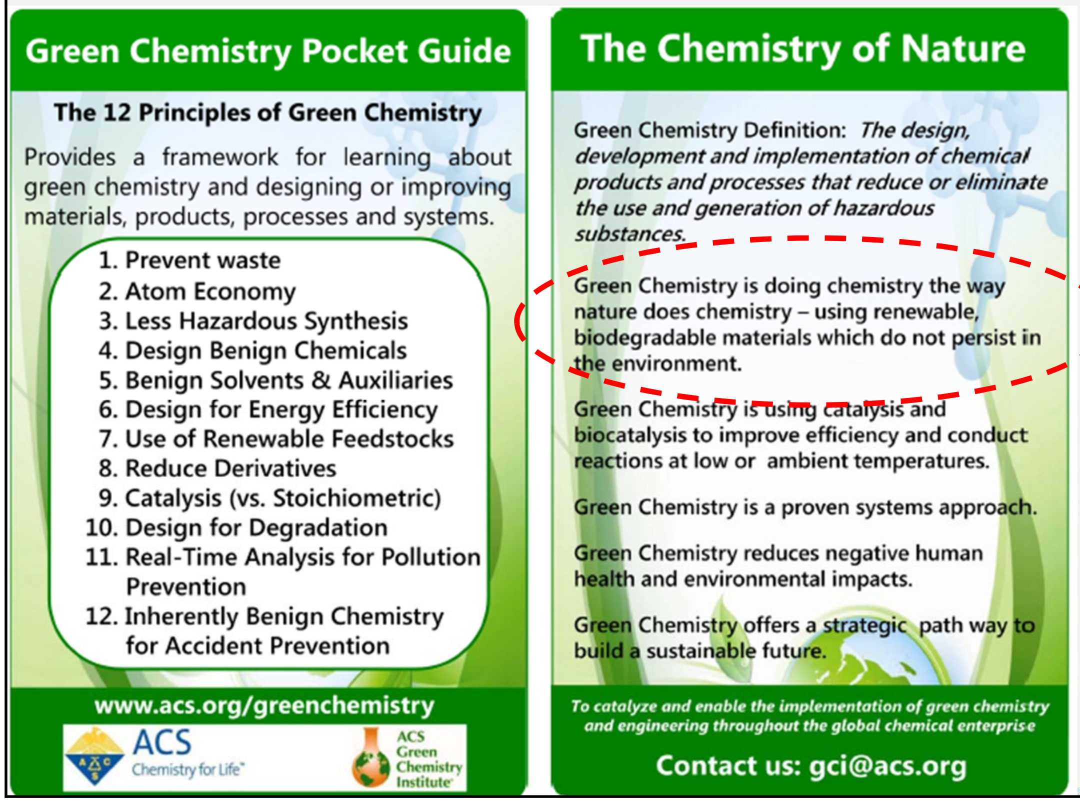
Sekcije za analitičku kemiju i spektroskopiju
Hrvatskog kemijskog društva

Case studies related to analytics of biogenic organic compounds: new green approaches, environmental impact and forensic challenges

Slavica Ražić
University of Belgrade, Faculty of Pharmacy
Department of Analytical Chemistry

Bioactive compounds

- to take the best from nature
- in accordance with principles of green chemisty
- plants as a promissing source



Green Chemistry Pocket Guide

The 12 Principles of Green Chemistry

Provides a framework for learning about green chemistry and designing or improving materials, products, processes and systems.

1. Prevent waste
2. Atom Economy
3. Less Hazardous Synthesis
4. Design Benign Chemicals
5. Benign Solvents & Auxiliaries
6. Design for Energy Efficiency
7. Use of Renewable Feedstocks
8. Reduce Derivatives
9. Catalysis (vs. Stoichiometric)
10. Design for Degradation
11. Real-Time Analysis for Pollution Prevention
12. Inherently Benign Chemistry for Accident Prevention

www.acs.org/greenchemistry

ACS Chemistry for Life™

ACS Green Chemistry Institute

The Chemistry of Nature

Green Chemistry Definition: *The design, development and implementation of chemical products and processes that reduce or eliminate the use and generation of hazardous substances.*

Green Chemistry is doing chemistry the way nature does chemistry – using renewable, biodegradable materials which do not persist in the environment.

Green Chemistry is using catalysis and biocatalysis to improve efficiency and conduct reactions at low or ambient temperatures.

Green Chemistry is a proven systems approach.

Green Chemistry reduces negative human health and environmental impacts.

Green Chemistry offers a strategic path way to build a sustainable future.

To catalyze and enable the implementation of green chemistry and engineering throughout the global chemical enterprise

Contact us: gci@acs.org

How to extract bioactive compounds ?

Distillations & Extractions

- HD
- SD
- ...

- SPME
- SE
- SDE
- PSE
- SFE
- SWE
- UAE
- SFE
- MAE
- SHS
- DHS
- ...

Advantages & limitations

- ✓ recoveries
- ✓ (polarity of analytes, ...)
- ✓ thermal and hydrolytic effect
- ✓ (possible oxidation/degradation)
- ✓ lower sensitivity for traces
- ✓ solvent(s) residues
- ✓ time and energy consumption
- ✓ less “green”
- ✓ automation

To keep in mind...

- depending on the plant origin and sample prep., extracts may produce **different effects.**

- **extraction efficiency** depends on the method used, the sample particle size, solvents' selectivity, temperature, extraction time, pH, composition of particular matrix, presence of interfering substances, *etc., etc.*, …

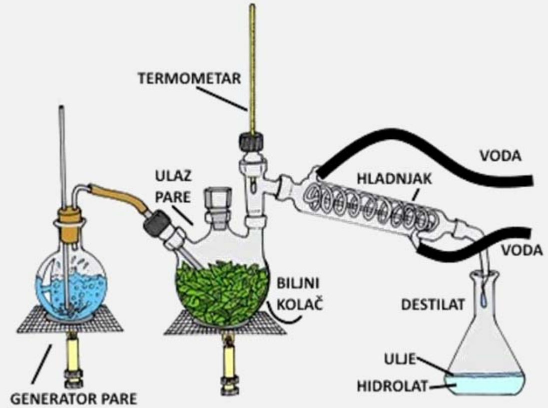
A few selected techniques...

- Soxhlet Extraction (SE)
- Ultrasound-assisted Extraction (UAE)
- Microwave-assisted Extraction (MAE)
- Subcritical water extraction (SCW)

... were compared

Recoveries of main bioactive cmpds. and antioxidant, anti-microbial and cytotoxic activity of plant extracts (dwarf elder and aronia).

pros & cons



- SE

  Traditional standard technique, economical value.
  Limitations: large amounts of solvents act at the temp below their boiling points, time consuming,...

- UAE

  The relatively good efficiency of ultrasound-assisted extraction can be attributed to cavitation phenomenon which, on the other hand, can also provoke a degradation of analytes due to free-radicals formation.

pros & cons

- MAE

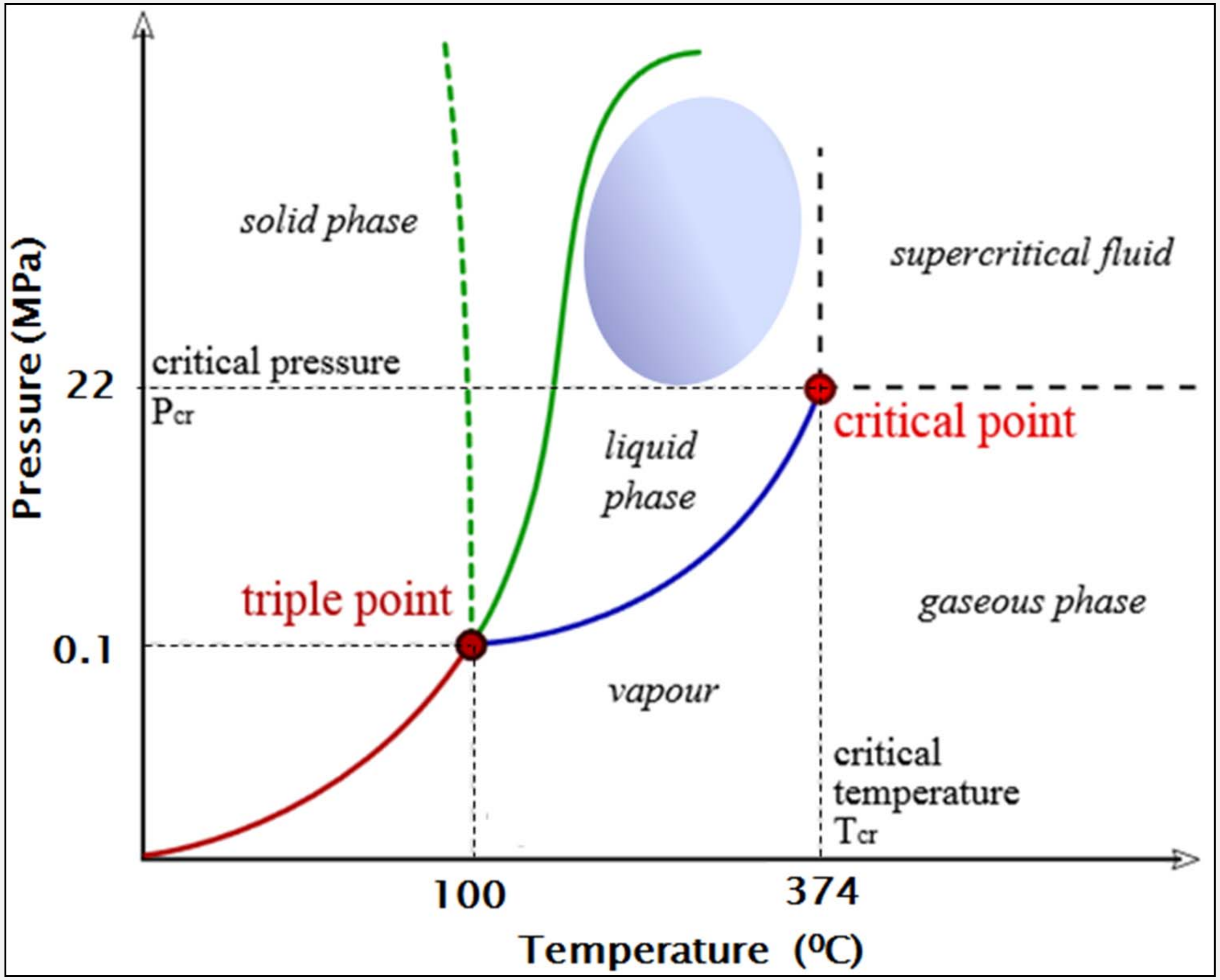
Reduction of extr. time and amounts of solvents

Microwaves induce sudden increase in temp. inside the cellular structure, which might result in an eventual rupturing of cell walls an rapid release of plant constituents into surrounding medium.

Let's think „green"...

- Sub- and supercritical fluid extraction
  - Replacing toxic solvents with benign fluids (CO_2 or H_2O)
  - Supercritical CO_2 is powerful in extraction of small molecules and non-polar cmpds.
  - Extension to more polar molecules, with a long side chains - addition of co-solvents

Let's continue thinking „green"…



Phase diagram of water

dielectric constant *vs.* temperature

Solventmo	t (^{0}C)	D
acetone	25.0	20.7
ethyl alcohol	25.0	24.3
methyl alcohol	20.0	33.1
propyl alcohol	20.0	21.8
chloroform	20.0	4.8
hexane	–90.0	2.0
ethylenamine	21.1	6.3
ethylene glycol	20.0	37.0
ether	20.0	4.3
water	25.0	80.4

water	
t (^{0}C)	D
25	80
250	27
350	13

It is important ...

- hydrolytic potential
  - to find optimal conditions for selective extraction of different classes of cmpd.
  - more polar cmpd at lower and less polar at higher temps.
  - obtaining extracts with the highest content of biol. active cmpd. and the lowest content of interfering subst.

Case study 1

Sambucus ebulus L. (dwarf elder)

… was analyzed in terms of chemical and biological fingerprints.

Let’s see what is inside!

Dwarf elder berries

Optimisation of the SCW extraction

- For each extract/case study and particular cmpd. of interest
  - Influence of the sample-to-solvent-ratio
  - Influence of the agitation rate
  - Influence of the pressure
  - Influence of the temperature
  - Influence of the extraction time

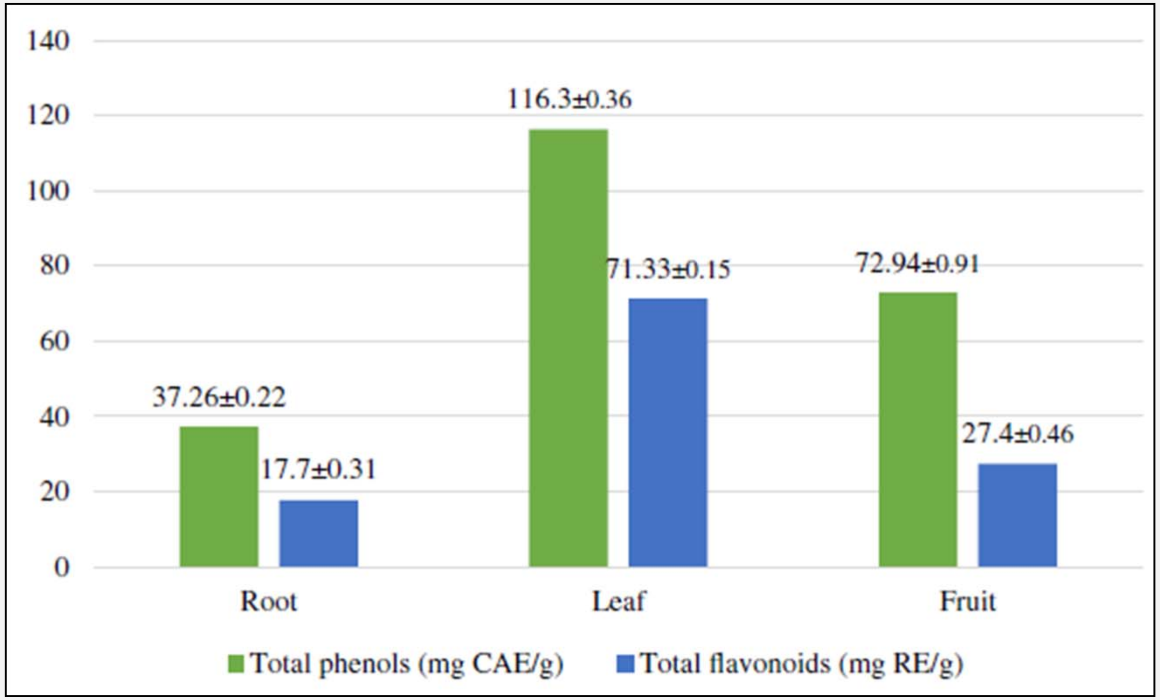
... in favor to the max yield of the key cmpds.

