People's Democratic Republic of Algeria
The Ministry of Higher Education and Scientific Research

Amar Telidji University – Laghouat
Faculty of Sciences

Biology department

Laboratory:
Fundamental sciences laboratory

THESIS

Study of the inhibitory effect of a few pure molecules and natural extracts on four key enzymes of Alzheimer's disease with molecular docking and ADMET analysis

A thesis submitted in fulfillment of the requirements for the degree of

DOCTOR OF SCIENCE

In Biology

by

SERSEG Talia

President	Dr. ALLAL Farida	University of Amar Telidji, Laghouat
Supervisor	Pr. BENAROUS Khedidja	University of Amar Telidji, Laghouat
Examinator	Dr. BOUSSOUSSA Hadjer	University of Amar Telidji, Laghouat
Examinator	Pr. BENHAMMOU Ep BELYAGOUBI Nabila	University of Abou Bekr Belkaid, Tlemcen
Examinator	Dr. ZIANE Mohamed	University of Belhadj Bouchaib, Ain Temouchent

2020/2021

To my parents

My brothers and sisters

My family

And all my friends

ACKNOWLEDGMENT

First of all, I would like to thank ALLAH almighty, the most merciful and compassionate, for his support, help, and generosity.

*I would like to express my gratitude to my supervisor, **Professor Dr. BENAROUS Khedidja**, who guided me throughout this work, by her continuous caring and guidance discussion. I am very indebted to her, for her continuous encouragement, unforgettable help, and kind feeling throughout the course of this study.*

*I would like express a special appreciation to **Professor Dr. YOUSFI Mohamed**, the director of sciences fundamentals laboratory, for his support, generosity, and help, and all laboratory staff.*

*My sincere thanks to the honorable members of the jury: **Dr. ALLAL Farida**, **Dr. BOUSSOUSSA Hadjer**, **Professor Dr. BENHAMMOU Ep BELYAGOUBI Nabila** and **Dr. ZIANE Mohamed** for accepting to examine this work.*

*All thanks to my **parents**, **brothers**, and **sisters** for their continuous encouragement, love, patience, support, prayers, and their presence by my side throughout the writing of this work.*

*I would also like to thank **Professor Dr. Mehmet ÖZTÜRK**, Director of the Organic Chemistry Laboratory (Sitki Koçman University of Mugla-Turkey), for his generosity and help by sharing the information and protocols.*

I would like to thank ***Mr. Khalifa Kouider****, Director of the local public health establishment in Djelfa, the manager and engineers of polyclinic of boukerdenna said, djelfa.*

I would also like to warmly and infinitely thank my dear colleague ***Dr. Gacemi Safia*** *for her invaluable help and support.*

All thanks to my dear friend, ***Ms. Hammou Asma*** *for her moral support and encouragement, which were was very helpful throughout the past three year of the work.*

I deeply appreciate and thank the collaboration from my colleague and friend ***Ms. Hfeid Fatiha****.*

I would like to thank, ***Dr. Harrath Mohamed*** *and* ***Dr. Hachani Soumeya*** *for their helpful advices.*

I would also like to thank ***Mr. Bouzidi Amdjed*** *and* ***Mr. Benalia*** *for their help in getting information and obtaining the local plants.*

My sincere thanks to ***my colleagues****,* ***engineers****, and High school of teachers Laghouat* ***staff****.*

Finally, I wish to express my love and my gratitude to ***my family*** *for their constant support, encouragement, never-ending patience, without whose love, this work would never be possible.*

Abstract

The search for active compounds from plants – for the development of new potent anti-AD drugs or as an alternative strategy for treatment of Alzheimer's disease- is the subject of many scientific and medical research. From this perspective, we aimed through this study to screen the antiacetycholinesterase effect of the crude extract of seven plants, and explored the possibility and predicted the activity of the major compounds of these extracts using in silico methods against Acetylcholinesterase (AChE), Butyrylcholinesterase (BuChE), Beta-secretase (BACE1), and Monoacylglycerol lipase (MAGL). Hence, determining the main interactions in the inhibitor enzyme complex. **In the first part**, we estimated the amount of phenolic compounds and flavonoids in these plants. The results showed that these plants contain a considered amount of phenolic compounds whose contents in hexane, ethyl acetate, and methanol extracts vary between 0.026±0.004 to 29.11±0.504 mgGAE/gDM. For flavonoids, the levels in the extracts vary between 0.00 to 7.690±0.481 mgQE/gDM. **In the second part**, we studied the inhibitory effect of *Arbutus unedo* L*, Coriandrum sativum, Juniperus oxycedrus, Juniperus phoenicea, Lavandula stoechas, Saussurea costus and Lepidium sativum* extracts on acetylcholinesterase using the Ellman method. The results confirmed the capacity of these plants to inhibit the enzyme with a value of IC_{50} 0.358±0.005 to 2.489±0.154 mg/mL. With the exception of A.unedo L (EtOAc), L. stoechas (Meth) which has no inhibitory activity. **In the third part**, we achieved a molecular docking study using Autodock Vina. Furthermore, the drug-likeness and ADMET properties of the major compounds in studied plants were also evaluated. All the complexes of studied molecules with the four enzymes show significant, several, and different interactions. The major components published in the literatures- of *J.oxycedrus, J.phoenicea, and L.sativum* namely: Catechin, Myricetin-O-pentoside and Lepidine B respectively bind tightly to AChE and BuChE as much as galantamine and donepezil. Expect the major molecules of *S.costus* that exhibit a low affinity to studied enzymes. We suggest that Lepidine B is a non-competitive inhibitory by interacting with PAS of AChE and BuChE, therefore it is capable to prevent the HuAChE-induced Aβ aggregation. The results of our study indicate that the studied plants are promising resources for anti-AD drugs such as Catechin, Myricetin-O-pentoside, and Lepidine B and might use to prevent Alzheimer's disease due to their roles as potent inhibitors for AChE, BuChE, BACE1, and MAGL. Indeed, they could inhibit Aβ fibrillogenesis.

Keywords: Algerian medicinal plants, Alzheimer's disease, Acetylcholinesterase, enzyme inhibition, in silico, pure molecules, natural extracts.

الملخص

أصبح البحث عن المركبات النشطة من مصادر نباتية –كمصادر لتطوير أدوية مضادة لمرض ألزهاير أو استراجية علاج بديلة– موضوع العديد من البحوث العلمية والطبية. من هذا المنظور ، نهدف من خلال هذه الدراسة لتقييم التأثير المضاد للأستيل كولين إستراز لمستخلصات سبعة نباتات طبية، واستكشاف باستخدام طرق المعلوماتية الحيوية إمكانية امتلاك مركاباتها الرئيسية قدرة على تثبيط الإنزيمات: أستيل كولين استراز، بيتريل كولين استراز، الليباز أحادي الجليسرين وبيتا سكريتاز 1. ومن ثم، تحديد التفاعلات الرئيسية في المعقد إنزيم–مثبط. **في الجزء الأول،** قمنا بتقدير كمية المركبات الفينولية والفلافونويد في هذه النباتات. أظهرت النتائج أن هذه النباتات تحتوي على كمية معتبرة من المركبات الفينولية التي تختلف محتوياتها في مستخلصات الهكسان وخلات الإيثيل والميثانول بين 0,026±0,004 و 29,11±0,504 مغ مكافئ حمض الغاليك/غ مادة جافة، بالنسبة للفلافنويدات، تتراوح مستوياتها في المستخلصات من 0,00 إلى 7,690±0,481 مغ مكافئ كورستين/غ مادة جافة. **في الجزء الثاني،** درسنا التأثير التثبيطي لمستخلصات اللنج، الكسبر، الطاقة، العرعار، العريعيرة، القسط الهندي وحب الرشاد، على انزيم أستيل كولين إستراز باستخدام طريقة إلمان. أكدت النتائج قدرة مستخلصات هذه النباتات على تثبيط الإنزيم بقيمة تثبيط نصفي (IC_{50}) تتراوح بين 0,358±0,005 مغ/مل و2,489±0,154 مغ/مل. بإستثناء مستخلص خلات الإيثيل لنبات اللنج ومستخلص الميثانول لنبات *العريعيرة* حيث أظهرت النتائج عدم وجود نشاط تثبيطي. **في الجزء الثالث،** قمنا بدراسة الإرساء الجزيئي باستخدام أوتودوك فاينا. كما تم تقييم خصائص أدميت وخصائص تشابه الأدوية للمركبات الرئيسية في النباتات المدروسة. تُظهر جميع المعقدات للجزيئات المدروسة مع الإنزيمات الأربعة تفاعلات مهمة، متعددة ومختلفة. ترتبط المكونات الرئيسية لنباتات: الطاقة الشوكية، العرعار وحب الرشاد المتمثلة في: كاتشين وميريستين بنتوزيد واللبيدين بي على التوالي، بالإنزيمين الأستيل كولين استراز وبيتريل كولين استراز بقدر غلانتامين ودونزبيزيل. كما أظهرت الدراسة أن المركبات الرئيسية من القسط الهندي تظهر ألفة منخفضة للإنزيمات المدروسة، كما أننا نقترح أن لبيدين بي مثبط غير تنافسي من خلال ارتباطه مع الموقع الموقع الأنيوني المحيطي للأستيل كولين استراز وبيتريل كولين استراز، وبالتالي فهو قادر على منع تراكم ببتيد بيتا أميلويد الناجم عن الأستيل كولين استراز. تشير نتائج دراستنا إلى أن النباتات المدروسة تمثل موارد واعدة للأدوية المضادة لمرض الزهايمر مثل كاتشين وميريستين بنتوزيد واللبيدين بي، وقد تستخدم للوقاية من مرض الزهايمر بسبب أدوارها كمثبطات قوية للأستيل كولين استراز و بيتريل كولين استراز، الليباز أحادي الجليسرين وبيتا سكريتاز 1، كما يمكنها أن تمنع تكوين ألياف بيتا أميلويد.

الكلمات المفتاحية: نباتات طبية جزائرية، مرض ألزهايمر، أستيل كولين استراز، بيتريل كولين استراز، الإرساء الجزيئي، جزيئات نقية، التثبيط الإنزيمي، مستخلصات طبيعية.

List of scientific works completed within the framework of this thesis

International publications:

1. **Serseg**, **T**., Benarous, K., Lamrani, M., Yousfi, M., "Lepidine B from Lepidium Sativum Seeds as Multi-Functional Anti-Alzheimer's Disease Agent: In Vitro and In Silico Studies," Current Computer-Aided Drug Design, **2020**; 16 (1), DOI: 10.2174/1573409916666200302120305 Pmid: 32116197.

Out of the thesis topic

2. **Serseg**, **T**., Benarous, K., Yousfi, M."The Inhibitory Effect Of Terpenes From Cannabis Sativa", International Journal of Human Settlements, **2021** (Accepted).

3. Benarous, K., **Serseg**, **T**., Yousfi, M. "Amoxicillin Trihydrate-Clavulanate Potassium as Potential Inhibitor Of 2019 Novel Coronavirus Main Protease With Strong Receptor-Binding Domain (RBD), Molecular Docking And SAR Study", **2020**, Preprint.

4. **Serseg**, **T**., Benarous, K., Yousfi, M. "Hispidin and Lepidine E: Two Natural Compounds and Folic Acid as Potential Inhibitors Of 2019-Novel Coronavirus Main Protease (2019-Ncovmpro), Molecular Docking And SAR Study" Current Computer-Aided Drug Design, **2020**, 16 (1), 1-15, DOI: 10.2174/1573409916666200422075440 PMID: 32321407

5. **Serseg**, **T**., Benarous, K., Yousfi, M. "The Inhibitory Effect of Three Essential Oils On Candida Rugosa Lipase: In Vitro And In Silico Studies." The Natural Products Journal 10.3 (**2020**): 208-215.

6. Nbeg, H., Benarous K., **Serseg**, **T**., Lazreg, A., Hassani, H., Yousfi, M., "Inhibition of Candida Rugosa Lipase And Human Pancreatic Lipase By Polar Extracts From Seeds, Leaves And Roots of Thapsia Garganica : In Vitro and In Silico Studies". Endocrine, Metabolic & Immune Disorders - Drug Targets. Vol 19, **2019**, DOI : 10.2174/1871530319666190128122211.

7. **Serseg**, **T**., Benarous, K., "The Inhibitory Effect of Some Drugs on Candida Rugosa Lipase And Human Pancreatic Lipase: In Vitro and In Silico Studies," Endocrine, Metabolic & Immune Disorders - Drug Targets, **2018**;18(6):602-609, DOI: 10.2174/1871530318666180319093342.

International communications

1. **Serseg**, **T**., Benarous, K., Yousfi, M."The Anti-Alzheimer's Effect of The Essential Oil of Cannabis Sativa" Séminaire International Sur Les Sciences Naturelles et de la Vie, International Journal of Human Settlements, 19-20 February 2021.

Out of the thesis topic

2. **Serseg**, **T**., Benarous, K., Yousfi, M., "In Silico Identification of Potential Inhibitors of SARS-CoV-2 Main Protease From Punica Granatum and Nigella Sativa: A Molecular Docking Study, International Conference: Impact of COVID 19 on The Environment 1 (Icovid.19e20), The University of Blida: Saad Dahleb, 30-31 December 2020.

3. **Serseg**, **T**., Benarous, K., "Inhibition of Candida Rugosa Lipase by Globularia Alypum L For The Treatment Of Candidiasis Infections", SIPM-2018: Séminaire International Sur Les Plantes Médicinales, Echahid Hamma Lakhdar University, El-Oued, 17-18 January 2018.

4. **Serseg**, **T**., Benarous, K., "Inhibition of Candida Rugosa Lipase By Teucrium Polium L: In Silico And In Vitro Studies", IPA.ICV17: Séminaire International Sur Phytodiversité Et Plantes D'intérêt Ecologique Et

Economique En Algérie - Inventaire, Conservation Et Valorisation, University of M'sila, 29-30 October 2017.

5. **Serseg**, **T**., Benarous, K., "The Inhibitory Effect of Some Drugs On Candida Rugosa Lipase and Human Pancreatic Lipase: In Vitro And In Silico Studies", International Conference On (ICCESEN), Kemer-Antalya-Turkey, 4-8 October 2017

TABLE OF CONTENTS

CHAPTER I : LITERATURE REVIEW

INTRODUCTION **2**

I. Alzheimer's disease 6

I.1. General 6

I.2. Neurodegenerative Diseases and Dementia 6

I.3. Alzheimer’s Disease 7

I.3.1. Subtypes of Alzheimer’s disease 9

I.3.2. Neuropsychological symptoms of Alzheimer’s disease 9

I.3.3. Pathophysiology: Brain changes associated with Alzheimer's disease 9

I.3.4. Diagnosis of Alzheimer’s disease 11

I.3.5. Risk factors 13

I.4. Hypotheses of Alzheimer's disease pathology 14

I.4.1. Amyloid cascade hypothesis 14

I.4.2. The revitalized tau hypothesis 15

I.4.3. Genetics of Alzheimer's disease 16

I.4.4. The inflammatory hypothesis 16

I.4.5. The apolipoprotein E hypothesis 17

I.4.6. Oxidative stress hypothesis 18

I.5. Obesity, Diabetes and Alzheimer’s disease 19

I.6. Treatment 20

I.5.1. Specific treatments 20

I.5.2. Non-specific treatments 21

II. Cholinesterases **22**

II.1. Structure and function 22

II.2. Cholinesterase inhibitors 23

III. Monoacylglycerol lipase **24**

III.1. Structure and function 24

III.2. Monoacylglycerol lipase inhibitors 25

IV. Beta-secretase (BACE1) **26**

IV.1. Structure and function 26

IV.2. Beta-secretase inhibitors 26

V. Studied medicinal plants **27**

V.1. *Arbutus unedo* L 27

V.2. *Coriandrum sativum*........ 28

V.3. *Juniperus oxycedrus*........ 28

V.4. *Juniperus phoenicea* 29

V.5. *Lavandula stoechas* 30

V.6. *Saussurea costus* 31

V.7. *Lepidium sativum*........ 32

VI. Secondary metabolites **33**

VI.1. Terpenes 33

VI.2. Phenolic compounds 34

VI.3. Alkaloids 34

VII. Natural products for Alzheimer's disease **35**

VII. The number of publications about Alzheimer's disease in literature **36**

CHAPTER II : MATERIALS AND METHODS

I. Plant material **41**

II. Instrumentation **41**

III. Extraction **41**

III.1. Crude extracts preparation 41

III.2. Alkaloid extract preparation........ 42

IV. Phytochemical screening of the plant extracts **42**

V. Quantification of secondary metabolites **43**

V.1. Determination of total phenolic compounds ... 43
V.2. Determination of total flavonoids ... 45
VII. Acetylcholinesterase assay ... 46
VIII. In silico study ... 47
VIII.1. Receptors and molecules selection ... 47
VIII.1.1. Selection of receptors ... 47
VIII.1.1. Selection of ligands ... 48
VIII.2. ADMET and drug-likeness evaluation ... 52
VIII.3. Preparation of molecules and receptors ... 52
VIII.4. Molecular docking ... 53
X. Statistical analysis ... 53

CHAPTER III : RESULTS AND DISCUSSION

I. Extraction yield ... 55
II. Phytochemical screening of the plant extracts ... 58
III. Quantification of secondary metabolites ... 61
IV. Acetylcholinesterase assays ... 66
V. In silico study ... 72
V.1. ADMET and drug-likeness evaluation ... 72
V.2. Molecular docking ... 77
CONCLUSION ... 117
REFERENCES ... 120
APPENDICES n1 ... i

LIST OF TABLES

Table 1. The Major Components Of Studied Plants And Their Pubchem Ids. 49

Table 2. Target Drug Enzymes And Docking Parameters. 53

Table 3. The Weight, Yield And Appearance Of The Extracts 55

Table 4. Extraction Yield Of Studied Plants In Literature. 57

Table 5. The Results For The Presence Of Secondary Metabolites In Various Extracts 59

Table 6. The Results For The Presence Of Secondary Metabolites In Various Extracts In The Literature 60

Table 7. Total Phenols And Flavonoids Contents In Plant Extracts. 61

Table 8. Total Phenols And Flavonoids Contents In Plants Extracts In Literature 65

Table 9. Inhibitory Effects Of Plant Extract Against Acetylcholinesterase. 66

Table 10. Inhibitory Effects Of Plant Extracts Against Cholinesterases In Literatures 71

Table 11. Admet And Drug-Likeness Evaluation Of Some Molecules 74

Table 12. The Results Of Interactions Between Major Compounds And Drugs (Galantamine, Donepezil) And (Ache). 98

Table 13. The Results Of Interactions Between Major Compounds And Drugs (Galantamine, Donepezil) And (Bche). 102

Table 14. The Results Of Interactions Between Major Compounds And Drugs (Galantamine, Donepezil) And Magl 107

Table 15. The Results Of Interactions Between Major Compounds And Drugs (Galantamine, Donepezil) And Bace1 112

LIST OF FIGURES

Figure 1. Percentage change in selected causes of death between 2000 and 2018 (Alzheimer's association, 2020). 7
Figure 2. The defining pathological hallmarks of Alzheimer's disease (Congdon et Sigurdssen, 2018). 8
Figure 3. The Amyloid β plaques (in brown) and neurofibrillary tangles of tau protein (in black) (Nelson et al. 2012) 10
Figure 4. Examples of MR images of brain 12
Figure 5. The effect of overproduction (pathologic) of amyloid-b (Morley et al., 2018). 14
Figure 6. A possible cascade of events connecting peripheral metabolic dysregulation to dementia (Ferreira et al., 2018). 19
Figure 7. Reaction catalyzed by acetylcholinesterase. 22
Figure 8. *Arbutus unedo* L (a) whole plant, (b) used part (inaturalist, 2021a) 27
Figure 9. *Coriandrum sativum*; (a) whole plant, (b) used part (inaturalist, 2021b) 28
Figure 10. *Juniperus oxycedrus*; (a) whole plant, (b) used part. 29
Figure 11. *Juniperus phoenicea*; (a) whole plant, (b) used part (inaturalist, 2021c) 30
Figure 12. *Lavandula stoechas*; (a) whole plant, (b) used part (inaturalist, 2021d,e) 31
Figure 13. *Saussurea costus*; (a) whole plant, (b) used part (Nadda et al., 2020) 31
Figure 14. *Lepidium sativum*; (a) whole plant, (b) used part (inaturalist, 2021f) 32
Figure 15. Nature publisher publications about alzheimer's disease 37
Figure 16. Number of publication in 2021 according to keywords (1-5) in databases 38
Figure 17. Number of publication in 2021 according to keywords (6-17) in databases 38
Figure 18. Publications about alzheimer's disease (Affiliation: algeria) 39
Figure 19. The standard curve of gallic acid. 44
Figure 20. The standard curve of quercetin. 46
Figure 21. Total phenolic content 62
Figure 22. Total flavonoid content 63
Figure 23. The inhibitory curves of studied extracts against AChEee 67
Figure 24. The binding regions of the *Torpedo californica* ache gorge (Houghton et al., 2006) ... 77
Figure 25. Binding mode of gallocatechin at the active site of HuAChE 78
Figure 26. Binding mode of myricetin at the active site of HuAChE 80

Figure 27. Binding mode of hesperidin at the active site of HuAChE 81
Figure 28. Binding mode of 3-p-coumaroylquinic acid at the active site of HuAChE 83
Figure 29. Binding mode of rosmarinic acid at the active site of HuAChE 85
Figure 30. Binding mode of lepidine b (a.) And lepidine e (b.) At the active site of HuAChE 88
Figure 31. Binding mode of galantamine (a.) And donepezil (b.) At the active site of HuAChE ... 90
Figure 32. Binding mode of lepidine b (a) and lepidine e (b) at the active site of BuAChE........... 93
Figure 33. Binding mode of galantamine (a) and donepezil (b) at the active site of BuAChE 94

LIST OF ABBREVIATIONS

2–AG: 2–Arachidonoylglycerol
Aβ: Amyloid– beta
ABP: Acyl binding pocket
ACh: Acetylcholine
AChE: Acetylcholinesterase
AChEee: Acetylcholinesterase electric eel
AD: Alzheimer's disease
ADI: Alzheimer's Disease International
ADMET: Absorption, distribution, metabolism, and excretion – toxicity
AMTS: Abbreviated mental test score
APOE: Apolipoprotein E
APP: Amyloid precursor protein
AS: Anionic sub–site
BACE1: β–secretase
BBB: Blood–brain barrier
BCh: Butyrylcholine
BChE: Butyrylcholinesterase
ChIs: Cholinesterase inhibitors
CNS: Central nervous system
CSF: Cerebrospinal fluid
CT: Computerized tomography
DNA: Deoxyribonucleic acid
DTNB: 5,5'–dithiobis–(2–nitrobenzoic acid
FDA: US Food and Drug Administration
FDG: Fluorodeoxyglucose
FFAs: free fatty acids
GAE.g^{-1}/DW: Gallic acid equivalents per g of dry weight
GBD: The global burden of diseases
GSK–3β: Glycogen synthase kinase 3 beta
H–bond: Hydrogen bond
HSL: Hormone Sensitive Lipase
IC_{50}: The half maximal inhibitory concentration
IL: Interleukin
MAGL: Monoacylglycerol lipase
MMSE: Mini–mental state examination
MRI: Magnetic resonance imaging
ND: Neurodegenerative
NFT: Neurofibrillary tangles
NLRP: Nucleotide–binding oligomerization domain, Leucine rich Repeat and Pyrin domain containing
OS: oxidative stress
PAS: Peripheral anionic site
PET: Positron emission tomography
QE.g^{-1}/DW: Qercetin equivalents per g of dry weight
RNA: Ribonucleic acid
ROS: Reactive oxygen species
SMILES: Simplified Molecular–Input Line–Entry System.
UV: Ultraviolet

Introduction

In recent decades, the increasing interest in natural products has encouraged the scientific community to obtain information about secondary metabolites and their importance in medicine, human nutrition, and the food industry (**Oliveira et al**., **2009**); Across many nations, medicinal plants have been attracting attention as a prospective alternative resource of therapy for many diseases (**Sheng et al**., **1997; Che et al**., **2013; Batiha et al**., **2020**), due to the perception that these plants being natural products have lesser side effects and improved efficacy than their synthetic counterparts (**Che et al**., **2013; Batiha et al**., **2019**), and the multiple risks of chemical compounds such as environmental problems, drug resistance, side effects, and high production costs. (**Rajaian et al**., **2002; Zarei et al**., **2015**). Many plant species have been reported to have pharmacological activities attributable to their constituents such as flavonoids glycosides, tannins, saponins, alkaloids, steroids, terpenes, etc. (**Rocha et al**., **2005; Adeyemi et al**., **2019; Batiha et al**., **2020**).

In Algeria, medicinal plants in all their forms are still widely used. Due to its climate variability, Algeria has rich and varied medicinal plants, and as a result, has had a long history in traditional medicine, this fact makes it so important to pay attention to these precious resources.

Alzheimer's disease (AD) is the most common form of progressive dementia in the elderly (**Atlam et al**., **2018**). AD affects more than 50 million people worldwide. Every year, there are nearly 10 million new cases (**Maitre et al**., **2017; WHO**, **2018**). It is a neurodegenerative disorder characterized by intracellular neurofibrillary tangles (NFT) and extracellular amyloid plaques in vulnerable brain regions (**Terry et al**., **1980; Sennvik et al**., **2000**). The onset of Alzheimer's Disease is usually subtle, with lessened initiative and some forgetfulness. A decline in judgment, progressive difficulty with word-finding, and loss of memory (especially recent) soon become evident, leading to

disorientation and behavioral abnormalities with changes in the mood (**Terry et al**., **1980; Maitre et al**., **2017**).

Acetylcholinesterase (AChE) is a key enzyme involved in the termination of nerve signals through the hydrolysis of acetylcholine (**Alpan et al**., **2013**). AChE participates in other functions related to neuronal development, differentiation, adhesion, and Aβ processing (**Fernández-Bachiller et al**., **2010**). Butyrylcholinesterase (BChE) is an enzyme involved in cholinergic neurotransmission (**Fernández-Bachiller et al**., **2010**). A deficiency in levels of the neuromediators acetylcholine (ACh) and butyrylcholine (BCh) has been observed in the brains of AD patients due to the loss of cholinergic neurons. Hence, inhibitors of Acetylcholinesterase and BChE such as tacrine, galantamine, rivastigmine, and donepezil enhance the acetylcholine concentration in the brain (**Schneider**, **2001; Pilotto et al**., **2009; Alptüzün et al**., **2010; Şenol et al**., **2010**). They have been introduced to the market for treating mild-to-moderate AD, but with limited success and efficiency in the current clinical therapy (**Bautista-Aguilera et al**., **2014**).

The understanding of the interaction between molecules to form a complex was an issue of concern for many researchers until the advent of molecular docking and other bioinformatics tools (**Simard**, **2012**), Molecular docking methodologies can be used to identify the interaction between a small drug-like ligand, and a target molecule to predict a reliable binding affinity, in which the best possible physicochemical prediction of how the target and ligand will interact; a strategy to enhance the selection of drug candidate ligands is based on the scores obtained from *insilico* approaches; these scores decrease the amount of unnecessary biological tests and significantly reduce the number of inefficient compounds synthesized by taking into account valuable information about crucial binding elements in a given ligand-receptor conglomerate (**Meng et al**., **2011; Menchaca et al**., **2020**). The scientific community has progressively shown an increasing interest in molecular docking methods, demonstrated by the increase in publications number in the field.

Although the previously published studies about the anticholinesterase effect of medicinal plants, a few studies have been conducted to evaluate the anticholinesterase, MAGL and BACE1 effect of medicinal plants.

Therefore, in our present work, we have explored the possibility of inhibiting cholinesterase by Algerian medicinal plants.

The thesis is divided into four experimental parts: i) Extraction of secondary metabolites, ii) Qualification and quantification of secondary metabolites such as total phenolic compounds and flavonoids, iii) Evaluation of the inhibitory effect of obtained extracts against acetylcholinesterase enzyme, and finely iv) Explore and prediction the interactions between major compounds of the studied plants and the main AD targets using the molecular docking.

All these contents were arranged in three chapters, as follow:

The first chapter is a theoretical framework outlined earlier in the thesis which describes a brief review from literature about Alzheimer's disease, main AD targets, a short presentation of the studied plants, and secondary metabolites.

The second chapter lists all materials and methodology used throughout the work.

In **the third chapter**, all the experimental results are presented, examined and discussed in detail.

Lastly, a **conclusion** summarizes all the obtained results of our work as well as recommendations and perspectives for further and complementary studies.

Chapter I: Literature Review

I. Alzheimer's disease

"I have lost my self!" Auguste D, 1901.

I.1. General

The disease was named by Kraepelin Emil after the German physician Alois Alzheimer (**Kraepelin, 1910**) who, in 1907, described for the first time the anatomical changes observed in the brain of a patient of 51 years, Auguste D (**Alzheimer, 1906**).

In 1901, Auguste D was asked by Dr. Alzheimer about her name, she could not remember, then looks at the doctor and said, "*I have lost myself*" (German: "*Ich habe mich verloren.*"), and she ran out from the isolated room screaming, "*I will not be cut. I do not cut myself.*". She became completely demented after many years (**Maurer et al., 1997**).

I.2. Neurodegenerative Diseases and Dementia

Neurodegenerative diseases are a wide range of neurological disorders, with heterogeneous pathological and clinical expressions (**Przedborski et al., 2003**). They feature by relentlessly progressive loss of neurons, provoked by unknown reasons. The most typical observed symptoms of ND are Dementia and movement disorders (**Shastry, 2003**). ND are usually classified based on the location and type of neuropathological damage or change, predominant clinical feature, or a combination of both (**Przedborski et al., 2003**).

Dementia is a general term, which is defined as a group of symptoms that results from the disruption of brain functions such as memory, thinking, language, perception, behavior, personality, and social relationships and is not regarded as a normal sign of aging. It can be obviously observed dementia in at least 50 different diseases (**Tomlinson, 1977**). Where dementia is not confined to ND; it is also commonly observed in ischemic, toxic, metabolic, infectious, and traumatic brain injury (**Tomlinson, 1977; Przedborski et al., 2003**). Furthermore, Parkinson's disease and Alzheimer's disease are considered among the most specific causes of dementia (**Han et al., 2018**).

In 2019, ADI (Alzheimer's Disease International) reports that approximately 50 million people are living with dementia worldwide. There are nearly 10 million new cases each year worldwide (**WHO**, **2020**). The number is projected to nearly triple by 2050 reaching 131.5 million people worldwide (**Prince et al**., **2015**).

I.3. Alzheimer's Disease

Alzheimer's disease is the most common cause of dementia, which accounts for 60-90% of the cases (**Sulkava et al**., **1983; Alzheimer's Association**, **2020**). According to a 2015 report by Alzheimer's Disease International 46 million people worldwide are estimated to suffer from AD (**Santamaría et al**., **2020**). Furthermore, this number is supposed to increase to twice every 20 years (**Yiannopoulou et al**., **2020**).

Dementia is the fifth leading cause of death globally (fig. 1), According to GBD (The global burden of diseases) study, 2,382,129 globally deaths have been recorded between 1990 and 2016. While, 11,868 deaths in Algeria in the same period (**Nichols et al**., **2019**).

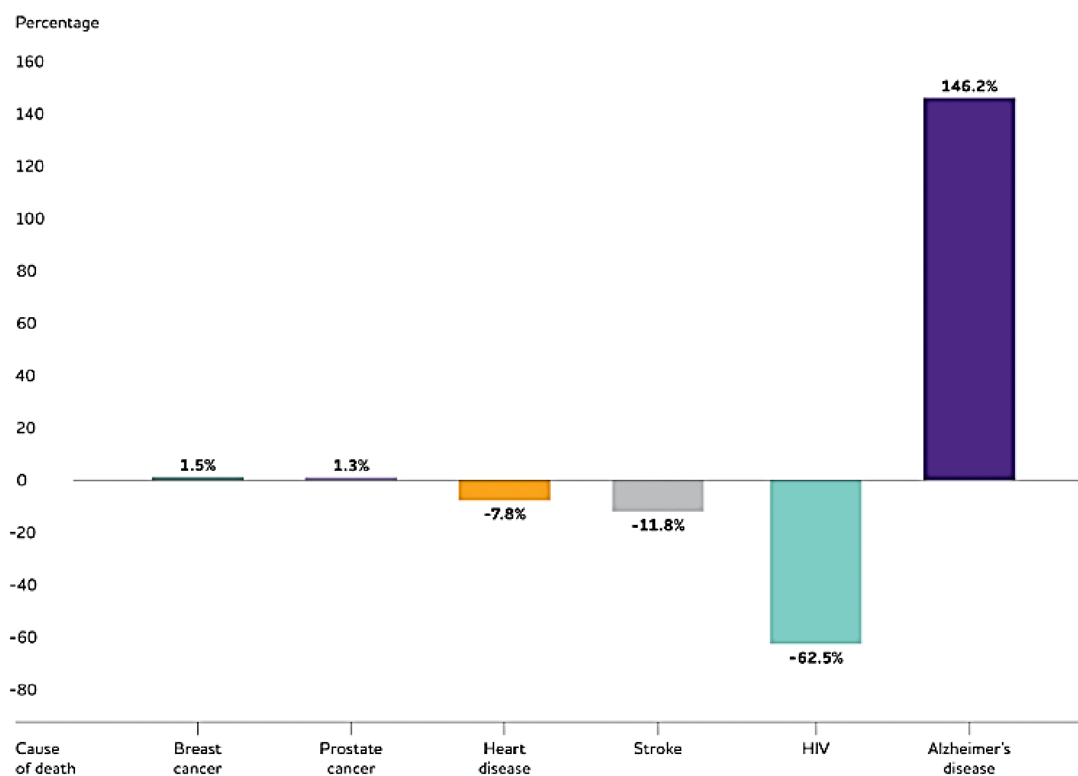


Figure 1. Percentage change in selected causes of death between 2000 and 2018 (**Alzheimer's Association**, 2020).

AD is defined as a neurodegenerative disease that affects different areas of the brain. (**Chomel-Guillaume et al**., **2010; Daily**, **2007**) marked by reduced neuronal function, leading to synaptic loss and neuronal death, this leads to permanent and irreversible cognitive impairment, impacts functional capacity, and steadily gives rise to dependence and disability (**Morris et al**., **2014; Cummings et al**., **2016; Dubois et al**., **2016; Santamaría et al**., **2020**).

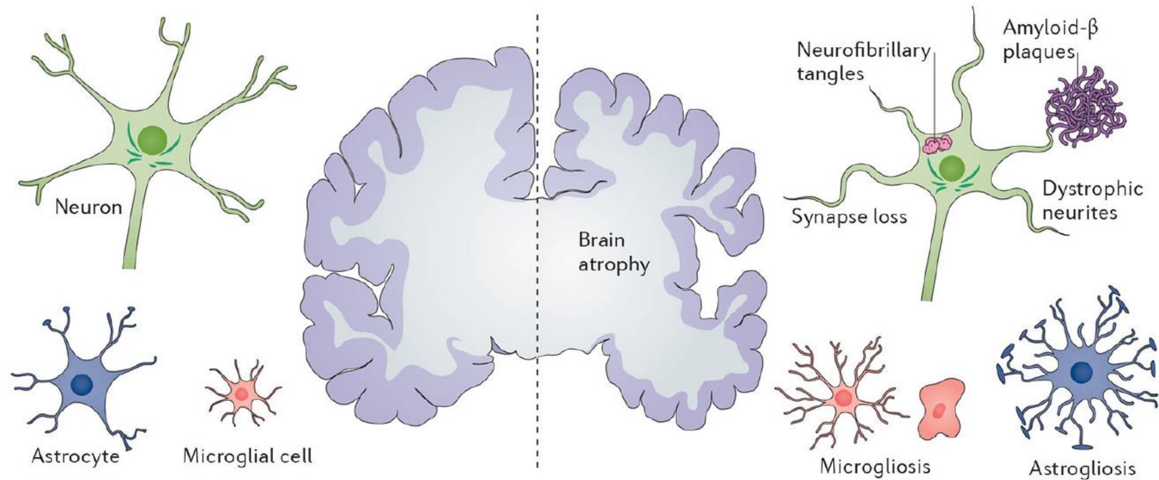


Figure 2. **The defining pathological hallmarks of Alzheimer's disease** (**Congdon et Sigurdssen**, **2018**).

Current research suggests that the two main hallmarks of the disease are: the extracellular amyloid plaques, composed mainly of amyloid-β (Aβ) and the intraneuronal neurofibrillary tangles (NFTs), the main constituent of which is the tau protein, these observations are associated with dystrophic neurites and loss of synapses as well as microgliosis and astrogliosis (fig. 2)(**Yiannopoulou et al**., **2013; Congdon and Sigurdsson**, **2018**).

Yet, In spite of all strenuous research endeavors, currently, there is no effective treatment for the disease (**Scheltens et al**., **2016**, **Cummings et al**., **2019**, **Yiannopoulou et al**., **2020**).

I.3.1. Subtypes of Alzheimer's disease

Four distinct subtypes of AD have been identified in several neuropathology and neuroimaging studies, based on the distribution of tau-related pathology and regional brain atrophy: Typical, Limbicpredominant, Hippocampal-sparing, and Minimal atrophy, had a pooled frequency of 55%, 21%, 17% and 15% respectively (**Ferreira et al., 2017; Ferreira et al., 2020**).

It has been found several differences between-subtype in age at onset, age at assessment, disease duration, sex distribution, global cognitive status, years of education, APOEe4 (apolipoprotein E) genotype, and CSF (cerebrospinal fluid) biomarker levels (**Scheltens et al., 2015; Ferreira et al., 2020**).

I.3.2. Neuropsychological symptoms of Alzheimer's disease

The basic symptoms of AD are impairment in memory and cognitive function (**Shinosaki et al., 2000**). The neuropsychological symptoms of Alzheimer disease include (**Donovan et al., 2018; Morley et al., 2018**):

- **Cognitive symptoms**: ***Memory:*** Amnesia, delayed recall failure, inability to recall over-learned facts, and working memory (ability to remember information in the face of distraction (**Baddeley et al., 2010**)); ***Language:*** Deficits in language abilities such as paraphasias, deficit in verbal fluency, and semantic categorization.

- **Behavioral symptoms**: Attention deficits, failure of dual-tasking, visuospatial deficits, functional impairment, development of apathy early in the disease, and agitation and other behavioral disturbances later in the disease.

I.3.3. Pathophysiology: Brain changes associated with Alzheimer's disease

The brain of AD patients can lose by 100–200 grams of its weight (**Budson et al., 2016**). The formation of amyloid plaques between neurons which are called senile

plaques, and the tau protein aggregates (neurofibrillary tangles) are the processes responsible for the disease (Fig.3) (**Yiannopoulou et al**., **2013; Congdon and Sigurdsson**, **2018**), the mechanism by which this occurs is unknown (**Small and McLean**, **1999**), but Aβ oligomers appears to associate with membrane proteins in the synapse, astrocytes and microglia, the synaptic receptors increase the Ca^{2+} concentration in the post-synapse leading to inflammation and cell death (**Sengupta et al**., **2016**), Besides, it has believed that the spreads of pathological tau between connected neurons, causes eventually accumulating in the somatic compartment where catastrophic nuclear damage ensues (**Ray and Buggia-Prevot**, **2021**).

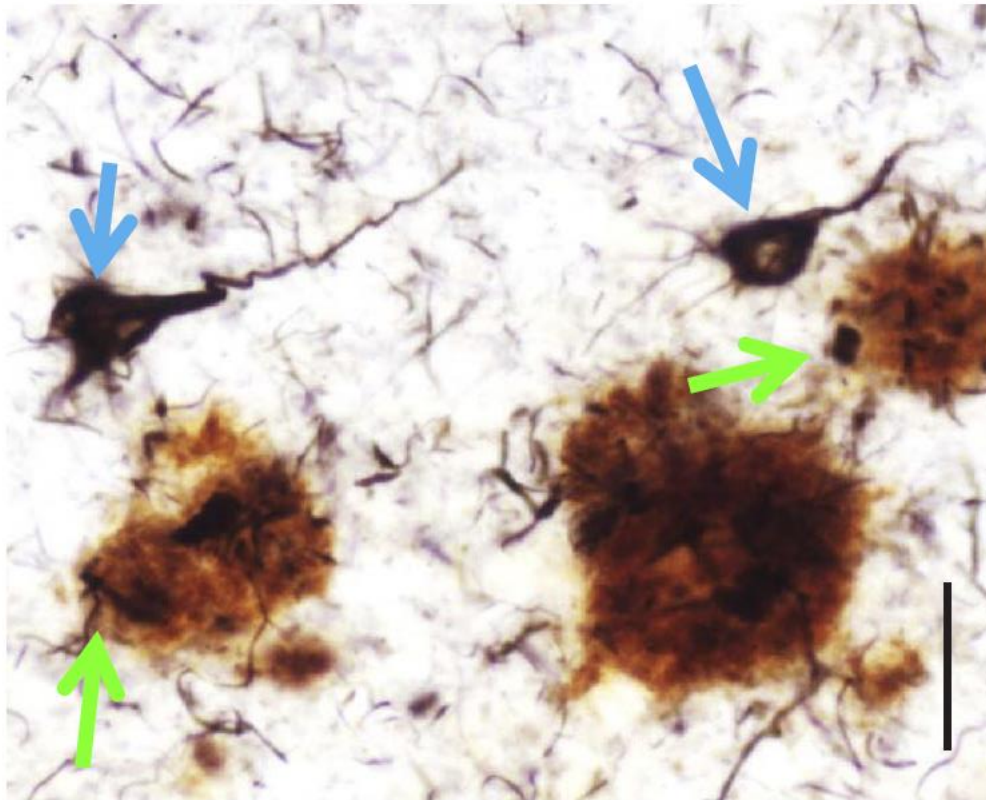


Figure 3. **The amyloid β plaques (in brown) and neurofibrillary tangles of tau protein (in black) (Nelson et al. 2012)**

I.3.4. Diagnosis of Alzheimer's disease

In addition to different symptoms, Alzheimer's disease can diagnosis two complementary methods, survey tests, and brain imaging:

Clinical diagnosis: The diagnosis remains essentially clinical during the patient's life. The clinical diagnosis is based on the history of the disease, questioning of the entourage, neuropsychological and behavioral exploration, and clinical examination (**Guériot-Milandre et al**., **1997**). It can be validated by cognitive and neuropsychological tests, which are used to assess memory, language skills, problem-solving, and other abilities related to mental functioning (**Ouldjaoui**, **2011**). These tests include: The Folstein test, known as the Mini-mental state examination (MMSE) (**Morley et al**., **2018**), The Grober and Buschke test (**Grober et al**., **1988; Pasquier**, **1990**), The Hodkinson abbreviated mental test score (AMTS)(**Hodkinson**, **1972**), Geriatric mental state examination (GMS)(**McWilliam et al**., **2018**). Currently, both the Montreal cognitive assessment (MoCA) (**Nasreddine et al**., **2005**) and the St. Louis university mental status exam (SLUMS) (**Cruz-Oliver et al**., **2012**) are better tests (**Morley et al**., **2018**). The diagnostic criteria used in general are those of Mc Khann *et al.* (NINCDS-ADRDA) (**McKhann et al**., **1984**).

Brain imaging: The imaging techniques are used to help confirm the diagnosis of Alzheimer's disease. These include:

- Structural Magnetic resonance imaging (MRI) detects the decrease in hippocampal volume, the reduction in gray matter density, atrophy in the temporal lobe and angulate gyrus, and hippocampus (**fig.4**) (**Rathore et al**., **2017**).
- Functional MRI detects the abnormalities in the default mode network through the examination of blood flow to areas of the brain (**Buckner et al**., **2008**).

- Diffusion tensor maximum signal intensity ratio detects the breakdown of the brain barriers that regulate fluid diffusion through the measurement of water diffusion in the brain (**Xie et al**., **2008; Morley et al**., **2018**).
- Computerized tomography (CT) uses to obtain cross-sectional images of the brain using X-rays (**Jacoby et al**., **1980; Khachaturian**, **1985**).
- Retinal scans: Neurodegenerative changes occur in the retina, these include a choroidal thinning, a decrease in the retinal nerve fiber layer, and optic nerve volume, amyloid-b is present in drusen and the retina of persons with Alzheimer's disease. These changes can be detected using photographically or by using curcumin, which binds to amyloid-b, which has been used with fluorescence imaging to detect amyloid-b plaques in the retina (**Mahajan et al**., **2017**).
- Positron emission tomography (PET) scan is typically used in the research setting. It uses a radioactive substance to detect substances in the brain. It can be used to (a) identify brain regions with decreased glucose metabolism (Fluorodeoxyglucose (FDG) PET) (**Mosconi et al**., **2008**), (b) to find the increased amyloid burden in persons with Alzheimer's disease (Amyloid PET) (**Klunk et al**., **2004**), and (c) to measure hyperphosphorylated tau in paired helical fragments (Tau PET) (**Masdeu et al**., **2017; Xia et al**., **2017**).

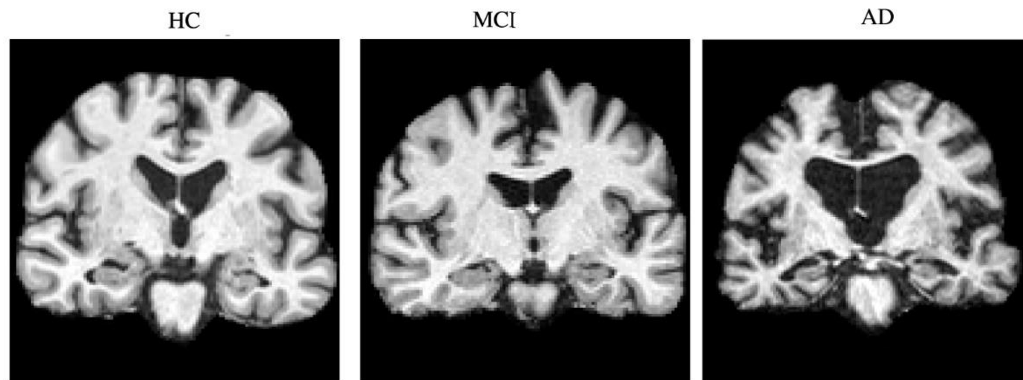


Figure 4. **Examples of MR images of brain**

HC: a healthy control subject (male, 84.8 years); MCI: mild cognitive impairment subject (female, 71.8 year); AD: Alzheimer's disease patient (male, 77.5 years) (**Ledig et al., 2018**).

Biomarkers: The two abnormally folded proteins: β-amyloid (Aβ) (extracellular plaques) and tau in (intracellular neurofibrillary tangles) are considered disease-specific biomarkers. Identification of these proteins in the brain (post-mortem) confirms the definite diagnosis of AD (**Kelényi et al**., **1967; Selkoe et al**., **2016; Bistaffa et al**., **2020**).

The definitive diagnosis of Alzheimer's disease is made post mortem. It is based on the presence of two types of lesions in large quantities in the hippocampal and associative cortical regions: these are neurofibrillary tangles and amyloid deposits. Cerebral atrophy and neuronal loss are usually observed (**Ball**, **et al**., **1977; Hyman et al**., **1984; Hyman et al**., **1990; Bobinski et al**., **1999**).

I.3.5. Risk factors

Although the exact causes of Alzheimer's disease are still unknown (**Lin et al**., **2013**), several factors are known to increase the risk of developing the condition. These include Age (**Guerreiro and Bras**, **2015**), gender: female (**Speck et al**., **1995**), family and genetic background (**Donix et al**., **2010; Khanahmadi et al**., **2015**), DNA mutations in at least four genes (APP, PSEN1, PSEN2, and MAPT) predisposing to AD (**Chartier-Harlin et al**., **1991; Cruchaga et al**., **2012**), the ApoE4 gene (**Riedel et al**., **2016**), environmental factors (aluminum and heavy metals including mercury (**McLachlan**, **1986; Campbell**, **2002; Virk and Eslick**, **2015; Hussien et al**., **2018; Lee et al**., **2018; Bakulski et al**., **2020**), cardiovascular disease: All risk factors for cardiovascular disease (**Rosendorff et al**., **2007**), diabetes (**Arvanitakis et al**., **2004; Xu et al**., **2009**), obesity (**Kivipelto et al**., **2005; Naderali et al**., **2009; Alford et al**., **2018**) and head injuries (**Rasmusson et al**., **1995; Hicks et al**., **2019; Frigerio et al**., **2019**).

I.4. Hypotheses of Alzheimer's disease pathology

I.4.1. Amyloid cascade hypothesis

According to the "amyloid hypothesis" Aβ production in the brain start up a cascade of events conducive to the clinical syndrome of AD, the elements of the cascade include tau hyperphosphorylation, local inflammation, excitoxicity (excessive glutamate), and oxidation (**Yiannopoulou et al., 2013; Yiannopoulou and Papageorgiou, 2020**). It has been found that injecting pathologic amounts of amyloid peptide directly into the mouse brain causes amnesia (**Flood et al., 1991; Flood et al., 1994; Morley et al., 2018**). Other studies suggest that the role of amyloid is to enhance memory by increasing the release of acetylcholine in the hippocampus (**Morley et al., 2010**), and it inhibits memory only when their levels become pathologic (**fig.5**) (**Morley et al., 2018**).

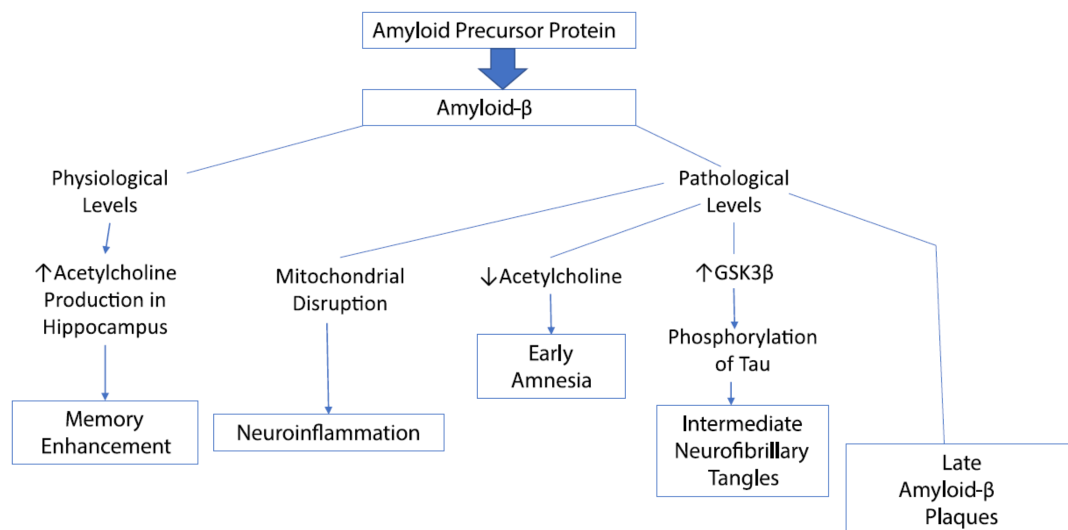


Figure 5. The effect of overproduction (pathologic) of amyloid–b (Morley et al., 2018).

