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PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT AND ANTICHOLINESTERASE ACTIVITIES OF *CLITORIA TERNATEA* ROOTS EXTRACT

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Abstract: *Clitoria ternatea* is also known as 'Bunga telang' in Malay. The roots of *C. ternatea* were traditionally used as a brain tonic to improve memory. This raises the possibility of using the roots extract as a cholinesterase inhibitor in treating Alzheimer's disease symptoms. Other than cholinesterase inhibitors, its antioxidant properties would also help in inhibiting the symptoms of Alzheimer's disease, as oxidative stress is one of the contributing factors for this disease. Therefore, this study was conducted to identify the phytochemical constituents in *C. ternatea* roots extract by using phytochemical screening and GC-MS analysis, to evaluate the antioxidant properties of *C. ternatea* roots through DPPH radical scavenging assay and total phenolic content and also to investigate the cholinesterase inhibition potential of *C. ternatea* roots extract. The phytochemical screening revealed the presence of saponin, phenols, terpenoids and steroids. While GC-MS analysis revealed n-hexadecanoic acid as the major constituent (12.95%). The IC_{50} value of DPPH radical scavenging assay for the extract was 85.31g/ml and the phenolic content in the extract was 10.84 ± 0.12 mg GAE/g. The IC_{50} value of *C. ternatea* roots extract against AChE was 118.5 ± 8.6 µg/ml and 83.59 ± 6.23 µg/ml against BChE. The roots extract showed potential cholinesterase inhibition and antioxidant activity.

Keywords: *Clitoria ternatea*, antioxidant, anticholinesterase.

Introduction

Clitoria ternatea is a perennial twinning leguminous herb from the Fabaceae family and commercially known as butterfly pea or blue pea in English, 'Bunga telang' in Malay and 'Aparajita' or 'Shankhpushpi' in Hindi (Lee *et al*., 2021). This species originates in tropical Asia and is distributed widely to South and Central America, West Indies, China, and India (Jeyaraj *et al*., 2020). In traditional Ayurvedic medicine, the extract of *C. ternatea* roots is commonly used as a brain tonic to increase cognitive abilities and can act as a memory enhancer for neurological disorders (Lee *et al*., 2021). Besides that, the extract of *C. ternatea* roots has been proven to increase apical and basal dendritic branches, acetylcholine content and acetylcholinesterase (AChE) activity in rats (Damodaran *et al*., 2018). The pentacyclic triterpenoids, such as taraxerol and taraxerone, are the major phytoconstituents found in this plant. According to Vasisht *et al*. (2016), taraxerol from *C. ternatea* roots extract can inhibit AChE. Lee *et al*. (2021) found that taraxerol, (Z)-9,17-octadecadienal and n-hexadecanoic in *C. ternatea* roots extract can prevent neurological illnesses and has been utilised as a therapeutic alternative for neurodegenerative diseases. Other than that, phytochemical screening analysis of the *C. ternatea* roots extract shows the presence of ternatins, alkaloids, flavonoids, saponins, tannins, carbohydrates, proteins, resins, and starch. Subsequently, the presence of terpenoids, flavonoids, tannins, and steroids might act as antioxidants (Lijon *et al*., 2017).

