); diterpenoids (Mohamed, 2015); triterpenoids (Torres *et al.*, 1998); essential oils (Yan *et al.*, 2011), and phenolic compounds (Juarez *et al.*, 1995). Asteraceae (Compositae) is an advanced and botanically highly specialized family of primarily herbaceous plants. In many respects, evolution has occurred, and the main development lines are grouped into tribes by related genera (Benson, 1957). The genus *Senecio* is the biggest genus in the family of the Asteraceae and includes more than 1500 species (Heywood *et al.*, 1977). Our present

study aimed to evaluate the antimicrobial and antioxidant activity and measuring the reducing power of secondary metabolites. In addition to, a phytochemical screening of main secondary metabolites present in *S. glaucus* extracts.

II. MATERIALS AND METHODS

The plant of *S. glaucus* subsp. *coronopifolius* was collected in the flowering stage, in March 2018 from Aswan University region. Plant was dried in room temperature, powdered using mechanical grinder and used for phytochemical and biological investigation.

A. Extraction Procedure

Plant materials were macerated with different systems: pure *n*-hexane; *n*-hexane: methylene chloride (75:25); (1:1); and (25:75) respectively; pure methylene chloride and methylene chloride: methanol (95:5); and (80:20), respectively; and methanol pure (MeOH) for 24 h two times for each system. The organic layers were filtered, concentrated using Rotary evaporator till dryness, and stored less than 4 °C for analysis.

B. Phytochemical Qualitative Examination

Qualitative examinations of different phytochemicals present in aqueous methanolic 80% with methylene chloride of *S. glaucus* subsp. *coronopifolius* extract has done using standard methods. Visual inspection of the appearance of

color or frothiness has been used as indicator of the presence or absence of a particular phytochemical group (Harborne, 1984).

C. Quantitative of Phenolic and Flavonoid Compounds

1) The Total Content of Flavonoids

The total content of flavonoids of the plant sample was established by colorimetric method using aluminum chloride (Chong *et al.*, 2002). In brief, (0.5 mL) of sample was transferred to a test tube then 0.3 mL of 5% sodium nitrite solution; 0.3 mL of 10% aluminum chloride solution were added after five min of incubation, and the mixture was kept standing for 6 min. Then, 2 mL of 1 M sodium hydroxide were added. The mixture was allowed to stand for 15 min, and absorbance was measured at 510 nm. From the calibration curve, the content was calculated, and the result was expressed as mg quercetin equivalent per gm dry weight (DW) and fresh weight.

2) Total Content of Phenolic Compounds

According to the Folin-Ciocalteu method, the total phenolic compounds were fixed spectrophotometrically (Qugh&Amerine, 1988). 0.5 mL of the sample extract was transmitted to a test tube, and 1 mL of 80% methanol and 0.5 mL of Folin-Ciocalteu phenol reagent were added. After incubation time of 5 min, 1 mL of 5% sodium carbonate was added, mixed well and allowed for an hour. The samples were vortexed and absorbance measured at 700 nm. Phenolic content was reported as mg Gallic acid equivalent per gm dry weight (DW).

D. Antioxidant Activities

1) DPPH Radical Scavenging Activity

The mixture of reaction (total volume, 3 mL), consisting of 1 mL of 0.2 mM DPPH in ethanol, and 0.5 mL of methanolic extract, was shaking hard (Blois, 1958). After incubation at room temperature for 30 min, the DPPH remaining was checked by measuring absorbance at 517 nm.

2) Total Antioxidant Capacity

The experiment was done according (Prieto, 1999). The tubes which containing the fraction and the reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 90°C for 90 min. The capacity of antioxidant was expressed as ascorbic.

3) Reducing Power Activity

According the method of (Oyaizu, 1986), the reducing power was measured. Briefly, phosphate buffer (2.5 mL, 0.2 M, pH 6.6) containing different concentrations of the bark fractions were prepared. Then, it was added to 2.5 mL of 1% (w/w) potassium ferricyanide, and mixed. After incubation at 50°C for 20 min, the mixtures were mixed with 2.5 mL of 10% (w/w) trichloroacetic acid, followed by centrifugation at 650 g for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of dist. water and 0.5 mL of 0.1% ferric chloride. The absorbance of solution was measured at 700 nm. Mean values were obtained from triplicate experiments. Ascorbic acid (40 µg/mL) served as positive control.

