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Effect of habitat variations on physiological response, chemical compositions and antioxidant, of *Ephedra alata* (stapf) Andr

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Abstract: *Ephedra alata* is a widespread shrub found in various countries that originates from Southwestern North America, Southern Europe, and North Africa. This medicinal plant is part of the Ephedra genus, family Ephedraceae. The plant was collected from Wadi Arar, Northern Regions, and Wadi Hagul, Cairo/Suez Road, to investigate how the plant's chemical composition and antioxidant capacities are affected by habitat variation. The findings revealed notable variations in the total phenolic, total flavonoid, antioxidant activity, and soil analysis aspects between the two regions under investigation. The reducing ability of ferric ion of the plant extracts is present as 858.0 ± 38.95 µMTE/mg extract of plant growing in Arar region, while the reducing ability of *E. alata* growing in Egypt has Ec50 (650.0 ± 29.85) by FRAB methods. The plants *extract of E. alata* quench both ABTS and DPPH free radical. The sample showed higher ABTS scavenging activity (228± 195 μMTE / mg for the plant growing in Arar region while the plant ephedra growing in Wadi Hagul showed lower scavenging activity 185± 285 Umte/MG) in addition the two-plant showed high antioxidant activity when evaluated by DPPH radical scavenging test with IC_{50} (458.0 ± 38.95 ug/mL and 425± 28.9/mL). Total phenolic was determined by Folin-Ciocalteau method, However, the aluminum chloride consider was additionally employed to estimate the total flavonoid., the results showed high phenolic content (62.177 mg GAE), and high flavonoid content (39.6148± 3.816mg/g extract) for plant growing in Arar region than plant growing in Wadi Hagul, which have (45.60 mg GAE and 29.615 mg rutin/ mg Extract). Finally, the study reflects high variation between two habitats, this corresponding to two variations in climate change and different environmental conditions.

Key words: Habitats variation, *Ephedra alata*, antioxidant, secondary metabolite, total phenolic, total flavonoid.

1. Introduction

Saudi Arabia is a vast country with a variety of landscapes, including rocky and sandy deserts, valleys, and mountains. (**Alsherif, *et al.*, 2013).** Furthermore, the country's variation in temperature from 2000 to 2024 is characterized by three separate regions: the northwest experiences temperatures below 10°C, the center experiences temperatures between 10° and 15°C, and the eastern and western coastal regions experience temperatures between 15° and 20°C during the winter. Temperatures rise statewide in the spring, from 20° to 35°C, with the north experiencing a faster rate of growth. In contrast, the summer months are warm nationwide, with highs above 35 °C. In the summer, the south-east region has the greatest temperatures, with a mean of more than 35°C. During fall, mild temperatures are reported around the country, ranging from 25° to 30°C, except in the northwest. (**Almazroui, 2012)**

A variety of secondary metabolites are produced by plants in their environment of growth that perform a range of cellular functions necessary for physiological processes. Increasingly recent research has linked the synthesis of SMs to stress and defense response signaling. A plant's species, genetics, physiology, developmental stage, and growing environment all influence the kind and concentration of secondary compounds it produces. This implies the physiological adaptations developed by different taxonomic groups of plants to deal with defensive stimuli and stress. **(Isah, 2019)**

Native in North Africa, Southern Europe, and Southwestern North America, Ephedra alata is a shrub that is widely distributed throughout several nations. The genus Ephedra, which includes this medicinal plant, belongs to the family Ephedraceae, one of the three families that comprise the order Gnetales. (**Elhadef *et al.*, 2020; Iqbal *et al.*, 2020).**

Ephedra alata leaves have a variety of phytochemicals, such as flavonoids, alkaloids, and phenolic compounds, which are known to have therapeutic benefits when extracted from the leaves. The leaves of the plant have capping and reducing activity which can employing as capping and reducing agent been employed as capping and reducing agents in the production of copper oxide nanoparticles, silver, and gold **(Al-Radadi, 2023).**

Changes in the environmental conditions of different habitats are expected to affect photosynthetic rates and plant metabolism, with significant effects on the plant's primary and secondary metabolites. Therefore, the goal of the current study was to examine how the geographical differences between the wadi Arar North region Saudi Arabia and wadi Hagul Cairo/Suez road in Egypt affected the antioxidant and chemical compositions of the plant *Ephedra alata.*

2. Material and Methods

2.1. Plant Material

During the spring of 2023, the flowering aerial part of Ephedra alata were gathered from two locations: The Wadi Hagul, Cairo/Suez Road in Egypt and the Wadi Arar region in the Northern border of Saudia Arabia. The plants' identities have been generously confirmed at Northern Border University's Faculty of Science. Ea. F.E.1.23 voucher specimens were placed at the Faculty of Science girl section.

