

# Isolation and Structural Characterization of Bioactive Compounds from *Vitis vinifera* L. Peel

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## Abstract

**Background:** *Vitis vinifera* L. (Vitaceae) is an important medicinal and nutritional plant rich in polyphenolic compounds, particularly trans-resveratrol, a naturally occurring stilbene with well-documented antioxidant and anti-inflammatory properties. Grape peel, an abundant agro-industrial by-product, represents a sustainable and economical source of this valuable phytoconstituent.

**Objective:** The present study aimed to extract, isolate, purify, and characterize trans-resveratrol from *Vitis vinifera* peel and evaluate its in vitro antioxidant, anti-inflammatory, and cytotoxic activities.

**Materials and Methods:** Dried grape peel powder was extracted using 70% ethanol by Soxhlet extraction, followed by ethyl acetate partitioning and silica gel column chromatography for isolation of resveratrol. The purified compound was characterized using thin-layer chromatography (TLC), UV-visible spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, high-performance liquid chromatography (HPLC), and liquid chromatography–tandem mass spectrometry (LC–MS/MS). Antioxidant activity was evaluated by DPPH, ABTS, and ferric reducing antioxidant power (FRAP) assays. Anti-inflammatory activity was assessed using albumin denaturation, proteinase inhibition, and human red blood cell membrane stabilization assays. Cytotoxicity was determined by the MTT assay using RAW 264.7 macrophage cells.

**Results:** Hydroalcoholic extraction followed by chromatographic purification successfully yielded trans-resveratrol with chromatographic and spectroscopic characteristics comparable to the authentic standard. The isolated compound exhibited concentration-dependent antioxidant activity in DPPH, ABTS, and FRAP assays and demonstrated significant inhibition of protein denaturation, proteinase activity, and erythrocyte membrane lysis. MTT analysis indicated low cytotoxicity toward RAW 264.7 cells within the tested concentration range.

**Conclusion:** The findings suggest that *Vitis vinifera* peel is a promising and sustainable source of naturally occurring trans-resveratrol. The isolated compound exhibited favorable antioxidant, anti-inflammatory, and cellular safety profiles, supporting its potential application in pharmaceutical, nutraceutical, and functional food formulations. Further in vivo studies and formulation development are warranted.

**Keywords:** *Vitis vinifera* L.; trans-resveratrol; grape peel; column chromatography; HPLC; LC–MS/MS; antioxidant activity; anti-inflammatory activity; MTT assay.

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## 1. Introduction

Natural products have been a useful source of medicinal compounds for ages and continue to play an important role in modern drug development. Approximately half of all currently licensed pharmacological medicines are either directly from natural sources or produced using naturally existing

bioactive compounds. Medicinal plants produce a wide range of secondary metabolites, including alkaloids, flavonoids, tannins, terpenoids, phenolic acids, and stilbenes, which have antioxidant, anti-inflammatory, antibacterial, cardioprotective, neuroprotective, and anti-cancer properties. Plant-

derived phytochemicals have received a lot of attention for pharmaceutical and nutraceutical applications because of their structural diversity, pharmacological activity, and relative low toxicity. As a result, the isolation and identification of bioactive chemicals from medicinal plants remains a critical area of pharmaceutical research. [1–4]

Polyphenols are one of the most abundant classes of naturally occurring phytochemicals, found in fruits, vegetables, grains, medicinal plants, and drinks. Grapes (*Vitis vinifera* L.) are one of the best dietary sources of polyphenols, particularly flavonoids, anthocyanins, tannins, phenolic acids, and stilbenes. These chemicals contribute to grapes' sensory characteristics as well as their amazing biological activity. Numerous experimental studies have found that grape polyphenols effectively scavenge reactive oxygen species, inhibit lipid peroxidation, regulate inflammatory mediators, and protect against oxidative stress-related disorders, lowering the risk of cardiovascular disease, diabetes, neurodegenerative disorders, and cancer. [5–8]

*Vitis vinifera* L., a member of the Vitaceae family, is one of the oldest cultivated fruit crops, with a vast distribution in Europe, Asia, Africa, Australia, and America. In India, it is grown extensively in Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu, and Punjab. Aside from its nutritional significance, many parts of the grapevine, including fruits, peels, seeds, leaves, and stems, have long been used in traditional medicine to treat inflammatory disorders, cardiovascular diseases, diabetes, gastrointestinal issues, and skin conditions. The plant contains a wide variety of phytochemicals, including catechin, epicatechin, quercetin, rutin, gallic acid, caffeic acid, proanthocyanidins, anthocyanins, tannins, organic acids, vitamins, minerals, and stilbenes, with resveratrol being the most bioactive. [5,7,9]

Resveratrol (trans-3,5,4'-trihydroxystilbene) is a naturally occurring polyphenolic stilbene that plants produce as a phytoalexin in response to fungal infection, UV irradiation, and environmental stress. It is mostly found in grape skins and is generated via the phenylpropanoid route via stilbene synthase. Resveratrol is chemically composed of two aromatic phenolic rings linked by an ethylene bridge, and it exists mostly in the physiologically active trans-isomer. Resveratrol has been shown in numerous pharmacological studies to have powerful antioxidant, anti-inflammatory, anticancer, cardioprotective, neuroprotective, hepatoprotective, antibacterial, anti-

aging, and antidiabetic properties. Its biological actions include neutralizing reactive oxygen species, inhibiting inflammatory cytokines, modulating NF- $\kappa$ B and MAPK signaling pathways, activating SIRT1, and regulating apoptosis-related proteins. [6,10–13]

