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Physicochemical, nutritional, antinutritional and antioxidant properties of juice and wines from *Rhododendron arboreum* Sm. petals

Hari Achhami [a,b], Bishwa Bandhu Pachhai [a], Sujan Chaudhary [a], Prakash Manandhar [b,*], Lok Ranjan Bhatt [a,*]

[a] Biological Resources Unit, Faculty of Science, Nepal Academy of Science & Technology, Khumaltar, Lalitpur 44700, Nepal
[b] Department of Microbiology, St. Xavier's College, Maitighar, Kathmandu 44600, Nepal

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ABSTRACT

Rhododendron arboreum Sm. is widely distributed in Nepal across the mid-hills and lower mountain region. Analyzing the petals of *R. arboreum* for juice and wine production may reveal potential health benefits and novel sensory experiences. The present study aims to analyze and compare physicochemical properties, proximate composition, phytochemical content, antioxidant activity, mineral content, antinutritional factors, antimicrobial activities, and sensory attributes of juice and three types wine (H_1, H_2, and H_3) based on different methodology. It was observed that wine H_2 has a maximum TPC (9.96 ± 0.15 mg/mL), TFC (7. 11 ± 0.05 mg/mL), and TAC (162.16 ± 2.3 mg/mL), correlating with its strong antioxidant activity (82.01 % DPPH scavenging) and improving colour and antioxidant properties, compared to wine H_1 and H_3. The highest concentration of minerals was seen in the juice, followed by wine (H_2). Antimicrobial screening revealed that juice and wine extract both have antimicrobial activity, with wine extract H_2 having the best activity against gram-positive bacteria. The preparation method chosen can have a significant impact on the quality of the wine H_2, which was significantly better in comparison to wine H_1 and H_3. In conclusion, the present study has demonstrated how the optimization of fermentation condition acts as a positive tool in upgrading the physicochemical, nutritional, and functional properties of *R. arboreum* juice. The findings highlighted the potential of *R. arboreum* wine, especially wine H_2, as functional beverages. These insights can guide local beverage development in Nepal, offering economic benefits and preserving traditional practices.

1. Introduction

Rhododendron arboreum Sm. is the national flower of Nepal, which belongs to the family Ericaceae. It is locally known as "Lali gurans". It is found at elevations between 4800 and 10,500 ft and has 33 species, 10 sub-species, and 14 varieties in Nepal (Paudel et al., 2011). The flowers of *R. arboreum* can be seen in various colors, including red, deep scarlet, white, pink, and especially bright red varieties typically thriving at lower elevations (Paul et al., 2005). These flowers contain polyphenols, coumaric acids, resins, amino acids, flavones, ursolic acids and carbohydrates (Nitika et al., 2021; Pathak et al., 2021). They have therapeutic properties that comprise antioxidant, anti-inflammatory, cholinergic, antidiabetic, anticancer, antihyperlipidemic, and cardioprotective properties (Paul et al., 2005; Shrestha & Budhathoki, 2012; Devi & Vats, 2017; Subba et al., 2023). Besides health benefits, *R. arboreum* anthocyanin-infused films can be used as an intelligent packaging material for monitoring meat freshness (Shahi et al., 2025). People from hilly places utilize *R. arboreum* flowers to make local alcoholic squash, jellies, juice, jam and brews. It is a popular and enjoyable drink as a refreshing appetizer and tonic (Kashyap, 2019; Sharma et al., 2021).

Wine is commonly prepared from the fruits that include grapes, peaches, plums, and apricots, that have a high content of sugar. Aside from fruit-based wine, there are flowers which have medicinal properties can be used for wine making. These wines have an alcohol content ranging from 6–13 % and are rich in minerals, antioxidants, and phytonutrients like carotenoids and phenolic compounds (Yang et al., 2009; Yadav et al., 2021). *R. arboreum* not only produces enough alcohol but also increases the beneficial aspects to health (Kashyap, 2019).

Fermentation is a low energy method of food preservation that is relatively efficient for preserving foods without the need for refrigeration or other types of preservatives (Saranraj & Ray, 2019). It enhances the nutritional and bioactive characteristics of food, improving human

* Corresponding authors.
E-mail addresses: prakashmanadhar7@gmail.com (P. Manandhar), lokranjan2000@yahoo.com (L.R. Bhatt).

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health through enzyme activity and microbial metabolism (Wilburn & Ryan, 2017). It also helps to improve digestion, gut health, immune function, mental wellness, while lowering the risks of developing chronic diseases (Leeuwendaal et al., 2022). Alcoholic fermentation is a bioprocess where saccharides like glucose, sucrose and fructose are transformed to cellular energy and produce ethanol and carbon dioxide as the products (Sablayrolles, 2009). This process is mainly carried out by few bacteria and yeasts and plays an important role in the production of alcoholic products like beer and wine (Eliodorio et al., 2019). Wine is an alcoholic drink made from fruit juice that ferments naturally or with the help of yeasts, undergoing key processes like fermentation and aging (Archibong et al., 2015). Yeast growth and fermentation rates are influenced by the initial sugar concentration, pH, fermentation temperature, and yeast strains (MacFarlane & MacFarlane, 1993; Sumby et al., 2019; Diaz-Muñoz & De Vuyst, 2022). Depending on temperature, fermentation durations could take between three days to six weeks, while wine aging lasts between six months and two years (Mohanty et al., 2006; Costello et al., 2015). Wine production especially depends on fermentation process where metabolomics can observe alteration in biochemical content of wines and the quality of wines from different sources and regions can be monitored and improved by analyzing their phyto-constituents (Emwas et al., 2021; Alharbi et al., 2024).

The production of wine from different fruits using sophisticated tools and equipment is well studied and documented. However, there is limited information available on wine production from flowers and especially from the petals of Rhododendron (Kashyap, 2019; Yadav et al., 2021). Production of wine from the flowers of *Madhuca latifolia* (Kashyap, 2019), *Camellia japonica* (Majumder et al., 2022), *Hibiscus rosa-sinensis* (Morya & Kishor, 2016), coffee flower (Liu et al., 2024), *Jasminum sambac* (Liang et al., 2024), and *Hibiscus sabdariffa* (Nawhia & Opara, 2012) have been reported from the various parts of the world. Among 33 *Rhododendron* spp. in Nepal, there is no published literature related to the production of wine from their petals. Thus, the objective of this study was to determine the effects of fermentation conditions and methods on physicochemical properties, proximate composition, phytochemical content, antioxidant activity, mineral content, anti-nutritional factors, antimicrobial activities, and sensory characteristics of Rhododendron-derived red wine and juice, while also devising an appropriate technique for Rhododendron wine manufacturing.

2. Materials and methods

2.1. Sample collection

Fresh and mature flowers of *R. arboreum* were collected from Phoolchowki, Lalitpur, Nepal, at an altitude of 2782 m and analyzed at the Biological Resource Laboratory, Nepal Academy of Science and Technology, Khumatar, Lalitpur.

2.2. Activation of yeast strain

Saccharomyces cerevisiae (Lalvin EC-1118) was used for fermentation as a yeast strain. One gram of dry yeast was activated by soaking in 10 mL of sterilized glucose solution (50 g/lit) for 30 min at 28.5 °C and 40 rpm as described previously (Sevda et al., 2011) with some modifications.

2.3. Preparation of juice and must

2.3.1. Juice preparation

The harvested petals of *R. arboreum* were cleaned in running tap water and then with distilled water. The petals were ground finely by mechanical grinder (Herbal Medicine Disintegrator), followed by pressing using squeezer (Aluminium Hand Press Juicer) and filtered using muslin cloth. It was stored in a bottle in the refrigerator at 2–4 °C until further analysis.

2.3.2. Must preparation for wine (H_1)

Freshly collected petals were crushed using mechanical crusher and mixed with water in a ratio of 1:3 (flower: water). The obtained infusion was used for the fermentation.

