1st International Electronic Conference on Medicinal Chemistry

2-27 November 2015

chaired by Dr. Jean Jacques Vanden Eynde

sponsored by pharmaceuticals

Biomolecules and Natural Medicine Preparations: Analysis of New Sources of Bioactive Compounds from *Ribes* and *Rubus* spp.

Dario Donno*, Maria Gabriella Mellano, Alessandro K. Cerutti, and Gabriele L. Beccaro

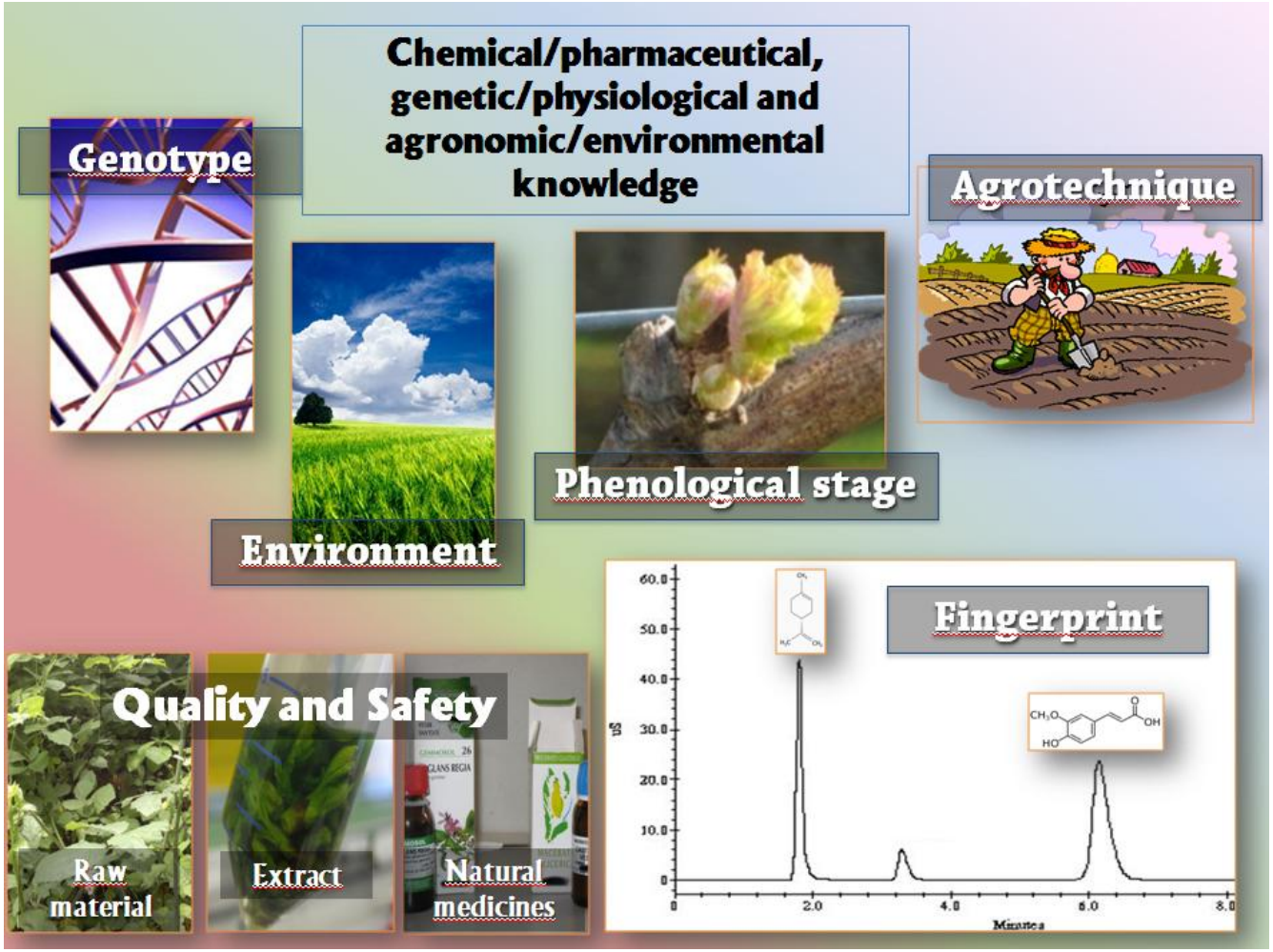
Department of Agriculture, Forestry and Food Science, University of Torino, Largo Braccini 2, 10095 – Grugliasco (TO);

* Corresponding author: dario.donno@unito.it

DISAFA

UNIVERSITÀ DEGLI STUDI DI TORINO

Biomolecules and Natural Medicine Preparations: Analysis of New Sources of Bioactive Compounds from *Ribes* and *Rubus* spp.



Abstract:
It is well known that plants are important sources for the preparation of natural remedies as they contain many biologically active compounds: in particular, polyphenols, terpenic compounds, organic acids, and vitamins are the most widely occurring groups of phytochemicals. Some endemic species may be used for the production of herbal preparations containing phytochemicals with significant bioactivity, as antioxidant activity and anti-inflammatory capacities, and health benefits: blackberry sprouts and blackcurrant buds are known to contain appreciable levels of bioactive compounds, including flavonols, phenolic acids, monoterpenes, vitamin C, and catechins, with several clinical effects.
The aim of this research was to perform an analytical study of blackcurrant and blackberry bud-preparations, in order to identify and quantify the main biomarkers, obtaining a specific phytochemical fingerprint to evaluate the single botanical class contribution to total phytocomplex and relative bioactivity, using a High Performance Liquid Chromatograph − Diode Array Detector; the same analyses were performed both on the University laboratory and commercial preparations.
Different chromatographic methods were used to determine concentrations of biomolecules in the preparations, allowing for quantification of statistically significant differences in their bioactive compound content both in the case of *Ribes nigrum* and *Rubus ulmifolius*.
Chemical, pharmaceutical and environmental knowledge could be a useful tool for obtaining label certifications for the valorization of specific genotypes, with high clinical and pharmaceutical value: this study allowed to develop an effective tool for the natural preparation quality control and bioactivity evaluation through the chemical fingerprinting of bud preparations.

