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Evaluation of the food, nutrition value, and α-glucosidase inhibitory activity of the ripe and unripe fruit of *Rubus steudneri* Schweinf

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Abstract

Rubus steudneri Schweinf. (Rosaceae) is one of the three unstudied *Rubus* species that grow in Ethiopia. The present work was aimed to determine the physicochemical characteristics, micronutrients, anthocyanins, and antinutrients of ripe and unripe *R. steudneri* fruits using a range of analytical protocols. The present work also investigated the α-glucosidase inhibitory activity of ripe and unripe fruits. The total and acid-insoluble ash contents of ripe fruits were higher than those of unripe fruits. Changes in the micronutrient contents and antinutrients were also observed during ripening. Ripe fruits contained anthocyanins, mineral elements, and water-soluble vitamins, namely niacin and pyridoxine, in higher quantities than in unripe fruits. Toxic heavy metals were not detected in both ripe and unripe fruits. There were lower amounts of tannin and phytic acid in ripe fruits than in unripe fruits. Both ripe and unripe fruit extracts displayed inhibitory activity against α-glucosidase. Greater inhibitory activity was shown by ripe fruits than unripe fruits, as indicated by the IC_{50} values. The total phenolics and flavonoids were slightly higher in unripe fruits. The lower contents of antinutrients (tannin and phytic acid), and the absence of toxic heavy metals indicate the edibility of *R. steudneri* fruits. The ripe and unripe fruits can also be used to manage diabetes mellitus as the extracts displayed inhibitory potential against α-glucosidase. The observed inhibitory activity could be ascribed to the phenolics and flavonoids of the fruits.

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Introduction

Fruits are healthy food resources, and consumed worldwide as nutrient sources including minerals. Fruits also contain many bioactive substances such as phenolics, flavonoids, glycosides, anthocyanins, tannins, and others which have several beneficial effects on health. Fruits are among the richest reservoirs of vitamins, minerals, and other key nutrients, and they have medicinal effects as well. Furthermore, fruits form an integral part of the economic income of many countries (Nandal and Bhardwaj, 2015; Belwal *et al.,* 2019). Upon ripening, fruits become softer and more palatable while also exhibiting textural modifications. Ripening involves a modification of pectin and hemicellulose in the cell wall, and an increased solubilisation of the pectic substances, together with a progressive loss of tissue firmness. During ripening, many other changes also occur such as changes in anthocyanins, ascorbic acid, sugars, and organic acids along with colour changes (Kim *et al.,* 2013; Ghai *et al.,* 2016). *Rubus steudneri* Schweinf (locally known as *gora*) is an important edible plant belonging to the family Rosaceae. This plant is a scandent shrub, and characterised by deeply furrowed stems covered with stellate hairs or sometimes prickles. The fruits of *R. steudneri* are edible, and traditionally consumed as food and medicine (Giday *et al.,* 2009; Yineger *et al.,* 2013; Kefalew *et al.,* 2015). The leaf, stem, and fruit of

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R. steudneri also form a diet for gorillas in Bwindi Impenetrable National Park, Uganda (Rothman *et al.,* 2006). In a previous study, we showed antiradical and lipid peroxidation inhibitory activity of the ripe and unripe fruit extract of *R. steudneri* (Raghavendra and Kekuda, 2018). To the best of our knowledge, there is no report available on the micronutrient analysis and α-glucosidase inhibitory activity of *R. steudneri* fruits. Keeping in mind the ethnobotanical details regarding the edible nature of the fruit, in the present work, we analysed the physicochemical parameters and anthocyanins, antinutrients, and micronutrients such as vitamins and minerals, as well as the α-glucosidase inhibitory activity of ripe and unripe *R. steudneri* fruits.

Materials and methods

Plant collection and identification

The ripe and unripe *R. steudneri* fruits were collected in Nekemte, East Wollega zone, Oromia region, Ethiopia (9°5′N 36°33′E to 9.083°N 36.550°E) with an elevation of 2123 meters above sea level. The plant materials were identified and authenticated at the Ethiopian Biodiversity Institute, Addis Ababa, Ethiopia.

