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4 **BOTANICALS IN *RIBES NIGRUM* L. BUD-PREPARATIONS: AN ANALYTICAL FINGERPRINTING TO**

5 **EVALUATE THE BIOACTIVE CONTRIBUTION TO TOTAL PHYTOCOMPLEX**

6

7 **Running head:** BOTANICAL CHARACTERIZATION IN *RIBES NIGRUM* BUD-PREPARATIONS

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15

16 **ABSTRACT**

17 Context: *Ribes nigrum* L. (Family: *Grossulariaceae*) is among the most commonly used herbal
18 medicines and it is popularized for its alleged tonic effect and curative and restorative
19 properties. The current practice of identifying herbal extracts is by measuring the concentration

1 of the main botanicals; their concentrations are used to characterize the herbal preparations
2 and fingerprinting is recommended by the main Pharmacopeias as a potential and reliable
3 strategy for the quality control of complex mixtures.

4 Objective: The aim of this research was to perform an analytical study of *Ribes nigrum* bud-
5 preparations, in order to identify and quantify the main bioactive compounds, obtaining a
6 specific chemical fingerprint to evaluate the single class contribution to herbal preparation
7 phytocomplex.

8 Materials and methods: The same analyses were performed using a High Performance Liquid
9 Chromatograph-Diode Array Detector both on University lab preparations and on commercial
10 preparations from different Italian locations: different chromatographic methods were used to
11 analyse the macerated samples, two for polyphenols and one for terpenic compounds.

12 Results: *Ribes nigrum* was identified as a rich source of anti-inflammatory and
13 antioxidant compounds: the observed analytical fingerprint demonstrated that these bud-
14 preparations represent a rich source of terpenic and polyphenolic compounds, especially
15 catechins and phenolic acids.

16 Discussion and conclusion: Analytical fingerprinting could be an important tool to study
17 the assessment of chemical composition and bioactivities of the plant-derived products, helping
18 in find out new sources of natural health-promoting compounds: this study allowed to develop
19 an effective tool for the quality control through the botanical fingerprinting of bud preparations.

20

21 Keywords: blackcurrant, flavonoids, phenolic acids, monoterpenes, bioactive profile, HPLC

22 **INTRODUCTION**

1 *Ribes nigrum* L. (Family: *Grossulariaceae*) is commonly used as herbal medicine and it is
2 popularized for its alleged tonic effect and possible curative and restorative properties (Tabart
3 et al., 2011; Tabart et al., 2012); *Ribes nigrum* is a shrub spontaneously growing in the cold and
4 temperate climate zones and today many orchards with different genetic materials are realized
5 in order to produce fruit, leaves and buds. The most important industrial product of *Ribes*
6 *nigrum* is fruit; however, due to their particular chemical composition and excellent flavor,
7 leaves and buds are also used in some applications as a raw material for the herbal and cosmetic
8 industries: many people use the buds as medicinal preparation for their anti-inflammatory
9 activity and anti-dermal diseases (eczema and psoriasis) (Dvaranauskaite et al., 2008).

10 For this reason, bud-preparations, derived from embryonic fresh plant tissues, are
11 important therapeutic remedies, prescribed in hepatic, respiratory, circulatory and
12 inflammatory disorders, but data on their chemical composition are lacking as, until now,
13 phytochemical studies have principally been performed on barks, roots and root exudates,
14 leaves, fruit and seeds (Peev et al., 2007; Donno et al., 2012a).

15 Polyphenols and terpenes are the dominant majority of biologically active plant
16 compounds with antioxidative and anti-inflammatory properties: these secondary plant
17 metabolites may be nutritionally important and play critical roles in human health in the
18 prevention of chronic diseases such as pulmonary inflammation, cancer, cardiovascular and
19 neurodegenerative diseases (Zhang et al., 2009; Komes et al., 2011; Mattila et al., 2011; Donno
20 et al., 2012b; Tabart et al., 2012).

21 By nature herbal preparations are complex matrices, comprising a multitude of
22 compounds, which are prone to variation due to environmental factors and manufacturing
23 conditions (Komes et al., 2011; Steinmann & Ganzera, 2011; Donno et al., 2012a; Edwards et al.,

1 2012). The analysis of plant and herbal preparation secondary metabolites is a challenging task
2 because of their chemical diversity: low variability is usually observed even within the same
3 species and an herbal preparation detailed chemical profile is certainly necessary also to ensure
4 the reliability and repeatability of clinical and pharmacological studies (Mok & Chau, 2006).

5 It is estimated that 100,000–200,000 metabolites occur in the plant kingdom (Oksman-
6 Caldentey & Inze, 2004), and only highly selective and sensitive methods will be suitable for
7 controlling their composition and quality because many traditional herbal preparations contain
8 several medicinal plants, (Steinmann & Ganzera, 2011): the most important chromatographic or
9 electrophoretic techniques coupled to different detectors are employed for this purpose. High
10 Performance Liquid Chromatography (HPLC) is still the preferred separation technique for the
11 analysis of natural products (Gray et al., 2010).

12 The current practice for herbal extract identification is by measuring the concentration
13 of the main bioactive compounds, called “markers”: the concentrations of the main chemical
14 components are used to characterize the herbal preparation (Mok & Chau, 2006) and referred
15 to as the “fingerprint”: indeed, some studies showed that synergistic or additive biological
16 effects of different phytochemicals (phytocomplex) contribute to disease prevention better than
17 a single compound or a group of compounds (Jia et al., 2012).

18 Chromatographic fingerprinting is recommended by the main national and international
19 Pharmacopoeias as a potential and reliable strategy for the quality control of complex mixtures
20 like herbal medicines: however, it should be noted that many traditional preparations are
21 composed of multiple herbs, so that the analysis of selected constituents might not reflect their
22 overall quality or efficacy (Zhou et al., 2008; Zhao et al., 2009; Qiao et al., 2010; Steinmann &
23 Ganzera, 2011). Different kind of features can be selected to characterize the herbal

1 preparations, and referred to as the overall fingerprint: genetic, quality, sensory or
2 morphological features could be used to create a fingerprint as showed in other studies
3 (Canterino et al., 2012; Mellano et al., 2012): in this study, polyphenolic and terpenic
4 composition was referred to as a chemical fingerprint.

5 The aim of this research was to perform an analytical study of *Ribes nigrum* bud-
6 preparations, in order to identify and quantify the main bioactive polyphenolic and terpenic
7 compounds, obtaining a specific profile of the main polyphenols and terpenes and the total
8 bioactive compound content; the same analyses were performed using an HPLC-DAD both on
9 University lab preparations and on commercial preparations in order to obtain a chemical
10 fingerprint for the assessment of the single bioactive class contribution to total bud-preparation
11 phytocomplex.

