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Phytochemical Analysis of the Desert Date *Balanites aegyptiaca*

Ibrahim Bayoumi Abdel-Farid[(1,2)#], Magdi Abdel-Radi El-Sayed[(2, 3)]
[(1)]Biology Department, College of Science, Jouf University, Sakaka, KSA; [(2)]Botany Department, Faculty of Science, Aswan University, Aswan, Egypt; [(3)]Molecular Biotechnology Program, Field of Advanced Basic Sciences, Galala University, New Galala City, Egypt.

PHYTOCHEMICAL analysis of the mesocarp of eleven morphologically different individuals of *Balanites aegyptiaca* was assessed. The antioxidant activity of their methanol extracts was also evaluated. Principal component analysis (PCA) and hierarchical clustering analysis (HCA) were used in combination with the metabolome detected by spectrophotometer of the evaluated individuals to distinguish between them. HCA separated the eleven individuals of *Balanites aegyptiaca* into three groups according to the metabolites content and total antioxidant capacity of their extracts. Group 1 is characterized with higher content of carbohydrates and lower content of other detected metabolites and antioxidant capacity. Group 2 is characterized with moderate contents of the detected metabolites, whereas Group 3 is characterized with higher contents of all detected secondary metabolites, total antioxidant capacity and strong DPPH radical scavenging. Total antioxidant capacity showed a positive correlation with total proteins, phenolics, saponins and DPPH radical scavenging activity. DPPH radical scavenging activity showed a positive correlation with total proteins, total phenolics, total flavonoids, total saponins, and negatively correlated with total carbohydrates. To our knowledge, this is the first report underlying the metabolomics of morphologically different *B. aegyptiaca* fruits using metabolome combined with multivariate data analysis.

Keywords: *Balanites egyptiaca*, DPPH, Metabolomic, PCA, Phenolics.

Introduction

Balanites aegyptiaca (L.) Delile (Zygophyllaceae) is distributed at southern part of the Sahara in dry land regions in Africa and Asia (Saboo et al., 2014). The plant is utilized in food preparation and in folk medicine in many regions in Africa and Asia (Obidah et al., 2009). In Egypt, *B. aegyptiaca* grows in both Eastern and Western deserts and at the borders between Egypt and Sudan. The plant is known as Hegleig and is considered a multipurpose medicinal plant (Al Ashaal et al., 2010). It is expected that *B. aegyptiaca* will be a very promising medicinal plant in near future. This is reflected nowadays by the use of different *B. aegyptiaca* parts such as leaves, barks, roots, fruits, and kernels in folk medicine in many countries (Kamel et al., 1991; Obidah et al., 2009; Kusch et al., 2011; Mayba Gnana Suky et al., 2011; Abdallah et al., 2012). The wide use of *B. aegyptiaca* in folk medicine is attributed to its unique chemical composition. Different parts from *B. aegyptiaca* showed high contents of saponins, alkaloids, flavonoids, tannins, fixed oils and phenolics (Al Ashaal et al., 2010; Kumawat et al., 2012; Gajalakshmi et al., 2013; Al-Thobaiti & Abu Zeid, 2017). *B. aegyptiaca* extracts showed anti-diabetic property (Zaahkouk et al., 2003), insecticidal potential (Elamin & Satti, 2013), antimicrobial activity against many strains of bacterial and fungal pathogens (Al Ashaal et al., 2010; Hena et al., 2010; Abdallah et al., 2012), antiviral and antiparasitic activity (Al Ashaal et al., 2010), anti-plasmodial activity (Kusch et al., 2011), hepato protective effect and antioxidant potentiality (Mayba Gnana Suky et al., 2011)

#Corresponding author email: bayoumi2013@aswu.edu.eg Tel. 00966 535040657
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and anti-asthmatic and anti-anaphylactic activity (Patil et al., 2011).

