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Polyphenolic Composition and Antioxidant Capacity of Home-brewed Plum, Cherry, Rhododendron, and Grape Wines

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Fruit and flower wines have been studied for their various polyphenols. Among them, red wines are the most widely studied for their flavonoid and polyphenolic content. Thus,we aimed to assess the polyphenolic contents and antioxidant capacity of home-brewed plum, cherry, grape, and rhododendron wines. The total polyphenolic contents and flavonoids of the wine samples were quantified using Folin-Ciocalteu, Folin-Denis, and aluminum chloride methods, respectively. Antioxidant activity was assessed through ABTS and DPPH assays. Additionally, the ability of the wine samples to mitigate lipopolysaccharide-induced reactive oxygen and nitrogen species was investigated in a RAW 264.7 murine macrophage cell line using dichlorodihydrofluorescein diacetate and Griess reagents, respectively. Rhododendron wine displayed the highest content of total polyphenolic compounds (383.33±18.75 μg/mL tannic acid equivalent) and the highest flavonoid content (167.75±9.53 μg/mL quercetin equivalent). Rhododendron and plum wines showed significant reducing power (1723.83±143.19 μg/mL and 1675.66±10.29 μg/mL quercetin equivalent antioxidant capacity, respectively) and free radical scavenging activity (82.16±7.38% and 78.2±9%, respectively). All four wines significantly reduced the reactive oxygen and nitrogen species formation in lipopolysaccharide-induced macrophages. Our findings indicate that plum, cherry, and rhododendron wines exhibit notable in vitro antioxidant potential, highlighting their capacity to enhance revenue within the fruit wine market.

Keywords: Antioxidant; Cherry wine; Grape wine; Plum wine; Rhododendron; RAW246.7.

For centuries, traditionally made grape wine has been enjoyed worldwide. However, many other fruits such as bananas, cherries, kiwis, plums, and papayas are also used in winemaking. These fruits are not only nutritious but also gain additional polyphenols and volatile compounds through the process of fermentation[1].

Excessive alcohol intake is linked to the progression of diseases such as chronic liver disease[2,3], liver cancer[2], hypertension, and cardiovascular diseases[4] and an increased risk of colorectal malignancies[5], In contrast, moderate alcohol intake is associated with a low risk of coronary heart disease[6,7]. Numerous *in vitro* and *in vivo* studies, along with epidemiological surveys, suggest that moderate wine consumption, despite its ethanol content, is related to a low risks of type 2 diabetes[6], cardiovascular diseases,

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neurodegenerative disorders[7], platelet aggregation, and oxidative damage, largely owing to the polyphenols present in wine[8]. Polyphenols are regarded as key compounds responsible for wine's potential health benefits[9].

Bioactive compounds, particularly polyphenols, form a major component of wine[10]. The polyphenolic content of wines depends on the type and variety of fruits selected for winemaking[11]. Polyphenols comprise a large class of phytochemical compounds that consist of many subclasses, namely flavonoids, phenolic acids, stilbenes, and lignans[12]. Flavonoids are the major polyphenols present in wine and can be further subdivided into groups such as flavan-3-ols (catechin and epicatechin), flavonols (quercetin, kaempferol, and myricetin), flavones, isoflavones, and anthocyanins (malvin and petunin)[8, 9, 12]. In red grape wine, the most abundant phenolic antioxidants include catechin, proanthocyanidins, resveratrol, epicatechin, quercetin, anthocyanins, and rutin[13]. Cherry wine is reported to contain naringenin and apigenin as the main compounds[14]. *Rhododendron mucronulatum* flowers which are rich in myricetin, quercetin, and kaempferol, have been used to make wine in the past.

Dietary polyphenols, especially those found in wines, play a significant role in shaping the composition and function of the human gut and oral microbiota[10]. Wine-derived polyphenols exhibit prebiotic properties that help in the proliferation of beneficial gut bacteria[15]. They also exhibit antimicrobial effects against pathogenic bacteria[16]. Grape-derived antioxidants have been demonstrated to possess antitumor properties through various *in vitro* and *in vivo* models. Studies on red wine indicate that polyphenols, such as quercetin, resveratrol, catechin, and gallic acid, are possible cancer chemopreventive representatives[17]. Additionally, polyphenols exhibit anti-inflammatory and antimutagenic activities[18].

Polyphenols in wine have garnered significant attention for their potent antioxidant properties. Studies have shown strong correlations between total phenolic content (TPC) and antioxidant capacity. Phenolic acids, such as hydroxycinnamic and hydroxybenzoic acids, demonstrate effective free radical scavenging, helping to sustain the balance of reactive oxygen intermediates *in vivo*[19]. The flavonoids in wine also exhibit dominant scavenging abilities against reactive oxygen, and nitrogen species[20]. While the health benefits of polyphenols in grape wines are well-documented, the potential of other fruit and flower wines remains underexplored. In the present study, we aimed to evaluate and compare the polyphenolic content and antioxidant properties of home-brewed wines derived from plum, cherry, rhododendron, and grape.

