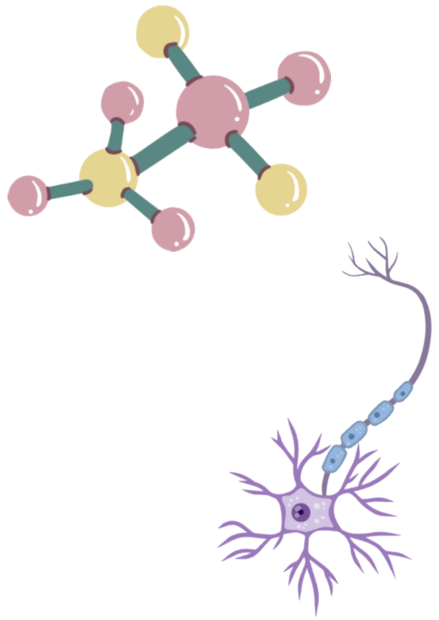
Foodomics study of bioactive compounds from plants, algae and agrifood by-products against Alzheimer Disease

J.D. Sánchez-Martínez, G. Alvarez, A. Valdés, E. Ibáñez, A. Cifuentes

a.cifuentes@csic.es

Foodomics Lab, CIAL, CSIC, Madrid, Spain



Thank you!

2010 2022

130 students from 26 countries at the Foodomics Lab

That is the best part, but…
How is the daily work in a laboratory going?

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How is the daily work in a laboratory going?... a good example may be this one →

How is the daily work in a laboratory going?... a good example may be this one →

…or when you receive the comments to your paper from this kind of reviewers →

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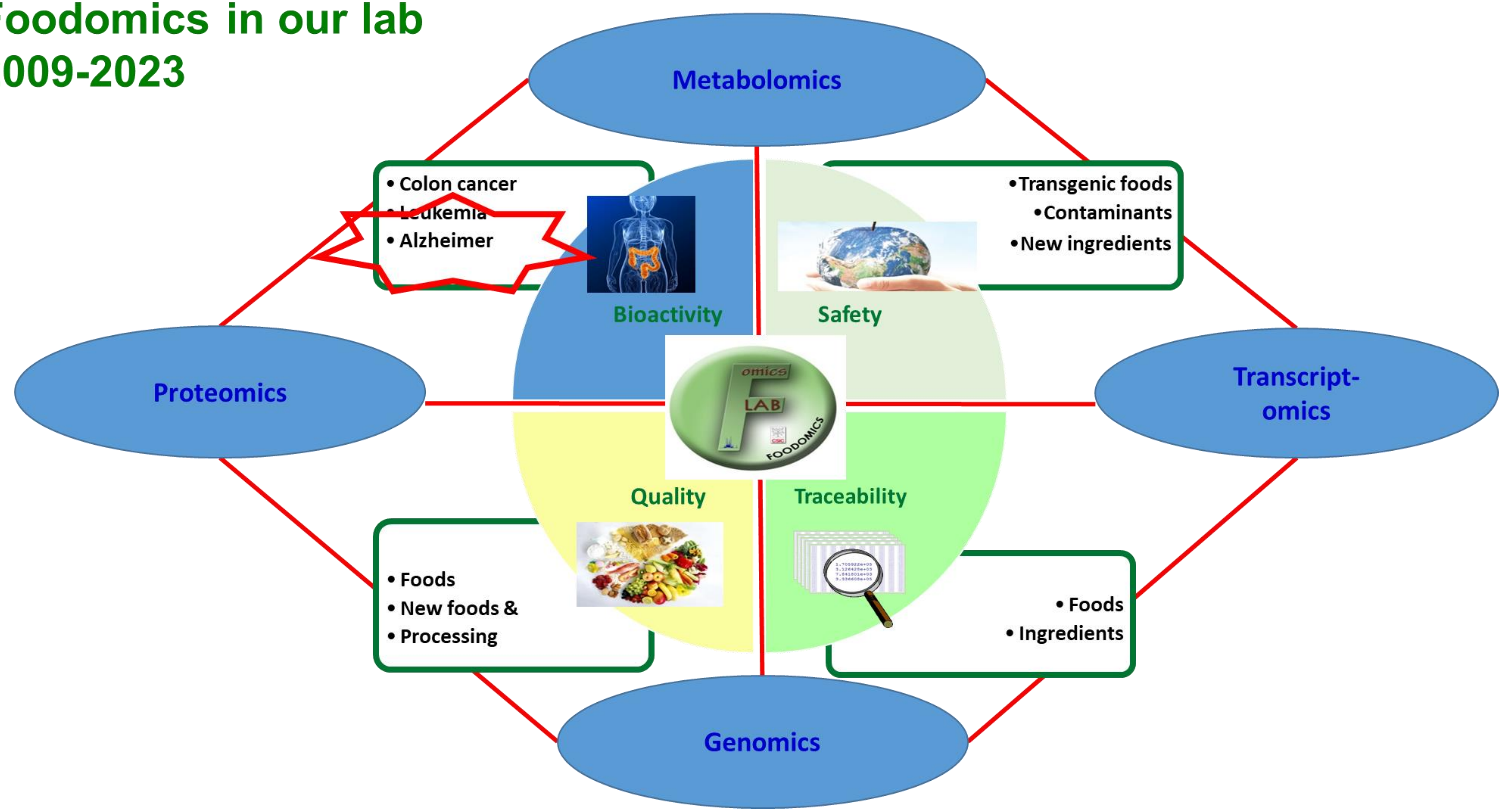
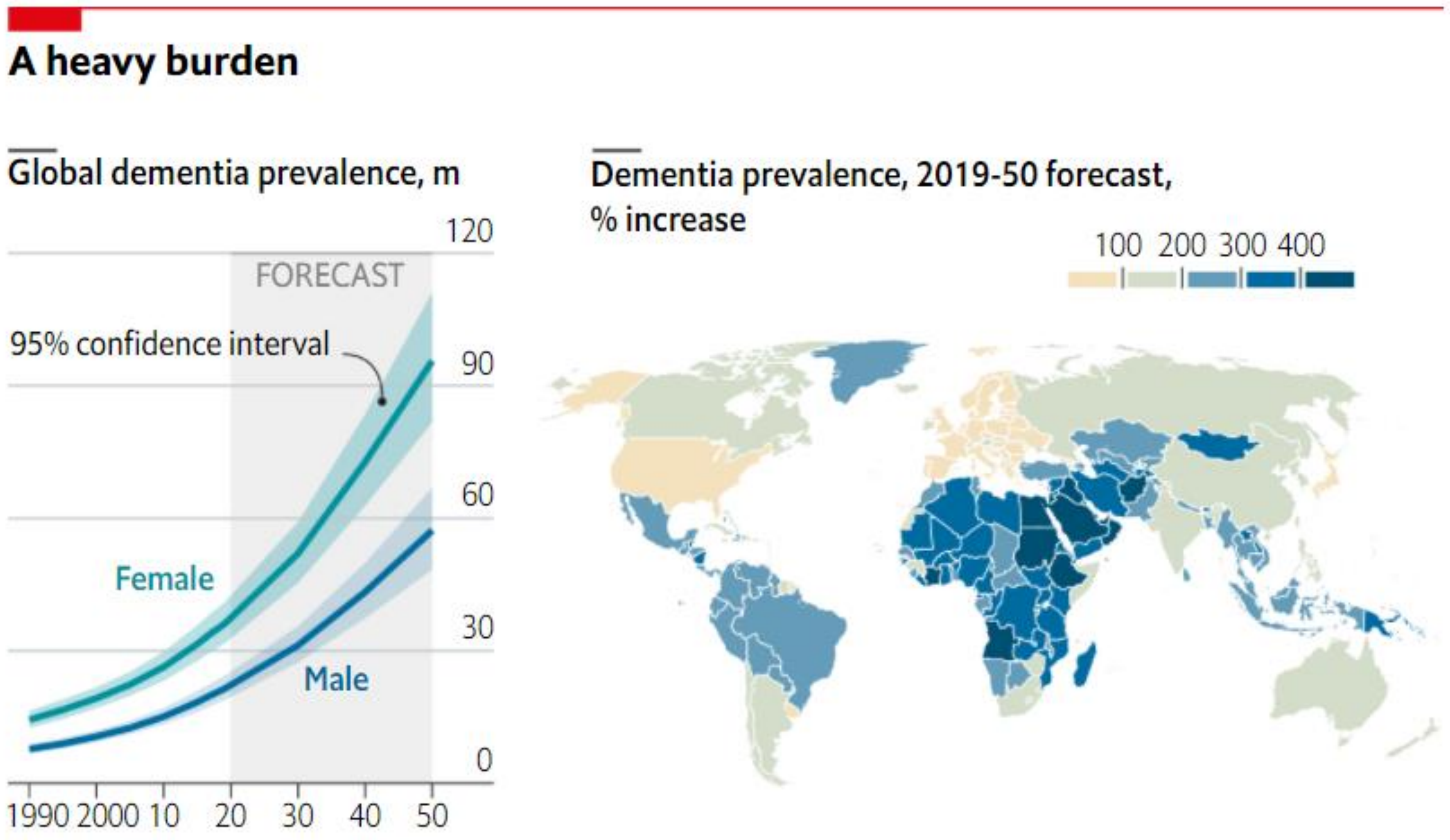
Foodomics in our lab 2009-2023





Source: "Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019", by Emma Nichols et al., *Lancet*, 2022

- Around 55 million of people were diagnosed with dementia in 2020, with forecasts reaching 78 million by 2030 and 153 million in 2050.
- Alzheimer's disease (AD) is the main neurodegenerative disorder and represents nearly 60–70% of dementia cases.

No cure for AD

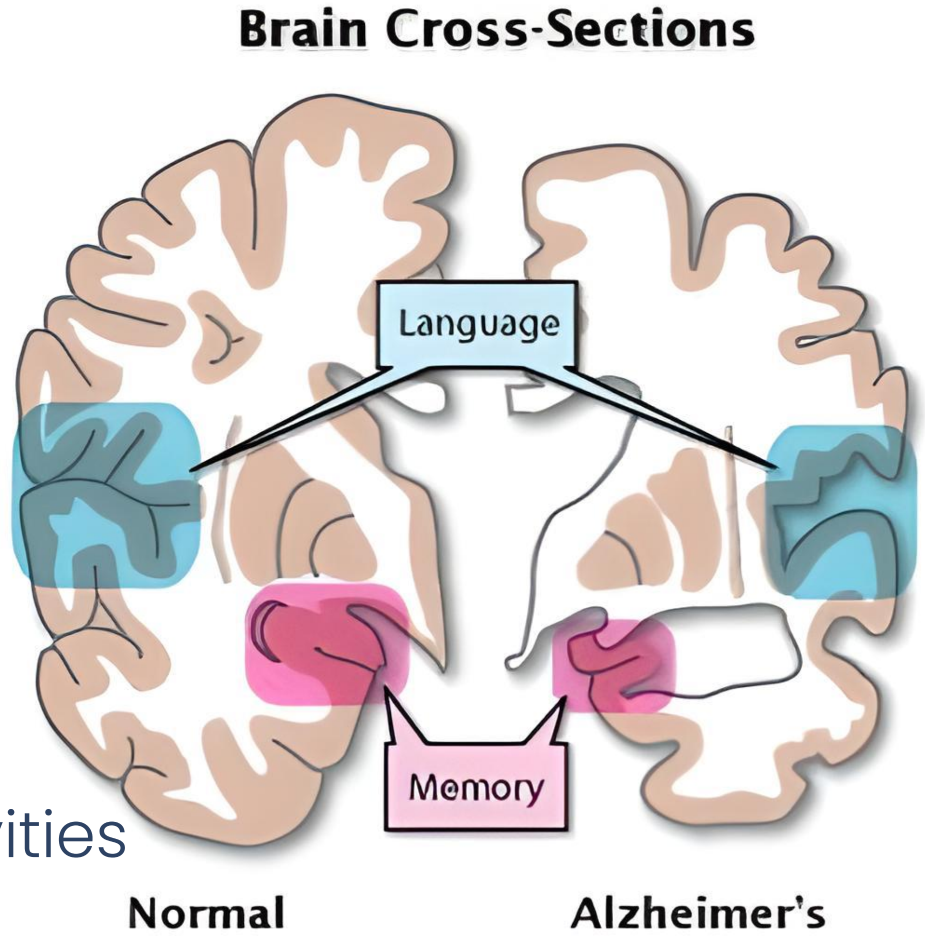
Existing **palliative treatment** consists of the use of drugs such as **galantamine** and rivastigmine, used to increase the **acetylcholine neurotransmitter**.

AD affects to …

Cognitive function

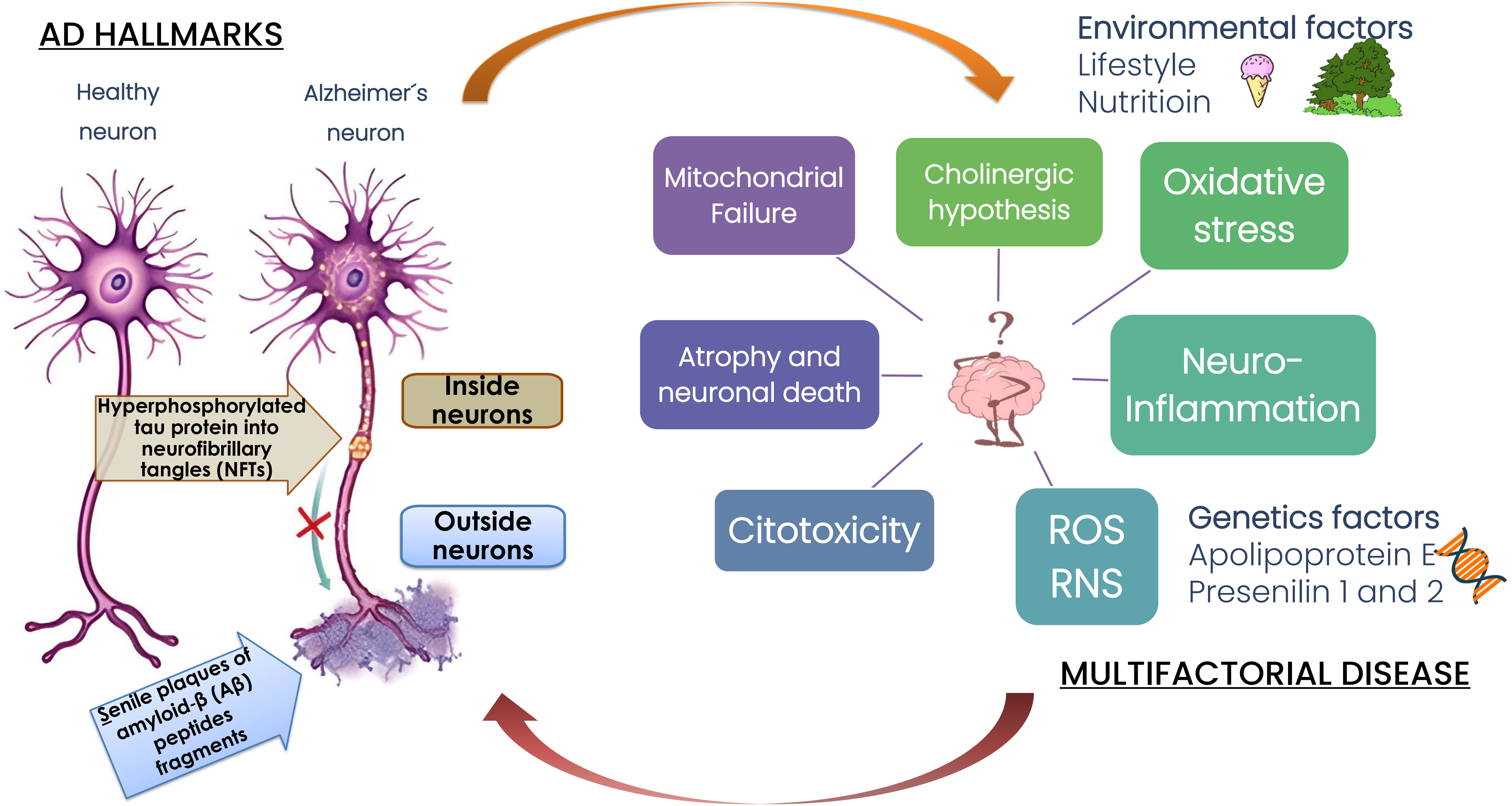
Thinking abilities

Performing daily activities



Multifactorial disease

Nowadays, **AD** is considered a **progressive**, **irreversible** and **incurable** disease, mainly due to their complex and multifactorial nature.


AD HALLMARKS
Healthy neuron
Alzheimer´s neuron
Hyperphosphorylated tau protein into neurofibrillary tangles (NFTs)
Inside neurons
Outside neurons
Senile plaques of amyloid-β (Aβ) peptides fragments
Environmental factors
Lifestyle
Nutritioin
Mitochondrial Failure
Cholinergic hypothesis
Oxidative stress
Atrophy and neuronal death
Neuro-Inflammation
Citotoxicity
ROS
RNS
Genetics factors
Apolipoprotein E
Presenilin 1 and 2
MULTIFACTORIAL DISEASE

In this context…

Natural products from plants are gaining importance as a source of bioactive molecules with high pharmacological and/or nutraceutical value and neuroprotective potential

In particular, some **by-products from agri-food industry** have shown great potential for **revalorization** and generation of high added value products, due to their content in **bioactive molecules** with beneficial effects on health.

Andrade et al. (2019) *Int. J. Mol. Sci*, **20**, 2313
Zhang T et al. (2020) *Alzheimer's Dis* **78**, 887–904

LOW-GENETIC FACTORS

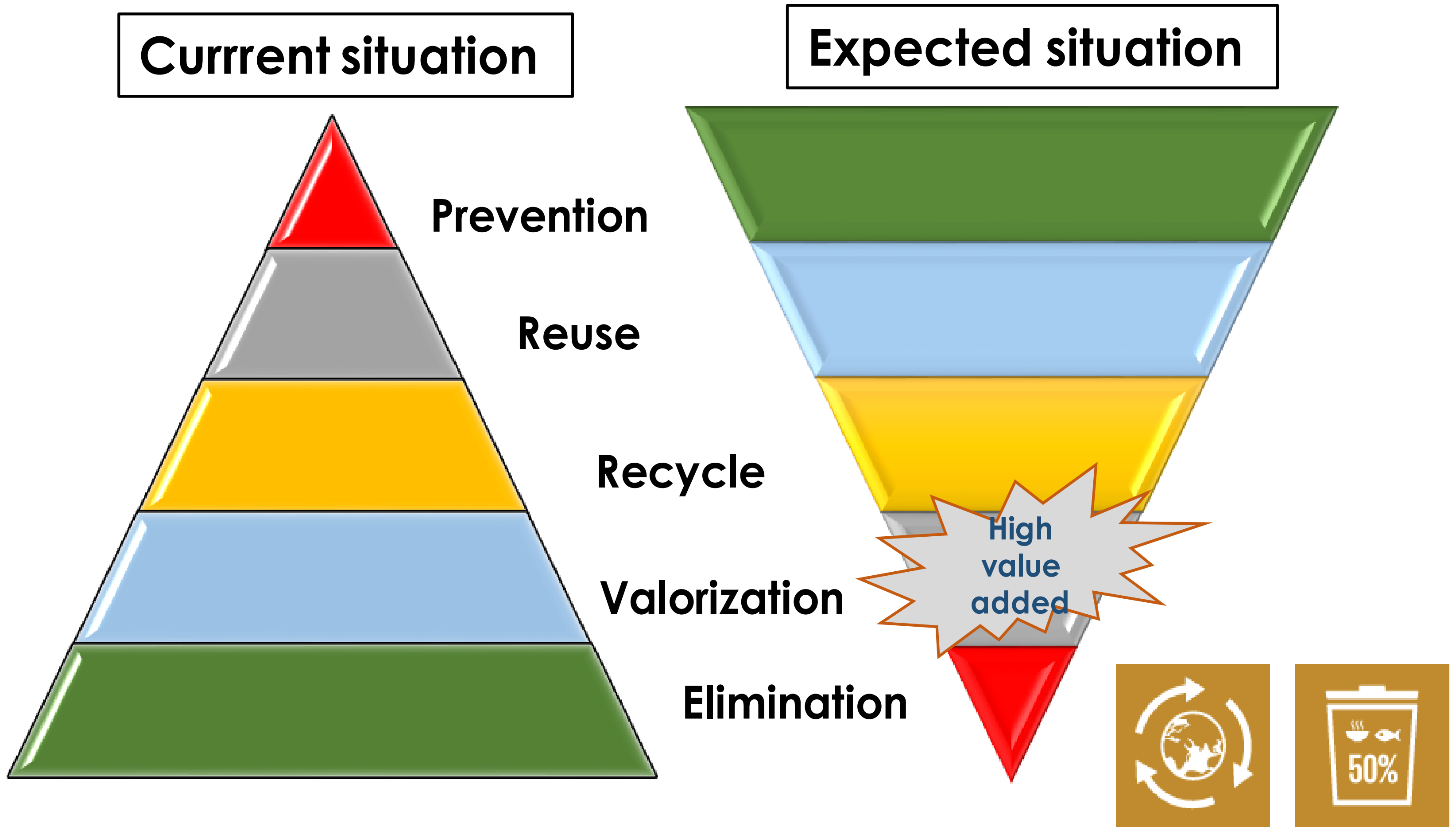
Lifestyle, diet, surrounding environment

According to the UN Environment Programme's Food Waste Index Report 2021, nearly one billion tonnes of food is wasted globally each year.

Traditionally, food waste is incinerated or disposed in landfills with the subsequent **air/water pollution**, and soil/food contamination

Revalorization of **agricultural by-products** has been suggested as a major opportunity to reduce the **environmental impact** and to improve the economical exploitation of **new products** such as nutraceuticals or functional foods

By-products use behavior



OBJECTIVE

To evaluate the **neuroprotective** potential against **AD** of extracts rich in bioactive compounds obtained from **food by-products, plants and algae.**

METHODOLOGY

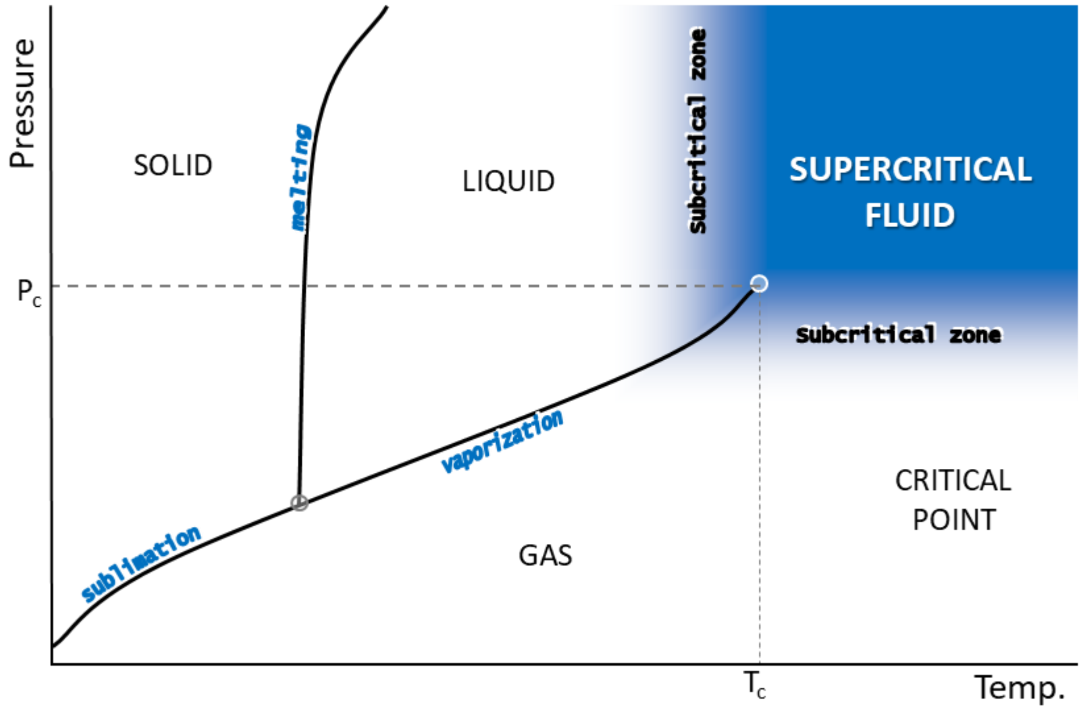
Extraction of bioactive compounds

Green Technologies

Pressurized liquid Extraction **(PLE)**

Supercritical Fluid Extraction **(SFE)**

Ultrasound -Assisted Extraction **(UAE)**



GRAS SOLVENTS (CO_2, EtOH, water, ethyl acetate…)

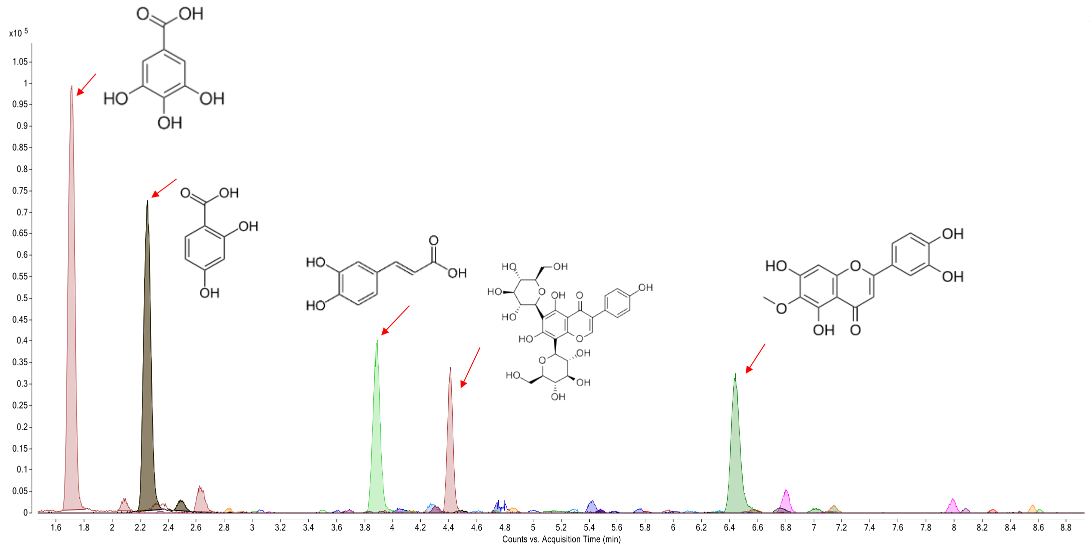
Thermodynamic properties of Liquid & Gas

- Low viscosity
- Diffusivity
- Density to liquids

Characterization of bioactive compounds

Advanced analytical techniques

UHPLC-QTOF-MS/MS

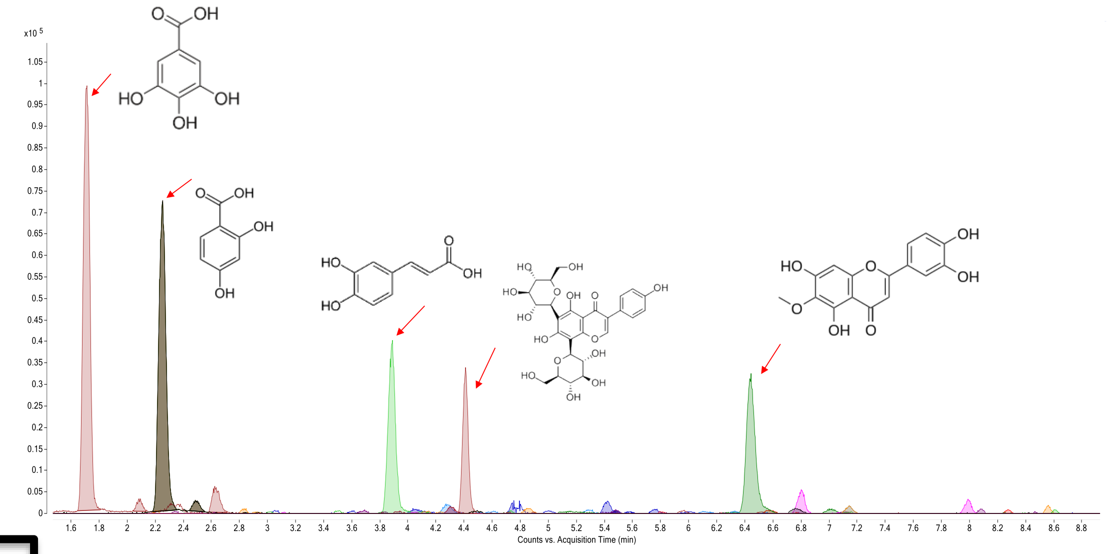


GC-QTOF-MS/MS

Characterization of bioactive compounds

Advanced analytical techniques

UHPLC-QTOF-MS/MS



Polar extract

Non-polar extract

LC-ESI(+/-)-Q-TOF-MS/MS Analysis

Full scan MS data

Peak list (m/z values)

MDF
(Peak detection in HREIC)

Molecular formula

MS/MS fragmentation data

NLF

DPIF

Structural information

HRMS/MS databases
Massbank
Pubmed
HMDB
Metlin
Bibliographic search

GC-Q-TOF-MS Analysis

Full scan MS data

Peak detection

Deconvolution

Library search
NIST Library

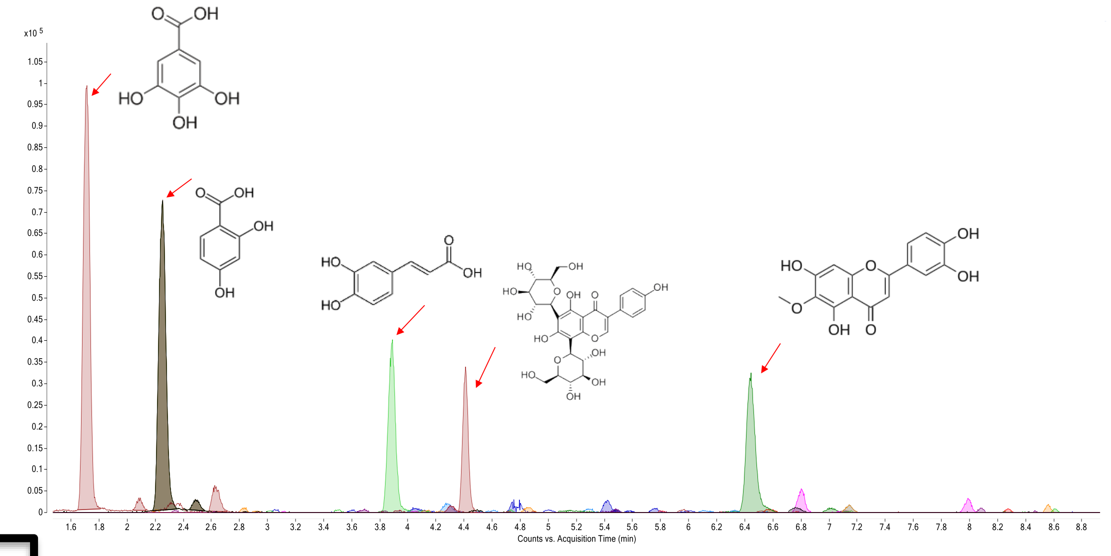
Unknown analysis
Agilent MassHunter Unknown Analysis Tool

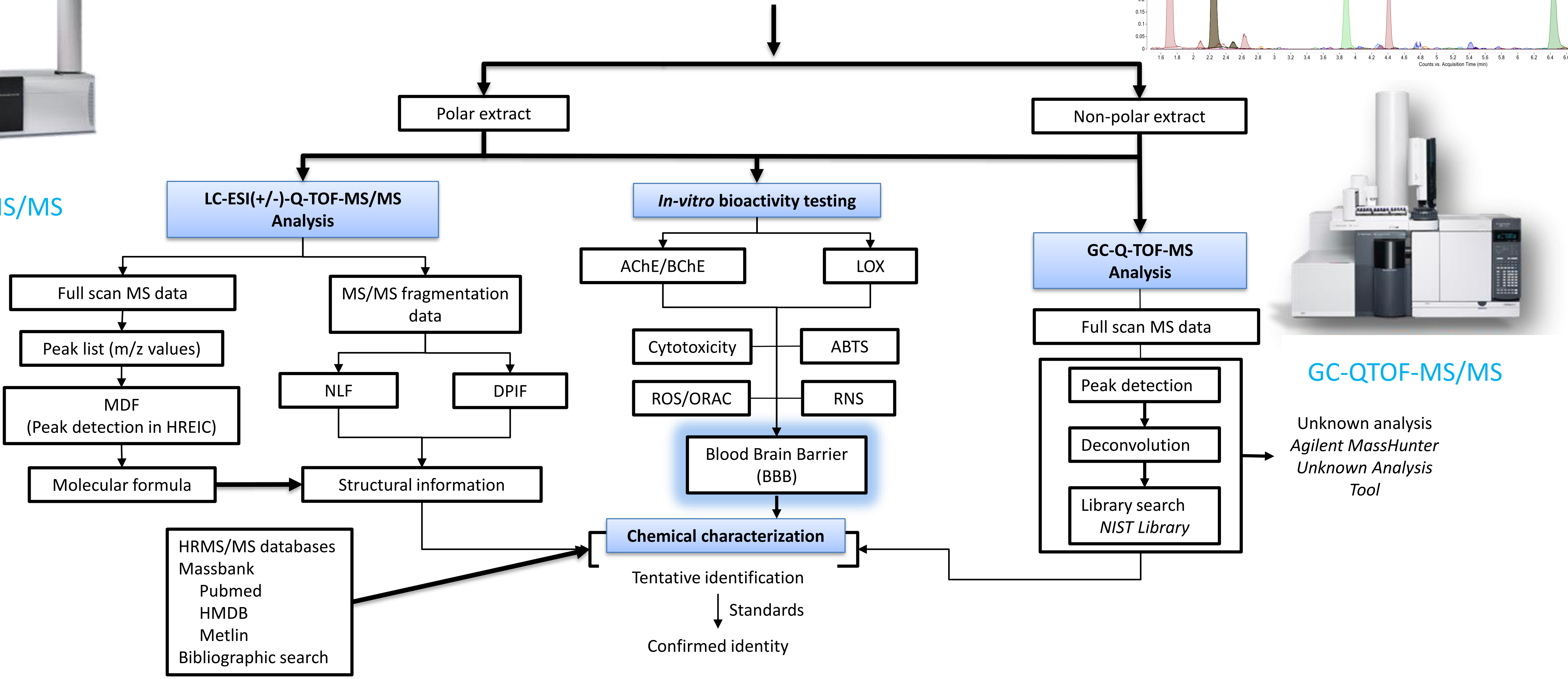
GC-QTOF-MS/MS

Characterization of bioactive compounds

Advanced analytical techniques

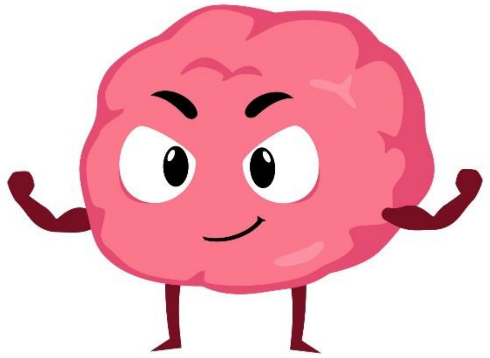
UHPLC-QTOF-MS/MS



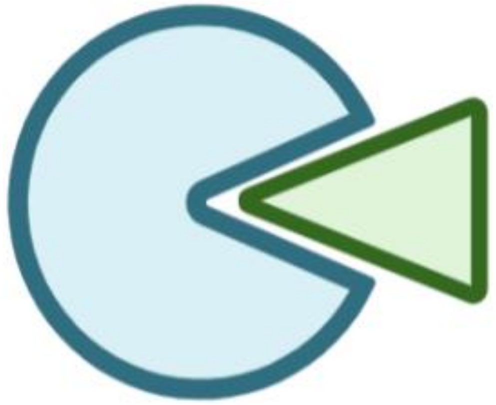


NEUROPROTECTIVE ASSESSMENT

Biological evaluation of bioactive compounds against Alzheimer´s disease hallmarks



1 Cholinergic enhancement



AChE / BChE Inhibition

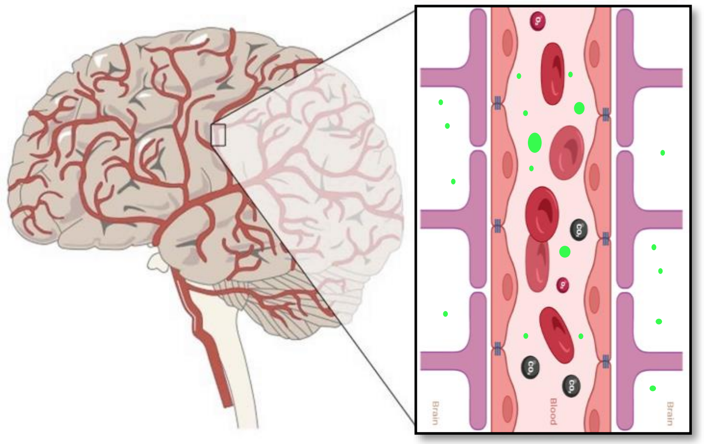
2 Neuroinflammation

LOX Inhibition

3 Antioxidants

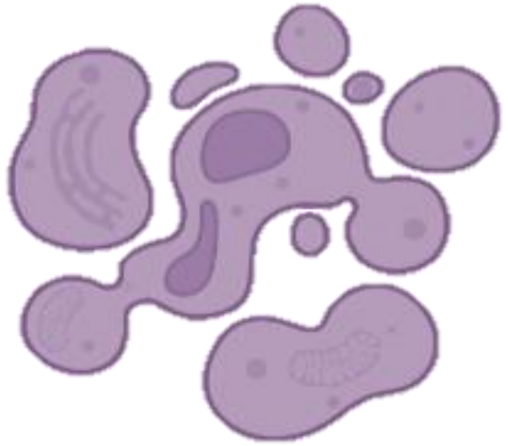
ABTS – ROS - RNS

4 CNS accessible



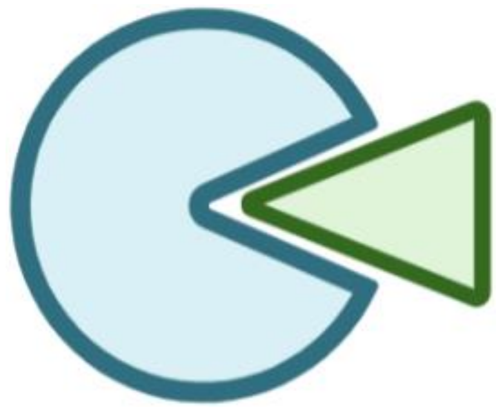
Blood–brain barrier (BBB) MODEL

5 Cytotoxicity assay



6 *C.elegans in vivo* AD model

1 Cholinergic enhancement



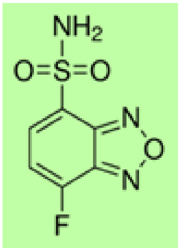
AChE / BChE Inhibition

$$\text{acetylthiocholine} \xrightarrow{\text{(enzyme)}} \text{thiocholine} + \text{acetate}$$