B. aegyptiaca in Niger showed phenotypic variation in fruits and morphological difference in seeds (Abasse et al., 2011). The phytochemical composition and antioxidant activity of fruits of thirty individual trees in Mauritania were evaluated showing difference in phytochemical composition of the naturally growing trees (Abdelaziz et al., 2020). The effect of ecological origin and provenance on the primary and secondary metabolites distribution in leaves of *B. aegyptiaca* was assessed. Provenance had affected the nutritional values, metabolomic composition and antioxidant activity of the evaluated leaves (Khamis et al., 2020). Oil, protein and some minerals were evaluated in *B. aegyptiaca* kernel (Elfeel, 2010) showing variation among different trees and different locations in the content of the previously mentioned parameters.

B. aegyptiaca is cultivated by seeds and vegetative propagation. Seeds collected from different locations in Egypt were cultivated in the desert garden located in Aswan University campus. The resulting trees started producing fruits after 5 years of plantation. Because of the morphological fruits and seeds differences, we will study the variation in metabolomic composition of each tree to evaluate the similarity and difference between these individuals based on their metabolomic characterization. In this study, the metabolomic characterization of eleven different individuals from *B. aegyptiaca* will be evaluated by spectrophotometer combined with MVDA. The aims will also be extended to evaluate if there is a correlation between antioxidant capacity and DPPH radical scavenging activity of *B. aegyptiaca* mesocarps and the content of the evaluated metabolites.

Materials and Methods

Samples collection

Morphologically different fruits of *B. aegyptiaca* were collected from eleven individual trees growing in the desert garden at the university campus, 15km southwest of Aswan city (the seeds of individual trees were collected from different locations allover Egypt before cultivation of these seeds in the desert garden). Samples were shade dried at room temperature. The fruits were weighted, pericarp was removed and the weight of mesocarp and kernel was determined. Dried mesocarp was powdered using electrical grinder and used directly for determination of metabolites and antioxidant activity.

Spectrophotometer analysis

Carbohydrates were estimated using anthrone reagent (Morris, 1948). The developed blue-green color was read at the wave length of 620nm against a blank containing only water and anthrone reagent. A standard was constructed using glucose and was measured at the same wavelength. Folin Ciocalteau reagent was used to estimate protein content (Lowry et al., 1951) and the content of protein was calculated from the constructed standard using egg albumin. Both samples and standard were read at 650nm.

Folin-Ciocalteau method (Singleton et al., 1999) was used to estimate the total content of phenolics in *B. aegyptiaca* extracts. Samples and standard (gallic acid) were measured at 700nm. Total flavonoids concentration was determined using the aluminum chloride colorimetric method in presence of quercetin as a standard and the absorbance was read at 510nm (Zhishen et al., 1999). Vanillin solution was used to determine the content of saponins at 473nm (Ebrahimzadeh & Niknam, 1998). Phosphomolybednum method was used to determine the total antioxidant capacity (TAC) (Prieto et al., 1999). Briefly, the plant extract was mixed with a reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) and incubated at 95ºC for 90min. Series of ascorbic acid concentrations was prepared as above. The absorbance of reaction mixture of samples and standard was measured at 695nm against blank after cooling to room temperature. The total antioxidant activity was expressed as (mg) ascorbic acid per g of sample.

DPPH radical scavenging activity was assessed by spectrophotometer using diphenyl picryl hydrazyl (Blois, 1958) as percentage of inhibition (Wang & Mazza, 2002). Different concentrations of plant extract were mixed vigorously with a mixture of DPPH in ethanol, acetic acid buffer and ethanol. After incubation at room temperature for 30min in dark, the amount of DPPH remaining was determined by measuring the absorbance at 517nm. Control without extract in presence and absence of DPPH was used. The concentration of ascorbic acid was measured at the same wavelength. The scavenging activity

on the DPPH radical was expressed as inhibition percentage using the following equation:

% radical scavenging activity= (Ac - As/Ac) x 100

where Ac= Absorbance of negative control at 517 nm and As= Absorbance of sample at 517nm (Wang & Mazza, 2002). IC_{50} was calculated from the inhibition percentage of DPPH of different concentrations from the fruits of each individual tree.

Data analysis

Spectrophotometer data of the eleven individuals of *B. aegyptiaca* was subjected to principal component analysis (PCA) and hierarchical clustering analysis (HCA) of the multivariate data analysis (MVDA) of the SIMCA-P software (v. 11.0, Umetrics, Umeå, Sweden). ANOVA was used to assess the statistical difference of metabolites contents among the resulted groups from MVDA and also between the content of metabolites in individual *B. aegyptiaca*. Correlation between total antioxidant capacity and DPPH radical scavenging activity with different metabolites detected in the samples was evaluated using Pearson's correlation from Minitab (ver. 12.21).

