



History:

Received: October 9, 2017
Accepted: December 3, 2017
First Published: December 17, 2017
Collection year: 2017
Confirmation of publication: Published

Identifiers and Pagination:

Year: 2017
Volume: 9
First Page: 17
Last Page: 30
Publisher ID: 10.21065/19204159.9.17
Doi: <http://dx.doi.org/10.21065/19204159.9.17>

Corresponding author:

Hossain Faruquee, Department of Biotechnology and Genetic Engineering, Faculty of Applied Science and Technology, Islamic University, Kushtia-7003, Bangladesh.
E.: faruquee@btge.iu.ac.bd

Citation:

Rezanur Rahman, Tania Islam, Hossain Faruquee*, Sharmin Akhter, and Anwarul Haque. Evaluation of antioxidant, cholinesterase inhibitory properties, and antibacterial potentials of glycomis pentaphylla leaf extract relevant to the treatment of alzheimer's disease. J App Pharm. Vol. 9: 2017.17-30

Original Research Article

EVALUATION OF ANTIOXIDANT, CHOLINESTERASE INHIBITORY PROPERTIES, AND ANTIBACTERIAL POTENTIALS OF GLYCOMIS PENTAPHYLLA LEAF EXTRACT RELEVANT TO THE TREATMENT OF ALZHEIMER'S DISEASE

Rezanur Rahman^{1,a}, Tania Islam^{1,a}, Hossain Md Faruquee^{1,*}, Sharmin Akhter², and Md Anwarul Haque¹

¹ Department of Biotechnology and Genetic Engineering, Faculty of Applied Science and Technology, Islamic University, Kushtia-7003, Bangladesh.

² Department of Applied Nutrition and Food Technology, Faculty of Applied Science and Technology, Islamic University, Kushtia-7003, Bangladesh.

Abstract

There is a tremendous unmet need to discover more potent and safe drugs for the treatment of Alzheimer's disease (AD). Reduced cholinergic activity and oxidative stress have been recognized as a major contributing factor in the pathogenesis of AD. Therefore, inhibition of cholinesterase and oxidation are the two promising strategies in the development of a drug for AD. This study determined the anti-acetylcholinesterase (AChE) activity, anti-butyrylcholinesterase (BChE) activity, DPPH free radical scavenging and antioxidant properties of *Glycomis pentaphylla* (*Rutaceae*). The objective of this study is to measure *G. pentaphylla* anti-AChE, anti-BChE, DPPH free radical scavenging, lipid peroxidation inhibition, antibacterial potentials to find out the MIC (minimum inhibitory concentration) against different pathogenic bacteria. *G. pentaphylla* leaf extract (GPEx) is exploited in the presented research to estimate its anticholinesterase, antioxidant potentials, and antibacterial properties. The cholinesterase inhibitory properties was quantified by modified Ellman method, and antioxidant potentials were evaluated by the assay of radical scavenging, and inhibition of lipid peroxidation. The antibacterial activity and minimum inhibitory concentration (MIC) were determined using agar well diffusion method. The methanolic extract exhibited significant dual acetylcholinesterase (AChE) and butyryl cholinesterase (BChE) effect. The IC₅₀ values of AChE and BChE were 325.1±0.91, and 42.14±3.31 µg/ml. Furthermore, the extract showed radical scavenging ability, and lipid peroxidation inhibitory effect. The IC₅₀ values of the extract for DPPH and hydroxyl free radical scavenging, and lipid peroxidation inhibition assay were 95.6±0.68, 198.0±1.39, and 288.7±0.91 µg/ml, respectively. Phytochemical screening of the extract revealed the presence of significant total phenolics and flavonoids contents. Additionally, the extract showed good effect with the zone of inhibition ranging 12–16 mm in diameter against *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*. The tested sample reflects potential antioxidative and anticholinesterase inhibitory potentiality which may warrant its effectiveness in the treatment of AD along with good antibacterial properties.

Keywords: Alzheimer's disease; *Glycomis pentaphylla*; anticholinesterase; anti-oxidant; antibacterial; radical scavenging; lipid peroxidation.

***Corresponding author:** Hossain Md Faruquee, Department of Biotechnology and Genetic Engineering, Faculty of Applied Science and Technology, Islamic University, Kushtia-7003, Bangladesh. E.: faruquee@btge.iu.ac.bd

1. INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by gradual memory loss, cognitive deficit and behavioural aberration, and the most predominant cause of dementia in the elderly having age more than 65 years[1–3]. It has been estimated that around 35 million people are now afflicted by the AD, and currently it is the fourth leading cause of death in the elderly person [3,4] and it is inferred that it may be reached to 65.7 million by 2030 [2].

Funding:

The authors received no direct funding for this research.

Competing Interests:

The authors declare no competing interests

Additional information is available at the end of the article.

Many factors are hypothesized to be involved in the pathophysiology of the AD such as oxidative stress, deposition of senile plaques, the formation of neurofibrillary tangles of the microtubule-associated protein tau, and profound deficits in cholinergic transmission is to be the hallmarks of neuropathophysiology of AD [2,5–8]. Understanding the etiology and pathogenesis of AD has been progressed, effective drugs remain limited yet. Reduced cholinergic activity and oxidative stress have been recognized as a major contributing factor in the pathogenesis of AD [2,9]. The deficit of cholinergic neurons and the associated decrease in levels of acetylcholine (ACh) has been found to correlate well with the cognitive impairment seen in AD patients [10].

The cognitive impairment observed in the AD is correlated with the loss of cholinergic neurons and its associated acetylcholine reduction [2,10]. The undue breakdown of acetylcholine by the increased activity of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) is implicated in the progression of the AD. Thus, inhibition of these two enzymes are the main strategy followed for AD and currently majority of drugs such as tacrine, donepezil, rivastigmine and galantamine are available for AD treatment are cholinesterase inhibitors (ChEI) despite it is only used for mild to moderate AD and could not reverse the disease progression simply they improve the symptoms [7,11,12]. In addition, these available drugs have adverse effects such as hepatotoxicity and gastrointestinal disturbances [13]. Therefore, in recent years much attention has been drawing to develop safe, cost-effective and active anti-cholinesterase from natural sources.

It has been reported that the reactive oxygen species (ROS) and other free radicals generated from activated neutrophils and macrophages during oxidative stress play an important role in the pathogenesis of AD [14]. A number of studies indicated that oxidative stress and A β protein are linked each other because A β induces oxidative stress in vivo and in vitro [15,16] and oxidative stress increases the production of A β [17,18]. Therefore, antioxidant therapy has been suggested to be successful in improving cognitive function and behavioural deficits in patients with mild to moderate AD [19]. Natural products are the important source of antioxidants which can be beneficial for AD therapy.

