OP-012 [Apitherapy]

Assessment In-vivo effect of the binary mixture of Moroccan propolis and Olea europaea leaf extracts on type 2 diabetes induced by fructose and streptozotocin in rats: formulation by design of experiments (DOE)

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[3]3 Laboratory of Medical sciences and translational research, Sidi Mohamed Ben Abdellah University, Fez, Morocco
[4]4 Laboratory of Microbial Biotechnology and Bioactive Molecules, Sidi Mohamed Ben Abdellah University, Fez, Morocco

The current investigation aimed to explore the impact of combining Moroccan propolis (MPME) and Olea europaea leaf (OELME) methanolic extracts of on the dynamic changes of key biochemical markers associated with type 2 diabetes (T2D) inducted in rats with 10 % fructose supplementation combined to the intraperitoneal injection of Streptozotocin (STZ). The study focused on parameters such as fasting blood glucose (FBG), rat's plasma insulin (INS), HOMA-IR index, glycated hemoglobin (HbA1C), and body weight (BW) in a rat model. Additionally, a binary mixture design specifically, a simplex lattice design, was employed to optimize the optimal combination of these extracts, enhancing their anti-diabetic activity by achieving optimal changes in T2D markers. The preliminary phytochemical analysis revealed total phenolic content (TPC) and total flavonoids content (TFC) values of 89.53 - 35.01 mgGAE/g and 31.91- 18.99 mgQE/g for MPME and OELME, respectively. Furthermore, HPLC-DAD analysis identified and quantified 14 phenolic compounds in propolis extract, in which Apigenin (0.342 mg/g), kaempferol (0.310 mg/g) and Epicatechin (0.222 mg/g) being the major quantified compounds. While, Olea europaea leaf contained 6 phenolic compounds, with Oleuropein being the most abundant molecule with a concentration of 29.91 mg/g DW.

The application of the experimental design facilitated the optimization of change rates, resulting in improvements of 68.95%, 148.08%, 30.36%, 23.00%, and 19.24% for FBG, INS, HOMA-IR index, HbA1C, and BW, respectively under optimal mixture setting of 49% of MPME and 51% of OELME.

Using the experimental design methodology for propolis and Olea europaea leaf has proved interesting for formulating mixtures with high biological values enabling a better In-vivo anti-diabetic effect, which represents an efficient and safe alternative for the management of T2D.

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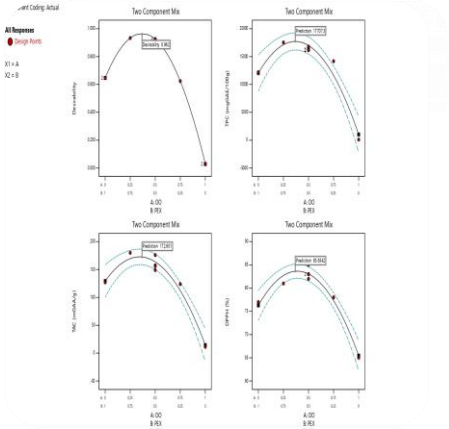
FACULTE DES SCIENCES DHAR EL MEHRAZ FES



" The Assessment *In-vivo* effect of the binary mixture of Moroccan propolis and *Olea europaea* leaf extracts on type 2 diabetes induced by fructose and streptozotocin in rats: formulation by design of experiments (DOE) "

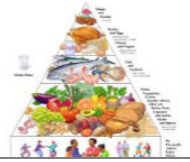
Pr Badiaa Lyoussi

Sidi Mohamed Ben Abdallah University of Fez
Morocco

Food as Medicine ?

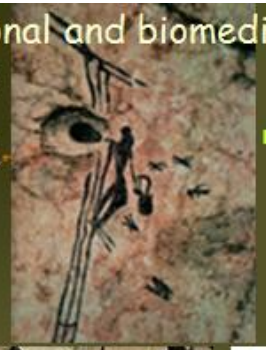
Prevention

is better than

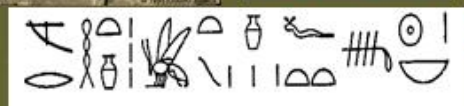


Cure

...traditional and biomedical history of the bee products



- Bee products have been used since 3000 b.c.
  - Ebers Papire
  - Spanish "spider" caves



- "Remedy for stomach ache reduction - honey should be ingested".

HISTORY, TRADITION...

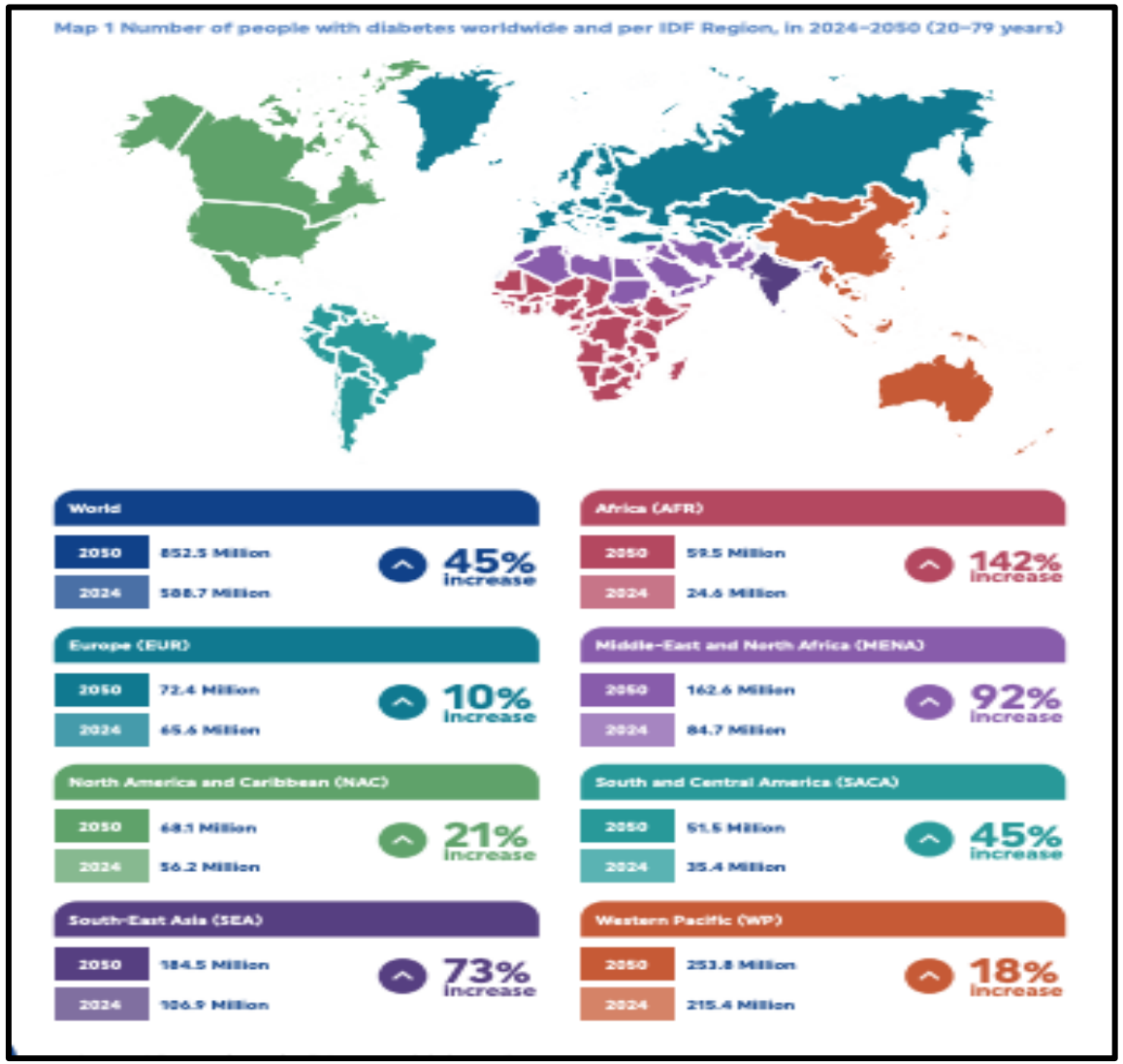
The Holy Quran and sayings of Prophet Muhammad refer to honey as **"a remedy".**

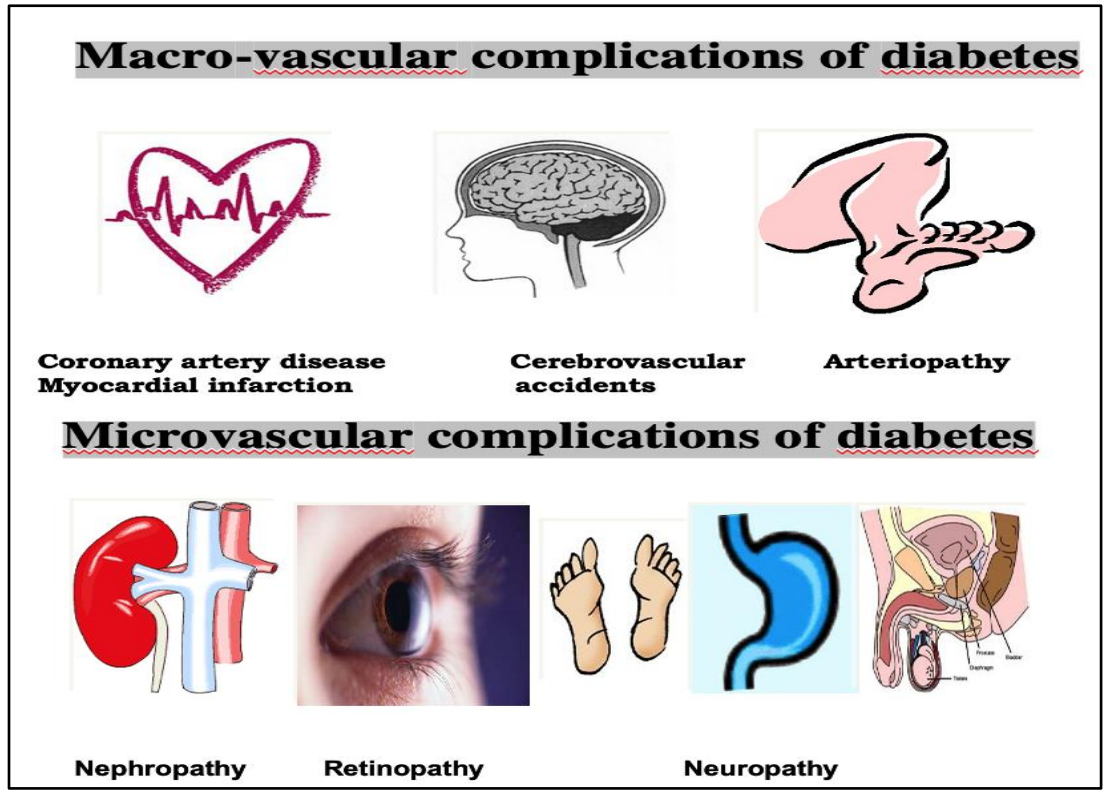
"Al-Qanun book"
A rare manuscript made in Iran in the 15th century

