



# Antioxidant and Acetylcholinesterase Inhibitor Potentials of the Stem Extract of *Pternandra galeata*

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## ABSTRACT

**Background:** *Pternandra galeata* belongs to the family Melastomataceae. It is a native flowering plant in Borneo Island that serve as food for monkey habitat. There has been limited study on the medicinal and chemical properties of this plant. **Objectives:** We investigated the acetylcholinesterase inhibitory activity and evaluated the antioxidant activity of the ethanolic extract of *Pternandra galeata* stem. The total phenolic content in the sample was also determined. **Methods:** The acetylcholinesterase inhibitory assays were performed using Ellman's method. Two different methods were used to evaluate the antioxidant activity of the extract by 2,2-diphenyl-1-picryl hydrazyl (DPPH) and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. The total phenolic content was determined by the Folin-Ciocalteu method by employing gallic acid as a reference. **Results:** The ethanolic extract of the *P. galeata* stems inhibited the AChE enzyme with an IC<sub>50</sub> value of 74.62 ± 0.89 µg/mL. The sample exhibited antioxidant activity in the DPPH assay with an IC<sub>50</sub> value of 20.21 ± 0.08 µg/mL and 7.68 ± 0.09 µg/mL in the ABTS scavenging assay. The total phenolic content was 164.71 ± 3.33 mg GAE/g extract. **Conclusion:** The ethanolic extract of the *P. galeata* stem can be a promising cholinesterase inhibitor and antioxidant for treating Alzheimer's disease.

**Keywords:** *Pternandra galeata*, Alzheimer's disease, Acetylcholinesterase inhibitor, Antioxidant, Phenolic compound.

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## RESUMEN

**Antecedentes:** *Pternandra galeata* pertenece a la familia Melastomataceae. Se trata de una planta con flores nativa de la isla de Borneo que sirve de alimento para el hábitat de los monos. Se han realizado pocos estudios sobre las propiedades medicinales y químicas de esta planta. **Objetivos:** Se investigó la actividad inhibitoria de la acetilcolinesterasa y se evaluó la actividad antioxidante del extracto etanólico del tallo de *Pternandra galeata*. También se determinó el contenido fenólico total de la muestra. **Métodos:** Los ensayos de inhibición de la acetilcolinesterasa (AChE) se realizaron mediante el método de Ellman. Se utilizaron dos métodos diferentes para evaluar la actividad antioxidante de los ensayos de 2,2-difenil-1-picril hidrazilo (DPPH) y 2,2'-azinobis-(ácido 3-etilbenzotiazolina-6-sulfónico) (ABTS). El contenido fenólico total se determinó por el método de Folin-Ciocalteu empleando el ácido gálico como referencia. **Resultados:** El extracto etanólico de los tallos de *P. galeata* inhibió la enzima AChE con un valor  $IC_{50}$  de  $74.62 \pm 0.89 \mu\text{g/mL}$ . La muestra mostró actividad antioxidante en el ensayo DPPH con un valor  $IC_{50}$  de  $20.21 \pm 0.08 \mu\text{g/mL}$  y  $7.68 \pm 0.09 \mu\text{g/mL}$  en el ensayo de barrido ABTS. El contenido fenólico total fue de  $164.71 \pm 3.33 \text{ mg GAE/g}$  de extracto. **Conclusión:** El extracto etanólico del tallo de *P. galeata* puede ser un prometedor inhibidor de la colinesterasa y antioxidante para el tratamiento de la enfermedad de Alzheimer.

