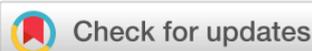


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Antioxidant, enzyme inhibition, toxicity, and molecular docking analysis of *Melaleuca cajuputi* leaf extract and fractions

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ABSTRACT: *Melaleuca cajuputi* plant has piqued the interest of researchers due to its pharmacological properties. However, there is scarce information regarding its enzyme inhibitory effects and possible mechanism of action. Thus, this study aimed to investigate the antioxidant, enzyme inhibitory, and potential binding interactions of bioactive components in *M. cajuputi* extract and fractions with the target enzymes. The 1,1-diphenyl-2-picrylhydrazyl radical scavenging (DPPH) assay was used for the antioxidant activity. The enzyme inhibition potential was assessed using tyrosinase and acetylcholinesterase (AChE) enzymes. *M. cajuputi* methanolic extract (MCME) demonstrated the most potent scavenging activity in comparison to *Melaleuca* fraction (MF1) and (MF2), as evidenced by IC₅₀ values of 28.14 ± 0.96, 35.26 ± 0.64, and 80.90 ± 0.17 µg/mL, respectively. Similarly, the results of the enzyme inhibition assay showed that the MCME (IC₅₀ 65.81 ± 0.39 µg/mL) had the highest potency on tyrosinase enzyme compared to MF1 (IC₅₀ 131.3 ± 0.43 µg/mL) and MF2 (IC₅₀ 215.3 ± 0.48 µg/mL). However, MF2 (IC₅₀ 3.35 ± 0.37 µg/mL) showed a remarkable AChE enzyme inhibition than MCME and MF1. On the other hand, MCME possessed a low toxicity effect with an LC₅₀ of 781 ± 0.07 µg/mL, whereas MF1 and MF2 were non-toxic. The 10-methylanthracene-9-carboxaldehyde, 2-isopropyl-10-methylphenanthrene, 2-tert butylanthracene, β-eudesmol, and α-eudesmol showed a strong binding propensity ranging from -5.1 to -6.2 and -6.4 to -7.6 kcal/mol towards tyrosinase and AChE respectively. The leaf extract of *M. cajuputi* could be considered a novel source of naturally occurring antioxidants and promising enzyme inhibitors.

1. INTRODUCTION

There has been a growing interest in natural products due to their phytochemical diversity, low toxicity, and diverse pharmacological properties (Shahrivari et al., 2022). Plants have historically been used as a valuable reservoir of bioactive molecules, intensively explored for pharmaceuticals and

functional ingredients for various health conditions and food preservation (Pandey et al., 2022). Among these, the *Melaleuca cajuputi* (commonly known as Gelam or Cajuput tree) stands out as a plant species with attractive pharmacological properties (Bua et al., 2020; My, 2020; Xu et al., 2020). *M. cajuputi* is a plant species that belongs to the Myrtaceae family. It is

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indigenous to Southeast Asia, specifically Malaysia, Thailand, Indonesia, and the Philippines. Different *M. cajuputi* parts have been used in folk medicine to manage digestive ailments, cough, anxiety, stress, and skin infections (Isah et al., 2022). Previous studies have documented diverse bioactive components from medicinal plants with promising therapeutic potential (Alfred et al., 2022). These compounds include polyphenols, terpenes, glycosides, alkaloids, and phenolics (Anza et al., 2021; Mera et al., 2019). Antioxidants are essential in reducing oxidative stress, a factor associated with numerous chronic conditions, such as cardiovascular diseases, cancer, and neurodegenerative disorders, thereby neutralizing harmful free radicals and protecting against cellular damage (Yang et al., 2021). Interestingly, plant-based compounds have been shown to exhibit promising antioxidant activities (Isah et al., 2022). Tyrosinase is an enzyme responsible for melanin production, and its overactivity can lead to hyperpigmentation disorders, such as melasma and age spots. The primary therapeutic approach for these disorders involves inhibiting tyrosinase activity (Liu et al., 2021). Hydroquinone and kojic acid, for example, are well-established and effective anti-tyrosinase agents. However, these compounds have been associated with many adverse effects, including skin cancer, inflammation, dermatitis, and melanocyte destruction. Thus, exploring alternative agents from natural products is imperative (Jia et al., 2023). On the other hand, the enzyme acetylcholinesterase is essential in the pathogenesis of neurodegenerative disorders. Hence, suppressing the activity of these enzymes has been linked to enhanced cognitive performance and holds significance in managing Alzheimer's disease (Jia et al., 2023). Identifying alternative anti-cholinesterase agents with improved pharmacological profiles from natural sources is necessary to supplant synthetic compounds associated with numerous adverse effects (Saber et al., 2021). Nevertheless, in addition to investigating the biological activities of plant extracts and compounds, safety evaluation is critical to verify that these plant extracts do not cause detrimental effects when applied topically or administered systematically (Sintayehu et al., 2022). Furthermore, certain plant-based compounds are being recognized for their antioxidant, antimicrobial, and enzyme inhibition properties. This phenomenon has increased researchers' focus on these compounds in drug discovery and development (Eyupoglu et al., 2022). Hence, this research examines the antioxidant, anti-tyrosinase, anti-cholinesterase, and toxicological properties of the methanolic leaf extract and its fractions from *M. cajuputi* leaf. Furthermore, the binding affinities of the active compounds towards the target enzymes were investigated using molecular docking studies.

