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Ecotype Difference in Bioactive Constituents and *In vitro* Antioxidant Activities of Some Saudi Medicinal Plants

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The bioactive phyto constituents of endemic and acclimated plants have been used for the bioprospecting of novel compounds, throughout the world. However, the constituents of bioactive phytochemicals and the antioxidant activity are influenced to a great extent by several variables such as altitude, sunlight, soils, season and region of cultivation. We collected *Retama raetam Salsola inermis*, *Hyoscyamus albus* and *Fagonia arabica* plants from two different regions; Jabal-al-Lawaz (JAE) and Wadi-e-Dissa (WDE) of Tabuk, Saudi Arabia. Phytochemical analysis of Methanolic leaf extracts of all the plants revealed the existence of alkaloids, phenols, flavonoids, terpenoids, tannins and carbohydrates. All the screened phytochemicals were higher in content in the JAE plants than WDE, except flavonoids in *S. inermis* (WDE). Results validate that these plants from Jabal-al-Lawz have considerable amount of bioactive constituents. Methanolic extracts of *H. albus* exhibited maximum DPPH antiradical, nitric oxide scavenging and metal chelating activities; however H_2O_2 scavenging activity was highest in *R. raetam*. It is concluded that the plants collected from Jabal-al-Lawz are rich sources of bioactive phytochemicals and antioxidants and they could be used in the treatment of oxidative-stress induced degenerative diseases and disorders.

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1. INTRODUCTION

The pathogenesis of several degenerative diseases such as cancer, cardiovascular diseases, atherosclerosis, inflammation, Alzheimer's and Parkinson's diseases are linked to the reactive oxygen species induced oxidative damages [1,2]. To counteract the oxidative stress the life forms are equipped with the enzymatic and non-enzymatic systems. This includes the enzymes (ascorbate peroxidase, superoxide dismutase, glutathione peroxidase, catalase), antioxidants (ascorbic acid, glutathione, α-tocopherol, β-carotene) and hormones (estrogen, angiotensin) which protect the organisms from oxidative damage [3]. Of late, medicinal plants have attracted a huge interest due to the inherent antioxidant potential of the phytochemicals that reduces the free-radical induced oxidative damage. Phytochemical screening by several workers has unveiled the presence of various bioactive constituents such as phenols, flavonoids, alkaloids, terpenoids and saponins [4,5]. Among various phytochemicals, phenolic compounds are widely distributed and the largest groups of plant metabolites [6]. The biological activities of phenolic compounds as antioxidants and free radical scavengers have been explored by several workers [7,8]. In addition to antioxidant capacity, they are also known to possess metal-chelating properties. The antiradical capacity of phenolics have been attributed to the redox properties of these compounds that enable them to act as hydrogen donors, reducing agents and uncoupled oxygen quenchers [9].

Saudi Arabia a vast arid country that occupies the four-fifths of Arabian Peninsula, surrounded by Arabian Sea on the east and red sea on the west with the large stretch of deserts and a bay between Mediterranean Sea on the north [10] represents a very varied climatic condition. The bioactive phytoconstituents of endemic and naturalized plants have been used for the bioprospecting of novel compounds. However, the content of bioactive phytochemicals and the antioxidant capacity of the plants are largely influenced by numerous factors such as altitude, sunlight, soils, season and region of cultivation [11, 12].

We have collected the plants of *Retama raetam*, *Salsola inermis*, *Hyoscyamus albus* and *Fagonia arabica*, which have been employed in traditional medicine since long ago. *Retama raetam* (Fabaceae) has long been used as an abortifacient, purgative and vermifuge. The local people of Saudi Arabia use *Salsola inermis* (Chenopodiaceae) in contraception and as anthelmintic, cathartic and diuretic [13]. However, *Hyoscyamus albus* (Solanaceae) is traditionally applied as a anticholinergic and sedative [14]. The traditional medicinal use of *Fagonia arabica* (Zygophyllaceae) includes the treatment of several illnesses such as sore mouth, small pox, fever, cough, cold, asthma and urinary infections [15].