Qvalitative & Quantitative analysis of SCW extract

- HPLC-DAD – analysis of phenolics and polyphenolic cmpds.

- total phenolics (Folin-Ciocalteu's method with clorogenic acid as a cal. stand.); CAE
- total flavonoids (Markham's method with rutin as a cal. stand.); RE

Total phenols and flavonoids in roots, leaves and fruits extracts of *Sambucus ebulus* L.



- photosynthesis,
- transpiration and
- gas exchange

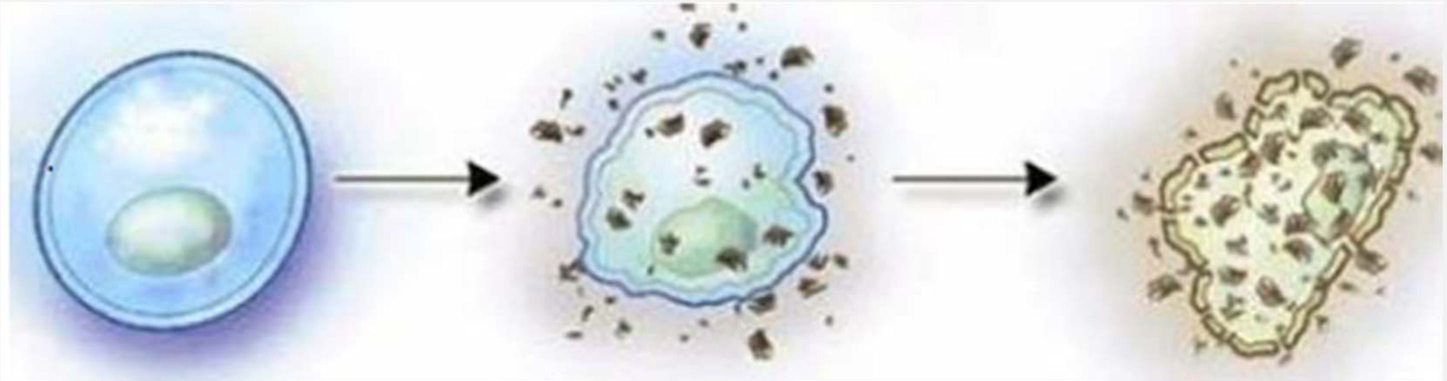
HPLC-DAD: Content of phenolic compounds (*mg/L*)

Compound	Roots	Leaves	Fruits
gallic acid	208.84	141.35	868.98
protocatechuic acid	12.23	19.56	39.16
catechin	55.98	16.20	n.d.
chlorogenic acid	5.96	274.69	36.82
vanillic acid	n.d.	n.d.	n.d.
caffeic acid	4.28	128.88	17.21
epicatechin	<LOQ (1.49)	<LOQ (1.46)	<LOQ (0.94)
syringic acid	n.d.	<LOQ (3.60)	n.d.
p-coumaric acid	<LOQ (2.21)	4.66	<LOQ (1.29)
ferulic acid	<LOQ (2.36)	<LOQ (1.79)	3.38
sinapic acid	<LOD (1.47)	<LOD (0.84)	<LOD (0.49)
naringin	1.82	5.31	1.97
rutin	n.d.	24.37	6.53
cinnamic acid	n.d.	2.44	n.d.
naringenin	n.d.	n.d.	n.d.
quercetin	n.d.	<LOQ (2.33)	<LOD (1.24)

n.d. – not detected; **LOQ – limit of quant., ***LOD – limit of det.

Free-radicals activity

beginning of illness



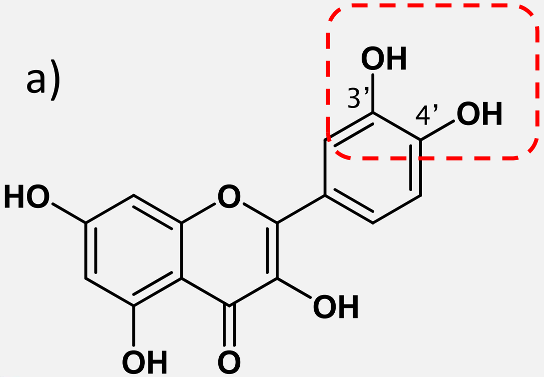
normal cell

Cell attacked by free radicals

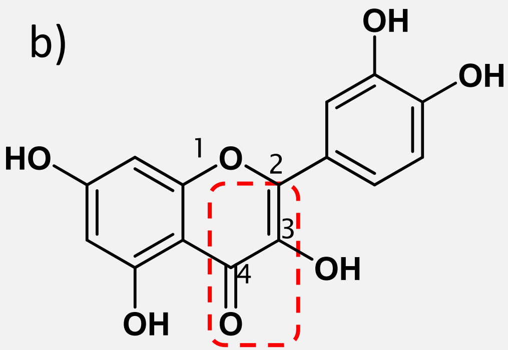
cell under oxidative stress

Free-radical scavenging activity and …

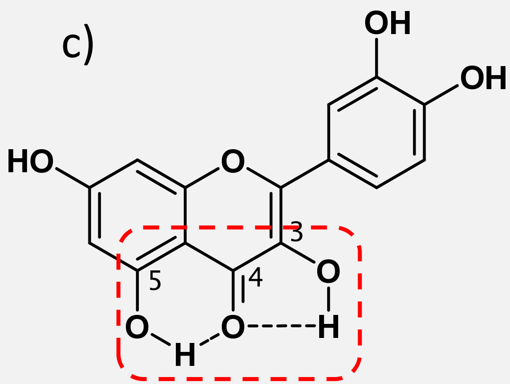
a)



b)



c)



flavonole quercetin

… responsible groups in flavonoids

Free- radical scavenging capacity

DPPH assay

- 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical $\Rightarrow\Rightarrow$ DPPH-H

$$\%RSC = 100 - \frac{A_{sample}}{A_{blank}} \cdot 100$$

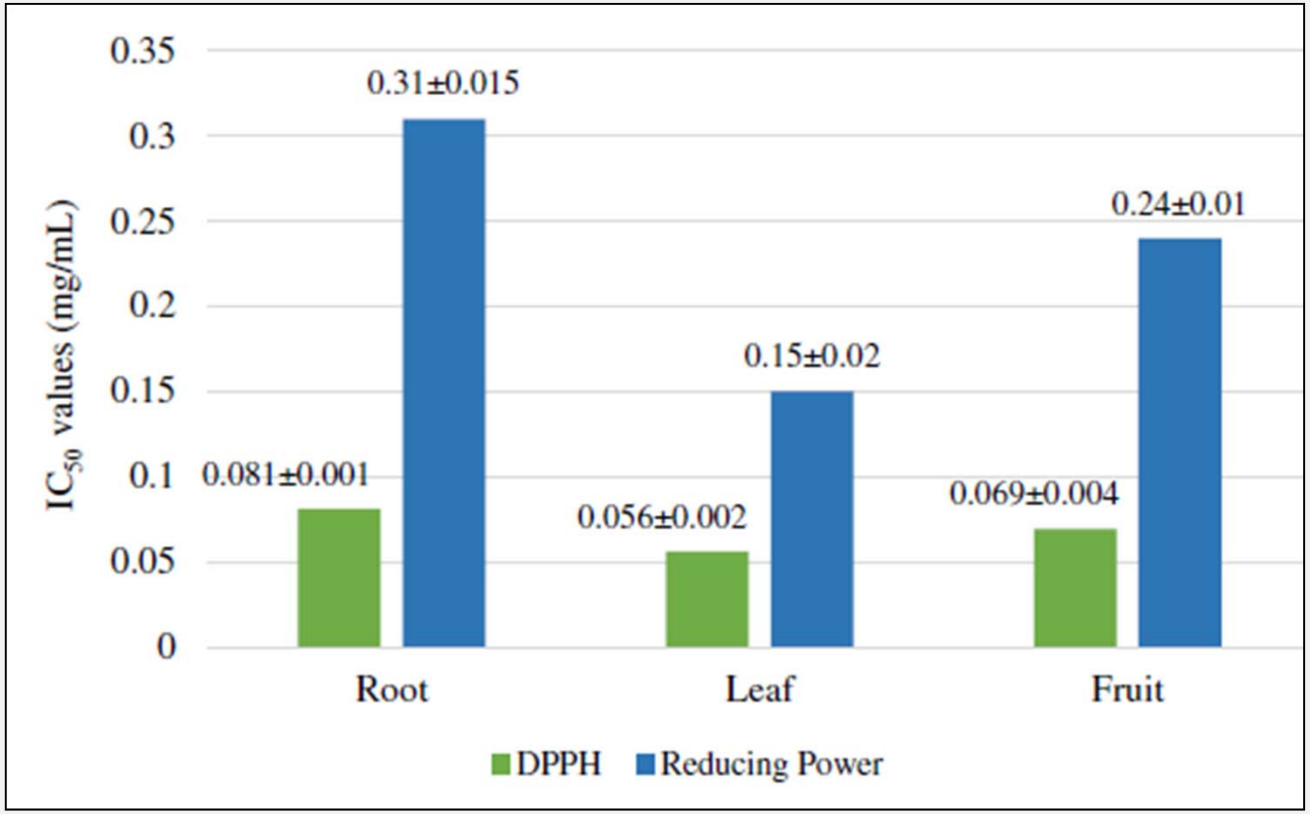
IC_{50} - inhibitory conc. at 50%
(conc. of test sol. for achieving 50% scavenging capacity

FRAP (Ferric Reducing Antioxidant Power)

- reduction of Fe^{3+} with 2,4,6-Tris(2-pyridyl)-s-triazine (*TPTZ*)

EC_{50} - conc. of extract which gives half-maximal response

Antioxidant activity – DPPH and FRAP tests



Cytotoxic activity – MTT assay

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide

- HeLa (human cervix adeno carcinoma cell line)
- A-549 (human lung adenocarcinoma) and
- S-174T (human colorectal adenocarcinoma)

	IC_{50} value (*μg/mL*)		
Sample	HeLa	A-549	LS-174T
roots	2.86±0.11	4.42±1.77	8.10±2.09
leaves	0.58±0.18	0.76±0.11	1.89±0.49
fruits	1.03±0.00	2.85±1.00	1.89±0.20

IC_{50} – conc. which inhibits 50% of the cell growth [μg/mL]

American National Cancer Institute: IC_{50} < *30 μg/mL*

Enzyme inhibitory activity

Diabetes mellitus (DM) is one of the most common types of metabolic syndrome and the prevalence of it is increasing day by day.

- the inhibition of carbohydrate digestive enzymes (α-amylase and α-glucosidase)
- the enzymes catalyze the hydrolysis of glycoside bonds in carbohydrates resulting in free glucose.
- control blood glucose level in DM.

Enzyme inhibitory activity

- Tyrosinase is a copper-containing enzyme and plays a key role in the synthesis of melanin.
- The inhibition of tyrosinase is considered an effective therapeutic tool for pigmentation disorders.

Enzyme inhibitory activity

Sample	α- amylase inhibiton (mmol ACAE/g extract)	α- glucosidase inhibition (mmol ACAE/g extract)	Tyrosinase inhibition (mg KAE/g extract
roots	0.48 ±0.02	0.37 ±0.11	9.32 ±0.55
leaves	0.42 ±0.02	2.04 ±0.05	19.67 ±5.26
fruits	0.45 ±0.01	1.40 ±0.15	10.94 ±0.89

**ACE- Acarbose equivalent , **KAE – Kojic acid equivalent*

- very high concentrations of chlorogenic and caffeic acids
- anti-diabetic and anti-tyrosinase agents

The present work is the first study on enzyme inhibitory properties of *Sambucus ebulus*, which could be considered as valuable source of naturally-occurring enzyme inhibitory agents.

Case study 2

Chemical and biological characterization of stems, leaves and berries *Aronia melanocarpa*'s extracts obtained by SWE

Total phenols and flavonoids in Total phenols and flavonoids of Aronia samples (mean±SD)

Sample	Total phenols (mg CAE/g extract)	Total flavonoids (mg RE/g extract)
Stems	49.96±0.15	25.10±0.38
Leaves	131.53±0.96	88.64±0.31
Berries	13.88±0.02	10.00±0.25

CAE – Chlorogenic acid equivalents; RE – Rutin equivalents.

HPLC-DAD: Phenolics profile of aronia

Compound	berries	leaves	stems
	(*mg/g* of dry extract)		
Protocatehuic acid	nd	nd	0.150
p-Hydroxybenzoic	0.469	0.128	0.161
Caffeic acid	n.d.	n.d.	0.019
Vanillic acid	0.238	0.041	n.d.
Chlorogenic	0.171	0.029	0.050
Syringic acid	0.419	0.055	0.056
p-coumaric acid	0.175	0.093	0.033
Ferulic acid	0.173	0.046	0.051
Sinapic acid	1.072	0.547	0.290
Rutin	**5.544**	0.693	1.264
Rosmarinic acid	0.236	0.155	0.115
Quercetin	1.396	0.105	0.247
Luteolin	0.154	0.334	0.364
Naringenin	0.172	0.026	0.065
Kaempferol	0.271	0.069	0.049
Apigenin	0.242	0.210	0.151

Antioxidant capacity of aronia samples

	IC$_{50}$ ($\mu g/mL$)		
Samples	DPPH scavenging activity	ABTS scavenging activity	Inhibitory activity against lipid peroxidation
Stems	35.43±0.25	34.57±0.29	20.41±0.45
Leaves	30.45±0.94	32.94±0.54	21.36±0.20
Berries	32.40±0.53	31.43±0.57	24.49±0.38
Ascorbic acid	6.05±0.34	2.39±0.93	>1000
BHT	15.61±1.26	19.32±0.72	1.00±0.23
α-tocopherol	–	–	0.48±0.05

- complex nature of phytochemicals or their synergetic/antagonist interactions
- different mechanisms of action, and different compound may participate in scavenging different radicals
- usually, second metabolites, such as polyphenols, are responsible for activity.

