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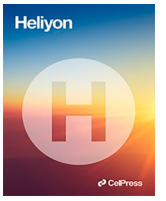

Research article

Influence of fruit stages on chemical compositions, phytochemicals, and antioxidant activity of wood apple (*Feronia limonia* (L.) Swingle)

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ABSTRACT

The *Feronia limonia* (L.) Swingle is an underappreciated tropical fruit, contains several vitamins, minerals, and bioactive substances, yet it has received significantly less attention. The fruit is edible at all stages (unripe, intermediate, and ripe); however, it is only utilized in very limited cuisine recipes. The study's goal is to examine the fruit's chemical compositions, phytochemical content, and antioxidant activity over three stages. Since the fruit is consumed from unripe to ripe, our research demonstrates the scientific validity of its medical properties at each stage. The chemical composition of *F. limonia* fruit was examined at three stages, including nutritional composition, carbohydrate profile, and vitamin and mineral content. The fruit's phytochemicals (Total phenolic and total flavonoid content) were assessed using a spectrophotometer. The antioxidant properties of DPPH (2, 2-diphenyl-1-picrylhydrazylradical scavenging), FRAP (ferric reducing antioxidant power), MCA (metal chelating activity), and RC (reducing capacity) were measured. Pearson's correlation coefficients and multiple linear regressions were used to investigate the link between phytochemical components and antioxidant activity. The study found that protein, fiber, ash, calcium, phosphorus, iron, and vitamin C content declined by 44.7 percent, 47.3 percent, 18.16 percent, 20.3 percent, 8.7 percent, 32.4 percent, and 20.0 percent, respectively, as full ripening progressed. Sucrose (1377.2 mg/100 g) was the predominant sugar in the ripe stage, but fructose (668.72 mg/100 g) was prominent in the unripe stage. During ripening, sucrose concentration rose from 288.1 mg/100g to 1377.2 mg/100g, whereas other sugar contents fell. Similarly, the unripe stage demonstrated increased antioxidant activity, followed by the intermediate and ripe stages. Individually, phenol and flavonoid compounds shown a strong Pearson's association with the antioxidant activity of the fruit, including DPPH scavenging activity (0.945, 0.915), ferric reducing antioxidant power (FRAP) (0.980, 0.907), metal chelating activity (MCA) (0.953, 0.914), and reducing capacity (RC) (0.981, 0.906). The current study's findings could help the pharmaceutical and food processing sectors determine the optimal stage for bioactive ingredient extraction and direct intake.

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1. Introduction

Fruits are pivotal in promoting health due to their essential nutrients, antioxidants, dietary fibers, and phytochemicals. Fruit consumption, therefore, has been associated with preventing chronic and degenerative diseases. *F. limonia* is an edible fruit of the Rutaceae family's genus *Feronia limonia* (synonyms: *Limonia acidissima* L., *Feronia elephantum* L.) [1]. It is frequently cultivated in its wild state or as regional variants that have adapted to local conditions. Instead of precise, separate varietal designations, wood apple grown in different places are most usually referred to by local names or adjectives linked to its fruit attributes (such as large-fruited or sweet-pulp kinds). As a result, the naming and classification of *F. limonia* variants remains relatively underdeveloped when compared to more commercialized fruits. The genus *F. limonia* includes three different types grown in India. Thar Gaurav, Ellora, and Dharwar are grown in various ecological zones around the country [2].

F. limonia is a deciduous species that grows in various parts of India, Pakistan, China, and Southeast Asia [3]. The tree is characterized by its slow growth and typically reaches heights of about 9 m. The tree has an erect growth pattern with slender branches covered in rough and spiny bark, which protects it from diverse environmental conditions. The tree yields a spherical fruit about 5–12.5 cm in diameter, called kaitha, monkey fruit, elephant apple, and wood apple [4]. The fruit is enclosed in a hard, woody shell known as the rind. The rind is grayish-white, incredibly tough (about 6 mm thick), and difficult to crack open without a hammer. The fruit's pulp is brown, aromatic, resinous, and embedded with numerous seeds [5]. Its unique flavor profile offers a delightful blend of sweet, tangy, and slightly bitter tastes. This distinct taste has made it a versatile ingredient in various culinary preparations, such as pickles, chutney, jam, jelly, beverages, etc. The pulps of ripe fruit are used for liver and cardiac tonics. Due to its high fiber content, unripe fruit is used to treat persistent diarrhea and dysentery [6]. Pulp with honey and pipli is an effective treatment for gums diseases, hiccups, and sore throats. The fruit also exhibits other therapeutic activities such as cholesterol-lowering, anti-diabetic, detoxification, anti-asthmatic, and tumor-inhibitory activity [7–9].

The fruit is rich in nutritious compounds like protein, phosphorus, calcium, fiber, and carbohydrates, as well as wide bioactive compounds such as phenols, flavonoids, tannin, saponin, steroids, terpenoids, etc. [6]. It was reported that the nutritional and phytochemical makeup of fruits varies with cultivar, genotypes, soil conditions, environmental conditions, and maturity stages. The fruit matures during October and November, with ripening occurring from early January through June in India. It's evident from previous studies that the stage of maturity significantly influences the nutritional and phytochemical composition of fruits [10]. Research has shown dynamic alterations in phytochemicals such as polyphenols, flavonoids, and triterpenes as fruits progress through different maturity stages. It was observed that the content may increase or decrease with different stages of maturity. For example, red, ripe strawberries have a higher phenolic content than green, immature ones, whereas apples show the opposite pattern [10–12]. Phytochemical compounds are renowned for their strong antioxidant qualities, which counteract oxidative stress and inflammation, potentially lowering the risk of chronic diseases. Consuming the fruit at its optimum stage is important for utilizing its high nutritional and antioxidant properties. However, there is scarce data on the nutritional composition, phytochemicals, and antioxidant attributes of *F. limonia* fruit at different stages. People recognized their medicinal values from personal experience, but they did not understand the scientific rationale and operation of their medications. This work contributes to a better understanding of the scientific rationale for fruit potential at each stage. Although the fruit is consumed in three stages, each with medicinal promise, it is still neglected. Fruit is

