## Research Article

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# Nutraceutical potential of *Mesembryanthemum* forsskaolii Hochst. ex Bioss.: Insights into its nutritional composition, phytochemical contents, and antioxidant activity

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Abstract: Mesembryanthemum forsskaolii Hochst. ex Bioss is a resilient succulent plant in the Aizoaceae family. This plant has been recognized for its nutritional and metabolic benefits, but its potential remains underexplored. The aim of this research is to analyze the nutritional composition, phytochemical content, and antioxidant potential of M. forsskaolii. The protein content, total sugars, macro-, and micronutrients were estimated in seeds, leaves, and stems of the studied plant. To investigate the phytochemical profiles and antioxidant capacity, GC-MS analysis, determination of total phenolic, flavonoid, and tannin contents, ferric reducing antioxidant power (FRAP) and total antioxidant activity (TAC) tests, and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assays were conducted. The soluble protein (147.55 mg  $g^{-1}$  DW), total protein (341.23 mg  $g^{-1}$ DW), total carbohydrate (0.258 mg mL<sup>-1</sup>), and nitrogen  $(26.152 \text{ mg g}^{-1})$  contents of M. forsskaolii seeds were the highest. Phytochemical analysis revealed the presence of several compounds, such as β-sitosterol, phthalic acid, and phytol, which have potential antioxidative, anti-inflammatory, and antimicrobial properties. Seeds showed the greatest presence of phenols, flavonoids, and tannins, indicating high antioxidant activity in FRAP and TAC tests. The ABTS and DPPH scavenging assays showed that the antioxidant activity increased proportionally with concentration in all plant parts. However, seeds consistently demonstrated the greatest capacity. This study provides a detailed analysis

**Keywords:** protein content, DPPH, ABTS, flavonoids, macronutrients, micronutrients

# 1 Introduction

Mesembryanthemum forsskaolii Hochst. ex Bioss. is a succulent plant species in the Aizoaceae family. The family comprises about 127 genera and 1,860 species and is widely dispersed throughout tropical and sub-tropical regions of Africa. M. forsskaolii is native to arid and coastal regions of Southern Africa and is highly adaptable to extreme environmental conditions, such as high temperatures, limited water availability, and saline soils [1]. Due to its remarkable ability to withstand harsh conditions, M. forsskaolii has gained attention from researchers and horticulturists. Additionally, it displays numerous intriguing nutritional and metabolic properties, making it a subject of scientific interest. This plant has a long-standing history of traditional use by indigenous communities in Southern Africa. Natural products are becoming more popular due to their various health benefits. Therefore, it is necessary to investigate the phytochemical constituents and biological activities of this unique plant.

Numerous studies have emphasized the significance of phytochemicals as bioactive compounds found in different plant species [2–9]. These secondary metabolites not only aid in the plant's defense mechanisms but also exhibit a vast array of biological activities, including antioxidant, anti-inflammatory, and anticancer properties [2]. *M. forsskaolii* has potential as a significant source of valuable phytochemicals due to its adaptation to the adverse environmental conditions and distinct physiological attributes [3].

Saudi Arabia

on the dietary protein, carbohydrates, essential nutrients, and antioxidants that can be obtained from *M. forsskaolii*, highlighting its potential as a valuable source of nutrition and phytochemicals.

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Antioxidants neutralize reactive oxygen species (ROS) and protect cells from oxidative damage [4]. Oxidative stress, caused by an imbalance between ROS production and the body's antioxidant defense mechanisms, has been linked to the development of chronic diseases such as cardiovascular disorders, neurodegenerative diseases, and cancer [5]. Therefore, the search for natural sources of antioxidants has gained significant attention in recent years.

The Aizoaceae family is known for its various biological activities, including antioxidant, antimicrobial, larvicidal, anti-inflammatory, anticancer, antihyperlipidemic, antipyretic, analgesic, anticholera, and anti-rheumatic properties [3,6-9]. However, research on the phytochemical composition and antioxidant potential of M. forsskaolii is limited. The aim of this manuscript is to fill this knowledge gap by conducting a comprehensive analysis of the phytochemical contents and antioxidant activity of this plant species. Various analytical techniques, such as spectrophotometry and mass spectrometry, are used to detect and measure the primary phytochemical constituents present in the plant extract. In addition, we will evaluate the extract's antioxidant capacity using in vitro assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assays. The aim of this study is to enhance the current understanding of the potential health benefits associated with M. forsskaolii by clarifying its phytochemical composition and antioxidant capabilities. The results of this investigation may have significant implications for developing natural antioxidant treatments and discovering new bioactive substances.

# 2 Materials and methods

# 2.1 Sample collection and preparation

Plant samples of *Mesembryanthemum forsskaolii* Hochst. ex Bioss were collected from Al-Jouf city in northern Saudi Arabia. The samples were transferred to the lab where they were washed thoroughly with tap water and left to dry under room temperature for 48 h before using for further analysis.

## 2.2 Nutritional composition analysis

#### 2.2.1 Protein content determination

The total protein content was determined according to the method described by Lowry et al. [10], using Bovine serum

albumin as a standard. To analyze the water-soluble protein, 0.1 g (dry weight) of tissue samples were immersed in 10 mL of distilled water and held at 90°C for 2 h. For total protein analysis, 50 mg of tissue samples were treated with 10 mL of 0.1 N NaOH at 90°C for 2 h. After centrifugation, the supernatants were collected. 1 mL of the extract was thoroughly mixed with 5 mL of alkaline reagent. This reagent is composed of 50 mL of 2% Na<sub>2</sub>CO<sub>3</sub> prepared in 0.1 N NaOH and 1 mL of 0.5% CuSO<sub>4</sub>·5H<sub>2</sub>O prepared in 1% Sodium Potassium Tartrate. The mixture was left to stand for 10 min, after which 0.5 mL of Folin phenol reagent (diluted 1:1 with distilled water) was added. The mixture was immediately mixed again, left for 30 min then the absorbance was measured at 700 nm against a blank. The outcomes were denoted as mg g<sup>-1</sup> dry mass. Insoluble proteins were determined by subtracting the quantities of complete and water-soluble proteins.

