Bokuchava N., Murtazashvili T., Sivsivadze K., Tatanashvili M., Masiukovichi T.

DETERMINATION OF THE TOTAL PHENOLIC CONTENT IN GRAPEVINE SHOOTS WITH FOLIN CIOCALTEU METHOD

TSMU, DEPARTMENT OF PHARMACEUTICAL AND TOXICOLOGICAL CHEMISTRY

INTRODUCTION

Georgia is one of the oldest wine regions in the world. The earliest remains found so far indicate the development of viticulture and production of wine and therefore, the existence of wine culture in Georgia about 8 thousand years ago [6].

Wine industry generates a high amount of by-products, such as shoots, canes, pomace etc., left from grape processing. The interest to these products is growing, as they can be used as a cheep and easily available source of raw materials for the recovery of a lot of bioactive substances for pharmaceutical and cosmetic applications instead of being treated as waste [4,11]. Polyphenolic compounds are the most valuable of bioactive substance derived from vineyard/wine by-products due to their well-documented biological activities. Phenolic compounds are important for plant growth and reproduction, also acting as antipathogens (stress conditions such as infections and wounds) and protection against UV radiation [1]. Pharmacological benefits of Polyphenols, such as antioxidant, cardioprotective, antimicrobial, anti-inflammatory, antiaging, and anticancer, are also well-known [2,9,10].

Polyphenols are chemically characterized as compounds with phenolic structural features, this group of natural products is highly diverse and contains several sub-groups of phenolic compounds. More than 8.000 types of polyphenols have been identified which include compounds containing carbon of C 6 to C30. Polyphenols are characterized by the presence of large multiples of phenol structural units. The number and characteristics of these phenol structures underlie the unique physical, chemical, and biological properties of particular members of the class [8].

The aim of the study was selection of the optimal extraction conditions for TPC (total polyphenol content) from grapevine shoots, to optimize a method for calculation of TPC and evaluation of possible variations of the Folin-Ciocalteu method applied to the shoot raw material and determine TPC in shoots of different samples of Georgian grapevine.

Generally, available methods of quantification of total phenolic content in food products or biological samples are based on the reaction of phenolic compounds with a colorimetric reagent, which allows measurement in the visible portion of the spectrum [5,7]. The Folin-Ciocalteu (F-C) assay is one of this kind of methods. The F-C assay relies on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes to form blue complexes that are determined spectroscopically at approximately 765 nm [3].

EXPERIMENTAL

Materials and method

The analytical standard and reagents were purchased: Rutin (cat. number PHL89270) and Folin Ciocalteu (cat. number F9252) from SIGMA-ALDRICH; Solvents - Ethanol and Methanol - from MERCK.

Three different samples of raw material shoots were collected in June-July 2019 in Kahketi region (Georgia) - Rkatsiteli (Arkhilo), Rkatsiteli (Alvani) and Saperavi (Arkhilo). The extraction conditions of polyphenols were selected and total quantity was determined expressed as Rutin content. Analyse was based on complex formation reaction, for which F–C reagent was used. Total Polyphenol quantity was measured with Spectrophotometric method on spectrophotometer i9; Hanon instruments.

Selection of optimal extraction conditions for TPC

Extraction of polyphenol compounds was conducted with different solvent systems and extraction conditions. Solvent systems were 50% Ethanol and Methanol; extraction conditions – Ultrasonic bath and water steam bath. Data is provided in table N1.

Table N1: Extraction conditions of total phenolic compound

	Solvent	Solvent volume (ml)	Raw material (g)	Extraction type	Extraction time (min)	*Vitis Variety*	Optical Density
Condition 1	50% Ethanol	100	10	Ultrasonic bath	10	Rkatsiteli Arkhilo	1.2
						Rkatsiteli Alvani	1.06
						Saperavi Arkhilo	1.08
Condition 2	50% Ethanol	100	10	Extraction on water bath	30	Rkatsiteli Arkhilo	0.67
						Rkatsiteli Alvani	0.61
						Saperavi Arkhilo	0.62
Condition 3	Methanol	100	10	Ultrasonic bath	10	Rkatsiteli Arkhilo	0.72
						Rkatsiteli Alvani	0.67
						Saperavi Arkhilo	0.69

TP (total polyphenols) content was extracted from different samples Georgian grapevine - Rkatsiteli (Arkhil), Rkatsiteli (Alvani) and Saperavi (Arkhilo), with selected optimal extraction conditions. Optimal analytical conditions were defined for the determination of TPC with Folin - Ciocalteu reagent in the derived extracts. Rutin standard solution was used for selection of optimal conditions.