Alzheimer's disease (AD) is a multifactorial neurodegenerative disease which is mainly described by the progressive decline of cognitive functions, including the inability to perform daily life tasks and memory impairment. Its incidence is found to rise significantly among the elderly population over 65 years (Se Thoe *et al*., 2021). One of the main factors that affect AD is the decreasing acetylcholine (ACh) level in the human brain. Butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) are the two cholinesterase enzymes that break down acetylcholine into acetate and choline (Kumaran *et al*., 2019). Besides acetylcholine decline, oxidative stress has also been established as one of the factors in AD. Excessive oxidative stress is observed as the levels of reactive oxygen species (ROS) increase. This will cause an increase in free radicals due to the loss of cell homeostasis and neurotoxicity (Oboh *et al*., 2020). Currently, there are six drugs approved by the Food and Drug Administration (FDA) to increase the number of neurotransmitters in the brain. It includes rivastigmine, galantamine, tacrine, donepezil, and NMDA receptors (Hashemi & Zali, 2017). However, these drugs were gradually discovered to be ineffective in removing the root of AD pathogenesis. These commercial drugs also have some adverse effects, such as sleep disturbances, hepatotoxicity, and gastrointestinal-related outcomes (Kumaran *et al*., 2019; Yang *et al*., 2021). Among these drugs, only galantamine comes from natural sources. Therefore, since *C. ternatea* roots extract has been widely used as a memory enhancer and possesses antioxidant activity, it could be the next potent anti-Alzheimer drug. Although the roots extract has been widely used traditionally, there are limited studies on its acetylcholinesterase and butyrylcholinesterase inhibition activity and antioxidant activity. Hence, this study was designed to determine the antioxidant activity of *C. ternatea* roots extract and to evaluate the acetylcholinesterase and butyrylcholinesterase inhibition activity of *C. ternatea* roots extract.

Materials and Methods

Extraction of Clitoria Ternatea Roots

Fresh *Clitoria ternatea* plants were collected from Kangar, Perlis. The roots were washed thoroughly with double distilled water to remove dust debris and soil and then dried in a hot air oven at 60°C. The roots were then made into powder using an electric blender. To prepare the extract, 27 g of powdered roots were extracted using a Soxhlet extraction unit using 95% ethanol as the solvent for 12 hours (Khaw *et al*., 2020). The extract was filtered using Whatman No. 1 filter paper. The filtrate was then concentrated to evaporate the ethanol completely by using a rotary evaporator. The percentage yield of the extract was calculated with respect to the starting material.

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis was carried out by splitless injection of 1 µL of the sample into Agilent 7890A gas chromatography (GC) directly coupled to the mass spectrometer system (MS) of an Agilent 5975C inert MSD with a triple-axis detector. The following parameters were used during the analysis of the roots extract of *C. ternatea* (Lee *et al*., 2021). The ethanolic roots extract of *C. ternatea* was passed through the DB5-MS UI 0.25 mm x 30 m x 0.25 µm column (Agilent Technologies) with the initial flow rate of 1.3 mL/min. Initial temperature of the oven was programmed at 70°C and held for 2 minutes, ramp at 20°C/minute to reach the temperature of 280°C. The final holding time was kept at 20 minutes. Helium was selected as the carrier gas and the inlet temperature was 250°C. The mass selective detector transfer line heater was set at 250 and 150°C for the mass spectrometer quadrupole. MS scan was at the range of 45-600, and the mass spectrum was interpreted in reference to the database of NIST/EPA/NIH version 2.0. Various volatile phytochemicals in the root extract were determined by the compound's name and its molecular weight and structure.

Phytochemical Screening

The ethanolic extract of *C. ternatea* roots was subjected to different tests to identify the nature of chemical constituents present in the plant material. The crude extracts were screened qualitatively for the phytochemical constituents utilising the standard methods of analysis (Tiwari *et al.,* 2011; Shaikh & Patil, 2020).

DPPH Free Radical Scavenging Assay

The antioxidant activity of roots extract of *C. ternatea* was measured by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (Elufioye *et al*., 2019). Briefly, 1000 µg/ml of stock solution of the extract in methanol was prepared. Then, 1 ml of 0.1 mM DPPH was added to 1 ml of roots extract at different concentrations ranging from 20 to 100 µg/ml. The mixture was shaken and incubated in a dark place for 30 minutes at 37°C. The negative control was prepared using 1 ml of DPPH and 1 ml of methanol without extract and standard. Ascorbic acid was used as the standard with concentrations ranging from 0.06 to 1.00 µg/ml. The decrease in absorbance of each sample was measured by using UV-Visible spectrophotometer at 517 nm. The test was conducted in triplicate. The DPPH radical scavenging activity was calculated as the percentage inhibition by using Equation (1). The results were reported as IC_{50} value, where a lower IC_{50} value represents a stronger DPPH scavenging capacity. IC_{50} value is the concentration of sample required to scavenge 50% DPPH free radical and was calculated from the graph of radical scavenging activity against the concentration of extract.

$$\text{Percentage inhibition (\%)} = (A_{control} - A_{test})/A_{control} \times 100 \quad (1)$$

where $A_{control}$ is the absorbance of the negative control and A_{test} is the absorbance of the sample/standard.