2. MATERIALS AND METHODS

2.1. Sample collection and identification of plant materials

Melaleuca cajuputi leaf samples were collected from Bachok District (6°0'N 102°22'E) Kota Bharu, Kelantan, Malaysia. Plant materials were identified at the International Islamic University of Malaysia (IIUM), and the specimen number (PIIUM 0304) was deposited at the IIUM herbarium unit. The

plant samples were dried in the oven at 45°C and then ground into a fine powder using an electric grinder. The samples were stored in a freezer at 4°C until needed for the experiments.

2.2. Preparation of crude methanolic extract

Fifty grams of powdered leaves from the *M. cajuputi* plant were immersed in 500 mL of methanol at room temperature for 72 hours. The mixture was stirred using a magnetic stirrer to ensure homogeneity. The sample was filtered using Whatman No. 1 filter paper, followed by solvent evaporation using a vacuum evaporator at 50°C under reduced pressure. This process yielded the crude methanolic extract of *M. cajuputi* leaf (Uba et al., 2022).

2.3. Fractionation of the crude methanolic extract

The methanolic extract (10 g) was subjected to fractionation using column chromatography on silica gel 60 (0.040-0.063 mm). The column was sequentially eluted using n-hexane, ethyl acetate (EtOAc), and methanol (MeOH). The polarity of the eluting solvent mixture was increased from 100% hexane by gradually increasing the volume of ethyl acetate (EtOAc) by 10% increments up to 100%. The solvent mixture was subsequently changed to a combination of EtOAc and MeOH, with an initial ratio of 10% MeOH and 90% EtOAc. This ratio gradually increased until reaching 100% MeOH, resulting in twenty-two distinct fractions. The fractions with similar TLC profiles were pooled into four *Melaleuca* fractions (MF1, MF2, MF3, and MF4). These fractions were screened for bioassays (Erhabor et al., 2021).

2.4. DPPH assay

The antioxidant activities of MCME and fractions were measured using the DPPH assay, as described by Pandey et al. (2022). Methanol (100 mL) was used to dissolve DPPH (3.9432 mg) to prepare a 100 mM DPPH solution in methanol. Various concentrations of the extract and fractions were prepared, spanning from 15.625 µg/mL to 1 mg/mL. Methanol and ascorbic acid were used as the blank and positive controls, respectively. The mixture was kept in the absence of light at room temperature for 30 min, absorbance was measured at a wavelength of 517 nm. The data was presented as IC₅₀ values and expressed as milligrams of ascorbic acid equivalent per gram of extract (mg AE/g extract). The estimation of DPPH scavenging percentage was conducted using the following equation.

$$\text{DPPH Radical Scavenging} = \frac{A-B}{A} \times 100\%$$

The variable A represents the absorbance value of the control, while the variable B represents the absorbance value of the test. The IC₅₀, which represents the inhibitory concentration at which absorbance reaches 50%, was determined through a regression analysis of the average percentage of DPPH scavenging.

2.5. Tyrosinase inhibition assay

All the plant samples were evaluated for tyrosinase inhibition, as described by Alfred et al. (2022). Each plant extract and fractions (40 μ L) of different concentrations ranging from 1000 μ g/mL– 15.625 μ g/mL, phosphate buffer (80 μ L at pH 6.8, 100 mM), and tyrosinase enzyme (40 μ L) from mushroom (25 KU; 1mg= 1881 units) were added in 96-well microplate flat bottom and kept for 10 min at 25°C. A L-Dopa (l-3,4-dihydroxyphenylalanine) (40 μ L) was added, and the mixture was kept in the dark for 30 min. Subsequently, an ELISA plate reader (BIO-RAD) was used to take the absorbance at 515 nm. The reference compound was kojic acid, while dimethyl sulfoxide (DMSO) was the blank control. The formula for calculating tyrosinase inhibition is as follows:

$$\text{Percentage Tyrosinase Inhibition} = (A(\text{Control}) - A(\text{Test})) / A(\text{Control}) \times 100\%$$

The variable A(Control) represents the absorbance of the control solution. On the other hand, A(Test) refers to the absorbance of the plant extract and fractions tested at different concentrations. The linear regression analysis determined the inhibitory concentrations at which the absorbance reaches 50% (IC₅₀) for the tyrosinase inhibition.

