

## Comparative nutraceutical analysis and antimicrobial activity of commonly eaten *Ficus* fruits in Bangladesh

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### Abstract

Several *Ficus* species showed potential antimicrobial, antidiabetic, antioxidant, wound healing, anti-inflammatory, anticarcinogenic, antidiarrheal, and hepatoprotective properties. Hence, this study examined the nutraceutical composition, antioxidant, and antimicrobial activity of two commonly consumed *Ficus* fruits (*Ficus racemosa* and *Ficus hispida*), from southeast Bangladesh, considering the growing demand for natural food supplements and preservatives. Proximate analysis revealed that *F. hispida* had significantly higher moisture (85.73±0.75%), crude fiber (26.53±0.15%), and inorganic content (8.87±0.10%). Conversely, *F. racemosa* contained higher fat (9.30±0.02%) and carbohydrate (48.57±0.13%) levels, while protein content was similar between the two species. Mineral analysis revealed that *F. racemosa* was rich in sodium, potassium and copper whereas *F. hispida* had higher calcium, magnesium, phosphorus and iron; zinc content was comparable in both species. Phytochemical screening indicated the presence of tannins and alkaloids in both fruits, though saponins were highly present in *F. racemosa* but absent in *F. hispida*. The total phenol content (mg GAE/g) and flavonoid content (mg QE/g) were notably higher in *F. racemosa* (10.42±0.03 and 20.05±0.15, respectively) compared with *F. hispida* (5.74±0.06 and 15.97±0.75, respectively). Antioxidant capacity, measured by DPPH scavenging activity, was significantly stronger in *F. racemosa* (IC<sub>50</sub> = 16.80±1.08) than in *F. hispida* (IC<sub>50</sub> = 35.83±1.75). Multivariate analysis revealed a distinct separation between the two species based on principal components, highlighting *F. racemosa* as rich in antioxidants, while *F. hispida* had higher mineral content. However, extracts from both fruits showed no inhibition zones against foodborne pathogens, *Staphylococcus aureus* and *Escherichia coli*. These findings suggested that *Ficus* fruits may serve as natural sources of essential nutrients to address malnutrition and oxidative stress-related diseases.

## 1. Introduction

In recent times, nutritional deficiency diseases have become a weighty problem around the world, especially in third-world countries. The most vulnerable groups are children and pregnant women (Müller and Krawinkel, 2005). Similarly, malnutrition in children under five years and pregnant women is prominent in Bangladesh (Rahman *et al.*, 2019). Approximately 2.6 million

children die each year from malnutrition around the world (International Centre for Diarrhoeal Disease Research, Bangladesh [ICDDR,B], 2022). Furthermore, the prevalence of nutrition-related non-communicable diseases (NCDs) such as cardiovascular disease, metabolic disorders, hypertension, fatty liver, as well as several cancers has increased over the last decade (Fahim *et al.*, 2021). As a result, the death rate is increasing, and

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67% of all fatalities in Bangladesh (Rasul *et al.*, 2022) and nearly 41 million (71% of all deaths) worldwide are related to NCDs (Gowshall and Taylor-Robinson, 2018). Among numerous causal factors, an unhealthy diet and physical inactivity mostly trigger the onset of this daunting health condition. Additionally, it has been shown that reactive oxygen species (ROS)-induced oxidative stress is strongly linked to NCDs (Grosso, 2018). To prevent this vicious reaction in the body, natural antioxidants' demand increases over synthetic antioxidants that have toxic and carcinogenic properties (Nasri *et al.*, 2014). Different types of naturally grown wild fruits showed rich sources of phytochemicals and antioxidants (Morales *et al.*, 2013; Li *et al.*, 2016; Hegazy *et al.*, 2019). A previous study has shown that nutraceutical and antioxidant-rich foods can play a role in the treatment and prevention of innumerable diseases (Bruins *et al.*, 2019). Ficus fruits are used as a vegetable and ayurvedic medicine in rural and hilly parts of Bangladesh (Ahmed and Urooj, 2010). However, detailed information regarding the antioxidant content and nutraceutical composition of these native fruits of Bangladesh is scant. Therefore, current research focuses primarily on commonly eaten Ficus fruits in Bangladesh for the treatment of malnutrition and oxidative stress.

