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Diffusion-ordered Spectroscopy of Flavonol Mixture from *Mesembryanthemum forsskaolii* (Aizoaceae)

Abeer Moawad[1*], Elham Amin[1,2] and Rabab Mohammed[1]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Beni-Suef University, Beni-Suef, 62514, Egypt.
[2]Department of Pharmacognosy, Faculty of Pharmacy, Al Jouf University, Sakaka, KSA.

Authors' contributions

This work was carried out in collaboration between all authors. All authors designed the study, performed the spectroscopic analysis, chromatographic isolation, wrote the protocol, wrote the first draft of the manuscript, and managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Phytochemical investigation of phenolic content in *Mesembryanthemum forsskaolii* Hochst. Ex. Boiss (family Aizoaceae).
Methodology: Chromatographic isolation of the total alcohol extract of the herb followed by the spectroscopic identification of the isolated compounds was performed adopting 1D, 2D NMR techniques including Diffusion-Ordered Spectroscopy (DOSY). Quantification of the phenolic content and evaluation of the antioxidant potential of *M. forsskaolii* was also performed. The total phenolic content (TPC) was determined using the Folin-Ciocalteu method and the total flavonoids content (TFC) was measured via complexation with aluminum chloride and the antioxidant activity was evaluated with DPPH (2.2-diphenyl-1-picrylhydrazyl) assay.
Results: Apigenin (**1**), kaempferol-3-*O*-glucoside (**2**), isorhamnetin-3-*O*-glucoside (**3**), apigenin-7-*O*-glucoside (**4**) and rutin (**5**) were isolated and identified. A mixture of isorhamnetin-3-*O*-rutinoside (**6**) and kaempferol-3-*O*-rutinoside (**7**) was obtained and its composition was identified without the need for further separation. 2D-DOSY experiment was performed, however very poor separation of

**Corresponding author: E-mail: abeer.2132006@yahoo.com, abeer.moawad@pharm.bsu.edu.eg;*

peaks was obtained, and that may be attributable to close molecular weights of the mixture components and their structure similarity. It is noteworthy that, all the isolated compounds are reported for the first time from this plant. The TPC was 0.090±0.001 gallic acid equivalents in mg/g plant material. The TFC was 0.033±0.006 rutin equivalent in mg/ g plant material. The plant exhibited a very low antioxidant property as DPPH scavenging activity was 0.085±0.002 mg ascorbic acid equivalent antioxidant capacity in 1 g plant material.

Conclusion: Results provide a new addition to the chemical literature of *M. forsskaolii*, in addition it increases the importance of NMR techniques in flavonoids structure elucidation.

Keywords: Mesembryanthemum; apigenin; isorhamnetin; kaempferol; Aizoaceae; 2D-DOSY.

1. INTRODUCTION

Aizoaceae is considered as one of the most widely distributed plant families in Africa, it is poorly described in terms of chemical composition and biological activities. The genus *Mesembryanthemum* is native to Saudi Arabia, the Mediterranean region, California, Atlantic Islands, South Australia and South Africa [1]. It contains about 70 species among which three are recorded in Egyptian flora, that are (*M. crystallinum* L., *M. nodiflorum* L. and M. *forsskaolii* Hochst. ex Bioss.). *Mesembryanthemum* species like *M. edule, M. crystallinum* and *M. nodiflorum* exhibited antioxidant properties and their phenolic constituents were previously determined [2] antiviral and antimicrobial activities [3,4]. *M. forsskaolii* Hochst. Ex. Boiss is cultivated in several Arabian countries and its seed - known as Samh- are used in many food recipes in Suadia Arabia [5]. The Samh seeds have some influence in lipid profile and liver antioxidant enzymes of diabetic rats [6]. Quantification of the phenolic content and evaluation of the antioxidant potential of *M. forsskaolii* is presented in this article.

Diffusion NMR experiments resolve different compounds spectroscopically in a mixture based on their differing diffusion coefficients, depending on the size and shape of the molecules. The analysis of a complex mixture can be simplified by the use of Diffusion-Ordered Spectroscopy (DOSY), in which the introduction of a second dimension allows a diffusion coefficient-based separation of the components [7]. The spectra produced resemble chromatograms is some aspects while also providing NMR information that can be used for assignment of individual components. 2D-DOSY experiment was performed to the mixture of compounds **6** and **7** but only the signals of solvent were separated and the mixture components were not separated because of their structure similarity and close molecular weights so detailed analysis of 1D, 2D NMR spectra was performed and allowed us to identify flavonol mixture without the need for further separation.

