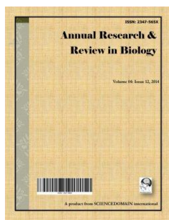

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Evaluation of Antioxidant and Cholinesterase Inhibitory Activities of *Hoya parasitica* Variegata - An *In-vitro* Study

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Authors' contributions

This work was carried out in collaboration between all authors. Author IJB designed and wrote the research protocol. Authors SBF and MA performed the experiments, managed the literature searches and wrote the manuscript. Overall review of the manuscript has done by author IJB. Author IJB performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Oxidative stress is responsible for the development of different neurological disorders such as Alzheimer's disease (AD). In the present study, *Hoya parasitica* variegata belonging to the family Apocynaceae was evaluated for its cholinesterase inhibitory and antioxidant activities with an objective of searching a new natural source to treat different neurological disorders.
Study Design: The methanolic extract of *H. parasitica* was subjected for *in-vitro* evaluation which included antioxidant and cholinesterase inhibitory activities.
Place and Duration of Study: Department of Pharmacy, Southeast University, Banani, Dhaka-1213, Bangladesh, from July to December 2016.
Methodology: The cholinesterase inhibitory and antioxidant activities were assessed by total phenol content, flavonoid content, total antioxidant, iron reducing power capacity, DPPH and hydroxyl radical scavenging capacity, lipid peroxidation inhibition, metal chelating activity as well as acetylcholinestrase (AChE) and butyrylcholinestrase (BChE) inhibitory activities.

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Results: Total phenolic and flavonoid content of the extract were 53.31 mg of gallic acid and 129.88 mg of quercetin equivalent respectively. The methanolic extract of *H. parasitica* (MEHP) showed considerable total antioxidant activity and reducing capacity. In DPPH and hydroxyl radical scavenging assay, the MEHP showed IC_{50} of 485 μg/mL and 39.65 μg/mL respectively. In lipid peroxidation inhibition activity MEHP showed IC_{50} value of 857.63 μg/mL and exhibited metal chelating activity with IC_{50} value 961.21 μg/mL. The MEHP represented inhibition of bovine brain acetylcholinesterase and human blood butyrylcholinesterase and the IC_{50} value was 269.5 μg/mL and 343.14 μg/mL respectively.

Conclusion: The results obtained from present study revealed that MEHP has considerable amount of antioxidant activity as well as anti-acetylcholinesterase and anti-butyrylcholinesterase activity suggesting its potential use in different neurological disorders such as AD.

Keywords: Antioxidant; oxidative stress; free radicals; acetylcholinesterase; butyrylcholinesterase; alzheimer's disease; Hoya parasitica.

1. INTRODUCTION

Alzheimer's disease (AD) is a chronic neurodegenerative disease that usually starts slowly and worsens over time [1,2]. Abnormal pathologic features of Alzheimer's disease include extracellular amyloid plaques and intraneuronal neurofibrillary tangles as well as a decrease in levels of the neurotransmitter acetylcholine (Ach) [3]. These changes result in the development of the typical symptoms of AD characterized by gross and progressive damage of cognitive function, often accompanied by behavioral disturbances such as memory loss, aggression, depression, and wandering [4].

These phenomena are mainly initiated and enhanced by oxidative stress, a process referring to an imbalance between antioxidants and oxidants in favor of oxidants. This imbalance can occur as a result of increased free radicals or a decrease in antioxidant defense, free radicals being a species that contains one or more unpaired electrons in its outer shell. The major source of potent free radicals is the reduction of molecular oxygen in water that produces the superoxide radical first, then hydrogen peroxide and finally highly reactive oxygen species (ROS) by reduction. This ROS can react with lipids, proteins, nucleic acids, and other molecules and may also alter their structures and functions. Thus, tissues and organs, particularly the brain are affected by ROS due to its composition with oxidizable lipids [5].

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are responsible for the metabolic hydrolysis of acetylcholine (ACh) at cholinergic synapses. AChE inhibitors such as Donepezil, Rivastigmine, and Galanthamine promote increase in the concentration and duration of action of synaptic Ach [6], thus helping as treatment strategy for AD, senile dementia, ataxia, myasthenia gravis and Parkinson's disease [7].

But non-selectivity, limited efficacy, poor bioavailability and adverse cholinergic side effects in the periphery, such as nausea, vomiting, diarrhoea, dizziness, and hepatotoxicity are the several limitations of these drugs [1]. Therefore researchers continue the search for better AChE inhibitors from natural sources including plants [7, 8].

H. parasitica variegata commonly known as waxvine, waxflower or simply Hoya, porcelain flower or bayupriya, is an evergreen tropical perennial shrub native to tropical wet forests and humid climate of southern Asia (India cast to southern china and southward), Australia, and Polynesia. The plant is traditionally used as antirheumatic and in acute renal failure [9]. The ethanol extract showed the presence of flavonoids, reducing sugars, tannins, gums and saponins [10]. An androstanoid, hoyasterone, a sesquiterpene, 15-bulnesolic acid, and a phenolic compound, 1-(4-hydroxy-3-methoxyphenyl)-1-methoxypropan-2-ol, together with a known triterpene, dihydrocanaric acid, were isolated from *H. parasitica* [11]. It is reported that the plant contains triterpenic 3, 4-seco acid 3, 4-secolup-20(29)-en-3-oic acid, along with lupeol and lupenone from stem [12]. However, no antioxidant and anti cholinesterase activity has been reported so far on this plant. Thus, our main objective of the present study was to evaluate the antioxidant and neuroprotective potential of *H. parasitica* to treat the AD and other neurodegenerative diseases.