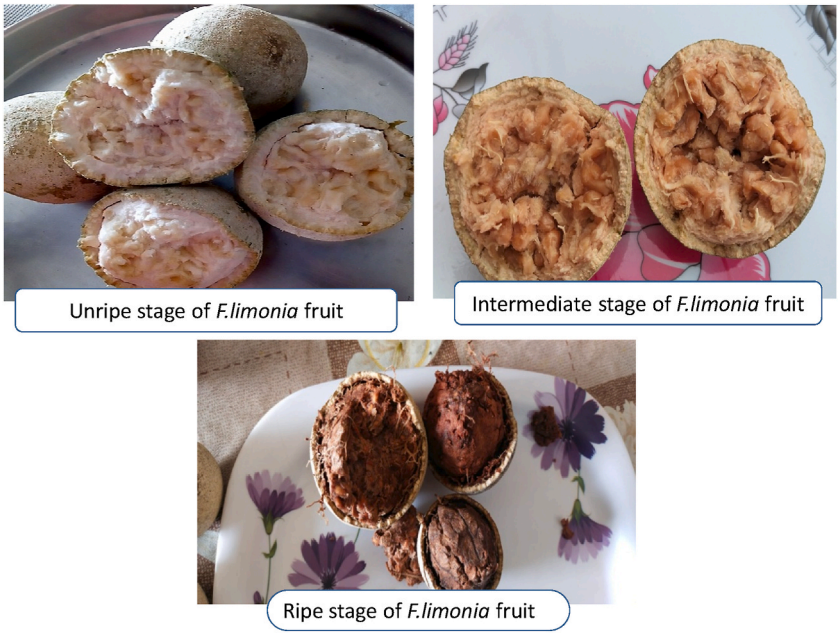

Fig. 1. Three ripening stage of *F. limonia* fruit.

generally utilized fresh; no popular commercially preserved version of this fruit is currently available on the market. This study can help the food sector develop commercially viable preserves from this fruit. There is currently no information on the optimal stage of the *F. limonia* fruit; therefore, our study could be useful for accurately identifying the best stage of the *F. limonia* fruits during processing in terms of chemical compositions, phytochemicals, and antioxidant activity.

In this respect, the work aim to investigate the chemical, phytochemical, and antioxidant properties of *F. limonia* fruits at three stages. The study also determined the associations between the fruit's bioactive compounds and antioxidant activity. This study would determine the best harvest stage of the fruit for extracting of bioactive components with high nutritional values.

2. Materials and methods

2.1. Collection of the samples

The samples were collected from *F. limonia* (var. Thar Gaurav) trees (FS/WA-13) from Kushrobagh (25.44242° or 25° 26′ 33″ North and 81.8208° or 81° 49′ 15″ East), Prayagraj district, India. The fruits were collected at three stages: unripe (October to December 2018), intermediate (January to March 2019), and ripe stage (April to June 2019). The unripe fruit has whitish to cream pulp, the intermediate stage is characterized by light brown colour of pulp and ripe stage has a dark brown colour of pulp (Fig. 1.). Thirty fruits at each stage (unripe, intermediate and ripe) were randomly collected from plants on the same day. This study was conducted in the Department of Family and Community Science, University of Allahabad, Prayagraj, Uttar Pradesh, India, in the year 2019.

2.2. Sample preparation

The fruits at distinct stages were kept separately in a properly labelled clear polyethene bag. Firstly, the rinds of the fruit were removed, and then the pulp was removed. A hot air oven (Cat no: MSW211) was used to dry fresh pulp for 48 h at 40 °C [13]. Following through drying, the pulp was ground into powder with a kitchen milling machine and passed through a 60-mesh screen. Powdered pulp stored in air-tight polyester containers at 4 °C for six months [11]. The dried sample was blended into a fine powder and refrigerated at 4 °C for future investigations [11]. Powdered samples were used to analyze of chemical compositions, carbohydrate profiling, phytochemicals and antioxidant analysis of each stage of fruit (Fig. 2.).

2.3. Reagent and chemicals

Sigma-Aldrich GmbH (Sternheim, Germany) supplied all of the chemicals used in the study including ethanol, ammonium oxalate, acetic acid, sulphuric acid, potassium thiocyanate, sodium hydroxide, sodium nitrate, aluminum chloride, trichloroacetic acid (TCA) and excreta. The standard includes gallic acid, quercetin dihydrate, DPPH (2,2-diphenylpicrylhydrazyl), TPTZ (2,4,6-tri(2-pyridyl)-*s*-triazine), ferrozine, ascorbic acid excreta utilized in the study were obtained from, Merck (Darmstadt, Germany) and Sigma Aldrich (St. Louis, MO, USA).

