P-Y-09

Exploring the potential biological activities and chiral composition of *Lippia origanoides* Kunth essential oil: inhibition of enzymes linked to Alzheimer's disease and Type 2 diabetes

Marta Pavarino[1,2*], Cecilia Cagliero[1], Arianna Marengo[1], Carlo Bicchi[1], Humberto R. Bizzo[2], Francisco C. M. Chaves[3], Patrizia Rubiolo[1], Barbara Sgorbini[1]

[1] *Department of Drug Science and Technology, University of Turin, Turin, ITALY*
[2] *Embrapa Food Agroindustry, Rio de Janeiro, BRAZIL*
[3] *Embrapa Western Amazon, Amazonas, BRAZIL*

*E-mail: m.pavarino@unito.it

Keywords: *Lippia origanoides* Kunth, essential oil, enzymatic inhibition, chiral analysis.

Abstract

Diabetes mellitus and Alzheimer's disease (AD) are increasingly common in ageing population. Type 2 diabetes is an important risk factor for AD due to shared phenotypes, including mitochondrial dysfunction [1]. The insulin resistance characteristic of diabetes alters the AD pathology by affecting amyloid precursor protein (APP) metabolism, contributing to plaque formation [2]. Impaired glucose and lipid metabolism, which is often associated with obesity, further exacerbate AD progression. In addition, there is new evidence of a link between the composition of the gut microbiota and the risk of obesity-related diseases [3]. Inhibition of enzymes such as acetylcholinesterase (AChE), butyrylcholinesterase (BChE), α-glucosidase and lipase offers potential therapeutic opportunities for both AD and diabetes. Essential oils (EOs), complex mixtures of volatile compounds obtained from individual plant species by hydro distillation and steam distillation or mechanical processes have diverse applications in perfumery, cosmetics, and pharmaceuticals [4], with recent focus on their potential in inhibiting disease-associated enzymes. This study therefore focused on a remarkable Brazilian native essential oil, *Lippia origanoides* Kunth EO, which has been investigated for some biological activities such as its antimicrobial, insecticidal, acaricidal and larvicidal properties [5,6,7], but not for its promising enzyme inhibitory activity with the exception of a paper dealing with AChE inhibition [6]. *Lippia origanoides* EO was chemically characterised, subjected to chiral analysis, and tested for its inhibitory effect on AChE, BChE, α-glucosidase and lipase. The results showed significant activity against all four enzymes, suggesting that the role of this EO as an adjuvant treatment with a broad spectrum of activity could be further investigated in addition to conventional therapies.

1.Introduction

Research interest in studying the biological properties of essential oils has increased due to their complex composition and widespread use in various fields such as cosmetics, food and pharmaceuticals. A particular focus is on investigating their influence on enzyme functions, especially those associated with critical human diseases like Alzheimer's and type 2 diabetes. In this framework, the present study centers on *Lippia origanoides* Kunth essential oil (family Verbenaceae), a slender, highly aromatic shrub or tree reaching heights of up to 3 meters. Indigenous to Central and Southern American countries like Mexico, Guatemala, Cuba, and Amazon region in countries such as Guiana, Venezuela, Brazil, and Colombia, it is known in northern Brazil as "Salva de Marajo" and "Alecrim d'Angola". In Brazil its leaves are traditionally used for culinary and medicinal purposes [5]. In this study, it was recognised as the only active essential oil showing remarkable activity against a spectrum of enzymes - AChE, BChE, α-glucosidase, and lipase - among numerous Brazilian indigenous plant essential oils investigated. To further explore the biological activity of this EO, preliminary tests of individual compounds from the essential oil are currently being carried out after determining its chemical composition and chiral characterisation by GC-MS and GC-FID to allow a more thorough investigation of the compounds responsible for the activity.

2. Material and Methods

Lippia origanoides Kunth samples were from Embrapa Western Amazon germplasm bank, in Manaus (AM), Brazil. The plant leaves were subjected to hydrodistillation in a Clevenger type apparatus. Access to biodiversity was authorized by CGEN under registry AC6AC63.