12

13 **MATERIAL AND METHODS**

14 *Plant material*

15 University lab preparations and commercial preparations were evaluated. In February
16 2012, samples of *Ribes nigrum* L. buds were picked up in a germplasm repository in San Secondo
17 di Pinerolo, Turin Province (Italy): two different varieties (Rozenhal and Daniels) were sampled,
18 in order to test the genotype effect on the chemical composition of the final product. Buds were
19 used fresh to prepare herbal preparations.

20 Commercial products from five different Italian herbal companies were also considered:
21 the companies are located in San Gregorio di Catania (Catania Province), Predappio (Forlì-
22 Cesena Province), Colleparado (Frosinone Province), Cambiasca (Verbania Province) and Binasco

1 (Milano Province). University lab and commercial preparations were labelled with a code (Table
2 1).

3

4 Macerated sample preparation protocol

5 The protocol of bud-preparations is detailed in the monograph "Homeopathic
6 preparations", quoted in the French Pharmacopoeia, 8th edition, 1965 (Pharmaciens, 1965).
7 Bioactive compounds were extracted through a cold maceration process for 21 days, in a
8 solution of ethanol (95%) and glycerol, followed by a first filtration (Whatman Filter Paper,
9 Hardened Ashless Circles, 185 mm Ø), a manual pressing and, after two days of decanting, a
10 second filtration (Whatman Filter Paper, Hardened Ashless Circles, 185 mm Ø). Macerated
11 samples were then stored at N.A., at 4°C and 95% R.H.

12

13 Solvents and chemicals

14 The maceration solvents, ethanol and glycerol, were purchased from Fluka Biochemika
15 (Switzerland) and Sigma Aldrich (USA) respectively. Analytic HPLC grade solvents, methanol and
16 formic acid, were purchased from Sigma Aldrich (USA) and Fluka Biochemika (Switzerland)
17 respectively; potassium dihydrogen phosphate was also purchased from Sigma Aldrich (USA).
18 Milli – Q ultrapure water was produced by using Sartorius Stedium Biotech mod. Arium.

19 All calibration standards were purchased from Sigma Aldrich (USA): caffeic acid,
20 chlorogenic acid, coumaric acid, ferulic acid, hyperoside, isoquercitrin, quercetin, quercitrin,

1 rutin, gallic acid, ellagic acid, catechin, epicatechin, limonene, phellandrene, sabinene, γ -
2 terpinene and terpinolene.

3

4 Standard preparation

5 Chemical structures of all the compounds are showed in Fig. 1.

6 Stock solutions of cinnamic acids and flavonols with a concentration of 1.0 mg/mL were
7 prepared in methanol: from these solutions, four calibration standards were prepared by
8 dilution with methanol; stock solutions of benzoic acids and catechins with a concentration of
9 1.0 mg/mL were prepared in 95% methanol and 5% water: from these solutions, four calibration
10 standards were prepared by dilution with 50% methanol–water.

11 Stock solutions of monoterpenes with a concentration of 1.0 mg/mL were prepared in
12 methanol: from these solutions, four calibration standards were prepared by dilution with
13 methanol.

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18 HPLC sample preparation and storage

1 Macerated University lab and commercial preparations were filtered with circular pre-
2 injection filters (0.45 μm , polytetrafluoroethylene membrane, PTFE) and then stored for a few
3 days at N.A., 4°C and 95% R.H..

4

5 Apparatus and chromatographic conditions

6 An Agilent 1200 High Performance Liquid Chromatograph, equipped with a G1311A
7 quaternary pump, a manual injection valve and a 20 μl sample loop, coupled to an Agilent
8 G1315D UV-Vis diode array detector, was used for the analysis.

9 Three different chromatographic methods were used to analyse the macerated samples,
10 two for polyphenols and one for terpenic compounds. The first method (A) was used for the
11 analysis of cinnamic acids and flavonols; bioactive compound separation was achieved on a
12 ZORBAX Eclipse XDB – C18 column (4.6 x 150 mm, 5 μm), while the mobile phase consisted of
13 methanol and a solution of 40 mM potassium dihydrogen phosphate in water. The flow rate was
14 1.0 mL min^{-1} (gradient analysis, 60 minutes) and the detector wavelength was 330 nm (Peev et
15 al., 2007; Donno et al., 2012a). The second method (B) was used for the analysis of benzoic acids
16 and catechins; bioactive molecules were separated on a ZORBAX Eclipse XDB – C18 column (4.6
17 x 150 mm, 5 μm), while the mobile phase consisted of a solution of methanol/water/formic acid
18 (5:95:0,1 v/v/v) and a mix of methanol/formic acid (100:0,1 v/v). The flow rate was 1.0 mL min^{-1}
19 (gradient analysis, 35 minutes) and the detector wavelengths were 250, 280 and 320 nm (Moller
20 et al., 2009; Donno et al., 2012a).

21 The third method (C) was used for the analysis of monoterpenes; chromatographic
22 separation was performed using a ZORBAX Eclipse XDB – C18 column (4.6 x 150 mm, 5 μm). The

1 liquid flow rate was 1.0 mL min⁻¹ using water and methanol as mobile phase with a linear
2 gradient of 75 minutes; UV spectra were recorded at 220 and 235 nm (Zhang et al., 2009).

3

4 Identification and quantification of bioactive compounds

5 All single compounds were identified in samples by comparison of their retention times
6 and UV spectra with those of standards in the same chromatographic conditions. Quantitative
7 determinations were performed using an external standard method. Calibration curves in the
8 125 – 1000 mg/L range with good linearity for a four point plot were used to determine the
9 concentration of polyphenolic and terpenic compounds in bud-preparation samples: the
10 linearity for each compound was established by plotting the peak area (y) versus the
11 concentration (x) of each analyte. The limit of detection (LOD) and the limit of quantification
12 (LOQ) of the three chromatographic methods were defined as the lowest amount of analyte that
13 gives a reproducible peak with a signal-to-noise ratio (S/N) of 3 and 10, respectively. Calibration
14 curve equations, linearity (R²), LOD and LOQ for all of the compounds are summarized in (Table
15 2).

16 All samples were analysed in triplicate (three repetitions for three plants for each
17 University lab sample and three repetitions for three products for each commercial sample), and
18 all data are given in order to assess the repeatability of the used methods (standard deviation).
19 Accuracy was checked by spiking samples with a solution containing each bioactive compound in
20 a concentration of 10 mg/mL.