2.3.3. Must preparation for wine (H_2)

Freshly picked flowers were cleaned, separated, crushed, and pressed to obtain juice. The juice was filtered through a muslin cloth and used for fermentation.

2.3.4. Must preparation for wine (H_3)

Fresh petals of the flower were boiled with water at ratio 1:3 (flower: water) and the slurry (aqueous solution) was filtered with muslin cloth and used for fermentation (Kashyap, 2019).

2.4. Fermentation

After filtration, the freshly prepared must was mixed with 200 ppm potassium metabisulfite (KMS) and left for 24 hours. Before inoculation, all parameters such as sugar and pH were examined. The pH of the Rhododendron must was corrected with 0.1 N calcium carbonate, and the TSS of the must was adjusted 23.5 °Brix with table sugar, and the solution was well stirred with a magnetic stirrer. The mixture was introduced to the fermenter along with the prepared activated yeast at a room temperature of approximately 28 °C and kept constant for the rest of the duration. Following the completion of the fermentation, the wine was filtered via a Millipore vacuum pump cum filter apparatus. The fermentation process for wine H_1, H_2, and H_3 are visualized in the flow chart (Fig. 1).

2.5. Physico-chemical analysis

The pH of the juice and wine was determined by a digital pH meter (Thermo-Scientific, ORION STAR A111), which was previously calibrated with phosphate buffer pH 4 and pH 7. The total soluble solid (TSS) of wine was calculated using digital refractometer (Cole-Parmer, an antylia scientific company) (AOAC, 2006). The total titratable acidity (TA) of juice and carbon treated wine was measured by titrating against the standardized 0.1 N NaOH with 1 % phenolphthalein, and the result was expressed as % (w/v) acetic acid (Tsegay, 2020).

2.6. Proximate analysis

2.6.1. Alcoho1 content and volatile acidity

The alcohol content in a wine was determined by mass ratio or specific gravity method at 20 °C by following AOAC (2000) method. The volatile acidity of wine was measured by slightly modified distillation method, followed by titration of the 10 mL distillate against standardized 0.1 NaOH using a 1 % phenolphthalein indicator solution (Idise & Odum, 2011).

2.6.2. Total fat content and total protein content

The total fat content of juice and wine was determined by the separating funnel extraction method (AOAC, 2006). The total protein content of juice and wine was analysed as described by Bradford (1976). In brief 100 µL of wine sample was added into 1.5 mL microfuge tubes in the same volume as BSA solution and control (water), in each microfuge tube, 1000 µl of newly made Bradford reagent was added at a 1:10 ratio. All samples and controls were tested in triplicate, and all tubes were properly vortexed and incubated at room temperature for at least 5 min but no >30 min. The absorbance of the samples was measured at 595 nm using a spectrophotometer.

2.6.3. Carbohydrate content

The phenol sulphuric acid technique is commonly used to determine the carbohydrate content of a solution (Masuko et al., 2005). One mL of

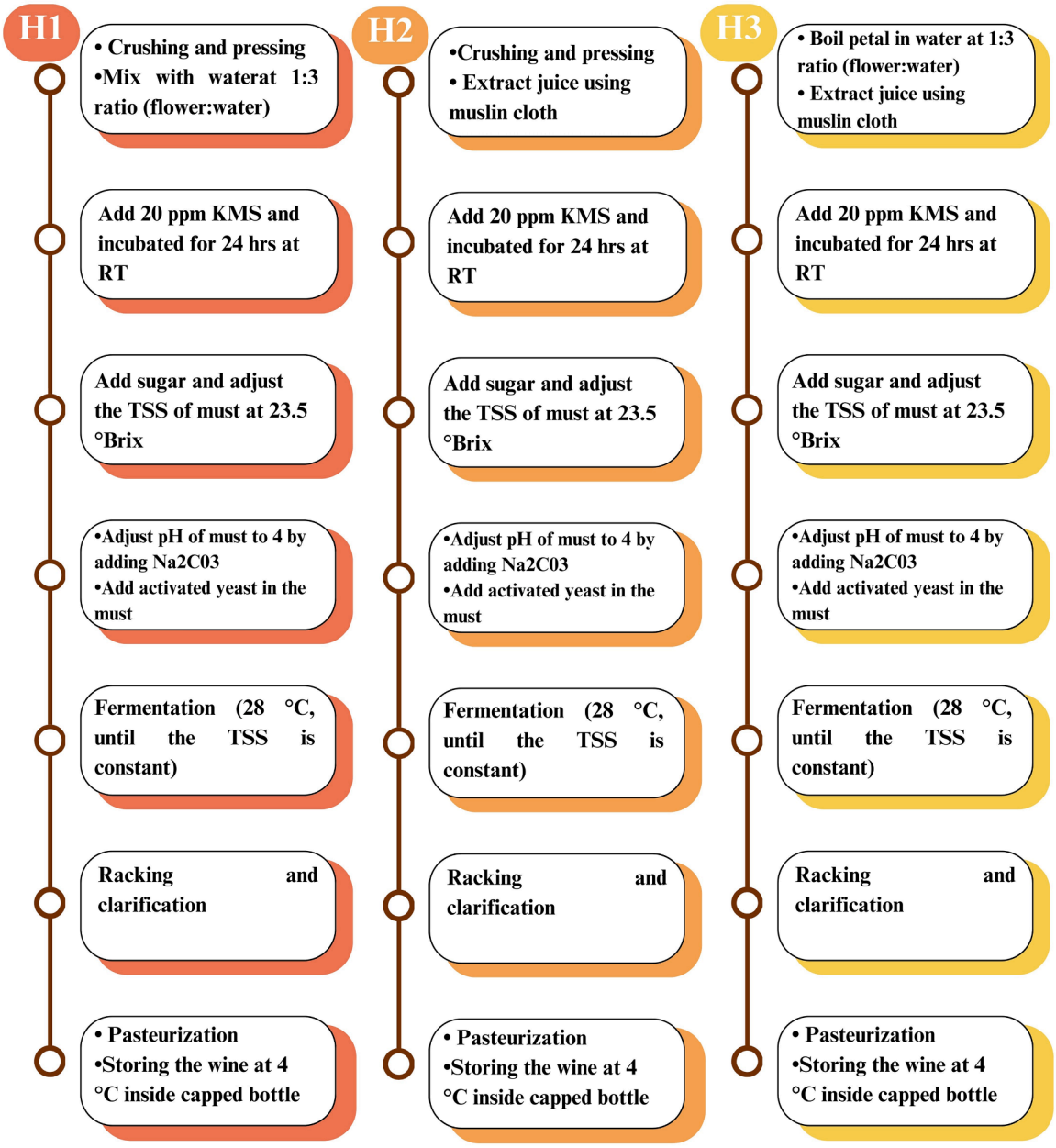

Fig. 1. Flowchart for making different types of Rhododendron wines.

5 % aqueous phenol solution was mixed with 1 mL of sample. Subsquently, 5 mL of concentrated sulphuric acid was swiftly added to the mixture. The test tubes were left to stand for 10 min and were vortexed for 30 s, followed by 20 min of water bath observation for color development. The glucose was used as the standard and the absorbance of the solution was measured at 480 nm.

2.7. Phytochemical analysis

2.7.1. Total phenolic content

Total phenolic content (TPC) of the wine was ascertained by slight modification of the Folin- Ciocalteu colorimetric method described by Cliff et al. (2007). The final volume of 5 mL was created by mixing 0.5 mL of juice and wine samples with 0.25 mL Folin-Ciocalteu reagent and finally with 4.25 mL of distilled water. After 5 min, 5 mL of 20 % sodium carbonate was added and thoroughly mixed. The solution was incubated at 37 °C for 90 min. A spectrophotometer (Multiskan skyhigh microplate spectrophotometer) was used to measure the absorbance at 750 nm. Gallic acid was used as a standard.

