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Multifaceted biological activities of culinary herbs and spices extracts on Alzheimer's disease prevention, focusing on *in vitro* and *in silico* molecular docking simulations

MASTER DISSERTATION

Nance Carina Hontman Gonçalves
MASTER IN APPLIED BIOCHEMISTRY

UNIVERSIDADE da MADEIRA
A Nossa Universidade
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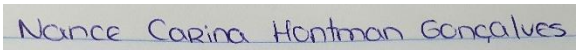
November 2024

Nance Carina Hontman Gonçalves



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Abstract

Culinary herbs and spices are commonly used worldwide, valued not only for flavor and odor but also for their medicinal and therapeutic benefits. These ingredients are composed of a broad spectrum of secondary bioactive metabolites, including polyphenols, terpenoids, and phytosterols, among others, which are involved in plant defenses against biotic or abiotic stresses, and also exhibit health-protecting or disease-preventing effects associated to anticarcinogenic, anti-inflammatory, antioxidant, and cognitive-enhancing properties. This study aimed to establish the volatile fingerprint of culinary herbs (lemon verbena, chives, basil, sage, coriander, and parsley) and spices (curcuma, nutmeg, cumin, black pepper, Jamaica pepper, and juniper berry) using headspace solid-phase microextraction combined with gas chromatography-mass spectrometry (HS-SPME/GC-MS). The predominant volatile organic metabolites (VOMs) identified were subjected to *in silico* molecular docking simulations of anti-Alzheimer (e.g., acetylcholinesterase (AChE), butyrylcholinesterase (BChE)), antioxidants (e.g., monoamine oxidase B (MAO-B), inducible nitric oxide synthase (iNOS)), and anti-inflammatory receptors (e.g., 5-lipoxygenase (5-LOX), cyclooxygenase-2 (COX-2)). The culinary herb and spice extracts were also subjected to *in vitro* assays to evaluate their potential as antioxidant (DPPH, ABTS, and ORAC) and anti-inflammatory (% protein denaturation) agents. A total of 121 VOMs were identified in the culinary herbs and spices, with the predominant chemical families being monoterpenoids (48.3%), sesquiterpenoids (14.0%), esters (11.9%), and carbonyl compounds (8.8%). *In silico* molecular docking simulations revealed that cuminaldehyde, β-caryophyllene, γ-curcumene, germacrene D, and τ-cadinol exhibited the strongest inhibitory activities against the selected receptors (AChE, BChE, MAO-B, 5-LOX, and COX-2). Among the extracts, Jamaica pepper showed the highest antioxidant and anti-inflammatory activities, while lemon verbena exhibited the lowest antioxidant and anti-inflammatory activities.

Keywords: Culinary herbs; Spices; HS-SPME/GC-MS; Antioxidant; Anti-inflammatory; Anti-Alzheimer

Resumo

As ervas culinárias e as especiarias são comummente utilizadas em todo o mundo, valorizadas não só pelo seu sabor, mas também pelos seus benefícios medicinais e terapêuticos. Estes ingredientes são compostos por um largo espetro de metabolitos secundários, incluindo polifenóis, terpenóides e fitoesteróis, entre outros, que estão envolvidos nas defesas das plantas contra stresses bióticos ou abióticos, e também exibem efeitos protetores de saúde ou preventivos de doenças associados a propriedades anticarcinogénicas, anti-inflamatórias, antioxidantes e de melhoria cognitiva. Este estudo teve como objetivo estabelecer perfil volátil de ervas culinárias (lúcia-lima, cebolinho, manjericão, salva, coentros e salsa) e especiarias (curcuma, noz-moscada, cominhos, pimenta preta, pimenta da Jamaica e bagas de zimbro) utilizando a microextração em fase sólida em modo espaço de cabeça combinada com a cromatografia em fase gasosa e espetrometria de massa (HS-SPME/GC-MS). Os metabolitos orgânicos voláteis (VOMs) predominantes identificados foram submetidos a simulações de *docking in silico* de recetores anti-Alzheimer (i.e., acetilcolinesterase (AChE), butirilcolinesterase (BChE)), antioxidantes (i.e., monoamina oxidase B (MAO-B), óxido nítrico sintase induzível (iNOS)) e recetores anti-inflamatórios (i.e., 5-lipoxigenase (5-LOX), ciclo-oxigenase-2 (COX-2)). Os extratos de ervas culinárias e especiarias foram também submetidos a ensaios *in vitro* para avaliar o seu potencial como agentes antioxidantes (DPPH, ABTS e ORAC) e anti-inflamatórios (% de desnaturação proteica). 121 VOMs foram identificados nas ervas culinárias e especiarias, sendo as famílias químicas predominantes os monoterpenóides (48,3%), sesquiterpenóides (14,0%), ésteres (11,9%) e compostos carbonílicos (8,8%). As simulações de *docking in silico* revelaram que o cuminaldeído, o β-cariofileno, o γ-curcumeno, o germacreno D e o τ-cadinol apresentaram as atividades inibitórias mais fortes contra os recetores selecionados (AChE, BChE, MAO-B, 5-LOX e COX-2). Entre os extratos, a pimenta da Jamaica apresentou as atividades antioxidantes e anti-inflamatórias mais elevadas, enquanto a lúcia-lima apresentou as atividades antioxidantes e anti-inflamatórias mais baixas.

Palavras-chave: Ervas culinárias; Especiarias; HS-SPME/GC-MS; Antioxidante; Anti-inflamatório; Anti-Alzheimer

Scientific output

Article

Abreu, T.; Sousa, P.; Gonçalves, J.; Hontman, N.; Teixeira, J.; Câmara, J.S.; Perestrelo, R. Grape Pomace as a Renewable Natural Biosource of Value-Added Compounds with Potential Food Industrial Applications. *Beverages* 2024, *10*, doi:10.3390/beverages10020045.

Book Chapter

Perestrelo, R.; Sousa, P.; Hontman, N.; Câmara, J.C. Composition of Different Species of Lamiaceae Plant Family: A Potential Biosource of Terpenoids and Antifungal Agents. In Advances in Antifungal Drug Development; Manzoor, N., Ed.; Springer Nature Singapore: Singapore, 2024; pp. 41–63 ISBN 978-981-97-5164-8.

Poster communication

Hontman N., Gonçalves J., Soares A., Câmara J.S, Perestrelo R. Spice Volatilomic Pattern – A Suitable Approach to Explore their Antioxidant Property. 16th National Chemistry Meeting + 5th Computational Chemistry Symposium; 2024 July 17-19; Lisboa, Portugal.

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Abbreviatures

5-LOX	5-lipoxygenase
ABTS	2,2’-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)
AChE	Acetylcholinesterase
AD	Alzheimer's disease
ADT	AutoDockTools
APP	Amyloid precursor protein
Aβ	Amyloid-beta
BChE	Butyrylcholinesterase
C3GE	Cyanidin-3-glucoside equivalents
COX-2	Cyclooxygenase-2
CPO	Cold-pressed oil
DPPH	1,1-diphenyl-2-picrylhydrazyl
DVB/CAR/PDMS	Divinylbenzene/carboxen/polydimethylsiloxane
DW	Dry weight
FDA	Food and drug administration
GAE	Gallic acid equivalent
GC-MS	Gas chromatography-mass spectrometry
HCA	Hierarchical cluster analysis
HS-SPME	Headspace solid-phase microextraction
iNOS	Inducible nitric oxide synthase
KI	Kovat index
MAO-B	Monoamine oxidase B
ORAC	Oxygen radical absorbance capacity
PBS	Phosphate-buffered saline
PDB	Protein data bank
PDBQT	Protein data bank, partial charge and atom type
PLS-DA	Partial least squares-discriminant analysis
QE	Quercetin equivalent
ROS	Reactive oxygen species
RT	Retention time
TAC	Total anthocyanin content
TE	Trolox equivalents
TFC	Total flavonoid content
TPC	Total phenolic content
VIPs	Variable importance in projections
VOMs	Volatile organic metabolites
ΔG	Gibbs free energy

Chapter I

INTRODUCTION

1. Introduction

1.1. Alzheimer's disease

Alzheimer's disease (AD) is a complex neurodegenerative disorder and a significant global health concern. The neuropathologies associated with AD are shown in Figure 1. In the coming decades, the prevalence of AD is expected to increase significantly as the population ages steadily [1]. Approximately 50 million people worldwide were estimated to have dementia in 2018, according to Alzheimer's Disease International. This number is expected to reach 131.5 million by 2050 [2]. According to the World Health Organization, at present, approximately 55 million people worldwide suffer from dementia, AD being the most prevalent cause of dementia with an incidence of 60 to 70% of the cases. Every 20 years, this number is expected to nearly double, rising to 82 million in 2030 and 152 million in 2050 [3–5].

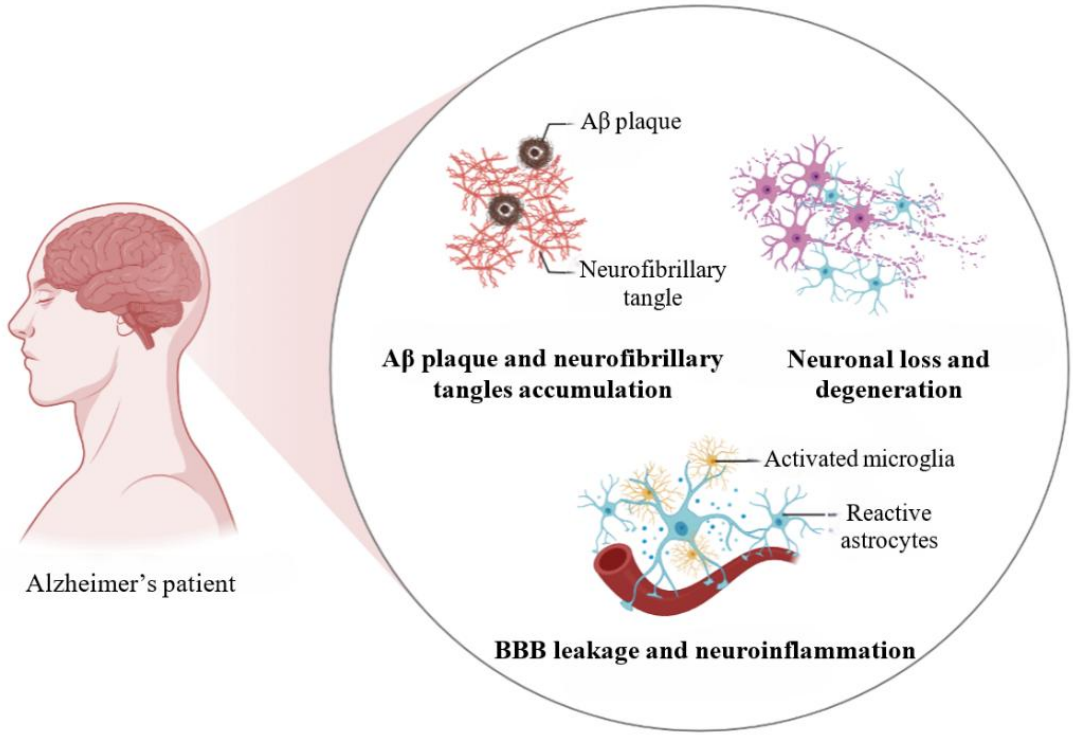


Figure 1. Neuropathological features of Alzheimer's disease. Created in BioRender.

Progressive memory loss, cognitive decline, and behavioral changes are hallmarks of AD, which ultimately causes a sharp decline in the quality of life of affected individuals [6]. AD is caused by several complex interactions between genetic, environmental, and lifestyle factors. Despite decades of intensive research, there are few effective therapeutic

interventions for AD, highlighting the urgent need to investigate alternative prevention and treatment methods.

Several risk factors for AD have been identified. They can be divided into non-modifiable and modifiable risk factors. Non-modifiable are those for which there is no control, such as age, family history of dementia, gender, existence of one apolipoprotein E ε4 allele, genetic variations, and alteration in gene regulation [7]. Modifiable risk factors are those that can be controlled in daily life, these include a healthy lifestyle, ingestion of foods rich in antioxidants and omega-3, balanced diet, physical activity, and appropriate sleep schedule [8].

Current treatments for AD still do not achieve a cure for it, they only act by disguising the symptoms generated by the disease [2]. The absence of an effective treatment for AD is partly attributed in part to the lack of a clear underlying mechanism [9].

1.1.1. Amyloid beta plaques

Several hypotheses have been proposed regarding the cause of AD. One of them is the accumulation of amyloid beta (Aβ) protein in the brain, which is a fragment of amyloid precursor protein (APP) [10].

In the healthy brain, α-secretase is responsible for the cleavage of APP, resulting in non-amyloidogenic fragments. However, in AD, cleavage of this protein by β-secretase and γ-secretase, instead of α-secretase, results in abnormal processes originating Aβ peptides (Figure 2) [10,11]. These Aβ peptides can fold in an irregular way and aggregate, forming plaques that accumulate in the extracellular medium around neurons.

As the condition progressed over time, these plaques spread to different areas of the brain. In the first place, they deposit in the neocortex, which is the center of higher mental functions, and the plaques appear in limbic regions that are responsible for learning and memory; after, they move to subcortical areas that regulate attention, emotions, and other activities; in later stages, it is possible to see the plaques in the midbrain, pons, and medulla oblongata that control motor movements and signal transmission; and in the end stages, the cerebellar cortex is affected, leading to the presence of dementia [12]. In the first two stages, the disease is asymptomatic; however, once homeostasis collapses, symptoms begin to appear [1,12].

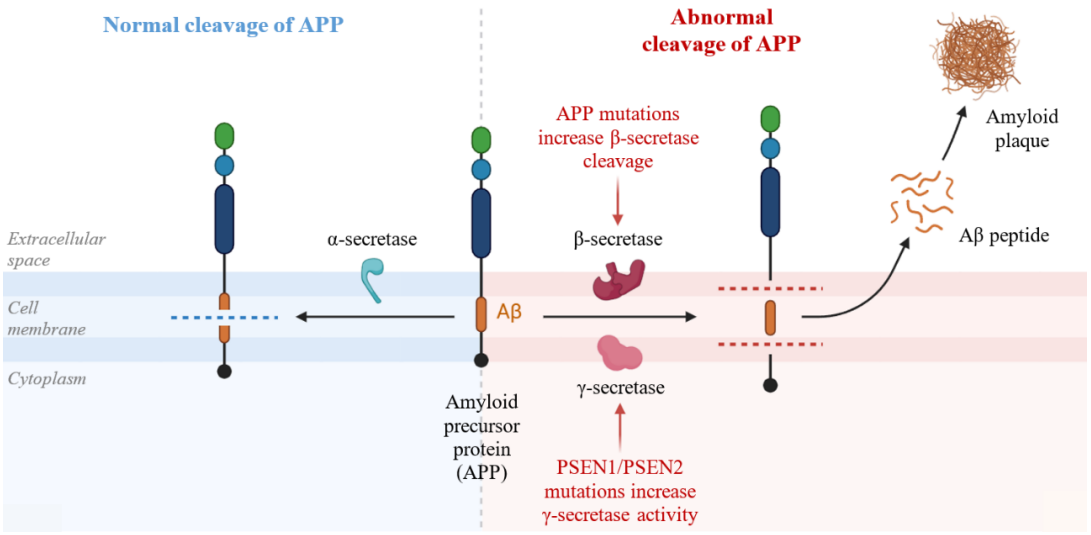


Figure 2. Cleavage of APP. Created in BioRender.

1.1.2. Tau protein and neurofibrillary tangles

Within neurons, microtubules play a crucial role in the formation of cell shape and transport of nutrients. Under normal conditions, tau protein is present in microtubules, maintaining their integrity. However, in AD, this protein suffers hyperphosphorylation, leading to its inactivation, consequently, the microtubules lose their integrity and collapse, leading to the disruption of numerous cellular processes [13]. With this, tau starts to form aggregates, forming paired helical fragments and then neurofibrillary tangles that accumulate inside neurons, resulting in the impairment of physiological functions, neuronal loss, and apoptosis [8,13].

1.1.3. Oxidative stress

Oxidative stress plays a major role in AD pathogenesis. This process causes neuronal damage and involves several pathways. Studies have revealed that Aβ peptides can increase reactive oxygen species (ROS) levels, leading to the induction of oxidative stress [14,15]. The major issues in this process are that lead to cell damage and mitochondrial dysfunction, which are the major producers of ROS [14,15]. ROS are responsible for regulating processes such as cell survival, cellular response to stress, and the activation of pro-inflammatory mechanisms [15].

Oxidative stress results from disequilibrium between pro-oxidants and antioxidants. Antioxidants are vital to compensate for the injuries caused by ROS. If there are not enough antioxidants, these molecules start to reach cells damaging important biological

macromolecules such as the membranes, proteins, and nucleic acids [14]. It was discovered that oxidative stress favors the wrong cleavage of APP, the hyperphosphorylation of tau by activating an enzyme called glycogen synthase kinase-3β, potentiates inflammatory processes such as microglia and astrocytes activation, the release of pro-inflammatory cytokines, and the recruitment of immune cells [14].

1.1.4. Neuroinflammation

Neuroinflammation is considered a central factor in the pathology of AD. Over time, the sustained presence of an immune response in the brain has become one more core pathology of AD. Also, it was verified that the persistent activation of immune cells in the brain aggravates Aβ and tau pathologies [13].

Inflammation can be either acute or chronic. Acute inflammation is a short-term response and is beneficial, fighting against infection, toxins, and injuries. However, when it gets out of control and passes to a persistent form, called chronic inflammation, the recruitment of pro-inflammatory molecules does not stop even when the pathogens that initiated the inflammation process is clear, leading to the destruction of their own organs. Inflammatory processes involve the release of cytokines, which stimulate more inflammatory cells to act against the pathogen [13,16]. Among the cytokines, interleukin 1 is associated with the increase of APP production, and other cytokines such as interleukin 6 consequently lead to the activation of cyclin-dependent kinase 5, responsible for the hyperphosphorylation of tau [13].

Microglia is a cell type that works in the clearance of foreign substances from the brain. In the first stages of AD, they are effective, but as the illness aggravates, they become inefficient [17]. Several stimuli, including Aβ and phosphorylated tau, can activate microglia. The overexpression of its biomarker, ionized calcium-binding adaptor molecule 1, is linked to the cognitive decline shown in AD patients [17].

1.1.5. Cholinergic hypothesis

The cholinergic hypothesis was one of the first theories developed for AD. This hypothesis proposes that low levels of acetylcholine are related to cognitive decline, leading to faster development of neurodegenerative diseases [18]. The neurons use neurotransmitters, such as acetylcholine, to communicate. This neurotransmitter is key in several activities including memory, learning, and behavior. The mechanism involves the

synthesis of acetylcholine by the enzyme acetylcholine transferase, its release from the presynaptic neuron, and its binding to the receptor in the postsynaptic neuron. Once its function is concluded, acetylcholine is subjected to hydrolysis by AChE, converting the neurotransmitter into acetate and choline, completing the signal transmission cycle (Figure 3) [18].

