

Determination of Vitamins and Pharmacological Properties of *Vitis Vinifera L.* Plant Fruit Part (Mixed Varieties) Syrup-Honey

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This paper presents the method of obtaining fruit syrup from *Vitis vinifera L.* plants, along with the analysis of its chemical composition, specifically focusing on the vitamins in terms of both quality and quantity. The study includes chromatographic data illustrating the relevant indicators of the syrup's components. In addition, the toxicity level of the fruit syrup from *Vitis vinifera L.* plants growing in Uzbekistan has been determined. The paper also investigates the pharmacological effects of the grape fruit syrup, particularly its potential for reducing inflammation in the body. The methods used in this research are thoroughly discussed, providing a comprehensive understanding of the syrup's composition, safety, and therapeutic properties.

Keywords: Ethanol; Fructose; Glucose; HPLC; Inflammation; Sucrose; Syrup; Vitamins; *Vitis vinifera L.*

Vitis vinifera L., the scientific name for grapes, is one of the most widely consumed fruits worldwide.¹ This ancient flowering or angiosperm plant is a member of the Juss family's *Vitis* genus and is indigenous to temperate, subtropical, and tropical parts of the world. There are over 1,000 species in the 15 genera that make up the vine family.² Since ancient times, grapes have been used extensively in medicine to treat a wide range of illnesses, including heart disease,

gastrointestinal disorders, anemia, poor energy, and tuberculosis.³ Ampelotherapy (Greek: ampelos grape, therapela treatment) is a novel scientifically based approach to grape-based treatment that is widely employed in medicine.⁴ Apple, wine, lemon, amber, sable, formic, and a number of other organic acids, as well as mineral salts like potassium, calcium, phosphorus, and sodium, coloring agents (pigments), and tannins in the fruit peel, are all found in newly harvested grapes and

are essential for human health.⁵ Useful vitamins like A, C, P, PP, B₁, B₂, B₆, B₁₂, and particularly vitamins of group B are abundant in grapes. The amount of amino acids stored in grapes varies depending on the grape variety's ripening period, whether or not the clusters are seeded, the vine's growth strength, weather, and care practices.⁶ Their uses and biological characteristics set them apart from one another. The high quality, disease, and pest resistance of table grapes have also received attention recently due to the growing demand for eco-friendly products. Because the quality of table grapes is evaluated based on their sugar content, transportability, durability, and acidity (they should have a lot of juice and low acidity). Therefore, table grapes are mainly used for consumption in the place of cultivation, transportation to other places where grapes are not grown, and storage in refrigerators during the winter. Depending on the relief of each place, the amount of heat and light can change the quality of the crop. Therefore, the main goal of the study is to study the chemical composition of the juice of the fruit of the zoned technical grape varieties (Saparevi, Nimrang, Toyipi, Muscat Uzbek, Rizamat and White Kishmish) and their biological activity, which are grown in Uzbekistan. Among the Central Asian republics, Uzbekistan, Tajikistan, and Turkmenistan are especially in the forefront in the cultivation of table grape varieties.⁷ It is worth noting that the importance of metabolites in fruits and medicinal plants is considered high, and their location, the ecological conditions of the countries (hot and dry climate, long growing season, abundance of food varieties with different ripening periods, etc.) are very important factors for producing abundant and high-quality crops.⁸ Grape seeds, skins, stems, and pomace are among the by-products that the wine business obtains; these materials are gathered in vast quantities and are frequently regarded as agricultural trash. Protein, fat, and fatty acids are abundant in grapes and their byproducts. Numerous phytochemicals, including as resveratrol, quercetin, anthocyanins, procyanidins, malvidin-3-glucoside, catechin, kaempferol, isorhamnetin, etc., are found in significant amounts in them.⁹ Numerous pharmacological properties, including cardioprotective, antiproliferative, antioxidant, anti-inflammatory, anti-aging, anti-candidiasis, anti-diabetic, anti-obesity, antibacterial, and anti-

contractile effects^{10,11}, have been demonstrated for grapes and their constituents.

MATERIALS AND METHODS

Animal Experiments

The European Directive 2010/63/EU regarding the welfare of animals used in scientific research was followed in all animal-based experimental procedures (European Union, 2010). The Institute of Bioorganic Chemistry, AS RUz's Animal Ethics Committee examined and approved the study protocol (Protocol No. 133/1a/h, dated August 4, 2014).