Results

Phytochemical analysis of B. aegyptiaca by spectrophotometer and multivariate data analysis and chemometrics

Eleven morphologically different individuals of *B. aegyptiaca* (Table 1 and Fig. 1) were analyzed by spectrophotometer for total carbohydrates, total proteins, total flavonoids, saponins and phenolics (Table 1). The weight of fruits, weight of mesocarp and weight of kernels were measured for the fruits of each individual tree (Table 1 and Fig. 1).

Metabolomic data of spectrophotometer together with the morphological data such as fruit, mesocarp and kernel weight was subjected to MVDA (Fig. 2). Hierarchical clustering analysis was used to reveal the similarity and difference among the fruits of eleven individuals of *B. aegyptiaca* from the point of the phytochemical and morphological data. Dendrogram classified the eleven individuals into three groups (Fig. 2 A). The first group includes four individual trees (No. 1, 2, 4 and 11), the second group includes two individuals (No. 8 and 9) and the third group includes five individuals (No. 3, 5, 6, 7, 10) (Fig. 2 A).

TABLE 1. Morphological and phytochemical analysis of the fruits of eleven trees of *B. aegyptiaca*

Trees	Fruits weight	Mesocarp weight	Kernel weight	Carbohy.	Proteins	Saponin	Phenolics	Flavonoid
1	5.07±1.02^{a}	3.16 ± 0.65^{a}	1.91±0.37^{a}	51.49±0.55^{a}	40.19±3.72^{a}	53.10±0.31^{a}	0.80±0.02^{a}	0.60±0.10^{a}
2	4.41±0.94^{a}	2.37 ± 0.59^{a}	2.03±0.35^{a}	51.67±0.65^{a}	38.96±0.54^{a}	55.5±0.23^{a,b}	0.86±0.02^{a}	0.79±0.09^{a}
3	6.11±0.31^{b}	3.41±0.13^{a}	2.70±0.35^{a,b}	51.54±0.34^{a}	47.12±3.53^{b}	57.88±0.23^{b}	1.16±0.01^{b}	1.27±0.11^{b}
4	4.86±0.61^{a}	2.47± 0.65^{a}	2.40±0.13^{a,b}	51.63±0.14^{a}	36.73±3.46^{a}	56.94±0.42^{b}	1.03±0.14^{b}	0.98±0.10^{a}
5	5.62±0.41^{a,b}	2.72±0.48^{a}	2.91±0.18^{a,b}	51.31±0.32^{a}	39.32±1.27^{a}	57.01±0.2^{b}	0.92±0.01^{a,b}	1.77±0.17^{b}
6	4.69±0.52^{a}	2.25±0.37^{a}	2.44±0.15^{a,b}	50.76±0.29^{a}	49.73±0.29^{b}	57.14±0.12^{b}	1.01±0.01^{b}	1.55±0.15^{b}
7	5.43±0.32^{a,b}	2.67±0.13^{a}	2.76±0.19^{a,b}	50.67±0.6^{a}	41.54±1.9^{a}	57.41±0.35^{b}	1.03±0.01^{b}	1.04±0.17^{a}
8	2.52±0.12^{c}	1.13±0.17^{c}	1.39±0.13^{c}	51.03±0.08^{a}	44.08±1.93^{a}	57.55±0.12^{b}	1.09±0.01^{b}	1.23±0.02^{b}
9	3.94±0.58^{a,c}	1.96±0.37^{a,c}	1.98±0.2^{a,b,c}	50.90±0.08^{a}	44.95±1.99^{a}	54.72±0.2^{a,b}	1.15±0.01^{b}	1.29±0.06^{b}
10	4.75±0.5^{a}	2.17±0.20^{a,b}	2.58±0.3^{a,b}	50.94±0.0^{a}	44.98±1.88^{a}	57.07±0.23^{b}	1.15±0.0^{b}	1.27±0.03^{b}
11	5.35±0.22^{a,b}	2.69±0.41^{a,b}	2.66±0.4^{a,b}	51.40±0.16^{a}	41.06±1.9^{a}	52.42±2.07^{a}	1.08±0.0^{b}	0.92±0.07^{a}

- The readings are the mean of three replicates ± standard deviations.

