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Comparison of Egyptian and Saudi *Mesembryanthemum forskalii* Hochst. ex Boiss. as an unconventional alternative protein of wheat and barley

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Mesembryanthemum forskalii, commonly called Forskal Fig-marigold and locally, *Samh*, is a wild plant, grown naturally in Saudi and Egyptian deserts and in many countries around the world. However, nutrition value, especially its protein content, has not been studied until now. Here, we explored this plant as a new source of protein, particularly in drought or salted lands that can't be cultivated with grains and to meet the increasing of human requirements for protein. Analysis of amino acids contents showed seven essential amino acids in Egyptian and Saudi *M. forskalii*. Moreover, both *M. forskalii* have some amino acids their concentrations higher than that of wheat and barley. In addition, SDS-PAGE protein results revealed the appearance of 7 monomorphic bands in all samples; two bands appeared only in *M. forskalii* and not found in wheat or barley. HDN protein profile exhibited Rubisco band in all samples. The CDDP analysis indicated that *M. forskalii* to be a potential new source of protein. It has many important amino acids and a valuable content of protein and can be combined or substituted with flour from cereals to enrich the diets of Saharan communities.

Keywords: Alternative protein source, Forskal Fig-marigold, Native Protein, Plant protein, Samh

Mesembryanthemum forskalii Hochst. Ex Boiss. (Fam. Aizoaceae), commonly called as Forskal Figmarigold, is a succulent, drought and salt tolerant plant^{1,2}. Stem is erect, short and branching from the base. Leaves are sessile, thick, opposite and fleshy. Flowers are axillary. Calyx with subequal conical lobes. Petals are creamy. It produces large amount of tiny dark brown and round shaped seeds³. In Egypt, *M. forskalii* is distributed along the Mediterranean coast and extended to the sand plains of deserts while, In Saudi Arabia, it is grown in the northern parts and locally known as "Samh" used by Bedouins for making bread or mixing it with dates^{3,4}.

M. forskalii seeds contain about 5.60% fat, 49% carbohydrates and 23% protein as good as lentil, corn and rice, particularly, the protein content. In addition it contains other important minerals such as sodium, calcium, potassium, magnesium, iron, zinc, manganese and copper⁵. Rubisco is an important protein complex in plant that regulates photosynthesis and photorespiration⁶. It contains about 50% of the chloroplast protein with a

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molecular weight of 560 kDa⁷. The Rubisco protein sequence length consists of 166 to 477 amino acids. It also has good foam stabilizing features, which could be utilizing in food industry^{8,9}.

M. forskalii has also been reported to have medicinal properties. The seeds have phytosterols and can minimize the level of cholesterol and can use to treat hair fungi¹⁰. High intake of samh seeds decrease triglycerides and glucose concentration¹¹. *M. forskalii* have citric, malic and oxalic acids and high potential of polyols which are known as health-promoting agents^{12,13}. *M. forskalii* is also used in meat industry. Samh flour is mixed with minced meat as a substitute for soybean meal (SBM) (3.5%) to make beef patties¹⁴. The samh seeds are used as feed for fishes and poultry^{15,16}.

In Egypt, family Aizoaceae is poorly studied¹⁷, and *M. forskalii* is just known as a wild plant in the Egyptian deserts and there are no previous studies available on its nutritional value or the analysis of its protein content. Also, there is no molecular markers used before to characterize Saudi *M. forskalii*. In this context, it is the first study to use CDDP technique to characterize *M. forskalii* plant. Conserved DNA-

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Derived Polymorphism (CDDP) is a plant DNA marker based on a selection of well characterized plant genes that involved in plant development or response to abiotic and biotic stresses¹⁸. Talebi *et al.*¹⁹ demonstrated that CDDP marker gave the highest percentage of polymorphism when compared with other markers.

