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Phytochemical investigation and *in vitro* evaluation of cholinesterase inhibitory and antioxidant properties of *Aglaonema hookerianum* stems

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ABSTRACT

Inhibition of acetyl- and butyryl-cholinesterase is the promising strategy for treatment of Alzheimer's disease (AD). Medicinal herb is an important source of cholinesterase inhibitors. *Aglaonema hookerianum* has been used in Bangladesh as a folk medicine in neurological problem and other ailments. Therefore, the current study was designed to determine the phytochemical content and to explore the anticholinesterase and antioxidant activity of the crude methanol extract and its n-hexane, chloroform, ethylacetate and aqueous fractions. Phytochemical analysis showed that the chloroform fraction contained the highest amount of phenolics (65.92 ± 0.67 mg GAE/gm of dried extract) and flavonoids (224.38 ± 1.67 mg CE/gm dried extract). *In vitro* assay revealed that the chloroform fraction exhibited the highest inhibition against both the acetylcholinesterase and butyrylcholinesterase with the IC_{50} values of 105.63 ± 0.32 and 111.00 ± 0.70 µg/ml, respectively. The fraction also exhibited strong antioxidant activity with an IC_{50} value of 10.07 ± 0.01 µg/ml for DPPH scavenging. These findings suggest the considerable cholinesterase inhibitory and antioxidant activity of the chloroform fraction which support the traditional use of this plant in the neurological disorder.

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INTRODUCTION

Acetylcholine is a cholinesterase neurotransmitter that mediates neurotransmission. It is involved in the acquisition of memory and learning (Chen *et al.*, 2022). In AD, it is gradually declined leading to the loss of memory and cognition (Rumiana *et al.*, 2024). Acetylcholinesterase (AChE) is the key enzyme involved in the hydrolysis of acetylcholine and terminates the nerve impulse at the cholinergic synapses (Hampel *et al.*, 2018). Besides AChE, butyrylcholinesterase (BCHE) can break down the Ach, but to a less extent. Therefore, inhibitors of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) that elevate the concentration of acetylcholine has become the primary treatment for the cognitive defect in AD (Li *et al.,* 2000). Only three cholinesterase inhibitors donezepil, rivasigmine and galantamine are used currently to treat AD (Pang *et al*., 2025). The handful of FDA approved cholinesterase inhibitors and their associated side effects led the researchers to discover new and safe inhibitors. Medicinal plants are recognized as the source of AD drugs. A good number of phyto-constituents have already been isolated and identified that augmented cholinesterase inhibition and enhanced cognitive function (Gregory *et al*., 2021).

Oxidative stress (OS) is an important feature of a variety of neurodegenerative diseases including Alzheimer's disease (AD), Parkinson's disease. In AD, OS develops due to excessive generation of free radicals such as hydroxyl radicals, superoxide free radicals etc as well as due to weakening of enzymatic antioxidative systems (Kao *et al.,* 2020). It is evident that amyloid Abeta protein, the major component of senile plaque in AD, increases free radical generation in neuronal cell, resulting in oxidative stress. This in turn caused oxidative damage leading to neuro-degeneration (Bai *et al*., 2022). Antioxidants can effectively scavenge the free radicals, therefore useful in the treatment of AD (Pritam *et al*., 2022).

Aglaonema hookerianum (Synonym- *Aglaonema clarkei* Hook.f.), locally known as habinishak, patabahar is an evergreen perennial herb belonging to the family araceae. In Bangladesh, it is mostly found in the forests of Sylhet, Bandarban, Chittagong and Chittagong Hill Tracts (Motaleb *et al.,* 2013**)**. The plant is also distributed in the north-eastern area of India, Bhutan, and Myanmar. Traditionally, the plant is used to treat the neurological problems including depression, hysteria and also a variety of ailments such as sexual problem, dropsy, fever, cirrhosis, flatulence, hyper acidity (gastritis), tetanus, conjunctivitis constipation and hysteria (Motaleb *et al.,* 2013; Gani *et al.,* 2021; Rahman *et al*., 2007). Survey of literature has shown multiple pharmacological activities including thrombolytic effect, anti-arthritic effect, antimicrobial and cytotoxic properties (Biozid *et al.,* 2015; Alam *et al.,* 2015; Roy *et al*., 2011). Till now no phytochemical study has been done on this plant. In the present study, we carried out an investigation of the anticholinesterase and antioxidant activity of the crude extract and its four fractions.

