horticulturae

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Evaluation of the Nutritional, Phytochemical, and Antioxidant Potential of *Rourea minor* Fruits: An Underutilized Species

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Abstract: The present study focuses on the nutritional, phytochemical composition, and antioxidant activities of the fruits of *Rourea minor* (Gaertn.) Alston., an underutilized plant species. The ripened seeds contained 0.28%, 12.39%, 25.70%, 1.4%, and 3.4% of fat, protein, carbohydrate, ash, and fiber, respectively, whereas ripened pulp possessed 0.19%, 0.34%, 0.90%, 0.35%, and 0.98% of fat, protein, carbohydrates, ash, and fiber, respectively. The ripened seed and pulp were also rich in mineral elements and especially microelements. The pulp, when ripe, had high levels of microelements such as boron, iron, zinc, copper, and manganese with values of 25.98, 2523.56, 499.12, 33.62, and 40.30 µg/g DW, respectively. Phytate and oxalate content were comparatively higher in ripened seeds (6.91 and 31.88 mg/g FW) than in the pulp. Acetone, absolute methanol, water, and 70% methanol were used for the extraction of phytochemicals, and 70% methanol extract contained the highest phytochemicals. The total phenolic, flavonoid, and alkaloid content of unripe seeds was 180.47 mg gallic acid equivalent (GAE)/DW, 68.95 quercetin equivalent (QE)/g DW, and 0.35 atropine equivalent (AE)/g DW, respectively, and that of unripe pulp was 8.21 mg GAE/g DW, 2.97 mg QE/g DW and 1.20 mg AE/g DW, respectively. All the extracts showed remarkable antioxidant activity, proved by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, total antioxidant activity, and ferric-reducing antioxidant power (FRAP) assay, following a similar trend to the phytochemical composition. The study concludes that *Rourea minor* fruits, both seeds, and pulp, could be an excellent source of nutrients, microelements, and antioxidants.

Keywords: antioxidants; minerals; nutrients; phytochemicals; *Rourea minor*; underutilized fruit

Citation: Murthy, H.N.; Yadav, G.G.; Kadapatti, S.S.; Pote, A.H.; Jagali, R.; Yarashi, V.; Dewir, Y.H. Evaluation of the Nutritional, Phytochemical, and Antioxidant Potential of *Rourea minor* Fruits: An Underutilized Species. *Horticulturae* **2023**, *9*, 606. https://doi.org/10.3390/horticulturae9050606

Academic Editor: Valentina Schmitzer

Received: 20 April 2023
Revised: 12 May 2023
Accepted: 18 May 2023
Published: 22 May 2023

1. Introduction

Around the world, plants constitute a significant source of food and medicine. Despite the fact that a substantial section of the population consumes animal food products, the world health organization advises choosing plant-based food over food from animal sources to reduce the risk of non-communicable diseases [1]. A plant-based diet is proven to help prevent obesity, diabetes, cardiovascular disease, cancer, and other chronic diseases [2]. However, it is challenging to feed the increasing human population while ensuring an adequate intake of nutrients. Despite knowing 30,000 edible plant species, only four species, rice, wheat, maize, and potato, account for 60% of the human energy supply [3]. Severe malnutrition issues are caused by the absence of diversity in agriculture and our diet. According to estimates, two billion people are deficient in one or more micronutrients [4].

Underutilized plants are the species that are wild or semi-domesticated and adapted to a particular climatic condition providing immense opportunities to combat malnutrition. They have been given very little or no attention by researchers and policymakers [3,5–7]. *Rourea minor* (Gaertn.) Alston (Family: Connaraceae) is one such fruit-yielding species

(Figure 1a) distributed in India, Bangladesh, Sri Lanka, Cambodia, Vietnam, Malaysia, Indonesia, Tibet, China, and Africa [6]. *Rourea* Aubl. comprises 91 species and *R. minor* is one of the three species distributed in India, whereas *R. prainiana* and *R. mimosoides* are the other two [8,9]. *R. minor* is well known for various medicinal uses, such as roots and twigs are used in diabetes, rheumatism, pulmonary complaints, scurvy, ulcers, and skin diseases; the decoction of the stem is used as a febrifuge and also given to women after childbirth [10,11]. The leaves are used in treating abrasions and lesions [12]. Many pharmacological studies have confirmed their traditional use. The methanolic [12], ethanolic and water [13] extracts of root significantly reduced the serum glucose level in the streptozotocin-induced diabetic rats. He et al. [14] isolated two compounds, rourinoside, and rouremin, from the stem and confirmed their antimalarial activity against *Plasmodium falciparum*. Apart from this, the fruits (Figure 1b,c) are found edible and used in the preparation of a variety of dishes. The fruit pulp (Figure 1d,e) is the edible portion that tastes sour and is used in culinary preparation such as "Appasehuli" and "Chutney" (in the Kannada language), in Uttara Kannada district of Karnataka state, India. Supriatna et al. [15] reported this fruit as one of the major dietary sources of Marron leaf monkeys in the Central Indonesian Borneo region.

