Bull. Pharm. Sci., Assiut University, Vol. 46, Issue 2, 2023, pp. 927-941.

Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://bpsa.journals.ekb.eg/
e-mail: bullpharm@aun.edu.eg

INFLUENCES OF ENVIRONMENTAL FACTORS ON THE ACTIVE METABOLITES AND CERTAIN BIOACTIVITIES OF DESERT DATE LEAF AND FRUIT (*BALANITES AEGYPTIACA* L. DELILE) GROWN IN EGYPT

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Balanites aegyptiaca (L.) Delile is a medicinal wild tree, naturally distributed in wide regions of Africa and South Asia. The fruits are commercially available in Egypt as antidiabetic natural products. The current study aimed to determine the effects of environmental factors on the metabolites and the bioactivities of fruit and leaf extracts. The main ecological differences between the sites of collection, Wadi El−Gemal and Baris Oasis, are temperature, aridity, humidity, and location. The fruit extract of Baris Oasis showed higher saponin content and saponin metabolites (LC−MS analysis) than the fruit extract of Wadi El−Gemal. Baris Oasis fruit extract showed twice the inhibition of α−Glucosidase than Wadi El−Gemal fruit extract. Twelve phenolics and flavonoids were detected in Baris Oasis leaf extract, while ten compounds were detected in Wadi El−Gemal leaf extract using HPLC. The higher quality and quantity of flavonoids and phenolics of Baris Oasis leaf extract than Wadi El−Gemal leaf extract, have significant effects on the antioxidant and acetylcholinesterase inhibitory activities. In conclusion, elevation of temperature and aridity have significant positive effects on the synthesis and accumulation of B. aegyptiaca saponins, phenolics and flavonoids. The bioactivities of the fruit and leaf extracts are directly proportional to the variation in metabolites due to abiotic stresses.

Key word: *Balanites aegyptiaca L, Ecological Factors, Saponins, Flavonoids, Phenolics.*

INTRODUCTION

Balanites aegyptiaca (L.) Delile is a popular medicinal wild tree, endogenous to Africa and South Asia[1]. It has a diverse ecological distribution in almost all African countries according to the temperature, rainfall, soil type, and location[1]. In Egypt, it is naturally distributed in all desert regions except the Mediterranean and north Red Sea strips[2-4]. *B. aegyptiaca* organs have been used worldwide in folk medicine. The fruits and their aqueous extract are commonly used as antidiabetic natural drugs in Egypt[5]. Other organs such as seeds, leaves, bark, and roots of *B. aegyptiaca* are used in African and Asian countries for the treatment of various diseases such as asthma, epilepsy, jaundice, malaria, fever, wounds, intestinal worms, constipation, dysentery, stomach aches, and hemorrhoids[6&7].

Several biological and phytochemical studies on the different *B. aegyptiaca* organs have been previously reported. In vivo, in vitro and clinical studies on the herbal tea, capsules,

Received in 15/6/2023 & Accepted in 24/7/2023

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aqueous, methanol and butanol fruit extracts as well as their isolated saponin compounds confirmed the hypoglycemic and antidiabetic activities by several mechanisms of action[8-14]. The ethanol and aqueous extract of leaves displayed antioxidant, antibacterial, acetylcholinesterase, and xanthine oxidase inhibitory activities[15&16]. Previous phytochemical studies on the leaf extract led to the identification of flavonoids, coumarin and saponins[6]. Other biological and phytochemical studies on the roots and seeds have been reported[6].

Several compounds of different chemical classes were isolated from *B. aegyptiaca* organs (fruit, leaves, stem bark, galls, root, and seeds), such as saponins (Furostanol, spirostanol, and other steroidal saponins), alkaloids (trigonelline, N-cis-feruloyl tyramine, and N-trans-feruloyl tyramine), polyphenols (flavonoids, coumarins, and simple phenolics), and pregnane glycosides[17&18].

Balanitins (1-7), deltonin, (3*β*,20S,22R,25R)-spirost-5-en-3-yl, *β* -D-xylopyranosyl-(1-3)- *β* -D-glucopyranosyl-(1-4)-[*α*-Lrhamnopyranosyl-(1-2)]-*β*- D-glucopyranoside and (3β,20S,22R,25S)-spirost-5-en-3-yl -D-xylopyranosyl-(1-3)- *β*-D-glucopyranosyl-(1-4)-[α-Lrhamnopyranosyl-(1-2)]-*β*-D-glucopyranoside were spirostanol saponins isolated from the fruit, root, seed, and stem bark of *B. aegyptiaca*[19-24].

Several furostanol steroid saponins were isolated from *B. aegyptiaca* fruit, including balanitesin, balanitoside, 22R and 22S epimers of 26-(O-*β*-D-glucopyranosyl)-3-*β* -[4-O-(*β* -D-glucopyranosyl)- 2-O-(*α* -L-rhamnopyranosyl)-*β* -D-glucopyranosyloxy]-22,26-dihydroxyfurost-5-ene, xylopyranosyl derivative of 26-(O-*β*-D-glucopyranosyl)-3-*β*-[4-O-(β-D-glucopyranosyl)-2-O-(*α*-L-rhamnopyranosyl) *β*-D-glucopyranosyloxy]-22,26-dihydroxyfurost-5-ene, 3-O-(2,4-di-O-*α*-L-rhamnopyranosyl)-*β*-D-glucopyranoside-22-methyl ether of 26-O-*β*-D-glucopyranosyl-(25R)-furost-5-ene-3*β*,22,26-triol and 3-O-(2,4-di-O-*α*-L-rhamnopyranosyl)-*β*-D-glucopyranoside-26-O-*β*-D-glucopyranosyl-(25R)-furost-5-ene-3,22,26-triol[25-29]. Six furostanol saponins with molecular masses of 1224, 1210, 1196, 1064, 1046 and 1078 Da were identified in the kernel extract by using LC-MS[30].