2. MATERIALS AND METHODS

2.1 List of Chemicals

Folin–Ciocalteu reagent, Methanol, Gallic acid, Ascorbic acid, DPPH, 2-deoxy-D-ribose, Thiobarbituric acid (TBA), (+)-Catechin, 5,5´-dithio-bis-(2-nitro) benzoic acid (DTNB), Acetylthiocholine iodide, S-Butyrylthiocholine iodide, Donepezil, Ferrozine monosodium,Trichloro acetic acid (TCA) and Triton X-100 were purchased from Sigma-Aldrich, USA. Butylated hydroxyl toluene (BHT) and Tris–HCl buffer were purchased from Merck, Germany.

2.2 Collection and Authentication of Plant Material

The plant sample of *H. parasitica* leaves were collected in September, 2015 from Sylhet hill track, Bangladesh. The plant was identified by Bangladesh National Herbarium, Dhaka, where a voucher specimen has been deposited (DACB Accession no. 42022). Plants were then washed properly to remove dirty materials and shade dried for several days with occasional sun drying. These were then dried in an oven for 24 hours at considerably low temperature (not exceeding 30°C to 40°C) for better grinding. The dried plants were ground into coarse powder.

2.3 Extraction of Plant Material

About 500 g of powdered plant was taken in a flat bottom glass container and soaked in methanol. The container with its content was sealed with aluminum foil and kept at room temperature for a period of 7 days accompanying occasional shaking and stirring. The extract was filtered through fresh cotton plug followed by Whatman No.1 filter paper (Bibby RE-200, Sterilin Ltd., UK). The filtrate (methanol extract) obtained was evaporated by Rotary evaporator (RE300, Stuart, Japan) at 5 to 6 rpm and at 50°C temperature. The resultant concentrated extract was evaporated to dryness and the dark brown residues then stored in refrigerator as methanol extract of *H. parasitica* (MEHP) for further use.

2.4 Determination of Phytoconstituents

2.4.1 Determination of total phenolics

Total phenolic content of *H. parasitica* was determined with minor modifications of the method of Singleton using Folin-Ciocalteu reagent [13]. Each test tube contained 0.5 mL of plant extract (prepared by dissolving 0.1 mg of MEHP in 10 mL distilled water) or standard Gallic acid solution at different concentrations 2.5 mL of Folin–Ciocalteu reagent solution (10 times diluted with water) and 2.5 mL of sodium carbonate (7.5%) solution. After adding all of the reagents the test tubes were incubated for 25 min at 25°C to complete the reaction and the absorbance of the solution was measured at 760 nm. A standard curve was prepared using Gallic acid as standard ($Y = 0.0151x + 0.059$, $R^2 = 0.9913$) for expressing the total content of phenol compounds in plant extract and shown as mg of Gallic acid equivalent (GAE)/g of dried extractives and the experiments was repeated for three times.

2.4.2 Determination of total flavonoids

Total flavonoid content was determined by the aluminum chloride colorimetric method described by Kumaran and quercetin was used as standard [14]. In 3 mL of methanol, 1.0 mL aliquot of *H. parasitica* or standard of different concentrations, 0.2 mL of 10% $AlCl_3$, 0.2 mL of 1 M potassium acetate and 5.6 mL of distilled water were added in test tubes. After incubation for 25 min the absorbance was taken at 420 nm. A quercetin standard curve was prepared ($Y = 0.009x + 0.036$, $R^2 = 0.972$) to express the result as mg of quercetin equivalent (QE)/g of dried extractives.

2.5 Antioxidant Ability Assay

2.5.1 Determination of total antioxidant capacity

Total antioxidant capacity was determined according to the method as described by Prieto with some modifications [15]. In this experiment, 0.5 mL aliquot of *H. parasitica* or standard (Catechin) of different concentrations (100 – 1000 µg/mL) was added to 3 mL of reaction mixture (containing 0.6 M sulphuric acid, 28 mM sodium phosphate and 1% ammonium molybdate) into the test tube. For completing the reaction the test tubes were incubated for 15 min at 90°C. Finally by cooling at room temperature the absorbance was measured at 695 nm.

2.5.2 Reducing power capacity assessment

The reducing power was evaluated by the method of Oyaizu [16]. In this method, various concentrations of MEHP or standard solutions

(1.0 mL) were mixed with 2.5 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1%) solution and 2.5 mL of potassium buffer (0.2 M, pH 6.6). Incubation carried out at 50°C, after 30 min of incubation 2.5 mL of trichloro acetic acid (10%) solution was added into the test tube. The total mixture was centrifuged at 3000 g for 10 min. Then 2.5 mL supernatant solution was taken from the mixture and mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1 %) solution. Then, the absorbance of the solution was measured at 700 nm and ascorbic acid (AA) was used as standard.