#### 2.2.2 Total sugar estimation

The total sugar content of the samples was evaluated using the method described by DuBois et al. [11]. The sample was treated consecutively with 1 mL of 5% phenol and 5 mL of 96% sulfuric acid. The reaction mixture was incubated in a water bath set at 30°C for 20 min, and the absorbance was measured spectrophotometrically at 490 nm. The total soluble sugar content was calculated by applying the equation (y = 10.334x - 0.0906 with  $R^2 = 0.993$ ) to a glucose standard ranging between 0.025 and 0.3 µg mL<sup>-1</sup>, which was used to prepare a calibration curve. The total soluble sugar content was expressed as glucose (mg g<sup>-1</sup> DW).

## 2.2.3 Macro- and micronutrient analysis

The plant specimen was digested using a combination of hydrogen peroxide  $(H_2O_2)$  and sulfuric acid  $(H_2SO_4)$ . Specifically, 0.3 g of the specimen were accurately weighed and placed into a 50 mL crucible. Then, concentrated 18 M (98%)  $H_2SO_4$  and  $H_2O_2$  (5 mL) were added. The resulting mixture was then heated on a hot plate at approximately 250°C until it became clear and transparent. After completing this step, the crucible was taken off the hot plate and allowed to cool down. Next it was filtered through Whatman filter paper (No. 42). The resulting filtrate was then transferred quantitatively to a 25 mL volumetric flask and distilled water was added to reach the required volume. Finally, the solution was stored in a 50 mL polyethylene bottle at a temperature of 4°C until analysis. The total

nutrients were determined using the methodology described by Estefan et al. [12].

## 2.3 Phytochemicals screening

## 2.3.1 Gas chromatography-mass spectrometry (GC-MS) analysis

The leaf, stem, and seed samples were oven-dried at 50°C for 48 h. Subsequently, the powdered material was extracted with 50 mL of methanol over a 24 h period. with gradual shaking at room temperature. The resulting methanolic extracts were then filtered through Whatman filter paper (Grade 1) and the solvent was evaporated using a rotary evaporator (IKA Werke GmbH & Co. KG, Staufen im Breisgau, Germany). The phytochemical composition of M. forsskaolii extracts from different parts was analyzed using GC-MS (Turbomass, PerkinElmer Inc., Waltham, MA). Each sample was injected individually into the Elite-5MS column (30 m long, 0.25 m thick, and 0.25 m in diameter) with Helium gas as the carrier at a flow rate of 1 mL min<sup>-1</sup>. The temperature was initially set at 40°C and maintained for 2 min before gradually increasing to 200°C at a rate of 5°C min<sup>-1</sup>. It was then kept at that temperature for an additional 2 min. Subsequently, the temperature was raised to 300°C at a rate of 5°C min<sup>-1</sup> in and held there for another 2 min. The compounds were identified by cross-referencing the obtained mass spectra with the Adams and Wiley databases. The potential biological activities of the identified compounds were determined based on available literature. Moreover, the chemical structure of each compound was generated using ChemDraw® v22.2.

## 2.3.2 Total phenolic content (TPC) determination

The method of Ainsworth and Gillespie [13] was used to determine the TPC. The plant extracted material (0.1 mL), Folin-Ciocalteu reagent (100 µL), and 20% sodium carbonate solution (300 µL) were mixed and incubated in the dark at room temperature for 30 min. The absorbance was measured at 765 nm using a UV-visible spectrophotometer. The TPC was determined using the equation (y =0.0021x + 0.0203, with an  $R^2$  value of 0.999), derived from a standard curve established with gallic acid (GAE). The TPC measurement was expressed as mg g<sup>-1</sup> GAE based on the dry weight of the sample.

#### 2.3.3 Total flavonoid content (TFC) determination

The TFC was estimated following the method of Ordoñez et al. [14]. The methanol extract (0.5 mL) was mixed with a

2% water solution of AlCl<sub>3</sub> (0.5 mL). After being kept in the dark and incubated at room temperature for 2 h, the absorbance was measured at 420 nm. The TFC was calculated using the equation (y = 0.00144x - 0.0886) based on a calibration curve prepared with a quercetin standard ranging from 50 to 400 µg mL<sup>-1</sup>. TFC was expressed as quercetin (mg  $g^{-1}$  DW).

#### 2.3.4 Total tannin content (TTC) determination

TTC was measured using the Folin-Ciocalteu method with slight modifications, as described by Chandran and Indira [15]. A total of 100 µL of the extracted plant material was added to a 2 mL tube containing 1.5 mL of deionized water and 100 µL of Folin-Ciocalteu phenol reagent. After 8 min, 300 µL of 35% sodium carbonate solution was added to the mixture. The mixture was shaken well and kept in the dark at room temperature for 20 min. Absorbance was recorded at 700 nm. TTC was calculated using the equation (Y =0.0052x - 0.0013) on the basis of a calibration curve prepared with tannic acid. TTC was expressed as mg g<sup>-1</sup> DW.

## 2.4 Antioxidant capacity

## 2.4.1 DPPH assay

DPPH scavenging activity was determined using the DPPH assay method [16]. A solution of 0.1 mmol L<sup>-1</sup> DPPH in methanol was prepared. 750 µL of the sample was added to 750 µL of the DPPH solution. The mixture was incubated for 30 min at 25°C and the absorbance was measured at 517 nm.

