Organic Chemistry

Phenolic Compounds in Grape Bunch and Wine of Georgian Autochthonal Vine Variety Tsolikauri

Armaz Shalashvili[*], Eka Tsutskiridze[**], Nino Beridze[**], Iraida Targamadze[*], Bezhan Chankvetadze[§]

* *Georgian Agrarian Univesity, Tbilisi*
** *I. Javakhishvili Tbilisi State University, Tbilisi*
§ *Academy Member, Georgian National Academy of Sciences, I. Javakhishvili Tbilisi State University, Tbilisi*

ABSTRACT. The objective of the study was to determine qualitative composition and quantitative content of phenolic compounds in grape bunch (stem, grape skin, seeds) and wine of Georgian autochthonal vine variety Tsolikauri by high-performance liquid chromatography (HPLC). Tsolikauri bunch stem contains the following phenolic compounds: gallic acid (0.096 mg/g), protocatechuic acid (0.071 mg/g), (+)-catechin (0.021 mg/g), caffeic acid (0.026 mg/g), (-)-epicatechin (0.007mg/g), rutin (0.112 mg/g) and quercetin (0.064 mg/g); trace quantities of vannilic acid, dihydroquercetin, *o*-coumaric acid. Syringic acid, ferulic acid, resveratrol and *p*-hydroxybenzoic acid were not found. Content of phenolic compounds in the grape skins is the following: protocatechuic acid (0.037 mg/g), caffeic acid (0.005mg/g), (-)-epicatechin (0.014 mg/g), rutin (0.149 mg/g) and *p*-hydroxybenzoic acid (0.063 mg/g); trace quantities of (+)-catechin, vannilic acid and *o*-coumaric acid. Gallic acids, syringic acid, ferulic acid, dihydroquercetin, resveratrol, quercetin were not found. Phenolic compounds content in the grape seeds is the following: gallic acid (0.535 mg/g), protocatechuic acid (0.212 mg/g), (+)-catechin (3.131 mg/g), syringic acid (0.313 mg/g), (-)-epicatechin (0.211 mg/g), ferulic acid (0.02 mg/g) and quercetin (0.102 mg/g); trace quantities of vanilic acid and *o*-coumaric acid. Caffeic acid, dihydroquercetin, rutin, resveratrol, *p*-hydroxybenzoic acid were not found. Phenolic compounds of Tsolikauri wine are: gallic acid (23.287 mg/l), protocatechuic acid (10.33 mg/l), (+)-catechin (23.987 mg/l), caffeic acid (0.616 mg/l), syringic acids (1.591mg/l), (-)-epicatechin (1.13 mg/l), dihydroquercetin (0.556) mg/l), rutin (9.103 mg/l) and quercetin (1.629 mg/l); trace quantities of vannilic acid, ferulic acid and *o*-coumaric acid. Resveratrol and *p*-hydroxybenzoic acid were not found. *© 2015 Bull. Georg. Natl. Acad. Sci.*

***Key words**: phenolic compounds, grapewine bunch, wine, hydroxybenzoic acids, hydroxycinnamic acids, catechins, flavanonol, flavonols, HPLC.*

Chemistry of grape and wine studies several thousands of compounds identified from the plants of *Vitis* family. These compounds belong to three general classes of natural compounds: phenolics, isoprenoids and alkaloids. The compounds play an important role in pharmaceutical and food industry. It should be especially noted that multiple biological activity of phenolic compounds have cardioprotective, antiinflammatory, anticancerogen and antibacterial action, caused by their antioxidant

and antibacterial properties. Those substances have physiological function in plants. They promote plant adaptation to the environment, strengthen their resistance to pests and diseases, condition symbiotic relationship with microorganisms. Phenols contribute to the characteristics of grape and wine. Color, taste, body, fragrance and antimicrobial activity of wine depend on phenolic and allied compounds. Phenolic compounds occur in fruits (skin and seeds), stem and other parts (organs) of the vine. In the process of wine making the number of phenolic compounds increases under the conditions of fermentation and prolonged maceration. Phenolic compounds in grapevine and wine are divided into two main subgroups: flavonoids (catechins, proanthocyanidins, flavonols and anthocyanins) and non-flavonoids (hydroxybenzoic acids, hydroxycinnamic acids and stilbens) [1-3].

