UDC 66

# THE GRAPE SKINS AND SEED POLYPHENOLIC EXTRACTS

# ПОЛИФЕНОЛЬНЫЕ ВИНОГРАДНЫЕ ЭКСТРАКТЫ

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*Abstract.* The paper dwells on identification of phenolic compounds and anthocyanins from newly–squeezed grape skins and seed ethanol extracts of Zeibeli and Izabela color grape varieties cultivated in viticulture–winemaking micro–zone of western Georgia, by combined gas chromatography mass–spectrometry, and their determination by method of high–pressure liquid chromatography, but antioxidant activity has been assessed by DPPH method.

For the experiment, we used newly–squeezed grape skins and seed without drying, mechanical and thermal treatment.

Total phenolic compounds content in grape skins and seed ethanol extracts of Zeibeli variety ($> 423mg$ /100 ml) was greater than in grape skins and seed extracts of Izabela variety ($>$ 394.2 mg/100 ml).

The same results have been registered when assessing antioxidant activity of extracts. In particular, in the conditions of the same dissolution of extracts, grape skins and seed ethanol extracts of Zeibeli variety had higher antioxidant activity (up to 67–79.4%) than grape skins and seed extracts of Izabela variety (49.5–63.9%). Studies have shown that total phenols and anthocyanins quantitative content correlates with antioxidant activity of extracts.

The polyphenolic contents and the antioxidant activity of the skins and pulps of different grape cultivars were estimated using HPLC and DPPH antioxidant assay, respectively. The phenolics and flavonoids identified were quercetin, kaempferol, caffeic acid, p–coumaric acid, cinnamic acid, and (−)–epicatechin. The total phenolic contents were found to be the highest in the grape skin of Flouxa (>400 mg/100 g), followed by Campbell Early and Tamnara (>300 mg/100 g), and then by Red Globe and Ruby Seedless (>250 mg/100 g), and the total phenolic content was the lowest in Italia and Delaware (<60 mg/100 g). The antioxidant activities of the grape extracts varied from 12.5% (Ruby Seedless) to 60.2% (Hongiseul) for skins, whereas the antioxidant activities of the grape extracts varied from 35.4% (Campbell Early) to 84.5% (Hongiseul) for pulps. The grape pulps have stronger antioxidant activities than those of the grape skins. Our results suggest that the phenolic and flavonoid contents in extracts of grape skins and pulps showed statistically significant correlations with the free radical scavenging activity.

*Аннотация.* В статье рассматривается идентификация фенольных соединений и антоцианов из только что выжатых этанольных экстрактов виноградной кожицы и косточек цветных сортов винограда Зейбели и Изабеллы, культивируемых в виноградно–

винодельческой микрозоне западной Грузии, с помощью метода определения высоко эфективной газовой и жидкостной хроматографии высокого давления, а антиоксидантная активность была оценена методом DPPH.

Для эксперимента мы использовали недавно выжатую виноградную кожицу и косточки без сушки, механической и термической обработки.

Общее содержание фенольных соединений в этанольных экстрактах виноградной кожицы и косточек сорта Зейбели (> 423 мг / 100 мл) было больше, чем в экстрактах виноградной кожицы и косточек сорта Изабеллы (> 394,2 мг / 100 мл).

Те же результаты были зарегистрированы при оценке антиоксидантной активности экстрактов. В частности, в условиях такого же получения экстрактов виноградной кожицы и косточек, этанольные экстракты сорта Зейбели имели более высокую антиоксидантную активность (до 67–79,4%), чем экстракты виноградной кожицы и косточек сорта Изабеллы (49,5–63,9%). Исследования показали, что количественный состав фенолов и антоцианинов коррелирует с антиоксидантной активностью экстрактов.

*Keywords:* extracts, grape, Izabela, Zeibeli, bio-flavonoids, color varieties, experimental equipment.

*Ключевые слова:* экстракты, виноград, Изабелла, Зейбели, биофлавоноиды, цветные сорта, экспериментальное оборудование.

