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Research article

Study of phenolic content using LC–MS/MS technic in Saperavi grapevine shoots growing in Georgia

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ABSTRACT

Georgia is one of the world's oldest wine areas. Interest to by-products of vine processing is rising because they may be exploited as source of bioactive compounds for pharmaceutical and cosmetic purposes, rather than being discarded. The aim of the present research was to study polyphenolic compounds content in Saperavi grapevine shoots extract. Shoots of Saperavi grapevine were collected in Kakheti, Georgia. Extract was preliminary prepared and concentrated 4-fold. Polyphenols were extracted using Methanol and total phenolic content (TPC) was measured with Folin-Ciocalteu (F-C) reagent in terms of Gallic acid. The qualitative composition and content of phenolic compounds was studied with Liquid Chromatography - tandem Mass Spectrometry (LC–MS/MS). The identification was achieved by comparison of the retention times (tR) and spectra characteristics of individual compounds with those in data library. As a result, 14 different phenolic compounds were isolated. Received results demonstrated possibility of using Georgian Saperavi grapevine shoots as a source of biologically active ingredients, for which studies should be proceeded accordingly. This research № PHDF-21-1607 has been supported by Shota Rustaveli National Science Foundation (SRNSFG).

Keywords: Waste products, Polyphenols, Saperavi shoots, Folin-Ciocalteu, LC–MS/MS.

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INTRODUCTION

Georgia is one of the world's oldest wine areas. Grapevine agriculture and wine making have been practiced in the fertile valleys and protected slopes of Transcaucasia for at least 8000 years. Wine has played a significant economic role in Georgian history for millennia, and its traditions are regarded intertwined with and inseparable from the national identity [1]. In modern Georgia, there are over 500 grape species due to its diverse and distinct climate.

By-products from grape processing, such as shoots, canes, and pomace, are abundant in the wine business. These products are gaining popularity because they may be used as a low-cost, easily available source to recover wide range of bioactive chemicals for farther uses rather than being wasted [2,3,4]. Polyphenolic compounds are the most useful bioactive component recovered from vineyard/wine by-products due to their well-documented biological action [5].

Because of their value in human health, dietary polyphenols have gotten a lot of attention from nutritionists, food scientists, and consumers. Polyphenols have been linked to a lower risk of degenerative illnesses such as cancer, cardiovascular disease, and neurological problems [6]. Polyphenols are strong antioxidants that, in conjunction with antioxidant vitamins and enzymes, defend tissues from oxidative stress caused by reactive oxygen species (ROS). Although *in vitro* research provides the majority of evidence for polyphenol antioxidant activity, there is rising evidence that they may function in ways other than antioxidant activities *in vivo*. The modification of cell signaling pathways by polyphenols may aid in understanding the mechanism of polyphenol-rich diet effects [7,8].

Polyphenols, in fact, are cluster of phenols that include at minimum two phenyl rings and one or more hydroxyl substituents [9]. With regard to complexity, this description encompasses significant number of heterogeneous substances. Polyphenols can therefore be segregated as flavonoids or nonflavonoids, also grouped into many subclasses based on phenol components amount in chemical structure, substituent groups, along with the kind of linkage between phenol units [10]. Plants produce polyphenols, which are often found among their different elements and appear more as glycosides. Although flavonoids' fundamental structures are aglycones, flavonoids can also be glycosides or aglycones. They have fundamental structure of diphenyl propanes, in which phenolic rings are connected by heterocyclic ring [11,12]. Variations in the

Hydroxylation pattern and oxidation state variations result in a diversified classification: flavanols, isoflavones, flavonols, antho cyanins, flavanones, flavones, anthocyanidins, and flavanonols. The existence or absence of bond between C2 and C3, as well as carbonyl group production by C4, determines the hydroxylation pattern and oxidation state. Flavonols, flavones, flavanones, and flavanonols are the most abundant class of polyphenols, accounting for the majority of flavonoid molecules [13].

The goal of present research was to study polyphenolic content in Saperavi grapevine shoots extract using LC–MS/MS technic.

MATERIALS AND METHODS

Chemicals

The analytical standard and reagents were purchased: Gallic acid and Folin-Ciocalteu from SIGMA-ALDRICH; Solvents – Ethanol and Methanol - from MERCK.