The emergence of multi-drug resistance pathogenic bacterial strains is a major medical concern globally and these antibiotics have an adverse reaction such as hypersensitivity, immune-suppression and allergic reactions lead to a search for new and active antibacterial compounds to combat infections against these pathogenic bacteria [19–21]. Therefore, plants or natural products would be an enormous resource to discover new compounds or drug-like molecule which will be helpful for antibiotic discovery and development.

The *G. pentaphylla* (Orange berry) is an evergreen shrub and it grows usually 4m tall and widely distributed throughout the world including various parts of Bangladesh [22]. It is widely distributed in Bangladesh, India Malaysia and Southern China to the Philippine Islands but this plant is native to eastern, southern, and southeastern Asia and north-eastern Australia [23]. Traditionally, this plant is used to reduce fever, liver complications, various intestinal parasites [23], and toothache and bleeding [22], all forms of cancers, hepatic disorder, rheumatic fever [24]. This traditional use should be supported by the scientific evaluations. In addition, it is reported that *G. pentaphylla* possessed anti-hepatocellular carcinoma activity, hepatoprotective activity, and antibacterial activity [23].

Therefore, the present study was carried out with an aim to explore the cholinesterase (AChE and BChE) inhibitory activity and antioxidant properties of *G. pentaphylla* as well as antibacterial activity with particular emphasis in order to treat AD.

2. MATERIALS AND METHODS

2.1 Plant material

The plant leaves of *G. pentaphylla* were collected from the Islamic University premises, Kushtia, Bangladesh in February 2017 by the authors and collected plant sample was identified by an expert taxonomist. A voucher specimen has been deposited at the Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia, Bangladesh. The freshly collected plants were

cleaned to remove dust.

2.2 Extraction of plant material

The leaves of *G. pentaphylla* plant were air dried and ground. The powdered plant (500 g) was subjected to extraction with methanol at room temperature for 7 days with sporadic shaking. The extractive solution was filtered and concentrated under vacuum in a rotary evaporator at 45 °C to yield 17 g of the crude extract (GPEX).

2.3 Enzyme sources

Adult Long-Evans rat (125-150g) brain homogenate was used as the source of acetylcholinesterase enzyme and human blood was used as the source of butyrylcholinesterase enzyme. The rats were purchased from the animal research branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The approval for this study was taken from the Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia, Bangladesh.

2.4 Chemicals

The chemicals used in this study were analytical grade. DPPH (2, 2'-diphenyl-1-picrylhydrazyl), aluminum chloride, potassium ferricyanide, ascorbic acid, Folin-Ciocalteu reagent, Tris-HCl and triton X-100 were obtained from Sigma-Aldrich (India). Gallic acid was obtained from Wako Pure Chemical Company Ltd., Japan. 5,5'-dithio-bis-(2-nitro) benzoic acid (DTNB), acetylthiocholine iodide (ATCI), S-butyrylthiocholine, galantamine, and donepezil were obtained from Sigma-Aldrich, Japan. Unless otherwise specified, all other chemicals were of analytical grade.

2.5 Determination of total phenolic content

The concentration of total phenolics of GPEX was determined by the method Folin-Ciocalteu [25]. Briefly, a 0.5 ml of plant extract was mixed to 2.5 ml of Folin-Ciocalteu reagent which was diluted 10 times with water and 2.5 ml of sodium carbonate (7.5%) solution and the reaction mixture was allowed to incubate for 20 min at room temperature (25°C) to complete the reaction thereafter the absorbance of the reaction mixture was measured at 760 nm by spectrophotometer. To quantify the total phenolics, the Gallic acid standard curve was exploited and the results were represented as mg of Gallic acid equivalent (GAE)/g of dried extract.

2.6 Determination of total flavonoid content

The aluminium chloride colourimetric method with slight modification was employed to determine the total flavonoid content of GPEX [26]. In short, 1.0 ml of each concentration of crude extract of GPEX was added with 3.0 ml of methanol, 0.2 ml of 10% AlCl₃, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water was added to the resulting mixture which was then incubated at room temperature for 30 min to complete the reaction. At 420 nm, the absorbance was recorded of the reaction mixture by spectrophotometer. Gallic acid was used as standard and the results were expressed as milligrams of gallic acid equivalents per gram of dried fraction [2].

2.7 Determination of DPPH radical scavenging activity

DPPH assay was used to assess the free radical scavenging activity of the extract according to the method described by Choi et al. with slight modifications [27]. Briefly, 3 ml of a methanol solution of DPPH and 2 ml of a methanol solution of plant extract or reference standard catechin at various concentrations was mixed into the test tube and allowed to incubate at room temperature (25°C) for 30 min in dark place to complete the reaction. The absorbance of the solution was measured out by spectrophotometer at 517 nm. DPPH free radical scavenging ability (%) was calculated by using the formula:

$$[(\text{An absorbance of control} - \text{An absorbance of sample}) / \text{An absorbance of control}] \times 100$$

2.8 Determination of hydroxyl radical scavenging activity

The hydroxyl free radical scavenging activity of the methanol crude extract of GPEx was determined by the method as described by Elizabeth and Rao with a slight modification [28]. At various concentrations the plant extract or reference compound was mixed with a reaction mixture contained, in a final volume of 1 ml: 2-deoxy-2-ribose (2.8 mM); KH₂PO₄- KOH buffer (20 mM, pH 7.4); FeCl₃ (100 µM); EDTA (100 µM); H₂O₂ (1.0 mM); and catechin (100 µM). The mixture was then incubated for 1 h at 37 °C and 0.5 ml of the reaction mixture was heated at 90 °C for 15 min after addition of 1 ml of 2.8 % TCA and 1 ml of 1 % aqueous TBA to develop the colour. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution [2]. Hydroxyl radical scavenging ability (%) was calculated by using the formula:

$$[(\text{An absorbance of control} - \text{An absorbance of sample}) / \text{An absorbance of control}] \times 100$$

