

# Phytochemical Screening and Acetylcholinesterase and Butyrylcholinesterase Inhibitory and Thrombolytic Activities of *Grewia abutilifolia* Vent. & Juss. Leaf

Md. Rajdoula Rafe<sup>1</sup>, Rayhanus Salam<sup>2</sup>, Md. Monir Hossain<sup>1</sup>  
and Mohammad Mehedi Masud<sup>3</sup>

<sup>1</sup>Department of Pharmacy, Jagannath University, Dhaka, Bangladesh

<sup>2</sup>Department of Pharmacy, Southeast University, Dhaka, Bangladesh

<sup>3</sup>Department of Pharmaceutical Chemistry, University of Dhaka, Bangladesh

(Received: .23 January, 2018; Accepted: 25 March, 2018; Published (web): 10 June, 2018)

**ABSTRACT:** Cholinesterase inhibitors offer the possible pathway to fight against various types of neurodegenerative diseases. *Grewia abutilifolia* is used extensively in traditional and folk medicines mainly to improve intellectual capabilities and memory enhancement purposes which support its possible effects against neurodegenerative disorders. We performed preliminary phytochemical screening to observe the presence of potential phytoconstituents of *G. abutilifolia* leaf extracts and evaluated acetylcholinesterase and butyrylcholinesterase inhibitory properties to measure its anti-cholinesterase activities. Preliminary phytochemical screening confirmed the presence of saponins, tannins, flavonoids, terpenoids, steroids, carbohydrates, coumarins, alkaloids etc. The petroleum ether (PESF), chloroform (CSF) and aqueous soluble fractions (AQSF) of the crude extract of the plant leaf were screened for bioactivities. The highest inhibition of acetylcholinesterase was exhibited by aqueous soluble fraction (AQSF) with  $IC_{50} = 5.73 \pm 0.59 \mu\text{g/ml}$ , while the highest inhibition of butyrylcholinesterase was shown by petroleum ether soluble fraction (PESF) with  $IC_{50} = 6.57 \pm 1.81 \mu\text{g/ml}$  in a dose-dependent manner. During assay for thrombolytic activity, the chloroform soluble fraction (CSF) revealed highest activity with  $25.75 \pm 1.62\%$  clot lysis. These results of the present study suggested the effectiveness of *G. abutilifolia* against various neurological diseases.

**Key words:** Phytochemical screening, cholinesterase inhibition, thrombolytic, *Grewia abutilifolia*.

## INTRODUCTION

In general, chronic breakdown and deterioration of neurons, particularly, those of central nervous system caused neurodegenerative disorders.<sup>1</sup> The common example of neurodegenerative diseases are Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis, multiple sclerosis, and spongiform encephalopathy<sup>2</sup> hence, the most familiar neurodegenerative disease is AD.<sup>3</sup> Neurological disorders are mainly increased with the increasing amount of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes in our body because these enzymes are responsible to break acetylcholine.<sup>4</sup> Recently, the importance of both

types of cholinesterase enzymes in our body makes them main purpose of research on this field for the treatment of neurological disorders.<sup>5,6</sup> Therefore, AChE and BChE inhibitors are the main targets for the drug discovery and design to fight against neurodegenerative disorders. A lot of recent researches exhibited that medicinal plants are used for the treatment of diseases in a specific part of the body like the nervous system and cardiovascular systems.<sup>7</sup> Thrombus formed in blood vessels lead to atherothrombotic diseases such as myocardial or cerebral infarction. Thrombolytic agents are used to dissolve the already formed clots in the blood vessels; however, these drugs sometimes cause serious and fatal consequences.<sup>8</sup> Previously, less attention has been focused on clot lysis activity of *Grewia abutilifolia*. Thus, the objective of this study

**Correspondence to:** Mohammad Mehedi Masud

Email: mehedi33@du.ac.bd

Phone No. : +8801758064654

Dhaka Univ. J. Pharm. Sci. 17(1): 81-86, 2018 (June)

was to investigate the *in vitro* thrombolytic potential of this medicinal plant of Bangladesh.