## 2.4.2 ABTS radical scavenging assay

The ABTS scavenging activity was determined using the ABTS+ radical cation decolorization assay with some modifications [17]. A solution of 5 mL of 7 mmol L<sup>-1</sup> ABTS was mixed with 88 µL of 140 mM potassium persulfate solution to produce ABTS<sup>+</sup>. The mixture was incubated in the dark at room temperature for 16 h. The prepared ABTS+ solution was then diluted with methanol to give an initial absorbance of 0.7 at 734 nm. Next 500 µL of the extract was mixed with 1,000 µL of the diluted ABTS solution and incubated in the dark for 10 min at room temperature. The absorbance was then measured at 734 nm.

#### 2.4.3 Total antioxidant activity (TAC) determination

The TAC of the extracts was determined according to the method of Prieto et al. [18]. A volume of 0.15 mL of the sample (4 mg mL<sup>-1</sup>) was mixed with 1.5 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The reaction mixture was incubated at 95°C for 90 min in a water bath. The absorbance of the sample mixtures was measured at 695 nm. TAC was expressed as milligrams of ascorbic acid equivalents per gram of extract.

#### 2.4.4 Ferric reducing antioxidant power (FRAP) assay

Iron reducing power was determined by the method described by Zhao et al. [19]. In this assay,  $500\,\mu\text{L}$  of the extract (4 mg mL<sup>-1</sup>) was mixed with 2.5 mL of 200 mmol L<sup>-1</sup> phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at  $50^{\circ}\text{C}$  for 20 min. Then, 2.5 mL of 10% trichloroacetic acid was added and the tubes centrifuged at 10,000 rpm for 10 min. 5 mL of the upper layer were mixed with 5.0 mL of distilled water and 1 mL of 0.1% ferric chloride, and the absorbance of the reaction mixtures was measured at 700 nm. The ferric reducing power assay was expressed as milligrams of ascorbic acid equivalents per gram of extract.

# 3 Results

## 3.1 Nutritional potential of M. forsskaolii

#### 3.1.1 Protein and carbohydrate content

Table 1 displays the protein content (mg g $^{-1}$  DW) of various M. forsskaolii parts such as seeds, stems, and leaves. Soluble proteins, insoluble proteins, and total proteins were measured for every plant part. The results demonstrate substantial differences in protein levels among the diverse plant parts. The highest content of soluble proteins is found in the seeds, with a mean concentration of

147.55 mg g<sup>-1</sup> DW. This indicates that seeds are a rich source of readily available proteins. On the other hand, stems have the lowest soluble protein content at 111.64 mg g<sup>-1</sup> DW, while leaves contain intermediate levels at 129.14 mg g<sup>-1</sup> DW. Insoluble proteins follow a similar pattern, with seeds having the highest content (193.68 mg g<sup>-1</sup> DW), stems having the lowest (34.28 mg g<sup>-1</sup> DW), and leaves falling in between (82.43 mg g<sup>-1</sup> DW). The total protein content represents the combination of soluble and insoluble proteins. Seeds have the highest total protein content at 341.23 mg g<sup>-1</sup> DW, indicating their potential as a protein-rich component of the plant. Leaves also contain a substantial amount of total proteins at 211.58 mg g<sup>-1</sup> DW, while stems have the lowest total protein content at 145.92 mg g<sup>-1</sup> DW. Total carbohydrates are essential energy sources in human diets. M. forsskaolii seeds have the highest total carbohydrate content at 0.258 mg mL<sup>-1</sup>, while leaves and stems showed substantially lower with 0.121 and 0.101 mg mL<sup>-1</sup>, respectively. The previous data indicate that M. forsskaolii possesses significant nutritional potential, especially in terms of protein content. Seeds, with their high levels of soluble and insoluble proteins, represent a valuable source of dietary protein, which is essential for growth and tissue repair in humans. Leaves also contain substantial protein content, making them a potentially nutritious component.

#### 3.1.2 Nutrient contents

The mineral composition of M. forsskaolii was analyzed in three distinct plant parts, viz. seeds, stems, and leaves (Table 2). Nitrogen is a fundamental component of amino acids, proteins, and chlorophyll, essential for plant growth and photosynthesis. Among the analyzed plant parts, seeds exhibited the highest nitrogen content, with a mean concentration of 26.152 mg g $^{-1}$ . This observation aligns with the well-established role of seeds as the primary storage site for protein. Leaves contained a moderate amount of protein (20.081 mg g $^{-1}$ ). This observation aligns with the well-established role of leaves as the primary site for

**Table 1:** Comparative analysis of total carbohydrate and soluble, insoluble, and total protein contents in different parts of *Mesembryanthemum forsskaolii* Hochst. ex Bioss

Plant part	Soluble proteins (mg g <sup>–1</sup> DW)*	Insoluble proteins (mg g <sup>–1</sup> DW)	Total proteins (mg g <sup>–1</sup> DW)	Total carbohydrates (mg mL <sup>-1</sup> )
Seeds	147.55 ± 5.29 <sup>a</sup>	193.68 ± 2.36 <sup>a</sup>	341.23 ± 3.83 <sup>a</sup>	$0.258 \pm 0.0045^{a}$
Stems	111.64 ± 0.99 <sup>c</sup>	$34.28 \pm 0.52^{c}$	145.92 ± 1.22 <sup>c</sup>	$0.101 \pm 0.0024^{c}$
Leaves	129.14 ± 1.49 <sup>b</sup>	$82.43 \pm 0.48^{b}$	211.58 ± 1.39 <sup>b</sup>	$0.121 \pm 0.0004^{b}$

<sup>\*</sup>Values are shown as the mean value of five replicates  $\pm$  SD. Values followed by different superscript letters are significantly different (p < 0.05).

