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Phytochemical Analysis and in Vitro Antioxidant Properties of *Sisymbrium irio* L Growing in Saudi Arabia: A Comparative Study.

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ABSTRACT

The current study was conducted to investigate the total phenolic and total flavonoid contents, the antioxidant activity and to clarify the chemical composition of different extracts of different plant organs of *Sisymbrium irio* L as a comparative study. *Sisymbrium irio* L is characterized chemically by the presence of various secondary metabolites such as flavonoids, triterpenoids/steroids, tannins, carbohydrates, saponins and alkaloids at different levels in different extracts of plant organs and the absence of cardiac glycosides and Anthraquinones. The antioxidant activity was determined in accordance to 1,1-diphenyl-2-picryl-hydrazyl radical scavenging protocol and using trolox as standard. Moreover, the total phenolic and total flavonoid contents were evaluated using Folin-Ciocalteu reagent. The results showed that the extracts of ethyl acetate, butanol and aqueous of leaves, stems and flowers exhibited a higher antioxidant activity which further confirmed by its higher amounts of phenolic and flavonoid contents among all the tested extracts. These results revealed that the ethyl acetate, butanol and aqueous extracts of different plant organs of *Sisymbrium irio* L could be used as a potential nutraceutical antioxidant natural product formulation.

Keywords: *Sisymbrium irio*, DPPH, total phenolic, total flavonoid.

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INTRODUCTION

The medicinal plants and herbal products are considered one of the most important curing agents to treat various diseases in Saudi culture. Saudi Arabia flora is rich in diversity of medicinal and wild growing plants. These plants are abundant during rainy season between January and April. This is very clear and noticeable through the appearance of these plants in beautiful flowering sight all over Saudi regions. Many of those plants are edible by local people while others are used in folk medicine to cure some diseases [1-2].

Family cruciferae has many applications in both pharmaceutical and commercial aspects as an important source of food or oil products. In addition, most of family members have important applications in folk medicine. The genus *Sisymbrium* species is one of cruciferae members which is used in treatment of inflammation, voice disorders and rheumatoid as well as some members showed antipyretic, analgesic, antioxidant and anti-microbial activities. Literature reviewing revealed that, this genus is characterized by presence of many metabolites such as; flavonoids, alkaloids, anthraquinones, oils and steroids [3-4].

Sisymbrium irio L (London rocket) is widely distributed worldwide and in Saudi Arabia. Reviewing the available literature, the seeds and leaves were used as febrifuge, expectorant in asthma, in fevers, antibacterial, antipyretic and analgesic. Reports were found dealing with isolation of β -sitosterol and flavonoid derivatives [1, 3, 5-8].

The previous studies were carried out on aerial parts all together and seeds of *Sisymbrium irio* L regarding phytochemical and biological screening without taking in consideration that may be the different plant parts could be possibly contain different constituents or even similar constituents with different proportions. Hence, there is no record on any comparative study on different organs of *Sisymbrium irio* L regarding their phytochemical contents and antioxidant activities. Therefore the current research was carried out to investigate of the phytochemical contents and to determine the antioxidant activity of different plant organs extracts as a separate extracts.

METHODS

Plant material

Sisymbrium irio L (fig. 1) was collected from campus of King Faisal University. Different parts (leaves, stems, flowers and roots) of plant were separated and subjected to air-drying. A voucher specimen was kept in Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University, Al-Hasa, Saudi Arabia.



Figure 1: Photo of *Sisymbrium irio* L

Extraction and fractionation

The powdered air dried plant material [leaves, stems, flowers and roots of *Sisymbrium irio* L, 400, 150, 100 and 100 g respectively] were exhaustively extracted twice at room temperature (each for 1 week) using 4 L 70% MeOH/H₂O at room temperature. The extracts were concentrated through distillation under vacuum

using Rota vapor to give the total extracts of different plant parts (leaves, stems, flowers and roots weighting 25, 8, 4 and 6 g respectively) which were kept in refrigerator for the next steps.