21 Examples of *Ribes nigrum* bud-preparation chromatographic profiles are reported in Fig.
22 2 and Fig. 3. Total bioactive compound content (TBCC) were determined as the sum of the most

1 important classes of polyphenols and terpenic compounds present in the samples. Four
2 polyphenolic classes were considered: benzoic acids (gallic acid and ellagic acid), catechins
3 (catechin and epicatechin), cinnamic acids (caffeic acid, chlorogenic acid, coumaric acid and
4 ferulic acid) and flavonols (hyperoside, isoquercitrin, quercetin, quercitrin and rutin); one
5 terpenic class was considered: monoterpenes (limonene, phellandrene, sabinene, γ -terpinene,
6 terpinolene). All results were expressed as mg per 100 g of buds fresh weight (FW).

7

8 Statistical Analysis

9 Results were subjected to ANOVA and t Student Test for mean comparison (SPSS 18.0
10 Software) and HSD Tukey multiple range test ($P < 0.05$). Principal Component Analysis (PCA) was
11 performed on the single botanical concentration data.

12

13 **RESULTS**

14 Total bioactive compound content (TBCC)

15 The content of total bioactive compounds in the evaluated bud-preparations is reported
16 in Figure 4. Statistically significant differences were observed among the analysed samples, with
17 a lower TBCC value of 3478.95 mg/100 g_{FW} (sample C1) and an higher value of 6507.29 mg/100
18 g_{FW} (sample UL2).

19 Principal Component Analysis was performed on all samples and it reduced the initial
20 variables (single bioactive compound concentration) into four principal components (83.15% of
21 total variance) and the initial seven groups into four groups, confirming the statistically

1 significant differences in TBCC (ANOVA Test): the new groups were called A (UL1), B (UL2), C (C1,
2 C2, C3, C5) and D (C4) (Fig. 5). PCA variable graph (Fig. 6) showed a correlation between the
3 most of polyphenols and PC1 (32.62% of total variance) and a correlation between
4 monoterpenes, except limonene and sabinene, and PC2 (24.77% of total variance).

5

6 Single bioactive compound profile

7 All data are reported in Table 3 (method A), 4 (method B) and 5 (method C).

8 *Ribes nigrum* bud-preparations showed the following botanical composition: four
9 cinnamic acids (caffeic acid, chlorogenic acid, coumaric acid, ferulic acid), one flavonol
10 (quercetin), one benzoic acid (gallic acid), two catechins (catechin, epicatechin) and five
11 monoterpenes (limonene, phellandrene, sabinene, γ -terpinene, terpinolene); hyperoside,
12 isoquercitrin, quercitrin, rutin and gallic acid were not detected. Single bioactive compound
13 concentration ranged from 0.84 mg/100 g_{FW} (chlorogenic acid, C1 sample) to 1309.19 mg/100
14 g_{FW} (γ -terpinene, UL2 sample).

15 Statistically significant differences were observed both in the University lab bud-
16 preparations and in commercial bud-preparations: the most important differences were
17 observed in the concentration of catechin, limonene and terpinolene.

18

19 Fingerprinting

20 Chemical fingerprint of *Ribes nigrum* bud-preparations was reported: in total, 13
21 botanicals were identified by HPLC/DAD. By single bioactive compound profile, botanicals were

1 grouped into polyphenolic and terpenic classes to evaluate the contribution of each class to
2 total phytocomplex composition.

3 Chemical fingerprint showed the prevalence of monoterpenes and catechins in chemical
4 composition of the all analyzed samples (mean values were considered): the most important
5 class was monoterpenes (82.94%), followed by catechins (9.46%), cinnamic acids (3.64%),
6 flavonols (2.67%) and benzoic acids (1.29%) (Tab. 6).

7 Therefore, monoterpenes and catechins were two main groups of bioactive compounds
8 in the evaluated bud-preparations: monoterpene contribution ranged from 77.75% in C4 sample
9 to 87.01% in UL2 sample, while catechins contributed to total phytocomplex in a different
10 range, from 6.67% (UL2) to 13.52% (C2).

11

12 **DISCUSSION**

13 The HPLC analysis of botanicals is nowadays a widespread and well developed
14 characterization tool and some analytical reports were found in literature. These compounds
15 are very interesting because of their wide structural variability (5,000 derivatives are known up
16 to now), which explains their broad spectrum of pharmacological effects and medicinal uses
17 (Ganzera, 2008): in most reports comparable analytical conditions were described, which are
18 based on reverse-phase (RP) stationary phases and acid mobile phases (Matsui et al., 2007; Guo
19 et al., 2008).

20 Reports on the analysis of phenolic acids (e.g. caffeic acid and its derivatives) by HPLC
21 coupled to diode array or mass detectors have been published. They describe phenolic acid
22 determination in medicinal plants and preparations, as *Ribes nigrum* bud-preparations (Urpi-

1 Sarda et al., 2009; Castro et al., 2010), according to single bioactive compound concentrations
2 showed in this research. Among other identified classes, flavonols and catechins were also
3 selected for quantitative studies (Huang et al., 2008; Yi et al., 2009; Surveswaran et al., 2010).
4 Based on the obtained results, the most of researches pointed out that the identified
5 polyphenolic compounds significantly contribute to the total phytocomplex of these herbal
6 preparations: the obtained fingerprints were useful for authentication and quality control
7 purposes (Amaral et al., 2009; Dugo et al., 2009); present study confirmed these results, adding
8 as well as the terpenic compounds also significantly contributed to the *Ribes nigrum* bud-
9 preparation phytocomplex, such as anti-inflammatory constituents in herbal preparations
10 (Zhang et al., 2009): few studies emphasized on the identification of single terpenoids in plant
11 material by HPLC analysis (Steinmann & Ganzera, 2011).

12 It is well-known that chemical composition of secondary plant metabolites highly
13 depends on some factors such as climate conditions, harvesting time and plant genotype
14 (Dvaranauskaite et al., 2008; Donno et al., 2012a), and the results of this research confirmed this
15 hypothesis: ANOVA and PCA results showed that the *Ribes nigrum* bud-preparation composition
16 (different locations) was similar in all the samples but the single compound concentrations were
17 different; moreover, observing the chemical composition, results showed that few compounds
18 were not detected in herbal medicines: chromatographic fingerprinting could be applied in the
19 differentiation of *Ribes nigrum* bud-preparations by other species (Zhao et al., 2009; Donno et
20 al., 2012a).