Total Phenolic Content

The total phenolic content of the roots extract was determined by using Folin-Ciocalteu assay (Elufioye *et al*., 2019; Johari & Khong, 2019). The external calibration was done using different concentrations of gallic acid (100, 50, 25, 12.5 and 6.25 µg/ml). In brief, 0.5 ml of extracts (1000 µg/ml) and 2.5 ml of 10% Folin-Ciocalteu reagent were mixed and after 5 minutes, 2 ml of 2% Na_2CO_3 was added. The solution was incubated for 90 minutes at 37°C and its absorbance was measured using UV-Visible spectrophotometer at 760 nm. The gallic acid was used as standard and distilled water was used as blank. All experiments were conducted in triplicate. The total phenolic content was calculated by using Equation (2). The result was reported as mM gallic acid equivalent (mM GAE) by using gallic acid calibration curve.

$$C = cV/m \quad (2)$$

where C is the total phenolic content mg GAE/g dry extract, *c* is the concentration of gallic acid obtained from the calibration curve in mg/ml, *V* is the volume of extract in ml, and m is the mass of extract on gram.

Cholinesterase Inhibition Assay

Cholinesterase inhibitory activity was measured by using a modified 96-well microplate assay based on Ellman colourimetric method (Lee *et al*., 2021). The enzyme hydrolyses the substrate acetylcholine resulting in the production of thiocholine which reacts with Ellman's reagent, 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) to produce 2-nitrobenzoate-5-mercapto thiocholine and 5-thio-2-nitrobenzoate which can be detected at 412 nm. 0.05 M sodium phosphate with pH 7.4 was used as a buffer throughout the experiment. Acetylcholinesterase (AChE) enzyme used in the assay was from an electric eel. In this method, 160 µl (blank) and 140 µl (reaction) of 0.05 M sodium phosphate buffer, 20 µl of root extract were added using a multichannel automatic pipetted into a 96-well microplate. Serial dilution was used to prepare a series of different concentrations of roots extract and followed by the addition of 20 l of 0.09 units/mL of AChE enzyme for the reaction only. The microplate was incubated for 15 minutes at 37°C. After incubation, 10 µl of 10

mM of DTNB and 10 µl of 14 mM acetylcholine iodide (ATCl) was added as substrate, and AChE activity was measured at 412 nm using microplate Spectrometer for 30 minutes. Physostigmine was used as positive control. All the reactions were performed in triplicate. The percentage inhibition was calculated using Equation (3). The test for butyrylcholinesterase enzyme was conducted using the same procedure and by using butyrylcholinesterase (BChE) enzyme and butyrylthiocholine iodide.

$$\text{Percentage inhibition (\%)} = (A_{control} - A_{test})/A_{control} \times 100 \quad (3)$$

where $A_{control}$ is the absorbance of the negative control and A_{test} is the absorbance of the sample/standard.

Statistical Analysis

Data were expressed as mean ± standard deviation for determinations in triplicates. The calculation of linear correlation coefficient was carried out by using Microsoft Excel for Windows (Office 365).

Results and Discussion

Extraction of Clitoria Ternatea Roots

Clitoria ternatea roots weighing 27.8886 g were extracted by using Soxhlet method with 95% ethanol for 12 hours. The weight of the dark brown crude extract obtained was 4.4326 g. Therefore, the percentage yield of *C. ternatea* roots extract is 15.89%.

