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CHARACTERIZATION OF EXTRACTIVES FROM SOME RAW MATERIALS PROCESSED BY BIOREFINING

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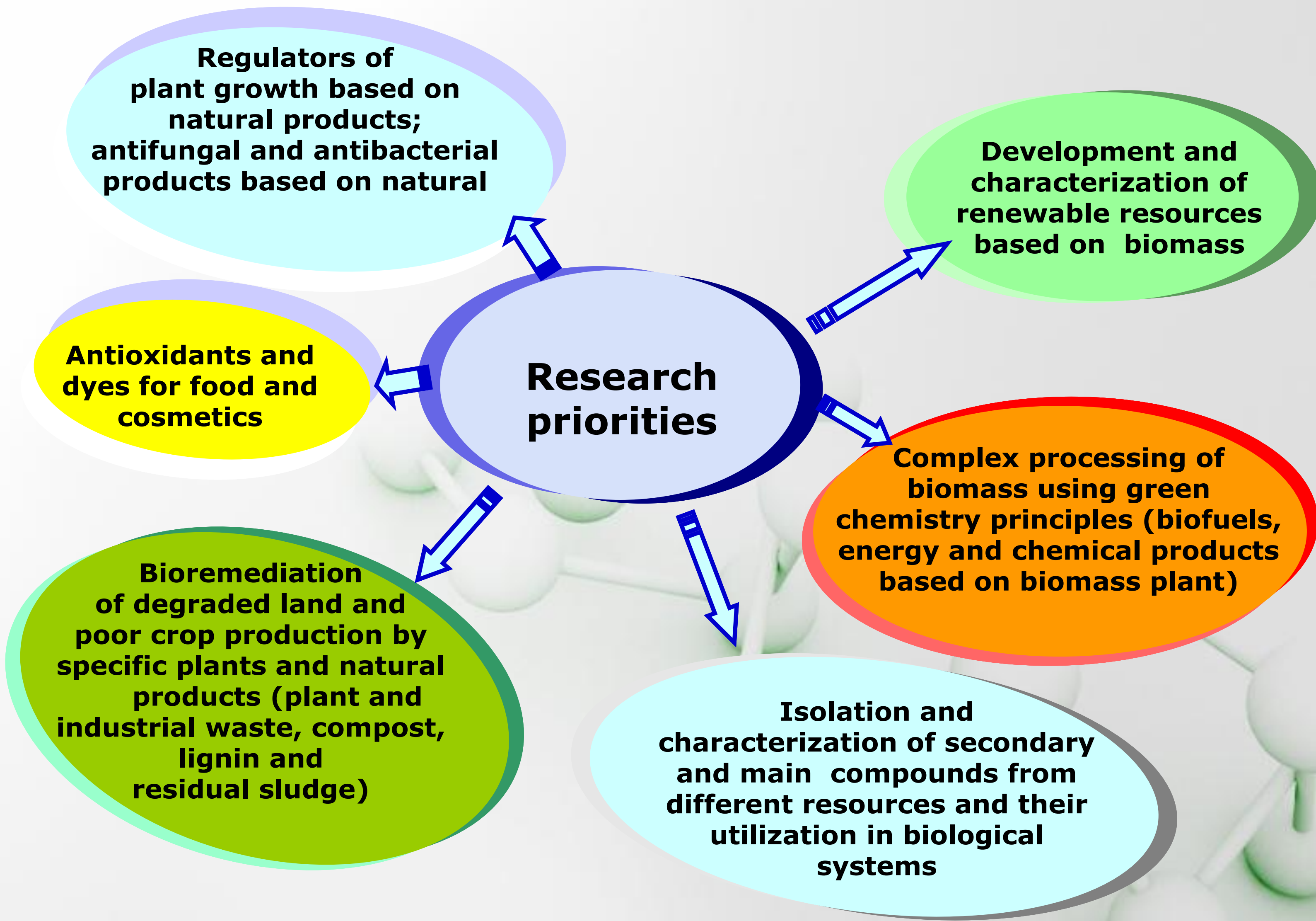
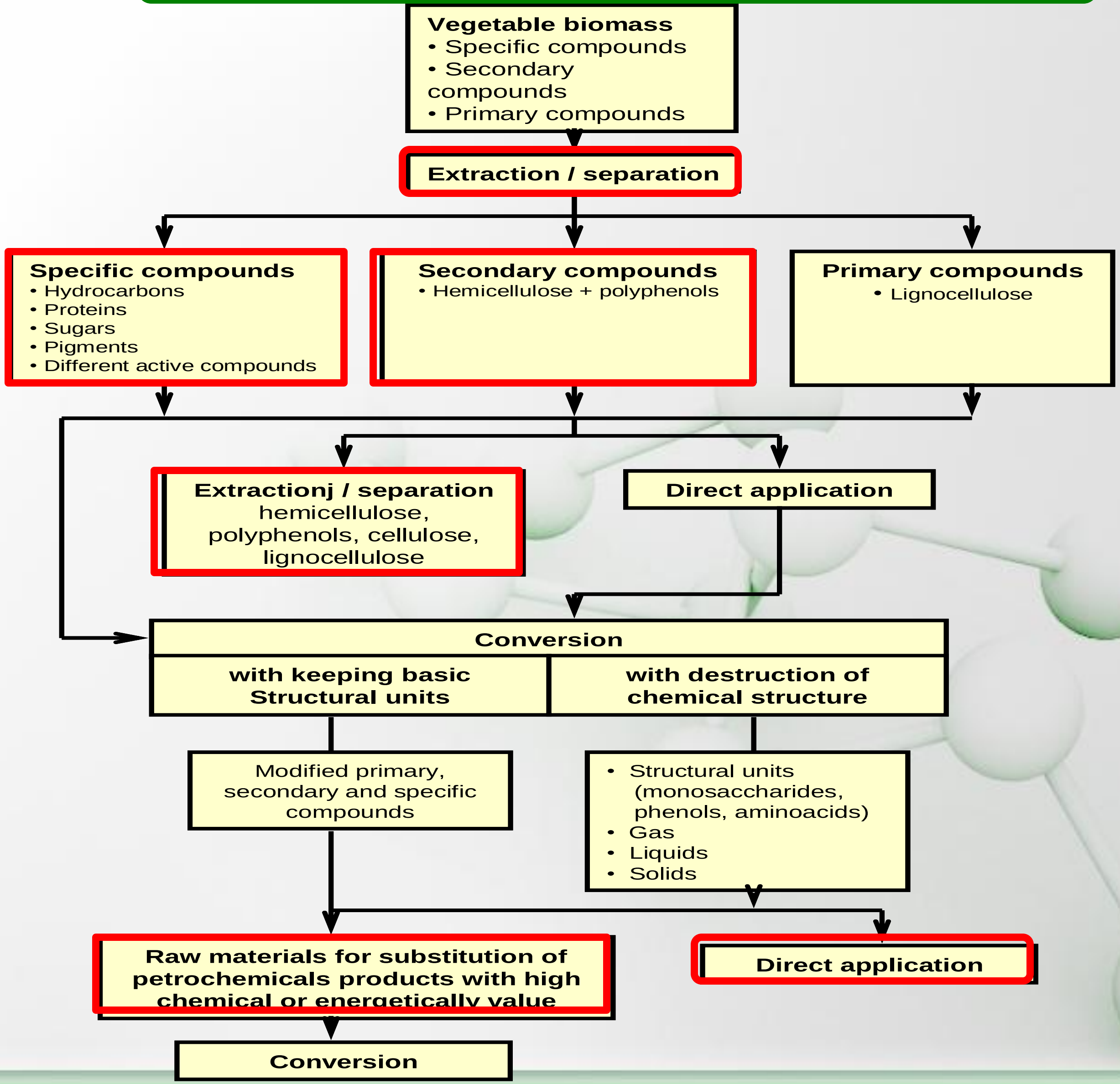