21 In this study, effective HPLC–DAD methods were developed for fingerprint analysis and
22 component identification of *Ribes nigrum* bud-preparations from different locations. Comparing
23 with other analytical studies (Tsao & Yang, 2003; Dugo et al., 2009), the chromatographic

1 conditions were optimized to obtain an effective fingerprint containing enough information of
2 constituents with good resolution and reasonable analysis time. For optimizing the elute
3 conditions, linear gradients in different slope were used for the compound separation, because
4 some constituents were similar in the structure with each other. In the macerated samples,
5 most constituents was also weakly acid, so adding formic acid was necessary for enhancing the
6 resolution and eliminating peak tailing (Zhao et al., 2009). The choice of detection wavelength
7 was a crucial step for developing a reliable fingerprint (Zhou et al., 2008; Zhao et al., 2009). A
8 full-scan on the chromatogram from 190 to 400 nm was performed and only selected
9 wavelengths were suitable to achieve more specific peaks as well as a smooth baseline.

10 The methods showed a good resolution for most peaks and could be routinely used to
11 evaluate bud-preparation overall quality. The results indicated that the developed methods
12 were feasible for comprehensive authentication and quality control of *Ribes nigrum* bud-
13 preparations. Knowledge of molecular structure, composition and quantity is necessary to
14 understand botanical role in determining potential health effects, because many traditional
15 preparations contain multiple herbs; moreover, pretending to have a natural origin, these
16 preparations sometimes contain a mixture of synthetic adulterants (e.g. sildenafil, diazepam,
17 captopril and amoxicillin), which explains their (unexpected) power but is also responsible for
18 side effects of “unknown” reason (Liang et al., 2006; Uchiyama et al., 2009; Kesting et al., 2010):
19 so that only highly selective, sensitive and versatile analytical techniques will be suitable for
20 quality control purposes (Hager et al., 2008).

21 This study is only a preliminary research about *Ribes nigrum* bud-preparation chemical
22 composition; by hyphenating High Performance Liquid Chromatography and mass spectrometry,

1 the high quality demand of the consumer is fulfilled, providing the lab technicians with a
2 multitude of technical options and applications (Gray et al., 2010; Steinmann & Ganzera, 2011).

3

4 **CONCLUSIONS**

5 Regarding the bud-preparations evaluated in this study, *Ribes nigrum* was identified as a
6 rich source of anti-inflammatory and antioxidant compounds: the observed analytical fingerprint
7 demonstrated that these bud-preparations represent a rich source of terpenes and polyphenolic
8 compounds, especially catechins; this research suggested that identified botanicals might
9 contribute to the total phytocomplex of these herbal preparations.

10 With gaining popularity of herbal remedies worldwide, the need of assuring safety and
11 efficacy of these products increases as well. Analytical fingerprinting could be an important tool
12 to assess the chemical composition and bioactivities of the plant-derived products, helping in
13 find out new sources of natural health-promoting compounds: only in this way it will be possible
14 to develop a new generation of standardized products which fulfill today's standards for quality,
15 safety and efficiency of herbal preparations.

16

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6 **Tables**

7 Tab. 1. Source and identification code of the analysed samples.

| Sample | City | Province | Region | Identification code |
|------------------|-------------------------|-----------------|----------------|----------------------------|
| University lab 1 | San Secondo di Pinerolo | Torino | Piemonte | UL1 |
| University lab 2 | San Secondo di Pinerolo | Torino | Piemonte | UL2 |
| Company 1 | San Gregorio di Catania | Catania | Sicilia | C1 |
| Company 2 | Predappio | Forli-Cesena | Emilia-Romagna | C2 |
| Company 3 | Colleparado | Frosinone | Lazio | C3 |
| Company 4 | Cambiasca | Verbania | Piemonte | C4 |
| Company 5 | Binasco | Milano | Lombardia | C5 |

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10 Tab. 2. Calibration curve equations, R^2 , LOD and LOQ of the used chromatographic methods for each
11 calibration standard.

| Class | Standard | Method | Calibration curve equations (peak area = y; concentration = x) | R ² | LOD (mg/L) | LOQ (mg/L) |
|----------------|---------------------|--------|---|----------------|---------------|---------------|
| Cinnamic acids | caffeic acid | A | $y = 10.155x + 13.008$ | 0.985 | 1.232 | 4.107 |
| | chlorogenic acid | A | $y = 7.165x + 95.749$ | 0.995 | 0.627 | 2.091 |
| | coumaric acid | A | $y = 10.904x + 187.144$ | 0.999 | 1.037 | 3.456 |
| | ferulic acid | A | $y = 6.181x - 273.562$ | 1.000 | 1.012 | 3.373 |
| Flavonols | hyperoside | A | $y = 14.315x - 262.753$ | 1.000 | 0.549 | 1.829 |
| | isoquercitrin | A | $y = 11.437x + 100.974$ | 0.998 | 0.475 | 1.585 |
| | quercetin | A | $y = 5.505x - 418.512$ | 0.996 | 1.897 | 6.323 |
| | quercitrin | A | $y = 5.162x - 168.272$ | 0.996 | 1.072 | 3.575 |
| | rutin | A | $y = 8.213x + 105.923$ | 0.999 | 0.672 | 2.241 |
| Benzoic acids | gallic acid | B | $y = 10.703x + 59.149$ | 0.998 | 0.283 | 0.944 |
| | ellagic acid | B | $y = 5.766x + 281.063$ | 0.988 | 1.881 | 6.271 |
| Catechins | catechin | B | $y = 6.567x - 178.554$ | 0.999 | 1.755 | 5.850 |
| | epicatechin | B | $y = 6.104x - 172.263$ | 0.997 | 1.749 | 5.829 |
| Monoterpenes | limonene | C | $y = 1.347x + 30.797$ | 0.997 | 2.108 | 7.026 |
| | phellandrene | C | $y = 4.488x - 39.986$ | 1.000 | 1.312 | 4.374 |
| | sabinene | C | $y = 29.237x - 296.283$ | 1.000 | 0.026 | 0.087 |
| | γ -terpinene | C | $y = 2.461x + 205.211$ | 0.993 | 2.758 | 9.194 |
| | terpinolene | C | $y = 0.056x - 1.809$ | 0.995 | 7.479 | 24.930 |

1 Tab. 3. Single polyphenolic profile of University bud-preparations and commercial bud-preparations (method A). Different letters for each sample indicate the
 2 statistically significant differences at $P<0.05$.

