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Nutritional Composition and In Vitro Antioxidant Activities of Seed Kernel and Seed Oil of *Balanites roxburghii*: An Underutilized Species

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Abstract: The seed kernel of *Balanites roxburghii* Planch., an underutilized species, yields a substantial amount of oil and has an impressive fatty acid profile. Both seed cake and seed oil have the potential to be rich nutritive sources. With this view, in the present study, nutritive profiling of seed kernel and seed oil including HPLC quantification of tocopherols and phytosterols has been done. Further, the total phenolic content and antioxidant activities of seed kernel and oil have also been analyzed. The seed kernel showed good proximate composition with 43.20% of oil and 10.96% of protein with a good amount of macro- and microelements. The seed oil possesses 5.36 mg/kg of carotenoids and 7.29, 1.79, and 0.72 mg/100 g of α, β + γ (together), and δ-tocopherols, respectively. The β-Sitosterol is the major phytosterol in the oil with 126.90 mg/100 g oil followed by stigmasterol and campesterol together (40.78 mg/100 g oil). It is also rich in squalene (17.45 mg/100 g oil). Oleic acid (56.38%), linoleic acid (18.77%), and palmitic acid (17.79%) were found to be the major fatty acids. Seed cake and seed oil possess significant antioxidant activities with 2.72 mg Gallic acid equivalent (GAE)/g and 8.90 mg GAE/100 g total phenolic content, respectively. Even though seed kernels contain some amount of anti-nutritional factors, they can be minimized by practicing appropriate food processing techniques. Considering all these facts, such as availability, good quality oil, proteins, and elemental composition, seeds of *B. roxburghii* could be considered a reliable food source to increase the food base of people and to get a variety of nutrients.

Keywords: edible oil source; carotenoids; tocopherols; phytosterols; nutritional elements

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1. Introduction

Population growth has created many threats including hunger, malnutrition, and poor health for humans. According to the FAO [1], the number of people suffering from acute food insecurity and in need of urgent assistance increased to 193 million in 2021 among the 53 countries studied. It also reported weather extremes, such as severe droughts and floods, as one of the major drivers of the food crisis. Among the 30,000 species of known edible plants, only four species—rice, wheat, maize, and potato account for 60% of the total human energy supply [2]. Lack of species diversity, as well as genetic diversity within the species of these few crops, make them more vulnerable to pests and diseases, abiotic stress, and extreme weather conditions, which in turn make food security very problematic [2].

Underutilized species that are wild or partially domesticated and adapted to the regional environmental conditions could be effectively utilized to combat poverty, malnutrition, and hunger [2,3]. Feeding the ever-growing population with healthy and nutritious food, while ensuring environmental protection could be achieved by diversifying the agriculture and food systems with underutilized species. *Balanites roxburghii* Planch. (family Zygophyllaceae) is one such underutilized species having numerous health and nutritional

benefits (Figure 1A). As an Indian native plant, it grows wildly in dry and arid regions, throughout India [4,5]. Fruits, seeds, stem bark, and roots are reported to be very useful in curing varied ailments [5]. In many parts of Southern India, leaves are used as a vegetable during the food scarcity period. Seed kernels possess a high amount of oil, i.e., 40–42% [6], which has the potential to be used for various applications including edible purposes. The seed oil is rich in unsaturated fatty acids such as oleic acid (32.31%), linoleic acid (28.61%), and linolenic acid (2.84%) [6]. Both seed kernel and oil of its allied species, *B. aegyptiaca*, are reported to be edible in various parts of Africa as a famine food during food scarcity times because of their high oil and protein content [7]. The seed kernel of *B. aegyptiaca* possesses an impressive nutritive profile with 32.40% of proteins, and 49% of oil which in turn has 70.91% of unsaturated fatty acids and a high amount of nutritional elements [8]. In the same way, there is a scope to develop *Balanites roxburghii* seed kernel as an additional food source to provide nutritional supplements for local people, as this plant also grows plentifully in low rainfall areas. Further, if we could bring this species into cultivation, we can probably get the best edible oil source as it possesses a high amount of oil and the left-over seed cake as animal feed.

