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Balanites aegyptiaca (L.) delile defatted seed flour: A promising ingredient for antioxidant-rich functional foods

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Abstract
The study explores the use of agro-industrial by-products, specifically Desert date (*Balanites aegyptiaca*) seed cake, as a potential ingredient for pharmaceutical product. The defatted seed flour was found to be nutritionally rich, containing 24.5% protein and 18.3% dietary fiber. In addition, it exhibited strong antioxidant activity, demonstrated by DPPH radical scavenging (73.26%), ABTS capacity (42.18 µmol TE/g), and FRAP value (58.12 µmol Fe^{2+}/g). The flour also showed a low predicted glycemic index (48.35), indicating its potential for sustained energy release and suitability in health-oriented diets. These findings highlight Desert date flour as a sustainable and value-added ingredient for functional foods and pharmaceutical formulations, supporting both nutritional security and circular bioeconomy initiatives.

Keywords: *Balanites aegyptiaca*, oilseed by-product, high-protein flour, food fortification, bakery product, wild edible plants

Introduction

Food security and sustainability challenges have led to growing interest in agro-industrial by-product valorization [1]. Sustainable food systems ensure global food security and waste reduction by exploring alternative sources like industrial, by-products, without depleting the agricultural sector. [2]. The Food and Agriculture Organization (FAO) notes that a large portion of food is wasted, with the amount varying by product. Repurposing these materials not only allows for the extraction of extra nutrients or bioactive components but also lessens the environmental impact associated with their disposal [3]. One such underutilized resource is the residual oilseed cake from *Balanites aegyptiaca* (L.) Delile, a drought-resistant tree native to arid zones of Africa and South Asia, which is widely used in food and medicine [4, 5]. Various parts of the plant have gained attention for their nutritional and therapeutic properties [6, 7]. The seeds are rich in oil and traditionally used in ethnomedicine and agroforestry for medicinal and nutritional purposes [8, 9]. Desert date seeds are primarily known for their high oil content, but the defatted seed flour, often treated as waste after oil extraction, remains underexplored despite being rich in proteins, phenolics, and other bioactive compounds [10].

De-oiled seed cake produced after oil extraction is often discarded or used as animal feed, prompting increased exploration of underutilized plant materials with potential health benefits [11]. Antioxidants are essential in mitigating oxidative stress by neutralizing these reactive molecules. Consequently, identifying novel, plant-based sources of antioxidants has become a key area of nutritional and pharmaceutical research. Desert date seed flour, with its antioxidant potential and functional properties, is underexplored due to limited scientific literature. The study evaluates the antioxidant capacity and functional potential of Desert date seed flour, derived from residual seedcake after oil extraction.

Graphical Abstract

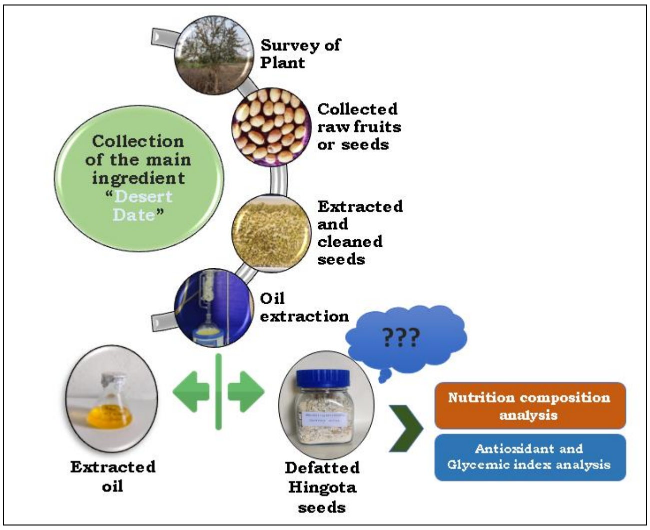

Fig 1. Integrated workflow for Desert date seeds valorization

Materials and Methods

1. Material Collection and Oil Extraction

Desert date fruits were collected, separated, cleaned, and soaked in boiling water. The kernels were dried, ground into a powder, and defatted using a Soxhlet apparatus for 3 hours, following AOAC's standard procedure [12]. After the extraction of oil, the hexane was removed from the defatted material using a rotary evaporator.

2. Proximate Composition Analysis

The nutritional composition of Desert date seed flour was analyzed using standard AOAC [12] methods, including estimation of moisture, crude protein, fat, ash, fiber, and total carbohydrate content.

2.1 Moisture Content

The moisture content of the sample was determined using the standard oven-drying method [12]. The sample, weighing 5g, was dried in a hot air oven for 4-6 hours, then cooled in a dessicator, and its moisture content was calculated using a formula.