Enzyme inhibitory effects of aronia samples

Alzheimer's disease (AD) and new therapeutic strategies
- Cholinergic hypothesis: inhibition of hydrolysis of acetylcholine in synaptic cleft may alleviate the symptoms of AD

Aging and anti-age treatment
- Elastase is the main enzyme which causes the breakdown of elastin, which is an important protein in the extracellular matrix.

Enzyme inhibitory effects of aronia samples

Samples	AChE Inhibition (*mg GALAE/g extract*)	BChE Inhibition (*mg GALAE/g extract*)	Elastase (*mmol CAE/g extract*)
Berries	0.814±0.016	0.605±0.066	3.549±0.113
Stems	0.713±0.030	n.a.	3.118±0.392
Leaves	0.774±0.023	n.a.	2.345±0.519

GALAE – Galantamine equivalent; CAE – Catechin equivalent; n.a. – not active

Ability of aronia SWE extracts to inhibit cholinesterase and elastase has not been reported yet.

Cytotoxic activity of aronia samples– MTT assay

MTT (3–[4,5–dimethylthiazol–2–yl]–2,5 diphenyl tetrazolium bromide

- A–549 (human lung adenocarcinoma)
- LS–174T (human colorectal adenocarcinoma)
- HeLa (human cervix adeno carcinoma cell line) and
- MRC–5 (**normal** human embryonic lung fibroblast)

IC_{50} – conc. which inhibits 50% of the cell growth [$\mu g/mL$]

Samples	A–549	LS–174T	HeLa	MRC–5
Stems	/	/	/	/
Leaves	2.01±1.05	1.38±0.28	**0.69±0.08**	1.72±0.06
Berries	4.21±1.06	5.44±0.24	3.41±0.01	5.50±0.07
Cisplatin	2.83±0.18	7.46±1.02	1.56±0.14	2.48±0.24

For the first time, demonstrated antitumor properties of the SWE aronia extracts.

Selectivity index in antitumor action of extracts and compounds

Samples	IC_{50} MRC5/IC_{50}A–549	IC_{50} MRC5/IC_{50} LS–174T	IC_{50} MRC5/IC_{50} HeLa
Leaves	0.86	1.24	2.49
Berries	1.3	1.01	1.61
Cisplatin	0.88	0.33	1.59

Antimicrobial activities of aronia samples- Microdilution method

Microbial strains/ Fungi	stems	leaves	berries	Amracin	Nistatin
	(mg/mL)				
Staphylococcus aureus	156.25	39.10	78.20	19.53	/
Klebsiella pneumoniae	625.00	312.50	312.50	39.10	/
Escherichia coli	19.53	312.50	312.50	19.53	/
Proteus vulgaris	78.12	39.10	78.20	156.25	/
Proteus mirabilis	312.50	19.53	78.12	312.50	/
Bacillus subtilis	625.00	78.12	312.50	78.12	/
Candida albicans	39.10	39.10	78.20	/	39.10
Aspergillus niger	19.53	312.50	312.50	/	19.53

- Amracin – control the sensitivity of the tested bacteria
- Nystatin – control against the tested yeast

Minimum inhibitory concentration (MIC), *mg/mL*

- *Extraction is a crucial step in the preparation of plant products.*
- Subcritical water in addition to its safety, green character and low price, offers competitive solvating properties and very good selectivity.
- *At this point, SCW could be considered as novel non-toxic and green procedure.*
- Both *Sambucus ebulus* and *Aronia melanocarpa SWE* extracts exhibited remarkable biological activity.
- Great potential for preparing new phyto-pharmaceuticals and functional food ingredients.

Research Article

Received: 11 July 2017 Revised: 27 September 2017 Accepted article published: 5 October 2017 Published online in Wiley Online Library: 24 November 2017

J Chem Technol Biotechnol 2018; **93**: 1097–1104

A new source for developing multi-functional products: biological and chemical perspectives on subcritical water extracts of *Sambucus ebulus* L.

Aleksandra Cvetanović,[a*] Zoran Zeković,[a] Jaroslava Švarc-Gajić,[a] Slavica Razić,[b] Ana Damjanović,[c] Gokhan Zengin,[d] Cristina Delerue-Matos[e] and Manuela Moreira[e]

University of Novi Sad, Faculty of Technology, Department for Biotechnology and Pharmaceutical Engeneering

Institute of Oncology and Radiology of Serbia, Belgrade

Food and Chemical Toxicology 121 (2018) 458–466

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Comparative *in vitro* studies of the biological potential and chemical composition of stems, leaves and berries *Aronia melanocarpa's* extracts obtained by subcritical water extraction

Aleksandra Cvetanović[a,*], Gökhan Zengin[b], Zoran Zeković[a], Jaroslava Švarc-Gajić[a], Slavica Ražić[c], Ana Damjanović[d], Pavle Mašković[e], Milan Mitić[f]

BVOCs

Volatile organic compounds and metals in thyme samples – from chromatography and spectroscopy *via* chemometrics to biosynthesis

Case study 3

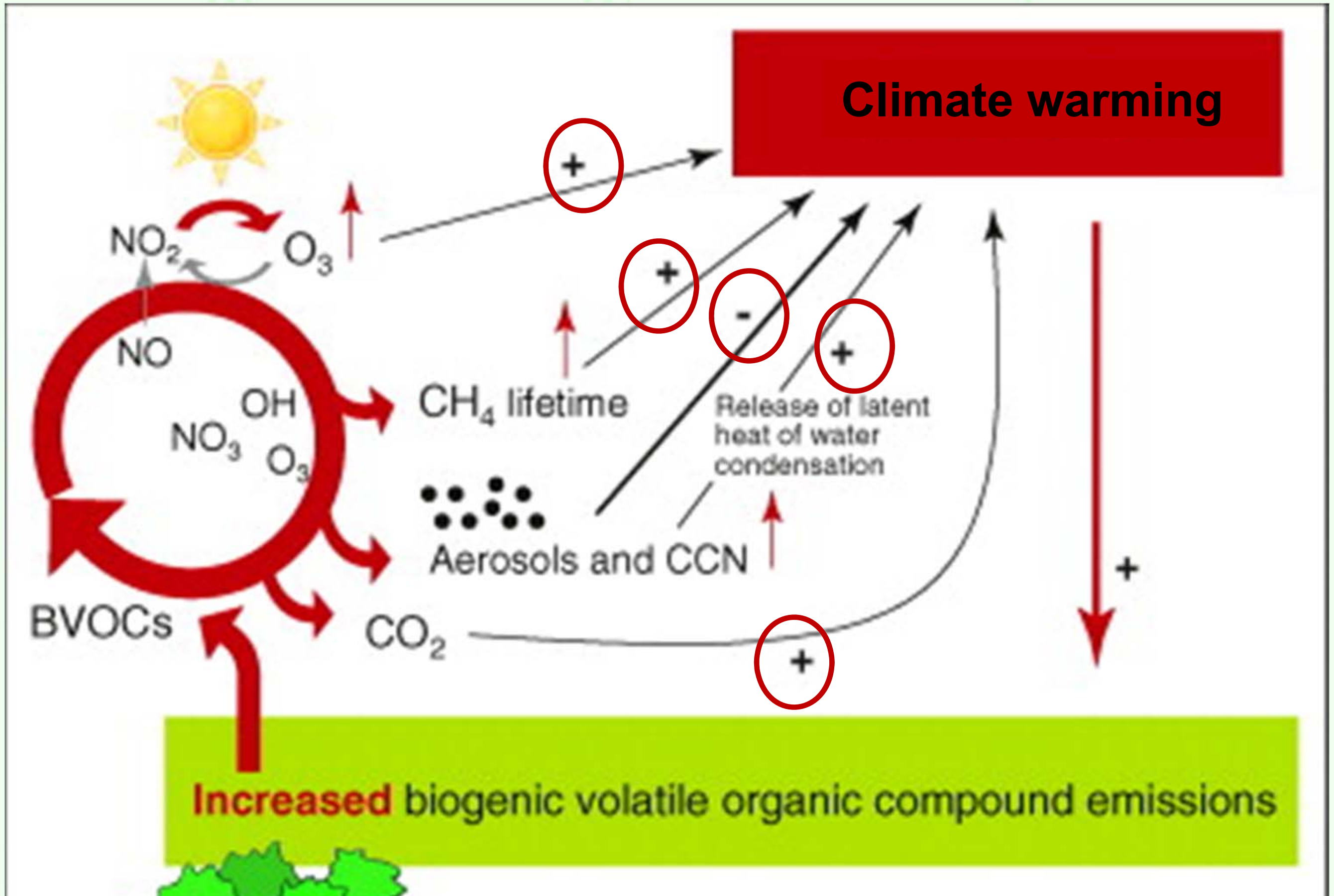
i. Biogenic volatile organic compounds (BVOCs) and emisson to atmosphere
ii. Environmental impact
iii. Analytics and technique dependant factors
iv. Theory of static headspace extraction
v. Biosynthesis of BVOCs influenced by metals

Terpenoids: a plant language

- carbon fixed by plants is emitted back into the atmosphere as BVOCs
  - plant-to plant signalling
  - pathogen defence
  - ozone quenching
  - ...

On a global scale, biogenic VOCs account for about 90% of total VOCs emissions.

Effects of increased BVOC emissions on atmospheric chemistry and climate



CCN – cloud condensation nuclei

Responses to a warming world

Think globally, act locally...

$\Downarrow$

Go to analytical lab !

Technique-dependent factors

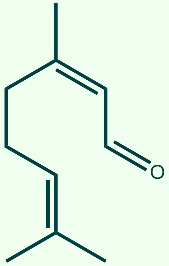
Hydrodistillation (HD)

☺ environmentally friendly
☺ no solvent residue
☺ safe to operate
☹ time and energy consuming
☹ thermal and hydrolytic effect
☹ loss of most polar and very vol. comp.
☹ no automation

Static headspace extraction (SHS)

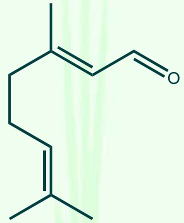
☺ good recovery of very vol. comp. (α-pinene, 1,8-cineole,...)
☹ low sensitivity for traces
☹ low recovery of low vol. comp.

SHS extraction - optimization

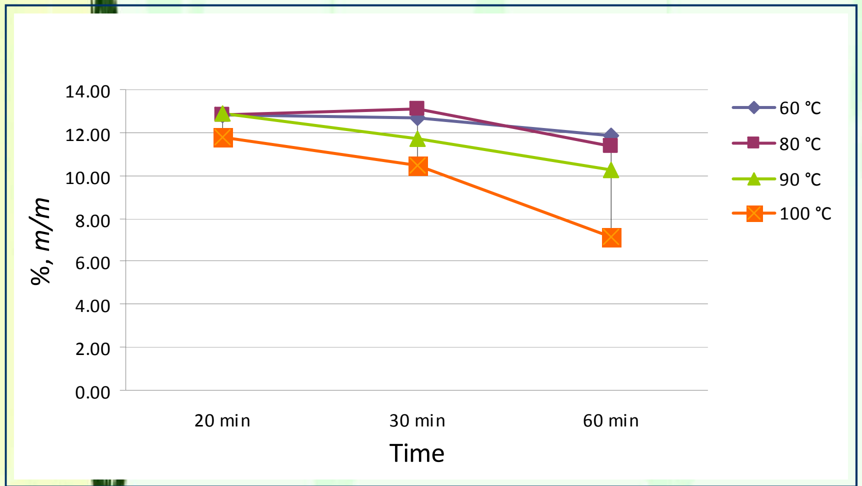


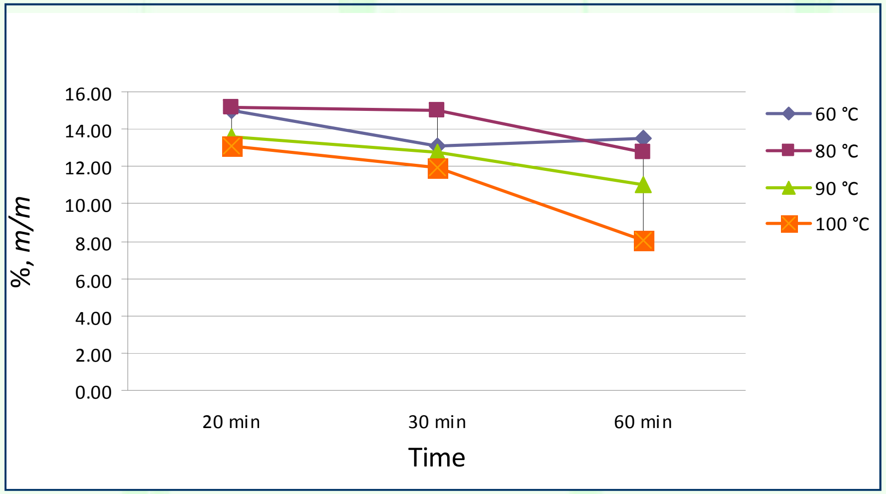
neral

Influence of temperature and time of equilibration on relative amount of neral and geranial in HS extracts



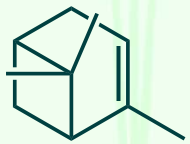
geranial



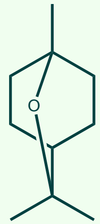


HS equilibration under 90 °C for 30 min gave HS extract which correlated the best with the composition of hydrodistilled EO.