Phytochemical Screening

Six phytochemical screening tests of *C. ternatea* roots extract were conducted to determine the presence of six classes of secondary metabolites, which are alkaloids, saponin, phenols, terpenoids, steroids and glycosides. The results showed that the extract contains saponin, phenols, terpenoids and steroids (Table 1). The test for alkaloids and glycosides showed negative results. A study by Jiji and Muralidharan (2021) reported the presence of phenols, steroids, triterpenoids, alkaloids, glycosides and saponins in ethanolic extract of *C. ternatea* roots. Taur and Patil (2011) reported the presence of steroids, saponin, and glycosides in ethanolic roots extract of *C. ternatea*. The result is slightly different from other studies because there is no specific solvent that can reliably extract all phytochemicals in plants. Besides that, phytochemical constituents in plants are influenced by several factors, including time, temperature, solvent concentration, and solvent polarity (Nawaz *et al*., 2020). Therefore, the phytochemical constituents observed in *C. ternatea* from Perlis, Malaysia might be different from phytochemical constituents observed in *C. ternatea* in India. However, our study reported the presence of phenols, terpenoids, flavonoids, tannins and steroids, which might act as antioxidants (Lijon *et al*., 2017).

GC-MS Analysis

GC-MS analysis was conducted on the ethanolic roots extract of *C. ternatea* to identify the phytoconstituents responsible for anticholinesterase activity. Among the

Table 1: Qualitative phytochemical analysis of ethanolic extract of *Clitoria ternatea* roots

Phytochemical Constituents	Phytochemical Test	Ethanolic Extract
Alkaloids	Mayer's test	-
Saponin	Foam test	+
Phenols	Ferric (III) chloride test	+
Terpenoids	Salkowski's test	+
Steroids	Salkowski's test	+
Glycosides	Keller-killani test	-

phytochemical compounds detected in the roots extract are phytosteroids, phenols, fatty acids, and triterpenoids. Table 2 shows 27 phytochemical compounds identified from the GC-MS analysis. From these phytochemical compounds, n-hexadecanoic acid, stigmasterol, β-sitosterol, eugenol, 9,12-octadecadienoic acid, 9-octadecenoic acid and (Z)-13-docosenamide were reported to possess neuroprotective properties. The major compound in the roots extract is n-hexadecanoic acid with a percentage area of 12.95%. The n-hexadecanoic acid or palmitic acid was reported to exhibit moderate cholinesterase inhibition activity (Kebbi *et al*., 2021; Dash *et al*., 2022). The peak area for stigmasterol is 1.2%, which is similar to area percentage reported by Lee *et al*. (2021). Stigmasterol was reported to effectively ameliorate memory disorder in mice induced by scopolamine and inhibit AChE with an IC_{50} value of 644.0 ± 11.7 µM (Lee *et al*., 2021). Another sterol, which is β-sitosterol, also exhibits cholinesterase inhibition activity with IC_{50} value of 55 and 50 µg/ml for AChE and BChE respectively (Ayaz *et al*., 2017). Ayaz and colleagues concluded that β-sitosterol is a potential compound in treating Alzheimer's disease. Eugenol, also known as clove oil, has a wide range of pharmaceutical applications, including as a neuroprotective agent in neuro cells (Moreira Vasconcelos *et al*., 2020). Moreover, Chowdhury and Kumar (2020) reported that eugenol is a better multi-targeted-directed-ligand than synthesised drugs used in the treatment of Alzheimer's disease. Long chain polyunsaturated fatty acids are known to be nutritionally important fatty acids. They are essential for neurocognitive development and normal brain function (Zhang *et al*., 2014). Two long-chain polyunsaturated fatty acids were found in the roots extract, which are 9,12-octadecadienoic acid (linoleic acid) (2.91%) and 9-octadecenoic acid (oleic acid) (2.46%). Youn *et al*. (2014) reported that both linoleic acid and oleic acid are potential chromophores in inhibiting β-secretase (BACE1) enzymes for Alzheimer's disease. Medicinally, fatty amides, such as erucamide or (Z)-13-docosenamide, are used in the prevention of metabolic disorders, respiratory diseases, neurodegenerative diseases, such as Parkinson's disease and Alzheimer's disease (Abel-Anyebe *et al*., 2020). In addition, Kim *et al*. (2018) also reported that erucamide may have preventive effects on memory deficits related to Alzheimer's disease. Saponins also possess the ability to inhibit cholinesterase (Khalil *et al.,* 2016). Although saponins were present in phytochemical screening, unfortunately, it was not observed using the GC-MS method in this study. Structurally, saponin comprises two parts: The sapogenin and the sugar part. The sapogenin is usually a triterpenoid (C30) or a steroid (C27). One of the triterpenoids saponin is the skeletal pentacyclic lupane (Xu & Yu, 2021). Based on the GC-MS result, lupenone with molecular formula $C_{30}H_{48}O$ could be present as saponin. Other than lupenone, Lee *et al.* (2021) reported the presence of β-amyrin, a pentacyclic triterpenes of the oleanane, which also may be present as saponins in the root extract (Burnouf-Radosevich *et al.,* 1985; Lee et al. 2021). The discovery of these valuable phytochemicals in the roots extract further justified the neuroprotective potential of *C. ternatea* roots extract.