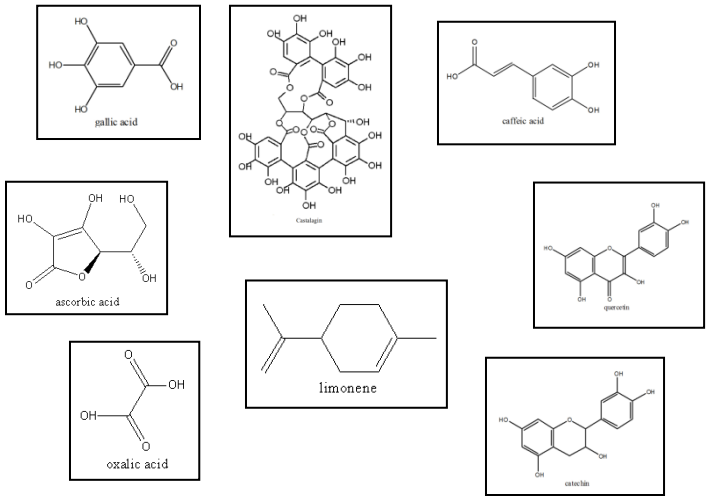
Keywords: biomarkers, *Ribes nigrum*, *Rubus ulmifolius*, bioactivity, phytochemical fingerprint

Introduction

PHYTOTHERAPY

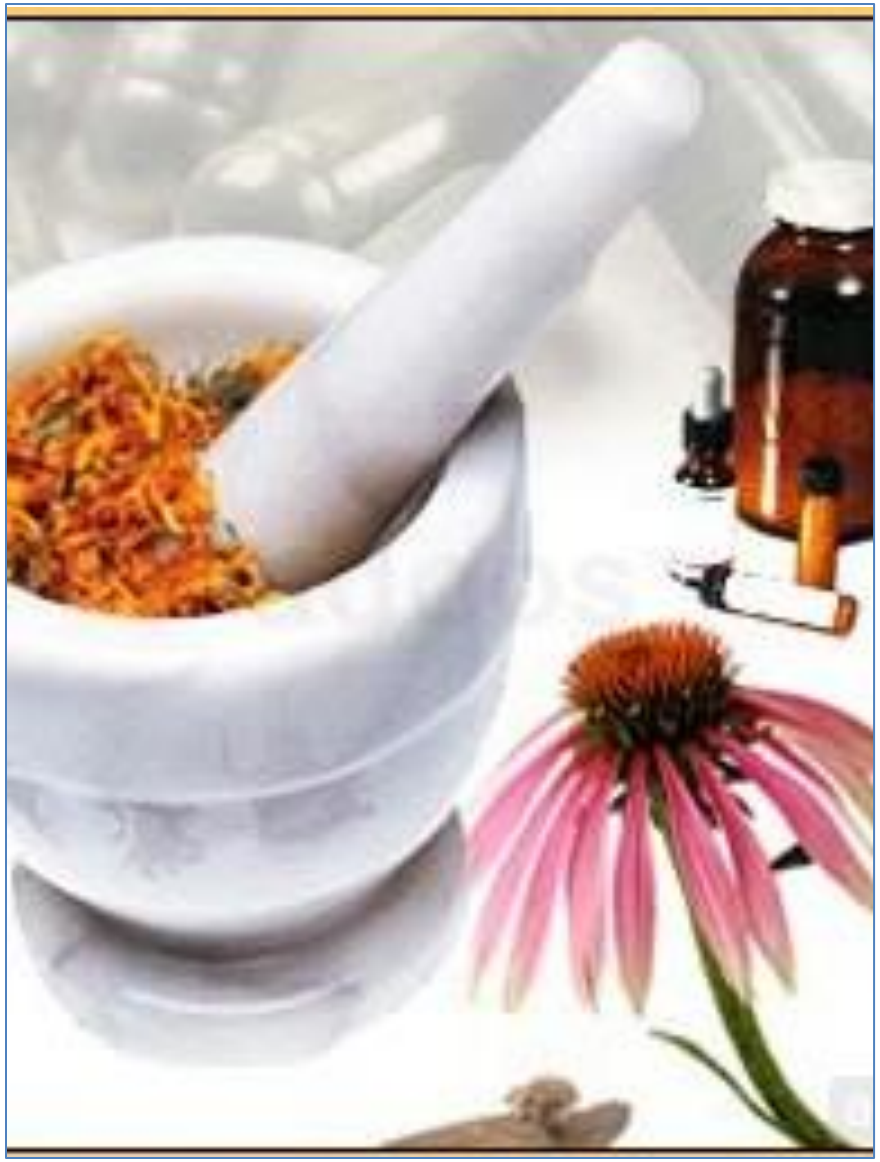
Study of natural extracts used as health-promoting agents for medical care

BOTANICALS



Low molecular weight secondary plant metabolites

Bioactive compounds play critical roles in human health and may be nutritionally important.



Herbal preparation
from different plant parts

seeds

leaves

flowers

buds

sprouts

Bud-preparations

Herbal products derived from meristematic fresh plant tissues (buds and sprouts)

Gemmotherapy is the most recent of several therapeutic techniques developed on the basis of the medical properties of plants.

Research on bud-preparations, until now, has been only focused on their clinical effects.

Researches on raw material origin, cultivation and quality still lack.

Medical benefit

Ready-for-use

Easy to administer

Homeopathy distribution in the world --> 70% Europe

30% Other countries

Prescribers of homeopathic medicines --> 200,000 physicians

Patients that use of homeopathic medicines --> 200,000,000 in the world

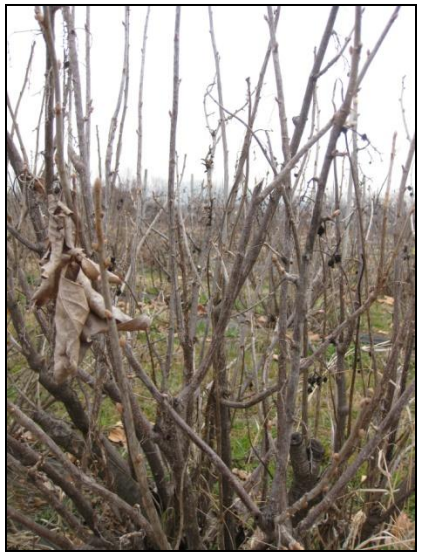
Homeopathic industry incoming --> 20 billion USD/year

Critical points:

- errors in botanical nomenclature (e.g. *Castanea vesca*);
- **few researches on the chemical composition related to their quality;**
- **few information on the effect of environment and genotype on the product quality;**
- differences in preparation protocols.

