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Valorization of NWFP's for tannin and antioxidants production: Study of pine and oak barks and pine nut shells

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Outline:

- ❑ Introduction
- ❑ Materials and Methods
- ❑ Results
- ❑ Conclusions

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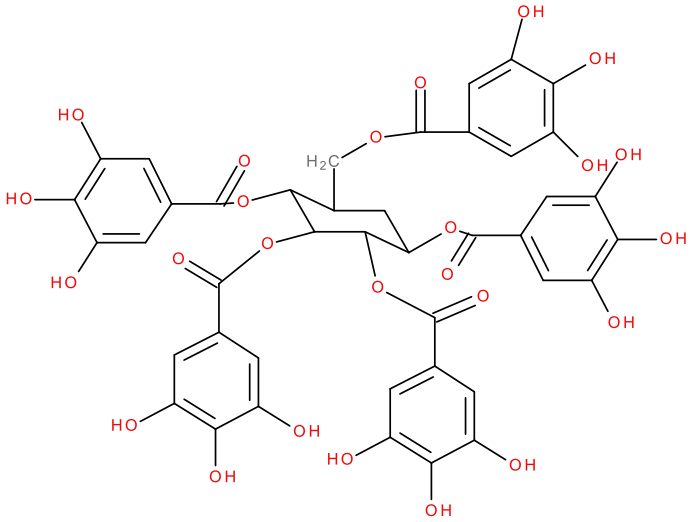
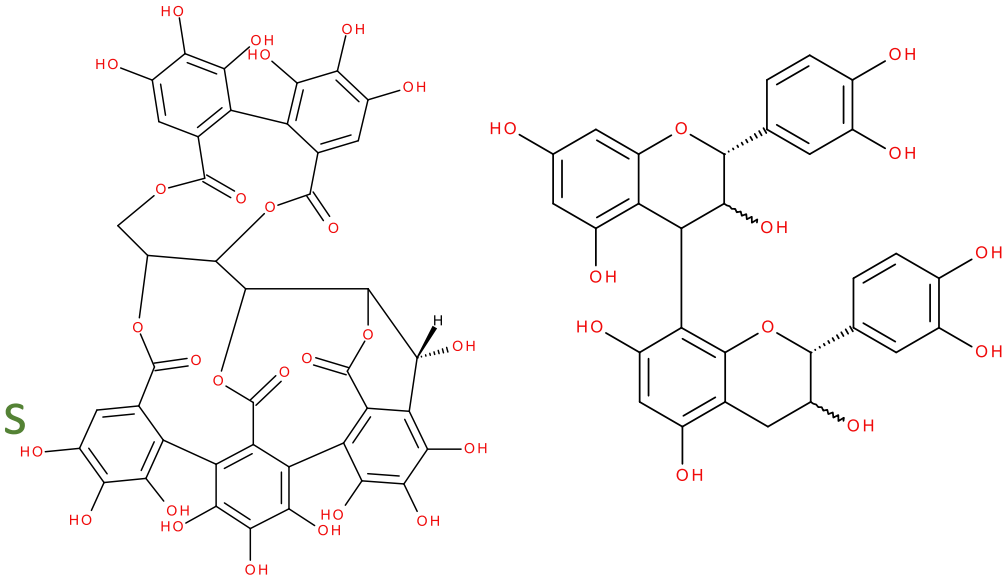
Introduction

Tannins:



- ✓ Water-soluble organic compounds
- ✓ Molecular weights between 500 and 3000 Da
- ✓ Classified as hydrolyzable or condensed tannins
- ✓ Tree barks contain importante amount of tannins
- ✓ Important antioxidant properties



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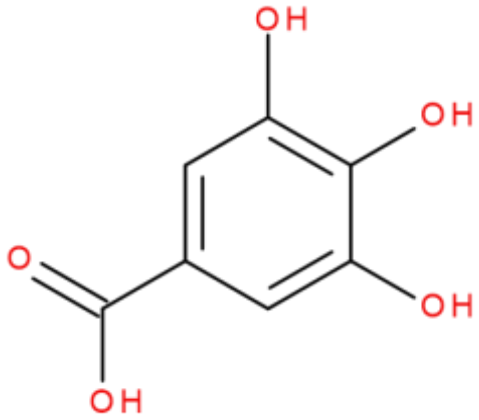
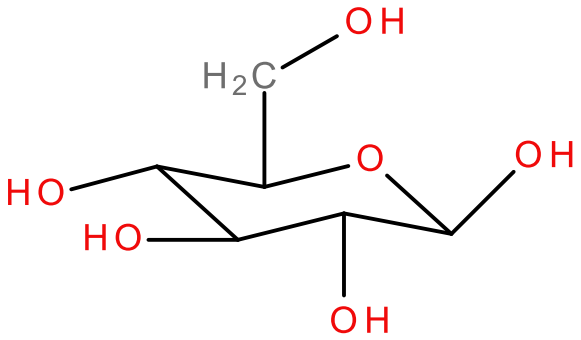
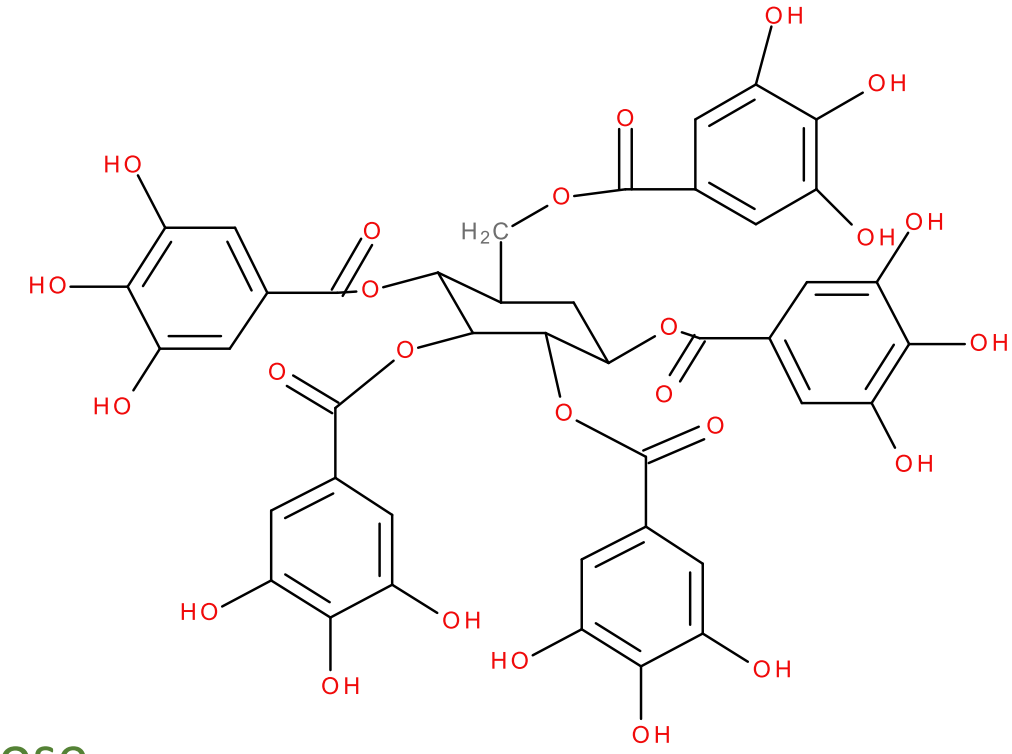
Hydrolyzable Tannins

