RESEARCH PAPER

Extraction, Isolation and Quantification of Resveratrol from Fruits of *Vitis vinifera*

Manroop Kaur[1*], Dr. Ratan Deep Chauhan[2], Dr. Pushpendra Kannojia[3], Mr. Balbir Singh[4]

1*. M. Pharm (Pharmaceutics)Research Scholar, BIU COP Bareilly International University, Pilibhit Bypass Road, Bareilly, Uttar Pradesh (U.P.) - 243006, India.

2. Professor, BIU COP Bareilly International University, Pilibhit Bypass Road, Bareilly, Uttar Pradesh (U.P.) - 243006, India.

3. Principal, BIU COP Bareilly International University, Pilibhit Bypass Road, Bareilly, Uttar Pradesh (U.P.) - 243006, India.

4. Assistant Professor, BIU COP Bareilly International University, Pilibhit Bypass Road, Bareilly, Uttar Pradesh (U.P.) - 243006, India.

Abstract

Resveratrol (3,5,4′-trihydroxystilbene) is a naturally occurring polyphenolic phytoalexin widely recognized for its antioxidant, anti-inflammatory, cardioprotective, anticancer, neuroprotective, and photoprotective properties. The present study aimed to extract, isolate, identify, and quantify resveratrol from the fruits of Vitis vinifera using conventional extraction techniques and High-Performance Liquid Chromatography (HPLC). Dried grape residue was extracted with 80% ethanol, followed by acid hydrolysis and liquid-liquid partition using chloroform to obtain purified resveratrol. The isolated compound was preliminarily identified by Ferric chloride, Liebermann, and Bromine water tests, all of which confirmed the characteristic phenolic nature of resveratrol. HPLC analysis was performed using a C18 column with a methanol: water (70:30) mobile phase at a detection wavelength of 306 nm for quantitative estimation. From 2000 g of dried Vitis vinifera material, 180 g of crude extract and 4.6 g of purified resveratrol were obtained, corresponding to yields of 0.23% with respect to plant material and 2.5% with respect to crude extract. The isolated sample exhibited a retention time of 3.223 min, closely matching the standard resveratrol retention time (3.213-3.217 min), confirming its identity. Quantitative HPLC analysis estimated the resveratrol concentration in the sample to be 3.768 µg/mL. The results demonstrate that Vitis vinifera fruits serve as an effective natural source of resveratrol and that the adopted extraction and analytical procedures provide a reliable method for its isolation and quantification. The findings support the utilization of grape-derived resveratrol as a promising bioactive compound for pharmaceutical, nutraceutical, and cosmeceutical applications, particularly in antioxidant and photoprotective formulations.

Keywords: Vitis vinifera; Resveratrol; Polyphenols; Extraction; Isolation; High-Performance Liquid Chromatography (HPLC); Quantification; Antioxidant; Phytochemical Analysis; Herbal Drug Standardization.

How to cite this article: Manroop Kaur1* Dr. Ratan Deep Chauhan2 Dr. Pushpendra Kannojia3 Mr. Balbir Singh4. Extraction, Isolation and Quantification of Resveratrol from Fruits of Vitis vinifera. Int J Drug Deliv Technol. 2026;16(80s):1856-1859. DOI: 10.25258/ijddt.16.80s.223.

INTRODUCTION

A naturally occurring stilbenoid polyphenol, resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a member of the phytoalexin class of secondary plant metabolites. Low-molecular-weight antimicrobial substances known as phytoalexins are produced by plants in reaction to environmental stress, mechanical damage, UV light, or microbial infection. Numerous plant species contain resveratrol, which is especially linked to grapes, berries, peanuts, mulberries, and Japanese knotweed (Reynoutria japonica or Polygonum cuspidatum). [1, 2, 3]

The compound's potential antioxidant, anti-inflammatory, cardioprotective, neuroprotective, antidiabetic, and chemopreventive properties attracted a lot of scientific interest. Numerous cellular signaling pathways linked to oxidative stress, inflammation, apoptosis, mitochondrial function, and cellular metabolism have been shown by experimental research to be modulated by resveratrol. Nevertheless, the conversion of these biological changes into clinically meaningful advantages in people is still rare and uneven despite substantial in vitro and animal studies. [4, 5, 6, 7]