Aim of the research

Performing an analytical study of blackcurrant and blackberry bud-preparations, in order to identify and quantify the main biomarkers, obtaining a specific phytochemical fingerprint to evaluate the single botanical class contribution to total phytocomplex and relative bioactivity, using a High Performance Liquid Chromatograph – Diode Array Detector.



Blackcurrant (*Ribes nigrum* L.)
The most important industrial product of black currant is fruits; however, leaves and buds, due to their characteristic chemical composition and excellent flavor, have also found some applications as a raw material for the herbal and cosmetic industries: many people use its buds as medicinal preparation for its anti-inflammatory activity and anti-dermal diseases (eczema and psoriasis).

Blackberry (*Rubus ulmifolius* Schott)
The main importer is U.S.A., which is supplied from Chile, Costa Rica, Guatemala and Mexico: sprouts have been used in traditional medicine for their many medicinal properties, as anti-inflammatory activity and anti-haemorrhoids and diarrhoea activity.

Buds were picked up in three different phenological stages: **bud sleeping**, **bud break**, and **first leaves**.

University bud-preparations

Species	Genotype	Year	Germplasm repository	Identification code
Ribes nigrum L.	Rozenthal	2014	San Secondo di Pinerolo, Torino, Italy	RR
	Tenah			RT
Rubus ulmifolius Schott	Black Pearl	2014	Grugliasco, Torino, Italy	RRBP
	Kiowa			RRK
	Wild variety			RRW

Commercial bud-preparations

Species	Company	Year	Germplasm repository	Identification code
Ribes nigrum L.	Company 1	2013	San Gregorio di Catania, Catania, Italy	RC1
	Company 2		Predappio, Forlì-Cesena, Italy	RC2
Rubus ulmifolius Schott	Company 1	2013	San Gregorio di Catania, Catania, Italy	RRC1
	Company 2		Predappio, Forlì-Cesena, Italy	RRC2

Ribes nigrum, Rozenthal

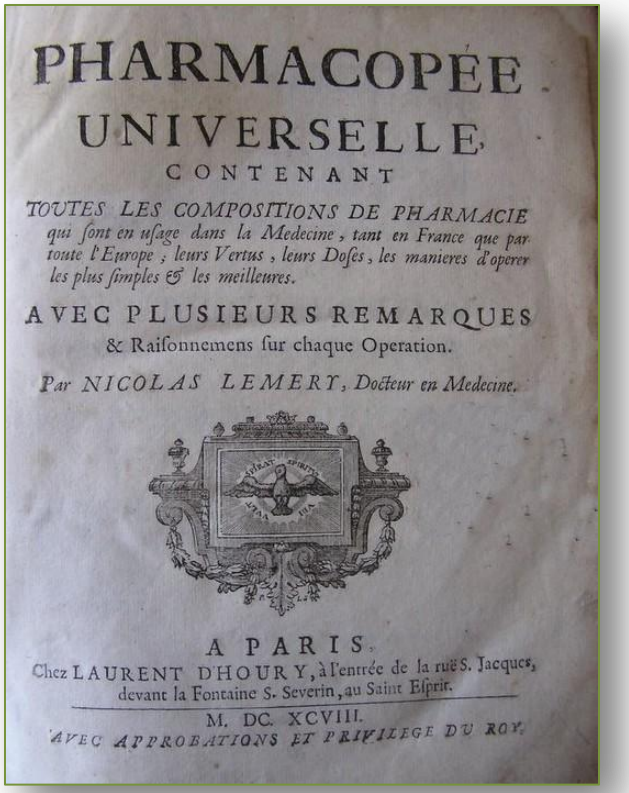
Ribes nigrum, Tenah

Rubus ulmifolius, Black Pearl

Rubus ulmifolius, Kiowa

Rubus ulmifolius, Wild variety

Bioactive compounds were extracted through a process of cold maceration (21 days), in a solution of ethanol (95%) and glycerol, followed by a filtration, a pressing and a second filtration.


PHARMACOPÉE
UNIVERSELLE
CONTENANT
TOUTES LES COMPOSITIONS DE PHARMACIE qui ſont en uſage dans la Medecine, tant en France que par toute l'Europe, leurs Vertus, leurs Doſes, les manieres d'operer les plus ſimples & les meilleures.
AVEC PLUSIEURS REMARQUES
& Raiſonnemens ſur chaque Operation.
Par NICOLAS LEMERY, Docteur en Medecine.
A PARIS.
Chez LAURENT D'HOURY, à l'entrée de la ruë S. Jacques, devant la Fontaine S. Severin, au Saint Eſprit.
M. DC. XCVIII.
AVEC APPROBATIONS ET PRIVILEGE DU ROY.

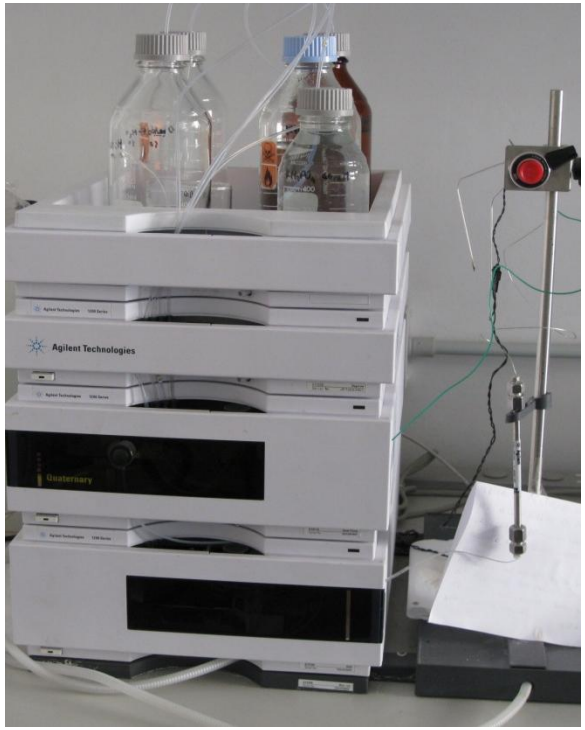
Monograph "Homeopathic preparations", French Pharmacopoeia, 8th edition, 1965

Bioactive compound classes were identified on the basis of the corrispondence between the observed clinical effects of the different species and the chemical composition of common drugs with the same therapeutical effects.