*Introduction*

Growth in radionuclide environmental background and chemicals use in food products are charactereized by upset of antioxidant balance of human organism and pathologies of the immune system. Treatment and prevention of the mentioned pathologies and excretion of toxic substances from the body are possible only by using the drastic antioxidant, antitoxic and foodborn disease preventing plant preparations [1, p. 256; 2]. The best raw material base for producing such preparations and nutritional supplements is represented by environmentally safe, i.e. cultivated without using chemicals, colored grape varieties. Of particular interest is a grape stone, which contains 65–70% of total amount of phenol compounds existing in grapes, and is mostly represented by bio–flavonoids. They prevent the development of thromobotic proceses, improve lipid metabolism that in turn reduces pathologies of cardiac ischemic diseases and extends life expectancy (1). Proanthocyanidins, which represent the catechins' polymeric chain were obtained for the first time by Professor Jacques Masquelier in 1936, and he named these compounds P vitamin.

Therapeutic–preventive daily norm of flavonoids for adults is 80–85 mg, but the upper acceptance limit in medicine is 120–130 mg/per day. They strengthen eliminating signals for cancer cells in human organism, so that they do not harm the healthy cells. In 2006, American scientists found proteins in the grapeseed ethanol extract, which foster the destruction of cancer cells [3, p. 218].

It has been experimentally established that nutritional supplements containing total polyphenols of grape skins and stone are characterized by strong antioxidant synergism [4, p. 70; 5, p. 292]. and they are activated even further in the presence of ascorbic acid. Thus, it is desirable to create such supplements from the compositions of therapeutic plant–based extracts concentrated by vacuum, and they will not be toxic even in the conditions of their long–term consumption.

There are not studied in fact raw materials of colored chemical–free grape varieties (Izabela and Zeibeli) cultivated in the Imereti region's viticulture–winemaking micro–zone, each kilogram

of which presumably contains about 10 g of phenolic compounds, and most of them (3% of a dry mass) are concentrated in grape–stone [6, p. 65; 7, p. 334].

The grape polyphenols represent drastic natural antioxidants and their biological activity in the presence of C vitamin it event grows further. They prevent free–radical destruction of bio–membrane cells structure, protect human organism in such pathological states, as atherosclerosis, stress, anemia, bronchitis, early ageing, intensive chemotherapy results, postoperative status, chronic fatigue syndrome and so on [8, p. 638; 9, p.79].

Therefore, of high topicality is the development of innovative technologies of extracting dissoluble total polyphenols from raw materials of colored chemical–free grape varieties cultivated in the Imereti region's viticulture–winemaking micro–zone, for producing biologiclly active drastic antioxidant, antiradiant antiradiation, antitoxic and foodborn diseases preventing preparations.

Within the World Health Organization program MONICA and according to subsequent studies carried out in France, USA and other countries, it has been established that the therapeutic effect of red grape wine on human organism and its recommended daily norm is 70–100 ml, but not everybody can to intake this dose every day (children, patients, athlets, hardly working and religious people), consequently, the development of innovative technologies for producing from environmentally safe raw materials high antioxidant, antitoxic and foodborn diseases preventing polyphenol concentrate has a high pragmatic value [10, p. 92; 11, p. 221].

*Materials and Methods*

The selected raw materials for the study were Zeibeli and Izabela color grape varieties cultivated in Baghdati viticulture–winemaking micro–zone.

The process of cultivating the mentioned grape varieties does not involve the use of pesticides, chemicals and inorganic enriching agents.

To provide maximum extraction of the phenolic complex from newly–squeezed grape skins and stone, we arranged a series of experiments.

Extraction of grape secondary resources was carried out in two stages separately, independently of each other.

Based on the carried out studies and analysis of batch–type extraction equipment, we developed the innovative batch–type industrial and experimental extractor, which was used for performing experimental works, the principal scheme of which is shown below in Figure 1.

*At the first stage*, the newly–squeezed skins and stone, without any drying and crushing, are supplied to the extraction equipment, where per 1000 g of the grape skins and stone composition were added with 1000 ml of 80–82% ethanol, which is oxidized to 1% by citric acid.