2.7.2. Total flavonoid content

The total flavonoid content (TFC) of juice and wine samples was measured using the spectroscopy method described by Hosu et al. (2014). One mL of sample was diluted with 4 mL of distilled water, then 0.3 mL of 5 % sodium nitrite was added for flavonoid measurement. The reaction mixture was then allowed to settle for 5 min before adding 0.3 mL of 10 % aluminium chloride and incubated for another 5 min. The resultant liquid was then thoroughly mixed with 2 mL of 1 N sodium hydroxide. To make the final combination 10 mL, 2.4 mL of distilled water was added. The absorbance of the sample was measured at 510 nm. Quercetin was utilized as a control.

2.7.3. Total anthocyanin content

Total anthocyanin content was determined by using the method described by Ribéreau-Gayon et al. (2006) with slight modification. 0.5 mL of juice and wine samples were mixed with 0.5 mL 0.1 % HCl in

ethanol, and 10 mL 2 % HCl aqueous solution. The first sample was treated with 4.4 mL of distilled water, and the second with 4.4 mL of 13 % sodium bisulfite solution. The mixture was diluted (1:1). The sample absorbance was measured at 520 nm using a blank solution consisting of 4.9 mL pure water, 0.5 mL of 0.1 % HCl in ethanol, and 10 mL of 2 % HCl aqueous solution. The difference (DA) between the absorbance readings of samples obtained with and without sodium bisulfite was calculated. The anthocyanin content was expressed in mg/L of wine and was calculated by multiplying the DA value by 875.

2.8. Antioxidant assay (DPPH assay)

Antioxidant activity of juice and wine samples was determined as described previously by Patel et al. (2010) with slight modifications. One mL of the sample was mixed with 3 mL of 0.1 mM DPPH in methanol. The mixture was incubated in the dark for 30 min and the absorbance was measured at 517 nm. Ascorbic acid was used as standard, while the blank was 1:3 solution of solvent and DPPH.

$$Scavengingactivity(\%) = [1 - A / B)] \times 100$$

2.9. Anti-nutritional analysis

The tannin content was estimated according to Folin-Ciocalteu procedure using of a standard solution of tannic acid (Mythili et al., 2014). The absorbance was measured at 725 nm using a spectrophotometer and result were expressed as mg of tannic acid per mL of sample (mg TAE/mL). The phytate was determined using Oriolowo et al. (2019) with slight modifications. Four mL of each sample was mixed with 20 mL of distilled water and incubated in shaking incubator for 3 hours at room temperature. Then, 5 mL of the wine extract was added to the conical flask, followed by 4 mL of reagents comprising 2 mL of 0.03 % $FeCl_3$ solution and 2.0 mL of 0.3 % sulfosalicylic acid until a brownish yellow color remained for 5 min. Using a spectrophotometer, the absorbance of the supernatant was measured at 500 nm. Sodium phytate was used as standard. The total saponin content in the sample was estimated using the method described by Le et al. (2018), with slight amendment. 0.25 mL of the sample was combined with 0.25 mL of 8 % vanillin in ethanol and 2.50 mL of 72 % sulphuric acid in water. The above solution was kept at 60 °C for 15 min in shaker. Diosgenin was used as a standard.

2.10. Methanol content

The methanol content in a wine was evaluated by using three qualitative tests: the choloform test, the iodoform test, and the silver nitrate test (Engstron, 1988; Wenzel, 2013).

2.11. Mineral content

Minerals content in juice and wine samples were analyzed using Atomic Absorption Spectroscopy (AAS) with a procedure set by Gleisner et al. (2010). First, the juice and wine samples were digested in a muffle furnace at 550 °C until white to grey ash developed. The digested samples were then filtered and the obtained filtrates were measured for mineral content using AAS. The results were expressed by comparing calibration curves of standard minerals.

2.12. Antimicrobial activity

2.12.1. Disc diffusion method

The antimicrobial activity of juice and wine extracts was evaluated by agar disc diffusion method (Nowak et al., 2022). Test microorganisms viz- *Bacillus subtilis* (ATCC 6051), *Escherichia coli* (ATCC 25,922), *Klebsiella pneumoniae* (ATCC 6380), *Staphylococcus aureus* (ATCC 25,923), *Pseudomonas aeruginosa* (ATCC 9027) and *Candida albicans* (ATCC 10, 231) were obtained from Sukra Raj Tropical & Infectious Disease Hospital, Teku, Kathmandu, Nepal. Above microbial strains were subcultured on nutrient broth (bacteria) and potato dextrose broth (*Candida albicans*). The freshly generated microbial inoculums were compared and matched with 0.5 MacFarland standards before being swabbed over Mueller-Hinton agar and potato dextrose agar using the carpet culture method. A sterile, 6 mm-diameter borer was used to drill four wells into the medium containing inoculums, which were then correctly labelled. The test wells were filled with 50 µl samples at varying concentrations (25–100 mg/mL) and kept at room temperature to allow the extracts to distribute in the medium. The plates were then incubated for 24 hours at 37 °C. The antimicrobial activity of the samples was determined by measuring their zone of inhibition. The standard antibiotic disc of the respective organism mentioned above was used as the positive control.

2.12.2. Minimum inhibitory concentration (MIC) determination

The minimum inhibitory concentration of the juice and wine extract was determined using the broth dilution method (Santoro et al., 2020). The test samples were dissolved in 10 % DMSO and were two-fold diluted to get series of concentrations from 50 mg/mL to 1.56 mg/mL. The culture inoculums of 0.5 MacFarland standard were freshly prepared in sterile nutrient broth for bacteria and potato dextrose broth for fungi. In each well of 96 well microtiter plate, 95 µL of diluted plant extract and 5 µL of broth containing microorganism suspension was added (Nielsen et al., 2012). Tetracycline (0.05 %) and cycloheximide (0.05 %) were used as positive controls for bacteria and fungi, respectively. The negative control well consisted of 195 µL of media and 5 µL of the standard inoculum. The plates were incubated for 24 hours at 37 °C for bacteria and 28 °C for fungi. After incubation, wells of samples with different dilutions were compared with positive and negative growth control. The lowest concentration of the samples that inhibited microbial growth was denoted as MIC value.

2.13. Sensory evaluation of wine

The sensory evaluation test was performed at Nepal Academy of Science and Technology (NAST). The analysis was accomplished after six months of wine production, under the distraction free environmental conditions at room temperature. The wines were submitted to a sensory evaluation using 50 untrained panel members (30 male and 20 female) between the ages of 23 to 52 years with preinformed written consent. The panel members were taught about each attribute and asked to read the questionnaires carefully. Sensory attributes were evaluated for five parameters in each sample such as appearance, flavour, odor, overall acceptability, and taste. These parameters were given nine-point hedonic scale from 1 to 9, where 9 indicates extreme like, 5 indicates neither like nor dislike, and 1 indicates extreme dislike. The panel members were instructed to rinse their mouth with water, eat a slice of bread rinse again, and wait one minute before proceeding to the next sample. A stopwatch was used to ensure strict adherence to the time.

2.14. Statistical analysis

Data analysis was done using R programming and MS Excel 2007 to compare juice and wines using physicochemical, proximate, and phytochemical composition. A one-way ANOVA was conducted to determine significant differences in the physicochemical, proximate, and phytochemical parameters among the juice and wines. The principle component analysis (PCA) was used to determine the distribution of phytochemical parameters among juice and three types of wines. The confidence interval of 95 % was used in ANOVA and PCA to infer the significant difference. To analyze this advanced multiple correlation heat map of phytochemical parameters with IC_{50} was developed. PCA was also conducted to observe the distribution of mineral contents among juice and wines. All the inferential statistics were done via the R programming version 4.1.3 (R Core Team, 2023).

