Future Journal of Pharmaceutical Sciences

RESEARCH

Open Access

Chemical profile, antioxidant and anti-Alzheimer activity of leaves and flowers of *Markhamia lutea* cultivated in Egypt: in vitro and in silico studies

Mai Magdy[1], Ahmed H. Elosaily[1], Engy Mohsen[2] and Hala M. EL Hefnawy[2*]

Abstract

Background Nowadays Alzheimer's disease and its treatment methods are global concerns. Patients with this disease have poor prognosis and need supportive treatment. The antioxidant activity, anti-acetylcholinesterase (anti-AChE), anti-butyryl cholinesterase (anti-BChE) and Aβ-amyloid-42 inhibition activities of the ethanolic extracts of both leaves and flowers (LEE and FEE) of *Markhamia lutea* were assessed. The antioxidant activity of LEE and FEE was evaluated using 2,2-diphenyl-1-picryl-hydrazyl-hydrate, oxygen radical absorbance capacity and ferrozine iron metal chelation assays. Additionally, their total flavonoids and total phenolics were determined. The phytochemicals of LEE were analysed using LC–MS/Q-TOF in both positive and negative modes. Also, molecular docking was done for phytochemicals identified in LEE.

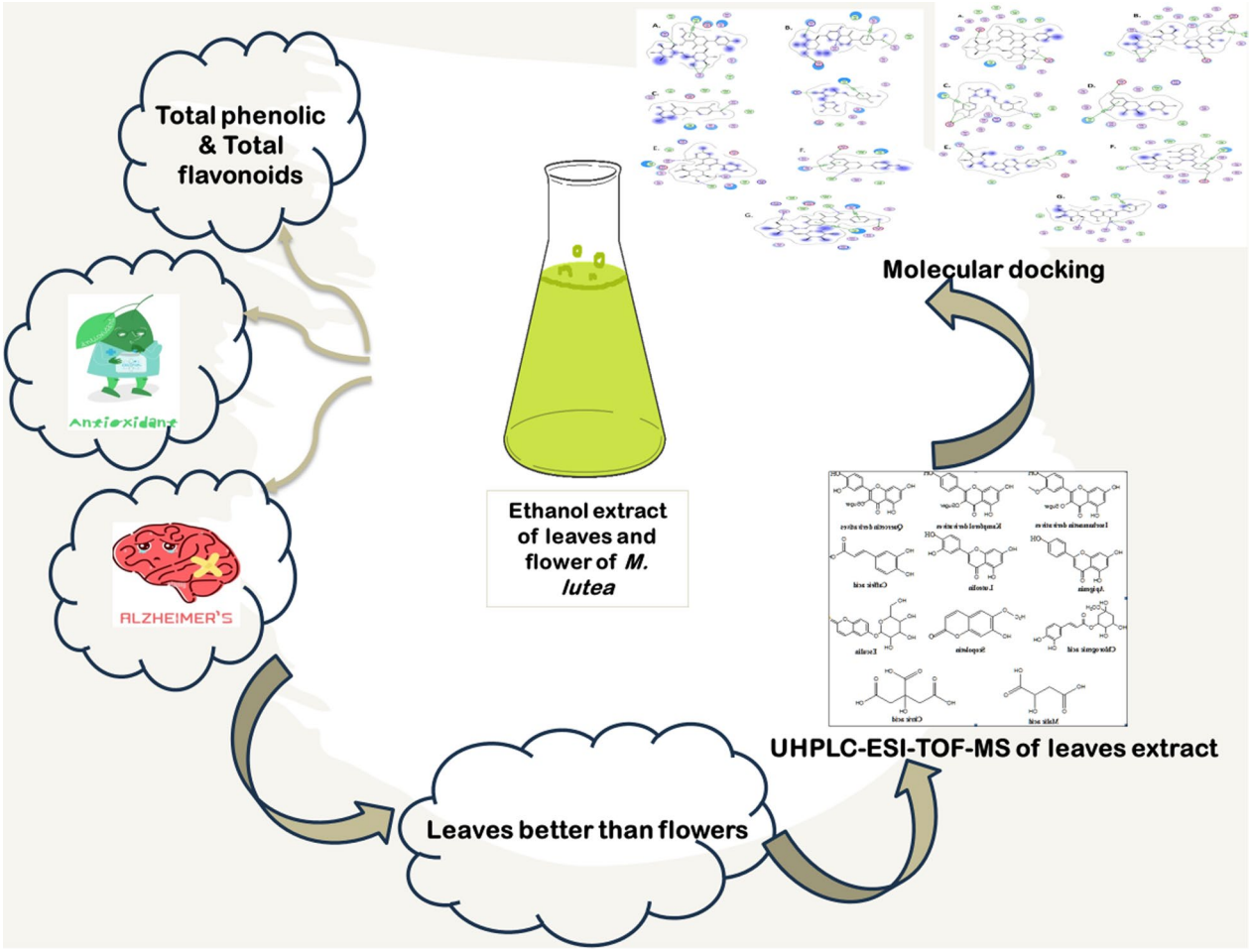
Result LEE exhibited higher antioxidant and anti-Alzheimer activities in all techniques due to its high flavonoids content. LEE showed better activity than donepezil in case of anti-butyryl cholinesterase than both donepezil and rivastigmine in case of Aβ-amyloid-42 inhibition. A total of 62 compounds were tentatively identified using Ultra-performance Liquid Chromatography-Electrospray Ionization-Quadrupole Time-of-Flight Mass Spectrometry (UHPLC–ESI–TOF–MS), viz. 35 flavonoids, 11 phenolic acids, 2 terpenoids, 2 phenylpropanoids derivatives, 7 polyphenols, 3 coumarins and 2 organic acids. The molecular docking of some constituents showed that isorhamnetin-*O*-rutinoside, sissotrin, 3,5,7-trihydroxy-4′-methoxyflavone (diosmetin), rosmarinic acid, kaempferol hexoside, kampferol-7-neohesperoside, acacetin, taxifolin and apigenin-*O*-hexoside exert a promising activity as anti-Alzheimer drugs.

Conclusion The LEE of *Markhamia lutea* contains secondary metabolites that is promising to act as natural antioxidants, acetylcholinesterase, butyryl cholinesterase and Aβ-amyloid-42 inhibition inhibitors, which can aid in the treatment of Alzheimer's.

Keywords Anti-Alzheimer, Antioxidant, *Markhamia lutea* (Benth) K. Schum, *M. lutea*, Flower, Leaves, Molecular docking, Q-TOF LC/MS/MS

*Correspondence:
Hala M. EL Hefnawy
Hala.elhefnawy@pharma.cu.edu.eg
Full list of author information is available at the end of the article

Graphical Abstract



Background

AD is the most common cause of dementia, especially in the elderly, who account for about 60–70% of cases [1]. Discovering improved disease modifying therapies against dementia remains a major challenge [2]. Acetylcholine (Ach) neurotransmitter levels are shown to be lowered in AD patients with cognitive and behavioural impairments, which are linked to the disruption of cholinergic pathways in the cerebral cortex and basal forebrain [1]. According to the amyloid hypothesis, neuropathology in Alzheimer's disease, such as neuronal cell death, is linked to the extracellular build-up of amyloid-β (Aβ) and neurotoxicity result from it [3]. Although the role of butyryl cholinesterase (BCh) remains incompletely understood, selective butyryl cholinesterase inhibitors (BChEI) have been developed to enhance ACh levels and diminish aberrant amyloid formation in AD [4]. Consequently, oxidative stress-reducing substances, acetyl cholinesterase inhibitor (AChI), butyryl cholinesterase inhibitor (BChI) and Aβ-amyloid-42 inhibitor are being investigated as potential therapeutic or preventive options for neurodegenerative diseases like Alzheimer's.

Natural products remain an important source of medication development. Herbs are gaining recognized for their health advantages, prompting the World Health Organization to urge for their usage over chemicals as part of the return to nature movement. This tendency is further supported by research into nontraditional raw components, such as plant and animal by-products [5]. Plant extracts were evaluated for actions related to AD treatment. A literature survey revealed that there is a potential of *M. lutea* to be used in treatment of dementia by using Prediction of Activity Spectra for Substances (PASS) online programme due to presence of two

Fig. 1 Photograph of leaves and flowers of *M. lutea*

cycloartane triterpenoids and their corresponding glycosides. The upright evergreen tree *M. lutea* belonging to family *Bignoniaceae* can reach 10–16 m in height bearing dark green leaves and bright yellow flowers appearing as showy terminal clusters (Fig. 1). It is commonly called the Bell Bean tree, Nile Tulip tree and Siala. Flavonoids, terpenoids, phenylpropanoids and their glycosides were identified from different parts of the plant. Traditionally, several plant parts are used to cure a number of illnesses, including anaemia, diarrhea, microbial and parasitic infections. Biological studies reported the antimicrobial, antiviral, anticancer and antioxidant activities of leaves or aerial parts of *M. lutea* [6]. On the other hand, little is known about the plant's ability to prevent Alzheimer's disease in the literature. For this reason, the study focuses on the neuroprotective effects and antioxidant activity of *M. lutea* leaves and flowers. LEE and FEE were tested for their antioxidant activity using 2,2-diphenyl-1-picryl-hydrazyl (DPPH), ferrozine iron metal chelation and oxygen radical absorbance capacity (ORAC), as well as their anti-cholinesterase activity, anti-butyryl cholinesterase and anti-Aβ amyloid-42 in management of AD. UHPLC–ESI–TOF–MS was also employed for analysing metabolites of *M. lutea* leaves in order to find components that would be useful for the plant's neuroprotective properties. Ultra-high-performance liquid chromatography is a hyphenated chromatographic techniques that is increasingly applied in plant phenolics profiling. It provides shorter time for analysis with higher sensitivity and resolution that allows the detection of minor metabolites providing a broad insight of plant metabolite profile. Both positive and negative ionization mode of were used to provide a comprehensive coverage of *M. lutea* metabolome. Also, in silico techniques were utilized to find chemical constituents in *M. lutea* that may contribute to the inhibition of acetylcholinesterase (AChE) and butyryl cholinesterase (BChE). Phenols and flavonoids are recognized to benefit human health and have antioxidant and anti-Alzheimer effects, and hence, the phenolic and total flavonoids content of LEE and FEE were evaluated.

Materials and methods

Materials

Plant material collection

A plant taxonomy consultant for the Agriculture Ministry, Therease Labib, is an agriculture engineer who was the director of El-Orman Botanic Garden in Giza, Egypt, verified fresh leaves and blossoms collected in June 2020 from the Botanical Garden, a voucher specimen (No. 22.10.2023).

Chemicals and reagent

Fisher Scientific, USA, provided the sodium hydroxide, formic acid and methanol needed to alter the pH for UHPLC–ESI–TOF–MS analysis. The supplier of DPPH, rutin, gallic acid, ammonium formate and acetonitrile was Sigma-Aldrich, Germany. The source of the water for UHPLC–ESI–TOF–MS analysis was Millipore, USA. Other chemicals and reagents used in this study were obtained from standard sources.

