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Nutritional composition, antioxidant potential, and antimicrobial activity of selected wild edible fruits from Champadevi community forest, Kathmandu, Nepal

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Abstract

Wild edible fruits are abundant in the natural environment and found in different parts of Nepal, varying in elevation from low to high lands. The present study aimed to evaluate the nutrient content, antioxidant potential, and antimicrobial activity of fruits of *Pyracantha crenulata, Rubus paniculatus, Berberis asiatica,* and *Myrsine semiserrata* collected from Champadevi community forest, Kathmandu. The carbohydrate, protein, and fat content were determined by Clogg-anthrone, Bradford, and Soxhlet extractor methods, respectively. Similarly, antioxidant potential was determined by DPPH radical scavenging assay and antimicrobial activity by agar well diffusion method. All the fruits were found to contain variable amounts of nutrients. The carbohydrate content ranged from 2.4±0.24% (*P. crenulata*) to 10.64±0.13% (*R. paniculatus*) of the dry weight of the sample. Fat content ranged from 0.84±0.69% (*P. crenulata*) to 10.83±0.11% (*R. paniculatus*), and protein content ranged from 0.08±0.01% (*M. semiserrata*) to 0.66 ± 0.01% (*P. crenulata*). Among the tested samples, *B. asiatica* showed the strongest antioxidant activity, with an IC_{50} value of 12.03 µg/mL. All the fruit extracts exhibited a zone of inhibition against the tested seven pathogens, while *R. paniculatus* and *M. semiserrata* showed a higher zone of inhibition against *Candida albicans*. The results indicate that wild edible fruits examined in the present study vary in their nutritional composition, antioxidant, and antimicrobial activities.

Keywords: Antimicrobial activity, Antioxidant property, Nutritional composition, Wild edible fruits

1. Introduction

Naturally occurring, uncultivated plants that possess nutritional properties suitable for meeting dietary requirements are known as wild edible plants. These plants are utilized by the peoples of different regions of the world as a part of their culture, and the people especially in rural and suburban areas use these resources during famine and scarcity (Pinela et al., 2017). Through trial and error, primitive people picked various edible wild plants, domesticated them, and consumed them (Niveditha, 2017). The Rural residents still rely on harvesting wild edible fruits for their nutritional needs, financial aspects, and primary medical care. These wild edible fruits substantially impact rural people's livelihood and food security (Islam et al., 2019). Many ethnic people in Nepal, particularly those who live in rural areas, still gather fruits from wild plants and consume them in various ways. Some even sell these fruits in local markets to supplement their income (Gautam et al., 2020). Wild edible plants give communities access to basic foods and sources of revenue, which contribute significantly to food security and livelihood. Even in the modern world, such plants are important for impoverished, rural, and ethnic communities. These plants are still found in the wild, despite their significance for economic growth, livelihood, and food security (Shirsat et al., 2023). Very few species

have been domesticated for use in medicine or religion. The public is still ignorant about the number, abundance, and availability of many of these plants because they are in their natural state, except for a small number of researchers and the communities using them regularly for their livelihood and well-being (Tadesse et al., 2024). The present study evaluates the nutritional composition, antioxidant potential, and antimicrobial activity of four wild edible fruits collected from Champadevi community forest of Kathmandu Valley, Nepal.

2. Materials and Methods

2.1 Plant Material

Four fruit samples were collected from Champadevi community forest, Kathmandu, Nepal. The prepared herbarium was sent to the National Herbarium and Plant Laboratories (KATH), Godawari, Lalitpur, for official identification. The voucher specimens were deposited in the Amrit Campus (ASCOL) Herbarium for future reference. The details about the collection of wild edible fruits are mentioned in Table 1.

2.2 Preparation of Samples

Healthy, harvested fruits were collected and cleaned with distilled water to remove the dust particles from the fruits. Then, pulp and seeds were separated with forceps and needles. Then, they were placed in a hot air oven set at 40°C for about a week to dry out and remove the moisture. Following drying, they were ground with a grinder to a fine powder and kept in plastic vials, sealed tightly, and labeled appropriately until further analysis.

2.3 Nutritional Analysis

Total Carbohydrate Content

The carbohydrate content of the fruit sample was determined by the colorimetric anthrone method (Osborne & Voogt, 1978). One gram of the fruit samples was digested with 13 mL of 52% perchloric acid. The prepared fruit sample was treated with anthrone reagent in 1:5 ratio and incubated in a water bath for 12 minutes. The absorbance of the above reaction mixture (0.2 mL in a 96-well plate) was measured at 630 nm using Agilent Technology Cary UV–Vis spectrophotometer, with a blank containing only water and anthrone reagent. Glucose solution (10–500 µg/mL) was used as a standard, and the results were expressed in g/100g of sample dry weight. The carbohydrate content of each sample was determined using the standard curve of glucose ($y = 0.0008x + 0.1018$, $R^2 = 0.99$).