Physicochemical characteristics of ripe and unripe R. steudneri fruits

The physicochemical properties namely moisture, total ash, acid-insoluble ash content, and pH of ripe and unripe *R. steudneri* fruits were evaluated.

Moisture content

The weight-difference method as described by Arambewela and Arawwawala (2010) was employed to determine the moisture content of the ripe and unripe fruits. Briefly, a known quantity of the fruit material (5 g) was taken in a pre-weighed weighing dish, spread uniformly, and kept in an oven at 100°C for drying. Following this, the dish was cooled and weighed again. The moisture content was determined using Eq. 1:

$$\text{Moisture (\%)} = \frac{\text{Loss of weight}}{\text{Weight of sample}} \text{X} 100 \quad \text{(Eq. 1)}$$

Total ash

The ash content of ripe and unripe fruits was determined using the methodology described by Arambewela and Arawwawala (2010). Briefly, a known quantity of the fruit material (2 g) was taken in a pre-weighed silica crucible, and ignited by gradually raising the temperature to 500°C until the sample turned white (indicating the absence of carbon). The crucible was cooled and weighed. The total ash content was determined using Eq. 2:

$$\text{Total ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \text{X} 100 \quad \text{(Eq. 2)}$$

Acid-insoluble ash

Briefly, 25 mL 2 N HCl was added to the crucible containing total ash, covered with a watch glass, and boiled gently for 5 min. Upon cooling, the acid-insoluble content of the ash was collected on ashless filter paper. The filter paper was washed with hot water until the filtrate was neutral in reaction. The filter paper with acid-insoluble material was taken in a pre-weighed crucible, dried, followed by ignition at high temperature, cooling, and weighing (Arambewela and Arawwawala, 2010). The acid-insoluble ash content was determined using Eq. 3:

$$\text{Acid insoluble ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \text{X} 100 \quad \text{(Eq. 3)}$$

pH

Briefly, 5% (w/v) aqueous solution of ripe and unripe fruits was prepared. The pH was determined using a calibrated digital pH meter (Sigma-27 DP) following AOAC method (AOAC, 2000). The pH meter was calibrated using 4, 7, and 9 pH standard solutions prior to the determination of pH.

Mineral

The inductively coupled plasma with optical emission spectroscopic technique (ICP-OES, Agilent Technologies 700 series, US) was employed to estimate the content of major elements [calcium (Ca), potassium (K), sodium (Na), and magnesium (Mg)], minor elements [iron (Fe), zinc (Zn), nickel (Ni), and copper (Cu)], and heavy metals [mercury (Hg), tin (Sn), cadmium (Cd), arsenic (As), and lead (Pb)] in microwave-digested samples (using ultrapure metal-free nitric acid) of ripe and unripe fruits (Dileep *et al.,* 2013). The calibration standards were prepared by diluting the standard solution of elements (1,000 mg/L) in nitric acid. Table 1 gives further details of the instrumental configuration and general experimental conditions used.

Vitamins

Agilent Technologies 1260 Infinity Series liquid chromatography system (Agilent, USA) was used for the determination of vitamins (vitamin C,

Table 1. ICP-OES operational conditions.

Parameter	Value
Power (kW)	1.20
Plasma flow (L/min)	15.00
Auxiliary flow (L/min)	1.50
Nebulizer flow (L/min)	0.75
Sample flow rate (L/min)	1.50
Replicate read time (s)	3.00
Instrument stabilisation delay (s)	15.00
Sample uptake delay (s)	10.00
Pump rate (rpm)	15.00
Rinse time (s)	10.00
Spray chamber	Cyclonic type
Element, wavelength (nm)	Ca (422.673), Cu (327.395), Na (589.592), Fe (238.204), K (766.491), Mg (279.553), Ni (231.604), Zn (213.857), As (193.696), Cd (226.502), Hg (253.652), Pb (220.353), Sn (189.925)