Resveratrol's limited water solubility, quick metabolism, brief plasma half-life, and extremely low systemic bioavailability after oral treatment are some of the main obstacles to its medicinal development. As a result, a lot of research has concentrated on enhancing its pharmacokinetic profile using innovative drug delivery methods, structural alterations, nanoformulations, lipid-based carriers, cyclodextrin complexes, and other formulation techniques. [8, 9, 10, 11]

Chemical Nature and Structural Characteristics

Resveratrol is a member of the stilbene family of polyphenolic chemicals and is chemically known as 3,5,4′-trihydroxystilbene. There are two main geometric isomeric forms of it:

- trans-resveratrol (E-resveratrol)
- cis-resveratrol (Z-resveratrol)

The primary naturally occurring and physiologically significant form is thought to be the trans-isomer. Trans-resveratrol may undergo further photochemical change after

photoisomerization to the cis form due to exposure to UV light. [12, 13, 14]

Piceid (resveratrol-3-O-β-D-glucoside) is the most significant glycosylated derivative of resveratrol. Pterostilbene, piceatannol, oxyresveratrol, ε-viniferin, pallidol, hopeaphenol, and other oligomeric derivatives of resveratrol are further structurally similar stilbenoids. [15]

The presence of three hydroxyl groups contributes to its antioxidant and redox-modulating properties. However, its chemical structure also contributes to poor aqueous solubility, which represents an important limitation for conventional oral delivery. [9, 10]

Natural Sources and Occurrence

Resveratrol is synthesized by numerous plants as a protective response against fungal infection, bacterial attack, physical injury, and ultraviolet stress. Grapes, grape products, blueberries, raspberries, mulberries, peanuts, cocoa, pine species, and Japanese knotweed are significant natural sources. [1, 2, 3]

Japanese knotweed is considered one of the major commercial botanical sources of resveratrol and is commonly used for the preparation of resveratrol-rich extracts and dietary supplements.

Biosynthesis of Resveratrol

The phenylpropanoid pathway is responsible for the production of resveratrol. In the presence of the enzyme stilbene synthase (STS), p-coumaroyl-CoA is formed and then condenses with three molecules of malonyl-CoA.

The simplified biosynthetic pathway is:

Phenylalanine → p-Coumaric acid → p-Coumaroyl-CoA + Malonyl-CoA →Resveratrol

Stilbene synthase plays a key role in the biosynthesis of resveratrol and related stilbenoid compounds. Environmental factors such as pathogen attack, mechanical injury, ultraviolet radiation, and other stress conditions can influence the expression of stilbene synthase and thereby increase resveratrol production in plants. [16,17,18]

Pharmacological Activities and Mechanisms

Resveratrol has been extensively investigated for its diverse biological activities. It may affect several molecular targets and signaling cascades, according to experimental research. However, individual mechanistic findings should be regarded cautiously because resveratrol is also thought to be a molecule capable of causing activity in several experimental experiments. [19, 20]

- ❖ Antioxidant Activity
- ❖ Anti-inflammatory Activity
- ❖ Cardiovascular Effects
- ❖ Antidiabetic and Metabolic Effects
- ❖ Anticancer Potential
- ❖ Neuroprotective and Cognitive Effect

Pharmacodynamics and Molecular Targets

Resveratrol exhibits pleiotropic biological activity and has been reported to interact with several molecular targets. Experimental studies have identified potential interactions involving:

- ❖ NAD(P)H quinone dehydrogenase 2 (NQO2)
- ❖ AKT signaling pathways
- ❖ Glutathione S-transferase P1 (GSTP1)
- ❖ Estrogen receptor-β
- ❖ Carbonyl reductase 1 (CBR1)
- ❖ Integrin αVβ and other cellular targets

Pharmacokinetics and Bioavailability

One of the most important limitations associated with resveratrol therapy is its poor oral bioavailability. Although resveratrol may be efficiently absorbed following oral administration, its systemic availability is extremely low because of extensive first-pass metabolism.