2.9 Determination of cholinesterase (ChE) inhibitory activities

The acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory assay were performed according to the colourimetric Ellman method with slight modification [2,29]. For the AChE enzyme source, rat brain was homogenized in a homogenizer with 5 volumes of a homogenization buffer [10 mM Tris-HCl (pH 7.2), which contained 1 M NaCl, 50 mM MgCl₂ and 1 % Triton X-100], and centrifuged at 10,000 g for 30 min. The resulting supernatant was used as an enzyme source. For the BChE enzyme source, human blood from anonymous healthy men subject was provided by the Islamic University Medical Center, Kushtia, Bangladesh and collected in EDTA treated (1 mg/ml) glass tubes. The tubes were centrifuged at 2000 g for 10 min to eliminate the red blood cells. The resulting plasma (supernatant) was then recuperated, diluted (1/200) with 50mM phosphate buffer (pH 7.4) and was used immediately for studying butyrylcholinesterase activity. The rates of hydrolysis by acetylcholinesterase were monitored spectrophotometrically. Each extract or standard (500 µl) was mixed with an enzyme solution (200 µl) and incubated at 37 °C for 15 min. Absorbance at 405 nm was read immediately after adding an Ellman's reaction mixture [3.5 ml; 0.5 mM acetylthiocholine iodide (ATCI), 1 mM DTNB in a 50 mM sodium phosphate buffer (pH 8.0)] with the incubated reaction mixture. The hydrolysis of ATCI was monitored by the formation of yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholine. 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. Percentage of inhibition of AChE/BChE was determined by comparison of rates of reaction of samples relative to blank sample using the formula (E-S)/E × 100, where E is the activity of enzyme without test sample and S is the activity of the enzyme with the test sample. The experiments were done in triplicates. Donepezil (anticholinesterase drug) was used as a standard. Assessment of BChE inhibition was performed as described above except that the enzyme solution was 50 µl and acetylthiocholine iodide was replaced by butyrylthiocholine iodide. Galantamine was used as positive control. The percentage inhibition of butyrylcholinesterase activity was calculated using the same formula as mentioned above for acetylcholinesterase activity.

2.9 Antibacterial Susceptibility Test and Minimum Inhibitory Concentration (MIC) Determination Test

To assess the antibacterial potentials of the crude methanol extract of GPEx, in vitro antibacterial susceptibility test was performed by agar well diffusion technique [30]. Human pathogenic bacterial culture (0.1%) was swabbed uniformly over the surface of solid nutrient agar media using sterile cotton swab under aseptic condition. After complete drying of the media, 4 mm small bore is filled with the crude extracts at the different concentration (0.5-10 mg/ml) with appropriate control, the inoculated plates were allowed for 24 h incubation. Finally, inhibition zones formed around the well were measured using a calibrated scale within millimeter. All the tests for antibacterial activity were carried out in triplicate.

The methanol crude GPEx extracts were subjected to serial dilution technique (0.5-10mg/ml) to prepare various concentrations for the determination of MIC. The various diluted extracts were applied to wells posed in the nutrient agar media containing inoculums and incubated for 24 h. After

overnight incubation, the growth of the test organisms was observed to determine the MIC.

2.10 Statistical analysis

The data were analyzed by one-way ANOVA followed by Tukey's test to estimate significant differences between the test and control groups, and IC₅₀ was determined with GraphPad Prism Data Editor for Windows, Version 5.0 (GraphPad Software Inc., San Diego, CA). Values were expressed as a mean $\pm$ Standard error of the mean ($\pm$ SEM). * $p < 0.05$ were considered as statistically significant.

3. RESULTS

3.1 Antioxidant activity

3.1.1 Total phenolics and flavonoid contents of *G. pentaphylla*

This research aims to evaluate the antioxidative and anticholinergic effects of the GPEx. For assessing the antioxidative effects, total phenolic and flavonoid contents of GPEx, DPPH radical scavenging capacity, hydroxyl radical scavenging effect and lipid peroxidation inhibitory effects were screened. Total phenolic and flavonoid contents were calculated using the standard curve for Gallic acid. Phenolic compounds have been reported to show anti-A β (amyloid β protein) aggregation effects in AD indicating the pivotal role of phenolics in preventing the Alzheimer's progression [2,10,31]. Flavonoids are known to serve as potential antioxidants for their radical scavenging, an ion chelating and lipid peroxidation inhibiting properties [10,32] preventing or slowing the progression of the AD by interfering the generation of amyloid- β peptides and its polymerization into neurotoxic oligomeric aggregates thereby reducing aggregation of tau proteins [31]. Higher content of total phenolic (28.23 mg of GAE/g of dried extract) and flavonoid (181.76 mg of GAE/g of dried extract) in TGEx, therefore, may carry an immense importance to evaluate its role in controlling AD.

3.1.2 DPPH radical scavenging activity of *G. pentaphylla*

Radical scavenging activity is very important to prevent the deleterious role of free radicals in AD [2,33]. DPPH is a stable free radical contains an odd electron and DPPH antioxidant assay is based on the ability of DPPH to decolourize in the presence of antioxidants. Decolorization of DPPH after accepting an electron donated by an antioxidant compound is quantitatively measured by the change in absorbance and % of scavenging activity. DPPH radical scavenging assay for *G. pentaphylla* is shown in Figure 1(A). The results of this study showed very significant DPPH scavenging activity with an IC₅₀ value of 95.6 ± 0.68 μ g/ml and found to be comparable with that of the standard antioxidative agent ascorbic acid showing IC₅₀ value 1.97 ± 0.27 μ g/ml.

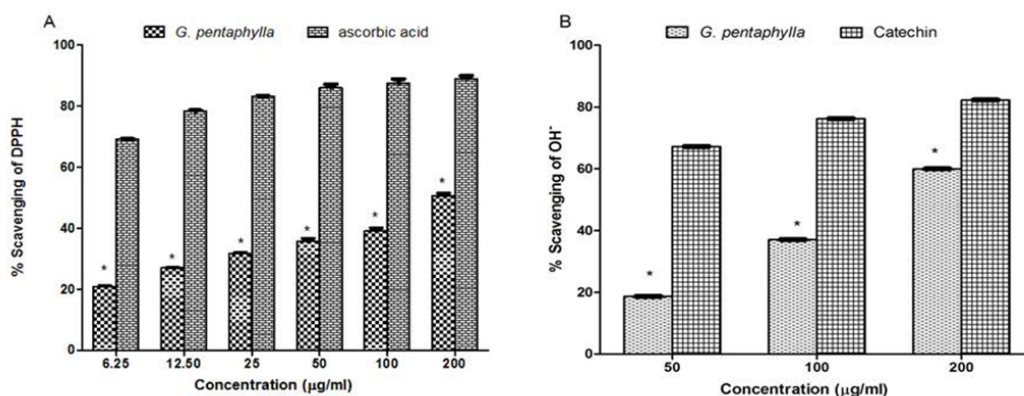


Figure 1: Radical scavenging activity of *G. pentaphylla* extract compared with the standard. (A) Percent of DPPH radical scavenging by different concentrations of the extract and reference standard ascorbic acid as assessed by spectrophotometric method using DPPH free radicals. (B) Percent of hydroxyl radical scavenging by different concentrations of the extract and reference standard (+)-catechin as assessed by the Fenton-reaction initiated deoxyribose degradation

method. Notes: Results represent mean $\pm$ SEM ($n = 3$). * $p < 0.05$ significantly different as compared with control.