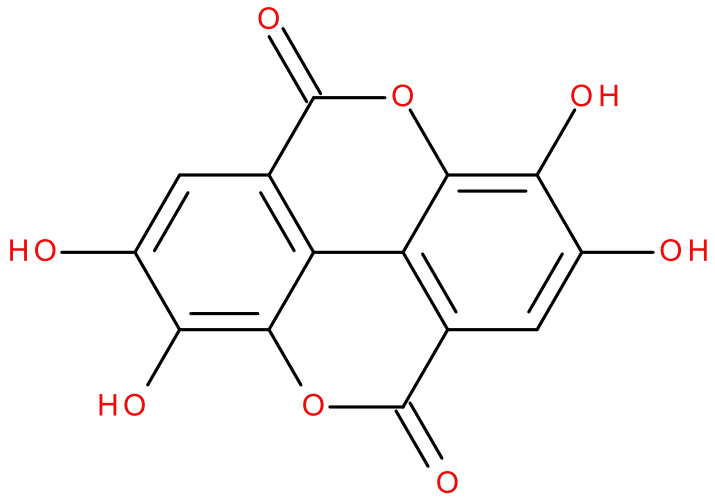


Gallic acid

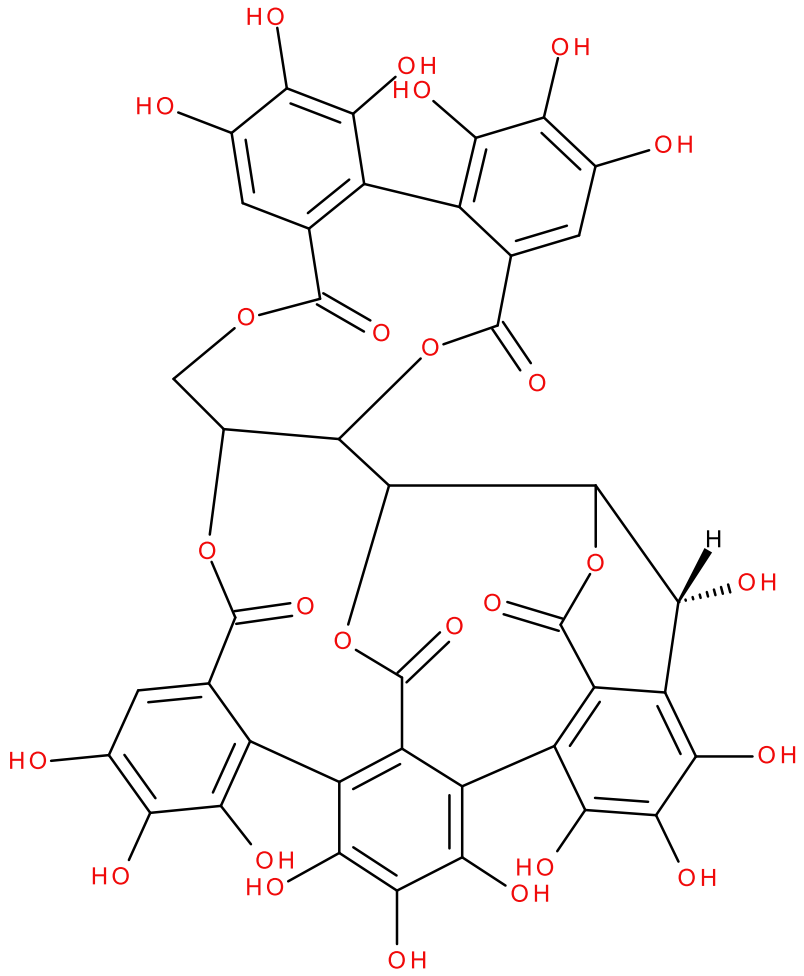


Core sugar: Glucose





Ellagic acid



Castalagin

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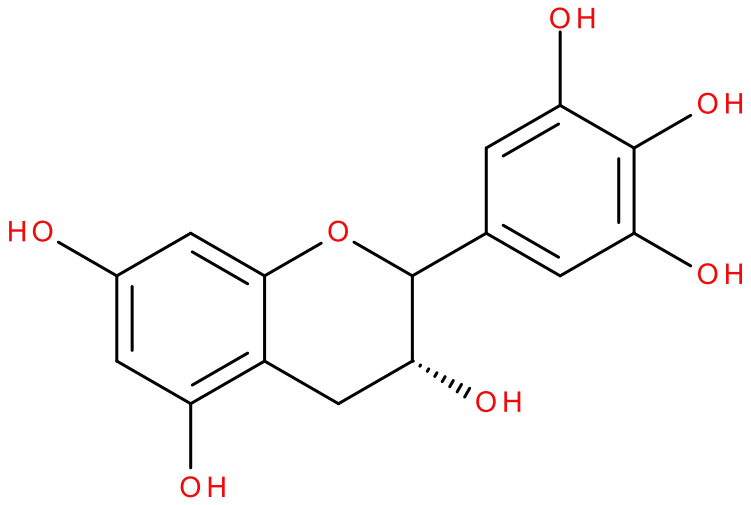
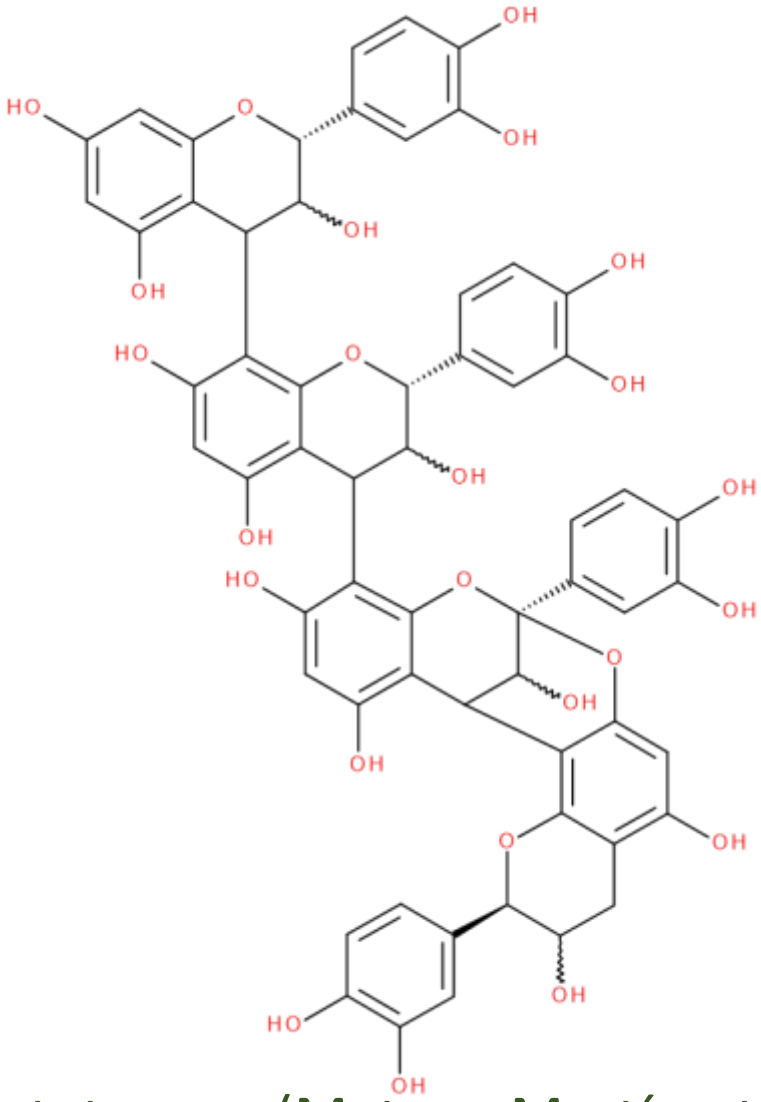
Condensed Tannins