2.6. Acetylcholinesterase inhibition assay

The acetylcholinesterase inhibition assay was performed according to the methodology described by Shahrivari et al. (2022) to assess the inhibitory potential of MCME and its fractions. A volume of 140 μ L of a 0.1 M sodium phosphate buffer solution at pH 8.0 and 7.0 was combined with 20 μ L of plant extract and fractions at various concentrations ranging from 1000 μ g/mL to 15.625 μ g/mL. Additionally, 15 μ L of a 0.28 U/mL solution of AChE enzyme was added to the mixture. The resulting solution was kept for 15 min at room temperature. Next, 10 μ L of a 0.075 M solution of acetylcholine iodide (ATCI) and 10 μ L of a 0.01 M of dithionitrobenzoic acid (DTNB) were added under dark conditions. The absorbance measurement was obtained using a microplate reader at a wavelength of 412 nm. The equation for calculating acetylcholinesterase (AChE) inhibition is as follows:

$$\text{Percentage AChE Inhibition} = [(\text{Blank Value} - \text{Sample Value}) / \text{Blank Value}] \times 100.$$

2.7. Brine shrimp lethality test

The lethal effects of various concentrations of MCME and its fractions on brine shrimp were assessed according to the method described by Zahra et al. (2021). The plant samples were reconstituted in 1% dimethyl sulfoxide (DMSO). *Artemia salina* eggs (0.5 g) were sprinkled in synthetic seawater (3.8% w/v sea salt) at pH 7 for 48 hours to hatch the shrimp eggs. Five milliliters (5 mL) of various sample concentrations (62.5 μ g/mL - 1000 μ g/mL) were added to the wells containing 5 mL sea salt solution. Ten nauplii were transferred in each well and were incubated at room temperature under constant light for 24 hours. After the incubation, LC₅₀ values and percentage

mortality of nauplii were calculated. The experiments were repeated three times.

2.8. GC-MS analysis

The GC-MS analysis was conducted using an Agilent, 19091S-443, USA instrument equipped with an HP-5MS column (30 m 0.25 mm, with a film thickness of 0.25 μ m) to analyze the chemical compositions of the MCME and most potent fractions. The oven temperature was set to 70°C and maintained for 3 min. It was then gradually increased to 120°C and held for 2 min. Subsequently, the temperature was further raised to 150°C and maintained for 2 min. Finally, the temperature was increased to 240°C and held for 5 min. The injector and transfer line temperatures were configured to 230°C and 240°C, respectively. A volume of 1 μ L of the diluted samples was injected into the GC-MS instrument. Bioactive compounds were identified by comparing their mass spectra with analogous compounds from the NIST libraries (Al-Dhahli et al., 2020).

2.9. Molecular modeling

The crystal protein structure of AChE (PDB ID: 6O52) (Gerlits et al., 2019) was retrieved from the protein data bank (<https://www.rcsb.org/>) (Kurumbail et al., 1996). The homology model of human tyrosinase was built using the crystal structure of tyrosinase from *Priestia megaterium* (PDB ID: 6QXD) (Ielo et al., 2019). The protocol used was described by Zengin et al. (2022). The two proteins were prepared using the PlayMolecule webserver. A program that predicts the pKa of the titratable residues and uses it to protonate each protein. The three-dimensional structure of each ligand was obtained from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov>), and geometry optimization was performed using UCSF Chimera (Pettersen et al., 2004). All prepared proteins and ligands were converted into Protein Data Bank, Partial Charge (Q), and Atom Type (T)) (PDBQT) format using MGLTools 1.5.6 (<https://autodock.scripps.edu>). Docking was carried out using AutoDock vina (PyRx) (<https://vina.scripps.edu/>) (Allouche, 2009). with the docking grid coordinates for human AChE (X = -0.0074 Å, Y = 39.14 Å, Z = 2.07 Å) and for human tyrosinase (X = 8.84 Å, Y = 14.28 Å, Z = 121.37 Å). The binding energy scores were calculated for each ligand pose (exhaustiveness = several runs: 8) against each protein and the best pose was analyzed using Biovia DS Visualizer v4.5 (BIOVIA, San Diego, CA, USA).

Table 1
Antioxidant activity of *M. cajuputi* leaf extract and fractions.

S/no.	Sample	DPPH IC ₅₀ (μ g/mL)
1.	MCME	28.14 \pm 0.96
2.	MF1	35.26 \pm 0.64
3.	MF2	80.90 \pm 0.17
4.	Ascorbic acid	7.40 \pm 0.47

MCME= *Melaleuca cajuputi* methanolic extract, MF= *Melaleuca* fraction

Table 2Enzymes inhibition potential and toxicity study of *M. cajuputi* extract and fractions.