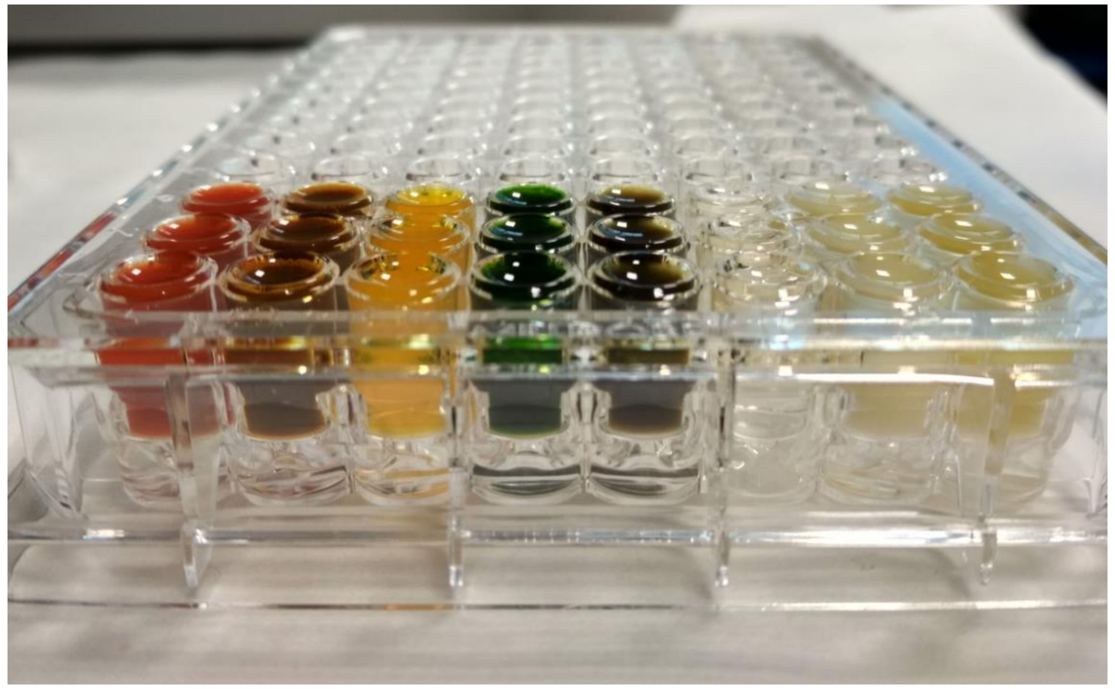
$$\text{thiocholine} + \text{dithiobisnitrobenzoate} \longrightarrow \text{yellow color}$$

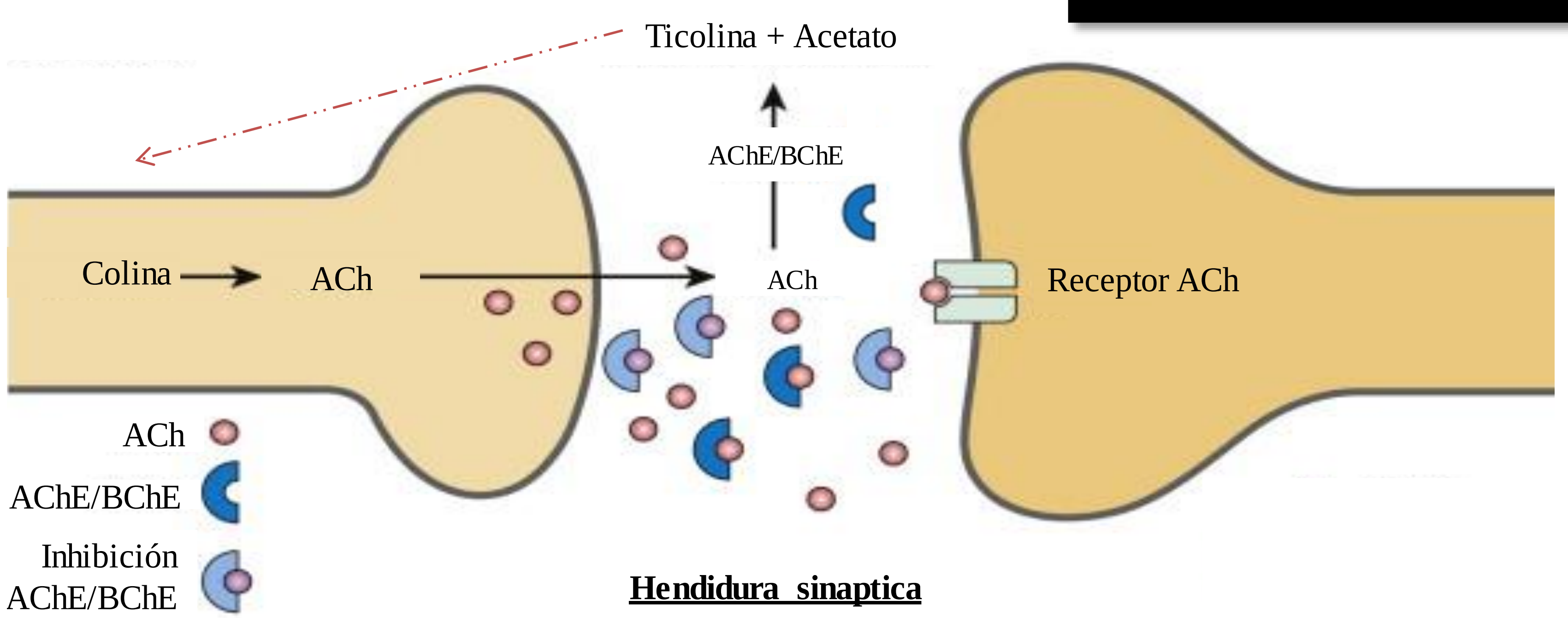
Ellman colorimetric method 1961

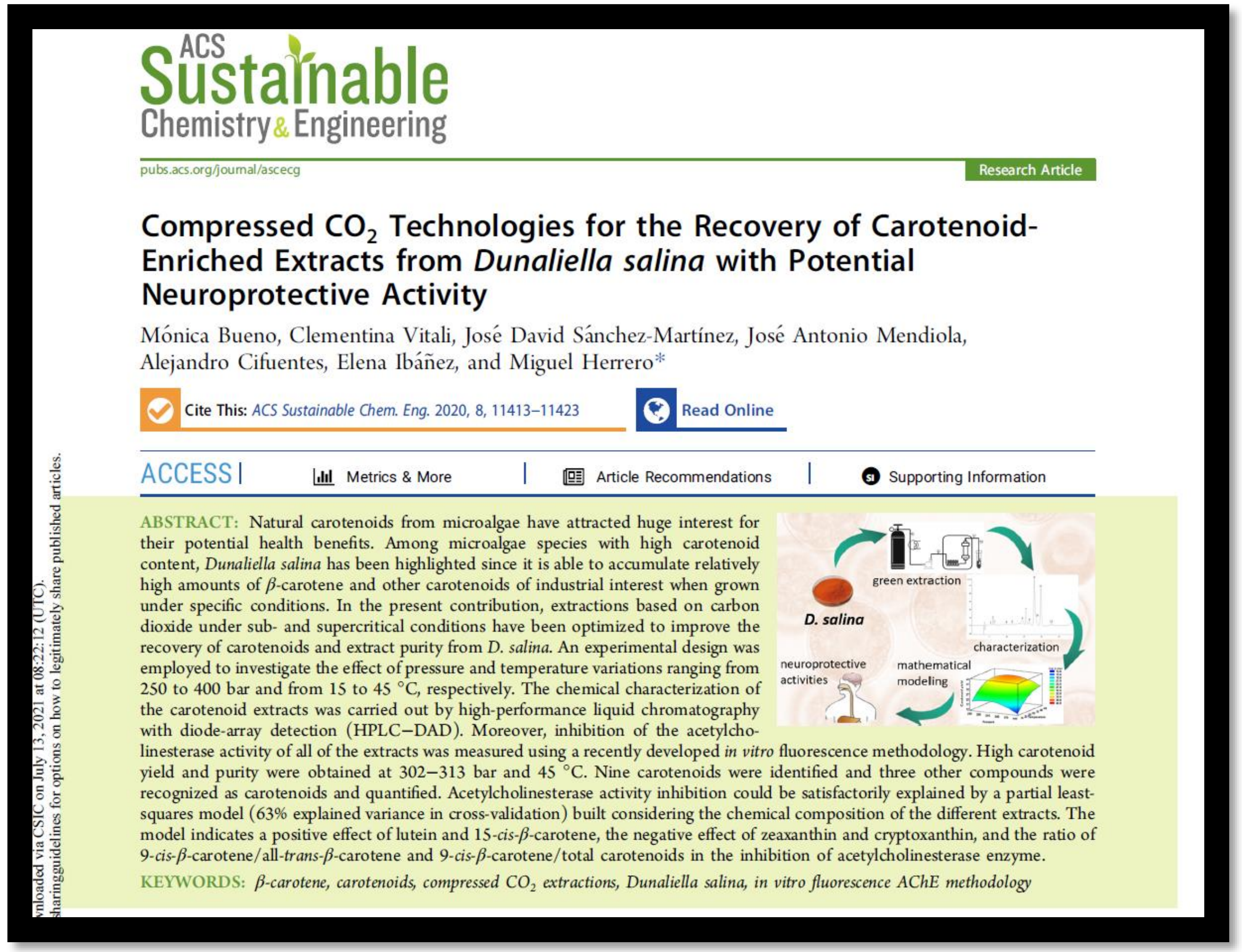
ABD-F



Sinapsis colinérgica







ACS Sustainable Chemistry & Engineering

pubs.acs.org/journal/ascecg — Research Article

Compressed CO_2 Technologies for the Recovery of Carotenoid-Enriched Extracts from *Dunaliella salina* with Potential Neuroprotective Activity

Mónica Bueno, Clementina Vitali, José David Sánchez-Martínez, José Antonio Mendiola, Alejandro Cifuentes, Elena Ibáñez, and Miguel Herrero*

Cite This: *ACS Sustainable Chem. Eng.* 2020, 8, 11413–11423 — Read Online

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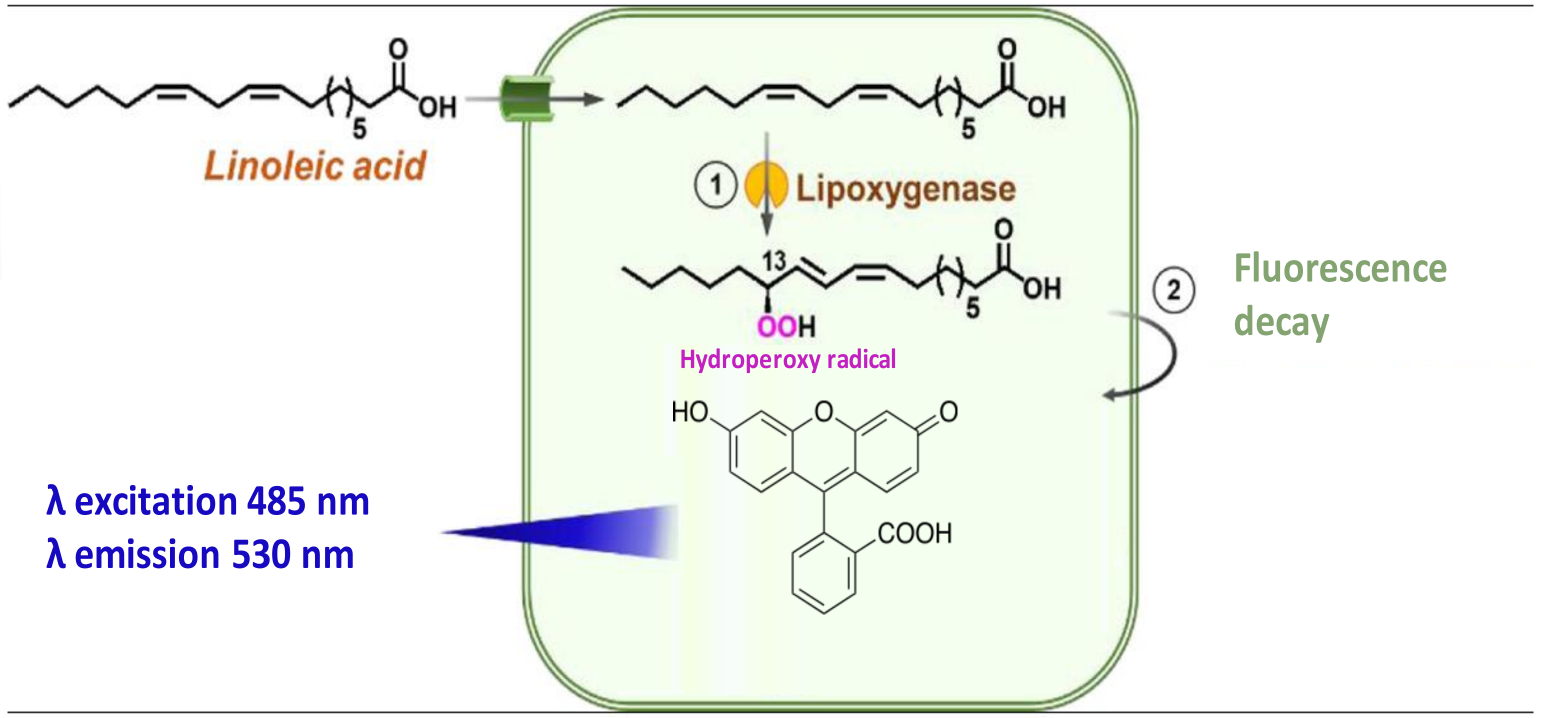
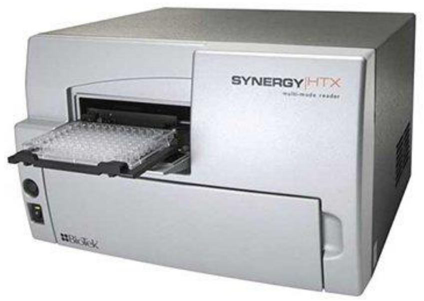
ABSTRACT: Natural carotenoids from microalgae have attracted huge interest for their potential health benefits. Among microalgae species with high carotenoid content, *Dunaliella salina* has been highlighted since it is able to accumulate relatively high amounts of β-carotene and other carotenoids of industrial interest when grown under specific conditions. In the present contribution, extractions based on carbon dioxide under sub- and supercritical conditions have been optimized to improve the recovery of carotenoids and extract purity from *D. salina*. An experimental design was employed to investigate the effect of pressure and temperature variations ranging from 250 to 400 bar and from 15 to 45 °C, respectively. The chemical characterization of the carotenoid extracts was carried out by high-performance liquid chromatography with diode-array detection (HPLC−DAD). Moreover, inhibition of the acetylcholinesterase activity of all of the extracts was measured using a recently developed *in vitro* fluorescence methodology. High carotenoid yield and purity were obtained at 302−313 bar and 45 °C. Nine carotenoids were identified and three other compounds were recognized as carotenoids and quantified. Acetylcholinesterase activity inhibition could be satisfactorily explained by a partial least-squares model (63% explained variance in cross-validation) built considering the chemical composition of the different extracts. The model indicates a positive effect of lutein and 15-*cis*-β-carotene, the negative effect of zeaxanthin and cryptoxanthin, and the ratio of 9-*cis*-β-carotene/all-*trans*-β-carotene and 9-*cis*-β-carotene/total carotenoids in the inhibition of acetylcholinesterase enzyme.

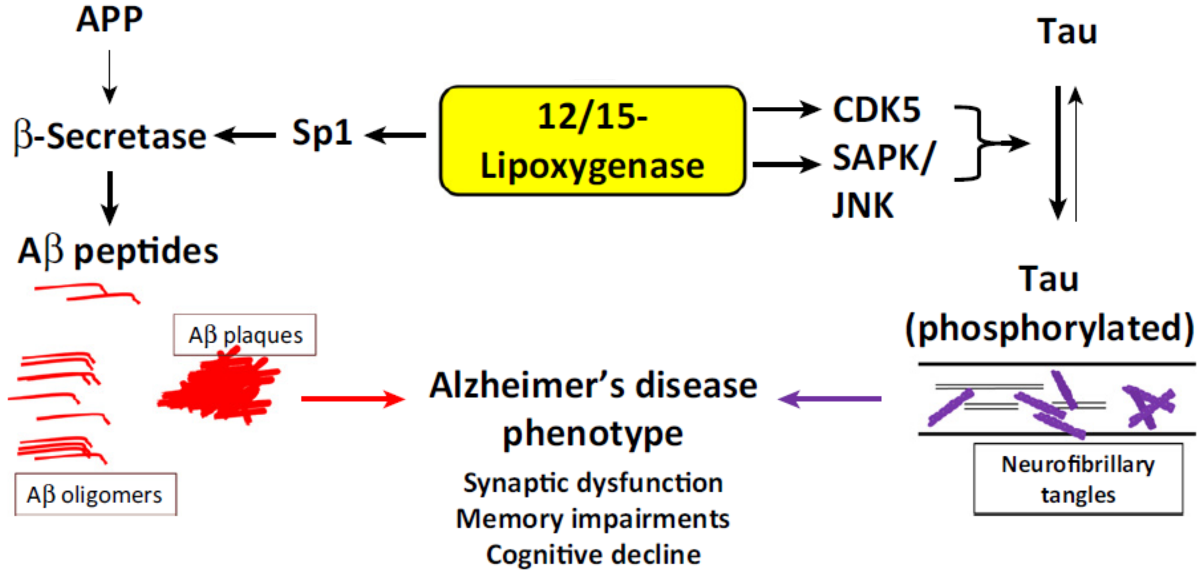
KEYWORDS: *β-carotene, carotenoids, compressed CO_2 extractions, Dunaliella salina, in vitro fluorescence AChE methodology*

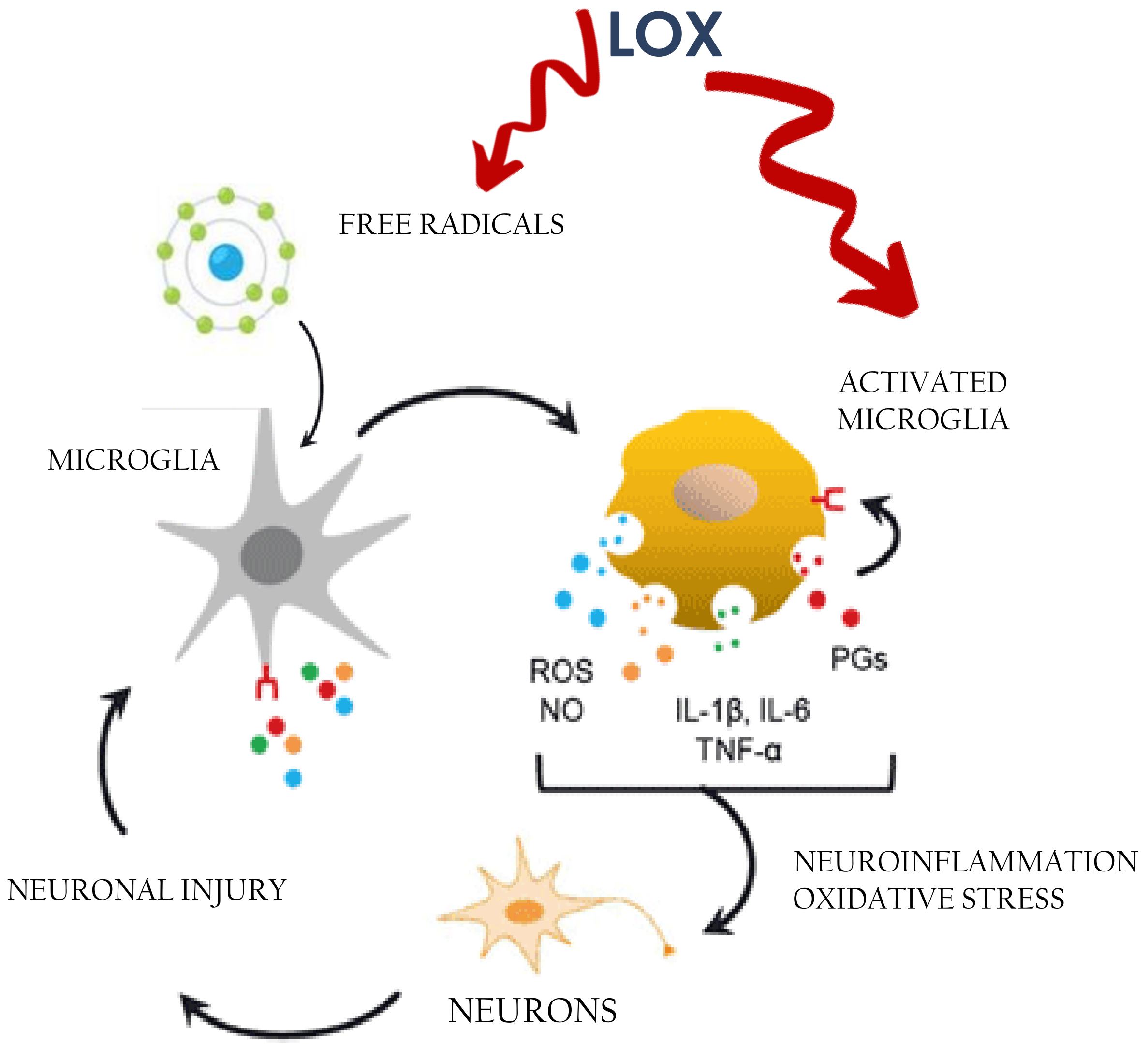
2 Neuroinflammation

LOX Inhibition



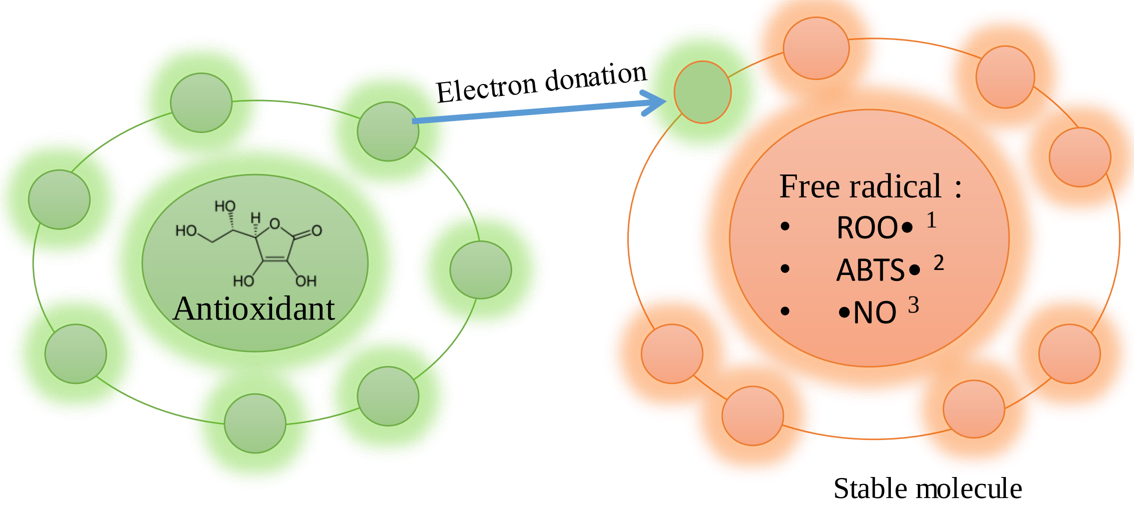






3 Antioxidants

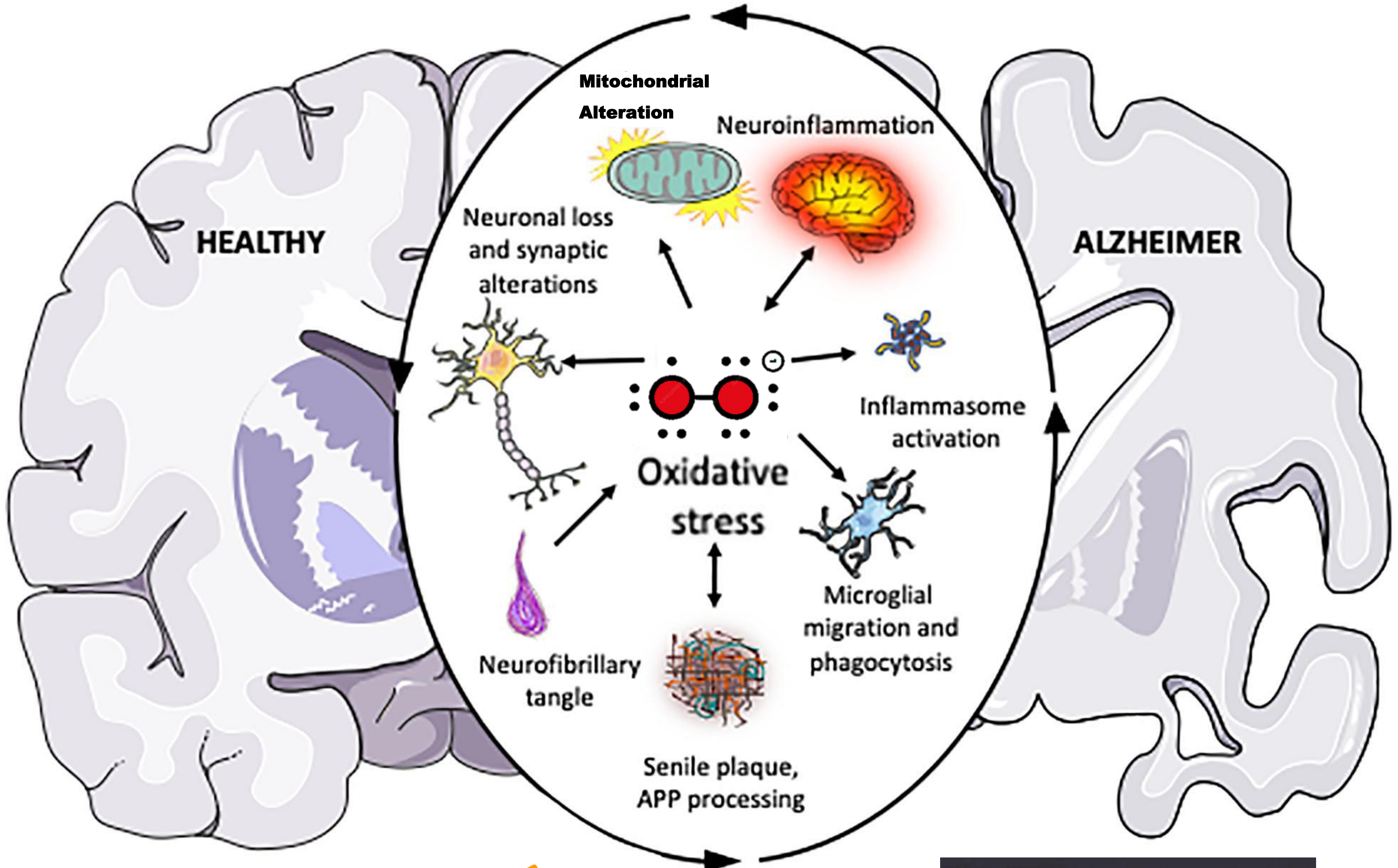
Antioxidant   Free radical   Healthy cell

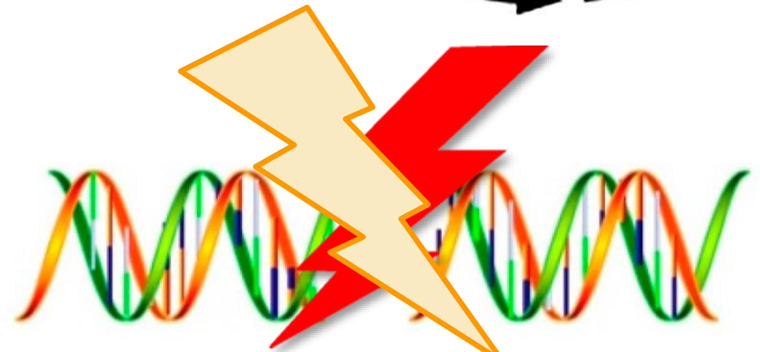


ROS + RNS

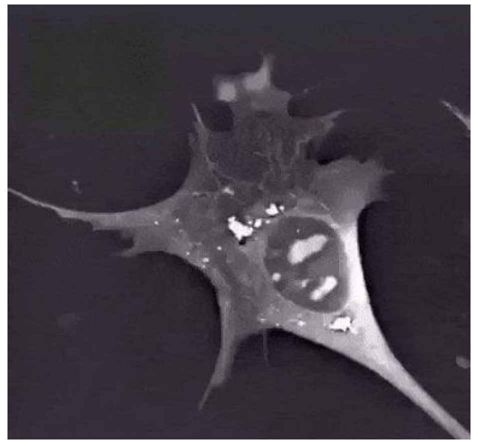
$\bullet NO + O_2\bullet^- = ONOO^-$

Peroxynitrite





Oxidative DNA damage



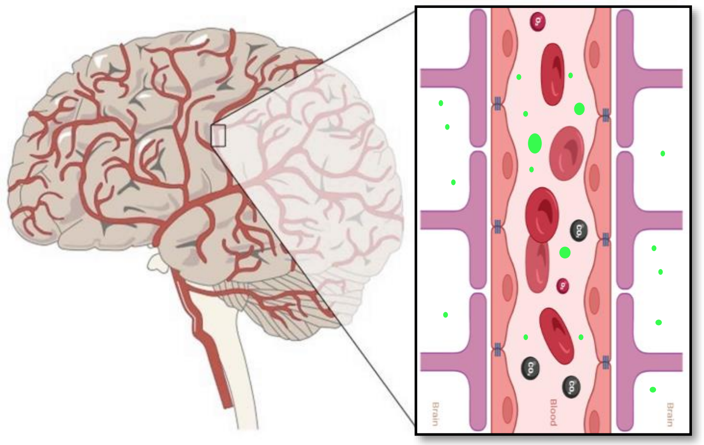
Neuronal cell death

Article **nature**

Somatic genomic changes in single Alzheimer's disease neurons

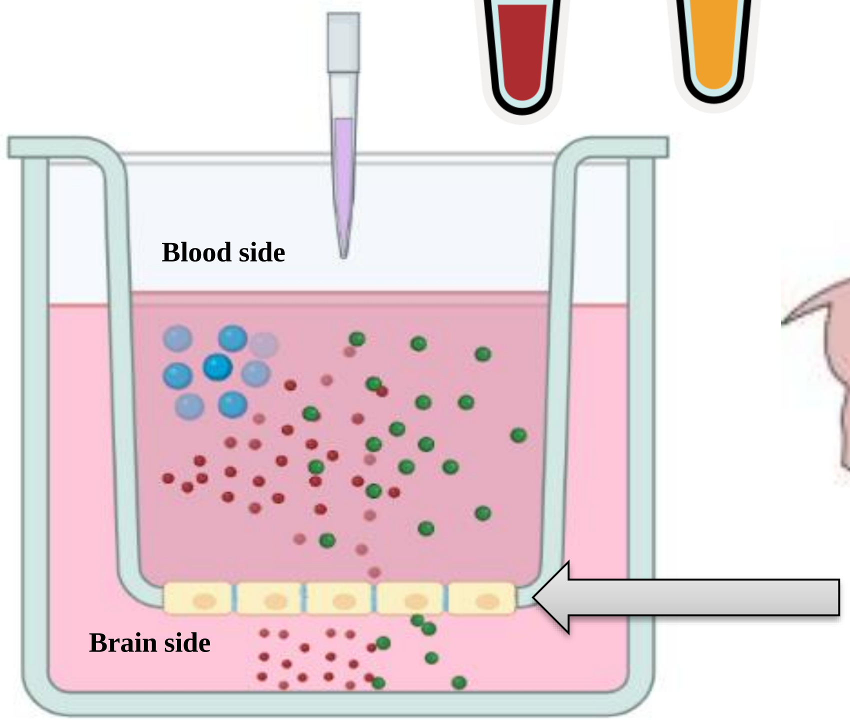
Miller et al., (2022) *Nature*, **604(7907)**, 714–722

4 CNS accessible

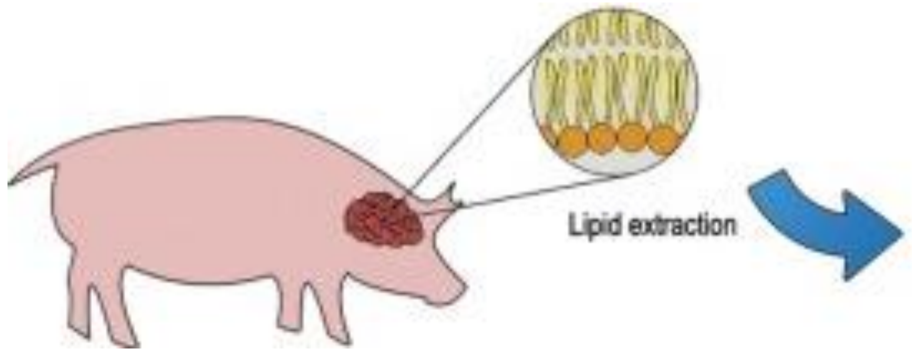


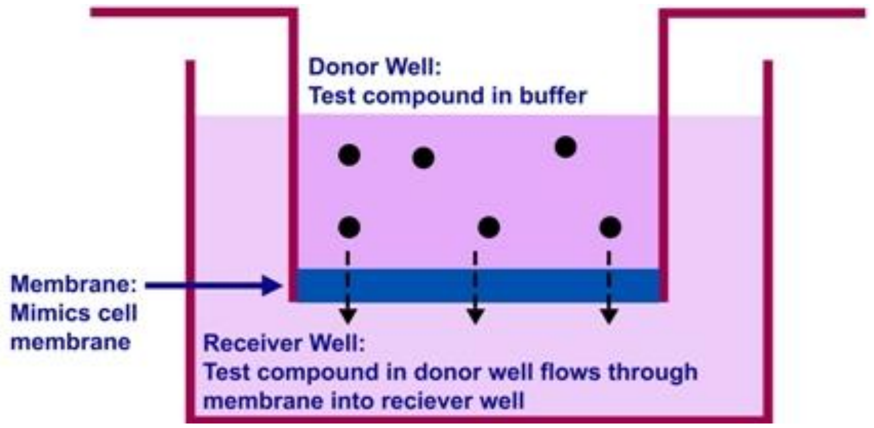
Blood–brain barrier (BBB) MODEL

Extracts



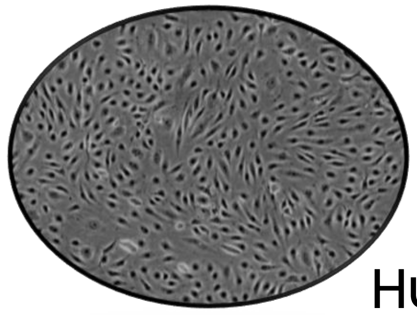
Brain Polar Extract Phospholipid Profile	
Component	wt/wt%
PC	12.6
PE	33.1
PI	4.1
PS	18.5
PA	0.8
Unknown	30.9
Total	100.0



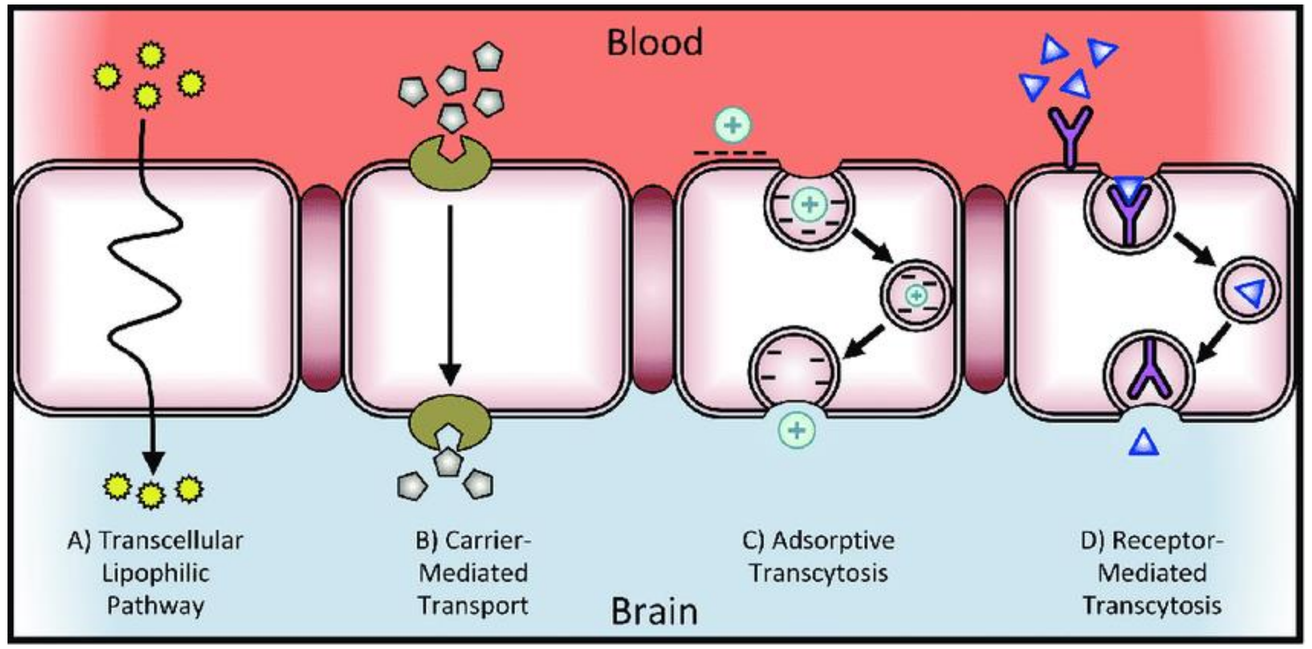


PAMPA-BBB (Specific porcine Brain Polar Lipid Extract)

HBMEC monolayer



Human brain microvascular endothelial cells



PAMPA-BBB

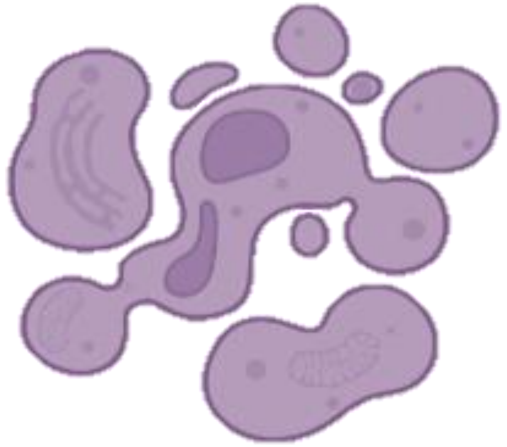
HBMEC-BBB

LISBOA UNIVERSIDADE DE LISBOA

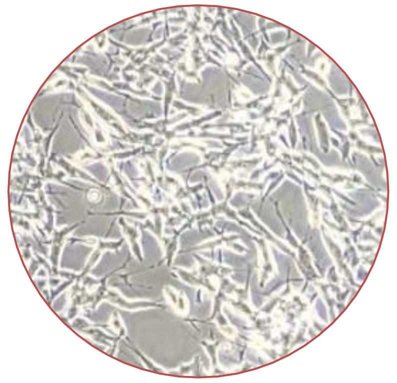
FACULDADE DE FARMÁCIA Universidade de Lisboa