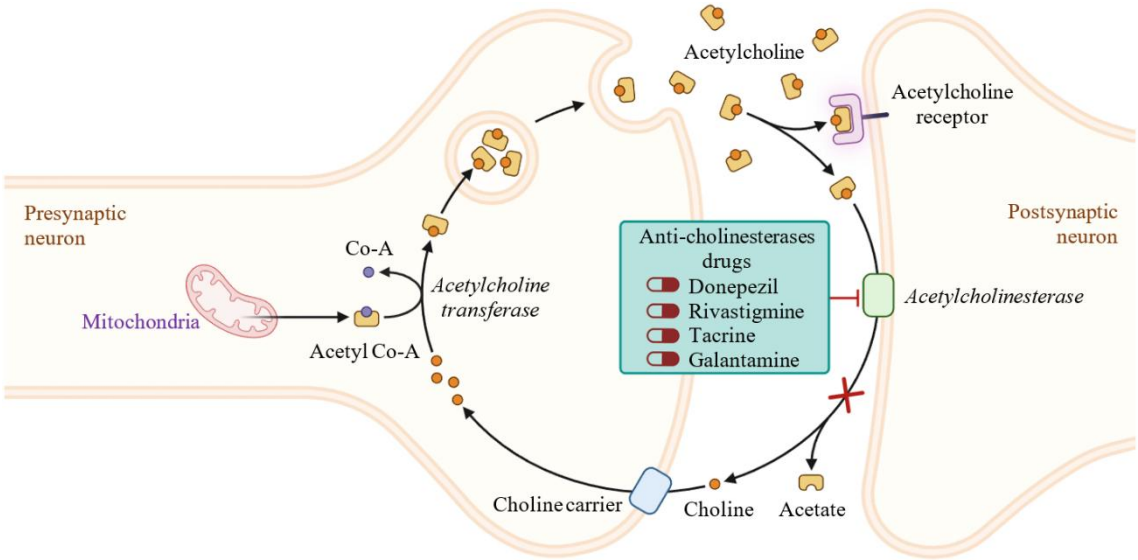


Figure 3. Mechanism of action of anti-cholinesterase drugs. Created by BioRender.

Over the years, the number of cholinergic neurons decrease, leading to a decrease in acetylcholine levels. Strategies such as the inhibition of AChE are used to maintain neurotransmitter levels. Some of the known and approved by the United States Food and Drug Administration (FDA) AChE inhibitors are donepezil, rivastigmine, and galantamine [18,19]. To find other inhibitors it is possible to compare the structures and properties of the well-known inhibitors and search for new candidates.

1.2. Secondary bioactive metabolites in culinary herbs and spices

A growing body of research supports the use of culinary herbs and spices as preventive and therapeutic agents in medicine, emphasizing their numerous health benefits. These benefits are primarily attributed to the abundance of phytochemicals, particularly secondary bioactive metabolites. These metabolites, such as VOMs, polyphenols, anthocyanins, tannins, alkaloids, and vitamins, have demonstrated the ability to prevent and alleviate both acute and chronic diseases due to their antioxidant,

anti-inflammatory, and antimicrobial properties [20–24]. In addition, current research supports the idea that culinary herbs and spices offer a variety of health benefits, including mood and cognition enhancement, antitumorigenic and anticarcinogenic effects, and glucose- and cholesterol-lowering properties. These effects are attributed to the diversity of secondary bioactive metabolites, including VOMs, identified in culinary herbs and spices, which influence various biological pathways [25] and are responsible for various health-promoting properties (Figure 4).

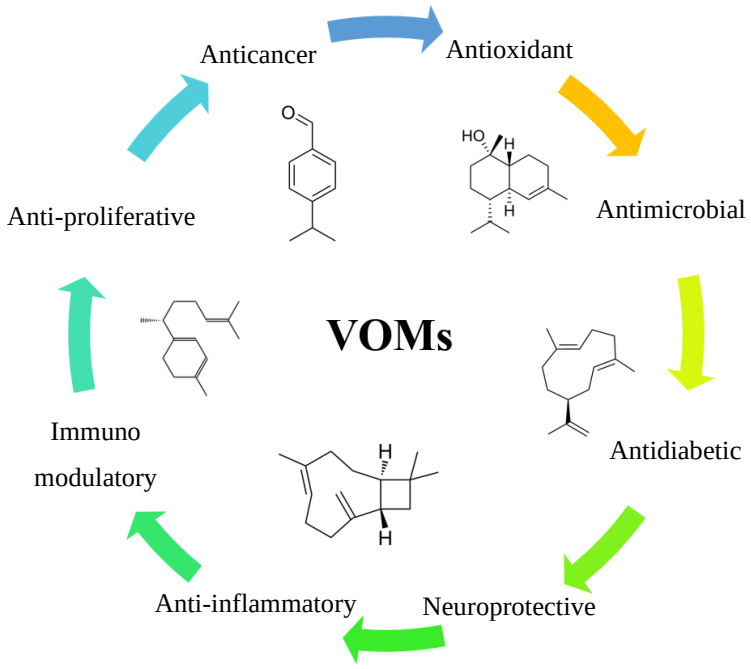


Figure 4. Properties of VOMs found in culinary herbs and spices.

A summary of key VOMs identified in culinary herbs and spices considered in the current study, along with their known biological effects will be highlighted: citral, geraniol and limonene identified in lemon verbena (*Aloysia citrodora*) are useful for reducing oxidative stress, improving sleep, and acting as a natural antimicrobial agent; methyl allyl disulfide and diallyl disulfide found in chives (*Allium schoenoprasum* L.) may help in cardiovascular health, cancer prevention, and fighting infections; eugenol, linalool and methyl chavicol reported in basil (*Ocimum basilicum* L.) supports immune health, reduces inflammation, and combats microbial infections; thujone, 1,8-cineole and camphor identified in sage (*Salvia officinalis*) may offer protection to the nervous system, while also acting as an anti-inflammatory and antimicrobial agents; linalool, α-pinene and geranyl acetate reported in coriander (*Coriandrum sativum* L.) may help in reducing

inflammation, fighting infections, and alleviating pain; myristicin and limonene present in parsley (*Petroselinum crispum*) have potential for cancer prevention, liver protection, and general antioxidant activity; turmerone and Ar-turmerone identified in curcuma (*Curcuma longa* L.) are known for their potential to reduce inflammation, protect the liver, and inhibit tumor growth; sabinene, myristicin and elemicin identified in nutmeg (*Myristica fragrans*) offers antioxidant protection and antimicrobial activity, though caution is advised with psychoactive compounds; cuminaldehyde, *p*-cymene and β-pinene identified in cumin (*Cuminum cyminum*) supports metabolic health, helps in preventing infections, and combats oxidative damage; limonene, pinene and β-caryophyllene found in black pepper (*Piper nigrum* L.) reduces inflammation, protects the digestive system, and offers antioxidant support; eugenol, methyl eugenol, and 1,8-cineole detected in Jamaica pepper (*Pimenta dioica* L.) are effective in pain relief, reducing oxidative stress, and fighting microbial infections; α-pinene, sabinene and myrcene identified in juniper berry (*Juniperus communis* L.) may act as a diuretic, helps reduce inflammation, and fights microbial infections [26–34]. Several extraction procedures have been proposed to extract VOMs from culinary herbs and spices, such as hydrodistillation [35], microwave-assisted hydrodistillation [34,35], Soxhlet [36], maceration [36], and supercritical fluid extraction [37]. Advancements in technology have shown weaknesses in prior extraction procedures, such as low stability, time-consuming, and solvent-intensive, among others [34]. Therefore, it is critical to optimize a useful and effective instrument to extract and detect VOMs in culinary herbs and spices. HS-SPME has gained popularity for the extraction of VOMs from culinary herbs and spices because of its ease of use, lack of solvents, high sensitivity, and reproducibility [28,29,38]. Wei et al. [34] found that integrating sampling, isolation, concentration, and injection into a single phase significantly reduced the time required to determine the volatile fingerprints in culinary herbs and spices. Moreover, HS-SPME can be combined with GC-MS, an analytical approach that integrates the characteristics of gas chromatography with the sensitivity and selective capacity of the mass detector, allowing for the identification and quantification of VOMs in complex mixtures. This effective analytical approach improves the recovery of VOMs through adsorption on fused silica fibers with minimal sample preparation [39]. HS-SPME combined with GC-MS is the most effective method for determining the volatile fingerprint in culinary herbs and spices compared to other analytical approaches [40]. Kim & Lee [38] studied lemon verbena and

identified 14 VOMs using HS-SPME/GC-MS methodology. Geranial and neral were the predominant VOMs identified in lemon verbena, whereas α-pinene, β-pinene, β-caryophyllene, and curcumene were identified in smaller amounts. Mahmoud et al. [28] used HS-SPME/GC-MS to establish the volatile fingerprint of basil and 30 VOMs were identified of which β-linalool, methyl eugenol, and methyl cinnamate were the most abundant. According to Wei et al. [34], the most abundant VOMs in coriander identified using HS-SPME/GC-MS are decanal, (*E*)-2-decenal, undecanal, and dodecanal. HS-SPME/GC-MS was performed in a study by Milenković et al. [29] using black pepper as a sample, and 45 VOMs were identified, with (*E*)-caryophyllene, limonene, and sabinene being the most abundant. In addition, the key VOMs identified in culinary herbs and spices can be subjected to *in silico* molecular docking simulations to predict their interactions with Alzheimer's-related targets (e.g., AChE, β-secretase) and oxidative/inflammatory pathways (e.g., iNOS, 5-LOX, COX-2). This approach, combined with *in vitro* assays (e.g., antioxidant and anti-inflammatory assays), could streamline the discovery of bioactive compounds for neurodegenerative and related diseases by prioritizing candidates with the highest predicted efficacy for further experimental testing.

1.3. Molecular modelling

Molecular modelling is a computational technique that involves the use of software and algorithms to simulate the behavior of molecules and their interactions. The use of this strategy has several advantages, such as the provision of spatial and temporal resolution that is difficult to reach in experimental studies, which can help understand phenomena that are not well understood and can be used to study rather complex systems at a fraction of the cost or time requirements of experimental studies. It is a useful screening tool for identifying promising candidates for further experimental studies and for designing new protein structures and complexes for various applications in research and medicine to predict the extent of enzyme activation and facilitate the development of prodrugs [41].

Docking and molecular dynamics simulations are very useful for studying the inhibition process of ligands, enzymes, and proteins. For this purpose, software such as Avogadro2 or similar is used for the design of the molecules for the study, AutoDockTools (ADT) to choose the target site in the receptor and to prepare the

molecules for docking simulation, AutoDock Vina for docking calculations, and ChimeraX to view and analyze the results. Molecular modelling is a powerful tool that can be used to study a wide range of molecular systems and phenomena. It offers several advantages over experimental studies and can be used to guide the design of new molecules and materials for various applications.

Mostafa et al. [42] evaluated the neuroprotective effects of black pepper cold-pressed oil (CPO) against scopolamine-induced oxidative stress and memory impairment in rats, using a combination of GC-MS and *in silico* molecular docking studies. Their findings revealed that CPO administration reduced AChE levels in the hippocampi of scopolamine-treated rats by 51%, which was corroborated by molecular docking studies that highlighted the potential of key VOMs in CPO to interact with Alzheimer's-related targets and to counteract oxidative stress.

AChE, BChE, MAO-B, iNOS, 5-LOX, and COX-2 are among the most significant enzymes associated with Alzheimer's disease. These enzymes play crucial roles in the biochemical pathways that contribute to the development and progression of this neurodegenerative disorder. Together, these enzymes highlight the complex interplay of neurotransmitter regulation, oxidative stress, and inflammation in Alzheimer's disease, making them critical targets for research and therapeutic intervention. AChE and BChE are closely associated with AD, as they play key roles in regulating acetylcholine levels in the body. Inhibiting these enzymes reversibly can increase acetylcholine levels in the brain, potentially easing some Alzheimer’s symptoms [43]. MAO-B and iNOS are linked to oxidative stress. MAO-B breaks down neurotransmitters, resulting in the release of ROS that can lead to neuronal cell death. iNOS is responsible for the production of reactive nitrogen species, which further contributes to oxidative stress [44,45]. 5-LOX and COX-2 are associated with inflammation. 5-LOX and COX-2 are crucial enzymes involved in inflammatory processes. 5-LOX is responsible for the production of leukotrienes from arachidonic acid, whereas COX-2 converts arachidonic acid into prostaglandins. The inhibition of 5-LOX has been associated with a decrease in Aβ production and tau phosphorylation, which are key factors in AD. Similarly, the inhibition of COX-2 helps lower inflammation by reducing the production of prostaglandins, which are powerful mediators of immune and inflammatory responses [46]. In AD, COX-2 is overexpressed, contributing to tau phosphorylation and the formation of neurofibrillary tangles [47].

1.4. *In vitro* assays to assess antioxidant activities

Total phenolic content (TPC) assay the phenolic compounds interact with the Folin-Ciocalteu reagent (phosphomolybdenum/phosphotungsten complex), which has a yellow color, through their oxidizable hydroxyl groups by transferring electrons to the complex, giving rise to a blue color. This reaction assesses the ability of phenolic compounds to reduce the acid complexes of the Folin-Ciocalteu reagent [48,49]. The reaction mechanism is shown in Figure 5.

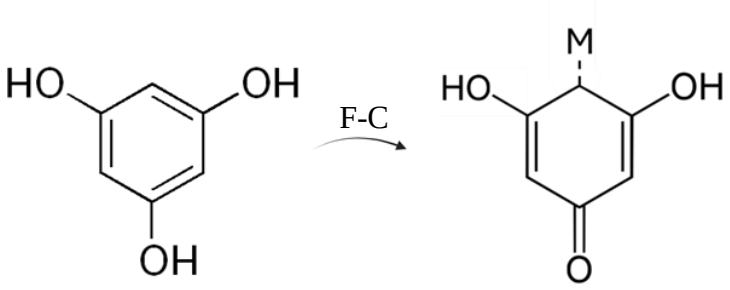


Figure 5. TPC method. F-C reagent: Folin-Ciocalteu reagent. Adapted from Shi et al. [49].

Total flavonoid content (TFC) is a method that assesses the approximate amount of flavonoids in the sample. In this reaction, a complex is formed between the carbonyl and hydroxyl groups of the flavonoid and the aluminum ion (Al^{3+}). The more flavonoids detected, the darker the solution [48,50]. The reaction mechanism is shown in Figure 6.

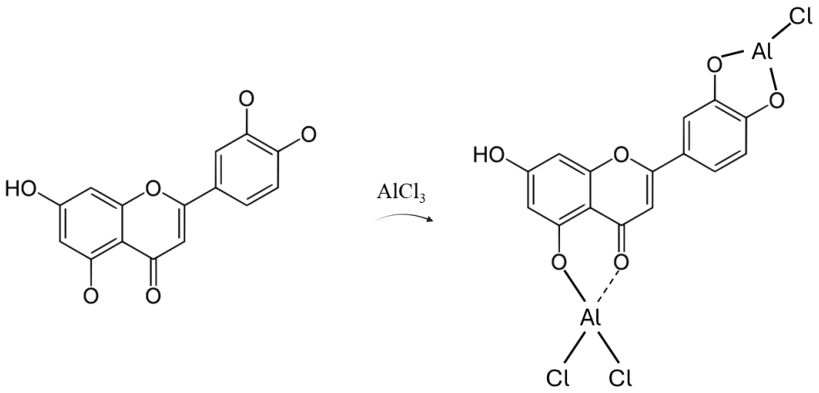


Figure 6. TFC method. Mechanism of the aluminum complexation reaction with the flavonoid. Adapted from Makuasa & Ningsih [50].

Total anthocyanin content (TAC) is a method that measures the amount of anthocyanins present in a sample. It is assessed through the pH differential method based on the structural modifications in the anthocyanin structure by measuring the absorbance

at pH 1 and 4.5 [51,52]. The structural changes of the anthocyanin in the assay are represented in Figure 7.

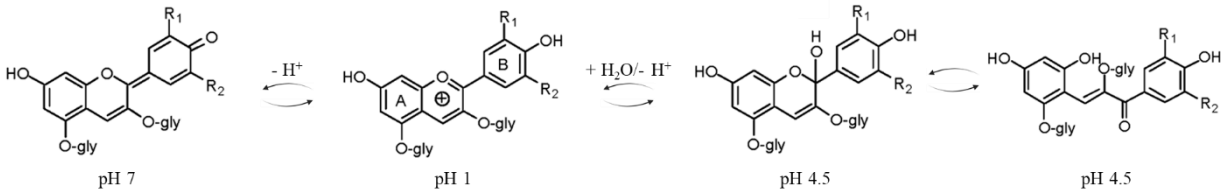


Figure 7. Mechanism of TAC assay. Adapted from Lee et al. [52].

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is a highly sensitive method that assesses samples' antioxidant capacity in eliminating the free radical DPPH. The DPPH solution is colored purple. This method is based on the transfer of electrons from the radical to the antioxidant and the donation of hydrogen atoms from the antioxidant to the radical [48,53,54]. As it is reduced, its characteristic color disappears, in other words, the solution becomes clearer. The reaction mechanism is shown in Figure 8.

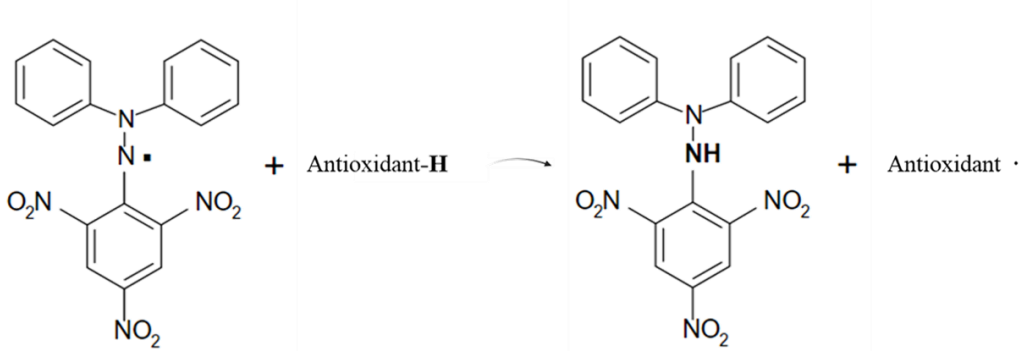


Figure 8. Mechanism of the DPPH reaction with the antioxidant. Adapted from Paixão et al. [54].

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay is a method for evaluating antioxidant capacity in the same way as DPPH. The ABTS solution is colored green. Phenolic compounds reduce their free radicals and the greater the reduction of these radicals, the clearer the solution [55]. The mechanism of the ABTS reaction is shown in Figure 9.

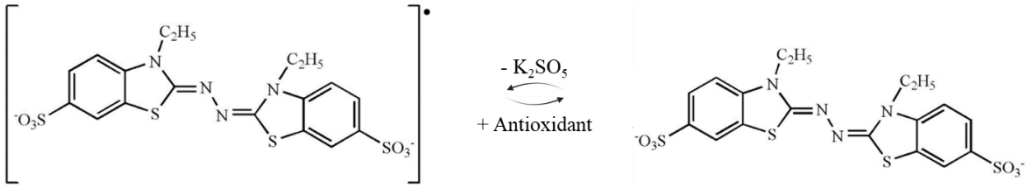


Figure 9. Mechanism of the ABTS reaction with the antioxidant. Adapted from Cruz et al. [55].