*At the second stage,* per 1000 g of the extraction cake remaining in the extractor (i. e. skins and stone remaining after primary extraction) were added with 1000 ml of 12–18% ethanol without oxidation, and extraction was carried out.

The composition of extracts of both stages was centrifuged and filtered by means of German–made wine layered filters, and condensation–concentration of a part of the obtained extracts was carried out in a vacuum at the temperature of not higher than 50 °C until 57–63% of dry substances content.

For analysis, we used both uncondensed and condensed extracts for both grape raw materials separately.

Studies of physical–chemical characteristics of extracts were carried out in compliance with international standards (ISO — International Organization for Standardization). In particular, as follows:

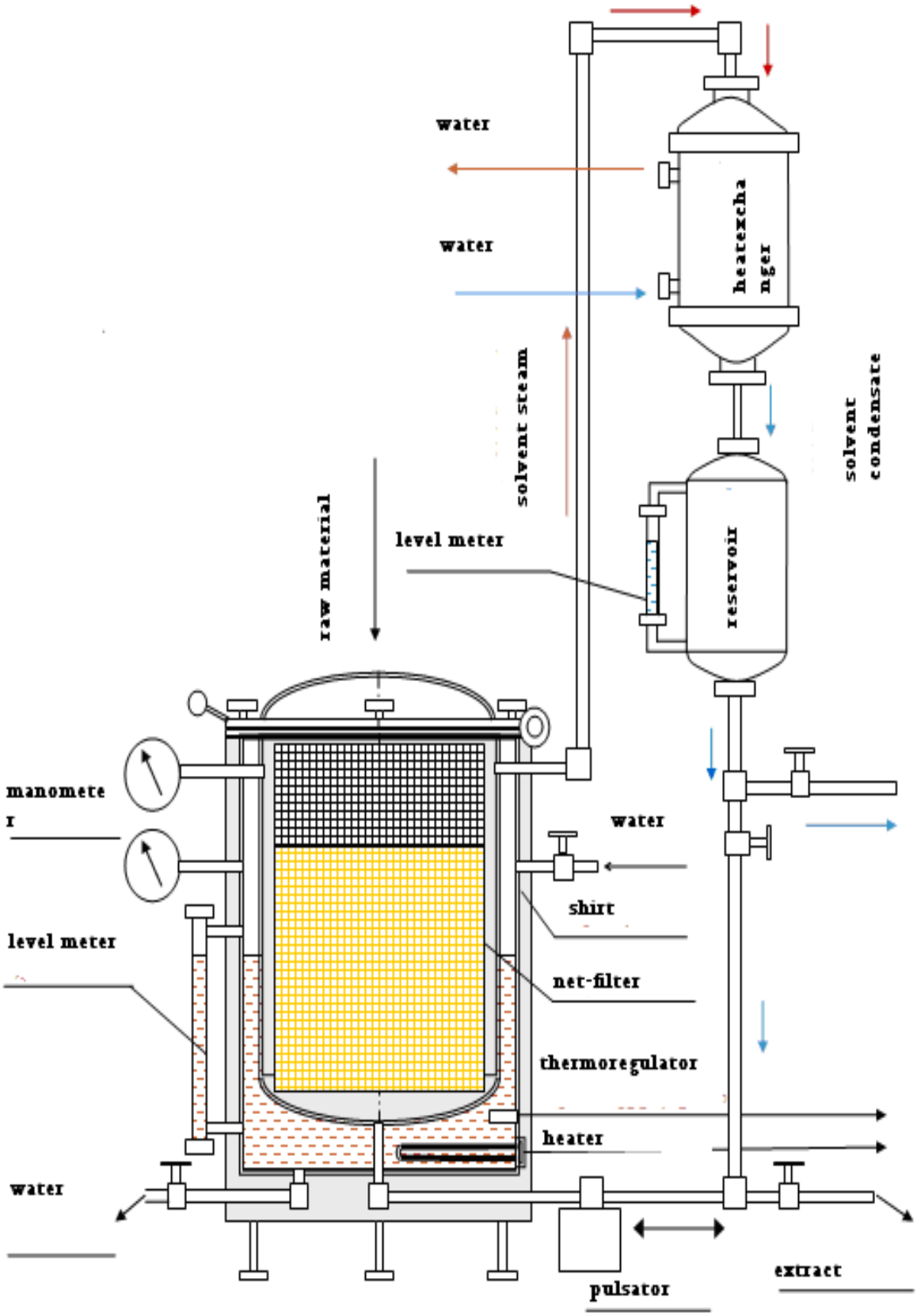



Figure 1. Principal Scheme of Industrial and Experimental equipment
At both stages, the extraction was carried out at the temperature of 45–50 °C within 4–4,5 hours under conditions of periodical pulsation

–the content of water–soluble extracts: *ISO 9768:1994*, which envisages extraction in the conditions of reflux, filtration, drying of non-dissolved waste and weighing with further calculation of extracting substances;

–determination of phenolic compounds in the extracts was carried out by colorimetric (Folin–Ciocalteu) method, which is based on the capacity of phenolic compounds to recover phosphatotungstic and phosphatomolybdic acids, and to measure the coloration intensity of a blue–color molybdenum to a rusting color by using colorimetric method;

–1 $cm^3$ of extract, 15 $cm^3$ of water, 1 Folin Ciocalteu reagent and 20 $cm^3$ of 20% sodium carbonate solution were placed in the flask with a capacity of 100 $cm^3$, and 30 minutes later we measured optical density by using the ditch with 10 mm thick and the wavelength of 670 nm. For comparison, we prepared the second solution, in which 1 $cm^3$ of sample of the analyzed extract was replaced by water.

The concentration of phenolic compounds was determined by a calibration curve of standard solution of gallic acid [12–13].

