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Headspace Gas Chromatography/Mass Spectrometry Analysis Endorses *Melaleuca* Species as an Abundant Source of Medicinal Eucalyptol and its Proposed Anti-Obesity Activity

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Abstract

In the present study, the essential oils of *Melaleuca bracteata, Melaleuca styphelioides, Melaleuca nesophila, Melaleuca leucadendra, and Melaleuca ericifolia* were analyzed *via* headspace gas chromatography/ mass spectrometry (HS-GC/MS). A consecutive molecular network was constructed *via* the GNPS (Global Natural Products Social Molecular Networking) for the visual exploration of the volatile constituents among *Melaleuca* species. The constructed molecular network divulged eucalyptol (1,8 cineole) as the prevailing volatile, especially in *M. leucadendra* oil. Quantitative estimation of eucalyptol content in the studied species proved *M. leucadendra* oil as a potential source for its isolation with a content of 6031.8 µg/g dry weight. In addition, the effect of eucalyptol on the activity of digestive enzymes (*α*-amylase and pancreatic lipase) was assessed. Eucalyptol showed IC_{50} values of 75.3±2.1 and 64.6± 1.4 µg/ml for *α*-amylase and pancreatic lipase inhibition, compared with acarbose and orlistat 34.71±1.3 and 23.8± 0.82 µg/ml, respectively with significant difference of p <0.05. Lastly, the molecular docking confirmed the less activity of the eucalyptol towards the tested enzymes revealing the less binding affinity of eucalyptol towards both *α*-amylase (free binding of energyΔG = -5.7 Kcal/mol, compared to reference ligand = -8.3 Kcal/mol), and lipase (ΔG = -5.5 Kcal/mol, compared to reference ligand=-6.1Kcal/mol). Further target prediction studies for eucalyptol were applied and proposed the Cytochrome 450 19A1 aromatase enzyme as a possible target of eucalyptol that could be a potential alternative therapy in the management of obesity.

Keyword: Digestive Enzymes; Eucalyptol*;* HS-GC/MS analysis; *Melaleuca*, Molecular Networking; Myrtaceae; Volatile Oil

1. Introduction

Myrtaceae is well recognized as an abundant source of medicinal volatile oils, compromising around 6000 species distributed into 145 genera [1]. Among these plants, the genus *Melaleuca* is well known for its volatile-rich species; in particular, *M. alternifolia* which is commercially known as "tea tree oil" and proved to exert antibacterial [2], antifungal [3], and antiviral [4] activities. Globally, around 220 species of *Melaleuca* are present native to the Oceania region [5] and were recently introduced to Egypt [6].

Melaleuca plants are shrubs or trees, commonly found along the watercourses and the edges of swamps. They show compelling phenotypic diversity in different ecosystems and accommodate climatic change [7, 8].

For decades, *Melaleuca* plants have been used in folk medicines in different civilizations. The most recognized is *Melaleuca alternifolia* (*i.e.* tea tree oil) which has been used traditionally in Asia, Australia, and America for the alleviation of bruises, insect bites, and skin infections [9]. Further, it is incorporated in many cosmetic preparations such as soaps, shampoos, and sunscreens along with numerous pharmaceutical preparations for skin infections [10, 11]. While bark and leaves of *M. leucadendron* (paperbark tree) were used for the relief of cold and flu symptoms [12], for psoriasis [13], vertigo, and rheumatism [14], obesity, and

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hyperlipidemia [15].

Several reports exist on the chemical composition of the volatile oils of various *Melaleuca* species from Benin, Brazil, Egypt, India, Thailand, and Tunisia [6, 16-18]. They are known to afford volatile oils rich mainly in monoterpenes (*i.e.*1, 8-cineole, and terpinen-4-ol). Nevertheless, variation in the volatile oil composition among *Melaleuca* species exists. For instance, methyl eugenol was reported in *M. ericifolia* oil as the predominant constituent *versus* caryophyllene oxide and spathulenol in *M. stypheloides* [16].

Interestingly, essential oils were reported as a bountiful source for potential enzyme inhibitors. For instance, compelling studies reported the promising activity of monoterpenes found in various essential oils against *α*-amylase and *α*-glucosidase [19]. Inhibition of the digestive enzymes (i.e. α-amylase and pancreatic lipase) could be a possible mechanism of action in the management of obesity *via* reducing the digestion and absorption of carbohydrates and lipids [20]. In that context, it seemed of interest to investigate the discrepancy of aroma constituents among five *Melaleuca* species through the employment of state-of-the-art molecular networking based on the HS-GCMS (Head Space- Gas Chromatography / Mass Spectrometry) *via* the GNPS (Global Natural Products Social Molecular Networking). Followed by quantification of eucalyptol (1,8-cineole) in the obtained oils and assessment of its *in vitro* digestive enzymes inhibitory activities (*i.e.,* α-amylase, and pancreatic lipase).