2.4. Extraction procedure

In a conical flask, 2g of dried sieved powder from three stages of fruit was blended with 200 mL of 80 percent ethanol. Fruit was

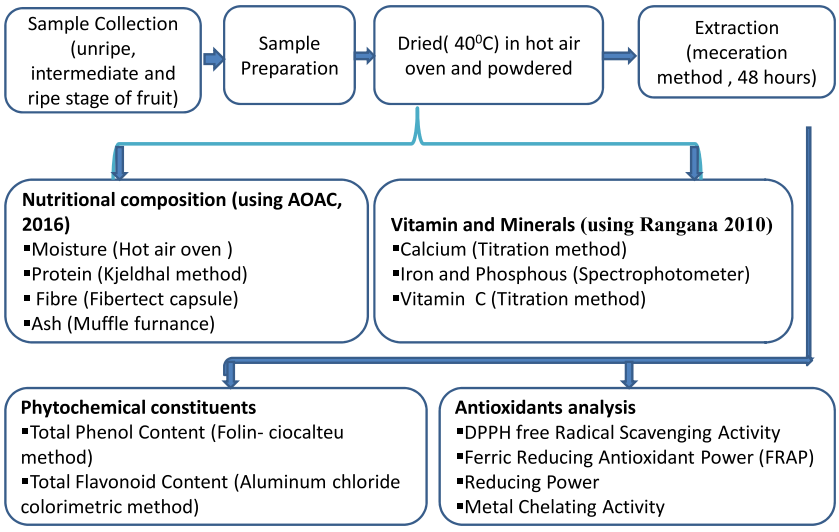

Fig. 2. Experimental procedure.

extracted using the maceration method, which involved leaving a conical flask at room temperature for 48 h. The mixture was then centrifuged at 1000 rpm (Remi) for 10 min (Fig. 2.). The mixture's supernatant was collected in an amber-coloured glass bottle and refrigerated at 4 °C for further investigation of phytochemicals and antioxidants in the fruit at each stage. They were recorded in triplicate.

2.5. Chemical composition analysis

2.5.1. Nutritional composition

Nutritional composition includes moisture, ash, protein, and fiber of *F. limonia* fruit at three stages was determined using the standard Association of Official Analytical Chemists (AOAC, 2016) techniques [12]. The moisture content was determined and the result is reported as g/100 g dw. The ash concentration was evaluated by placing the sample in a muffle furnace (Germany, Naffletherm, L (T) 15/12) at 550 °C, and finding were presented as g/100 g dw. The protein in the samples were tested using the Kjeldahl method (KEL PLUS Kes12L), and results were represented as percentages (g/100g). To assess the amount of fiber present, the sample was boiled in acid, then base, dried, and lastly burned at 550 °C (AOAC 925.10) [12] (Fig. 2.).

2.5.2. Determination of carbohydrate profiling

Carbohydrate profiling was determined by using metrohm 817 Bioscan. The Metrosep carb-150 column (size 2.0 × 150 mm); particle size 5.0 μm; flow rate 1 ml per min; temperature 31±1 °C were used to test the sample after it was extracted using reflux in 80 percent ethanol. The standard solutions were prepared in 18 M NaOH solution and used for calibration curves at different concentrations (25–300 μg/mL). The peak surface was calculated by using Shimadzu lab solutions software version 5.42.

2.5.3. Vitamin and minerals

The vitamin C and calcium content of the fruit were measured by iodine titration [13,14]. The iodine reagent was standardized by titrating it against 5 mL of 1.0 percent ascorbic acid solution (to which three drops of 1.0 percent starch were added) until the blue starch-iodine color appeared; the findings were stated as mg/100 g dw. Phosphorus and iron were determined using the method reported by Rangana, 2010 [13] with slight modification using a spectrophotometer (Model Evolution 600,Thermo Scientific, US) at 650 and 480 nm respectively [15] (Fig. 2.)

2.6. Determination of phytochemical components

The fruit extracts were analyzed for total phenol (TPC), and total flavonoid content (TFC).

2.6.1. Total phenol content (TPC)

TPC was measured using the Folin–Ciocalteau method [14]. Briefly, 0.2 mL of extract was added to 10.0 percent diluted Folin–Ciocalteau phenol reagent (0.5 mL), followed by 7.5 percent sodium carbonate solution (4 mL). For 60 min, the mixture was incubated in the dark. The absorbance was measured at 765 nm with a spectrophotometer (Model Evolution 600, Thermo Scientific, US) and compared to a standard curve made with gallic acid. The TPC result was expressed as mg of Gallic acid equivalents (GAE)/g of extract (mg GAE/g).

2.6.2. Total Flavonoid content (TFC)

TFC was determined using a colorimetric test with aluminium chloride. To begin, add 0.2 mL of extract to 150 μL of 5 percent NaNO2 and then 150 μL of 10 percent $AlCl_3$. After 10 min, 1 mL of 1 M sodium hydroxide was added to the mixture and the total volume was increased to 10 mL using distilled water. The absorbance at 510 nm was measured against a blank and compared to a calibration curve for quercetin solution (20, 40, 60, 80, and 100 mg/L). The results were represented in mg quercetin equivalent (QCE)/g of dry sample [16].

2.7. Antioxidant activity of fruit

2.7.1. DPPH free radical scavenging activity

Scavenging activity of the fruit was evaluated by using 2, 2-Diphenyl-1-picrylhydrazylradical as described by Ref. [16]. Fruit extract (100 μL) was combined with 0.1 mmol DPPH methanol solutions (150 μL) and incubated for 15 min at 515 nm using the spectrophotometer (Model Evolution 600, Thermo Scientific, US).

DPPH activity was calculated using the formula:

$$\frac{(\text{Absorbance control} - \text{Absorbance sample}) \times 100}{\text{Absorbance control}}$$

2.7.2. Ferric reducing antioxidant power (FRAP) assay

To test the ferric reducing power of the fruit extracts, 200 μL fruit extract was mixed with 1.3 ml of FRAP reagent (sodium acetate buffer, 10 mmol TPTZ solution, and 20 mmol iron (III) chloride solution in a volume ratio of 10:1:1 respectively). A spectrophotometer (Model Evolution 600, Thermo Scientific, U.S.) was used to measure the absorbance at 593 nm after the mixture had been incubated for

30 min at 37 °C. The standard curve for $FeSO_4.7H_2O$ solution (200, 400, 600, 800, 1000 μmol) was generated and results were computed in terms of μmol of ferrous equivalent Fe (II)/g of material on a dry basis [17].