Chemicals

- 500ml of molasses sample from mixed grape varieties (nimrang, toyipi, saparevi, Rizamat, aq kishmish)
- analytical balance
- volumetric flasks
- 40% ethanol
- reactor (boiler)
- Vitamins (Water-Soluble Standards): Vitamin B₂, (PP)B₃, B₆, B₉ and C, analytical grade (1 mg/ml stock solutions prepared in 40% ethanol, Sigma-Aldrich).
- Acetonitrile (HPLC grade): Used as the mobile phase in HPLC analysis (Merck)6.
- Acetate buffer: pH 5.0, used sodium acetate and acetic acid for HPLC separation.
- HPLC System: Agilent-1200 with Diode Array Detector (DAD) and Eclipse XDB C18 column (5 μm, 4.6 × 250 mm).
- Samples of grape concentrate at dosages of 100 and 200 mg/kg were collected.
- The reference medication was 25 mg/kg of indomethacin (Sopharma).
- In outbred white laboratory mice with a body weight of 20±2.0 g
- An electronic caliper (Insize, model 7140, Digital Caliper, 0-150mm/0-6, China) was used to measure the paw diameter (PV...) 1, 2, 3, 4, 5 and 24 hours after carrageenan injection (PVt).
- To evaluate the anti-inflammatory activity of the samples, the animals were divided into 14 groups, each with 6 rats.
- Special probe, etc.

Method for obtaining syrup (molasses) from the fruit part of *Vitis vinifera L.* plants

The following steps are involved in

making grape molasses. Well-ripened grape heads are smashed with a pestle and rinsed in cold water to make molasses. The grape liquid, or sap, is then filtered. The grape juice (sap) is filtered, excess particles (dust, grape pulp) are precipitated, and the filtered grape juice is put in a reactor (boiler) and boiled over low heat for one to two hours at 80 to 90 °C until the color becomes clear and pourable.¹² This prevents the grape juice (sap) from spoiling (separating). Every now and then, it is agitated. The final molasses has a reddish-brown hue. Even after being preserved for a long period, molasses made using this method maintains its chemical composition.

Determination of Water-Soluble Vitamins in *Vitis vinifera* L. Root Using High-Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography was used to identify the water-soluble vitamins in grape syrup as well as molasses. A 300 ml round-bottomed flask was filled with 5–10 g of grape molasses, which had been weighed on an analytical scale. It was mixed with 50 milliliters of a 40% ethanol solution. A magnetic stirrer and reflux condenser were used to heat the mixture. It was then boiled for an hour while being vigorously swirled, and then allowed to sit at room temperature for two hours. After cooling, the mixture was filtered. 25 milliliters of 40% ethanol were used twice to remove the remaining amount. The filtrates were mixed together and 40% ethanol (5–10%) was added till the mark was reached. For ten minutes, the resultant solution was centrifuged at 7000 rpm. The resultant solution's supernatant was removed for examination.¹³

Water-soluble vitamin working solutions were made at a concentration of 1 mg/ml. This was accomplished by precisely weighing 50.0 mg of each vitamin standard on an analytical scale, dissolving it in 40% ethanol, and then filling a 50 ml volumetric flask to the appropriate level.¹⁴

The eluents for the HPLC study of water-soluble vitamins in *Vitis vinifera* L. root were acetonitrile and an acetate buffer system.¹⁵ The following were the chromatographic conditions:

Agilent-1200 chromatograph (autosampler included) Diode array detector (DAD), detected at 250 nm; column: Exlipse XDB C18 (reverse-phase), 5 µm, 4.6 x 250 mm 0.8 ml/min is the flow rate. The composition of the eluent

is Acetate buffer: 96:4 for 0–5 minutes, 90:10 for 6–8 minutes, 80:20 for 9–15 minutes, and 96:4 for 15–17 minutes; thermostat temperature: 25°C

• Injection volume: 5 µl

Determination of toxicological (LD₅₀) properties of *Vitis vinifera* L. plants

Grapes have long been recognized for their therapeutic qualities, and both ripe and raw grapes, together with their juice, vinegar, leaves, and stems, have all been utilized as medicinal plants in Eastern medicine. The use of medicinal plants is based on their safe role in the body's metabolism, ability to treat a variety of illnesses, and relative lack of adverse effects when compared to synthetic medications. Studying the acute toxicity and overall impact of concentrated syrup (molasses) samples of natural grape fruits on the animal body was the primary goal of the project. The experiments were conducted on male outbred white laboratory mice with a body weight of 20±2.0 g. The studies were carried out according to the generally accepted method, 6 mice were taken for each group, and the total number of these animals for each dose was 18. All pharmacological studies were conducted on healthy, sexually mature mice kept in quarantine for 10-14 days. Samples of dry grape fruit extract were administered to the stomach of mice using a special probe at a dose of 5000 mg/kg in various concentrations. At the end of the experiment, the median lethal dose (LD₅₀) and toxicity class of the tested drug were determined.¹⁶