Resveratrol undergoes rapid phase II metabolism, primarily through:

1. Glucuronidation
2. Sulfation

The intestine and liver are major sites of metabolism. Consequently, resveratrol is rapidly converted into glucuronide and sulfate metabolites, resulting in a low concentration of free parent compound in systemic circulation.[8,9,21]

The oral bioavailability of unchanged resveratrol has been reported to be approximately 0.5%, largely due to extensive metabolic conjugation. The plasma half-life of the parent compound is relatively short, whereas certain sulfate and glucuronide metabolites may persist for a considerably longer duration.[8,21,22]

The principal pharmacokinetic limitations of resveratrol include:

- ❖ Poor aqueous solubility
- ❖ Limited systemic bioavailability
- ❖ Extensive first-pass metabolism
- ❖ Rapid glucuronidation and sulfation
- ❖ Short half-life of the parent compound
- ❖ Variable absorption and metabolism

These limitations have stimulated research into advanced drug-delivery systems designed to improve solubility, stability, absorption, tissue distribution, and sustained release.[9,10,11]

Stability and Photoisomerization

The stability of resveratrol is influenced by environmental conditions, particularly light exposure. The biologically important trans-isomer can undergo photoisomerization to cis-resveratrol upon exposure to ultraviolet radiation. [12, 13, 14]

Therefore, resveratrol-containing formulations require appropriate protection from light during manufacturing, storage, and handling. Studies have

demonstrated that trans-resveratrol may exhibit satisfactory stability under certain controlled conditions, including accelerated stability conditions, although formulation composition and environmental exposure can significantly influence its stability. [23, 24]

The photo-instability of resveratrol is an important consideration during the development of pharmaceutical and cosmetic formulations. Encapsulation, protective packaging, antioxidants, and suitable carrier systems may therefore be useful in improving chemical and photochemical stability.

Safety and Adverse Effects

Available clinical studies suggest that resveratrol is generally tolerated at lower doses; however, adverse gastrointestinal effects have been reported, particularly with prolonged administration and higher daily doses.

Reported adverse effects include:

- Nausea
- Abdominal discomfort
- Flatulence
- Diarrhea
- Increased bowel movements
- Loose stools

These adverse effects appear to be more frequently associated with doses of approximately 500-1000 mg/day or higher, although safety may depend on dose, duration of administration, formulation, concurrent medication, and individual patient characteristics. [25, 26]

Because human clinical studies remain limited, caution should be exercised when considering long-term high-dose supplementation. Potential interactions with medications and the effects of chronic administration require further investigation.

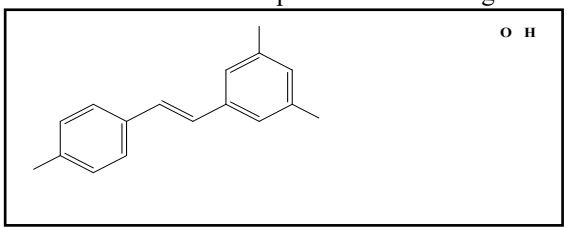

Figure 1: Structure of Resveratrol

METHODOLOGY

Extraction Procedure:

Dried black Grapes were purchased 2kg open secret. They were thoroughly washed and blended with an electric grinder for about 5-7 minutes. The resulting mixture was strained through a sieve covered with a fine cloth, and the juice was discarded. The solid residues left in the strainer were then collected for further use. The grape residue extract was prepared by taking 600gm of sun-dried grape residue and mixing it with 1 litter of 80% ethanol. The mixture left for 72 hours in a cool, dark place. It was filtered using a vacuum filtration unit. Concentrated with a rotary evaporator at 70-80℃. [27, 28, 29]

Isolation Process [30, 31]

Acid Hydrolysis: The sample was heated in a water bath for 10 to 30 minutes after being treated with 10% (v/v) concentrated HCl. The glycosidic linkages were hydrolyzed as a result of this process, releasing the aglycone portion. The mixture was heated and then let to cool. In order to extract the particles from the solution containing aglycone, the cooled mixture was filtered.

Liquid-Liquid Partition: A separatory funnel was filled with the filtrate. Chloroform, an organic solvent, was introduced in a volume equivalent to the aqueous phase. The extraction was carried out three times after the funnel was gently shaken. After that, the organic (chloroform) layers were mixed together. To get rid of any last traces of acid, distilled water was used to wash the mixed organic fraction. A rotary evaporator was used to concentrate the pooled chloroform layers to dryness at low pressure at around 30°C. The residue that was left over looked like a thick, green oil.

4.1.1. Identification of Resveratrol [32]

1. **Ferric Chloride Test:** 2 ml of the test solution were combined with a solution containing 5% ferric chloride. A favourable response was indicated by the appearance of a blue, blue-black, or blue-green colour.
2. **Liebermann Reaction:** A phenolic sample was placed in a dry test tube. Then, 1 mL of concentrated H_2SO_4 was added to the sample. A few crystals of $NaNO_2$ were introduced afterward. A blue-green or blue-violet colour developed immediately. Upon dilution, the colour changed to red.
3. **Bromine water Test:** The test solution was combined with water containing bromine. A favourable response was indicated by the solution's original colour fading.