To determine anthocyanins, we used the method of high–pressure liquid chromatography by using the American–made chromatograph *(Waters (UV/Visible Detector 2489, Binary HPLC Pump1525).* The analyzed extract was filtered with a special–purpose chromatograph's filter (0,45 μm), and we used chromatograph column Symmetry C18, 3,5 μm 4,6×75 mm, detection was carried out at 510 and 524 nm, the solvent systems: 1% phosphorus acid (A), acetonitrile (B) and 5% formic acid (A), methanol (B) (Merk; Aldrich) in a linear gradient. Dissolution rate — 1 ml/min, sample quantity — 20 μl. Duration of chromatography — 45 minutes [14–15].

To determine antioxidant activity, i. e. radical binding activity, 1 ml of the analyzed extract is added with 3 ml of DPPH alcohol solution (0.1 mM DPPH — in 0.004 g/100 ml of ethyl alcohol), and 30 minutes later, there was carried out spectrophotometric determination of optical density of analyzed sample on 515 nm. The reference solution is represented by DPPH solution, but the background — by 96% ethyl alcohol. During the research process, the optimal concentration of solution was determined for inactivation of DPPH radical for each standard compound [16–18].

## *Results and Discussion*

An analysis of the research results has shown that many factors have a significant influence on the extraction process, including the ratio of extraction raw materials and extracting agent, extraction temperature, extracting agent polarity, degree of raw materials crushing, extraction method, pulsation frequency and so on.

Based on the analysis of available literature sources, it has been established that the extracts obtained from grape skins and stone without preliminary drying and crushing mostly possess health–promoting properties. Based on this, we selected for the extraction the newly–squeezed environmentally safe color grape skins and stone, and the extraction was carried out without drying and crushing.

The two–stage extraction at the temperature of 45–50 °C within 4–4.5 hours under pulsation conditions, is conditioned for increasing periodical pulsation and for maximal extraction of phenolic complex.

Table and Figure 2 show the quantitative content of phenolic compounds in the grape skins and stone ethanol extract of Zeibeli and Izabela varieties.

The conducted research has shown a very high bio–flavanoid composition of eco–extracts, which in turn points to high antioxidant activity of extracts. The total phenols content is high

enough in the grape skins and seed extracts of Zeibeli variety (≥423/100 ml), and is relatively low in the similar extracts of Izabela (≥ 394,2/100 ml).