In addition, the molecular docking prediction was employed to explain the obtained *in-vitro* results and introduce another promising target enzyme that is implicated in risk factors for the obesity problem.

2. Experimental

2.1. Plant Materials

Five *Melaleuca species* were collected from Mazhar Botanical Garden and authenticated by the taxonomist Mrs. Therese Labib, a consultant at the Ministry of Agriculture and the former director of El-Orman Botanical Garden (**Table 1**). Plant material was air-dried in shade then crushed before analysis.

2.2. Chemicals and Reagents

Standard eucalyptol and chemicals for the biological assays were purchased from Sigma-Aldrich (Merck, USA).

Table 1: *Melaluca* accessions used in the study

Latin plant name	Sample Code	Sample origin	Herbariun code
Melaluca bracteata	M-1	Mazhar Botanical Garden	3/4/2/9
Melaluca styphelioides	M-2	Mazhar Botanical Garden	3/4/4/2
Melaluca nesophila	M-3	Mazhar Botanical Garden	3/4/3/8
Melaluca leucadendra	M-4	Mazhar Botanical Garden	3/4/3/6
Melaluca ericifolia	M-5	Mazhar Botanical Garden	3/4/2/20

2.3. Headspace GC-MS for volatile Analysis

Three grams of the air-dried and crushed *Melaleuca* sample were placed into a 20 ml headspace vial and immediately sealed with silicone rubber septa and aluminum caps for the absorption of the volatile compounds. They were transferred to the headspace and heated up to 80°C for 20 min while being agitated, and then introduced directly into the GC injector with a loop temperature of 120°C, and transfer line temperature of 140°C.

GC-MS analysis was executed on an Agilent Technologies system (7890B), equipped with a mass spectrometer detector (5977A), headspace sampler (7697A), and HP-5MS capillary column (30 m x 0.25 mm internal diameter and 0.25 μm film thickness). Analyses were carried out using hydrogen as the carrier gas at a flow rate of 1.0 ml/min with a splitless mode and the following temperature program: 50 °C for 2 min; rising at 10 °C /min to 250 °C; rising at 15 °C/min to 300 °C and held for 10 min. The injector and detector were held at 280 °C and 300 °C, respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV; using a spectral range of m/z 30-550 and solvent delay 5 min. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

2.4. Quantification of Eucalyptol in Melaleuca Species Understudy

Melaleuca species (0.5 g) were analyzed in triplicates by HS-GCMS for the quantification of eucalyptol using a standard curve (**Fig. 1**). Eucalyptol concentrations ranging from (2-1200 nL) were analyzed *via* HS-GCMS under the same conditions to construct the standard curve. The regression coefficient (R2) was 0.9822 and the plot has a slope (m) = 56025. The equation of the constructed standard curve was y = 56025x (**Fig. 1**).

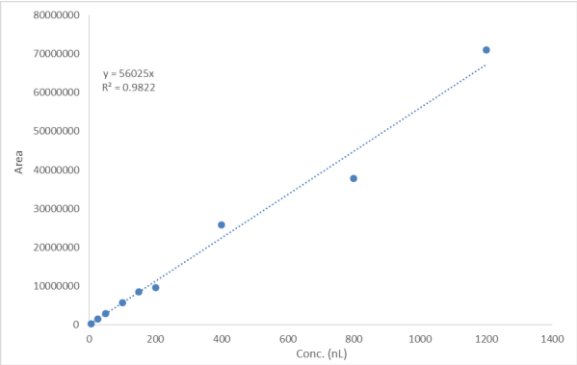

Fig. 1: Eucalyptol standard curve.

2.5. GC-MS Molecular Networking

A molecular network (MN) for the GC-MS data of the studied *Melaleuca* species was constructed following the workflow described by [21]. Raw data files (.d format) were converted into the open format (.mzML) supported by the GNPS platform (Global Natural Products Social Molecular Networking); using MSconvert available at (http://proteowizard.sourceforge. net/download.html).

The spectra in the network were then searched against GNPS-GCMS spectral libraries. The created MN was investigated and visualized using Cytoscape (ver. 3.8.2), which is open-source software for the analysis and exploration of the MNs [22].

2.6. *In-vitro* Study

2.6.1. α- Amylase Inhibitory Assay

Serial concentrations of eucalyptol (1000-7.81 µg/ml) were prepared and mixed with 1ml of prepared enzyme solution (α-amylase in 20mM phosphate buffer (6.9) at the concentration of 0.5 mg/ml) and incubated at 25°C for 10 min. After that, 1ml of starch (0.5%) solution was added to the prepared mixture and further incubation for 10 min at the same degree (25°C). To stop the chemical reaction, 2 mL of dinitro salicylic acid (DNS, color reagent) was added, then the mixture was heated in a boiling water bath for 5 min. After cooling, the absorbance (abs) was calculated calorimetrically at λ_{max} 565 nm. The inhibition percentage (%) was computed using the following formula;

$$\% \text{ inhibition} = [(1 - As/Ac) \times 100].$$

As: Abs of the test sample, Ac: control Abs (containing all reagents except the test sample), and Acarbose was used as a standard drug.

