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Research Article

PHYTOCHEMICAL PROPERTIES, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES, AND COLORIMETRIC pH RESPONSIVENESS OF *Rhododendron arboreum* SM. FLOWER EXTRACT FROM MANMA-KALIKOT, NEPAL

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ABSTRACT

Rhododendron arboreum Sm. is widely found in the hilly regions of Asia and is recognized as the national flower of Nepal. This study examines the extracts of *R. arboreum* flowers for total phenolic and flavonoid contents, antioxidant and antimicrobial properties, and pH-responsive color sensing properties. The phenolic content of the extract was assessed using the Folin–Ciocalteu method, flavonoid levels were measured with the aluminum trichloride assay, and antioxidant activity was evaluated through the DPPH radical scavenging test. The antimicrobial properties of the flower extract were evaluated using the agar well diffusion method, while pH responsiveness was examined across the buffer solutions spanning pH 1 to 14. The extract contained total phenolic level of 11±0.15 mg gallic acid equivalent and flavonoid level of 13.36±0.11 mg quercetin equivalent per gram of dry extract. For antioxidant potential, IC_{50} value was determine which was found to be 20.6±1.18 µg/mL. A strong correlation was observed between phenolic level and antioxidant potential. The extracts showed bactericidal effects against Gram-positive and Gram-negative bacterial strains and moderate antifungal activity. Furthermore, anthocyanin pigments in the extracts displayed notable color variations in buffer solution under different pH conditions. These findings highlight the potential of *R. arboreum* flowers as a source of natural antioxidants, antimicrobials, and most importantly pH responsive color sensing anthocyanins, encouraging further exploration of their bioactive compounds. This study highlights the untapped potential of *R. arboreum,* promoting its broader application in scientific research and commercial development.

Keywords: Antimicrobial effect, antioxidant activity, pH sensitivity, *Rhododendron arboreum*

INTRODUCTION

Plants have been the rich source of therapeutic compounds with significant applications for centuries. *Rhododendron arboretum* Sm., an evergreen ornamental plant in the Ericaceae family, is abundantly found throughout Nepal, especially in the subtropical and temperate regions ranging from 1200 to 3300 meters. It is national flower of Nepal and commonly known as 'Lali Gurans'. This medium-sized tree produces vibrant red flowers and is highly valued for its ethnic, medicinal, nutritional, commercial, and aesthetic significance. The flowering season in Nepal spans from March to June. Its flowers are a promising source of bioactive compounds, making them useful as functional foods, antioxidants, and nutraceuticals. The flowers are used to prepare various products such as pickles, juice, jam, squash (Achhami *et al.*, 2025). The flowers of *Rhododendron* species are rich in anthocyanins and studied for several pharmacological properties (Gautam *et al.*, 2020, Shahi *et al.*, 2025). Despite being recognized as the national flower of Nepal and its prevalence in hilly regions, the potential of *R. arboreum* remains underutilized, both scientifically and commercially. Local communities often lack awareness of its medicinal and nutritional significance, resulting in the underuse of these valuable resources. Phytochemical, antioxidant, antimicrobial along with pH responsive sensing properties of the extracts from *R. arboreum* flower can highlight their significance.

Phenolics are major secondary metabolites in most of the plants and are utilized in fields such as medicine, flavoring, beverage production, dyeing, insect repellents, perfumery, and cosmetics (Dai & Mumper, 2010; Djeridane *et al.*, 2006). Crude extracts from plant rich in phenolics are increasingly valued in the food industry due to their ability to prevent lipid oxidation, enhancing both the quality and nutritional value of food products (Wojdyło *et al.*, 2007). Plant phenolics encompass compounds such as phenolic acids, flavonoids, tannins, and rarer compounds like stilbenes and lignans. Among these, flavonoids are the most prevalent polyphenols in our diet and are divided into six groups: flavones, flavanols, flavanones, isoflavones, and anthocyanins (Dai & Mumper, 2010; Vuolo *et al.*, 2019). Flavonoids are linked to various health benefits and are essential

components in many nutraceutical, pharmaceutical, medicinal, and cosmetic formulations (Panche *et al.*, 2016). An antioxidant is a substance that prevents or slows the oxidation of other substances, protecting cells from damage or aging caused by free radical unstable molecules produced during metabolism. Antioxidant neutralizes or eliminates free radicals by donating electrons. 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 (Santos-Sánchez *et al.*, 2019). 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 (Handique *et al.*, 2012; Waladkhani & Clemens, 1998) .

Anthocyanins are a specific class of flavonoids, distinguished by their basic flavylium cation structure, and are responsible for the variety of colors found in flowers, fruits, and vegetables. They are considered as significant group of visible plant pigments, after chlorophyll (Khoo *et al.*, 2017; Kong *et al.*, 2003). The color of anthocyanin pigments is highly affected by factors like molecular structure, pH, temperature, enzymes, UV light, co-pigmentation, and the presence of oxygen. 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 (Santos-Sánchez *et al.*, 2019; Waladkhani & Clemens, 1998) . Anthocyanins from various plants have been explored for pH responsive colour variation; however, the anthocyanins from *R. arboreum* still remained unexplored for their pH responsive sensing properties. The study specifically focuses on evaluating the pH-responsive sensing properties, total flavonoid and phenolic levels, as well as the antioxidant and antimicrobial activities of methanol extracts from *R. arboreum* flowers.