3. Results and discussion

3.1. Physico-chemical analysis

The result revealed that the pH of the must was consistently acidic during the fermentation (Fig. 2). The pH of wine decreased gradually over time. On day 1, the pH of the juice and all three wines was 3.21 and 4.0, while on day 19, the pH of the juice and wines (H_1, H_2, and H_3) were 3.15, 3.45. 3.53, and 3.71, respectively. This observation was in agreement with the study of Ferreira and Mendes-Faia (2020). Wine becomes more acidic with the increase in fermentation period due to the production of organic acids during sugar metabolism by yeast (Vamvakas & Kapolos, 2020). The changes in pH, even within a small range can affect the function of biomolecules (Duarte et al., 2020) and upholding it within the standard limit is an important for maintaining the quality of wine.

It was observed that TSS content in juice, H_1, H_2, and H_3 were reduced from 9.1 to 8.7, 23.5 to 9.7, 23.5 to 8.9, and 23.5 to 12.0, respectively (Fig. 3). The decrease in TSS of the must during the fermentation period was primarily due to the conversion of sugars into alcohol and carbon dioxide by yeast (Jordao et al., 2015). TSS level decreased from 15 % to 1 % during the fermentation while working on the wine production from *Agave americana* (Steinkraus, 1995). The decrease in TSS is associated with the alcoholic fermentation of juice into wine, showing that sugar is used during fermentation (Udeagha et al., 2020). The decrease in TSS indicates the end of the fermentation process and can be explained by the increased fermentability of the juice, which, in turn, can be attributed to the availability of more sugar at the beginning. The wine (H_2) had lower TSS, probably due to high sugar utilization for the synthesis of alcohol (Nikhanj & Kocher, 2015). Where the dilution rate is concerned, higher dilution levels promoted increased fermentation rates (Joshi et al., 2012).

3.2. Proximate analysis

The physicochemical and proximate analysis revealed the significant variations in the value of every parameter among the juice and wines (Table 1). The highest moisture content was seen in juice with 92.31 % and the lowest in the H_3 with 84.34 %. The moisture content of the juice in the present study was slightly higher compared to the previous study (Devi & Vats, 2017). Wine has a lower moisture content than fresh juice because the presence of alcohol in wine decreases the overall water content, leading to a decrease in moisture compared to the original juice (Varela et al., 2020). Wines with high moisture content are considered refreshing beverages that effectively quench thirst, which is a hallmark of a good beverage (Ezemba et al., 2022). The ash content of fresh juice of *R. arboreum* and wine was found to be 0.31 %, 0.19 %, 0.16 %, and 0.25, respectively. This variation was closely associated with the total soluble solids in juice and wine (Udeagha et al., 2020). These results showed that the ash content of the juice decreased after fermentation due to the microorganisms, such as yeast or bacteria, that metabolize sugars in the juice, transforming them into a variety of byproducts. Tatah et al., 2022 reported 0.15 % of ash in *Cucumis melo* wine. Similarly, 0.70 % of the ash was found in banana wine, 0.30 % in pawpaw wine, and 0.02 % in red wine (Awe & Nnadoze, 2015).

The protein content in wine — H_1, H_2, and H_3 was 0.027 %, 0.037 %, and 0.026 %, respectively, whereas juice had a comparatively higher protein concentration of 0.063 %. Okokon and Okokon (2019) had found that protein concentration in wine varies between 0.12 % and 0.60 %. A clarifying process is sometimes done to wines after fermentation to have the wine free from particles or proteins, among others. This is due to the fact that bentonite and other fining agents help in the process of the elimination of proteins and other unwanted particles. This clarification step plays a big role in the elimination of proteins in the final wine, as noted by the previous study (Roger, 1998). The total carbohydrate content of the fresh juice and wine samples ranged from 0.71 % to 5.85 %. Total carbohydrates gradually decrease during the fermentation stage as the carbohydrate is transformed into alcohol and carbon dioxide (Kashyap, 2019). The present result was in agreement with the previous literature (Ajit et al., 2018). The highest fat content (0.15 %) was recorded in the fresh juice sample, whereas in the fermented juices, it ranged between 0.1 and 0.13 %. Tatah et al., 2022 reported that the crude fat content in the *Cucumis melo* fruit wine was 0.27 %, which was slightly higher than the current study. After fermentation, total fat concentration in juice samples was slightly decreased in comparison to fresh juice samples. Wine undergoes filtering and clarifying processes that remove suspended particles, as well as lipids (fats) from the juice (Jackson, 2020).

Crude fiber content in juice and wines (H_1, H_2, and H_3) was 0.12 %, 0.11 %, 0.12 %, and 0.11 %, respectively. The crude fiber content in the present study was lower than that of the previous study (Ramzija et al., 2022). The total titratable acidity percentage in juice and wine samples ranged between 0.25 % and 0.54 %. The present study revealed a consistent increase in the total acidity of wine samples throughout the primary fermentation period, which was supported by the study of Tsegay (2020). The acids (citric acid, tartaric acid, and some amount of

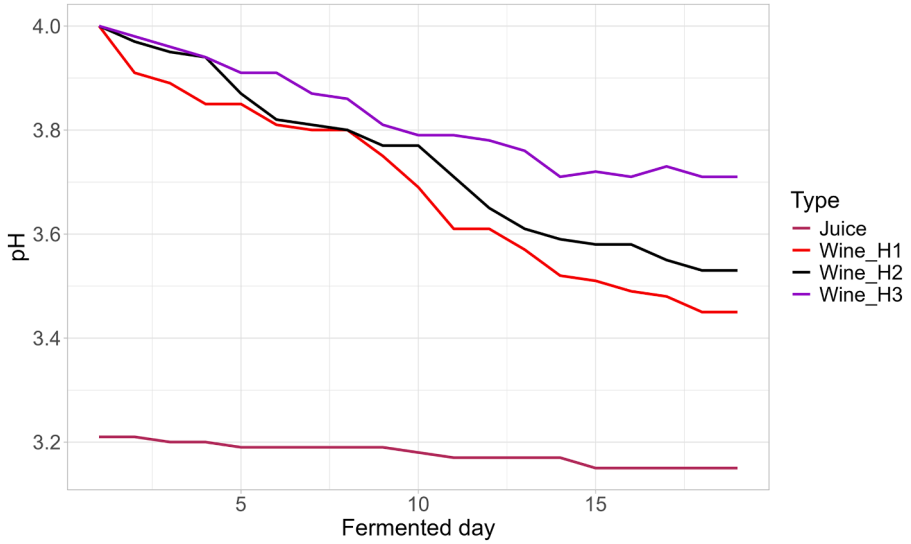

Fig. 2. pH variation in juice and wines with the fermentation days.

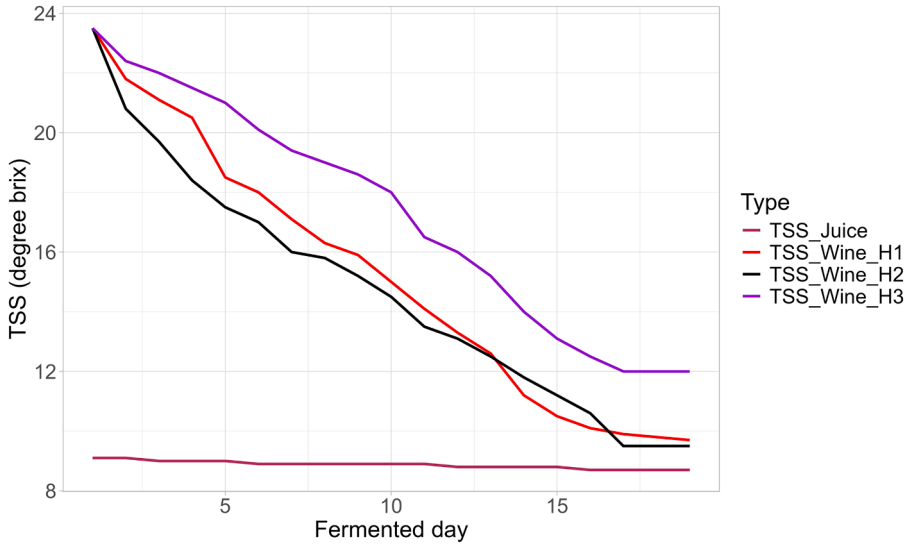

Fig. 3. TSS variation of juice and wine H_1, H_2, and H_3 during fermentation period.