Oxygen Radical Absorbance Capacity (ORAC) is a very sensitive assay that evaluates the antioxidant activity through the measure of the fluorescence of a target protein. Its decrease represents the change in the protein's conformation when peroxyl radicals cause its oxidation [56]. The mechanism of ORAC is represented in Figure 10.

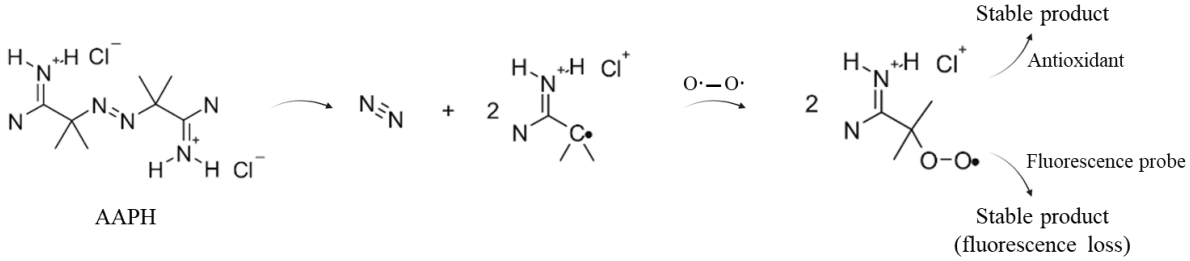


Figure 10. Mechanism of AAPH reaction with the antioxidant. Adapted from Zulueta et al. [56].

Aims

The current study aimed to establish a volatile fingerprint of culinary herbs and spices using the HS-SPME/GC-MS method and to evaluate the antioxidant and anti-inflammatory effects of their extracts through *in vitro* assays. Furthermore, the most abundant VOMs identified in the samples were correlated with their antioxidant and anti-inflammatory activities using Pearson's correlation. *In silico* molecular docking simulations were performed on targets related to AD, such as anti-Alzheimer (e.g., AChE, BChE), antioxidants (e.g., MAO-B, iNOS), and anti-inflammatory receptors (e.g., 5-LOX, COX-2) using the predominant VOMs identified in culinary herbs and spices. An experimental design is shown in Figure 11.

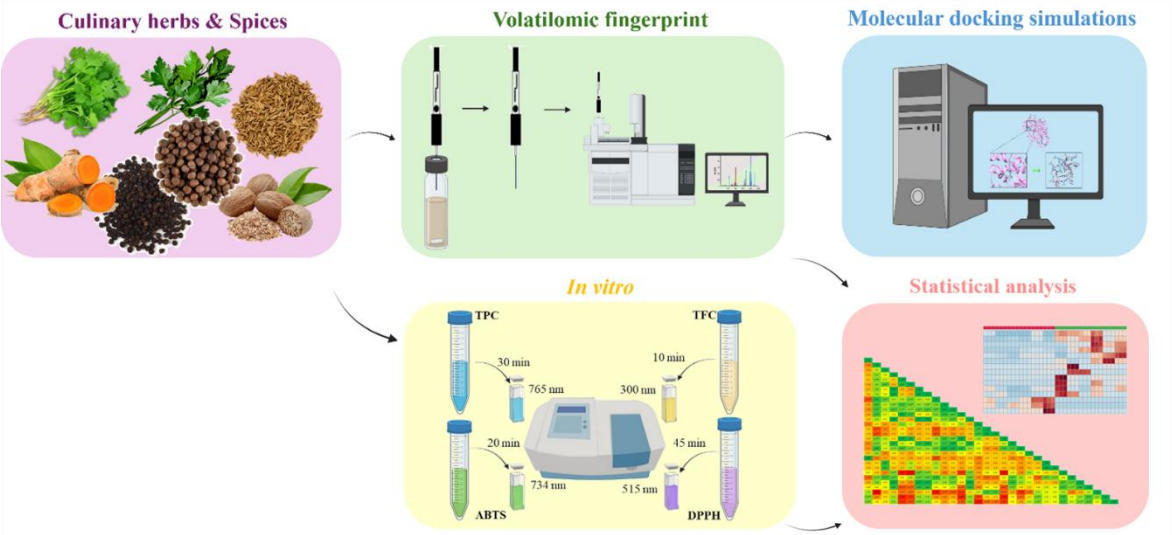


Figure 11. Experimental design.

Chapter II

MATERIAL AND METHODS

2. Material and methods

2.1. Chemicals

All the chemicals and reagents used were of analytical grade. HPLC-grade methanol was obtained from Fischer Scientific (Loughborough, UK). Gallic acid (purity ≥ 99%), quercetin (≥ 98%), sodium chloride (NaCl, 99.5%), 3-octanol (internal standard, 99%), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox, 98.0%), hydrochloric acid (HCl, 37% v/v), fluorescein, sodium acetate (CH_3COONa), 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH), albumin (≥ 99%), and anhydrous sodium carbonate (Na_2CO_3, 99.8%) were sourced from Sigma-Aldrich (St. Louis, MO, USA). Aluminum chloride ($AlCl_3$) and potassium chloride (KCl, 99.5%) were purchased from Riedel-de Haën (Seelze, Germany). The Folin-Ciocalteu reagent (FR, 2 N), 1,1-diphenyl-2-picrylhydrazyl (DPPH· ≈ 90%) in its free radical form, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS, 98.0%), potassium persulfate (99.0%), and an alkane series (C8 to C20, 40 mg/L in n-hexane) were supplied by Fluka (Buchs, Switzerland). The SPME fiber coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (50/30 µm), an SPME holder for manual sampling, and glass vials were purchased from Supelco (Bellefonte, PA, USA). Ultrapure water (18 MΩ cm) was obtained using a Milli-Q water purification system (Millipore, Milford, MA, USA).

2.2. Samples

Six culinary herbs: lemon verbena (*Aloysia citrodora*), chives (*Allium schoenoprasum* L.), basil (*Ocimum basilicum* L.), sage (*Salvia officinalis*), coriander (*Coriandrum sativum* L.), parsley (*Petroselinum crispum*) and six spices: curcuma (*Curcuma longa* L.), nutmeg (*Myristica fragrans*), cumin (*Cuminum cyminum*), black pepper (*Piper nigrum* L.), Jamaica pepper (*Pimenta dioica* L.), and juniper berry (*Juniperus communis* L.), were freshly obtained from a local market in Funchal (Madeira Island, Portugal) in 2024. All samples were milled and homogenized using a grinder (A11 Basic analytical mill; IKA, Staufen, Germany) and stored at room temperature until analysis.

2.3. HS-SPME procedure to extract volatile organic metabolites

HS-SPME extraction was carried out following the method described by Izcara et al. [57] with minor modifications. Briefly, 2 g of fresh sample, 0.5 g of NaCl, 5 mL of deionized water, and 5 µL of 3-octanol (102 µg/mL) along with a stirring bar were added to a 20 mL glass vial, which was then sealed with a polytetrafluoroethylene/silicone septum. The DVB/CAR/PDMS fiber was exposed to the headspace of the vial for 45 min at 40 ± 1 °C. After exposure, the fiber was retracted into the needle and the VOMs were thermally desorbed at 250 °C for 6 min by introducing it into the GC injection port. Prior to use, the SPME fibers were thermally conditioned according to the manufacturer's guidelines. Each analysis was performed in triplicate (n = 3), and a blank run was conducted between samples to ensure no carryover of the analytes.

2.4. GC-MS conditions

The volatile fingerprints of the culinary herbs and spices were analyzed using GC-MS. The analysis was conducted using an Agilent Technologies 6890N gas chromatograph connected to an Agilent 5975 quadrupole mass selective detector. A BP20 capillary column (60 m × 0.25 mm I.D. × 0.25 µm) was utilized for this analysis. The temperature program began at 40 ºC and increased at a rate of 2.7 ºC/min until reaching 220 ºC, with a total run time of 73.67 minutes. Helium of 5.0 purity was used as the carrier gas at a constant flow rate of 1.3 mL/min to separate analytes. Data acquisition was conducted over a mass range of 30–350 m/z using electron ionization at an energy level of 70 eV. VOMs were identified by comparing their GC retention times (RT), Kovat index (KI), and mass spectra with those of available standards, as well as by matching their mass spectra with the National Institute of Standards and Technology MS 05 spectral database (Gaithersburg, MD, USA), with a matching probability greater than 80%. The Kovat index values were calculated using the van den Dool and Kratz equation and compared to values reported in the literature for similar columns [58]. The relative area was determined by adding 3-octanol (internal standard, IS) and using the following equation: Relative area = (VOM GC peak area / IS GC peak area).

2.5. Molecular modelling

2.5.1. Preparation of target proteins

The crystal structure of the target proteins (receptors) was obtained through RCSB Protein Data Bank (https://www.rcsb.org/): AChE (code: 4PQE) with resolution 2.90 Å; BChE (code: 6ESY) with resolution 2.80 Å; MAO-B (code: 2V5Z) with resolution 1.60 Å; iNOS (code: 4NOS) with resolution 2.25 Å; 5-LOX (code: 6N2W) with resolution 2.71 Å; COX-2 (code: 5KIR) with resolution 2.70 Å. ChimeraX 1.2.5. [59] was used to verify and remove the presence of non-standard residues, and ADT-1.5.7 [60] was used to convert the receptors from Protein Data Bank (PDB) to Protein Data Bank, partial charge (Q) and atom type (T) (PDBQT).

2.5.2. Preparation of small-molecule inhibitors

For each enzyme, substrates and known inhibitors were chosen. Among them, the VOMs with variable importance in projections (VIPs) higher than 1.5 and the most representative of culinary herbs and spices of each sample were selected for further investigation with docking studies. The molecules were drawn in Avogadro2 (version 1.91.0) [61] their energy was optimized using the force field general AMBER force field, and the molecules were then converted to PDBQT with ADT. Figure S1 shows the structures of the compounds (ligands) used in this study.

2.5.3. Molecular docking analysis

A series of docking simulations were conducted for six receptors: anti-Alzheimer (AChE, BChE), antioxidant (MAO-B, iNOS), and anti-inflammatory (5-LOX, COX-2). A grid box is built to delimit the search space. The molecule files were converted to PDBQT using ADT and configuration files were created to provide commands to Vina. Finally, the AutoDock Vina (version 1.2.3.) software [62,63] was used to generate the detailed ligand-receptor interactions, in which the Gibbs free energy (ΔG) was expressed as kcal/mol. The results were analyzed using ChimeraX. All softwares used in the current study are open source.

2.6. *In vitro* assessment of total phenolic content, total flavonoids content, antioxidant, and anti-inflammatory of culinary herbs and spices extracts

An aqueous solution was prepared to evaluate the total phenolic content (TPC), total flavonoid content (TFC), total anthocyanin content (TAC), as well as the antioxidant and anti-inflammatory activities of the culinary herbs and spices being studied. All measurements were conducted in triplicate. Specifically, 2 g of each sample was boiled in 100 mL hot water for 15 min. Following this, the mixture was centrifuged at 5000 rpm for 5 min (using a SIGMA 1–7 centrifuge with a maximum capacity of 6 × 15 mL and a maximum relative centrifugal force of 6153 × g). The supernatant was collected and stored at -80 ºC for subsequent *in vitro* assays.

2.6.1. Total phenolic content (TPC)

TPC was measured spectrophotometrically using the Folin-Ciocalteu test, as outlined by Abreu et al. [64]. A calibration curve was established using gallic acid as the reference standard, covering a concentration range of 15 to 76 mg/L, to quantify the TPC in culinary herb and spice extracts. The results were reported as milligrams of gallic acid equivalent per gram of sample [mg GAE/g]. Spectrophotometric readings were obtained at 765 nm using a UV-Vis spectrophotometer (Lambda 25, Perkin Elmer, Waltham, MA, USA).

2.6.2. Total flavonoids content (TFC)

TFC was determined using the $AlCl_3$ colorimetric assay according to Abreu et al. [64]. In brief, 3 mL of the appropriately diluted extract and 3 mL of a 2% (w/v) $AlCl_3$ solution in methanol were combined in a 10 mL screw-capped centrifuge tube and vortexed for 30 seconds. A slight yellow color developed, and the mixture was incubated for 10 min in the dark at 25 ± 1 °C. The absorbance of all the samples was measured at 300 nm using a spectrophotometer. Quercetin served as the reference standard for constructing a calibration curve within the concentration range of 5–25 mg/L. The results were reported as milligrams of quercetin equivalent per gram of sample [mg QE/g].

2.6.3. Total anthocyanin content (TAC)

The TAC of the culinary herb and spice extracts was calculated using the pH differential assay reported by Ribeiro et al. [65]. This spectroscopic method involved measuring the absorbance of the extracts at 510 and 700 nm at two different pH levels: 1.0 (using KCl, 0.025 mol/L) and 4.5 (using CH_3COONa, 0.40 mol/L). For the assay, 0.5 mL of the extract was placed in a 5 mL screw-capped centrifuge tube, resulting in two dilutions: one with pH 1.0 buffer and the other with pH 4.5 buffer. The absorbance values were then converted using a molar absorption coefficient of 26,900 L/mol·cm, and the results were expressed as total milligrams of cyanidin-3-glucoside per gram of sample, denoted as mg C3GE/g. Water was used as the control.

2.6.4. Antioxidant activity

Different i*n vitro* assays were carried out to evaluate the antioxidant activity of culinary herb and spice extracts.

i) 2,2-Diphenyl-1-picrylhydrazyl scavenging assay (DPPH)

The antioxidant activity of $DPPH^{\bullet}$ free radical-scavenging capacity (A_{AR}) was determined according to the procedure proposed by Abreu et al. [64]. Briefly, a stock solution of $DPPH^{\bullet}$ radical in methanol (400 µM) was prepared and maintained at 25 ± 1 ºC in the dark. For the experiment, this stock solution was diluted in methanol to attain a working solution with an absorbance of 0.900 (± 0.030) at 515 nm. A calibration curve was constructed using trolox at concentrations ranging from 25 to 600 mg/L. Results were expressed as mg of trolox equivalents per gram of sample, mg TE/g. DPPH quenching assays were performed in triplicate. Methanol was used as a control.

ii) 2,2′ - Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) scavenging assay (ABTS)

The ABTS assay was performed according to Izcara et al. [66]. A 50 mL solution of 2,2′ - azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet}$) (20 mM) was prepared in phosphate-buffered saline (PBS pH 7.4) and 200 µL of potassium persulfate solution (70 mM) was added to the $ABTS^{\bullet}$ solution. The solution was stored at 25 ± 1 ºC for 16 h to obtain a stable radical cation. After that, the solution was diluted

in PBS to achieve a working solution with an absorbance of 0.900 (± 0.030) at 734 nm. For the reaction, 12 µL of extract was added to 3 mL of the working solution and incubated, in the dark, for 20 min at 25 ± 1 ºC. A calibration curve was done by plotting with concentrations of trolox ranging from 100 to 1200 mg/L. Results were expressed as mg TE/g. ABTS assays were performed in triplicate. PBS was used as a control.

iii) Oxygen radical absorbance capacity (ORAC)

ORAC was conducted as Zulueta et al. [56] with slight modifications. In a plate with 96 white flat-bottom wells, 50 µL of fluorescein (1.56 nM), 50 µL of the extract, and 25 µL of AAPH (221 mM) were added. The plates were then incubated at 37 °C for 15 min. The fluorescence was read at 485 nm and 520 nm (excitation and emission wavelengths, respectively) in a Victor3 Multilabel Plate Counter 1420 fluorescence reader at 5 min intervals until the fluorescence decayed and the absorbance became constant. PBS and trolox (20 µM) were used as control and reference, respectively. The results are expressed as µmol TE/g.

2.6.5. Anti-inflammatory activity

In vitro anti-inflammatory activity was assessed by measuring the inhibition of albumin denaturation, following the method described by Gunathilake et al. [67]. For the assay, 0.5 mL of 1% bovine serum albumin in PBS at pH 6.4 was combined with 0.5 mL of the culinary herb and spice extract in a 5 mL screw-capped centrifuge tube. The reaction mixture was vortexed for 30 seconds and then incubated at 37 ºC for 15 minutes. It was subsequently heated at 70 ºC for 5 minutes. After cooling, the turbidity was measured at 660 nm using a UV/VIS spectrophotometer, with PBS serving as the control. The results were expressed as the percentage inhibition of albumin denaturation.

2.7. Statistical analysis

Statistical analysis was conducted using the MetaboAnalyst 6.0 web-based tool [68]. The raw GC-MS data and results from *in vitro* assays were pre-processed, normalized (through cubic root transformation and autoscaling), and then subjected to one-way analysis of variance (ANOVA), followed by Fisher's test for post-hoc multiple comparisons. A significance level of $p < 0.05$ was used to identify meaningful differences in the data from the culinary herbs and spices. Additionally, partial least squares-

discriminant analysis (PLS-DA) was applied to the volatile fingerprint dataset of the culinary herbs and spices to analyze sample separations and pinpoint VOMs that contribute to the differentiation of sample sets, focusing on those with VIPs scores greater than 1.5. Pearson correlation analysis was performed to examine the relationships among key VOMs and the *in vitro* assay results.

Chapter III

RESULTS AND DISCUSSION

3. Results and discussion

This section presents the key results of our study on assessment of the volatile fingerprint of culinary herbs and spices, in addition to the molecular docking analysis and evaluation of the bioactive properties of the investigated samples related to the prevention of AD. The findings are organized according to the main research questions outlined in Chapter II, and their implications are discussed in relation to existing literature.

3.1. Volatile fingerprint of culinary herbs and spices

The volatile fingerprint of culinary herbs and spices was established using HS-SPME/GC-MS, a robust method for assessing authenticity and quality due to their nutritional value, high consumer demand, and distinctive flavors. In total, 121 VOMs were identified across the culinary herbs and spices analyzed, including 43 monoterpenoids, 13 sesquiterpenoids, 4 norisoprenoids, 6 alcohols, 18 carbonyl compounds, 11 esters, 4 volatile phenols, 3 furanic compounds, 8 sulfur compounds, and 11 other categories. The contribution of terpenoids and esters to the volatile fingerprint was nearly double in spices compared to culinary herbs, while carbonyl compounds had a contribution nearly four times higher in culinary herbs than in spices. Other chemical families contributed less than 6% on average to the total volatile fingerprint.

3.1.1. Volatile fingerprint of culinary herbs

Table 1 presents the retention time, Kovat index, chemical families, and relative peak area of each VOM identified in the culinary herbs.

Table 1. Relative peak area of VOMs identified in culinary herbs using HS-SPME/GC-MS.