S/no.	Sample	Tyrosinase IC ₅₀ (µg/mL)	AChE IC ₅₀ (µg/mL)	Toxicity (BLST) LC ₅₀ (ppm)
1.	MCME	65.81±0.39	282.6±0.70	781±0.07
2.	MF1	131.3±0.43	26.7±0.66	6621±0.7
3.	MF2	215.3±0.48	3.35±0.37	1165±0.04
4.	Kojic acid	198.4±0.78	NT	NT
5.	Galantamine	NT	5.50±0.41	NT

NT= Not tested, MCME= *Melaleuca cajuputi* methanolic extract, MF= *Melaleuca* fraction**Table 5**

GC-MS analysis result of MF2

Peak No.	R.T. (min.)	Compound name	Molecular formula	Total area %
1.	1.277	Ethylamine	C ₂ H ₅ NH ₂	0.28
2.	9.046	3,4,5-Trimethoxybenzoic acid	C ₁₀ H ₁₂ O ₅	2.60
3.	12.249	10-Methylanthracene-9-carboxaldehyde	C ₁₆ H ₁₂ O	10.95
4.	12.466	3,4-Diphenylpyrazole	C ₁₂ H ₁₂ O ₄	2.10
5.	12.842	2-Isopropyl-10-methyl phenanthrene	C ₁₈ H ₁₈	83.09

RT= Retention time

Table 3

GC-MS analysis results of MCME

S/no.	R.T. (min)	Compound name	Molecular formula	Total area %
1.	5.92	Caryophyllene	C ₁₅ H ₂₄	2.6
2.	7.82	α-Guaiene	C ₁₅ H ₂₄	8.96
3.	7.95	5-methoxy-2-phenyl-1H-1,3-Benzimidazole	C ₁₄ H ₁₂ N ₂ O	2.84
4.	8.16	Cycloisolongifolene, 8,9-dehydro-	C ₁₆ H ₂₂ O	2.44
5.	8.22	1H-Cycloprop[e]azulene	C ₁₁ H ₈	5.02
6.	8.45	Valencene	C ₁₅ H ₂₄	19.61
7.	8.59	Azulene	C ₁₀ H ₈	3.03
8.	10.24	1H-Cyclopropa[a]naphthalene	C ₁₁ H ₈	13.06
9.	12.20	10-Methylanthracene-9-carboxaldehyde	C ₁₆ H ₁₂ O	4.57
10.	12.44	1,4-Dimethoxy-6,7,8,9-tetrahydro-5-benzocycloheptenone	C ₁₃ H ₁₆ O ₃	5.27
11.	12.74	2-tert-Butylanthracene	C ₁₈ H ₁₈	13.48

RT= Retention time

Table 4

GC-MS analysis result of MF1

Peak No.	R.T. (min.)	Compound name	Molecular formula	Total area %
1.	8.214	γ-Eudesmol	C ₁₅ H ₂₆ O	9.21
2.	8.447	α-Eudesmol	C ₁₅ H ₂₆ O	18.83
3.	10.237	β-Eudesmol	C ₁₅ H ₂₆ O	71.96

RT= Retention time

3. RESULTS AND DISCUSSION

The findings demonstrated that the crude extract of *M. cajuputi* leaf and its fractions (MF1 and MF2) possess promising antioxidant and enzyme-inhibitory properties. However, MF3 and MF4 showed no activity in all the bioassays. While fractions MF1 and MF2 did not exhibit any toxicity effect, MCME demonstrated mild toxicity on *Artemia salina*. These results indicated that the bioactive compounds from *M. cajuputi* leaf could be used as alternative antioxidants and enzyme inhibitors. It has been reported that polyphenols, tannins, terpenes, and alkaloids are plant-active components and exogenous antioxidants with promising efficacy (Pandey et al., 2022).

3.1. Antioxidant activity

The antioxidant properties of MCME and its fractions, as determined by DPPH free radical scavenging assays, are presented in Table 1. The MCME exhibited significant antioxidant activity with the IC₅₀ value of 28.14 µg/mL, whereas fractions MF1 and MF2 demonstrated moderate antioxidant activity, with IC₅₀ values of 35.26 µg/mL and 80.90 µg/mL, respectively, and the reference compound (ascorbic acid) had an IC₅₀ value of 7.40 g/mL. Phytochemical analysis of the MCME revealed the presence of terpenes compounds and phenolics, which could be attributed to its remarkable free radical scavenging activity (Paari et al., 2019). According to a report, the antioxidant activity of natural products is significantly impacted by their phenolic contents (Al-Abd et al., 2015). The results of this study corroborate the antioxidant activity of *Melaleuca* species by Khalaf et al. (2021), which scored IC₅₀ values ranging from 60.97 µg/mL to 34.60 µg/mL. It has been highlighted that natural products could be regarded as potential alternative antioxidants, as synthetic antioxidants are associated with adverse effects (Jia et al., 2023). Consequently, the leaf of *M. cajuputi* could be considered a valuable source of natural antioxidants for industrial applications.

