CHARACTERIZATION OF EXTRACTIVES FROM SOME RAW MATERIALS PROCESSED BY BIOREFINING

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Research priorities

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

Vegetable biomass
- Specific compounds
- Secondary compounds
- Primary compounds

Extraction / separation

Specific compounds
- Hydrocarbons
- Proteins
- Sugars
- Pigments
- Different active compounds

Secondary compounds
- Hemicellulose + polyphenols

Primary compounds
- Lignocellulose

Extraction / separation
hemicellulose, polyphenols, cellulose, lignocellulose

Direct application

Conversion
- with keeping basic Structural units
- with destruction of chemical structure

Modified primary, secondary and specific compounds
- Structural units (monosaccharides, phenols, aminoacids)
- Gas
- Liquids
- Solids

Raw materials for substitution of petrochemicals products with high chemical or energetically value

Direct application
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.
- Current 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.
Lipophilic extraction

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

Figure 1. GC-MS chromatogram of Asclepias syriaca extract
<table>
<thead>
<tr>
<th>Compounds</th>
<th>Asclepias syriaca</th>
<th>Crategus monogyna</th>
<th>Spruce bark</th>
<th>Grape seeds</th>
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</thead>
<tbody>
<tr>
<td>Arachidic acid</td>
<td>-</td>
<td>-</td>
<td><strong>8.65</strong></td>
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<td>Azelaic acid</td>
<td>0.28</td>
<td>-</td>
<td>1.27</td>
<td>0.85</td>
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<td>Eicosanoic acid</td>
<td>1.53</td>
<td>1.37</td>
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<td>9-duodecenoic acid</td>
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<td>Tetradecanoic acid</td>
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<td>-</td>
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<td>Pentadecanoic acid</td>
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<td>Octodecanoic acid</td>
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<td>Beheric acid</td>
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<td>1.01</td>
<td><strong>34.22</strong></td>
<td>0.10</td>
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<td>0.26</td>
<td>0.25</td>
<td>-</td>
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<tr>
<td>Lignoceric acid</td>
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<td>-</td>
<td>-</td>
<td>1.36</td>
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<tr>
<td><strong>Linoleic acid</strong></td>
<td><strong>21.05</strong></td>
<td><strong>44.60</strong></td>
<td>-</td>
<td><strong>65.33</strong></td>
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<tr>
<td>Margaric acid</td>
<td>1.63</td>
<td>0.11</td>
<td>-</td>
<td>0.13</td>
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<td>Myristic acid</td>
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<td>0.20</td>
<td>0.87</td>
<td>0.09</td>
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<tr>
<td><strong>Oleic acid</strong></td>
<td><strong>19.29</strong></td>
<td><strong>33.78</strong></td>
<td>1.25</td>
<td><strong>12.80</strong></td>
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<td><strong>Palmitic acid</strong></td>
<td><strong>38.44</strong></td>
<td><strong>13.21</strong></td>
<td><strong>11.91</strong></td>
<td><strong>10.35</strong></td>
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<td>Stearic acid</td>
<td>3.65</td>
<td>2.23</td>
<td>3.00</td>
<td>6.67</td>
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<td>Tricosanoic acid</td>
<td>3.11</td>
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<td>0.03</td>
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<tr>
<td>Methylerucate</td>
<td>2.66</td>
<td>-</td>
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<tr>
<td><strong>Methyl lignocerat</strong></td>
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<td>-</td>
<td><strong>35.60</strong></td>
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<tr>
<td>Methylpalmitoleat</td>
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<td>2.20</td>
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</table>
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 phenolic content mg GAE/100g

Concentration, mg GAE/100g

Aqueous extract

Methanolic extract

Ethanolic extract

Grape seeds extracts

Spruce bark extracts

Hawthorn extracts

Asclepias Syriaca extracts
Total content of flavonoids and anthocyanins

![Bar chart showing the concentration of flavonoids and anthocyanins in different extracts.](chart.png)
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.
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 analysis
FTIR spectrum of grape seeds extract

- glycosidic groups ($\nu$O-H 3350 cm$^{-1}$)
- complex $\nu$C-O vibrations between 1400-1050 cm$^{-1}$
- 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
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

Cinnamic acids

- p-coumaric acid
- Sinapic acid
- Ferulic acid
- Caffeic acid
- Catechine

Benzoic acids

- Benzoic acid
- Sinapic acid
- Siringic acid
- Vanillic acid
- Gallic acid
Cromatographic profile of standard polyphenols: 1- gallic acid; 2- catechin; 3- vanillic 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- catechin;

Cromatographic profile of spruce bark ethanolic extract: 1- gallic acid; 2- catechin; 3- vanillic acid
<table>
<thead>
<tr>
<th>Raw material</th>
<th>Extract</th>
<th>Gallic acid</th>
<th>Catechine</th>
<th>Vanilic acid</th>
<th>Caffeic acid</th>
<th>Syringic acid</th>
<th>p-coumaric acid</th>
<th>Ferulic acid</th>
<th>Sinapic acid</th>
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<tr>
<td>Grape seeds</td>
<td>Aqueous extract</td>
<td>6.12±0.2</td>
<td>44.36±0.1</td>
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<td>Ethanol extract</td>
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<td>Alkaline hydrolysis before</td>
<td>38.20±0.5</td>
<td>21.05±0.9</td>
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<td>63.30±1.5</td>
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<td>42.19±1.2</td>
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<tr>
<td>Hawthorne</td>
<td>Aqueous extract</td>
<td>23.42±0.9</td>
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<td>Methanolic extract</td>
<td>9.19±0.3</td>
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<td>2.39±0.6</td>
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<td>Ethanol extract</td>
<td>10.98±0.7</td>
<td>89.52±2.1</td>
<td>2.95±1.1</td>
<td>3.59±0.4</td>
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<td>Alkaline hydrolysis before</td>
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<td>19.23±1.3</td>
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<tr>
<td>Asclepias syriaca</td>
<td>Aqueous extract</td>
<td>-</td>
<td>-</td>
<td>0.87±0.1</td>
<td>0.98±0.09</td>
<td>0.11±0.1</td>
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<td>Methanolic extract</td>
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<td>3.72±0.9</td>
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<td>Ethanol extract</td>
<td>0.65±0.4</td>
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</table>
GC-MS analysis

- Qualitative analysis

- Silylation is an ideal procedure for the GC analysis of non-volatile and thermolabile 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](image)

1- vanilic acid; 2- gentisic acid; 3- gallic acid; 4- p-cumaric acid; 5- cafeic acid; 6-catechine

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 significantly higher amounts of phenolic compounds compared to water extracts.

- At the same time ethanol as extraction solvent provided a similar efficiency 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 systems 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, epicatechin) that were not identified by HPLC analysis according to standards.
Thank you for your attention!