Exactly 20, 5, 3 and 5 g of leaves, stems, flowers and roots total extracts respectively were taken and suspended in distilled water (200 ml) using a separating funnel and partitioned with n-hexane (6×250 ml). The resulting n-hexane layers were collected and combined to be concentrated to the least amount using rotary evaporator and then dried to give 10, 1.2, 1 and 0.5 g respectively then were stored in a deep freezer in well-closed container. The remaining aqueous part was subjected to partition with chloroform (6×250 ml). The obtained chloroform fractions were also combined and its amount was reduced to the minimal amount through using rotary evaporator and then freeze dried to give 2.3, 0.5, 0.3 and 0.6 g, respectively, and then all were put in fridge in a strong-tight container for later use.

In the same way of fractionation, the ethyl acetate and n-butanol extracts were also obtained using the same above-mentioned method to give 2.5, 1, 0.9 and 1.1 g respectively for ethyl acetate fraction and to give 1.5, 0.4, 0.3 and 1.2 g respectively for n-butanol fraction. The remaining mother liquor (aqueous fraction) was also freeze-dried to powder to give 2, 1, 0.5 and 1.5 g respectively and kept cooled for further use in an air-tight container [9].

Phytochemical Screening

Preliminary phytochemical testes were done on the n-hexane, chloroform, ethyl acetate, butanol and aqueous extracts of different organs using standard methods to identify the possible nature of its chemical contents [9-12].

Test for flavonoids

Part of dried extracts of each organ was boiled with 10 ml of distilled water for 5 min and filtered while hot. Few drops of 20% sodium hydroxide solution were added to 1 ml of the cooled filtrate. A change to yellow color, which on addition of acid changed to colorless solution, depicts the presence of flavonoids.

Test for saponins

Part of dried extracts of each organ was separately boiled with 10 ml of distilled water in a bottle bath for 10 min. The mixture was filtered while hot and allowed to cool. Demonstration of frothing: 2.5 ml of filtrate was diluted to 10 ml with distilled water and shaken vigorously to form a stable persistent froth.

Test for steroids and/or triterpenoids

Part of dried extracts of each organ was separately boiled with 10 ml of distilled water in a bottle bath for 10 min. The mixture was filtered while hot and allowed to cool. Five milliliters of each extract was mixed in 2 ml of chloroform. Three milliliters of concentrated H₂SO₄ was then added to form a layer. A reddish brown precipitate coloration at the interface formed indicated the presence of steroids and/or triterpenoids.

Test for alkaloids

Part of dried extracts of each specimen was separately boiled with water and 10 ml hydrochloric acid on a water bath and filtered. The pH of the filtrate was adjusted with ammonia to about 6-7. A very small quantity of Dragendorff's reagent (potassium iodide 0.11 M, bismuth nitrate 0.6 M in acetic acid 3.5 M), the test tubes were observed for orange to brown turbidity.

Test for anthraquinones

Part of dried extracts of each organ was boiled with 2 ml of 10% hydrochloric acid for 5 min. The mixture was filtered while hot and filtrate was allowed to cool. The cooled filtrate was partitioned against equal volume of chloroform and the chloroform layer was transferred into a clean dry test tube using a clean pipette. Equal volume of 10% ammonia solution was added into the chloroform layer, shaken and allowed to

separate. The separated aqueous layer was observed for any color change; delicate rose pink color showed the presence of anthraquinone.

Test for tannins

Part of each dried extracts of each organ was separately boiled with 20 ml distilled water for 5 min in a water bath and was filtered while hot. One milliliter of cool filtrate was distilled to 5 ml with distilled water and a few drops (2-3) of 10% ferric chloride were observed for any formation of precipitates and any color change. A bluish-black or brownish-green precipitate indicated the presence of tannins.