Total Phenolic Content

The Folin-Ciocalteu method was used to determine the total phenolic content of *C. ternatea* roots extract by using gallic acid as standard. Polyphenols are compounds found in plant extract with redox properties and can act as antioxidants which can change from yellow to blue when reacting with Folin-Ciocalteu reagent. The reaction produces a blue chromophore of a phosphotungstic-phosphomolybdenum complex with the maximum absorption of the chromophores varying depending on the alkaline solution and phenolic compound concentration (Blainski *et al*., 2013; Hudz *et al*., 2019). The absorbance was measured at 760 nm which is considered suited and stable to produce maximum absorption of the compounds as recommended by the European Pharmacopoeia (Blainski *et al*., 2013; Hudz *et al*., 2019). The

total phenolic content of roots extract was calculated from the regression equation of calibration curve (y = 0.0049x -0.0178; R^2 = 0.9713) of gallic acid and expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). Thus, the content of phenolic compounds in ethanol extracts of *C. ternatea* roots was 10.84 ± 0.12 mg GAE/g. Madhu (2013) reported that the ethanolic extract of *in vivo* and *in vitro* grown *C. ternatea* plants were 25.5 ± 0.360 mg GAE/g and 19.5 ± 0.458 mg GAE/g. This shows that the total phenolic content of *C. ternatea* from Perlis, Malaysia is quite similar to *C. ternatea* grown in Muzaffarpur, India. The higher the total phenolic content the higher its antioxidant activity.

DPPH Free Radical Scavenging Assay

The DPPH free radical scavenging assay is one of the most used methods for screening antioxidant activity due to the short time required to evaluate the activity (Škrovánková *et al*., 2012). DPPH free radical is a purple-coloured compound that gives a strong absorption maximum at 517 nm (Ahmad & Abdullah, 2013). The absorption of DPPH solution decreases when the odd electron is coupled off in the presence of a free-radical scavenger, and changes from deep purple to light yellow. Five different concentrations of roots extract (20, 40, 60, 80 and 100 µg/ml) and ascorbic acid as standard (0.06, 0.13, 0.25, 0.50, 1.00 µg/ml) were evaluated. The concentration of sample and standard is different because ascorbic acid is considered one of the most powerful antioxidants (Ahmad & Abdullah, 2013).

The roots extract showed concentration-dependent increases in radical scavenging capacity. The IC_{50} value of the roots extract is 85.31 µg/ml and is compared with the IC_{50} value of ascorbic acid which is 3.35 µg/ml (Figure 1). The root extract's inhibition capacity is lower than ascorbic acid, as it is a strong antioxidant. However, the antioxidant activity of ethanolic roots extract of *C. ternatea* in this study is higher than the ethanolic extract of *C. ternatea* plant. Madhu (2013) reported that the highest DPPH radical scavenging was 67% and 70% of *in vitro* and *in vivo* grown *C. ternatea* at 600 µg/ml. The present recorded 50% inhibition at 85.31 µg/ml. Previous studies have found that high total phenolic content is responsible for antioxidant activity (Aryal *et al*., 2019). Although Madhu (2013) reported slightly higher total phenolic content, nevertheless the present study found stronger antioxidant activity. This is because, other than phenolic compounds, flavonoids and steroids also contribute to the antioxidant ability of an extract (Madhu, 2013; Lijon *et al*., 2017). The chemical constituent from GC-MS analysis in Table 2 shows the presence of steroids, which are campesterol, stigmasterol and β-sitosterol.