Methods

Sample preparation

Fresh leaves (1000 g) and blossoms (120 g) were both finely chopped before being fully extracted using 70% ethanol maceration. Following filtering, the extracts were vacuum-evaporated at a temperature not to exceed 50 °C using a rotary evaporator (Buchi, Switzerland). As a result, 80.5 g of dry LEE residue and 40.5 g of dried

FEE residue were produced, respectively. For additional research, the desiccator held the dried residues.

Analytical methods

There are in vitro tests for antioxidants and anti-Alzheimer's

DPPH assay

Using the DPPH free radical assay, the LEE and FEE were evaluated, as detailed in [7]. In summary, in 96-well plates ($n=6$), 100 µL of the newly prepared DPPH reagent (0.1% in methanol) was combined with 100 µL of the sample. After incubation for 30 min in the dark at ambient temperature, the DPPH colour intensity was measured at 540 nm to determine its decrease.

ORAC assay

Following [8] method, with a few minor adjustments, the ORAC test was performed on the LEE and FEE. Specifically, 30 min at 37 °C were spent incubating 12.5 µL of the prepared samples with 75 µL fluorescein (10 nM). Three 90-s fluorescence cycles (485 EX, 520 EM and nm) were carried out in order to quantify the background. Following this, 12.5 µL of freshly prepared 2, 2'-Azobis (2-amidinopropane) dihydrochloride (AAPH) at a concentration of 240 mM was immediately added to each well. The measurements of fluorescence (485 EX, 520 EM, and nm) were taken for 2.5 h.

FIC assay (ferrozine iron metal chelation)

Based on [9], the assay was performed for both LEE and FEE, with a few minor adjustments that needed to be made in microplates. Specifically, 50 mL of the sample or substance was mixed with 20 mL of newly prepared ferrous sulphate (0.3 mM) on 96-well plates ($n=6$). Following that, 30 µL of ferrozine (0.8 mM) was introduced into every well and allowed to incubate for ten minutes at ambient temperature. At the end of time of incubation, the decrease in the generated colour intensity at 562 nm was measured.

Acetyl cholinesterase inhibitory activity

Using a slightly modified spectrophotometric technique, the inhibitory activity of acetylcholinesterase was determined [10]. The enzyme used was AChE; the substrate was acetylthiocholine iodide (AChI); the colouring agent was 5,5-Dithiobis-(2-nitrobenzoic acid) (DTNB) used as the colouring agent; and control was donepezil as positive. Potential AChE activity inhibitors are screened using BioVision's Acetylcholinesterase Inhibitor Screening Kit (RayBio_Quantichrom ACHE inhibitors screening Kit). Measurements of absorbance at 412 nm can be used to identify the yellow chromophore that is produced by an active human AChE enzyme after it has hydrolysed the provided colorimetric substrate. The following formulas were used to calculate the percentage of both activity and inhibition I (%):

$$\%\ \text{of inhibition} =: \frac{\text{slopeofcontrol} - \text{slopeofsample}}{\text{slopeofcontrol}} * 100$$

$$\%\ \text{of activity} = \frac{\text{slopeofsample}}{\text{slopeofcontrol}} * 100$$

Butyryl cholinesterase inhibitory activity

Butyryl cholinesterase inhibitory activity was measured using a slightly modified spectrophotometric method. BChE was used as an enzyme, acetylthiocholine iodide (BChI) as a substrate, 5,5-Dithiobis-(2-nitrobenzoic acid) (DTNB) as a colouring agent and donepezil and rivastigmine as positive controls. BioVision's Acetylcholinesterase Inhibitor Screening Kit (RayBio_Quantichrom BCHE inhibitors screening Kit) is used for screening potential inhibitors of BChE activity. Through the process of hydrolysing the colorimetric substrate provided, the active human BChE enzyme is capable of generating a yellow chromophore that can be identified by measuring the absorbance at 412 nm. The following formulas were used to calculate the percentage of both activity inhibition I (%):

$$\%\ \text{of inhibition} =: \frac{\text{slopeofcontrol} - \text{slopeofsample}}{\text{slopeofcontrol}} * 100$$

$$\%\ \text{of activity} = \frac{\text{slopeofsample}}{\text{slopeofcontrol}} * 100$$

Aβ-amyloid-42 inhibitory assay

Based on the thioflavin T fluorescence method reported by [11], the inhibitory experiment was conducted. To prevent evaporation, 200 lL of ThT samples were sealed in black Clear 96-microwell plates and incubated at 37 °C. Every five minutes the ThT fluorescence intensity of each sample was monitored using a FLUOstar OPTIMA plate reader (BMG Lab technologies, Melbourne, VIC, Australia) with 440 / 490-nm excitation /emission filters set. The change in ThT fluorescence (in arbitrary units, a.u.) and DF, as defined by the equation, were plotted to normalize the ThT data. DF is equal to the average ThT fluorescence measurement (in arbitrary units, a.u.) from triplicate trials (F)—the average

ThT fluorescence measurement (in arbitrary units, a.u.) following a 30-min incubation period (F30).

Total phenolic content

The total phenolic content of LEE and FEE was determined according to [12]. 100 µL of the standard or sample and 10 µL of the sample were combined in a 96-well microplate of diluted Folin–Ciocalteu reagent (1:10). Next, 80 µL of 4 N Na_2CO_3 was added. After that, the microplate was left in the dark for 20 min at room temperature (25 °C). Using the FluoStar Omega microplate reader, the absorbance of the blue colour produced was measured at 630 nm at the end of time of incubation. The reference standard was gallic.

Total flavonoid content

The total flavonoid content of LEE and FEE was estimated using the aluminium chloride colorimetric method according to [13]. In summary, 1.5 ml of methanol, 0.1 ml of aluminium chloride, 0.1 ml of potassium acetate solution, 2.8 ml of distilled water, and 0.5 ml of each extract stock solution were added and thoroughly mixed. A similar procedure was used to prepare the sample blank, using distilled water instead of aluminium chloride. All extracts were prepared as samples and sample blanks, and the FluoStar Omega microplate reader was used to analyse the absorbance of the samples at 630 nm. The reference standard utilized was rutin.

UHPLC–ESI–TOF–MS analysis and metabolite identification

The LEE was analysed using UHPLC–ESI–TOF–MS in accordance with the methodology outlined by [14]. The ExionLC system (AB Sciex, Framingham, MA, USA) equipped with an autosampler system and an X select HSS T3 column (2.5 µm, 2.1×150 mm, Waters Corporation, Milford, MA, USA) was used for the UHPLC–ESI–TOF–MS investigation. To create a gradient flow of the mobile phase, acetonitrile (ACN) and water were combined with 0.1% formic acid in each solvent. A 10% increase in ACN was initiated after the first 20 min of the experiment and then increased to 90% from 21 to 25 min. From 25.01 min onwards, the ACN level was maintained at 10% until the end of the experiment at 28 min, with a constant flow rate of 0.3 ml/min. Using a DuoSpray source in the electrospray ionization (ESI) mode, the Triple TOF 5600 + system (AB SCIEX, Concord, ON, Canada) was utilized for the mass spectrometry. The LC-Triple TOF control was performed using the Analyst-TF 1.7.1 program [14].

Molecular modelling for acetylcholinesterase and butyryl cholinesterase receptor

Docking simulations were carried out with the structure preparation programme in Molecular Operating Environment (MOE), 2014.10 33–35. The structure of X-ray crystallography AChE (PDB code: 4moe) and BChE (PDB code: 4aqd) crystals was gathered from the Protein Data Bank on the [www.rcsb.org] 36 website of the Research Collaboration for Structural Bioinformatics (RCSB). The default strategy of the MOEDock application was utilized to identify the best binding configurations for the ligands that were being studied. The optimum poses were ordered based on their GBVI/WSA DG free energy calculations. Visual inspection and analysis were conducted on docking postures and interactions with binding pocket residues.

Statistical analysis

For DPPH GraphPad Prism 5® was used to calculate the inhibitory concentration 50 (IC_{50}) value. For ORAC and FIC, the data were recorded using the FluoStar Omega microplate reader (85 cycles, each lasting 90 s). For AChE, BCHE and Aβ-Amyloid-42 inhibition activities were recorded as mean ± SD, and the results were expressed as an IC_{50} value (µg/mL). Results of total phenolic and total flavonoids were expressed as mg per g extract and as means ± SD, and the data were represented.

For UHPLC-ESI-TOF–MS analysis and metabolite identification, PeakView2.2 software (Sciex) was utilized for the extraction and identification of metabolites. Furthermore, peaks based on their fragments were identified using an open-source programme named MS-DIAL 4.6 and datasets such as ReSpect positive (2737 records) and ReSpect negative (1573 records). Through comparison with the reference literature, the compounds' preliminary identification was also verified.

Results and discussion

Antioxidant and anti-Alzheimer activities of *M. lutea*

Experimentally, LEE demonstrated stronger antioxidant activity than FEE in the employed assays, even when compared to the standard Trolox. (IC_{50} 24.42 ± 0.87 µg/ml). The IC_{50} of LEE was 35.69 ± 1.02 µg/ml compared to 38.39 ± 1.02 µg/ml for flowers utilizing DPPH assay. The leaves had a greater antioxidant potential (16,694.4 ± 2526.7µM TE/mg extract and 70.7 ± 5.4 µM EDTA eq/mg extract) than the flowers (5213.3 ± 517.8 µM TE/mg extract and 7.8 ± 0.7 µM EDTA eq/mg extract), respectively (Table 1).

Similarly, LEE exhibited superior anti-Alzheimer action compared to FEE. LEE indicated a higher level of inhibition of acetylcholinesterase and butyrylcholine

Table 1 DPPH, ORAC and ferrozine iron metal chelation antioxidant results for LEE and FEE

M. lutea extracts	DPPH (µg/ml)	ORAC (µM TE/mg extract)	Ferrozine iron metal chelation (µM EDTA eq/mg extract)
LEE	35.69 ± 1.02	16,694.4 ± 2526.7	70.7 ± 5.4
FEE	38.39 ± 1.02	5213.3 ± 517.8	7.8 ± 0.7

DPPH: 2,2-diphenyl-1-picryl-hydrazyl-hydrate assay, ORAC: Oxygen radical absorbance capacity assay

Table 2 IC_{50} for acetylcholinesterase, butyryl cholinesterase and Aβ amyloid-42 inhibitory activities for FEE and LEE

M. lutea extracts	Acetyl cholinesterase (µg/ml)	Butyryl cholinesterase (µg/ml)	Aβ amyloid-42 inhibitory (µg/ml)
LEE	5.252 ± 0.28	0.025 ± 0.008	12.02 ± 0.56
FEE	17.69 ± 0.93	0.112 ± 0.105	47.99 ± 2.25

esterase with IC_{50} value 5.252 ± 0.28 ug/ml and 0.025 ± 0.008 ug/ml than that of FEE with IC_{50} value 17.69 ± 0.93 ug/ml and 0.112 ± 0.022 ug/ml compared to donepezil with IC_{50} value 2.031 ± 0.11 ug/ml and 0.026 ± 0.003 ug/ml as a positive control, respectively (Table 2), Figs. 2 and 3. In Aβ-Amyloid-42 inhibition assay, LEE showed a greater inhibition IC_{50} of 12.02 ± 0.56 ug/ml than FEE (IC_{5O} 47.99 ± 2.25 ug/ml) and donepezil (IC_{50} 40.59 ± 1.9 ug/ml) Fig. 4 and (Table 2).