NEUROVASCULAR LABORATORY

5 Cytotoxicity assay

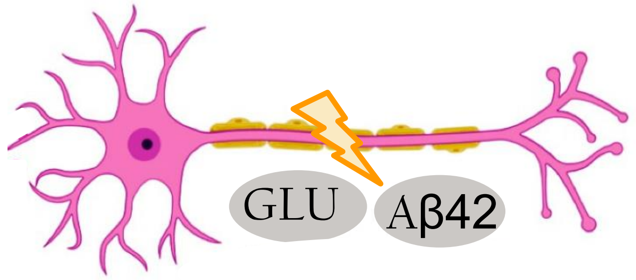


SH-SY5Y



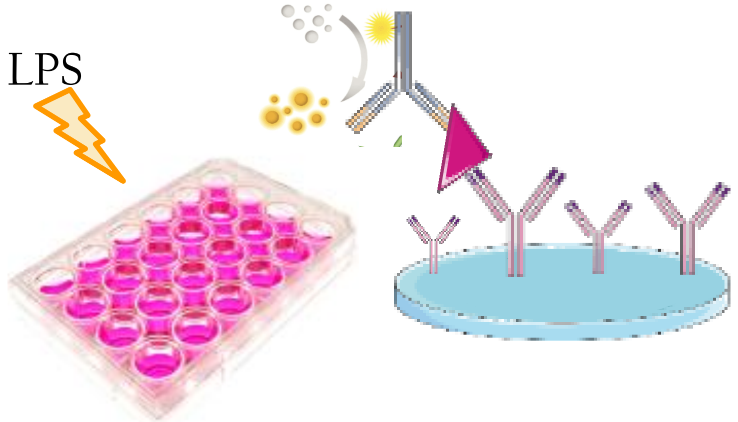
Neuroblastoma

NEUROPROTECTIVE POTENTIAL AGAINST NEUROTOXIC AGENTS



THP-1

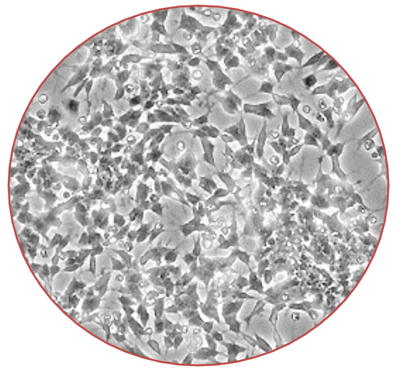
THP-1 monocytes



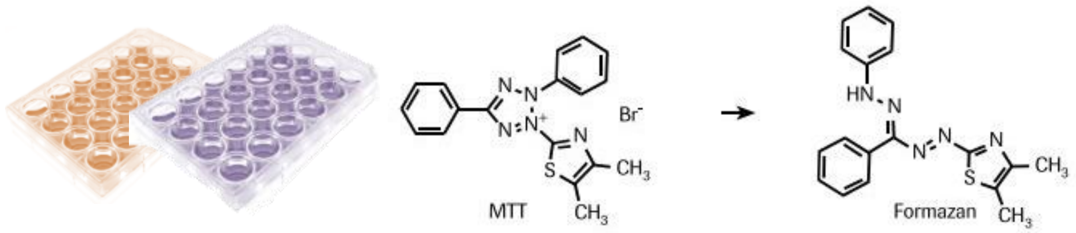
INFLAMMATORY RESPONSES

IL-6, IL-1β and TNF-α

HK-2



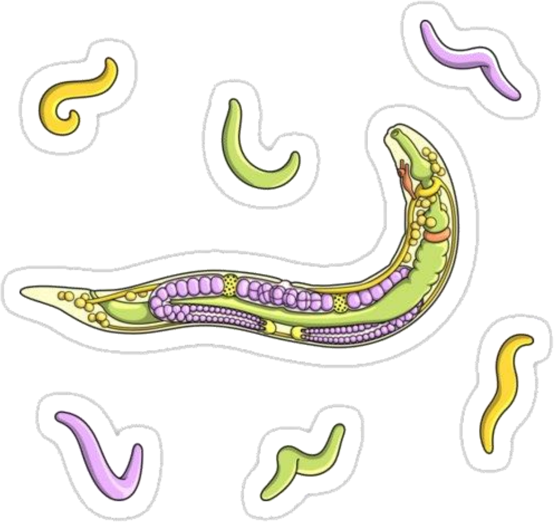
Proximal tubular epithelial cells



6 *C.elegans in vivo* AD model

BIOLOGICAL PROPERTIES

1 mm



Short life cycle

Adulthood in 3 days

Easy cultivation

Life expectancy

21 days at 25°C

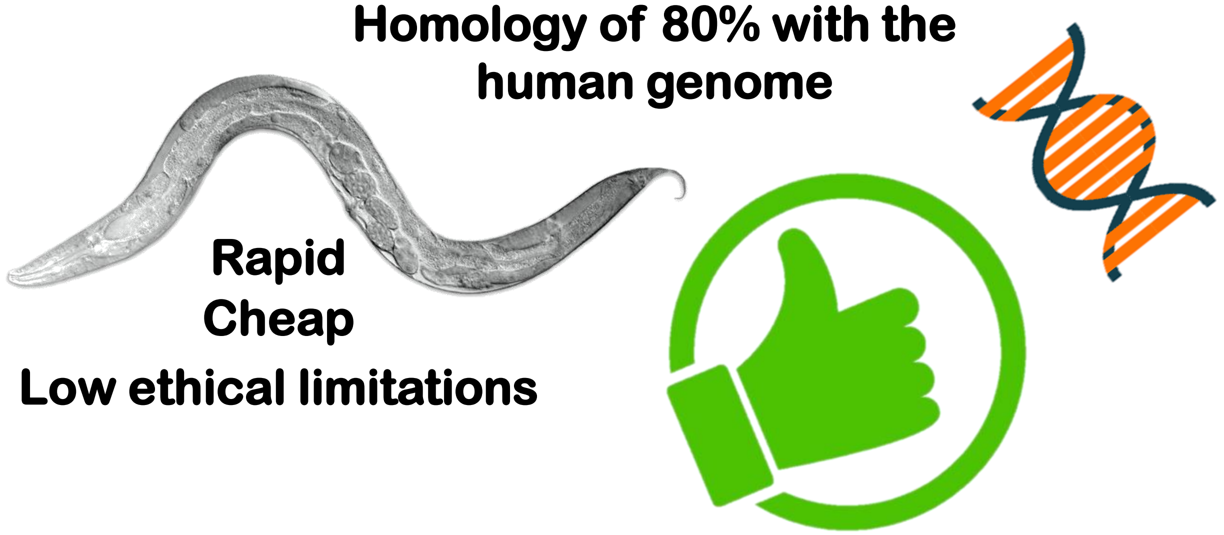
Rapid reproduction

Hermaphrodite

Caenorhabditis elegans

Invertebrate animal

Homology of 80% with the human genome



Rapid
Cheap
Low ethical limitations

Mammalian models

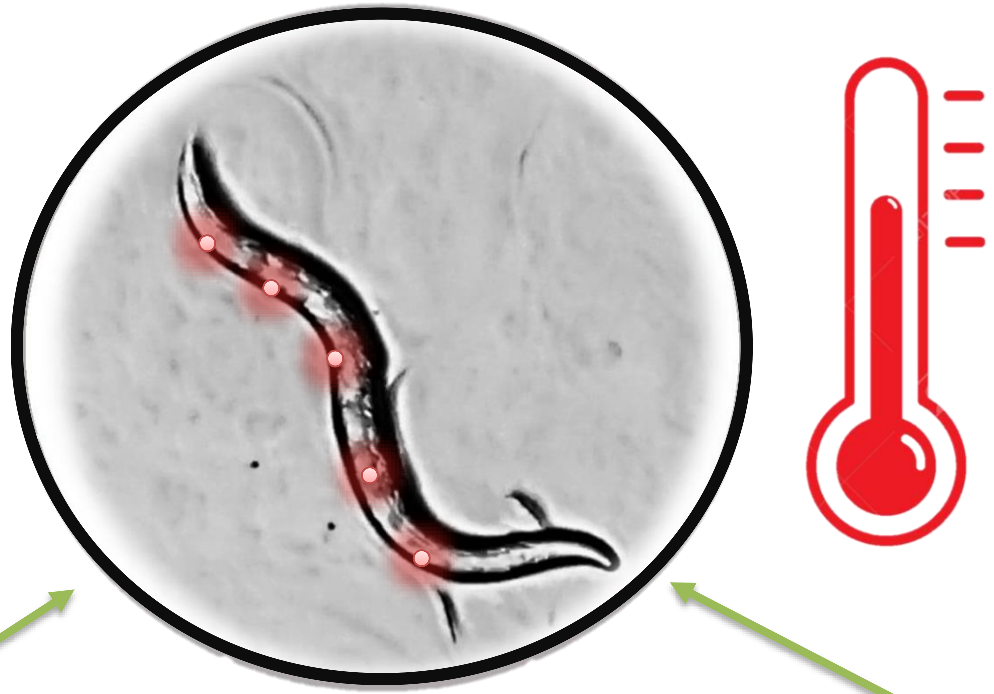
Laborious
Expensive
Ethical limitations

II Methodology

6 *C.elegans in vivo* AD model

CL4176 STRAIN

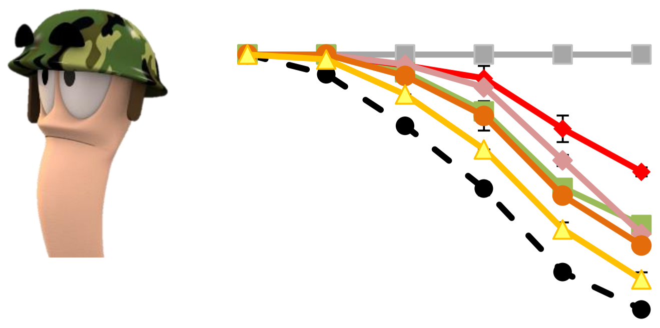
Human Aβ_{42} Expression



Mechanism of disease

Test drugs and therapies

Paralysis assay



Foodomics evaluation

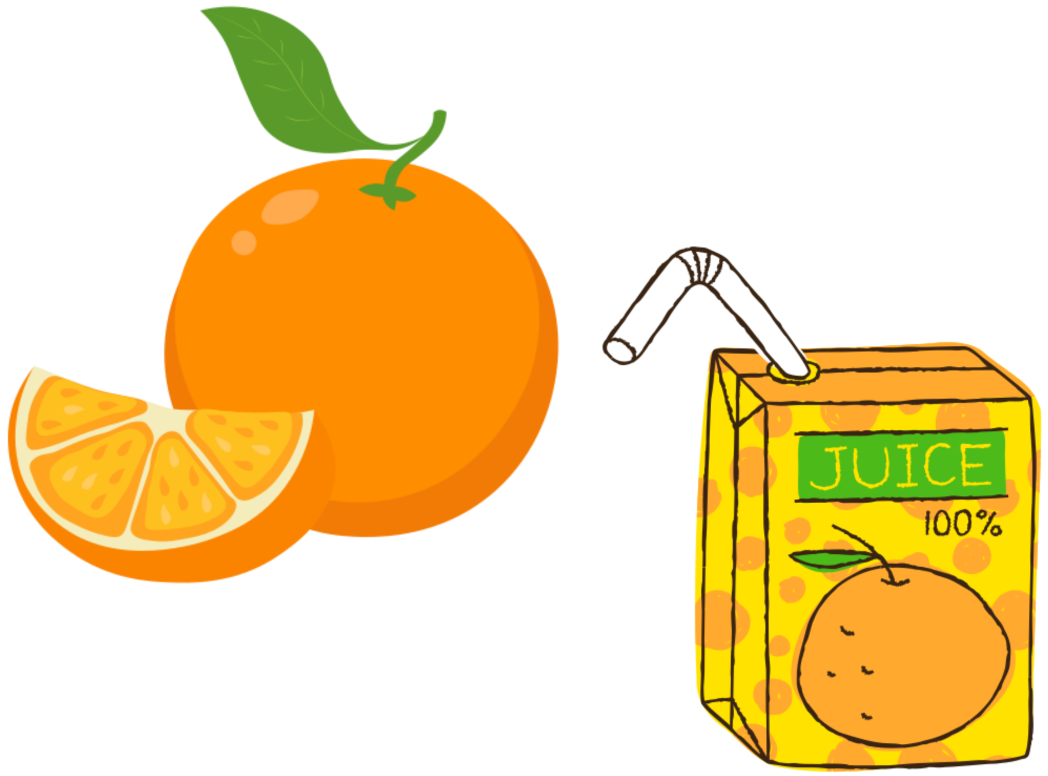
Transcriptomics	Lipidomics	Metabolomics
MetaboAnalyst 5.0	MS-DIAL	NIST MS

WormBase

III RESULTS AND DISCUSSION

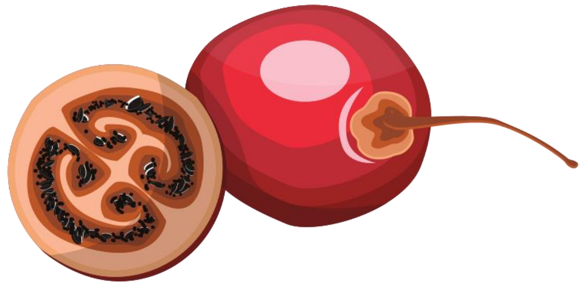
PART I

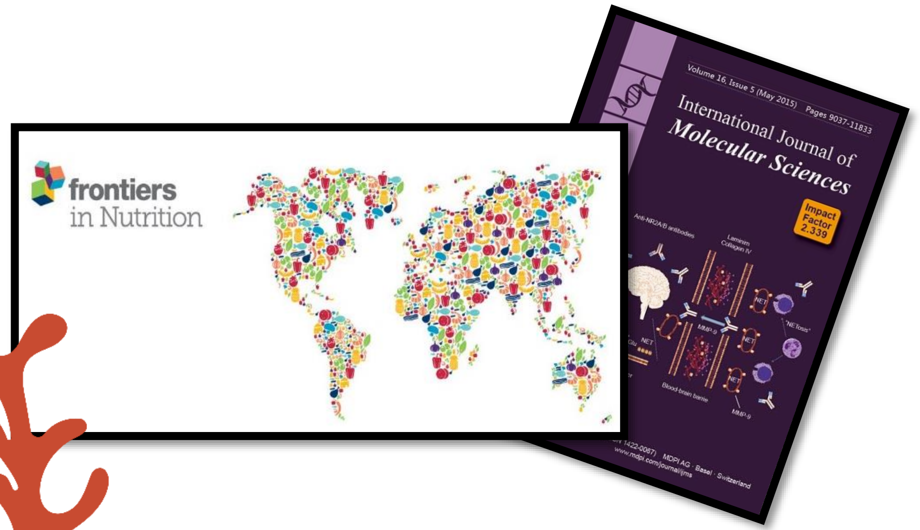
Study of the **neuroprotective** potential of extracts from **orange by-products**.



PART II

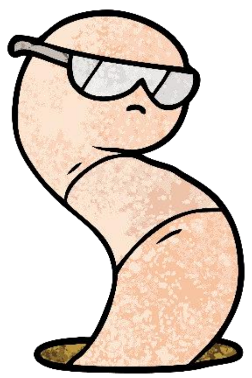
Other **natural matrices** as a source of **neuroprotective** compounds and their transport across the **BBB**.





PART III

Foodomics study of the neuroprotective effect of extracts on *C. elegans* as *an in vivo* model of AD



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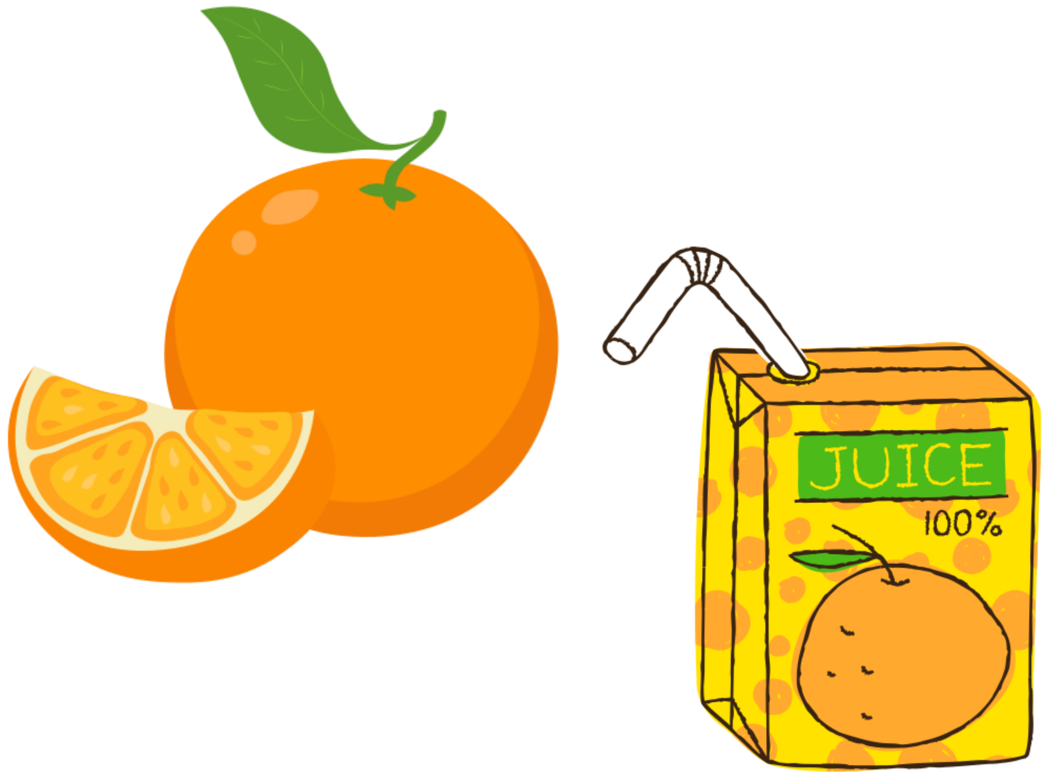

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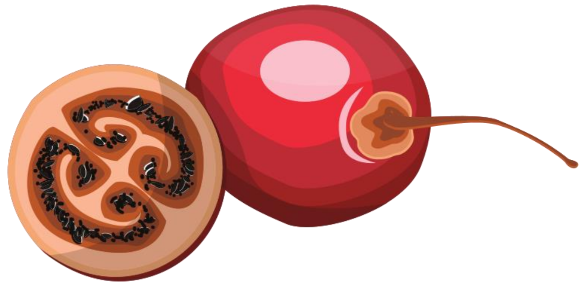
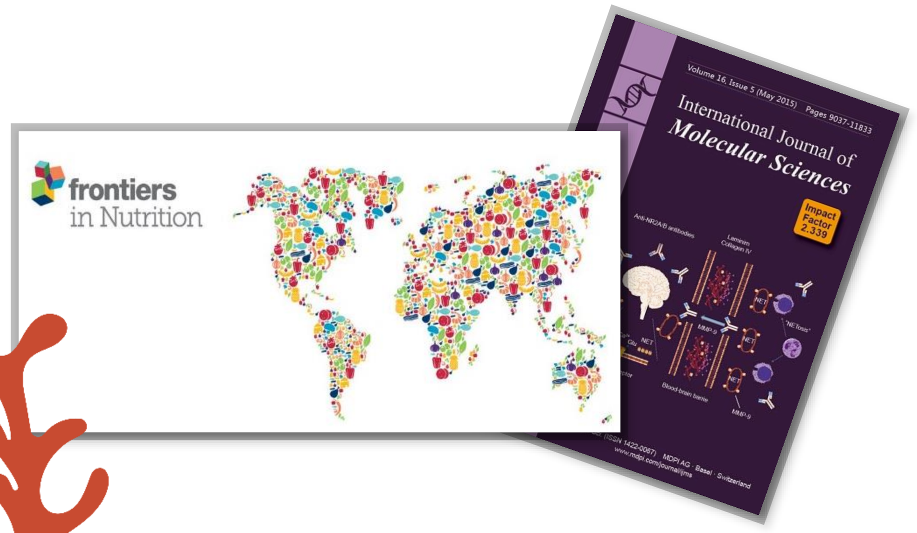
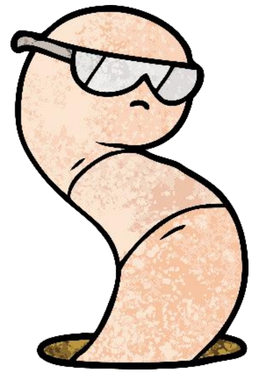




PART III

Foodomics study of the neuroprotective effect of extracts on *C. elegans* as *an in vivo* model of AD



Spain is the **largest** producer in the European Union and the **sixth** in the world.

Orange juice production process generated high amount of pulp, peel and seeds.

Citrus wastes reach **24.3 million tons per year** from which, **1.3 million tons correspond to Spain**

R & D: Part I Study of the neuroprotective potential of extracts from orange by-products

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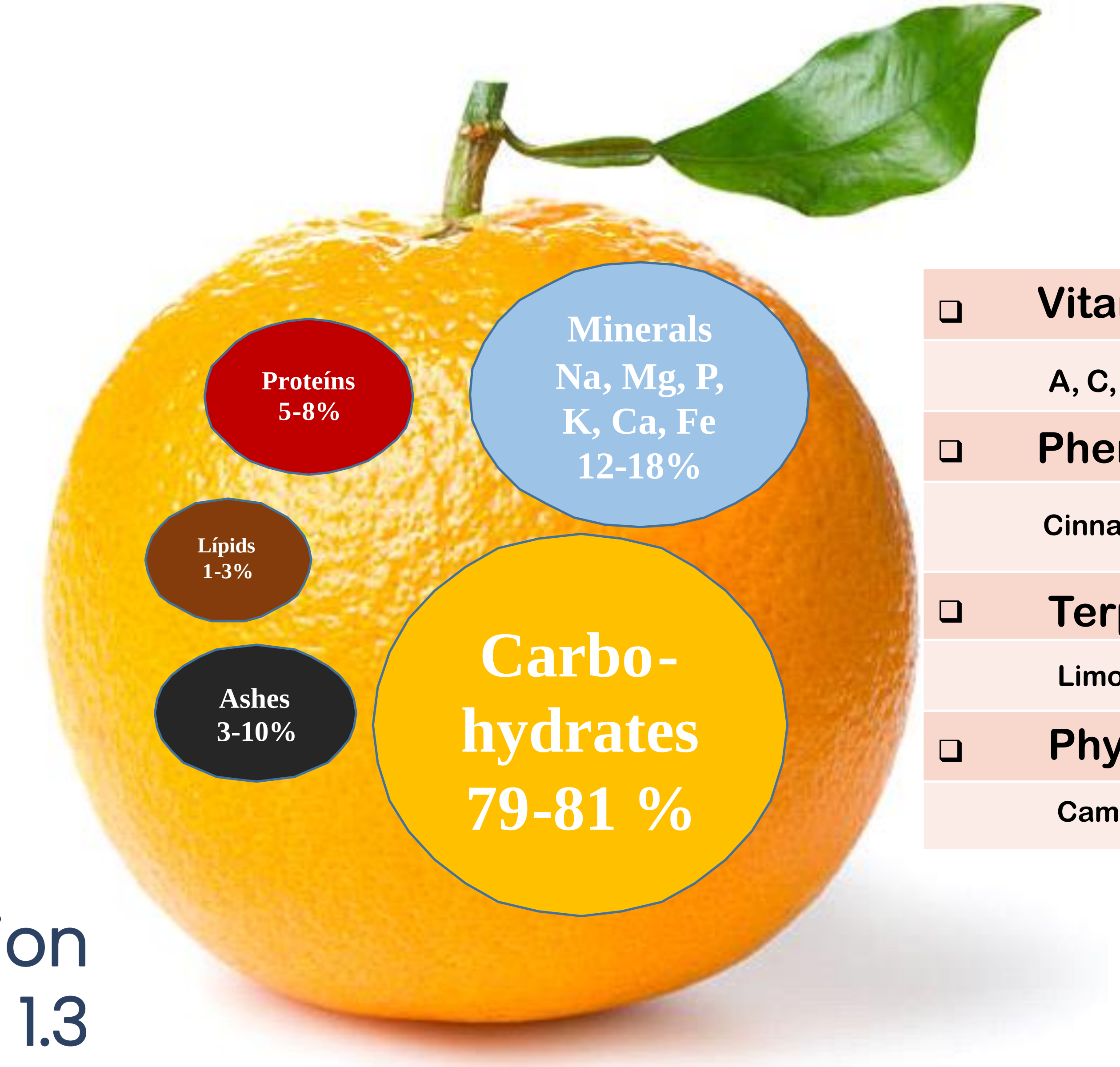

- **Vitamins**
  - A, C, B3, B2, B1, E (tocopherols)
- **Phenolic compounds**
  - Cinnamic acid, hesperidin, tangeritin
- **Terpenes**
  - Limonene, valencene, squalene
- **Phytosterols**
  - Campesterol, sitosterol, stigmasterol

Orange juice by-products have been considered a wide source of bioactive compounds

R & D: Part I Study of the neuroprotective potential of extracts from orange by-products

Conventional extraction
Maceration

Ethyl Acetate Ethanol Acetone N-heptane

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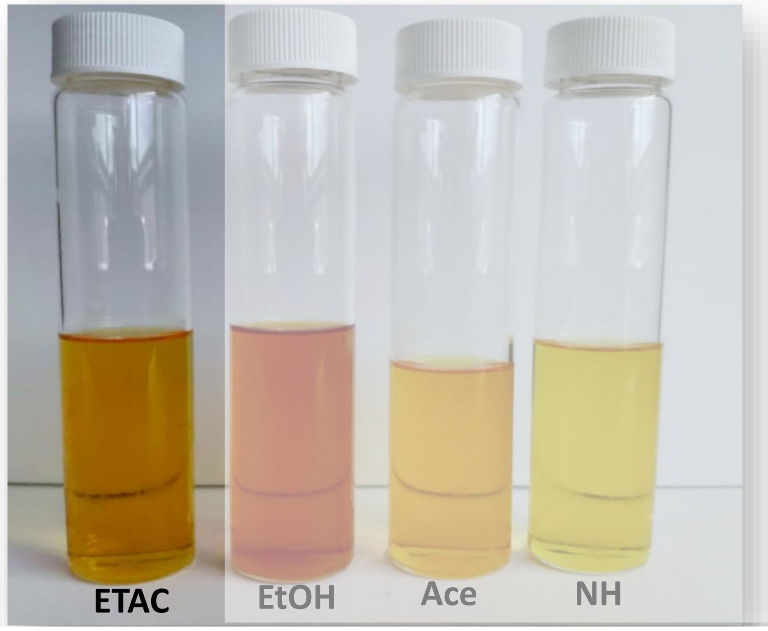

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R & D: Part I Study of the neuroprotective potential of extracts from orange by-products

Conventional extraction
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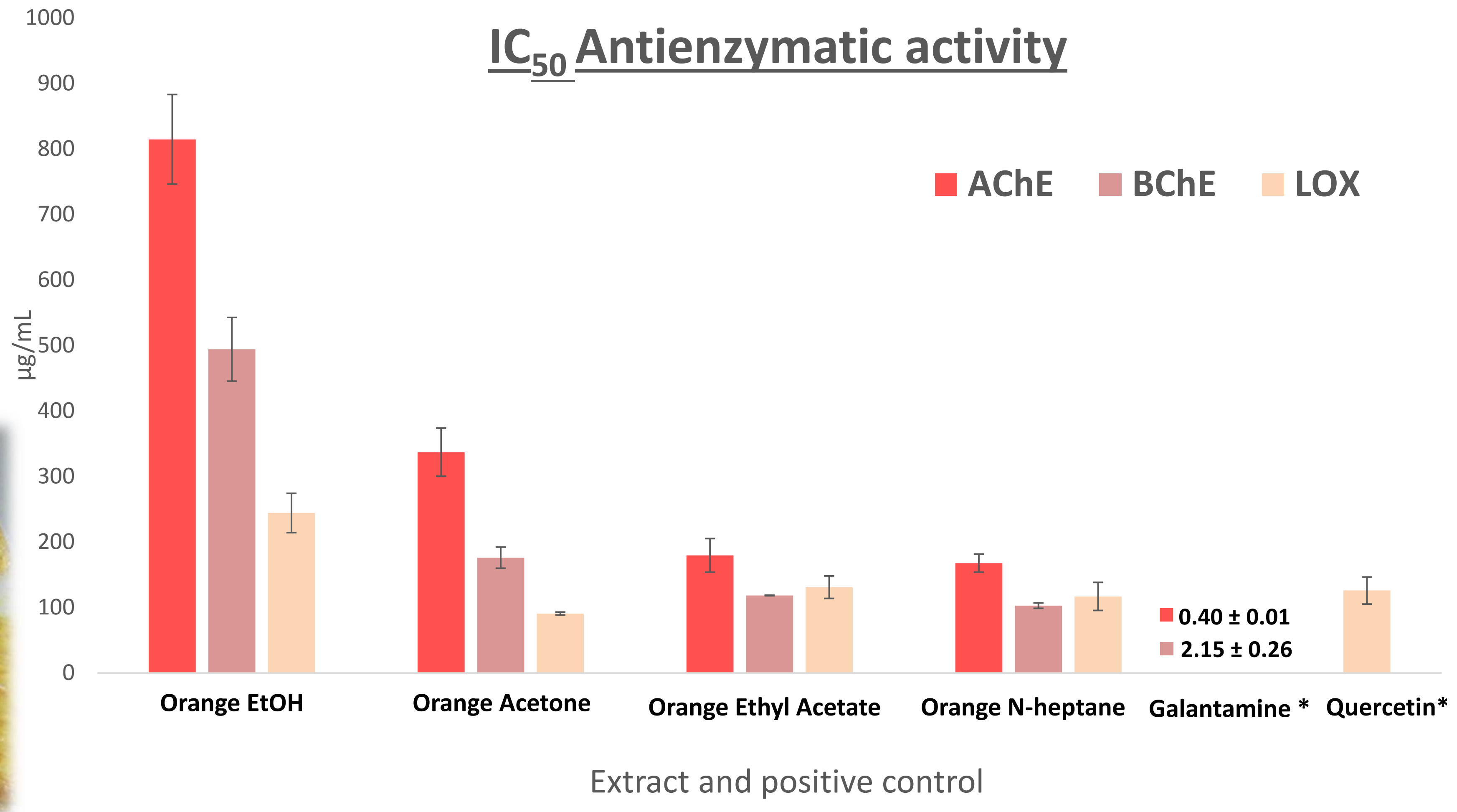

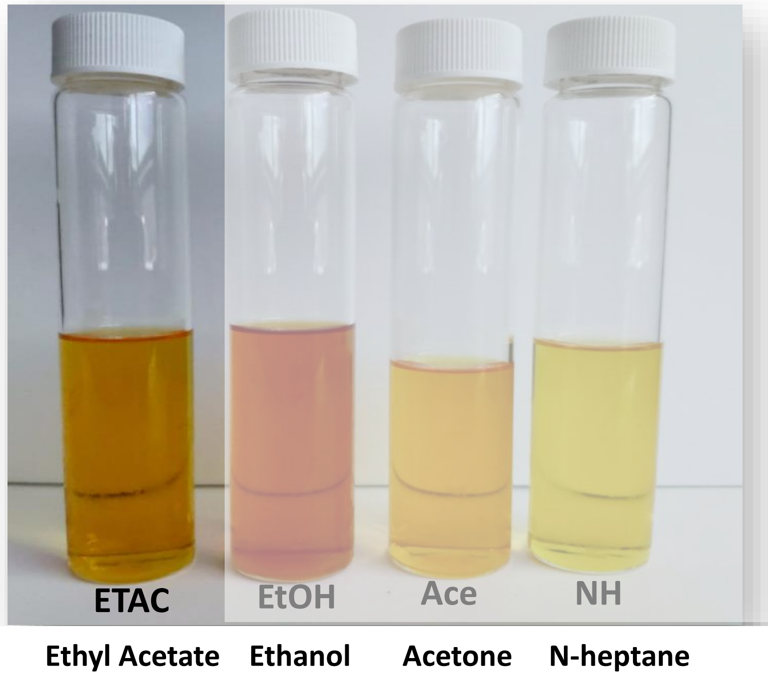

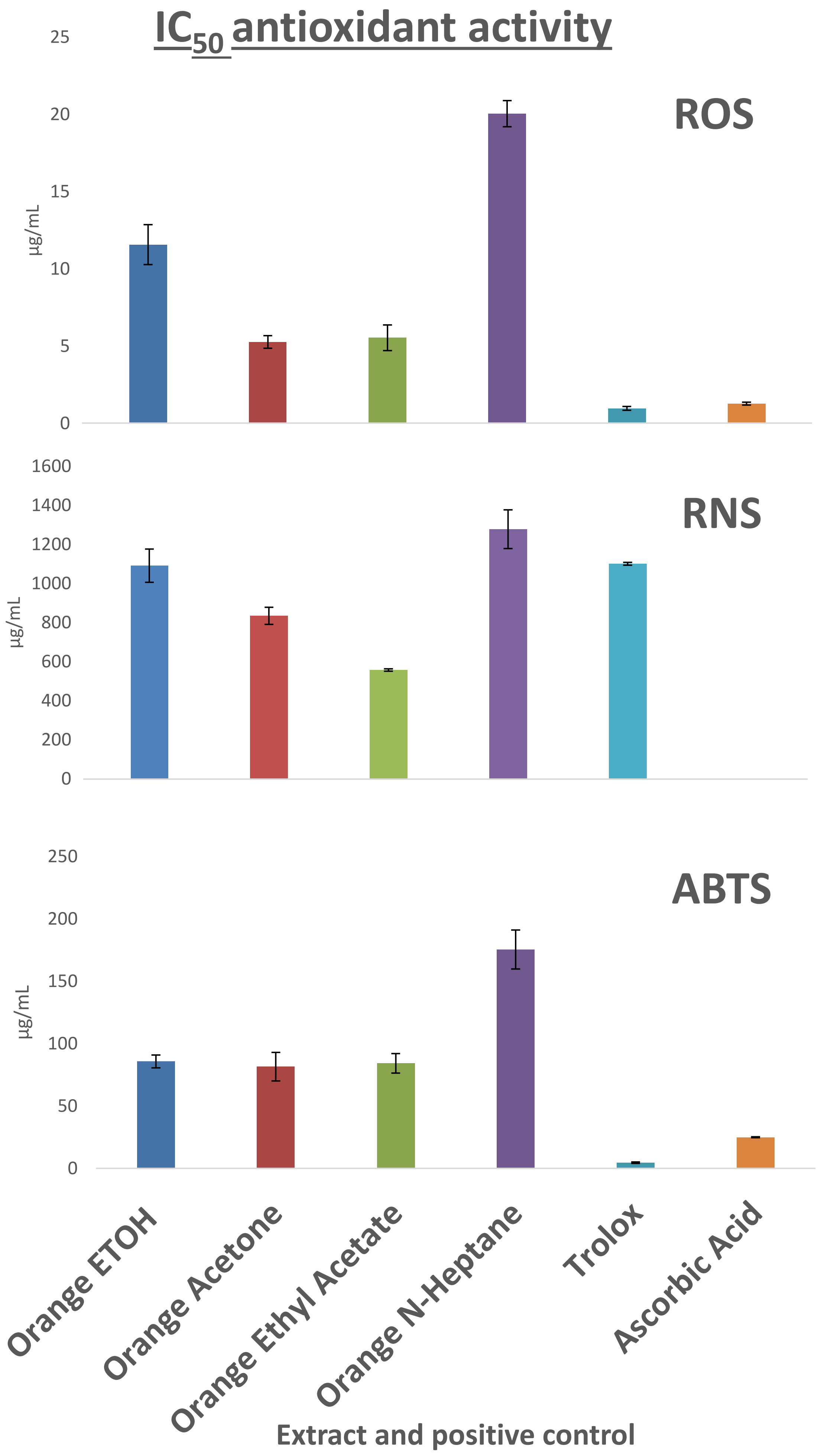

R & D: Part I Study of the neuroprotective potential of extracts from orange by-products

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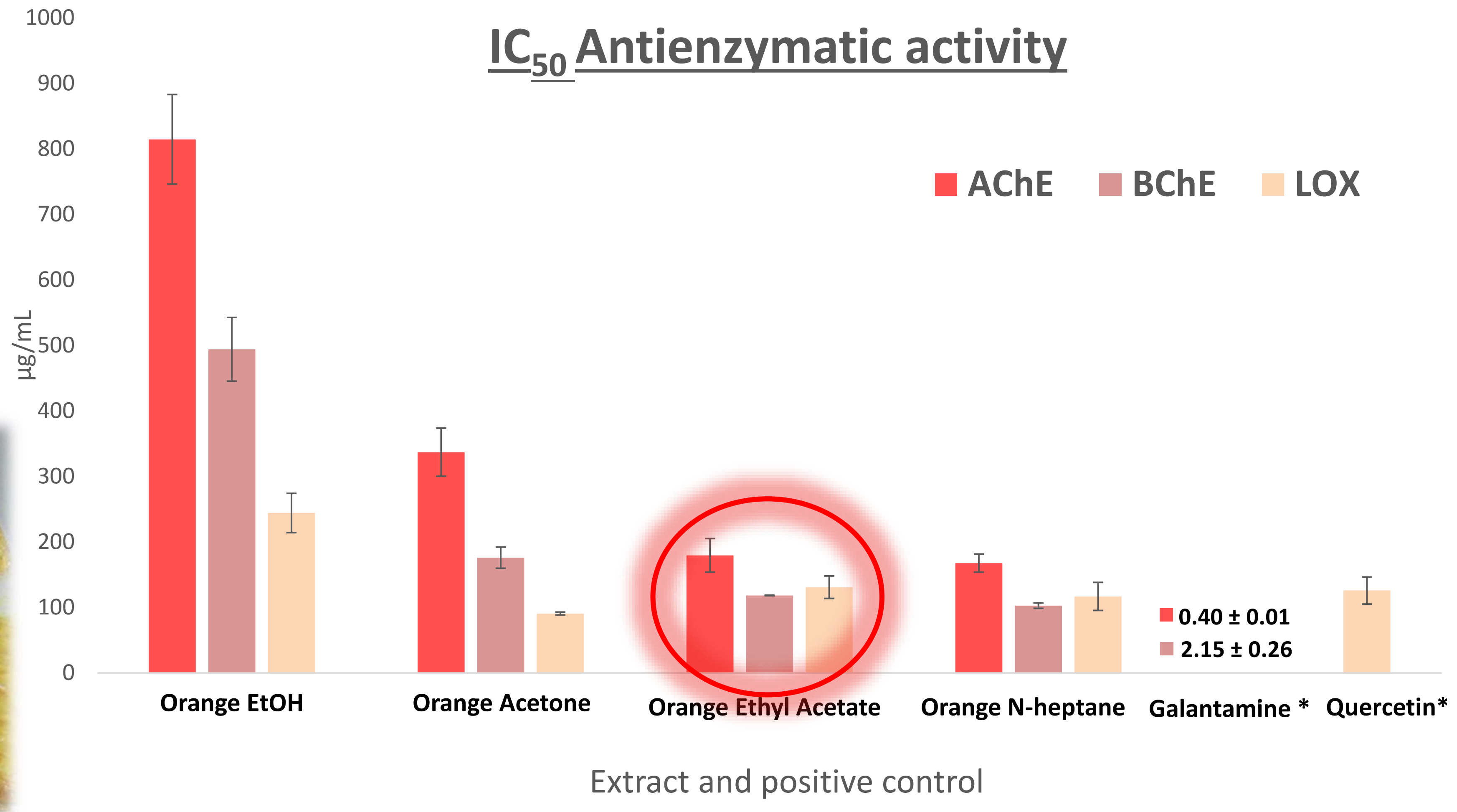

