Proximate analysis, phytochemical Properties, Antioxidant, Antimicrobial Properties, and pH-Responsive Colorimetric Behavior of *Pyracantha crenulata* (Ghangaru) Fruit Juice Extract.

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Abstract

Pyracantha crenulata (Ghangaru), a perennial evergreen shrub native to the Himalayan region, produces nutrient-rich berries traditionally consumed for food and medicine but remains under-explored in scientific and commercial applications. This study examined the nutritional composition, phytochemical profile, antioxidant, antimicrobial activities, and pH-responsive properties of *P. crenulata* fruit juice. The fruit showed high moisture content (75.13%), and measurable levels of protein (0.47%), carbohydrate (1.82%), and fat (0.51%). Phytochemical analysis revealed substantial total phenolic content (25.05 mg GAE/mL) and flavonoid content (21.01 mg QE/mL). The extract demonstrated strong antioxidant activity with an IC_{50} of 35.74 µg/mL and significant antimicrobial activity, particularly against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. FTIR analysis confirmed the presence of phenolic and flavonoid functional groups. The extract showed distinct pH-responsive color transitions from pinkish red to brown across acidic to alkaline ranges, highlighting its potential as a natural pH indicator. These findings establish *P. crenulata* as a rich source of bioactive compounds with notable antioxidant and antimicrobial efficacy, as well as promising applications in nutraceuticals, pharmaceuticals, and intelligent food packaging. Promoting its utilization could support both scientific advancement and economic opportunities for Himalayan communities.

Introduction

Pyracantha crenulata, belonging to the Rosaceae family, is a perennial evergreen shrub naturally found in the temperate Himalayan regions of Nepal, and also in areas of Bhutan and northern India [1]. Locally referred to as 'Ghangaru,' it is a thorny, densely branched plant. It blooms in April, with its small, red, nutrient-rich berries ripening around July [2]. These edible fruits are packed with bioactive compounds, making them a valuable source for functional foods, antioxidants, and nutraceutical applications. Rich in vitamins, minerals, phenolics, flavonoids, and anthocyanins, the berries are traditionally consumed raw an d are known for their nutritional and medicinal properties [1]. Traditionally, *P. crenulata* has been used to stimulate appetite, particularly in anorexia, and to address health issues such as anxiety, burns, and cardiovascular conditions [2]. The powdered roots are

regularly blended into bath water to aid in treating skin infections, reducing body pain, and managing various skin issues [3-5]. The fruits have long been a staple in Himalayan diets and are also used to produce value-added products like juice, squash, sauces, and jams [6].Despite its widespread presence and recognized edibility in Nepal's hilly regions, the full potential of *P. crenulata* remains underexploited both in scientific research and commercial use. Many local communities are unaware of its medicinal and nutritional benefits. Promoting scientific studies on its therapeutic value and the pH-sensitive properties of its anthocyanins could enhance public knowledge, boost demand, and drive the development of related products. This would open doors to economic opportunities through the commercialization of its value-added products.

Phenolic compounds are secondary metabolites which are highly important bioactive molecules due to their notable medicinal benefits for humans. Their basic structure typically includes a phenolic ring (C_6H_5OH), along with carboxylic acid (-COOH) and hydroxyl (-OH) functional groups [7, 8]. Plant phenolics comprise a diverse group of compounds, including phenolic acids, flavonoids, tannins, stilbenes, and lignans. Among these, flavonoids are the most prevalent polyphenols in the human diet and are categorized into subgroups like flavones, flavanols, flavanones, isoflavones, and anthocyanins [9, 10]. Flavonoids are well-known for their antioxidant, anti-inflammatory, anti-mutagenic, and anti-carcinogenic effects, making them essential ingredients in nutraceuticals, pharmaceuticals, cosmetics, and various medical applications [11].

Phenolic compounds function as antioxidants through various mechanisms. These hydroxyl groups in flavonoids act as efficient hydrogen donors, allowing them to neutralize reactive oxygen and nitrogen species through a termination reaction that stops the propagation of new radicals [12].When the body lacks sufficient antioxidant, it can rely on external sources from food, dietary supplements, or medications. Key exogenous antioxidant include phenolic compounds, carotenoids, vitamin C, and certain minerals such as selenium and zinc [13]. These include neutralizing oxidative agents, enhancing immune function, regulating genes involved in cell growth and death, controlling hormone levels, and displaying antibacterial and antiviral properties [14, 15]. The antihypertensive effect of its fruits has been scientifically proven and tested in several clinical trials, showing their ability to alter the progression of cardiovascular diseases and reduce the risk of related deaths [16]. Preclinical studies have demonstrated that *P. crenulata* exhibits antiurolithiatic activity, along with strong antimicrobial and antioxidant properties in vitro [17-19]. Anthocyanins, the most common water-soluble pigments found in plants, are responsible for the vivid hues seen in flowers, fruits, and leaves [20, 21]. The intensity and shade of color in anthocyanins are affected by the quantity and arrangement of hydroxyl and methoxyl groups within their molecular structure [22]. Being polar, anthocyanins dissolve easily in polar solvents like methanol, ethanol, and water. Their color varies with the pH of the solution, due to their ionic structure. In acidic conditions, some anthocyanins appear red, and they are more stable in lower pH solutions [13, 14]. Anthocyanins from various plants have been explored for pH responsive colour variation; however, the anthocyanins from *P. crenulata* still remained unexplored for their pH responsive sensing properties.

