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Acetylcholinesterase and Butyrylcholinesterase Enzyme Inhibitory Effect of *Alangium salviifolium (L. f.) Wang* pericarp Extracts with Their Phytochemical and Antioxidant Values

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MN and AH designed the study. Author AH performed the statistical analysis. Author MN wrote the protocol, and wrote the first draft of the manuscript. Authors MAU, ZY, MSU and AK managed the analyses of the study. Authors SAA and YQA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *Alangium salviifolium (L. f.) Wang* is a medicinal plant of the *Alanginaceae* family which was used as a traditional medicine to cure or prevent a variety of ailments. The aim of the study was to investigate the anticholinesterase effects and compare the phytochemical and antioxidant values of ethanol, dichloromethane, chloroform and aqueous extracts of *A. salviifolium* pericarp.

Place and Duration of Study: The study was done in Department of Pharmacy, Comilla University, Comilla, Bangladesh and Southeast University, Dhaka, Bangladesh, between January 2015 to December 2016.

Materials and Methods: The pericarp of *A. salviifolium* was extracted with different solvents. Ellman's assay was applied to investigate acetylcholinesterase and butyrylcholinesterase enzyme inhibitory effect. Phytochemical screening has been done by using qualitative methods whereas total phenol content, total flavonoid content and total flavonol content were determined by Folin-Ciocalteau reagent, aluminum trichloride and sodium acetate solution methods, respectively. Antioxidant activities were assessed by DPPH radical scavenging, FRAP and TAC assay.

Results: Donepezil, a standard drug, showed maximum inhibitory effect of AChE (IC_{50}: 6.30±1.23 µg/mL) and BChE (IC_{50}: 8.70±0.21 µg/mL). EASP followed by AASP had potent inhibitory effects while CASP and DASP had mild inhibitory effects of the enzymes. Preliminary phytochemical screening exposed the presence of valuable phytochemicals with significantly (P*<0.05, P**<0.01, P***<0.001) different content of TPC, TFC and TFIC. EASP, among the extracts, had shown the highest TPC (524.28±7.14 mg/g gallic acid), followed by TFC (231.90±14.86 mg/g quercetin) and TFIC (139.44±5.55 mg/g quercetin). In fact, maximum antioxidant potential, FRAP (EC_{50}: 50.33±4.93 µg/mL) and TAC (352.40±19.51 mg/g ascorbic acid) was found in the EASP.

Conclusion: This research has revealed that different pericarp extracts of the *A. salviifolium* have moderate to potent antioxidant and enzyme inhibitory effects and further analysis may find potential candidate for healing Alzheimer's disease.

Keywords: Acetylcholinesterase; butyrylcholinesterase; antioxidant values; Alangium salvifolium (L.f) wang pericap.

1. INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease that is characterized by the impairment of memory and a weakening in cognitive function [1,2]. An adequate amount of (Ach) is necessary for proper functioning of the brain [3,4]. An enzyme acetylcholinesterase (AChE) catalyzes the hydrolysis reaction of the Ach, and another enzyme butyrylcholinesterase (BChE) potentiates the catalyzing activity of the AChE, which causes a decreased level of Ach in the brain. Further, it leads to neurodegeneration and as well as poor cognitive function [5]. Therefore, inhibition of AChE and BChE may be the significant way of protecting the Ach to treat Alzheimer's disease. Oxidative stress causes an imbalance between the intracellular production of free radicals/reactive oxygen species (ROS) [6]. Antioxidants are used to prevent damage caused by the ROS [7]. Antioxidant may scavenge a highly reactive free radical or may inactivate it by donating a proton atom or by accepting an electron from the radical, and eventually prevents the free radical-induced diseases [8-10]. The antioxidant potential of nervous system cells can be exploited as a therapeutic manner for preventing neurodegeneration [11].

A. salviifolium wang belongs to the family of *Alanginaceae* [12]. An array of ailments including diabetes, jaundice, gastric disorders, protozoal diseases, rheumatic pain, burning sensation, hemorrhages, lung cancer, poisonings, leprosy and many inflammatory patches have been treated by using various parts of the plant [13,14]. A literature review of the plant indicates the presence of coumarins, triterpenoids, and some potent alkaloids in it [15,16]. The objective of this study was to evaluate anticholinesterase and antioxidant effects of various extracts of the *A. salviifolium* pericarp.

