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Original Article

Evaluation of Potential Antioxidant Activity of Leaves of *Bauhinia Acuminate*

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Abstract

The methanol extracts of leaves of *Bauhinia acuminata* (MESF) and their different fractions obtained from modified Kupchan partitioning method i.e. methanol, pet ether, carbon tetrachloride, chloroform, and aqueous soluble fractions were subjected to biological screening such as total phenolic content and antioxidant activity screening.The amount of total phenolic content differed in different extractives and ranged from 15.90 mg of GAE /gm of extractives to 124.80 mg of GAE /gm of extractives of *B. acuminate*. Among all extractives of *B. acuminate* the highest phenolic content was found in AQSF (124.80 mg of GAE /gm of extractives) followed by CSF (103.78 mg of GAE /gm of extractives).Significant amount of phenolic compounds also present in CTCSF (75.59 mg of GAE /gm of extractives), MESF (66.20 mg of GAE /gm of extractives) and PETSF (15.90 mg of GAE /gm of extractives) were also found. The antioxidant activity of IC_{50} values in DPPH method differed in different extractives and ranged from 22.01 to 77.79. Among all extractives of *B. acuminata* the highest free radical scavenging activity was given by CTCSF (absorbance 22.01) followed by AQSF (absorbance 29.83).Significant free radical scavenging activity was also exhibited by CSF (absorbance 45.09), MESF (absorbance 43.78), PESF (absorbance 77.79).

Key words: Bangladesh, *Bauhinia acuminate,* DPPH, Free radical scavenging, Total phenolic compound Leaves.

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1. Introduction

Bauhinia acuminata belonging to the family; Fabaceae, an evergreen large shrub, grows in disturbed areas of Southeast Asia such as Indonesia, Malaysia or the Philippines [1]. In Bangladesh, it grows in hilly forests of Sylhet and Chittagong. It grows 2 to 3 meters tall.

Leaves with petioles 1.5-4 cm long; broadly ovate or sub orbicular, blades ovate, divided about 1/3 their length, membranous, densely puberulent abaxially, glabrous adaxially, apex of lobes acute, base cordate to rounded. They are apical cleft up to 5 cm deep with 6 to 15 centimeters long and broad. The flowers are 8 to 12 centimeters in diameter with fragrant, with five white petals, ten yellow-tipped stamens and a green stigma [2]. The species occurs in deciduous forests and scrub. Several chemical compounds including palmitic acid, three phthalic acid esters, phthalic acid, gallic acid, ursolic acid were identified from the leaves of *B. acuminata* [3]. In this study, we report the anti-oxidant activity of the methanol leaf extracts of this plant.

2. Materials and Methods

2.1. Plant Materials

The leaves of *Bauhinia acuminata* were collected in August 2013 from the Batali hill, Lalkhan bazaar, Chittagong. A voucher specimen no. 38305 for this plant has been maintained in Bangladesh National Herbarium, Dhaka, Bangladesh. 500 gm sun dried powdered leaves of *Bauhinia acuminata* was macerated in 2.5 L of methanol for 7 days and then filtered through a cotton plug followed by Whatman filter paper number 1. At low temperature (40-45 ºC) and reduced pressure, all extracts were concentrated with a rotary evaporator. Using modified Kupchan partitioning method [4] the concentrated methanolic extract (ME) was fractionated and the resultant fractions i.e., pet-ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF), and aqueous (AQSF) soluble fractions were used for the experimental processes.

2.2. *Total Phenolic Compound Analysis*

Total phenolic content of leaves of *Bauhinia acuminata* extractives was measured employing the method as described[5] using Folin-Ciocalteu reagent as oxidizing agent and gallic acid as standard described by Md. Reyad-ul-ferdous et al., 2014 [6] with some modifications.

2.2.1. Standard Curve Preparation

Gallic acid was used here as standard. Different gallic acid solution were prepared having a concentration ranging from 100 µg / ml to 0 µg / ml. 2.5 ml of Folin-Ciocalteu reagent diluted 10 times with distilled water with 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 at room temperature for 20 minutes. The absorbance was measured at 760 nm, after 20 minutes. 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.

2.2.2. Sample Preparation

2 mg of the extractives was taken and dissolved in the distilled water to get a sample concentration of 2 mg / ml in every case. The

samples along with their concentration for the total phenolic contents were measured.

2.2.3. Total Phenolic Compound Analysis

To 0.5 ml of extract solution (conc. 2 mg/ml) and 2.5 ml of Folin-Ciocalteu reagent (diluted 10 times with water) as well as 2.0 ml of Na_2CO_3 (7.5 % w/v) solution was added. The mixture was incubated at room temperature for 20 minutes. The absorbance was measured at 760 nm by UV-spectrophotometer after 20 minutes and using the standard curve prepared from gallic acid solution with different concentration. The total phenols content of the sample was measured. The phenolic contents of the sample were expressed as mg of GAE (gallic acid equivalent) / gm of the extract.

