Nutritional composition, phytochemical analysis, and *in vitro* antioxidant activities of *Balanites roxburghii*Planch. leaves; an underutilized plant species

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Abstract

Leaves of *Balanites roxburghii* Planch., an underutilized species, is used as a nutrition source during food scarcity periods, in many parts of Southern India and it has the potential to be a rich nutritive source. With this view, in the present study, nutritive profiling of leaves has been done. Further, the phytochemical composition and antioxidant activities of acetone, methanol, and water extract leaveshave also been analyzed. The leaves showed good proximate composition with 61.67% of moisture, 3.61% of proteins, 3.88% of carbohydrates, and 21.12% of fiber. It is rich in both macro and microelements, especially, calcium (18 mg/g DW), magnesium (6.6 mg/100 g DW), and iron (1346.01 µg/g DW). Among all the extracts, methanol showed the highest phenolic and flavonoid content with the values of 242.22 mg GAE/g extract and 36.57 mg QE/g extract, respectively. Acetone extract is rich in alkaloids with a content of 8.93 mg TE/g extract. Even though acetone extract containsa low amount of phenolic and flavonoid content, it showed the highest antioxidant activity in all the methods tested. DPPH radical scavenging activity, TAA, and FRAP activity of acetone extract are found to be 53.84 mg GAE/g extract, 1717.1 mg AAE/g extract, and 84.03 mg AAE/g extract, respectively. Even though leaves contain some amount of anti-nutritional factors, they can be minimized by practicing appropriate food processing techniques. Considering all these facts, such as availability, proteins, fiber, mineral composition, and bioactive compounds, leaves of *B. roxburghii* could be considered a good food source to increase the food base of people and to get a variety of nutrients.

Keywords: *Balanites roxburghii*, Underutilized Species, Nutrition, Antioxidant Activities

1. Introduction

Population growth has created many threats such as hunger, malnutrition, and poor health to the existence of humans. As per the report of FAO (2022), in 2021, there are 193 million people who are suffering from acute food insecurity and in need of urgent assistance, among the 53 countries studied. It also reported that 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 (Padulosi et al. 2013). 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 difficult (Padulosi et al. 2013).

Underutilized species which are wild or partially domesticated and adapted to the regional environmental conditions could be effectively utilized to combat poverty, malnutrition, and hunger (Padulosi et al. 2013; Murthy and Bapat 2020). 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. As an Indian native plant, it grows wildly in dry and arid regions, throughout India (Sands 2001; Yadav and Murthy 2022). Fruits, seeds, stem bark, and roots are reported to be very useful in curing varied ailments (Yadav and Murthy 2022). In many parts of Southern India, leaves are used as a vegetable during the food scarcity period. Leaves of its allied species, *Balanites aegyptiaca* is reported to be edible in many parts of Africa and more popular in Nigeria (Kubmarawa et al. 2008). *B. aegyptiaca* leaves are rich in proteins (15.86 g/100g DW), carbohydrates (32.38 g/100 g DW), fiber (30.75 g/100 g DW), and ash (9.26 g/100 g DW; Kubmarawa et al. 2008). Further, Khamis et al. (2020) proved *in vitro* antidiabetic property with α-amylase and β-glucosidase inhibiting experiments. In the same way, there is a scope to develop *Balanites roxburghii*leaves as an additional food source to provide nutritional supplements for local people as well as animal feed, asthis plant grows luxuriously in low rainfall areas also.

However, information regarding the nutritional composition of leavesof *B. roxburghii* is lacking. Therefore, in the present study, the nutritional composition of leaves of *Balanites roxburghii* including proximate analysis and mineral compositionhas been analyzed. Further, anti-nutritional components of leaves and phytochemical composition, and antioxidant capacities of leaf extractshave also been studied and its suitability as a nutritive material has also been discussed.

2. Materials and Methods

2.1 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 TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) was purchased from Sigma-Aldrich, Bengaluru, India. All other solvents and chemicals were of analytical grade.

