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IDENTIFICATION OF SELECTED POLYPHENOLS IN GRAPE (VITIS VINIFERA) LEAVES

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Abstract
Grape (*Vitis vinifera*) leaves have long been utilized in various traditional medical practices due to their wide spectrum of therapeutic properties. In addition to being a common dietary component in some cultures, grape leaves are known to contain a diverse array of bioactive compounds, particularly polyphenols, which are associated with antioxidant, anti-inflammatory, and antimicrobial effects. This study focuses on the preliminary identification of selected polyphenolic compounds present in grape leaves and evaluates their potential pharmacological relevance. The chemical composition of the leaves was analyzed using spectrophotometric methods to determine the presence of key phenolic constituents. The findings contribute to a growing body of evidence supporting the ethnomedicinal use of grape leaves in the prevention and management of chronic diseases, including cardiovascular disorders, metabolic syndrome, and gastrointestinal ailments. Furthermore, the study emphasizes the importance of grape leaves as a sustainable natural resource with potential applications in functional food and phytopharmaceutical development.
Keywords: grape, grape leaf, chemical composition, traditional medicine, beneficial properties.

Introduction

Grape (*Vitis vinifera*), a member of the genus *Vitis* from the family *Vitaceae*, is considered one of the oldest flowering or angiosperm plants cultivated by humans. The *Vitaceae* family comprises approximately 14 genera and nearly 1,000 species [1]. The geographic origin of grape is believed to span from northeastern Afghanistan to the southern coastal zones of the Black and Caspian Seas. Grapes were domesticated as early as 4000 BCE in this region and subsequently spread to the Mediterranean basin, Western Europe, India, China, and Japan. Spanish colonizers introduced the grapevine to the Americas, and today, grape cultivation is widespread across the globe. In some regions, local varieties have evolved

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through hybridization with indigenous *Vitis* species, adapting to specific environmental conditions [2, 3].

The medicinal use of grape leaves has long been recognized in traditional medical systems. These leaves are rich in vitamins A, K, and PP, as well as ascorbic acid and several representatives of the B-vitamin complex. A 100-gram portion of grape leaves provides nearly half the recommended daily intake of sodium, manganese, and copper. Additionally, they are a source of essential minerals such as calcium, iron, phosphorus, and zinc, contributing significantly to their biochemical and therapeutic value [4, 5].

Grape leaves are particularly rich in vitamin C (ascorbic acid), which plays a crucial role in boosting the immune system and protecting against oxidative stress. This vitamin is also vital for blood coagulation and bone health. The B-complex vitamins found in grape leaves—including B2, B3, B6, and folic acid—are involved in energy production, nervous system functioning, and the formation of red blood cells. Moreover, the presence of magnesium supports muscular and neural activity, alleviates stress, and contributes to skin health and immune function. These attributes make grape leaves a valuable dietary and medicinal plant.

Beyond vitamins and minerals, grape leaves are abundant in polyphenolic compounds and flavonoids, which exhibit strong antioxidant and anti-inflammatory activity. Among them, flavonols and flavanols represent the major subclasses identified in grape leaves [6, 7, 8]. These compounds are widely distributed in various fruits and vegetables and contribute to the health-promoting properties of grape leaves.

This study focuses on identifying selected polyphenolic compounds in the leaves of the locally grown grape variety "Katta Qo‘rg‘on" in Uzbekistan using high-performance liquid chromatography (HPLC). The results aim to provide a scientific basis for the traditional use of grape leaves and highlight their potential in nutraceutical and pharmacological applications.

Materials and Methods

Reagents and Equipment

Gallic acid was obtained from Macklin (China), and salicylic acid from Rhydburg Pharmaceuticals (Germany). Quercetin, apigenin, and kaempferol standards were purchased from Regal (China), while rutin was isolated from natural sources using extraction followed by column chromatography. High-purity HPLC-grade reagents were used throughout the analysis, including water, acetonitrile, glacial acetic acid, and sodium hydroxide.

