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Free Radical Scavenging Activity of *Andrographis paniculata* and *Anthocephalus chinensis*

Md. Alamgir Hossain[1], A.M. Sarwaruddin Chowdhury[1], Mohammad A. Rashid[2] and Choudhury M. Hasan[2,3]

[1]*Department of Applied Chemistry and Chemical Engineering, Dhaka University, Dhaka-1000, Bangladesh*

[2]*Department of Pharmaceutical Chemistry, Dhaka University, Dhaka-1000, Bangladesh*

[3]*Centre for Biomedical Research, Dhaka University, Dhaka-1000, Bangladesh*

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Abstract

Andrographis paniculata and *Anthocephalus chinensis*, the two Bangladeshi plants were studied for investigating free radical scavenging potentiality. The crude ethanolic extract of the aerial part of *Andrographis paniculata* as well as its n-hexane, carbon tetrachloride, dichloromethane and aqueous soluble fractions and the methanolic extract of the stem bark of *Anthocephalus chinensis* and its n-hexane, carbon tetrachloride, chloroform and aqueous soluble fractions were subjected to this study with 2,2-Diphenyl-1-picrylhydrazyl (DPPH). The dichloromethane soluble fraction of *A. paniculata* exhibits the highest free radical scavenging activity (antioxidant effect) having IC_{50} value of 19.33 µg/ml and the crude methanolic extract of *A. chinensis* showed the potential antioxidant activity having IC_{50} value of 22.68 µg/ml.

Key words: *Andrographis paniculata, Anthocephalus chinensis*, Free radicals, Antioxidants.

I. Introduction

There have some information that ancient scientists were much effort to invent medicine to overcoming the death. But they did not know the actual causes of damaging biomolecules In recent years, it has known that 'free radicals' are the main culprit for accelerate aging, cancer, malaria, neurodegenerative diseases and other several pathological events in living organisms[1]. Antioxidants which scavenge free radicals are known to posses an important role in preventing these free radical induced-diseases. There is an increasing interest in the antioxidants effects of compounds derived from plants, which could be relevant in relations to their nutritional incidence and their role in health and diseases[2,3,4,5,6]. A number of reports on the isolation and testing of plant derived antioxidants have been described during the past decade. Natural antioxidants constitute a broad range of substances including phenolic or nitrogen containing compounds and carotenoids.[7,8,9] Some synthetic free radical scavergers such as *tert*-butyl-1-hydroxytoluene (TBHT), *tert*-butylhydroquinone (TBHQ) butylated hydroxyanisole (BHA) and propyl gallate (PG) are widely used as food additives, which save the food quality, especially lipid and lipid containing products by retarding the process of lipid peroxidation. However, TBHT and BHA are known to have not only toxic and carcinogenic effects on humans[10] but abnormal effects on enzyme systems[11]. Thus, Herbalism and folk medicine, both ancient and modern, have been the source of much useful therapy.[12] Therefore, the demand for effective antioxidant agents from natural sources are increased gradually.. Within this context, considerable interest has arisen in the possibility that the impact of several major diseases may be either ameliorated or prevented by improving the dietary intake of natural nutrients with antioxidant properties, such as β-carotene, vitamin C, vitamin E and plant phenolics such as flavonoids and tannins.

In order to identify plant species having antioxidant activity, the preliminary free radical scavenging activity of various fractions of the aerial part of *A. paniculata* and the stem bark of *A. chinensis* have been reported in the present work.

II. Materials and Methods

A. paniculata (Kalomegh), belonging to Acanthaceae family, is an annual herb that grows wild in wasteland throughout Bangladesh (particularly in Chittagong Hill Tracts) and occasionally planted in gardens.[13] Traditional village doctors used the plant for making various medicines, specially '*Kalomegh'* is made from fresh leaves of this plant, which is given the village people in Bangladesh to children suffering from liver and stomach complaints. Juice of fresh leaves is used in the treatment of jaundice, and also as blood purifier by the *Chakma* and other ethnic community of Patuakhali district.[14]

A. chinensis (Kadam, Family-Rubiaceae) is a moderate to large-sized tree, is found to grow wild in jungles in almost all areas of the country and also planted as a timber and shade tree..[15] The bark is used as a febrifuse and tonic and the decoction of leaves used as a gargle in the cases of aphthae and stomatitis.[16]