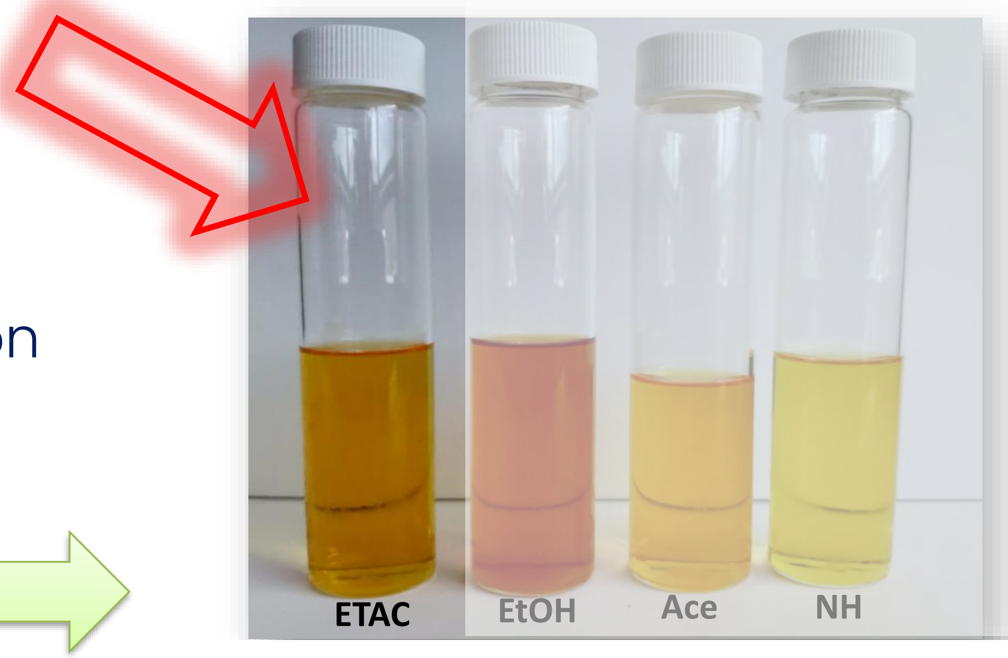

Ethyl Acetate Ethanol Acetone N-heptane

III R & D: Part I Study of the neuroprotective potential of extracts from orange by-products

GC-Q-TOF-MS/MS

III

R & D: **Part I** Study of the neuroprotective potential of extracts from orange by-products

GC-Q-TOF-MS/MS

PHYTOCHEMICAL PROFILING

Peak no	Ret. time (min)	Family	Tentative identification	Formula	Match factor (%)
1	5.856	Monoterpene	Limonene	C10H16	86
2	6.688	Monoterpene	3-Carene	C10H16	75
3	7.164	Monoterpene	(−)Myrtenol	C10H16O	88
4	8.046	Monoterpene	L-α-Terpineol	C10H18O	68
5	8.395	Monoterpene	Nerol	C10H18O	92
6	10.075	Monoterpene	Limonene epoxide	C10H16O	73
7	10.480	Sesquiterpene	α-Copaene	C15H24	82
8	10.631	Sesquiterpene	β-Elemen	C15H24	91
9	11.074	Sesquiterpene	β-Caryophyllene-1	C15H24	86
10	11.191	Sesquiterpene	Farnesene	C15H24	85
11	11.342	Sesquiterpene	7-Prop[b]	C15H24	73
12	11.419	Sesquiterpene	β-Caryophyllene-2	C15H24	72
13	11.778	Sesquiterpene	β-Panasinsene	C15H24	76
14	11.875	Sesquiterpene	(−)-Aristolene	C15H24	85
15	11.998	Sesquiterpene	Valencene	C15H24	93
16	12.033	Sesquiterpene	γ-Selinene	C15H24	81
17	12.116	Sesquiterpene	δ-Cadinene	C15H24	80
18	12.267	Sesquiterpene	Isoledene	C15H24	80
19	12.324	Sesquiterpene	(−)-α-Panasinsen	C15H24	87
20	12.649	Sesquiterpene	Elemol	C15H26O	84
21	13.598	Sesquiterpene	Guaiol	C15H26O	70
22	14.068	Sesquiterpene	α-Gurjunenepoxide	C15H24	85
23	14.292	Sesquiterpene	β-Sinensal	C15H22O	78
24	14.410	Sesquiterpene	β-Oplopenone	C15H24O	69
25	15.214	Sesquiterpene	Isololiolide	C11H16O3	81
26	15.617	Sesquiterpene	Nootkatone	C15H22O	86
27	15.751	Sesquiterpene	Ylangenal	C15H22O	77
28	24.062	Tritepene	Squalene	C30H50	92
29	25.759	Tritepene	γ-Tocopherol	C28H48O2	89
30	26.272	Tritepene	α-Tocopherol	C29H50O2	94
31	26.992	Tritepene	Campesterol	C28H48O	86
32	27.157	Tritepene	Stigmasterol	C29H48O	89
33	27.548	Tritepene	γ-Sitosterol	C29H50O	88
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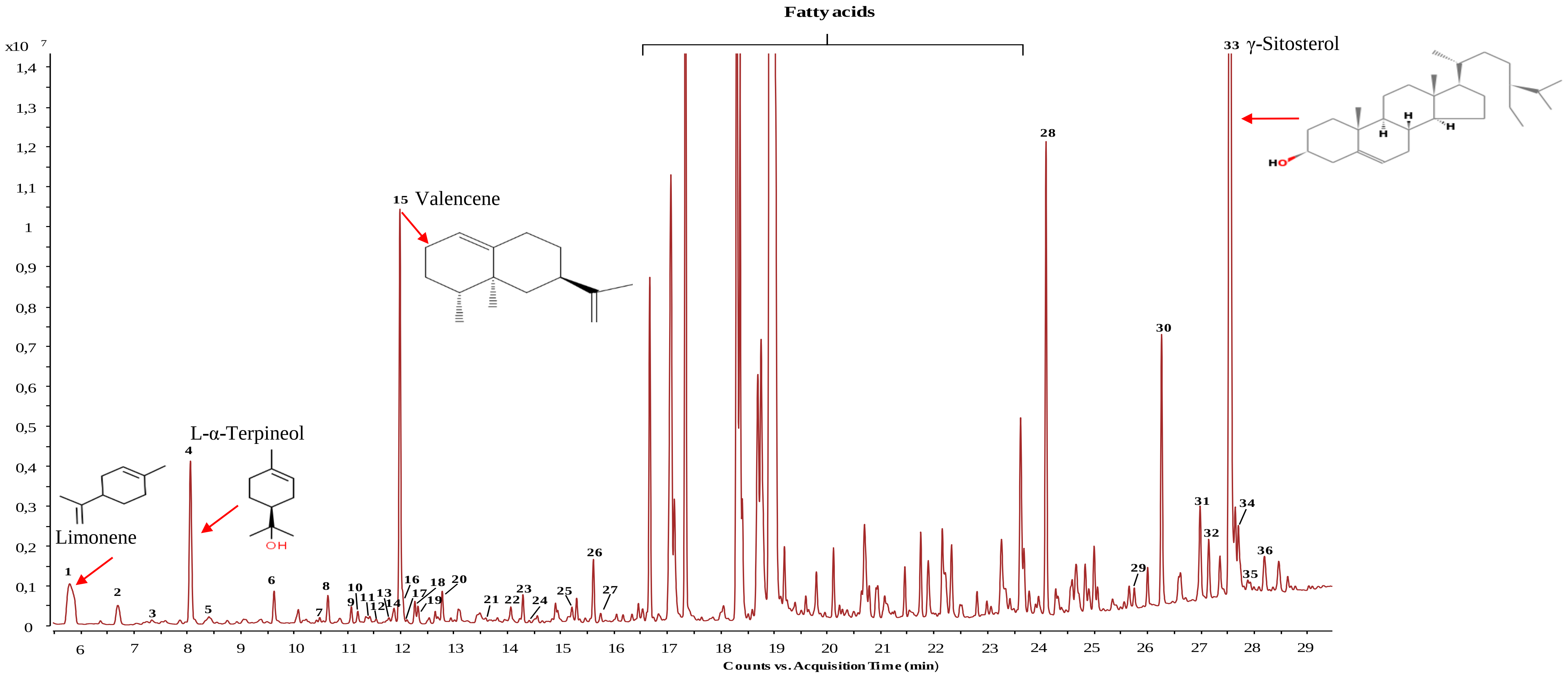
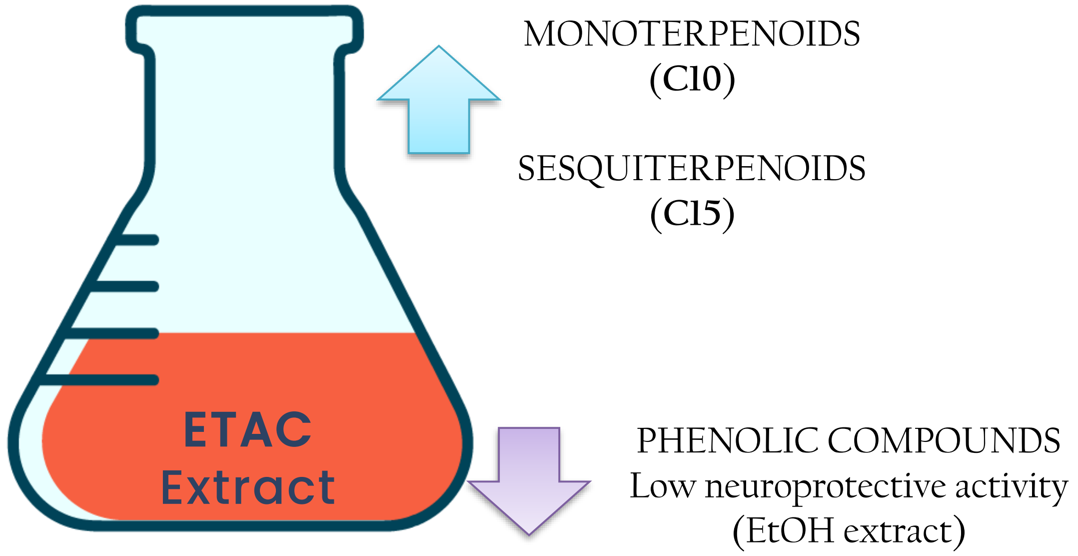
35	27.874	Tritepene	Lupeol	C30H50O	65
36	28.186	Tritepene	β-Amyrin	C30H50O	87
37	35.471	Tritepene	δ-Tocopherol[c]	C28H48O2	72



R & D: **Part I** Study of the neuroprotective potential of extracts from orange by-products



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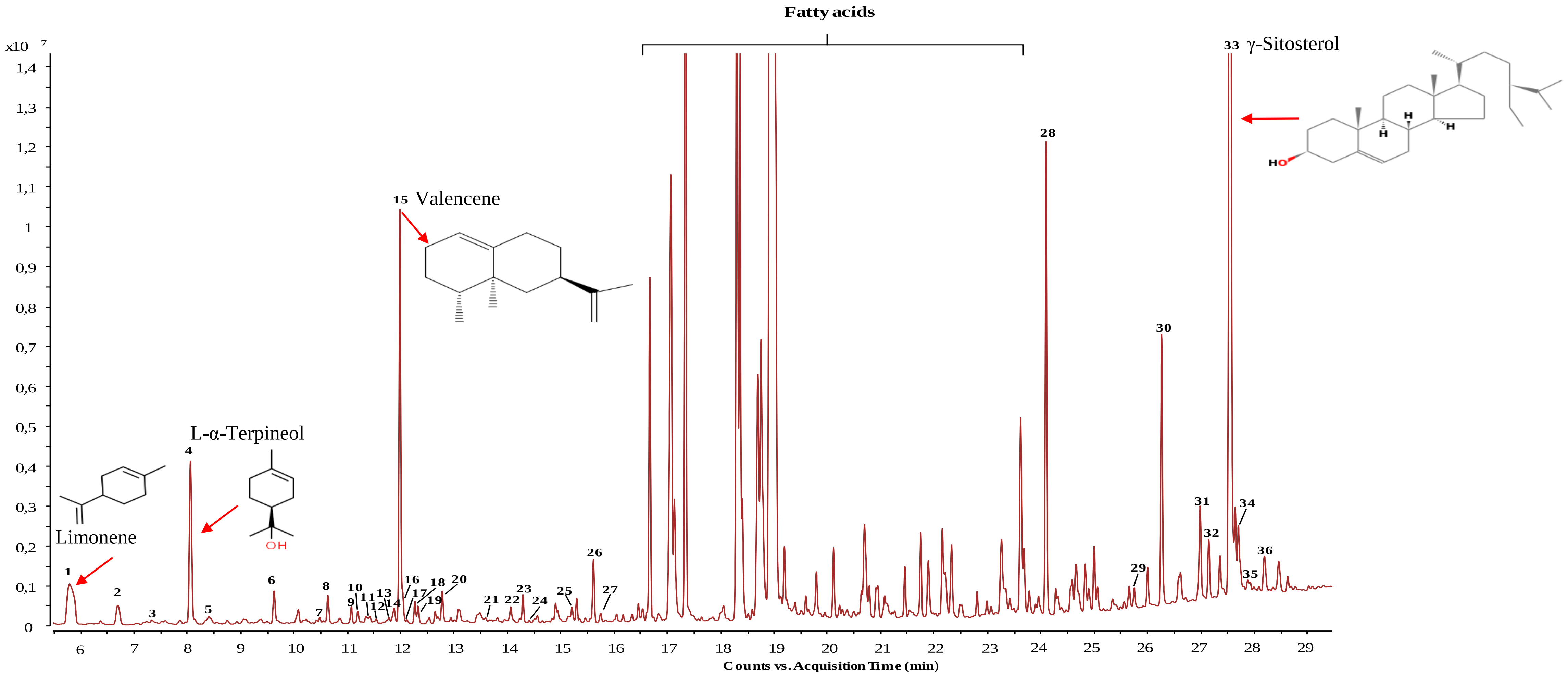
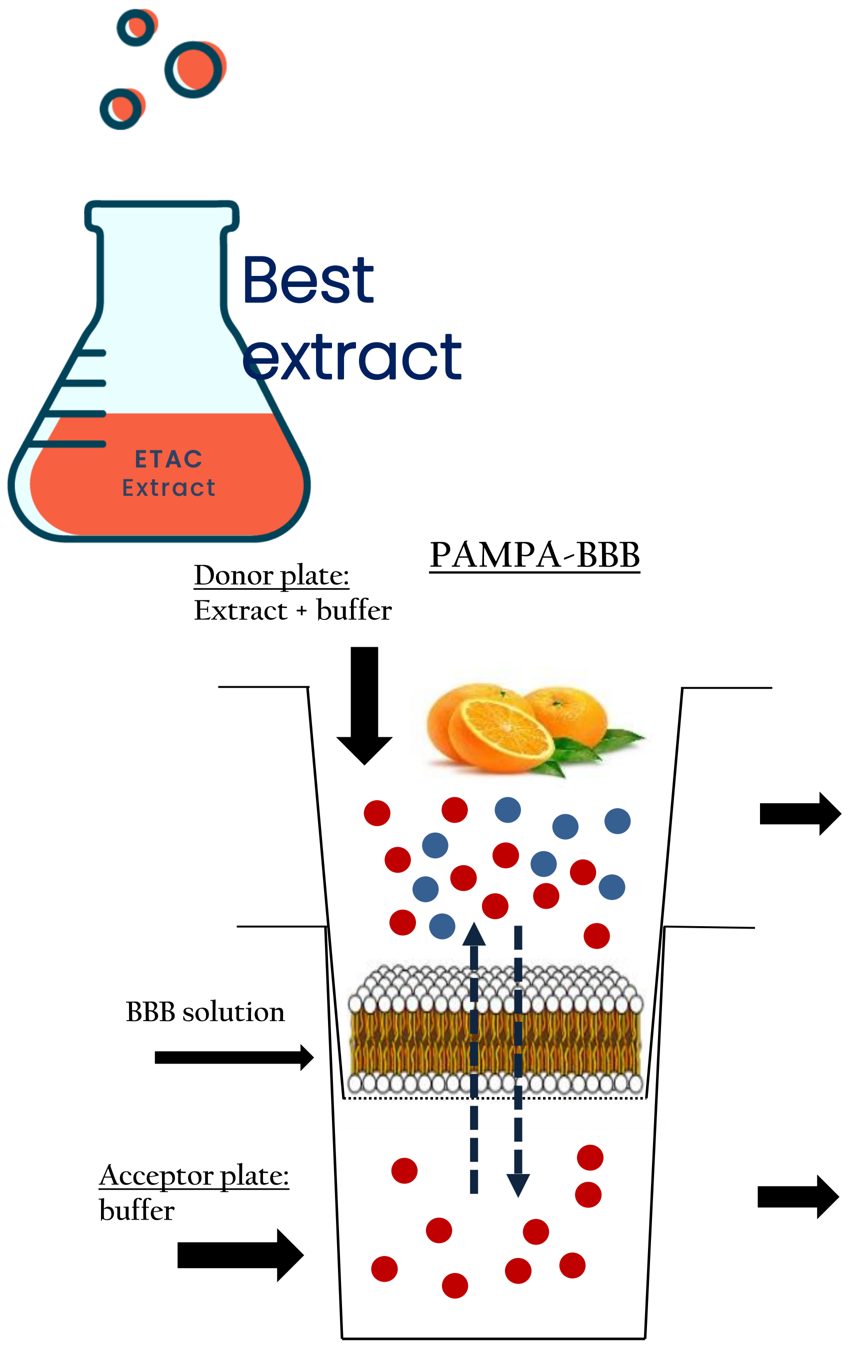
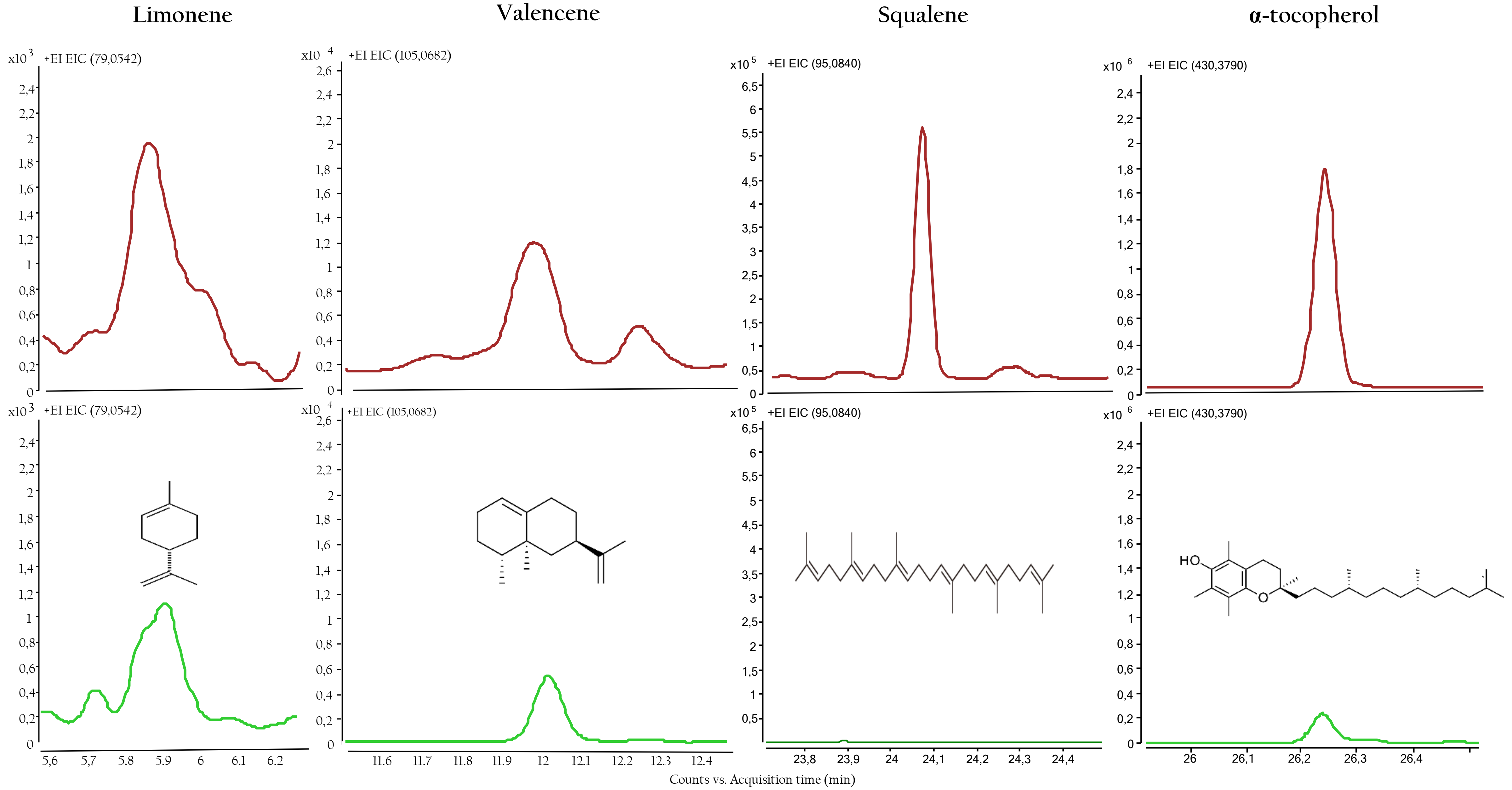
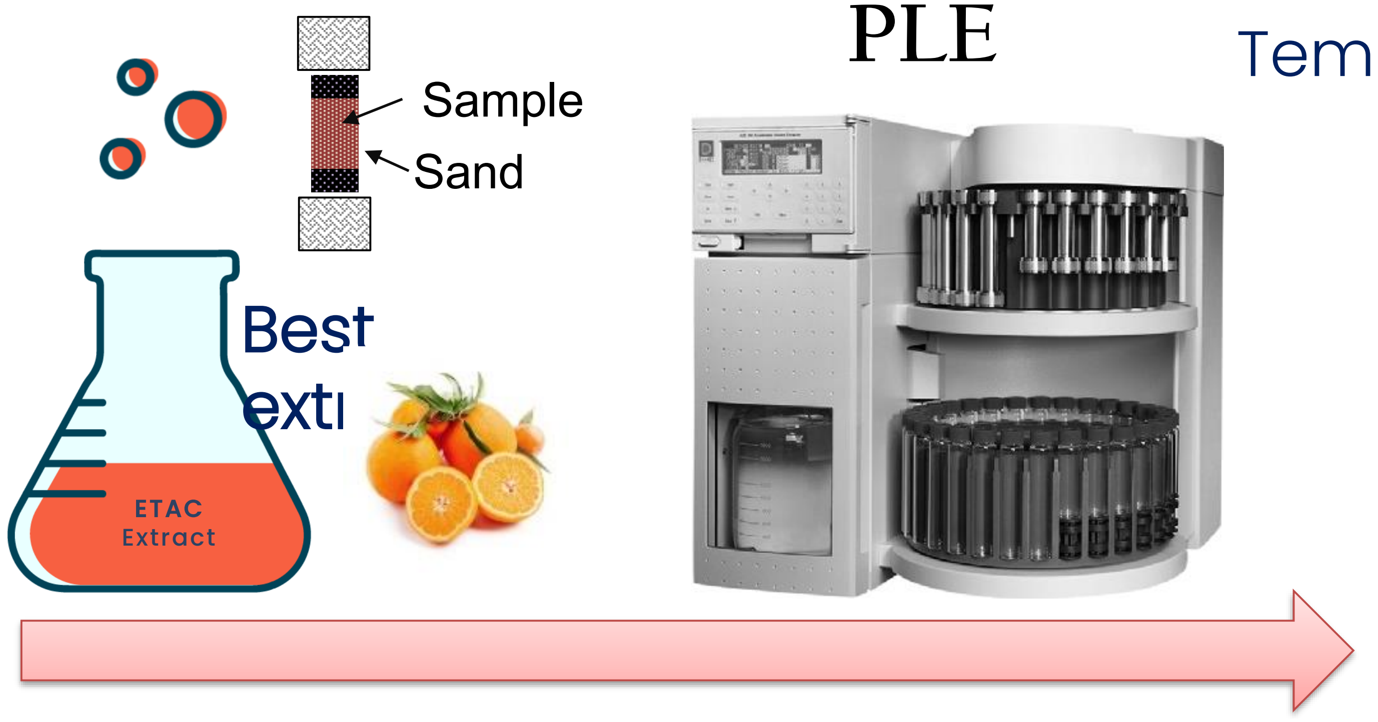




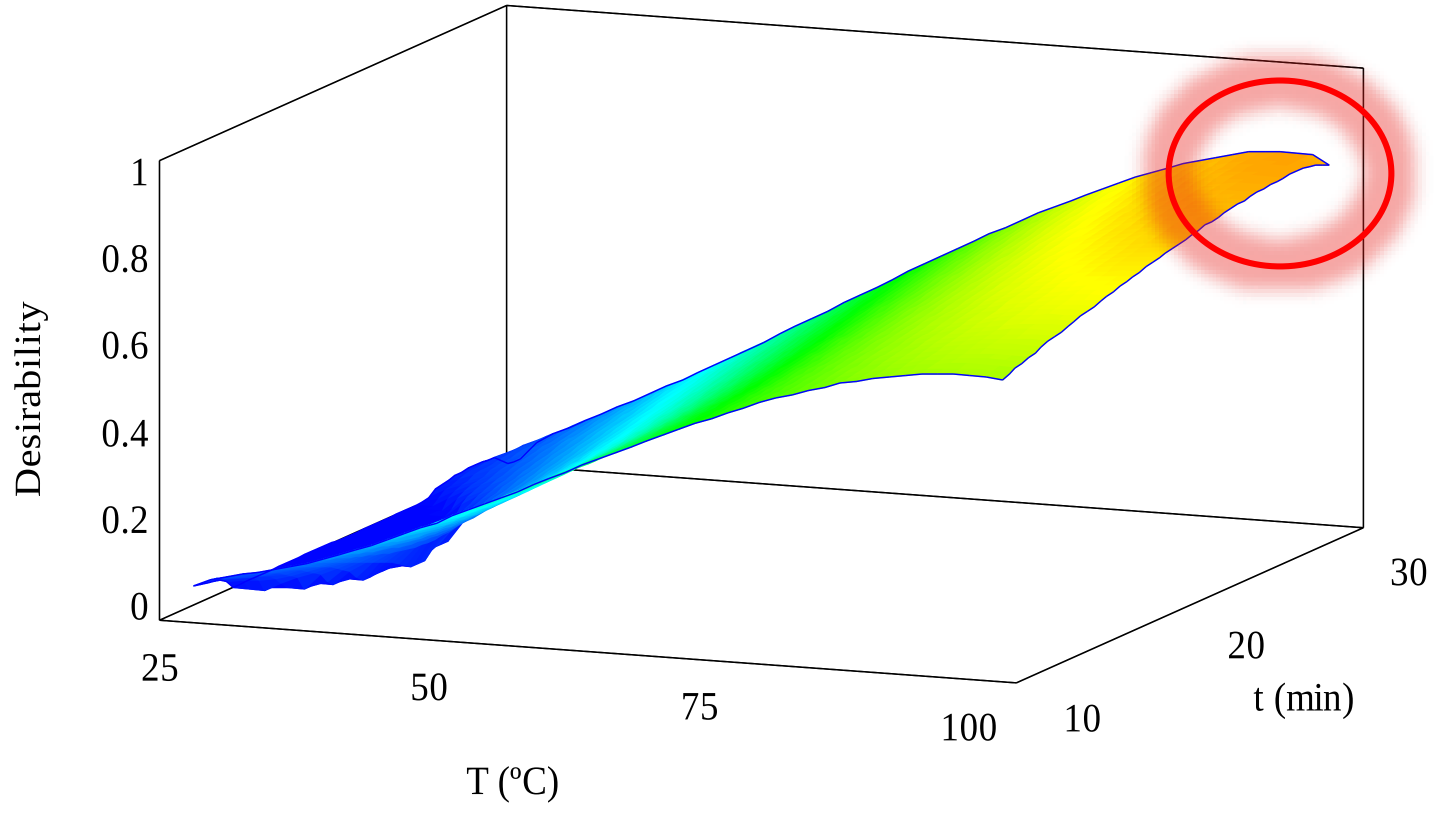
Parallel artificial membrane permeability assay for the blood-brain barrier (**PAMPA-BBB**)



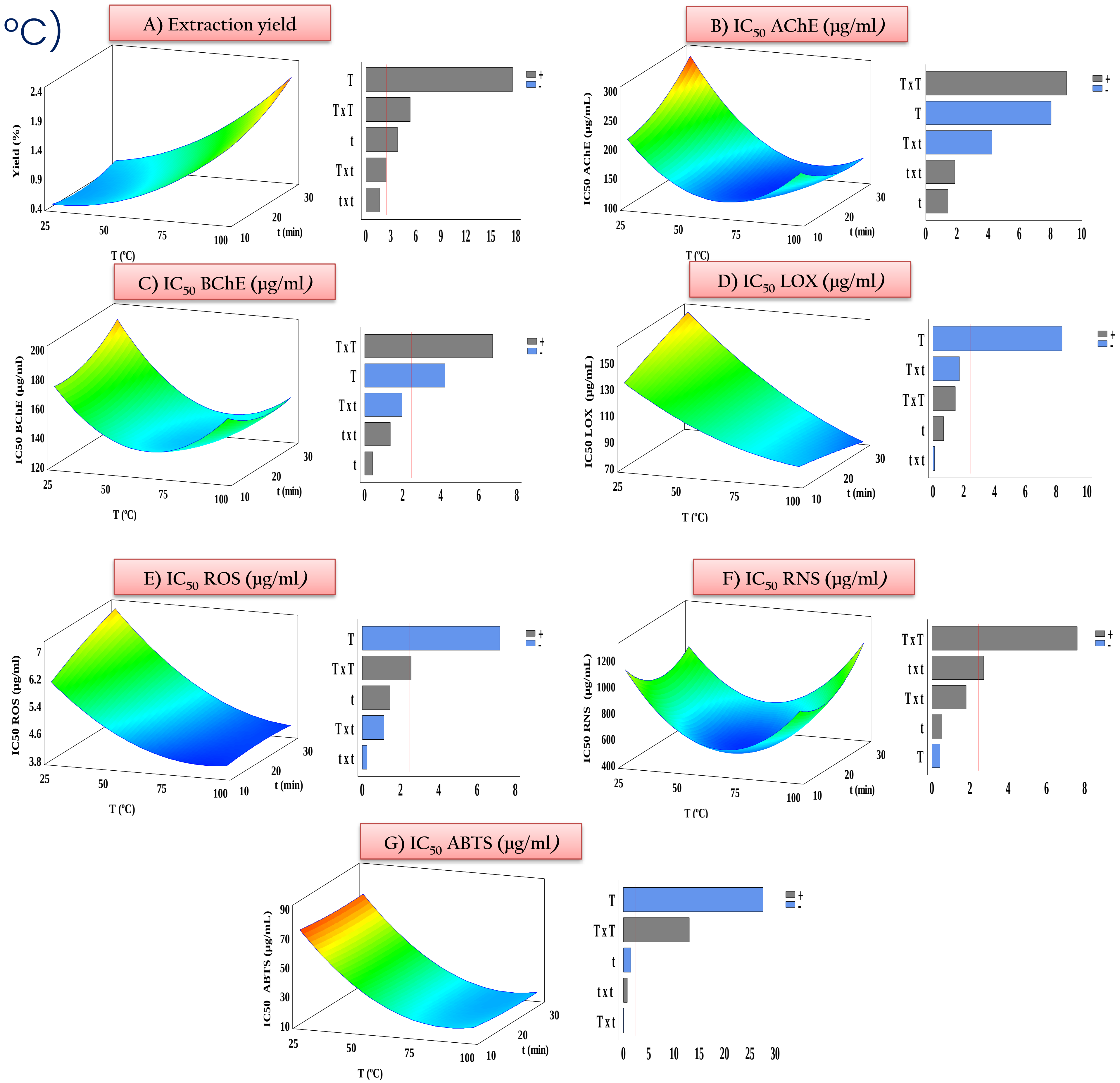
III R & D: Part I Study of the neuroprotective potential of extracts from orange by-products



Time (t, min)
Temperature (T, °C)



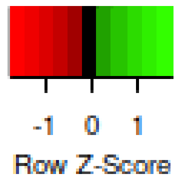
Response surfaces of each response variables and their corresponding Standardized Pareto charts.



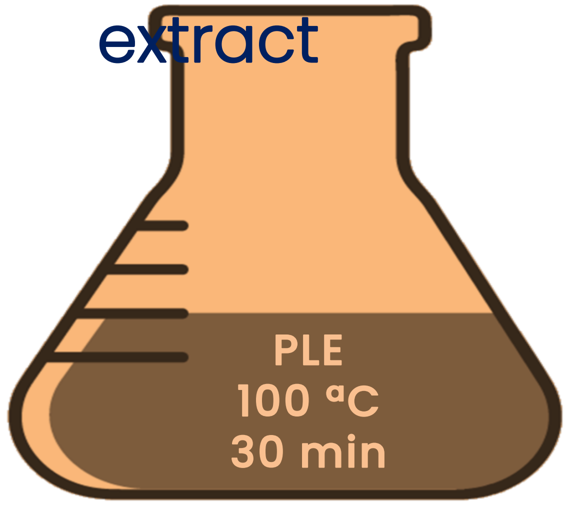
	(IC 50 µg/mL)						%
Extract	AChE	BChE	LOX	ABTS	ROS - ORAC	RNS	Yield
Optimal PLE PLE100	137.1[de] ± 8.1	147.0[cde] ± 7.5	76.1[f] ± 10.4	13.5[e] ± 0.8	4.4d[ef] ± 0.4	1199[a] ± 98	2.1[b] ± 0.2
Conventional	179.2[c] ± 25.1	102.2[f] ± 4.5	130.7[c] ± 7.0	84.1[a] ± 7.7	5.5[c] ± 0.1	556.9[ef] ± 11	0.5[f] ± 0.0

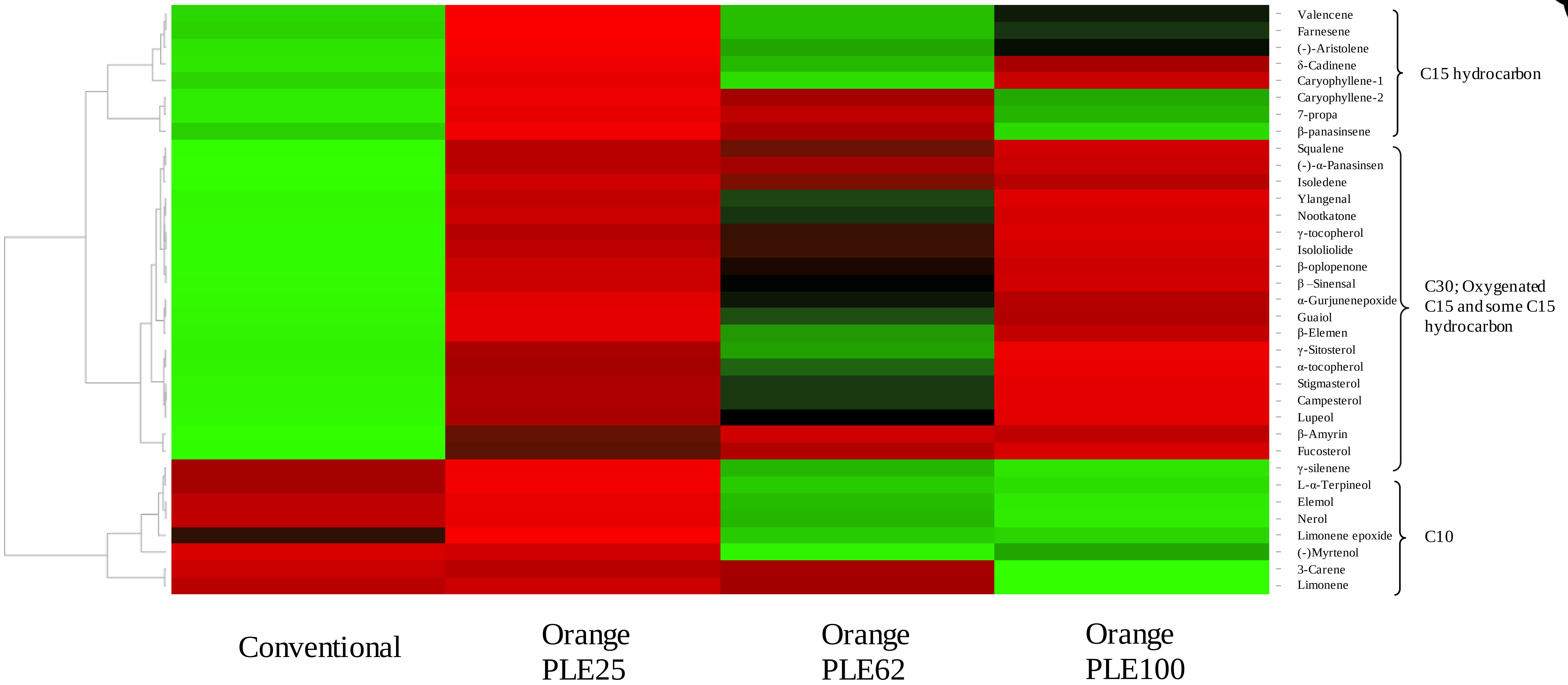
30 min vs 24h

Heat map from Orange PLE extract and their terpenoid composition

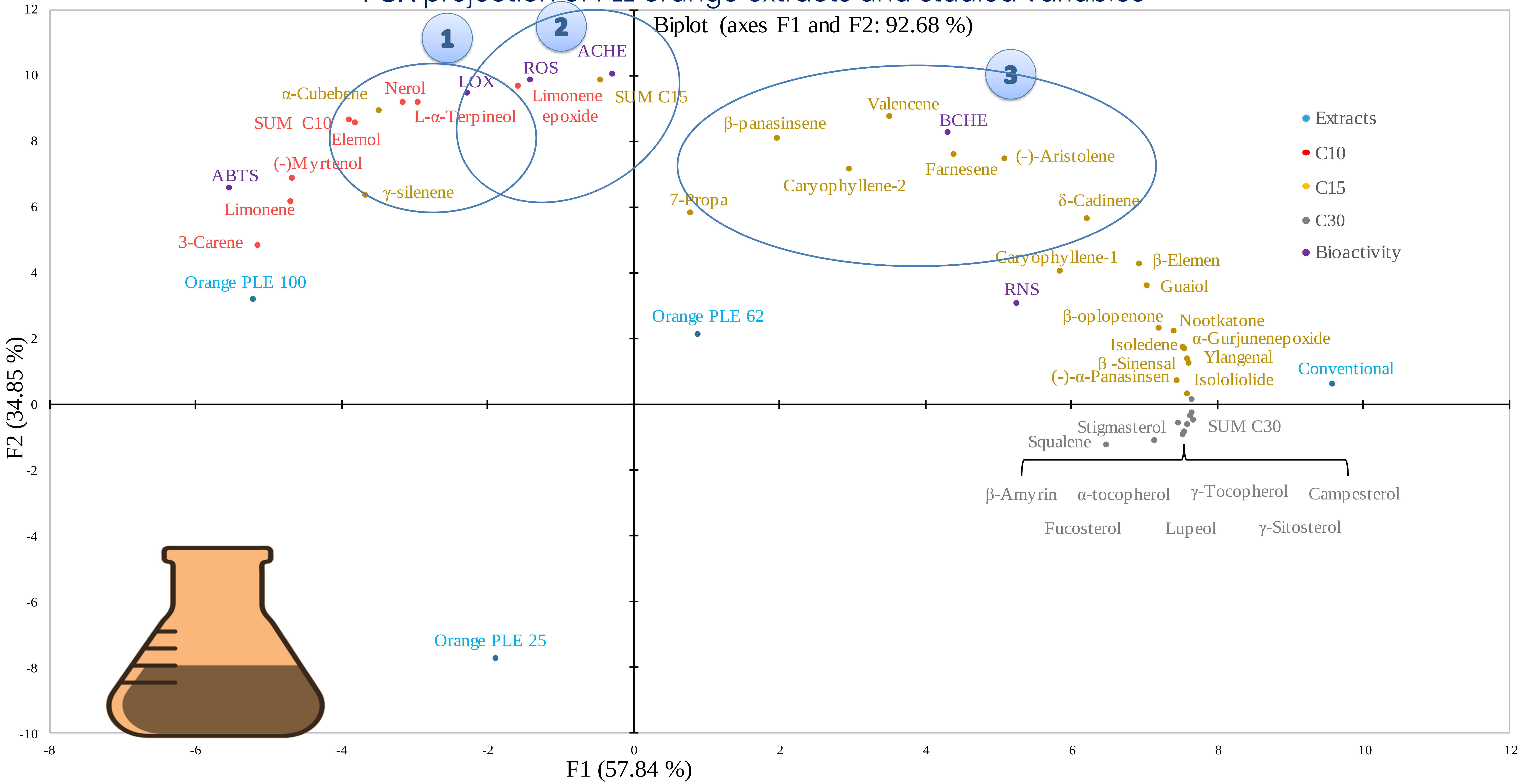


Best extract

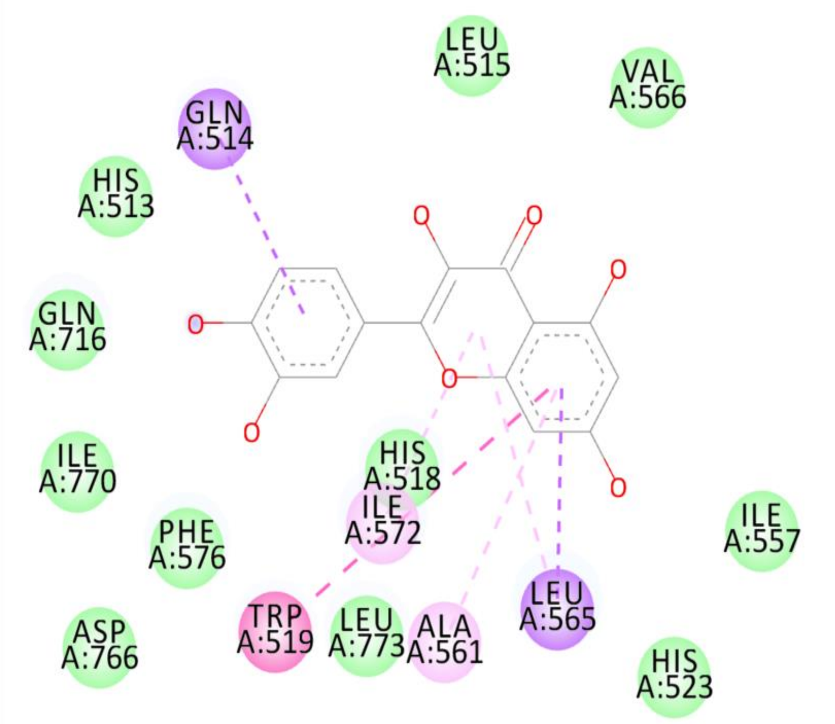




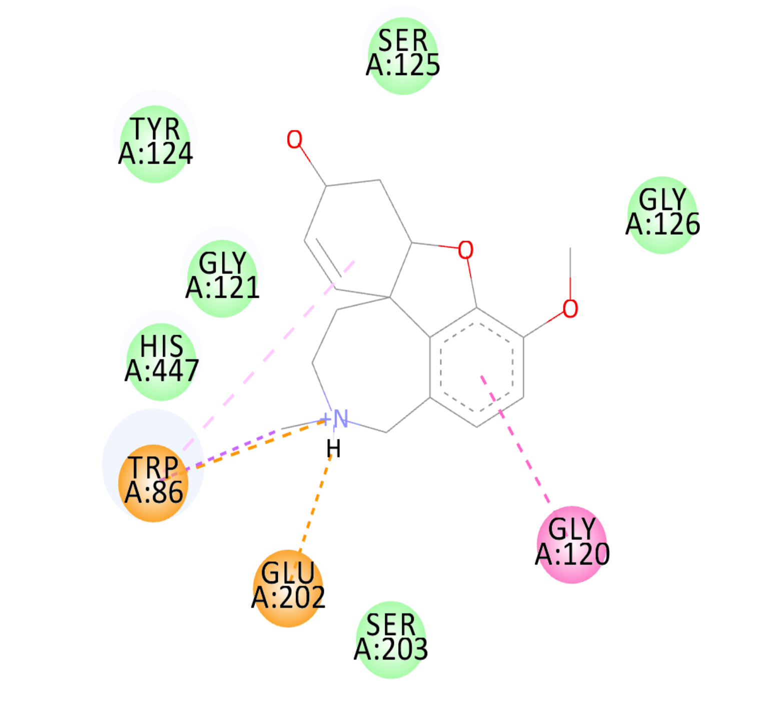
PCA projection of PLE orange extracts and studied variables