Table 2: Mineral composition of Mesembryanthemum forsskaolii Hochst. ex Bioss. seeds, stems, and leaves

Plant part	N (mg g <sup>-1</sup> )*	P (mg g <sup>-1</sup> )	K (mg g <sup>-1</sup> )	Fe (mg L <sup>-1</sup> )	Cu (mg L <sup>-1</sup> )	Zn (mg L <sup>-1</sup> )	Mn (mg L <sup>-1</sup> )
Seeds	26.152 ± 1.13 <sup>a</sup>	$0.583 \pm 0.05^{a}$	$25.431 \pm 2.30^{a}$	180.486 ± 30.30 <sup>c</sup>	$0.333 \pm 0.01^{b}$	20.646 ± 1.68 <sup>b</sup>	115.551 ± 6.18 <sup>b</sup>
Stems	13.076 ± 0.88c	$0.567 \pm 0.09^{a}$	$3.533 \pm 0.80^{c}$	265.734 ± 33.18 <sup>b</sup>	$0.014 \pm 0.01^{c}$	75.591 ± 8.12 <sup>a</sup>	287.379 ± 8.12 <sup>a</sup>
Leaves	20.081 ± 1.01 <sup>b</sup>	$0.367 \pm 0.08^{b}$	$14.633 \pm 1.50^{b}$	598.068 ± 70.11 <sup>a</sup>	$1.665 \pm 0.26^{a}$	111.555 ± 7.99 <sup>a</sup>	298.701 ± 9.95 <sup>a</sup>

<sup>\*</sup>Values are shown as the mean value of five replicates  $\pm$  SD. Values followed by different superscript letters are significantly different (p < 0.05).

photosynthesis and protein synthesis in plants. The relatively lower nitrogen content in stems (13.076 mg g<sup>-1</sup>) reflects their differing physiological functions. High nitrogen contents in seeds and leaves, accompanied by high contents of proteins, contribute to the potential of M. forsskaolii as a valuable source of dietary protein. Phosphorus and potassium are crucial macronutrients involved in various metabolic processes, including energy transfer and cell division. Seeds and stems demonstrated the highest phosphorus content at 0.583 and 0.567 mg g<sup>-1</sup>, respectively. Furthermore, seeds had the highest potassium content (25.431 mg g<sup>-1</sup>) while leaves showed intermediate content (14.633 mg g<sup>-1</sup>). In contrast, stems had the lowest potassium content.

Micronutrients, such as iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn), are essential for enzyme activities and various physiological functions in humans. Leaves exhibited significantly higher concentrations of these micronutrients compared to seeds and stems (Table 2). Leaves contained the highest iron content at 598.068 mg  $L^{-1}$ , emphasizing their role in chlorophyll synthesis and photosynthetic electron transport. Cu, Zn, and Mn were also most abundant in leaves, indicating their participation in enzyme activities associated with photosynthesis and stress response. The significant presence of essential micronutrients in leaves suggests that M. forsskaolii may hold potential health benefits related to these micronutrients, which are crucial for vital physiological functions in humans.

The data presented in Table 2 contribute to a deeper understanding of the nutritional aspects of M. forsskaolii and can have implications for its potential utilization in agriculture and nutrition. However, it is important to consider factors affecting mineral bioavailability, such as the chemical form of minerals and food processing methods, which may influence their overall nutritional impact. Further research is needed to assess the bioavailability of these minerals from M. forsskaolii and to determine the extent to which they can contribute to human nutrition. Specifically, it is important to determine the concentrations of possible antinutritional factors, e.g., phytic acid and oxalate, in different plant tissues. Additionally, methods for predicting mineral bioavailability, e.g., molar ratios of antinutrient/minerals [20] could be useful.

# 3.2 Phytochemical profile

M. forsskaolii leaves were analyzed using GC-MS (Table 3), revealing significant compounds such as  $\beta$ -sitosterol (18.80%), Phthalic acid (16.35%), and phytol (10.73%). These compounds contribute to the leaves' antioxidant, anti-inflammatory, and potentially antimicrobial properties. Phthalic acid (33.10%) was also among the key compounds in the seed extract, along with 7-hexadecenoic acid (24.07%) and Hexadecenoic acid (13.25%). The compounds indicate potential nutritional benefits, including antimicrobial and stress response effects, which can be attributed to the significant role of essential fatty acids. Upon analyzing the stem extract, it was found that methyl β-D-mannofuranoside (23.35%), cis-Vaccenic acid (8.68%), and 9-Octadecenoic acid (8.58%) were present in significant concentrations. These compounds highlight potential nutritional benefits particularly with mannose-derived compounds and essential fatty acids. The varied phytochemical composition of M. forsskaolii is underscored by these findings; each plant component offers a distinct array of compounds that enrich its nutritional and bioactive characteristics.

## 3.3 Antioxidant activity

Phenolic compounds are known for their antioxidant properties and potential health benefits. Among the plant parts analyzed, seeds have the highest phenol content at 62.56 mg GAE <sup>-1</sup> DW (Table 4), followed by leaves (39.13 mg GAE g<sup>-1</sup> DW), and stems (26.44 mg GAE g<sup>-1</sup> DW). The high phenolic content in seeds and leaves suggests their potential to contribute to the plant's overall antioxidant capacity, which can be beneficial for human health. Flavonoids are a subgroup of phenolic compounds known for their antioxidant and anti-inflammatory properties. Similar to phenols, seeds contain the highest flavonoid content at 53.57 mg QE g<sup>-1</sup> DW, followed by leaves (15.35 mg QE g<sup>-1</sup> DW), and stems (10.35 mg QE g<sup>-1</sup> DW). Similarly, seeds have the highest tannin content at 31.81 mg TAE per gram DW, followed by leaves (17.26 mg TAE g<sup>-1</sup> DW), and stems (11.15 mg TAE  $g^{-1}$  DW). Tannins are polyphenolic

 
 Table 3: Phytochemical compounds identified in the extracts of leaves, seeds, and stem of Mesembryanthemum forsskaolii Hochst. ex Bioss. via GC-MS
 analysis and their potential biological activities