MATERIALS AND METHODS

Materials

Fresh fruits such as green grapes (*Vitis vinifera*), cherries (*Prunus avium*), and plums (*Prunus salicina*) were procured from the local market (Silvassa). Rhododendron flowers (dried) were purchased from the Paraman store through Amazon. Absolute ethanol, methanol, AlCl3, Folin Ciocalteau reagent, Folin–Denis reagent, ferric ion reducing antioxidant power (FRAP), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), lipopolysaccharide (LPS), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) reagent, dichlorofluorescein diacetate (DCFDA), and tannic acid were purchased from Sigma-Aldrich. Dulbecco's modified Eagle medium (DMEM) and fetal bovine serum (South American origin) were purchased from MP Biomedicals. Trypsin EDTA solution, nutrient agar, and yeast extract-peptone-dextrose media were purchased from HiMedia.

Manufacturing of wine

Wines were prepared according to published protocol[21, 22] after several modifications. such as Green grapes (*Vitis vinifera*), cherries (*Prunus avium*), and plums (*Prunus salicina*) were cleaned with distilled water. The fruits were mashed, and the pulp and skin were used for fermentation. Ten kilograms of fruits were chopped into small pieces and juiced using a mortar and pestle. They were not mashed for a long time to avoid pectin release. Two kilograms of powdered table sugar was added to the fruit pulp/flower juice. Subsequently, 30 g of *Saccharomyces cerevisiae* was added in 100 mL of warm water with 5 g of glucose and after 15 to 20 mins, bubbles or foam were observed, indicating the activation of the yeast. This mixture was added to the fruit pulp/

flower juice. The volume was adjusted to 10 L with distilled water, and the mixture was transferred to an amber-colored bottle with adequate headspace, after which the fermentation rate was monitored by counting bubbles per minute. The mixture was kept for 21 days. Two hundred milliliters of diluted egg white (1:10) was prepared using water as the diluent and added to further clarify the wine. After a week, the wine was decanted and filtered through two layers of muslin cloth and stored in a glass bottle in a refrigerator (Flowchart 1). The method was slightly different for manufacturing flower wine. In the case of rhododendron (*Rhododendron arboreum*) wine, 100 g of dried flowers were soaked in 500 mL of boiling water and the mixture was kept for 24 h at room temperature and then kept at 4 °C for 24 h before being strained. Then, 300g of powdered table sugar and 3gms of activated yeast were added to the flower juice. The volume was adjusted to 1 L with sterile distilled water. The mixture was kept for 21 days in an air-lock container at 25°C. The wine was decanted and filtered through two layers of muslin cloth and stored in a glass bottle in a refrigerator (Flowchart 2).

Determination of physicochemical properties

Estimation of pH

pH was measured using a pH meter (Thermo Fisher Orion Versa Star Pro).

Estimation of Titratable acidity

Titratable acidity was determined using the alkaline titration method with 0.1 N NaOH solution and phenolphthalein as an indicator. A 3 mL sample of wine was placed in a flask, and the volume was then brought up to 25 mL with distilled water. The sample was titrated until a pink color appeared. Titratable acidity was calculated in terms of tartaric acid(g/L) using the following formula[23]:

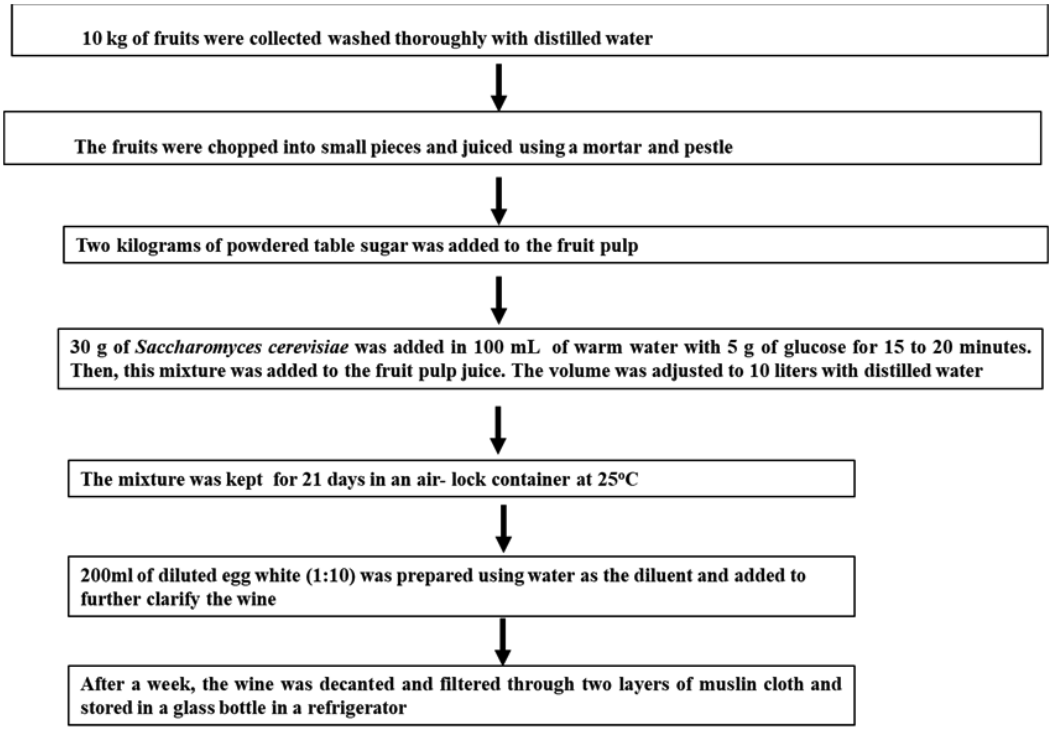
Titratable acidity (g/L) = 75 × Normality of NaOH × Titrant volume (mL) / Volume of sample (mL)