Lippia origanoides Kunth EO was subjected to an AChE and BChE *in vitro* colourimetric assay according to the Ellman method, the protocol used in this study was optimized by Pavarino et al. [8] by modifying that of Rhee et al. [9], a α-glucosidase *in vitro* colourimetric assay according to the protocol of Oboh et al. [10] and a lipase *in*

vitro colourimetric assay according to the protocol of Slanc et al. [11], all with slight modifications.

Qualitative and quantitative chemical characterisation of the essential oil was performed by GC-MS and GC-FID analyses, using both an apolar and a polar column, respectively a MEGA5 (95% polydimethylsiloxane, 5% phenyl, 30 m x 0.25 mm x 0.25 μm) and a MEGAWAX (polyethylene glycol, 60 m x 0.25 mm x 0.25 μm) to resolve coelutions.

For chiral analysis, two enantioselective capillary columns with stationary phases of 2,3-di-O-ethyl-6-O-tert-butyldimethylsilyl-β-cyclodextrin and 2,3-di-O-methyl-6-O-tert-butyldimethylsilyl-β-cyclodextrin were used.

3. Results

Lippia origanoides EO Kunth was obtained in 2.4% yield, and was chemically characterised by GC-MS and GC-FID using an apolar and a polar column, revealing carvacrol (41.8%), p-cymene (14.9%), γ-terpinene (8.5%), trans-β-caryophyllene (4.8%) and thymol (4.2%), as the main compound, percentages referring to the apolar column. In addition, enantioselective analysis revealed the presence of (1S,4R)-(-)-camphene, (S)-(+)-α-phellandrene, (1R,6S)-(-)-δ-3-carene, (1S,4S)-(-)-camphor, (1S,2R,4S)-(-)-borneol, (1Z,6Z,8S)-(-)-germacrene D and (1R,9R,E)-(-)-β-caryophyllene as enantiomerically pure compounds. On the other hand, α-thujene, α-pinene, β-pinene, sabinene, limonene, linalool and terpinen-4-ol were scalemic mixtures.

The EO of *L.origanoides* inhibits AChE by 61.9% ± 3.79 (at a concentration of 38.4 μg/ml in the final mixture), BChE by 21.3% ± 0.372 (at a concentration of 38.4 μg/mL in the final mixture), lipase by 84.8% ± 1.11 (at a concentration of 33.3 μg/mL in the final mixture) and α-glucosidase by 29.1% ± 2.27 (at a concentration of 50 μg/mL in the final mixture). To better compare the inhibitory activities of the *L.origanoides* EO over the four enzymes, the inhibitory concentration that halves the enzyme activity under the experimental conditions considered (IC_{50}) was determined, as shown in Table 1. The IC_{50} values confirmed the interesting inhibitory activity towards these enzymes. However, the IC_{50} value for BChE could not be calculated, even if the enzyme has an interesting inhibitory activity, since 50% inhibition was never achieved under the assumed experimental conditions. Preliminary tests of some compounds characterising the composition of the EO revealed that mainly oxygenated compounds of the EO such as carvacrol are responsible for the activity over AChE, with a contribution from minor compounds such as 1,8-cineole. Carvacrol is also active on BChE, while 1,8-cineole was inactive in this case. The compounds contributing to the biological activity against lipase and α-glucosidase were mainly hydrocarbon compounds. For the former, myrcene and limonene were active, for the latter limonene, α-pinene and β-pinene.

Table 1 IC_{50} values of *L.origanoides* EO towards AChE, BChE, α-glucosidase and lipase enzymes with their standard deviation value (n = 3).

Enzyme	**IC_{50} *L.origanoides* (μg/ml)**
AChE	22.9 ± 0.907
BChE*	Activity lower than 50%
α-glucosidase	14.6 ± 0.580
lipase	74.9 ± 3.84

*IC_{50} value of *L. origanoides* towards BChE was not calculated, because 50% inhibition was never achieved under the assumed experimental conditions.