Table 1
Physicochemical and proximate analysis of juice and wines represented by mean ± standard error value. For each physicochemical and proximate parameter, columns with the similar shared superscript above the mean value represents no significant difference between each other and different shared superscript denotes significant difference between each other.

Parameters	Juice	H_1	H_2	H_3	P value
Total solid (%)	4.87 ± 0.1^a	6.68 ± 0.2^b	6.49 ± 0.3^b	9.66 ± 0.3^c	< 0.001
Moisture content (%)	92.13 ± 0.1^a	87.32 ± 0.2^b	87.51 ± 0.1^b	84.34 ± 0.1^d	< 0.001
Ash content (%)	0.31 ± 0.01^a	0.19 ± 0.01^b	0.25 ± 0.01^c	0.16 ± 0.01^d	< 0.001
Protein content (%)	0.063^a	0.027^b	0.037^c	0.026^b	< 0.001
Carbohydrate content (%)	0.71 ± 0.1^a	5.58 ± 0.5^b	4.84 ± 0.2^b	5.85 ± 0.1^b	< 0.001
Fat content (%)	0.15 ± 0.01^a	0.1 ± 0.01^b	0.13 ± 0.01^c	0.12 ± 0.01^d	< 0.001
Crude fibre (%)	0.12 ± 0.01^a	0.11 ± 0.01^b	0.12 ± 0.01^a	0.11 ± 0.01^b	< 0.001
TA (%)	0.25^a	0.45^b	0.54^c	0.50^d	< 0.001
Alcohol (%)	–	7.36^a	8.23^b	7.12 ^{a}	< 0.001
VA (g/L)	–	0.7^a	0.7^a	0.06^b	< 0.001

TA = Titrable acidity, VA = Volatile acidity.

lactic acid) have a direct impact on the quality of the wine by increasing titratable acidity and, by improving the wine's flavor, fragrance, and preservative characteristics (Kashyap, 2019).

The maximum alcohol content (8.23 %) was observed in H_2 and the minimum (7.12 %) in H_3, which was in accordance with the finding of Ajit et al. (2018) that reported 7 to 13 % of alcohol in their sample. The alcohol content in wine may range approximately between 9 % and 15 % (O'Keefe et al., 2014), which nearly matches the alcohol percentage of Wine (H_2). The wine prepared from petals in the present study might be the reason of lower alcohol content because sugar content in petals is comparatively lower than the fruits. Dilution of must with water has the ability to lower alcohol content by over 1 % v/v (Varela et al., 2020). The present study observed similar findings, as the fermentation process came to an end, yeast activity gradually declined because of a progressive rise in alcohol content and a reduction in pH levels (Berbegal et al., 2022). The highest volatile content of 0.7 g/L was found in H_2 and H_1 which was followed by H_3 (0.6 g/L). Volatile acidity of the banana wine ranged from 0.29 g/L to 0.58 g/L which is closely similar to previous findings (Idise & Odum, 2011). Acetic acid bacteria can cause volatile acidity by converting ethanol into acetic acid.

3.3. Phytochemical analysis

The phytochemical parameters varied significantly among the juice and wine samples (Table 2). The amount of total phenolic content in juice and wines had significant variation, ranging from 3.87 GAE/mL to 9.96 mg GAE/mL. The total phenolic content was highest in wine (H_2) but decreased in H_1 and H_3. Dilution during must preparation might be responsible for lowering the phenolic content of H_1 and H_3 (Varela et al., 2015). The increment in TPC can be attributed to the enhanced mobility of molecules, a decrease in the viscosity of the medium, and an increase in the diffusion coefficient at higher temperatures (Gao et al., 1997). In contrast, the decrease in TPC might be a result of yeast's inefficiency to transport the phenolic compounds that were chelated in the cell wall, hence delaying the polymerization process (Ghan et al., 2015).

The highest TFC values were obtained in juice, followed by H_2 (Table 2). TFC levels in wines H_1 and H_3 may have decreased due to quercetin glycoside degradation and glucosinolate oxidation, pH variations, tannin-forming polymeric pigment, and alcohol concentration-related joint alterations (Yang & Xiao, 2013). The total flavonoid content was highest in wine (H_2) but decreased in H_1 and H_3. This may be due to the dilution of H_1 and H_3 during the preparation of must (Varela et al., 2015). Anthocyanin content in wine samples decreased continuously after the fermentation except H_2. The anthocyanin content in a wine ranged between 34.19 mg/L and 388.79 mg/L Hosu et al. (2014), which fits with the juice and wines of the present study except H_1. Air exposure, alteration in pH level, and enzymatic activity during fermentation are major factors that lead to anthocyanin degradation (Bhatt et al., 2022). The health benefits of wine phenolics, notably their antioxidant and anti-inflammatory characteristics, are also considered in terms of preventing and reducing the risk of noncommunicable diseases such as heart disease, malignancies, and neurological problems (El Rayess et al., 2024).

The study revealed that H_2 had the highest tannin content with 13.42 mg/mL, while H_3 had the least at 4.28 mg/mL. Tannin has antinutritional effects because it creates the compounds with minor elements (phosphorus, calcium, magnesium) as well as large elements (carbohydrate, proteins) that prevent the body from using them (Mangan, 1988). It was observed that the phytate content of the juice and wine of the

Table 2
Phytochemical parameters of juice and wines.

SN	Name of Sample	TPC (mg GAE/mL)	TFC (mg QE/mL)	TAC (mg/mL)	Tannin Content (mg/ml)	Phytate Content (mg/ml)	Saponin Content (mg/ml)
1	Juice	8.022 ± 0.13^{a}	13.38 ± 0.37^{a}	135.625 ± 1.23^{a}	11.9 ± 0.12^{a}	0.036 ± 0.01^{a}	7.84 ± 0.08^{a}
2	H_1	3.87 ± 0.07^{b}	2.52 ± 0.02^{b}	19.833 ± 0.57^{b}	12.27 ± 0.1^{b}	0.061 ± 0.01^{b}	4.34 ± 0.05^{b}
3	H_2	9.96 ± 0.15^{c}	7.11 ± 0.09^{c}	162.166 ± 2.3^{c}	13.42 ± 0.09^{c}	0.048 ± 0.02^{c}	5.42 ± 0.03^{c}
4	H_3	5.34 ± 0.01^{d}	2.64 ± 0.04^{b}	57.458 ± 0.97^{d}	4.28 ± 0.03^{d}	0.061 ± 0.01^{b}	6.88 ± 0.02^{d}
	P value	0.001	0.001	0.001	0.001	0.001	0.001

TPC= Total phenolic content; TFC= Total flavonoid content, TAC= Total Anthocyanin content.

R. arboreum ranged between 0.21 and 0.37 mg/mL. Wine (H_3) possessed the highest phytate content, followed by H_1, H_2, and juice. The quantity of phytate content in wine ranged between 0.029 and 0.092 g/L (Iwuozor, 2019). In the present study, saponin content in juice and wine ranged from 4.34 mg/mL to 7.84 mg/mL. Saponin content in the wine sample slightly decreased after the fermentation, which was supported by Tatah et al., 2022) and Ebana et al. (2019), which reported that saponin content in *Cucumis sativus* L. (cucumber) wine was between 1.35 and 1.70 mg/mL which was slightly lower than this study. Saponin-rich diets assist in controlling plasma cholesterol, prevent peptic ulcers, osteoporosis, and reduce the risk of heart disease (Gemede & Ratta, 2014). High concentrations of saponin in the human diet can reduce nutritional bioavailability, enzyme activity, and protein digestibility (Braide et al., 2012).