Food poisoning is another issue of grave concern worldwide, along with nutritional diseases (Patel *et al.*, 2022). It is highly prevalent in third-world countries like Bangladesh due to poor sanitation, insufficient infrastructure, and overpopulation (Al Banna *et al.*, 2022). Globally, 600 million people become infected, and 420,000 people die as a result of chemical and/or toxin contamination (Zhao and Talha, 2021). To prevent these contaminations, food producers generally use chemicals as preservatives that are detrimental to health (Gupta and Yadav, 2021). Recurring and excessive use of chemical preservatives leads to microbial resistance against chemicals (Mostafa *et al.*, 2018). Regarding the above concerns, research has been looking into alternative preservatives that are safe, natural, and environmentally friendly (Gonelimali *et al.*, 2018; Rubab *et al.*, 2018; Bedaida *et al.*, 2020; Hemeg *et al.*, 2020). In rural and hilly areas of Bangladesh, Ficus leaves have been used in milk to increase the shelf life, and the scientific validity of it is inadequate. Several research has shown that Ficus leaf extracts (Tanveer *et al.*, 2016; Mandal *et al.*, 2000; Dafalla, 2022) and bark extracts (Kuete *et al.*, 2009; Teinkela *et al.*, 2017) have antibacterial efficacy against foodborne microbes. Furthermore, some Ficus species showed potential properties of antimicrobial (Stephen, 2020), antidiabetic and antioxidant (Misbah *et al.*, 2013; Degollado *et al.*, 2014), wound healing (Khémiri *et al.*, 2019; Koshak *et al.*, 2021), anti-inflammatory and anticarcinogenic

(Lansky *et al.*, 2008), antidiarrheal (Patil *et al.*, 2012), and hepatoprotective (El-hawary *et al.*, 2019). Therefore, the current study focuses on the estimation of nutraceuticals and antibacterial activity of fruit extracts of *F. racemosa* and *F. hispida* grown in Bangladesh. In addition, it is worth investigating the phytochemicals and antioxidant potentiality of both fruit species.

## 2. Materials and methods

### 2.1 Sample collection and preparation

Fresh mature fruits were collected from Chattogram district, Bangladesh. Then fruits were cleaned and subsequently cut into small pieces. Afterwards, dried in a cabinet dryer (Genlab 1000-L, UK) at 60°C for two days and finally ground into powder by the grinder (Walton WBL-VK01-Mixer Grinder) and stored into a zip-lock plastic bag at 4°C for further examinations.

### 2.2 Nutritional composition

The moisture content was determined on a fresh basis and crude protein, fat, fiber, and ash content were analyzed on a dry basis following the standard methods of Association of the Analytical Collaboration (AOAC) International (2016).

### 2.3 Metallic composition

Initially, the powder sample was digested with acid ( $\text{HNO}_3:\text{HClO}_4 = 2:1$ ), and then macro and micro minerals were evaluated by a biochemical analyzer (Humalyzer 3000, Germany) following the standard method (Uddin Zim *et al.*, 2021).

### 2.4 Anti-nutrient screening

The presence of anti-nutrients (tannin and saponin) was assessed by following the standard method described by Akter *et al.* (2018). One gram sample was heated with 10 mL methanol until boiling, and then 3 mL solution was transferred into a test tube and shaken vigorously. Foam formation in solution is considered as the presence of saponin. Similarly, 1 g sample was heated with the mixture of butanol-HCl solution until boiling, if the solution turned into pink color, consider flavonoid presence in the sample. The presence of alkaloids was detected by Hager's test (Ahmed *et al.*, 2007). The presence of alkaloids was confirmed by adding a few drops of Hager's reagent (saturated picric acid solution) into 3 mL of sample extract, and yellow color precipitation proved that alkaloids were present in the sample (Godlewska *et al.*, 2022).

### 2.5 Total phenol content

The Folin-Ciocalteu method was used to determine

the total phenol concentration (Khan *et al.*, 2022). Initially, 1 g of sample was homogenized with 10 mL of methanol (80%). Then 2 mL of Folin-Ciocalteu reagent was added to 1 mL of supernatant. Afterwards, 10 mL sodium carbonate (20%) was added and incubated at room temperature for 24 hrs. Finally, the absorbance was measured at 765 nm using UV-visible spectroscopy (Shimadzu-2600, Germany). To quantify total phenol in the sample, a standard gallic acid curve ( $R^2 = 0.9986$ ) was used, and the unit was determined as mg of gallic acid equivalent (GAE) per gram of dried weight (DW).