2.5.3 Determination of DPPH radical scavenging activity

DPPH radical scavenging activity was determined according to the method as described by Choi [17]. A 2 mL aliquot of *H. parasitica* or standard (AA) at different concentration was mixed with 3 mL (0.02 %) of methanol solution of DPPH. We kept the mixture in dark place for 30 min to complete the reaction, the absorbance was taken at 517 nm against methanol as blank.

2.5.4 Determination of hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was determined by the method of Elizabeth [18]. Hydroxyl radical was generated by the Fe^{3+}-ascorbate-EDTA-H_2O_2 system (the Fenton reaction). A 1 mL reaction mixture was prepared by adding 2-deoxy-D-ribose (2.8 mM), KH_2PO_4-KOH buffer (20 mM, pH 7.4), $FeCl_3$ (100 µM), EDTA (100 µM), H_2O_2 (1.0 mM), AA (100 µM) and various concentrations of MEHP or reference compound [(+) - catechin)]. After incubation for 1 h at 37°C, 0.5 mL of the reaction mixture was mixed with 1 mL of 2.8% TCA and 1 mL of 1% aqueous TBA, to develop the color the mixture was incubated at 90°C for 15 min. After cooling, the mixture's absorbance was measured at 532 nm against an appropriate blank solution.

2.5.5 Determination of metal chelating activity

The chelating activity of MEHP for ferrous ion (Fe^{2+}) was measured according to the method of Sabate [19], using ferrozine (substrate) and ferrous chloride ($FeCl_2$). In this method, 0.5 mL of extract or standard was added to 1.6 mL of $FeCl_2$ (2 mM). After incubation for 30 s, 0.1 mL ferrozine (5 mM) was added and kept 10 min at room temperature then the absorbance of the Fe^{2+} – Ferrozine complex was measured at 562 nm. A typical blank solution contained all reagents except plant extract or standard (EDTA) solution.

2.5.6 Determination of lipid peroxidation inhibition activity

The inhibition of lipid peroxidation activity was determined according to the method as described by Liu [20], with a slight modification. The brain of bovin was dissected and homogenized with a homogenizer in ice-cold Phosphate buffer (50 mM, pH 7.4) to produce a 1/10 homogenate. The homogenate was centrifuged at 10,000 g for 30 min at 4°C. The supernatant was used as liposome for in vitro lipid peroxidation assay. The ability of MEHP to inhibit lipid peroxidation was studied by incubating bovin brain homogenates treated with hydrogen peroxide (10 µM) and different concentrations of extract or standard solution. Hydrogen peroxide induced lipid peroxidation in bovin brain homogenates. 1 mL of 0.15 M KCl and 0.5 mL of liposome containing brain homogenate were added with different concentrations of plant extract or standard solution. The reaction was started by adding 100 µl of 0.2 mM ferric chloride with the above mentioned mixture then incubated at 37°C for 30 min. The reaction was stopped by adding 2 mL of 0.25 N HCl, 15% TCA, 0.5% BHT and 0.38% TBA solution. Lipid peroxides reacted with TBA to form a pink product, thiobarbituric acid reacting substances (TBARS), measurable absorbance at 532 nm. The difference between the control and the test sample is the measurement of decrease in TBARS formation, reflecting reduced hydroxyl radical induced lipid peroxidation. As standard for comparison we used (+)-Catechin.

2.6 Determination of AChE Inhibitory Activity

The AChE inhibitory activity was performed according to the method of Doctor and Ellman [21,22] using acetylthiocholine iodide as a substrate. For the enzyme source, the bovin brains were homogenized in a homogenizer with 5 volumes of a homogenization buffer [10 mM Tris-HCl (pH 7.2), which contained 50 mM $MgCl_2$, 1% Triton X-100 and 1 M NaCl], and centrifuged at 10,000 *g* for 30 min. The resulting supernatant was used as an enzyme source. All

of the extraction steps were carried out at 4°C. Protein concentration was determined using the BCA kit (bicinchoninic acid; Sigma Co., USA) with bovine serum albumin (BSA) as a protein standard. Each MEHP or standard solution (500 µl) was mixed with an enzyme solution (500 µl). After incubation at 37°C for 15 min the absorbance was measured at 412 nm immediately after adding the Ellman's reaction mixture (3.5 mL; 0.5 mM acetylthiocholine iodide, 1 mM DTNB) in a 50 mM sodium phosphate buffer (pH 8.0) to the above reaction mixture. Reading was repeated for 10 min at 2 min intervals to verify that the reaction occurred linearly. The blank reaction was measured by substituting saline for the enzyme. Donepezil was used as standard.

2.7 Determination of BChE Inhibitory Activity

The BChE assay was performed according to the method of Ellman and Doctor [21, 22] with some modifications using s-butyrylthiocholine iodide as a substrate. For the enzyme source, the human blood was homogenized in a homogenizer with 5 volumes of a homogenization buffer [10 mM Tris–HCl (pH 7.2), which contained 50 mM $MgCl_2$, 1% Triton X-100 and 1 M NaCl], and centrifuged at 10,000 g for 30 min. The resulting supernatant was used as an enzyme source. All of the extraction steps were carried out at 4°C. The rates of hydrolysis by BChE were monitored spectrophotometrically. Each MEHP or standard solution (500 µl) was mixed with an enzyme solution (50 µl) and incubated at 37°C for 15 min. Absorbance at 412 nm was read immediately after adding the Ellman's reaction mixture (3.5 mL; 0.5 mM S-butyrylthiocholine iodide, 1 mM DTNB) in a 50 mM sodium phosphate buffer (pH 8.0) to the above reaction mixture. Reading was repeated for 10 min at 2 min intervals to verify that the reaction occurred linearly. The blank reaction was measured by substituting saline for the enzyme. Donepezil was used as standard.