RT (min)[a]	Peak Nº	KI cal[b]	KI lit[c]	Volatile organic metabolites	Culinary herbs					
					Lemon verbena	Chives	Basil	Sage	Coriander	Parsley
				Monoterpenoids						
17.23	1	1013	1013	α-Pinene	1.81 ± 0.19	0.43 ± 0.04	0.07 ± 0.01	0.09 ± 0.02	0.12 ± 0.01	0.74 ± 0.05
19.01	3	1052	1052	Camphene	-	-	-	0.57 ± 0.07	-	-
20.95	5	1090	1090	β-Pinene	2.14 ± 0.19	-	0.13 ± 0.01	-	-	-
21.29	6	1096	1096	Sabinene	5.20 ± 0.48	0.84 ± 0.11	-	-	-	7.36 ± 0.63
22.24	7	1115	1114	3-Carene	-	-	-	-	-	2.21 ± 0.32
22.76	9	1126	1128	β-Myrcene	14.5 ± 1.29	2.70 ± 0.46	1.43 ± 0.04	0.99 ± 0.18	0.22 ± 0.03	2.97 ± 0.48
22.97	10	1130	1140	α-Phellandrene	1.30 ± 0.19	0.93 ± 0.15	-	0.18 ± 0.03	-	12.0 ± 0.92
24.49	12	1159	1155	Limonene	41.6 ± 4.55	35.5 ± 2.86	0.18 ± 0.01	2.47 ± 0.39	0.44 ± 0.01	8.76 ± 1.10

24.71	13	1163	1163	β-Phellandrene	2.34 ± 0.31	-	0.20 ± 0.01	-	-	6.28 ± 0.04
25.17	14	1172	1173	Eucalyptol	13.9 ± 0.79	2.58 ± 0.30	1.76 ± 0.03	12.9 ± 2.35	-	-
26.59	17	1197	1195	β-Ocimene	1.09 ± 0.09	-	1.65 ± 0.11	-	0.37 ± 0.07	-
26.86	18	1201	1200	γ-Terpinene	4.28 ± 0.69	-	-	-	0.52 ± 0.04	-
27.67	20	1218	1220	*p*-Menth-4(8)-ene	-	-	0.30 ± 0.04	0.12 ± 0.01	-	-
27.90	21	1222	1225	α-Terpinene	-	-	-	-	0.44 ± 0.06	9.11 ± 0.24
28.40	22	1232	1232	*p*-Cymene	24.2 ± 3.42	6.59 ± 1.21	-	-	-	-
28.57	23	1235	1248	*o*-Cymene	13.2 ± 0.68	-	-	1.24 ± 0.16	0.43 ± 0.05	170 ± 16.0
30.53	25	1272	1284	δ-Terpinene	46.5 ± 8.71	-	-	0.23 ± 0.04	0.31 ± 0.03	23.3 ± 0.48
33.64	29	1330	1330	Alloocimene	0.64 ± 0.05	-	-	0.32 ± 0.04	-	-
35.92	34	1374	1383	Fenchone	-	-	-	-	-	18.3 ± 1.79
37.11	39	1396	1420	α-Cyclocitral	0.97 ± 0.06	31.2 ± 3.64	-	-	-	-
38.27	42	1421	1421	α-Thujone	-	-	-	9.71 ± 1.56	-	-
38.47	43	1425	1425	β-Thujone	7.09 ± 0.34	-	5.71 ± 0.33	17.6 ± 2.15	-	-
42.18	52	1503	1501	(+)-Camphor	-	-	-	25.6 ± 2.19	-	-
42.34	53	1506	1506	Linalool	-	-	1.49 ± 0.11	1.07 ± 0.06	-	-
43.48	55	1531	1542	Limonene oxide	8.72 ± 0.98	-	-	-	-	-
48.52	68	1641	1649	Estragole	52.1 ± 4.50	-	3.53 ± 0.29	1.75 ± 0.31	-	-
49.55	72	1665	1665	α-Terpineol	64.1 ± 5.71	-	-	1.63 ± 0.22	-	-
49.62	73	1666	1664	Isoborneol	13.6 ± 0.82	-	2.24 ± 0.38	68.8 ± 0.10	-	6.26 ± 0.47
51.03	76	1698	1700	Phellandral	147 ± 9.01	-	-	-	-	-
51.18	77	1701	1706	Geranial	126 ± 16.4	56.4 ± 3.86	13.5 ± 0.80	9.20 ± 1.23	-	-
53.53	87	1759	1756	β-Citronellol	157 ± 14.1	-	4.83 ± 0.35	9.49 ± 1.16	-	-
54.04	88	1771	1771	Cuminaldehyde	8.24 ± 0.38	-	-	0.50 ± 0.08	-	-
55.61	94	1808	1809	*p*-Cymen-8-ol	8.21 ± 0.45	-	-	0.34 ± 0.06	-	-
56.93	99	1838	1842	Geraniol	-	-	0.09 ± 0.01	-	-	-
69.11	116	2155	2173	Carvacrol	-	5.81 ± 0.79	-	6.81 ± 1.06	-	-
				Sesquiterpenoids						
42.76	54	1515	1529	β-Bourbonene	10.8 ± 0.71	6.91 ± 0.96	-	0.61 ± 0.08	-	3.09 ± 0.51
44.49	57	1553	1558	β-Cubebene	10.1 ± 0.19	-	45.0 ± 7.67	-	-	-
45.77	60	1579	1579	α-Bergamotene	-	-	-	6.23 ± 0.85	-	-
46.09	62	1586	1589	Aromandrene	-	-	2.28 ± 0.40	0.90 ± 0.16	-	-
47.84	65	1625	1623	β-Caryophyllene	203 ± 13.8	20.8 ± 1.71	3.28 ± 0.04	16.4 ± 1.15	-	-
49.00	69	1652	1653	β-Farnesene	44.9 ± 2.84	15.1 ± 1.68	1.53 ± 0.28	-	-	-
51.90	81	1719	1718	Germacrene D	53.0 ± 1.96	-	1.72 ± 0.05	0.78 ± 0.05	-	-
55.98	95	1816	1816	Calamenene	3.95 ± 0.22	-	7.43 ± 0.19	-	-	-
59.31	104	1889	1901	α-Calacorene	45.0 ± 2.66	10.2 ± 1.89	2.33 ± 0.20	1.27 ± 0.09	-	7.99 ± 0.18
60.30	107	2010	2008	Nerolidol	8.49 ± 0.56	35.7 ± 5.37	0.21 ± 0.01	0.19 ± 0.03	1.79 ± 0.18	-
70.22	118	2177	2188	Cadalene	21.1 ± 2.85	6.44 ± 0.91	2.60 ± 0.30	1.37 ± 0.14	-	5.85 ± 0.65
71.48	120	2186	2187	τ-cadinol	-	-	-	0.58 ± 0.03	-	-
				Norisoprenoids						
54.83	91	1790	1797	β-Damascenone	4.01 ± 0.24	1.73 ± 0.23	-	0.68 ± 0.11	-	-
56.13	96	1820	1825	α-Ionene	38.2 ± 3.09	-	-	3.94 ± 0.72	-	28.5 ± 2.45
56.19	97	1821	1820	Geranylacetone	-	-	-	0.33 ± 0.06	-	-
59.65	105	1896	1896	β-Ionone	11.7 ± 2.11	-	0.16 ± 0.02	-	-	-

				Alcohols							
34.87	30	1354	1354	(*Z*)-3-Hexen-1-ol	-	-	-	-	5.90 ± 0.32	1.25 ± 0.12
35.84	33	1373	1373	(*E*)-2-Hexen-1-ol	3.87 ± 0.61	-	0.44 ± 0.02	0.46 ± 0.06	1.08 ± 0.07	-
49.96	74	1674	1692	(*E*)-2-Nonen-1-ol	-	-	-	-	1.77 ± 0.23	-
54.49	90	1782	1792	(E)-2-Decen-1-ol	51.1 ± 2.93	2.67 ± 0.45	2.88 ± 0.16	1.10 ± 0.06	77.6 ± 9.16	-
58.09	102	1863	1863	Phenylethyl alcohol	4.28 ± 0.38	1.56 ± 0.26	0.42 ± 0.06	0.15 ± 0.03	13.9 ± 1.27	-
62.32	109	2063	2076	1-Tridecanol	8.10 ± 1.35	2.35 ± 0.33	0.23 ± 0.04	0.67 ± 0.07	15.1 ± 0.95	-
				Carbonyl compounds						
26.36	16	1193	1192	2-Hexenal	-	-	-	-	6.29 ± 0.51	-
32.72	28	1312	1312	6-Methyl-5-hepten-2-one	0.62 ± 0.05	-	-	-	-	-
35.52	32	1367	1367	Nonanal	-	-	-	-	1.21 ± 0.08	-
41.11	49	1481	1480	Decanal	-	-	-	0.26 ± 0.05	434 ± 41.2	2.77 ± 0.07
41.87	51	1496	1496	Benzaldehyde	-	-	0.33 ± 0.01	-	0.67 ± 0.06	-
45.90	61	1582	1583	Undecanal	-	-	-	-	33.9 ± 5.49	-
47.88	66	1626	1628	(*E*)-2-Decenal	-	-	-	-	443 ± 50.3	2.33 ± 0.14
50.76	75	1692	1695	Dodecanal	-	-	-	-	11.1 ± 1.23	-
52.51	84	1734	1737	(*Z*)-7-Dodecenal	-	-	-	-	2.18 ± 0.20	-
52.58	85	1736	1743	(*E*,*Z*)-2,4-Decadienal	-	-	-	-	1.78 ± 0.22	-
55.12	92	1797	1794	Tridecanal	-	-	-	-	3.58 ± 0.31	-
57.00	100	1839	1830	(*E*)-2-Dodecenal	-	-	-	-	133 ± 17.5	-
59.22	103	1887	1888	(*Z*)-Cinnamaldehyde	2.37 ± 0.28	-	0.36 ± 0.04	0.11 ± 0.02	2.41 ± 0.27	-
63.27	110	2087	2084	(*E*)-Cinnamaldehyde	-	3.67 ± 0.30	-	0.37 ± 0.05	3.76 ± 0.44	-
70.07	117	2165	2171	Piperonal	-	-	-	0.27 ± 0.05	19.8 ± 0.20	-
71.55	121	2180	2205	Myristicin	1.96 ± 0.12	-	-	-	-	-
				Esters						
31.47	26	1288	1290	(*Z*)-3-Hexen-1-ol acetate	-	-	-	-	4.40 ± 0.45	2.85 ± 0.49
39.18	46	1441	1454	Octyl acetate	-	-	0.40 ± 0.02	-	-	-
45.01	58	1563	1554	Isobornyl acetate	63.6 ± 8.35	28.6 ± 1.10	3.64 ± 0.42	6.45 ± 0.71	-	-
46.25	63	1589	1581	Nonyl acetate	145 ± 7.42	21.3 ± 2.05	4.08 ± 0.48	22.6 ± 1.22	63.1 ± 7.39	-
49.20	71	1657	1650	Decyl acetate	-	-	-	-	2.02 ± 0.24	-
51.66	79	1713	1722	9-Decenyl acetate	-	-	-	-	2.77 ± 0.31	-
52.22	83	1727	1728	Geranyl acetate	54.7 ± 3.38	-	-	0.83 ± 0.02	-	-
53.08	86	1748	1745	Methyl salicylate	-	-	-	-	-	59.7 ± 1.64
62.16	108	2059	2059	(*Z*)-Methyl isoeugenol	91.1 ± 0.91	-	0.26 ± 0.03	0.37 ± 0.03	-	-
65.13	111	2115	2114	Bornyl benzoate	0.99 ± 0.08	2.44 ± 0.33	0.93 ± 0.08	0.50 ± 0.05	79.6 ± 9.08	-
68.05	113	2145	2126	(*E*)-Methyl isoeugenol	11.0 ± 1.11	-	4.31 ± 0.58	0.34 ± 0.02	-	-
				Volatile phenols						
59.83	106	1947	1959	*o*-Cresol	-	-	0.08 ± 0.01	-	-	-
67.51	112	2140	-	3-Allylguaiacol	1.03 ± 0.07	-	32.4 ± 1.24	1.33 ± 0.06	-	-
68.93	115	2154	2158	Eugenol	-	5.85 ± 0.76	0.30 ± 0.05	-	-	-
70.59	119	2170	2183	(*E*)-Isoeugenol	-	-	1.30 ± 0.20	-	-	-
				Furanic compounds						
37.90	40	1413	-	2-Methyl-2,3-dihydrobenzofuran	-	-	-	-	1.60 ± 0.23	229 ± 3.13
38.96	45	1436	1436	2-Furfural	-	-	-	-	0.57 ± 0.06	-
47.47	64	1616	1613	2-Furanmethanol	54.3 ± 2.43	-	0.88 ± 0.04	2.43 ± 0.07	-	-

				Sulphur compounds							
22.50	8	1121	-	Allyl isopropyl sulfide	-	2.42 ± 0.37	-	-	-	-
25.42	15	1176	1181	2,4-Dimethylthiophene	-	1.00 ± 0.02	-	-	-	-
27.57	19	1216	1218	Methyl propyl disulfide	-	2.08 ± 0.05	-	-	-	-
28.66	24	1237	1240	3,4-Dimethylthiophene	-	18.7 ± 1.98	-	-	-	-
35.31	31	1363	1365	Dipropyl disulfide	-	82.8 ± 7.32	-	1.69 ± 0.23	-	-
49.08	70	1654	1662	Dipropyl trisulfide	-	74.2 ± 13.6	-	-	-	-
52.09	82	1724	1723	2-Vinyl-1,3-dithiane	-	8.27 ± 1.22	-	-	-	-
54.34	89	1779	1775	*cis*-3,5-Diethyl-1,2,4-trithiolane	-	7.70 ± 0.98	-	-	-	-
				Others						
20.57	4	1083	1100	Undecane	-	-	-	-	2.60 ± 0.25	-
37.00	37	1394	-	3-Aminorhodanine	-	22.6 ± 2.11	-	-	-	-
37.03	38	1395	1394	α-*p*-Dimethylstyrene	54.3 ± 4.74	-	1.52 ± 0.28	0.94 ± 0.14	-	-
41.63	50	1492	-	*m/z* 105, 119, 161	42.5 ± 2.52	22.3 ± 3.72	2.49 ± 0.09	3.37 ± 0.45	-	25.0 ± 1.56
56.50	98	1828	-	Propanethioamide	-	3.50 ± 0.32	-	-	-	-
57.42	101	1848	-	4-Ethyl-*m*-xylene	5.07 ± 0.95	5.53 ± 0.88	0.20 ± 0.02	0.21 ± 0.02	-	-
68.39	114	2148	-	Iproniazid	-	6.59 ± 0.92	-	-	-	-

-: Not detected. [a] RT: Retention time. [b] Kovat index of relative n-alkanes (C8–C20) on a BP20 capillary column. [c] Relative Kovat index reported in literature for equivalent capillary columns [58].

Figure 12 illustrates the contribution of each chemical family to the overall volatile fingerprint of all analyzed culinary herbs. Monoterpenoids accounted for an average of

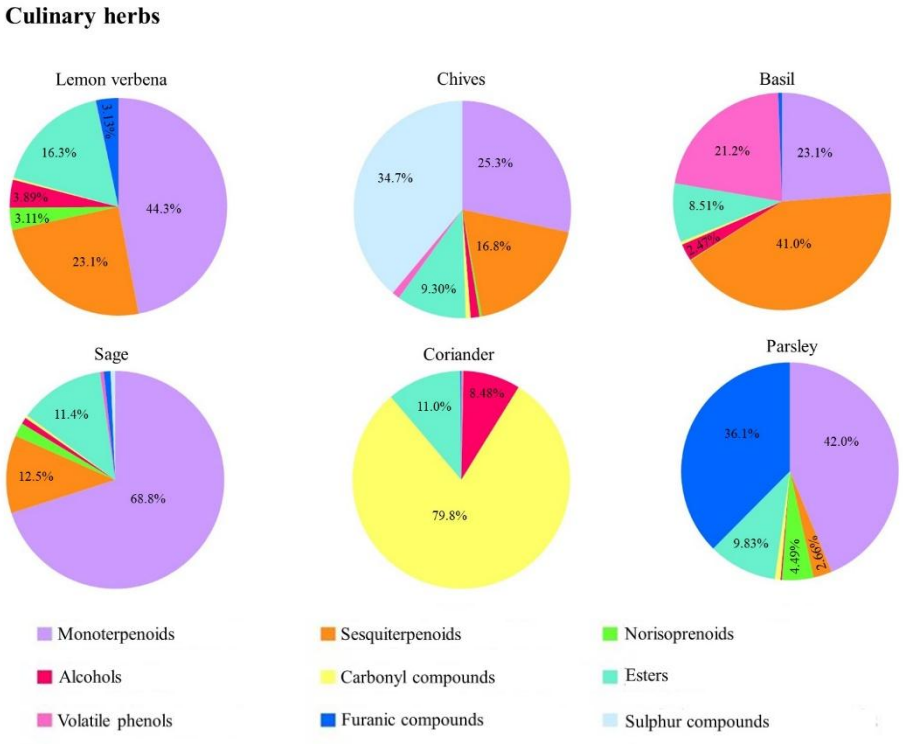


Figure 12. Contribution of each chemical family to the total volatile fingerprint of culinary herbs analyzed.

34.0 ± 0.99% of the total volatile fingerprint, whereas esters contributed 6.58 ± 1.27%, sesquiterpenoids 10.1 ± 2.21%, and carbonyl compounds 14.9 ± 1.41%.

From the obtained results, some of the VOMs were unique to specific culinary herbs. The specific VOMs identified in the different culinary herbs are listed in Table 2.

Table 2. Specific compounds identified in culinary herbs.

Lemon verbena	Chives	Basil	Sage	Coriander	Parsley
Limonene	Allyl isopropyl sulfide	Octyl acetate	Camphene	Decyl acetate	β-Myrcene
oxide	2,4-Dimethylthiophene	(*E*)-Isoeugenol	α-Thujone	9-Decenyl acetate	Fenchone
Phellandral	Methyl propyl disulfide	*o*-Cresol	(+)-Camphor	2-Furfural	Methyl salicylate
Myristicin	3,4-Dimethylthiophene				
	Dipropyl disulfide				
	Dipropyl trisulfide				
	cis-3,5-Diethyl-1,2,4-trithiolane				

Lemon verbena is a medicinal herb and source of antioxidant compounds (for example, VOMs, and polyphenols). In total, 55 VOMs were identified in lemon verbena, with the majority being monoterpenoids (42.2%), followed by sesquiterpenoids (22.0%), and esters (20.2%). The most prevalent VOMs were β-caryophyllene, β-citronellol, phellandral, geranial, nonyl acetate, and (*Z*)-methyl isoeugenol, contributing 47.9% of the total volatile fingerprint. Kim & Lee [38] and Rashid et al. [33] reported fewer VOMs using HS-SPME/GC-MS, identifying only 14 and 27, respectively, which align with the current findings. Chives, known for their use in food seasoning and health benefits, have 38 identified VOMs, predominantly sulfur compounds (34.8%), monoterpenoids (25.3%), and sesquiterpenoids (16.8%), comprising 76.8% of the volatile fingerprint. The key VOMs included dipropyl disulfide, dipropyl trisulfide, geranial, limonene, β-caryophyllene, and nerolidol, which accounted for 54.0% of the total volatile fingerprints. These results are consistent with those of the earlier studies by Dai et al. [27] and Hanif et al. [69]. Basil, known for its medicinal uses, has 45 identified VOMs, primarily sesquiterpenoids (41.2%), monoterpenoids (23.0%), and volatile phenols (21.2%). The major VOMs were 3-allylguaiacol, β-cubebene, and geranial, contributing 27.9%, 20.1%, and 8.4% of the total volatile fingerprint, respectively, which is in agreement with the findings of Du et al. [70] and Mahmoud et al. [28]. Sage, another herb with healing properties, had 54 VOMs, mostly monoterpenoids (68.8%), esters (12.5%), and sesquiterpenoids (11.4%), making up 92.3% of its volatile fingerprint. The predominant

VOMs, including isoborneol, (+)-camphor, β-thujone, eucalyptol, β-caryophyllene, and nonyl acetate, represented 65.7% of the total volatile fingerprint. These results align with those of Pachura et al. [31]. Coriander, used in both cooking and traditional medicine, had 37 VOMs identified, mostly carbonyl compounds (79.9%), esters (11.1%), and alcohols (8.40%). The dominant VOMs included decanal, (*E*)-2-decenal, (*E*)-2-dodecenal, (*E*)-2-decen-1-ol, bornyl benzoate, and nonyl acetate, which made up 79.9% of the volatile fingerprint, consistent with Wei et al. [34]. Parsley, which is widely used in food and pharmaceuticals, had 23 VOMs identified, predominantly monoterpenoids (42.1%), furanic compounds (36.0%), and esters (9.84%). The main VOMs were *o*-cymene, 2-methyl-2,3-dihydrobenzofuran, and methyl salicylate, representing 72.2% of the volatile fingerprint.