3.4. Antioxidant and IC_{50}

The maximum scavenging activity (82.01 %) was observed in H_2, while the minimum activity (42.96 %) was observed in H_1 at the concentration of 25 µg/mL (Fig. 4). These results observed that juice and H_2 had higher antioxidant activity whereas H_1 and H_3 had lower antioxidant activity compared with the standard (ascorbic acid). Yadav et al. (2021) reported that the antioxidant activity of *R. arboreum* wine ranged in between 55 % and 75 %, which is slightly lower compared to the present results. The antioxidant compounds in wine, such as polyphenols, flavonoids, and resveratrol, contribute to its antioxidant activity (Hur et al., 2014). Wine H_2 was prepared from the petals of *R. arboreum* juice, without further dilution. However, wine H_1 and H_3 were diluted with water during must preparation, which might be responsible for their decline in the antioxidant activity. Fermentation implies great antioxidant activity by upraising phenolic substances and flavonoids through microbial catabolism. Phenolic glycosides are cleaved by microbial enzymes, and the component known as the aglycone exhibits antioxidant activity (Santos et al., 2016). According to Hosu et al. (2014), the growth of the yeasts may be facilitated by low pH through enhancement of antioxidants and inhibition of the enzymatic oxidation. The juice and wine H_2 exhibited the higher level of antioxidants that help the body fight infection and disease more effectively by supporting the immune system from oxidative damage and enhancing their capabilities (Knight, 2000). Clinical and experimental studies have also revealed that moderate consumption of red wine can lower the risk of heart disease (Castaldo et al., 2019; Covas et al., 2010; Li et al., 2023; Nova et al., 2019), increase good cholesterol and reduce bad cholesterol (Di Renzo et al., 2015).

The IC_{50} value, which represents the amount of antioxidant activity required to reduce the initial DPPH concentration by 50 %, is a typical measure of antioxidant activity (Khan et al., 2012). A lower IC_{50} value suggests greater antioxidant potency (Brighente et al., 2007). The study revealed *R. arboreum* juice and H_2 showed the greater antioxidant property with IC_{50} values of 15.39 µg/mL and 11.31 µg/mL respectively,

Table 3
IC_{50} value of juice, wine and ascorbic acid.

SN	Samples	IC_{50} Value (µg/ml)
1	Juice	15.39
2	H_1	27.70
3	H_2	11.31
4	H_3	25.25
5	Ascorbic Acid (Standard)	17.10

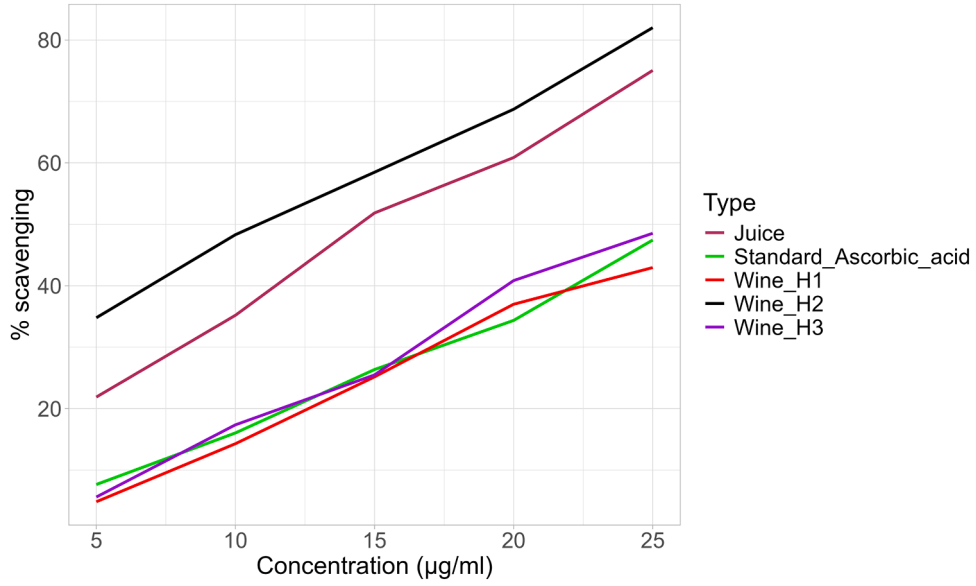

Fig. 4. DPPH scavenging activity of juice and wine samples at different concentrations.

as compared with ascorbic acid (17.10 μg/mL), whereas the least antioxidant property was found in H_1 with an IC_{50} value of 27.70 μg/mL (Table 3). The lower the IC_{50} value, the more powerful the substance in scavenging DPPH radicals, implying stronger antioxidant activity (Wang et al., 2019).

3.5. Methanol content and mineral content

The juice and wine of *R. arboreum* were subjected to chloroform, silver nitrate and iodoform tests for detection of methanol. All the qualitative tests showed negative results i.e. absence of methanol (Table 4).

The PCA disclosed the varied pattern in the distribution of the minerals among the juice and wines. The PC_1 and PC_2 described 88.4% and 8 % variance respectively (Fig. 5). The biplots displayed how nutrients correlated with the principal components, illustrating their relative importance. The results reveal that juice and wine (H_2) distinctively possess higher nutrient levels compared to wines (H_1 and H_3). Among the wines, H_2, appeared to have a favorable nutrient profile with the higher level of Zn and Mn, which could be beneficial depending upon the nutritional needs. The mineral content in wines in this study also decreased after fermentation (Awe & Nnadoze, 2015). Different post-fermentation processes like filtration, fining, and clarification remove most of the suspended particulates, including minerals (Braide et al., 2012). Also, some minerals form complexes, which can either precipitate or get entrapped in the lees and are therefore skimmed off during racking. These combined processes contribute to a decline in mineral content in the wine produced compared to that of the initial must (Shimizu et al., 2020).

3.6. Antimicrobial activity

All the sample extracts displayed antimicrobial activities. The zone inhibition diameter and MIC obtained against the Gram-positive (*B. subtilis,* and *S. aureus*) and Gram-negative bacteria (*E. coli, P. aeruginosa,* and *K. pneumoniae*) and fungi (*C. albicans*) are shown in Tables 5 and 6. The extract of juice and wine H_2 induced inhibitory activities against all the tested organisms, with zone inhibition diameter varying from 10 to 20 mm and MIC from 50 mg/ml to 6.25 mg/ml. *B. subtilis* and *S. aureus* were most affected by juice and all wine extracts, with the maximum inhibition zone (20 mm) and most sensitive are Gram positive bacteria *B. subtilis* and *S. aureus* (ID = 20 mm, and MIC = 6.25 mg/ml) in the case of H_2. The higher zone of inhibition against Gram positive bacteria was shown by H_2 followed by juice. However, H_1 and H_3 showed poor activity against Gram positive bacteria and not effective against fungi and Gram negative bacteria. Tetracycline (0.05 %) and cycloheximide (0.05 %) used as positive control for MIC, showed no growth of microorganisms. Papadopoulou et al. (2005) also reported that the wine extracts were more effective against *S. aureus* as compared to *E. coli* and *C. albicans.*

Wine extracts and juice exhibited a larger zone of inhibition against gram positive bacteria than Gram-negative bacteria, indicating the greater sensitivity towards former ones (Santoro et al., 2020). Compared to Gram-positive bacteria, which have a relatively simpler outer lipid membrane, Gram-negative bacteria have a more complicated structure and a less permeable membrane. This difference makes Gram-positive bacteria more susceptible to antimicrobial substance (Kashyap, 2019).