No.	RT (min)*	Name of compound	Area %	Molecular weight (g mol <sup>-1</sup> )	Chemical structure	Biological activity
Leaf	extract					
1	5.182	<i>p</i> -Xylene	1.990	106.078		
2	14.602	Carbonic acid, monoamide, N-methyl-N-phenyl-, allyl ester	1.376	191.095	, i	
3	19.025	Hexadecanoic acid, methyl ester	2.191	270.256		Antibacterial, antiacne, anti- inflammatory, antihistaminic, and antieczemic activities [20,21]
4	19.5	Phthalic acid, butyl undecyl ester	16.350	376.261		Antimicrobial, and anti- inflammatory activities [22]
5	20.076	Methanol, tris (methylenecyclopropyl)-	9.513	188.12	НО	
_	20.720	04045.0 // 1 // 1 // 1	2.442	202.24		
6	20.739	9,12,15-Octadecatrienoic acid, methyl ester, ( <i>Z</i> , <i>Z</i> , <i>Z</i> )-	2.443	292.24		
7	20.857	Phytol	10.736	296.308	но	Antinociceptive, antioxidant, antitumor, anti-diabetic, anti-inflammatory, and anticancer activities [23–25]
8	24.148	2-Undecanone, 6,10-dimethyl-	2.549	198.345	l a l a l	
9	24.392	Sulfurous acid, butyl heptadecyl ester	0.929	376.301		Antibacterial [26]
10	24.686	Hexadecanoic acid, 2-hydroxy-1- (hydroxymethyl)ethyl ester	7.923	330.277	) on	Antioxidant, anthelmintic, and anti-inflammatory activities [27]
11	26.356	2(1 <i>H</i> )-Naphthalenone, octahydro-4a-methyl-7-(1- methylethyl)-, (4a.alpha.,7.beta.,8a.beta.)-	18.967	208.183	H	
12	26.863	Tetracosapentaene, 2,6,10,15,19,23-hexamethyl-	4.834	412.407	=	
13	27.795	β-Sitosterol	18.800	414.386		Anticancer, antioxidant, cardioprotective, and antidiabetic effects [28]

 Table 3: Continued

No.	RT (min)*	Name of compound	Area %	Molecular weight (g mol <sup>–1</sup> )	Chemical structure	Biological activity
14	28.295	26-Hydroxycholesterol	0.787	402.35	OH	
15	28.464	1-Methoxy-3-(2-hydroxyethyl)	0.610	202.193	HO OH	Antifungal, antioxidant [29]
Seed	extract	nonane				
1	5.182	1,3-Cyclopentadiene, 5-(1-methylethylidene)-	7.384	106.078		
2	18.568	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	6.401	334.214		
3	19.037	Hexadecanoic acid, methyl ester	13.259	270.256		
4	19.519	Phthalic acid, isobutyl octadecyl ester	33.102	474.371		Antidiabetic activity [30]
5	20.119	Preg-4-en-3-one, 17.alpha hydroxy-17.betacyano-	11.494	313.204	HO	
6	20.726	7-Hexadecenoic acid, methyl	24.073	268.24		Antioxidant, anticancer, and
7	20.945	ester, ( <i>Z</i> )- Cyclopentanetridecanoic acid, methyl ester	4.287	296.272		antiviral [31]
Stem	extract					
1	10.525	Methyl beta-p-mannofuranoside	23.35	194.079	HOOOH	
2	11.206	Hexadecanoic acid, methyl ester	2.25	270.256	HO 0	

Table 3: Continued

No.	RT (min)*	Name of compound	Area %	Molecular weight (g mol <sup>–1</sup> )	Chemical structure	Biological activity
3	11.438	<i>n</i> -Hexadecanoic acid	6.53	256.24	) on	Anti-inflammatory, anticancer, anti-androgenic, antipsychotic, and hypocholesterolemic properties [32–34]
4	12.126	9-Octadecenoic acid ( <i>Z</i> )-, methyl ester	8.58	296.272		Anti-inflammatory, antioxidant, antiandrogenic cancer preventive, anemiagenic, 5-alpha reductase inhibitor, insectifuge [20,35]
5	12.401	<i>cis</i> -Vaccenic acid	8.68	282.256	о́ он	Antibacterial activity, hypolipidemic [36]
5	14.497	Hexadecanoic acid, 2-hydroxy-1- (hydroxymethyl)ethyl ester	7.40	330.277	) ON ON	
7	15.491	2-(Adamantan-1-yl)- <i>N</i> -[1- (adamantan-1-yl)ethyl] acetamide	43.21	355.288	NH	

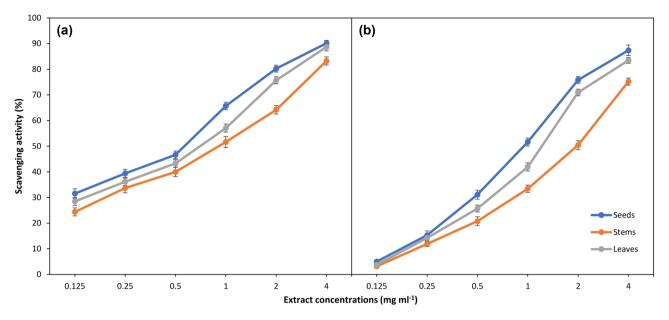
<sup>\*</sup>RT: Retention time.

compounds that have been associated with various health benefits, including antioxidant and anti-inflammatory effects.