The aim of the present work is to study qualitative and quantitative content of phenolic compounds by high-performance liquid chromatography in the grape bunch (stem, grape skin and seed) and wine of Georgian autochthonal vine variety Tsolikauri. Tsolikauri is a local Imeretian vine variety spread almost everywhere in Western Georgia. From Tsolikauri various kinds of table wine are produced characterized by high mouthfeel properties and rich chemical content. Besides the relatively high level of alcohol, Tsolikauri wine possesses rich body and ample amount of acids, which eventually improves wine in aging and its duration [4].

Materials and Methods

Samples of Tsolikauri grape (*Vitis vinifera* L.) were gathered for analysis in Keda municipality of Ajara, Western Georgia, in autumn 2012 at the stage of technical maturity. The bunches, skins and seeds were scoured by hand, washed well in running water and dried at the room temperature. The dry mass was ground in a coffee grinder. Two grams of each samples were extracted 3-times over the boiling water bath with 70% ethanol (duration of each extraction was 20 min, ratio of plant material and extragent - 1:20). Extracts were combined, filtered through a paper filter and distilled in rotary evaporator under vacuum at 40° C to remove ethanol. Extraction of phenolic compounds from the remained aqueous solution was performed in the separation funnel with water-saturated ethyl acetate 5-times. Extracts of ethyl acetate were combined and treated with anhydrous sodium sulphate with the aim of dehydration; the obtained ethyl acetate extract was filtered through a paper filter and distilled in the rotary evaporator under vacuum to obtain a dry residue, which was solved in methanol and filtered through a 0.45 μm membrane filter (Waters).

For making Tsolikauri grape wine, 5 kg grapes were pressed together with stems and placed in enamel ware. Fermentation was performed using endemic yeast *(Saccaromyces cerevisiae*). Fermentation lasted for 5 days with open cap stirring several times a day. On the 7th day of grape pressing the wine was poured into glass and placed in the refrigerator (2 l). The wine was analyzed after 1.5 years of storage. 50 ml of Tsolikauri grapewine was extracted 4-times with ethyl acetate. Extracts were combined (100 ml), dehydrated with anhydrous sodium sulphate, filtered through a paper filter, distilled on rotary evaporator at 30° C to obtain a dry residue. The residue was solved in 7 ml methanol and filtered through a 0.45 μm membrane filter (Waters). Identification of phenolic compounds was performed using HPLC (Agilent 1260) equipped with a pump, automatic device to reject the sample, thermostat and detector (in light UV and visible areas). Detection was made at 280 nm, and the data were computed with an apropriate software (Chemstation). Division was performed on the reversed phase Agilent-Eclipse XDB-C18-01 column (150 mm x 4.6 mm, 5 μm), at the room temperature. To divide individual phenolic compounds a modified method was used (Jordao et al.) [5]. A gradient consisting of (A) acetic acid/bidistilled water (2:98) and (B) acetonitryle/bidistilled water/ acetic acid (90:8:2) was used as mobile phase. Content of mobile phase changed as follows: 0 min 100% A, 0% B; 10 min 90% A, 10% B; 30 min 85% A, 15% B;

Table 1. Phenolic compounds in grape bunch and wine of vine variety Tsolikauri

№	Phenolic compounds	Retention time (min)	Parts of grape bunch (mg/g dry weight)			Wine Mg/L
			Stem	Skin	Seeds	
1	Gallic acid	5.057	0.096±0.007	nd	0.535±0.194	23.287±7.59
2	Protocatechuic acid	8.855	0.071±0.008	0.037±0.003	0.212±0.012	10.33±5.34
3	(+)-Catechin	12.965	0.021±0.025	traces	3.131±0.057	23.987±9.04
4	Vannilic acid	1.682	traces	traces	traces	traces
5	Caffeic acid	15.750	0.026±0.006	0.005±0.013	nd	0.616±0.169
6	Syringic acid	17.514	nd	nd	0.313±0.187	1.591±0.066
7	(-)-Epicatechin	19.454	0.007±0.005	0.014±0.011	0.211±0.274	1.13±0.601
8	Ferulic acid	27.108	nd	nd	0.02±0.003	traces
9	Dihydroquercetin	29.285	traces	nd	nd	0.556±0.007
10	Rutin	36.433	0.112±0.028	0.149±0.134	nd	9.103±3.26
11	*o*-Coumaric acid	39.474	traces	traces	traces	traces
12	Resveratrol	47.683	nd	nd	nd	nd
13	Quercetin	52.653	0.064±0.027	nd	0.102±0.059	1.629±0.24
14	*p*-Hydroxybenzoic acid	69.759	nd	0.063±0.009	nd	nd