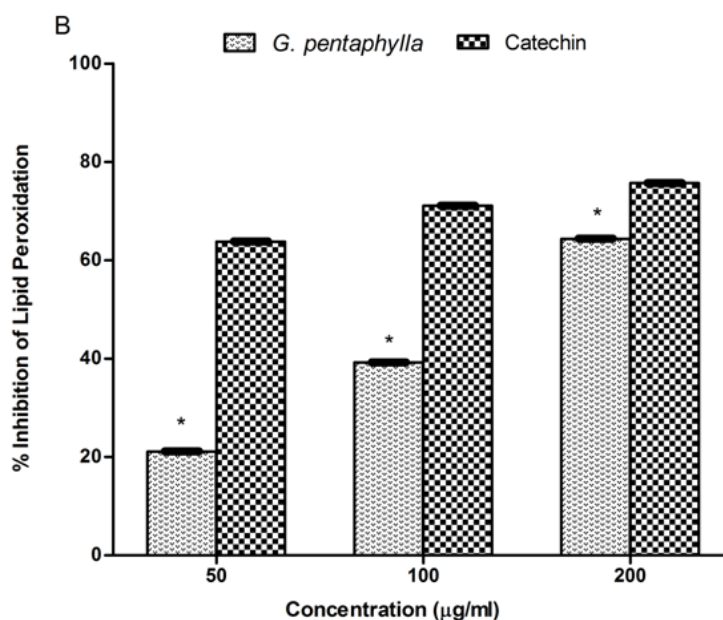


Figure 2: Percentage of lipid peroxidation inhibition at different concentrations of *G. pentaphylla* extract and the reference standard (+)-catechin.

Notes: Results represent mean $\pm$ SEM ($n = 3$). * $p < 0.05$ significantly different as compared with control.

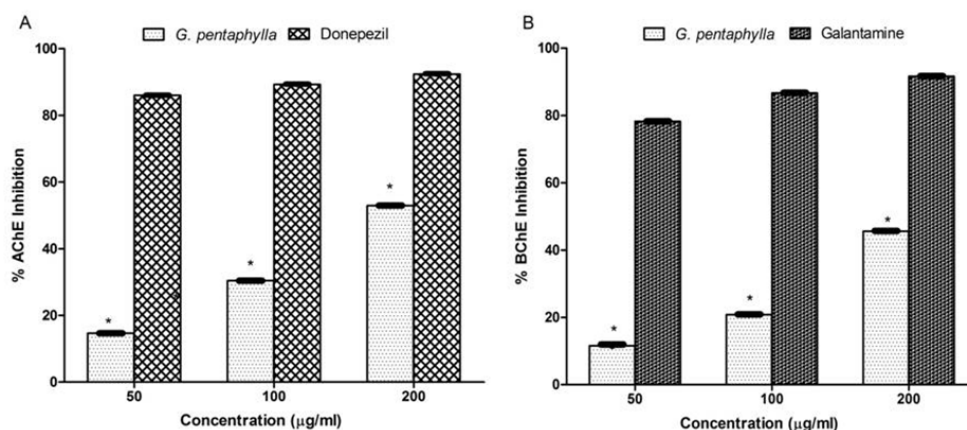


Figure 3: Percentage of inhibition of acetylcholinesterase (A) and butyrylcholinesterase (B) activity at different concentrations of *G. pentaphylla* extract and the reference standard donepezil and galantamine, respectively.

Notes: Results represent mean $\pm$ SEM ($n = 3$). * $p < 0.05$ significantly different as compared with control.

Table1. IC50 value of the test extract and standards obtained in the radical scavenging and enzyme inhibitory activity assays

Sample	IC50 (µg/ml)				
	DPPH radical scavenging	Hydroxyl radical scavenging	Inhibition of lipid peroxidation	AChE	BChE
Ascorbic Acid	1.97±0.27	-	-	-	-
(+)-Catechin	-	25.44±0.63	19.50±0.56	-	-
Donepezil	-	-	-	85.13±0.57	-
Galantamine	-	-	-	-	11.37±0.56
Extract	95.6±0.68	198.0±1.39	288.7±0.91	325.1±0.91	42.14±3.31

Note: Values are expressed as a mean ± SEM ($n = 3$).

Table2. Minimum Inhibitory Concentration (MIC) (mg/mL) of methanolic extract of *G. pentaphylla* L. leaves.

Conc. (mg/ml)	Amount in µg	St (DIZ)	Pa (DIZ)	Sa (DIZ)	Ec (DIZ)	Ec14 (DIZ)
.5	50	NG	SG	NG	NG	NG
1	100	NG	SG	NG	NG	NG
2	200	NG	12.01±0.06	NG	NG	NG
3	300	12.1±0.15	12.06±0.06	SG	NG	NG
4	400	12.1±0.20	16.19±0.36	12.1±0.05	NG	NG
5	500	12±0.16	16.20±0.41	12.18±0.09	NG	NG
6	600	14.10±0.06	12±0.17	14.03±0.09	NG	NG
7	700	14.1±0.15	14.05±0.10	12.09±0.04	NG	NG
8	800	11.08±0.04	SG	12.10±0.05	NG	NG
9	900	12.04±0.04	12.12±0.06	14.04±0.04	NG	NG
10	1000	14.1±0.05	12.01±0.08	15.13±0.06	NG	NG
Control	-	NG	NG	NG	NG	NG

Note: St: *Salmonella typhi*; Pa: *Pseudomonas aeruginosa*; Sa: *Staphylococcus aureus*; Ec: *Escherichia coli*; Ec14: *Escherichia coli* 14; SG: Slight growth; NG: No growth. Conc.: Concentration; DIZ: Diameter of Zone of Inhibition in mm. Note: Values are expressed as a mean ± SEM ($n = 3$).