Furthermore, seeds exhibit the highest antioxidant activity in both the FRAP (89.48 mg AAE g<sup>-1</sup> DW) and TAC (297.04 mg AAE g<sup>-1</sup> DW) assays (Table 4), highlighting their potential to provide strong antioxidant protection. The antioxidant activity of different parts of M. forsskaolii, including seeds, stems, and leaves, was further investigated using the ABTS and DPPH scavenging assays at various concentrations. In both assays, there was a clear concentration-response relationship (Figure 1). This indicates that the plant extracts become more effective at neutralizing free radicals with higher concentrations. As the concentration of plant extracts increased from 0.125 to 4 mg mL<sup>-1</sup>, the ABTS scavenging capacity also rose significantly (Figure 1a). At the highest concentration (4 mg mL<sup>-1</sup>), seeds exhibited a remarkable ABTS scavenging capacity of 90.12%, followed by leaves (87.64%) and stems (83.24%). Similar to the ABTS assay, there is a concentration-dependent increase in DPPH scavenging capacity across all plant parts (Figure 1b). Seeds consistently exhibit the highest DPPH scavenging capacity,

reaching 87.37% at the highest concentration (4 mg mL<sup>-1</sup>), followed by leaves (75.19%) and stems (83.47%). These results reinforce the idea that seeds are a robust source of antioxidants in *M. forsskaolii*. Leaves and stems also display antioxidant activity, though at slightly lower levels compared to seeds.

The presence of phenols, flavonoids, and tannins in different parts of *M. forsskaolii* aligns with its potential nutritional value, particularly in terms of antioxidant and bioactive compounds. These compounds are known for their ability to combat oxidative stress and inflammation in the human body, which are linked to various chronic diseases. Seeds, with their high phenol, flavonoid, and tannin contents, may offer substantial antioxidant capacity, making them a valuable addition to diets aimed at improving overall health. Leaves also contain significant levels of these compounds, suggesting their potential as a nutritional component. The differences in phenol, flavonoid, and tannin content and antioxidant capacity among plant parts emphasize the need for a holistic approach in utilizing the nutritional potential of *M. forsskaolii*, where



**Figure 1:** Antioxidant activity assessment of *Mesembryanthemum forsskaolii* Hochst. ex Bioss.: a comprehensive analysis of ABTS (a) and DPPH (b) scavenging activity in seeds, stems, and leaves.

different parts of the plant may serve various dietary and health-related purposes.

# 4 Discussion

In this study, we have comprehensively examined the nutritional composition, phytochemical content, and anti-oxidant potential of *Mesembryanthemum forsskaolii* Hochst. ex Bioss. Our findings not only contribute to the scientific understanding of this plant but also shed light on its potential as an alternative nutritional resource, particularly in regions where unconventional food sources can play a vital role in addressing nutritional challenges.

One of the primary aspects of our investigation focused on the nutritional composition of *M. forsskaolii*, including protein and carbohydrate content across different plant parts (seeds, stems, and leaves). These parameters are essential indicators of the nutritional value of a plant and its

potential role as a food source. Our results revealed significant variation in these nutritional components among different plant parts, with seeds, stems, and leaves showing distinct profiles. This variation is a critical consideration for optimizing the utilization of *M. forsskaolii* as a nutritional resource.

Comparing our findings with previous research (e.g., [3]) revealed that previous studies focused on the antioxidant potential of different *Mesembryanthemum* species; however, our study delves into a more comprehensive analysis of nutritional components. Our results suggest that *M. forsskaolii* contains appreciable levels of soluble and insoluble proteins, as well as carbohydrates, which are crucial for dietary diversity and nutrition.

Furthermore, we conducted an in-depth analysis of the phytochemical composition of *M. forsskaolii*, including phenols, flavonoids, tannins, and antioxidant activity (FRAP, TAC, ABTS, DPPH assays). Phytochemicals are known to contribute not only to the nutritional quality of plants but also to their potential health benefits. Our findings indicate that

Table 4: Phytochemical profile and antioxidant activity of different parts of Mesembryanthemum forsskaolii Hochst. ex Bioss

Plant part	Phenols (mg GAE g <sup>–1</sup> DW)*	Flavonoids (mg QE g <sup>-1</sup> DW)	Tannins (mg TAE g <sup>–1</sup> DW)	FRAP (mg AAE g <sup>-1</sup> DW)	TAC (mg AAE g <sup>–1</sup> DW)
Seeds	62.56 ± 0.99 <sup>a</sup>	53.57 ± 0.25 <sup>a</sup>	31.81 ± 0.29 <sup>a</sup>	89.48 ± 4.49 <sup>a</sup>	297.04 ± 6.35 <sup>a</sup>
Stems	$26.44 \pm 0.37^{c}$	10.35 ± 0.36 <sup>c</sup>	11.15 ± 0.19 <sup>c</sup>	17.88 ± 2.65°	183.92 ± 1.45 <sup>b</sup>
Leaves	39.13 ± 0.48 <sup>b</sup>	15.35 ± 0.11 <sup>b</sup>	17.26 ± 0.52 <sup>b</sup>	22.76 ± 3.31 <sup>b</sup>	87.64 ± 1.35 <sup>c</sup>

<sup>\*</sup>Values are shown as the mean value of five replicates ± SD. Values followed by different superscript letters are significantly different (p < 0.05).

M. forsskaolii is rich in these bioactive compounds, which are associated with antioxidant properties. This suggests that the plant may have a role beyond basic nutrition in promoting health and well-being. Comparing our phytochemical results with Falleh et al. [9], who studied Mesembryanthemum edule, we observe similarities in the presence of phenolic compounds. Falleh et al. [9] identified quercitrin and avicularin as the main phenolic compounds in M. edule leaves, while our analysis reveals the presence of phenolic compounds, including flavonoids and tannins, in various parts of M. forsskaolii. This similarity suggests that Mesembryanthemum species, including M. forsskaolii, may share common phytochemical attributes with potential health benefits.

