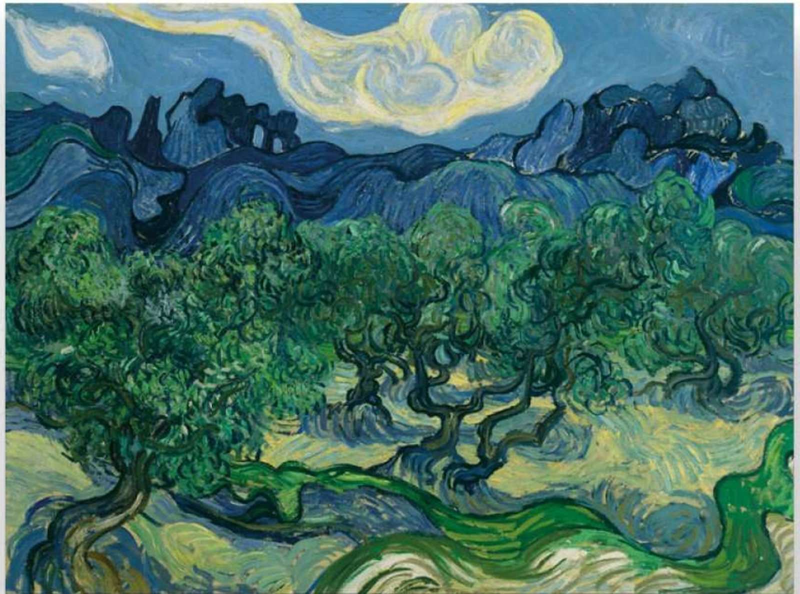
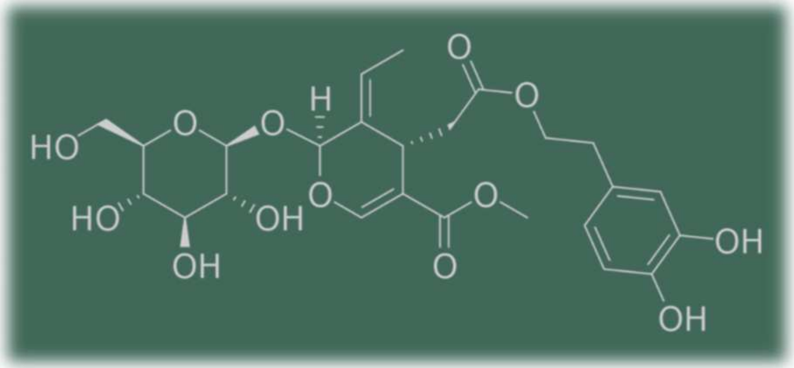
HELLENIC REPUBLIC
National and Kapodistrian University of Athens
EST. 1837

Department of Pharmacy
Laboratory of valorization of bioactive natural products



Exploitation of olive oil industry products and by-products for pilot isolation and semi-synthesis of promising medicinal agents

Leandros A. Skaltsounis



Olea Europaea L.

The domesticated olive tree seems to have coexisted with humans for about 8.000 years

Zohary, D., Hopf, M., & Weiss, E. 2012. Domestication of plants in the old world, 4th ed. Oxford University Press,New York.

O.O. apart its nutritional value has important medicinal properties

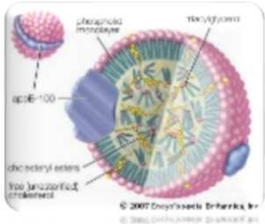
Olive harvest. Pot of the 6th century BC, *British Museum.*

Hippocrates used olive oil in more than 60 remedies

First report on olive oil production in 6000 BC

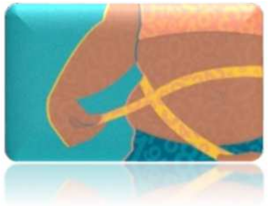
Hippocrates treating a patient (*Kos Archaeological Museum*)

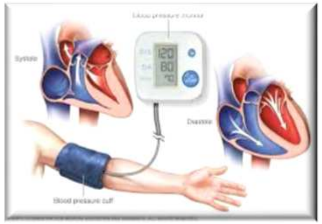
Zohary, D., Hopf, M., & Weiss, E. 2012. Domestication of plants in the old world, 4th ed. Oxford University Press, New York



Lipid metabolism: LDL, total cholesterol, triglycerides

Glucose metabolism: prevention of diabetes type II

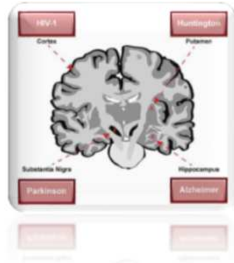




Blood pressure: diminish the risk of hypertension, vasodilator capacity

Osteoporosis: favorable effect on bone calcification and bone mineralization

Reduction of the risk of neurodegenerative diseases: preventing age-related cognitive decline, memory loss, dementia and Alzheimer’s disease



Digestive system: inhibits gastric motility, stimulates, the digestion of lipids and prevents the onset of gallstones

Prevent platelet aggregation: improves blood circulation

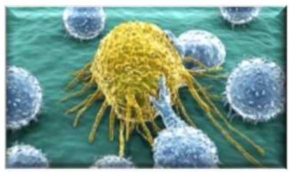
Biological Impact of Olive Oil

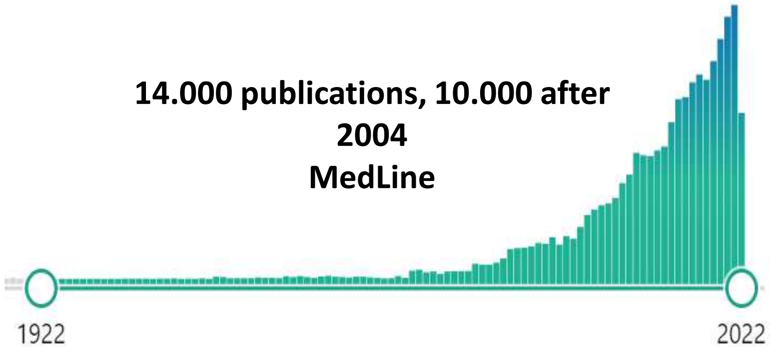
Prevention oxidative stress: prevents the development of certain types of skin cancer

Antimicrobial properties: reduction of the microbial activity

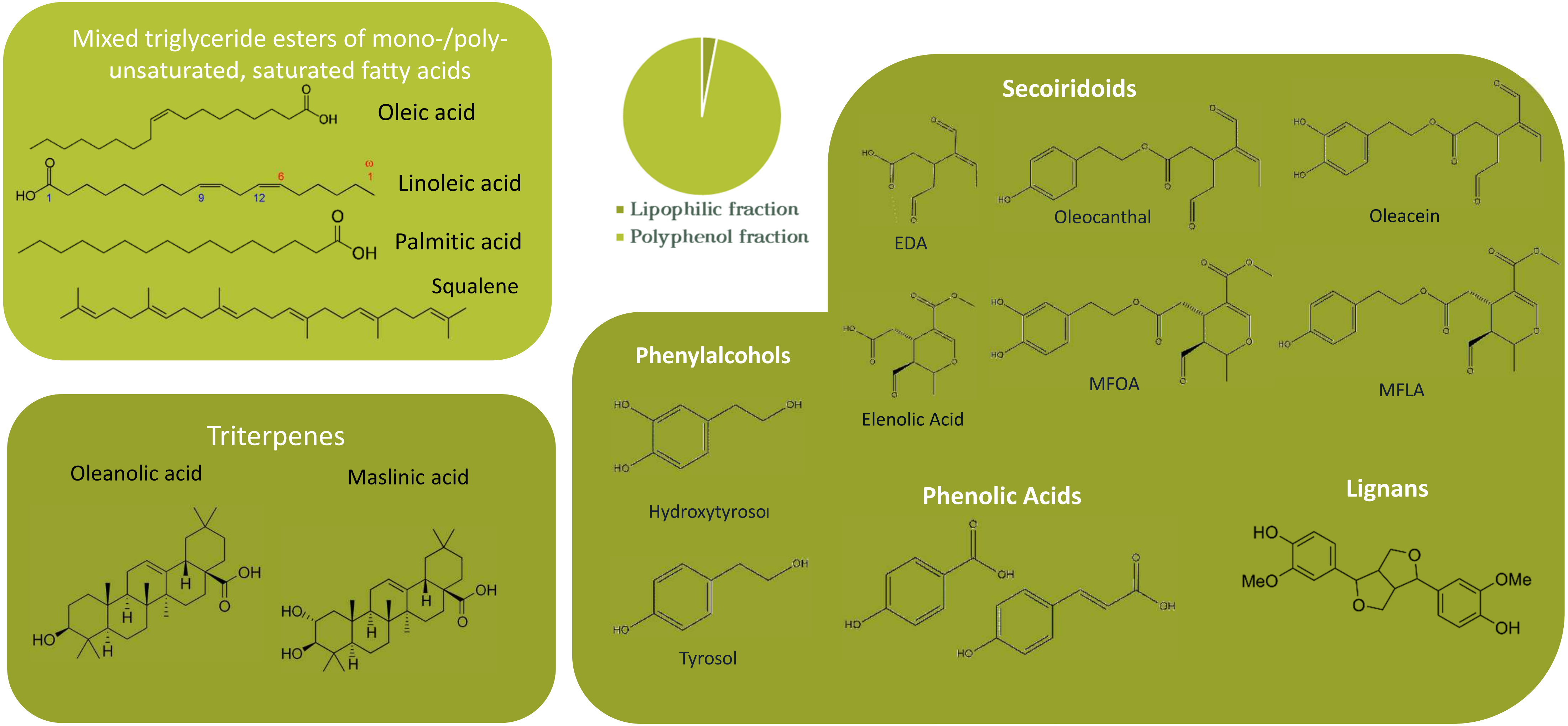
Anti-inflammatory properties: decreasing C reactive proteins and pro-inflammatory genes

Antitumor properties: breast, prostate, endometrium, digestive tract, etc.





Chemical Composition Of Olive Oil



Olive Oil health benefits – recent advances

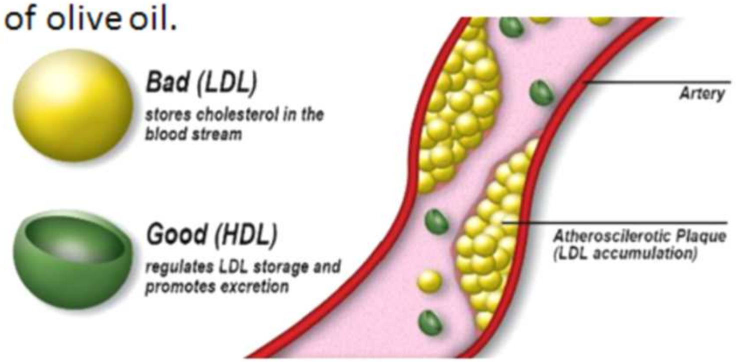
efsa

European Food Safety Authority

- ID 1706: “squalene idrocarburo” and
- “Antioxidant activity, with protection of body tissue
- and skin from oxidant agents (UV rays)”

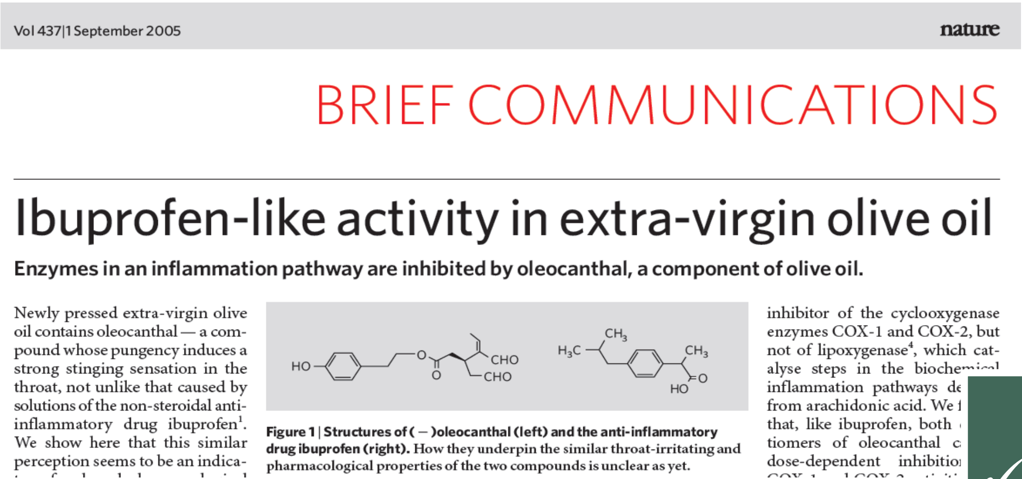
EFSA Scientific opinion (2011):
Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress.

Conditions for use:
The claim may be used only for olive oil which contains at least **5 mg of hydroxytyrosol** and its derivatives (e.g. oleuropein complex and tyrosol) per 20 g of olive oil.



http://www.efsa.europa.eu/fr/efsajournal/pub/2033

OO major dialdehyde bioactives



Vol 437|1 September 2005 nature

BRIEF COMMUNICATIONS

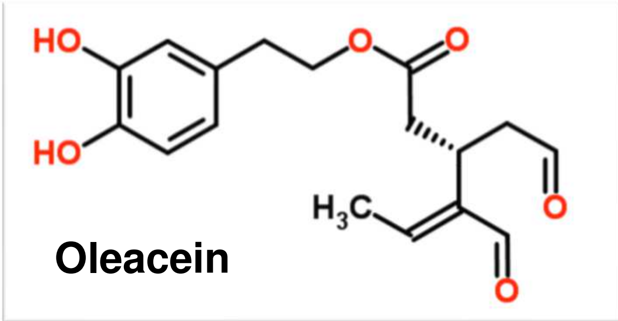
Ibuprofen-like activity in extra-virgin olive oil

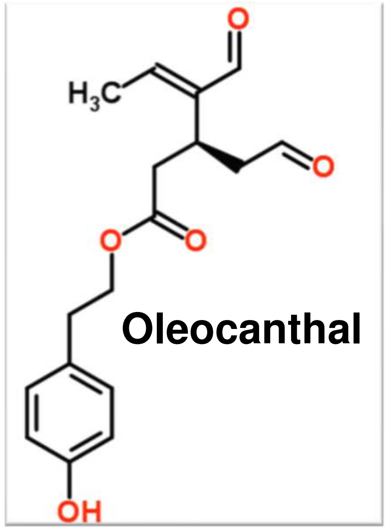
Enzymes in an inflammation pathway are inhibited by oleocanthal, a component of olive oil.

Newly pressed extra-virgin olive oil contains oleocanthal — a compound whose pungency induces a strong stinging sensation in the throat, not unlike that caused by solutions of the non-steroidal anti-inflammatory drug ibuprofen[1]. We show here that this similar perception seems to be an indica-

Figure 1 | Structures of (−)oleocanthal (left) and the anti-inflammatory drug ibuprofen (right). How they underpin the similar throat-irritating and pharmacological properties of the two compounds is unclear as yet.

inhibitor of the cyclooxygenase enzymes COX-1 and COX-2, but not of lipoxygenase[4], which catalyse steps in the biochemical inflammation pathways de from arachidonic acid. We f that, like ibuprofen, both tiomers of oleocanthal c dose-dependent inhibitio

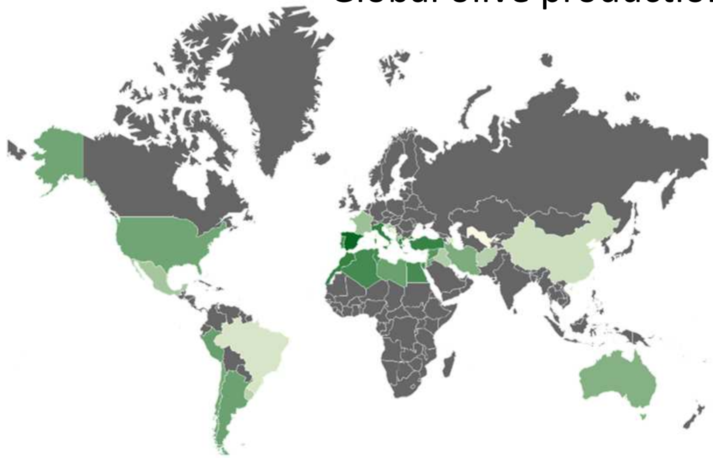




✓ Olive oil dialdehydes
✓ Anti-inflammatory

Oleacein: decarboxylate aglycon of oleuropein
Oleocanthal: decarboxylate aglycon of ligstroside

Global olive production

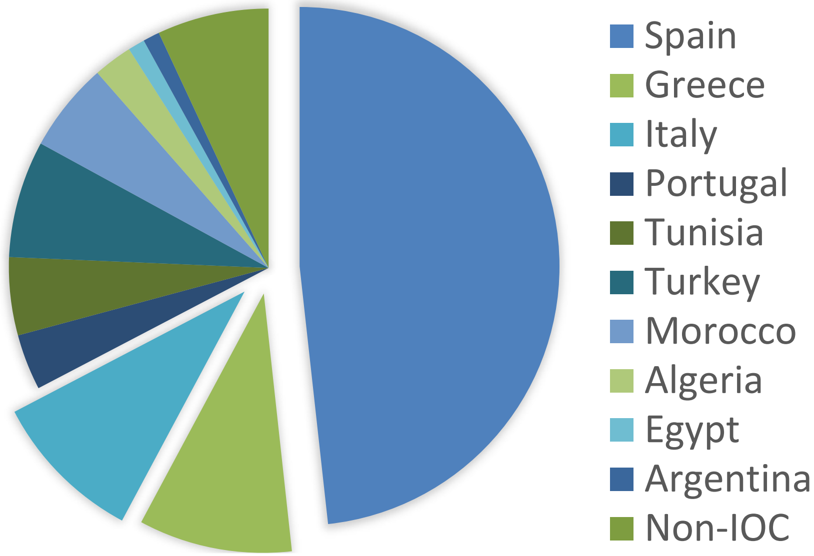


Region	% in global OO production
EU	80
AFRICA	9
ASIA	6
AMERICA	3
AUSTRALIA	2

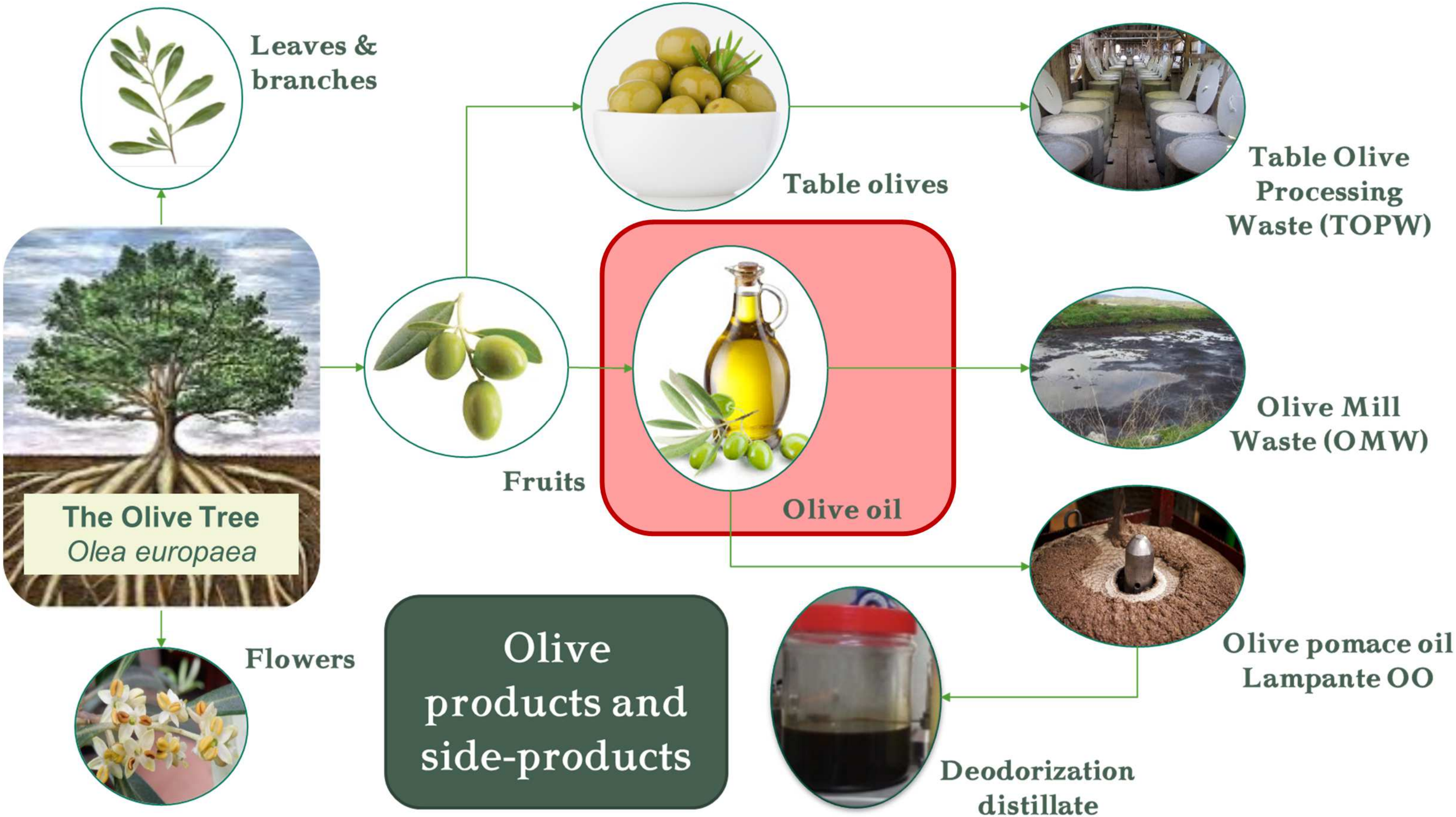
3 millions tons of olive oil

600 millions olive trees

2020-21 Olive Oil global production



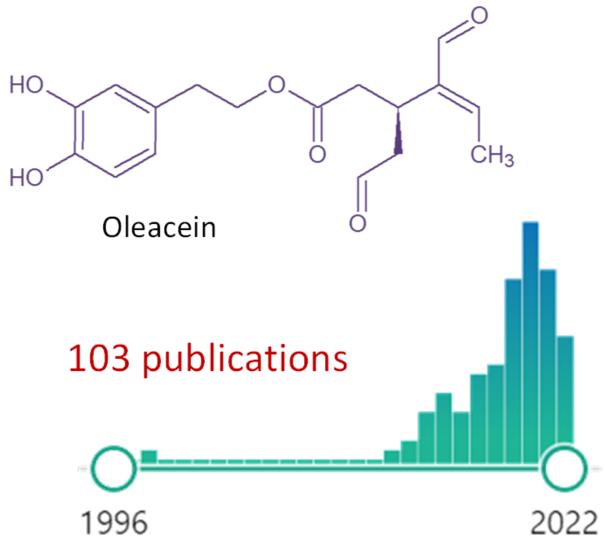
- Olive tree: **main crop globally** utilized for table olives and olive oil production
- **Greece: 2nd producer in the world** (2020)
- More than **130 million olive trees** are cultivated for **olive oil** production (about 300.000 tns annually)
- **20 million olive trees** are cultivated **for table olives production**

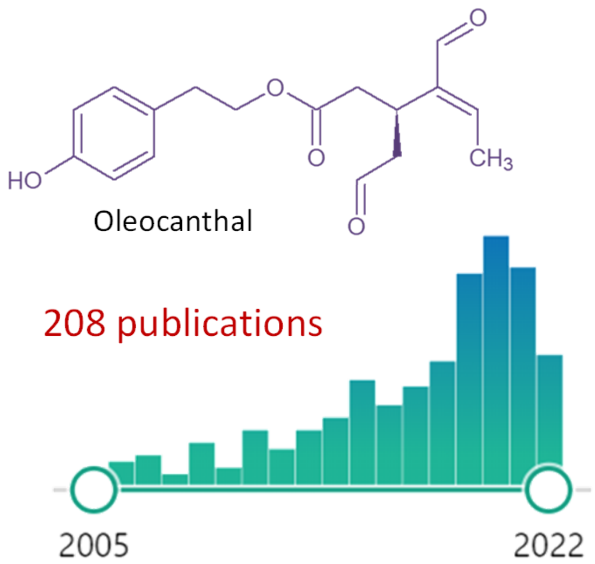

Leaves &
branches
The Olive Tree
Olea europaea
Flowers
Fruits
Table olives
Olive oil
Table Olive
Processing
Waste (TOPW)
Olive Mill
Waste (OMW)
Olive pomace oil
Lampante OO
Deodorization
distillate
Olive
products and
side-products

- The limited availability of these secoiridoids hinders their more in-depth pharmacological studies.

OO as a source of unique secoiridoid dialdehydes

- Oleocanthal and oleacein are unique secoiridoid dialdehydes present in olive oil
- Extensive research on their bioactivity started after the 2000s



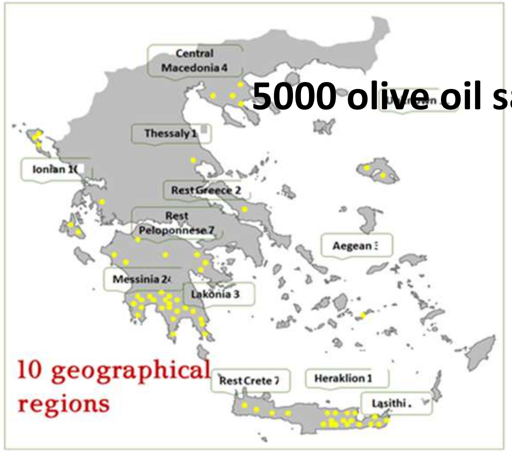


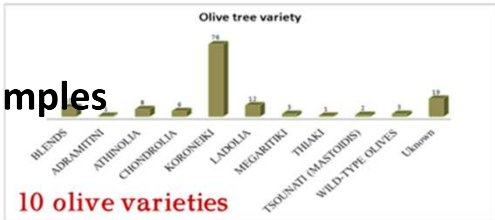
1g of oleocanthal 30.000 euros

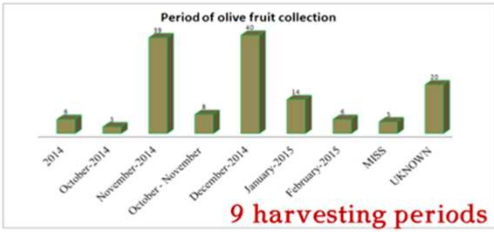
Rodríguez-Juan, Elisa, et al. "From Low-Quality Olive Oils to Valuable Bioactive Compounds: Obtaining Oleacein and Oleocanthal from Olive Oils Intended for Refining." Journal of Agricultural and Food Chemistry 70.1 (2021): 333-342.