- Carbohy.= Carbohydrates.

- Different letters in the same column referes to a significant difference.

Fig. 1. Morphological differences of fruits and kernels in eleven individuals of *B. aegyptiaca*

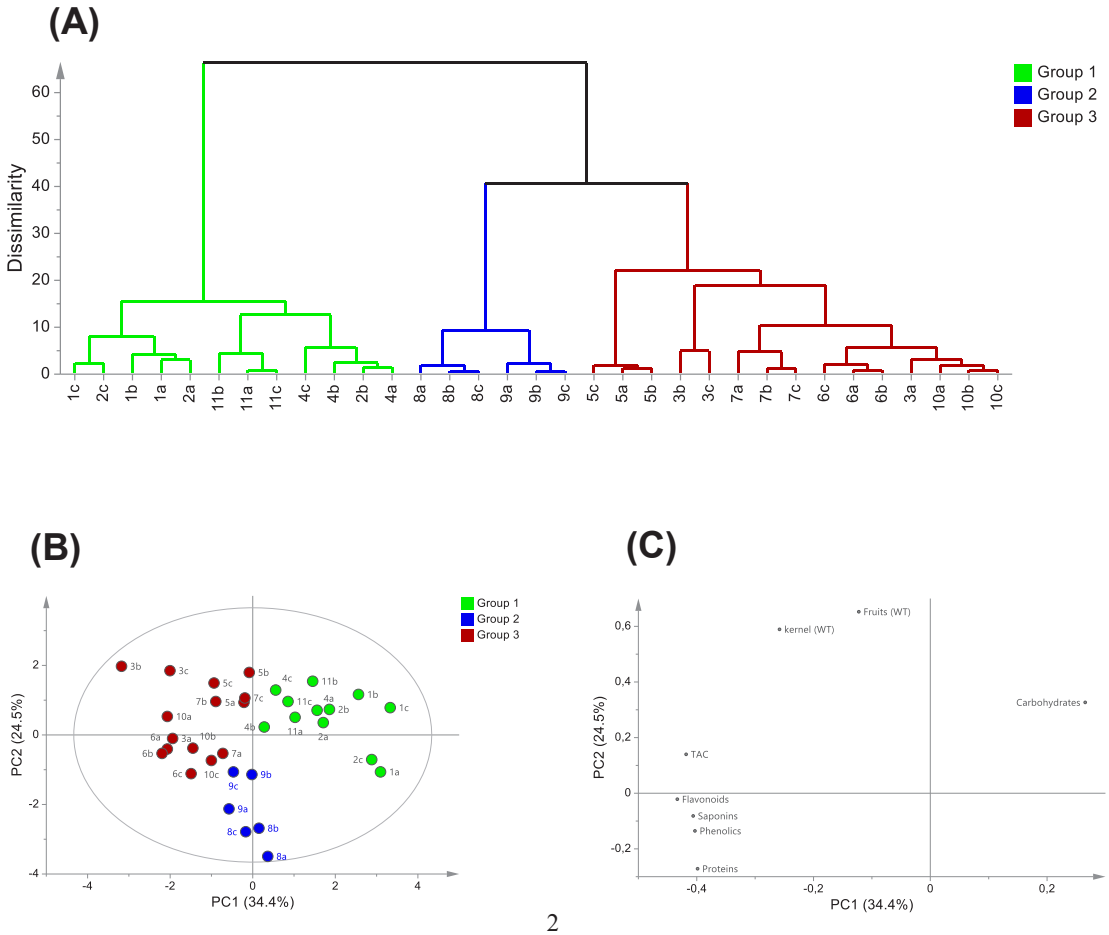

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Fig. 2. Hierarchical clustering analysis (HCA) (A), Score scatter (B) and score loading plot (C) of PC1 vs. PC2 of eleven individuals of *B. aegyptiaca*