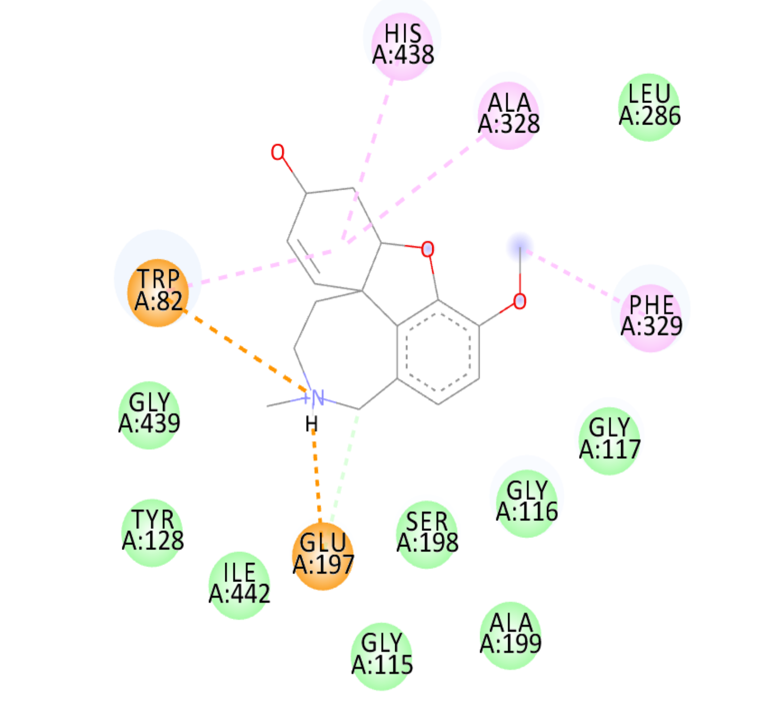
Control



Quercetin – Lipoxygenase - 5,84



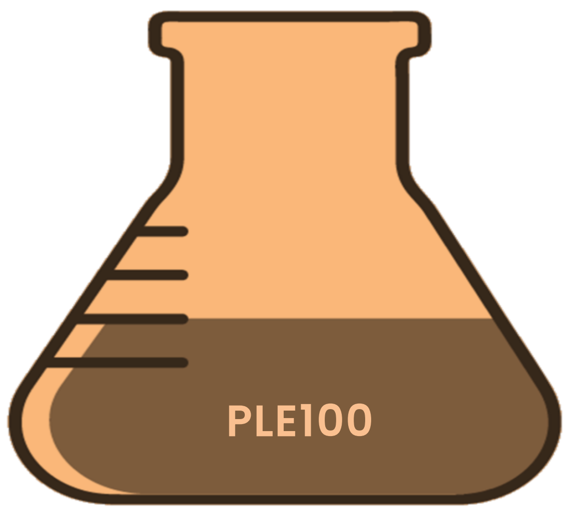
Galantamine – AChE - 6,59



Galantamine – BChE - 6,67

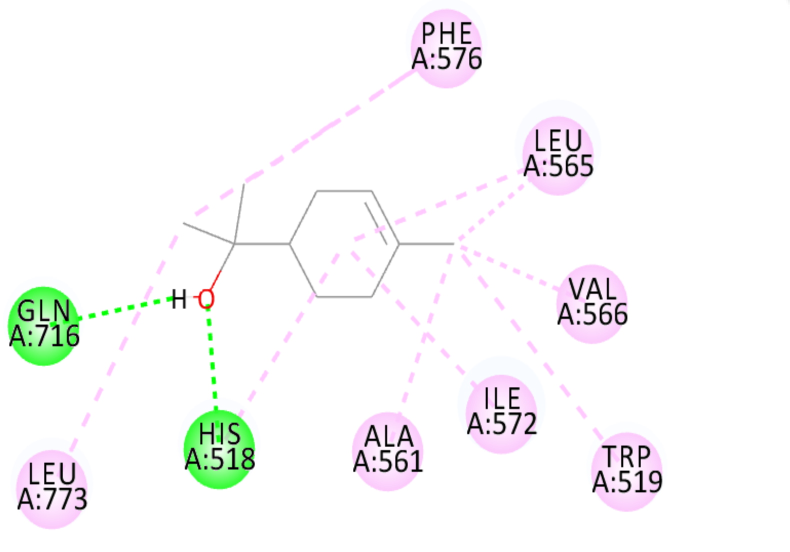
Bound
Colour legends

- van der Waals
- Pi-Sigma
- Alkyl
- Pi-Alkyl
- Salt Bridge
- Carbon Hydrogen Bond
- Pi-Cation
- Conventional Hydrogen Bond



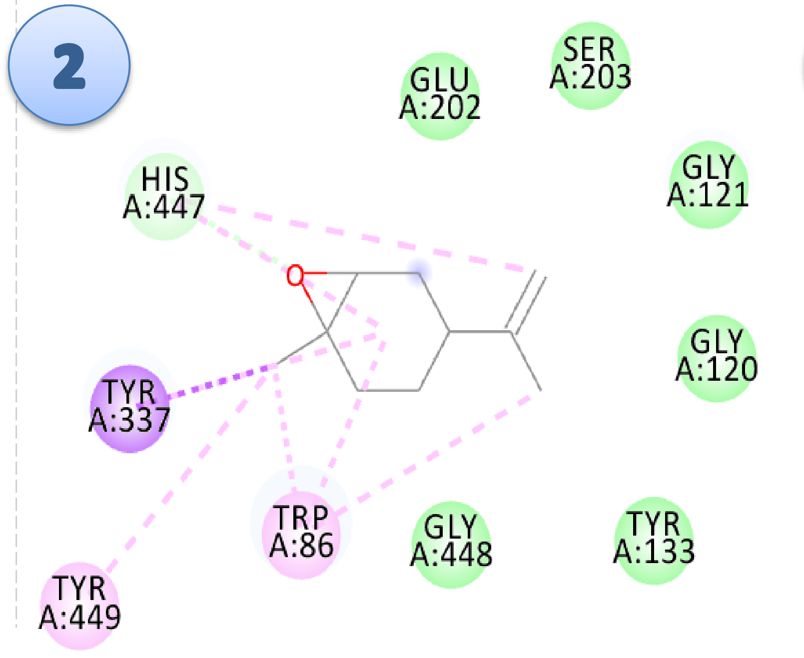
Natural terpenoids

1



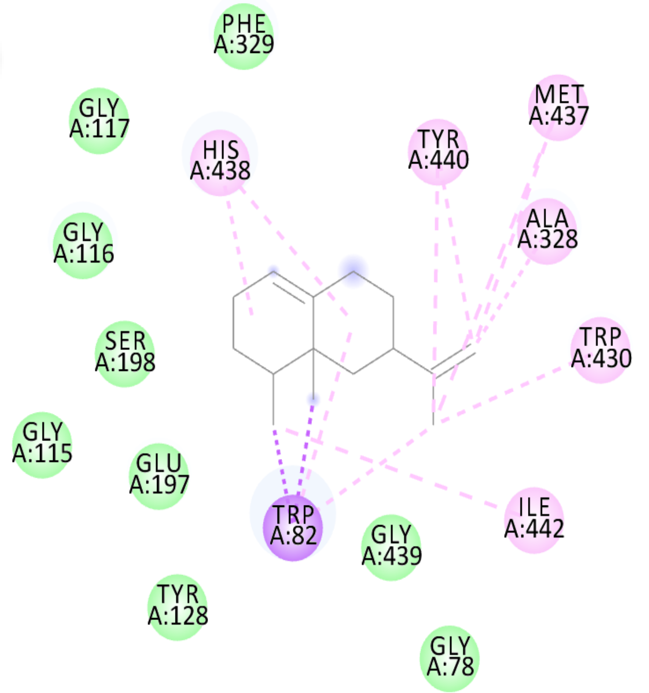
Alpha terpineol – Lipoxygenase -6,04

2



Limonene epoxide – AChE -5,54

3



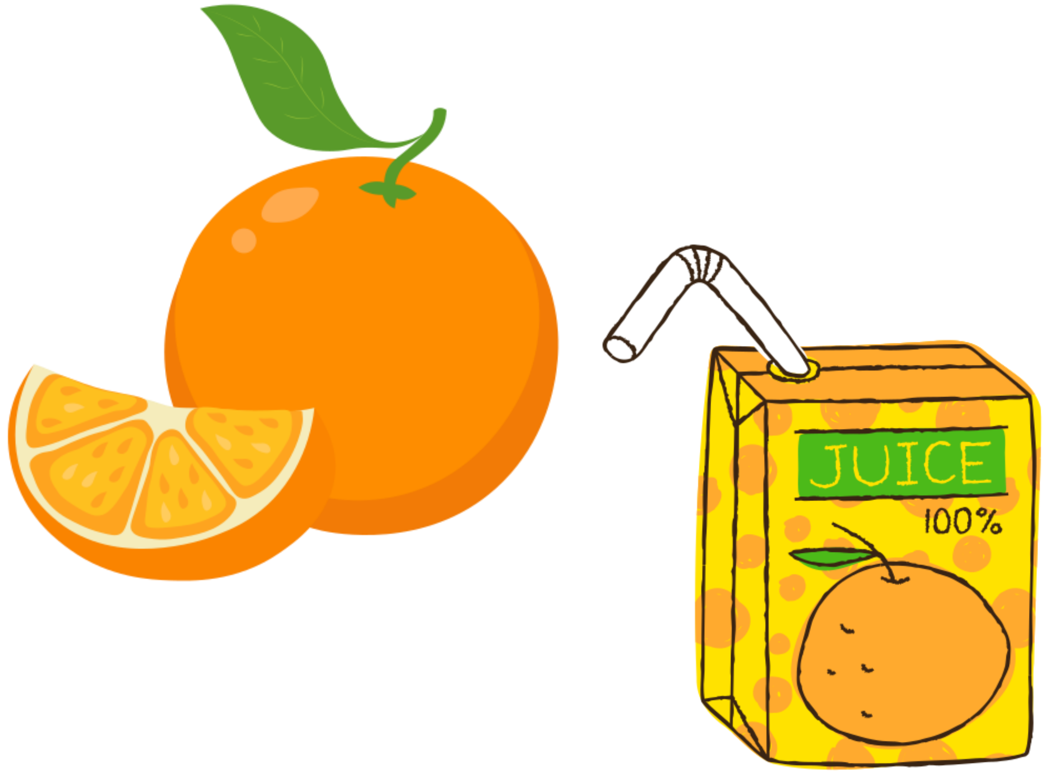
Valencene – BChE -6,54

IN SILICO MOLECULAR DOCKING MODEL

The enzymes **LOX**, **AChE** and **BChE** **docking complexes** with different ligands, the controls, that are commonly known inhibitors, and the new complexes performed with different terpenoids. In red colour are the **different binding energies acquired.**

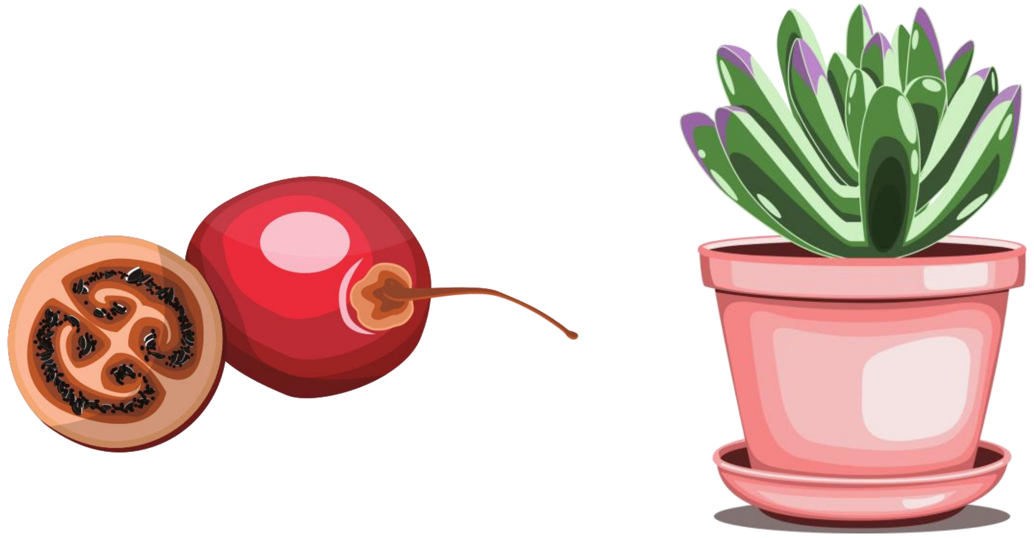
PART I

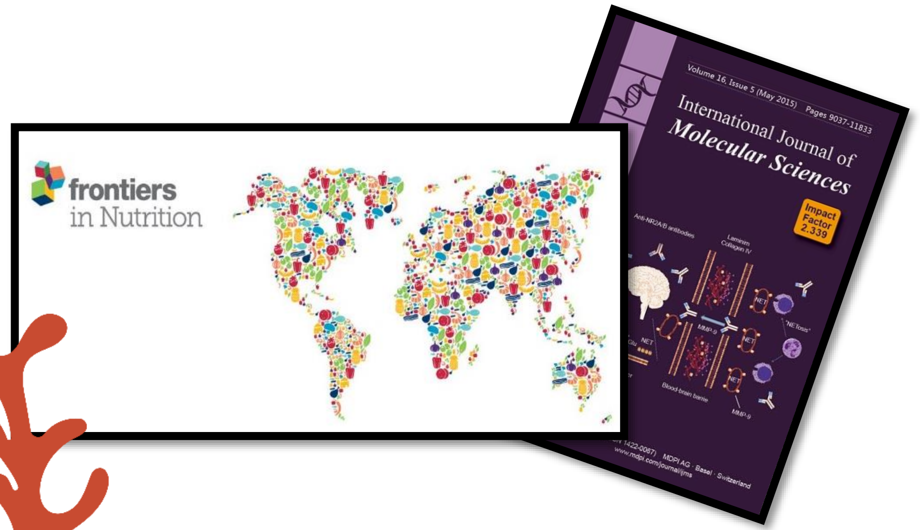
Study of the **neuroprotective** potential of extracts from **orange by-products** .



PART II

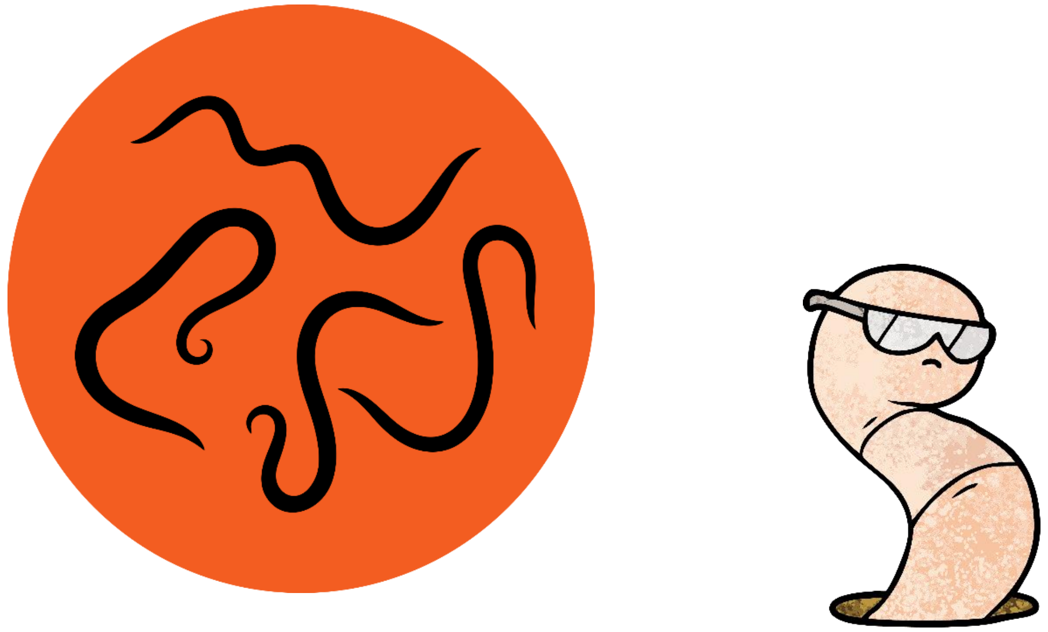
Other **natural matrices** as a source of **neuroprotective** compounds and their transport across the **BBB**.



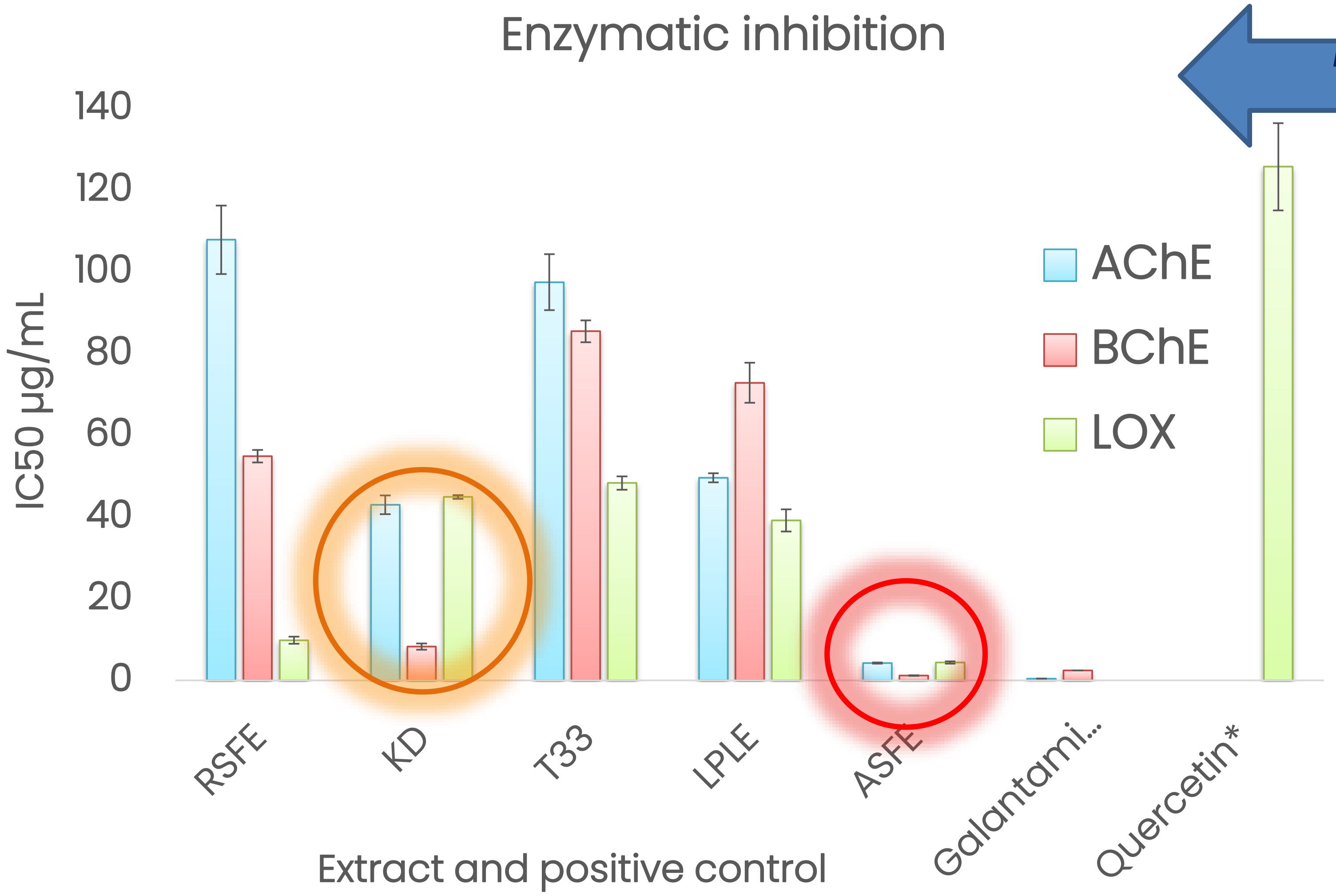


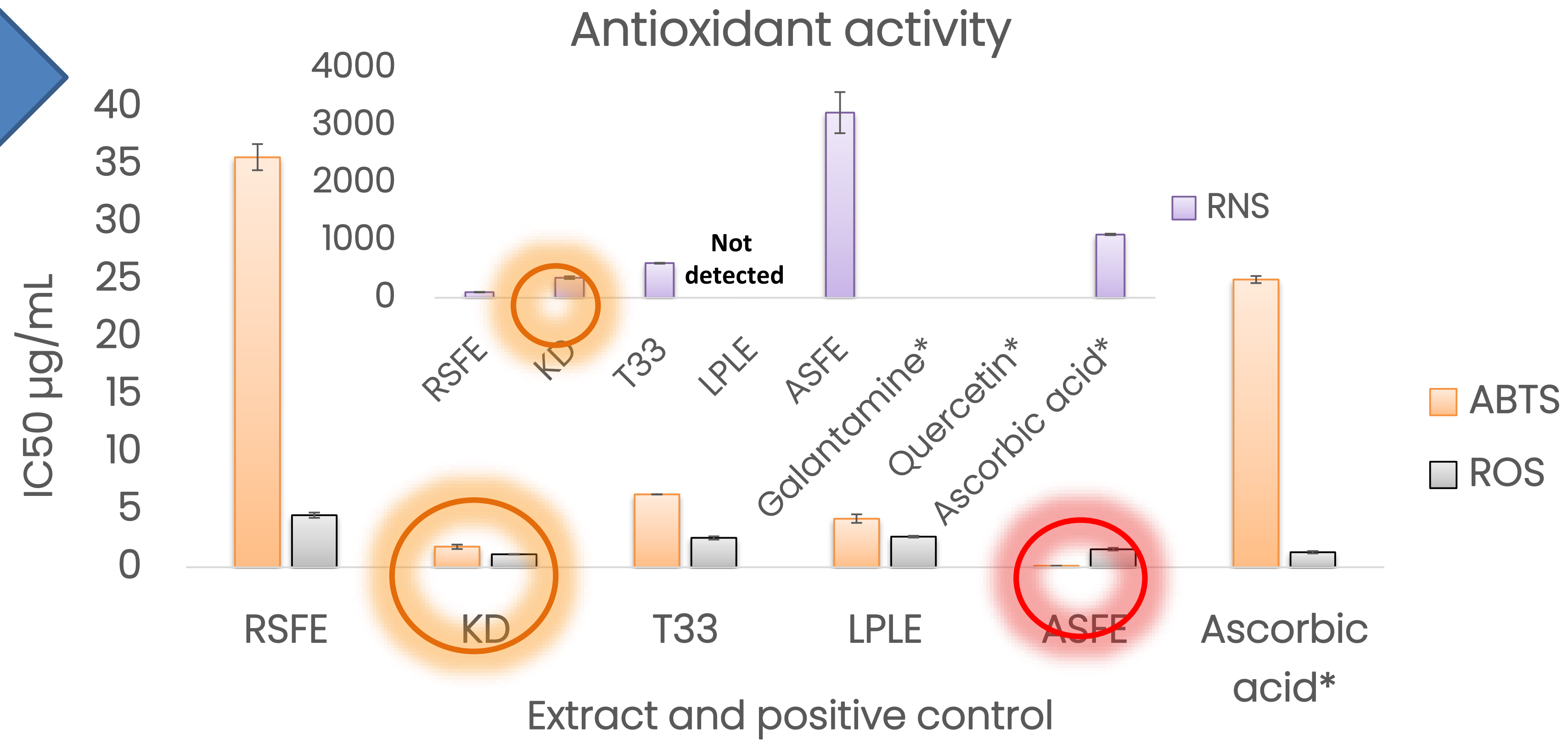
PART III

Foodomics study of the neuroprotective effect of extracts on *C. elegans* as *an in vivo* model of AD



III R&D: Part II Other natural matrices as a source of neuroprotective compounds and transport across the BBB.



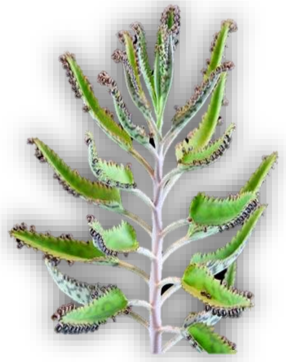


By-products

Cooperage woods

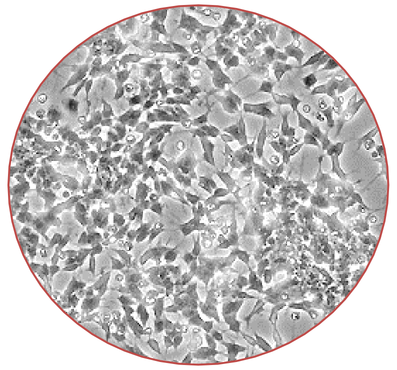
Plants

Extract	RSFE	KD	T33	LPLE	ASFE
Plant material	*Rosmarinus officinalis* L. (Rosemary) (Leaves)	*Kalanchoe daigremontiana* (Roots)	*Cyphomandra betacea* (Tamarillo) (Peel)	*Nothofagus pumilio* (Lenga)	*Robinia pseudoacacia* (Acacia)
Extraction method	SFE	UAE	PLE	PLE	SFE