Quantification of polyphenolic compounds in grape leaves was performed using a high-performance liquid chromatography system (LC-40 Nexera Lite) manufactured by Shimadzu (Japan).

Preparation of Standard Solutions

Standard stock solutions were prepared by dissolving 5.2 mg of gallic acid, 5.2 mg of salicylic acid, and 5 mg each of rutin, quercetin, apigenin, and kaempferol in 96% ethanol. The compounds were sonicated in an ultrasonic bath for 20 minutes and transferred to 50 mL volumetric flasks, which were then filled to the mark with ethanol. Aliquots of 200 µL from each standard solution were combined and serially diluted to prepare four different working solutions. Each was transferred into separate vials and used for HPLC analysis.

Preparation of Grape Leaf Extract

To extract phenolic compounds from grape leaves, 1 g of the dried and ground sample was accurately weighed using an NV222 analytical balance (OHAUS, USA) with a precision of 0.01 g. The sample was placed into a 50 mL conical flask and mixed with 25 mL of 96% ethanol. The mixture was subjected to ultrasonic extraction in a GT SONIC-D3 ultrasonic bath

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(China) at 60 °C for 20 minutes. After cooling, the extract was filtered and diluted to 25 mL with ethanol in a volumetric flask.

A 1.5 mL aliquot of the final extract was centrifuged using a Mini-7 centrifuge (BIOBASE, China) at 7000 rpm. The supernatant was filtered through a 0.45 µm syringe filter and then used for chromatographic analysis.

Identification of Phenolic Compounds in Grape Leaf Extract

The detection of phenolic compounds in the grape (*Vitis vinifera*) leaf extract was carried out using a reversed-phase Shim-pack GIST C18 analytical column (150 × 4.6 mm, 5 µm particle size; Shimadzu, Japan). The mobile phase consisted of a gradient mixture of acetonitrile (A) and 0.5% aqueous acetic acid (B), as detailed in Table 1. The injection volume was set to 10 µL, with a constant flow rate of 0.5 mL/min. The column temperature was maintained at 40 °C using a built-in thermostat. Detection of the analytes was performed at a wavelength of 300 nm, where the analytical signals (peak areas) of the phenolic compounds were recorded (Figure 1).

Table 1. Gradient program for the mobile phase.

Time, min	Acetonitrile (A), %	0.5% acetic acid (B), %
0	5	95
5	5	95
17	40	60
22	40	60
22,1	5	95
40	Finish	

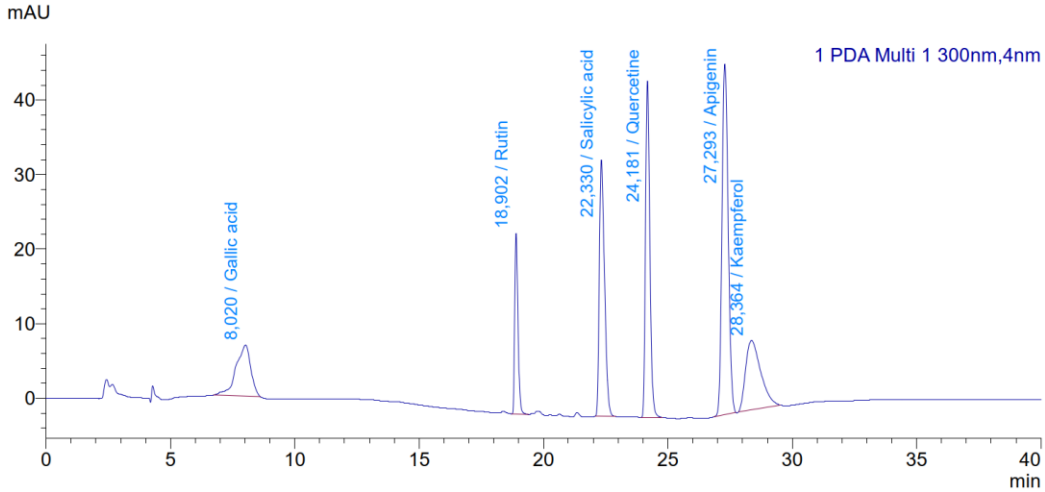

Figure 1. Chromatogram of standards at 300 nm.