The IC_{50} value was defined as sample concentration that inhibits 50% of the enzyme activity under the assay conditions [23].

2.6.2. Pancreatic Lipase Inhibitory Assay

The lipase inhibition activity of the eucalyptol compound was determined as described by Kim *et al* [24]. In which, *p*-nitrophenyl butyrate (NPB) was used as a substrate in this assay. Eucalyptol serial concentrations (1000 to 7.81 μg/mL) were pre-incubated with 100 µg/mL of lipase (100 µg/mL in a 0.1 mM potassium phosphate buffer "pH 6.0") for 10 min at 37 °C. The reaction starts with the addition of 0.1 mL of NPB, and is left for 15 min at 37 °C. The released amount of *p*-nitrophenol in the reaction was measured using Multiplate Reader. Each experiment was conducted in triplicates. The results were expressed as percentage inhibition (%), calculated by the following formula; Inhibitory activity (%) = (1 − As/Ac) ×100. As: the absorbance of the test substance and Ac:

2.7. Statistical Analysis

α-amylase and pancreatic lipase inhibitory determinations were carried out in a triplicate manner and values are expressed as the mean ± SD. The IC_{50} value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assayed conditions. The obtained data were analyzed using an unpaired t-test. Results were considered significantly different at p <0.05. The data analysis was be done using graph pad instate 5.0 (Inc. La Jolla, CA, USA) software.

2.8. In Silico Study

The structures of all tested compounds including co-crystallized ligand and eucalyptol were modeled using the Chemsketch software (http://www.acdlabs.com/resources/freeware/). The structures were optimized and energy minimized using VEGAZZ software [25]. The optimized compounds were used to perform molecular docking against pancreatic α-amylase, and pancreatic lipase. The three-dimensional structure of the molecular target was obtained from Protein Data Bank (PDB) (www.rcsb.org): (for α-amylase, PDB: 1OSE, https://www.rcsb.org/structure/1ose,(for lipase, PDB: 1LPA, https://www.rcsb.org/structure/1lpa).The steps for receptor preparation included the removal of heteroatoms (water and ions), the addition of polar hydrogen, and the assignment of charge. The active sites were defined using grid boxes of appropriate sizes around the bound cocrystal ligands. The docking study was performed using Autodock vina

[26] and Chimera for visualization [27]. All docking procedures and scoring were recorded according to our previous publications [28, 29].

2.8.1. **Target Prediction, Pharmacokinetics, and ADME activity**

For the identification of eucalyptol biological targets, a database search was performed based on SwissTargetPrediction function integrated on Swiss institute Bioinformatics web-based tools [30]. Further, the absorption, distribution, metabolism, and excretion "ADME" were calculated hypothetically based on Swiss ADME [31, 32].

3. Results and Discussion

3.1. HS-GC-MS Analysis of *Melaleuca* Essential Oil

Headspace extraction was performed prior to the GC-MS analysis of the essential oils of the *Melaleuca* species under study. The essential oil composition of the Melaleuca accessions is presented in Table 2 along with quantitative data. Compounds identification was endorsed on the mass spectral data (MS) and relative retention time in comparison with those of Wiley spectral library collection and NIST library databases. The GC-MS analysis unveiled considerable differences among the five *Melaleuca* accessions. For instance, oxygenated monoterpenes dominated in *M. styphelioides* (84.73%), *M. nesophila* (97.63%), and *M. leucadendra* (97.14%) *versus* monoterpene hydrocarbons in *M. bracteata* (63.23%), and phenyl propene in *M. ericifolia* (36.01%), (Table 2 & Fig. 2).

Following, a molecular network was constructed from the GCMS data to allow for the visual exploration and comparison of their constitutive volatiles. The created MN (**Fig. 3a)** divulged the predominance of eucalyptol (1, 8-cineole) with the highest content found in *M. leucodendra* (**Fig. 3b**) which can be revered as an abundant source of medicinal eucalyptol. Twelve volatile constituents were identified in the essential oils of the studied *Melaleuca* accessions.

Oxygenated monoterpene exemplified by eucalyptol was the main constituent in *M. leucadendra* and *M. nesophila* oils representing 97.62% and 95.08% of their aroma correspondingly. Followed by *M. styphelioides* (56.10 %), *M. ericifolia* (29%), and lastly *M. bracteata* (10.71%). Research on eucalyptol proved its anti-inflammatory [33], antioxidant [34], and antinociceptive activities [35].