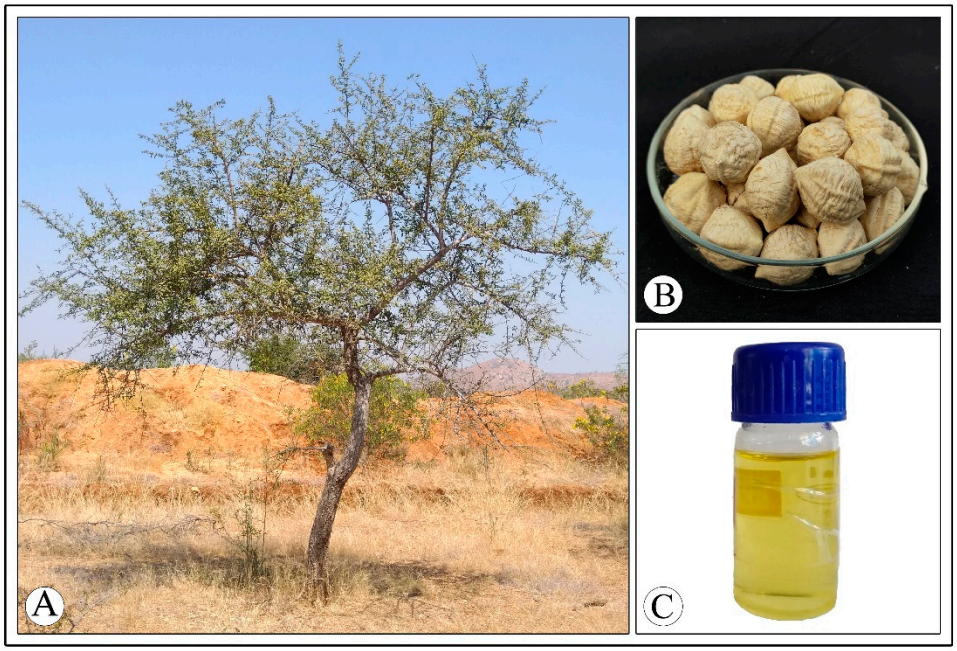

Figure 1. (**A**) Habit of *Balanites roxburghii*; (**B**) Seed kernel; (**C**) Seed oil.

However, information regarding the nutritional composition of seed kernel and seed oil of *B. roxburghii* is lacking. Therefore, in the present study, the nutritional composition of *B. roxburghii* including proximate analysis, the composition of nutritional elements, physicochemical properties, and fatty acid profiling of seed oil have been analyzed. Further, anti-nutritional components, phytochemical composition, and antioxidant capacities of seed cake and oil have been studied and its suitability as a nutritive material has also been discussed.

2. Materials and Methods

2.1. Plant Material

Fruits of *Balanitis roxburghii* were collected from Moka reserved forest, Ballari district, Karnataka, India (15°15′11.5″ N 77°04′16.9″ E). Species were identified by using *The Flora of the presidency of Bombay* by Cooke [9] and a monograph by Sands [4]. The specimen was deposited at Herbarium, Department of Botany, Karnatak University, Dharwad, India. Fruits were collected at their ripened stage indicated by their complete yellow color during February 2021. Twenty matured plants were selected randomly and 10–15 fruits

were collected from each plant, pooled together, and selected for analysis. Epicarp and mesocarp were removed and hard endocarp was broken mechanically to get the seed kernel (Figure 1B); they were then ground to make a fine powder, transferred to an air-tight polythene cover, and stored at 4 °C until further used.

2.2. Chemicals and Reagents

Chemicals used in the present study such as bovine serum albumin, anthrone reagent, sodium phytate, tannic acid, FC (Folin–Ciocalteu) reagent, gallic acid, DPPH (2,2-diphenyl-1-picrylhydrazyl), sodium phosphate, ammonium molybdate, and ascorbic acid were purchased from Himedia laboratories, Mumbai, India whereas BF_3-methanol, heptadecanoic acid, FD (Folin–Denis) reagent, TPTZ (2,4,6-tris(2-pyridyl)-s-triazine), ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)], Trolox, and tocopherols, phytosterols and squalene standard compounds were purchased from Sigma-Aldrich, Bengaluru, India. All other solvents and chemicals were of analytical grade.

2.3. Proximate Analysis

Moisture content was determined gravimetrically as mentioned in AOAC method 930.15, by drying the fresh seed kernels at 135 °C in an oven for 4 h [10]. Oil content was analyzed gravimetrically by extracting seed kernels with petroleum ether in a Soxhlet apparatus at 65 ± 2 °C for 8 h. Petroleum ether fraction was evaporated using a rotary evaporator, and oil was collected and stored at −20 °C until further analysis (Figure 1C). The seed cake obtained after extracting the oil was used for all further analysis. Proteins were estimated by modifying Lowry's method as described by Hartree [11] using BSA as standard. Carbohydrates were quantified by the Anthrone reagent method as described by Sadashivam and Manickam [12]. Ash content was determined according to AOAC method 942.05 by igniting 0.5 g of seed cake in a crucible at 600 °C for 8 h in a muffle furnace and the ash remaining was expressed as g/100 g FW [10]. Fiber content was determined according to the AOAC method 978.10 by digesting the sample with 1.25% of H_2SO_4 and 1.25% of NaOH [10]. Energy values were calculated using Atwater-specific factors calculated for legumes and nuts [13].