Study of the anti-inflammatory activity of the fruit syrup of *Vitis vinifera* L. plants

Aqueous extracts of grape syrup concentrate (molasses) at levels of 100 and 200 mg/kg were chosen as plant objects that contain biologically active chemicals with anti-inflammatory effects in order to establish strategies for stabilizing the design of pharmaceutical products with anti-inflammatory effects. The percentage decrease in carrageenan-induced edema in the rats' paws was used to measure the samples' anti-inflammatory efficacy *in vivo*.

Before starting the research to determine the anti-inflammatory activity of the samples *in vivo*, the animals were kept in a closed place for 10 days in quarantine for adaptation. In the room where the animals were kept, all regulatory requirements were observed: room temperature (25±3°C), 12-hour cycle "darkness/light" and

humidity (30-70%). The rats were fed ad libitum and had no water restrictions. After adaptation, they were randomly assigned to groups of 6 animals each.

Acute inflammation model induced by carrageenan in rat paws

To evaluate the anti-inflammatory activity of the studied samples, the animals were divided into 14 groups, each with 6 rats. To study acute inflammation, a paw edema model induced by carrageenan was induced. For this purpose, 0.1 ml of 1% sodium carrageenan salt solution was injected into the aponeurosis of the right hind paw of rats.¹⁷ One hour prior to the carrageenan injection, the rats in the experimental groups were given the compounds under study orally. Simultaneously, rats in the control group received 5 ml/kg of saline. The positive control group was given a dose of 25 mg/kg of the comparable medication indomethacin, while the experimental groups were given doses of 100 and 200 mg/kg of the drugs under study.

The percentage of swelling reduction of the substances in the paws of rats was calculated by taking the value of 0% swelling reduction in the control group. An electronic caliper (Insize, model 7140, Digital Caliper, 0-150mm/0-6, China) was used to measure the paw diameter (PV...) 1, 2, 3, 4, 5 and 24 hours after carrageenan injection (PVt).

The swelling in the treated groups compared to the control group was calculated using the following formula:

Swelling reduction % = $\frac{(PVt - PV^{(c)})}{(PVt - PV^{(c)}) - (PV^{(t)} - PV^{(c)})} \times 100$, where PVt is the paw diameter after carrageenan, PV... is the paw swelling diameter of the treated rat paw before carrageenan¹⁸.

RESULTS

The amount of vitamins was ascertained by comparing a solution made from grape molasses with a working standard solution that had first been prepared in the chromatograph. (Figures 1–2)

According to the chromatographs, water-soluble vitamins B₂=0.194487 mg, B₆=0.194487 mg, B₉=3.666393 mg, and (PP) B₃=0.063445 mg are present in the chemical composition of grape molasses^{19,20}. Vitamin-rich molasses is used in medicine as a blood-boosting, appetite-stimulating, energy-giving, and anemia-reducing agent^{21,22}.

Table 1 displays the study's findings, which demonstrated the anti-inflammatory properties of the fruit syrup of the grape *Vitis vinifera L.* at dosages of 100 and 200 mg/kg. According to the data presented in the table, in the animals of the control group, edema reaches a maximum after 3 hours, with an increase in paw volume of 121±8.0,