The data showed that LEE is the most promising drug for preventing Alzheimer's disease and serving as an antioxidant, and that it should undergo further chemical investigation.

Chemical profiling of *M. lutea*

Analysis of total flavonoids and phenolics revealed that FEE had a greater total phenolic content (101.9 mg/g extract ± 3.5) than LEE (94.3 mg/g extract ± 5.9). Interestingly, the highest level of total flavonoids was detected in LEE (51.8 mg/g extract ± 2.8) than FEE with (31.7 mg/g extract ± 1) (Table 3). Several research works have demonstrated the diverse pharmacological properties of flavonoids, including their anti-inflammatory, hepatoprotective, antioxidant and anti-angiogenic properties [15]. The flavonoid content of LEE was analysed by UHPLC–ESI–TOF–MS to identify and correlate with the biological activities.

Sixty-two phytoconstituents of different chemical classes were tentatively recognized in the LEE using

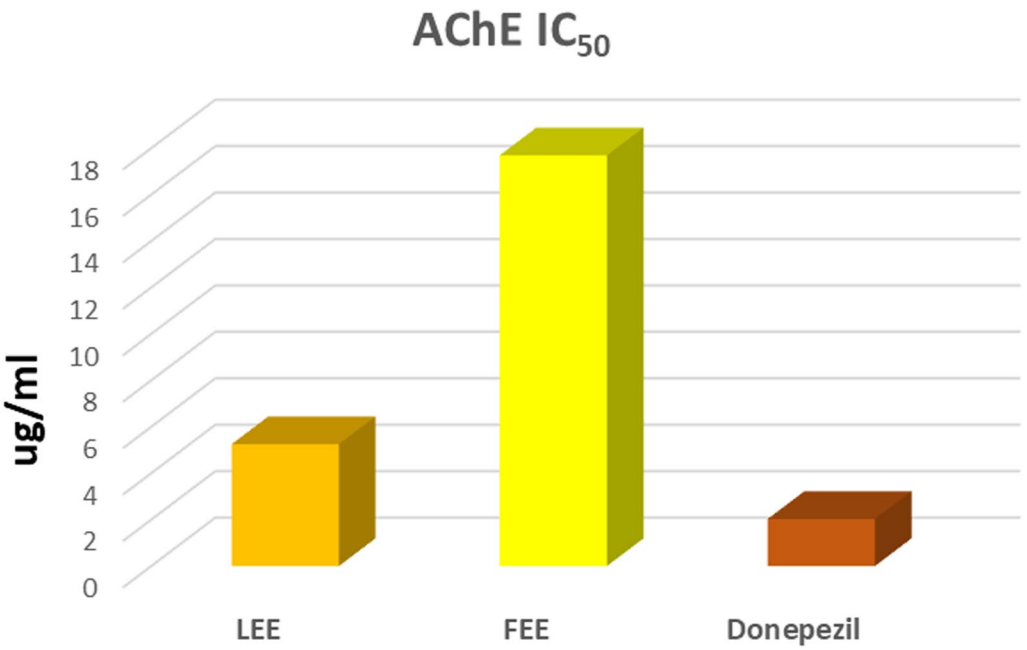


Fig. 2 Acetyl cholinesterase inhibition activities of different extracts of *M. lutea* and donepezil

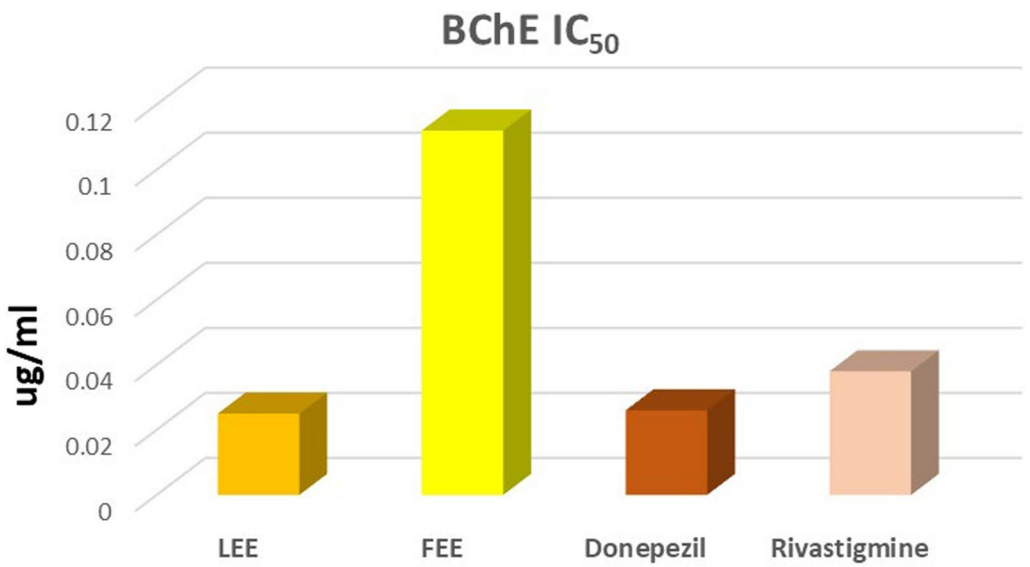


Fig. 3 Butyryl cholinesterase inhibition activities of LEE, FEE of *M. lutea,* donepezil and rivastigmine

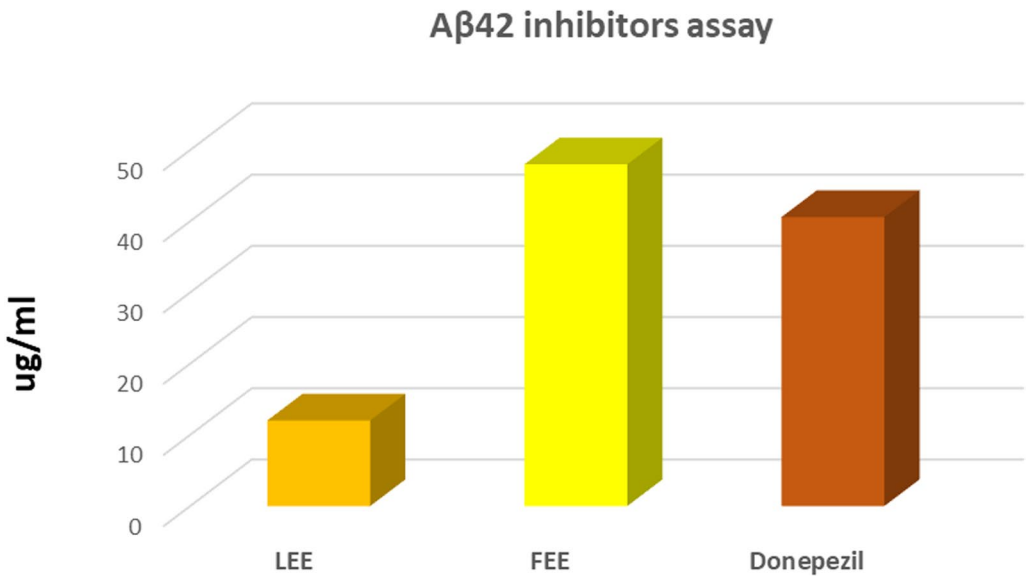


Fig. 4 Aβ-amyloid-42 inhibition activities of LEE, FEE of *M. lutea* and donepezil

positive and negative modes (Table 4, Figs. 5, 6). The successfully identified compounds were 36 flavonoids, 11 phenolic acids, 2 terpenoids, 2 phenylpropanoids, 6 polyphenols, 3 coumarins and 2 organic acids placed in order of retention duration (R_T). Structures of some compounds identified in UPLC–ESI–TOF–MS are shown in Figs. 7 and 8. For more information about identified compound fragmentation, kindly check (Table 4) in supplementary data.

Table 3 Total phenolics and flavonoids contents for *M. lutea* LEE and FEE

M. lutea extracts	Total phenolic (mg/g extract ± SD)	Total flavonoids (mg/g extract ± SD)
LEE	94.3 mg/g extract ± 5.	51.8 mg/g extract ± 2.8
FEE	101.9 mg/g extract ± 3.5	31.7 mg/g extract ± 1

