

**PHYTOCHEMICAL PROFILE AND ANTIOXIDANT ACTIVITY OF *RHODODENDRON*
ARBOREUM FLOWER VINEGAR AND ITS BASIL-INFUSED VARIANT**

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ABSTRACT

Background: *Rhododendron arboreum* flowers are rich in phenolics, flavonoids, and anthocyanins and are traditionally valued for their antioxidant and medicinal properties. Acetic fermentation may further enhance their bioactive potential and functional food value.

Objective: To develop vinegar from *R. arboreum* flowers and a basil-infused variant, and to evaluate their phytochemical composition and antioxidant activity.

Methods: Vinegar was produced by fermenting cold-pressed *R. arboreum* flower juice using *Saccharomyces cerevisiae* followed by *Acetobacter aceti*. A portion was infused with basil. Qualitative phytochemical screening was performed, and total phenolic content (TPC), total flavonoid content (TFC), β -carotene, quercetin, rutin, and L-ascorbic acid were quantified using spectrophotometric and HPLC methods. Antioxidant activity was assessed by the DPPH assay, and data were statistically analyzed.

Results: Both vinegars contained diverse phytochemicals. Basil infusion significantly increased TPC (384 ± 11.85 mg GAE/100 mL) and TFC (102 ± 7.21 mg QE/100 mL) compared with plain vinegar (357.53 ± 7.80 mg GAE/100 mL and 68.50 ± 8.17 mg QE/100 mL, respectively). β -Carotene showed a non-significant increase following basil infusion, while L-ascorbic acid remained comparable between samples. In contrast, plain vinegar contained significantly higher rutin and quercetin levels. MANOVA and ANOVA confirmed a significant effect of infusion type on antioxidant profiles, primarily driven by basil addition.

Conclusion: Basil infusion enhanced total phenolics, flavonoids, and antioxidant activity, whereas plain *R. arboreum* vinegar retained higher levels of rutin and quercetin, highlighting the complementary functional value of both formulations.

Keywords: *Acetobacter aceti*, rutin, quercetin, flavonoids, β -carotene

INTRODUCTION

Rhododendron arboreum (Ericaceae), widely distributed in the Indian Himalayan region, is valued for its nutritional and therapeutic properties. Its flowers and leaves are rich in bioactive secondary metabolites, including flavonoids, tannins, saponins, glycosides, and phenolic compounds, which contribute to strong antioxidant activity. Traditionally, *R. arboreum* flowers have been used in herbal beverages and medicinal preparations to manage ailments such as fever, asthma, cardiovascular disorders, and liver dysfunction. Previous studies have

demonstrated high polyphenol and flavonoid content and potent free-radical scavenging activity in *R. arboreum* extracts, supporting its potential as a functional food or nutraceutical (Ahmad et al., 2022; Kur et al., 2023). Basil (*Ocimum basilicum*) is an aromatic herb widely recognized for its therapeutic and culinary applications. It contains abundant phenolics, flavonoids, and carotenoids, including β -carotene, and has been reported to enhance antioxidant defense, immune response, and cardiovascular health. Beyond flavour enhancement, basil incorporation can improve the nutritional and functional quality of food and beverage products.

Vinegar is produced through a two-stage fermentation process involving alcoholic fermentation by *Saccharomyces* species followed by acetic acid formation by *Acetobacter* species. Depending on the substrate, vinegars may be derived from fruits, grains, or other carbohydrate-rich materials and are valued for their preservative, culinary, and health-promoting properties (Ezemba et al., 2021; Bisht and Sharma, 2023). Recent studies on fruit- and herb-infused vinegars have shown that acetic fermentation and botanical infusion can enhance phenolic content and antioxidant activity; however, research has largely focused on common fruits such as grapes and apples.