Aβ peptides (Aβ38, Aβ40, and Aβ42 variants) are produced after the sequential cleavage of the large precursor protein amyloid precursor protein (APP) by two enzymes, β–secretase (BACE1) and γ–secretase. However, Aβ is not formed if APP is first cleaved by α–secretase instead of β–secretase (**Anand et al., 2010; Sakono and Zako,**

2010). Aβ monomers aggregate to form assemblies that exist in several forms including oligomers, protofibrils, and amyloid fibrils. Amyloid oligomers are soluble, while amyloid fibrils are larger, insoluble, and highly proteolysis-resistant, and they can further assemble into amyloid plaques (**Ngoungoure et al**., **2015; Chen et al**., **2017; Cline et al**., **2018**).

Clinical trials' results have shown that removing the amyloid plaques will not reverse the damage or stop Alzheimer's disease (**Cappai and Barnham**, **2008; Hardy**, **2009; Kayed and Lasagna-Reeves**, **2010**).

I.4.2. The revitalized tau hypothesis

Neurofibrillary tangles (NFTs) which composed of hyperphosphorylated tau protein (τ) (**Grundke-Iqbal et al**., **1986; Nukina and Ihara**, **1986**) have been proposed as the second pathological hallmark of AD (**Ittner and Götz**, **2011; Cai and Tammineni**, **2017**). Increasing evidence suggests that abnormally hyperphosphorylated tau plays a crucial role in the pathogenesis of AD (**Cheng and Bai**, **2018**). Tau protein as a major microtubule-associated protein plays a vital role in the neuronal transport system by binding microtubules in cells (**Amos**, **2004; Cheng and Bai**, **2018; Yiannopoulou and Papageorgiou**, **2020**). Abnormally hyperphosphorylated and aggregated tau seems to damage the axonal transport and synaptic dysfunction (**Eckert et al**., **2014**) by separating from microtubules which inhibit transport (**Anand et al**., **2017**), and leads to shortage and imbalance between neurotransmitters (eg, acetylcholine, dopamine, serotonin) (**Yiannopoulou and Papageorgiou**, **2013; Yiannopoulou and Papageorgiou**, **2020**).

It has been observed that the pathological tau can be developed in the brain decades before the onset of symptoms (**Braak et al**., **2006; Braak et al**., **2011; Wharton et al**., **2016; Congdon and Sigurdsson 2018**). Even though, AD mice models exhibited cognitive impairment before observation of NFTs (**Mondragón-Rodríguez et al**., **2013**).

I.4.3. Genetics of Alzheimer's disease

More than 30% of AD cases can be attributed to the APOE-ε4 allele (**Louwersheimer et al**., **2017**), and approximately 5% of AD case is caused by mutations in three genes that involved in Aβ processing, for either APP (chromosome 21) or enzymes that control its metabolism including presenilin 1 (chromosome 14) and presenilin 2 (chromosome 1) (**Goate et al**., **1991; Levy-Lahad et al**., **1995; Sherrington et al**., **1995; Louwersheimer et al**., **2017; Santamaría et al**., **2020**), These mutations linked to familial forms, inherited in an autosomal dominant fashion, and cause early-onset disease (**Morley et al**., **2018; Santamaría et al**., **2020**). Whereas, 8% of AD cases can be attributed to any of the additional genetic risk loci (SORL1, BIN1, CR1, CLU, PICALM, ABCA7, FERMT2, CAAS4, MS4A6A, EPHA1, HLA-DRB5/HLA-DRB1 locus, PTK2B, CD2AP, ZCWPW1 locus, SLC24A4/RIN3 locus, INPP5D, MEF2C, NME8 locus, CELF1 locus, and CD33) detected in the genome-wide association studies (**Cauwenberghe et al**., **2016**, **Louwersheimer et al**., **2017**).

I.4.4. The inflammatory hypothesis

It is believed that microglia play a vital role in AD pathogenesis (**Van Furth**, **1982; Perlmutter et al**., **1990; McGeer and Rogers**, **1992**).

Several in vitro studies found that microglia secrete a large group of inflammatory factors including ROS (reactive oxygen species) (**Coraci et al**. **2002; Wyss-Coray and Rogers**, **2012**), the elevation of these factors was found in pathologically-vulnerable regions of the AD brain (**Akiyama et al**., **2000; Rogers et al**., **2007; Landreth and Reed-Geaghan 2009**). The inflammatory factors: Interleukin 1β, TNFα, and other cytokines were believed to impair neuronal function by suppression of long-term potentiation of synaptic transmission (**Heneka et al**., **2013**), and the elevated concentrations of the proinflammatory cytokine TNFα and decreased concentrations of anti-inflammatory

TGF-β in the CSF increase the risk for conversion from mild cognitive impairment to the dementia stage of Alzheimer's disease patients (**Tarkowski et al**., **2003**).

Besides the multiple stimuli for the inflammatory responses of microglia in the AD brain (**Wyss-Coray and Rogers**, **2012**), deposition of the amyloid beta peptide (Aβ) also activates microglia (**Lucin and Wyss-Coray**, **2009; Prinz et al**., **2011**), where microglia are able to bind to soluble amyloid β (Aβ) oligomers and Aβ fibrils via cell-surface receptors (**Paresce et al**., **1996; Bamberger et al**., **2003; Heneka et al**., **2015; Sikorska et al**., **2020**). Aβ activates NLRP3 inflammasome in microglia leading to IL-1β maturation and subsequent inflammatory events (**Halle et al**., **2008**), where the uptaking of Aβ causes lysosomes' damage, which leading to the assembly of the NLRP3 inflammasome and the activation of caspase-1. Caspase-1 then cleaves pro-IL-1β to releases IL-1β (**Heneka et al**., **2013; Li et al**., **2019; Sun et al**., **2019; Guan et al**., **2020**). Furthermore, the synergistic action of Aβ with either interferon γ or CD40 ligand triggers TNFα secretion and production of neurotoxic reactive oxygen species (**Heneka et al**., **2015**).

Thus, NLRP3 inflammasome inhibition is considered a novel therapeutic intervention for AD (**Tan et al**., **2013; Zhang et al**., **2020**). However, treatment trials with anti-inflammatory drugs have been disappointing (**Wyss-Coray and Rogers**, **2012**). Additionally, the suppressing of classical inflammatory proteins and cytokines may cause more harm than good (**Wyss-Coray and Rogers**, **2012**).

I.4.5. The apolipoprotein E hypothesis

Apolipoprotein E (ApoE) is a secreted apolipoprotein (**Kara et al**., **2018**), produced by astrocytes in the central nervous system (CNS) and by the liver and macrophages in peripheral tissues (**Liu et al**., **2013**). It plays a vital role in lipid transport and redistribution among the different organs and cells (**Corbo and Scacchi**, **1999**). ApoE gene is polymorphic (**Singh et al**., **2006**), with three main alleles ε2, ε3, and ε4 (**Kara et al**., **2018**).

Considering the ability of ApoE to bind to Aβ (**Jiang et al**., **2008; Carlo et al**., **2013**), several studies suggest that the ApoE protein plays a role in Aβ clearance (**Cole and Ard**, **2000; Hanson et al**., **2013**), yet the ApoE4 isoform is not effective as other isoforms, it is less able to bind to Aβ and clear it (**LaDu et al**., **1994; Walker et al**., **2000**), resulting in an increased risk of developing AD in individuals with ε4 allele (**Sando et al**., **2008**).

However, despite the many AD cases attributed to the ApoE-ε4 allele (**Van Cauwenbergh et al**., **2016**), some ApoE-ε4 homozygotes reach over 100 years and never lost their cognitive health (**Louwersheimer et al**., **2017**), up to 50% of people with AD do not carry the high-risk ε4 allele (**Farrer et al**., **1997**).

I.4.6. Oxidative stress hypothesis

Although oxidative stress (OS) is recognized to be associated with the normal aging process (**Kregel and Zhang**, **2007**), it has been observed an increase in oxidative stress in AD brain (**Kuo et al**., **2014; Uddin and Kabir**, **2019a**), which leads to proposed the important role of OS in the pathogenesis of neuron degeneration and death in AD (**Markesbery**, **1997**). Besides, biomarkers of OS: Lipide peroxidation, DNA, and protein oxidation was found in AD brains (**Praticò**, **2008; Uddin and Kabir**, **2019b**).

Reactive oxygen species (ROS) are formed in 1) mitochondria through cellular respiration, 2) immune system cells in the inflammatory response (**Cadenas and Davie**, **2000; Shadel and Horvath**, **2015; Videira and Castro-Caldas**, **2018**), 3) different environmental stressors including exogenous (e.g., UV, ionizing radiation, drugs, air pollution) and endogenous (e.g., mitochondrial and non-mitochondrial enzymes) (**Federico et al**., **2012; Kim et al**., **2015; Yeung et al**., **2020**). Due to the excessive generation of ROS and their high reactivity, they could interact with DNA, RNA, and proteins (oxidation) or lipids (peroxidation) (**Hannan et al**., **2020**), which lead to mitochondrial dysfunction, ROS overproduction, and eventually cell death and initiation of neurodegeneration (**Mancuso et al**., **2006; Wang et al**., **2014; Lee et al**., **2019; Yeung et al**., **2020**).

I.5. Obesity, Diabetes and Alzheimer's disease

Lately, the dysregulation of insulin signaling pathway is observed in Alzheimer's disease (AD) brain associated with downstream neuronal survival and plasticity (**Tramutola et al., 2020; Kellar and Craft, 2020**). This abnormality is known as insulin resistance which is linked to obesity and diabetes (**fig.6**). Strong evidence (**Despres and Lemieux, 2006; Goossens, 2008; Andersson, et al., 2008**) has proven that obesity represents the most powerful cause that leads to the development of insulin resistance and type 2 diabetes (**Murdolo et al., 2013**).

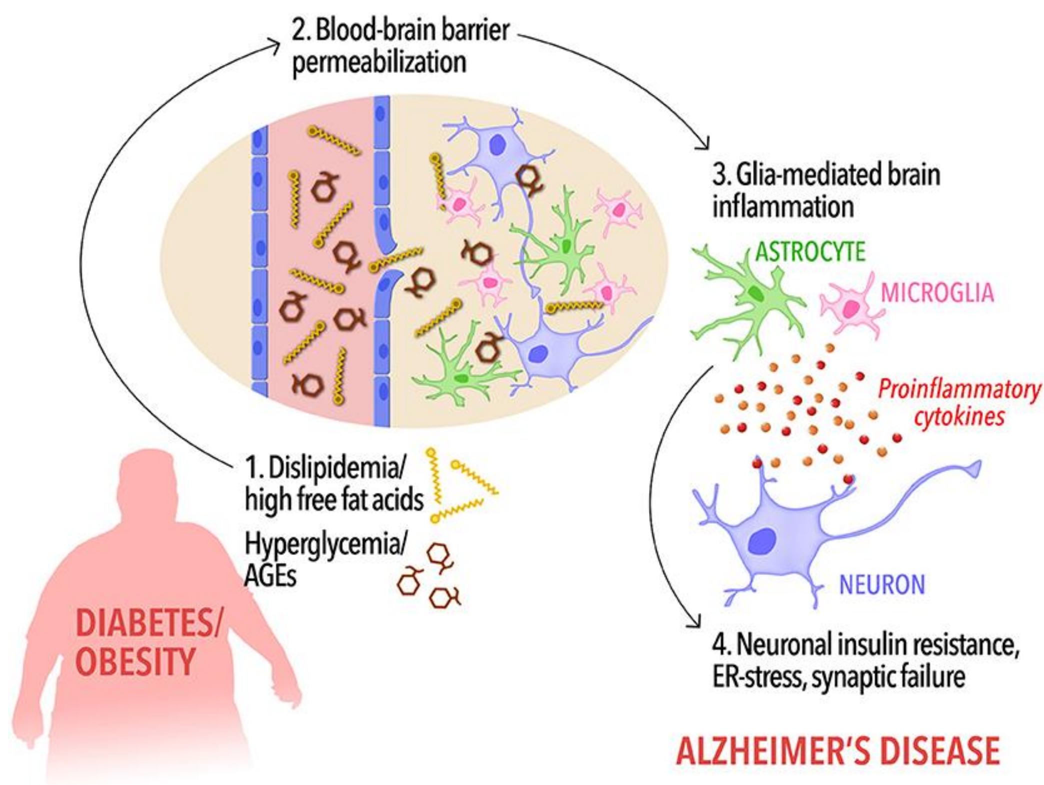


Figure 6. A possible cascade of events connecting peripheral metabolic dysregulation to dementia (**Ferreira et al.**, 2018).

It is well-known that Insulin inhibits adipose Hormone Sensitive Lipase (HSL) (**Gonzalez-Yanes and Sanchez-Margalet, 2006; Bustanji et al., 2010**). HSL is a lipase that catalyzes the hydrolysis of triglycerides in adipocytes leading to the release of free fatty acids (FFAs) (**Anthonsen et al., 1999; Osterlund, 2001**). Therefore, the development of

insulin resistance leads to disruption in the regulatory inhibition of HSL activity, which eventually leads to an increase in the levels of FFAs (**Postic and Girard**, **2008**). However, high FFAs levels are known to cause mitochondrial dysfunction (**Kuo et al**., **2012**), activate the inflammation pathway (**de Mello et al**., **2018**), increase the permeability of the blood-brain barrier (BBB) (**Patil and Chan**, **2005; Ferreira et al**., **2018**), contribute to the inhibition of glucose uptake, and suspected to augment insulin resistance (**Bergman and Ader**, **2000; Erion and Shulman**, **2010; Pfeiffer**, **2007**). These effects are exacerbated by obesity (**de Mello et al**., **2018**). From this point, the increase of FFAs is thought to be an early factor or initiator of the pathogenesis of AD (**Wilson and Binder**, **1997; Ferreira et al**., **2018**).

I.6. Treatment

Currently, there is no cure for AD (**Szczechowiak et al**., **2019**), However, several potential anti-AD drugs and strategies are currently being evaluated.

I.5.1. Specific treatments

The different hypothesis of AD pathology present multiple points where therapeutic intervention is possible, these include:

- Cholinesterase inhibitors: They inhibit the activity of acetylcholinesterases AChE or butyrylcholinesterase (BChE). Several AChE inhibitors were approved by the US Food and Drug Administration (FDA) for the treatment of AD patients such as donepezil, rivastigmine, tarcine, galanthamine (**Zhang et al**., **2018; Kabir et al**., **2019a; Sharma**, **2019**).
- NMDA antagonists' blockers: N-methyl-D-aspartate (NMDA) neural receptors are glutamate receptors that play an important role in synaptic plasticity (**Blanke and VanDongen**, **2009; Li and Ju**, **2012; Waqar and Batool**, **2017**), The inhibiting

its activity has been introduced as a possible target for treatment of AD (**Glynn-Servedio and Ranola**, **2017; Kabir et al**., **2019b**).

- Secretase inhibitors: β-secretase inhibitors (**Cole et al**., **2006; Moussa**, **2017**) and γ-secretase inhibitors (**Wolfe**, **2011; Golde et al**., **2013; Steiner et al**., **2018**) have been introduced in clinical studies to reduce the amyloid plaques in the brain (**Dovey et al**., **2001**).

- Monoacylglycerol lipase (MAGL) inhibitors: It is considered a promising drug for the treatment of neurodegenerative (**Glass et al**., **2010; Chen et al**., **2012; Deng et li**, **2020**). MAGL plays an important role in the regulation of endocannabinoid and eicosanoid signaling pathways (**Long et al**., **2009; Chanda et al**., **2010; Schlosburg et al**., **2010**) by degradation of 2-AG (2-Arachidonoylglycerol) (**Baggelaar et al**., **2018**). This latter plays an essential role in the regulation of many physiological processes such as neuroprotection (**Sánchez et García-Merino**, **2012**).

- Targeting tau protein modifications: Kinase inhibitors (**Noble et al**., **2005**), Inhibiting tau acetylation (**Min et al**., **2010; Min et al**., **2015**), Inhibiting tau deglycosylation (**Liu et al**., **2005; Smith et al**., **2016**) and Inhibiting tau truncation (**Congdon and Sigurdsson 2018; VandeVrede et al**., **2020**).

I.5.2. Non-specific treatments

It deals with the behavior and risk factors without targeting the disease itself:

- Several drugs are given to AD patients in order to treat the emotional behavior such as anxiety, irritability, aggression, sleep-wake, and circadian disruption or agitation states of patients (**Todd**, **2020**).

- Nutrition and other drugs targeting the risk factors of the disease can be an alternative therapeutic option in AD such as antioxidants, anti-inflammatory (**Szczechowiak et al**., **2019**), curcumin (**Congdon and Sigurdsson**, **2018; Pluta et al**., **2018;**

Mukherjee et al., 2019), Vitamin B complex (**Smith et al., 2016**), Vitamin D3 (**Annweiler et al., 2015**), Coffee and caffeine (**Madeira et al., 2017**). Some evidence shows that the Mediterranean diet (**Petersson et al., 2016; Anastasiou et al., 2017**), Extra virgin olive oil (**Farr et al., 2012; Martinez-Lapiscina et al., 2013**), omega-3 polyunsaturated fatty acids (**Petursdottir et al., 2008; Hooper et al., 2017; De Souto Barreto et al., 2018**), uridine, phospholipids (**Onakpoya et al., 2017**) ketone bodies (**Farr et al., 2012; Farr et al., 2016; Syarifah-Noratiqah et al., 2017**), alipoic acid, and polyphenols (**Ciavardelli et al., 2017**) may have some benefit against developing Alzheimer disease (**Morley et al., 2018**).

II. Cholinesterases

II.1. Structure and function

Acetylcholinesterase (AChE) (EC 3.1.1.7) is a key enzyme involved in the termination of nerve signals through the hydrolysis of acetylcholine (**fig. 7**) (**Alpan et al. 2013**) in both the autonomic and peripheral nervous systems (**Santamaría et al., 2020**). It also participates in other functions related to neuronal development, differentiation, adhesion, and Aβ processing (**Fernández-Bachiller et al., 2010**).

AChE isolated and sequenced for the first time from Pacific electric ray (*Torpedo californica*) and its 3D structure was elucidated in 1991 (**Sussman et al., 1991; Harel et al., 1994**).

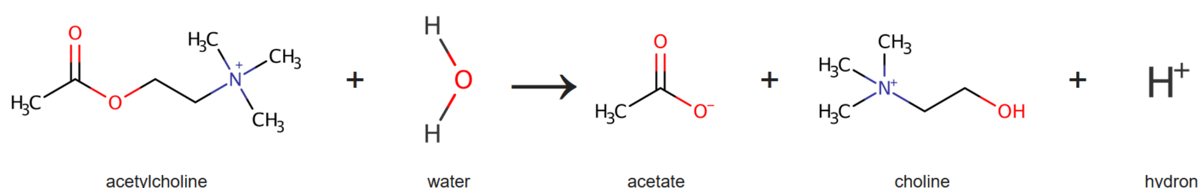


Figure 7. Reaction catalyzed by acetylcholinesterase.

It is thought that the initial binding of acetylcholine with the active site is occur at its outer rim of the gorge in a region called the "peripheral site". While the actual hydrolysis occurs at the bottom of the gorge, where, there are four main subsites, these

being the "esteratic site", the "oxyanion hole", the "anionic subsite" and the "acyl pocket". The esteratic subsite, where acetylcholine is hydrolyzed to acetate and choline, contains the catalytic triad of three amino acids: serine, histidine and glutamate (**Houghton et al**., **2006**).

Butyrylcholinesterase (BChE) (EC 3.1.1.8) also known as the "pseudo" or "non-neuronal" cholinesterase (**Mesulam et al**., **2002**), is an enzyme involved in cholinergic neurotransmission (**Fernández-Bachiller et al**., **2010**). BChE prefer to hydrolyze butyrylcholine over acetylechine (**Westfall**, **2009**). Previous studies have been reported the presence of BChE activity in the AD brains, which is associated with amyloid plaques and neurofibrillary tangles (**Geula and Mesulam**, **1989; Guillozet et al**., **1997; Nordberg et al., 2013**).

A deficiency in levels of the neuromediators acetylcholine (ACh) and butyrylcholine (BCh) has been observed in the brains of AD patients due to the loss of cholinergic neurons, Hence inhibitors of Acetylcholinesterase and BchE the key enzymes which hydrolyze acetylcholine and butyrylcholine, respectively, enhancing the acetylcholine concentration in the brain, have been introduced to the market for treating mild-to-moderate AD, but with limited success and efficiency in the current clinical therapy (**Bautista-Aguilera et al**., **2014**). AChE inhibitors are also used in the treatment of myasthenia garavis, glaucoma, and as insecticides (**Maggi and Mantegazza**, **2011; Cholkar et al**., **2015; Gupta et al**., **2019**).

II.2. Cholinesterase inhibitors

Well-known examples of FDA-approved AD medications are tacrine, galantamine, rivastigmine, and donepezil (**Alptüzün et al**., **2010; Şenol et al**., **2010**). According to **Miranda et al**. (**2015**), the rate of response to these drugs is only 27.8% (**Santamaría et al**., **2020**).

The most common gastrointestinal side effects of Donepezil include nausea, diarrhea, vomiting, and anorexia. Additionally, some patients developed muscle dizziness, cramps, syncope, insomnia, flushing, weakness, drowsiness, and fatigue (**Schneider et al**., **2001; Pilotto et al**., **2009**).

Galantamine (Reminyl), an alkaloid extracted from *Galanthus woronowi* and related species belonging to the Amaryllidaceae family. It is a reversible, competitive inhibitor of AChE with relatively less butyrylcholinesterase activity, adverse effects of Galantamine are nausea, diarrhea, vomiting, anorexia, weight loss, and dizziness (**Harvey**, **1995; Bores et al**., **1996; Schneider et al**., **2001; Konrath et al**., **2013**). Both donepezil and galantamine present highly affinity for AChE then to BChE (**Cacabelos**, **2007; Campos et al**., **2016; Santamaría et al**., **2020**).

Cholinesterase inhibitors (ChIs) effectively increase the amount of acetylcholine available for intrasynaptic cholinergic receptor stimulation (**Schneider et al**., **2001**). BChE activity seems to correlate with AChE activity in AD and a cognitive improvement could be reached therefore one of the promising approaches is the development of dual inhibitors for AChE and butyrylcholinesterase (BChE) (**Alptüzün et al**., **2010; Şenol et al**., **2010**).

Since 2003, No new drug for AD has been approved by FDA, and there are more than 200 research projects in the last decade have failed or have been abandoned (**Atri**, **2019; Yiannopoulou**, **2020**).

III. Monoacylglycerol lipase

III.1. Structure and function

Monoacylglycerol lipase (MAGL) is an enzyme belonging to the serine hydrolase superfamily metabolizing lipids (**Zechner et al**., **2009; Labar et al**., **2010**). As a member of serine hydrolase family, MAGL catalytic site is formed by the triad Ser-Asp-His, which are Ser122, Asp239, and His269 in the human MAGL (**Gil-Ordóñez et al**., **2018**).

MAGL hydrolyze the endogenous cannabinoid 2-arachidonoylglycerol (2-AG) (**Dinh et al**., **2002; Blankman et al**., **2007**) into arachidonic acid (AA) (**Dinh et al**., **2002**). 2-AG functions as a retrograde messenger in regulation or modulation of synaptic transmission and plasticity (**Alger**, **2009; Heifets and Castillo**, **2009; Kano et al**., **2009**), exhibits anti-inflammatory and neuroprotective properties (**Panikashvili et al**., **2001; Panikashvili et al**., **2005; Centonze et al**., **2007; Zhang and Chen**, **2008; Arevalo-Martin et al**., **2010; Scotter et al**., **2010; Chen et al**., **2011**) and promotes neurogenesis (**Gao et al**., **2010**). Moreover, β-amyloid promotes MAGL overexpression (**Stasiulewicz et al**., **2020**). Therefore, MAGL inhibition prevents neuroinflammation and decreases neurodegeneration (**Chen et al**., **2012**).

III.2. Monoacylglycerol lipase inhibitors

Most of the reported MAGL inhibitors share similar structural motifs. For instance, piperidine or piperazine rings and urea or carbamate groups are often connected together (**Deng et al**., **2020**). A couple of molecules have been reported as MAGL inhibitors so far, which are: (a) irreversible inhibitors: maleimides, disulfides, carbamates, ureas and arylthicarmide, (b) reversible inhibitors: tetrahydrolipstatin-based derivatives (β-lactones), isothiazolines, natural terpenoids, and amide-based derivatives, etc (**Dinh et al**., **2002**). It is found that MAGL interacts with sulfhydryl-sensitive inhibitors, due to the active cysteine residues near its active site (Cys201, Cys208, and Cys242) (**Saario et al**., **2015**). Remarkably, until now only one selective inhibitor of MAGL has entered to clinical trials, which was on June 2020 under number: NCT04419636.

IV. Beta-secretase (BACE1)

IV.1. Structure and function

The β-site amyloid precursor protein (APP)-cleaving enzyme 1 (BACE1), also known as β-secretase, is a key enzyme involved in Alzheimer's disease (**Sinha et al., 1999; Vassar et al., 1999; Yan et al., 1999; Botteri et al., 2018**). BACE1 belongs to aspartic proteases family, which typically have two active aspartate motifs, located in a separate lob (**Szecsi, 1992; Yan, 2016**). BACE1 is the principal neuronal protease required to cleave between residues Met671 and Asp672 that generate and extracellular release of the soluble APP-β fragment (**Seubert et al., 1993; Cai et al., 2001; Kimura et al., 2016**).

IV.2. Beta-secretase inhibitors

At present BACE1 Inhibitors are being considered for their ability to reduce cerebral Aβ concentrations and to treat and prevent Alzheimer's disease. Due to the large BACE1 catalytic pocket, the intense efforts to produce BACE1 inhibitors led to few compounds with in vitro half-maximal inhibitory concentrations (IC_{50}) in the nM range (**Luo and Yan, 2010; Yan, 2016**).

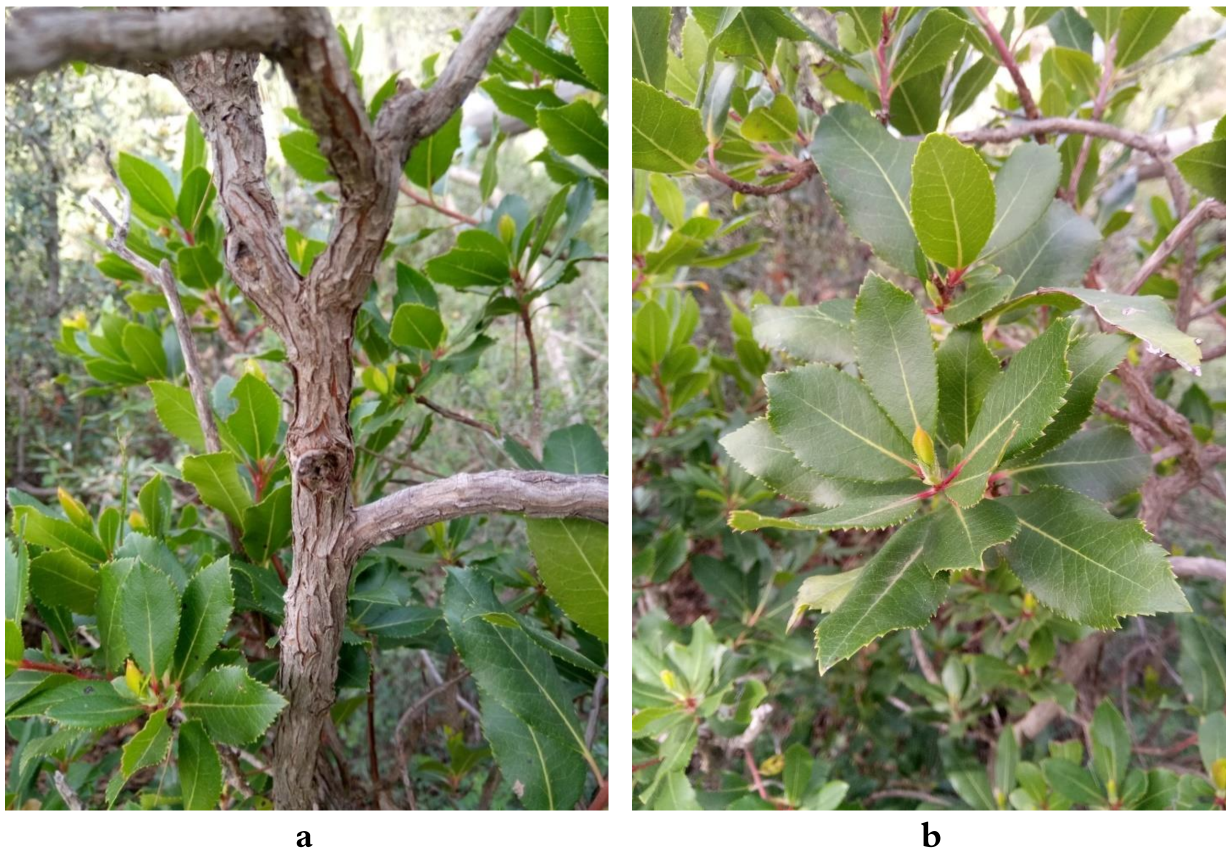
There is clear evidence that BACE1 inhibitors reduce Aβ generation and likely amyloid deposition (**Swahn et al., 2012**). Several promising BACE1 inhibitors are being tested in human clinical trials (**Ghosh et al., 2008; Yan and Vassar, 2014**). Many laboratories have terminated the development of BACE1 inhibitors, also some compounds were terminated during clinical trials, due to safety fears (**Yan and Vassar, 2014**), where It is reported to cause unacceptable side effects such as liver toxicity in patients (**Vassar, 2014**). Despite the concerns about the toxicity of BACE1 inhibitors and considering the relatively mild phenotypes exhibited by BACE1-null mice (**Luo**

et al., **2003**), it is thought that BACE1 is perhaps the best target for reducing Aβ generation (**Yan**, **2016**).

V. Studied medicinal plants

V.1. *Arbutus unedo* L

A. unedo, (Strawberry tree) (**fig**. **8**), belongs to the Ericaceae family, and it is native of the Mediterranean climate. It has been traditionally used as food. In folk medicine, the fruits and leaves of *A. unedo* are used to treats several diseases (**Ziyyat et al**., **1997**) such as: antiseptic, laxative, diuretic, astringent, urinary, antidiarrheal, depurative (**Oliveira et al**., **2009**).



a **b**

Figure 8. **Arbutus unedo L** (**a**) **whole plant**, (**b**) **used part** (**Inaturalist**, **2021a**)

V.2. *Coriandrum sativum*

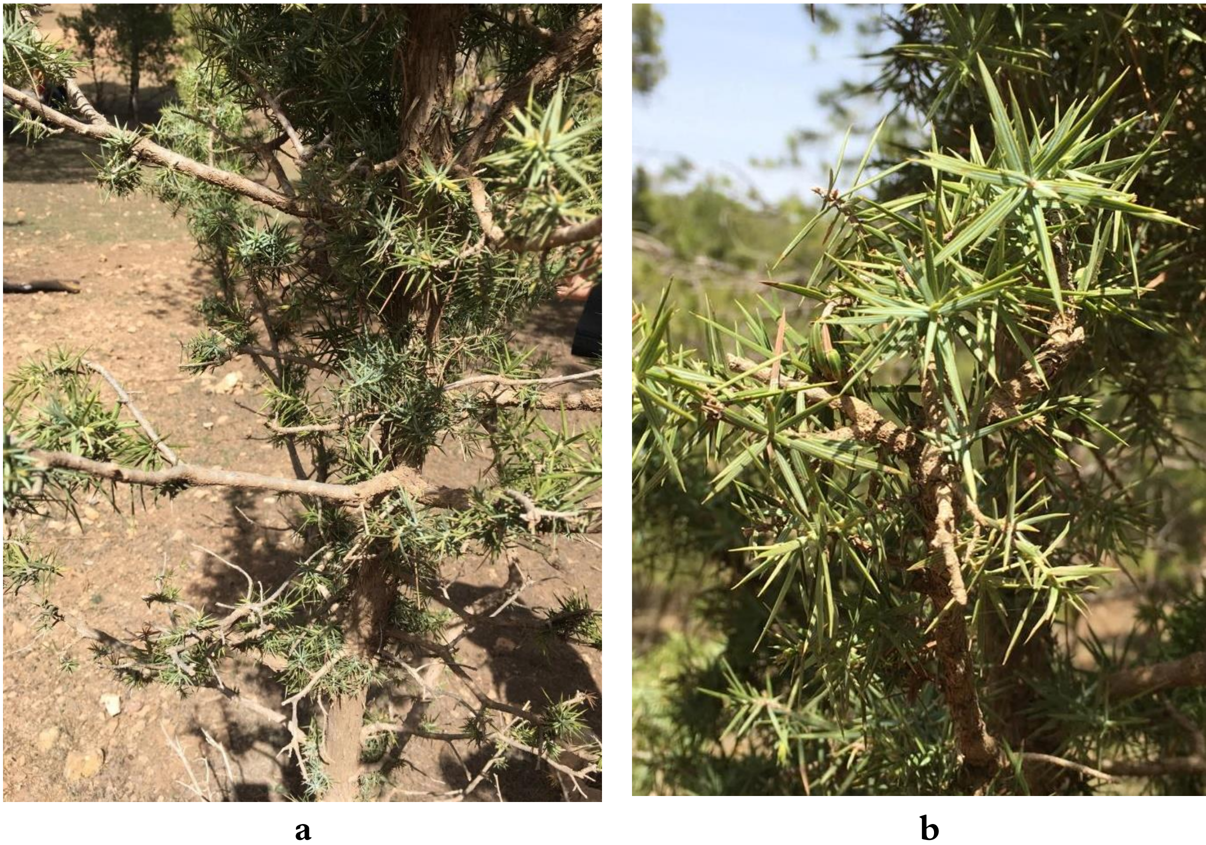
Coriander (*Coriandrum sativum* L.) is an aromatic annual herb (**fig. 9**), belonging to Apiaceae family, widely grown in North Africa and the Middle East. It is commonly used for its fresh leaves and dry powder of its fruits (commonly known as coriander seeds) for its organoleptic and flavoring properties (**Barros et al., 2012**).

a **b**

Figure 9. Coriandrum sativum; (a) whole plant, (b) used part (Inaturalist, 2021b)

V.3. *Juniperus oxycedrus*

Juniperus oxycedrus L. (Cupressaceae) (**fig. 10**), a dioecious species widely distributed in the Mediterranean region (**Ortiz et al., 1998**), appeared as a shrub or small tree (**Tavianoet et al. 2011**), used widely in folk medicine (**Ben Mrid et al., 2019**) and food as a spice and flavor. Besides, *J. oxycedrus is* used for parasitic disease, urinary infections and bronchitis (**Loizzoet al. 2007; Djebaili et al. 2013**).



a b

Figure 10. Juniperus oxycedrus; (a) whole plant, (b) used part (Serseg 2018).

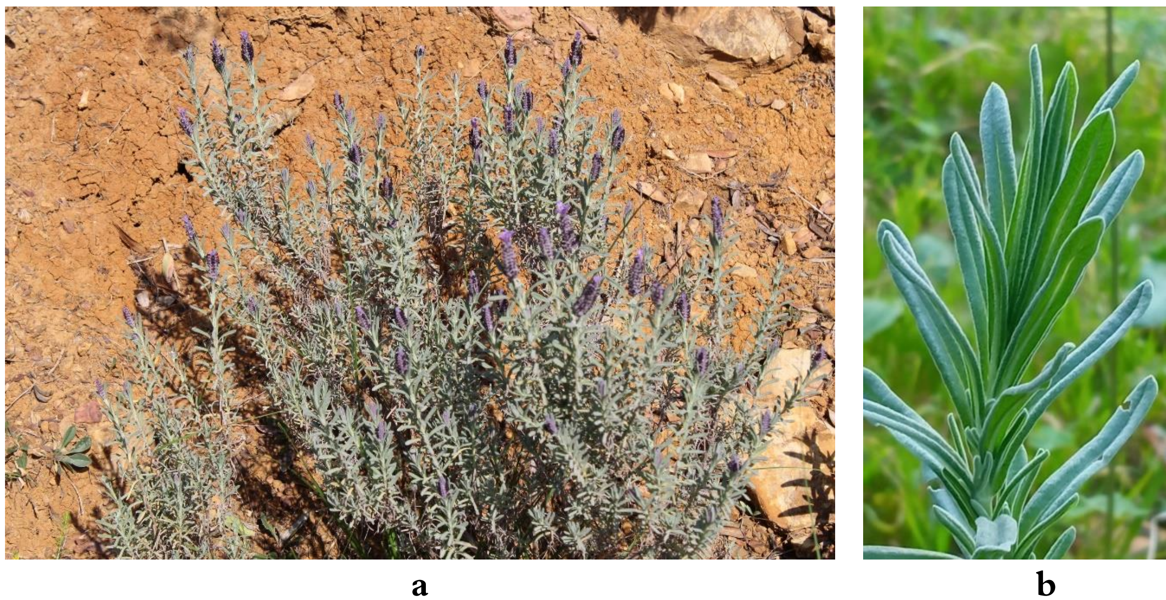
V.4. *Juniperus phoenicea*

Juniperus phoenicea (family Cupressaceae) (**fig. 11**) is a small Mediterranean tree considered as an important medicinal plant largely used in traditional medicine (**Meloni et al., 2006; Mazari et al., 2010**). Its distribution, ranging from Portugal to Saudi Arabia, covers the whole Mediterranean basin (**Meloni et al., 2006**). It is also native to North Africa in Algiers and Morocco, as well as the Canary Islands (**Adams et al., 1996**)

Figure 11. Juniperus phoenicea; (a) whole plant (Serseg, 2018), (b) used part (Inaturalist, 2021c)

V.5. *Lavandula stoechas*

Lavandula stoechas belongs to the family of Lamiaceae (**fig. 12**), is one such plant with potential therapeutic effects. Ethnobotanical used for rheumatic diseases, polyarthritis, nephritis, cystitis and gastric infections have traditionally been treated with *L. stoechas* extracts (**Ez zoubi et al. 2020; El-Hilaly et al. 2003**).



a b

Figure 12. Lavandula stoechas; (a) whole plant, (b) used part (Inaturalist, 2021d,e)

V.6. *Saussurea costus*

Saussurea costus (*S. costus*) belongs to Asteraceae family (**fig. 13**), is well known in Islamic medicine. It is known in Arab countries as "Al-Kost Al-Hindi" and used by traditional healers since the era of the Islamic civilization. It was used traditionally as a stimulant, antiseptic, carminative, sedative, bronchodilator and astringent agent (**Wani et al., 2011; Abdallah et al., 2017**).

a b

Figure 13. Saussurea costus; (a) whole plant, (b) used part (Nadda et al., 2020)

V.7. *Lepidium sativum*

Lepidium sativum (Brassicaceae (Cruciferae)) (**fig. 14**), known as, garden cress in English, is an annual, herbaceous edible plant, native to Egypt and Southwest Asia (**Doke et al., 2014; Imade et al., 2018; Šamec et al., 2019**). It has a major therapeutic significance (**Altay et al., 2018**). The seedlings are consumed in Europe as salad and spice (**Maier et al., 2015**). The edible whole seed is known to have health-promoting properties and possessing a number of pharmacological properties such as treatment for bronchitis, asthma, and cough. It is considered diuretic, antibacterial, gastrointestinal stimulant and gastroprotective (**Gokavi et al., 2004; Karazhiyan et al., 2009; Jain et al., 2016**).

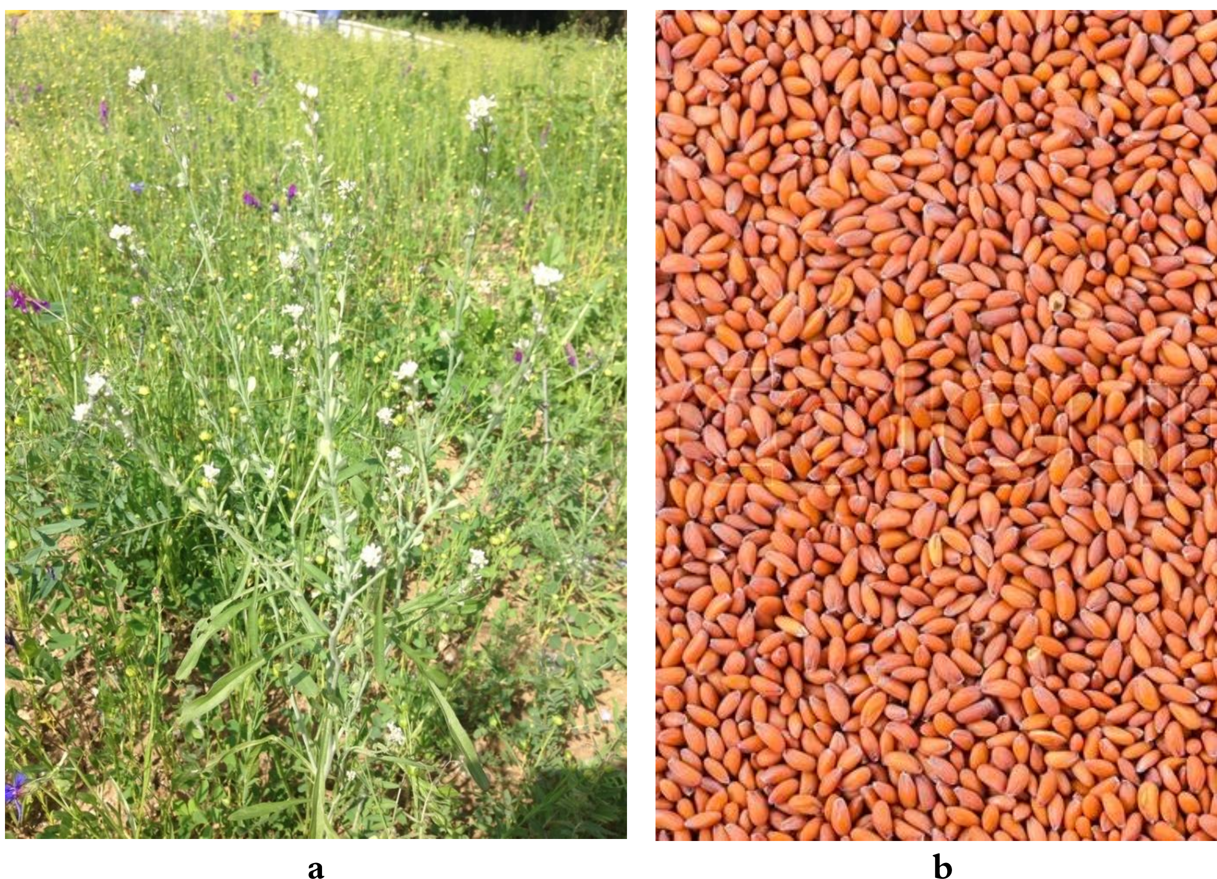


Figure 14. Lepidium sativum; (a) whole plant, (b) used part (Inaturalist, 2021f)

VI. Secondary metabolites

Secondary metabolites are organic compounds produced by living organisms such as bacteria, fungi, plants, and animals (**James**, **2017**) that are not involved directly in the normal growth, development and reproduction of the organism (**Anulika et al**., **2016**).

About 500,000 secondary metabolites have been described to date, 350,000 of these are from plants (**Bills and Gloer**, **2017**). Plant secondary metabolites can be found in the leaves, flowers, stem, root, or bark of the plant (**Anulika et al**., **2016**). They are key components of active and potent defense mechanisms (**Bennett and Wallsgrove**, **1994**). Due to their large biological activities, plant secondary metabolites have been used for centuries in traditional medicine (**Bourgaud**, **et al**, **2001**). Plants' secondary metabolites can be classified into three distinctive groups namely: terpenes, phenolic compounds and nitrogen-containing compounds such as alkaloids (**Anulika et al**., **2016; Guerriero et al**., **2018**).

VI.1. Terpenes

Terpenes and terpenoids are a widely distributed family of natural products (**Davis and Croteau**, **2000**), about 55,000 molecules were isolated so far (**Chen and Baran**, **2009**). Terpenes are isoprene (2-methylbuta-1,3-diene) oligomers (**Grabarczyk et al**., **2020**), classified depending on the size into hemiterpenes, monoterpenes, sesquiterpenes, diterpenes, triterpenes, tetraterpenes, and polyterpenes (**Paduch et al**., **2007**). Their role in plants is not fully explained, but as the main components of essential oils (**Grabarczyk et al**., **2020**), they appear to be responsible for the fragrance that can attract pollinating insects (**Schwab et al**., **2008**), and as a defense against abiotic and biotic stresses, for instance against bacterial or fungal infection, insect or plant-eating animals (**Berenbaum and Feeny**, **1981; Schilling et al**., **2015; Yazaki et al**., **2017**).

Terpenes are considered among the most important substances with various medical properties (**Camargo et al**., **2018; Cox-Georgian et al**., **2019**) It has been

described the use of several diterpene compounds such as andrographolide in humans diagnosed with Alzheimer's disease, for preventing and treating cognitive impairment (**Orozco and Aguilera**, **2005; Sabnis**, **2018**). Andrographolide is a diterpene that binds to GSK-3β to prevents changes in the neuropathology of Alzheimer's models (**Inestrosa and Orozco**, **2015**).

VI.2. Phenolic compounds

Phenolic compounds are bioactive molecules with great potential therapeutic value (**Luna-Guevara et al**., **2019**), defined chemically as a substance that possesses an aromatic ring contained one or more hydroxy substituents (**Velderrain-Rodríguez et al**., **2014**), and include mainly flavonoids, simple phenolics, phenolic acids, tannins, stilbenes, anthocyanins, coumarins, xanthines and lignans (**Khalaf et al**., **2019**). Typically, phenolic compounds are related to defense responses in the plant and as a response to environmental and functional stress such as exposure to pathogenic factors, insect attack, UV radiation, and injury (**Yang et al**., **2018**; **Naczk and Shahidi 2004; Khoddami et al**., **2013**). However, phenolic metabolites play an important part in other processes, for instance incorporating attractive substances to accelerate pollination, coloring for camouflage and defense against herbivores, as well as antibacterial and antifungal activities (**Lin et al**., **2016**).

Phenolic compounds have been receiving increasing interest due to their potential to improve human health and prevent different diseases which are proved after continuous consumption (**Luna-Guevara et al**., **2019**), Phenolic compounds also have been investigated widely for treating Alzheimer's disease (**Sabnis**, **2018**).

VI.3. Alkaloids

Alkaloids is a term used to describe a large and diverse group of chemicals that have at least one nitrogen atom in a heterocyclic ring structure and alkali-like

properties (**Taylor and Hefle**, **2017**). Most alkaloids are derived amino acids such as phenylalanine, tyrosine, tryptophan, ornithine, andlysine (**Verpoorte**, **2005**). The majority of alkaloids are colorless with a bitter taste (**Kukula-Koch and Widelski**, **2017**).

Alkaloids have a wide range of biological activities (**O'Connor**, **2010**), therefore they have a commercial interest as medicines and tools in pharmacological studies. These substances have unique properties for medicinal use, due to their solubility in water under acidic conditions and in lipid under neutral and basic conditions (**Verpoorte**, **2005**). In addition, most alkaloids able to cross the blood-brain barrier (BBB) and interact with neurotransmitter receptors, which gives it depressant or stimulation effects on the central nervous system (CNS) (**Kerry and Simon**, **2013**).

VII. Natural products for Alzheimer's disease

There are many published papers, patents, and patent applications on natural products used for diagnosing, preventing, and treating Alzheimer's disease (**Sabnis**, **2018**). In particular, but not exclusively, the use of curcumins (curcumin, demethoxycurcumin, bisdemethoxycurcumin, or tetrahydrocurcumin) for treating Alzheimer's disease has been described (**Miller and Mitchell**, **2009**), due to the ability of curcumin to link to Aβ plaque and its fluorescence properties, it is suggested to use as fluorescent marker for diagnosing Alzheimer's disease (**Koronyo et al**., **2011**), Eight other patents filed about the uses of curcumin and its derivatives to treat or diagnostic Alzheimer's disease (**Sabnis**, **2018**). Also, the use of phenolic compounds from coffee extract for preventing/treating Alzheimer's disease has been patented (**Bel-Rhlid et al**., **2011 and 2013**). Eleven patents filed for the use of galantamine and its derivatives as Cholinesterase inhibitors to treat AD (**Sabnis**, **2018**).

Several patents have been filed about the uses of **diterpenes** such as Labdane, isosteviol, Steviol (**Zhao et al**., **2012**), tanshinone derivatives synthetized (**Dev and Subbiah**, **2007**) or extracted from salvia genus as acetylcholinesterase inhibitors (**Shaw et**

al., **2004 and 2006**), and **triterpenes** such as lupeol, betulin, betulinic acid, erythrodiol, oleanolic acid, maslinic acid, uveol, ursolic acid, hydroxytyrosol, verbascoside, oleuropein, echinacoside, martynoside, leucosceptoside and mixtures thereof (**Quintela Fernandex et al**., **2014 and 2016**), oleanolic acid, hederagenin, ursolic acid (**Choi et al**., **2007a**,**b**, **2011 and 2014**), celastrol, pristimerin (**Vigo-Pelfrey et al**., **1999**), ginsenoside compounds (dammarane) (**Kim and Chung**, **2005 and 2008**), oleanane and glycosides of oleanane triterpenes, namely, D-Glu(OMe)$_4$, L-Ara-(OAc)$_3$, and D-Gal(OAc)$_4$ (**Honda et al**., **2005**), alpha-irigermanal, gama-irigermanal (**Ichinose and Kakuo**, **2014 and 2015**), gypensapogenin A (**Shi et al**., **2013a**) and gypensapogenin B (**Shi et al**., **2013b**), methyl toonapubesate A (**Guo et al**., **2012**) for preventing and treating Alzheimer's disease.

VII. The number of publications about Alzheimer's disease in literature

There is a gradual and rapid growth in the number of publications about Alzheimer's disease through the years, especially in recent years.