Tabuk, the northern province of Saudi Arabia is a region largely characterized by asymmetrical topography ranging from plains to low and high mountains that create a highly distinct environmental variable of extreme cold to extreme hot. Diversified topography coupled with varied environmental conditions supports the growth of several medicinally important plants in this region. Therefore, the present study was carried out to evaluate and compare the bioactive phytochemicals and antiradical capacity of four medicinal plants (*Retama raetam*, *Salsola inermis*, *Hyoscyamus albus* and *Fagonia arabica*) from Jabal-Al-Lawz (2580 m asl) and Wadi-e-Dissa (945 m asl), the two geographically distinct regions of Tabuk with disparate climatic conditions.

2. MATERIALS AND METHODS

2.1 Plant collection

The plants were collected from Jabal Al-Lawz (28°39′N, 35°18′E) and Wadi-e-Dissa (27°63′N, 36°52′E) the two different regions of Tabuk, the Northern Province of Saudi Arabia (latitude 28°22´59" N; 36°34´59" E, altitude 773m). The region is bounded by Red sea on the west to the Hufa depressions in the east. The climate of Wadi-e-Dissa is characterized by comparatively cool weather where presence of several oases serves as a huge reserve of water. However, the Jabal-al-Lawz region is typified by hyper-arid climate with higher mean temperatures and very low humidity. The plant materials were authenticated by Dr. Mohammad Nasir Khan from Department of Biology, University of Tabuk. Voucher specimens (TUDB201-208/2014) were deposited at the herbarium of the Department of

Biology. Collected plants were dried in shade under dark. Air-dried leaf samples were ground to a fine powder (80 mesh) using an electric blender and stored in a clean labeled air-tight containers.

2.2 Plant extracts

100 g powdered leaf sample of each plant were extracted with methanol for 24 h by using soxhlet apparatus. The extracts were separated from the solids by filtration with Whatman No. 1 filter paper. The remaining solids were be extracted twice with the same methanol and extracts combined. The extracts were concentrated under reduced pressure at 45 °C, in a rotary evaporator (EYELA, Tokyo, Japan) and kept in a refrigerator at 4 °C) until analyzed.

2.3 Preliminary phytochemical screening

The Leaf extracts of *Retama raetam*, *Salsola inermis*, *Hyoscyamus albus* and *Fagonia arabica* were administered to different chemical tests for the analysis of bioactive phytoconstituents such as alkaloids, phenol, flavonoids, steroids, terpenoids, tannins, saponins, anthraquinones, carbohydrates and cardiac glycosides following the methods described by [16] and [17]. The tests for phytochemical screening were depended upon the visual observation of relative change in the color formation or the formation of precipitates.

2.4 Total Phenol

The content of total phenols was estimated by the Folin-Ciocalteu method with little modification of [18]. From each sample, 0.5 ml of methanolic extract was added to 2.5 ml of Folin-Ciocalteu reagent and 2 ml of 1 M sodium carbonate. The tubes were incubated at 45°C for 30 min. The absorbance of total phenolics was measured at 765 nm using Hewlett Packard, UV/visible light. Total phenolics content was expressed as mg gallic acid equivalents per g dry weight.

2.4 Total Flavonoids

Total flavonoid content in the methanolic extracts was measured spectrophotometrically following the method of [19]. 4 ml of water was added to 1ml (500 mg/ml) extract or standard catechin solution and 0.3 ml of 5% $NaNO_2$. After keeping it for 5 min, 0.3 ml 10% $AlCl_3$ was added. To this mixture, after 6 min 2 ml 1M NaOH was added and the total volume was made up to 10 ml with water. The solution was thoroughly mixed and the absorbance was recorded against a prepared reagent blank at 510 nm. Total flavonoid content of was expressed as catechin equivalents in mg per g dry weight.

2.5 Determination of antiradical activity

2.5.1 Determination of DPPH free radical scavenging

The free radical scavenging capacity of methanolic extracts of different plantswas recorded using DPPH method as described by [20]. DPPH, stable free radicals that accepts an electron or hydrogen radical and get converted in to yellow-coloured diphenylpicylhydrazine, a diamagnetic molecule. The reduction capacity of DPPH is estimated by the decrease in the absorbance at 517 nm induced by antioxidants. 5ml of 0.004% freshly prepared methanolic solution of DPPH (2, 2-diphenyl-1-picrylhydrazyl) were added to 50 µl of different concentrations of sample. After 30 min in the dark at room temperature, the absorbance was recorded spectrophotometrically against a blank at 517nm. DPPH free radical scavenging activity was expressed as the percentage of inhibition.