In this study, we characterized the Egyptian and Saudi *M. forskalii* as natural and wild plants inhabiting Egyptian and Saudi deserts with (CDDP-PCR) molecular marker, and studied the protein value of *M. forskalii* as a new source of protein by analysis of individual amino acids, separation of total cellular protein using SDS-PAGE and analysis of Histidine Deoxycholate Native (HDN) protein of both the samples of *M. forskalii* and also compared them with the protein of Egyptian wheat and barley.

Materials and Methods

Materials

Saudi *M. forskalii* seeds were collected from Al Jouf in the northern region of Saudi Arabia and Egyptian *M. forskalii* seeds were collected from the Fossilized Forest Reserve located 18 Km East of Maadi, Cairo. The two samples collected in August 2017 and had been identified following (Al-Hassan; Collenette)^{20,21}.

Grains of Egyptian Hordeum vulgare (Giza-134) and Egyptian Triticum aestivum (Shandaweel-1) were provided from Field Crops Research Institute, Agriculture Research Center (ARC), Giza, Egypt. The experiments were conducted using 9 samples as follows: Gr. I, Seeds of Egyptian Mesembryanthemum forskalii only; Gr. II, Seeds of Saudi M. forskalii only; Gr. III, Grains of Triticum aestivum (Shandaweel-1) only; Gr. IV, Grains of Hordeum vulgare (Giza-134) only; Gr. V, Mixture of 70% Egyption M. forskalii and 30% Triticum aestivum (Shandaweel-1); Gr. VI, Mixture of 70% Saudi M. forskalii and 30% Triticum aestivum (Shandaweel-1); Gr. VII, Mixture of 70% Egyption M. forskalii and 30% Hordeum vulgare (Giza-134); Gr. VIII, Mixture of 70% Saudi M. forskalii and 30% Hordeum vulgare (Giza-134); and Gr. IX with mixture of 70% Triticum aestivum (Shandaweel-1) and 30% Hordeum vulgare (Giza-134).

CDDP Method

Conserved DNA-Derived Polymorphism (CDDP) is a plant DNA marker developed by Collard & Mackill¹⁸. Fifteen primers of (CDDP) genomic molecular marker were used for this study (Table 1).

DNA extraction and purification and estimation of DNA concentration

DNA extraction and purification were carried out using a DNAeasy Plant Mini Kit (QIAGEN, Santa Clarita, CA) and according to the manufacturer's protocol. The DNA samples (2 μ L) were run on 1.5 % agarose gel in comparison to 10 μ L of a DNA marker (lambda DNA *Hind III* digest and *Phi* X 174/*Hae*III digest), and the DNA concentration was estimated by comparing the DNA sample fluorescence degree with the bands of DNA marker.

CDDP-PCR reactions

The amplification reaction (25 μ L) had 30 ng template DNA, 1 μ M primer (Promega, USA), 1 U Taq DNA polymerase GoTaq® Flexi DNA polymerase, 1X PCR buffer, 1.5 mM MgCl₂ and 0. mM dNTP.

Thermocycling profile and detection of the PCR products

PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to 35 cycles after an initial denaturation cycle for 5 min at 94°C. Each cycle consisted of a denaturation at 94°C for 1 min, an annealing at 45°C for 1 min, and an elongation at 72°C for 1.5 min. The primer extension was for 7 min at 72°C in the final cycle. The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5 μ g/mL) in 1X TBE buffer at 95 volts. A 1kb DNA ladder was used as a molecular size standard. PCR products were visualized on UV light and photographed using a Gel Documentation System (BIO-RAD 2000).