Naturally isolated resveratrol has become more popular due to consumer desire for plant-derived bioactive chemicals and its potential usage in pharmaceutical, nutraceutical, and cosmetic formulations, even though chemically synthesized resveratrol is commercially available. A cheap, sustainable, and renewable source of natural resveratrol is grape peel, a plentiful agro-industrial by-product produced during the manufacturing of juice and wine. In addition to making it easier to use agricultural waste, the effective extraction and purification of resveratrol from grape peels encourages the creation of value-added products with improved medicinal potential. [5,6,8]

Few studies have thoroughly examined the optimal extraction of resveratrol from *Vitis vinifera* peel, followed by chromatographic isolation, structural characterization using spectroscopic techniques, and systematic biological evaluation within a single experimental study, despite a large body of research on the pharmacological activities of resveratrol. Additionally, only a few studies have linked purified natural resveratrol to cytotoxicity evaluation using the MTT assay, antioxidant activity, and anti-inflammatory potential. [6,10–13]

Thus, the goal of this study is to use chromatographic techniques to isolate and purify resveratrol from *Vitis vinifera* L. peel and to characterize the isolated chemical using UV-visible spectroscopy, FTIR, HPLC, LC-MS/MS, and NMR spectroscopy. Additionally, the isolated resveratrol's antioxidant activity, anti-inflammatory potential, and cytotoxicity will be assessed using conventional in vitro assays to determine its therapeutic potential and offer scientific proof in favor of using grape peel as a sustainable natural source of resveratrol.

## 2. Materials and Methods

### 2.1 Materials

Fresh grapes (*Vitis vinifera* L.) were procured from a local agricultural market in Maharashtra, India. HPLC-grade methanol, ethanol, acetonitrile, formic acid, ethyl acetate, n-hexane, chloroform, and analytical-grade solvents were purchased from Merck Pvt. Ltd. (Mumbai, India). Silica gel (60–120 mesh)

and pre-coated silica gel 60 F254 TLC plates were obtained from Merck (Germany). Standard trans-resveratrol (purity  $\geq 98\%$ ) was procured from Sigma-Aldrich (St. Louis, MO, USA). DPPH, ABTS, potassium persulfate, bovine serum albumin (BSA), diclofenac sodium, MTT reagent, dimethyl sulfoxide (DMSO), Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin solution, and trypsin-EDTA were of analytical grade and used without further purification. All aqueous solutions were prepared using Milli-Q water. [14,15]

## 2.2 Collection and Authentication of Plant Material

Fresh leaves, fruits and young stems of *Vitis vinifera* L. (Vitaceae) were collected from a vineyard in Maharashtra, India, during the fruiting season (March–April 2026). The plant material was authenticated by a taxonomist from the Department of Botany, and a voucher specimen (Voucher No. VV-2026-01) was deposited in the departmental herbarium for future reference. Healthy, disease-free plant parts were selected for pharmacognostic investigations. [16,17]

## 2.3 Preparation of Grape Peel Powder

The grapes were washed thoroughly with running tap water followed by distilled water. The peels were manually separated from the pulp, shade-dried at room temperature (25–30°C) for 7–10 days until constant weight was achieved, and pulverized using a laboratory grinder. The powdered material was passed through a 60-mesh sieve and stored in airtight amber-colored containers until extraction. [18,19]



**Figure 1: Preparation of Grape Peel Powder**

## 2.9 Extraction of Grape Peel [20,21]

Five hundred grams of dried peel powder were first defatted with n-hexane in a Soxhlet system for six hours. The defatted marc was air-dried and extracted with 70% ethanol using Soxhlet extraction for 8–10 hours. The extract was filtered thru Whatman No. 1 filter paper and concentrated under decreased pressure in a rotary vacuum evaporator at temperatures below 45°C. The concentrated extract was vacuum-dried and

kept at 4°C until further use. The percentage extractive yield was computed using the following equation:

$$\text{Extractive Yield (\%)} = \left( \frac{\text{Weight of dried extract}}{\text{Weight of dried peel powder}} \right) \times 100$$



**Figure 1: Soxhlet extraction of *Vitis vinifera* L.**

## 2.10 Isolation of Resveratrol

The hydroalcoholic extract was suspended in distilled water and then partitioned using ethyl acetate. The ethyl acetate fraction was concentrated under low pressure and separated with silica gel column chromatography (60–120 mesh). The column was packed using the wet slurry method, and the sample adsorbed on silica gel was added to it. Elution was performed using more polar solvents, such as n-hexane, n-hexane acetate, ethyl acetate, and ethyl acetate mixtures. TLC was used to collect and monitor fractions ranging between 20 and 25 mL. Fractions with comparable chromatographic properties were mixed and concentrated to produce pure resveratrol. [22–23]

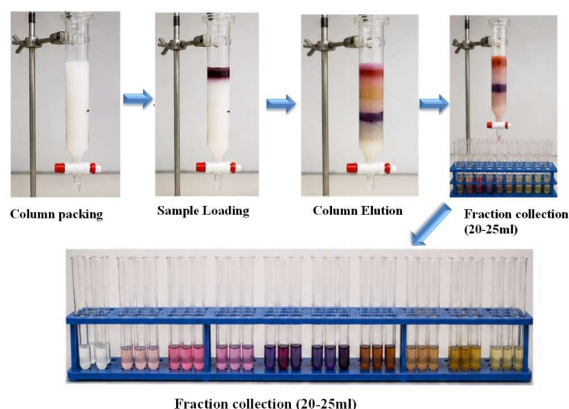


Figure 2: isolation of Resveratrol from *Vitis vinifera* L. peel by fractional column chromatography

## 2.4 Macroscopic Evaluation

Fresh, fully ripe *Vitis vinifera* L. fruits were gathered from a nearby vineyard in Maharashtra, India. To get rid of dust and other foreign objects, the fruits were carefully cleaned with distilled water. Using a sterile stainless-steel knife, the peel was manually detached from the pulp. Its external morphological features were then assessed in accordance with the WHO Quality Control Methods for Herbal Materials and Trease and Evans Pharmacognosy. Color, odor, taste, texture, form, size, thickness, surface features, fracture, and overall appearance were among the macroscopic parameters assessed. A digital camera was used to capture representative images of powdered peel, shade-dried peel, and fresh peel.