Quantification of Resveratrol by High-performance liquid chromatography (HPLC): [33, 34]

Preparation of Mobile Phase: Methanol: Water (70:30) was used as mobile phase. The mobile phase was degassed in an ultra Sonicator for 15 min.

Stock solution of standard: 1 mg of the standard resveratrol was dissolved in 1 ml water. From the Stock solutions, working standard solutions of the standard were prepared by appropriate dilution with water. Standard calibration curve was prepared with using concentrations: 2.5ug/ml, 5ug/ml & 10ug/ml.

Preparation of Sample: Each sample was prepared at a concentration of 1 mg/mL in methanol. A working solution of 100µg/mL was then prepared by diluting the stock solution with methanol (e.g., 100 µL stock + 900 µL methanol). The solution was sonicated for 10 minutes and

filtered through a 0.2 µm, 13 mm nylon membrane filter prior to injection into the system.

Chromatographic Conditions:

Table: Quantification of Resveratrol by High-performance liquid chromatography (HPLC)

S. No.	Parameters	Standard	Condition
1.	Stationary phase	Resveratrol	Agilent TC C18(2), 4.6x250mm, 5um
2.	Mobile phase	Resveratrol	Methanol: Water (70:30)
3.	Detection Wavelength	Resveratrol	306nm
4.	Flow rate	Resveratrol	1 ml/min
5.	Injection volume	Resveratrol	10 µl
6.	Temperature	Resveratrol	30°C
7.	LC System	Resveratrol	Agilent test system and Open Lab CDS2

RESULTS

Extraction of Resveratrol from Fruits of *Vitis vinifera*

Figure No. 4: Ethanolic Extracts of *Vitis vinifera*

Figure No. 5: Concentration of Ethanolic Extracts by Rotary Evaporator

Figure No. 2: Fruits of *Vitis vinifera*

Figure No. 3: Fruits pulp of *Vitis vinifera*

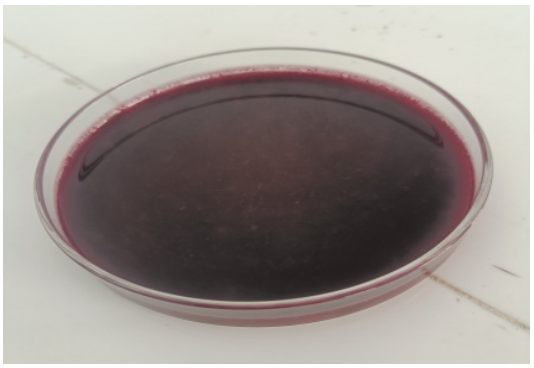

Figure No.6: Crude resveratrol Extract Figure No. 7: Acid hydrolysis Crude resveratrol Extract

Figure No. 8: Liquid-liquid partition of Crude resveratrol extract Figure No. 9: Resveratrol

Table No. 1: Yield of Resveratrol from Fruits of Vitis vinifera.

Polyphenols	**Plant**	**Weight of dried plant material**	**Weight of crude extract obtained**	**Weight of purified & Isolated**	**Yield with respect to plant material**	**Yield with respect to extract**
Resveratrol	*Vitis vinifera*	2000 gm.	180 gm.	4.6 gm.	0.23 %	2.5 %

Identification of Resveratrol

Table No. 2: Identification of Resveratrol

Polyphenols	**Observation**	**Result**
Ferric chloride test	Blue	++
Liebermann Reaction	Red	+
Bromine water test	Initial colour fade	+

Quantification of Resveratrol by High-performance liquid chromatography (HPLC):

TEST REPORT

Resveratrol standard:

Table No. 3: High-performance liquid chromatography (HPLC) of Resveratrol standard

S. No.	**Type**	**Code**	**Conc. (ug/ml)**	**RT**	**Area**
1.	Standard	Std-10ug/ml	10	3.213	33038772
2.	Standard	Std-5ug/ml	5	3.217	7482599
3.	Standard	Std-2.5ug/ml	2.5	3.213	2008847