Research priorities
Regulators of plant growth based on natural products; antifungal and antibacterial products based on natural
Development and characterization of renewable resources based on biomass
Antioxidants and dyes for food and cosmetics
Complex processing of biomass using green chemistry principles (biofuels, energy and chemical products based on biomass plant)
Bioremediation of degraded land and poor crop production by specific plants and natural products (plant and industrial waste, compost, lignin and residual sludge)
Isolation and characterization of secondary and main compounds from different resources and their utilization in biological systems

Flow sheet of integral and complex processing of phytomass



Objectives

- Lipophilic extraction of fatty acids

- Extraction of phenolic compounds from different raw materials using ethanol 80%, methanol 80%, water and NaOH as extraction agents.

- Identification and characterization of phenolic compounds in the obtained extracts:

⇨ Spectrophotometric methods (UV-VIS, FTIR)

⇨ HPLC determination of individual compounds

⇨ GC-MS analysis

RAW MATERIALS

***Crataegus Monogyna* (hawthorn)**

✓Traditional medicinal plant with many health benefits.

✓ The current use of hawthorn is essentially as a heart medicine.

✓ The main constituents responsible for most of the pharmacological activity of hawthorn are polyphenols: catechin, epicatechin, oligomeric proanthocyanidins and flavonoids.

Asclepias syriaca

✓ Very complex composition: cellulose and lignin, hemicelluloses, and polyphenols, sugars, alkaloids and hydrocarbons.

✓Curent use of this plant is for production of cellulose fibers, floss, pulp and paper

✓Hydrocarbons and unsaturated fatty acids which could be used as an alternative triglyceride source.

RAW MATERIALS

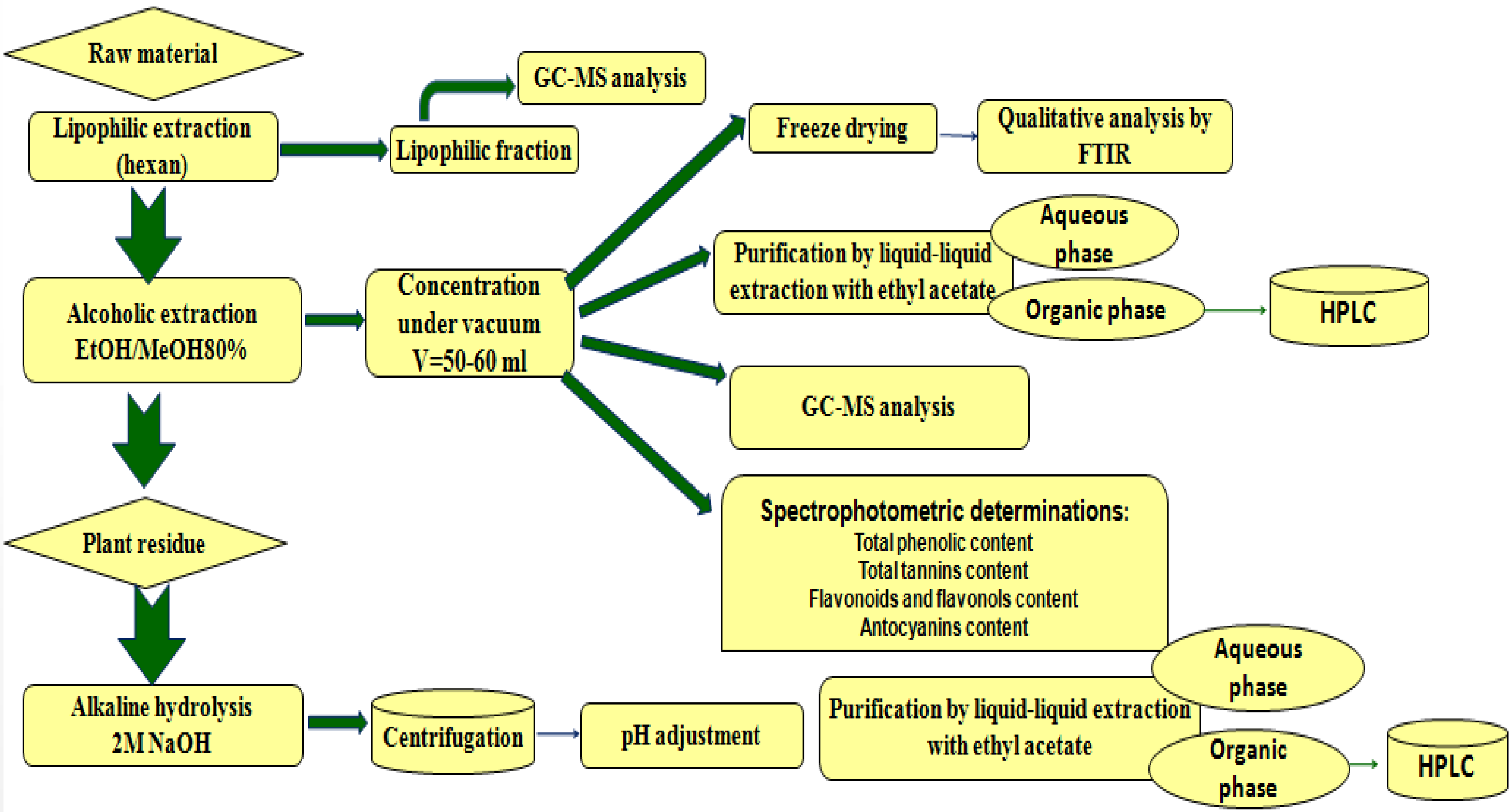
Spruce bark

- ✓ Valued medicinally for its rich content of proanthocyanidins.
- ✓ Spruce and pine bark extract has been used as a folk medicine and is used as a dietary supplement.
- ✓ Abundant waste source in the wood industry

Grapes seeds

- ✓ In the recent years naturally occurring plant phenolics in grapes by-products have raised a lot of attention considering that Grapes (*Vitis vinifera*) belong to the world's largest fruit crops.
- ✓The seeds constitute a considerable proportion of the pomace, amounting to 40–50% on a dry matter basis

Experimental flowchart



Lipophilic extraction

- Conditioning of the raw material
- Prior stage before the alcoholic extraction
- Elimination of liphofilic compounds