Phytocomplex (total bioactive compound content, TBCC) was determined as sum of the most important classes present in the samples.

- benzoic acids
- catechins
- cinnamic acids
- flavonols
- monoterpenes
- organic acids
- vitamins

Results: mg per 100 g of fresh weight (FW)



Agilent 1200 High Performance Liquid Chromatograph coupled to an Agilent UV-Vis diode array detector

Different chromatographic methods were used for the analysis of macerated samples.

Method A: analysis of cinnamic acids and flavonols

Method B: analysis of benzoic acids and catechins

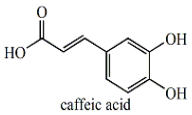
Method C: analysis of monoterpenes

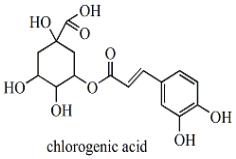
Method D: analysis of organic acids

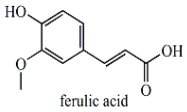
Method E: analysis of vitamins

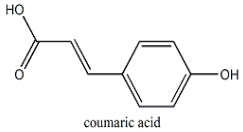
Selected biomolecules used as biomarkers for phytocomplex evaluation

CINNAMIC ACIDS

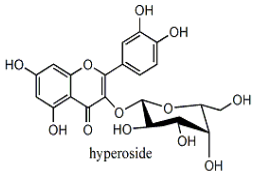

caffeic acid

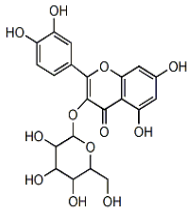

chlorogenic acid

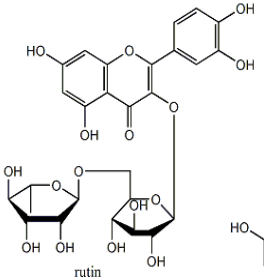

ferulic acid

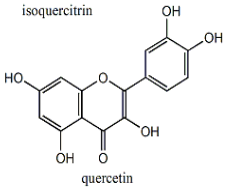

coumaric acid

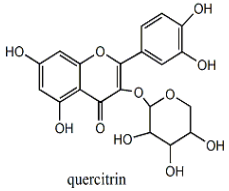
FLAVONOLS


hyperoside