All samples of fresh aerial parts (50 gm plant powder) were extracted with the maceration process using a methanol-water mixture (80/20), then filtered after three days. Additionally, the same procedure was used to remove the raised marc three times. At a temperature of no more than 40 oC, the mixed methanol extracts were concentrated until they were dry under reduced pressure. Each sample's 10 and 12 g of crude methanolic plant extract were separated using 200 ml of chloroform and then filtered. The residue that had air-dried was filtered after being repeatedly cleaned with 100 milliliters of methanol. To the residue, extracts, tannins, flavonoids, and other compounds isolated in a neutral layer were mixed with 20 milliliters of water, 10 milliliters of 1 N HCl, and 100 milliliters of methylene chloride. A 10% Na2co3 (5 ml) solution was added to neutralize the aqueous layer, which was then stored in a freezer for a day before being filtered. For the next tests, all these fractions were stored in desiccators **(Elsharkawy *et al.*, 2021)**.

Table (1). Meteorological data covering areas studied during the last three months before samples collection

Habitat	Seasons	Total Precipitation	Max Temp. °C	Minimum temp. °C	RH %	Wind speed W/S
Wadi Arar Saudia Araiba	Wet	47.41	10.8	6.3	65.7	5.43
	Dry	21.97	35.0	25.7	56.2	4.33
Wadi Hagul/Cairio-Suez Road	Wet	38.82	19.2	12.7	67.0	3.38
	dry	12.04	29.8	22.6	63.0	3.82

2.2. The study area

The current study's territory is in Saudi Arabia's northern region (30 550 1300 N, 41 00 300 E). One of the principal wadis in Saudi Arabia's northeast is Wadi Arar. It stretches alongside Arar City and occupies an area of roughly 9500 km². This area has a Mediterranean desertic continental climate, this region is classed as a dry zone, with an average annual temperature of 21.5 degrees Celsius and a hot, dry, desert climate. The winter months frequently receive the most precipitation, with an average of 20.2 mm per year; extreme rainfall exceeding 90 mm happens between January and May. **(Osman *et al*., 2014, Elsharkawy *et al*., 2020)**

While Wadi Hagul is in Egypt's Northern Eastern Desert, within the Cairo-Suez district. According to Abdelaal (2017), it covers around 350 km2 or 0.16% of Egypt's Eastern Desert. The area is limited by latitudes 29°48'28"- 29°57'43" N. and longitudes 32°09'32"- 32°17'27" E. (Fig. 1). The main waterway is approximately 40 km long, 6-10 km wide, and ends in the Gulf of Suez. The Wadi Hagul region is characteristic of an arid desert climate with minimal rainfall, high temperatures, and significant evaporation rates. According to (**Zahran and Willis, 2009 and Nayira *et al*., 2019).**

2.3. Soil Analysis

Kilmer and Alexander used the pipette method to do a mechanical examination of the soil (without CaCO3 restriction). Electrical conductivity (EC), pH value, cations (Na^+, K^+, Ca^{++}), and anions (Cl^- m SO_4^{--}, and CO_3^{--}) were determined in soil water extract (1:5) and soil moisture content according to **(Rowell, 1994)** chlorides were measured by titrating 0.01N $AgNO_3$ with drops of potassium chromate at 5%. The turbidity method was used to determine sulphates (**Estefan *et al*., 2013**). Titration with sulfuric acid 0.01N was used to measure bicarbonate (**Reitemeier, 1943)**, and meq/L was used to express cations and anions.

Table (2). physical and chemical characters of soil supporting Ephedra alata at two regions

Regions	Moisture content %	pH	EC dsm^{-1}		Soluble anions meq\ L				Soluble cations meq\ L		
				Texture	Mg++	Ca++	K+	Na+	Cl-	$SO4^{--}$	HCO3-
Wadi Arar wadi	15.35	7.3	10.6	silty sand	7.6	3.8	2.05	45.32	4.5	20	0.7
Wadi Hagul	20.92	8.5	9.6	Sandy loam	4.5	11	1.02	34.78	3.0	12.8	1.20

2.4. Determination of total phenolic content (TPC)

The Folin-Ciocalteu's reagent (FCR) was used to determine the total phenolic compound content of all *E. alata* samples. Folin's phenol reagent, or FCR, is a colorimetric test for phenolic and polyphenolic antioxidants. It is a combination of phosphomolybdate and phosphotungstate. is a technique explained by (**Attard, 2017)** and gallic acid used as stander solution.