B. aegyptiaca is a rich source of flavonoids and phenolics. Kaempferol, myricetin, quercetin, hyperoside, quercetin 3-glucoside, and rutin are examples of flavonoids previously detected in the leaf extract [31]. Phenolics such as caffeic, ferulic, gentisic, p-coumaric, sinapic, and syringic acids were also detected [31].

The abiotic stresses such as light, temperature, water, soils type, and locations have direct effects on the synthesis and accumulation of the secondary metabolites [32] and, hence, the quality of the medicinal plant raw materials for the pharmaceutical industry of herbal products. Therefore, our study aims to detect the effects of environmental factors on *B. aegyptiaca* leaf and fruit metabolites. The effects of the variation in metabolites on the bioactivity of fruit and leaf extracts are discussed in the current study.

MATERIALS AND METHODS

Plant materials

The plant used in the current study was identified by Prof. Fawzy M. Salama, professor of Botany, Faculty of Science, Assiut University, Egypt.The fruits and leaves of *B. aegyptiaca* were collected from Wadi El−Gemal, South−Eastern desert, and Baris village Oasis, New Valley, South−Western desert, Egypt in March 2021. The leaves and fruit pulps were dried at room temperature, grounded into powders and stored at room temperature. Voucher specimens of the fruit and leaf were placed in the herbarium of the Department of Botany, Faculty of Science, Assiut University, Egypt with numbers F−2221983 and L−2221984.

Environmental conditions of the areas of plant collections

The fruits and leaves of *B. aegyptiaca* were collected from two climatically different areas **(Fig. 1).** The first area was Wadi El−Gemal, in the southeastern desert of Egypt[2]. The second area was the Baris Oasis, New Valley, in the South−Western desert of Egypt. Wadi El−Gemal is in Egypt's South−Eastern desert at 24° 39′ 25.84″N and 35° 05′ 34.86″E. It is near to the Red Sea and its altitude is at the same sea level. According to meteorological data obtained from Marsa Alam station in the Eastern desert, the temperature is regular in the four seasons. The winter and summer months are hot. In the last five years (2017−2022), the average lowest minimum temperature was 26

°C in 2020, and the average highest maximum temperature was 35 °C in 2017, 2018, and 2019.The highest mean relative humidity in the last 5 years was 51.0% recorded in 2022 and the lowest mean was 42.0% recorded in 2019. Rainfall was rare and occurred in 2017 (2.4 mm) only in the last 5 years (Meteorological Marsa Alam station, Egypt (Meteorological data Egypt, 2022).

Baris Oasis is located at 24° 40'−26° N and 30° 07'−30° 47' E, in the South Western desert of Egypt. Its altitude is 115 m above the Red Sea. According to meteorological data obtained from New Valley station in the western desert, the winter and summer months are very hot. The average minimum temperature over the last five years (2017−2022) was 29 °C in 2022, and the average maximum temperature was 45 °C in 2022. The highest mean relative humidity over the last 5 years is 18%, recorded in 2017, 2019 and 2021, and the lowest mean is 15%, recorded in 2020. There has been no rainfall during the last 5 years (hyper arid weather).

Extraction of secondary metabolites

The fruit powders (100 g) were decocted separately in 0.5 L of methanol (70%) for 2 hrs, three times. The same methanol extracts were mixed and concentrated under vacuum pressure. The dried extracts (20 g) of fruits collected from the eastern and western deserts of Egypt were kept at −20 ºC until phytochemical and biological studies. The leaf powders (150 g) were defatted by maceration in ether for 48 hrs. The leaf marc was then macerated twice in ethanol (80%) for 48 hrs. The same ethanol extracts were mixed and concentrated by using rotavapor. The dried ethanol extracts of leaves (40 g) collected from the eastern and western deserts of Egypt were kept at −20 ºC until phytochemical and biological studies.

Fig.1: A Google map of the sites of plant collection; Baris Oasis, South Valley, West Desert of Egypt; and Wadi El−Gemal, East Desert of Egypt.