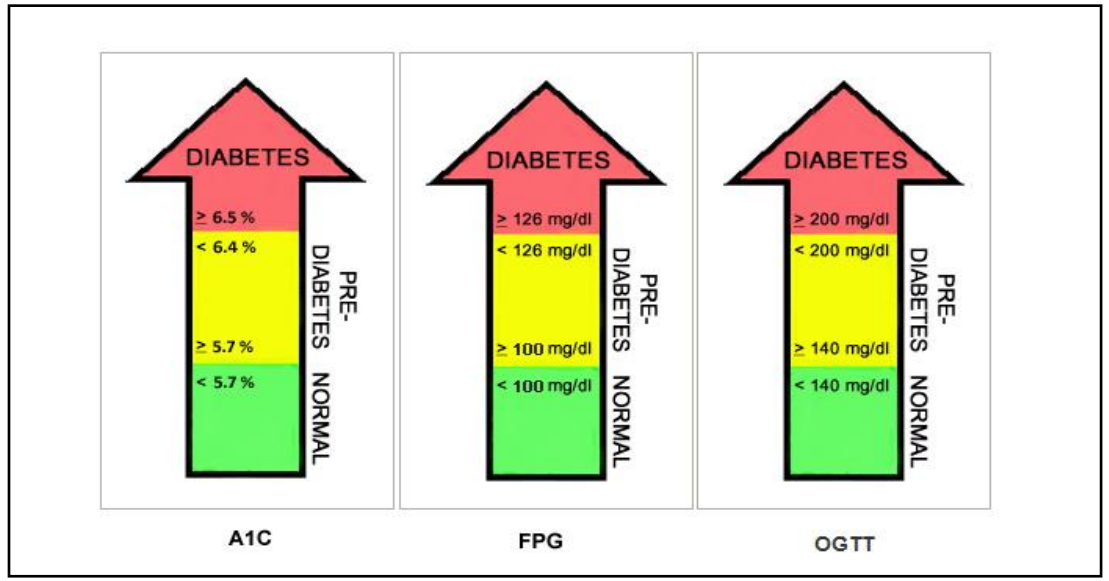
Canon (al-Qanun fi al-Tibb)

The Primary Law of Healing
"Let the food be the medicine"

Diabetes is a major health issue that has reached alarming levels. Diabetes is one of the fastest-growing global health emergencies of the 21st century



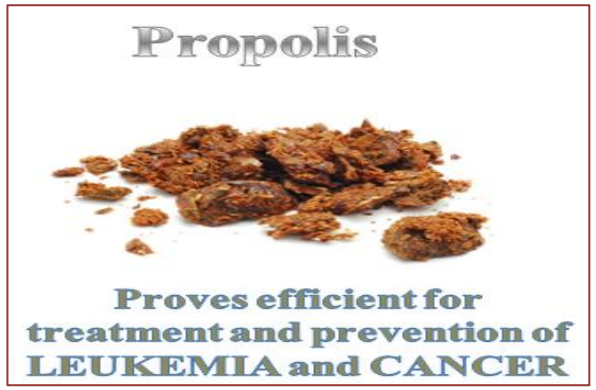




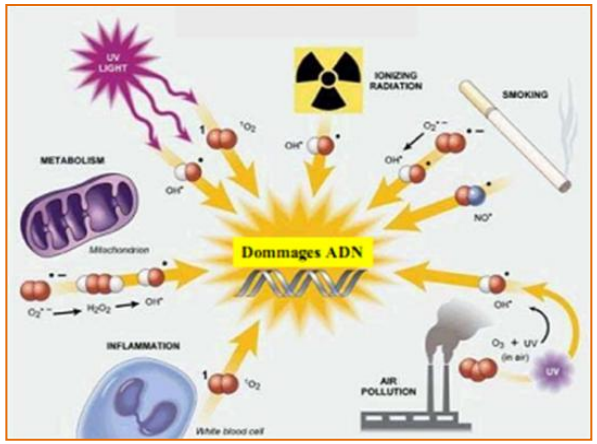
Propolis ...

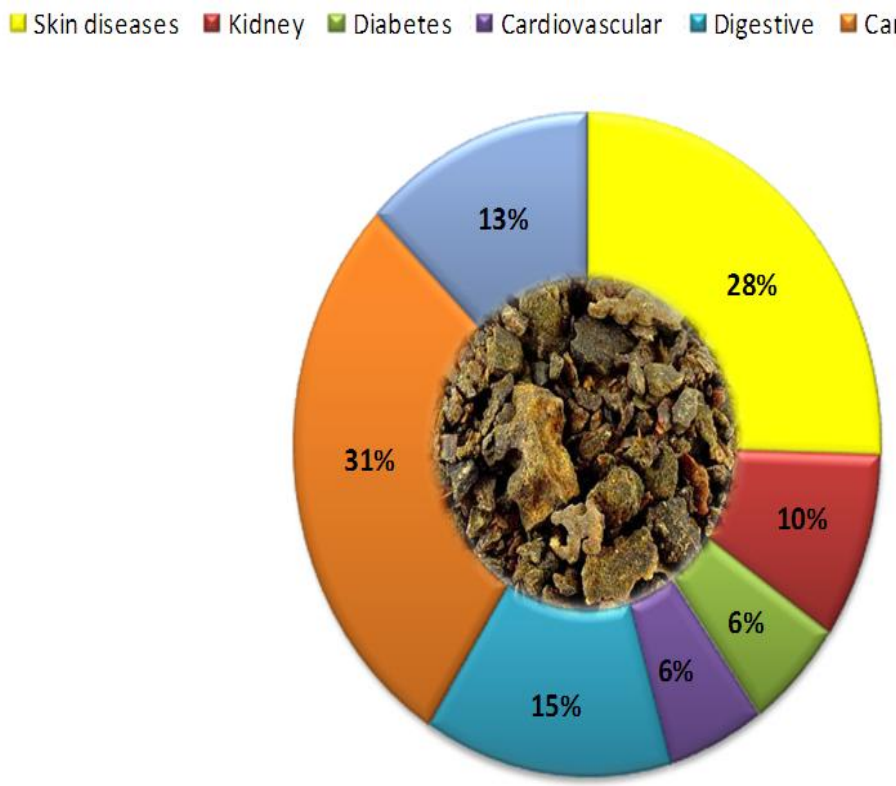
- is the external immune system of the bee
- strengthens the human defences against disease
- is used for detoxification of heavy metals
- protects against toxicity of environmental toxins
- protects cells

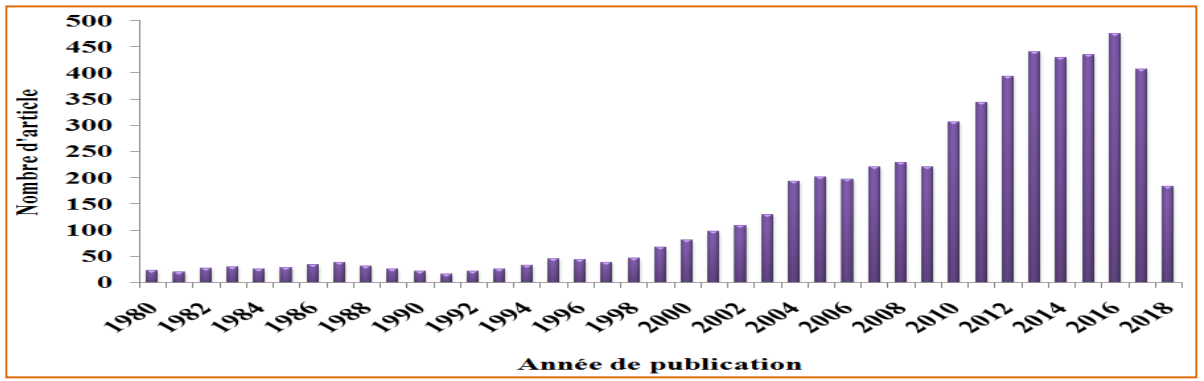
Propolis and pathologies

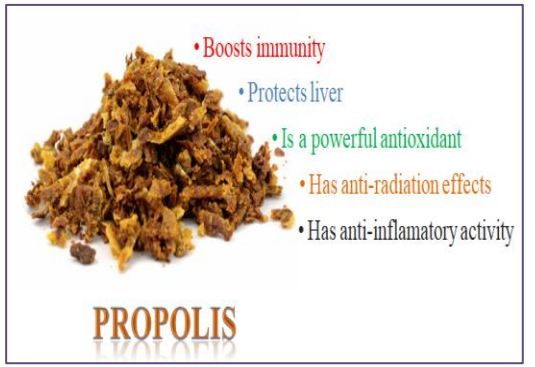


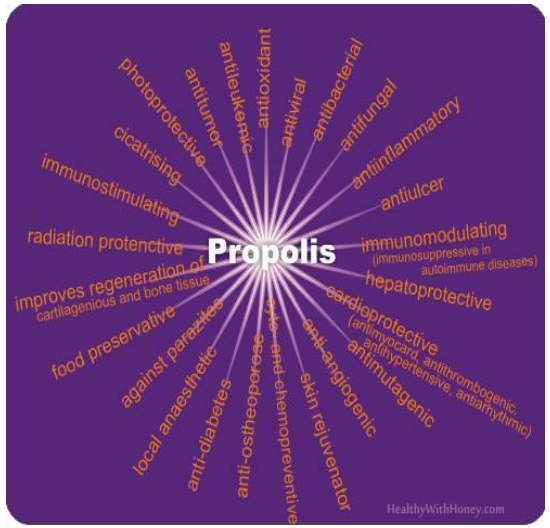
Effect against free radicals
Potent free radical scavenging activity of dicaffeoyl quinic acid derivatives from propolis

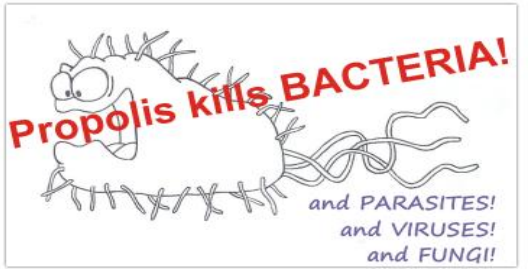












Major bioactive class of compounds

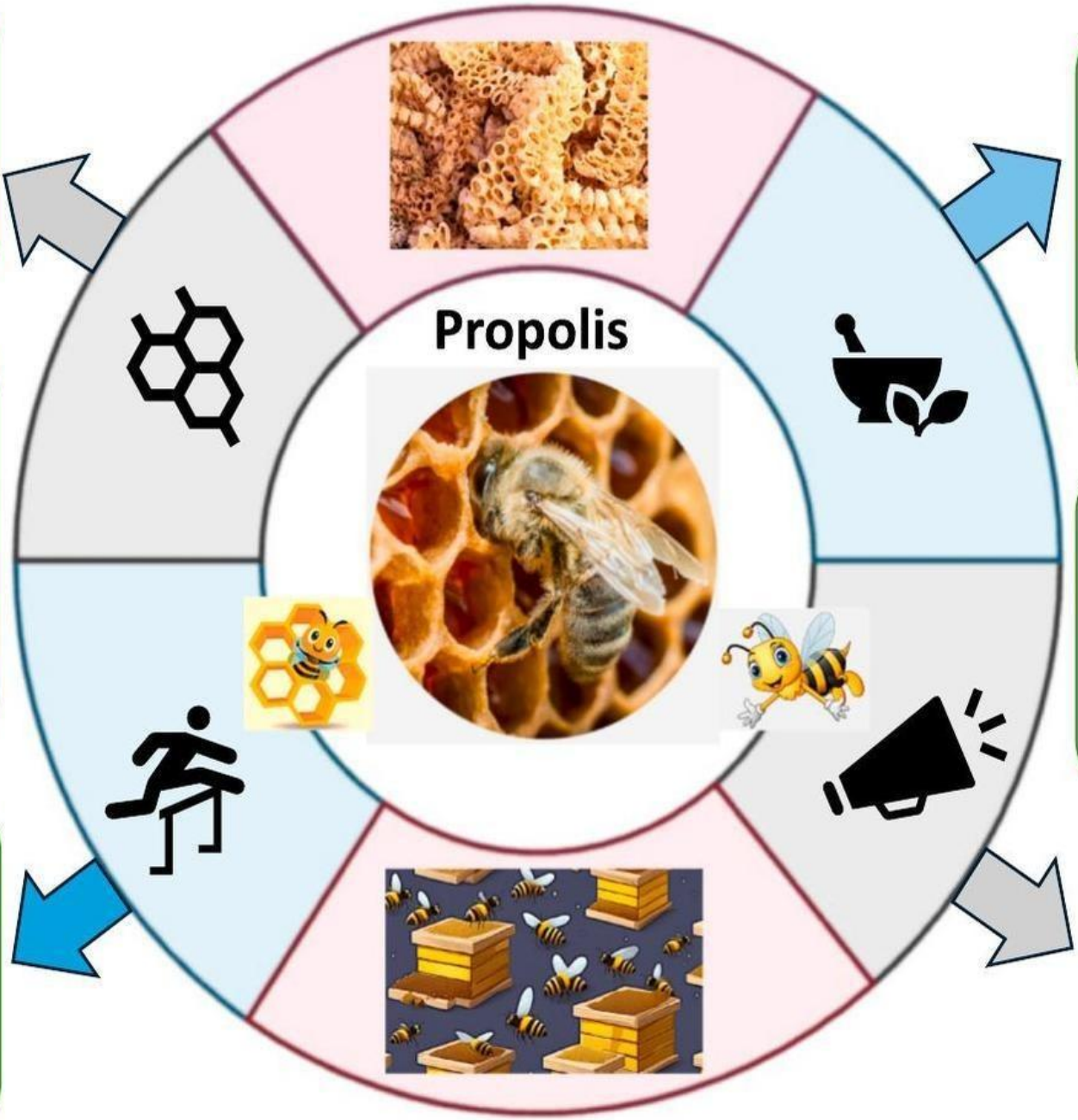
Flavonoids, Phenolic Acids and their Esters, Terpenoids, Aromatic Aldehydes, Waxes, Fatty Acids, Essential Oils, Amino Acids, Vitamins and Minerals