3.1.2. Volatile fingerprint of spices

Table 3 presents the retention time, Kovat index, chemical families, and relative peak area of the VOMs identified in spices.

Table 3. Relative peak area of VOMs identified in spices using HS-SPME/GC-MS.

RT (min)[a]	Peak Nº	KI cal[b]	KI lit[c]	Volatile organic metabolites	Spices					
					Curcuma	Nutmeg	Cumin	Black pepper	Jamaica pepper	Juniper berry
				Monoterpenoids						
17.23	1	1013	1013	α-Pinene	16.0 ± 0.63	-	5.67 ± 0.14	454 ± 33.6	8.75 ± 1.66	8.52 ± 0.46
17.88	2	1028	1021	α-Thujene	7.47 ± 0.40	121 ± 7.39	3.87 ± 0.37	3.19 ± 0.42	6.57 ± 0.64	-
19.01	3	1052	1052	Camphene	-	16.5 ± 2.61	-	8.63 ± 1.02	0.76 ± 0.14	35.2 ± 6.42
20.95	5	1090	1090	β-Pinene	11.7 ± 0.73	321 ± 33.1	90.5 ± 14.6	-	8.52 ± 0.44	210 ± 17.8
21.29	6	1096	1096	Sabinene	-	28.9 ± 4.02	7.25 ± 1.18	21.2 ± 0.55	9.07 ± 0.36	742 ± 21.0
22.24	7	1115	1114	3-Carene	5.55 ± 0.68	17.3 ± 2.38	-	1499 ± 210	4.23 ± 0.32	12.3 ± 1.90
22.76	9	1126	1128	β-Myrcene	8.91 ± 1.25	119 ± 19.9	4.88 ± 0.39	-	523 ± 33.3	1325 ± 125
22.97	10	1130	1140	α-Phellandrene	189 ± 10.3	61.1 ± 5.88	2.73 ± 0.16	325 ± 49.9	5.65 ± 0.86	4.69 ± 0.52
23.71	11	1145	1149	4-Carene	12.0 ± 1.91	303 ± 32.9	-	865 ± 58.7	5.56 ± 0.54	25.6 ± 0.87
24.49	12	1159	1155	Limonene	21.5 ± 2.72	251 ± 30.8	5.44 ± 0.21	1350 ± 114	36.4 ± 3.89	375 ± 33.2
24.71	13	1163	1163	β-Phellandrene	-	-	-	-	15.6 ± 2.15	38.1 ± 3.31
25.17	14	1172	1173	Eucalyptol	59.1 ± 9.71	10.7 ± 1.64	5.30 ± 0.78	-	154 ± 3.59	9.45 ± 1.27
26.59	17	1197	1195	β-Ocimene	-	146 ± 18.2	6.28 ± 1.16	-	4.81 ± 0.22	1.82 ± 0.02
26.86	18	1201	1200	γ-Terpinene	20.9 ± 1.73	618 ± 119	127 ± 9.61	56.8 ± 4.16	10.1 ± 1.10	76.3 ± 4.18
27.67	20	1218	1220	*p*-Menth-4(8)-ene	-	-	-	-	78.0 ± 4.62	-
28.40	22	1232	1232	*p*-Cymene	150 ± 4.88	262 ± 40.7	93.9 ± 18.2	120 ± 15.7	40.2 ± 4.23	76.1 ± 4.73
28.57	23	1235	1248	*o*-Cymene	-	-	-	-	-	-
30.53	25	1272	1284	δ-Terpinene	15.8 ± 2.66	172 ± 33.9	3.71 ± 0.11	33.3 ± 2.31	15.8 ± 1.82	69.0 ± 1.99
33.64	29	1330	1330	Alloocimene	1.86 ± 0.08	2.37 ± 0.30	-	1.30 ± 0.04	1.25 ± 0.17	43.4 ± 0.46

35.92	34	1374	1383	Fenchone	-	-	-	1.14 ± 0.18	-	5.86 ± 0.38
36.58	36	1387	1405	Perillene	-	-	-	-	1.40 ± 0.22	6.22 ± 0.37
38.27	42	1421	1421	α-Thujone	-	-	-	-	-	4.68 ± 0.52
38.47	43	1425	1425	β-Thujone	-	-	-	1162 ± 185	-	-
38.93	44	1435	1437	β-Terpineol	-	38.3 ± 4 .14	0.62 ± 0.04	1.52 ± 0.12	-	27.6 ± 0.72
42.34	53	1506	1506	Linalool	-	14.8 ± 2.24	1.35 ± 0.18	-	19.7 ± 0.79	14.4 ± 0.14
43.48	55	1531	1542	Limonene oxide	13.9 ± 0.77	10.2 ± 1.27	-	-	1.73 ± 0.23	-
44.47	56	1552	1555	α-Fenchol	-	-	63.1 ± 10.3	-	-	-
45.57	59	1575	1573	Hotrienol	11.2 ± 1.80	396 ± 34.9	5.34 ± 0.99	-	49.8 ± 5.73	165 ± 5.35
48.52	68	1641	1649	Estragole	-	-	-	25.4 ± 1.08	48.3 ± 7.07	-
49.55	72	1665	1665	α-Terpineol	-	32.0 ± 5.89	-	-	-	-
51.03	76	1698	1700	Phellandral	-	-	17.3 ± 1.90	-	-	-
51.39	78	1707	1705	(*E*)-Piperitol	-	12.6 ± 0.82	-	-	-	-
53.53	87	1759	1756	β-Citronellol	46.8 ± 5.84	-	-	-	3.23 ± 0.31	-
54.04	88	1771	1771	Cuminaldehyde	2360 ± 199	-	2439 ± 162	-	-	-
55.21	93	1799	1798	Anethole	4.87 ± 0.35	4.61 ± 0.21	-	-	1.90 ± 0.35	19.7 ± 0.99
55.61	94	1808	1809	*p*-Cymen-8-ol	8.09 ± 0.29	4.09 ± 0.30	-	6.34 ± 0.77	3.26 ± 0.57	-
56.93	99	1838	1842	Geraniol	13.2 ± 1.99	430 ± 37.0	24.4 ± 1.50	7.12 ± 0.48	-	-
69.11	116	2155	2173	Carvacrol	-	5.90 ± 1.06	12.3 ± 0.58	-	-	-
				Sesquiterpenoids						
42.76	54	1515	1529	β-Bourbonene	-	-	-	730 ± 91.6	-	-
45.77	60	1579	1579	α-Bergamotene	21.2 ± 1.62	-	-	-	-	-
46.09	62	1586	1589	Aromandrene	-	-	-	-	-	167 ± 19.7
49.00	69	1652	1653	β-Farnesene	74.1 ± 13.5	-	8.93 ± 0.37	-	-	-
51.68	80	1714	1704	γ-Curcumene	239 ± 10.1	-	-	-	-	-
51.90	81	1719	1718	Germacrene D	-	-	-	-	-	637 ± 22.2
59.31	104	1889	1901	α-Calacorene	-	2.54 ± 0.21	-	4.91 ± 0.03	-	8.16 ± 0.58
71.48	120	2186	2187	τ-cadinol	2184 ± 280	-	-	10.4 ± 1.27	12.4 ± 2.19	-
				Norisoprenoids						
56.13	96	1820	1825	α-Ionene	-	8.79 ± 0.63	-	24.2 ± 2.18	-	19.9 ± 0.39
				Alcohols						
54.49	90	1782	1792	(*E*)-2-Decen-1-ol	-	-	528 ± 46.9	-	-	-
58.09	102	1863	1863	Phenylethyl alcohol	-	-	-	-	2.25 ± 0.23	-
				Carbonyl compounds						
31.82	27	1294	1291	2-Heptenal	-	-	1.04 ± 0.19	-	1.51 ± 0.26	-
32.72	28	1312	1312	6-Methyl-5-hepten-2-one	2.40 ± 0.07	1.10 ± 0.21	-	-	-	1.46 ± 0.10
36.29	35	1381	1388	3-Octen-2-one	0.84 ± 0.04	-	1.62 ± 0.01	-	-	-
41.87	51	1496	1496	Benzaldehyde	6.03 ± 0.15	-	4.30 ± 0.53	-	4.04 ± 0.73	-
59.22	103	1887	1888	(*Z*)-Cinnamaldehyde	32.7 ± 1.54	-	6.52 ± 0.57	8.35 ± 1.07	-	-
63.27	110	2087	2084	(*E*)-Cinnamaldehyde	-	21.2 ± 3.03	4.02 ± 0.30	6.70 ± 1.18	-	-
70.07	117	2165	2171	Piperonal	-	-	-	27.5 ± 2.88	9.89 ± 1.49	-
71.55	121	2180	2205	Myristicin	-	1021 ± 76.5	12.0 ± 1.67	-	-	-
				Esters						
39.18	46	1441	1454	Octyl acetate	-	-	-	-	3.20 ± 0.35	-
45.01	58	1563	1554	Isobornyl acetate	-	21.6 ± 2.02	3.79 ± 0.12	-	-	33.2 ± 1.46

52.22	83	1727	1728	Geranyl acetate	-	23.7 ± 2.87	-	28.6 ± 2.44	4.43 ± 0.77	295 ± 40.9
53.08	86	1748	1745	Methyl salicylate	-	-	-	-	12.4 ± 1.26	-
62.16	108	2059	2059	(*Z*)-Methyl isoeugenol	-	343 ± 53.9	4.80 ± 0.60	37.6 ± 7.10	2976 ± 416	194 ± 4.60
65.13	111	2115	2114	Bornyl benzoate	-	1.75 ± 0.06	68.6 ± 3.48	-	-	-
68.05	113	2145	2126	(*E*)-Methyl isoeugenol	-	57.4 ± 4.27	4.10 ± 0.19	-	39.0 ± 6.11	-
				Volatile phenols						
67.51	112	2140	-	3-Allylguaiacol	-	41.9 ± 3.33	-	-	8.05 ± 1.28	-
68.93	115	2154	2158	Eugenol	-	-	-	-	1626 ± 282	79.0 ± 2.23
70.59	119	2170	2183	(*E*)-Isoeugenol	-	-	-	-	13.8 ± 2.53	-
				Furanic compounds						
47.47	64	1616	1613	2-Furanmethanol	-	-	-	-	2.25 ± 0.25	-
				Others						
37.03	38	1395	1394	α-*p*-Dimethylstyrene	16.7 ± 1.41	17.4 ± 2.90	3.77 ± 0.22	13.2 ± 1.09	6.80 ± 0.98	10.2 ± 0.81
38.06	41	1417	1417	Acetic acid	-	-	-	3.35 ± 0.36	17.4 ± 2.38	7.79 ± 1.22
39.49	47	1447	1449	Tetramethyl pyrazine	5.37 ± 0.49	-	-	-	-	-
39.73	48	1452	-	2,4-Quinolinediol	-	14.8 ± 2.86	-	-	-	173 ± 3.57
47.97	67	1628	-	2,6-Dimethyl-2,6-octadiene	-	15.7 ± 1.80	-	-	-	-

-: Not detected. a RT: Retention time. b Kovat index of relative n-alkanes (C8–C20) on a BP20 capillary column. c Relative Kovat index reported in literature for equivalent capillary columns [58].

Monoterpenoids represented 62.6 ± 1.31% of the total volatile fingerprint, esters 12.2 ± 1.80%, sesquiterpenoids 10.8 ± 3.27% and carbonyl compounds 3.47 ± 2.78% (Figure 13).

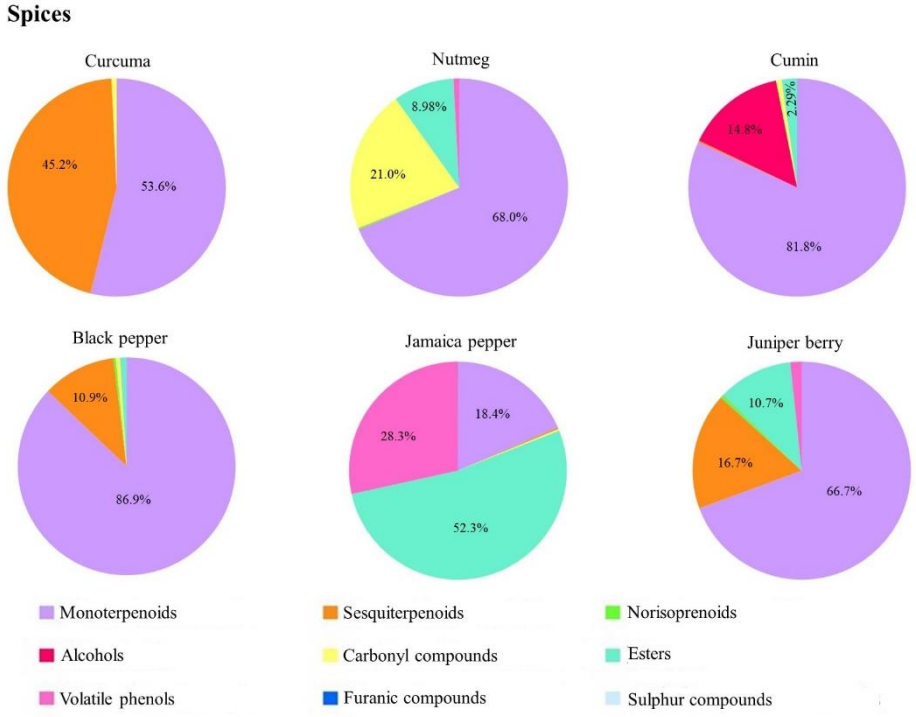


Figure 13. Contribution of each chemical family to the total volatile fingerprint of spices analyzed.

Based on the results obtained, certain VOMs were exclusive to particular spices. In Table 4 presents the specific VOMs identified in the spices.

Table 4. Specific compounds identified in spices.

Curcuma	Nutmeg	Cumin	Black pepper	Jamaica pepper	Juniper berry
α-Bergamotene	α-Terpineol	Phellandral	β-Thujone	*p*-Menth-4(8)-ene	Aromandrene
γ-Curcumene	(*E*)-Piperitol	(*E*)-2-Decen-1-ol	β-Bourbonene	Phenylethyl alcohol	Germacrene D
		α-Fenchol		Octyl acetate	
				Methyl salicylate	
				(*E*)-Isoeugenol	
				2-Furanmethanol	

A total of 30 VOMs were identified in curcuma, where the chemical families of monoterpenoids (53.6%) and sesquiterpenoids (45.3%) were the most abundant, representing 98.9% of their total volatile fingerprint. The most abundant VOMs in the analyzed curcuma were cuminaldehyde, τ-cadinol, and γ-curcumene, representing 42.4, 39.3, and 3.43% of the total volatile fingerprint, respectively. Qiang et al. [32] used the HS-SPME/GC-MS methodology to establish the volatile fingerprint of curcuma essential oil and identified curcumene as one of the most predominant VOM. Nutmeg is a traditional spice commonly used worldwide. Myristicin, present in nutmeg, has antioxidant, anti-inflammatory, and neuroprotective properties [30]. A total of 39 VOMs were tentatively identified, mainly monoterpenoids (68.1%), carbonyl compounds (20.9%), and esters (8.97%). Myristicin, γ-terpinene, hotrienol, and (*Z*)-methyl isoeugenol represented 20.5%, 12.4%, 7.94, and 6.87% of their total volatile fingerprints, respectively. Cumin, another popular spice, has been studied because of its favorable antioxidant, anti-inflammatory, antibacterial, and antidiabetic properties. In total, 33 VOMs were identified in cumin. The total volatile fingerprints of this spice were highly influenced by monoterpenoids, representing 81.8% of the total volatile fingerprints. Alcohols and esters were also identified but contributed to a lesser extent to the volatile fingerprint of cumin (14.8% and 2.29%, respectively). Cuminaldehyde, γ-terpinene, β-pinene, and *p*-cymene accounted for 77.0% of the total volatile fingerprints. A total of 29 VOMs were identified in black pepper, mainly monoterpenoids (86.9%) and sesquiterpenoids (10.9%), representing 97.8% of their total volatile fingerprints. The dominant VOMs identified in black pepper were 3-carene, β-thujone, limonene, 4-carene, and β-bourbonene, representing 82.0% of the total volatile fingerprints. Several VOMs

identified in our study agreed with the data provided in a previous study [29]. Because of its volatile fingerprint, Jamaica pepper is used in cooking and traditional medicine. The two major VOMs in Jamaica pepper are eugenol and methyl eugenol, and their content is dependent on the individual tree and harvest time [26]. Forty-two VOMs were tentatively identified in Jamaica pepper. Esters (52.4%), volatile phenols (28.4%), and monoterpenoids (18.2%) were the most abundant chemical families detected in Jamaica pepper, representing 99.0% of the total volatile fingerprints. (*Z*)-Methyl isoeugenol and eugenol were the main VOMs identified in Jamaica pepper, accounting for 51.3% and 28.1% of the total volatile fingerprints, respectively. Thirty-five VOMs were identified in juniper berry. Monoterpenoids (67.0%), sesquiterpenoids (16.5%), and esters (10.6%) were the main chemical families found in juniper berry, representing 94.1% of their total volatile fingerprints (Figure 13). This contribution was mainly provided by β-myrcene, germacrene D, and sabinene, which accounted for 26.9%, 12.9%, and 15.1% of the total volatile fingerprints of juniper berry, respectively.

3.2. Statistical analysis

In the current study, the VOMs identified in the culinary herbs and spices were analyzed using PLS-DA to explore the differences between the two groups. As illustrated in Figure 14, the analysis revealed a clear distinction between culinary herbs and spices. Based on VIP scores greater than 1.5 (Figure 14b), the key VOMs responsible for distinguishing between the two groups were β-pinene (#5), 3-carene (#7), β-myrcene (#9), α-phellandrene (#10), 4-carene (#11), limonene (#12), γ-terpinene (#18), *p*-cymene (#22), hotrienol (#59), nonyl acetate (#63), β-caryophyllene (#65), isoborneol (#73), cuminaldehyde (#88), (*Z*)-methyl isoeugenol (#108), and τ-cadinol (#120).

Figure 15 presents the hierarchical cluster analysis (HCA) and heat map for the VOMs with VIP values greater than 1.5, using the average algorithm and Pearson distance analysis. This visualization highlights the dataset's patterns, often used to identify samples or features with notably high or low values. The heatmap indicated that nonyl acetate (#63), β-caryophyllene (#65), and isoborneol (#73) were positively correlated with culinary herbs. In contrast, spices were strongly linked to VOMs such as β-pinene (#5), 3-carene (#7), β-myrcene (#9), α-phellandrene (#10), 4-carene (#11), limonene (#12), γ-terpinene (#18), *p*-cymene (#22), hotrienol (#59), cuminaldehyde (#88), (*Z*)-methyl isoeugenol (#108), and τ-cadinol (#120).