MATERIALS AND METHODS

Chemicals and reagents

The Folin-Ciocalteu reagent was obtained from Research Lab Fine Chem Industry, while ascorbic acid was sourced from Central Drug House. Gallic acid was purchased from Loba Chemie. Sodium carbonate, hydrochloric acid, and disodium hydrogen phosphate were acquired from Thermo Fischer Scientific. 2,2-diphenyl-1-picrylhydrazyl (DPPH) from Glentham Life Sciences and quercetin was obtained from Sigma Aldrich. Methanol from Avantor Performance Materials India Ltd and ethanol was acquired from Changshu Hongsheng Fine Chemical Co. Ltd.

Sample collection and identification

The *R. arboreum* flowers were collected from Manma, Kalikot, Karnali, Nepal, at an elevation ranging from 2133 to 2200 meters during its natural flowering season (April 2024). The plant was identified by one of the authors (L.R. Bhatt) and verified by National Herbarium and Plant Laboratories (KATH), Godawari, Nepal. The freshly collected red flowers were shade-dried naturally. After drying, the flowers were ground into a fine powder, which was then stored for subsequent analysis.

Preparation of methanol extract

The extract was prepared using a modified version of the method by Dawadi *et al.* (2023). Briefly, seven grams of flower powder was mixed with 70 mL of methanol and incubated at 37°C in a shaking incubator for 72 hours. The sample was then filtered through Whatman filter paper, and the filtrate was evaporated using a rotary evaporator until a sticky mass formed. This was transferred to a sterile, capped vial and refrigerated for subsequent analysis.

Antimicrobial activity

A stock solution was prepared by dissolving 1 g of the plant extract in 1 mL of 10% v/v Dimethyl sulfoxide (DMSO). The antimicrobial activity of the flower extract was evaluated using a modified agar-well diffusion method, adopted from previously established protocol (Gandhiraja *et al.*, 2009). Fresh bacterial and fungal inoculum were standardized to a 0.5 MacFarland turbidity and evenly spread on Mueller-Hinton Agar plates using carpet culture technique. The antimicrobial efficacy of the extract was assessed at concentrations of 25, 50, 75, and 100 mg/ml. Wells of 6 mm in diameter were aseptically created in the agar using a sterile borer and appropriately labeled. Each well was loaded with 60 µL of the test extract or control solution (10% DMSO). The plates were allowed to stand at room temperature for diffusion before being incubated at 37°C for 24 hours. The antibacterial activity was determined by measuring the diameter of zone of Inhibition formed around the wells. This study examined the antimicrobial effects of the flower extract against five bacterial strains: *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6051), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 6380), and *Pseudomonas aeruginosa* (ATCC 9027), as well as one fungal strain, *Candida albicans* (ATCC 10231). The above microbial strains were obtained from the Sukraraj Tropical and Infectious Disease Hospital in Teku, Kathmandu, Nepal. The bacterial isolates were sub-cultured in Nutrient Agar (NA) and subsequently grown in Nutrient Broth (NB) and fungal isolates were sub cultured in potato dextrose agar and potato dextrose broth for experimental use. Commercially available antibiotic discs (Ciprofloxacin (5µg), Chloramphenicol (30µg), Ceftriaxone (30µg), Tetracycline (30µg), and Itraconazole (30µg) from HiMedia, Mumbai, India) were used as positive controls for corresponding test microorganisms.

pH responsive color sensing activity

One gram of dried flower powder was immersed in 20 mL of 50% ethanol and filtered through Whatman paper. Then, 1 mL of the extract was combined with 3 mL of buffer solutions with pH values ranging from 1 to

14. The variation of colors was captured using a smart phone camera, Redmi Note 8 Pro Mobile Phone.

Antioxidant property

The antioxidant potential of the sample was assessed using DPPH (2,2-Diphenyl-1-Picrylhydrazyl) free radical scavenging assay, following a previously reported protocol with slight modifications (Bhatt *et al.*, 2018). A stock solution (1 mg/mL) was prepared, and then diluted to concentrations ranging from 0 to 50 µg/m. A 500 µL aliquot of the extract was combined with an equal volume of methanolic DPPH solution (0.3 mM), mixed thoroughly, and incubated in the dark at room temperature for 30 minutes. Absorbance was recorded at 517 nm using the solvent as a blank. Ascorbic acid was used as standard (positive control). A control containing methanol and DPPH was used for comparison, and scavenging activity (%) was determined using following equation (1)

$$\text{Scavenging activity (\%)} = \frac{A-B}{A} X100 \quad (1)$$

Where A and B are absorbance of control and sample, respectively.

The IC_{50} value, or the concentration required to reduce DPPH by 50%, was determined from a graph plotting scavenging activity against concentrations (µg/mL).

Total phenolic content

Folin-Ciocalteu colorimetric method was employed to determine the total phenolic content (TPC) of the extract as discussed in previous studies (Dawadi *et al.*, 2022). One mg/mL gallic acid stock solution (10mL) was prepared and diluted to obtain standard solutions ranging from 0 to 500 µg/mL. For TPC measurement, 50 µL of the extract was mixed with 150 µL of Folin-Ciocalteu reagent in an Eppendorf tube and incubated at room temperature for 3 minutes. Then, 150 µL of a 6% sodium carbonate solution was added followed by dilution to 1000 mL with distilled water and re-incubated for 90 minutes. The absorbance was recorded at 725 nm using a UV-vis spectrophotometer. The standard solutions were processed similarly, with water as the blank. A standard curve was generated to quantify TPC, expressed as milligrams of Gallic acid equivalents (GAE) per gram of sample.

Total flavonoid content

The flavonoid in extract was assessed using a spectrophotometry method outlined in the literature (Bhusal *et al.*, 2020). Quercetin stock solution (10 mg/mL) was prepared and diluted to obtain standard solutions ranging from 0 to 500 µg/mL. For analysis, 500 µL of the sample was mixed with an equal volume of 2% $AlCl_3$ solution in an Eppendorf tube and incubated at room temperature for 60 minutes. Absorbance was then measured at 515 nm using an ELISA plate reader. Standard solutions were processed similarly, with water as the blank. The experiment was performed in triplicate, and flavonoid content was calculated using a calibration curve, expressed as milligrams of quercetin equivalents per gram of sample.