After a rapid search in publication databases: **Springer**, **ScienceDirect**, **PubMed**, and **Nature**, we have found a significant number of publications about Alzheimer's disease. we have counted a range of 0 to **730** publications about the topics related to drugs or targets used in the treatment of AD (**fig**. **17**). While the number of publications about the pathology and physiology of the disease and reviews (**fig**. **16**) is more by 10 times the previously mentioned topics, the number of publications in 3 months reach 4871 publications in ScienceDirect database only.

Generally, we note that the highest number of publications was in the ScienceDirect database.

For Nature database, according to **figure 15**; we have observed a fast growth in publications number about Alzheimer's disease started from 2015 until the current year, where the number of publications in 2020 was **1775** publication. On the other hand, according to **figures 16 and 17**, the major publications on nature database were generally about understanding this disease and its relation to physiological and pathological changes in the organism, in contrast, there was a lack of publications about therapeutic topics, uses of natural product-derived compounds and molecular docking studies.

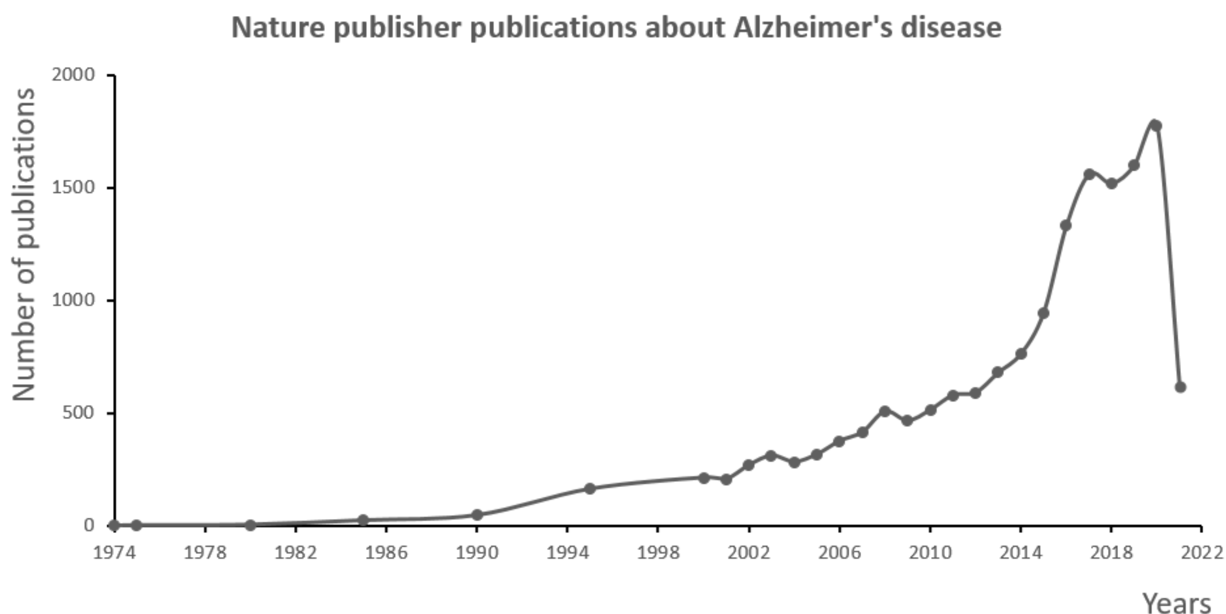


Figure 15. **Nature publisher publications about Alzheimer's disease**

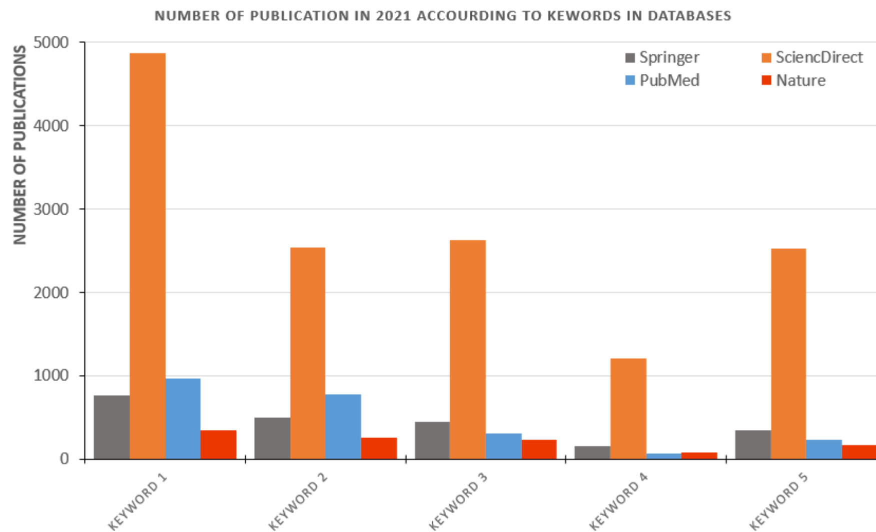


Figure 16. Number of publications in 2021 according to keywords (1–5) in DATABASES

Keywords: 1: Alzheimer's disease+"review"; 2: Alzheimer's disease+"pathology" ;3: Alzheimer's disease+"inflammatory"; 4: Alzheimer's disease+"Obesity"; 5: Alzheimer's disease+"Diabetes".

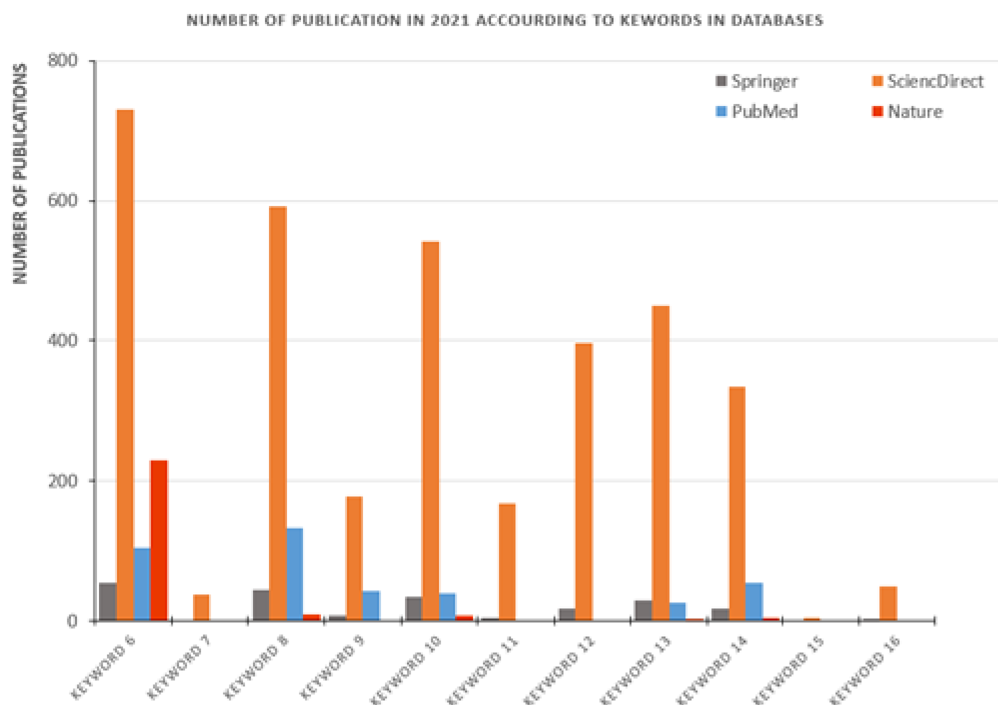


Figure 17. Number of publications in 2021 according to keywords (6–17) in DATABASES

Keywords: 6: Alzheimer's disease+"BACE1"+"β–secretase"+"Amyloid";7: Alzheimer's disease+"MAGL"+"Monoacylglycerol lipase";8: Alzheimer's disease +"AcetylCholinesterase" ;9: Alzheimer's disease+"ButyrylCholinesterase";10: Alzheimer's disease+"Natural products";11: Alzheimer's disease+"terpenes";12: Alzheimer's disease+"Phenolic compound";13: Alzheimer's disease+"Alkaloids";14: Alzheimer's disease+"Molecular docking";15: Alzheimer's disease+"Antioxydant"; 16: Alzheimer's disease+"Algeria".

Despite the vast number of publications from the different countries (showed in **figures 15–18**), unfortunately, the numbers of the publications about Alzheimer's disease affiliated by Algeria still very little (**fig. 18**), where:

In PubMed database: in 2021, there is one publication from 4316 publications in general (0,0231%), whereas, in 2020, there were 8 Algerian publications from 13904 (0,0575%).

In ScienceDirect database: in 2021, there is 26 publications from 7241 publication in general (0,359%), where as in 2020, there was 27 Algerian publications from 14966 (0,180%).

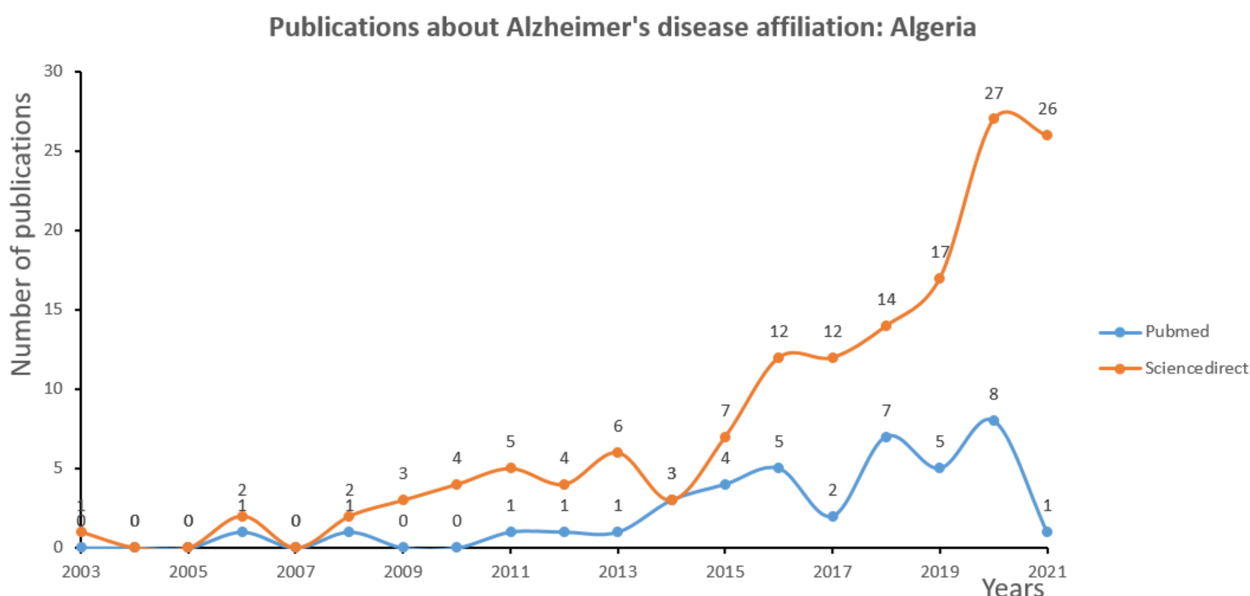


Figure 18. **Publications about Alzheimer's disease** (**Affiliation**: **Algeria**)

Chapter II: Materials and Methods

I. Plant material

Lepidium sativum and *Coriandrum sativum* seeds, and *Saussurea costus* roots were obtained from a local store in Mars 2018, *Juniperus phoenicea*, *Juniperus oxycedrus, Arbutus unedo* L, and *Lavandula stoechas* were collected from Djelfa region in April 2018 and taxonomically identified in the department of Biology, University of Laghouat.

The aerial parts of *A. unedo* L, *J. phoenicea*, *J. oxycedrus,* and *L. stoechas* were air-dried at room temperature, the dried plant material of all plants was ground into powder using a mechanical grinder. All plant materials were packed and kept until use.

II. Instrumentation

All experiments are based on absorbance methods. Absorbances were determined either on Shimadzu 1800 UV/Vis spectrophotometer or microplates reader of type DialabELX 800 UV. For in silico studies, we have used a 8 and 12 CPU workstations in Laboratory of sciences fundamentals, Amar Telidji university.

III. Extraction

III.1. Crude extracts preparation

The crude extracts of *A. unedo* L, *C. sativum, J. phoenicea*, *J. oxycedrus*, *L. steochas,* and *S. costus* were obtained as follows: 25g of plant materials were successively macerated in three solvents: Hexane (250ml) and Ethyl acetate (250ml), Methanol (250ml) for 24 hours twice, then filtered on Whatman filter paper. The extracts were collected, and concentrated under vacuum at 40°C. The final dry weight was used to calculate extraction yield. The obtained crude extracts contain phenolic compounds and terpenes, kept at +4°C until use (**Harborne**, **1973a**).

III.2. Alkaloid extract preparation

The alkaloids were extracted according to **Gacemi *et al.* (2020)**. As follows: a quantity of 25g of *L. sativum* seeds powder was macerated in 250ml of HCl (10%) for 48h. Then after filtration, the liquid phase was homogenized with 250ml NH_4OH (25%), the alkaloids were extracted by adding chloroform to the aqueous phase. Finally, we separated the organic mixture and dried it using rotary evaporation at 40°C to have the dry crude extract of alkaloids.

IV. Phytochemical screening of the plant extracts

The therapeutic effect of plants is due to the wide variety of secondary metabolites synthesized by plants, such as flavonoids, tannin, alkaloids, saponin, terpenoids(**Yang et al., 2018**). We have conducted a phytochemical screening of all the extracts to confirm the presence of absence of these secondary metabolites, alkaloids, flavonoids, saponins, tannins, and terpenoids, by the method described by **Harborne** and **Sazada** (**1973**) as follows:

A volume of 1mL of different extracts were used to perform the phytochemical screening by using standard methods for the detection of the following:

- Flavonoids: Treatment by a few drops of $AlCl_3$. The formation of yellow color indicates the presence of flavonoids (**Mabry et al., 1970**).
- Tannins: Using ferric chloride reagent ($FeCl_3$), the Formation of intensive green or blue color indicates the presence of tannins, as described by **Trease and Evans** (**1989**).
- Alkaloids: Using Wagner and Mayer reagents, the formation of brown-red colored precipitate and yellow colored precipitate respectively, indicates the presence of alkaloids (**Harborne, 1973b; Kokate et al., 2003**).

- Diterpenes: Treatment by few drops of cupric acetate, Formation of emerald green color, indicates the presence of diterpenes (**Harborne**, **1998; Rao et al**., **2002; Kokate et al**., **2003**).
- Triterpenoids and steroids: Using Salkowski's test: treatment chloroform followed by concentrated H_2SO_4 shaken and allowed to stand. The formation of a golden yellow layer at the bottom indicates the presence of triterpenoids, if the layer is red sterol is present (**Prashanth and Krishnaiah**, **2014**).

An amount of 0.1 g of the plant of each species was mixed into distilled water then heated in a bain-marie until boiling, then filtered. The aqueous extract was screened for saponins as follows:

- Saponins (Frothing test): the aqueous extracts were stirred for few seconds. The formation of a stable soapy foaming substance indicates the presence of saponins (**Kokate et al**., **2003; Kumar et al**., **2009**).

V. Quantification of secondary metabolites

V.1. Determination of total phenolic compounds

Phenolics were determined according to **Singleton and Rossi 1965**, which was based on Folin-ciocalteu reagent, and spectrophotometer UV-Visible apparatus. This method is widely used to determine the total phenolic content in plant extracts.

Principle: the principle of this technique is based on the change of the color of Folin-Ciocalteu reagent to blue, due to its reduction by the phenols on the extract. This reagent is composed of phosphotungstic acid ($H_3PW_{12}O_{40}$), et phosphomolybdic acid ($H_3PMo_{12}O_{40}$), which give blue of tungsten and blue of molybdenum in presence

of phenols. Those can be detected by a spectrophotometer at 760nm. We have used gallic acid as a standard phenol.

Protocol: For the standard curve of gallic acid, we prepare a serial solution with a croissant concentration of gallic acid. A volume of 250µl of 10% Folin-Ciocalteu reagent, was added to test tubes contain 50µl of plant extracts alongside with serial concentrations of standard gallic acid. After 2min, a volume of 1 ml of 4% sodium carbonate solution (Na_2CO_3) was added. The solutions were incubated in dark at room temperature (25°C) for 30 minutes. Then, the absorbance was recorded against blank (prepared as same as the test but instead of sample, 20 µl of distilled water was added) at 760 nm using Shimadzu 1800 UV/Vis spectrophotometer. The obtained standard curve of gallic acid is represented in **figure 19**.

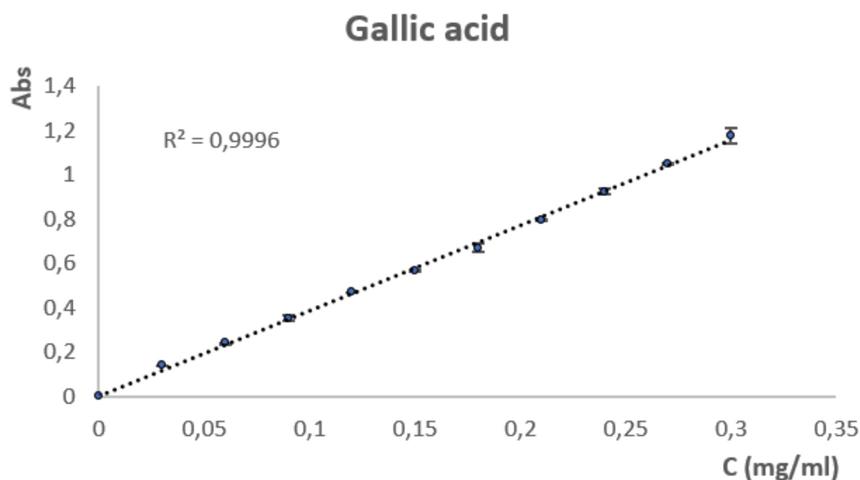


Figure 19. **The standard curve of gallic acid**.

The total phenolics content expressed as mg gallic acid equivalent (mg GAE.g^{-1} of dry weight), and was calculated from the standard curve according to the equation:

$$TPC = \left[\left[\left(\frac{Abs}{\alpha}\right) * (d) * \left(\frac{V}{W}\right)\right]\right] mg\ GAE.\, g^{-1}$$

Where:

TPC: Total phenolics content

Abs: Absorption

α: The slope

d: Dilution ratio

V: Total volume (mL)

W: Total dry weight of the sample (g)

V.2. Determination of total flavonoids

The flavonoids in extracts were estimated by the aluminum chloride colorimetric method according to **Lamaison and Carnat (1991)** method, which is based on aluminum chloride as a reagent, and spectrophotometer UV-Visible apparatus. This method is widely used to determine the total flavonoid content in plant extracts.

Principle: The principle of this technique is based on the formation of a yellow-colored complex that can absorb at 430 nm using a spectrophotometer. Aluminum chloride form a stable complex with carbonyl group (C4) and hydroxyls groups (C3 and C5) of the flavones and flavonols, and non-stable compounds with dihydroxyls ortho of cycle A or B of flavonoids (**Mabry et al., 1970**). We have used quercetin as a standard flavonoid for the standard curve.

Protocol: A volume of 500 µl of each sample was taken in a test tube alongside with different standard quercetin solutions, 500 µl of 2% aluminum chloride ($AlCl_3$) was added to each tube. The blank was prepared by adding the same amount of ethanol instead of the extracts. After incubation at dark and room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm using Shimadzu 1800 UV/Vis spectrophotometer. The obtained standard curve of quercetin is represented in **figure 20**.

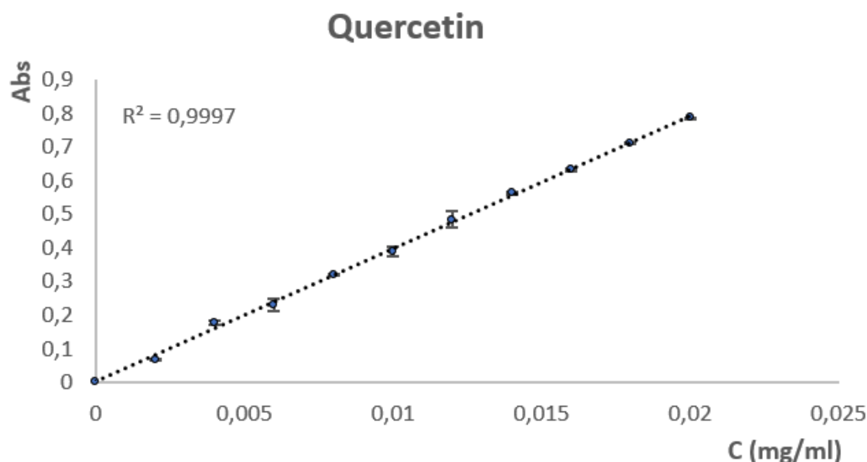


Figure 20. **The standard curve of quercetin**.

The total flavonoids content was expressed as mg quercetin equivalent (mg QE.g^{-1} of dry weight), and was calculated from the standard curve according to the equation:

$$TFC = \left[\left[\left(\frac{Abs}{\alpha}\right) * (d) * \left(\frac{V}{W}\right)\right]\right] mg\ QE.g^{-1}$$

Where:

TFC: Total flavonoids content

Abs: Absorption

α: The slope

d: Dilution ratio

V: Total volume (mL)

W: Total dry weight of the sample (g)

VII. Acetylcholinesterase assay

The activity of AChE electric eel) was achieved as described by **Tel et al**. (**2012**), a slightly modifying the spectrophotometric method developed by **Ellman et al**. (**1961**). 130µL of sodium phosphate buffer (pH 8.0), 20 µL AChE solution in buffer and 10µL of the solution of the tested compounds dissolved in ethanol at different concentrations were mixed and incubated for 15 min at 25°C, and 20 µL of DTNB was added. The

reaction was then initiated by the addition of 20 μL of 2 mg/ml acetylthiocholine iodide. The mixture was incubated at 37°C for 10 min. The hydrolysis of the substrate was monitored spectrophotometrically by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine iodide, at 405 nm using a 96-well microplate reader. We have used donepezil as the reference compound (**Ellman et al**., **1961; Tel et al**., **2012**).

VIII. In silico study

Molecular docking is a computational simulation approach used to predict the affinity of binding of the small molecule to their protein targets by using scoring functions (**Nisha et al**., **2016**). The inhibitory effect of studied compounds on hAChE, hBChE, hMAGL, and hBeta-secretase was investigated by flexible docking experiments.

VIII.1. Receptors and molecules selection

VIII.1.1. Selection of receptors

We have chosen four enzymes that are involved in brain function and were admitted as pharmacological targets for the development of new drugs to cure Alzheimer's disease. Those enzymes are Acetylcholinesterase, Butyrylcholinesterase, Monoacylglycerol lipase, and Beta-secretase 1. The role of these receptors was mentioned in the review chapter (Pages: 22-26).

For human acetylcholinesterase, human butyrylcholinesterase, human monoacylglycerol lipase, and human beta-secretase 1, we have chosen PDB files IDs: 6O4W, 1P0M, 5ZUN, and 6EJ3, respectively, based on the date of publication of their X-ray crystal coordinates, those are the newest crystallized structures published with the highest resolution and were crystallized with known ligands: donepezil,

choline molecule, 9JX (PubChem ID: 134820912), and B7T (PubChem ID: 363591852) respectively.

VIII.1.1. Selection of ligands

The major components of the studied plant from the methanolic or alkaloid fraction were chosen as ligands from molecular docking study to test their ability to interact with the enzymes involved in Alzheimer's disease. Those molecules are presented in **table 1**, and 2D structures are presented in the appendices n:1.

Table 1. the major components of studied plants and their PubChem IDs.

PLANT	MOLECULE Name	Type	PubChem CID	Canonical SMILES	REFERENCE
A. unedo	Gallocatechin	Flavonol	65084	C1C(C(OC2=CC(=CC(=C21)O)O)C3=CC(=C(C(=C3)O)O)O)O	Fiorentino et al., 2007
	Ethyl gallate	Phenolic acid	13250	CCOC(=O)C1=CC(=C(C(=C1)O)O)O	
	Arbutin	hydroquinone	440936	C1=CC(=CC=C1O)OC2C(C(C(C(O2)CO)O)O)O	
C. sativum	p-coumaric acid	Phenolic acid	637542	C1=CC(=CC=C1C=CC(=O)O)O	Laribi et al., 2015
	Ferulic acid	Phenolic acid	445858	COC1=C(C=CC(=C1)C=CC(=O)O)O	
	Chlorogenic acid	Phenolic acid	1794427	C1C(C(C(CC1(C(=O)O)O)OC(=O)C=CC2=CC(=C(C=C2)O)O)O)O	
	Kaempferol (Leaves)	Flavonol	5280863	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O	
	Quercetin (Leaves)	Flavonol	5280343	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O	
	Stigmasterol	Sterol	5280794	CCC(C=CC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C	
	β-sitosterol	Sterol	222284	CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C	
	γ-tocopherol	Tocols	92729	CC1=C(C=C2CCC(OC2=C1C)(C)CCCC(C)CCCC(C)CCCC(C)C)O	
	α-tocotrienol	Tocols	5282347	CC1=C(C2=C(CCC(O2)(C)CCC=C(C)CCC=C(C)CCC=C(C)C)C(=C1O)C)C	
	γ-tocotrienol	Tocols	5282349	CC1=C(C=C2CCC(OC2=C1C)(C)CCC=C(C)CCC=C(C)CCC=C(C)C)O	

J. oxycedrus	Catechin	Flavan-3-ol	9064	C1C(C(OC2=CC(=CC(=C21)O)O)C3=CC(=C(C=C3)O)O)O	Yaglioglu and Eser, 2017
	Quercetin	//	//	//	Özdemir, 2016
	Myricetin	Flavonol	5281672	C1=C(C=C(C(=C1O)O)O)C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O	
	Rutin	Flavonol	5280805	CC1C(C(C(C(O1)OCC2C(C(C(C(O2)OC3=C(OC4=CC(=CC(=C4 C3=O)O)O)C5=CC(=C(C=C5)O)O)O)O)O)O)O)O	Ben Mrid et al., 2019
	Naringenin	Flavanone	932	C1C(OC2=CC(=CC(=C2C1=O)O)O)C3=CC=C(C=C3)O	
	Hesperidin	Flavanone	10621	CC1C(C(C(C(O1)OCC2C(C(C(C(O2)OC3=CC(=C4C(=O)CC(OC4 =C3)C5=CC(=C(C=C5)OC)O)O)O)O)O)O)O)O	
	Salicylic acid	Phenolic acid	338	C1=CC=C(C(=C1)C(=O)O)O	
	Thymoquinone	Terpene	10281	CC1=CC(=O)C(=CC1=O)C(C)C	
J. phoenicea	3-p-Coumaroylquinic acid	Phenolic acid	9945785	C1C(C(C(CC1(C(=O)O)O)OC(=O)C=CC2=CC=C(C=C2)O)O)O	Ghouti et al., 2018
	Quercetin-O-pentoside	Flavonol	5878729	C1C(C(C(C(O1)OC2=C(OC3=CC(=CC(=C3C2=O)O)O)C4=CC (=C(C=C4)O)O)O)O)O	
	Myricetin-O-pentoside	Flavonol	21477996	C1C(C(C(C(O1)OC2=C(OC3=CC(=CC(=C3C2=O)O)O)C4=CC (=C(C(=C4)O)O)O)O)O)O	
L. stoechas	Rosmarinic acid	Phenolic acid	5281792	C1=CC(=C(C=C1CC(C(=O)O)OC(=O)C=CC2=CC(=C(C=C2)O)O)O)O	Celep et al., 2018
	Chlorogenic acid	//	//	//	
	Apigenin 7-glucoside	Flavonoid	5280704	C1=CC(=CC=C1C2=CC(=O)C3=C(C=C(C=C3O2)OC4C(C(C(C(O4)CO)O)O)O)O)O	

	Luteolin 7-O-β-D-glucoside	Flavonoid	12015660	No available structure	
L. sativum	Lepidine B	Alkaloid	100927764	C1=CC(=CC(=C1)OC2=C(C=CC=C2O)CC3=NC=CN3)CC4=N C=CN4	Maier et al., 1998
	Lepidine E	Alkaloid	100927767	C1=CC(=CC(=C1)OC2=C(C=CC(=C2)CC3=NC=CN3)O)CC4= NC=CN4	
S. costus	Dehydrocostus lactone	Sesquiterpene lactone	73174	C=C1CCC2C(C3C1CCC3=C)OC(=O)C2=C	Pandey et al., 2007
	Malic acid		525	C(C(C(=O)O)O)C(=O)O	
	Chlorogenic acid	//	//	//	Yang et al., 2017
	Quinic acid	Phenolic acid	6508	C1C(C(C(CC1(C(=O)O)O)O)O)O	

VIII.2. ADMET and drug–likeness evaluation

The vast majority of drug candidates fails due to their toxicity. ADME/Tox studies play a crucial role in the success of a drug candidate (**Li**, **2001**). The evaluation of ADMET and the drug-likeness prediction of the studied compounds has been carried out using webservers: admetSAR 2.0 (http://lmmd.ecust.edu.cn/admetsar2/) and SwissADME server (http://www.swissadme.ch/index.php). The canonical SMILES of the compounds were obtained from PubChem Database to calculate ADMET properties.

VIII.3. Preparation of molecules and receptors

All molecules were obtained from PubChem database (**Kim**, **2016**) and assembled with Discovery Studio visualizer v4.0. The PDB files of four enzymes (PDB IDs: 6O4W, 1P0M, 5ZUN, and 6EJ3) were downloaded from the Protein Data Bank (PDB) (**Berman et al**., **2000**).

To regard the flexibility of side-chain during docking, flexible torsions in the ligands were assigned, and the acyclic dihedral angles were allowed to rotate freely (**Martins et al**., **2011**).

For Proteins, they were prepared by removing all heteroatoms, any ligands, co-crystallized solvent, and water molecules, expect the involved water molecules in the interactions between inhibitors and enzymes, they have been set for docking. Polar hydrogens and partial charges were added to the structure using Autodock tools (ADT) (version 1.5.4) (**Morris et al**., **2009**). The software uses rectangular boxes for the binding site, the center of the box has been set and displayed using ADT. The Docking was flexible where Trp286, Tyr124, Tyr337, Tyr72, Asp74, Thr75, Trp86, and Tyr341 receptor residues of hAChE were selected to keep flexible during docking simulation (**Bartolini et al**., **2011**). The motion of these residues facilitates the access of ligand to the

catalytic site by increase the size of the gorge (**Chioua et al**., **2018**). The PDB IDs of four enzymes with their different docking parameters are shown in **table 2**:

Table 2. Target drug enzymes and docking parameters.

Enzyme	PDB ID	Number of Water Molecules	x*y*z Center of Grid Box	x*y*z Size of Grid Box
Acetylcholinesterase	6O4W	-	90.473*84.969*-7.352	24*25*22
Butyrylcholinesterase	1P0M	HOH772, HOH811, HOH 890	130.099*120.256*40.93	22*16*18
β-secretase	6EJ3	HOH601, HOH625, HOH671	-20.009*-37.666*-8.61	20*20*18
Monoacylglycerol lipase	5ZUN	HOH365,HOH377,HOH406,HOH441	-13.93*22.411*-9.731	24*22*22

VIII.4. Molecular docking

Molecular docking was performed by the AutoDock Vina program (**Morris et al**., **2009**), in a 12 and 8 CPU stations. The default settings were used, except that the number of output conformations was set to 1. The number of docking runs was set at 50 runs. The number of solutions obtained is equal to 50 conformations. All these solutions are very well handled. The "random seed" is random. The preferred conformations were those of lower binding energy within the active site (**Benarous et al**., **2015; Serseg et al**., **2018**). Finally, the generated docking results were directly loaded into Discovery Studio visualizer, v 4.0 (**Systèmes**, **2016**). We have used LigPlot+ to generate images with the precise hydrogen bond length (**Laskowski and Swindells**, **2011**).

X. Statistical analysis

The experiments were replicated three or six times for each of a range of concentrations and the results were expressed as an average with standard deviation.

Chapter III: Results and discussion

I. Extraction yield

After the extraction and evaporation of the extraction solvent, the residue was weighted and used to calculate the extraction yield. The yield was calculated as follows:

$$\text{Yield\%} = \frac{\text{Extract weight} \times 100}{\text{Dry plant}}$$

The weight, yield and appearance of the different extracts are represented in **table 3**.

Table 3. The weight, yield and appearance of the extracts

Plant	Solvent	Weight (g)	Yield (%)	Color	Aspect
Arbutus unedo L	Hexane	1.064	4.256	Green	Past-Solid
	Ethyl Acetate	0.636	2.544	Green	Dry matter
	Methanol	2.330	9.322	Dark green	Dry matter
Coriandrum sativum	Hexane	1.945	7.779	Brown Green	Oily
	Ethyl Acetate	0.932	3.729	Green yellowish	Liquid
	Methanol	1.490	5.960	Brown	Past
Juniperus oxycedrus	Hexane	0.5337	2.135	Dark green	Dry matter
	Ethyl Acetate	0.6921	2.768	Dark green	Dry matter
	Methanol	3.284	13.136	Dark Brown	Dry matter
Juniperus phoenicea	Hexane	0.952	3.809	Dark green	Dry matter
	Ethyl Acetate	1.293	5.170	Dark green	Dry matter
	Methanol	1.713	6.853	Dark green	Dry matter
Lavandula stoechas	Hexane	0.126	0.504	Yellow-green	Past
	Ethyl Acetate	1.156	4.624	Green	Past
	Methanol	1.728	6.912	Green	Past
Saussurea costus	Hexane	1.078	4.315	Yellow	Oily thick
	Ethyl Acetate	0.483	1.931	Green	Liquid
	Methanol	2.034	8.136	Brown	past
Lepidium sativum	Alkaloids	0.25	1.000	White	Dry matter

The results showed that all plant extract showed different amounts of extractable soluble compounds, and it was an expected result. We observed that the methanol exhibits the highest yield, except *C. Sativum*, where, the hexane extract is higher than methanol extract, 7.78% and 5.96% respectively; for *C. Sativum* we have used seeds, for that, we think the hexane gives a high yield because of the seeds are rich in oils, where the aspect of hexane extract was oily.

In comparisons between different methanolic extracts*, J. oxycedrus* exhibits the highest yield (13.14%), followed by *A. unedo*, *S. costus, L. stoechas, J. phoenicea, C. sativum* respectively. Whereas, for ethyl acetate extracts, the highest extraction yield was exhibited by *J. phoenicea* (5.17%), followed by *L. stoechas, C. sativum, J. oxycedrus, A. unedo and S. costus,* respectively..

Our results are a little different from those in the literature as shown in **Table 4**. For *A. unedo,* our results are less than that found by **Andrade et al., 2009; Oliveira et al., 2009; Pavlović et al., 2009**; and **Malheiro et al., 2012**, where the extraction yield of leaves was 10.01, 45.05, 22.85, 61.1% respectively. In addition, the extraction yield *of J. oxycedrus* in this study was less than that mentions by **El Jemli, et al., 2016 and Živić et al., 2019** where they found 18.01, 49.80 and 15.6% respectively.

Whereas, our results for *S. costus* are higher than that found by **Ahmed et al., 2016**, we can explain the differences between our results and the results in literature by the part of the plant used, the solvents used in the extraction process, and finally the place of harvest, where the change in the environment leads to change in the content on the metabolites in the plant (**John et al., 2006**).

Table 4. Extraction yield of studied plants in literature.

Plant	Place	Used part	Solvent	Yield %	References
A.undo	Portugal	Arial part	Ethanol	10.04	Andrade et al., 2009
	Portugal	Leaves	Boiling water	61.1-27.8	Malheiro et al., 2012
	Portugal	Leaves	Methanol	22.85	Oliveira et al., 2009
	Serbia	leaves	Ethanol	45.05	Pavlović et al., 2009
J. oxycedrus	Serbia	berries	Ethanol	49.80	Živić et al., 2019
		berries	Ethyl acetate	15.60	Živić et al., 2019
	morocco	Leaves	Boiling water	18.01± 0.08	El Jemli, et al., 2016
	Algeria	Root bark	Hexane	3.5	Chaouche et al., 2015
	Algeria	Root bark	Methanol	12.1	Chaouche et al., 2015
J. phoenicea	morocco	Leaves	Boiling water	20.13± 0.05	El Jemli, et al., 2016
	Morocco	Leaves	Chloroform	3.37	Amalich et al., 2016
			Ethyl acetate	1.89	
			Methanol	9.08	
	Tunisia	Leaves	Hexane	4,80	Amalich et al., 2016
			Ethyl acetate	5,12	
			Methanol	16,76	
S. costus	India	Roots	Methanol	2.4±0.265	Ahmed et al., 2016

II. Phytochemical screening of the plant extracts

The phytochemical tests have enabled us to demonstrate the presence of some secondary metabolites in our studied plants by qualitative characterization (precipitation, coloring with specific reagents, or by examination under the light. UV). The results of the phytochemical test are summarized in **Table 5**.

According to the results showed in **Table 5**, preliminary phytochemical screening of different extracts of studied plants revealed the presence of various bioactive components such as alkaloids, flavonoids, terpenoids, steroids, diterpenoids, and tannins.

The phytochemical results reveal that hexane is not an ideal solvent for the preparation of plant extracts rich in phenolic compounds, but it is ideal to extract terpenoids as much as ethyl acetate.

The tests of flavonoids, tannins, alkaloids for all plants conducted with the hexane extract gave a negative result, with the two remaining solvents, the secondary metabolites results show positive results for the presence of flavonoids, tannins, diterpenoids, triterpenoids, and steroids, except the ethyl acetate extract of *S. costus* which gives negative results for both flavonoids and tannins tests. The ethyl acetate extract of *C. sativum* gives also negative results for tannins, diterpenoids, triterpenoids and steroids tests. Only the methanol extract of *L. stoechas* shows the absence of diterpenoids.

The alkaloids are present in small quantities in methanol extract of *A. unedo, C. sativum* and *S. costus*, while they are absent in the other methanol extracts and all hexane and ethyl acetate extracts of all studied plants.

As shown in **Table 6**, the results of our study did not differ significantly from those reported in literature where it is reported the presence of flavonoids, tannins, terpenoids, and steroids in studied plants, except the methanol extract of *J. phoenicea* and *A. unedo* where it is reported the absence of alkaloids. And the absence of saponins in *J. phoenicea* ,*A. unedo, J. oxycedrus, S. costus.*

Table 5. The results for the presence of secondary metabolites in various extracts

Plant	Extract	Flavonoids	Tannins	Saponins	Alkaloids		Diterpenoids	Triterpenoids and steroids
					W	M		
Arbutus unedo L	*Hexane*	–	–	n.d.	–	–	++	–
	Ethyl Acetate	+	+	n.d.	–	–.	+++	+
	Methanol	+++	+++[b]	n.d.	+	++	+++[*]	++[R]
	Water	n.d.	n.d.	+++	n.d.	n.d.	n.d.	n.d.
Coriandrum sativum	*Hexane*	–	–	n.d.	–	n.d.	+	–
	Ethyl Acetate	+	–	n.d.	–	n.d.	–	–
	Methanol	++	+	n.d.	+	++	+++	++
	Water	n.d.	n.d.	–	n.d.	n.d.	n.d.	n.d.
Juniperus oxycedrus	*Hexane*	–	–	n.d.	–	–	+	+
	Ethyl Acetate	+	++	n.d.	–	–	+	+
	Methanol	+++	+++	n.d.	–	–	+	+
	Water	n.d.	n.d.	++	n.d.	n.d.	n.d.	n.d.
Juniperus phoenicea	*Hexane*	–	–	n.d.	–	–	+	++[R]
	Ethyl Acetate	+	+	n.d.	–	–	+++	++[R]
	Methanol	+	++[d]	n.d.	–	+	+++[*]	+++[R]
	Water	n.d.	n.d.	+++	n.d.	n.d.	n.d.	n.d.
Lavandula stoechas	*Hexane*	–	–	n.d.	–	–	+++	++
	Ethyl Acetate	++	+	n.d.	–	–	++	++
	Methanol	++	+++[d]	n.d.	–	+	–	+++[R]
	Water	n.d.	n.d.	++	n.d.	n.d.	n.d.	n.d.
Saussurea costus	*Hexane*	–	–	n.d.	–	–	+	+++[P]
	Ethyl Acetate	–	–	n.d.	–	–	++	+++[p]
	Methanol	+	+++[b]	n.d.	+	+	+++[*]	++[R]
	Water	n.d.	n.d.	+++	n.d.	n.d.	n.d.	n.d.

+++: important presence, ++: average presence, +: weak presence and –: absence; b: blue-green (the presence of gallic tannins); d: dark green (the presence of catechic tannins); *Brown precipitate, W: Wagner's test; M: Mayer's test.

Table 6. The results for the presence of secondary metabolites in various extracts in the literature

Plant	Used part	solvent	Flavonoids	Tannins	Saponins	Coumarins	Alkaloids	Steroid	Triterpenes	References
A unedo	Leaves	Boiling water	++	+++	–	n.d.	–	n.d.	n.d.	Kachkoul et al., 2018
	Roots	Methanol	+	+	–	–	–	n.d.	n.d.	Dib et al., 2013
	Fruit	Ethyl acetate	+	+	n.d.	+	–	+	–	El Cadi et al., 2020
	Fruit	Methanol	+	+	n.d.	–	–	+	+	El Cadi et al., 2020
C. sativum	Fruit	Methanol	++	+	+	+	–	++	–	Ahmed et al., 2018
J. oxycedrus	Berries	Ethanol	+	+	–	+	+	+	+	Živić et al., 2019
	Berries	Ethyl acetate	+	–	–	–	+	+	+	Živić et al., 2019
J. phoenicea	Leaves	Methanol	+++	+	–	n.d.	–	+	°	Amalich et al., 2016
L. stoechas	Arial Parts	Ethanol-water	+	+	n.d.	n.d.	n.d.	+	+	Ez Zoubi et al., 2020
	Leaves	Ethyl acetate	–	++	n.d.	++	–	+++	+++	Boufellous et al., 2017
	Leaves	Ethanol-water	+	+	+	n.d.	++	+	+	Mushtaq et al., 2018
S. costus	India	Ethanol	+	+	–	+	+	+	+	Abdallah et al., 2017
		Hexane	–	–	–	n.d.	–	+	+	Ahmed et al., 2018
		Methanol	+	+	–		+	+	+	

III. Quantification of secondary metabolites

Phenolic compounds in plants constitute a major class of secondary plant metabolites with bioactive potential attributed to antioxidant and antibacterial activities. Free phenolic acids or derivatives present in ester or ether form, are found in varying quantities throughout plant tissues in response to characteristic synthesis patterns resulting from encounters with different forms of environmental stress (**Saha and Pal**, **2021**).

All extracts were analyzed quantitatively by spectrophotometer for their total polyphenol contents using the Folin-Ciocalteu method and flavonoid contents using $AlCl_3$ method. The results obtained are expressed in mg gallic acid equivalents (mg GAE) per g of dry weight for phenols and mg quercetin equivalents (mg QE) per g of dry weight **Table 7**, summarized the results of total phenolics and flavonoids quantifications.

Table 7. Total phenols and flavonoids contents in plant extracts.

Plant	Extract	TPC (mgGAE/gDW)	TFC (mgQE/gDW)
Arbutus unedo L	Hexane	0.762±0.043	0.099±0.003
	Ethyl Acetate	1.187±0.092	0.430±0.016
	Methanol	15.913±0.179	2.208±0.073
Coriandrum sativum	Hexane	0.707±0.075	0
	Ethyl Acetate	0.547±0.040	0.018±0.007
	Methanol	2.246±0.027	1.244±0.022
Juniperus oxycedrus	Hexane	0.610±0.067	0.030±0.010
	Ethyl Acetate	0.914±0.061	0.588±0.041
	Methanol	29.106±0.504	7.699±0.481
Juniperus phoenicea	Hexane	1.105±0.066	0.085±0.008
	Ethyl Acetate	2.857±0.043	0.439±0.024
	Methanol	8.631±0.013	0.573±0.078
Lavandula stoechas	Hexane	0.125±0.053	0.004±0.002
	Ethyl Acetate	1.446±0.017	0.848±0.007
	Methanol	3.503±0.123	0.973±0.028
Saussurea costus	Hexane	0.156±0.047	0.016±0.003
	Ethyl Acetate	0.584±0.057	0.040±0.002
	Methanol	11.279±0.045	0.286±0.005

From the results presented in **Table 7** and **figures 21**, we found that all the prepared extracts contain phenolic compounds but in widely varying concentrations. The extract prepared by methanol has presented the highest concentration of total phenols in comparison to ethyl acetate and hexane. On the other hand, the extracts prepared by hexane presented the lowest level, the concentration of which was not exceeded 1.015 mgEGA/gDW.

Whereas, Total flavonoids contents were determined as quercetin equivalents in milligrams per gram of dry weight respectively. As shown on **figure 22**, the highest total flavonoid contents in *J.oxycedrus* methanol extract. In methanol extracts, total flavonoid contents ranged between 7.70 and 0.02 mgEQ/gDW. Low quantities of flavonoids were observed in ethyl acetate and hexane extracts of all studied plants.

In addition, we observed an absence of flavonoids in the hexane extract of *C. sativum*.

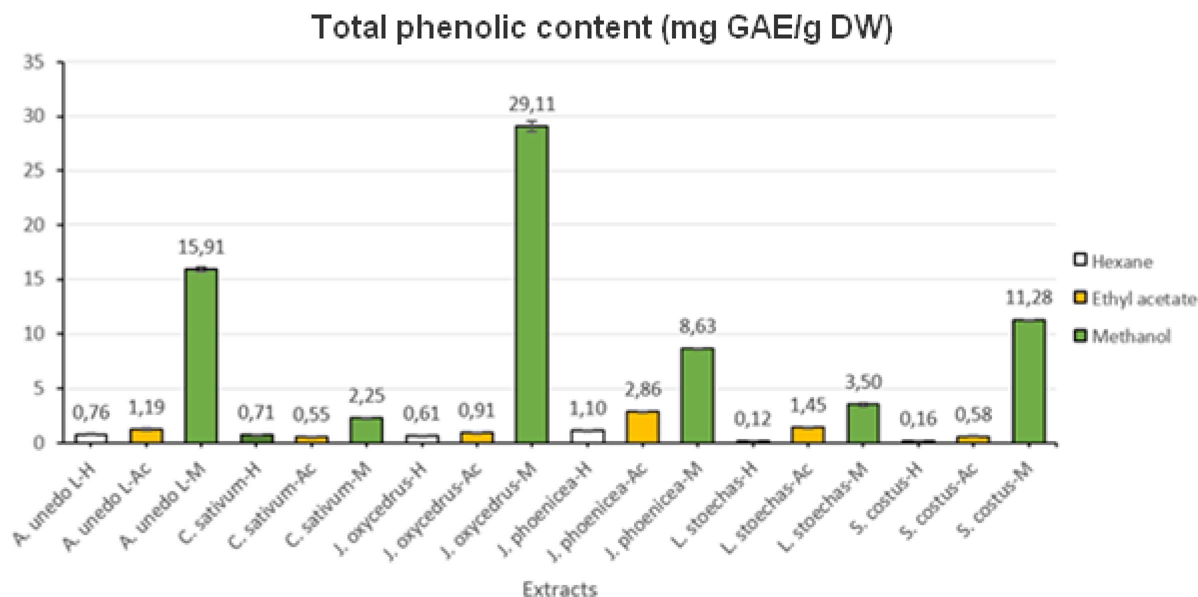


Figure 21. **Total phenolic content**

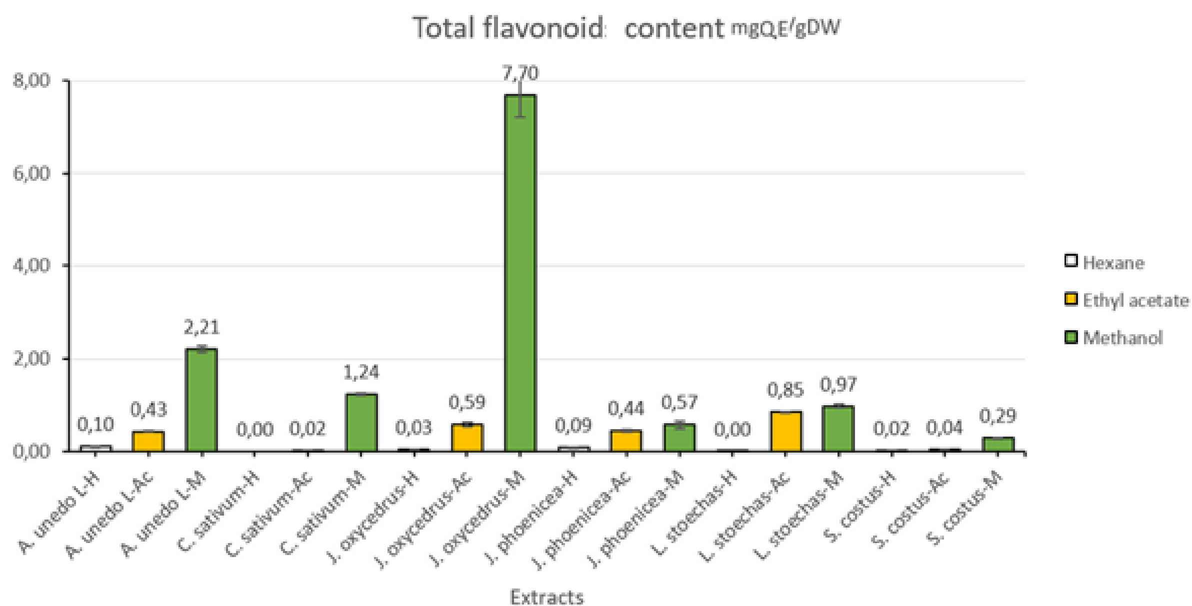


Figure 22. Total flavonoid content

The results of the quantification of total phenolic and flavonoids content in our study are slightly different from the results reported in the literature, which are also different between each other.

The change in the biotope and biocenosis around the plant lead to a change in the secondary metabolite quantitatively and qualitatively.

According to **Table 8**, **Msaada et al. 2017** reported that the coriander extract had a 1.09 and 1.00 mgGAE/gDW of total phenolic polyphenol content in Syrian and Tunisian varieties. Indeed, the methanolic extract of *C. sativum* has been previously reported to present a polyphenol content of 1.89 mg GAE/g DW in the Norwegian variety.