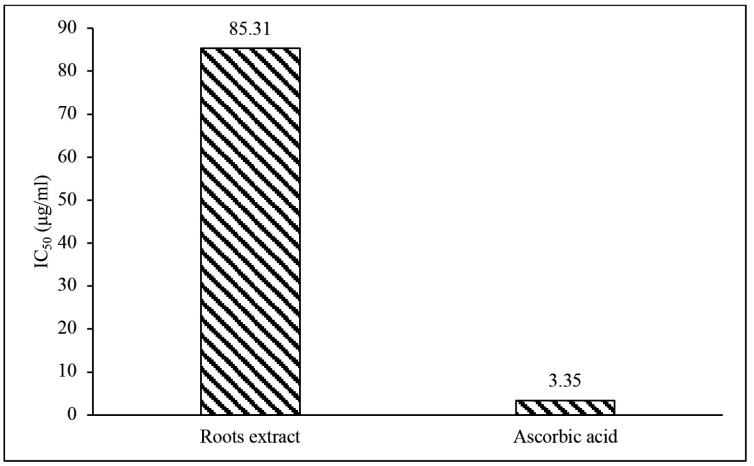

Figure 1: Comparison of DPPH radical scavenging activity of ascorbic acid and *C. ternatea* roots extract

Table 2: Phytochemical profile of ethanolic extract of *Clitoria ternatea* roots obtained via GC-MS analysis

Peak	RT	Peak area (%)	Name of Compound	Molecular Formula	MW
1	7.1972	0.1839	Eugenol	$C_{10}H_{12}O_2$	164.2
2	7.9479	0.0993	Cyclodecane	$C_{10}H_{20}$	140.27
3	8.1877	0.7075	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{17}H_{30}OSi$	278.5
4	9.7144	1.1207	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180.2
5	10.749	12.9539	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4
6	10.8499	0.8389	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.5
7	11.2032	0.1961	Heptadecanoic acid	$C_{17}H_{34}O_2$	270.45
8	11.3546	0.0952	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.47
9	11.3798	0.0952	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296.49
10	11.5817	2.9156	9,12-Octadecadienoic acid (Z,Z)-(Linoleic acid)	$C_{18}H_{32}O_2$	280.45
11	11.607	2.4604	9-Octadecenoic acid, (E)- (trans-oleic acid)	$C_{18}H_{34}O_2$	282.46
12	11.6638	0.3707	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308.5
13	11.689	1.118	Octadecanoic acid	$C_{18}H_{36}O_2$	284.48
14	11.7962	0.1815	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312.53
15	13.1526	0.2347	Carbonic acid, octadecyl 2,2,2-trichloroethyl ester	$C_{21}H_{39}Cl_3O_3$	445.9
16	13.2914	0.4615	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330.5
17	13.3671	0.1784	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.56
18	13.5374	0.3007	Docosanoic acid	$C_{22}H_{44}O_2$	340.58
19	14.1872	0.5459	6H-Benzofuro[3,2-c][1]benzopyran, 6a,11a-dihydro-3,9-dimethoxy-, (6aR-cis)-	$C_{17}H_{16}O_4$	284.31
20	14.3638	0.8648	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	298.9
21	14.8433	0.1474	Tetracosanoic acid	$C_{24}H_4O_2$	368.63
22	14.9884	0.2281	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$	337.58
23	15.0326	0.2492	Decanedioic acid, bis(2-ethylhexyl) ester	$C_{26}H_{50}O_4$	426.67
24	20.6725	0.6237	Campesterol	$C_{28}H_{48}O$	400.68
25	21.1709	1.1988	Stigmasterol	$C_{29}H_{48}O$	412.69
26	22.5398	3.9179	beta-Sitosterol	$C_{29}H_{50}O$	414.71
27	23.8079	0.4473	Lup-20(29)-en-3-one	$C_{30}H_{48}O$	424.7