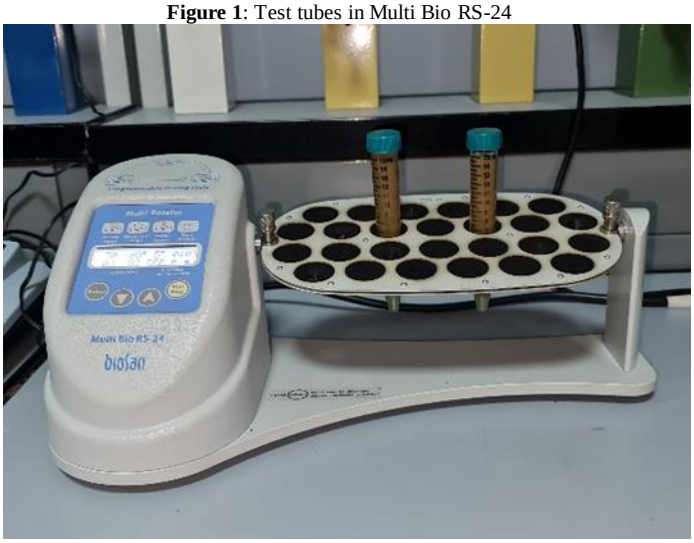
Plant material

Shoots of Saperavi grapevine were collected in 07.2021 in Kakheti region, Georgia. Extract was preliminary prepared and concentrated 4-fold.

Extraction process

20g concentrated extract of Saperavi shoots were placed in 2 test tubes. 5-5ml of chloroform was added per each and shaked on Multi Bio RS-24 (Figure 1) for 10 minutes. After shaking test tubes were placed in refrigerator. following separation, upper parts of test solutions were combined, placed on the porcelain bowl and evaporated on the water bath till dry residue. Dry residue was placed in chemical cup and 10ml of methanol was added for extraction. Received extract was filtered through the Millipore Filter. Extraction process was repeated twice.

Figure 1: Test tubes in Multi Bio RS-24

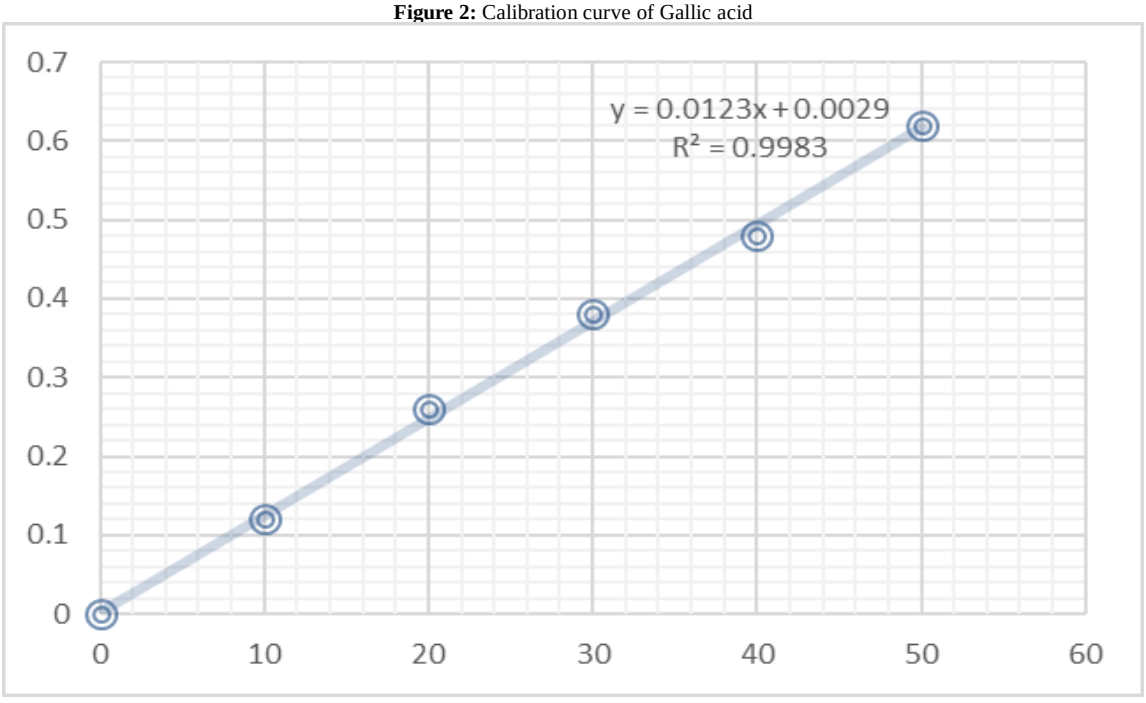


Total phenolic content (TPC) was measured via Folin-Ciocalteu (F-C) in terms of Gallic acid.