B1, B_3, B_6, and B_{12}) in ripe and unripe fruits. The chromatographic system was equipped with G1312C binary pump, a degasser, an Agilent Technologies S-6020 injector, a variable-wavelength UV, photodiode array (PDA) detector, and a column thermostat. The Prevail™ C18 column (5 µm particle size, 250 × 4.6 mm) was used. The instrument control, and data acquisition and analysis were performed using Chemstation Software (Agilent Technologies). The in-house method of ITC Life Sciences and Technology Centre, Bangalore, Karnataka, India was used to determine the vitamin contents.

Vitamin C

The vitamin C content was estimated in the ripe and unripe fruits (0.1 g/mL fruit material sonicated to dissolve in water and filtered) using an isocratic 25 mm KH_2PO_4 (in water adjusted to pH 2.5 with diluted orthophosphoric acid) mobile phase at a flow rate of 1 mL/min. The temperature of the analytical column was kept at 35°C. The wavelength of the detection was set at 250 nm to detect the eluent, and the run time was 30 min. The vitamin C content was estimated by comparing the retention time/peak areas of the standard vitamin C (0.5 mg/mL; Sigma Aldrich, USA) using Eq. 4.

$$\text{Vitamin content (mg/kg)} = \frac{A1}{B1} X \frac{C1}{C2} X \text{purity of vitamin C} \quad \text{(Eq. 4)}$$

where, A1 = average area of the sample in duplicate injections, B1 = average area of standard in duplicate injections, C1 = standard concentration in g/mL, and C2 = sample concentration in g/mL.

Vitamins B_1, B_3, B_6, and B_{12}

For the estimation of thiamine (vitamin B_1), nicotinic acid (vitamin B_3), and pyridoxine (vitamin B_6), 0.2 g/mL fruit powder and a pinch of amylase in acidified water were allowed to stand for 30 min at 40°C. The mixture was then centrifuged at 5,000 rpm for 10 min, and filtered through 0.45 mm syringe filters. The filtrate was used to estimate vitamins B_1, B_3, and B_6. For cobalamin (vitamin B_{12}), 0.3 g/mL fruit powder was vortexed in KH_2PO_4 solution (1 g KH_2PO_4 in 500 mL water) for 10 min, and centrifuged. The filtrate was used to estimate vitamin B_{12}.

A sample volume of 20 µL was injected into the column. The gradient was made of either (A) 500 mg heptane sulphonic acid and 1 mL glacial acetic acid in 1 L of water, or (B) 100% methanol. The solvent gradient employed was as follows: 0 - 8 min, 90% A and 10% B; 8 - 12 min, 80% A and 20% B; 12 - 16 min, 70% A and 30% B; 16 - 20 min, 90% A and 10% B at a flow rate of 0.7 mL/min. The temperature of the analytical column was maintained at 40°C. The wavelength of detection was set to 254, 265, 280, and 361 nm to detect the eluent. The desired vitamin content was quantified by comparing

the retention time/peak areas of the standard vitamin B_1, B_3, B_6, and B_{12} (500 ppm, Sigma Aldrich, USA) using Eq. 5.

$$TAC (\% w/w) = (A \times MW \times DF \times 100)/(\varepsilon x \ell) \quad (Eq. 5)$$

Anthocyanin

The total anthocyanin content (TAC) of ripe and unripe fruit powders was estimated by the pH differential method (Price *et al.,* 1978). Briefly, the fruit powders were diluted in two buffer solutions namely 0.025 M potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5) at a 1:5 ratio. After 15 min, the absorbance was measured simultaneously at 516 and 700 nm. Absorbance was measured against distilled water as a blank. The TAC was expressed as cyanidin-3-glucoside (% w/w) equivalents (CYE) in 100 g of dried powder (mg CYE/100 g dried powder) using Eq. 6:

$$\text{Vitamin (mg/kg)} = \frac{\text{Sample area X Standard concentration X Dilution factor X Purity}}{\text{Standard area X Weight of the sample}} \quad (Eq. 6)$$

where, $A = (A_{516nm} - A_{700nm})_{pH1.0} - (A_{516nm} - A_{700nm})_{pH4.5}$; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside; DF = dilution factor; ε = molar absorbance; and ℓ= path length in cm.

