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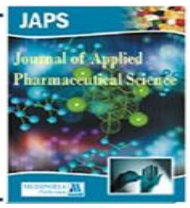


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# Evaluation of the Antioxidant and Anti-Cholineesterase activities of the Stem, Barks and Leaves of the Plant *Vernonia Cinerea* (Family: Asteraceae)

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**ABSTRACT**

The aim of this study was to evaluate the anti-oxidant and anti-choline esterase activity of the stem, barks and leaves of the plant *V. cinerea*. The stems, barks and the leaves of the plant *V.Cineria* was sun dried and extracted using methanol. The anti-oxidant activity of the crude methanolic extract was measured by the DPPH free radical scavenging activity. The crude methanolic extract showed significant anti-oxidant activity by the DPPH free radical scavenging method. Evaluation of ChEs enzyme activity of the crude methanolic extract was done by Ellman method. The methanolic extract of *V. cinerea* leaves exerted significant AChE and BChE inhibitory effects.

**Keywords:** *Vernonia cinerea*, anti-oxidant, DPPH, Kanamycin, acetylcholinesterase (AChE), butyryl-cholinesterase (BChE).

## INTRODUCTION

Plants represent a rich source of natural antioxidants (Halliwell *et al.*,, 1995). Many of the plant materials used in traditional medicines are readily available in rural areas at relatively cheaper than modern medicines (Mann *et al.*,, 1985). Plants generally produce many secondary metabolites which constitute an important source of microbicides, anti-oxidants and many pharmaceutical drugs used in traditional medicine. Many natural substances having anti-oxidant and anti-choline esterase activity have been used in health foods for medicinal and preservative purposes (Reynolds *et al.*,, 1985). *Vernonia cinerea* (Family: Asteraceae) is a terrestrial annual erect herb. It grows up to 80 cm high. It can be found in roadside, open waste places, dry grassy sites and in perennial crops during plantation. It is located especially in different Asian countries such as India, Bangladesh and Nepal. *V. cinerea* is an important medicinal plant having application in abortion, cancer and various gastrointestinal disorders (Yusuf *et al.*, 1994). The carbon tetrachloride fraction of methanolic extract of the plant possesses significant antioxidant properties (Kumar and Kuttan *et al.*, 2009) but whether this plant extract could affect anticholinesterase and thus finally be used for treating Alzheimer disease because of antioxidant property is not reported.

Therefore, the present study was designed to investigate the phytochemical bioactive compounds of the methanolic extract of *V. cinerea* and its antioxidative, anticholinesterage property.

## MATERIALS AND METHOD

### Collection of the plant sample

Fresh plant of *Vernonia cinerea* was collected from Gabtoli, Dhaka, Bangladesh in October, 2007. This plant was identified by Bangladesh National Herbarium. The reference sample for the plant was DACB Accession Number 32126.

### Preparation of plant extract

The stem-bark and leaves were sundried for 5 days. The plant materials were then oven dried for 24 hours at low temperature. 960 gm of powdered material (Stem-bark and leaves) was macerated with 7.5 L of methanol in two 4 L round bottom flask. The containers were sealed with cotton plug and aluminum foil at room temperature for 15 days with occasional shaking. The mixture was filtered through cotton and then evaporated to dryness ($45^0$C) under reduced pressure by rotary evaporator. The obtained crude extract was 49.54 grams.

### Evaluation of antioxidant activity

Brand-Williams (Brand-Williams *et al.*, 1995) method was used to estimate free radical scavenging activities of the methanolic extracts of stem-bark and leaves of the plant on stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH). 2.0 mg of the extracts was dissolved in methanol for the experiment. Solution of different concentrations such as 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.50 µg/ml, 31.25 µg/ml, 15.62 µg/ml, 7.8125 µg/ml, 3.91 µg/ml, 1.95 µg/ml and 0.98 µg/ml were obtained by serial dilution technique. 50 µl of methanol solution of the extract of each concentration was mixed with 5 ml of a DPPH-methanol solution (40µg/ml). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm and from these values the corresponding percentage of inhibitions were calculated by using the following equation:

$$\% \text{ inhibition} = [1- (ABS_{sample} / ABS_{control})] \times 100 \%$$

Where $ABS_{sample}$ is the absorbance of the sample material and $ABS_{control}$ is the absorbance of the control reaction (containing all reagents except the test material). Then percent inhibitions were plotted against respective concentrations. $IC_{50}$ values were calculated as the concentration of each sample required to give 50% DPPH radical scavenging activity from the graph. Tert-butyl-1-hydroxytoluene (BHT) and Ascorbic acid (AS) were used as positive control. The experiment was performed thrice and the result was expressed as mean ± Standard Error of Mean (SEM) in every case.

