

POST-FERMENTATION BOTANICAL ENRICHMENT OF SHIRAZ WINE WITH SELECTED LEAF EXTRACTS: QUALITY CHARACTERISTICS AND STORAGE STABILITY

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ABSTRACT

Post-fermentation botanical infusion provides a strategy for modifying wine composition without altering primary vinification. This study evaluated Shiraz wine infused after fermentation with 70% (v/v) ethanolic leaf extracts of drumstick (*Moringa oleifera*), elephant foot leaf (*Elephantopus scaber*) and betel (*Piper betle* L.) at 5, 10 and 15 mL per 100 mL formulation. Ten formulations, including an uninfused control, were evaluated in a completely randomized design with three replications for physicochemical, antioxidant, phenolic, mineral, volatile, and sensory characteristics. The 15 mL drumstick formulation (T1) recorded the highest total phenols (165.40 mg GAE/100 mL), ferric-reducing antioxidant activity (286.45 mg AAE/100 mL), calcium (12.40 mg/100 mL), magnesium (9.35 mg/100 mL), zinc (0.42 mg/100 mL), copper (2.58 mg/L), and iron (9.20 mg/L). The highest initial sensory score was obtained for the 5 mL betel formulation (T9; 17.65/20). In a separate storage experiment, selected 15 mL formulations and the control were aged for three months and subsequently stored under cold conditions for six months. Total phenols and antioxidant activity decreased during storage but remained higher in enriched wines than in the control, whereas mineral concentrations showed comparatively limited changes. Post-fermentation botanical infusion modified the measured compositional and sensory characteristics of Shiraz wine, with compositional enrichment retained during six months of storage.

KEYWORDS: Shiraz wine; post-fermentation infusion; *Moringa oleifera*; *Elephantopus scaber*; *Piper betle*; ferric-reducing antioxidant activity; total phenols; mineral composition; volatile compounds; storage stability

1. INTRODUCTION

Wine is a complex fermented beverage in which phenolic compounds, organic acids, ethanol, sulfur dioxide, minerals, and volatile constituents collectively determine its physicochemical and sensory characteristics [1]. Phenolic compounds are particularly important because of their contribution to colour, flavour, astringency, oxidative stability, and antioxidant capacity. Incorporation of plant-derived materials into wine provides an approach for modifying its chemical composition and sensory properties [2]. Plant leaves contain diverse phenolic compounds and other phytochemicals, making them relevant sources for enrichment of food and beverage products. *Moringa oleifera* leaves, in particular, contain phenolic constituents and exhibit antioxidant activity [3].

The stage and level of botanical incorporation are important determinants of the resulting wine composition. Pre-fermentation or co-fermentation addition exposes plant constituents to the biochemical changes occurring during alcoholic fermentation, whereas post-fermentation incorporation introduces the extract into an established wine matrix. This approach allows plant-derived constituents to be incorporated after completion of alcoholic fermentation and provides a defined means of modifying the composition of finished wine [4]. Previous studies have demonstrated that incorporation of botanical materials can increase phenolic content and antioxidant capacity, with responses influenced by the botanical source and stage of addition [5]. The proportion of extract incorporated is also important because it determines the relative contribution of botanical constituents to the final wine matrix.

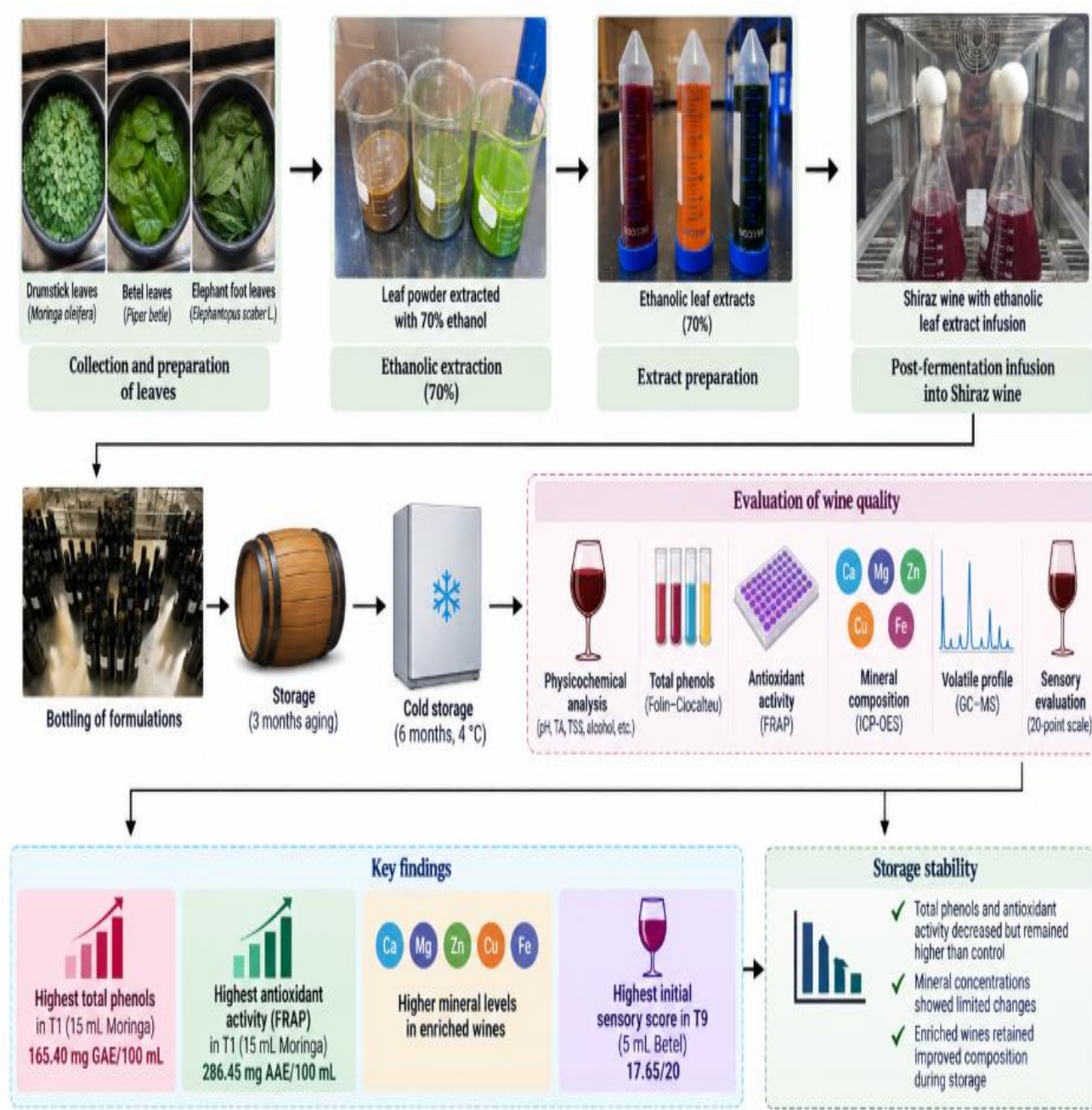
The botanical sources selected for the present study represent distinct phytochemical resources. *M. oleifera* leaves are characterized by phenolic and antioxidant constituents [2], while betel (*Piper betle* L.) leaves contain phenolic compounds associated with antioxidant activity [6]. *Elephantopus scaber* has also been reported to