*G. abutilifolia* plant belongs to *Grewia* genus and Tiliaceae family. Nearly 40 species of this genus are found in India, some of which are renowned for their medicinal values.<sup>9</sup> Many bioactivity studies of genus *Grewia* have been reported earlier on different species. Some of them are used in folk medicine for the treatment of malaria, diarrhea, typhoid fever, dysentery, small pox, cough, irritable condition of bladder and intestine, eczema and rheumatism.<sup>10</sup> Already the use of *G. abutilifolia* for the treatment of malarial<sup>11</sup>, bacterial infections<sup>12</sup> and anemia<sup>13</sup> have been reported in addition to the roots of *G. abutilifolia* to treat abscesses. Furthermore, recent reports suggest that the plant extracts usually from bark can show toxicity like liver injury. Its seed extract and seed oil exhibited anti-fertility activity too.<sup>14</sup> There is no report of its activities against neurodegenerative diseases and oxidative stress. Thus it was our main objective to find out any possible treatment outcome for neurological disorders and cardioprotective activity by assessing cholinesterase enzymes inhibitory and thrombolytic activities of *G. abutilifolia* leaf.

## MATERIALS AND METHODS

**List of drugs.** Galantamine, donepezil and streptokinase were used as standard drugs. Unless otherwise specified, all other chemicals were of analytical grade.

**Collection of plant.** The fresh plant material (leaves) was collected in January 2015 from Comilla Hill track and the plant was verified by an authorized person of Bangladesh National Herbarium, Dhaka.

**Preparation of extract.** The collected leaves were dried with exposing to sun after washing properly to eliminate other particles followed by oven drying at 40°C temperature for almost 24 hour. The dried leaves were ground into a coarse powder by a grinding machine in the Department of Pharmacy, Southeast University. About 500 gm of powder was taken in an amber colored reagent bottle and soaked in 1.5 liters of methanol. The bottle with its contents

was sealed and kept for 10 days with intermittent shaking and stirring. After that, the mixture was filtered with cotton bed and filter paper and concentrated with a rotary evaporator under reduced pressure to obtain crude methanol extract (ME), final weight of which was 45.39 gm. The crude methanol extract (ME) was then partitioned according to the modified Kupchan method<sup>15</sup> by using petroleum ether and chloroform.

**Qualitative phytochemical analysis.** The preliminary phytochemical analysis was done to detect different classes of compounds in the plant extract. Phytochemical screening for major constituents was performed using the standard method.<sup>16,17</sup>

**Acetylcholinesterase (AChE) inhibitory activity.** Acetylcholinesterase inhibitory activity was determined by the method of Ellman's *et al.*<sup>18</sup> for several fractions of plant extract in various concentrations. In this enzymatic reaction, acetylthiocholine iodide and 5,5'-dithiobis(2-nitrobenzoic acid) were used as substrate to estimate the AChE activity. A homogenization buffer consisting of 10 mM Tris-HCl (pH 7.2), along with MgCl<sub>2</sub> (50 mM), NaCl (1 M), Triton X-100 (1%) was used. Homogenization of buffer and the bovine brain (5:1) was carried out through centrifugation (15,000 g, 30 minutes) and the supernatant was separated, which was bovine brain AChE. Collected AChE was then standardized with BCA kit (Bicinchoninic acid; Sigma Co., USA) comparing with the standard (bovine serum albumin). UV-Vis spectrophotometer (Shimadzu Scientific Instruments) was used to measure the hydrolysis rate of acetylcholinesterase for plant extract or standard (Donepezil) sample at various concentrations (500 µl) by adding 50 µl enzyme solution. Then, it was incubated (15 minutes, 37°C) and absorbance ( $\lambda_{\text{max}} = 0.405 \text{ nm}$ ) was measured after adding Ellman's reaction mixture instantly in a 50 mM sodium phosphate buffer (pH 8.0). All the observed readings were repeated for 10 min at 2 min intervals to verify that the reaction occurred linearly. In this method the blank reaction was measured by substituting saline for the enzyme.