where 75= milliequivalent factor for tartaric acid.

Estimation of alcohol content

Using hydrometer

The concentration of alcohol in wines was assessed using a hydrometer. The initial fruit/ flower juice was filled in a hydrometer tube, and the



Flowchart 1. Wine preparation using Plum, Cherry, and Grape fruits

hydrometer was immersed in the liquid (allowing it to freely float). Subsequently, the initial specific gravity was recorded. After fermentation, the same procedure was repeated, and the final reading was noted. The percent alcohol concentration was estimated using the following equation[24]:

(Initial specific gravity- Final specific gravity) × 131.25

Using the dichromate method

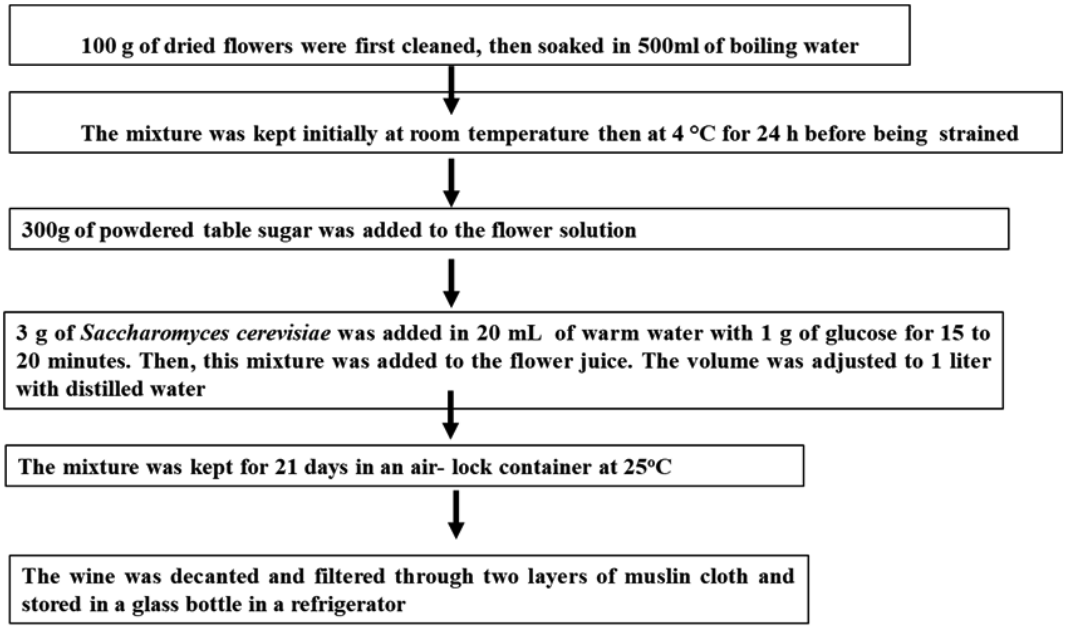
The potassium dichromate reagent was used to estimate the alcohol concentration of the wine samples. Absolute ethanol was used as the standard (3-6% v/v) for this assay. One milliliter of the standard or wine sample was added to a 100 mL flask, followed by 10 mL of 0.1 N potassium dichromate reagent and 10 mL of 50% v/v sulfuric acid. After incubating the flask at 60°C for 20 min and allowing it to cool, the solution was diluted to 50 mL with distilled water. The absorbance was then measured at 587 nm using a spectrophotometer (Epoch II, BioTek). A standard graph was plotted, and alcohol concentrations were calculated using a linear equation obtained from the standard curve[25].