For *A. unedo*, it has been reported to be rich in phenolics and flavonoids according to **Andrade et al., 2009**, **Oliveira et al., 2009**, **Pavlović et al., 2009**, **Malheiro et al., 2012 and Kachkoul et al., 2018 (Table 8)**. In the studies of **Laribi et al., 2015**, **Živić et al., 2019**, **Živić et al., 2019**, **El Jemli, et al., 2016**, **Chaouche et al., 2015**, **Chaouche et al., 2015**, **Chaouche et al., 2013**, **Djeridane et al., 2006**, it has been found a significant amount

of phenolics and flavonoids in *J. oxycerus*. In addition, we found several studies focused on the quantification of phenols and flavonoids of *J. phoenicea* have some good quantities of these compounds, this explains the different biological activities exhibited by this plant.

Table 8. Total phenols and flavonoids contents in plants extracts in literature

Plant	Place	Used part	Solvant	TPC (mgGAE/g DW)	TFC (mgQE/g DW)	References
A.undo	Portugal	Arial part	Ethanol	254.50±4.60a	30.30±1.62b	Andrade et al., 2009
	Portugal	Leaves	Boiling water	215.0–148.0a	–	Malheiro et al., 2012
	Portugal	Leaves	Methanol	149.28±5.33a	–	Oliveira et al., 2009
	Morocco	Leaves	Boiling water	95.83 ± 2.083	–	Kachkoul et al., 2018
	Serbia	Leaves	Ethanol	11.08±0.28%c	1.92±0.07%c	Pavlović et al., 2009
C. *sativum*	n.m.	Arial part	Methanol	110 ± 9.9d	–	Wong and Kitts, 2006
	Portugal	Vegetative parts	Methanol:water	6273.47± 1.16e	5259.52±69.91e	Barros et al., 2012
	Syria	Fruits	Methanol	1.09	2.51 ± 0.08	Msaada et al., 2014
	Tunisia	Fruits	Methanol	1.00 ± 0.06	2.03 ± 0.04	Msaada et al., 2014
	Norwegian	Fruits	Ethyl acetate	1.89f	–	Laribi et al., 2015
J. oxycedrus	Serbia	Berries	Ethanol	58.73 ± 0.14	21.39 ± 0.33	Živić et al., 2019
		Berries	Ethyl acetate	27.20 ± 0.08	18.43 ± 0.12	Živić et al., 2019
	morrocc	Leaves	Boiling water	278.56 9.67g	20.81±0.63h	El Jemli, et al., 2016
	Algeria	Root bark	Hexane	3.2±0.1	2.0±0.1	Chaouche et al., 2015
			Methanol	76.2±2.8	39.0±2.5	Chaouche et al., 2015
		Needles	Methanol:H_2O	133.08 ± 4.1	61.5 2 ± 3.1	Chaouche et al., 2013
	Algeria	n.m.	Ethanol : H_2O	12.66 ± 0.41	3.50 ± 0.50	Djeridane et al., 2006
J. phoenicea	morocco	Leaves	Boiling water	116.35 9.71g	6.69 0.22h	El Jemli, et al., 2016
	Morocco	Leaves	Chloroform	1.32	0.54	Amalich et al., 2016
			Ethyl acetate	2.91	2.36	
			Methanol	1.17	0.36	
	Tunisia	Leaves	Hexane	162.3±3.2	96.00±0.48	Amalich et al., 2016
			Ehtyl acetat	180.8±3.6	104.00±0.80	
			Methanol	265.0±5.8	176.00±0.52	
S. costus	India	Roots	Methanol	20i	59j	Ahmed et al., 2016

a:GAE/g plant extract; b:QE/g plant extract; c:%Plant material; d:mg caffeic acid/100g fresh weight; e:mg/kg,dw ; f: GAE/100g extract; g:µg GAE/mg extract; h:µg QE/mg extract; i:µg GAE/g dried material; j: µg Rutin Equivalent/g dried material.

IV. Acetylcholinesterase assays

Inhibitors of Acetylcholinesterase (AChE) have been introduced to the market as AD drugs (**Zhang et al**., **2018; Kabir et al**., **2019a; Sharma**, **2019**). In the current study, in vitro inhibitory activities of seven plants were tested against AChE using a microplate-reader assay based on the Ellman method. The obtained results are presented in **Table 9** and **figure 23**.

Table 9. Inhibitory effects of plant extract against Acetylcholinesterase.

Plant	Extract	IC_{50} (mg/ml)
Arbutus unedo L	Hexane	2.489±0.154
	Ethyl Acetate	NA
	Methanol	**0.695±0.059**
Coriandrum sativum	Hexane	1.775±0.067
	Ethyl Acetate	n.d.
	Methanol	2.792±0.740
Juniperus oxycedrus	Hexane	**0.401±0.027**
	Ethyl Acetate	**0.599±0.057**
	Methanol	1.077±0.062
Juniperus phoenicea	Hexane	1.253±0.096
	Ethyl Acetate	**0.615±0.031**
	Methanol	**0.358±0.005**
Lavandula stoechas	Hexane	1.011±0.221
	Ethyl Acetate	n.d.
	Methanol	NA
Saussurea costus	Hexane	1.506±0.069
	Ethyl Acetate	n.d.
	Methanol	n.d.
Lepidium sativum	Alkaloids	**0.391±0.067**
Donepezil	FAD-Drug	**0.18±0.002***

n.d. not determined, NA not active, *µg/ml

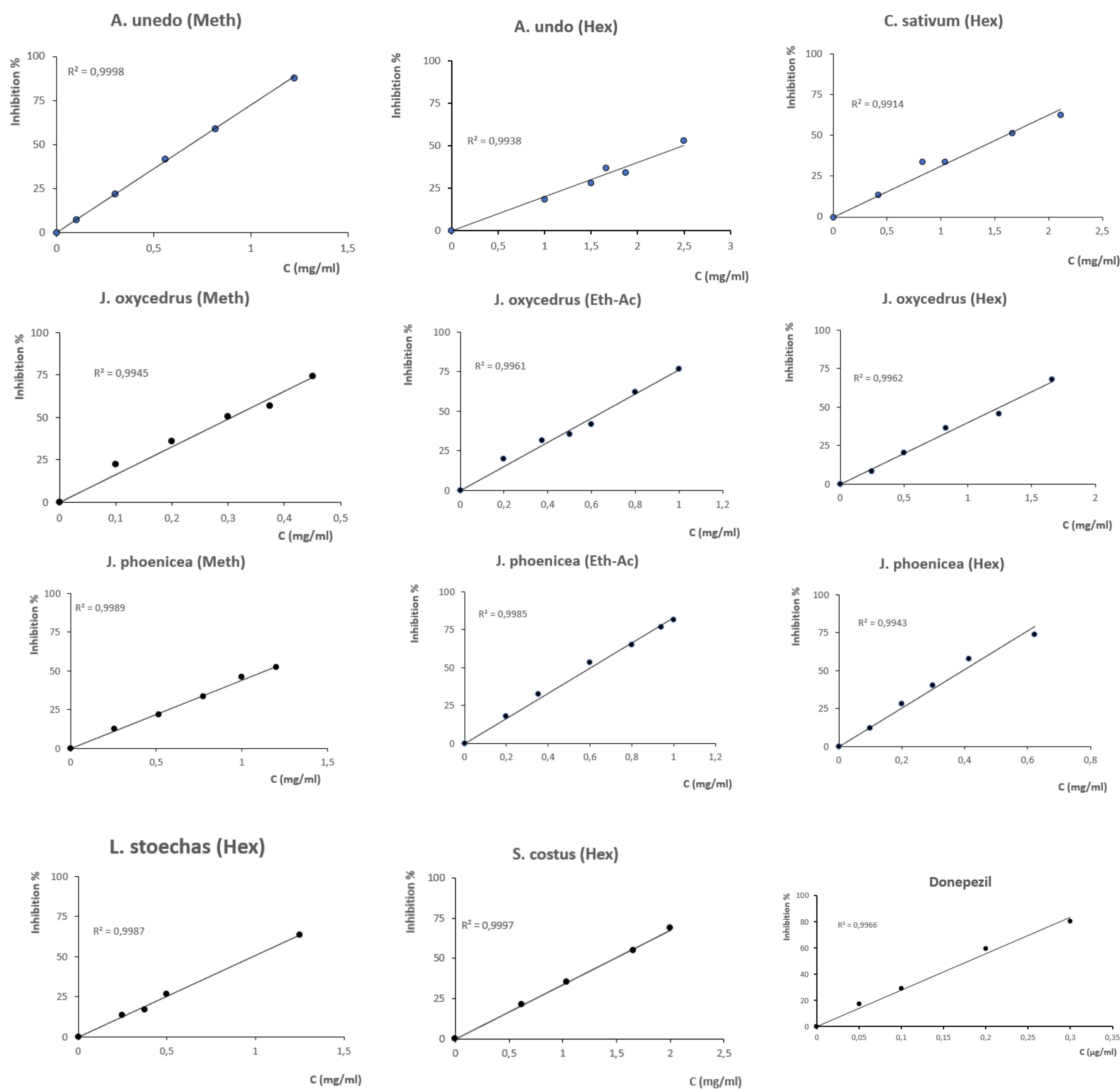


Figure 23. The inhibitory curves of studied extracts against AChEee

The extracts show a wide different range of IC_{50} values; Where, the good inhibitory effect against AChE with values 0.358, 0.401, 0.599, 0.615 and 0.659 mg/ml showed by *J. phoenicea* (Meth), *J. oxycedrus* (Meth), *J. oxycedrus* (EtOAC), *J. phoenicea* (EtOAc) and *A. unedo* (Meth) extracts respectively, compared to the positive control donepezil with IC_{50}: 0.18µg/ml. Considering that these extracts are not pure compound. Followed by a low inhibitory effect translated by high (more than 1mg/ml) IC_{50} values, those values are 1.011, 1.077, 1.253, 1.506, 1.775, 2.489, and 2.792 mg/ml for the extracts: *L. stoechas* (Hex), *J. oxycedrus* (Meth), *J. phoenicea* (Hex), *S. costus* (Hex), *C. sativum* (Hex), *A.unedo* (Hex) and *C. sativum* (Meth) respectively.

The rest of the extracts were either inactive: *A.unedo* (EtOAC), *L. stoechas* (Meth) or insignificantly active (mentioned in the table as n.d.). In this case, we find a very low activity where I% was less than 10%, for all tested concentrations or the percent inhibition reach the stability phase before I%=100%, which mean these extracts could not inhibit the enzyme 100% whatever was the concentration of the extract in the reaction mixture, those extracts are: *C. sativum* (EtOAC), *L. stoechas* (EtOAC), *S. costus* (EtOAC and Meth).

It has been found that the ethyl gallate, as one of the major compounds of *A. unedo* (**Fiorentino et al**., **2007**), has an IC_{50} value of 1.907±0.071 mM (**Odontuya et al**., **2016**). Although we have found that all extracts of *C. sativum* neither not active or insignificantly active. Its major compounds (**Laribi et al**., **2015; Al-Rimawi et al**., **2017; Corina et al**., **2018; Mighri et al**., **2019**) have good inhibitory activity against AChE, namely: Quercetin, Kaempferol, ferulic acid, and Chlorogenic acid with IC_{50} values of 5.98µg/mL (**Jung and Park**, **2007**), 30.4µM (**Min et al**., **2010**), 1.370mM (**Odontuya et al**., **2016**) and 196µg/ml (**Hernandez et al**., **2010**), respectively.

We have found that *Juniperus* species are rich of phenolics and flavonoinds, we suggest that the inhibitory effect of *J. phoenicea* extract is due to quercetin derivatives

which were declared to be the main compounds in this plant (**Ghouti et al**., **2018**), also according to **Jung and Park**, **2007** and **Orhan et al**., **2007** studies, quercetin derivatives have been identified as good inhibitors of AChE (IC_{50}=5.98µg/mL). It has been demonstrated that quercetin is a competitive inhibitor of AChE (**Khan et al**., **2009**). While the inhibitory effect of *J. oxycedrus*, is due to their major compounds as: Catechin, Myricetin, Rutin and Naringenin (**Özdemir**, **2016; Yaglioglu and Eser**, **2017; Ben Mrid et al**., **2019**). Based on previous studies, those flavonoids have a significantly anti-cholinesterase effect. **Odontuya et al**., **2016** have found the IC_{50} of Catechin 1.042±0.027mM, and **Katalinić et al**., **2010** also declared that Ki of Myricetin is 37,8µM. And IC_{50} of Rutin was 86 µg/ml (**Hernandez et al**., **2010**). The data of the study of **Heo et al**., **2004** show a significant anti-amnesic effect in mice injected by naringenin and it has an inhibitory effect on AChE.

Up to date, quite a lot of studies have reported affirmative effects of plant extracts in neurodegenerative disease but a few have been examined the Algerian plants.

In comparison with previous results (**Table 10**): **Nunes and Carvalho**, **2017**, After using *A. unedo* leaves extract extracted in different temperatures degrees, declare that the leaves contain compounds with high inhibitory activity can only be extracted using temperatures above 40°C. Nevertheless, other extracts obtained using lower temperatures show a considerable inhibitory activity towards AChE. Moreover the leaves extracts show the best effect in comparison with fruits and flowers of the same plant. **Politeo and Ercegović**, **2019** tested the volatile oil of *A. undo* where they found that this oil showed low to moderate AChE activity (48.5 %) for 1 mg/mL.

In general, and based on the results of **Murata et al**., **2015**, **Adhami et al**., **2011** studies, extracts of *C. sativum* showed a low inhibitory effect against AChE.

Tavares et al., **2012** tested AChE inhibitory capacity by four *Juniperus*, all tested extracts show significant activity. The inhibitory percent of tested concentrate 0.8mg/ml was estimated by 38.78 ± 4.07, 65.48 ± 3.03, 72.65 ± 17.06, and 55.60 ±

5.2% for *J. oxycedrus, J. phoenicea, J. turbinate,* and *J. navicularis,* respectively. Where *J. turbinate* was the most effective species, followed by *J. phoenicea, ,J. navicularis* and *J. oxycedrus*.

Comparing these results with values obtained by **Moraga-Nicolás et al**. **2018**, we found that AChE inhibitory activity of Hexanic alkaloidal extract, Chloroformic alkaloidal extract and Buthanolic alkaloidal extract of *Rhodolirium andicola* is higher than our results with IC_{50} values of 11.25± 0.04, 17.34±1.13, and 57.78±1.92 (µg/ml), respectively, as matched with IC_{50} value of 0.17±0.15 (µg/ml) for Galantamine (Reminyl®). In the same report, authors described that alkaloid extracts from *Rhodolirium andicola*, containing thirteen alkaloid compounds, include galantamine and other galantamine-type alkaloids that have AChE inhibitory activity, which explain the strong inhibitory effect of these alkaloid extracts (**Moraga-nicolás et al**., **2018**). The same note was suggested by **Cortes and his colleagues** about the notable inhibitory activity as shown by the alkaloidal extract from *Zephyranthes carinata* (5.97 ± 0.24 µg/ml) which could be related to the presence of galantamine, lycorine, and lycoramine (**Moraga-nicolás et al**., **2018**; **Cortes et al**., **2015**). The basic $CHCl_3$ extract of *Hippeastrum argentinum*, alkaloids showed modest activity against AChE (IC_{50} = 50.2 µg/mL) and weak activity against BuChE (IC_{50} = 115.5 µg/mL) (**Ortiz**, **et al**., **2016**). Butanoic alkaloidal extracts from bulbs of *Ammocharis coranica* showed a high AChE inhibitory activity with an IC_{50} value of 0.05 ± 0.02 µg/mL while ethyl acetate alkaloidal extract from same plant shows a low AChE inhibitory activity with an IC_{50} value of 43.1 ± 1.22 g/ml (**Elisha et al**., **2013**).

In this regard, **Shukla et al**. **2011** evaluated the neurobehavioral effects of the total alkaloid from *L. sativum* seeds in Swiss Albino mice and Wistar albino rats. The animals were intraperitoneally injected with total alkaloids from *L. sativum* seeds (50, 150 and 250 mg/kg body weight). Total Alkaloids demonstrate sedative, myorelaxant, analgesic and anxiolytic like effect in mice, as the increased preference to plus-maze open arm (**Shukla et al**., **2011; Doke and Guha**, **2011**).

Table 10. Inhibitory effects of plant extracts against Cholinesterases in literatures

plant	Place	Used part	Solvent	AChE ee µg/mL	BuChE (*equine*) µg/mL	References
A unedo	n.m.	Leaves	Ethanol:water	173.86 ± 13.12	518.43 ± 22.67	Nunes and Carvalho, 2017
	n.m.	Volatile oil	Ethanol:water	48.5%*	–	Politeo and Ercegović, 2019
C. sativum	India	Fruits	Methanol	3.9%**	–	Murata et al., 2015
	Iran	Fruits	Methanol DCM	Not Active Not Active	–	Adhami et al., 2011
J. oxycedrus	Portugal	Leaves	Ethanol:water	38.78 ± 4.07***	–	Tavares et al., 2012
J. phoenicea	Portugal	Leaves	Ethanol:water	65.48 ± 3.03***	–	Tavares et al., 2012
J. turbinata	Portugal	Leaves	Ethanol:water	72.65 ± 17.06***	–	Tavares et al., 2012
J. navicularis	Portugal	Leaves	Ethanol:water	55.60 ± 5.2***	–	Tavares et al., 2012
L. viridis	Portugal	Arial parts	Ethanol	52.81 ± 1.22%****	51.19 ± 1.52%****	Costa et al., 2013
S. costus	India	Root	Ethanol	58.68 ± 0.86	94.46 ± 0.5	Kadyala, et al., 2014

Ee: electric eel; *1mg/ml; **0.5 mg/ml; ***0.8 mg/ml; ****2.5 mg/ml

V. In silico study

V.1. ADMET and drug–likeness evaluation

ADMET and drug-likeness evaluation allows to selection of drug candidates with good pharmacological properties such as toxicological potential, drug-drug interaction potential, metabolic stability and intestinal permeability. The prediction of drug properties based on chemical structure. This method will reduce unecessary time and cost in drug development.

The most properties evaluated are oral delivery, which is the most desirable route of drug administration. Therefore, it is important to check if the developed drugs can be absorbed effectively through the intestinal epithelium, the used model to evaluate this property in Caco-2, a derived cell from human colon adenocarcinoma. Also, Inhibitor-Substrat called also Drug-drug interactions, if there is an interaction between two drugs, this can affect the metabolic stability of these drugs; Drug toxicity is another crucially important drug property, which generally examinate hepato-toxicity of the developed drug (**Li**, **2001**).

Lipinski et al., **1997** proposed the "Rule of Five", which was a filter of drug-likeness distinguishing whether a molecule is orally absorbed well or not. According to the Rule of Five, a molecule would not be orally active if it violates two or more of the five rules, namely: molecular weight (MW) $\leq$ 500 Da, octanol/water partition

coefficient (A log P) ≤ 5, number of hydrogen bond donors (HBDs) ≤ 5 and number of hydrogen bond acceptors (HBAs) ≤ 10.

Only a few molecules could pass through BBB, which is an important step for the molecule to reach the cholinesterase enzymes in the brain. These molecules are Ethyl gallate, Arbutin, p-coumaric acid, ferulic acid, Salicylic acid, Thymoquinone, Dehydrocostus lactone and FAD-drugs: Donepezil and Galanthamine. All mentioned molecules passed the different drug-likeness criteria successfully and were predicted to be orally bioavailable, they respect Lipinski rules, have high human intestinal absorption, are not toxic nor Carcinogens nor mutagenesis. These observations indicate that these molecules are good choices to develop new drugs against Alzheimer's disease of other diseases.

Based on the results presented in **Table 11**, not all other studied molecules could pass through BBB. Whereas, Quercetin and Rutin are suspected to be a mutagenesis agent; while Quercetin-O-pentoside, Myricetin-O-pentoside, Rutin, hesperidin violate the Lipinski rules.

Table 11. ADMET and drug–likeness evaluation of some molecules

		DRUGLIKENESS	ABSORPTION					METABOLISM							TOXICITY		
PLANT	Molecule	Lipinski	Blood–Brain Barrier	Human Intestinal Absorption	Caco–2	Human oral bioavailability	Skin Permeation	P–glycoprotein Inhibitor	P–gp Substrate	CYP450 1A2 Inhibitor	CYP450 2C9 Inhibitor	CYP450 2D6 Inhibitor	CYP450 2C19 Inhibitor	CYP450 3A4 Inhibitor	AMES mutagenesis (probability)	Carcinogens (probability)	Hepatotoxicity (probability)
A. unedo	Gallocatechin	+	-	High	-	-	–8.17 cm/s	-	-	-	-	-	-	-	–(0.630)	–(0.928)	+(0.525)
	Ethyl gallate	+	+	High	+	+	–6.59 cm/s	-	-	-	-	-	-	-	–(0.910)	–(0.685)	–(0.675)
	Arbutin	+	+	High	-	-	–8.92 cm/s	-	-	-	-	-	-	-	–(0.810)	–(0.957)	–(0.850)
C. sativum	Quercetin	+	-	High	-	-	–7.05 cm/s	-	-	+	-	+	-	+	+(0.900)	–(1.000)	+(0.735)
	Kaempferol	+	-	High	-	-	–6.70 cm/s	-	-	+	-	+	-	+	+(0.730)	–(1.000)	+(0.775)
	P–coumaric acid	+	+	High	+	-	–6.26 cm/s	-	-	-	-	-	-	-	–(0.940)	–(0.788)	–(0.600)
	Ferulic acid	+	+	High	+	+	–6.41 cm/s	-	-	-	-	-	-	-	–(0.990)	–(0.803)	–(0.700)
	Chlorogenic acid	+	-	Low	-	-	–8.76 cm/s	-	-	-	-	-	-	-	–(0.990)	–(0.929)	–(0.575)
	Stigmasterol	+	-	Low	+	-	–2.74 cm/s	-	+	-	+	-	-	-	–(0.830)	–(0.857)	–(0.775)

	β-sitosterol	+	-	**Low**	+	+	**−2.20 cm/s**	-	+	-	-	-	-	-	−(**0.870**)	−(0.971)	−(**0.750**)
	γ-tocopherol	+	-	**Low**	+	+	**−1.51 cm/s**	-	-	-	-	-	-	-	−(**0.800**)	−(0.943)	−(**0.800**)
	α-tocotrienol	+	-	**Low**	+	-	**−2.28 cm/s**	+	-	-	-	-	-	-	−(**0.700**)	−(0.943)	−(**0.625**)
	γ-tocotrienol	+	-	**Low**	+	-	**−2.46 cm/s**	+	-	-	-	-	-	-	−(**0.750**)	−(0.943)	−(**0.600s**)
J.oxycedrus	Catechin	+	-	**High**	-	-	**−7.82 cm/s**	-	-	-	-	-	-	-	+(**0.630**)	−(0.929)	−(**0.500**)
	Myricetin	+	-	**Low**	-	-	**−7.40 cm/s**	-	-	+	-	-	-	+	+(**0.530**)	−(1.000)	+(**0.675**)
	Rutin	-	-	**Low**	-	-	**−10.26 cm/s**	-	-	-	-	-	-	-	+(**0.800**)	−(0.986)	+(**0.700**)
	Naringenin	+	-	**High**	-	-	**−6.17 cm/s**	-	-	+	-	-	-	+	−(**0.870**)	−(0.986)	+(**0:675**)
	Hesperidin	-	-	**Low**	-	-	**−10.12 cm/s**	-	-	-	-	-	-	-	−(**0.660**)	−(0.971)	+(**0.650**)
	Salicylic acid	+	+	**High**	+	+	**−5.54 cm/s**	-	-	-	-	-	-	-	−(**0.990**)	−(0.637)	−(**0.825**)
	Thymoquinone	+	+	**High**	+	+	**−5.74 cm/s**	-	-	-	-	-	-	-	−(**0.890**)	−(0.675)	−(**0.550**)

J. phoenicea	3-p-Coumaroylquinic acid	+	-	**Low**	-	-	**–8.41 cm/s**	-	-	-	-	-	-	-	–(0.920)	–(0.901)	–(0.525)
	Quercetin-O-pentoside	-	-	**Low**	-	-	**–8.64 cm/s**	-	-	-	-	-	-	-	+(0.750)	–(0.986)	+(0.625)
	Myricetin-O-pentoside	-	-	**Low**	-	-	**–9.00 cm/s**	-	-	-	-	-	-	-	+(0.550)	–(0.986)	+(0.525)
L. sativum	Lepidine B	+	-	**High**	+	+	**–6.08 cm/s**	-	-	+	+	+	+	+	–(0.530)	–(0.957)	+(0.625)
	Lepidine E	+	-	**High**	+	-	**–6.08 cm/s**	+	-	+	+	+	+	+	–(0.640)	–(0.957)	+(0.600)
L. stoechas	Rosmarinic acid	+	-	**Low**	-	-	**–6.82 cm/s**	-	-	-	-	-	-	-	–(0.857)	–(0.650)	+(0.625)
	Apigenin 7-glucoside	+	-	**Low**	-	-	**–7.65 cm/s**	-	-	-	-	-	-	-	–(0.985)	–(0.610)	+(0.550)
S. costus	Dehydrocostus lactone	+	+	**High**	+	+	**–5.84 cm/s**	-	-	-	+	-	+	-	–(0.700)	–(0.943)	–(0.675)
	Malic acid	+	-	**High**	-	+	**–8.01 cm/s**	-	-	-	-	-	-	-	–(0.870)	–(0.871)	–(0.750)
	Quinic acid	+	-	**Low**	-	+	**–9.15 cm/s**	-	-	-	-	-	-	-	–(0.960)	–(0.957)	–(0.800)
Drug	Donepezil	+	+	**High**	+	+	**–5.58 cm/s**	+	+	-	-	+	-	+	–(0.600)	–(0.986)	–(0.775)
	Galanthamine	+	+	**High**	+	+	**–6.75 cm/s**	-	+	-	-	+	-	-	–(0.710)	–(0.957)	–(0.875)

V.2. Molecular docking

Molecular docking is a popular method in medicinal chemistry research (**Ramírez et al**., **2018**). It is an important tool for drug discovery to predict the binding mode of small molecules to target proteins and the binding energy of the complex (**Moraga-nicolás et al**, **2018; Taghipour et al**., **2018; Serseg et al**., **2020**). With the aim to confirm the experimental results, we have conducted a molecular docking study to simulate the interactions of studied compounds in the catalytic site of AChE, BChE, MAGL and BACE1. It is established that the hydrolysis reaction of ACh is mediated by the anionic site that presents ACh molecule to the catalytic site; at which hydrolysis occurs (**Abdel Bar et al**.,**2018**). Oxyanion hole plays an important role in the stabilization of high-energy intermediates through hydrogen bonding (**fig. 24**) (**Zhang et al**., **2002**, **Yang et al**., **2013**)

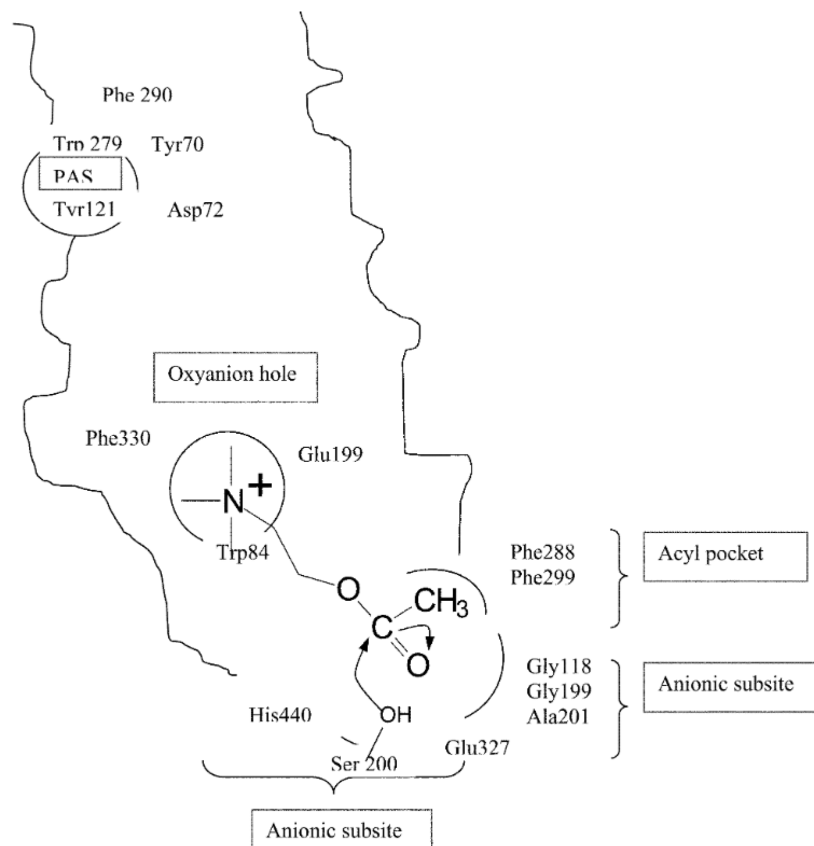


Figure 24. The binding regions of the *Torpedo californica* AChE gorge (**Houghton et al**., **2006**)

Arbutus unedo L

We have found that only gallocatechin has a good affinity toward AChE, where ΔG was -8.3 Kcal/mol, the other compounds have ΔG less than -6 Kcal/mol (**Table12**). The same compounds have also a weak affinity toward BChE (**Table13**), and all hydrogen bonds are larger than 2 Å. Whereas gallocatechin has a good affinity toward MAGL with ΔG equals -9.5 Kcal/mol and forms 8 hydrogen bonds with residues: HOH542, HOH585, HOH564, SER122, ALA51, GLU53, GLU190 and SER181 (**fig.25**). The shortest hydrogen bonds were with SER181 with a length of 1,692 Å (**Table14**). The same compound forms three hydrogen bonds (**Table15**) with HOH625, TRP115, GLN73 and GLY230 of BACE1 with a length of 3.782, 1.842, 2.702 and 1.727 Å respectively.

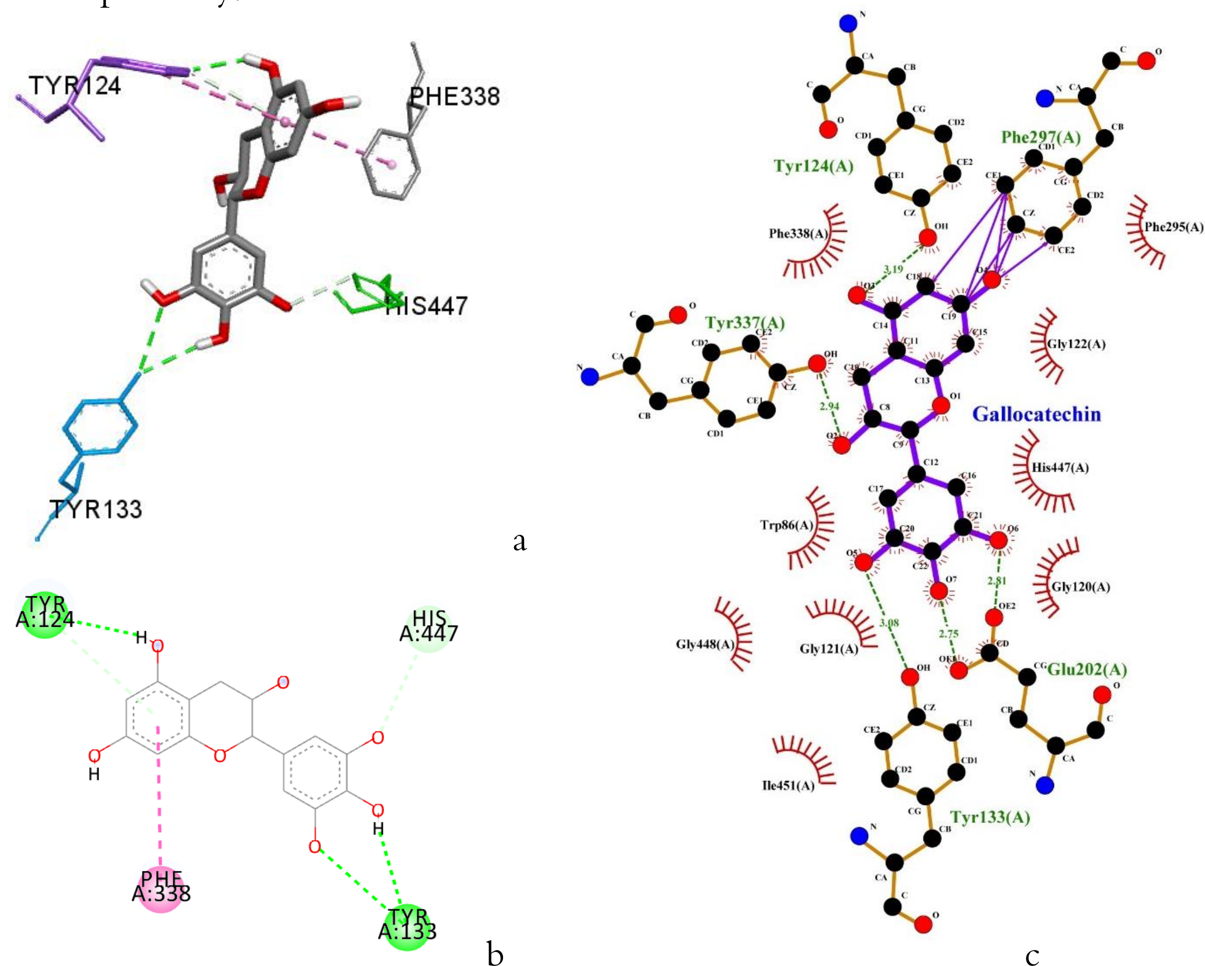


Figure 25. Binding mode of gallocatechin at the active site of HuAChE

Gallocatechin is illustrated in gray sticks. Catalytic triad of the active site was colored in green. Green dashed lines present hydrogen bond interactions. Pink dashed lines present hydrophobic interactions. a: 3D presentation obtained by DSV; b: 2D presentation obtained by DSV; c: the length of hydrogen bonds obtained by LigPlot+.

Based on those results, we think that gallocatechin is a good choice to develop an inhibitor for AChE and MAGL, in comparison to other *A. unedo* major molecules, where the predicated ΔG was -8.3 and -9.5Kcal/mol respectively. Moreover, it can form short H-bonds with MAGL active site residues: 1.692 Å.

These observations were identical to previous results, **Kennedy et al**., **2011** prove that gallocatechins could improve cognitive performance, while **Bastianetto et al**., **2006**, found that gallocatechins show several neuroprotective effects, thus include amelioration of β-amyloid-induced neurotoxicity. In other in silico study gallocatechin needs -11.01 kcal/mol to bind to AChE (**Ali et al**., **2016**).

Coriandrum sativum

We have found insignificant results in-vitro, which is similar to the docking results. Where almost of molecules did not form hydrogen bonds with the active site residues of AChE, if there are hydrogen bonds, the length is higher than two Å. We observe the same results from the three enzymes: BChE, MAGL and BACE1. There is an exception, where stigmasterol forms hydrogen bonds with THR122 of BChE with a length of 1,549 Å, but the repeating ratio of this pose was only 26%. In addition, quercetin and kaempferol bind tightly with all enzymes, these results confirmed in silico and in-vitro tests, but in our study, we did not get a significate result. We have used the seeds extract while these compounds are present in the leaves.

Juniperus oxycedrus

All molecules have 100% repeated poses, we think the obtained pose is the most stable one in the complex ligand-enzyme.

The amount of energy to bind with AChE needed by naringenin was -9.4Kcal/mol followed by myricetin (-9.0Kcal/mol) and catechin (-8.7Kcal/mol). Myricetin forms four hydrogen bonds with Ser203, Phe295, Tyr341 and Phe338 with length of 3.224, 3.120, 2.370 and 2.466Å, respectively (**fig**.**26**). Moreover, naringenin forms only two hydrogen bonds with Ser293 and Tyr337 (2.343 and 2.405 Å).

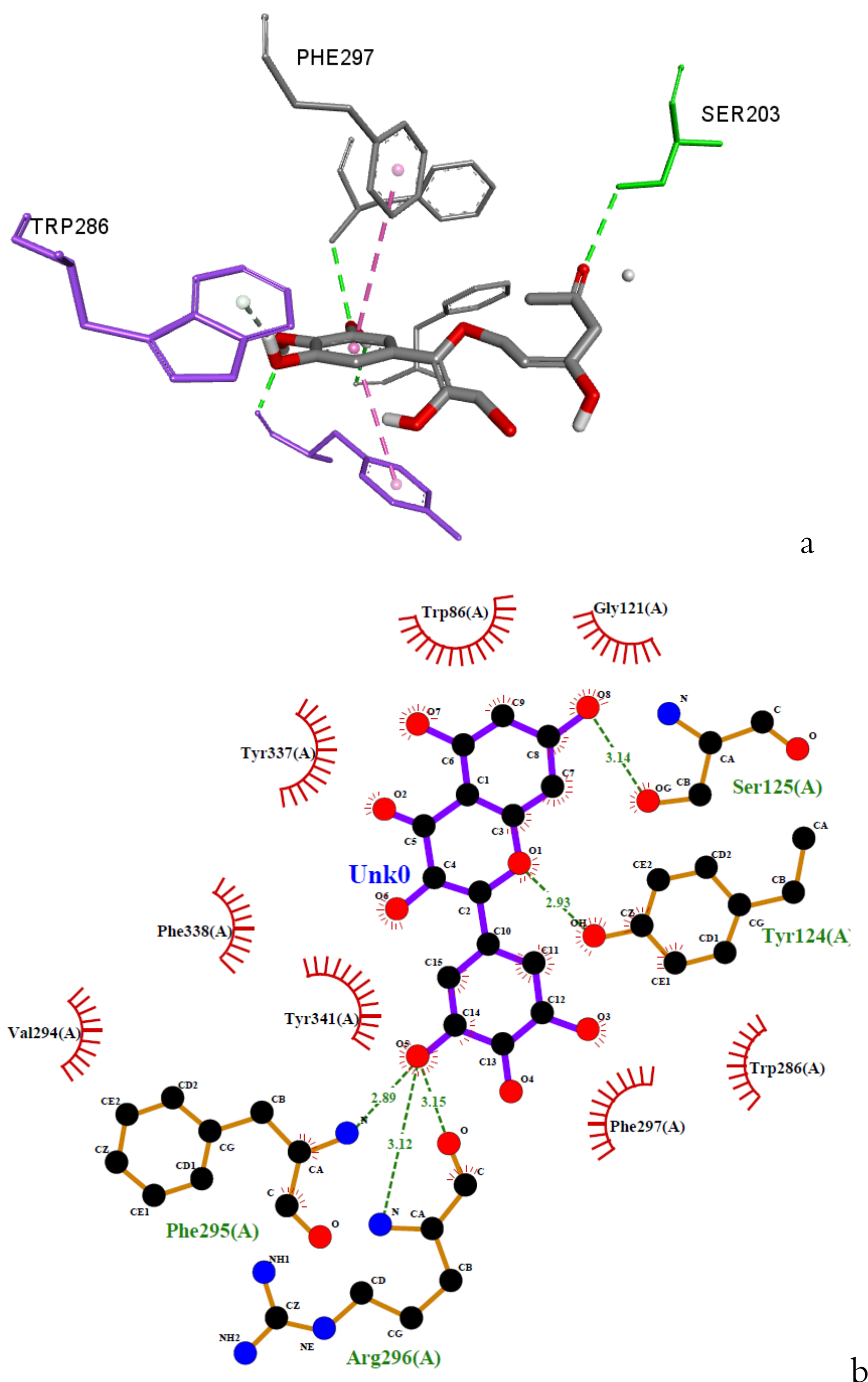


Figure 26. Binding mode of myricetin at the active site of HuAChE

Myricetin is illustrated in gray sticks. Catalytic triad of the active site was colored in green. Green dashed lines present hydrogen bond interactions. Pink dashed lines present hydrophobic interactions. a: 3D presentation obtained by DSV; b: the length of hydrogen bonds obtained by LigPlot+.

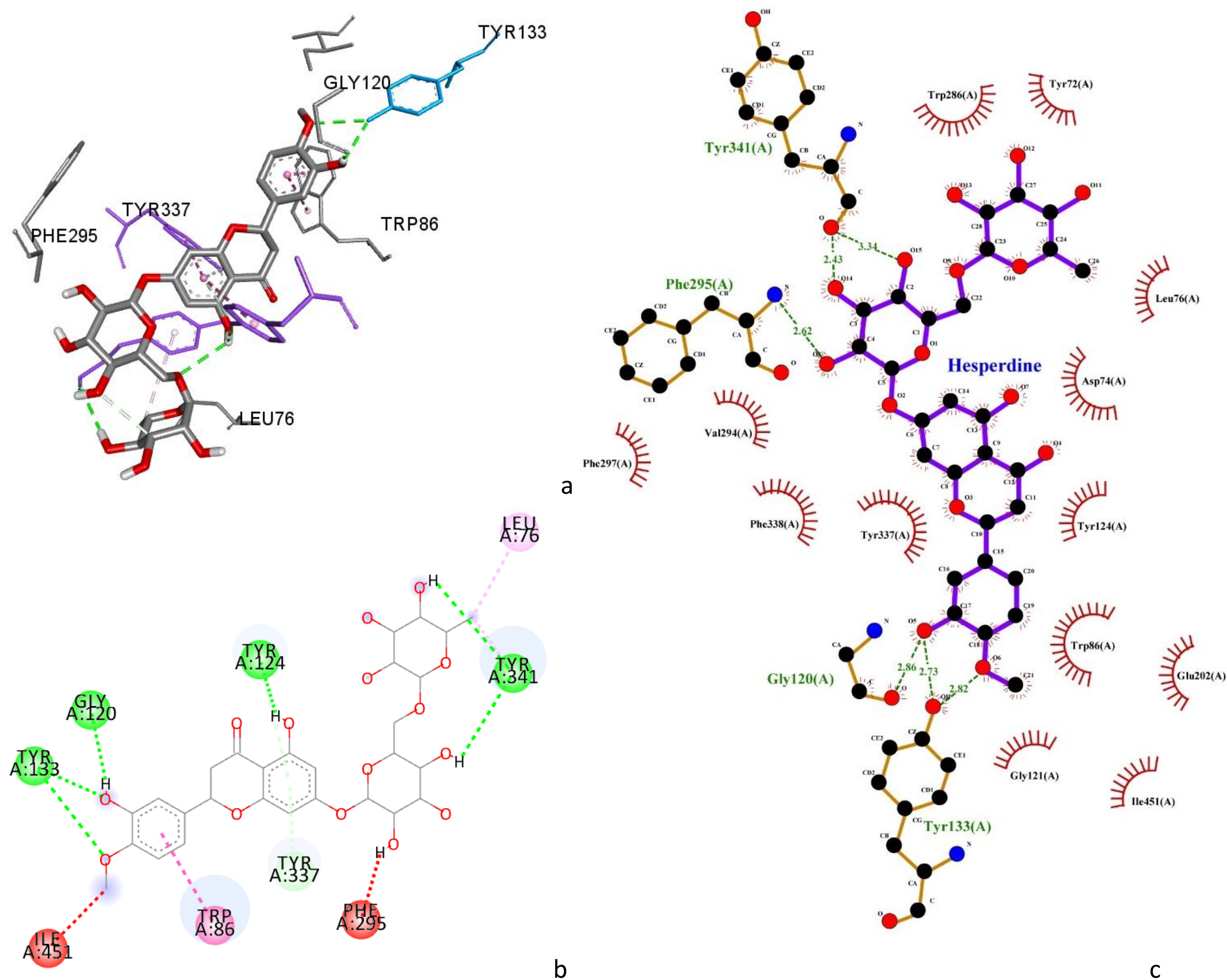


Figure 27. **Binding mode of hesperidin at the active site of HuAChE**
Hesperidin is illustrated in gray sticks. Catalytic triad of the active site was colored in green. Green dashed lines present hydrogen bond interactions. Pink dashed lines present hydrophobic interactions. a: 3D presentation obtained by DSV; b: 2D presentation obtained by DSV; c: the length of hydrogen bonds obtained by LigPlot+.

For BChE, the lowest binding energy was -8.2Kcal/mol of naringenin followed by -7.2Kcal/mol of catechin. Catechin as a major compound in *J. oxycedrus*, forms five hydrogen bonds with HOH811, HOH890, TYR332, TYR128 and GLN67 of BChE, the lengths of these bonds are 2.976, 3.486, 2.856, 1.780 and 3.000 Å. While hesperidin forms six hydrogen bonds with HOH772, HOH811, HOH890, THR122, GLN67 and TYR128, with a length of 2.818, 2.706, 3.374, 1.783, 2.062 and 2.759 Å.

For MAGL, we get one pose for all studied molecules with 100% repetition, the binding energy of all studied molecules is between -10.9 and -7.3Kcal/mol, but the majority of interaction was hydrophobic interactions. While for BACE1, hesperidin forms nine hydrogen bonds with HOH601, HOH625, HOH625, HOH671, ARG128, ARG128, ARG128, PRO70 and THR329 with lengths of 3.274, 2.888, 2.978, 2.68, 2.493, 2.641, 1.979, 2.534 and 2.268Å. In addition, thymoquinone forms only one hydrogen bond with TRP76 but the length of this bond is 1.829 Å.

We conclude that all molecules catechin, myricetin, rutin, naringenin, hesperidin (**fig.27**), salicylic acid and thymoquinone represent a good choice to develop anti-AD drugs with fewer side effects.

All those molecules have been reported as inhibitors of AChE, **Wang et al., 2017** confirmed that myricetin potently ameliorated memory deficits in mice, through inhibiting AChE activity and reducing oxidative stress. The IC_{50} of rutin was 242.00 ± 2.91 µM based on **Yan et al., 2018** study. Moreover, **Anesti et al., 2020** prove that rutin exhibits an important anticholinesterase activity in specific brain areas. **Umukoro et al., 2018**, suggest that naringenin attenuated memory deficits, via mechanisms related to inhibition of acetyl-cholinesterase activity, neuroinflammation and oxidative stress. In another study, the authors found that naringenin inhibited AChE activity, they also evaluated the anti-amnesic activity of naringenin, by the administration to mice at 4.5 mg/kg body weight, and they have found that naringenin significantly ameliorated scopolamine-induced amnesia as measured in both the passive avoidance and the Y-maze test (**Heo et al., 2004**). **Kim et al., 2019** declared that hesperidin plays an important neuroprotective role through its antioxidant and anti-inflammatory activities. In addition, hesperidin has an ani-AChE effect, where IC_{50}=134.44µM (**Liu et al., 2020**). They have found that IC_{50} of thymoquinone equals 0.14mg/mL (**Jukic et al., 2007**).

Juniperus phoenicea

For AChE: All studied molecules form hydrogen bonds with active site residues of AChE, but only 3-p-coumaroylquinic acid has a good affinity toward the enzyme with predicated ΔG equals -9.5Kcal/mol. The shortest hydrogen bonds were between 3-p-coumaroylquinic acid and Tyr337 (1.980Å; **fig.28**) and myricetin-O-pentoside and Ser203 (1.787Å). Furthermore, we also remarked that the amino acids of the catalytic site form hydrogen bonds as follows: His447 with 3-p-coumaroylquinic acid (2.670 Å), Ser203 with quercetin-O-pentoside (2.914Å) and myricetin-O-pentoside (1.787Å).

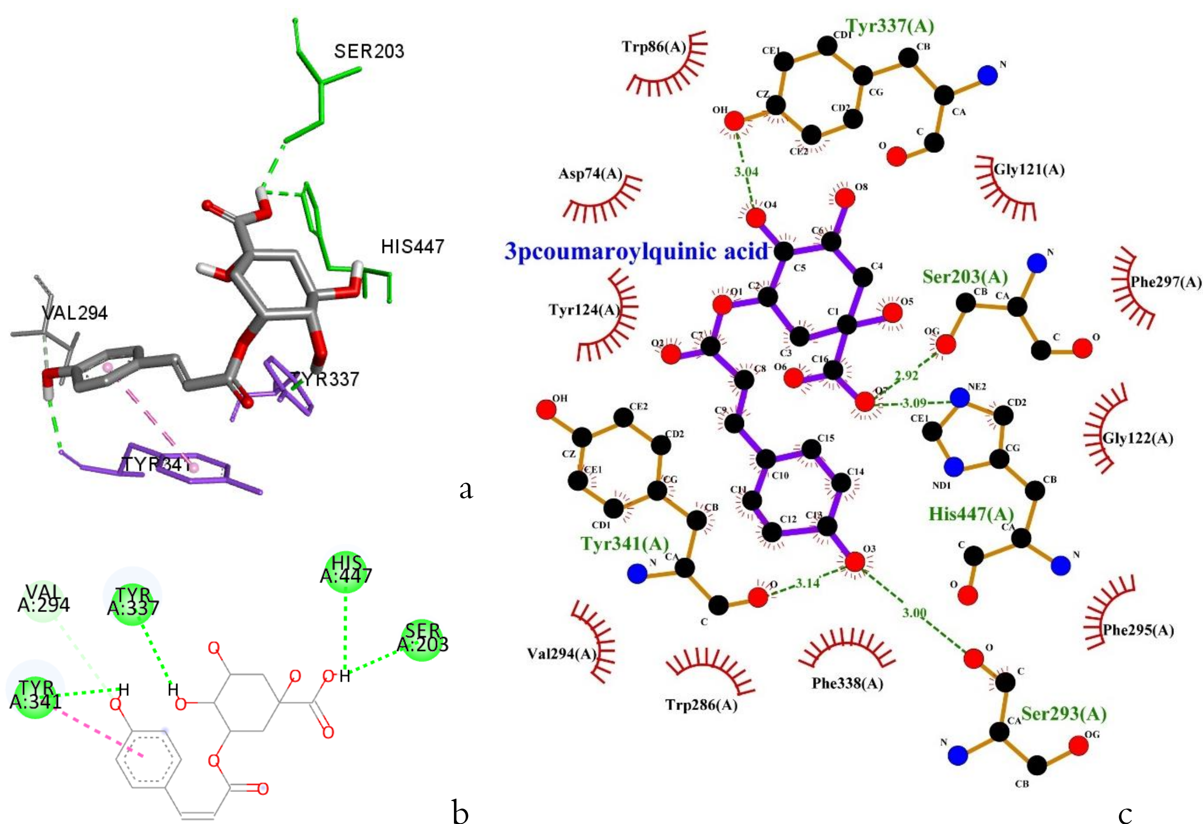


Figure 28. Binding mode of 3-p-Coumaroylquinic acid at the active site of HuAChE
3-p-Coumaroylquinic acid is illustrated in gray sticks. Catalytic triad of the active site was colored in green. Green dashed lines present hydrogen bond interactions. Pink dashed lines present hydrophobic interactions. a: 3D presentation obtained by DSV; b: 2D presentation obtained by DSV; c: the length of hydrogen bonds obtained by LigPlot+.