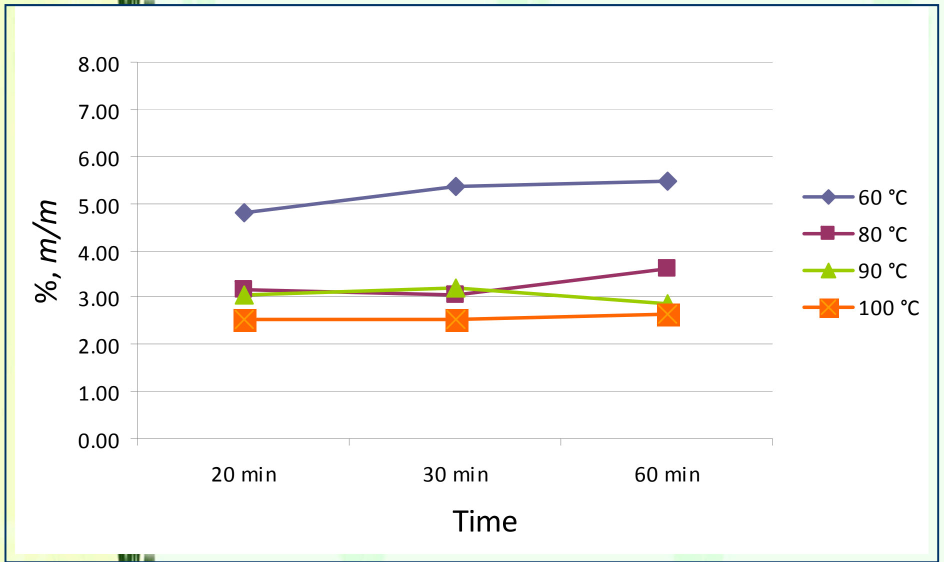
SHS extraction - optimization

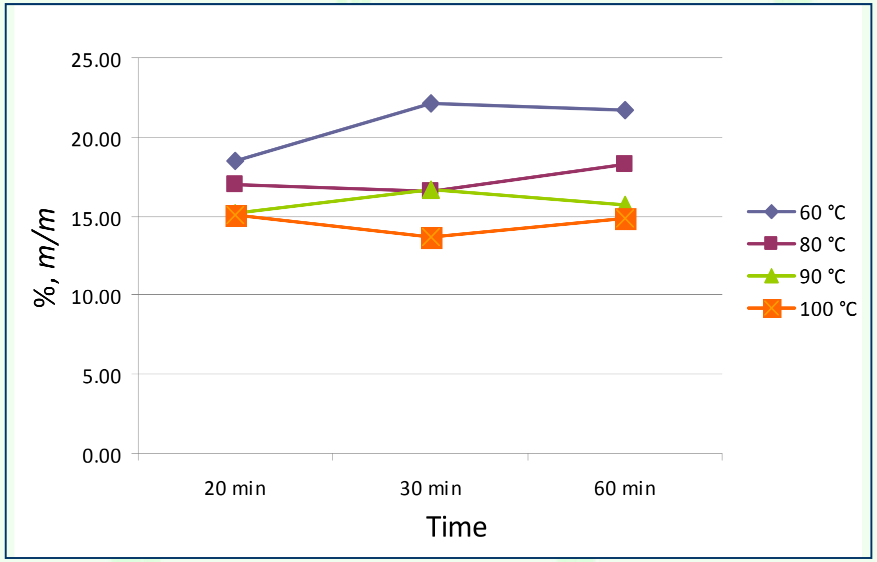


***α*-pinene**



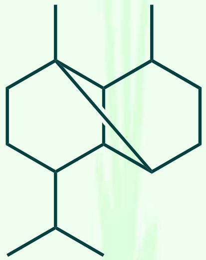
1,8-cineole



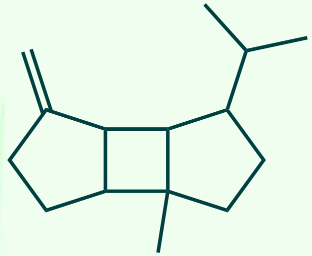


Influence of temperature and time of equilibration on relative amount of *α*-pinene and 1,8-cineole in HS extracts

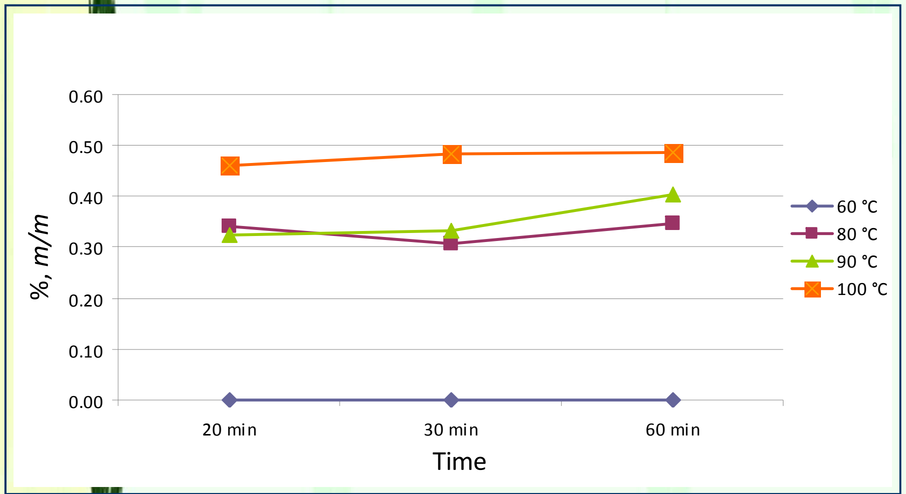
SHS extraction - optimization

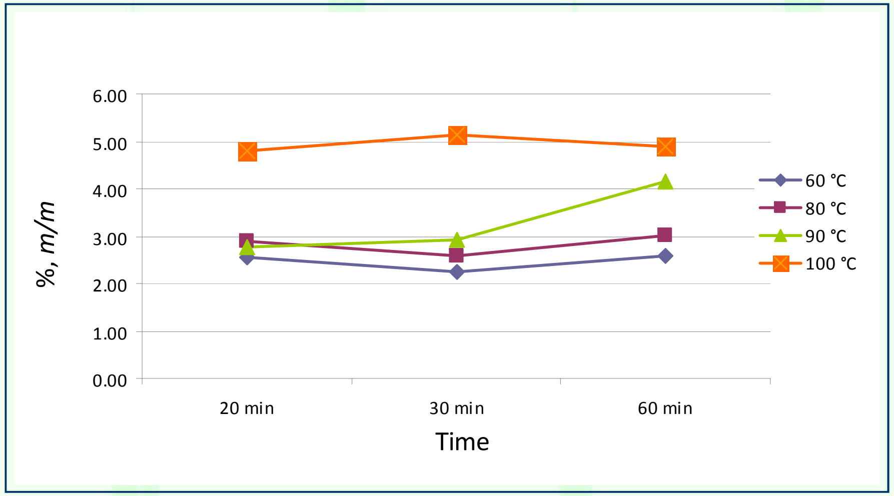


α-copaene



β-bourbonene





Influence of temperature and time of equilibration on relative amount of *α*-copaene and *β*-bourbonene in HS extracts

Identification and quantification of BVOCs

GC-MS

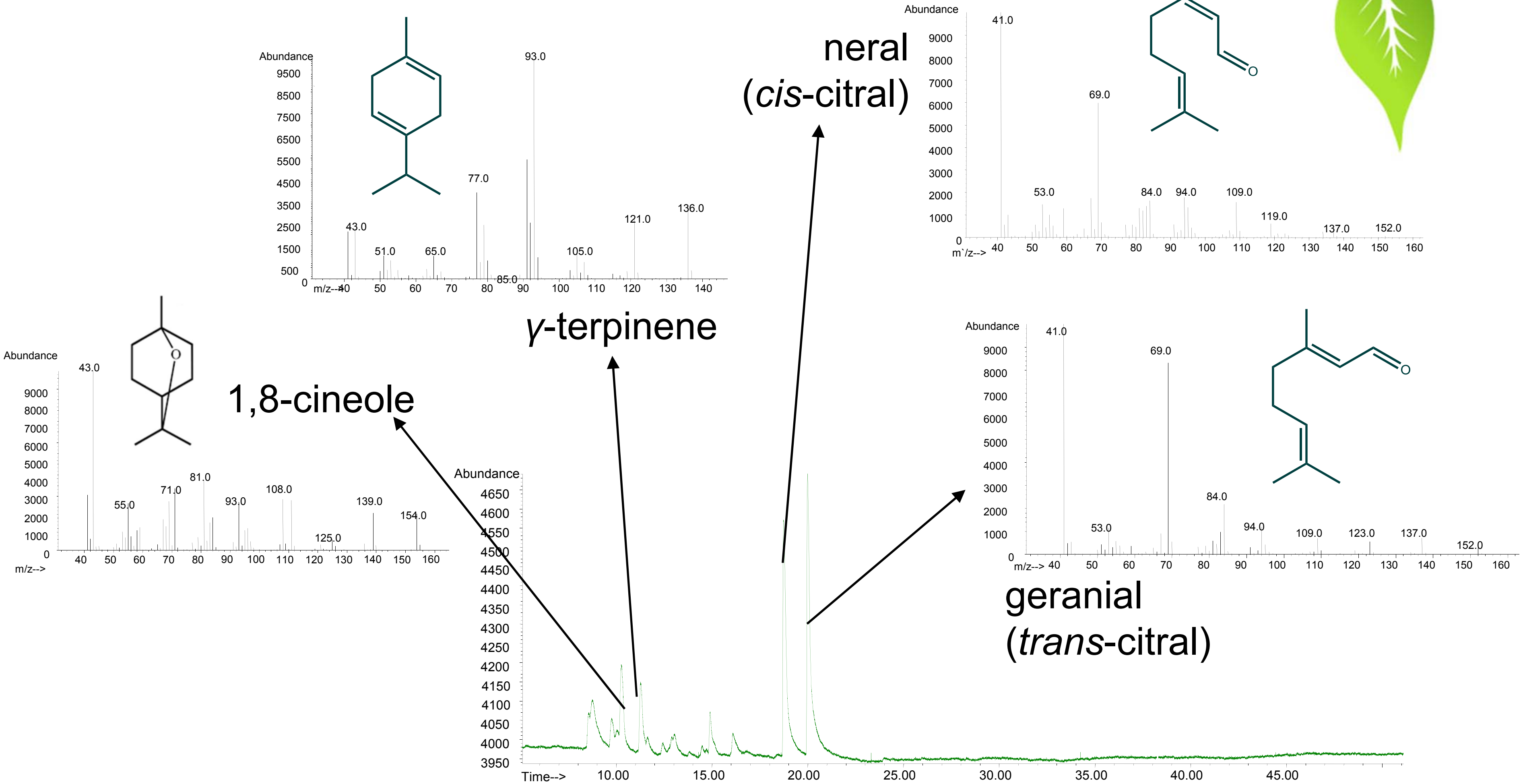
Identification of constituents - comparing calculated Kovats RI and MS spectra obtained from authentic samples, NIST/Wiley spectra libraries and literature data (Adams, 2007).

The linear RIs were determined in relation to a homologues series of n-alkanes (C9–C24) under the same operating conditions.

GC-FID

Quantitative analysis of HD oil and HS extracts

SHS-GC-MS



Static headspace extraction @ 70 ^{0}C, 30 min

Inorganic analysis: sample prep. (MW) and measurements (F-AAS, GF-AAS, ICP-OES)

Oxigenated monoterpenes (5%) and metals

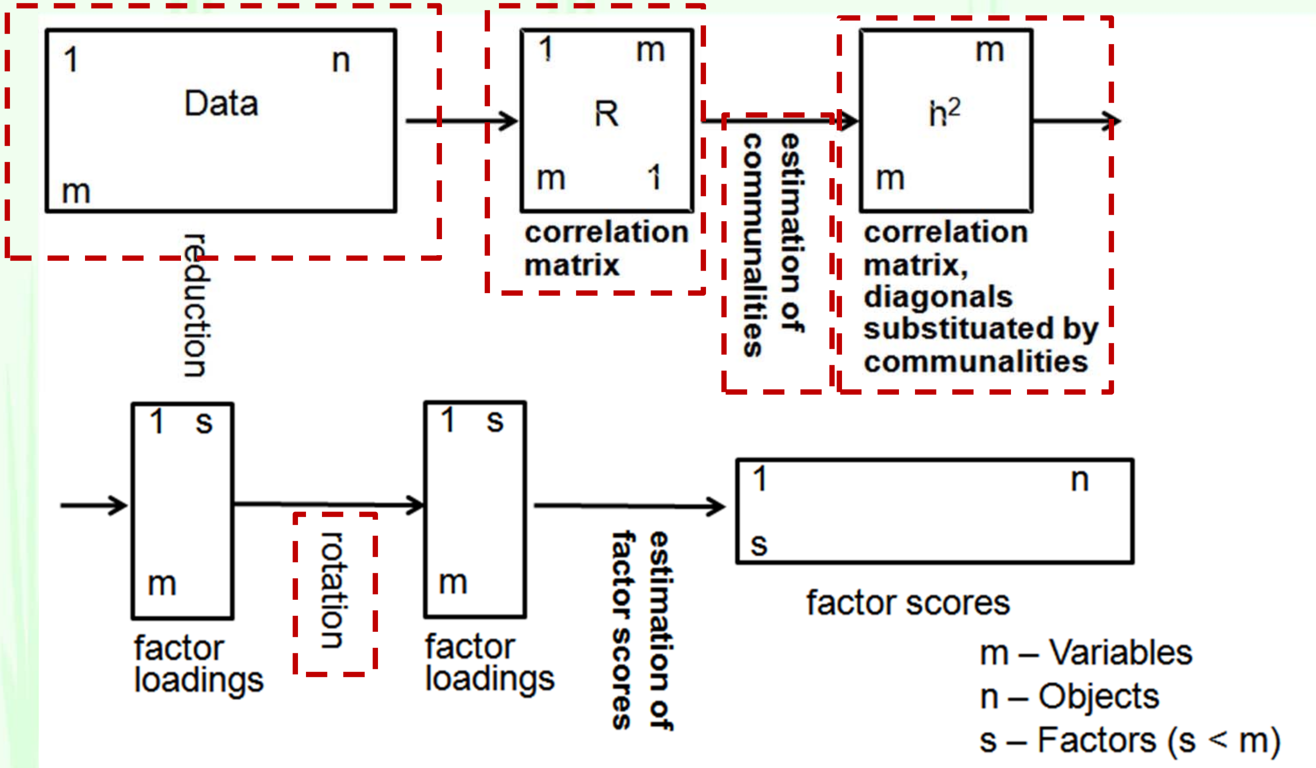
variables (features)