FTIR spectroscopy

FTIR spectra were recorded using an infrared spectrometer (Shimadzu, Japan), recorded from 4000 to 450 cm^{-1} at a resolution of 1 cm^{-1}, conducting 100 scans (scan rate: 0.5 cm^{-1}/s).

Statistical analysis

The data presented are the averages of triplicate measurements. Microsoft Excel 2016 was utilized to calculate the means, standard deviations, and regression.

RESULTS AND DISCUSSION

Antimicrobial activity

The antimicrobial potential of *R. arboreum* flower extract was evaluated against commonly encountered bacterial strains and fungal strains. The extract demonstrated notable inhibitory effect against *K. pneumonia* and *P. aeruginosa* across all tested concentrations (25-100 mg/mL) (Fig. 1, Table 1). At 75 mg/mL and 100 mg/mL, significant growth inhibition was observed for *B. subtilis, C. albicans*, and *S. aureus*. However, the extract exhibited limited inhibitory effect against *E. coli* at all tested concentrations. The reduced efficacy against *E. coli* may be attributed to the insufficient concentration of active compounds or the presence of interfering substances that compromise antibacterial activity (Akhtar & Mirza, 2018). The highest zone of inhibition (17.7±0.26 mm) was noted at a concentration of 100 mg/mL against *K. pneumonia*. These findings suggested that *R. arboreum* exhibits moderate antimicrobial potential. The antimicrobial activity of flower extracts of *Etlingera elatior* and *Magnolia* species also supports this study (Cristea *et al.*, 2024; Lachumy *et al.*, 2010) .

pH responsive sensing properties R. *arboreum* flower extract

The color of the buffer solutions varied distinctly with pH upon the addition of Rhododendron extract (Fig. 2). It can be seen that the color of the solution changes from pink to dark greenish with an increasing pH of buffer from 1 to 14. Specifically, it appeared pink at pH 1-3, fain pink at pH 4-5, light green at pH 6-8, reddish brown at pH 9-11, and dark brown at pH 12-14. The color changes were attributed to structural changes in anthocyanins pigment: the flavylium cation in acidic environments to chalcone in alkaline environments (Roy & Rhim, 2021). Particularly, anthocyanin molecules undergo structural changes with varying pH: forming flavylium cations at pH 3, carbinol pseudo-bases at pH 4-5, quinoidalanhydro-bases at pH 6-8, and chalcones at pH greater than 10 (M. Duan *et al.*, 2021). Two major anthocyanins, cyanidin-3-*O*-*β*-galactoside and cyanidin-3-*O*-*a*-arabinoside are mainly present in *R. arboretum* flower extract (Bhatt *et al.*, 2022). Anthocyanins contribute to vibrant coloration of flowers, fruits, and vegetables, playing a crucial role in attracting insects that facilitates pollination and seed dissemination (Harborne & Williams, 2001). 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 as pH-responsive colorimetric sensors in smart food packaging. Given the widespread distribution of *R. arboreum* across the hilly regions of Nepal and other Asian countries, investigating the pH-responsive properties of *R. arboreum* flower extract presents valuable opportunities for both scientific innovation and benefits to local communities.

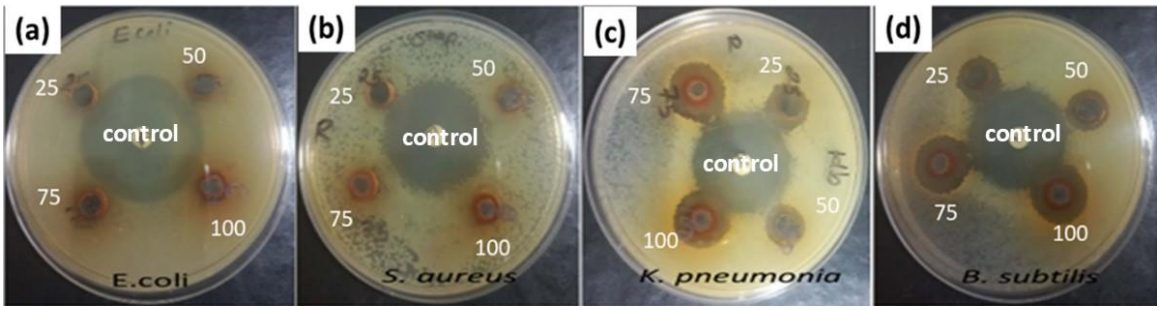

Figure 1. Antimicrobial activity of *R. arboreum* flower extract against (a) *E. coli*, (b) *S. aureus*, (c) *K. pneumonia*, and (d) *B. subtilis* at concentration ranging from 25mg/mL to 100 mg/mL.

Table 1. Zone of inhibition in (mm) of extract sample and antibiotics against different organisms

Organism	Zone of inhibition (mm) in different concentration				Positive control (mm)	Antibiotic used
	25mg/ml	50mg/ml	75mg/ml	100mg/ml		
E. coli	-	-	-	-	29.7±0.6	Ciprofloxacin (5µg)
B. subtilis	-	-	12.8±0.2	14.9±0.4	29.4±0.5	Chloramphenicol (30µg)
P. aeruginosa	12.8±0.2	14.8±0.2	15.7±0.2	16.4±0.5	21.5±0.4	Ceftriaxone (30µg)
K. pneumoniae	13.8±0.3	14.8±0.7	16.4±0.5	17.7±0.2	34.0±1.0	Tetracycline (30µg)
S. aureus	-	-	14.7±0.6	16.8±0.3	25.5±0.5	Chloramphenicol (10µg)
C. albicans	-	-	12.9±0.1	14.5±0.5	29.8±0.7	Itraconazole (30µg)

Figure 2. Color variation of *R. arboreum* flower extract in buffer solution across different pH levels (1-14).