Protein Content

A modified Bradford test was performed to determine the protein content (Bradford, 1976). A conical flask containing 200 mg of powdered materials and 20 mL of distilled water was

Table 1. Wild edible fruits with their geographical locations in Champadevi community forest

S.N.	Plant Name	Elevation (m.)	GPS Coordinates
1	*Pyracantha crenulata* (D. Don) M. Roem	1512	27° 39' 08.64" N, 85° 15' 58" E
2	*Rubus paniculatus* Sm.	1987	27° 38' 36.55" N, 85° 13' 33.25" E
3	*Berberis asiatica* Roxb. Ex DC	1987	27° 38' 36.45" N, 85° 13' 33.35" E
4	*Myrsine semiserrata* Wall.	2002	27° 38' 27.58" N, 85° 15' 31.25" E

maintained at 50°C and 40 rpm for a full day (Bhusal et al., 2020). After that, Whatman No. 1 filter paper was used to filter it, and the filtrate was utilized to analyze the protein. The microfuge tube was filled with the sample filtrate and a freshly made Bradford reagent in a 1:10 ratio. It was then placed in a dark room for no more than an hour. After that, 200 µL of each mixture was put into a 96-well plate, and the absorbance was taken at 595 nm in a spectrophotometer. Bovine Serum Albumin (BSA) solution in water (10–500 µg/mL) was used as a standard solution. The protein content of the fruit was determined using the standard curve of BSA ($y = 0.0007x+0.1652$, $R^2 = 0.9886$).

Fat content

Fat content was determined by solvent extraction method (Tee et al., 1996) with slight modifications. Five grams of dry sample was weighed and kept in thimble paper, then placed into a Soxhlet extractor. Before the installation of the extractor, a dry, clean round-bottom flask was weighed, and 250 milliliters of petroleum ether solvent was added to the same round-bottom flask. After that, the extraction unit was assembled and heated at 40–60°C to boil the solvent. The extraction process was continued for about 6 hours. Then the flask was removed from the extraction unit and heated in an oven at 70°C to remove the excess solvent. The flask was cooled in a desiccator, and weight was taken. Fat % was calculated using the formula:

Crude Fat % = $(W_1 - W_2) / W_3 \times 100$

Where,

W_1 = Weight of the flask with extracted fat

W_2 =Weight of empty flask

W_3 = Weight of sample taken

Ash Content

The ash content was determined using the dry ash method (Tee et al., 1996). Five grams of the sample was placed in a clean and dried crucible. The sample was then incinerated at 550°C for approximately 6 hours or until the biological matter burned out and appeared yellowish or grayish ash. The remaining inorganic residue was weighed after being cooled in a desiccator.

Moisture content

The moisture content of the fresh fruit samples was estimated using the direct drying method (Bradley, 2010) with minor modifications. Each fresh sample was weighed five grams, put in a crucible that had been cleaned, and heated to 110ÚC using a hot air oven. The crucible's final weight was recorded every two hours until it reached a consistent value. The moisture content was calculated using the formula below:

Moisture % = $(A - B) / C \times 100$

Where,

A = Initial weight of crucible with sample

B = Final weight of crucible with sample

C = Weight of the sample

2.4 Antioxidant Activity

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity of fruit extracts was evaluated as described previously (Pavlov et al., 2002). In brief, 900 µL of 0.1 mM DPPH was mixed with 300 µL of methanolic extracts of different concentrations (5-50 µg/mL) of fruit samples. The above mixture was mixed well using a vortex. After 30 minutes of incubation in the dark, the absorbance of the reaction mixture was measured at 517 nm using a spectrophotometer. Ascorbic acid was used as positive control and the blank was prepared using methanol and DPPH only. The antioxidant

activity of the extracts was calculated using a linear equation curve and expressed as an IC_{50} value (Thapa et al., 2024).

2.5 Antimicrobial Activity

Crude extract preparation

Five grams of powdered dry fruits were dissolved in 60 mL of methanol at 150 rpm at 37°C for 48 hours in a shaking incubator, then filtered using Whatman filter paper no. 1. The filtrate was evaporated in a water bath heated to 50°C. The dry extract was kept at 4°C for later examination.

Preparation of stock/working solutions

A stock solution with a concentration of 100 mg/mL was made by dissolving 100 mg of the fruit sample's dry methanolic extract in 1 mL of dimethyl sulfoxide (DMSO). Likewise, concentrations of 25 mg/mL, 50 mg/mL, and 75 mg/mL were prepared and worked with the antimicrobial test.