2.5.2 Nitric oxide scavenging activity

To estimate nitric oxide radical inhibition activity, method described by [21] was followed with little modifications. Briefly, sodium nitropruside (5mM, pH 7.4) in phosphate buffer saline was mixed with 3ml of different concentrations of methanolic plant extracts and incubated at 25°C for 150 min. From this incubated solution, 0.5 ml was taken and mixed with 0.5ml Griess reagent [(1.0 ml sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5 min with 1 ml of naphthylethylenediamine dichloride (0.1% w/v)]. After 30 min of incubation, absorbance was recorded at 540 nm. A standard solution of ascorbic acid was treated in the same way with the Griess reagent as a positive control. Nitric oxide scavenging activity was expressed as the percentage of inhibition.

2.5.3 Hydrogen peroxide scavenging activity

The ability of the extracts to scavenge hydrogen peroxide was estimated based on the method of [22]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was

determined by absorption at 230 nm using a spectrophotometer. Plant extracts in methanol were added to a hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was noted after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as positive control. Hydrogen peroxide scavenging activity was expressed as the percentage of inhibition.

2.5.4 Metal chelating activity

The formation of ferrous ion-ferrozine complex was recorded to estimate the ferrous level according to [23]. The reaction mixture consisted of different concentration of plant extracts and 0.1 ml of 2 mM ferrous chloride. 0.2 ml of 5 mM ferrozine was added to this mixture to initiate the reaction and left to stand at room temperature for 10 min. The absorbance of the solution was measured at 562 nm. Ascorbic acid was used as positive control. The chelating activity was expressed as percentage of inhibition.

2.6 Data analysis

IC_{50} values were calculated by employing linear regression analysis. Results were expressed as Mean ± SD.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

The methanolic extracts of aerial part of the all the investigated plants from the two different regions were screened for the presence of bioactive phytochemicals. The phytochemical screening of the extracts revealed the presence of alkaloids, phenols, flavonoids, terpenoids, tannins and carbohydrates in all the leaf samples (Table 1). However, saponins were absent in *Retama raetam* and steroids in *Salsola inermis* in both the ecotypes, JAE (Jabal-al-Lawz ecotype) and WDE (Wadi-e-Dissa ecotype). Anthroquinones were absent in *Hyoscyamus albus* and *Salsola inermis*. Phenols and Tannins in *Retama raetam*, flavonoids in *Salsola inermis* and alkaloids and tannins in *Hyoscyamus albus*were strongly positive in the JAE samples (Table 1).

3.2 Total Phenol and Flavonoid Content

According to [24] and [25], the content of phenol is hugely influenced by environmental factors and genotypic variations, selection of the parts, time of the sampling and analytical methods. As in Fig. 1, methanolic leaf extracts of both the ecotypesof *Retama raetam*, *Salsola inermis*, *Hyoscyamus albus* and *Fagonia arabica* showed substantial amount of phenolic compounds. The total phenol content of the investigated plants differed greatly in both the ecotypes. The JAE of *Retama raetam*, *Salsola inermis*, *Hyoscyamus albus* and *Fagonia arabica,* has 22.2%, 6.7%, 15.1% and 15.9% more phenol than the WDE, respectively. Highest content of phenol was found in the leaves of Jabal-al-Lawz ecotype of *Retama raetam* (93.7± 3.9 mg GAE/g DW)which was 49.3%, 43.2 % and 53.4% more than *Salsola inermis*, *Hyoscyamus albus* and *Fagonia arabica,* respectively. The Wadi-e-Dissa ecotype displayed a similar trend where we observed the highest phenol content in *Retama raetam* which was 42%, 39.8% and 50.8% higher than *Salsola inermis*, *Hyoscyamus albus* and *Fagonia arabica,* respectively. We have noted a higher amount of total phenol in JAE of *Retama raetam*, *Hyoscyamus albus* and *Salsola inermis* than those observed by [26] and [27] in other members of Chenopodiaceae, respectively.