Antioxidant property

Antioxidant property of *R. arboretum* flower extract was studied by determining the radical scavenging activity (RSA) from concentration 10 µg/mL to 50 µg/mL (Fig. 3). The RSA (%) exhibited a linear increase with increasing the extract concentration, demonstrating strong antioxidant potential. At 50 µg/mL, the extract achieved an RSA of 85.31±1.28 %. The antioxidant activity of the sample was comparable to reference standard ascorbic acid at all tested concentrations. Jha *et al.* (2024) reported that the ability of extracts to neutralize free radicals improves proportionally with increasing concentration (Jha *et al.*, 2024). The IC_{50} value of the *R. arboretum* flower extract was determined to be 20.6±1.18 µg/mL (Table 2), further confirming its strong antioxidant activity. Upon interaction with DPPH, the deep violet color of the solution gradually

shifts to yellow due to donation of hydrogen atoms, leading to a reduction in free radicals (Kashyap *et al.*, 2017). A more pronounced color change corresponds to higher antioxidant activity (Naik *et al.*, 2003). Antioxidant property in the *R. arboreum* flower extract (Jha *et al.*, 2024; Kashyap *et al.*, 2017; Sharma *et al.*, 2022,) and juice (Achhami *et al.*, 2025) have been reported previously. Likewise, flower extracts of *Tenucium stocksianum* and *Etlingera elatior* exhibited significant dose dependent radical scavenging activity (Lachumy *et al.*, 2010; Rahim *et al.*, 2013). Natural antioxidants present in fruits, vegetables, spices, leaves, cereals, and flowers offer significant health benefits, particularly in healthcare. Their ability to neutralize free radicals plays a crucial role in preventing infectious disease (Shrestha *et al.*, 2021, Kumari *et al.*, 2023).

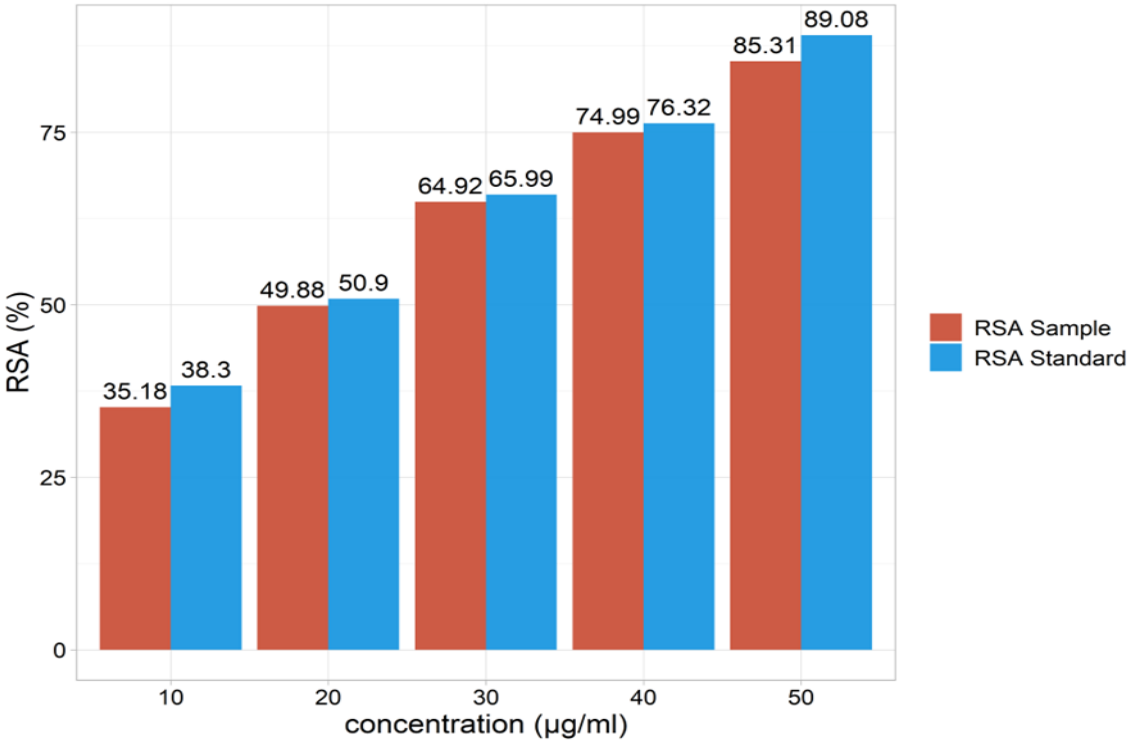

Figure 3. Graph showing the radical scavenging activity of sample and standard (ascorbic acid) at different concentrations.