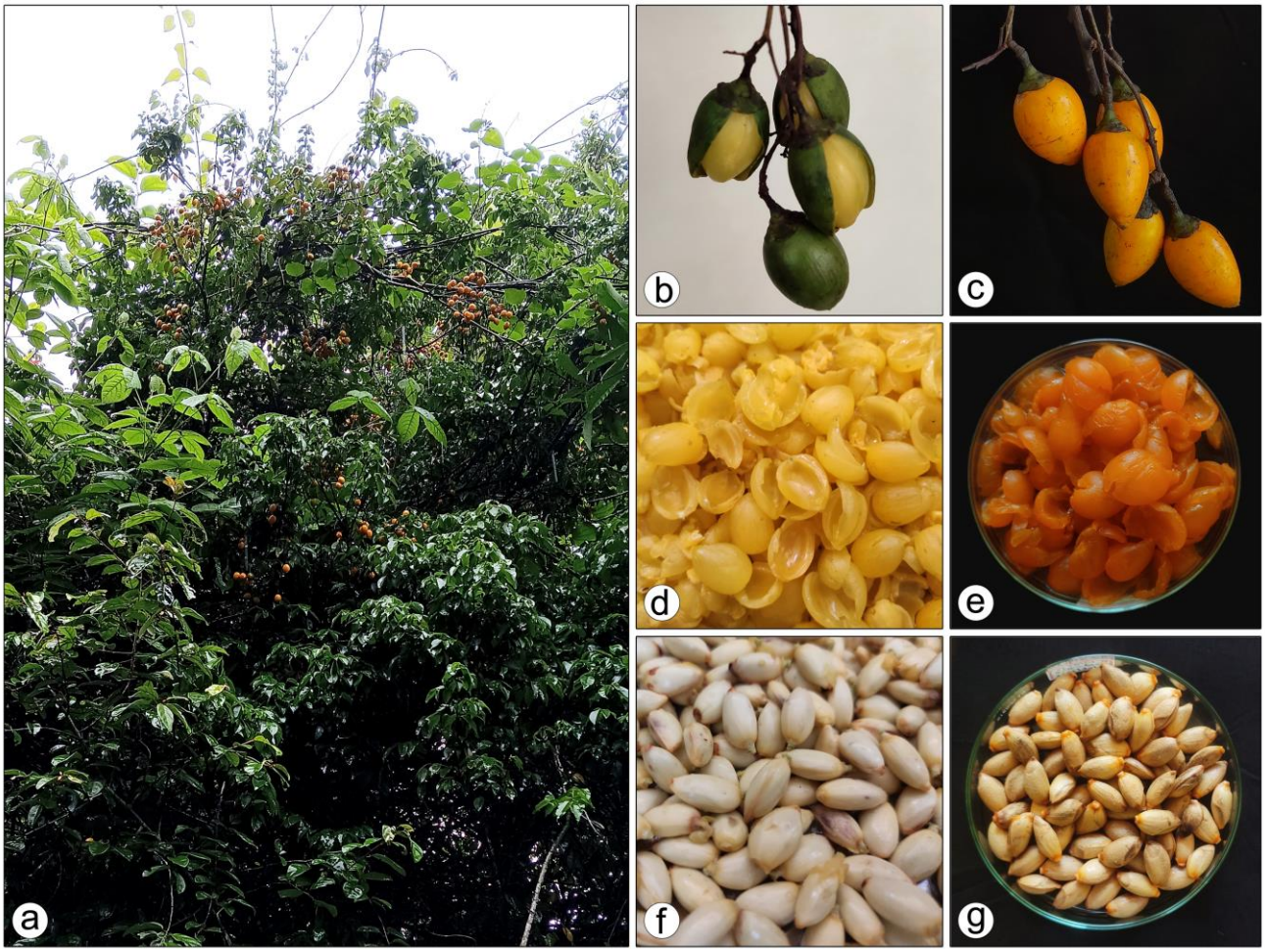

Figure 1. Morphology of *Rourea minor* (Gaertn.) Alston. (**a**). Habit. (**b**) Unripe fruits. (**c**) Ripened fruits. (**d**) Unripe pulp. (**e**) Ripened pulp. (**f**) Unripe seeds. (**g**) Ripened seeds.

The unavailability of accurate data regarding the nutritional composition and consumption data of underutilized species is one of the major constraints holding them from full potential utilization [3,16]. Hence, it is essential to know the species' usage, availability status, and nutritional composition to raise public awareness of their value. Additionally, each fruit has a unique period of maturity at which its nutritional quality is best. The

ripening of fruit is a complex process involving changing morphological, sensory, and phytochemical characteristics [17]. Hence, it is necessary to know the appropriate stage at which harvesting should be done to obtain maximum nutritional benefits. Additionally, the type of solvent is one of the critical factors, along with duration, temperature, pH, and physical agents, that determines the efficiency of obtaining valuable phytochemicals [18]. Concerning the facts mentioned above, no reports are available on the nutritional status, phytochemical composition, and biological activities of *Rourea minor* fruits. Hence, in the present study, we are reporting the proximate, elemental composition, anti-nutritional contents, and phytochemicals of seed (Figure 1f,g) and pulp (Figure 1d,e) of unripe and ripened fruits of *R. minor*. The present study also accounts for the effect of fruit maturity and the phytochemical composition and antioxidant activities of seed and pulp.

2. Materials and Methods

2.1. Plant Material

The *Rourea minor* fruits were collected from Kanchuguli village, Uttara Kannada, Karnataka, India (14.4822860 N, 74.8081505 E). The unripe fruits (Figure 1b) were collected during April, whereas ripened ones (Figure 1c) were collected in June 2022. The epicarp was removed, and the pulp (Figure 1d,e) and seeds (Figure 1f,g) were separated and dried in an oven at 40 ± 2 °C until completely dried. They were powdered using a mechanical grinder and stored airtight using polythene bags at 4 °C until further use.

2.2. Chemicals and Reagents

The chemicals and solvents used in this study, such as the DPPH (2,2-diphenyl-1-picrylhydrazyl), $FeCl_3 \cdot 6H_2O$, bromocresol green, $NaNO_3$, sodium phosphate, ammonium molybdate, Folin–Ciocalteu (FC) reagent, Anthrone, bovine serum albumin, gallic acid, quercetin, ascorbic acid, and atropine, were purchased from HiMedia Laboratories, Mumbai, India, whereas $AlCl_3$ and 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) were purchased from Sigma-Aldrich, Bengaluru, India. All other chemicals and solvents were of analytical grade.

2.3. Extraction of Phytochemicals from Pulp and Seeds

The epicarp was removed from unripe and ripened fruits and pulp and seeds were separated for further analysis. The unripe and ripened pulp and seeds were extracted with different solvents for phytochemical analysis and antioxidant activities in two patterns. In the first pattern, they were extracted with 70% methanol containing 0.1% HCl with constant shaking at room temperature for 16 h, and the solvent was removed to obtain the extract, which is directly taken for analysis. In the second pattern, the samples were extracted with three solvents, viz., acetone, methanol, and water in increasing order of their polarity (i.e., acetone < methanol < water) by using a Soxhlet apparatus for 8 h with each solvent. The residue obtained from each extract was dried and subsequently used for the extraction with the next solvent. The organic solvents were separated from the extracts using a rotary evaporator (Buchi, Rotavapor R-100, Flawil, Switzerland), and the water content from the aqueous extract was removed by keeping the extract in an oven at 40 ± 2 °C. The extracts were stored in sterile glass vials at −20 °C till further analysis.