2.6 Total flavonoid content

Flavonoid was measured against a standard quercetin curve ( $R^2 = 0.9985$ ) by following the colorimetric method (Shah and Hossain, 2014). Approximately 50 g of dried sample was extracted with 70% ethanol and then dried. Then the extracted dried sample was again extracted with a solvent mixture (hexane, chloroform, ethyl acetate, and butanol) and then dried. Afterwards, 4 mg of extracted powder was mixed with 50 mL of deionized water. Subsequently, 0.5 mL sample solution was taken in a test tube and 2.8 mL of water, 1.5 mL of methanol, 0.1 mL of aluminum chloride, and 0.1 mL of 1 M potassium acetate were added and kept at room temperature for 30 mins. Finally, the absorbance was measured using a UV-visible spectrophotometer (Shimadzu-2600, Germany) at 415 nm, and the results were expressed as mg of quercetin equivalent (QE) per gram.

2.7 Antioxidant capacity

The antioxidant capacity of *F. racemosa* and *F. hispida* was determined by the DPPH (2, 2- Diphenyl-1-picrylhydrazyl) method, where ascorbic acid (AA) solution was considered as a reference described by Akter *et al.* (2021). Initially, 10 mg of dried sample was mixed with 10 mL methanol and then centrifuged. The supernatant was diluted and then 3 mL of 0.004% DPPH solution (4 mg DPPH in 100 mL 95% methanol) was added to 1 mL of each diluted solution. After 30 mins incubation in a dark place at room temperature, the absorbance was taken at 517nm using a UV-spectrophotometer (Shimadzu UV-2600, Germany). The free radical scavenging capacity was calculated by following:

$$\% \text{ of scavenging of DPPH} = \frac{(A_0 - A_s)}{A_0} \times 100$$

where,  $A_0$  = DPPH absorbance;  $A_s$  = absorbance of sample or standard solutions.

The half maximal inhibitory concentration ( $IC_{50}$ ) value was deliberated from the percentage of inhibition graph (Figure 1) by the following equation:

$$IC_{50} = \frac{50 - I}{S}$$

where I = intercept value and S = slope of line.

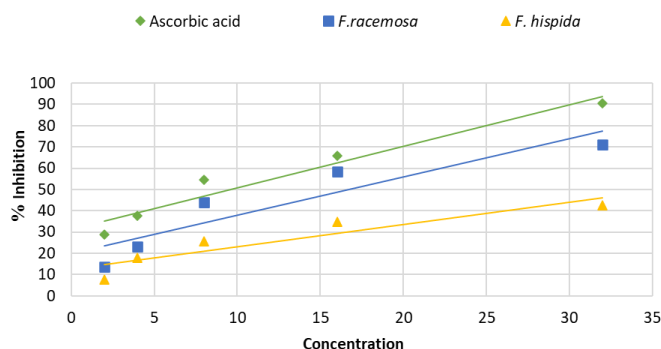


Figure 1. Comparative scavenging capacity of ascorbic acid *F. racemosa* and *F. hispida* fruit extracts.

2.8 Antimicrobial activity

The antimicrobial activity of fruit extracts (aqueous and diethyl ether) was assessed using the disk diffusion method (Mostafa *et al.*, 2018). In aqueous extraction, 10 g dried fruit powder was extracted in 100 mL distilled water at 60°C for 2 hrs. The supernatant was then concentrated to one-fourth of the final volume after being filtered and centrifuged (Parekh *et al.*, 2005). Diethyl ether extraction (10 g sample in 100 mL diethyl ether solution) was prepared, and both solutions were stored at 4°C. The antibacterial effectiveness of fruit extracts was evaluated using common food poisoning pathogens, namely *Staphylococcus aureus* and *Escherichia coli* bacterial strains, acquired from the culture collection of the Department of Microbiology and Veterinary Public Health, Chattogram Veterinary and Animal Sciences University, Bangladesh. The bacteria were cultured on blood agar. The pure cultures were then transferred to 3 mL of phosphate buffer solution and incubated for 24 hrs. After that, the broth was mixed and adjusted to 0.5 McFarland standards. The broth culture was then spread in a zigzag pattern on a Muller Hinton agar plate for 15 mins. The sterile filter paper discs containing fruit extract were then inoculated on the agar plate. The standard antibiotic (Ciprofloxacin, 5 mg) and blank disc were used as a positive and negative control. The antibacterial activities were determined by measuring the zone of inhibition in millimeters.