Preperation of Rutin standard and calibration solutions

Rutin standard solution

0.025g Rutin standard was placed in 25 ml measuring flask. Ethanol was added for dissolution. The volume was filled up with Ethanol (A solution). 1 ml of A solution was placed in 10 ml measuring flask and the volume was filled up with distilled water (B solution). Concentration of B solution is 0.1 mg/ml.

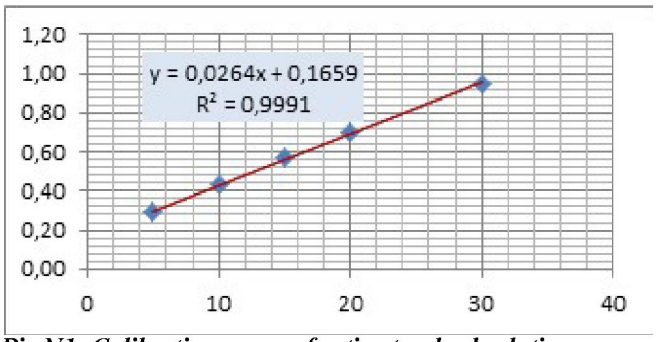
Serial dilution for calibration curve

0.5, 1.0, 1.5, 2.0 and 3.0ml of 0.1% Rutin solution (B solution) was placed in 10ml measuring flasks. Required amount of distilled water and 0.25ml of 1N F-C reagent were added. After retention of about 7-8 minutes on room temperature, the volume was filled up with Sodium Carbonate till flask neck. Solution was stored in a dark place for 40 minutes and absorption ability was measured with Spectrophotometer on 730 nm wavelength. Calibration curve is shown on the picture 1.

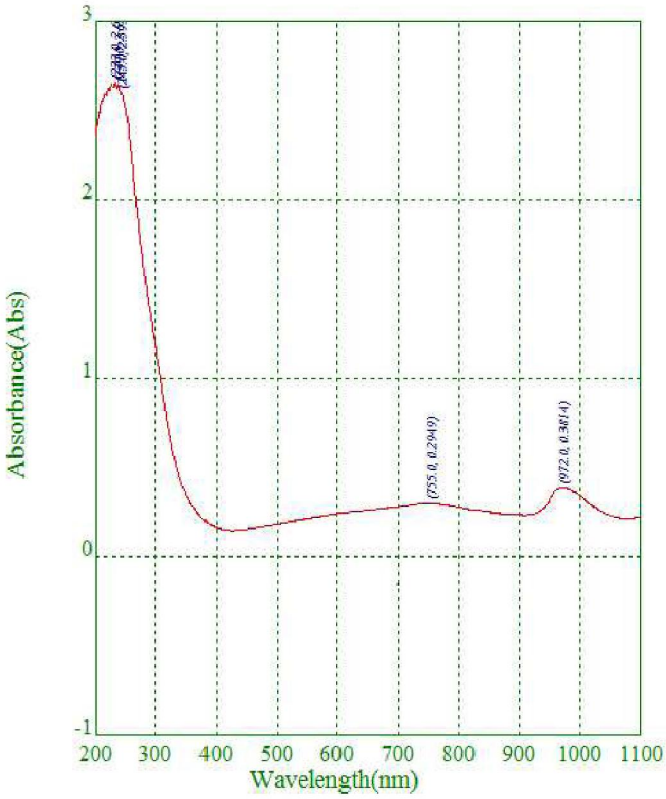
Based on preliminary studies were selected optimal conditions for calculation of TPC with the Folin-Ciocalteu reagent in shoots extract, see table N2:

Table N2: Optimal conditions for determination of total phenolic compound in shoots extracts