objects

Sample	1,8-cineole	cis-sabinene hydrate	neral	geranial	Cr	Ni	Mo	Cu	Zn	Mn	Fe	Mg	Ca	K	Na
A21	2.21E-03	1.50E-01	6.81E-07	6.81E-07	0.511	1.459	0.662	10.490	42.454	52.160	195.849	2589	11521	14683	17.78
A22	1.13E+01	1.20E-01	6.81E-07	6.81E-07	0.474	2.225	0.357	13.363	52.673	58.585	332.159	2337	10060	12816	8.40
A23	2.97E-03	1.87E-01	2.32E-01	4.94E-01	0.752	3.048	0.407	7.194	33.855	41.808	460.689	2424	9493	16082	17.44
B21	9.10E+00	1.92E+00	1.91E+01	1.99E+01	0.702	4.453	0.505	11.560	63.399	133.099	578.803	3230	12167	15110	55.10
B22	2.92E+00	1.11E+00	1.78E+01	1.83E+01	0.727	1.350	1.247	10.396	77.664	20.542	240.066	1820	11816	18883	10.42
B23	4.07E+00	2.65E+00	1.59E+01	1.60E+01	0.828	1.390	0.151	7.045	106.663	140.935	206.546	2466	8572	17924	6.20
C21	9.30E+00	5.98E+00	1.65E+01	1.69E+01	0.753	3.793	0.055	12.301	65.808	185.030	368.038	2639	10855	23067	114.01
C22	6.01E+00	3.42E+00	2.21E+01	2.34E+01	0.726	2.072	0.274	9.219	68.571	163.648	191.877	2427	10557	21113	112.66
C23	3.36E+00	5.51E+00	1.91E+01	1.97E+01	0.410	1.438	0.161	8.041	62.092	88.693	89.501	2104	7886	13191	2.53
D21	1.25E+01	2.05E-01	1.90E+01	1.99E+01	0.558	4.474	0.556	14.590	55.474	45.503	233.810	2773	11221	13457	9.34
D22	1.12E+01	8.50E-01	1.87E+01	1.87E+01	0.853	2.795	0.845	10.005	91.179	41.187	352.195	3803	10588	8006	10.97
D23	8.73E+00	3.88E+00	1.15E+01	1.21E+01	0.703	2.795	0.171	9.608	82.313	219.092	749.914	2685	10307	13323	14.72
E21	4.25E+00	9.01E-01	6.81E-07	6.81E-07	0.881	3.556	0.446	15.670	49.540	43.306	387.087	1827	10475	11192	24.28
E22	7.56E+00	1.02E+00	6.81E-07	6.81E-07	0.960	4.010	1.115	11.610	43.823	62.837	406.713	2553	11989	11129	14.67
E23	4.69E+00	1.61E-01	6.81E-07	6.81E-07	0.705	3.067	1.163	12.817	48.792	45.238	282.115	2668	11318	13133	14.98

≥5%

Principal Component Analysis and Factor Analysis



Chemometrics approach

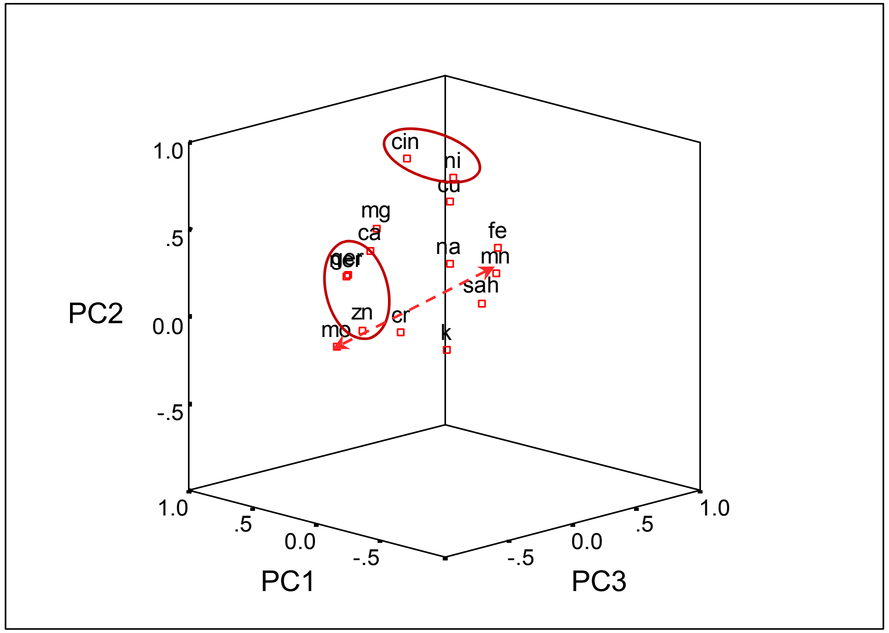
Rotated Component Matrix

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
Rotation converged in 9 iterations

	Component				
	1	2	3	4	5
cin	.435	**.795**	.135	-.154	.051
sah	.365	-.116	.657	.465	-.023
ner	**.905**	.032	.138	.286	-.152
ger	**.896**	.040	.143	.299	-.157
Cr	.043	-.044	-.296	.123	**.810**
Ni	-.124	**.824**	-.049	.063	.351
Mo	-.076	.012	**-.919**	-.195	.112
Cu	-.313	.772	-.272	.022	-.173
Zn	**.821**	-.267	.178	-.058	.203
Mn	.304	.057	**.707**	.435	.334
Fe	-.139	.363	.283	-.109	**.754**
Mg	.497	.420	-.034	-.303	.374
Ca	-.080	.519	-.663	.258	.273
K	.126	-.243	.150	**.881**	-.081
Na	.142	.240	.183	**.864**	.118

cin - 1,8-cineole; sah - cis-sabinene hydrate; ner – neral; ger - geranial

Component Plot in Rotated Space

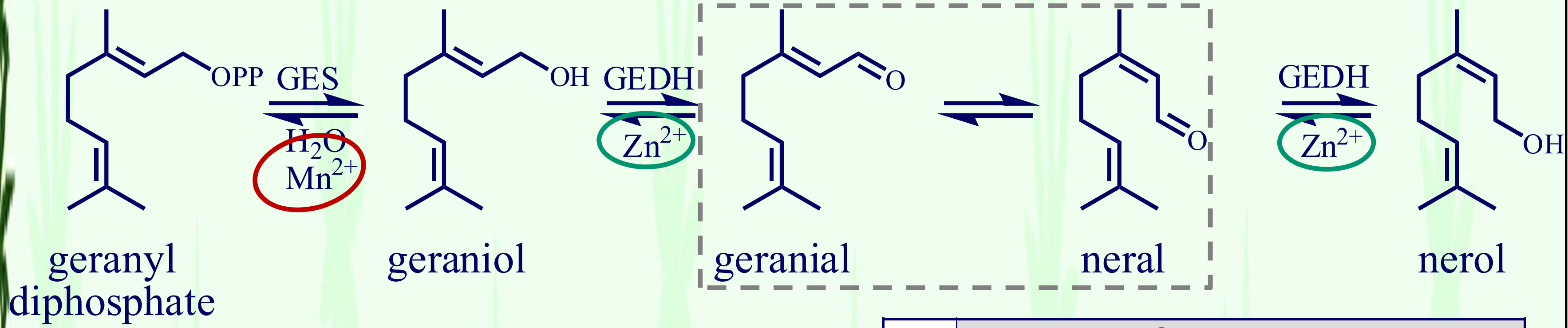


1: neral, geranial, Zn
2: 1,8-cineole, Ni
3: Mn / Mo
4: K, Na
5: Cr, Fe

46

Chemometrics approach

The proposed role of metals in the biosynthetic route of citral in *Th. pannonicus*



GES – Geraniol Synthase

GEDH – Geraniol Dehydrogenase

1: neral, geranial, Zn

3: Mn / Mo

	Component				
	1	2	3	4	5
cin	.435	**.795**	.135	-.154	.051
sah	.365	-.116	.657	.465	-.023
ner	**.905**	.032	.138	.286	-.152
ger	**.896**	.040	.143	.299	-.157
Cr	.043	-.044	-.296	.123	**.810**
Ni	*-.124*	***.824***	*-.049*	*.063*	*.351*
Mo	-.076	.012	**-.919**	-.195	.112
Cu	-.313	.772	-.272	.022	-.173
Zn	**.821**	-.267	.178	-.058	.203
Mn	.304	.057	**.707**	.435	.334
Fe	-.139	.363	.283	-.109	**.754**
Mg	.497	.420	-.034	-.303	.374
Ca	-.080	.519	-.663	.258	.273
K	.126	-.243	.150	**.881**	-.081
Na	.142	.240	.183	**.864**	.118

- monoterpene hydrocarbons
- oxigenated monoterpenes
- sesquiterpene hydrocarbons

variables (features)

objects

Sample	α-pinene	*p*-cymene	1,8-cineole	cis-sabinene hydrate	*β*-bourbonene	Cr	Ni	Mo	Cu	Zn	Mn	Fe	Mg	Ca	K	Na
A21	4,850	7,768	0,002	0,150	1,923	0,511	1,459	0,662	10,490	42,454	52,160	195,849	2589	11521	14683	17,78
A22	10,296	0,685	11,298	0,120	0,836	0,474	2,225	0,357	13,363	52,673	58,585	332,159	2337	10060	12816	8,40
A23	7,963	11,852	0,003	0,187	2,140	0,752	3,048	0,407	7,194	33,855	41,808	460,689	2424	9493	16082	17,44
B21	1,553	0,731	9,103	1,919	1,396	0,702	4,453	0,505	11,560	63,399	133,099	578,803	3230	12167	15110	55,10
B22	1,581	0,992	2,915	1,112	1,223	0,727	1,350	1,247	10,396	77,664	20,542	240,066	1820	11816	18883	10,42
B23	2,175	0,393	4,067	2,647	2,097	0,828	1,390	0,151	7,045	106,663	140,935	206,546	2466	8572	17924	6,20
C21	2,101	2,747	9,297	5,981	1,698	0,753	3,793	0,055	12,301	65,808	185,030	368,038	2639	10855	23067	114,01
C22	0,726	2,389	6,009	3,418	0,812	0,726	2,072	0,274	9,219	68,571	163,648	191,877	2427	10557	21113	112,66
C23	2,485	3,126	3,357	5,509	0,990	0,410	1,438	0,161	8,041	62,092	88,693	89,501	2104	7886	13191	2,53
D21	1,307	0,719	12,525	0,205	3,590	0,558	4,474	0,556	14,590	55,474	45,503	233,810	2773	11221	13457	9,34
D22	1,242	0,789	11,208	0,850	1,849	0,853	2,795	0,845	10,005	91,179	41,187	352,195	3803	10588	8006	10,97
D23	3,606	4,279	8,733	3,882	2,138	0,703	2,795	0,171	9,608	82,313	219,092	749,914	2685	10307	13323	14,72
E21	11,906	1,670	4,252	0,901	0,613	0,881	3,556	0,446	15,670	49,540	43,306	387,087	1827	10475	11192	24,28
E22	4,506	3,261	7,561	1,021	0,877	0,960	4,010	1,115	11,610	43,823	62,837	406,713	2553	11989	11129	14,67
E23	35,584	0,381	4,687	0,161	0,399	0,705	3,067	1,163	12,817	48,792	45,238	282,115	2668	11318	13133	14,98

present in all samples

Chemometrics approach

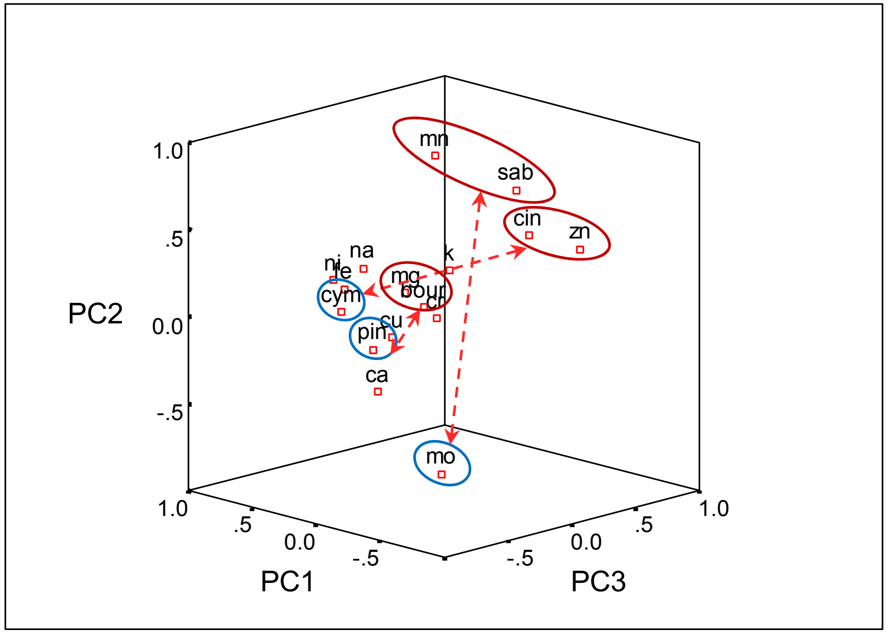
Rotated Component Matrix

Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization. a Rotation converged in 14 iterations

	Component					
	1	2	3	4	5	6
pin	.233	-.172	-.318	**-.695**	-.443	-.033
cym	-.049	.197	**-.852**	.053	.128	.058
cin	.233	.252	**.897**	-.082	-.040	.065
sah	-.244	**.711**	.322	.085	.286	.296
Bour	-.061	.108	-.214	**.867**	.001	-.084
Cr	.162	-.059	.104	-.027	.082	**.928**
Ni	**.894**	.037	.022	.058	-.101	.210
Mo	.069	**-.926**	.052	-.072	-.151	.175
Cu	**.729**	-.320	.323	-.305	.081	-.223
Zn	-.369	.324	**.704**	.363	.054	.216
Mn	.145	**.883**	.079	.175	.181	.059
Fe	.647	.063	-.136	.104	-.126	.573
Mg	.426	.031	.126	**.696**	-.261	.135
Ca	.607	-.564	.085	.085	.405	.224
K	-.192	.330	-.146	-.035	**.808**	-.090
Na	.530	.198	-.103	.009	.689	.305

pin - α-pinene; cym – p-cymene; sah- sabinene hydrate; bour – β-bourbonene

Component Plot in Rotated Space

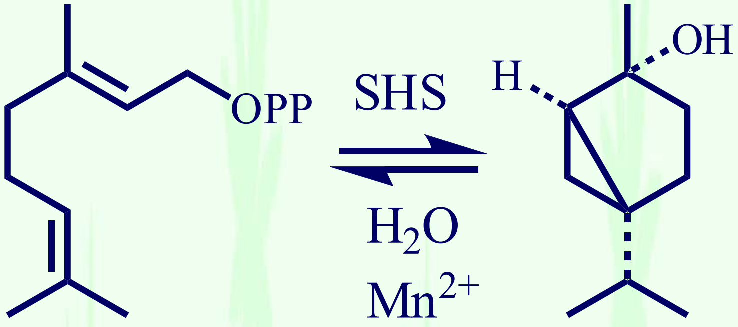


2: *cis*-sabinene hydrate, Mn / Mo

3: 1,8-cineole, Zn / p-cymene

4: β-bourbonene, Mg / α-pinene

2: *cis*-sabinene hydrate and Mn / Mo



geranyl diphosphate

cis-sabinene hydrate

SHS - Sabinene Hydrate Synthase catalyzes Mn^{2+} dependent cyclization of geranyl diphosphate to the bicyclic monoterpene alcohols - sabinene hydrate.