Test for cardiac glycosides

Part of dried extracts of each organ was separately boiled with 10 ml of distilled water in a bottle bath for 10 min. The mixture was filtered while hot and allowed to cool. Five milliliters of each extract was treated with 2 ml of glacial acetic acid containing one drop of 10% ferric chloride solution. This was underplayed with 1 ml of concentrated H_2SO_4 . A brown ring at the interface indicated the deoxy-sugar characteristics of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may be formed.

Test for carbohydrates

Part of various extracts were dissolved separately in 4 ml of distilled water and filtered. The filtrate was treated with 2-3 drops of alcoholic alpha-naphthol and 2 ml of concentrated H_2SO_4 was added along the sides of the test tube. Appearance of brownish violet ring at the junction of the two liquids indicates the presence of carbohydrates.

Determination of the total phenolic content

The total phenolic content was estimated using the Folin-Ciocalteu index protocol [13]. Stock solutions (1 mg/ml) of different extracts will be prepared in methanol. Half ml of Folin-Ciocalteu reagent and Six milliliters of double distilled deionized water were successively added to 0.1 ml of stock solution of each extracts. In addition, 1.5 ml of a 20% Na_2CO_3 solution and water was added to obtain 10 ml. A reaction will take place within 2 hrs. at normal room temperature. Then, the absorbance was recorded at 760 nm. Calibration was done using serial dilution of Gallic acid as a standard (0.5, 0.4, 0.3, 0.2 and 0.1 mg/ml in distilled water, $y = 0.897x + 0.0243$ $r^2 = 0.9741$). The amount of phenolic components was demonstrated as the equivalence of milligrams of standard Gallic acid per gram of dried plant extract (mg GAE/g).

Determination of the total flavonoid content

The total flavonoid content was calculated in accordance to Heimler et al. [14]. Ten mg of extracts will be diluted in 100 ml of deionized water and acetone with ratio of (1:1 v/v). A solution of 0.25 ml of the serially diluted sample was added to 0.75 μ l of a $NaNO_2$ (5% w/v) solution, as well as 0.15 ml of a recently prepared aluminum chloride (10% w/v) solution, together with 0.5 ml of 1 M NaOH solution. Then the total volume of reactants was completed to 10 ml with deionized double distilled water. The resultant components were kept for 5 min and the absorption was observed at 510 nm against the same components lacking of the sample. Calibration was done using quercetin as reference substance, from that a standard calibration curve got with solutions of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml ($y = 0.811x + 0.0612$, $r^2 = 0.8901$). The results were shown as the equivalence of milligrams of quercetin per gram of dried plant extract (mg QE/g).

DPPH radical scavenging activity

Radical scavenging activity was evaluated using the protocol described by Khalil et al. 2017[14] with some modifications. The absorbance of various dilutions of the test extracts which were previously dissolved in Methanol (1ml) was recorded at 515 nm at zero time as **Ab blank**. Then, 1 ml of 200 μ M 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution in methanol was add to every test tube, then was kept at room temperature 30 minutes. Followed by, measurement of the absorbance again as **Ab sample**. Trolox was used as positive control. Each test was performed in triplicates. The percentage of inhibition was measured using this equation:

$$\% \text{ of inhibition} = (1 - [\text{Ab sample} - \text{Ab blank}] / \{\text{Ab control} - \text{Ab blank}\}) \times 100$$

Where **Ab control** is the absorbance of mixture (with methanol and all other reactant without test extracts) [15]. **IC₅₀** was recorded as the sample concentration that is essential to cause inhibition of DPPH radical to be formed by 50%.

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screening of different extracts showed the presence of variety of important constituents such as flavonoids, triterpenoids/steroids, carbohydrates, alkaloids, saponins and tannins at different levels in different extracts of plant organs and the absence of cardiac glycosides and anthraquinones as shown in Table 1.

Table1: phytochemical screening of different fractions of different plant organs.