2. MATERIALS AND METHODS

2.1 Plant

For the study, *A. salviifolium* wang pericarp was taken from Rajshahi, Bangladesh in January

2015 and acknowledged by a responsible person from Bangladesh National Herbarium, Dhaka. A voucher specimen number was reserved with an accession no. DACB-40214. The pericarp was cleaned, dried for one week and crushed into a coarse powder using mortar-pestle. Then powders were preserved in an airtight bottle and stored in a cool, dark, and dry place until further investigation.

2.2 Extract Preparation

500 g of pericarp powder was poured separately in four flat-bottomed clean glass containers. Then they are soaked in ethanol, dichloromethane, chloroform and distilled water. After 7 days, extraction was carried out using ultrasonic sound bath accompanied by sonication (40 min). Then soaked extracts were filtrated by Whatman filter 45. Filtrates were dried using rotary evaporator at 40°C temperature and a low pressure. Then crude ethanolic extracts (EASS) (10.3 g), dichloromethane extracts (DASS) (11.5 g), chloroform extracts (CASS) (12.1 g) and aqueous extracts (AASS) (9.4 g) were obtained and stored at 4°C for next investigations.

2.3 Drugs and Chemicals

Required enzymes like AChE electric eel (type-VI-S), (BChE) equine serum lyophilized, substrates acetylthiocholine iodide (ATCI), butyrylthiocholine iodide (BuTCI), chromogen 5, serine and 5-dithio-bis (2-nitrobenzoic) acid (DTNB) were bought from Sigma-Aldrich, USA. Also, dimethyl sulfoxide (DMSO), 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin, trichloroacetic acid (TCA), ascorbic acid, gallic acid, ferric chloride were purchased from Merck, GmbH. Other chemicals and diluents used for the study were of highest pureness and commercially available.

2.4 Anticholinesterase (AChE and BChE) Assays

AChE from Electric eel and BChE from equine serum were used to explore the enzymes inhibitory potential of *A. salviifolium* pericarp extracts by using Ellman's assay [17]. The assay is based on the hydrolysis of ATCI or BuTCI by the respective enzymes and the formation of 5-thio-2-nitrobenzoate anion followed by complexation with DTNB to give a yellow color compound which is detected with spectrophotometer beside the reaction time.

2.4.1 Preparation of solutions

A phosphate buffer solution (0.1 M and 8.0 ± 0.1 pH) was prepared by adding K_2HPO_4 (17.4 g/L) and KH_2PO_4 (13.6 g/L) in distilled water. Various concentrations (25, 50, 100, 200, 400, 800 µg/mL) of the extracts and standard drug, Donepezil were prepared by series dilution. AChE (518 U/mg solid) and BChE (7 to 16 U/mg) were diluted by adding the freshly prepared buffer solution to obtain 0.03 and 0.01 U/mL concentration of the enzymes, respectively. Solutions of DTNB (0.2273 mM), ATChI and BTChI (0.5 mM) were prepared in distilled water and were kept in eppendorf tubes in the refrigerator at 8°C temperature.

2.4.2 Spectroscopic analysis

For these assays, 5 µL of AChE/BChE enzymes were taken in different cuvettes followed by addition of 205 µL sample (extracts/standard solution) and 5 µL DTNB reagent solutions. The solution mixture in each cuvette was mixed gently and maintained at 30°C for 15 min using a water bath with the subsequent addition of 5 µL substrate solution (ATChI in AChE containing cuvette and BTChI in BChE containing cuvettes). Absorbance was read against a blank solution by using a UV-Visible spectrophotometer. The absorbance of each solution along with the reaction time was taken for four minutes at 30°C. The enzyme activity and enzyme inhibition by control and tested samples were calculated from the rate of absorbance change with time ($V = \Delta Abs / \Delta t$) as follows: Enzyme inhibition (%) = 100 - percent enzyme activity. Enzyme activity (%) = $100 \times V/Vmax$. Where V is the enzyme activity in the presence of standard drug or extracts and Vmax is the enzyme activity in the absence of extracts or standard drug.