Total phenolic content

The total phenolic content of *R. arboreum* flower extract was determined using standard calibration curve of gallic acid (y = 0.0027x + 0.0655) (Fig. 4) and was found to be 11±0.15 mg GAE/g of dry extract (Table 2)**.** The TPC observed in this study was lower than the previously reported values (Bhatt *et al.*, 2022), likely due to variation in extraction techniques, processing methods, plant maturity, and geographical factors (Marcos-Gómez *et al.*, 2024). This result further supports and confirms the phytochemical analysis of *R. arboreum* flower extracts conducted by Kiruba *et al.* (2011). Phenolic compounds are crucial plant constituents known for their redox properties which contribute to their antioxidant activity (Soobrattee *et al.*, 2005). Previous studies reported that TPC is a key determinant to scavenge free radicals, directly influencing its antioxidant potential (Jha *et al.*, 2024, Karki *et al.*, 2024; Achhami *et al.*, 2025) . As dietary antioxidants, plant polyphenols play a crucial role in mitigating oxidative damage, with broad implications for human health and disease prevention (Lin *et al.*, 2016). Due to their high phenolic content, many plants are of pharmacological interest, particularly for their potential in managing inflammatory conditions and promoting wound healing (Petti & Scully, 2009). The findings of this study further emphasize the significance of *R. arboreum* as a potential source of bioactive phenolic compounds with promising health benefits (Achhami *et al.*, 2025).

Total flavonoid content

The total flavonoid content of *R. arboreum* flower extract was determined using standard calibration curve of quercetin solution (y = 0.0006x + 0.0551) (Fig. 5) and was found to be 13.36±0.11 mg QE/g of dry extract (Table 2). Flavonoids are a major class of polyphenolic compounds widely distributed in fruits vegetables and nuts. Phytochemical analysis of methanol flower extract of medicinal plants like *H.isora*, *S. companulata*, *A. leptopus*, and *T. grandiflora* showed the presence of various bioactive compounds including phenolics and flavonoids (Marimuthu *et al.*, 2012). These bioactive phytochemicals possess diverse chemical structures are known for their strong antibacterial properties, enabling them to combat various microbial pathogens (Jha *et al.*, 2024). Phytochemicals, including flavonoids, interact synergistically with dietary fibers and essential minerals playing a crucial role in disease prevention (Pareek,

2016). 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 (S.-G. Duan *et al.*, 2021). Their ability to neutralize free radicals helps mitigate oxidative stress, which is linked to chronic diseases such as cancer, neurodegenerative disorders, and cardiovascular conditions. 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.

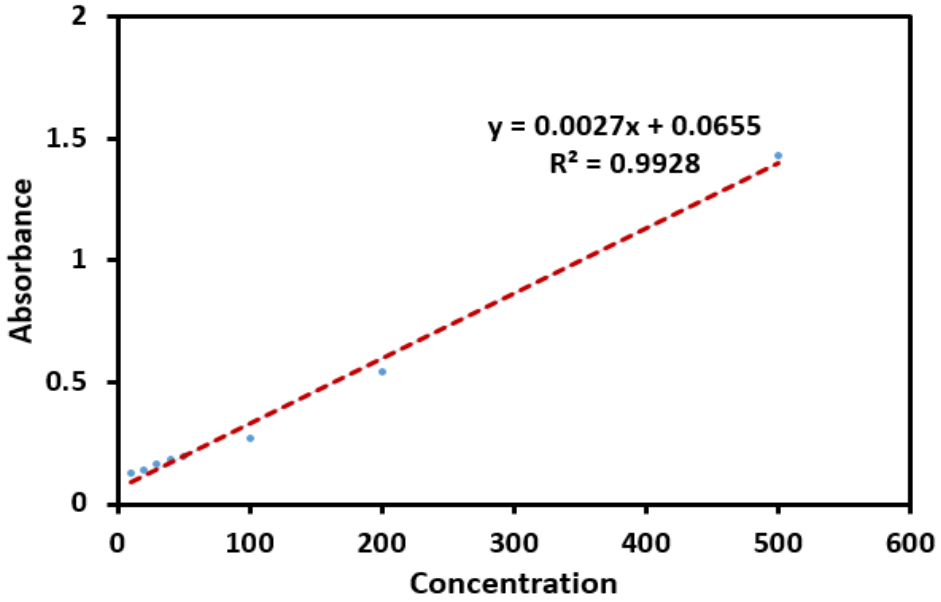

Figure 4. Standard curve of Gallic acid to determine the total phenolic content

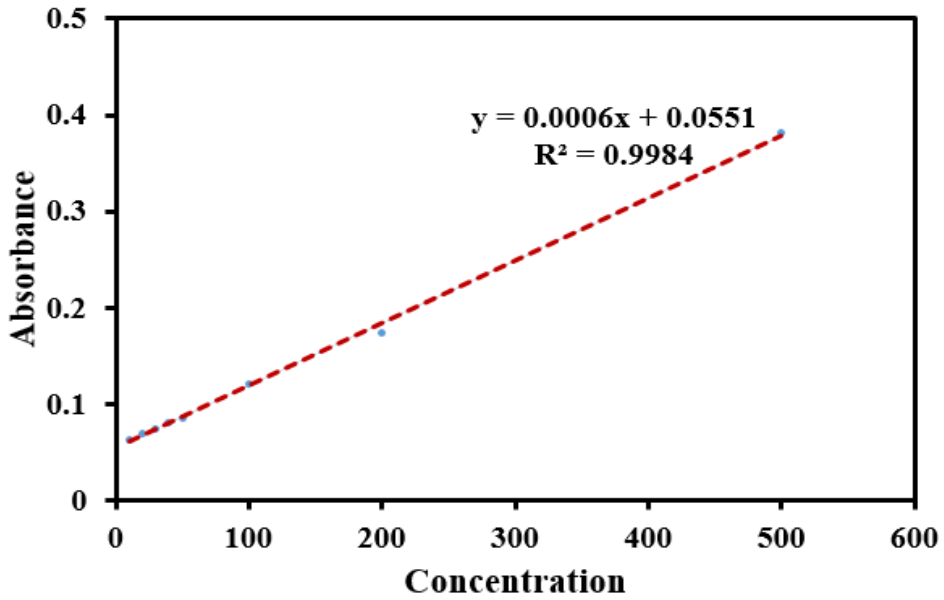

Figure 5. Standard curve of quercetin solution to determine the total flavonoid content

Spectroscopic analysis of extract

FTIR spectra were used to identify the chemical bonds and functional groups in the flower extract (Bhushan *et al.*, 2023). FTIR spectrum of anthocyanin (Fig. 6) showed a strong peak at 3295cm^{-1} for O-H bond-stretching and the peak at 2925 cm^{-1} for C-H bond-stretching (Shi *et al.*, 2024).