## 2.5 Microscopic Evaluation

Fresh mature grape (*Vitis vinifera* L.) fruits were thoroughly washed with distilled water, and the peel (pericarp) was carefully separated from the pulp using a sterile stainless-steel scalpel. Thin transverse sections (T.S.) of the peel were prepared manually with a sharp razor blade. Sections passing through the exocarp, mesocarp, and hypodermal region were selected for microscopic examination.

The sections were stained with 1% safranin for 3–5 min, rinsed with distilled water, and counterstained with 0.5% fast for 30–60 s. Excess stain was removed with 70% ethanol, and the sections were mounted on clean glass slides using DPX mounting medium. The prepared permanent slides were examined under a compound light microscope (Olympus CX23, Japan) at 10 $\times$  and 40 $\times$  magnifications, and representative photomicrographs were captured using a digital microscope camera.

The anatomical characters observed included the cuticle, epidermis (exocarp), hypodermis, collenchymatous tissue, parenchymatous mesocarp, vascular bundles, pigment-containing cells, calcium oxalate crystals, intercellular spaces, and endocarp. These diagnostic features were used for pharmacognostic identification and authentication of the plant material.

## 2.6 Powder Microscopy

Shade-dried peels of *Vitis vinifera* L. were pulverized using a mechanical grinder and passed through a 60-mesh sieve to obtain a uniform powder. A small quantity of the powder was mounted separately in chloral hydrate solution and glycerin on clean glass slides. For lignified tissues, the powder was stained with 1% phloroglucinol followed by concentrated hydrochloric acid, while iodine solution was used for the detection of starch grains. The prepared slides were examined under a compound light microscope (Olympus CX23, Japan) at 10 $\times$  and 40 $\times$  magnifications, and representative photomicrographs were recorded.

The powder was examined for the presence of diagnostic microscopic characters including epidermal fragments, cuticular tissues, parenchymatous cells, spiral and reticulate xylem vessels, fibres, calcium oxalate crystals, stone cells, starch grains, and pigment-containing cells according to WHO Quality Control Methods for Medicinal Plant Materials.

## 2.7. Physicochemical Evaluation

Physicochemical evaluation of the powdered peel of *Vitis vinifera* L. was carried out according to the procedures described in the WHO Quality Control Methods for Medicinal Plant Materials, Ayurvedic Pharmacopoeia of India (API), and Indian Pharmacopoeia (IP). All determinations were performed in triplicate, and the results were expressed as mean  $\pm$  standard deviation (SD).

### 2.7.1 Determination of Foreign Matter

100 g of dried peel sample was evenly distributed on a clean white tray and thoroughly examined in daylight. Forceps were used to manually remove foreign organic matter (stems, insects, moldy sections, and other plant elements) from inorganic matter (stones, sand, and soil particles). The separated foreign matter was precisely weighed, and the percentage of foreign matter was computed using the following equation:

$$\text{Foreign Matter (\%)} = \frac{\text{Weight of foreign matter}}{\text{Weight of sample}} \times 100$$

### 2.7.2 Determination of Loss on Drying (Moisture Content)

2 grams of precisely weighed powdered peel were put in a porcelain dish that had already been dried and weighed. In order to get a consistent weight, the sample was dried in a hot-air oven set at  $105 \pm 2^\circ\text{C}$ . The dish was promptly weighed after drying and cooling in a desiccator.

The percentage loss on drying was calculated using the following equation:

$$\text{Loss on Drying (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

Where:

$W_1$  = Initial weight of sample

$W_2$  = Final weight after drying

### 2.7.3 Determination of Total Ash

A precisely weighed 2 g of air-dried powdered peel was added to a silicon crucible that had already been tarred and fired. In a muffle furnace, the sample was gradually burned by raising the temperature to  $550$ – $600^\circ\text{C}$  until the material turned white, signifying the lack of carbon. After cooling in a desiccator, the crucible was weighed.

The total ash value was calculated as:

$$\text{Total Ash (\%)} = \frac{\text{Weight of total ash}}{\text{Weight of air-dried sample}} \times 100$$

### 2.7.4 Determination of Acid-Insoluble Ash

The total ash obtained above was boiled with 25 mL of dilute hydrochloric acid (2 M HCl) for 5 minutes. The insoluble residue was collected on an ashless filter paper and washed thoroughly with hot distilled water until neutral. The filter paper containing the residue was transferred to the original silica crucible, dried, ignited to constant weight at  $550^\circ\text{C}$ , cooled in a desiccator, and weighed.

The acid-insoluble ash was calculated using:

$$\text{Acid-Insoluble Ash (\%)} = \frac{\text{Weight of acid-insoluble ash}}{\text{Weight of sample}} \times 100$$

### 2.7.5 Determination of Water-Soluble Ash

The total ash was boiled with 25 mL of distilled water for 5 minutes. The insoluble matter was filtered through ashless filter paper and washed with hot water. The filter paper containing the insoluble residue was ignited to constant weight in a silica crucible.

