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Research Article

Solvent Extraction and Phytochemical Screening of *Vitis vinifera* L. Leaves using Acetone, Alcohol and Benzene

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ABSTRACT

The present study investigates phytochemical composition and quantitative phenolic–flavonoid profiles of Vitis vinifera L. leaf extracts obtained using acetone, alcohol, and benzene to evaluate their potential for industrial valorisation. Preliminary phytochemical screening revealed solvent-dependent variations, with acetone and alcohol extracts exhibiting a broader spectrum of bioactive constituents, particularly flavonoids, phenols, and saponins. Quantitative assays demonstrated a concentration-dependent increase in total flavonoid and phenolic contents across the 20–100 µg/mL range, with acetone consistently yielding the highest values, followed by alcohol and benzene. The strong linearity observed in standard calibration curves confirms the reliability of the colorimetric methods employed. These findings highlight the significant phytochemical richness of grapevine leaves, an underutilized agro-industrial residue, and underscore their suitability as a sustainable source of antioxidant compounds. The study provides a scientific basis for integrating Vitis vinifera leaves extract into functional food, nutraceutical, cosmetic, and biobased product applications, contributing to value addition within vineyard by-product chains.

INTRODUCTION

Vitis vinifera L. is one of the most economically important perennial crops worldwide, has been extensively cultivated for fruits, wines, and various industrial commodities. Beyond its agronomic value, the species has attracted considerable scientific interest due to the rich bioactive profile of its leaves, which possess notable antioxidant, anti-inflammatory, and therapeutic properties (Orhan et al., 2018). These phytochemical attributes make grape leaves a promising raw material for developing value-added products within the functional food, nutraceutical, cosmetic, and natural dye sectors (Vankar, 2007).

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Phytochemicals such as flavonoids, phenolic acids, tannins, and alkaloids are known to play crucial roles in plant physiology and offer broad health-promoting benefits to humans, including antioxidant defense and anti-inflammatory responses (Pourcel et al., 2007). Accurate characterization of these compounds relies on standardized phytochemical screening approaches, extraction protocols, and advanced analytical techniques, as established in classical and modern phytochemical methodologies (Harborne, 1998; Trease & Evans, 2002). Among these, solvent extraction remains a fundamental step, influencing both the yield and spectrum of bioactive constituents recovered from plant matrices (Tiwari et al., 2011).

The efficiency of extraction is largely dependent on solvent polarity, which determines the solubility of phenolic compounds and flavonoids within plant tissues (Stalikas, 2007). Several studies have demonstrated that variations in solvent type can significantly alter total phenolic content, flavonoid concentration, and antioxidant potential of plant extracts (Sultana et al., 2009). For instance, polar solvents such as alcohols often enhance the extraction of hydrophilic phenolics, while less polar solvents such as acetone or benzene may be effective for non-polar phytoconstituents (Do et al., 2014). Given this variability, solvent selection becomes a critical determinant of industrial applicability, influencing both extract quality and downstream product performance.

Although *Vitis vinifera* leaves have been examined for their polyphenolic composition (Keskin et al., 2025), comparative studies focusing on solvent-dependent extraction efficiency remain limited. Systematic evaluation of solvents with different polarities can generate essential insights for optimizing extraction processes, particularly for industrial sectors relying on plant-derived antioxidants and bioactive compounds. Furthermore, assessing qualitative and quantitative phytochemical profiles using validated screening methods is indispensable for ensuring reproducibility, quality assurance, and commercial scale-up (Harborne, 1998; Tiwari et al., 2011; Trease & Evans, 2002).

The present study aims to comparatively analyze the extraction efficiency and phytochemical composition of *Vitis vinifera* L. leaves using solvents of varying polarity alcohol, acetone, and benzene. By integrating classical phytochemical assays with contemporary analytical approaches, this research seeks to identify solvent-specific differences in bioactive profiles and contribute to the development of optimized extraction strategies for industrial applications in the functional, pharmaceutical, and natural product sectors.