Gallic acid stock solution, serial dilution and calibration curve

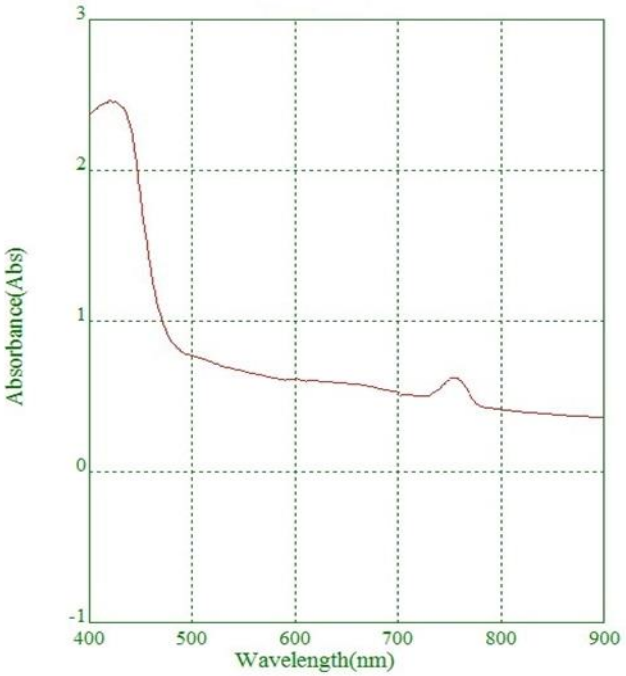
To prepare a stock solution In a 100ml measuring flask, 0.1063g of Gallic acid standard was inserted, distilled water was added, and dissolution was performed on an ultrasonic water bath. After the dissolving process was completed, the volume was filled. The stock solution was used to prepare the serial dilution.1-1ml of diluted solutions were placed in 10ml measuring flasks, 5ml 10% F-C reagent was added and retained for 10 minutes. Afterwards, 4ml of 7.5% Na_2CO_3 was added to the solution and the flasks were hold for 1 hours in the dark place. Absorption ability was assessed with Spectrophotometer i9; Hanon instruments on 765 nm wavelength. Calibration curve is shown on the figure 2.

Figure 2: Calibration curve of Gallic acid



For determination of TPC 0.5ml of prepared Saperavi shoots extract was placed in 10ml measuring flask, 0.25ml Folin-Ciocalteu reagent and 8ml distilled water and was added and solution was retained for 10 minutes. Afterwards, 1.25ml of 7.5% Na_2CO_3 was added and the flask was hold for 1 hr in the dark place. Optical density of the solution was analyzed on 765nm wavelength, and the result is demonstrated on the Figure 3.

Figure 3: UV Spectra of Saperavi extract



The TPC was calculated in terms of Gallic acid according to equation and the result amounted to 35.585 mg.

The qualitative composition and content of phenolic compounds in Saperavi shoots extract was studied on LC system, consisted - Agilent Technologies 1290 Infinity Agilent Technologies 6460 Triple quad LC-MS/MS. For the analysis, a chromatographic column Zorbax Eclipse C18 (100.0mm, 1.8µm), pre-column guard Zorbax Eclipse C18 (5.0 mm, 1.8µm) was used. Current analysis was carried out under the following conditions: the mobile phase: 0.1% aqueous solution of formic acid (Solution B) : 0.1% Formic acid in Acetonitrile (Solution A) with the ratio 95 : 5, gradient elution : 5 – 25% solution B – 0-7 min, 25-70% solution B – 7-20 min and 5% solution B – 20-25 min; mobile phase flow rate was 0.8 ml/min, column thermostat temperature - 30°C, ionization – ESI^-, scanning options – Total Ion Chromatogram (TIC) and multiple reactions monitoring (MRM), sample volume 5µl and duration of analysis - 25 minutes. Conditions for MS/MS detection were as following: gas temperature - 310°C, gas flow rate – 8 ml/min, nebuliser – 40 ml, capillary voltage – 4000 V, fragmentation amplitude – 100 V, variable collision energy. The identification was achieved by analysing retention times (t_R) and spectra characteristics of individual compounds with those in data library. Achieved results are demonstrated on LC-MS/MS and MRM chromatograms on figures 4 and 5. Identified phenolic profile of Saperavi shoots extract is summarized in table 1.