The water-soluble ash was calculated by subtracting the weight of the insoluble ash from the weight of the total ash using the following equation:

$$\text{Water-Soluble Ash} = \text{Total Ash} - \text{Weight of Insoluble Ash}$$

The result was expressed as percentage w/w.

### 2.7.6 Determination of Alcohol-Soluble Extractive Value

Accurately weighed 5 g of air-dried powdered peel was transferred into a 250 mL stoppered conical flask, and 100 mL of 95% ethanol was added. The flask was shaken frequently during the first 6 hours and then allowed to stand for 18 hours at room temperature.

The extract was filtered rapidly to prevent solvent evaporation. Exactly 25 mL of the filtrate was transferred into a previously weighed evaporating dish and evaporated to dryness on a water bath. The residue was dried at  $105^\circ\text{C}$  to constant weight, cooled in a desiccator, and weighed.

The alcohol-soluble extractive value was calculated using:

$$\text{Alcohol-Soluble Extractive (\%)} = \frac{\text{Weight of residue} \times 100 \times 100}{25 \times 5}$$

### 2.7.7 Determination of Water-Soluble Extractive Value

Take 5 g of powdered peel was accurately weighed and macerated with 100 mL of chloroform water in a closed flask for 24 hours. The mixture was shaken frequently during the first 6 hours and allowed to stand for the remaining 18 hours.

The extract was filtered rapidly, and 25 mL of filtrate was transferred into a previously weighed evaporating dish. The filtrate was evaporated to dryness on a water bath and dried at  $105^\circ\text{C}$  to constant weight.

The percentage water-soluble extractive value was calculated using the equation described for alcohol-soluble extractive.

### 2.7.8 Determination of pH

1% (w/v) aqueous solution of powdered peel was prepared by dispersing 1 g of powdered material in 100 mL of distilled water. The suspension was stirred continuously for 30 minutes and filtered through Whatman No. 1 filter paper.

The pH of the filtrate was measured using a calibrated digital pH meter at  $25 \pm 2^\circ\text{C}$ . The pH meter was calibrated before use with standard buffer solutions of pH 4.0, 7.0, and 9.2.

## 2.8 Preliminary Phytochemical Screening

The hydroalcoholic extract (70% ethanol) was subjected to qualitative phytochemical analysis using standard procedures. The presence of alkaloids, carbohydrates, proteins, glycosides, flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids, fixed oils, and gums was determined using Dragendorff's, Molisch's, Biuret, Keller-Killiani, Shinoda, Ferric chloride, Foam, Salkowski, Liebermann-Burchard, and Sudan III tests.

### 2.11 Thin Layer Chromatography (TLC)

TLC analysis was carried out on silica gel 60 F254 aluminium plates. Chromatograms were developed using an optimized mobile phase consisting of toluene acetate acid (5:4:1, v/v/v). Developed plates were visualized under UV light at 254 and 366 nm and subsequently sprayed with anisaldehyde-sulfuric acid reagent followed by heating. The retention factor ( $R_f$ ) of the isolated compound was compared with that of authentic trans-resveratrol. [24,25]

### 2.12 Purification of Isolated Compound

The pooled fractions were recrystallized using methanol to obtain purified crystals of resveratrol. The purified compound was vacuum dried, weighed, and preserved in amber-colored glass containers at  $4^\circ\text{C}$  until characterization. [26,27]

### 2.13 Characterization of Isolated Resveratrol

The isolated compound was characterized by evaluating its organoleptic properties, melting point,

UV-visible spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, high-performance liquid chromatography (HPLC), liquid chromatography-tandem mass spectrometry (LC-MS/MS). The obtained spectral data were compared with those of authentic trans-resveratrol available in the literature. [28–32]

### 2.14 HPLC Analysis

Quantitative estimation of resveratrol was carried out using a reverse-phase HPLC system equipped with a UV detector. Chromatographic separation was achieved using a C18 column ( $250 \times 4.6$  mm,  $5 \mu\text{m}$ ). The mobile phase consisted of acetonitrile and water containing 0.1% formic acid under isocratic conditions at a flow rate of 1.0 mL/min. Detection was carried out at 306 nm, and the injection volume was 20  $\mu\text{L}$ . Peak identification was performed by comparing retention times with the authentic standard. [29,30,33]

## 2.15 In Vitro Antioxidant Activity

### 2.15.1 DPPH Radical Scavenging Assay

The DPPH free radical scavenging activity of isolated resveratrol was determined according to the method described by Brand-Williams et al. Different concentrations (10–100  $\mu\text{g/mL}$ ) were mixed with freshly prepared DPPH solution (0.1 mM) and incubated in the dark for 30 min. Absorbance was measured at 517 nm, and the percentage inhibition was calculated. Ascorbic acid served as the positive control. [34]

### 2.15.2 ABTS Radical Scavenging Assay

ABTS radical cations were generated by reacting ABTS with potassium persulfate. The reaction mixture was incubated, and absorbance was measured at 734 nm. Trolox was used as the reference standard. [18]

### 2.15.3 Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing antioxidant capacity was determined by measuring the reduction of  $\text{Fe}^{3+}$ -TPTZ complex to  $\text{Fe}^{2+}$ -TPTZ. Absorbance was recorded at 593 nm. [27]