E. Antimicrobial Activity References

1) Pathogenic Fungal and Bacterial strains

Strains were obtained from mycology lab of Faculty of

Science, Aswan University. Extracts were tested against bacteria, including Gram negative (*Escherichia coli*, *Klebsiella pneumoniae*); Gram positive strains (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*); Fungi strains (*Candida albican*, *Candida tropicalis*, *Candida tamata*, *Candida ciferri*).

2) Disc Diffusion Method

The method of disc diffusion was used for testing the antibacterial effect of extract. The different dried extracts were respectively dissolved in sterile (1 n-Hexane: 1 CH₂Cl₂: drop MeOH). A suspension of the tested bacteria, 2×10⁶ cfu/mL, was spread on the solid media plates. Filter paper discs of 5 mm in diameter were individually impregnated with 50 micro of the extract and then placed on the previously inoculated agar plates. The petri dishes were allowed at 4 °C for 2 h and then incubated at 37 °C for 24 h. The of inhibition zones diameters were measured and expressed in millimeters gentamycin (was used as a positive control), sterile disc impregnated with solvent is used as negative control and antibacterial tap used as positive control (Dulge&Gonuz, 2004; Parek&Chanda, 2007).

F. Statistical analytics

The tests were done in triplicate and results are given as the mean standard deviation. The data in all tests were analyzed for statistical significance using Student is Tukey's test and differences were considered significant at p < 0.05.

III. RESULTS AND DISCUSSION

A. Phytochemical Screening

In the present study, the qualitatively identified phytochemicals of *S. glaucus subsp. coronopifolius* parts have been tabulated in Table I. In the identification process, the extracts showed many types of chemical constituents in different solvents. The results obtained for the quantitative estimation of some secondary metabolites are presented in Table I.

TABLE I: PHYTOCHEMICAL QUALITATIVE SCREENING OF DIFFERENT EXTRACTS OF *SENECIO GLAUCUS*

Phytochemical screening	Methylene Chloride	MeOH	MeOH 80%
Alkaloids	-	+	++
Flavonoids	+	+	++
Phenolics	+	+	++
Tannins	-	+	++
Terpenoids	+	+++	++
Proteins	-	-	-
Saponins	-	-	+

a. p: aerial parts; +: Positive; ++: Moderate; +++: Strongly positive; - : Not detected

B. Total Phenolic

Our results showed significant variations in phenolic content ($P \leq 0.05$) in plant between different polarities of solvent. The highest amount of phenolic content in case of methanolic extract (0.11 µg/g GAE), while other extracts observed equal value (0.02, 0.013, 0.03, 0.11 and 0.025, respectively) (Fig.1).

C. Total Flavonoid

Total flavonoid content of plant showed that the highest

number of flavonoids were noticed in (Hexane: CH₂Cl₂, 1:1) extract (1.17 µg/g QE) and other extracts were equal (0.113, 0.118, 0.256 and 0.187, respectively), while the fresh Lablab beans showed no flavonoid content (Fig. 1).

Fig. 1. Total flavonoid and total phenolic of various extracts of *Senecio glaucus subsp. coronopifolius*. All values are mean $\pm$ SD, Values in the same group with various superscript letters are significantly different, $P < 0.05$ (ANOVA after Tukey's test analysis).

D. Antioxidant Activities

1) DPPH Scavenging Ability

The DPPH% scavenging activity of extracts are presented in (Fig. 2) significant variation ($P < 0.05$) in the results of the DPPH assay between extract at same concentrations of crude was observed the highest DPPH scavenging activity was in MeOH (10 µg by 69.35%) and the lowest was at n-Hexane (10 µg by 30.42%).

2) Total Antioxidant Capacity (TAC)

Total antioxidant capacity (TAC) of different extracts was varied significantly as function of concentrations ($P < 0.05$), the TAC increased with increasing the concentration of extract. Hex: CH₂Cl₂ (1:1) extract showed higher capacities, it was 0.11 µg while the other was 0.05, 0.03, 0.11 and 0.12 at 10 µg similarly (Fig. 2).

Figure 2. Total antioxidant and reducing power of various extracts of *Senecio glaucus subsp. coronopifolius*. All values are mean $\pm$ SD, Values in the same group with different superscript letters are significantly different, $P < 0.05$ (ANOVA after Tukey's test analyses).

3) Reducing Power Activity

The reducing power increased significantly with increasing concentrations, methanolic extract was higher reducing power on the extract effect 58.5% and other extract show low concentration (0.01, 0.4, 0.36 and 0.29, respectively) (Fig. 2).