MATERIALS AND METHODOLOGY

a) Plant Material Collection and Preparation

Fresh, disease-free leaves of *Vitis vinifera* L. were collected, rinsed with distilled water, and air-dried under shade at 25 ± 2 °C. Dried leaves were ground into fine powder using a sterile mechanical grinder and stored in airtight amber bottles to prevent photodegradation. All steps followed standard pharmacogenetic procedures for botanical material preparation (Mukherjee, 2002; Khandelwal, 2008).

b) Solvent Extraction Using Benzene, Acetone, and Alcohol

To examine solvent-dependent extraction efficiency, three solvents of differing polarity benzene (nonpolar), acetone (moderately polar), and alcohol (highly polar) were used. Soxhlet extraction of leaf powder was carried out using

standard phytochemical extraction procedures. Briefly, a known quantity of air-dried, finely powdered leaf material was placed in a thimble and extracted in a Soxhlet apparatus using an solvents (acetone, alcohol and benzene) for 6–8 hours until the siphoning cycle rendered the solvent in the thimble colorless, indicating exhaustive extraction. During the process, the solvent was continuously heated, vaporized, condensed, and passed through the plant matrix, ensuring efficient and repeated washing of the sample. After completion, the solvent extract was concentrated using a rotary evaporator under reduced pressure at temperatures below 40 °C to prevent degradation of thermo-labile phytochemicals. The dried extract was stored in airtight containers for further phytochemical analysis (López-Bascón et al., 2020)

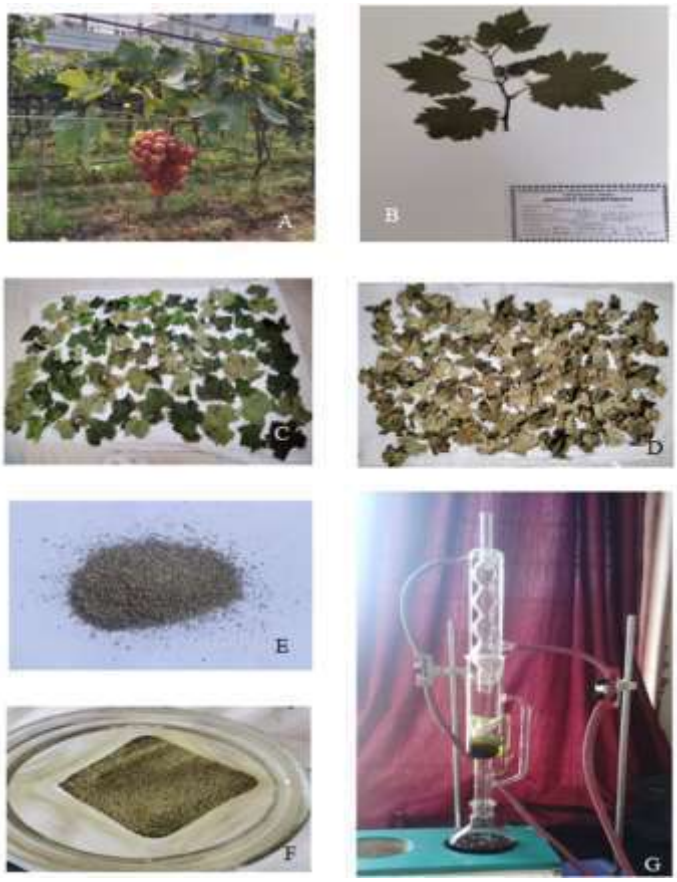

Fig. 1: A - Plant of *Vitis vinifera* L.; B - Herbarium of *Vitis vinifera* L.; C - Freshly collected leaves of *Vitis vinifera* L.; D - Shed dried leaves of *Vitis vinifera* L.; E and F - Ground powder from dried leaves of *Vitis vinifera* L.; G - Assembly of Soxhlet apparatus.