2.8 Calculations and Statistical Analysis

The percentage inhibitions or scavenging of DPPH radicals, hydroxyl radicals, metal chelating, lipid peroxidation, AChE and BChE inhibitory activity of the MEHP were calculated by using the formula:

$$\text{Percentage inhibition or scavenging} = \{(Ao-A1)/Ao\} \times 100$$

Where,

A_0 is the absorbance of the control, and
A_1 is the absorbance of the extract/standard.

All the experiments were carried out for three times and the results were calculated as the mean of three replicates. The percentage inhibition or scavenging was calculated by above mentioned equation. By plotting % inhibition or scavenging against the concentration, the IC_{50} value (the concentration of the extract required to scavenge 50% of radicals or to inhibit 50% of enzyme activity) was calculated for the standard and MEHP. The IC_{50} values of different studies shown in Table 1.

3. RESULTS AND DISCUSSIONS

3.1 Determination of Phytoconstituents

3.1.1 Total phenolics

According to pharmacological studies, the prolonged use diet containing poly phenols can provide protection from many chronic diseases including AD [23]. By using Folin-Ciocalteu reagent the total phenolic content of MEHP was assessed. Due to presence of hydroxyl group phenolic compounds possess primary antioxidant property with free radical neutralizing activity [17]. Gallic acid was used as standard for calculating the total phenolic content of the sample was calculated and the results were expressed as mg of gallic acid equivalent (GAE)/g of dried extractives. The phenolic content of MEHP was 53.31 mg of GAE/g of dried extract. In a previous study, the phenolic content of methanolic fruit extract of *P. acidus* (Family- Euphorbiaceae) was found to be 58.12 mg of GAE/g of dried extract. MEHP's phenolic content is much similar with amount of *P. acidus* [24].

3.1.2 Total flavonoids

Flavonoids are well known compound used as antioxidants because of their radical scavenging property, it also has metal ion chelating and lipid peroxidation inhibiting activities. Flavonoids may cure or slow down the progression of AD by interfering with the production and polymerization of amyloid-β peptides into neurotoxic oligomeric aggregates and also by decreasing aggregation of tau proteins [25]. Total flavonoids content of MEHP was assessed by using aluminum chloride colorimetric method. Flavonoid content of the

samples was calculated on the basis of the standard curve for quercetin and the results were expressed as mg of quercetin equivalent (QE)/g of dried extractives. The flavonoid content of MEHP was 129.88 mg of QE/g of dried extract which is less in amount with the flavonoid content of methanol fruit extract of *P. acidus* reported earlier which is 168.24 mg of QE/g of dried extract [24].

3.2 Antioxidant Activity

3.2.1 Total antioxidant capacity

By phosphor-molybdenum method, total antioxidant capacity of MEHP was determined. This method was based on the lightening of Mo (VI) to Mo (V) by the antioxidant compound and the formation of green phosphate/ Mo (v) complex with a maximal absorption at 695 nm [26]. The MEHP showed considerable antioxidant activity compared to catechin. At the concentration 1000 µg/mL, the absorbance of MEHP and catechin were 0.825 and 1.694 respectively. At 500 µg/mL concentration, the absorbance of MEHP and catechin were only 0.486 and 1.043 respectively as depicted in Fig. 1. From the figure we can presume that *H. parasitica* plant may have antioxidant capacity [24].

3.2.2 Reducing power capacity

The iron reducing capacity of a compound is an effective indicator of its potential antioxidant activity because of its ability to break the free radical chain through release of a hydrogen atom. By the method of Oyaizu, the Fe^{3+} reducing power of MEHP was assessed [16]. The reducing activity of MEHP is less than that of AA (a standard antioxidant). The extract showed 0.338 absorbance at 1000 µg/mL, where AA showed much higher 3.922 µg/mL which shown in Fig. 2. From this figure

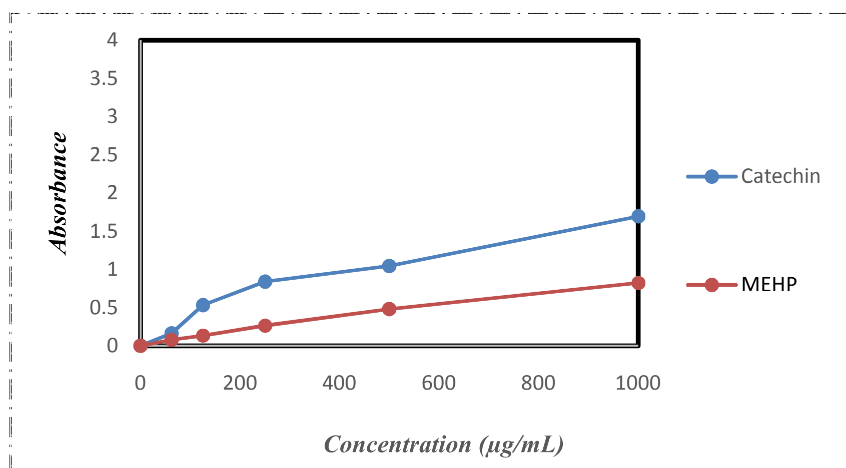

Fig. 1. Total antioxidant activity of the MEHP and catechin at different concentrations