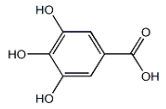

isoquercitrin

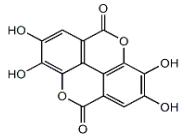

rutin


quercetin

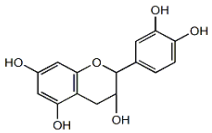

quercitrin

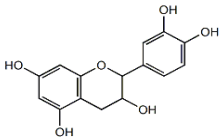
BENZOIC ACIDS


gallic acid

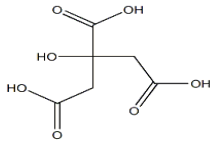

ellagic acid

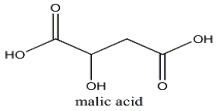
CATECHINS

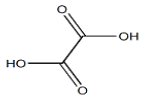

catechin

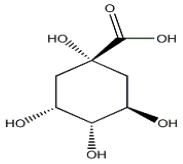

epicatechin

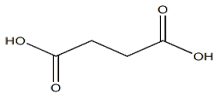
ORGANIC ACIDS

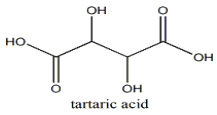

citric acid


malic acid

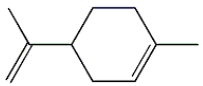

oxalic acid

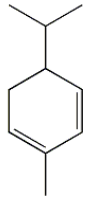

quinic acid

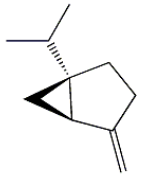

succinic acid

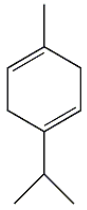

tartaric acid

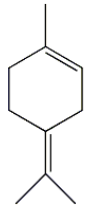
MONOTERPENES


limonene

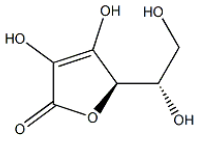

phellandrene

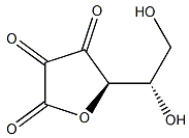

sabinene


γ-terpinene


terpinolene

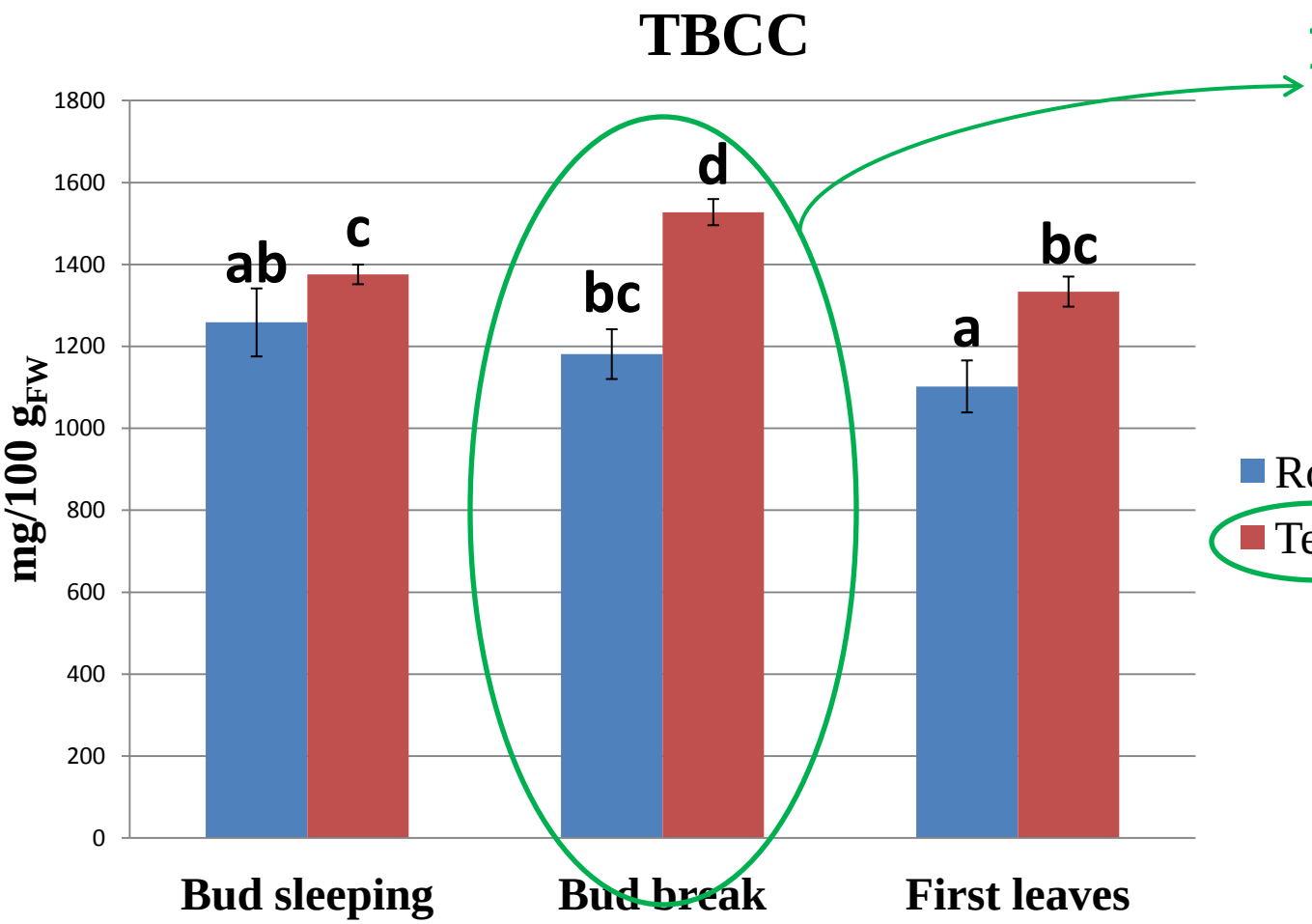
VITAMINS


ascorbic acid


dehydroascorbic acid

Results and discussion

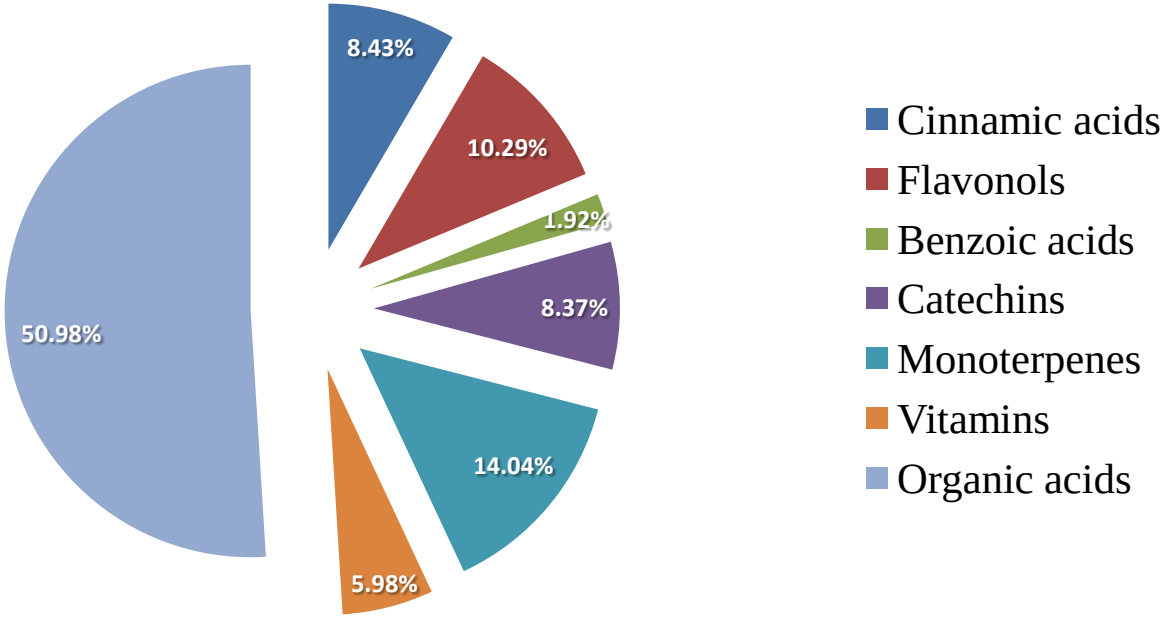
University lab-preparations, *Ribes nigrum*