2.9 Data analysis

Experimental data were stored in Microsoft Excel 2016 and scrutinized for missing values. IBM SPSS 25 was used for statistical analysis, and independent sample T-test was used to compare mean values at 95% confidence intervals. The data is presented in the form of means and standard deviations (SD). A web-based tool was used to perform multivariate analysis (metaboanalyst.ca).

### 3. Results

#### 3.1 Nutritional composition

The moisture content was measured on a fresh weight basis and the other parameters were on a dry basis. All parameters except protein content were significantly different between the two species. *Ficus hispida* contains a higher amount of protein (10.07±0.08%), fiber (26.53±0.15%), ash (8.87±0.10%), and moisture (85.73±0.75%), whereas *F. racemosa* contains higher fat (9.30±0.02%) and carbohydrate (48.57±0.13%) represented in Table 1.

#### 3.2 Mineral composition

From the result, it was observed that both *Ficus* species are rich sources of macro and micro minerals. *F. racemosa* contained a significantly copious amount of sodium (144.95±5.33 mg/100 g), potassium (1269.46±8.95 mg/100 g), and copper (2.52±0.18 mg/100 g), in contrast, *F. hispida* had a higher amount of Ca (762.85±5.66 mg/100 g), Mg (213.86±4.71 mg/100 g), P (970.27±1.29 mg/100 g), and Fe (68.80±0.50 mg/100 g) depicted in Figure 2. However, there was no significant difference in Zn content between the two species.

#### 3.3 Screening of anti-nutrients

This study performs screening for some common anti-nutrients, namely tannin, alkaloid, and saponin. Saponin was highly present in *F. racemosa*, whereas absent in *F. hispida* shown in Table 2. Tannins and alkaloids were present in both species of fruits.

#### 3.4 Phytochemical content and antioxidant activity

The methanolic extract of both fruit samples was

Table 2. Anti-nutrient screening test.

Sample	Tannins	Alkaloids	Saponin
<i>F. racemosa</i>	+	+	++
<i>F. hispida</i>	+	+	-

+ indicates presence, ++ indicates highly present and - indicates absence of anti-nutrient.

subjected to measure total flavonoid and phenol content. From Table 3, it was observed that total phenol and flavonoid were significantly higher in *F. racemosa* than in *F. hispida*. The half inhibitory concentration (IC<sub>50</sub>) of the methanolic extract of *F. racemosa* (16.80 µg/mL) was lower than *F. hispida* (35.83 µg/mL) illustrated in Table 3.

Table 3. Phytochemical (phenol and flavonoid) content and antioxidant activity.

Fruit	Total phenol (mg GAE/g)	Total flavonoid (mg QE/g)	Antioxidant activity (IC <sub>50</sub> ) (µg/mL)
<i>F. racemosa</i>	10.42±0.03 <sup>b</sup>	20.05±0.15 <sup>b</sup>	16.80±1.08 <sup>a</sup>
<i>F. hispida</i>	5.74±0.06 <sup>a</sup>	15.97±0.75 <sup>a</sup>	35.83±1.75 <sup>b</sup>

Values are presented as the mean±SD (n = 3). Values with different superscripts within the same column are statistically significantly different (p<0.05).

#### 3.5 Nutraceutical differences of *Ficus racemosa* and *Ficus hispida*

The multivariate analysis revealed that *F. racemosa* has a superior nutritional quality to *F. hispida* due to a higher concentration of phytochemicals and essential nutrients. *Ficus racemosa* and *F. hispida* both are distinct based on the principle component scores plot (Figure 3a). Ca and K are significant contributing variables for this distinction of both species as their VIP

Table 1. Nutritional composition of *F. racemosa* and *F. hispida*.

Sample	Moisture (%)	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	CHO (%)
<i>F. racemosa</i>	81.17±1.25	9.88±0.09	9.30±0.02	18.08±0.11	7.62±0.06	48.57±0.13
<i>F. hispida</i>	85.73±0.75	10.07±0.08	4.38±0.21	26.53±0.15	8.87±0.10	40.67±0.23
P-value	0.006	0.052	0.001	0.001	0.001	0.001

Values are presented as mean±SD. P-value <0.05 is regarded as statistically significant.