Colorimetric Assay for quantification of phenolics, flavonoids and saponins

Folin−Ciocalteu's method[33] was used for the determination of the total phenolics of the leaf extracts. Gallic acid solutions (10 − 100 ppm) **(Fig. S1 A)** as well as the leaf extracts (10 mg/mL) were prepared. Folin−Ciocalteu's phenol reagent (0.2 mL), 1 mL of sodium carbonate, and 1.6 mL of distilled water were added to 1 mL of each gallic acid concentration and tested samples. The tubes were kept in the dark for 10 min. The intensity of colour was measured at 750 nm using a UV2000 spectrophotometer (Ray Wild Limited Company, L569 Gottingen, Germany). The flavonoid content of the extracts of leaves was determined by using quercetin as a reference compound (**Fig.S1 B**) and $AlCl_3$ 2% reagent (0.6 mL) as a colorimetric reagent[34]. The intensity of colour was measured at 415 nm using a UV2000 spectrophotometer (Ray Wild Limited Company, L569 Gottingen, Germany). The vanillin sulfuric method[35&36] was used to quantify the saponin content of fruit extracts. As a reference compound, digoxin **(Fig.S1 C)** was used. The spectrophotometric wavelength of the measurements of saponins and digoxin was 450 nm. The saponin, phenolic, and flavonoids contents of fruit and leaf extracts were calculated as a mean of triplicate measurements ± SD.

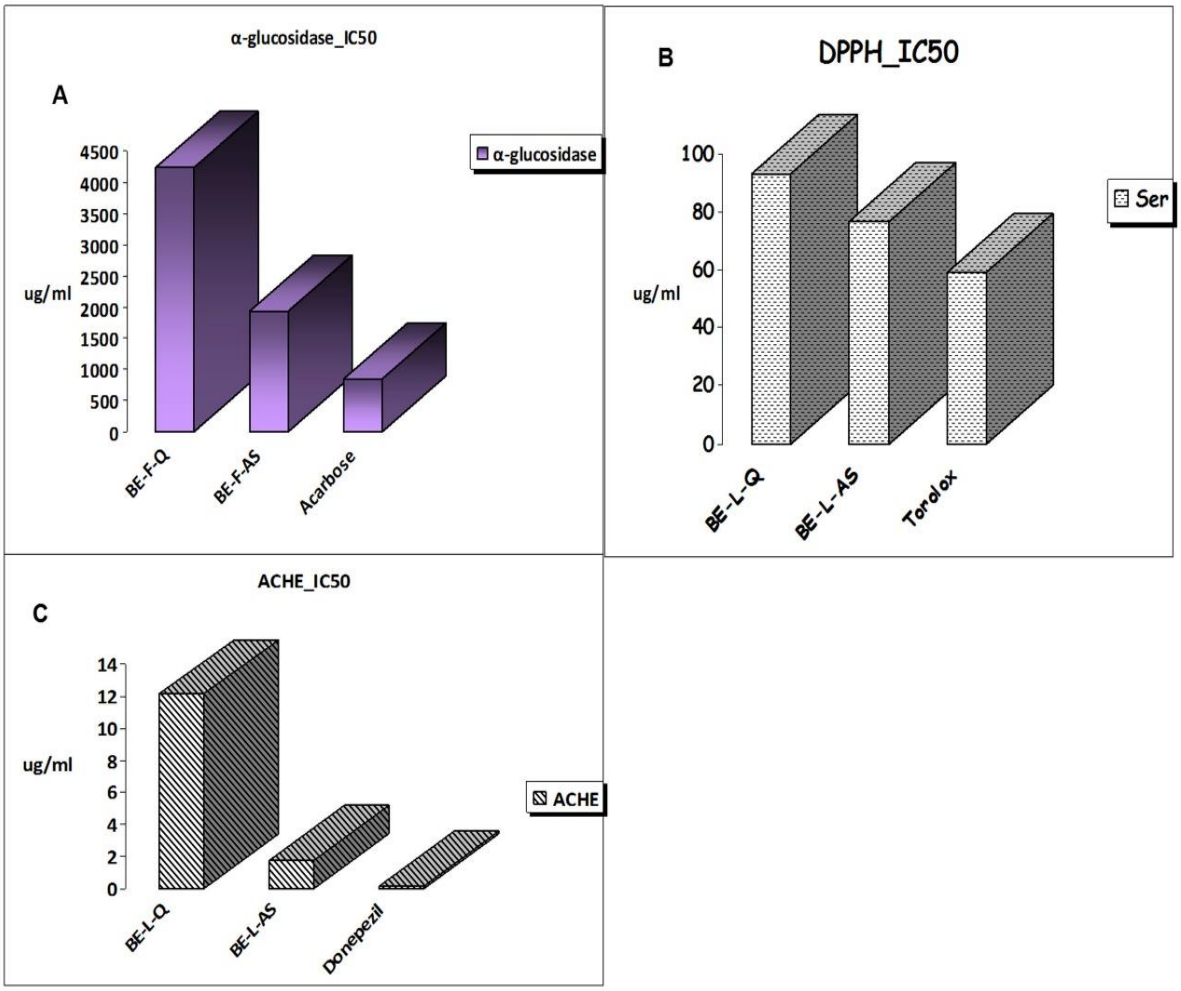

Fig.2: Bioactivities of leaf and fruit extracts of *B. aegyptiaca.*

A: α−Glucosidase inhibitory activities (IC_{50}) of Baris Oasis fruit extract (BE−F−AS) and Wadi El−Gemal fruit extract (BE−F−Q).

B: Antioxidant (DPPH scavenging IC_{50}) activities of Baris Oasis leaf extract (BE−L−AS) and Wadi El−Gemal leaf extract (BE−L−Q).

C: Acetylcholinesterase inhibitory activities (IC_{50}) of Baris Oasis leaf extract (BE−L−AS) and Wadi El−Gemal leaf extract (BE−L−Q).