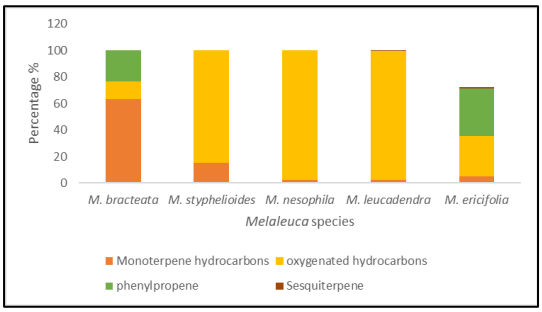

Fig.2. Percentages of the main volatile classes in the five *Melaleuca* species as revealed from the HS-GC-MS analysis

It is incorporated in many pharmaceutical products for the management of bronchitis, sinusitis, and chronic rhinitis, and asthma [36, 37]. Similarly, cyclohexanol, 2-methyl-5-(1-methylethenyl) was found with fluctuating levels of 28.63%, 2.79 %, 0.71%, and 0.04% in *M. styphelioides, M. bracteata, M. ericifolia,* and *M. leucadendra,* respectively. Other oxygenated monoterpene was α-terpineol present at much lower levels (1.85%), E-sabinene hydrate (0.18%), and *Z*-sabinene hydrate (0.1%) in *M. leucadendra.*

Interestingly, α-terpineol is known to exert a broad range of biological activities to include antioxidant, antiulcer, antihypertensive, anticancer, and anticonvulsant [38]. Conversely, monoterpene hydrocarbons were the prevailing hydrocarbons in *M. bracteata* represented by α-pinene (63.23%), and being present in the other *Melaleuca* samples at lower levels, *i.e.* 15.27 %, 3.5%, 2.37 %, and 1.72 % in *M. styphelioides, M. ericifolia, M. nesophila,* and *M. leucadendra,* correspondingly. α-pinene is documented to have antioxidant and anti-inflammatory properties [39]. Likewise, sesquiterpenes were present such as caryophyllene (0.12%), humulene (0.02%) in *M. leucadendra,* and longipinene epoxide (0.72%) in *M.* styphelioides disagreeing with previous reports stating their prevalence in Tunisian samples [40]. These observed discrepancies in the volatile composition could be attributed to environmental factors, the plant developmental stage, and or sample preparation.
the absorbance of control. The IC_{50} value was defined as the concentration of the sample that inhibits 50% of the enzyme activity under the assay conditions.

Table 2: Composition of the essential oil of *Melaleuca* accessions as analyzed by HS-GC-MS.

No.	Compound class	Name*	RRt[a]	Formula	M-1	M-2	M-3	M-4	M-5
1	Monoterpene hydrocarbons	*α*.-Pinene	0.818	$C_{10}H_{16}$	63.2 %	15.2 %	2.37 %	1.72 %	3.5 %
2	Monoterpene hydrocarbons	*β*-Pinene	0.896	$C_{10}H_{16}$	-	-	-	0.88 %	0.95 %
3	Oxygenated monoterpene	Eucalyptol, (1,8-cineole)	1	$C_{10}H_{18}O$	10.7 %	56.1 %	97.6 %	95.0 %	29.6 %
4	Oxygenated monoterpene	*E*-Sabinene hydrate	1.037	$C_{10}H_{18}O$	-	-	-	0.18 %	-
5	Oxygenated monoterpene	Cyclohexanol, 2-methyl-5-(1-methylethenyl), (carvomenthol)	1.089	$C_{10}H_{18}O$	2.79 %	28.6 %	-	0.03 %	0.71 %
6	Oxygenated monoterpene	*Z*-Sabinene hydrate	1.253	$C_{10}H_{18}O$	-	-	-	0.09 %	-
7	Phenylpropene	Methyleugenol	1.582	$C_{11}H_{14}O_2$	23.1 %	-	-	-	36.0 %
8	Oxygenated monoterpene	*α*-Terpineol	1.263	$C_{10}H_{18}O$	-	-	-	1.85 %	-
9	Sesquiterpene hydrocarbons	Caryophyllene	1.613	$C_{15}H_{24}$	-	-	-	0.12 %	-
10	Sesquiterpene hydrocarbons	Humulene	1.661	$C_{15}H_{24}$	-	-	-	0.01 %	-
11	Sesquiterpene	Longipinene epoxide	1.726	$C_{15}H_{24}O$	-	-	-	-	0.72 %
12	Monoterpene hydrocarbons	*β*-Citronellene	2.184	$C_{10}H_{18}$	-	-	-	-	0.46 %

*Identification of volatile constituents was based on the mass spectral data (MS with those of Wiley spectral library collection and NIST library databases. [a]RRt: retention time relative to Eucalyptol standard.