Plant Material. The experimental studies were done in 'Centre for Biomedical Research', University of Dhaka. The aerial part of *A. paniculata* was collected from the garden of BCSIR Laboratories, Chittagong and the stem bark of *A. chinensis* was collected from Boalmari, Faridpur. Both the plants were identified at the Plant Taxonomy Division of BCSIR and Bangladesh National Herbarium, Mirpur, Dhaka (Accession no: DACB 32888 for *A. paniculata* and DACB 31749 for *A. chinensis*) The samples were cut into small pieces, dried at room temperature for some days and then ground to a coarse powder.

Extraction and isolation. The powder of *A. paniculata* and *A. chinensis* were soaked separately in 2 L of ethanol and 2 L methanol respectively for 10 days and then filtered through a cotton plug followed by Whatman filter paper number 1. The extract was concentrated with the help of a rotary evaporator. A portion of the concentrated ethanol/methanol extract (5 g) were fractionated by the modified Kupchan partitioning method[17] using the following solvents (table 1).

Table. 1. Test samples of Experimental Plants1

Plant	Test samples	Code	Measured amount (mg)
Andrographis paniculata	Ethanol soluble aerial part extract (crude)	ESAE	2.00
	n-Hexane soluble partitionate of ESAE	HXSP	2.00
	Carbon tetrachloride soluble partitionate of ESAE	CTSP	2.00
	Dichloromethane soluble partitionate of ESAE	DMSP	2.00
	Aqueous soluble partitionate of ESAE	AQSP	2.00
Anthocephalus chinensis	Methanol soluble bark extract (crude)	MSBE	2.00
	n-Hexane soluble partitionate of MSBE	HXSP	2.00
	Carbon tetrachloride soluble partitionate of MSBE	CTSP	2.00
	Chloroform soluble partitionate of MSBE	CFSP	2.00
	Aqueous soluble partitionate of MSBE	AQSP	2.00

The free radical scavenging activity of the plant extractives on the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was estimated by the method of Brand-Williams.[18] Here 2.0 ml of a methanol solution of the samples at different concentration were mixed with 3.0 ml of DPPH methanol solution (20 µg/ml). The antioxidant potential was assayed from the bleaching of purple colored methanol solution of DPPH radical by plant extract as compared to that produced by the standard antioxidant agents of *tert*-butyl hydroxytoluene (TBHT) and ascorbic acid (ASA) by UV spectrophotometer.

Choi *et al.,* 2000[19] and Desmarchelier *et al.,* 1997[20] also used the following method to evaluate the free radical scavenging activity (antioxidant potential) of various compounds and medicinal plants

- 2.0 ml of a methanol solution of the extract at different concentration (500 to 0.977 µg/ml) was mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml).
- After 30 min reaction period at room temperature in dark place absorbance was measured at 517 nm against methanol as blank by spectrophotometer.
- Inhibition free radical DPPH in percent (I%) was calculated as follows: (I%) = $\{(A_{blank} - A_{sample})/ A_{blank}\} \times 100$

  Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test material).
- Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration.
- TBHT was used as positive control.
- Tests carried out in triplicate and average value was taken.

III. Results and Discussion

Different partitionates of ethanolic extract of aerial part of *A, paniculata* and methanol extract of *A. chinensis* were subjected to investigating free radical scavenging activity. In this investigation, the dichloromethane soluble part (DMSP) of *A, paniculata* showed the highest free radical scavenging activity with IC_{50} value 19.33 µg/ml. At the same time carbon tetrachloride soluble part (CTSP) and crude ethanolic extract (ESAE) also exhibit strong antioxidant potential having IC_{50} values 21.25 and 23.06 µg/ml, respectively (table 2, figure 1).