the best phenological stage for bud harvesting

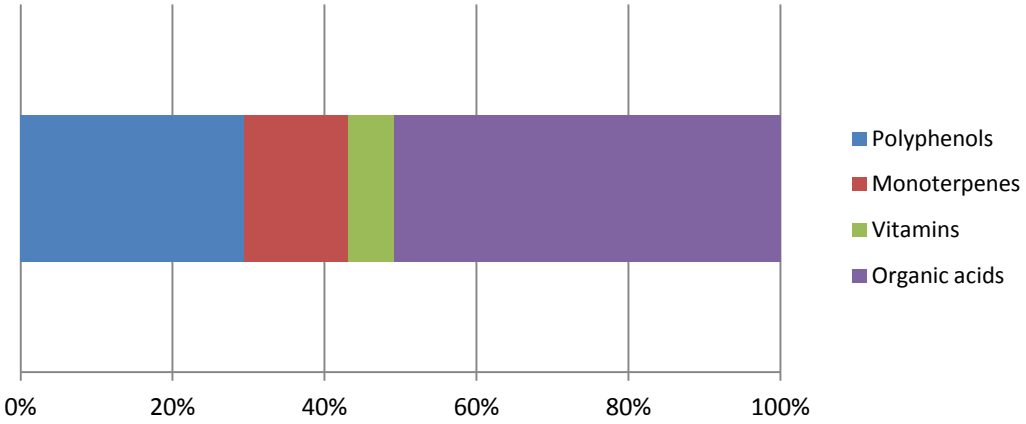
the best genotype

Phytocomplex

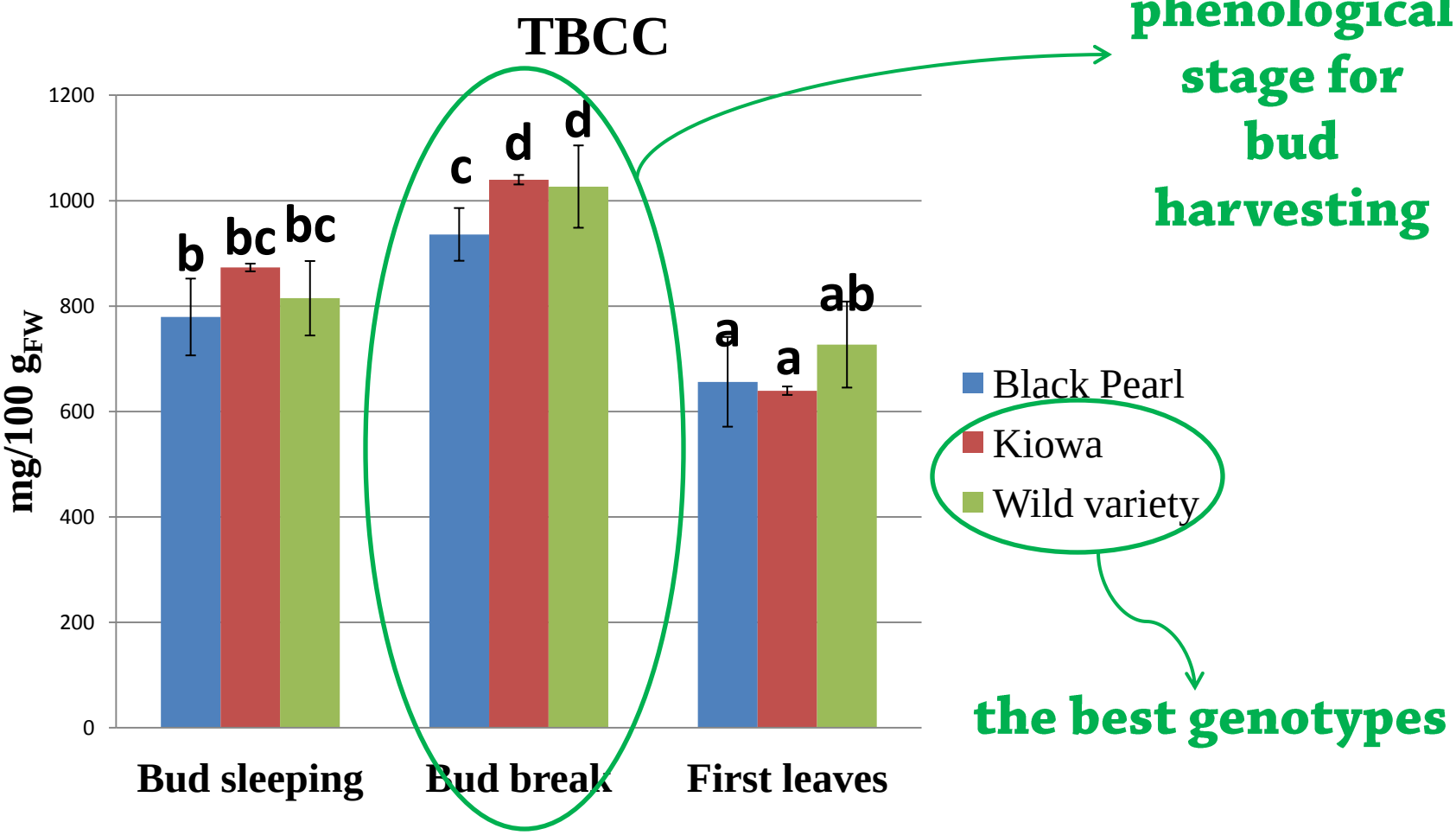


Identified biomarkers

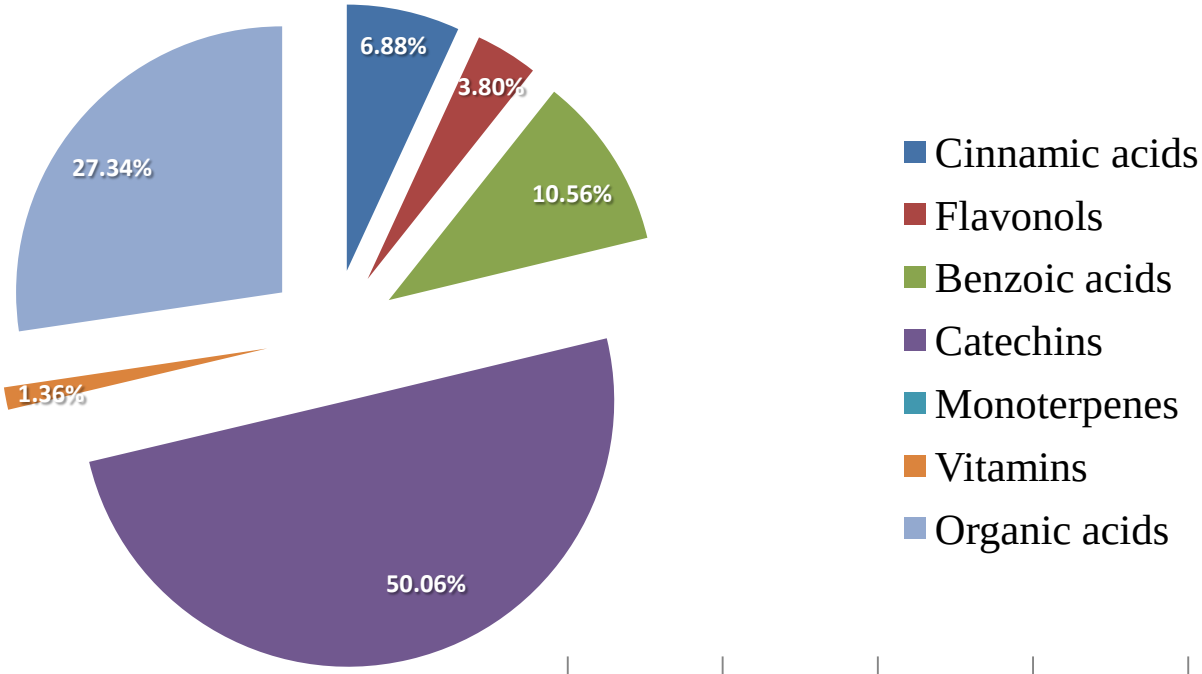
caffeic acid, **chlorogenic acid**, **coumaric acid**, **ferulic acid**
isoquercetin, **quercetin**, **quercitrin**, **rutin**
ellagic acid, **gallic acid**
catechin, **epicatechin**
limonene, **phellandrene**, **sabinene**, **γ-terpinene**, **terpinolene**
vitamin c
malic acid, **oxalic acid**, **quinic acid**, **succinic acid**, **tartaric acid**