Table.

PHENOLIC COMPOUNDS CONTENT IN GRAPE SKINS AND SEED ETHANOL EXTRACTS OF ZEIBELI AND IZABELA VARIETIES

| *No* | *Name* | *Concentration of phenolic compounds, mg/dm³* | | | |
|---|---|---|---|---|---|
| | | *Total quantity* | *Monomeric forms* | | *Polymeric forms* |
| | | | *Sum total* | *including anthocyanins* | |
| 1. | Grape skins and seed ethanol extract of Zeibeli variety | 4230 | 1932 | 451 | 1847 |
| 2. | Grape skins and seed ethanol extract of Izabela variety | 3942 | 1823 | 405 | 1714 |

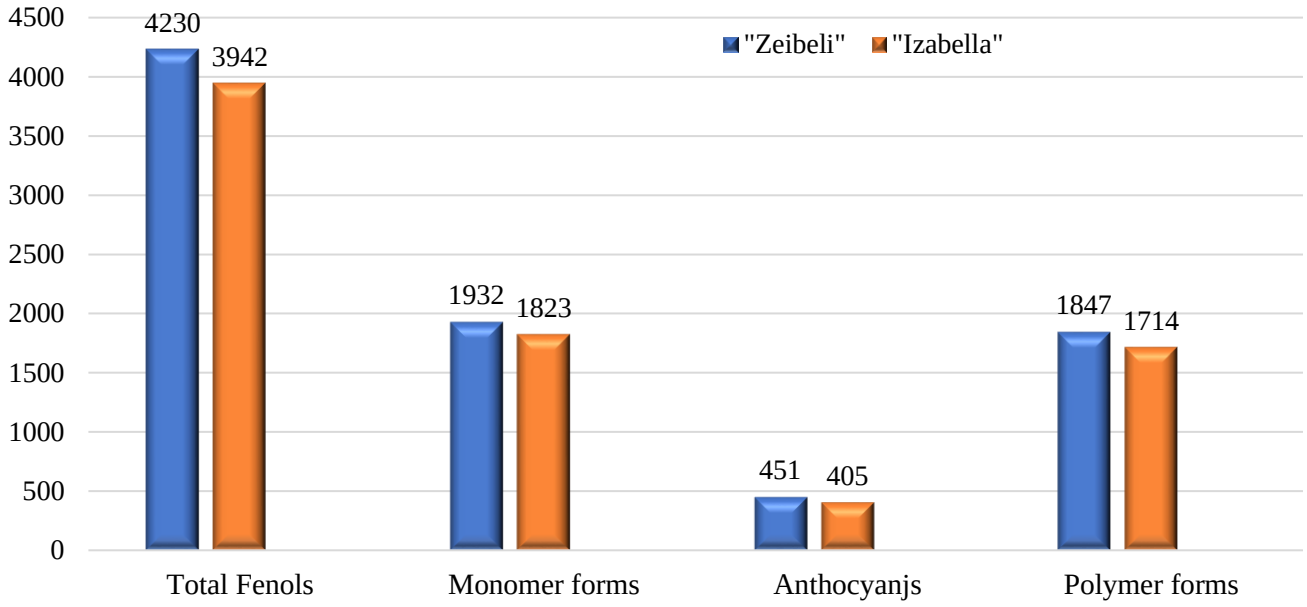



Figure 2. Phenolic compounds content in the extract s, mg/dm³

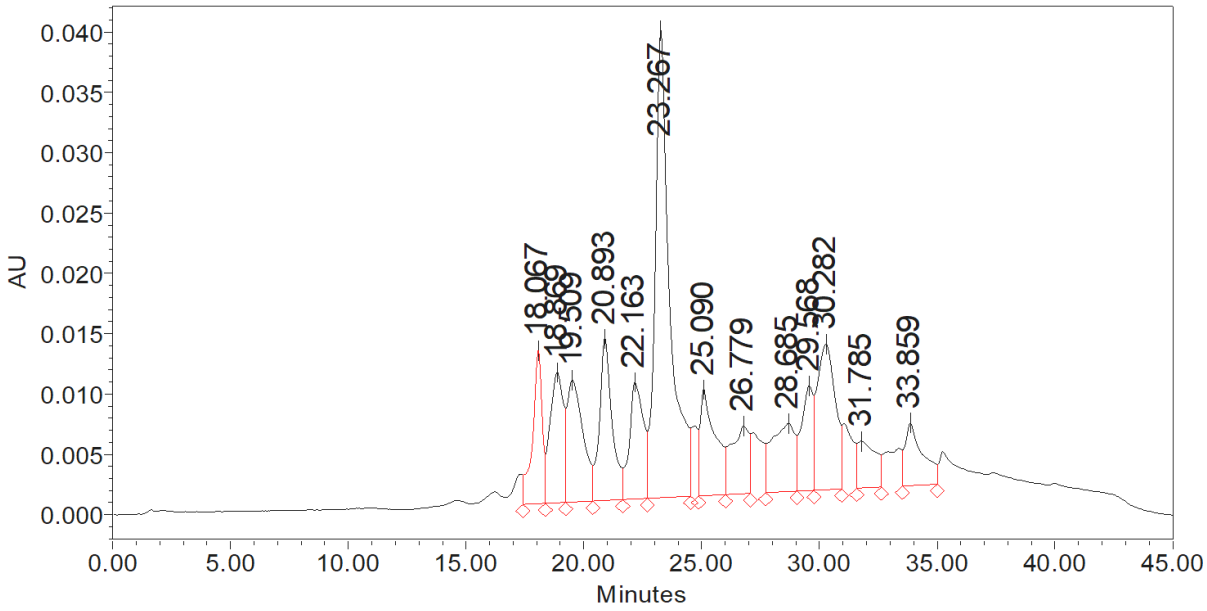
Figure 3 illustrates the chromatogram of the anthocyanins content in the grape skins and stone ethanol extracts of Zeibeli variety, where it is clearly seen that 63.04% of anthocyanins are represented by mono–glucosides, and the rest are the diglucosite and unknown forms.

Both types of extracts are characterized by high values of antioxidant activity (see Figure 4).

The first and second samples illustrated in Figure 4 are the grape skins and seed extracts of Zeibeli variety, non-concentrated and concentrated, accordingly, but the third and fourth samples are the grape skins and seed extracts of Izabela variety, non-concentrated and concentrated, accordingly.

Antioxidant activity of both non-concentrated and concentrated eco–extracts is very high, and therefore, of high relevance is the use of hydrophilic and lipophilic extracts of secondary resources