Table 3: Results of eucalyptol mean content in the different *Melaleuca spp*.

Eucalyptol	*M-1*	*M-2*	*M-3*	*M-4*	*M-5*
(nL/0.5 g)	31.93	4.58	96.27	3269.25	41.43
(µg/ g dry leaves)	58.9	8.5	177.6	6031.8	76.5

Finally, phenylpropene manifested by methyleugenol was the main constitutive volatile in *M. ericifolia* (36.01%) followed by *M. bracteata* (23.15%) agreeing with previous reports by [6]. Methyl eugenol is found in many fruits and plants (*i.e.* blackberries, citrus, walnuts, clove, and others) and is used as a flavouring agent in food industries. Nevertheless, human exposure to high doses of methyl eugenol has a toxicological burden owing to its structural similarity to the carcinogenic phenylpropanoids estragole and safrole [41].

3.2. Quantification of eucalyptol in *Melaleuca* species under study

Eucalyptol (1, 8- cineole) has a distinctively fresh and camphoraceous fragrance and a pungent taste. It is broadly employed used in food-flavor, pharmaceutical, and cosmetic industries. Moreover, it is cited to have several health benefits such as anti-inflammatory, anti-microbial, anticancer, and antimicrobial properties [42]. It is registered in Germany in the form of 100 mg capsules as a remedy for acute and chronic bronchitis, sinusitis, and respiratory infections [43]. Eucalyptol content in different *Melaleuca* species was calculated from the constructed standard curve (**Fig. 1**) and confirming the high eucalyptol content in *M. leucadendra* 6031.8 µg/ g dry leaves (**Table 3**, **Fig. 3b**). Conclusively, *M. leucadendra* could be an economic source for the isolation of medicinal eucalyptol and could be propagated and processed for its production.

3.3. Digestive Enzymes Inhibitory Activities

Currently, diverse reports have demonstrated the potentiality of natural products to diminish the activity of digestive enzymes such as α-amylase and pancreatic lipase [20,44,45]. Such attenuating activity may aid in obesity management through delaying carbohydrates and lipids digestion and hence glucose and triglycerides levels.

In this context, the in *vitro* inhibitory activity of eucalyptol on the digestive enzymes (α-amylase and pancreatic lipase) was assessed in comparison to acarbose and orlistat as positive controls. The inhibitory effects of eucalyptol, acarbose, and orlistat on α-amylase and pancreatic lipase are illustrated in Fig. 4. The IC_{50} values for the inhibition of the α-amylase enzyme by eucalyptol were 75.3±2.1 µg/ ml in comparison to 34.71±1.3 µg/ ml for the acarbose. Comparably, the activity of the pancreatic lipase enzyme was reduced with an IC_{50} value of 64.6± 1.4 µg/ ml for eucalyptol versus 23.8± 0.82 µg/ ml for orlistat.