Data analysis

The banding patterns generated by SDS-PAGE protein, HDN protein and CDDP-PCR were compared

Table 1 –	- Names and sequences of primers used						
in CDDP-PCR analysis							
Primer Name	Sequence						
CDDP-1	5'-TGGCGSAAGTACGGCCAG-3'						
CDDP-2	5'-GTGGTTGTGCTTGCC-3'						
CDDP-3	5'-GCCCTCGTASGTSGT-3'						
CDDP-4	5'-GCASGTGTGCTCGCC-3'						
CDDP-5	5'-TGSTGSATGCTCCCG-3'						
CDDP-6	5'-CCGCTCGTGTGSACG-3'						
CDDP-7	5'-GGCAAGGGCTGCCGC-3'						
CDDP-8	5'-GGCAAGGGCTGCCGG-3'						
CDDP-9	5'-CACTACCGCGGSCTSCG-3'						
CDDP-10	5'-GCSGAGATCCGSGACCC-3'						
CDDP-11	5'-TGGCTSGGCACSTTCGA-3'						
CDDP-12	5'-AAGGGSAAGCTSCCSAAG-3'						
CDDP-13	5'-CACTGGTGGGAGCTSCAC-3'						
CDDP-18	5'-CTSTGCGACCGSGAGGTG-3'						
CDDP-19	5'-ACSCCSATCCACCGC-3'						

to determine the differences and similarities between samples under study. Clear and distinct amplification bands were scored as (1) for presence and (0) for absence. Bands of the same mobility were scored as identical. The genetic similarity coefficient (GS) between samples was estimated according to Dice coefficient²².

Amino acids anlysis

Amino acids content of samples was determined using amino acid analyzer (Sykam S 433) according to the method of AOAC²³. Sample was digested with 25 mL of 6N HCl at 110°C for 24 h, and HCl was removed by evaporation. The remaining solid fraction was dissolved with 0.2N sodium citrate buffer (pH 2.2). One mL of the solution was filtered through 0.45 μ M Millipore membrane filters. The standard amino acids (17 amino acids) was treated as same as the samples. Amino acids were expressed as g/100 g protein on dry weight basis.

Total soluble protein

Protein extraction

The samples were ground in liquid N_2 and homogenized in Trizma buffer pH= 8.0 (W/V) (Sigma- Aldrich, St. Louis, MO, USA), 0.1 mM phenyl methyl sulfonyl fluroide (PMSF) as protease inhibitor, and 100 mM mercaptoethanol as a reducing agent. The extraction was performed on ice. Total soluble proteins were collected by centrifugation at 4°C at 15000 rpm for 15 min²⁴. Protein concentrations of the different samples were measured spectrophotometrically²⁵.

SDS-PAGE

Proteins were separated on 12% polyacrylamide gel in the presence of SDS-PAGE according to Laemmli²⁶. Equal percentage of protein extracts were loaded onto the gel (2% of extracted volume/lane). Electrophoresis was performed at 70 V for 6 h in Bio-Rad mini vertical electrophoresis cell. Protein markers (Geneaid, Taiwan) were loaded which covering a wide range of molecular weights (10-245 kDa). The protein bands were stained by Coomassie brilliant blue- G-250 and destained to clear the gel background from the stain. Gel was photographed by Gel Doc System - Bio-Rad.

Histidine deoxycholate native (HDN-PAGE)

The procedure of HDN-PAGE was conducted according to $Ladig^{27}$. All extraction steps were performed at 4°C. The composition of different buffer systems is given in Ladig procedure. Here, 20%

glycerol was used for gradient formation. All gels were prepared in a cold room with pre-cooled solutions. Casting of the HDN gels were performed without stacking gel with a 3.5-12% (v/v) acrylamide gradient from bottom to top by the aid of a Hoefer gradient mixer (GE Healthcare) and with Native molecular weight standards (HMW Native Marker kit, GE Healthcare).

Results and Discussion

In this study, we characterized *Mesembryanthemum forskalii* collected from the deserts of Egypt and Saudi Arabia as a natural and wild plant with (CDDP-PCR) marker and measured its protein value. Although the genus *Mesembryanthemum* comprises about 72 species and it is native to the Mediterranean region, South Africa, Saudi Arabia, Atlantic Islands, California and South Australia²⁸, Egypt and other world countries have a little knowledge about its nutrition value. This study highlights the importance of this wild plant as a new source of protein especially in drought or salted lands that cannot be cultivated with grains like wheat, corn or barley.