2.5 Phytochemical screening

Phytochemical screening of the extracts was done by applying some previously established methods. Alkaloids, saponins, terpenoids, and steroids were detected by applying Harborne method [18]. Flavonoids and tannins were examined by applying methods of Sofowara [19]. Reducing sugar and resins were evaluated by following methods of Dipali [20]. Coumarins, anthraquinones, cardiac glycosides and phlobatannins were detected by applying the methods of Trease and Evans [21].

2.5.1 Determination of total phenolic content (TPC)

TPC of the extracts was determined by using the Folin-Ciocalteau method [22] with slight modification. Briefly, the extracts and standard gallic acid solution (1 mL) were mixed with 2.58 mL of Folin-Ciocalteu's phenol reagent. 0.3 mL of saturated sodium carbonate solution was added to the mixture after 3 min and incubated at room temperature (25°C) for 20 min. Then, the absorbance of each sample was measured at 760 nm with a spectrophotometer. TPC of the extracts was calculated from the regression equation (r^2= 0.958) of the standard gallic acid, and the results were expressed as milligram per gram of gallic acid equivalent of the dried extracts.

2.5.2 Determination of total flavonoid content (TFC)

1 mL extract in methanol (200 mg/mL) was mixed with 1 mL aluminum trichloride in ethanol (20 mg/mL, and a drop of acetic acid), and then the mixture was diluted by the addition of ethanol up to its 25 mL volume. Blank samples were prepared by adding all the reagents with equal volume used in the sample, except the extract. The absorbance of the solution was read at 415 nm after 40 min of incubation at room temperature. Using the same procedure for the absorbance of quercetin, a standard compound of flavonoid was read, and TFC of the extracts was calculated from the standard curve (r^2 = 0.902) of the quercetin (12.5 to 200 mg/mL). Total flavonoid content was expressed as mg/g of quercetin equivalent [23].

2.5.3 Estimation of total flavonol content (TFIC)

TFIC was determined by applying a method previously described by Mbaebie et al. [24], with slight modification (Mbaebie et al., 2012). According to the method, 1 mL of the extracts (200 µg/mL) was taken separately in different test tubes. 2 mL ethanol solution of AlCl3 and 3 mL of (50 g/l) sodium acetate solution were added to the test tubes. After gently mixing, all the test tubes were allowed to stand for 2.5 h at 20°C temperature. Then, absorbance was determined by using a spectrophotometer at a wavelength of 440 nm. Quercetin was used as standard flavonol compound. Following the aforementioned procedure, the absorbance of the quercetin was taken at various concentrations (25 to 400 µg/mL) of series dilution. TFIC of the extracts was calculated from regression equation (r^2 = 0.951) of the standard quercetin, and the results were expressed as milligram per gram of quercetin equivalent of the dried extracts.

2.6 Antioxidant Assay

2.6.1 Determination of total antioxidant content (TAC)

TAC of the extracts was evaluated by phosphomolybdenum complex method with slight modification, which was described by Prieto et al. [25]. Briefly, a reagent solution was prepared having 0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate in distilled water. 1 mL of each extract was combined with the reagent solution in separate test tubes. After shaking gently, the test tubes were incubated for 90 min at 95°C temperature. Then after cooling at room temperature, absorbance was measured at 695 nm wavelength using a spectrophotometer. Similarly, ascorbic acid, a standard antioxidant, was run through the process at different concentration gradient (25 to 400 µg/mL). Using this absorbance value, a standard calibration curve and a regression equation (r^2 = 0.964) was derived, from which TAC of each of the extracts was determined and expressed as mg/g of the ascorbic acid equivalent of the dried extracts.

2.6.2 Determination of 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH free radical scavenging activity was measured by an established method described by Braca et al. [26]. Briefly, 50mM of DPPH radical solution was prepared in methanol, and then 900 µL of this solution was mixed with 100 µL of extract or standard ascorbic acid solution (12.5 to 200 µg/mL) and kept in a dark place for 30 minutes. Then, absorbance was measured at 517 nm. Scavenging capacity of DPPH radicals (% Inhibition) was measured by the following formula.

$$\text{Inhibition (\%)} = (A_0 - A_s) / A_0 \times 100$$

Where A_0 = Absorbance of control group, As = Absorbance of sample.