2.4. Elemental Composition Analysis

Analysis of phosphorus, potassium, sulphur, sodium, calcium, magnesium, boron, zinc, iron, manganese, and copper was carried out on NOVA 400 atomic absorption spectrophotometer (model Analytic Jena AG, Jena, Germany) with an air or acetylene flame and absorbance was carried out by using respective hollow-cathode lamps [14,15]. Further, nitrogen was estimated according to Liu et al. [16] by using the two-step digestion-UV spectrophotometric method. To be brief, 100 mg of seed cake was added with 100 µL of distilled water, and the tube was heated in an oil bath at 150 °C for 5 min followed by the addition of sulphuric acid and hydrogen peroxide (30%). The temperature of digestion was increased to 360 °C and kept constant for 40 min and then decreased to 150 °C and kept constant for 15 min. After reaching room temperature the solution was made up to 50 mL, and 5 mL aliquot of the diluted sample was taken separately and added to 5 mL of NaOH-potassium persulfate solution prepared by adding 1.5 g each of NaOH and potassium persulfate into 100 mL of distilled water. Tubes were again digested in an autoclave at 125 °C for 30 min. After cooling to room temperature, tubes were added with 1 mL of 3.7% HCl and absorbance of the solutions was taken at 220 and 275 nm using a UV-Vis spectrophotometer.

2.5. Seed Oil Characterization

2.5.1. Physicochemical Characterization

The extracted oil was kept at room temperature for 4 h and observed for its color and state. Density and refractive index were determined by using a specific gravity bottle and Abbe refractometer, respectively [14]. Free fatty acid content, peroxide value, iodine

value, and unsaponification value were assessed according to AOCS [17]. Carotenoids were quantified by diluting 100 mg of oil in 1 mL of acetone and reading the absorbance at 446 nm using a UV-Vis spectrophotometer. A diffusion coefficient of 383 was used to calculate the carotenoid content and expressed as mg/kg of oil [18]. Tocopherols and phytosterols were quantified by using an HPLC system (Nexera X-2 LC-30A, Shimadzu Corporation, Kyoto, Japan) connected with a reverse-phase C18 column (Chromasol, 250 × 4.6 mm, 5 µm) as reported by Rogers et al. [19] and Sánchez-Machado et al. [20], respectively. For tocopherols, 15 mg of oil was diluted with hexane and filtered through a 0.22 µm syringe filter and injected into the HPLC system. An isocratic solution containing methanol and water, at a ratio of 95:5, was used as a mobile phase with a 1.5 mL/min flow rate. An RF 20A fluorescence detector was used to detect the compounds. For phytosterols and squalene, 10 mg of the unsaponifiable matter was dissolved in acetone and filtered through a 0.22 µm syringe filter and injected into the HPLC system. An isocratic solution of methanol and acetonitrile (30:70) was the mobile phase with a flow rate of 1.2 mL/min and a column temperature of 30 ± 0.1 °C was set. A PDA detector was used to detect the compounds at 205 nm.

2.5.2. Fatty Acid Profiling

Initially, the fatty acids were converted into fatty acid methyl esters (FAME) according to AOCS [17]. An amount of 15 mg of oil was added with 1 mL of BF_3-methanol and incubated for 30 min at 60 °C, before the tube was instantly transferred to an ice bath and kept there for 5 min. Then 1 mL of hexane was added to the solution followed by 1 mL of distilled water and then vortexed. Lastly, the undisturbed top layer was taken and injected into a GC-MS system (PerkinElmer, Turbo-mass Gold, Mass spectrometer) equipped with a flame ionization detector and fused silica Rtx-2330 column (Restek, 30 m 90.32 mm ID and 0.20 mm film thickness). The injector port and detector temperature were set at 230 and 250 °C, respectively, with N_2 as the carrier gas. The initial column temperature was 120 °C, further increased to 220 °C over 20 min and held for 10 min. FAME was detected by comparing the fragmentation pattern and retention time with that of standards and the NIST library. Heptadecanoic acid was used as an internal standard.

2.6. Determination of Anti-Nutritional Factors

Phytate content was determined according to the method described by Gao et al. [21]. Briefly, 0.5 g of seed cake was extracted with 10 mL of 2.4% HCl and kept for 16 h with constant shaking; the solution was then filtered, followed by the addition of 1 g NaCl to filtrate, and kept for shaking for 20 min followed by centrifuging at 1000 g for 20 min at 10 °C when supernatant was collected. The known volume of this solution was taken and diluted to 3 mL using distilled water followed by the addition of 1 mL of Wade reagent (0.03% $FeCl_3 \cdot 6H_2O$ + 0.3% sulfosalicylic acid). The absorbance of the color was read at 500 nm with a UV-Vis spectrophotometer. A control was prepared without the addition of a sample. Sodium phytate was used as standard.