Phenolic components present in the samples might exhibited strong antibacterial characteristics (Leyva-Jimenez et al., 2019). Phenolic compounds have broad-spectrum antimicrobial activity against Gram-positive bacteria and their modes of action against bacterial membranes, cell walls, enzymes, DNA, and quorum sensing. Moreover, their effectiveness to produce the oxidative stress, chelate metals, and bust biofilms contributes to the broad-spectrum activity (Alvarez-Martínez et al., 2020). Due to the variety of the described modes of action, phenolic compounds are promising to use for the treatment of the infections, which are caused by Gram-positive bacteria, especially with the growth of antibiotic resistance (Cetin-Karaca & Newman, 2015). The main antibacterial mechanism of phenolics is associated with their ability to target the cytoplasmic and outer lipid membranes of Gram-negative bacteria (Vaquero et al., 2007).

Table 4
Qualitative test for the methanol in juice and Wine.

SN	Name of Sample	Qualitative methods		
		Chloroform test	Silver nitrate test	Iodoform test
1	Juice	Negative	Negative	Negative
2	H_1	Negative	Negative	Negative
3	H_2	Negative	Negative	Negative
4	H_3	Negative	Negative	Negative

3.7. Sensory evaluation

The average scores of wine for appearance, odor, taste, flavour, and overall acceptability are highlighted in Fig. 6. These results suggest that, H_1 stands out for its flavor and overall acceptability, making it the most favored wine sample among the three. A wide range of volatile and non-volatile chemicals contribute to the distinct taste and odor of alcoholic beverages (Dooley, Threlfall, Meullenet, & Howard, 2012; Jung et al., 2014). Sensory analysis is highly significant in our daily lives and is crucial in oenology. It serves as the primary criterion for making informed decisions throughout the winemaking (Okokon & Okokon, 2019; Nunes et al., 2020).

3.8. Phytochemical parameters in juice and wines

The principle component 1 (PC_1) and principle component 2 (PC_2) explained 70.1 % whereas 20.2 % were variables, respectively (Fig. 7). This analysis revealed that TAC and TPC are strong predictors of IC_{50}, TFC and PC were moderate, and TC and SC had minimal impact on the variability of IC_{50}. The IC_{50} value for DPPH exhibited significant ($p < 0.001$) and extremely strong negative association with TPC and TAC, showing that greater quantities of these chemicals correlate to a lower concentration needed to attain IC_{50}. Lower IC_{50} values generally indicate greater efficacy in terms of antioxidant, antibacterial, and anti-inflammatory activity (Fejliciano et al., 2009). The higher TPC and TAC levels contribute to health benefits by reducing oxidative stress which slows aging processes while decreasing the probability of cancer development and cardiovascular and neurodegenerative diseases (Pham-Huy et al., 2008). Also, protective effects of antioxidants and phenolics supporting gut health, immune function, and overall cellular protection (Cardona et al., 2013; Zhang & Tsao, 2016).

TPC and TC were positively linked to H_2, and IC_{50} was not significantly present in it. In contrast, IC_{50} was seen in a higher amount in H_1 and H_3 compared to juice and H_2. Juice contained a higher amount of SC, TFC, and TAC while having a lower amount of IC_{50} and PC. PCA was complimented by the correlation heat map (Fig. 8) and values of the linear regression (Table 7). The above findings showed a different level of correlation between IC_{50} and several phytochemicals when subjected to PCA and linear regression analysis. Consequently, TAC and TPC have the significant correlationship with IC_{50} with R square values of 0.985 and 0.925, respectively, which showed that TAC and TPC phytochemicals account for a large amount of variance in the IC_{50}. We also observed significant positive relationship between TFC and PC with R-squared of 0.497, 0.595, and p-values of 0.006 and 0.001, respectively. In contrast, the relationship between TC & SC and IC_{50} was very weak and insignificant, as seen from R square = 0.202 and 0.029 and $p = 0.08$ and 0.275, respectively.

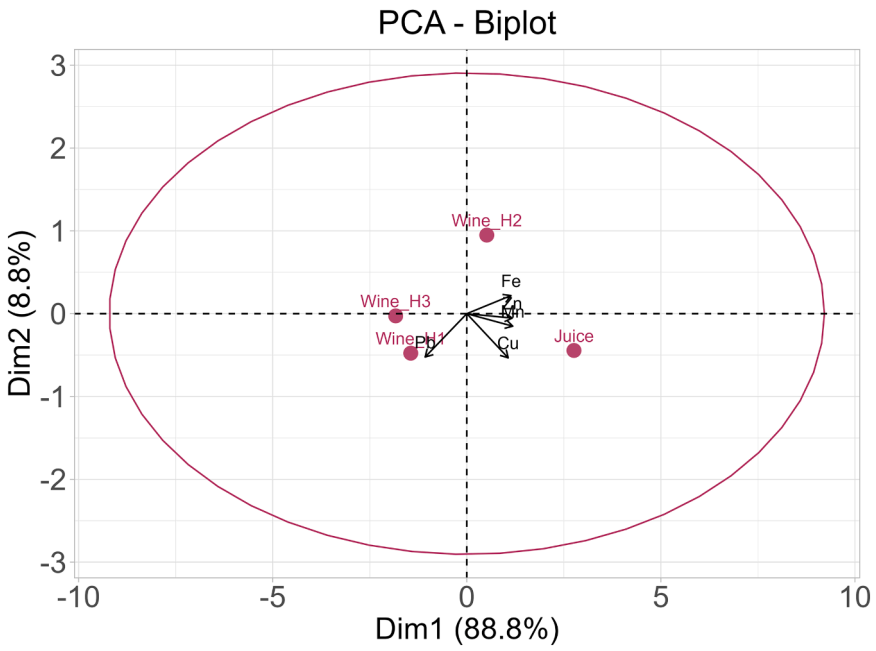

Fig. 5. Principle component analysis for the distribution of minerals among the juice and wines.

Table 5
The antimicrobial property of juice and wine extracts against common human pathogen at different concentration by disc diffusion method.

Sample	Concentration (mg/ml)	Zone of inhibition (mm)					
		E. coli	*B. subtilis*	*P. aeruginosa*	*K. pneumoniae*	*C. albicans*	*S. aureus*
Juice	25	10	12	13	9	–	10
	50	12	14	15	13	–	12
	75	14	16	16	14	11	14
	100	16	18	17	15	13	18
Wine(H1)	25	–	–	10	–	–	9
	50	–	10	12	10	12	11
	75	10	12	14	12	14	13
	100	12	14	16	15	16	17
Wine(H2)	25	–	10	–	9	–	11
	50	10	12	10	13	9	15
	75	12	15	12	15	11	17
	100	14	18	14	17	13	20
Wine(H3)	25	–	10	–	–	–	10
	50	–	12	–	10	9	12
	75	–	13	10	12	11	14
	100	11	14	12	14	13	16
Positive control	–	30	30	22	35	30	26

Positive control used: *E. coli*= Ciprofloxacin-5mcg; Bacillus subtilis= Chloramphenicol-30mcg; Pseudomonas aeruginosa = Ceftriaxone-30mcg; K. pneumonia= Tetracycline-30mcg; Candida albicans= Itraconazole-10mcg; Staphylococcus aureus= Chloramphenicol-30mcg.

Table 6
Minimum inhibitory concentration of Juice and wine sample (mg/ml).

Sample	Test organism					
	E. coli	*B. subtilis*	*P. aeruginosa*	*K. pneumoniae*	*C. albicans*	*S. aureus*
Juice	12.5	6.25	12.5	25	>50	25
Wine($H_{1)}$	>50	50	50	50	50	25
Wine($H_{2)}$	>50	12.5	>50	25	25	6.25
Wine($H_{3)}$	50	25	50	50	25	12.5

Tetracycline (0.05 %) for bacteria and Cycloheximide (0.05 %) for fungi as positive control.

4. Conclusion

This research work particularly focused on the effect of the fermentation method on the physicochemical, proximate, phytochemical, anti-nutritional, and antimicrobial qualities of *R. arboreum* wines and juice. In the present study, the specified parameters were found to undergo significant changes during the fermentation process, and these changes improved the quality and functionality of the wine, especially wine H_2. Moreover, the wine H_2 exhibited the highest antioxidant and antimicrobial capacity, correlated with phenolic content, indicating that an increased consumption of *R. arboreum* wine could have certain health benefits because of its higher antioxidant and antimicrobial abilities.