Clinical Trials

Oral Health
Respiratory Infections
Gastrointestinal Health
Wound Healing
Dermatology
Allergic Rhinitis
Type 2 Diabetes
Antioxidant Activity
Immunomodulatory Effects
Cancer Support

Major challenges

Standardization and quality control , Extraction and purification, Bioavailability and delivery systems, Toxicological studies, Sustainable production and protection of bee populations, Commercialization



Novel drug delivery systems

Nanoparticles
Liposomal Formulations
Hydrogels
Cyclodextrin Complexes
Nanoencapsulation
Micelles
Transdermal Delivery Systems
Electrospun Fibers
Mucoadhesive Systems

Therapeutic potential

Wound healing
oral health
Cardiovascular health
Neuroprotective effects
Hepatoprotective benefits
Gastrointestinal benefits

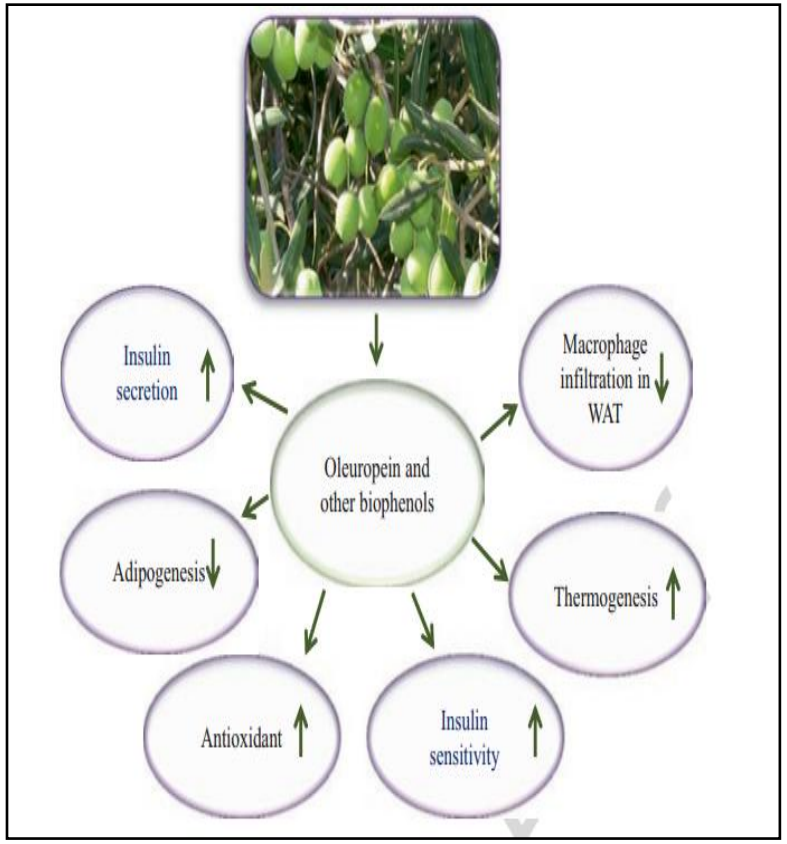
Biological activities

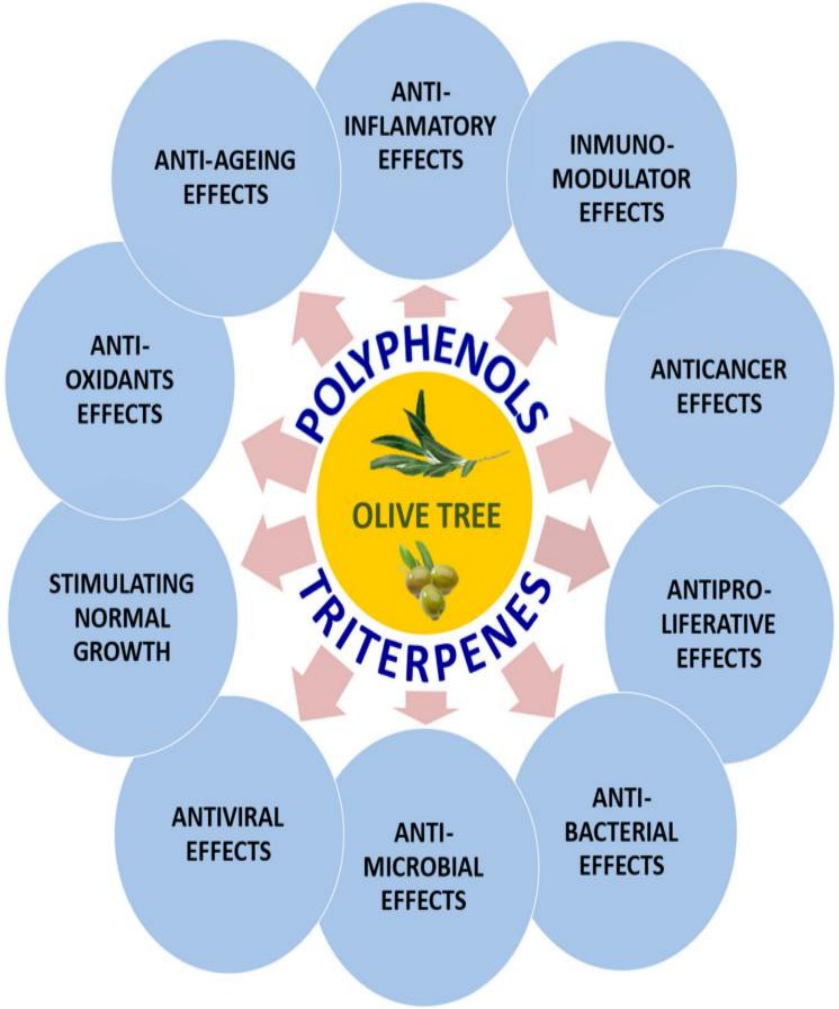
Antibacterial
Antioxidant
Anti-inflammatory
Anti-cancer
Anti-diabetic
Immunomodulatory

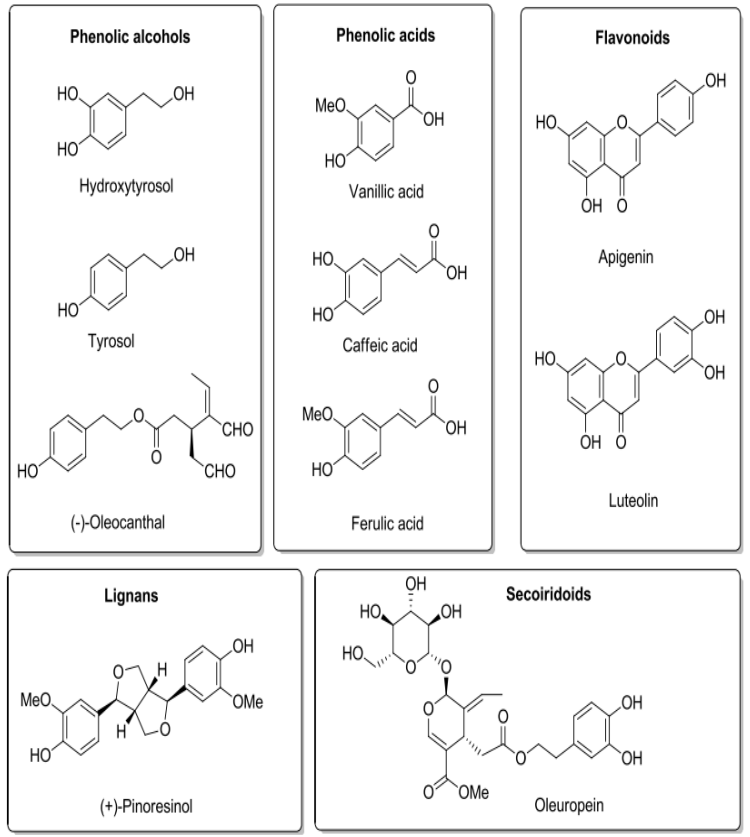
Fam : Oleaceae

Genre : Olea

Es : Olea europaea L







processes

MDPI

Article

Simultaneous Optimization of Extraction Yield, Phenolic Compounds and Antioxidant Activity of Moroccan Propolis Extracts: Improvement of Ultrasound-Assisted Technique Using Response Surface Methodology

Abderrazak Aboulghazi [1], Meryem Bakour [1], Mouhcine Fadil [2] and Badiaa Lyoussi [1,*]

WJEM World Journal of Experimental Medicine

Submit a Manuscript: https://www.f6publishing.com

World J Exp Med 2025 September 20; 15(3): 99330

DOI: 10.5493/wjem.v15.i3.99330

ISSN 2220-315x (online)

ORIGINAL ARTICLE

Basic Study

Effect of propolis and honey in hyperglycemia-induced kidney and liver injuries, proteinuria, and oxidant and antioxidant parameters

Soumaya Touzani, Noori Al-Waili, Hassan Laaroussi, Abderrazak Aboulghazi, Nawal Hamas, Hamada Imtara, Asmae El Ghouizi, Ilham ElArabi, Ahmed Al-Waili, Badiaa Lyoussi

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journal homepage: www.elsevier.com/locate/matpr

Comparative assessment of total phenolics content and *in vitro* antioxidant capacity variations of macerated leaf extracts of *Olea europaea* L. and *Argania spinosa* (L.) Skeels

Aziza Lfitat [a,*], Hind Zejli [a], Fatima Zohra Bousraf [b], Abdelkamel Bousselham [a], Yassine El Atki [a], Abdelkader Gourch [b], Badiaa Lyoussi [a], Abdelfattah Abdellaoui [b]

Wiley

Journal of Food Biochemistry

Volume 2024, Article ID 8246224, 16 pages

https://doi.org/10.1155/2024/8246224

Research Article

Phenolic Screening and Mixture Design Optimization for *In Vitro* Assessment of Antioxidant and Antimicrobial Activities of Honey, Propolis, and Bee Pollen

Abderrazak Aboulghazi [1], Mouhcine Fadil [2], Soumaya Touzani [1], Lahbib Hibaoui [3], Christophe Hano [4] and Badiaa Lyoussi [1]

Unraveling the Chemical Composition, Antioxidant, α-amylase and α-glucosidase Inhibition of Moroccan Propolis

Hassan Laaroussi , Pedro Ferreira-Santos ; Zlatina Genisheva; Meryem Bakour; Driss Ousaaid; José Antonio Teixeira; Badiaa Lyoussi