Amino acids have an important role in every metabolic pathway²⁹. Amino acid content is an important factor affecting the nutritional value of seed crops and it determines the quality of the protein³⁰. In order to assess the nutritional value of the protein in Egyptian and Saudi *M. forskalii*, we compared the amino acids composition of them with amino acids content in wheat and barley as famous sources of protein.

The amino acids profile (Table 2) indicated that the content of amino acids in both samples of *M. forskalii* are almost similar with the amino acids content in wheat and Barley. The amino acid glycine concentration was higher in Saudi and Egyptian *M. forskalii* than its concentration in wheat and barley. The total amino acids concentration in the mixture of Saudi or Egyptian *M. forskalii* with wheat are higher the mixture between them and barley, and higher than the mixture between wheat and barley.

Among the 17 amino acids, seven essential amino acids were detected (isoleucine, leucine, methionine, lysine, phenylalanine, valine and threonine) in Saudi and Egyptian *M. forskalii*. Nutritionally, human body cannot make essential amino acids and it must be obtained from food. The major amino acids in *M. forskalii* seeds were glutamic acid, proline, glycine, histidine and aspartic acid. Methionine and cystine were found in trace amounts, while tryptophan was absent. Egyptian

	Table 2 — Individual amino acids profile										
Type of Amino Acid	Concentration (mg/g)										
	Egypt. M. forskalii	Saudi M. forskalii	Egypt. Wheat	Egypt. Barley	Egypt. <i>M. forskalii</i> & Wheat	Saudi <i>M. forskalii</i> & Wheat	Egypt. <i>M. forskalii</i> & Barley	Saudi <i>M. forskalii</i> & Barley	Egypt. Wheat & Barley	Standard Deviations (SD)	
Aspartic	11.70	19.50	8.56	7.37	11.00	12.50	8.00	10.00	8.25	±3.73	
Threonine	3.80	5.75	3.62	3.72	3.30	4.50	2.10	4.00	3.75	±0.97	
Serine	5.40	10.50	4.86	4.07	5.70	7.00	3.40	5.50	5.25	± 2.05	
Glutamic	46.10	62.75	59.81	40.34	45.20	62.00	27.70	41.00	47.50	±11.64	
Proline	36.60	5.50	12.46	11.20	27.50	11.75	14.30	9.00	11.75	±9.93	
Glycine	13.10	30.75	0.75	4.20	14.30	12.00	13.90	10.25	5.00	± 8.66	
Alanine	3.50	7.25	4.55	4.00	2.80	6.25	1.40	5.25	5.25	±1.79	
Cystine	1.30	3.25	1.32	0.80	1.20	1.25	1.30	1.00	1.00	±0.72	
Valine	4.60	5.50	55.44	48.76	3.60	6.00	2.00	5.00	5.25	± 21.06	
Methionine	2.90	3.50	1.85	1.35	2.60	1.50	1.70	1.75	1.50	±0.75	
Isoleucine	3.40	5.25	4.21	3.33	2.90	5.00	1.70	3.75	4.00	1.08	
Leucine	8.20	8.25	7.99	6.72	6.70	8.75	3.50	6.75	7.50	±1.56	
Tyrosine	5.90	7.00	2.60	2.18	5.50	3.50	3.80	3.25	2.50	± 1.70	
Phenylalanine	5.50	7.25	5.70	5.33	4.30	6.25	2.30	5.25	6.00	±1.39	
Histidine	18.30	12.75	6.09	3.21	18.60	6.00	15.10	5.00	3.50	±6.33	
Lysine	3.50	4.75	4.43	3.83	3.30	5.00	1.80	3.75	4.50	±0.97	
Arginine	9.00	22.75	6.91	5.55	11.00	10.75	8.30	9.75	6.50	±5.12	
Total	182.80	222.25	199.78	161.77	169.50	170.00	112.30	130.25	129.00	± 79.45	