Table 4 Metabolites identified in *M. lutea*LEE using Q-TOF LC/MS/MS

Peak no	RT (min)	ESI ±	Precursor *m/z*	Molecular formula	Error (ppm)	Identified compound	Product ions
1	1.072	$[M–H]^-$	133.0136	$C_4H_6O_5$	0.4	D-(+)-Malic acid	71, 115
2	1.121	$[M–H]^-$	209.0647	$C_6H_{10}O_8$	3.4	Mucate (Galactarate)	147
3	1.134	$[M–H]^-$	191.0562	$C_6H_8O_7$	− 0.5	Citric acid	111, 129, 173
4	1.195	$[M–H]^-$	153.0192	$C_7H_6O_4$	0.6	2,5-Dihydroxy benzoic acid (Protocatechuic acid)	109
5	1.336	$[M–H]^-$	447.1141	$C_{21}H_{20}O_{11}$	0.8	Quercitrin	251, 341, 301
6	1.363	$[M–H]^-$	153.0175	$C_7H_6O_4$	4.5	3,4-dihydroxy benzoic acid	109
7	1.402	$[M–H]^-$	163.039	$C_9H_8O_3$	0.5	3-(4-Hydroxy phenyl) prop-2-enoic acid (Coumaric acid)	119
8	3.039	$[M–H]^-$	461.1685	$C_{21}H_{18}O_{12}$	− 2.9	Kaempferol hexoside	285, 324,392
9	3.456	$[M–H]^-$	445.1363	$C_{21}H_{18}O_{11}$	− 0.7	Baicalein-*O*-hexoside	143, 161, 268, 283
10	4.106	$[M–H]^-$	609.1465	$C_{27}H_{30}O_{16}$	− 0.4	Luteolin-di-*O*-hexoside	285, 447
11	4.216	$[M–H]^-$	419.1555	$C_{21}H_{24}O_9$	0.7	4-deoxy phloridzin	257, 282
12	4.240	$[M–H]^-$	301.0626	$C_{16}H_{14}O_6$	− 0.2	Hesperetin	301
13	4.240	$[M–H]^-$	339.0716	$C_{15}H_{16}O_9$	0.9	Esculin	159, 161, 177
14	4.362	$[M+H]^+$	137.0246	$C_7H_6O_3$	− 0.1	P-hydroxy benzoic acid	93
15	4.597	$[M–H]^-$	593.1507	$C_{27}H_{30}O_{15}$	2.2	Kaempferol-7-neohesperidoside	285, 351, 353, 383, 473, 503, 589, 591
16	5.148	$[M–H]^-$	353.0867	$C_{16}H_{18}O_9$	2.2	Chlorogenic acid	191
17	5.285	$[M+H]^+$	305.06558	$C_{15}H_{12}O_7$	4.4	(+-)-Taxifolin	153,163
18	5.950	$[M–H]^-$	359.0984	$C_{18}H_{16}O_8$	− 0.4	Rosmarinic acid	150,169,314
19	6.411	$[M+H]^+$	579.1451	$C_{30}H_{26}O_{12}$	0.5	Procyanidin B2	561
20	6.523	$[M–H]^-$	507.15	$C_{23}H_{24}O_{13}$	0.9	Syringetin-*O*-hexoside	323, 354, 394, 462, 489
21	6.534	$[M+H]^+$	595.1621	$C_{27}H_{31}O_{15}$	2.8	Cyanidin-*O*-rutinoside (Antirrhinin)	287, 441, 449
22	6.560	$[M–H]^-$	593.155	$C_{27}H_{30}O_{15}$	− 2.9	Datiscin	285
23	6.610	$[M–H]^-$	463.0869	$C_{21}H_{20}O_{12}$	0.9	Quercetin hexoside	301
24	6.771	$[M–H]^-$	623.1973	$C_{28}H_{32}O_{16}$	1.3	Isorhamnetin-*O*-rutinoside	315
25	6.794	$[M+H]^-$	317.0762	$C_{15}H_{10}O_8$	− 0.5	Myricetin	271, 179,137,151
26	6.891	$[M–H]^-$	447.0961	$C_{21}H_{20}O_{11}$	− 3	Luteolin-*O*-hexoside	284, 285
27	7.035	$[M–H]^-$	477.1435	$C_{22}H_{22}O_{12}$	− 3.9	Isorhamnetin-*O*-hexoside	271, 315, 409
28	7.059	$[M–H]^-$	431.1552	$C_{21}H_{20}O_{10}$	0.8	Kaempferol-3-*O*-hexoside (Afzelin)	363
29	7.132	$[M–H]^-$	595.2059	$C_{27}H_{32}O_{15}$	− 1.4	Eriodictyol-7-*O*-neohesperidoside (Neoeriocitrin)	289, 593, 594
30	7.333	$[M+H]^+$	609.2134	$C_{28}H_{32}O_{15}$	− 3.7	Diosmin	325
31	7.432	$[M–H]^-$	447.0958	$C_{21}H_{20}O_{11}$	− 2.3	Maritimetin-*O*-hexoside	285
32	7.620	$[M+H]^+$	593.1724	$C_{28}H_{32}O_{14}$	− 0.6	Acacetin-*O*-rutinoside (Linarin)	286,243
33	7.633	$[M+H]^+$	433.1094	$C_{21}H_{20}O_{10}$	1.5	Apigenin-*O*-hexoside	271, 431
34	7.646	$[M+H]^+$	449.1093	$C_{21}H_{21}O_{11}$	− 0.9	Cyanidin-*O*-hexoside	267, 287
35	7.828	$[M+H]^+$	177.0186	$C_9H_6O_4$	1	Daphnetin	121, 133, 149
36	8.157	$[M–H]^-$	405.1031	$C_{20}H_{22}O_9$	− 0.4	E-3,4,5′-Trihydroxy-3′-glucopyranosylstilbene	191
37	8.458	$[M+H]^+$	417.155	$C_{21}H_{20}O_9$	0.5	Puerarin (Daidzein-*C*-hexoside)	191
38	8.618	$[M–H]^-$	609.2725	$C_{28}H_{34}O_{15}$	1.8	Hesperetin-*O*-neohesperidoside	285, 607
39	8.630	$[M–H]^-$	577.1915	$C_{27}H_{30}O_{14}$	1.3	Rhoifolin (Apigenin-*O*-neohesperidoside)	133, 161, 576
40	9.171	$[M+H]^+$	193.0483	$C_{10}H_8O_4$	2.5	Scopoletin	133, 151, 153, 175
41	9.523	$[M+H]^+$	303.04993	$C_{15}H_{10}O_7$	− 0.2	Quercetin	122, 259
42	9.718	$[M+H]^+$	287.0538	$C_{15}H_{10}O_6$	0.1	Luteolin	287, 137,153, 241

Table 4 (continued)

Peak no	RT (min)	ESI ±	Precursor *m/z*	Molecular formula	Error (ppm)	Identified compound	Product ions
43	9.973	$[M-H]^-$	315.0509	$C_{16}H_{12}O_7$	0.4	3′-Methoxy-4′,5,7-trihydroxy flavonol	300
44	9.997	$[M-H]^-$	463.123	$C_{22}H_{23}O_{11}$	0.5	Peonidin-3-*O*-hexoside chloride	258, 287, 301, 302, 342
45	10.050	$[M+H]^+$	447.1275	$C_{22}H_{22}O_{10}$	0.6	Sissotrin	145, 242, 271, 285
46	10.264	$[M+H]^+$	465.1155	$C_{21}H_{20}O_{12}$	0.7	Hyperoside (Quercetin-*O*-hexoside)	303
47	10.318	$[M+H]^+$	493.1346	$C_{23}H_{25}O_{12}$	− 5	Malvidin-3-hexoside	331
48	10.563	$[M-H]^-$	269.0457	$C_{15}H_{10}O_5$	0.1	Apigenin	117, 149, 151, 159, 181, 201, 225, 227, 269
49	10.662	$[M+H]^+$	273.184	$C_{15}H_{12}O_5$	− 0.6	Naringenin	91
50	12.256	$[M-H]^-$	623.3208	$C_{29}H_{36}O_{15}$		Verbascosides (acetosides)	461, 315
51	12.733	$[M+H]^+$	181.123	$C_9H_8O_4$	− 1.8	Caffeic acid	107, 135, 145, 163, 181
52	13.203	$[M-H]^-$	623.3208	$C_{29}H_{36}O_{15}$		Isoverbascosides (isoacteosides)	461, 315
53	13.525	$[M+H]^+$	225.1943	$C_{13}H_{20}O_3$	2.5	Methyl Jasmonate	83, 85, 100
54	13.617	$[M+H]^+$	271.0595	$C_{15}H_{10}O_5$	0.4	Genistein	271
55	14.074	$[M+H]^+$	387.1796	$C_{17}H_{22}O_{10}$	0.4	1-*O*-b-D-glucopyranosyl sinapate	91, 103, 105, 121
56	14.297	$[M+H]^+$	289.182	$C_{15}H_{12}O_6$	− 4.8	Eriodictyol (3′ 4′5 7-tetrahydroxyflavanone)	271
57	14.297	$[M+H]^+$	149.0232	$C_9H_8O_2$	− 0.7	*Trans*-Cinnamate(Cinnamic acid)	65, 121
58	14.537	$[M+H]^+$	285.0739	$C_{16}H_{12}O_5$	2.9	Acacetin	285, 124, 167, 187, 197, 213, 242, 270
59	14.616	$[M+H]^+$	433.1504	$C_{21}H_{20}O_{10}$	0	Apigenin-C-hexoside (vitexin)	164, 313, 367
60	19.972	$[M-H]^+$	301.1418	$C_{16}H_{12}O_6$	−2.2	3,5,7-Trihydroxy-4′-methoxyflavone (diosmetin)	211, 239, 258, 283, 286
61	20.663	$[M-H]^-$	471.3474	$C_{30}H_{48}O_4$	1	Pomolic acid	427, 453
62	22.977	$[M+H]^+$	455.3459	$C_{30}H_{48}O_3$	4.3	Oleanolic acid	149, 163, 203, 391, 407

Flavonoids

Most common compounds found in LEE were flavonoids, mostly identified as *O*-glycosides. The sugar residues hexose, deoxyhexose, pentose and rutinoside moieties were identified by the mass loss of 162, 146, 132 and 308 amu, respectively [16, 17]. Flavonoids had 36 peaks, belonging to different subclasses: flavanonols, flavonols, flavanones, flavones and isoflavonoids. Table 4 shows the tentatively identified flavonoids and their aglycones. The type of sugar in the glycosidic bond was identified by the mass loss that matched the eliminated sugar residues. **(Peak 24)** $[M-H]^-$ at *m/z* 623.1973 calculated for $C_{28}H_{32}O_{16}$ and **(peak 27)** $[M-H]^-$ at *m/z* 477.1435 calculated for $C_{22}H_{22}O_{12}$– were determined to be isorhamnetin rutinoside and isorhamnetin hexoside, respectively, using the negative mode of ionization, with characteristic fragments at *m/z* 315 counted for the aglycone [16, 17]. This isorhamnetin derivative was previously identified in family *Bignoniaceae*. Numerous pharmacological properties, including antioxidant, anti-inflammatory and anti-tumour properties, have been found for isorhamnetin [18]. Isorhamnetin glycosides could be considered a main reason for antioxidant activity reported in this study.

The presence of hydroxyl groups in the structure of flavonoids has previously been reported to exhibit antioxidant properties, especially flavonols, such as quercetin, which were reported to possess the highest IC_{50} values due to their higher hydroxyl groups [19]. Various quercetin derivatives **(peaks 23, 41, 46)** were also detected. The typical quercetin product ion was produced at *m/z* 301 or 303, respectively, when quercetin derivatives were ionized in a positive or negative mode [15]. As a matter of fact, quercetin hexoside **(peak 23)**, quercetin **(peak 41)** and hyperoside (quercetin-*O*-hexoside) **(peak 46)** have previously been identified in the family [20, 21].