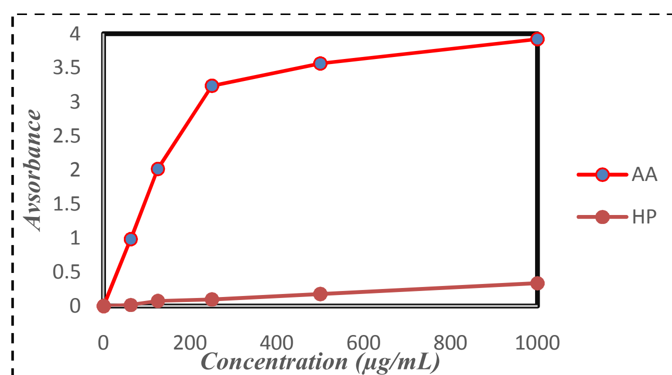

Fig. 2. Reducing power capacity of the MEHP and ascorbic acid at different concentrations

comparing the absorbance against concentration it was observed that *H. parasitica* has poor reducing power capacity.

3.2.3 DPPH radical scavenging activity

Radical scavenging activities of antioxidants are very important to obstruct the noxious role of free radicals in AD. Based on the ability of DPPH- a stable free radical, to decolorize in the presence of antioxidants [27]. The antioxidant activity of the extract of MEHP was evaluated by DPPH radical scavenging activity. The results of DPPH radical scavenging activity of MEHP and Ascorbic acid (AA) as standard are shown in Fig. 3, and the scavenging activity of the MEHP was found less than that of AA. The IC_{50} of AA and MEHP were 50.6 µg/mL and 485 µg/mL respectively shown in Table 1. These results suggest that MEHP has moderate radical scavenging activity.

3.2.4 Hydroxyl radical scavenging activity

By incubating Fe^{3+}-EDTA with H_2O_2 and (+) catechin at pH 7.4, hydroxyl radicals can be generated in free solution and detected by their ability to degrade 2-deoxy-2-ribose into fragments that at low pH forms a pink chromogen by heating with TBA [28,29]. In the hydroxyl radical scavenging activity the ability of *H. parasitica* to remove the formed hydroxyl radical in solution was evaluated quantitatively by colorimetric method. When plant extract and the reference compound, (+)-catechin, were added to the reaction mixture, they removed hydroxyl radicals from the sugar and prevented degradation. The reaction mixture decolorized pink chromogen due to removal of hydroxyl radicals which can be quantitatively measured from the change in absorbance at 532 nm. The results demonstrate that the MEHP possesses hydroxyl radical scavenging activity in comparison with the standard (+)-catechin and the activity increased by increasing the concentration of the plant extractive which shown in Fig 4. IC_{50} value of MEHP and (+)-catechin were 15.83 µg/mL and 39.65 µg/mL respectively shown in Table 1. The hydroxyl radical scavenging activity of methanolic fruit extract of *P. acidus* was performed in a previous study and the result demonstrated that MEHP has nearly similar hydroxyl radical scavenging activity like *P. acidus* [24].

3.2.5 Metal chelating activity

Oxidative damage of brain found in AD patients which is due to free radical reactions can be catalyzed by Fe^{2+} According to the method as described by Sabate the chelating activity of MEHP for ferrous ions was determined [19]. In this method free iron binds in the blood stream and increasing metal elimination in the urine which reduces the damage done to various organs and tissues, such as the liver and nerves and estimates chelating using ferrous chloride ($FeCl_2$) and Ferrozine (substrate). The inhibitory activity of MEHP enhanced with increasing concentration and the highest activity (47.89 %) was obtained at 1000 µg/mL concentration but at the same concentration EDTA (Standard) gave

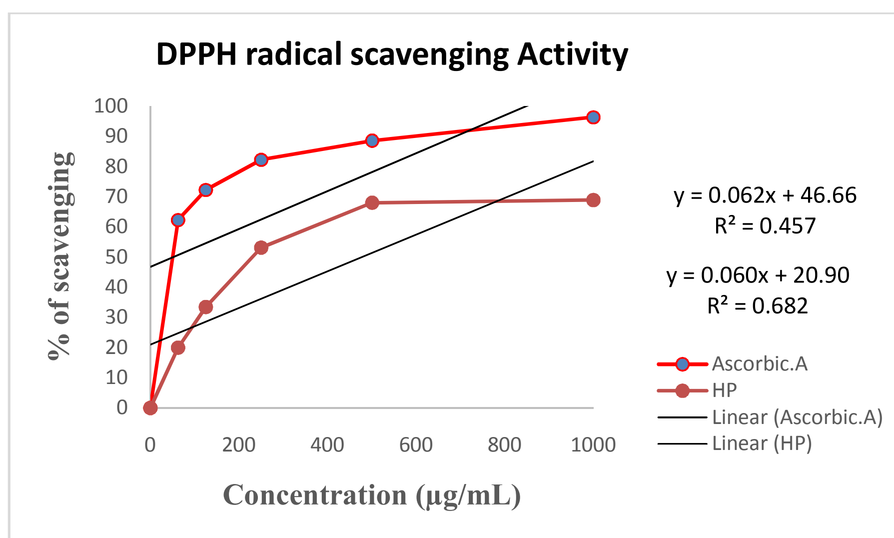

Fig. 3. DPPH radical scavenging activity of MEHP and AA at different concentrations