Plant organ		Flavonoids	Saponins	Triterpenoids/steroids	Alkaloids	Anthraquinones	Tannins	Cardiac glycosides	Carbohydrates
Leaves	nH	-	+	+	-	-	-	-	-
	CH	+	+	+	+	-	-	-	-
	EA	+	+	+	+	-	+	-	+
	BT	+	+	+	+	-	+	-	+
	AQ	+	-	-	-	-	+	-	+
Stems	nH	-	+	+	-	-	-	-	-
	CH	+	+	+	+	-	-	-	-
	EA	+	+	+	+	-	+	-	+
	BT	+	+	+	+	-	+	-	+
	AQ	+	-	-	-	-	+	-	+
Flowers	nH	-	+	+	-	-	-	-	-
	CH	+	+	+	+	-	-	-	-
	EA	+	+	+	+	-	+	-	+
	BT	+	+	+	+	-	+	-	+
	AQ	+	-	-	-	-	+	-	+
Roots	nH	-	-	+	-	-	-	-	-
	CH	+	+	+	+	-	-	-	-
	EA	+	+	+	+	-	+	-	+
	BT	-	+	-	+	-	+	-	+
	AQ	-	-	-	-	-	+	-	+

nH, n-Hexane; CH, chloroform ; EA, Ethyl acetate; BT, Butanol; AQ, Aqueous. + (present); - (absent).

Determination of total phenolic constituents

Carrying out determination of total phenolic constituents showed that its level varies from extract to extract of different organs ranging from 0.0.019±0.728 to 45.295±0.931 mg GAE/g of dry extract (Fig. 2). Ethyl acetate fraction contains the highest percentage of total phenolic components, followed by butanol fraction then the aqueous fraction. Chloroform fraction contains the least amount while n. hexane extract contains very less of phenolic contents.

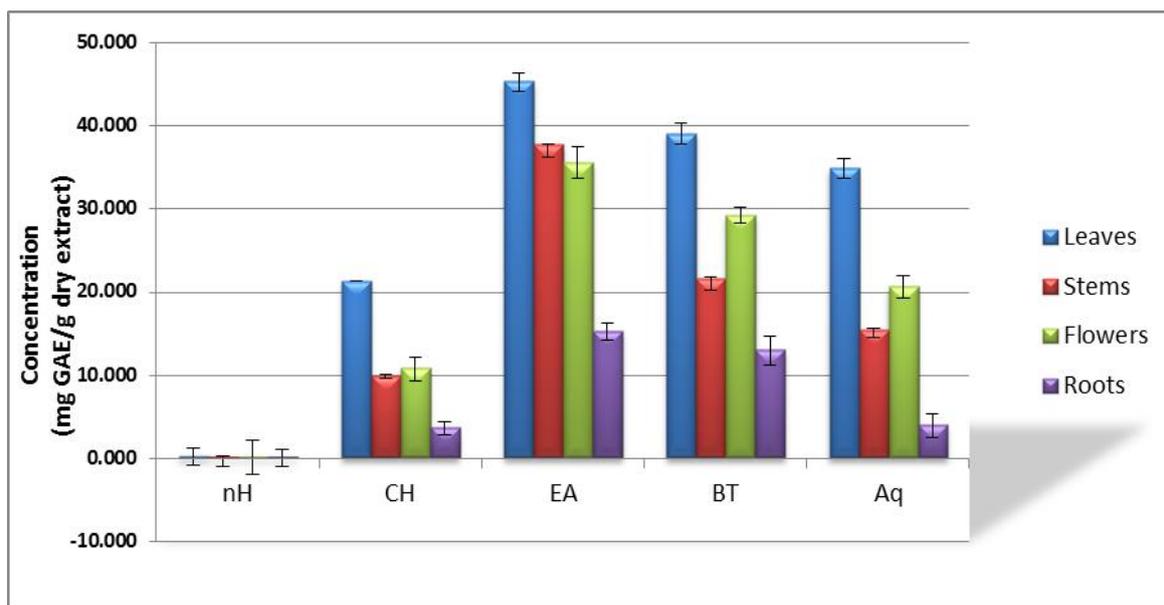


Figure 2: Total phenolic contents of different extracts of *Sisymbrium irio* L. nH; n-Hexane fraction, CH; Chloroform fraction, EA; Ethyl acetate fraction, BT; Butanol fraction, Aq.; Aqueous fraction, GAE; Gallic acid equivalent. Data are the means \pm standard deviation of three replicates.