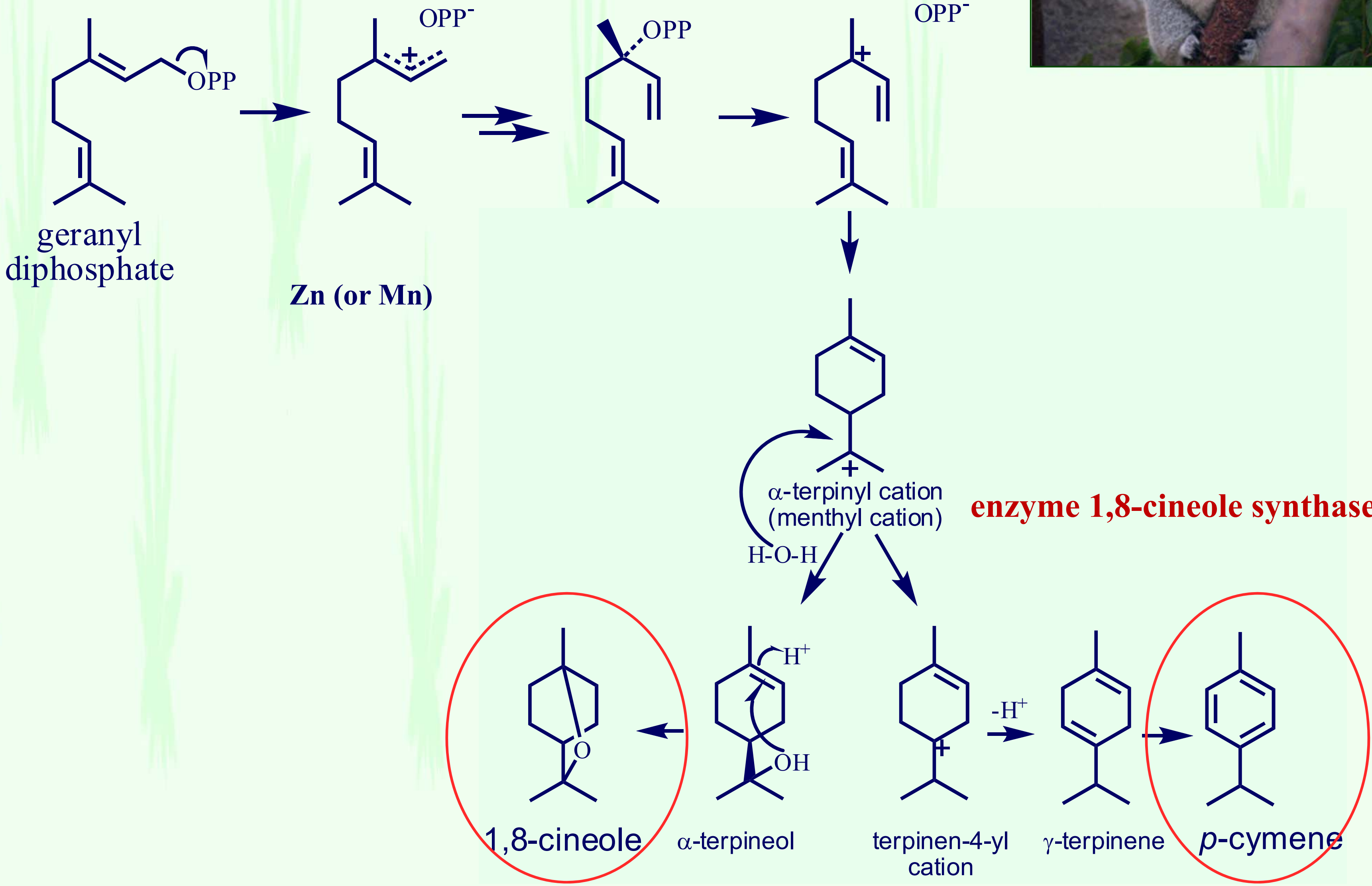
- hydroxyl group is derived from water.

Enzymatic synthesis of *cis*-sabinene hydrate in *Thymus pannonicus* could be Mn^{2+}-dependent process.

Chemometrics approach

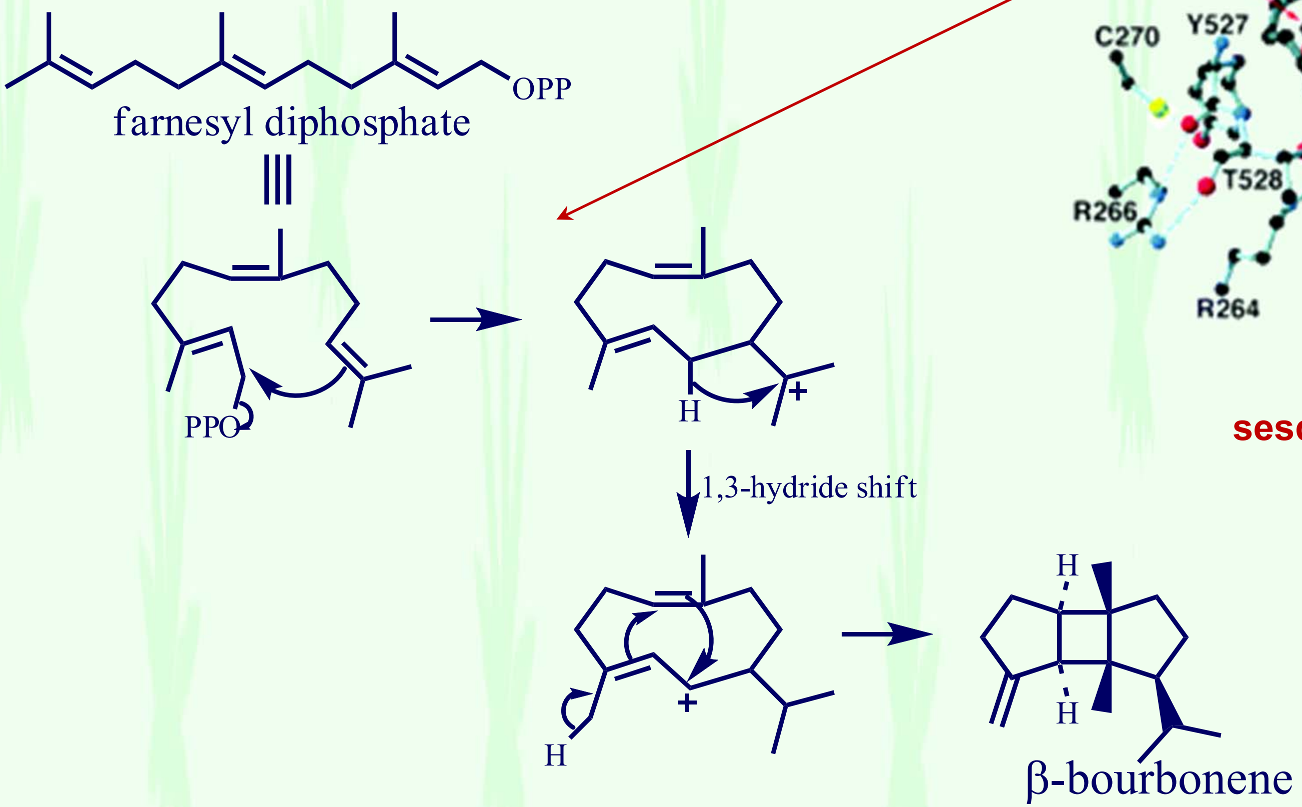
Cineole's got an unusual structure, how is it made?

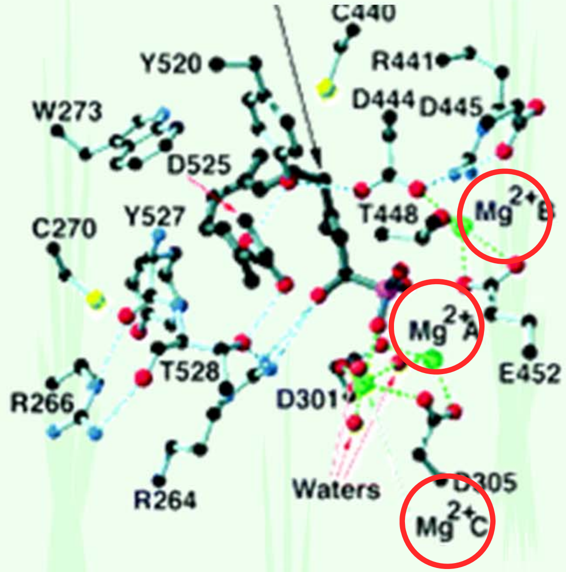
3: 1,8-cineole, Zn / *p*-cymene



Chemometrics approach

4: *β*-bourbonene, Mg / *α*–pinene





sesquiterpene cyclase from tobacco

Dalton D.R., Foundations of Organic Chemistry: Unity and Diversity of Structures, Pathways, and Reactions, Wiley, 2011.

SHS-GC-MS / SHS-GC-FID

- BVOCs and **environmental chemistry**

- HS analysis – **analytical challenge**

- knowledge of the composition of headspace of great interest from a **chemotaxonomic** and **biogenetic** point of view.

- significant correlations BVOCs – metals and **the role of metals in the biosynthesis of volatiles**.

Plant Physiology and Biochemistry 71 (2013) 298–306

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

A chemometrics as a powerful tool in the elucidation of the role of metals in the biosynthesis of volatile organic compounds in Hungarian thyme samples

Jelena Arsenijević[a,*], Jelena Marković[b], Ivan Šoštarić[c], Slavica Ražić[d]

[a] University of Belgrade – Faculty of Pharmacy, Department of Pharmacognosy, Vojvode Stepe 450, 11221 Belgrade, Serbia
[b] University of Belgrade – Vinča Institute of Nuclear Sciences, P.O. Box 522, 11001 Belgrade, Serbia
[c] University of Belgrade – Faculty of Agriculture, Nemanjina 6, 11080 Belgrade-Zemun, Serbia
[d] University of Belgrade – Faculty of Pharmacy, Department of Analytical Chemistry, Vojvode Stepe 450, 11221 Belgrade, Serbia

University of Belgrade, Faculty of Pharmacy – Dept. for Pharmacognosy

Short story of of cannabis

“Sampling”

Cannabis sativa

Cannabis ruderalis

Cannabis indica

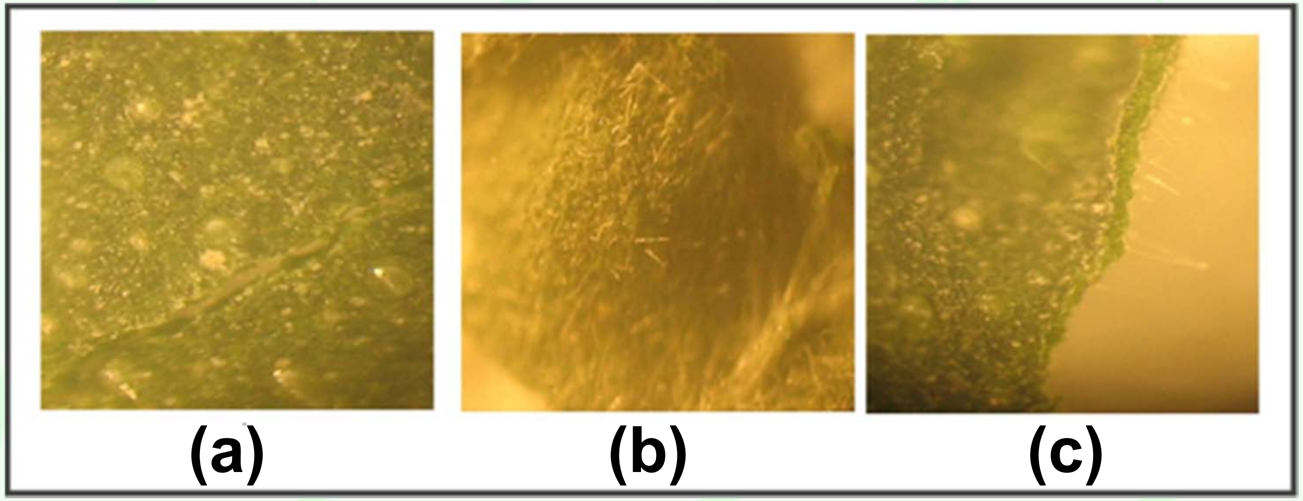
Case study 4

Young hemp plants were seized by the Police authorities as plant material, grown illegally under controlled indoor conditions.

Qualitative analysis of plant material

The positive forensic identification of herbal material as C. sativa by **Recommended Methods for Identification and Analyses of Cannabis and its products. United Nations Office on Drugs and Crime. New York instructions (2009).**

Macroscopically and microscopically

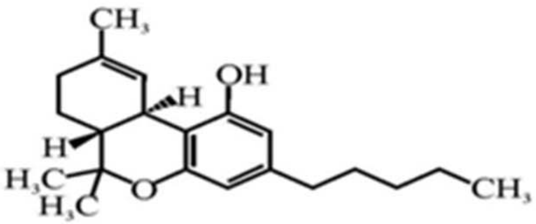


Microscopic images for a) upper leaf surface b) lower leaf surface and c) leaf cross-section of *Cannabis sativa* L.