E. Antimicrobial Activity

The general screening result for antibacterial activity are shown different effect on type of *Candida* species were investigated. The highest activity was demonstrated by pure

hexane extract; while the lowest activity was in MeOH extract (inhibition zone diameter is 0.5 mm). The results of present investigation clearly the antibacterial activity vary with the solvent extract of the plants.

TABLE II: ANTIBACTERIAL ACTIVITY SCREENING OF SEVERAL EXTRACTS EXPRESSED AS ABSOLUTE UNIT (AU)

Sample	<i>Candida albicans</i>	<i>Candida auris</i>	<i>Escherichia coli</i> (G)
n-Hexane	1.9	0.0	0.5
CH ₂ Cl ₂	1.3	0.0	0.0
MeOH	0.0	0.9	0.0
n-Hexane: CH ₂ Cl ₂ (1:1)	0.8	0.0	0.0
Control	0.0	0.0	0.0

IV. PUBLICATION PRINCIPLES

There is growing interest in using bioactive phytochemicals from natural origin. Phytochemicals obtained from a different part of plants have different effects on human health (Avato&Argentieri, 2018). The extraction efficiency of biologically active compounds depended on a large number of parameters-extraction method such as type of solvents, temperatures and extraction times (Linchewa, 2017). This result showed that plant have large amount of terpenes, alkaloids, flavonoids, phenolics and tannins in methanol 80% and methanol pure, while absence of anthraquinones, saponins and proteins. Phenolic compounds are secondary metabolites and synthesized during normal growth and development (Benincasa, 2015), they are more powerful and bioavailable as antioxidant, involved in regulation of ROS, these natural antioxidant compounds have ability for free radicals removing, chelate metal catalysts, and activate antioxidant enzymes (Dravie *et al.*, 2020). Moreover, these compounds help in lower risks of degenerative diseases, especially cardiovascular diseases (Vural *et al.*, 2020). The total content of phenolic compounds for the same species exist in Egypt, also showed comparable values (Mohamed, 2015), while the species growing in Turkey showed that high total phenolic contents that ranged from 11.63 ± 2.1 mg GAE/g extract in *S. viscosus* to 117.45 ± 1.8 mg GAE/g in *S. cilicius* (Albayrak *et al.*, 2014). Flavonoids are widespread plant secondary metabolites and act as a natural antioxidant provide protection against oxidative stress (Bhagyawand *et al.*, 2019). It can be exploited for prevention of several chronic diseases by intake of flavonoid rich food (Ginwala *et al.*, 2019). DPPH radical scavenging activity is a measurement of non-enzymatic antioxidant activity. The antioxidant ability of various seeds is closely related to phenolic content and is an important part of the human guard system (Khang *et al.*, 2016). The current results showed that *Senecio* exhibited higher content of phenolics attained increased level of DPPH radical scavenging activity. The antioxidant activity of *Senecio* extracts with different type of solvents was studied. The radical scavenging activity of *Senecio* against DPPH radical was higher and has more protective effects. Moreover, the methanolic root extract of *S. glaucus* growing in Egypt had an antioxidant activity with $IC_{50} = 79.57 \pm 0.74$ µg/mL using the DPPH method (Mohamed, 2015). The ferric reducing power activity of *Senecio* extract was also determined. The reducing capacity

of a compound may serve as a considerable indicator of its potential antioxidant activity (Lourenco *et al.*, 2019). Major improvement in the reduction power was observed at 10 µg for both methylene and methanolic extracts of examined plant. The interest of natural antioxidants from plants has increased because of the safety of these antioxidants in comparison with synthetic antioxidants (Xu *et al.*, 2017). Natural phenolic antioxidants have an essential role in reducing the harmful effects of free radicals (Wang *et al.*, 2010). The correlation between phenolics and scavenging activity was reported previously (Moukette *et al.*, 2015). The antioxidative power of plants can be estimated through their ability to scavenge free radicals. In present study, we showed that *S. glaucus* displayed a potent antioxidant activity through two screening methods.

V. CONCLUSION

Our study fixed the secondary compounds; phenolics and flavonoids of *S. glaucus* found in south of Egypt. Our data indicated that *S. glaucus* is a perfect potential source of natural antioxidants, like GA and VA. Our study support researchers in order to fully elucidate the effective constituents of *S. glaucus*, which leads to the discovery of new and effective drugs.

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CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

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