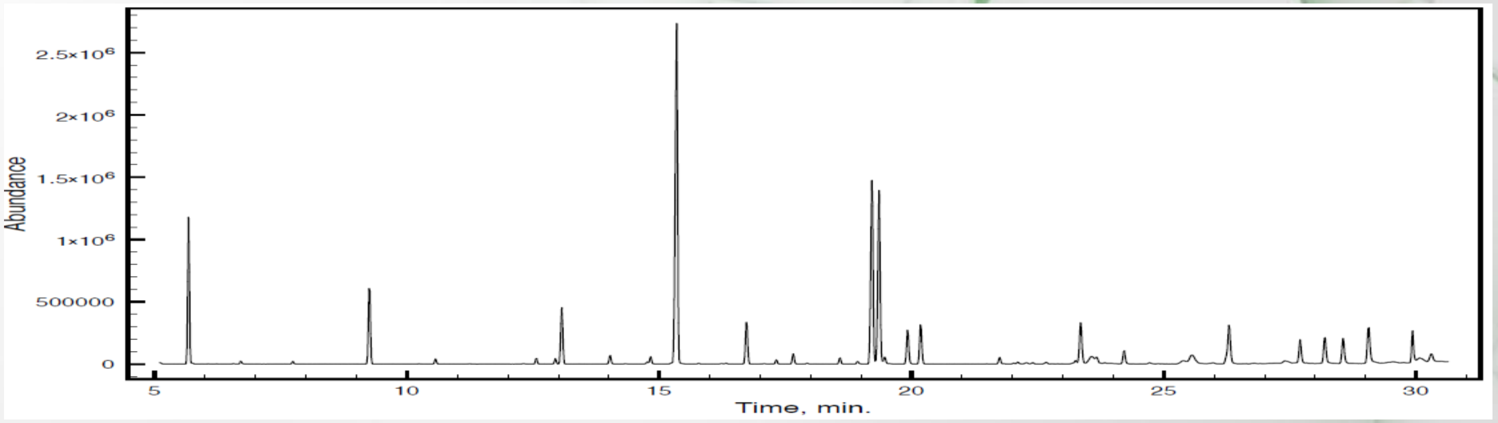


Figure 1. GC-MS chromatogram of Asclepias syriaca extract

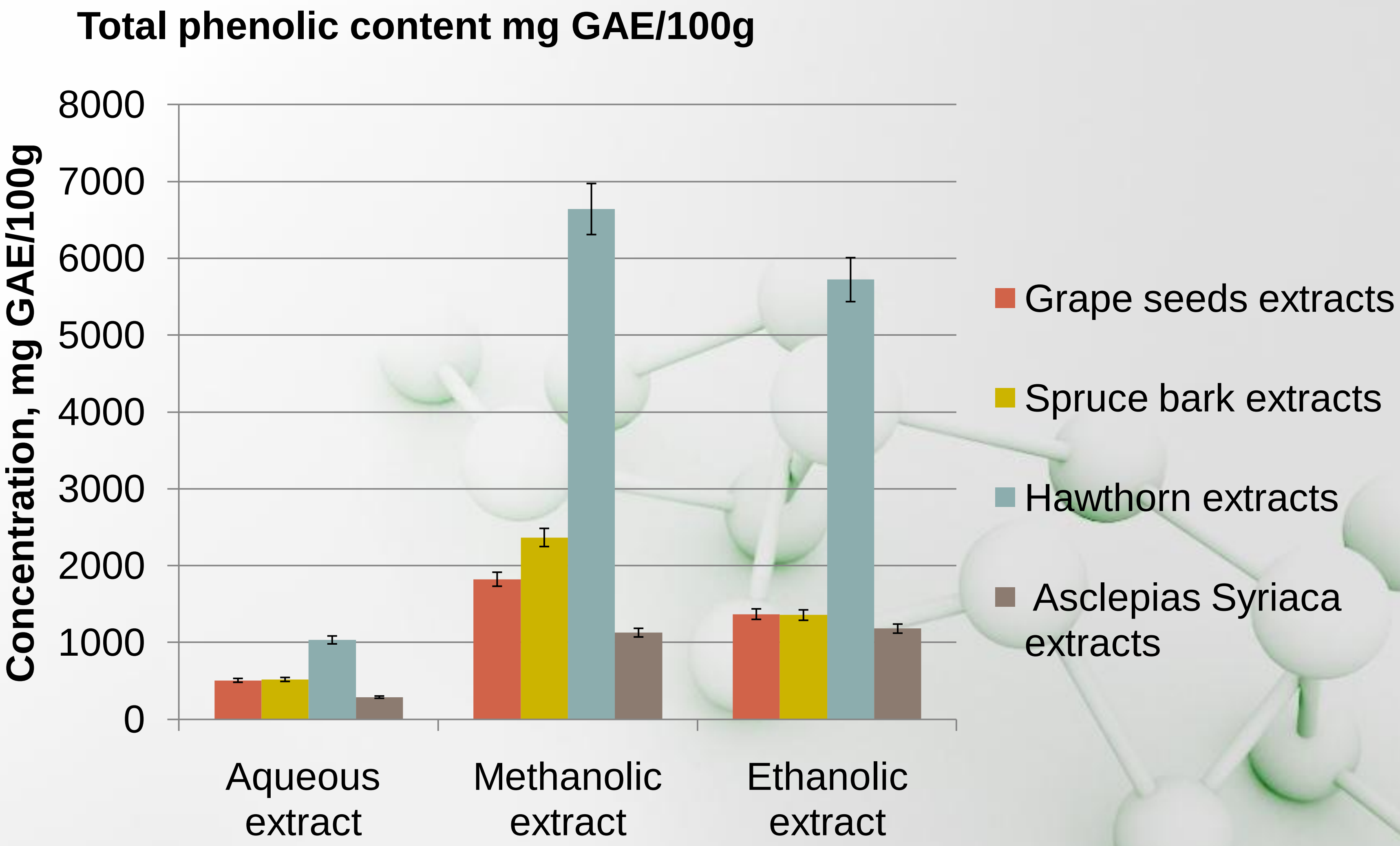
Compounds	Asclepias syriaca %	Crategus monogyna %	Spruce bark %	Grape seeds %
Arachidic acid	-	-	**8.65**	-
Azelaic acid	0.28	-	1.27	0.85
Eicosanoic acid	1.53	1.37	-	0.38
9-dodecanoic acid	0.20	-	-	0.035
9-duodecenoicacid	0.40	-	-	-
Tetradecanoic acid	-	-	0.12	-
Pentadecanoic acid	0.48	-	0.80	0.02
9-hexadecanoic acid	0.71	-	1.50	0.18
Octodecanoic acid	-	-	0.50	-
Beheric acid	3.39	1.01	**34.22**	0.10
Lauric acid	-	0.26	0.25	-
Lignoceric acid	2.66	-	-	1.36
Linoleic acid	**21.05**	**44.60**	-	**65.33**
Margaric acid	1.63	0.11	-	0.13
Myristic acid	-	0.20	0.87	0.09
Oleic acid	**19.29**	**33.78**	1.25	**12.80**
Palmitic acid	**38.44**	**13.21**	**11.91**	**10.35**
Stearic acid	3.65	2.23	3.00	6.67
Tricosanoic acid	3.11	-	-	0.03
Methylerucate	2.66	-	-	-
Methyl lignocerat	-	-	**35.60**	-
Methylpalmitoleat	-	2.20	-	-