2.5. Determination of total flavonoid content:

The total flavonoid of plant samples was determined by Aluminum chloride methods using Rutin as a reference [Belhaj, *et al*., (2016)]. Rutin s was used as a stock solution. The results were expressed in milligrams equivalent to rutin per milligram of extract.

2.6. Antioxidant activity

2.6.1. ABTS Assay

The plant extracts sample was dissolved in methanol 2 mg/2 ml methanol and prepared stock solution of Trolox 1 m/M in methanol five serial dilution was prepared in concentration 600, 500m 400, 300, 200, 100, and 50 uM then the assay was carried out according to by**(Arano *et al*., 2001)** the color intensity was measured at 734 nm and all data recorded as means SD as the following equation.

Scavenging activity = Ac-Ab/Ac × 100

Ac: is Absorbance of control; Ab is Absorbance of sample

2.6.2. FRAP Assay

The ferric reducing ability was assayed according to the method of **(Benzi *et al*., 1996).** FluoStar Omega, a microplate reader, was used to measure the ensuing blue color at 593 nm. The means ± SD are used to represent the data. Using the linear regression equation taken from the calibration curve (the linear dose-response curve of Trolox), the samples' ferric reducing capacity is displayed as a μM TE/mg sample.

2.6.3. DPPH Assay

The DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical assay was conducted using the Boly *et al*. (2016) methodology. To put it briefly, 100μL of the sample and 100μL of newly made DPPH reagent (0.1% in methanol) were placed in a 96-well plate (n=3), and the reaction was allowed to sit at room temperature for 30 minutes in the dark. The subsequent decrease in DPPH color intensity was measured at 540 nm at the conclusion of the incubation period. Means are used to depict data. **(Elsharkawy *et al*., 2018)**.

Scavenging activity = Ac-Ab/Ac × 100

Ac: is Absorbance of control; Ab is Absorbance of sample

2.7. Statistical analysis

The study used a randomized full block design with combined analysis and comprised two sites with three replicates, two species, and two seasons. The MSTAT software package was used to evaluate the data collected for total flavonoids and total phenols (1991).

3. Results

The present study was aimed to study the effects of environmental condition between two habitats Wadi Hagul, Cairo/Suez Road region in Egypt and the Wadi Arar region in the Northern Region of Saudia Arabia), on the accumulation of secondary metabolites, and antioxidant of plant *E. alata* the plant sample were collected manually in triplicate at random from two habitat region at wet season.

3.1. Soil analysis

The data in Table 2 shows the chemical analysis of soil in two regions the results confirm the variation between two habits where the soil in wadi Arar is silty sand where other region are sandy loam, soil moisture content show significant vibration as in table according to type soil where silty sand are not apple to keep water and water content is very low. Soluble cation and anions showed higher concentrations in wadi Arar than in Wadi Hagul.

3.2. Total Phenolic and Flavonod content

The total phenolic compounds and total flavonoid content of *E. alata* extracts at two regions were shown in table 3. TPC and TFC were assayed according to method described above, the results showed high phenolic content (62.177 mg GAE), and high flavonoid content (39.6148± 3.816mg/g extract) for plant growing in Arar region than plant growing in Suez / Cairo Road region, which have (45.60 mg GAE and 29.615 mg rutin/ mg).

Table (3). Total phenolic and flavonoid content of *E. alata* at different habitat

Sample	**Total phenolic** **GAE mg./g extract**	**Total Flavonod content** **Rutin mg./g extract**
E.alata Sa	62.1775± 3.1792	39.6148± 3.81
E. alata Eg	45.60 ± 2.187	29.615± 2.12

3.3. Antioxidant activity

Ferric reducing antioxidant Power: FRAP method

This is a basic and simple technique for determining the reducing power activity of plant extracts. The FRAP Assay was designed to determine antioxidant capability by reducing Fe 3+ -TPTZ to FE2+ TPTZ using an antioxidant component detected in an analytical sample (phenolic fraction) of methanol extract of *E. alata* the reducing power activity of the samples is presented as 858.0 ± 38.95µMTE/mg extract of plant growing in Arar region, while the reducing ability of Ephedra growing in Egypt has Ec50 (650.0 ± 29.85) using the linear regression equation extracted from the calibration curve (linear dose-response curve of Trolox). As shown in Table 4, the plant grown in the Arar region has a higher reducing power.