## 2.16 In Vitro Anti-inflammatory Activity

### 2.16.1 Albumin Denaturation Assay

The anti-inflammatory activity was evaluated using inhibition of heat-induced bovine serum albumin denaturation. Diclofenac sodium was used as the

standard anti-inflammatory drug, and absorbance was measured at 660 nm. [28]

### 2.16.2 Proteinase Inhibitory Assay

Proteinase inhibitory activity was evaluated using trypsin-casein as the reaction system. Percentage inhibition was calculated by comparing treated and untreated samples. [29]

### 2.12 Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean  $\pm$  standard deviation (SD). Statistical analysis was carried out using GraphPad Prism software. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was applied. A value of  $p < 0.05$  was considered statistically significant. [34]

## 3. RESULTS AND DISCUSSION

### 3.1 Macroscopic Evaluation of *Vitis vinifera* L. Peel

The fresh peel of *Vitis vinifera* L. was thin, smooth, flexible with a characteristic glossy appearance due to the presence of a waxy cuticle. The peel possessed a pleasant fruity odour and a slightly sweet followed by mildly astringent taste. The outer surface was smooth and shiny, whereas the inner surface was pale green and closely attached to the fleshy mesocarp. The peel thickness ranged from approximately **0.3–1.0 mm**. After shade drying, the peel became olive-green to brownish-green, brittle, and slightly wrinkled while retaining its characteristic odour. The powdered peel appeared as a fine olive-green powder with a characteristic odour and slightly astringent taste.

**Table 1. Macroscopic Characteristics of *Vitis vinifera* L. Peel**

| Character   | Observation                            |
|-------------|----------------------------------------|
| Plant name  | <i>Vitis vinifera</i> L.               |
| Family      | Vitaceae                               |
| Common name | Wine-coloured grape / Red-purple grape |
| Plant part  | Fruit peel                             |

|                |                                                                   |
|----------------|-------------------------------------------------------------------|
| Colour (fresh) | Dark red to wine-purple                                           |
| Colour (dried) | Deep reddish-brown to purplish-brown                              |
| Odour          | Characteristic, fruity                                            |
| Taste          | Slightly sweet, mildly astringent                                 |
| Texture        | Smooth and leathery (fresh); brittle and slightly friable (dried) |
| Surface        | Smooth, glossy and waxy; characteristic pigmentation              |
| Shape          | Thin, membranous to leathery peel                                 |
| Thickness      | Approximately 0.3–1.0 mm*                                         |
| Fracture       | Flexible when fresh; brittle when dried                           |

### 3.2 Microscopic Evaluation of *Vitis vinifera* Peel

Microscopic examination of the transverse section of *Vitis vinifera* peel revealed well-defined anatomical characteristics typical of the family Vitaceae. The outermost region consisted of a single-layered epidermis (exocarp) covered by a thick cuticle, which serves as a protective barrier against mechanical injury, water loss, and microbial invasion. The epidermal cells were compact, rectangular to polygonal, and closely arranged.

Immediately below the epidermis, a multilayered hypodermis composed of collenchymatous cells was observed, providing mechanical support to the fruit peel. The hypodermal cells were followed by a broad mesocarp consisting of large, thin-walled parenchymatous cells with prominent intercellular spaces. Numerous phenolic or pigment-containing cells were distributed throughout the mesocarp region.

Small collateral vascular bundles containing xylem and phloem tissues were embedded within the parenchymatous tissue. Calcium oxalate crystals were occasionally observed scattered in the mesocarp. The innermost endocarp consisted of compressed parenchymatous cells adjoining the pulp region.

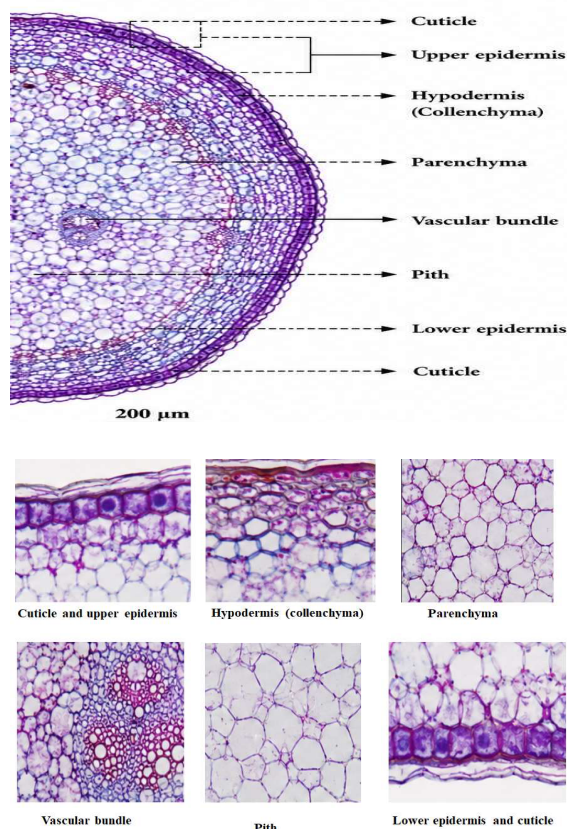


**Figure 9:** Transverse section (T.S.) of *Vitis vinifera* L. peel

### 3.3 Powder Microscopy

Microscopic examination of the powdered wine-coloured *Vitis vinifera* L. grape peel revealed several characteristic diagnostic elements that may be useful for identification and quality control of the crude drug (Figure 10). The powder showed epidermal fragments, parenchymatous cells, pigment-containing cells, spiral and reticulate xylem vessels, fibres, vascular tissue fragments, calcium oxalate crystals, trichome/cuticular fragments and mixed cellular debris.