Although *Rhododendron arboreum* has been widely investigated in the form of solvent extracts and non-fermented beverages, vinegar produced directly from its flowers has not yet been documented. Furthermore, basil-infused floral vinegars derived from Himalayan edible flowers remain unexplored. To address these gaps, the present study developed plain *R. arboreum* flower vinegar and a basil-infused variant using a two-stage fermentation process involving yeast-mediated alcoholic fermentation followed by acetic acid bacterial oxidation. The phytochemical profiles of both vinegars were comparatively evaluated in terms of total phenolic content, total flavonoid content, L-ascorbic acid, β -carotene, quercetin, and rutin. In addition, their in vitro antioxidant potential was assessed using DPPH radical scavenging activity and IC_{50} determination to elucidate the relationship between bioactive composition and antioxidant efficacy. It was hypothesized that *R. arboreum* flower vinegar would exhibit antioxidant potential comparable to conventional fruit vinegars and that basil infusion would further enhance phenolic and flavonoid content, modulate specific bioactive compounds, and improve radical-scavenging capacity.

MATERIALS AND METHODS

Procurement of raw material: Fresh flowers of *Rhododendron arboreum* Sm. var. *arboreum* (Ericaceae) were collected during peak flowering (March–April 2024) from Rudraprayag district, Uttarakhand, India, while fresh basil (*Ocimum basilicum* L., Lamiaceae) leaves were procured from a local market in Jaipur, Rajasthan. Plant materials were washed thoroughly with distilled water to remove dirt and contaminants. Fully expanded, mature, non-senescent rhododendron flowers and healthy basil leaves without visible damage were selected. Taxonomic identification was carried out by Dr. Parul Sharma, Assistant Professor, Department of Food Science and Nutrition, Banasthali Vidyapith.

Rhododendron flower juice was extracted by cold pressing to preserve thermosensitive phytochemicals. The juice was adjusted to 10 °Brix using sucrose and subjected to alcoholic fermentation with *Saccharomyces cerevisiae* at 28–30 °C for 7 days until residual soluble solids decreased to ≤ 1 °Brix. This was followed by acetic fermentation using *Acetobacter aceti* at 30 °C for 21 days, terminated when titratable acidity reached $\geq 4.0\%$ (as acetic acid) and ethanol content dropped below 0.5% (v/v). All fermentations were conducted in duplicate.

After fermentation, vinegar was clarified, bottled, and pasteurized at 75–80 °C for 30–40 min. For the basil-infused treatment, fresh basil leaves (8% w/v) were added to the finished rhododendron vinegar and infused at room temperature for 48–72 h before removal of leaves and pasteurization. Both plain and basil-infused vinegars were subsequently analyzed for phytochemical composition, bioactive compounds, and antioxidant activity.

Aqueous extract preparation: Antioxidant extracts were prepared from the final vinegar products (plain *Rhododendron arboreum* vinegar and basil-infused Rhododendron vinegar), not from dried flowers. Each vinegar sample was mixed with 80% (v/v) ethanol at a ratio of 1:4 (vinegar: solvent) and shaken on an orbital shaker for 2 h at ambient temperature (≈ 25 °C), then filtered. The residue was re-extracted twice under the same conditions and all supernatants were combined; the ethanol was removed under reduced pressure at ≤ 40 °C, and the extract was dried to constant weight. The dried extract was redissolved in methanol to obtain a stock solution (e.g. 10–50 mg/mL), filtered through a 0.45 μm syringe filter, and stored at -20 °C until analysis of TPC, TFC and DPPH.

Qualitative screening of phytochemicals: Qualitative phytochemical screening of *R. arboreum* revealed the presence of tannins, saponins, flavonoids, alkaloids, glycosides, steroids, and terpenoids, with variations depending on extraction yield. Polar solvents such as methanol and

ethanol were most effective in extracting bioactive compounds, yielding higher levels of phenolics, flavonoids, tannins, terpenoids, and glycosides, indicating the plant's pharmacological potential. Phytochemical analyses were performed using standard qualitative tests, including ferric chloride (tannins), froth test (saponins), Dragendorff's (alkaloids), Shinoda (flavonoids), Legal (glycosides), Salkowski (steroids), and Liebermann–Burchard (terpenoids) methods (Vidra and Németh, 2018).