c) Preliminary Phytochemical Screening

Each solvent extract (benzene, acetone, alcohol) was subjected to qualitative phytochemical screening to identify major classes of secondary metabolites, including flavonoids, phenolics, alkaloids, tannins, glycosides, terpenoids, and saponins. Standard reagent-based phytochemical tests were performed following classical pharmacognostic manuals (Khandelwal, 2002; Mukherjee, 2002). Observations were recorded in triplicate to ensure reproducibility.

d) Determination of Total Phenolic Content (TPC)

Total phenolic content was quantified using the Folin–Ciocalteu colorimetric assay. A 0.5 mL

aliquot of each solvent extract was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent and 2 mL of 7.5% sodium carbonate. Samples were incubated for 30 min at room temperature, and absorbance was measured at 765 nm. Gallic acid served as the standard. The procedure followed established phenolic quantification methodologies used in herbal antioxidant studies (Ammor et al., 2018).

e) Determination of Total Flavonoid Content (TFC)

Flavonoid content in benzene, acetone, and alcohol extracts was determined using the aluminum chloride colorimetric assay described by Chang et al. (2002). One milliliter of extract was reacted sequentially with 5% sodium nitrite, 10% aluminum chloride, and 1 M sodium hydroxide. Absorbance was measured at 510 nm, and quercetin was used as the calibration standard. This method is widely recommended for quantifying flavonoids in plant extracts with varying polarities.

f) Quality Control and Standardization

All assays were performed in triplicate, and results were expressed as mean ± standard deviation. Instrument calibration, reagent preparation, and analytical validation were conducted per botanical quality-control standards (Mukherjee, 2002; Khandelwal, 2008). Extract handling and processing followed established pharmacognostic principles to ensure integrity and reproducibility.

RESULT

The preliminary phytochemical screening of the acetone, alcohol, and benzene extracts of *Vitis vinifera* L. leaves revealed distinct differences in constituent profiles depending on the solvent system employed (Table 1). Although acetone and alcohol extracts showed the same number of phytochemical groups, their composition differed. The acetone extract contained steroids, triterpenoids, saponins, flavonoids, phenols, and flavanols, while the alcohol extract showed the presence of steroids, carbohydrates, proteins, flavonoids, phenols, and flavanols. Benzene extract, owing to its non-polar nature, displayed a comparatively narrow profile and tested positive primarily for saponins, flavonoids, phenols, and flavanols. Notably, glycosides, alkaloids, tannins, starch, and anthocyanins were absent across all three solvent extracts, indicating their limited solubility or negligible abundance in the leaf matrix under the tested extraction conditions. The differential solubility patterns observed highlight the critical role of solvent polarity in selectively extracting secondary metabolites from *Vitis vinifera* foliage.

Table 1. Preliminary phytochemical analysis of acetone, alcohol, and benzene extract of leaves of *Vitis vinifera* L.

Sr. No.	Phytochemical Constituents	Acetone	Alcohol	Benzene
1	Steroids	+	+	-
2	Triterpenoids	+	-	-
3	Glycosides	-	-	-
4	Saponins	+	-	+
5	Carbohydrates	-	+	-
6	Alkaloids	-	-	-
7	Flavonoids	+	+	+
8	Phenol	+	+	+
9	Proteins	-	+	-
10	Tannins	-	-	-
11	Starch	-	-	-

12	Flavanol	+	+	+
13	Anthocyanin	-	-	-

The calibration data generated for quercetin at 510 nm demonstrated a clear linear relationship between standard concentration and absorbance across the tested range of 20–100 µg/mL (Table 2). All three solvent systems acetone, alcohol, and benzene exhibited identical absorbance values for the corresponding quercetin concentrations, indicating uniform spectrophotometric response and methodological consistency. The absorbance increased progressively from 0.343 at 20 µg/mL to 0.677 at 100 µg/mL, confirming the reliability of the aluminium chloride colorimetric method for flavonoid quantification. The linear progression observed in the standard curve validates the suitability of quercetin as the reference standard and ensures accurate estimation of total flavonoid content in the respective *Vitis vinifera* leaf extracts.