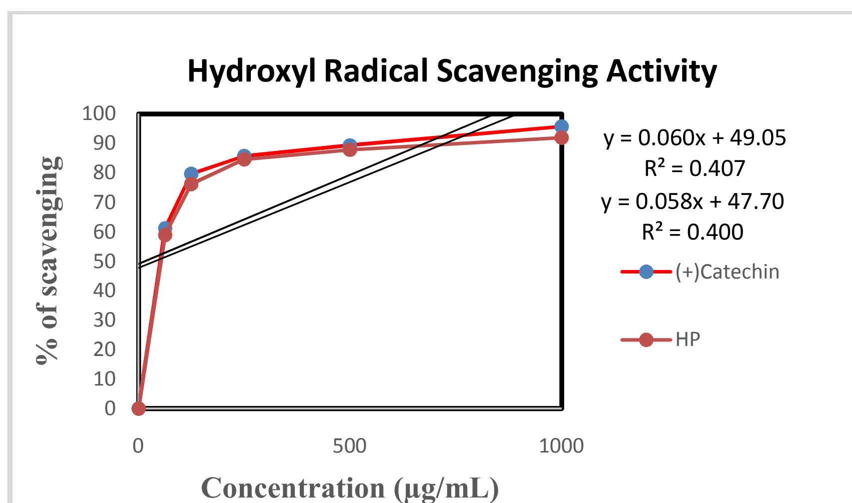

Fig. 4. Hydroxyl radical scavenging activity of MEHP and (+)-catechin at different concentrations

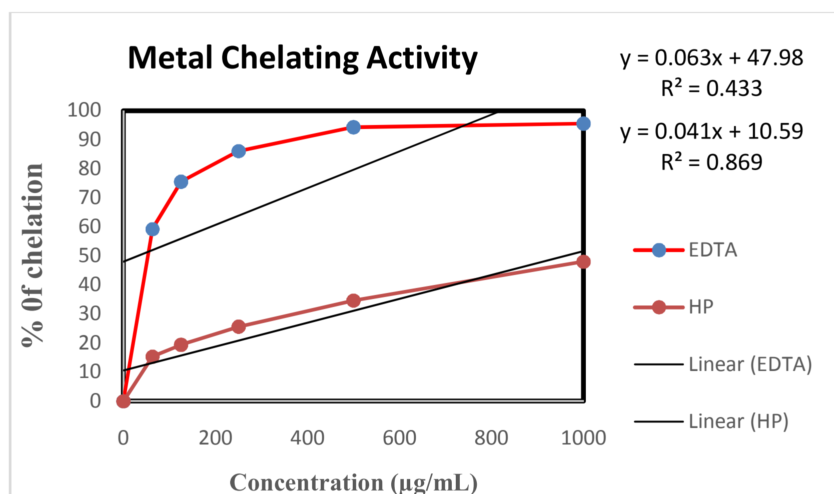

Fig. 5. Metal chelating activity of MEHP and EDTA at different concentrations

95.68% inhibitory activity that shown in Fig. 5. The IC_{50} value of EDTA and MEHP were 32.06 µg/mL and 961.21 µg/mL respectively shown in Table 1. In comparison with a previous report on metal chelating activity of methanolic fruit extract of *Phyllanthus acidus* and with standard EDTA, MEHP has low ferrous ion chelating activity [24].

3.2.6 Lipid peroxidation inhibition activity

Reactive oxygen species generated by ultraviolet light, ionizing radiation, chemical reactions, and metabolic processes have many pathological effects, such as causing lipid peroxidation, protein peroxidation, DNA damage, and cellular degeneration related to a variety of diseases including AD [30,31]. Lipid peroxidation has been reported to be elevated in the brain of AD. During lipid peroxidation, low molecular weight end products, generally malonaldehyde, are formed by oxidation of poly-unsaturated fatty acids that may react with two molecules of thiobarbituric acid to give a pinkish red color [32,26]. In the lipid peroxidation inhibition activity, the activity of MEHP against non-enzymatic lipid peroxidation in bovin brain homogenate was evaluated. Addition of Fe^{2+}- ascorbate to the brain homogenate caused an increase in lipid peroxidation which can be quantitatively assessed from the change in absorbance at 532 nm and % of inhibition activity of different concentrations were shown in Fig. 6. The IC_{50}

value of (+)-catechin and MEHP were 115.92 µg/mL and 857.63 µg/mL respectively shown in Table 1. The lipid peroxidation inhibition activity of methanolic fruit extract of *Phyllanthus acidus* was performed in an earlier study and the results report that MEHP has much higher inhibitory activity against lipid peroxidation [24].