3.2. Enzyme inhibition and toxicity studies

The enzyme inhibition and toxicity effects of MCME and its fractions are presented in Table 2, and the lethality curves for the BSLT are obtainable in supplementary material, Appendix A. Based on the results, it was observed that MCME demonstrated significant anti-tyrosinase activity (IC₅₀ value of 65.81 µg/mL), followed by MF1 (IC₅₀ value of 131.3 µg/mL) and MF2 (IC₅₀ value of 215.3 µg/mL). The positive control (Kojic acid) demonstrated an IC₅₀ value of 198.4 µg/mL. Tyrosinase is an essential enzyme in melanogenesis. Therefore, inhibiting these enzymes is an important treatment strategy for hyperpigmentation disorders (Shahrivari et al., 2022). One of the most critical concepts in drug development for hyperpigmentation disorders is suppressing the tyrosinase enzyme by natural competitive inhibitors to avoid the skin-related issues associated with synthesized compounds like hydroquinones and kojic acid (Cheraif et al., 2020). The anti-tyrosinase activity of MCME could be due to the terpenes

and phenolic compounds identified in the extract. The results are consistent with that of [Saber et al. \(2021\)](#), who reported the anti-tyrosinase activity of *Feijoa sellowiana* (a member of the Myrtaceae family) with an IC_{50} value of 115.85 mg/mL. Flavonoids and phenolic substances have been shown to have considerable tyrosinase inhibitory activity ([Saidi et al., 2019](#)). Thus, the enormous bioactive components identified in the plant extract and fractions such as 3,4,5-trimethoxybenzoic acid, 2-isopropyl-10-methylphenanthrene, α -eudesmol, and 2-tert-butylanthracene could be responsible for the observed inhibitory effects. Interestingly, MCME and MF1 showed better anti-tyrosinase activity than kojic acid (positive control). Therefore, *M. cajuputi* leaf might be an alternative source of tyrosinase inhibitors.

The results of the anticholinesterase assay indicate that MF2 demonstrated the highest potency against cholinesterase enzyme, with an IC_{50} value of 3.35 μ g/mL. Followed by MF1 with an IC_{50} value of 26.7 μ g/mL. The least activity was observed on MCME (IC_{50} value of 282.6 μ g/mL). The IC_{50} value for the control compound (Galantamine) was 5.50 μ g/mL. Alzheimer's disease is usually managed by inhibiting AChE activity, which helps restore cholinergic stability ([Shahrivari et al., 2022](#)). Compared with galantamine (positive control), MCME and MF1 exhibited weak inhibitory activity on AChE. However, it is noteworthy that MF2 showed higher efficacy than galantamine. [Sareedenchai et al. \(2019\)](#) reported a remarkable percentage inhibition of the AChE enzyme by *M. cajuputi* essential oil ($21.18 \pm 0.54\%$). Similarly, oxygenated monoterpenes derived from *Mentha pulegium* and *Lavandula officinalis* exhibited remarkable AChE inhibition ([Cheraif et al., 2020](#)). The strong anticholinesterase activity of MF2 could be attributed to the major compounds 10-methylanthracene-9-carboxaldehyde and 2-isopropyl-10-methyl phenanthrene or the synergistic effect of all the bioactive components. The observed cholinesterase inhibition by MCME and fractions suggests that *M. cajuputi* has the potential to be a cognitive enhancer and may aid in the management of Alzheimer's disease.

On the other hand, the toxicity testing of the MCME and fractions on *Artemia salina* showed that MCME had LC_{50} values of 781 μ g/mL, whereas MF1 and MF2 recorded LC_{50} values of 6621 and 1165 μ g/mL, respectively. According to Clarkson's toxicity classification index, LC_{50} more than 1000 μ g/mL is considered non-toxic, LC_{50} between 500 μ g/mL and 1000 μ g/mL is termed low toxicity, whereas from 100 μ g/mL to 500 μ g/mL is moderately toxic and high toxicity is recorded at less than 100 μ g/mL ([Noor et al., 2020](#)). In light of these considerations, MCME (781 μ g/mL) showed low toxicity, whereas MF1 (6621 μ g/mL) and MF2 (1165 μ g/mL) were non-toxic against *Artemia salina*. In a previous study, it has been reported that MCME showed toxicity at LC_{50} 427 μ g/mL. However, *M. cajuputi* essential oil (MCEO), *M. cajuputi* aqueous extract (MCAE), and *M. cajuputi* decoction extract (MCDE) with LC_{50} values 2131.1, 1062, and 2477 μ g/mL respectively were non-toxic ([Noor et al., 2020](#)). In

contrast, [Daud et al. \(2018\)](#) reported that MCME had no toxic effects on the reproductive systems of male and female Sprague Dawley rats, perhaps due to several factors like varying solvent percentage, simultaneous physiological changes of the extract, and different exposure times in experiments.