Journal of Food Science

IFT

REVIEW ARTICLE OPEN ACCESS

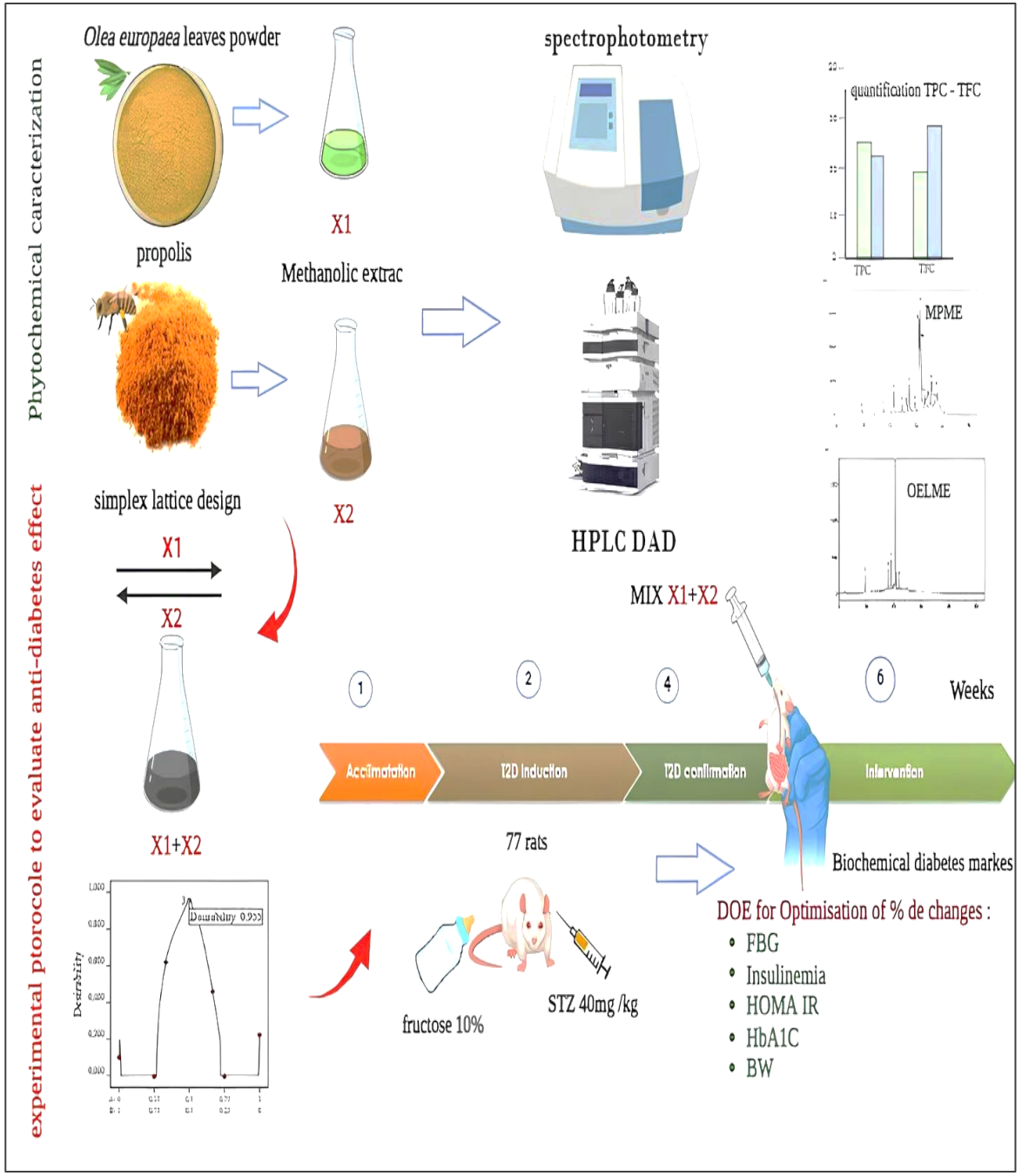
Concise Reviews and Hypotheses in Food Science

Sustainable Valorization of Olive Oil By-Products: Green Extraction of Phytochemicals, Encapsulation Strategies, and Food Applications

Laura Aracely Contreras-Angulo [1] | Hassan Laaroussi [2] | Driss Ousaaid [2,3] | Meryem Bakour [2,4] | Badiaa Lyoussi [5] | Pedro Ferreira-Santos [6,7]

The objective of this study is to evaluate the anti-diabetic effect of combined chemically characterized extracts from propolis and *Olea europaea* leaf in a rat model of Type 2 Diabetes (T2D) by applying **mixture design methodologies** to identify the most effective formulation that maximizes the biological activity, focusing on their synergistic effects by optimizing

- ✓ **Fast Blood Glucose**
- ✓ **Insulinemia**
- ✓ **HBA_1C**



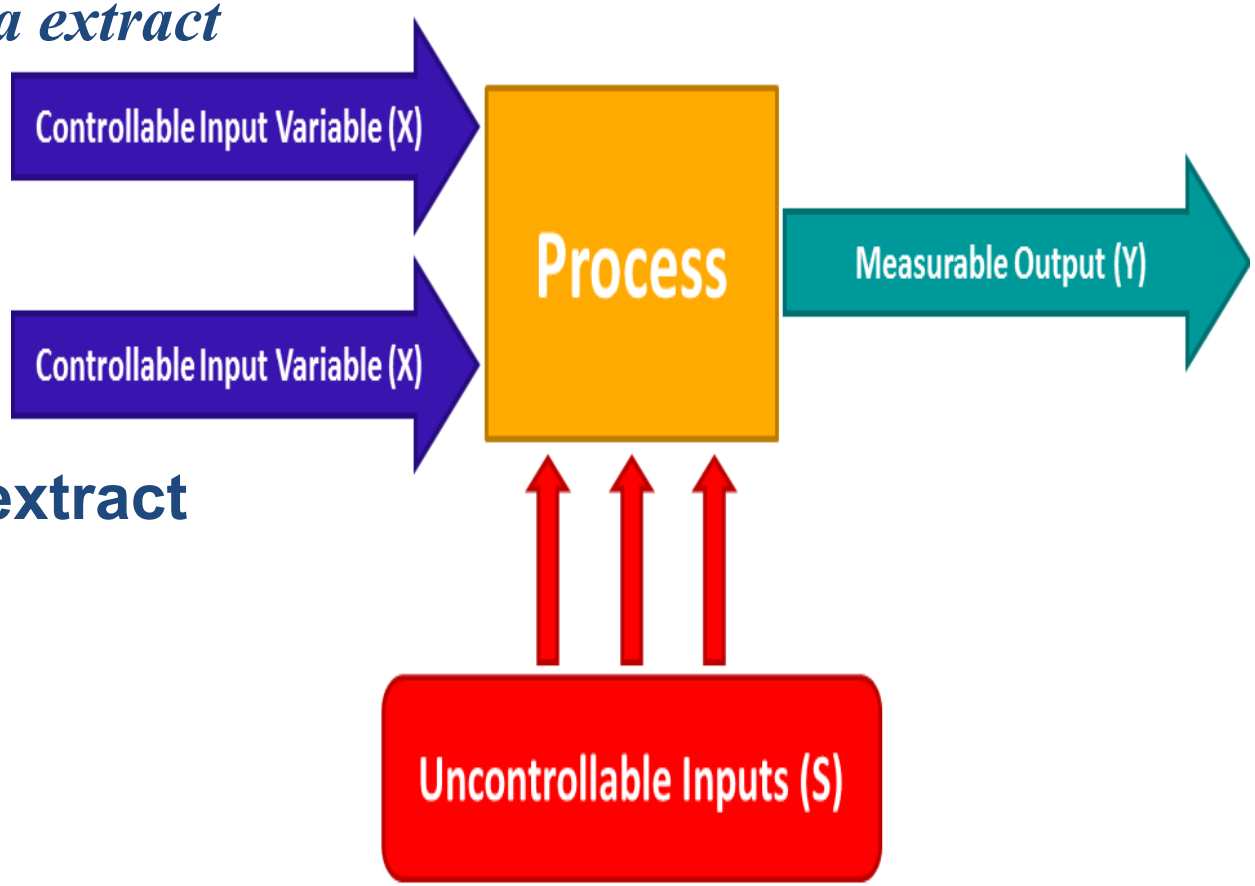
Propolis

Olea europaea leaves extract

Formulation Prop'Olea

Application of the mixture design to predict the optimal combination of propolis and *Olea europaea* extracts allowing:

Olea europaea extract



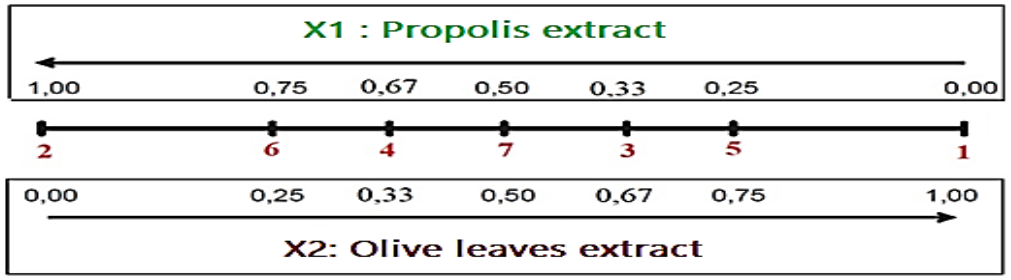
Propolis extract

Optimization :

- Fast Blood Glucose
- Insulinemia
- HBA_1C

Mixting design

File Version	13.0.1.0
Study Type	Mixture
Design Type	Simplex Lattice
Design Model	Quadratic



factors	Comp	Coded low	Coded high
X_1	MPME	0	1
X_2	OELME	0	1
Sum		1	

% of change

FBG, INS, HOMA-IR index, HbA_1C, and BW

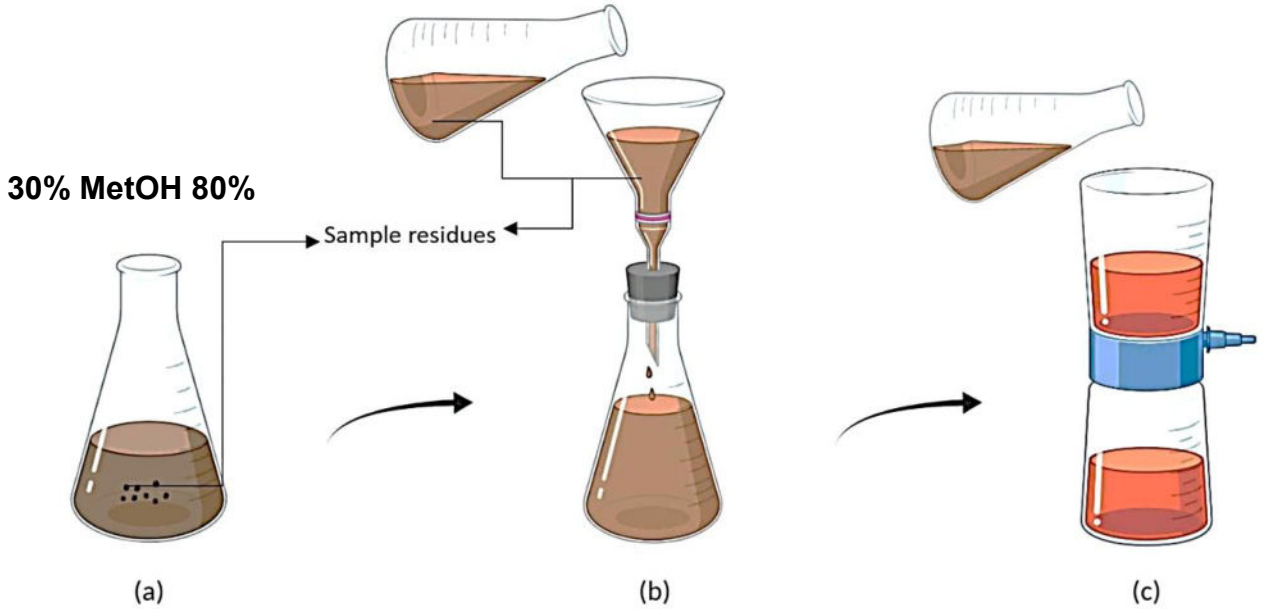
Mathematical Model

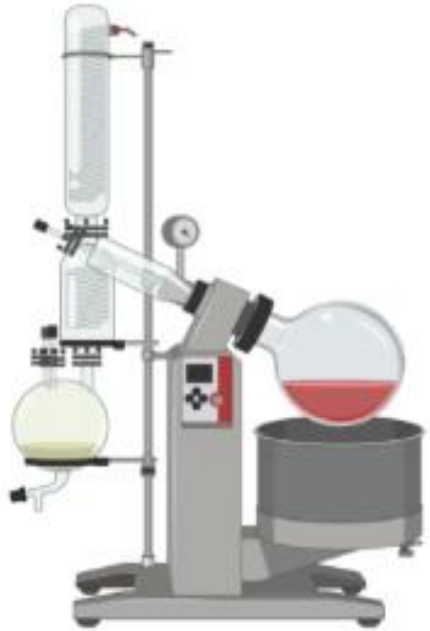
The postulate mathematical model was a quadratic Scheffe model, represented by the following equation