Determination of total phenolic content: Reaction conditions and wavelength: Total phenolic content (TPC) of vinegar was determined by the Folin–Ciocalteu method with minor modifications. Appropriately diluted vinegar extract (e.g. 0.5 mL) was mixed with 2.5 mL Folin–Ciocalteu reagent (diluted 1:10, v/v with water), allowed to react for 5 min, then 2.0 mL of 7.5% (w/v) Na_2CO_3 was added. After incubation for 30 min at room temperature in the dark, absorbance was read at 765 nm in a 1 cm path-length quartz cuvette using a UV–Vis spectrophotometer.

Calibration range and regression: A gallic acid calibration curve (0–500 mg/L) was prepared (e.g. standards at 0, 50, 100, 150, 250, and 500 mg/L), and the regression equation was $y = ax + b$ (e.g. $y = 0.0012x + 0.010$), with coefficient of determination $R^2 \geq 0.99$.

Units and expression of results: TPC was expressed as mg gallic acid equivalents (GAE) per 100 mL vinegar sample (mg GAE/100 mL), using the calibration curve and accounting for all dilution factors. Measurements were performed in 1 cm quartz cuvettes in standard 1 mL cells (Gul et al., 2017).

Determination of total flavonoids: Total flavonoid content (TFC) was determined using the NaNO_2 – $\text{Al}(\text{NO}_3)_3$ – NaOH colorimetric method with quercetin as the standard, following Deng et al. (2013) with minor modifications. Briefly, diluted vinegar extract (1.0 mL) was mixed with distilled water and sequentially reacted with 5% NaNO_2 , 10% $\text{Al}(\text{NO}_3)_3$, and 1 M NaOH . The final volume was adjusted to 10 mL, and absorbance was measured at 510 nm using a UV–Vis spectrophotometer. A quercetin calibration curve (0–100 mg/L) showed excellent linearity ($R^2 \geq 0.99$). TFC was expressed as mg quercetin equivalents per 100 mL vinegar (mg QE/100 mL), corrected for dilution.

Determination of quercetin and rutin: Ethanolic extracts of vinegar samples were filtered through 0.45 μm PTFE syringe filters and analyzed for quercetin and rutin by HPLC–UV following Li et al. (2015) with minor modifications. Separation was achieved on a C18 reversed-phase column using an acidified aqueous solvent and acetonitrile/methanol under isocratic conditions at a flow rate of 1.0 mL/min, with detection at 360–370 nm. Identification was based on retention time and UV spectral

matching with authentic standards, and quantification was performed using external calibration curves (5–50 µg/mL; $R^2 \geq 0.999$). Results were expressed as mg/100 mL vinegar after dilution correction.

Estimation of β -carotene: Vinegar samples were extracted using an organic solvent mixture containing BHT to prevent carotenoid oxidation, and the organic phase was dried under nitrogen, reconstituted in the mobile phase, and filtered (0.45 µm PTFE) prior to analysis. β -Carotene was quantified by RP-HPLC using a C18 column at 30 °C with detection at 450 nm. Separation was achieved using an acetonitrile–methanol–dichloromethane (or ethyl acetate) mobile phase under optimized isocratic or gradient conditions. Quantification was performed using external calibration curves (0.5–10 µg/mL; $R^2 \geq 0.999$), and results were expressed as µg/mL vinegar after dilution correction. β -Carotene concentration was additionally verified spectrophotometrically at 450 nm using a standard curve in hexane (Pandey et al., 2020).

Plain and basil-infused *Rhododendron* vinegars were analyzed in three independent fermentation batches, with each batch measured in triplicate. Data are reported as means of three biological replicates ($n = 3$).

L-ascorbic determination: Vinegar samples were stabilized in 3% metaphosphoric acid and filtered prior to analysis. L-ascorbic acid was quantified by redox titration with standardized 2,6-dichlorophenolindophenol (DCPIP), following Wu et al. (2008) with minor modifications. Sample aliquots were titrated to a persistent light pink endpoint, and calibration curves were constructed using standard ascorbic acid solutions (2–20 mg/100 mL; $R^2 \geq 0.99$). L-ascorbic acid content was calculated from the calibration curve, corrected for dilution, and expressed as mg/100 mL vinegar.