Abbreviation: nd, not detected;

40 min 80% A, 20% B; 60 min 60% A, 40% B; 80 min 60% A, 40% B; 81 min 100% A, 0% B. Volume rate of mobile phase was 0.7 ml/min. Volume of analyzed sample solution was 5 µl. The compounds were identified using retention times of standard phenolic compounds. For quantification of individual compounds the calibration curves on HPLC were constructed for the following phenolic compounds: (+)-catechin, (-)-epicatechin and gallic acid (Sigma), quercetin and rutin (Chemapol), dihydroquercetin (Austrowaren), resveratrol (Bio-Tech Co.), protocatechuic, vannilic, caffeic, syringic, ferulic, *o*-coumaric and *p*-hydroxybenzoic acids (Reachim). Experimental results were processed statistically by MS-Excel-AVERAGEIF program. All determinations were made in triplicate.

Results and Discussion

According to the data presented in Table 1 the following hydroxibenzoic acids were found in the Tsolikauri grape bunch: gallic, protocatechuic, vannilic, syringic and *p*-hydroxibenzoic acids. In the stem, the gallic and protocatechuic acids were in the amount of 0.096 and 0.071 mg/g, respectively; vannilic acid was in trace quantities, and *p*-hydroxybenzoic acid was not found. In the grape skin, the amount of protocatechuic and *p*-hydroxybenzoic acids was 0.037 and 0.063 mg/g, respectively; vannilic acid was in trace quantities while gallic and syringic acids were not found. As for quantitative content of hydroxibenzoic acids, there is significant difference in the grape seed, stem and skin. The seed contains 5.5-times more gallic acid than the stem, and the amount of protocatechuic acid in it is 3- and 5.7-times more than in the stem and skin, respectively. Syringic acid content in the seed is 0.313 mg/g, vannilic acid is in trace quantities and *p*-hydroxybenzoic acid is not found.

From hydroxycinnamic acids there were found caffeic acid, ferulic acid and *o*-coumaric acid in the Tsolikauri grape bunch (Table 1). In the stem, the caffeic acid reaches 0.026 mg/g, *o*-cumaric acid is in trace quantities and ferulic acid is not found. Caffeic acid is 5.2-times less in the skin compared to the stem (0.005 and 0.026 mg/g, respectively), but it was not found in the seed. Ferulic acid was not found in skin,