MATERIALS AND METHODS

General

Solvent methanol, n-hexane, chloroform, benzene, ethylacetate, acetonitrile, acetone, dichloromethane, cyclohexane are obtained from Duksan pure chemicals Ltd., Korea Republic. Reagent 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma (Merck KGaA, Darmstadt, Germany). Acetylcholinesterase (AChE) from electric eel and butyrylcholinesterase enzyme from equine serum, acetylthiocholine iodide (ATCI) and S-butyrylthiocholine iodide were purchased from Sigma-Aldrich, Germany. Silica gel 60 F_{254} and silica gel (40–60 µm, 60 Å, 230–400 mash) were from Merck, India. Nuclear magnetic resonance (NMR) spectra were recorded in methanol (CD_3OD) on a Bruker Avance spectrometer operating at 600 MHz (for ^1H NMR) and 400 MHz (for ^{13}C NMR). A Shimadzu UV-1800 spectrophotometer was used for obtaining the absorbance of samples

Plant material collection, extraction and solvent partitioning

The stem of *A. hookerianum* was collected from Chittagong district, Bangladesh and was identified by taxonomist Mr. A.H.M. Mahbubur Rahman, Dept. of Botany, University of Rajshahi and the voucher specimen was deposited at the herbarium of same department (RR-136). The collected part of *A. hookerianum* was washed, dried and grinded in to coarse powder using mortar and was made ready for further analysis. Powdered plant material was extracted with sufficient amount of methanol according to cold extraction method. The extract was then filtered, concentrated in vacuo to obtain the crude methanol extract (MAH). The extracts were stored at 4 degree centigrade in the refrigerator until use. The methanol extract of plant was partitioned with three solvents such as n-hexane, chloroform, and ethyl-acetate solvents by the modified Kupchan method (Kupchan, 1970**)** that yielded the corresponding fractions such as n-Hexane fraction (AHH) Chloroform fraction (AHC), ethylacetate fraction (AHE) and aqueous fraction (AHA).

Estimation of total phenolic content (TPC)

The TPC of the crude methanol extracts of four plants were determined using the Folin-Ciocalteu reagent (Majhenic *et al.,* 2007). Plant extract (0.5 ml) at different concentrations was mixed with 2.5 ml of Folin-Ciocalteu (diluted 10 times with water) reagent and 2.5 ml of sodium carbonate (7.5%) solution. Following incubation for 20 minutes at 25^{o}C, the absorbance of the mixture was measured at 760 nm. The phenolic content was determined using the standard curve obtained for gallic acid and the results were expressed as mg of gallic acid equivalent (GAE) per gm of dried extract. Each of the sample was tested for three times and mean value had taken as the total content.

Estimation of total flavonoid content (TFC)

The TFC of plant extract were measured by the aluminum chloride colorimetric assay as described by Dewanto method (Dewanto *et al.*, 2002) using catechin as a standard. 1 ml plant extract with a concentration of 500 µg/ml was taken in a 10 ml of volumetric flask, and then 5 ml of distilled water followed by 0.3 ml of 5% sodium nitrite were added to it. About 0.6 ml of 10% aluminum chloride was added after 5 minutes. Again after 5 minutes 2 ml of 1M sodium hydroxide was added and volume was made up to the mark with distilled water. The solution was under through mixing and absorbance was taken as 510 nm wavelength. The flavonoid content was determined using the standard curve obtained for catechin and the results were expressed as mg of catechin equivalent (CE) per gm of dried extract. Each of the samples was tested for three times and readings so obtained were averaged.

Evaluation of the anticholinesterase activity

The cholinesterase (AchE and BuChE) inhibitory activity was determined by Ellman’s method (Ellman *et al.,* 1961) using acetylthiocholine iodide (ATCI) and S-butyrylthiocholine iodide (BTCI) as substrates. About 200 µl of sample/standard of various concentration, 200 µl of enzyme extract and 2.00 ml of 50 mM Tris-HCl buffer, pH 7.4 were taken in test tubes, mixed and kept for one hour at room temperature. Afterwards, 200 µL of 0.7 mM DTNB and 400 µl of 0.35 mM ATCI/BTCI were added to it and then the absorbance of all samples were taken at a wavelength of 412 nm every 30 sec for 3 minutes against a blank solution. Donepezil and galantamine were used as positive control for the estimation of AchE and BuChE inihibitory activity of test samples respectively. The percentage (%) of inhibition, I%, was calculated by using the following equation.

$$I\%=\{(A_0-A_1)/A_0\}\times 100$$

Where, A_0 represents rate of the absorbance change of the control solution per minute and A_1 represents rate of the absorbance change of the sample per minute. IC_{50} value was obtained by plotting the percentage of inhibition value against the respective concentrations. The relevant assessments were performed thrice.