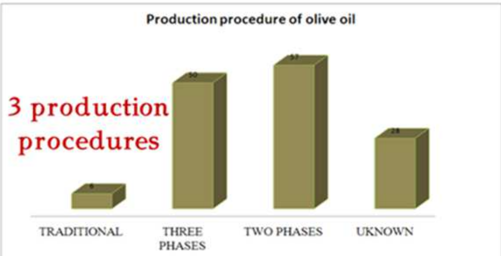
Mapping of Greek olive oil for the discovery of OO with a high percentage of phenols and particularly Oleocanthal and Oleacein

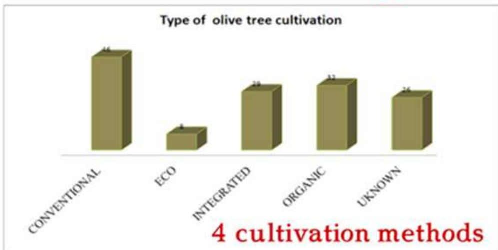
5000 olive oil samples

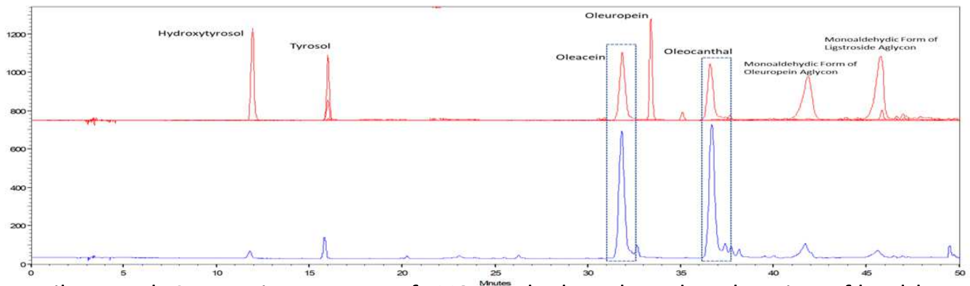




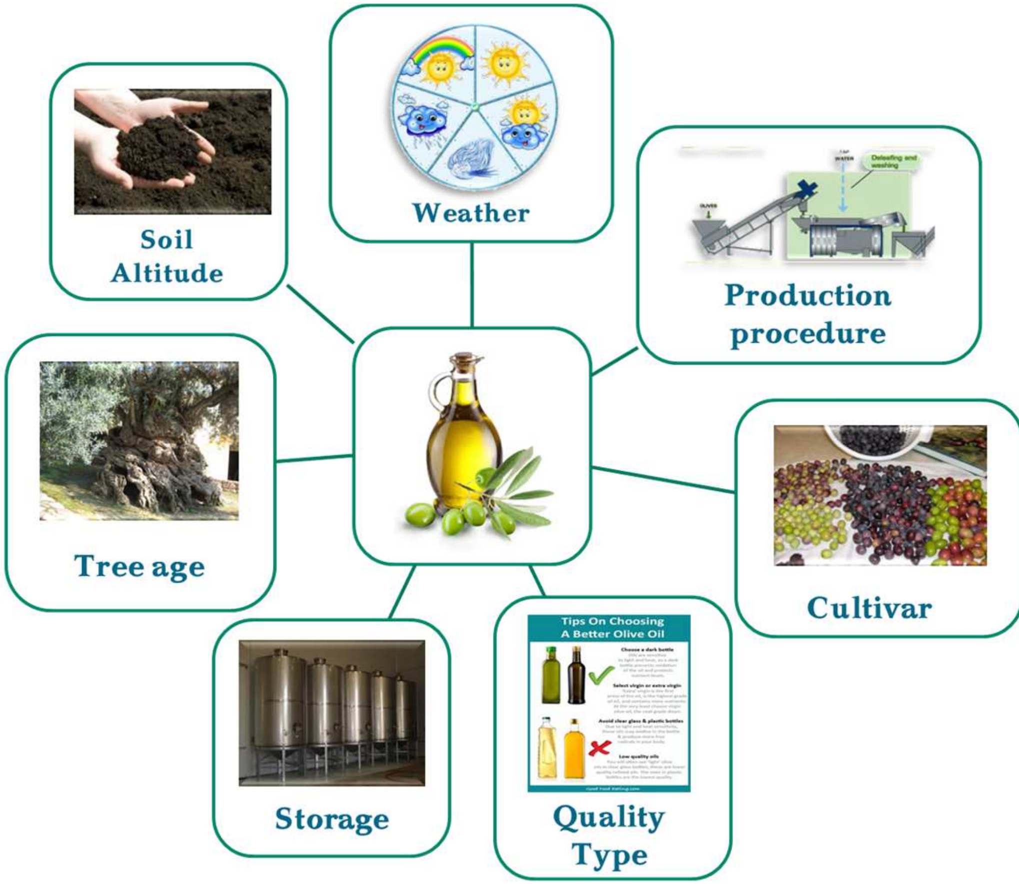








Nikou et al. Comparison survey of EVOO polyphenols and exploration of healthy aging-promoting properties of oleocanthal and oleacein, **Food and Chemical Toxicology** 2019/1/21



✓**58%** of OO samples meet the EU regulation 432/2012, containing more than 250 mg of HT/Kg

Recovery of OO polyphenols (Total Polyphenolic Fractions), in collaboration with prof. J.H. Renault, Reims University

100 Kg OO

Annular Centrifugal Extraction

ACE

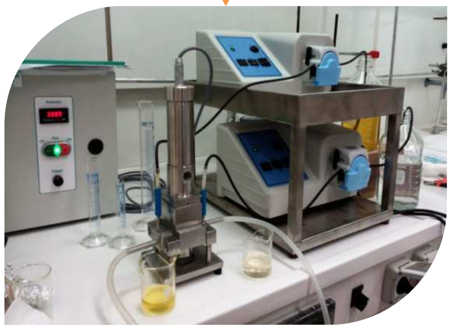
TPF 2 kg

FCPC prepHPLC-DAD

Pure compounds

- Major compounds in high yield
- Fractions enriched in minors

Efficient scale-up to pilot



Lab scale extractor BXP012®

Pilot scale extractor BXP190®

Suitable for industrial scale applications

The use of ACE allowed:

- ✓ The development of a continuous process
- ✓ The treatment of large volume of OO
- ✓ Very high process productivity
- ✓ Recycling of solvents

Extracting conditions

Upper phase:

- Olive oil: 75 Kg
- Hexane: 100 Kg
- Upper phase feed: 3 L/min

Lower phase:

- Isopropanol: 100 Kg
- Water: 75 Kg
- Lower phase feed: 3 L/min

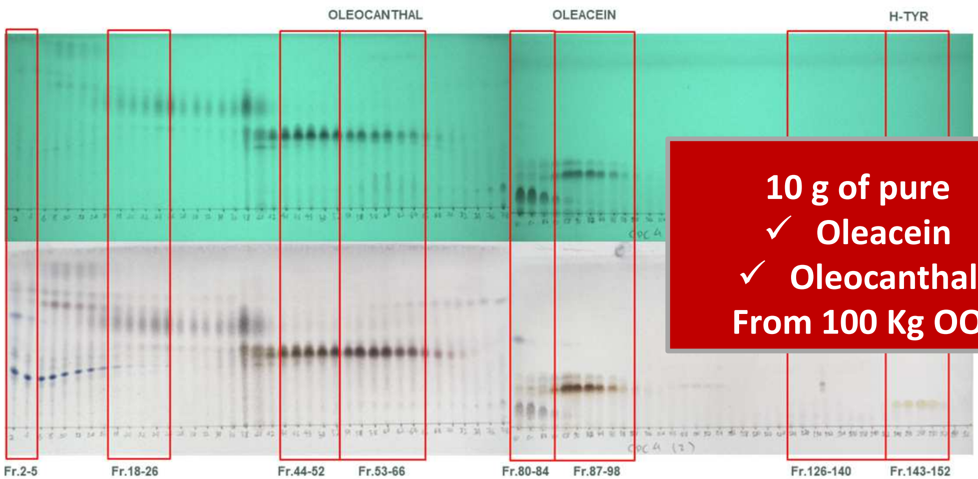
Rotation speed: 750 RPM

Angelis, Apostolis, et al. "Pilot continuous centrifugal liquid-liquid extraction of extra virgin olive oil biophenols and gram-scale recovery of pure oleocanthal, oleacein, MFOA, MFLA and hydroxytyrosol." *Separation and Purification Technology* 255 (2021): 117692.

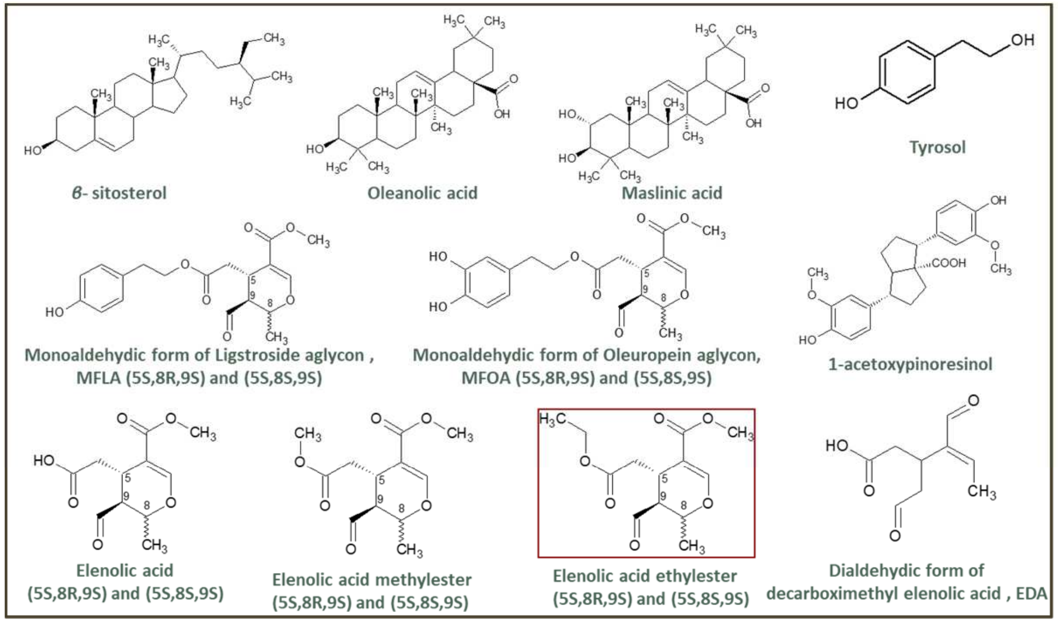
FCPC1000 fractionation of TPF

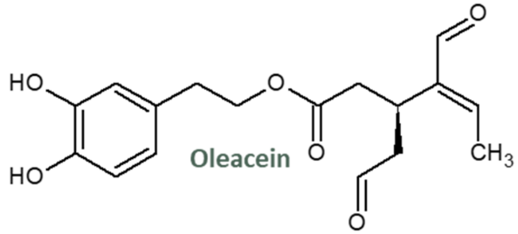
Column volume	1000 mL
Pumping mode	Asceding
Flow rate (mL/min)	20 mL/min .
Rotation speed (rpm)	900 rpm
Injection Volum	5 g

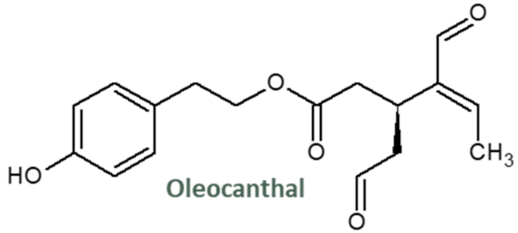
	Volume composition of used solvent systems				Mobile phase (mL)
System	*n*-hex	EtOAc	EtOH	H_2O	FCPC1000
S_1	4	1	2	3	500
S_2	3	2	2	3	500
S_3	2	3	2	3	2000
S_4	1	4	2	3	1000



10 g of pure
✓ Oleacein
✓ Oleocanthal
From 100 Kg OO







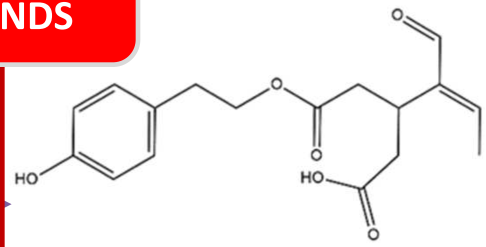
Angelis, Apostolis, et al. "An integrated process for the recovery of high added-value compounds from olive oil using solid support free liquid-liquid extraction and chromatography techniques." *Journal of chromatography A* 1491 (2017): 126-136.

Isolation of new minor compounds from OO

Fractions from CPC with **unknown** composition **and minor compounds**

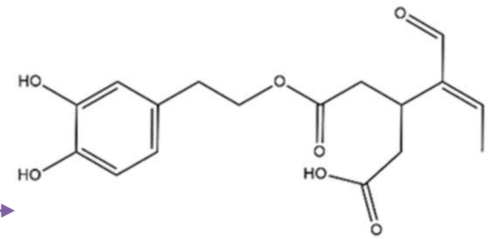
prepHPLC-DAD

NEW COMPOUNDS



Oleocanthalic Acid

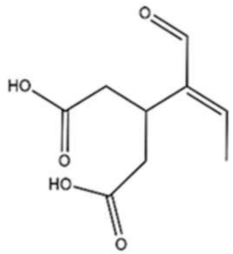
Recovery : approx.
0.2mg/100ml EVOO



Oleaceinic Acid

Recovery : approx.
0.11mg/100ml EVOO

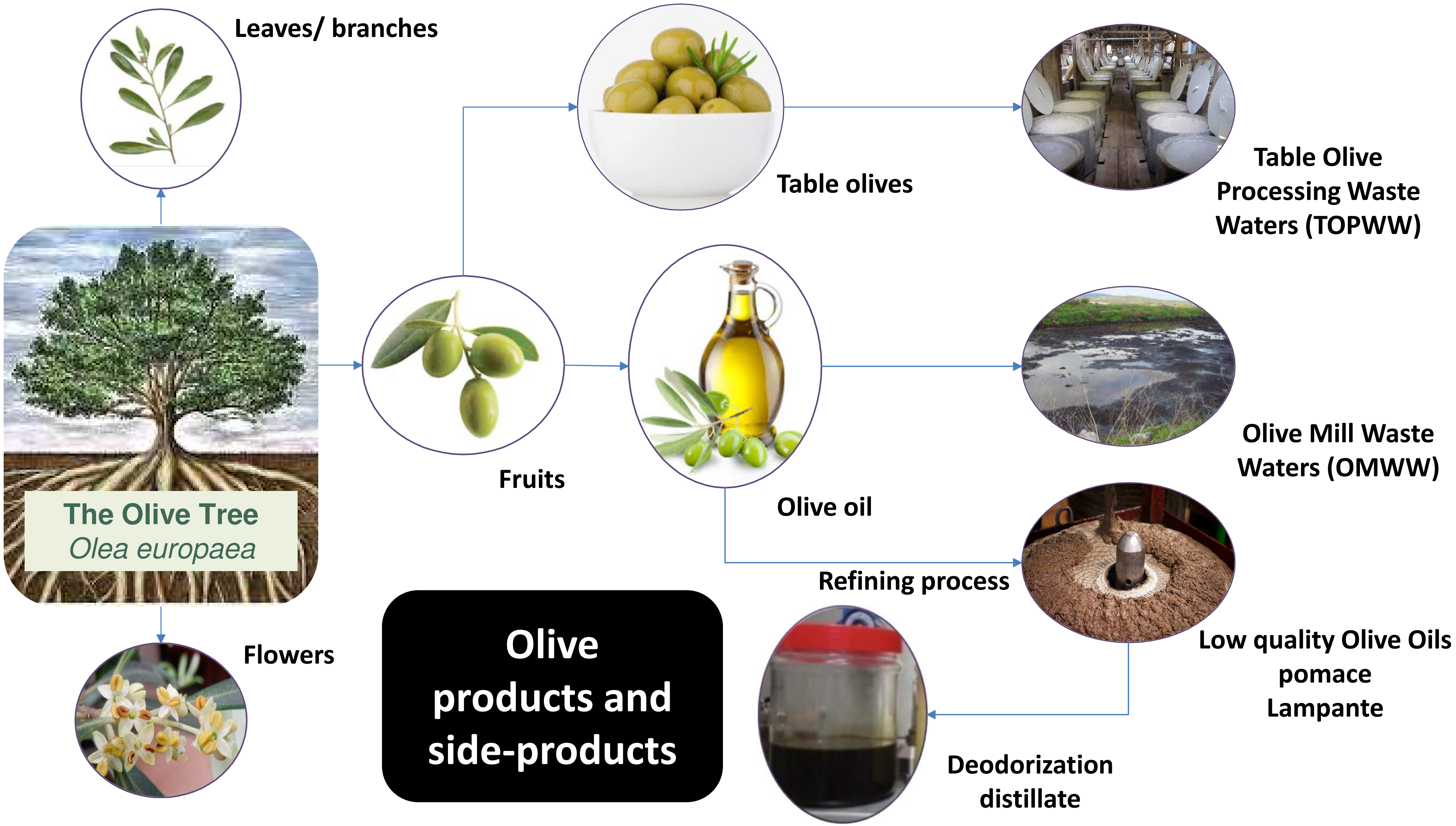
Isolation of
EDA acid for the first time
in Olive Oil



EDA Acid

Recovery : approx.
0.14mg/100ml EVOO

Apostolis ANGELIS et al. “Oleocanthalic and Oleaceinic acids: New compounds from Extra Virgin Olive Oil (EVOO).” ***Phytochemistry Letters***, 2018, 26, 190-194.



Leaves/ branches
Table olives
Table Olive Processing Waste Waters (TOPWW)
The Olive Tree
Olea europaea
Fruits
Olive oil
Olive Mill Waste Waters (OMWW)
Refining process
Low quality Olive Oils pomace Lampante
Flowers
Olive products and side-products
Deodorization distillate

Olive side-products –environmental impact

5 lt of OMWW are produced / 1 lt of olive oil

15 m. tons/year

1.2 lt of TOPWW are produced/1 Kg of table olives

2 m. tons/year

The biophenols in olive oil and table olives is 1-2% of the available pool of biophenols in the untreated olive fruit

The rest 98% is lost in the side products

OMW lagoon

Difficult management

Olive mill waste are disposed into:

- streams, which end up to the sea
- the soil forming lagoons

Olive side-products –environmental impact

10-30 kg of olive leaves and branches/tree during the olive oil production and during olive tree pruning

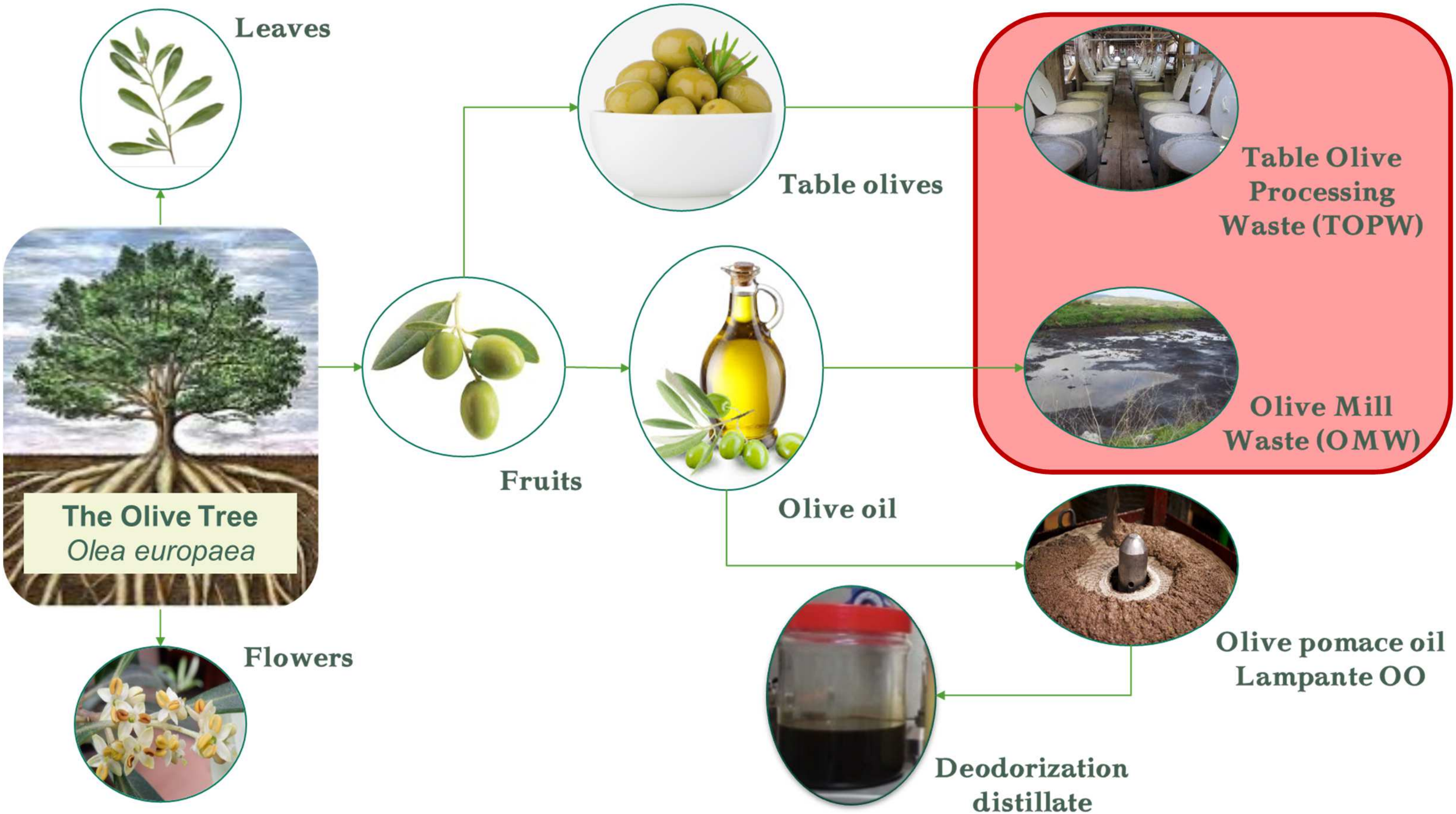
80% of the production becomes untreated biomass

20% are used as compost or animal feed

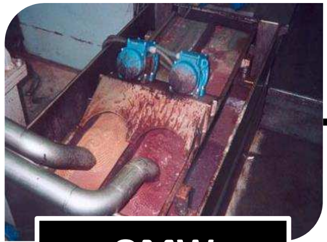
Burning of olive tree leaves is a major source of organic aerosol production in the Mediterranean

Liangou, Aikaterini, et al. "A Method for the Measurement of the Water Solubility Distribution of Atmospheric Organic Aerosols." *Environmental Science & Technology* 56.7 (2022): 3952-3959.

Despite their harmful effects, olive tree side-products could be a valuable source of bioactive compounds and chemicals with proved bioactivity

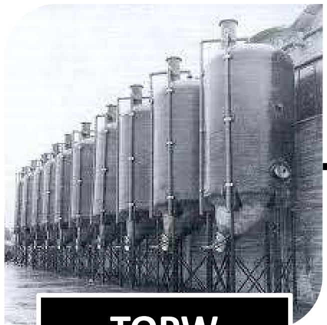

Leaves
Table olives
Table Olive Processing Waste (TOPW)
The Olive Tree
Olea europaea
Fruits
Olive oil
Olive Mill Waste (OMW)
Flowers
Olive pomace oil
Lampante OO
Deodorization distillate

Recovery of biophenols from OMWW/TOPWW



OMW

or

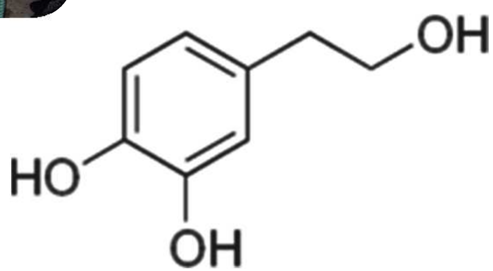


TOPW

2-stage filtration (100/25 μm)

Adsorption on XAD7 resin

Recovery of polyphenols with propanol/water



solvent concentration recovery

Maltodextrin addition

Spray drying

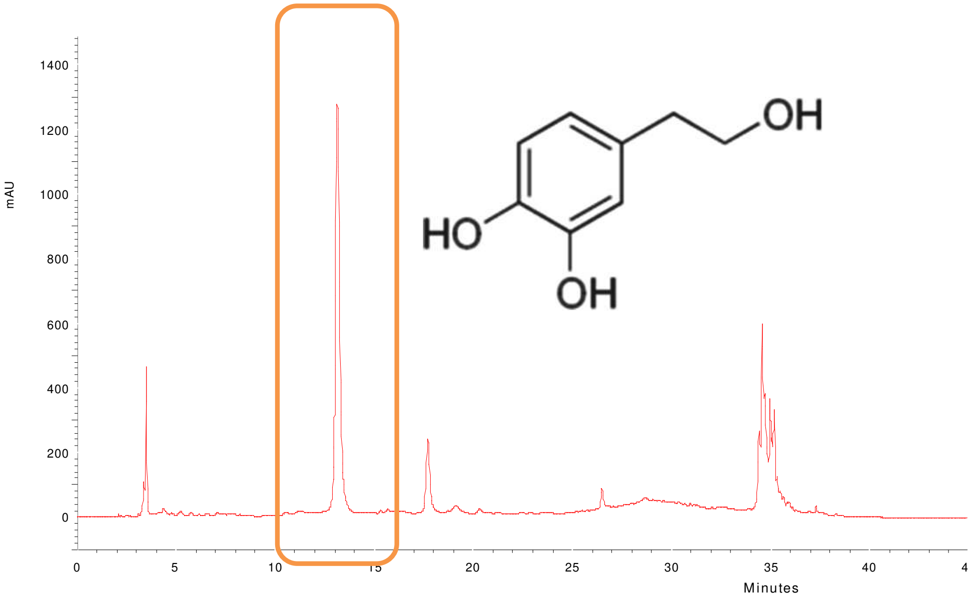
Inlet temperature: 170 °C
Outlet temperature: 80-82 °C
Spraying pressure: 2 bar
Feed: 3.5 lt/hr
Maltodextrin content in final powder: 40%

Dry standardized extract 10% in HT

DPPH IC50 64 μg/ml

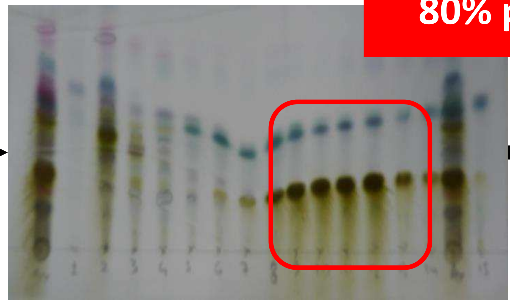
Recovery of biophenols from OMW/TOPW

- ✓ **1000 Lt of TOPWW → 3,3 Kg of phenols extract**
- ✓ **1000 Lt of OMWW → 4 Kg of phenols extract**



After treatment with adsorption resins the remaining waste is: an odorless yellowish wastewater with a 99.5% reduced content in polyphenols and low toxicity

High purity HT from OMW/TOPW



80% purity

Automated preparative RP-HPLC

40 g of phenolic fraction

3 g of hydroxytyrosol (80%)

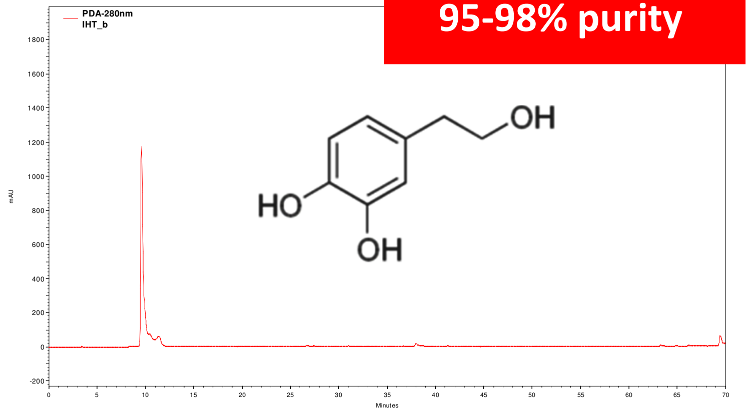
Fast Centrifugal Partition Chromatography (FCPC)

Cyclohexane / Ethyl acetate / Ethanol / Water
2/8/1/9, v/v/v/v

higly pure HT

(200 g from 1000 lt waste water)

95-98% purity

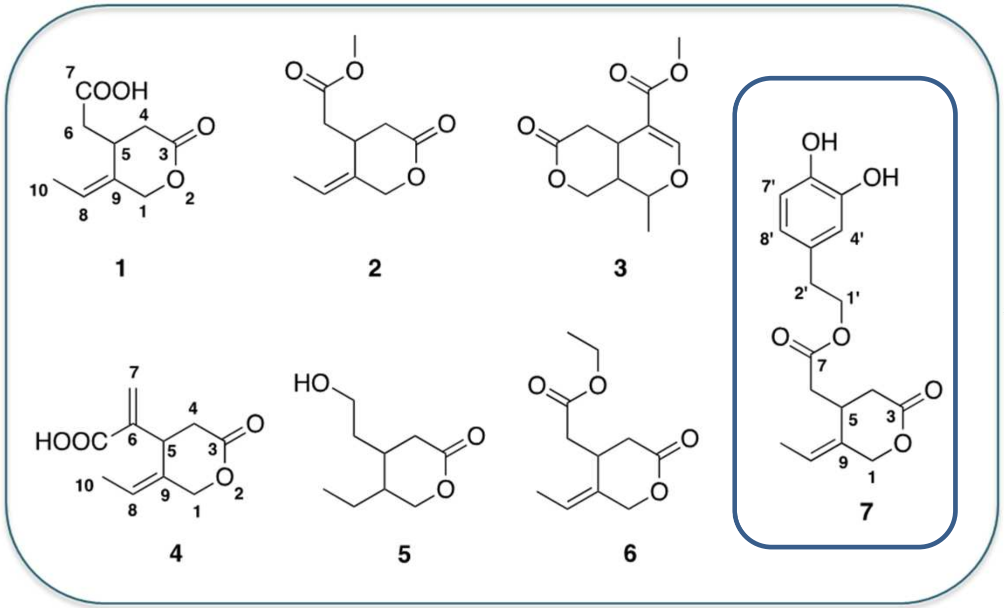


Xynos, Nikos et al. “Development of a Sustainable Procedure for the Recovery of Hydroxytyrosol from Table Olive Processing Wastewater Using Adsorption Resin Technology and Centrifugal Partition Chromatography.” *Planta medica* vol. 81,17 (2015): 1621-7.