Oxalate content was determined according to the method of Dye [22]. In brief, 2 g of seed cake was heated in a water bath at 90 °C with 190 mL distilled water and 10 mL of 6 N HCl for 4 h. The solution was filtered, made up to 250 mL, and 50 mL aliquot of this solution was titrated with concentrated ammonia using methyl orange indicator and heated to 95 °C followed by the addition of 10 mL of 5% $CaCl_2$. After 10 min, 6 N NH_4OH was added until the color changed, and it was kept overnight to precipitate calcium oxalate. The precipitate was filtered and dissolved in hot sulfuric acid, the filtrate was made up to 125 mL, heated to 95 °C, and titrated against 0.05 N $KMnO_4$. Oxalate was determined using the following equation:

$$\text{Oxalate } (\%) = \frac{(\text{mL } KMnO_4)(0.05)(45.02)(100)(5)}{(1000)(\text{Wt of sample})}$$

Tannin content was determined as described by Bajaj and Devsharma [23] with some modifications. Briefly, seed cake was extracted with 70% methanol containing 0.1% HCl and the known volume of extract was taken in a test tube followed by making up the volume to 3 mL with distilled water followed by the addition of 0.25 mL of FD (Folin–Denis) reagent. Then 0.5 mL of 30% Na_2CO_3 was added and after incubation for 30 min at room temperature the color developed was measured at 700 nm. Tannic acid was used as a standard compound.

2.7. Total Phenolic Content Analysis

Both seed cake and oil were extracted with 70% methanol containing 0.1% HCl and extract was used for the analysis of total phenolic content and antioxidant activities. Total phenolic content was estimated by using FC (Folin–Ciocalteu) reagent method as described in Murthy et al. [24], with slight modification. Briefly, a known amount of sample was taken and made up to 3 mL with distilled water. Then 0.1 mL of 2 N FC reagent was added followed by incubation for 6 min and the addition of 0.5 mL of 20% Na_2CO_3 to each tube. Tubes were kept in warm water for 30 min and the absorbance of the color developed was read at 760 nm using a UV-Vis spectrophotometer. Gallic acid was used as the standard compound.

2.8. Antioxidant Activities

2.8.1. DPPH Radical Scavenging Activity

DPPH radical scavenging activity was determined according to Manasa et al. [25] with some modifications. A known volume of extract was taken and diluted to 0.5 mL. Solutions were added with 3 mL of freshly prepared 0.1 mM DPPH in methanol and kept in the dark for 30 min. Absorbances were read at 517 nm using a UV-Vis spectrophotometer. Percentage inhibition activity was calculated and compared with the gallic acid standard.

2.8.2. Total Antioxidant Activity (TAA)

The total antioxidant activity of the samples was determined by the phosphomolybdenum method [26]. A reagent containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate was prepared. A known volume of extract was taken in different tubes, made up to 0.5 mL using distilled water, and added with 3 mL of reagent followed by incubation at 95 °C for 90 min. The absorbance of the color developed was measured at 695 nm. Ascorbic acid was used as the standard.

2.8.3. Ferric Reducing Antioxidant Power (FRAP)

FRAP activity of the samples was analyzed according to the method developed by Benzie and Strain [27]. FRAP reagent was prepared by mixing 300 mM acetate buffer of pH 3.6, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ in the ratio of 10:1:1. A known volume of extract was taken in different tubes, made up to 0.5 mL using distilled water and added with 3 mL of reagent followed by incubation at room temperature for 6 min. The absorbance of the color developed was read at 593 nm. Trolox was used as the standard.

2.8.4. ABTS

ABTS activity of the samples was analyzed according to the method developed by Re et al. [28]. ABTS free radical stock solution was prepared by mixing 7 mM ABTS with 2.45 mM potassium persulfate and allowing the mixture to incubate for 16 h before use. ABTS working solution was prepared by diluting the stock solution with methanol until the absorbance of the diluted solution reaches 0.7 ± 0.02 at 734 nm. A known volume of extract was taken in different tubes, made up to 0.5 mL using methanol, and added with 3 mL of reagent followed by incubation at room temperature for 6 min. Absorbances of the decolorized solutions were read at 734 nm. Trolox was used as the standard.

2.9. Statistical Analysis

Descriptive statistics (mean, standard deviation, and standard error) were calculated using Microsoft Excel 2019, and results were presented as the mean ± standard error of three replicates.

3. Results and Discussion

3.1. Proximate Composition

Proximate analysis is the estimation of major essential nutrients such as protein, carbohydrate, fat, ash, and crude fiber; they provide most of the calories needed by the body. The proximate composition of *Balanites roxburghii* seed kernels revealed it as the finest source of oil along with protein and fiber (Table 1). *B. roxburghii* seed kernel consists of 43.20% of oil, which is more than that of well-known edible oil seeds such as soybean (18.3%), rapeseed (38.6%), sunflower (40.9%), and ground nut (4.03%) [29]. Protein and fiber make the seed kernels more valuable by constituting 10.96 and 15.12% of the total weight, respectively. The moisture, carbohydrate, and ash values were found to be 12.87%, 5.74%, and 4.60%, respectively.

Table 1. Proximate composition of *Balanites roxburghii* seed kernel.