Characterization of polyphenolic extracts

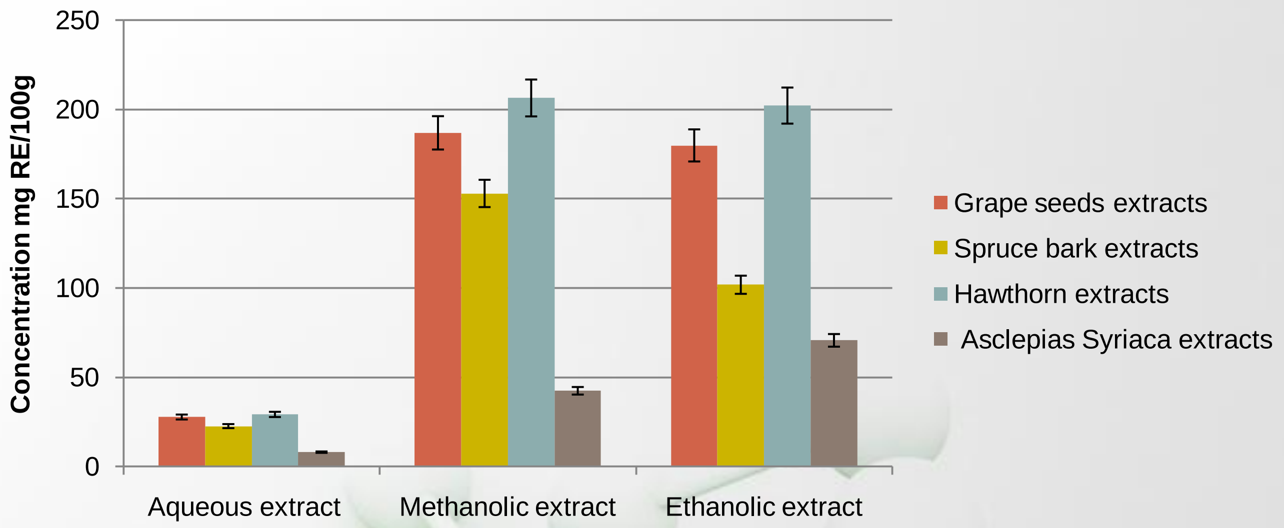
Colorimetric methods:

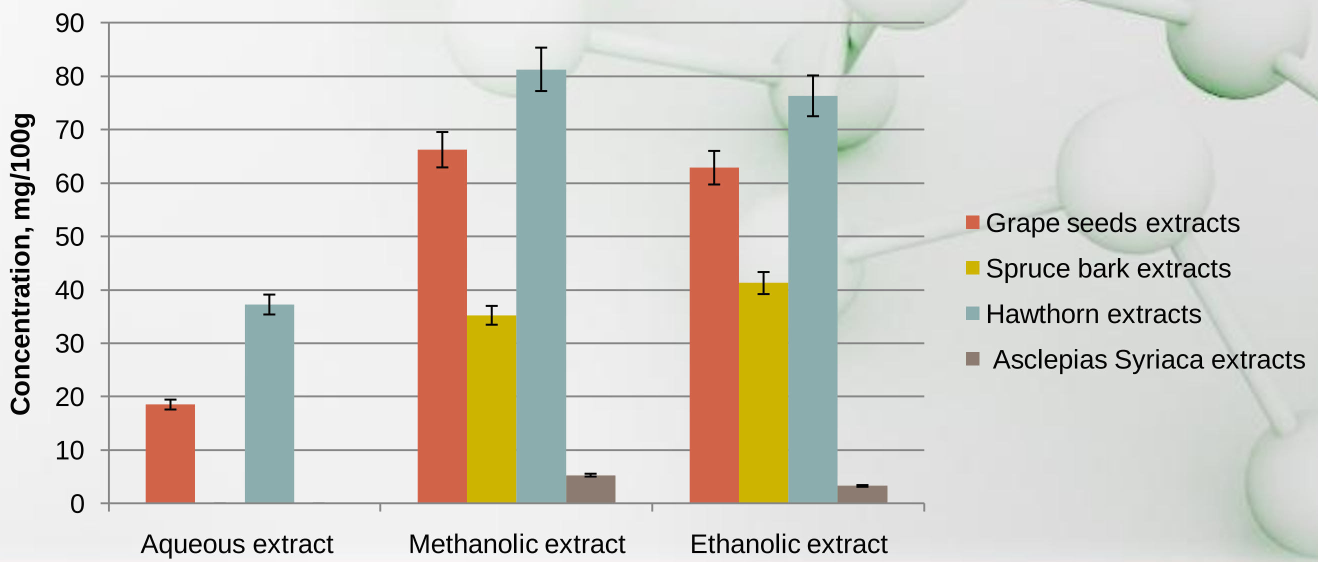
- Total phenolic content (Folin- Ciocateu method)
- Total tannins content (selective precipitation of tannins with casein, Folin- Ciocalteu method for the initial solution and filtrate)
- Total flavonoids and flavonols content (aluminum chloride method using rutin as a reference compound)
- Anthocyanins content (pH differential method)

Separation and characterization of polyphenolic extracts



Total content of flavonoids and anthocyanins





FTIR analysis

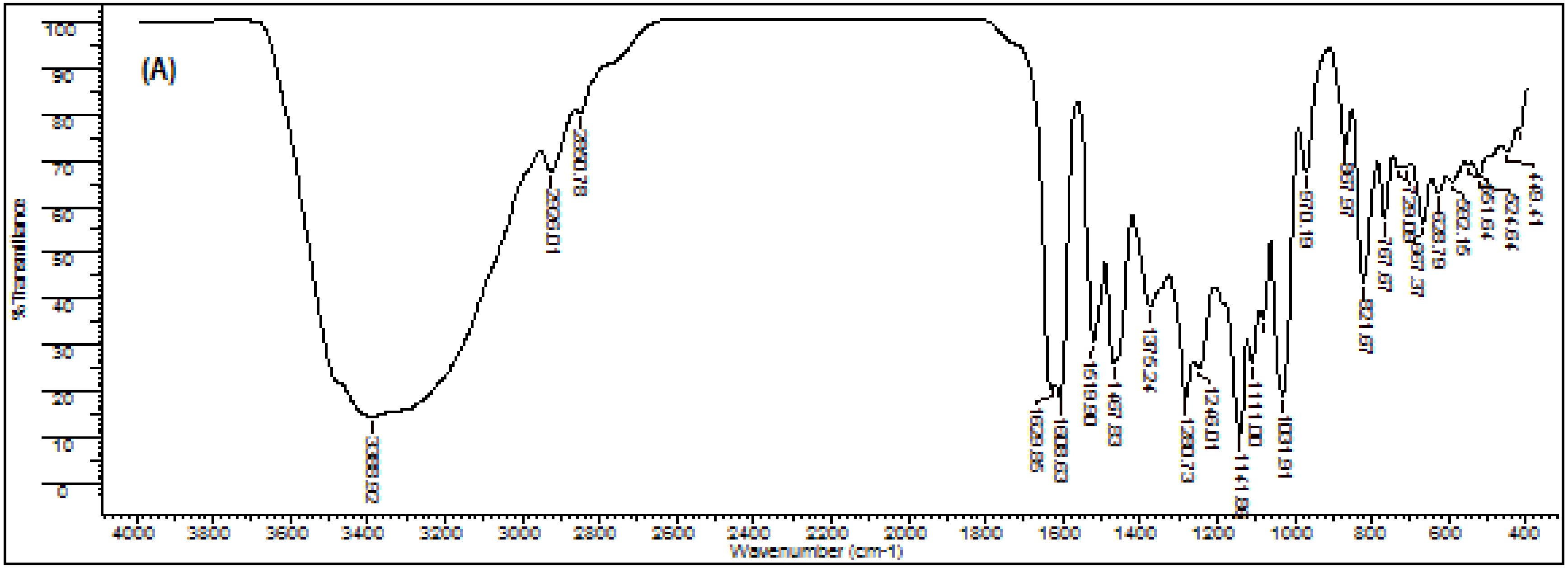
- Bruker Vector 22 FT-IR spectrometer equipped with a diamond reflection accessory was used to record IR spectra.