According to [28] and [29], among polyphenolic groups, flavonoids are known to possess a broad spectrum of biological and antiradical activities. Furthermore, flavonoids have been acclaimed for exemplary anti-microbial and anti-inflammatory activities among several others [30]. Maximum level of flavonoids were recorded in the *Retama raetam* (48.8± 2.7 mg CE/g DW) of JAE which was 68%, 47.3% and 61.7% higher than *Salsola inermis*, *Hyoscyamus albus* and *Fagonia arabica,* respectively. In WDE, the flavonoid content of *Retama raetam* was 37.5%, 35.7% 55.8% more than *Salsola inermis*, *Hyoscyamus albus* and *Fagonia arabica,* respectively. Among two ecotypes, the flavonoid contents of all the plants of JAE were higher than WDE, except for WDE of *Salsola inermis* which was 51.2% higher than JAE of *Salsola inermis*. Our data on flavonoid content of *Retama raetam, Hyoscyamus albus* and *Fagonia arabica* was different to those of [31], [32] and [26]. The observed differences in the phenol and flavonoid content of JAE and WDE growing in two geographically distant habitats could be ascribed to the effect of environmental variables on physiology and specific biosynthetic pathways [33].

3.3 Antiradical Activity

3.3.1 DPPH free radical scavenging activity

The free radical scavenging activities of methanolic leaf extracts of *Retama raetam*, *Salsola inermis*, *Hyoscyamus albus* and *Fagonia arabica* from Jabal-al-Lawz and Wadi-e-Dissa were assessed by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) method. As shown in Figs. 2A-B, the DPPH radical scavenging activities of methanolic leaf extracts of different plants shown to occur in a dose-dependent manner. The concentration required to inhibit 50% radical-scavenging activity (IC_{50}) was established from the results of a series of concentrations evaluated. A lower IC_{50} value corresponds to a larger scavenging activity. The rank order of potency showed that the methanolic leaf extracts of *Hyoscyamus albus* (JAE) was 1.7-, 1.8-, 1.9- , 2.5-, 2.7-, 3- and 3.3-fold more powerful than the *F. arabica* (JAE), *H. albus* (WDE), *F. arabica* (WDE), *R. raetam* (JAE), *R. raetam* (WDE), *S. inermis* (JAE) and *S. inermis* (WDE), respectively. The scavenging effect of methanolic extracts on the DPPH radical expressed as IC_{50} values was in the following order: *H. albus* (JAE) >*F. arabica* (JAE) >*H. albus* (WDE) >*F. arabica* (WDE) >*R. raetam* (JAE) >*R. raetam* (WDE) >*S. inermis* (JAE) >*S. inermis* (WDE) (Table 2). The substantial antioxidant activity of *R. raetam*, *S. inermis*, *H.albus* and *F. arabica* could be assigned to the presence of sesquiterpene lactones and flavonoids in the methanolic extracts of these plants. We recorded 43.2% more phenol in *R. raetam* than *H. albus* in Jabal-al-Lawz ecotype. However, the leaf extracts of *H. albus* (JAE) showed 2.5 times more potent free radical scavenging activity than *R. raetam* (JAE). The relationship between the antiradical activity and the total phenol may be interpreted in several ways; in fact, the total phenol content may not incorporate all the free radicals. Furthermore, the communion between the antioxidants and the antioxidant activity seems to be relied not only on the concentration, but also on the structure and the interaction between antioxidants (Table 2). Our results are in agreement with the findings of [34] and [35], who also noted a high antiradical activity with low phenol contents.

3.3.3 H_2O_2 scavenging activity

Hydrogen peroxide is not very reactive to the biomolecules, but, its ability to cross the biological membrane and serve as a precursor for potentially toxic hydroxyl radical [36] makes it very deleterious. The hydroxyl radical is an extremely reactive free radical capable of damaging almost every molecule found in living cells [37]. As in Figs. 3A-B, the methanolic extracts of the aerial parts of all the plants investigated, have shown a concentration dependent hydrogen peroxide scavenging activity. Lowest hydrogen peroxide scavenging activity was recorded for *R. raetam* (WDE) which was 1.3-, 1.6-, 1.9-, 2.1-, 2.2-, 2.9- and 3-fold lower than *R. raetam* (JAE), *S. inermis* (JAE), *S. inermis* (WDE), *H. albus* (JAE), *H. albus* (WDE), *F. arabica* (JAE) and *F. arabica* (WDE), respectively (Figs. 3A-B). The H_2O_2 scavenging activity of methanolic extracts expressed as IC_{50} values was in the following order: *R. raetam* (WDE) >*R. raetam* (JAE) >*S. inermis* (JAE) >*S. inermis* (WDE) >*H. albus* (JAE) >*H. albus* (WDE) >*F. arabica* (JAE) >*F. arabica* (WDE) (Table 2). According to [29], H_2O_2 scavenging activity of these methanolic extracts could be assigned to the presence of active constituents that that may donate electrons to H_2O_2 and thereby neutralizing it to water. Particularly the higher H_2O_2 scavenging activity of the *R. raetam* may be due to the presence flavonoids such as daidzein, daidzein 7, 4′-dimethyl ether, chrysoeriol 7-O-glucoside and orientin in the leaves [38].