DPPH scavenging activity: The antioxidant activity of vinegar samples was evaluated using the DPPH radical scavenging assay. A 0.1 mM DPPH solution was prepared in methanol and kept in the dark. Sample or standard solutions were mixed 1:1 (v/v) with DPPH solution and incubated at room temperature (≈ 25 °C) for 30 min in the dark, after which absorbance was measured at 517 nm with a UV–Vis spectrophotometer. All measurements were carried out in 1 cm path-length quartz cuvettes (standard 1 mL cuvettes). Vinegar extracts were tested at a series of concentrations (e.g. 10–50 µg/mL) prepared in methanol. For each concentration, the percentage of DPPH inhibition was calculated as:

$$\% \text{Inhibition} = \frac{A_{\text{control}} - (A_{\text{sample}} - A_{\text{sample blank}})}{A_{\text{control}}} \times 100$$

A plot of % inhibition versus sample concentration was constructed and fitted by non-linear regression (or linear regression in the linear range) to estimate the concentration giving 50% inhibition (IC_{50} , µg/mL) (Dinesh et al., 2015).

Experimental Design

The study followed a completely randomized design with two treatment groups (plain and basil-infused vinegar). Vinegar production was performed in three independent fermentation batches per treatment. All analytical determinations were carried out in triplicate, and results are reported as mean $\pm$ standard deviation (SD). Batch effects were considered during statistical evaluation.

Statistical Analysis: To compare bioactive components between plain *Rhododendron* vinegar (R) and basil-infused *Rhododendron* vinegar (B), multivariate analysis of variance (MANOVA) was first performed using SPSS 16.0. Univariate normality (Shapiro–Wilk test, Q–Q plots), homogeneity of variances (Levene’s test), and covariance matrices (Box’s M test) were assessed, and multicollinearity was checked via Pearson correlations. With balanced groups and no major violations, data were analyzed without transformation. MANOVA tested the overall effect of vinegar type on total phenolic content (TPC), total flavonoid content (TFC), quercetin, rutin, β -carotene, L-ascorbic acid, and DPPH IC₅₀. Following a significant multivariate effect, separate one-way ANOVAs were conducted for each variable. For DPPH IC₅₀, three groups (standard antioxidant, R, B) were compared using ANOVA followed by Fisher’s LSD or Tukey’s test. ANOVA results (F values, significance codes) are reported in Tables 2 and 3, with means considered significantly different at $p < 0.05$.

RESULT AND DISCUSSION

Qualitative screening of phytochemicals:

Table1. Qualitative phytochemical analysis of *Rhododendron* vinegar and basil-infused *Rhododendron* vinegar

Phytochemicals	R	B
Tannins	+++	++
Saponins	–	–
Flavonoids	–	–
Alkaloids	–	–
Glycosides	+	++
Steroids	++	–
Terpenoids	+	++

+: low in abundance; ++: moderate in abundance; +++: high in abundance; -: absent. (R- *Rhododendron arboreum* vinegar; B- Basil infused *Rhododendron arboreum* vinegar)

Naturally occurring compounds found in plants are called phytochemicals or phytonutrients. These compounds have been shown to exhibit antioxidant activity and to be good for human health. Phytochemicals may have anti-inflammatory and antioxidant properties. It is essential for the body's detoxification of toxic and damaging substances.

According to the findings of the qualitative screening of phytochemicals, Rhododendron vinegar had a positive reaction from tannins, glycosides, steroids, and terpenoid but a negative response from saponins, flavonoids, and alkaloids (Table 1). On the other hand, basil-infused Rhododendron vinegar has tannins, glycosides, and terpenoids, but lacks saponins, flavonoids, alkaloids, and steroids. These antioxidants lessen metal chelation and oxidative damage brought on by free radicals. By halting the growth of cancer cells, saponins may have antitumor and antimutagenic qualities and lower the risk of cancer in people, according to research. The antioxidant activity and phytochemical characteristics of vinegar derived from pineapple fruit and peel were examined. The study found that terpenoids, flavonoids, tannins, quinones, and saponins are present in both pineapple peel and fruit vinegars (Haririan et al., 2023).