- FTIR spectroscopy can be used as an additional tool to screen vegetal samples for their content of phenolic compounds.

- The FT-IR spectres of the samples were obtained and the effective peaks the functional groups were compared with that of the standards.

- The registered spectres showed band assignments for polyphenols in the standards and investigated samples.

FTIR analysis



FTIR spectrum of: (A)- catechine;

The wavelengths numbers of FTIR spectra for catechine

817: C–H alkenes

1,029: –C–O alcohols

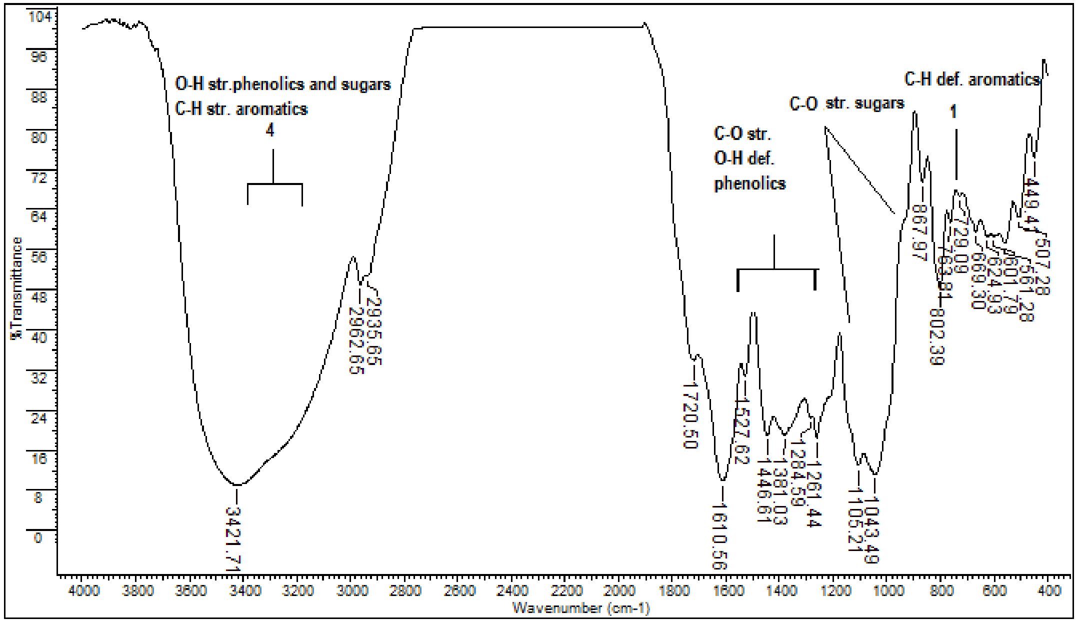
1,141: C–OH alcohols

1,280: –OH aromatic

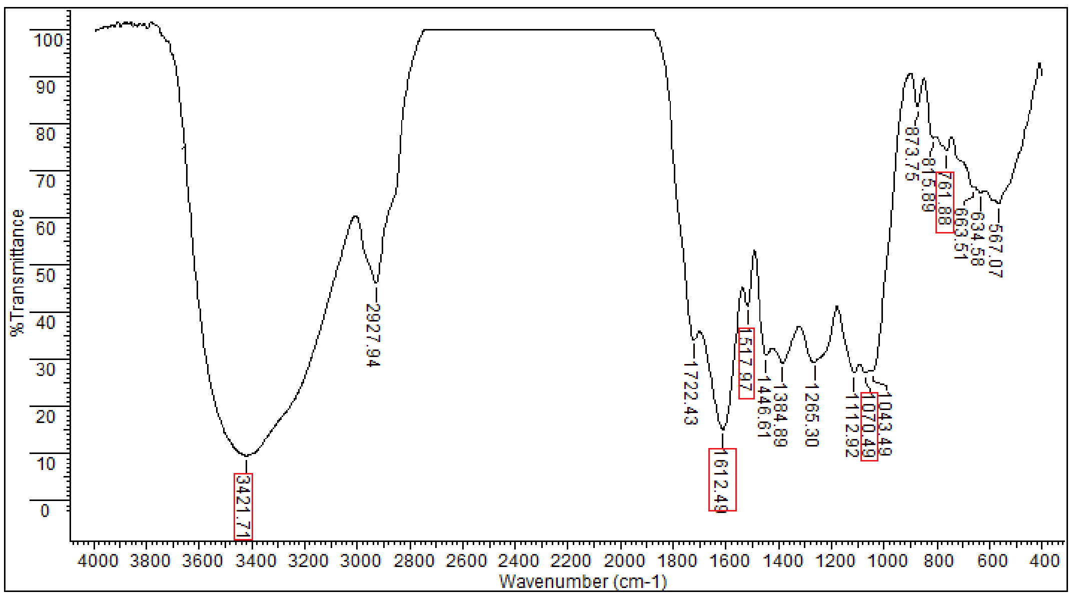
1,453: C–O alcohols

1,518:C–H alkanes,

1,603 cm^{-1} C=C aromatic ring and C=C alkenes.



FTIR spectrum of grape seeds extract



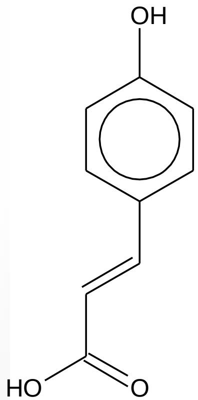
FTIR spectrum of spruce bark extract

✓glycosidic groups (νO-H 3350 cm^{-1}
✓complex νC-O vibrations between 1400-1050cm^{-1})
✓phenyl rings (1515 cm^{-1})
✓carbonyl substituents (1700, 1626, 1599 cm^{-1})
✓strong absorption bands at 3,385, 1,612, and 1,067 cm^{-1} were attributed to those of the characteristic functional groups of polyflavonoids (Yazaki and Hillis 1977)
✓1,522 and 777 cm^{-1} were attributed to aromatic ring breathing mode and CH out-of-plane deformation with two adjacent free hydrogen atoms, respectively, indicating the prominent presence of procyanidin (PC) structure (Chang Sub Ku, 2007)).