Determination of total flavonoid contents

Determination of total flavonoids content illustrated similarity with those of total phenolic where the amount of total flavonoid constituents varies from 0.009 ± 0.420 to 30.572 ± 1.005 mg QE/g of dry extract (Fig. 3). Ethyl acetate fraction is the richest fraction in flavonoid components, next to it the butanol fraction then the aqueous fraction. Similarly, to phenolic contents, the chloroform fraction contains the least amount compared to other fractions, while n-hexane fraction hardly contains any flavonoid constituents.

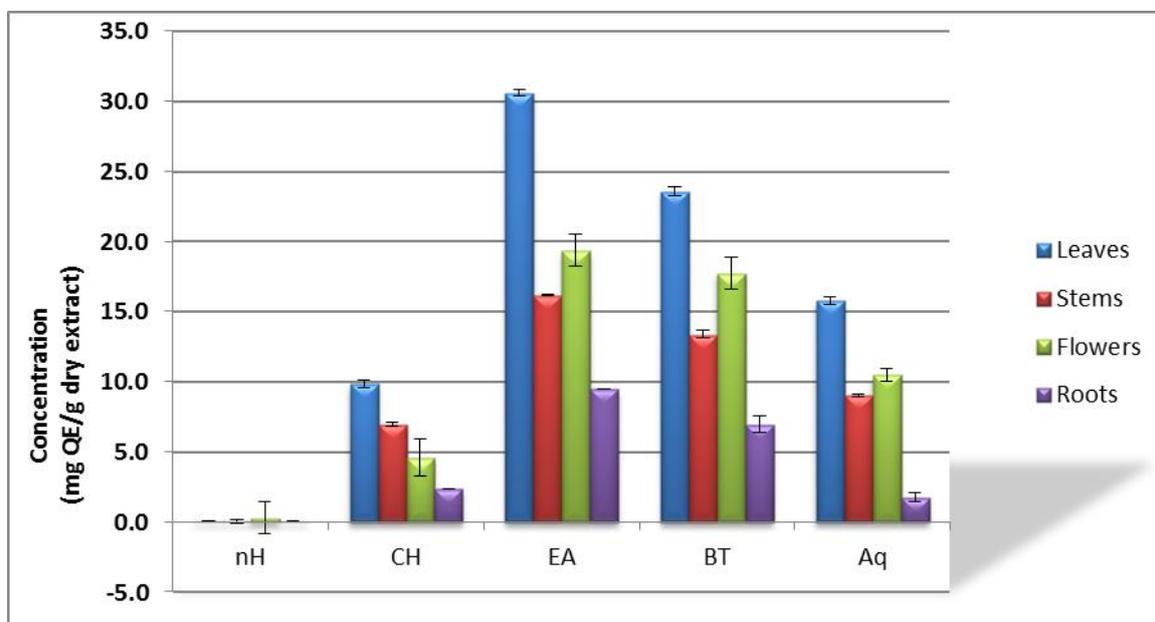


Figure 3: Total flavonoid contents of different extracts of *Sisymbrium irio* L. nH; n-Hexane fraction, CH; Chloroform fraction, EA; Ethyl acetate fraction, BT; Butanol fraction, Aq.; Aqueous fraction, QE; Quercetin Equivalent. Data are the means \pm standard deviation of three replicates.