For BChE, all studied molecules form a significant number of hydrogen bonds: 3-p-coumaroylquinic acid (7 H-bonds and binding affinity equal -7.9Kcal/mol),

quercetin-O-pentoside (7 H-bonds), and myricetin-O-pentoside (7 H-bonds). The shortest hydrogen bonds were between quercetin-O-pentoside and HOH890 (1.922Å); and myricetin-O-pentoside and ASN83 (1.898Å) but binding affinity was -2.2 and -2.5Kcal/mol, respectively.

For MAGL, Both 3-p-coumaroylquinic acid and myricetin-O-pentoside form hydrogen bonds with lengths less than 2Å: 1.868 Å with ASP180 and 1.872 Å with GLY177. While quercetin-O-pentoside is stabilized in the active site only by hydrophobic interactions.

For BACE1, all molecules (3-p-coumaroylquinic acid, quercetin-O-pentoside and myricetin-O-pentoside) bind tightly to active site residues of BACE1, where they have good affinity with binding energy equals -8.1, -8.8 and -9.4Kcal/mol respectively. Moreover, it forms hydrogen bonds as follows: 3-p-coumaroylquinic acid forms six H-bonds with HOH625, HOH671, TRP76, GLY34, GLY34, and ILE126 with a length of 2.099, 1.994, 2.228, 2.549, 2.447, and 2.628 Å, respectively. quercetin-O-pentoside forms three H-bonds with HOH671, HOH625, and THR72 with a length of 3.073, 2.940 and 2.466 Å, respectively.

From these results, we think that the inhibitor effect of the *J.phoenicea* extract is due to 3-p-coumaroylquinic acid because it is the only molecule that give us good results for cholinesterase enzymes. To the best of our knowledge, there is no previous studies evaluate the effect of 3-p-coumaroylquinic acid, quercetin-O-pentoside and myricetin-O-pentoside on AChE activity.

Lavandula stoechas

The docking results were a little different to those of in-vitro experiments, where we have found low energy requested to bind rosmarinic acid and apigenin 7-glucoside with AChE, -10 and -9.7Kcal/mol, which means that they have a good affinity towards the enzyme, and the presence of a significant number of hydrogen bonds

formed between AChE residues and molecules (**fig**.**29**) but they are longer than 2 Å, which we think that those molecules are bind strongly to AChE active site.

The results for BChE, are different from those of AChE. We have found that rosmarinic acid might form a very short hydrogen bond with TRP82 residue with a length of 1.666 Å. From these results, and ΔG was higher than those obtained for AChE, -6.6 and 1.1 Kcal/mol.

For MAGL-rosmarinic acid complex, the predicated ΔG was -10.0Kcal/mol, and there are eight H-bonds with HOH542, HOH585, HOH542, HIS121, HIS121, SER122, HIS269 and SER181. All those bonds are larger than 2 Å. ΔG of apigenin 7-glucoside also -10.6Kcal/mol, and it could form 5 H-bonds with HOH542, ALA51 and ASP180, we can expect that both rosmarinic acid and apigenin 7-glucoside are good inhibitor for MAGL.

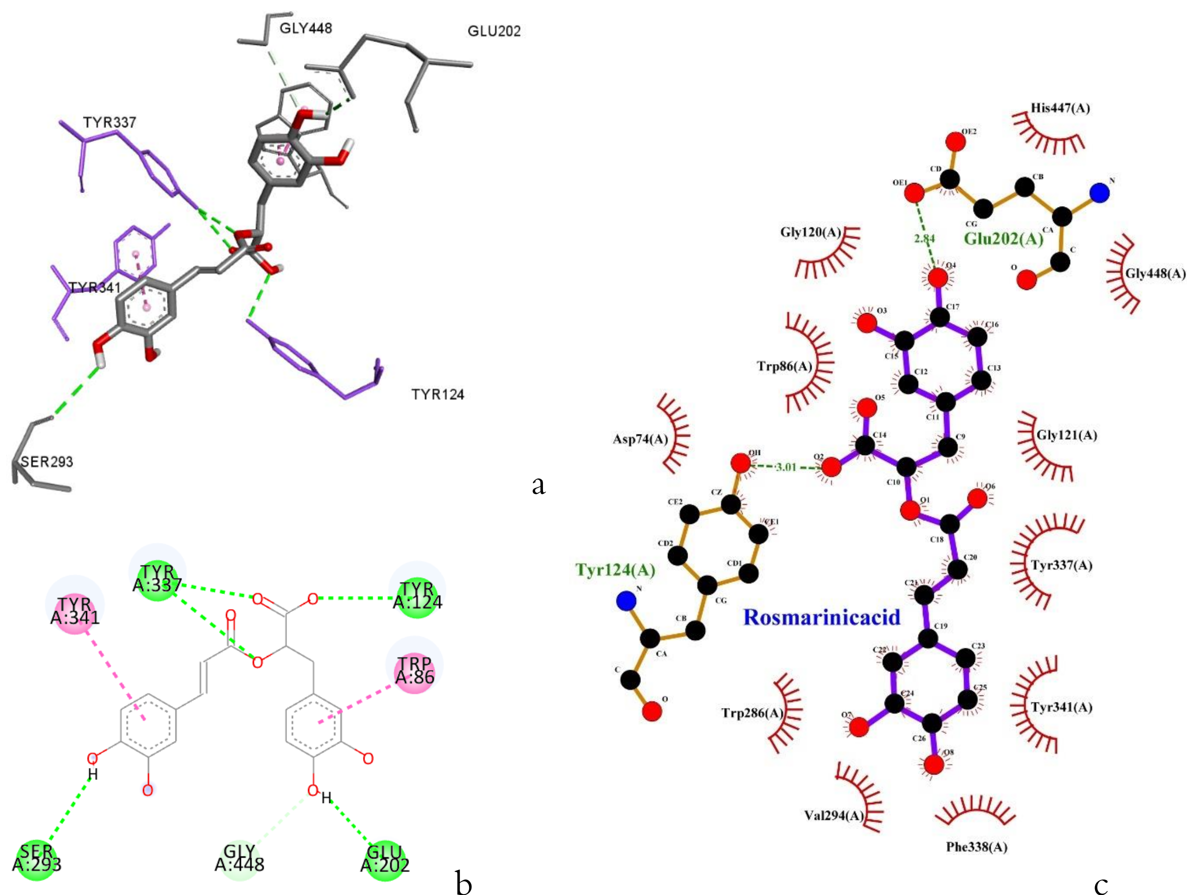


Figure 29. **Binding mode of rosmarinic acid at the active site of HuAChE**

Rosmarinic acid is illustrated in gray sticks. Catalytic triad of the active site was colored in green. Green dashed lines present hydrogen bond interactions. Pink dashed lines present hydrophobic interactions. a: 3D presentation obtained by DSV; b: 2D presentation obtained by DSV; c: the length of hydrogen bonds obtained by LigPlot+.

For BACE1, we did not find any repeated pose for both major molecules: rosmarinic acid and apigenin 7-glucoside. We have chosen one pose randomly and there was only one hydrogen bond for both ligands, which means that these molecules have a weak affinity for BACE1, and the complex ligand-enzyme, in this case, is unstable. In general, the two molecules showed a strong interaction with AChE and MAGL but the interaction with BChE and BACE1 was weak.

In a previous study, the inhibitory constant Ki of rosmarinic acid was 42.52 picoM for AChE 121.60 picoM for BChE (**Gülçin et al**., **2016**). Whereas in another study performed by **Orhan et al**.**2008**, rosmarinic acid decreases the activity of AChE by 47.3±1.05% and BChE activity by a value of 85.8±1.31% at a concentration of 1mg/ml. Moreover, *in vivo* evidence **Hamaguchi et al**., **2009**, reveals that oral administration of rosmarinic acid affecting various amyloid-β aggregation pathways, which prevented the development of Alzheimer's disease. There are no available studies about the effect of Apigenin 7-glucoside on any of the studied enzymes.

Saussurea costus

The docking results was similar to *in vitro* results for *S. costus*, all poses for all major molecules show a weak interaction with AChE residues, for example, we did not find any H-bond with a length less than 2 Å. In addition, predicated ΔG were -7.8, -5.3 and -6.8 Kcal/mol for dehydrocostus lactone, malic acid and quinic acid. In comparison with other studied molecules where we found -11 and -10 Kcal/mol (**Table 12**). Same results for BChE, except for malic acid, where it formed six H-bonds with HOH890, HOH772, THR120, SER198 and GLU197, the length of these bonds also larger than 2 Å.

For MAGL, dehydrocostus lactone, interacts with this enzyme only by hydrophobic interactions of type Alkyl. Whereas the other molecules did not form any hydrophobic interactions with this enzyme, they bind to the active site only by H-bonds. We get the same results for BACE1, dehydrocostus lactone stabilized mostly in

the active site with hydrophobic interactions of type Alkyl, while of the molecules malic acid and quinic acid stabilized in the active site only by means of H-bonds, where their length larger than 2 Å.

We conclude that the molecules malic acid and quinic acid are not good choice to develop a new anti-Alzheimer's disease drug, because they could not form strong interactions with the active site of all AD targets: AChE, BChE, MAGL, and BACE1, their binding to these enzymes is weak, which means that are weak inhibitors.

Lepidium sativum

Molecular docking calculation from 6O4W predicate that binding energy of HuAChE-lepidine B and HuAChE-lepidine E is -11.1 and -10.4 kcal/mol, respectively, whereas galantamine and donepezil are -9.6 and -11.7 kcal/mol, respectively.

These results show that the affinity of HuAChE to Lepidine B is more than galantamine (the current commercial drug), Lycoramine, 6α-Deoxy-tazettine, and Norpluvine diacetate found by **Moraga-nicolás et al** (**2018**) with values of -8.58, -8.83, -8.57 and -8.92 kcal/mol, respectively.

Table 12 shows the binding energy of studied complexes, **Moraga-nicolás et al**., **2018; Dubey et al**. **2018** studied the predicated energy binding of HuAChE to Galantamine and Donepezil that was -9.7 and -10.9 Kcal/mol, respectively, these results are similar to the obtained results in this study (**Dubey et al**., **2018**).

Molecular docking results show that the three molecules interact with HuAChE, in the same way, forming interactions with Trp86 and Phe338 (AS), Phe295 (ABP), Tyr72, Tyr124, Trp286 and Tyr341 (PAS) and other residues such as Tyr337. On the other hand, 96% of poses indicate that Lepidine B stabilized in the active site by hydrogen bond while only 14% and 10% of poses of galantamine and Donepezil, respectively, contain hydrogen bonds. In blind docking, 96% of poses with the lowest

binding energy shows that Lepidine B bind to HuAChE in the Active site, whereas Donepezil and Galantamine are 76.19% and 56%, respectively.

This confirms that Lepidine B is more stable in the active site than Galantamine. It should be noted that the molecular interactions with HuAChE obtained for Lepidine B are stronger than those observed for Galantamine and Donepezil.

We have done the docking with these parameters with Lepidine B for the first time.

The docking study predicts that Lepidine B binds strongly in the active site, which is presented in **figure 30a**. In the most frequented pose of the HuAChE-Lepidine B complex, OH group establishes H-bond interaction with the hydroxyl group of Tyr124 (2.31Å). The hydrophobic interactions between Lepidine B and HuAChE active site are most with the oxyanion hole, Anionic site, and Peripheral anionic site residues. The benzene ring interacts by means of π-π-stacking interaction with the aromatic rings of Trp86, Tyr124 of PAS. The other benzene ring interacts by π-π-stacked interaction with the aromatic ring of Trp86, and π-T-shaped interaction with Try337 and Phe338. The imidazole ring interacts by π-π-stacked interaction with benzene ring of Tyr341 of PAS. **Figure 30a** shows the main interactions of this alkaloid at the binding pocket of HuAChE.

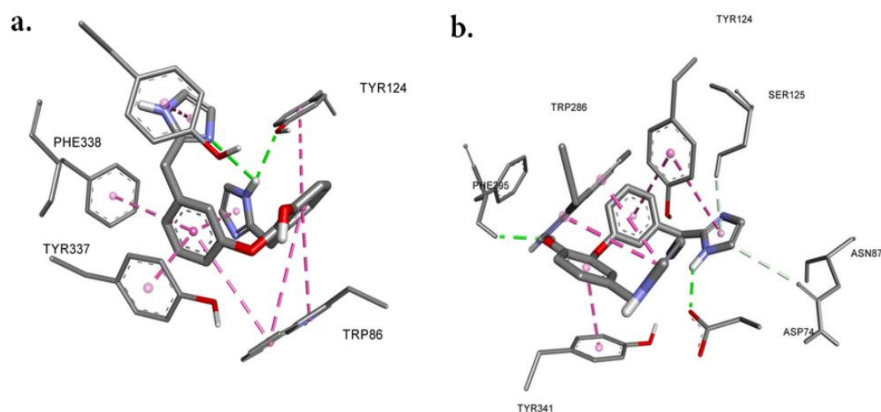


Figure 30. Binding mode of Lepidine B (a.) and Lepidine E (b.) at the active site of HuAChE
Lepidine B and E are illustrated in gray. Catalytic triad of the active site was colored in green. Green dashed lines present hydrogen bond interactions. Pink dashed lines present hydrophobic interactions.

Lepidine E binds strongly in the active site of HuAChE by two hydrogen bonds. The first between NH group of lepidine E and the hydroxyl group of Asp74 (2.21Å), the second is between the hydroxyl group of lepidine E and NH main group of Phe295 (2.26Å). This interaction was reinforced by hydrophobic interactions with PAS residues. The benzene ring of Tyr124 forms hydrophobic interaction of type π-π-stacked with the benzene ring of lepidine E. The other benzene ring interacts with Tyr341 by means π-π-stacking. The imidazole ring forms two interactions of type π-π-stacked with Trp286. The formed interactions are presented in **figure 30b**.

As depicted in **figure 31a**, there are two H-bonds interactions between Galantamine and HuAChE residues in PDB file of crystal structure of AChE with galantamine (PDB ID: 4EY6: HuAChE-Galantamine complex). The hydrogen atom at OCH_3 group is oriented toward Ser204 forming H-bond (2.9Å), a second hydrogen bond was formed between the hydroxyl group of galantamine and Glu202 (2.65 Å). Additional H-bonds were formed between galantamine and HOH molecules such as: HOH707, HOH815, HOH820, HOH860, HOH860, HOH862, HOH892 with length 3.30, 3.23, 3.44, 3.83, 3.03, 3.16, and 3.50 Å, respectively. This complex is further intensified by hydrophobic interactions. π-alkyl interactions have been observed between Trp86 and Tyr337 and the benzene ring and azepane ring of galantamine. The Gly122 of oxyanion hole interacts with benzene ring of galantamine by means of amide-π stacking. F**igure 31b** shows that Donepezil was well accommodated in the PAS through hydrophobic interactions of π-alkyl type between the piperidine ring and the benzene ring of Tyr341 and Tyr337, and π-stacked interaction between indole ring of Trp86 and benzene ring of donepezil. Both benzene rings and the cyclopentane ring of donepezil interact with Trp286 and Tyr341 by means of a π-alkyl type contact. This interaction was reinforced by a hydrogen bond between the hydroxyl group of donepezil and Phe295 (2.90Å). Additional hydrogen bonds have been found in the complex HuAChE-Donepezil (Crystal structure of

AChE with Donepezil PDB ID:6O4W), between donepezil and water molecules such as: HOH708, HOH788, HOH797, HOH832, HOH832, HOH875 (3.03, 3.72, 3.69, 3.01, 3.01, and 3.39, respectively).

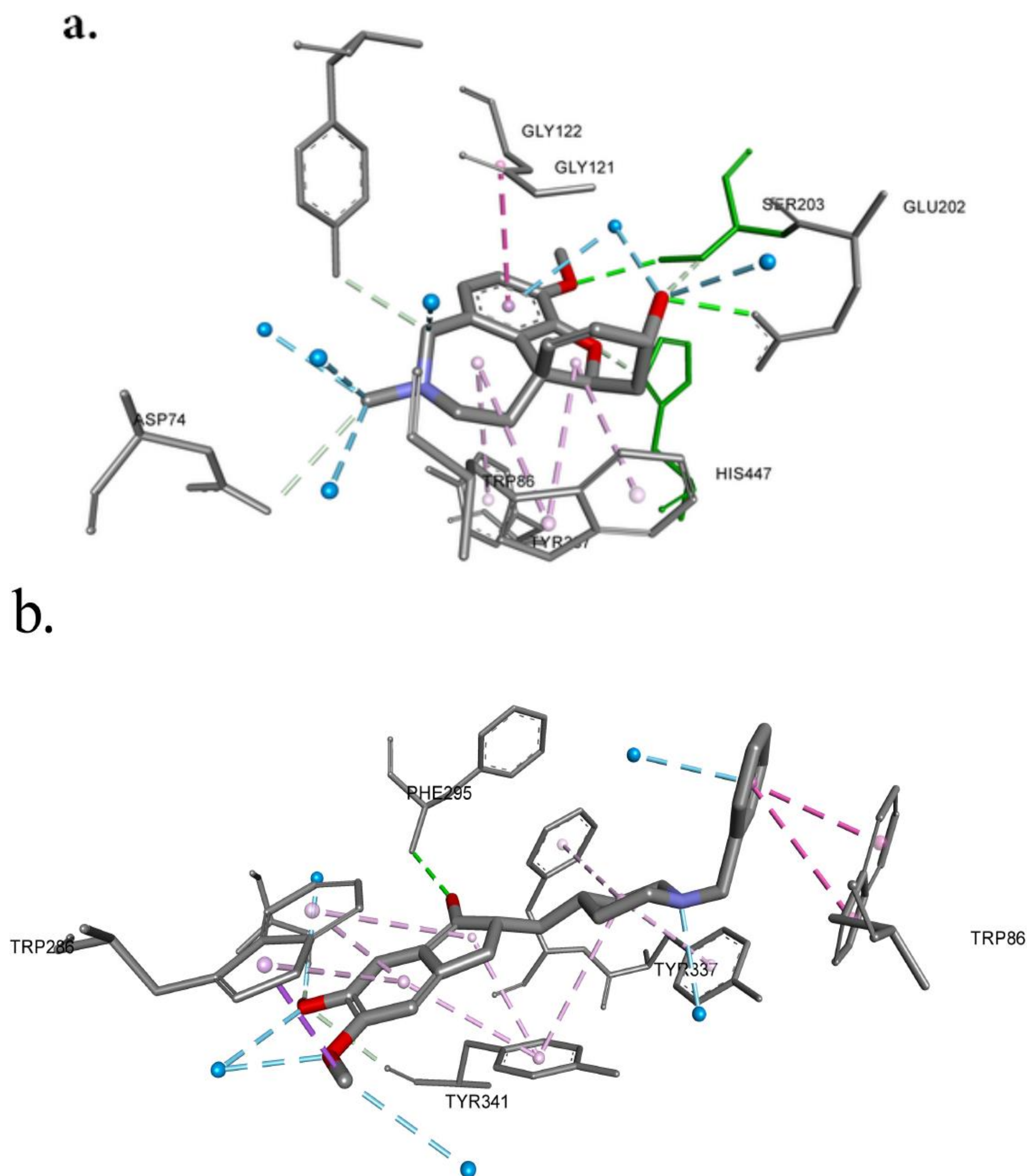


Figure 31. **Binding mode of Galantamine (a.) and Donepezil (b.) at the active site of HuAChE**
Galantamine and Donepezil are illustrated in gray. Catalytic triad of the active site were colored in green. Green dashed lines present hydrogen bond interactions. Pink dashed lines present hydrophobic interactions

It has been reported that replacement of OCH3 group by OH in Galantamine (the extra hydroxyl group of sanguinine (9-O-demethylgalanthamine) available for interaction with AChE, could explain the strong inhibitory activity of this alkaloid,

while the lack of AChE inhibitory activity of lycoramine and epinorlycoramine the galanthamine-type alkaloids could be due to the occurrence of a double bond in ring C (**López et al**., **2002**, **Ortiz et al**., **2018**). Our results support such hypothesis; it is rational to think that the stronger affinity observed for Lepidine B might be attributed to hydroxyl group which can establish H-bond shorter than that observed in Donepezil and galantamine and imidazole group which forms hydrophobic interactions as well as to the stronger molecular interactions observed for Lepidine B in comparison to those of Donepezil and galantamine for the Tyr124.

Chioua et al. **2018** founded that 4k compound interacts with residues at the PAS and not with those at the catalytic triad where free binding energy was -10Kcal/mol. While QPT16, a synthesized molecule can interact with the acyl binding pocket and PAS (binding affinity: −12.4 kcal/mol, IC_{50}= 1.10 ±0.15 mM). The key interactions involved in the binding are with Trp286, Leu289, and Tyr341 by π-stacked interaction, and with Leu289 by π-alkyl interaction (**Chioua et al**., **2018a;b**).

These interactions are similar to the found interactions in this study. In addition, **Dickerson et al**. **2005** have found that most of the studied compound binding in PAS, where Tyr residues (72, 124, 337 and 341) interact with the Ride chain of these compounds.

In another study performed using Molecular Operating Environment (MOE) software. A compound (16) has moderate inhibitory activity against AChEee with IC_{50} =0.89 ± 0.02 μM. Molecular modeling studies showed that the compound was a dual site binding inhibitor for HuAChE, it can simultaneously interact with PAS and the catalytic triad of HuAChE, the phenyl of chroman-4-one was binding in PAS through a π-π stacking interaction with Trp286 (3.76Å).

While the protonated nitrogen of N-dimethylamino can bind to CAS with the indole ring of Trp86 (4.07Å) via a cation-π interaction (**Lui et al**., **2017**).

In a comparing study between in vitro and in silico studies about the type of inhibition, the kinetic data confirm that a non-competitive inhibitor shows interactions with the peripheral anionic site (PAS) of AChE (**Boulebd et al**., **2016**), which leads us to suggest that Lepidine B displays a non-competitive inhibition (**Samadi et al**., **2012**). Donepezil interacts with PAS residues which indicate that the inhibition mode of Donepezil is noncompetitive, this observation is similar to previous studies (**Sugimoto et al**., **2002**, **Bacalhau et al**., **2018**). Peripheral anionic site (PAS) is an important part of AChE, due to its involvement in the proaggregating action of AChE on Aβ as well as its ability to regulate the esterase activity (**Bartolini et al**., **2003**). Donepezil was found to inhibit the HuAChE-induced Aβ aggregation (**Bartolini et al**., **2003**). Lepidine B as a possible non-competitive inhibitor and his ability to interact with PAS might prevent the AChE-induced amyloid aggregation.

Likewise, the results obtained for HuBuChE are in agreement with the experimental data described previously in many studies; Galantamine and Donepezil are dual inhibitors for HuAChE and HuBuChE (**Mohamed et al**., **2012; Akbarzadeh et al**., **2018; Chierrito et al**., **2018; Kurt et al**., **2018; Gurjar et al**., **2018**). Molecular docking explains the differential behavior of these compounds Lepidine B, Lepidine E, Galantamine, and Donepezil. All these compounds bind in the same region of the active site of HuBuChE. The docking study predicts that Lepidine B showed the same binding profile as Galantamine unless the length on H-bond was stronger and shorter in HuBuChE-LepidineB with a length of 1.7Å, which prefaced to covalent bond in this complex, while the length of H-bond in HuBuChE-Galantamine was 2.88Å. Lepidine E forms two hydrogen bonds with HuBuChE (2.2 and 2.73Å). The complex HuBuChE-Donepezil is stabilizing only by hydrophobic interactions.

Figure 32a shows the main stabilizing interactions for the complex HuBuChE-Lepidine B. NH group of the imidazole ring can form H-bond with the hydroxyl group of Tyr128 (1.7Å). The same interaction has been observed in the complex

HuBuChE-Galantamine (2.88Å) as shown in **figure 33a**. Additional H-bond in the complex HuBuChE-Galantamine between the hydroxyl group of Thr120 and OCH_3 of galantamine. NH group of the imidazole ring of Lepidine E forms H-bond with Asp (2.23Å), another H-bond was observed between hydroxyl group of Lepidine E and Trp82 (2.73Å), as shown in **figure 32b**.

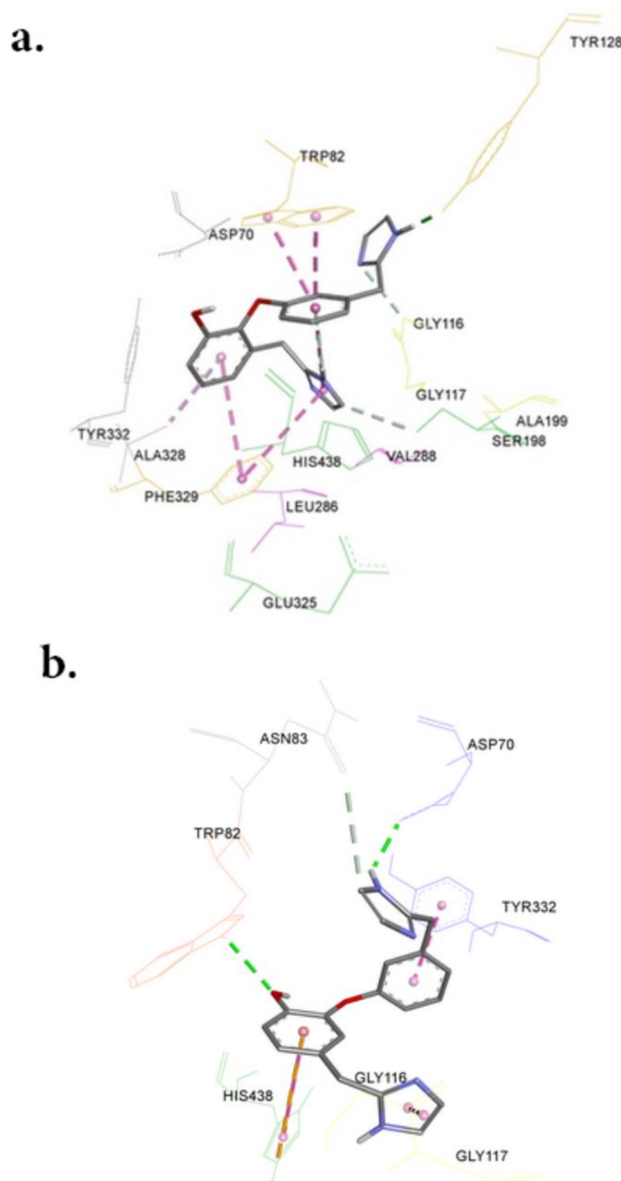


Figure 32. **Binding mode of Lepidine B (a) and Lepidine E (b) at the active site of HuBuChE**
Lepidine B and E are illustrated in gray. Ligand is rendered as sticks. Different subsites of the active site were colored: catalytic triad (CT) in green, oxyanion hole (OH) in yellow, anionic sub-site (AS) in orange, acyl binding pocket (ABP) in pink, and peripheral anionic subsite (PAS) in blue. Green dashed lines present hydrogen bond interactions. Pink dashed lines present hydrophobic interactions.

It should be noted that Donepezil did not form any H-bond with HuBuChE, it is stabilized in the active site only by hydrophobic interactions (**fig. 33b**). Benzene ring of Lepidine B interacts with the indole ring of Trp82, Ala328, and Phe329 by means π-stacking, π-alkyl and π-T-shaped, respectively. Benzene rings of Phe329 and Tyr332 also form hydrophobic interactions of type π-stacked with imidazole ring of lepidine B. In addition to H-bonds, Lepidine E stabilizes in the active size by hydrophobic interactions between benzene rings and Gly116-Gly117, Tyr332 and His438 of type Amide- π-stacked, π-stacked and π-Tshaped, respectively.

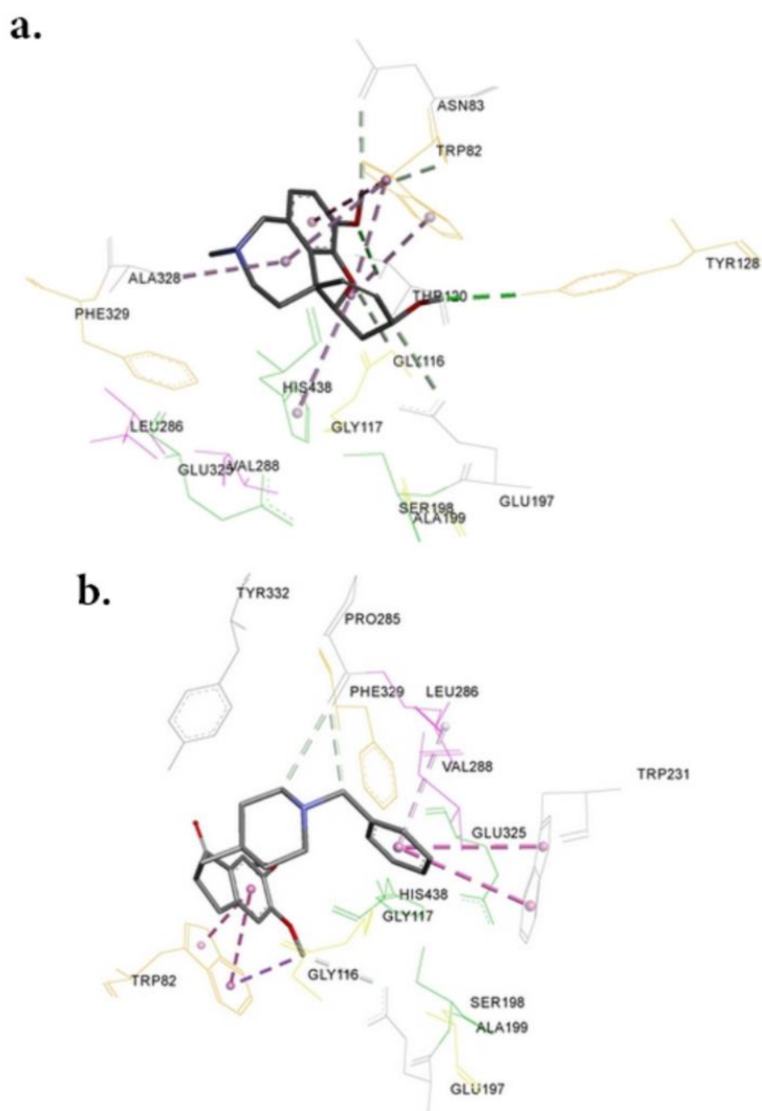


Figure 33. Binding mode of Galantamine (a) and Donepezil (b) at the active site of BuChE
Galantamine and Donepezil are illustrated in gray. Ligand is rendered as sticks. Different subsites of the active site were colored: catalytic triad (CT) in green, oxyanion hole (OH) in yellow, anionic sub-site (AS) in orange, acyl binding pocket (ABP) in pink, and peripheral anionic subsite (PAS) in blue. Green dashed lines present hydrogen bond interactions. Pink dashed lines present hydrophobic interactions.

On the other hand, Trp82 interacting with galantamine in three ways, by forming π-stacked interaction between indole ring and benzene ring of galantamine, and π-alkyl interaction between the indole ring of Trp82 and the benzene ring of galantamine or the imidazole ring of galantamine (**fig. 33a**).

Otherwise, Donepezil stabilizes in the active site only by hydrophobic interactions, which might explain the high ability and selectivity of Donepezil to inhibit AChE more than BuChE (**Dickerson et al**., **2005; Ghobadian et al**., **2018**). As shown in **figure 33b**, Indole ring of Trp82 interacts with the benzene ring by means π-stacking interaction, and with the methyl group of Donepezil by means π-sigma interaction. Benzene ring of Donepezil forms hydrophobic interaction of type T-shaped with the indole ring of Trp231. An additional hydrophobic interaction of type π-alkyl was observed between the methyl group of Leu286 and the benzene ring of donepezil. 4K molecule from **Chioua et al**. **2018** study, binds very strongly to HuBuChE. It can form hydrogen bonds with the catalytic amino acids His438 and Ser198 (**Berman et al**., **2000**). Enzyme kinetic studies showed that 3b (1–butanoyl–3–(2, 6–dimethylphenyl) thiourea) has an uncompetitive binding with AChE and interacts with Trp86 and Tyr337 (PAS residue) as molecular docking studies had shown. While 3e (1–butanoyl–3–(3–methoxyphenyl)Thiourea) showed a mixed inhibition of HuBuChE. Molecular docking studies showed a binding affinity with Trp82 and His438 when it is docked with HuBuChE(**Larik et al**., **2017**).

We have performed specific docking for these molecules with the other two Alzheimer's-related enzymes: β-Secretase-1 (BACE1) and Monoacylglycerol lipase (MAGL). The results are summarized in **Table 14** and **15**. For β-Secretase-1 (BACE1) with PDB ID: 6EJ3 (**Johansson et al**.,**2018**), all poses of the lepidine B-BACE complex show the same interactions (100%), one hydrogen bond has been recorded between OH of lepidine B and HOH671 with ID of 2.11Å.

Benzene ring forms hydrophobic interactions of type π-πTshaped with Trp76, π-Cation with Arg128. Imidazole ring forms hydrophobic interactions of type Amide-π stacked with Gly34 and Ser35. Another hydrophobic interaction has saved π-sigma interaction between Lepidine B and Tyr71 (**Table 15**).

Lepidine E shows a strong binding in the active site of BACE1. A H-bond with Gly34 of length 1.89Å has been observed, additional H-bonds were formed with HOH601 and HOH 671 molecules (3.05 and 2.51Å). Imidazole ring forms hydrophobic interactions of type πT-shaped, π-Alkyl and π-Anion with Tpr76, Ile118 and Asp228, respectively. In comparison with Donepezil and Galantamine, we propose that galantamine has a weak interaction with β-Secretase-1, which means that galantamine could not be a good inhibitor for this enzyme, while Donepezil has a strong interaction with β-Secretase-1. Two hydrogen bonds have been saved in the complex Donepezil-BACE, first with HOH625 with ID=3.61Å, second with Trp76 with ID=2.23Å. Hydrophobic interactions with different types as Alkyl with Ile118, π-Alkyl with Try71 & Leu30 a π-Cation with Arg128 (**Table 15**).

For Monoacylglycerol lipase (MAGL) PDB ID: 5ZUN (**HATTORI et al**., **2019**), with repeating ratio of 72.5%, 100% 92.5% of Lepidine B, Donepezil and galantamine, respectively, it has been found that Lepidine B binds tightly into the catalytic cavity more than galantamine. One hydrogen bond has been found for Lepidine B with ID=2.14Å, and hydrophobic interactions of type π-Alkyl with Ala151, Leu213, Leu214, Leu148, Ile179 & Leu205 and of type π-sigma with Leu213, Leu241. The repeating ratio of Lepidine E was 50%. However, Lepidine E binds strongly in the active site by H-bonds and hydrophobic interactions. H-bonds were observed between imidazole ring and Ser181 & HOH564 (2.65, 3.74Å, respectively). Hydrophobic interactions were between imidazole rings and Ala51, Val 191, Leu213, Leu 241, Val207, Lys273 of tye π-Alkyl and with Leu 148, Tyr194, Cyc242 of type π-Sigma, π-π-Stacked and π-Sulfur, respectively. Benzene ring interacts with Ala51,

Ile179, Leu241 forming a π-Alkyl interaction and π-Sigma interaction with Val270. No hydrogen bond has been recorded for galantamine. The latter forms hydrophobic interactions with Monoacylglycerol lipase MAGL with different types as π-Sigma with Leu 213 & Leu 241, Alkyl with ALA51, Ile179, Ilz205 & Leu241 and π-Alkyl with Ala51. For complex MGLL-Donepezil, in all poses (100%), three hydrogen bonds have been found, with HOH542 (3.06Å), Ser122 (2.72Å) & Met123(2.74Å). Hydrophobic interactions between the three rings of Donepezil and MAGL residues as by different types as π-sigma with Leu241 & Val270, π- π Stacked with Tyr194, Alkyl with Ile179 & Val270 and π-Alkyl with Ala51 & Lys273, The β-site amyloid cleavage enzyme 1 (BACE1) is a promising target as a pharmacological treatment for AD (**Fang et al**., **2019**, **LIANG et al**.,**2019**). Several studies suggested that BACE1 inhibitors have high therapeutic potential for the prevention of the long-term progression of Alzheimer's disease (**PONZONI et al**., **2019**).

MAGL is currently being considered as a promising drug target for several disorders including neurodegenerative, cancer and inflammatory diseases (**FERRISI et al**., **2019**). Our results show promising results for Lepidine B as a multifunctional potential drug for Alzheimer’s disease, where it can be a good inhibitor for AChE, BuChE, BACE, and MAGL.

Table 12. The results of interactions between major compounds and Drugs (Galantamine, Donepezil) and (AChE).

	MOLECULE	BUTYRYLCHOLINESTERASE (1P0M)					
		RR%	**ΔG(Kcal/mol)**	**Closest Residues**	**Hydrophobic interactions**	**H bonds**	**Length**
A. unedo	Gallocatechin	70	-8.3	His447, Tyr124, Phe338	Pi-Pi T-shaped	Tyr133 Tyr133 Tyr124	3.081 2.634 2.322
	Ethyl gallate	100	-6.6	Gly448, Trp86	Pi-Pi Stacked	Tyr337 Glu202	3.051 3.076
	Arbutin	100	-7.4	-	-	TYR124 SER125	2.910 3.238
C. sativum	Quercetin*	100	-9.3	PHE295, TRP236, VAL294	Pi-Pi Stacked, Pi-Alkyl, Pi-Pi T-shaped	SER203 SER203	2.875 2.701
	Kaempferol	100	-8.7	A:PHE295, TRP286, TRP236, VAL294	Pi-Pi Stacked, Pi-Alkyl, Pi-Pi T-shaped	SER203 ARG296	1.804 2.441
	p-coumaric acid	100	-8.5	Gly448, Trp86	Pi-Pi Stacked	Tyr337 Glu202 Tyr124 Ser125	2.894 2.107 2.660 3.079
	ferulic acid	100	-7.5	Tyr133, Trp86	Pi-Pi Stacked	Tyr124 Tyr337 Glu202	3.062 2.964 2.779
	3-O-caffeoylquinic acid*	100	-9.7	Trp86, Tyr341, Phe297	Pi-Pi Stacked, Pi-Pi T-shaped	Tyr337 Ser293	2.834 2.895
	Stigmasterol	96	-9.0	Trp286, Phe297, Tyr337, Phe338, Tyr341, His447	Pi-Alkyl	-	-
	β-sitosterol	98	-8.3	Tyr341, Trp286, Tyr72, Phe297, Tyr337, Phe338, Tyr341, His447	Pi-Alkyl, Pi-Sigma	-	-
	γ-tocopherol	66	-9.7	Trp86, Tyr337,	Pi-Sigma, Pi-Alkyl	-	-

				Phe297, Phe338, Tyr341, His447			
	α-tocotrienol	100	-9.8	Tyr341, Trp286, Tyr337, Trp86, Tyr72, Phe297, Phe338, His447, Tyr449	Pi-Sigma, Pi-Pi Stacked, Pi-Alkyl	-	-
	γ-tocotrienol	100	-11.0	Tyr341, Tyr337, Val294, Trp86, Tyr124, Trp286, Phe297, Phe338, His447, Tyr449	Pi-Alkyl, Pi-Sigma, Alkyl	-	-
J. oxycedrus	Catechin	96	-8.7	Phe338	Pi-Pi T-shaped	Ser203 Tyr337	3.102 2.145
	Myricetin	82	-9.0	Trp286, Tyr341, Phe297	Pi-Pi Stacked, Pi-Pi T-Shaped	Ser203 Phe295 Tyr341 Phe338	3.224 3.120 2.370 2.466
	Rutin	100	-3.6	Gly122, Trp286, Tyr341, Trp86	Pi-Alkyl, Pi-Pi Stacked	Tyr124 Tyr133 Tyr341 Tyr337 Tyr341 Glu202 Glu202	2.800 2.918 3.165 2.461 2.062 2.691 2.273
	Naringenin	100	-9.4	Tyr124, Trp286, Tyr337	Pi-Pi T-Shaped, Pi-Pi Stacked	Ser293 Tyr337	2.343 2.405
	Hesperidin	70	-3.3	Tyr124, Tyr337, Trp86, Leu76, Tyr341	Pi-Alkyl, Alkyl, Pi-Pi T-Shaped, Pi-Pi Stacked	Tyr133 Tyr133 Gly120 Tyr124 Tyr341 Tyr341	2.730 2.818 1.876 2.725 2.562 2.796
	Salicylic acid	100	-7.2	Trp86	Pi-Pi Stacked	Tyr133	2.963
	Thymoquinone	84	-6.7	Trp86, Tyr124, Phe297, Phe338	Pi-Alkyl, Pi-Sigma	Tyr337	3.087

J. phoenicea	3-p-Coumaroylquinic acid	56	-9.5	Val294, Tyr341	Pi-Pi Stacked	Tyr341 Tyr337 Ser203 His447	2.223 1.980 2.122 2.670
	Quercetin-O-pentoside	100	-5.3	Gly121, Tyr337, Tyr124, Trp286, Phe338, Tyr341	Pi-Pi T-Shaped, Pi-Pi Stacked	Ser125 Ser203 Ser203 Tyr337 Asp74 Tyr337 Ser293	2.914 3.224 2.959 2.908 2.840 2.683 2.908
	Myricetin-O-pentoside	100	-6.3	Ser125, Tyr341, Trp286, Phe338	Pi-Anion, Pi-Pi Stacked, Pi-Pi T-Shaped	Ser203 Tyr337 Tyr337 Ser125 Ser203 Ser293	3.257 2.660 2.900 2.376 1.787 2.643
L. sativum	Lepidine B	100	-11.1	Trp86, Tyr124, Tyr337, Phe338	-	Tyr124	2.310
	Lepidine E	100	-10.4	Asn87, Ser125, Trp286, Tyr341,	-	Phe295 Asp74	2.200 2.210
L. stoechas	Rosmarinic acid	100	-10.0	Gly448, Trp86, Tyr341	Pi-Pi Stacked	Tyr124 Tyr337 Tyr337 Ser293 Glu202	3.006 2.985 2.993 3.061 2.219
	Apigenin 7-glucoside	100	-9.7	His447, Tyr124, Trp86, Phe338	Pi-Sigma, Pi-Pi T-Shaped	Tyr337 Ser125 Tyr341	3.033 2.732 2.446
S. Costus	Dehydrocostus lactone	100	-7.8	Trp86, Phe297, Tyr124, Tyr337, Phe338, His447	Pi-Alkyl, Pi-Sigma	Ser203 Ser203	3.167 3.121
	Malic acid	78	-5.3	-	-	Glu202 Glu202	2.750 2.195
	Quinic acid	100	-6.8	-	-	Ser125	3.042

						Tyr337 Tyr124 Ser203	2.872 2.346 2.865
Drugs	Donepezil	100	-11.7	Trp86, Trp286, Ser293, Tyr337, Phe338, Tyr341	-	Phe295	2.90
	Galanthamine	100	-9.6	Asp74, Trp86, Gly121, Gly122, Tyr124, **SER203**, Tyr337, His447	-	Glu202 Ser203	2.65 2.90

Table 13. The results of interactions between major compounds and Drugs (Galantamine, Donepezil) and (BChE).