3.1.3 Hydroxyl radical scavenging activity

Hydroxyl radicals are the major reactive oxygen species that severely damage the neurons in AD [34]. In the hydroxyl radical scavenging activity, the ability of GPEx to remove the formed hydroxyl radical in solution was evaluated quantitatively by Fenton-reaction initiated deoxyribose degradation assay and the result has been shown in Figure 1(B).

The test sample significantly scavenged the hydroxyl radicals generated in the reaction in a dose-dependent manner with an IC50 value of 198.0±1.39 µg/ml which was significant compared to that (25.44±0.63 µg/ml) of the reference standard (+)-catechin. Removal of hydroxyl radicals from the reaction mixture decolourized the pink chromogen which was quantitatively measured by the change in absorbance at 532 nm. The results demonstrate very promising hydroxyl radical scavenging potentials for therapeutic uses. because the cut-off value for antioxidative compounds is 1000 µg/ml [35].

3.1.4 Lipid Peroxidation inhibition activity of *G. pentaphylla*

Reactive oxygen species produced by ultraviolet light, ionizing radiation, chemical reactions, and metabolic processes have numerous pathological effects, such as causing lipid peroxidation, protein peroxidation, DNA damage, and cellular degeneration related to a variety of diseases including Alzheimer's disease [36–38]. In the lipid peroxidation inhibition activity, the activity of GPEx against non-enzymatic lipid peroxidation in rat brain homogenate was evaluated. Addition of Fe²⁺-ascorbate to the brain homogenate caused an increase in lipid peroxidation which was quantitatively measured by the change in absorbance at 532 nm and % of inhibition activity of different concentrations were shown in Figure 2. The GPEx showed that the inhibition of lipid peroxidation was correlated with the increasing concentration of the extract. The IC₅₀ values of the methanol extract and the reference standard (+)-catechin were 288.7±0.91 and 19.50±0.56 µg/ml, respectively.

3.2 Cholinesterase inhibitory activity

3.2.1 AChE inhibitory activity of *G. pentaphylla*

The inhibitory activity of GPEx against rat brain AChE was determined by widely accepted modified Ellman's method [29] which estimates the level of AChE using acetylthiocholine iodide (substrate) and DTNB. The inhibitory activity of GPEx was increased with the increasing concentration and showed for 50, 100 and 200 µg/ml concentration of GPEx provided 14.63±0.026 %, 30.4±0.026, and 52.92±0.02 % AChE inhibition respectively, and the IC₅₀ was 325.1±0.91 µg/ml (Figure 3(A)). This concentration-dependent inhibition of GPEx was significant ($p < 0.05$) compared to control but the IC₅₀ values were higher than that (IC₅₀ = 85.13±0.57) of the reference drug donepezil (Table1).

3.2.2 BChE inhibitory activity of *G. pentaphylla*

All the cholinesterase inhibitors currently licensed for the AD, inhibit AChE and, to a varying extent, BChE [11,39]. This research has studied the inhibitory actions of GPEx against BChE using the similar methods used for AChE. Similar to AChE inhibitory activity, the GPEx also had very good inhibitory actions against BChE, 11.6±0.35, 20.83±0.05, and 45.61±0.08 % BChE inhibition respectively (Figure 3(B)) with an IC₅₀ value of 42.14±3.31 µg/ml whereas galantamine showed the IC₅₀ value of 11.37±0.56 µg/ml (Table1).

Our results demonstrate a dual activity of *G. pentaphylla*. However, methanol extract showed higher activity against BChE compared with AChE.

3.3 Minimum Inhibitory Concentrations (MIC)

A total of five bacterial species were examined for antibacterial screening with methanolic extract of GPEx. It has been observed that the extract under study only effective against *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* with the zone of inhibition ranges from 12-14 mm, 12-16mm, and 12-15mm in diameter respectively., and found ineffective against *Escherichia coli*, *Escherichia coli* 14 . The findings are summarized in Table 2.

The highest MIC of *G. pentaphylla* was found against *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* with 500 microgram (5mg/ml).

4. DISCUSSION

AD is the degenerative neuro disorder and prime cause of death in elderly persons, and oxidative imbalance, cholinergic dysfunction is considered to be the major causative factors in the pathogenesis of AD [5,6,8]. Thus, a drug candidate that would inhibit the oxidative stress and cholinergic dysfunction would be useful for the drug development and treatment of AD. The search for new drug molecules with increased effectiveness, safety and lesser adverse effect for neuro-protection would be feasible and possible from the natural sources such as plant extracts and plant-originated compounds; for instance, plant natural alkaloid galantamine was first discovered from

Galanthus sp., which has been approved by FDA in 2001 for treatment of AD.

G. pentaphylla has been reported to possess anti-hepatocellular carcinoma activity, hepatoprotective activity, antibacterial activity [23]. Our findings indicate that *G. pentaphylla* also possesses acetylcholinesterase and butyrylcholinesterase inhibitory properties with antioxidative activities. In AD patient the cholinergic neurotransmitter is reduced, the acetyl choline breakdown prevention through the inhibition of AChE activity, and increased concentration of acetylcholine enhances the communication between the nerve cells will give a therapeutic benefit in a patient with AD [3]. It has been revealed in our study that the methanol crude extract demonstrated inhibitory activity against both the cholinesterases, AChE and BChE. The IC₅₀ value of this extract were found 325.1±0.91 µg/ml, 42.14±3.31 µg/ml respectively, indicating that methanolic extract has higher specificity for BChE. Dual inhibition of AChE and BChE might more improve the signs and symptoms of AD. However, our results demonstrated the dual anticholinesterase activity of *G. pentaphylla*.

The brain consumes a lot of oxygen and contains easily oxidizable fatty acids as well as the low amount of antioxidants, therefore, more susceptible to free radical attacks [40]. Several assays such as DPPH, hydroxyl radical scavenging activity, and lipid peroxidation assay were employed to evaluate the antioxidant potentials of *G. pentaphylla* because a single assay is insufficient to reflect the antioxidant property of a compound. The stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples [1,41]. The DPPH radical scavenging ability of the *G. pentaphylla* extracts revealed that scavenged DPPH radicals significantly in a dose dependent manner (Fig. 1).

