Full Length Research Paper

Phytochemical screening, antioxidant and anticholinesterase effects of *Alangium salvifolium* (L.F) Wang root extracts

Md. Nasrullah¹, Anamul Haque²*, Zerina Yasmin¹, Mohammad Ashraf Uddin¹, Kushal Biswas³ and Mohammed Saiful Islam³

¹Department of Pharmacy, Southeast University, Banani, Dhaka, Bangladesh.
²Department of Pharmacy, Comilla University, Comilla, Bangladesh.
³Department of Pharmacy, Rajshahi University, Rajshahi, Bangladesh.

Received 4 August, 2015; Accepted 19 October, 2015

*Alangium salvifolium* *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 and compare the phytochemical profiles, antioxidant and anticholinesterase effects of ethanol (EASR), dichloromethane (DASR), chloroform (CASR) and aqueous (AASR) extracts of *A. salvifolium* root. Phytochemical screening was done by using qualitative methods whereas total phenol content (TPC), total flavonoid content (TFC) and total flavonol content (TFlC) were determined by Folin-Ciocalteau reagent, aluminium trichloride and sodium acetate solution methods, respectively. Antioxidant activities were assessed by DPPH radical scavenging, ferric reducing antioxidant power (FRAP) and total antioxidant content (TAC) assay. Ellman's assay was applied to investigate acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzyme inhibitory effect. Preliminary phytochemical screening revealed the presence of valuable phytochemicals with significantly (*P*<0.05, *P***<0.01, *P****<0.001) different content of TPC, TFC and TFlC. CASR, among the extracts, had shown the highest TPC (492.38±22.34 mg/g gallic acid), followed by TFC (276.25±17.23 mg/g quercetin) and TFlC (332.92±7.07 mg/g quercetin). Moreover, maximum antioxidant potential, including DPPH radical scavenging (*IC*₅₀: 11.26±1.29 µg/ml), FRAP (*EC*₉₀: 26.64±2.17 µg/ml) and TAC (639.55±10.51 mg/g ascorbic acid) was found in the CASR. Donepezil, a standard drug, showed maximum inhibitory effect of AChE (*IC*₉₀: 7.94±1.12 µg/ml) and BChE (*IC*₉₀:12.58±2.15 µg/ml). CASR followed by DASR had potent inhibitory effects while AASR had mild and EASR practically had no inhibitory effects of the enzymes. The present study has demonstrated that the root extracts of the *A. salvifolium* have moderate to potent antioxidant and enzyme inhibitory effects.

**Key words:** *Alangium salvifolium*, antioxidant, Anticholinesterase effect, phenol content, flavonoid content, 2,2-diphenylpicrylhydrazyl (DPPH), reducing power.

INTRODUCTION

Free radical damage and oxidative stress are considered as important causative factors for generation as well as exacerbation of various ailments like cancer, diabetes, asthma, and the pathogenesis of alzheimer’s disease.
(AD) (Asmat et al., 2015). Oxidative stress, a potential source of damage to DNA, lipids, sugars and proteins, causes an imbalance between the intracellular production of free radicals/reactive oxygen species (ROS) and antioxidant defense mechanisms, resulting in cellular injury (Gjumrakch et al., 2008). The brain consumes a large proportion of the inhaled oxygen, and therefore produces a comparatively large quantity of free radical by-products (Yongxin et al., 2013). However, less quantity of the reactive oxygen (ROS) species are the precondition to keep the integrity of the neuronal cells and subsequently their normal functioning, since the elevated level of the radicals can lead to neuronal cell death (Yongxin et al., 2013). In contrast, antioxidants, being the defensive agents against the oxidative stress, have multiple functions in biological systems, including maintenance of cell integrity and cell signaling pathways (Kumar et al., 2008). One principal cellular function of antioxidants is to prevent damage caused by the ROS. Various studies have proved that an 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 (Jiaoji et al., 2012).

Alzheimer, the most common among the neurodegenerative disorders and dementia, is a major challenge of the modern era, and is a slowly progressive disease of the brain that is characterized by the impairment of memory (Rahmat et al., 2012). For normal functioning of brain, sufficient level of acetylcholine (Ach) is necessary which is essential for proper neurotransmission. Acetylcholinesterase (AChE) enzyme catalyzes hydrolysis reaction of the Ach and butyrylcholinesterase (BChE) potentiates the catalyzing activity of the AChE, resulting in a decreased level of Ach in the brain (Zeb et al., 2014). This condition leads to neurodegeneration and subsequently cognition. So, inhibition of AChE and BChE may be the most effective way of protecting the Ach to prevent or to improve dementia.