This research seeks to address that knowledge gap by examining the fruit extract of *P. crenulata*, specifically focusing on its pH-sensitive color-changing properties, total flavonoid and phenolic

content, as well as its antioxidant and antimicrobial activities. By examining these properties in methanol and ethanol extracts, this research enhances our understanding of the plant's phytochemical and pharmacological value. The findings support its potential application in food intelligent food packaging functional foods, nutraceuticals, and therapeutic products, contributing to its broader recognition and utilization in both scientific and commercial arenas.

Materials and methods

Chemicals and reagents

Disodium hydrogen phosphate and 2,2-diphenyl-1-picrylhydrazyl (DPPH), Quercetin were acquired from Glentham Life Sciences. Disodium hydrogen phosphate (Na2HPO4) were used to prepare various buffer solutions with a pH range of 2.0 to 12.0. The Folin-Ciocalteu reagent was obtained from Research Lab Fine Chem Industry, and ascorbic acid from Central Drug House. Gallic acid was sourced from Loba Chemie, while sodium carbonate, hydrochloric acid, and citric acid were provided by Fischer Scientific. Ethanol was sourced from Changshu Hongsheng Fine Chemical Co. Ltd. Citric acid ($C_6H_8O_7$).

Sample collection, identification and juice preparation

P. crenulata berries were harvested from Chhuwala, Ward No. 10, Dullu Municipality, Dailekh, Nepal. The plant was verified by National Herbarium and Plant Laboratories, Godawari, Nepal. The harvested berries were cleaned with tap water then distilled water. The freshly collected fruits were ground with mechanical grinder and filtered with muslin cloth. The resulting juice was stored at 4 °C under refrigeration for further analysis.

FTIR spectroscopy

FTIR spectra were recorded using an infrared spectrometer (Shimadzu, Japan), recorded from 4000 to 400 cm^{-1} at a resolution of 1 cm^{-1}.

pH responsive color sensing activity

A series of buffer solutions covering the pH range from 2 to 12 were prepared, and 3 mL of each was transferred into separate vials. Subsequently, 100 µL of the pyracantha fruit juice was added to each buffer solution, and the resulting color changes were recorded through photographic documentation.

Proximate analysis

Total fat content, protein content and carbohydrate content

The total fat content in the juice was assessed using the separating funnel extraction techniques [23]. Protein quantification was conducted following the method outlined by Bradford [185]. Briefly, 100 µL of the juice sample, Bovine Serum Albumin (BSA) standard solution, and control (water) were dispensed into separate 1.5 mL micro centrifuge tubes. Subsequently, 1000 µL of freshly prepared Bradford reagent, at a 1:10 dilution ratio, was added to each tube. All samples and controls were analyzed in triplicate, thoroughly vortexed, and incubated at room temperature for a minimum of 5 minutes and no longer than 30 minutes. Absorbance was then measured at 595 nm using a spectrophotometer.

The phenol–sulfuric acid assay is a commonly used method for quantifying carbohydrate content in liquid samples [24]. In this method, 1 mL of 5% aqueous phenol solution is mixed with 1 mL of the test sample, followed by the immediate addition of 5 mL of concentrated sulfuric acid. The mixture is

allowed to stand for 10 minutes, then vortexed for 30 seconds, and subsequently incubated in a water bath for 20 minutes to allow color development. Glucose is used as the reference standard, and the absorbance of the resulting solution is recorded at 480 nm using a spectrophotometer.

Phytochemical analysis

Total phenolic content

The total phenolic content (TPC) of juice sample was determined using a modified version of the Folin–Ciocalteu colorimetric method, based on the protocol by Cliff et al [25] In this procedure, 0.5 mL of the juice sample was mixed with 0.25 mL of Folin–Ciocalteu reagent and 4.25 mL of distilled water to obtain a final volume of 5 mL. After a 5-minute reaction period, 5 mL of 20% sodium carbonate solution was added, and the mixture was thoroughly blended. The solution was then incubated at 37 °C for 90 minutes to allow for color development. Absorbance was measured at 750 nm using a Multiskan SkyHigh microplate spectrophotometer, with gallic acid serving as the calibration standard

Total flavonoid content

The total flavonoid content (TFC) of the juice sample was determined following the spectrophotometric method outlined by Hosu et al. [26]. One milliliter of the sample was diluted with 4 mL of distilled water, followed by the addition of 0.3 mL of 5% sodium nitrite solution. After standing for 5 minutes, 0.3 mL of 10% aluminum chloride solution was added, and the mixture was incubated for an additional 5 minutes. Subsequently, 2 mL of 1 N sodium hydroxide was added, and the final volume was adjusted to 10 mL by adding 2.4 mL of distilled water. The absorbance was then measured at 510 nm using a spectrophotometer, with quercetin employed as the standard reference compound.