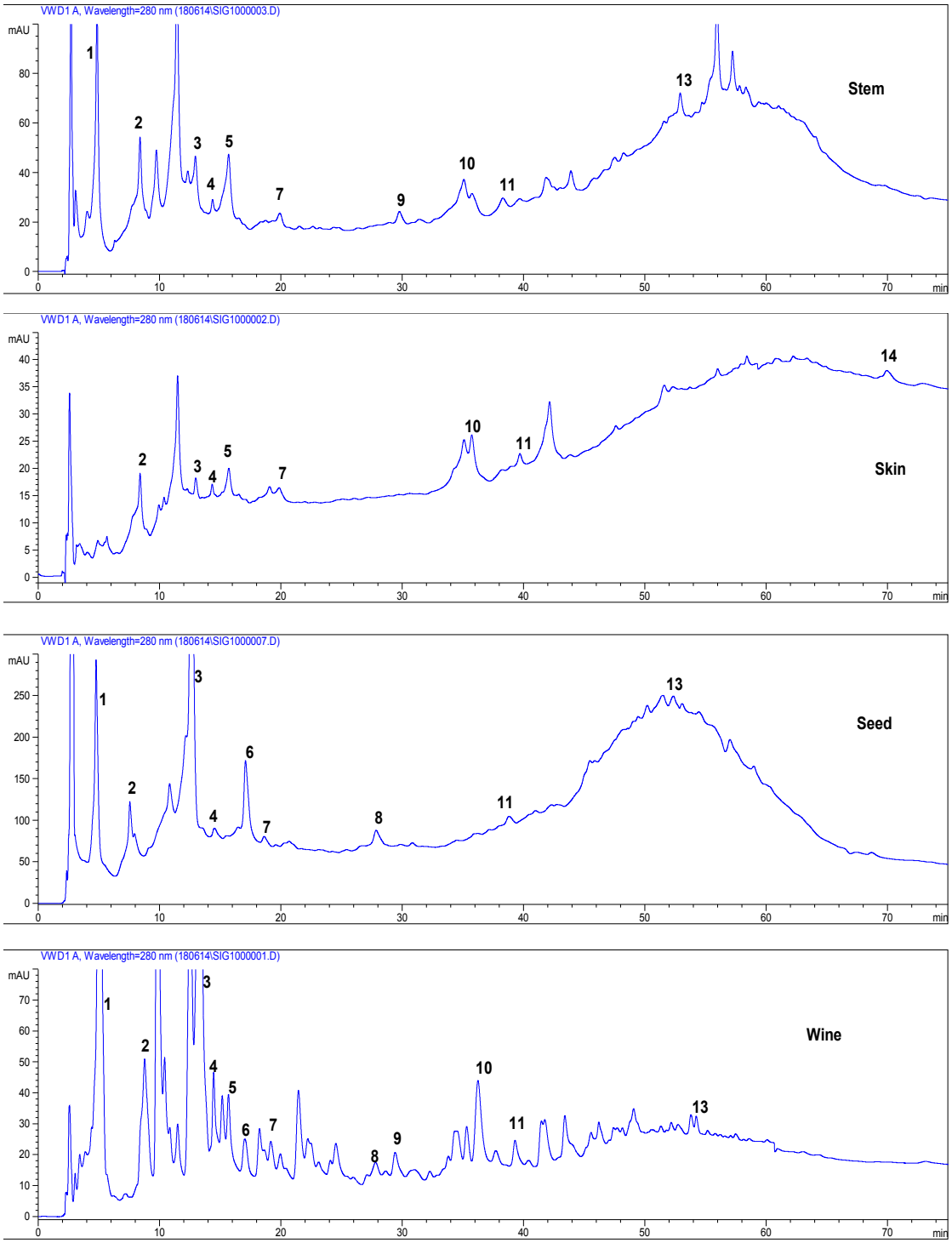

Fig: 1. Chromatograms of phenolic compounds of Tsolikauri grapewine bunch and wine (see Table 1 for peak identification)

whereas seed contains 0.02 mg/g of it. *o*-Cumaric acid in the stem, skin and seed is in trace quantities.

In the Tsolikauri grape bunch, from catechins (+)-catechin and (-)-epicatechin were revealed (Table 1). In the stem, the quantities of these compounds are 0.021 and 0.007 mg/g, respectively. In the grape skin (+)-catechin is in trace quantities, and the amount of (-)-cepicatechin makes 0.014 mg/g. The amount of these substances is much more in the seeds - 3.131 and 0.211 mg/g, respectively. At the same time the amount of (+)-catechin 14.8-times exceeds that of (-)-epicatechin in the seeds. Flavanonol dihydroquercetin (taxifolin) was in trace quantities in the stem, but it was not found in the skin and seed. Likewise, stilben resveratrol was not found in the Tsolikauri grape bunch components (stem, skin, seed).

From flavonols rutin and quercetin were found in the Tsolikauri grape bunch (Table 1). The content of these compounds in the stem makes 0.112 and 0.064 mg/g, respectively. The amount of rutin in the skin reaches 0.149 mg/g, and quercetin is not found. On the contrary, rutin was not found in the seed, and quercetin quantity made 0.102 mg/g.