University lab-preparations, *Rubus ulmifolius*

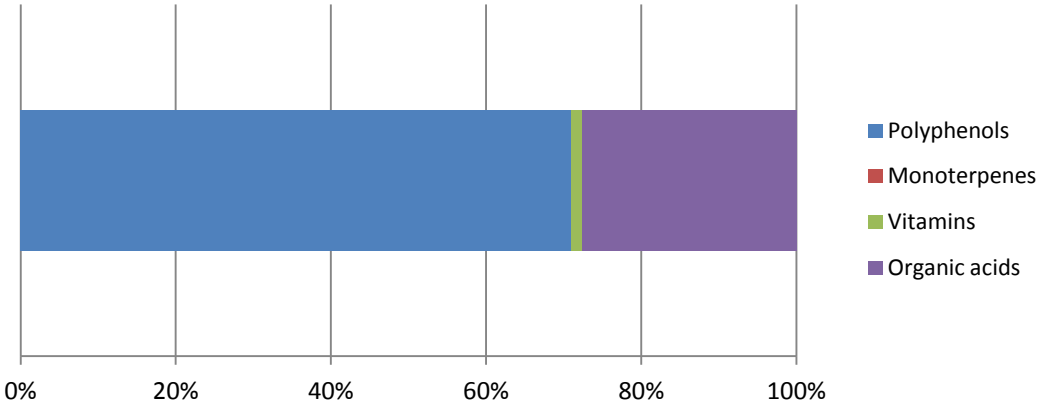


Phytocomplex

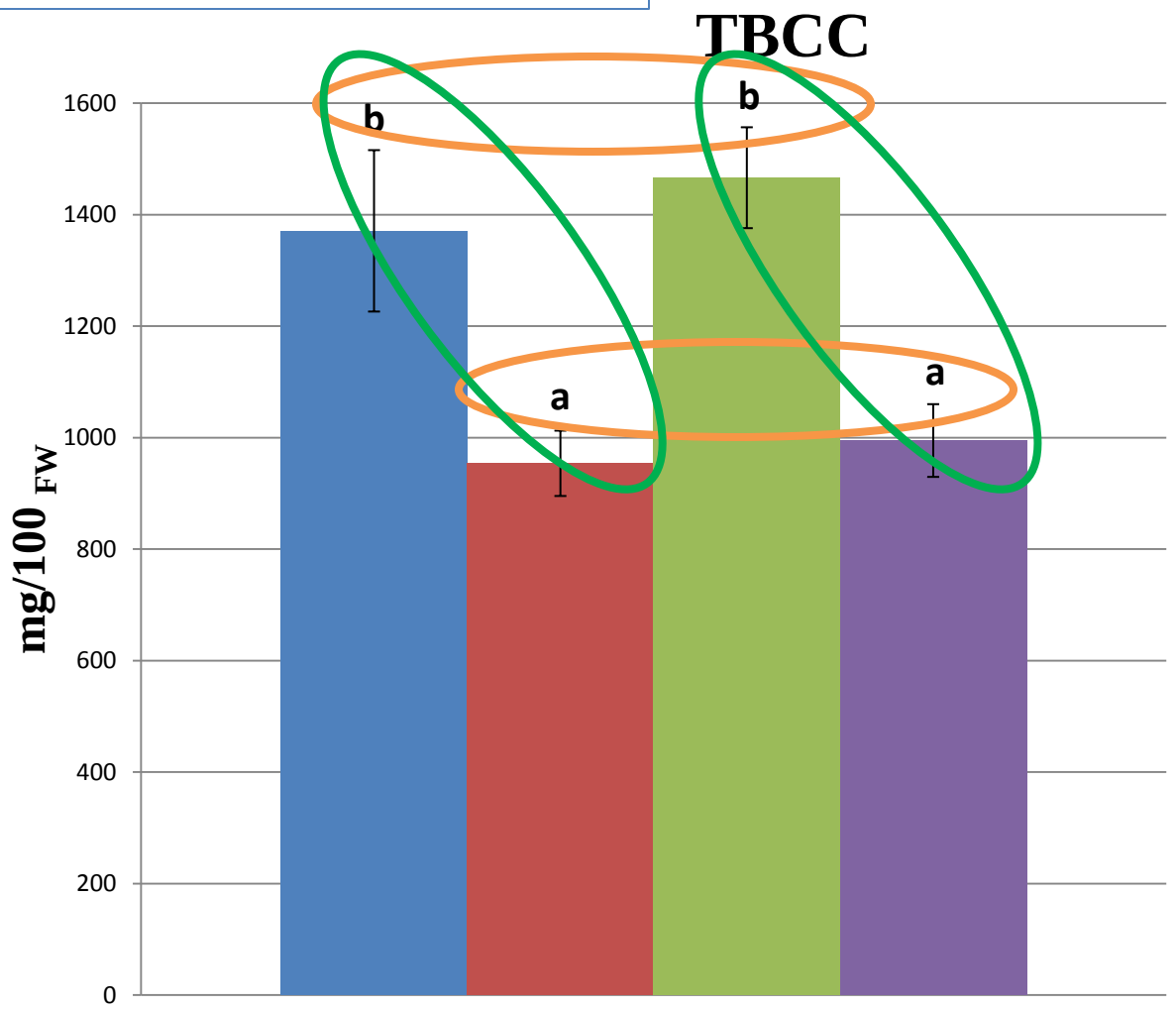


Identified biomarkers

caffeic acid, chlorogenic acid, coumaric acid, ferulic acid
hyperoside, isoquercetin, rutin
ellagic acid, gallic acid
catechin, epicatechin
vitamin c
malic acid, oxalic acid, quinic acid, tartaric acid