2.2 Plant material collection and extraction

Leaves of *Balanitis roxburghii* were collected from Moka reserved forest, Ballari district, Karnataka, India (15°15'11.5"N 77°04'16.9"E). Leaves were shade dried until reaching constant weight and ground to make a fine powder, transferred to an air-tight polythene cover, and stored at 4 ℃ until further used. The leaf powder was extracted successively with acetone, methanol, and water with their increasing order of polarity by using the Soxhlet apparatus for 8 h each. The extract collected was storedat -20 ℃ until further used.

2.3 Proximate analysis

Moisture content was determined gravimetrically as mentioned in AOAC method 930.15, by drying the fresh leaves at 135 ℃ in an oven for 4 hours (AOAC 1990). Fat content was analyzed gravimetrically by extracting leaves with petroleum ether in a Soxhlet apparatus at 65 ± 2 ℃ for 8 hours. Proteins were estimated by modifying Lowry's method according to Hartree (1972) using BSA as standard. Carbohydrates were quantified by the Anthrone reagent method as described by Sadashivam and Manickam (2008). Ash content was determined according to AOAC method 942.05 by igniting 0.5 g of leaf powder in a crucible at 600 ℃ for 8 hours in a muffle furnace and the ash remained was expressed as g/100 g (AOAC 1990). 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 (AOAC 1990). Energy values were calculated using Atwater-specific factors calculated for vegetables(FAO 2003).

2.4Elementalcomposition 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. (AOAC 2000; Fernandez-Hernandez et al. 2010). Further, nitrogen was estimated according to Liu et al. (2013) by using the two-step digestion-UV spectrophotometric method.

2.5 Determination of anti-nutritional factors

2.5.1 Phytate

Phytate content was determined according to the method described by Gao et al. (2007). Briefly, 0.5 g leaf powderwas extracted with 10 mL of 2.4% HCl and kept for 16 h with constant shaking, and the solution was filtered followed by the addition of 1 g NaCl to filtrate and kept for shaking for 20 minutes followed by centrifuging at 1000 g for 20 minutes at 10 ℃ and 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.

2.5.2 Oxalate

Oxalate content was determined according to the method of Dye (1956). To brief, 2 g of leaf powder was heated in the water bath at 90 ℃ with 190 mL distilled water and 10 mL of 6 N HCl for 4 h. 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 ℃ followed by the addition of 10 mL of 5% $CaCl_2$. After 10 min, 6 N NH_4OH was added till the color changed and kept for 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 ℃, and titrated against 0.05 N $KMnO_4$. Oxalate was determined using the following equation;

$$\text{Oxalate (\%)} = \frac{(\text{mL KMnO4})(0.05)(45.02)(100)(5)}{(1000)(\text{Wt of sample})}$$

2.6Phytochemical analysis

2.6.1 Total phenolic content

The total phenolic content of extracts was estimated by using FC (Folin-Ciocalteu) reagent method as described in Murthy et al. (2022)with slight modification. Briefly, a known amount of extract was taken and made up to 3 mL with distilled water. Then added 0.1 mL of 2 N FC reagent followed by incubation for 6 minutes and the addition of 0.5 mL of 20% Na_2CO_3 to each tube. Tubes were kept in warm water for 30 minutes 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.

2.6.2 Flavonoid content

The flavonoid content of extracts was analyzed as described by Pekal and Pyrzynskaet(2014). To brief, a known amount of extract was taken and made up the volume to 3ml by using distilled water followed by the addition of 0.15 ml of $NaNo_3$and incubated for 5minutes at room temperature. Then add 0.3 ml of 10% $AlCl_3$and 2ml of 1M NaOH after 5 mins of incubation at room temperature. Solutions were vortexed and absorbance was measured at 510 nm. Quercetin was used as standard.