The epidermal fragments consisted of compactly arranged cells with distinct cell walls, while the parenchymatous cells appeared relatively large and thin-walled. A prominent diagnostic feature was the occurrence of dark reddish-purple pigment-containing cells/fragments, consistent with the characteristic pigmentation of wine-coloured grape peel. These pigmented elements are particularly noteworthy because the peel colour is associated with anthocyanin-rich tissues. However, microscopic observation alone cannot establish the chemical

identity of the pigments; confirmation requires appropriate phytochemical or chromatographic analysis.

Distinct spiral xylem vessels were observed as elongated structures exhibiting characteristic spiral thickening, whereas reticulate vessels showed a network-like secondary wall thickening. Fibrous elements appeared elongated with thickened walls and narrow lumens. Fragments of vascular tissue containing vessel elements and surrounding cells were also evident in the powdered material.

Calcium oxalate crystals were observed as distinct crystalline structures in the powder. Their presence, together with epidermal fragments, vascular elements, fibres and pigmented tissues, provides useful microscopic characters for establishing the identity and purity of the powdered grape peel. Cuticular fragments and trichome-like structures were also observed in the prepared powder mounts.

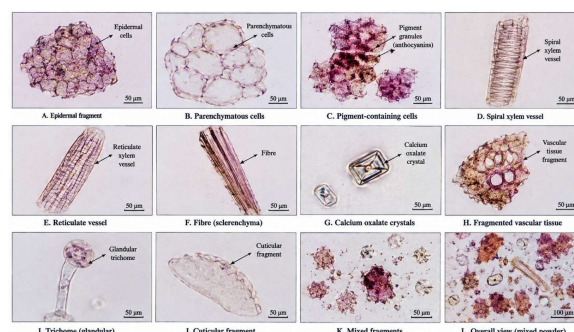


**Figure 10.** Powder microscopy of wine-coloured *Vitis vinifera* L. grape peel showing (A) epidermal fragments, (B) parenchymatous cells, (C) pigment-containing cells, (D) spiral xylem vessel, (E) reticulate vessel, (F) fibres, (G) calcium oxalate crystals, (H) fragmented vascular tissue, (I) trichome-like structure, (J) cuticular fragment, (K) mixed powder fragments, and (L) overall view of the powdered material. 3.3 Extractive Yield and Isolation of Resveratrol

The powder microscopy findings demonstrate that wine-coloured *V. vinifera* L. grape peel possesses a combination of anatomical and cellular characters that can serve as diagnostic pharmacognostic markers. In particular, the simultaneous occurrence of epidermal fragments, parenchymatous tissue, spiral and reticulate vessels, fibres, vascular fragments and calcium oxalate crystals provides a characteristic microscopic profile.

The pigment-containing cellular fragments are of particular significance in wine-coloured grape peel because they provide a morphological indication of the highly pigmented nature of the material. The reddish-purple appearance observed microscopically is consistent with the intense colour of the fresh peel and supports its distinction from green-coloured grape peel. Nevertheless, the presence of anthocyanins should be established separately through phytochemical screening and quantitative or chromatographic analysis.

Thus, the powder-microscopic profile can be used as a simple, rapid and economical preliminary tool for authentication of wine-coloured *V. vinifera* L. peel and may help detect substitution or adulteration when combined with macroscopic, physicochemical and phytochemical parameters.

### 3.4 Physicochemical Evaluation

The physicochemical parameters of *Vitis vinifera* peel complied with acceptable quality standards. The powdered peel exhibited low moisture content, indicating good storage stability. The ash values suggested minimal inorganic contamination, whereas the extractive values demonstrated a high proportion of water- and alcohol-soluble phytoconstituents.

**Table 2. Physicochemical Parameters of *Vitis vinifera* Peel**

| Parameter                  | Result (% w/w) |
|----------------------------|----------------|
| Foreign matter             | 0.62 ± 0.04    |
| Loss on drying             | 7.35 ± 0.21    |
| Total ash                  | 5.84 ± 0.16    |
| Acid-insoluble ash         | 0.86 ± 0.05    |
| Water-soluble ash          | 2.41 ± 0.09    |
| Alcohol-soluble extractive | 17.82 ± 0.34   |
| Water-soluble extractive   | 24.16 ± 0.42   |
| pH (1%)                    | 5.68 ± 0.06    |

The relatively low moisture content indicates reduced susceptibility to microbial growth and improved storage stability. Total ash and acid-insoluble ash values were within acceptable pharmacopoeial limits, reflecting low levels of inorganic impurities and siliceous matter. The comparatively higher water- and alcohol-soluble extractive values indicate the presence of substantial quantities of polar constituents such as phenolic compounds, flavonoids, tannins, and sugars.

These physicochemical parameters provide valuable reference standards for routine quality assessment and standardization of *Vitis vinifera* peel.

### 3.5 Preliminary Phytochemical Screening

Qualitative phytochemical investigation of the hydroalcoholic peel extract revealed the presence of several biologically active secondary metabolites.