Alangium salvifolium wang belongs to the family of Alanginaceae. Ankola and Alangi are its common name in India, and Stone Mango in English. It is a small deciduous thorny tree or shrub (Uthiraselvam et al., 2012) which is distributed in tropical and subtropical region such as Bangladesh, India, China Phillipines, Africa, Srilanka and Indochina (Ronok et al., 2013). An array of ailments including diabetes, jaundice, gastric disorders, protozoal diseases, rheumatic pain, burning sensation, haemorrhages, lung cancer, poisonings, leprosy and many inflammatory patches have been treated by using various parts of the plant (Meera et al., 2013). Many bioactive phytochemicals such as several flavanoids, phenolic compounds, irridoid glycosides and oxyuglucosides have been isolated by phytochemical screening of it (Gopinath, 2013). Literature review of the plant indicates the presence of coumarins, triterpenoids, and some potent alkaloids in it (Savithramma et al., 2012). The aim of the present study was to evaluate antioxidant and anticholineesterase effects of various extracts of the A. salvifolium root.

MATERIALS AND METHODS

Plant

For the investigation, A. salvifolium wang root was collected from Rajshahi, Bangladesh between January and June, 2013 and identified by an expert of the Bangladesh National Herbarium, Dhaka, where a voucher specimen number was retained with an accession no. 40214. The collected plant part was cleaned, dried for one week and pulverized into a coarse powder using a suitable grinder. Powdered material was stored in an airtight container and kept in a cool, dark, and dry place until further analysis was taken.

Extract preparation

Approximately 500 g of powdered root was placed separately in four clean and flat-bottomed glass containers and soaked in ethanol, dichloromethane, chloroform and distilled water. All the containers with their contents were sealed and kept for 7 days. Then extraction was carried out using ultrasonic sound bath accompanied by sonication (40 min). The entire mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then the extract was filtered through Whatman filter paper and concentrated by using a rotatory evaporator at reduced pressure. The gummy extracts were then dried by using an electric oven, and finally obtained EASR (12.25 g), DASR (9.5 g), CASR (7.5 g) and AASR (14.17 g). The dried extracts were separately stored in air tight containers until completion of the analysis.

Drugs and chemicals

Enzymes including acetylcholinesterase (AChE) electric eel (type-VI-S), butyrylcholinesterase (BChE) equine serum lyophilized substrates acetylthiocholine iodide (ATCI), butyrylthiocholine iodide (BuTCl), chromogen 5, 5-dithio-bis (2-nitrobenzoic) acid (DTNB) and serine were purchased from Sigma-Aldrich, USA, 1,1-Diphenyl-1-2-picrylhydrazyl (DPPH), dimethyl sulfoxide (DMSO), trichloroacetic acid (TCA), quercetin, ascorbic acid, gallic acid, ferric chloride, and glacial acetic acid were purchased from Merck.

*Corresponding author. E-mail: pharmaripon@gmail.com. Tel: +8801912894766. Fax: +880-8170035.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License.
Germay. All other reagents and solvents used for the study were of highest purity grade and commercially available.

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 (Harborne, 1973) method. Flavonoids and tannins were examined by applying methods of Sofowara (Sofowara, 1993). Reducing sugar and resins were evaluated by following methods of Dipali (Dipali et al., 2013). Coumarins, anthraquinones, cardiac glycosides and phlobatannins were detected by applying the methods of Trease and Evans (Trease and Evans., 1989).

Determination of total phenolic content (TPC)

TPC of the extracts was determined by using the Folin-Ciocalteau method with slight modification (Gao et al., 2000). Briefly, the extracts and standard gallic acid solution (1 ml) was mixed with 2.58 ml of Folin-Ciocalteau’s phenol reagent. After 3 min, 0.3 ml of saturated sodium carbonate solution was added to the mixture and incubated at room temperature (25°C) for 20 min. Then, 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.

Determination of total flavonoid content (TFC)

1 ml extract in methanol (200 mg/ml) was mixed with 1 ml aluminium 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 absorbance of quercetin, 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 (Kumaran and Karunakaran, 2007).

Antioxidant assay

**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. (1999). 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 ascorbic acid equivalent of the dried extracts.