2.6.3 Ferric reducing antioxidant power (FRAP) assay

The Fe3+ reducing power was determined by the method of Oyaizu et al. [27] with slight

modifications. Shortly, 1 mL of extract or standard ascorbic acid solution was taken in a test tube and mixed with 2.5 mL of phosphate buffer solution (0.2 M, pH 6.6). Then 2.5 mL of potassium ferricyanide (1%) was added and incubated at 50°C for 30 min. After that, 2.5 mL of trichloroacetic acid (10%) was added and centrifuged at 4000 rpm for 10 min. Finally, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.1 mL of $FeCl_3$ (0.1%) solution followed by incubation at 35°C for 10 min. The absorbance was measured at 700 nm, and the reducing power of the extracts was compared with the standard ascorbic acid. From standard calibration curve, median effective concentration (EC_{50}) was calculated. The EC_{50} value (µg/mL) is the effective concentration giving an absorbance of 0.5.

2.7 Statistical Analysis

All the data were presented as the mean value of triplicate experiment (n=3) along with standard deviation (Mean±SD). P* < 0.05, P** < 0.01 and P*** < 0.001 were considered as significance level. ANOVA, followed by Dunnett's test was done in SPSS version 15.0. IC_{50} and EC_{50} values were calculated by using the MS-excel program. TPC, TFC, and TFIC were calculated from regression equation of each standard sample by using the program (MS-excel). All the figures were prepared by using Graph Pad Prism software, version 7.03.

3. RESULTS AND DISCUSSION

3.1 Anticholinesterase Inhibitory Effectt

All extracts and the standard drug donepezil showed AChE and BChE inhibitory effect in a concentration gradient manner. Among the extracts, EASP and AASP showed strong effect which displayed an IC_{50} value of 278.66±10.2 µg/mL and 387.33±12.5 µg/mL in AChE inhibition, and 91.88±1.74 µg/mL and 82.19±1.99 µg/mL in BChE inhibition, respectively. The donepezil showed 6.30±1.23 µg/mL and 8.70±0.21 µg/mL IC_{50} value in the AChE and BChE inhibition, respectively (Fig. 1 and Fig. 2).

3.2 Preliminary Phytochemical Screening

Preliminary phytochemical screening of the extracts revealed the important bioactive metabolites which are presented in Table 1.

3.3 Phytoconstituents

3.3.1 Total phenol content (TPC)

All the extracts showed phenolic content with significant (P**<0.01, P***<0.001) difference among them which are summarized in Table-2. EASP, among the extracts, showed the highest phenolic content followed by AASP. The order of TPC among the extracts was EASP > CASP > DASP > AASP (Table 2).

3.3.2 Total flavonoid content (TFC)

TFC was significantly (P*<0.05, P**<0.01, P***<0.001) different among the extracts. The EASP had the highest content while CASP had the lowest content, and the order of TFC was EASP > AASP > DASP > CASP (Table 2).

3.3.3 Total flavonol content (TFIC)

Both the DASP and CASP showed poor content, having 69.07±8.48 and 28.33±5.55 mg/g quercetin equivalent of TFIC, respectively. On the other hand, significantly more content of the TFIC was found in EASP (139.44±5.55 mg/g quercetin equivalent) and AASP (28.33±5.55 mg/g quercetin equivalent). Here, the order of TFIC was EASP > AASP > DASP > CASP (Table 2).

3.4 *In vitro* Antioxidant Activity

3.4.1 Total antioxidant content (TAC)

The phosphomolybdate method, another quantitative method of antioxidant effect measurement, is based on the reduction of molybdenum (VI) to molybdenum (V) which takes place in the presence of the antioxidant compound in the extracts. In the present study, all experimented samples had good TAC. EASP had the highest (352.40±19.51 mg/g AA content) while AASR had the lowest TAC (200.55±16.66 mg/g AA content). The order of TAC among the extracts was EASP > DASP > CASP > AASP (Table 2).

