

CHARACTERIZATION OF SEVERAL WEST GEORGIAN AUTOCHTHONOUS GRAPES AND THEIR WINE STILBENES

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Abstract: The present study was conducted to determine the content of Stilbenes in the several autochthonous West Georgian grapes and wines. Stilbenes were analyzed using HPLC-UV samples and were prepared using resin (amberlite XAD2) column, C18 Cartridge Solid Phase Extraction (SPE) Waters Sep-Pak C18 (500 mg). The identification was achieved using UPLC-PDA and MS analysis. The study was conducted on the following Stilbenes and their derivatives – Astringin, Piceid, Trans/cis resveratrol, and ϵ -Viniferin. The amount of stilbenes in grapes was determined as: Aladasturi-131.9 \pm 4.8 mg kg⁻¹, Aleksandrouli 148.2 \pm 4.9 mg kg⁻¹, Mujuretuli 215.3 \pm 7.9 mg kg⁻¹, Chkhaveri 77.5 \pm 2.5 mg kg⁻¹, Ojaleshi 260.71 \pm 9.1mg kg⁻¹. In red wines the content of Stilbenes was Aladasturi-19.17 \pm 0.5 mg kg⁻¹, Aleksandrouli 14.87 \pm 0.4 mg kg⁻¹, Mujuretuli 21.36 \pm 0.5 mg kg⁻¹, Chkhaveri 14.5 \pm 0.4 mg kg⁻¹, Ojaleshi 60.8 \pm 2.2 mg kg⁻¹.

UDC Classification: 547.1, **DOI:** 10.12955/cbup.v7.1479

Keywords: stilbenes, red wines, HPLC-UV, UPLC-MS, PDA

Introduction

Stilbenes are non-flavonoid phenolic compounds present in plants (Shen et al., 2009). They are considered phytoalexins. They protect plants from many diseases and ultraviolet radiation (Alonso-Villaverde et al., 2011). The recent interest in them is explained by the fact that they are a powerful antioxidant with great activity (Gulcin, 2010; Marel et al., 2018; Hao et al., 2004). Against the backdrop of modern environmental problems, it is important that the human diet includes products with antioxidant properties. Such products can be grapes and wines, which contain various types of phenolic compounds (Rimando et al., 2004). The biological activity of phenolic compounds of the class of stilbenes, as physiologically active compounds, are tested in different directions (Wen et al., 2018). After the wine making process, the residues – the grape must (Anastasiadi et al., 2010; Tang et al., 2018), are often used for the production of dietary supplements. The research of Stilbenes is conducted by HPLC UV, MS, and PDA detection methods (Careri et al., 2003; Sánchez et al., 2005; Ji et al., 2014).

To prepare a sample, an extraction is carried out using different solvents and concentrates. The best option for extracting from grapes is alcohol mixed with water, and in the case of wine, it is a concentrated using Amberlite resin (Hurtado-Gaitán et al., 2017; López-Hernández et al., 2016; Decendit, 2017). The interest in studying stilbenes is great due to its antioxidant properties. Even though there are hundreds of autochthonous grapes and wines in Georgia there is no prior research done on the determination of stilbene content in them. The goal of our project was to study stilbenes in some autochthonous West Georgian grapes and the wines made from them.

Methods and materials

High-performance liquid chromatography analysis was carried out on a Waters (Breeze, USA) HPLC system equipped with a model 1525 pump and UV detectors (2489). Separations were performed on a C18 column (250 x 4.6 mm; 5 μ m particle size) column temperature 30°C. The mobile phases consisted of water 0.1 % formic acid (solvent A) and acetonitrile 0.1 % formic acid (solvent B). 200 μ L of each sample was injected and analyzed at 30 °C. A elution program was performed at 2.7 mLmin⁻¹ was 10 % B (0-2 min), 10-60 % B (2-14 min), 60 % B (14-16 min) followed by a 2 min wash with 100 % B and a 5 min re-equilibration step. at a flow rate of 1 mL min⁻¹ The detection wavelengths were set at 290 and