DPPH radical scavenging activity

All fractions were examined for their radical scavenger activity using the DPPH free radical scavenging assay (Fig. 4). Ethyl acetate, butanol fractions of leaves, stems and flowers and aqueous fractions of leaves and flowers showed marked scavenging activities ranging from IC_{50} : 74.2 to 89.6 $\mu\text{g/ml}$, chloroform fraction of leaves and stems showed effect with IC_{50} of 87.6 and 98.7 $\mu\text{g/ml}$ respectively, on the other hand, the rest of chloroform fractions of other plant parts (leaves, stems and roots), hexane fractions of all plant parts and all fractions of root demonstrated much weaker effects with IC_{50} above 100 $\mu\text{g/ml}$ comparable with the standard trolox (IC_{50} : 22.9 μM).

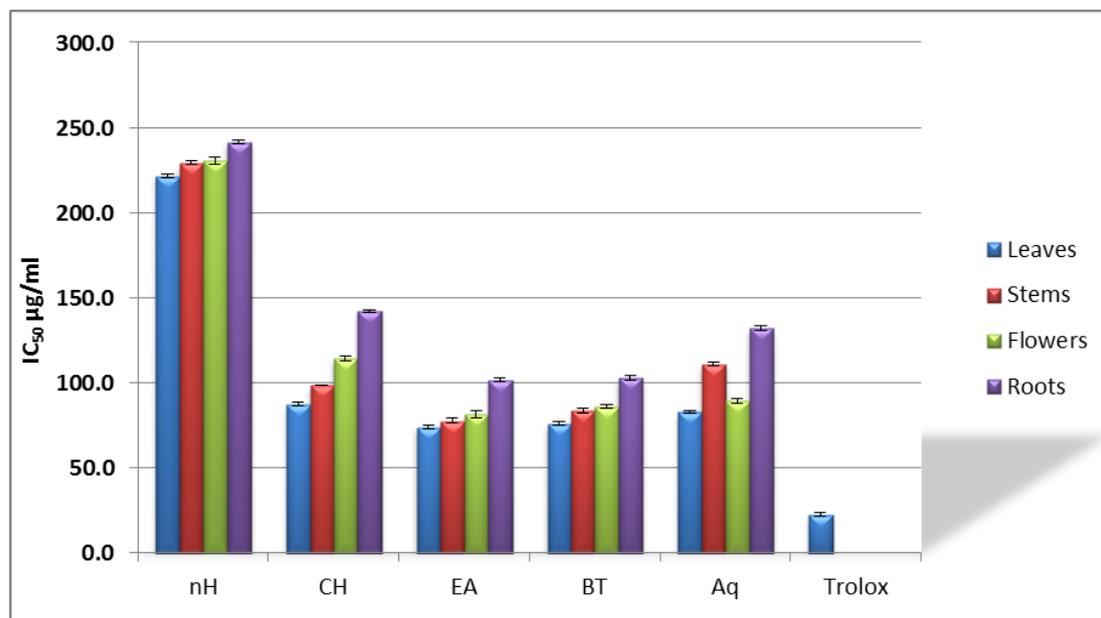


Figure 4: IC_{50} values of DPPH assay of different extracts of *Sisymbrium irio* L. nH; n-Hexane fraction, CH; Chloroform fraction, EA; Ethyl acetate fraction, BT; Butanol fraction, Aq.; Aqueous fraction. Data are the means \pm standard deviation of three replicates.

CONCLUSION

In conclusion, the current study revealed that *Sisymbrium irio* L phytochemically characterized by its contents of various chemical constituents as well as various organ extracts exhibited good antioxidant activities specially the ethyl acetate, butanol and aqueous fractions which could be attributed to the its higher contents of phenolic and flavonoid constituents. Furthermore, this study demonstrated the importance of leaves and stems compared to that of flowers and roots. So this study could identify a good source of herbal remedy with higher antioxidant activity.

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