3.4.2 DPPH free radical scavenging activity

All the extracts inhibited DPPH radicals at concentration gradient manner (more concentration more inhibition). Ascorbic acid, the standard antioxidant compound, exhibited maximum inhibition such as 28.36±3.25% to 92.36±6.58% at 12.5 to 200 µg/mL concentration range. DASP, among all the extracts, have

shown the highest inhibition which was shown 27.96±2.15, 51.97±2.24, 63.80±2.67, 70.61±3.47, 74.19±5.36 percent inhibition at 12.5, 25, 50,100 and 200 µg/mL concentration where its IC_{50} value is 35.45±3.17. However, AA, EASP and CASP have shown antioxidant effect with the IC_{50} value of 19.50±4.83 µg/mL, 55.50±2.35 µg/mL and 91.23±1.45 µg/mL, respectively. On the other hand, AASP should least antioxidant effect with IC_{50} value 120.22±3.12 (Table 3).

3.4.3 Ferric reducing power assay

Reducing power of all the extracts and the standard compound ascorbic acid was increased with the gradual increase of concentration. Ascorbic acid, a standard reducing agent, showed the highest absorbance (0.32±0.01 to 1.58±0.07) at concentrations ranging from 12.5 to 200 µg/mL. 50% effective concentration (EC_{50}) of this was 32.45±4.12 µg/mL. Among the extracts, EASP showed maximum reducing potential (0.37±0.02 to 0.95±0.03 absorbance value) at concentrations ranging from 12.5 to 200 µg/mL, and EC_{50} value of it was 50.33±4.93 µg/mL. The reducing capability order of the extracts was EASP > AASP > CASP > DASP (Table 4).

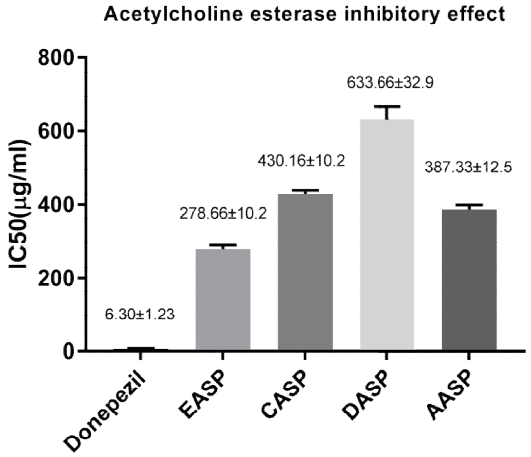

Fig. 1. Acetylcholine esterase inhibitory effect

Values were expressed as mean±SD (n= 3)

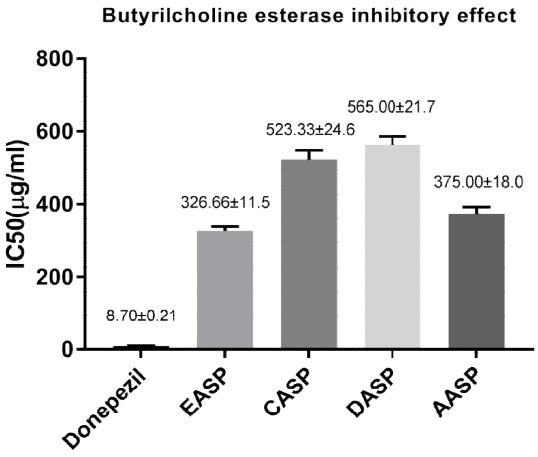

Fig. 2. Butyrilcholine esterase inhibitory effect

Values were expressed as mean±SD (n= 3)

Table 1. Result of phytochemical screening of the extracts

Phytoconstituents	Test	Different extracts and extant of their phytoconstituents			
		EASP	DASP	CASP	AASP
Alkaloids	Mayer's test	++	+	++	+
Saponins	Froth test	+	+	+	+
Terpenoids	Salkowski tests	+	+	+	-
Flavonoids	Ammonia test	++	+	++	+
Tannins	Ferric chloride test	+	+	+	+
Steroids	Liebermann-Burchard's test	-	-	-	-
Reducing sugar	Benedict's test	+	-	+	+
Resins	Acetone, Distill water	+	-	-	+
Anthraquinones	Borntrager's test	-	-	-	-
Cardiac glycosides	Keller-Killiani test	-	-	-	-
Coumarins	NaOH	+	-	+	-
Phlobatannins	HCl	-	-	-	-

Where based on the intensity of the characteristic color, + = Present in mild amount, ++ = Present in moderate amount, −= Not present.