DPPH and ABTS Assay

An elegant technique for examining the antioxidant activity of hydrogen-donating and chain-breaking antioxidants is the ABTS assay. Table 4 shows the outcomes of various sample and standard solution (torolox) concentrations. The findings demonstrated that *E. alata* plant extract quenches both ABTS and DPPH free radical. The sample showed higher ABTS scavenging activity (228± 195 μMTE / mg for the plant growing in Arar region while the plant ephedra growing in Wadi Hagul show lower scavenging activity 185± 285 uMTE/mg) in addition the two plant showed high antioxidant activity when evaluated by DPPH radical scavenging test with IC_{50} (458.0 ± 38.95 ug/mL and 425± 28.9) respectively for Ephedra plant collected from Arar and Egypt compared with control Trolox.

Table (4). Antioxidant activity DPPH, ABTS and FRAB assays of *E. alata* at two regions

Sample	ABTS µMTE / mg	FRAB EC_{50} µM TE/mg extract	DPPH IC_{50} ug/mL
E. alata Wadi Arar	228± 195	858.0 ± 38.95	458.0 ± 38.95
E. alata Wadi Hagul	185± 285	650± 18.95	425± 28.9

4. Discussion

The present study was carried out for investigation the effect of two different Arar and Wadi Hagul Cairo/Suez Road on the total phytochemical components and antioxidants of plant extract. Firstly, the environment in both regions exhibited considerable changes in climate parameters.

While the Suez region has unique features with a notable drop in temperature and a decrease in rainfall, the Arar region is characterized by very variable environmental conditions that range from extremely cold in the winter to high temperatures in the summer and more rainfall at last years. The chemical components of plants taken from different regions are anticipated to change because of these variations, which are likely to affect plant metabolism as an adaptation strategy. Certain chemicals, such antioxidants and total phenolic, build up more because of this dryness. The present study revealed a higher accumulation of polyphenolic compounds. Our findings showed that drastic condition, which was associated with lower temperatures and high winds speed, increased the production of total phenols and flavonoids in plant samples collected from the Arar region of Saudi Arabia. Total phenolic and flavonoids were found to accumulate the most in *E. alata* growing in the Arar region when soil moisture content decreased. Furthermore, raising salt levels in the plant's soil might result in an increase in total phenols and flavonoids.

Both drought response pathways depend heavily on phenols and flavonoids, which are the main constituents of secondary metabolites in plants. The findings concur with numerous authors. (**Abd El maboud and Elsharkawy, 2021**), who observed increased accumulation of total phenols in Salicornia represent the effect of environmental conditions in a different habitat during the dry season.

The study of abiotic variables that affect secondary metabolism during plant growth in vitro and in vivo has attracted increasing attention in recent decades. With applications in metabolic engineering of biosynthetic pathway intermediates, the use of molecular biology tools and techniques is making it easier to comprehend the signaling pathways and processes involved in the production of secondary metabolites during vivo and in vitro growth. **(Isah, 2019)**

In a previous study by **Elsharkawy *et al*. (2021),** it was shown that the total phenolic and alkaloid compounds of the plant *Achelia fragmmentissma* are accumulated in higher amounts in the Arar region, and some compounds, such as ferulic acid Eugenol and salicylic acid ester, are affected by calamitic change in the Northern region.

The current investigation revealed that *E. alata* collected from Wadi Ara has considerably greater antioxidant activity assayed by DPPH. The reducing power of plants which is determined by FRAP method also reflects the high antioxidant activity of the plant in two regions. The increased levels of antioxidant activity of the plant gathered from wadi Arar can be related mostly to the higher amounts of polyphenolic compounds. That is consistent with the previously issued results. Phenolic compounds' capacity to scavenge free radicals by donating hydrogen atoms and electrons and chelating metal cations explains their antioxidant action. **(Elsharkawy *et al*., 2018).**

5. Conclusion

The current study concluded that plant E. alata, which is spread in the northern region of Saudi Arabia and Wadi Hagul Suez/Cairo Road in Egypt, showed that changes in habitat between Wadi Arar and Wadi Hagul had a substantial impact on the chemical compositions and antioxidant activities of plants taken from both environments. This difference is mostly due to the increased quantities of polyphenolic compounds found in the plant growing in the Arar region because of the effect of the drastic condition. This demonstrates the influence of geographical location on plant chemical composition and biological activity. The diversity of climatic parameters and soil analysis represent the change in the accumulation of secondary metabolites of the plant species, which reflect the role of phenolic compounds in adaptation to such conditions.

Conflicts of Interest

The authors declare no conflicts of interest

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