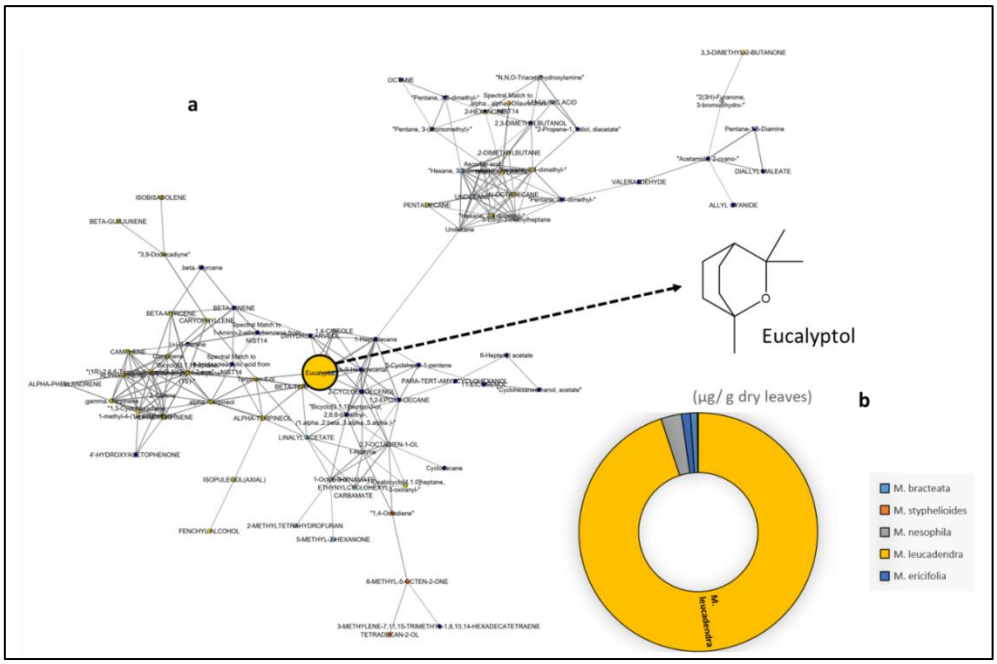

Fig.3a. Molecular network of the HS-GC-MS analysis of *Melaleuca* accessions: *M. bracteata, M. styphelioides, M. nesophila, M. leucadendra, and M. ericifolia*. Node size: the total sum of the intensity of the corresponding ion. Nodes color: distribution of ions among different *Melaleuca* species. Nodes are labelled with the spectral matches from the GNPS-GC-MS spectral libraries. **Fig. 3b**: Eucalyptol content (µg/gm dry weight) in the five *Melaleuca* species as determined quantitatively.

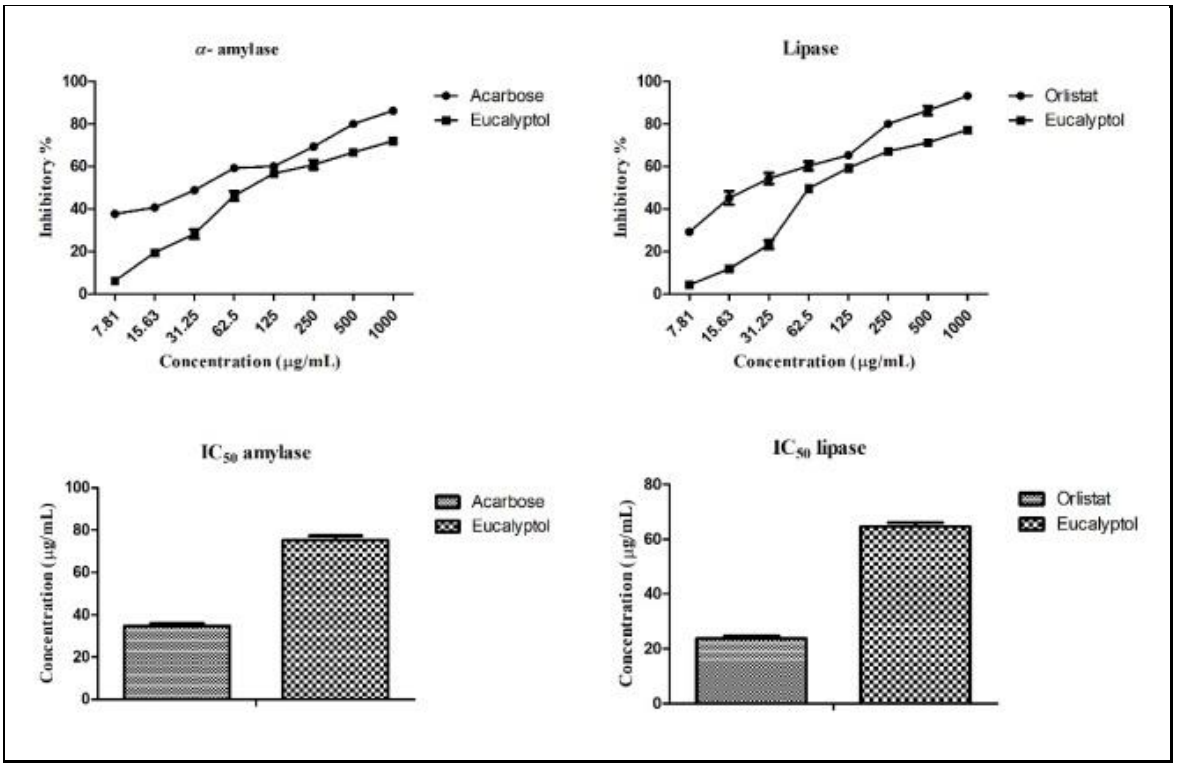

Fig. 4: *In-vitro* α-amylase and pancreatic lipase inhibitory activity (%), and IC_{50} (µg/mL) values of eucalyptol compared with acarbose and orlistat. All values represent Mean ±SD, n = 3, and the unpaired t-test showed significant differences with $p < 0.05$ compared with standards

3.4. Molecular Docking Results

In an attempt, to extrapolate more on the potential use of eucalyptol as an anti-obesity agent, molecular docking was done against both lipase (PDB: 1LPA), and *α*-amylase (PDB: 1OSE) enzymes. Docking study revealed a less binding affinity for eucalyptol against both lipase (free binding of energy=-5.5 Kcal/mol, compared to reference ligand= - 6.1Kcal/ mol), and amylase enzymes (free binding of energy = -5.7Kcal/mol, compared to reference ligand = -8.3 Kcal / mol) (**Tables 4 & 5**).