Minor compounds - secoiridoid lactones

OOMW Crude → (XAD7 resin) → OOMW Treated → (partition AcOEt/Acetone/Water 3:3:4) → OMW aqueous / OMW organic

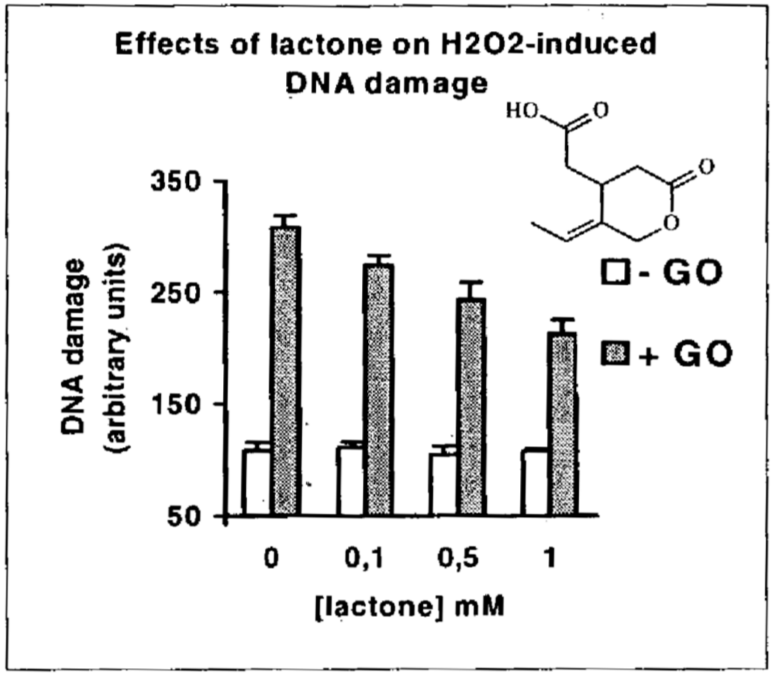
Gradient FCPC
Ascending mode
Lower phase (stationary):
Water/MeOH 9:1
Upper phase (mobile):
cHex (1h) → AcOEt in 2h → AcOEt



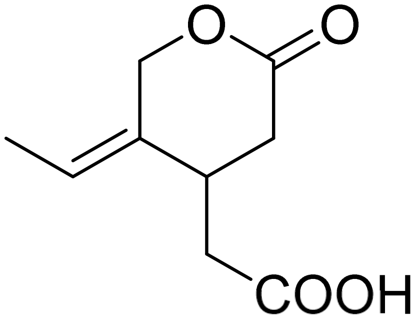
Compound 7 is the first secoiridoid lactone found in OMW that is conjugated with a hydroxytyrosol moiety

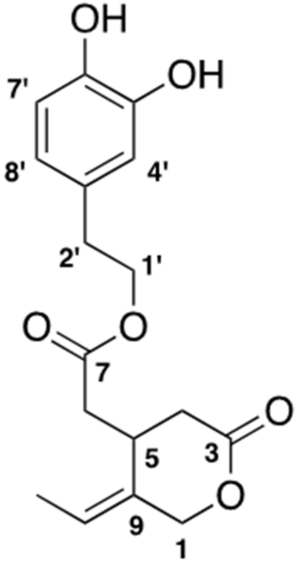
Vougogiannopoulou, Konstantina et al. "Chemical and Biological Investigation of Olive Mill Waste Water - OMWW Secoiridoid Lactones." *Planta medica* vol. 81,12-13 (2015): 1205-12.

Antioxidant effect of OMW extract – protection of DNA from oxidative damage (Jurkat cells)

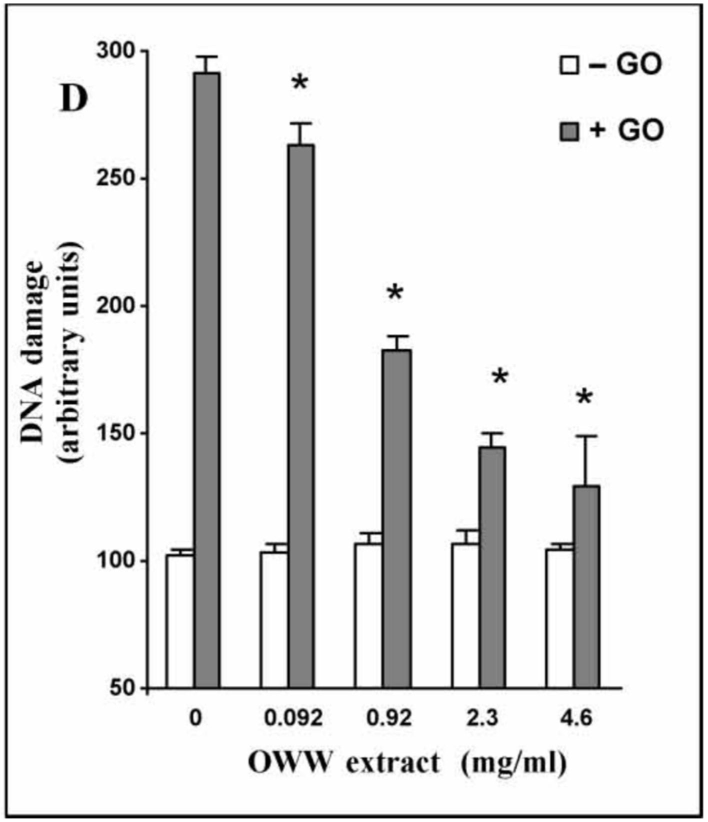


Effect of OMWW lactone on DNA damage, with the presence of glucose oxidase (GO)

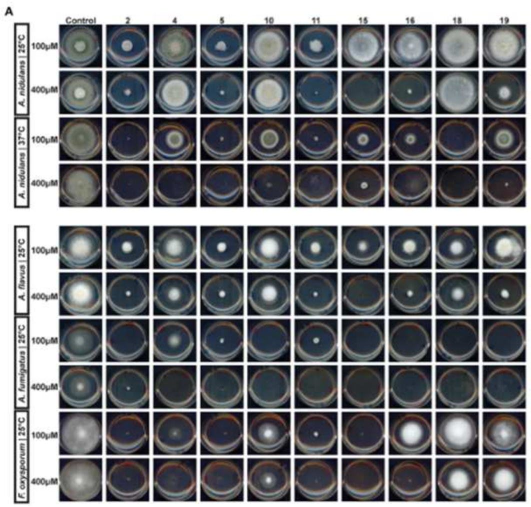


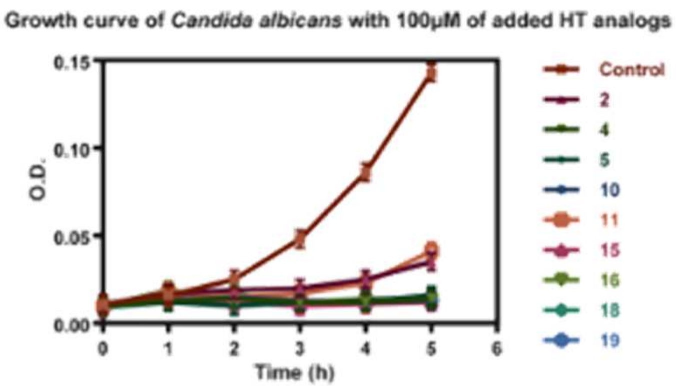


OMW extract offers >50% protection



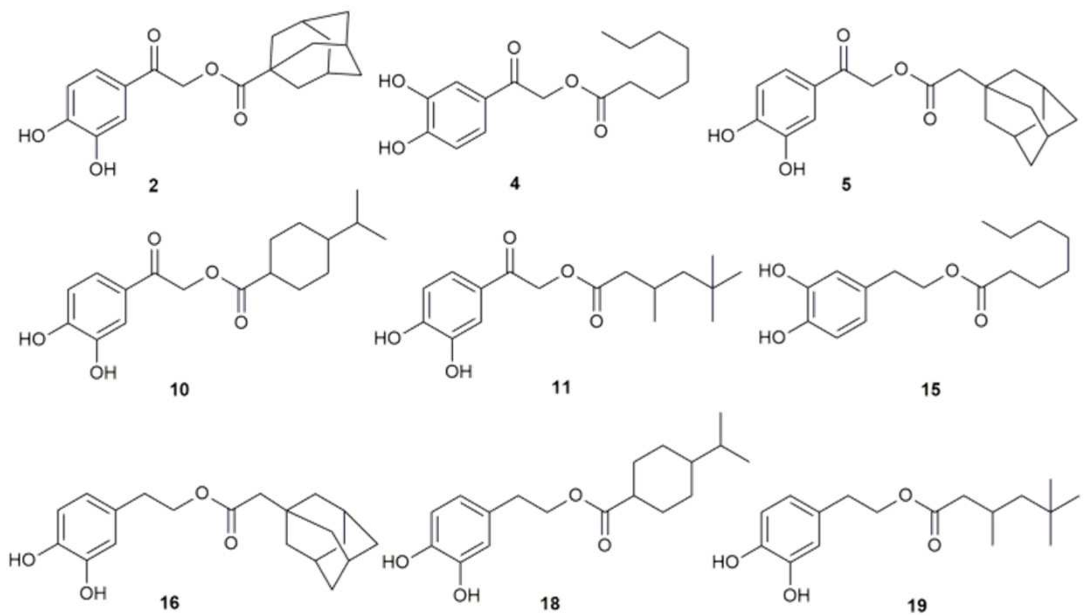
Synthesis of 27 HT analogs with increased lipophilicity





❖ Synthesis of HT esters bearing lipophilic chains

❖ Evaluation against *Aspergillus sp.*, *Fusarium oxysporum*, and *Candida albicans.*



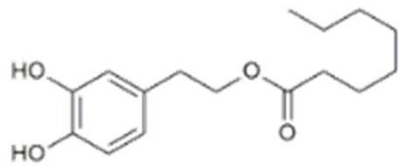
HT analogs more potent antifungals than HT

Approximate concentrations of HT analogs that lead to 50% reduction of fungal growth (IC50)

	HT	2	4	5	10	11	15	16	18	19
A. nidulans 37°C	>400	~50	<100	~50	~100	~50	< 100	<100	~50	~100
A. nidulans 25°C	>400	<100	<200	<100	<200	~100	< 200	<200	< 200	< 200
A. fumigatus	>400	~50	<100	~50	~50	<100	<50	<100	~50	~50
A. flavus	>400	<100	>100	<100	>100	<100	<100	~100	~100	>100
F. oxysporum	>400	<50	<50	<50	~100	<50	<50	<200	<200	<200
C. albicans	>400	~100	~100	~100	~100	~100	~100	~100	~100	~100

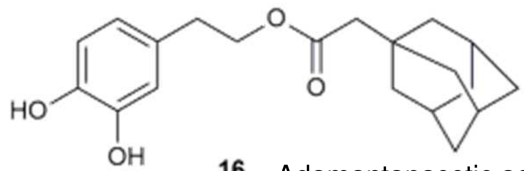
Most active analogs showed **no toxicity** in mammalian cell lines

Using *Aspergillus nidulans* as an *in vivo* cellular model system, we found that HT analogs act as potent antifungals, by direct disruption of the fungal cell membrane



15

Caprylic acid



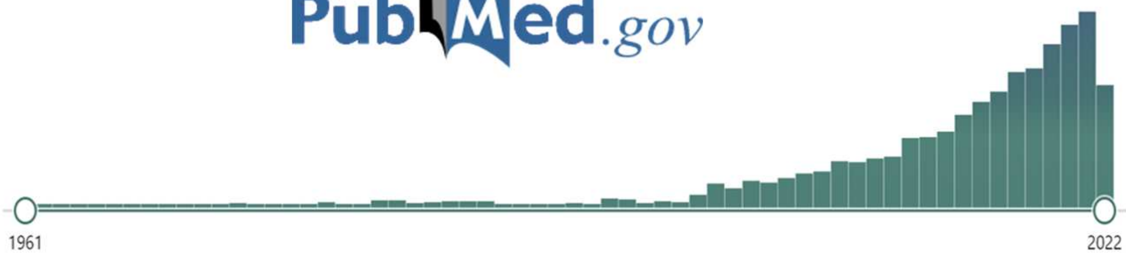
16 Adamantanacetic acid

Diallinas, George, et al. "Hydroxytyrosol (HT) analogs act as potent antifungals by direct disruption of the fungal cell membrane." *Frontiers in microbiology* 9 (2018): 2624.

From a harmful waste to the scale up production of HT

- The claim of EFSA spurred a new interest for the use and bioactivity of hydroxytyrosol
- One of the most potent natural antioxidant agent
- HT is the subject of more than 1700 scientific publications since the 60s
- Multitude of biological impact

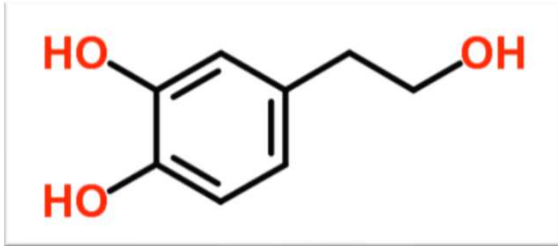
PubMed.gov



Anti-inflammatory

Anti-cancer

Cardioprotective

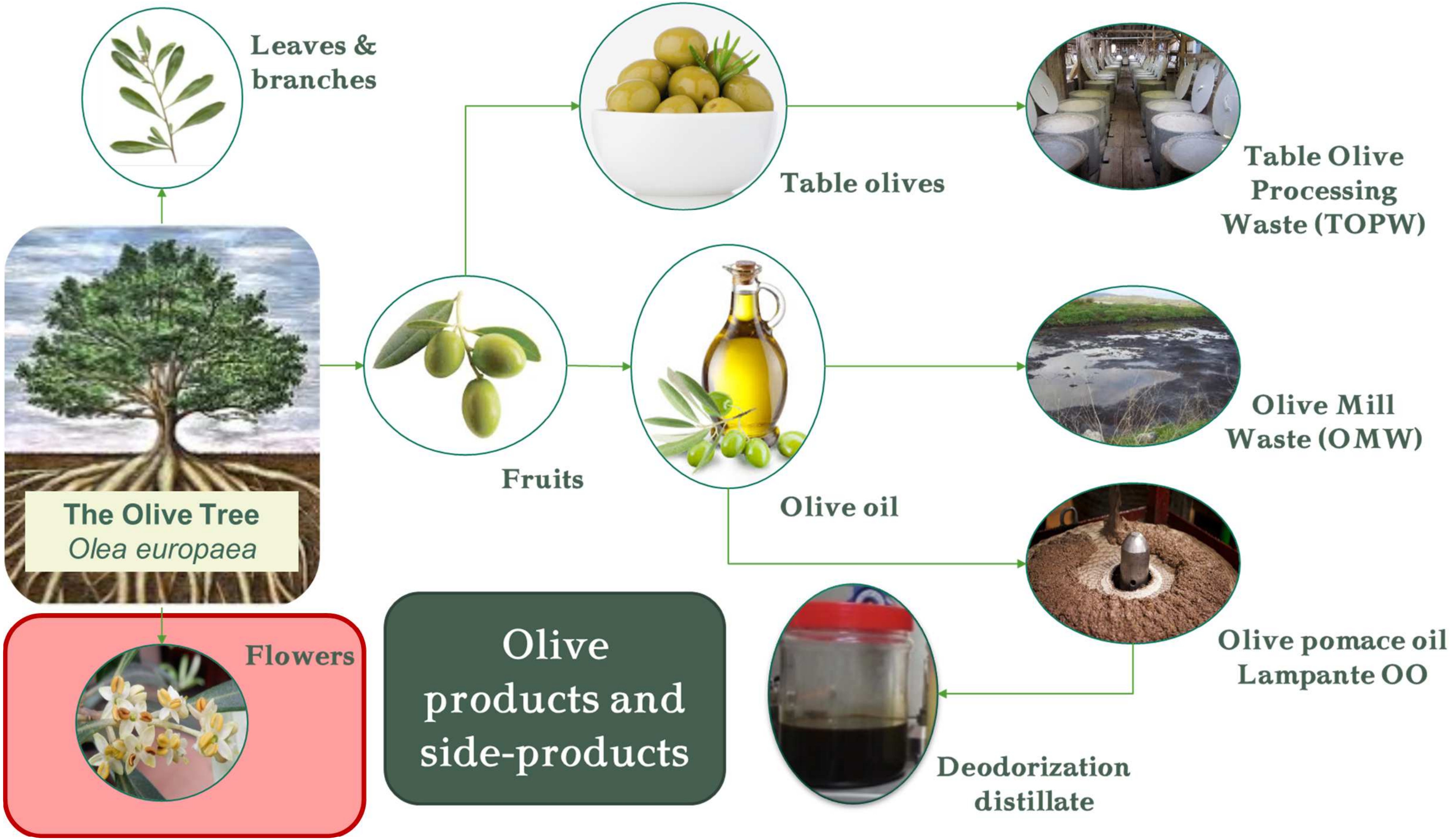


Hydroxytyrosol (HT)

Anti-atherogenic

Antimicrobial

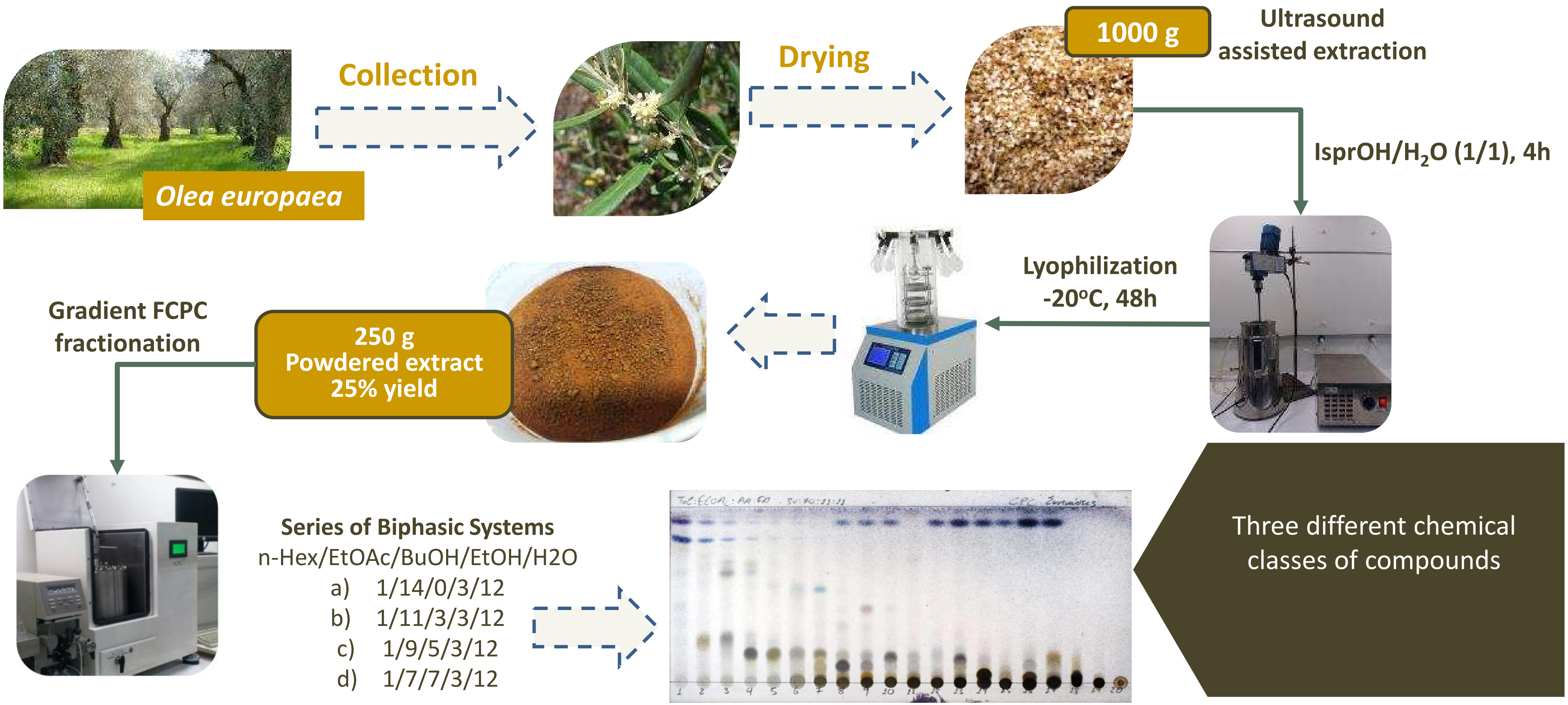
Antioxidant


Leaves & branches
The Olive Tree
Olea europaea
Flowers
Fruits
Table olives
Olive oil
Table Olive Processing Waste (TOPW)
Olive Mill Waste (OMW)
Olive pomace oil
Lampante OO
Deodorization distillate
Olive products and side-products

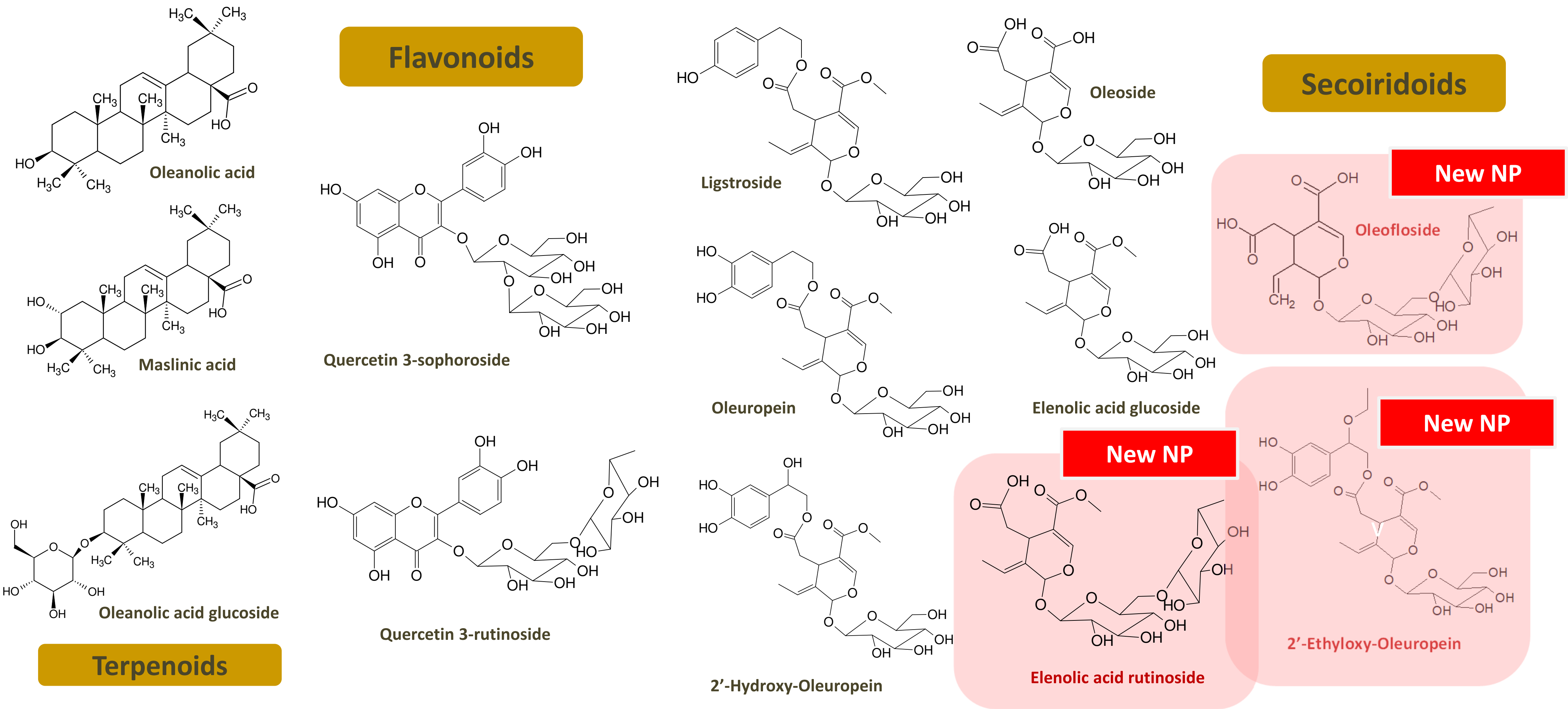
Olive flowers are not an environmental threat However is an important by product given that 80% of olive flowers fall to the ground before fruiting

F. Famiania, The cost of flowering in olive (Olea europaea L.)
Scientia Horticulturae, 2019, 252, 208-273
0.5 kg/ tree

Olive flowers as a valuable source of antioxidants



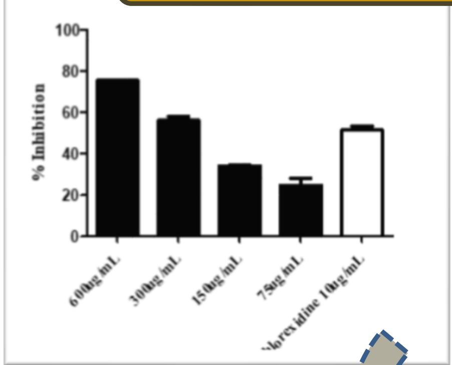
Isolation & Identification of Main Components in Flowers



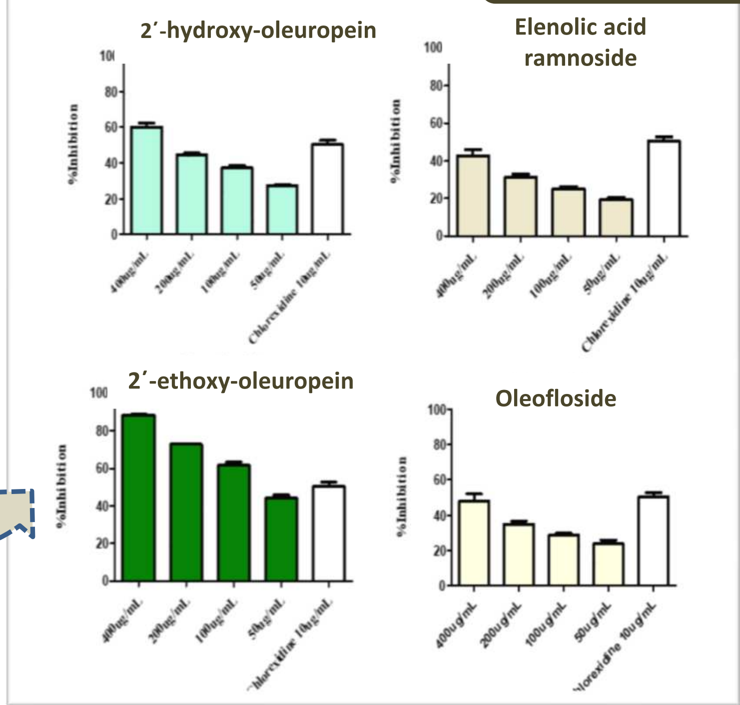
Angelis, Apostolis, et al. "Phytochemical analysis of olive flowers' hydroalcoholic extract and in vitro evaluation of tyrosinase, elastase and collagenase inhibition activity." *Fitoterapia* 143 (2020): 104602.