Estimation of total polyphenolic content using Folin–Ciocalteau and Folin–Denis methods

The total polyphenolic content of the wine samples was assessed using the Folin–Ciocalteau and Folin–Denis methods with slight modifications[26]. Twenty microliters of undiluted wine samples were mixed with 100 µL of Folin–Ciocalteau or Folin–Denis reagent in a 96-well plate, followed by 80 µL of sodium bicarbonate (0.1M) after 10 min. Absorbance at 760 nm was measured after 30 min of incubation at 25°C using a plate reader (Epoch II, BioTek). Tannic acid (10-100 µg/mL) served as the reference standard. The TPC was calculated using a calibration curve and expressed as µg/mL of tannic acid equivalent.

Estimation of flavonoid content by $AlCl_3$ assay

An aluminum chloride ($AlCl_3$) assay was used to measure the flavonoid content in the wine samples[27]. In a 96-well plate, 100 µL of 2% $AlCl_3$ was mixed with 50 µL of the wine sample and kept for 30 min at 25°C. Absorbance at 420 nm was recorded using a plate reader (Epoch II, BioTek). Flavonoid content was determined using a quercetin standard curve prepared with water as a solvent (0-200 µg/mL) and expressed as µg/mL of quercetin equivalent.



Flowchart 2. Wine preparation using rhododendron flower

Estimation of antioxidant activity using the FRAP method

The FRAP assay was performed according to the reported protocol[28] with some modifications. Briefly, 150 µL of FRAP solution was mixed with 50 iL of each wine sample in a 96-well plate. A range of standard concentrations of quercetin (0-50 µg/mL) was prepared using water as the solvent. The absorbance was taken at 593 nm was measured using a plate reader (Epoch II, BioTek). The antioxidant properties of wines were calculated based on the linear equation obtained from quercetin standard curve.

Estimation of antioxidant activity using the ABTS method

The free radical scavenging activity of the wines was assessed using the ABTS method as described earlier[29]. Aqueous quercetin (50 µg/mL) served as a positive control. Absorbance was measured at 734 nm with a plate reader (Epoch II, BioTek), and the antioxidant activity was calculated based on the percentage inhibition of the ABTS radical.

Estimation of antioxidant activity using the DPPH method

The free radical scavenging ability of the wine samples was evaluated using the DPPH assay as outlined by a previous study.[30] Briefly, 50 µL of each wine sample was mixed with 150 µL of 200 µM methanolic DPPH solution and incubated in the dark at room temperature for 30 min Aqueous solution of quercetin (50 µg/mL) was used as a positive control, and methanol served as a negative control. Absorbance was taken at 517 nm using a microplate reader (Epoch II, BioTek).

Cell culture and cell viability assay

RAW 264.7 macrophages, sourced from the National Centre for Cell Science (Pune, India), were cultured at 37°C in DMEM with 10% fetal bovine serum under a 5% CO_2 atmosphere. Cell viability was assessed using an MTT assay, as described earlier[31].

Nitric oxide production

RAW 264.7 cells were cultured in 96-well plates at 5×10^5 cells/mL and incubated overnight. After the incubation, the cell supernatant was

Table 1. Physicochemical characteristics of fruit and flower wines

Characteristics	Wines			
	Grape	Cherry	Plum	Rhododendron
pH	3.6±0.06	3.5±0.04	3.4±0.1	3.4±0.05
Titratable acidity, g/L	5.2±0.02	5.7±0.02	5.6±0.03	4±0.02
Alcohol (Hydrometer) %	8±0.24	6±0.97	7±0.86	4±0.42
Alcohol (Dichromate method) %	7.2±0.51	4.7±0.42	5.4±0.09	5.8±0.3

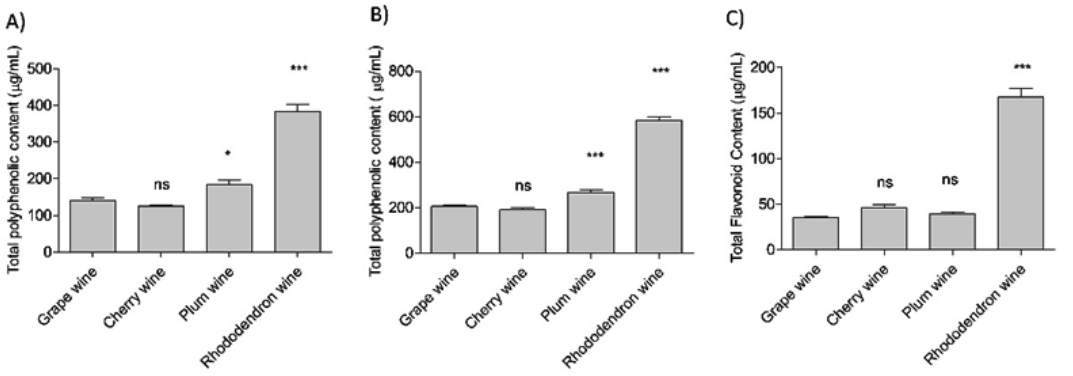


Fig. 1. *In vitro* assays for determination of total polyphenolic content and total flavonoid content of Grape, Cherry, Plum, and Rhododendron wine samples usinf a)Folin–Ciocalteau assay, b) Folin–Denis assay, and c) $AlCl_3$ assay (where, *** $P < 0.0001$; ns, not significant [on comparison with grape wine])