Antinutrients

Tannin

The tannin content of ripe and unripe fruit powders was determined following the method of Price *et al.* (1978) using the reaction between condensed tannin and vanillin in the presence of mineral acid, resulting in the development of a red colour. The absorbance was read at 500 nm, and a standard curve was constructed using catechin. The results were expressed as catechin equivalents.

Phytic acid

The phytic acid content of ripe and unripe fruit powders was determined by analysing the phytate phosphorus (Ph-P) following the method described by Wheeler and Ferrel (1971). The phytic acid was estimated by multiplying the amount of Ph-P by a factor of 3.55 based on the empirical formula $C_6P_6O_{24}H_{18}$ (660 g) and the phytic phosphorus (P6) molecular mass (186 g) (*i.e.,* phytate = P × 3.55), and the results were expressed as phytic acid in mg per 100 g (db).

Extraction of ripe and unripe R. steudneri fruits

The whole fruit powder was subjected to Soxhlet extraction following the method described by Bhagath *et al.* (2013). Methanol was used as solvent. The filtrate containing extracted material was subjected to evaporation using rotary flash evaporator to obtain the extract of the ripe and unripe fruits.

α-glucosidase inhibitory activity of fruit extracts

The method described by Ohta *et al.* (2002) was employed to evaluate the α-glucosidase inhibitory activity of ripe and unripe fruit extracts (10 - 50 µg/mL). Acarbose (0.5 - 2 µg/mL) was used as reference compound. Absorbance was measured at 510 nm (Molecular Devices, Versamax Microplate Reader). The inhibition of enzyme activity was calculated using Eq. 7:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100 \quad (Eq. 7)$$

The IC_{50} value was determined from the % inhibition versus concentration plot using non-linear regression formulae.

Total phenolic contents of fruit extracts

The total phenolic contents were estimated using Folin-Ciocalteau's reagent (FCR) method as described by Badejo *et al.* (2016). Gallic acid was used as reference compound. The total phenolic contents were expressed as milligrams of gallic acid equivalents (GAE)/100 g of fruit material.

Total flavonoid contents of fruit extracts

The total flavonoid contents were estimated by the aluminium chloride colorimetric estimation method as described by Badejo *et al.* (2016). Catechin was used as reference compound. The total flavonoid contents were expressed as milligram catechin equivalents (CE) per 100 g of fruit material.

Statistical analysis

The experiments were performed in triplicates. All data collected were subjected to descriptive and *t*-test analysis using GraphPad Prism 7.0 (GraphPad Software, Inc., San Diego, CA). The data were expressed as means ± standard deviations (S.D.) at a confidence level of determination of $p < 0.05$. The IC_{50} value was determined from the % inhibition versus concentration plot using non-linear regression formulae ($y = a + bx$; where x = concentration, and y = % inhibition).

Results

The physicochemical parameters of ripe and

Table 2. Physicochemical parameters of ripe and unripe *R. steudneri* fruits.

S. No.	Parameter	Unripe fruit	Ripe fruit	*t*-value	*p*-value
1	Moisture (%)	5.91 ± 0.32	6.42 ± 0.21	2.27	0.085
2	Total ash (%)	3.88 ± 0.25	4.69 ± 0.16	4.59	**0.010**
3	pH	6.40 ± 0.10	5.60 ± 0.10	9.66	**0.006**
4	Acid-insoluble ash (%)	0.40 ± 0.06	0.56 ± 0.09	2.66	0.056

Table 3. Major and minor elements of ripe and unripe *R. steudneri* fruits.