Gallo catechin



(epi)catechin tetramer (Mateos-Martín *et al*. 2012)

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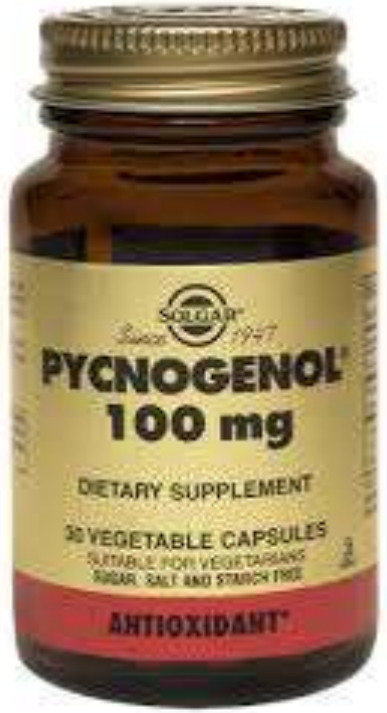
Pycnogenol:

- *Pinus pinaster (*French Maritime Pine*)* bark extract
- Composed of mainly catechin units
- Powerful antioxidant
- Used for treating circulation problems, asthma, diabetes, etc.



Source: www.solgar.com

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Materials and Methods

Materials

Barks were obtained from two hardwood species (*Quercus suber* and *Q. cerris*) and two softwood species (*Pinus pinaster* and *P. pinea*)

Pine nut shells were obtained from *P. pinea*

Bark and pine nut shells were grinded into 40-60 (0.25-0.42 mm) mesh granule size

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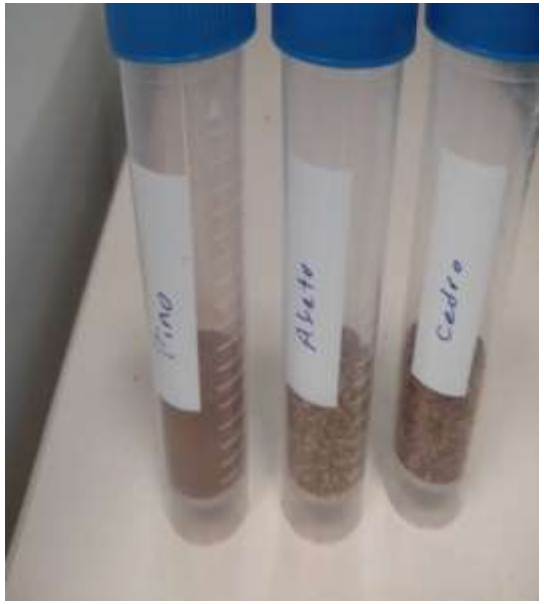
Materials and Methods

Methods

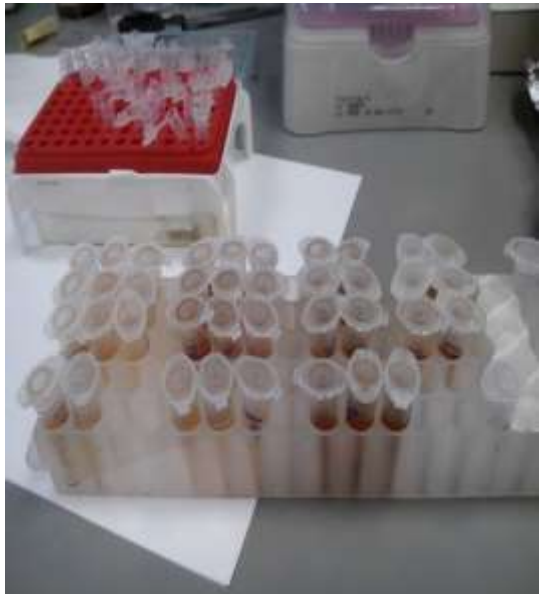
1. Extraction

2. MALDI Tof Analysis

3. EPR Analysis



1



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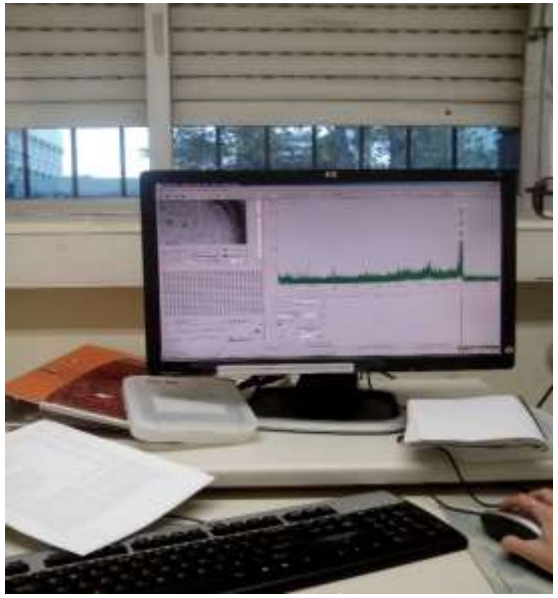

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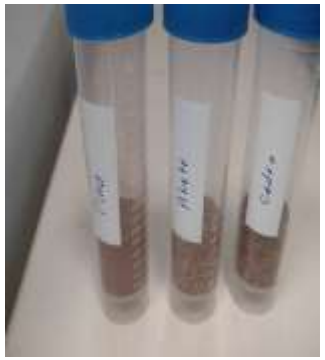
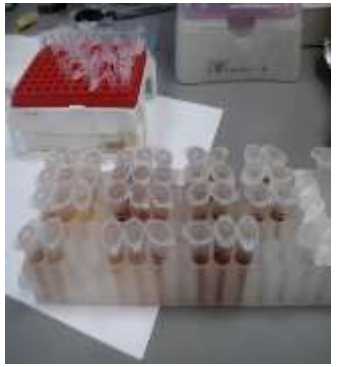


1. Extraction:

- ✓ 40-60 mesh bark samples were defatted with hexane to remove lipophilic extractives. The extracted bark samples were treated with water: acetone: acetic acid (70: 29.5: 0.05 v/v) solution

- ✓ The obtained polyphenol extracts were freeze-dried and kept at -20 ^{o}C until the time of MALDI and EPR analyses.



2. MALDI Tof Analysis:

A tandem mass spectrometer (AutoFLEX III, BrukerDaltonics, Bremen, Germany) and the following conditions were applied (Mateos *et al*. 2012):

- ✓ The accelerating voltage was 20 kV and the reflectron voltage 21 kV.
- ✓ Spectra were the sum of 500 shots with a frequency of 200 Hz. Both positive and negative reflectron modes were tried.
- ✓ The MS/MS spectra were obtained in the collusion-induced dissociation (CID) mode using Argon as the collusion gas.