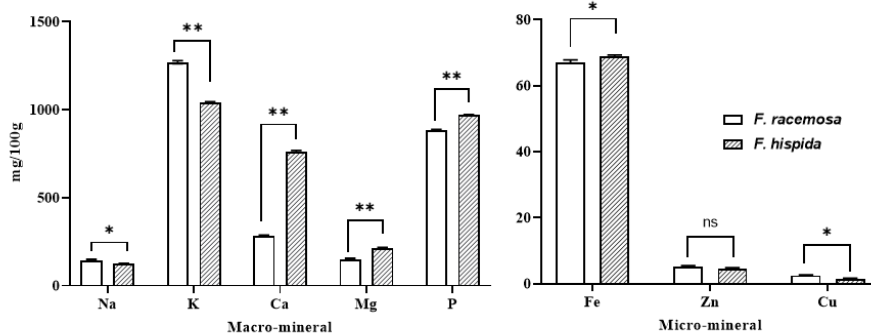


Figure 2. Mineral content of *Ficus* sample on dry basis. \*\* indicates P-value <0.001, \* indicates P-value <0.005, ns = no significance.

score is greater than 1 (Figure 3b). From Spearman's correlation heatmap dendrogram graph, it is also clearly observed that both species are distinct from one another (Figure 3c).

### 3.6 Antibacterial activity

Figure 4 depicts the antibacterial activity of fruit extracts and control over two food poisoning-causing bacterial strains. No zone of inhibition on bacterial culture was exhibited in both aqueous and ether extracts. Only the positive control (T) indicated the zone of inhibition.

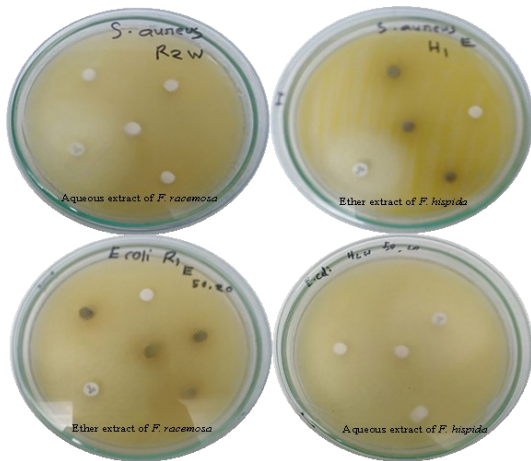


Figure 4. Antibacterial activity of aqueous and ether extracts of *F. racemosa* and *F. hispida* fruits.

## 4. Discussion

In the current study, the nutraceutical composition, antioxidant potential, and antibacterial activity of *F.*

*racemosa* and *F. hispida* were assessed to reveal their potentiality in terms of nutritional and medicinal value. The results showed that both species are reliable sources of essential nutrients, namely fiber, protein, fat, P, Ca, K, Fe, and free radical scavengers. However, the multivariate study revealed that *F. racemosa* is better than *F. hispida* based on antioxidant activity, while minerals are higher in *F. hispida*. Furthermore, fruit extracts in aqueous or ether had no antibacterial impact on the two strains of bacteria (*E. coli* and *S. aureus*) that are commonly responsible for food poisoning.

Bangladesh has progressed tremendously in different sectors such as agricultural production, poverty and malnutrition reduction. Despite this progress, still, approximately 35% of people are food insecure, 23% of under-five children are underweight, and 10% are acutely malnourished (Bangladesh Bureau of Statistics (BBS) United Nations Children's Fund (UNICEF), 2019). To ensure the food and nutrition security of the vast population, developing and undeveloped countries are looking into low-cost, nutrient-dense alternative sources of foods; wild and so-called neglected fruits can alleviate this grave concern. From the nutritional composition of Table 1, it is observed that both species are good sources of protein, range  $9.88 \pm 0.09$  to  $10.07 \pm 0.08\%$ , which is slightly higher than reported in the previous study ( $6.31 \pm 0.08$  to  $8.60 \pm 0.96\%$ ) on a dry basis in different species of the same genus (Sadia et al., 2014). Our results are comparable to the Food and Agriculture Organization (FAO) datasheet, which states that *F. racemosa* contains 10.1% to 11.2% protein (FAO,