2.4. Proximate Analysis

The moisture content was analyzed by keeping fresh samples in an oven at 135 °C for 4 h, and the weight difference was noted [19]. The fat content was analyzed by extracting the powdered samples with petroleum ether in a Soxhlet apparatus for 8 h, and the obtained fat was determined gravimetrically [20]. The protein content was quantified by Lowry's method, as mentioned by Hartree [21], using bovine serum albumin as the standard. The carbohydrates were estimated by the Anthrone reagent method, according to Sadashivam and Manickam [22]. Ash content was determined by igniting a known amount of sample in a crucible at 600 °C for 8 h in a muffle furnace, and the weight difference was determined gravimetrically [20]. Fiber content was estimated by acid (1.25% H_2SO_4) and alkali (1.25%

NaOH) digestion of samples [20]. Water-specific factors for fruits were used to calculate the energy values [23].

2.5. Elemental Analysis

The elements phosphorus, potassium, sodium, sulfur, magnesium, calcium, boron, iron, zinc, copper, and manganese were analyzed by using a NOVA 400 atomic absorption spectrophotometer (Analytic Jena AG, Jena, Germany) with an air or acetylene flame, and absorbance was carried out by using respective hollow-cathode lamps [24,25]. The nitrogen was quantified by a two-step digestion-UV spectrophotometric (Hitachi U-3310, Tokyo, Japan) method described by [26].

2.6. Determination of Anti-Nutritional Factors

2.6.1. Phytate

The phytate was quantified by the Wade reagent method, as described by Gao et al. [27]. The samples (0.5 g each) were extracted with 2.4% HCl (10 mL) by keeping in constant shaking for 16 h. The extract was filtered using Whatman filter paper, and NaCl (1 g) was added to the filtrate and kept for shaking for 20 min. The solution was centrifuged at 1000× *g* for 20 min at 10 °C, and the supernatant was used for the phytate estimation. Then, 0.5 mL of extract of known concentration was made up to 3 mL with distilled water and 1 mL of Wade reagent was prepared by adding 0.03% $FeCl_3 \cdot 6H_2O$ and 0.3% sulfosalicylic acid. The reduction in the solution color indicates the presence of phytate. The absorbance was read at 500 nm using a UV-Vis spectrophotometer (Hitachi U-3310, Tokyo, Japan). Sodium phytate was used as the standard compound to draw a calibration curve (Conc. 0.5–2.5 mg/mL; R^2 = 0.991).

2.6.2. Oxalate

The oxalate content of the samples was analyzed as described by Dye [28]. Briefly, 2 g of sample was mixed with 190 mL distilled water and 10 mL of 6 N HCl at 90 °C for 4 h. The solution was filtered and added with distilled water to make it up to 250 mL. Next, 50 mL of this solution was titrated with concentrated ammonia, and then we observed color change with methyl orange indicator and added 10 mL of $CaCl_2$ after it was heated to 95 °C. Then, 6 N NH_4OH was added after 10 min till the color changed and was left at room temperature overnight to precipitate calcium oxalate. The calcium oxalate precipitated was filtrated out, dissolved in hot sulfuric acid, and made to 125 mL, followed by heating to 95 °C and titrating with 0.05 N $KMnO_4$. The following equation was used for oxalate determination:

$$\text{Oxalate } (\%) = \frac{(\text{mL of } KMnO_4 \text{ used})(0.05)(45.02)(100)(5)}{(1000)(\text{Weight of sample in gram})} \quad (1)$$

2.7. Phytochemical Analysis

2.7.1. Total Phenolic Content

Estimation of total phenolic content was carried out according to Murthy et al. [29]. To be brief, the extract of known concentration (0.05 to 0.5 mg/mL) was taken in a test tube and made to be 3 mL with distilled water. Then, 0.1 mL of 2 N FC reagent was added and incubated for 6 min, followed by adding 0.5 mL of 20% Na_2CO_3. The tubes were incubated at room temperature for 30 min, and the developed color was measured at 760 nm using a UV-Vis spectrophotometer (Hitachi U-3310, Tokyo, Japan). Gallic acid was used as the standard compound to draw a calibration curve (Conc. 0.1–0.5 mg/mL; R^2 = 0.9988).

2.7.2. Total Flavonoid Content

Estimation of flavonoid content was carried out according to Pękal and Pyrzynska [30]. Briefly, the extract of known concentration (0.5 to 5.0 mg/mL) was taken in a test tube and made to be 3 mL with distilled water. Then, 0.15 mL of $NaNO_3$ was added to the test

tubes and kept at room temperature for 5 min. Next, 0.3 mL of 10% $AlCl_3$ was added and incubated at room temperature for 5 min, followed by the addition of 2 mL of 1 M NaOH. The solutions were mixed well, and the color was measured at 510 nm using a UV-Vis spectrophotometer (Hitachi U-3310, Tokyo, Japan). Quercetin was used as the standard compound to draw a calibration curve (Conc. 0.2–1.0 mg/mL; R^2 = 0.9999).

2.7.3. Total Alkaloid Content

Estimation of alkaloids was carried out as Shamsa et al. [31] mentioned. Initially, bromocresol green solution was prepared by dissolving 6.98 mg of bromocresol green powder in 0.3 mL of 2 N NaOH and diluted to 100 mL with distilled water. The phosphate buffer containing 2 M sodium phosphate and 0.2 M citric acid was prepared and adjusted to a pH of 4.7. The extract of known concentration (5 to 10 mg/mL) was taken in a test tube, we added 5 mL of bromocresol green solution, and 5 mL of phosphate buffer was added followed by the addition of 5 mL of chloroform. The solution was vortexed for 1 min, taken out of the denser chloroform layer, and its absorbance was read at 470 nm using a UV-Vis spectrophotometer (Hitachi U-3310, Tokyo, Japan). Atropine was used as the standard compound to draw a calibration curve (Conc. 0.1–0.5 mg/mL; R^2 = 0.9869).