2.7.3. Metal chelating activity (MCA)

MCA was determined as outlined by Ref. [17] with slight modification. Fruit extracts (0.5 mL) was mixed with ferrous sulfate (50 μl). After 5 min, add 80.0 percent ethanol (1.6 mL) and ferrozine (100 μl) and then vortex for 1 min. After 10 min, absorbance was measured at 562 nm [18]. The following ratio was used to determine the metal chelating activity:

$$(1 - \text{absorption of sample} / \text{absorption of control}) \times 100$$

The results were expressed as percentage of inhibition of the samples's ferrous sulfate on a dry basis.

2.7.4. Reducing capacity (RC)

Reducing capacity was determined as described by Ref. [19] with slight modification. Fruit extracts were combined with 2.5 mL sodium phosphate buffer (pH 6.6) and 2.5 mL potassium ferricyanide (1.0 % w/v) for 20 min before adding 2.5 ml of TCA (10 % v/v). The solution was centrifuged at 10,000 rpm for 10 min and the supernatant was combined with 2.5 mL of distilled water and 0.5 mL of ferric chloride (0.1 % v/v). The absorbance was then measured at 700 nm using a spectrophotometer [19]. The results were represented as μmol of ascorbic acid equivalents (AAE)/g of dry weight.

2.8. Statistical analysis

The mean ± standard deviation of triplicates was used to present the experimental data. The Statistical Package for the Social Sciences (SPSS) software (Version 16.0) was used to analyze the data. All graphs were drawn in Origin 18. (GraphPad Software, San Diego, CA, USA, Version 8.4.1). ANOVA was used to compare the mean, followed by a one-way analysis of variance to evaluate whether there were statistically significant differences between stages. A post-hoc Duncan test was performed at a 5.0 percent level of significance. The link between phytochemical components and antioxidant activity was analyzed using Pearson's correlation coefficients ($p < 0.05$) in SPSS 16.0 version. The antioxidant activity of *F. limonia* fruit was predicted using multivariate regression ($p < 0.05$) in SPSS. The antioxidant activity (Y) was employed as the dependent variable, with total phenol content (X1) and total flavonoid content (X2) serving as independent variables. Multiple linear regression models were created, as shown in Equation $Y = \beta X1 + \beta X2 + \beta 0$, where Y is the expected response and β0 is the intercept value. Initially, all of the variables were auto-scaled, and the models were created.

3. Results and discussion

3.1. Nutritional composition of F. limonia fruit at three stages

Fig. 3 illustrates the variations in the fruit's nutritional composition at various stages, including moisture, ash, protein, and fiber levels. According to the research, moisture content increased significant ($p < 0.05$) as growth continued. Highest moisture content is seen in the ripe *F. limonia* (6.3 ± 0.5g/100g), which is followed by the intermediate (5.9 ± 0.1g/100g) and unripe stages (4.87 ± 0.2 g/100g). This pattern confirms a linear increase in moisture content and is consistent with earlier findings [20]. Fruits' moisture content indicates their perishability and self life. As a result, the rate of microbial deterioration of ripe fruits is higher than that of the intermediate and unripe phases.

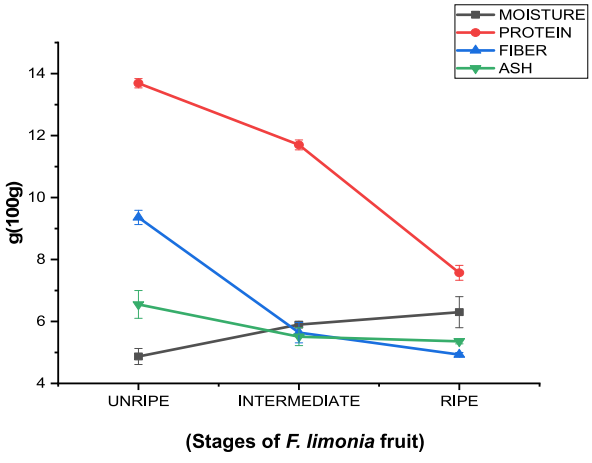

Fig. 3. Nutritional composition of *F. limonia* fruit at three stages.

Additionally, when the fruit ripened from unripe to ripe, the study found that the amounts of protein (13.69–7.57 g/100 g), ash (6.55–5.36 mg/100 g), and fiber (9.36–4.9 g/100 g) decreased. Consistent with observations in date powder [21], there is a considerable drop in protein content across the phases, declining by 14.5 percent at the intermediate stage and 35.2 percent from the intermediate to ripe stages. Comparable patterns in fruit protein content have been documented in earlier research; certain fruits exhibit higher quantities in their unripe phases, while other fruits show elevated levels in their ripe stages [22]. There has been a noticeable change in the metabolic activity of fruit tissues, as observed in earlier research. The overall protein composition of the fruit may decrease as a result of these enzymatic modifications, which may make it easier for proteins to be converted into other substances that are necessary for the fruit to mature [23].