Antioxidant assay (DPPH assay)

The antioxidant activity of the juice was evaluated using a modified version of the DPPH assay as described by Patel *et al*.[27].In this method, 1 mL of the sample was mixed with 3 mL of 0.1 mM DPPH solution prepared in methanol. The reaction mixture was incubated in the dark for 30 minutes to allow for radical scavenging activity(RSA), after which the absorbance was recorded at 517 nm.

$$\mathrm{RSA}(\%) = \frac{\mathrm{A_{DPPH}} - \mathrm{A_S}}{\mathrm{A_{DPPH}}} \mathrm{X100}$$

where A_S is the absorbance of the sample-containing solution and A_{DPPH} is the absorbance of the DPPH control. The concentration of the extract required to achieve 50% scavenging activity (EC_{50}) was derived from the plot of RSA percentage versus extract concentration. Ascorbic acid was used as the reference standard, and a blank consisting of 1:3 ratio of solvent to DPPH solution was employed for baseline correction.

Total moisture content

The moisture content was determined by direct drying method [28]. Clean 3 g sample was taken in a crucible and heated in hot air oven maintained at 110^{o}C. The crucible was weighted at one-hour interval until the constant weight was achieved, indicating complete removal of moisture. The moisture content was determined as:

$$\text{Moisture content } (\%) = \frac{\mathrm{W_1} - \mathrm{W_2}}{\mathrm{W_1}} X100$$

Where,

W_1= Weight of sample before heating and

W_2= Constant weight of sample after heating

Total ash content

The total ash content was determined by dry ashing method [29]. A muffle furnace was used to burn the sample. Clean 3 g sample was taken in a crucible and heated up to 600 ºC until yellowish grey ash was obtained. The remained inorganic residue was weighted after cooling the crucible in desiccator.

Antimicrobial activity

The antimicrobial potential of the fruit juice was evaluated using the agar well diffusion technique. The assay was performed against seven microbial strains, comprising two Gram-positive bacteria (*Enterococcus faecalis*, *Staphylococcus aureus*), four Gram-negative bacteria (*Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*), and one fungal strain (*Candida albicans*). The bacterial cultures were maintained in nutrient broth, while *C. albicans* was propagated in potato dextrose broth. Fresh microbial suspensions were adjusted to the 0.5 McFarland turbidity standard and uniformly inoculated onto Mueller–Hinton agar (for bacteria) and potato dextrose agar (for *C. albicans*) using the spread plate technique. Wells of 6 mm diameter were aseptically bored into the agar plates, properly labeled, and filled with 50 µL of fruit juice samples at concentrations ranging from 50 to 100 mg/mL. The plates were kept at ambient temperature to facilitate diffusion of the extract and subsequently incubated at 37 °C for 24 hours. Antimicrobial efficacy was determined by measuring the diameters of inhibition zones surrounding each well, while standard antibiotic discs corresponding to each test organism served as positive controls.

Statistical analysis

The data presented are the averages of triplicate measurements. Origin 2018 and Microsoft Excel 2016 were utilized for data analysis.

Results and Discussion

Spectroscopic analysis of extract

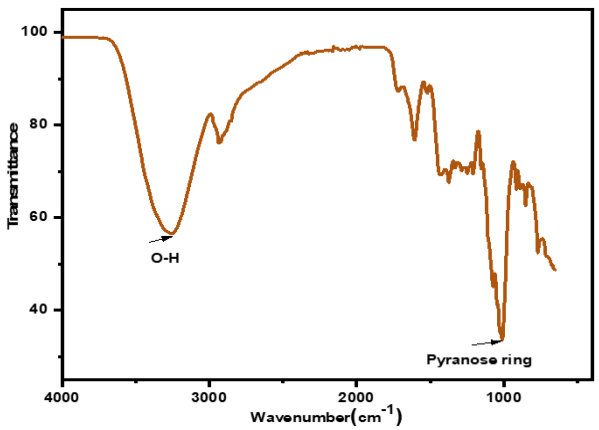

Figure 1. FTIR spectra of *P.crenulata fruit extract*

FTIR is a highly effective method employed for the analysis of various compounds, offering essential insights into the molecular structure found in the sample [30].FTIR spectra were used to identify the chemical bonds and functional groups in the fruit extract. The FTIR bands observed in the 4000–1500

