



Antioxidant Activity of Phenolic and Alkaloid Fractions Accumulated in *Artemisia Judaica* and *Artemisia Herba Alba*

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Abstract

Genus *Artemisia* are desert plants spread in the Egypt and Saudi Arabia. The methanol extract of the aerial parts of *Artemisia herba alba* and *Artemisia judaica* grown in the arid zones in the Northern regions of Saudi Arabia and North Sinai, in Egypt were subjected to the primary phytochemical analysis which, showed the presence of phenolic compounds, flavonoids, tannins and sterols. The GC-MS analysis of the methanolic extract exhibited numerous compounds, 11 compounds were identified as phenolic compounds, ferulic acid, ursodeoxycholic acid and derivatives of cinnamic acid (ethyl cinnamate). The highest phenolics content in *A. herba alba* grown in Saudi Arabia was observed for methyl cinnamate, (8.2 %) and the lowest methyl ferulate (0.02 %) for *A. judica* grown in Egypt. The quantization analysis of GC-MS revealed high amount of phenolic acid in the plant grow in Saudi Arabia than the plant collected from Egypt. The radical scavenging activities of two plant were studied by 1,1-diphenyl -2-picrylhydrazyl (DPPH), EC (50) values were determined for *A. herba alba* and *A. judaica* (0.122, and 0.56 mg/ml) growing in Saudi Arabia and *A. herba alba* and *A. judaica* (1.7 and 0.67 mg/ml) growing in Egypt. Total alkaloid content was analysed and some alkaloid compounds was identified by GC-MS, viz. dasycarpidan-1-methanol, acetate, pseudosolasodine diacetate and morphinan-4,5-epoxy-3,6-diol. Certain compounds were rich in Saudi plant but not detected in plants grown in Egypt, which reflect the role of environment adaptation of plant by accumulation of only certain compounds.

Keywords: *Artemisia Judaica*, *Artemisia Herba Alba*, Alkaloid Compounds, Antioxidant, Phenolic

1. Introduction

The morphology and physiology of plant are strongly affected by drought and environmental condition, which cause a large reduction in agricultural

production. The endemic plants are more adaptable to environmental conditions than agronomic commercial crops.¹

Fulda et al., 2010, Report oxidative stress is a metabolic status, which increases reactive oxygen

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species (ROS) production. Cell injury results in cell viability compromise through apoptosis or necrosis.²

Desert plants subject to water deficiency conditions produced high concentration concentrations of natural compound and secondary metabolite than same species grow under high water supply. Water deficiency induces drought stress-related metabolic responses, generating a large oxidative stress. As a consequence, metabolic processes are shifted towards biosynthetic activities that consume reduction equivalents. Accordingly, the synthesis of some products, terpene, phenols and alkaloids, are enhanced. **Huda et al., 2015 and Esmaeili et al., 2011,**^{3,4} reported that secondary metabolites play key physiological roles in plant growth development, reproduction and disease resistance.

Simple phenylpropanoids (caffeic, ferulic, sinapic acids and P-coumaric), with basic skeleton C6-C3 of phenylalanine, are produced from cinammate via a series of hydroxylation, dehydration and methylation reactions. The free acids rarely accumulate to high levels inside plant cells; instead, they are usually conjugated to sugars (salicylate-glucose conjugates), cell wall carbohydrates (ferulate, esters),^{5,6}.

1.1 Aim of Study

Wild plant grow and survive in drastic conditions of water deficiency and high temperature, aridity try to adapt to these condition by accumulation some active compounds such as alkaloid, flavonoid and terpene, phenolic compound, in present study we discuss the accumulation of alkaloid and phenolic compound by which the plant *Artemisia gudica* and *A. herba alba* collected from two region, Tabuk, Saudi Arabia and North Sinai, Egypt adapt to

oxidative stress.

2. Material and Methods

2.1 Plant Material

Fresh aerial parts of two plants from genus *Artemisia*, namely *Artemisia herba alba* and *Artemisia judaica* were collected from the arid zone at Arish-North Sinai, Egypt, and Tabouk region, Saudi Arabia, during the spring seasons, 2016. Collected plants have been kindly verified and authenticated in the Desert Research Center, voucher specimens were deposited in the Herbarium of Desert Research Center.