**Butyrylcholinesterase (BChE) inhibitory activity.** Butyrylcholinesterase (BChE) inhibitory activity was determined using *s*-butyrylthiocholine iodide as a suitable substrate according to the method described by Ellman's *et al.*<sup>18</sup>, with slight changes (Galantamine standard). Homogenization buffer of 5 volumes [10 mM Tris-HCl (pH 7.2), which contained 1 M NaCl, 50 mM MgCl<sub>2</sub> and 1% Triton X-100] was mixed with human blood and then centrifuged for 30 minutes at 10,000 rpm. Then the supernatant was separated and this supernatant was applied as enzyme source. In this whole process temperature was maintained at 4°C. Hydrolysis rate by BChE were observed through spectrophotometric method. Plant extracts or standard solution (500 µl) at different concentrations were mixed with an enzyme solution (50 µl) and incubated at 37°C for 15 minutes. Absorbance was monitored at 405 nm after addition of Ellman's reaction mixture [3.5 ml; 0.5 mM butyrylthiocholine, 1 mM 5, 5'-dithiobis (2-nitrobenzoic acid)] in 50 mM sodium phosphate buffer (pH 8.0) to the above reaction mixture. Reading was repeated for 10 min at 2 min intervals to verify that the reaction occurred linearly. The blank reaction was measured by substituting saline for the enzyme.

**Statistical analysis.** For different experimental purposes (AChE and BChE inhibitory activity), the percentage of inhibition was calculated by using the following formula:

$$\text{Rate (Inhibition/scavenging) \%} \\ = \{(A_B - A_S)/A_B\} \times 100$$

Where,  $A_B$  = Absorbance observed without sample (Control) and  $A_S$  = Absorbance observed with sample (Extract/Standard).

The IC<sub>50</sub> value was calculated (Table 2) for the standard and plant extracts. Correlation analysis had been carried out. All results are expressed as mean ± standard deviation (SD) from 3 independent experiments.

**Thrombolytic activity.** Thrombolytic activity study was carried out by the method developed earlier.<sup>19</sup> In this study, adequate amount of blood drawn from volunteers was taken in sterile

microcentrifuge tubes which were pre-weighed and incubated at 37°C for 45 minutes. After that serum from the tube was completely separated and weighed the clot weight. 100 µl of aqueous solutions of different soluble fractions were then added to the microcentrifuge tube. 100 µl of streptokinase was added to the positive control tube and 100 µl of distilled water was added to the negative control tube. All tubes were incubated at 37°C for 90 minutes before the observation of clot lysis. Released fluid was then removed from the tube. Percentage of clot lysis was calculated from the weight changes before and after the clot lysis.

## RESULTS AND DISCUSSION

**Determination of phytoconstituents.** The existence of saponins, tannins, flavonoids, terpenoids, steroids, phlobatannins, carbohydrates, coumarins, alkaloids, proteins, emodins, and alkaloids have been shown qualitatively in table 1.

**Acetylcholinesterase (AChE) inhibitory activity.** Inhibition rate for different extractive or standard was seen to increase at an increasing rate (Table 2). IC<sub>50</sub> value for donepezil (Standard) and crude methanol extract were observed as 3.74±0.74 and 8.43±1.21 µg/ml, respectively. On the other hand, the anticholinesterase activity of all the fractions of crude methanol extracts such as PESF, CSF and AQSF have been observed at 7.125-250 µg/ml concentrations. Among the fractions, the highest activity was found for AQSF with IC<sub>50</sub> value of 5.73±0.59 µg/ml followed by CSF with IC<sub>50</sub> value of 15.19±2.02 µg/ml, while the PESF revealed IC<sub>50</sub> value of 6.36±1.76 µg/ml (Table 2).