Moreover, eucalyptol has not any hydrogen bond interaction with both lipase (compared to 5 hydrogen bonds formed by reference ligand), and amylase (compared to 5 hydrogen bonds formed by reference ligand) (**Fig. 5 & 6**). This explains the low activity observed for eucalyptol against both enzymes, suggesting another possible mechanism of action against obesity.

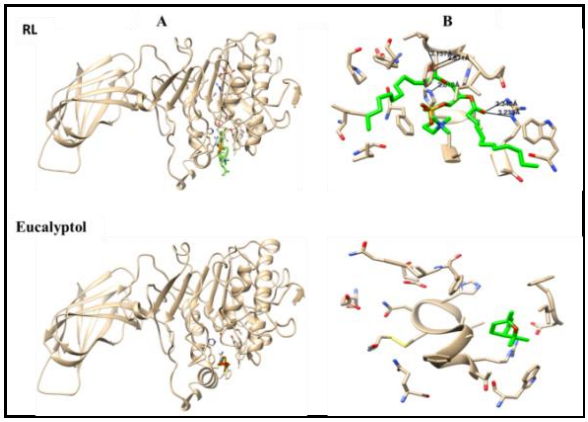

Fig.5. The interaction of the eucalyptol with 1LPA protein compared to reference ligand (RL), **A)** 3D interaction

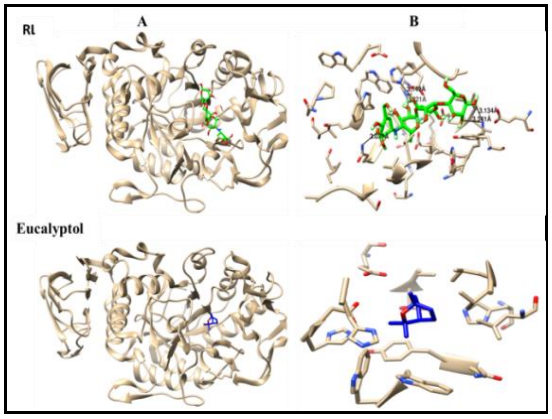

Fig.6. The interaction of the eucalyptol with 1OSE protein compared to reference ligand (RL), **A**) 3D interaction and **B**) hydrogen bond formation (Black solid lines).

Table 4: Results of the docking study of the Eucalyptol compound against lipase binding pocket in comparison to the co-crystallized ligand.

Comp. No	*1LPA* Energy of free binding ΔG_b [a]	H-Bond No.	Amino acid	Length Å	Hydrophobic interaction
Eucalyptol	-5.5	0	-	-	PHE77, PHE215, PHE258, LEU213. LEU264
Reference Ligand	-6.1	5	ARG256 ARG256 HIS263 LEU153 PHE77	3.233 3.340 2.819 3.157 2.974	PHE77, PHE215, LEU153. LEU213. LEU264, ILE72, ILE209

Table 5: Results of the docking study of the eucalyptol compound against α-amylase binding pocket in comparison to the co-crystallized ligand.

Comp. No	*1OSE* Energy of free binding ΔG_b [a]	H-Bond No.	Amino acid	Length Å	Hydrophobic interaction
Eucalyptol	-5.7	0	-	-	LEU162, LEU165, VAL163
Reference ligand	-8.3	5	LYS200 LYS200 HIS305 GLN63 HIS299	3.134 3.341 3.149 3.031 3.221	ILE49, ILE148, ILE235, LEU162, LEU165, VAL51, VAL163

3.4.1. Target prediction

Molecular insight into the mode of action of bioactive small molecules is key to understanding observed phenotypes, predicting potential side effects or cross-reactivity. Based on target prediction software, eucalyptol was found to target Cytochrome P450 family 19 subfamily A member 1 gene (Cytochrome 450 19A1) (**Table 6**). The monooxygenases protein encoded by this gene localizes in endoplasmic reticulum [46], and is crucial in many vital biological process involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. Mutations in this gene can result in disturbance in aromatase activity; which is clinically correlated with cancer, and obesity [47]. Moreover, based on cohort study, the CYP19A1 genetic polymorphisms is associated also as risk factors for obesity [48]. Therefore, targeting this enzyme using eucalyptol could be a potential alternative therapy in the management of obesity.

3.4.2. ADME Prediction

To predict the physicochemical and drug-likeness properties, a bioinformatics study for eucalyptol was carried out. Bioavailability Rada has been generated to check the suitable physicochemical properties for oral bioavailability, based on six characters. This to include lipophilicity: XLOGP3 between − 0.7 and + 5.0, size: MW between 150 and 500 g/mol, polarity: TPSA between 20 and 130 Å, solubility: log S not higher than 6, saturation: a fraction of carbons in the *sp* 3 hybridization not less than 0.25, and flexibility: no more than 9 rotatable bonds). Eucalyptol exhibited good physicochemical properties, as it relies on the colored area in the bioavailability Radar **(Fig. 7)**.