Component	% Composition
Moisture	12.87 ± 1.23
Fat	43.20 ± 0.46
Protein	10.96 ± 0.09
Carbohydrate	5.74 ± 0.03
Ash	4.60 ± 0.12
Fiber	15.12 ± 1.21
Energy (Kcal/100 g)	422.81

Each value represents the mean ± standard error of three replicates.

Oil and protein components of seed kernels of *B. aegyptiaca*, an allied species of *B. roxburghii*, are found to be 49.0% and 32.4%, respectively [30]. With all these primary nutrients, *B. roxburghii* seed kernel has an energy value of 422.81 Kcal/100 g, which is comparable with that of *B. aegyptiaca* seed kernel which has the energy value of 605.40 Kcal/100 g [8] and higher than that of some major pulses such as cowpea (320.57 Kcal/100 g), green gram (326.08 Kcal/100 g), red gram (331.1 Kcal/100 g), and soybean (381.82 Kcal/100 g) [31]. Because of its good quality oil and more protein, *B. aegyptiaca* seed is considered a useful edible product in many parts of Africa during dry seasons and drought periods [7]. *B. roxburghii* is reported to yield 8.5 tons of seeds per acre [32], and hence it could be an efficient source of edible oil.

3.2. Elemental Composition Analysis

Nutritional elements are essential to the proper functioning of the body as they are involved in various physiological and biochemical activities. Though they are required in small amounts, they must be present in a regular diet because failing with this may cause severe health problems [33]. Ash is the inorganic residue that remained after the complete combustion of organic matter and represents the total elemental content. A good ash content (4.60%) portrays *B. roxburghii* seed cake as a better source of nutritional elements, which in turn is proved by individual elemental analysis (Table 2).

Table 2. Elemental composition of *Balanites roxburghii* seed cake.

Element	Composition
Macroelements (mg/g)	
Nitrogen	28.40
Phosphorous	7.62
Potassium	14.00
Sulphur	5.60
Sodium	0.32
Calcium	6.05
Magnesium	6.00
Microelements (µg/g)	
Boron	28.96
Zinc	431.00
Iron	1419.00
Manganese	44.50
Copper	36.80

Nitrogen (28.40 mg/g) is the most abundant element in the seed cake of *B. roxburghii,* followed by potassium (14 mg/g) and phosphorous (7.62 mg/g). Calcium, magnesium, and sodium content are found to be 6.05, 6, and 0.32 mg/g, respectively. Seed cake is also rich in microelements such as iron (1419 µg/g), zinc (431 µg/g), manganese (44.50 µg/g), copper (36.80 µg/g), and boron (28.96 µg/g). These values are very high when compared with the two most important oil seed crops, ground nut and soybean, which have an iron content of 34.4 and 82.9 µg/g, zinc content of 31.8 and 40.1 µg/g, manganese content of 16.2 and 26.9 µg/g, copper content of 9.2 and 12.9 µg/g, calcium content of 0.54 and 2.39 mg/g and magnesium content of 1.97 and 2.59 mg/g, respectively [31].

3.3. Physicochemical Properties of Oil

Fats and oils are an essential part of the human diet as they are the major dietary sources of fat-soluble nutraceuticals such as carotenoids, tocopherols, phytosterols, and lignans along with essential fatty acids. Hence, different physical and chemical parameters are used to assess the quality and nutraceutical richness of the oil [34]. The physicochemical properties of *B. roxburghii* seed oil is presented in Table 3. *B. roxburghii* seed oil is yellowish in color and liquid at room temperature with a density of 0.915 g/cm^3 and a refractive index of 1.33. It has low free fatty acid content and peroxide value, which are found to be 0.31% and 69.98 meq O_2/kg, respectively. The iodine value indicates the unsaturation level of the oil which in turn is related to fatty acid composition. The iodine value of *B. roxburghii* is found to be 98.88 I_2/100 g. The unsaponifiable matter is a non-glyceridic part and is a mixture of various components such as phytosterols, pigments, and fat-soluble vitamins and hence the indicator of oil-based nutraceuticals [35]. *B. roxburghii* seed oil is rich in unsaponifiable matter with a value of 1.93%, and also with a good amount of other nutraceuticals. Carotenoids are triterpenoid pigments that have a beneficial role in preventing eye-related problems. *B. roxburghii* seed oil contains 5.36 mg/kg of carotenoid content, which is higher than that of sunflower oil, a popular edible oil that had a carotenoid content of 1.1–1.6 mg/kg oil [36]. Tocopherols are fat-soluble natural antioxidants and are commonly called vitamin E; α-Tocopherol (7.29 mg/100 g) is the predominant type in *B. roxburghii* seed oil followed by β and γ-tocopherols together (1.79 mg/100 g) and δ-tocopherol (0.72 mg/100 g) (Table 3; Figure 2A,B). Similarly, *B. roxburghii* seed oil is also rich in phytosterols such as ergosterol, stigmasterol, campesterol, and β-sitosterol. The β-sitosterol is the prominent type with an amount of 126.90 mg/100 g followed by 40.78 mg/100 g of stigmasterol and campesterol together and 5.16 mg/100 g of ergosterol (Table 3; Figure 2C,D). The amount of squalene is found to be 17.45 mg/100 g, which is the precursor molecule in the synthesis of steroids and steroidal hormones. The well-known edible oils such as olive oil and coconut oil are reported to contain 5.9 and 9.3 mg/100 g of campesterol, 2.7 and 12 mg/100 g of stigmasterol, and 133 and 45 mg/100 g of β-