Aβ protein, main hallmark of AD, triggers the generation of free radicals, which leads to the oxidation of protein, lipid and DNA [16,42]. Hydroxyl radicals (OH•) are the major reactive oxygen species that severely damage the neurons in AD. The hydroxyl radical scavenging abilities of the extracts of the plant are presented in Fig. 2. Results revealed that the methanol extract has potential hydroxyl radical scavenging activity with an IC₅₀ of 198.0±1.39 µg/ml, which appeared to higher to that of the reference standard catechin whose IC₅₀ was found to be 25.44±0.63 µg/ml under the same condition.

The ChE inhibitory property and antioxidant activity of *G. pentaphylla* were supported by the presence of endogeneous polyphenols and flavonoids. Polyphenols are the most abundant antioxidants in the plant kingdom. Recent studies have shown that polyphenols are able to cross the blood-brain barrier [43,44] and play role in neuroprotection [45]. The polyphenolic compound is believed to scavenge free radicals and activate antioxidant enzymes [46] and the antioxidant activity of phenolics has been attributed from their redox properties [47]. Flavonoids are the secondary metabolites of plant and have good antioxidant and neuroprotective potentials [48]. Our results indicate that this extract derived from *G. pentaphylla* is a noteworthy source of phenolics and flavonoids which may contribute to the inhibition of AChE, BChE and oxidative activities.

The zone of inhibition observed of GPEx against *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* were in the range of 12-16 mm against, while the two strain *E. coli* and *E. coli 14* were found not affected by the extract. It was found at the antibacterial activity of the extracts correlated strongly with the DPPH activity due to the availability of the antioxidative compounds to exert different inhibitory effect against tested organisms [49]. The well-diffusion technique is useful for the qualitative antibacterial screening and it is also important for the quantitative minimum inhibitory concentration (MIC) determination. The MIC is referred to as a gold standard for determining the susceptibility of organisms against antimicrobial [50]. A lower MIC indicates the necessity of less amount of that compound/drug to inhibit the growth of the microorganisms.

5. CONCLUSION

The results obtained from this study clearly indicated that the methanol extract of *G. pentaphylla*, possibly due to its polyphenolic compounds, displayed a combination of cholinesterase inhibitory activities as well as antioxidant properties and thus may be useful for an effective and safe treatment for Alzheimer's disease. Besides, it showed promising antibacterial activity. This study provides a

basis to the claim traditional use for the treatment of nervous system disorders including the AD and infectious disease. Thus, the results of presented work support the traditional use of *G.pentaphylla*.

ACKNOWLEDGEMENT

We are grateful to the Department of Biotechnology and Genetic Engineering, Islamic University, Bangladesh for providing facilities of this research work.

Authors' Contributions

MRR, TI, and HMF conceived and designed the experiments; MRR, TI, SA, and MA.H carried out the anticholinesterase and antioxidant tests. MRR, TI, HMF conducted the antibacterial assay experiments; MRR wrote the manuscript; MRR, and TI performed statistical analysis. All authors read and approved the final manuscript.

Competing interest

The authors declare that they have no conflict of interest.

Funding

The authors received no direct funding for this research.

Abbreviations

AD	Alzheimer's disease
DPPH	1,1-Diphenyl 2-picrylhydrazyl
AChE	Acetylcholinesterase
BChE	Butyrylcholinesterase
EDTA	Ethylenediaminetetraacetic acid
TCA	Trichloroacetic acid
TBA	Thiobarbituric acid
DTNB	5,5' dithio-bis (2-nitrobenzoic acid)
Tris-HCl	tris(hydroxymethyl)aminomethane hydrochloride
MIC	Minimum Inhibitory Concentration

REFERENCES

1. Uddin, M. J.; Abdullah-Al-Mamun, M.; Biswas, K.; Asaduzzaman, M.; Rahman, M. M. Assessment of anticholinesterase activities and antioxidant potentials of *Anisomeles indica* relevant to the treatment of Alzheimer's disease. *Orient. Pharm. Exp. Med.* **2016**, *16*, 113–121, doi:10.1007/s13596-016-0224-z.
2. Uddin, J.; Alam, M. N.; Biswas, K.; Rahman, A. In vitro antioxidative and cholinesterase inhibitory properties of *Thunbergia grandiflora* leaf extract. *Cogent Food Agric.* **2016**, *955*, 1–11, doi:10.1080/23311932.2016.1256929.
3. Singhal, A.; Bangar, O.; Naithani, V. Medicinal plants with a potential to treat Alzheimer and associated symptoms. *Int. J. Nutr. Pharmacol. Neurol. Dis.* **2012**, *2*, 84, doi:10.4103/2231-0738.95927.
4. Abou-Donia, A. H.; Darwish, F. A.; Toaima, S. M.; Shawky, E.; Takla, S. S. A new approach to develop a standardized method for assessment of acetylcholinesterase inhibitory activity of different extracts using HPTLC and image analysis. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2014**, *955–956*, 50–57, doi:10.1016/j.jchromb.2014.02.013.
5. Masters, C. L.; Selkoe, D. J. Biochemistry of amyloid β -protein and amyloid deposits in Alzheimer disease. *Cold Spring Harb. Perspect. Med.* **2012**, *2*, 1–24, doi:10.1101/cshperspect.a006262.
6. Wang, J.-Z.; Xia, Y.-Y.; Grundke-Iqbal, I.; Iqbal, K.; Vingtdeux, V.; Davies, P. Tau in Alzheimer disease and related tauopathies. *Front. Neurol.* **2011**, *4*, 122,

doi:10.3389/fneur.2013.00122.