Phytochemical analysis of fruit extracts by LC−MS

A GL−Science−C18 column (Torrance, CA, USA) (3 µm, 2.1 × 100 mm) was used for metabolite separation. The mobile phase solvents, (A) 0.1% formic acid in water and (B) acetonitrile, were used for separation of fruit metabolites at 25 °C with a flow rate of 0.35 mL/min in 25 min. The chromatographic separation of fruit metabolites was performed by using gradient elution of solvents (A and B), starting from 100 % of solvent A at 0 min until 100 % of solvent B at 25 min. Identification of compounds was attained by using an X500 QTOF mass spectrometer equipped with a Turbo Ion spray source ESI used for ionization. MS analysis was performed with a capillary voltage of 4000 V in both negative and positive modes.

Phytochemical analysis of leaf extracts by HPLC

Detection of phenolic compounds

The HPLC−Agilent 1100 (Agilent Technology, USA) was used for the identification of the compounds. A C18 column (125 mm × 4.60 mm, 5 µm) was used in the separation of compounds using solvent A: acetic acid in water (1:25) and solvent B: 100% methanol mobile phase in a gradient elution in 15 min. Phenolic compounds were detected at 280 nm. They were detected by using Agilent ChemStation software (Agilent Technology, USA) and were confirmed by using authentic samples.

Detection of flavonoids compounds

The HPLC−Agilent 1100 (Agilent Technology, USA) was used for the identification of the compounds. A C18 column (125 mm × 4.60 mm, 5 µm) was used for the separation of compounds using solvent A: formic acid (0.1%) in water and solvent B: 100% acetonitrile mobile phase in isocratic elution with 70% solvent B in 15 min. Phenolic compounds were detected at 320 nm. The compounds were identified by using authentic samples.

In vitro antidiabetic activity of fruit extracts

The extracts of fruits collected from Wadi El−Gemal and Baris were tested separately for their inhibitory activity against α−Glucosidase using colorimetric assay[37]. Different concentrations of fruit extracts (100 − 10000 µg/mL) in ethanol were prepared. α−Glucosidase inhibitor screening kit (Biovesion, Tokyo, Japan) was used as the detection reagent. Preparation of a 20−fold of enzyme was performed by adding 2 µL of α−Glucosidase with 38 µL of α−Glucosidase assay buffer, mixing and keeping the diluted enzyme on ice. Then, 10 µL dilluted enzyme was added to 96 well plates previously divided into 4 groups; group A contained 10 µL tested samples in triplicates for each concentration, group B contained 10 µL Acarbose in triplicates for each concentration, group C contained 10 µL and 20 µL α−Glucosidase buffer and group D contained 10 µL ethanol (Negative control). The next steps were mixing the enzymes with the tested groups, adjusting each well to contain 80 µL of buffer, and keeping them for 15 min on dark. The reaction was started by adding 20 µL reaction substrate solution (17 µL assay buffer & 3 µL α−Glucosidase substrate) to each well. Spectrophotometric measurement of the p−nitrophenol (End product of the reaction of α−Glucosidase with the substrate) in the 95 tested plates was performed at 410 nm using a plate reader (Thermo−Fisher Scientific, MA, USA). Determination of the relative inhibition and relative activity was achieved by these equations:

$$\%\ \text{Relative inhibition} = \frac{\text{Slope of Enzyme control} - \text{Slope of tested sample} \times 100}{\text{Slope of Enzyme control}}$$

$$\%\ \text{Relative activity} = \frac{\text{Slope of tested sample}}{\text{Slope of Enzyme control}}$$

In vitro antioxidant and acetylcholinesterase inhibitory activities of leaves.

The extracts of *B. aegyptiaca* leaves collected from Wadi El−Gemal and Baris were tested separately for their antioxidant and cholinesterase inhibitory activities. The antioxidant activities were performed by using trolox (Positive control) in the well−known DPPH assay method[38]. The scavenging degree of the tested samples was calculated by the following equation:

$$\%\ \text{Scavenging effect} = \frac{\text{Absorbance of control} - \text{Absorbance of tested sample} \times 100}{\text{Absorbance of control}}$$

In the acetylcholinesterase assay, different concentrations of leaves extracts (0.1– 100 µg/mL) in ethanol were prepared. Assay reagent: Acetylcholinesterase inhibitor screening kit (Biovesion, Tokyo, Japan) was used as the detecting reagent. Preparation of 50−fold of enzyme was performed by adding 2 µL of acetylcholinesterase (10 µM) to 98 µL of acetylcholinesterase assay buffer, mixing and keeping the diluted enzyme on ice. Then, 10 µL diluted enzyme was added to 96 well plates previously divided into 3 groups; group A contained 10 µL tested samples in triplicates for each concentration; group B contained 10 µL donepezil in triplicates for each concentration and group C contained 100 µL of acetylcholinesterase assay buffer. Mixing the enzymes with the tested groups, adjusting each well to contain 160 µL with the buffer and kept for 15 min on dark were the next steps. The reaction was started by adding 40 µL reaction substrate solution (25 µL assay buffer, 5 µL prob Mix, & 10 µL acetylcholinesterase substrate) to each well. Spectrophotometric measurement of the 95 tested plates was performed at 412 nm using a plate reader (Thermo−Fisher Scientific, MA, USA). Determination of the relative inhibition and relative activity was achieved by these equations:

$$\%\ \text{Relative inhibition} = \frac{\text{Slope of Enzyme control} - \text{Slope of tested sample} \times 100}{\text{Slope of Enzyme control}}$$

$$\%\ \text{Relative activity} = \frac{\text{Slope of tested sample} \times 100}{\text{Slope of Enzyme control}}$$

Statistical analysis

The variation in the quantity of secondary metabolites and collected areas was assessed by one−way ANOVA, followed by Duncan's Post−Hoc multiple comBarisons test. The differences between the means seemed to be significant at $P < 0.05$B. All these analyses were performed using SPSS software (version 26).