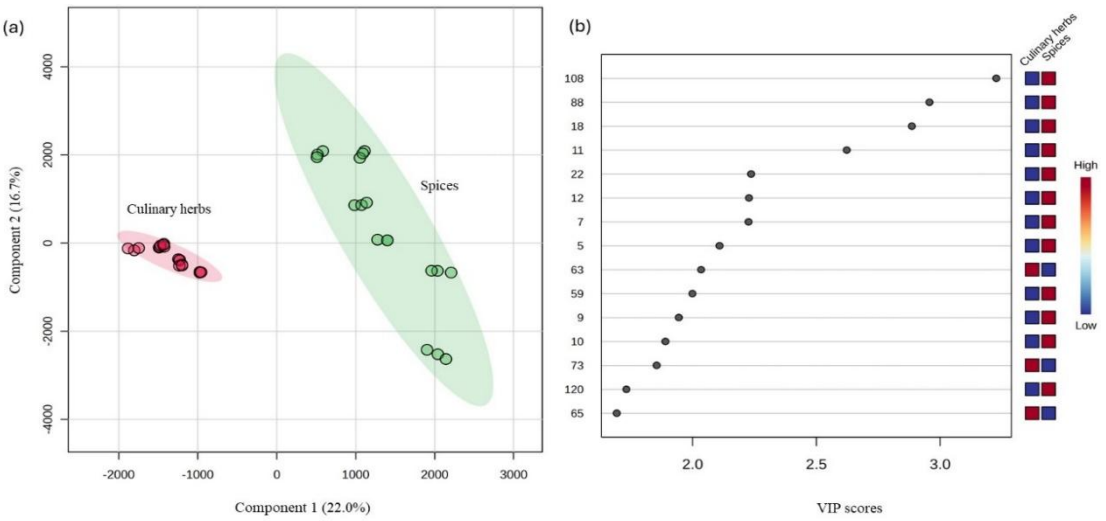


Figure 14. PLS-DA of the total volatile fingerprint of the culinary herbs and spices (n = 3 for each data point): (a) score scatter plot and (b) VIP scores.

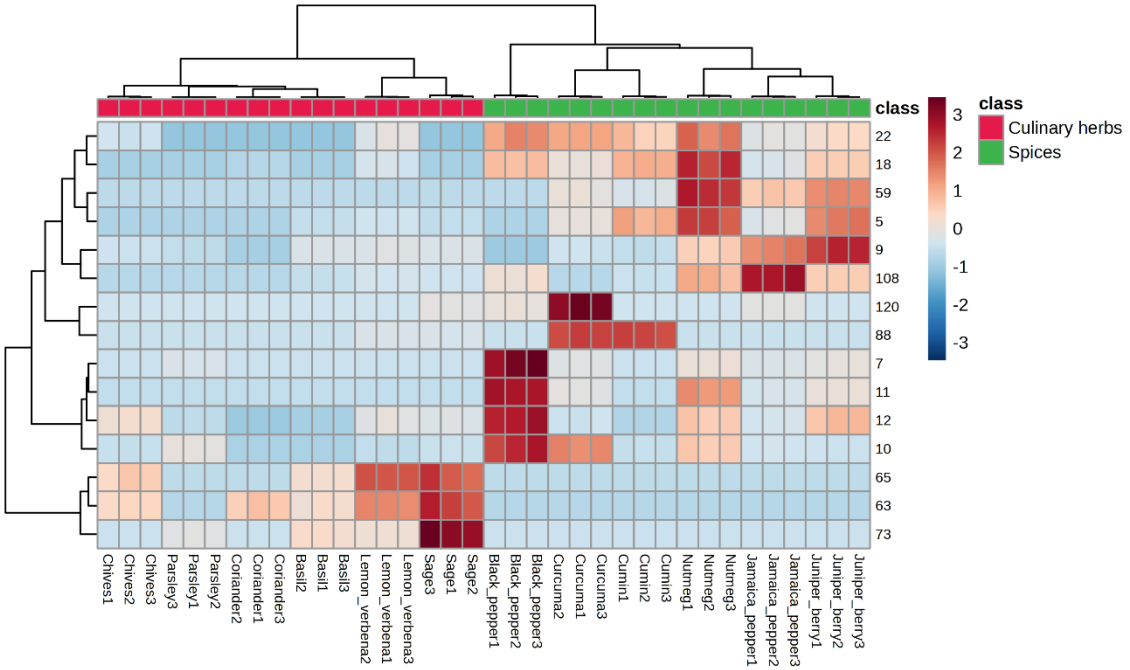


Figure 15. HCA and heatmap of the investigated culinary herbs and spices, generated by the average algorithm and Pearson distance analysis.

3.3. Molecular docking analysis

The primary VOMs found in culinary herbs and spices exhibited diverse binding energies for the target proteins (AChE, BChE, MAO-B, iNOS, 5-LOX, and COX-2), as indicated by ΔG. Contemporary drug design protocols suggest that suitable candidates

should have ΔG values below -6.0 kcal/mol. However, there is no agreed-upon range for the binding energies of physiologically active substances [71]. Table 5 reveals that cuminaldehyde, β-caryophyllene, γ-curcumene, germacrene D, and τ-cadinol displayed ΔG values under -6.0 kcal/mol for all examined receptors, suggesting their potential as anti-Alzheimer, antioxidant, and anti-inflammatory inhibitors. Nonetheless, these VOMs did not interact with COX-2 at docking scores lower than those of its inhibitors, specifically celecoxib (-8.666 kcal/mol) and meclofenamic acid (-8.477 kcal/mol). Additionally, as shown in Figure 16, cuminaldehyde interacted with 5-LOX at a docking score lower than nordihydroguaiaretic acid (-6.077 kcal/mol), while β-caryophyllene, γ-curcumene, germacrene D, and τ-cadinol exhibited docking scores lower than both nordihydroguaiaretic acid (-6.077 kcal/mol) and zileuton (-6.714 kcal/mol). β-caryophyllene, germacrene D, and τ-cadinol exhibited stronger interactions with AChE and BChE compared to the inhibitor rivastigmine, as evidenced by their lower docking scores of -6.359 and -6.367 kcal/mol, respectively. τ-cadinol formed multiple hydrogen bonds with AChE (three bonds: SER 293 and TYR341) and BChE (one bond: THR 120), enhancing the stability of the complex, as shown in Figure 17. β-caryophyllene demonstrated superior interaction with AChE (-8.240 kcal/mol) compared to other inhibitors like donepezil (-7.439 kcal/mol), galantamine (-7.535 kcal/mol), and huperzine A (-7.557 kcal/mol). Regarding antioxidant receptors, γ-curcumene was the only compound that interacted with MAO-B at a lower docking score (-8.208 kcal/mol) than safinamide (-8.125 kcal/mol), as illustrated in Figure 18. Although there are no FDA-approved inhibitors for iNOS, however nitroarginine and 1400 W are commonly used despite the lack of FDA approval, possibly owing to efficacy or safety concerns [72]. Notably, cuminaldehyde (-7.220 kcal/mol), γ-curcumene (-7.975 kcal/mol), and τ-cadinol (-7.695 kcal/mol) showed stronger interactions with iNOS compared to the ΔG values of nitroarginine (-5.584 kcal/mol) and 1400W (-6.761 kcal/mol).

Conversely, although (*E*)-2-decen-1-ol, decanal, (*E*)-2-decenal, nonyl acetate, dipropyl disulfide, and dipropyl trisulfide were identified as primary VOMs in certain examined samples, but computational molecular docking simulations failed to indicate any potential for these VOMs to function as inhibitors of the receptors chosen in this investigation.

Table 5. Interacting residues, binding affinities (ΔG: kcal/mol), the number of hydrogen bonds (H-b), and residues H-bonding (Residues H-b) obtained through docking simulations.

	Anti-Alzheimer receptors						Antioxidants receptors						Anti-inflammatory receptors					
	AChE			BChE			MAO-B			iNOS			5-LOX			COX-2		
Substrate	**ΔG**	**H-b**	**Res. H-b**	**ΔG**	**H-b**	**Res. H-b**	**ΔG**	**H-b**	**Res. H-b**	**ΔG**	**H-b**	**Res. H-b**	**ΔG**	**H-b**	**Res. H-b**	**ΔG**	**H-b**	**Res. H-b**
Acetylcholine	-3.634	1	HIS 405	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Butyrylcholine	-	-	-	-4.033	1	TYR 128	-	-	-	-	-	-	-	-	-	-	-	-
2-Phenylethylamine	-	-	-	-	-	-	-6.266	2	ALA 263 TYR 393	-	-	-	-	-	-	-	-	-
L-arginine	-	-	-	-	-	-	-	-	-	-6.564	10	CYS 15 CYS 110 CYS 115 GLY 117 SER 118 ALA 459 TRP 461 MET 480	-	-	-	-	-	-
Arachidonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-4.494	6	ARG 370 SER 447 THR 545	-5.425	0	-
Inhibitor																		
Donepezil	-7.439	0	-	-8.835	1	THR 120	-	-	-	-	-	-	-	-	-	-	-	-
Galantamine	-7.535	5	TYR 124 SER 293 PHE 295 TYR 341	-8.230	3	ALA 199 TYR 440	-	-	-	-	-	-	-	-	-	-	-	-
Rivastigmine	-6.359	1	TYR 341	-6.367	1	THR 120	-	-	-	-	-	-	-	-	-	-	-	-
Huperzine A	-7.557	4	ASP 74 TYR 124 TRP 286 PHE 295	-8.960	7	ASP 70 THR 120 GLU 197 SER 198 ALA 199	-	-	-	-	-	-	-	-	-	-	-	-
Safinamide	-	-	-	-	-	-	-8.125	1	GLY 434	-	-	-	-	-	-	-	-	-

Nitroarginine	-	-	-	-	-	-	-	-	-	-5.584	7	SER 118 ILE 201 THR 376 ARG 381 TRP 461 ILE 462	-	-	-	-	-	-
1400W	-	-	-	-	-	-	-	-	-	-6.761	1	PRO 350	-	-	-	-	-	-
Zileuton	-	-	-	-	-	-	-	-	-	-	-	-	-6.714	3	HIS 225 GLN 656 LEU 657	-	-	-
Nordihydroguaiaretic acid	-	-	-	-	-	-	-	-	-	-	-	-	-6.077	6	HIS 360 GLN 363 THR 364 HIS 550	-	-	-
Celecoxib	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-8.666	9	ARG 61 SER 121 HIS 122 ILE 124 ASP 125 LYS 546
Meclofenamic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-8.477	1	VAL 344
VOMs																		
3-Carene	-6.627	0	-	-5.619	0	-	-6.966	0	-	-6.714	0	-	-6.073	0	-	-6.356	0	-
β-Myrcene	-5.655	0	-	-4.643	0	-	-5.298	0	-	-5.931	0	-	-5.361	0	-	-5.326	0	-
Limonene	-6.501	0	-	-5.854	0	-	-6.853	0	-	-7.103	0	-	-6.607	0	-	-6.710	0	-
Eucalyptol	-5.211	0	-	-5.657	0	-	-6.520	3	SER 59 TYR 60 LYS 296	-5.263	0	-	-5.802	0	-	-6.174	0	-
γ-Terpinene	-6.505	0	-	-5.89	0	-	-6.845	0	-	-7.247	0	-	-6.283	0	-	-6.561	0	-
p-Cymene	-6.759	0	-	-5.931	0	-	-6.890	0	-	-7.331	0	-	-6.656	0	-	-6.555	0	-
β-Thujone	-6.148	0	-	-5.769	0	-	-6.387	3	SER 59 GLN 65 MET 436	-6.201	1	TRP 90	-5.582	0	-	-6.320	0	-
Isoborneol	-5.180	2	ASN 233	-5.669	1	TYR 332	-6.506	3	ILE 198 GLY 205 GLN 206	-5.483	2	THR 376 VAL 465	-5.508	0	-	-5.782	1	VAL 349
Geranial	-4.572	0	-	-4.727	0	-	-5.819	1	TYR 188	-6.431	1	TYR 491	-5.816	1	ARG 221	-5.625	1	TYR 348

Cuminaldehyde	-6.344	0	-	-6.131	1	TYR 440	-6.932	0	-	-7.220	0	-	-6.582	0	-	-6.516	0	-
β-Caryophyllene	-8.240	0	-	-7.083	0	-	-7.792	0	-	-6.981	0	-	-6.874	0	-	-7.439	0	-
γ-Curcumene	-6.988	0	-	-6.192	0	-	-8.208	0	-	-7.975	0	-	-7.099	0	-	-6.897	0	-
Germacrene D	-6.631	0	-	-6.721	0	-	-7.935	0	-	-6.561	0	-	-6.882	0	-	-6.658	0	-
τ-Cadinol	-7.552	3	SER 293 TYR 341	-7.310	1	THR 120	-7.516	2	TYR 60 LYS 296	-7.695	1	VAL 352	-6.736	0	-	-7.521	0	-
(*E*)-2-Decen-1-ol	-4.821	4	TYR 124 TYR 337 TYR 341	-4.289	3	PHE 371 HIS 372 ASP 375	-5.473	2	CYS 172 TYR 435	-4.464	2	SER 118 GLN 478	-4.689	2	ARG 221 GLN 656	-4.549	1	SER 353
Decanal	-4.600	0	-	-3.948	0	-	-4.698	1	CYS 172	-4.773	2	GLY 371 MET 374	-4.409	1	ILE 320	-4.943	0	-
(*E*)-2-Decenal	-4.923	1	PHE 295	-4.144	1	THR 120	-5.267	1	CYS 397	-4.641	1	THR 376	-5.112	0	-	-4.921	2	ALA 516 ILE 517
Myristicin	-6.155	3	TYR 124 PHE 295 ARG 296	-6.290	1	THR 120	-6.815	4	ARG 42 LYS 296 CYS 397	-7.203	0	-	-5.911	3	TYR 81 TYR 383	-6.782	1	PHE 529
Nonyl acetate	-5.071	3	TYR 124 PHE 295 ARG 296	-4.204	0	-	-4.906	1	CYS 397	-4.664	0	-	-4.111	1	THR 366	-5.546	1	PHE 529
(*Z*)-Methyl isoeugenol	-5.724	1	PHE 295	-5.579	3	THR 120 SER 198	-6.483	1	TYR 435	-6.351	1	CYS 200	-5.654	2	THR 366 ARG 457	-6.272	0	-
3-Allylguaiacol	-5.870	2	PHE 295 ARG 296	-5.721	2	SER 198 ALA 199	-6.718	1	VAL 235	-6.855	3	CYS 200 SER 242 ASN 370	-5.331	4	ARG 370 SER 447 THR 545	-6.068	0	-
2-Methyl-2,3-dihydrobenzofuran	-6.216	1	TYR 124	-6.018	0	-	-7.138	2	GLY 12 ALA 35	-7.223	1	SER 242	-5.972	0	-	-6.183	0	-
Dipropyl disulfide	-3.116	0	-	-3.210	0	-	-4.086	0	-	-3.880	0	-	-3.529	0	-	-3.614	0	-
Dipropyl trisulfide	-3.160	0	-	-3.351	0	-	-3.935	0	-	-4.125	0	-	-3.565	0	-	-3.826	0	-

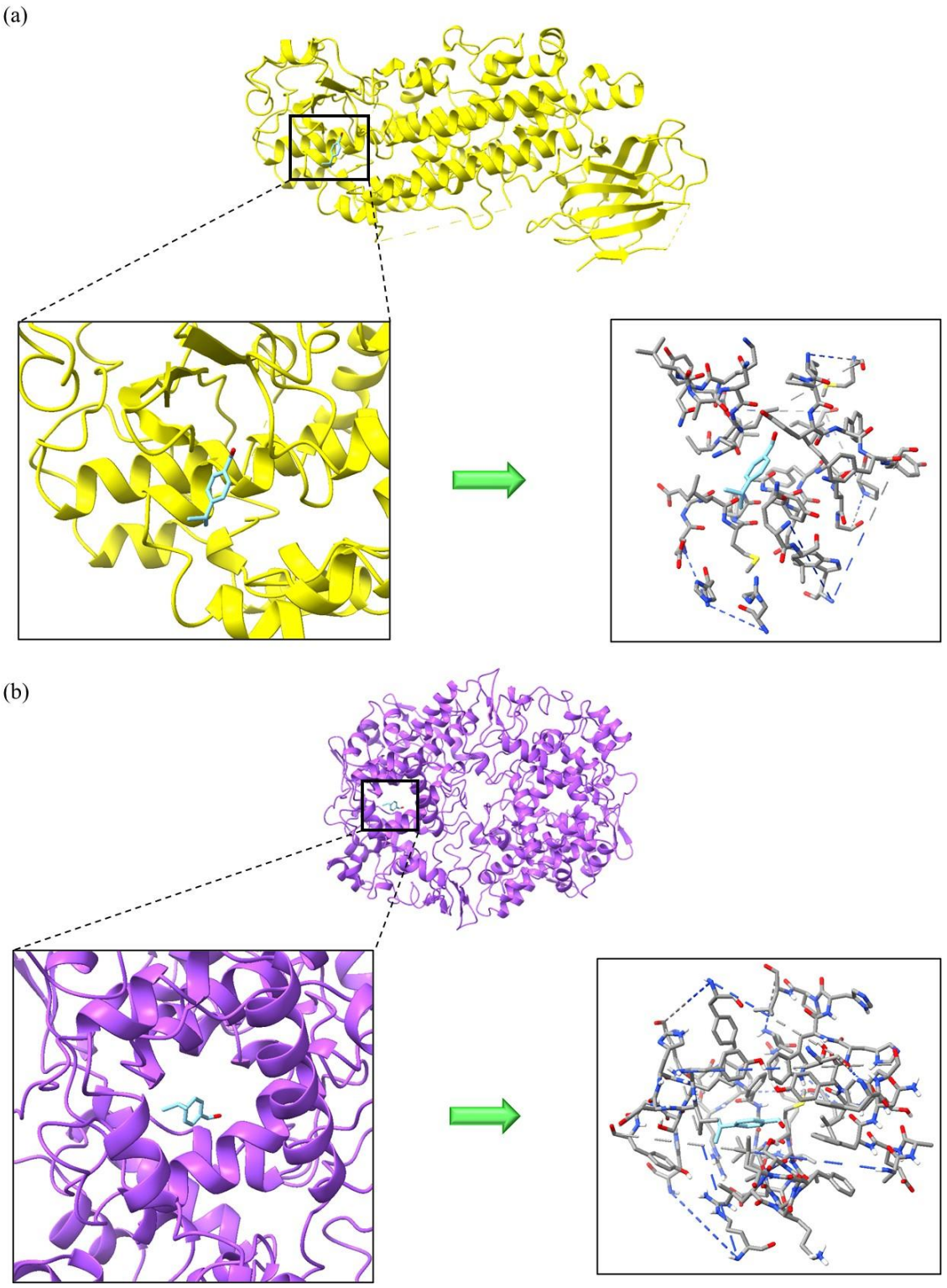


Figure 16. 5-Lipoxygenase (a) and cyclooxygenase-2 (b) in complex with cuminaldehyde.