S. No.	Parameter (mg/kg)	Unripe fruit	Ripe fruit	*t*-value	*p*-value
1	Calcium (Ca)	4075.70 ± 91.50	5615.65 ± 102.78	19.20	**0.000**
2	Potassium (K)	12062.70 ± 215.44	13034.50 ± 195.00	5.91	**0.004**
3	Magnesium (Mg)	2408.70 ± 55.17	2795.90 ± 43.10	10.50	**0.001**
4	Sodium (Na)	120.00 ± 8.50	160.80 ± 10.57	2.88	**0.045**
5	Iron (Fe)	200.30 ± 10.31	315.90 ± 25.65	6.11	**0.004**
6	Nickel (Ni)	4.70 ± 1.00	6.40 ± 0.54	2.32	0.081
7	Copper (Cu)	34.70 ± 4.78	39.11 ± 2.40	1.19	0.302
8	Zinc (Zn)	38.44 ± 2.36	41.70 ± 1.57	1.74	0.156
9	Arsenic (As)	BDL	BDL	-	-
10	Cadmium (Cd)	BDL	BDL	-	-
11	Mercury (Hg)	BDL	BDL	-	-
12	Lead (Pb)	BDL	BDL	-	-
13	Tin (Sn)	BDL	BDL	-	-

BDL = below detection limit.

unripe fruits are shown in Table 2. The moisture, total ash ($p < 0.010$), and acid-insoluble ash contents of ripe fruit were higher than those of unripe fruits. The pH of ripe fruit ($p < 0.006$) was slightly lower than that of unripe fruit.

The major and minor elements of ripe and unripe fruits are shown in Table 3. There were more mineral elements in ripe fruit than in unripe fruit. Potassium ($p < 0.004$) and nickel were the highest among the major and minor elements, respectively, in both ripe and unripe fruits. Heavy metals were not detected in both ripe and unripe fruits.

Table 4 shows the vitamin contents of ripe and unripe fruits. Thiamine and cobalamin were not detected in both ripe and unripe fruits. Vitamin C was detected only in ripe fruit. Niacin ($p < 0.023$) and pyridoxine ($p < 0.038$) were detected in both ripe and unripe fruits, but to a greater degree in ripe fruit than in unripe fruit.

The content of anthocyanins was shown to be significantly ($p < 0.004$; $t = 6.14$) higher in ripe fruit (0.34 ± 0.009 mg CYE/100 g dried powder) than in unripe fruit (0.29 ± 0.005 mg CYE/100 g dried powder).

Table 5 shows the antinutrients, namely, tannin and phytic acid, in ripe and unripe fruits. Tannin content was slightly higher in unripe fruit than in ripe fruit. Phytic acid content in ripe fruit was significantly lower ($p < 0.007$) than that in unripe fruit.

Figure 1 shows the effect of ripe and unripe fruit extracts on the activity of α-glucosidase. The extracts displayed dose-dependent inhibitory activity against the enzyme. Among the extracts, marked inhibitory activity was displayed by ripe fruit extract (IC_{50} = 24.18 µg/mL) relative to the unripe fruit extract (IC_{50} = 32.67 µg/mL). The reference drug acarbose was more effective (IC_{50} = 0.76 µg/mL) relative to both fruit extracts.

The total phenolic and flavonoid contents of ripe and unripe fruit extracts are shown in Table 6. The unripe fruit extract was shown to contain higher total phenolic and flavonoid contents than the ripe fruit extract.

Table 4. Vitamins of ripe and unripe *R. steudneri* fruits.

Vitamin	Unripe fruit	Ripe fruit	*t*-value	*p*-value
Thiamine (B_1) (g/100 g)	-	-	-	-
Niacin (B_3) (g/100 g)	0.05 ± 0.00	0.09 ± 0.02	3.57	**0.023**
Pyridoxine (B_6) (g/100 g)	0.06 ± 0.01	0.08 ± 0.00	3.05	**0.038**
Cyanocobalamin (B_{12}) (g/100 g)	-	-	-	-
Vitamin C (mg/1000 g)	-	0.12 ± 0.03	-	-

Table 5. Antinutrients of ripe and unripe *R. steudneri* fruits.