The GC-MS analysis demonstrated various phytochemical compounds present in the extracts of M. forsskaolii. Leaves, seeds, and stems were particularly rich in these beneficial compounds which enhance the plant's nutritional and potential health benefits. Notably, the presence of Hexadecanoic acid, methyl ester, phytol, β-sitosterol, and phthalic acid in leaf extract suggests promising antioxidant, anti-inflammatory, and antimicrobial properties [21–26,28,29]. The seeds' extract contains compounds like phthalic acid which suggest potential antimicrobial and stress response capabilities [23,31]. Additionally, the seeds possess essential fatty acids, including 7-Hexadecenoic acid, that highlight their nutritional significance [32]. The stem extract highlights potential nutritional benefits, including Methyl β-D-mannofuranoside and n-Hexadecanoic acid [33-35]. Additionally, essential fatty acids such as 9-Octadecenoic acid and cis-Vaccenic acid contribute to the overall health-promoting qualities of the stems [21,36,37]. These findings align with the nutritional and phytochemical profiles discussed earlier, emphasizing the plant's potential as a diverse and alternative nutritional resource with many health benefits. The identified compounds play critical roles in antioxidant defense, antimicrobial activity, and overall nutritional quality.

In regions where access to diverse and nutrient-rich foods may be limited, the identification of alternative nutritional resources can be of paramount importance. *M. forsskaolii*, with its varied nutritional composition and phytochemical content, offers a potential solution to address nutritional challenges. The diversity of plant parts, each with its unique nutritional profile, provides an opportunity for local communities to incorporate this plant into their diets, potentially enhancing their nutritional intake. Nevertheless, it is essential to recognize that while our study lays the foundation for understanding the nutritional and phytochemical composition of *M. forsskaolii*, further

research is needed to elucidate the specific health benefits and culinary applications of this plant. Exploring traditional uses and conducting sensory evaluations can help guide its integration into local diets. Additionally, identifying the bioactive compounds responsible for the observed antioxidant potential can open doors to various applications in functional foods and nutraceuticals.

# 5 Conclusion

Our study highlights *M. forsskaolii* as a promising alternative nutritional resource due to the high protein and low carbohydrate contents found in its seeds, leaves, and stems. Furthermore, the plant contains significant amounts of macro- and micronutrients, particularly nitrogen and iron, which contribute to its nutritional benefits. The results indicate the strong antioxidant capacity of the different parts of *M. forsskaolii*, highlighting its potential as an antioxidant agent. By considering the nutritional and phytochemical richness of this plant, we can take steps towards addressing nutritional challenges in regions where such resources are needed. Further research and community engagement are essential to harness the full potential of *M. forsskaolii* in promoting both nutrition and health.

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# References

- Hartmann HE. Illustrated handbook of succulent plants. Aizoaceae FZ: Springer Science & Business Media; 2002.
- Tiwari R, Rana C. Plant secondary metabolites: a review. Int | Eng [2] Res Gen Sci. 2015;3(5):661-70.
- El-Amier YA, Alghanem SM, Al-hadithy ON, Fahmy AA, El-Zayat MM. Phytochemical analysis and biological activities of three wild Mesembryanthemum species growing in heterogeneous habitats. I Phytol. 2021:13:1-8. doi: 10.25081/jp.2021.v13.6403.
- He L, He T, Farrar S, Ji L, Liu T, Ma X. Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. Cell Physiol Biochem. 2017;44(2):532-53. doi: 10.1159/000485089.
- Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, et al. Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. Front Physiol. 2020;11:694. doi: 10.3389/fphys.2020.00694.
- Kalicharan B, Naidoo Y, van Staden J. Ethnopharmacology and biological activities of the Aizoaceae. J Ethnopharmacol. 2023;303:115988. doi: 10.1016/j.jep.2022.115988.
- Moawad A, Amin E, Mohammed R. Diffusion-ordered spectroscopy of flavonol mixture from Mesembryanthemum forsskaolii (Aizoaceae). Eur | Med Plants. 2016;16(1):1-8. doi: 10.9734/EJMP/ 2016/27794.
- Ibtissem B, Abdelly C, Sfar S. Antioxidant and antibacterial properties of Mesembryanthemum crystallinum and Carpobrotus edulis extracts. Adv Chem Eng Sci. 2012;2(3):359-65. doi: 10.4236/aces.
- 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 Crop Prod. 2011;34(1):1066-71. doi: 10.1016/j.indcrop.2011.03.018.
- [10] Lowry O, Rosebrough N, Farr AL, Randall R. Protein measurement with The folin phenol reagent. J Biol Chem. 1951;193(1):265-75. doi: 10.1016/S0021-9258(19)52451-6.
- [11] DuBois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem. 1956;28(3):350-6. doi: 10.1021/ac60111a017.
- [12] Estefan G, Sommer R, Ryan J. Methods of soil, plant, and water analysis: a manual for the West Asia and North Africa region. Beirut, Lebanon: International Center for Agricultural Research in the Dry Areas (ICARDA); 2013. p. 244.
- [13] Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nat Protoc. 2007;2(4):875-7. doi: 10.1038/ nprot.2007.102.
- [14] Ordoñez AAL, Gomez JD, Vattuone MA, Isla MI. Antioxidant activities of Sechium edule (Jacq.) Swartz extracts. Food Chem. 2006;97(3):452-8. doi: 10.1016/j.foodchem.2005.05.024.
- [15] Chandran KC, Indira G. Quantitative estimation of total phenolic, flavonoids, tannin and chlorophyll content of leaves of Strobilanthes kunthiana (Neelakurinji). J Med Plants Stud. 2016;4(4):282-6.
- [16] Sogi DS, Siddiq M, Greiby I, Dolan KD. Total phenolics, antioxidant activity, and functional properties of 'Tommy Atkins' mango peel