Collection of test organisms

The six American Type Culture Collection (ATCC) strains of bacteria, namely *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6051a, *Samonella enterica* subsp. *enterica* serovar Typhi ATCC 19430, and *Enterococcus faecalis* ATCC 29212, as well as one strain of the fungus, *Candida albicans* ATCC 10231, were collected from the National Public Health Laboratory, Teku, Kathmandu, Nepal. The above microorganisms were then sub-cultured on Nutrient Broth and further cultivated on Nutrient Agar petri plates.

Antimicrobial assay by agar well diffusion method

The sterile Muller Hinton Agar media measuring 9 cm was prepared. An updated version of the approach was used to conduct an antimicrobial assay of fruit extract utilizing the agar-well diffusion method (Nowak et al., 2022). Using a sterile cotton swab, the freshly made bacterial inoculums were compared and matched with 0.5 MacFarland standards before being swabbed over Mueller-Hinton agar. Four wells were drilled into the medium containing inoculums and appropriately labeled using a sterile, 6 mm-diameter borer. Tetracycline (30 µg) for *Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis*; chloramphenicol (30 µg) for *Bacillus subtilis, Staphylococcus aureus, Salmonella* Typhi, and itraconazole (10 µg) for *Candida albicans* were used as positive controls in the tested organisms. A positive control was positioned in the center of the plates, and 40 µL each of the test and negative control solutions (10% DMSO) were added to a test well. Then the plates were left at room temperature to allow the extracts to diffuse in the medium for about 30 minutes. After that, the plates were incubated at 37°C for 24 hours. The zone of inhibition of the extract was measured in millimeters (mm) including the well to measure its antibacterial activity.

2.6 Data Analysis

All the data except for antimicrobial activity were collected in triplicates. The data analysis was performed using Microsoft Excel.

3. Results

3.1 Nutritional composition

As a part of nutritional variability, parameters like moisture content, carbohydrate content, protein content, fat, and ash content evaluated for four different wild edible fruits are shown in Table 2. Among the evaluated parameters, moisture content was found highest (88.45%)

in the fruits of *R. paniculatus* and lowest (52.52%) in the fruits of *M. semiserrata*. The carbohydrate content was again found highest (10.64%) for the dry weight of the fruits of *R. paniculatus* and lowest (2.4%) for *P. crenulata.* Similarly, the protein content was highest in the fruits of *P. crenulata* (0.66%) and lowest in the fruit samples of the remaining species (0.08 to 0.09%). All the fruits except *P. crenulata* (0.84%) were found to contain higher fat content which ranged from 7.64-10.83%. Furthermore, the ash content evaluated for different fruits was found highest (1.90%) for *M. semiserrata* and lowest (0.36%) for *R. paniculatus*.

3.2 Antioxidant Activity

The results of IC_{50} values are presented in Figure 1. Compared to the standard ascorbic acid (21.30µg/mL), all fruit extracts revealed free radical scavenging activity. However, among the fruits, *B. asiatica* showed the lowest IC_{50} value i.e. highest radial scavenging activity.

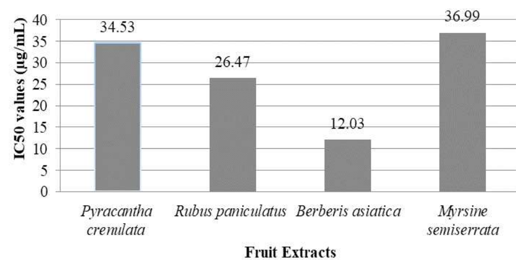

Fig 1. IC_{50} values of different fruits samples

3.3 Antimicrobial Activity

The antimicrobial activity of wild edible fruits was determined by the agar-well diffusion technique and examined on ATCC cultures of seven common human pathogens: *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Samonella enterica* subsp. *enterica* serovar Typhi*, Bacillus subtilis, Enterococcus faecalis,* and *Candida albicans* (Table 3 and Table 4). The fruit extract of four species at higher concentrations (100 mg/mL) showed a higher zone of inhibition, compared to the lower concentration of fruit extracts. At lower concentrations (25 mg/mL), only the fruit extract of *Rubus paniculatus* showed a zone of inhibition against all three gram-negative bacteria. In the case of gram-positive bacteria and *C. albicans*, all the fruit extracts except *B. asiatica*, at lower concentrations (25 mg/mL), showed a clear zone of inhibition.