possess phenolic constituents and antioxidant properties [7]. Comparative evaluation of these botanical materials in a common wine matrix is therefore relevant for determining their effects on wine composition and quality.

The incorporation of botanical extracts can also influence the chemical stability of wine through interactions with its existing constituents. Sulfur dioxide, phenolic compounds, iron, and copper are involved in oxidation–reduction processes that influence wine stability [8,9]. In addition, differences in extract incorporation levels alter the relative proportions of wine and botanical extract and can consequently affect measured volatile and non-volatile constituents. Therefore, evaluation of botanical-enriched wine requires consideration of physicochemical composition, antioxidant-related attributes, mineral constituents, volatile compounds, and sensory quality. Sensory assessment is particularly important because increases in chemical constituents do not necessarily correspond to improved aroma, flavour, astringency, or overall quality. Furthermore, storage can modify both chemical and sensory characteristics, making evaluation of compositional stability an important component of product assessment.

Despite increasing interest in plant-enriched beverages, comparative information on post-fermentation incorporation of different botanical leaf extracts at defined levels into a common Shiraz wine matrix remains limited. In particular, information integrating chemical, mineral, volatile, sensory, and storage characteristics is insufficient.

The present study aimed to evaluate the effects of post-fermentation incorporation of 70% (v/v) ethanolic leaf extracts of drumstick (*Moringa oleifera* Lam.), elephant-foot yam (*Elephantopus scaber* L.), and betel (*Piper betle* L.) at different levels on the physicochemical, chemical, mineral, volatile, and sensory characteristics of Shiraz wine and to determine the stability of selected quality attributes during storage.



2. MATERIALS AND METHODS

2.1. Plant material and preparation of ethanolic extracts

Fresh, disease-free drumstick (*Moringa oleifera* Lam.), elephant-foot yam (*Elephantopus scaber*.) and betel (*Piper betle* L.) leaves were used. Leaves were processed into powder. For extraction, 5% dried leaf powder was mixed separately with 50%, 70% or 90% (v/v) ethanol, homogenized, covered with aluminium foil and maintained in the dark at ambient temperature for 24 h. The crude extracts were centrifuged and filtered through qualitative filter paper. The clear filtrates were collected and stored at -20°C until use.

2.2. Preparation of Shiraz base wine

Shiraz grapes (*Vitis vinifera* L. cv. Shiraz) were de-stemmed, washed and crushed. Potassium metabisulfite was added at 200 mg/kg. After 4 h, the must soluble-solids concentration was adjusted to 24 °Brix and the pH was adjusted according to the standard winemaking procedure. *Saccharomyces cerevisiae* was subsequently added. Alcoholic fermentation was conducted for 48 h with the cap present, followed by pressing and a further 7 d of fermentation without the cap. Activated charcoal was added at 100 mg/L, followed by filtration and bottling. The prepared base wine was stored at 12°C until formulation.

2.3. Experimental design and treatment formulation

The formulation experiment comprised 10 treatments with three replications in a completely randomized design (CRD). The three 70% ethanolic leaf extracts were incorporated into fermented Shiraz wine at 5, 10 and 15 mL per 100 mL final formulation. The uninfused Shiraz wine (100 mL) served as the control. Because the infusion was performed after alcoholic fermentation, treatment effects represented post-fermentation compositional changes rather than effects on primary fermentation kinetics.

2.4. Physicochemical and chemical analyses

Alcohol content was determined by ebulliometry after calibration with distilled water and expressed as % (v/v). Total acidity was determined by titration of 10 mL wine diluted with distilled water against 0.1 N sodium hydroxide using phenolphthalein and expressed as g/L tartaric acid. Free sulfur dioxide was determined by the Ripper method using automatic titration with oxidation–

Table 1: Treatment combinations used for infusion of 70% ethanolic leaf extracts into Shiraz wine.