Collagenase inhibitory activity of flower extracts and compounds

Total extract

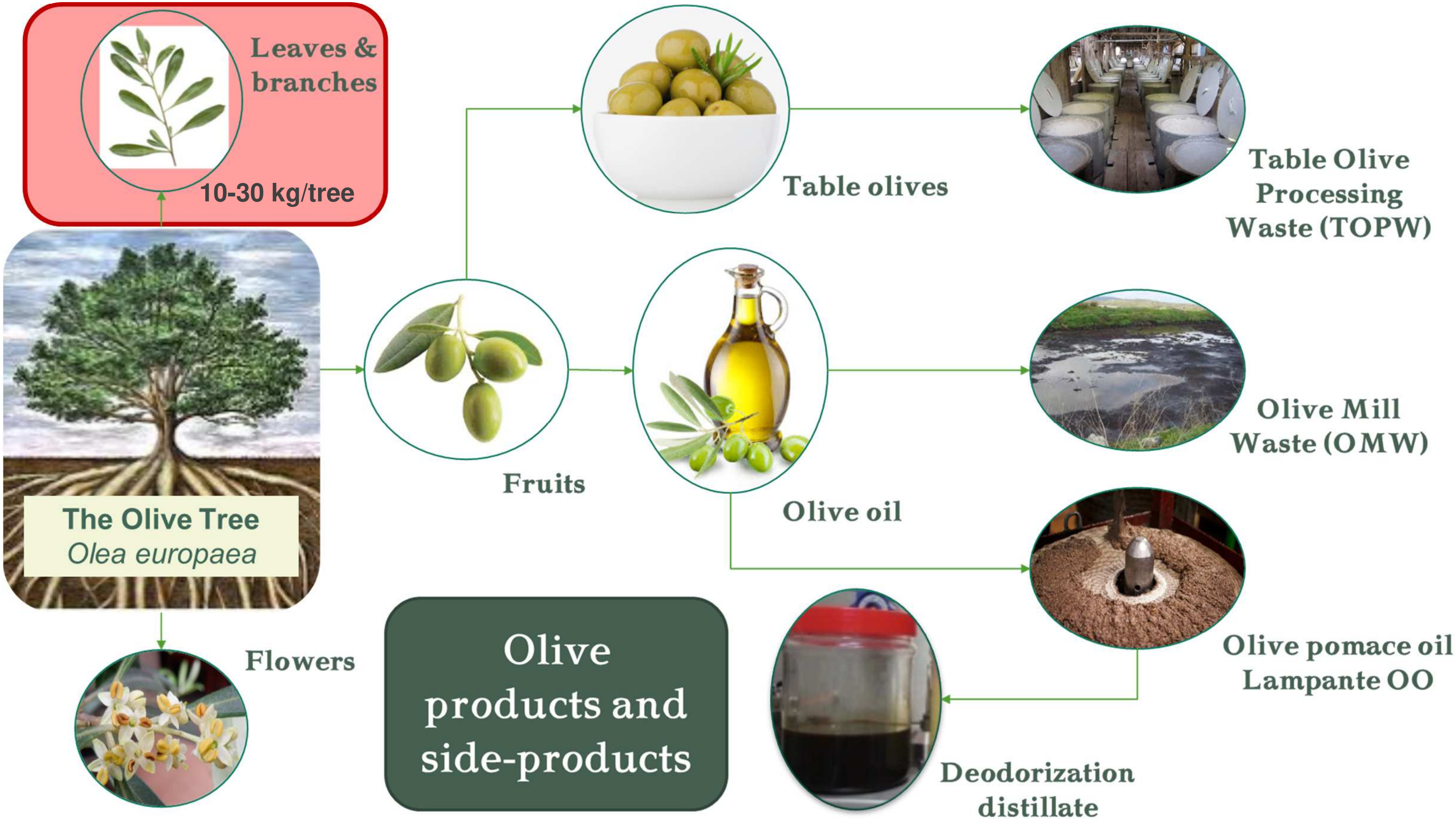


Pure compounds



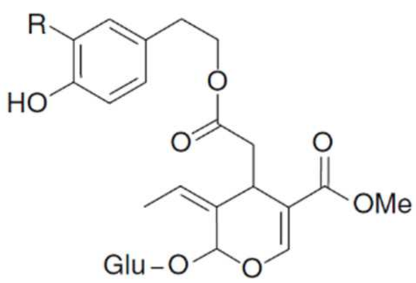
Fluorescence Measurement-Parameters:
Excitation maximum: 320 nm and emission maximum: 405 nm
Positive control: Chlorexidine

Olive branches are a valuable source of bioactives, with proved antioxidant activity, and their valorization, could offer an alternative source of biophenols and antioxidants, as well as novel applications in food, cosmetic, pharmaceutical and agricultural industry.


Leaves & branches
10-30 kg/tree
The Olive Tree
Olea europaea
Flowers
Fruits
Table olives
Table Olive Processing Waste (TOPW)
Olive oil
Olive Mill Waste (OMW)
Olive pomace oil
Lampante OO
Deodorization distillate
Olive products and side-products

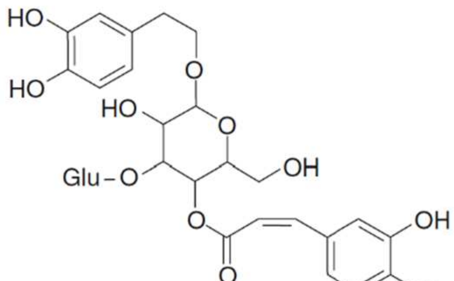
Chemical composition of leaves & branches

Secoiridoids



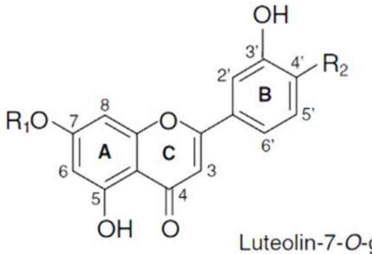
Oleuropein : R=OH
Ligstroside : R=H

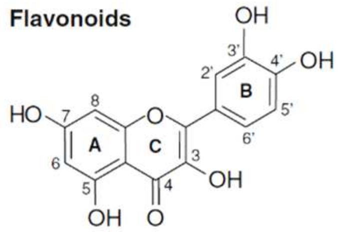
Phenyl ethanoid glucoside



Verbascoside

Flavonoids



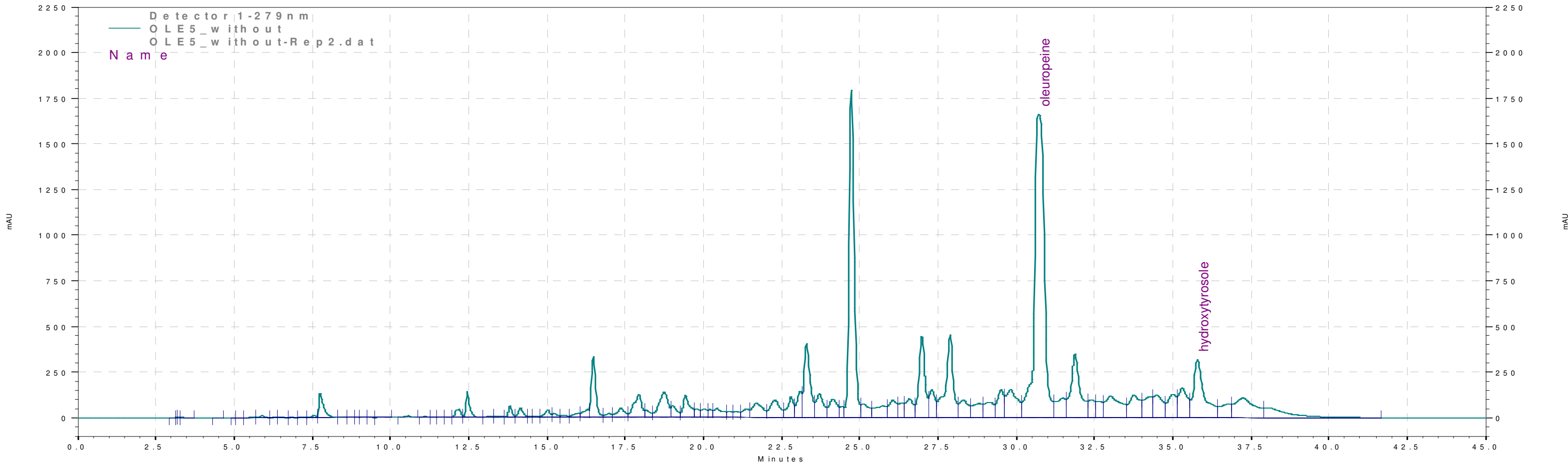


Quercetin (flavonol)

Luteolin-7-*O*-glucoside (flavone): R_1=Glucose, R_2=OH
Diosmetin (flavone): R_1=H, R_2=OCH_3

Oleuropein could be 10% on dry leaves weight

> 100 compounds identified based on LC-HRMS



Michel, Thomas, et al. "UHPLC-DAD-FLD and UHPLC-HRMS/MS based metabolic profiling and characterization of different Olea europaea organs of Koroneiki and Chetoui varieties." *Phytochemistry Letters* 11 (2015): 424-439.

Extraction of olive leaves for the recovery of oleuropein

1

- ACETONE EXTRACTION
- LIMONENE EXTRACTION, ENRICHMENT IN OLEUROPEIN
- FCPC PURIFICATION OF OLEUROPEIN

>85% purity

2

- METHANOL EXTRACTION Ultrasound
- MPLC PURIFICATION OF OLEUROPEIN

>90% purity

Extraction of olive leaves for the recovery of oleuropein (1)

100 kg leaves (dried) 4% oleuropein

Acetone 800lt maceration, 4h

Solvent evaporation

Crude oleuropein extract

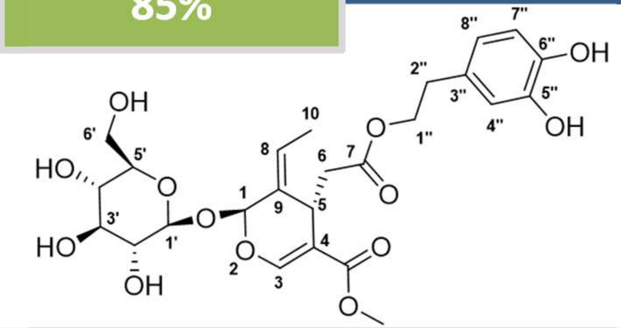
Limonene treatment (elimination of chlorophylls and triterpenoids)

Enriched oleuropein extract

9 kg extract 50% oleuropein

FCPC separation

3 Kg Oleuropein 85%



Extraction of olive leaves for the recovery of oleuropein (2)

150 kg leaves (dried)

Ultrasound assisted methanol maceration, 2h, 600lt

Crude oleuropein extract

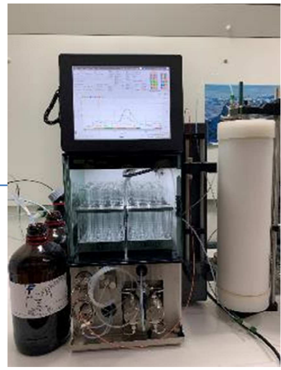
Spray drying

30 kg extract 20% oleuropein

Dry standardized Oleuropein Extract 20%

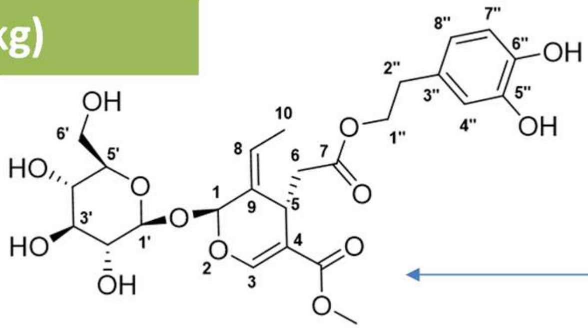
Inlet temperature: 170 °C
Outlet temperature: 80-82 °C
Spraying pressure: 2 bar
Feed: 3.5 lt/hr
Maltodextrin content in final powder: 40%

Name: FlashPure Ecoflex C18 3000g
Vcolumn: 5600 mL
Max pressure 84 psi
Max flow 200 mL/min
Starting pressure: 71 psi
Solvent H2O /MeOH



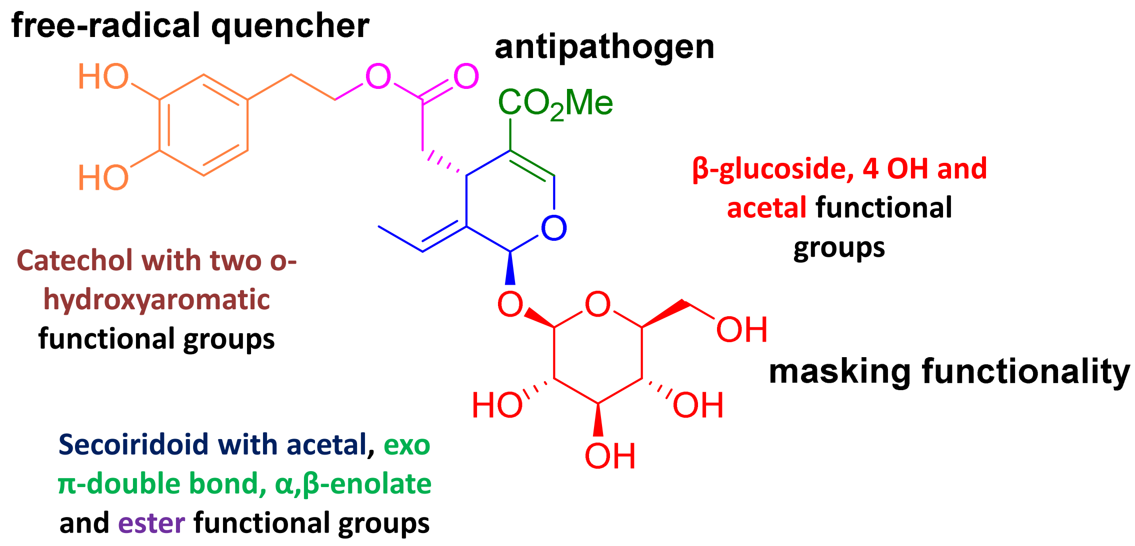
MPLC-DAD/ELSD

Oleuropein 90% (4 kg)



From a harmful waste to the scale up production of Oleuropein

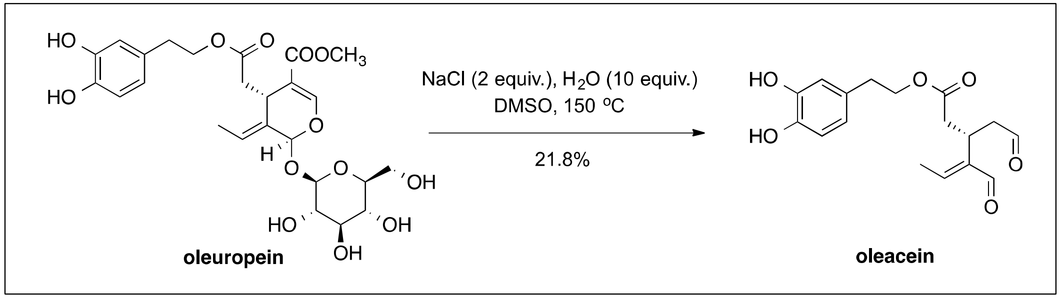
- ❖ Different functional groups define pharmacological activity
- ❖ Oleuropein has a wide spectrum of biological properties such as:
  - ✓ Cardioprotective, antiatherogenic
  - ✓ Antioxidant, anti-ageing
  - ✓ Anti-inflammatory
  - ✓ Antiviral, antimicrobial
  - ✓ Skin protective
  - ✓ cytotoxic (ER negative)

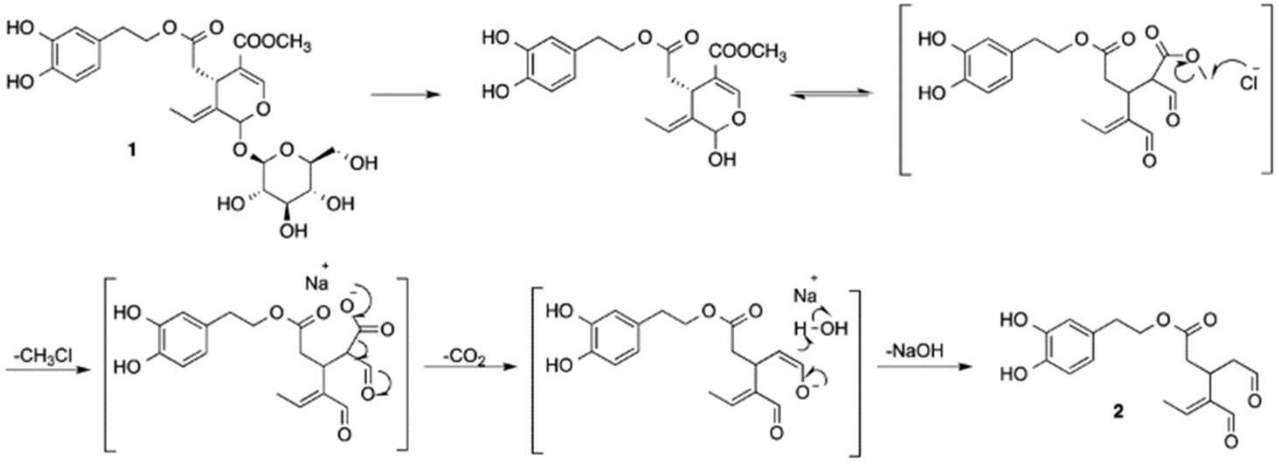


Versatile scaffold for chemical modifications!

One-step conversion of oleuropein to oleacein

- Oleacein was produced in one step, by **Krapcho decarbomethoxylation** reaction, in an aprotic solvent (DMSO) and elevated temperature (150^{o}C)
- Ligstroside (isolated from *Fraxinus* sp) was used for oleocanthal synthesis



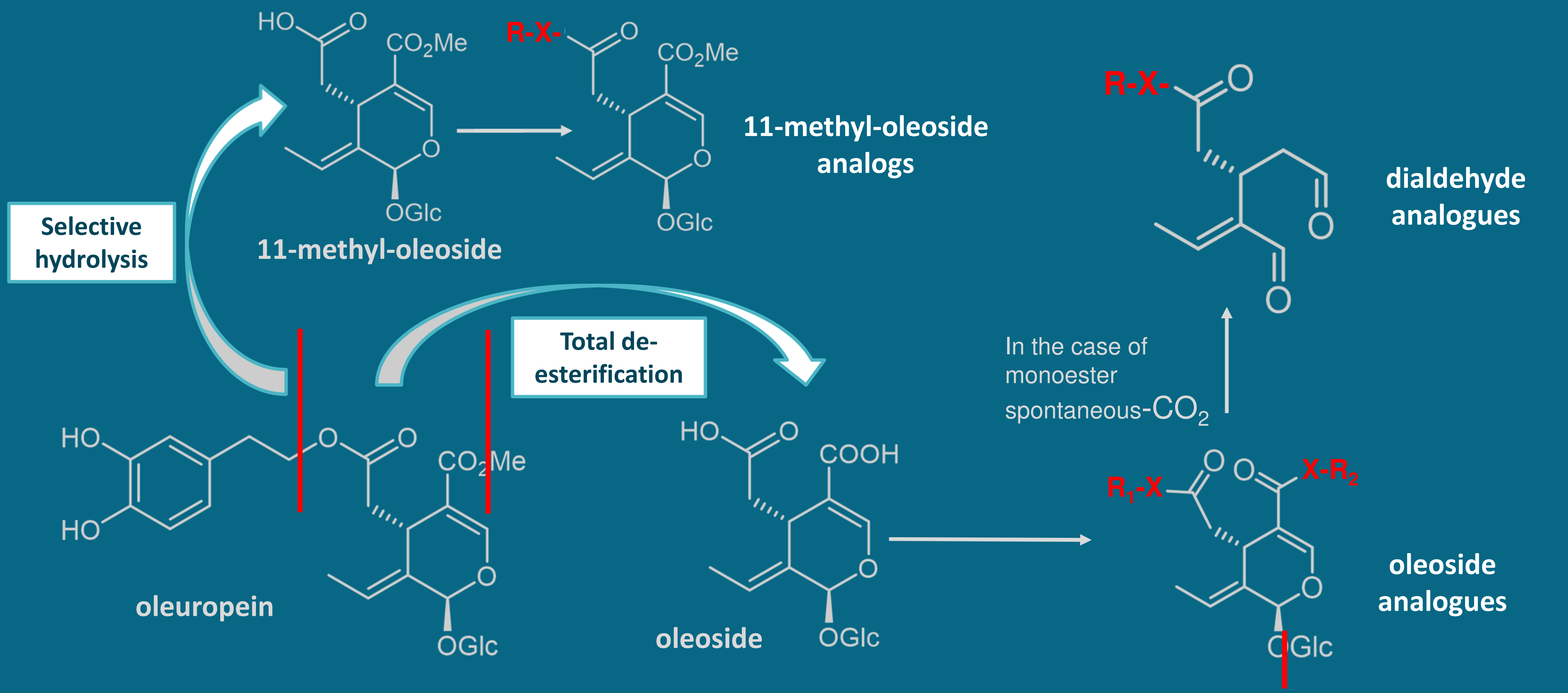


1-step conversion of oleuropein to oleacein

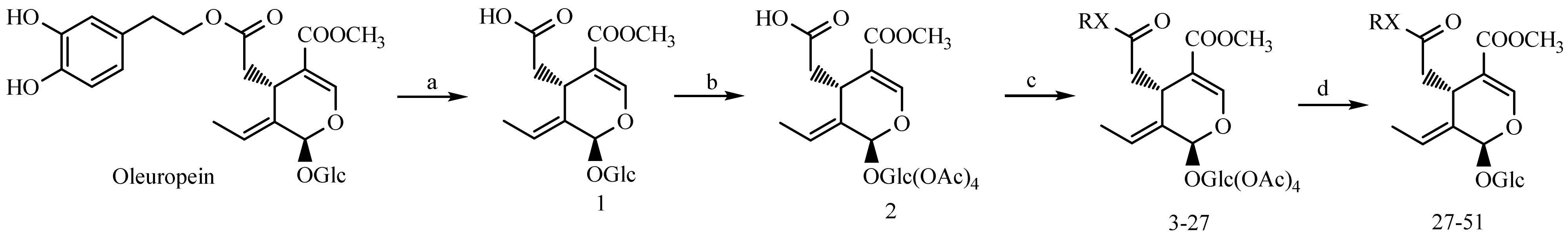
1-step conversion of ligstroside to oleocanthal

Vougogiannopoulou, Konstantina, et al. "One-step semisynthesis of oleacein and the determination as a 5-lipoxygenase inhibitor." *Journal of natural products* 77.3 (2014): 441-445.