2.8. Antioxidant Activities

2.8.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

DPPH radical scavenging activity of the extracts was analyzed according to Yadav et al. [32]. To be brief, 0.5 mL of extract of known concentration (0.05 to 1 mg/mL) was taken and added with 3.0 mL of 0.1 mM DPPH prepared in methanol. The tubes were shaken well and incubated in the dark for 15 min, and the absorbance of the solution was measured at 517 nm using a UV-Vis spectrophotometer (Hitachi U-3310, Tokyo, Japan). The activity of the extracts was expressed as gallic acid equivalents.

2.8.2. Total Antioxidant Activity (TAA)

The total antioxidant activity of the extracts was analyzed by the phosphomolybdenum method, as mentioned in Prieto et al. [33]. A reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate was prepared. A 0.5 mL of the extract of known concentration (0.1 to 1 mg/mL) was added with 3.0 mL of reagent solution, shaken well, and incubated for 90 min at 95 °C in a water bath. The solution's absorbance was measured using a UV-Vis spectrophotometer (Hitachi U-3310, Tokyo, Japan) at 695 nm. The activity of the extracts was expressed as ascorbic acid equivalents.

2.8.3. Ferric Reducing Antioxidant Power (FRAP) Activity

The FRAP activity of the extracts was performed according to Benzie and Strain [34]. Initially, FRAP reagent was prepared by mixing 300 mM acetate buffer of pH 3.6, 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ in 10:1:1 ratio. Then, 0.5 mL of the extract of known concentration (0.01 to 2.5 mg/mL) was added with 3.0 mL of reagent solution, shaken well, and kept at room temperature for 6 min, and the absorbance was read with a UV-Vis spectrophotometer (Hitachi U-3310, Tokyo, Japan) at 593 nm. The activity of the extracts was expressed as ascorbic acid equivalents.

2.9. Statistical Analysis

All experiments were performed in triplicates. The descriptive statistics, such as mean and standard error, were calculated. One-way ANOVA and Duncan's multiple range test were conducted in IBM SPSS Statistics Version 20.

3. Results

3.1. Proximate Composition

The proximate compositions of *Rourea minor* seed and pulp are presented in Table 1. Both the seed and pulp had high moisture content. The unripened seed had 82.66%

moisture, whereas it was reduced to 52.55% when ripened. The moisture content of the unripened and ripened pulp was 94.03 and 93.86%, respectively, and did not change much. The fat content is comparatively less in both the seed and pulp. The ripened seed had the highest of 0.28% fat. The seed accommodated the highest amount of protein, i.e., 4.30 and 12.39% in unripe and ripened seeds, respectively. The protein content was 0.31 and 0.34% in unripe and ripened pulp, respectively. The ripened seed had the highest amount of carbohydrates, i.e., 25.70%, followed by 6.17% in unripe seed, 0.90% in ripened pulp, and 0.63% in unripe pulp. Ash content was highest in ripened seed, i.e., 1.4%, and all other parts ranged between 0.28 and 0.35%. Similarly, fiber content was also highest in the unripe seed, i.e., 3.4%, while it ranged from 0.30 to 0.98% in the remaining parts. The energy value of the seed increased from 41.70 kcal/100 g in the unripe stage to 149.93 kcal/100 g in the ripe. However, it did not change much in pulp, which was 4.98 and 5.97 kcal/100 g in unripe and ripe, respectively.

Table 1. Proximate composition of *Rourea minor* fruits.

Fruit Parts	Seed Unripe	Ripe	Pulp Unripe	Ripe
Moisture (g/100 g FW)	82.66 ± 0.50^{b}	52.55 ± 0.55^{c}	94.03 ± 0.82^{a}	93.86 ± 0.75^{a}
Fat (g/100 g FW)	0.20 ± 0.02^{b}	0.28 ± 0.08^{a}	0.20 ± 0.03^{b}	0.19 ± 0.04^{b}
Protein (g/100 g FW)	4.30 ± 0.06^{b}	12.39 ± 0.53^{a}	0.31 ± 0.01^{c}	0.34 ± 0.01^{c}
Carbohydrate (g/100 g FW)	6.17 ± 0.08^{b}	25.70 ± 1.47^{a}	0.63 ± 0.02^{c}	0.90 ± 0.03^{c}
Ash (g/100 g FW)	0.31 ± 0.06^{b}	1.4 ± 0.23^{a}	0.28 ± 0.04^{b}	0.35 ± 0.07^{b}
Fiber (g/100 g FW)	0.82 ± 0.09^{b}	3.4 ± 0.30^{a}	0.30 ± 0.01^{c}	0.98 ± 0.12^{b}
Energy (Kcal/100 g)	41.7	149.93	4.98	5.97

Each value represents the mean ± standard error of three replicates. Mean values followed by different letters in their superscript are significantly different from each other ($p = 0.05$) in the respective row according to Duncan's multiple range test; FW—Fresh weight.

3.2. Elemental Composition

In the present study, we analyzed seven macroelements and five microelements in the seed and pulp of *R. minor*, and the results are presented in Table 2. The results showed that the pulp and seeds are an impressive source of minerals. Ripened pulp is a rich source of minerals compared to the others. The ripened pulp consists of 0.92 mg/g DW of phosphorous, 4.98 mg/g DW of nitrogen, 12.20 mg/g DW of potassium, 0.96 mg/g DW of sodium, 5.80 mg/g DW of sulphur, 7.20 mg/g DW of magnesium and 6.80 mg/g DW of calcium. The boron, iron, zinc, copper, and manganese content of ripened pulp was 25.98, 2523.56, 499.12, 33.62, and 40.30 µg/g DW, respectively. The unripened pulp consists of all the elements on par with the ripened pulp, especially boron (32.50 µg/g DW) and iron (2627.28 µg/g DW), which were higher than that of the latter. The seeds were rich in zinc and manganese, where their values were 535.12 and 118.40 µg/g DW in unripe seed and 542.26 and 98.70 µg/g DW in ripened, respectively.