cm−1 range indicate the presence of functional groups, while the strong absorption bands in the 1500–500 cm−1 region correspond to the fingerprint region [31].FTIR spectrum of anthocyanin (Fig. 1) showed a strong peak at 3270cm^{-1} for O-H bond-stretching and the peak at 2940 cm^{-1} for C-H bond-stretching [32, 33]. The OH group plays a significant role in antidiabetic, antioxidant, and antibacterial properties [34].The peak observed at 1710 cm^{-1} corresponds to the stretching vibration of the carbonyl (C=O) group present in the compound [33].The peak at 1608 cm^{-1} indicated the C=C stretch of the aromatic hydrocarbon skeleton [35]. The band at 1020 cm^{-1} can be attributed to C-O-C stretching vibrations [36]. The IR peaks from 993 to 1149 cm^{-1} can be assigned to the typical pyranose ring of glucose [37]. Overall, the analysis revealed the presence of O-H, C=O, C=C, and C-O-C functional groups, which are characteristic of phenolic compounds such as flavonoids and anthocyanins, along with an additional peak below 800 cm^{-1} indicating the presence of a benzene ring [32, 38].

pH responsive properties of *P.crenulata* fruit extract

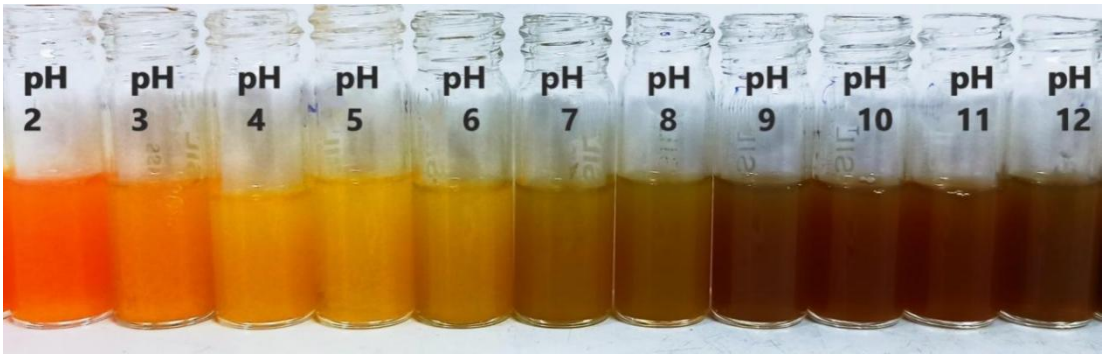

Figure 2. Variation of colors of fruit extract of *P.crenulata* in different pH(2-12).

Anthocyanins are responsible for the coloration found in various parts of plants and their derived products. The color they produce is influenced by several factors, including their molecular structure, the surrounding environment, physical conditions, and interactions with other substances [39].They are unstable molecules because of their conjugated double bonds and the associated electron resonance, making them highly sensitive to factors like pH, temperature, light, metal ions, and solvents. In acidic conditions, the flavylium cation is the dominant form. As the pH slightly increases, deprotonation occurs rapidly, leading to the formation of blue quinonoid structures. However, water can also react with anthocyanins, producing colorless hemiketal forms, which may further convert into chalcone structures under alkaline conditions [40]. Fig.2 displays the color variation of ghangaru fruit extract in buffer solutions of different pH(2-12). The color of the extract solution shifted from pinkish-red to brown as the pH of the buffer increased from 2 to 12. More specifically, it was orange-red at pH 2–3, turned yellow at pH 4–6, changed to greenish yellow at pH 7–9, and became brown at pH 10–12. Anthocyanin molecules experience structural transformations in response to changes in pH. Particularly, these color variations were due to structural transformations of the anthocyanin pigment, particularly involving the flavylium cation [21, 41]. Anthocyanins contribute to vibrant coloration of flowers, fruits, and vegetables, attracting insects that facilitates pollination and seed dissemination [42]. In addition to their ecological function, anthocyanins protect DNA and the photosynthetic system from strong radiation exposure and exhibit strong antioxidant properties. Recently, anthocyanins

derived from various plant sources have gained considerable attention on natural food colorant and smart food packaging. Considering the widespread presence of *P. crenulata* in the hilly regions, exploring the pH-responsive properties of its fruit extract offers valuable opportunities for scientific advancement and potential benefits to local communities.

Proximate analysis

Its moisture content was measured at 75.13 %, while the ash content was found to be 0.78 %. The levels of fat, protein, and carbohydrates were 0.51 %, 0.47 %, and 1.82 %, respectively which is presented in Table 1.

Table 1: Proximate analysis of juice (Data with ± standard Deviation).