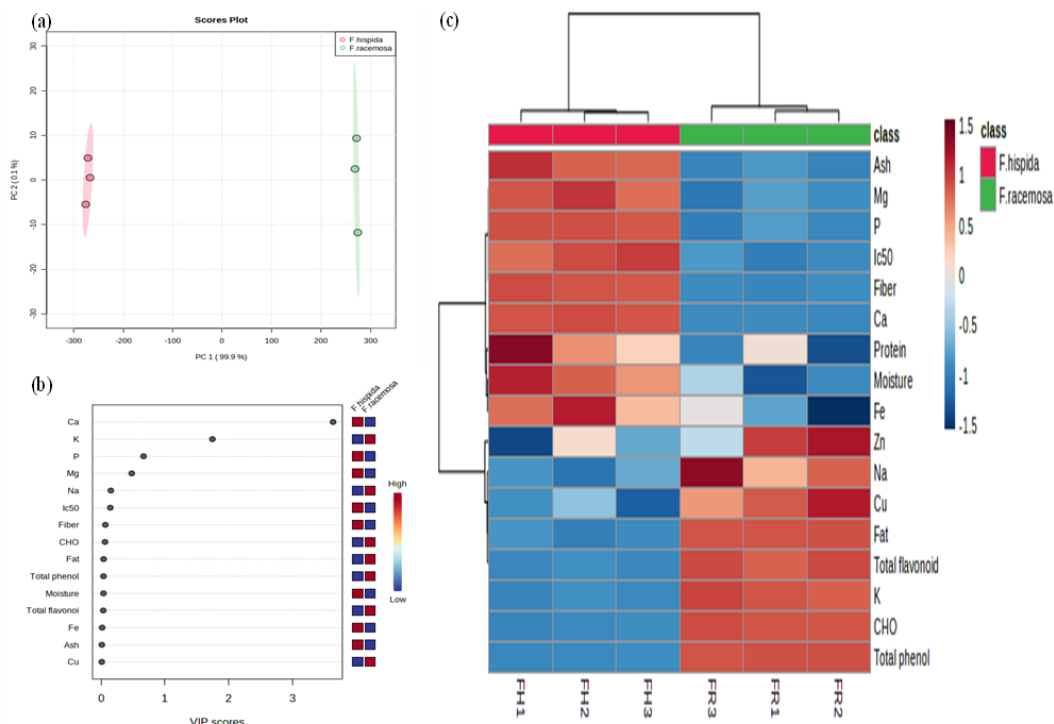


Figure 3. Principal component analysis (PCA) scores plot (a), VIP scores (b), and Spearman's correlation heatmap, where correlation coefficients are represented by colors, with red specifying a significant correlation and blue denoting a negative association (c) from nutraceutical components of *F. racemosa* and *F. hispida* fruit extracts.

2012); nonetheless, an Indian investigation revealed that the amount is 28.23% (Bhogaonkar *et al.*, 2014). The protein content variation may be due to the geographical location, state and season of harvesting, and nutrient uptake from soil (Hornick, 1996). A high-protein diet helps to combat protein-energy malnutrition that impedes the growth and development of children (Kar *et al.*, 2008). Dietary fiber is another macronutrient in the daily diet that increases bulk volume and prevents constipation, irritable bowel syndrome (IBS), and colorectal cancer (Barber *et al.*, 2020; Singh and Paul, 2021). According to Food and Drug Administration (FDA), an adult person's diet should possess 25-34 g of fiber based on their age, health status, and gender (Fernando, 2022); despite that, recent meals have low fiber as people are more inclined to fast food, high in sugar and salt, and less in fiber. Table 1 shows both fruits are rich sources of crude fiber,  $18.08 \pm 0.11$  and  $26.53 \pm 0.15\%$  in *F. hispida* and *F. racemosa* respectively. The findings resemble the results of previous studies conducted in other parts of the world (FAO, 2012; Sadia *et al.*, 2014). Fruits and vegetable fat is considered good for our health as it contains more polyunsaturated fat, essential fatty acid, and fat-soluble vitamins. The mean crude fat content of *F. racemosa* ( $9.30 \pm 0.02\%$ ) was about double that of the content ( $4.38 \pm 0.21\%$ ) of *F. hispida* fruit. The reported fat percentage is within the range (2.4 - 12.5%) recorded by FAO's datasheet (FAO, 2012), but higher than other species of *Ficus* genus ( $1.02 \pm 0.19$  -  $2.71 \pm 0.03\%$ ) on a dry weight basis (Sadia *et al.*, 2014).