2.6.3 Alkaloids

The alkaloid content of extracts was estimated by using the method of Shamsa et al. (2008). 6.98 mg of bromocresol green powder was dissolved in 0.3 mL of 2N NaOH and diluted to 100 mL with distilled water. A known amount of sample was added with 5 mL of bromocresol green solution followed by the addition of 5 mL of phosphate buffer containing 2 M sodium phosphate and 0.2 M citric acid adjusted to a pH of 4.7. The solutions were added with 5 mL of chloroform, shaken vigorously, chloroform layer was collected, and read absorbance at 470 nm. Tramadol was used as the standard.

2.7 Antioxidant activities

2.7.1 DPPH radical scavenging activity

DPPH radical scavenging activity was determined according to Manasa et al. (2020) with some modifications. A known volume of sample 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 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.

2.7.2 Total Antioxidant activity (TAA)

The total antioxidant activity of the samples was determined by the phosphomolybdenum method (Prieto et al. 1999). A reagent containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate was prepared. A known volume of sample was taken in different tubes, made up to 0.5 mL using distilled water, added with 3 mL of reagent followed by the incubation at 95 °C for 90 minutes. The absorbance of the color developed was measured at 695 nm. Ascorbic acid was used as the standard.

2.7.3 Ferric reducing antioxidant power (FRAP)

FRAP activity of the samples was analyzed according to the method developed by Benzie and Strain (1999). 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 $FeCl_3.6H_2O$ in the ratio of 10:1:1. A known volume of sample was taken in different tubes, made up to 0.5 mL using distilled water and added with 3 mL of reagent followed by the incubation at room temperature for 6 min. The absorbance of the color developed was read at 593 nm. Trolox was used as the standard.

2.9 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 analysis

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*leaves revealed it as the finest source of protein, carbohydrate, and fiber (Table 1). It has a fiber content of 21.12% whereas protein and carbohydrate content was found to be 3.61 and 8.38%, respectively. It contains a good amount of ash, i.e., 3.88%, which is an indication of the presence of a good amount of minerals. The fat content is found to be 0.85%. The present study suggests that the proximate composition of *B. roxburghii* leaves is having more nutritional benefits than that of many well-known leafy vegetables. For instance, protein, carbohydrate, ash, fiber, and fat content of *Basella alba* leaves was reported to be 1.57, 2.01, 1.09, 2.21, and 0.45%, respectively, with an energy value of 19.62 Kcal/100 g FW (Longvah et al., 2017). The energy value of *B. roxburghii* leaves is found to be 45.84 Kcal/100 g FW.

3.2 Elemental composition

Minerals are very essential to the proper functioning of the body as they involve in various physiological and biochemical activities. Though they are required in small amounts, they must be present in a regular diet failing which may cause severe health problems (Harris and Marshall, 2017). Ash is the inorganic residue that remained after the complete combustion of organic matter and represents the total mineral content. A good ash content (3.88%) portrays *B. roxburghii*leaves as a better source of minerals which in turn is proved by individual elemental analysis (Table 2).Potassium and calcium are the two most abundant minerals in the leaves of *B. roxburghii* with an amount of 20.3 and 18 mg/g DW respectively. Nitrogen, phosphorus, sulphur, sodium, and magnesium content are found to be 11.43, 1.23, 3.65, 3.07, and 6.60 mg/g DW, respectively. The leaves are seemingto bean excellent source of microelements, such as iron, copper, zinc, manganese, and boron with an amount of 1346.01, 22.90, 344.02, 67.9, and 34.78 µg/g DW, respectively. Calciumis very important for blood clotting, muscle contraction, and various enzymes for metabolic activities, whereas iron is very essential to prevent anemia. The excellent elemental profile of *B. roxburghii* leaves suggests that it could be the best nutritional supplement for rural people who are having mineral deficiencies as well as fodder for cattle.