Oleuropein – a robust scaffold leading to bioactive secoiridoids



Semi-synthesis of Oleuropein analogs from 11-methyl-oleoside



a) NaOH, 1 M, MW, 10 min b) Ac2O, Pyr, c) i) 2,4,6-trichlorobenzoyl chloride (Yamaguchi reagent), Et3N, ii) RX, DMAP, d) Et2NH, MeOH, RT

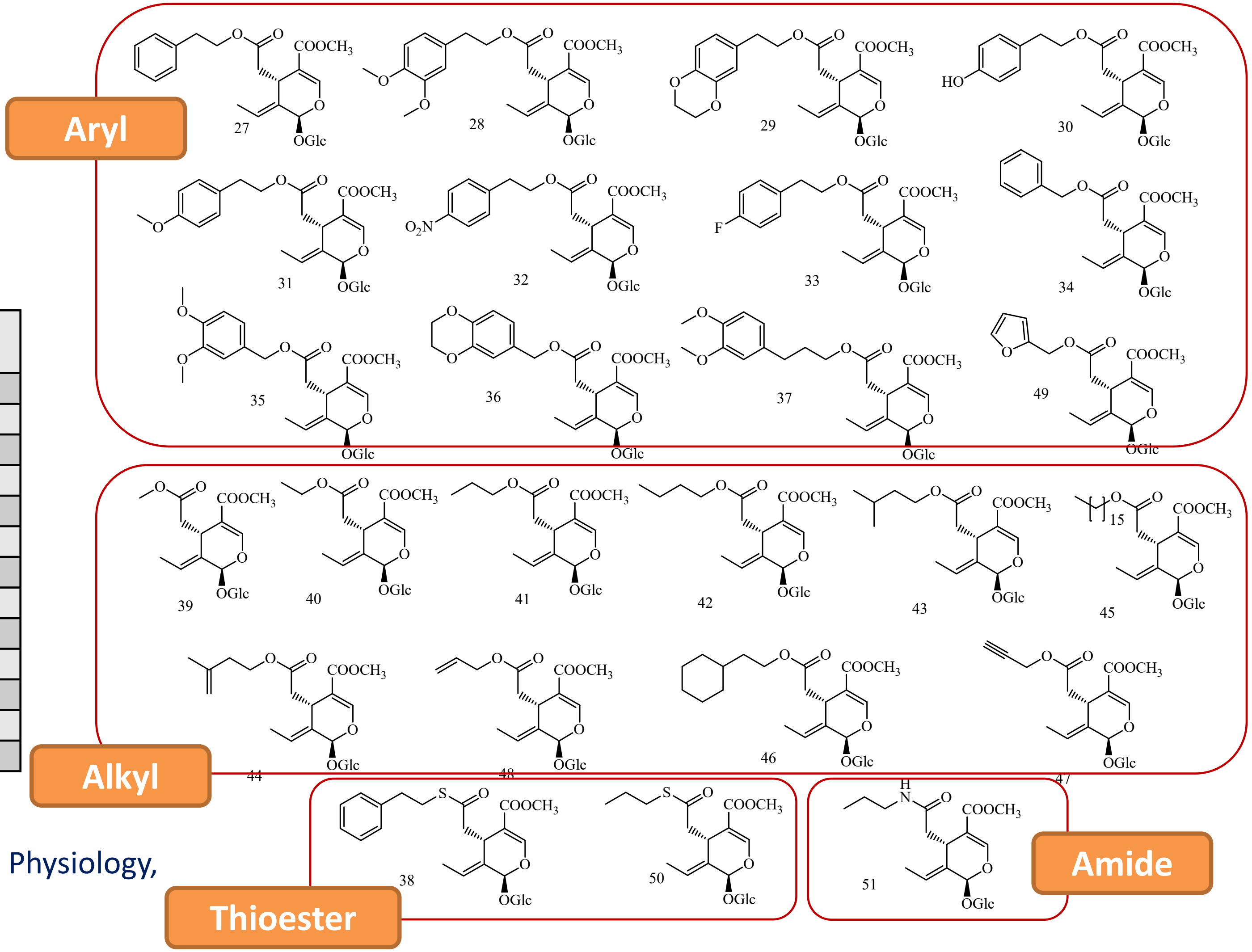
- ✓ **51 compounds synthesized**
- ✓ ***in vitro* screened in SKBR3 Breast Cancer**
- ✓ **The most active against seven cancer cell lines**
- ✓ **The most potent was further tested in an *in vivo* melanoma model C57BL/6 mice.**

R= alcohols, thiols, amines as nucleophiles

Samara, Pinelopi, et al. "New semi-synthetic analogs of oleuropein show improved anticancer activity in vitro and in vivo." *European Journal of Medicinal Chemistry* 137 (2017): 11-29.

Oleuropein analogs

Screening of oleuropein analogs against the human breast cancer cells SKBR3.



COMPOUND	SKBR3 IC_{50} ± SD (μM)	COMPOUND	SKBR3 IC_{50} ± SD (μM)
27	11.02 ± 0.71	**40**	>15
28	>15	**41**	>15
29	>15	**42**	>15
30	>15	**43**	9.02 ± 0.71
31	>15	**44**	>15
32	>15	**45**	1.60 ± 0.42
33	>15	**46**	2.00 ± 0.71
34	>15	**47**	>15
35	>15	**48**	>15
36	>15	**49**	>15
37	>15	**50**	>15
38	>15	**51**	>15
39	>15		

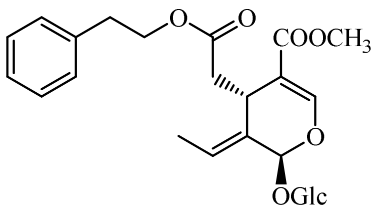
- Department of Biology, Section of Animal & Human Physiology, University of Athens – Prof. O. Tsitsilonis group

IC_{50} (µM) of the most active semi-synthetic analogs of oleuropein

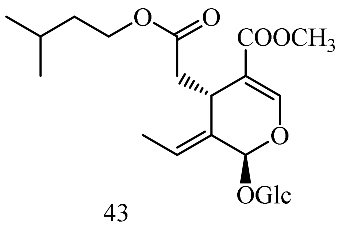
Alkyl group or aromatic without substitution increase lipophilicity enhancing **cell membrane permeability**

	IC_{50} ± SD (in µM)						
Comp.	**FM3**	**HCT-116**	**HeLa**	**MCF-7**	**SKBR3**	**HL-60**	**K562**
27	28.00 ± 2.00	25.33 ± 2.89	18.51 ± 4.04	19.33 ± 4.04	11.02 ± 0.71	10.33 ± 0.76	20.04 ± 1.20
43	27.67 ± 2.52	26.67 ± 2.08	19.50 ± 2.24	21.33 ± 2.89	9.02 ± 0.71	9.83 ± 0.76	18.00 ± 0.89
45	8.83 ± 1.61	12.33 ± 2.52	2.70 ± 0.23	2.00 ± 0.00	1.60 ± 0.42	0.38 ± 0.04	0.70 ± 0.08
46	9.33 ± 1.53	13.67 ± 3.21	5.60 ± 0.60	5.00 ± 1.00	2.00 ± 0.71	0.48 ± 0.04	0.85 ± 0.07
Oleuropein	268.82 ± 3.1	181.86 ± 2.89	275.60 ± 2.1	161.57 ± 2.08	160.44 ± 1.50	54.26 ± 4.04	64.73 ± 3.02

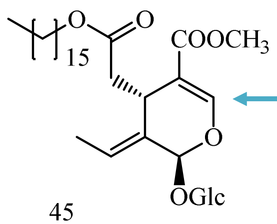
FM3: Melanoma Cell Line; **HT-116**: Colorectal carcinoma cell line; **HeLa**: Cervical carcinoma cell line; **MCF-7**: Breast cancer cell line; **SKBR**3: Breast cancer cell line that overexpresses Her2; **HL-60**: Leukemia cell line; **K562**: Chronic myelogenous leukemia cell line.



27

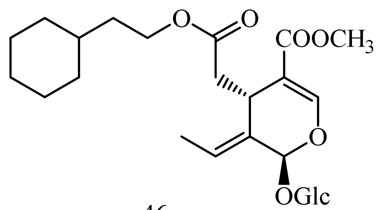


43



45

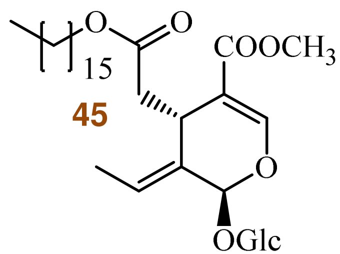
Compound 45 with a palmitic ester group is active in low µM and nM level

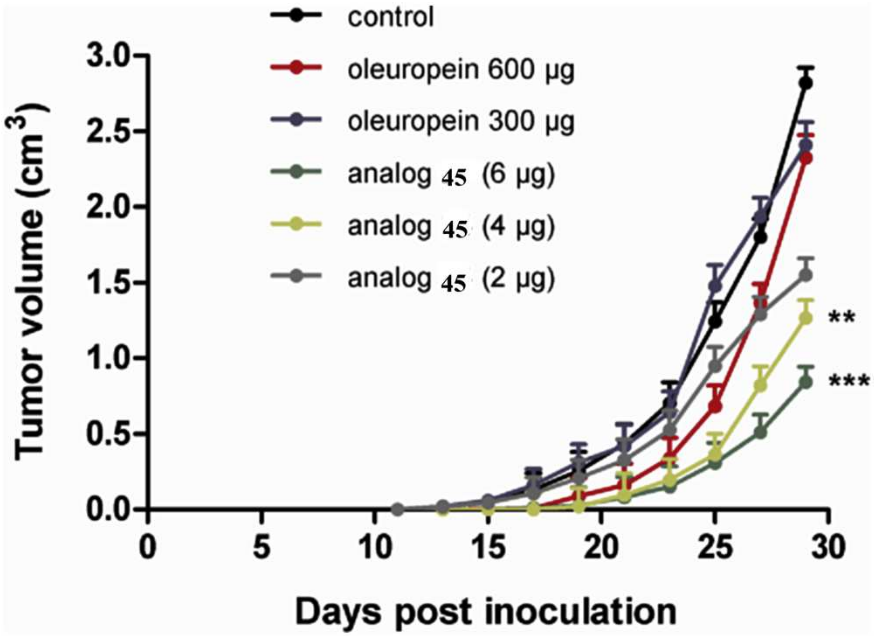


46

Effect of 45 on melanoma cancer tumor growth in a B16.F1 mouse model

IC_{50} ± SD (in μM)	
Comp.	**B16.F1**
27	41.00 ± 1.44
43	39.12 ± 1.10
45	1.50 ± 0.18
46	5.04 ± 0.31
Oleuropein	283.90 ± 1.82



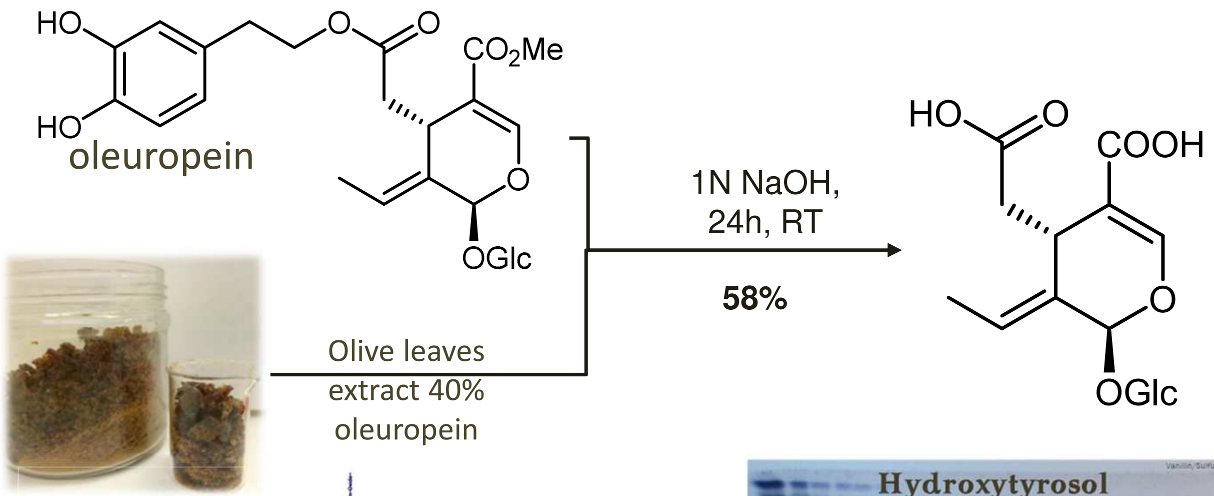


IC_{50} values (in μM) of the most active semi-synthetic analogs of oleuropein against the mouse melanoma cell line B16.F1

Compound 45 reduces melanoma tumor growth *in all three groups compares to oleuropein and control*

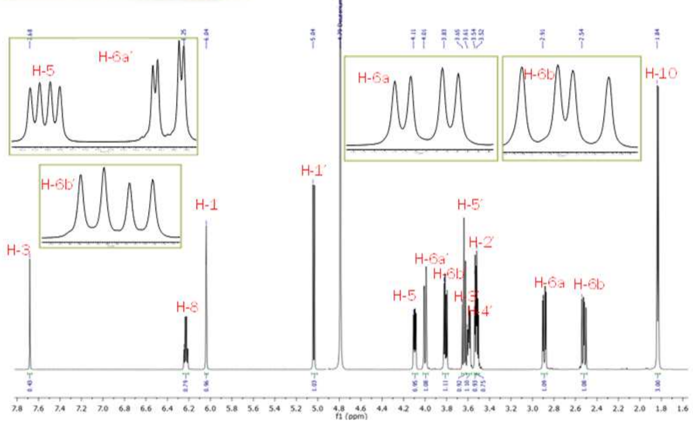
Samara, Pinelopi, et al. "New semi-synthetic analogs of oleuropein show improved anticancer activity in vitro and in vivo." *European Journal of Medicinal Chemistry* 137 (2017): 11-29.

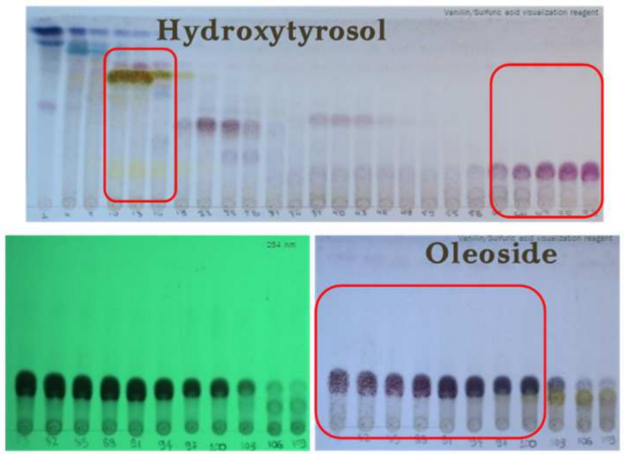
Semi-synthesis of oleoside and its purification



FCPC purification

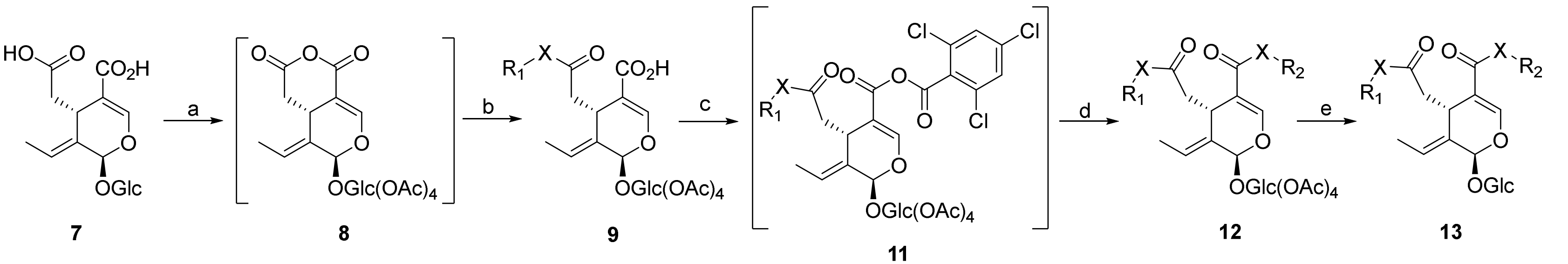
Recovery of **Oleoside 500 g** from **50 kg** Olive leaves in purity >90%





Prep CPC conditions	
Biphasic system	EtOAc/2-Propanol/EtOH/H_2O/AA 8:2:1:10:0,5 v/v/v/v
Column volume	1000 mL
Rotation	800 rpm
Mode	Ascending
Flow rate	15 mL/min
Elution	500 mL,
Extrusion	1000 mL
Inject volume	20 g/ 50 mL 1:1 v/v upper/lower phase
Number of fraction	120 of 30 mL

Semi-synthesis of Oleoside Di-esters



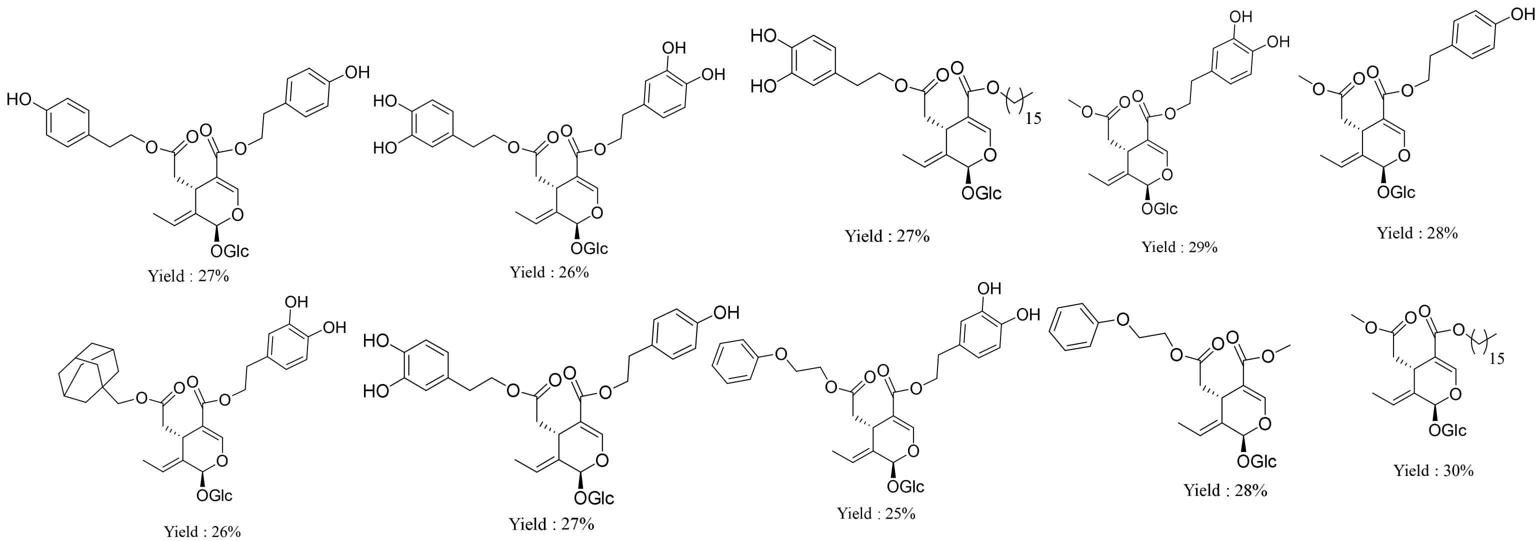
22 : R_1 = methyl , X = O, R_2 = 3-hydroxytyrosolyl, X = O
23 : R_1 = methyl , X = O, R_2 = tyrosolyl, X = O
24 : R_1 = methyl , X = O, R_2 = hexadecanyl, X = O
25 : R_1 = 3-hydroxytyrosolyl, X = O, R_2 = 3-hydroxytyrosolyl, X = O
26 : R_1 = 3-hydroxytyrosolyl, X = O, R_2 = tyrosolyl, X = O
27 : R_1 = 3-hydroxytyrosolyl, X = O, R_2 = hexadecanyl, X = O
28 : R_1 = tyrosolyl, X = O, R_2 = tyrosolyl, X = O
29 : R_1 = adamantanemethyl, X = O, R_2 = 3-hydroxytyrosolyl, X = O
30 : R_1 = phenoxyethyl, X = O, R_2 = 3-hydroxytyrosolyl, X = O
31 : R_1 = phenoxyethyl, X = O, R_2 = methyl, X = O

10 synthetic compounds

a) pyridine, acetic anhydride, RT, 3 hours, b) Et_3N, 4-DMAP, R_1= alkyl, aryl, X= O, S, DCM, RT, 3h, c) Et_3N, 2,4,6-trichlorobenzoyl chloride, DCM, 0°C→RT, 3h, d) 4-DMAP, R_2X (R_2= alkyl, aryl, X= O, S), DCM, RT, 3h, e) Et_2NH, MeOH, RT, 6 h.

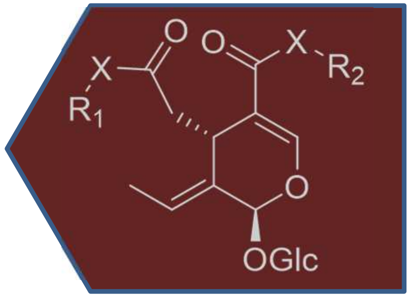
Secoiridoids including two phenethyl moieties have been isolated as minors (**Oleaceae**, **Cyperaceae**)

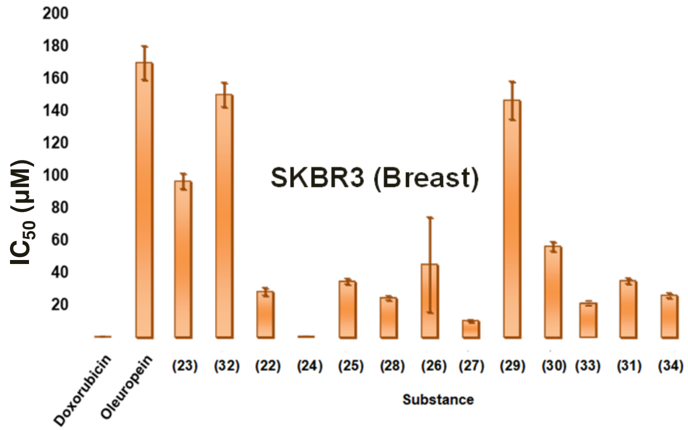
Synthesis of un-natural di-ester analogs



In vitro cytotoxicity of oleoside di-esters

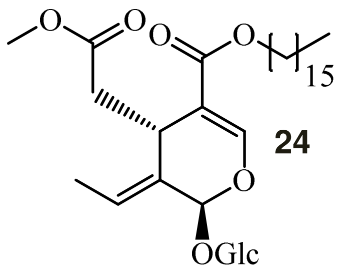
No	R_1	X_1	R_2	X_2	C10-C9-C8
(22)		O		O	
(23)		O		O	
(24)		O		O	
(25)		O		O	
(26)		O		O	
(27)		O		O	
(28)		O		O	
(29)		O		O	
(30)		O		O	
(31)		O		O	
(32)		O		O	
(33)		S		O	
(34)		O	H	O	





Selective cytotoxicity depending on the substituent R1 and R2

- Compound **24** one palmitic ester group and one methyl group
- Compound **27** one palmitic and one hydroxytyrosol group
- Compound **28** two tyrosol groups



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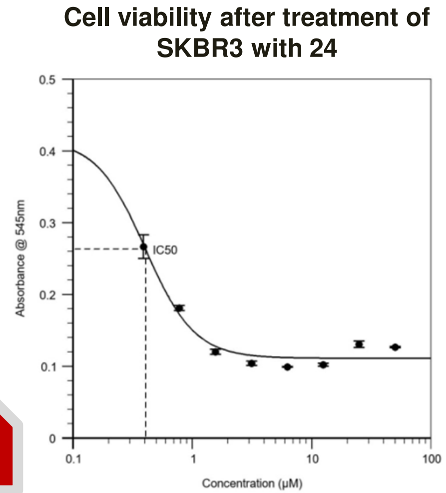
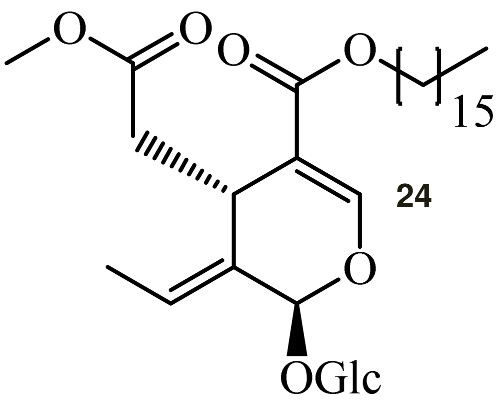


IC_{50} of 24 in the **nanomolar range**

- Department of Biology, Section of Animal & Human Physiology, University of Athens – Prof. O. Tsitsilonis group

In vitro cytotoxicity of oleoside di-esters



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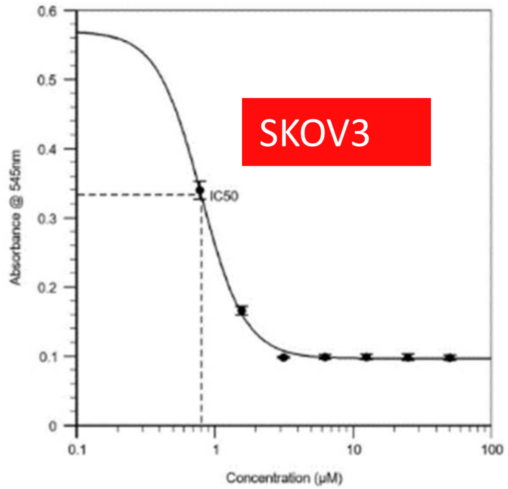
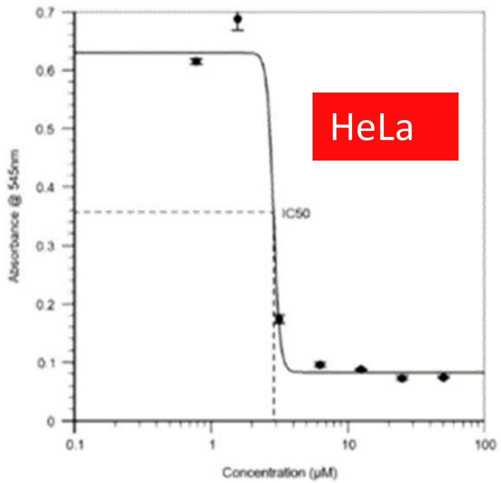
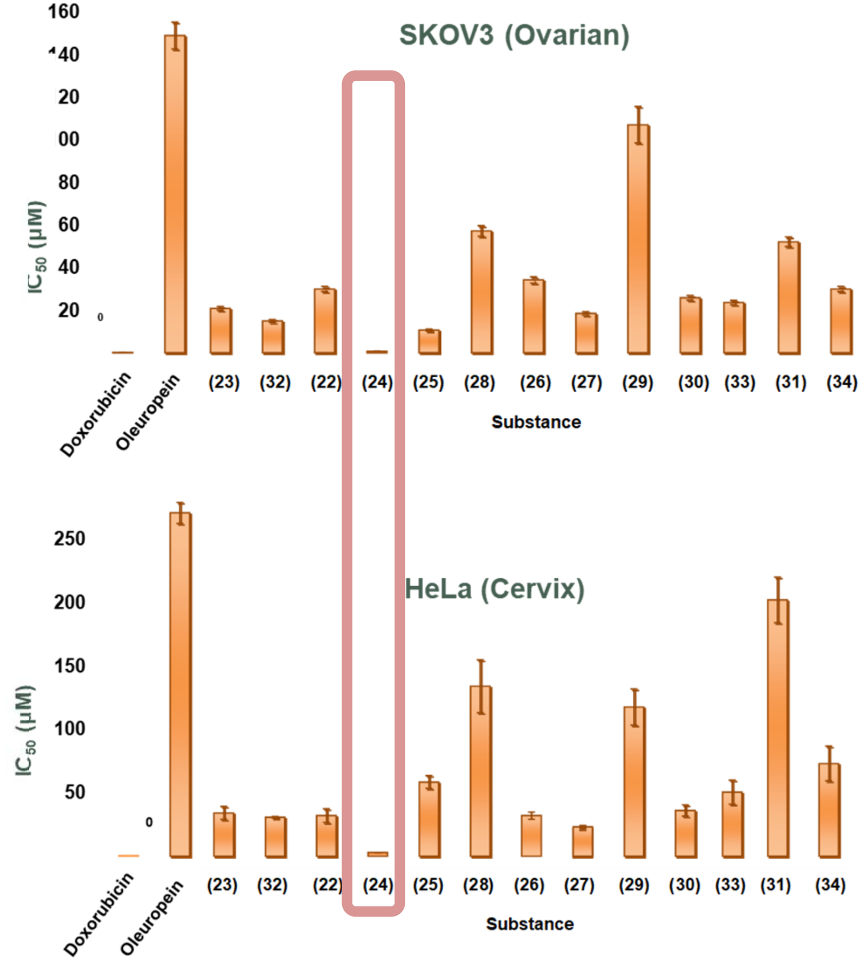