Kaempferol was reported to have a good antioxidant activity that help in myocardial ischaemia [22]. The product ion at *m/z* 285 that kaempferol glycosides displayed is only seen in negative mode of kaempferol aglycone [23]. For example, the molecular ion [M–H] at *m/z* 461.1685

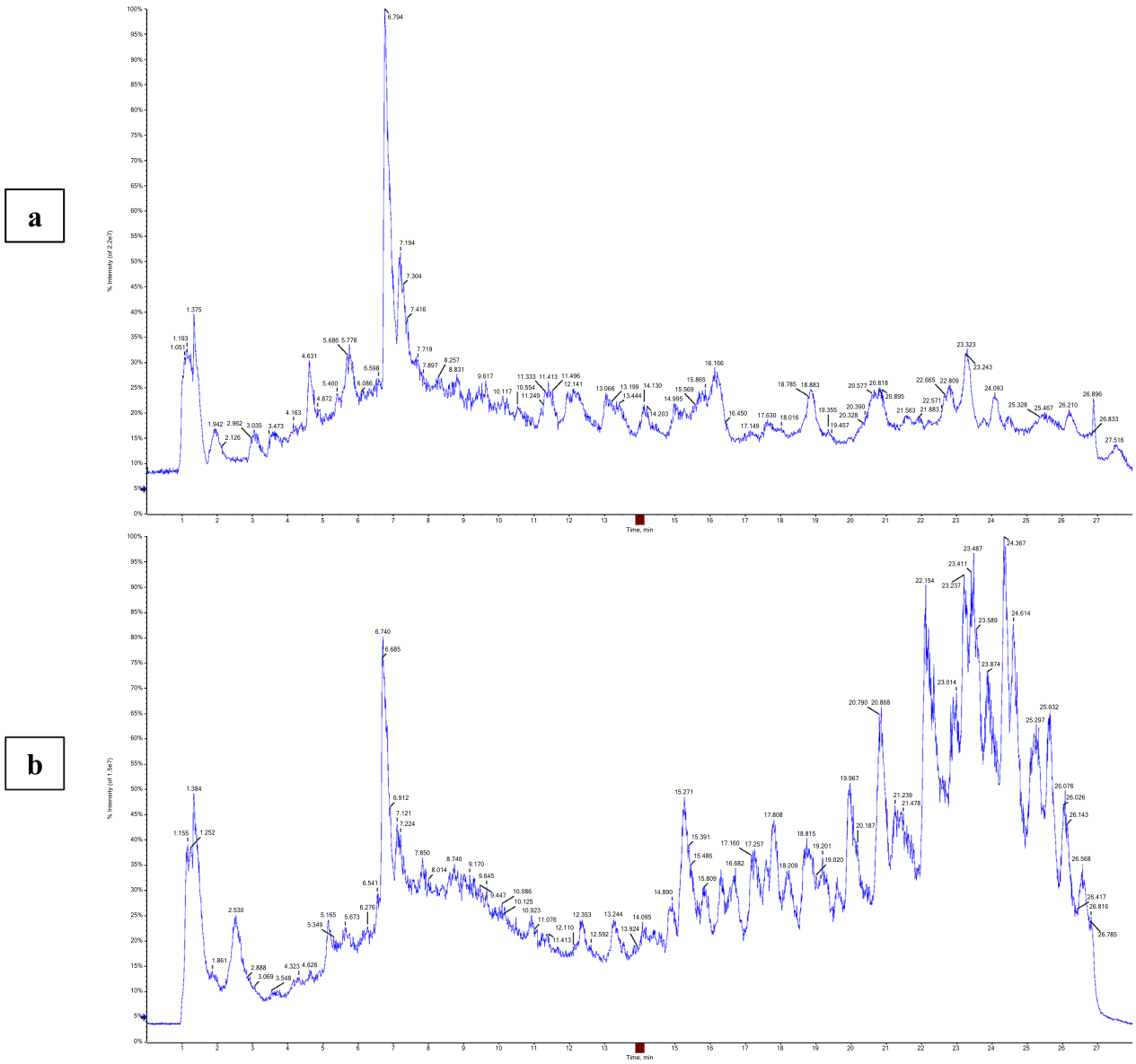


Fig. 5 *M. lutea* LEE total ion chromatogram. **a**. Negative mode chromatogram, **b**. Positive mode chromatogram

and the product ion at *m/z* 285.04199 were used provisionally to identify kaempferol hexoside **(peak 8)**.

In addition, apigenin and luteolin derivatives were identified. A variety of apigenin derivatives **(peak 39)** and **(peak 48)** were identified, as rhoifolin (apigenin-*O*-neohesperidoside) as previously reported in the family [24]. Apigenin was also previously identified in *M. Platycalyx* [25] at *m/z* 577.1915 and 269.0457, respectively [26], while (**peak 33** and **peak 59**) showed molecular ions at *m/z* 433.1504 and 433.1094, respectively, suggesting that they are, apigenin-*O*-hexoside and apigenin-*C*-hexoside (vitexin), respectively. Following that, unique ion peak fragments of aglycone were identified at *m/z* 271 and 311 owing to the loss of *O*-glucoside and *C*-glycoside, respectively [27, 28]. Apigenin-*O*-hexoside and Apigenin-*C*-hexoside(vitexin) were identified for first time in this genus.

Also, luteolin-*O*-hexoside was identified previously in family *Bignoniaceae* [24]. Both luteolin-*O*-hexoside **(peak 26)** and luteolin-di-*O*-hexoside **(peak 10)** were identified at *m/z* value 447.09 and 609.13, with neutral ion loss of couple of hexose molecules (324 *m/z*) and the characteristic fragmentation for aglycone at 285 [26]. Earlier studies have identified luteolin and its derivatives from genus

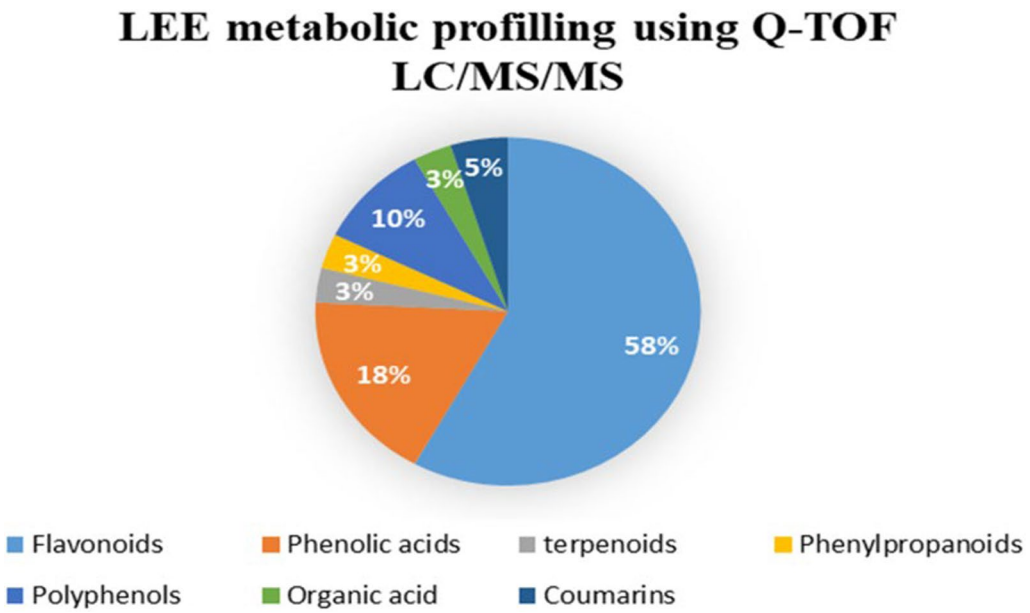


Fig. 6 Phytochemical composition of *M. lutea* LEE determined using LC–MS/Q-TOF

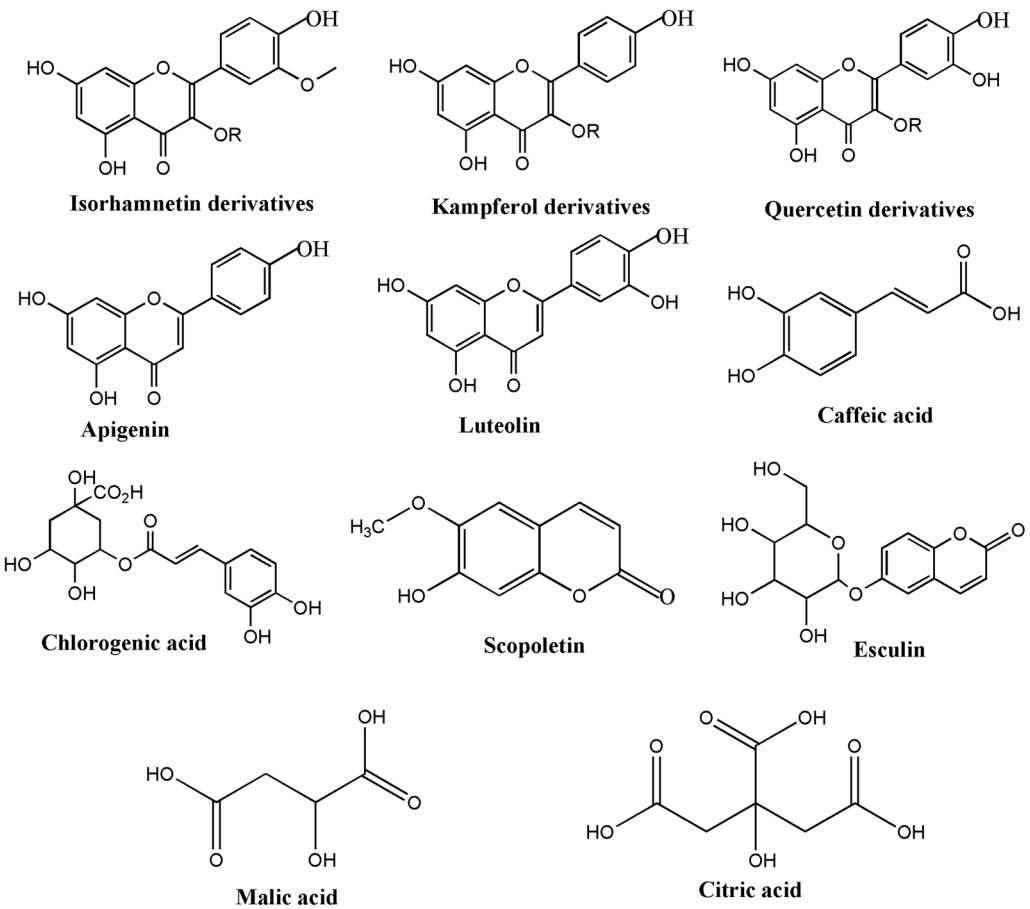


Fig. 7 Selected compounds from different chemical classes identified in *M. lutea* leaves extract

