International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

PHYTOCHEMICAL SCREENING, CUPRIC REDUCING ANTIOXIDANT CAPACITY (CUPRAC), NITRIC OXIDE SCAVENGING ASSAY, SCAVENGING OF HYDROGEN PEROXIDE AND REDUCING POWER CAPACITY ASSESSMENT OF LEAF EXTRACT OF *AVERRHOA BILIMBI* (FAMILY- OXALIDIACEAE)

Mohammad Mooneem Mannan[1], Mohiuddin Ahmed Bhuiyan[1], Mohammad Shahriar[1], Fariha Nowshin Ara Khan[1], Milton Kumar Kundu[2]

[1]Phytochemistry Research Laboratory, Department of Pharmacy, University of Asia Pacific, Dhaka, Bangladesh
[2]Pharmacy Discipline, Khulna University, Khulna, Bangladesh

***Corresponding author e-mail:** 3m.rupok@gmail.com

ABSTRACT

The leaf extracts of *Averrhoa bilimbi* were subjected to a comparative evaluation of the phytochemical screening, cupric reducing antioxidant capacity, nitric oxide scavenging assay, scavenging of hydrogen peroxide and reducing power capacity assessment. The preliminary phytochemical screening of three different extracts of *Averrhoa bilimbi* leaves was carried out according to the standard methods and found tannin, phenol, carbohydrate, flavonoid, cardiac glycoside, alkaloid and saponin. In addition, the three different extracts of *Averrhoa bilimbi* leaves were screened to evaluate cupric reducing antioxidant capacity, nitric oxide scavenging assay, scavenging of hydrogen peroxide and reducing power capacity assessment. It is demonstrated that the three different extracts of *Averrhoa bilimbi* leaves showed different level of antioxidant activity and is a potential source of antioxidants and thus could prevent many radical related diseases.

Key words: *Averrhoa bilimbi*, Cupric Reducing Antioxidant Capacity (CUPRAC), Nitric Oxide Scavenging Assay, Scavenging of Hydrogen Peroxide and Reducing Power Capacity

INTRODUCTION

Medicinal plants usually called nature's gift for a disease free healthy life and play an important role in preserving our health [1]. Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value. Medicinal herb is considered to be a chemical factory as it contains multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene, lactones and oils (essential and fixed) [2]. There are a number of evidence of an association between the oxidative stress resulting from radical generation and antioxidant insufficiency and tissue damage. The most common forms of ROS include superoxide radical, hydrogen peroxide, hydroxyl free radical, singlet oxygen and nitric oxide, which have significantly high biological activities in vivo and in vitro. They can directly lead to DNA mutation, alteration of gene expression, modification of cell signal transduction, cell apoptosis, lipid peroxidation and protein degradation [3]. *Averrhoa bilimbi* is a multipurpose, long-lived tropical plant locally known as belembu, belemburi; In English, this is also known as- bilimbi, cucumber tree, tree sorrel etc. belonging

to family Oxalidaceae [4, 5] . *Averrhoa bilimbi* is medicinally utilized as a folk therapy for many symptoms. It is employed for the treatment of fever, mumps, pimples, inflammation of the rectum and diabetes, itches, boils, rheumatism, syphilis, bilious colic, whooping cough, hypertension, stomach ache, ulcer and as a cooling [6, 7] . However there is no research work for the assessment of phytochemical screenings, cupric reducing antioxidant capacity (CUPRAC), nitric oxide scavenging assay, scavenging of hydrogen peroxide and reducing power capacity assessment of leaf extract of *Averrhoa bilimbi* using its different fractions. So our present study was aimed to investigate anti-oxidant activity of *Averrhoa bilimbi* leaf extracts.

MATERIALS AND METHODS

***Plant materials collection and identification*:** The whole plants of *Averrhoa bilimbi* were collected during July, 2014 from Sunamgonj, Sylhet, Bangladesh. Then the plant sample was submitted to the National Herbarium of Bangladesh, Mirpur, Dhaka. One week later its voucher specimen was collected after its identification (Accession No. 39642).

Preparation of extract: The sun dried and powdered leaves (20 gm) of *Averrhoa bilimbi* was successively extracted in a Soxhlet extractor at elevated temperature using 200 ml of ethanol (40-60)°C which was followed by chloroform and methanol. All extracts were filtered individually through filter paper and poured on Petri dishes to evaporate the liquid solvents from the extract to get dry extracts. Then dry crude extracts were weighed and stored in air-tight container with necessary markings for identification and kept in refrigerator (0-4)°C for future investigation.