Results and discussion

Determination of the amount of phenolic compounds in grape leaf extract. A chromatogram of a sample extract weighing 1 g was obtained (Figure 2), and based on the results, the amount of phenolic compounds in 100 g of the sample was calculated using the following formula and is presented in Table 2.

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$$X = \frac{C_{phen} \cdot V_{extract}}{m_{sample}} \cdot 100\ g$$

Here, X – The amount of phenolic compounds in 100 grams of fruit, mg;

C_{phen} – concentration of phenolic compounds in the extract determined by the HPLC method, mg/l;

$V_{extract}$ – volume of sample extract, l;

m_{sample} – mass of sample taken for extract preparation.

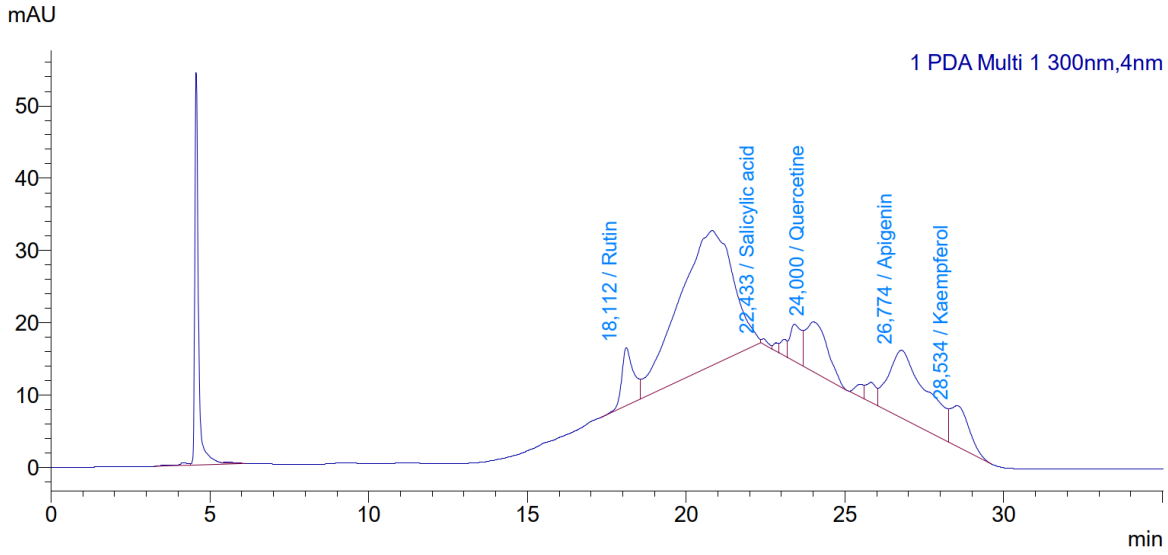

Figure 2. Chromatogram of polyphenols in the sample extract.

Table 2. Amount of polyphenols in the extract and retention times.

Phenol compound name	Holding time, sec	Concentration, mg/l	Amount in 100 ml of sample, mg
Gallic acid	Not specified	0	0,000
Routine	18,112	15,613	39,033
Salicylic acid	22,433	0,418	1,045
Quercetin	24	10,008	25,020
Apigenin	26,774	16,753	41,883
Kaempferol	28,534	9,796	24,490

As shown in the chromatographic analysis, the grape leaf extract contained significant amounts of polyphenolic compounds such as apigenin, rutin, quercetin, and kaempferol. The relative abundance of these compounds in the extract was observed in the following descending order: apigenin > rutin > quercetin > kaempferol > salicylic acid. Among these, salicylic acid was present in the lowest concentration, while gallic acid was not detected in the sample.

