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Evaluation of total phenolic content, free radical scavenging activity and phytochemical screening of different extracts of *Averrhoa bilimbi* (fruits)

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ABSTRACT

The present study was designed to investigate the phytochemical screening, the free radical scavenging activity and to determine the total phenolic content of methanolic extract and different solvent soluble fractions of *Averrhoa bilimbi* Linn. (Oxalidiaceae) fruits. The free radical scavenging activity was evaluated by analyzing the bleaching rate of 1,1-diphenyl-2-picrylhydrazyl (DPPH), and total phenolic content was determined by using Folin-Ciocalteau reagent, which results were expressed in gallic acid equivalent (mg of GAE/g of sample). The phytochemical screening revealed the potent source of different phytochemical constituents on different extractives including, phenol, flavonoid, tannin that are responsible for antioxidant action. In the determination of total phenolic content, different extractives showed a significant content of phenolic compounds ranging from 50.23-68.67 mg of GAE/g of extractive. The plant sample displayed significant DPPH free radical scavenging activity with highest IC_{50} value in crude methanolic extract (30.365 µg/ml) followed by chloroform, carbon tetrachloride, pet-ether and aqueous soluble fractions having value of 32.852 µg/ml, 36.708 µg/ml, 50.35 µg/ml, and 79.918 µg/ml, respectively as opposed to that of the scavenging effects of BHT of 19.656 µg/ml.

Key Words: Phytochemical screening, antioxidant activity, total phenolic content, gallic acid, DPPH, and IC_{50}.

INTRODUCTION

Auto-oxidation of lipids, as well as reactive nitrogen species (RNS) is the main source of reactive oxygen species (ROS) in the forms of superoxide anions, hydroxyl radicals and hydrogen peroxide (Aruoma, 1996). Generation of these excess ROS and RNS by Ultraviolet (UV) radiation, smoking and drug metabolisms are likely to damage several cellular components such as lipids, proteins, nucleic acids, and DNAs through the oxidation or nitration processes (Sawa *et al.*, 2000). In addition, these reactive oxygen species cause inflammation or lesion on different organs and are related with various degenerative diseases, including cancer, ageing, arteriosclerosis, and rheumatism (Choi *et al.*, 2002).

All aerobic organisms, including human beings, possesses antioxidant defenses that protect against oxidative damages, frequent damage removal and repair enzymes to eliminate or repair damaged molecules (Yildirim *et al.*, 2001). However, many have been reported that these natural antioxidant mechanisms can be inefficient (Halliwel, 1994; Terao *et al.*, 1994; Moure *et al.*, 2011.). Although some synthetic antioxidant, including butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are commonly used in processed foods, which have some adverse effects (Kehrer *et al.*, 1990). Therefore, recent search to discover natural originated antioxidant has been increased.

Averrhoa bilimbi Linn. belonging to the family Oxalidiaceae has some local name include belembu, belemburi; In English, this is also known as- bilimbi, cucumber tree, tree sorrel etc. This is attractive, long-lived tropical tree, reaches 16 to 33 ft. (5-10 m) in height; has a short trunk soon dividing into a number of upright branches. Probably, *A. bilimbi* is native of Moluccas in Indonesia. This plant is also found semi-wild throughout, Brazil, Cuba, Philippines, Sri Lanka, Bangladesh, Myanmar (Burma) and Malaysia. *A. bilimbi* is used as traditional

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medicine for treating cough, cold, itches, boils, rheumatism, syphilis, diabetes, whooping cough, and hypertension in Asia (Goh *et al*., 1995; Mackeen *et al*., 1997). *A. bilimbi* is also used in the treatment of children's cough (syrup of flowers), stomach ache (fruits) and as a cooling drink (juice of preserved fruits). Earlier studies showed that ethanolic leaf extract of *A. bilimbi* and its semi–purified fractions possesses hypoglycemic and hypolipidemic properties in Type I diabetic rats when administered intraperitoneally (Tan *et al*., 1996) as well as orally (Pushparaj *et al*., 2000).

A survey of the published literature shows that there is a number of research works for the assessment of total phenolic content determination and antioxidant activity of *Averrhoa bilimbi* fruits using its crude extracts; however there is no research work for the assessment of total phenolic content determination and antioxidant activity of *Averrhoa bilimbi* fruits using its different fractions. So our present study is aimed to investigate total phenolic content and antioxidant activity of methanolic extract of *Averrhoa bilimbi* fruits and its different fractions.