Figure 4: LC-MS chromatogram of Saperavi shoots extract

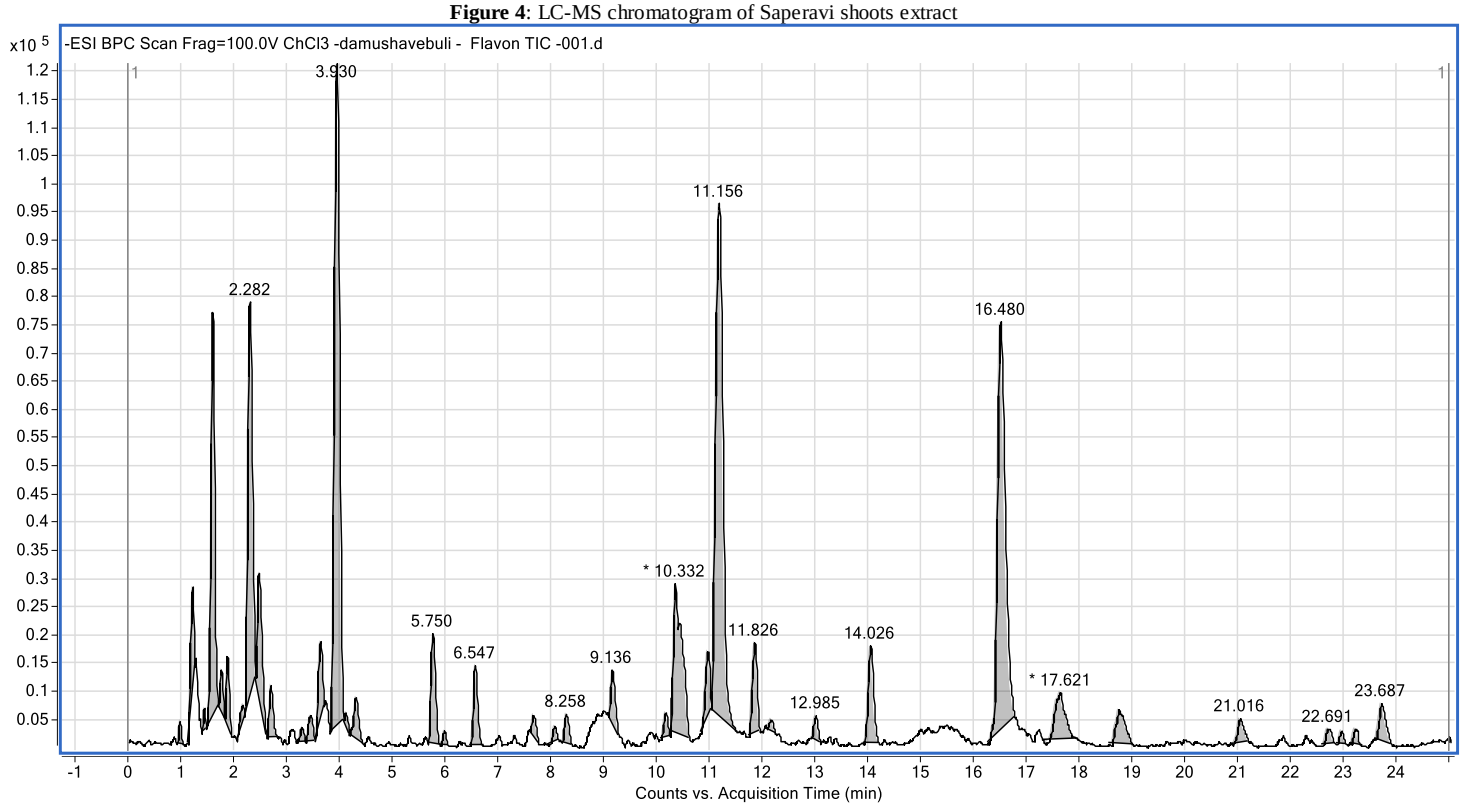
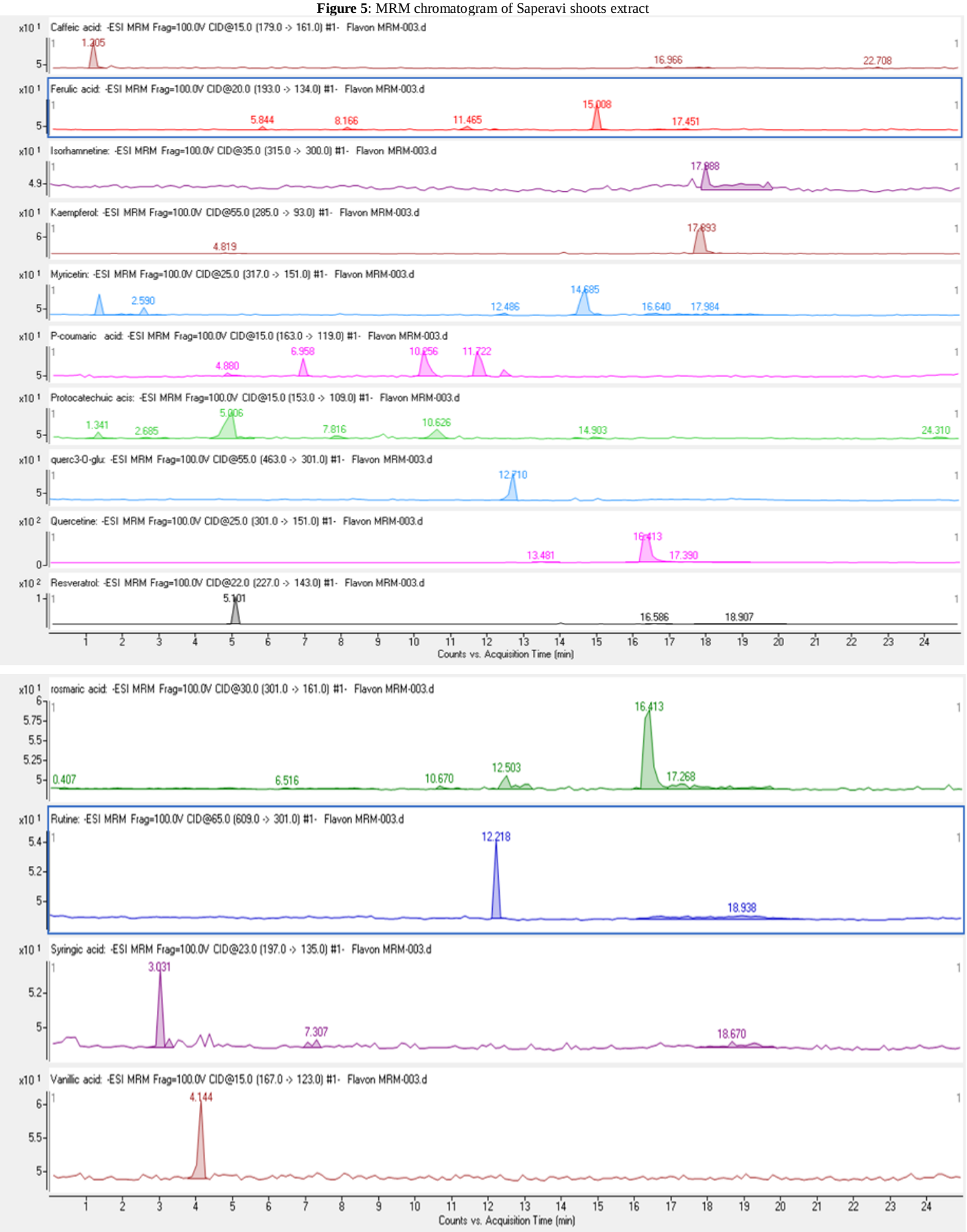