4. Discussion

Wild edible fruits are highly appreciated fruits due to their distinct flavors, textures, and colors. Wild edible fruits have recently been demonstrated to have considerable health advantages due to their high antioxidant content, vitamins and minerals, fiber, and folic acid. Rural and tribal groups depend heavily on various wild edible fruits for their livelihood (Ercýslý & Sagbas, 2017). However, human

Table 2. Nutritional composition of fruit samples

S.N.	Fruit Samples	Moisture (%)	Carbohydrates (%)	Proteins (%)	Fat (%)	Ash content (%)
1	*P. crenulata*	84.12±0.65	2.4±0.24	0.66±0.01	0.84±0.69	0.80±0.56
2	*R. paniculatus*	88.45±0.43	10.64±0.13	0.08±0.04	10.83±0.11	0.36±0.11
3	*B. asiatica*	74.47±0.59	8.14±0.29	0.09±0.03	7.68±0.55	1.08±0.20
4	*M. semiserrata*	52.51±0.10	3.13±0.28	0.08±0.01	7.64±0.54	1.90±0.33

All data are means ± SD

Table 3: Zone of inhibition (in mm) of fruit extracts against gram negative bacteria

S.N.	Samples	Pathogens	Zone of inhibition (in mm)				
			100 mg/mL	75 mg/mL	50 mg/mL	25 mg/mL	*Positive controls
1	*Pyracantha crenulata*	*E. coli*	14	14	13	11	24 (Tetr.)
		*S.*Typhi	9	7	7	0	25 (Chlo.)
		K. pneumoniae	8	8	8	0	26 (Tetr.)
2	*Rubus paniculatus*	*E. coli*	13	11	11	10	24 (Tetr.)
		S. Typhi	15	13	10	8	25 (Chlo.)
		K. pneumoniae	13	12	10	8	27 (Tetr.)
3	*Berberis asiatica*	*E. coli*	8	0	0	0	25 (Tetr.)
		S. Typhi	9	8	0	0	26 (Chlo.)
		K. pneumoniae	9	8	8	8	26 (Tetr.)
4	*Myrsine semiserrata*	*E. coli*	15	14	14	13	24 (Tetr.)
		S. Typhi	11	9	8	0	25 (Chlo.)
		K. pneumoniae	13	11	11	8	26 (Tetr.)

* Positive controls (antibiotics) Tetr.: Tetracycline, Chlo.: Chloramphenicol

Table 4. Zone of inhibition (in mm) of fruit extracts against gram-positive bacteria and *C. albicans*

S.N.	Samples	Pathogens	Zone of inhibition (in mm)				
			100 mg/mL	75 mg/mL	50 mg/mL	25 mg/mL	*Positive controls
1	*Pyracantha crenulata*	*E. faecalis*	9	9	8	8	24 (Tetr.)
		B. subtilis	12	11	9	7	27 (Chlo.)
		S. aureus	13	10	10	8	28 (Chlo.)
		C. albicans	14	13	12	11	28 (Itra.)
2	*Rubus paniculatus*	*E. faecalis*	12	11	9	9	24 (Tetr.)
		B. subtilis	11	11	11	9	28 (Chlo.)
		S. aureus	16	15	12	9	28 (Chlo.)
		C. albicans	17	15	14	12	26 (Itra.)
3	*Berberis asiatica*	*E. faecalis*	9	8	8	7	25 (Tetr.)
		B. subtilis	9	9	8	0	27 (Chlo.)
		S. aureus	11	9	8	0	29 (Chlo.)
		C. albicans	7	7	0	0	26 (Itra.)
4	*Myrsine semiserrata*	*E. faecalis*	12	11	11	10	25 (Tetr.)
		B. subtilis	14	12	9	8	27 (Chlo.)
		S. aureus	12	11	9	8	29 (Chlo.)
		C. albicans	17	17	16	14	26 (Itra.)

* Positive controls (antibiotics) Tetr.: Tetracycline, Chlo.: Chloramphenicol, Itra.: Itraconazole

activities, such as changes in land use, including the expansion of the agricultural sector, leading to deforestation, have resulted in the loss of wild edible fruit diversity (Beckmann et al., 2019).

