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QUALITATIVE AND QUANTITATIVE DETERMINATION OF TOTAL FLAVONOIDS IN THE CANES OF DIFFERENT VITIS VARIETY

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INTRODUCTION

Georgia is one of the oldest winemaking region in the world and grapes have a long and abundant history. The fertile valleys and protective slopes of the Transcaucasia were home to grapevine cultivation and wine production for at least 8000 years. Due to the many millennia of wine in Georgian history and its prominent economic role, the traditions of winemaking are considered entwined with and inseparable from the national identity [3].

People often enjoy the various grape products, such as fruit, raisins, juice and wine. Grape fruit contains various nutrient elements, such as vitamins, minerals, carbohydrates, edible fibers and phytochemicals [5].

Polyphenols are the most important phytochemicals in grapevine, because of possessing many biological activities and health-promoting benefits. The phenolic compounds mainly include anthocyanins, flavanols, flavonols, stilbenes (resveratrol) and phenolic acids [2].

Flavonoids, a group of secondary metabolites widely distributed in plants that represent a huge portion of the soluble phenolics present in grapevine. These compounds play different physiological roles and are often involved in protection against biotic and abiotic stress [4].

Based on the scientific research, in grape, flavonoids are the major portion of soluble phenolic compounds and represent the most concentrated natural antioxidants in the fruit berry. Polyphenolic compounds such as flavonoids have been used in various medicines and food products due to their potential health benefits and are still relevant and popular [7].

Nowadays, there is a growing interest in the waste management. It is very important to use natural wastes in a convenient and economical way. In particular, grapevine's waste products could be an alternative source for obtaining natural flavonoids [1].

The phenolic compounds – flavonoids of the canes of grapevines, particularly the sum of flavonoids have been the center of attention of recent studies.

According to the structures and the properties of these bioactive components were performed experiments to develop thin layer chromatography (TLC) for quality and photocolorimetric methods for quantitative determination.

EXPERIMENTAL

Chemicals and Material

The analytical standard and reagents were purchased: Rutin, Aluminum Chloride (AlCl3), 2-Aminoethyl diphenylborinate (spray reagent) from SIGMA-ALDRICH; Solvents - Ethanol, Chloroform, Methanol and glacial acetic acid from MERCK.

Five different types of material were collected in April - May 2017. Samples of cane were prepared from different areas in Georgia: Ojaleshi and Aladasturi from Imereti region, Sachkhere; Rkatsiteli and Dirbula from Kakheti region,Akhmeta; Adesa was gathered in Tbilisi, Georgia. Material was dried in ventilated facility during 2 weeks.

Instrumentation

Thin layer chromatography (TLC) was performed on 20 × 20 cm TLC silica gel 60 F254 plates (Merck, Germany). Chloroform-methanol-water in volume ratio 26 : 14 : 3 was used as mobile phase.

Photocolorimetric measurements were performed on a 364 nm wavelength on photo colorimeter KFK 2 M (LOMO, Russia), was used cuvette with a layer thickness of 10 mm.

Methods

Extraction procedure

The crushed samples of plant material (10 g) different varieties were transferred to a round-bottom flask of 250 mL and added 200 mL 50% ethanol. The mixture was heated up in water bath, under reflux during 30 min. The residue of herbal material was resubmitted to reflux with 100 mL of the same solvent during 30 min. Received extractive was collected, cooled at room temperature and filtered into a volumetric flask of 250 mL through the filter paper. Filtrate was evaporated on the water bath during 20 minutes and received extract was transferred into separation funnel. Purification was done by double step liquid-liquid extraction, using 15 - 15 mL chloroform as purification agent. After finishing the isolation procedure was collected and filtered to originate the stock solution.

Qualitative and quantitative analysis

Identification of total flavonoids by TLC

Standard solution

0.05 g of the rutin standard was transferred to a 100 mL volumetric flask and filled the volume up with 96% ethanol. The standard was dissolved by heating in a water bath and after cooling the volume of the solution was adjusted with 96% ethanol. Analytical sample 5.0 mL from each SS was transferred to a 10 mL volumetric flask and filled the volume with 96 % ethanol.

Analytical procedure

TLC was performed using silica plate. Standard solution of rutin and analytical samples were applied on the TLC silica plates at 1 cm from the bottom (as spots) using a capillary tube. Suitable mobile phase was loaded into a tank. At least half an hour solvent vaporized inside the running tank. After the balance was achieved, plates with sample and standard solution were transferred in a running tank. The running process was allowed to leave the mobile phase to reach the top point of up to 10 cm. After finishing of the process plates were removed from the tank and then dried in a fume cupboard until the smell of solvents disappeared. Plates were visualized directly after drying. There were developed colored and colorless spots on the plate under the UV light at 254 nm. Then all spots on the dried plate were treated with spray reagent under the UV light at 365 nm. On chromatograms, flavonoids appeared as orange-yellow spots.

Flavonoids were determined by comparison of *Rf* values of each species and color characteristics of the standards. *Rf* values of the standards are given in Table 1.