Estimation of antioxidant activity *in vitro*

Antioxidant potential of the test samples were estimated by using DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay as described by Braca method (Braca *et al.,* 2001). Briefly, 2 mL of methanol solution of test samples at different concentration were taken in a test tube. 2 ml 0.004% methanol solution of DPPH was added to each of the test tube and incubated in dark place for 60 minutes to complete the reaction. Then the absorbance of the sample solutions were measured as 517 nm using a spectrophotometer. Catechin was used as reference compound for comparison purpose. The experiment was done thrice and the results were averaged. The percentage of scavenging (I %), was reckoned by using the following equation.

$$I\% = \{(A_0 - A_1)/A_0\} \times 100$$

Where, A_0 is the absorbance of the control solution and A_1 is the absorbance of the samples. The results so obtained were expressed in terms of IC_{50} value by plotting the percentage of scavenging value against the respective concentrations.

Statistical analysis

In this study, each of the respective test was conducted three times. The data are expressed as average ± SD. The statistical and graphical analysis were done using GraphPad Prism (10.0.1) and Microsoft Excel 2013. With a view to determine the statistical significance (p value< 0.05) between mean values, T-test was assumed. Pearson correlation test was done for correlation analysis.

RESULTS

Phytochemical content

The total phenolic content (TPC) of the extract and fractions from *A. hookerianum* was measured by Folin-Ciocalteu method and the result has been presented in the Table 1. The crude methanol extract was found to possess a considerable amount of phenolics which was 52.18 ± 0.24 mg GAE/g dried extract. Among the fractions, chloroform fraction was the richest in phenolics followed by ethylacetate, n-hexane and aqueous fractions. The TPC was 65.92 ± 0.67, 48.34 ± 0.41, 34.56 ± 0.33 and 26.15 ± 0.28 mg GAE/g dried extract, respectively. Similarly, the methanol extract had a large amount of flavonoids (338.17 ± 7.39 mg CE/g dried extract). Of the fractions, the chloroform fraction contained the highest content of flavonoids followed by ethylacetate, aqueous and n-hexane fractions which were 224.38 ± 1.67, 180.51 ± 1.94, 78.64 ± 0.71 and 63.62 ± 0.58 mg CE/g dried extract, respectively.

Table 1. Total phenolic content and total flavonoid content of *A. hookerianum* methanol extract and its four solvent fractions

Samples	Total phenolic content mg GAE/g dried extract	Total flavonoid content mg CE/g dried extract
MAH	52.18 ± 0.24 [b]	338.17 ± 7.39 [a]
AHH	34.56 ± 0.33 [d]	63.62 ± 0.58 [d]
AHC	65.92 ± 0.67 [a]	224.38 ± 1.67 [b]
AHE	48.34 ± 0.41 [c]	180.51 ± 1.94 [c]
AHA	26.15 ± 0.28 [e]	78.64 ± 0.71 [c]

Table 2. IC50 value of extract and fractions for cholinesterase enzyme inhibition and antioxidant activity

Samples	IC_{50}±SD value for AChE inhibition (µg/ml)	IC_{50} ±SD value for BuChE inhibition (µg/ml)	IC_{50} ±SD value for antioxidant activity (µg/ml)
DON	0.42 ± 0.02	-	-
GAL	-	1.35 ± 0.06	-
CAT	-	-	4.66 ± 0.07
MAH	124.64 ± 0.46	173.70 ± 0.64	11.63 ± 0.61
AHH	444.83 ± 0.21	>500	89.72 ± 0.45
AHC	105.63 ± 0.32	111.0 ± 0.70	10.07 ± 0.01
AHE	194.83.± 0.25	>500	24.09 ± 0 .41
AHA	>1000	>500	60.51 ± 0.94

Cholinesterase inhibitory activity

The crude methanol extract and its four fractions were evaluated for acetylcholinesterase inhibition by Ellman's method and the results were compared with the standard drug donezepil. The crude methanol extract showed dose dependent inhibition of acetylcholinestease with an IC_{50} value of 124.64 ± 0.46µg/ml (Fig. 1A, Table 2).