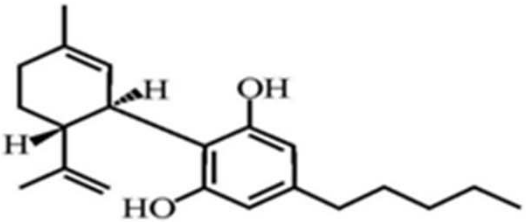
Approximately 70 cannabinoids are known to exist, classified into 11 cannabinoid types.

Cannabinoids are terpenophenolic compounds unique to cannabis.

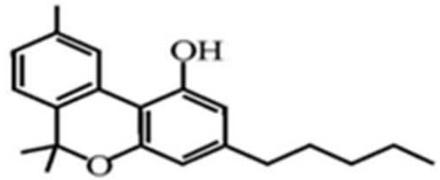
The most important in the sense of forensic examination



(a)



(b)



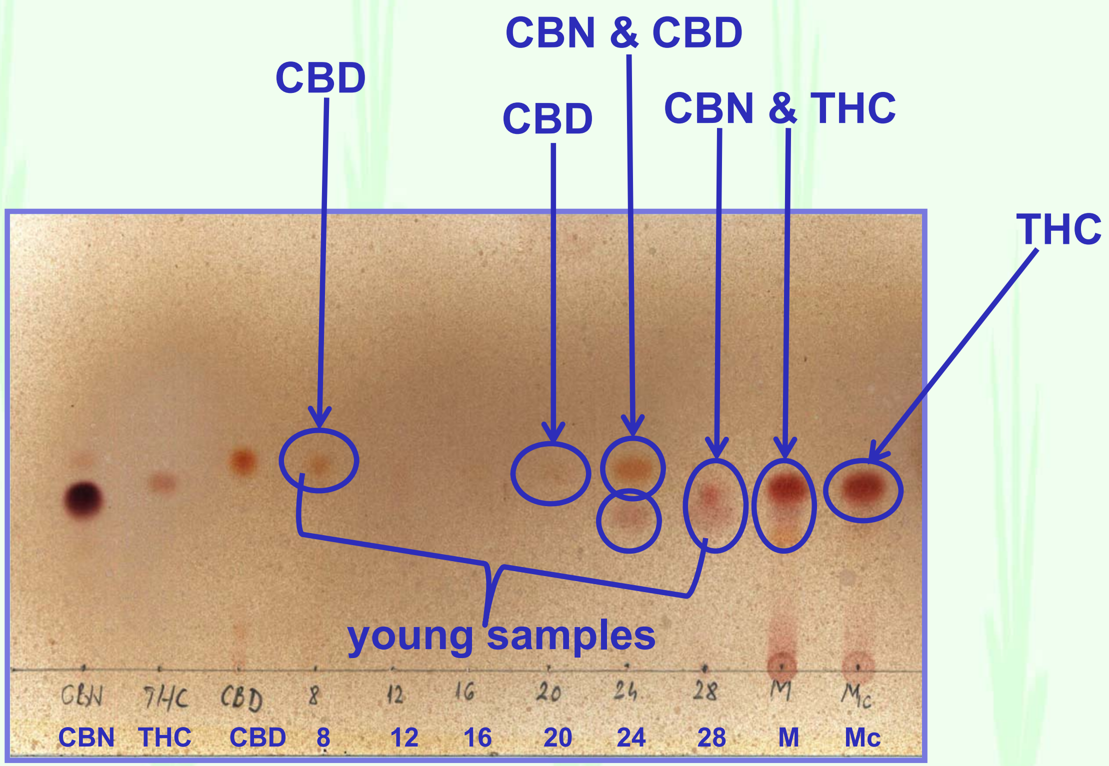
(c)

Δ⁹-*tetrahydrocannabinol* *(*Δ⁹-THC*)*

cannabidiol *(CBD)*

cannabinol *(CBN)*

Semiquantitative analysis of cannabinoids in hemp's samples by TLC



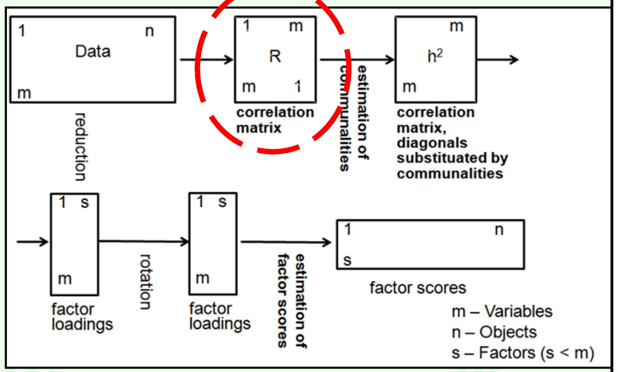
Cannabinoids standards (CBN, THC and CBD);
young cannabis samples (8, 12, 16, 20, 24 and 28);
mature plants analyzed after 3 years (M) and immidiatelly analyzed (Mc)

Chemometrics approach

1. **Quantitative analysis of cannabinoids in Cannabis leaves (*mg/mL*) by GC-FID**

2. **Quantitative analysis of metals in hemp's samples by F-AAS and ET-AAS**

Chemometrics approach



The correlation matrix of the elements and cannabinoids data (Pearson correlation)

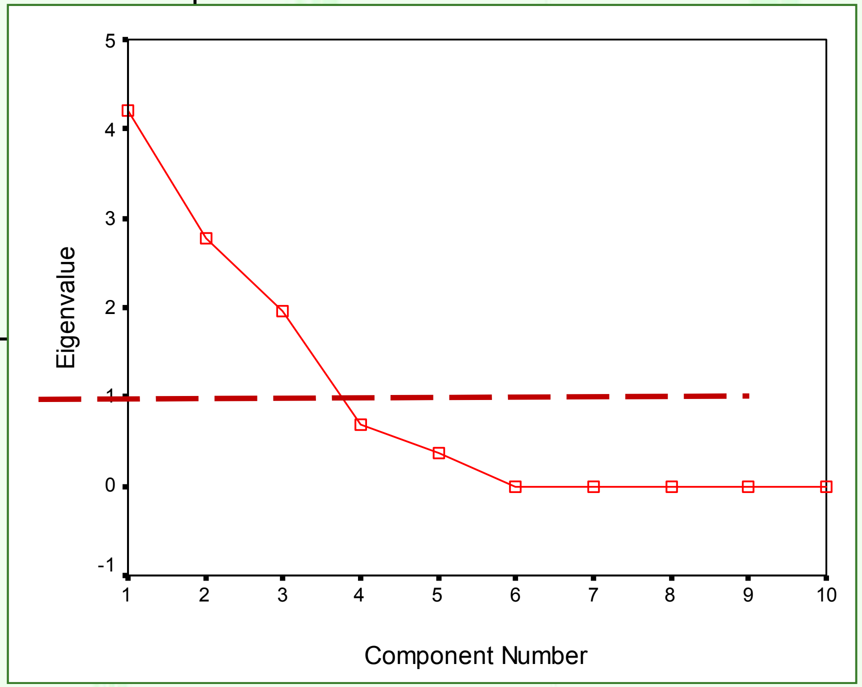
	Cu	Fe	Mn	Zn	Cr	Ca	Mg	THC	CBD
Fe	-0.344								
	0.505								
Mn	0.484	-0.134							
	0.331	0.800							
Zn	0.201	-0.675	0.496						
	0.702	0.142	0.317						
Cr	-0.097	0.628	-0.097	-0.775					
	0.855	0.182	0.855	0.070					
Ca	0.452	0.464	0.443	-0.224	0.649				
	0.368	0.354	0.379	0.670	0.163				
Mg	-0.724	0.272	-0.176	-0.148	-0.190	-0.622			
	0.104	0.602	0.739	0.780	0.719	0.188			
THC	0.061	-0.379	0.791	0.618	-0.373	-0.145	0.285		
	0.909	0.459	0.061	0.191	0.467	0.784	0.584		
CBD	-0.113	-0.411	-0.331	0.596	-0.659	-0.378	-0.176	-0.209	
	0.831	0.418	0.522	0.212	0.155	0.460	0.379	0.691	
CBN	0.332	-0.602	0.813	0.802	-0.545	-0.111	0.000	0.929	0.011
	0.520	0.206	0.049	0.055	0.263	0.835	1.000	0.007	0.984

Principal Component Analysis (PCA)

Eigen analysis

Comp.	Total	% of Variance	Cumulative %
1	4.209	42.086	42.086
2	2.779	27.786	69.872
3	1.955	19.553	**89.425**
4	.686	6.861	96.286
5	.371	3.714	
6	4.647E-16	4.647E-15	
7	2.900E-16	2.900E-15	
8	2.918E-17	2.918E-16	
9	-9.370E-17	-9.370E-16	
10	-6.144E-16	-6.144E-15	

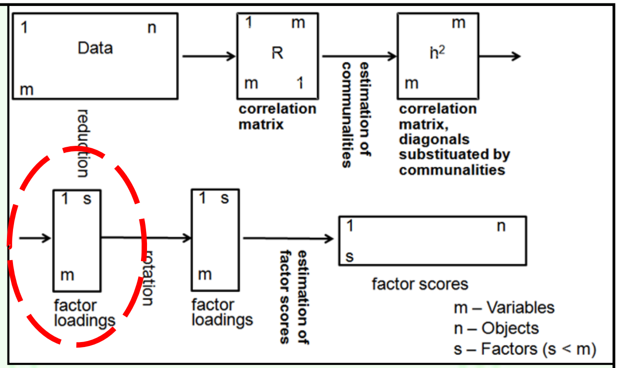
Eigen values ≥ 1.0



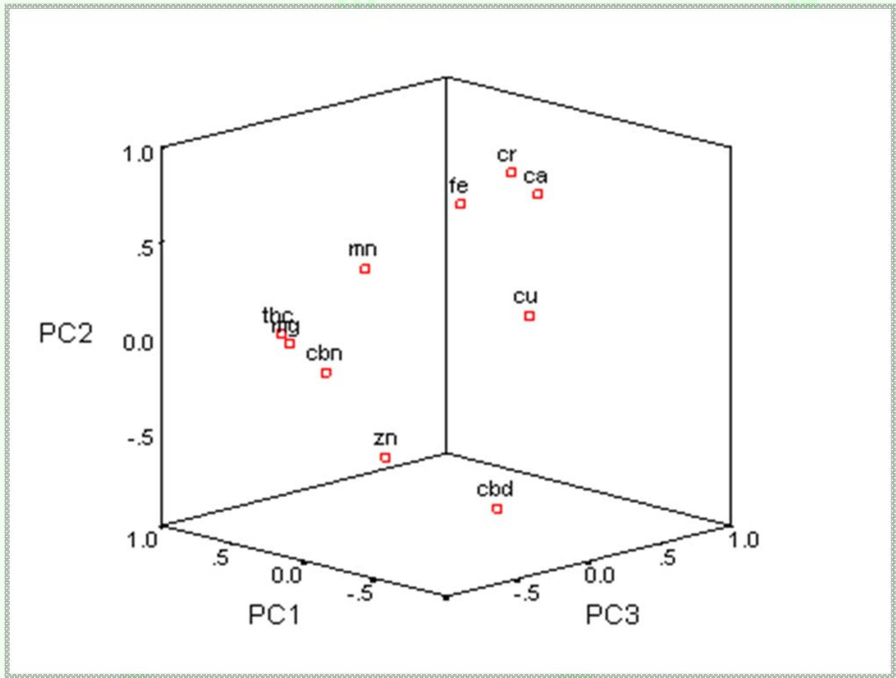
Scree plot

Chemometrics approach

Rotated component matrix
Extraction Method: PCA.
Rotation Method: Varimax with Kaiser Normalization.
Rotation converged in 5 iterations.

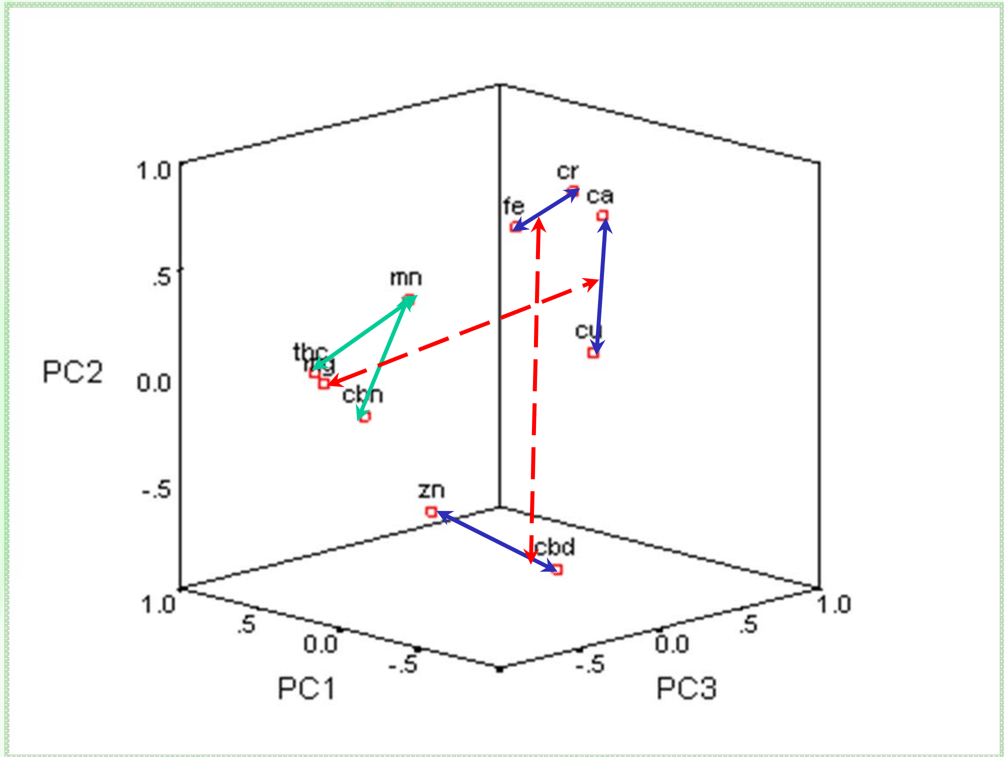


	PC1	PC2	PC3
Cu	0.258	-0.091	**0.842**
Fe	-0289	**0.790**	-0.177
Mn	**0.912**	0.123	0.345
Zn	0.548	**-0.765**	0.129
Cr	-0.266	**0.878**	0.194
Ca	0.071	0.601	**0.721**
Mg	0.142	0.115	**-0.965**
THC	**0.966**	-0.134	-0.191
CBD	-0.334	**-0.851**	0.021
CBN	**0.920**	-0.380	0.081