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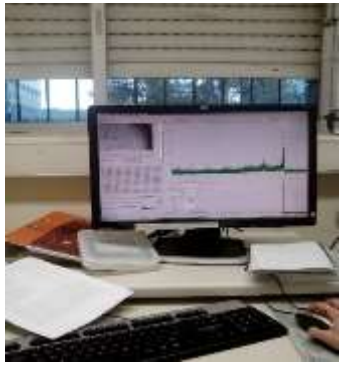

3. EPR (Electron paramagnetic resonance) Analysis:

The following conditions were used:

- ✓ EPR Spectrometer (Bruker EMX-Plus 10/12), microwave frequency 9.876 GHz, microwave power 30.27 mW, modulation frequency 100 kHz, modulation amplitude 1.86 G

- ✓ Spin trap was: 5,5-dimethyl-1-pyrolline-*N*-oxide (DMPO)

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Results

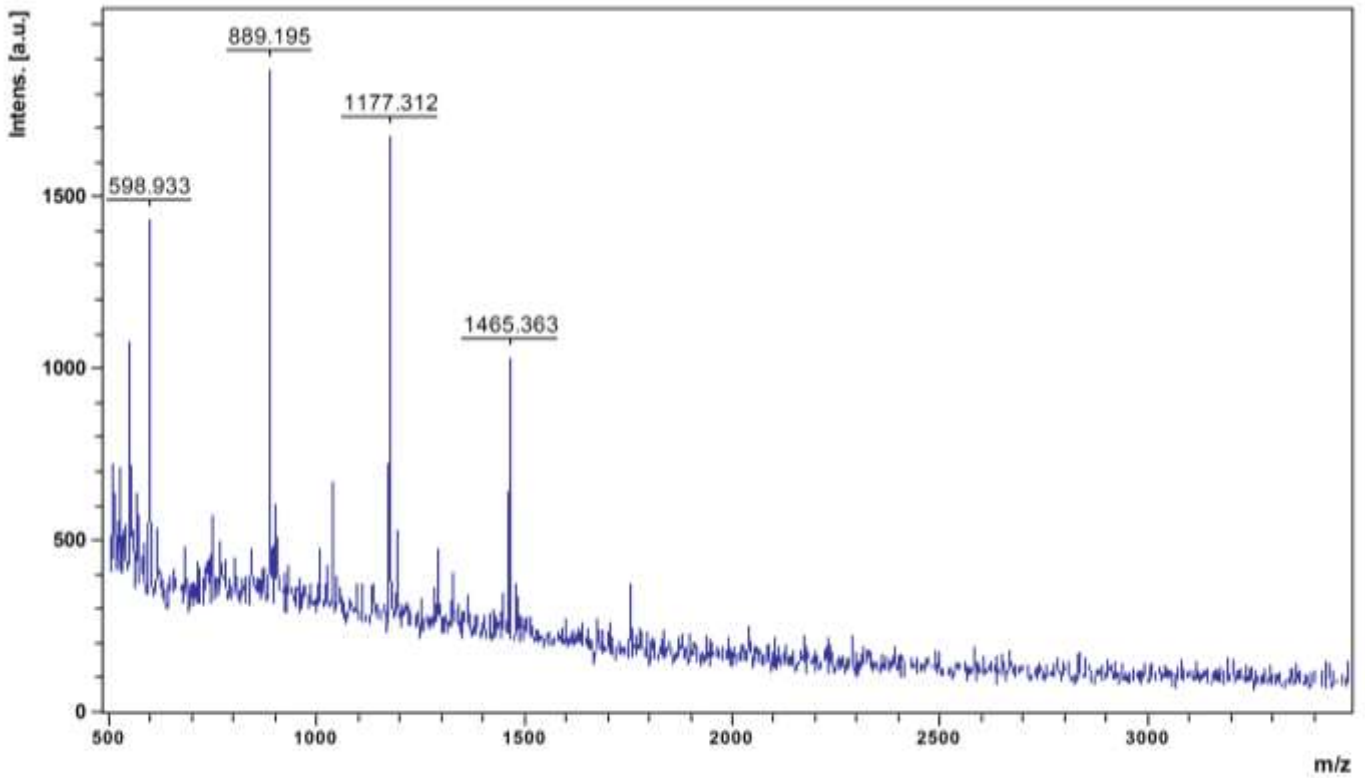


Fig 1. MaldiTOF spectrum of *P. pinaster* bark extract

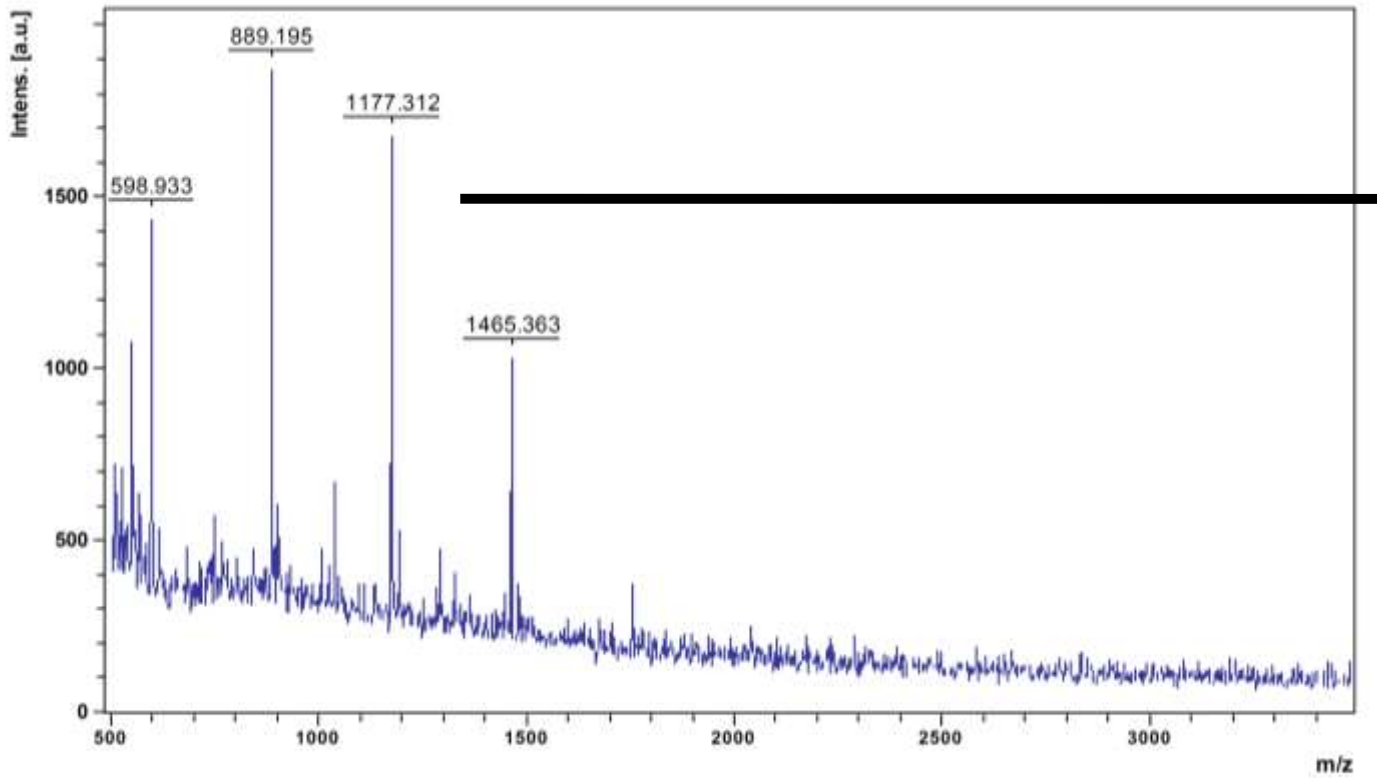
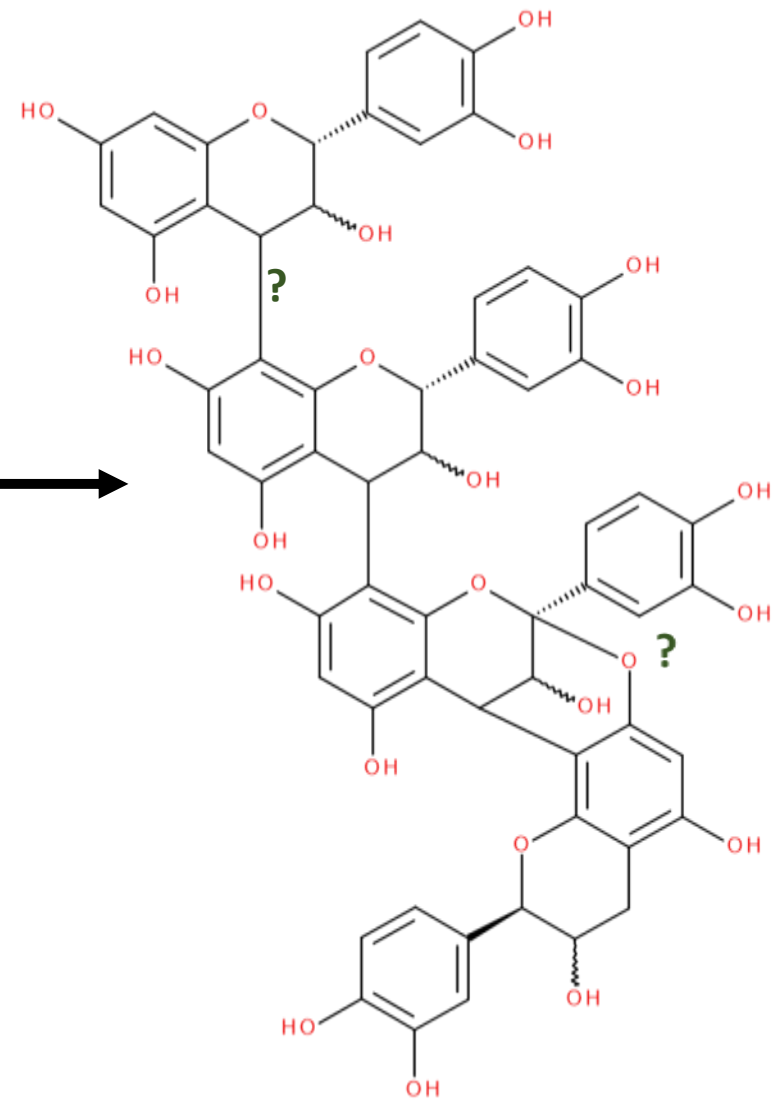


Fig 1. MaldiTOF spectrum of *P. pinaster* bark extract



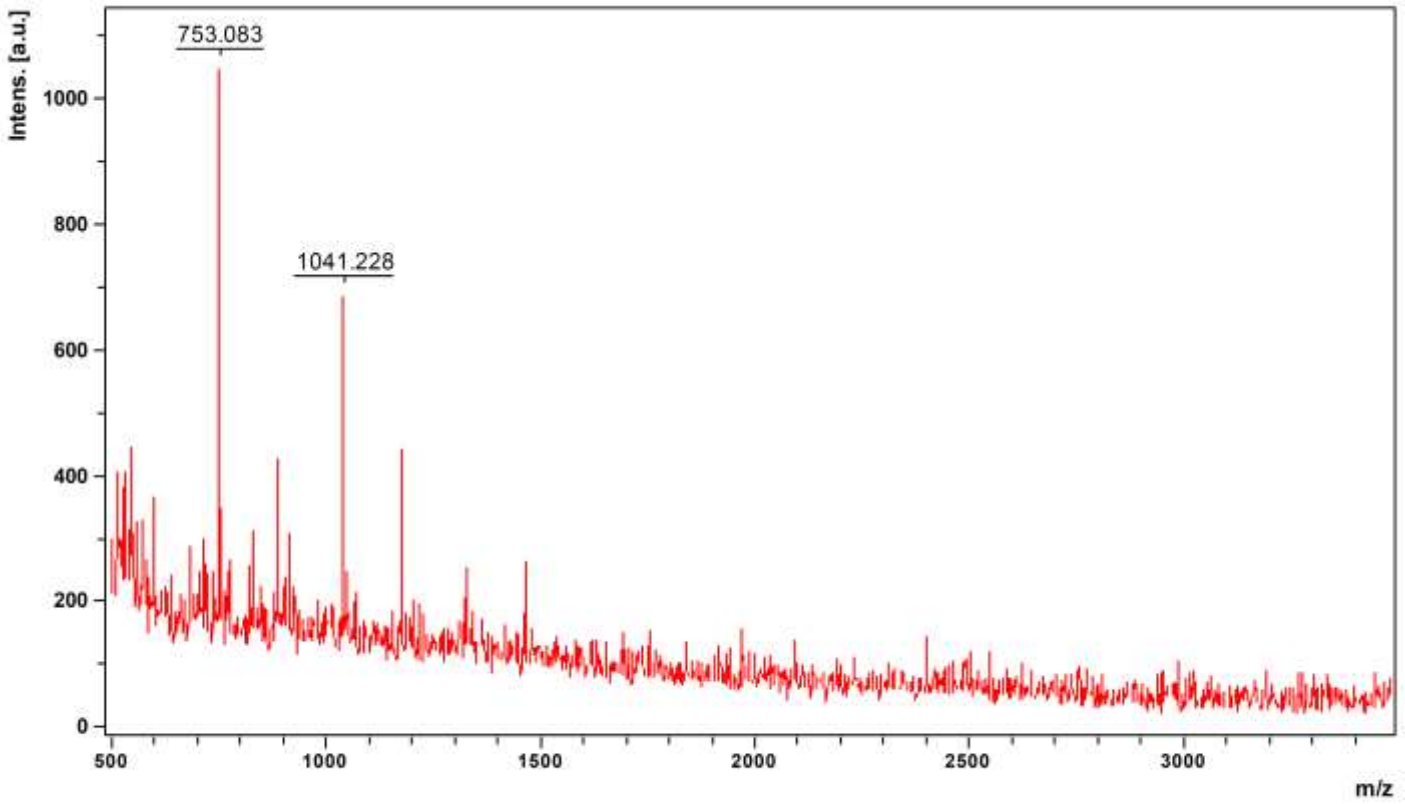
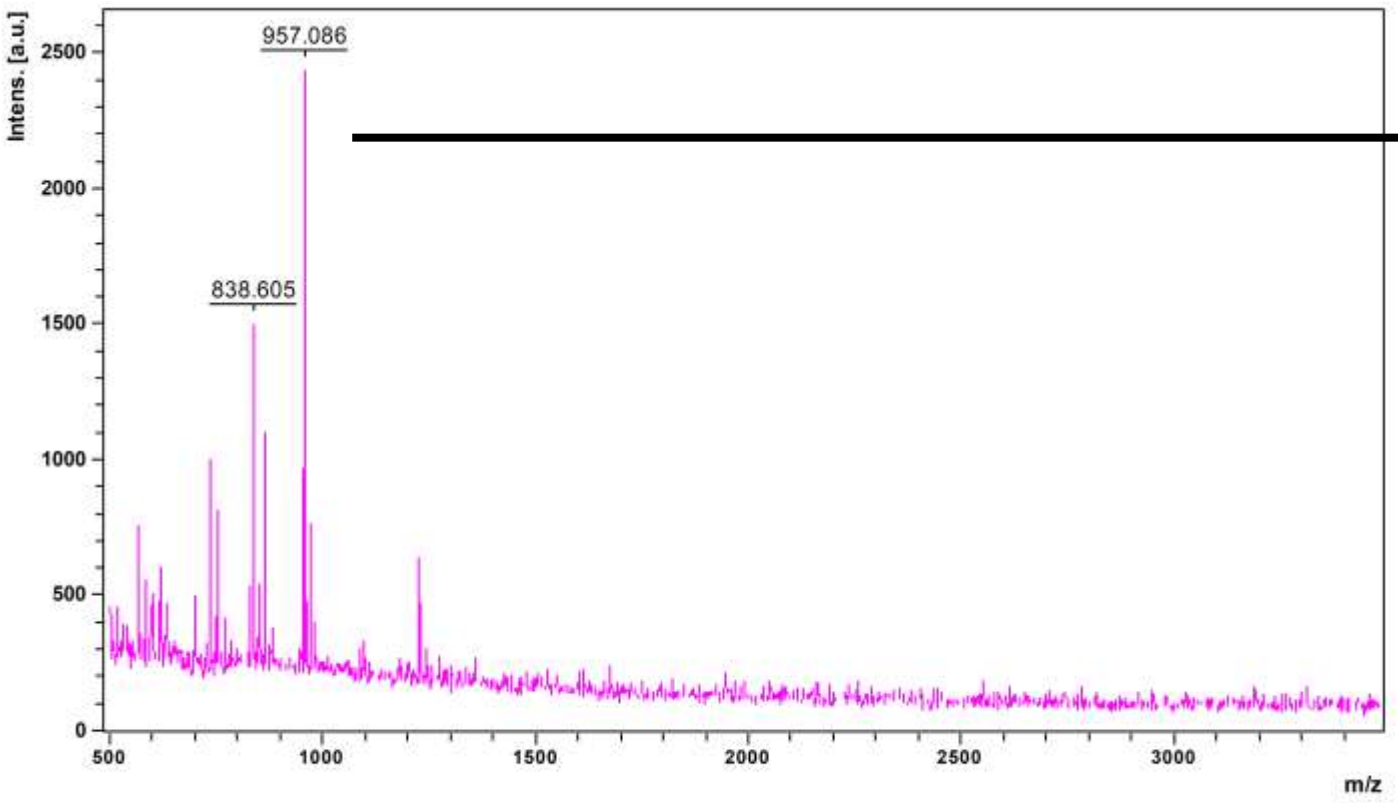