Treatment	Formulation
T1	85 mL Shiraz wine + 15 mL drumstick leaf extract
T2	90 mL Shiraz wine + 10 mL drumstick leaf extract
T3	95 mL Shiraz wine + 5 mL drumstick leaf extract
T4	85 mL Shiraz wine + 15 mL elephant foot leafleaf extract
T5	90 mL Shiraz wine + 10 mL elephant foot leafleaf extract
T6	95 mL Shiraz wine + 5 mL elephant foot leafleaf extract
T7	85 mL Shiraz wine + 15 mL betel leaf extract
T8	90 mL Shiraz wine + 10 mL betel leaf extract
T9	95 mL Shiraz wine + 5 mL betel leaf extract
T10	100 mL Shiraz wine (control)

reduction potential endpoint detection. Total sulfur dioxide was determined by the modified Ripper procedure after alkaline treatment to release bound sulfur dioxide. Tannin concentration was determined by the Folin–Denis spectrophotometric method described in IS 7585:1995 and expressed as g/L tannic acid equivalent. Reducing sugars were quantified by the Lane–Eynon volumetric method using Fehling's solution and expressed as g/L. Extract content was determined gravimetrically after evaporation and drying to constant mass and expressed as g/L. Volatile acidity was determined by steam distillation followed by titration of the distillate with standardized sodium hydroxide and expressed as g/L acetic acid.

2.5. Bioactive compounds and antioxidant activity

Total phenolic content was determined using the Folin–Ciocalteu method with gallic acid as the reference standard, and the results were expressed as mg gallic acid equivalents (GAE) per 100 mL. Antioxidant activity was determined using the ferric-reducing antioxidant power (FRAP) assay described by Benzie and Strain [6] and expressed as mg ascorbic acid equivalents (AAE) per 100 mL. β -Carotene content was determined spectrophotometrically, while ascorbic acid content was estimated by 2,6-dichlorophenolindophenol (2,6-DCPIP) dye titration.

2.6. Mineral analysis

Wine samples were prepared for mineral analysis according to the procedures specified in the Compendium of International Methods of Analysis of Wines and Musts of the International Organisation of Vine and Wine (OIV). Calcium and magnesium contents were determined by complexometric titration using ethylenediaminetetraacetic acid (EDTA). Zinc and iron concentrations were determined by atomic absorption

spectrophotometry, whereas copper was estimated by the sodium diethyldithiocarbamate colorimetric method. Calcium, magnesium and zinc concentrations were expressed as mg/100 mL, while copper and iron concentrations were expressed as mg/L.

2.7. Volatile compound analysis

Volatile compounds, including esters, methanol, and aldehydes, were analysed by gas chromatography coupled with a flame-ionization detector (GC–FID). Esters were analysed using a CP-Wax 57 CB capillary column (50 m × 0.32 mm × 0.2 µm) with helium as the carrier gas at 1.8 mL/min. Samples were degassed, filtered through a 0.45 µm membrane, and 4-methyl-2-pentanol was used as the internal standard. The injector and detector temperatures were maintained at 240 and 250 °C, respectively. The oven temperature was held at 40 °C for 5 min, increased at 5 °C/min to 220 °C, and held for 5–10 min. Individual esters were identified by comparison of retention times with authentic standards and quantified using the internal-standard method [11]. Methanol was determined by GC–FID following the AOAC method for alcohols in wine, with slight modification. Samples were degassed, filtered when necessary, and diluted where required before injection. Methanol was identified by comparison with an authentic standard and quantified using a calibration curve. Aldehydes were analysed by GC–FID using a DB-WAX capillary column under the chromatographic conditions described for the method. Individual aldehydes were identified by comparison of retention times with authentic standards and quantified using the internal-standard method.

2.8. Sensory evaluation

Sensory evaluation of the Shiraz wine samples was conducted using the Davis 20-point scorecard described by Amerine and Roessler [12]. The prepared wine samples were coded and presented to the panel members for evaluation. The samples were assessed for appearance, aroma, acidity, sweetness, body, flavour, astringency, and overall quality. The maximum scores assigned to these attributes were 2, 4, 2, 1, 1, 2, 2, and 6, respectively, giving a maximum possible score of 20. Panel members recorded individual scores based on sensory perception, and the total sensory score was calculated for each treatment.

2.9. Storage studies

The storage experiment comprised four selected treatments: T1 (85 mL Shiraz wine + 15 mL Moringa oleifera leaf extract), T4 (85 mL Shiraz wine + 15 mL Elephantopus scaber leaf extract), T7 (85 mL Shiraz wine + 15 mL Piper betle leaf extract), and T10 (100 mL control wine). The experiment was conducted with five replications in a completely randomized design. The wines underwent three months of ageing followed by six months of cold storage. Physicochemical, bioactive, mineral, volatile, and sensory parameters were analysed at monthly intervals using the procedures described above.

2.10. Statistical analysis

The formulation and storage experiments were analysed using a completely randomized design (CRD). The experimental data were subjected to analysis of variance (ANOVA) to determine the significance of treatment effects. When the F-test indicated significant treatment differences, treatment means were separated using Duncan's multiple range test (DMRT) at the 1% significance level. The results were expressed as treatment means with standard error of the mean (SEM ±) and critical difference (CD). Statistical significance was considered at $p < 0.01$ for the treatment comparisons presented in the formulation experiment. All statistical analyses were performed using R statistical software (R Foundation for Statistical Computing, Vienna, Austria).