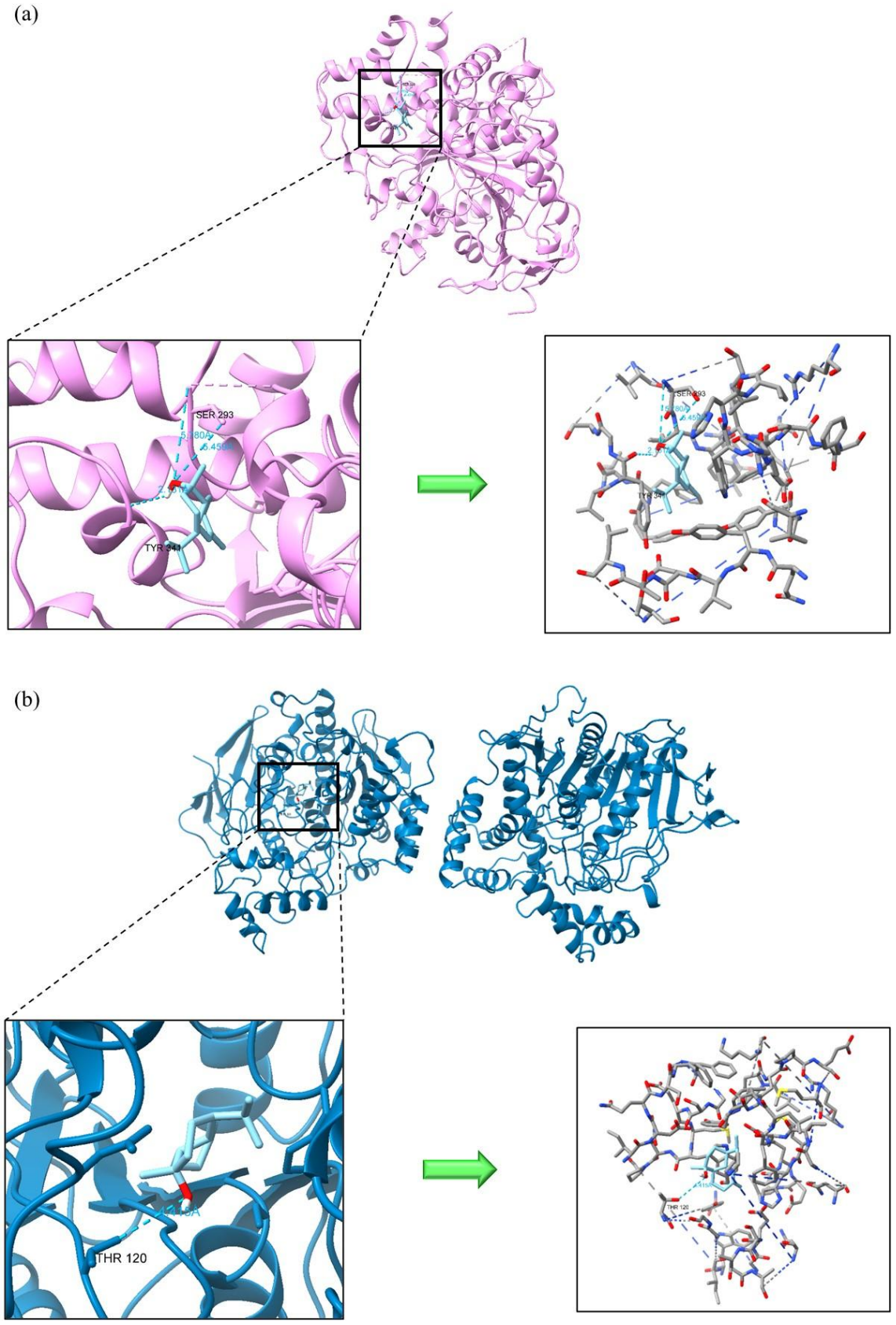


Figure 17. Acetylcholinesterase (a) and butyrylcholinesterase (b) in complex with τ-Cadinol.

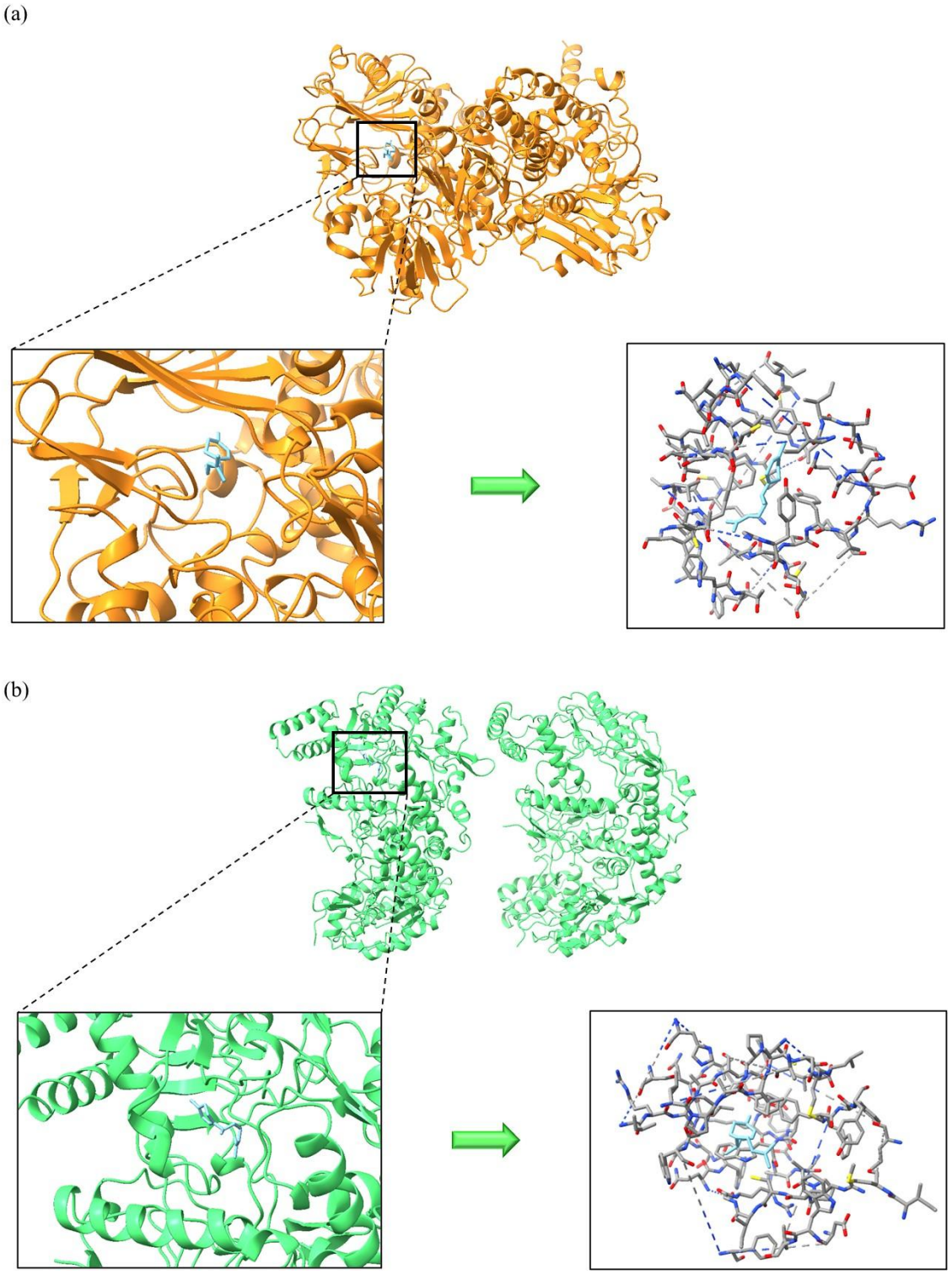


Figure 18. Monoamine oxidase B (a) and inducible nitric oxide synthase (b) in complex with γ-curcumene.

3.4. Assessment of the total phenolic, flavonoids, antioxidant and anti-inflammatory activities of culinary herbs and spices

The total phenolic, flavonoids, antioxidant and anti-inflammatory activities of the culinary herbs and spices were analyzed through *in vitro* assays (TPC, TFC, TAC, DPPH, ABTS, ORAC, and inhibition of protein denaturation). The TPC of aqueous extracts of the culinary herbs and spices using the Folin-Ciocalteu assay is presented in Table 6. Jamaica pepper demonstrated the highest TPC level, 65.4 mg GAE/g DW, and lemon verbena the lowest, 2.50 mg GAE/g. On average, the TPC levels in spices (22.3 ± 0.45 mg GAE/g DW) are 4.37 times higher than those determined in culinary herbs (5.11 ± 0.15 mg GAE/g DW). No statistically significant differences ($p < 0.05$) in TPC levels were observed between chives, coriander and curcurma, as well as between basil, sage, curcuma, and nutmeg.

Table 6. Total phenolic content (TPC), total flavonoid content (TFC), total anthocyanin content (TAC), antioxidant capacity (DPPH, ABTS, ORAC), and inhibition of protein denaturation of the culinary herbs and spices. The values are expressed as mean ± SD per gram of dry weight (DW).

Samples	TPC mg GAE/g	TFC mg QE/g	TAC mg C3GE/g	DPPH mg TE/g	ABTS mg TE/g	ORAC µM TE/g	Protein denaturation (%)
Lemon verbena	2.50 ± 0.01^{a}	2.28 ± 0.02^{a}	n.d.	1.97 ± 0.05^{a}	5.85 ± 0.48^{a}	1.70 ± 0.09^{a}	3.65 ± 0.17^{a}
Chives	4.65 ± 0.12^{b}	$3.35 \pm 0.20^{a,b}$	n.d.	6.78 ± 1.05^{b}	20.7 ± 1.40^{b}	63.9 ± 2.40^{b}	32.2 ± 1.58^{b}
Basil	$6.06 \pm 0.02^{b,c}$	$2.02 \pm 0.01^{a,b}$	0.70 ± 0.01^{a}	4.79 ± 0.44^{b}	$7.78 \pm 0.75^{a,c}$	48.1 ± 0.82^{c}	37.9 ± 0.57^{b}
Sage	6.73 ± 0.13^{c}	$3.49 \pm 0.23^{a,b}$	2.06 ± 0.06^{b}	11.8 ± 0.82^{c}	$15.9 \pm 1.93^{b,c}$	87.1 ± 6.90^{d}	47.3 ± 1.10^{c}
Coriander	$5.39 \pm 0.36^{b,c}$	$1.16 \pm 0.05^{a,c}$	n.d.	5.72 ± 0.25^{b}	34.8 ± 1.32^{d}	46.7 ± 0.63^{c}	10.3 ± 0.35^{d}
Parsley	$5.36 \pm 0.24^{b,c}$	$1.10 \pm 0.01^{a,c}$	n.d.	5.70 ± 0.79^{b}	$25.4 \pm 2.61^{b,c}$	$94.4 \pm 9.60^{d,e}$	48.9 ± 1.94^{c}
Curcuma	$6.16 \pm 0.21^{b,c}$	$0.99 \pm 0.01^{a,c}$	23.9 ± 0.22^{c}	6.14 ± 0.19^{b}	35.4 ± 2.61^{d}	62.7 ± 0.76^{b}	45.5 ± 2.07^{c}
Nutmeg	6.33 ± 0.10^{c}	0.62 ± 0.03^{c}	1.18 ± 0.06^{d}	11.2 ± 0.08^{c}	35.2 ± 4.77^{d}	82.5 ± 0.37^{d}	47.6 ± 4.54^{c}
Cumin	26.4 ± 0.25^{d}	$3.31 \pm 0.02^{a,b}$	0.31 ± 0.03^{e}	25.5 ± 0.52^{d}	79.7 ± 1.61^{e}	$56.5 \pm 1.15^{b,c}$	77.0 ± 0.39^{e}
Black pepper	15.3 ± 0.24^{e}	13.1 ± 1.24^{d}	n.d.	10.5 ± 1.12^{c}	48.9 ± 2.79^{f}	46.3 ± 0.60^{c}	70.6 ± 0.35^{f}
Jamaica pepper	65.4 ± 1.38^{f}	36.9 ± 0.64^{e}	8.45 ± 0.16^{f}	87.3 ± 0.12^{e}	187 ± 4.87^{g}	107 ± 2.84^{e}	76.6 ± 0.23^{e}
Juniper berry	14.6 ± 0.50^{e}	10.6 ± 0.64^{d}	0.61 ± 0.02^{a}	19.3 ± 1.10^{f}	62.6 ± 3.66^{h}	63.3 ± 2.94^{b}	$74.5 \pm 0.37^{e,f}$

n.d. – not detected; GAE – gallic acid equivalents; QE – quercetin equivalents; C3GE – cyanidin-3-glucoside equivalents; TE – trolox equivalents. The same letter indicates no significant difference at $p < 0.05$.

The obtained values for TFC, conducted by the $AlCl_3$ assay, ranged from 0.62 to 36.9 mg QE/g DW, with the lowest values found in the nutmeg (0.62 mg QE/g) and curcuma (0.99 mg QE/g) and the highest values in Jamaica pepper (36.9 mg QE/g) and

black pepper (13.1 mg QE/g DW). On average, the TFC levels in spices (10.9 ± 0.43 mg QE/g) are 4.91 times higher than those determined in culinary herbs (2.22 ± 0.08 mg QE/g). No statistically significant differences ($p < 0.05$) in TFC levels were observed between lemon verbena and basil; between chives, sage, and cumin; and among coriander, parsley, and curcuma.

Several values have been reported for TPC and TFC levels in culinary herbs and spices, most of them referring to hydroalcoholic, methanol, or ethanolic extracts [33,73]. The extraction of phenolic compounds in general is more efficient using alcohol and/or alcohol mixed with water as solvent than water as was demonstrated by others [33,74,75], since promotes the degradation of lipid cell membranes and releases the bioactive compounds from plant cells. In the current study, hot water was used as a solvent to extract phenolic compounds to simulate teas and infusions, and to mimic the consumption of spices when seasoning food. Considering aqueous extracts, Ferreira et al. [76] reported lower levels of TPC for parsley and chives, 2.10 and 0.64 mg GAE/g DW, respectively. On the other hand, higher levels of TFC for parsley and chives were reported by Ferreira et al. [76], 8.89 and 11.9 mg QE/g DW, respectively. For basil, higher TPC and TFC levels were reported by Muzolf-Panek & Stuper-Szablewska [75], who found 11.4 mg GAE/g DW and 3.25 mg QE/g DW, respectively. Kiani et al. [77] found lower levels of TPC for basil and coriander in aqueous extracts, 3.15 and 3.02 mg GAE/g, respectively. In nutmeg, lower TPC and TFC levels were reported by Muzolf-Panek & Stuper-Szablewska [75], 2.17 mg GAE/g DW and 0.59 mg QE/g DW, respectively.

The TAC levels of the culinary herbs and spices investigated ranged from 0.30 to 23.9 mg C3GE/g DW, with the lower value corresponding to cumin and the higher value to curcuma. No statistically significant differences ($p < 0.05$) in TAC levels were observed between basil and juniper berry. On the other hand, TAC levels of lemon verbena, chives, coriander, parsley, and black pepper were null in the current study (Table 6). This result is not in agreement with those reported in the literature for coriander and cumin, since Tashtoush et al. [78] determined TAC levels in coriander and cumin using different solvents (ethanol, methanol, acetone) and temperatures (20, 40 and 60 ºC), ranging from 0.03 to 0.19 mg C3GE/g DW and from 0.02 to 0.13 mg C3GE/g DW, respectively. These results suggest that hot water may not be the most suitable solvent for extracting anthocyanins from culinary herbs and spices since the TAC levels in hot water were lower than reported earlier from ethanolic or hydroalcoholic extracts [79], this might

be related to changes in the polarity of solvents, extraction procedures, as well as other environmental factors (e.g., light exposure, temperature, soil compositions).

Several *in vitro* assays have been proposed to measure the antioxidant activities of culinary herbs and spices. The lack of consensus stems from the fact that *in vitro* assays, such as DPPH, ABTS, and ORAC, each target a specific antioxidant mechanism (e.g., electron donation, radical scavenging, or reducing power), and different antioxidants in a complex matrix may behave differently across these assays. As a result, relying on a single assay can overlook the broad spectrum of antioxidant interactions, highlighting the importance of using a combination of assays for a more comprehensive evaluation of antioxidant activity in culinary herbs and spices [76,79]. Consequently, three *in vitro* assays, namely DPPH, ABTS, and ORAC, based on different principles were applied to attain more precise results (Table 6). It was possible to observe that independently of *in vitro* assay, Jamaica pepper again had the highest antioxidant activity and lemon verbena the lowest.

In DPPH the highest value was found in Jamaica pepper (87.3 mg TE/g), followed by cumin (25.5 mg TE/g), juniper berry (19.3 mg TE/g), sage (11.8 mg TE/g), nutmeg (11.2 mg TE/g), black pepper (10.5 mg TE/g), chives (6.78 mg TE/g), curcuma (6.14 mg TE/g), coriander (5.72 mg TE/g), parsley (5.70 mg TE/g), basil (4.79 mg TE/g), and lemon verbena (1.97 mg TE/g). The antioxidant activity for chives, basil, coriander, parsley, and curcuma did not present statistically significant differences ($p < 0.05$). In ABTS, Jamaica pepper again had the highest value, with 187 ± 4.87 mg TE/g, and lemon verbena the lowest, with 5.85 ± 0.48 mg TE/g. Statistically significant differences ($p < 0.05$) were observed in lemon verbena, cumin, black pepper, Jamaica pepper, and juniper berry. The results obtained by Trifan et al. [80] revealed higher antioxidant activity for nutmeg using DPPH and ABTS, 49.1 and 66.2 mg TE/g, respectively. In ORAC, similar to ABTS, Jamaica pepper, and lemon verbena had the highest and the lowest values, 107 and 1.71 µM TE/g, respectively. No statistically significant differences ($p < 0.05$) in ORAC values were observed among basil, coriander, cumin, black pepper, sage, parsley, and nutmeg, or among chives, curcuma, and juniper berry (Table 6). Results obtained by Ferreira et al. [76] revealed lower and higher antioxidant activity for parsley (110 µM TE/g DW) and chives (31.49 µM TE/g DW) aqueous extracts, respectively.

The anti-inflammatory activity of culinary herbs and spices was evaluated using the denaturation of the albumin assay. Albumin denaturation occurs when external

substances, such as acids, bases, heat, or organic solvents, destroy the tertiary and secondary structures of a protein. As a result, denaturation of tissue proteins is recognized as an inflammatory marker. Table 6 shows the percentage inhibition by culinary herbs and spices. Literature indicates that anti-inflammatory agents must suppress protein denaturation by at least 20% [81]. In this sense, lemon verbena (3.65%) and coriander (10.3%) cannot be considered potential anti-inflammatory agents, since their protein denaturation was lower than 20%. The chives (32.2%) and basil (37.9%), as well as sage, parsley, curcuma, and nutmeg, showed an inhibition percentage of protein denaturation that was not significantly different ($p < 0.05$) when compared to the remaining extracts investigated. The inflammatory activity of the extracts can be attributed to the presence of VOMs, phenolic compounds, flavonoids, and other bioactive compounds present in culinary herbs and spices. Related to VOMs (e.g., limonene, linalool, isoborneol, camphor, α-pinene, β-caryophyllene, (*Z*)-methyl isoeugenol, eugenol, myristicin) several studies highlighting their potential therapeutic applications in treating inflammatory diseases, due to their antioxidant properties [82,83]. Perhaps these VOMS show promise, but some adverse effects have been reported, such as cytotoxicity and allergic reactions associated with certain compounds (e.g., α-pinene, camphor, and myristicin). This emphasizes the need for further research to determine safe concentrations for therapeutic use [82].