2. MATERIALS AND METHODS

2.1 General Experimental

NMR spectra were recorded on a Bruker Avance III 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C NMR (Bruker AG, Switzerland) with BBFO Smart Probe and Bruker 400 MHz AEON Nitrogen-Free Magnet. Data were analyzed using Topspin 3.1 Software. CD_3OD and DMSO-d_6 were purchased from Cambridge Isotope Laboratories, Inc., (Andover, MA, USA). Column chromatography was performed with silica gel (230–400 mesh), Sephadex LH-20 (Pharmacia Biotech, Uppsala) and Diaion® HP-20SS (Sigma Aldrich Chemicals-Germany). UV absorbance was recorded on Shimadzu UV-visible (UV-1650) spectrophotometer. Absolute ethanol, $NaNO_2$, NaOH were of analytical grade. $AlCl_3$, Folin Ciocalteu reagent, DPPH, gallic acid and rutin were purchased from Sigma Aldrich Chemicals, Germany.

2.2 Plant Material

The entire herb of *M. forsskaolii Hochst. Ex. Boiss* was collected from Cairo-Suez road on August 2008 and identified as described [8].

2.3 Extraction and Isolation

A dried plant sample (1 g) was extracted for 2 h with 5 ml of 80% MeOH at room temperature on an orbital shaker set at 200 rpm [9]. The mixture was centrifuged at 1400 g for 20 min and the supernatant was decanted into a 10 ml measuring flask. The residue was re-extracted under identical conditions. Supernatant was combined and used for determination of total

antioxidant activity, total phenolic and total flavonoid contents. For chromatographic isolation of the flavonoids; the air-dried herb of *M. forsskaolii* (1.5 kg) was powdered, crushed and steeped in 70% EtOH at room temperature for 48 h, the extract was collected and the solvent was removed under reduced pressure at below 45ºC to give a residue (150 g). The residue was suspended in water and then partitioned with *n*-hexane, DCM, EtOAc, and *n*-butanol saturated with water to yield the corresponding fractions (25 g, 5 g, 2 g and 6 g respectively).

The chloroform fraction (5 g) was chromatographed on silica gel column using gradient elution technique starting from hexane, DCM and MeOH collecting 50 ml fractions to get 30 fractions that were screened by TLC and similar fractions were combined. The sub-fraction C2 eluted with 15% MeOH in DCM was rechromatographed over silica gel column to get 12 mg of compound **1**.

The EtOAc fraction (2 g) was subjected to column chromatography using silica gel column eluted with DCM and MeOH in gradient technique. The collected fractions were TLC monitored and similar fractions were combined to get eight sub-fractions (E1-E8). The sub-fraction E4 showed flavonoid spots and was chromatographed on Sephadex LH-20. The sub-fraction obtained was subjected to HPLC to get a mixture of two flavonoids. This mixture was rechromatographed on Sephadex LH-20 using 80% aqueous MeOH as eluent to get 3 mg compound **2** and 2 mg compound **3**. The *n*-butanol extract (8 g) was chromatographed over HP-20SS eluted with water and decreasing the polarity by 20% MeOH increments. The fractions (B8 and B11) eluted with 40% and 60% aqueous MeOH respectively showed flavonoid spots on TLC using solvent system EtOAc-DCM-MeOH-H_2O (12:8:8:2). B8 was fractionated over Sephadex LH-20 several times eluted with 80% aqueous MeOH and collecting 1 ml fraction to get 12 mg compound **4** and 15 mg a mixture from compound **6** and **7**. The fraction (B11) was filtered over Sephadex-LH20 in the same way to get 23 mg compound **5.**

2.4 Characterization of the Isolated Compounds

*Apigenin (**1**)*

Yellow powder, 12 mg. ^{1}H NMR (400 MHz, MeOD) δ 7.85 (d, *J* = 8.6 Hz, H-2′, H-6′), 6.93 (d, *J* = 8.6 Hz, H-3′, H-5′), 6.60 (s, H-3), 6.46 (d, *J* = 1.7 Hz, H-8), 6.21 (d, *J* = 1.7 Hz, H-6). ^{13}C NMR (100 MHz, DMSO) δ 182.2, 164.7, 164.2, 161.9, 161.6, 157.7, 128.9, 121.6, 116.4, 104.1, 103.3, 99.3, 94.4. These data were consistent with that reported for apigenin [10].