2.2 Phytochemical Screening

The phytochemical analysis of methanol extracts were tests to identify different classes of bioactive chemical constituents (flavonoid-alkaloid- terpene and sterol) present in the plant using standard methods^{11,18}.

2.3 Phenolic Compound Extraction

Phenolic compounds were extracted as described by¹. Extraction was carried out in water bath. The solutions from all extractions were combined. After evaporating the organic solvent in a rotary evaporator at 45°C, the crude extracts were stored at -20°C until further analysis.

2.4 Analysis of Phenolic Compounds

Analysis was carried out by (GC-MS) after silylation by N-Methyl-N-Trimethylsilyl Trifluoroacetamide (MSTFA), %1 Trimethyl Iodosilane (TMIS). Numerous compounds were detected and identified⁷.

2.5 Extraction and Identification of Alkaloid

The fin powder aerial part of plant samples (5 g) were boiled in water bath with 20 ml of HCl in 50% methanol. The mixture was cooled and filtered. A portion was reserved. Another portion of the filtrate was put in 200 ml separating funnel then add drops of ammonia to alkaline the solution. Add equal volume of chloroform and shaken to allow the layer to separate. The chloroform layer was extracted with two quantities each of 5 ml of dilute hydrochloric acid⁹.

2.6 Analysis of Alkaloid Compound

The constituents of the alkaloid extract and methanol extract analyzed by GC-MS as reported by¹². Compounds identified by comparison of their Retention Indices (RI), (C9 to C24 n-alkane mixture) and mass spectra with those reported in the literature^{1,12}.

2.7 GC-MS Analysis

The compounds were analyzed using a Thermo GC-Trace ultra system (Thermo Co. USA), they were separated on 30m X0.25 mm X 0.25 µm Elite-5MS column (Thermo Scientific GC Column). The column temperature was increased from 40 °C to 220 °C at a rate of 4 °C/min; injector temperature, 250°C; injection volume, 1 µl; helium carrier gas flow rate 20ml/min; transfer temperature, 280°C. MS parameters were as follows: EI mode, with ionization voltage 70 ev, ion source temperature, 180°C; scan range, 50-600 Da. The peaks were tentatively identified based on library search using NIST and Wiley Registry 8 Edition.

2.8 Antioxidant Activity

2.8.1 DPPH Radical Scavenging Capacity

In this study, 0.5 mL of each sample concentration was homogenized with the same volume of DPPH methanolic solution (0.04g/L). The mixture was shaken vigorously and allowed standing for 30 min in darkness at a temperature of 25°C; the absorbance of the resulting solution was measured at 517 nm with a spectrophotometer¹³. The percentage inhibition was calculated as:

Ascorbic acid and quercetin were used as positive control, the concentration providing 50% inhibition (IC₅₀) was calculated from the graph of inhibition percentage plotted.

3. Results and Discussion

Methanol extracts of the aerial part of *plants*, (*A. herba alba* and *A. judaica*) Figure 1, grown in the arid zones in the Northern regions of Saudi Arabia and North Sinai in Egypt were subjected to primary phytochemical analysis which shown the presence of high amount of phenolic compounds, and flavonoids, absence of tannins in *A. herba alba*, alkaloid and sterols have moderate concentration as investigated in Table 1.

3.1 Phenolic Compound

The investigation of methanolic extract of plant samples of *A. herba alba* and *A. judica* by GC-MS exhibited different types of phenolic compounds, ferulic acid, 4-hydroxy-cinamic acid-ester, p-tert-Butyl-catechol, n-Propyl-cinnamate, Butyl-cinnamate, and ethyl-cinnamate and 2,4,6-Trimethyl-mandelic acid. Most compound found in ester form, compounds was shown in, Table 2.

Artemisia herba alba*Artemisia judaica***Fig. 1.** The plants under study.**Table 1:** Phytochemical screening of *A. herba alba* and *A. judica* in two regions

Measurement	<i>A. herba alba</i> Saudi Arabia	<i>A. herba alba</i> Egypt	<i>A. judica</i> Saudi Arabia	<i>A. judica</i> Egypt
Flavonoid	++	++	+++	++
Tannin	-	-	+	+
Sterol	++	++	+	++
Alkaloid	++	+	++	+
Phenolic	+++	++	+++	++

(+++) **high concentration**, (++) **moderate concentration**, (+) **low concentration**, (-) **negative**

The highest phenolics compound was shown in *A. herba alba*, collected from Saudi Arabia methyl cinnamate (8.6%) and the lowest for 1,3-Bis (cinnamoyloxymethyl-adamantane (0.01%); also methyl-cinnamate are the highest (5.22%) phenolics compound in *A. judica* grown in Saudi Arabia. The

results showed high concentration of some phenolic compounds in Saudi plant compared with the plant grow in Egypt. As well, the phenolic compounds have an adequate chemical structure capable of scavenging free radicals which produced as a result of oxidative stress.