7. Tanaka, T.; Kazui, H.; Morihara, T.; Sadik, G.; Kudo, T.; Takeda, M. Post-marketing survey of donepezil hydrochloride in Japanese patients with Alzheimer's disease with behavioral and psychological symptoms of dementia (BPSD). *Psychogeriatrics* **2008**, *8*, 114–123, doi:10.1111/j.1479-8301.2008.00250.x.
8. Terry Jr, A. V.; Buccafusco, J. J. The Cholinergic Hypothesis of Age and Alzheimer's Disease- Related Cognitive Deficits: Recent Challenges and Their Implications for Novel Drug Development. *J. Pharmacol. Exp. Ther.* **2003**, *306*, 821–827, doi:10.1124/jpet.102.041616.For.
9. Agarwal, R.; Gupta, S. K.; Agarwal, P.; Srivastava, S.; Alyayutdin, R. Anticholinesterase, Antioxidant and Nitric Oxide Scavenging Activity of the Aqueous Extract of Some Medicinal Plants. *Br. J. Pharm. Res.* **2013**, *3*, 807, doi:10.9734/BJPR/2013/4833.
10. Asaduzzaman, M.; Uddin, M. J.; Kader, M. A.; Alam, A. H. M. K.; Rahman, A. A.; Rashid, M.; Kato, K.; Tanaka, T.; Takeda, M.; Sadik, G. In vitro acetylcholinesterase inhibitory activity and the antioxidant properties of Aegle marmelos leaf extract: Implications for the treatment of Alzheimer's disease. *Psychogeriatrics* **2014**, *14*, 1–10, doi:10.1111/psyg.12031.
11. Massoud, F.; Gauthier, S. An update on the pharmacological treatment of Alzheimer disease. *Curr. Neuropharmacol.* **2010**, *8*, 69–80, doi:/S0034-98872005000200012.
12. Neugroschl, Judith; Sano, M. Current Treatment and Recent Clinical Research in Alzheimer's Disease. *Mt. Sinai J. Med.* **2010**, *76*, 173–181, doi:10.1002/MSJ.
13. Schulz, V. Ginkgo extract or cholinesterase inhibitors in patients with dementia: What clinical trials and guidelines fail to consider. *Phytomedicine* **2003**, *10*, 74–79, doi:10.1078/1433-187X-00302.
14. Fawole, O. A.; Amoo, S. O.; Ndhlala, A. R.; Light, M. E.; Finnie, J. F.; Van Staden, J. Anti-inflammatory, anticholinesterase, antioxidant and phytochemical properties of medicinal plants used for pain-related ailments in South Africa. *J. Ethnopharmacol.* **2010**, *127*, 235–241, doi:10.1016/j.jep.2009.11.015.
15. Murakami, K.; Irie, K.; Ohigashi, H.; Hara, H.; Nagao, M.; Shimizu, T.; Shirasawa, T. Formation and stabilization model of the 42-mer A β radical: Implications for the long-lasting oxidative stress in Alzheimer's disease. *J. Am. Chem. Soc.* **2005**, *127*, 15168–15174, doi:10.1021/ja054041c.
16. Tabner, B. J.; El-Agnaf, O. M. A.; Turnbull, S.; German, M. J.; Paleologou, K. E.; Hayashi, Y.; Cooper, L. J.; Fullwood, N. J.; Allsop, D. Hydrogen peroxide is generated during the very early stages of aggregation of the amyloid peptides implicated in Alzheimer disease and familial British dementia. *J. Biol. Chem.* **2005**, *280*, 35789–35792, doi:10.1074/jbc.C500238200.
17. Tamagno, E. Oxidative Stress Increases Expression and Activity of BACE in NT2 Neurons. *Neurobiol. Dis.* **2002**, *10*, 279–288, doi:10.1006/nbdi.2002.0515.
18. Tong, Y.; Zhou, W.; Fung, V.; Christensen, M. A.; Qing, H.; Sun, X.; Song, W. Oxidative stress potentiates BACE1 gene expression and AB generation. *J. Neural Transm.* **2005**, *112*, 455–469, doi:10.1007/s00702-004-0255-3.
19. Gutzmann, H.; Hadler, D. Sustained efficacy and safety of idebenone in the treatment of Alzheimer's disease : update on a 2-year double-blind multi centre study of patients with dementia of the Alzheimer type (DAT) of mild to moderate degree . A total of 450 patients were ran. 2–3.
20. Rahman, S.; Parvez, A.; Islam, R.; Khan, M. Antibacterial activity of natural spices on

- multiple drug resistant *Escherichia coli* isolated from drinking water, Bangladesh. *Ann. Clin. Microbiol. Antimicrob.* **2011**, 10, 10, doi:10.1186/1476-0711-10-10.
21. Abubacker, M. R. R. Antibacterial Activities of *Argemone mexicana* L. (Papaveraceae) Leaf. *Drug Invent. Today* **2012**, 4, 385–387.
 22. Hasan, M. M.; Annay, E. A.; Sintaha, M.; Khaleque, H. N.; Noor, F. A.; Nahar, A.; Seraj, S.; Jahan, R.; Chowdhury, M. H.; Rahmatullah, M. A survey of medicinal plant usage by folk medicinal practitioners in seven villages of Ishwardi Upazilla, Pabna district, Bangladesh. *Am. J. Sustain. Agric.* **2010**, 4, 334–340.
 23. Asha, D.; S, S. P.; J, P. R.; V, A. V A review on the pharmacology and phytochemistry of traditional medicinal plant, *Glycosmis pentaphylla* (Retz.) Correa. *J. Pharm. Res.* **2012**, 55, 2723–2728.
 24. Haque, M. A.; Bari, L.; Hasan, M. M.; Sultana, M. M.; Reza, S. A. A survey on medicinal plants used by the folk medicinal practitioners in Tangail Sadar Upazilla, Tangail, Bangladesh. *J. Environ. Sci. Nat. Resour.* **2014**, 7, 35–39.
 25. Singleton, V. L.; Orthofer, R.; Lamuela-Raventós, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol.* **1998**, 299, 152–178, doi:10.1016/S0076-6879(99)99017-1.
 26. Zhishen, J.; Mengcheng, T.; Jianming, W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* 1999, 64, 555–559.
 27. Choi, H. Y.; Jhun, E. J.; Lim, B. O.; Chung, I. M.; Kyung, S. H.; Park, D. K. Application of flow injection-chemiluminescence to the study of radical scavenging activity in plants. *Sect. Title Biochem. Methods* **2000**, 14, 250–253, doi:10.1002/1099-1573(200006)14:4<250::AID-PTR587>3.0.CO;2-J.
 28. Kunchandy, E.; Rao, M. N. A. Oxygen radical scavenging activity of curcumin. *Int. J. Pharm.* **1990**, 58, 237–240, doi:10.1016/0378-5173(90)90201-E.
 29. Ellman, G. L.; Courtney, K. D.; Andres, V.; Featherstone, R. M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* **1961**, 7, 88–95, doi:10.1016/0006-2952(61)90145-9.
 30. Balouiri, M.; Sadiki, M.; Ibnsouda, S. K. Methods for in vitro evaluating antimicrobial activity: A review. *J. Pharm. Anal.* **2016**, 6, 71–79, doi:10.1016/j.jpha.2015.11.005.
 31. Dhalwal, K.; Shinde, V. M.; Namdeo, A. G.; Mahadik, K. R. Antioxidant Profile and HPTLC-Densitometric Analysis of Umbelliferone and Psoralen in *Aegle marmelos*. *Pharm. Biol.* **2008**, 46, 266–272, doi:10.1080/13880200701741088.
 32. Kumarasamy, Y.; Byres, M.; Cox, P. J.; Jaspars, M.; Nahar, L.; Sarker, S. D. Screening seeds of some Scottish plants for free radical scavenging activity. *Phyther. Res.* **2007**, 21, 615–621, doi:10.1002/ptr.2129.
 33. Moniruzzaman, M.; Asaduzzaman, M.; Hossain, M. S.; Sarker, J.; Rahman, S. M. A.; Rashid, M.; Rahman, M. M. In vitro antioxidant and cholinesterase inhibitory activities of methanolic fruit extract of *Phyllanthus acidus*. *BMC Complement. Altern. Med.* **2015**, 15, 403, doi:10.1186/s12906-015-0930-y.
 34. Kenche, V. B.; Barnham, K. J. Alzheimer's disease & metals: therapeutic opportunities. **2011**, 211–219, doi:10.1111/j.1476-5381.2011.01221.x.
 35. Chew, A. L.; Jessica, J. J. A.; Sasidharan, S. Antioxidant and antibacterial activity of different parts of *Leucas aspera*. *Asian Pac. J. Trop. Biomed.* **2012**, 2, 176–180, doi:10.1016/S2221-1691(12)60037-9.