*Kaempferol-3-O-glucoside (**2**)*

Yellow powder, 3 mg. ^{1}H NMR (400 MHz, DMSO) δ 8.02 (d, *J* = 8.5 Hz, H-2′, H-6′), 6.87 (d, *J* = 8.8 Hz, H-3′, H-5′), 6.26 (s, H-8), 6.06 (s, H-6), 5.43 (d, *J* = 7.0 Hz, H-1") - 3.08-3.58 remaining sugar proton overlapped with water of DMSO. The ^{1}H NMR were consistent with literature [10].

*Isorhamnetin 3-O-β-glucopyranoside (**3**)*

Yellow powder, 2 mg. ^{1}H NMR (DMSO, 400 MHz): δ 7.53 (1H, d, *J* = 2.0 Hz, H-2'), δ 7.50 (1H, dd, *J* = 1.84, 9.0 Hz, H-6'), δ 6.80 (1H, d, *J* = 8.24 Hz, H-5') δ 6.29 (1H, d, *J* = 2.0 Hz, H-8), δ 6.08 (1H, d, *J* = 1.8 Hz, H-6), δ 5.45 (1H, d, *J* = 7.32 Hz, H-1"), δ 3.86 (3H, s, OCH3, δ 3.01-3.68 (6H, m, H-2", H-3", H-4", H-5", H-6"). Data were typical for isorhamnetin 3-*O*-β-glucopyranoside [8].

*Apigenin-7-O-glucoside (**4**)*

Yellow powder, 12 mg. ^{1}H NMR (400 MHz, MeOD) δ 7.89 (d, *J* = 8.6 Hz, 2H), 6.94 (d, *J* = 8.7 Hz, 2H), 6.82 (d, *J* = 1.9 Hz, 1H), 6.66 (s, 1H), 6.50 (d, *J* = 1.8 Hz, 1H), 5.08 (d, *J* = 7.2 Hz, 1H). DEPT-135 NMR (100 MHz, D_2O) δ 129.13(d), 118.30(d), 116.54(d), 103.51(d), 100.19(d), 99.92(d), 99.86(d), 95.67(d), 94.61(d), 76.51(d), 75.91(d), 73.13(d), 69.59(d), 60.98. NMR was consistent with the reported data [11].

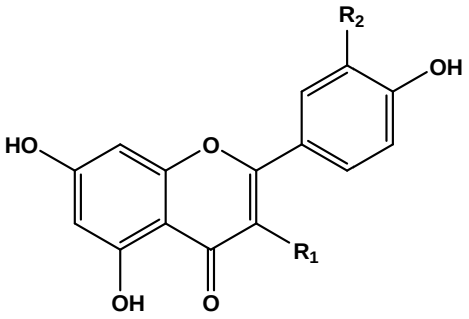
*Rutin (**5**)*

Yellow powder, 23 mg, ^{13}C NMR (100 MHz, MeOD) δ 176.2 (s), 163.1(s), 159.8(s), 155.76(s), 155.4(s), 147.8(s), 145.2(s), 132.5(s), 129.4(d), 120.9(s), 119.9(d), 113.2(d), 113.1(d), 111.5(d), 102.6(s), 101.6(d), 99.5(d), 99.4(d), 97.1(d), 92.0(d), 75.2(d), 74.3(d), 72.9(d), 70.8(d), 69.3(d), 69.0(d), 68.6(d), 66.8(d), 65.5 (t) 14.9(q). These NMR data was consistent with the reported data [10].

2.5 Determination of Total Phenolic Contents

Total phenolic content was determined using Folin–Ciocalteu reagent described by [12]. Three

hundred microliters of extract were mixed with 2.25 ml of Folin–Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min; 2.25 ml of sodium carbonate (60 g/l) solution was added to the mixture. After 90 min at room temperature, absorbance was measured at 725 nm using spectrophotometer. Results were expressed as mg gallic acid equivalents in 1 g of dried sample (mg GAE/g).