Table 2. Antioxidant activity, total phenolic and flavonoid content of flower extract of *R. arboreum*

Antioxidant activity (IC_{50})	Total phenolic content (mg GAE /g)	Total flavonoid content (mg QE/g)
20.6±1.18 µg/mL	11.00±0.15	13.36±0.11

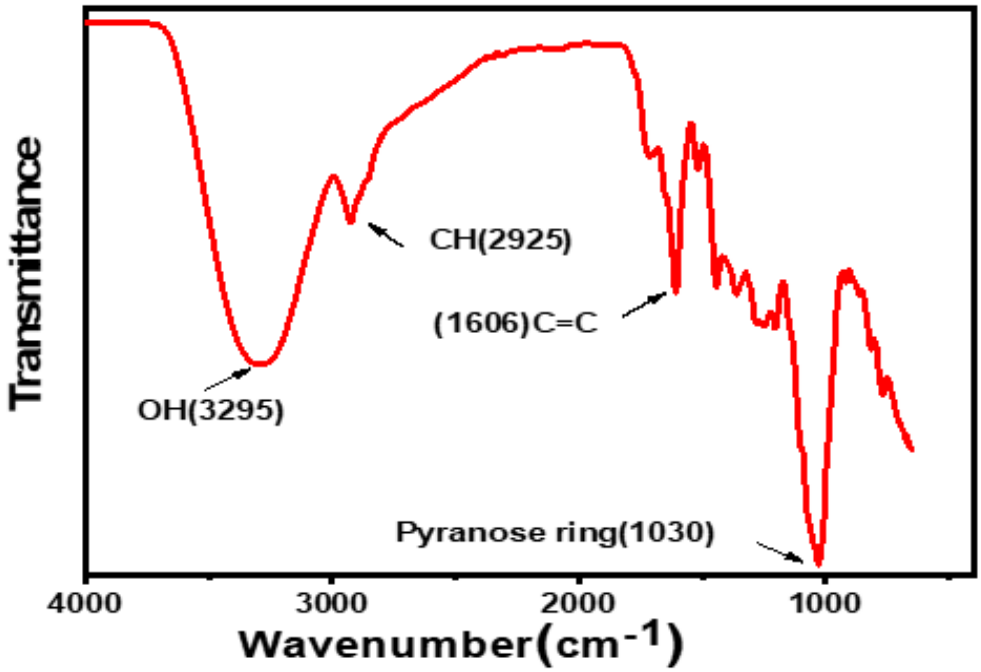

Figure 6. FTIR spectra of *R. arboreum* anthocyanin.

The peak at 1606 cm^{-1} indicated the C=C stretch of the aromatic hydrocarbon skeleton (Qin *et al.*, 2019). A peak near 1600 cm^{-1} represents a carbon-oxygen double bonds. The bands at 1500 cm^{-1} and 1445 cm^{-1} were linked to carbon–carbon double bonds vibrations (Tong *et al.*, 2020). C-O stretching band was seen at 1200 cm^{-1} and the band at 1028 cm^{-1} can be attributed to C-O-C stretching vibrations (Sarkar *et al.*, 2014). The IR peaks from 993 to 1149 cm^{-1} can be assigned to the typical pyranose ring of glucose (Wu *et al.*, 2018). Overall, the analysis showed O-H, C=O, C=C, and C-O-C groups, typical of flavonoids and anthocyanin with additional peak below 800 corresponding to benzene ring (Tong *et al.*, 2020; Shi *et al.*, 2024).

CONCLUSIONS

This study investigated the phytochemical constituents, antimicrobial, and antioxidant activities of R. arboreum flower extracts. The flower extract contained significant amounts of phenolic compounds and flavonoids. The flower extracts demonstrated strong antioxidant activity and showed significant antibacterial effects against Gram- negative and Gram-positive bacteria as well as fungi that cause food spoilage. The anthocyanin pigments in the extracts displayed notable color variations in buffer solution under different pH conditions. Due to these effects, they may be useful in biotechnology, food, and/or pharmaceutical industries. These findings highlight the antibacterial, antioxidant, and pH responsive colorimetric sensing properties of R. arboreum flower extracts, indicating their potential use in biomedical research and agriculture as a source of bioactive compounds. Further studies, such as the use of other separation and purification methods of the bioactive compounds of R. arboreum flower extracts and in vivo investigations might be helpful in clarifying the mechanism of action of the noted positive benefits.

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AUTHOR CONTRIBUTION STATEMENT

DKS: Writing-original draft, methodology, investigation, formal analysis, conceptualization; HA: Methodology, investigation; NK: Writing-review & editing; BBP: Writing original draft, methodology; ER: Writing-review; DG: Writing-review & editing; DN: Writing-review & editing; SRR: Review & editing; RJ: Writing-review & editing; LRB: Conceptualization, writing-review & editing, supervision; MKJ: Conceptualization, writing-review & editing, validation, supervision.

CONFLICT OF INTEREST

The authors confirm that they have no recognized financial conflicts of interest or personal relationships that may have influenced the research presented in this paper.

DATA AVAILABILITY STATEMENT

Upon reasonable request, the corresponding authors will provide the data supporting the study's conclusions.