of Zeibeli and Izabela varieties in production of food supplements fortified with biologically active compounds.




| | SampleName | Acq Method Set | Channel Description | ColumnType |
|---|---|---|---|---|
| 2 | Extract-1 | Anthociane $CH_3CN$ 85% | W2489 ChB 524nm | C 18 |

| | *Name* | *Retention Time* | *Area* | *% Area* | *Height* | *Int Type* |
|---|---|---|---|---|---|---|
| 1 | delphinidin-3-monogalactoside | 15.389 | | | | Missing |
| 2 | Peak2 | 16.048 | | | | Missing |
| 3 | delpinidin-3-monoglucoside | 17.034 | | | | Missing |
| 4 | Peak4 | 18.067 | 357189 | 5.61 | 12693 | VV |
| 5 | cyanidin-3-monogalactoside | 18.869 | 430186 | 6.75 | 10831 | VV |
| 6 | delphinidin-3-monoarabinoside | 19.509 | 474603 | 7.45 | 10125 | VV |
| 7 | Peak7 | 20.893 | 501864 | 7.88 | 13422 | VV |
| 8 | cyanidin-3-monoglucoside | 22.163 | 402541 | 6.32 | 9676 | VV |
| 9 | malvidin-3-monoarabinoside | 23.267 | 1692237 | 26.57 | 38710 | VV |
| 10 | petynidin-3-monoglucoside | 25.090 | 398121 | 6.25 | 8830 | VV |
| 11 | peonidin-3-monogalactoside | 25.370 | | | | Missing |
| 12 | petunidin-3-monoarabinoside | 26.779 | 294660 | 4.63 | 5625 | VV |
| 13 | petynidin-3-monogalactoside | 28.685 | 389792 | 6.12 | 5644 | VV |
| 14 | peonidin-3-monoglucoside | 29.568 | 297645 | 4.67 | 8682 | VV |
| 15 | | 30.282 | 650573 | 10.21 | 12074 | VV |
| 16 | peonidin-3-monoarabinoside | 31.785 | 209002 | 3.28 | 3884 | VV |
| 17 | | 33.859 | 271669 | 4.26 | 5199 | VV |
| 18 | Peak16 | 38.941 | | | | Missing |