| METHOD A | cinnamic acids (mg/100 g _{FW}) | | | | | | | | | | | |
|-------------|---|-----------|-------|------------------|-----------|-------|---------------|-----------|-------|--------------|-----------|--------|
| | caffeic acid | | | chlorogenic acid | | | coumaric acid | | | ferulic acid | | |
| sample name | mean | Tukey HSD | SD | mean | Tukey HSD | SD | mean | Tukey HSD | SD | mean | Tukey HSD | SD |
| UL1 | 5.986 | a | 1.145 | 4.013 | a | 1.754 | 2.143 | a | 1.486 | 126.939 | b | 33.288 |
| UL2 | 8.468 | a | 0.689 | 1.960 | a | 2.336 | 8.243 | b | 1.983 | 205.919 | c | 16.170 |
| C1 | 5.818 | a | 0.435 | 0.844 | a | 0.646 | 2.982 | a | 1.038 | 133.842 | b | 12.331 |
| C2 | 6.151 | a | 0.813 | 1.177 | a | 0.636 | 3.315 | a | 0.822 | 134.176 | b | 10.132 |
| C3 | 22.869 | b | 2.472 | 0.864 | a | 0.075 | 4.152 | a | 0.209 | 42.244 | a | 4.207 |
| C4 | 85.039 | d | 1.565 | 13.127 | b | 2.493 | 39.817 | c | 1.735 | 153.000 | b | 0.899 |
| C5 | 37.987 | c | 2.234 | 24.406 | c | 3.158 | 7.757 | b | 0.805 | 69.421 | a | 0.723 |

| METHOD A | Flavonols (mg/100 g _{FW}) | | | | | | | | | | | | | | |
|-------------|--|-----------|----|---------------|-----------|----|-----------|-----------|--------|------------|-----------|----|-------|-----------|----|
| | hyperoside | | | isoquercitrin | | | quercetin | | | quercitrin | | | rutin | | |
| sample name | mean | Tukey HSD | SD | mean | Tukey HSD | SD | mean | Tukey HSD | SD | mean | Tukey HSD | SD | mean | Tukey HSD | SD |
| UL1 | n.d. | / | / | n.d. | / | / | 106.813 | ab | 15.691 | n.d. | / | / | n.d. | / | / |
| UL2 | n.d. | / | / | n.d. | / | / | 125.270 | b | 13.023 | n.d. | / | / | n.d. | / | / |
| C1 | n.d. | / | / | n.d. | / | / | 93.399 | a | 6.247 | n.d. | / | / | n.d. | / | / |
| C2 | n.d. | / | / | n.d. | / | / | 94.399 | a | 2.290 | n.d. | / | / | n.d. | / | / |
| C3 | n.d. | / | / | n.d. | / | / | 165.806 | c | 4.678 | n.d. | / | / | n.d. | / | / |
| C4 | n.d. | / | / | n.d. | / | / | 105.961 | ab | 2.259 | n.d. | / | / | n.d. | / | / |
| C5 | n.d. | / | / | n.d. | / | / | 117.957 | b | 1.081 | n.d. | / | / | n.d. | / | / |

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 5 Tab. 4. Single polyphenolic profile of University bud-preparations and commercial bud-preparations (method B). Different letters for each sample indicate the
 6 statistically significant differences at $P<0.05$.

| METHOD B | Benzoic acids (mg/100 g FW) | | | | | | Catechins (mg/100 g FW) | | | | | |
|-------------|--------------------------------|-----------|----|-------------|-----------|--------|----------------------------|-----------|--------|-------------|-----------|--------|
| | ellagic acid | | | gallic acid | | | catechin | | | epicatechin | | |
| sample name | mean | Tukey HSD | SD | mean | Tukey HSD | SD | mean | Tukey HSD | SD | mean | Tukey HSD | SD |
| UL1 | n.d. | / | / | 20.424 | a | 3.857 | 249.537 | ab | 75.127 | 104.801 | ab | 32.633 |
| UL2 | n.d. | / | / | 61.105 | b | 8.400 | 292.256 | ab | 63.608 | 141.965 | bc | 20.902 |
| C1 | n.d. | / | / | 55.036 | b | 4.420 | 157.306 | a | 11.401 | 101.777 | ab | 6.074 |
| C2 | n.d. | / | / | 46.440 | b | 21.191 | 329.133 | b | 88.014 | 191.961 | d | 3.608 |
| C3 | n.d. | / | / | 56.262 | b | 1.670 | 238.588 | ab | 2.420 | 132.134 | bc | 1.827 |
| C4 | n.d. | / | / | 106.575 | c | 0.503 | 490.686 | c | 7.120 | 154.602 | cd | 4.388 |
| C5 | n.d. | / | / | 54.121 | b | 3.836 | 274.767 | ab | 1.388 | 67.504 | a | 3.824 |

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2 Tab. 5. Single terpenic profile of University bud-preparations and commercial bud-preparations (method C). Different letters for each sample indicate the
3 statistically significant differences at $P < 0.05$.

| METHOD C | Monoterpenes (mg/100 g FW) | | | | | | | | | | | | | | |
|-------------|-------------------------------|-----------|---------|--------------|-----------|--------|----------|-----------|--------|---------------------|-----------|---------|-------------|-----------|---------|
| | limonene | | | phellandrene | | | sabinene | | | γ -terpinene | | | terpinolene | | |
| sample name | mean | Tukey HSD | SD | mean | Tukey HSD | SD | mean | Tukey HSD | SD | mean | Tukey HSD | SD | mean | Tukey HSD | SD |
| UL1 | 380.473 | ab | 100.139 | 64.732 | a | 13.187 | 30.970 | a | 16.271 | 28.497 | a | 15.760 | 3382.533 | d | 290.008 |
| UL2 | 1298.200 | d | 310.905 | 74.571 | a | 17.809 | 196.103 | b | 39.423 | 1309.192 | b | 370.441 | 2784.041 | c | 171.753 |
| C1 | 649.515 | bc | 31.710 | 504.298 | d | 7.209 | 27.498 | a | 2.690 | 48.770 | a | 1.631 | 1697.864 | a | 70.449 |
| C2 | 224.658 | a | 6.342 | 149.762 | b | 8.001 | 217.870 | b | 5.883 | 60.147 | a | 0.349 | 2395.201 | bc | 57.017 |
| C3 | 859.927 | c | 7.222 | 149.573 | b | 6.283 | 39.484 | a | 5.896 | 143.154 | a | 1.132 | 2151.387 | b | 18.609 |
| C4 | 911.788 | c | 8.498 | 327.717 | c | 6.748 | 182.977 | b | 5.296 | 65.508 | a | 2.910 | 2527.106 | bc | 118.068 |
| C5 | 644.830 | bc | 3.576 | 528.999 | d | 5.669 | 222.978 | b | 4.146 | 164.950 | a | 4.373 | 1611.298 | a | 134.242 |