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[Amino acids were expressed as g/100 g protein on dry weight basis. ± SD of three separate determinations]

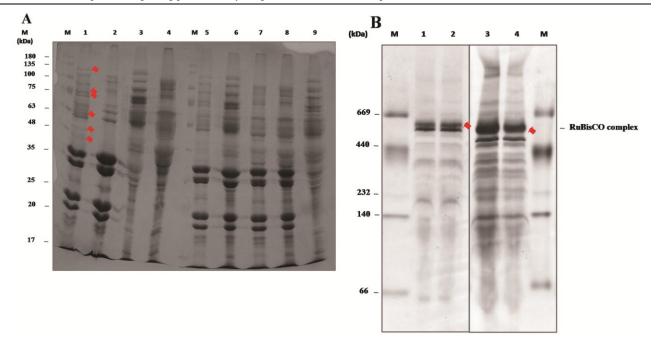


Fig. 1 — (A) Electrophoretic banding profiles of total cellular protein using SDS-PAGE. Lanes: (M) Marker, (1) Egyptian *M. forskalii*, (2) Saudi *M. forskalii*, (3) Egyptian wheat, (4) Egyptian barley, (5) mixture of Egyptian *M. forskalii* and wheat, (6) mixture of Saudi *M. forskalii* and wheat, (7) mixture of Egyptian *M. forskalii* and barley, (8) mixture of Saudi *M. forskalii* and barley, (9) mixture of wheat and barley. Arrows refer to unique polypeptides in Egyptian *M. forskalii*, (3) Egyptian wheat, (4) Egyptian wheat, (4) Egyptian barley, (7) mixture of total cellular protein using HDN-PAGE between (1) Egyptian *M. forskalii*, (2) Saudi *M. forskalii*, (3) Egyptian wheat, (4) Egyptian barley, (M) Marker. [Arrows refer to Rubisco complex]

M. forskalii have proline and histidine higher than Saudi one. Proline helps in fertility of pollen and increase resistance to unfavorable environmental conditions. Histidine helps fruit ripening. This indicated the high fertility and the ability of Egyptian *M. forskalii* to resistant adverse climatic conditions and produce mature and healthy seeds. Glycine is an important amino acid used as a bread enhancer in bakery products³¹.

The protein conducted from plants serves as a primary source of protein in human diet. The results of SDS-PAGE protein profile (Table 3 and Fig. 1A) revealed the appearance of 7 monomorphic bands