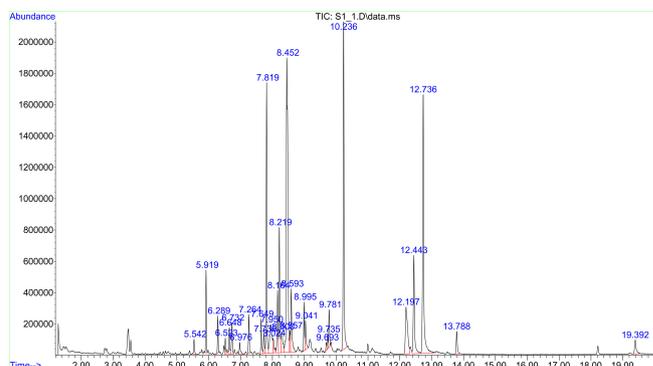


Figure 1. MCME total ion chromatograms from GC-MS analysis

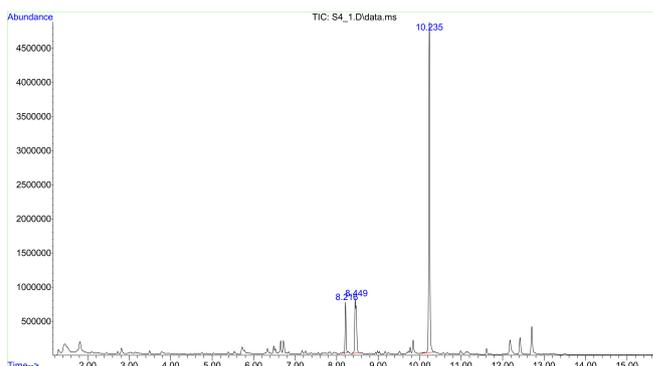


Figure 2. Fraction MF1 total ion chromatograms from GC-MS analysis

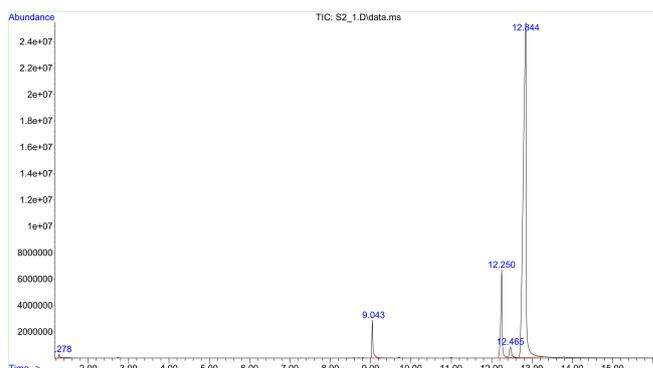


Figure 3. Fraction MF2 total ion chromatograms from GC-MS analysis

3.3. The GC-MS analysis of MCME, MF1, and MF2

The total ion chromatograms (TIC) of MCME are shown in [Figure 1](#). A total of thirty compounds were identified. Based on the TIC profile, 11 prominent peaks were observed

within the retention time range of 5.92 to 12.74 minutes, as indicated in Table 3. Valencene (sesquiterpenes) occurred at a retention time of 8.45 min and recorded the highest total area percentage (19.61%). The GC-MS analysis of MF1 identified three compounds, as presented in Table 4. The total ion chromatogram (TIC) of MF1 is shown in Figure 2. These compounds include β -eudesmol (71.96%), α -eudesmol (18.83%), and γ -eudesmol (9.21%). The GC-MS analysis of MF2 identified five compounds, as indicated in Table 5. The total ion chromatograms (TIC) are shown in Figure 3. The major compound is 2-isopropyl-10-methylphenanthrene, with a total area percentage of (83.09%). The presence of these bioactive compounds in the leaf extract of *M. cajuputi* corresponds with previous findings that reported similar phytoconstituents in *M. cajuputi* leaf extracts (Hassan et al., 2022; Sahimi et al., 2022). However, minor differences in the total area percentages of the compounds could be influenced by factors including geographical location, harvest time, plant parts used, and extraction processes (Hassan et al., 2022). These bioactive compounds can act synergistically or independently to provide various pharmacological effects, such as enzyme inhibition, antimicrobial, anti-inflammatory, and antioxidant properties (Cheraif et al., 2020). For example, the antioxidant activity of natural products like plant extracts is significantly linked to their phenolic contents (Al-Abd et al., 2015). Furthermore, flavonoids and phenolic substances such as 3,4,5-trimethoxybenzoic acid have been shown to have considerable tyrosinase and AChE inhibitory activity (Saidi et al., 2019). Terpenes compounds were also highlighted as novel antioxidants and enzyme inhibitors (Cheraif et al., 2020).