3.3. Anti-Nutritional Components

The phytate and oxalate of the *R. minor* fruit and the results are presented in Table 3. Seeds accumulate greater amounts of phytate and oxalate compared to pulps. At the same time, both the phytate and oxalate content increased when the fruits ripened except in the pulp, where the oxalate content is less in the ripened condition. The phytate content of unripe and ripe seeds was 3.00 and 6.91 mg/g, respectively, whereas that of unripe and ripe pulp was 0.37 and 0.59 mg/g, respectively. The oxalate content was 6.19 and 31.88 mg/g in unripe and ripe seeds, respectively, whereas it was 11.11 and 6.50 mg/g in unripe and ripe pulp, respectively.

Table 2. Elemental composition of *Rourea minor* fruits.

Fruit Parts		Seed Unripe	Ripe	Pulp Unripe	Ripe
Major elements (mg/g DW of sample)	P	0.98 ± 0.09 ^{ab}	1.04 ± 0.09 ^{a}	0.91 ± 0.07 ^{b}	0.92 ± 0.11 ^{b}
	N	5.53 ± 0.12 ^{b}	6.25 ± 0.30 ^{a}	4.43 ± 0.20 ^{d}	4.98 ± 0.30 ^{c}
	K	4.80 ± 0.13 ^{c}	5.30 ± 0.22 ^{c}	10.30 ± 0.28 ^{b}	12.20 ± 0.40 ^{a}
	Na	0.27 ± 0.05 ^{c}	1.05 ± 0.08 ^{a}	0.58 ± 0.06 ^{b}	0.96 ± 0.05 ^{a}
	S	2.10 ± 0.15 ^{c}	4.56 ± 0.18 ^{b}	1.08 ± 0.08 ^{d}	5.80 ± 0.20 ^{a}
	Mg	3.60 ± 0.12 ^{c}	5.80 ± 0.14 ^{b}	6.00 ± 0.24 ^{b}	7.20 ± 0.22 ^{a}
	Ca	4.02 ± 0.10 ^{c}	5.20 ± 0.23 ^{b}	5.04 ± 0.21 ^{b}	6.80 ± 0.12 ^{a}
Minor elements (µg/g DW of sample)	B	22.98 ± 2.30 ^{b}	33.65 ± 1.86 ^{a}	32.50 ± 2.25 ^{a}	25.98 ± 1.50 ^{b}
	Fe	780.50 ± 15.10 ^{b}	798.34 ± 14.40 ^{b}	2627.28 ± 22.20 ^{a}	2523.56 ± 21.20 ^{a}
	Zn	535.12 ± 12.5 ^{a}	542.26 ± 13.3 ^{a}	492.23 ± 11.6 ^{b}	499.12 ± 9.20 ^{b}
	Cu	17.30 ± 0.80 ^{d}	42.56 ± 1.34 ^{a}	22.90 ± 0.90 ^{c}	33.62 ± 2.32 ^{b}
	Mn	118.40 ± 5.60 ^{a}	98.70 ± 4.12 ^{b}	38.30 ± 3.32 ^{c}	40.30 ± 1.23 ^{c}

Each value represents the mean ± standard error of three replicates. Mean values followed by different letters in their superscript are significantly different from each other (p = 0.05) in the respective row according to Duncan's multiple range test; FW—Fresh weight.

Table 3. Anti-nutritional factors of *Rourea minor* fruits.

Fruit Parts		Phytate (mg/g FW of Sample)	Oxalate (mg/g FW of Sample)
Seed	Unripe	3.00 ± 0.09 ^{b}	6.19 ± 0.12 ^{c}
	Ripe	6.91 ± 0.58 ^{a}	31.88 ± 1.50 ^{a}
Pulp	Unripe	0.37 ± 0.01 ^{c}	11.11 ± 0.43 ^{b}
	Ripe	0.59 ± 0.03 ^{c}	6.50 ± 0.20 ^{c}

Each value represents the mean ± standard error of three replicates. Mean values followed by different letters in their superscript are significantly different from each other (p = 0.05) in the respective column according to Duncan's multiple range test; FW—Fresh weight.

3.4. Phytochemical Composition

The fruits of *R. minor* were investigated for the three major phytochemical groups, phenolics, flavonoids, and alkaloids, by extracting with different solvent systems, and the presented results are in Table 4. Unripen fruits accommodate more phytochemicals than ripened ones. Seeds contain the highest amount of phenolics and flavonoids, whereas pulp surpasses seeds slightly in alkaloid accumulation. Among all the extracts, the 70% methanol solvent system consisted of the highest phytochemicals in all the individual fruit parts except in a few cases, such as acetone and the water extract of ripened pulp, accommodating the highest alkaloid and flavonoid content, respectively.

The total phenolic content (TPC) of unripe seeds was 180.47, 82.79, and 56.40 mg GAE/g DW in 70% methanol, acetone, and methanol extracts, respectively. It was 119.45, 14.10, and 100.82 mg GAE/g DW in 70% methanol, acetone, and methanol extracts of ripened seeds, respectively. When the fruits ripened, the phenolic compounds that are soluble in acetone significantly decreased, while those that are soluble in methanol rose in the seeds. TPC was 8.21, 6.04, and 2.64 mg GAE/g DW, respectively, for 70% methanol, methanol, and acetone extracts of unripe pulp, but it was 5.67, 2.97, and 2.44 mg GAE/g DW, respectively, for ripened pulp. Ripened pulp had less phenolics in the acetone extract than unripened pulp.

The total flavonoid content (TFC) also followed a similar trend as that of TPC. The flavonoid content of seeds was highest in 70% methanol, i.e., 68.95 and 44.10 mg QE/g DW in unripe and ripened, respectively. Unripe and ripened pulp had 2.97 and 1.61 mg QE/g DW, respectively, in 70% methanol extract. As an exception, water-soluble flavonoids were highest in the water extract of ripened pulp with a value of 3.25 QE/g DW. The alkaloid content of pulp was slightly higher than that of seeds. Combining the acetone, methanol,

and water extracts, unripe and ripened pulp accommodated 2.06 and 3.20 mg AE/g DW, respectively, whereas it was 1.84 and 0.94 mg AE/g DW in unripe and ripened seeds, respectively.