$$Y = b_1X_1 + b_2X_2 + b_{12}X_1X_2 + \varepsilon$$

propolis

olea europaea





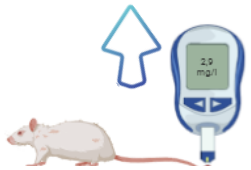
2
OEME

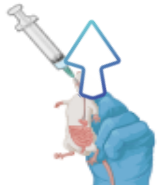
1
MPME

Acclimatation → induction DT2 → confirmation → Intervention

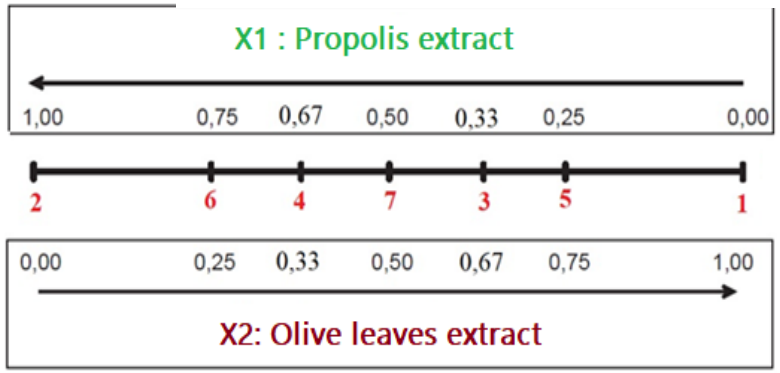
1 — 2 — 3 — 4 — 5 — 6

77



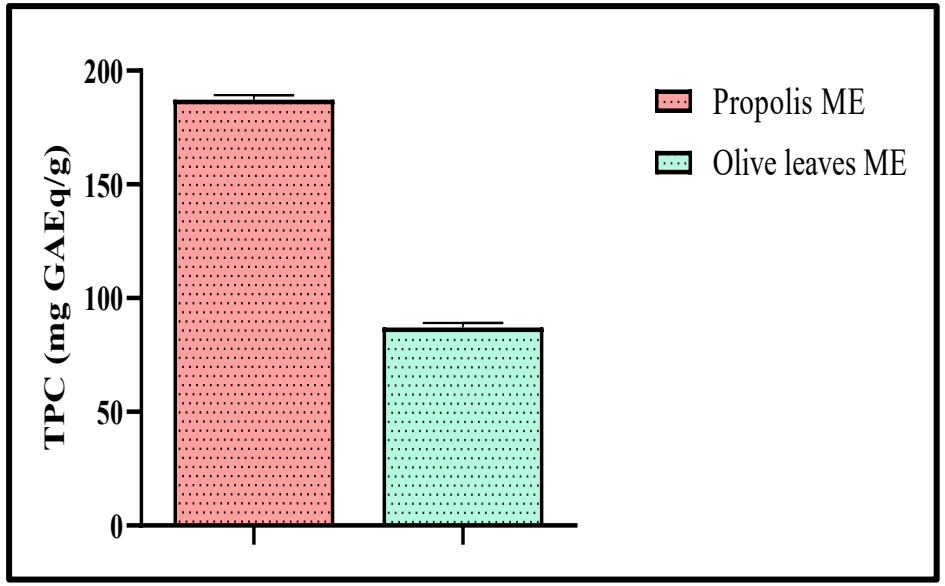


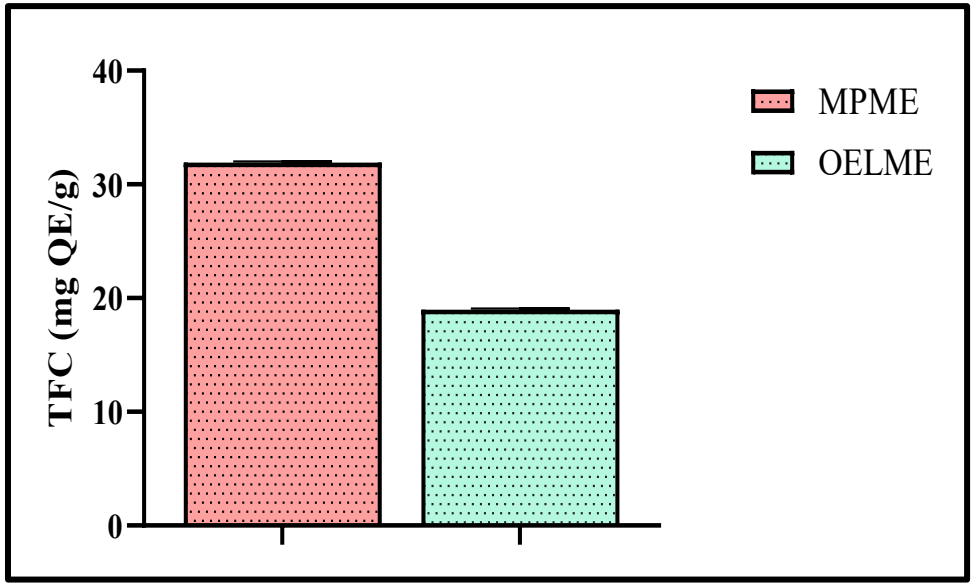
G1	G2	G 3	G 4	G 5	G 6	G7 x 3	GMET	GT
EEP 500mg/kg PC	EFO500mg/kg PC	2/3 EP 1/3 EFO	2/3 EFO 1/3PE	¼ EFOP ¾ EP	3/4 EFO¼ EP	½EFO ½ EP	5mg/kg PC	5ml H2O

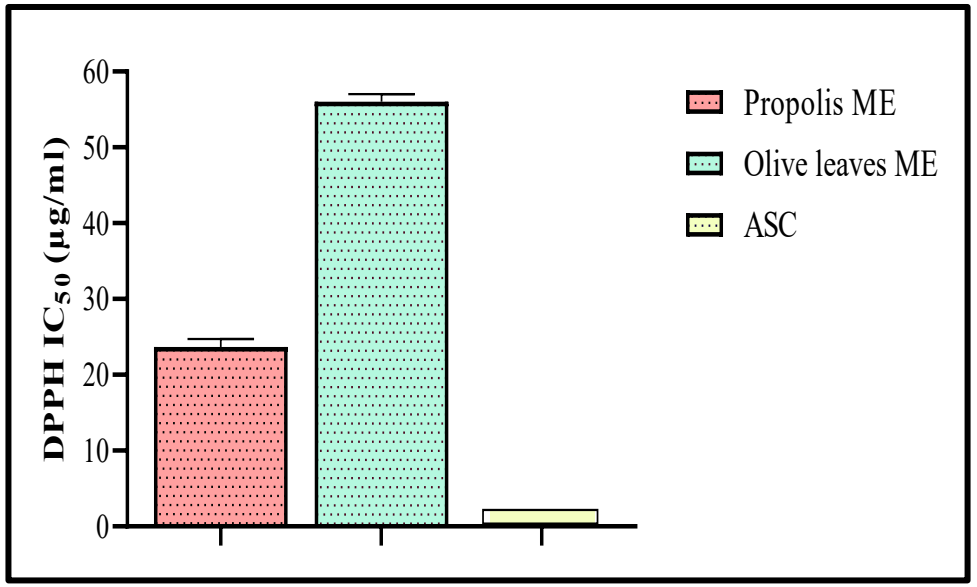


Evaluation DT2

FBG ,INS, HB1C

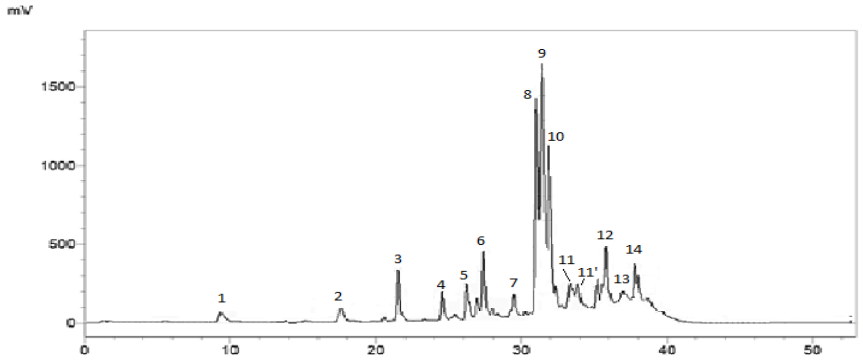

TPC (mg GAEq/g)
200
150
100
50
0
Propolis ME
Olive leaves ME


TFC (mg QE/g)
40
30
20
10
0
MPME
OELME


DPPH IC50 (µg/ml)
60
50
40
30
20
10
0
Propolis ME
Olive leaves ME
ASC

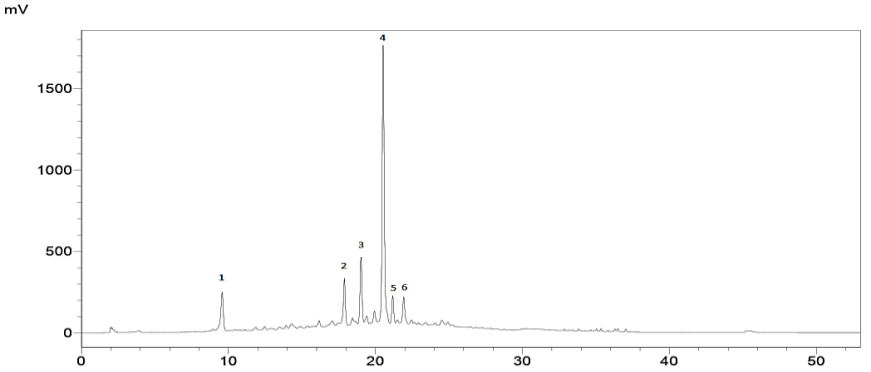
Phenolic screening by HPLC-DAD

Propolis extract



Phenolic Compounds	Values (mg/g)
Catechin	Nd
Vanilic Acid	Nd
Epicatechin	0.222 ± 0.001
o-coumaric Acid	0.0008 ± 0.00
Ferulic Acid	0.0002 ± 0.00
Ellagic Acid	0.055 ±0.00
Narginin	0.077± 0.00
Hesperidin	0.098 ± 0.00
Apigenin	0.342± 0.001
Cinnamic Acid	0.026 ± 0.00
Resveratrol	0.023 ± 0.00
rosmarinic Acid	0.037 ± 0.00
Rutin	0.025 ± 0.00

Olea europaea leaves Extract



Phenolic Compounds	Values (mg /g)
Hydroxytyrosol	2.00± 0.05
Quercetin	0.88± 0.09
Rutin	0.18± 0.00
Oleuropein	29.91± 0.16
Oleocanthal	0.71± 0.02
Luteolin	1.33 ± 0.05