PCA was used to confirm these results and to detect the responsible factors (metabolites and morphological parameters) contributed in this classification and clustering. PC1 vs. PC2 explain 59% of the variation among data. Scatter plot of PC1 and PC2 also discriminated the eleven varieties into the same three groups shown by HCA (Fig. 2 B). The score loading plot of PC1 and

PC2 showed the parameters responsible for this clustering (Fig. 2 C). Group one was characterized by very high amounts of carbohydrates and also by high fruit, mesocarp and kernel weight but has lower content of secondary metabolites and TAC. The second group was characterized by moderate content of all detected metabolites and also moderate weight of fruit and kernel. The third group was characterized by very low content of carbohydrates but higher content of secondary metabolites (phenolics, flavonoids and saponins) and TAC (Fig. 2 C).

Antioxidant activity of B. aegyptiaca and relation between antioxidant activity and determined metabolites

The two groups which were characterized by the moderate and the highest content of total secondary metabolites such as phenolics, flavonoids and saponins were characterized by the highest values of TAC and DPPH radical scavenging (Table 2). The highest TAC and DPPH of these two groups were reflected in the IC_{50} of their extracts (Table 2). The significant difference in fruit weight, mesocarps weight, kernels weight, total phenolics, flavonoids, saponins, TAC and DPPH radical scavenging activity among the three groups resulted from hierarchical clustering analysis and principal component analysis was evaluated using ANOVA. The three groups showed significant difference from each other in the content of all detected metabolites, TAC, DPPH radical scavenging activity, fruit, mesocarp and kernel weights ($P< 0.05$ for carbohydrates, $P< 0.001$ for proteins, $P< 0.001$ for saponins, $P< 0.01$ for phenolics, $P< 0.001$ for flavonoids, $P< 0.05$ for TAC, $P< 0.001$ for DPPH, $P< 0.001$ for fruit weight, $P< 0.001$ for the weight of mesocarp and $P< 0.001$ for the weight of kernel. Pearson's correlation revealed that TAC was positively correlated with protein, saponin and phenolics contents, whereas DPPH radical scavenging activity was positively correlated with protein, saponin, phenolics and flavonoids contents (Table 3).

TABLE 2. Antioxidant activity of eleven individual trees of *B. aegyptiaca*

Individual	TAC	DPPH (400µg/mL)	IC_{50} (µg/mL)
1	1.09 ± 0.10^{a}	52.5 ± 1.27^{a}	269 ± 5.3
2	1.23 ± 0.05^{a}	50.0 ± 1.97^{a}	271 ± 21.5
3	2.08 ± 0.65^{b}	70.0 ± 1.41^{b}	208 ± 8.5
4	$1.37 \pm 0.26^{a,c}$	56.0 ± 2.55^{c}	247 ± 15.6
5	1.16 ± 0.16^{a}	61.0 ± 3.91^{c}	228 ± 7.2
6	$1.61 \pm 0.19^{a,c}$	66.0 ± 2.0^{b}	215 ± 11.0
7	1.33 ± 0.21^{a}	59.0 ± 3.81^{c}	23 1± 6.0
8	1.2 ± 0.28^{a}	67.0 ± 1.55^{b}	204 ± 3.0
9	1.33 ± 0.23^{a}	61.0 ± 3.11^{c}	224 ± 9.5
10	$1.57 \pm 0.16^{a,c}$	70.0 ± 3.59^{b}	198 ± 18.0
11	1.23 ± 0.12^{a}	50.0 ± 4.39^{a}	274 ± 25.0

- TAC= Total antioxidant capacity

- Different letters in the same column means there is a significant difference.

TABLE 3. Correlation coefficient (r) and probability (P) between the detected metabolites and both total antioxidant capacity (TAC) and DPPH radical scavenging activity

	Carbohydrates	Proteins	Saponins	Phenolics	Flavonoid	TAC	Fruits weight	Mesocarp weight	Kernel weight
TAC	ns	r = 0.39 P (0.024)	r = 0.41 P (0.017)	r = 0.46 P (0.007)	ns		ns	ns	ns
DPPH	r = -0.39 P (0.025)	r = 0.66 P (0.00)	r = 0.61 P (0.00)	r = 0.59 P (0.00)	r = 0.58 P (0.00)	r = 0.49 P (0.004)	ns	ns	ns

ns= Non-significant at $P< 0.05$

Discussion

Phytochemical analysis of Balanites aegyptiaca by spectrophotometer combined with multivariate data analysis