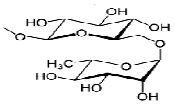
Compound	R_1	R_2
6		OCH_3
7		H

Fig. 1. Flavonoids in mixture isolated from *Mesembryanthemum forsskaolii Hochst*

2.6 Determination of Total Flavonoid Content

Total flavonoid content was determined using colorimetric method previously described [12]. Half milliliter of the extract was mixed with 2.25 ml of distilled water in a test tube followed by addition of 0.15 ml of 5% $NaNO_2$ solution. After 6 min, 0.3 ml of a 10% $AlCl_3$ solution was added and allowed to stand for another 5 min before 1.0 ml of 1 M NaOH was added. The mixture was mixed well with vortex. The absorbance was measured immediately at 510 nm using spectrophotometer. Results were expressed as mg rutin equivalents in 1 g of dried sample (mg RE/g).

2.7 DPPH Free Radical Scavenging Assay

The scavenging activity of the extracts was estimated by using 1,1-diphenyl-2-pycrylhydrazyl (DPPH) as a free radical model and a method adapted from [13]. An aliquot of 300 µL of samples or control (80% MeOH) were mixed with 3.0 ml of 500 µM (DPPH) in absolute ethanol. The mixture was shaken vigorously and left to stand at room temperature for 30 min in the dark. The mixture was measured spectrophotometrically at 517 nm. The free radical scavenging activity was calculated as follows: Scavenging effect (%) = [1-{absorbance of sample/absorbance of control}]*100. A standard of ascorbic acid was run using several concentrations ranging from 0.05 to 0.25 mg/ml. A standard curve was then prepared by plotting the percentage (%) of free radical scavenging activity of ascorbic acid versus its concentration. The final result was expressed as mg ascorbic acid equivalent antioxidant capacity in 1 g of sample (mg AEAC/g).

2.8 Statistical Analysis

All experiments were carried out in 3 replicates and presented as mean ± standard deviation of (SD).

3. RESULTS AND DISCUSSION

During the course of isolation of flavonoids from *M. forsskaolii* Hochst. Ex. Boiss (family Aizoaceae); seven flavonoids were identified as apigenin [10], kaempferol-3-*O*-glucoside [10], isorhamnetin 3-*O*-β-glucopyranoside [8], apigenin-7-*O*-glucoside [11] and rutin [10]. A mixture of isorhamnetin-3-*O*-rutinoside (**6**) and kaempferol-3-*O*-rutinoside (**7**) was obtained and its composition was identified by adopting 1D, 2D NMR techniques. The mixture showed a proportion of 1:0.75; the difference in intensity allowed us to differentiate between their corresponding signals in ^{1}H and ^{13}C NMR spectra compiled in Table 1.

3.1 2D DOSY Experiment

The spectral separation of the two mixture components according to their self-diffusion Coefficients (D) is shown in Fig. 2. D generally decreases with increasing molecular weight (mw). The difference between the two mw_s must be sufficient to get different D_s and so a separation of the signals. It is generally admitted that the ratio m/ mean(mw) ($m = mw_a - mw_b$) must not be lower than 0.10–0.15 to observe separated signals along the diffusion axis [14]. In this mixture the mws for isorhamnetin-3-*O*-rutinoside (**6**) and kaempferol-3-*O*-rutinoside (**7**) are 624.5 and 594.5 respectively with m= 30. The ratio equal to 0.049, thus poor separation

was obtained and intensive study of the HMQC and HMBC of the mixture was done which facilitated the assignment of both compounds.

3.2 Structure Elucidation of the Mixture Components

Compounds **6** and **7** mixture showed two anomeric doublets at δ_H 5.22 and 5.12 ppm with different integration values in addition to another anomeric singlet at δ_H 4.54 and two overlapped doublets at δ1.12 ppm corresponding to two rhamnose sugar residues. These anomeric protons were confirmed by their $^{1}J_{CH}$ correlation to anomeric carbons at δ_c103.15, 103.34, 101.01 ppm respectively.

