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POTENTIAL EX-VIVO ANTIOXIDANT INVESTIGATIONS OF *PARATHELYPTERIS BEDDOMEI* LEAVES

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ABSTRACT

The crude methanolic extract of leaves of *Parathelypteris beddomei* and its different organic soluble partitionates were screened for total phenolic content, and free radical scavenging activity. The amount of total phenolic content differed in different extractives and ranged from 50.58 mg of GAE / gm of extractives to 266.38 mg of GAE / gm of extractives of leaves of *P. Beddomi*. Among all extractives of leaves of *P. Beddomei* the highest phenolic content was found in PESF (12.41 mg of GAE / gm of extractives) which is followed by CSF (11.20 mg of GAE / gm of extractives). Significant amount of phenolic compounds were also present in CTCSF (2.08 mg of GAE / gm of extractives). The phenolic contents were observed in lesser amount in ME (2.08 mg of GAE / gm of extractives) followed by AQSF (3.11 mg of GAE / gm of extractives). In this investigation, AQSF showed the highest free radical scavenging activity. The IC_{50} value was 0.36 µg/ml. The other partionates like PESF, CSF, CTCSF and ME exhibited good scavenging activity having IC_{50} values 62.31 µg/ml, 72.02 µg/ml, 8.51 µg/ml, and 5.31 µg/ml, respectively. Further studies may lead to isolation of potent drugs moieties from *P. Beddomei*.

KEYWORDS: *Parathelypteris beddomei;* Biological Investigation, Total Phenolic Content, Antioxidant, DPPH.

1. INTRODUCTION

Numerous vegetables, crops, spices and medicinal herbs have been tested in an effort to identify new and potentially useful antioxidants. More recently, it has become evident that phenolic natural products may reduce oxidative stress by indirect antioxidant action. For example, various flavonoids, which are found naturally in fruits, vegetables and some beverages, have been demonstrated to exert antioxidant effects through a number of different mechanisms.

Majority of the diseases/disorders are mainly linked to oxidative stress due to free radicals. Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals, and also by acting as oxygen scavengers. 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.[1] A number of reports on the isolation and testing of plant derived antioxidants have been described during the past decade. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability.

Different synthetic antioxidant such as tert-butyl-1-hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG) and tert-butylhydroquinone (tBHQ) used as food additives to increase self life are known to have not only toxic and carcinogenic effects and humans, but abnormal effects on enzyme systems[2] Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer. Therefore, the interest in natural antioxidant, especially of plant origin, has greatly increased in recent years.[3] Plant polyphenols have been studied largely because of the possibility that they might underlie the protective effects afforded by fruits and vegetables intake against cancer and other chronic diseases. The purpose of this study was to evaluate different extractives of *p. beddomei* as new potential sources of natural antioxidants and phenolic compounds. *P. beddomei*, or Ferns or fern allies, is an evergreen tree native from Anhui, Chongqing, Guangxi, Guizhou, Henan, Hunan, Jiangxi, Shaanxi, Sichuan, Taiwan, Yunnan, Zhejiang.[4]

2. MATERIALS AND METHODS

2.1. Plant Material

The leaves of *p. beddomei* were collected from Mirpur Botanical Garden, Dhaka, Bangladesh, in the month of November 2011. A voucher specimen for this plant has been maintained in Bangladesh National Herbarium, Dhaka, Bangladesh (Accession no. 38305). The fruit were picked and washed with water to remove all unwanted plant materials and sand, air dried under light exposure (27°C-30°C for 7 days), pulverized in a mill and stored in an airtight container for further study.[5]

2.2. Preparation of Extract

The air dried and powdered fruit (500 gm) of *p. beddomei* 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. All the extracts were concentrated with a rotary evaporator at low temperature (40-45 ºC) with reduced pressure. The concentrated methanolic extracts (me) were partitioned by modified kupchan method and described by md. Reyad-ul-ferdous[6,7,8] the resultant partitionates which are pet-ether (pesf), chloroform (csf), carbon tetrachloride (ctcsf), and aqueous (aqsf) soluble fractions were used for the experimental processes.

2.3. Total Phenolic Compound Analysis

To 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 were 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 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.[5]

2.4. Antioxidant Activity: DPPH Assay

2.4.1. Principle

The free radical scavenging activities (antioxidant capacity) of the plant extracts on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were estimated by the method of Brand-Williams).[9]

2.0 ml of a methanol solution of the extract at different concentration were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml). The antioxidant potential was assayed from the

bleaching of purple colored methanol solution of DPPH radical by the plant extract as compared to that of *tert*-butyl-1-hydroxytoluene (BHT) and ascorbic acid (ASA) by UV spectrophotometer.

2.4.2. Control Preparation for Antioxidant Activity Measurement

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

2.4.3. Test Sample Preparation

Calculated amount of different extractives was 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.

2.4.4. 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.

2.4.5. Assay of Free Radical Scavenging Activity

2.0 ml of a methanol solution of the sample (extractives/ control) at different concentration (500 µg/ml to 0.977 µg/ml) were mixed with 3.0 ml of a DPPH methanol solution (20 µg/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.[9]

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

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

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

Total Phenolic Content

The extract of properly dried and grinded leaves of *P. beddomei* using methanol (ME) and its different partitionates i.e. petroleum ether (PESF), carbontetrachloride (CTCSF), chloroform (CSF) and aqueous soluble fractions (AQSF) were tested for total phenolic content. Folin-Ciocalteu reagent was used for the test. Based on the absorbance values of the various extract solutions the colorimetric analysis of the total phenolics of different extracts were determined and compared with the standard solutions of gallic acid equivalents. Total phenolic content of the samples are expressed as mg of GAE (gallic acid equivalent)/ gm of extractives and are given in fig-1.