3.3 Anti-nutritional factors

Anti-nutritional components are those which make the bioavailability of nutrients very difficult. Phytate and oxalate are both considered major anti-nutritional factors as they hinder the bioavailability of minerals by binding with them(Kaushik et al. 2018). Phytate and oxalatecontent of *B. roxburghii*leaves is found to be 7.37 and 22.10 mg/g DW, respectively(Table 3). Phytate is the major stored form of phosphorus in plants and 50-85% of phosphorus is stored in this form. Usually, seeds contain a high amount of phytate than other parts (Samtiya et al. 2020). However, the phytate content of *B. roxburghii* leaves iscomparable with that of drumstick leaves, which havea content of 1.28 mg/g FW(Longvah et al., 2017). The oxalate content is very less when compared with that of spinach leaves and green amaranth leaves, which have values of 125.76 and 100.56 mg/g DW, respectively(Radek and Savage 2008). Different cooking processes such as milling, roasting, and soaking significantly reduced the phytateand oxalate content in different legume species (Udensi et al. 2009; Shi et al. 2018; Samtiya et al. 2020). Thus, different food processing strategies can be followed to minimize the anti-nutritional compounds from the leaves of *B. roxburghii*.

3.4 Phytochemical composition

Phytochemicals have protective or disease-resistant properties along with their antioxidant activities. Some of the health benefitsof providing phytochemicals are polyphenols, flavonoids, isoflavonoids, phytoestrogens, terpenoids, carotenoids, phytosterols, andglucosinolates. These arethe phytochemicalsthat show goodantioxidant properties. The successive extraction of leaves with acetone, methanol, and water resulted in a yield of 7.30, 20.93, and 15.39 mg/g DW, respectively (Table 4). Phenolics and flavonoids, with various biological activities, increase the nutritional value of the food with their antioxidant properties. Water extract contains the highest amount of total phenolics, followed by methanol and acetone extract. The total phenolic content of water, methanol, and acetone extracts was found to be 259.08, 242.22, and 72.11 mg GAE/g extract. Both water and methanol extracts were rich in flavonoid content with the values of 34.44 and 36.57 mg QE/g extract, respectively, whereas acetone extract contains 22.46 mg QE/g extract. Alkaloids represent an important group of phytochemicals that are considered

very helpfully in providing health as well as economic benefits (Shamsa et al. 2008). Alkaloids are very rich in acetone extract with an amount of 8.93 mg TE/g extract followed by methanol extract (3.47 mg TE/g extract). Water extract contains a very less amount of alkaloids. Thus, the leaves of *B. roxburghii* are very rich in phytochemicals, along with the essential nutritional components. These phytochemicals contribute to various biological activities, especially, antioxidant activities.

3.5 Antioxidant activities

Oxidative stress causes various diseases such as cancers, autoimmune disorders, Alzheimer's disease, and Parkinson's disease and these diseases can be prevented by the intake of an adequate amount of antioxidants (Pizzino et al. 2017). Hence,the present work attempted to assess a wide range of antioxidant potential of *B. roxburghii* leaf extracts with DPPH radical scavenging activity (DPPH), total antioxidant activity (TAA), andferric reducing antioxidant power (FRAP) and the results are presented in Table 5. Acetone extract showed the highest antioxidant activity, followed by methanol and water extracts, in all the methods tested. Acetone extract has 53.84 mg GAE/g extract of DPPH radical scavenging activity, whereas methanol and water extracts have 7 and 4.3 mg GAE/g extract activity, respectively. TAA exhibited by acetone, methanol, and water extracts were 1717.1, 261.26, and 90.73 mg AAE/g extract, respectively. In FRAP activity, acetone extract showed 84.03 mg AAE/g extract activity, methanol extract showed 40.49 mg AAE/g extract activity and water extract showed 38.78 mg AAE/g extract activity. Even though acetone extract contains a low amount of phenolics and flavonoid compounds, it showed the best antioxidant activity in all the methods analyzed. This is probably due to the presence of more potent antioxidant compounds than that present in water and methanol extracts. Hence, further studies are needed to identify the more potent compounds from the extracts of *B. roxburghii* leaves. By considering its availability, nutritional profile, elemental composition, bioactive compounds, and antioxidant activity, leaves of *B. roxburghii* could be used as a potent nutritional source.