3.3.4 Nitric Oxide scavenging activity

According to [39], nitric oxide regulates many pathological conditions, especially acute inflammatory condition. Nitrite and peroxynitrite anions are generated when oxygen reacts with the excess nitric oxide that may acts as free radicals [40]. In the present study the methanolic leaf extracts of all the investigated plants competes with oxygen to react with nitric oxide and thus inhibits the generation of the anions in a dose dependent manner. As in Figs. 4A-B, the highest nitric oxide scavenging activity among the analyzed plant extracts were displayed by *H. albus* (JAE) (IC_{50}= 138 µg/ml). It should be noted that the nitric oxide scavenging activity of *H. albus* (JAE) and *S. inermis* (WDE) was almost equal but 1.1-, 1.1-, 1.2-, 1.2-, 1.3- and 1.4-fold less than *F. arabica* (JAE), *F. arabica* (WDE), *S. inermis* (JAE), *R. raetam* (JAE), *H. albus* (WDE) and *R. raetam* (WDE), respectively (Table 2). The bioactive constituents of *Hyoscyamus* species includes flavonoids, chlorogenic acid, tannins, and coumarins [41] while *Salsola* species are rich in flavonoids [42], alkaloids [43], saponins [44], sterols [45] and coumarins [46].

The ubiquity of these bioactive substances makes them powerful scavengers.

3.3.5 Metal chelating activities

The metal chelating ability of the methanolic leaf extracts of *R. raetam*, *S. inermis*, *H. albus* and *F. arabica* from two different regions were quantified by the formation of ferrous ion-ferrozine complex of red colour which absorbs at 562 nm [47]. The ability of a chelating agent to form σ bond with a metal, may act as effective scavenger as they lower the redox potential and stabilize the oxidised metal ion [48]. As in Figs. 5A-B, the highest and lowest metal chelating activity was noted in *H. albus* (JAE) and *F. arabica* (WDE), respectively. Based on IC_{50} value, the rank order of potency for metal chelating activity of methanolic leaf extracts of all the samples were; *H. albus* (WDE) >*S. inermis*(WDE) >*R. raetam* (JAE) >*H. albus* (JAE) >*R. raetam* (WDE) >*S. inermis* (JAE) >*F. arabica* (JAE) >*F. arabica* (WDE) (Table 2). The results of our study demonstrated that metal chelating activities of methanolic leaf extracts of all the samples were reacted in a dose-dependent manner where the absorbance of Fe^{2+}-ferrozine complex was linearly decreased with increasing concentration. Fe^{2+}, a transition metal can transfer a single electron and can trigger a series of radical reactions even with non-transition metals. The polyphenolic contents of our samples did not show relation with metal chelating activities (data not shown). The methanolic leaf extract of *H. albus* (WDE) has lower phenol content than *R. raetam* (JAE) but proved to be very efficient metal- chelater. Similar to our results, [49,50,51] also fail to find any relation between phenol content and metal-chelating activities. [52,53] suggested that Fe^{2+}-chelating activity rely on flavonoid structures and position of the hydroxyl ion in the molecule determines the proton donating and radical scavenging activity.