Simplex lattice design results

Experimental matrix

% of change = [final value – initial value]/ initial value x 100

EXP	Propolis ME	Olive leaves ME	% en change		
			GLY	INSULIN	HbA_1C
1	1	0	63 ±2.32[a]	52 ± 2.78[a]	14 ± 0.34[a]
2	0	1	66 ±2.60[a]	62 ± 2.35[a]	29 ± 0.93[a]
3	0.5	0.5	68 ±2.91[a]	145 ± 6.32[ab]	23 ± 0.91[ab]
4	0.5	0.5	70 ± 4.82[b]	159 ± 7.72[bcd]	24 ± 0.90[bc]
5	0.5	0.5	70 ± 2.37[bc]	142 ± 6.16[ab]	21 ± 0.89[bc]
6	0.25	0.75	65 ± 3.17[bcd]	47 ± 2.91[bc]	20 ± 0.99[bc]
7	0.75	0.25	45 ± 2.32[b]	66 ± 3.30[bc]	10 ± 0.96[bc]
8	0.67	0.33	55 ± 2.54[bc]	130 ± 5.31[abc]	16 ± 0.91[a]
9	0.33	0.67	65 ± 3,32[bc]	70 ± 5.90[bcd]	24 ± 0.92[a]

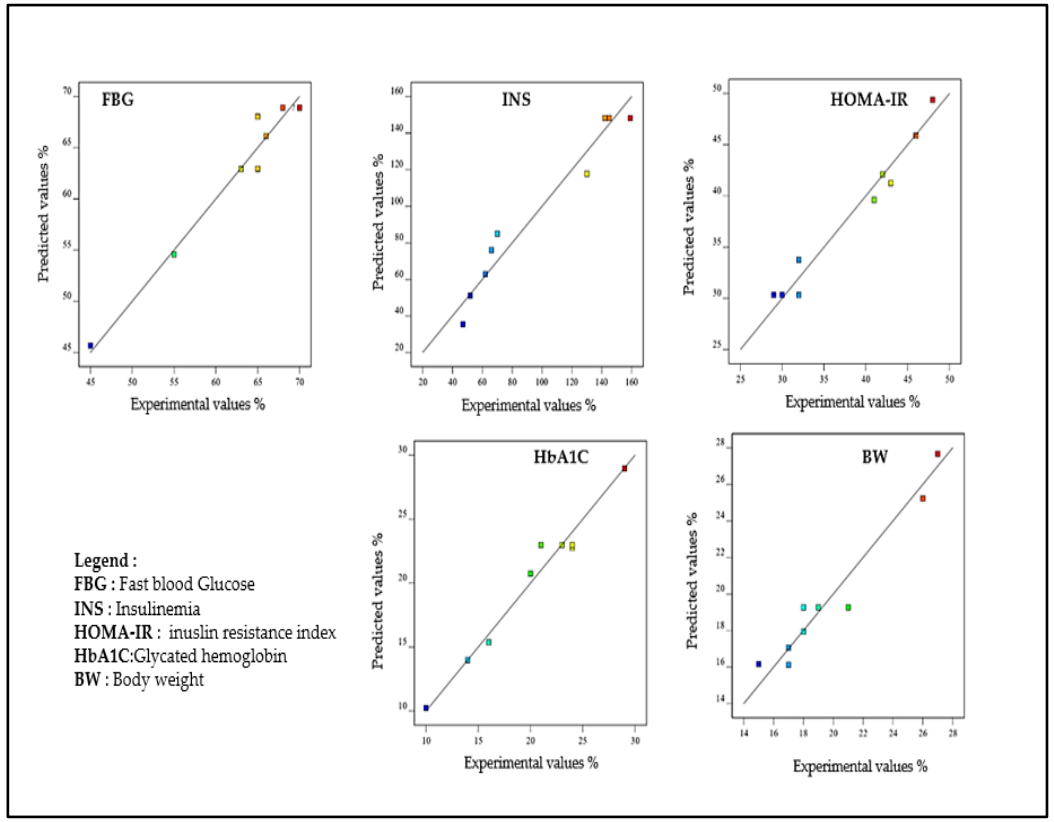
Results demonstrate rates of change :

- **FBG** : 45 to 70 %
- **INS**: 47 to 159%
- **HBA_1C**: 10 to 29 %

These results confirm the influence of binary mixtures on all the responses studied

Validation of Mathematical Model

Response	Source	DDL	SC	CM	F-value	p-value
% change FBG	Regression	4	510.72	127.68	29.56	0.0031
	Résidus	4	17.28	4.32		
	LOF	2	14.61	7.31	5.48	0.15
	Pur Error	2	2.67	1.33		
	Total	8	528			
	R^2			0.96		
% change INS	Régression	4	15927.15	3981.79	20.56	0.01
	Résidus	4	774.85	193.71		
	LOF	2	610.18	305.09	3.71	0.21
	Erreur pure	2	164,67	82.33		
	Total	8	16702.00			
	R^2			0.95		
% change HbA_1C	Régression	4	267.38	66.84	35.60	0.002
	Residus	4	7.51	1.88		
	LOF	2	2.84	1.42	0.61	0.62
	Erreur pure	2	4.67	2.33		
	Total	8	274.89			
	R^2			0.97		



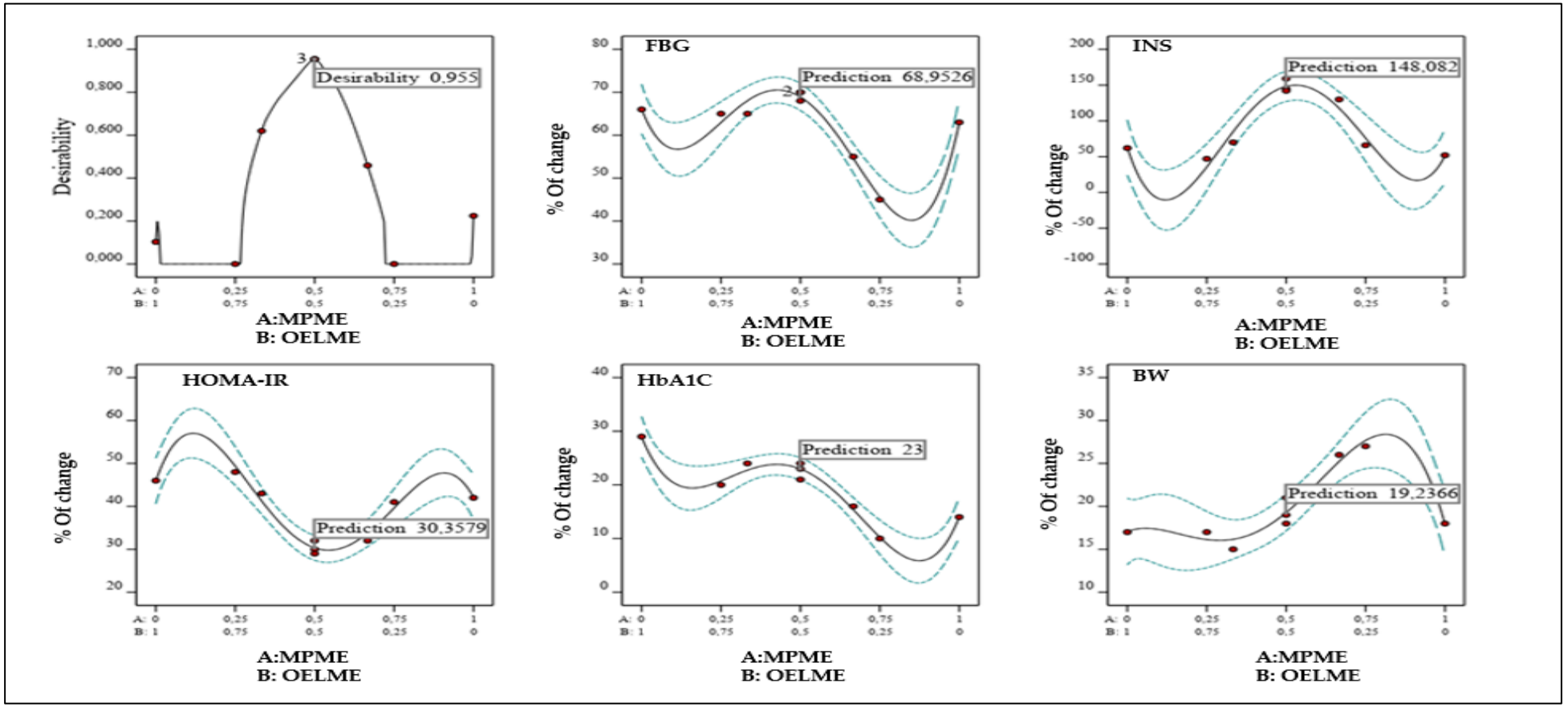
Curves of observed values VS predicted values for FBG, INS, HOMA-IR, HbA_1C and BW changes

Mathematical model coefficients

Coefficient	Term	FBG		INS		HbA_1C	
		Est.	p-value	Est.	p-value	Est.	p-value
A	β_1	62.92	0.009*	51.19	0.44	14.00	0.001*
B	B_2	66.13	0.009*	62.87	0.44	28.96	0.001*
AB	β_{12}	17.50	0.077	364.72	0.002*	5.98	0.288
AB(A-B)	δ	-83.56	0.004*	247.33	0.06	-16.09	0.164
AB(A-B) ²	γ	-287.95	0.001*	-1485.79	0.004*	-151.65	0.003*

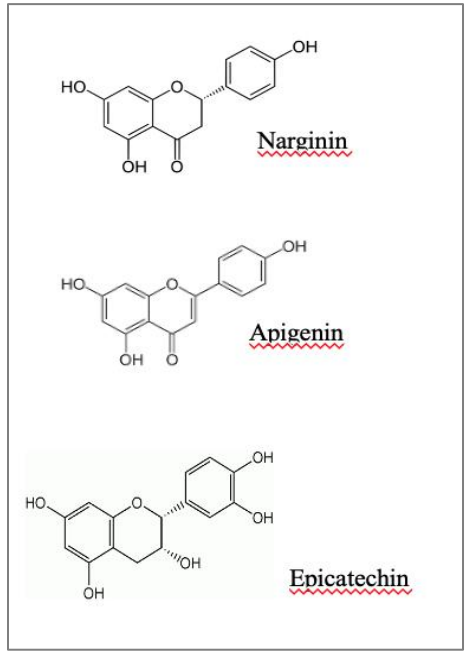
Effects of binary mixture on :

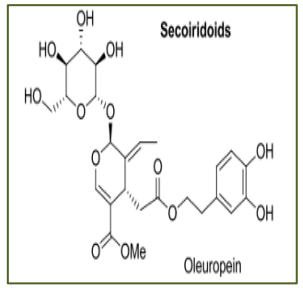
- **FBG** : $Y = 62{,}92X_1 + 66{,}13X_2 - 83{,}56X_1X_2(X_1-X_2) - 287{,}95X_1X_2\,(X_1-X_2)^2+\varepsilon$
- **INS** : $Y = 364{,}72X_1X_2 - 1485{,}79X_1X_2\,(X_1-X_2)^2+\varepsilon$
- **HbA_1C** : $Y = 14X_1 + 28{,}96X_2 - 151{,}65X_1X_2\,(X_1-X_2)^2+\varepsilon$