3.5. Pearson correlation between main volatile organic metabolites and biological activities

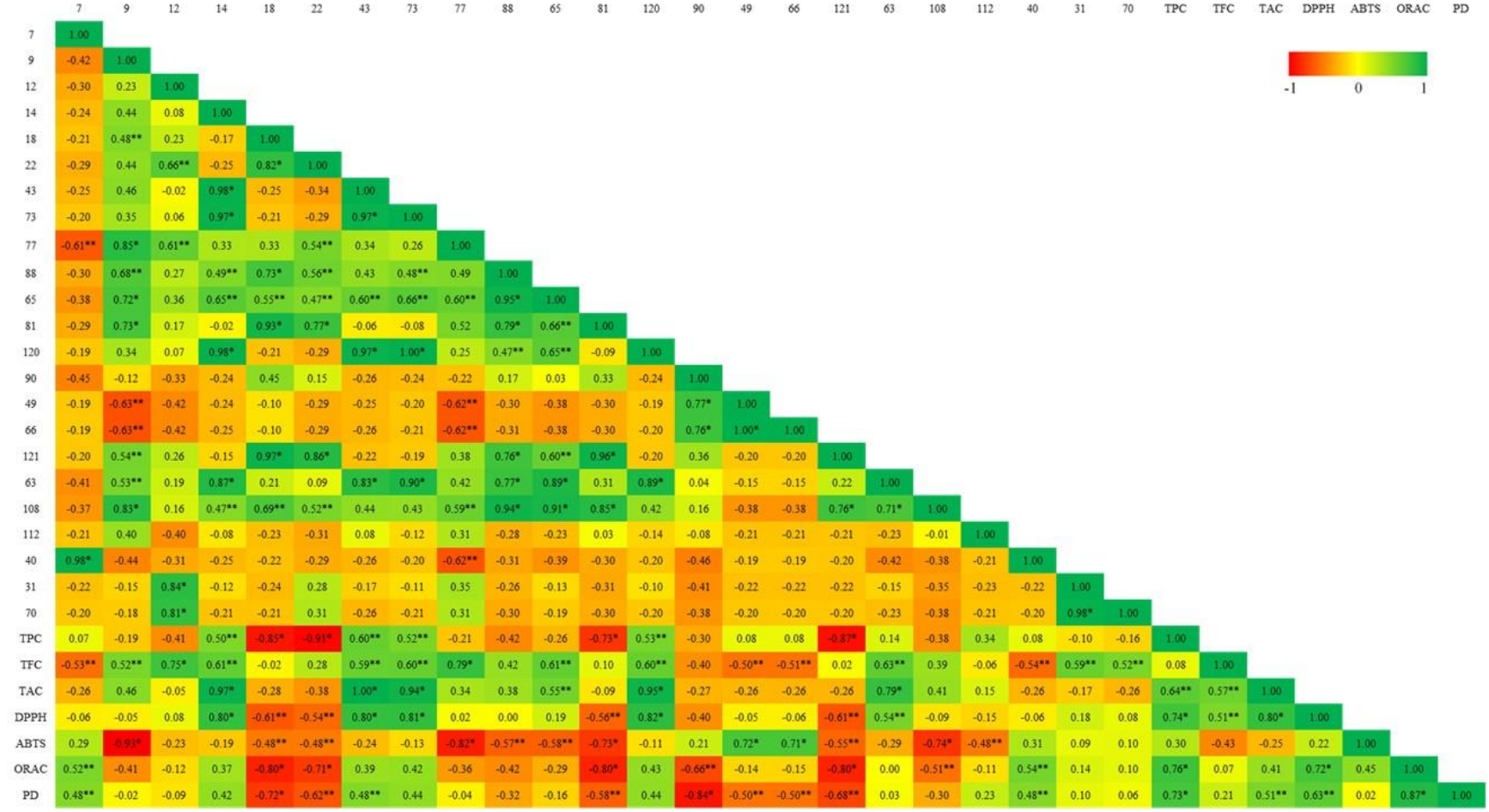
To determine which primary VOMs in culinary herbs and spices might be linked to antioxidant and anti-inflammatory properties, Pearson correlation analysis was conducted. The results of this analysis for various *in vitro* assays are shown in Tables 7 and 8 for culinary herbs and spices, respectively. The strength and direction of the relationships were interpreted using correlation coefficient (r). Positive correlations were indicated by values greater than zero, whereas negative correlations were indicated by values less than zero. The strength of the correlation was categorized as follows: weak (0 – 0.3), moderate (0.3 – 0.7), and strong (0.7 – 1).

The analysis of culinary herbs revealed strong, statistically significant ($p < 0.001$) positive correlations between several antioxidant measures: TPC–DPPH ($r = 0.74$), TPC–ORAC ($r = 0.76$), TAC–DPPH ($r = 0.80$), and DPPH–ABTS ($r = 0.72$). Similarly, spices

exhibited robust and significant ($p < 0.001$) positive correlations among the following antioxidant parameters: TPC–TFC ($r = 0.91$), TPC–DPPH ($r = 0.98$), TPC–ABTS ($r = 0.99$), TFC–DPPH ($r = 0.91$), TFC–ABTS ($r = 0.91$), DPPH–ABTS ($r = 0.99$), DPPH–ORAC ($r = 0.79$), and ABTS–ORAC ($r = 0.72$). Regarding anti-inflammatory activities, spice extracts showed moderate, significant ($p < 0.05$) positive correlations with TPC-PD ($r = 0.62$), TFC-PD ($r = 0.58$), DPPH–PD (0.53), and ABTS–PD ($r = 0.60$). In contrast, culinary herbs showed strong correlations between TPC and PD ($r = 0.73$) and ORAC-PD ($r = 0.87$, $p < 0.001$). These results indicate that bioactive compounds in culinary herbs and spices may play a substantial role in their antioxidant and anti-inflammatory properties. Additionally, antioxidant activity might be attributed to the chemical structure of these compounds, as well as their combined additive, synergistic, or antagonistic effects.

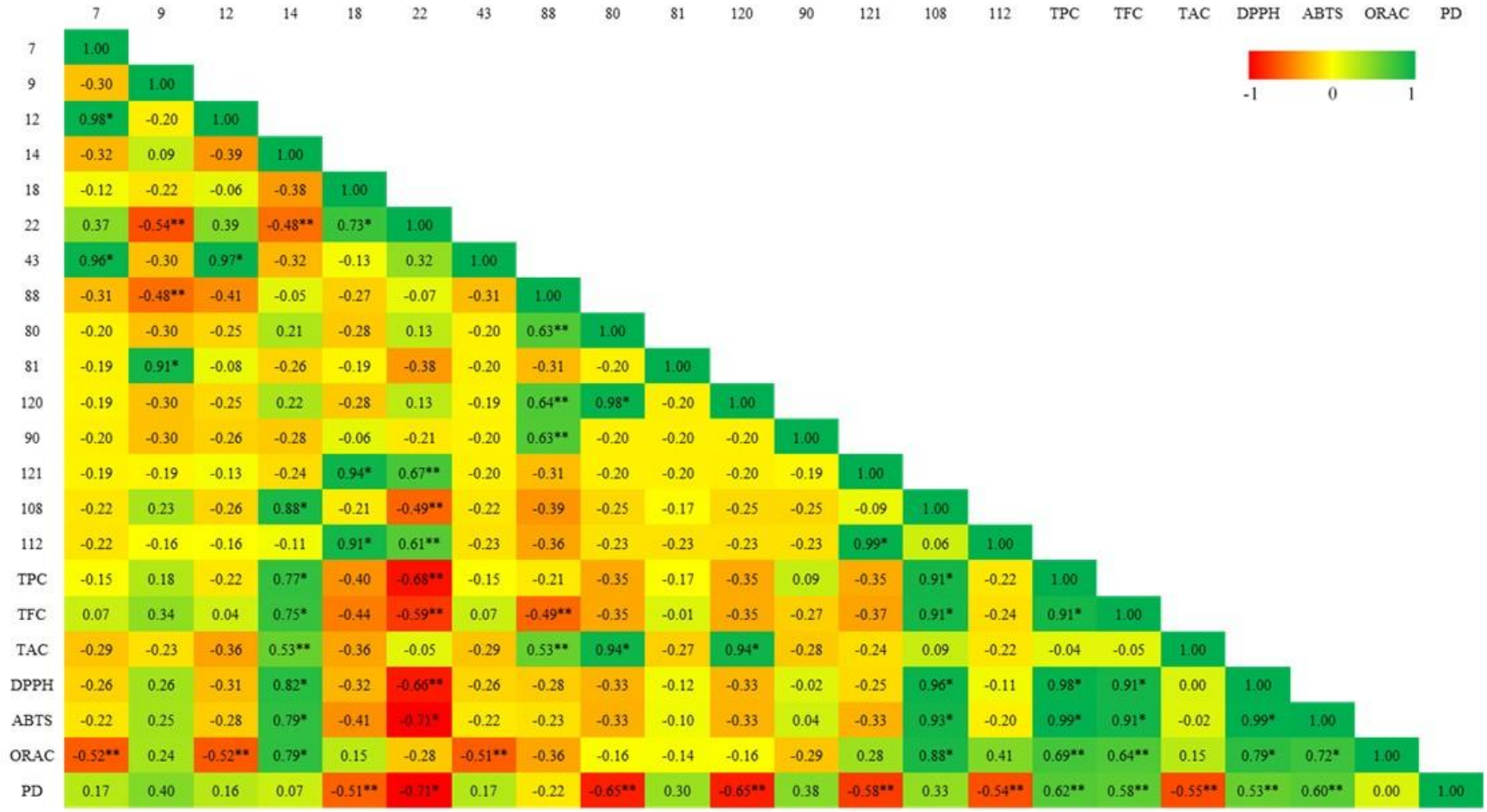
Among the main VOMs identified in spices, eucalyptol (#14) and (*Z*)-methyl isoeugenol (#108) showed a strong positive and significant ($p < 0.001$) correlation with TPC, TFC, DPPH, ABTS, and ORAC. γ-Curcumene (#80) and τ-cadinol (#120) showed a strong and significant ($p < 0.001$) correlation with TAC, while eucalyptol (#14) and cuminaldehyde (#88) showed a moderate correlation with TAC. On the other hand, for culinary herbs eucalyptol (#14), β-thujone (#43), isoborneol (#73) and τ-cadinol (#120) showed a strong and significant ($p < 0.001$) correlation with TAC and DPPH assays. In addition, decanal (#49) and (*E*)-2-decenal (#66) strongly correlated with ABTS ($p < 0.001$). Eucalyptol (#14), β-thujone (#43), isoborneol (#73), and τ-cadinol (#120) demonstrated a moderate and significant ($p < 0.001$) correlation with TPC and TFC. β-caryophyllene (#65), nonyl acetate (#63) showed a moderate and significant ($p < 0.001$) correlation with TFC and TAC. Regarding anti-inflammatory activities, a moderate positive and significant ($p < 0.05$) correlation was observed for the following main VOMs identified in culinary herbs: 3-carene (#7), β-thujone (#43), and 2-methyl-2,3-dihydrobenzofuran (#40), while a moderate correlation was observed in spices without significance.

Table 7. Pearson correlation between VOMs and bioactive activities of the culinary herbs (* significant difference at $p < 0.001$; ** significant difference at $p < 0.05$).



Peak identification: 7 – 3-Carene; 9 – β-Myrcene; 12 – Limonene; 14 – Eucalyptol; 18 – γ-Terpinene; 22 – *p*-Cymene; 43 – β-Thujone; 73 – Isoborneol; 77 – Geranial; 88 – Cuminaldehyde; 65 – β-Caryophyllene; 81 – Germacrene D; 120 – τ-Cadinol; 90 – (*E*)-2-Decen-1-ol; 49 – Decanal; 66 – (*E*)-2-Decenal; 121 – Myristicin; 63 – Nonyl acetate; 108 – (*Z*)-Methyl isoeugenol; 112 – 3-Allylguaiacol; 40 – 2-Methyl-2,3-dihydrobenzofuran; 31 – Dipropryl disulfide; 70 – Dipropyl trisulfide; PD – protein denaturation

Table 8. Pearson correlation between VOMs and bioactive activities of the spices (* significant difference at $p < 0.001$; ** significant difference at $p < 0.05$).



Peak identification: 7 – 3-Carene; 9 – β-Myrcene; 12 – Limonene; 14 – Eucalyptol; 18 – γ-Terpinene; 22 – *p*-Cymene; 43 – β-Thujone; 88 – Cuminaldehyde; 80 – γ-Curcumene; 81 – Germacrene D; 120 – τ-Cadinol; 90 – (*E*)-2-Decen-1-ol; 121 – Myristicin; 108 – (*Z*)-Methyl isoeugenol; 112 – 3-Allylguaiacol; PD – protein denaturation.

Chapter IV

CONCLUSIONS

4. Conclusions

The HS-SPME/GC-MS method successfully establishes the volatile fingerprint of culinary herbs and spices, revealing 121 VOMs. Limonene was the only VOM consistently present in all samples. The predominant chemical families were monoterpenoids (34.0 ± 0.99% in culinary herbs and 62.6 ± 1.31% in spices), esters (6.58 ± 1.27% in culinary herbs and 12.2 ± 1.80% in spices), sesquiterpenoids (10.1 ± 2.21% in culinary herbs and 10.8 ± 3.27% in spices), and carbonyl compounds (14.9 ± 1.41% in culinary herbs and 3.47 ± 2.78% in spices). In *in silico* molecular docking, VOMs like cuminaldehyde, β-caryophyllene, γ-curcumene, germacrene D, and τ-cadinol exhibited ΔG values below -6.0 kcal/mol across multiple receptors, suggesting their potential as anti-Alzheimer, antioxidant, and anti-inflammatory agents. *In vitro* assays of aqueous extracts showed that Jamaica pepper, juniper berry, black pepper, and cumin had the highest antioxidant and anti-inflammatory activities, while lemon verbena had the lowest. Notably, eucalyptol (#14) and (*Z*)-methyl isoeugenol (#108) showed strong correlations ($p < 0.001$) with various antioxidant metrics in spices, while eucalyptol (#14), β-thujone (#43), isoborneol (#73), and τ-cadinol (#120) were closely related to total anthocyanin content and DPPH assays in culinary herbs. Additionally, 3-carene (#7), β-thujone (#43), and 2-methyl-2,3-dihydrobenzofuran (#40) were moderately correlated ($p < 0.05$) with anti-inflammatory activities.

These findings indicate that culinary herbs and spices are rich in bioactive metabolites that could be valuable as functional foods, nutraceuticals, and dietary supplements. They offer potential benefits for cognitive health, especially in preventing or delaying neurodegenerative diseases, such as Alzheimer's disease. Their antioxidant and anti-inflammatory properties make them suitable for cosmetic formulations aimed at protecting against oxidative stress and ageing. Overall, culinary herbs and spices hold promise for applications in preventive health and developing products targeting AD and related conditions.

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Appendix

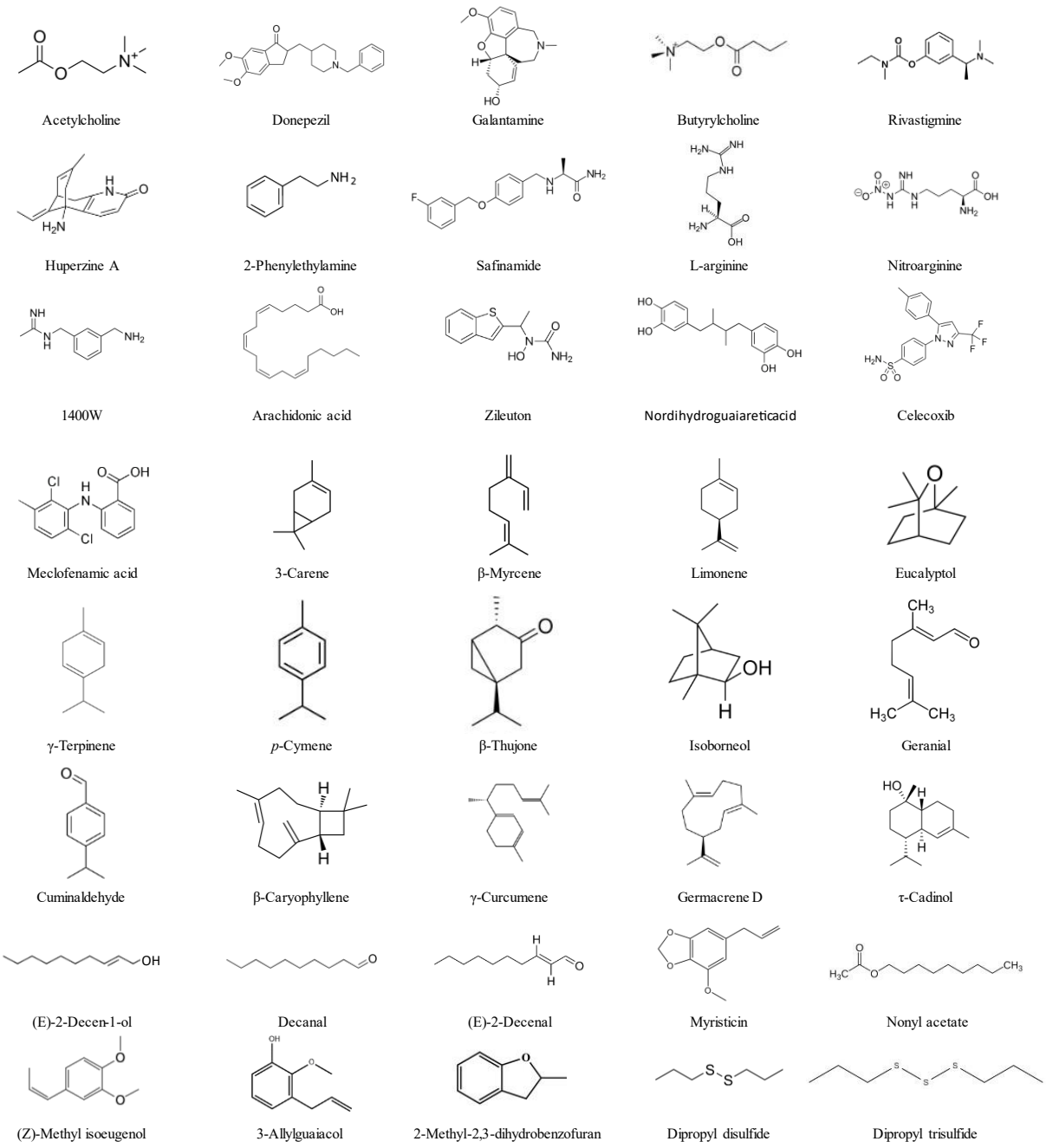

Figure S1. Chemical structures of the compounds used in the molecular modelling study.