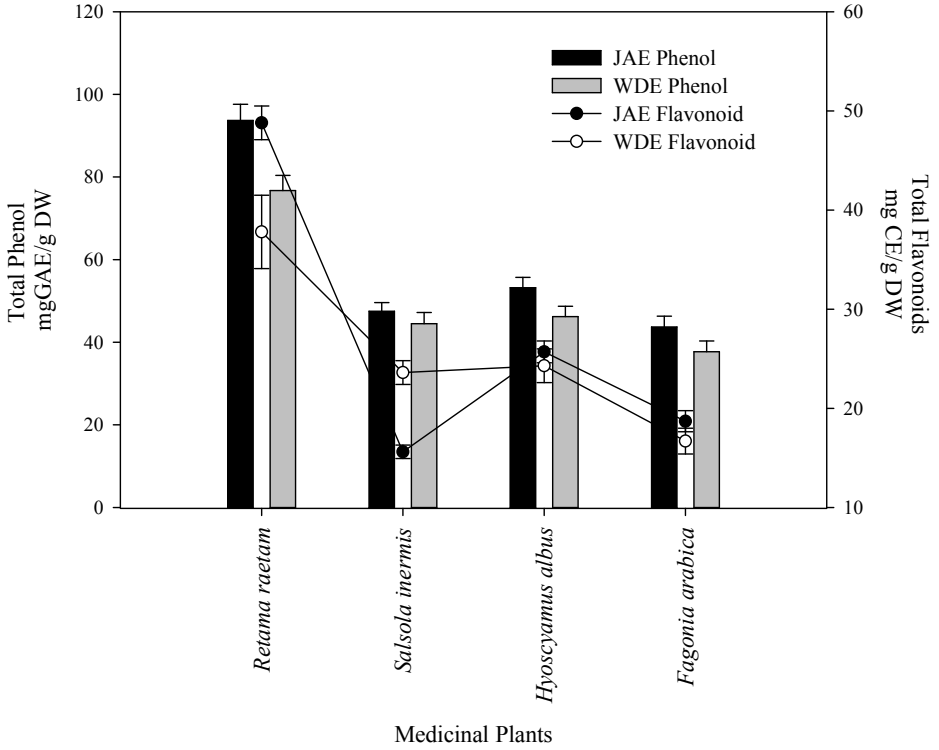

Fig. 1. Total phenol and flavonoid contents of methanolic extracts of Jabal Al-Lawz ecotype (JAE) and Wadi-Dissa ecotype (WDE) of Saudi medicinal plants of *Retama raetam*, *Salsola inermis*, *Hyoscyamus albus* and *Fagonia arabica*

Table 1. Phytochemical profiles of methanolic extracts of the Jabal Al-Lawz ecotype (JAE) and Wadi-Dissa ecotype (WDE) of Saudi medicinalplants of *Retama raetam*, *Salsola inermis*, *Hyoscyamus albus* and *Fagonia Arabica*

Phytochemicals	Medicinal plants							
	Wadi-E-Dissa Ecotype (WDE)				Jabal-Al-Lawz Ecotype (JAE)			
	Retamaraetam	***Salsolainermis***	***Hyoscyamus albus***	***Fagonia arabica***	***Retama raetam***	***Salsolainermis***	***Hyoscyamus albus***	***Fagoniaarabica***
Alkaloids	++	+	++	+	+	+	+++	+
Phenol	++	++	+	+	+++	++	++	+
Flavonoids	+	+	++	+	++	+++	+	+
Steroids	+	-	+	+	+	-	+	+
Terpenoids	+	++	++	+	+	+	++	+
Tannins	+	++	++	+	+++	++	+++	+
Saponins	-	+	+	++	-	++	+	++
Anthroquinones	++	-	-	+	+	-	-	+
Carbohydrates	+	+	++	+	+	++	+	+
Cardiac glycosides	-	+	+	++	-	+	+	++

(+++) = Strongly positive; (++) = Moderately positive; (+) = Positive; (-) = Absent

Table 2. IC_{50} value (µg/ml) of DPPH radical scavenging, hydrogen peroxide (H_2O_2) scavenging, nitric oxide (NOX) scavenging and metal chelating activities of the methanolic extracts of Jabal Al-Lawz ecotype (JAE) and Wadi-Dissa ecotype (WDE) of Saudi medicinal plants of *Retama raetam*, *Salsola inermis*, *Hyoscyamus albus* and *Fagonia Arabica*