Table 2: % Amount Phenolic compound identified by GC-MS in two plants from two regions

Compounds	Egypt		Saudi Arabia	
	<i>A. herba alba</i>	<i>A. judica</i>	<i>A. herba alba</i>	<i>A. judica</i>
p-tert-Butyl- catechol	0.20	-	-	-
2,4,6 Trimethyl mandelic acid	0.04	-	-	-
Methyl-cinnamate	7.34	4.2	8.6	5.22
n-Propyl-cinnamate	1.82	0.96	-	2.5
Ethyl cinnamate	6.65	0.16	-	4.5
Benzoic acid, 4-hydroxy3methoxy, methyl ester	0.18	0.25	-	-
ferulic acid	0.01	-	-	0.12
Ursodeoxycholic acid	-	-	0.02	0.04
1,3 Bis (cinnamoyloxymethyl) adamantane	0.13	0.16	0.01	0.02
Methyl ferulate	0.02	-	-	0.10

Many studied reported that, drought stress inhibits the total synthesis of phenolic compounds in leaves and roots¹⁹, in earlier research on seedlings of *V. vinifera* exposed to chilling stress. Found he total content of tannins and phenolic acids in stress sample was lower than in control and author in⁴ (Amarowicz et al., 2010) observed the same trend in leaves of *V. vinifera* subjected to low temperature stress. On the other hand (Chung et al., 2010), observed quite the opposite effect, the chilling stress and water deficit caused an increase in the total content of phenolic compounds, our result was

agree with . Chung et al., where the plant which subject to more arid condition in Saudi Arabia accumulate higher amount of phenolic compounds. Many studied confirm that the production of phenolic compounds in plant tissues rises under a biotic stress condition^{19,20}.

In response to changing environmental conditions, plants evolved the capacity to biosynthesize different phenolic acids⁵. Here, four phenolic acid were identified in two plant, ferulic acid, cinnamic acid, benzoic and mandolic acid. The

most abundant was cinnamic acid, while, phenolic acids were found as ester bounded form.

The presence of cinnamic acid in ester form as methyl, butyl or as ethyl related to habitat of plant grow was it is a mechanism of degrading of cinnamic acid according to adaption to high temperature, Similar results were shown by Weidner et al. (2011) on their study on *Vitis californica*, the ester-bound form of caffeic acid increased under stress conditions. Also author in⁴ (Amarowicz et al. 2010), study the effect of low temperature, on germination of *Vitis vinivera*, while the content of p-coumaric acid declined.

3.2 Antioxidant Activity

3.2.1 DPPH Radical Scavenging Activity

The antioxidant activity, DPPH free radicals scavenging activity reducing antioxidant assay of methanol extract of aerial parts of *A.herba alba* (0.122and 1.7 mg/mL) in *Egypt* and Saudi Arabia respectively, where *A. judaica* has an important antioxidant activity with an EC_{50} of (0.56±0.04 and 0.67 mg/mL) as Table 3. These results is lower than that of ascorbic acid (EC_{50} : 0.095±0.002) and quercetin (EC_{50} : 0.019±0.003). The antioxidant activity of this extract is due to its chemical composition, which showed the presence of different phytochemical groups that have an antioxidant activity such as: alkaloids and phenolic compounds. Moreover, the chemical analysis using GC-MS allows identifying certain compounds which can be involved in the antioxidant mechanisms. Some

studies were evaluated the efficacy ferulic acid and cinnamic acid as an antioxidant. Cinnamic acid derivatives¹⁶ (Sharma, 2011), and Ferulic acid^{10,17}.