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7 Tab. 6. Contribution (%) of botanical classes to the phytocomplex in analysed *Ribes nigrum* bud-preparations.

| Sample | cinnamic acids | flavonols | benzoic acids | catechins | monoterpenes |
|-------------------|-----------------------|------------------|----------------------|------------------|---------------------|
| <i>UL1</i> | 3.09% | 2.37% | 0.45% | 7.86% | 86.23% |
| <i>UL2</i> | 3.45% | 1.93% | 0.94% | 6.67% | 87.01% |
| <i>C1</i> | 4.12% | 2.68% | 1.58% | 7.45% | 84.16% |
| <i>C2</i> | 3.76% | 2.45% | 1.20% | 13.52% | 79.07% |
| <i>C3</i> | 1.75% | 4.14% | 1.40% | 9.25% | 83.45% |
| <i>C4</i> | 5.63% | 2.05% | 2.06% | 12.50% | 77.75% |
| <i>C5</i> | 3.65% | 3.08% | 1.41% | 8.94% | 82.91% |
| <i>mean value</i> | 3.64% | 2.67% | 1.29% | 9.46% | 82.94% |

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Figures

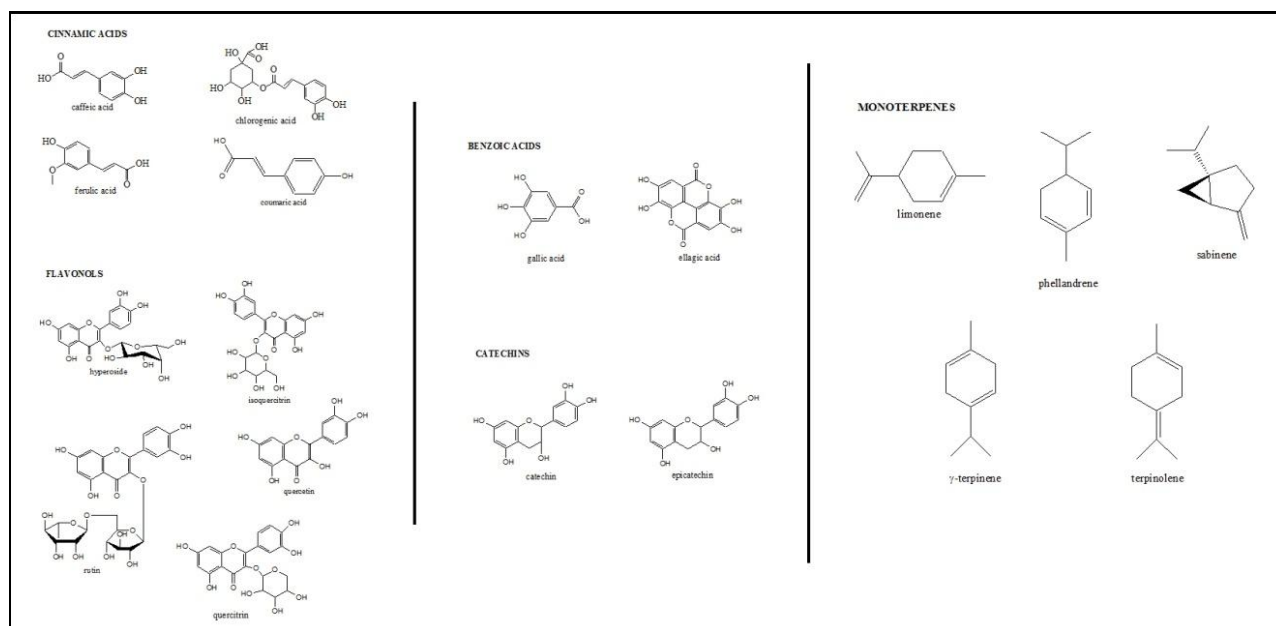


Fig. 1. Chemical structures of the detected bioactive compounds.