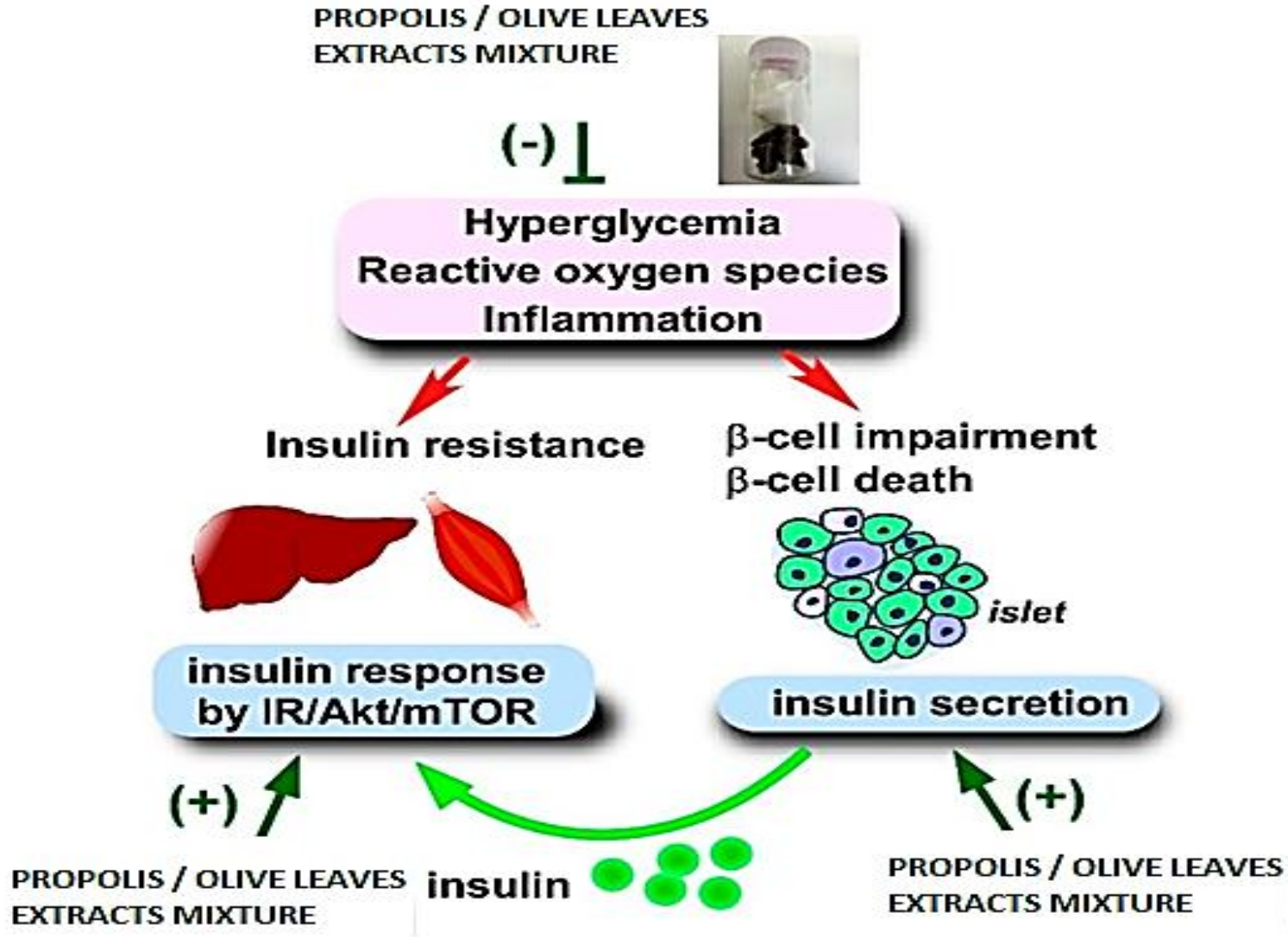


Simultaneous desirability graph for FBG , INS, HOMA-IR HbA_1C and BW percentages of changes

Des (%)	MPME	OELME	Percentage of change (%)				
			FBG	INS	HOMA-IR	HbA_1C	BW
95.5	0.49	0.51	68.95	148.08	30.36	23.00	19.24


Narginin
Apigenin
Epicatechin

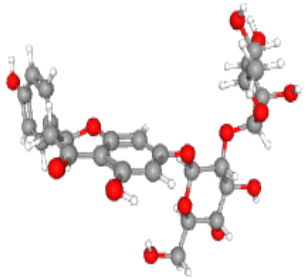

Secoiridoids
Oleuropein


PROPOLIS / OLIVE LEAVES
EXTRACTS MIXTURE
(-)
Hyperglycemia
Reactive oxygen species
Inflammation
Insulin resistance
β-cell impairment
β-cell death
islet
insulin response
by IR/Akt/mTOR
insulin secretion
(+)
(+)
PROPOLIS / OLIVE LEAVES
EXTRACTS MIXTURE
insulin
PROPOLIS / OLIVE LEAVES
EXTRACTS MIXTURE

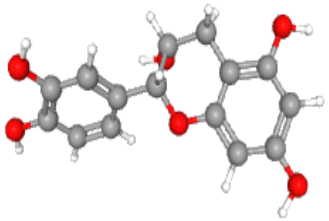
Ligands studied

Main components of the mixture of propolis and olive leaves

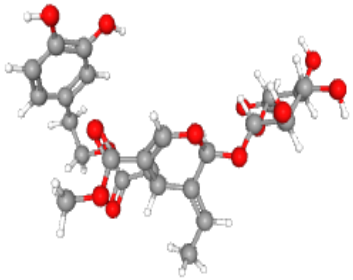
Narginin



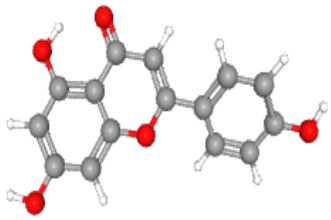
Epicatechin



Oleuropein



Apigenin

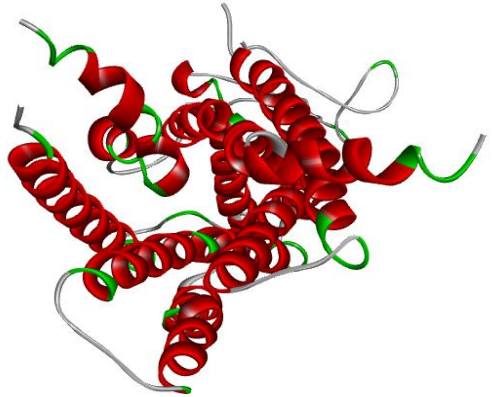


Interaction

Identifies proteins that contribute to the antidiabetic effect

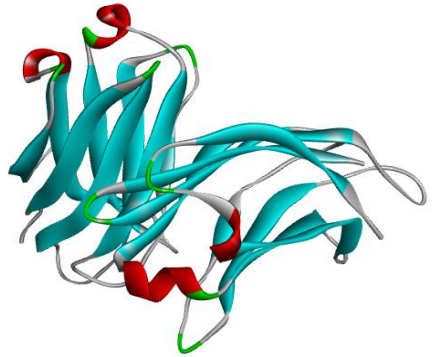
Enhances insulin secretion

Glucagon-like Peptide-1 Receptor (7S15)

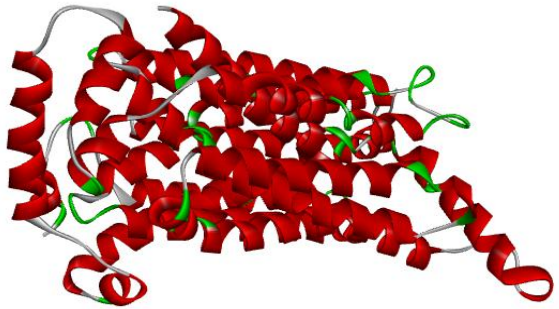


Critical in the insulin signaling cascade

Phosphoinositide 3-kinase (6BTY)



Glucose transporter type 4 (7WSM)



The main glucose transporter in muscle and adipose tissue

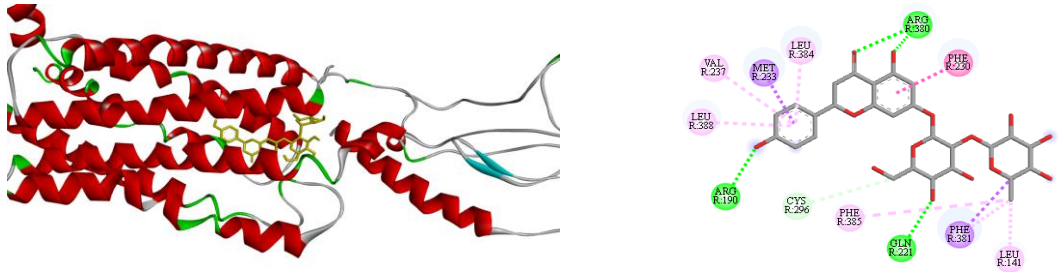
Table 1: Interaction scores between bioactive compounds and

Glucagon-like Peptide-1 Receptor

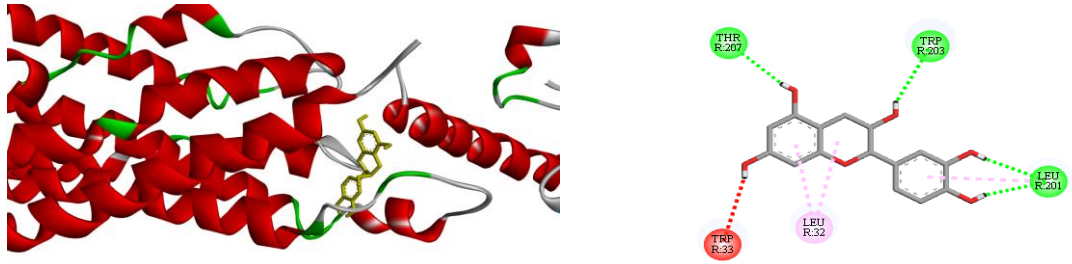
Ligands	Binding energy	Inhibition constant
Narginin	–6.85 Kcal/mol	9.59 µM
Epicatechin	–6.92 Kcal/mol	8.47 µM
Oleuropein	–6.73 Kcal/mol	11.66 µM
Apigenin	–7.02 Kcal/mol	7.19 µM

- ✓ The four compounds examined show **a specific interaction** with **essential residues** of the **active site** of the **Glucagon-like Peptide-1 Receptor.**
- ✓ These values suggest a **strong ability to stabilize** the ligand-protein complex, translated by low inhibition constants, indicative of an effective interaction.

Narginin



Epicatechin



Oleuropein

Apigenin

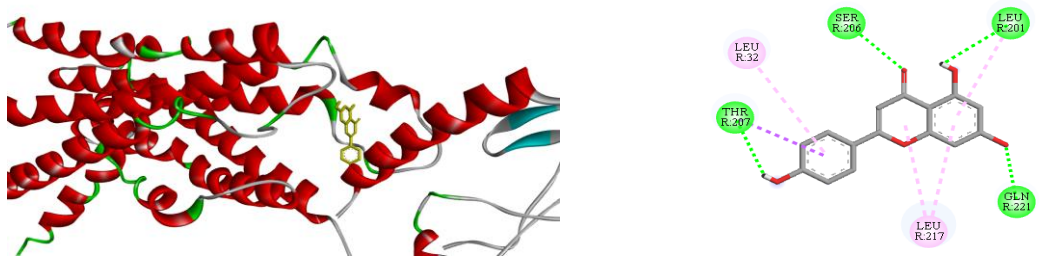


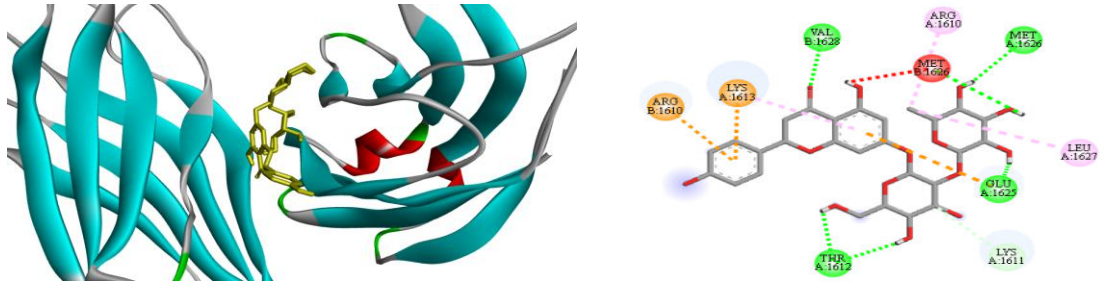
Table 2: Interaction scores between bioactive compounds and