**Determination of 1, 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. (2002). Briefly, 0.004% w/v 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 thirty minutes. Then, absorbance was measured at 517 nm. Scavenging capacity of DPPH radicals (% Inhibition) was measured by the following formula and finally the 50% inhibition concentration (IC\(_{50}\)) was calculated using MS-Excell software.

\[
\text{Inhibition} \% = \left( \frac{A_0 - A_e}{A_0} \right) \times 100
\]

Where \( A_0 = \text{Absorbance of control group} \), \( A_e = \text{Absorbance of sample} \).

**Ferric reducing power (FRAP) assay**

The Fe\(^{2+}\) reducing power was determined by the method of Oyaizu (1986) 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.

**Anticholinesterase (AChE and BChE) assays**

AChE from Electric eel and BChE from equine serum were used to explore the enzymes inhibitory potential of A. salivilium root extracts by using Ellman’s assay (Classics et al., 1961). The assay is based on the hydrolysis of acetylthiocholine iodide or...
butyrylthiocholine iodide by the respective enzymes and the formation of 5-thio-2-nitrobenzoate anion followed by complexation with DTNB to give a yellow colour compound which is detected with spectrophotometer beside the reaction time.

**Preparation of solutions**

A phosphate buffer solution (0.1 M and 8.0 ± 0.1 pH) was prepared by adding K₂HPO₄ (17.4 g/L) and KH₂PO₄ (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 up to obtain 0.03 and 0.01 U/ml concentration of the enzymes, respectively. Solutions of DTNB (0.0002273 M), ATChI and BTChI (0.0005 M) were prepared in distilled water and were kept in appedor caps in the refrigerator at 8°C temperature.

**Spectroscopic analysis**

For these assays, 5 μl of AChE/BChE enzymes were taken in different cuvette 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 water bath with subsequent addition of 5 μl substrate solution (ATChI in AChE containing cuvette and BTChI in BChE containing cuvette). 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 = ΔAbs / Δt) as follows: Enzyme inhibition (%) = 100 - percent enzyme activity. Enzyme activity (%) = 100 x V/V_max, Where, V is the enzyme activity in the presence of standard drug or extracts and V_max is the enzyme activity in the absence of extracts or standard drug. 50% inhibition concentration (IC₅₀) values were calculated by using MS-Excel software.

**Determination of correlation (r²) between antioxidant activities and phytochemical assay**

MS-excel program was used to determine the correlations between antioxidant activities and phytochemical contents. IC₅₀ values of DPPH, EC₅₀ values of FRAP and TAC were put against TPC, TFC and TFIC values of the extracts. In each set, pearson correlation (r² value) was determined from the regression equation.

**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 and 95% confidence of interval was calculated from it. IC₅₀ and EC₅₀ 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 5.0.

**RESULTS**

**Preliminary phytochemical screening**

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

**Phytoconstituents**

**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 Figure 1A. DASR, among the extracts, showed the highest phenolic content followed by CASR. The order of TPC among the extracts was DASR > CASR > AASR > EASR. 95% confidence interval (CI) was 76.34 to 154.48 in EASR, 436.88 to 547.88 in DASR, 230.51 to 308.65 in CASR and 139.41 to 170.52 in AASR.

**Total flavonoid content (TFC)**

TFC was significantly (P*<0.05, P**<0.01, P***<0.001) different among the extracts. The CASR had the highest content while EASR had the lowest content, and the order of TFC was CASR > DASR > EASR > AASR (Figure 1B). The 95% confidence interval (CI) was 24.07 to 58.10 in EASR, 53.91 to 105.89 in DASR, 233.42 to 219.07 in CASR and 10.23 to 44.48 in AASR.

**Total flavonol content (TFIC)**

Both the EASR and AASR showed poor content, having 21.2 ± 4.63 and 78.29 ± 7.07 mg/g ascorbic acid equivalent of TFIC, respectively. On the other hand, significantly more content of the TFIC was found in DASR (294.35 ± 13.89 mg/g ascorbic acid equivalent) and CASR (332.92 ± 7.07 mg/g ascorbic acid equivalent). Here, the order of TFIC was CASR > DASR > AASR > EASR (Figure 1C). The 95% confidence interval (CI) was 9.69 to 72.70 in EASR, 259.84 to 328.85 in DASR, 315.34 to 350.49 in CASR and 60.72 to 95.86 in AASR.