replaced with fresh medium containing 100 µL of 1 µg/mL LPS (prepared in DMEM medium), with or without 100 µL of wine samples, and incubated for another 24 h. Nitrite levels in the culture supernatant, indicative of NO production, were measured using the Griess reagent. Equal volumes of the culture supernatant and Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthyl ethylenediamine-HCl) were mixed and incubated for 10 min, and absorbance was measured at 540 nm. Fresh culture medium served as the blank, and nitrite concentration was determined using a sodium nitrite standard curve.

Measurement of reactive oxygen species production

For measuring reactive oxygen species (ROS), 5×10^5 cells/mL of cell suspension was seeded in a black 96-well plate. The experiment was performed as described earlier[28]. Briefly, the cells were cultured and treated with LPS, as described above. Then, the medium was replaced with 10 µM DCFDA,, and the cells were incubated for 30 min at 37 °C and 5% CO2. The medium was discarded, and the cell layer was washed with phosphate-buffered saline. Subsequently, 200 µL of serum-free medium was added to each well, and the fluorescence intensity was measured with an excitation wavelength of 485 nm and an emission wavelength of 535 nm using a spectrophotometer (iD3 SpectraMax, Molecular Devices, San Jose, CA, USA).

High-performance liquid chromatography

The wine samples were analyzed using a Eurosphere C-18 reversed-phase cartridge (dimensions: 300 mm in length and 4 mm in diameter, particle size: 5µm; KNAUER HPLC, Germany). Standard stock solutions of catechin, quercetin, and gallic acid were prepared separately in methanol. Then, the final stock solutions of the standards (10-100 µg/mL was prepared in the mobile phase which was a mixture of 28% acetonitrile and 2% aqueous acetic acid v/v. The sample injection volume was 10 µL. The polyphenols were monitored at 360 nm and identified based on their retention times. ChromGate software was used for data analysis.

Statistical analysis

All the experiments were performed at least three times in triplicate. Statistical analyses were performed using Microsoft Excel and GraphPad Prism version 5.0. Data are shown as mean ± standard deviation. One-way analysis of variance, followed by Tukey's post-hoc test, was applied to identify significant differences between means. $P<0.05$ was considered significant. Pearson's correlation coefficient (r) was used to determine correlations between different parameters.

Table 2. Total polyphenolic content and flavonoid content of the wine samples

Fruit wines	Total polyphenolic content: Folin–Ciocalteau assay (Tannic acid equivalent)	Total polyphenolic content: Folin–Denis assay (Tannic acid equivalent)	Total flavonoid content: AlCl3 (Quercetin equivalent)
Grape	141.16±7.9	205.66±3.7	35.33±1.28
Plum	183.16±12.5	267±12.51	39.41± 1.66
Cherry	124.83±2.6	190.4±8.4	46.25±3.3
Rhododendron	383.33±18.75	383.33±18.75	167.75 ±9.5

Table 3. Polyphenolic content of fruit and flower wines

Fruit wines	Catechin (mg/L)	Gallic acid (mg/L)	Quercetin (mg/L)
Grape	62.41±5	1.31±0.3	1.07±0.06
Plum	11.99±1.02	0.60±0.02	7.83±0.05
Cherry	89.58±7.2	1.63±0.2	52.88±2.2
Rhododendron	64.20±5.3	1.75±0.3	7.94±0.82

RESULTS AND DISCUSSION

Determination of physicochemical properties: Alcohol content, pH, titratable acidity

The alcohol concentration of wines was estimated using the hydrometer and dichromate method and was found to range between 4% and 8% (Table 1). The highest alcohol concentration was observed in grape wine, whereas the lowest was found in rhododendron wine. Kashyap and Deepshikha have reported an alcohol level of 6.3% in rhododendron and mahua flower wines[32]. Li et al and colleagues reported an average alcohol content of 10.9% in cherry wine[32], which is higher than the values obtained in this study. This difference may be attributed to the fact that home-brewed wines