Our investigation found that fiber content decreased considerably ($p < 0.05$) from unripe to intermediate stage by 39.7 percent and 12.5 percent to ripe stage. The findings closely mirror prior research [24], which found a declining trend in fiber concentration with ripening. The result could be attributed to fiber breakdown into smaller molecules by enzymes such as pectinase, which increases during ripening [25]. High fiber content has a variety of therapeutic benefits and is an attractive feature for functional foods. The ash content of the fruit ranges from 6.55 ± 0.45 to 5.36 ± 0.29 mg/100g (Fig. 3). During the intermediate stage, ash content decreased by 15.8 percent ($p < 0.05$), but there was no significant difference between intermediate and fully ripe. Adeyemi and Oladiji (2009) discovered that young fruits contain more ash than mature ones [26]. However, the nutritional composition trends observed during fruit ripening were inconsistent across different fruit species, implying that factors influencing nutrient changes during maturation could differ significantly depending on the individual characteristics and metabolic pathways inherent in each fruit species [27].

3.2. Carbohydrate profiling of *F. limonia* fruit at three consuming stages

Carbohydrates are essential for both energy production and food quality assessment. Table 1 further illustrates the variations in the carbohydrate profile of *F. limonia* fruit. Table 1 shows that whereas fructose and other sugar alcohols such as mannitol (139.24 mg/100 g) and inositol (110 mg/100 g) are more prevalent in the unripe stage, sucrose content peaks in the ripe stage. This may be related to the process that turns sugar alcohol into sucrose when it ripens from the unripe to the ripe state. This is consistent with Suketi's [28] observations about papaya. Fructose and other sugar alcohols are abundant in unripe fruits, which give the fruit cells a source of energy to promote development and metabolism.

Furthermore, compared to their mature counterparts, the sugars in unripe fruits contribute to their taste profile, making them slightly less sweet but still pleasant. In terms of health, unripe fruits that contain fructose and other sugar alcohols (inositol and mannitol) have low glycemic indices, which may help diabetic patients manage their blood sugar levels. Inositol is a sugar-like substance that functions in cellular signaling and has insulin-mimetic characteristics. Its potential to treat insulin resistance and mood disorders like anxiety and sadness has been researched.

3.3. Vitamin and minerals of *F. limonia* fruit at three stages

Fig. 4 shows the vitamin and mineral content of fruit at three different phases. According to the findings, fruit's vitamin and mineral content is highest in its unripe stage, followed by its intermediate and ripe stages. In the current study, as ripening advanced, the vitamin C concentration dropped by 20.0 percent. Fruit at the intermediate stage had no discernible difference in vitamin C content between the ripe and unripe stages ($p < 0.05$). Previous research, however, produced a range of findings, reporting either a steady or rising vitamin C concentration as fruit matured. The current trend of declining vitamin C levels is consistent with the observations of Sharma et al. [29] in *Feronia limonia* and Kachhwaha and Gehlot in *Cordia myxa* and *Carissa carandas* fruit during ripening [30].

The maximum calcium concentration (188.8 mg/100 g) is found in the unripe stage of *F. limonia* fruit followed by the intermediate (159.2 ± 8.96 mg/100 g) and ripe stage (150.3 ± 5.51 mg/100 g). Additionally, iron content was higher in the early stages (10.66 ± 0.5) and gradually decreased to 8.5–7.2 mg/100 g in the later phases of growth. As a result, the calcium content significantly decreased, first by a significant 15.6 percent at the intermediate stage and then by a steady further 5.5 percent from the intermediate to the completely ripe stage. Similar to this, fruit's phosphorus content is much greater in the unripe stage (98.9 mg/100 g), significantly lowers ($p < 0.05$) by 6.7 percent at the intermediate stage. Nevertheless, during its complete ripening, it did not exhibit any notable alterations. The results are in close agreement with [31], who calculated that the unripe stage of papaya displays a greater value of

Table 1
Carbohydrates profiling of the *F. limonia* fruit at three stages (unripe, intermediate and ripe).

Parameter (mg/100g)	Three stages of *F. limonia* fruit		
	Unripe	Intermediate	Ripe
Inositol	110 ± 2.51	37.00 ± 2.3	26.88 ± 0.59
Mannitol	139.24 ± 1.19	90.46 ± 3.5	5.93 ± 0.02
Dextrose	960 ± 5.2	ND*	9.51 ± 0.08
Fructose	668.72 ± 3.2	443.26 ± 2.9	621.34 ± 2.9
Sucrose	288.1 ± 1.19	359.53 ± 3.9	1377.2 ± 7.5
Sorbitol	ND[a]	ND[a]	ND[a]
Arabinose	ND[a]	ND[a]	ND[a]

[a] Not Detected.

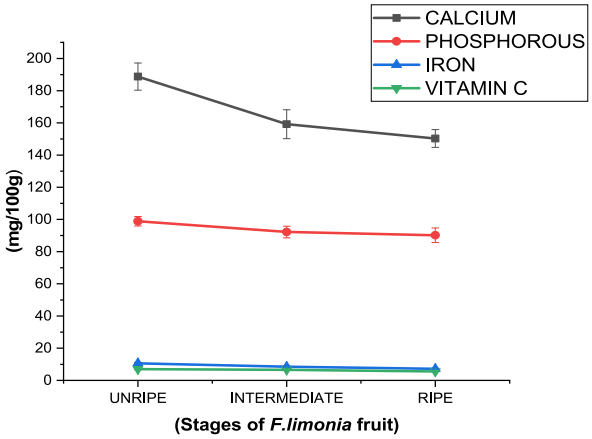

Fig. 4. Minerals and vitamin content of *F. limonia* fruit at three stages.

calcium and phosphorus among the three stages. Phosphorus is necessary for bone health, energy metabolism, and cell structure, whereas iron is essential for the transportation of oxygen and the synthesis of energy. Since fruits are a dietary source of these minerals, it is imperative to monitor fluctuations in their mineral concentrations at different fruit stages. However, further research is needed to understand how the nutritional components of *F. limonia* fruits change as they ripen.