Quantitative analysis of total flavonoids by Photocolorimetry

Analytical procedure

Standard solution

0.05g of the rutin standard was transferred to a 100 mL volumetric flask and the volume was filled with 96% ethanol. It was dissolved by heating in a water bath, after cooling the volume of the solution was adjusted with 96% ethanol (Standard solution A). Was taken 2 mL of standard solution A transferred to a 10 mL volumetric flask, added 2 ml AlCl3 2% solution in 96 % ethanol, 1 ml 30 % glacial acetic acid and filled the volume with 96 % ethanol.

Blank of standard solution

2 mL standard solution A was transferred to a 10 mL volumetric flask, added 1 ml 30 % glacial acetic acid and the volume was filled with 96 % ethanol.

Analytical sample

2.0 mL SS of each species was transferred to a 10 mL volumetric flask, was added 2 ml AlCl3 2% solution in 96 % ethanol, 1 ml 30 % glacial acetic acid and the volume was filled with 96 % ethanol.

Blank solution for sample

2.0 mL SS of each species was transferred to a 10 mL volumetric flask, was added 1 ml 30 % glacial acetic acid and the volume was filled with 96 % ethanol.

The absorbance of analytical sample against blank solution was determined on Photocolorimeter at 364 nm.

RESULTS AND DISCUSSION

Total flavonoids content (TFC) of different species of grapevine cane were identified and determined. Flavonoids were identified using chromatographic techniques. On the TLC plates extracts were applied together with rutin standard and were compared for their *Rf* values and spot color intensity.

Table 1. *Rf* values and their colors of total flavonoids identified on TLC chromatogram

	Rf values	Color under UV 365 nm
Rutin	0.47	Orange to Yellow
Ojaleshi	0.49	Orange to Yellow
Aladasturi	0.44	Orange to Yellow
Rkatsiteli	0.51	Light Yellow
Dirbula	0.48	Orange to Yellow

Adesa	0.46	Orange to Yellow

As a result of the comparison of *Rf* values (take in account possible deviation) and colors under UV light, the total flavonoid compounds were identified in the different species grapevine cane.

TFC in different species of Georgian grapevine canes were calculated, as rutin, using Equation

$$X=\frac{D_x * M_{st} * 100 * 100 * 100}{D_{st} * 100 * P(100-w)}$$

Where:

Dx - Optical density of samples

Dst - Optical density of rutin standard solution

Mst – weight of the standard of rutin, (g)

P – weight of the crushed plant material (canes), (g)

W – content of moisture in plant material (canes), (%) which was 3 %.

Obtained results of Photocolorimetery determination are given in Table 2.

Table 2. Optical density (D) of solutions and respective content of total flavonoids

Sample	Optical density (D)	Content of total flavonoids
Rutin	0.30	-
Ojaleshi	1.20	2.11
Aladasturi	0.95	1.67
Rkatsiteli	1.30	2.28
Dirbula	0.93	1.63
Adesa	0.98	1.72

CONCLUSION

Was applied an optimized extraction conditions to maximize the extraction of flavonoids from different species of the grapevine canes. This study allowed to identify total TFC in canes as a rutin by using TLC. Was developed simple and fast photocolorimetry method condition to determine TFC grapevine canes.

The results presented in this study demonstrate that waste product grapevine canes have content of flavonoids. This will give an opportunity to use Georgian grapevines canes as a source of flavonoid compounds.

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სხვადასხვა ჯიშის ვაზის ყლორტში ფლავონოიდების ჯამის თვისობრივი და რაოდენობრივი განსაზღვრა

1თსსუ, ფარმაცევტული და ტოქსიკოლოგიური ქიმიის დეპარტამენტი; 2თსსუ, ფარმაკოგნოზიის და ბოტანიკის მიმართულება

ფლავონოიდები, ფენოლური ნაერთების დიდი ჯგუფი, წარმოადგენს მნიშვნელოვან ბიოლოგიურად აქტიურ ნივთიერებებს, რომლებიც აუცილებელია ადამიანის ორგანიზმის ნორმალური ფუნქციონირებისათვის. ფლავონოიდები ფართოდ არის გავრცელებული მცენარეულ სამყაროში. მათ ძირითადად შეიცავს მცენარის ნაყოფები, ფოთლები და ყვავილები. ფლავონოიდების ერთ-ერთ მნიშვნელოვან წყაროს წარმოადგენს, ვაზი და ვაზის პროდუქტები. მოცემული კვლევის ფარგლებში შემუშავებული იყო სხვადასხვა ჯიშის ქართული ვაზის ყლორტებიდან ფლავონეიდების ექსტრაქციის ოპტიმალური პირობები. ფლავონეიდების გამოყოფისათვის გამოიყენებოდა ორჯერადი ექსტრაქცია 96 % ეთანოლის გამოყენებით, ხოლო გასუფთავება ხდებოდა ქლოროფორმით. მიღებულ ექსტრაქტში ფლავონოიდების ჯამის განსაზღვრისათვის შემუშავებული იყო ფოტოკოლორიმეტრული მეთოდის ოპტიმალური პირობები, ხოლო იდენტიფიცირება მოხდა თხელფენოვანი ქრომატოგრაფიული მეთოდით, მოძრავი ფაზა ქლოროფორმი-

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