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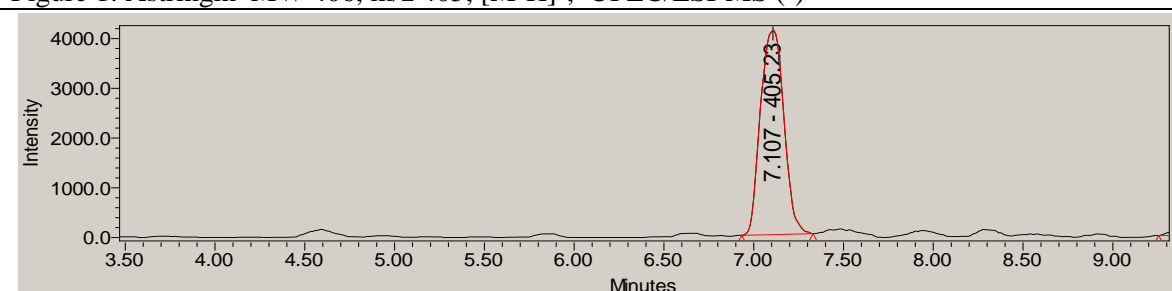
306 nm. The elution program involved gradient elution from 35% B for 3 min to reach 50% B at 10 min, 70% B at 20 min and 100% at 23 to 28 min. UPLC-PDA, MS, column BEN C18 (100x2.1 mm 1.7 μ m). mobile phase 0.1 % Formic acid in DW (A), 0.1 % Formic acid in Acetonitrile (B), gradient solvent B 20 % 13 min, B 99 % in 16 min, B 1% in 18 min, equilibrate 3 min. Flow 0,4 mlmin⁻¹, column temp 40 °C, MS- scan 40-1200 da, Probe 500 °C, Positive 0,8 kV, Capillary 1,5 kV, CV -15, PDA scan 210-500 nm. For identification and quantification purposes, standards of trans-resveratrol were used.

Wine samples were concentrated from 100 to 90 mL in a vacuum at a temperature of not more than 40°C (before alcohol removal). The concentrated wine was poured over Amberlite XAD-16 resin (Sigma-Aldrich), in an open column (2.5 cm id \times 14 cm), and rinsed with 1L of water to remove sugars, small organic acids and other non-polyphenol compounds. The polyphenols were then eluted with 1 L of methanol and 0.5 L of acetone. To prepare a sample of wine, it was concentrated from 100 mL to 10 mL a vacuum at no more than 40°C. The resulting concentrate (4 mL) was subjected to column chromatography using Sep-pac C18 500 mg (SPE-Waters). The column was equilibrated with the mixtures of 4 mL methanol and 0.1 % formic acid and 4 mL water and 0.1 % formic acid, before injection of 5 mL samplers). Elution was performed with a mixture of water and 0.1 % formic acid (chemical grade). Stilbenes extraction was performed with 4 mL of ethyl acetate. This fraction was concentrated and dissolved with a 90-10 ratio of A-B solvent (0.5 mL). All the samples were filtered (Waters Acrodisc LC PVDF Filter 13 mm 0,45 μ m) before the chromatography.

Results and their review

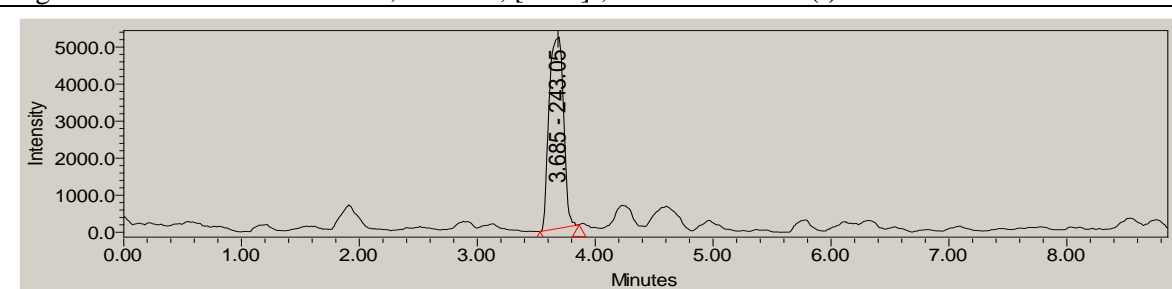
Using a HPLC preparation column, a chromatography was made of the obtained fractions UPLC- PDA, MS and the results were compared to the <https://metlin.scripps.edu> database as well as using literary data. It then became possible to identify the following compounds. A substance 1 m/z 407, [M-H]⁺, fragment m/z 196 [M-H]⁺ λ max 307 according to the obtained results, the substance 1 is Astringin MW 406; a substance 2 m/z 245, [M-H]⁺, fragment m/z 135 [M-H]⁺ λ max 306 and 322 according to the obtained results a substance 2 is Piceatannol MW 244; a substance 3 m/z 391, [M-H]⁺, fragment m/z 229 [M-H]⁺ λ max 308 and 319 according to the obtained results a substance 3 is Piceid MW 390; a substance 4 m/z 227, [M-H]⁻, fragment m/z 107 [M-H]⁻, λ max 306 and 317 according to the obtained results a substance 4 is Trans/cis resveratrol MW 228; a substance 5 m/z 453, [M-H]⁻, fragment m/z 107 [M-H]⁻ λ max 306 according to the obtained results a substance 5 is ϵ -Viniferin MW 454.