sitosterol, respectively; *B. roxburghii* is found to be rich in all these phytosterols when compared with olive and coconut oil [37]. Similarly, ergosterol, the precursor of vitamin-D_2, is higher in *B. roxburghii* seed oil than in sunflower oil (0.79–1.74 mg/100 g), avocado oil (0.42–2.34 mg/100 g), and wheat grain oil (2.21–3.42 mg/100 g) [38]. With all these facts, *B. roxburghii* seed oil could be considered for inclusion in the diet to avail its rich nutraceutical components.

Table 3. Physicochemical characteristics of *Balanites roxburghii* seed oil.

Parameter	Values
Color	Yellow
State at room temperature	Liquid
Density (g/cm^3)	0.915
Refractive index	1.33
Free fatty acid content (%)	0.31 ± 0.01
Peroxide value (meq O_2/kg)	69.98 ± 5.18
Iodine value (I_2/100 g)	81.19 ± 0.72
Unsaponification value (%)	1.93 ± 0.14
Carotenoids (mg/kg)	5.36 ± 0.39
Tocopherols (mg/100 g)	
α	7.29 ± 0.03
β + γ	1.79 ± 0.01
δ	0.72 ± 0.01
Phytosterols (mg/100 g)	
Ergosterol	5.16 ± 0.53
Stigmasterol + campesterol	40.78 ± 1.60
β-Sitosterol	126.90 ± 1.08
Squalene	17.45 ± 0.03

Each value represents the mean ± standard error of three replicates.

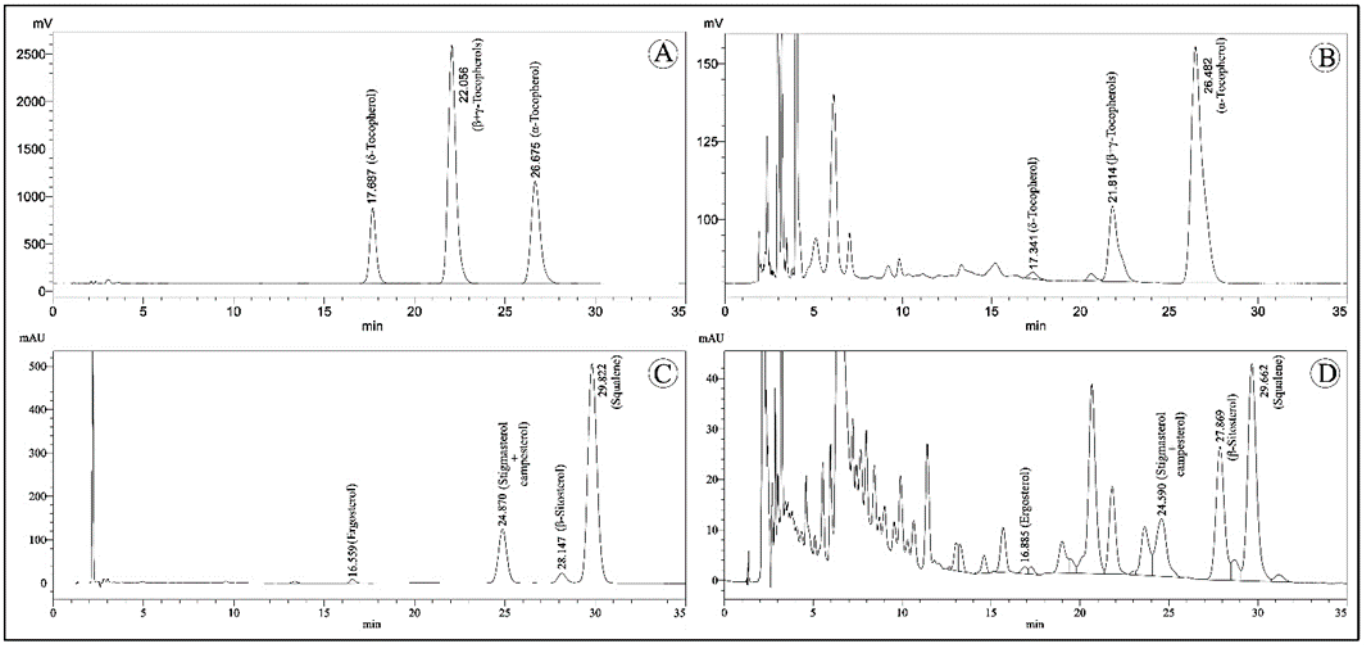

Figure 2. HPLC chromatograms of tocopherol and phytosterol analysis—(**A**) Tocopherol standards; (**B**) Tocopherols in seed oil; (**C**) Phytosterol standards; (**D**) Phytosterols in seed oil.