It is interesting to compare phenolic compound content in Tsolikauri grape bunch with that of the white grape species cultivated in different geographic environment. According to the existing literature data, there are some similarities between phenolic compounds contents in grape stems of two white cultivars of Greece Asyrtiko and Aidani [6] and Tsolikauri grape stem. The grape stems of all three species contain gallic acid, (+)-catechin and quercetin. Besides, stems of Asyrtiko and Tsolikauri grapes contain (-)-epicatechin, ferulic and caffeic acids. The difference is that in the Tsolikauri stem syringic acid and resveratrol was not found, and in the Aidani stem (-)-epicatechin, caffeic and syringic acids are below the detection limit. (+)-Catechin and (-)-epicatechin were found in the grape skin and seed of white cultivar Weisser Riesling cultivated in Germany [7] and Chardonnay cultivated in Georgia, USA [8] and, also, in the seeds of Chardonnay and Riesling grape cultivated in Canada [9] and of three white species cultivated in Serbia [10]. Gallic acid was not found in the the Tsolikauri grape skin whereas its content in the Chardonnay grape skin is 5 mg/100 g dry material [8]. Grape skins of white grape variety Merzling cultivated in Germany [7] and Tsolikauri contain protocatechuic acid, *p*-hydroxybenzoic and caffeic acids; syringic and ferulic acids were not found. Flavonol rutin is found in the grape skins of Tsolikauri and Moscato white grape [11]. As a result of comparison of the contents of hydroxybenzoic and hydroxycinnamic acids in the grape seeds of Tsolikauri and three white varieties cultivated in Serbia [10] it was revealed that gallic acid is characteristic of all four varieties and only the Traminer variety grape seed contains caffeic acid. Apart from Tsolikauri, the seeds of Italian Riesling and Traminer also contain syringic acid in trace quantities, and *p*-hydroxybenzoic acid is found only in the Smederevka seeds. Thus, according to the data, obtained certain difference is observed in phenolic compound content among vine cultivars of different geographic environments.

Tsolikauri wine (Table 1) hydroxybenzoic acids content is the following: gallic acid 23.287 mg/l, protocatechuic acid 10.33 mg/l and syringic acid 1.591 mg/l, vannilic acid is in trace quantities, and *p*-hydroxybenzoic acid is not found. From hydroxicinnamic acid the caffeic acid was found in Tsolikauri wine (0.616 mg/l) while ferulic and *o*-coumaric acids were in trace quantities. From catechins the Tsolikauri wine contains (+)-catechin (23.987 mg/l) and (-)-epicatechin (1.13 mg/l). Unlike the Tsolikauri grape bunch 0.556 mg/l dihydroquercetin (taxifolin) was revealed in the wine, it was also found in Riesling wine [12]. From Chardonnay white grape variety Trousdale and Singleton [13] isolated chromatographically pure flavanonol astilbin, which presents dihydroquercetin 3-ramnozid. Astilbin is also found in trace quantities in Italian Riesling and Traminer grape seeds [10]. Ap-

parently, in the winemaking process astilbin hydrolysis takes place that is why the wine contains dihydroquercetin. From flavonols Tsolikauri wine contains rutin (9.103 mg/l) and quercetin (1.629 mg/l) while stilben resveratrol is not found in it. According to the data obtained, Tsolikauri grape wine is rich in such biologically active compounds as gallic acid, protocatechuic acid, caffeic acid and (+)-catechin, which are characterized by high antiradical effectiveness (AE) [14, 15].

Comparison of qualitative composition and quantitative content of phenolic compounds of Tsolikauri grape wine and that of Rkatsiteli and other white grape wines is of special interest. Unlike Rkatsiteli wine, caffeic acid, syringic acid, rutin and quercetin were found inTsolikauri wine [16]. Different catechin content was observed in these two wines. (+)-Catechin and (-)-epicatechin content in Rkatsiteli wine exceeds their content in Tsolikauri wine, 1.33- and 51.8-times, respectively. As is known, European white wine made without solid parts of bunch and without maceration contains phenolic compounds in a small amount [17]. In particular, in ten French white wines [18] the amount of (+)-catechin, (-)-epicatechin, gallic acid and caffeic acid varies in the following ranges: 4.1-9.1 mg/l, 1.5-7 mg/l, 1.1-12 mg/l and 1-7.6 mg/l, respectively. In Portuguese white wines [19], the amount of gallic acid, (+)-catechin and rutin changes in ranges of 1.0-17 mkg/ml, 1.6-3.8 mkg/ml and 3.1-3.4 mkg/ml, respectively. In eight white wines produced in Czech Republic [20] the amount of gallic acid, protocatechuic acid, *p*-hydroxybenzoic acids, (+)-catechin, caffeic acid, ferulic acid, quercetin, resveratrol, syringic acid and rutin amount changes in the range of 0.02-0.29 mg/l, 0.03-0.42 mg/l, 0.02-0.06 mg/l, 1.01-9.59 mg/l, 0.08-0.27 mg/l, 0.16-0.49 mg/l, 0.17-0.41mg/l, 0.15-0.25 mg/l, 0.04-0.67 mg/l and 0.04-0.39 mg/l, respectively.