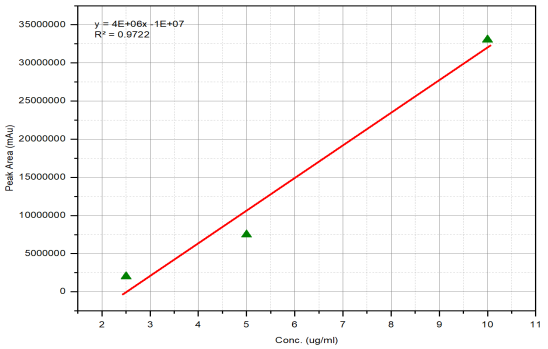

Figure No. 10: Graph shows Peak Area and Concentration

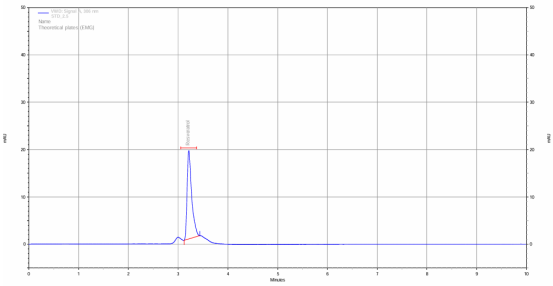

Figure No. 11: Chromatogram of resveratrol 2.5ug/ml showing peak of resveratrol

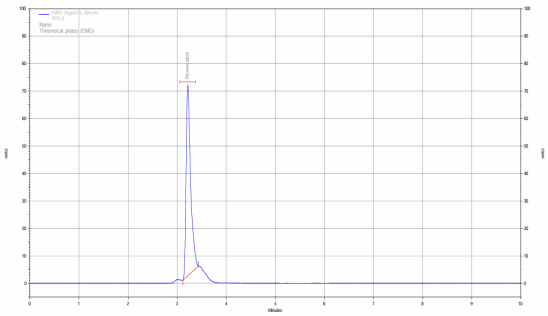

Figure No. 12: Chromatogram of resveratrol 5ug/ml showing peak of resveratrol

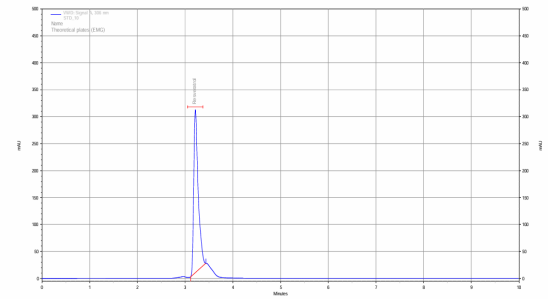

Figure no. 13: Chromatogram of resveratrol 10ug/ml showing peak of resveratrol

Resveratrol Sample:

Table: No. 4 High-performance liquid chromatography (HPLC) of Resveratrol sample

S. No.	Type	Code	Resveratrol RT	Resveratrol Area	Concentration (ug/ml)
1.	Sam	VV	3.223	259562	3.768
	ple	Me		95	

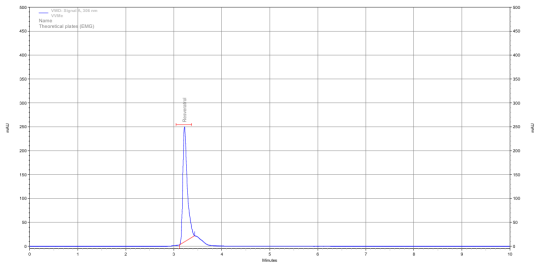

Figure No. 14: Chromatogram of sample- VVMe showing peak of resveratrol

Discussion and Conclusion:

The present study successfully demonstrated the extraction, purification, and isolation of the selected polyphenolic compound, namely resveratrol, were successfully carried out from *Vitis vinifera*. The percentage yield obtained at different stages of the isolation process provided an indication of the abundance of the target polyphenol in the selected plant material as well as the efficiency of the adopted extraction and purification procedure.

From 2000 g of dried *Vitis vinifera* plant material, 180 g of crude extract was obtained, followed by the isolation of 4.6 g of purified resveratrol. The yield of resveratrol was 0.23% with respect to dried plant material and 2.5% with respect to the crude extract. The relatively low yield may be attributed to the limited natural abundance of resveratrol and variations in its distribution within plant tissues.