The ^{13}C Chemical shift of the sugar residues were typical for rutinose in addition to HMBC correlation of the rhamnose anomeric proton with C-6″ of the two other sugar residues thus confirming the rutinose structure of the sugar residue of both mixture components. Two spin systems of ring B could be identified; an AA'BB' and AMX indicating a para –substituted B-ring in one component and 1,3,4 substituted B-ring in the other. Only two singlets were traced corresponding to H-6 (δ_H 6.19) and H-8 (δ 6.30) of both components indicating flavonol structure in addition to two C-4 at δ_C 177.8 and 177.9 and two C-3 at δ_c134.14 and 134.08 with different intensities, a singlet at δ_H 3.90 ppm corresponding to a methoxy signal. Careful HSQC and HMBC study of the long range coupling of each proton signal allowed the identification of the flavonol aglycons as kaempferol and isorhamnetin. The attachment site of the rutinose residues was confirmed by the $^{3}J_{CH}$ correlation of the two glucose anomeric protons to C-3 at δc134.14 and 134.08 ppm. Although the two mixture components have different B-ring substitution, still some signals have the same chemical shift in both compounds; like the H-6, H-8 and the anomeric proton of the rhamnose sugar and their corresponding carbons. The presence of the two mixture components in different proportions allowed us to unambiguously assign their ^{1}H and ^{13}C NMR signals, data are showed in Table 1. Spectroscopic analysis and identification of a mixture of β-sitosterol and stigmasterol glucosides was previously reported [15] and their assignment was performed in mixture. In a similar way, identification and unambiguous assignment of isorhamnetin-3-*O*-rutinoside (**6**) and kaempferol-3-*O*-rutinoside (**7**) was carried out in this research subject.

3.3 Total Phenolic Content

Folin–Ciocalteu reagent was used to determine total polyphenol in sample extract. This reagent oxidizes phenolates, resulting in the production of complex molybdenum-tungsten blue which can be detected spectrophotometrically at 725 nm. TPC for *M. forsskaolii* was (0.09±0.001) gallic acid equivalents in mg/g plant material (Table 2).

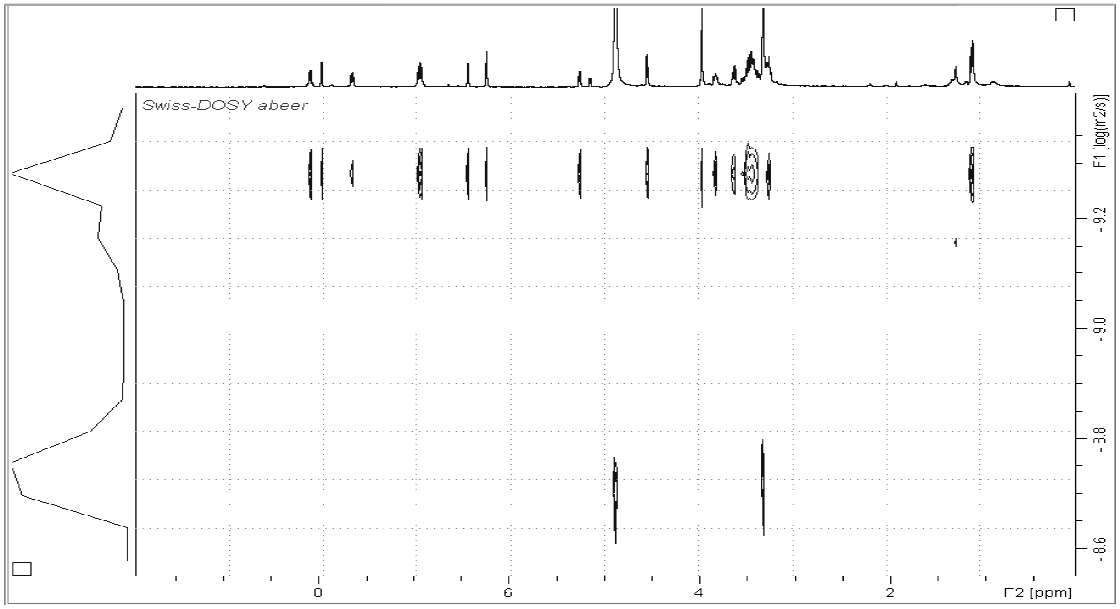


Fig. 2. 2D DOSY spectrum of mixture (6 and 7)