**Palabras claves:** *Pternandra galeata*, Enfermedad de Alzheimer, Inhibidor de Acetilcolinesterasa, Antioxidante, Compuestos fenólicos

## INTRODUCTION

Neurodegenerative disease, such as Alzheimer's disease (AD), has become a concern, especially in developed countries with a high aging population. This disease has created social and health problems since it burdens the patient, family, and health system (1). Most people with Alzheimer's are 65 and older, but the early stage of AD can be found at younger ages. AD mainly affects brain function, which causes memory loss, diminished judgment capacity, language impairment, and behavioral changes (2). This disease is progressive and, over the years, can develop dementia (60-70% of dementia is caused by AD) (3). Multiple factors are involved in the pathogenesis of AD, including deficiency of neurotransmitter acetylcholine (ACh) and oxidative stress. The regulation of acetylcholine is controlled by acetylcholinesterase (AChE) (4). This enzyme catalyzes the breakdown of ACh into choline and acetate. Therefore, inhibiting this enzyme will help maintain the level of ACh in the brain. The use of cholinesterase inhibitors has shown positive effects in the improvement of cognitive function of AD patients (5). Studies have revealed that oxidative stress plays a significant role in the pathogenesis of neurodegenerative diseases, including AD. Oxidative stress causes the loss of neurons and AD progression to dementia (6-8). Oxidative stress also involves the accumulation of a toxic peptide,  $\beta$ -amyloid, in the brain of AD patients (8,9).

*Pternandra galeata*, locally known as Tiju, is a plant found on Borneo Island in Indonesia and Malaysia. It is a small to medium size tree with a height of up to 27 m. The leaves and flowers of *P. galeata* are the food source for Borneo's monkeys (10). A literature search showed a limited study of *P. galeata* regarding its metabolites and bioactivities. The methanolic extract of these plant leaves showed

potential antiamoebic activity against *Entamoeba histolytica* (11). There is no report on the antioxidant and cholinesterase inhibitory activity of this plant. In our continuing study searching for potential medicinal plants and marine organisms to contend with neurodegenerative diseases, we screened several plants from Borneo for antioxidant and cholinesterase activity. The current research focuses on the antioxidant and cholinesterase activity of the ethanolic extract of the stem of *P. galeata*.

## MATERIAL AND METHODS

### Reagents

The chemical used for cholinesterase assays were acetylcholinesterase from electric eel (AChE type VI-S), acetylthiocholine iodide (ATCI), horse-serum butyrylcholinesterase (BChE), butyrylthiocholine iodide (BTCI), 5,5'-dithiobis[2-nitrobenzoic acid] (DTNB), bovine serum albumin (BSA), tris buffer, and galantamine. The chemicals used for antioxidant assays were 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and potassium persulfate. Folin-Ciocalteu's phenol reagent, sodium carbonate, and gallic acid were used to determine total phenolics. All reagents were purchased from Sigma-Aldrich.

### Preparation of plant extract

The stems of *Pternandra galeata* (Korth.) Ridl. was obtained from Landau Village, Nanga Taman District, Sekadanau, West Kalimantan, Indonesia. The plant was identified by Purwodadi Botanic Garden, Indonesian Institute of Sciences, with identification letter number: B-262/IPH.06/KS.02/VII/2020. The stems of *Pternandra galeata* were cut

into small pieces, then dried in the shade for seven days, and finally grounded. A hundred grams of the powdered stem was soaked with 600 mL ethanol for 24 hours; then, the filtrate was separated under vacuum. The residue was re-extracted using the same procedure three times. All collected filtrates were evaporated under *vacuo* to obtain 0.55 gram ethanolic extract.

### Acetylcholinesterase inhibitory assay

The assay was performed based on the modified Ellman's method (12-14). The extract was dissolved in 10% methanol at 1-500 µg/mL. Twenty-five microliters of sample solutions were mixed with 1.5 mM ATCI (25 µL), 3 mM DTNB (125 µL), Tris buffer (50 µL), and 0.22 U/mL AChE (25 µL) in a 96-well microplate. The formation of yellow colored product, 5-thio-2-nitrobenzoate, was monitored in a microplate reader (Thermo Scientific Multiskan FC) at 405 nm every 5 s for 2 mins. Experiments were carried out in triplicates. Galantamine was used as a standard, and 10% methanol was used as a control/blank. The percent of inhibition was then calculated as follows:

$$\% \text{Inhibition} = \frac{(\text{Mean velocity of control} - \text{Mean velocity of sample})}{\text{Mean velocity of control}} \times 100$$