RESULTS AND DISCUSSION

Results

***B. aegyptiaca* secondary metabolites**

The current study showed the effect of some environmental factors (temperature, location, air humidity, and aridity) on the synthesis and accumulation of fruit saponins and leaf polyphenols. The fruit saponins were selected in the current study due to their previous isolation in both furostanol and spirostanol types as major compounds responsible for the antidiabetic activity of the fruit [17,18,22,28,30,39,40]. The leaf produced phenolics and flavonoids that were responsible for antioxidant and acetylcholinesterase inhibition [16]. The antioxidant activity of leaf extract and fractions was in direct proportion to the total phenolic and total flavonoid contents [16]. This encourages us to study the effect of abiotic stresses on the leaf polyphenols, antioxidants, and anticholinesterase activities. The differences in location, temperature, aridity, and humidity between the sites of plant collection, Wadi El−Gemal and Baris Oasis have excessive effects on both the quality and quantity of *B. aegyptiaca* var. aegyptiaca fruit saponins and leaf polyphenols, as shown in Tables 1, 2, and 3. The difference in quantities of fruit saponins and leaf polyphenols is listed in Table 1. The LC−MS metabolite profiling of fruit extract in the negative (−) and positive (+) ESI modes of ionization led to the identification of saponins, an alkaloid (trigonelline), and citric acid, as shown in **Table 2 and Figs. S2, S4, S5, S6, and S7**. The identification of compounds was depended on the molecular weight, molecular formula, and comparison with previously reported data and the Metlin database. HPLC−UV was used to analyse the leaf extract, and the detected polyphenols are listed in **Table 3** and shown in **Figs. S3, S8−S10**. The detected flavonoids and phenolics were identified by using authentic samples.

Bioactivity of *B. aegyptiaca* fruits and leaves extracts

B. aegyptiaca fruit extract, butanol fraction, and isolated furostanol saponin have been shown to inhibit the α−Glucosidase [41]. The antioxidant and acetylcholinesterase inhibitory activities of the leaf extract in correlation with the polyphenol content have been previously described [42]. These reported activities compel us to investigate the effects of environmental factors (location, aridity, air humidity, and temperature) on the fruit extract's α−Glucosidase inhibition of the fruit extract and the DPPH antioxidant, as well as the acetylcholinesterase inhibitory activity of the

leaf extract. The bioactivities are listed in **Table S1** and shown in **Fig. 2**.

Discussion

The quantity of fruit saponins and leaf polyphenols of *B. aegyptiaca* var. aegyptiaca (Table 1) was influenced by the abiotic stresses of the collected area. The elevation in temperature, lowering of air humidity, and drought stress in the Baris Oasis area increased the accumulation of fruit saponins (19.92 ± 3.85% mg/g D.W.) in comparison with Wadi El−Gemal (13.70 ± 3.09 mg/g D.W.). The increase in the accumulation of saponin (glycyrrhizin) under drought stress in *Glycyrrhiza glabra* was previously reported [43]. The drought stress stimulated the expression of genes responsible for the synthesis of glycyrrhizin (saponin)[43]. Similarly, drought stress and temperature increases stimulated the gene expression of *B. aegyptiaca* var. aegyptiaca saponins. Therefore, twenty saponins were detected in Baris Oasis fruit and seventeen saponins in Wadi El−Gemal fruit (Table 2). The detected saponins were classified as ten furostanols, five spirostanols, and five triterpenoid saponins of the oleanane type. Three furostanol saponins: 26-(O-*β*-$_D$-glucopyranosyl)-3-*β*-[4-O-(3-*β*-$_D$-xylopyranosyl-*β*-$_D$-glucopyranosyl)-2-O-(*α*-$_L$-rhamnopyranosyl)-*β*-$_D$-glucopyranosyloxy]-22,26-dihydroxyfurost-5-ene (**4**), (3,20S,22R,25R)-26-(*β*-$_D$-glucopyranosyloxy)-22-methoxyfurost-5-en-3-yl-D-xylopyranosyl-(1-3)- *β*-$_D$-glucopyranosyl-(1-4)-[*α*-$_L$-rhamnopyranosyl-(1-2)]-*β*-$_D$-glucopyranoside (**5**), and balanitoside (**6**) were previously reported in the *B. aegyptiaca* fruit [25,27,28]. Five known spirostanol saponins (**7**, **8**, **14**, **18**, and **21**) were detected in Baris Oasis fruit for the first time in the plant. Spirostanol saponins (**8** and **14)** were not detected in Wadi El−Gemal fruit. Five known oleanane type triterpene saponins (**2**, **9**, **12**, **16**, and **20**) were first reported in Baris Oasis fruit. Compound **9 (**Assamsaponin E) was not detected in the Wadi El−Gemal fruit sample. The plant sources for the first time detected saponins (**2, 7, 8, 9, 12, 14, 16, 18, 20, 21**) were previously reported[44-52].