Table 1. ^{1}H and ^{13}C NMR data of compounds 6 and 7 (MeOD)

Carbon no.	6		7	
	δc	δ_H	δc	δ_H
2	157.07		157.07	
3	134.08		134.14	
4	177.79		177.89	
5	161.46		161.46	
6	98.68	6.19(s)	98.68	6.19(s)
7	164.9		164.9	
8	93.6	6.37(s)	93.6	6.37(s)
9	156.94		156.94	
10	104.17		104.13	
1′	121.51		121.30	
2′	113.13	7.95 (s)	130.99	8.06 (d, *J* = 8.8 Hz)
3′	146.83		114.73	6.90 (*overlapped)*
4′	149.39		160.04	
5′	114.67	6.89 *(overlapped)*	114.73	6.90 (*overlapped)*
6′	122.56	7.61(d, *J*=8.4)	130.99	8.06 (d, *J* =8.8 Hz)
1″	103.15	5.22(d, *J*=6.8 Hz)	103.34	5.12 (d, *J*=6.8 Hz)
2″	74.51		74.63	
3″	75.89		75.74	
4″	70.64		70.64	
5″	76.73		76.73	
6″	67.12		67.19	
1‴	101.10	4.54 (s)	101.01	4.54 (s)
2‴	70.17		70.02	
3‴	70.86		70.89	
4‴	72.44		72.51	
5‴	68.37		68.32	
6‴	16.50	1.12(d, *overlapped)*	16.54	1.13 (d, *overlapped)*)
3′-OCH3	55.3	3.95(s)		

Carbon chemical shift sometimes displayed in two digits format to differentiate between close signals

Table 2. Total phenolic, total flavonoids and DPPH scavenging ability of *Mesembryanthemum forsskaolii Hochst*

Plant material	TPC [A]	TFC [B]	DPPH assay [C]
M. forskaolii herb	0.090±0.001	0.033±0.006	0.085±0.002

Values are presented in mean ± SD (n = 3). [A] Total phenolic was expressed as mg gallic acid equivalent in 1 g of dry sample. [B] Total flavonoid was expressed as mg rutin equivalent in 1 g of dry sample. [C] DPPH free radical scavenging activity was expressed as mg ascorbic acid equivalent antioxidant capacity in 1 g of dry sample

3.4 Total Flavonoid Content

Flavonoids are the most common and widely distributed group of plant phenolic compounds that are characterized by a benzo-γ-pyrone structure. Total flavonoid can be determined in the sample extracts by reaction with sodium nitrite, followed by the development of colored flavonoid-aluminum complex formation using aluminum chloride which can be monitored spectrophotometrically at 510 nm. TFC for *M. forsskaolii* was (0.033±0.006) rutin equivalent in mg/ g plant material (Table 2). It is considered a low flavonoid content compared to many fruits and vegetables [16].

3.5 DPPH Free Radical Scavenging Assay

In the present study, investigation of total antioxidant capacity was measured as the cumulative capacity of the compounds present in the sample to scavenge free radicals, using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) reaction. The presence of antioxidant in the sample leads to the disappearance of DPPH radical chromogens which can be detected

spectrophotometrically at 517 nm. The plant exhibited a very low antioxidant property as DPPH scavenging activity was 0.085±0.002 mg ascorbic acid equivalent antioxidant capacity in 1 g plant material (Table 2). Analysis of correlation revealed that the DPPH free radical scavenging activity was strongly correlated with the total flavonoid content and total phenolics. Although many flavonoids were isolated from the plant but their yield is low compared to many traditional foods may be due to the high salt content of these plants. Compared to other *Mesembryanthemum* species [2] the reported antioxidant activity is very low due to low total phenolics and flavonoids.