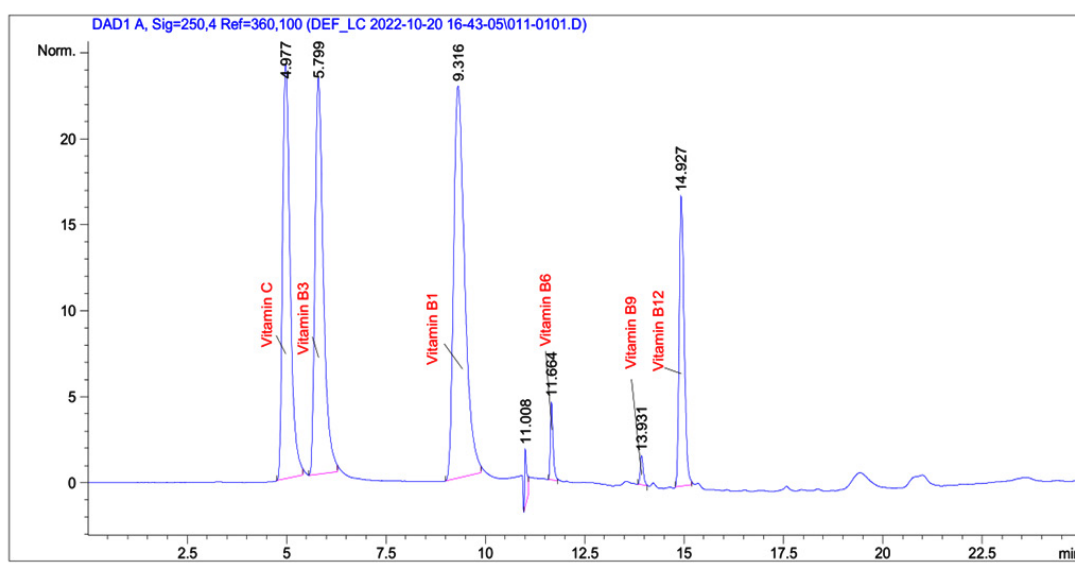


Fig. 1. Shows the chromatography of the working standard solution used to measure the vitamin content of *Vitis vinifera L.* grape molasses

and after 24 hours it decreases to $44 \pm 3.1\%$. Similar dynamics are observed in most of the studied substances, but a lower degree of edema of the rat paw is observed in them^{23,24}. In the study group, the maximum edema was also observed at 3 hours after the introduction of carrageenan, but the effect of the drugs almost approached the norm after 24 hours, and Table 1 presents the results of 5 hours in percentages for each hour^{25,26}.

According to the data presented in Table 2, the anti-exudative effect of all studied preparations

varies between (55.7%) and (44.6%) carrageenan in different variants of concentrated syrup of fruit parts of *Vitis vinifera L.* plants (mixed varieties)^{27,28}.

At doses of 100 and 200 mg/kg, the anti-exudative properties of many concentrated syrup variations of the fruit portion of *Vitis vinifera L.*²⁹ plants (mixed types) were examined in comparison to the control³⁰.

Table 1. Effect of concentrated syrup of *Vitis vinifera L.* fruit parts (mixed varieties) with different variants of carrageenan on leg edema (in % of baseline, $M \pm m$; $n=5$).

	One hour	Two hours	Three hours	Four hours	Five hours	24-hour
Control group	$40 \pm 2,7$	$67 \pm 4,5$	$121 \pm 8,0$	$80 \pm 5,4$	$67 \pm 4,5$	$44 \pm 3,1$
G.S, 100 mg/kg	$26,8 \pm 1,8$	$40,2 \pm 2,7$	$53,6 \pm 3,6$	$47,3 \pm 3,2$	$40,2 \pm 3,0$	$19,6 \pm 2,1$
G.S, 200 mg/kg	$26,8 \pm 1,8$	$40,2 \pm 3,7$	$67,0 \pm 4,5$	$53,6 \pm 3,6$	$40,2 \pm 2,7$	$13,4 \pm 0,9$

Table 2. Anti-exudative activity of different variants of concentrated syrup of fruit parts of *Vitis vinifera L.* plants (mixed varieties) at the same dose of 200 mg/kg ($M \pm m$; $n=5$)

Substance name	Swelling volume after 3 hours, %	“Anti-exudative effect in percent”
Control group	$121 \pm 8,0$	—
G.S, 100 mg/kg	$53,6 \pm 3,6$	55,7
G.S., 200 mg/kg	$67,0 \pm 4,5$	44,6
Indomethacin	$40,4 \pm 5,8$	39,3