3. RESULTS

3.1. pH, alcohol content and total acidity

Leaf-extract infusion significantly affected pH, alcohol content, and total acidity of the Shiraz wine (Table 2). The pH ranged from 3.55 to 3.64, with the highest value recorded in T1 (3.64) and the lowest in T7 (3.55), compared with 3.58 in the control (T10). Alcohol content ranged from 13.00 to 14.70% (v/v), with the highest value in T7 (14.70%) and the lowest in the control. Total acidity ranged from 6.24 to 6.58 g/L, with the lowest value in T1 and the highest in T7. Within the drumstick and elephant-foot treatments, increasing extract volume was associated with an increase in pH and a decrease in total acidity, whereas the betel treatments showed an inverse trend.

Table 2. Effect of 70% ethanolic leaf-extract infusion on pH, alcohol content and total acidity of Shiraz wine.

Treatment	pH	Alcohol (% v/v)	Total acids (g/L)
T1	3.64 ^a	14.50 ^{ab}	6.24 ^h
T2	3.62 ^{ab}	14.10 ^{cd}	6.29 ^g
T3	3.60 ^{bcd}	13.70 ^f	6.34 ^f
T4	3.61 ^{bc}	14.60 ^a	6.35 ^{ef}
T5	3.60 ^{bcd}	14.20 ^{cd}	6.38 ^{def}
T6	3.59 ^{cde}	13.80 ^{ef}	6.41 ^{cd}
T7	3.55 ^g	14.70 ^a	6.58 ^a

T8	3.56 ^{fg}	14.30 ^{bc}	6.51 ^b
T9	3.57 ^{efg}	14.00 ^{de}	6.45 ^c
T10	3.58 ^{def}	13.00 ^g	6.39 ^{de}
Mean	3.59	14.09	6.39
SEm ±	0.006	0.058	0.012
CD (1%)	0.02	0.23	0.05

Note: Values are treatment means of three replications. Within a column, means bearing different superscript letters differ significantly according to Duncan's multiple range test at $p < 0.01$. SEm ±, standard error of mean; CD, critical difference; NS, non-significant.

3.2. Sulfur dioxide and tannins

Free SO₂ concentrations ranged from 58.00 to 78.00 ppm, with the highest concentration in the control (T10) and the lowest in T7 (Table 3). Within each botanical series, free SO₂ decreased with increasing extract volume. Total SO₂ ranged from 182.00 to 238.00 ppm, with the highest value in T10 and the lowest in T9. Tannin concentration increased markedly following extract infusion, ranging from 0.08 g/L in the control to 1.18 g/L in T7.

Table 3. Effect of 70% ethanolic leaf-extract infusion on free SO₂, total SO₂ and tannin concentration.

Treatment	Free SO ₂ (ppm)	Total SO ₂ (ppm)	Tannins (g/L)
T1	60.00 ^h	232.00 ^b	1.05 ^b
T2	67.00 ^e	205.00 ^c	0.82 ^c
T3	73.00 ^c	191.00 ^f	0.58 ^h
T4	62.00 ^g	206.00 ^c	0.92 ^d
T5	69.00 ^d	195.00 ^d	0.70 ^f
T6	75.00 ^b	184.00 ^h	0.48 ⁱ
T7	58.00 ⁱ	193.00 ^e	1.18 ^a
T8	65.00 ^f	187.00 ^g	0.95 ^c
T9	72.00 ^c	182.00 ⁱ	0.66 ^g
T10	78.00 ^a	238.00 ^a	0.08 ^j
Mean	67.90	201.30	0.74
SEm ±	0.289	0.289	0.006
CD (1%)	1.16	1.16	0.02

Note: Values are treatment means of three replications. Within a column, means bearing different superscript letters differ significantly according to Duncan's multiple range test at $p < 0.01$. SEm ±, standard error of mean; CD, critical difference; NS, non-significant.

3.3.Reducing sugars, β-carotene, ascorbic acid, antioxidant activity and total phenols

Reducing sugars ranged from 5.40 to 6.32 g/L and increased with extract volume within each botanical series. T7 recorded the highest concentration (Table 4). β-Carotene ranged from 0.03 to 0.78 mg/100 mL, and ascorbic acid from 0.62 to 3.24 mg/100 mL; T1 recorded the highest value for both variables. FRAP antioxidant activity increased from 112.80 mg AAE/100 mL in the control to 286.45 mg AAE/100 mL in T1. Total phenols increased from 78.40 mg GAE/100 mL in the control to 165.40 mg GAE/100 mL in T1.

Table 4. Effect of 70% ethanolic leaf-extract infusion on reducing sugars, β-carotene, ascorbic acid, antioxidant activity and total phenols.

Treatment	Reducing sugars (g/L)	β-Carotene (mg/100 mL)	Ascorbic acid (mg/100 mL)	Antioxidant activity (mg AAE/100 mL)	Total phenols (mg GAE/100 mL)
T1	6.25 ^b	0.78 ^a	3.24 ^a	286.45 ^a	165.40 ^a
T2	5.95 ^c	0.56 ^b	2.82 ^b	238.60 ^c	135.75 ^c
T3	5.65 ^h	0.34 ^c	2.36 ^d	192.75 ^f	107.60 ^f
T4	6.10 ^c	0.38 ^d	2.18 ^e	224.30 ^d	128.45 ^d
T5	5.85 ^f	0.27 ^f	1.91 ^g	186.85 ^g	106.80 ^f
T6	5.60 ^h	0.17 ^h	1.58 ^h	148.20 ⁱ	88.30 ^h
T7	6.32 ^a	0.52 ^c	2.87 ^b	268.90 ^b	154.20 ^b
T8	6.02 ^d	0.36 ^{de}	2.48 ^c	218.40 ^e	126.50 ^e
T9	5.72 ^g	0.22 ^g	2.09 ^f	173.65 ^h	98.75 ^g
T10	5.40 ⁱ	0.03 ⁱ	0.62 ⁱ	112.80 ^j	78.40 ⁱ
Mean	5.89	0.36	2.22	205.09	119.02
SEm ±	0.02	0.006	0.02	0.32	0.24