Cultivars	Ecotypes	DPPH	H_2O_2	NOX	Metal chelating
Retama raetam	JAE	257.3	139.3	165.1	214
	WDE	273.2	109	189.3	238
Salsola inermis	JAE	314.4	179	161.5	302
	WDE	339.4	208	140	211.1
Hyoscyamus albus	JAE	101.8	228	138	218
	WDE	187.2	233.4	172	179
Fagonia arabica	JAE	179	319.3	150	396
	WDE	195	317	157	415

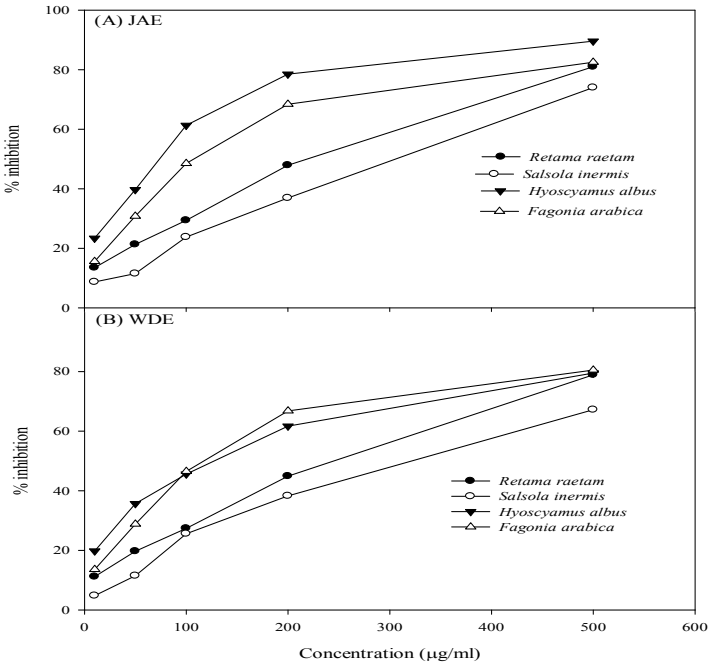

Fig. 2. DPPH radical scavenging activities of the methanolic extracts of Jabal Al-Lawz ecotype (JAE) and Wadi-Dissa ecotype (WDE) of Saudi medicinal plants of *Retama raetam*, *Salsola inermis*, *Hyoscyamus albus* and *Fagonia arabica*

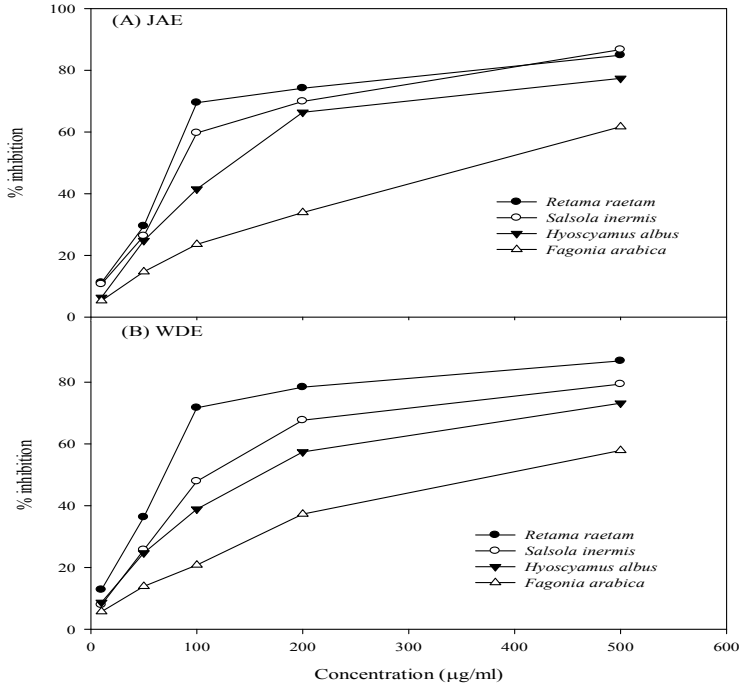

Fig. 3. H_2O_2 scavenging activities of the methanolic extracts of Jabal Al-Lawz ecotype (JAE) and Wadi-Dissa ecotype (WDE) of Saudi medicinal plants of *Retama raetam*, *Salsola inermis*, *Hyoscyamus albus* and *Fagonia arabica*