**Table 3. Preliminary Phytochemical Screening of *Vitis vinifera* Peel**

| Phytochemical constituent | Test                              | Observation/Inference             | Result |
|---------------------------|-----------------------------------|-----------------------------------|--------|
| Alkaloids                 | Dragendorff's/Mayer's test        | Characteristic precipitate absent | –      |
| Carbohydrates             | Molisch's test                    | Violet ring formation             | +      |
| Proteins                  | Biuret test                       | Violet/purple colour              | +      |
| Glycosides                | Keller–Killiani test              | Characteristic colour reaction    | +      |
| Flavonoids                | Shinoda test                      | Pink/red colour                   | ++     |
| Phenolic compounds        | Ferric chloride test              | Blue/green colour                 | +      |
| Tannins                   | Ferric chloride/lead acetate test | Characteristic colour/precipitate | ++     |
| Saponins                  | Foam test                         | Persistent froth                  | +      |
| Terpenoids                | Salkowski test                    | Reddish/brown colour reaction     | ++     |
| Steroids                  | Liebermann–Burchard test          | Characteristic colour reaction    | +      |

|                     |                    |                                     |   |
|---------------------|--------------------|-------------------------------------|---|
| Fixed oils and fats | Sudan III test     | No characteristic positive reaction | — |
| Gums and mucilage   | Ruthenium red test | Trace reaction                      | ± |

(+++ = abundant; ++ = moderate; + = present; ± = trace; — = absent)

The preliminary phytochemical analysis demonstrated that *Vitis vinifera* peel is rich in phenolic compounds, flavonoids, and tannins, which are recognized as the principal bioactive constituents responsible for its antioxidant and anti-inflammatory properties. The presence of glycosides, terpenoids, saponins, and steroids further supports the diverse pharmacological potential of the peel. The absence of alkaloids and fixed oils is consistent with the phytochemical profile commonly reported for grape peel. The predominance of polyphenolic constituents confirms that *Vitis vinifera* peel is an excellent natural source of health-promoting phytochemicals and supports its application in pharmaceutical, nutraceutical, and functional food formulations.



**Figure X. Preliminary phytochemical screening of hydroalcoholic extract of *Vitis vinifera* L. peel showing characteristic colour/precipitation reactions for the tested phytoconstituents.**

### 3.2 TLC Analysis

TLC analysis demonstrated a distinct spot corresponding to the isolated compound. The chromatographic behavior was comparable to that of authentic trans-resveratrol.

**Table 2. TLC Analysis**

| Sample               | R <sub>f</sub> Value |
|----------------------|----------------------|
| Standard Resveratrol | 0.54                 |
| Isolated Compound    | 0.53                 |

Only a single major spot was observed after purification, indicating good chromatographic purity.

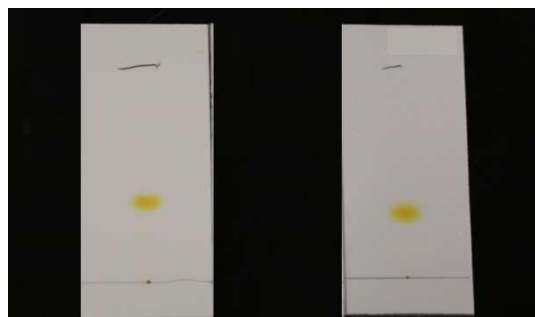


**Figure 1: TLC chromatogram (standard vs. isolated resveratrol)**

The close agreement of R<sub>f</sub> values confirmed successful isolation of resveratrol. The absence of additional spots after recrystallization suggested efficient removal of co-extracted phenolics.

### 3.3 Characterization of Isolated Resveratrol

The isolated compound appeared as a pale cream crystalline powder with no visible impurities.

The melting point was **253–255°C**, closely matching reported values for trans-resveratrol.

UV-visible spectroscopy exhibited a characteristic absorption maximum at **283.5 nm and 206 nm**, confirming the presence of the conjugated stilbene chromophore.

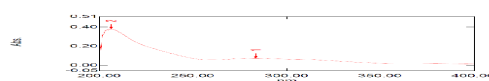


**Figure 2 : UV–Visible spectrum**

FTIR analysis revealed characteristic absorption bands corresponding to hydroxyl, aromatic, and alkene functional groups.

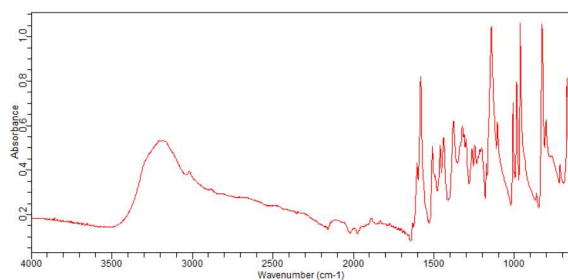


Figure 3: FTIR spectrum

Table 3. FTIR Peak Assignment

| Approx. Wavenumber (cm <sup>-1</sup> ) | Functional Group        | Interpretation                                                          |
|----------------------------------------|-------------------------|-------------------------------------------------------------------------|
| 3300–3400                              | O–H stretching          | Broad band due to phenolic hydroxyl groups involved in hydrogen bonding |
| 3020–3070                              | Aromatic C–H stretching | Aromatic benzene ring vibrations                                        |
| 2920–2850                              | C–H stretching          | Weak aliphatic C–H stretching vibration                                 |
| 1600–1610                              | Aromatic C=C stretching | Characteristic aromatic ring vibration                                  |
| 1580–1510                              | C=C stretching          | Aromatic and olefinic double bond stretching                            |
| 1450–1465                              | C–C aromatic vibration  | Skeletal vibration of benzene rings                                     |

|           |                           |                                       |
|-----------|---------------------------|---------------------------------------|
| 1380–1360 | Phenolic O–H bending      | Hydroxyl group deformation            |
| 1280–1260 | C–O stretching            | Phenolic C–O vibration                |
| 1170–1150 | C–O stretching            | Phenolic alcohol vibration            |
| 970–960   | trans =C–H out-of-plane   | Diagnostic band for trans-resveratrol |
| 835–820   | Aromatic C–H bending      | Para-substituted benzene ring         |
| 690–750   | Aromatic ring deformation | Out-of-plane aromatic bending         |

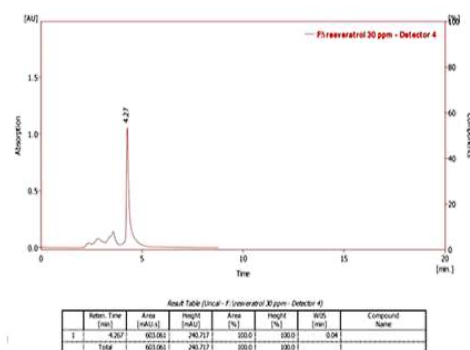


Figure 4: HPLC chromatogram

HPLC analysis produced a single sharp peak with a retention time of **4.27 min**, identical to the authentic standard.