Bioactive compounds in vinegar:

Table 2. Quantitative analysis of Bioactive compounds (Mean $\pm$ SD)

Compounds	R (Mean $\pm$ SD)	B (Mean $\pm$ SD)	Significance
TPC (mg GAE /100ml)	357.53 $\pm$ 7.80 ^a	384.80 $\pm$ 11.85 ^a	*
TFC (mg QE/100 mL)	68.50 $\pm$ 8.17 ^b	102.00 $\pm$ 7.21 ^b	**
Quercetin (mg/100ml)	1.77 $\pm$ 0.35 ^c	0.73 $\pm$ 0.25 ^d	*
Rutin (mg/100ml)	2.80 $\pm$ 0.56 ^d	0.14 $\pm$ 0.02 ^e	***
β -carotene (mg/100ml)	0.12 $\pm$ 0.13 ^f	0.61 $\pm$ 0.29 ^d	NS
L-Ascorbic acid (mg/100ml)	10.91 $\pm$ 1.75 ^c	8.94 $\pm$ 1.52 ^c	NS

Values are presented as mean $\pm$ standard deviation ($n = 3$). Mean values within the same column followed by different superscript letters differ significantly according to Duncan's Multiple Range Test ($p < 0.05$). TPC = Total Phenolic Content; TFC = Total Flavonoid Content. Significance levels: * $p < .05$, ** $p < .01$, *** $p < .001$, NS = not significant.

Total phenolic content: Statistical analysis using Duncan's Multiple Range Test (DMRT) revealed a significant difference ($p < 0.05$) in total phenolic content between samples R and B, as indicated by different superscript letters. This confirms that the observed increase in TPC in sample B is statistically meaningful and not due to random variation. Polyphenols are the main bioactive components and antioxidant molecules found in vinegars, and they have several positive health effects (Fouda-Mbanga and Tywabi-Ngeva, 2022). This study used the straightforward, dependable, and repeatable Folin–Ciocalteu method to determine the total phenolic content of vinegars. The TPC of R and B lies within/above the range reported for apple and grape vinegars 84.2 mg GAE/100 mL, 102.51 mg GAE/100 mL for grape vinegar, 45.9 mg GAE/100 mL, 52.12 mg GAE/100 mL, 158.37 mg GAE/100 mL, as well as between 73.45 and 111.06 mg GAE/100 mL, 269 mg GAE/100 mL, and 98.80 mg GAE/100 mL for apple vinegar; (Bakir et al., 2016; Kara et al., 2022; Guzel, 2024) .

(110.35 mg GAE/100 mL and 760 mg GAE/100 mL), and is comparable to rosehip or berry vinegars, indicating that *Rhododendron* flowers are a polyphenol-rich substrate (M. Guzel, 2024). The increase in TPC in Sample B likely results from the extraction of additional basil-derived phenolics (such as rosmarinic acid, caffeic acid, chicoric acid, and other polyphenols) during the 48–72 h infusion. The application of DMRT further validates the influence of basil infusion on enhancing phenolic concentration in the vinegar matrix.

Total flavonoids: Statistical analysis using Duncan's Multiple Range Test (DMRT) showed a highly significant difference ($p < 0.01$) in total flavonoid content between *Rhododendron* vinegar (R) and basil-infused *Rhododendron* vinegar (B), as indicated by different superscript letters in Table 2. The total flavonoid concentration of the vinegars ranged from 68.50 ± 8.17 to 102.00 ± 7.21 mg QE/100 mL of basil-infused *Rhododendron* vinegar (B) and *Rhododendron* vinegar (R), respectively. When compared to *Rhododendron* vinegar, sample B exhibited the highest flavonoid content (Table 2). Due to the additional flavonoids that basil contributes and potential synergistic interactions during infusion, the flavonoid content of vinegar infused with basil may have increased. In contrast, oxidative deterioration, extended fermentation, heat exposure, or enzymatic breakdown during processing can all lead

to reductions in the flavonoid concentration of various vinegars. Variations in fruit type, age, and processing conditions can also lead to variation. In studies, flavonoid contents were reported as 24.45 mg QE/100 mL (M. Guzel, 2024), 2.03 mg QE/100 mL (Budak et al., 2022), between 79 and 153 mg CE/100 mg (M. Guzel, 2024), 29.8 mg CE/100mL (Yun et al., 2016) and 22.18 mg CE/100mL of grape vinegars, as between 1.87 and 13.10 mg QE/100 mL, 0.3 mg QE/100 mL, between 42 and 240 mg CE/100 mg) and 17.48 mg CE/100mL of apple vinegars (M. Guzel, 2024). According to the literature research, vinegars vary greatly in their overall phenolic content. Bioactive substances like flavonoids and phenolics are heavily reliant on the raw materials as well as the type of acetic acid bacteria and yeast that cause fermentation (Kara et al., 2022).