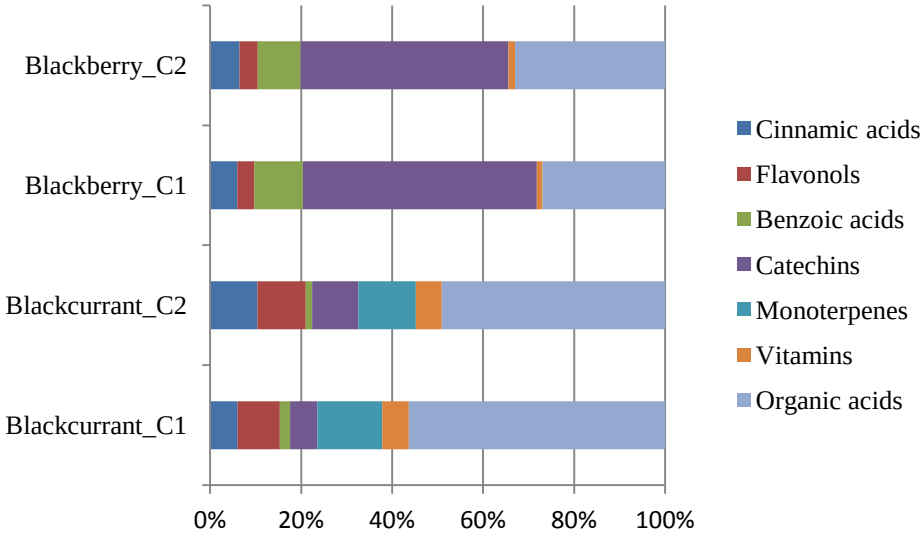


Commercial products



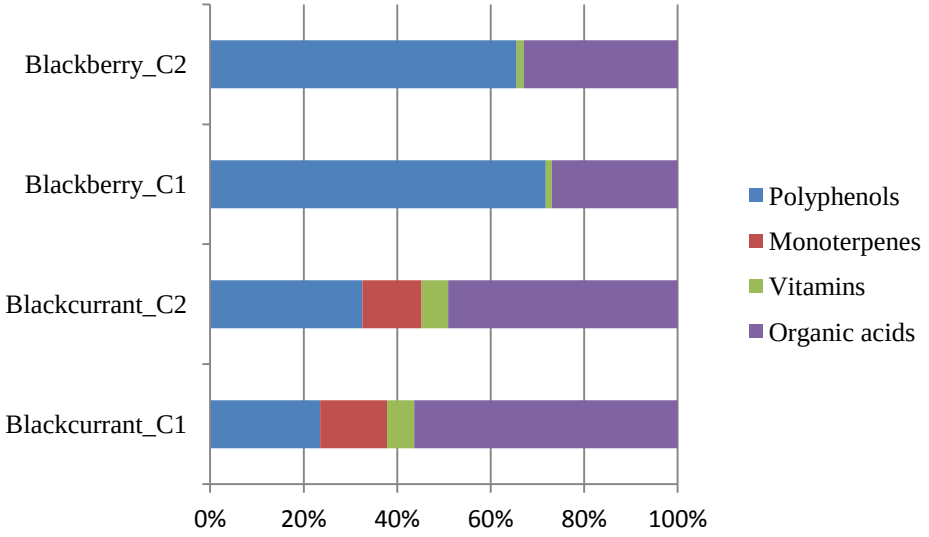
Statistical differences between species

No statistical differences between companies



Phytocomplex

The commercial bud-preparations show similar contribution of each bioactive class to the total phytocomplex in accordance with University lab-preparations.



Conclusions

The results indicate that secondary plant metabolite concentration in bud preparations highly depends on **pedoclimatic conditions**, **harvesting time** and **plant genotype**.

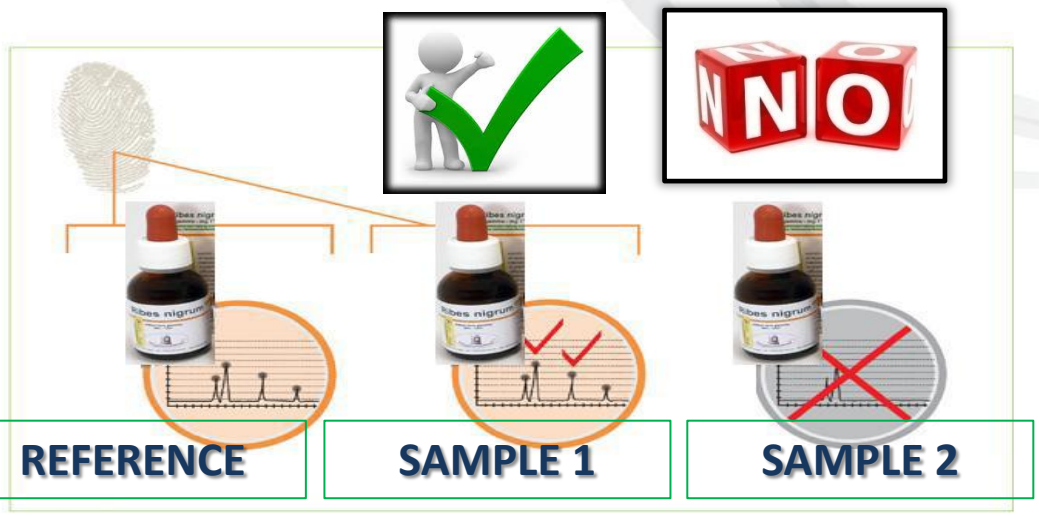
The concentrations of principal bioactive compounds in buds, and consequently in bud-preparations, can be opportunely defined on the basis of chemical-pharmaceutical, agricultural and environmental knowledge.

Definition of chemical, pharmaceutical, agronomic and environmental parameters for product quality

Safety and quality of natural medicines

***Ribes* and *Rubus* spp. as new sources of natural antioxidants and other health-promoting compounds for use in herbal products**

Plant/product fingerprinting



Effective tool for the natural preparation quality control and bioactivity evaluation

1. herbal product characterization and authentication;
2. stability and safety of the preparations (against contamination and adulteration);
3. quality control and standardization of all the supply chain steps;
4. quality certification for use of local plant material with high clinical and pharmaceutical value.

Creation of specific cultivation protocols for the production of buds specifically grown for herbal preparations:

- selecting the best genotypes;
- growing them in the most suitable environment with the best agrotechniques;
- taking into account the phenological stage of the buds at the time of harvesting.

Herbal and pharmaceutical companies

LABEL CERTIFICATIONS

- security in composition
- scientific validation
- quality control
- product standardization

Thanks for your attention!