4. CONCLUSION

Phytochemical investigation of the *M. forsskaolii* afforded the isolation of seven flavonoids for the first time in the plant and this enriches the chemical data of this halophyte. Due to the high salt content of this plant, the yield of the isolated flavonoids was very low and this was confirmed by the low TPC and TFC which consequently affected the antioxidant properties of the plant.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hartmann HEK. Illustrated handbook of succulent plants: Aizoaceae F-Z [Internet]. Springer Science & Business Media. 2002;422.
   [Cited 2015 Jul 23]
   Available:https://books.google.com/books?id=8zLlxBJ8m-EC&pgis=1
2. Falleh H, Ksouri R, Medini F, Guyot S, Abdelly C, Magné C. Antioxidant activity and phenolic composition of the medicinal and edible halophyte *Mesembryanthemum edule* L. Ind Crops Prod [Internet]. Elsevier B.V. 2011;34(1):1066–71.
   [Cited 2013 Nov 19].
   Available:http://linkinghub.elsevier.com/retrieve/pii/S0926669011000781
3. Rahman SMA, Abd-ellatif SA, Deraz SF, Khalil AA. Antibacterial activity of some wild medicinal plants collected from western mediterranean coast, Egypt: Natural alternatives for infectious disease treatment. African J Biotechnol. 2011; 10(52):10733–43.
4. Bouftira I, Abdelly C, Sfar S. Antioxidant properties of *Mesembryanthemum crystallinum* and *Carpobrotus edulis* extracts. Asian J Chem. 2009;21(1):549–59.
5. Al-Qahiz NM. The impact of Samh seeds on blood parameters of experimental animals. Pakistan Journal of Nutrition. 2009;872–6.
6. Al-Faris NA, Al-Sawadi AD, Alokail MS. Effect of samh seeds supplementation (*Mesembryanthemum forsskalei* Hochst) on liver enzymes and lipid profiles of streptozotocin (STZ)-induced diabetic Wistar rats. Saudi J Biol Sci [Internet]. King Saud University. 2010;17(1):23–8.
   [Cited 2014 Mar 6].
   Available:http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3730945&tool=pmcentrez&rendertype=abstract
7. Balayssac S, Delsuc MA, Gilard V, Prigent Y, Malet-Martino M. Two-dimensional DOSY experiment with Excitation Sculpting water suppression for the analysis of natural and biological media. J Magn Reson. 2009;196(1):78–83.
8. Mohammed R, El-hawary SS, Abo-youssef AM. Journal of Natural Products Biological investigation of some wild Aizoaceae and Chenopediaceae species growing in Egypt. J Nat Prod India. 2012;5:193–206.
9. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant Activity and total phenolics in selected fruits, vegetables, and grain products. J Agric Food Chem [Internet]. 1998;46(10):4113–7.
   Available:http://dx.doi.org/10.1021/jf9801973
10. Gao BB, She GM, She DM. Chemical constituents and biological activities of plants from the genus *Ligustrum.* Chem Biodivers. 2013;10:96–128.
11. Ersöz T, Harput Ü Ş, Saracoğlu I, Çaliş I, Ogihara Y. Phenolic compounds from *Scutellaria pontica.* Turkish J Chem. 2002;26(4):581–8.
12. Abu Bakar MF, Mohamed M, Rahmat A, Fry J. Phytochemicals and antioxidant

activity of different parts of bambangan *(Mangifera pajang*) and tarap (*Artocarpus odoratissimus*). Food Chem [Internet]. Elsevier Ltd. 2009;113(2):479–83. Available:http://dx.doi.org/10.1016/j.foodchem.2008.07.081

13. Magalhães LM, Segundo MA, Reis S, Lima JLFC. Automatic method for determination of total antioxidant capacity using 2,2-diphenyl-1-picrylhydrazyl assay. Anal Chim Acta. 2006;558(1-2):310–8.
14. Balayssac S, Trefi S, Gilard V, Malet-Martino M, Martino R, Delsuc MA. 2D and 3D DOSY 1H NMR, a useful tool for analysis of complex mixtures: Application to herbal drugs or dietary supplements for erectile dysfunction. J Pharm Biomed Anal. 2009;50(4):602–12.
15. De-eknamkul W, Potduang B. Biosynthesis of b -sitosterol and stigmasterol in *Croton sublyratus* proceeds via a mixed origin of isoprene units. 2003;62:389–98.
16. Moussa AM, Emam AM, Diab YM, Mahmoud ME, Mahmoud AS. Evaluation of antioxidant potential of 124 Egyptian plants with emphasis on the action of *Punica granatum* leaf extract on rats. Int Food Res J. 2011;18:535–42.

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