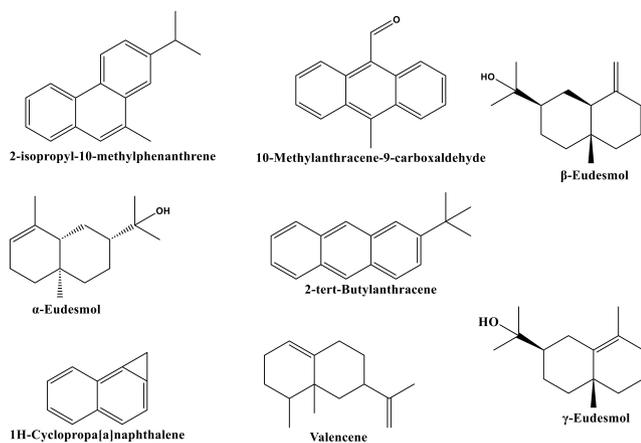


Figure 4. Chemical structures of the most active compounds from MCME, MF1, and MF2

3.4. Molecular docking studies

The predicted binding energy scores suggest that all the ligands studied potentially bound to AChE and tyrosinase (Table 6) and Figure 4. Analysis of ligand-enzyme interaction in some selected complexes reveals the details of the interaction mode. For instance, 10-methylantracene-9-carboxaldehyde bound to the AChE, mainly via π -sigma interactions with

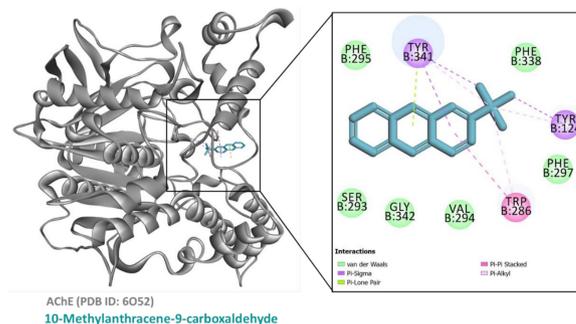


Figure 5. Interaction between AChE (PDB ID: 6O52) and 10-Methylantracene-9-carboxaldehyde

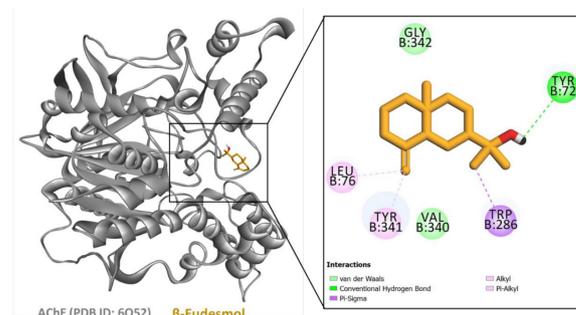


Figure 6. Interaction between AChE (PDB ID: 6O52) and β -Eudesmol

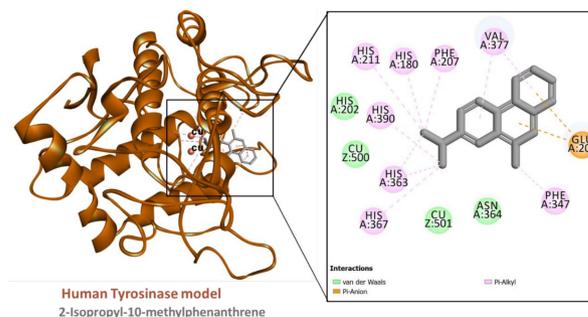


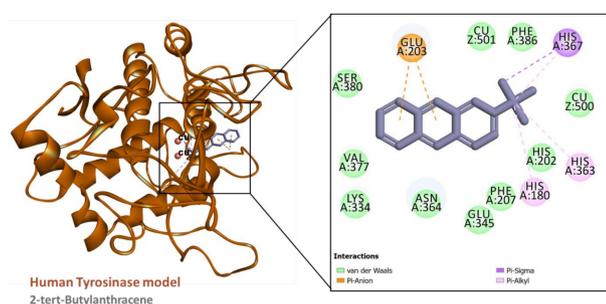
Figure 7. Interaction between the human tyrosinase model (built using PDB ID:6QXD) and 2-Isopropyl-10-methylphenanthrene

Tyr124 and Tyr341; a π - π stacked interaction with Trp286; hydrophobic interactions with Tyr124, Tyr341, and Trp286; as well as a few van der Waals interaction with amino acids residues lining the active site (Figure 5). The interaction between AChE and β -eudesmol is rather interesting: one H-bond with Tyr72, a π -sigma interaction with Trp286, two hydrophobic interactions with Leu76 and Tyr341, with two van der Waals interactions reinforcing the binding (Figure 6). The key interactions between tyrosinase and 2-isopropyl-10-methylphenanthrene are multiple hydrophobic contacts with residues deep inside the pocket and π -anion interactions with Glu203 near the entrance