Quercetin and rutin: Statistical analysis using Duncan's Multiple Range Test (DMRT) indicated a significant difference in quercetin content ($p < 0.05$) and a highly significant difference in rutin content ($p < 0.001$) between *Rhododendron* vinegar (R) and basil-infused *Rhododendron* vinegar (B), as denoted by different superscript letters in Table 2. *Rhododendron* vinegar (R) had the highest quercetin levels, whereas basil-infused *Rhododendron* vinegar (B) had the lowest (Table 2). The third most prevalent flavonol, rutin, was found in significant amounts in the vinegar sample 2.80 ± 0.56 mg/100ml in R and 0.14 ± 0.02 mg/100ml in B. A reduction in quercetin and rutin in vinegars can be brought on by oxidation, exposure to heat or light, extended fermentation, or enzymatic breakdown. Lower or higher observed concentrations may occasionally be the consequence of interactions between chemicals during infusion and processing, variations in the makeup of the raw materials, or the addition of additional constituents. The reduction of quercetin and rutin is most plausibly explained by a combination of degradation and matrix interaction during infusion. Extended exposure to oxygen, light, and mildly acidic conditions can promote hydrolysis of rutin into quercetin followed by oxidative degradation of quercetin itself. Basil also contains polyphenol oxidase and peroxidase enzymes, which may accelerate oxidation of flavonols during the prolonged infusion step.

β -carotene: Statistical analysis using Duncan's Multiple Range Test (DMRT) revealed no significant difference ($p > 0.05$) in β -carotene content between *Rhododendron* vinegar (R) and basil-infused *Rhododendron* vinegar (B), as indicated by identical superscript letters in Table 2. Statistical analysis using Duncan's Multiple Range Test (DMRT) revealed no significant difference ($p > 0.05$) in β -carotene content between *Rhododendron* vinegar (R)

and basil-infused *Rhododendron* vinegar (B), as indicated by identical superscript letters in Table 2. Basil infusion tended to increase β -carotene levels, although the difference compared with R was not statistically significant at $p < 0.05$. Orange juice samples had 0.053–0.069 mg/100ml of β -carotene, apple juice had 0.014–0.023 mg/100ml, and vitamin-supplemented drinks had 0.13–0.19 mg/100 ml (Kelebek et al., 2017). Carotenoids are typically degraded by oxidative and enzymatic processes during fermentation. On the other hand, carotenoids from basil may contribute to the greater β -carotene level in vinegar infused with basil, and interactions with other bioactive ingredients during infusion may stabilize these molecules. Differences in the content of raw materials, processing conditions, exposure to heat and light, and the degree of fermentation can also cause variations in β -carotene levels.

L-ascorbic acid: Statistical analysis using Duncan's Multiple Range Test (DMRT) indicated no significant difference ($p > 0.05$) in L-ascorbic acid content between *Rhododendron* vinegar (R) and basil-infused *Rhododendron* vinegar (B), as evidenced by the same superscript letters in Table 2. In *Rhododendron* vinegar (R), the L-ascorbic concentration and in basil-infused *Rhododendron* vinegar (B) is stated as in Table 2. The R sample outperformed the B sample in terms of value. The amount of ascorbic acid in black currant vinegar was 104.17 ± 0.02 mg/100 mL, about double that of red currant vinegar (56.2 ± 0.03 mg 100 mL⁻¹) and gooseberry vinegar (48.9 ± 0.01 mg 100 mL⁻¹). Scientists discovered that apple vinegar had 10.89 mg of vitamin C per 100 mL⁻¹. They also noted that throughout the acidic fermentation process, *Acetobacter aceti* strains create more ascorbic acid than the amount normally present in the substrate (Aamer and El-Kholy, 2017). In certain samples, like B, the reduction in L-ascorbic acid may be due to heat during processing, oxidation, lengthy fermentation, or light exposure. The utilization of vitamin-rich raw materials, effective fermentation by particular microbial strains, or stabilization by other bioactive chemicals, on the other hand, may be the cause of increased amounts in some samples. The values in R and B are in the same order as apple vinegar and lower than berry vinegars, and that oxidation during fermentation and pasteurization likely causes partial vitamin C loss, explaining the modest levels and non-significant difference between treatments.