Phytochemical analysis and multivariate data analysis discriminated the eleven morphologically different fruits of *B. aegyptiaca* based on the metabolomic composition and antioxidant activity of their extracts. The individual trees showed different metabolomic composition and some individuals showed some similarity among each other. Thirty trees of *B. aegyptiaca* growing naturally in hyper-arid and arid regions of Mauritania were evaluated for their fruits polyphenols, tannins, flavonoids content and antioxidant activities of their extracts. Principal component analysis was used with the metabolomic data and also some morphological data such as fruit weight, length and width. PCA showed significant difference in the metabolites content and antioxidant activity among the trees of different regions (Abdelaziz et al., 2020). It was reported that *B. aegyptiaca* different parts have many bioactive metabolites such as saponins, terpenoids, alkaloids, flavonoids and steroids which have significant roles in the potentiality of *B. aegyptiaca* as traditional medicine as antimicrobial, antidiabetic, antiviral, antihelminthic, anticancer agent (Al-Thobaiti & Abu Zeid, 2018). In a recent study, the metabolomic profiling of *B. aegyptiaca* leaves showed high contents of bioactive secondary metabolites which was positively reflected in the antioxidant, antimicrobial and anticancer activity of their extracts (Khamis et al., 2020). Flowers of *B. aegyptiaca* showed high content flavonoids and phenolics which affect positively the antioxidant activity of flower extracts (Amadou et al., 2019). Very few studies were performed to use the phytochemical analysis from spectrophotometer combined with MVDA in metabolomic differentiation among different species of plants. Spectrophotometric data coupled with MVDA was used to discriminate between three Acacia species (*A. seyal*, *A. nilotica* and *A. laeta*) (Abdel-Farid et al., 2014). Three species were separated from each other according to the metabolome of each species and also the antioxidant capacity of their extracts. Recently, differentiation between different parts of the same plant was evaluated using metabolomic data and TAC obtained from spectrophotometer (Taha et al., 2020). These types of studies may open an avenue for more metabolomic studies using spectrophotometer data combined with MVDA.

Antioxidant activity and correlation between antioxidant activity and the detected metabolites

The resulted groups in our study (Group 2 and 3) which were characterized with moderate to high content of secondary metabolites and protein in addition to TAC showed high DPPH and lower IC_{50} of their extracts. TAC was highly correlated with the content of total phenolics in many plants used as antidiabetic (Basar et al., 2013). Saponins content was positively correlated with TAC in Soybean (Lee et al., 2011), in shallot (Vu et al., 2013) and in Acacia (Abdel-Farid et al., 2014). Protein content was positively correlated with antioxidant capacity in Acacia. Lipid oxidation is prevented by protein through its role in removing of the free radicals (Elias et al., 2008). Correlation of the detected metabolites such as proteins, saponins, phenolics and flavonoids with DPPH and TAC is in line with our work on the antioxidant activity of Acacia (Abdel-Farid et al., 2014). The significant correlation between total antioxidant capacity and DPPH radical scavenging activity with proteins, saponins, phenolics and flavonoids indicates the importance of these metabolites in antioxidant activity. The association of different metabolites with the TAC and DPPH of some Balanites individuals was accompanied with the less IC_{50} of these individuals. Positive correlation of polyphenolics with the antioxidant activity and consequently the IC_{50} was documented (Abdel-Farid et al., 2014; Taha etal., 2020). The use of *B. aegyptiaca* as antidiabetic (Zaahkouk et al., 2003) and also as anti-inflammatory (Mayba Gnana Suky et al., 2010) may be attributed to the presence of these bioactive metabolites which work in individual manner or through synergism.