The high content of naturally occurring flavonoids in grape leaves is directly associated with their notable biological activity and therapeutic potential. Flavonoids are well-known for their antioxidant properties, playing a crucial role in neutralizing free radicals and reducing

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oxidative stress. Additionally, they exhibit antiviral, antibacterial, and anti-inflammatory activities, making them valuable in both preventive and therapeutic applications.

For instance, quercetin is recognized for its ability to inhibit the synthesis of nucleic acids in pathogenic bacteria and viruses, thereby slowing their replication. Rutin, on the other hand, has demonstrated anti-inflammatory effects and contributes to vascular protection and the prevention of capillary fragility. These findings are consistent with earlier studies that have linked dietary flavonoids to reduced incidence of inflammatory and infectious diseases [10, 11, 12].

Conclusion

In summary, the presence of bioactive polyphenolic compounds such as rutin, salicylic acid, quercetin, apigenin, and kaempferol in grape leaves underscores their potential as a natural source for health-promoting agents. These compounds have been associated with the prevention and treatment of conditions such as inflammation, viral and bacterial infections, respiratory disorders, vascular constriction, thrombosis, and metabolic dysfunctions.

Given these findings, grape leaves represent a promising candidate for the development of natural, non-toxic, eco-friendly nutraceuticals and functional food additives. Their incorporation into therapeutic formulations may serve as an alternative or complementary approach to synthetic pharmaceuticals. Future research and development efforts should focus on optimizing extraction methods, evaluating pharmacodynamics, and conducting clinical studies to validate their efficacy and safety in medical and nutritional applications.

References

1. Tojiboev, Sh. (2010). *O‘simliklar sistematikasi* [Plant Systematics]. Tashkent: O‘zbekiston Milliy Ensiklopediyasi nashriyoti. [in Uzbek].
2. Mamatkov, M., & Karimov, A. (2012). *Uzumchilik asoslari* [Fundamentals of Viticulture]. Tashkent: Mehnat nashriyoti. [in Uzbek].
3. Abdullayev, A. (2008). *Mevanavodchilik va tokchilik* [Pomology and Viticulture]. Tashkent: O‘qituvchi. [in Uzbek].
4. Janick, J., & Paull, R. E. (2008). *The encyclopedia of fruit and nuts*. Wallingford, UK: CABI Publishing.
5. This, P., Lacombe, T., & Thomas, M. R. (2006). Historical origins and genetic diversity of wine grapes. *Trends in Genetics, 22*(9), 511–519. https://doi.org/10.1016/j.tig.2006.07.008.
6. Ashurov, A. Sh., & Yunuskhojaev, N. O‘. (2005). *Vitaminlar va ularning ahamiyati* [Vitamins and Their Importance]. Tashkent: O‘qituvchi. [in Uzbek].
7. Hamraev, A. (2014). *Biologik faol moddalar: vitaminlar, fermentlar va gormonlar* [Biologically Active Substances: Vitamins, Enzymes and Hormones]. Tashkent: Universitet. [in Uzbek].
8. Gülçin, İ. (2012). Antioxidant activity of food constituents: An overview. *Archives of Toxicology, 86*(3), 345–391. https://doi.org/10.1007/s00204-011-0774-2.
9. United States Department of Agriculture (USDA). (n.d.). *FoodData Central: Grape leaves, raw*. Retrieved from https://fdc.nal.usda.gov/ [online resource].
10. Ibn Sino, A. A. (1960). *Tib qonunlari kitobi* [The Canon of Medicine]. Tashkent: O‘zbekiston FA nashriyoti. [in Uzbek].
11. Asqarov, I. R. (2019). *Tabobat qomusi* [Encyclopedia of Healing]. Tashkent: Mumtoz so‘z. [in Uzbek].

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ISSN (E): 2942-1918 Open Access| Peer Reviewed

12. Asqarov, I. R. (2021). *Sirli tabobat* [Mysterious Healing]. Tashkent: Fan va texnologiyalar nashriyoti-matbaa uyi. [in Uzbek].