When vinegar samples R and B were compared for bioactive chemicals, there were notable variations in TPC ($p = 0.029$), TFC ($p = 0.006$), Quercetin ($p = 0.014$), and Rutin ($p = 0.001$). Sample R had higher amounts of Quercetin and Rutin, whereas sample B had higher levels of TPC and TFC. While there was no significant difference in L-ascorbic acid levels ($p = 0.214$), sample B had greater β -carotene levels, indicating a non-significant difference ($p =$

0.057). These results suggest that the main cause of the variation between the two vinegar varieties is polyphenolic chemicals, although the levels of carotenoids and L-ascorbic acid are comparatively constant.

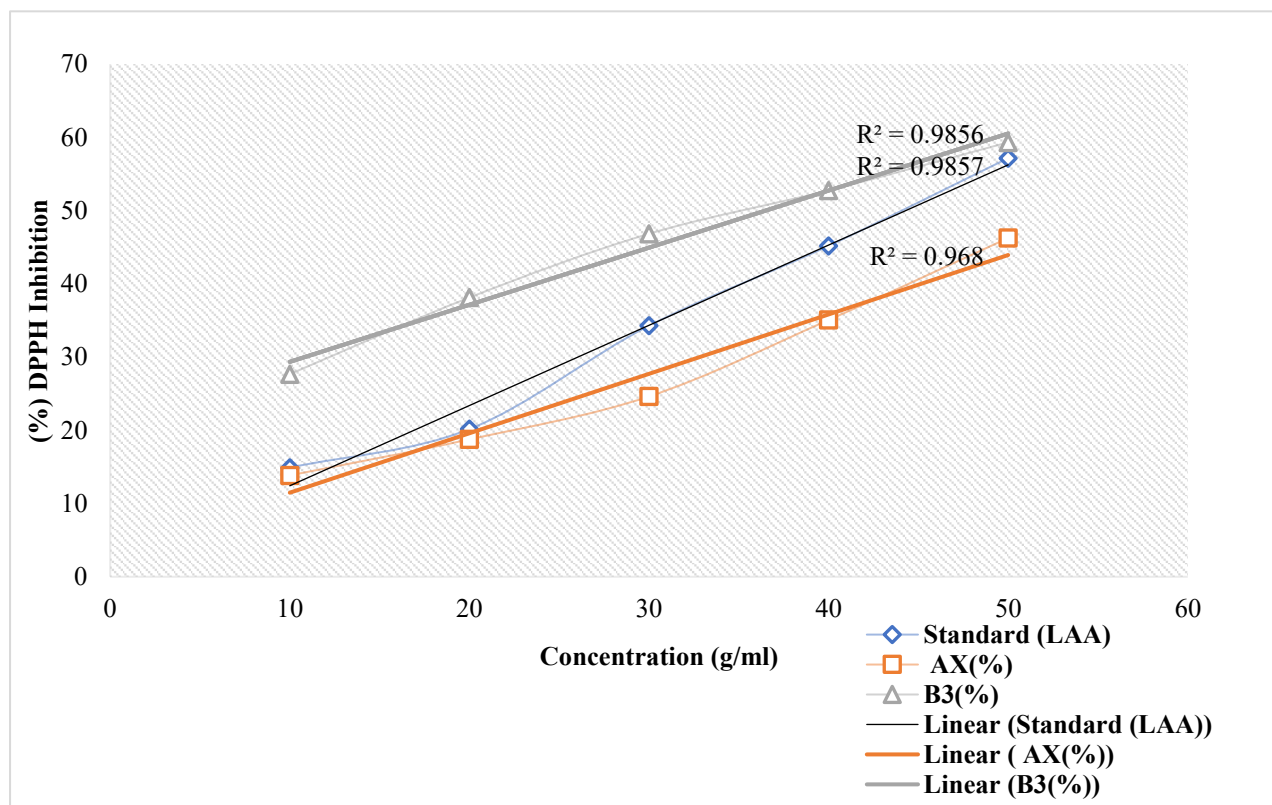


Fig. 1: DPPH radical scavenging activity of vinegar

Fig.1. DPPH radical-scavenging activity of standard antioxidant (Std), Rhododendron vinegar (R) and basil-infused Rhododendron vinegar (B). Data are means $\pm$ SD ($n = 3$ independent batches). Curves show % DPPH inhibition plotted against extract concentration ($\mu\text{g/mL}$), from which IC_{50} values were derived by regression. Linear regression lines were fitted to the linear portion of each curve to calculate IC_{50} values. The coefficient of determination (R^2) for each regression was 0.9857 for the standard (LAA), 0.968 for AX, and 0.9856 for B3, indicating a strong linear relationship between concentration and percentage inhibition.

Table 3. IC₅₀ percentage inhibition value of standard and vinegar samples

Sample	IC ₅₀ value (µg/ml)
Standard	44.47±0.93 ^b
R	58.29±0.40 ^c
B	36.19±0.51 ^a

(R= *R. arboreum* vinegar, B= Basil infused *R. arboreum* vinegar) IC₅₀ values were calculated by linear regression of DPPH inhibition (%) versus concentration, with lower IC₅₀ values indicating higher antioxidant potency. Ascorbic acid was used as the reference standard. Values are expressed as mean ± SD of three independent batches (n = 3). Different superscript letters within the column indicate significant differences as determined by one-way ANOVA followed by Duncan's Multiple Range Test (DMRT) at $p < 0.05$.