Figure 3. The chromatogram of the anthocyanins content
in the grape skins and seed ethanol extracts of Zeibeli variety

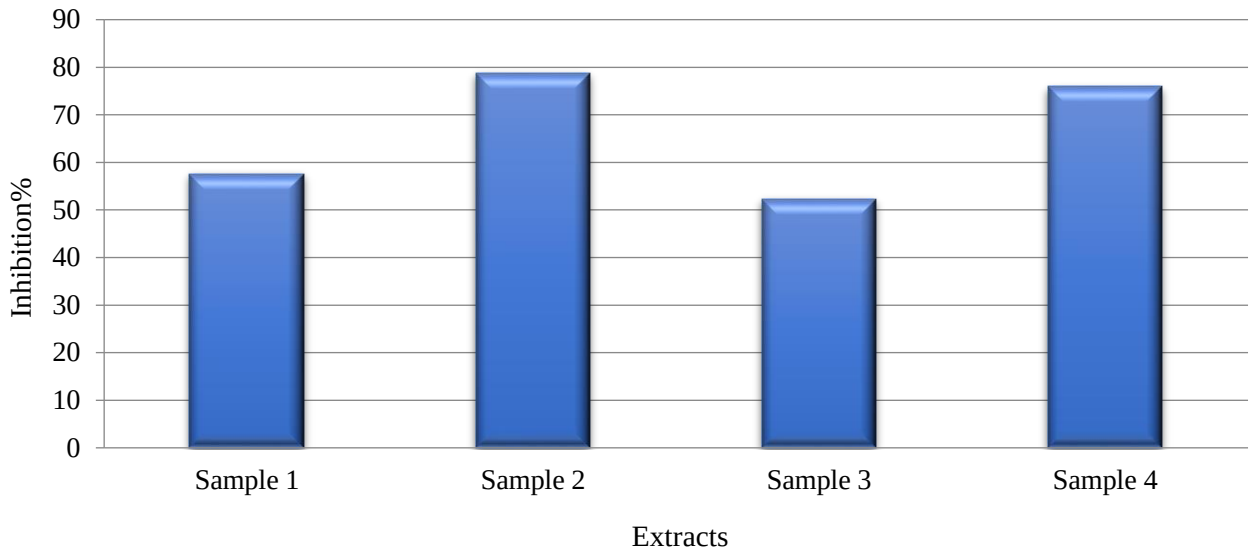



Figure 4. Binding of DPPH radical by the grape skins and seed extracts of Zeibeli (Sample1 and 2) and Izabela, (Sample 3 and 4) varieties.

The average annual polyphenol reserves of the grape harvest in Georgia, which is mostly accumulated in a practically unused grape–seed, exceeds several hundreds of tons that in monetary terms makes up millions of US dollars. Because of fact that such natural wealth is not used, the health losses in the population are considerable and obvious, although it is difficult to assess them properly. In addition, we propose environmentally safe raw materials of colored chemical–free grape varieties cultivated in the region, which are practically unused, and the local population seriously sensible about that, since one of the main fields of their activities in a viticulture

*Conclusion*

By using the method of high–pressure liquid chromatography, in the color grape skins and seed extracts cultivated without use of chemical pesticides, there have been established a high content of total phenols and anthocyanins and antioxidant activity.

These resources are the best, ecologically pure raw materials for production of the drastic antioxidant polyphenolic concentrates.

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