**In vitro antioxidant activity**

**DPHH 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: 40.14 ± 2.24% to 87.10 ± 2.15% at 6.25 to 200 µg/ml concentration range. CASR, among all the extracts have shown the highest inhibition which was 46.59 ± 2.71 to 79.93 ± 1.64% at 12.5 to 200 µg/ml concentration range. AA, CASR and DASR have shown potent antioxidant effect with the IC₅₀ value of 12.58 ± 1.45 µg/ml (5.88 to 15.05 CI), 11.26 ± 1.29 µg/ml (8.06 to 14.47 CI) and 16.48 ± 1.12 µg/ml (13.70 to 19.26 CI), respectively. The remaining extracts showed moderate antioxidant potential. The order of antioxidant effect was CASR > DASR > AASR > EASR (Table 2).

**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 (0.460 ± 0.01 to 2.13 ± 0.23) at concentrations ranging from 12.5 to 200 µg/ml. 50% effective concentration (EC₅₀) of it was 8.95 ± 1.03 µg/ml (5.04 to 12.86 CI). Among the extracts, CASR showed maximum reducing potential (0.350 ± 0.06 to 1.350 ± 0.13 absorbance value) at concentrations ranging from 12.5 to 200 µg/ml, and EC₅₀ value of it was 26.64 ± 2.17 µg/ml (22.83 to 30.45 CI). The reducing capability order of the extracts and the ascorbic acid was AA > CASR > DASR > AASR > EASR (Table 2).

**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 for the presence of antioxidant compound in the extracts. In the present study, all experimented samples had good TAC but in significantly (P* < 0.05, P** < 0.01 and P*** < 0.001) different extent. CASR had the highest (639.55 ± 10.51) while EASR had the lowest TAC (114.11 ± 12.83). The order of TAC among the extracts was CASR > DASR > AASR > EASR (Figure 1D). The 95% confidence interval (CI) was 82.21 to 146.00 in EASR, 452.34 to 489.74 in DASR, 613.44 to 665.66 in CASR and 159.19 to 184.70 in AASR.

**Anticholinesterase inhibitory effect**

All extracts, except EASR, and the standard drug donepezil showed AChE and BChE inhibitory effect in a concentration gradient manner. Among the extracts, CASR and DASR showed strong effect which displayed an IC₅₀ value of 152.73 ± 9.94 µg/ml (128.02 to 177.44 CI) and 192.28 ± 12.52 µg/ml (161.16 to 223.40 CI) in AChE inhibition, and 178.60 ± 20.53 µg/ml (127.60 to 229.60 CI) and 212.39 ± 12.23 µg/ml (182.00 to 242.78 CI) in BChE inhibition, respectively. The donepezil showed 7.94 ± 1.12 µg/ml (4.36 to 11.53 CI) and 12.58 ± 2.15 µg/ml (7.01 to 18.15 CI) IC₅₀ value in the AChE and BChE inhibiton, respectively (Table 3).

**Correlation between antioxidant effects and phytochemicals**

The correlation analysis was performed to investigate the

---

**Table 1.** Phytochemical screening of *A. salvifolium* root extracts.

| Phytoconstituents | Test                     | EASR | DASR | CASR | AASR |
|-------------------|--------------------------|------|------|------|------|
| 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                      | -    | -    | -    | -    |

++ = low content, ++ = moderate content, +++ = high content, - = no response.
Table 2. DPPH radical scavenging and FRAP of A. salvifolium root extracts.