The qualitative phytochemical evaluation confirmed the presence of polyphenolic compounds in the isolated samples. Resveratrol exhibited a strong positive ferric chloride test (++), producing a blue colour, while the Liebermann reaction and bromine water test showed positive responses, indicating the presence of phenolic hydroxyl groups and unsaturation. Overall, these characteristic reactions supported the successful isolation and preliminary identification of resveratrol from the selected plant sources.

The HPLC analysis was carried out for the qualitative identification and quantitative estimation of resveratrol in the *Vitis vinifera* methanolic extract (VVMe). Standard resveratrol solutions at concentrations of 2.5, 5, and 10µg/ml exhibited retention times of 3.213, 3.217, and 3.213 min, respectively, indicating consistent chromatographic behaviour of the reference compound. The VVMe sample showed a distinct chromatographic peak at a retention time of 3.223 min, which was closely comparable with the retention time of standard resveratrol. The minor variation in retention time may be attributed to the complexity of the plant extract and chromatographic conditions. The sample exhibited a peak area of 25,956,295, corresponding to a calculated resveratrol concentration of 3.768µg/ml.

The close agreement between the retention times of the standard and sample confirmed the presence of resveratrol in the extract. Thus, the HPLC findings supported the successful identification and quantitative estimation of resveratrol in *Vitis vinifera*.

Overall, the findings establish that Vitis vinifera fruits are a valuable natural source of resveratrol and that the adopted extraction and analytical procedures provide an efficient approach for its isolation and quantification. The study provides a scientific basis for utilizing grape-derived resveratrol as a bioactive ingredient in pharmaceutical, nutraceutical, and cosmeceutical formulations, particularly in antioxidant and photoprotective products. Furthermore, the validated HPLC method can be employed as a routine quality control tool for standardization of resveratrol-containing herbal extracts and formulations. Future studies should focus on optimizing extraction conditions, improving recovery through advanced extraction technologies, evaluating the purity of the isolated compound using complementary spectroscopic techniques (LC-MS, FTIR, and NMR), and investigating its biological efficacy and formulation stability to enhance its therapeutic and commercial applications.

Acknowledgement:

The author sincerely acknowledges Bareilly International University, Bareilly, particularly the College of Pharmacy (BIU-COP), for providing the academic support and facilities required for this research. The author is also grateful to CytoGene Research & Development, Lucknow, for providing laboratory facilities and technical assistance in carrying out the experimental work. Heartfelt thanks are extended to all faculty members, staff, and well-wishers for their valuable support and encouragement throughout the study.

REFERENCES:

1. Linus Pauling Institute. Resveratrol. Micronutrient Information Center, Oregon State University; 2015.
2. Frémont L. Biological effects of resveratrol. *Life Sciences*. 2000; 66(8):663–673.
3. Sales JM, Resurreccion AV. Resveratrol in peanuts. *Critical Reviews in Food Science and Nutrition*. 2014; 54(6):734–770.
4. Vang O, Ahmad N, Baile CA, et al. What is new for an old molecule? Systematic review and recommendations on the use of resveratrol. *PLoS One*. 2011; 6(6):e19881.
5. Singh AP, Singh R, Verma SS, et al. Health benefits of resveratrol: Evidence from clinical studies. *Medicinal Research Reviews*. 2019; 39(5):1851–1891.
6. Zeraattalab-Motlagh S, Jayedi A, Shab-Bidar S. The effects of resveratrol supplementation in patients with type 2 diabetes, metabolic syndrome, and nonalcoholic fatty liver disease. *American Journal of Clinical Nutrition*. 2021; 114(5):1675–1685.
7. Jeyaraman MM, Al-Yousif NS, Singh Mann A, et al. Resveratrol for adults with type 2 diabetes mellitus. *Cochrane Database of Systematic Reviews*. 2020; 1:CD011919.
8. Walle T, Hsieh F, DeLegge MH, Oatis JE, Walle UK. High absorption but very low bioavailability of oral resveratrol in humans. *Drug Metabolism and Disposition*. 2004; 32(12):1377-1382.
9. Chimento A, De Amicis F, Sirianni R, et al. Progress to improve oral bioavailability and beneficial effects of resveratrol. *International Journal of Molecular Sciences*. 2019; 20(6):1381.
10. Gambini J, Inglés M, Olaso G, et al. Properties of resveratrol: In vitro and in vivo studies about metabolism, bioavailability, and biological effects in animal models and humans. *Oxidative Medicine and Cellular Longevity*. 2015; 2015:837042.
11. Baur JA, Sinclair DA. Therapeutic potential of resveratrol: The in vivo evidence. *Nature Reviews Drug Discovery*. 2006; 5(6):493-506.
12. Lamuela-Raventos RM, Romero-Perez AI, Waterhouse AL, de la Torre-Boronat MC. Direct HPLC analysis of cis- and trans-resveratrol and piceid isomers in Spanish red wines. *Journal of Agricultural and Food Chemistry*. 1995; 43(2):281–283.
13. Bernard E, Britz-McKibbin P, Gernigon N. Resveratrol photoisomerization: An integrative guided-inquiry experiment. *Journal of Chemical Education*. 2007; 84(7):1159.
14. Yang I, Kim E, Kang J, et al. Photochemical generation of a new, highly fluorescent compound from non-fluorescent resveratrol. *Chemical Communications*. 2012; 48(32):3839-3841.
15. Mattivi F, Reniero F, Korhammer S. Isolation, characterization, and evolution in red wine vinification of resveratrol monomers. *Journal of Agricultural and Food Chemistry*. 1995; 43(7):1820-1823.
16. Valletta A, Iozia LM, Leonelli F. Impact of environmental factors on stilbene biosynthesis. *Plants*. 2021; 10(1):90.