***Preliminary phytochemical screening*:** Qualitative phytochemical analyses were done using the methods described by Tiwari *et al.* 2011 and Trease and Evans, 2002 [8, 9]. The presence of carbohydrates, alkaloids, flavonoids, glycosides, tannins, phenols, saponin, and phytosterols were analyzed.

Cupric Reducing Antioxidant Capacity (CUPRAC): The assay was conducted as described previously Apak et al., 2004 [10]. 0.5 ml of plant extract or standard of different concentrations solution, 1 ml of copper (II) chloride solution (0.01 M prepared from $CuCl_2.2H_2O$), 1 ml of ammonium acetate buffer at pH 7.0 and 1 ml of neocaproin solution (0.0075 M) were mixed. The final volume of the mixture was adjusted to 4.1 ml by adding 0.6 ml of distilled water and the total mixture was incubated for 1 hour at room temperature. Then the absorbance of the solution was measured at 450 nm using a spectrophotometer against blank. Ascorbic acid was used as a standard.

Nitric Oxide Scavenging Capacity Assay: Nitric oxide scavenging assay was carried by using sodium nitroprusside [11]. This can be determined by the use of the Griess Illosvoy reaction. 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract/sub-fraction at various concentrations and the mixture was incubated at 25°C for 150 minutes. From the incubated mixture 0.5 ml was taken out and added into 1.0 ml sulphanilamide solution (0.33% in 20% glacial acetic acid) and further incubated at room temperature for 5 minutes. Finally, 1.0 ml naphthyl ethylenediamine dihydrochloride (0.1% w/v) was mixed and maintained at room temperature for 30 minutes. The absorbance was measured at 546 nm. A typical blank/control solution contained the same solution mixture without plant extract or standard. The absorbance of the blank/control solution was measured at 546 nm. The percentage inhibition was calculated according to the following equation:

% Inhibition = $(1- A_1/A_0) \times 100$
Where, A_1 = Absorbance of the extract or standard, A_0 = Absorbance of the control

Scavenging of Hydrogen Peroxide: Scavenging activity of extract and its sub-fractions were evaluated by hydrogen peroxide [12]. 1 ml of various concentrations of the extract, sub-fractions and standards in ethanol was added to 2 ml of hydrogen peroxide solution in phosphate buffered saline (PBS, pH 7.4). Then finally the absorbance was measured at 230 nm after 10 minutes. Ascorbic acid was used as standard. Control sample was prepared containing the same volume without any extract and standard and the absorbance was read at 230 nm using a spectrophotometer.

Reducing Power Capacity Assessment: Assay of reducing power was carried out by potassium ferricyanide method [13]. 1 ml of extract and its sub-fractions (final concentration 5- 200 µg/ml) were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide. The mixture was then incubated at 50°C for 20 minutes. To this mixture 2.5 ml of trichloroacetic acid was added, which was then centrifuged at 3000 rpm for 30 minutes. Finally, 2.5 ml of the supernatant solution was collected and mixed with 2.5 ml of distilled water and 0.5 ml ferric chloride and absorbance was

measured at 700 nm. Ascorbic acid was used as standard and phosphate buffer as blank solution.

RESULTS AND DISCUSSIONS

***Preliminary phytochemical screening*:** Preliminary phytochemical screening of the extracts of leaves of *Averrhoa bilimbi* revealed the presence of different bioactive components that are summarized in Table 1. It was observed from preliminary phytochemical screening of the leaf extracts of *Averrhoa bilimbi* that all leaf extracts showed the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenols and steroids. While saponins are absent in ethanol as well as chloroform extracts and tannins are absent in chloroform extracts.

Cupric Reducing Antioxidant Capacity (CUPRAC): Reduction of Cu^{2+} ion to Cu^{+} was found to rise with increasing concentrations of the different extracts. The standard ascorbic acid showed highest reducing capacity. Among the extracts the ethanol extract of *Averrhoa bilimbi* showed maximum reducing capacity that is comparable to ascorbic acid (Figure 1).This method is based on the principle of increase in the absorbance of the reaction mixtures. Increase in the absorbance indicates increase in the antioxidant activity hence indicates the reducing power of the samples [14]. Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity [15] with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid per-oxidation processes, so that they can act as primary and secondary antioxidants [16].