2.3. Antioxident Activity: DPPH Assay

In antioxident activity (DPPH) was used to evaluate the free radical scavenging activity (antioxidant potential) of various compounds and medicinal plant [6,7,8].

2.3.1. Control Preparation for Antioxidant Activity Measurement

Tert-butyl-1-hydroxytoluene (BHT) as well as ascorbic acid (ASA) was used as positive control. Calculated amount of ASA and BHT were dissolved in methanol to get a mother solution (1000 µg/ml). Serial dilution was made using the mother solution to get different concentration ranging from 500.0 to 0.977 µg /ml.

2.3.2. Test Sample Preparation

Calculated amount of different extractives were measured and dissolved in methanol to get the mother solution (Conc. 1000 µg/ml). Serial dilution of the mother solution gave different concentration ranging from 500.0 to 0.977 µg /ml which were kept in the marked flasks.

2.3.3. DPPH Solution Preparation

20 mg DPPH powder was weighed and dissolved in methanol to get a DPPH solution having a concentration 20 µg/ml. The solution was prepared in the amber reagent bottle and kept in the light proof box.

2.3.4. Assay of Free Radical Scavenging Activity

2.0 ml of a methanol solution of the sample (extractives/ control) at different concentration (500µg/ml to 0.977µg/ml) were mixed with 3.0 ml of a DPPH methanol solution (20µg/ml). After 30 min reaction period at room temperature in dark place the absorbance was measured at 517 nm against methanol as blank by UV spetrophotometer.

Inhibition of free radical DPPH in percent *(I%)* was calculated as follows:

$(I\%) = (1 - A_{sample}/A_{blank}) \times 100$

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.

3. Results and Discussion

The methanol extract of *Bauhinia acuminate* (MESF) and different fractions i.e. Pet Ether (PetSF), carbon tetrachloride (CTCSF), chloroform (CSF) and aqueous (AQSF) soluble fractions were subjected to total phenolic content determination. Based on the absorbance values of the various extract solutions, reacted with Folin-Ciocalteu reagent and compared with the standard solutions of gallic acid equivalents, results of the colorimetric analysis of the total phenolics are given in figure-1. Total phenolic content of the samples are expressed as mg of GAE (gallic acid equivalent)/ gm of extractives.

The amount of total phenolic content differed in different extractives and ranged from 15.90 mg of GAE /gm of extractives to 124.80 mg of GAE /gm of extractives of *Bauhinia acuminate*. Among all extractives of *Bauhinia acuminate* the highest phenolic content was found in AQSF (124.80 mg of GAE /gm of extractives) followed by CSF (103.78 mg of GAE /gm of extractives). Significant amount of phenolic compounds were also present in CTCSF (75.59 mg of GAE /gm of extractives), MESF (66.20 mg of GAE /gm of extractives) and PETSF (15.90 mg of GAE /gm of extractives) are also found.

The Methanol extract of leaves of *Bauhinia acuminata* (MESF), and different fractions i.e. pet ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF) and aqueous (AQSF) soluble fraction of the methanol extract of leaves of *Bauhinia acuminata* were subjected to free radical scavenging activity. Here, *tert*-butyl-1-hydroxytoluene (BHT) and ascorbic acid (ASA) was used as reference standard.

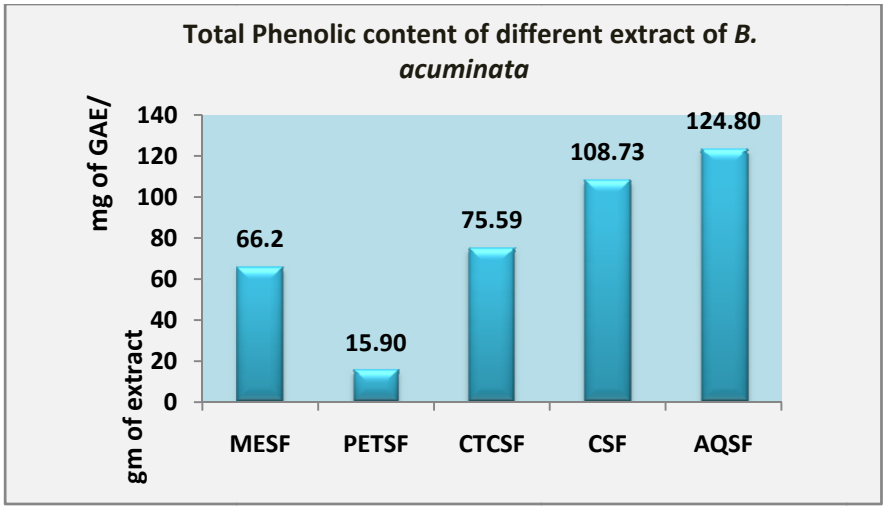

Figure 1. Total phenolic content (mg of GAE / gm of extractives) of different extractives of Leaves of *B. acuminata.* (MESF = Methanolic extract soluble fraction; PESF = Pet-ether soluble fraction; CTCSF = Carbon tetrachloride soluble fraction; CSF= chloroform soluble fraction; AQSF =Aqueous soluble fraction of the methanolic extract of *B. acuminata*).