17. Dubrovina AS, Kiselev KV. Regulation of stilbene biosynthesis in plants. *Planta*. 2017; 246(4):597-623.
18. Wang C, Zhi S, Liu C, et al. Characterization of stilbene synthase genes in mulberry and metabolic engineering for the production of resveratrol. *Journal of Agricultural and Food Chemistry*. 2017; 65(8):1659-1668.
19. Baell J, Walters MA. Chemistry: Chemical con artists foil drug discovery. *Nature*. 2014; 513(7519):481-483.
20. Vang O. Resveratrol: Challenges in analyzing its biological effects. *Annals of the New York Academy of Sciences*. 2015; 1348(1):161-170.
21. Luca SV, Macovei I, Bujor A, et al. Bioactivity of dietary polyphenols: The role of metabolites. *Critical Reviews in Food Science and Nutrition*. 2020; 60(4):626-659.
22. Sharan S, Nagar S. Pulmonary metabolism of resveratrol: In vitro and in vivo evidence. *Drug Metabolism and Disposition*. 2013; 41(5):1163-1169.
23. Prokop J, Abrman P, Seligson AL, Sovak M. Resveratrol and its glycon piceid are stable polyphenols. *Journal of Medicinal Food*. 2006; 9(1):11-14.
24. Pantusa M, Bartucci R, Rizzuti B. Stability of trans-resveratrol associated with transport proteins. *Journal of Agricultural and Food Chemistry*. 2014; 62(19):4384-4391.
25. Cottart CH, Nivet-Antoine V, Beaudeux JL. Review of recent data on the metabolism, biological effects, and toxicity of resveratrol in humans. *Molecular Nutrition & Food Research*. 2014; 58(1):7–21.
26. Liu Y, Ma W, Zhang P, He S, Huang D. Effect of resveratrol on blood pressure: A meta-analysis of randomized controlled trials. *Clinical Nutrition*. 2015; 34(1):27–34.
27. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, et al. Effect of extraction solvent on total phenol content, total flavonoid content and antioxidant activity of *Limnophila aromatica*. J Food Drug Anal. 2014; 22(3):296-302.
28. Dent M, Dragović-Uzelac V, Penić M, Bosiljkov T, Levaj B. The effect of extraction solvents, temperature and time on the composition of polyphenolic extracts. Food Technol Biotechnol. 2013; 51(1):84-91.
29. Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010; 15(10):7313-7352.
30. Khoddami A, Wilkes MA, Roberts TH. Techniques for analysis of plant phenolic compounds. Molecules. 2013; 18(2):2328-2375.
31. Stalikas CD. Extraction, separation and detection methods for phenolic acids and flavonoids. J Sep Sci. 2007; 30(18):3268-3295.
32. Evans WC. Trease and Evans Pharmacognosy. 16th ed. London: Saunders Elsevier; 2009. p. 225-242.
33. Guideline, I. H. T. (2005). Validation of analytical procedures: text and methodology. Q2 (R1), 1(20), 2.
34. Katsagonis, A., Atta-Politou, J., & Koupparis, M. A. (2005). HPLC method with UV detection for the determination of trans-resveratrol in plasma. Journal of liquid chromatography & related technologies, 28(9), 1393-1405.