Cholinesterase Inhibition Assay

Cholinesterase inhibitory activity of ethanolic *C. ternatea* roots extracts were evaluated using Ellman's colourimetric methods. This method is used to determine the effectiveness of natural extracts as anti-acetylcholinesterase agents based on the final product 5-mercapto-2-nitrobenzoic acid which is a yellow-coloured compound with a maximum absorption coefficient of 412 nm. The reaction between 5,5'-dithiobis-2-nitrobenzoic acid (DTNB, Ellman's reagent) with thiocholine produced 5-mercapto-2-nitrobenzoic acid (Lee *et al*., 2021). Table 3 shows the percentage inhibition (%) and IC_{50} values (µg/ml) for *C. ternatea* roots extract and physostigmine against AChE and BChE. The percentage inhibition (%) of *C. ternatea* roots extract against AChE and BChE at the concentration of 800 µg/ml are 86.57 ± 1.92 and 91.74 ± 0.84%, respectively (Table 3). Meanwhile, physostigmine at a concentration of 0.2 µg/ml showed 83.26 ± 2.51 and 70.59 ± 0.93% of AChE and BChE percent inhibition, respectively. The IC_{50} value of ethanolic roots extract of *C. ternatea* for AChE is 118.5 ± 8.6 µg/ml and for BChE it is 83.59 ± 6.23 µg/ml. While the IC_{50} value for physostigmine is 0.03 ± 0.006 µg/ml for AChE and 0.09 ± 0.007 µg/ml for BChE. This shows that the *C. ternatea* roots extract could inhibit the cholinesterase enzyme even though the inhibition strength is weaker than physostigmine. Besides that, *C. ternatea* roots extract is highly selective for butyrylcholinesterase, whereas physostigmine demonstrated greater potency for acetylcholinesterase over butyrylcholinesterase. A previous study by Lee *et al*. (2021) reported that the IC_{50} value of *C. ternatea* roots extract was 70.37 ± 0.01 µg/ml while physostigmine was 0.025 ± 0.00049 µg/ml. Therefore, it shows *C. ternatea* roots extract has weaker cholinesterase inhibition than the standard. In 2014, Shahnas also reported that alcoholic roots extracts of *C. ternatea* have weaker inhibition compared to the standard.

Conclusion

In this study, ethanolic extract of *C. ternatea* roots was evaluated for the presence of phytochemicals, antioxidants, and anticholinesterase activities. GC-MS analysis revealed the presence of n-hexadecanoic acid, stigmasterol, β-sitosterol, eugenol, 9,12-octadecadienoic acid, 9-octadecenoic acid and (Z)-13-docosenamide, which was linked to memory enhancing properties and neuroprotective properties of *C. ternatea*. In addition, the extract of the root also showed inhibition against AChE and BChE enzymes, which are responsible for the progression of Alzheimer's disease. This showed that the roots extract has potential in treating Alzheimer's disease as it showed inhibition for both enzymes. The roots extract also exhibits moderate antioxidant activity. Due to oxidative stress being a contributing factor in Alzheimer's disease, the antioxidant properties of the extract would further enhance Alzheimer's disease treatment. Therefore, further studies are needed to isolate the active compounds with neuroprotective activity and compare the enzyme inhibition with the extracts.

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Table 3: AChE and BChE inhibitory activity of *Clitoria ternatea* roots extract

Sample	Concentration (µg/ml)	% Inhibition		IC_{50} (µg/ml)		Selectivity	
		AChE	BChE	AChE	BChE	AChE	BChE
CTR EtOH	800	86.57 ± 1.92	91.74 ± 0.84	118.5 ± 8.6	83.59 ± 6.23	0.71	1.42
Physostigmine	0.2	83.26 ± 2.51	70.59 ± 0.93	0.03 ± 0.006	0.09 ± 0.007	3	0.33

(Data are expressed as mean ± SD, n = 3)

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