Fig 2. MaldiTOF spectrum of *P. pinea*bark extract

Glucosyl catechin chain ?



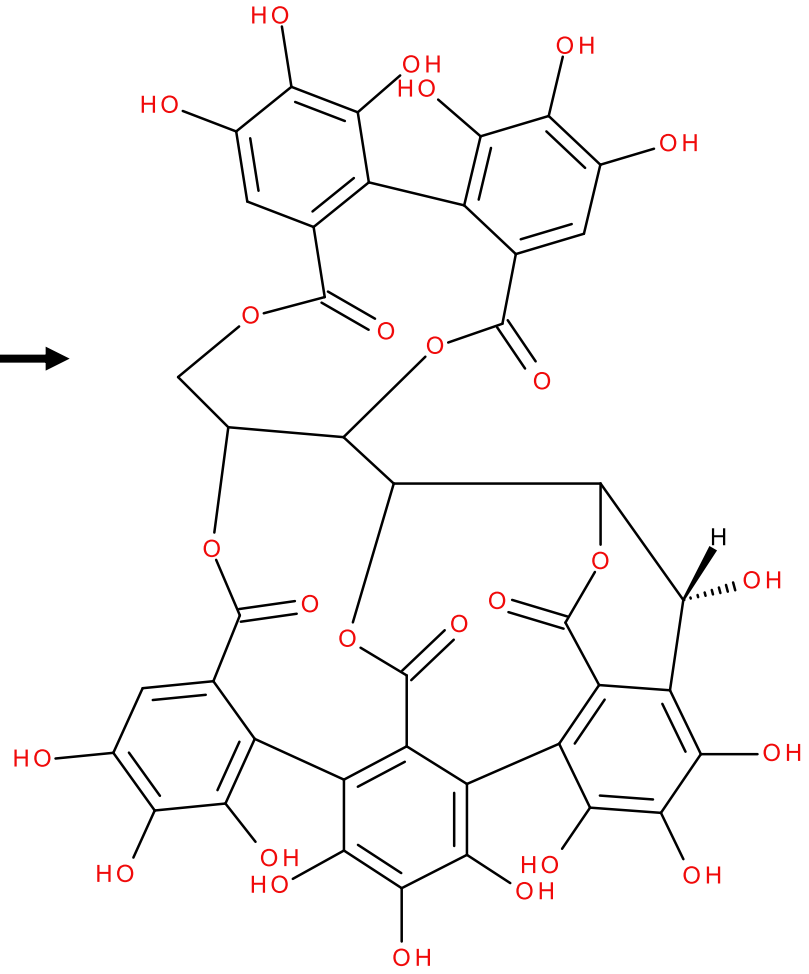


Fig 3. MaldiTOF spectrum of *Q. suber*bark extract

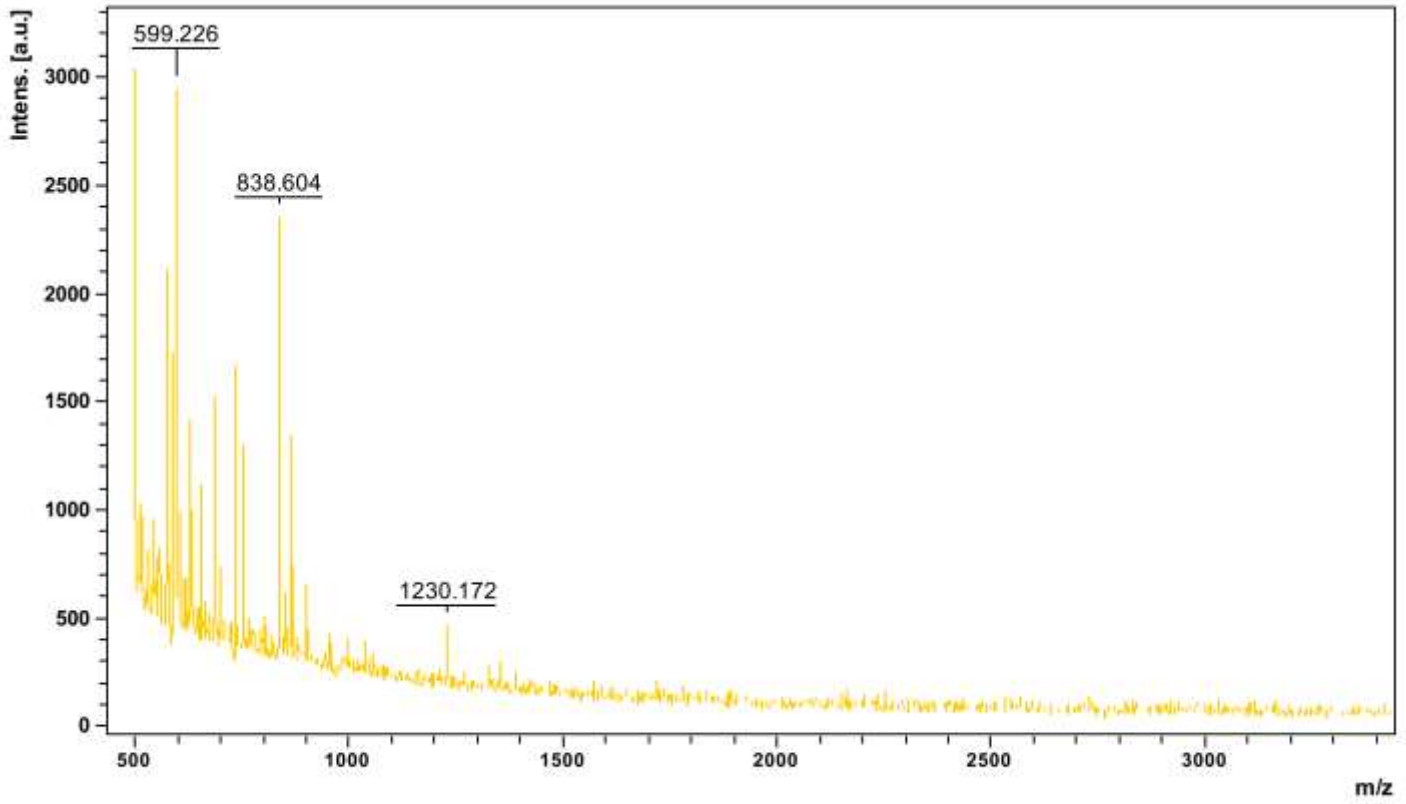


Fig 4. MaldiTOF spectrum of *Q. cerris*bark extract

Contains condensed tannins?