Conclusion

B. aegyptiaca is used in a large scale in folk medicine in different regions all over the world. For this reason there was an urgent need to a metabolomic study revealing the metabolomic composition of *B. aegyptiaca* and also to evaluate the similarities and differences among individual trees particularly when collected from different places of different environmental conditions. This metabolomic of *B. aegyptiaca* is a very important issue as it will contribute for a well exploitation of the plant in the future. MVDA including PCA and HCA was effectively used in combination with the

metabolomic data in exploring the similarity and difference among the morphologically different individuals of *B. aegyptiaca*. The individual trees of the desert date showed significant difference among each other regarding the metabolomic profiling and antioxidant activity. The resulted three groups from multivariate data analysis showed significant difference in the content of the detected metabolites particularly secondary metabolites. Metabolome and multivariate data analysis will be a promising tool in such types of studies and will have a significant role in metabolomic characterization of different taxa of a particular genus or species.

Conflict of interest: The authors declare that there is no conflict of interest.

Author contribution: Ibrahim Abdel-Farid: Conceptualization, formal analysis, methodology, investigation, software and visualization and writing the original draft of the manuscript. Magdi El-Sayed: Conceptualization, formal analysis, methodology, data curation, validation, writing - review & edit. Both authors have read and agreed to the published version of the manuscript.

Ethical approval: Not applicable

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التحليل الفيتوكيميائي لبلح اللالوب الصحراوي

إبراهيم بيومي عبد الفريض[1, 2]، مجدي عبد الراضي السيد[2, 3]

[1]قسم الأحياء - كلية العلوم - جامعة الجوف سكاكا - المملكة العربية السعودية، [2]قسم النبات - كلية العلوم - جامعة أسوان - أسوان - مصر، [3] برنامج التقنية الحيوية - مجال العلوم الاساسية المتقدمة - جامعة الجلالة مدينة الجلالة الجديدة - مصر.

تم تقييم التحليل الفيتوكيميائي للميزوكارب لثمار أحد عشر شجرة مختلفًة مورفولوجيا من نبات اللالوب الصحراوي (البلانيتس ايجيبتياكا). تم أيضًا تقييم النشاطات المضادة للأكسدة لمستخلصات الميثانول لثمار تلك الأشجار. وقد تم استخدام التحليل متعدد العوامل (تحليل المكون الرئيسي (PCA) وتحليل المجموعات الهرمية (HCA) للمركبات الايضية لثمار الأحد عشر شجرة والتي تم تقديرها بواسطة مقياس المطياف الضوئي (الاسبكتروفوتوميتر) وذلك للتفرقة بين تلك الافراد من ناحية تركيبهم الايضي وكذلك قدرة مستخلصاتهم المضادة للأكسدة. وقد فصل تحليل المجموعات الهرمية HCA ثمار الأفراد الأحد عشر من نبات اللالوب إلى ثلاث مجموعات وفقًا للمحتوى الايضي والقدرة الكلية المضادة للأكسدة في مستخلصاتهم. ولقد تميزت المجموعة الأولى بمحتوى أعلى من المواد الكربوهيدراتية ومحتوى أقل من المركبات الايضية الأخرى كالفينولات والفلافونويدات والمواد الصابونينية والبروتينات وكذلك قلة القدرة المضادة للأكسدة لمستخلصاتها. وقد تميزت المجموعة الثانية بمحتويات متوسطة من المركبات الايضية المقدرة، بينما تميزت المجموعة الثالثة بمحتويات أعلى من جميع المركبات الايضية المقدرة من مواد فينولية وفلافونويدات ومواد صابونينية وبروتينات، وكذلك ارتفاع القدرة الكلية المضادة للأكسدة وارتفاع النشاط الكاسح للجذور الحرة لمستخلصاتها. أظهرت القدرة الكلية المضادة للأكسدة ارتباطًا إيجابيًا مع محتوى كل من البروتينات والفينولات والمواد الصابونينية وكذلك النشاط الكاسح للجذور الحرة. كما أظهر النشاط الكاسح للجذورالحرة إرتباطًا إيجابيًا بمحتوى البروتينات الكلية والفينولات والفلافونيدات والمواد الصابونينية الكلية وارتباطًا سلبيًا بمحتوى المواد الكربوهيدراتية الكلية. على حد علمنا، تعتبر هذه الدراسة هي الأولى التي تناولت دراسة محتويات وتوزيع المركبات الايضية لثمار نبات اللالوب في أشجار مختلفة مورفولووجيا باستخدام اللتحليل متعدد العوامل.