Table 4. Phytochemical composition of *Rourea minor* fruit extracts.

Fruit Parts		Solvent	Total Phenolics (mg GAE/g DW of the Sample)	Flavonoids (mg QE/g DW of the Sample)	Alkaloids (mg AE/g DW of the Sample)
Seed	Unripe	Acetone	82.79 ± 6.44 ^{d}	27.43 ± 1.23 ^{c}	0.68 ± 0.12 ^{def}
		Methanol	56.40 ± 3.41 ^{e}	24.56 ± 0.20 ^{d}	1.09 ± 0.01 ^{bc}
		Water	3.16 ± 0.02 ^{g}	1.84 ± 0.01 ^{e}	0.07 ± 0.01 ^{h}
		70% Methanol	180.47 ± 7.53 ^{a}	68.95 ± 3.30 ^{a}	0.35 ± 0.03 ^{g}
	Ripe	Acetone	14.40 ± 0.66 ^{f}	2.99 ± 0.05 ^{e}	0.61 ± 0.04 ^{ef}
		Methanol	100.82 ± 1.24 ^{c}	12.80 ± 0.48 ^{e}	0.32 ± 0.01 ^{g}
		Water	2.78 ± 0.23 ^{g}	1.55 ± 0.03 ^{e}	0.01 ± 0.00 ^{h}
		70% Methanol	119.45 ± 4.29 ^{b}	44.10 ± 1.50 ^{b}	0.88 ± 0.01 ^{cd}
Pulp	Unripe	Acetone	2.64 ± 0.24 ^{g}	0.92 ± 0.1 ^{e}	0.88 ± 0.08 ^{cd}
		Methanol	6.04 ± 0.21f ^{g}	2.42 ± 0.17 ^{e}	0.64 ± 0.06 ^{def}
		Water	1.58 ± 0.03 ^{g}	0.54 ± 0.02 ^{e}	0.54 ± 0.06 ^{fg}
		70% Methanol	8.21 ± 0.20f ^{g}	2.97 ± 0.04 ^{e}	1.20 ± 0.11 ^{b}
	Ripe	Acetone	2.44 ± 0.02 ^{g}	1.37 ± 0.15 ^{e}	1.61 ± 0.10 ^{a}
		Methanol	2.97 ± 0.16 ^{g}	1.27 ± 0.02 ^{e}	0.77 ± 0.01 ^{def}
		Water	2.33 ± 0.16 ^{g}	3.25 ± 0.08 ^{e}	0.82 ± 0.10 ^{de}
		70% Methanol	5.67 ± 0.18f ^{g}	1.61 ± 0.01 ^{e}	0.67 ± 0.01 ^{def}

Each value represents the mean ± standard error of three replicates. Mean values followed by different letters in their superscript are significantly different from each other (p = 0.05) in the respective column according to Duncan's multiple range test. GAE—Gallic acid equivalent; QE—Quercetin equivalent; AE—Atropine equivalent.

3.5. Antioxidant Activities

The impressive phytochemical profile of seeds and pulp of *R. minor* leads to the anticipation of their excellent antioxidant activity. Hence, we analyzed their antioxidant capacity with three in vitro methods, DPPH radical scavenging activity, total antioxidant activity, and FRAP assay, and the results are presented in Table 5. Parallel to the phytochemical composition, unripe seeds exhibit the highest antioxidant potential in all the methods, whereas pulp showed comparatively less activity and the least was shown by ripened pulp. The 70% methanol showed more activity in all the tested methods than other samples' extracts, except in unripe pulp, where acetone extract surpassed it.

In DPPH radical scavenging activity, the 70% methanol extract of unripe and ripened seeds showed 210.47 and 174.01 mg GAE/g DW, respectively, whereas unripe and ripened pulp exhibited 3.46 and 2.15 mg GAE/g DW activity, respectively. The activity was 5.62 and 1.14 mg GAE/g DW in the acetone extracts of unripe and ripened pulp, respectively. The total antioxidant activity of unripe and ripened seeds with 70% methanol extract was 237.98 and 177.21 mg AAE/g DW, respectively. The unripe and ripened pulp with acetone extract manifested 102.97 and 22.04 mg AAE/g DW, respectively, whereas, with 70% methanol extract, it was 32.81 and 39.60 mg AAE/g extract, respectively. In FRAP activity, the 70% methanol extract of unripe seed outperformed the standard ascorbic acid with an activity of 1783.87 mg AAE/g DW. The activity was 363.51, 6.24, and 4.13 mg AAE/g DW for ripened seed and unripe and ripened pulp's 70% methanol extracts, respectively.

Table 5. Antioxidant activities of *Rourea minor* fruit extracts.