	MOLECULE	BUTYRYLCHOLINESTERASE (1P0M)					
		RR%	ΔG(Kcal/mol)	Closest Residues	Hydrophobic interactions	H bonds	Length
A. unedo	Gallocatechin	100	-6.8	Trp82, Phe329, Ala328	Π-Sigma, Π-π Stacked, Π-π T-shaped, Π-Alkyl	HOH811 HOH772 TRP82 TYR440 ALA328	3.384 2.758 2.528 2.689 2.956
A. unedo	Ethyl gallate	90	-6.7	Trp82	Π-π Stacked	HOH811 HOH772 GLY115 GLU197	3.398 2.487 2.435 2.402
A. unedo	Arbutin	70	-7.1	Ser198, Trp82, Phe329, Ala328	Π-π T-shaped, Π-Alkyl	HOH811 HOH890 THR120 GLY115 GLY115	3.145 3.105 2.536 2.061 2.448 2.408
C. sativum	Quercetin*	90	-6.6	Phe329, Trp82, Ala328	Π-Sigma, Π-π Stacked, Π-π T-shaped, Π-Alkyl	HOH811 TYR128 GLN67	3.107 2.788 3.043
C. sativum	Kaempferol	76	-6.5	Trp82, La328	Π-Sigma, Π-π Stacked, Π-π T-shaped, Π-Alkyl	GLY121 TYR128 GLY115 TRP82	2.417 2.779 2.120 2.314
C. sativum	p-coumaric acid	82	-7.9	Ser198, Trp82, Ala328	Π-Sigma, Π-π T-shaped, Π-Alkyl	HOH811 HOH772 SER198 HIS438 GLY115	3.511 2.674 2.721 2.592 2.675
C. sativum	ferulic acid	86	-6.7	Gly116, Trp82, His438	Π-π Stacked	HOH772 TYR332 GLU197	2.015 2.625 2.863

	3-O-caffeoylquinic acid (Chlorogenic acid)**	58	-7.9	Gly121, Tyr332, Asp70	Π-Alkyl	HOH772 THR120 TYR128 GLY78 TRP82 GLY115 GLY115	3.049 2.239 2.374 2.839 2.485 2.120 2.046
	Stigmasterol	26	4.6	Tyr114, Trp82, Phe329, Trp430, Ala328, Leu125, Met437, Tyr440	Π-Alkyl, Alkyl, Π-Sigma	THR122 THR122	**1.549** 2.743
	β-sitosterol	18	8.8	Trp82, Trp430, Ala328, Leu125, Met437, Tyr128, Phe329, His438, Tyr440	Π-Sigma, Alkyl, Π-Alkyl	THR122	2.015
	γ-tocopherol	92*	-3.3	Trp82, Trp231, Ala328, Val288, Phe329, Tyr332, Phe398, His438	Π-Sigma Alkyl Π-Alkyl	GLY121	2.356
	α-tocotrienol	8	-1.7	Asp70, Phe329, Trp82,Ala328, Tyr332, Trp430, His438	Π-Anion, Π-Sigma, Π-π T-shaped, Alkyl, Π-Alkyl	HOH811 HOH89	4.092 3.373
	γ-tocotrienol	88	-2.7	Trp82, Trp231, Leu286, Trp231, Phe329, Tyr332, Phe398, His438	Π-Sigma, Alkyl, Π-Alkyl	-	-
J. oxycedrus	Catechin	86	-7.2	Trp82, Ala328	Π-Sigma, Π-π Stacked, Π-π T-shaped, Π-Alkyl	HOH811 HOH890 TYR332 TYR128 GLN67	2.976 3.486 2.856 **1.780** 3.000
	Myricetin	78	-5.8	Gly121, Trp82	Π-Sigma, Π-π Stacked	HOH890 HOH811 TRP82 ASN83 TRP430	2.505 3.739 2.550 2.149 2.753

						TYR440 GLY115 GLN67	2.470 2.622 2.873
	Rutin	100*	-	Ser79, His438	Π-Alkyl	HOH890 HOH811 HOH811 TRP82 GLY116 PRO285	3.318 2.535 2.332 3.094 2.022 2.110
	Naringenin	88	-8.2	Trp82, Ala328	Π-π Stacked, Π-π T-shaped, Π-Alkyl, Π-Sigma	HOH772 HOH811 TRP82	3.106 3.546 2.245
	Hesperidin	100	3.4	Ser198, Gly439, Tyr128, Trp82, Trp430, His438, Ala328	Π-Sigma, Π-Alkyl	HOH772 HOH811 HOH890 THR122 GLN67 TYR128	2.818 2.706 3.374 **1.783** 2.062 2.759
	Salicylic acid	98	-6.4	Gly115, Gly116	Amide-Pi Stacked	HOH890 HOH772 GLY116 TYR128	2.874 2.535 3.037 2.524
	Thymoquinone	100	-6.2	Trp82, His438	Π-π Stacked, Π-Alkyl, Π-Sigma	HOH890	4.148
J. phoenicea	3-p-Coumaroylquinic acid	62	-7.9	Ala328	Π-Alkyl	HOH811 HOH890 SER198 SER198 HIS438 TRP82 GLU197	3.491 3.164 2.372 2.328 2.782 3.065 2.007
	Quercetin-O-pentoside	64	-2.2	Gly439, Gln67, His438	-	HOH811 HOH890 HOH811	2.700 3.150 3.351

						HOH811 HOH890 HIS438 GLU197	2.828 **1.922** 2.380 2.448
	Myricetin-O-pentoside	58	-2.5	Gly116, His438, Trp82, Ala328	Π-Alkyl, Π-π Stacked	HOH811 HOH890 TRP82 ASN83 SER198 HIS438 HIS438 GLY115	3.335 3.097 2.794 **1.898** 2.619 2.296 2.485 2.355
L. sativum	Lepidine B	100	-9.4	Trp82, Thr120, Tyr128, Ala328, Phe329, Tyr332	-	Tyr128	**1.70**
	Lepidine E	100	-9.0	Gly116, Gly117, Asn83, Tyr332, **His438**	-	Asp70 Trp82	2.23 2.73
L. stoechas	Rosmarinic acid	40	-6.6	Trp82, His438, Asp70	Π-π Stacked, Π-π T-shaped, Π- Anion	HOH811 TYR332 TRP82 THR120	2.844 2.871 1.666 2.365
	Apigenin 7-glucoside	80	1.1	Thr120, Trp430, Ala328	Π-Alkyl	HOH890 HOH890 GLY121 THR122 TYR128	2.577 2.110 2.251 2.030 2.350
S. Costus	Dehydrocostus lactone	92	1	Trp82, Phe329, His438	Π-Sigma, Π-Alkyl	THR120	2.264
	Malic acid	90	1	Gly116	-	HOH890 HOH772 THR120 SER198 SER198 GLU197	3.367 2.647 2.201 2.498 2.332 2.551
	Quinic acid	100	1	Gly116, Trp82	Π-Sigma	HOH772 HOH811	2.641 2.705

				HOH890 TYR128 GLU197	2.976 2.652 2.571
Drugs	Donepezil	-9.5	Trp82, Trp231, Phe286	-	-
	Galanthamine	-8.5	Trp82, Thr120, Tyr128, Ala328, His438	Tyr128 Thr120	2.88 3.26

Table 14. The results of interactions between major compounds and Drugs (Galantamine, Donepezil) and MAGL

	MOLECULE	MONOACYLGLYCEROL LIPASE (5ZUN)					
		RR%	**ΔG(Kcal/mol)**	**Closest Residues**	**Hydrophobic interactions**	**H bonds**	**Length**
A. unedo	Gallocatechin	100	-9.5	Ser122, Ser185, His269, Tyr194 , Val270, Val191, Lys273, Ile179	Π-Alkyl, Π- Π-T-shaped, Π-Π-Stacked, Π-Sigma, Π-Cation	HOH542 HOH585 HOH564 SER122 ALA51 GLU53 GLU190 SER181	3.055 2.837 2.012 2.218 2.902 2.311 2.414 1.692
	Ethyl gallate	100	-7.2	Ser181, Val191, Val270, Tyr194	Π-Sigma, Π-π Stacked	HOH542 HOH585 HOH564	3.214 1.982 2.491
	Arbutin	90	-6.7	ALA51, LEU241	Π-Sigma	SER122 MET123 ASP180 ASP180 PRO178	2.540 2.359 2.221 2.280 2.012
C. sativum	Quercetin*	100	-10.4	Gly50, Ser122, His269, Val270, Tyr194, Ile179, Ala51, Leu241, Leu184	Π-Cation, Π-Sigma, Π-Alkyl, Π-π Stacked, Π-π T-shaped	HOH542 ARG57	4.009 2.255
	Kaempferol	100	-10.3	Gly50, Ser122, His269, Val270, Tyr194, Ile179, Ala51, Leu241, Leu184	Π-Cation, Π-Sigma, Π-π Stacked, Π-π T-shaped, Π-Alkyl	ALA51	2.267
	p-coumaric acid	96	-7.9	Val270, Tyr194	Π-Sigma, Π-π Stacked	HOH542 HIS121 HIS269 ALA51	2.745 1.944 2.748 2.131
	ferulic acid	100	-7.7	Ser181, Val270, Tyr194	Π-Sigma,	HOH542	2.885

					Π-π Stacked	HOH564 SER122 HIS269	2.934 **1.908** 2.332
	3-O-caffeoylquinic acid	86	-8.1	Tyr194, Val270, His121, His269	Alkyl, Π-Alkyl	ALA51 MET123 MET123	2.077 2.193 2.772
	Stigmasterol	100	-9.9	Ala51, Ile179, Leu184, Leu241, Met88, Leu205, Leu213, Leu214, Phe159	Alkyl, Π-Alkyl	-	-
	β-sitosterol	100	-10.1	Ala51, Leu148, Ala151, Ala156, Leu213, Leu241, Leu205, Ile179, Leu214, Phe159	Π-Alkyl, Alkyl	HOH542 ALA51	2.627 **1.841**
	γ-tocopherol	64	-8.7	Val183, Leu184, Arg240, Leu241, Leu205, Leu213, Leu148, Cys242, Met88, Ile179, Phe159, Phe209	Alkyl, Π-Alkyl	ASP180	**1.965**
	α-tocotrienol	64	-10.9	Leu205, Tyr194, Ala51, Ala156, Ile179, Leu213, Leu241, Leu184, Val270, Lys273, Phe159, Phe209	Π-Alkyl, Alkyl, Π-Sigma	-	-
	γ-tocotrienol	64	-10.0	Phe159, Ala51, Leu241, Leu205, Val183, Leu184, Leu213, Leu148, Met88, Ile179	Alkyl, Π-Alkyl, Π-Sigma	-	-
J. oxycedru	Catechin	100	-9.7	Ser185, His269, Val270, Tyr194, Val191, Lys273, Ile179, Leu184	Π-Sigma Π-π Stacked Π-π T-shaped Π-Alkyl	HOH564 ARG57 SER122 SER185 GLU190	2.428 2.061 2.198 3.093 3.061
	Myricetin	100	-9.4	Gly50, Val270, Tyr194	Π-Sigma, Π-π Stacked	HOH585 ALA51	2.701 3.049

						HIS121	2.422
	Rutin	100	-10.3	Leu213, Arg240, Leu148, Ala151	Π-Sigma, Alkyl, Π-Alkyl	HOH542 MET123 LEU213 GLY177 GLY177	3.286 2.397 2.949 3.352 2.299
	Naringenin	100	-10.0	Gly50, His121, Ser122, His269, Leu241, Val270, Tyr194, Ala51, Ile179, Leu184	Π-Cation, Π-Sigma Π-π Stacked, Π-π T-shaped, Π-Alkyl	HOH542 HOH542 MET123	3.080 4.138 2.309
	Hesperidin	100	-10.9	Leu241, Val270, Tyr194, Ala156, Leu214, Phe159, Ala51, Ile179	Π-Sigma, Π-π Stacked, Alkyl, Π-Alkyl	HOH542 HOH585A LA51 MET123 GLU53	3.435 2.673 2.495 2.370 2.823
	Salicylic acid	100	-7.3	Ser185, Val270, Tyr194, Leu184	Π-Sigma, Π-π Stacked, Π-Alkyl	HOH585	2.611
	Thymoquinone	100	-7.6	Ser181, Val270, Tyr194, Lys273, Leu184, Ile179, His121, His269	Π-Sigma, Π-Alkyl Π-π Stacked, Alkyl	HOH585	3.351
J. phoenicea	3-p-Coumaroylquinic acid	48	-7.5	Phe159, Ala156	Π-π Stacked Π-Alkyl	ASP180 GLY177	**1.868** 2.547
	Quercetin-O-pentoside	76	-8.9	Ser155, Leu213, Leu241, Phe159, Ala151	Π-Sigma, Π-Alkyl Π-π T-shaped	-	-
	Myricetin-O-pentoside	100	-9.2	Leu241, Ala51, Ile179, Leu205	Π-Alkyl Π-Sigma	HOH542 SER155 GLY210 GLY177 ALA51	2.602 2.848 3.062 **1.872** 2.367

L. sativum	Lepidine B	72	-8.1	Leu148, Ala151, Ile179, Leu205, Leu213, Leu214, Leu241	π-Alkyl, π-Sigma	SER155	2.14
	Lepidine E	50	-9.5	Val191, Tyr194, Leu213, Leu241, Cys242, Val270, Lys273	π-Alkyl, π-Sigma, π-π-Stacked,π-Sulfur	SER181	2. 65
L. stoechas	Rosmarinic acid	80	-10.0	Ser185, Leu241, Val270, Tyr194, Ala51	π-Alkyl, π-Sigma, π-π-Stacked	HOH542 HOH585 HOH542 HIS121 HIS121 SER122 HIS269 SER181	2.991 3.354 2.031 2.309 2.212 2.476 2.430 2.352
	Apigenin 7-glucoside	100	-10.6	Gly50, His269, Ser122, Leu241, Val270, Tyr194, His269 , Ile179, Ala51, Leu184	π-Alkyl, π-Sigma, π-π-Stacked,π-Cation, Π-π T-shaped	HOH542 ALA51 ASP180 ASP180 ASP180	3.217 2.104 2.536 2.257 2.754
S. costus	Dehydrocostus lactone	100	-8.5	Ala51, Leu213, Leu241, Leu205, Ile179, Leu148	Alkyl	-	-
	Malic acid	100	-5.6	Ser185	-	HOH585 ARG57 SER181 GLU53	2.323 2.052 2.224 2.802
	Quinic acid	98	-5.2	-	-	HOH585 ALA51 GLU53	2.186 2.288 2.603
Drugs	Donepezil	100	-10.7	Ala51, His121, Ile179, Tyr194, Leu241, Val270, Lys273	Alkyl, π-Alkyl, π-π-Stacked, π-Sigma	SER122 MET123	2.72 2.74

	Galanthamine	92	-7.6	Ala51, Leu205, Ser155, Ile179, Leu213, Leu241	Alkyl, π-Alkyl, π-Sigma	-	-

Table 15. The results of interactions between major compounds and Drugs (Galantamine, Donepezil) and BACE1

	MOLECULE	BETA-SECRETASE (6E3J)					
		RR%	ΔG(Kcal/mol)	Closest Residues	Hydrophobic interactions	H bonds	Length
A. unedo	Gallocatechin	-	-	Leu30	Pi-Sigma	HOH625 TRP115 GLN73 GLY230	3.782 1.842 2.702 1.727
	Ethyl gallate	-	-	Arg128, Tyr71	Pi-Cation, Pi-Pi T-shaped, Pi-Alkyl	-	-
	Arbutin	-	-	-	-	HOH601 GLY117 GLN12	3.059 2.891 3.006
C. sativum	Quercetin*	100	-8.5	Trp76, Tyr71, Val69, Ile118	Π-π Stacked, Π-Alkyl	HOH671 HOH625 HOH625	3.027 2.317 2.364
	Kaempferol	100	-8.1	Trp76, Tyr71, Val69, Ile118	Π-π Stacked, Π-Alkyl	HOH671 HOH625 HOH601	3.007 2.439 2.958
	p-coumaric acid	100	-6.2	Ser35, Phe108, Trp76, Tyr71, Ile118	Π-Donor Hydrogen Bond, Π-Alkyl, Π-π T-shaped	HOH625 HOH601 TYR71	3.260 3.000 2.538
	ferulic acid	100	-6.5	Tyr71	Π-π Stacked	HOH625 HOH601 HOH671 TRP76	2.854 3.576 3.795 2.137
	3-O-caffeoylquinic acid	60	-8.2	Phe108, Tyr71	Π-Cation, Π-Alkyl	HOH625 TRP76 ARG128 PHE108 GLY34	2.050 2.336 2.444 2.001 2.496

	Stigmasterol	100	-9.1	Leu30, Ile110, Tyr71, Trp76, Trp115	Alkyl, Π-Alkyl	HOH671	3.278
	β-sitosterol	100	-9.5	Tyr71, Ile110, Leu30, Phe108, Trp115	Π-Alkyl, Alkyl, Π-Sigma	-	-
	γ-tocopherol	42	-7.4	Leu30, Tyr71, Trp115	Alkyl, Π-Alkyl	TRP76	2.083
	α-tocotrienol	24	-7.7	Ser35, Val69, Ile118, Tyr71, Trp76, Tyr198	Alkyl, Π-Alkyl	TYR198	2.273
	γ-tocotrienol	62	-8.1	Tyr71, Ile118, Trp76, Phe108	Π-Sigma, Alkyl, Π-Alkyl	-	-
J. oxycedru	Catechin	100	-8.3	Trp76, Val69, Ile118	Π-Donor Hydrogen Bond, Π-Alkyl	HOH671 HOH625 ARG128 PHE108	3.006 2.214 2.738 2.717
	Myricetin	100	-8.3	Ile118	Π-Alkyl	HOH601 HOH625 ARG128 GLY230	3.344 2.401 2.814 2.318
	Rutin	100	-9.9	Trp76, Val69	Π-Donor Hydrogen Bond, Π-Alkyl	HOH671 ARG128 PHE108 ASN37 ASP228	2.108 2.844 2.483 2.39 2.566
	Naringenin	100	-8.9	Tyr71, Val69	Π-π Stacked, Π-Alkyl	HOH601 HOH601 HOH625 HOH671 ASN37	3.232 3.224 1.983 2.165 3.054
	Hesperidin	98	-10.4	Tyr71, Val69	Π-π Stacked, Π-Alkyl	HOH601 HOH625 HOH625	3.274 2.888 2.978

						HOH671 ARG128 ARG128 ARG128 PRO70 THR329	2.68 2.493 2.641 1.979 2.534 2.268
	Salicylic acid	100	-5.4	Trp76, Val69	Π-Donor Hydrogen Bond, Π-Alkyl, Π-π T-shaped	HOH601 HOH671 SER35 TRP76	2.850 2.957 2.143 2.550
	Thymoquinone	100	-5.9	Tyr71, Ile118, Val69, Phe108	Π-π Stacked, Alkyl, Π-Alkyl	TRP76	1.829
J. phoenicea	3-p-Coumaroylquinic acid	100	-8.1	Tyr71	Π-π Stacked	HOH625 HOH671 TRP76 GLY34 GLY34 ILE126	2.099 **1.994** 2.228 2.549 2.447 2.628
J. phoenicea	Quercetin-O-pentoside	100	-8.8	Tyr71, Ile118	Π-π Stacked, Π-Alkyl	HOH625 ARG128 ASN37	2.998 2.800 2.848
J. phoenicea	Myricetin-O-pentoside	100	-9.4	Val69, Ile118	Π-Alkyl	HOH671 HOH625 THR72	3.073 2.940 2.466
L. sativum	Lepidine B	100	-7.7	Asp32, Gly34, Ser35, Tyr71, Gly230, Trp76, Arg128, Asp228	Amide-π-Stacked, π-Cation, π-Sigma, π-π Stacked, π-π-Tshaped	-	-
L. sativum	Lepidine E	100	-8.0	Trp76, Ile118, Arg128, Asp228	π-Alkyl, π-Anion, π-Cation, π- π-Tshaped	Gly34	**1.89**
L. stoe	Rosmarinic acid	-	-	-	-	TYR71	2.824
L. stoe	Apigenin 7-glucoside	-	-	Val69, Arg128	Π-Alkyl	HOH601	2.705

						HOH671	2.749
						GLN73	2.132
						GLY74	2.042
S. costus	Dehydrocostus lactone	100	-8.0	Val69, Ile118, Tyr71, Trp76	Alkyl, Π-Alkyl	ARG128	2.490
	Malic acid	94	-5.6	-	-	HOH671	2.939
						HOH601	2.267
						HOH671	2.434
						ARG128	2.708
	Quinic acid	100	-5.4	-	-	HOH601	3.069
						HOH671	2.544
						HOH671	2.427
						TRP76	2.565
						ASP32	2.559
						ASN37	2.597
Drugs	Donepezil	100	-8.9	Leu30, Ile118, Tyr71, Ile126, Arg128	Alkyl, π-Alkyl, π-Cation	TRP76	2.23
	Galanthamine	100	-7.5	Tyr71	π-Alkyl, π-π-Stacked	-	-

Conclusion

During this thesis, we studied the inhibitory effect of seven plant extracts against cholinesterase and other enzymes which describes as targets for AD treatments and carry out a phytochemical study for those plants, in this case: *Arbutus unedo* L, *Coriandrum sativum, Juniperus oxycedrus, Juniperus phoenicea, Lavandula stoechas, Saussurea costus and Lepidium sativum*.

In this conclusion, we will compare the previous work with our results in order to highlight the contribution of our work.

First, we conducted a phytochemical screening for several medicinal plants, we found that all plants are rich in flavonoids, tannins, and terpenoids; whereas alkaloids do not present in all plants. Then, we have quantified the total phenolic and flavonoids in those plants, we found that these results confirmed the results of phytochemical screening, the highest phenolic and flavonoids were found in methanolic extract of *J. oxycedrus* with values of 29.106 mgGAE/gDW, and 7.699 mgQE/gDW, respectively. The hexane extract of *C. sativum* does not contain flavonoids.

Then, we evaluate the inhibitory effect of all extracts, we found a significant difference between the extracts. The most active extract was *J. phoenicea* followed by *J. oxycedrus, S. costus then A.undo*. While *C. sativum* is not active or has a low effect against Acetylcholinesterase.

Finally, the molecular docking results confirm the results of in vitro studies, where all major molecules of *C. sativum*, have weak interactions with cholinesterase enzymes. However, the molecules from *A. unedo, J. phoenicea and J. oxycedrus*, form strong hydrogen bonds with those enzymes.

We conclude that a particular attention should be devoted to the plants growth in Algeria, to be treated as an important resource for drug candidates against Alzheimer's disease. Moreover, we suggest conducting further researches and studies

to isolate the bioactive components in these different plants, and evaluate their inhibitory effect against cholinesterase from different origins, and purchasing the in vivo assays to confirm the in vitro and the in silico obtained results.

References

A

Abd El-Wahab, A. E., Ghareeb, D. A., Sarhan, E. E., Abu-Serie, M. M., & El Demellawy, M. A. (2013). In vitro biological assessment of Berberis vulgaris and its active constituent, berberine: antioxidants, anti-acetylcholinesterase, anti-diabetic and anticancer effects. BMC complementary and alternative medicine, 13(1), 1-12.

Abdallah, E. M., Qureshi, K. A., Ali, A. M., & Elhassan, G. O. (2017). Evaluation of some biological properties of Saussurea costus crude root extract. Biosci. Biotechnol. Res. Commun, 10(4), 601-611.

Abdel Bar, F.M.; Elimam, D.M.; Mira, A.S.; El-Senduny, F.F.; Badria, F.A. Derivatization, molecular docking and in vitro acetylcholinesterase inhibitory activity of glycyrrhizin as a selective anti-Alzheimer agent. Nat. Prod. Res., 2018, 6419, 1-9.

Abraham, M. J., Murtola, T., Schulz, R., Páll, S., Smith, J. C., Hess, B., & Lindahl, E. (2015). GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. SoftwareX, 1, 19-25.

Adams, R.P. A. F. Barrero, and A. Lara. "Comparisons of the Leaf Essential Oils of Juniperus phoenicea, J. phoenicea subsp. eumediterranea Lebr. & Thiv. and J. phoenicea var. turbinata (Guss.) Parl. J. Essent. Oil Res., 1996, 8(4), 367-371.

Adeyemi, O. S., Atolani, O., Awakan, O. J., Olaolu, T. D., Nwonuma, C. O., Alejolowo, O., ... & Batiha, G. E. S. (2019). Focus: Organelles: In vitro screening to identify anti-Toxoplasma compounds and in silico modeling for bioactivities and toxicity. The Yale journal of biology and medicine, 92(3), 369.

Adhami, H. R., Farsam, H., & Krenn, L. (2011). Screening of medicinal plants from Iranian traditional medicine for acetylcholinesterase inhibition. Phytotherapy Research, 25(8), 1148-1152.

Ahmed, A., Ahmad, S., Soni, K., Lapa, B., Afzal, M., Sharma, K., & Kumar, G. (2016). Suitable solvent and drying condition to enhance phenolics and extractive value of Saussurea costus. Journal of Ayurvedic and Herbal Medicine, 2(5), 165-170.

Ahmed, E. H., Abadi, R. S., & Mohammed, A. M. (2018). Phytochemical screening, chemical composition and antioxidant activity of seeds essential oil of

Coriandrum sativum L. from the Sudan. International journal of Herbal medicine, 6(1), 1-4.

Akbarzadeh, T.; Sharifzadeh, M.; Bukhari, S.N.A.; Amini, M. Novel tetrahydrocarbazole benzyl pyridine hybrids as potent and selective butryl cholinesterase inhibitors with neuroprotective and β-secretase inhibition activities. Eur. J. Med. Chem., 2018, 155, 49-60.

Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, Cooper NR, Eikelenboom P, Emmerling M, Fiebich BL, et al. 2000. Inflammation and Alzheimer's disease. Neuroinflammation Working Group. Neurobiol Aging 21: 383–421.

Alexandre, A. M., Matias, A. A., Bronze, M. R., Cocero, M. J., & Mato, R. (2020). Phenolic Compounds Extraction of Arbutus unedo L.: Process Intensification by Microwave Pretreatment. Processes, 8(3), 298.

Alford, S., Patel, D., Perakakis, N., & Mantzoros, C. S. (2018). Obesity as a risk factor for Alzheimer's disease: weighing the evidence. Obesity reviews, 19(2), 269-280.

Alger, B. E. (2009). Endocannabinoid signaling in neural plasticity. Behavioral neurobiology of the endocannabinoid system, 141-172.

Ali, B., MS Jamal, Q., Shams, S., A Al-Wabel, N., U Siddiqui, M., A Alzohairy, M., ... & A Kamal, M. (2016). In silico analysis of green tea polyphenols as inhibitors of AChE and BChE enzymes in Alzheimer's disease treatment. CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders), 15(5), 624-628.

Alpan, A. S., Parlar, S., Carlino, L., Tarikogullari, A. H., Alptüzün, V., & Güneş, H. S. (2013). Synthesis, biological activity and molecular modeling studies on 1H-benzimidazole derivatives as acetylcholinesterase inhibitors. Bioorganic & medicinal chemistry, 21(17), 4928-4937.

Alptüzün, V., Prinz, M., Hörr, V., Scheiber, J., Radacki, K., Fallarero, A., Vuorela, P.; Engels, B.; Braunschweig, H.; Erciyas, E. & Holzgrabe, U. (2010). Interaction of (benzylidene-hydrazono)-1, 4-dihydropyridines with β-amyloid, acetylcholine, and butyrylcholine esterases. Bioorganic & medicinal chemistry, 18(5), 2049-2059.

Altay, A.; Koktepe, T.; Durmaz, L.; Topal, F.; Gülçin, İ.; Köksal, E. Purification and selected biochemical properties of peroxidase from cress (Lepidium sativum sub sp. sativum). Int. J. Food Prop., 2018, 21(1), 2610-2621.

Alzheimer A (1906) Über einen eigenartigen, schweren Erkrankungsprozess der Hirnrinde. Neurol Zbl vol.25, p.1134.

Alzheimer's Association. 2020 Alzheimer's disease facts and figures. Alzheimers Dement 2020; 16: 391– 460

AMALICH, S., Fadili, K., Fahim, M., Hilali, F. E., & Zaïr, T. (2016). Polyphenols content and antioxidant power of fruits and leaves of Juniperus phoenicea L. From Tounfite (Morocco). Moroccan Journal of Chemistry, 4(1), 4-1.

Amos, L. A. (2004). Microtubule structure and its stabilisation. Org. Biomol. Chem. 2, 2153–2160

Anand A, Patience A, Sharma N, Khurana N. The present and future of pharmacotherapy of Alzheimer's disease: a comprehensive review. Eur J Pharmacol. 2017; 815:364-375

Anastasiou CA, Yannakoulia M, Kosmidis MH, et al. Mediterranean diet and cognitive health: initial results from the Hellenic longitudinal investigation of ageing and diet. PLoS One 2017;12(8):e0182048.

Andersson, C. X., Gustafson, B., Hammarstedt, A., Hedjazifar, S., & Smith, U. (2008). Inflamed adipose tissue, insulin resistance and vascular injury. Diabetes/metabolism research and reviews, 24(8), 595-603.

Andrade, D., Gil, C., Breitenfeld, L., Domingues, F., & Duarte, A. P. (2009). Bioactive extracts from Cistus ladanifer and Arbutus unedo L. Industrial Crops and Products, 30(1), 165-167.

Anesti, M., Stavropoulou, N., Atsopardi, K., Lamari, F. N., Panagopoulos, N. T., & Margarity, M. (2020). Effect of rutin on anxiety-like behavior and activity of acetylcholinesterase isoforms in specific brain regions of pentylenetetrazol-treated mice. Epilepsy & Behavior, 102, 106632.

Annweiler, C., et al., 2015. Vitamin D and cognition in older adults': updated interna-tional recommendations. J. Intern. Med. 277 (1), 45–57.

Anthonsen MW , Contreras JA, Fourel D, Holst LS, Karlsson M, Laurell H, Osterlund T, Holm C (1999). Hormone-sensitive lipase - structure, function and regulation. Prog. Obes. Res., 8: 89-96.

Anulika, N. P., Ignatius, E. O., Raymond, E. S., Osasere, O. I., & Abiola, A. H. (2016). The chemistry of natural product: Plant secondary metabolites. Int. J. Technol. Enhanc. Emerg. Eng. Res, 4, 1-8.

Arevalo-Martin, A., Garcia-Ovejero, D., & Molina-Holgado, E. (2010). The endocannabinoid 2-arachidonoylglycerol reduces lesion expansion and white matter damage after spinal cord injury. Neurobiology of disease, 38(2), 304-312.

Arvanitakis, Z., Wilson, R. S., Bienias, J. L., Evans, D. A., & Bennett, D. A. (2004). Diabetes mellitus and risk of Alzheimer disease and decline in cognitive function. Archives of neurology, 61(5), 661-666.

Atlam, F.; Awad, M.; Salama, R. Factors Influencing the Potency of Alzheimer Inhibitors: Computational and Docking Studies. Am. J. Alzheimers Dis. Other Demen., 2018, 33(3), 166-175.

Atri, A. (2019, April). Current and future treatments in Alzheimer's disease. In Seminars in neurology (Vol. 39, No. 02, pp. 227-240). Thieme Medical Publishers.

B

Bacalhau, P. In silico, NMR and pharmacological evaluation of an hydroxyoxindole cholinesterase inhibitor. Bioorg. Med. Chem., 2018, 1-10.

Baddeley, A. (2010). Working memory. Current biology, 20(4), R136-R140.

Bakulski, K. M., Seo, Y. A., Hickman, R. C., Brandt, D., Vadari, H. S., Hu, H., & KyunPark, S. (2020). Heavy metals exposure and Alzheimer’s disease and related dementias. Journal of Alzheimer's Disease, (Preprint), 1-28.

Ball M. J. (1977) Neuronal loss, neurofibrillary tangles and granulovacuolar degeneration in the hippocampus with ageing and dementia.Acta neuropath.,Berlin37,111–118

Bamberger ME, Harris ME, McDonald DR, Husemann J, Landreth GE. A cell surface receptor complex for fibrillar beta-amyloid mediates microglial activation. J Neurosci 2003; 23: 2665–74.

Barros, L., Dueñas, M., Dias, M. I., Sousa, M. J., Santos-Buelga, C., & Ferreira, I. C. (2012). Phenolic profiles of in vivo and in vitro grown Coriandrum sativum L. Food Chemistry, 132(2), 841-848.

Bartolini, M., Bertucci, C., Cavrini, V., & Andrisano, V. (2003). β-Amyloid aggregation induced by human acetylcholinesterase: inhibition studies. Biochemical pharmacology, 65(3), 407-416.

Bartolini, M.; Pistolozzi, M.; Andrisano, V.; Egea, J.; López, M.G.; Iriepa, I.; Moraleda, I.; Gálvez, E.; Marco-Contelles, J.; Samadi, A. (2011) Chemical and pharmacological studies on enantiomerically pure pmethoxytacripyrines, promising multi-target-directed ligands for the treatment of Alzheimer's disease. ChemMedChem, 6(11), 1990-1997.

Bastianetto S, Yao Z-X, Papadopoulos V, Quirion R. Neuroprotective effects of green and black teas and their catechin gallate esters against beta-amyloid-induced toxicity. Eur J Neurosci. 2006;23:55-64.

Batiha, G. E. S., Beshbishy, A. M., Adeyemi, O. S., & Nadwa, E. H. (2020). Phytochemical screening and antiprotozoal effects of the methanolic Berberis vulgaris and acetonic Rhus coriaria extracts. Molecules, 25(3).

Batiha, G. E. S., Beshbishy, A. M., Tayebwa, D. S., Shaheen, H. M., Yokoyama, N., & Igarashi, I. (2019). Inhibitory effects of Syzygium aromaticum and Camellia sinensis methanolic extracts on the growth of Babesia and Theileria parasites. Ticks and tick-borne diseases, 10(5), 949-958.

Bautista-Aguilera, O. M., Esteban, G., Bolea, I., Nikolic, K., Agbaba, D., Moraleda, I., Iriepa, I.; Samadi, A.; Soriano, E.; Unzeta, M.; Marco-Contelles, J. (2014). Design, synthesis, pharmacological evaluation, QSAR analysis, molecular modeling and ADMET of novel donepezil–indolyl hybrids as multipotent cholinesterase/monoamine oxidase inhibitors for the potential treatment of Alzheimer's disease. European journal of medicinal chemistry, 75, 82-95.

Bautista-Aguilera, O.M.; Esteban, G.; Bolea, I.; Nikolic, K.; Agbaba, D.; Moraleda, I.; Iriepa, I.; Samadi, A.; Soriano, E.; Unzeta, M.; Marco-Contelles, J. Design, synthesis, pharmacological evaluation, QSAR analysis, molecular modeling and ADMET of novel donepezil-indolyl hybrids as multipotent

cholinesterase/monoamine oxidase inhibitors for the potential treatment of Alzheimer's disease. Eur. J. Med. Chem., 2014, 75, 82-95.

Bel-Rhlid, R., Kraehenbuehl, K., Cavin, C., Raab, T. W., & Page, N., Products comprising, and uses of, decarboxylated phenolic acids derived from chlorogenic acids of coffee, Eur. Patent EP 2282642,2013

Ben Mrid, R.; Bouchmaa, N.; Bouargalne, Y.; Ramdan, B.; Karrouchi, K.; Kabach, I.; El Karbane, M.; Idir, A.; Zyad, A.; Nhiri, M. Phytochemical Characterization, Antioxidant and In Vitro Cytotoxic Activity Evaluation of Juniperus oxycedrus Subsp. oxycedrus Needles and Berries. Molecules, 2019, 24(3), 502.

Benarous, K., Bombarda, I., Iriepa, I., Moraleda, I., Gaetan, H., Linani, A., ; Tahri, D.; Sebaa, M.; Yousfi, M. (2015). Harmaline and hispidin from Peganum harmala and Inonotus hispidus with binding affinity to Candida rugosa lipase: In silico and in vitro studies. Bioorganic chemistry, 62, 1-7.

BENNETT, R. N., & WALLSGROVE, R. M. (1994). Secondary metabolites in plant defence mechanisms. New Phytologist, 127(4), 617–633.

Berenbaum, M., & Feeny, P. (1981). Toxicity of angular furanocoumarins to swallowtail butterflies: escalation in a coevolutionary arms race?. Science, 212(4497), 927-929.

Bergman RN, Ader M (2000). Free fatty acids and pathogenesis of type 2 diabetes mellitus. Trends Endocrinol. Metab., 11: 351-356

Berman, H.M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T.N.; Weissig, H.; Shindyalov, I.N.; Bourne, P.E. (2000) The Protein Data Bank. Nucleic Acids Res., 28(1), 235-242.

Bills, G. F., & Gloer, J. B. (2017). Biologically Active Secondary Metabolites from the Fungi. The Fungal Kingdom, 1087–1119.

Bistaffa, E., Tagliavini, F., Matteini, P., & Moda, F. (2020). Contributions of Molecular and Optical Techniques to the Clinical Diagnosis of Alzheimer's Disease. Brain Sciences, 10(11), 815.

Blanke ML, VanDongen AMJ. Activation mechanisms of the NMDA receptor. In: Van Dongen AM, editor. Biology of the NMDA receptor. Boca Raton (FL): CRC Press; 2009.

Blankman, J. L., Simon, G. M., & Cravatt, B. F. (2007). A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. Chemistry & biology, 14(12), 1347-1356.

Bobinski, M., De Leon, M. J., Wegiel, J., Desanti, S., Convit, A., Saint Louis, L. A., Rusinek, H., and Wisniewski, H. M. (1999). The histological validation of post mortem magnetic resonance imaging-determined hippocampal volume in Alzheimer's disease. Neuroscience, 95(3), 721-725

Bores, G. M., Huger, F. P., Petko, W., Mutlib, A. E., Camacho, F., Rush, D. K., Selk, D.E.; Wolf, V.; Kosley, R.W., Jr; Davis, L. & Vargas, H. M. (1996). Pharmacological evaluation of novel Alzheimer's disease therapeutics: acetylcholinesterase inhibitors related to galanthamine. Journal of Pharmacology and Experimental Therapeutics, 277(2), 728-738.

Botteri, G., Salvadó, L., Gumà, A., Hamilton, D. L., Meakin, P. J., Montagut, G., Ashford, M.L.J.; Ceperuelo-Mallafré, V.; Fernández-Veledo, S.; Vendrell, J.; Calderón-Dominguez, M.; Serra, D.; Herrero, L.; Pizarro, J.; Barroso, E.; Palomer, X. & Vázquez-Carrera, M. (2018). The BACE1 product sAPPβ induces ER stress and inflammation and impairs insulin signaling. Metabolism, 85, 59-75.

Boufellous, M., Lrhorfi, L. A., Berrani, A., Haoud, H. E., Zaher, A., Bouhaddioui, B., & Bengueddour, R. (2017). Phytochemical screening of a medicinal plant: Lavandula stoechas (Lamiaceae). Journal of Pharmacognosy and Phytochemistry, 6(2), 56-62.

Boulebd, H.; Ismaili, L.; Bartolini, M.; Bouraiou, A.; Andrisano, V.; Martin, H.; Bonet, A.; Moraleda, I.; Iriepa, I.; Chioua, M.; Belfaitah, A.; Marco-Contelles, J. Imidazopyranotacrines as Non-Hepatotoxic, Selective Acetylcholinesterase Inhibitors, and Antioxidant Agents for Alzheimer's Disease Therapy. Molecules, 2016, 21(4), 400.

Bourgaud, F., Gravot, A., Milesi, S., & Gontier, E. (2001). Production of plant secondary metabolites: a historical perspective. Plant Science, 161(5), 839–851.

Braak H, Alafuzoff I, Arzberger T, Kretzschmar H & Del Tredici K Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. Acta Neuropathol. 112, 389–404 (2006).

Braak H, Thal DR, Ghebremedhin E & Del Tredici K Stages of the pathologic process in Alzheimer disease: age categories from 1 to 100 years. J. Neuropathol. Exp. Neurol 70, 960–969 (2011).

Buckner RL, Andrews-Hanna JR, Schacter DL. The brain's default network: anatomy, function, and relevance to disease. Ann N Y Acad Sci 2008; 1124:1–38

Budson, A. E., & Solomon, P. R. (2016). Alzheimer's disease dementia and mild cognitive impairment due to Alzheimer's disease. Memory loss, Alzheimer's disease, and dementia.

Bustanji, Y., Issa, A., Mohammad, M., Hudaib, M., Tawah, K., Alkhatib, H., ... & Al-Khalidi, B. (2010). Inhibition of hormone sensitive lipase and pancreatic lipase by Rosmarinus officinalis extract and selected phenolic constituents. Journal of Medicinal Plants Research, 4(21), 2235-2242.

C

Cacabelos, R. (2007). Donepezil in Alzheimer's disease: From conventional trials to pharmacogenetics. Neuropsychiatric Disease and Treatment.

Cadenas E, and Davies KJ. Mitochondrial free radical generation, oxidative stress, and aging. Free Radic Biol Med 29: 222-230, 2000.

Cai, H., Wang, Y., McCarthy, D., Wen, H., Borchelt, D. R., Price, D. L., & Wong, P. C. (2001). BACE1 is the major β-secretase for generation of Aβ peptides by neurons. Nature neuroscience, 4(3), 233-234.

Cai, Q., and Tammineni, P. (2017). Mitochondrial aspects of synaptic dysfunction in Alzheimer's disease. J. Alzheimer's Dis. 57, 1087–1103.

Camargo, S. B., Simoes, L. O., de Azevedo Medeiros, C. F., de Melo Jesus, A., Fregoneze, J. B., Evangelista, A., ... & Silva, D. F. (2018). Antihypertensive potential of linalool and linalool complexed with β-cyclodextrin: Effects of subchronic treatment on blood pressure and vascular reactivity. Biochemical pharmacology, 151, 38-46.

Campbell, A. (2002). The potential role of aluminium in Alzheimer's disease. Nephrology Dialysis Transplantation, 17(suppl_2), 17-20.

Campos, C., Rocha, N. B. F., Teles Vieira, R., Rocha, S. A., Telles-Correia, D., Paes, F., ... & Caixeta, L. (2016). Treatment of cognitive deficits in Alzheimer's disease: A psychopharmacological review. Psychiatria danubina, 28(1), 0-12.

Cappai R, Barnham KJ (2008) Delineating the mechanismof Alzheimer’s disease A beta peptide neurotoxicity.Neu-rochem Res33, 526-532.

Carlo, A.-S., Gustafsen, C., Mastrobuoni, G., Nielsen, M. S., Burgert, T., Hartl, D., … Willnow, T. E. (2013). The Pro-Neurotrophin Receptor Sortilin Is a Major Neuronal Apolipoprotein E Receptor for Catabolism of Amyloid- Peptide in the Brain. Journal of Neuroscience, 33(1), 358–370.

Celep, E., Akyüz, S., İnan, Y., & Yesilada, E. (2018). Assessment of potential bioavailability of major phenolic compounds in Lavandula stoechas L. ssp. stoechas. Industrial Crops and Products, 118, 111–117. doi:10.1016/j.indcrop.2018.03.041

Centonze, D., Finazzi-Agro, A., Bernardi, G., & Maccarrone, M. (2007). The endocannabinoid system in targeting inflammatory neurodegenerative diseases. Trends in pharmacological sciences, 28(4), 180-187.

Chaouche, T. M., Haddouchi, F., Atik-Bekara, F., Ksouri, R., Azzi, R., Boucherit, Z., ... & Larbat, R. (2015). Antioxidant, haemolytic activities and HPLC–DAD–ESI–MSn characterization of phenolic compounds from root bark of Juniperus oxycedrus subsp. oxycedrus. Industrial Crops and Products, 64, 182-187.

Chaouche, T. M., Haddouchi, F., Ksouri, R., Medini, F., & Atik-Bekara, F. (2013). In vitro evaluation of antioxidant activity of the hydro-methanolic extracts of Juniperus oxycedrus subsp. oxycedrus. Phytothérapie, 11(4), 244-249.

Chartier-Harlin, M. C., Crawford, F., Houlden, H., Warren, A., Hughes, D., Fidani, L., ... & Mullan, M. (1991). Early-onset Alzheimer's disease caused by mutations at codon 717 of the β-amyloid precursor protein gene. Nature, 353(6347), 844-846.

Che, C. T., Wang, Z. J., Chow, M. S. S., & Lam, C. W. K. (2013). Herb-herb combination for therapeutic enhancement and advancement: theory, practice and future perspectives. Molecules, 18(5), 5125-5141.

Chen, G. F., Xu, T. H., Yan, Y., Zhou, Y. R., Jiang, Y., Melcher, K., & Xu, H. E. (2017). Amyloid beta: structure, biology and structure-based therapeutic development. Acta Pharmacologica Sinica, 38(9), 1205-1235.

Chen, K., & Baran, P. S. (2009). Total synthesis of eudesmane terpenes by site-selective C–H oxidations. Nature, 459(7248), 824-828.

Chen, R., Zhang, J., Wu, Y., Wang, D., Feng, G., Tang, Y. P., Teng, Z. & Chen, C. (2012). Monoacylglycerol lipase is a therapeutic target for Alzheimer's disease. Cell reports, 2(5), 1329-1339.

Chen, X., Zhang, J., & Chen, C. (2011). Endocannabinoid 2-arachidonoylglycerol protects neurons against β-amyloid insults. Neuroscience, 178, 159-168.

Cheng, Y., & Bai, F. (2018). The association of tau with mitochondrial dysfunction in alzheimer's disease. Frontiers in neuroscience, 12, 163.

Chierrito, T.P.C.; Pedersoli-Mantoani, S.; Roca, C.; Sebastian Pérez, V.; Martínez-Gonzalez, L.; Pérez, D.I.; Perez, C.; Canales, A.; Cañada, F.J.; Campillo, N.E.; Carvalho, I.; Martinez, A. Chameleon like behavior of indolylpiperidines in complex with cholinesterases targets: Potent butyrylcholinesterase inhibitors. Eur. J. Med. Chem., 2018, 145, 431-444.

Chioua, M. Synthesis, Biological Assessment and Molecular Modeling of Racemic QuinoPyranoTacrines for Alzheimer’s Disease Therapy. ChemistrySelect, 2018, 3(2), 461-466a.

Chioua, M.; Buzzi, E.; Moraleda, I.; Iriepa, I.; Maj, M.; Wnorowski, A.; Giovannini, C.; Tramarin, A.; Portali, F.; Ismaili, L.; López-Alvarado, P.; Bolognesi, M.L.; Jóźwiak, K.; Menéndez, J.C.; Marco-Contelles, J.; Bartolini, M. (2018) Tacripyrimidines, the first tacrine-dihydropyrimidine hybrids, as multi-target-directed ligands for Alzheimer’s disease. Eur. J. Med. Chem., 155, 839-846b.

Choi W., Han C., Kim T. K., Im G., Jung C. M., Yun S. J., Kim B., Lee S., Kwak W., Triterpene compounds which are effective on improvement of brain function, Repub. Korea KR 100751071, 2007.

Choi W., Han C., Kim T. K., Im G., Jung C. M., Yun S. J., Kim B., Lee S., Kwak W., Triterpene Compounds which are Effective on Improvement of Brain Function, U.S.Pat. Appl. Publ. US 20070249711, 2007.

Choi W., Han C., Kim T. K., Im G., Jung C. M., Yun S. J., Kim B., Lee S., Kwak W., Triterpene compounds which are effective on improvement of brain function, U.S.Patent US 8790687, 2014.

Choi W., Han C., Kim T. K., Im G., Jung C. M., Yun S. J., Kim B., Lee S., Kwak W., Triterpene compounds with brain function improving effect, Jpn.Tokkyo Koho JP 4675903, 2011.

Cholkar, K., Trinh, H. M., Pal, D., & Mitra, A. K. (2015). Discovery of novel inhibitors for the treatment of glaucoma. Expert opinion on drug discovery, 10(3), 293-313.

Chomel-Guillaume, S., Leloup, G., & Bernard, I. (2010). Les aphasies. Evaluation et rééducation.

Ciavardelli D, Piras F, Consalvo A, et al. Medium-chain plasma acylcarnitines, ketone levels, cognition, and gray matter volumes in healthy elderly, mildly cognitively impaired, or alzheimer's disease subjects. Neurobiol Aging 2016;43:1–12.

Cline, E. N., Bicca, M. A., Viola, K. L., & Klein, W. L. (2018). The amyloid-β oligomer hypothesis: Beginning of the third decade. Journal of Alzheimer's Disease, 64(s1), S567-S610.

Cole GM, Ard MD. Influence of lipoproteins on microglial degradation of Alzheimer's amyloid beta-protein. Microsc Res Tech. 2000;50(4):316-324.

Cole, D. C., Manas, E. S., Stock, J. R., Condon, J. S., Jennings, L. D., Aulabaugh, A., ... & Harrison, B. L. (2006). Acylguanidines as small-molecule β-secretase inhibitors. Journal of medicinal chemistry, 49(21), 6158-6161.

Congdon, E. E., & Sigurdsson, E. M. (2018). Tau-targeting therapies for Alzheimer disease. Nature Reviews Neurology, 14(7), 399-415.

Coraci IS, Husemann J, Berman JW, Hulette C, Dufour JH, Campanella GK, LusterAD, Silverstein SC, El-Khoury JB. 2002. CD36, a class B scavenger receptor, is expressed on microglia in Alzheimer's disease brains and can mediate production of reactive oxygen species in response to b-amyloid fibrils. Am J Pathol 160: 101–112.

Corbo, R. M., & Scacchi, R. (1999). Apolipoprotein E (APOE) allele distribution in the world. Is APOE* 4 a 'thrifty'allele?. Annals of human genetics, 63(4), 301-310.

Cortes, N.; Alvarez, R.; Osorio, E.H.; Alzate, F.; Berkov, S.; Osorio, E. Alkaloid metabolite profiles by GC/MS and acetylcholinesterase inhibitory activities with binding-mode predictions of five Amaryllidaceae plants. J. Pharm. Biomed. Anal., 2015, 102,222-228

Costa, P., Gonçalves, S., Valentão, P., Andrade, P. B., & Romano, A. (2013). Accumulation of phenolic compounds in in vitro cultures and wild plants of Lavandula viridis L'Hér and their antioxidant and anti-cholinesterase potential. Food and Chemical Toxicology, 57, 69-74.

Cox-Georgian, D., Ramadoss, N., Dona, C., & Basu, C. (2019). Therapeutic and medicinal uses of terpenes. In Medicinal Plants (pp. 333-359). Springer, Cham.

Cruchaga, C., Chakraverty, S., Mayo, K., Vallania, F. L., Mitra, R. D., Faber, K., ... & Boeve, B. F. (2012). Rare variants in APP, PSEN1 and PSEN2 increase risk for AD in late-onset Alzheimer's disease families. PloS one, 7(2), e31039.

Cruz-Oliver, D. M., Malmstrom, T. K., Allen, C. M., Tumosa, N., & Morley, J. E. (2012). The Veterans Affairs Saint Louis University Mental Status exam (SLUMS exam) and the Mini-mental status exam as predictors of mortality and institutionalization. The journal of nutrition, health & aging, 16(7), 636-641.

Cummings J, Aisen PS, DuBois B, Frölich L, Jack CR, Jones RW,et al. Drug development in Alzheimer's disease: the path to2025. Alzheimers Res Ther. 2016;8:39.

Cummings, J, Lee, G, Ritter, A, Sabbagh, M, Zhong, K. Alzheimer's disease drug development pipeline: 2019. Alzheimers Dement. 2019;5:272-293.

D

Daily, C. E. (2007). Understanding the Brain: The Birth of a Learning Science Centre for Educational Research and Innovation.

Davis, E. M., & Croteau, R. (2000). Cyclization enzymes in the biosynthesis of monoterpenes, sesquiterpenes, and diterpenes. In Biosynthesis (pp. 53-95). Springer, Berlin, Heidelberg.

de Mello, A. H., Costa, A. B., Engel, J. D. G., & Rezin, G. T. (2018). Mitochondrial dysfunction in obesity. Life sciences, 192, 26-32.

De Souto Barreto P, Andrieu S, Rolland Y, et al. Physical activity domains and cognitive function over three years in older adults with subjective memory complaints: secondary analysis from the MAPT trial. J Sci Med Sport 2018;21:52–7.

Deng, H., & Li, W. (2020). Monoacylglycerol lipase inhibitors: modulators for lipid metabolism in cancer malignancy, neurological and metabolic disorders. Acta Pharmaceutica Sinica B, 10(4), 582-602

Després, J. P., & Lemieux, I. (2006). Abdominal obesity and metabolic syndrome. Nature, 444(7121), 881-887.

Dev, I. K., & Subbiah, V. , Diterpene derivatives for the treatment of cardiovascular, cancer and inflammatory diseases, U.S. Pat. Appl. Publ. US 20070207989, 2007

Dib, M. E. A., Allali, H., Bendiabdellah, A., Meliani, N., & Tabti, B. (2013). Antimicrobial activity and phytochemical screening of Arbutus unedo L. Journal of Saudi Chemical Society, 17(4), 381-385.

Dinh, T. P., Carpenter, D., Leslie, F. M., Freund, T. F., Katona, I., Sensi, S. L., Kathuria, S. & Piomelli, D. (2002). Brain monoglyceride lipase participating in endocannabinoid inactivation. Proceedings of the national Academy of sciences, 99(16), 10819-10824.

Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., & Vidal, N. (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food chemistry, 97(4), 654-660.

Doke, S.; Guha, M. "Garden cress (Lepidium sativum L.) Seed - An Important Medicinal Source : A Review," J. Nat. Prod. Plant Resour, 2014, 4(1), 69-80.

Donix, M., Burggren, A. C., Suthana, N. A., Siddarth, P., Ekstrom, A. D., Krupa, A. K., ... & Small, G. W. (2010). Family history of Alzheimer's disease and hippocampal structure in healthy people. American Journal of Psychiatry, 167(11), 1399-1406.

Donovan NJ, Locascio JJ, Marshall GA, et al. Longitudinal association of Amyloid Beta and anxious-depressive symptoms I cognitively normal older adults. Am J Psychol 2018.

Dovey, H. F., John, V., Anderson, J. P., Chen, L. Z., de Saint Andrieu, P., Fang, L. Y., ... & Hu, K. L. (2001). Functional gamma-secretase inhibitors reduce beta-amyloid peptide levels in brain. Journal of neurochemistry, 76(1), 173-181.

Dubey, B. N., Bashary, R., Mehta, M., Satija, S., Khurana, N., Sharma, N., & Khatik, G. L. (2018), Identification of Possible Molecular Targets of Potential Anti-

Alzheimer Drugs by Predicting their Binding Affinities Using Molecular Docking Technique; International Journal of Green Pharmacy, pp. 16- 21.

Dubois B, Hampel H, Feldman HH, Scheltens P, Aisen P,Andrieu S, et al. Preclinical Alzheimer's disease: definition,natural history, and diagnostic criteria. Alzheimer's Dement.2016;12:292—323.8

E

Eckert, A., Nisbet, R., Grimm, A., and Götz, J. (2014). March separate, strike together - Role of phosphorylated TAU in mitochondrial dysfunction in Alzheimer's disease. Biochim. Biophys. Acta 1842, 1258–1266.

El Cadi, H., El Cadi, A., Kounnoun, A., El Majdoub, Y. O., Lovillo, M. P., Brigui, J., ... & Cacciola, F. (2020). Wild strawberry (Arbutus unedo): Phytochemical screening and antioxidant properties of fruits collected in northern Morocco. Arabian Journal of Chemistry, 13(8), 6299-6311.

El Jemli, M., Kamal, R., Marmouzi, I., Zerrouki, A., Cherrah, Y., & Alaoui, K. (2016). Radical-scavenging activity and ferric reducing ability of Juniperus thurifera (L.), J. oxycedrus (L.), J. phoenicea (L.) and Tetraclinis articulata (L.). Advances in pharmacological sciences, 2016.

El Sayed, M., Ghareeb, D., Sarhan, E., & Khalil, A. (2011). Therapeutic bio-screening of the bioactive ingredients of Berberis vulgaris. Functional Plant Science and Biotechnology, 5(1), 63-68.

Elisha, I.L.; Elgorashi, E.E.; Hussein, A.A.; Duncan, G.; Eloff, J.N. Acetylcholinesterase inhibitory effects of the bulb of Ammocharis coranica (Amaryllidaceae) and its active constituent lycorine. S. Afr. J. Bot., 2013, 85, 44-47.

Ellman, G. L., Courtney, K. D., Andres Jr, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical pharmacology, 7(2), 88-95.

Erion DM, Shulman GI (2010). Diacylglycerol-mediated insulin resistance. Nat. Med., 16: 400-402

F

Fang, W.S.; Sun, D.Y.; Yang, S.; Cheng, C.; Moschke, K.; Li, T.; Sun, S.; Lichtenthaler, S.F.; Huang, J.; Wang, Y. Discovery of a series of selective and cell permeable beta-secretase (BACE1) inhibitors by fragment linking with the assistance of STD-NMR. Bioorg. Chem., 2019, 92103253

Farr SA, Niehoff ML, Ceddia MA, et al. Effect of botanical extracts containing carnosic acid or rosmarinic acid on learning and memory in SAMP8 mice. Physiol Behav 2016;165:328–38.

Farr SA, Price TO, Banks WA, et al. Effect of alpha-lipoic acid on memory, oxidation, and lifespan in SAMP8 mice. J Alzheimers Dis 2012;32:447–55.

Farr SA, Price TO, Dominguez LJ, et al. Extra virgin olive oil improves learning and memory in SAMP8 mice. J Alzheimers Dis 2012;28:81–92.

Farrer LA, Cupples LA, Haines JL, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. JAMA 1997;278:1349–1356

Federico A, Cardaioli E, Da Pozzo P, Formichi P, Gallus GN, and Radi E. Mitochondria, oxidative stress and neurodegeneration. J Neurol Sci 322: 254-262, 2012.