REFERENCES

Achhami, H., Pachhai, B.B., Chaudhary, S., Manandhar, P., & Bhatt, L.R. (2025). Physicochemical, nutritional, antinutritional and antioxidant properties of juice and wines from *Rhododendron arboreum* Sm. petals. *Applied Food Research*, 100929.

Akhtar, N., & Mirza, B. (2018). Phytochemical analysis and comprehensive evaluation of antimicrobial and antioxidant properties of 61 medicinal plant species. *Arabian Journal of Chemistry, 11*(8), 1223-1235.

Bhatt, L.R., Wagle, B., Adhikari, M., Bhusal, S., Giri, A., & Bhattarai, S. (2018). Antioxidant activity, total phenolic and flavonoid content of *Berberis aristata* DC. and *Berberis thomsoniana* CK Schneid. from Sagarmatha National Park, Nepal. *Pharmacognosy Journal*, 10(6s).

Bhatt, V., Sendri, N., Swati, K., Devidas, S.B., & Bhandari, P. (2022). Identification and quantification of anthocyanins, flavonoids, and phenolic acids in flowers of *Rhododendron arboreum* and evaluation of their antioxidant potential. *Journal of Separation Science, 45*(14), 2555-2565.

Bhusal, S., Pant, D.R., Joshi, G.P., Adhikari, M., Raut, J.K., Pandey, M.R., & Bhatt, L.R. (2020). Antioxidant activity and nutraceutical potential of selected Nepalese wild edible fruits. *Scientific World, 13*(13), 8-13.

Bhushan, B., Bibwe, B., Pal, A., Mahawar, M.K., Dagla, M.C., Yathish, K., . . . & Singh, A. (2023). FTIR spectra, antioxidant capacity and degradation kinetics of maize anthocyanin extract under variable process conditions. *Applied Food Research, 3*(1), 100282.

Cristea, R.M., Sava, C., Căpăţână, C., & Kanellou, A. (2024). Phytochemical analysis and specific activities of bark and flower extracts from four Magnolia plant species. *Horticulturae, 10*(2), 141.

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

Dawadi, P., Shrestha, R., Mishra, S., Bista, S., Raut, J.K., Joshi, T.P., & Bhatt, L.R. (2022). Nutritional value and antioxidant properties of *Viburnum mullaha* Buch.-Ham. ex D. Don fruit from central Nepal. *Turkish Journal of Agriculture and Forestry, 46*(5), 781-789.

Dawadi, P., Siddiqui, M.A., Belbase, S., Syangtan, G., Kronenberg, B., Rana, K., ... & Bhatt, L.R. (2023). Characterizing nutritional, antioxidant and antimicrobial values of *Diploknema butyracea* (Roxburgh) HJ Lam from the Chepang community, Makwanpur, Nepal. *Nepal Journal of Biotechnology, 11*(2), 65-74.

Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., & Vidal, N. (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry, 97*(4), 654-660.

Duan, M., Yu, S., Sun, J., Jiang, H., Zhao, J., Tong, C., Hu, Y., Pang, J., & Wu, C. (2021). 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, 187*, 332-340.

Duan, S.-G., Hong, K., Tang, M., Tang, J., Liu, L.-X., Gao, G.-F., Shen, Z.-J., Zhang, X.-M., & Yi, Y. (2021). Untargeted metabolite profiling of petal blight in field-grown *Rhododendron agastum* using GC-TOF-MS and UHPLC-QTOF-MS/MS. *Phytochemistry, 184*, 112655.

Gandhiraja, N., Sriram, S., Meenaa, V., Srilakshmi, J.K., Sasikumar, C., & Rajeswari, R. (2009). Phytochemical screening and antimicrobial activity of the plant extracts of *Mimosa pudica* L. against selected microbes. *Ethnobotanical Leaflets, 2009*(5), 8.

Gautam, V., Sharma, A., Arora, S., Bhardwaj, R., Ahmad, A., Ahamad, B., & Ahmad, P. (2020). In-vitro antioxidant, antimutagenic and cancer cell growth inhibition activities of *Rhododendron arboreum* leaves and flowers. *Saudi Journal of Biological Sciences, 27*(7), 1788-1796.

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

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

Jha, A.K., Khalid, M.A., & Labh, S.N. (2024). In vitro antioxidant and antibacterial activities of medicinal flower Laligurans *Rhododendron arboreum* collected from Kathmandu Valley, Nepal. *International Journal of Food Science, 2024*(1), 6073042.

Karki, N., Achhami, H., Pachhai, B.B., Bhattarai, S., Shahi, D.K., Bhatt, L.R., & Joshi, M.K. (2024). Evaluating citrus juice: A comparative study of physicochemical, nutraceutical, antioxidant, and antimicrobial properties of citrus juices from Nepal. *Heliyon*, 10(23).

Kashyap, P., Anand, S., & Thakur, A. (2017). Evaluation of antioxidant and antimicrobial activity of *Rhododendron arboreum* flowers extract. *International Journal of Food and Fermentation Technology, 7*(1), 123-128.

Khoo, H.E., Azlan, A., Tang, S.T., & Lim, S.M. (2017). Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food & Nutrition Research, 61*(1), 1361779.

Kiruba, S., Mahesh, M., Nisha, S., Paul, Z.M., & Jeeva, S. (2011). Phytochemical analysis of the flower extracts of *Rhododendron arboreum* Sm. ssp. nilagiricum (Zenker) Tagg. *Asian Pacific Journal of Tropical Biomedicine, 1*(2), S284-S286.

Kong, J.-M., Chia, L.-S., Goh, N.-K., Chia, T.-F., & Brouillard, R. (2003). Analysis and biological activities of anthocyanins. *Phytochemistry, 64*(5), 923-933.