Conclusion

Hydroxybenzoic acids (gallic, protocatechuic, vannilic, syringic acids) and hydroxycinnamic acids (caffeic, ferulic and *o*-coumaric acids), catechins ((+)-catechin and (-)-epicatechin), flavanonol (dihydroquercetin) and flavonols (rutin and quercetin) are identified and quantified in Georgian autochthonal vine variety Tsolikauri grape bunch (stem, grape skin, seed) and wine using HPLC.

ორგანული ქიმია

საქართველოს ავტოქთონური ვაზის ჯიშის ცოლიკაურის ყურძნის მტევნის და ღვინის ფენოლური ნაერთები

ა. შალაშვილი[*], ე. ცუცქირიძე[**], ნ. ბერიძე[**], ი. თარგამაძე[*], ბ. ჭანკვეტაძე[§]

** საქართველოს აგრარული უნივერსიტეტი, თბილისი*
***ი. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი, თბილისი*
§ აკადემიის წევრი, საქართველოს მეცნიერებათა ეროვნული აკადემია; ი. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი, თბილისი

შესწავლილია საქართველოს ავტოქთონური ვაზის ჯიშის ცოლიკაურის ყურძნის მტევნის (კლერტი, მარცვლის კანი, წიპწა) და ღვინის ფენოლური ნაერთების თვისებრივი შედგენილობა და რაოდენობრივი შემცველობა მაღალეფექტური სითხური ქრომატოგრაფიის მეთოდით. ცოლიკაურის კლერტი შეიცავს შემდეგ ფენოლურ ნაერთებს: გალმჟავას (0,096 მგ/გ), პროტოკატექმჟავას (0,071 მგ/გ), (+)-კატექინს (0,021 მგ/გ), ყავამჟავას (0,026 მგ/გ), (-)-ეპიკატექინს (0,007 მგ/გ), რუტინს (0,112 მგ/გ) და კვერცეტინს (0,064 მგ/გ); კვალის სახით – ვანილინმჟავას, დიჰიდროკვერცეტინს, ო-კუმარმჟავას, მაგრამ არ შეიცავს იასამანმჟავას, ფერულმჟავას, რესვერატროლს, პ-ჰიდროქსიბენზომჟავას. ყურძნის კანი შეიცავს შემდეგ ფენოლურ ნაერთებს: პროტოკატექმჟავას (0,037 მგ/გ), ყავამჟავას (0,005 მგ/გ), (-)-ეპიკატექინს (0,014 მგ/გ), რუტინს (0,149 მგ/გ) და პ-ჰიდროქსიბენზომჟავას (0,063 მგ/გ); კვალის სახით -- (+)-კატექინს, ვანილინმჟავას, ო-კუმარმჟავას, მაგრამ არ შეიცავს გალმჟავას, იასანმანმჟავას, ფერულმჟავას, დიჰიდროკვერცეტინს, რესვერატროლს, კვერცეტინს. წიპწა შეიცავს შემდეგ ფენოლურ ნაერთებს: გალმჟავას (0,535 მგ/გ), პროტოკატექმჟავას (0,212 მგ/გ), (+)-კატექინს (3,131 მგ/გ), იასამანმჟავას (0,313 მგ/გ), (-)-ეპიკატექინს (0,211 მგ/გ), ფერულმჟავას (0,02 მგ/გ) და კვეცეტინს (0,102 მგ/გ); კვალის სახით – ვანილინმჟავას, ო-კუმარმჟავას, მაგრამ არ შეიცავს ყავამჟავას, დიჰიდროკვერცეტინს, რუტინს, რესვერატროლს, პ-ჰიდროქსიბენზომჟავას. ცოლიკაურის ღვინო შეიცავს შემდეგ ფენოლურ ნაერთებს: გალმჟავას (23,287 მგ/ლ), პროტოკატექმჟავას (10,33 მგ/ლ), (+)-კატექინს (23,987 მგ/ლ), ყავამჟავას (0,616 მგ/ლ), იასამანმჟავას (1,591 მგ/ლ), (-)-ეპიკატექინს (1,13 მგ/ლ), დიჰიდროკვერცეტინს (0,556 მგ/ლ), რუტინს (9,103 მგ/ლ) და კვერცეტინს (1,629 მგ/ლ); კვალის სახით -- ვანილინმჟავას, ფერულმჟავას, ო-კუმარმჟავას, მაგრამ არ შეიცავს რესვერატროლს, პ-ჰიდროქსიბენზომჟავას.

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