Flavonoids such as kaempferol, myricetin, quercetin, hyperoside, quercetin 3−glucoside, and rutin were previously detected in the *B. aegyptiaca* leaf extract[31]. Caffeic, ferulic, gentisic, p-coumaric, sinapic, and syringic phenolic acids were also detected[31]. These polyphenols were responsible for the antioxidant activity of the leaf [42]. Drought stress and temperature elevation influenced *B. aegyptiaca* leaf flavonoids and phenolic content. The flavonoid content (Table 1) of Baris Oasis leaf (1.91 ± 0.03 mg/g D.W.) was twice as high as that of Wadi El−Gemal leaf (0.97 ± 0.09 mg/g D.W.). The phenolic content of Baris Oasis leaf (13.44 ± 1.02 mg/g D.W.) was increased in response to drought stress and an increase in temperatures. The effects of these two abiotic stresses (drought and temperature) on the synthesis and accumulation of the polyphenols were previously confirmed [53]. Drought stress, for example, increased the flavonoid and phenolic content of Hypericum brasiliense [53]. The elevation in temperatures increased the concentration of phenolics in *Astragalus compactus*[54].

Table 1: Phenolics and flavonoid content of the leaf's extracts, and saponin content of the fruit extracts.

Metabolite contents	Baris Oasis fruit extract (mg/g D w)	Wadi El−Gemal fruit extract (mg/g D w)	Baris Oasis leaves extract (mg/g D w)	Wadi El−Gemal leaves extract (mg/g D w)	F. Value
Saponins	19.92 ± 3.85	13.70 ± 3.09	−	−	1.59
Flavonoids	−	−	1.91 ± 0.03	0.97 ± 0.09	106.03**
Phenolics	−	−	13.44 ± 1.02	9.40 ± 1.07	7.54*

*: $p < 0.05$; significant difference, **: $p < 0.001$; high significant difference.

Table 2: LC−MS based metabolite profiling of the fruit extract collected from Wadi El−Gemal (BEF-Q) and Baris oasis (BEF-ASS) in both positive (+) and negative (-) modes of ionization.

Peak no.	Retention time (Rt)	Ionized mass from LC−MS	Molecular formula	Presence (+) / Absence (-)		Compound name
				BEF-ASS	BEF-Q	
1	0.8	191.0204 $[M-H]^-$	$C_6H_8O_7$	(+)	(+)	Citric acid
2	13.05	1411.6406 $[M+CH_3COO]^-$	$C_{63}H_{100}O_{31}$	(+)	(+)	Foetidissimoside B
3	13.3	1225.5849 $[M-H]^-$	$C_{57}H_{94}O_{28}$	(+)	(+)	Trigoneoside XIII
4	13.47	1195.5741 $[M-H]^-$	$C_{56}H_{92}O_{27}$	(+)	(+)	26-(O-β-$_D$-glucopyranosyl)-3-β-[4-O-(3- β-$_D$-xylopyranosyl-β -$_D$-glucopyranosyl)-2-O-(α-$_L$-rhamnopyranosyl)- β -$_D$-glucopyranosyloxy]-22,26-dihydroxyfurost-5-ene.
5	13.49	1209.5891 $[M-H]^-$	$C_{57}H_{94}O_{27}$	(+)	(+)	(3,20S,22R,25R)-26-(β-$_D$-glucopyranosyloxy)-22-methoxyfurost-5-en-3-yl-D-xylopyranosyl-(1-3)- β-$_D$-glucopyranosyl-(1-4)-[α-$_L$-rhamnopyranosyl-(1-2)]-β-$_D$-glucopyranoside.
6	13.55	1063.5315 $[M-H]^-$	$C_{51}H_{84}O_{23}$	(+)	(+)	Balanitoside
7	13.7	1353.6335 $[M-H]^-$	$C_{63}H_{102}O_{31}$	(+)	(+)	Disogenin-3-[glucosyl-(1-4)-[glucopyranosyl-(1-6)]-glucopyranosyl-(1®4)-rhamnosyl-(1-4)-[rhamnosyl-(1®2)]-glucoside].
8	13.79	1237.5861 $[M+FA-H]^-$	$C_{57}H_{92}O_{26}$	(+)	(−)	Disogenin-3-[glucosyl-(1®6)-glucosyl-(1-4)-rhamnosyl-(1-4)[rhamnosyl-(1-2)]-glucoside].
9	13.8	1237.5619 $[M+Na-2H]^-$	$C_{59}H_{92}O_{26}$	(+)	(−)	Assamsaponin E
10	13.82	901.4821 $[M-H]^-$	$C_{45}H_{74}O_{18}$	(+)	(+)	Trigofoenoside A
11	13.87	933.5077 $[M-H]^-$	$C_{46}H_{78}O_{19}$	(+)	(+)	Anemarsaponin E (3 β,5 β,22 α,25R)-Furostane-22-methoxy-3,26-diol 3-[glucosyl-(1®2)-glucoside] 26-glucoside.