| Sample | DPPH radical scavenging activity | | | Ferric reducing antioxidant power (FRAP) | |
|---|---|---|---|---|---|
| | Concentrations (µg/ml) | Percent inhibition | IC<sub>50</sub> (µg/ml) | Absorbance | EC<sub>50</sub> value (µg/ml) | |
| Ascorbic acid | 6.25 | 40.14±2.24 | 12.58±1.45*** | 0.40±0.05 | 8.95±1.57*** | |
| | 12.5 | 53.41±1.64 | 0.62±0.03 | | | |
| | 25 | 66.67±1.86 | 0.84±0.04 | | | |
| | 50 | 71.33±1.64 | 1.45±0.03 | | | |
| | 100 | 82.44±1.24 | 1.67±0.03 | | | |
| | 200 | 87.10±2.15 | 1.75±0.03 | | | |
| | 12.5 | 31.54±1.64 | 0.20±0.02 | | | |
| | 25 | 37.63±1.86 | 0.25±0.02 | | | |
| EASR | 50 | 47.67±5.52 | **61.33±10.45** | 0.20±0.02 | **136.75±18.81** | |
| | 100 | 59.50±2.24 | 0.45±0.01 | | | |
| | 200 | 64.16±2.24 | 0.58±0.02 | | | |
| | 12.5 | 53.76±2.15 | 0.27±0.02 | | | |
| | 25 | 57.35±3.10 | 0.33±0.02 | | | |
| DASR | 50 | 65.95±1.64 | **16.48±1.12*** | 0.47±0.02 | **43.24±5.94*** | |
| | 100 | 72.04±1.07 | 0.66±0.02 | | | |
| | 200 | 76.34±2.15 | 0.93±0.03 | | | |
| | 12.5 | 46.59±2.71 | 0.31±0.01 | | | |
| | 25 | 65.23±2.24 | 0.42±0.01 | | | |
| CASR | 50 | 70.61±1.64 | **11.26±1.29*** | 0.65±0.01 | **26.64±1.53*** | |
| | 100 | 77.42±1.08 | 0.82±0.02 | | | |
| | 200 | 79.93±1.64 | 1.08±0.01 | | | |
| | 12.5 | 25.09±1.64 | 0.25±0.03 | | | |
| | 25 | 36.56±1.08 | 0.28±0.03 | | | |
| AASR | 50 | 48.03±1.24 | **56.47±6.49** | 0.35±0.03 | **79.99±0.97*** | |
| | 100 | 60.93±1.64 | 0.56±0.01 | | | |
| | 200 | 65.95±2.24 | 0.65±0.02 | | | |

Data are expressed as mean ± standard deviation (n = 3). P*< 0.05, P**<0.01 and P***<0.001 are considered as significant difference of IC<sub>50</sub>/EC<sub>50</sub> value compared with the highest value.

Relationship between the phytochemicals and antioxidant activity of the extracts. Among the phytochemicals, TFC showed strong positive correlation with DPPH \( (r^2 = 0.913) \), FRAP \( (r^2 = 0.803) \) and TAC \( (r^2 = 0.782) \). TFC had well positive correlation with TAC \( (r^2 = 0.764) \) while weak correlation with DPPH and FRAP effects. TPC showed weak correlation with FRAP and TAC but moderate correlation with the DPPH test (Table 4).

**DISCUSSION**

Oxidative stress plays a vital role for generation and progression of AD, where nerve cells or cellular components are oxidized by some free radicals that are considered as powerful oxidizing agents. Among these, the ROS (\( \cdot O_2^-, \cdot OH, H_2O_2, O_3 \)) are very potential to induce lipid peroxidation and subsequently cell death. These are generated mostly by mitochondrial oxidation and moderately by the influence of environmental pollutants, smoking and harmful radiations (Lobo et al., 2010). We have a self protective mechanism against the radicals, namely antioxidant defense system, composed of some enzymatic antioxidants, main function of which is to protect our body from the oxidative stress. Here, antioxidants, enzymatic or non enzymatic, show their...
Table 3. AChE and BChE inhibitory effect of *A. salvifolium* root extracts.

| Sample | 50% inhibitory concentration (IC$_{50}$) value (µg/ml) |
|--------|-------------------------------------------------------|
|        | AChE inhibition | BChE inhibition |
| Donepezil | 7.94±1.44*** | 12.58±2.24*** |
| EASR | No effect | No effect |
| DASR | 192.28±12.52*** | 212.39±12.23*** |
| CASR | 152.73±9.94*** | 178.60±20.53*** |
| AASR | 1081.34±70.44 | 1172.61±149.48 |

Data are expressed as mean ± standard deviation (n = 3). P*< 0.05, P**<0.01 and P***<0.001 are considered as significant difference of IC$_{50}$ value compared with the highest value.