Table. 2. IC_{50} values of standard and the test samples of *A.. paniculata*

Test samples/Code	Equation of Regression line	R^2	**IC_{50} (µg/ml)#**
TBHT	y = 14.666Ln(x) + 10.202	0.946	15.08±0.52
ESAE	y = 11.135Ln(x) + 14.706	0.9727	23.79±1.17
HXSP	y = 8.796Ln(x) + 15.194	0.9341	52.26±2.1
CTSP	y = 7.1105Ln(x) + 28.262	0.9773	21.25±0.59
DMSP	y = 10.469Ln(x) + 18.988	0.976	**19.33±1.08**
AQSP	y = 9.9965Ln(x) + 14.005	0.9658	36.6±1.63

#The values of IC_{50} are expressed as mean±SD (n=3)
(Code- as mentioned in table 1)

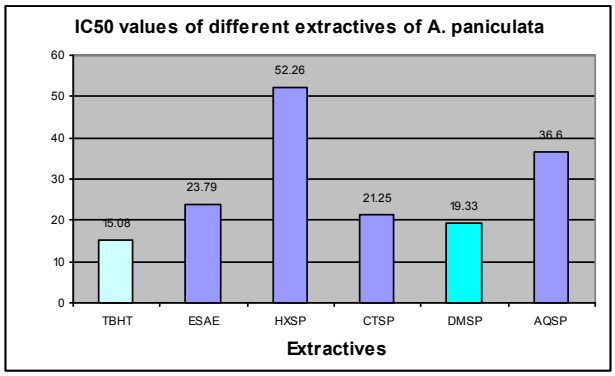

Fig. 1. IC_{50} values of standard and different partitionates of *A. paniculata*

In the case of *A, chinensis* the values for methanol soluble crude extract and aqueous soluble part exhibited significant antioxidant capacity having IC_{50} values of 22.68 µg/ml and 24.54 µg/ml. The chloroform part also showed high antioxidant potential (.IC_{50} = 27.21µg/ml.). The carbon tetrachloride soluble and n-hexane soluble partitionates of the methanolic extract showed moderate and very low antioxidant properties having IC_{50} of 53.37 µg/ml and 157.15 µg/ml, respectively (table 3, figure 2).

IC50 values

IC50 values (micrograms ml)

Table. 3. IC_{50} values of standard and test samples of *A. chinensis*

Test samples/Code	Equation of Regression line	R^2	IC_{50} (µg/ml)
TBHT	y = 14.666Ln(x) + 10.202	0.946	15.08±0.52
MSBE	y = 14.405Ln(x) + 5.0287	0.9426	**22.68±1.12**
HXSP	y = 10.108Ln(x) – 1.1272	0.853	157.15±2.08
CTSP	y = 10.535Ln(x) + 8.0922	0.9457	53.37±0.68
CFSP	y = 11.3Ln(x) + 12.661	0.9738	27.21±2.3
AQSP	y = 12.022Ln(x) + 11.518	0.9629	24.54±1.47

#The values of IC_{50} are expressed as mean±SD (n=3)
(Code- as mentioned in table 1)

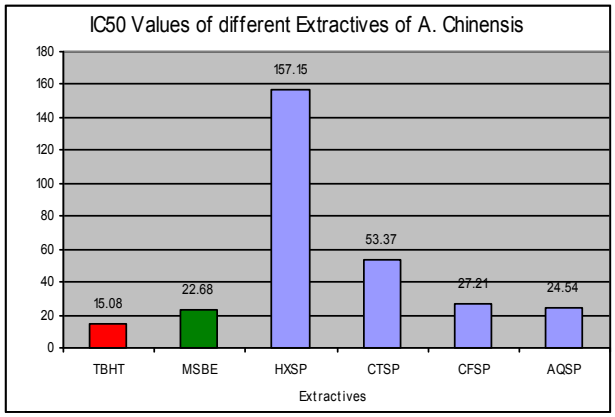

Fig. 2. IC_{50} values of the standard and partitionates of *A. chinensis*

IV. Conclusion

The results of dichloromethane, carbon tetrachloride soluble fractions and ethanol extract of *A. paniculata* showed potential antioxidant activities. Aqueous and n-hexane soluble fractions also showed moderate free radical scavenging activities.In the case of *A. chinensis*, crude methanol extract exhibited potential antioxidant activities. Chloroform part also showed moderate free radical scavenging activities. Further work especially bioassay-guided fractionation is warranted in order to isolate and characterize the free radical scavenging constituents responsible for the antioxidant property.

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