Table 2: Continued.

12	13.96	1105.5439 $[M-H]^-$	$C_{53}H_{86}O_{24}$	(+)	(+)	Neomacrostemonoside D.
13	14.09	1357.6237 $[M-H_2O-H]^-$	$C_{62}H_{104}O_{33}$	(+)	(+)	Capsicoside D.
14	14.56	1379.6473 $[M-H_2O-H]^-$	$C_{65}H_{106}O_{32}$	(+)	(−)	Soyasapogenol B "3-O-[α -$_L$-rhamnosyl-(1-2)-[β,-$_D$-glucosyl-(1-3)]- β,-$_D$-galactosyl-(1-2)- β,-$_D$-glucuronide] 22-O-[β,-$_D$-glucosyl-(1-2)- α -$_L$-arabinoside]".
15	14.67	1177.5663 $[M-H_2O-H]^-$	$C_{56}H_{92}O_{27}$	(+)	(+)	Trigofoenoside G.
16	14.75	1381.6610 $[M-H]^-$	$C_{65}H_{106}O_{31}$	(+)	(+)	Acutoside D.
17	14.82	1045.5224 $[M-H]^-$	$C_{51}H_{82}O_{22}$	(+)	(+)	Melongoside.
18	14.97	1193.5933 $[M-H_2O-H]^-$	$C_{57}H_{96}O_{27}$	(+)	(+)	3-O-(Rha-3-Glc1-2-(Xyl1-3)-Glc1-4-Gal)-(25R)-5- α -spirostan-3 β−ol.
19	15.17	1047.5359 $[M-H_2O-H]^-$	$C_{51}H_{86}O_{23}$	(+)	(+)	3-β-[4-O-(-$_D$-glucopyranosyl)-2- O-(- $_L$-rhamnopyranosyl)- $_D$−glucopyranosyloxy]-22,26-dihydroxyfurost-5-ene.
20	15.18	1377.6338 $[M-H]^-$	$C_{65}H_{102}O_{31}$	(+)	(+)	Goyasaponin I.
21	16.36	1089.586 $[M+CH_3COO]^-$	$C_{52}H_{86}O_{20}$	(+)	(+)	Parrisaponin.
1	0.82	138.0549 $[M+H]^+$	C7H7NO2	(+)	(+)	Trigonelline.

(+) present, (−) absent.

HPLC analysis of Baris Oasis leaf extract **(Table 3 and Fig. S3, and S8)** led to the identification of rutin, quercetin, kaempferol, luteolin, apigenin, and catechin flavonoids, as well as syringic acid, cinnamic acid, caffeic acid, pyrogallol, ellagic acid, and gallic acid (phenolics) **(Table 3, and Figs. S3 and S10).** Luteolin, apigenin, catechin, cinnamic acid, pyrogallol, gallic acid, and ellagic acid were first reported. The other flavonoids (rutin, quercetin, and kaempferol) and phenolics (syringic acid, and caffeic acid) were previously detected in *B. aegyptiaca* leaf extract [31].

HPLC analysis of Wadi El−Gemal leaf extract **(Table 3 and Fig. S3, S9, S11)** led to the identification of naringin, quercetin, kaempferol, apigenin, and catechin flavonoids, as well as syringic acid, ferulic acid, caffeic acid, coumaric acid, and ellagic acid (phenolics).

Table 3: Phenolics and flavonoids were detected in the extracts of leaves by HPLC.

Compound no.	Rt (min)	Compound	Baris leaf extract (Conc. µg/g)	Wadi El−Gemal leaf extract (Conc. µg/g)
Phenolics				
1	4.8	Syringic	1.58	3.56
2	6	P−coumaric	−	4.26
3	7	Cinnamic	2.11	−
4	8	Caffeic	14.36	14.38
5	9	Pyrogallol	8.69	−
6	9.7	Gallic acid	1.98	−
7	11	Ferulic acid	−	1.58
8	13	Ellagic acid	5.74	15.63
Flavonoids				
1	4.1	Naringin	−	3.55
2	4.6	Rutin	13.25	−
3	6.8	Quercetin	4.52	3.48
4	8	Kaempferol	1.47	13.69
5	9	Luteolin	3.48	−
6	10	Apigenin	4.11	7.85
7	12	Catechin	2.77	9

(−) Absent.

B. aegyptiaca fruit extract α-Glucosidase inhibition and leaf extract antioxidant activity, as well as the leaf extract's acetylcholinesterase inhibition have previously been reported[16,39]. Drought stress, aridity, and temperature elevation increased the α-Glucosidase inhibition of Baris Oasis fruit extract (IC_{50} = 1924 ± 88.3 µg/mL) compared to Wadi El-Gemal fruit extract (IC_{50} = 4243 ± 195 µg/mL) as shown in Table S1 and Fig. 2A. The increase in Baris Oasis fruit activity was due to an increase in saponin synthesis and accumulation.