Parameters	Moisture content (%)	Ash content (%)	Protein content (%)	Carbohydrate content (%)	Fat content (%)
Value	75.13 ± 0.31	0.78 ± 0.48	0.47 ± 0.1	1.82 ± 0.15	0.51 ± 0.2

Phytochemical analysis

Total phenolic content, Total flavonoid content, and total anthocyanincontent

The TPC of was determined using calibration curve of Gallic acid and was found to be 25.053±0.43 mg GAE /mL of juice. Phenolic compounds are essential plant components recognized for their redox properties, which play a key role in their antioxidant activity [43]. Previous studies reported that TPC is a key determinant to scavenge free radicals, directly influencing its antioxidant potential [44]. As dietary antioxidants, plant polyphenols play a crucial role in mitigating oxidative damage, with broad implications for human health and disease prevention [8]. Due to their high phenolic content, many plants are of pharmacological interest, particularly for their potential in managing inflammatory conditions and promoting wound healing [45].

The TFC was determined using standard calibration curve of quercetin solution and was found to be 21.01 ±0.42mg QE/mL of juice. Flavonoids are a prominent group of polyphenolic compounds commonly found in fruits and vegetables. These bioactive phytochemicals possesses diverse chemical structure and are known for their strong antibacterial properties, enabling them to combat various microbial pathogens [44]. Phytochemicals, including flavonoids, interact synergistically with dietary fibers and essential minerals, playing crucial role in disease prevention [46]. In addition, to their antimicrobial effects, flavonoids offer various health benefits, including antiviral, anticancer, and antioxidant effects, as well as protective effects on the cardiovascular and nervous systems [47]. Their capacity to neutralize free radicals helps reduce oxidative stress, which is associated with different serious health problems. Given these extensive health benefits, incorporating flavonoid-rich fruits and vegetables into the daily diet is highly recommended to support overall well-being and disease prevention.

Antioxidant property mineral content

Antioxidant property of *P.crenulata fruit* juice extract was studied by determining the radical scavenging activity (RSA) from concentration 5 to 50 µg/mL and presented in Fig.3.The RSA (%) exhibited a linear increase with increasing the juice concentration, demonstrating strong antioxidant

potential. At 50 µg/mL, the extract achieved an RSA of 69.21 %. The IC50 value, which represents the amount of antioxidant activity required to reduce the initial DPPH concentration by 50 %, is a typical measure of antioxidant activity [48]. A lower IC_{50} value suggests greater antioxidant potency [49]. The study revealed *P.crenulata fruit* juice showed the greater antioxidant property with IC_{50} values of 35.74±0.19µg/mL as compared with ascorbic acid (17.10 µg/mL), further confirming its strong antioxidant activity. Natural antioxidants present in fruits, vegetables, spices, leaves, cereals, flowers offer significant health benefits, particularly in healthcare. Their capacity to neutralize free radicals is essential in helping to prevent infectious diseases [50]. The antioxidant activity of phenolic compounds and flavonoids is strongly related to the number of hydroxyl (-OH) groups in the sample. Furthermore, the exact positioning of these hydroxyl groups is crucial in boosting their ability to scavenge free radicals [51, 52]. The hydroxyl groups in these compounds function as effective hydrogen donors, enabling them to neutralize reactive oxygen and nitrogen species. This process occurs through a termination reaction that halts the formation of new free radicals [12].

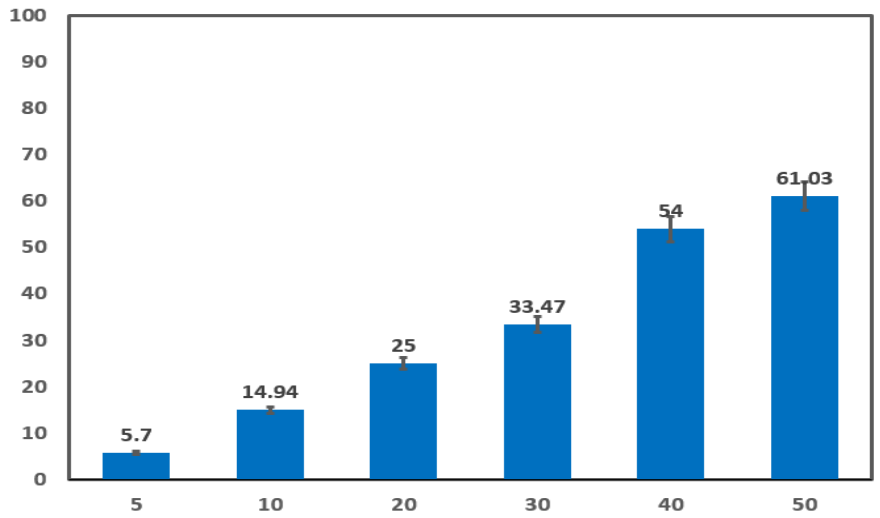

Figure 3: DPPH radical scavenging activity of *P.crenulata* fruit extract sample with different concentrations.