Fruit Parts		Solvent	DPPH (mg GAE/g DW of the Sample)	TAA (mg AAE/g DW of Sample)	FRAP (mg AAE/g DW of the Sample)
Seed	Unripe	Acetone	46.77 ± 2.57 ^{c}	58.30 ± 1.08 ^{de}	104.21 ± 2.12 ^{c}
		Methanol	43.67 ± 3.41 ^{c}	69.59 ± 3.92 ^{d}	73.05 ± 7.88 ^{d}
		Water	1.33 ± 0.16 ^{d}	4.45 ± 0.15 ^{i}	11.81 ± 0.61 ^{f}
		70% Methanol	210.47 ± 8.28 ^{a}	237.98 ± 17.77 ^{a}	1783.87 ± 45.15 ^{a}
	Ripe	Acetone	13.68 ± 1.69 ^{d}	9.72 ± 0.62 ^{i}	2.63 ± 0.25 ^{f}
		Methanol	12.99 ± 3.85 ^{d}	50.89 ± 5.20 ^{ef}	34.84 ± 1.06 ^{e}
		Water	3.07 ± 0.37 ^{d}	6.58 ± 0.51 ^{i}	0.55 ± 0.04 ^{f}
		70% Methanol	174.01 ± 23.69 ^{b}	177.21 ± 15.03 ^{b}	363.51 ± 8.25 ^{b}
Pulp	Unripe	Acetone	5.62 ± 0.40 ^{d}	102.97 ± 3.91 ^{c}	8.80 ± 0.55 ^{f}
		Methanol	1.00 ± 0.04 ^{d}	7.59 ± 0.59 ^{i}	2.67 ± 0.25 ^{f}
		Water	0.59 ± 0.03 ^{d}	6.10 ± 0.49 ^{i}	1.11 ± 0.03 ^{f}
		70% Methanol	3.46 ± 0.34 ^{d}	32.81 ± 1.62 ^{gh}	6.24 ± 0.35 ^{f}
	Ripe	Acetone	1.14 ± 0.12 ^{d}	22.04 ± 0.96 ^{hi}	3.17 ± 0.33 ^{f}
		Methanol	1.37 ± 0.13 ^{d}	36.80 ± 4.30 ^{fgh}	3.03 ± 0.29 ^{f}
		Water	0.96 ± 0.08 ^{d}	15.62 ± 0.66 ^{i}	6.15 ± 0.10 ^{f}
		70% Methanol	2.15 ± 0.10 ^{d}	39.60 ± 0.92 ^{fg}	4.13 ± 0.29 ^{f}

Each value represents the mean ± standard error of three replicates. Mean values followed by different letters in their superscript are significantly different from each other (p = 0.05) in the respective column according to Duncan's multiple range test. Each value represents the mean ± standard error of three replicates. GAE—Gallic acid equivalent; AAE—Ascorbic acid equivalent.

4. Discussion

Providing people with a safe, healthy, and nourishing food source today has proven to be difficult, especially for low-income groups and undernourished populations in developing nations. Due to food scarcity, high prices, and an unstable supply of healthy food in emerging and underdeveloped nations, finding affordable and alternative sources of good and nourishing food has become a major concern. Unlocking the potential of underutilized wild edible plants will enable us to offer a significant answer to the problem of food insecurity [5]. Underutilized fruits and nuts are rich sources of nutrients, minerals, and other bioactive compounds such as phenolics, flavonoids, terpenoids, and alkaloids [5]. The exploration of nutritional and phytochemical analysis and their utilization can solve hunger and malnutrition in certain regions of the world [35–40]. Researchers are generating information on the nutritional and phytochemical properties of several underutilized fruits such as *Baccarurea rmiflora* [38], *Bridelia stipularis* [39], *Carrisa carandas*, *Dovyalis hebecarpa*, *Flocourtia indica*, *Malphighia emerginata*, *Slacia chinensis*, *Syzygium indica* [36], *Diospyros chloroxylon* [29], *Garcinia livingstonei* [41], and *Garcinia morella* [42,43]. Such information is helpful in the proper utilization of unexplored wild fruits, and their cultivation, improvement, and utilization. In the current study, we carried out the nutritional, phytochemical, and antioxidant evaluation of unripe and ripened fruits of *Rourea minor*, which is an underutilized fruit of the tropical region. Proximate analysis revealed that ripened pulp possessed 0.19%, 0.34%, 0.90%, 0.35%, and 0.98% of fat, protein, carbohydrates, ash, and fiber, respectively. The protein, fat, carbohydrate, ash, and fiber content of *Rourea minor* pulp is comparable to the nutrients of wild fruits of Africa such as Kei apple (0.4%, 0.4%, 4.7%, 0.3%, 0.3% of protein, fat, carbohydrate, ash, and fiber), wild plum (0.9%, 0.4%, 19.2%, 0.5%, 1.3% of protein, fat, carbohydrate, ash, and fiber), wild apricot (1.0%, 0.2%, 33.1%, 0.6%, 0.8% of protein, fat, carbohydrate, ash, and fiber), and sour plum (3.1%, 1.3%, 26.3%, 1.4%, 0.7% of protein, fat, carbohydrate, ash, and fiber) [35]. The fat, protein, and fiber values of *Rourea minor* fruit pulp were equivalent to the chemical composition of major fruits such as lemon (0.8%, 0.9%, and 1.6% fat, protein, and fiber, respectively) [44] and strawberry (0.11%, 0.43 and 2.1% fat, protein and fiber, respectively) [44,45]. *Rourea minor* pulp comprised 0.92 mg/g DW of phosphorus, 4.98 mg/g DW of nitrogen, 12.20 mg/g

DW of potassium, 0.96 mg/g DW of sodium, 5.80 mg/g DW of sulfur, 7.20 mg/g DW of magnesium, and 6.80 mg/g DW of calcium as part of its mineral composition. Ripe pulp had 25.98, 2523.56, 499.12, 33.62, and 40.30 g/g DW of boron, iron, zinc, copper, and manganese, respectively. The composition of minerals of the pulp of *Rourea minor* was similar to those of wild fruits such as *Carrisa carandas*, *Dovyalis hebecarpa*, *Flocourtia indica*, *Malphighia emerginata*, *Slacia chinensis*, and *Syzygium indica* [36]. The findings of the nutritional study show that matured fruit pulp from *Rourea minor*, which is crucial for human nutrition and health, is a significant source for meeting nutritional needs. We evaluated the nutritional value of *Rourea minor* seeds in addition to the pulp's nutritional value. The ripened seed had 0.28% fat, 12.39% protein, 25.70% carbohydrates, 1.4% ash, and 3.4% fiber. These values are similar to those of *Garcinia morella* seeds, except for fat values, which were 38.08%, 8.50%, 36.25%, 3.90%, and 16.58%, respectively [42,43]. The ash and fiber values of *Rourea minor* seeds were comparatively lower than seeds of apple (3.7–5.20% ash and 3.92–4.32% ash and fiber) [46]. Phytate and oxalate were the major antinutritional elements considered in this investigation. Due to their capacity to bind with and hinder the bioavailability of iron from plant-based diets, these antinutritional factors were chosen [47]. The amount of phytate and oxalate in the ripened pulp were 0.59 and 6.50 mg/g and with the seed were 6.91 and 31.99 mg/g. Pulp comparatively has lesser values of antinutrients and could be used for human consumption, whereas the seeds were having higher value antinutrients and therefore could be used for roughage for animal feeding.