On the other hand, no research on wood apples at all three ripening stages was discovered. The fruit's physiological changes throughout maturation may also be connected to the lowering trend in vitamin C, calcium, iron, and phosphorus content [32,33]. These minerals may be concentrated or redistributed throughout the fruit during the ripening proces in order to support different metabolic activities that are necessary for the fruit to ripen. Important minerals that play a variety of functions in the body include calcium, iron, and phosphorus.

3.4. Phytochemical content and antioxidant activity of F. limonia fruit at three stages

The three stages significantly ($p < 0.05$) affected the total phenol and flavonoid content of *F. limonia* fruit (Table 2). Plants release phenol chemicals as secondary metabolites to guard against UV rays, herbivores, and infections. The fruit's phenolic content is highest in the unripe stage (51.89 ± 2.5 mg GAE/100 g), followed by the intermediate (44.16 ± 1.16 mg GAE/100 g) and the ripe stage (30.62 ± 1.5 mg GAE/100 g). Polyphenol oxidase may oxidize various phenolic compounds to polyphenols, which would account for the changes in phenolic content of *F. limonia* fruit from an unripe to ripe stage [34]. The current study's findings, which were corroborated by Eichholz et al. [35] and Barreca et al. [36], showed a gradual decline in TPC content.

By neutralizing free radicals and reactive oxygen species (ROS), generated during metabolic processes or under environmental strain, these substances assist plants in managing oxidative stress. According to our data, TFC drops by 1.9 percent between the unripe and intermediate stages and by 24.15 percent between the intermediate and ripening stages (Table 2). The outcome, which demonstrated a declining trend in flavonoids during blackberry ripening, was consistent with our observations [37]. The research findings verified that fruit stages had a noteworthy influence on both TPC and TFC ($p < 0.05$). This effect results from the metabolic conversion of bioactive substances found in fruit, wherein early fruit growth is associated with higher amounts of phenolic acids [38]. Fruits may experience color, texture, and flavor changes when they ripen. These changes may be the result of biological processes that break down or alter bioactive chemicals into other molecules. Furthermore, some bioactive substances may have protective effects on unripe fruits that fade as the fruit ages and hardens. As fruits ripen, there is a documented decrease in bioactive chemicals, which is frequently caused by these collective processes.

Fruit's antioxidant activity was assessed using DPPH activity, FRAP, MCA and RC; the results are presented in Table 2. The results

Table 2
Phytochemicals and Antioxidant analysis of *F. limonia* fruit at three stages.

	Parameter	*Feronia limonia* fruit		
		Unripe	Intermediate	Ripe
Phytochemical constituents of *F.limonia* fruit	Total phenol content (mg GAE/100g)	51.89 ± 2.5a	44.16 ± 1.9b	30.62 ± 1.5c
	Total Flavonoid content (mg QCE/g)	32.47 ± 0.9a	31.83 ± 0.9a	24.14 ± 0.5c
Antioxidant activity of *F.limonia* Fruit	DPPH (%)	58.32 ± 1.76a	54.90 ± 2.67a	35.46 ± 0.56b
	FRAP(mmol Fe(II)/g)	14.87 ± 0.77a	11.82 ± 0.91b	5.46 ± 0.62c
	MCA(%)	85.00 ± 4.25a	80.34 ± 5.16b	50.18 ± 3.05c
	RC (μmol AAE/g)	69.60 ± 3.15a	56.69 ± 1.39b	18.50 ± 0.9c

*Values are presented as mean ± SD which are statistically analyzed by ANOVA ($p < 0.05$). Different letters in the same rows indicate that mean values differ significantly whereas same values shows no significant difference between the values.

demonstrate that as the stages advanced, antioxidant activity decreased. In a similar vein, cornelian fruits exhibit noticeably more antioxidant activity during their first ripening stage than they do during their final stage [39]. The maximum free radical scavenging activity is observed in the unripe stage (58.32 percent), which is followed by the intermediate stage (54.90 percent) and the ripening stage (35.46 percent). The capacity to lower ferric ions is verified by FRAP analysis. Over the course of the three stages, the FRAP-reducing activity dropped from 14.87 to 5.46 μmol of ferrous equivalent Fe (II)/g. The current study's findings were consistent with Gull et al.'s [40] observation that the FRAP value decreased as the guava fruit ripened. Fruit that is 85 percent unripe has the maximum metal chelating activity, followed by fruit that is 80.34 percent intermediate and 50.18 percent ripe. Our findings show that reducing power of *F. limonia* fruit varies significantly ($P < 0.05$) with maturation stage. Unripe stage of the fruit shows maximum reducing activity (69.60 μmol AAE/g), which was reduced by 18.5 percent in the intermediate stage and then drastically decreased by 73.41 percent at the complete ripening stage. Allaith's previous investigations [41] reported similar results to the current study, which found a significant decline in antioxidant activity with progress.

3.5. Correlation between the phytochemicals and antioxidant activities of F. limonia

Flavonoids and phenolics are renowned for their powerful antioxidant effects and health benefits [42]. Figs. 5 and 6 show the relationship between antioxidant activity and phytochemical constituents (TPC and TFC). Fig. 5 illustrates the significant ($p < 0.05$) strong association between total phenol content and DPPH, FRAP, MCA, and RC, as determined by Pearson's correlation coefficients of 0.945, 0.980, 0.953, and 0.981, respectively. With Pearson's correlation coefficients of 0.915, 0.907, 0.914, and 0.906, respectively, it was discovered that there was a considerably ($p < 0.05$) strong association between the total flavonoid content and DPPH, FRAP, and MCA (Fig. 6.). Compared to flavonoids, TPC appeared to correlate more strongly with all antioxidants. As a result, the results of the investigation demonstrated a strong and substantial ($p < 0.05$) association between antioxidants and phenolic and flavonoid chemicals in terms of DPPH, FRAP, MCA and RC.