One-way analysis of variance (ANOVA) revealed a significant difference in IC₅₀ values among the standard and vinegar samples ($p < 0.05$). Following ANOVA, Duncan's Multiple Range Test (DMRT) was applied for mean separation, which showed that all samples differed significantly from each other. Different superscript letters in Table 3 indicate statistically significant differences, with lower IC₅₀ values corresponding to higher antioxidant activity. Among the samples, basil-infused vinegar (B) exhibited the lowest IC₅₀, indicating the strongest radical scavenging activity, followed by ascorbic acid and plain *Rhododendron* vinegar. The enhanced activity of B likely reflects synergistic effects between *Rhododendron* phenolics and basil-derived compounds, consistent with its higher total phenolic and flavonoid contents. These findings align with previous reports showing that antioxidant capacity in vinegars varies with phenolic composition and bioactive compounds (Davalos et al., 2005; Ozdemir et al., 2022).

CONCLUSION

Rhododendron arboreum flower vinegar and its basil-infused variant were successfully produced by a two-stage yeast–acetic acid bacteria fermentation and characterized for key phytochemicals and in vitro DPPH radical-scavenging activity. Basil infusion significantly increased total phenolic and flavonoid contents and was associated with a lower DPPH IC₅₀ compared with plain *Rhododendron* vinegar, whereas the non-infused vinegar retained higher levels of quercetin and rutin, indicating that herb infusion modifies both the quantity and profile of measurable bioactive compounds in these vinegars. The observed phenolic contents

and antioxidant capacities are comparable to or higher than those reported for several fruit vinegars, suggesting that Rhododendron flower vinegar, particularly with basil infusion, has potential as an antioxidant-rich beverage within the limits of in vitro assays only. Further studies on storage stability, sensory acceptability, bioaccessibility and in vivo effects are needed before any claims regarding health benefits, nutraceutical applications or clinical efficacy can be made.

Taken together, these in vitro data indicate that Rhododendron flower vinegar, particularly when infused with basil, may be considered a promising candidate for development as an antioxidant-rich functional beverage; however, comprehensive studies on compound bioavailability, product safety, sensory properties and in vivo biological effects are still required before any nutraceutical positioning or explicit health claims can be justified.

CONFLICT OF INTEREST:

The authors declare no conflicts of interest.

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