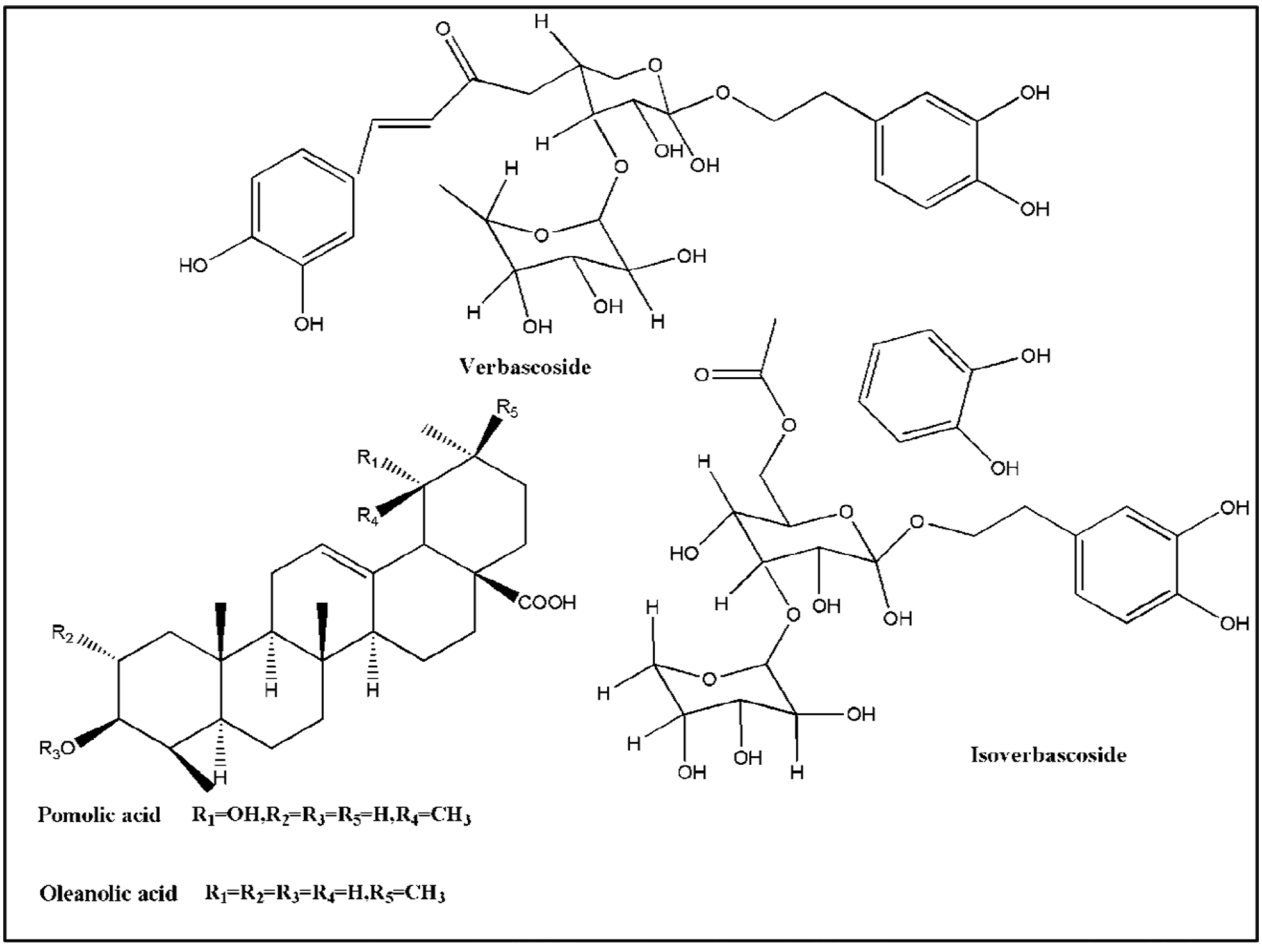


Fig. 8 Terpenoids and phenylpropanoids identified in *M. lutea* leaves extract

Markhamia, viz. *M. tomentosa* [29], *M. platycalyx* [25] and *M. zanzibarica* [30], while luteolin-di-*O*-hexoside has been identified in *M. zanzibarica* [30] and *M. tomentosa* [31]. Luteolin was reported to ameliorate oxidative and nitrosative stress and suppress the expression of NF-B, an inflammatory factor [32].

Phenolic acids

Plant phenolic acids are being studied for their potential to be anti-inflammatory, liver protective, antioxidant, anti-bacterial, cardioprotective, anti-diabetic, anticancer and neuroprotective properties [15]. Our study identified several phenolic acids, as well as their derivatives (11 phenolic acids in leaves), mainly hydroxy cinnamic acid and hydroxyl benzoic acid and their derivatives. A characteristic fragment ion peak at *m/z* 191.0546 corresponding to $[C_7H_{11}O_6]^-$ residue and a molecular ion peak $[M-H]^-$ at *m/z* 353.0872 are detected for chlorogenic acid **(peak 16)** [18, 33]. Caffeic acid **(peak 51)** and protocatechuic acid **(peak 4)** were also identified at *m/z* 181.123 and *m/z* 153.0192, respectively. All of these peaks were accompanied by distinct fragments at *m/z* 109.0180, 135.0395, confirming a CO_2 neutral loss [15]. Both caffeic and protocatechuic acid are previously identified in *M. platycalyx* [25]. Nonetheless, it is interesting to note that this is the first report of both acids in *M. lutea*. The molecular ion peak $[M-H]^-$ at *m/z* 359.0984 corresponds to rosmarinic acid [32], which was previously reported in *M. tomantosa* [31] but identified for first time in *M. lutea*. **Peak 7** was determined to be coumaric acid, because it had the typical fragment of coumaric acid at *m/z* 119 and base peak 163.039 [34]. Coumaric acid was previously identified in family Bignoniaceae but identified for first time in *M. lutea.* Interestingly, this is the first study to detect phenolic acids in *M. lutea* detected using Q-TOF LC/MS/MS.

As an effective antioxidant, caffeic acid terminates the chain reaction of lipid peroxidation as well as minimizes its detrimental effects by quenching free radicals and inhibiting their formation. It was also discovered to boost the activities of antioxidant enzymes. Also, both

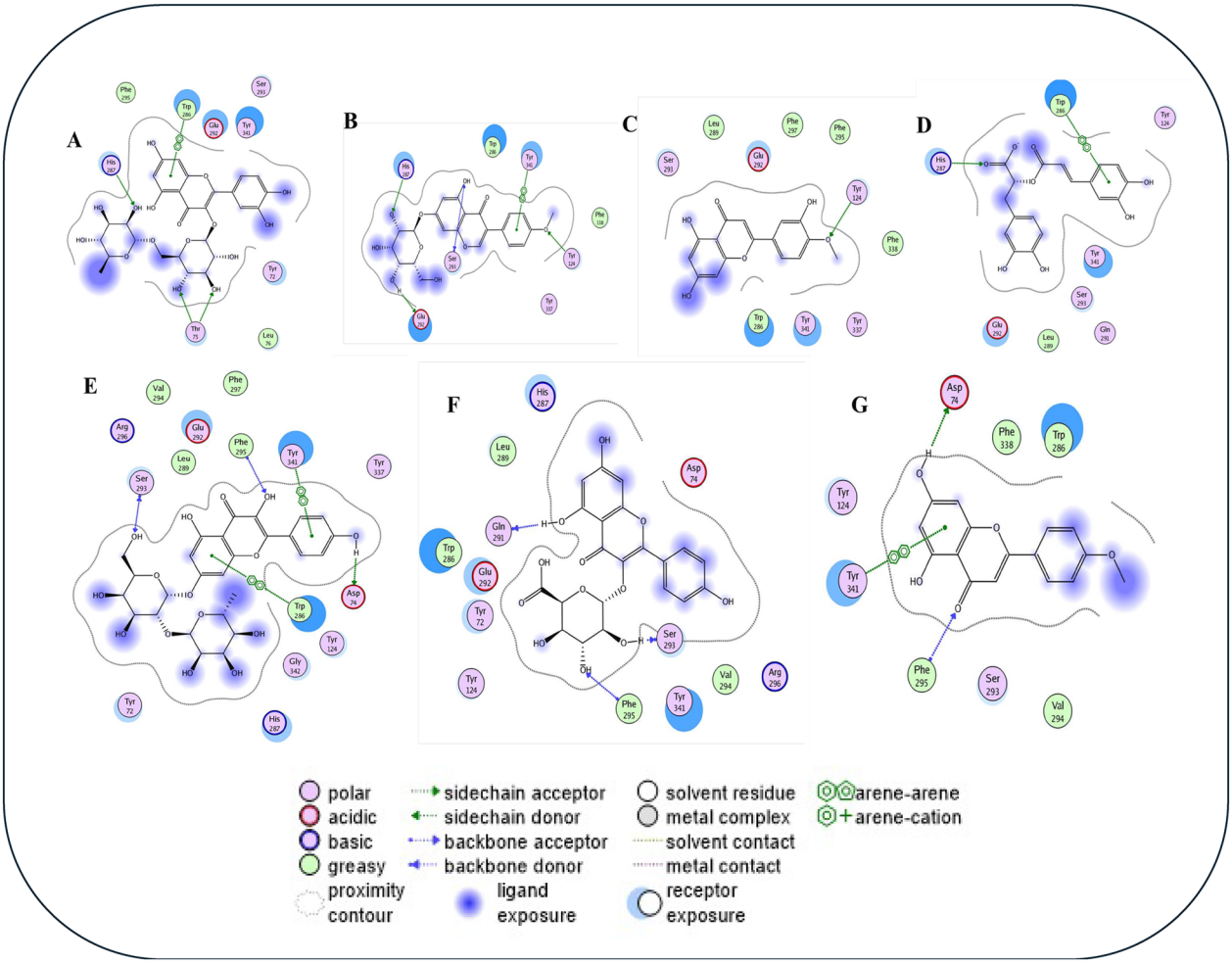


Fig. 9 2D binding interaction of AChE with most promising compounds, **A** Isorhamnetin-o-rutinoside, **B** sissotrin, **C** 3,5,7-trihydroxy-4-methoxyflav one, **D** rosmarinic acid, **E** kaempferol-7-neohesprosides, **F** kaempferol hexoside, **G** acacetin

rosmarinic acid and chlorogenic acid are reported to reduce oxidative stress [32].

Polyphenols

M. lutea LEE contained seven polyphenolic components, three of which were anthocyanidins **(peaks 21, 34 and 47)**, one bioflavonoids **(peak 19)**, one stilbene glycoside **(peak 36)** and Cyanidin-*O*-rutinoside known as antirrhinin **(peak 21)** exhibited a pseudo-molecular ion $[M+H]^+$ at *m/z* 595.1667, followed by a peak at *m/z* 287 due to cyanidin nucleus (− 308 Da). The molecular ion peak of cyanidin-*O*-hexoside (**peak 34**) was observed at 449.1086, while the main fragment ion was observed at 287 [(M + H)-162] [15]. Malvidin-hexoside (**peak 47**) produced a [M + H] + at *m/z* 493.1346; however, hexose loss caused the fragmentation to give a *m/z* 331 [35]. Procyanidin B_2 **(peak 19),** which was detected by a precursor ion at *m/z* 579.1451, was previously identified in *M. tomantosa* [31] and *M. platycalyx* [36], but fortunately it was first time to be identified in *M. lutea*. E-3,4,5′-trihydroxy-3′-glucopyranosyl stilbene (**peak 36**) was found to exhibit $[M–H]^-$ at *m/z* 405.1031, indicating the presence of stilbene derivatives. The polyphenolic components found in *M. lutea* leaf extract were identified for the first time in this study.