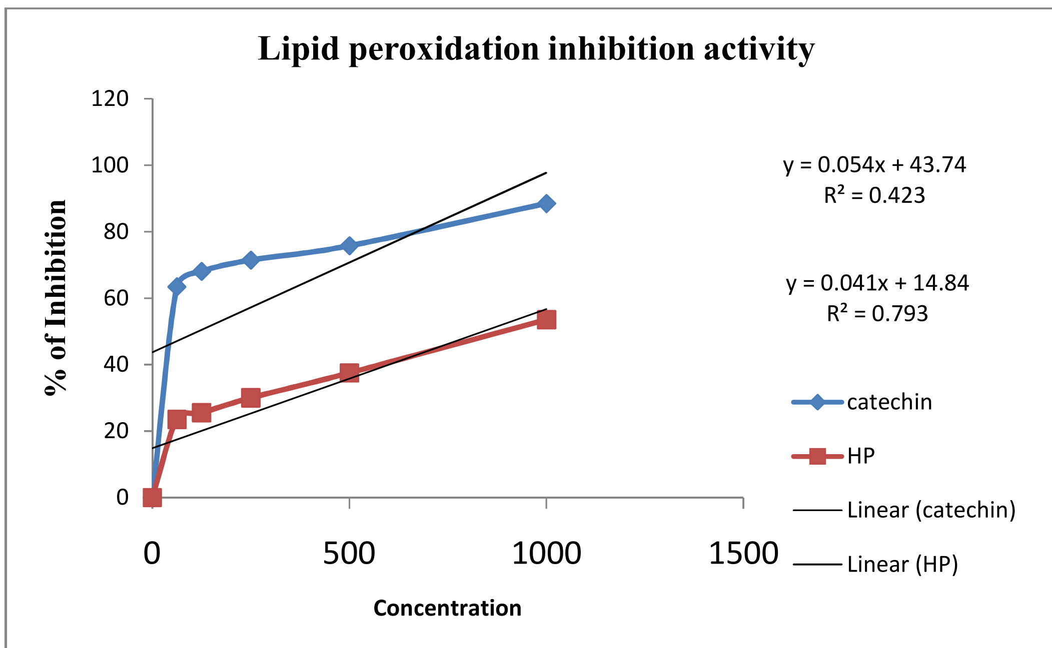

Fig. 6. Lipid peroxidation inhibition activity of MEHP and (+)-catechin at different concentrations

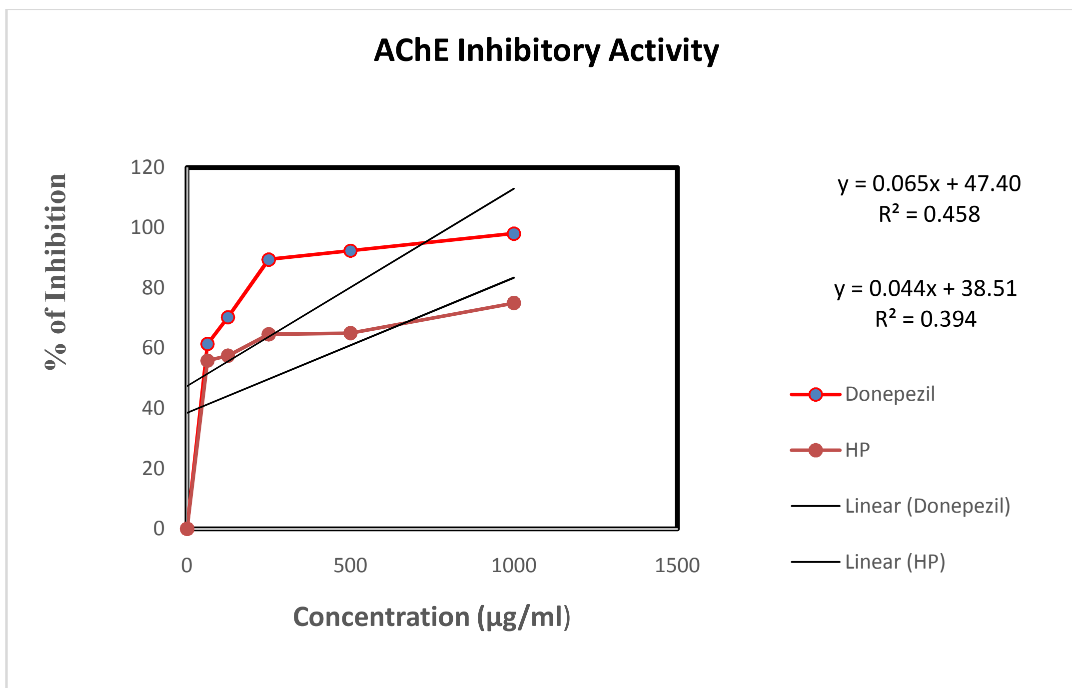

Fig. 7. AChE inhibitory activity of MEHP and Donepezil at different concentrations