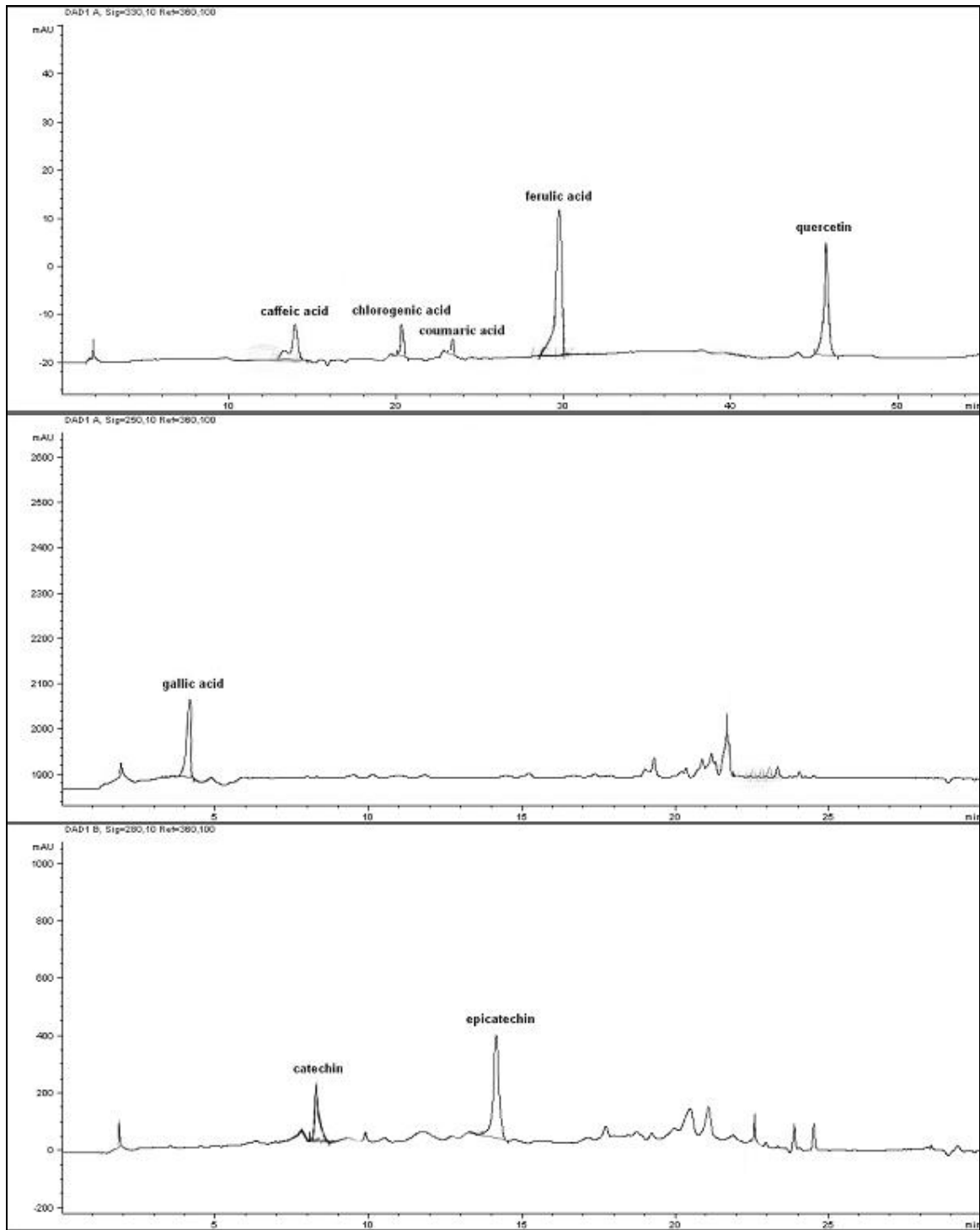


Fig. 2. HPLC/DAD *Ribes nigrum* bud-preparation polyphenolic profile.

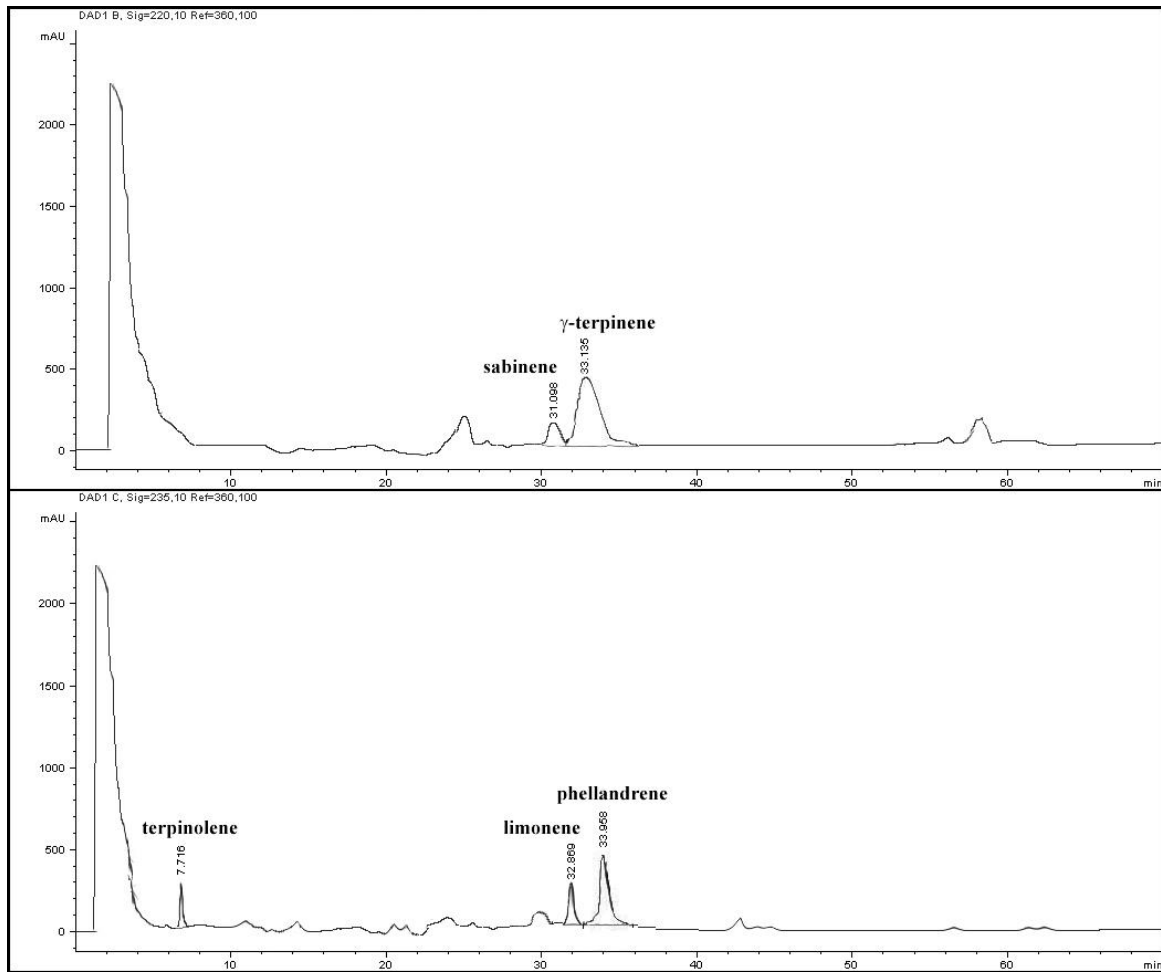


Fig. 3. HPLC/DAD *Ribes nigrum* bud-preparation terpene profile.

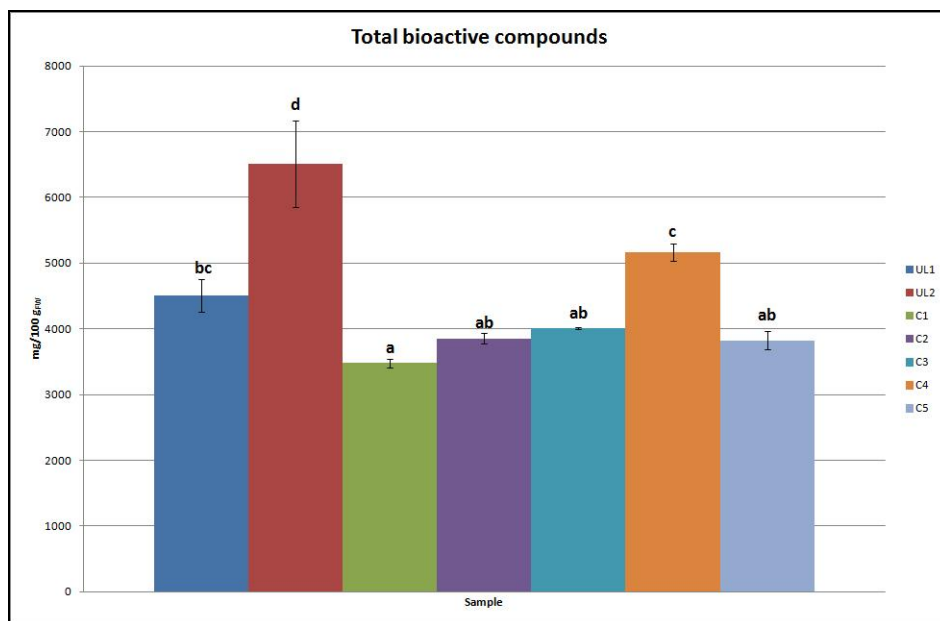


Fig. 4. TBCC in University lab and commercial bud-preparations. Different letters for each sample indicate the significant differences at $P < 0.05$.