Table 3 — Total number of monomorphic and polymorphic bands of total cellular protein using SDS-PAGE											
MW (kda)	Egypt. M. forskalii	Saudi M. forskalii	Egypt. Wheat	Egypt. Barley	Egypt. <i>M. forskalii</i> & Wheat	Saudi <i>M. forskalii &</i> Wheat	Egypt. <i>M. forskalii</i> & Barley	Saudi <i>M. forskalii</i> & Barley	Egypt. Wheat & Barley		
117.80	1	0	1	0	1	1	0	0	1		
105.23	1	1	1	1	1	1	1	1	0		
101.24	0	1	0	0	0	0	1	1	1		
94.57	1	0	0	1	0	0	1	1	0		
90.44	0	1	1	1	1	1	1	1	1		
84.74	0	0	0	1	0	0	1	1	0		
76.86	1	0	0	0	0	0	1	0	0		
75.54	1	0	1	0	1	1	0	0	1		
72.52	0	0	1	0	1	1	0	0	1		
68.55	0	1	1	0	1	1	0	1	1		
65.99	1	1	1	1	1	1	1	1	1		
64.10	1	0	0	0	1	0	1	1	1		
62.42	1	1	1	1	1	1	0	0	1		
61.93	0	0	0	0	1	0	1	1	0		
60.26	0	0	0	1	0	0	0	1	0		
59.09	0	0	0	0	0	0	1	1	0		
53.69	0	0	0	1	0	1	0	1	0		
48.86	1	0	1	0	1	1	1	1	1		
43.37	1	0	1	1	0	1	1	1	1		
39.19	0	0	0	1	0	0	0	1	0		
34.66	1	1	0	1	1	1	1	1	1		
32.91	0	0	1	1	0	0	0	0	0		
30.04	1	1	0	0	1	1	0	1	1		
27.33	0	0	1	1	0	1	0	1	1		
25.76	0	0	1	1	0	1	0	1	0		
24.10	1	1	1	1	1	1	1	1	1		
22.23	0	0	1	1	0	1	0	0	0		
19.77	0	0	0	1	0	0	0	0	0		
19.10	1	1	1	1	1	1	1	1	0		
20.11	0	1	0	0	0	0	0	0	0		
16.08	1	1	1	1	1	1	1	1	0		
12.46	1	1	1	1	1	1	1	1	1		
10.60	1	1	0	0	1	1	1	1	0		
9.87	0	0	1	1	0	0	0	0	0		
5.71	0	1	1	1	0	1	1	1	1		
7.36	0	0	1	0	0	0	0	0	0		
Total No. of bands	17	15	21	22	18	22	19	25	17		

between the four samples (Egyptian *M. forskalii*, Saudi *M. forskalii*, Egyptian wheat, Egyptian barley) with molecular weights 117.80, 94.57, 76.86, 75.54, 64.10, 48.86 and 43.37 kDa, respectively. Two unique bands molecular weights are 76.86 and 64.10 kDa observed in the Egyptian *M. forskalii* were not found in the remaining samples. Also, there were six unique bands appeared in the Egyptian *M. forskalii* with molecular weights are 117.80, 94.57, 76.86, 75.54, 48.86 and 43.37 kDa, respectively were not found in Saudi *M. forskalii*. Further, the two bands with molecular weightse 30.04 and 10.60 kDa observed in Egyptian and Saudi *M. forskalii* were not found in wheat or barleyhad,.

The SDS-PAGE protein pattern of the mixtures between *M. forskalii* and wheat or barley revealed the appearance of 10 monomorphic bands their molecular

weights are 105.23, 90.44, 65.99, 48.86, 34.66, 24.10, 19.10, 16.08, 12.46 and 10.60 kDa, respectively.

The results of HDN protein (Table 4 and Fig. 1B) showed a similarity between Egyptian & Saudi *M. forskalii* and Egyptian wheat and barley with the appearance of six monomorphic bands in all samples with molecular weights 567.86, 473.17, 385.55, 213.77, 94.96 and 55.58 kDa, respectively. The rubisco band was found in all the samples at the same molecular weight of 567.862 kDa. Rubisco is the most abundant soluble protein of plant leaves responsible for carbon fixation, has many good features for food nutritional value; it is well digestible and has good emulsification features³².

Mustafa *et al.*³³ have shown samh flour to improve the nutritional value of the bread and cookies due to its content of protein, fat, fiber and its content of minerals.