Moisture (%) = (Initial weight –Final weight)/Sample weight×100

2.2 Crude Protein Content

The protein content of a sample was quantified using the Bradford assay [13], which involves homogenizing 150 mg of seed flour in chilled phosphate buffer (0.1 M, pH 7.5) and centrifuging it for 15 min at 10,000 g. The supernatant contains soluble proteins, estimated using Bradford reagent, and protein concentration calculated using absorbance values on a calibration curve, ensuring accuracy and reproducibility.

2.3 Crude Fat Content

The crude fat content of a sample was determined using the Soxhlet extraction method, following the AOAC's protocol [12]. The study involved extracting seed flour from a cellulose-sealed flask using petroleum ether, which was evaporated at 40-60 °C, leaving only residual oil. Crude fat content was calculated using a dry weight formula.

Crude Fat Content (%) = (Weight of extracted fat / Weight of dry sample) ×100.

2.4 Ash Content

Ash was estimated by incinerating the sample in a muffle furnace at 550–600 °C for 5–6 hours until a white/gray residue was obtained [12]. This residue represents the total mineral content.

Ash (%) = Weight of ash/Sample weight×100

2.5 Crude Fiber Content

The crude fiber content was estimated using the acid and alkali digestion method [12]. The sample underwent acid and alkaline digestion, was dried, weighed, and incinerated to determine its crude fiber content, which was then calculated using a formula.

Crude fiber (%) = (weight after digestion - weight after incineration)/Sample weight×100.

2.6 Carbohydrate Content

Total carbohydrate content was determined using the phenol-sulfuric acid colorimetric method, as described by Dubois *et al*. [14]. The 50 mg of sample was hydrolyzed with HCl, treated with 1 ml of 5 % phenol, and then 5 ml of concentrated sulfuric acid was added. The absorbance was measured at 490 nm, and carbohydrate content was quantified using a glucose standard curve and expressed as a percentage on a dry weight basis.

Carbohydrate (%) = {Concentration from standard curve (mg)/Sample weight (m g)} ×100

3. Antioxidant and Glycemic Index Assays

3.1 DPPH Radical Scavenging Activity

The antioxidant activity of Desert date seed flour was tested using (DPPH) assay, following by Hatano *et al*. [15] method. The reaction mixture consisted of acetic acid buffer (0.5 ml; pH 5.5), Desert date flour extract (1 mg/ml in ethanol), DPPH solution (1.0 ml; 0.2 mM) and 1.0 ml of 50% (v/v) aqueous ethanol. The absorbance was measured at 517 nm using a UV-Visible spectrophotometer after incubation at room temp. for 30 min in dark. The following formula wasused to determine the scavenging activity.

DPPH radical scavenging activity (%) = [(Ac − As) / Ac] × 100

(Ac: absorbance of the control (without extract); As: absorbance of the sample)

3.2 ABTS Radical Cation Scavenging Assay

The ABTS assay was conducted as per the method described by Re *et al*. [16], generating ABTS•⁺ radicals by mixing 7 mM ABTS with 2.45 mM potassium persulfate. The mixture was incubated in dark for 16 hours. Ethanol was added to the solution to dilute ABTS Solution until its absorbance at 734 nm was 0.70 ± 0.02, and then 100 µL of flour extract was added to the 3.9 ml ABTS working solution. Absorbance was recorded at 734 nm, and Trolox equivalents per gram of flour were expressed as µmol TE/g.

3.3 Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP activity was evaluated based on the method of Benzie and Strain [17]. The antioxidant potential based on the reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions under acidic conditions. The FRAP reagent is prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ in a 10:1:1 ratio. The 100 µL flour extract is mixed with the 3 ml FRAP reagent and incubated at 37°C for 4 minutes. The absorbance was measured at 593 nm. The results were expressed as:

FRAP activity = µmol Fe^{2+} equivalents per gram of dry flour

3.4 Superoxide Dismutase (SOD)-Like Activity

The superoxide radical scavenging activity of Desert date seed flour was evaluated based on the inhibition of nitroblue tetrazolium (NBT) reduction, as described by Jain *et al*. [18], with minor modifications. The reaction mixture, with a total volume of 750 µl, consisted of 2.5 µl of hydrolysate sample, 185 µl of phosphate buffer (0.1 M, pH 7.4), 187.5 µl of NADH solution (468 µM), 187.5 µl of NBT solution (150 µM), and 187.5 µl of PMS solution (60 µM). The mixture was incubated for 5 min and absorbance was measured at 560 nm using a spectrophotometer, and the scavenging activity was expressed as a percentage inhibition of superoxide radical formation relative to the control, calculated using the DPPH equation.