Table 4. Correlation of antioxidant activity with phytochemicals.

| Antioxidant test | Pearson correlation (r$^2$) |
|-----------------|----------------------------|
|                 | TPC           | TFC           | TFIC          |
| IC$_{50}$ value of DPPH | 0.634        | 0.588        | 0.913        |
| EC$_{50}$ value of FRAP  | 0.488        | 0.487        | 0.803        |
| TAC             | 0.438        | 0.764        | 0.782        |

Acetylcholine, an organic molecule, acts as a neurotransmitter, 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. 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% (Faiyaz et al., 2013). This abnormality, increased break down rate of Ach, leads to decrease the availability of the ACh than its normal physiological scale. Furthermore, the reduced level of ACh adversely affects the physiological functions of the brain. In addition, AChE and BChE potentiates neuronal degeneration by forming some protein complexes such as: neurofibrillary tangles (NFT) and extracellular neurotoxic deposits of Aβ, respectively (Dominik and Kamila, 2012). 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. This stable complex directly interacts with ACh receptors and therefore, interferes with their signal transductions and potentiates ultrafast hydrolysis of ACh (Swetha et al., 2013). 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 (Taiwo et al., 2010). 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 (Faiyaz et al., 2013). 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 (Fadwa et al., 2012). Majority of the phytochemicals having potent AChE and BChE inhibitory effects, are alkaloids followed by terpinoids, steroids, flavonoids, glycosides, saponins and essential oils (Seyed et al., 2014). Since most of the natural or synthetic products, having enzyme inhibitory effects are known to contain nitrogen atom, the promising effect of the medicinal plants could be due to their high alkaloidal contents (Seyed et al., 2014).

*Alangium salvifolium* is rich with biologically active phytochemicals where various types of alkaloids have
been isolated and identified. Among these alangimaridine, mehy1-1H pyrimidine-2, 4-dione, alangine A and B, alangicine, markidine, lamarkcinine and emetine are important. Besides, phytochemical screening of it revealed the presence of flavonoids, phenolics, glycosides etc (Ashalatha and Gopinath, 2013; Ronok et al., 2013; Savithramma et al., 2013). So, these compounds may be considered for the antioxidant effect and enzyme (AcH and BcH) inhibitory activities of the extracts.

**Conclusion**

*A. salvifolium* wang is extensively used as folk medicine. The present study showed that the plant is important for its phytochemical constituents. It has significant amount of phenolics, flavonoid and flavonol. Root 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 disease. However, further analysis is necessary to isolate the key compounds and to find out the actual mechanism of action.

**Conflict of Interests**

The authors have not declared any conflict of interests.

**REFERENCES**

Asmat U, Abad K, Ismail K (2015). Diabetes mellitus and oxidative stress-A concise review. Saudi Pharm. J. In Press.

Braca A, Sortino C, Politi M (2002). Antioxidant activity of flavonoids from *Licania licaniae* flor. J. Ethnopharmacol. 79:379-38.

Classics EGL, Courtney KD, Andres V, Featherstone RM (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7:88-95.

Dipali OS, Vilas A, Kamble (2013). Phytochemical screening of some important medicinal plants. Inter. J. Pharm. Bio. Sci. 4(2):383-389.

Dominik S, Kamila B (2012). Screening for cholinesterase inhibitors in selected fruits and vegetables. Elec. J. Polish Agric. 15 (2):06.

Fadwa C, Jihed B, Amira L, Aicha N, Somaya K, Kamel G, Leila C (2012). Antioxidant, genotoxic and antigenotoxic activities of *Daphne gnidium* leaf extracts. BMC Complement. Altern. Med. 12:153.

Faiyaz A, Raza MG, Saikala P, Mueen AK (2013). Cholinesterase inhibitors from botanicals. Pharmacogn. Rev. 7(14):121-130.

Gao X, Ohlander M, Jeppsson N, Björk L, Trajkovski V (2000). Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophae rhamnoides*) during maturation. J. Agric. Food Chem. 48:1485-1490.

Gjumrakch A, Mark EO, Prakash RV, Justin CS, Paul M, Akihiko N, Xiongwei Z, Mark AS, George P (2008). Antioxidant therapy in alzheimer’s disease: Theory and practice. Mini Rev. Med. Chem. 8(13):1395-406.

Gopinath SM (2013). Broad spectrum antimicrobial activities and phytochemical analysis of *Alangium salvifolium* flower extract. Global J. Res. Med. Plants Indigen. Med. 2(3):135-141.

Harborne JB (1973). Phytochemical methods. London: Chapman and Hall, Ltd. pp. 49-188.

Iwaki H, Namoto M (2014). The adverse effects of anticholinergic drugs. Brain Nerve 66(5):551-560.

Jiaoqiao Z, Yicun C, Fen Y, Wei Zhou C, Ganggang S (2012). Chemical composition and antioxidant/antimicrobial activities in supercritical carbon dioxide fluid extract of *Gloiopters tenax*. Mar. Drugs 10(12):2634-2647.