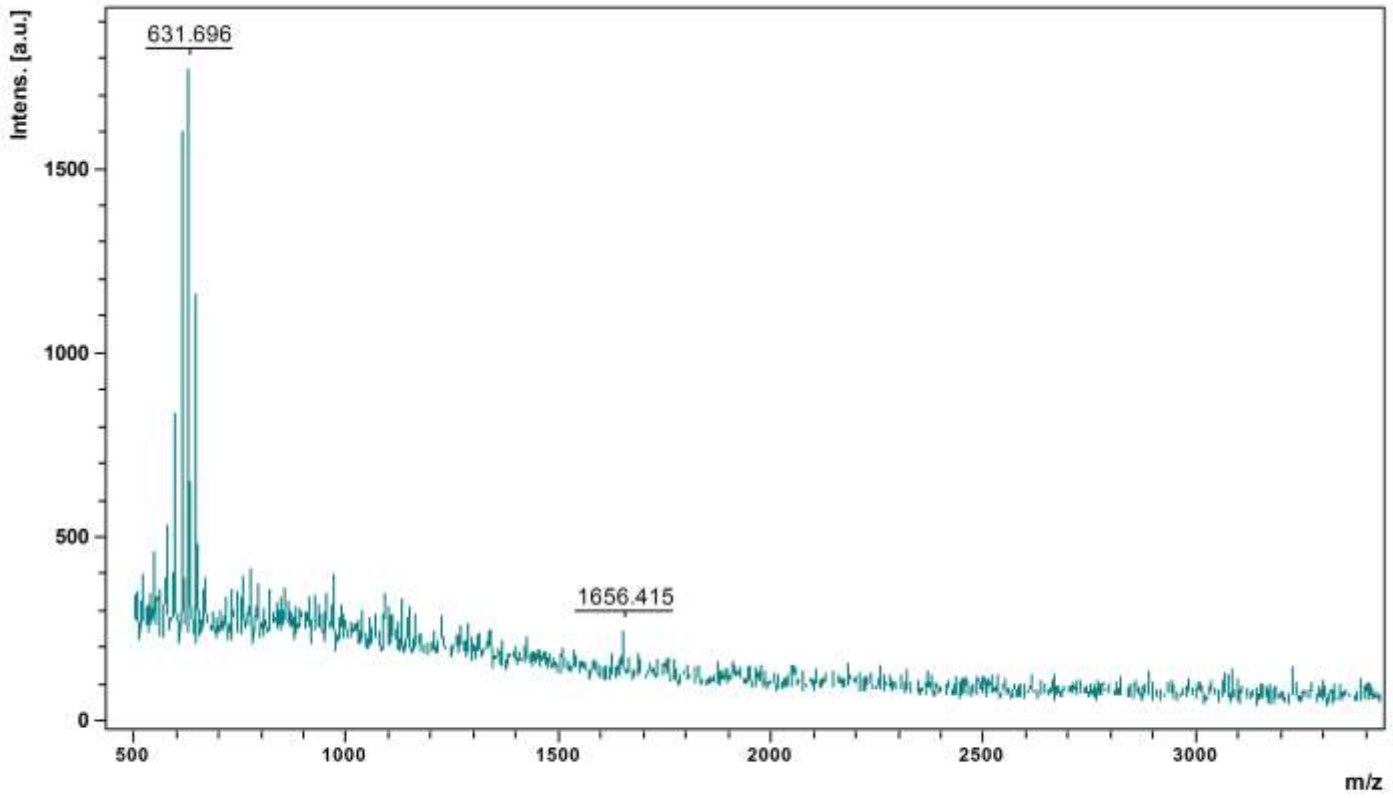
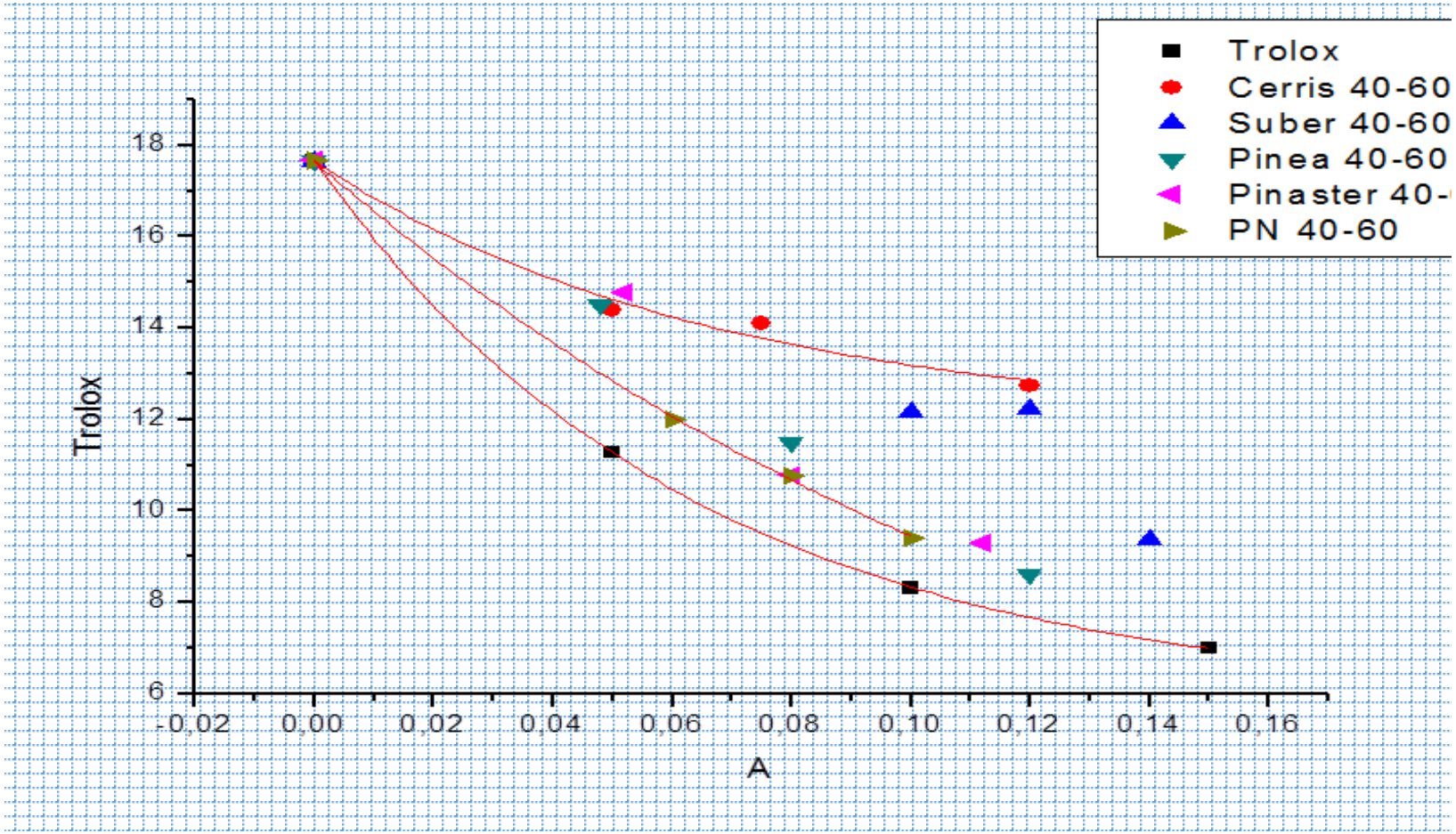
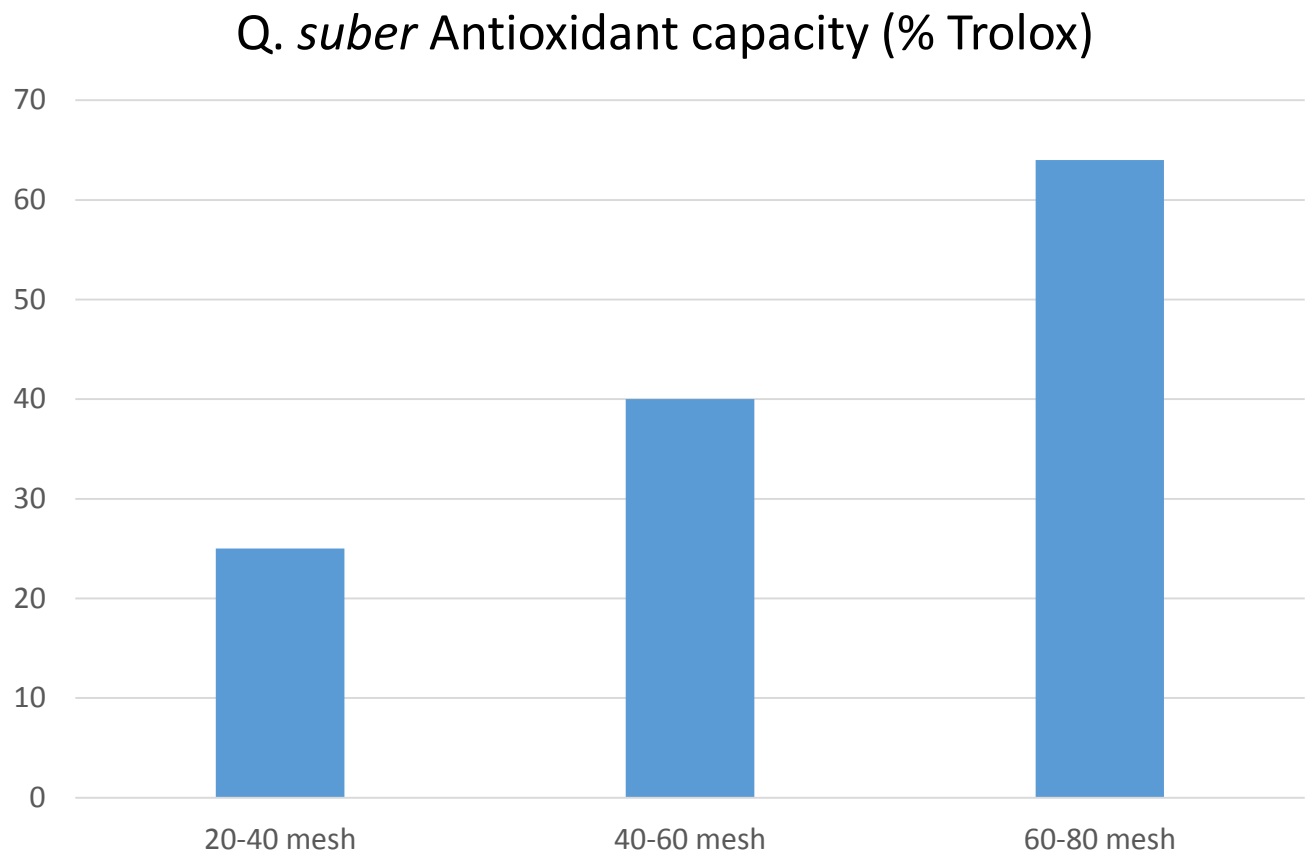


Fig 5. MaldiTOF spectrum of pine nut shell extract

Important amount of N compounds?


Trolox
Cerris 40-60
Suber 40-60
Pinea 40-60
Pinaster 40-
PN 40-60
Trolox
18
16
14
12
10
8
6
-0,02
0,00
0,02
0,04
0,06
0,08
0,10
0,12
0,14
0,16
A


Q. suber Antioxidant capacity (% Trolox)
70
60
50
40
30
20
10
0
20-40 mesh
40-60 mesh
60-80 mesh

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Conclusions

1. MALDI Tof and EPR analyses of bark extracts showed that they have a good potential for the production of phenolics and antioxidants

2. Biomasses vary in polyphenol composition

3. *P. pinea* and *P. pinaster* have similar polyphenol monomers but they differ in catechin series and polymerization degrees

4. *Q. suber* and *Q. cerris* contain ellagi tannins but their composition is diferent. The main ellagi tannin is velcalagin (castalagin)

5. Pine nut shells contain low molecular weight polyphenols with significant antioxidant properties

6. Antioxidant activites of pine barks are higher than those of oak barks

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Thank you for your attention!

Hvala lijepa!