Table 2. Phytoconstituents status in the pericarp extracts of *Alangium salviifolium*

Sample	TAC mg/g AA content	TPC mg/g GA content	TFC mg/g querceetin content	TFIC mg/g quercetin content
EASP	352.40±19.51	524.28±7.14	231.90±14.86	139.44±5.55
DASP	269.07±25.05	317.14±14.28	110.47±14.86	69.07±8.48
CASP	239.44±16.66	369.52±10.914	67.61±10.91	28.33±5.55
AASP	**200.55±16.66**	**281.42±7.14**	**162.85±14.28**	**107.96±8.48**

Data are presented as mean±SEM, ANOVA is done in SPSS version15.0

Table 3. DPPH radical scavenging effect of the extracts

Concentration (µg/mL)	AA	EASP	CASP	DASP	AASP
12.5	28.36±3.25	28.67±1.64	20.79±1.24	27.96±2.15	15.41±0.62
25	56.89±4.23	43.01±1.10	44.09±1.08	51.97±2.24	40.86±2.14
50	70.36±4.85	60.57±3.23	53.41±1.64	63.80±2.67	47.31±3.25
100	85.36±5.26	66.31±2.20	56.63±0.62	70.61±3.47	52.69±4.25
200	92.36±6.58	74.19±3.58	62.72±2.71	74.19±5.36	57.35±5.36
IC_{50}	**19.50±4.83**	**55.50±2.35**	**91.23±1.45**	**35.45±3.17**	**120.22±3.12**

Data are presented as mean±SEM, ANOVA is done in SPSS version15.0

Table 4. Ferric reducing power of the extracts

Concentration (µg/mL)	AA	EASP	CASP	DASP	AASP
12.5	0.32±0.01	0.37±0.02	0.21±0.01	0.12±0.03	0.22±0.01
25	0.45±0.02	0.39±0.04	0.28±0.02	0.20±0.02	0.29±0.03
50	0.65±0.03	0.52±0.01	0.48±0.04	0.40±0.04	0.44±0.02
100	0.98±0.05	0.75±0.05	0.66±0.02	0.43±0.05	0.78±0.04
200	1.58±0.07	0.95±0.03	0.72±0.05	0.68±0.03	0.94±0.06
EC_{50}	**32.45±4.12**	**50.33±4.93**	**88.33±7.69**	**127.00±8.54**	**68.00±6.57**

Data are presented as mean±SEM, ANOVA is done in SPSS version15.0

4. DISCUSSION

Oxidative stress plays a vital role for generation and progression of the AD, where nerve cells or cellular components are oxidized by some free radicals that are considered as powerful oxidizing agents. Among these, the ROS (●O_2-, ●OH, H_2O_2, O_3) are very potential to induce lipid peroxidation and subsequently cell death [28,29]. These are generated mostly by mitochondrial oxidation and moderately by the influence of environmental pollutants, smoking and harmful radiations [30,31]. We have a self-protective mechanism against the radicals, namely antioxidant defense system, composed of some enzymatic antioxidants, the main function of which is to protect our body from the oxidative stress. Here, antioxidants, enzymatic or no enzymatic, show their effect either by scavenging or by deactivating the free radicals [32,33]. The coordinate action of the antioxidant system is very critical for the detoxification of the radicals. Superoxide dismutase acts on highly reactive superoxide radical (●O_2-) and converts it to less reactive H_2O_2 radical. Then catalase and glutathione peroxidase convert the H_2O_2 to water, and thus brain tissues are protected from the reactive radicals [34]. However, in the case of stress condition, defensive power of the natural antioxidant system declines sharply, since the brain cells consume the large proportion of the inhaled oxygen which consequently generates the increased number of free radicals due to high metabolic rate in it [35]. Moreover, ascorbate and transient metals, largely present in the nerve tissues, acts as pro-oxidant and potentiates the oxidative damage of the nerve cells due to their high content of polyunsaturated fatty acids [23,24]. So, when free radicals exceed their normal threshold level, then oxidative stress proceeds abundantly. And the cells fail to function effectively and consequently. As a result cellular degeneration takes place which is a way of the AD progression