**Butyrylcholinesterase (BChE) inhibitory activity.** Inhibition rate (against butyrylcholinesterase) for different extractive or standard was seen to increase at an increasing rate (Table 2). Anti-butyrylcholinesterase activity of standard galantamine (GAL), crude methanolic extract and all the fractions of crude methanol extract such as PESF, CSF and AQSF have been investigated at 7.125-250 µg/ml concentration. Among the fractions of crude methanol extract, PESF showed the

most potent activity with  $IC_{50}$  value of  $6.57 \pm 1.81$   $\mu\text{g/ml}$  which was very close that of GAL (Standard) showing  $IC_{50}$  value of  $3.49 \pm 0.98$   $\mu\text{g/ml}$  (Table 2). On

the other hand, ME, CSF and AQSF showed activity with  $IC_{50}$  value of  $6.61 \pm 1.03$   $\mu\text{g/ml}$ ,  $7.52 \pm 0.87$   $\mu\text{g/ml}$  and  $8.26 \pm 1.61$   $\mu\text{g/ml}$  respectively (Table 2).

**Table 1. Phytochemical test results of different extractives of *G. abutilifolia*.**

Phytochemical tests	Methanol extract	Petroleum ether soluble fraction	Chloroform soluble fraction	Aqueous soluble fraction
Saponins	++	-	++	+
Tannins	+++	+	+	++
Flavonoids	+	+	++	+
Terpenoids	+	-	-	+
Steroids	+++	+++	+++	++
Phlobatannins	++	-	-	++
Carbohydrates	+	-	-	-
Coumarins	+++	-	+	++
Alkaloids	+++	++	-	+
Proteins	+	+	-	-
Emodins	+	-	+++	++
Anthraquinones	-	-	-	-
Anthocyanins	-	-	-	-

- = Absent, + = Mildly present, ++ = Moderately present, +++ = Highly present

**Table 2. Results of acetylcholinesterase inhibitory, butyrylcholinesterase inhibitory and thrombolytic activities of *G. abutilifolia*.**

Name of fractions	Inhibition of acetyl cholinesterase ( $IC_{50}$ $\mu\text{g/ml}$ )	Inhibition of butyrylcholinesterase ( $IC_{50}$ $\mu\text{g/ml}$ )	Thrombolytic activity (% of clot lysis)
ME	$8.43 \pm 1.21$	$6.61 \pm 1.03$	$12.56 \pm 1.98$
PESF	$6.36 \pm 1.76$	$6.57 \pm 1.81$	$18.65 \pm 1.21$
CSF	$15.19 \pm 2.02$	$7.52 \pm 0.87$	$25.75 \pm 1.62$
AQSF	$5.73 \pm 0.59$	$8.26 \pm 1.61$	$16.16 \pm 0.98$
Donepezil	$3.74 \pm 0.78$	-	-
Galanthamine	-	$3.49 \pm 0.98$	-
SK	-	-	$69.76 \pm 2.10$
Blank	-	-	$6.04 \pm 0.88$

Values are mean of triplicate experiments and represented as mean  $\pm$  STD. AChE = acetylcholinesterase, BChE = butyrylcholinesterase,  $IC_{50}$  = Inhibitory concentration 50, AD = Alzheimer's disease, ACh = acetylcholine, BCh = butyrylcholine, GAL = galanthamine, PESF = Petroleum ether soluble fraction, CSF = Chloroform soluble fraction, AQSF = Aqueous soluble fraction, ME = Methanol extract.

**Thrombolytic activity.** After treating with 100  $\mu\text{l}$  SK, a positive control (30,000 I.U.) to the clots along with 90 minutes of incubation at  $37^\circ\text{C}$ , showed

69.76% clot lysis (Table 2). Clots when treated with 100  $\mu\text{l}$  of sterile distilled water (negative control) showed only negligible clot lysis (6.04%). After

treatment of clots with 100 µl of aqueous solutions of ME, PESF, CSF and AQSF have shown mild clot lysis i.e., 12.56%, 18.65%, 25.75%, and 16.16% respectively as shown in table 2, but the mean percentage of clot lysis was more than that of water.