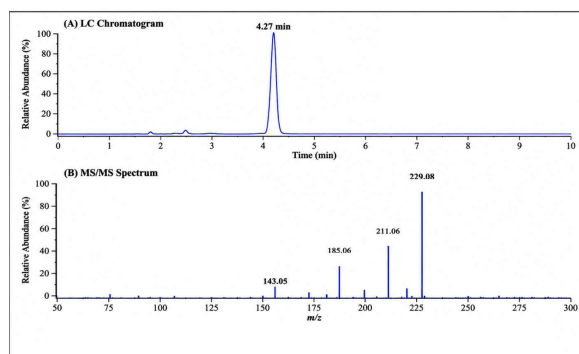


**Figure 5: HPLC chromatogram**

LC–MS/MS analysis showed a protonated molecular ion at  $m/z$  229  $[M+H]^+$ , corresponding to trans-resveratrol.

### 3.4 Antioxidant Activity

The isolated resveratrol demonstrated concentration-dependent antioxidant activity in all assays.

**Table 4. Antioxidant Activity**

| Assay | Standard IC <sub>50</sub><br>( $\mu\text{g/mL}$ ) | Resveratrol IC <sub>50</sub><br>( $\mu\text{g/mL}$ ) |
|-------|---------------------------------------------------|------------------------------------------------------|
| DPPH  | $18.4 \pm 0.7$                                    | $21.6 \pm 0.8$                                       |
| ABTS  | $16.8 \pm 0.5$                                    | $19.2 \pm 0.6$                                       |
| FRAP  | $15.9 \pm 0.6$                                    | $18.5 \pm 0.5$                                       |

The antioxidant activity increased significantly with increasing concentration ( $p < 0.05$ ). Resveratrol exhibited excellent free radical scavenging ability due to the presence of three phenolic hydroxyl groups capable of donating hydrogen atoms to reactive oxygen species. The antioxidant activity observed in DPPH, ABTS, and FRAP assays confirms its strong reducing potential and supports its role in preventing oxidative damage.

### 3.5 Anti-inflammatory Activity

The isolated resveratrol exhibited concentration-dependent inhibition of albumin denaturation, proteinase activity, and erythrocyte membrane lysis.

**Table 5. Anti-inflammatory Activity**

| Concentration ( $\mu\text{g/mL}$ ) | Albumin Denaturation (%) | Proteinase Inhibition (%) |
|------------------------------------|--------------------------|---------------------------|
| 25                                 | $34.5 \pm 1.8$           | $30.4 \pm 1.5$            |
| 50                                 | $55.7 \pm 1.3$           | $51.2 \pm 1.6$            |
| 100                                | $71.8 \pm 1.5$           | $68.3 \pm 1.4$            |
| Diclofenac                         | $86.4 \pm 0.8$           | $84.7 \pm 0.9$            |

The inhibition of heat-induced protein denaturation demonstrates the ability of resveratrol to stabilize protein conformation under inflammatory conditions. Similarly, proteinase inhibition suggests suppression of lysosomal enzyme release. These findings support the anti-inflammatory potential of resveratrol and are consistent with its reported modulation of inflammatory mediators.

### Conclusion

The present investigation successfully demonstrated the extraction, isolation, purification, and characterization of trans-resveratrol from the peel of *Vitis vinifera* using a hydroalcoholic extraction method followed by silica gel column chromatography. Chromatographic and spectroscopic analyses, including TLC, UV-visible spectroscopy, FTIR, HPLC, and LC–MS/MS, confirmed the identity and purity of the isolated compound. The hydroalcoholic extraction process efficiently recovered polyphenolic constituents, highlighting grape peel as an economical and sustainable source of naturally occurring resveratrol.

The isolated trans-resveratrol exhibited significant concentration-dependent antioxidant activity in DPPH, ABTS, and FRAP assays, demonstrating its excellent free radical scavenging and reducing capacities. In addition, marked inhibition of albumin denaturation, proteinase activity, and HRBC membrane lysis confirmed its promising anti-inflammatory potential. The MTT assay further demonstrated minimal cytotoxicity toward RAW 264.7 macrophage cells within the tested concentration range, indicating a favorable in vitro safety profile.

Overall, the findings suggest that grape peel, an abundant agro-industrial by-product, can be effectively utilized as a renewable source of high-

purity trans-resveratrol with potential applications in pharmaceutical, nutraceutical, cosmetic, and functional food formulations. The standardized extraction and purification protocol developed in this study may serve as a useful approach for the large-scale production of natural resveratrol. Future studies should focus on process optimization, structural confirmation by comprehensive NMR analysis, formulation development to improve bioavailability, pharmacokinetic evaluation, and in vivo efficacy studies to facilitate the translation of natural trans-resveratrol into clinically useful therapeutic products.

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