Antinutrient	Unripe fruit	Ripe fruit	*t*-value	*p*-value
Tannin (%)	0.15 ± 0.03	0.14 ± 0.05	0.36	0.739
Phytic acid (mg/100 g)	10.26 ± 1.15	5.85 ± 0.95	5.11	**0.007**

Table 6. Total phenolics and flavonoids of ripe and unripe *R. steudneri* fruits.

Parameters	Unripe fruit (mg/100g)	Ripe fruit (mg/100 g)	*t*-value	*p*-value
Total phenolics	172.21 ± 6.84	149.71 ± 5.25	4.52	**0.011**
Total flavonoids	64.72 ± 3.69	52.61 ± 1.98	5.00	**0.008**

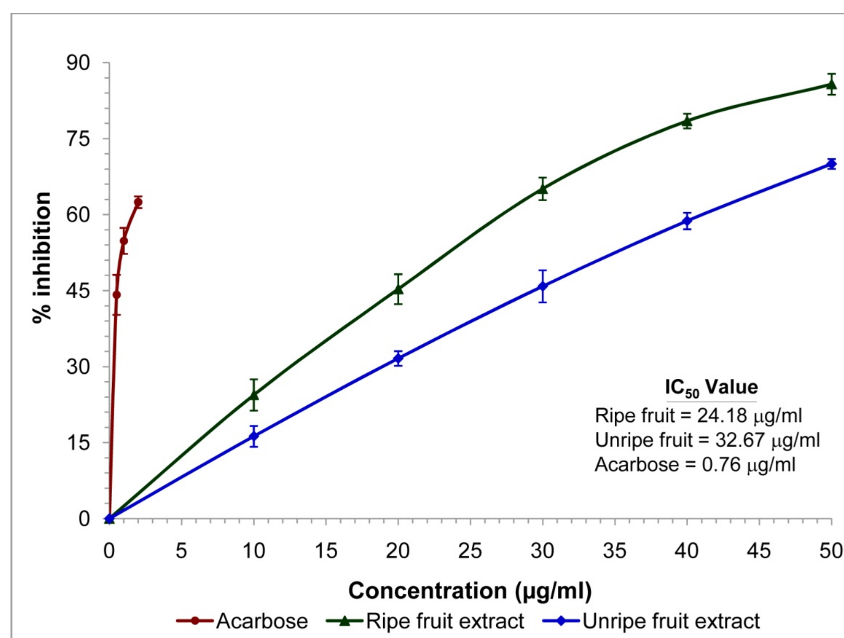

Figure 1. α-glucosidase inhibitory activity of ripe and unripe *R. steudneri* fruit extracts.

Discussion

The present work showed that the moisture content of the studied fruits considerably increased during ripening (5.91 to 6.42%). A similar result was observed in earlier study by Appiah *et al.* (2011), in which the moisture content of ripe *Mangifera indica* fruit was higher than that of unripe fruit. The ash content reflects the quantity of its inorganic constituents and other impurities. The determination of total ash is important as far as the detection of metals, salts, and silica is concerned. Acid-insoluble ash content reflects the presence or contamination of silica and other materials (Alam and Us Saqib, 2015;

Shirsat *et al.,* 2017). The ash content of ripe fruit was higher ($p < 0.010$) than that of unripe fruit. In a similar study, Badejo *et al.* (2016) observed a slightly higher content of total ash in ripe tomato than in unripe tomato. In the present work, the low value of acid-insoluble ash indicated that *R. steudneri* fruit is not contaminated by sand or similar material. Ripe fruit had slightly lower pH values ($p < 0.006$) than unripe fruit, which indicated a decrease in pH during ripening. Similarly, in a study by Adi *et al.* (2019), a decrease in pH during ripening was observed in plantains (*Musa* ABB).