Coumarins

According to the study, three peaks were tentatively identified as coumarins (**Peaks 13, 35 and 40)** that are esculin, daphnetin [31] and scopoletin [37] depending on precise molecular weights and compared to literature. The compound daphnetin is dihydroxycoumarins that has been identified by MS/MS analysis at *m/z* 133, 149 and 177 [26]. A coumarin glycoside with MS/MS

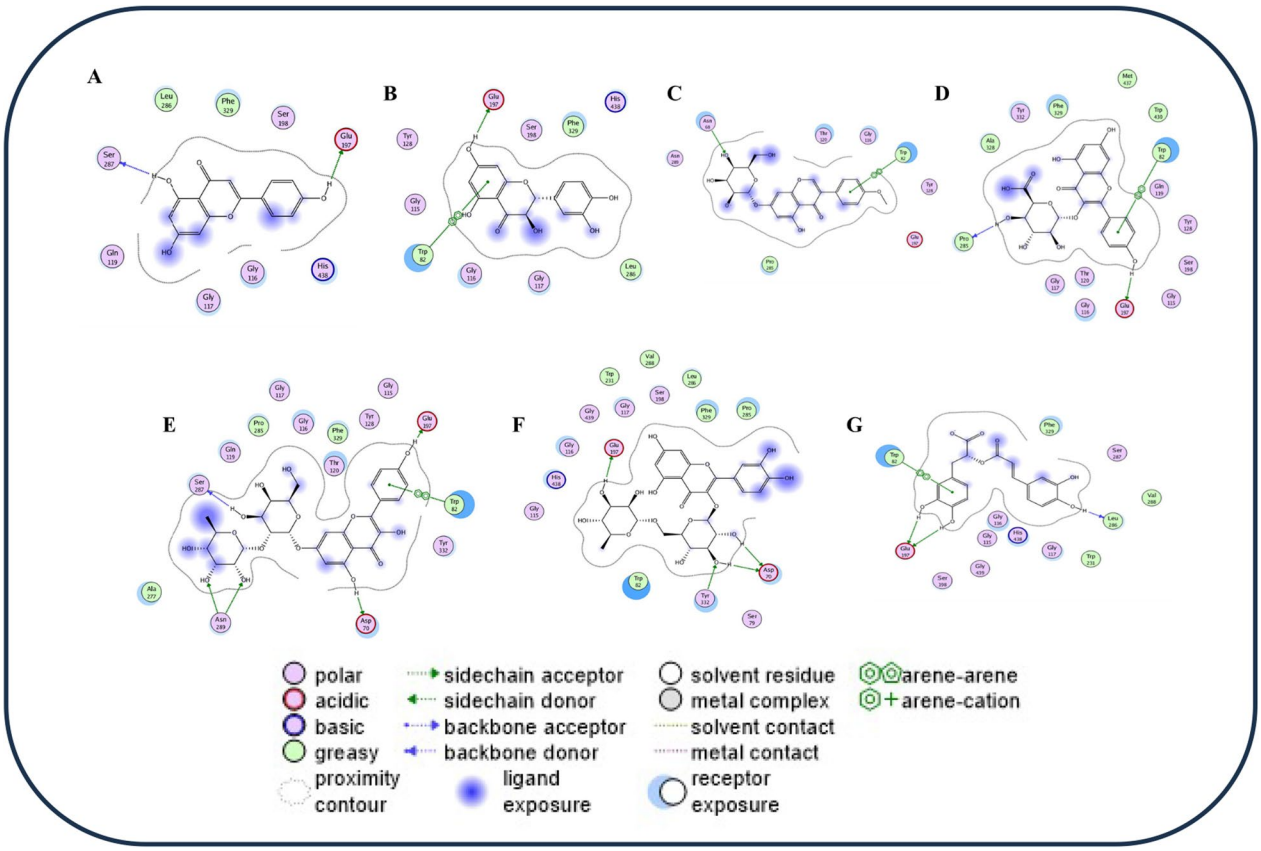


Fig. 10 2D binding interaction of BChE with most promising compounds, **A** Apigenin-o-glycoside, **B** taxifolin, **C** sissotrin, **D** kaempferol hexoside, **E** kaempferol-7-neohesprosides, **F** isorhamnetin-o-rutinoside, **G** rosmarinic acid

fragments at *m/z* 133 was identified as scopoletin [18]. All these coumarins have been identified in *M. lutea* for the first time.

Phenylpropanoids glycosides

Among the chemical classes identified in the genus *Markhamia* were phenylpropanoids glycosides. In *M. lutea* extract, two phenylpropanoids glycosides were tentatively detected as verbascosides (acetosides) and isoverbascosides (isoacteosides), which were assigned to (**peaks 50** and **52**), respectively. The two isomers are different in the caffeoyl moiety position isoverbascosides, and verbascosides showed the same $[M–H]^-$ ion at *m/z* 623.3208 with characteristic fragments ion peak at *m/z* 461 due to $[C_9H_6O_3]^-$ residue and *m/z* 315 for further loss of rhamnose sugar moiety loss [38].

Terpenoids

One of the characteristic chemical classes in the genus *Markhamia* were terpenoids. In *M. lutea* extract, two terpenoids were tentatively detected as pomolic acid and oleanolic acid. Pomolic acid (*m/z* 471.3474) and oleanolic acid (*m/z* 455.3459) were attributed to **peaks 61 and 62**, respectively, and both showed characteristic fragments at *m/z* 453 and 407, respectively, due to loss of water (− 18 Da) and (− 60 Da) due to loss of acetate loss, respectively [39]. All these compounds have been isolated from *M. lutea* [6] but, interstitially, it is the first time to be identified by Q-TOF LC/MS/MS.

Organic acid

A product ion at *m/z* 115 due to water loss and a molecular ion [M-H] at *m/z* 133.0136 were found in **(Peak 1)**, and these were tentatively identified as malic acid. The molecular ion of [M–H] at *m/z* 191.0562 was tentatively recognized as citric acid (**peak 3**) [40].

Top docking phytoconstituents interaction with AChE and BChE

Phytoconstituents identified by UHPLC–ESI–TOF–MS were screened against both AChE and BChE inhibition using molecular docking. Seven characteristic

phytoconstituents showed a higher binding affinity to allosteric site of AChE and BChE with low binding energy compared to the binding energy of donepezil and rivastigmine as Isorhamnetin-*O*-rutinoside (− 22.0042 kcal/mol), sissotrin (− 20.7694 kcal/mol), 3,5,7-Trihydroxy-4′-methoxyflavone (diosmetin) (− 19.5477 kcal/mol), rosmarinic acid (− 19.3211 kcal/mol), kaempferol hexoside (− 19.0069 kcal/mol), kampferol-7-neohesperosides (− 16.9908 kcal/mol) and acacetin (− 16.4584 kcal/mol) for AChE and isorhamnetin-*0*-rutinoside (− 29.904 kcal/mol), kampferol-7-neohesperosides (− 23.5882 kcal/mol), rosmarinic acid (− 22.7783 kcal/mol), taxifolin (− 21.4458 kcal/mol), sissotrin (− 19.8249 kcal/mol), kaempferol hexoside (− 20.7694 kcal/mol) and apigenin-*O*-hexoside (− 19.3567 kcal/mol) for BChE versus donepezil (− 16.01522 kcal/mol), rivastigmine (− 15.38282 kcal/mol) for AChE and donepezil (− 28.6337 kcal/mol), rivastigmine (− 18.5409 kcal/mol) for BChE. The results for both molecular docking against both BChE and AChE are shown in Figs. 9 and 10, respectively. High binding affinity and selectivity were shown by these phytoconstituents with both AD treatment targets, making them a promising lead molecule for anti-AD effects.

Conclusion

In conclusion, *M. lutea* LEE includes a variety of bioactive substances with antioxidant and anti-Alzheimer properties. As LEE showed the best result in antioxidant activity by DPPH, ORAC, FIC and also the highest inhibition for AChE, BChE and Aβ-amyloid. Phenolic acids, coumarins, polyphenols and some flavonoids are identified for the first time in LEE. A computational investigation (in silico molecular docking) also identified seven promising phytoconstituents with affinity for two AD targets. Consequently, further insight and additional investigation could be done on such valuable metabolites as possess drug-like qualities. As a result, *M. lutea* can be considered a prospective source of compounds used in the management of Alzheimer's disease. This study encourages further research on *M. lutea* leaves to investigate their in vivo antioxidant activity and its promising neuroprotective potential.

Abbreviations

LEE	Leaves ethanolic extract
FEE	Flowers ethanolic extract
M. lutea	*Markhamia lutea*
PASS	Prediction of activity spectra for substances
M. tomantosa	*Markhamia tomentosa*
M. platycalyx	*Markhamia platycalyx*
DPPH	2,2-Diphenyl-1-picryl-hydrazyl-hydrate
ORAC	Oxygen radical absorbance capacity
FIC	Ferrozine iron metal chelation
Q-TOF LC/MS/MS	Quadrupole time-of-flight liquid chromatography mass spectrometer
IC_{50}	Inhibitory concentration 50
µM TE/mg extract	Micromolar trolox equivalent per milligram extract
µM EDTA eq/mg extract	Micromolar ethylenediaminetetraacetic acid per milligram extract
AChE	Acetyl cholinesterase
AchI	Acetyl cholinesterase inhibitor
BChE	Butyryl cholinesterase
BChI	Butyryl cholinesterase inhibitor
AD	Alzheimer's disease

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43094-024-00679-1.

Supplementary Material

Acknowledgements
None.

Author contributions
Mai Magdy performed the investigation, resources, visualization and writing the original draft. Hala M. El Hefnawy, Ahmed H. Elosaily and Engy Mohsen were involved in conceptualization, supervision, visualization and writing review and editing of the manuscript.

Funding
Open Access funding was enabled by agreement between science and technology development fund (STDF) Egypt and Springer OA.

Availability of data and materials
All data generated and analysed during the current study are available in this published article.

Declarations

Plant authentication
A plant taxonomy consultant for the Agriculture Ministry, Therease Labib, is an agriculture engineer who was the director of El-Orman Botanic Garden in Giza, Egypt, verified fresh leaves and blossoms collected in June 2020 from the Botanical Garden. A voucher specimen (NO. 22.10.2023) was deposited at Department of Pharmacognosy, Faculty of Pharmacy Cairo University.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Pharmacognosy, Faculty of Pharmacy, Ahram Canadian University, Cairo, Egypt. [2]Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, Cairo 11562, Egypt.