Baris Oasis leaf extract, which has more polyphenol content, showed higher antioxidant activity (IC_{50} = 76.904 ± 4.6 µg/mL) **(Table S1 and Fig. 2B)**. and highly potent acetylcholinesterase inhibitory activity (IC_{50} = 1.694 ± 0.09 µg/mL) than Wadi El−Gemal leaf extract (IC_{50} = 93.182 ± 5.6 & 12.14 ± 0.643 µg/mL respectively) **(Table S1 and Fig. 2C).** The leaf's bioactivity was influenced by abiotic stresses such as aridity, temperature, and drought.

Conclusion

Elevated temperatures, drought stress, decreasing air humidity, and location all promote the synthesis and accumulation of *B. aegyptiaca* saponins, flavonoids, and phenolics. The LC-MS analysis of the fruit extract led to the identification of first reported saponins in *B. aegyptiaca*. The bioactivities of *B. aegyptiaca* fruits and leaves extracts are in direct proportion to their phytochemical and quantitative analyses. The West Desert of Egypt is the best area for the collection of *B. aegyptiaca* fruits and leaves to produce herbal products.

List of Abbreviations

HPLC; High Performance Liquid Chromatography, LC−MS; Liquid Chromatography Mass Chromatography, DPPH; 2,2−diphenyl−1−picrylhydrazyl, °C; Celsius temperature degree, nm; nanometer, UV/Vis; Ultraviolet/Visible, GAE; Gallic acid Equivalent, QE; Quercetin Equivalent, SD; Standard Deviation, µL; microliter, ppm; part per million, ANOVA; Analysis of Variance, SPSS; Statistical Package for the Social

Sciences, QTOF; Quadrupole Time of Flight, ESI; Electrospray, µg/mL: microgram per milliliter, µm; micrometer, mm; millimeter.

Consent to publish

Corresponding author submits the current manuscript on behalf of all authors agreements.

Availability of data and materials

Correspondence and requests for materials should be addressed to A.M.Z.

Competing interests

The authors declare no conflict of interest.

Author contributions

- **A.M.Z.** Responsible for the aim of the study, interpretation of the data and writing the manuscript.
- **F.M.S:.** Responsible for selecting the plant of study and collection areas.
- **N.A.E:** Collecting the plant materials and climatic data from Wadi El−Gemal
- **S.H.E:** Drying, extraction and fractionation of plant materials.
- **R.S.:** Participate in determination of flavonoid, phenolic and saponin contents.
- **A.E.G.**: Collecting the plant materials and climatic data from Baris Oasis.
- **F.M.S., A.E.G. and A.M.Z.** reviewed the manuscript.

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Bull. Pharm. Sci., Assiut University, Vol. 46, Issue 2, 2023, pp. 927-941.

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تاثيرات العوامل البيئية علي المواد الفعالة و التاثيرات البيولوجية لورقة وثمرة نبات بلح الصحراء النامي في مصر

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بلح الصحراء هي شجرة برية طبية ، منتشرة بشكل طبيعي في مناطق واسعة من افريقيا وجنوب اسيا. تستخدم ثمار هذه الشجرة شعبيا في مصر كمنتجات طبيعية لعلاج مرض السكر. هدفت الدراسة الحالية الي تحديد تاثير العوامل البيئية علي المواد الفعالة والنشاط الحيوي لمستخلصات الاوراق والثمار. تم تجميع الاوراق والثمار من كلا من وادي الجمال بالصحراء الشرقية وواحة باريس بالوادي الجديد بالصحراء الغربية. وتعد درجة الحرارة و الرطوبة و الجفاف اهم الاختلافات البيئية الرئيسية بين مواقع التجميع. أظهر مسخلص ثمار واحة باريس اختلاف كمي وكيفي لمركبات الصابونين عن مستخلص ثمار وادي الجمال باستخدام اجهزة .(LC–MS and UV spectroscopy) وقد اثر هذا الاختلاف الكمي والكيفي علي التاثيرات البيولوجية لمستخلص ثمار نبات الصحراء حيث اظهر مستخلص ثمار واحة باريس بالصحراء الغربية ضعف تثبيط انزيم ألفا جلوكوزيداز مقارنة بمستخلص ثمار وادي الجمال بالصحراء الشرقية. وكذلك أظهر مستخلص اوراق واحة باريس اختلاف كمي وكيفي علي الاحماض الفينولية و مركبات الفلافونويدات عن مستخلص اوراق وادي الجمال. حيث تم الكشف عن اثني عشر مادة فينولية وفلافونويدية في مستخلص اوراق واحة باريس مقارنة بعشرة مركبات في مستخلص اوراق وادي الجمال باستخدام اجهزة HPLC. and UV spectroscopy أثرت هذة الاختلافات الكمية والكيفية بين مستخلصات الاوراق علي التاثيرات البيولوجية مثل مضاد الاكسدة و تسبيط وأسيتيل الكولينستراز تاثيرا كبيرا. لذا فان الاختلاف في العوامل البيئية مثل ارتفاع درجة الحرارة و الجفاف بين صحراء مصر الشرقية والغربية كان لهما تاثير فعال علي تكوين المواد الفعالة لثمار واوراق نبات بلح الصحراء وقد اثر ذلك علي التاثيرات البيولوجية. لذا توصي هذه الدراسه علي تجميع ثمار نبات بلح الصحراء المستخدمة في مصر كمنتجات طبيعية لعلاج السكر من الصحراء الغربية وليست من الصحراء الشرقية.