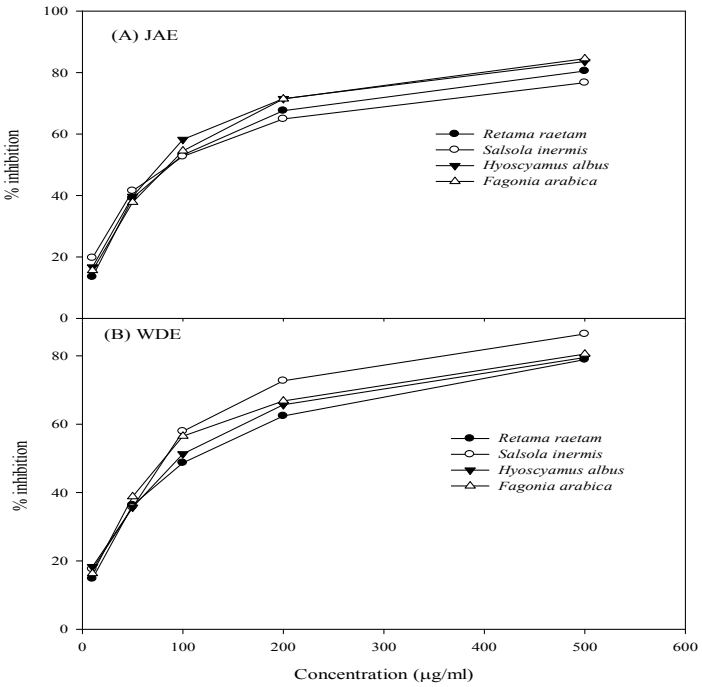

Fig. 4. Nitric oxide scavenging activities of the methanolic extracts of Jabal Al-Lawz ecotype (JAE) and Wadi-Dissa ecotype (WDE) of Saudi medicinal plants of *Retama raetam, Salsola inermis, Hyoscyamus albus* and *Fagonia arabica*

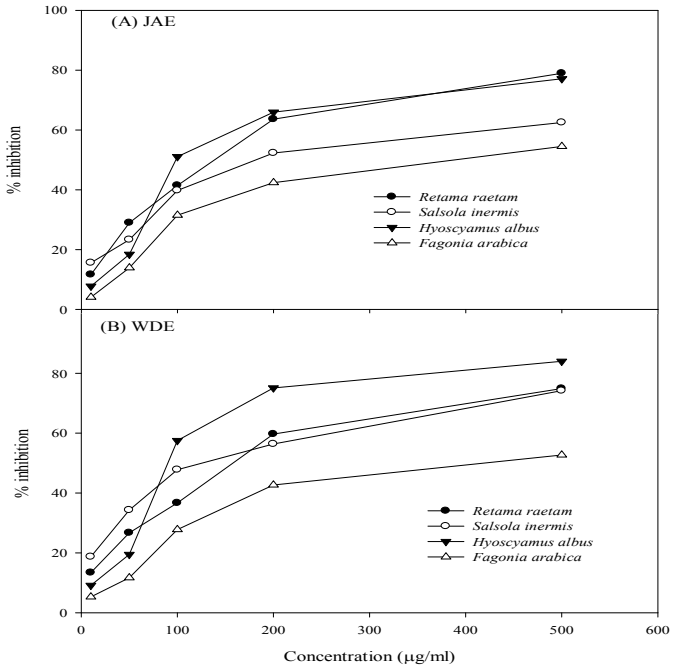

Fig. 5. Metal chelating activities of the methanolic extracts of Jabal Al-Lawz ecotype (JAE) and Wadi-Dissa ecotype (WDE) of Saudi medicinal plants of *Retama raetam*, *Salsola inermis*, *Hyoscyamus albus* and *Fagonia arabica*

4. CONCLUSIONS

The findings of the present study suggest that all the plants screened from two different regions have some common bioactive phytochemicals, but the contents of phenol and flavonoids were higher in the plants collected from Jabal-al-Lawz than the plants from Wadi-e-Dissa, The DPPH antiradical, nitric oxide scavenging and metal chelating activities were higher in *H. albus* than all the other plants included in this study. This investigation has provided the biochemical evidences for the ethno-pharmacological applications of these plants in the prevention of several degenerative diseases and disorders. However, further work regarding the isolation and identification of bioactive constituents responsible for strong antioxidant activity of these plants should be carried.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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