Fernández, J. C. Q., García, E. D. L. F., Pugliese, M., Aroca, P. M., Santafé, J. B., Parrilla, J. F. E., ... & Battestini, B. W. (2019). U.S. Patent No. 10,376,529. Washington, DC: U.S. Patent and Trademark Office.

Fernández, J. C. Q., García, E. D. L. F., Pugliese, M., Aroca, P. M., Santafé, J. B., Parrilla, J. F. E., ... & Battestini, B. W. (2019). U.S. Patent No. 10,376,529. Washington, DC: U.S. Patent and Trademark Office.

Fernández-Bachiller, M.I.; Pérez, C.; González-Muñoz, G.C.; Conde, S.; López, M.G.; Villarroya, M.; García, A.G.; Rodríguez-Franco, M.I. Novel tacrine-8-hydroxyquinoline hybrids as multifunctional agents for the treatment of Alzheimer's disease, with neuroprotective, cholinergic, antioxidant, and copper-complexing properties. J. Med. Chem., 2010, 53(13), 4927-4937.

Ferreira, D., Verhagen, C., Hernández-Cabrera, J. A., Cavallin, L., Guo, C. J., Ekman, U., ... & Westman, E. (2017). Distinct subtypes of Alzheimer's disease based

on patterns of brain atrophy: longitudinal trajectories and clinical applications. Scientific reports, 7(1), 1-13.

Ferreira, D., Nordberg, A., & Westman, E. (2020). Biological subtypes of Alzheimer disease: A systematic review and meta-analysis. Neurology, 94(10), 436-448.

Ferreira, L. S., Fernandes, C. S., Vieira, M. N., & De Felice, F. G. (2018). Insulin resistance in Alzheimer's disease. Frontiers in neuroscience, 12, 830.

Ferrisi, R. Synthesis and optimization of benzoylpiperidine derivatives as Monoacylglycerol Lipase (MAGL) inhibitors, 2019.

Fiorentino, A., Castaldi, S., D'Abrosca, B., Natale, A., Carfora, A., Messere, A., & Monaco, P. (2007). Polyphenols from the hydroalcoholic extract of Arbutus unedo living in a monospecific Mediterranean woodland. Biochemical Systematics and Ecology, 35(11), 809.

Flood JF, Morley JE, Roberts E. Amnestic effects in mice of four synthetic peptides homologous to amyloid beta protein from patients with Alzheimer disease. Proc Natl Acad Sci U S A 1991;88:3363–6.

Flood JF, Roberts E, Sherman MA, et al. Topography of a binding site for small amnestic peptides deduced from structure-activity studies: relation to amnestic effect of amyloid beta protein. Proc Natl Acad Sci U S A 1994;91:380–4.

Freitas, A. P., Santos, C. R., Sarcinelli, P. N., Silva Filho, M. V., Hauser-Davis, R. A., & Lopes, R. M. (2016). Evaluation of a brain acetylcholinesterase extraction method and kinetic constants after methyl-paraoxon inhibition in three Brazilian fish species. Plos one, 11(9), e0163317.

Frigerio, C. S., Wolfs, L., Fattorelli, N., Thrupp, N., Voytyuk, I., Schmidt, I., ... & Möller, T. (2019). The major risk factors for Alzheimer’s disease: age, sex, and genes modulate the microglia response to Aβ plaques. Cell reports, 27(4), 1293-1306.

Furrianca, M. C., Alvear, M., Zambrano, T., Fajardo, V., & Salazar, L. A. (2017). Phytochemical constituents of the root of Berberis microphylla. Asian J. Pharm. Clin. Res, 10, 225-227.

G

G.H. Goossens, The role of adipose tissue dysfunction in the pathogenesis ofobesity-related insulin resistance, Physiol. Behav. 94 (2008) 206e218.

Gacemi, S., Benarous, K., Imperial, S., & Yousfi, M. (2020). Lepidine B & E as new target inhibitors from Lepidium sativum seeds against four enzymes of the pathogen Candida albicans: in vitro and in silico studies. Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders), 20(1), 127-138.

Gao, Y., Vasilyev, D. V., Goncalves, M. B., Howell, F. V., Hobbs, C., Reisenberg, M., Shen, R.; Zhang, M.Y.; Strassle, B.W.; Lu, P.; Mark, L.; Piesla, M.J.; Deng, K.; Kouranova, E.V.; Ring, R.H.; Whiteside, G.T.; Bates, B.; Walsh, F.S.; Williams, G.; Pangalos, M.N.; Samad, T.A. & Doherty, P. (2010). Loss of retrograde endocannabinoid signaling and reduced adult neurogenesis in diacylglycerol lipase knock-out mice. Journal of Neuroscience, 30(6), 2017-2024.

Geula, C., & Mesulam, M. (1989). Special properties of cholinesterases in the cerebral cortex of Alzheimer's disease. Brain research, 498(1), 185-189.

Ghobadian, R.; Mahdavi, M.; Nadri, H.; Moradi, A.; Edraki, N.; Akbarzadeh, T.; Sharifzadeh, M.; Bukhari, S.N.A.; Amini, M. Novel tetrahydrocarbazole benzyl pyridine hybrids as potent and selective butryl cholinesterase inhibitors with neuroprotective and β-secretase inhibition activities. Eur. J. Med. Chem., 2018, 155, 49-60.

Ghosh, A. K., Gemma, S., & Tang, J. (2008). β-Secretase as a therapeutic target for Alzheimer's disease. Neurotherapeutics, 5(3), 399-408.

Ghouti, D., Rached, W., Abdallah, M., Pires, T. C., Calhelha, R. C., Alves, M. J., ... & Ferreira, I. C. (2018). Phenolic profile and in vitro bioactive potential of Saharan Juniperus phoenicea L. and Cotula cinerea (Del) growing in Algeria. Food & function, 9(9), 4664-4672.

Gil-Ordóñez, A., Martín-Fontecha, M., Ortega-Gutiérrez, S., & López-Rodríguez, M. L. (2018). Monoacylglycerol lipase (MAGL) as a promising therapeutic target. Biochemical Pharmacology. 2018.07.036

Glynn-Servedio, B. E., & Ranola, T. S. (2017). AChE inhibitors and NMDA receptor antagonists in advanced Alzheimer's disease. The Consultant Pharmacist®, 32(9), 511-518.

Goate A, Chartier-Harlin MC, Mullan M, Brown J, Crawford F, Fidani L, Giuffra L, Haynes A, Irving N, James L, Mont R, Newton P, Rooke K, Roques P, Talbot C, Pericak-Vance M, Roses A, Williamson R, Rossor M, Owen M, Hardy J (1991) Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature 349, 704–706.

Gokavi, S.S.; Malleshi, N.G.; Guo, M. Chemical composition of garden cress (Lepidium sativum) seeds and its fractions and use of bran as a functional ingredient. Plant Foods Hum. Nutr., 2004, 59(3), 105-111.

Golde T.E., Koo E.H., Felsenstein K.M., Osborne B.A., and Miele L., "γ-Secretase inhibitors and modulators", Biochimica et Biophysica Acta (BBA) - Biomembranes, vol. 1828, pp. 2898-2907, 2013.

Gonzalez-Yanes, C., Sanchez-Margalet, V. 2006. Signalling mechanisms regulating lipolysis. Cell. Signal. 18:401-408

Grabarczyk, M., Mączka, W., Żołnierczyk, A. K., & Wińska, K. (2020). Transformations of Monoterpenes with the p-Menthane Skeleton in the Enzymatic System of Bacteria, Fungi and Insects. Molecules, 25(20), 4840.

Grober, E., Buschke, H. C. H. B. S., Crystal, H., Bang, S., & Dresner, R. (1988). Screening for dementia by memory testing. Neurology, 38(6), 900-900.

Grundke-Iqbal, I., Iqbal, K., Tung, Y.-C., Quinlan, M., Wisniewski, H. M., and Binder, L. I. (1986). Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. Proc. Natl. Acad. Sci. U.S.A. 83, 4913–4917.

Guan, X., Guan, Y., Shi, C., Zhu, X., He, Y., Wei, Z., ... & Hou, T. (2020). Estrogen deficiency aggravates apical periodontitis by regulating NLRP3/caspase-1/IL-1β axis. American journal of translational research, 12(2), 660.

Guériot-Milandre C, Semprez C, Poncet M. Aspects cliniques et diagnostic de la maladie d'alzheimer. Médecine Thérapeutique. 1997; 3: 343-352.

Guerreiro, R., & Bras, J. (2015). The age factor in Alzheimer's disease. Genome medicine, 7(1), 1-3.

Guerriero, G., Berni, R., Muñoz-Sanchez, J. A., Apone, F., Abdel-Salam, E. M., Qahtan, A. A., ... & Siddiqui, K. S. (2018). Production of plant secondary metabolites: Examples, tips and suggestions for biotechnologists. Genes, 9(6), 309.

Guillozet, A. L., Mesulam, M. M., Smiley, J. F., & Mash, D. C. (1997). Butyrylcholinesterase in the life cycle of amyloid plaques. Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society, 42(6), 909-918.

Gülçin, İ., Scozzafava, A., Supuran, C. T., Koksal, Z., Turkan, F., Çetinkaya, S., ... & Alwasel, S. H. (2016). Rosmarinic acid inhibits some metabolic enzymes including glutathione S-transferase, lactoperoxidase, acetylcholinesterase, butyrylcholinesterase and carbonic anhydrase isoenzymes. Journal of enzyme inhibition and medicinal chemistry, 31(6), 1698-1702

Guo Y., Zhang H., Wang J., Fu Y., Liu H., Methyl toonapubesate A and preparation method as well as application thereof, Faming Zhuanli Shenqing CN 102311481,2012.

Gupta, R. C., Mukherjee, I. R. M., Malik, J. K., Doss, R. B., Dettbarn, W. D., & Milatovic, D. (2019). Insecticides. In Biomarkers in toxicology (pp. 455-475). Academic Press.

Gurjar, A.S.; Darekar, M.N.; Yeong, K.Y.; Ooi, L. In silico studies, synthesis and pharmacological evaluation to explore multi-targeted approach for imidazole analogues as potential cholinesterase inhibitors with neuroprotective role for Alzheimer's disease. Bioorg. Med. Chem., 2018, 26(8), 1511-1522.

H

Halle, A., Hornung, V., Petzold, G. C., Stewart, C. R., Monks, B. G., Reinheckel, T., ... & Golenbock, D. T. (2008). The NALP3 inflammasome is involved in the innate immune response to amyloid-β. Nature immunology, 9(8), 857.

Hamaguchi T, Ono K, Murase A, Yamada M. 2009. Phenolic compounds prevent Alzheimer's pathology through different effects on the amyloid-beta aggregation pathway. Am J Pathol 175: 2557–2565.

Han, Z., Tian, R., Ren, P., Zhou, W., Wang, P., Luo, M., ... & Jiang, Q. (2018). Parkinson's disease and Alzheimer's disease: a Mendelian randomization study. BMC medical genetics, 19(1), 1-9.

Hannan, M. A., Dash, R., Sohag, A. A. M., Haque, M., & Moon, I. S. (2020). Neuroprotection against oxidative stress: Phytochemicals targeting TrkB signaling and the Nrf2-ARE antioxidant system. Frontiers in Molecular Neuroscience, 13, 116.

Hanson, A. J., Bayer-Carter, J. L., Green, P. S., Montine, T. J., Wilkinson, C. W., Baker, L. D., ... & Tsai, E. (2013). Effect of apolipoprotein E genotype and diet on apolipoprotein E lipidation and amyloid peptides: randomized clinical trial. JAMA neurology, 70(8), 972-980.

Harborne JB (1973)b Phytochemical methods: a guide to modern technique of plant analysis. Chapman & Hall, London

Harborne, A. J. (1998). Phytochemical methods a guide to modern techniques of plant analysis. springer science & business media.

Harborne, J. B. (1973). Phenolic compounds. In Phytochemical methods (pp. 33-88). Springer, Dordrecht.

Harborne, J. B. (1973)a. Methods of Plant Analysis. Phytochemical Methods, 1–32.

Harborne, J.B., 1998. Methods of extraction and isolation: Phytochemical Methods. 3rd edition, Chapman & Hall, London

Hardy J (2009) The amyloid hypothesis for Alzheimer's dis-ease: A critical reappraisal.J Neurochem110, 1129-1134.

Harel, M., Schalk, I., Ehret-Sabatier, L., Bouet, F., Goeldner, M., Hirth, C., ... & Sussman, J. L. (1993). Quaternary ligand binding to aromatic residues in the active-site gorge of acetylcholinesterase. Proceedings of the National Academy of Sciences, 90(19), 9031-9035.

Harvey, A. L. (1995). The pharmacology of galanthamine and its analogues. Pharmacology & therapeutics, 68(1), 113-128.

Hattori Y. Design, Synthesis, and Evaluation of (4 R)-1-{3-[2-(18F) Fluoro-4-methylpyridin-3-yl] phenyl}-4-[4-(1, 3-thiazol-2-ylcarbonyl) piperazin-1-yl] pyrrolidin-2-one ([18F] T-401) as a Novel Positron-Emission Tomography Imaging Agent for Monoacylglycerol Lipase. J. Med. Chem., 2019, 62(5), 2362-2375.

Heifets, B. D., & Castillo, P. E. (2009). Endocannabinoid signaling and long-term synaptic plasticity. Annual review of physiology, 71, 283-306.

Heneka, M. T., Carson, M. J., El Khoury, J., Landreth, G. E., Brosseron, F., Feinstein, D. L., ... & Herrup, K. (2015). Neuroinflammation in Alzheimer's disease. The Lancet Neurology, 14(4), 388-405.

Heneka, M. T., Kummer, M. P., Stutz, A., Delekate, A., Schwartz, S., Vieira-Saecker, A., ... & Gelpi, E. (2013). NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. Nature, 493(7434), 674-678.

Heo, H.J.; Kim, M.J.; Lee, J.M.; Choi, S.J.; Cho, H.Y.; Hong, B.S.; Kim, H.K.; Kim, E.; Shin, D.H. Naringenin from Citrus junos has an inhibitory effe²ct on acetylcholinesterase and a mitigating effect on amnesia. Dement. Geriatr Cog. Disor., 2004,17(3), 151-15

Hernandez, M. F., Falé, P. L. V., Araújo, M. E. M., & Serralheiro, M. L. M. (2010). Acetylcholinesterase inhibition and antioxidant activity of the water extracts of several Hypericum species. Food Chemistry, 120(4), 1076–1082.

Hicks, A. J., James, A. C., Spitz, G., & Ponsford, J. L. (2019). Traumatic brain injury as a risk factor for dementia and Alzheimer disease: critical review of study methodologies. Journal of neurotrauma, 36(23), 3191-3219.

Hodkinson, HM (1972). "Evaluation of a mental test score for assessment of mental impairment in the elderly". Age and Ageing. 1 (4): 233–8

Honda T., Honda Y., Gribble G. W., Sporn M. B., SuhN., Inhibitors and methods of use thereof, U.S. Patent US 6974801,2005.

Hooper C, De Souto Barreto P, Coley N, et al. Cognitive changes with omega-3 polyunsaturated fatty acids in non-demented older adults with low omega-3 index.J Nutr Health Aging 2017;21:988–93.

Houghton, P. J., Ren, Y., & Howes, M. J. (2006). Acetylcholinesterase inhibitors from plants and fungi. Natural Product Reports, 23(2), 181-199.

Hussien, H. M., Abd-Elmegied, A., Ghareeb, D. A., Hafez, H. S., Ahmed, H. E., & Abd El-moneam, N. (2018). Neuroprotective effect of berberine against environmental heavy metals-induced neurotoxicity and Alzheimer's-like disease in rats. Food and chemical toxicology, 111, 432-444.

Hyman B. T., Van Hoesen G. W. and Damasio A. R. (1990) Memory-related neural systems in Alzheimer's disease: an anatomic study.Neurology40,1721–1730.

Hyman B. T., Van Hoesen G. W., Damasio A. R. and Barnes C. L. (1984) Alzheimer's disease: cell-specific pathology isolates the hippocampalformation. Science 225,1168–1170

I

Ichinose S., Kakuo S., Jpn. Aromatase activator, Tokkyo Koho JP 5646358, 2014.

Ichinose S., Kakuo S., U.S. Patent US 9072695, 2015.

Imade, O.V.; Smith, O.F.; Gazal, O.S. Effects of dietary inclusion of Lepidium sativum (garden cress) seed on plasma luteinizing hormone and reproductive performance in female rabbits; , 2018, 6, pp. July;79-84.

Inaturalist, 2021a; Url: https://www.inaturalist.org/observations/42325161, (Last accessed in: 29/04/2021).

Inaturalist, 2021b; Url: https://www.inaturalist.org/observations/70657101, (Last accessed in: 29/04/2021).

Inaturalist, 2021c; Url: https://www.inaturalist.org/observations/73860028, (Last accessed in: 29/04/2021).

Inaturalist, 2021d; Url: https://www.inaturalist.org/observations/70274672, (Last accessed in: 29/04/2021).

Inaturalist, 2021e; Url: https://www.inaturalist.org/observations/68212989, (Last accessed in: 29/04/2021).

Inaturalist, 2021f; Url: https://www.inaturalist.org/observations/14884280, (Last accessed in: 29/04/2021).

Inestrosa, N. C., & Orozco, J. L. H. (2015). Treatment of alzheimer's and cognitive impairment with andrographolides, U.S. Patent Application No. 14/761,824.

Ittner, L. M., and Götz, J. (2011). Amyloid-β and tau - a toxic pas de deux in Alzheimer's disease. Nat. Rev. Neurosci. 12, 67–72.

J

Jacoby, R. J., Levy, R., & Dawson, J. M. (1980). Computed tomography in the elderly: 1. The normal population. The British Journal of Psychiatry, 136(3), 249-255.

Jain, T.; Grover, K. A Comprehensive Review on the Nutritional and Nutraceutical Aspects of Garden Cress (Lepidium sativum Linn.). Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci., 2016, 2-9.

James, K. D. (2017). Animal metabolites: from amphibians, reptiles, aves/birds, and invertebrates. In Pharmacognosy (pp. 401-411). Academic Press.

Jiang, Q., Lee, C. D., Mandrekar, S., Wilkinson, B., Cramer, P., Zelcer, N., ... & Richardson, J. C. (2008). ApoE promotes the proteolytic degradation of Aβ. Neuron, 58(5), 681-693.

John, K. M., Vijayan, D., Kumar, R. R., & Premkumar, R. (2006). Factors Influencing the Efficiency of Extraction of Polyphenols from Young Tea Leaves. Asian Journal of Plant Sciences.

Jukic, M., Politeo, O., Maksimovic, M., Milos, M., & Milos, M. (2007). In Vitroacetylcholinesterase inhibitory properties of thymol, carvacrol and their derivatives thymoquinone and thymohydroquinone. Phytotherapy Research, 21(3), 259–261. doi:10.1002/ptr.2063

Jung, M., & Park, M. (2007). Acetylcholinesterase inhibition by flavonoids fromAgrimonia pilosa.Molecules, 12(9), 2130–2139.

K

Kabir, M. T., Sufian, M. A., Uddin, M., Begum, M., Akhter, S., Islam, A., ... & Amran, M. (2019). NMDA Receptor Antagonists: Repositioning of Memantine as a Multitargeting Agent for Alzheimer's Therapy. Current Pharmaceutical Design, 25(33), 3506-3518. B

Kabir, M. T., Uddin, M., Begum, M., Thangapandiyan, S., Rahman, M., Aleya, L., ... & Ashraf, G. M. (2019). Cholinesterase inhibitors for Alzheimer's disease: multitargeting strategy based on anti-Alzheimer's drugs repositioning. Current Pharmaceutical Design, 25(33), 3519-3535. a

Kachkoul, R., Houssaini, T. S., El Habbani, R., Miyah, Y., Mohim, M., & Lahrichi, A. (2018). Phytochemical screening and inhibitory activity of oxalocalcic crystallization of Arbutus unedo L. leaves. Heliyon, 4(12), e01011.

Kadiyala, M., Ponnusankar, S., & Elango, K. (2014). Screening of siddha medicinal plants for its in-vitro acetylcholinesterase and butyrylcholinesterase inhibitory activity. Pharmacognosy magazine, 10(Suppl 2), S294.

Kano, M., Ohno-Shosaku, T., Hashimotodani, Y., Uchigashima, M., & Watanabe, M. (2009). Endocannabinoid-mediated control of synaptic transmission. Physiological reviews, 89(1), 309-380.

Kara, E., Marks, J. D., Roe, A. D., Commins, C., Fan, Z., Calvo-Rodriguez, M., ... & Hyman, B. T. (2018). A flow cytometry–based in vitro assay reveals that formation of apolipoprotein E (ApoE)–amyloid beta complexes depends on ApoE isoform and cell type. Journal of Biological Chemistry, 293(34), 13247-13256.

Karazhiyan, H. Rheological properties of Lepidium sativum seed extract as a function of concentration, temperature and time. Food Hydrocoll., 2009, 23(8), 2062-2068.

Katalinić, M., Rusak, G., Domaćinović Barović, J., Šinko, G., Jelić, D., Antolović, R., & Kovarik, Z. (2010). Structural aspects of flavonoids as inhibitors of human butyrylcholinesterase. European Journal of Medicinal Chemistry, 45(1), 186–192.

Kayed, R., & Lasagna-Reeves, C. A. (2013). Molecular mechanisms of amyloid oligomers toxicity. Journal of Alzheimer's Disease, 33(s1), S67-S78.

Kelényi, G. Thioflavin S fluorescent and congo red anisotropic stainings in the histologic demonstration of amyloid. Acta Neuropathol.1967,7, 336–348

Kellar, D., & Craft, S. (2020). Brain insulin resistance in Alzheimer's disease and related disorders: mechanisms and therapeutic approaches. The Lancet Neurology.

Kennedy DO, Wightman EL. Herbal extracts and phytochemicals: plant secondary metabolites and the enhancement of human brain function. Adv Nutr. 2011;2:32-50.

Kerry, B., Simon, M., 2 - Principles of herbal pharmacology, Principles and Practice of Phytotherapy (Second Edition), Churchill Livingstone, 2013.

Keskes, H., Mnafgui, K., Hamden, K., Damak, M., El Feki, A., & Allouche, N. (2014). In vitro anti-diabetic, anti-obesity and antioxidant proprieties of Juniperus phoenicea L. leaves from Tunisia. Asian Pacific journal of tropical biomedicine, 4, S649-S655.

Khachaturian, Z. S. (1985). Diagnosis of Alzheimer's disease. Archives of neurology, 42(11), 1097-1105.

Khacheba, I., Djeridane, A., & Yousfi, M. (2014). Twenty traditional Algerian plants used in diabetes therapy as strong inhibitors of α-amylase activity. International Journal of Carbohydrate Chemistry, 2014.

Khalaf, R. A., Alhusban, A. A., Al-Shalabi, E., Al-Sheikh, I., & Sabbah, D. A. (2019). Isolation and structure elucidation of bioactive polyphenols. In Studies in Natural Products Chemistry (Vol. 63, pp. 267-337). Elsevier.

Khan, M. T. H., Orhan, I., Senol, F. S., Kartal, M., Sener, B., Dvorská, M., et al. (2009).Cholinesterase inhibitory activities of some flavonoid derivatives and chosenxanthone and their molecular docking studies.Chemico-Biological Interactions,181(3), 383–389.

Khanahmadi, M., Farhud, D. D., & Malmir, M. (2015). Genetic of Alzheimer’s disease: A narrative review article. Iranian journal of public health, 44(7), 892.

Khoddami, A., Wilkes, M. A., & Roberts, T. H. (2013). Techniques for analysis of plant phenolic compounds. Molecules, 18(2), 2328-2375.

Kim GH, Kim JE, Rhie SJ, and Yoon S. The role of oxidative stress in neurodegenerative diseases. Exp Neurobiol 24: 325-340, 2015.

Kim T., Chung S., Compounds for treating Alzheimer's disease and for inhibiting beta-amyloid peptitde production, U.S. Pat. Appl. Publ. US 20050245465, 2005.

Kim T., Chung S., Compounds for treating alzheimer's disease and for inhibiting beta-amyloid peptitde production, Faming Zhuanli Shenqing CN 101133075, 2008.

Kim, J., Wie, M. B., Ahn, M., Tanaka, A., Matsuda, H., & Shin, T. (2019). Benefits of hesperidin in central nervous system disorders: a review. Anatomy & cell biology, 52(4), 369.

Kim, S. (2016) PubChem Substance and Compound databasesNucleic Acids Res; 44, pp. (Database issue)1202-1213. http://dx.doi.org/10.1093/nar/gkv951

Kimura, A., Hata, S., & Suzuki, T. (2016). Alternative selection of β-site APP-cleaving enzyme 1 (BACE1) cleavage sites in amyloid β-protein precursor (APP) harboring protective and pathogenic mutations within the Aβ sequence. Journal of Biological Chemistry, 291(46), 24041-24053.

Kivipelto, M., Ngandu, T., Fratiglioni, L., Viitanen, M., Kåreholt, I., Winblad, B., ... & Nissinen, A. (2005). Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. Archives of neurology, 62(10), 1556-1560.

Klunk WE, Engler H, Nordberg A, et al. Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. Ann Neurol 2004;55:306–19.

Kokate, C. K., Purohit, A. P., & Gokhale, S. B. (2003). Text book of Pharmacognosy. Pune: Nirali Prakashan, 8(66), 1-624.

Kolar, D., Wimmerova, L., & Kadek, R. (2010). Acetylcholinesterase and butyrylcholinesterase inhibitory activities of Berberis vulgaris. Phytopharmacol, 1, 7-11.

Konrath, E. L., Passos, C. D. S., Klein-Júnior, L. C., & Henriques, A. T. (2013). Alkaloids as a source of potential anticholinesterase inhibitors for the treatment of A lzheimer's disease. Journal of Pharmacy and Pharmacology, 65(12), 1701-1725.

Koronyo Y., Koronyo M., Black K. L., Schwartz M., Farkas D. L., Optical method for the detection of alzheimer's disease, U.S. Pat. Appl. Publ.US 20110286932, 2011

Kraepelin E (1910) Klinische Psychiatrie. Psychiatrie. Ein Lehrbuch für Studierende und Ärzte, vol 2. Johann Ambrosius Barth, Leipzig

Kregel, K. C., & Zhang, H. J. (2007). An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 292(1), R18-R36.

Kukula-Koch, W. A., & Widelski, J. (2017). Alkaloids. In Pharmacognosy (pp. 163-198). Academic Press.

Kumar A, Ilavarasn R, Jayachandran T, Decaraman M, Aravindhan P,Padmanaban N (2009) Phytochemical investigation on tropicalplant. Pakistan J Nutr 8(1):83–85

Kuo, H.-C., Yen, H.-C., Huang, C.-C., Hsu, W.-C., Wei, H.-J., & Lin, C.-L. (2014). Cerebrospinal fluid biomarkers for neuropsychological symptoms in early stage of late-onset Alzheimer's disease. International Journal of Neuroscience, 125(10), 747–754.

Kuo, J. J., Chang, H. H., Tsai, T. H., & Lee, T. Y. (2012). Curcumin ameliorates mitochondrial dysfunction associated with inhibition of gluconeogenesis in free fatty acid-mediated hepatic lipoapoptosis. International journal of molecular medicine, 30(3), 643-649.

Kurt, B.Z.; Gazioglu, I.; Kandas, O.; Sonmez, F. Synthesis, Anticholinesterase, Antioxidant, and Anti-Aflatoxigenic Activity of Novel Coumarin Carbamate Derivatives. Biol. Chem. Chem. Biol., 2018, 3, 3978-3983.

L

Labar, G., Wouters, J., & Lambert, D. M. (2010). A review on the monoacylglycerol lipase: at the interface between fat and endocannabinoid signalling. Current medicinal chemistry, 17(24), 2588-2607.

LaDu MJ, Falduto MT, Manelli AM, Reardon CA, Getz GS, Frail DE. Isoform-specific binding of apolipoprotein E to β-amyloid. J Biol Chem. 1994;269(38):23403-23406.

Lamaison, J. L., & Carnart, A. (1991). Teneurs en principaux falvonoïdes des fleurs et des feuilles de Crataegus monogyna Jacq. et de Crataegus laevigata (Poiret) DC. en fonction de la période de végétation. Plantes médicinales et phytothérapie, 25(1), 12-16.

Landreth GE, Reed-Geaghan EG. 2009. Toll-like receptors in Alzheimer's disease. Curr Top Microbiol Immunol 336: 137–153.

Laribi, B., Kouki, K., M'Hamdi, M., & Bettaieb, T. (2015). Coriander (Coriandrum sativum L.) and its bioactive constituents. Fitoterapia, 103, 9–26.

Larik, F. A. New cholinesterase inhibitors for Alzheimer's disease: Structure activity relationship, kinetics and molecular docking studies of 1–butanoyl–3–arylthiourea derivatives, Int. J. Biol. Macromol, 2018, 116(2017), 144-150.

Laskowski, R. A., & Swindells, M. B. (2011). LigPlot+: multiple ligand–protein interaction diagrams for drug discovery.

Ledig, C., Schuh, A., Guerrero, R., Heckemann, R. A., & Rueckert, D. (2018). Structural brain imaging in Alzheimer's disease and mild cognitive impairment: biomarker analysis and shared morphometry database. Scientific reports, 8(1), 1-16.

Lee, H. J., Park, M. K., & Seo, Y. R. (2018). Pathogenic mechanisms of heavy metal induced-Alzheimer's disease. Toxicology and Environmental Health Sciences, 10(1), 1-10.

Lee, J. G., Woo, Y. S., Park, S. W., Seog, D. H., Seo, M. K., & Bahk, W. M. (2019). The neuroprotective effects of melatonin: Possible role in the pathophysiology of neuropsychiatric disease. Brain sciences, 9(10), 285.

Levy-Lahad E, Wasco W, Poorkaj P, Romano DM, Oshima J, Pettingell WH, Yu CE, Jondro PD, Schmidt SD, Wang K, et al. (1995) Candidate gene for the chromosome 1 familial Alzheimer's disease locus. Science 269, 973–977.

Li ST, Ju JG. Functional roles of synaptic and extrasynaptic NMDA receptors in physiological and pathological neuronal activities. Curr Drug Targets. 2012;13(2):207–21.

Li, A. P. (2001). Screening for human ADME/Tox drug properties in drug discovery. Drug discovery today, 6(7), 357-366.

Li, X. Q., Yu, Q., Fang, B., Zhang, Z. L., & Ma, H. (2019). Knockdown of the AIM2 molecule attenuates ischemia-reperfusion-induced spinal neuronal pyroptosis by inhibiting AIM2 inflammasome activation and subsequent release of cleaved caspase-1 and IL-1β. Neuropharmacology, 160, 107661.

LIANG, Zhi-Man. The BACE1-Specific DNA Aptamer A1 Rescues Amyloid-β Pathology and Behavioral Deficits in a Mouse Model of Alzheimer's Disease. nucleic acid therapeutics, 2019.

Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., ... & Chen, H. (2016). An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. Molecules, 21(10), 1374.

Lin, L., Huang, Q. X., Yang, S. S., Chu, J., Wang, J. Z., & Tian, Q. (2013). Melatonin in Alzheimer's disease. International journal of molecular sciences, 14(7), 14575-14593.

Liu CC, Liu CC, Kanekiyo T, Xu H, Bu G (February 2013). "Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy". Nature Reviews. Neurology. 9 (2): 106–18.

Liu, C., Hou, W., Li, S., & Tsao, R. (2020). Extraction and Isolation of Acetylcholinesterase Inhibitors from Citrus limon Peel Using an in vitro Method. Journal of Separation Science. doi:10.1002/jssc.201901252

Liu, F., Shi, J., Tanimukai, H., Gu, J., Gu, J., Grundke-Iqbal, I., ... & Gong, C. X. (2009). Reduced O-GlcNAcylation links lower brain glucose metabolism and tau pathology in Alzheimer's disease. Brain, 132(7), 1820-1832.

Liu, Q.H.; Wu, J.J.; Li, F.; Cai, P.; Yang, X.L.; Kong, L.Y.; Wang, X.B. Synthesis and pharmacological evaluation of multi-functional homoisoflavonoid derivatives as potent inhibitors of monoamine 18 Current Computer-Aided Drug Design, 2020, Vol. 16, No. 1 Talia et al. oxidase B and cholinesterase for the treatment of Alzheimer's disease. MedChemComm, 2017, 8(7), 1459-1467.

Long, J. Z., Li, W., Booker, L., Burston, J. J., Kinsey, S. G., Schlosburg, J. E., ... & Cravatt, B. F. (2009). Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. Nature chemical biology, 5(1), 37-44.

López, S.; Bastida, J.; Viladomat, F.; Codina, C. Acetylcholinesterase inhibitory activity of some Amaryllidaceae alkaloids and Narcissus extracts. Life Sci., 2002, 71(21), 2521-2529.

Louwersheimer, E., Cohn-Hokke, P. E., Pijnenburg, Y. A., Weiss, M. M., Sistermans, E. A., Rozemuller, A. J., ... & Koene, T. (2017). Rare Genetic Variant in SORL1 May Increase Penetrance of Alzheimer's Disease in a Family with Several Generations of APOE-ε4 Homozygosity. Journal of Alzheimer's Disease, 56(1), 63-74.

Lucin KM, Wyss-Coray T. Immune activation in brain aging and neurodegeneration: too much or too little? Neuron. 2009;64:110–122.

Luna-Guevara, M. L., Luna-Guevara, J. J., Hernández-Carranza, P., Ruíz-Espinosa, H., & Ochoa-Velasco, C. E. (2019). Phenolic compounds: a good choice against chronic degenerative diseases. In Studies in natural products chemistry (Vol. 59, pp. 79-108). Elsevier.

Luo X, Yan R. Inhibition of BACE1 for therapeutic usein Alzheimer's disease. Int J Clin Exp Pathol. 2010;3:618–28

Luo, Y., Bolon, B., Damore, M. A., Fitzpatrick, D., Liu, H., Zhang, J., ... & Citron, M. (2003). BACE1 (β-secretase) knockout mice do not acquire compensatory gene expression changes or develop neural lesions over time. Neurobiology of disease, 14(1), 81-88.

M

M. Glass, M.E. Abood, E.L. Scotter, The endocannabinoid system as a target for the treatment of neurodegenerative disease, Br J Pharmacol, 160 (2010), pp. 480-498.

M.P. Baggelaar, M. Maccarrone, M. van der Stelt, 2-Arachidonoylglycerol: a signaling lipid with manifold actions in the brain. Prog Lipid Res, 71 (2018), pp. 1-17

M.S. Wolfe, "γ-Secretase inhibitors and modulators for Alzheimer's disease", Journal of Neurochemistry, vol. 120, pp. 89-98, 2011

Mabry, T., Markham, K. R., & Thomas, M. B. (2012). The systematic identification of flavonoids. Springer Science & Business Media.

Madeira, M.H., Boia, R., Ambrósio, A.F., Santiago, A.R., 2017. Having a coffee break: theimpact of caffeine consumption on microglia-mediated inflammation in neurode-generative diseases. Mediat. Inflamm. 2017.

Maggi, L., & Mantegazza, R. (2011). Treatment of myasthenia gravis. Clinical drug investigation, 31(10), 691-701.

Mahajan D, Votruba M. Can the retina be used to diagnose and plot the progression of Alzheimer's disease? Acta Ophthalmol 2017;95:768–77.

Maier, U. H., Gundlach, H., & Zenk, M. H. (1998). Seven imidazole alkaloids from Lepidium sativum. Phytochemistry, 49(6), 1791-1795.

Maitre, M.; Klein, C.; Mensah-Nyagan, A.G. Mechanisms, risk factors and therapeutic strategies in Alzheimer's disease. Npg, Neurol. Psychiatr. Geriatr., 2017, 17(102), 352-364.

Malheiro, R., Sá, O., Pereira, E., Aguiar, C., Baptista, P., & Pereira, J. A. (2012). Arbutus unedo L. leaves as source of phytochemicals with bioactive properties. Industrial Crops and Products, 37(1), 473-478.

Mancuso, M., Coppede, F., Migliore, L., Siciliano, G., & Murri, L. (2006). Mitochondrial dysfunction, oxidative stress and neurodegeneration. Journal of Alzheimer's Disease, 10(1), 59-73.

Markesbery, W. R. (1997). Oxidative Stress Hypothesis in Alzheimer’s Disease. Free Radical Biology and Medicine, 23(1), 134–147.

Martinez-Lapiscina EH, Clavero P, Toledo E, et al. Virgin olive oil supplementation and long-term cognition: the PREDIMED-NAVARRA randomized, trial. J Nutr Health Aging 2013;17:544–52.

Martins, C.; Carreiras, M.C.; León, R.; de los Ríos, C.; Bartolini, M.; Andrisano, V.; Iriepa, I.; Moraleda, I.; Gálvez, E.; García, M.; Egea, J.; Samadi, A.; Chioua, M.; Marco-Contelles, J. (2011) Synthesis and biological assessment of diversely substituted furo[2,3-b]quinolin-4-amine and pyrrolo[2,3-b]quinolin-4-amine derivatives, as novel tacrine analogues. Eur. J. Med. Chem., 46(12), 6119-6130.

Masdeu JC. Tau and cortical thickness in Alzheimer’s disease. JAMA Neurol 2017;74:390–2.

Maurer, K., Volk, S., Gerbaldo, H., Auguste D and Alzheimer's disease, The Lancet, 1997, 349(9064), P1546-1549.

Mazari, K.; Bendimerad, N.; Bekhechi, C. Chemical composition and antimicrobial activity of essential oils isolated from Algerian Juniperus phoenicea L. and Cupressus sempervirens L. J. Med. Plants Res., 2010, 4(10), 959-964.

McGeer, P. L., & Rogers, J. (1992). Anti-inflammatory agents as a therapeutic approach to Alzheimer’s disease. Neurology, 42(2), 447–447.

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., & Stadlan, E. M. (1984). Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA Work Group* under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology, 34(7), 939-939.

McLachlan, D. C. (1986). Aluminum and Alzheimer's disease. Neurobiology of aging, 7(6), 525-532.

McWilliam, Christopher; Copeland, John R. M.; Dewey, Michael E.; Wood, Neil (February 2018). "The Geriatric Mental State (GMS) used in the community: replication studies of the computerized diagnosis AGECAT". Br. J. Psychiatry. 152 (2): 205–208

Meloni, M.; Perini, D.; Filigheddu, R.; Binelli, G. Genetic variation in five Mediterranean populations of Juniperus phoenicea as revealed by inter-simple sequence repeat (ISSR) markers. Ann. Bot., 2006, 97(2), 299-304.

Menchaca, T. M., Juárez-Portilla, C., & Zepeda, R. C. (2020). Past, Present, and Future of Molecular Docking. In Drug Discovery and Development-New Advances. IntechOpen.

Meng, X. Y., Zhang, H. X., Mezei, M., & Cui, M. (2011). Molecular docking: a powerful approach for structure-based drug discovery. Current computer-aided drug design, 7(2), 146-157.

Mesulam, M., Guillozet, A., Shaw, P., & Quinn, B. (2002). Widely Spread Butyrylcholinesterase Can Hydrolyze Acetylcholine in the Normal and Alzheimer Brain. Neurobiology of Disease, 9(1), 88–93. doi:10.1006/nbdi.2001.0462

Miller J. C., Mitchell M. O., Method for the synthesis of curcumin analogues, U.S. Patent US 7507864, 2009

Min, B.S.; Cuong, T.D.; Lee, J.S.; Shin, B.S.; Woo, M.H.; Hung, T.M. Cholinesterase inhibitors from Cleistocalyx operculatus buds. Arch. Pharm. Res., 2010,33(10), 1665-167

Min, S. W., Chen, X., Tracy, T. E., Li, Y., Zhou, Y., Wang, C., ... & Gan, L. (2015). Critical role of acetylation in tau-mediated neurodegeneration and cognitive deficits. Nature medicine, 21(10), 1154-1162.

Min, S. W., Cho, S. H., Zhou, Y., Schroeder, S., Haroutunian, V., Seeley, W. W., ... & Gan, L. (2010). Acetylation of tau inhibits its degradation and contributes to tauopathy. Neuron, 67(6), 953-966.

Miranda, L. F., Gomes, K. B., Silveira, J. N., Pianetti, G. A., Byrro, R., Peles, P. R., ... & Caramelli, P. (2015). Predictive factors of clinical response to cholinesterase inhibitors in mild and moderate Alzheimer's disease and mixed dementia: a one-year naturalistic study. Journal of Alzheimer's Disease, 45(2), 609-620.

Mohamed, T.; Yeung, J.C.K.; Vasefi, M.S.; Beazely, M.A.; Rao, P.P.N. Development and evaluation of multifunctional agents for potential treatment of Alzheimer’s disease: application to a pyrimidine- 2,4-diamine template. Bioorg. Med. Chem. Lett., 2012, 22(14), 4707-4712.

Mokhber-Dezfuli, N., Saeidnia, S., Gohari, A. R., & Kurepaz-Mahmoodabadi, M. (2014). Phytochemistry and pharmacology of berberis species. Pharmacognosy reviews, 8(15), 8.

Mondragón-Rodríguez, S., Perry, G., Zhu, X., Moreira, P. I., Acevedo-Aquino, M. C., and Williams, S. (2013). Phosphorylation of tau protein as the link between oxidative stress, mitochondrial dysfunction, and connectivity failure: implications for Alzheimer's disease. Oxidative Med. Cell. Longevity 2013:940603.

Moraga-nicolás, F. Rhodolirium andicola : a new renewable source of alkaloids with acetylcholinesterase inhibitory activity, a study from nature to molecular docking. Rev. Bras. Farmacogn., 2018, 28(1), 34-43.

Morley JE, Farr SA, Banks WA, et al. A physiological role for amyloid-beta protein: enhancement of learning and memory. J Alzheimers Dis 2010;19:441–9.

Morley, J. E., Farr, S. A., & Nguyen, A. D. (2018). Alzheimer disease. Clinics in geriatric medicine, 34(4), 591-601.

Morris JC, Blennow K, Froelich L, Nordberg A, Soini-nen H, Waldemar G, et al. Harmonized diagnostic criteriafor Alzheimer's disease: recommendations. J Intern Med.2014;275:204—13.9.

Morris, G.M.; Huey, R.; Lindstrom, W.; Sanner, M.F.; Belew, R.K.; Goodsell, D.S.; Olson, A.J. (2009) AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J. Comput. Chem., 30(16), 2785-2791.

Mosconi L, Tsui WH, Herholz K. Multicenter standardized 18F-FDG PET diagnosis of mild cognitive impairment, Alzheimer's disease, and other dementias. J Nucl Med 2008;49:390–8.

Moussa, C. E. (2017). Beta-secretase inhibitors in phase I and phase II clinical trials for Alzheimer's disease. Expert opinion on investigational drugs, 26(10), 1131-1136.

Msaada, K., Jemia, M. B., Salem, N., Bachrouch, O., Sriti, J., Tammar, S., ... & Marzouk, B. (2017). Antioxidant activity of methanolic extracts from three coriander (Coriandrum sativum L.) fruit varieties. Arabian Journal of Chemistry, 10, S3176-S3183.

Mukherjee, A., Sarkar, S., Jana, S., Swarnakar, S., Das, N., 2019. Neuro-protective role ofnanocapsulated curcumin against cerebral ischemia-reperfusion induced oxidativeinjury. Brain Res. 1704, 164–173

Murata, K., Matsumura, S., Yoshioka, Y., Ueno, Y., & Matsuda, H. (2015). Screening of β-secretase and acetylcholinesterase inhibitors from plant resources. Journal of natural medicines, 69(1), 123-129.

Murdolo, G., Piroddi, M., Luchetti, F., Tortoioli, C., Canonico, B., Zerbinati, C., ... & Iuliano, L. (2013). Oxidative stress and lipid peroxidation by-products at the crossroad between adipose organ dysregulation and obesity-linked insulin resistance. Biochimie, 95(3), 585-594.

Mushtaq, A., Anwar, R., & Ahmad, M. (2018). Lavandula stoechas (L) a very potent antioxidant attenuates dementia in scopolamine induced memory deficit mice. Frontiers in pharmacology, 9, 1375.

N

Naczk, M., & Shahidi, F. (2004). Extraction and analysis of phenolics in food. Journal of chromatography A, 1054(1-2), 95-111.

Nadda, R. K., Ali, A., Goyal, R. C., Khosla, P. K., & Goyal, R. (2020). Aucklandia costus (syn. Saussurea costus): Ethnopharmacology of an endangered medicinal plant of the Himalayan region. Journal of Ethnopharmacology, 113199.

Naderali, E. K., Ratcliffe, S. H., & Dale, M. C. (2009). Obesity and Alzheimer's disease: a link between body weight and cognitive function in old age. American Journal of Alzheimer's Disease & Other Dementias®, 24(6), 445-449.

Nasreddine, Z. S., Phillips, N. A., Bédirian, V., Charbonneau, S., Whitehead, V., Collin, I., Cummings, J. L., and Chertkow, H. (2005). The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. Journal of the American Geriatrics Society, 53(4), 695-699.

Nelson, P. T., Alafuzoff, I., Bigio, E. H., Bouras, C., Braak, H., Cairns, N. J., ... & Duyckaerts, C. (2012). Correlation of Alzheimer disease neuropathologic changes with cognitive status: a review of the literature. Journal of Neuropathology & Experimental Neurology, 71(5), 362-381.

Ngoungoure, V. L. N., Schluesener, J., Moundipa, P. F., & Schluesener, H. (2015). Natural polyphenols binding to amyloid: A broad class of compounds to treat different human amyloid diseases. Molecular nutrition & food research, 59(1), 8-20.

Nicholsn et al., Global, regional, and national burden of Alzheimer's disease and other dementias, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016, The Lancet Neurology,2019, 18 (1), 88-106.

Nisha, C. M., Kumar, A., Nair, P., Gupta, N., Silakari, C., Tripathi, T., & Kumar, A. (2016). Molecular docking and in silico ADMET study reveals acylguanidine 7a as a potential inhibitor of β-secretase. Advances in bioinformatics, 2016.

Noble, W., Planel, E., Zehr, C., Olm, V., Meyerson, J., Suleman, F., ... & Duff, K. (2005). Inhibition of glycogen synthase kinase-3 by lithium correlates with reduced tauopathy and degeneration in vivo. Proceedings of the national academy of sciences, 102(19), 6990-6995.

Nordberg, A., Ballard, C., Bullock, R., Darreh-Shori, T., & Somogyi, M. (2013). A review of butyrylcholinesterase as a therapeutic target in the treatment of Alzheimer’s disease. The primary care companion for CNS disorders, 15(2).

Nukina, N., and Ihara, Y. (1986). One of the antigenic determinants of paired helical filaments is related to tau protein. J. Biochem. 99, 1541–1544.

Nunes, R., & Carvalho, I. S. (2017). Potential application of Arbutus unedo L. leaf, fruit and flower extracts in the treatment of Alzheimer’s and Parkinson’s diseases. RICARDO JORGE DOS SANTOS NUNES, 193.

O

O’Connor, S. E. (2010). 1.25-Alkaloids. Comprehensive Natural Products II, 1, 977-1007.

Odontuya, G. (2016). Anti-oxidative, acetylcholinesterase and pancreatic lipase inhibitory activities of compounds from Dasiphora fruticosa, Myricaria alopecuroides and Sedum hybridum. Mongolian Journal of Chemistry, 17(43), 42-49

Oliveira, I., Coelho, V., Baltasar, R., Pereira, J. A., & Baptista, P. (2009). Scavenging capacity of strawberry tree (Arbutus unedo L.) leaves on free radicals. Food and Chemical Toxicology, 47(7), 1507-1511.

Onakpoya IJ, Heneghan CJ. The efficacy of supplementation with the novel medical food, Souvenaid, in patients with Alzheimer's disease: a systematic review and meta-analysis of randomized clinical trials. Nutr Neurosci 2017;20:219–27.

Orhan, I., Aslan, S., Kartal, M., Şener, B., & Hüsnü Can Başer, K. (2008). Inhibitory effect of Turkish Rosmarinus officinalis L. on acetylcholinesterase and butyrylcholinesterase enzymes. Food Chemistry, 108(2), 663–668.

Orhan, I., Kartal, M., Tosun, F., & Sener, B. (2007). Screening of various phenolic acidsand flavonoid derivatives for their anticholinesterase potential.Zeitschrift furNaturforschung C, Journal of Biosciences, 62(11–12), 829–832

Orozco, J. L. H., & Aguilera, R. A. B. (2011). Composition of labdane diterpenes extracted from andrographis paniculata, useful for the treatment of autoimmune diseases, and alzheimer disease by activation of ppr-gamma receptors, U.S. Patent No. 8,084,495. Washington, DC: U.S. Patent and Trademark Office.

Ortiz, J.E.; Berkov, S.; Pigni, N.B.; Theoduloz, C.; Roitman, G.; Tapia, A.; Bastida, J.; Feresin, G.E. Wild Argentinian Amaryllidaceae, a new renewable source of the acetylcholinesterase inhibitor galanthamine and other alkaloids. Molecules, 2012, 17(11), 13473- 13482.

Ortiz, J.E.; Garro, A.; Pigni, N.B.; Agüero, M.B.; Roitman, G.; Slanis, A.; Enriz, R.D.; Feresin, G.E.; Bastida, J.; Tapia, A. Cholinesterase-inhibitory effect and in silico analysis of alkaloids from bulbs of Hieronymiella species. Phytomedicine, 2018, 39, 66-74.

Ortiz, J.E.; Pigni, N.B.; Andujar, S.A.; Roitman, G.; Suvire, F.D.; Enriz, R.D.; Tapia, A.; Bastida, J.; Feresin, G.E. Alkaloids from Hippeastrum argentinum and Their Cholinesterase-Inhibitory Activities: An in Vitro and in Silico Study. J. Nat. Prod., 2016, 79(5), 1241-1248.

Ortiz, P.L.; Arista, M.; Talavera, S. Low reproductive success in two subspecies of Juniperus oxycedrus L. Int. J. Plant Sci., 1998, 159(5), 843-847

Osterlund T (2001). Structure-function relationships of hormone-sensitive lipase. Eur. J. Biochem., 268: 1899-1907

Ouldjaoui, A., Etude de la tolerance au glucose, insuline, cortisol, homocysteine et determination du polymorphisme des genes APOE et MTHFR dans la maladie

d'alzheimer etude de population : nord Constantinois. Théses de Doctorat, Université Mahmoud Mentouri de Constantine, 2011.