Antimicrobial activity

Phenolic compounds, due to their bioactive nature, exhibit antimicrobial properties by acting through multiple mechanisms, impacting various components of microbial structure and function. Their effectiveness is probably linked to their ability to interact with extracellular and soluble proteins, as well as bind to bacterial cell walls [53]. The juice extract exhibited a significant inhibitory effect on *Escherichia coli* at all tested concentrations (50–100 mg/mL) which is presented in Table.2 The fungal strain *Candida albicans* also responded well to the extract. Notably, at a concentration of 100 mg/mL, there was a substantial inhibition of *E. coli*, *S. aureus* and *C. albicans* growth. In contrast, the extract had limited effects on *Enterococcus faecalis*, *Salmonella typhi*, and *Klebsiella pneumoniae*, which displayed relatively small zones of inhibition. The largest zone of inhibition was recorded at 100 mg/mL for *E. coli* (14mm) and *C. albicans* (13 mm). These results indicate that *P. crenulata* has promising antimicrobial properties, supporting its potential use in pharmaceutical applications.

Table 2: Antimicrobial activity with zone of inhibition (mm) of different concentration of extract(50-100mg/mL)

S.N.	Organism	Zone of inhibition (mm)			
		50 mg/mL	75 mg/mL	100 mg/mL	Control
1	*E. faecalis (ATCC 29212)*	7	8	8	29
2	*S. aureus (ATCC 25923)*	10	10	13	28
3	*E. coli (ATCC 25922)*	11	12	14	24
4	*S. Typhi (ATCC 19430)*	7	7	8	25
5	*K. pneumoniae (ATCC 6380)*	8	8	8	32
6	*P. aeruginosa (ATCC 9027)*	8	8	9	23
7	*C. albicans (ATCC 10231)*	11	12	13	28

Positive control: Tetracycline-30 µg (bacterial strain) and Itraconazole-10 µg (fungal strain), respectively.

Conclusion

This study investigated the proximate, phytochemical, antioxidant and antimicrobial activities of juice of *Pyracantha crenulata* (Ghangaru) fruit demonstrates significant nutritional and phytochemical richness and biological activities, positioning it as a valuable yet underutilized natural resource. Widely distributed in the temperate Himalayan region, the fruit is traditionally consumed and used for medicinal purposes, but scientific exploration has only recently begun to highlight its extensive potential. The juice of *P. crenulata* fruits revealed notable levels of TPC and TFC which cause antioxidant and antimicrobial activities. It also showed some level of carbohydrate, protein, fat content. Moreover, the fruit's anthocyanins exhibit distinctive pH-responsive colorimetric behavior, making it a promising candidate for natural food colorant and intelligent food packaging and functional product development. The spectroscopic study and biological evaluations reinforce the fruit's pharmacological significance and underscore the need for further research and commercialization. Due to its abundant availability across the hilly and mountainous regions of Nepal, *P. crenulata* holds great potential for juice and fermented beverage production, offering a promising source of income for local communities and entrepreneurs. By advancing knowledge of *P. crenulata*'s bioactive compounds and their roles in health and environmental applications, this research not only adds to scientific literature but also opens up new avenues for local economic development through the production of nutraceuticals, therapeutic agents, and pH-sensitive packaging solutions.

Authorship contribution statement

Dikpal Kumar Shahi: Writing-original draft, Methodology, Investigation, Formal analysis, Data analysis, Data curation, Conceptualization. **Shukra Raj Regmi:** Resources, Review & editing, Formal analysis. **Sanjaya Paudel:** Methodology, Editing, Review & editing, **Lok Ranjan Bhatta:** Writing-review &editing, Validation, Supervision. **Mahesh Kumar Joshi:** Writing-review &editing, Validation, Supervision, Investigation.

Conflict of interest statement

We declare that we have no conflict of interest

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References

Kewlani, P., et al., *Pyracantha crenulata (Roxb. ex D. Don) M. Roem*, in *Himalayan Fruits and Berries*. 2023, Elsevier. p. 319-330.

Sultana, S., M. Ali, and S.R. Mir, *Cinnamic acid and lanostenoic acid derivatives from the leaves of Pyracantha crenulata (D. Don) M. Roem.* Journal of Pharmaceutical and Biological Sciences, 2017. **5**(3): p. 91.

Kavidayal, H. and N. Uniyal, *A survey on traditional knowledge and the status of medicinal plants in Garhwal and Kumaon regions of Uttarakhand.* Biosci. Trends, 2020. **13**(2): p. 96-101.

Nautiyal, S., et al., *Medicinal plant resources in Nanda Devi Biosphere Reserve in the central Himalayas.* Journal of herbs, spices & medicinal plants, 2001. **8**(4): p. 47-64.