The amount of total phenolic content differed in different extractives and ranged from 50.58 mg of GAE / gm of extractives to 266.38 mg of GAE / gm of extractives of leaves of *P. Beddomi*. Among all extractives of leaves of *P. Beddomei* the highest phenolic content was found in PESF (12.41 mg of GAE / gm of extractives) which is followed by CSF (11.20 mg of GAE / gm of extractives). Significant amount of phenolic compounds were also present in CTCSF (2.08 mg of GAE / gm of extractives). The phenolic contents were observed in lesser amount in ME (2.08 mg of GAE / gm of extractives) followed by AQSF (3.11 mg of GAE / gm of extractives).

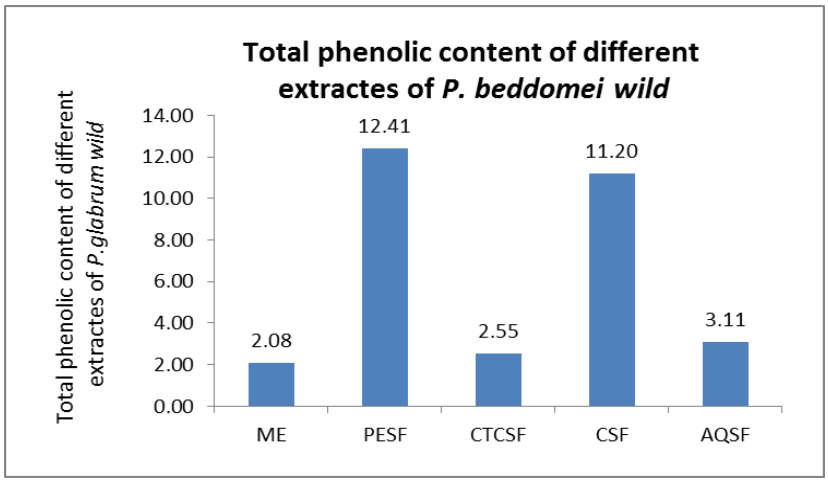

Figure-1: Total phenolic content (mg of GAE / gm of extractives) of different extractives of leaves of *P. beddomei*.

Antioxidant activity by DPPH Assay

The methanol extract (ME) of properly dried and grinded leaves of *P. beddomei* and its different partitionates i.e. petroleum ether (PESF), carbontetrachloride (CTCSF), chloroform

(CSF) and aqueous (AQSF) soluble fractions were subjected to free radical scavenging activity by the method of Brand-Williams[9] Here, *tert*-butyl-1-hydroxytoluene (BHT) and ascorbic acid (ASA) were used as reference standards.

In this investigation, AQSF showed the highest free radical scavenging activity. The IC_{50} value was 0.36 µg/ml. The other partionates like PESF, CSF, CTCSF and ME exhibited good scavenging activity having IC_{50} values 62.31 µg/ml, 72.02 µg/ml, 8.51 µg/ml, and 5.31 µg/ml, respectively fig-2.

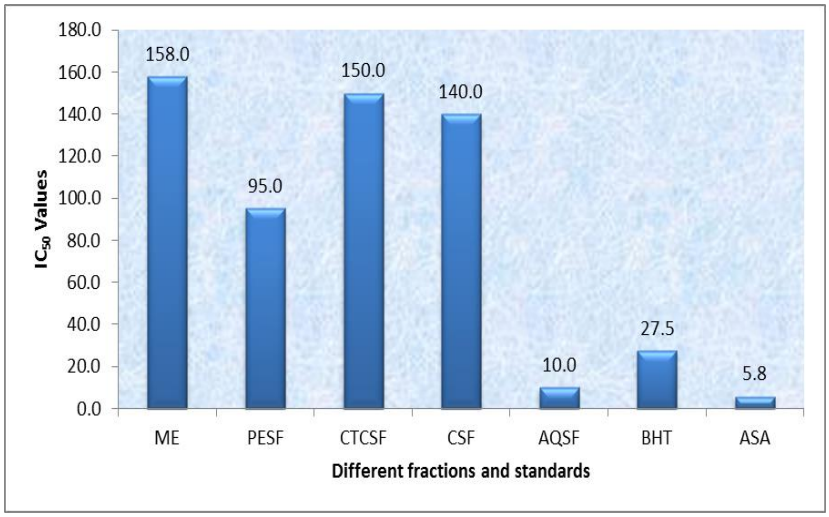

Figure-2: IC $_{50}$ values of the standards and partitionates of leaves of *P. beddomei*

CONCLUSION

The methanolic extract and its different partitionates of leaf of *Parathelypteris Beddomei* were subjected to different biological investigations such as cytotoxic activity, total phenolic content, antimicrobial activity, thrombolytic activity, membrane stabilizing activity and free radical scavenging activity.The investigation confirmed us that leaf of *P.beddomei* has strong antioxidant property and significant antimicrobial property. Pet ether soluble fraction (PESF) of *P,beddomei* leaves also has strong cytotoxic properties.The methanolic soluble fraction (MESF), pet ether soluble fraction (PESF), aqueous soluble fraction (AQSF), chloroform soluble fraction (CSF) of leaves of *p.beddomei* strongly protect the lysis of human erythrocyte membrane by both hypotonic solution and heat induced method, which confirms that the plant has membrane stabilizing activity.This is only a preliminary study and to make final comment the extract should be thoroughly investigated phytochemically and pharmacologically to exploit their medicinal and pharmaceutical potentialities.

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