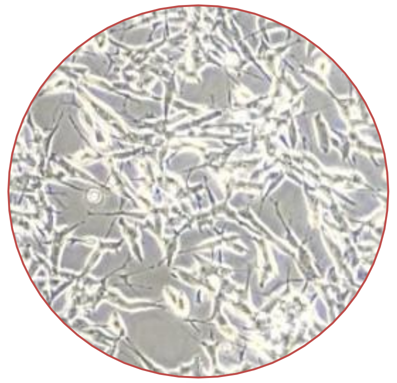

CYTOTOXICITY ASSAY

HK-2



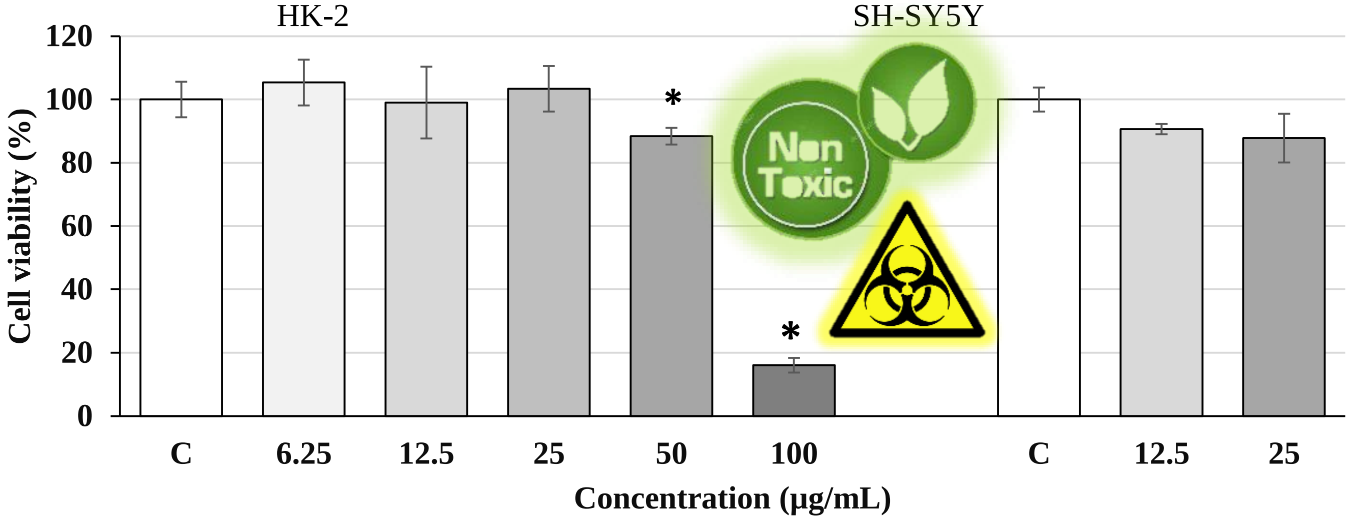
Proximal tubular epithelial cells

SH-SY5Y

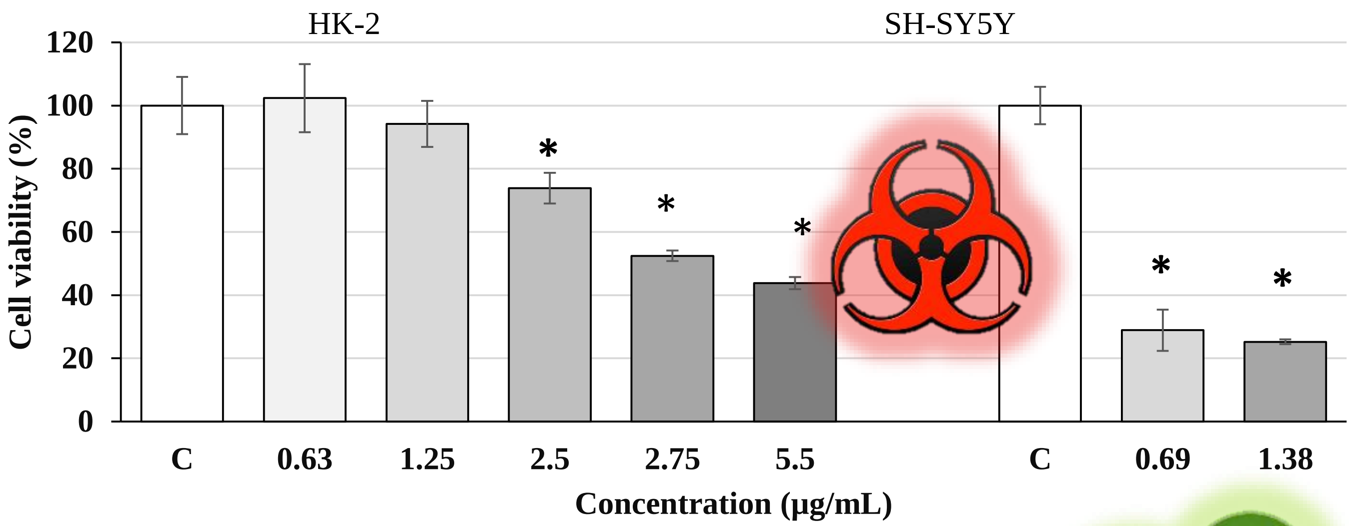


Neuroblastoma

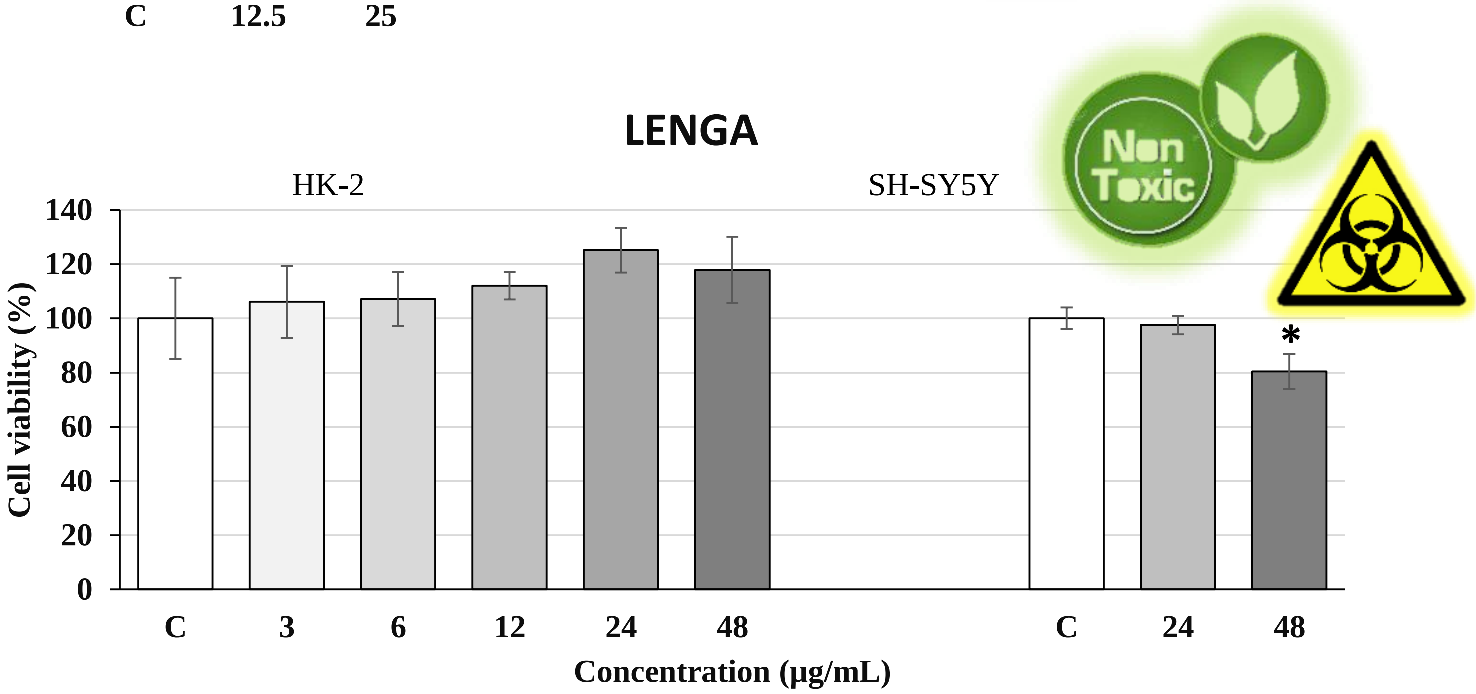
ROSEMARY



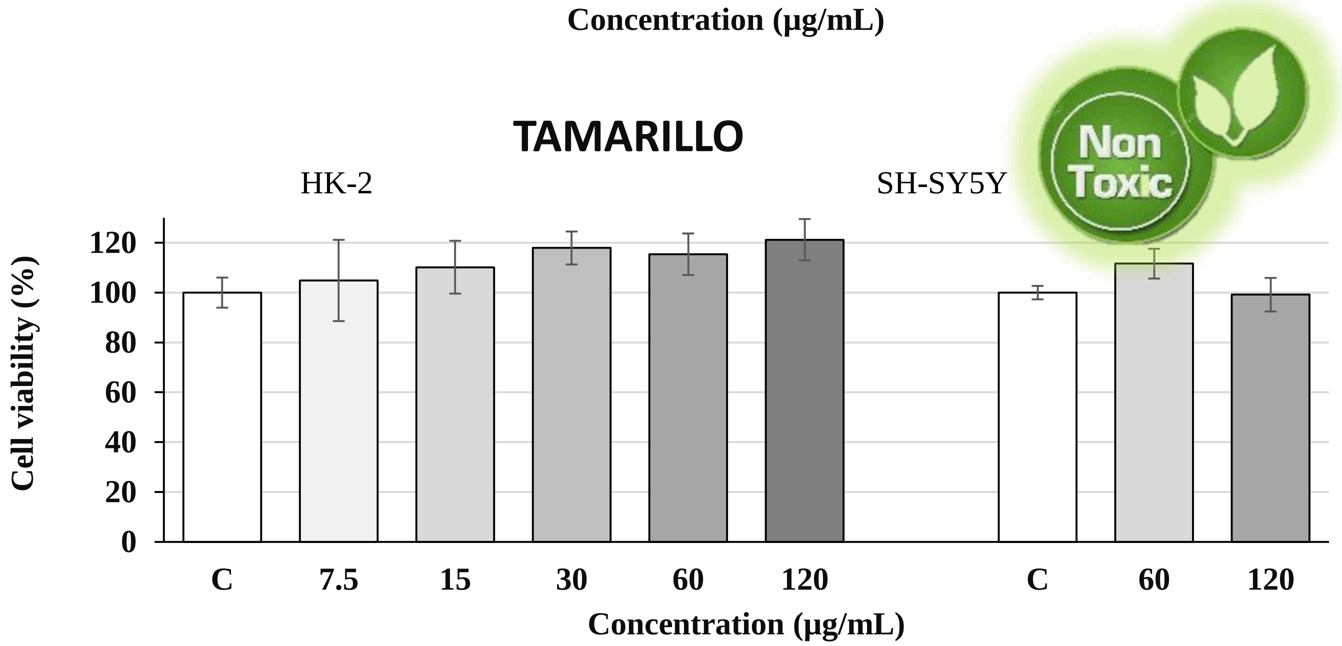
KALANCHOE



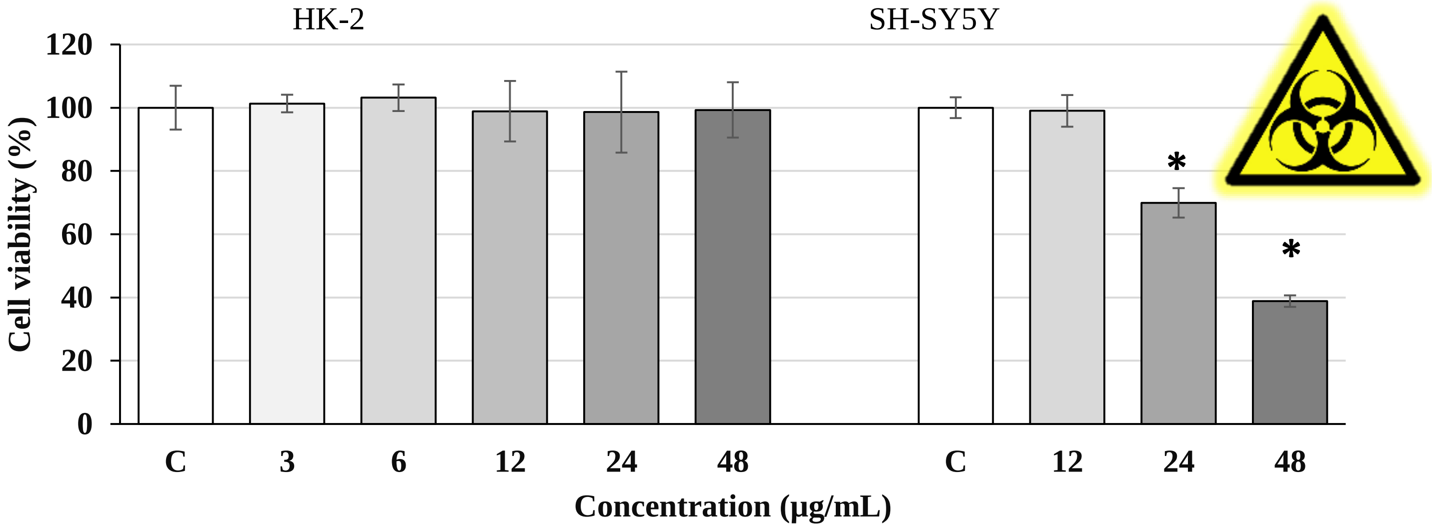
LENGA



TAMARILLO



ACACIA



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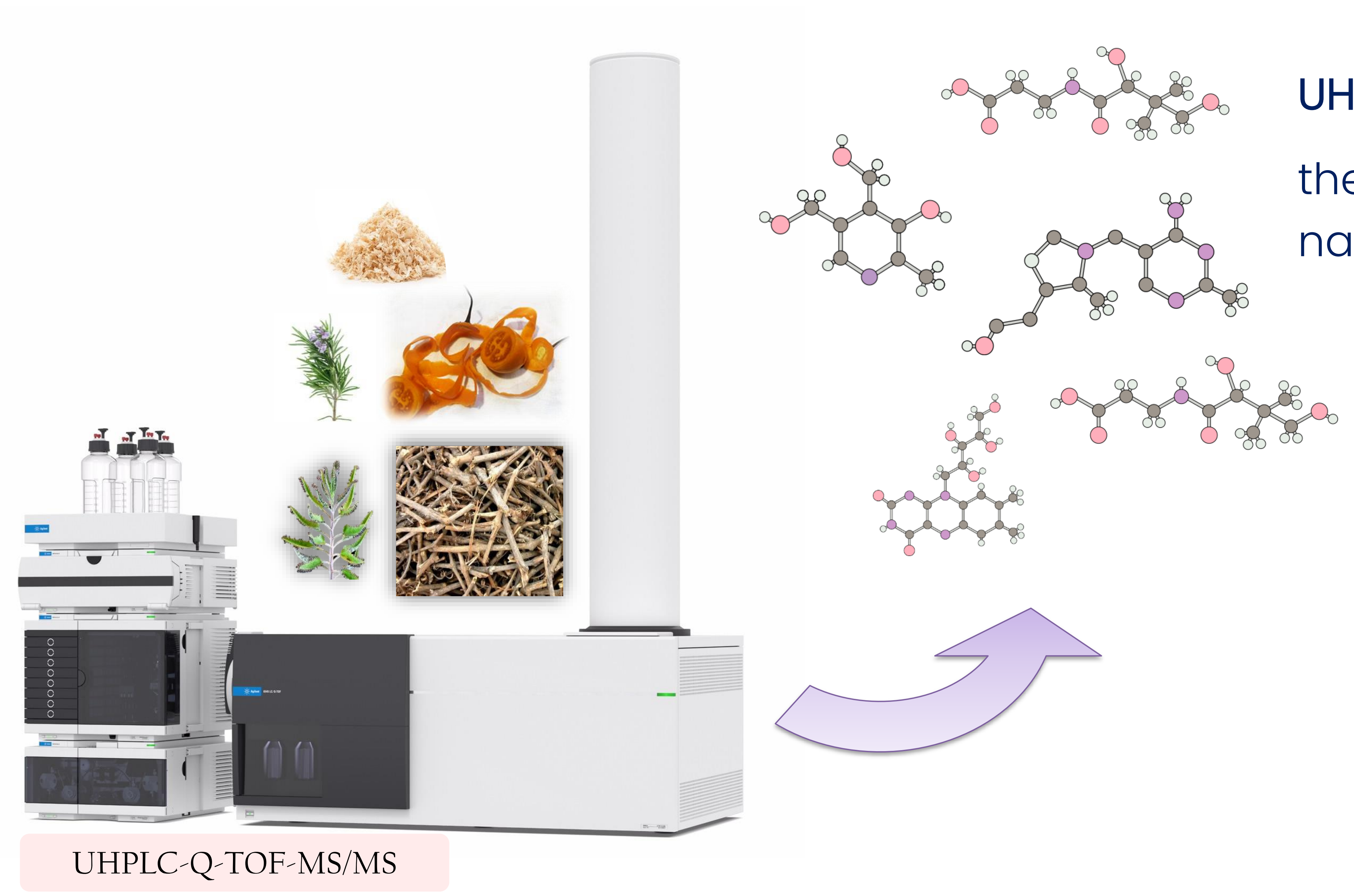
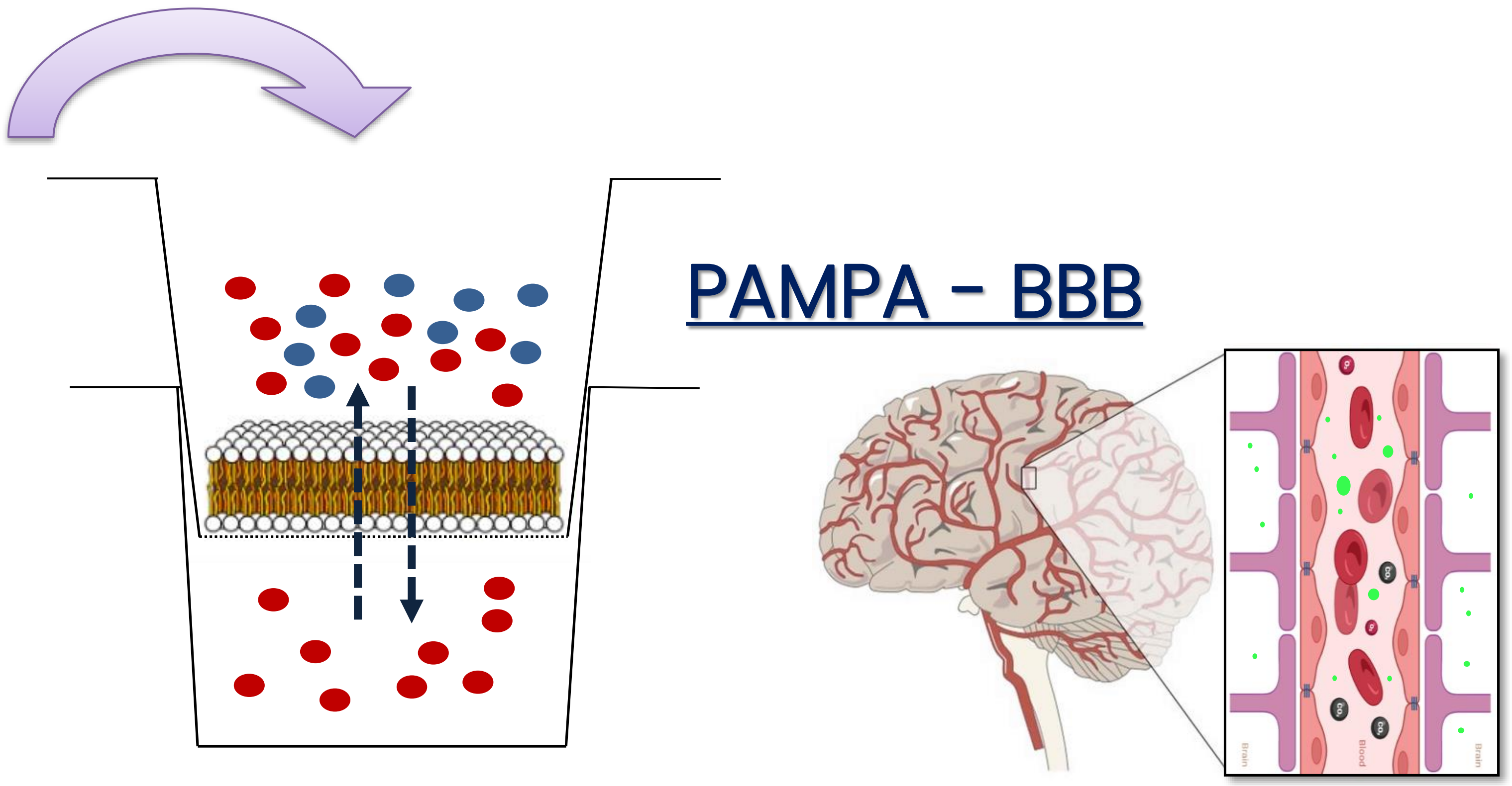
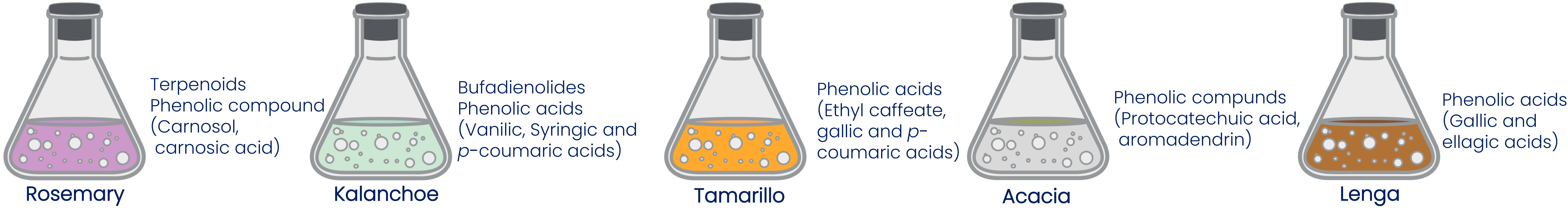
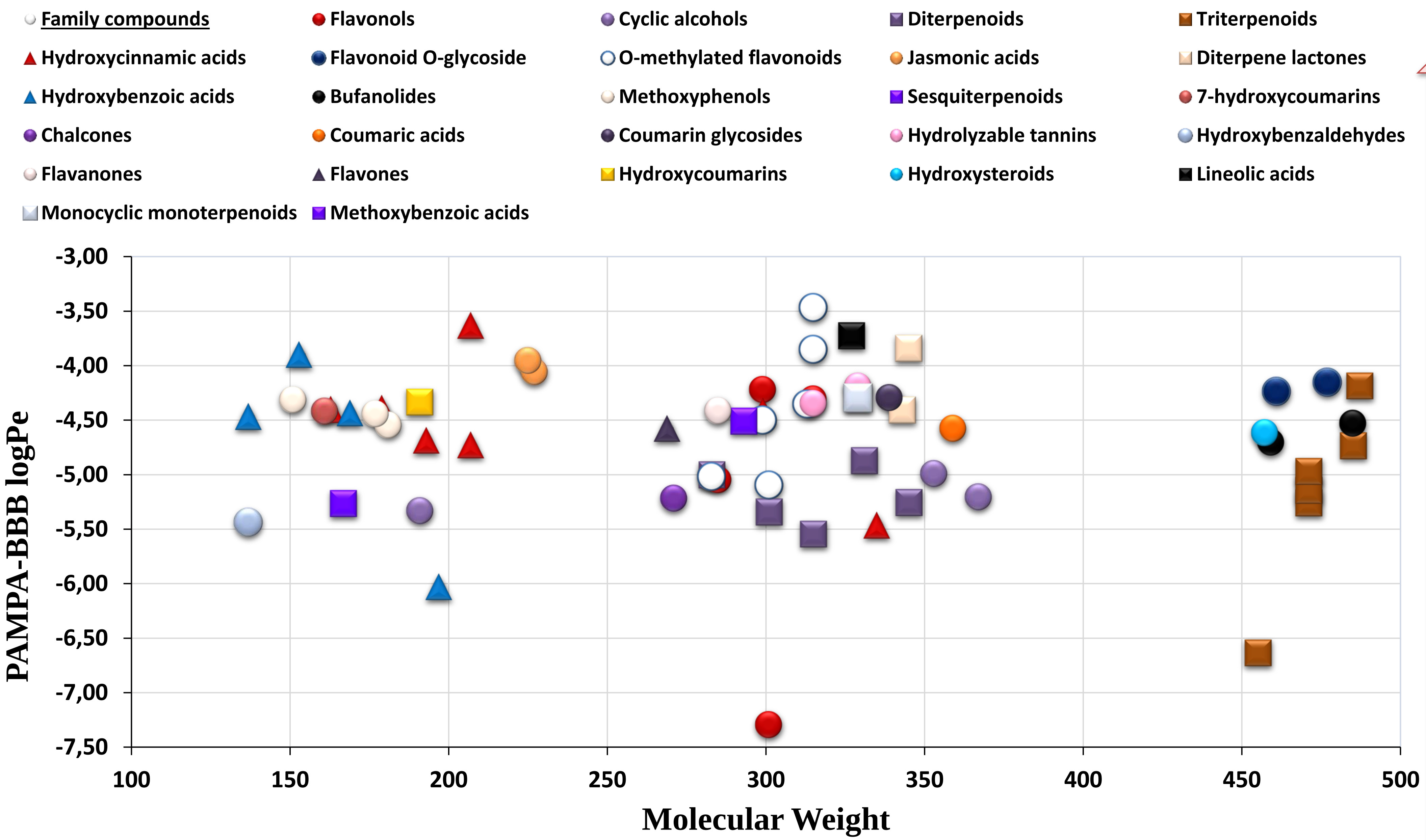


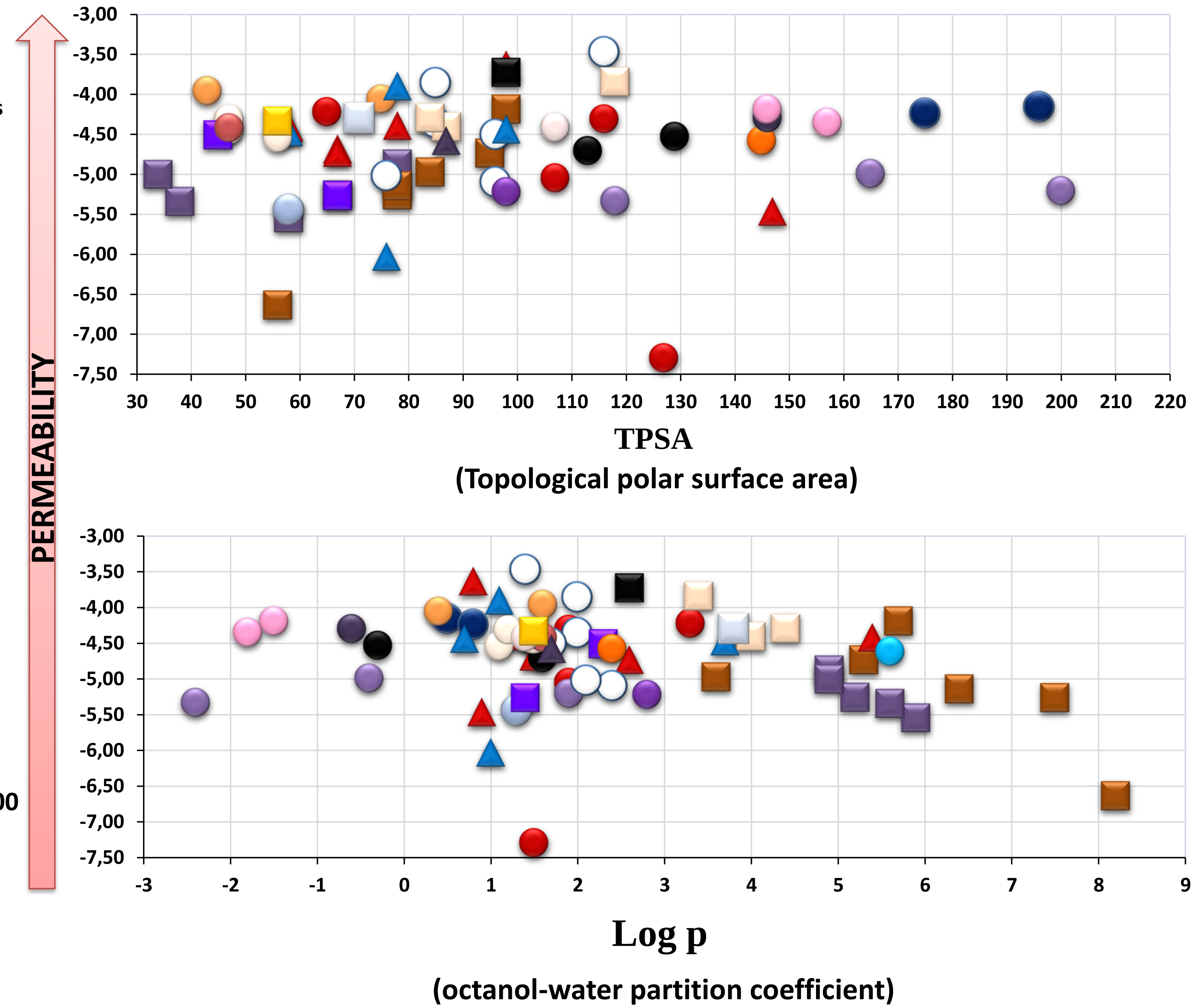
UHPLC-Q-TOF-MS/MS phytochemical proflling allowed the identifcation of **113 different** compounds from the natural matrices studied





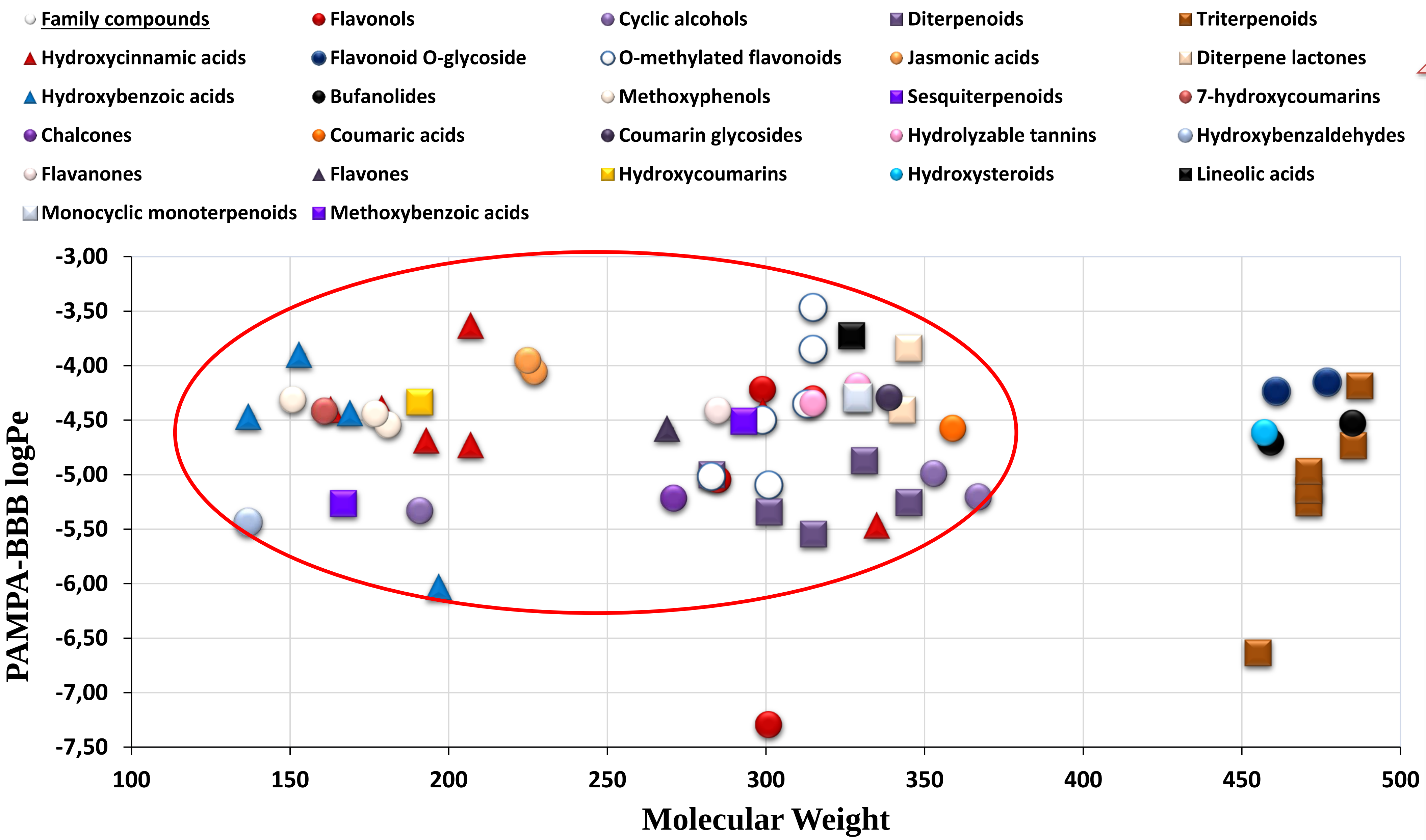
R&D: Part II Other natural matrices as a source of neuroprotective compounds and transport across the BBB.

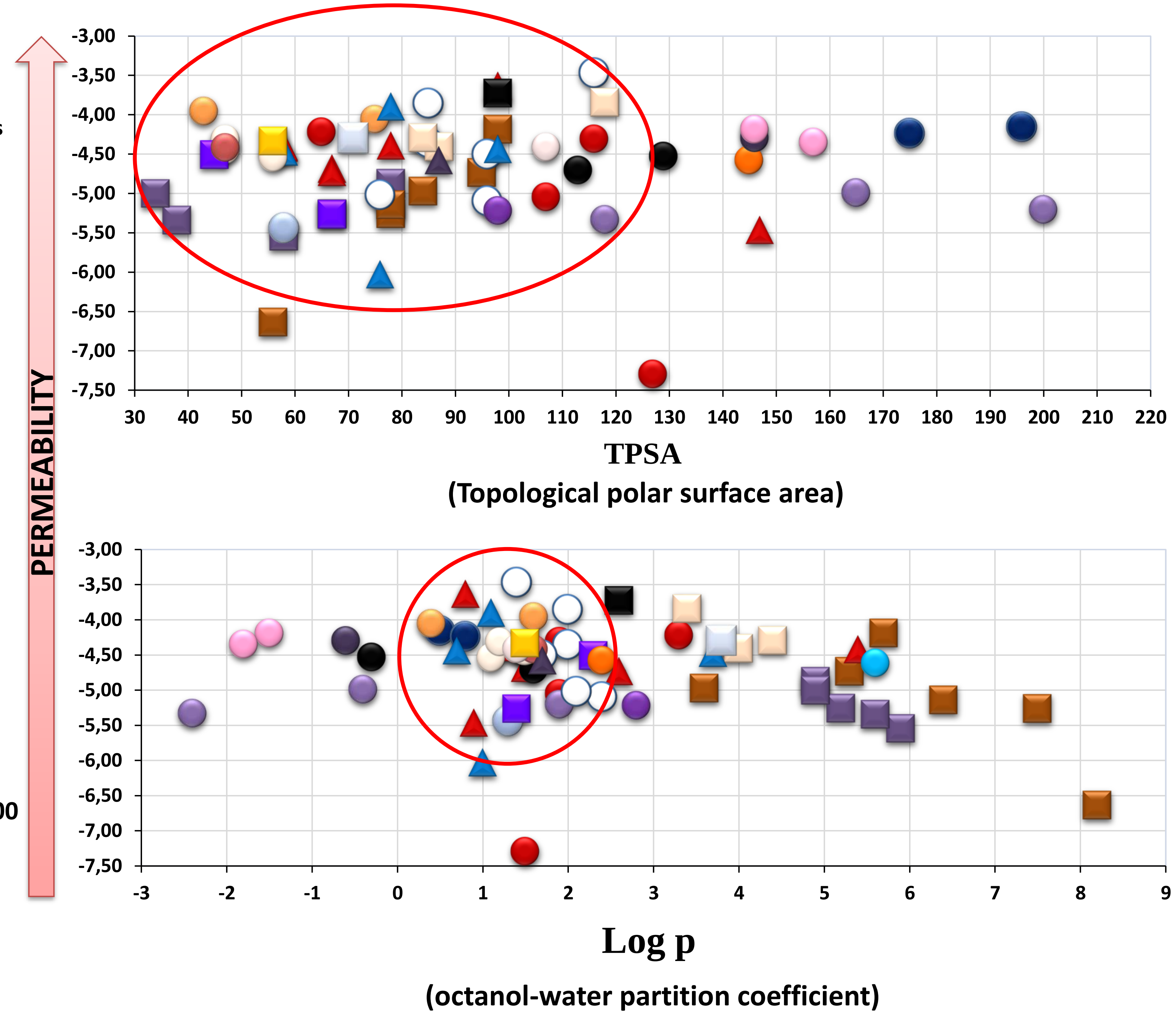




The **113** compounds classified into chemical families, and some physicochemical molecular parameters were considered to study their BBB permeability.

R&D: Part II Other natural matrices as a source of neuroprotective compounds and transport across the BBB.

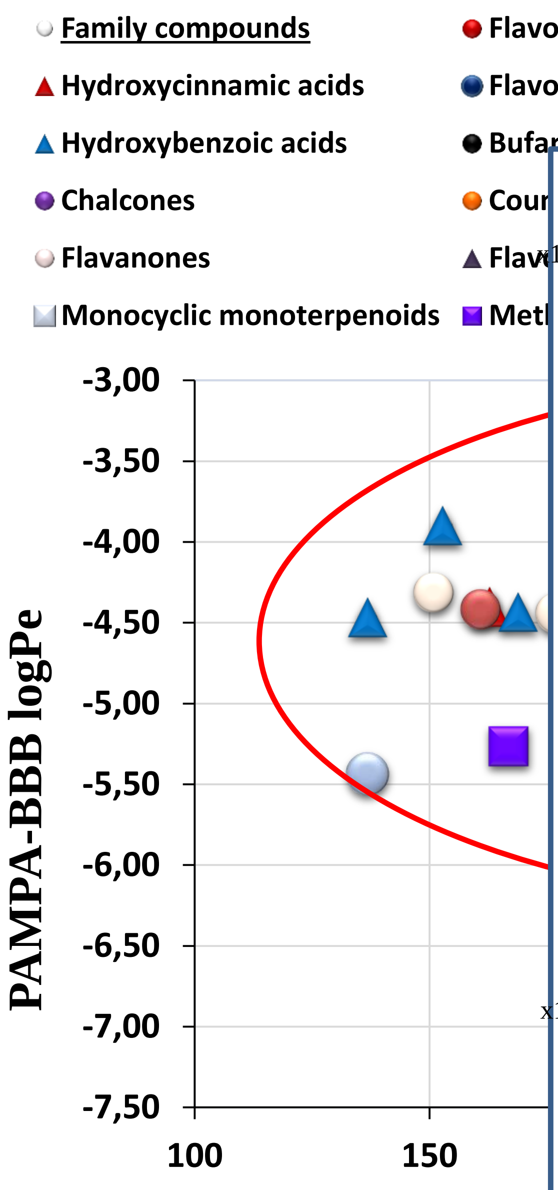


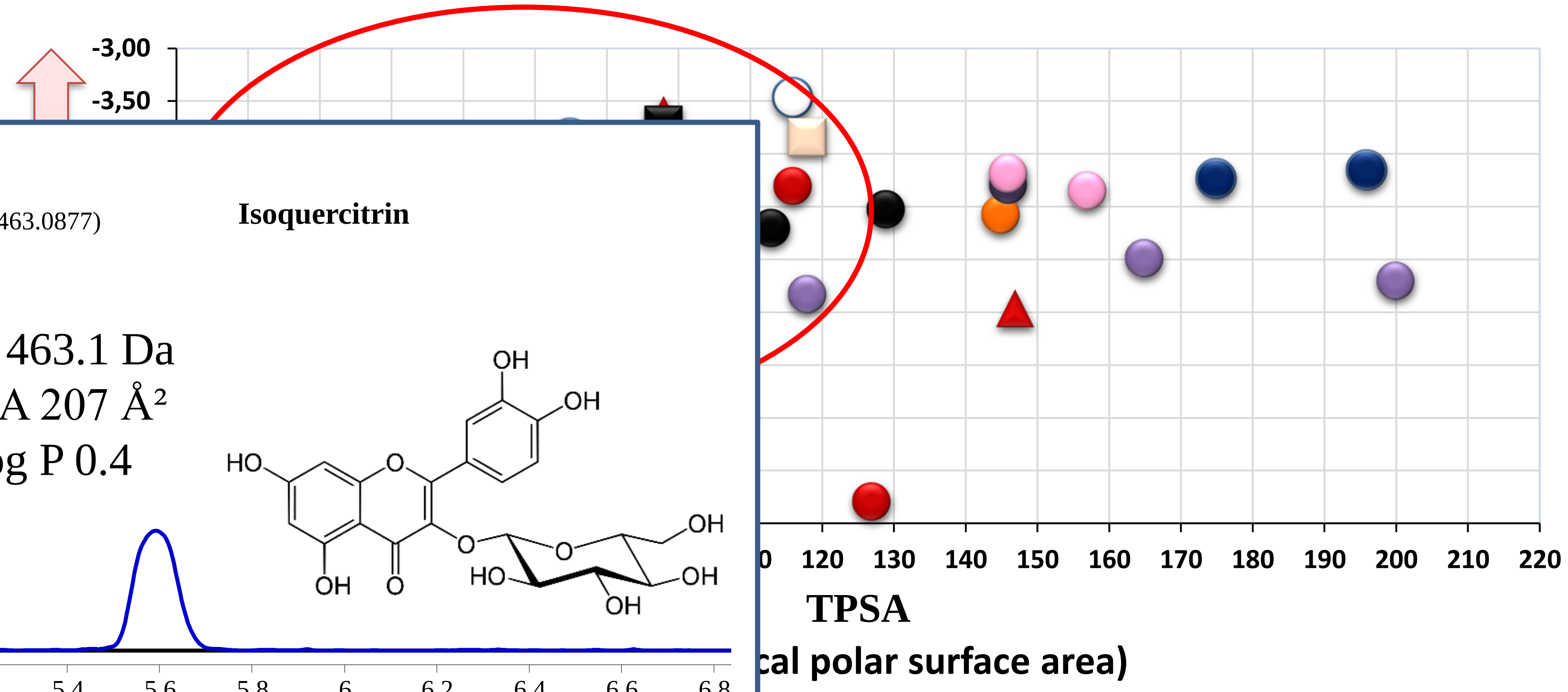


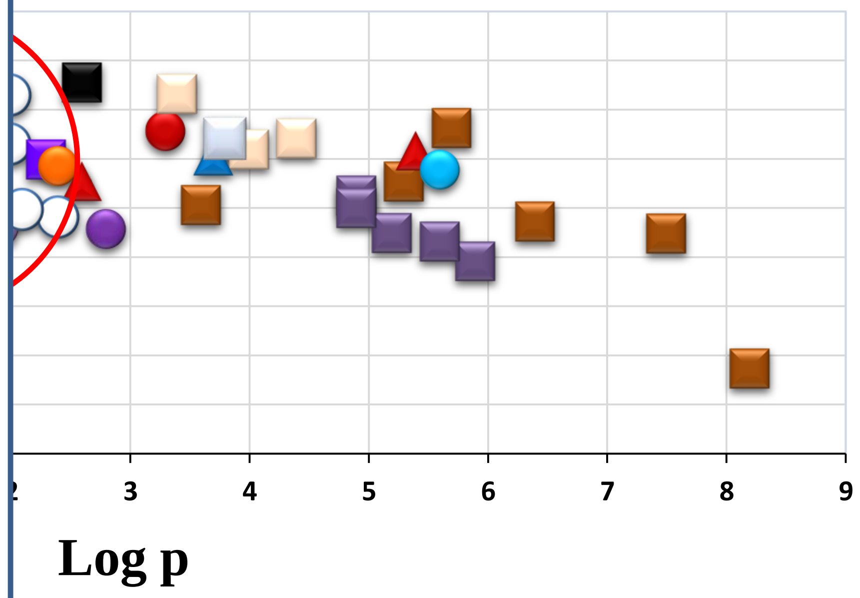
The **113** compounds classified into chemical families, and some physicochemical molecular parameters were considered to study their BBB permeability.

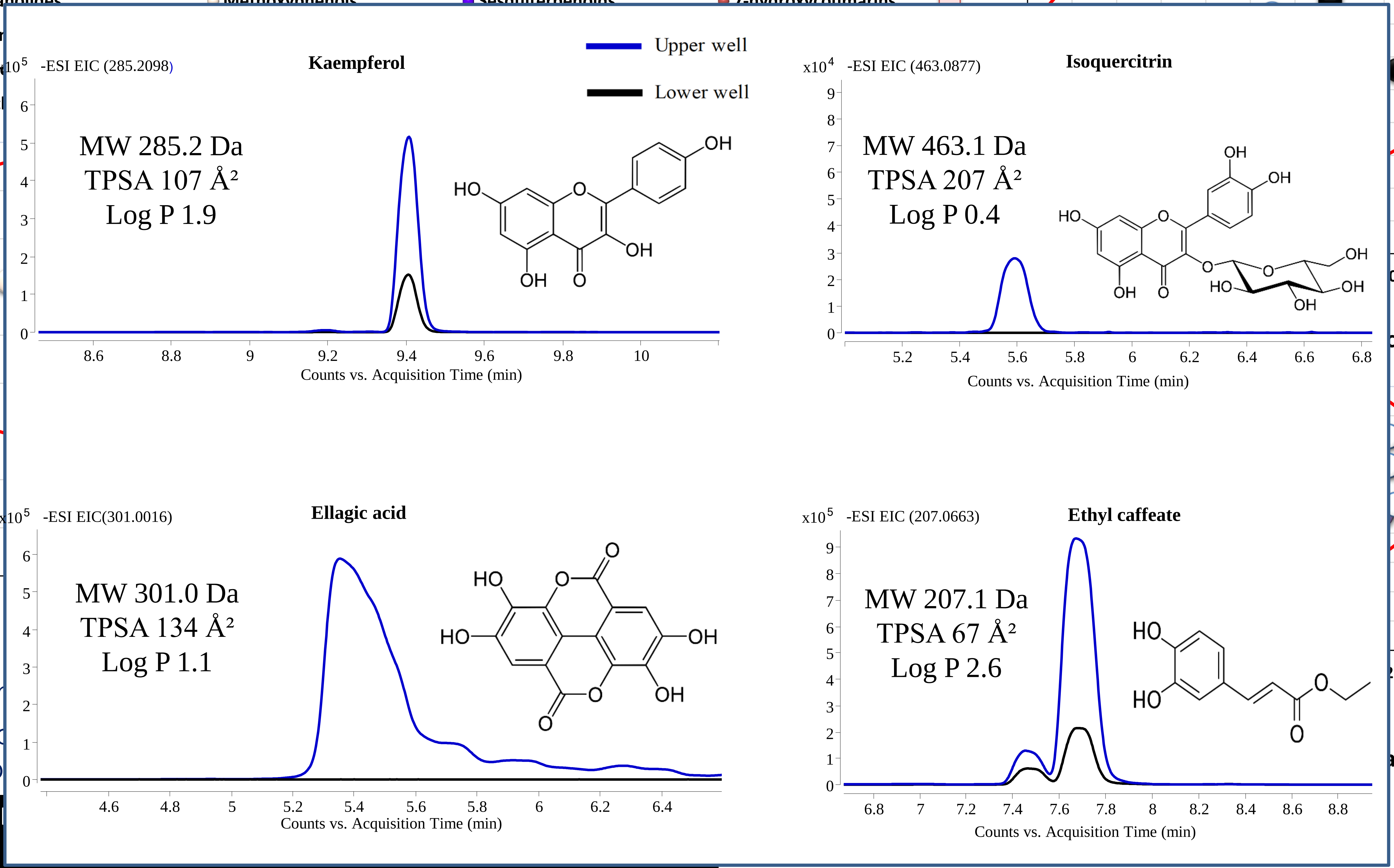
	Our results	Other authors (Hitchcock SA., 2008; Agatonovic-Kustrin S., et al., 2020; Waterhouse R N., 2003)
MW	< 500 Da	< 500 Da
TPSA	< 120 Å^2	< 90 Å^2
logP	0-2	0-3

III R&D: Part II Other natural matrices as a source of neuroprotective compounds and transport across the BBB.