3.5 *In vitro* Glycemic Index Determination

The method of Goni [19] was followed to predict glycemic index (GI). 1 gm of samples was incubated with pepsin in acidic condition adjusted with HCl (pH 1.5) for 60 minutes at 37°C. Following neutralization, pancreatin and amyloglucosidase were used to digest the material. The glucose oxidase-peroxidase (GOD-POD) method was used to measure the amount of released glucose at intervals of 0, 20, 60, 90, and 120 minutes. The Hydrolysis Index (HI) was calculated by comparing the area under the hydrolysis curve (AUC) for the test sample with that of white bread (ref= 100). GI was calculated by this formula:

pGI = 39.71 + (0.549 × HI)

4. Statistical analysis

All experimental results are presented as mean values ± standard deviation (SD) derived from three independent replicates. The study used SPSS Statistics software (version 17.0; IBM Corp., Armonk, NY, USA) to analyze experimental results, determining significant differences among means using one-way ANOVA and Duncan's Multiple Range Test, with a confidence level of $P \leq 0.05$.

Results and Discussion

The findings confirm earlier reports on the rich protein, fiber, and mineral content of Desert date seed kernels [20, 21], establishing a scientific foundation.

1. Proximate Composition of Desert date Flour

The Desert date flour showed high protein content (24.5 ± 0.4%), considerable fiber (18.3 ± 0.2%), moderate fat (6.2 ± 0.3%), and ash content (5.7 ± 0.1%). Carbohydrates were 45.3 ± 1.0%. The nutrient composition highlights its suitability for fortifying cereal-based formulations. The high protein content of Desert date seed flour (24.5%), comparable to that of legumes such as soybean and chickpea. The fiber content, measured at 18.3%, stands out as significant, exceeding the usual levels found in wheat flour. These findings point to potential roles in enhancing digestive wellbeing, prolonging satiety, and modulating glycemic response [22]. The relatively low-fat content (6.2%) post-extraction aligns with expectations from cold-pressing techniques and enhances its compatibility with low-fat food formulations.

2. Antioxidant Activity of Desert date Seed Flour

2.1 DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of Desert date seed flour extract was found to be 73.26 ± 2.15% at a concentration of 1 mg/mL. The flour's high scavenging activity indicates the presence of potent antioxidant compounds like polyphenols, flavonoids, and saponins, which neutralize free radicals and reduce oxidative stress [23, 24]. Similarly, desert-adapted plants such as *Ziziphus mauritiana* and *Prosopis cineraria* have shown high radical scavenging abilities due to their adaptation to oxidative stress in arid environments [25, 26]. These findings support the potential of Desert date seed flour as a natural antioxidant-rich ingredient for functional food applications. Although the DPPH assay indicates high radical scavenging potential, *in vitro* methods cannot fully predict physiological antioxidant effects. Bioavailability, metabolism, and food matrix interactions influence *in vivo* outcomes [27].

2.2 ABTS Radical Cation Scavenging Activity

The ABTS assay revealed a scavenging capacity of 42.18 ± 1.87 µmol Trolox equivalents/g, further validating the antioxidant profile of Desert date seed flour. The ABTS assay measures the total electron-donating capacity of antioxidant compounds in a sample, providing a broader assessment than DPPH, which focuses on hydrogen atom transfer [16]. Studies on plant-based flours and seed extracts show similar trends like *Moringa oleifera* seed flour and *Sesamum indicum* seed extracts exhibited ABTS capacities of 35–50 µmol Trolox/g, influenced by phenolic and flavonoid content [28]. Desert date flour, a traditional plant-based flour, has antioxidant potential, supporting its role in preventing oxidative damage linked to chronic diseases like diabetes and cardiovascular conditions [29].

2.3 Ferric Reducing Antioxidant Power (FRAP)

The reducing capacity of Desert date seed flour measured by the FRAP assay was 58.12 ± 2.05 µmol Fe^{2+}/g, indicating its ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The FRAP assay is a widely accepted indicator of electron-donating capacity of antioxidants in food matrices, influenced by the concentration and redox potential of phenolic and flavonoid compounds [30, 31]. Similar FRAP values have been reported in legume-based and seed-derived flours such as chickpea, lentil, and *Moringa oleifera*, which are rich in polyphenols and non-nutrient antioxidants [32]. For example, lentil flour extracts demonstrated FRAP values in the range of 50–70 µmol Fe^{2+}/g depending on genotype and extraction method [33]. Desert date flour's high reducing power not only neutralizes oxidative damage in biological systems but also enhances oxidative stability in food products during storage [34, 35].