MATERIALS AND METHODS

Collection and identification

The fruits of *Averrhoa bilimbi* were collected from daudkandi, Comilla, Bangladesh on July, 2012. After collection, fruits were thoroughly washed with water, sliced with a knife and dried under sun. The plant was identified and authenticated by Taxonomist of the National Herbarium of Bangladesh, Mirpur, Dhaka (Accession number: DACB 37752).

Preparation, extraction and fractionation

Cold maceration technique was used for extraction. The dried and powdered fruits (500g) were soaked in 2500 ml of methanol for about 15 days at room temperature with occasional stirring. After 15 days the solution was filtered using filter cloth and Whatman's filter paper. The filtrate (methanol extract) obtained was evaporated under ceiling fan and in a water bath below 40°C until dried. It rendered a brown granular compound. The brown granular compound was designated as crude extract of methanol.

The concentrated methanol extract was separately partitioned by the modified Kupchan method (Vanwagenen *et al*., 1993) using pet-ether, carbon tetrachloride, and chloroform. The aqueous methanolic fraction was preserved as aqueous fraction. All the four fractions were evaporated to dry by keeping 7 days in room temperature.

Phytochemical screening

Phytochemical properties of different extractives of plant materials were tested using the following chemicals and reagents according to the method of Trease and Evans (1989): Alkaloids with Mayer and Dragendoff's reagents, Tannin ($FeCl_3$), Saponins (foaming test), Flavonoids (chip of magnesium and HCl), Glycosids (NaCl, and Felhing's solutions A and B), Sterols and Triterpens (ethylic, sulphuric acid and anhydride acetic), Phenols -$FeCl_3$ and $K_3Fe(CN_6)$-, Cardiac glycosides (aceticacetic, $FeCl_3$, concentrate sulphuric acid, carbohydrate (alcoholic α-naphthol solution, Benedict's reagent).

Determination of total phenolic content

Total phenolic content of fruits of *A. bilimbi* extractives was measured employing the method (Demiray *et al*., 2009) involving Folin-Ciocalteu reagent as oxidizing agent and gallic acid as standard (Majhenic *et al*., 2007). Different Gallic acid solution were prepared having a concentration ranging from 50 µg/ml to 0 µg/ml. 2.5 ml of Folin-Ciocalteau reagent (diluted 10 times with water) and 2.0 ml of Na_2CO_3 (7.5 % w/v) solution was added to 0.5 ml of Gallic acid solution. The mixture was incubated for 20 minutes at room temperature. After 20 minutes the absorbance was measured at 760 nm. After plotting the absorbance in ordinate against the concentration in abscissa a linear relationship was obtained which was used as a standard curve for the determination of the total phenolic content of the test samples. In 0.5 ml of extract solution (conc. 2 mg/ml), 2.5 ml of Folin-Ciocalteu reagent (diluted 10 times with water) and 2.0 ml of Na_2CO_3 (7.5 % w/v) solution was added. The mixture was incubated for 20 minutes at room temperature. After 20 minutes the absorbance was measured at 760 nm by UV-spectrophotometer and using the standard curve prepared from gallic acid solution with different concentration, the total phenolic content of the sample was measured. The phenolic contents of the

Table 1: Results of phytochemical screening of different extractives of *Averrhoa bilimbi* fruits.

Test	MEF	PTSF	CTSF	CSF	AQSF
Alkaloid	+	+	-	+	+
Tanin	+	+	+	-	-
Saponins	+	+	-	-	+
Flavonoids	+	+	+	+	+
Cardiac glycosides	+	+	-	+	+
Glycosides	+	+	+	+	+
Phytosterols	-	-	-	-	-
Triterpenes	+	+	-	+	-
Phenols	+	+	+	+	+
Carbohydrate	+	-	+	+	+

+ = presence of phytoconstituents, - = absence of phytoconstituents, MEF= methanolic extract, PTSF= pet-ether soluble fraction, CTSF= carbon tetrachloride soluble fraction, CSF= chloroform soluble fraction, AQSF= aqueous soluble fraction.

sample were expressed as mg of GAE (gallic acid equivalent) / gm of the extractive.

DPPH free radical scavenging activity

The free radical scavenging activity of the extract, based on the scavenging activity of the stable 1, 1-diphenyl-2- picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca *et al.* (2001). 2.0 ml of a methanol solution of the extract at different concentration (500 to 0.977 µg ml) were mixed with 3.0 ml of a DPPH methanol solution (20µg/ml). After 30 minutes reaction period at room temperature in dark place the absorbance was measured against at 517 nm against methanol as blank by UV spectrophotometer. The percentage inhibition activity was calculated from $[(A_0–A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/ standard. Then the inhibition curves were prepared and IC_{50} values were calculated. BHT was used as positive control.