Negi, C., et al., *Ethnomedicinal plant uses in a small tribal community in a part of central Himalaya, India.* Journal of human ecology, 2002. **14**(1): p. 23-31.

Negi, V.S., R. Maikhuri, and L. Rawat, *Non-timber forest products (NTFPs): a viable option for biodiversity conservation and livelihood enhancement in central Himalaya.* Biodiversity and Conservation, 2011. **20**(3): p. 545-559.

Bernards, M., *Plant natural products: a primer.* Canadian Journal of Zoology, 2010. **88**(7): p. 601-614.

Lin, D., et al., *An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes.* Molecules, 2016. **21**(10): p. 1374.

Dai, J. and R.J. Mumper, *Plant phenolics: extraction, analysis and their antioxidant and anticancer properties.* Molecules, 2010. **15**(10): p. 7313-7352.

Vuolo, M.M., V.S. Lima, and M.R.M. Junior, *Phenolic compounds: Structure, classification, and antioxidant power*, in *Bioactive compounds*. 2019, Elsevier. p. 33-50.

Panche, A.N., A.D. Diwan, and S.R. Chandra, *Flavonoids: an overview.* Journal of nutritional science, 2016. **5**: p. e47.

Pereira, D.M., et al., *Phenolics: From chemistry to biology*. 2009, Molecular Diversity Preservation International. p. 2202-2211.

Santos-Sánchez, N.F., et al., *Antioxidant compounds and their antioxidant mechanism.* Antioxidants, 2019. **10**: p. 1-29.

Waladkhani, A. and M.R. Clemens, *Effect of dietary phytochemicals on cancer development.* International Journal of Molecular Medicine, 1998. **1**(4): p. 747-800.

Handique, J.G., M.P. Boruah, and D. Kalita, *Antioxidant activities and total phenolic and flavonoid contents in three indigenous medicinal vegetables of north-east India.* Natural Product Communications, 2012. **7**(8): p. 1934578X1200700815.

Holubarsch, C.J., et al., *The efficacy and safety of Crataegus extract WS 1442 in patients with heart failure: the SPICE trial.* Eur J Heart Fail, 2008. **10**(12): p. 1255-63.

Bahuguna, Y.M., et al., *Evaluation of Pyracantha crenulata Roem for antiurolithogenic activity in albino rats.* African journal of urology, 2009. **15**(3).

Saklani, S., et al., *Health promoting phytochemicals their concentration and antioxidant activity of wild edible fruits of Uttarakhand, India.* Asian Journal of Chemistry, 2012. **24**(12): p. 5558.

Saklani, S. and S. Chandra, *In vitro antimicrobial activity, nutritional value, antinutritional value and phytochemical screening of Pyracantha crenulata fruit.* Int J Pharm Sci Rev Res, 2014. **26**(1): p. 1-5.

Jackman, R.L., et al., *Anthocyanins as food colorants—a review.* Journal of food biochemistry, 1987. **11**(3): p. 201-247.

Roy, S. and J.-W. Rhim, *Anthocyanin food colorant and its application in pH-responsive color change indicator films.* Critical Reviews in Food Science and Nutrition, 2021. **61**(14): p. 2297-2325.

He, J. and M.M. Giusti, *Anthocyanins: natural colorants with health-promoting properties.* Annual review of food science and technology, 2010. **1**(1): p. 163-187.

Aoac, I., *Official methods of analysis of AOAC international, 18^ ed.* AOAC Int, 2010. **6**(382): p. 90022-5.

Masuko, T., et al., *Carbohydrate analysis by a phenol–sulfuric acid method in microplate format.* Analytical biochemistry, 2005. **339**(1): p. 69-72.

Cliff, M.A., M.C. King, and J. Schlosser, *Anthocyanin, phenolic composition, colour measurement and sensory analysis of BC commercial red wines.* Food Research International, 2007. **40**(1): p. 92-100.

Hosu, A., V.-M. Cristea, and C. Cimpoiu, *Analysis of total phenolic, flavonoids, anthocyanins and tannins content in Romanian red wines: Prediction of antioxidant activities and classification of wines using artificial neural networks.* Food chemistry, 2014. **150**: p. 113-118.

Patel, V.R., P.R. Patel, and S.S. Kajal, *Antioxidant activity of some selected medicinal plants in western region of India.* Advances in Biological research, 2010. **4**(1): p. 23-26.

Nielsen, S.S. and R.L. Bradley, *Moisture and total solids analysis.* Food analysis, 2010: p. 85-104.

Md Noh, M.F., et al., *Recent techniques in nutrient analysis for food composition database.* Molecules, 2020. **25**(19): p. 4567.

Patle, T.K., et al., *Phytochemical screening and determination of phenolics and flavonoids in Dillenia pentagyna using UV–vis and FTIR spectroscopy.* Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2020. **242**: p. 118717.