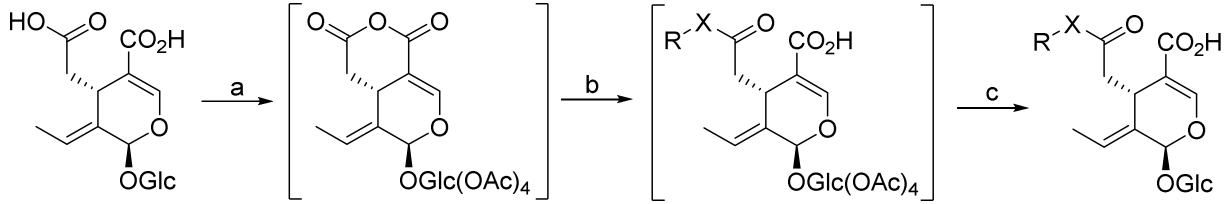


IC$_{50}$ of 24 in the
nanomolar range also for HeLa and SKOV3

Semi-synthesis of oleoside mono-esters

- ✓ Acetylation results in the formation of the mixed anhydride
- ✓ One-pot conjugation with various nucleophiles (DMAP) and deprotection of glucose

PROCESS FOR THE PRODUCTION OF OLEOCANTHAL, OLEACEIN AND THEIR ANALOGUES
European patent application EP 3 838 885 A1
23/06/2021

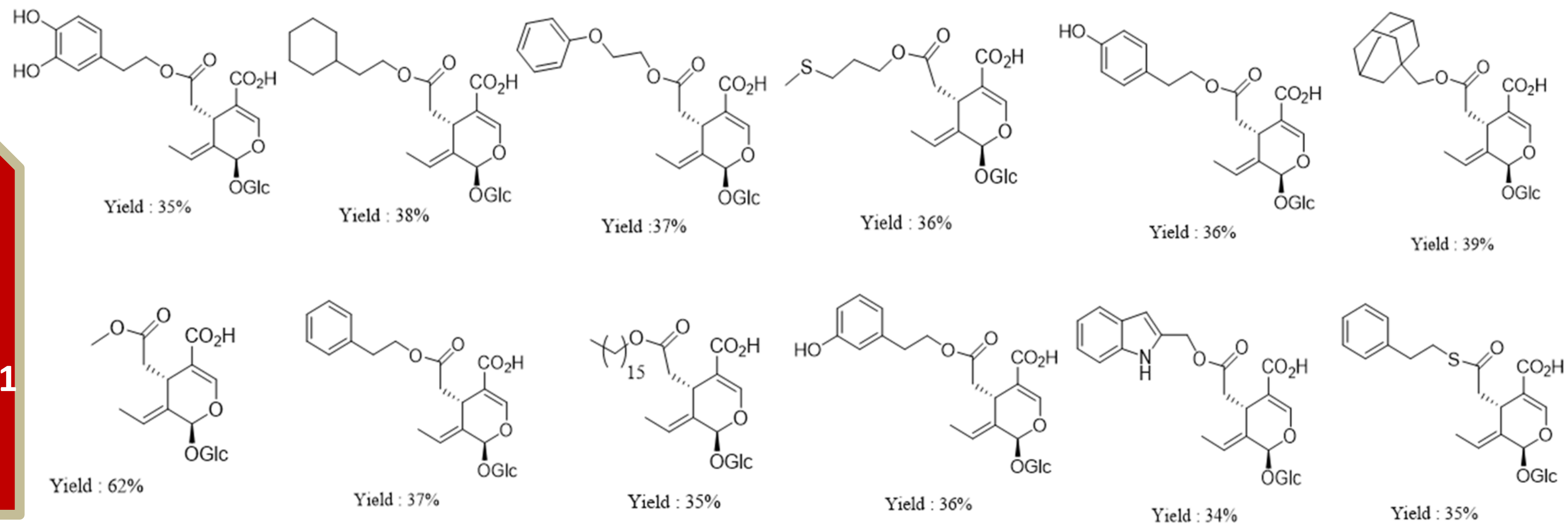


7 8 9 10-19

10 : R = methyl , X = O
11 : R = cyclohexylethyl, X = O
12 : R = adamantanemethyl, X = O
13 : R = phenylethyl, X = O
14 : R = phenoxyethyl, X = O
15 : R = (1H-Indole-2)-methyl , X = O
16 : R = hexadecanyl, X = O
17 : R = 3-(methylthio)-1-propyl, X = O
18 : R = phenethyl, X = S
19 : R = 3-Hydroxyphenethyl, X = O

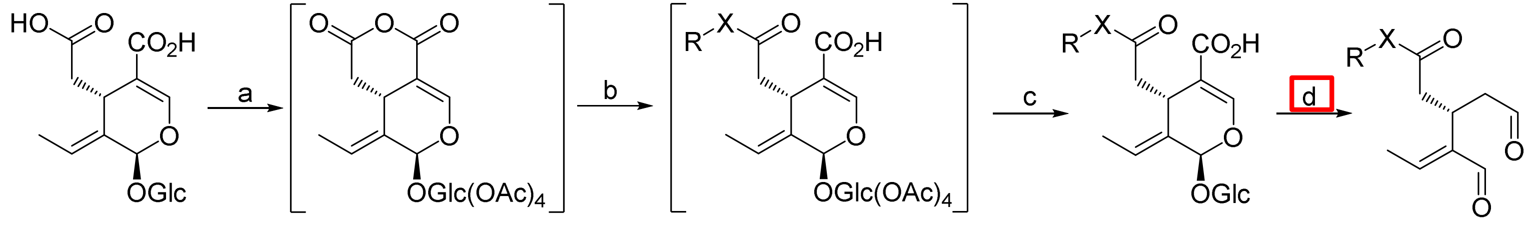
12 synthetic compounds

a) pyridine, acetic anhydride, RT, 3h, b) Et_3N, 4-DMAP, R= alkyl, aryl, X= O, S, DCM, RT, 3h, c) Et_2NH, MeOH, RT, 6 h



Semi-synthesis of dialdehyde analogs

Overall Yield: 21–32%

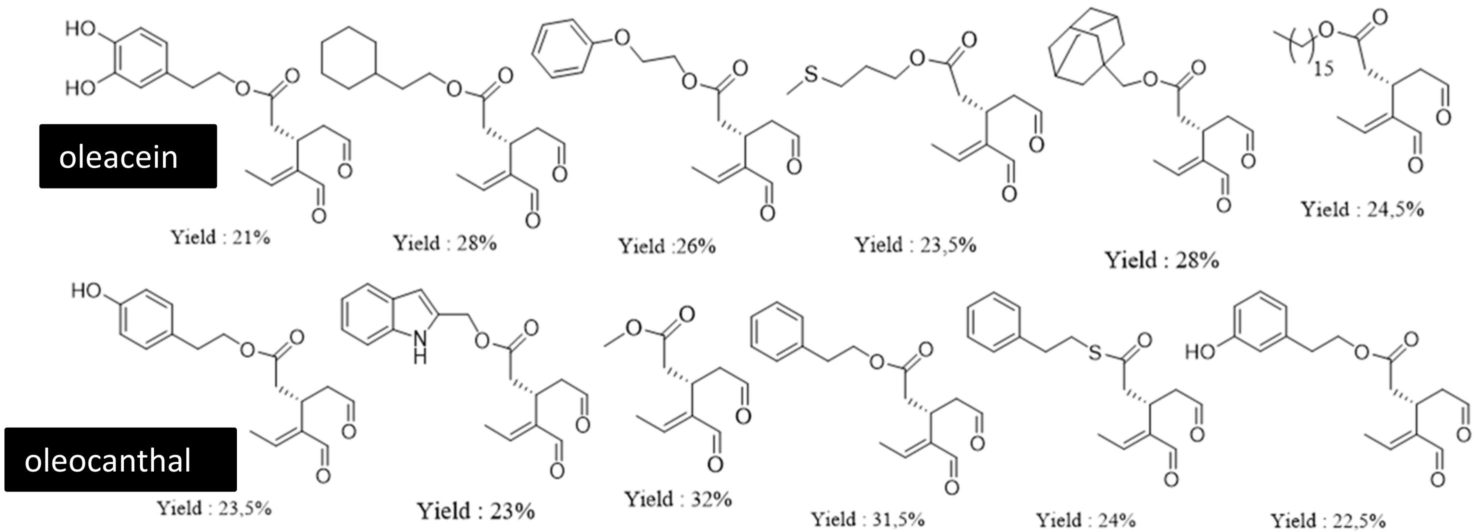


7 8 9 10-19 34-43

34 : R = methyl , X = O
35 : R = cyclohexylethyl, X = O
36 : R = adamantanemethyl, X = O
37 : R = phenylethyl, X = O
38 : R = phenoxyethyl, X = O
39 : R = (1H-Indole-2)-methyl , X = O
40 : R = hexadecanyl, X = O
41 : R = phenethyl, X = S
42 : R = 3-(methylthio)-1-propyl, X = O
43 : R = 3-hydroxyphenethyl, X = O

a) pyridine, acetic anhydride, RT, 3 hours, b) Et_3N, 4-DMAP, R= alkyl, aryl, X= O, S, DCM, RT, 3h, c) Et_2NH, MeOH, RT, 6 hours d) ***β*-glucosidase, CH_3COOH/CH_3COONa, pH 5, 37 °C, 3 h.**

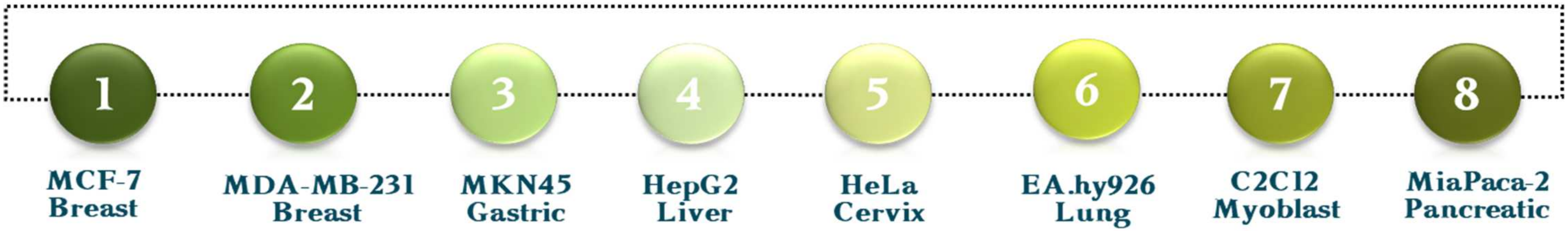
10 g of dialdehyde analogues can be obtained from 10 kg of olive leaves



Treatment with *β*-glucosidase → Sugar hydrolysis and ring opening by spontaneous decarboxylation and formation of dialdehyde

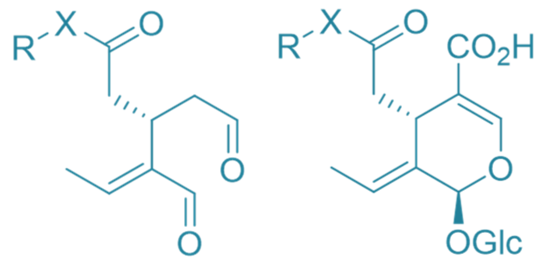
Sarikaki, Georgia, et al. "Biomimetic synthesis of oleocanthal, oleacein, and their analogues starting from oleuropein, a major compound of olive leaves." *Journal of Natural Products* 83.6 (2020): 1735-1739.

In vitro cytotoxicity evaluation of dialdehyde analogs



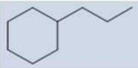
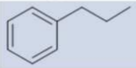
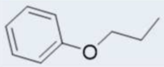
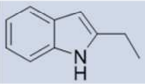
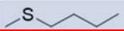
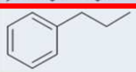
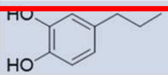
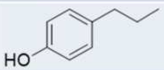
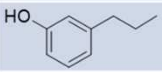
Department of Biochemistry and Biotechnology, University of Thessaly Greece, **prof. D. Kouretas group**

Attica Science Ltd, UK & USA, **prof. D. Iliopoulos group**



XTT assay on hormone-dependent & hormone-independent cell lines

In vitro cytotoxicity of dialdehyde analogues

No	R	X	MCF-7	MDA-MB -231	MKN45	HepG2	HeLa	EA.hy926	C2C12	MiaPaca-2
(3)	Oleuropein		>50	>50	>50	>50	>50	>50	>50	>50
(34)		O	>50	>50	>50	>50	40.87	>50	>50	>50
(35)		O	>50	>50	>50	41.12	49.31	43.81	>50	>50
(36)		O	16.9	>50	>50	>50	>50	>50	>50	>50
(37)		O	21.76	38.7	>50	>50	39.23	>50	45.24	>50
(38)		O	38.88	38.52	>50	45.3	37.33	>50	>50	>50
(39)		O	18.24	>50	>50	>50	>50	>50	>50	>50
(40)		O	4.17	44.91	>50	>50	>50	>50	>50	17.37
(42)		O	>50	43.43	15.97	28.41	27.69	35.19	14.23	5.67
(41)		S	3.57	5	>50	>50	>50	>50	>50	2.28
(43)		O	>50	>50	>50	>50	>50	>50	>50	>50
(44)		O	>50	41.21	>50	>50	30.29	>50	46.68	>50
(45)		O	>50	44.78	38.07	44.59	46	>50	44.09	>50

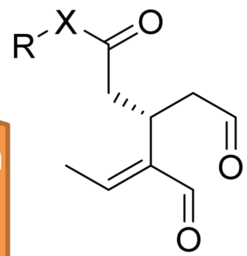
MiaPaca-2 (Pancreatic) cell line

The presence of sulfur:

- Phenylethyl thioyl (**41**) and 3-methylthio-1-propanyl (**42**),

Phenylethyl thioyl (41) good cytotoxicity in MCF-7 and MDA-MB-231 breast cell lines

Sulfur increases antiproliferative activity in cancer cell lines



Mechanism of Action of 41 on a panel of kinase and metabolic cancer pathways

p70-S6 (T389) kinase has essential roles in cancer, obesity, diabetes, and aging.
- phenylethylthioyl derivative (**41**) suppressed p70 S6 (T389) expression and thereby it could possibly induce autophagy of cancer cells

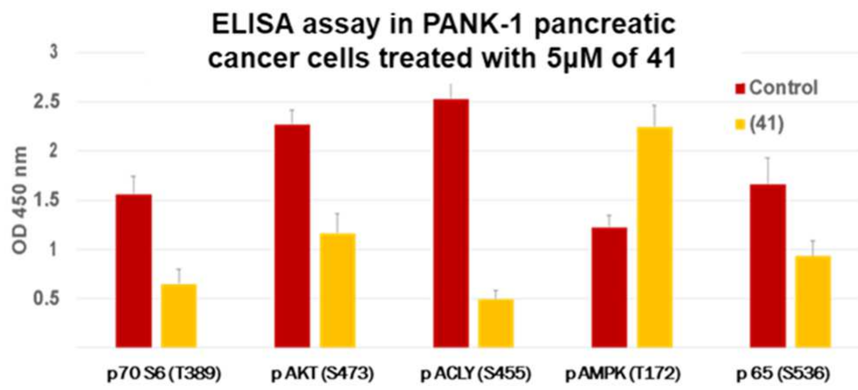
p AKT (S473) kinase plays a role in pancreatic and other types of cancer.
- phenylethylthioyl derivative (**41**) downregulated p AKT, and we could hypothesize an anticancer potential of the latter

p AMPK (T172) kinase could be utilized as a key target for the treatment of cancer.
- The dialdehyde derivative (**41**) led to increased p AMPK activation and could be utilized as a potent activator of AMPK

p 65 (S536) kinase could be utilized as a key target for the treatment of cancer.
- In response to the compound (**41**) treatment of pancreatic cells, the phosphorylation of p65 at S536 was decreased

p ACLY could be utilized as a key target for the treatment of cancer.
- phenylethylthioyl derivative (41) suppressed p ACLY (S455) expression

These data suggests that 41 is affecting key metabolic pathways, suppressing ACLY enzymatic activity



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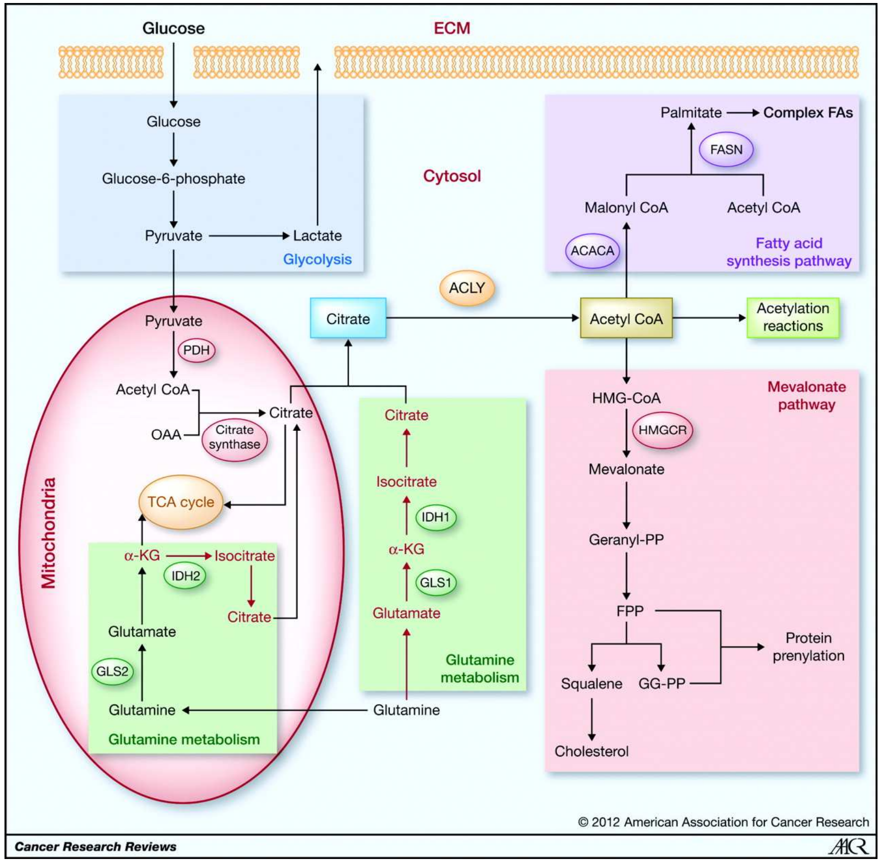
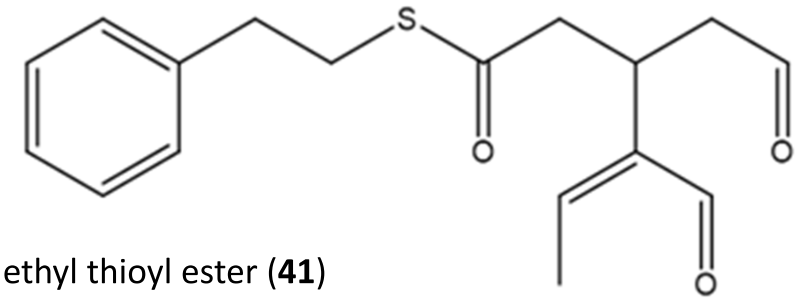
Role of ATP citrate lyase (ACLY)





Phenylethyl thioyl ester (**41**)

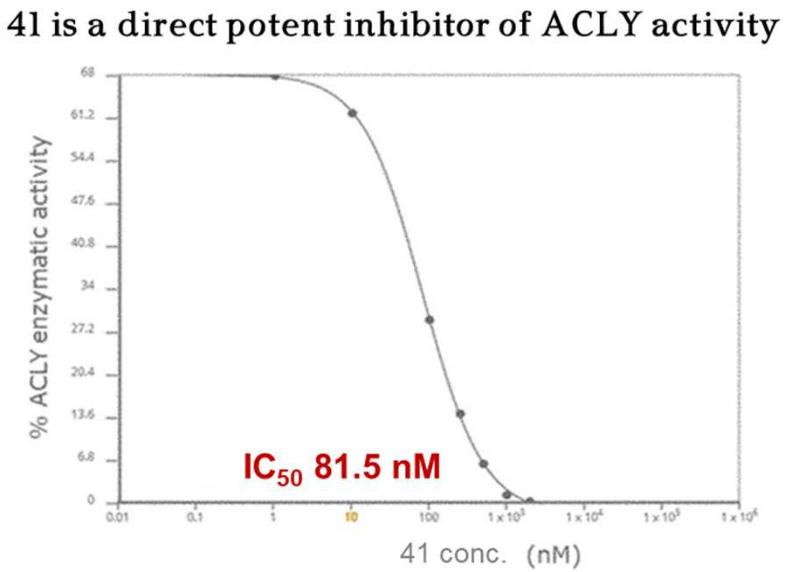
- **ACLY is an important enzyme involved in lipid biogenesis linked with glucose metabolism.**
- **This enzyme plays a significant role in cancer cell proliferation and progression**

- In many cancer types such as pancreatic cancer, colorectal cancer, breast cancer, non-small cell lung cancer, hepatocellular carcinoma etc., the level of ACLY has been found to be quite high as compared to normal cells

41 is a direct inhibitor ACLY enzymatic activity

- ✓ **41** is potent ACLY inhibitor with an **IC_{50} of 81.5 nM,** whilst Oleuropein and Oleocanthal are inactive (IC_{50} > 10 µM).

- ✓ An ACLY assay kit (BPS Bioscience) was used to evaluate **41** against ACLY enzymatic activity (Concentrations used: 1, 10, 100, 250, 500, 1000 and 2000 nM)



BMS303141 a well known synthetic commercialized ACLY inhibitor IC_{50} is 130 nM

Granchi, Carlotta. "ATP citrate lyase (ACLY) inhibitors: An anti-cancer strategy at the crossroads of glucose and lipid metabolism." *European journal of medicinal chemistry* 157 (2018): 1276-1291.

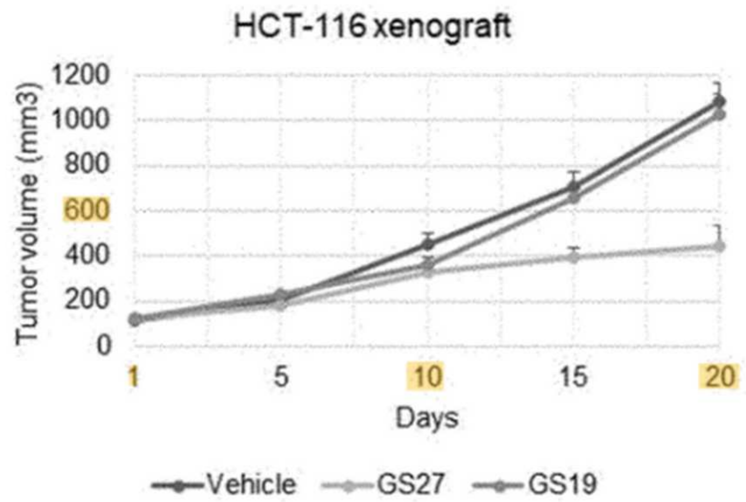
In vivo studies of 41 (GS27) – HCT-116

- The effect of 41 on **colon cancer tumor** growth in mice was evaluated in a **HCT-116 (human colorectal cancer cell line with a RAS proto-oncogene mutation)** xenograft mouse model.

41 treatment showed a 60% reduction of the tumor size

HCT-116 colon cancer tumor growth in mice

GS27 suppresses HCT-116 colon tumor growth in xenograph mice relative to GS19 which has no effect



- Xenograft tumors were generated by injecting HCT-116 colon cancer cells (5 x 10^6) into the right flank of female nu/nu mice. All mice developed tumors in 10 days with size approximately 100 mm^3. For each experiment, mice were randomly distributed into equal groups (4 mice per group) that were untreated or treated by **intraperitoneal injections** every 5 days with **20 mg/kg** of GS27 or oleocanthal. Tumor volume was evaluated until 20 days post-treatment.

Oleocanthal GS19, showed no in vivo effect

Attica Science Ltd, UK & USA, **prof. D. Iliopoulos group**

In vivo studies of GS27 (41) – HepG2

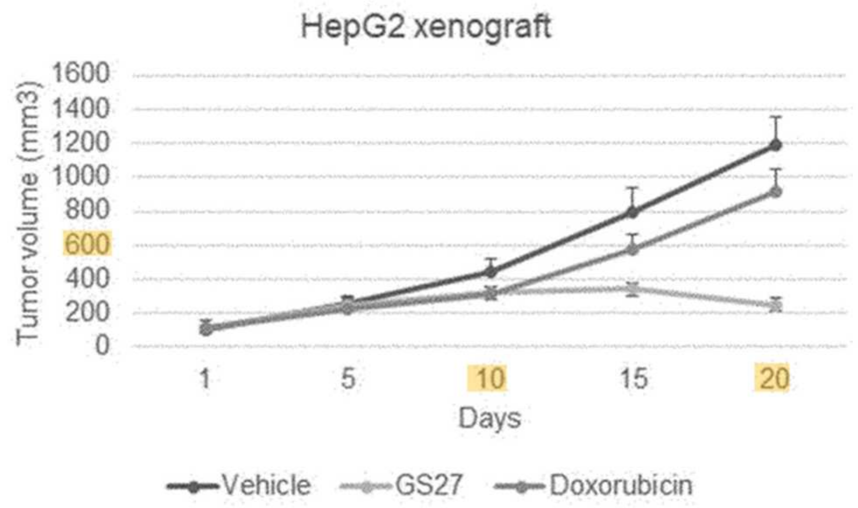
- The effect of GS27 on **liver cancer tumor** growth in mice was evaluated in a **HepG2 xenograft mouse model**.
- Since hepatocellular carcinoma is often treated with doxorubicin, **results were compared to those obtained with doxorubicin.**

41 treatment showed an 80% reduction of the tumor size in comparison to 13% of doxorubicin.

HepG2 liver cancer tumor growth in mice

GS 27 more active than doxorubicin

GS27 suppresses HepG2 liver tumor growth in mice more effectively than doxorubicin



- Xenograft tumors were generated by injecting HepG2 liver cancer cells (2.5 x 106) into the right flank of female nu/nu mice. All mice developed tumors in 10 days with size approximately 100 mm3. For each experiment, mice were randomly distributed into equal groups (4 mice per group) that were untreated or treated **by intraperitoneal injections** every 5 days with **20 mg/kg** of GS27 or doxorubicin. Tumor volume was evaluated until 20 days post-treatment.

In vivo studies of GS27 (41) – AsPC-1

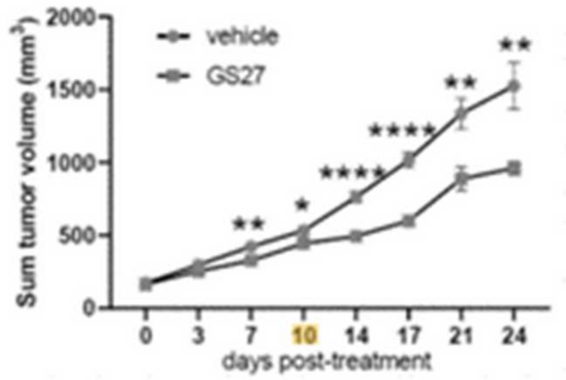
❖ The effect of GS27 on **pancreatic cancer tumor** growth in mice was evaluated in a **AsPC-1 xenograft mouse** model.