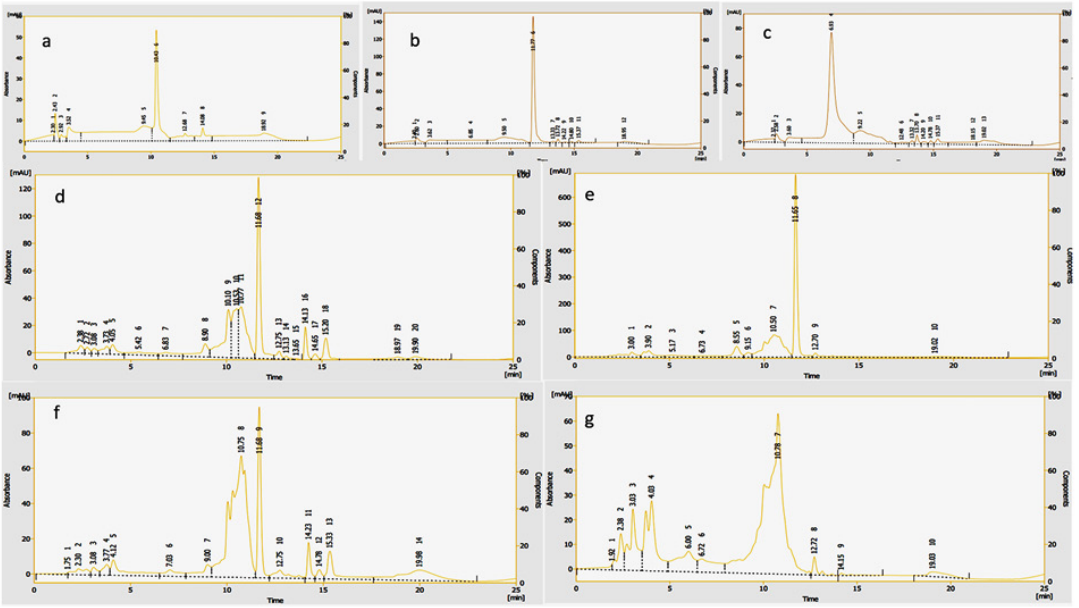


Fig. 2. High- performance liquid chromatogram of (a) standard catechin, (b) quercetin, (c) gallic acid, (d) Plum wine, (e) Cherry wine, (f) Rhododendron wine, and (g) Grape wine at 360 nm. The x-axis represents retention time and the y-axis represents absorbance (in milli absorbance unit) at 360 nm

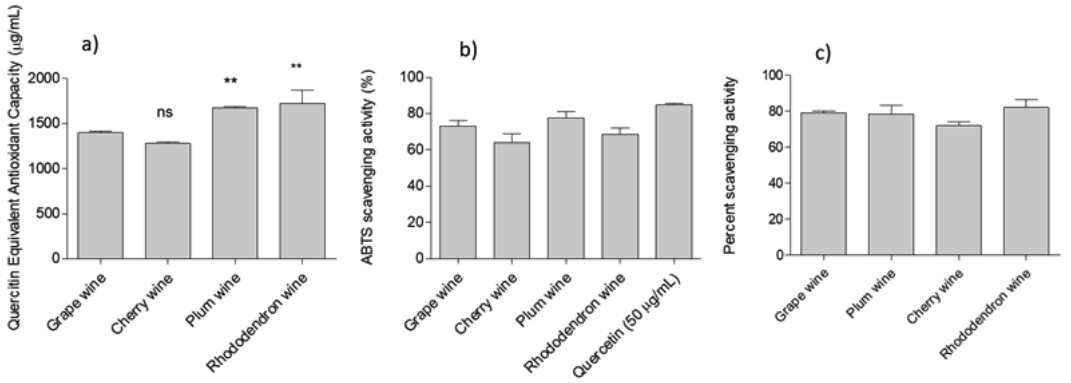


Fig. 3. *In vitro* assays for determination of antioxidant activity using a) FRAP assay, b) ABTS assay, and c) DPPH assay (*** $P < 0.001$; ns, not significant [on comparison with grape wine]) Abbreviations: FRAP, ferric ion reducing antioxidant power; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl

typically have low alcohol content. Our findings are consistent with the general range of 5-13% for home-brewed wines[29].

Titratable acidity and pH were determined for all wines and were found to be in the range of the standard values normally found in the respective fruit wines in the Indian subcontinent. The pH of the wine samples was in the range of 3.4 to 3.6 and cherry wine had the highest titratable acidity (Table 1). According to a study, the pH of red wine ranges between 3 and 4.5, which is comparable to the pH of home-brewed red wines in the present study[33].