Table 2. Standard Curve Data for Quercetin (Flavonoids) Measured at 510 nm

Sr. No.	Quercetin Concentration (µg/mL)	Absorbance (Acetone)	Absorbance (Alcohol)	Absorbance (Benzene)
1	20	0.343	0.343	0.343
2	40	0.470	0.470	0.470
3	60	0.552	0.552	0.552
4	80	0.610	0.610	0.610
5	100	0.677	0.677	0.677

Standard Curve Analysis for Quercetin

The standard calibration curve constructed for quercetin showed a strong linear correlation between concentration and absorbance within the range of 20–100 µg/mL (Table 2). The absorbance values for acetone, alcohol, and benzene extracts were identical for each standard concentration, demonstrating high analytical precision and consistent spectrophotometric performance across solvents. The calibration plot yielded a regression equation of:

$$\mathbf{y = 0.0035x + 0.273}$$

with a coefficient of determination:

$$\mathbf{R^2 = 0.996}$$

The high R^2 value indicates excellent linearity and confirms that the aluminium chloride method is suitable for accurate flavonoid quantification. The linear increase in absorbance from 0.343 at 20 µg/mL to 0.677 at 100 µg/mL supported reliable interpolation of sample absorbance values for total flavonoid content (TFC) determination. This validated calibration curve was subsequently used to calculate the TFC of acetone, alcohol, and benzene extracts of *Vitis vinifera* leaves, ensuring accuracy in comparative solvent extraction efficiency.

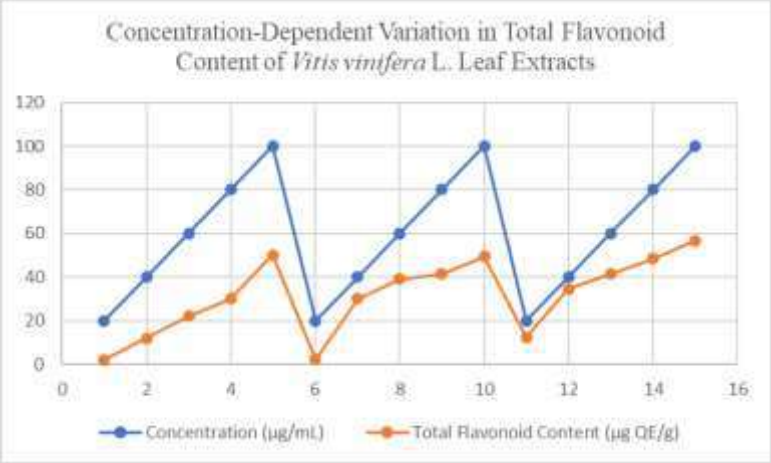

Fig. 2. Relationship Between Extract Concentration and Total Flavonoid Content in *Vitis vinifera* L. Leaf Extracts.

The graphical representation in Figure 2 shows a distinct concentration-dependent increase in total flavonoid content across the tested extract range (20–100 µg/mL). Higher extract concentrations consistently produced greater flavonoid values, demonstrating efficient solubilization and reliable detection of flavonoid compounds under the aluminum chloride colorimetric assay. The steady upward trend confirms a strong linear association between extract concentration and absorbance, supporting the sensitivity of the method for flavonoid estimation in *Vitis vinifera* leaf extracts. Minor fluctuations in the plotted values may arise from solvent-specific effects or natural variability in extract composition, yet these variations do not alter the overall linear pattern. Collectively, the results indicate that *Vitis vinifera* leaves exhibit a predictable concentration-response behavior, reinforcing their suitability as a viable and scalable source of natural flavonoids with potential applications in industrial bioproducts and antioxidant formulations.