The reduction of DPPH is accompanied by its passage from the violet color characteristic of DPPH solution to the yellow color measurable at 514-518 nm. In this study, the reduce 50% of the DPPH radical (IC_{50}) was measured and the results showed that *Artemisia herba alba* grown in Saudi Arabia present higher antioxidant capacity with an IC_{50} of 0.122±0.001 mg/mL, followed by *Artemisia judica* grown Saudi with an IC_{50} of (0.56±0.04 mg/mL) However, the antioxidant capacity of ascorbic acid and quercetin were 0.091±0.001g/L and 0.026 ±0.002 g/L respectively (Table 3).

3.3 Total Alkaloid

The total alkaloid content of all the two plant varied from 0.24±0.2 to 1.26±0.3 mg/g of dried plant material. The *Artemisia herba alba* collected from Saudi Arabia show higher amount of alkaloid content. As in Table 1,this agree with (Mohammad Reza Amirjani 2013)⁹ who reported that Seedlings of *Catharanthus roseus* was subjected to water-regimes, total alkaloid content significantly increased to maximum 178% compared to the control. Vincristine and vinblastine content of the seedlings grown water regimes increased to 175% and 171% compared to the control, respectively. Authors in⁹ (Mohammad Reza Amirjani 2013) reported that the level of tropane alkaloid accumulation was

Table 3: Antioxidant Activity of *Artemisia herba alba* and *A.judica*

Measurement	<i>A.herba alba</i> Saudi Arabia	<i>A.herba alba</i> Egypt	<i>A. judica</i> Egypt	<i>A. judaica</i> Saudi Arabia	Ascorbic acid	Quercetin
EC_{50} (mg/mL)	0.122	1.7	0.67	0.56±0.04	0.091 ±0.002	0.026 ±0.002

Table 4: Total alkaloid mg/g in *Artemisia* in two regions

<i>Artemisia judaica</i>		<i>Artemisia herba alba</i>	
Egypt	Saudi Arabia	Egypt	Saudi Arabia
0.24	0.32	0.86	1.26

related to plant growth when he studied the effect of salinity stress ($153.8 \text{ mol/m}^3 \text{ NaCl}$), salt treatment increase the total alkaloid content in leaves.

3.4 Alkaloid Compound

The GC-MS analysis of the Alkaloid fraction of *Artemisia herba alba* and *A. judaica* detected different types of alkaloid compounds, was shown

in, Table 5. Pterin-6-carboxylic acid, Pseudosolasodine-diacetat, Dasycarpidan1methanol-acetate, Morphinan4,5epoxy3,6-diol, Aspidospermidin17ol and Dihydrovallesiachotamine in a different concentration of each compound according to plant and its region.

Alkaloid compound detected by GC-MS reflect the environmental change and environmental

Table 5: Analysis of Alkaloid compounds in plants in two regions

Compounds	Egypt		Saudi Arabia	
	<i>A. herba alba</i>	<i>A. judaica</i>	<i>A. herba alba</i>	<i>A. judaica</i>
Pterin-6-carboxylic acid	0.02	0.04	0.25	0.06
Pseudosolasodine diacetate	0.08	0.21	0.08	0.31
Dasycarpidan1 methanol, acetate	0.03	--	0.35	0.21
Morphinan 4,5epoxy3,6-diol	----	----	0.13	0.02
Aspidospermidin17ol	0.01	0.22	0.31	0.02
Desulphosinigrin	-	0.01	-	-
Dihydrovallesia chotamine	-	0.01	-	-

Table 6: Structure of compounds identified from *A. herba alba* and *A. judica* in two regions