Perera et al. [36] investigated the total phenolic content (TPC) of six underutilized fruits (*Carrisa carandas*, *Dovyalis hebecarpa*, *Flocourtia indica*, *Malphighia emerginata*, *Slacia chinensis*, *and Syzygium indica*) and showed that values varied from 6.77 to 10.29 mg GAE/g on a fresh weight basis. Whereas the *Baccaurea sapida* fruits and total flavonoid content (TFC) was 32.78 µg GAE/g and 71.57 µg RE/g [38]. The TPC, TFC and alkaloid content obtained from fruits in this study were 119.45 mg GAE/g DW, 44.10 mg QE/g DW, 0.88 mg AE/g DW in ripe seeds, and 5.67 mg GAE/g DW, 1.61 mg QE/g DW and 0.67 mg AE/g DW in ripe pulp, respectively, in the 70% methanol extract. The value of TPC, TFC, and alkaloids was sequestered optimally in 70% methanol extract compared to acetone, absolute methanol, and water extracts. The fact mentioned above might be due to the serial extraction of a sample with different solvents, and subsequently, the phytocompounds were divided according to their affinity. However, as the fresh materials were used for 70% methanol, it dissolved the highest phytochemicals. The present study indicates that the TPC and TFC values were higher than the other wild fruits viz. *Aegle marmelos*, *Hamidesmus indicus*, *Cassia auriculata*, *Scoparia dulcis*, *Tinospora cordifolia*, *Aerva lanata*, and *Sida rhombifolia* [48]. The TPC and TFC of citrus fruits were reported to be 204.40 mg/GAE g and 27.50 mg QE/g and TPC values of *Rourea minor* fruit pulp were comparatively lower than citrus fruits [44]. The phytochemicals are also separated into solvents based on the polarity of their solvent [18]. Similar efforts to extract phytochemicals from the fruit of *Dacryodes rostrata* were conducted by Thavamoney et al. [49], who serially extracted pulp using the solvents hexane, ethyl acetate, butanol, ethanol, and water. They discovered a considerable variance in the distribution of phytochemicals among the solvents. In ethanol, butanol, and ethyl acetate, the TPC values were 11.35, 0.60, and 0.13 mg GAE/g DW, respectively. The flavonoid distribution, however, underwent a significant alteration. They discovered flavonoids in ethanol, butanol, and ethyl acetate at concentrations of 3.87, 0.30, and 92.89 mg QE/g DW, respectively. Other researchers have also demonstrated the solvent influence on phytochemical solubility [50,51]. In the current study, 70% methanol was found suitable for the extraction of TPC, TFC, and alkaloids.

The antioxidant activities assessed in *Rourea minor* fruit extracts carried out by DPPH, TAA, and FRAP assays and 70% methanol extract depicted better antioxidant activities, and seed extract demonstrated the highest activities (174.01 mg GAE/g DW, 177.21 AAE/G DW, 363.51 AAE/g DW as per DPPH, TAA and FRAP assays with ripe fruit extracts). The antioxidant activities were higher with seed extracts than with pulp extracts and it is obvious that the TPC and TFC contents were higher with seeds. The antioxidant

properties of wild and underused fruits have been successfully demonstrated by a number of additional experiments employing a variety of antioxidant assays. For instance, in terms of the ability to scavenge DPPH radicals in *Diospyros chloroxylon*, the unripe pulp performed better than the ripened one, and acetone extract performed better than methanol and water extracts [29]. In a related examination of the antioxidant activities of peel, pulp, and seed fractions of twenty-eight common fruits, the antioxidant values (FRAP test) of fruit pulp ranged from 0.14 to 13.42 mmol/g on a wet weight basis, which was greater than that of the studied fruits [52]. The IC_{50} values for DPPH radical scavenging activity in a study similar to the one carried out in order to determine the antioxidant activities of berries were as follows: blueberry, 0.70 mg/mL; raspberry, 0.80 mg/mL; blackberry, 1.40 mg/mL; and strawberry, 5.60 mg/mL, thus showing lower antioxidant activity than the fruits tested in the present study [53]. Based on the findings described here, it can be concluded that fruits are a rich source of bioactive chemicals and have the potential to be used to create value-added goods and other dietary applications that will improve health benefits.

5. Conclusions

In India, *Rourea minor* is a species that is not utilized to its full potential, but its fruits are still used in culinary preparations. However, in order to fully exploit the species, it is important to understand its nutritional value. The present study aims to comprehensively analyze the basic nutritional parameters, including proximate and elemental composition, anti-nutritional factors, phytochemical composition, and antioxidant activities using various solvent systems. The unripe and ripened seeds and pulp were found to have good proximate composition and elemental content, particularly microelements. The seeds, especially the unripe ones, and the pulp were rich in phytochemicals. Extracting the seeds and pulp with 70% methanol was the most effective method for obtaining high levels of phytochemicals. The antioxidant activity was consistent with the phytochemical composition, with both the seeds and pulp demonstrating impressive activity. According to the results of this study, the fruit of *Rourea minor* has the potential to be used in value-added products and other food applications, as it is a rich source of bioactive chemicals.

Author Contributions: Conceptualization, methodology, H.N.M.; formal analysis and data curation, H.N.M., G.G.Y. and S.S.K.; validation, G.G.Y. and S.S.K.; visualization, G.G.Y., S.S.K. and Y.H.D.; investigation, A.H.P., R.J. and V.Y.; writing, review and editing, H.N.M., G.G.Y. and Y.H.D. All authors have read and agreed to the published version of the manuscript.

Funding: Researchers Supporting Project number (RSP2023R375), King Saud University, Riyadh, Saudi Arabia.

Data Availability Statement: All data are presented in the article.

Acknowledgments: The authors acknowledge the Researchers Supporting Project number (RSP2023R375), King Saud University, Riyadh, Saudi Arabia.

Conflicts of Interest: The authors declare no conflict of interest.

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