The results showed that the *F. limonia* fruit's phenol and flavonoid levels both display antioxidant activity. The fruit's phenol content has a stronger linear relationship with antioxidant activities (DPPH, FRAP, MCA, and RC) than its flavonoid content. Previous research revealed that the fruit of *F. limonia* contains phenolic compounds like gallic, syringic, and vanillic acid [43] and flavonoid compounds like coumarin [44]. However, further research is needed to understand which phenolic and flavonoid components are linked to the fruit's antioxidant properties.

The R2 values in Table 3 show that the regression model explains roughly 95.0 percent of the variance in DPPH, 99.0 percent in

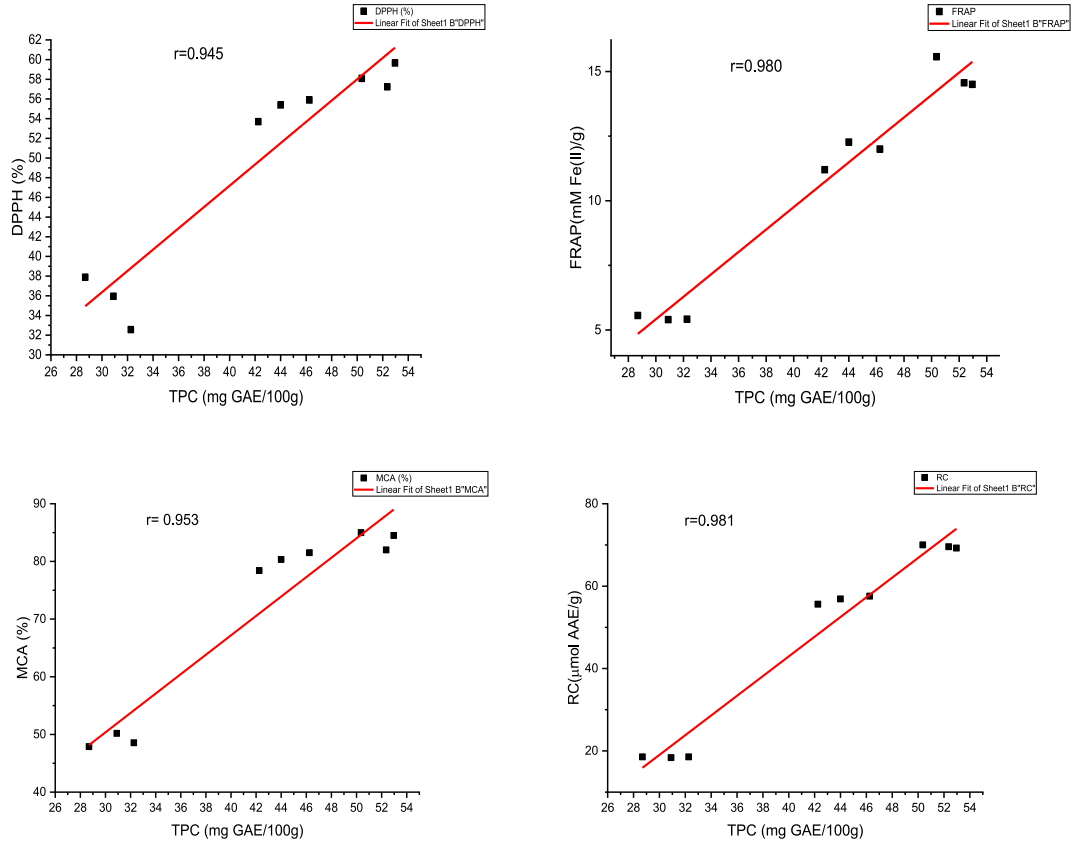

Fig. 5. Correlation between TPC and antioxidant activity.