RESULTS AND DISCUSSION

Phytochemical screening

The preliminary phytochemical investigation showed the presence of phytochemical constituents such as alkaloid, tanin, saponins, flavonoids, cardiac glycosides, triterpenes, phenols, carbohydrate but absence of phytosterols in different extractives (Table 1). Huda *et al.,* 2009 also found the presence of flavonoids, and triterpenes in the fruits extract of

Table 2: Results of total phenolic content of different extractives of *Averrhoa bilimbi* fruits.

Extractives	Total phenol content (mg of GAE /g of extractive)
MEF	65.16±0.52
CTSF	55.31±1.01
CSF	52.00±0.90
PTSF	68.67±0.94
AQSF	50.23±0.56

Values are the mean of duplicate experiments and represented as mean±SD. GAE= Gallic acid equivalents

A. bilimbi. The presence of phenols, flavonoids and tannins in all the plants is likely to be responsible for the free radical scavenging effects observed. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers (Polterait, 1997).

Total phenolic content

Previously, it is mentioned that the phytochemical screening of the extractives revealed the presence of flavonoid, tannin and phenol. Polyphenolic compounds, like flavonoids, tannins and phenolic acids, usually found in plants have been reported to have multiple biological effects, including antioxidant activity. Flavonoids and tannins present in the plant extract, as evident from phytochemical screening, may be responsible for the antioxidant action in the tested models. The result of total phenol content of the plant extractives is presented in Table 2. The result is expressed as the number of gallic acid equivalents per gram of the plant extractives. Different extractive possesses total phenolic content ranging from 50.23-68.67 mg of GAE /g of extractive.

DPPH free radical scavenging activity

The DPPH free radical scavenging activity is based on the ability of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to be decolorized in the presence of antioxidants (Kumarasamy *et al.*, 2007). The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance (Subhan *et al.,* 2008).

Table 3: Results of DPPH free radical scavenging activity of different extractives of *Averrhoa bilimbi* fruits with BHT.

Extractives	IC_{50} (µg/ml)
MEF	30.365
CTSF	36.708
CSF	32.852
PTSF	50.35
AQSF	79.918
BHT	19.656

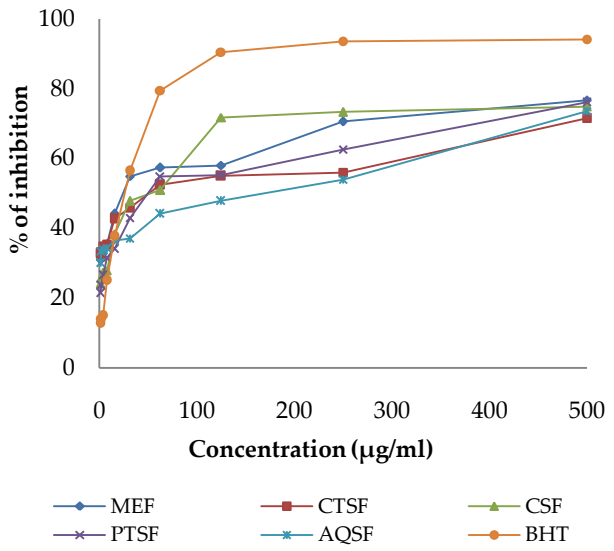

Figure 1: Comparative DPPH radical scavenging activity of different extractives of *Averrhoa bilimbi* fruits along with BHT.

Five extractives exhibited considerable DPPH free radical scavenging activity as indicated by their IC_{50} values and this has been showed in Table 3. IC_{50} Indicate the potency of scavenging activity. Standard BHT was found to have an IC_{50} of 19.656µg/ml. In comparison to BHT, methanol, carbon tetrachloride, pet-ether and chloroform soluble fraction displayed IC_{50} of 30.365µg/ml, 36.708µg/ml, 50.35µg/ml and 32.852µg/ml, respectively. Aqueous soluble fraction is seen to have the least free radical scavenging activity. From above experiment, it can be mentioned that, different extractives of *Averrhoa bilimbi* fruits may possess significant DPPH free radical scavenging activity due to presence of flavonoid and tannin. Figure 1 shows the DPPH free radical scavenging activity of BHT and different extractives.

CONCLUSION

From the study it may be concluded that *Averrhoa bilimbi* fruits is a good source of phytochemicals. Different extractives showed significant DPPH free radical scavenging activity and total phenolic content. Hence, further studies are suggested to be undertaken to pinpoint the exact compound(s) and to better understand the mechanism of such actions scientifically.

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