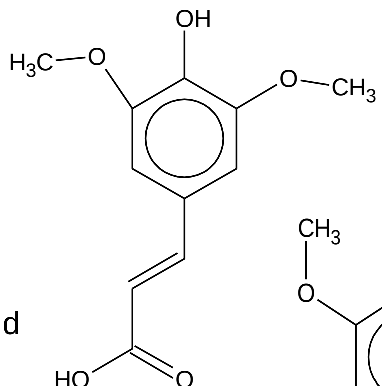
Yazaki and Hillis, (1977), Polyphenolic Extractives of Pinus radiata Bark, Holzforschung, 31 (1), p. 20–25
Chang Sub Ku and Sung Phil Mun, (2007), Characterization of proanthocyanidin in hot water extract isolated from *Pinus radiata* bark, Wood Science and Technology, 41(3), 235-247, DOI: 10.1007/s00226-006-0103-8

HPLC characterization

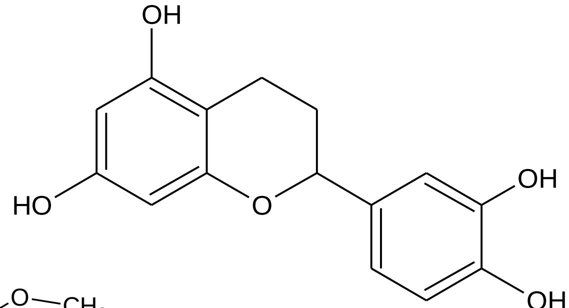
Conditions:
Mobile phase A: 1% acetic acid
Mobile phase B: 1% acetic acid in methanol
Gradient 10%B-40%B, 20 min
Stationary phase: Dionex C18 120x2.1, 5µm