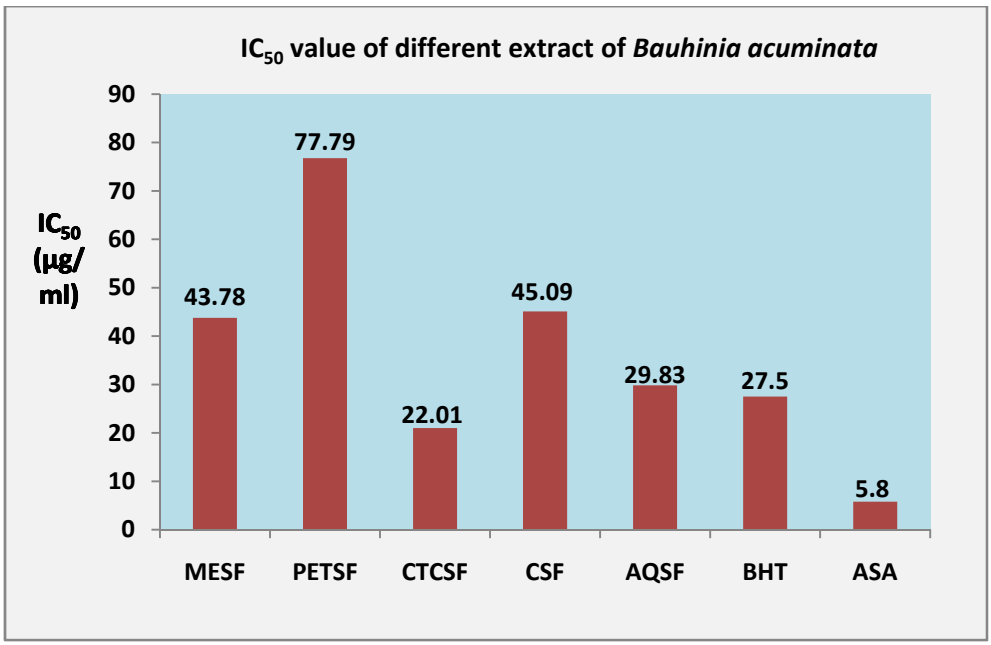

Figure 2. IC_{50} values of the standard and fractions of leaves of *Bauhinia acuminata*. (MESF = Methanolic extract soluble fraction; PESF = Pet-ether soluble fraction; CTCSF = Carbon tetrachloride soluble fraction; CSF= chloroform soluble fraction; AQSF =Aqueous soluble fraction of the methanolic extract of *B. acuminate*; BHT= *tert*-butyl-1-hydroxytoluene and ASA= ascorbic acid).

The antioxidant activity of IC_{50} values in DPPH method are differed in different extractives and ranged from 21.01 to 76.79. Among all extractives of *B. acuminata* the highest free radical scavenging activity was given by CTCSF (absorbance 21.01) followed by AQSF (absorbance 29.83).Significant free radical scavenging activity was also exhibited by CSF (absorbance 45.09), MESF (absorbance 43.78), PESF (absorbance 76.79). Results are given in figure-2.

This investigation indicates that the fraction (AQSF) which contained highest phenol compounds also exhibited highest antimicrobial activity due to presence of phenolic compounds. Several studies suggest that different classes of polyphenols, especially flavonoids are mostly responsible for many antioxidant effects of plant foods and medicinal plants [9-11]. This further supports the anti-oxidant activity of the plant extracts is due to a high content of phenolic compounds. Such antioxidant-based drug products may be helpful for the treatment and prevention of complicated diseases like diabetes, atherosclerosis, Alzheimer's disease, stroke and cancer and so on [12].

4. Conclusion

The results of the present investigation indicate that the leaves of *Bauhinia acuminata* possess significant anti-oxidant and total phenolic content activities and suggest that the

plant may be a safe, economical and easily available source of natural agents used in several disorders involving oxidation process and anti-cancer, anti-microbial agents. Further investigation on identification, isolation and purification of active moieties of the plant responsible for these therapeutic properties may lead to new drug development in future.

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