CD (1%)	0.07	0.02	0.06	1.27	0.96
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Note: Values are treatment means of three replications. Within a column, means bearing different superscript letters differ significantly according to Duncan's multiple range test at $p < 0.01$. SEm $\pm$, standard error of mean; CD, critical difference; NS, non-significant.

3.4. Mineral composition

Significant differences among treatments were observed for all five measured minerals (Table 5). Calcium concentration ranged from 7.02 to 12.40 mg/100 mL, magnesium from 6.10 to 9.35 mg/100 mL, zinc from 0.15 to 0.42 mg/100 mL, copper from 1.64 to 2.58 mg/L, and iron from 4.92 to 9.20 mg/L. T1 recorded the highest concentration of Ca, Mg, Zn, Cu, and Fe, whereas the control (T10) recorded the lowest concentration for each mineral.

Table 5. Effect of 70% ethanolic leaf-extract infusion on the mineral composition of Shiraz wine.

Treatment	Ca (mg/100 mL)	Mg (mg/100 mL)	Zn (mg/100 mL)	Cu (mg/L)	Fe (mg/L)
T1	12.40 ^a	9.35 ^a	0.42 ^a	2.58 ^a	9.20 ^a
T2	10.92 ^c	8.42 ^c	0.35 ^c	2.28 ^c	7.85 ^c
T3	8.82 ^f	7.35 ^f	0.27 ^e	1.96 ^g	6.40 ^f
T4	10.05 ^d	8.18 ^d	0.32 ^d	2.22 ^d	7.35 ^d
T5	8.72 ^g	7.42 ^f	0.26 ^e	2.03 ^f	6.25 ^g
T6	7.86 ⁱ	6.86 ^h	0.21 ^g	1.82 ⁱ	5.55 ⁱ
T7	11.36 ^b	8.82 ^b	0.38 ^b	2.40 ^b	8.10 ^b
T8	9.68 ^e	7.96 ^e	0.31 ^d	2.15 ^e	6.95 ^e
T9	8.35 ^h	7.16 ^g	0.24 ^f	1.88 ^h	5.90 ^h
T10	7.02 ^j	6.10 ⁱ	0.15 ^h	1.64 ^j	4.92 ^j
Mean	9.52	7.76	0.29	2.10	6.85
SEm $\pm$	0.02	0.02	0.003	0.01	0.03
CD (1%)	0.09	0.09	0.01	0.05	0.12

Note: Values are treatment means of three replications. Within a column, means bearing different superscript letters differ significantly according to Duncan's multiple range test at $p < 0.01$. SEm $\pm$, standard error of mean; CD, critical difference; NS, non-significant.

3.5. Extract content, methyl alcohol and volatile acidity

Extract content ranged from 24.06 to 34.98 g/L, with the highest value in T7 and the lowest in the control (T10) (Table 6). Methyl alcohol concentration ranged from 0.08 to 0.14 g/L, with the highest value in the control and the lowest values recorded in T1, T4 and T5. Volatile acidity ranged from 0.573 to 0.743 g/L, with the highest value in T7 and the lowest in T10.

Table 6. Effect of 70% ethanolic leaf-extract infusion on extract content, methyl alcohol and volatile acidity.

Treatment	Extract (g/L)	Methyl alcohol (g/L)	Volatile acidity (g/L)
T1	29.18 ^c	0.08 ^d	0.650 ^{de}
T2	28.52 ^c	0.11 ^{bc}	0.637 ^{ef}
T3	27.23 ^f	0.12 ^b	0.623 ^f
T4	32.32 ^{bc}	0.08 ^d	0.683 ^c
T5	31.77 ^c	0.08 ^d	0.663 ^d
T6	30.63 ^d	0.09 ^{cd}	0.633 ^{ef}
T7	34.98 ^a	0.11 ^b	0.743 ^a
T8	33.37 ^b	0.11 ^b	0.713 ^b
T9	32.88 ^b	0.12 ^b	0.700 ^{bc}
T10	24.06 ^g	0.14 ^a	0.573 ^g
Mean	30.49	0.105	0.662
SEm $\pm$	0.268	0.003	0.005
CD (1%)	1.079	0.013	0.019

Note: Values are treatment means of three replications. Within a column, means bearing different superscript letters differ significantly according to Duncan's multiple range test at $p < 0.01$. SEm $\pm$, standard error of mean; CD, critical difference; NS, non-significant.

3.6. Ester profile

Nine esters were detected in the Shiraz wine: ethyl acetate, isoamyl acetate, ethyl hexanoate, ethyl butyrate, phenethyl acetate, diethyl succinate, ethyl lactate, ethyl decanoate, and ethyl octanoate (Table 7). The concentrations of most esters were lower in the infused treatments than in the control. Within each botanical series, most ester concentrations decreased with increasing extract volume. Ethyl acetate was the predominant ester, ranging from 76 to 103 mg/L, with the highest concentration in the control. Isoamyl acetate ranged from 27 to 40 mg/L, ethyl hexanoate from 15 to 29 mg/L, ethyl butyrate from 12 to 19 mg/L, phenethyl acetate from 10 to 16 mg/L, diethyl succinate from 20 to 31 mg/L, ethyl lactate from 25 to 36 mg/L, ethyl decanoate from 13 to 21 mg/L, and ethyl octanoate from 23 to 32 mg/L.