Kumar KS, Ganesan K, Rao P (2008). Antioxidant potential of solvent extracts of *Kappaphycus alvarezii* (Doly) Doty-An edible seaweed. Food Chem. 107:289-295.

Kumaran A, Karunakaran RJ (2007). *In vitro* antioxidant activities of methanol extract of *Phyllanthus* species from India. LWT- Food Sci. Technol. 40:344-352.

Lauru B, Giovanna C, Cristina L, Giulia F, Chiara P, Stefano G, Erica B, Marco R, Maria B, Maria S, Aleksandra S, David AB, Maurizio M, Daniele P (2012). Conformational altered p53 as an early marker of oxidative stress in alzheimer’s disease. PLOs ONE 7(1):1-11.

Lin HM, Tseng HC, Wang CJ, Lin JJ, Lo CW, Chou FP (2008). Hepatoprotective effects of *Solanum nigrum* Linn extract against CCI(4)-induced oxidative damage in rats. Chem. Biol. Interact. 171(3):283-293.

Lobo V, Patil A, Phatak A, Chandra N (2010). Free radicals, antioxidants and functional foods: impact on human health. Pharmacogn. Rev. 4(8):118-126.

Mbaebie B, Edogha H, Afolayan A (2012). Phytochemical analysis and antioxidants activities of aqueous stem bark extract of *Schotia latifolia* Jacq. Asian. Pac. J. Trop. Biomed. 2(2):118-124.

Meera R, Shabina S, Devi P, Venkataraman S, Parameswari PT, Nagarajan K, Aruna A (2013). Anti-hyperglycemic effect of aqueous and ethanolic extracts of leaf and stem bark of *Alangium salvifolium* (L.F.) Wang in alloxan induced diabetic rats. Inter. J. Pharm. Res. Allied Sci. 2(4):28-32.

Oyaizu M (1986). Studies on product of browning reaction prepared from glucose amine. Jpn. J. Nutr. 44:307-315.

Prito P, Pineda M, Aguilar M (1999). Spectrophotometric quantitative of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem. 269:337-341.

Rahmat AK, Muhammad RK, Sumaira S (2012). Brain antioxidant markers, cognitive performance and acetylcholinesterase activity of rats: efficiency of *Sonchus asper*. Behav. Brain Funct. 8:21.

Ronok Z, Laizuman N, Luthfun NM (2013). Antinociceptive and anti-inflammatory activities of flower (*Alangium salvifolium*) extracts. Pak. J. Biol. Sci. 16(19):1040-1045.

Savithramma N, Ling RM, Ankanna S (2012). Preliminary phytochemical screening of some selected Nigerian medicinal plants. Int. J. Bio. Sci. 16(19):1040.

The adverse effects of anticholinergic drugs. PLOS ONE 7(1):1-11.

Taiwo OE, Efere MO, Afolake TS, Joseph M A, Saburi AA (2010). Antioxidant activities of flower (Alangium salvifolium) against the inflammatory pathway. Plos one 8(6):1-15.

Tiwro OE, Etere MO, Afolake TS, Joseph M A, Saburi AA (2010). Acetylcholinesterase and butyrylcholinesterase inhibitory activity of some selected Nigerian medicinal plants. Brazil. J. Pharmacogn. 20(4):472-477.

Trease GE, Evans WC (1989). Pharmacognosy. 11th edition. London: Brailiar Tiried Can Macmillan Publishers. pp. 60-75.

Uthirasevilam M, Asmathu FS, Peer MH, Babu SM, Kavitha G (2012). Pharmacognostical studies on the medicinal plant - *Alangium salvifolium* (Linn. F) Wang. (Alangiaceae). Asian J. Plant Sci. Res. 2(6):670-674.
Varcin M, Bentea E, Michotte Y, Sarre S (2012). Oxidative stress in genetic mouse models of Parkinson’s disease. Oxid. Med. Cell. Longeve. 624925.
Yongxin Z, Jun D, Tao W, Haji AA (2013). Investigating the antioxidant and Acetylcholinesterase inhibition activities of Gossypium herbaceum. Molecules 18:951-962.

Zeb A, Hammed A, Khan L, Khan I, Dalvandi K, Choudhury MI, Basha FZ (2014). Quinoxaline derivatives: Novel and selective butyrylcholinesterase inhibitors. Med. Chem. 10(7):724-729.