Following partitioning of the extract into four solvent fractions, the highest activity was found in the chloroform fraction with an IC_{50} value of 105.63 ± 0.32 µg/ml. The ethylacetate, n-hexane and aqueous fraction showed activity, but failed to show 50% enzyme inhibition at the same concentration.

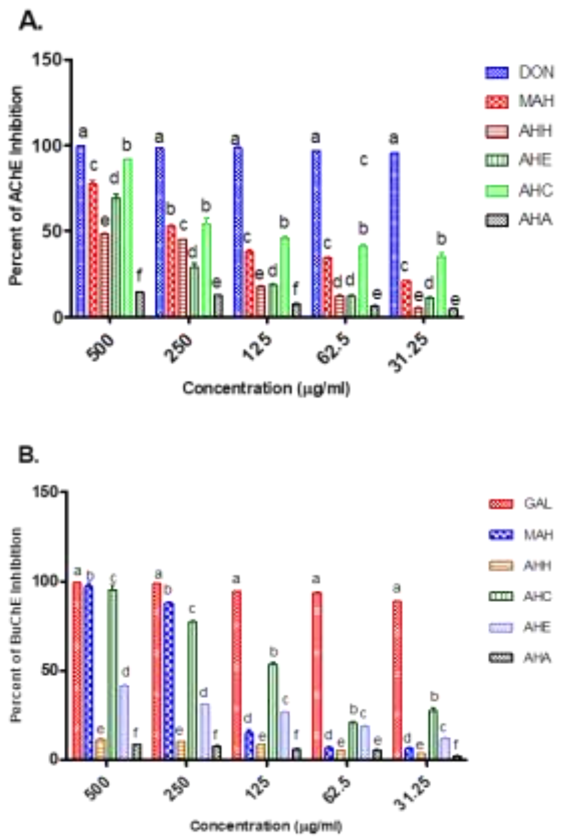

Fig. 1. Cholinesterase inhibitory activity of extracts and solvent fractions from *A. hokerianum*

A. Acetylcholinesterase inhibition, B. Butyrylcholinesterase inhibition. Mean with different letters (a-f) differ significantly ($p < 0.05$)

The inhibitory potential of the extract and fractions against butyrylcholinesterase was similarly evaluated by Ellman's method and the results have been presented in the Fig. 1B and Table 2. Similar to acetylcholinesterase inhibition, the crude methanol extract exhibited inhibition against butyrlcholinesterase with IC_{50} value of 173.70 ± 0.64 µg/ml. Among chloroform fraction showed the highest activity with IC_{50} value of 110.0 ± 0.70 µg/ml. The remaining n-hexane, ethylacetate and aqueous fraction had activity, but failed to reduce 50% of enzyme inhibition at the same concentration. These results suggested that the chloroform fraction was a dual cholinesterase inhibitor the fractions.

Antioxidant activity

The antioxidant activity of the extract and fractions from *A. hookerianum* was assessed by the DPPH free radical scavenging assay. DPPH model is widely used for evaluation of antioxidant activity due to its simplicity and rapidity (Braca *et al.*, 2001). The results (Fig. 2, Table 2) demonstrated the strong antioxidant activity of the crude methanol extract. The IC_{50} value of the extract was found to be 11.63 ± 0.61 µg/ml. Among the fractions, the potent activity was present in the chloroform fraction followed by the ethylacetate fraction with IC_{50} values of 0.07 ± 0.01 and 24.09 ± 0.41µg/ml. The chloroform fraction exhibited more potent activity than the standard catechin. The n-hexane and aqueous fractions showed poor activity.