3.4. Fatty Acid Composition of Oil

The physicochemical characteristics of oil such as viscosity, density, iodine value, and its physical state at room temperature are dependent on the fatty acid composition which in turn depends on the climate, soil type, genotype, and maturity of the sample [39].

Polyunsaturated fatty acids, or essential fatty acids such as α-linolenic acid and linoleic acid, must be supplied through the diet and the seed oils are the major source of such essential fatty acids. Evaluation of *B. roxburghii* seed oil revealed its good fatty acid composition which comprises 56.38% of monounsaturated fatty acids, 19.38% of polyunsaturated fatty acids, and 24.1% of saturated fatty acids (Table 4). Oleic acid is the major fatty acid which constitutes 56.38% of the oil followed by linoleic acid (18.77%), palmitic acid (17.79%), and stearic acid (6.31%). Linolenic acid and 1,19,17-docosatriene are present in small quantities and constitute 0.38 and 0.23% of the oil, respectively (Table 4). Arora and Tak [6] analyzed the fatty acid composition of *B. roxburghii* seed oil collected from the Jodhpur region of Rajasthan state, India, and they reported 32.31% of linoleic acid, 28.61% of oleic acid, 17.96% of palmitic acid, 11.45% of stearic acid and 2.84% of linolenic acid. The basic fatty acid pattern is same in both studies but the variation in the composition might be due to the geographic or genotypic effect or both. The linoleic acid and unsaturated fatty acid composition of *B. roxburghii* seed oil is comparable to common edible oils such as soybean, cottonseed, rapeseed, groundnut, and palm oil [40] and thus, considering its high oil content, rich nutraceuticals, and appreciable fatty acid composition, it could be exploited as a new edible oil source.

Table 4. Fatty acid composition of *Balanites roxburghii* seed oil.

Fatty Acid	% Composition
Palmitic acid (16:0)	17.79 ± 0.15
Stearic acid (18:0)	6.31 ± 0.01
Oleic acid (18:1)	56.38 ± 0.28
Linoleic acid (18:2)	18.77 ± 0.44
Linolenic acid (18:3)	0.38 ± 0.03
1,9,17-Docosatriene (22:3)	0.23 ± 0.03
Total saturated fatty acids (SFA)	24.10
Total monounsaturated fatty acids (MUSFA)	56.38
Total polyunsaturated fatty acids (PUSFA)	19.38

Each value represents the mean ± standard error of three replicates.

3.5. Anti-Nutritional Components

Anti-nutritional components are those which make the bioavailability of nutrients very difficult. Phytate and oxalate are both considered as major anti-nutritional factors as they hinder the bioavailability of nutritional elements by binding with them. Tannins are polyphenolic compounds, and they decrease protein digestibility by inhibiting digestive enzymes [41]. Phytate, oxalate, and tannin content of *B. roxburghii* seed kernel were found to be 21.71, 32.01, and 1.99 mg/g, respectively (Table 5).

Table 5. Anti-nutritional factors of *Balanites roxburghii* seed kernel.

Factor	Composition
Phytate (mg/g)	21.71 ± 1.87
Oxalate (mg/g)	32.01 ± 1.28
Tannins (mg TAE/g)	1.99 ± 0.02

Each value represents the mean ± standard error of three replicates.

Plant stores 50–85% of phosphorus in the form of phytate whereas tannins are produced as a defense in seeds; hence, along with the good nutritional profile, pulses also contain some amount of phytic acid [42]. Shi et al. [43] reported 12.4 mg/g, 17.1 mg/g, 22.85 mg/g, 14 mg/g, 18.82 mg/g, and 22.91 mg/g of phytic acid in pea, lentil, fava bean, chickpea, bean, and soybean, respectively. Similarly, soybean and peanut contain 1.93 and 8.9 mg/g of tannins, respectively [42]. However, the oxalic acid content is quite high in *B. roxburghii* seed kernels as compared with various pulses [43]. Different cooking processes such as milling, roasting, and soaking significantly reduced the phytate, tannin, and oxalate

content in different legume species [42–44]. Thus, different food processing strategies can be followed to minimize the anti-nutritional compounds from the seed kernels of *B. roxburghii*.