Estimation of TPC and flavonoid content of wines

Folin–Ciocalteau and Folin–Denis both methods were utilized to estimate the polyphenol content in wines (Fig. 1a-b and Table 2). Rhododendron wine contained significantly higher (P<0.05) concentrations of polyphenols than other fruit wines, as estimated using both methods. A positive correlation was observed between the Folin–Ciocalteau and Folin–Denis methods (r=0.9938, P<0.0001), validating both methods for the estimation of TPC. The Folin–Denis method yielded higher TPC values than the Folin–Ciocalteau method. A previous study also reported similar results[25]. The composition of wine varies with respect to the compounds present, depending on the fruit type, climate, terrain, conditions of winemaking, and reactions that occur during the aging of wine, which could account for the results obtained[34]. Literature data on rhododendron wine show a polyphenol content of 790 µg/mL, which is higher than that observed in the present study[32]. TPC in cherry wine was

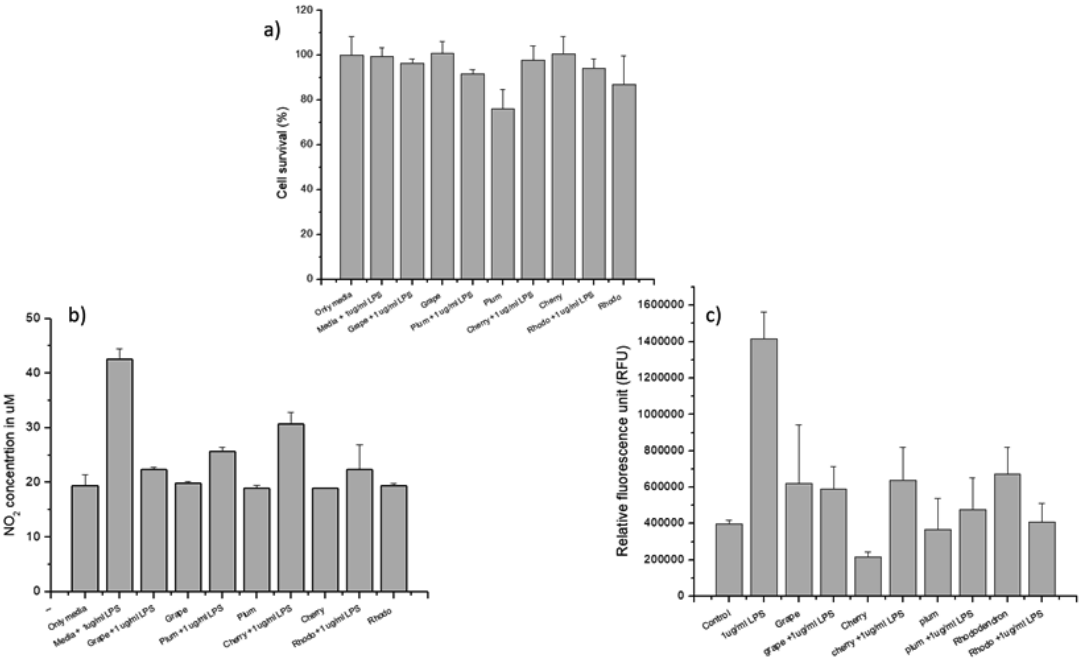


Fig. 4. *In vitro* assays using mouse macrophage cells (RAW 246.7) for the estimation of antioxidant activity

a) Effects of lipopolysaccharide (LPS) and wine samples on the viability of RAW 246.7 macrophage cells determined using the MTT assay

b) Measurement of nitrite concentration using Griess reagent: In this assay, RAW 264.7 macrophage cells were subjected to oxidative stress using 1 µg/mL LPS with or without wine samples and its stable conversion product nitrite (NO_2^-) was measured.

c) Measurement of ROS using DCFDA: Mouse macrophage cells (RAW 246.7) were treated with or without 1µg/mL LPS and wine samples for 24 h, and ROS production of the treated and untreated cells was determined using DCFDA staining. The relative fluorescence unit estimation was performed using a fluorescence plate reader (iD3 SpectraMax, Molecular Devices).

Abbreviations: LPS, lipopolysaccharide; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ROS, reactive oxygen species; DCFDA, dichlorofluorescein diacetate

lower than the previously reported value of 1940 µg/mL[14]. A previous study has indicated that plum wine contains more polyphenols then cherry wine, a result that aligns with the findings of this study[35]. The polyphenol content of wines may decrease owing to unfavorable biochemical reactions such as oxidation, degradation, formation of complexes with proteins, and precipitation with sugars present in wine[36].

The flavonoid content in wines was measured using the aluminum chloride assay (Fig. 1c). Rhododendron wine showed the highest flavonoid content (167.75±9.53 µg/mL quercetin equivalent) compared with other wines. Sometimes, fruit wine contains more flavonoids than the fruit itself because of the fermentation process[37]. Moreover, the bioavailability of the flavonoids and polyphenols are high in wines because of the presence of alcohol [38, 39].