Table 7. Ester profile of Shiraz wine after infusion with 70% ethanolic leaf extracts (mg/L).

Treatment	Ethyl acetate	Isoamyl acetate	Ethyl hexanoate	Ethyl butyrate	Phenethyl acetate	Diethyl succinate	Ethyl lactate	Ethyl decanoate	Ethyl octanoate
T1	86	34	21	13	11	23	28	13	25
T2	91	36	22	16	13	23	30	17	25
T3	98	37	25	17	13	26	33	20	30
T4	76	27	15	12	11	20	25	14	23
T5	85	31	20	13	11	23	30	16	24
T6	90	33	22	16	13	23	30	17	27
T7	80	29	19	13	10	20	27	14	23
T8	89	33	20	14	12	23	29	16	24
T9	94	37	24	16	13	27	32	19	27
T10	103	40	29	19	16	31	36	21	32
Mean	89.20	33.70	21.70	14.90	12.30	23.90	30.00	16.70	26.00
SEm ±	0.84	0.68	0.63	0.58	0.68	0.63	0.63	0.58	0.63
CD (1%)	3.37	2.75	2.55	2.32	2.75	2.55	2.55	2.32	2.55

Note: Values are treatment means of three replications. Within a column, means bearing different superscript letters differ significantly according to Duncan's multiple range test at $p < 0.01$. SEm ±, standard error of mean; CD, critical difference; NS, non-significant

3.7. Aldehyde profile

Eleven aldehydes were detected in the wine samples, with acetaldehyde as the predominant aldehyde (Table 8). Acetaldehyde concentrations ranged from 21.10 mg/L in T7 to 52.90 mg/L in the control (T10). The concentrations of the other measured aldehydes were also generally lower in the infused treatments than in the control. Within each botanical series, aldehyde concentrations generally decreased with increasing extract incorporation.

Table 8. Aldehyde profile of Shiraz wine after infusion with 70% ethanolic leaf extracts (mg/L).

Treat ment	Non anal	Furf ural	5-H M F	Benzald ehyde	Hex anal	Acetald ehyde	Formald ehyde	Propional dehyde	Butyral dehyde	Hept anal	Isobut yralde hyde
T1	1.70	1.50	2.10	2.30	1.90	23.80	1.20	2.20	1.50	1.30	1.20
T2	2.20	3.70	3.40	2.90	3.00	28.90	1.60	3.10	2.10	1.70	1.40
T3	3.00	5.00	4.40	3.70	3.90	40.60	2.40	3.70	2.60	2.20	1.90
T4	2.20	3.30	3.00	2.70	2.70	27.90	1.90	2.60	2.00	1.70	1.30
T5	2.70	4.10	3.90	3.20	3.20	34.10	2.10	3.30	2.50	2.20	1.80
T6	3.20	5.20	5.00	4.30	4.20	43.50	2.80	4.20	3.10	3.00	2.00
T7	1.50	2.70	2.40	2.20	2.10	21.10	1.20	2.00	1.60	1.30	1.10
T8	2.00	3.40	3.20	2.70	2.50	27.90	1.80	2.60	1.80	1.60	1.30
T9	2.90	4.70	4.00	3.60	3.20	37.70	2.20	3.30	2.50	2.30	1.70

T10	4.40	6.60	6.00	5.40	5.10	52.90	3.30	4.70	3.50	3.50	2.70
Mean	2.58	4.02	3.74	3.30	3.18	33.84	2.05	3.17	2.32	2.08	1.64
SEm $\pm$	0.06	0.11	0.06	0.07	0.07	0.62	0.06	0.07	0.07	0.06	0.06
CD (1%)	0.25	0.45	0.25	0.28	0.29	2.48	0.23	0.28	0.29	0.24	0.25

Note: Values are treatment means of three replications. Within a column, means bearing different superscript letters differ significantly according to Duncan's multiple range test at $p < 0.01$. SEm $\pm$, standard error of mean; CD, critical difference; NS, non-significant.

3.8.Sensory evaluation

Sensory scores differed among treatments for most attributes, whereas sweetness was not significantly different. T9 recorded the highest total sensory score (17.65/20), followed by T1 (17.45/20) and T2 (17.25/20). T7 recorded the lowest total score (15.60/20). T9 had the highest numerical scores for aroma, flavour, astringency, body and overall quality. The thesis reported T9 as statistically comparable with T1, T2, T4, T6 and T10 at $p < 0.01$.

Table 9. Sensory evaluation of Shiraz wine using the Davis 20-point score card.