Component plot in rotated space

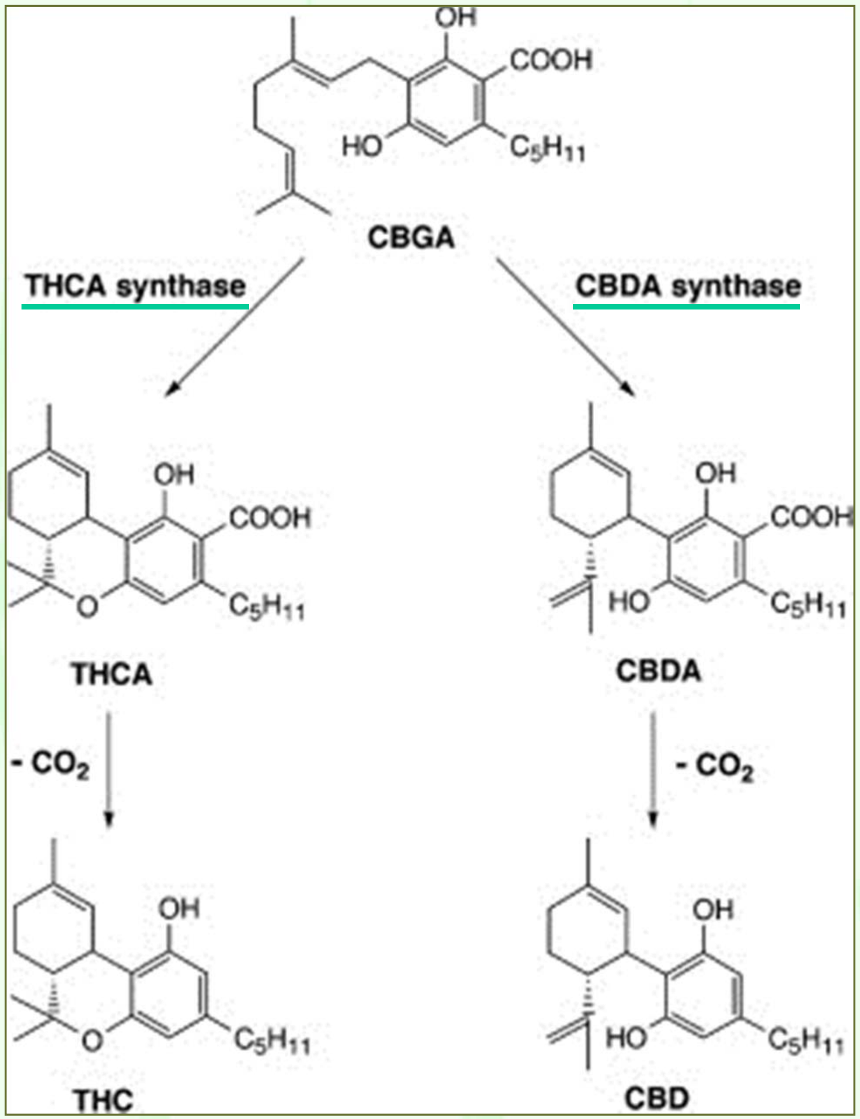
Chemometrics approach



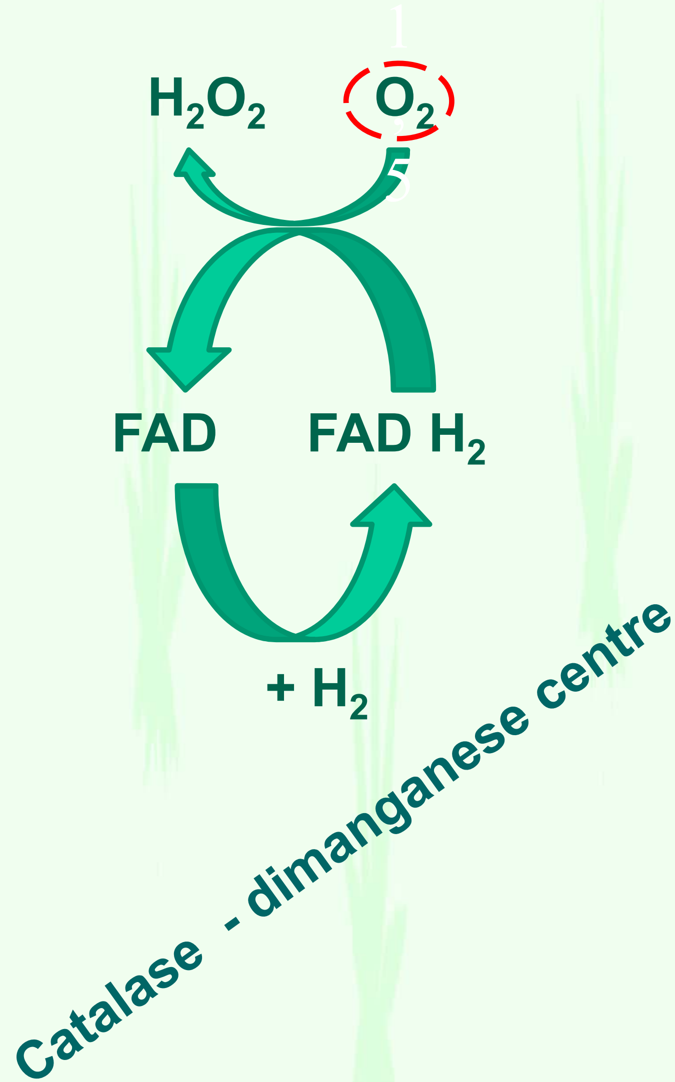
Component plot in rotated space

PC1: CBN, THC and Mn
PC2: Fe and Cr, Zn and CBD
PC3: Cu, Ca and Mg

FAD - Flavin adeninine dinucleotide



Biogenesis of THCA and CBDA and decarboxylation to THC and CBD



Catalase (haem Fe groups + di-Mn centre)

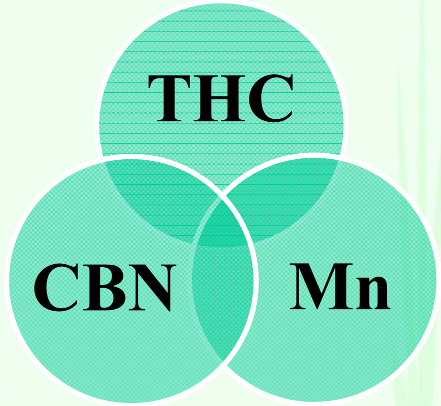
PC1

$$2H_2O_2 \rightleftharpoons H_2O + O_2$$

$$2Mn^{3+} + H_2O_2 \rightleftharpoons 2Mn^{2+} + O_2 + 2H^+$$

$$2Mn^{2+} + H_2O_2 + 2H^+ \rightleftharpoons 2H_2O + 2Mn^{3+}$$

$$\Delta G < 0$$

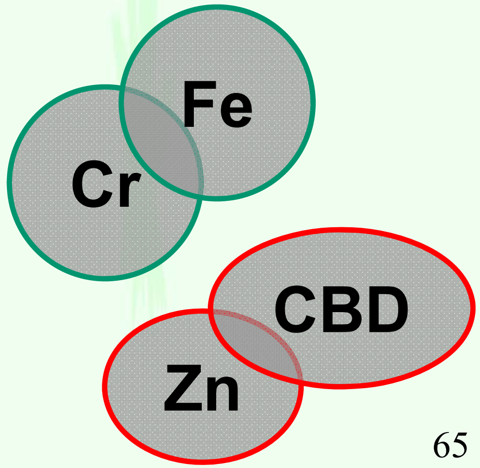


$$H_2O_2 + \textbf{Fe (III)-Enzyme} \rightleftharpoons H_2O + \textbf{O=Fe(IV)-Enzyme(+)}$$

$$H_2O_2 + \textbf{O=Fe(IV)-Enzyme(+)} \rightleftharpoons H_2O + \textbf{Fe (III)-Enzyme} + O_2$$

PC2

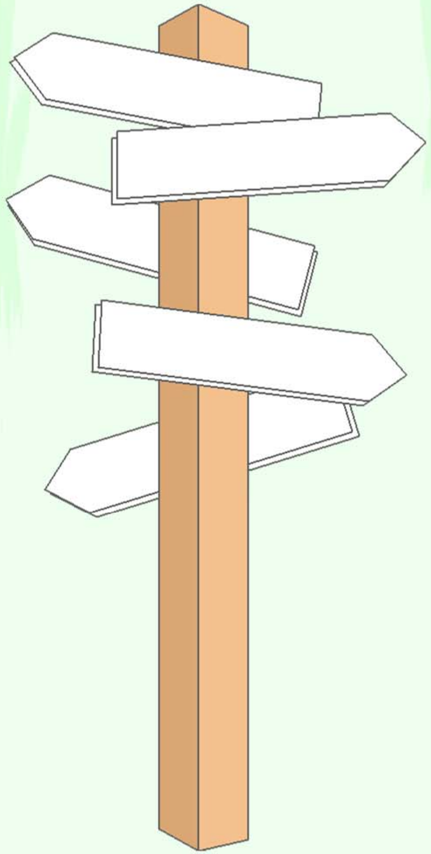
Transition state is energetically unstable



PC3: Cu, Ca and Mg

To be considered...

Bearing in mind all highlighted issues, content of metals in rhizosphere soil could be an indication of the cannabinoids content. The c**orrelations of metals and biogenic organic compounds contain a hidden information responsible for cannabinoids production***.*



Journal
Natural Product Research >
Formerly Natural Product Letters
Volume 28, 2014 - Issue 8

Articles

Metals and organic compounds in the biosynthesis of cannabinoids: a chemometric approach to the analysis of *Cannabis sativa* samples

Natasa Radosavljevic-Stevanovic, Jelena Markovic, Snezana Agatonovic-Kustrin & Slavica Razic

Pages 511-516 | Received 22 Nov 2013, Accepted 27 Dec 2013, Published online: 31 Jan 2014

Head of the forensic lab at
Ministry of Internal Affairs,
Republic of Serbia, Belgrade

A bit more... published this year:

University of Novi Sad,
Faculty of Science –
Dept. for Environmental
Protection

International Journal of Environmental Science and Technology
pp 1–10 | Cite as

Fate of bromine-containing disinfection by-products precursors during ozone and ultraviolet-based advanced oxidation processes

Authors Authors and affiliations

M. Petronijević, J. Agbaba, S. Ražić, J. Molnar Jazić, A. Tubić, M. Watson, B. Dalmacija

Original Paper
First Online: 08 February 2018

Review Article

Analytical Approaches to the Characterization of Solid Drug Delivery Systems with Porous Adsorbent Carriers

Author(s): Marko Krstić, Slavica Ražić*.

Journal Name: Current Medicinal Chemistry

Volume 33, Issue 33, 2018, 3956 – 3972

Univ. of Belgrade,
Faculty of Pharmacy –
Dept. for Analytical
Chemistry

Univ. of Belgrade,
Faculty of Pharmacy

LIGNOCELLULOSIC BIOMASS AS A SOURCE OF MICROCRYSTALLINE CELLULOSE – CHEMICAL AND TECHNOLOGICAL CHARACTERIZATION AND FUTURE PERSPECTIVES

MARKO KRSTIC,[*] ZORAN MAKSIMOVIC,[**] SVETLANA IBRIC,[*] TAMARA BAKIC,[***] JOVANA PRODANOVIC[**] and SLAVICA RAZIC[***]

[*]Department of Pharmaceutical Technology and Cosmetology, University of Belgrade, Faculty of Pharmacy, Vojvode Stepe 450, P.O. Box 146, 11221 Belgrade, Serbia
[**]Department of Pharmacognosy, University of Belgrade, Faculty of Pharmacy, Vojvode Stepe 450, P.O. Box 146, 11221 Belgrade, Serbia
[***]Department of Analytical Chemistry, University of Belgrade, Faculty of Pharmacy, Vojvode Stepe 450, P.O. Box 146, 11221 Belgrade, Serbia
✉ Corresponding author: Marko Krstic, mkrstic109@gmail.com

In the submission mission:

1. **Chemical profiles of essential oils and hydromethanol extracts of cultivated and wild growing *Thymus pannonicus* All.**

Jelena Arsenijević, Slavica Ražić *et al.*
University of Belgrade, Faculty of Pharmacy – Dept of Pharmacognosy

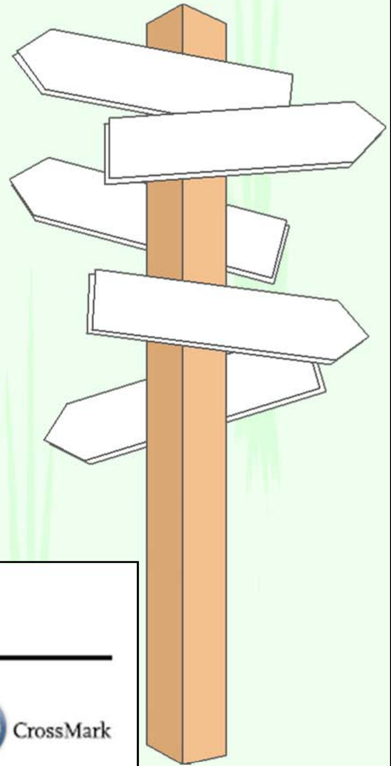
2. **Poly (ϵ–caprolactone) microspheres for prolonged release of selenium nanoparticles**

Nenad Filipović, Magdalena Stevanović, Slavica Ražić *et al.*
Institute of Technical Sciences of the Serbian Academy of Sciences and Arts, Belgrade

3. **Lead isotope ratios and chemometrics as tools for elucidation of chemical interactions of *Macrolepiota procera* with soil components**

Slađana Đurđić, Slavica Ražić, Jelena Mutić *et al.*
University of Belgrade, Faculty of Chemistry – Dept. of Analytical Chemistry

You're never too cool to learn something new.



Analytical and Bioanalytical Chemistry (2018) 410:6973–6974
https://doi.org/10.1007/s00216-018-1357-3

EDITORIAL

CrossMark

Be unafraid … to try something new or challenging

Adam T. Woolley[1]

Published online: 14 September 2018