Phosphoinositide 3-kinase

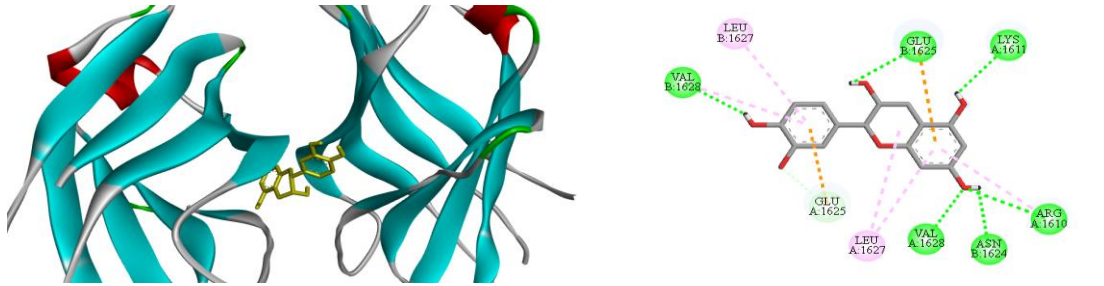
Ligands	Binding energy	Inhibition constant
Narginin	–7.95 Kcal/mol	1.50 µM
Epicatechin	–8.89 Kcal/mol	304.65 nM
Oleuropein	–4.88 Kcal/mol	265.27 µM
Apigenin	–8.64 Kcal/mol	461.53 nM

- ✓ The four analyzed compounds demonstrate precise interactions with essential residues of the active site of Phosphoinositide 3-kinase, exposing a diverse molecular recognition
- ✓ These values suggest a **strong ability to stabilize the ligand-protein complex,** translated by low inhibition constants

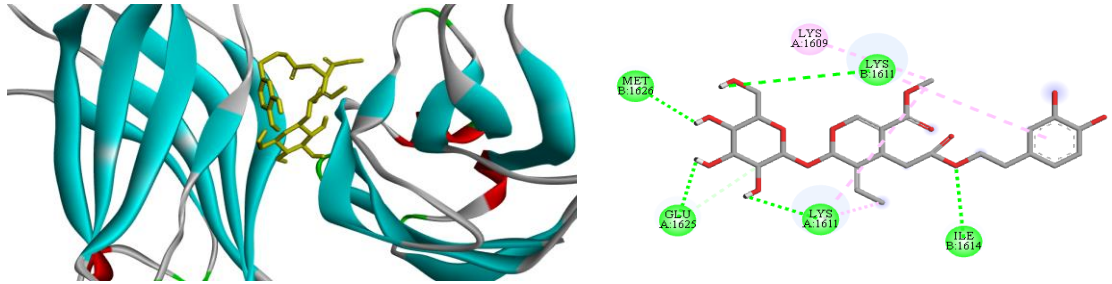
Narginin



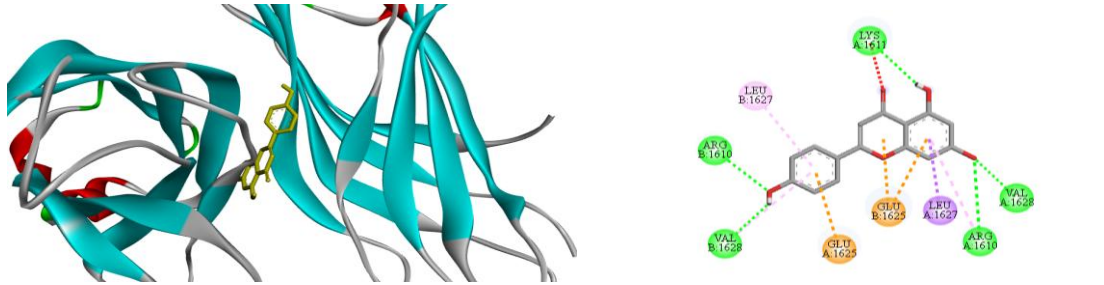
Epicatechin



Oleuropein



Apigenin



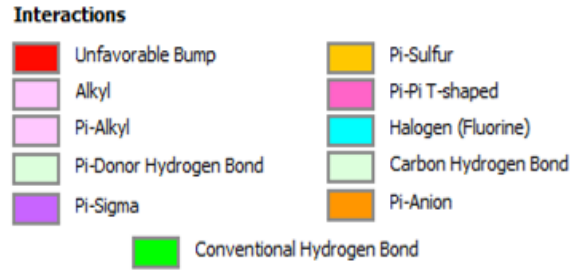
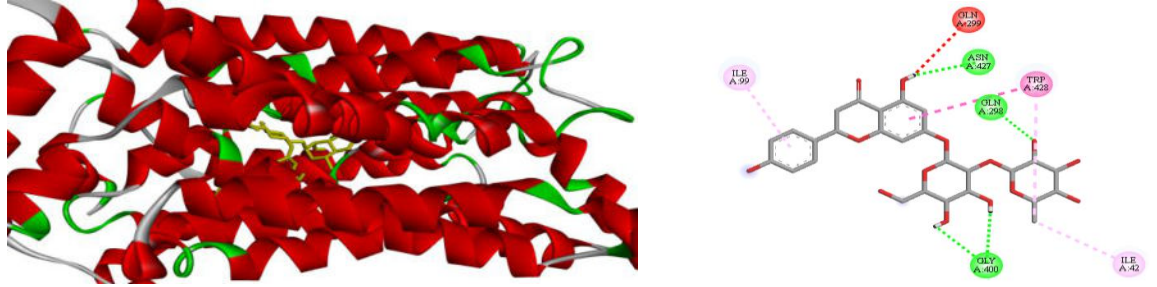


Table 3: Interaction scores between bioactive compounds and **Glucose transporter type 4**

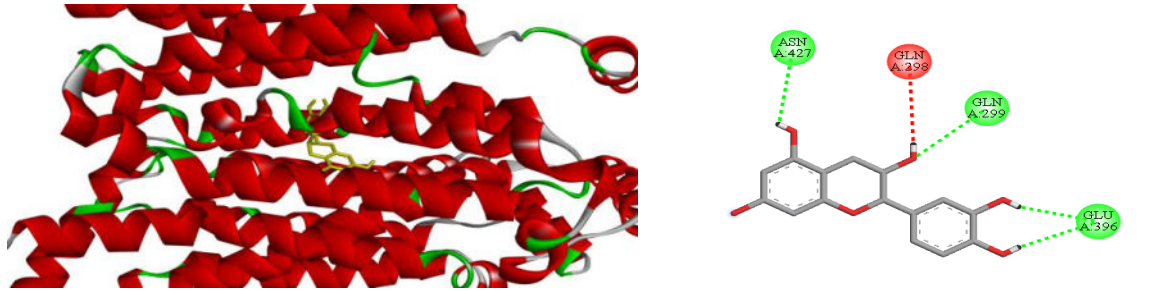
Ligands	Binding energy	Inhibition constant
Narginin	–7.08 Kcal/mol	6.44 µM
Epicatechin	–6.16 Kcal/mol	30.74 µM
Oleuropein	–6.76 Kcal/mol	11.09 µM
Apigenin	–6.59 Kcal/mol	14.74 µM

- ✓ The four analyzed compounds interact in a specific way with essential residues of the active site of type 4 Glucose.
- ✓ These values **suggest a strong ability to stabilize the ligand-protein complex**, translated by low inhibition constants, indicative of an effective interaction

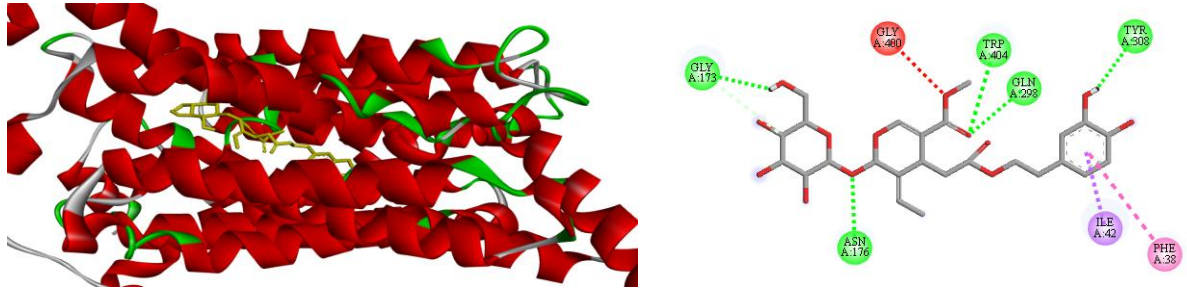
Narginin



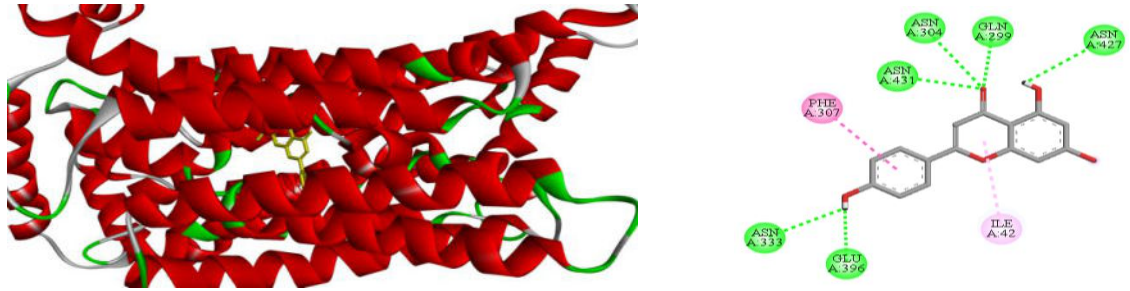
Epicatechin

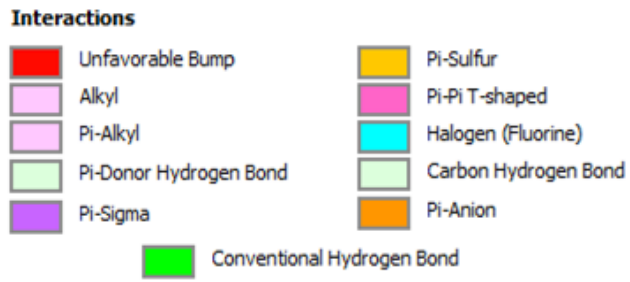


Oleuropein



Apigenin





- ✓ Molecular docking revealed that **Apigenin and Epicatechin** exhibited the strongest binding affinities to key antidiabetic targets (GLP-1R and PI3K), while **Naringenin** showed selective affinity toward GLUT4.

- ✓ Interaction analyses demonstrated **stable hydrogen bonding**, hydrophobic, and π–π interactions with essential residues, suggesting a **strong potential** to modulate glucose metabolism pathways.

- ✓ The *in silico* prediction experiments **confirmed** the *in vivo* antidiabetic effects of the propolis and olive leaves mixture with the selected compounds.

CONCLUSION

- Using an experimental design methodology to formulate an optimized mixture of propolis and *Olea europaea* leaf methanolic extracts within specified ranges can lead to significant variations in biomarkers associated with type 2 diabetes (T2D). This approach not only underscores the potential of natural products in managing diabetes but also promotes further investigation into their roles in dietary interventions and therapeutic formulations.

- The synergistic effects of these extracts may contribute to more effective strategies for glycemic control in diabetic patients. However, to validate these findings, further research is essential, particularly clinical studies utilizing standardized extracts. This will ensure the reproducibility of the pharmacological properties observed and aid in translating the results from various *in-vivo* studies to practical human applications.

Many thanks
for your attention!

Merci beaucoup

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