### Evaluation of ChEs enzyme activity

The inhibitory activities of stem-bark and leaves methanolic extract were measured in vitro by Ellman method (Eliman *et al.*, 1961) and the assay was done in triplicate. Acetylthiocholine iodide (ACh), butyrylthiocholine chloride (BCh) were used as the substrates to assay the inhibitory activities of acetylcholinesterase (AChE) and butyryl-cholinesterase (BChE) respectively. The assay contained 1 ml of 0.25 mM ACh or BCh and 0.25 mM DNTB in 50mM sodium phosphate buffer pH 8, 200µl of stem-bark and leaves extract in reaction concentration range of 0-250 µg/ml and 200 µL of AchE or BChE in different concentrations 0.01-0.243µg/ml. The final volume is adjusted to 3 ml with 50 mM sodium phosphate buffer pH 8.The tested sample and positive control Eserine was dissolved in 10% DMSO. With the help of AChE or BChE, hydrolysis of Ach and BCh lead to the formation of acetyl or butyl group and thiocholine as the product. Thiocholine then reacted with DTNB to form 5-thio2-nitrobenzoate which is a colored anion that absorbs UV at 412 nm. The absorbance was recorded every 1 min interval starting from 1 min. The rate of product formation was measured by the difference of absorbance in every 1 min time interval within 25 min. Then the product formation was calculated for each AchE and BchE concentration. The concentration of the extract that inhibited 50% of AchE activity [$IC_{50}$] was estimated by method by Alhomida et al (2000). The method was performed by plotting % activity and % inhibition of AchE or BchE versus extract [inhibitor] concentration on the same graph. The concentration at the intersection of these two curves was the $IC_{50}$ value.

### Statistical analysis

Results were presented as mean ±Standard Error of Mean (SEM) and the statistical analysis was done by using one way analysis of variance (ANOVA) followed by Tukey Post-hoc test. A p-value of $p < 0.05$ was considered to be statistically significant.

## RESULTS

### In vitro antioxidant activity

The antioxidant activity of the methanolic extract of *V. cinerea* was measured on the basis of its DPPH scavenging activity. The concentration of methanolic extract of the stem-bark and leaves needed for 50% scavenging ($IC_{50}$) of DPPH was found to be 82 ±3.40 µg/ml. Two positive controls were used- Butyl hydroxyl toluene (BHT) and Ascorbic acid (AS) for which the $IC_{50}$ values were found to be 22±0.39 and 5.0±0.003 µg/ml respectively.

### In vitro anticholinesterage enzyme activity

The methanolic extract of *V. cinerea* leaves exerted significant AChE and BChE inhibitory effects. The methanolic extract had $IC_{50}$ of 160.5 ± 1.1µg/ml and 205.4 ± 2.2 µg/ml for AChE and BChE respectively whereas Eserine had $IC_{50}$ of 0.018 ± 0.01 µg/ml and 0.038 ± 0.01 µg/ml for AChE and BChE respectively. In case of BChE, the methanolic extract had statistically ($p<0.05$) significant inhibitory effect.

## DISCUSSION

The anti-oxidant activity is generally due to the presence of phenolic compounds. The antioxidant activity exerted by the

stem-bark and leaves extract was statistically significant which supports previous finding of the antioxidant activity of the *V. cinerea* leaves (Kumar *et al.*, 2009). *V. cinerea* exhibited significant BChE inhibitory effect which might be responsible for increasing the level of neurotransmitter acetylcholine and thus might improve synaptic transmission in the Alzheimer Disease (AD). The anti-oxidant activity of the plant extract also implicated its neuroprotective effect in AD through the scavenging of reactive free radicals and ROS which otherwise play important role in the formation of neurofibrillary tangles and neurotic plaques (Butterfield *et al.*, 2007). Study on AD animal model for finding the role of *V. cinerea* on fibrillar amyloid plaques might give additional information regarding applicability of this plant in AD.

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