Nitric Oxide Scavenging Assay: Nitric oxide is a very unstable species and reacting with oxygen molecule produce stable nitrate and nitrite which can be estimated by using Griess reagent. In the presence of a scavenging test compound, the amount of nitrous acid will decrease which can be measured at 546 nm. Chloroform extract of *Averrhoa bilimbi* leaf has potent nitric oxide scavenging activity (IC_{50} value 23.197 µg/ml) and methanol extract has showed the least nitric oxide scavenging activity (IC_{50} value 159.512 µg/ml) (Table 2).The scavenging of NO by the extracts was increased in dose dependent manner. Figure 2 illustrates a significant decrease in the NO radical due to the scavenging ability of extracts and ascorbic acid. The chloroform extract showed maximum activity of 92.59% at 100 µg/ml, whereas ascorbic acid at the same concentration exhibited 94.44% inhibition.

Scavenging of Hydrogen Peroxide: Hydrogen peroxide, although not a radical species play a role to contribute oxidative stress. The generation of even low levels of H_2O_2 in biological systems may be important. Naturally-occurring iron complexes inside the cell believed to react with H_2O_2 in vivo to generate highly reactive hydroxyl radicals and this may be the origin of many of its toxic effects [17]. Scavenging of hydrogen peroxide of different extracts of *Averrhoa bilimbi* is presented in figure 3. Among three extracts Chloroform in the top position in depleting H_2O_2 with an IC_{50} value of 147.210 µg/ml (Table 3). The percentage of H_2O_2 scavenging activity of Chloroform extract was found to be 91.62 % which is highest among three extract at 200 µg/ml compared to antioxidant activity of standard ascorbic acid was 99.40 % at the same concentration.

Reducing Power Capacity Assessment: Reducing power of the fractions was assessed using ferric to ferrous reducing activity as determined spectrophotometrically from the formation of Perl's Prussian blue colour complex [18]. Reducing power of different extracts of *Averrhoa bilimbi* was compared with ascorbic acid (Figure 4). Among the extracts the ethanol extract exhibited the most reducing power. This result indicates that the extracts may consist of polyphenolic compounds that usually show great reducing power.

CONCLUSION

On the basis of above result and available reports, all three leaf extracts of *Averrhoa bilimbi* had potent antioxidant effects. The preliminary phytochemical screening of three different extracts of *Averrhoa bilimbi* leaves was carried out according to the standard methods. Chemical constituents of the extracts of the leaves were found to be tannin, phenol, carbohydrate, flavonoid, cardiac glycoside, alkaloid and saponin. In addition, three different extracts of *Averrhoa bilimbi* leaves were screened to evaluate cupric reducing antioxidant capacity. Among three extracts, the ethanol extract showed maximum reducing capacity comparable to ascorbic acid. In nitric oxide scavenging assay, chloroform showed better activity. Among three extracts, chloroform in the top position in depleting H_2O_2. In reducing capacity assessment ethanol showed better activity. To sum up, these findings together demonstrate that *Averrhoa bilimbi* is an excellent plant candidate for further investigation of in vivo antioxidant activity and the different antioxidant mechanisms and also appears to be a most promising candidate from which specific antioxidant bioactive products could be developed. Therefore, in depth extensive study should be an urgency to sort out bioactive compounds.

Table 1: Results of chemical group test of various leaf extracts of *Averrhoa bilimbi*

Name of Tests	Name of Extracts		
	Methanol	**Ethanol**	**Chloroform**
Alkaloids	+	+	+
Carbohydrates	++	++	+
Flavonoids	+	+	+
Glycosides	+	+	+
Phenols	+	+	+
Steroids	+	+	+
Saponins	+	-	-
Tannins	+	+	-

[+++: presence in high concentration; ++: presence in moderate concentration; +: presence in low concentration; - : Absence.]

Table 2: IC_{50} values of the different extracts of *Averrhoa bilimbi* leaf in nitric oxide scavenging assay

Extract/standard	IC_{50} µg/ml
Methanol	159.512
Ethanol	140.892
Chloroform	23.197
Ascorbic acid	13.120

Table 3: IC_{50} values of different extracts of *Averrhoa bilimbi* in H_2O_2 scavenging assay

Sample/standard	IC_{50} (µg/mL)
Methanol Extracts	159.821
Ethanol Extracts	166.102
Chloroform Extracts	147.210
Ascorbic acid	21.988