High-performance liquid chromatography

The concentrations of catechin, quercetin, and gallic acid in plum, cherry, grape, and rhododendron wines were estimated using high-performance liquid chromatography. The chromatograms of standard catechin, quercetin, and gallic acid, with retention times of 10.43 min, 11.76 min, and 6.93 min, respectively, are presented in Figure 2 (Fig. 2a–c). Standard curves were generated using known concentrations of each polyphenol and their corresponding area under the curve (AUC) values. Similarly, plum, cherry, rhododendron, and grape wine samples were analyzed, and the concentrations of the selected polyphenols were determined based on the standard curves (Fig. 2d–g, Table 3). The concentrations of catechin and quercetin present in the grape wine samples were within the range reported previously[40, 41, 42]. However, only a few reports are available on plum, cherry, and rhododendron wine samples. To our knowledge, this study is the first to quantify its catechin, quercetin, and gallic acid concentrations of rhododendron wine.

Estimation of antioxidant activity using FRAP, ABTS, and DPPH methods

The antioxidant capacity of the wine samples was assessed using the FRAP assay, revealing that Rhododendron wine had the highest antioxidant capacity (1723.8±143.19 µg/mL quercetin equivalent) (Fig. 3a). Both Rhododendron and plum wines exhibited significantly higher antioxidant capacities (P<0.05) than grape wine in the FRAP assay. Additional evaluations using the ABTS and DPPH assays confirmed that all four wines demonstrated robust free-radical scavenging activity (Fig. 3b and 3c), though no significant differences were found among them. Specifically, plum wine had the highest ABTS activity (77.5±3.64%), while Rhododendron wine showed the greatest DPPH scavenging ability (82.16±7.38%). These variations are likely attributed to differences in phenolic and flavonoid compounds, which significantly impact antioxidant capacity. For example, the antioxidant properties of flavonoids are influenced by factors such as the presence of hydroxyl groups, their hydrophobicity, and molecular planarity[43, 44]. Previous studies have reported varying antioxidant capacities for different wines. For example, cherry wine was found to have a high antioxidant capacity, and a further increase in TPC (2.73 g gallic acid equivalent/L) and antioxidant activity (22.07 mM Trolox equivalent/L) after adding green tea to it[44]. Kashyap and Deepshikha reported the antioxidant capacities of rhododendron wines[32]. Similarly, plum wine reported to have a phenolic content of 469 ± 7 mg/L gallic acid equivalents and a total antioxidant activity of 304.36±6.24 µg/L (Trolox equivalents)[45]. Correlations between the FRAP, ABTS, Folin–Ciocalteu, Folin–Denis, and AlCl3 assays were positive in the present study (i.e., FRAP-ABTS: r=0.52; Folin–Ciocalteau-FRAP: r=0.78). The literature reveals diverse results regarding the relationship between antioxidant capacity and phenolic or flavonoid contents of wine. Some studies indicate a linear correlation between antioxidant capacity and TPC[45], while others suggest that antioxidant capacity is closely related to specific flavonoid fractions. The antioxidant activity of these compounds relies on their proton-donating capacity and the number of hydroxyl groups, with glycosylation also affecting antioxidant potency[46].

Determination of antioxidant potential using RAW 264.7 cells

The antioxidant potential was assessed by measuring the reactive nitrogen species (RNS) and ROS in mouse macrophage cells (RAW 264.7) by inducing oxidative stress with LPS (1 µg/mL). LPS, which is predominantly found in the outer cell wall of gram-negative bacteria,

triggers an inflammatory response in the host, leading to elevated production of ROS/RNS and other proinflammatory mediators[47]. Macrophages exposed to 1 µg/mL LPS triggered ROS and RNS without inducing cytotoxicity. The cell viability was determined in the presence and absence of LPS and wine samples in macrophage cells using MTT assay (Fig. 4a). More than 86% cell viability was observed for all wine samples, except for plum wine, where the cell viability was relatively low (76%). The ability of wine samples to prevent RNS/ROS generation is shown in Fig. 4b-c. Wine samples, particularly grape and plum wines, significantly reduced nitrite concentration compared with samples treated with LPS only. None of the four wine samples showed significant RNS production in RAW 264.7 cells compared with the media control. LPS (1 µg/mL) induced a high level of ROS production in RAW 264.7 cells (1,415,300±147,303 RFU), which was significantly reduced by all four wine samples (ranging from 406,965 to 635,281 RFU). These results indicate that plum, cherry, and rhododendron wines demonstrate ROS/RNS scavenging potential similar to grape wine, without significant cytotoxic effects on macrophage cells.

CONCLUSIONS

In the present study, we performed a comparative analysis between traditional grape wine and the conventionally less-explored cherry, plum, and rhododendron wines. Our study showed that rhododendron wine possesses greater antioxidant activity than grape wine, using various *in vitro* assays. However, plum, cherry, and rhododendron wines showed significant antioxidant potential in macrophage cells treated with LPS compared with grape wine. Further investigation is needed to quantify additional individual bioactive compounds in wines and to elucidate the health benefits of wine polyphenols using a mouse model

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