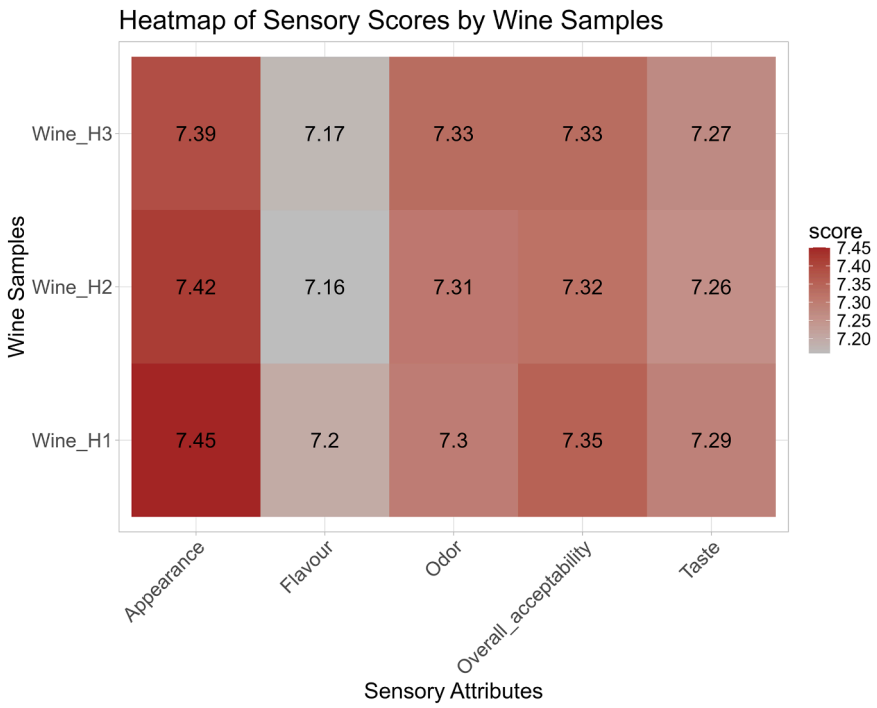

Fig. 6. Sensory evaluation of different wines based on sensory attributes.

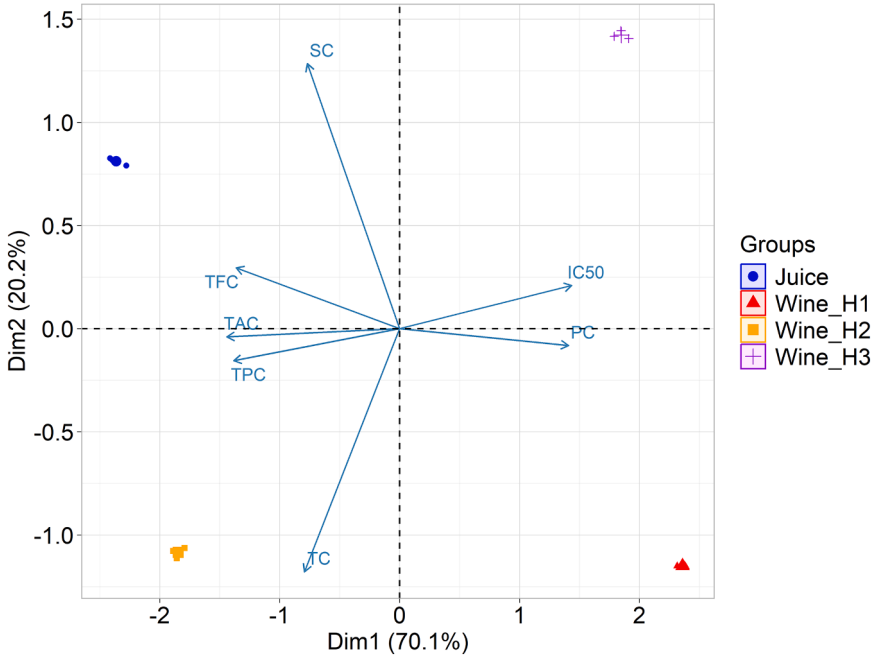

Fig. 7. Principle component analysis (PCA) showing the significant phytochemical parameters (TFC = Total flavonoid content; TAC = Total anthocyanin content; TPC = Total phenolic content; TC = Tannin content; PC = Phytate content; SC = Saponin content) associated with juice, wine (H_1), wine (H_2) and wine (H_3).

The observed enhancements in antioxidant and antimicrobial activities in wine H_2, along with the descriptive characteristics such as sensory profile indicated the strict regulation of the fermentation conditions is capable of generating enhanced nutritive *R. arboreum* wines with enhanced sensory qualities. The present findings not only revealed the potential health and functional benefits associated with wine H_2 but also highlighted its prospect for commercialization. Due to its abundant availability throughout the hilly and mountainous regions of Nepal, it has huge potential for juice and fermented beverages production and could be a good source of income for local people, entrepreneur and contribute national economy through additional revenue and employment generation. However, before commercial production and marketing, further optimization and clinical studies on its health benefits should be carried out.

CRediT authorship contribution statement

Hari Achhami: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Bishwa Bandhu Pachhai:** Writing – review &

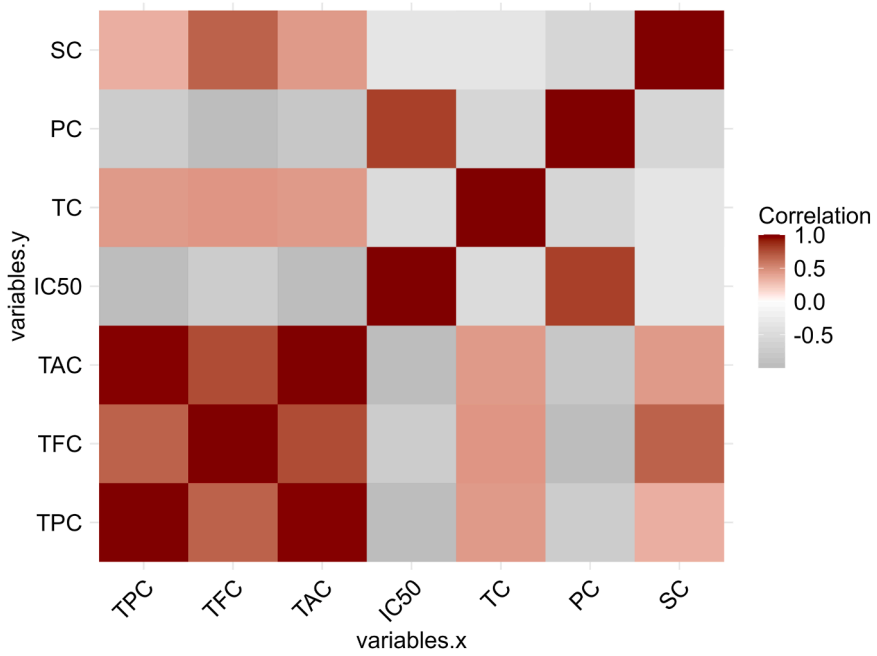

Fig. 8. Heat map showing the relationships between the phytochemical parameters.

Table 7
Linear regression between IC_{50} and selected phytochemicals.

	R square value	p value
TPC	0.925	< 0.001
TFC	0.497	0.006
TAC	0.985	< 0.001
TC	0.202	0.08
PC	0.595	0.001
SC	0.029	0.275

editing, Writing – original draft. **Sujan Chaudhary:** Writing – review & editing, Writing – original draft, Formal analysis. **Prakash Manandhar:** Supervision. **Lok Ranjan Bhatt:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical statement

Informed written consent was taken from all the participants during the sensory evaluation of the wine.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2025.100929.

Data availability

Data will be made available on request.

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