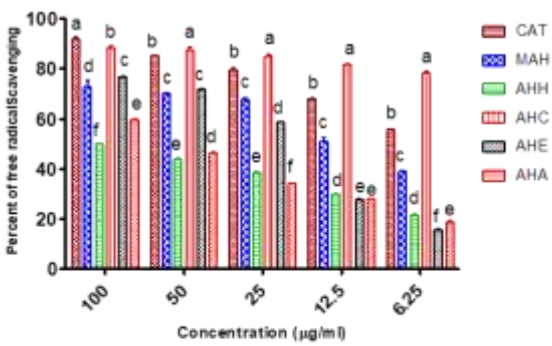

Fig. 2. DPPH free radical scavenging activity of the extract and fractions from *A. hookerianum*

Catechin was used as a reference standard. Mean with different letters (a-f) differ significantly ($p < 0.05$).

DISCUSSION

AD is considered as a complex neurodegenerative disease because of the involvement of multiple pathways in the pathogenesis. Amyloid-beta (Aβ) deposition, cholinergic dysfunction, formation of neurofibrillary tangles and oxidative stress are important pathways, among others (Chen *et al.*, 2022; Rumiana *et al.*, 2024; Hampel *et al.*, 2018; Li *et al.*, 2000). The current therapeutics such as cholinesterase inhibitors have been developed aiming a single target (Pang *et al.*, 2025). The drugs can effectively improve the condition of AD patients but do not halt the disease progression. This can be explained by the fact that the other pathogenic processes are involved in the progression of the disease. Therefore, a multifunctional agents have been suggested which will not only inhibit the cholinesterase, but modulate the other pathologic processes. Medicinal plant continues to provide modern drugs and phytochemicals that have diverse pharmacological properties. Apigenin, curcumin, catechin are compouns isolated from plants that reduce the AD pathology through multiple biological activities (Gregory *et al.*, 2021). *Aglaonema hookerianum* have been traditionally used in the treatment of neurological problems and other ailments (Motaleb *et al.,* 2013; Gani *et al.,* 2021; Rahman *et al.*, 2007). In this study, we determined the phytochemical contents and explored the anticholinesterase and antioxidant activity of the crude methanol extract and its four fractions.

Medicinal plants exhibit a diverse biological activity due to biosynthesis of a large number of phytochemicals. Polyphenolics, which include the phenolics and flavonoids, are common in medicinal plants that play important role in defensive function and other biological activities (Gregory *et al.*, 2021). Phytochemical analysis revealed a large amount of phenolics and flavonoids in the methanol extract. Following fractionation of the methanol extract, the highest polyphenolics were found in the chloroform fraction. These results indicated that the chloroform fraction is rich in phenolics and flavonoids which may provide potential antioxidant and cholinesterase inhibitory activity. A large number of polyphenolic compounds such as ferulic acid, caffeic acid, catechin and quercetin have been isolated from medicinal herbs (Pritam *et al.*, 2022).

Inhibitors of acetyl- and butyryl-chloinesterase are considered as the first line drugs for AD (Hampel *et al.*, 2018). *In vitro* assay of the extract demonstrated that the crude methanol extract possesses both the acetyl- and butyryl-cholinesterase inhibitory activity. Following fractionation, the activity was largely present in the chloroform fraction with IC_{50} values of 105.63 ± 0.32, and 110.0 ± 0.70 µg/ml for acetylcholinesterase and butyrlcholinesterase, respectively. The result indicated that the chloroform fraction is a dual cholinesterase inhibitor and may be used as a source of potential inhibitors.

Oxidative stress is implicated in the progression of AD (Kao *et al.,* 2020). By neutralizing the excess free radicals, antioxidants have shown an improvement of memory and cognition (Pritam *et al.*, 2022). Therefore, oxidative stress has been emerged as an important drug target for the treatment of AD. In DPPH assay, the crude extract showed strong activity with an IC_{50} value of 11.63 ± 0.61 µg/ml. Among the fractions, the most potent activity was found in the chloroform fraction that gave an IC_{50} value of 0.07 ± 0.01 µg/ml, even more potent than standard catechin havin IC_{50} value of 4.66 ± 0.07 µg/ml. The findings suggested that the chloroform fraction is a rich source of antioxidants.

CONCLUSION

In summary, the chloroform fraction obtained from *Aglaonema hookerianum* has considerable acetyl- and butyryl-cholinesterase inhibitory activity. The fraction also showed strong antioxidant activity. This study supports the traditional use of the plant in the treatment of neurological disorders. Further investigation on chloroform fraction may provide new cholinesterase inhibitors and antioxidants which may be used in the treatment of Alzheimer’s disease.

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