Kumari, R., Gupta, A., Pant, R., Vyas, P., & Bajpai, A. (2023). 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, 16*(12), 5765-5769.

Lachumy, S.J.T., Sasidharan, S., Sumathy, V., & Zuraini, Z. (2010). Pharmacological activity, phytochemical analysis and toxicity of methanol extract of *Etlingera elatior* (torch ginger) flowers. *Asian Pacific Journal of Tropical Medicine, 3*(10), 769-774.

Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., Liu, Y., Chen, H., Qin, W., Wu, H., & Chen, S. (2016). An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. *Molecules, 21*(10), 1374.

Marcos-Gómez, R., Vera-Guzmán, A.M., Pérez-Ochoa, M.L., Martínez-Martínez, L., Hernández-Delgado, S., Martínez-Sánchez, D., & Chávez-Servia, J.L. (2024). Phenolic compounds and antioxidant activity in edible flower species from Oaxaca. *Applied Sciences, 14*(8), 3136.

Marimuthu, J., Aparna, J.S., Jeeva, S., Sukumaran, S., & Anantham, B. (2012). Preliminary phytochemical studies on the methanolic flower extracts of some selected medicinal plants from India. *Asian Pacific Journal of Tropical Biomedicine, 2*(1), S79-S82.

Naik, G., Priyadarsini, K., Satav, J., Banavalikar, M., Sohoni, D., Biyani, M., & Mohan, H. (2003). Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. *Phytochemistry, 63*(1), 97-104.

Panche, A.N., Diwan, A.D., & Chandra, S.R. (2016). Flavonoids: an overview. *Journal of Nutritional Science, 5*, e47.

Pareek, S. (2016). Nutritional and biochemical composition of banana (*Musa* spp.) cultivars. In Simmonds, M.S.J., & Preedy, V.R. (Eds.), *Nutritional composition of fruit cultivars* (pp 49-81), Elsevier.

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

Qin, Y., Liu, Y., Yong, H., Liu, J., Zhang, X., & Liu, J. (2019). Preparation and characterization of active and intelligent packaging films based on cassava starch and anthocyanins from *Lycium ruthenicum* Murr. *International Journal of Biological Macromolecules, 134*, 80-90.

Rahim, G., Qureshi, R., Arshad, M., & Gulfraz, M. (2013). Phytochemical analysis and antioxidant properties of *Teucrium stocksianum* flower from Malakand Division, Pakistan. *International Journal of Agriculture and Biology, 15*(2), 377-381.

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

Santos-Sánchez, N.F., Salas-Coronado, R., Villanueva-Cañongo, C., & Hernández-Carlos, B. (2019). Antioxidant compounds and their antioxidant mechanism. *Antioxidants, 10*, 1-29.

Sarkar, A.K., Pal, A., Ghorai, S., Mandre, N., & Pal, S. (2014). Efficient removal of malachite green dye using biodegradable graft copolymer derived from amylopectin and poly (acrylic acid). *Carbohydrate Polymers, 111*, 108-115.

Sendri, N., Singh, S., Bhatt, V., Bhatt, P., & Bhandari, P. (2022). Valorization of red cabbage pomace for stabilization of anthocyanins in *Rhododendron arboreum*. *Industrial Crops and Products, 187*, 115371.

Shahi, D.K., Awasthi, G.P., Bahadur, G.R., Panthi, K.P. & Joshi, M.K., et. al. (2025). *Rhododendron arboreum* Sm. anthocyanin-infused starch, chitosan, and polyvinyl alcohol based composite films: Comparative analysis of physical, UV barrier, antioxidant and intelligent behavior. *International Journal of Biological Macromolecules*, 140532.

Sharma, S., Chaudhary, S., & Harchanda, A. (2022). *Rhododendron arboreum*: A critical review on phytochemicals, health benefits and applications in the food processing industries. *Current Nutrition & Food Science, 18*(3), 287-304.

Shi, Y., Chen, X., Gao, Q., Zou, Y., Xing, D., Chen, R., Li, Q. (2024). Preparation and chemical properties of microencapsulation developed with mulberry anthocyanins and silk fibroin. *Industrial Crops and Products, 212*, 118383.

Shrestha, R., Dawadi, P., Bhusal, S., & Bhatt, L.R. (2021). Nutritional value and antioxidant properties of *Diospyros malabarica* (Desr.) Kostel., fruit from midhills of western Nepal. *Nepal Journal of Science and Technology, 20*(1), 113-125.

Soobrattee, M.A., Neergheen, V.S., Luximon-Ramma, A., Aruoma, O.I., & Bahorun, T. (2005). Phenolics as potential antioxidant therapeutic agents: mechanism and actions. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 579*(1-2), 200-213.

Tong, Y., Deng, H., Kong, Y., Tan, C., Chen, J., Wan, M., Wang, M., Yan, T., Meng, X., & Li, L. (2020). Stability and structural characteristics of amylopectin nanoparticle-binding anthocyanins in *Aronia melanocarpa*. *Food Chemistry, 311*, 125687.

Vuolo, M.M., Lima, V.S., & Junior, M.R.M. (2019). Phenolic compounds: Structure, classification, and antioxidant power Bioactive compounds. In Campos, M.R.S. (Ed.), *Bioactive compounds: Health benefits and potential applications* (pp. 33-50): Elsevier.

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

Wojdyło, A., Oszmiański, J., & Czemerys, R. (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. *Food chemistry, 105*(3), 940-949.

Wu, S., Wang, W., Yan, K., Ding, F., Shi, X., Deng, H., & Du, Y. (2018). Electrochemical writing on edible polysaccharide films for intelligent food packaging. *Carbohydrate Polymers, 186*, 236-242.