Extract volume (ml)	Water volume (ml)	F-C volume (ml)	Na_2CO_3 volume (ml)	Retention time (min)	Wavelength (nm)
0.5	8.0	0.25	1.25	40	730



Pic.N1: Calibration curve of rutin standard solution



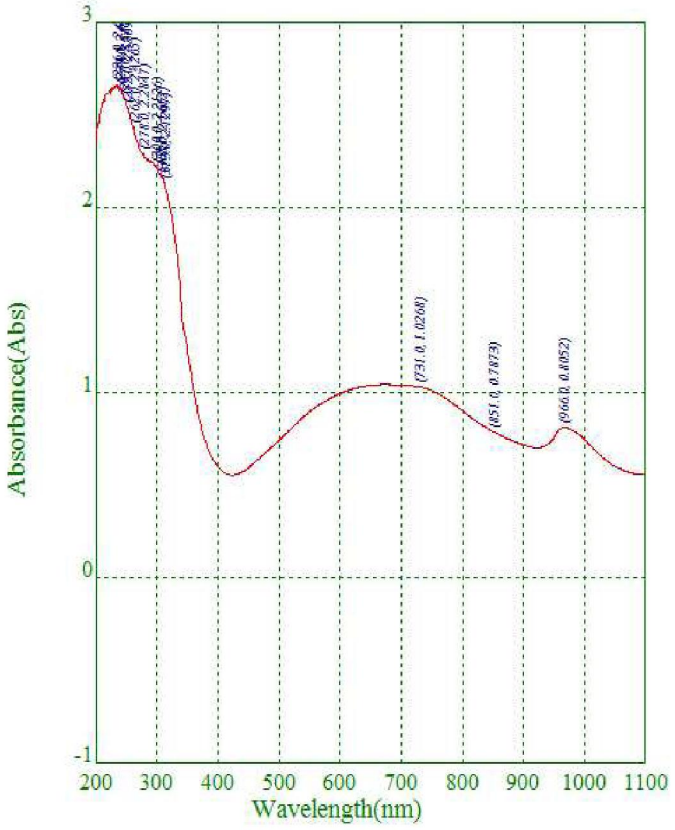
Pic.N2: UV spectra of Rutin Standard Solution

RESULTS AND DISCUSSION

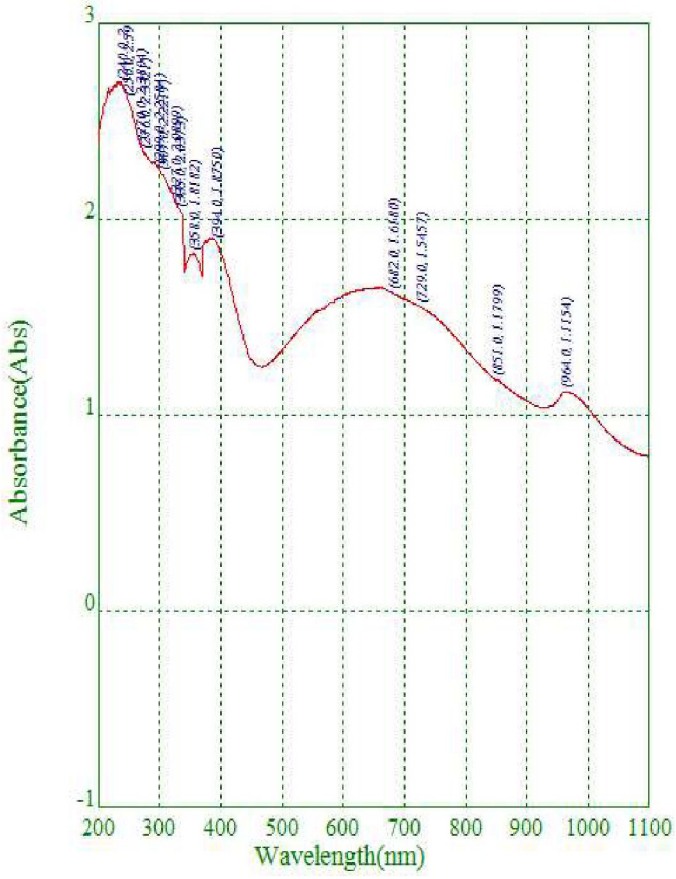
Based on the conducted experiment, extraction with 50% Ethanol on ultrasonic bath for 10 minutes was selected as an optimal condition for TP isolation from the selected samples. Selected condition were used for extraction of total Polyphenol content from the grapevine cane of different plant material. TPC were determine in grapevine cane extracts with Spectrophotometer on 730 nm wavelength, expressed as Rutin content, spectra is given on pictures 2,3,4 and 5.