Karpagasundari, C. and S. Kulothungan, *Analysis of bioactive compounds in Physalis minima leaves using GC MS, HPLC, UV-VIS and FTIR techniques.* Journal of Pharmacognosy and Phytochemistry, 2014. **3**(4): p. 196-201.

Shi, Y., et al., *Preparation and chemical properties of microencapsulation developed with mulberry anthocyanins and silk fibroin.* Industrial Crops and Products, 2024. **212**: p. 118383.

Pantoja-Castro, M.A. and H. González-Rodríguez, *Study by infrared spectroscopy and thermogravimetric analysis of tannins and tannic acid.* Revista latinoamericana de química, 2011. **39**(3): p. 107-112.

Sabandar, C.W., et al., *Medicinal uses, chemistry and pharmacology of Dillenia species (Dilleniaceae).* Phytochemistry, 2017. **134**: p. 6-25.

Qin, Y., et al., *Preparation and characterization of active and intelligent packaging films based on cassava starch and anthocyanins from Lycium ruthenicum Murr.* International journal of biological macromolecules, 2019. **134**: p. 80-90.

Sarkar, A.K., et al., *Efficient removal of malachite green dye using biodegradable graft copolymer derived from amylopectin and poly (acrylic acid).* Carbohydrate polymers, 2014. **111**: p. 108-115.

Wu, S., et al., *Electrochemical writing on edible polysaccharide films for intelligent food packaging.* Carbohydrate Polymers, 2018. **186**: p. 236-242.

Tong, Y., et al., *Stability and structural characteristics of amylopectin nanoparticle-binding anthocyanins in Aronia melanocarpa.* Food chemistry, 2020. **311**: p. 125687.

Cabrita, L., T. Fossen, and Ø.M. Andersen, *Colour and stability of the six common anthocyanidin 3-glucosides in aqueous solutions.* Food chemistry, 2000. **68**(1): p. 101-107.

Hrazdina, G., *Reactions of the anthocyanidin-3, 5-diglucosides: Formation of 3, 5-di-(O-β-d-glucosyl)-7-hydroxy coumarin.* Phytochemistry, 1971. **10**(5): p. 1125-1130.

Duan, M., et al., *Development and characterization of electrospun nanofibers based on pullulan/chitin nanofibers containing curcumin and anthocyanins for active-intelligent food packaging.* International journal of biological macromolecules, 2021. **187**: p. 332-340.

Harborne, J.B. and C.A. Williams, *Anthocyanins and other flavonoids.* Natural Product Reports, 2001. **18**(3): p. 310-333.

Soobrattee, M.A., et al., *Phenolics as potential antioxidant therapeutic agents: mechanism and actions.* Mutation Research/Fundamental and Molecular mechanisms of mutagenesis, 2005. **579**(1-2): p. 200-213.

Jha, A.K., M.A. Khalid, and S.N. Labh, *In Vitro Antioxidant and Antibacterial Activities of Medicinal Flower Laligurans Rhododendron arboreum Collected from Kathmandu Valley, Nepal.* International Journal of Food Science, 2024. **2024**(1): p. 6073042.

Petti, S. and C. Scully, *Polyphenols, oral health and disease: A review.* Journal of dentistry, 2009. **37**(6): p. 413-423.

Pareek, S., *Nutritional and biochemical composition of banana (Musa spp.) cultivars.* Nutritional composition of fruit cultivars, 2016: p. 49-81.

Duan, S.-G., et al., *Untargeted metabolite profiling of petal blight in field-grown Rhododendron agastum using GC-TOF-MS and UHPLC-QTOF-MS/MS.* Phytochemistry, 2021. **184**: p. 112655.

Khan, R.A., et al., *Evaluation of phenolic contents and antioxidant activity of various solvent extracts of Sonchus asper (L.) Hill.* Chemistry Central Journal, 2012. **6**: p. 1-7.

Brighente, I., et al., *Antioxidant activity and total phenolic content of some Brazilian species.* Pharmaceutical biology, 2007. **45**(2): p. 156-161.

Kumari, R., et al., *Qualitative Chemical Plant Characterization and Evaluation of the Antioxidant and Antimicrobial potential of Rhododendron, a high Attitudinal Medicinal Plant in Uttarakhand, India.* Research Journal of Pharmacy and Technology, 2023. **16**(12): p. 5765-5769.

Meenakshi, S., et al., *Total flavanoid and in vitro antioxidant activity of two seaweeds of Rameshwaram coast.* Global Journal of Pharmacology, 2009. **3**(2): p. 59-62.

Balasundram, N., K. Sundram, and S. Samman, *Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses.* Food chemistry, 2006. **99**(1): p. 191-203.

Cowan, M.M., *Plant products as antimicrobial agents.* Clinical microbiology reviews, 1999. **12**(4): p. 564-582.