41 treatment showed a 34% reduction of the tumor size

AsPC-1 pancreatic cancer tumor in mice

❖ Xenograft tumors were generated by injecting AsPC-1 pancreatic cancer cells (0.5 x 106) into the right flank of male NOD/SCID mice. All mice developed tumors in 10 days with size approximately 100 mm3. For each experiment, mice were randomly distributed into equal groups (5 mice per group) that were untreated or treated by **intraperitoneal injections** every 5 days with **100 mg/kg** of GS27. Tumor volume and the weight of the animals were evaluated until 24 days post-treatment.

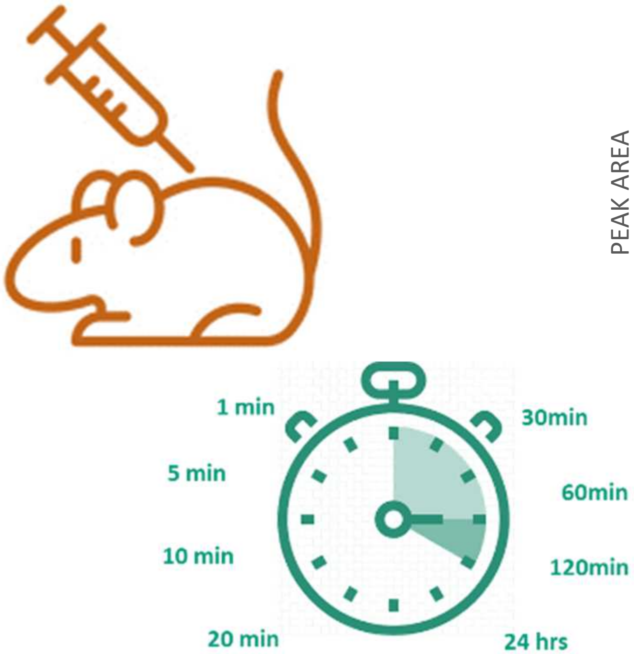
GS27 inhibits AsPC-1 pancreatic cancer tumor growth in mice

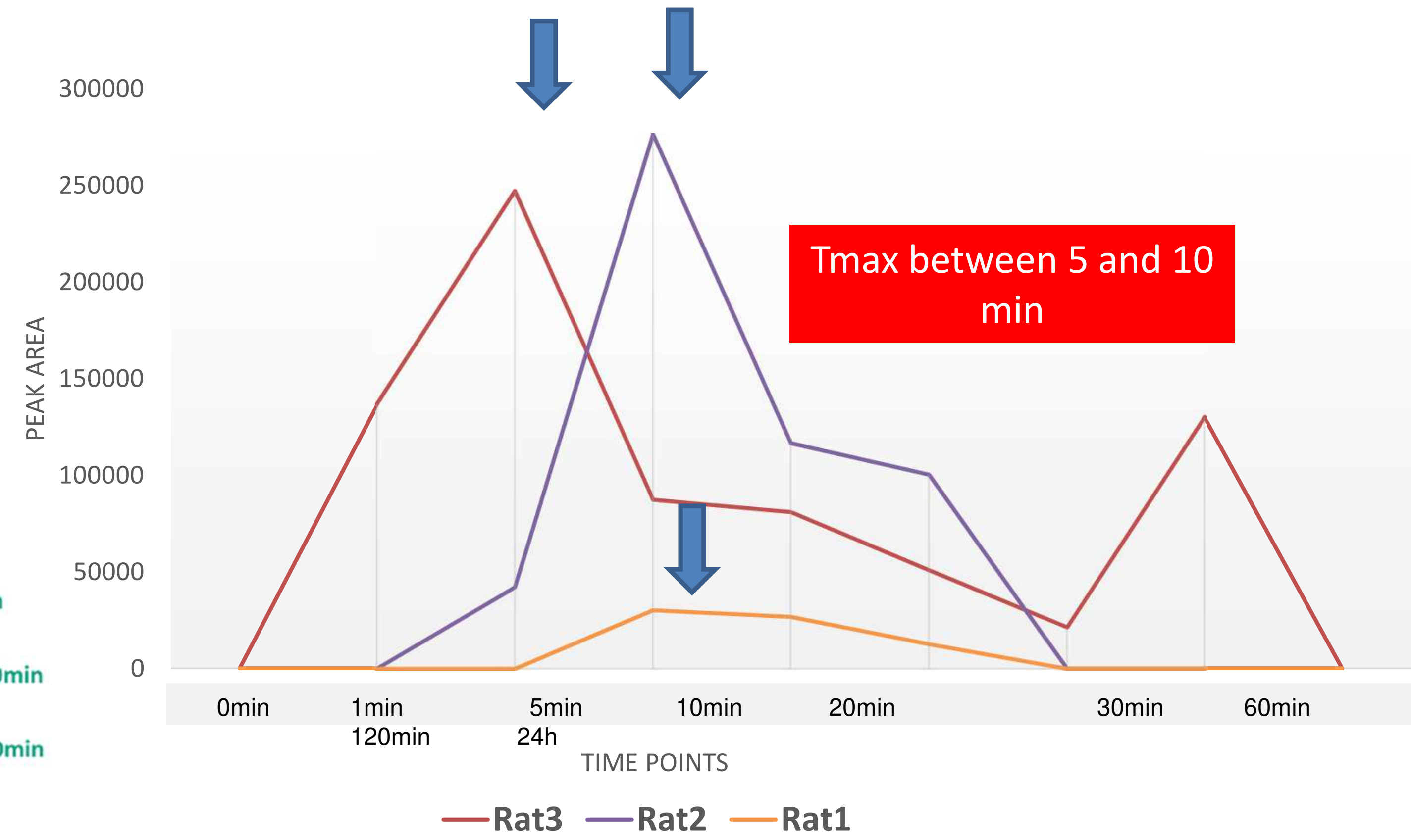


*p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001 vs vehicle

Monitoring of (41) in rat plasma samples by LC-HRMS/MS

3 male rats (Wistar) between 240-260 grams
100mg/kg of body weight GS27



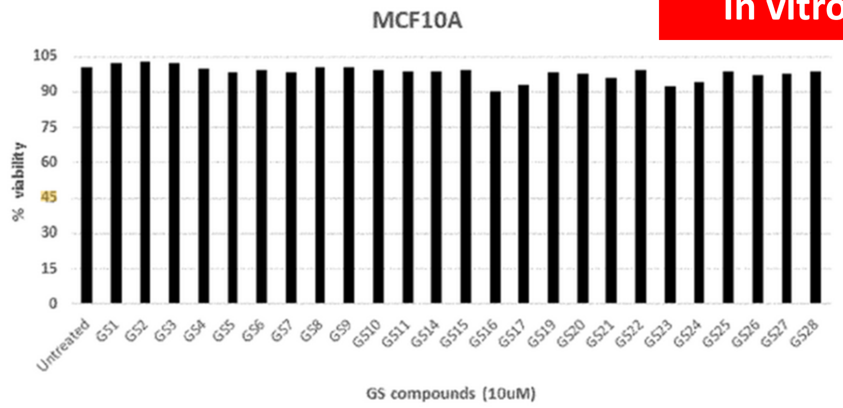


Safety studies of GS27 (41)

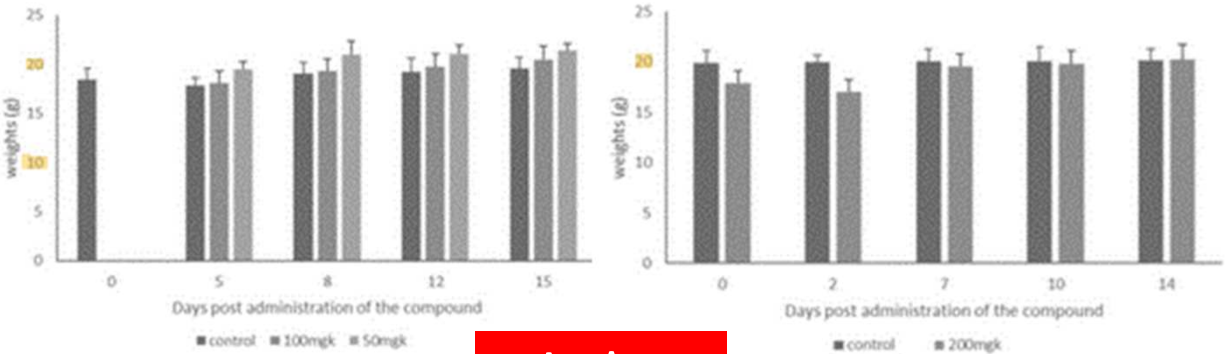
- A non-tumorigenic epithelial cell line (MCF-10A) was used as control to evaluate the effect of GS compounds on “normal” cells and evaluate potential toxicities. All compounds do not show any effect on MCF-10A cell growth (10 µM) except of GS16 which reduced by 10.1% the MCF-10A cell growth

Dialdehyde analogs do not affect the growth of MCF-10A normal epithelial cells

In vitro



GS27 maximum tolerated dose (MTD) studies in rodents

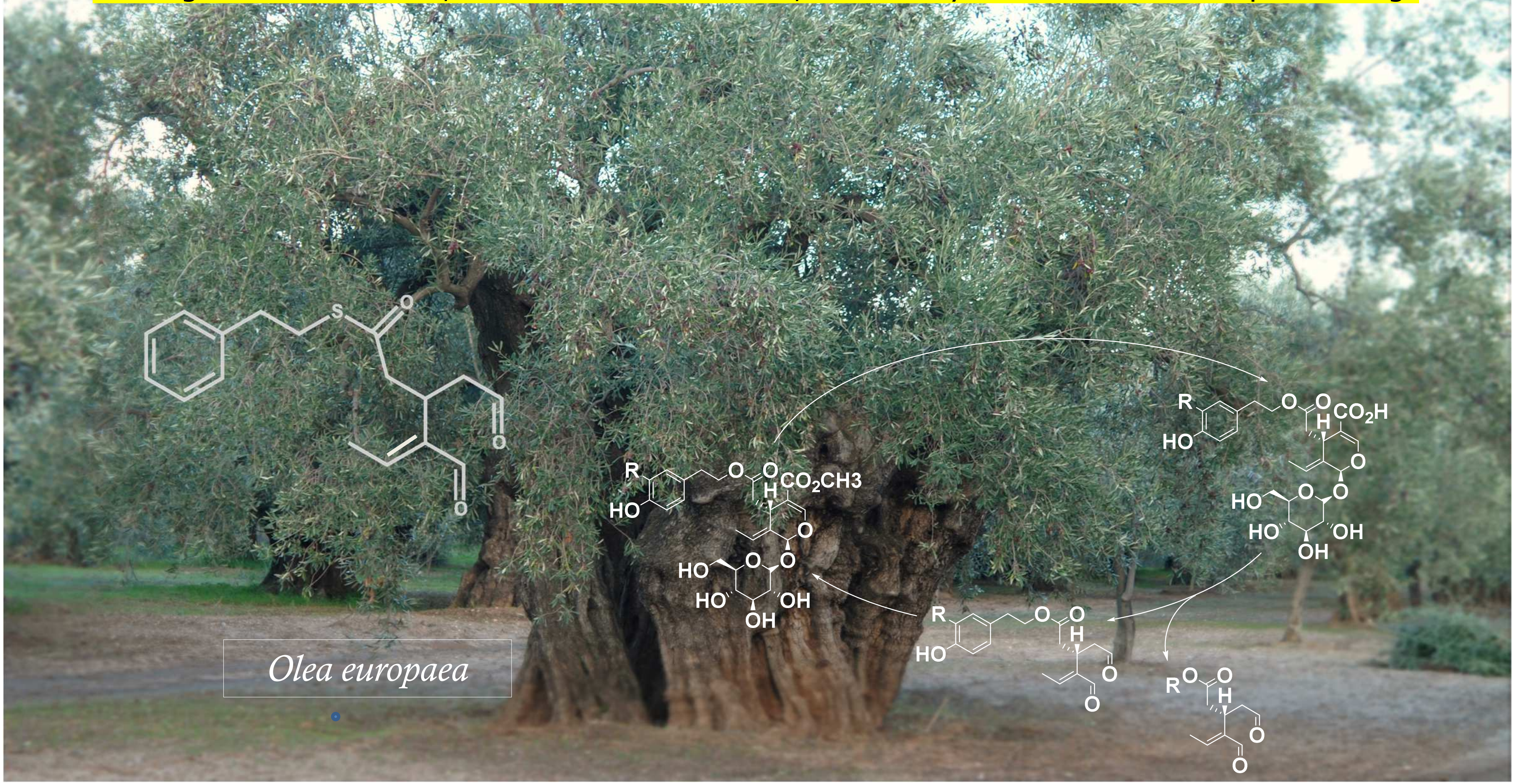


In vivo

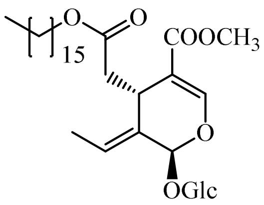
- GS27 Maximum Tolerated Dose (MTD) studies in male/female mice
- MTD Studies shows that GS27 is well tolerated up to 100mg/kg for two weeks.
- There were no changes in animal weight for the study period or neurological changes.
- Furthermore, there was no toxicity identified in mice treated with 200 mg/kg, thus the MTD is greater than 200 mg/kg

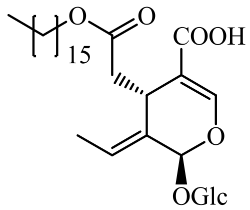
Summarizing….

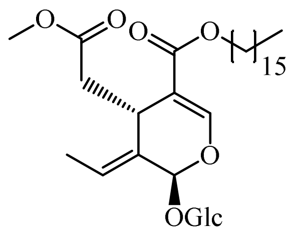
Starting from olive leaves, a biorenewable material, we have synthesized 105 Oleuropein analogs

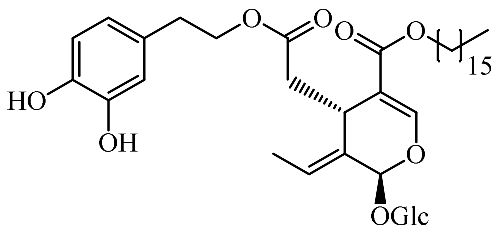


S.A.R

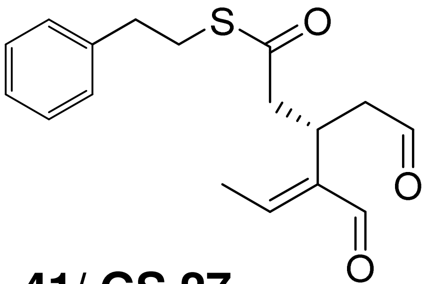








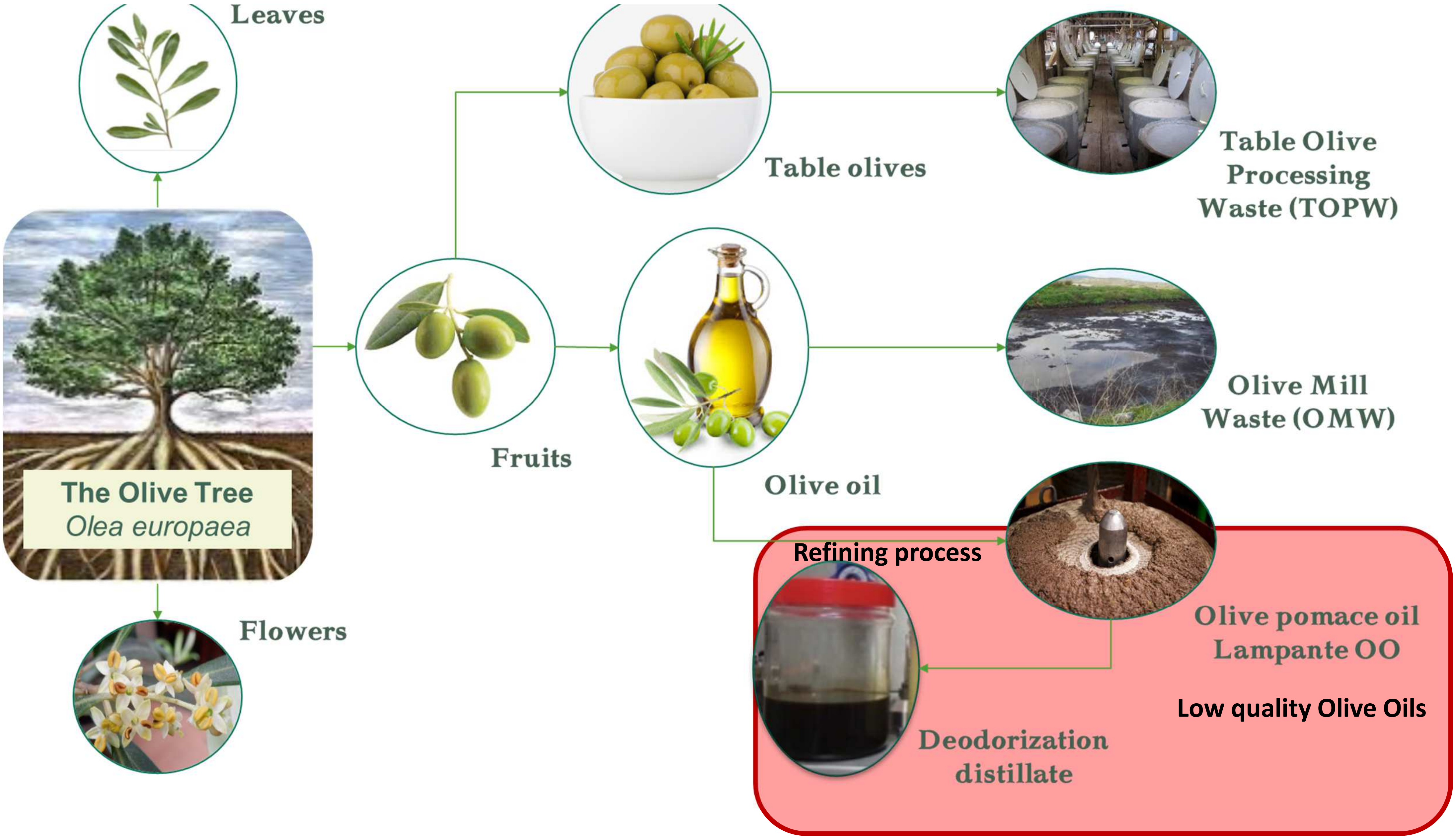
The presence of palmitic ester group increase the activity, in vitro & in vivo



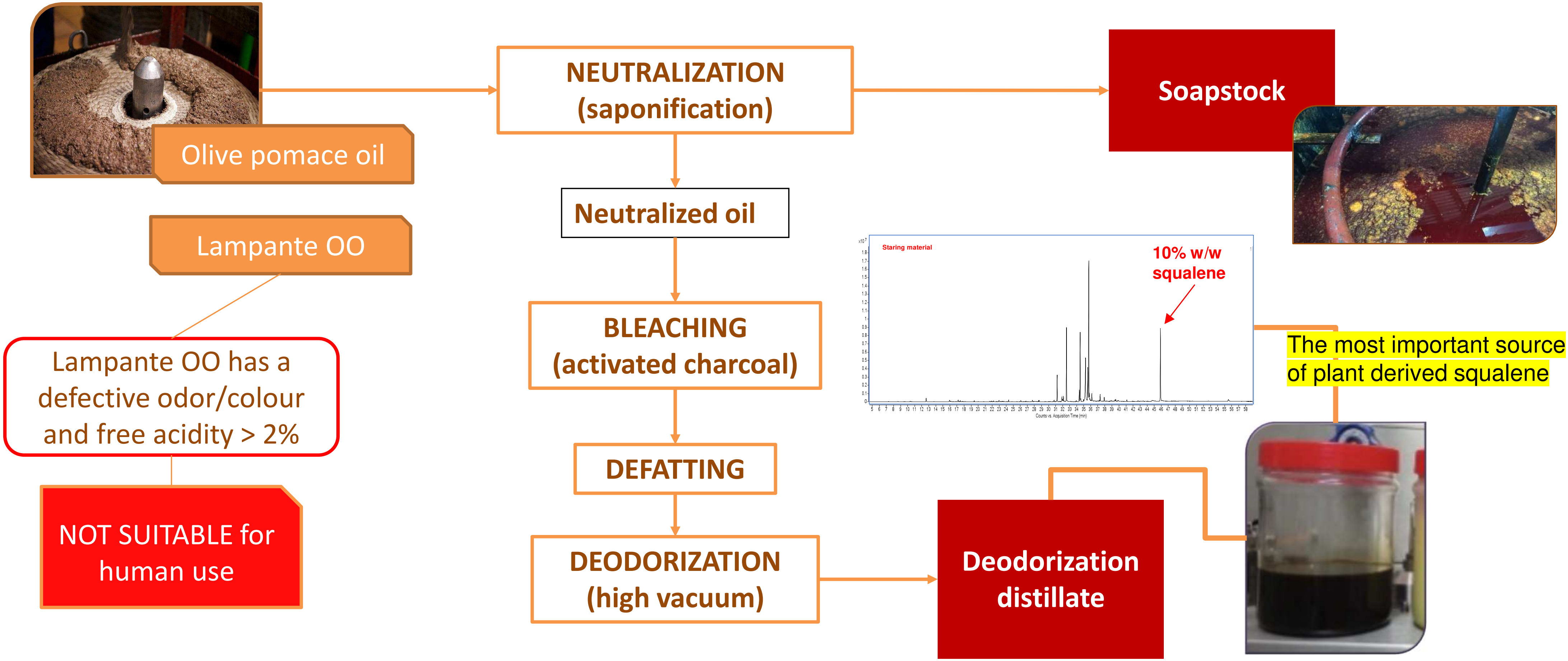
41/ GS 27

Phenylethyl thioyl ester

Potential for further development on clinical studies
Highly potent in vitro, in vivo
Non toxic
Defined mechanism of action in a specific target
Feasible synthesis 42% total yield from oleoside
1 Kg from 100 Kg olive leaves

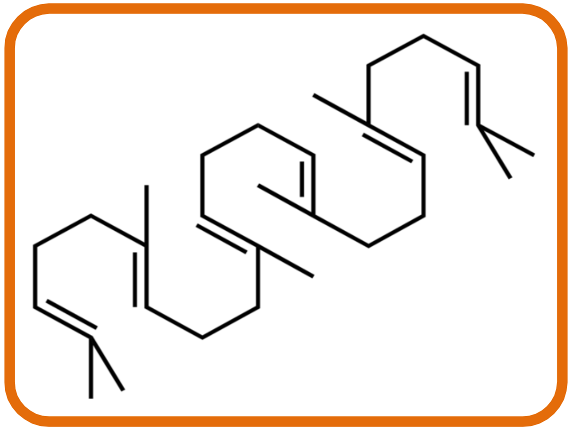

Leaves
Table olives
Table Olive Processing Waste (TOPW)
The Olive Tree
Olea europaea
Fruits
Olive oil
Olive Mill Waste (OMW)
Refining process
Olive pomace oil
Lampante OO
Low quality Olive Oils
Deodorization distillate
Flowers

Refining procedure of low quality OO



Uses and importance of squalene

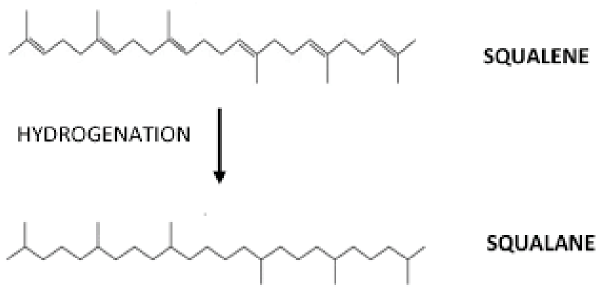
- **Squalene,** is a polyunsaturated triterpenoid
- Antioxidant activity and skin protective properties
- Used in sunscreen, eye makeup, lipstick, and many other cosmetic products



Pure squalene is also used as **adjuvant** in **common flu vaccines** containing viral proteins or parts of the virus

Five of the dozens of COVID-19 vaccines under development use squalene

When squalene adjuvants are added, they help stabilize the protein or virus so the vaccine can be stored and shipped without freezing.



Animal vs. plant derived squalene

- Squalene was originally obtained from shark liver oil
- The global demand for shark liver oil in 2012 was estimated at 2,200 tons
- **3,000 sharks are needed to produce 1 ton of squalene**
- **2 million sharks** are captured and killed for their livers
- The cosmetic industry has started to shift toward **plant-based squalane in the last decade**

High demand of high purity squalene from cosmetic/pharmaceutical industry

Unilever , L'Oréal, Beiersdorf, LVMH, Henkel, Boots, Clarins, Sisley and La Mer made a commitment to **replace shark squalane in their products with plant squalane.**

https://axiologybeauty.com/blogs/our-blog/everything-you-need-to-know-about-one-of-the-cosmetic-industrys-deadliest-ingredients-squalene

On the hunt for alternatives to shark squalene for vaccines

Advocates want vaccine makers to make adjuvants from plant sources instead

by **Melody M. Bomgardner**

December 6, 2020 | A version of this story appeared in **Volume 98, Issue 47**

c&en
CHEMICAL & ENGINEERING NEWS

Sharks use it to stay buoyant. Cosmetics makers use it to soften skin. But in the time of COVID-19, squalene's use in vaccine formulations is what's bringing attention—and some **controversy**—to this natural lipid.

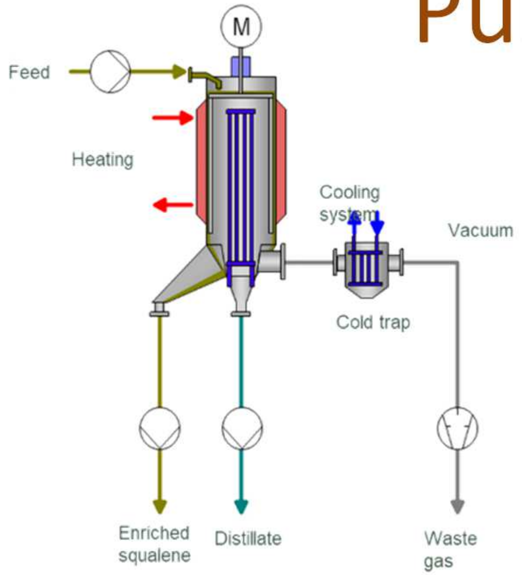
Squalene plays a powerful role in some of the additives, called adjuvants, that boost the body's immune response to a vaccine's active ingredient. And the most concentrated source of high-purity squalene is the livers of sharks, particularly in shark species that live in deep water.

The global trade in pharmaceutical-grade squalene, as for shark parts like fins, relies on sharks caught either on purpose or accidentally with other fish. Most squalene is used to make squalane for cosmetics; demand from the pharmaceutical industry is not known but is thought to be much smaller.