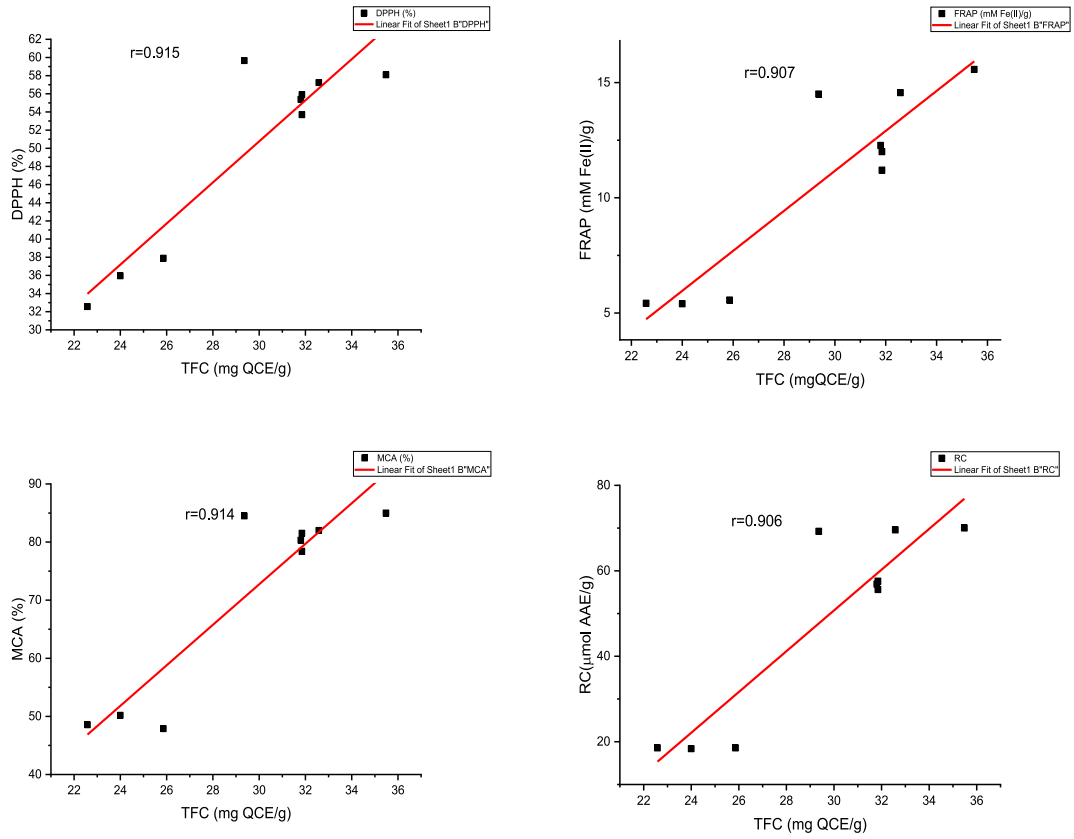

Fig. 6. Correlation between TFC and antioxidant activity.

Table 3
Multiple linear regression models of DPPH, FRAP, Metal chelating activity, RC with TPC and TFC ($p < 0.05$) of *F. limonia* fruit.

Model	Dependant variable (Y)	Independent variable (X)	Standardized Coefficients	Regression equation	R^2	Significant ($P < 0.05$)
1	DPPH[a]	[e]TPC(X1)	0.597	Y = 0.683X1+1.047X2-10.104	0.952	0.009
		[f]TFC(X2)	0.424			0.036
2	FRAP[b]	TPC(X1)	0.724	Y = 0.321X1+0.297X2-11.561	0.992	0.000
		TFC(X2)	0.310			0.003
3	MCA[c]	TPC(X1)	0.623	Y = 1.100X1+1.527X2-20.523	0.961	0.005
		TFC(X2)	0.400			0.031
4	RC[d]	TPC(X1)	0.731	Y = 1.781X1+1.598X2-74.053	0.992	0.000
		TFC(X2)	0.304			0.003

[a] DPPH radical scavenging activity.
[b] Ferric reducing antioxidant power.
[c] Metal chelating activity.
[d] Reducing capacity.
[e] Total phenol content.
[f] Total flavonoid content.

FRAP, 96.0 percent in MCA, and 98.0 percent in RC. Furthermore, it appears that changes in both independent variables (TPC and TFC) explain for 96.0 percent of the variance in the dependent variable, Y. The results indicate that phenol content is more responsible for antioxidant activity than flavonoid content, as indicated by the higher standardized coefficients of TPC (DPPH (0.597), FRAP (0.724), MCA (0.623), and RC (0.831)) compared to TFC (DPPH (0.424), FRAP (0.10), MCA (0.400), and RC (0.191).

For each mg GAE/100g TPC, the value of X1 in models 1, 2, 3, and 4 suggested that the following would increase with TFC held constant: 0.683 percent DPPH, 0.321 mM Fe (II)/g FRAP, 1.1 percent MCA, and 1.7 μM AAE/g RC; meanwhile, X2 suggested that the following would increase with each additional mg QCE/g TFC: 1.047 percent DPPH, 0.297 mM Fe (II)/g FRAP, 1.5 percent MCA, and 1.5 μM AAE/g RC. Multiple linear regression analyses allow us to state that fruit phenol content has a greater influence on antioxidant activity than flavonoid content. Multiple linear regression analyses revealed that the antioxidant activity was attributed to both

flavonoids and phenolic substances. Our results concurred with those of earlier research [39–41].

According to the aforesaid data, the phenol and flavonoid content of the *F. limonia* fruit both showed antioxidant activity [45,46, and47]]. Compared to flavonoid, the phenol content of *F. limonia* fruit exhibited a stronger, linear connection with DPPH, FRAP, MCA, and RC. Based on the findings of this study, additional research is needed to identify the bioactive compounds, such as specific flavonoids and phenols that are related with the antioxidant activity of the fruits.

4. Conclusion

The results of the investigation showed that the three stages had a substantial impact on the chemical composition, phytochemical content, and antioxidant activity of *F. limonia* fruit. The analysis's findings showed a discernible rise in moisture content as the fruit progressed from unripe to ripe, showing a quicker rate of microbial deterioration, followed by intermediate and unripe stages. A noteworthy observation was the decrease in the fruit's protein, ash, and fiber content as it ripened. Additionally, the amount of calcium, phosphorus, iron, and vitamin C is higher in unripe stages. In summary, the mature and intermediate stages have lower chemical compositions than the unripe stage. Fruit's profile of carbohydrates revealed that whereas ripe fruit has more sucrose, unripe fruit has higher levels of inositol, mannitol, dextrose, and fructose. Studies that would be helpful to the food business and consumers about the effects of three stages on the chemical compositions, phytochemicals and antioxidant activity of *F. limonia* fruits are scarce. More research is required to determine whether particular phytochemical components are controlling the fruit's antioxidant activity. These additional effects would give the pharmaceutical sector a fresh perspective on *F. limonia* fruit.

CRediT authorship contribution statement

Rashmi Srivastava: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Neha Mishra:** Writing – review & editing, Visualization, Validation, Data curation. **Arshi:** Writing. **Shraddha Tripathi:** Resources. **Smriti:** Writing. **Neha Taslim Fatima:** Resources. **Neetu Mishra:** Supervision, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Rashmi Srivastava reports equipment, drugs, or supplies was provided by Centre of Food Technology, University of Allahabad, Uttar Pradesh, India. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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