Özdemir, H. (2016). Dyeing Properties of Natural Dyes Extracted from the Junipers Leaves (J. excelsa Bieb. and J. oxycedrus L.). Journal of Natural Fibers, 14(1), 134–142.

Öztürk, M., Tümen, İ., Uğur, A., Aydoğmuş-Öztürk, F., & Topçu, G. (2011). Evaluation of fruit extracts of six Turkish Juniperus species for their antioxidant, anticholinesterase and antimicrobial activities. Journal of the Science of Food and Agriculture, 91(5), 867-876.

P

P. Shaw, M. T. Ho, K. Fung, D. C. Wan, Use of transhinone derivates as cholinesterase inhibitors in treating related diseases, U.S. Pat. Appl. Publ. US 20040191334, 2004

P. Shaw, M. T. Ho, K. Fung, D. C. Wan, Use of tanshinone derivates as cholinesterase inhibitors in treating related diseases, Faming Zhuanli Shenqing CN 1764447, 2006

P.K. Chanda, Y. Gao, L. Mark, J. Btesh, B.W. Strassle, P. Lu, et al., Monoacylglycerol lipase activity is a critical modulator of the tone and integrity of the endocannabinoid system, Mol Pharmacol, 78 (2010), pp. 996-1003.

Paduch, R., Kandefer-Szerszeń, M., Trytek, M., & Fiedurek, J. (2007). Terpenes: substances useful in human healthcare. Archivum immunologiae et therapiae experimentalis, 55(5), 315.

Pandey, A., Bigoniya, P., Raj, V., & Patel, K. K. (2011). Pharmacological screening of Coriandrum sativum Linn. for hepatoprotective activity. Journal of Pharmacy and Bioallied sciences, 3(3), 435.

Pandey, M. M., Rastogi, S., & Rawat, A. K. S. (2007). Saussurea costus: botanical, chemical and pharmacological review of an ayurvedic medicinal plant. Journal of ethnopharmacology, 110(3), 379-390.

Panikashvili, D., Mechoulam, R., Beni, S. M., Alexandrovich, A., & Shohami, E. (2005). CB1 cannabinoid receptors are involved in neuroprotection via NF-κB inhibition. Journal of Cerebral Blood Flow & Metabolism, 25(4), 477-484.

Panikashvili, D., Simeonidou, C., Ben-Shabat, S., Hanuš, L., Breuer, A., Mechoulam, R., & Shohami, E. (2001). An endogenous cannabinoid (2-AG) is neuroprotective after brain injury. Nature, 413(6855), 527-531.

Paresce DM, Ghosh RN, Maxfi eld FR. Microglial cells internalize aggregates of the Alzheimer's disease amyloid beta-protein via a scavenger receptor. Neuron 1996; 17: 553–65.

Pasquier F. Early diagnosis of dementia: neuropsychology. J Neurol. 1999 Jan; 246(1): 6-15.

Patil, S., & Chan, C. (2005). Palmitic and stearic fatty acids induce Alzheimer-like hyperphosphorylation of tau in primary rat cortical neurons. Neuroscience letters, 384(3), 288-293.

Pavlović, R. D., Lakušićć, B., Došlov-Kokoruš, Z., & Kovačević, N. (2009). Arbutin content and antioxidant activity of some Ericaceae species. Die Pharmazie-An International Journal of Pharmaceutical Sciences, 64(10), 656-659.

Perlmutter LS, Barron E, Chui HC. Morphologic association between microglia and senile plaque amyloid in Alzheimer disease. Neurosci Lett 1990;119:32-36.

Petersson SD, Philippou E. Mediterranean diet. Cognitive function, and dementia: a systematic review of the evidence. Adv Nutr 2016;7:889–904.

Petursdottir AL, Farr SA, Morley JE, et al. Effect of dietary n-3 polyunsaturated fatty acids on brain lipid fatty acid composition, learning ability, and memory of senescence-accelerated mouse. J Gerontol A Biol Sci Med Sci 2008;63:1153–60.

Pfeiffer AFH (2007). Adipose tissue and diabetes therapy: do we hit the target? Horm. Metab. Res., 39: 734-738.

Pilotto, A., Franceschi, M., D'Onofrio, G., Bizzarro, A., Mangialasche, F., Cascavilla, L., Paris, F.; Matera, M.G.; Pilotto, A.; Daniele, A.; Mecocci, P.; Masullo, C.; Dallapiccola, B. & Seripa, D. (2009). Effect of a CYP2D6 polymorphism on the efficacy of donepezil in patients with Alzheimer disease. Neurology, 73(10), 761-767.

Pluta, R., Ułamek-Kozioł, M., Czuczwar, S., 2018. Neuroprotective and neurological/cognitive enhancement effects of curcumin after brain ischemia injury withAlzheimer's disease phenotype. Int. J. Mol. Sci. 19 (12), 4002

Politeo, O., & Ercegović, I. (2019) Chemical composition and acetylcholinesterase inhibition activity of volatile oil from leaves of Arbutus unedo L. from Croatia. Asian journal of chemistry, 31(5), 1110-1112.

PONZONI, Ignacio. QSAR Classification Models for Predicting the Activity of Inhibitors of Beta-Secretase (BACE1) Associated with Alzheimer's Disease. Scientific Reports, 2019, 9.1, 9102.

Postic, C., & Girard, J. (2008). Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. The Journal of clinical investigation, 118(3), 829-838.

Prashanth, G. K., & Krishnaiah, G. M. (2014). Chemical composition of the leaves of Azadirachta indica Linn (Neem). International Journal of Advancement in Engineering and Technology, Management and Applied Science, 1(3), 21-31.

Praticò, D. (2008). Oxidative stress hypothesis in Alzheimer's disease: a reappraisal. Trends in Pharmacological Sciences, 29(12), 609–615.

Prince, Martin; Wimo, Anders ; Guerchet, Maelenn; Ali, Gemma-Claire; Wu, Yu-Tzu; Prina M. World Alzheimer Report 2015: The Global Impact of Dementia: An analysis of prevalence, incidence, cost and trends. Available from: https://www.alz.co.uk/research/world-report-2015 (accessed Jan 3, 2017)

Prinz M, Priller J, Sisodia SS, Ransohoff RM. Heterogeneity of CNS myeloid cells and their roles in neurodegeneration. Nat. Neurosci. 2011;14:1227–1235.

Przedborski S, Vila M, Jackson-Lewis V. Neurodegeneration: what is it and where are we? J Clin Invest 2003;111(1):3-10

R

Rahimi-Madiseh, M., Lorigoini, Z., Zamani-Gharaghoshi, H., & Rafieian-Kopaei, M. (2017). Berberis vulgaris: specifications and traditional uses. Iranian Journal of Basic Medical Sciences, 20(5), 569.

Rajaian, H., & Toranjipour, H. (2002). Antibacterial effects of the root of Berberis vulgaris. Iranian J Vet Res, 3, 175-178.

Ramírez, D.; Caballero, J. Is It Reliable to Take the Molecular Docking Top Scoring Position as the Best Solution without Considering Available Structural Data? Molecules, 2018, 23(5), 1-17

Rao, B.R., P.N. Kaul, K.V. Syamasundar and S. Ramesh. Water soluble fractions of rose scented geranium (Pelargonium species) essential oil. Bioresource Technol., 2002; 84: 243-246.

Rasmusson, D. X., Brandt, J., Martin, D. B., & Folstein, M. F. (1995). Head injury as a risk factor in Alzheimer's disease. Brain Injury, 9(3), 213-219.

Rathore S, Habes M, Iftikhar MA, et al. A review on neuroimaging-based classification studies and associated feature extraction methods for Alzheimer's disease and its prodromal stages. Neuroimage 2017;155:530–48

Ray, W. J., & Buggia-Prevot, V. (2021). Novel Targets for Alzheimer's Disease: A View Beyond Amyloid. Annual Review of Medicine, 72.

Riedel, B. C., Thompson, P. M., & Brinton, R. D. (2016). Age, APOE and sex: triad of risk of Alzheimer's disease. The Journal of steroid biochemistry and molecular biology, 160, 134-147.

Rocha, L. G., Almeida, J. R. G. S., Macedo, R. O., & Barbosa-Filho, J. M. (2005). A review of natural products with antileishmanial activity. Phytomedicine, 12(6-7), 514-535.

Rogers J, Mastroeni D, Leonard B, Joyce J, Grover A. 2007. Neuroinflammation in Alzheimer's disease and Parkinson's disease: Are microglia pathogenic in either disorder? Int Rev Neurobiol 82: 235–246.

Rosendorff, C., Beeri, M. S., & Silverman, J. M. (2007). Cardiovascular risk factors for Alzheimer's disease. The American journal of geriatric cardiology, 16(3), 143-149.

S

Saario, S. M., Salo, O. M., Nevalainen, T., Poso, A., Laitinen, J. T., Järvinen, T., & Niemi, R. (2005). Characterization of the sulfhydryl-sensitive site in the enzyme responsible for hydrolysis of 2-arachidonoyl-glycerol in rat cerebellar membranes. Chemistry & biology, 12(6), 649-656.

Sabnis, R. W. (2018). Patents on Natural Products for Diagnosing/Preventing/Treating Alzheimer's Disease. Studies in Natural Products Chemistry, 85–141.

Saha, S., & Pal, D. (2021). Elicitor Signal Transduction Leading to the Production of Plant Secondary Metabolites. In Bioactive Natural Products for Pharmaceutical Applications (pp. 1-39). Springer, Cham.

Sakono, M., & Zako, T. (2010). Amyloid oligomers: formation and toxicity of Aβ oligomers. The FEBS journal, 277(6), 1348-1358.

Samadi, A.; de los Ríos, C.; Bolea, I.; Chioua, M.; Iriepa, I.; Moraleda, I.; Bartolini, M.; Andrisano, V.; Gálvez, E.; Valderas, C.; Unzeta, M.; Marco-Contelles, J. Multipotent MAO and cholinesterase inhibitors for the treatment of Alzheimer's disease: synthesis, pharmacological analysis and molecular modeling of heterocyclicsubstituted alkyl and cycloalkyl propargyl amine. Eur. J. Med. Chem., 2012, 52, 251-262.

Šamec, D.; Salopek-Sondi, B. Cruciferous (Brassicaceae) Vegetables; Nonvitamin Nonmineral Nutr. Suppl, 2019, pp. 195-202.

Sánchez, A. J., & Garcia-Merino, A. (2012). Neuroprotective agents: cannabinoids. Clinical immunology, 142(1), 57-67.

Sando, S. B., Melquist, S., Cannon, A., Hutton, M. L., Sletvold, O., Saltvedt, I., ... & Aasly, J. O. (2008). APOE ε4 lowers age at onset and is a high risk factor for Alzheimer's disease; A case control study from central Norway. BMC neurology, 8(1), 9.

Santamaría, T. Z., Gómez, P. Y., Galindo, I. F., González, M. G., Vázquez, A. O., & López, M. L. (2020). Pharmacogenetic studies in Alzheimer disease. Neurología (English Edition).

Scheltens, N.M., Galindo-Garre, F., Pijnenburg, Y.A., van der Vlies, A.E., Smits, L.L., Koene, T., Teunissen, C.E., Barkhof, F., Wattjes, M.P., Scheltens, P. and van der Flier, W.M., 2016. The identification of cognitive subtypes in Alzheimer's disease dementia using latent class analysis. Journal of Neurology, Neurosurgery & Psychiatry, 87(3), pp.235-243.

Scheltens, P, Blennow, K, Breteler, MMB, et al. Alzheimer's disease. Lancet. 2016;388:505-517.

Schilling, J. V., Schillheim, B., Mahr, S., Reufer, Y., Sanjoyo, S., Conrath, U., & Büchs, J. (2015). Oxygen transfer rate identifies priming compounds in parsley cells. BMC plant biology, 15(1), 1-11.

Schlosburg, J. E., Blankman, J. L., Long, J. Z., Nomura, D. K., Pan, B., Kinsey, S. G., ... & Cravatt, B. F. (2010). Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system. Nature neuroscience, 13(9), 1113-1119.

Schneider, L. S. (2001). Treatment of Alzheimer's disease with cholinesterase inhibitors. Clinics in geriatric medicine, 17(2), 337-358.

Schneider, L.S. Treatment of Alzheimer's disease with cholinesterase inhibitors. Clin. Geriatr. Med., 2001, 17(2), 337-358.

Schwab, W., Davidovich-Rikanati, R., & Lewinsohn, E. (2008). Biosynthesis of plant-derived flavor compounds. The plant journal, 54(4), 712-732.

Scotter, E. L., Abood, M. E., & Glass, M. (2010). The endocannabinoid system as a target for the treatment of neurodegenerative disease. British journal of pharmacology, 160(3), 480-498.

Selkoe, D.J.; Hardy, J. The amyloid hypothesis of Alzheimer's disease at 25 years.EMBO Mol. Med.2016,8, 608–655

Sengupta, U., Nilson, A. N., & Kayed, R. (2016). The role of amyloid-β oligomers in toxicity, propagation, and immunotherapy. EBioMedicine, 6, 42-49.

Sennvik, K.; Fastbom, J.; Blomberg, M.; Wahlund, L.O.; Winblad, B.; Benedikz, E. Levels of α- and β-secretase cleaved amyloid precursor protein in the cerebrospinal fluid of Alzheimer's disease patients. Neurosci. Lett., 2000, 278(3), 169-172.

Şenol, F. S., Orhan, I., Yilmaz, G., Cicek, M., & Şener, B. (2010). Acetylcholinesterase, butyrylcholinesterase, and tyrosinase inhibition studies and antioxidant activities of 33 Scutellaria L. taxa from Turkey. Food and chemical toxicology, 48(3), 781-788.

Serseg, T., Benarous, K., Lamrani, M., & Yousfi, M. (2020). Lepidine B from Lepidium Sativum Seeds as Multi-Functional Anti-Alzheimer's Disease Agent: In Vitro and In Silico Studies. Current computer-aided drug design.

Serseg, T., & Benarous, K. (2018). The inhibitory effect of some drugs on Candida rugosa lipase and human pancreatic lipase: in vitro and in silico studies. Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders), 18(6), 602-609.

Seubert, P., Oltersdorf, T., Lee, M. G., Barbour, R., Blomquist, C., Davis, D. L., Bryant, K.; Fritz, L.C.; Galasko, D.; Thal, L.J. & Schenk, D. B. (1993). Secretion of β-amyloid precursor protein cleaved at the amino terminus of the β-amyloid peptide. Nature, 361(6409), 260-263.

Shadel GS, and Horvath TL. Mitochondrial ROS signaling in organismal homeostasis. Cell 163: 560-569, 2015

Sharma, K. (2019). Cholinesterase inhibitors as Alzheimer's therapeutics. Molecular medicine reports, 20(2), 1479-1487.

Shastry BS. Neurodegenerative disorders of protein aggregation. Neurochem Int 2003;43(1):1-7.

Sheng, W. D., Jiddawi, M. S., Hong, X. Q., & Abdulla, S. M. (1997). Treatment of chloroquine-resistant malaria using pyrimethamine in combination with berberine, tetracycline or cotrimoxazole. East African medical journal, 74(5), 283-284.

Sherrington R, Rogaev EI, Liang Y, Rogaeva EA, Levesque G, Ikeda M, Chi H, Lin C, Li G, Holman K, Tsuda T, Mar L, Foncin JF, Bruni AC, Montesi MP, Sorbi S, Rainero I, Pinessi L, Nee L, Chumakov I, Pollen D, Brookes A, Sanseau P, Polinsky RJ, Wasco W, Da Silva HA, Haines JL, Perkicak-Vance MA, Tanzi RE, Roses AD, Fraser PE, Rommens JM, St George-Hyslop PH (1995) Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. Nature 375, 754–760.

Shi H., Zhang G., Wu J., Application of gypensapogenin A to Alzheimer disease treatment drugs, Faming Zhuanli Shenqing CN 102872050, 2013a.

Shinosaki, K., Nishikawa, T., & Takeda, M. (2000). Neurobiological basis of behavioral and psychological symptoms in dementia of the Alzheimer type. Psychiatry and Clinical Neurosciences, 54(6), 611-620.

Shukla, A.; Singh, C.S.; Bigoniya, P. Phytochemical and CNS activity of Lepidium sativum Linn. seeds total alkaloid. Der Pharm. Lett., 2011, 3(2), 226-237.

Siddiqui, S., Verma, A., Rather, A. A., Jabeen, F., & Meghvansi, M. K. (2009). Preliminary phytochemicals analysis of some important medicinal and aromatic plants. Advances in biological research, 3(5-6), 188-195.

Sikorska, K., Grądzka, I., Wasyk, I., Brzóska, K., Stępkowski, T. M., Czerwińska, M., & Kruszewski, M. K. (2020). The Impact of Ag Nanoparticles and

CdTe Quantum Dots on Expression and Function of Receptors Involved in Amyloid-β Uptake by BV-2 Microglial Cells. Materials, 13(14), 3227.

Simard, J., 2012. Collaboration haptique étroitement couplée pour la déformation moléculaire interactive, thèse de doctorat, Université Paris-Sud, 263.

Singh, P. P., Singh, M., & Mastana, S. S. (2006). APOE distribution in world populations with new data from India and the UK. Annals of human biology, 33(3), 279-308.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture, 16(3), 144-158.

Sinha, S., Anderson, J. P., Barbour, R., Basi, G. S., Caccavello, R., Davis, D., Doan, M.; Dovey, H.F.; Frigon, N.; Hong, J.; Jacobson- Croak, K.; Jewett, N.; Keim, P.; Knops, J.; Lieberburg, I.; Power, M.; Tan, H.; Tatsuno, G.; Tung, J.; Schenk, D.; Seubert, P.; Suomensaari, S.M.; Wang, S.; Walker, D.; Zhao, J.; McConlogue, L. & John, V. (1999). Purification and cloning of amyloid precursor protein β-secretase from human brain. Nature, 402(6761), 537-540.

Small, D. H., & McLean, C. A. (1999). Alzheimer's Disease and the Amyloid Protein: What Is the Role of Amyloid?. Journal of neurochemistry, 73, 443-449.

Smith D, Refsum H, Oulhaj A, de Jager CA, Jerneren F. Beneficial interactions between Bvitamins and omega-3 fatty acids in the prevention of brain atrophy and of cognitivedecline in early stage Alzheimer's disease. FASEB J. 30(1): 407–6 (2016)

Smith, S. M., Struyk, A., Jonathan, D., Declercq, R., Marcus, J., Toolan, D., ... & Schoepp, D. (2016). O2-13-04: Early Clinical Results and Preclinical Validation of the O-Glcnacase (OGA) Inhibitor Mk-8719 as a Novel Therapeutic for the Treatment of Tauopathies. Alzheimer's & Dementia, 12, P261-P261.

Speck, C. E., Kukull, W. A., Brenner, D. E., Bowen, J. D., McCormick, W. C., Teri, L., ... & Larson, E. B. (1995). History of depression as a risk factor for Alzheimer's disease. Epidemiology, 366-369.

Stasiulewicz, A., Znajdek, K., Grudzień, M., Pawiński, T., & Sulkowska, J. I. (2020). A guide to targeting the endocannabinoid system in drug design. International journal of molecular sciences, 21(8), 2778.

Steiner, H., Fukumori, A., Tagami, S., & Okochi, M. (2018). Making the final cut: pathogenic amyloid-β peptide generation by γ-secretase. Cell Stress, 2(11), 292.

Sugimoto, H.; Ogura, H.; Arai, Y.; Limura, Y.; Yamanishi, Y. Research and development of donepezil hydrochloride, a new type of acetylcholinesterase inhibitor. Jpn. J. Pharmacol., 2002, 89(1), 7-20.

Sulkava, R. A. I. M. O., Haltia, M. A. T. T. I., Paetau, A. N. D. E. R. S., Wikström, J., & Palo, J. O. R. M. A. (1983). Accuracy of clinical diagnosis in primary degenerative dementia: correlation with neuropathological findings. Journal of Neurology, Neurosurgery & Psychiatry, 46(1), 9-13.

Sun, W., Lu, H., Lyu, L., Yang, P., Lin, Z., Li, L., ... & Lu, D. (2019). Gastrodin ameliorates microvascular reperfusion injury–induced pyroptosis by regulating the NLRP3/caspase-1 pathway. Journal of physiology and biochemistry, 75(4), 531-547.

Sussman, J. L., Harel, M., Frolow, F., Oefner, C., Goldman, A., Toker, L., & Silman, I. (1991). Atomic structure of acetylcholinesterase from Torpedo californica: a prototypic acetylcholine-binding protein. Science, 253(5022), 872-879.

Swahn, B.-M., Kolmodin, K., Karlström, S., von Berg, S., Söderman, P., Holenz, J., … Fälting, J. (2012). Design and Synthesis of β-Site Amyloid Precursor Protein Cleaving Enzyme (BACE1) Inhibitors with in Vivo Brain Reduction of β-Amyloid Peptides. Journal of Medicinal Chemistry, 55(21), 9346–9361.

Syarifah-Noratiqah S, Naina-Mohamed I, Zulfarina MS, et al. Natural polyphenols in the treatment of Alzheimer’s disease. Curr Drug Targets 2017.

Systèmes, D. (2016). Biovia, discovery studio modeling environment. Dassault Systèmes Biovia: San Diego, CA, USA.

Szczechowiak, K., Diniz, B. S., & Leszek, J. (2019). Diet and Alzheimer's dementia–Nutritional approach to modulate inflammation. Pharmacology Biochemistry and Behavior, 184, 172743.

Szecsi PB. The aspartic proteases. Scand J Clin Lab Invest Suppl. 1992;210:5–22

T

Taghipour, P.; Zakariazadeh, M.; Sharifi, M.; Dolatabadi, J. E. N.; Barzegar, A. Bovine serum albumin binding study to erlotinib using surface plasmon resonance and molecular docking methods, J. Photochem. Photobiol. B Biol, 2018

Tan, M. S., Yu, J. T., Jiang, T., Zhu, X. C., & Tan, L. (2013). The NLRP3 inflammasome in Alzheimer's disease. Molecular neurobiology, 48(3), 875-882.

Tarkowski E, Andreasen N, Tarkowski A, Blennow K. Intrathecal inflammation precedes development of Alzheimer's disease. J Neurol Neurosurg Psychiatry 2003; 74: 1200–05.

Tavares, L., McDougall, G. J., Fortalezas, S., Stewart, D., Ferreira, R. B., & Santos, C. N. (2012). The neuroprotective potential of phenolic-enriched fractions from four Juniperus species found in Portugal. Food chemistry, 135(2), 562-570.

Taylor, S. L., & Hefle, S. L. (2017). Naturally occurring toxicants in foods. In Foodborne Diseases (pp. 327-344). Academic Press.

Tel, G., Apaydın, M., Duru, M. E., & Öztürk, M. (2012). Antioxidant and cholinesterase inhibition activities of three Tricholoma species with total phenolic and flavonoid contents: the edible mushrooms from Anatolia. Food Analytical Methods, 5(3), 495-504.

Terry, R.D.; Davies, P. Dementia of the Alzheimer type. Annu. Rev. Neurosci., 1980, 3(1), 77-95.

Todd, W. D. (2020). Potential Pathways for Circadian Dysfunction and Sundowning-Related Behavioral Aggression in Alzheimer's Disease and Related Dementias. Frontiers in neuroscience, 14, 910.

Tomlinson, BE. The pathology of dementia. Contemp. Neurol. Ser. 1977. 15:113-153.

Tramutola, A., Lanzillotta, C., Di Domenico, F., Head, E., Butterfield, D. A., Perluigi, M., & Barone, E. (2020). Brain insulin resistance triggers early onset Alzheimer disease in Down syndrome. Neurobiology of Disease, 137, 104772.

Trease, G. E. (1989). Trease and Evans. Pharmacognosy, A Physician's Guide to Herbal Medicine, Bailliere Tindall, London, 13, 912.

U

Uddin, M. S., & Kabir, M. T. (2019). Biological, Diagnostic and Therapeutic Advances in Alzheimer's Disease.a

Uddin, M. S., & Kabir, M. T. (2019). Oxidative stress in Alzheimer's disease: molecular hallmarks of underlying vulnerability. In Biological, Diagnostic and Therapeutic Advances in Alzheimer's Disease (pp. 91-115). Springer, Singapore.b

Umukoro, S., Kalejaye, H. A., Ben-Azu, B., & Ajayi, A. M. (2018). Naringenin attenuates behavioral derangements induced by social defeat stress in mice via inhibition of acetylcholinesterase activity, oxidative stress and release of pro-inflammatory cytokines. Biomedicine & Pharmacotherapy, 105, 714–723. doi:10.1016/j.biopha.2018.06.016

V

Van Cauwenberghe, C., Van Broeckhoven, C., & Sleegers, K. (2016). The genetic landscape of Alzheimer disease: clinical implications and perspectives. Genetics in Medicine, 18(5), 421-430.

Van Furth R. Current view on the mononuclear phagocyte system. Immunobiology 1982;161:178-185. 17.

VandeVrede, L., Boxer, A. L., & Polydoro, M. (2020). Targeting tau: Clinical trials and novel therapeutic approaches. Neuroscience Letters, 134919.

Vassar, R. (2014). BACE1 inhibitor drugs in clinical trials for Alzheimer's disease. Alzheimer's research & therapy, 6(9), 89.

Vassar, R., Bennett, B. D., Babu-Khan, S., Kahn, S., Mendiaz, E. A., Denis, P., Luo, Y. ... & Citron, M. (1999). β-Secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. science, 286(5440), 735-741.

Velderrain-Rodríguez, G.R.; Palafox-Carlos, H.; Wall-Medrano, A.; AyalaZavala, J.F.; Chen, C.-Y.O.; Robles-Sanchez, M.; Astiazaran-García, H.; Alvarez-Parrilla, E.; González-Aguilar, G.A. Phenolic compounds: Their journey after intake. Food Funct. 2014, 5, 189–197.

Verpoorte, R. (2005). ALKALOIDS. Encyclopedia of Analytical Science, 56–61.

Videira PA, and Castro-Caldas M. Linking Glycation and Glycosylation With Inflammation and Mitochondrial Dysfunction in Parkinson's Disease. Front Neurosci12: 381, 2018.

Vigo-Pelfrey, C. (1998). The use of celastrol to treat Alzheimer's disease. International Patent Classification WO, 98(25573), 18.

Virk, S. A., & Eslick, G. D. (2015). Occupational exposure to aluminum and Alzheimer disease: a meta-analysis. Journal of occupational and environmental medicine, 57(8), 893-896.

W

Walker LC, Pahnke J, Madauss M, et al. Apolipoprotein E4 promotes the early deposition of Aβ42 and then Aβ40 in the elderly. Acta Neuropathol. 2000;100(1):36-42.

Wang, B., Zhong, Y., Gao, C., & Li, J. (2017). Myricetin ameliorates scopolamine-induced memory impairment in mice via inhibiting acetylcholinesterase and down-regulating brain iron. Biochemical and Biophysical Research Communications, 490(2), 336–342.

Wang, X., Wang, W., Li, L., Perry, G., Lee, H. G., & Zhu, X. (2014). Oxidative stress and mitochondrial dysfunction in Alzheimer's disease. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1842(8), 1240-1247.

Wangensteen, H., Samuelsen, A. B., & Malterud, K. E. (2004). Antioxidant activity in extracts from coriander. Food chemistry, 88(2), 293-297.

Waqar, M., & Batool, S. (2017). In silico analysis of binding interaction of conantokins with NMDA receptors for potential therapeutic use in Alzheimer's disease. Journal of Venomous Animals and Toxins including Tropical Diseases, 23(1), 42.

Westfall, T. C. (2009). Cholinergic neurotransmission in the autonomic and somatic motor nervous system.

Wharton SB et al. Epidemiological pathology of Tau in the ageing brain: application of staging for neuropil threads (BrainNet Europe protocol) to the MRC cognitive function and ageing brain study. Acta Neuropathol. Commun 4, 11 (2016).

WHO, "Dementia," WHO. [Online]. Available: https://www.who.int/news-room/factsheets/detail/dementia[Accessed: 26-Dec-2018]

WHO. Available from: https://www.who.int/news-room/fact-sheets/detail/dementia (accessed Jan 3, 2017)

Wilson, D. M., & Binder, L. I. (1997). Free fatty acids stimulate the polymerization of tau and amyloid beta peptides. In vitro evidence for a common effector of pathogenesis in Alzheimer's disease. The American journal of pathology, 150(6), 2181.

Wong, P. Y., & Kitts, D. D. (2006). Studies on the dual antioxidant and antibacterial properties of parsley (Petroselinum crispum) and cilantro (Coriandrum sativum) extracts. Food chemistry, 97(3), 505-515.

Wyss-Coray, T., & Rogers, J. (2012). Inflammation in Alzheimer disease—a brief review of the basic science and clinical literature. Cold Spring Harbor perspectives in medicine, 2(1), a006346.

X

Xia C, Makaretz SJ, Caso C, et al. Association of in vivo [18F]AV-1451 Tau PET imaging results with cortical atrophy and symptoms in typical and atypical Alzheimer disease. JAMA Neurol 2017;74:427–36.

Xie S, Xiao JX, Gong GL, et al. Voxel-based detection of white matter abnormalities in mild Alzheimer disease. Neurology 2006;66:1845–9

Xu, W. L., von Strauss, E., Qiu, C. X., Winblad, B., & Fratiglioni, L. (2009). Uncontrolled diabetes increases the risk of Alzheimer's disease: a population-based cohort study. Diabetologia, 52(6), 1031.

Y

Yaglioglu, A. S., & Eser, F. (2017). Screening of some Juniperus extracts for the phenolic compounds and their antiproliferative activities. South African Journal of Botany, 113, 29-33.

Yan, R. (2016). Stepping closer to treating Alzheimer's disease patients with BACE1 inhibitor drugs. Translational neurodegeneration, 5(1), 13.

Yan, R., & Vassar, R. (2014). Targeting the β secretase BACE1 for Alzheimer's disease therapy. The Lancet Neurology, 13(3), 319–329.

Yan, R., Bienkowski, M. J., Shuck, M. E., Miao, H., Tory, M. C., Pauley, A. M., Brashier, J.R.; Stratman, N.C.; Mathews, W.R.; Buhl, A.E.; Carter, D.B.;

Tomasselli, A.G.; Parodi, L.A.; Heinrikson, R.L.; & Gurney, M. E. (1999). Membrane-anchored aspartyl protease with Alzheimer's disease β-secretase activity. Nature, 402(6761), 533-537

Yan, X., Chen, T., Zhang, L., & Du, H. (2018). Study of the interactions of forsythiaside and rutin with acetylcholinesterase (AChE). International Journal of Biological Macromolecules, 119, 1344–1352.

Yang, H. J., Kim, M. J., Kang, S., Moon, N. R., Kim, D. S., Lee, N. R., … Park, S. (2017). Topical treatments of Saussurea costus root and Thuja orientalis L. synergistically alleviate atopic dermatitis-like skin lesions by inhibiting protease-activated receptor-2 and NF-κB signaling in HaCaT cells and Nc/Nga mice. Journal of Ethnopharmacology, 199, 97–105.

Yang, H.; Wong, M.W. Oxyanion Hole Stabilization by C-H ··· O Interaction in Transition State - A Three-Point Interaction Model for Cinchona Alkaloid-Catalyzed Asymmetric Methanolysis of Meso-Cyclic Anhydrides Oxyanion Hole Stabilization by C – H ••• O Interaction in Transit. J. Am. Chem. Soc., 2013, 1-32.

Yang, L., Wen, K. S., Ruan, X., Zhao, Y. X., Wei, F., & Wang, Q. (2018). Response of plant secondary metabolites to environmental factors. Molecules, 23(4), 762.

Yazaki, K., Arimura, G. I., & Ohnishi, T. (2017). 'Hidden'terpenoids in plants: their biosynthesis, localization and ecological roles. Plant and Cell Physiology, 58(10), 1615-1621.

Yeung, A. W. K., Tzvetkov, N. T., Georgieva, M. G., Ognyanov, I. V., Kordos, K., Jóźwik, A., ... & Atanasov, A. G. (2020). Reactive Oxygen Species and Their Impact in Neurodegenerative Diseases: Literature Landscape Analysis. Antioxidants & Redox Signaling.

Yiannopoulou KG, Papageorgiou SG. Current and future treatments for Alzheimer's disease. Ther Adv Neurol Disord. 2013;6:19-33.

Yiannopoulou, K. G., & Papageorgiou, S. G. (2020). Current and Future Treatments in Alzheimer Disease: An Update. Journal of Central Nervous System Disease, 12, 1179573520907397.

Yiannopoulou, KG, Papageorgiou, SG. Current and future treatments for Alzheimer's disease. Ther Adv Neurol Disord. 2013;6:19-33.

Z

Zarei, A., Changizi-Ashtiyani, S., Taheri, S., & Ramezani, M. (2015). A quick overview on some aspects of endocrinological and therapeutic effects of Berberis vulgaris L. Avicenna journal of phytomedicine, 5(6), 485.

Zechner, R., Kienesberger, P. C., Haemmerle, G., Zimmermann, R., & Lass, A. (2009). Adipose triglyceride lipase and the lipolytic catabolism of cellular fat stores. Journal of lipid research, 50(1), 3-21.

Zhang, J., & Chen, C. (2008). Endocannabinoid 2-arachidonoylglycerol protects neurons by limiting COX-2 elevation. Journal of Biological Chemistry, 283(33), 22601-22611.

Zhang, X., Rakesh, K. P., Bukhari, S. N. A., Balakrishna, M., Manukumar, H. M., & Qin, H. L. (2018). Multi-targetable chalcone analogs to treat deadly Alzheimer's disease: Current view and upcoming advice. Bioorganic chemistry, 80, 86-93.

Zhang, Y., Zhao, Y., Zhang, J., & Yang, G. (2020). Mechanisms of NLRP3 inflammasome activation: its role in the treatment of Alzheimer's disease. Neurochemical Research, 1-13.

Zhang, Y.; Kua, J.; McCammon, J.A. Role of the catalytic triad and oxyanion hole in acetylcholinesterase catalysis: an ab initio QM/MM study. J. Am. Chem. Soc., 2002, 124(35), 10572-10577.

Zhao Q., Leng Y., Deng X., Li M., Zhao Y., Shen Y., Jun H., Kaurene diterpene derivative, medicinal composition thereof and application of kaurene diterpene derivative to medicament, Faming Zhuanli Shenqing CN 102603519, 2012

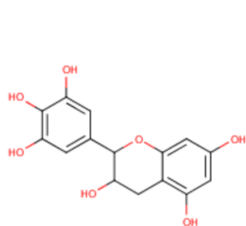
Živić, N., Milošević, S., Dekić, V., Dekić, B., Ristić, N., Ristić, M., & Sretić, L. (2019). Phytochemical and antioxidant screening of some extracts of Juniperus communis L. and Juniperus oxycedrus L. Czech Journal of Food Sciences, 37(5), 351-358.

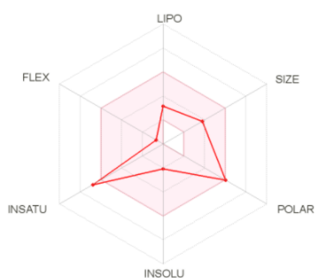
Zoubi Ez. Y., Farah, A., Zaroual, H., & Lalami, A. E. O. (2020). Antimicrobial activity of Lavandula stoechas phenolic extracts against pathogenic bacteria isolated from a hospital in Morocco. Vegetos, 33(4), 703-711

Appendices

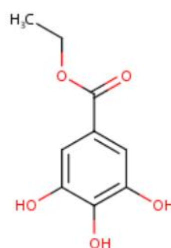
APPENDICES n1

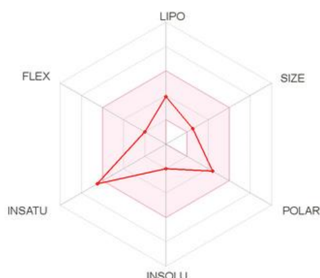
A. unedo molecules



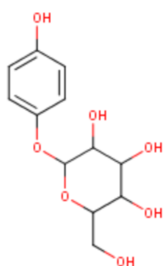


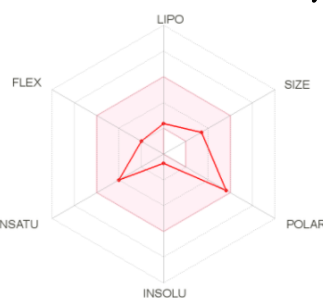
Gallocatechin





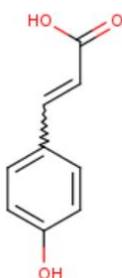
Ethyl gallate

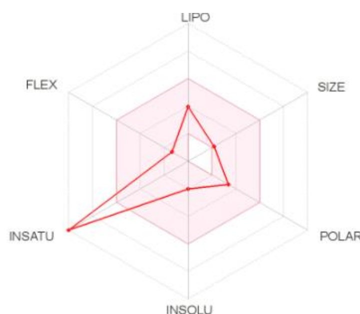




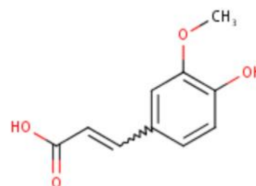
Arbutin

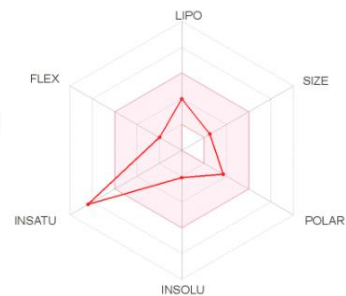
C. sativum molecules



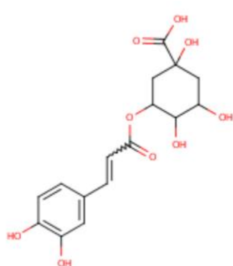


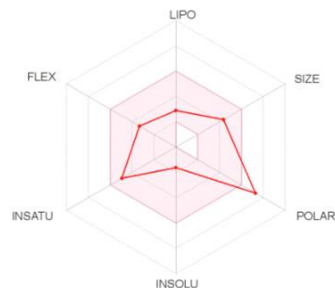
P-coumaric acid



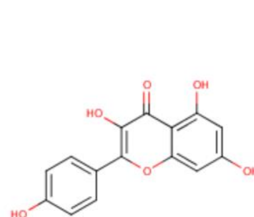


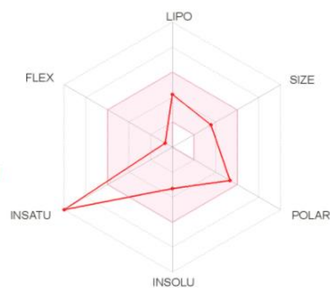
ferulic acid



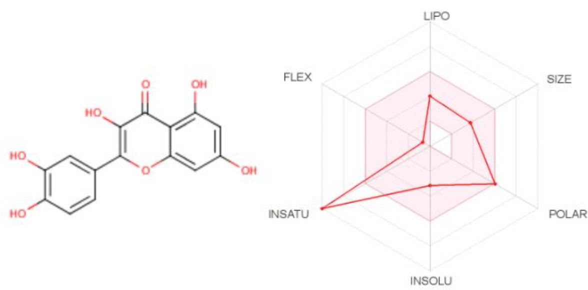


3-O-caffeoylquinic acid (Chlorogenic acid)

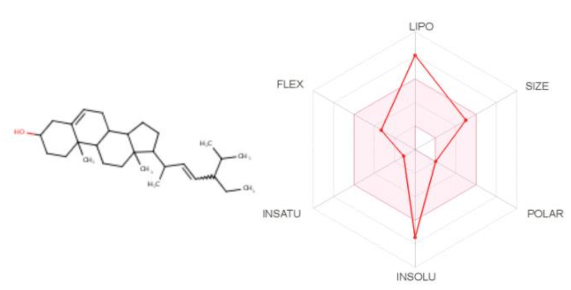




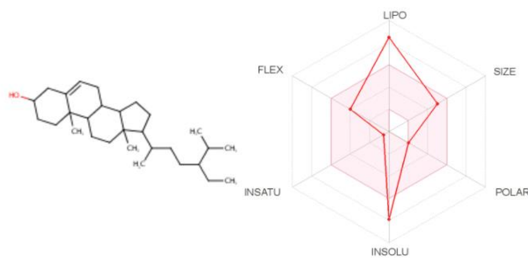
Kaempferol (Leaves)



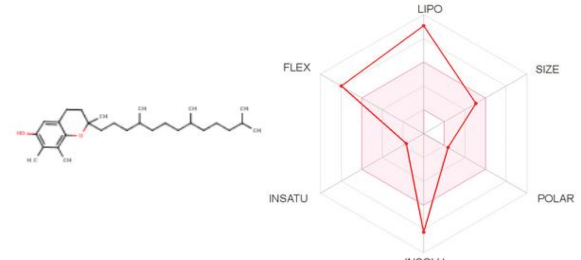
Quercetin (Leaves)



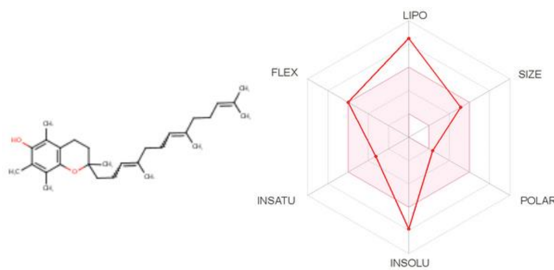
Stigmasterol



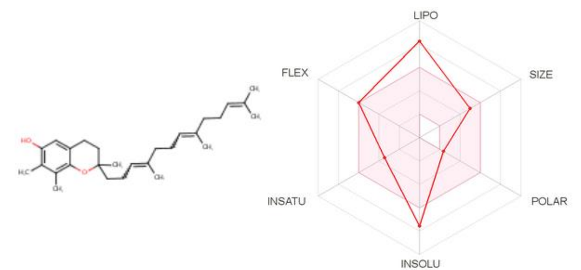
β–sitosterol



γ–tocopherol

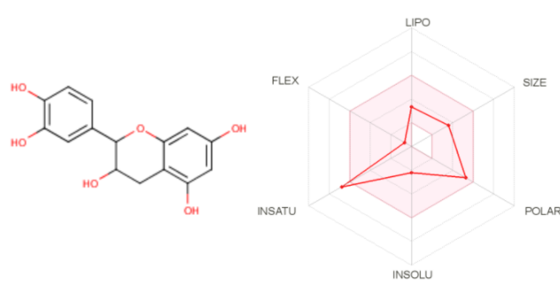


α–tocotrienol

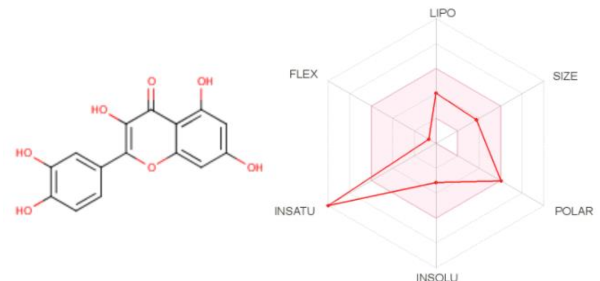


γ–tocotrienol

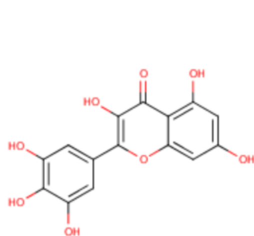
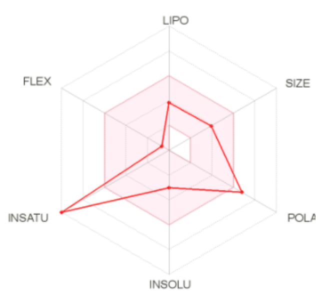
J. oxycedrus molecules

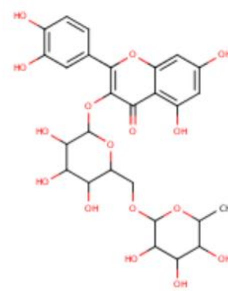
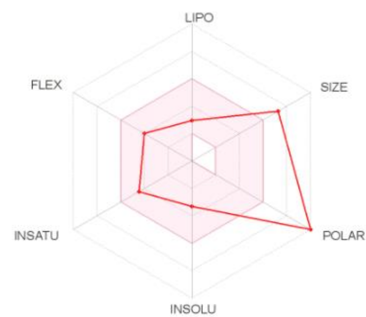


Catechin

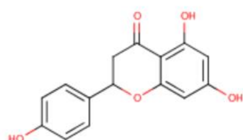
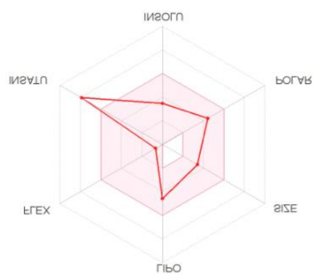
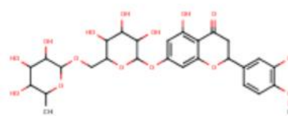
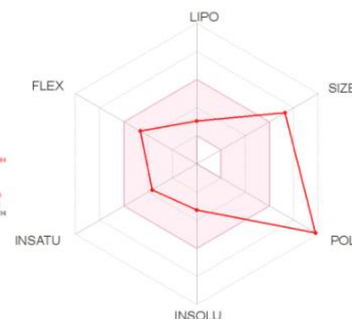


Quercetin

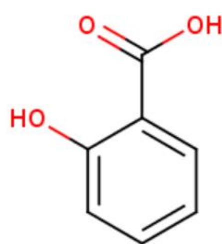



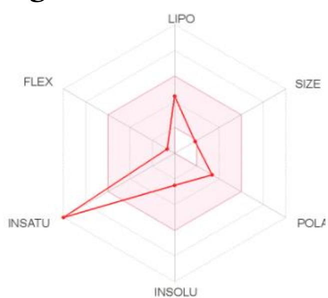



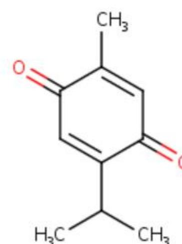
Myricetin **Rutin**

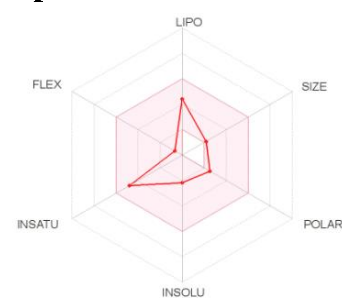





Naringenin **Hesperidin**



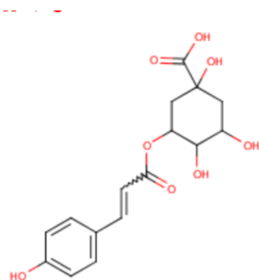
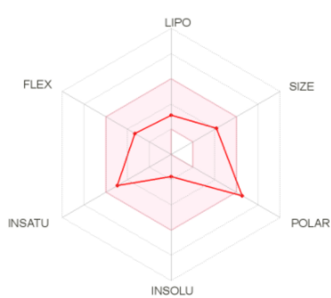


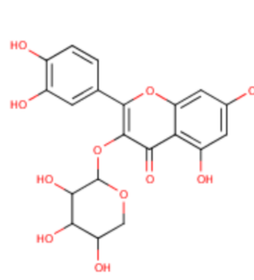
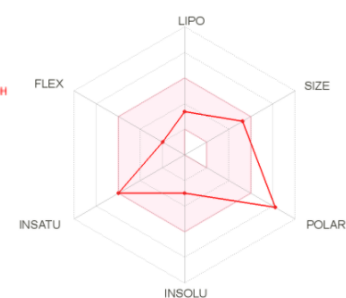




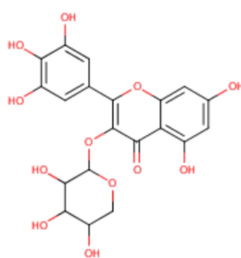
Salicylic acid **Thymoquinone**

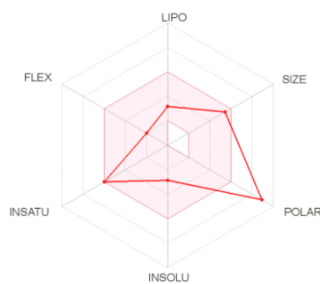
J. phoenicea molecules

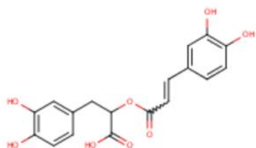
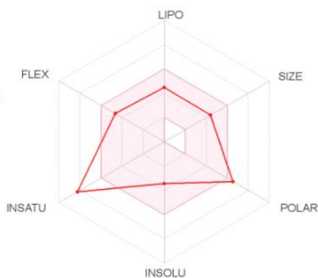
3–p–Coumaroylquinic acid **Quercetin–O–pentoside**

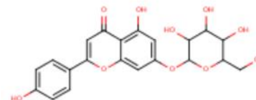
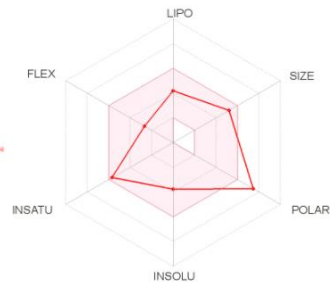




Myricetin–O–pentoside

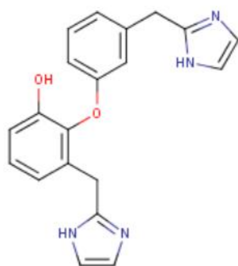
L. stoechas molecules

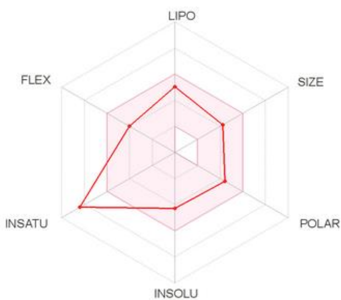



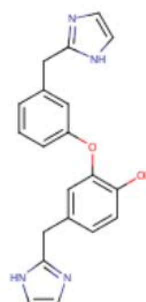



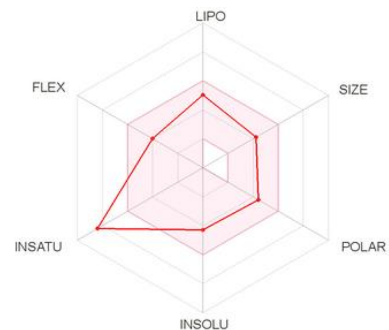
Rosmarinic acid **Apigenin 7–glucoside**

L. sativus molecules









Lepidine B **Lepidine E**

S. costus molecules

Dehydrocostus lactone

3-O-caffeoylquinic acid (Chlorogenic acid)

Malic acid

Quinic acid

APPENDICES n2

Figure 1. Chart representing the extraction yield