According to received analytical data, TPC with F-C reagent was calculated, which was 2.1% for Rkatsiteli (Arkhilo), 1.9% for Saperavi (Arkhilo) and 1.8% for Rkatsiteli (Alvani).

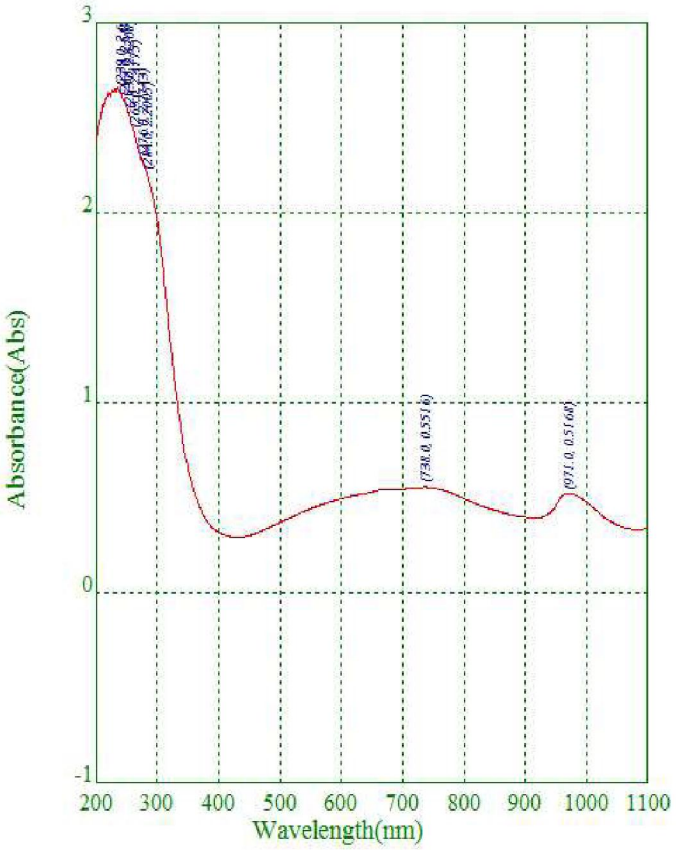
Selected method for determination of TPC was validated according to the international validation standards on following paramaters: lineraty ($R^2 = 0.9991$), Precision (0.65%), Accurency (89.9%), LOQ (100 µg/ml), LOD (30 µg/ml), method has shown selectivity.



Pic.N3: UV spectra Rkatsiteli (Arkhilo)



Pic.N4: spectra of Saperavi extract (Arkhilo)



Pic.N5: spectra of Rkatsiteli extract (Alvani)

CONCLUSION

An optimized extraction conditions were selected to maximize the extraction of TP from grapevine shoots of different samples of Georgian grapevine - Rkatsiteli (Arkhilo), Rkatsiteli (Alvani) and Saperavi (Arkhilo). For determination of TPC with Folin-Ciocalteu reagent an optimal analytical conditions were chosen. Developed method showed linearity, precision, accuracy and selectivity. Comparative determination of TPC in different samples of grapevine (by location and verities) was conducted according to the described method. Study results demonstrated, that shoots of Rkatsiteli (Arkhilo), Rkatsiteli (Alvani) and Saperavi (Arkhilo) contain sum of Polyphenols and the highest value was shown in Rkatsiteli (Arkhilo).