In regulation of cognitive functions one of the most significant neurotransmitter systems is central cholinergic system and the loss of cholinergic neuronal activity causes neurodegenerative disorders. Therefore, increasing central cholinergic activity by inhibiting cholinesterase enzymes is currently the main way of preventing neurodegenerative disorders.<sup>20, 21</sup>

One of the most widely used anti-cholinesterase drug galantamine is derived from plant sources.<sup>22</sup> Nicotinic and muscarinic receptor-modulating properties of galantamine contribute to the antipsychotic effect and development of cognitive dysfunction. Available drugs which are used to treat Alzheimer's disease are mainly the inhibitors of cholinesterase enzymes.<sup>23,24</sup> Our study results demonstrated that all fractions of the plant *G. abutilifolia* have high inhibitory activity against AChE in comparison to the standard donepezil. Similar activities was reported previously by Moniruzzaman *et al.*<sup>25</sup> for aqueous extract of *Phyllanthus acidus*.<sup>25</sup> The comparison between IC<sub>50</sub> values of *P. acidus* and *G. abutilifolia* revealed that *G. abutilifolia* has greater AChE inhibitory activity than *P. acidus*.

It has been reported that combined inhibition of AChE and BChE might improve the signs and symptoms of AD due to the key role of BChE in hydrolysis of acetylcholine.<sup>24</sup> According to our results, the plant extracts have high inhibitory activity against BChE in comparison with the standard galanthamine.

We also determined the thrombolytic activity of *G. abutilifolia* extract and different fractions by percentage of clot lysis using streptokinase as positive control. In comparison with negative and positive control the plant extracts showed negligible clot lysis activity.

## CONCLUSIONS

This study results showed that the crude methanol extract and other soluble fractions obtained from methanolic extract of leaves of *G. abutilifolia* contained saponins, tannins, flavonoids, terpenoids, steroids, phlobatannins, proteins, carbohydrates, coumarins etc. Crude extract and its fractions showed significant anti-cholinesterase activity. However, mild thrombolytic activity was discerned by the methanol extract of leaf of *G. abutilifolia* and its different fractions. Because of the presence of significant anticholinesterases activity of this plant, it could be a potential source of compounds to lead the development of drugs which may prevent diseases caused by neurological imbalance.

## Competing interests

The authors declare that they have no competing interests.

## REFERENCES

1. Adewusi, E.A., Moodley, N., and Steenkamp, V. 2010. Medicinal plants with cholinesterase inhibitory activity: A Review. *African J. Biotechnol.* **9**, 8257-8276.
2. Chiba, Tomohiro, Nishimoto, Ikuo, Aiso, Sadakazu, and Matsuoka, Masaaki. 2007. Neuroprotection against neurodegenerative diseases. *Mol. Neurobiol.* **35**, 55-84.
3. Citron, Martin. 2004. Strategies for disease modification in Alzheimer's disease. *Nat. Rev. Neurosci.* **5**, 677-685.
4. Ahmad, Waqar, Ahmad, Bashir, Ahmad, Manzoor, Iqbal, Zafar, Nisar, Muhammad, and Ahmad, Mansoor. 2003. *In vitro* inhibition of acetylcholinesterase, butyrylcholinesterase and lipoxygenase by crude extract of *Myricaria elegans* Royle. *J. Biol. Sci.* **11**, 1046-1049.
5. Lahiri, D.K., Rogers, J.T., Greig, N.H., and Sambamurti, K. 2004. Rationale for the development of cholinesterase inhibitors as anti-Alzheimer agents. *Curr. Pharm. Des.* **10**, 3111-3119.
6. Bahrani, H., Mohamad, J., Paydar, M.J., and Rothan, H.A. 2014. Isolation and characterisation of acetylcholinesterase inhibitors from *Aquilaria subintegra* for the treatment of Alzheimer's disease (AD). *Curr. Alzheimer Res.* **11**, 206-214.