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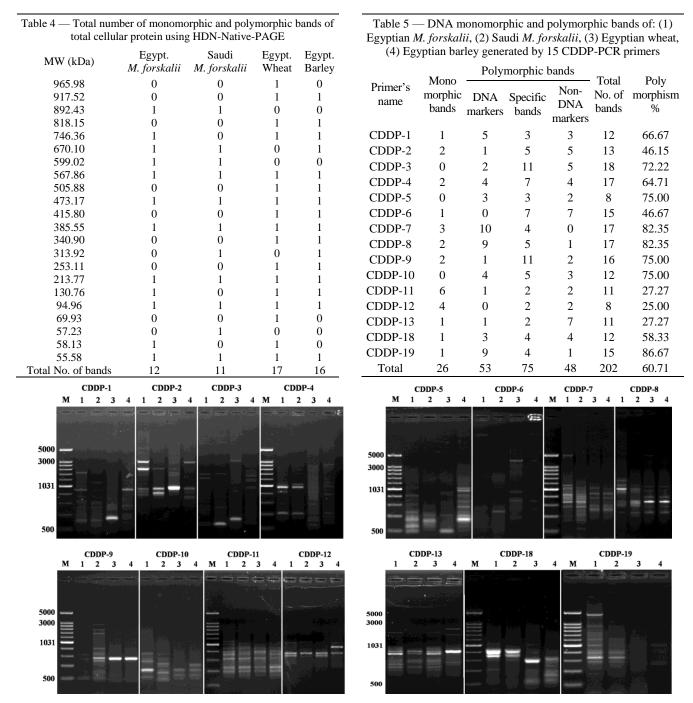


Fig. 2 —Differential amplicons pattern generated from 15 CDDP-PCR primers between: (1) Egyptian *M. forskalii*, (2) Saudi M. forskalii, (3) Egyptian wheat, (4) Egyptian barley, (M) DNA Marker.

They made bread with mixing wheat flour with samh flour up to 35% and found that it has the same characters of wheat bread. Also, they made cookies from 100% samh flour resembling chocolate cookies³³. Hamed *et al.*³⁴ have demonstrated that the samh seeds protein can replace yellow corn used in animal feeding especially in countries like Saudi Arabia that cannot cultivate these crops due to unfavorable conditions.

DNA molecular markers is useful tools for fast supplying genetic information about plants, analysis of genetic variability, species identification, molecular markers for economically important traits and genotyping of individuals^{35,36}. It is the first use of CDDP markers for identification and characterization of Egyptian and Saudi *Mesembryanthemum forskalii*. The CDDP analysis (Table 5 and Fig. 2) of Egyptian and Saudi M. forskalii with the 15 primers generated a total of 202 fragments of which 128 were polymorphic, corresponding to 60.71% level of polymorphism. The highest ratio of polymorphism was generated by CDDP-5 (86.67%), while the CDDP-4 primer produced the lowest polymorphism (25%). The total number of species-specific markers scored across samples is 75. Thirty-eight markers were positive specific markers (found in both Egyptian and Saudi samples) and 15 were negative specific markers (found in both wheat and barley). The highest number of specific markers generated from CDDP analysis was 11 (CDDP-3 and CDDP-9 primers), while the lowest number of specific markers 2 was generated from primers CDDP-11, CDDP-12 and CDDP-13.

The genetic similarities among the four samples based on Nei's method³⁷ resulted from CDDP analysis showed that the pairwise similarity indices of the Egyptian *M. forskalii* and wheat was 46%; the Egyptian *M. forskalii* and barley 44%; the Saudi *M. forskalii* and wheat 43%; and the Saudi *M. forskalii* and barley was 40%. This indicates that *M. forskalii* has a great similarity with wheat or barley and sharing some genes with them that may be related to abiotic and biotic stress in which the primers of CDDP PCR targeted them³⁸. These results demonstrate that *M. forskalii* comparable to other sources of protein such as grass, seaweed and fungi³⁹.

Conclusion

The above findings demonstrated that Mesembryanthemum forskalii Hochst (Samh) seeds have 17 amino acids, seven of them are essential for human feeding. In addition, protein analysis and CDDP results showed similarity between M. forskalii and wheat or barley. It could be concluded that M. forskalii has a nutrition value and it is a good rich source for protein and can be cultivated in regions that can't cultivate grains. The flour of it can be mixed or substitute with flour of cereals for producing high protein bakery products. Samh-based products represent the solution for consumers with allergenic problems. It has many medicinal benefits and can be used for the production of animal feeds and in medicinal and food industries.

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Conflict of interest

Authors declare no conflict of interests.

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