36. Arlt, S.; Beisiegel, U.; Kontush, A. Lipid peroxidation in neurodegeneration: new insights into Alzheimer's disease. *Curr. Opin. Lipidol.* **2002**, 13, 289–294, doi:10.1097/00041433-200206000-00009.
37. Baptista, F. I.; Henriques, A. G.; Silva, A. M. S.; Wiltfang, J.; Da Cruz E Silva, O. A. B. Flavonoids as therapeutic compounds targeting key proteins involved in Alzheimer's disease. *ACS Chem. Neurosci.* **2014**, 5, 83–92, doi:10.1021/cn400213r.
38. Nimmi, I.; Jahan, I. A.; Hossain, M. H.; Burhan, M.; Rana, M. S.; Haq, M. M. A Comparative Study on Antioxidant Properties of Two Phyllanthus Species Growing in Bangladesh. **2012**, 11, 191–197.
39. Bonda, D. J.; Wang, X.; Perry, G.; Nunomura, A.; Tabaton, M.; Zhu, X.; Smith, M. A. Oxidative stress in Alzheimer disease: A possibility for prevention. *Neuropharmacology* **2010**, 59, 290–294, doi:10.1016/j.neuropharm.2010.04.005.
40. Oboh, G.; Akinyemi, A. J.; Omojokun, O. S.; Oyeleye, I. S. Anticholinesterase and Antioxidative Properties of Aqueous Extract of *Cola acuminata* Seed *In Vitro*. *Int. J. Alzheimers. Dis.* **2014**, 2014, 1–8, doi:10.1155/2014/498629.
41. Kumar, R. S.; Raj Kapoor, B.; Perumal, P. Antioxidant activities of Indigofera cassioides Rottl. Ex. DC. using various in vitro assay models. *Asian Pac. J. Trop. Biomed.* **2012**, 2, 256–261, doi:10.1016/S2221-1691(12)60019-7.
42. Lyras, L.; Cairns, N. J.; Jenner, A.; Jenner, P.; Halliwell, B. An assessment of oxidative damage to proteins, lipids, and DNA in brain from patients with Alzheimer's disease. *J Neurochem* **1997**, 68, 2061–2069, doi:10.1046/j.1471-4159.1997.68052061.x.
43. Janle, E. M.; Lila, M. A.; Grannan, M.; Wood, L.; Higgins, A.; Yousef, G. G.; Rogers, R. B.; Kim, H.; Jackson, G. S.; Ho, L.; Weaver, C. M. Pharmacokinetics and tissue distribution of ¹⁴C-labeled grape polyphenols in the periphery and the central nervous system following oral administration. *J. Med. Food* **2010**, 13, 926–33, doi:10.1089/jmf.2009.0157.
44. Faria, A.; Pestana, D.; Teixeira, D.; Couraud, P.-O.; Romero, I.; Weksler, B.; de Freitas, V.; Mateus, N.; Calhau, C. Insights into the putative catechin and epicatechin transport across blood-brain barrier. *Food Funct.* **2011**, 2, 39–44, doi:10.1039/c0fo00100g.
45. Rice-Evans, C. a; Miller, N. J.; Bolwell, P. G.; Bramley, P. M.; Pridham, J. B. The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radic. Res.* **1995**, 22, 375–383, doi:10.3109/10715769509145649.
46. Mbaebie, B. O.; Edeoga, H. O.; Afolayan, A. J. Phy to chemical analysis and antioxidants activities of aqueous stem bark extract of *Schotia latifolia* Jacq. *Asian Pac. J. Trop. Biomed.* **2012**, 2, 118–124, doi:10.1016/S2221-1691(11)60204-9.
47. Tsao, R. Chemistry and biochemistry of dietary polyphenols. *Nutrients* **2010**, 2, 1231–1246, doi:10.3390/nu2121231.
48. Pietta, P. G. Flavonoids as antioxidants. *J. Nat. Prod.* 2000, 63, 1035–1042.
49. Roy, A.; Geetha, R. V; Lakshmi, T. Evaluation of the Antibacterial Activity of Ethanolic Extract of *Dioscorea Villosa* Tubers- an in Vitro Study. **2012**, 4, 2–4.
50. Azam, S.; Mahmud, M.; Naquib, M.; Hossain, S.; Alam, M.; Uddin, M.; Sajid, I.; Hossain, M.; Karim, M.; Hasan, M. In Vitro Anti-Oxidant and Anti-Microbial Potentiality Investigation of Different Fractions of *Caryota urens* Leaves. *Biomedicines* **2016**, 4, 17, doi:10.3390/biomedicines4030017.



© 2016 The Author(s). This open access article is distributed under a Creative Commons Attribution (CC-BY) 4.0 license.

You are free to:

Share — copy and redistribute the material in any medium or format

Adapt — remix, transform, and build upon the material for any purpose, even commercially.

The licensor cannot revoke these freedoms as long as you follow the license terms.

Under the following terms:

Attribution — You must give appropriate credit, provide a link to the license, and indicate if changes were made.

You may do so in any reasonable manner, but not in any way that suggests the licensor endorses you or your use.

No additional restrictions

You may not apply legal terms or technological measures that legally restrict others from doing anything the license permits