REFERENCES:

1. D. Rusjan, R. Veberic and M. Mikulic-Petkovsek. The response of phenolic compounds in grapes of the variety 'Chardonnay' (Vitis vinifera L.) to the infection by phytoplasma Bois noir. European journal of plant pathology 133, 1–10 (2012).

2. Doshi, P.; Adsule, P.; Banerjee, K.; Oulkar, D. Phenolic compounds, antioxidant activity and insulinotropic effect of extracts prepared from grape (Vitis vinifera L.) byproducts. J. Food. Sci. Technol. 2015, 52, 181–190

3. Elizabeth A Ainsworth, Kelly M Gillespie, Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent, Published online 12 April 2007; doi:10.1038/nprot.2007.102

4. González-Centeno, M.R.; Comas-Serra, F.; Femenia, A.; Rosselló, C.; Simal, S. Effect of power ultrasound application on aqueous extraction of phenolic compounds and antioxidant capacity from grape pomace (Vitis vinifera L.): Experimental kinetics and modeling. Ultrason. Sonochem. 2014, 22, 506–514.

5. Magalha˜es, L.M., Segundo, M.A., Reis, S., Lima, J.L. & Rangel, A.O. Automatic method for the determination of Folin–Ciocalteu reducing capacity in food products. J. Agric. Food Chem. 54, 5241–5246 (2006).

6. Putkaradze R., Perspectives Of Georgian Wine Export On The Global Market.

7. Robards, K. & Antolovich, M. Analytical chemistry of fruit bioflavonoids a review. Analyst 122, 11R–34R (1997).

8. Rong Tsao, Chemistry and Biochemistry of Dietary Polyphenols, Nutrients 2010, 2, 1231-1246.

9. Xia, L.; Xu, C.; Huang, K.; Lu, J.; Zhang, Y. Evaluation of phenolic compounds, antioxidant and antiproliferative activities of 31 grape cultivars with different genotypes. J. Food Biochem. 2019, 43, 12626.

10. Xu, Y.; Burton, S.; Kim, K.; Sismour, E. Phenolic compounds, antioxidant, and antibacterial properties of pomace extracts from four Virginia-grown grape varieties. Food Sci. Nutr. 2016, 4, 125–133.

11 Zilich, O.V.; Schweiggert-Weisz, U.; Eisner, P.; Kerscher, M. Polyphenols as active ingredients for cosmetic products. Int. J. Cosmet. Sci. 2015, 37, 455–464.

Bokuchava N., Murtazashvili T., Sivsivadze K., Tatanashvili M., Masiukovichi T.

DETERMINATION OF THE TOTAL PHENOLIC CONTENT IN GRAPEVINE SHOOTS WITH FOLIN CIOCALTEU METHOD

TSMU, DEPARTMENT OF PHARMACEUTICAL AND TOXICOLOGICAL CHEMISTRY

Georgia is one of the oldest wine regions in the world. Wine industry generates a high amount of by-products, such as shoots, canes, pomace etc., left from grape processing. The interest to these products is growing, as they can be used as a cheap and easily available source of raw materials for the recovery of a lot of bioactive substances for pharmaceutical and cosmetic applications instead of being treated as waste. Polyphenolic compounds are the most valuable of bioactive substance derived from vineyard/wine by-products. The aim of the study was selection of the optimal extraction conditions for TPC (total polyphenol content) from grapevine shoots, to optimize a method for calculation of TPC and evaluation of possible variations of the Folin-Ciocalteu method applied to the shoot raw material and determine TPC in shoots of different samples of Georgian grapevine.

Three different samples of raw material shoots were collected in June-July 2019 in Kahketi region (Georgia) - Rkatsiteli (Arkhilo), Rkatsiteli (Alvani) and Saperavi (Arkhilo). The total Polyphenol extraction optimal conditions were selected and quantity was determined expressed as Rutin content. Developed method showed linarity, Precision, Accuracy and Selectivity. Comparative determination of TPC in different samples of grapevine was conducted according to the described method. Study results demonstrated, that shoots of Rkatsiteli (Arkhilo), Rkatsiteli (Alvani) and Saperavi (Arkhilo) contain sum of Polyphenols and the highest value was shown in Rkatsiteli (Arkhilo).