The present investigation illustrates that both species are rich sources of macro-minerals and trace elements, which play a crucial role in maintaining the physiological process in the human body. Insufficient intake affects growth, development, immune function, and other nutrition-related diseases (Ahmed *et al.*, 2016). Essential minerals deficiency in children and women is an eminent problem in Bangladesh, where approximately 41% of women (Jana *et al.*, 2022) and 51% of under-five children are suffering from iron deficiency anemia; zinc deficiency among children and women is 45% and 51% respectively (United States Agency for International Development [USAID], 2021). Figure 2 depicts that Na, K, and Cu are significantly higher in *F. racemosa*, whereas Ca, Mg, P, and Fe are in *F. hispida*. However, there was no significant difference ( $P = 0.108$ ) in the Zn content of both species. The most abundant minerals in both fruits were potassium and phosphorus, and the least amount was Cu. This study observed that both fruits' sodium-potassium ratio was less than 1, which is preferable for hypertensive patients (Kogure *et al.*, 2021). Ca and P form the bone and teeth matrix, and Mg strengthens the matrix; deficits in the diet increase the

prevalence of osteomalacia and osteoporosis (Heron, 2018). Besides, Iron deficiency leads the fatigue, infection, impaired cognitive performance, and growth in children (Kumar *et al.*, 2022). According to the National Institute of Health, USA, the Recommended Dietary Allowances (RDAs) range from 7-27 mg/day based on age (National Institutes of Health [NIH], 2022). The estimated iron content (mg/100g) was  $66.87 \pm 0.95$  in *F. racemosa* and  $68.80 \pm 0.50$  in *F. hispida* sample (dry basis), which is within the range ( $31.20 \pm 0.19$  to  $82.18 \pm 0.03$  mg/100 g dry sample) for other species of *Ficus* fruits previous study reported (Sadia *et al.*, 2014). Trace elements are required in a small amount to regulate the metabolic processes and good health. Excess and deficit lead to an imbalance in the metabolic process (Afzal and Quinones, 2022).

Anti-nutrients are substances usually present in plant foods that reduce the bioavailability of essential nutrients, especially minerals (Abifarín *et al.*, 2021). A higher amount of these components in food may develop food toxicity; however, food processing techniques such as boiling, soaking, heating, sauteing, and autoclaving decrease the activity of antinutrients (Petroski and Minich, 2020). Table 2 showed that both species contain tannin and alkaloid, while the saponin test was highly positive for *F. racemosa*, but negative for *F. hispida*. Recently, secondary metabolite/anti-nutrients have drawn attention due to their health benefits. Tannin has antibacterial, antioxidant, and anti-cancerous properties (Sharma *et al.*, 2019), alkaloids show analgesic attributes (Bello *et al.*, 2014), saponin has anti-diabetic, anti-hyperlipidemic, anti-carcinogenic qualities (Kareem *et al.*, 2022). Table 3 results depict that total phenol ( $10.42 \pm 0.03$  mg GAE/g) and flavonoid ( $20.05 \pm 0.15$  mg QE/g) content are significantly higher in *F. racemosa* compared with *F. hispida* ( $5.74 \pm 0.06$  mg GAE/g and  $15.97 \pm 0.75$  mg QE/g respectively). Bangladeshi *Ficus* species contain lower phenol compared to Thai species,  $48.31 \pm 0.66$  and  $72.57 \pm 0.27$  mg GAE/g in *F. racemosa* and *F. hispida* respectively (Puangpradab *et al.*, 2018). This variation is influenced by the quality of the analyte and the extraction process used in the methodology (Dai and Mumper, 2010). Furthermore, environmental factors such as temperature (Alba *et al.*, 2022), the maturity stage of fruits, and soil qualities also influence the production of secondary metabolites or phytochemicals (Feduraev *et al.*, 2019). Recent studies divulged that phenolic components prevent oxidative stress, cancer, and chronic diseases (Dai and Mumper, 2010; Bhuyan and Basu, 2017). Another phytochemical, flavonoid has antioxidant, anti-allergic, and anti-ulcer properties (Muhammad and Oluwaniyi, 2022).