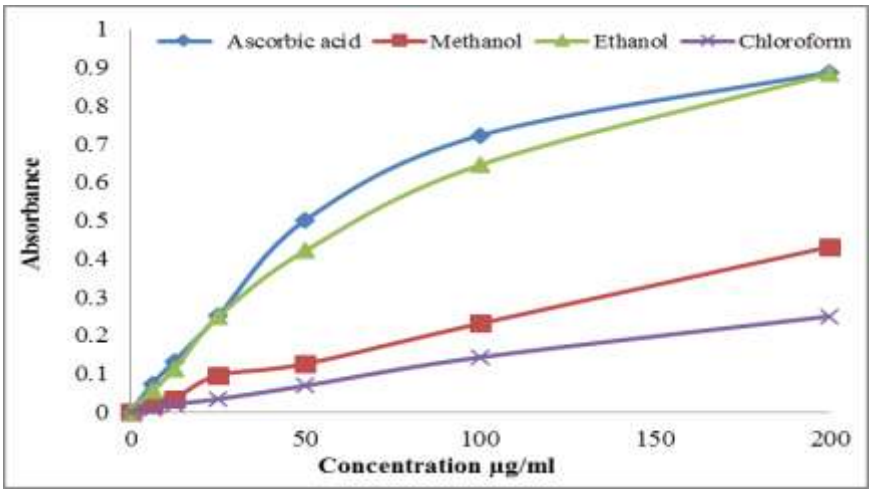

Figure 1: Comparative cupric reducing antioxidant capacity of *Averrhoa bilimbi* leaf extract, ascorbic acid

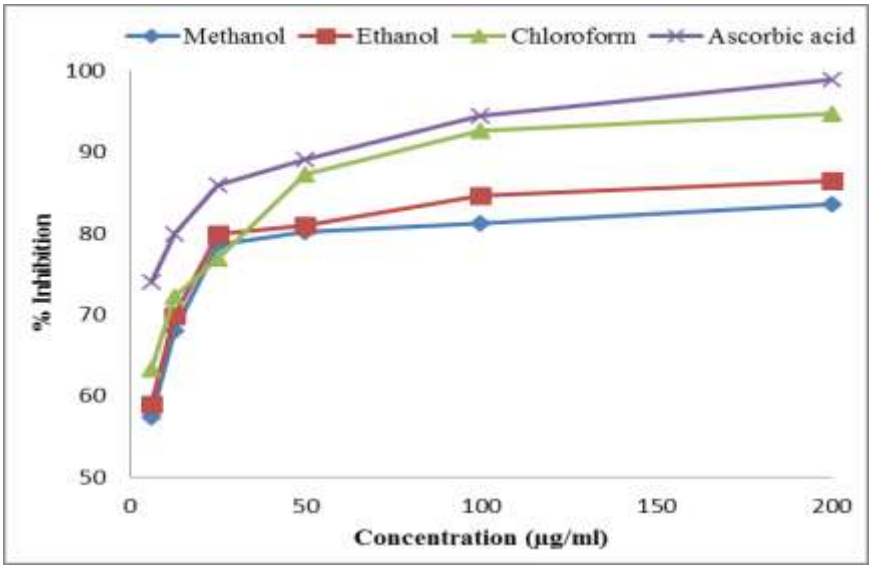

Figure 2: Comparative nitric oxide scavenging activity of Averrhoa bilimbi leaf extract, ascorbic acid

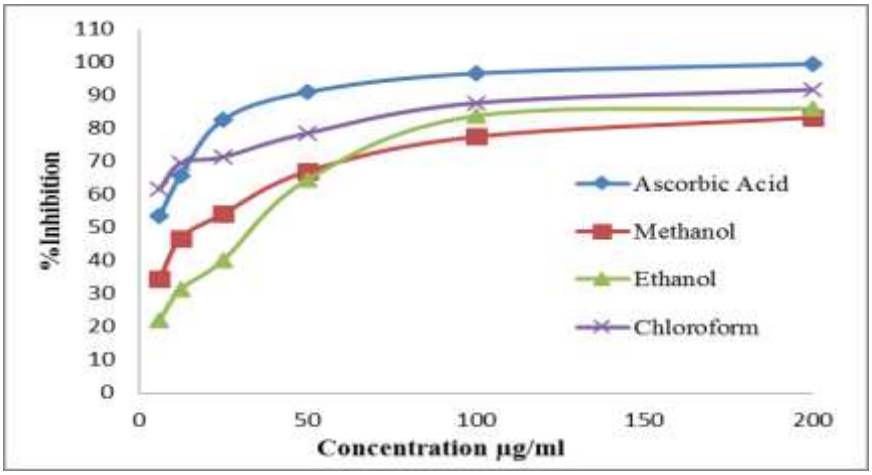

Figure 3: Comparative H_2O_2 scavenging activity of *Averrhoa bilimbi* leaf extract, ascorbic acid