- and kernel as affected by drying methods. Food Chem. 2013;141(3):2649-55. doi: 10.1016/j.foodchem.2013.05.053.
- [17] Tang J, Dunshea FR, Suleria HAR. LC-ESI-QTOF/MS characterization of phenolic compounds from medicinal plants (Hops and Juniper Berries) and their antioxidant activity. Foods. 2020;9(1):7. doi: 10. 3390/foods9010007.
- Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem. 1999;269(2):337-41. doi: 10.1006/abio.
- [19] Zhao H, Fan W, Dong J, Lu J, Chen J, Shan L, et al. Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties. Food Chem. 2008;107(1):296-304. doi: 10.1016/j. foodchem 2007 08 018
- [20] Morris ER, Ellis R. Usefulness of the dietary phytic acid/zinc molar ratio as an index of zinc bioavailability to rats and humans. Biol Trace Elem Res. 1989;19(1-2):107-17. doi: 10.1007/ bf02925452.
- [21] Krishnamoorthy K, Subramaniam P. Phytochemical profiling of leaf, stem, and tuber parts of Solena amplexicaulis (Lam.) Gandhi using GC-MS. Int Sch Res Not. 2014;2014:567409. doi: 10.1155/2014/ 567409.
- [22] Shaaban MT, Ghaly MF, Fahmi SM. Antibacterial activities of hexadecanoic acid methyl ester and green-synthesized silver nanoparticles against multidrug-resistant bacteria. J Basic Microbiol. 2021;61(6):557-68. doi: 10.1002/jobm.202100061.
- [23] Al-Gara awi NI, Abu-Serag NA, Alee Shaheed KA, Bahadly ZKA. Analysis of bioactive phytochemical compound of (Cyperus alternifolius L.) by using gas chromatography-mass spectrometry. IOP Conf Ser: Mater Sci Eng. 2019;571(1):012047. doi: 10.1088/1757-899X/571/1/012047.
- [24] Santos CCdMP, Salvadori MS, Mota VG, Costa LM, de Almeida AAC, de Oliveira GAL, et al. Antinociceptive and antioxidant activities of phytol in vivo and in vitro models. Neurosci J. 2013;2013:949452. doi: 10.1155/2013/949452.
- [25] Elmazar MM, El-Abhar HS, Schaalan MF, Farag NA. Phytol/Phytanic acid and insulin resistance: potential role of phytanic acid proven by docking simulation and modulation of biochemical alterations. PLoS One. 2013;8(1):e45638. doi: 10.1371/journal.pone.0045638.
- [26] Silva RO, Sousa FBM, Damasceno SRB, Carvalho NS, Silva VG, Oliveira FRMA, et al. Phytol, a diterpene alcohol, inhibits the inflammatory response by reducing cytokine production and oxidative stress. Fundam Clin Pharmacol. 2014;28(4):455-64. doi: 10.1111/fcp.12049.
- [27] Kumar RS, Anburaj G, Subramanian A, Vasantha S, Selvam AP. Preliminary phytochemical investigation, Antimicrobial activity and GC-MS analysis of leaf extract of Capparis zeylanica Linn. J Pharmacogn Phytochem. 2019;8(1):1399-405.
- Al-Marzoqi AH, Hameed IH, Idan SA. Analysis of bioactive chemical components of two medicinal plants (Coriandrum sativum and Melia azedarach) leaves using gas chromatography-mass spectrometry (GC-MS). Afr J Biotechnol. 2015;14(40):2812-30. doi: 10.5897/ AJB2015.14956.
- Khan Z, Nath N, Rauf A, Emran TB, Mitra S, Islam F, et al. Multifunctional roles and pharmacological potential of  $\beta$ -sitosterol: Emerging evidence toward clinical applications. Chem-Biol Interact. 2022;365:110117. doi: 10.1016/j.cbi.2022.110117.
- Ingallina C, Capitani D, Mannina L, Carradori S, Locatelli M, Di Sotto A, et al. Phytochemical and biological characterization of

- Italian "sedano bianco di Sperlonga" Protected Geographical Indication celery ecotype: A multimethodological approach. Food Chem. 2020;309:125649. doi: 10.1016/j.foodchem.2019.125649.
- [31] Sivajothi V, Shruthi S. In vitro and in silico anti-diabetic activity of phthalic acid isolated from phyllanthus rheedii. Int J Res Ayurveda Pharm. 2013;4(6):889-92.
- [32] Reza ASMA, Hague MA, Sarker J, Nasrin MS, Rahman MM, Tareq AM, et al. Antiproliferative and antioxidant potentials of bioactive edible vegetable fraction of Achyranthes ferruginea Roxb. in cancer cell line. Food Sci Nutr. 2021;9(7):3777-805. doi: 10.1002/
- [33] Tyagi T, Agarwal M. Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of Pistia stratiotes L. and Eichhornia crassipes (Mart.) solms. J Pharmacogn Phytochem. 2017;6(1):195-206.

- [34] Ravi L, Krishnan K. Cytotoxic potential of *n*-hexadecanoic acid extracted from Kigelia pinnata leaves. Asian | Cell Biol. 2017;12(1):20-7. doi: 10.3923/ajcb.2017.20.27.
- [35] Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C, Haridas M. Anti-inflammatory property of *n*-hexadecanoic acid: Structural evidence and kinetic assessment. Chem Biol Drug Des. 2012;80(3):434-9. doi: 10.1111/j.1747-0285.2012.01418.x.
- [36] Belakhdar G, Benjouad A, Abdennebi E. Determination of some bioactive chemical constituents from Thesium humile Vahl. J Mater Environ Sci. 2015;6(10):2778-83.
- Hamazaki K, Suzuki N, Kitamura K-I, Hattori A, Nagasawa T, [37] Itomura M, et al. Is vaccenic acid (18:1t n-7) associated with an increased incidence of hip fracture? An explanation for the calcium paradox. Prostaglandins Leukot Essent Fat Acids. 2016;109:8-12. doi: 10.1016/j.plefa.2016.04.001.