Received: 24 March 2024 Accepted: 4 August 2024
Published online: 19 August 2024

References

1. de Almeida RB et al (2023) Identification of a novel dual inhibitor of acetylcholinesterase and butyrylcholinesterase: in vitro and in silico studies. Pharmaceuticals 16(1):95

2. Adetuyi AR, Ayenero ME, Olaleye MT, Akindahunsi AA, Akinmoladun AC (2024) Antioxidant and acetylcholinesterase inhibitory activities, in silico analyses, and anti-Alzheimer's disease potential of leaf extracts of three Nigerian endemic medicinal plants (*Spondias mombin*, *Carica pa* paya and *Kalanchoe crenata*). FJPS 10(1):6
3. Althobaiti NA, Menaa F, Dalzell JJ, Albalawi AE, Ismail H, Alghuthaymi MA, Aldawsari RD, Iqbal H, McAlinney C, Green BD (2022) Ethnomedicinal plants with protective effects against beta-amyloid peptide (Aβ) 1–42 indicate therapeutic potential in a new in vivo model of Alzheimer's disease. Antioxidants 11(10):1865
4. Yeniçeri E, Altay A, Koksal E, Altın S, Taslimi P, Yılmaz MA, Cakir O, Tarhan A, Kandemir A (2024) Phytochemical profile by LC–MS/MS analysis and evaluation of antioxidant, antidiabetic, anti-Alzheimer, and anticancer activity of *Onobrychis argyrea* leaf extracts. Eur J Integr Med 66:102337
5. Fathallah N, El Deeb M, Rabea AA, Almehmady AM, Alkharobi H, Elhady SS, Khalil NJM (2023) Ultra-performance liquid chromatography coupled with mass metabolic profiling of *Ammi majus* roots as waste product with isolation and assessment of oral mucosal toxicity of its psoralen component Xanthotoxin. Mol 13(10):1044
6. Mohsen E et al (2023) Mini review on morphology, traditional uses, phytochemical and pharmacological potential of *Markhamia lutea*. Egypt J Chem 66(13):507–518
7. Boly R, Lamkami T, Lompo M, Dubois J, Guissou I (2016) DPPH free radical scavenging activity of two extracts from *Agelanthus dodoneifolius* (Loranthaceae) leaves. IJTPR 8(1):29–34
8. Liang Z et al (2014) Antioxidant and antiproliferative activities of twenty-four Vitis vinifera grapes. PLoS ONE 9(8):e105146
9. Santos JS, Brizola VRA, Granato D (2017) High-throughput assay comparison and standardization for metal chelating capacity screening: a proposal and application. Food Chem 214:515–522
10. Ellman GL, Courtney KD, Andres V Jr, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. J Biochem Pharmacol 7(2):88–95
11. Hudson SA, Ecroyd H, Kee TW, Carver JA (2009) The thioflavin *T. fluorescence* assay for amyloid fibril detection can be biased by the presence of exogenous compounds. FEBS J 276(20):5960–5972
12. Attard E (2013) A rapid microtitre plate Folin–Ciocalteu method for the assessment of polyphenols. Open Life Sci 8(1):48–53
13. Kiranmai M, Kumar CM, Mohammed I (2011) Comparison of total flavanoid content of Azadirachta indica root bark extracts prepared by different methods of extraction. Res J Pharm Biol Chem Sci 2(3):254–261
14. Mohammed HA, Khan RA, Abdel-Hafez AA, Abdel-Aziz M, Ahmed E, Enany S, Mahgoub S, Al-Rugaie O, Alsharidah M, Aly MS (2021) Phytochemical profiling, in vitro and in silico anti-microbial and anti-cancer activity evaluations and Staph GyraseB and h-TOP-IIβ receptor-docking studies of major constituents of *Zygophyllum coccineum* L. Aqueous-ethanolic extract and its subsequent fractions: An approach to validate traditional phytomedicinal knowledge. Molecules 26(3):577
15. Abbas HA et al (2022) Novel neuroprotective potential of *Bunchosia armeniaca* (Cav.) DC against lipopolysaccharide induced Alzheimers disease in mice. Plants 11(14):1792
16. Dang J, Zhang L, Wang Q, Mei L, Yue H, Liu Z, Shao Y, Gao Q, Tao Y (2018) Target separation of flavonoids from Saxifraga tangutica using two-dimensional hydrophilic interaction chromatography/reversed-phase liquid chromatography. J Sep Sci 41(24):4419–4429
17. Otify AM, El-Sayed AM, Michel CG, Farag MA (2019) Metabolites profiling of date palm (*Phoenix dactylifera* L.) commercial by-products (pits and pollen) in relation to its antioxidant effect: a multiplex approach of MS and NMR metabolomics. Metabolomics 15:1–17
18. Abbas EY, Ezzat MI, Ramadan NM, Eladl A, Hamed WH, Abdel-Aziz MM, Teaima M, El Hefnawy HM, Abdel-Sattar E (2023) Characterization and anti-aging effects of *Opuntia ficus-indica* (L.) Miller extracts in ad-galactose-induced skin aging model. Food Funct 14(7):3107–3125
19. Zuo A, Yanying Y, Li J, Binbin X, Xiongying Y, Yan Q, Shuwen C (2011) Study on the relation of structure and antioxidant activity of isorhamnetin, quercetin, phloretin, silybin and phloretin isonicotinyl hydrazone. Free Radic Antioxid 1(4):39–47
20. Hamed A, Samy MN, Mahmoud BK, Attia EZ, Ali TF, Afifi AH, Kamel MS (2021) Flavonoidal glycosides and in vitro antioxidant activity of *Bignonia binata* Thunb. leaves Family Bignoniaceae and in silico evidence of their potential anti-COVID-19 activity. J Adv Biomed Pharm Sci 4:98–106
21. Serra MB, Barroso WA, Rocha C, Furtado PG, Borges AC, Silva SN, Tangerina MM, Nascimento JRD, Vilegas W, Alves AC (2020) Chemical characterization and wound healing property of *Jacaranda decurrens* Cham. (Bignoniaceae): an experimental study based on molecular mechanisms. Evid Based Complement Alternat Med 2020:4749712
22. Zhou M, Ren H, Han J, Wang W, Zheng Q, Wang D (2015) Protective effects of kaempferol against myocardial ischemia/reperfusion injury in isolated rat heart via antioxidant activity and inhibition of glycogen synthase Kinase-3. Oxid Med Cell Longev 2015:1–8. https://doi.org/10.1155/2015/481405
23. de la Luz C-G, Fernández-Arroyo S, Joven J, Segura-Carretero A (2013) Comprehensive characterization by UHPLC–ESI–Q–TOF–MS from an *Eryngium bourgatii* extract and their antioxidant and anti-inflammatory activities. Food Res Int 50(1):197–204
24. Rivera-Mondragón A, Tuenter E, Ortiz O, Sakavitsi ME, Nikou T, Halabalaki M, Caballero-George C, Apers S, Pieters L, Foubert K (2020) UPLC-MS/MS-based molecular networking and NMR structural determination for the untargeted phytochemical characterization of the fruit of *Crescentia cujete* (Bignoniaceae). Phytochemistry 177:112438
25. El Dib R, Gaara A, El-Shenawy S, Micky J, Mohammed A, Marzouk M (2014) Leaf extract of *Markhamia platycalyx*: polyphenolic profile, acute toxicity, anti-inflammatory, hepatoprotective and in vitro antioxidant activities. Drug Res 64:680–689
26. Alqahtani MJ, Elekhnawy E, Negm WA, Mahgoub S, Hussein IA (2022) *Encephalartos villosus* Lem. Displays a strong in vivo and in vitro antifungal potential against *Candida glabrata* clinical isolates. J Fungi. 8(5):521
27. Bouaziz M, Sayadi S (2005) Isolation and evaluation of antioxidants from leaves of a Tunisian cultivar olive tree. EJMP 107(7–8):497–504
28. Guo Z, Zhu P, He X, Yan T, Liang X (2021) Components identification and isomers differentiation in pigeon pea (*Cajanus cajan* L.) leaves by LC–MS. J Sep Sci 44(13):2510–2523
29. Sofidiya MO, Agunbiade FO, Koorbanally NA, Sowemimo A, Soesan D, Familusi T (2014) Antiulcer activity of the ethanolic extract and ethyl acetate fraction of the leaves of *Markhamia tomentosa* in rats. J Ethnopharmacol 157:1–6
30. Ibrahim MB, Kaushik N, Sowemimo AA, Odukoya OA (2016) Review of the phytochemical and pharmacological studies of the genus *Markhamia*. Pharmacogn Rev 10(19):50
31. Alfred M (2020) A review of medicinal uses, phytochemistry and biological activities of *Markhamia tomentosa*. J Pharm Nutr Sci 10(4):140–147
32. Elhady SS, Abdelhameed RF, Mehanna ET, Wahba AS, Elfaky MA, Koshak AE, Noor AO, Bogari HA, Malatani RT, Goda MS (2022) Metabolic profiling, chemical composition, antioxidant capacity, and in vivo hepato-and nephroprotective effects of Sonchus cornutus in mice exposed to cisplatin. Antioxidants 11(5):819
33. Zhang X, Lv H, Li Z, Jiang K, Lee MR (2015) HPLC/QTOF–MS/MS application to investigate phenolic constituents from *Ficus pandurata H. aerial* roots. Biomed Chromatogr 29(6):860–868
34. Fogliano V, Corollaro ML, Vitaglione P, Napolitano A, Ferracane R, Travaglia F, Arlorio M, Costabile A, Klinder A, Gibson G (2011) In vitro bioaccessibility and gut biotransformation of polyphenols present in the water-insoluble cocoa fraction. Mol Nutr Food Res 55(S1):S44–S55
35. Choi JY, Lee SJ, Lee SJ, Park S, Lee JH, Shim JH, El-Aty AA, Jin JS, Jeong ED, Lee WS (2010) Analysis and tentative structure elucidation of new anthocyanins in fruit peel of *Vitis coignetiae* Pulliat (meoru) using LC–MS/MS: Contribution to the overall antioxidant activity. J Sep Sci 33(9):1192–1197
36. Hassaan Y, Handoussa H, El-Khatib AH, Linscheid MW, El Sayed N, Ayoub N (2014) Evaluation of plant phenolic metabolites as a source of Alzheimer's drug leads. Biomed Res Int 2014:1–10. https://doi.org/10.1155/2014/843263
37. He Y, Li Z, Wang W, Sooranna SR, Shi Y, Chen Y, Wu C, Zeng J, Tang Q, Xie H (2018) Chemical profiles and simultaneous quantification of Aurantii

fructus by use of HPLC-Q-TOF–MS combined with GC–MS and HPLC methods. Molecules 23(9):2189
38. Li L, Tsao R, Yang R, Liu C, Young JC, Zhu H (2008) Isolation and purification of phenylethanoid glycosides from *Cistanche deserticola* by high-speed counter-current chromatography. Food Chem 108(2):702–710
39. Sut S, Zengin G, Maggi F, Malagoli M, Dall'Acqua S (2019) Triterpene acid and phenolics from ancient apples of Friuli Venezia Giulia as nutraceutical ingredients: LC–MS study and in vitro activities. Molecules 24(6):1109
40. El Gizawy HA, El-Haddad AE, Attia YM, Fahim SA, Zafer MM, Saadeldeen AM (2022) In vitro cytotoxic activity and phytochemical characterization (UPLC/T–TOF–MS/MS) of the Watermelon (*Citrullus lanatus*) rind extract. Molecules 27(8):2480

Publisher's Note