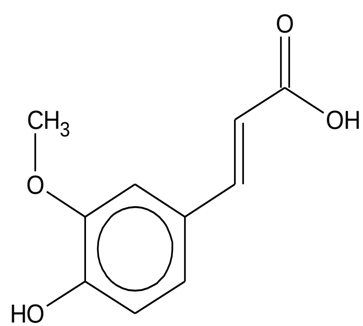
p-coumaric acid



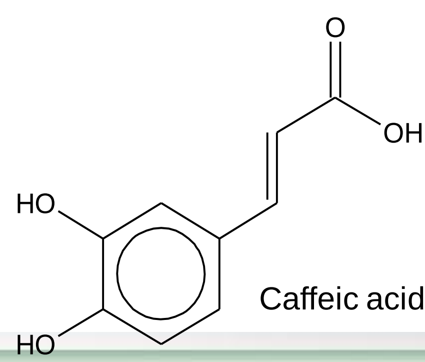
Sinapic acid



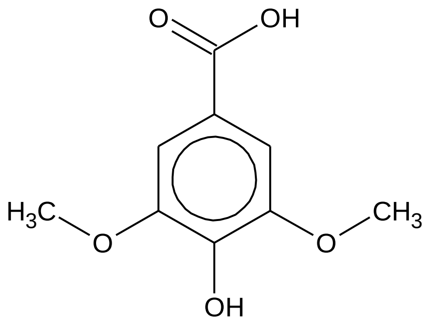
catechine



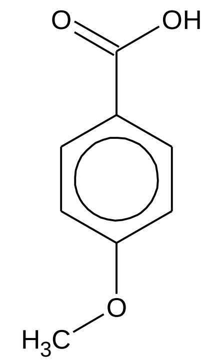
Ferulic acid



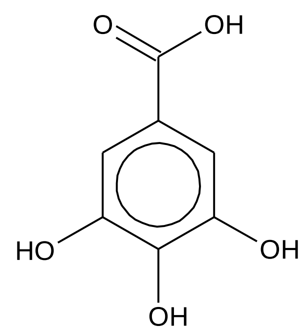
Caffeic acid



Siringic acid



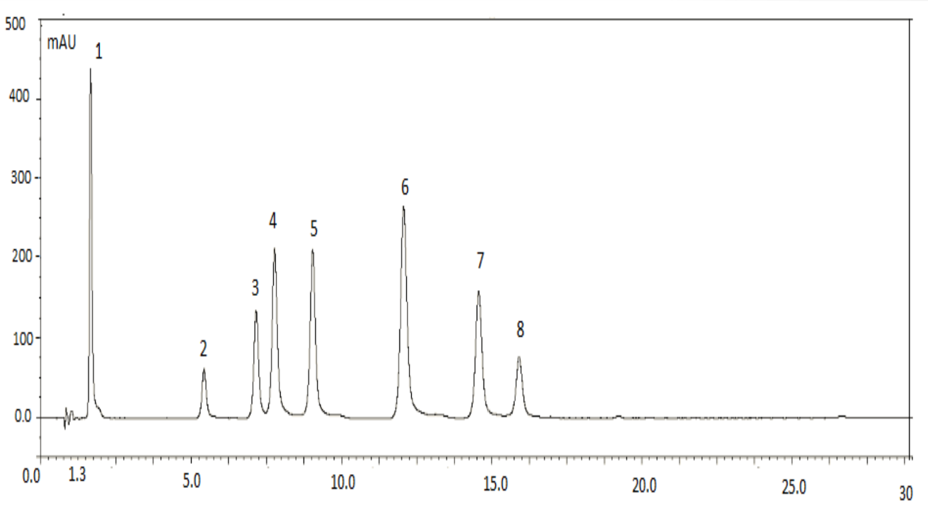
Vanilic acid



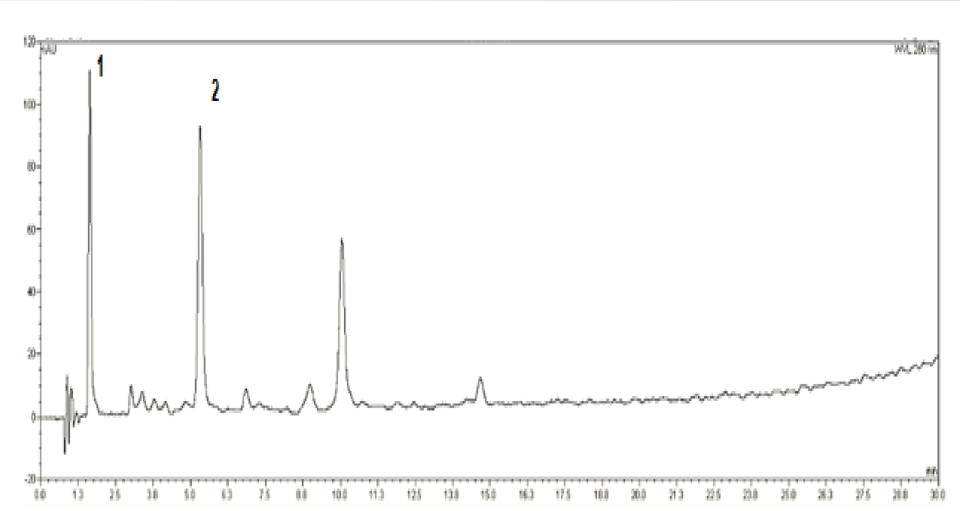
Gallic acid

Cinnamic acides

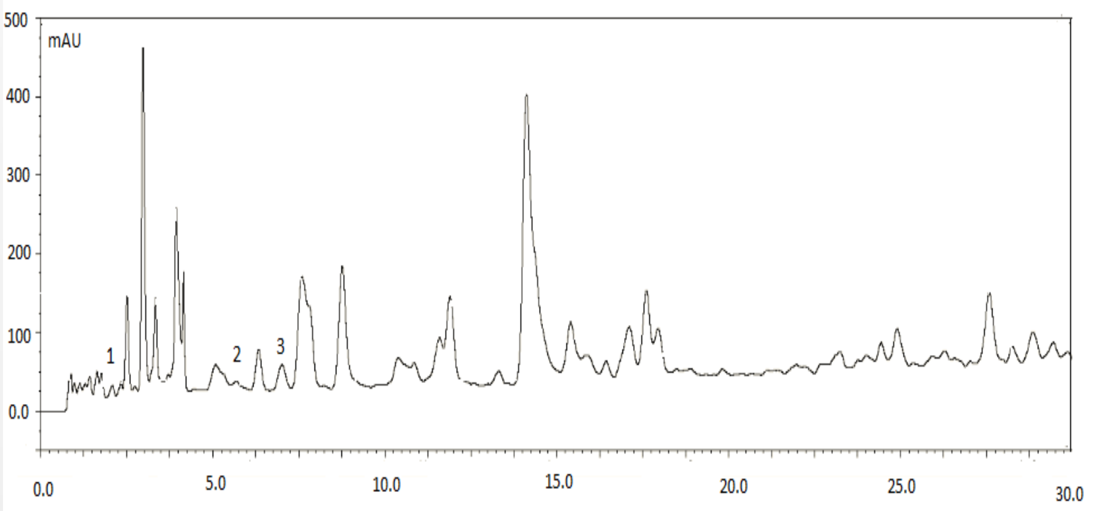
Benzoic acids



Cromatographic profile of standard polyphenols: 1- gallic acid; 2- catechine; 3- vanilic acid; 4- caffeic acid; 5- syringic acid; 6- p-coumaric acid; 7- ferulic acid; 8- sinapic acid



Cromatographic profile of grape seeds ethanolic extract: 1- gallic acid; 2- catechine;



Cromatographic profile of spruce bark ethanolic extract: 1- gallic acid; 2- catechine; 3- vanilic acid

Concentration of phenolic compounds (mg/100g dried plant) in the investigated samples

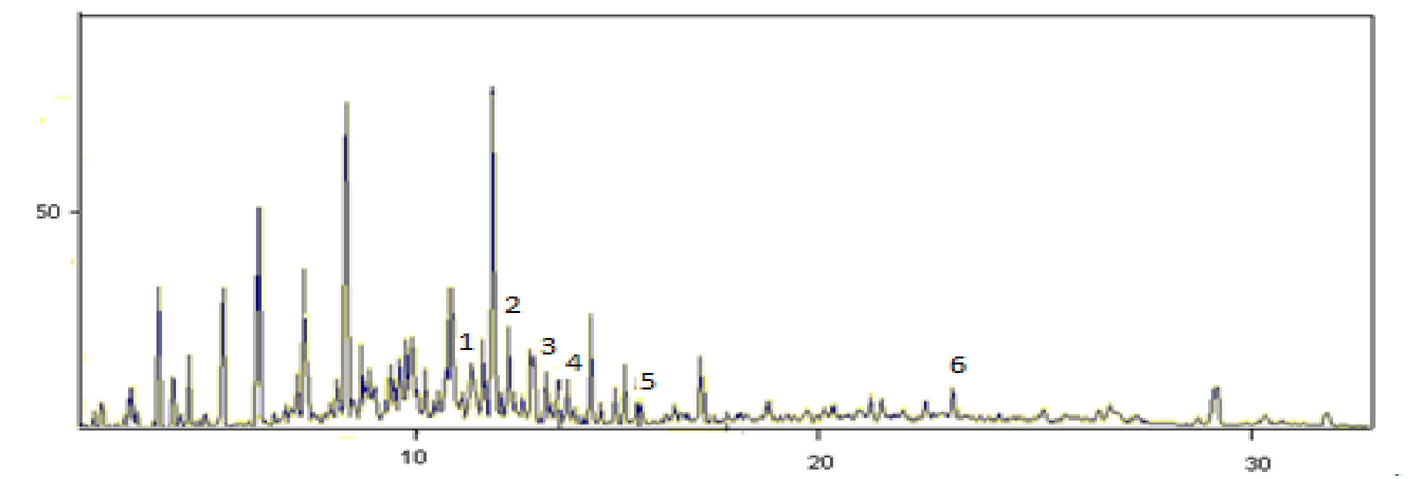
Raw material	Extract	Galic acid	Catechine	Vanilic acid	Caffeic acid	Syringic acid	p-coumaric acid	Ferulic acid	Sinapic acid
Grape seeds	Aqueous extract	6.12±0.2	44.36±0.1	-	-	-	-	-	-
	Methanolic extract	14.87±0.4	88.42±2.3	-	-	-	-	-	-
	Ethanolic extract	12.54±0.8	63.60±1.7	-	-	-	-	-	-
	Alkaline hydrolysis before ethanolic extraction	38.20±0.5	21.05±0.9	-	-	63.30±1.5	-	-	-
	Alkaline hydrolysis before methanolic extraction	41.52±0.9	19.81±2.1	-	-	42.19±1.2	-	-	-
Hawthorne	Aqueous extract		23.42±0.9	-	-	2.14±0.09	-	-	-
	Methanolic extract	9.19±0.3	56.64±1.2	-	-	2.91±1.1	2.39±0.6	0.58±1.1	-
	Ethanolic extract	10.98±0.7	89.52±2.1	-	-	2.95±1.1	3.59±0.4	2.25±1.4	-
	Alkaline hydrolysis before ethanolic extraction	-	-	-	-	19.23±1.3	-		-
	Alkaline hydrolysis before methanolic extraction	-	-	-	-	22.51±1.6	-		-
Spruce bark	Aqueous extract	-	31±1.9	39.4±0.2	-		-		-
	Methanolic extract	7.1±0.7	70.8±2.3	62.4±0.5	-		-		-
	Ethanolic extract	10.2±0.3	71.9±2.7	71.9±0.8	-		-		-
	Alkaline hydrolysis before ethanolic extraction	-	-	-	-	42.3±3.2	-	45.08±1.6	-
	Alkaline hydrolysis before methanolic extraction	-	-	3.93±0.1	-	42.87±2.9	-	42.91±1.3	-
Asclepias syriaca	Aqueous extract	-	-	0.87±0.1	-	0.98±0.09	0.11±0.1	-	-
	Methanolic extract	0.66±0.2	-	3.72±0.9	-	2.85±1.1	0.41±0.12	-	-
	Ethanolic extract	0.65±0.4	-	2.94±1.1	-	1.94±0.9	0.40±0.09	-	-
	Alkaline hydrolysis before ethanolic extraction	-	-	-	-	25.44±1.2	0.14±0.1	-	-
	Alkaline hydrolysis before methanolic extraction	-	-	-	-	17.17±1.4		-	-

GC-MS analysis

- Qualitative analysis

- Silylation is an ideal procedure for the GC analysis of non-volatile and thermolabil compounds.