4. Conclusions

Balanites roxburghii is an underutilized species having various medicinal properties. Along with that, seed kernels are used as the nutritive source in some Southern Indian regions as a famine food. Thus, leaves could also be used as a nutritional source as it contains a substantial amount of fiber and proteins. The present study sheds light on the nutritional aspects of the leaves of *B. roxburghii*. Leaves are rich in protein, fiber, and minerals. As a result of various phytochemicals along with phenolic compounds, all the extracts exhibited good antioxidant activities. Though the presence of anti-nutritional factors limits its nutritional benefits, leaves can be processed with suitable techniques to minimize those anti-nutritional factors. Exposing wild edible sources is the need of the day to expand our food base as well as agriculture base. Wild plants can combat weather extremes and also food from diverse sources can ensure people a balanced diet. Thus, *B. roxburghii*leaves can be a good source of nutrients and further work is needed to assess its micronutrient composition, various biological activities, and effects of intake.

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31. Table 1. Proximate composition of *Balanites roxburghii*leaves

Component	% Composition
Moisture	61.67 ± 0.14
Fat	0.85 ± 0.05
Protein	3.61 ± 0.13
Carbohydrate	8.38 ± 0.15
Ash	3.88 ± 0.01
Fiber	21.12 ± 0.24
Energy (Kcal/100 g)	45.84

Each value represents the mean ± standard error of three replicates

Table 2. Elemental composition of *Balanites roxburghii*leaves

Element	Composition
Macroelements (mg/g DW)	

Nitrogen	11.43 ± 0.12
Phosphorous	1.23 ± 0.06
Potassium	20.30 ± 0.14
Sulphur	3.65 ± 0.10
Sodium	3.07 ± 0.08
Calcium	18.00 ± 0.17
Magnesium	6.60 ± 0.09
Microelements (µg/g DW)	
Boron	34.78 ± 0.19
Zinc	344.02 ± 9.30
Iron	1346.01 ± 10.97
Manganese	67.90 ± 0.58
Copper	22.90 ± 0.57

Each value represents the mean ± standard error of three replicates

Table 3. Anti-nutritional factors of *Balanites roxburghii*leaves

Factor	Composition (mg/g FW)
Phytate	7.37 ± 0.30
Oxalate	22.10 ± 3.38

Each value represents the mean ± standard error of three replicates

Table 4. Phytochemical composition of *Balanites roxburghii*leaf extracts

Activity	Acetone (mg/g extract)	Methanol (mg/g extract)	Water (mg/g extract)
Extract yield (g/100 g DW)	7.30 ± 0.42	20.93 ± 0.74	15.39 ± 0.50
Total phenolics (GAE)	72.11 ± 2.70	242.22 ± 12.58	259.08 ± 25.20
Flavonoids (QE)	22.46 ± 0.68	36.57 ± 3.36	34.44 ± 0.60
Alkaloids (TE)	8.93 ± 0.25	3.47 ± 0.58	0.40 ± 0.06

Each value represents the mean ± standard error of three replicates. GAE – Gallic acid equivalent; QE – Quercetin equivalent; TE – Tramadol equivalent.

Table 5. Antioxidant activities of *Balanites roxburghii*leaf extracts

Activity	Acetone (mg/g extract)	Methanol (mg/g extract)	Water (mg/g extract)
DPPH (mg GAE)	53.84 ± 4.66	7.00 ± 0.55	4.30 ± 0.69
TAA (mg AAE)	1717.1 ± 15.5	261.26 ± 14.48	90.73 ±2.83
FRAP (mg AAE)	84.03 ± 11.95	40.49 ± 0.84	38.78 ± 0.66

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