4. Discussion and Conclusions

The aim of this study is to identify promising biological activities in plants traditionally used by the population. We specifically investigated the essential oil of *Lippia origanoides* Kunth due to its proven versatility, especially its inhibitory effect on enzymes such as AChE, BChE, lipase and α-glucosidase, which play a crucial role in diseases prevalent in the elderly. To summarise, this essential oil is a strong candidate for further research, which is currently being conducted, to investigate the compounds responsible for its inhibitory properties. Such exploration is promising for its potential use as a complementary treatment in these interrelated diseases.

ACKNOWLEDGEMENTS

This study was carried out within the project "Studio di composti biologicamente attivi del metabolismo secondario di matrici di origine vegetale" supported by 'Ricerca Locale' (Ex60%2023 of University of Turin, Turin (Italy). H. R. B. thanks CNPq (311021/2021-2) and FAPERJ (E-26/201.047/2022) for support.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

[1] Silzer T.K., Phillips N.R., (2018). Etiology of type 2 diabetes and Alzheimer's disease: Exploring the mitochondria. *Mitochondrion*, 43, 16-24.

[2] Ribe E.M., Lovestone S., (2016). Insulin signaling in Alzheimer′s disease and diabetes: From epidemiology to molecular links, *J. Intern. Med*., 280, 430–442.

[3] Choi Y.-Y., Osada K., Ito Y., Nagasawa T., Choi M.-R., Nishizawa N., (2005). Effects of Dietary Protein of Korean Foxtail Millet on Plasma Adiponectin, HDL-Cholesterol, and Insulin Levels in Genetically Type 2 Diabetic Mice, *Biosci. Biotechnol. Biochem*., 69, 31–37.

[4] Essential Oils General Monograph. In European Pharmacopoeia 11th Edition; EDQM Council of Europe: Strasbourg, France, 2023.

[5] Oliveira D.R., Leitão G.G., Bizzo R.H., Lopes D., Alviano S. D., Alviano S.C., Leitão G.S., (2007). Chemical and antimicrobial analyses of essential oil of Lippia origanoides H.B.K., *Food Chemistry*, 101, 236-240.

[6] Mar J.M., Silva L.S., Azevedo S.G., França L.P., Goesa A.F.F., dos Santos A.L., de A. Bezerrab J., de Cássia S. Nunomurab R., Machado M.B., Sanches E.A., (2018). Lippia origanoides essential oil: An efficient alternative to control Aedes aegypti, Tetranychus urticae and Cerataphis lataniae, *Industrial Crops & Products*, 111, 292-297.

[7] dos Santos F. J.B., Lopes J.A.D., Cito A.M.G.L., de Oliveira E.H., de Lima S.G., de A.M. Reis F., (2004). Composition and Biological Activity of Essential Oils from Lippia origanoides H.B.K., *Journal of Essential Oil Research*, 16, 504-506.

[8] Pavarino M., Marengo A., Cagliero C., Bicchi C., Rubiolo P., Sgorbini B., (2023). Elettaria cardamomum (L.) Maton Essential Oil: An Interesting Source of Bioactive Specialized Metabolites as Inhibitors of Acetylcholinesterase and Butyrylcholinesterase. *Plants*. 12, 3463.

[9] Rhee I.K., Van de Meent M., Ingkaninan K. ,Verpoorte R., (2001). Screening for acetylcholinesterase inhibitors from Amaryllidaceae using silica gel thin-layer chromatography in combination with bioactivity staining. *J. Chromatogr*. A, 915, 217–223.

[10] Oboh G., Akinbola I.A., Ademosun A.O., Sanni D.M., Odubanjo O. V., Olasehinde T.A., Oyeleye S.I., (2015). Essential Oil from Clove Bud (Eugenia aromatica Kuntze) Inhibit Key Enzymes Relevant to the management of type-2 diabetes and some pro-oxidant induced lipid peroxidation in rats pancreas in vitro. *J. Oleo Sci*., 64, 775–782.

[11] Slanc P., Doljak B., Kreft S., Lunder M., JaneS D., Ítrukelj B (2009). Screening of Selected Food and Medicinal Plant Extracts for Pancreatic Lipase Inhibition. *Phytotherapy Research*, 23, 874-877.