Together with vitamins, minerals exert physiological effect on human health. Mineral elements are considered micronutrients, and required in small amounts for normal physiology. Around 25 elements are indispensable for normal human health. Based on the quantity required, minerals are classified into major (required in a quantity of > 100 mg/day) and minor elements (< 100 mg/day). Many analytical methods are available for estimating mineral elements in plants (Dileep *et al.,* 2013; Satyanarayana and Chakrapani, 2013; Swathi *et al.,* 2019). In the present work, the ripe and unripe fruits were subjected to elemental analysis with ICP-OES, a recently developed and sophisticated methodology for determining the quantity of many elements at once (Dileep *et al.,* 2013). It was observed that ripe fruit contained minerals in greater quantity than unripe fruit. The levels of potassium ($p < 0.004$) and sodium ($p < 0.045$) were the highest and lowest, respectively, in both ripe and unripe fruits. Among the minor elements, iron ($p < 0.004$) was the highest while nickel the lowest. Interestingly, heavy metals namely arsenic, cadmium, mercury, lead, and tin were not detected in both ripe and unripe fruits.

Niacin is an important water-soluble vitamin, also known as nicotinic acid or pellagra preventive factor. Niacin acts as a coenzyme, and is important for dehydrogenase activity. Pyridoxine (vitamin B_6) is important in the synthesis of serotonin, histamine, and niacin coenzymes from amino acids. Pyridoxal phosphate, the coenzyme form of pyridoxine, participates in some reactions such as transamination, decarboxylation, deamination, and condensation (Satyanarayana and Chakrapani, 2013; Maqbool *et al.,* 2017). In the present work, both niacin ($p < 0.023$) and pyridoxine ($p < 0.038$) were identified in ripe and unripe fruits, being higher in ripe than in unripe fruit. Thiamine is a coenzyme in metabolism of carbohydrates and branched chain amino acids. Cobalamin is the largest member of the vitamin B complex, and important for proper functioning of many enzymes, and considered as antipernicious anaemia factor (Satyanarayana and Chakrapani, 2013; Maqbool *et al.,* 2017). In the present work, neither vitamin B_1 nor vitamin B_{12} were detected in ripe or unripe fruits. Vitamin C is a water-soluble vitamin, and plays an important role in the prevention of many diseases related to oxidative damage due of its ability to neutralise the deleterious action of free radicals (Badejo *et al.,* 2016). In the present work, vitamin C was not detected in unripe fruit, while ripe fruit was shown to contain it. The level of vitamin C in a fruit is usually positively correlated with ripening, as seen during the ripening of tomato (Badejo *et al.,* 2012).

Anthocyanins are colour pigments found in various parts of plants such as flowers, leaves, and fruits; these are used as dyes and food colorants. Anthocyanins exhibit various pharmacological features such as antimicrobial, antioxidant, antiobesity, anti-inflammatory, and anticancer activities (Miguel, 2011; Khoo *et al.,* 2017; Pervaiz *et al.,* 2017). In the present work, ripe fruit contained higher levels of anthocyanins ($p < 0.004$) than unripe fruit, thus indicating an increase in anthocyanin content during ripening. In a study by Chung *et al.* (2016), the total anthocyanins of *Vaccinium corymbosum* cv. Bluecrop was found to increase during ripening. Belwal *et al.* (2019) also reported an increase in total anthocyanins during ripening of wild edible fruits in the Himalayas. Acosta-Montoya *et al.* (2010) found an increase in total anthocyanins in *R. adenotrichus* during ripening.

Plants contain several antinutrients, namely tannin, phytic acid, and lectin. Phytic acid is the storehouse of phosphorus in many seeds and other parts of the plants. However, phytic acid is a chelator for many minerals such as calcium, iron, and zinc, thereby reducing the bioavailability of these minerals when present in high concentrations. It has also been reported that phytate chelates niacin, thus leading to vitamin B_3 deficiency (Onomi *et al.,* 2004; Papova and Mihaylova, 2019). In the present work, the phytic acid content was shown to be higher ($p < 0.007$) in unripe fruit than in ripe fruit, thus indicating a decrease in the content of phytic acid during ripening. Tannin is among the most important antinutrients found in various parts of plants. Tannin could lead to impaired digestion of several nutrients and the decrease in bioavailable nutrients. Tannin also forms complexes with proteins (Butler 1992; Papova and Mihaylova, 2019; Sharma *et al.,* 2019). In the present work, low tannin contents were detected. Both ripe and unripe fruits contained more or less similar quantity of tannin, with the unripe fruit containing a slightly higher quantity.