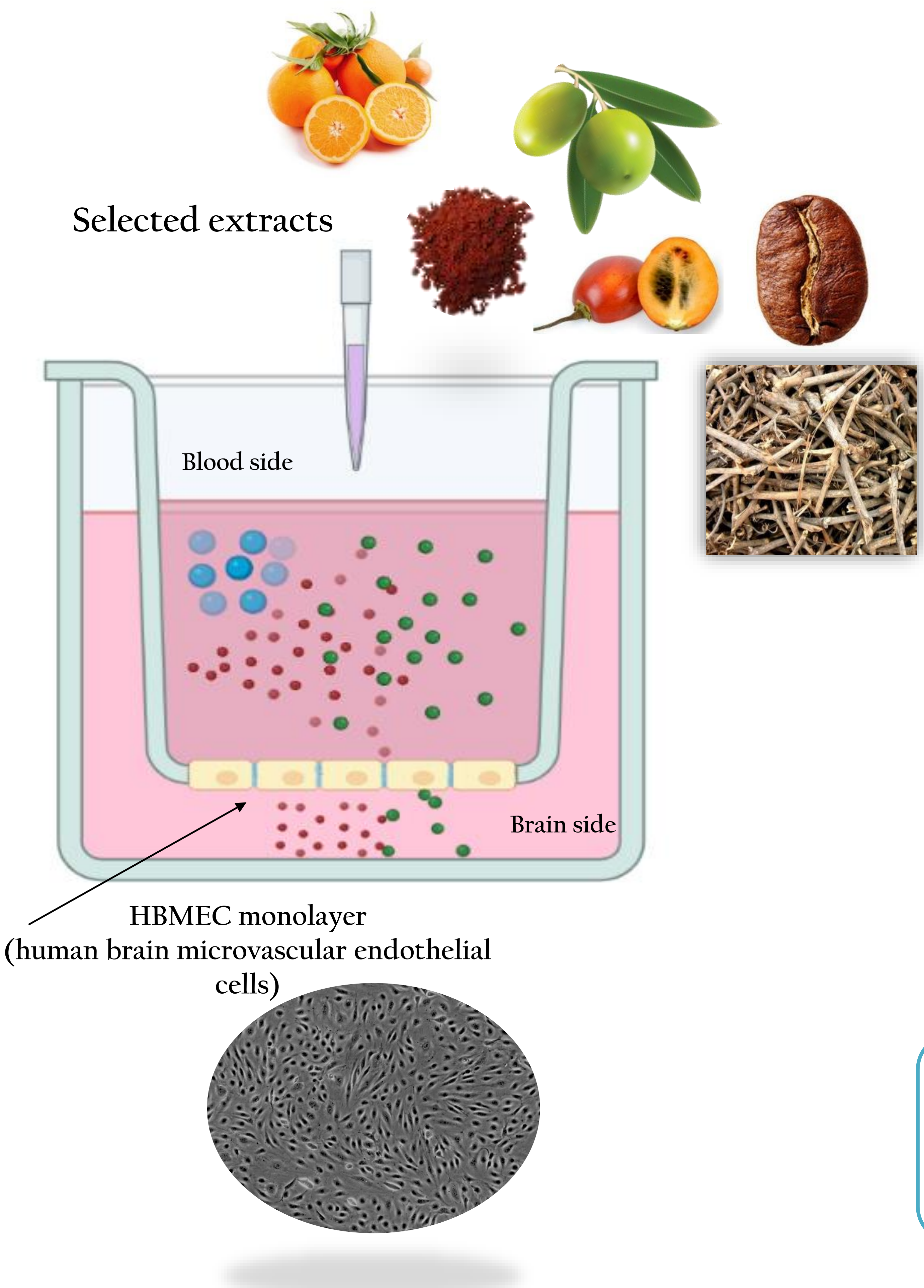


cal polar surface area)

ater partition coefficient)

The 113 compour
and some physicoc
to study their BBB p

	Our resul	Waterhouse R N., 2003)
MW	< 500 Da	< 500 Da
TPSA	< 120 Å^2	< 90 Å^2
logP	0-2	0-3

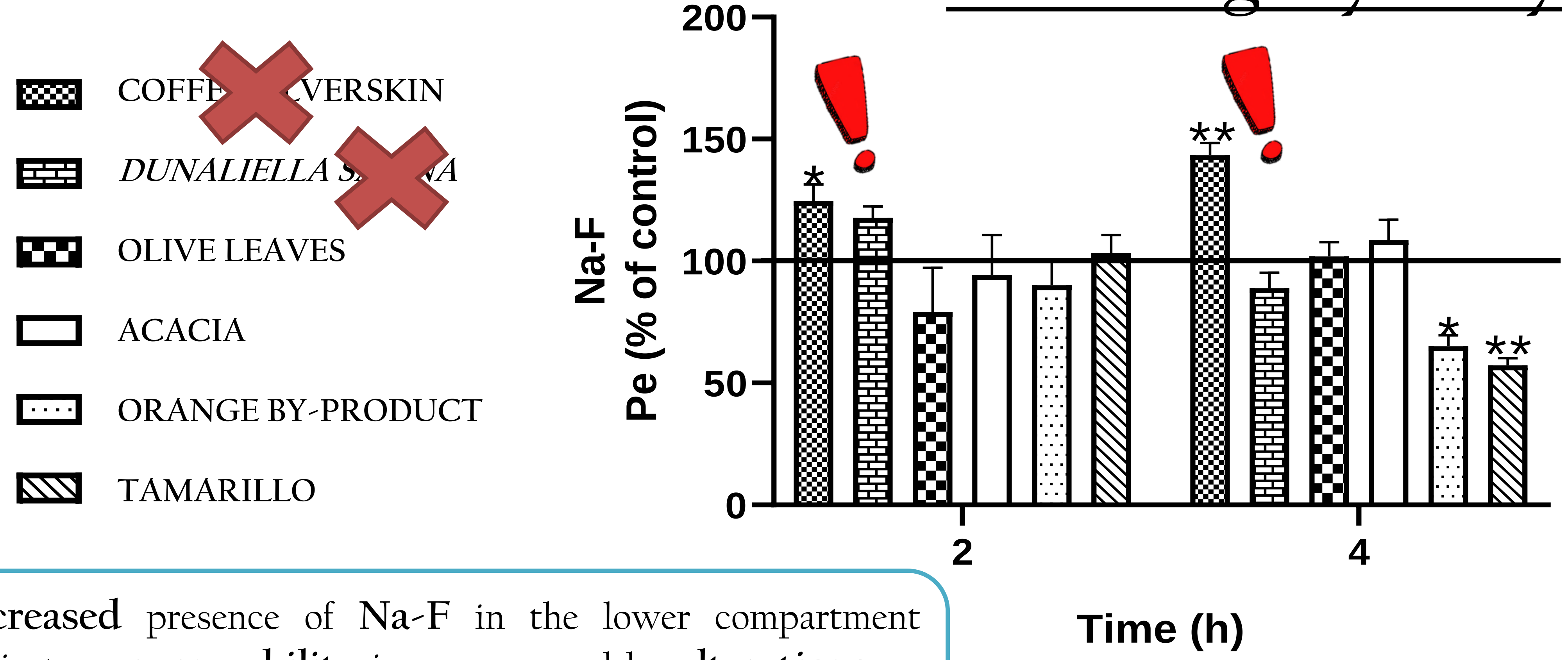


- ✓ ORANGE BY-PRODUCT
- ✓ TAMARILLO
- ✓ ACACIA
- ✓ *DUNALIELLA SALINA* — Gallego R., et al., *Anal Bioanal Chem* (2021) **414**:5357-5371.
- ✓ OLIVE LEAVES — Gallego R., et al., *Front Nutr* (2021) **0**:768.
- ✓ COFFEE SILVERSKIN

SH-SY5Y neuroprotection against $A\beta_{1-42}$

- *In vitro* **neuroprotective** potential
- *Low cytotoxic effect*
- *Innovative and promising source of bioactive compounds*

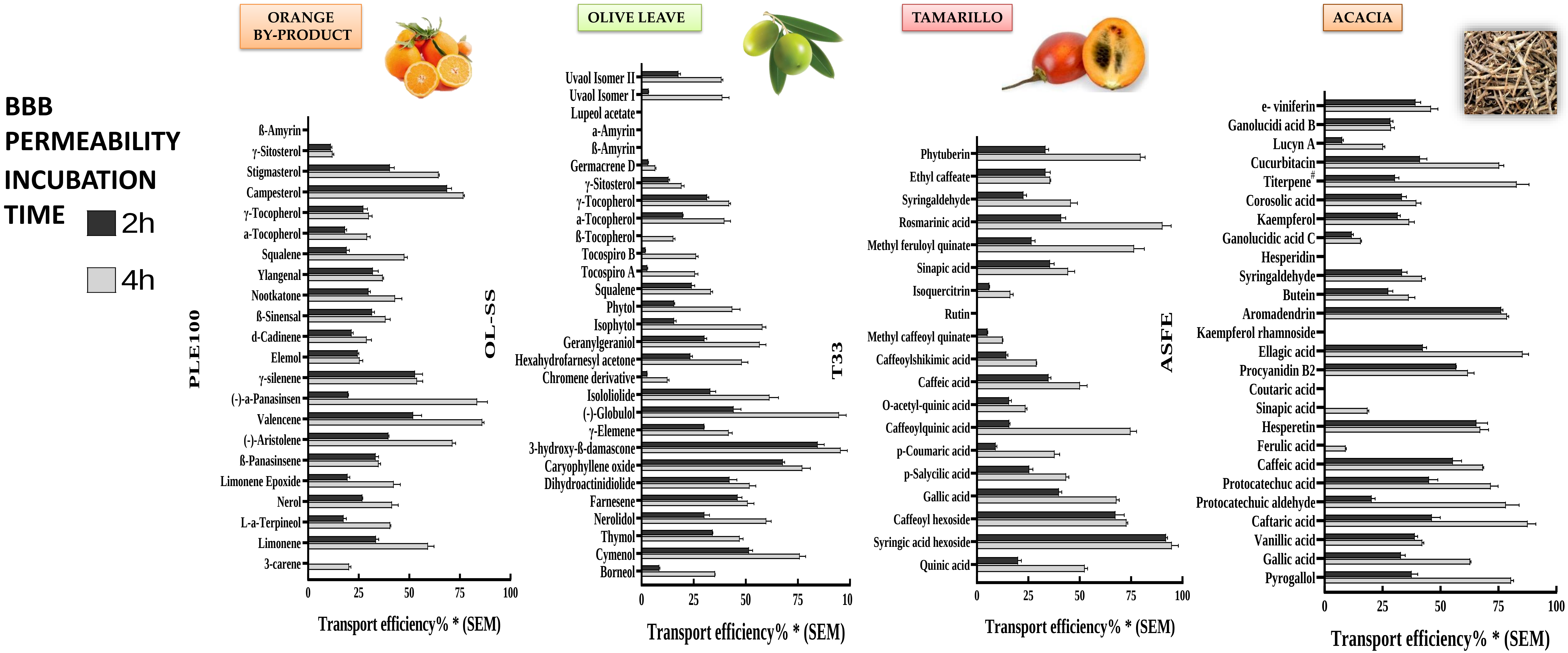
BBB Integrity assay



Increased presence of **Na-F** in the lower compartment indicates a **permeability** increase caused by **alterations** on the monolayer integrity and, as a consequence, the **loss of barrier functionality**

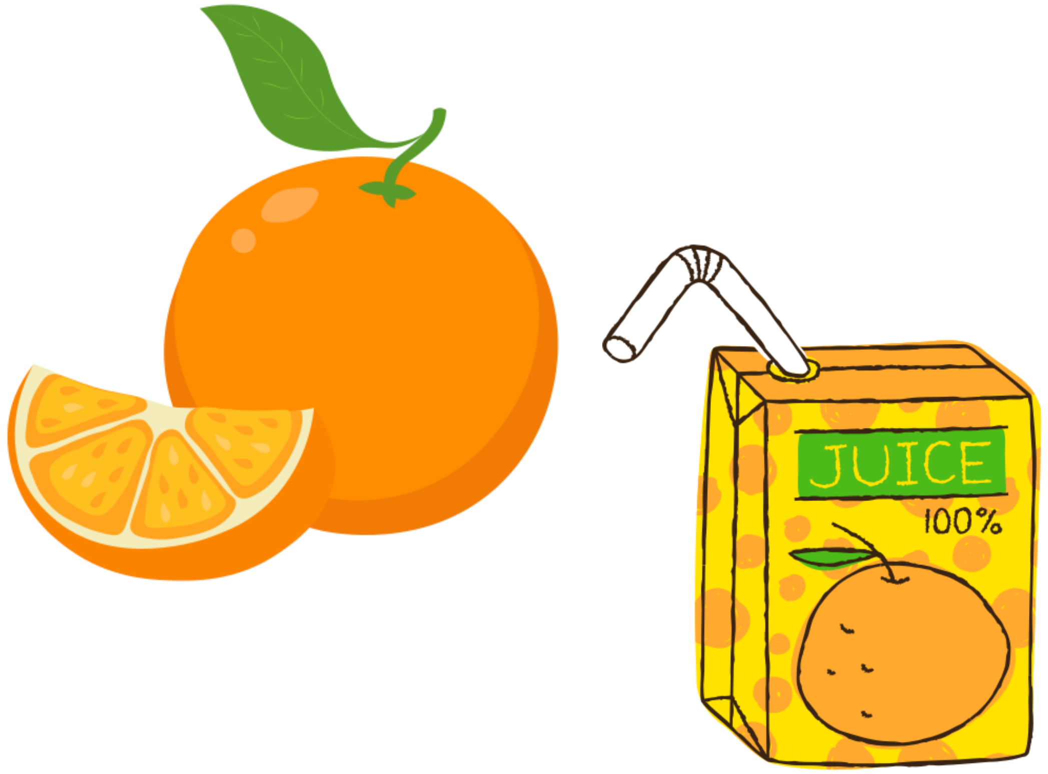
R&D: Part II Other natural matrices as a source of neuroprotective compounds and transport across the BBB.

- ✓ Along with **MW** and **TPSA**; the **logP** values, the presence of **HBD** and **HBA** should be also considered in BBB permeability.
- ✓ The results of this work reinforce the neuroprotective potential of bioactive molecules such as **low molecular weight terpenoids (C10 and C15) like limonene epoxide or valencene**, as well as phenolic acids and flavonoids from various natural sources, including **protocatechuic, ferulic and gallic acids, among other compounds.**



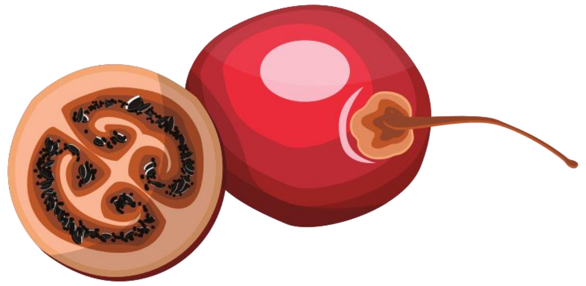
PART I

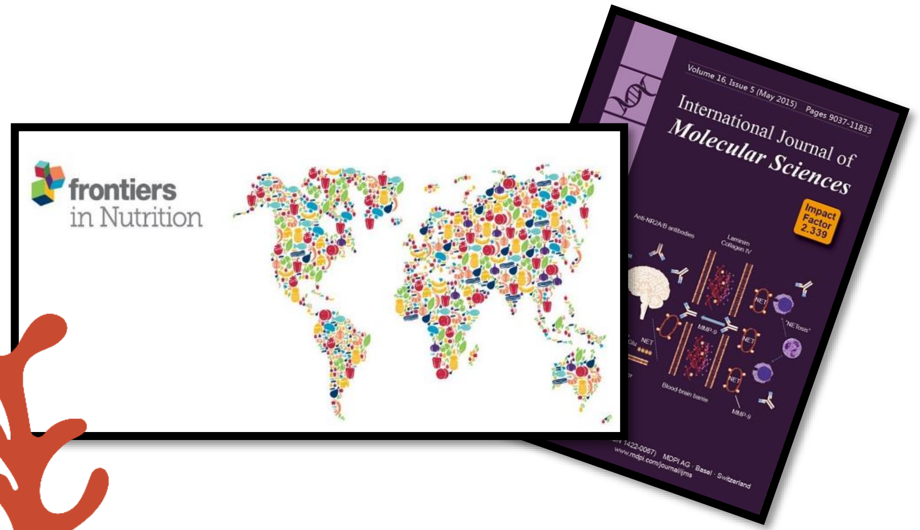
Study of the **neuroprotective** potential of extracts from **orange by-products** .



PART II

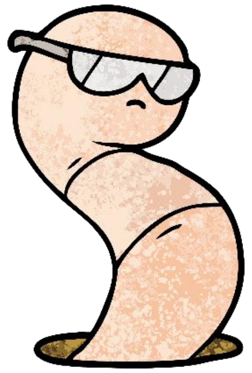
Other **natural matrices** as a source of **neuroprotective** compounds and their transport across the **BBB**.





PART III

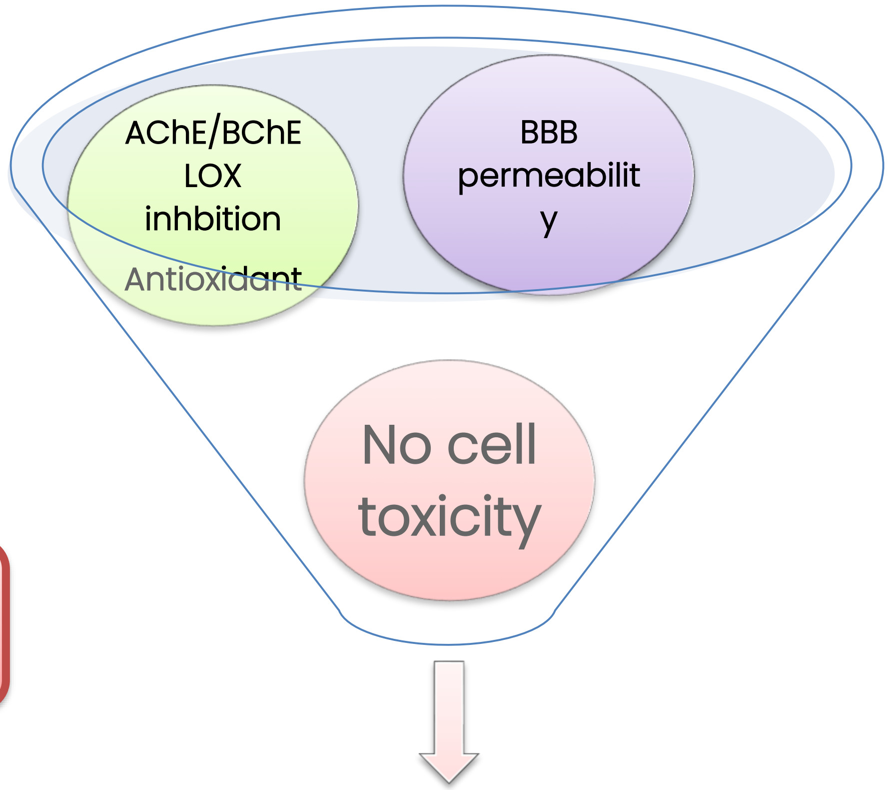
Foodomics study of the neuroprotective effect of extracts on *C. elegans* as *an **in vivo*** model of AD

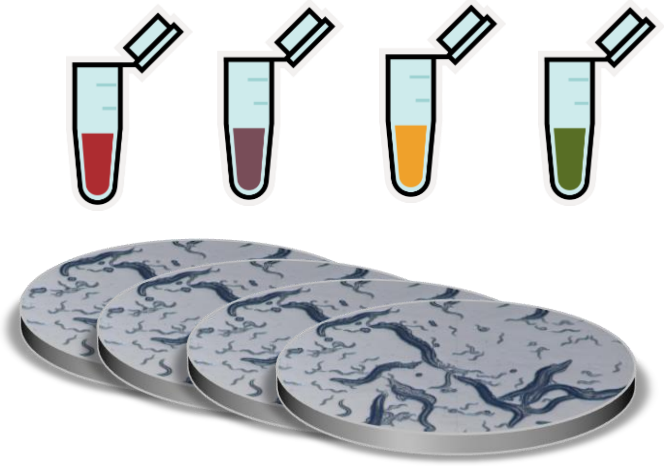


III R&D: Part III Foodomics study of the neuroprotective effect of extracts on *C. elegans* as an *in vivo* model of AD

Extractos	AChE	BChE	LOX	ABTS	ROS	RNS
	IC50 µg/mL (± Desviación estándar)					
Robinia pseudoacacia (ASFE)	4.23 (0.1)	1.20 (0.0)	4.37 (0.2)	0.11 (0.0)	1.56 (0.12)	3218 (358)
Dunaliella salina (DS)	18.9 (0.9)	113 (11)	63.3 (6.5)	16.3 (2.1)	3.41 (0.2)	698.2 (34)
Orange by product (PLE 100)	137 (8.1)	147 (7.5)	76.1 (10)	13.5 (0.8)	4.38 (0.4)	1199 (98)
Olive leaf by product Sand (OL-SS)	144 (29)	183 (22)	104 (11)	82.5 (1.1)	18.2 (0.5)	1036 (114)
Coffee Silverskin (PPC1)	67.2 (5.3)	150 (1.0)	52.2 (5.3)	6.86 (0.0)	6.95 (0.5)	838.2 (99)
Rosmarinus officinalis (RSFE)	107 (8.4)	54.9 (1.5)	9.82 (0.8)	35.6 (1.1)	4.51 (0.2)	95.35 (6.4)
Orange by product (maceration)	179 (1.2)	118 (0.3)	130 (7.1)	84.1 (7.8)	5.54 (0.1)	556.9 (11)
Kalanchoe daigremontiana (KD)	42.9 (2.3)	8.26 (0.7)	44.9 (0.4)	1.77 (0.2)	1.12 (0.0)	348.5 (25)
Tamarillo (T33)	97.5 (6.8)	85.5 (2.7)	48.3 (1.6)	6.33 (0.0)	2.54 (0.1)	599.7 (5.9)
Olive leaf by product Silica 150P SFE	270 (16)	NR	139 (11)	32.6 (0.11)	NR	NR
Passiflora mollissima by product PLE	194 (15)	206 (7.9)	146 (26)	121 (15)	9.15 (0.8)	292.1 (14)
Physalis peruviana L. by product PLE	803 (103)	930 (46)	406 (24)	54.2 (2.4)	153 (17)	621.3 (33)
Nannochloropsis oceanica PLE	269 (19)	267 (19)	135 (15)	112 (14)	41.3 (3.6)	747.9 (81)
Porphyridium cruentum PLE	668 (44)	768 (11)	278 (22)	145 (18)	95.2 (4.7)	902.5 (120)
Tisochrysis lutea PLE	47.0 (3)	146 (8.4)	28.4 (2.7)	14.6 (0.0)	NR	NR
Haematococcus pluvialis PLE	87.1 (2)	113 (12)	51.8 (4.5)	18.7 (0.4)	NR	NR
American oak PLE	205 (22)	180 (9.7)	97.5 (7.6)	5.58 (0.6)	1.81 (0.1)	3359 (156)
French oak PLE	151 (0.5)	151 (0.5)	33.8 (2.9)	1.7 (0.0)	2.22 (0.2)	ND
Chestnut PLE	121 (1.9)	85.8 (6.5)	83.3 (3.0)	4.15 (0.3)	1.96 (0.2)	4210 (286)
Cherry PLE	80.9 (5.9)	80.9 (5.9)	147 (10)	0.43 (0.0)	0.38 (0.0)	448.8 (11)
Robinia pseudoacacia PLE	7.94 (0.9)	1.09 (0.1)	4.64 (0.3)	0.11 (0.0)	1.59 (0.1)	3024 (121)
Lenga native (LPLE)	49.6 (1.0)	72.8 (4.9)	39.1 (2.7)	4.22 (0.4)	2.65 (0.1)	ND
Sarmiento PLE	368 (34)	381 (30)	167 (9.9)	8.81 (0.6)	1.75 (0.1)	1462 (194)
Raspón PLE	60.2 (3.8)	445 (36)	288 (10)	9.74 (0.4)	2.71 (0.0)	3193 (267)
American oak SFE	191 (12)	193 (13)	41.3 (2.2)	7.43 (0.8)	3.23 (0.2)	1217 (18)
French oak SFE	205 (6.1)	121 (9.8)	26.8 (2.4)	2.31 (0.1)	2.97 (0.0)	ND
Chestnut SFE	167 (3.8)	148 (12)	66.9 (4.2)	5.07 (0.6)	3.27 (0.3)	1301 (65)
Cherry SFE	80.1 (10)	67.9 (1.0)	55.6 (4.8)	1.51 (0.1)	0.59 (0.0)	670.8 (35)
Lenga native SFE	76.2 (0.1)	73.5 (4.7)	35.9 (0.4)	1.68 (0.2)	4.02 (0.1)	ND
Sarmiento SFE	368 (34)	722 (24)	118 (9.1)	15.1 (1.7)	2.97 (0.1)	1019 (72)
Raspón SFE	30.6 (2.2)	283 (14)	119 (8)	10.5 (1.2)	2.82 (0.1)	2451 (57)
Control positivo*	0.40 (0.0)	2.36 (0.0)	125 (10)	4.56 (0.4)	0.98 (0.1)	1100 (13)

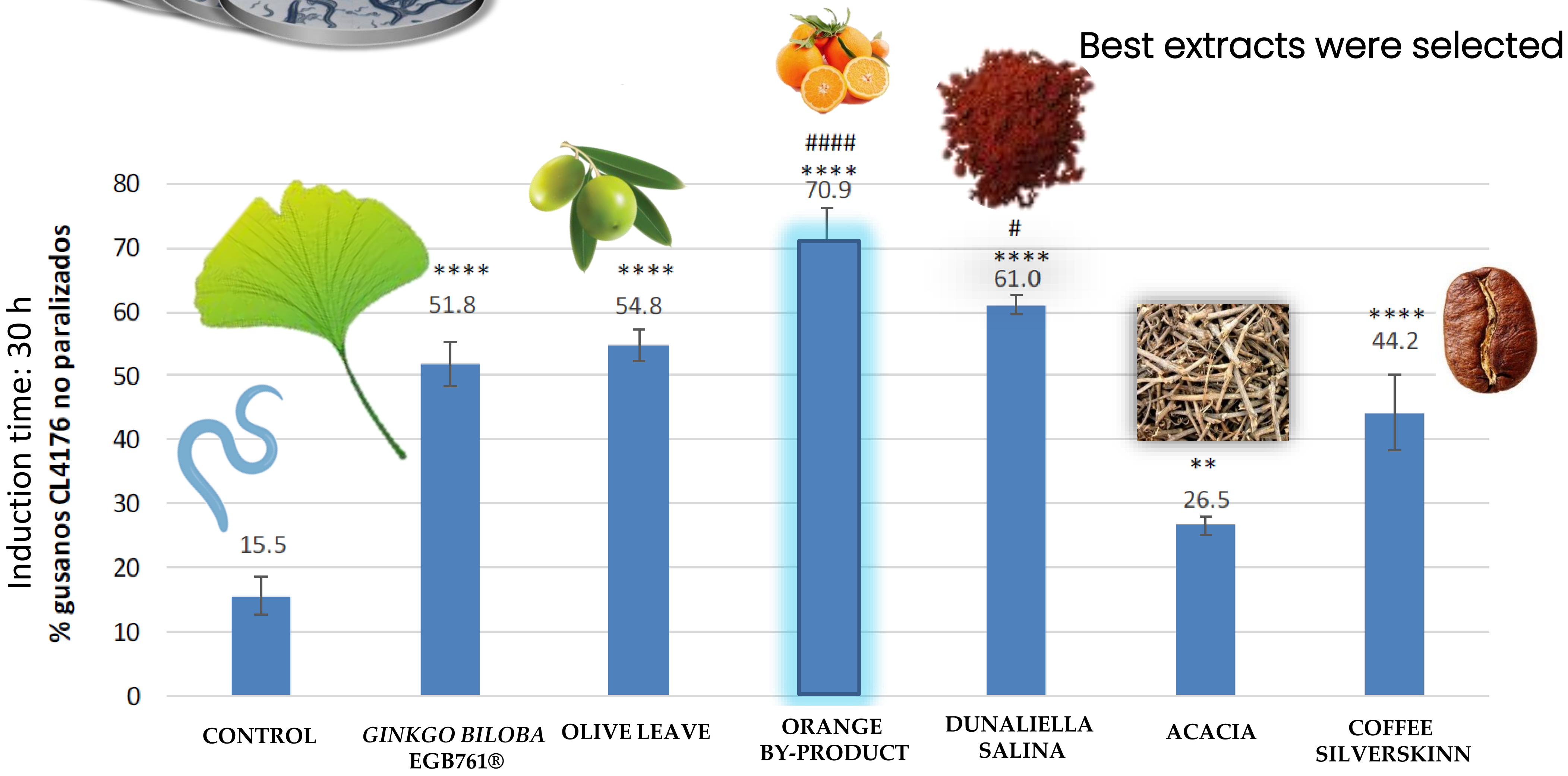
Several extracts were extensively evaluated in terms of *in vitro* neuroprotective capacity



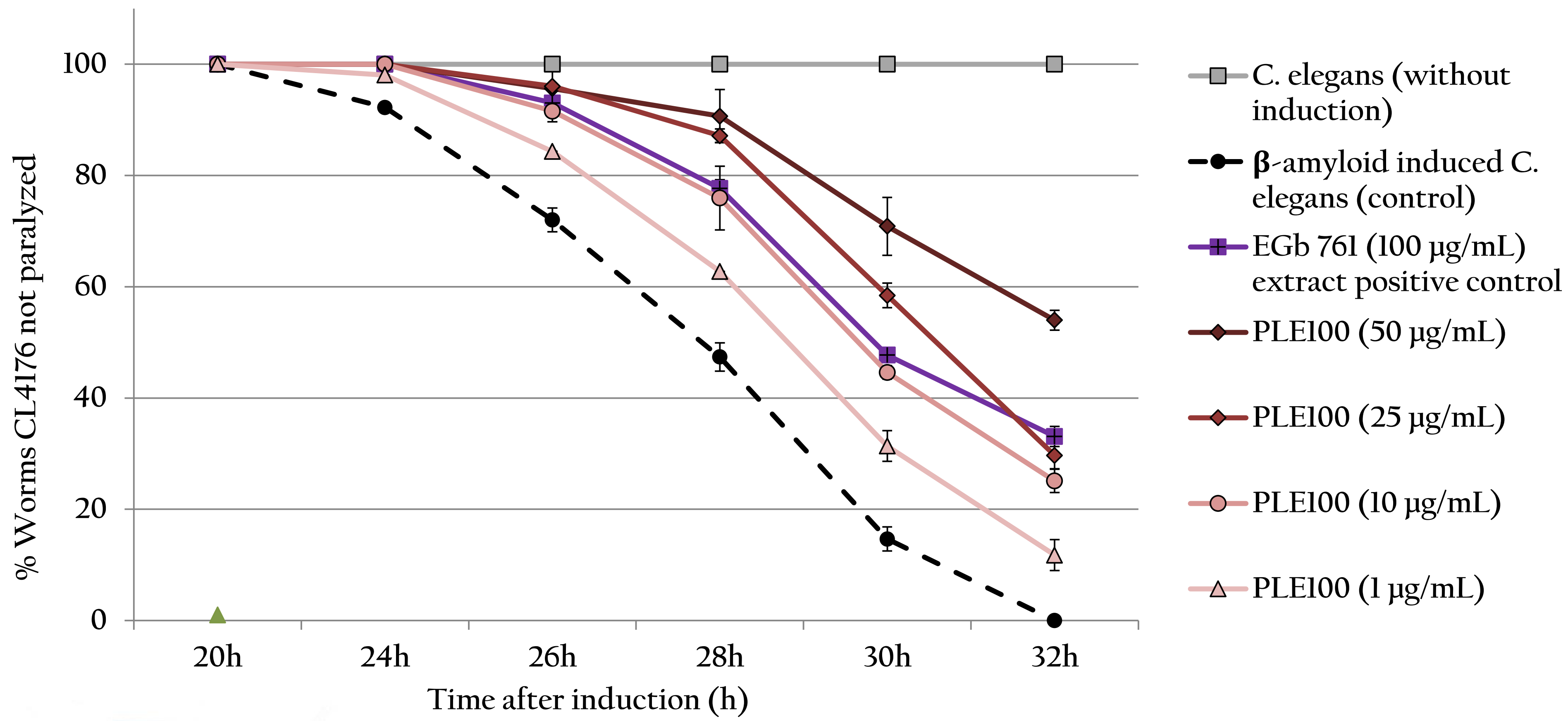


For *in vivo* AD model
C. elegans CL4176

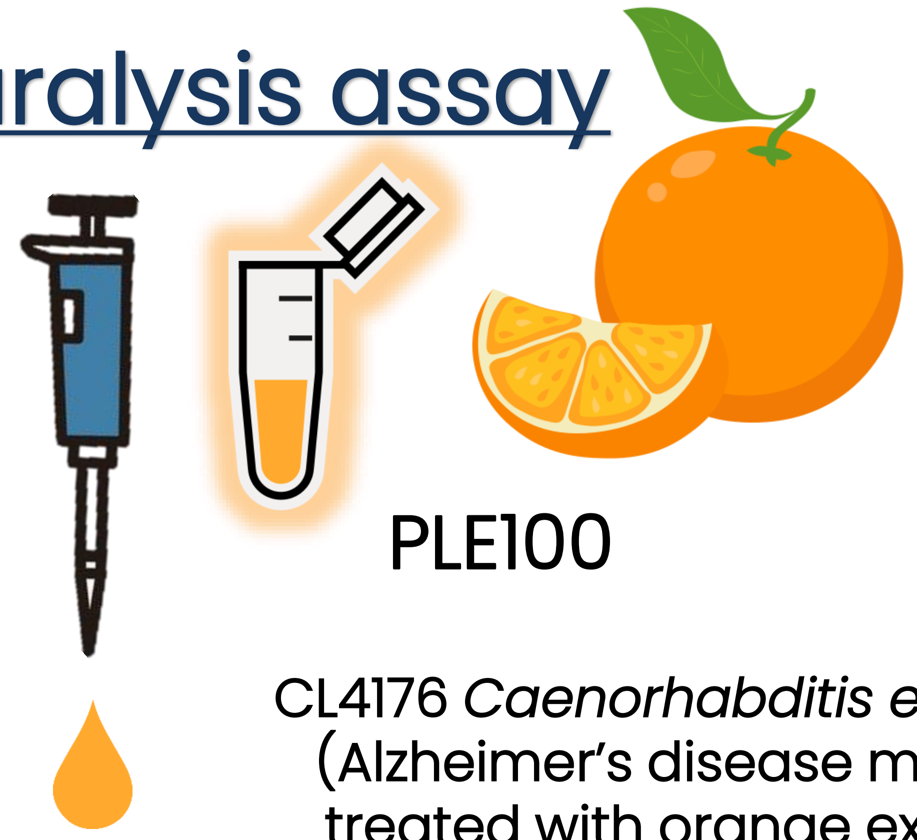
Best extracts were selected



III R&D: Part III Foodomics study of the neuroprotective effect of extracts on *C. elegans* as an *in vivo* model of AD



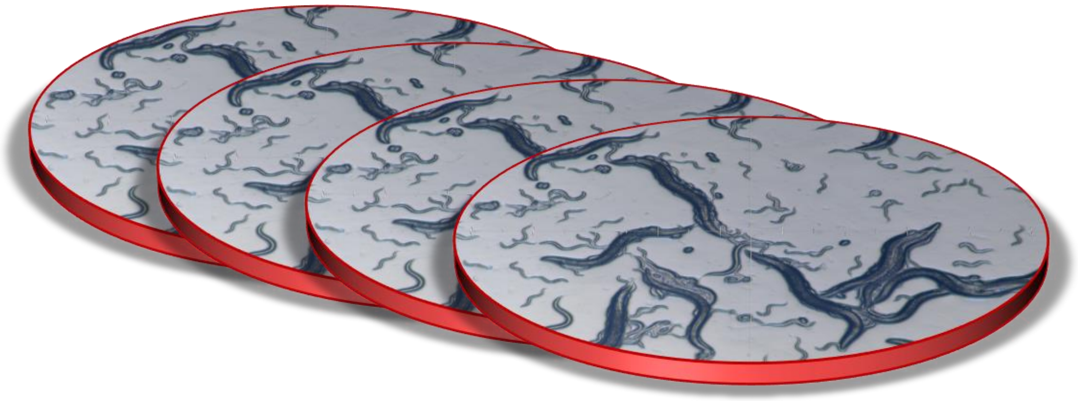
Paralysis assay



CL4176 *Caenorhabditis elegans* (Alzheimer's disease model) treated with orange extract

Orange extract neuroprotects in a dose-dependent manner against the **Aβ-peptide paralysis toxicity**

More efficient at 50 µg/mL compared to the **Ginkgo biloba EGb761®** control extract at 100 µg/mL



TRANSCRIPTOMICS

RNA-Seq

Illumina NextSeq 2000

METABOLITE ANALYSIS

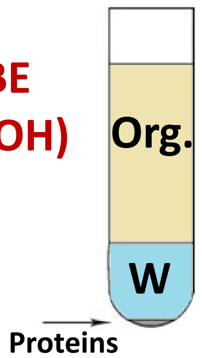
MTBE/MeOH extraction method

Lipid profiling

CSH-ESI-Q-TOF MS/MS

Ceramides (Cer); Diacylglycerols (DG); Free fatty acids (FA);Lysophosphatidylcholines (LPC); Phosphatidic acid (PA); Phosphatidylcholines (PC); Phosphatidylethanolamines (PE) ;Triacylglycerols (TG); Sphingomyelins (SM); Phosphatidylinositols (PI); Phosphatidylglycerols (PG)

MTBE (+ MeOH)



H_2O (+ MeOH)