3.3 AChE Inhibitory Activity

Preventing break down of ACh by the inhibition of AChE is responsible for the elevation of ACh level in the synaptic cleft is the most significant changes observed in AD [18]. The inhibitory activity of MEHP against rat brain AChE was determined by Ellman's method [22]. This method estimates the level of AChE using acetylthiocholine iodide (substrate) and DTNB. The enzymatic activity was measured by the yellow color compound produced by thiocholine when it reacts with dithiobisnitro benzoate ion. The inhibitory activity of MEHP increased with increasing concentration and the highest activity was obtained at 1000 µg/mL which is 75% but, at the same conditions donepezil gave 98.03% inhibitory activity shown in Fig. 7. The IC_{50} value of donepezil and MEHP were 40.00 µg/mL and 269.5 µg/mL respectively shown in Table 1. The AChE inhibitory activity of aqueous extract of *P. acidus* was reported in an earlier study [30]. The comparison between IC_{50} values of aqueous extract of *P. acidus* and MEHP demonstrates that it has much higher acetylcholinesterase inhibitory activity than *P. acidus* [24].

3.4 BChE Inhibitory Activity

The inhibitory activity of MEHP against human blood BChE was measured by Ellman's method with some modifications [22]. This method estimates BChE using DTNB and s-butyrylythiocholine iodide (substrate). The enzymatic activity was assessed by the yellow colour compound produced by thiocholine when it reacts with dithiobisnitro benzoate ion. The inhibitory activity of MEHP increased with increasing concentration and the highest activity was obtained at 1000 µg/mL which is 72.22% but, at the same conditions donepezil gave 96.03% inhibitory activity that shown in Fig. 8. The IC_{50} value of donepezil (Standard) and MEHP were 14.16 µg/mL and 343.14 µg/mL respectively shown in Table 1. The comparison between IC_{50} values of aqueous extract of *P. acidus* and MEHP demonstrates that it has lower BChE inhibitory activity than *P. acidus* [24].

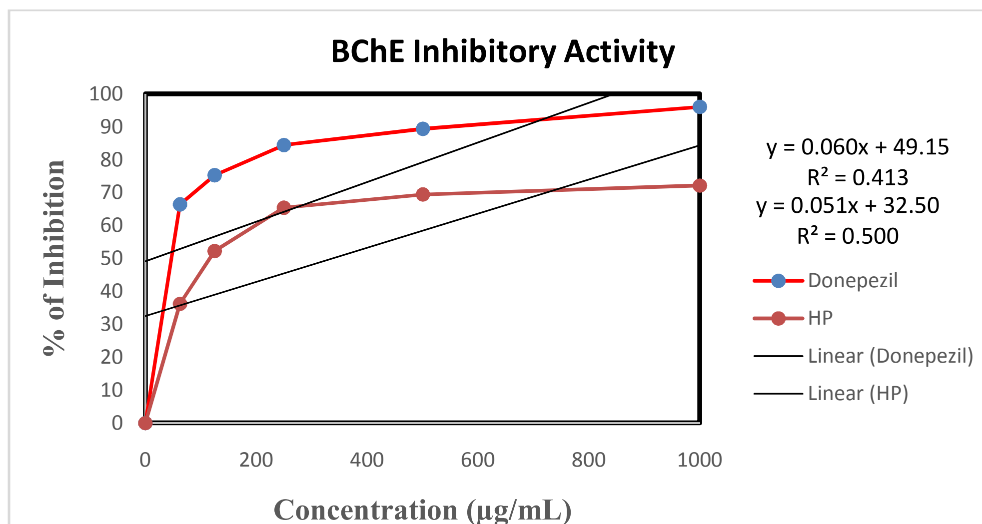

Fig. 8. BChE inhibitory activity of MEHP and Donepezil at different concentrations

Table 1. IC_{50} values obtained in the radical scavenging and enzyme inhibitory activity assays

Sample	IC_{50} (µg/mL)					
	DPPH scavenging	Hydroxyl radical scavenging	Metal chelating	Inhibition of lipid peroxidation	AChE	BChE
EDTA		-	32.06	-	-	-
(+)-Catechin	-	15.83	-	115.92	-	-
Donepezil	-	-	-	-	40.00	14.16
Ascorbic Acid	50.60					
MEHP	485.00	39.65	961.21	857.63	269.5	343.14

The IC_{50}value (the concentration of the extract required to scavenge 50% of radicals or to inhibit 50% of enzyme activity) was calculated for the standard and MEHP. The IC_{50} values of different studies shown in Table 1.

4. CONCLUSION

H. Parasitica has been used to treat a variety of diseases in Bangladesh as folk medicine. But there was no previous report about the antioxidant activity of this plant. We tried to investigate the antioxidant and cholinesterase inhibitory activity of MEHP. Our results clearly showed that MEHP had compound like phenol, flavonoides. It also has antioxidant capacity. It exhibited potential antioxidants and radical scavenging activities which are comparable with the standard drugs by scavenging various free radicals as well as effectively inhibits AChE and BChE activity. Hence, the methanolic extract from *H. parasitica* plant could be used as a health-care food supplement and in the pharmaceutical industry for the treatment of diseases resulting from oxidative stress like AD. Due to the presence of significant antioxidant activity of this plant, further studies are carried out for isolation and identification of lead compound(s) to cure the AD and other neurodegenerative diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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