During the metabolic process or external exposures

(smoking, food additives, X-ray, chemical), the body produces reactive oxygen species (ROS) and other free radicals (Lobo *et al.*, 2010) that damage DNA and cells by initiating a chain reaction (Sirisha *et al.*, 2010). Antioxidants act as reducing substances that terminate this reaction by transferring electrons to free radicals and neutralizing them. Suboptimal dietary intake of antioxidants leads to oxidative stress that is interrelated with numerous chronic diseases, namely cardiovascular disorders, kidney damage, diabetes, and some cancer (Liguori *et al.*, 2018). Nutritionists and medical professionals have recommended that an adult consume between 8,000 and 11,000 ORAC (oxygen radical absorption capacity) units daily to prevent oxidative stress, despite the fact that there is no standard recommended daily consumption of antioxidants (Greger, 2021). Synthetic antioxidants have been utilized extensively because of their stability, availability, and affordability (Lourenço *et al.*, 2019); nonetheless, studies revealed that they are carcinogens (Seifirad *et al.*, 2014; Kornienko *et al.*, 2019; George and Abrahamse, 2020). In recent years, in light of safety and health issues, intensive looking into alternative natural sources of antioxidants (Lourenço *et al.*, 2019). The current study found that the extract of *F. racemosa* has higher antioxidant activity (IC<sub>50</sub> 16.80 g/mL) than *F. hispida* (35.83 g/mL), where *F. hispida* fruits (IC<sub>50</sub> = 28.39 µg/mL) showed slightly higher activity than our finding (Sumsuzzman *et al.*, 2016). Another study reported methanolic extract of *F. racemosa* fruits (IC<sub>50</sub> = 8.59 µg/mL) had strong antioxidant capacity, with a standard ascorbic acid value of 4.15 µg/mL (Sumi *et al.*, 2016).

To further emphasize the variations in nutraceutical substances between the fruits, a multivariate analysis was carried out (Figure 3). The most widely used techniques for separating different groups in multivariable analysis are Principal component analysis (PCA) and PLS-based analyses (Kim *et al.*, 2021). The most significant factor, which accounted for 99.9% of the overall variance in the nutraceutical components between fruits, was the first principal component (PC1). Figure 3a shows that *F. racemosa* was positioned on the positive PC1 axis, indicating a positive correlation between the nutraceutical components, in contrast to *F. hispida*, which was situated on the negative PC1 axis, representing a negative correlation with PC1. In the PLS-DA model, the variable importance in projection (VIP) score is expressed as a substantially contributing variable (> 1 scores). The highest VIP score was displayed for Ca, followed by K, and P (Figure 3b). The nutraceuticals with the highest VIP scores were most ubiquitous in *F. hispida* except K, which was higher in *F. racemosa*. However, total phenol and total flavonoid were higher in *F. racemosa*. These two phenolic compounds are

positively correlated with free radical scavenging activity (Mustafa *et al.*, 2010; Shirazi *et al.*, 2014) that prevent degenerative diseases, cancer, and NCDs (Lutz *et al.*, 2019; Phuyal *et al.*, 2020). Spearman's correlation heatmap depicts the relationship between nutraceutical compound levels and fruit groups replication (Figure 3c). The dendrogram of the heatmap illustrates that the two fruits are distinct from one another. *F. hispida* shows a positive correlation with mineral contents, whereas *F. racemosa* correlates strongly with macronutrients and phenolic compounds.

*Escherichia coli* and *S. aureus* strains were shown resistant to Ficus extracts in our study. However, the standard antibiotic (Ciprofloxacin) inhibited both types of strains' growth. The antimicrobial properties of diethyl ether extract had not previously been documented. However, different solvent extracts from *F. racemosa* fruits demonstrated a zone of inhibition at various concentrations against 8 bacterial pathogens, including *E. coli* and *S. aureus* (Hasan *et al.*, 2017). Moreover, different parts of *Ficus* species such as bark, leaves, and roots also showed antibacterial activity (Mohamed and Hayssam, 2013; Chaware *et al.*, 2020). In our study, we found no antibacterial activity, the reason behind this may be the employment of extraction solvent types and the effectiveness of the extraction processes. Furthermore, the maturity stage, freshness, season and harvest time of fruits, as well as the drying method used before the extraction process also attributed to the efficacy of antimicrobial activity (Jouda *et al.*, 2015).

## 5. Conclusion

The findings in this study indicate that both Ficus fruits contain essential nutrients, antioxidants, and phytochemicals, with noticeable differences between the fruits, where *F. racemosa* being superior for antioxidant activity and *F. hispida* having higher levels of minerals. So, Ficus fruits could be used as raw materials in the nutraceutical industry to manufacture food supplements. However, zone of inhibition for *E. coli* or *S. aureus* was not visible in either the aqueous or diethyl ether extractions. Further research is needed to determine in vivo antioxidant and antibacterial activities of different extract solutions.

## Conflict of interest

The authors declare no conflict of interest.

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