Still, marine conservationists, suppliers of squalene from nonshark sources, and some scientists say vaccine adjuvant makers including GlaxoSmithKline, Merck KGaA, and Novartis should take steps to move to nonshark sources of squalene. "If you care about the ocean, you have to care about sharks," says Stefanie Brendl, executive director of the advocacy group Shark Allies.

https://cen.acs.org/pharmaceuticals/vaccines/hunt-alternatives-shark-squalene-vaccines/98/i47

Purification of squalene from OO



Molecular distillation is:

- ✓ **100% totally green technology**
- ✓ **Free of organic solvents**
- ✓ **Fast and easy procedure**

We are working in collaboration with a USA Pharmaceutical company to purify olive-oil squalene to meet pharmaceutical standards by combination of **Molecular distillation** and **FCPC** technologies

The whole procedure takes place in high temperatures and high vacuum in order to make the separation possible.
The wipers rotate at high speeds creating a thin film on the surface of the evaporator and combined with the low vacuums and high temperatures the transition of the liquid to a gas face is achieved.

Squalene enrichment from olive oil refinery side-products

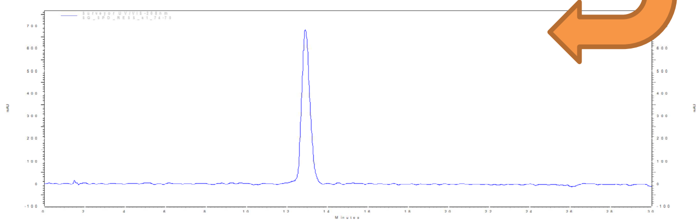
95% purity after FCPC

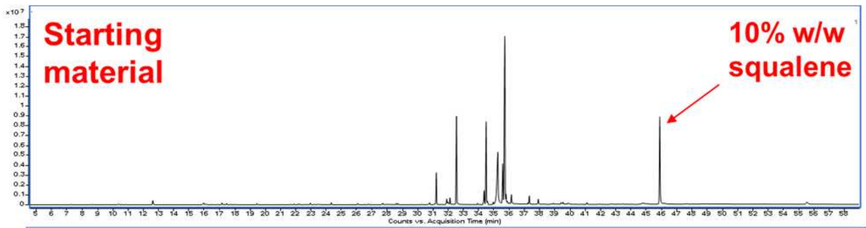
Using a biphasic solvent system consisting of heptane– acetonitrile–butanol (1.8:1.4:0.7, v/v/v)

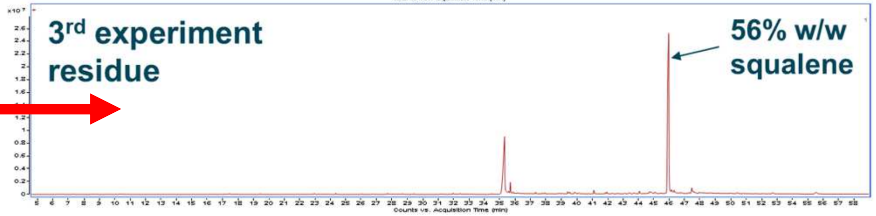
Molecular distillation

Sample	Starting material (2000 gr)	1st experiment residue	2nd experiment distillate	3rd experiment residue
Feed speed	20 Hz	20 Hz	20 Hz	20 Hz
Residue speed	18 Hz	13 Hz	18 Hz	10 Hz
Distillate speed	5 Hz	13 Hz	8 Hz	15 Hz
Rotation speed	350 rpm	350 rpm	350 rpm	350 rpm
Feed temperature	70 °C	70 °C	70 °C	70 °C
Evaporator temperature	130 °C	170 °C	130 °C	190 °C
Residue temperature	70 °C	70 °C	70 °C	70 °C
Cooler temperature	50 °C	50 °C	50 °C	50 °C
Pressure	$5x10^{-2}$ mbar	$1x10^{-3}$ mbar	$5x10^{-2}$ mbar	1 mbar
Residue mass	1347.03 g	671.65 g	373.92 g	180 g
Distillate mass	531.17 g	625.26 g	199.09 g	232.74 g

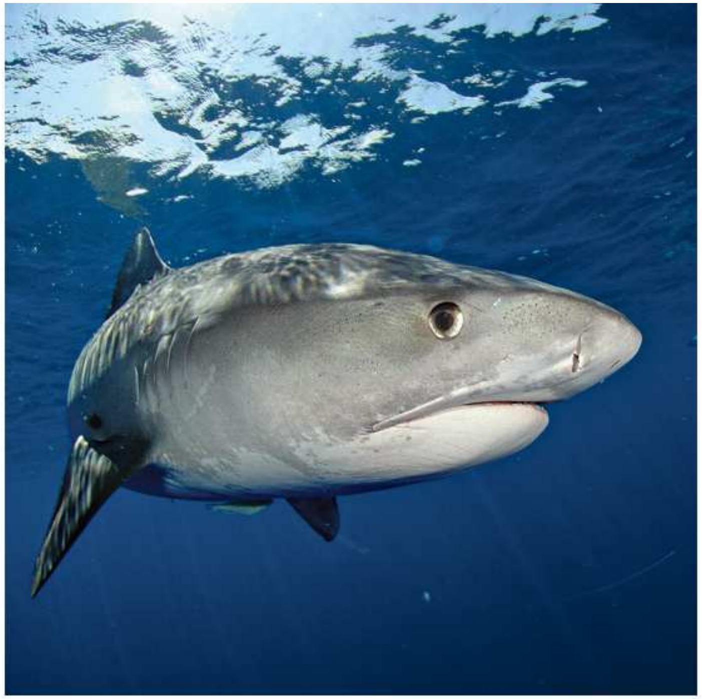
Xynos N. A single-step isolation of squalene from olive oil deodorizer distillates by using centrifugal partition chromatography Separation Science and Technology, 2016, 51, 830-835,





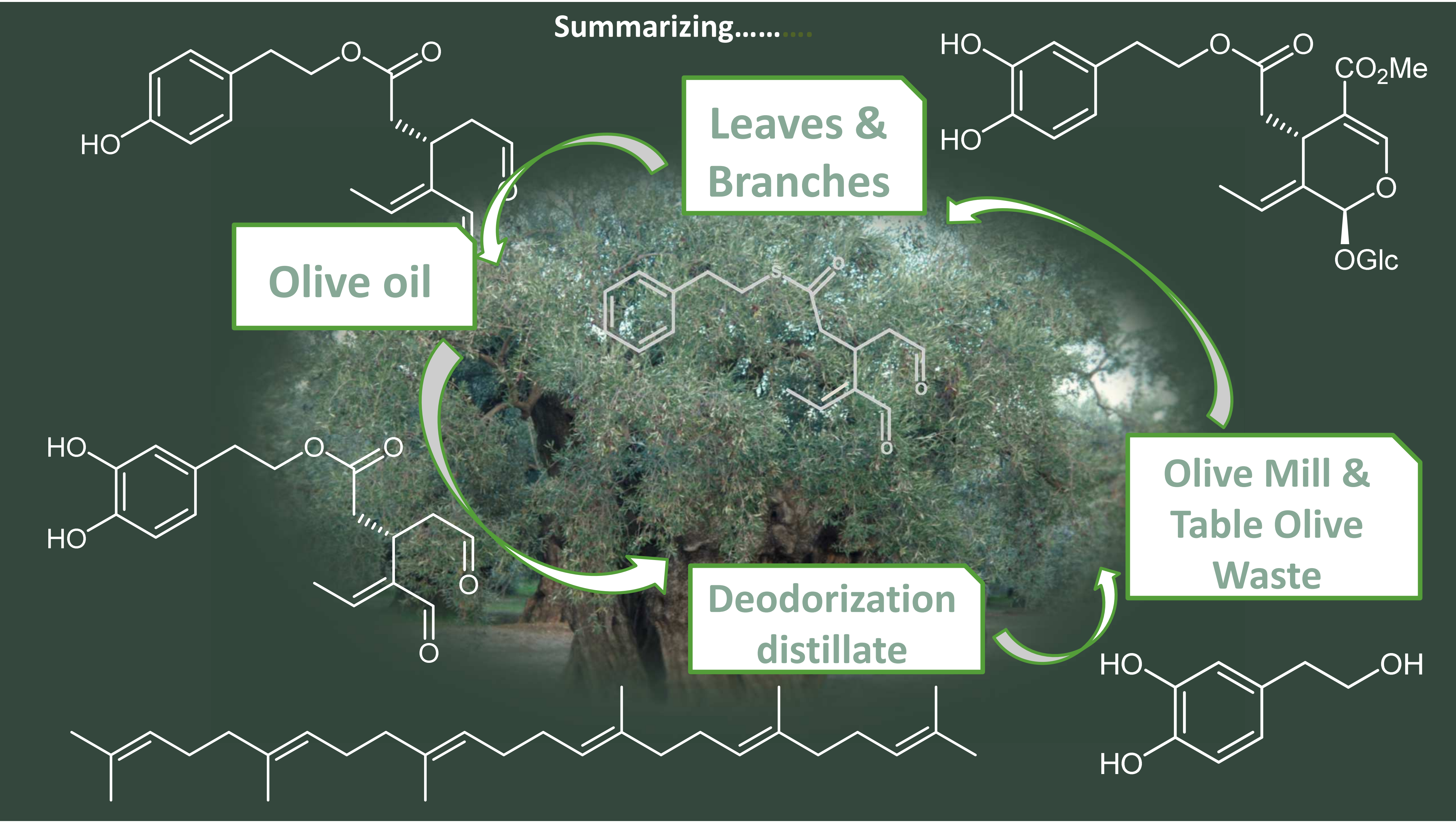


5.6 times enrichment



Credit: Shark Allies

HAPPY SHARKS!!!



Summarizing……
Leaves & Branches
Olive oil
Deodorization distillate
Olive Mill & Table Olive Waste
HO
O
O
HO
HO
CO2Me
O
OGlc
HO
HO
O
O
O
O
HO
OH
HO

BIOLOGICAL EVALUATION OF OLIVE, OLIVE POLYPHENOLS & OLEUROPEIN, OLEOCANTHAL, OLEACEIN and HYDROXYTYROSOL

In vivo cardioprotective activity

In collaboration with:
Department of Pharmacology, School of Pharmacy &
Attikon General Hospital, University of Athens
Prof. I. Andreadou and **Prof. E. Mikros groups**

I. ANDREADOU et al.
Acute administration of the olive constituent, oleuropein, combined with ischemic postconditioning increases myocardial protection by modulating oxidative defense.
Free Radical Biology and Medicine 2021, **166**, 18-32

E. K.ILIODROMITIS et al.
The natural olive constituent oleuropein induces nutritional cardio protection in normal and cholesterol-fed rabbits: Comparison with preconditioning.
Planta Medica, 2015, **81**, 655-663

I. ANDREADOU et al.
Effects of the olive tree leaf constituents on myocardial oxidative damage and atherosclerosis.
Planta Medica, 2015, **81**, 648-654

In collaboration with:

- Human and Animal Physiology, Wageningen University, The Netherlands, **Prof. J. Keijer group**

E. MIKROS et al. Metabonomic identification of novel biomarkers in doxorubicin cardiotoxicity and protective effect of the natural antioxidant oleuropein.
NMR Biomed., 2009, 22, 585-92

E. MIKROS et al. Assessment of the Nutraceutical Effects of Oleuropein and the Cytotoxic Effects of Adriamycin, When Administered Alone and in Combination, in MG-63 Human Osteosarcoma Cells
Nutrients, 2021, 13, 10.3390/nu13020354

J. KEIJER et al.
Nutraceutical oleuropein supplementation prevents high fat diet-induced adiposity in mice.
Journal of Functional Foods, 2015**, 14,** 702-715

D. KREMASTINOS et al.
Cardioprotective effect of a natural olive product, oleuropein, in normal and hypercholesterolemic rabbits.
Journal of Nutrition, 2006, **136**, 2213-2219

Anti-Leishmania activity in vitro and in vivo

In collaboration with:
Hellenic Pasteur Institute, **Dr H. Dotsika group**

E. DOTSIKA et al.
Exploring the Immunotherapeutic Potential of Oleocanthal against Murine Cutaneous Leishmaniasis.
Planta Med 2022, **88**, 783–793

K. KARAMPETSOU et al.
Total Phenolic Fraction (TPF) from Extra Virgin Olive Oil: Induction of apoptotic-like cell death in *Leishmania* spp. promastigotes and *in vivo* potential of therapeutic immunomodulation.
Plos Neglected Tropical Diseases, 2021,

E. DOTSIKA et al.
Evaluation of total phenolic fraction derived from extra virgin olive oil for its antileishmanial activity.
Phytomedicine, 2018, 47, 143-150

J.D. KYRIAZIS et al.
Leishmanicidal activity assessment of olive tree extracts.
Phytomedicine, 2013, 20, 275-281

Treatment of postmenopausal syndrome

In collaboration with:

- Institute of Zoology, Molecular Cell Physiology and Endocrinology, Technical University of Dresden, Germany, **Prof. G. Vollmer group**
- Metabolic Diseases and Micronutrients Unit, INRA Theix, France Prof. **V. Coxam group**
- School of Medicine, University of Athens, **Prof. I. Dontas group**

G. VOLLMER et al.
Impact of a functionalized olive oil extract on the uterus and the bone in a model of postmenopausal osteoporosis.
European Journal of Nutrition, 2014, **53**, 1073-1081

G. VOLLMER et al.
Oleocanthal Modulates Estradiol-Induced Gene Expression Involving Estrogen Receptor
Planta Medica, 2015, **81**, 1263-1269.

V. COXAM et al.
Dose-response study of effect of oleuropein, an olive oil polyphenol, in an ovariectomyl/inflammation experimental model of bone loss in rat.
Clinical Nutrition, 2006, **25**, 859-868

V. COXAM et al.
Major Phenolic Compounds in Olive Oil Modulate Bone Loss in an Ovariectomy/Inflammation Experimental Model.
J. Agric Food Chem., 2008, **56**, 9417-22

I.A. DONTAS et al.
The effect of table olive wastewater extract administration on the adult ovariectomized rat model of osteoporosis.
British Journal of Nutrition, 2021, **126**, 1761–1770

Extraction, separation, isolation

In collaboration with University of Reims **Prof. Renault group**

Pilot continuous centrifugal liquid-liquid extraction of extra virgin olive oil biophenols and gram-scale recovery of pure oleocanthal, oleacein, MFOA, MFLA and hydroxytyrosol
Separation and Purification Technology, 2021, 255, 117692

An Integrated Process for the Recovery of High Added-Value Compounds from Olive Oil using Solid Support Free Liquid-Liquid Extraction and Chromatography Techniques
Journal of Chromatography A, 2017, 1491, 126-136. DOI:10.1016/j.chroma.2017.02.046

In collaboration with GREEN Extraction Team, INRAE, UMR 408, Avignon University **Prof. F. Chemat group**
Higher Yield and Polyphenol Content in Olive Pomace Extracts Using 2-Methyloxolane as Bio-Based Solvent
Foods 2022, 11, 1357

Analytics, metabolomics and metabolism

In collaboration with **Assoc. Prof. M. Halabalaki group**

Availability and Metabolic Fate of Olive Phenolic Alcohols Hydroxytyrosol and Tyrosol in the Human GI Tract Simulated by the In Vitro GIDM–Colon Model.
Metabolites 12, 5, 391

Metabolic Fate of the Secoiridoids Oleacein and Oleocanthal in an In Vitro Continuous Dialysis System with Human Gut Microbiota.
Antioxidants, in press

Effect of Long-Term Hydroxytyrosol Administration on Body Weight, Fat Mass and Urine Metabolomics: A Randomized Double-Blind Prospective Human Study.
Nutrients 14, 7, 1525

From sample preparation to NMR-based metabolic profiling in food commodities: The case of table olives.
Phytochemical Analysis 33 (1), 83-93

Isotopic Traceability (13C and 18O) of Greek Olive Oil.
Molecules 25, 24, 5816

Olive oil quality and authenticity assessment aspects employing FIA-MRMS and LC-Orbitrap MS metabolomic approaches.
Frontiers in public health 8, 558226

NMR-based metabolic profiling of edible olives—Determination of quality parameters.
Molecules 25, 15, 3339

1998-2022: 25 years research on *Olea Europaea*, olive oil and by-products

http://oliverg.pharm.uoa.gr

70 publications
3 book chapters

- **5 international patents**
- **2 commercialized products as chemical/ biological reagents**
- **4 standardized extracts**
- **4 EU projects 11 National projects**
- **15 Masters**
- **9 Phd**

INTERNATIONAL NETWORK OF COLLABORATORS

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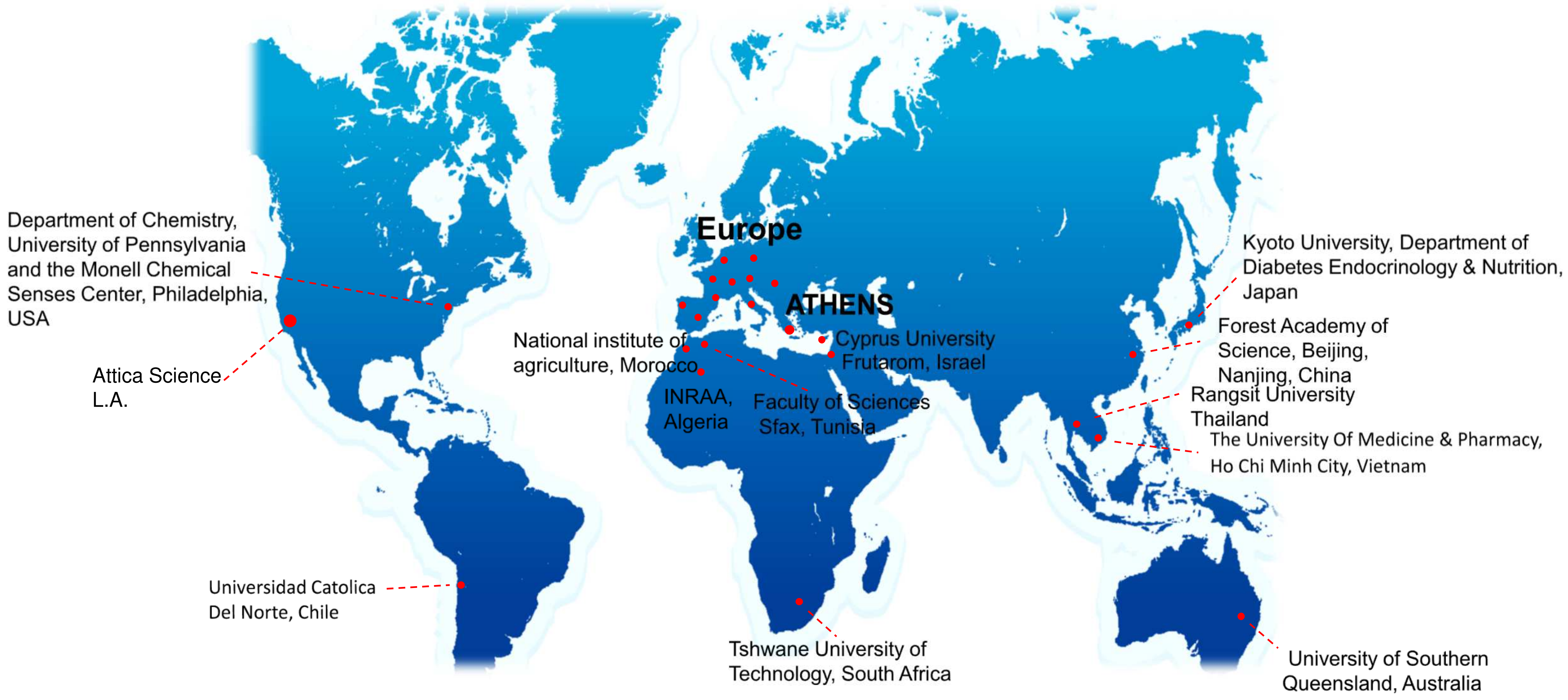


OLITEC (2009-2014)

Funded by the European Union

Bioactive natural compounds extracted and isolated from olive tree using modern technologies: Probing into their therapeutic potential

Coordinator: University of Athens, Prof. A.L. Skaltsounis

- Paris Descartes University – **Prof. S. Michel** (France)

- Frutarom S.A. – **Dr. D. Piscitello** (Switzerland/Israel)
- Hitex S.A. – **Dr. I. Lamour** (France)
- Lavipharm S.A. – **Dr. S. Fotinos** (Greece)

MediHealth (2016-2019)

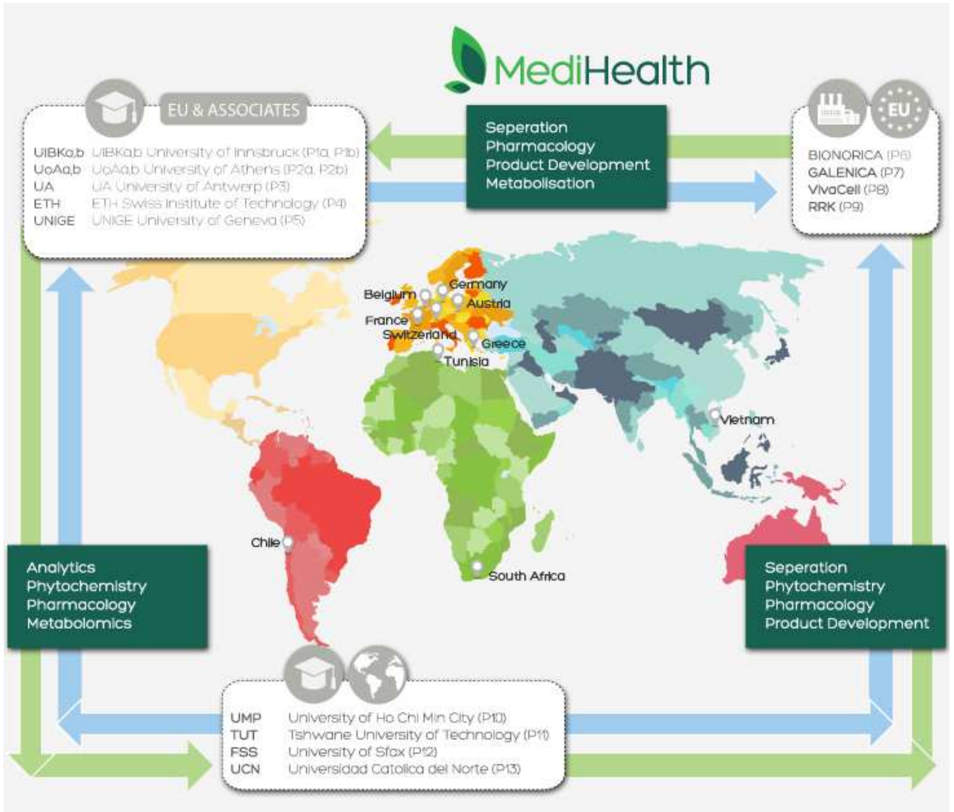
Novel natural products for healthy ageing from Mediterranean diet and food plants of other global sources

Coordinator: University of Innsbruck, Prof. H. Stupnner

- Swiss Federal University of Technology in Zurich – **Prof. C. Wolfrum** (Switzerland)
- University of Geneva – **Prof. J.-L. Wolfender** (Switzerland)
- University of Athens – **Prof. A.L. Skaltsounis** (Greece)
- University of Antwerp – **Prof. L. Pieters** (Belgium)

- Bionorica Research Gmbh – **Dr. D. Intelmann** (Austria)
- Galenica S.A. – **Dr. N. Adamopoulos** (Greece)
- Vivacell Biotechnology Gmbh – **Dr. B. Fiebich** (Germany)
- Rousselet Centrifugation S.A. – **Mr. J. Meucci** (France)

- Tshwane University of Technology – **Prof. A. Viljoen** (South Africa)
- Faculty of Sciences of Sfax – **Prof. N. Allouche** (Tunisia)
- Catholic University of the North – **Prof. V. Kesternich** (Chile)
- The University of Medicine & Pharmacy, Ho Chi Minh City – **Prof. H. Tran** (Vietnam)



Olive-Net (2017-2022)

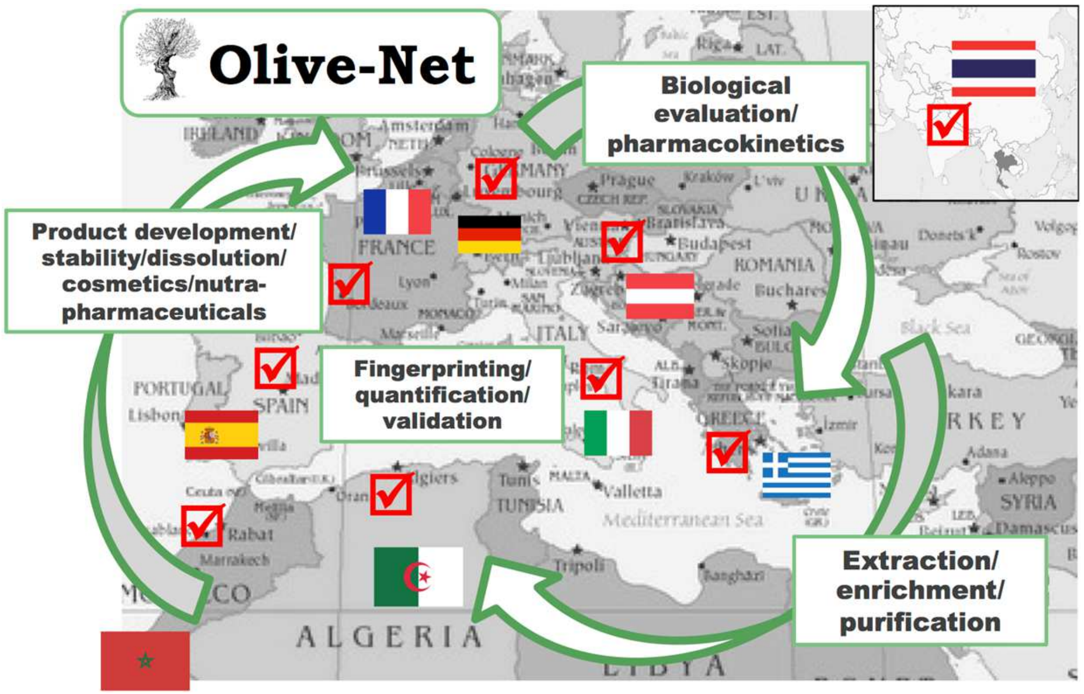
Bioactive compounds from *Olea europaea*: investigation and application in food, cosmetic and pharmaceutical industry

Coordinator: University of Athens, Prof. A.L. Skaltsounis

- French National Centre for Scientific Research – **Prof. E. Lesellier** (France)
- University of Orleans – **Prof. G. Leonard** (France)
- University of Avignon – **Prof. F. Chemat** (France)
- Complutense University of Madrid – **Prof. V. Lahera** (Spain)
- Galician Health Service – **Prof. O. Gualillo** (Spain)
- National Research Council – **Prof. D. Corradini** (Italy)
- Austrian Drug Screening Institute GmbH – **Prof. G Bonn** (Austria)

- PharmaGnose S.A. – **Dr. A. Argyropoulou** (Greece)
- Uni-Pharma Pharmaceutical Laboratories S.A. – **Dr. I. Tsetis** (Greece)
- Natac Biotech SL – **Dr. J. C. Quintela** (Spain)
- FoodQS GmbH - **Prof. S. Schwarzinger** (Germany)
- Neuro-Sys SAS – **Dr. Y. Jaudouin** (France)

- National School of Agriculture - **Prof. N. Ouazzani** (Morocco)
- National Institute of Agriculture Research – **Prof. M. Douzane** (Algeria)
- Rangsit University (OMEBCRC) – **Prof. S. Wongyai** (Thailand)



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http://oliveresearchgroup.com/

Thank you for your attention