Figure 5: MRM chromatogram of Saperavi shoots extract



RESULTS AND DISCUSSION

The present study was conducted for evaluation of phenolic compounds content in Saperavi shoots extract and isolation of individual polyphenols. Total phenolic content was analysed using Folin-Ciocalteu reactive and achieved result was 35.585mg, expressed as GAE. The qualitative composition and content of phenolic compounds in Saperavi shoots extract was studied with LC–MS/MS. As a result, 14 different phenolic compounds were isolated and detected (table 1).

Table 1: Detected phenolic content in Saperavi shoot extract

Compound	MS1	MS2	Identification
1	179	161	Caffeic acid
2	193	134	Ferulic acid
3	315	300	Isorhamnetine
4	285	93	Kaempferol
5	317	151	Myricetin
6	163	119	P-coumaric acid
7	153	109	Protocatechuic acis
8	463	301	Querc3-O-glu
9	301	151	Quercetine
10	227	143	Resveratrol
11	301	161	Rosmaric acid
12	609	301	Rutine
13	197	135	Syringic acid
14	167	123	Vanillic acid

CONCLUSION

Study of phenolic content in Saperavi grapevine shoots extract was carried out by calculating TPC with F-C reagent in terms of Gallic acid and identification of compounds on LC–MS/MS. Achieved results demonstrated rich content of phenolic agents in Saperavi grapevine shoots extract. 14 different phenolic compounds were isolated and detected during the current study: Caffeic acid, Ferulic acid, Isorhamnetine, Kaempferol, Myricetin, P-coumaric acid, Protocatechuic acis, Querc3-O-glu, Quercetine, Resveratrol, Rosmaric acid, Rosmaric acid, Rutine, Syringic acid, Vanillic acid. Received results demonstrated possibility of using Georgian Saperavi grapevine shoots as a source of biologically active ingredients, for which studies should be proceeded accordingly.

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Conflict of interest:

The authors declare no conflict of interest.

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