Type-2 diabetes is a prominent metabolic disorder. The enzyme α-glucosidase in the mucosal brush border of the small intestine carries out the end step of carbohydrate digestion which leads to an increase in the glucose level that can lead to hyperglycaemia. The inhibition of α-glucosidase is an attractive strategy in the management of type-2 diabetes mellitus, and a range of drugs of this type are currently in use such as acarbose, miglitol, and voglibose. However, the use of these drugs has the drawbacks of high cost and adverse effects on health. Natural products, especially those of plants, are promising sources of compounds including polyphenolic compounds that can act as α-glucosidase inhibitors (Hyun *et al.,* 2016; Bhatia *et al.,* 2019; Assefa *et al.,* 2019). In the present work, both ripe and unripe fruit extracts are promising with respect to their potential to cause the inhibition of α-glucosidase activity. Inhibitory activity of 50% and higher was observed at extract concentrations of 30 and 40 µg/mL in the case of ripe and unripe fruit extracts, respectively. Among the extracts, marked activity was shown by ripe fruit extract with an IC50 value of 24.18 µg/mL when compared to unripe fruit extract (IC_{50} = 32.67 µg/mL). Butyl isobutyl phthalate isolated from chloroform fraction of *R. steudneri* leaves (Shetty Hallur *et al.,* 2021) showed a potent α-glucosidase inhibitory (IC_{50} = 10.68 µg/mL) activity (Raghavendra *et al.,* 2021). In a similar study, the solvent extracts of *R. fruticosus* leaves were shown to display effects against α-amylase and α-glucosidase activities (Salehi *et al.,* 2013). It has been well established that phenolic compounds (Moradi-Afrapoli *et al.,* 2012), flavonoids (Proença *et al.,* 2017), and anthocyanins (Chen *et al.,* 2020) exhibit α-glucosidase inhibitory activity. The α-glucosidase inhibitory activity of the fruit extracts assessed in the present work indicate that they can potentially reduce postprandial hyperglycaemia by causing a delay in carbohydrate digestion.

Polyphenolic compounds, including flavonoids, are a diverse group of plant secondary metabolites that are known to be distributed in various parts of the plants such as the leaves, roots, seeds, and flowers. These compounds have multiple effects on the body. In the present work, the total phenolic and flavonoid contents of ripe and unripe fruits were estimated using FCR and aluminium chloride colorimetric estimation, respectively. The unripe fruit had higher levels of total phenolics (p < 0.011) and flavonoids (p < 0.008) than the unripe fruit. Da Silva *et al.* (2019) showed a decrease in the content of phenolics and flavonoids in apple during ripening. Belwal *et al.* (2019) reported a decrease in the content of phenolic compounds during the ripening of *R. ellipticus*. Acosta-Montoya *et al.* (2010) also found a decrease in total phenolic compounds and flavanols during ripening of *R. adenotrichus*.

Conclusion

Ripe and unripe *R. steudneri* fruits contained minerals such as calcium, magnesium, and iron, and did not contain toxic heavy metals, thus indicating their suitability for consumption. They also contained anthocyanin and water-soluble vitamins such as niacin, pyridoxine, and vitamin C, thus exhibiting strong potential health benefits. The observed α-glucosidase inhibitory potential of *R. steudneri* fruit extracts could be attributed to the presence of anthocyanins, phenolic, and flavonoid compounds that can be used in the treatment of diabetes mellitus. Overall, the results of the present work justified the traditional utilisation of *R. steudneri* fruits for consumption.

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