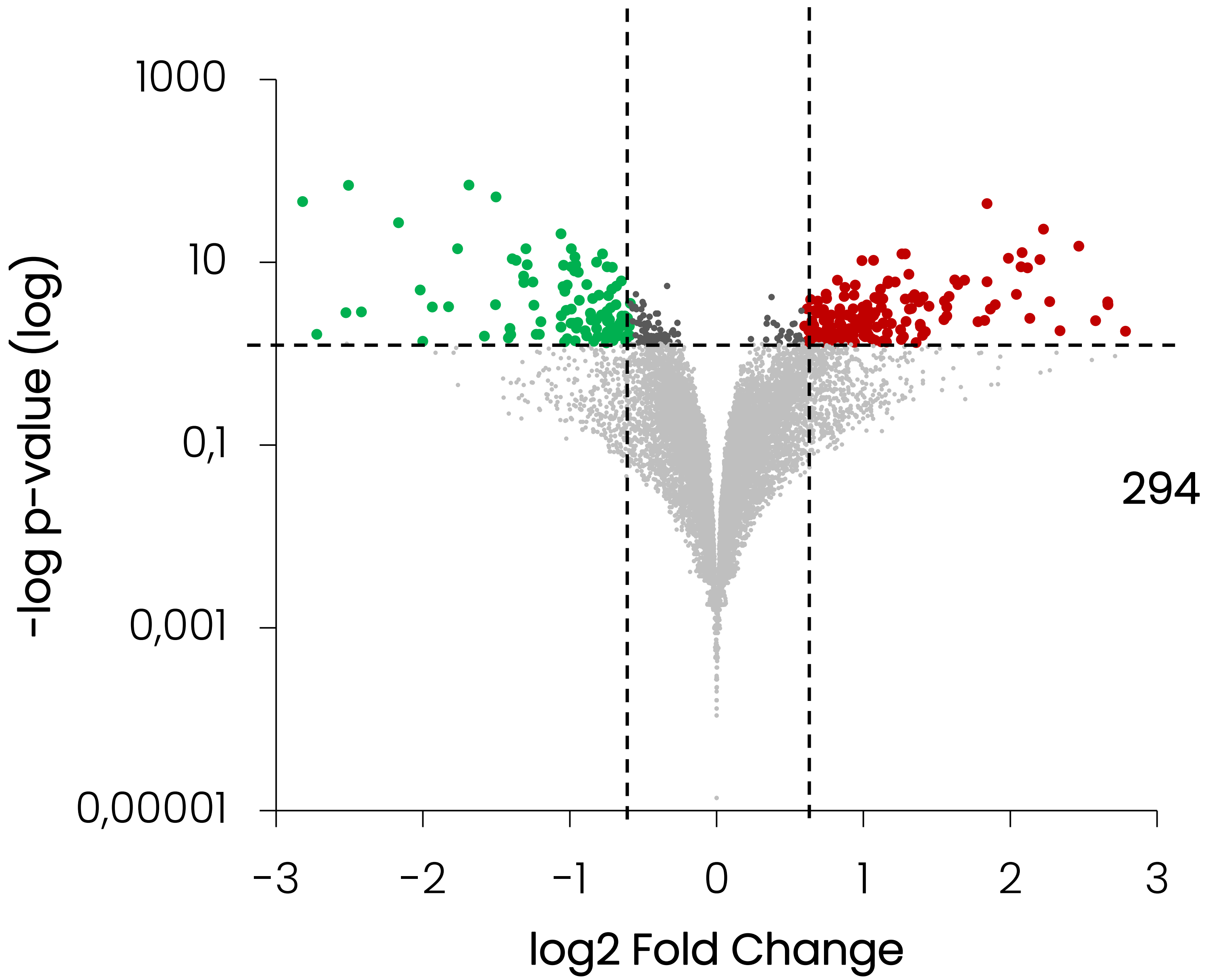
Primary metabolism profiling

- Carbohydrates
- Sugar alcohols
- Hydroxyl acids
- Polyamines
- Purines
- Pyrimidines
- Aromatics
- Ketone bodies
- Organic phosphates
- Inorganic ions
- Organic cations

HILIC-ESI-Q-TOF MS/MS
GC-EI-Q-TOF MS

PLE100-TREATED vs NON-TREATED

Transcriptomics analysis – RNA Seq



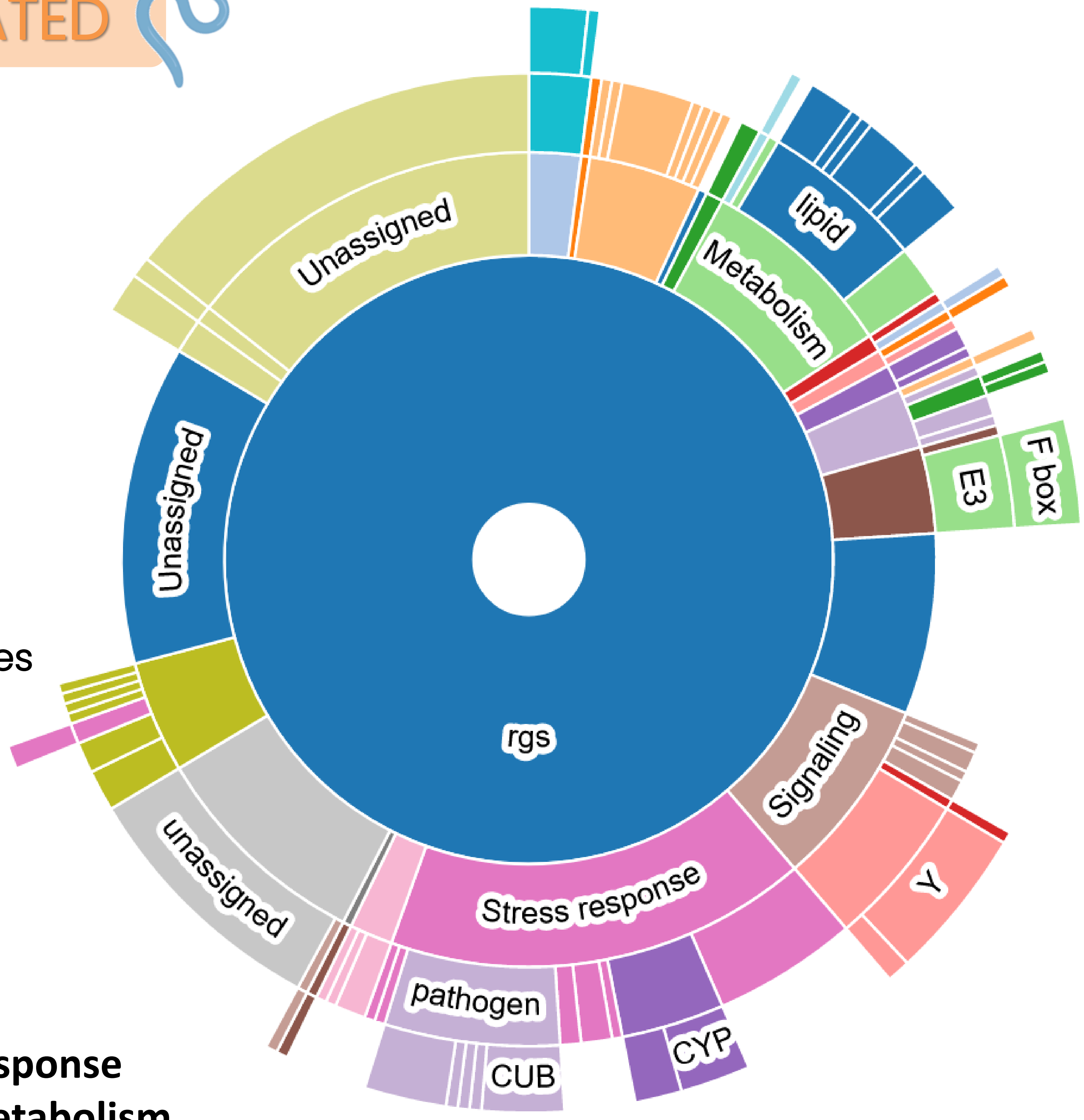
19,684 indentified genes

294 differentially expressed genes
(FDR < 0.05, FoldChange > 1.5)

186 up-regulated

108 down-regulated

Stress response
Nutrient metabolism
(Lipids)

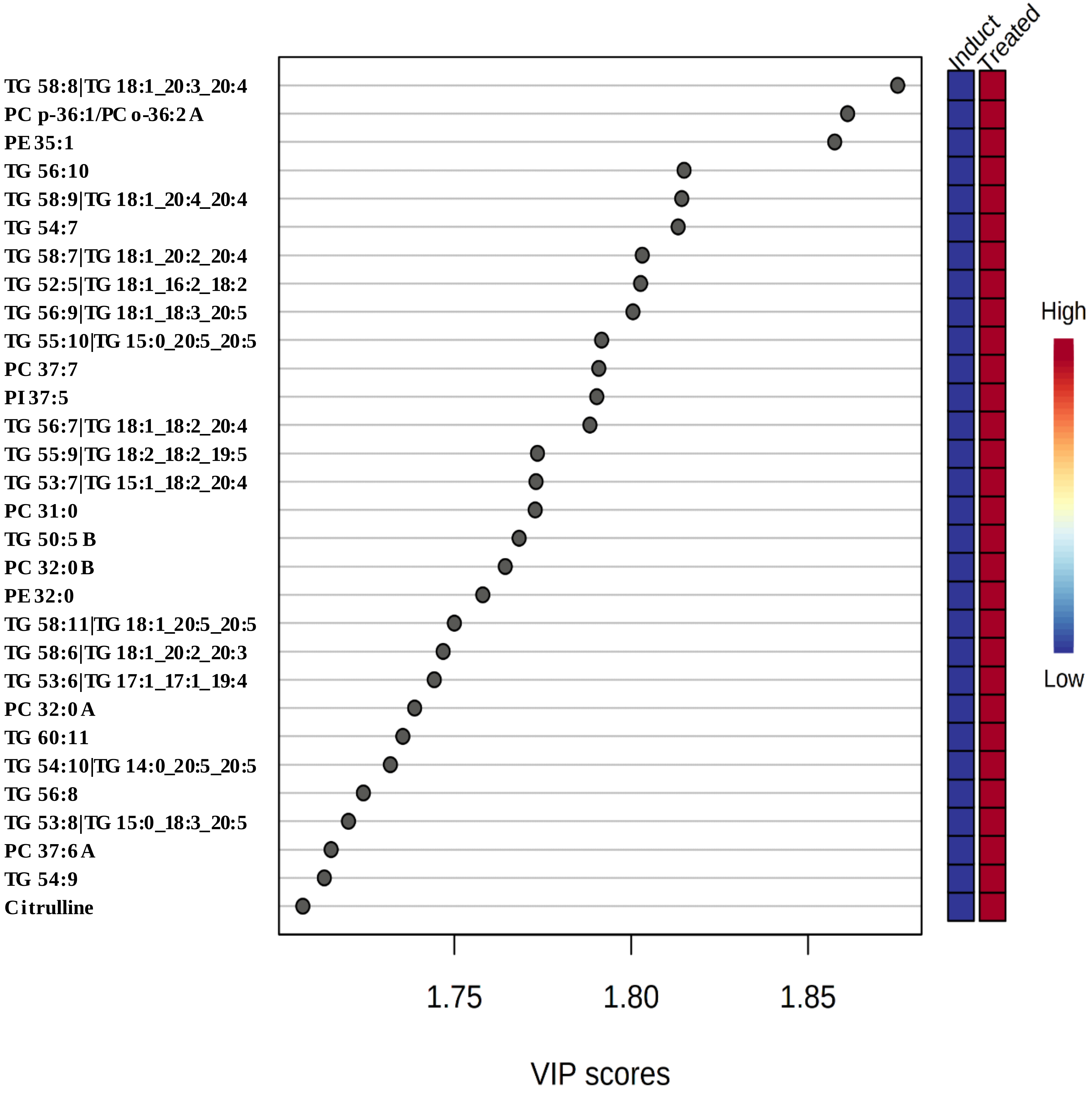


Gene Ontology Enrichment Analysis

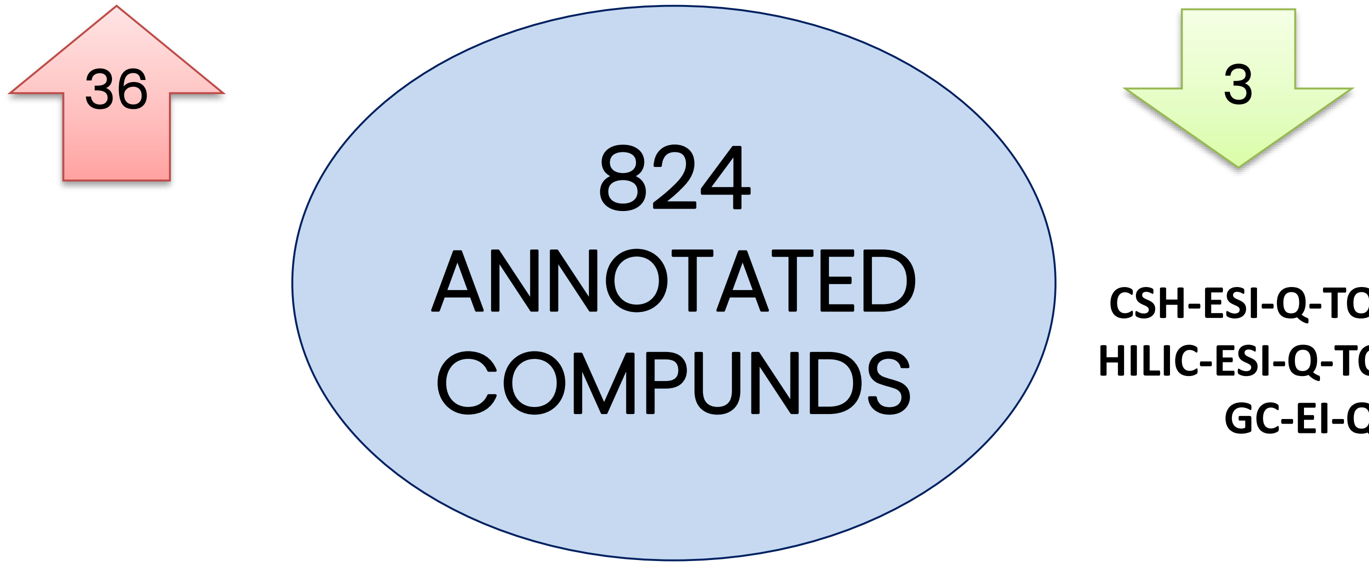
WORMCAT 2.0

Metabolomics analysis

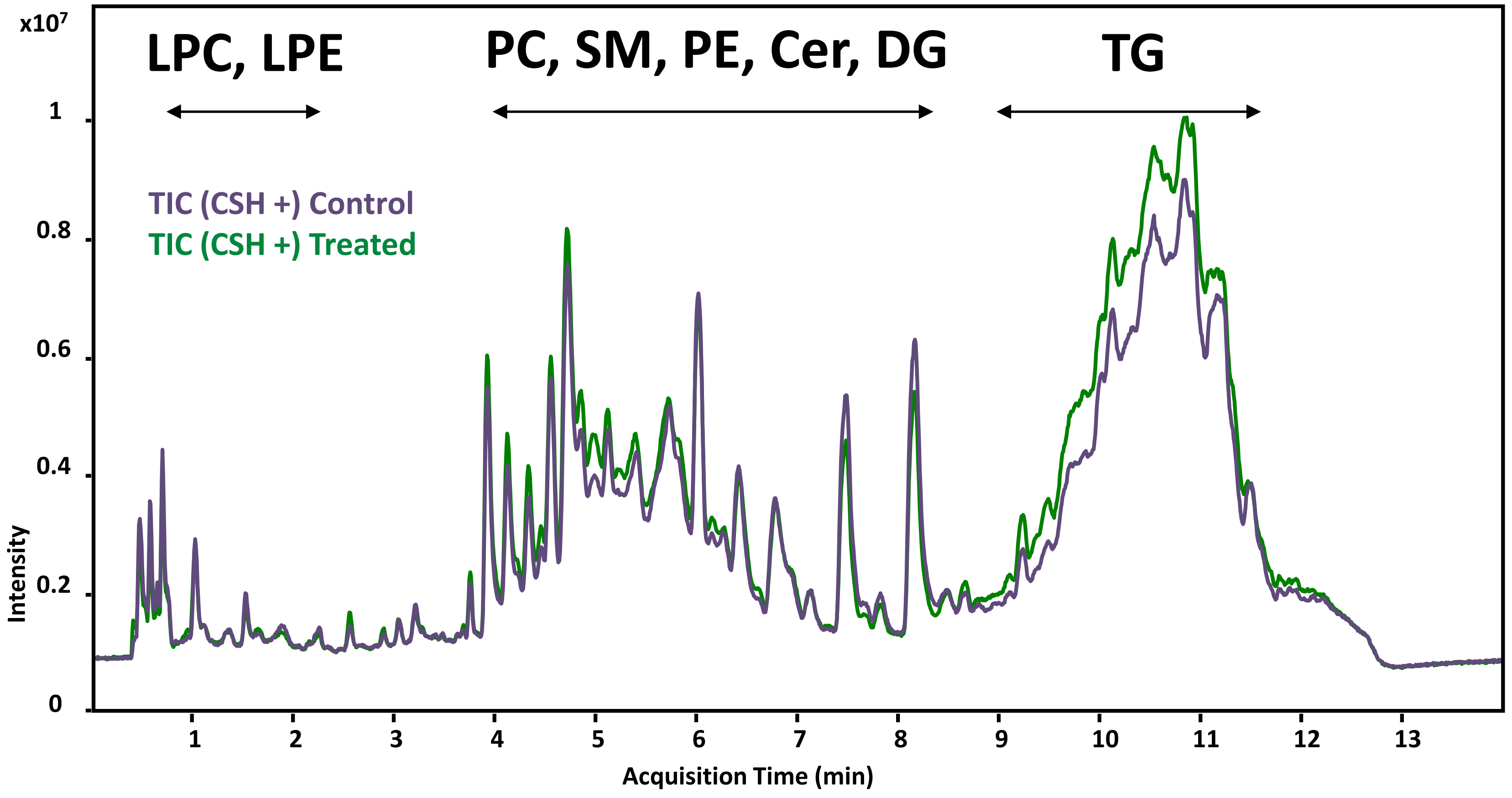
Phosphatidylcholines (PCs)
Unsaturated fatty acids
Sphingomyelins (SMs)



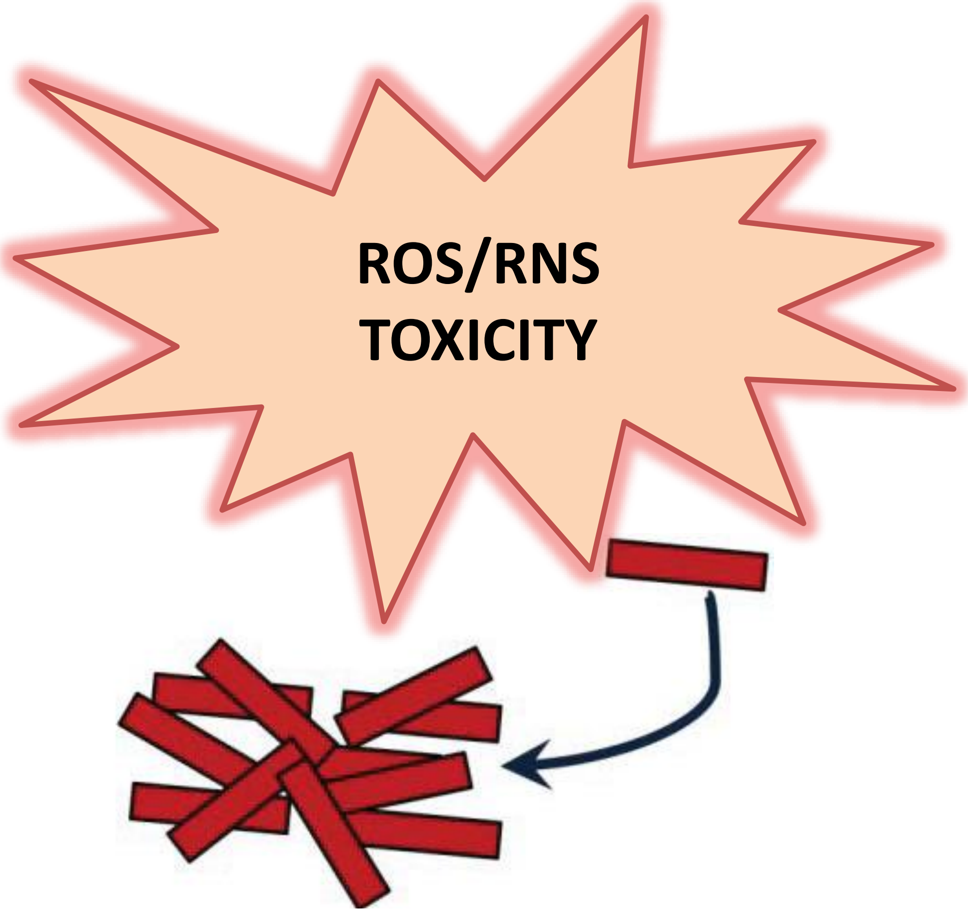
39 metabolites significantly altered after extract treatment



CSH-ESI-Q-TOF MS/MS (+/-)
HILIC-ESI-Q-TOF MS/MS (+/-)
GC-EI-Q-TOF MS

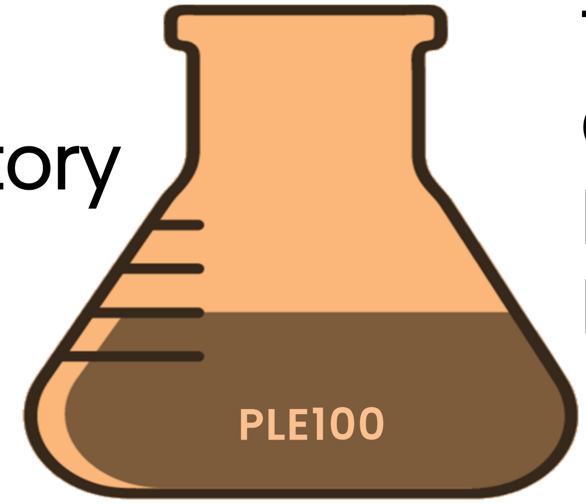


Neuroprotective mechanisms



Accumulation of Aβ plaques

Antioxidant
Anti-inflammatory
AChE inhibitor



Terpenoids
Carotenoids
PUFAs
Phytosterols

Sánchez-Martínez JD et al., (2021) *Food Funct.* **12**:302–314

Sánchez-Martínez JD et al., (2022). *Food Chem X.* **13**:100242

Cholinergic transmission
Anti Aβ agreggation
Neuronal stability and synapsis

Lesa et al., (2003). *J Cell Sci.* **116**:4965–4975

Shatshat et al., (2019). *Arch Biochem Biophys.* **663**:34–43

Cheng et al., (2019). *Int J Biol Sci.* **15**(13):2897-2910

Direct (ROS/RNS) | Indirect Hormesis

Antioxidant response

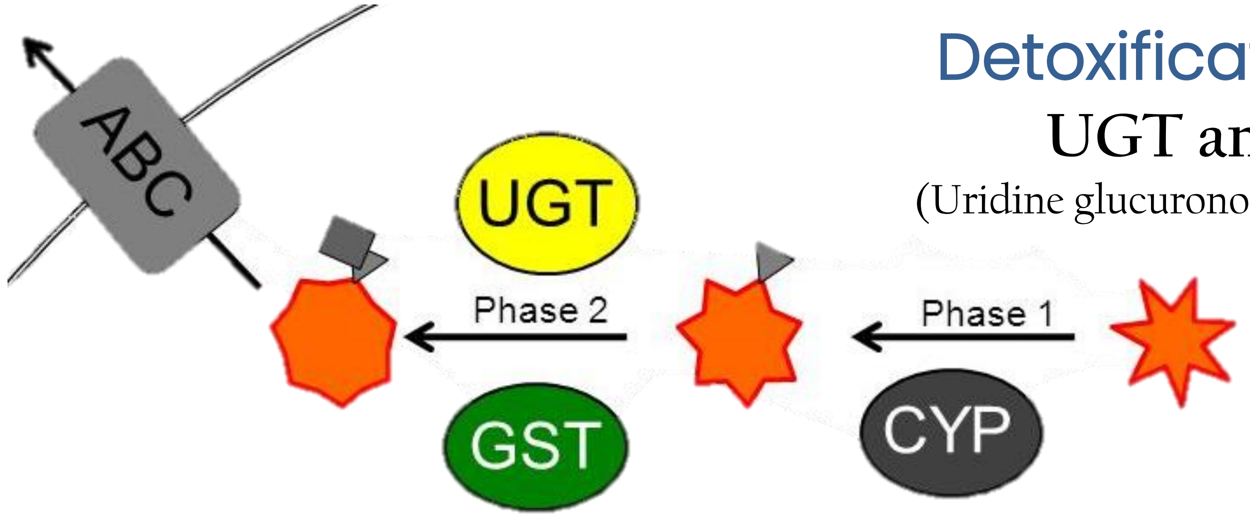
gst-14; sod-3

(Glutathione S-transferase; superoxide dismutase)

Detoxification mechanisms

UGT and CYPs families

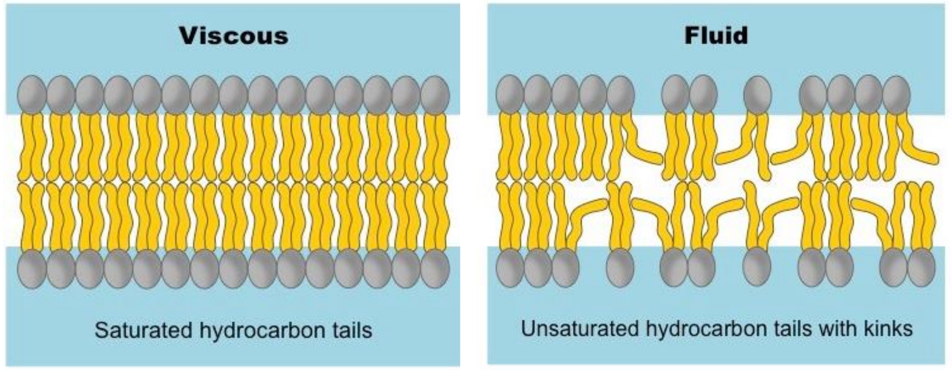
(Uridine glucuronosyltransferase; cytochrome P450)



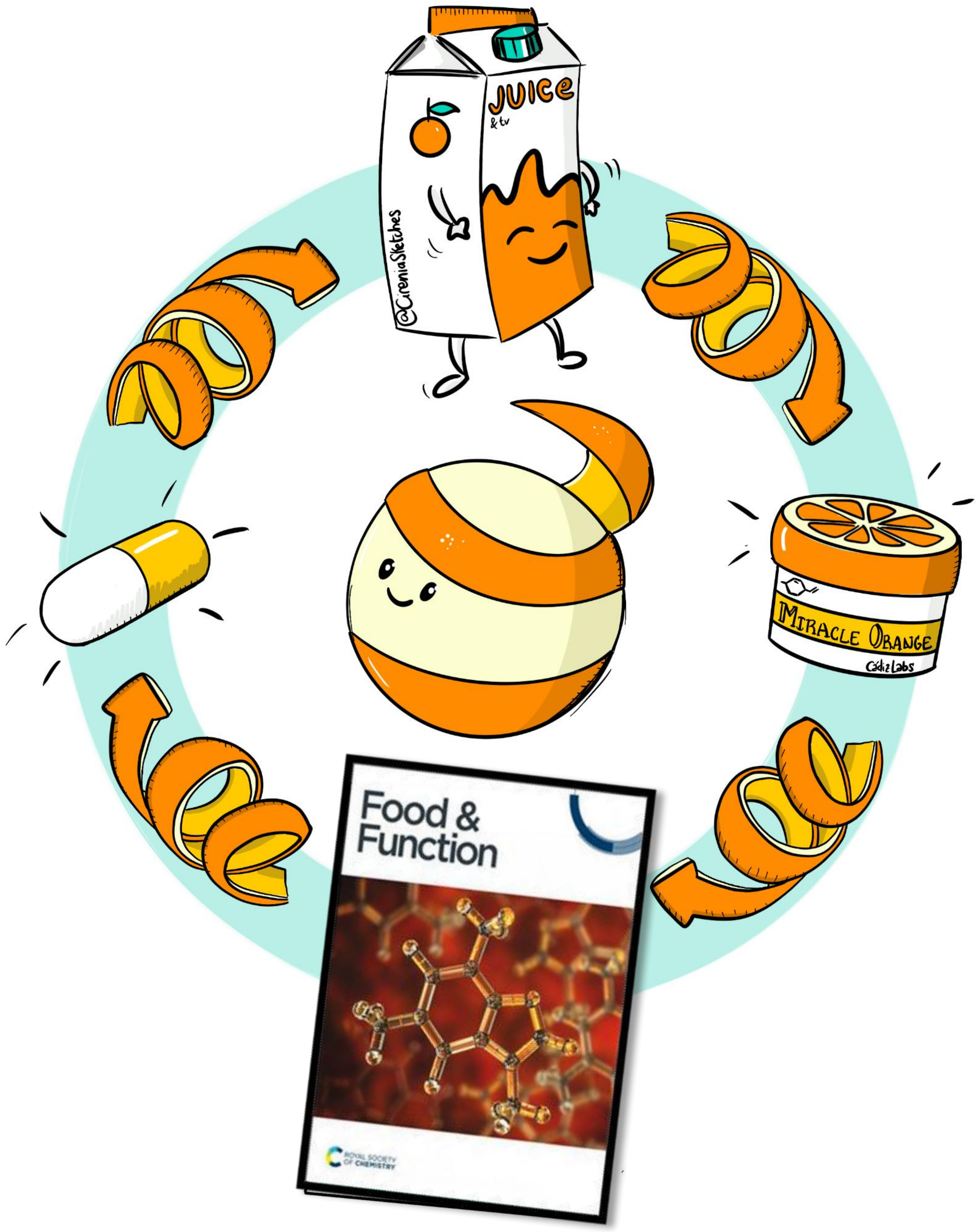
Lipid metabolism

Many gens altered related to:
beta-oxidation of lipids
acyl transferase enzymes
Sphingolipid metabolism

Phosphatidylcholines (PCs)
Unsaturated fatty acids
Sphingomyelins (SMs)



Membrane fluidity



GENERAL CONCLUSION

This work shows the potential of plant/algae biomasses and food by-products as renewable and sustainable sources of bioactive compounds. The neuroprotective potential of high value-added bioactive compounds from these biomasses has been verified using *in vitro* assays, permeability studies through the BBB, and Foodomics studies using an *in vivo* model.

FUTURE WORK

The promising neuroprotective capacity of extracts will be confirmed in experiments against AD in mammals; and if the positive results are confirmed, they will be studied in humans.

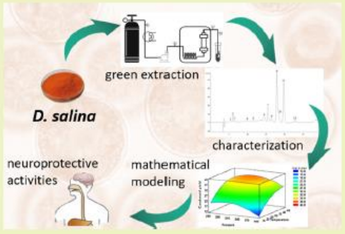
ACS Sustainable Chemistry & Engineering
pubs.acs.org/journal/ascecg
Research Article

Compressed CO_2 Technologies for the Recovery of Carotenoid-Enriched Extracts from *Dunaliella salina* with Potential Neuroprotective Activity

Mónica Bueno, Clementina Vitali, José David Sánchez-Martínez, José Antonio Mendiola, Alejandro Cifuentes, Elena Ibáñez, and Miguel Herrero*

Cite This: *ACS Sustainable Chem. Eng.* 2020, 8, 11413–11423 Read Online

ABSTRACT: Natural carotenoids from microalgae have attracted huge interest for their potential health benefits. Among microalgae species with high carotenoid content, *Dunaliella salina* has been highlighted since it is able to accumulate relatively high amounts of β-carotene and other carotenoids of industrial interest when grown under specific conditions. In the present contribution, extractions based on carbon dioxide under sub- and supercritical conditions have been optimized to improve the recovery of carotenoids and extract purity from *D. salina*. An experimental design was employed to investigate the effect of pressure and temperature variations ranging from 250 to 400 bar and from 15 to 45 °C, respectively. The chemical characterization of the carotenoid extracts was carried out by high-performance liquid chromatography with diode-array detection (HPLC−DAD). Moreover, inhibition of the acetylcholinesterase activity of all of the extracts was measured using a recently developed *in vitro* fluorescence methodology. High carotenoid yield and purity were obtained at 302–313 bar and 45 °C. Nine carotenoids were identified and three other compounds were recognized as carotenoids and quantified. Acetylcholinesterase activity inhibition could be satisfactorily explained by a partial least-squares model (63% explained variance in cross-validation) built considering the chemical composition of the different extracts. The model indicates a positive effect of lutein and 15-*cis*-β-carotene, the negative effect of zeaxanthin and cryptoxanthin, and the ratio of 9-*cis*-β-carotene/all-*trans*-β-carotene and 9-*cis*-β-carotene/total carotenoids in the inhibition of acetylcholinesterase enzyme.



KEYWORDS: *β-carotene, carotenoids, compressed CO_2 extractions, Dunaliella salina, in vitro fluorescence AChE methodology*

Food & Function
ROYAL SOCIETY OF CHEMISTRY
PAPER
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View Journal | View Issue
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Cite this: *Food Funct.*, 2021, **12**, 302

In vitro neuroprotective potential of terpenes from industrial orange juice by-products

José David Sánchez-Martínez,†[a] Mónica Bueno,†[a] Gerardo Alvarez-Rivera,[a] José Tudela,[b] Elena Ibañez[a] and Alejandro Cifuentes*[a]

Citrus sinensis (orange) by-products represent one of the most abundant citric residues from orange juice industrial production, and are a promising source of health-promoting compounds like terpenes. In this work, different extraction solvents have been employed to increase terpene extraction yield and selectivity from this orange juice by-product. A set of bioactivity assays including enzymatic (acetylcholinesterase (AChE), butylcholinesterase (BChE) and lipoxygenase (LOX)) as well as antioxidant (ABTS, reactive oxygen species (ROS) and reactive nitrogen species (RNS)) activity tests have been applied to investigate the neuroprotective potential of these compounds. New fluorescence-based methodologies were developed for AChE and BChE assays to overcome the drawbacks of these tests when used *in vitro* to determine the anticholinergic activity of colored extracts. Comprehensive phytochemical profiling based on gas chromatography coupled to quadrupole time of flight mass spectrometry (GC-qTOF-MS) analysis showed a high content of mono- and sesquiterpenes in the extracts obtained with ethyl acetate, whereas *n*-heptane extracts exhibited a large amount of triterpenes and carotenoids. From a neuroprotective activity point of view, ethyl acetate extract is the most promising due to its anticholinergic activity and antioxidant capacity. Finally, a multivariate data analysis revealed a good correlation between some monoterpenes (*e.g.* nerol or limonene) and the antioxidant capacity of the natural extract, while a group of sesquiterpenes (*e.g.* δ-Cadinene or nootkatone) showed correlation with the observed AChE, BChE and LOX inhibition capacity. Hydrocarbons mono- and sesquiterpenes reveal high capacity *in vitro* to cross the blood–brain barrier (BBB).

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Neuroprotective potential of terpenoid-rich extracts from orange juice by-products obtained by pressurized liquid extraction

José David Sánchez-Martínez[a,1], Gerardo Alvarez-Rivera[a,1], Rocio Gallego[a], Mariane Bittencourt Fagundes[b], Alberto Valdés[a], Jose A. Mendiola[a], Elena Ibañez[a,*], Alejandro Cifuentes[a]

[a] Laboratory of Foodomics, Institute of Food Science Research, CIAL, UAM-CSIC, Nicolás Cabrera 9, 28049 Madrid, Spain
[b] Department of Food Technology and Science, Federal University of Santa Maria, Santa Maria, Rio Grande do Sul, Brazil

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ABSTRACT

Pressurized liquid extraction (PLE) conditions were optimized to improve the recovery of orange (Citrus sinensis) by-products terpenoids. The neuroprotective potential of the PLE extracts were tested against a set of in-vitro assay (antioxidant (ABTS), reactive oxygen/nitrogen species (ROS/RNS)) as well as enzymatic tests (acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and lipoxygenase (LOX)). Gas chromatography coupled to high-resolution mass spectrometry (GC-q-TOF-MS) analysis revealed a higher enrichment in mono- and sesquiterpenoids of the PLE extracts with the highest neuroprotection capacity. *In-silico* molecular docking analysis showed the specific interaction of representative terpenoids with enzymes active sites. The results demonstrate that the selected extract at 100 °C and 30 minutes possesses high antioxidant (ABTSIC50 = 13.5 μg mL^{-1}; ROSIC50 = 4.4 μg mL^{-1}), anti-cholinesterase (AChEIC50 = 137.1 vg L^{-1}; BChEIC50 = 147.0 μg mL^{-1}) and anti-inflammatory properties (against IL-6 and LOXIC50 = 76.1 μg mL^{-1}), with low cytotoxicity and protection against L-glutamic acid in cell models.

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Blood–Brain Barrier Permeability Study of Potential Neuroprotective Compounds Recovered From Plants and Agri-Food by-Products

José David Sánchez-Martínez[1], Alberto Valdés[1], Rocio Gallego[1], Zully Jimena Suárez-Montenegro[1], Marina Alarcón[2], Elena Ibañez[1], Gerardo Alvarez-Rivera[1] and Alejandro Cifuentes[1]**

[1] Laboratory of Foodomics, Institute of Food Science Research, CIAL, Spanish National Research Council (CSIC) - Universidad Autónoma de Madrid (UAM), Madrid, Spain, [2] Area of Food Technology, Faculty of Chemical Sciences and Technologies, University of Castilla-La Mancha, Ciudad Real, Spain

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Plants and agri-food by-products represent a wide and renewable source of bioactive compounds with neuroprotective properties. In this research, various green extraction techniques were employed to recover bioactive molecules from *Kalanchoe daigremontiana* (kalanchoe), epicarp of *Cyphomandra betacea* (tamarillo), and cooperage woods from *Robinia pseudoacacia* (acacia) and *Nothofagus pumilio* (lenga), as well as a reference extract (positive control) from *Rosmarinus officinalis L.* (rosemary). The neuroprotective capacity of these plant extracts was evaluated in a set of *in vitro* assays, including enzymatic [acetylcholinesterase (AChE), butyrylcholinesterase

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In Vitro Study of the Blood–Brain Barrier Transport of Natural Compounds Recovered from Agrifood By-Products and Microalgae

José David Sánchez-Martínez[1,†], Ana Rita Garcia[2,3,†], Gerardo Alvarez-Rivera[1], Alberto Valdés[1], Maria Alexandra Brito[2,3,*] and Alejandro Cifuentes[1,*]

[1] Laboratory of Foodomics, Institute of Food Science Research, CIAL, UAM-CSIC, Nicolás Cabrera 9, 28049 Madrid, Spain
[2] Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal
[3] Department of Pharmaceutical Sciences and Medicines, Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal
* Correspondence: mariabrito@campus.ul.pt (M.A.B.); a.cifuentes@csic.es (A.C.); Tel.: +351-217946449 (M.A.B.); +34-910017955 (A.C.)
† These authors contributed equally to this work.

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Abstract: Agrifood by-products and microalgae represent a low-cost and valuable source of bioactive compounds with neuroprotective properties. However, the neuroprotective effectiveness of therapeutic molecules can be limited by their capacity to cross the blood–brain barrier (BBB) and reach the brain. In this research, various green extracts from *Robinia pseudoacacia* (ASFE), *Cyphomandra betacea* (T33), *Coffea arabica* (PPC1), *Olea europaea* L., (OL-SS), *Citrus sinensis* (PLE100) by-products and from the microalgae *Dunaliella salina* (DS) that have demonstrated in vitro neuroprotective potential were submitted to an in vitro BBB permeability and transport assay based on an immortalized human brain microvascular endothelial cells (HBMEC) model. Toxicity and BBB integrity tests were performed, and the transport of target bioactive molecules across the BBB were evaluated after 2 and 4 h of incubation using gas and liquid chromatography coupled to quadrupole-time-of-flight mass spectrometry (GC/LC-Q-TOF-MS). The HBMEC-BBB transport assay revealed a high permeability of representative neuroprotective compounds, such as mono- and sesquiterpenoids, phytosterols and some phenolic compounds. The obtained results from the proposed in vitro BBB cellular model provide further evidence of the neuroprotective potential of the target natural extracts, which represent a promising source of functional ingredients to be transferred into food supplements, food additives, or nutraceuticals with scientifically supported neuroprotective claims.

Keywords: neuroprotection; blood–brain barrier; green-extraction; LC/GC-q-TOF-MS; food waste; bioactive compounds

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