3.6. Total Phenolic Content (TPC) and Antioxidant Activities of Seed Cake and Seed Oil

Phenolic compounds are the most diverse plant secondary metabolites which perform a very broad range of physiological roles including the prevention of oxidative stress and its associated problems [45]. The total phenolic content (TPC) of *B. roxburghii* seed cake is found to be 2.72 mg GAE/g, whereas in oil it is found to be 8.88 mg GAE/100 g (Table 6). TPC of seed cake is high when compared with that of some pulses such as chickpea (0.98 mg GAE/g), cowpea (1.07 mg GAE/g), and pigeon pea (0.79–1.21 mg GAE/g) and comparable to that of lentil (4.9–7.8 mg GAE/g) [46]. Generally, seed oil will not contain polar compounds such as phenolics, but during the extraction process, a portion of seed phenolics gets transferred into oils, which provides various health benefits. Edible oils such as palm, ground nut, and sunflower oils are reported to contain 3.2, 3.2, and 1.5 mg GAE/100 g of phenolic compounds [47]. The present study suggests that *B. roxburghii* seed oil and cake are rich in polar nutrients such as phenolics and it is reflected in their antioxidant activities; those compounds can assist the diet with their beneficial roles.

Table 6. Total phenolic content and antioxidant activities of seed cake and seed oil of *Balanites roxburghii*.

Activity	Seed Cake (for 1 g)	Seed Oil (for 100 g)
Total phenolic content (mg GAE/g)	2.72 ± 0.03	8.90 ± 0.45
Antioxidant activities		
DPPH (mg GAE)	0.65 ± 0.03	2.80 ± 0.07
TAA (mg AAE)	20.27 ± 1.15	2.63 ± 0.07
FRAP (mM TE)	117.9 ± 8.2	13.09 ± 1.56
ABTS (mM TE)	178.7 ± 9.2	32.85 ± 0.79

Each value represents the mean ± standard error of three replicates. GAE—Gallic acid equivalent; AAE—Ascorbic acid equivalent; TE—Trolox equivalent.

Oxidative stress causes various diseases such as cancers, autoimmune disorders, Alzheimer's disease, and Parkinson's disease, but these diseases can be prevented by the intake of an adequate amount of antioxidants [48]. Thus, the present work attempted to assess a wide range of antioxidant potential of seed cake and seed oil with four different in vitro methods (Table 6). In all the methods, seed cake showed higher activity than oil, as evidenced by its total phenolic content. DPPH activity of seed cake is found to be 0.65 mg GAE/g whereas TAA, FRAP, and ABTS are found to be 20.27 mg AAE/g, 117 mM TE/g, and 178.7 mM TE/g, respectively (Table 6). Similarly, the DPPH activity of seed oil is found to be 2.80 mg GAE/100 g, whereas TAA is 2.63 mg GAE/100 g, FRAP activity is 13.09 mM TE/100 g, and ABTS activity is 32.85 mM TE/100 g. Wang et al. [49] studied the antioxidant activity of chickpea, cow gram, and soybean by using FRAP activity and they reported 2.41, 5.87, and 4.33 μM TE/g of antioxidant activity. Similarly, the seed oil is also having good antioxidant activity like commercial edible oils such as extra virgin olive oil (7.5 mg GAE/100 g) and corn oil (2.82 mg/100 g), as studied by Christodouleas et al. [50] using DPPH radical scavenging activity. The results of the present study demonstrate that seed oil is very rich in nutraceuticals as well as essential fatty acids that could be used for edible purposes. Seed kernel possesses good proximate composition, seed cake is rich in nutritional elements and phenolic compounds and showed significant antioxidant activities. However, the presence of anti-nutritional components may be an obstacle to including seed cake in the diet, but they can be reduced by practicing appropriate processing methods and thus enabling a nutrition-rich *B. roxburghii* seed cake to be so.

4. Conclusions

Balanites roxburghii is an underutilized species having various medicinal properties. Along with that, leaves are used as a vegetable in some southern Indian regions as a famine food. Thus, seeds could also be used as a nutritional source as it contains a substantial amount of oil. The present study sheds light on the nutritional aspects of seed kernel and seed oil of *B. roxburghii*. Kernels are rich in oil, protein, and nutritional elements, whereas oil has a justifiable fatty acid composition with rich nutraceuticals such as carotenoids, tocopherols, and phytosterols. Both seed cake and seed oil possess a considerable amount of total phenolic content. As evident by the various in vitro methods, such as DPPH radical scavenging activity, total antioxidant activity, FRAP activity, and ABTS radical scavenging activity, seed cake and seed oil showed impressive antioxidant properties and they could be included in the diet to reduce the oxidative stress-related problems. Though the presence of anti-nutritional factors limits the nutritional benefits of seed kernels, they can be processed with suitable techniques to minimize them. Nowadays, wild edible sources represent an opportunity to expand our food resources. Furthermore, they can also adapt to climate change, and hence they provide an opportunity to expand our agricultural base. Food from diverse sources can ensure people have a balanced diet. Thus, *B. roxburghii* seed kernels can be a good source of nutrients, but further work is needed to assess its micronutrient composition, various biological activities, and effects of intake.

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