In the current research, four wild edible fruits were selected to evaluate their nutritional and medicinal qualities. Several factors, like climatic conditions, temperature, parts of the plant used, extraction time, analytical methods, and extraction procedure, play an important role in isolating bioactive compounds that have pharmacological activity and influence the nutritional value of fruits (John et al., 2006; Niu et al., 2008). Among the nutrition composition of selected wild edible fruits, moisture content was highest (88.45%) in the fruits of *R. paniculatus* which was similar to the value (84%) reported earlier (Rana et al., 2018). Moisture content plays a major role in food preservation, processability, quality, and resistance to deterioration (Nielsen, 2010). The reason for its high moisture content could be attributed to an increase in the percentage of juice in fruits, and a decrease in the percentage of total dissolved solids indicates its appropriateness for manufacturing juices and jams (Gerasopoulos & Stavroulakis, 1997). The slight difference in the moisture value might be the fruit maturity time, harvesting time, storage duration, soil moisture, and humidity. Besides moisture, carbohydrates covered the highest percentage of nutrient composition. The value of the carbohydrate content of *P. crenulata* was lower than the amount reported by Singh et al. (2018) i.e. 5.90%, and Saklani and Chandra (2014) i.e. 24.88%. Among the studied fruits, the amount of protein was lowest (0.08-0.66%) compared to fats (0.84-10.83%). The fat content in the fruits of *P. crenulata* was more or less similar to the value reported earlier (Saklani & Chandra, 2014; Rana et al., 2018). In general, fruits have low lipid and protein content. However, fruits with high lipid content can regulate a wide range of functions, including blood clotting, blood lipid levels, and immune and inflammatory responses (Aune, 2016).

The mineral strength of wild edible fruits was assessed by their ash content, which ranged from 0.36±0.11% in *R. paniculatus* to 1.90±0.33% in *M. semiserrata* in the current study. The ash content of *P. crenulata* was 0.80±0.56%, which is somewhat similar to the value reported by Dincer and Temiz (2023) for *P. coccinea* var. *lalandi*, i.e., 0.85%. The ash content in food is related to the presence of macrominerals and microminerals which play a crucial role in human health (Dawadi et al., 2022; Rupérez, 2002). The differences in nutritional content might be attributed to changes in sample and processing procedures, geo-climatic conditions, plant age, fruit maturity, species variances, and harvesting environment (Ercisli & Orhan, 2007; Hegazy et al., 2019).

The DPPH assay is one of the most reliable techniques for evaluating the potential of antioxidants present in a sample to scavenge radicals through the hydrogen atom transfer mechanism (Huang et al., 2005). The quantity of sample needed to generate 50% of the DPPH free radical scavenging activity is referred to as IC_{50}, which determines the antioxidant activity of the sample. The study revealed that *B. asiatica* methanolic extract had maximum antioxidant potential with an IC_{50} value of 12.03 µg/mL (R^2 = 0.9828) but less than the value reported by (Dhungel et al., 2016) i.e. 90.73. The IC_{50} value of *P. crenulata* methanolic extract was found to be 34.53 µg/mL, whereas Pandey et al. (2023), and Tewari et al. (2020) reported 18.75 µg/mL and 115.29 µg/mL, respectively, from ethanolic extract. The

variation in IC_{50} may be due to environmental factors (water, air, soil, and elevation), genetic differences between species, extraction methods, and methods of measurement (Zargoosh et al., 2019). Antioxidants, secondary metabolites found in fruits, can protect against oxidative stress and cell damage. Consuming antioxidant-rich food may reduce the risk of diseases caused by free radicals (Sen & Chakraborty, 2011).

The antimicrobial activity of all wild edible fruit was determined by the agar-well diffusion technique. All four tested fruit species exhibit some zone of inhibition against tested human pathogens, which may be due to the presence of terpenoids, which exert antimicrobial effects through multiple mechanisms. These mechanisms may include disruption of microbial cell membranes, inhibition of essential enzymes or proteins, interference with microbial biofilm formation, and modulation of microbial gene expression. Terpenoids may also act synergistically with other antimicrobial compounds, such as antibiotics or other plant secondary metabolites (Huang et al., 2022). In this study, methanolic fruit extract of *P. crenulata* exhibited a zone of inhibition ranging from 8-13 mm against selected gram-positive bacteria and yeast species i.e. *E. coli*, *K. pneumonia*, *S. aureus,* and *C. albicans* at a concentration of 50 mg/mL which is similar (11 mm zone of inhibition) to the previous findings (Saklani & Chandra, 2014). The inhibitory property of wild edible fruit concentrates may be due to the presence of phytochemicals, organic acids, and polyphenols, which showed a significantly positive correlation with the value of phytochemicals observed in wild edible fruits (Rios & Recio, 2005). Terpenoids may also act synergistically with other antimicrobial compounds such as antibiotics or other plant secondary metabolites (Huang et al., 2022).

5. Conclusions

The present study revealed that the selected fruits vary in their nutritional composition, antioxidant, and antimicrobial properties. *R. paniculatus* and *B. asiatica* in particular, could be useful in the preparation of beverages and other value-added products due to their stronger antioxidant activity and higher carbohydrate contents.

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