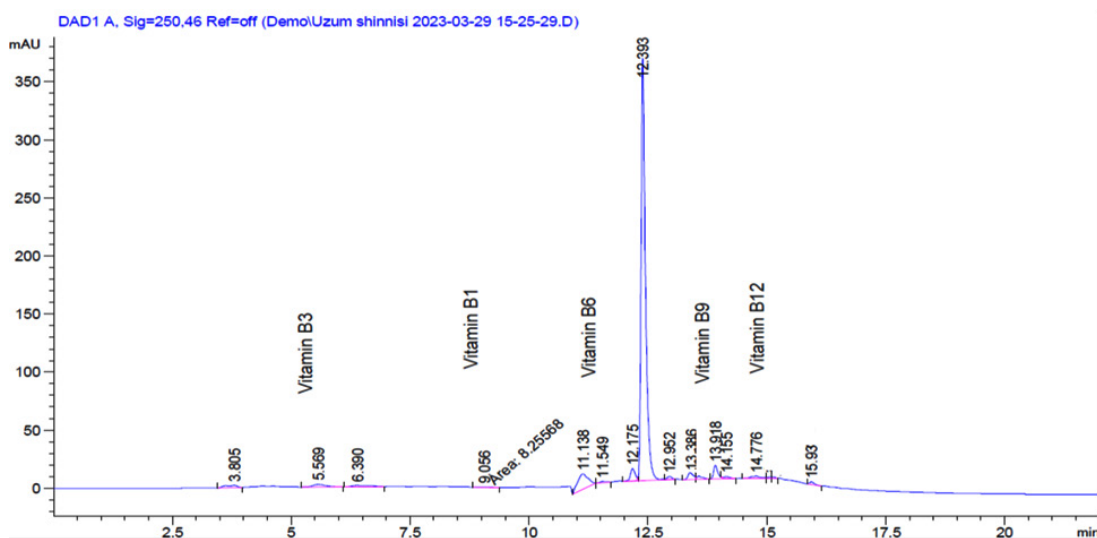


Fig. 2. Shows the chromatography of a grape molasses-based solution to ascertain the vitamin content of the mixture

DISCUSSION

The study highlights the significant presence of water-soluble vitamins in grape molasses, as determined through chromatographic analysis. Vitamins B2 (0.194487 mg), B6 (0.194487 mg), B9 (3.666393 mg), and B3 (PP, 0.063445 mg) were identified, underscoring the nutritional value of grape molasses. These findings support its traditional use in medicine for enhancing blood health, stimulating appetite, boosting energy, and reducing anemia. Such attributes make grape molasses a functional food with potential therapeutic applications.

Furthermore, the anti-inflammatory properties of grape syrup derived from *Vitis vinifera L.* were evaluated using an animal model. Table 1 demonstrates that in the control group, carrageenan-induced paw edema peaked at 3 hours post-administration, with an increase in paw volume of $121 \pm 8.0\%$. This inflammation reduced to $44 \pm 3.1\%$ after 24 hours. In contrast, the group treated with grape syrup exhibited a noticeable reduction in inflammation. While edema was still observed 3 hours post-carrageenan injection, its severity was significantly mitigated, with the effects nearly returning to baseline levels within 24 hours.

The anti-exudative effect of the syrup was further quantified (Table 2), with effectiveness ranging from 44.6% to 55.7%, depending on the variant of the concentrated syrup and dosage. The study suggests that the anti-inflammatory properties are dose-dependent, as the higher dosage of 200 mg/kg yielded more pronounced effects compared to the 100 mg/kg dosage.

These results reinforce the medicinal potential of grape-derived syrups, particularly for managing inflammation. The reduction in edema and exudative response at both dosages reflects the bioactive components' ability to modulate inflammatory pathways. This aligns with the traditional applications of *Vitis vinifera L.* in herbal medicine and suggests its utility as a complementary treatment for inflammatory conditions. Further studies are needed to isolate and characterize the specific compounds responsible for these effects and to confirm the findings in human models.

CONCLUSION

The study highlights the development of an efficient technique for producing *Vitis vinifera L.* fruit syrup (molasses) that retains its chemical composition even after prolonged storage, making it a stable and valuable nutritional product. The syrup is particularly rich in water-soluble vitamins, especially vitamin B9, which adds to its nutritional significance. Toxicological studies demonstrate that the syrup is safe, with an LD50 value exceeding 5000 mg/kg, classifying it as a non-toxic compound. Furthermore, the syrup exhibits significant anti-inflammatory activity at doses of 100 mg/kg, indicating its potential therapeutic application. These findings underscore the nutritional, safety, and pharmacological value of *Vitis vinifera L.* fruit syrup, paving the way for its use in both dietary and medicinal contexts. Further research could explore its broader health benefits and mechanisms of action.

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Conflict of interest

The authors declare that they have no conflict of interest regarding the publication of this article.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Author Contributions

Aziza Azimova – Conceptualization, methodology, investigation, and writing the original draft. Akmal Islomov and Abdullaev Izzatullo – Methodology, data analysis, and writing review and editing. Dilnoza Abdugafurova – Investigation, data curation, and visualization. Sevinch Siddiqova – Methodology and resources. Aziza Ishmuratova – Data analysis and interpretation. Lazizbek Mahmudov – Investigation and manuscript preparation. Salikhjan Maulyanov – Resources and data management. Ibrokhim Asqarov – Supervision of experiments and project administration.

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