2.4 Superoxide Dismutase (SOD)-Like Activity

The SOD-like activity of Desert date seed flour was recorded at 6.84 ± 0.31 units/mg protein, suggesting the presence of bioactive compounds capable of mimicking endogenous enzymatic defense systems. The SOD assay assesses compounds' ability to inhibit superoxide anion generation, a key reactive oxygen species in oxidative stress-related damage, often linked to peptides, polyphenols, and saponins from plant seed proteins [36]. Legumes like soy, chickpea, and mungbean produce protein hydrolysates with similar SOD-like activities due to short-chain peptides and antioxidative amino acid residues [36, 37]. These components can function by directly scavenging superoxide radicals or by upregulating intrinsic antioxidant enzymes *in vivo* [38, 39]. Desert date seed flour's high SOD-like activity suggests potential anti-inflammatory and cytoprotective effects, making it a promising functional ingredient for oxidative stress-related dietary interventions, especially for chronic diseases [40].

2.5 *In vitro* Glycemic Index

The predicted glycemic index (GI) of Desert date seed flour-based cookie samples was found to be 48.35 ± 1.42, which falls within the low GI category (<55). The method of Goni [19] validates starch-rich food digestibility, indicating slower absorption of carbohydrates due to resistant starch, dietary fiber, and phenolic compounds, causing gradual blood glucose rise [41]. Similar effects have been observed in other legume- and seed-based flours rich in bioactive compounds that modulate carbohydrate metabolism [42, 43]. Dietary fiber can act as a physical barrier, limiting enzyme-substrate interactions, while phenolics may inhibit digestive enzymes such as α-amylase and α-glucosidase [44]. A low GI value is desirable for diabetic-friendly and weight-management food formulations, as it helps prevent postprandial hyperglycemia and promotes satiety [45, 46].

Table 1. Antioxidant activities and predicted glycemic index of the formulated cookies, with values expressed as mean ± SD and normalized to a 0–1 scale for comparative analysis.

Parameter	Result (Mean ± SD)	Unit	Min-Max Normalized (0–1)
DPPH	73.26 ± 2.15	% Inhibition	1.00 *(highest)*
ABTS	42.18 ± 1.87	µmol Trolox Eq/g	0.48
FRAP	58.12 ± 2.05	µmol Fe^{2+}/g	0.79
SOD-like activity	6.84 ± 0.31	Units/mg protein	0.00 (lowest)
Predicted Glycemic Index	48.35 ± 1.42	% (Low GI category)	0.25 (lower= better)

2.6 Comparative Significance

The antioxidant activity and low glycemic index values of Desert date seed flour (Table 1; Fig 2) suggest its suitability in developing functional foods with dual health benefits: oxidative stress reduction and blood glucose management [47, 48]. Compared to conventional cereal flours, Desert date flour demonstrates superior nutraceutical potential, [49,50].

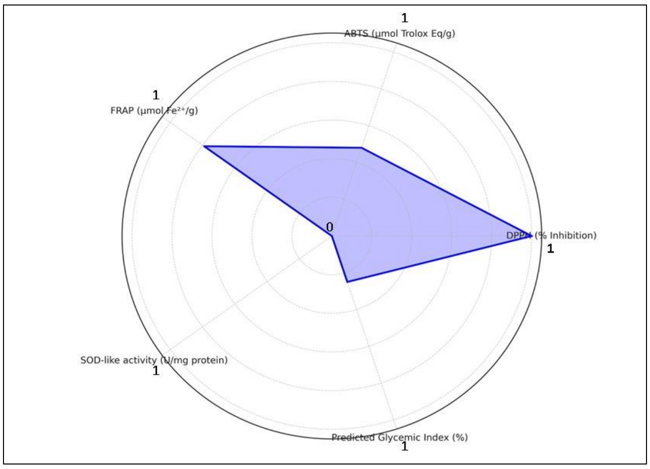

Fig 2. Antioxidant properities and Glycemic index of Desert date seed flour

Conclusion

This study highlights the potential of *Balanites aegyptiaca* (Desert date) seed cake flour, a by-product of oil extraction, as a valuable functional ingredient. Rich in protein, dietary fiber, and antioxidants, Desert date flour enhanced the nutritional and functional profile the valorization of Desert date seed flour not only contributes to waste reduction but also supports the development of health-promoting and diabetic-friendly applications. The findings support the integration of wild and underutilized crops into mainstream food systems, thereby addressing both nutritional and environmental sustainability.

Conflicts of interest: The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report.

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