[36]. In this condition, the antioxidant supplement is essential to combat with the radicals, and to protect the brain from the cell degeneration.

AD is developed by numerous pathogenic factors such as the formation of the abnormal compound, namely amyloid-β peptide (Aβ) and intracellular neurofibrillary tangles (NFTs), reduction of acetylcholine level and exacerbation of oxidative stress [37-39]. Acetylcholine, an organic molecule, acts as a neuro-transmitter and is associated with neuronal networking in central and peripheral nervous systems. Naturally, it is produced in some of our brain cells which are called cholinergic neurons. After a specific life span, ACh goes to break down by the AChE and BChE enzymes. In case of normal healthy people, the rate of synthesis and cleavage of the ACh remain steady to maintain its normal level [40,41]. In this case, the AChE is 1.5-fold to 60-fold more active than that of BChE. But, in the case of AD, enzyme performance shifts towards the BChE, where its activity increases up to 120%. In contrast, AChE loses its effectiveness by 10 to 15%. This abnormality, increased break down rate of Ach, leads to decrease the availability of the ACh than its normal physiological scale. AChE/BChE bind with Aβ and a protein called ApoE protein, resulting in the formation of a highly stable complex (AChE/BChE-Ab-ApoE complex) in cerebrospinal fluid (CSF) of the brain [42]. This stable complex directly interacts with ACh receptors and therefore, interferes with their signal transductions and potentiates ultrafast hydrolysis of ACh. Researchers, for example, from postmortem studies of AD patients, have found strongly reduced number of ACh receptors and loss of basal forebrain and cortical cholinergic neurons. Furthermore, the reduced level of ACh adversely affects the physiological functions of the brain. Also, AChE and BChE potentiate neuronal degeneration by forming some protein complexes such as neurofibrillary tangles (NFT) and neuritic plaques (NP) which are aggregates of hyperphosphorylated tau protein and extracellular neurotoxic deposits of Aβ, respectively [43]. Therefore, inhibition of AChE and BChE is the most effective therapeutic approach to treat the symptoms of AD. Consequently, cholinesterase inhibitors are the only approved drugs for treating patients with mild to moderately severe Alzheimer's disease.

Many phytochemicals have been reported to have satisfactory antioxidant and anticholinesterase effects. Among these phenolics and flavonoids, potent antioxidative compounds act as free radical scavengers. Majority of the phytochemicals having potent AChE and BChE inhibitory effects, are alkaloids followed by terpenoids, steroids, flavonoids, glycosides, saponins and essential oils. Since most of the natural or synthetic products, having enzyme inhibitory effects are known to contain a nitrogen atom, the promising effect of the medicinal plants could be due to their high alkaloidal contents. *Alangium salviifolium* is rich with biologically active phytochemicals where various types of alkaloids have been isolated and identified [44]. Among these alangimaridine, methyl-1H pyrimidine-2, 4-dione, alangine A and B, alangicine, markindine, lamarckinine and emetine are important. Besides, phytochemical screening of it revealed the presence of flavonoids, phenolics, glycosides, etc. So, these compounds may be considered for the antioxidant effect and enzyme (AChE and BChE) inhibitory activities of the extracts.

5. CONCLUSION

A. salviifolium wang is extensively used as folk medicine. The present study showed that the plant is important for its phytochemical constituents. It has the significant amount of phenolics, flavonoid, and flavonol. Pericarp extracts of the plant have shown moderate to potent antioxidant potential. These are also effective to inhibit AChE and BChE enzymes. So the plant is effective to protect from Alzheimer's disease. However, further analysis by isolation of the key compounds can find out the actual mechanism of action.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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