Table 6

Binding energy scores (kcal/mol) of the compounds towards the target enzymes

Compound	Binding energy (kcal/mol)		Key interacting residues (Tyrosinase)	Key interacting residues (AChE)
	Tyrosinase	AChE		
α -Eudesmol	-5.3	-6.4	Tyr124, Trp286, Tyr341	Phe347, Phe207, His367, Val377
β -Eudesmol	-5.1	-6.7	Tyr72, Leu76, Trp286, Tyr341	Phe347, His363, His367, Val377
γ -Eudesmol	-5.3	-6.4	Tyr124, Trp286, Tyr341	Phe347, Phe207, His367, Val377
Valencene	-5.1	-5.9	Leu76, Phe297, Tyr341	His367, Ile368, Val377
2-tert-Butylanthracene	-6.2	-7.5	Tyr72, Val340, Tyr341, Phe297	His180, Glu203, His363, His367, Val377, His390
1H-Cyclopropa[a]naphthalene	-5.1	-6.4	Tyr124, Trp286, Tyr341	Glu203, Phe207, His211, His367
2-Isopropyl-10-methyl phenanthrene	-6.2	-7.4	Tyr124, Trp286, Val340, Tyr341	His180, Glu203, Phe207, His211, Phe347, His363, His367, Val377, His390
10-Methylantracene-9-carboxaldehyde	-5.8	-7.6	Tyr124, Trp286, Phe297, Tyr341	Glu203, Phe207, His211, Phe347, His363, His367

**Figure 8.** Interaction between the human tyrosinase model (built using PDB ID:6QXD) and 2-tert-Butylanthracene

to the active site. In addition, the two catalytically essential Cu^{2+} ions, along with a couple of residues, were found to be involved in van der Waals interactions (Figure 7). It is also interesting to note that 2-tert-butylanthracene engaged tyrosinase via two π -anion interactions with Glu203, three hydrophobic contacts with His180, His363, and His367: a π -sigma interaction with His367. Moreover, the two active site Cu^{2+} ions and several amino acid residues were involved in van der Waals interactions with the molecules (Figure 8). This suggests that the bioactive compounds in *M. cajuputi* leaves extract and fractions potentially bind to and inhibit the activities of AChE and tyrosinase.

The binding scores from the molecular docking analysis demonstrate a strong correlation with the inhibitory potential of MCME and its fractions. The MCME and MF1 had higher inhibitory activity against the tyrosinase enzyme. Similarly, their active components showed a strong binding affinity for the enzyme, as evidenced by docking scores ranging from -5.1 kcal/mol to -6.2 kcal/mol. In the same vein, the compounds 10-methylantracene-9-carboxaldehyde and 2-isopropyl-10-methyl phenanthrene, identified in MF2, had a strong affinity for AChE, as indicated by their respective docking scores of -7.6 and -7.4 kcal/mol. This finding provides evidence for the effectiveness of MF2 in inhibiting the activity

of AChE. A previous study reported the binding affinities of the standard compounds kojic acid and galantamine towards tyrosinase and AChE, with docking scores of 5.4 kcal/mol and 7.6 kcal/mol, respectively (Dilshad et al., 2022). The findings of this study are similar to previous reports on the inhibitory potential of triterpene, steroid, and flavonoid compounds on tyrosinase and AChE enzymes (Saber et al., 2021; Yurtcu et al., 2022).

4. CONCLUSION

In conclusion, this is the first comprehensive study highlighting the antioxidant, anti-tyrosinase, anti-cholinesterase, and possible mechanism of action of *M. cajuputi* leaf extract and fractions. The results suggest that MCME and its fractions have significant potential as a source of active compounds with protective properties. Thus, it has promising applications to act as a co-adjuvant in cosmetics and neurodegenerative disease management. Further research is needed to isolate and purify the active components responsible for biological activities.

5. CONFLICTS OF INTEREST

Given his role as Associate Editor, Gokhan Zengin has not been involved and has no access to information regarding the peer review of this article. Full responsibility for the editorial process for this article was delegated to Editor-in-Chief Carlos L. Cespedes Acuña. There are no relevant conflicts of interest regarding this article.

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A. APPENDIX. SUPPLEMENTARY INFORMATION

The supplementary information associated with this article available at <https://doi.org/10.53365/nrfhh/176775>.

AUTHOR CONTRIBUTIONS

Musa Isah - Collection and/or assembly of data and writing the article. Gokhan Zengin - Critical revision of the article. Wan-Nor-Amilah Wan Abdul Wahab - Research concept and design. Hasmah Abdullah - Critical revision of the article. Mohd Dasuki Sul'ain - Final approval of the article. Abdullahi Ibrahim Uba - Data analysis and interpretation. Wan Rosli Wan Ishak - Research concept and design. Shajarahtunnur Jamil - Data analysis and interpretation.

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