Treatment	Appearance/2	Aroma/4	Acidity/2	Sweetness/1	Flavour/2	Astringency/2	Overall quality/6	Total/20	Body/1
T1	1.85 ^a	3.62 ^a	1.76 ^a	0.78 ^a	1.72 ^a	1.66 ^{ab}	5.20 ^{ab}	17.45 ^{ab}	0.86 ^{ab}
T2	1.78 ^{ab}	3.60 ^a	1.78 ^a	0.77 ^a	1.70 ^a	1.64 ^{ab}	5.16 ^{ab}	17.25 ^{abc}	0.82 ^{abc}
T3	1.65 ^{bc}	3.40 ^{bcd}	1.70 ^a	0.74 ^a	1.58 ^{bc}	1.56 ^b	4.67 ^c	16.05 ^{bcd}	0.75 ^{cd}
T4	1.76 ^{ab}	3.45 ^{abcd}	1.66 ^{ab}	0.75 ^a	1.64 ^{abc}	1.63 ^{ab}	5.13 ^{ab}	16.85 ^{abcd}	0.83 ^{abc}
T5	1.70 ^{abc}	3.36 ^{cd}	1.68 ^{ab}	0.74 ^a	1.55 ^{cd}	1.60 ^{ab}	4.73 ^c	16.15 ^{bcd}	0.79 ^{bcd}
T6	1.62 ^c	3.35 ^{cd}	1.64 ^{ab}	0.72 ^a	1.56 ^{bcd}	1.58 ^{ab}	5.07 ^{ab}	16.30 ^{abcd}	0.76 ^{cd}
T7	1.76 ^{ab}	3.20 ^d	1.40 ^c	0.74 ^a	1.46 ^d	1.38 ^c	4.84 ^{bc}	15.60 ^d	0.82 ^{abc}
T8	1.70 ^{abc}	3.28 ^{cd}	1.50 ^{bc}	0.74 ^a	1.50 ^{cd}	1.55 ^{bc}	4.80 ^{bc}	15.85 ^{cd}	0.78 ^{bcd}
T9	1.82 ^a	3.65 ^a	1.72 ^a	0.78 ^a	1.78 ^a	1.74 ^a	5.28 ^a	17.65 ^a	0.88 ^a
T10	1.65 ^{bc}	3.55 ^{ab}	1.68 ^{ab}	0.72 ^a	1.68 ^{ab}	1.58 ^{ab}	4.95 ^{abc}	16.55 ^{abcd}	0.74 ^d
Mean	1.73	3.45	1.65	0.75	1.62	1.59	4.98	16.57	0.80
SEm $\pm$	0.04	0.07	0.05	0.03	0.05	0.04	0.10	0.37	0.02
CD (1%)	0.16	0.27	0.19	NS	0.18	0.17	0.40	1.48	0.08

Note: Values are treatment means of three replications. Within a column, means bearing different superscript letters differ significantly according to Duncan's multiple range test at $p < 0.01$. SEm $\pm$, standard error of mean; CD, critical difference; NS, non-significant

3.9.Storage stability during six months of cold storage

The storage study demonstrated progressive chemical evolution without an abrupt deterioration in the measured quality attributes. The four stored formulations were the 15 mL drumstick, elephant-foot and betel leaf extract treatments and the unfortified control. The treatment ranking for the principal bioactive and mineral variables was largely retained throughout the six-month storage period.

Antioxidant activity declined progressively during storage. For S1 (15 mL drumstick extract), the value decreased from 286.45 to 250.80 mg AAE/100 mL, corresponding to 87.55% retention. The control decreased from 112.80 to 100.20 mg AAE/100 mL (88.83% retention). Total phenols in S1 decreased from 165.40 to 147.30 mg GAE/100 mL (89.06% retention), while the control decreased from 78.40 to 71.20 mg GAE/100 mL (90.82% retention). The treatment ranking remained unchanged, with S1 highest throughout the reported storage observations.

Mineral concentrations changed less than the phenolic and antioxidant measurements. In S1, calcium decreased from 12.40 to 12.02 mg/100 mL, zinc from 0.42 to 0.41 mg/100 mL, copper from 2.58 to 2.46 mg/L and iron from 9.20 to 8.72 mg/L over six months. The corresponding control values changed from 7.02 to 6.86 mg/100 mL, 0.15 to 0.15 mg/100 mL, 1.64 to 1.57 mg/L and 4.92 to 4.71 mg/L, respectively.

Volatile composition changed during storage. Ethyl acetate, isoamyl acetate, ethyl hexanoate, phenethyl acetate, ethyl decanoate and ethyl octanoate declined, whereas ethyl butyrate, diethyl succinate and ethyl lactate increased. The measured aldehydes increased during storage, while the infused wines generally retained lower aldehyde concentrations than the control. These changes are consistent with ongoing wine ageing chemistry, but the present dataset does not distinguish ester hydrolysis, esterification, oxidation, volatilization or other individual mechanisms.

Sensory quality showed a non-linear trajectory. The mean total sensory score increased from 16.61 initially to 17.18 at three and four months and then decreased slightly to 16.96 at six months. At six months, S1 recorded 17.76, followed by S2 (17.21), the control S4 (16.72) and S3 (16.13).

Table 10. Selected quality indicators of Shiraz wine before and after six months of cold storage.

Storage treatment	Antioxidant initial	Antioxidant 6 mo	Phenols initial	Phenols 6 mo	Ca initial	Ca 6 mo	Sensory initial	Sensory 6 mo
S1Drumstick 15 mL	286.45	250.80	165.40	147.30	12.40	12.02	17.45	17.76
S2Elephant foot leaf15 mL	224.30	196.20	128.45	114.90	10.05	9.74	16.85	17.21
S3Betel 15 mL	268.90	233.50	154.20	136.20	11.36	10.99	15.60	16.13
S4Control	112.80	100.20	78.40	71.20	7.02	6.86	16.55	16.72

Note: Selected initial and six-month values from the separate storage experiment. The storage treatments correspond to the 15 mL formulations selected from the initial experiment and the uninfused control.