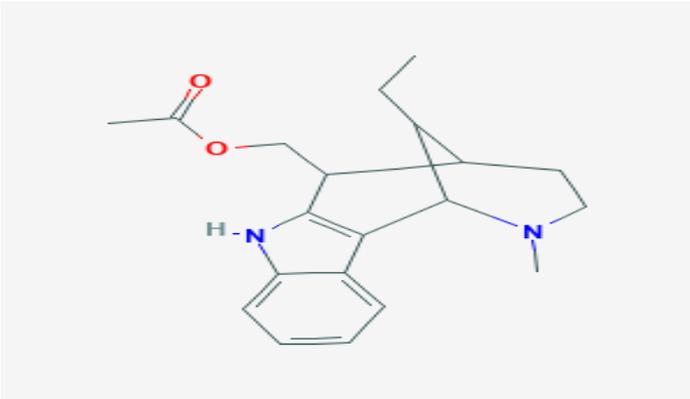
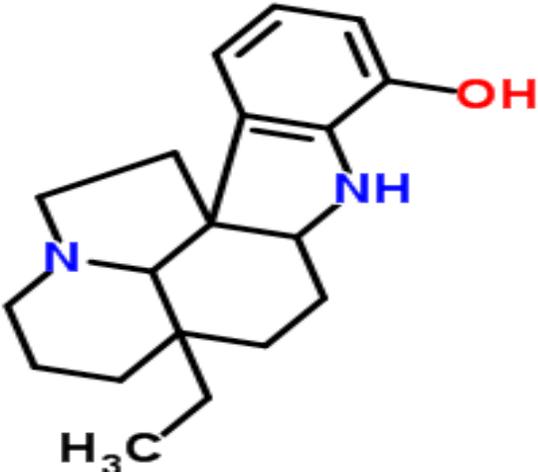
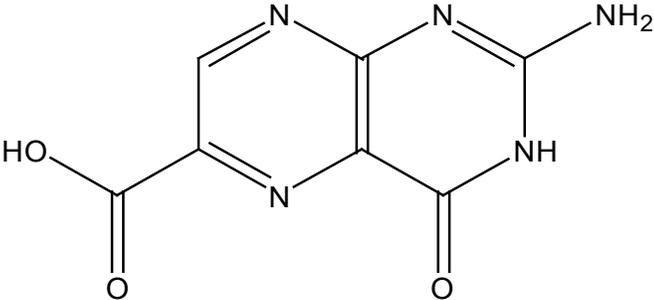
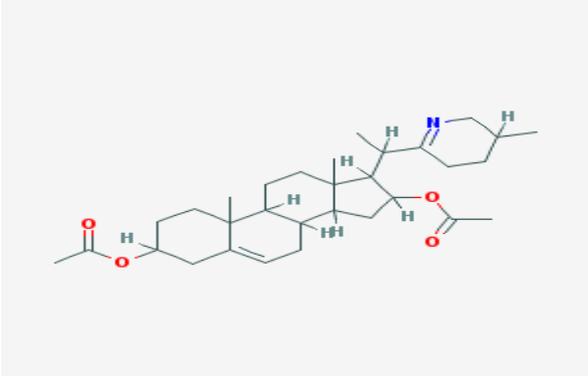
Rt	structure	Name
10.2		Dasycarpidan-1-methanol, acetate
12.06		Aspidospermidin-17-ol
14.86	 <p>Pterin-6-carboxylic acid</p>	Pterin-6-carboxylic acid

Table 6 Continued

32.86		Pseudosolasodine diacetate
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difference between two region from which the plant collected where some compounds are found in two plant in two region while other found in one habitat and not found in other, Morphinan-4,5-epoxy-3,6-diol compound was significantly showed in concentration (0.13 and 0.02 %) for two plant *A. herba alba* and *A. judaica* grown in Saudi Arabia and not detected in the plant collected from Egypt. Where Dasycarpidan-1-methanol, acetate was not detect in *A. judaica* grown in Egypt, furthermore Desulphosinigrin and Dihydrovallesiachotamine only detected in *A. judaica* and not detected in other samples.

The concentration of some alkaloid compound in plant grow in Saudi Arabia was more than the plant grow in Egypt as (Dasycarpidan-1-methanol, acetate and Pterin-6-carboxylic acid) in two plant *A. herba alba* and *A. judica* this agree with², **Hansan et al.**, reported that, Gramine concentration in the leaf of Barley grown in Arimar was affected by temperature in the same way, gramine concentration remained low at suboptimal and optimal growth temperatures, increased sharply as temperature became moderately sup optimal, and began to fall when the heat stress was severe

4. Conclusion

The antioxidant activity of genus *A. herba alba* and *A. judaica* growing in Egypt and Saudi Arabia reflect the ability of plant to survive under drastic condition by the accumulation of some secondary metabolite, phenolic and alkaloid compounds, GC-MS-analysis of methanolic extract exposed numerous phenolic compound which have high antioxidant activity, cinammic acid, ferulic acid and nButyl- cinnamate, where alkaloid fraction analysis revealed the presence of Pseudosolasodine diacetate, Pterin-6-carboxylic acid and Aspidospermidin-17-ol. These results proved the two plant species have high antioxidant activity espialy plant grow in Saudi Arabia which potent high antioxidant activity than plant grown in Egypt which reflexes the effect of environmental condition.

8. References

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