Figure 1: Astringin MW 406, m/z 405, [M-H]⁻, UPLC/ESI-MS (-)



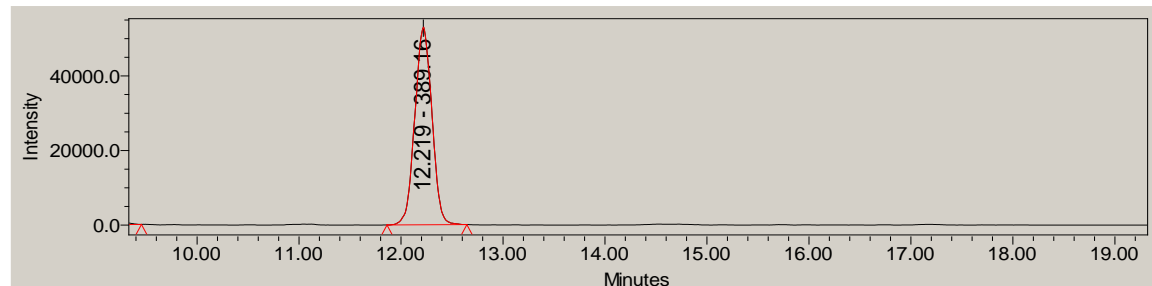
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Figure #2: Piceatannol MW 244, m/z 243, [M-H]⁻, UPLC/ESI-MS (-)



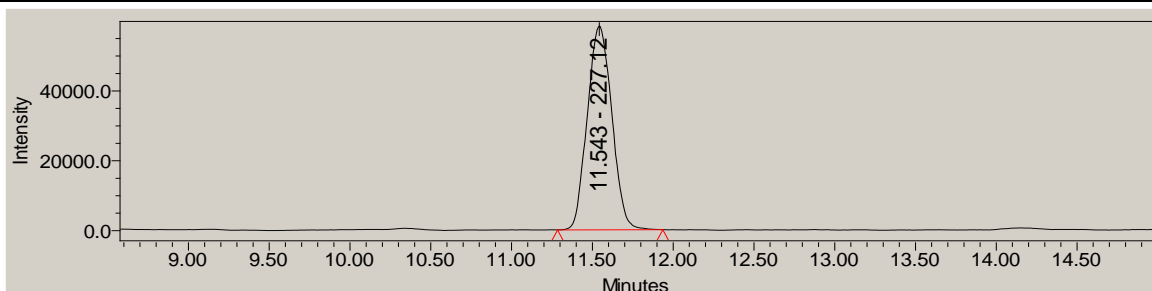
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Figure 3: Piceid MW 390, m/z 389, [M-H]⁻, UPLC/ESI-MS (-)



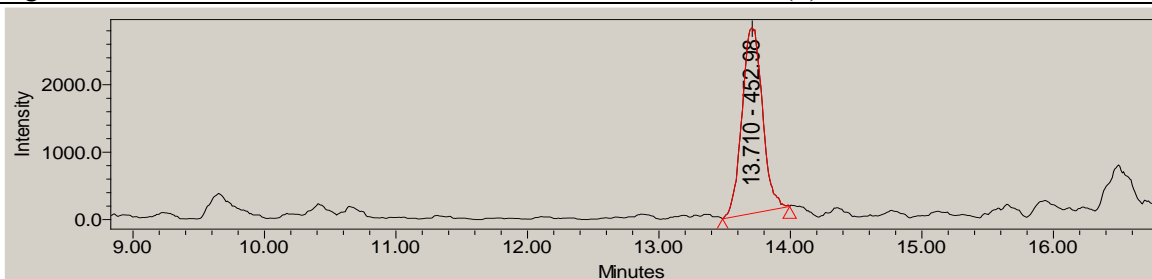
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Figure 4: Trans/cis resveratrol MW 228, m/z 227, [M-H]⁻, UPLC/ESI-MS (-)