4.DISCUSSION

4.1.Compositional effects of post-fermentation infusion

The principal compositional response was an increase in total phenols and FRAP reducing capacity after leaf-extract infusion. T1 increased total phenols from 78.40 to 165.40 mg GAE/100 mL and FRAP activity from 112.80 to 286.45 mg AAE/100 mL relative to the control. The parallel response is consistent with transfer of extractable redox-active constituents into the wine matrix and agrees with previous reports that post-fermentation herb addition can increase wine phenolic and antioxidant measurements [3,5]. However, Folin–Ciocalteu and FRAP are global assays; they do not establish the concentrations of individual phenolic compounds or their biological activity [12]. The results therefore demonstrate analytical enrichment of total reducing/phenolic-associated capacity rather than a health effect.

The dose response was also evident for minerals, with T1 recording the highest concentrations of Ca, Mg, Zn, Cu and Fe. This pattern is consistent with transfer of extractable mineral fractions from the leaf material. Nevertheless, the nutritional interpretation should remain cautious because the measured concentrations represent the final beverage composition and do not establish mineral bioavailability. The presence of Fe and Cu is also relevant to wine stability because these transition metals can participate in oxidative reactions involving phenolics and oxygen [8,9,13].

The increase in measured alcohol concentration with extract volume requires particular methodological caution. The thesis reports 13.00% (v/v) in the control and 13.70–14.70% in the 5–15 mL infusion treatments. Because the extracts were prepared with 70% ethanol, a direct solvent mass balance would require measurement of the actual ethanol concentration retained in the filtered extract before blending.

4.2.Volatile and sulfur-dioxide responses

The lower concentrations of many esters and aldehydes in infused wines are strongly confounded by formulation dilution: the enriched wines contained 85–95% fermented wine and 5–15% extract, whereas the control contained 100% fermented wine. Therefore, the data support a difference in final volatile composition but do not by themselves demonstrate that leaf phenolics degraded or chemically bound the volatile compounds. The same caution applies to sulfur dioxide. Free SO₂ decreased with increasing extract incorporation, whereas total SO₂ did not show a universal monotonic response across all treatments. This pattern is compatible with dilution and redistribution between free and bound fractions, but the experiment did not directly quantify the underlying binding or oxidation reactions. Wine SO₂ chemistry is strongly influenced by carbonyl compounds, polyphenols, oxygen and transition metals [8,9].

4.3.Sensory response and formulation balance

The sensory results provide an important counterpoint to the chemical enrichment data. T1 produced the strongest measured phenolic, FRAP and mineral enrichment, whereas T9 produced the highest initial sensory score of 17.65/20. Thus, the formulation optimum depended on the response criterion. The lower score of T7 at the highest betel dose indicates that increasing extract concentration can introduce a sensory penalty even when

chemical enrichment increases [3,11]. This supports the use of multi-attribute optimization rather than selecting a treatment solely on the basis of a single compositional variable.

4.4.Storage stability

The storage experiment showed that the compositional advantage of the enriched wines persisted for six months, although absolute phenolic and FRAP values declined. S1 retained 89.06% of its initial total phenols and 87.55% of its initial FRAP value. The decline is compatible with continuing oxidation, polymerization, precipitation and other ageing reactions in wine. Mineral concentrations showed smaller relative changes, whereas sensory scores remained within the numerical range reported for the initial samples. Comparable studies have also reported that plant-derived additions can modify phenolic and antioxidant characteristics during wine ageing [14,15].

The storage findings should nevertheless be interpreted within the design limitations. Only the three 15 mL extract formulations and the control were stored, so stability of the 5 and 10 mL treatments was not established. The supplied thesis also reports storage only as a cold condition without specifying the temperature, bottle closure, headspace or light exposure. Microbiological stability was not directly measured. Consequently, the study establishes chemical and sensory trends over the tested observation period but does not establish commercial shelf life.

4.5.Study limitations and implications

The principal limitations are incomplete documentation of the sensory panel and ethics procedure, missing yeast strain and inoculation information, incomplete specification of some analytical parameters, incomplete statistical-software information, and the unresolved taxonomic identity of the elephant-foot material. A further limitation is the absence of a solvent-matched control that would separate the effect of leaf constituents from the effect of the extraction medium. The lack of an independently quantified ethanol concentration in the final extracts also prevents a rigorous ethanol mass balance [2]. These limitations should be resolved from the laboratory records before submission.

Despite these constraints, the study provides a useful formulation comparison across three botanical sources and three infusion levels. The data indicate that 15 mL drumstick extract generated the strongest analytical enrichment, whereas 5 mL betel extract gave the highest initial sensory score. Future work should incorporate authenticated plant vouchers, solvent-matched controls, independent fermentation batches, detailed volatile-method validation, targeted phenolic profiling by LC-MS/HPLC, and microbiological shelf-life testing [10,11].

5.CONCLUSION

Post-fermentation infusion of 70% ethanolic drumstick, elephant-foot and betel leaf extracts substantially modified the measured quality attributes of Shiraz wine. The 15 mL drumstick formulation (T1) produced the highest total phenols (165.40 mg GAE/100 mL), FRAP reducing capacity (286.45 mg AAE/100 mL) and measured Ca, Mg, Zn, Cu and Fe concentrations. In contrast, the 5 mL betel formulation (T9) produced the highest initial sensory score (17.65/20), demonstrating that chemical enrichment and sensory preference were not maximized by the same formulation. In the separate six-month storage study, the selected 15 mL formulations retained their treatment ranking, while total phenols, FRAP activity and several other quality variables declined gradually. The results support post-fermentation botanical infusion as a formulation approach for modifying Shiraz wine composition, but the evidence is limited to analytical composition and sensory responses. Commercial shelf-life, microbiological stability, individual phenolic identity, bioavailability and health effects were not established

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