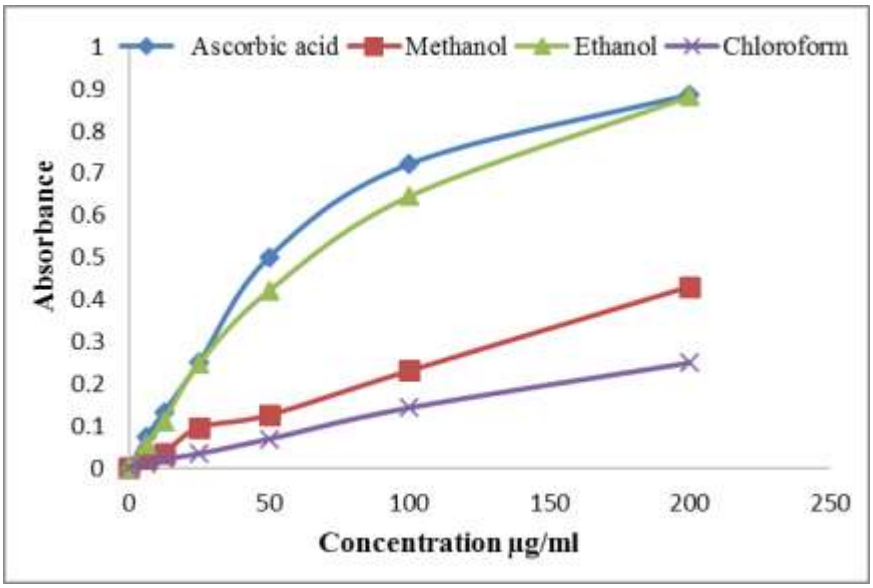

Figure 4: Comparative reducing power capacity of *Averrhoa bilimbi* leaf extract, Ascorbic acid

References:

1. Agwa OK, Akiene BP, Obichi EA. Int J Modern Agri, 2013; 2(2): 48-57.
2. Kumar RS, Venkateshwar C, Samuel G, Rao SG. Int J Engin Sci Inven, 2013; 2(8): 65-70.
3. Meher B, Satapathy T, Roy A, Dash DK. Int J Pharm, 2015; 5(2): 478-84.
4. Ashok KK, Gousia SK, Anupama M, Latha JNL. Int J Pharm & Pharm Sci Res, 2013; 3(4): 136-39.
5. Mannan MM, Akhter R, Shahriar M, Bhuiyan MA. Int J Pharm, 2015; 5(2): 357-60.
6. Hasanuzzaman M, Ali MR, Hossain M, Kuri S, Islam MS. Int Cur Pharma J, 2013; 2(4): 92-6.
7. Kumar AS, Kavimani S, Jayaveera KN. Int J Phytopharm, 2011; 2(2): 53-60.
8. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Int Pharma Sci, 2011; 1: 103-4.
9. Trease GE, Evans WC. Pharmacology. 15th ed., London; Saunders Publishers: 2002.
10. Apak R, Güçlü K, Özyürek M, Karademir SE. J Agri & Food Chem, 2004; 52(26): 7970-81.
11. Sreejayan, Rao MNA. J Pharma & Pharma, 1997; 49(1): 105-7.
12. Jayaprakasha GK, Singh RP, Sakariah KK. Food Chemistry, 2001; 73(3): 285-90.
13. Yildirim A, Mavi A, Kara AA. J Agri & Food Chem, 2001; 49(8); 4083-89.
14. Jayaprakasha GK, Rao LJ, Sakariah KK. Bioorganic & Med Chem, 2004; 12(19); 5141-46.
15. Oktay M, Gulcin I, Kufrevioglu OI. Food Sci & Tech, 2003; 36(2); 263-71.
16. Yen GC, Chen HY. J Agri & Food Chem, 1995; 43(1); 27-32.
17. Miller HE, Rigelhof F, Marquart L, Prakash A, Kanter M. J Amer Coll Nutri, 2000; 19(3); 312S-19S.
18. Yildirim A, Mavi A, Kara AA. J Agri & Food Chem, 2001; 49(8): 4083-89.