### DPPH radical scavenging assay

The assay was conducted in triplicates based on modified protocols of Herald *et al.* and Lee *et al.* (15,16). The DPPH solution (0.25 mM) was made in methanol. Different concentrations of extract (1.25 – 40 µg/mL) in methanol and standard gallic acid were incubated with DPPH solutions in 96 well plates and allowed to stand at room temperature in the dark for 30 minutes. The solutions were shaken for 30 s, and the absorbance was measured at 517 nm in a microplate reader. Gallic acid was used as a standard. The following formula calculated the percent DPPH scavenging effect:

$$\text{DPPH Radical Scavenging activity}(\%) = \frac{(\text{abs control} - \text{abs sample})}{\text{abs control}} \times 100$$

where *abs control* is the absorbance of DPPH radical + methanol and *abs sample* is absorbance DPPH radical + extract/standard.

### ABTS Radical Scavenging Assay

The bioassay was performed based on the previous method of Lee *et al.* with some modifications (16). ABTS radical was generated by mixing 5 mL ABTS (7 mM) with 88 µL potassium persulfate (140 nM) and allowed to stand for 16 hours in the dark at room temperature. Samples at a concentration range of 1.25 – 25 µg/mL in methanol were treated with 100 µL of ABTS in a 96-well microplate, followed by incubation for 6 mins in the dark condition at room temperature. The absorbance was read at 734 nm in a microplate reader. Gallic acid was used as a standard. Experiments were carried out in triplicates. The ABTS radical scavenging activity of the extract was calculated as above.

### Quantification of total phenolic content

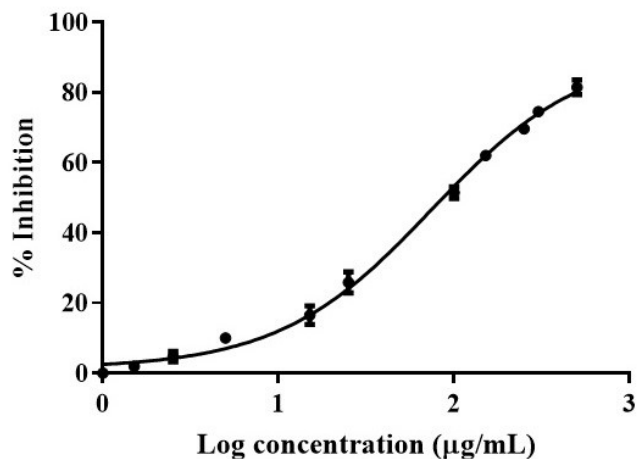
The total soluble phenolic compounds (TPC) in the extract were measured according to the method by Zhang *et al.* and Herald *et al.* with slight modification using gallic acid as a standard (15, 17). Briefly, 25 µL solution of extract (1000 µg/mL) or gallic acid (25 – 500 µg/mL) was mixed with water (75 µL) and Folin & Ciocalteu's phenol reagent (25 µL) in a 96-well microplate. The mixtures were allowed to stand for 6 minutes at room temperature, followed by adding 100 µL Na<sub>2</sub>CO<sub>3</sub> solution (75 g/L). The mixtures were then incubated for 90 minutes in the dark at room temperature. The absorbance was recorded at 765 nm, and the TPC was calculated based on the gallic acid standard calibration curve. The TPC of the extract was expressed as gallic acid equivalent (GAE) in milligrams per gram of dry extract.

## RESULTS

### Acetylcholinesterase inhibitory activity

The ethanolic extract of *P. galeata* was screened against the AChE enzyme using Ellman's method. In this assay, the enzyme AChE will hydrolyze the substrate acetylthiocholine iodide (ATCI), resulting in the product thiocholine, which will react with the Ellman's reagent (DTNB) to form a yellow-colored compound 5-thio-2-nitrobenzoate that can be monitored at 405 nm. The presence of the AChE inhibitor will prevent the hydrolysis of ATCI so that the yellow-colored product will not be formed (12). Various concentrations of *P. galeata* extract were prepared to evaluate the dose-response mode and the fifty percent inhibitory activity (IC<sub>50</sub>) of the extract. The result presented in Table 1 showed that the extract inhibited the AChE enzyme with

an  $IC_{50}$  value of 74.62  $\mu\text{g/mL}$ . Figure 1 showed that the extract inhibited AChE in a dose-dependent manner.