7. Rafe, M.R. 2017. A review of five traditionally used anti-diabetic plants of Bangladesh and their pharmacological activities. *Asian Pacific J. Trop. Med.* **10**, 933-939.
8. Rahman, M. Atiar, Sultana, Rabeya, Bin Emran, Talha, Islam, M. Saiful, Rahman, M. Ashiqur, Chakma, Joti Sankhar, Rashid, Harun ur and Hasan, Chowdhury Mohammad Monirul. 2013. Effects of organic extracts of six Bangladeshi plants on *in vitro* thrombolysis and cytotoxicity. *BMC Complement. Altern. Med.* **13**, 13-25.
9. Chopra, Ram Nath, Nayar, Sham Lal, and Chopra, Ishwar Chander. 1956. Glossary of Indian Medicinal Plants. *C SIR*.
10. Joshi, M.C., Patel, M.B., and Mehta, P.J. 1980. Some folk medicines of Dangs, Gujarat state. *Bull. Med. Ethnobot. Res.* **1**, 8-24.
11. Ma, Cuiying, Hong, Jie Zhang, Ghee, Teng Tan, Van Hung, Nguyen, Nguyen, Manh Cuong, Soejarto, D. Doel, and Fong, Harry H.S. 2006. Antimalarial compounds from *Grewia bilamellata*. *J. Nat. Prod.* **69**, 346-350.
12. Grierson, D.S. and Afolayan, A.J. 1999. Antibacterial activity of some indigenous plants used for the treatment of wounds in the Eastern Cape, South Africa. *J. Ethnopharmacol.* **66**, 103-106.
13. Sulieman, M.S. and Eldoma, A.M.A. 1994. Marketing of non-wood forest products (excluding the gum arabic) in the Sudan. Forest products consumption survey: Topic specific study report. no. 14.
14. Obidah, Wilson, Godwin, Julius L., Fate, Joseph Z., and Madusolumuo, Mike A. 2010. Toxic effects of *Grewia mollis* stem bark in experimental rats. *J. Am. Sci.* **6**, 1544-1548.
15. Kupchan, S.M., Uchida, I., Branfman, A.R., Dailey, R.G. and Fei, B.Y., 1976. Antileukemic principles isolated from Euphorbiaceae plants. *Science* **191**, 571-572.
16. Odebiyi, O.O. and Sofowora, E.A. 1991. Phytochemical screening of Nigerian medicinal plants II. *Lloydia J. Nat. Prod.* **41**, 234-246.
17. Fadeyi, M.O., Adeoye, A.O., and Olowokudejo, J.D. 1989. Epidermal and phytochemical studies in the genus *Boerhavia* (Nyctaginaceae) in Nigeria. *Int. J. Crude Drug Res.* **27**, 178-184.
18. Ellman, G.L., Courtney, K.D., Andres, V., and Feather-Stone, R.M. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* **7**, 88-95.
19. Prasad, Sweta, Kashyap, Rajpal S., Deopujari, Jayant Y., Purohit, Hemant J., Taori, Girdhar M., and Daginawala, Hatim F. 2006. Development of an *in vitro* model to study clot lysis activity of thrombolytic drugs. *Thromb. J.* **4**, 4-14.
20. Enz, A., Amstutz, R., Boddeke, H., Gmelin, G., and Malanowski, J. 1993. Brain selective inhibition of acetylcholinesterase: a novel approach to therapy for Alzheimer's disease. *Prog. Brain Res.* **98**, 431-438.
21. Siddiqui, Muhammad F. and Levey, Allan I. 1999. Cholinergic therapies in Alzheimer's disease. *Drugs Futur.* **24**, 417-424.
22. Ago, Yukio, Koda, Ken, Takuma, Kazuhiro, and Matsuda, Toshio. 2011. Pharmacological aspects of the acetylcholinesterase inhibitor galantamine. *J. Pharmacol. Sci.* **116**, 6-17.
23. Massoud, Fadi and Gauthier, Serge. 2010. Update on the pharmacological treatment of alzheimers disease. *Curr. Neuropharmacol.* **8**, 69-80.
24. Decker, Michael, Kraus, Birgit, and Heilmann, Jörg. 2008. Design, synthesis and pharmacological evaluation of hybrid molecules out of quinazolinimines and lipoic acid lead to highly potent and selective butyrylcholinesterase inhibitors with antioxidant properties. *Bioorg. Med. Chem.* **16**, 4252-4261.
25. Moniruzzaman, M., Asaduzzaman, M., Hossain, M.,S., Sarker, J., Rahman, S.M.A., Rashid, M. and Rahman, M.M. 2015. *In vitro* antioxidant and cholinesterase inhibitory activities of methanolic fruit extract of *Phyllanthus acidus*. *BMC Complement. Altern. Med.* **15**, 2-10.