Table 3. Total Phenolic Content (µg GAE/g) of *Vitis vinifera* L. Leaf Extracts Using Acetone, Alcohol, and Benzene

Sr. No.	Concentration (µg/mL)	Acetone Extract (µg GAE/g)	Alcohol Extract (µg GAE/g)	Benzene Extract (µg GAE/g)
1	20	9.91	7.50	8.21
2	40	18.88	18.05	10.20
3	60	21.18	27.30	16.80
4	80	38.87	34.40	28.30
5	100	47.20	40.30	39.40

The total phenolic content of *Vitis vinifera* L. leaf extracts varied distinctly with both solvent type and extract concentration. A consistent increase in phenolic content was recorded across the concentration gradient (20–100 µg/mL) for all extracts, demonstrating strong concentration-dependent behavior and confirming the reliability of the Folin–Ciocalteu assay in detecting phenolic constituents. Among the tested solvents, the acetone extract yielded the highest phenolic content, reaching 47.20 µg GAE/g at 100 µg/mL, whereas the alcohol and benzene extracts achieved 40.30 µg GAE/g and 39.40 µg GAE/g, respectively.

At lower concentrations (20–40 µg/mL), acetone and alcohol showed similar extraction capacities, while benzene consistently produced lower phenolic values. As concentrations increased (60–100 µg/mL), alcohol demonstrated slightly improved efficiency over benzene but did not surpass the extraction capacity of acetone. Overall, the extraction pattern followed the order: *Acetone = Alcohol > Benzene,* indicating that phenolic constituents of *Vitis vinifera* leaves are more readily solubilized in polar organic solvents. These solvent-dependent differences highlight the critical influence of solvent polarity on maximizing phenolic recovery for potential industrial and bioactive applications.

The comparative solvent extraction analysis revealed clear differences in the phytochemical composition, flavonoid content, and phenolic yield of *Vitis vinifera* L. leaf extracts. Acetone consistently demonstrated superior extraction efficiency, followed by alcohol and benzene, reflecting the strong influence of solvent polarity on metabolite recovery. The concentration-dependent trends observed in both phenolic and flavonoid assays further confirm the responsiveness of *V. vinifera* leaf constituents to quantitative colorimetric evaluation. Collectively, the results establish *V. vinifera* leaves as a chemically rich botanical resource and underscore their potential for development into antioxidant-based industrial products. These findings provide a solid foundation for subsequent discussions on extract functionality, industrial applicability, and targeted optimization of solvent-assisted phytochemical extraction.

DISCUSSION

The present investigation highlights clear solvent-dependent variations in the phytochemical profile and phenolic–flavonoid recovery from Vitis vinifera L. leaves, aligning with the extensive literature on the metabolic richness and extractability of grapevine foliage. Consistent with earlier metabolomic surveys demonstrating the biochemical complexity of healthy and diseased V. vinifera leaves (Weiller et al., 2024; Fortes et al., 2024), the current study confirms that leaves remain a valuable reservoir of diverse secondary metabolites, particularly phenolics and flavonoids. The strong extraction performance of acetone observed in this work parallels established evidence that solvents of intermediate polarity maximize phenolic solubilization across a range of botanical matrices (Sultana et al., 2009; Do et al., 2014; Stalikas, 2007). Furthermore, the superior recovery of flavonoids in polar organic solvents supports previous findings on grapevine leaves, where solvent polarity critically shaped metabolite yields and antioxidant potential (Katalinić et al., 2013; Moldovan et al., 2020).

The phytochemical profile obtained characterized by the consistent presence of flavonoids, phenols, and flavanols corresponds with documented reports showing that *V. vinifera* leaves accumulate a broad spectrum of phenolic constituents, including flavonols, anthocyanins, proanthocyanidins, stilbenes, and polyprenols (Goufo et al., 2020; Zokirova et al., 2013; Šuković et al., 2020). Notably, the detection of flavonoids across all solvents reflects the ubiquitous presence of these compounds in grapevine foliage, which has been widely associated with antioxidant, anti-inflammatory, and antiproliferative activities (Muñoz-Mingarro et al., 2025; Aouey et al., 2016; Tartaglione et al., 2018). These bioactivities often correlate with the redox behavior of flavonoids and their propensity for oxidative cycling, as outlined by Pourcel et al. (2007), which may partly explain the strong linear concentration–response relationship observed in the present flavonoid quantification.