**Figure 1.** Concentration-dependent response of *P. galeata* extract against AChE

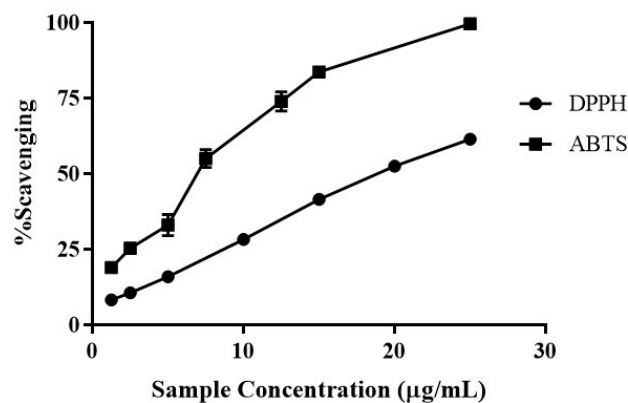
### Antioxidant Activity

The antioxidant property of *P. galeata* was examined using DPPH and ABTS assays. In the DPPH assay, the antioxidant provides a hydrogen atom that reacts with the stable radical DPPH to form a yellow-colored non-radical diphenylpicrylhydrazine (18,19). The principle of ABTS assay is similar to that of DPPH assay, in which the antioxidant acts as a hydrogen donor to form a non-radical ABTS. The reduction of a dark-bluish color of ABTS radical can be monitored by a spectrophotometer (20). The results presented in Table 1 and Figure 2 suggested that the extract of *P. galeata* exhibited concentration-dependent radical scavenging activities in both DPPH and ABTS assays with  $IC_{50}$  values of 20.21 and 7.68  $\mu\text{g/mL}$ , respectively.

**Table 1.** Radical scavenging effect and AChE inhibitory activities of *P. galeata* extract and standards

Samples	$IC_{50}$ ( $\mu\text{g/mL}$ )		
	AChE	DPPH radical scavenging	ABTS radical scavenging
<i>P. galeata</i> extract	74.62 $\pm$ 0.89	20.21 $\pm$ 0.08	7.68 $\pm$ 0.09
Galantamine	0.63 $\pm$ 0.05	-	-
Gallic acid	-	2.76 $\pm$ 0.02	0.97 $\pm$ 0.03

<sup>a</sup>Each value is the average of three analyses  $\pm$  standard deviation



**Figure 2.** DPPH and ABTS radical scavenging effects of different concentrations of *P. galeata* extract

### Total phenolic content

The total phenolic content of *P. galeata* extract was ascertained by the method of Folin-Ciocalteu and calculated as gallic acid equivalent (GAE). The total phenolic content of *P. galeata* extract was 164.71  $\pm$  3.33 mg GAE/g of dry extract (Table 2).

**Table 2.** Extract yield and total phenolic content of *P. galeata* extract

Sample	Extract Yield (%)	TPC (mg GAE/g extract) <sup>a</sup>
<i>P. galeata</i> extract	0.55	164.71 $\pm$ 3.33

<sup>a</sup>Value is the average of three analyses  $\pm$  standard deviation

### DISCUSSION

Alzheimer's disease is one of the most prevalent neurodegenerative diseases that mainly occurs in older adults. Cognitive decline is an apparent symptom of this disease, caused by the deficit of neurotransmitter acetylcholine, particularly affecting cholinergic neurons in the basal forebrain. Another factor involved in the etiology of AD is oxidative stress. It is associated with the mechanism of  $A\beta$ -induced cytotoxicity of cholinergic neurons. Currently, AChE inhibitors, such as galantamine, rivastigmine, and donepezil, have been proven to be the most effective therapy to improve AD patients' memory and cognitive function (21). In addition, antioxidants have shown benefits in improving cognitive function and behavioral deficits in AD animal models (22). Therefore, it is suggested that natural antioxidants with potent cholinesterase inhibitory activity can be a better drug for treating AD (23,24). In the current study, the stem of *Pternandra galeata* was extracted with ethanol. The

extract was then subjected to *in vitro* antioxidant and acetylcholinesterase inhibitory assays.

Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) are enzymes that are related to the level of acetylcholine (ACh) in the brain (25). However, AChE is reported as the major cholinesterase enzyme in the brain that catalyzes the hydrolysis of acetylcholine and shows higher specificity toward acetylcholine. Therefore, AChE enzyme has been an attractive target for AD therapy (26). In our study, *P. galeata* extract showed moderate inhibition against the AChE enzyme in a dose-dependent manner. Currently, we have not found a report from the genus *Pternandra* as a cholinesterase inhibitor. However, a plant from the same family Melastomataceae, *Miconia* sp., has been reported to have moderate AChE inhibitory activity (27). A triterpene, sumaresinolic acid, isolated from *Miconia stenostachya* showed AChE inhibitory activity (28,29).

Oxidative stress has been reported to play an essential role in AD pathogenesis. Therefore, preventing or reducing oxidative damage by using antioxidants for AD and another therapy may provide better results (24). Plant materials, such as fruits, vegetables, herbs, and spices, have been known as the source of natural antioxidants (30). The current study examined the antioxidant property of *P. galeata* by DPPH and ABTS methods. The DPPH and ABTS free radicals are stable free radicals widely used to estimate the radical scavenging activity of antioxidants. The results showed that *P. galeata* gave strong radical scavenging activities in both DPPH and ABTS assays. This is due to the different reactivity of compounds in the *P. galeata* extract to DPPH and ABTS radicals. The reaction of DPPH radicals depends on the steric accessibility of compounds to them. Small molecules generally react better with DPPH radicals compared to large molecules. ABTS radical can be used to measure the antioxidant capacity of both hydrophilic and lipophilic compounds (31, 32). The extract showed a lower  $IC_{50}$  value in the ABTS assay than in the DPPH assay. There has been no report of *P. galeata* as an antioxidant. However, a plant from the same genus, *P. azurea* exhibited radical scavenging activity against DPPH, ABTS, and nitric oxide (NO) (33).

There has been evidence of the correlation of phenolic compounds with antioxidant and cholinesterase inhibitory activities. The presence of hydroxyl groups in the phenolic compounds is believed to play a significant role in AChE inhibitory activity. The multiple hydroxyl groups in the phenolic

compounds can enhance the inhibitory action of AChE due to their stronger binding capacity (34,35). Several phenolic compounds have been reported to show inhibition against AChE and BChE in both *in vitro* and *in vivo* studies, such as quercetin, resveratrol, curcumin, gallic acid, and its derivatives (35). Phenolic compounds are also well known to be essential in the antioxidant activities of medicinal plants. The antioxidant potential of the phenolics is predominantly due to the redox capability so that it can absorb and neutralize free radicals, decompose peroxide, and quench singlet or triplet oxygen (36). The number and arrangement of the hydroxyl groups in phenolics are believed to be closely related to their antioxidant capacity.

## CONCLUSION

The stem extract of *P. galeata* exhibited antioxidant and acetylcholinesterase inhibitory activities. The presence of phenolic compounds in the extract may be responsible for the antioxidant and AChE activities. Further study is needed to investigate the chemistry of the phenolic compounds in the extract.

## CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation.

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## AUTHOR'S CONTRIBUTIONS

Concept – S.S.; Design – S.S.; Supervision – S.S., A.W., R.R.; Resources – S.S., K.I., A.W.; Materials – S.S., R.R.; Data Collection and/or Processing – S.S., D.N.I.; Analysis and/or Interpretation – S.S., D.N.I.; Writing – S.S., A.D.A.; Critical Reviews – S.S., D.N.I., A.W., R.R.

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