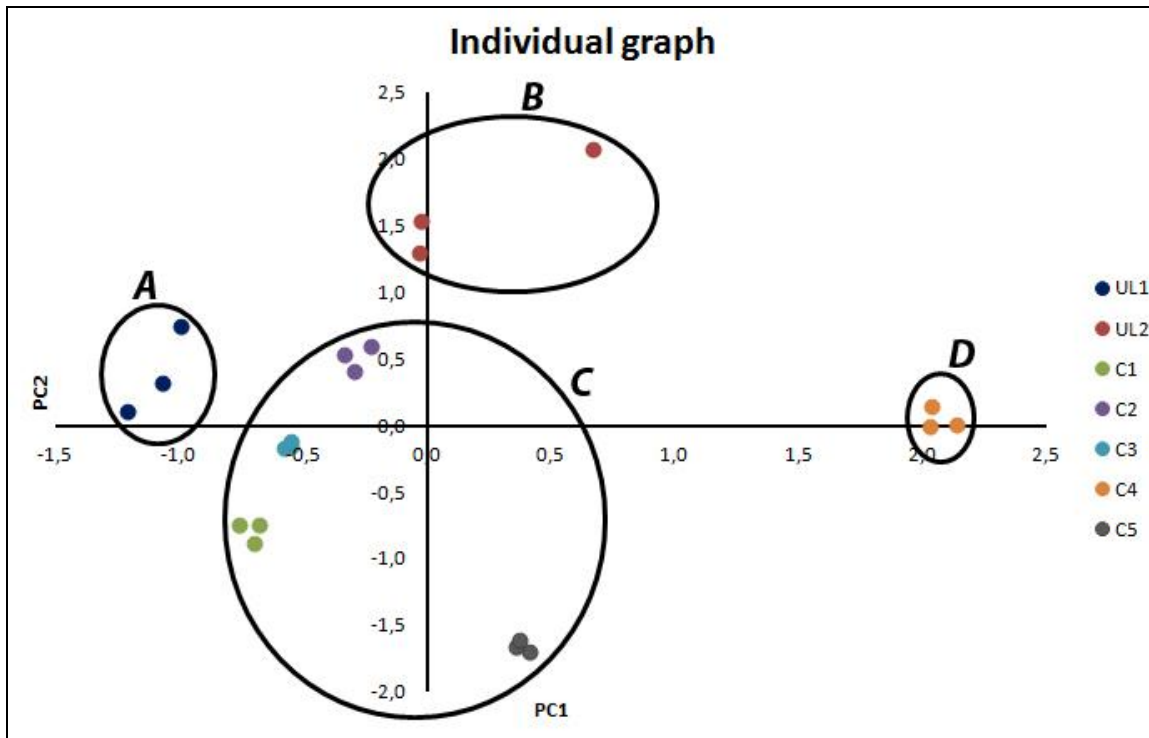


Fig. 5. PCA individual graph of bud-preparation samples.

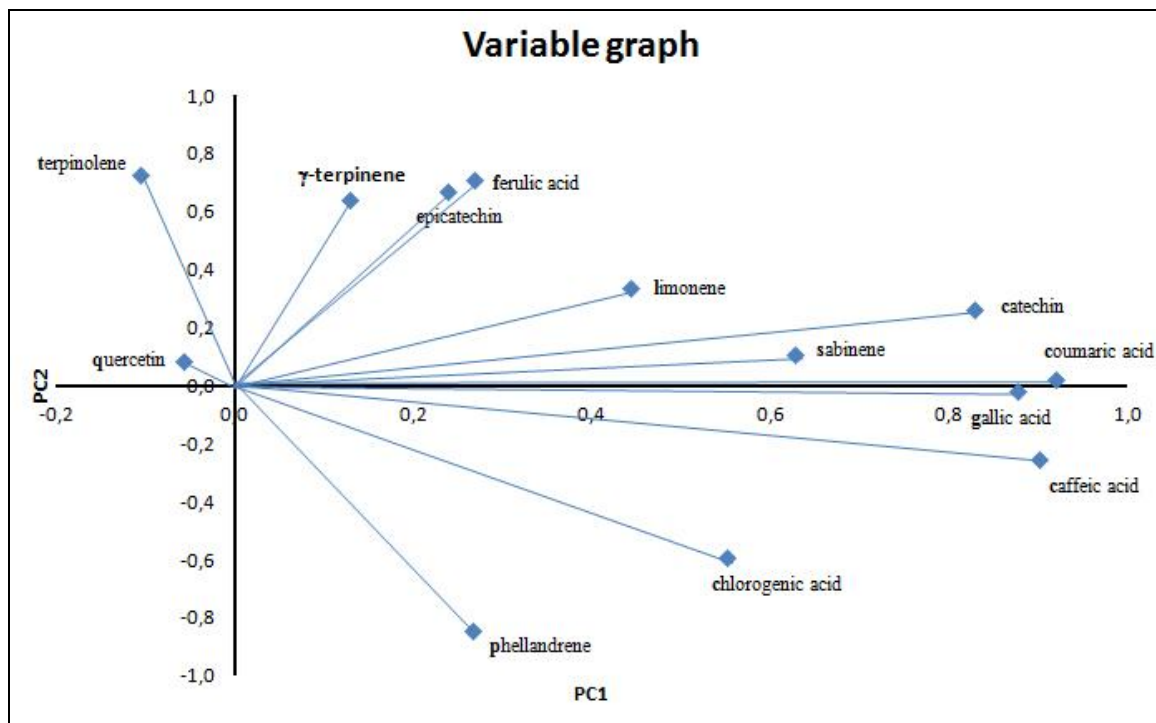


Fig. 6. PCA variable graph of bud-preparation samples.