In agreement with earlier reports on antioxidant-rich extracts obtained from *Vitis vinifera* leaves using conventional or microwave-assisted extraction (Djemaa-Landri et al., 2020), our findings underscore the pivotal role of solvent polarity in maximizing total phenolic content. Acetone, exhibiting the highest phenolic yield in this study, has been identified previously as an efficient solvent for extracting mid- to high-polarity phenolic acids, flavonols, and stilbene derivatives such as resveratrol and pterostilbene (Langcake et al., 1979; Căpruciu & Gheorghiu, 2025). The relatively lower yields observed with benzene align with the limited solubility of glycosylated flavonoids and phenolic acids in nonpolar solvents, as previously noted for grapevine tissues (Loizzo et al., 2019; Fernandes et al., 2013).

The solvent-driven extraction patterns observed also mirror the chemical diversity and biological potential documented in earlier studies. Extracts rich in phenolics and flavonoids have demonstrated antimicrobial (Ceyhan et al., 2012; Katalinic et al., 2009), anti-diabetic (Orhan et al., 2006), anti-leishmanial (Mansour et al., 2013), and neuroprotective effects (Borai et al., 2017), suggesting that the phytochemical richness confirmed in the present study may translate into broad therapeutic and industrial applicability. Recent investigations have also supported the valorization of grapevine leaf biomass in functional ingredients, green nanoparticle synthesis, and bioactive encapsulation systems (Saravanadevi et al., 2022; Pettinelli et al., 2024), reinforcing the relevance of efficient extraction strategies for industrial applications.

Taken together, the present findings corroborate the extensive phytochemical and biological potential of *Vitis vinifera* leaves reported across global studies and highlight the critical role of solvent polarity in determining extract quality and bioactive yield. By establishing acetone and alcohol as efficient extraction solvents for phenolic and flavonoid recovery, this study provides valuable insights for optimizing leaf-derived bioactive production within food, pharmaceutical, and industrial bioproduct sectors an approach consistent with the sustainable utilization strategies emphasised in modern grapevine by-product research (Fernandes et al., 2013; Nzekoue et al., 2022).

CONCLUSION

This study confirms that *Vitis vinifera* L. leaves are a rich source of phenolic and flavonoid compounds, with extraction efficiency strongly dependent on solvent polarity. Acetone and alcohol produced the highest yields, followed by benzene, demonstrating the superior capacity of polar solvents to recover bioactive constituents. The concentration-dependent increase observed across all assays indicates reliable extract responsiveness and validates the analytical methods used. Overall, the results support the potential of grapevine leaves as a sustainable raw material for antioxidant-rich industrial products and highlight the need for optimized solvent-based extraction strategies for future applications.

Industrial Application Implications

The demonstrated abundance of phenolic and flavonoid constituents in *Vitis vinifera* L. leaves positions this agricultural by-product as a valuable, low-cost raw material for multiple industrial sectors. The superior recovery achieved with acetone and alcohol underscores the feasibility of scalable solvent-based extraction systems for producing antioxidant-rich extracts suitable for incorporation into functional foods, nutraceuticals, phytopharmaceuticals, and cosmetic formulations. Given the strong

antioxidant potential associated with grapevine leaf metabolites, these extracts may also serve as natural preservatives and stabilizing agents in food and biopolymer industries. Furthermore, the valorization of grape leaves aligns with sustainable agro-industrial practices by transforming vineyard waste into high-value bioproducts, thereby contributing to circular bioeconomy models. Continued optimization of extraction protocols and process integration will enhance their commercial applicability in antioxidant-driven industrial applications.

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CONFLICT OF INTEREST:

The authors have no conflicts of interest.

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