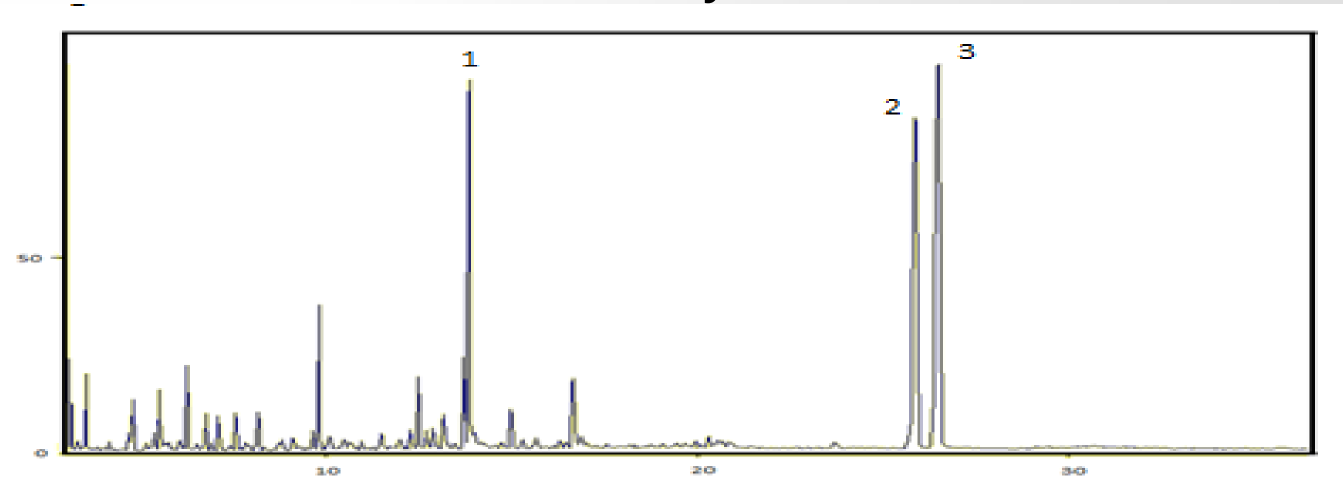
- Identification of compounds was achieved by comparing the retention times with those of authentic compounds and according to Proestos et.al., (2006).



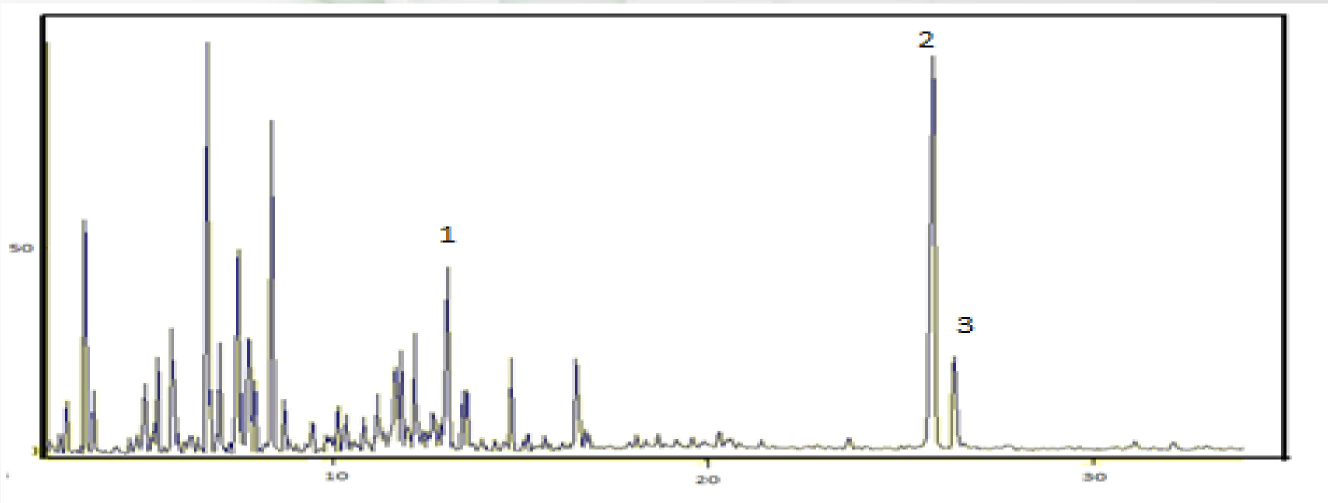
GC-MS chromatogram of spruce bark ethanolic extract
1- vanilic acid; 2- gentisic acid; 3- galic acid; 4- p-cumaric acid; 5- cafeic acid; 6-catechine

Proestos, C., Sereli, D., Komaitis, M., (2006) Determination of phenolic compounds in aromatic plants by RP-HPLC and GC-MS, Food chemistry 95: 44-42.

GC-MS analysis



GC-MS chromatogram of grape seeds ethanolic extract: 1- acid galic acid; 2- catehina; 3- epicatechina



GC-MS chromatogram of Crategus Monogyna ethanolic extract: 1- galic acid; 2- catechine; 3- epicatechine

CONCLUSIONS

- Selected raw materials contain considerable quantities of bioactive aromatic compounds that may have many industrial applications.

- It has been showed that the selected vegetal samples apart from being an important source of antioxidants they can also be a valuable source of fatty oils.

CONCLUSIONS

- Alcoholic extracts showed significantlly higher amounts of phenolic compounds compared to water extracts.

- At the same time ethanol as extraction solvent provided a similar effciency as methanol in terms of individual compounds extracted and also in terms of concentrations.

- Nevertheless, in food industry ethanol and water are preferred because of their nontoxic,environmentally safe and inexpensive features and also due to their compatibility with potential application in biological sistems and food industry.

- The alkaline extraction showed a significant liberation of phenolic acids (syringic acid) that exist as insoluble bound complexes.

CONCLUSIONS

- FTIR analysis even if it cannot provide separation nor quantification of individual compounds, the spectra achieved for polyphenolic vegetal extracts provided useful information concerning the content of different functional groups.

- Using HPLC technique gallic acid and catechine were the major compounds identified in all the vegetal samples, in high concentrations.

CONCLUSIONS

- Using HPLC technique gallic acid and catechine were the major compounds identified in all the vegetal samples, in high concentrations.

- GS-MS analysis confirmed the presence of certaines phenols in the natural extracts, some of them being previously determined on HPLC.

- On the other side, there were identified several phenolic compounds (gentisic acid, caffeic acid, epicatechine) that were not identified by HPLC analysis according to standards.

Thank you for your attention!