Source: Authors

Figure 5: ε-Viniferin MW 454, m/z 453, [M-H]⁻, UPLC/ESI-MS (-)



Source: Authors

Table 1: MS characterization of wine stilbenes

#	Compounds Stilbenes	MW	M/Z +	Fragm	M/Z -	Fragm	λ max	Sample
1	Astringin	406	407	196	405		307	Red Wine
2	Piceatannol	244	245	135	243		306, 322	Grapevine
3	Piceid	390	391	229	389	227	308, 319	Grapevine
4	Trans/cis resveratrol	228	229	107	227	185	306, 317	Red Wine
5	ε-Viniferin	454	455	107	453		306	Grapevine

Source: Authors

Table #2: Stilbenes mg kg⁻¹ in grapes (expressed as trans-resveratrol)

Wine Name	Piceid	Trans/cis resveratrol	Astringin	ε-Viniferin	Total stilbenes	Antioxidant activity (AA)
Aleqsandrouli	67.4	50.14	17.8	14.2	148.82	19.80
Mujuretuli	89.9	102.3	23.1	20.24	215.30	17.10
Aladasturi	75.74	22.15	9.9	24.2	131.99	18.81
Chkhaveri	86.25	10.61	3.13	7.51	77.50	20.14
Ojaleshi	98.9	124.6	6.61	30.6	260.71	7.36

Source: Authors

It is noteworthy that in the analyzed grape fruit and wine obtained from 5 red grape varieties (the Aleqsandrouli, the Mujuretuli, the Aladasturi, the Chkhaveri, the Ojaleshi), we have identified that the dominant compound is Trans/cis resveratrol and Piceid, which together make up more than 90% of the total content of Stilbenes.

Stilbenes are unevenly distributed in different grape varieties and, correspondingly, in the wine produced from them. Among the studied varieties, the highest total maximum amount of Stilbenes is in the Ojaleshi and the Mujuretuli samples (the total content is 260.71 and 215.3 mg kg⁻¹ respectively). 50% of the total content belong to Resveratrol. Relatively fewer stilbenes are in the Alexandrouli (148.82 mg kg⁻¹) and the Aladasturi (131.99 mg kg⁻¹), however, these samples are distinguished by the maximum content of Piceid in the total amount (67.4 mg kg⁻¹, 75.74 mg kg⁻¹). The lowest number of Stilbenes is presented in the Chkhaveri (77.5 mg kg⁻¹). The content of Stilbenes decreases by a certain proportion in the following wines: Aladasturi-19.17 mgkg⁻¹, Aleksandrouli 14. 87 mgkg⁻¹, Mujuretuli 21.36 mgkg⁻¹, Chkhaveri 14.5 mgkg⁻¹, Ojaleshi 60.8 mgkg⁻¹.

A direct proportionate relationship between the total composition and the antioxidant activity of Stilbenes has also been determined. In this respect, the Ojaleshi and the Mujuretuli wines have the highest rates (0.1 mM DPPH 50% inhibition from 7.36 mL and 17.1 mg respectively), while the Chkhaveri has a relatively low rate (20.14 mg). Moreover, there is a direct connection between the amount of stilbenes and the antioxidant properties of the sample. The higher the total stilbene amount the lower the amount of grape juice and wine is needed to inhibit 0.1 mM DPPH 50% which means that the antioxidant property is high. A similar relationship can also be observed with other types of grapes.

Conclusion

The Georgian Autochthonous red grape varieties of Aleqsandrouli, Mujuretuli, Aladasturi, Chkhaveri, and Ojaleshi and the red wines obtained from them are distinguished by their content of Stilbenes-trans/cis resveratrol, Piceid, Astringin, ε-Viniferin. Among the wines, produced from the above grape varieties, the highest content of Stilbenes is in the varieties of Ojaleshi and Mujuretuli.

A correlation was found between the total content of Stilbenes and antioxidant activity. The amount of stilbenes in the Georgian Autochthonous grapes and wines is directly coorelated with the antioxidant activity

Acknowledgment

The designated Project was fulfilled through the financial support of the Georgia National Science Foundation (Grant AP/96/13, Grant216816). Any idea in this publication is possessed by the author and may not represent the opinion of the Georgia National Science Foundation.

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