In addition, and according to Lipinski's rule of five for good drugs good absorption, and bioavailability, the compound should have the following criteria; M.wt. < 500, log P < 5, HBD < 5, and HBA < 10, eucalyptol exhibited good values for the entire rule principles with potential drug-like properties (**Table7**).

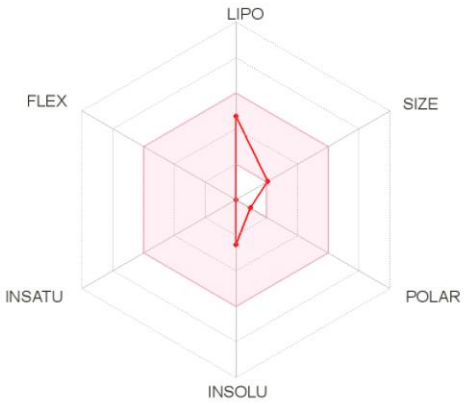

Fig. 7: The Bioavailability Radar shows that Eucalyptol represented by central red lines fits in the pink area, which is the optimal range for each property.

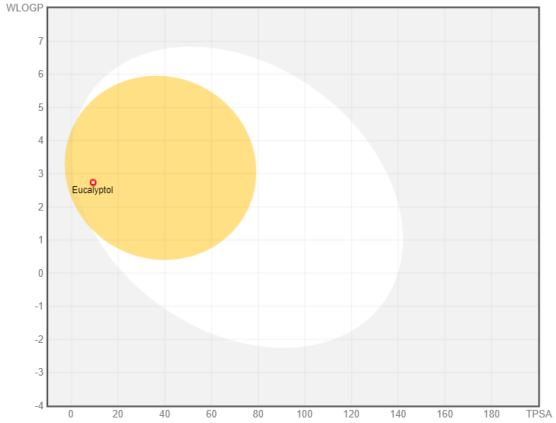

Fig. 8: Pharmacokinetics bioled egg model. Yellow colour represents blood brain barrier (BBB), while colour represents gastrointestinal absorption for the selected drugs. Red circel represents eucalyptol which has both gastrointestinal absorption, and blood brain barrier premability.

Further, pharmacokinetics studies showed in the boiled egg model that eucalyptol has high gastrointestinal absorption (GI), can inter blood-brain barrier (BBB), can't bind to P-glycoproteins, and has good skin permeability **(Fig. 8)**.

Table 6: Protein targets of Eucalyptol using Swiss Target prediction tool

Target	Common Name	Uniprot ID	ChEMBL ID	Target Class	Probability*	Known actives (3D/2D)
Cytochrome P450 19A1	CYP19A1	P11511	CHEMBL1978	Cytochrome P450	0.0822880706974	0/12
Sonic Hedgehog protein (by homology)	SHH	Q15465	CHEMBL5602	Unclassified protein	0.0439186325197	0/10
Cytochrome P450 51 (by homology)	CYP19A1	Q16850	CHEMBL3849	Cytochrome P450	0.0439186325197	0/1
Acetylcholinesterase	ACHE	P22303	CHEMBL220	Hydrolase	0.0439186325197	0/1

Table7: Physiochemical properties and drug-likeness

Properties	Eucalyptol
Formula	$C_{10}H_{18}O$
Molecular weight	154.25g/mol
Num. of heavy atoms	11
Num. of aromatic heavy atoms	0
Num. of rotatable bonds	0
Num. H-bond donors (HBD)	0
Num. H-bond acceptor (HBA)	1
Molar reactivity	47.12
Topological Polar Surface area (TPSA)	9.23 Å
Lipophilicity (Log P)	2.67
Water solubility (Log S)	-2.52

*Num: Number

Conclusion

The HS-GC/MS analysis of the five considered species of *Melaleuca* unearthed considerable discrepancies in their volatiles composition both qualitatively and quantitatively. As witnessed from the created MN, eucalyptol was found in all the studied species with the highest content in *M. leucadendra* (*i.e.* 6031.8 µg/ g dry leaves) recommending it as a potential source for its production.

Additionally, the *in vitro* and *silico* modulating activity of eucalyptol on the digestive enzymes (α-amylase and pancreatic lipase) was assessed. Eucalyptol showed weak inhibitory activity to the digestive enzymes as shown from its IC_{50} values, which could be attributed to its low binding affinity to the tested enzymes as revealed by the molecular docking studies.

Finally, Cytochrome 450 19A1 was introduced as a promising target for eucalyptol that could be an alternative target in the management of obesity.

Conflict of interest

The authors declare no conflict of interest.

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