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POTENTIAL EX-VIVO CARDIOPROTECTIVE EVALUATION OF LEAVES OF *P. BEDDOMEI*

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ABSTRACT

The crude methanolic extracts of leaves of *Parathelypteris beddomei* and its different organic soluble partitionates were screened for thrombolytic activity for isolation of cardioprotective drugs. The various extracts of *Parathelypteris beddomei* were tested to know the activity against lysis of human erythrocyte clot formation. All extracts exhibit mild to moderate thrombolysis activity. methanolic soluble fraction 13.47%, chloroform soluble fraction 19.55%, aqueous soluble fraction 18.56%, pet ether soluble fraction 10.94%, and carbon tetrachloride soluble fraction 13.18%, whereas standard and blank exhibit lysis 65.16% and 5.79% respectively. Further studies may lead to isolation, purification of lead compounds for treatment of various diseases.

KEYWORDS: *Parathelypteris beddomei*; Cardioprotetive, Biological Investigation.

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. Cardio-protective activity assay

2.3.1. Preparation of Sample

The thrombolytic activity of all extractives was evaluated by a method using streptokinase (SK) as standard substance. The dry crude extract (100 mg) was suspended in 10 ml of distilled water and it was kept overnight. Then the soluble supernatant was decanted and filtered.

2.3.2. Streptokinase (SK)

Commercially available lyophilized altepase (streptokinase) vial (Beacon Pharmaceutical Ltd.) of 1,500,000 I.U., was collected and 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100 µl (30,000 I.U) was used for *in vitro* thrombolysis.

2.3.3. Blood Sample

Whole blood (n=10) was drawn from healthy human volunteers without a history of oral contraceptive or anticoagulant therapy and 1ml of blood was transferred to the previously weighed microcentrifuge tubes and was allowed to form clots.

2.3.4. Thrombolytic Assay

The cardio-protective activity was evaluated by thrombolytic activity assy. The thrombolytic activity of all extracts was evaluated by the method describe by daginawala[9] and modified by kawsar[10] using streptokinase (sk) as the standard as well as described by reyad-ul-ferdous m.[7] 0.1 gm extract was suspended in 10ml distilled water and the suspension was shaken vigorously on a vortex mixer. The suspension was kept overnight and decanted to remove the soluble supernatant, by using a filter. 100 µl of this aqueous preparation of herbs was added to the alpine tubes containing the clots to check the thrombolytic activity. 10

alpine tubes were taken and each eppendrof tube containing clot was properly labelled and venous blood drawn from the healthy volunteers. That was transferred in 10 different pre weighed sterile alpine tube (0.5 ml /tube) and incubated at 37^{o}c for 45 minutes. Aspirated washout without disturbing the clot formed. Water was also added to one of the tubes containing clot and this serves as a negative thrombolytic control. All the tubes are than incubated at 37^{o}c for 90 minutes and observed for clot lysis. After incubation, fluid obtained was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis. Percentage of clot lysis was determined from below formula.

% of clot lysis = (weight of released clot /clot weight) ×100

3. RESULTS AND DISCUSSION

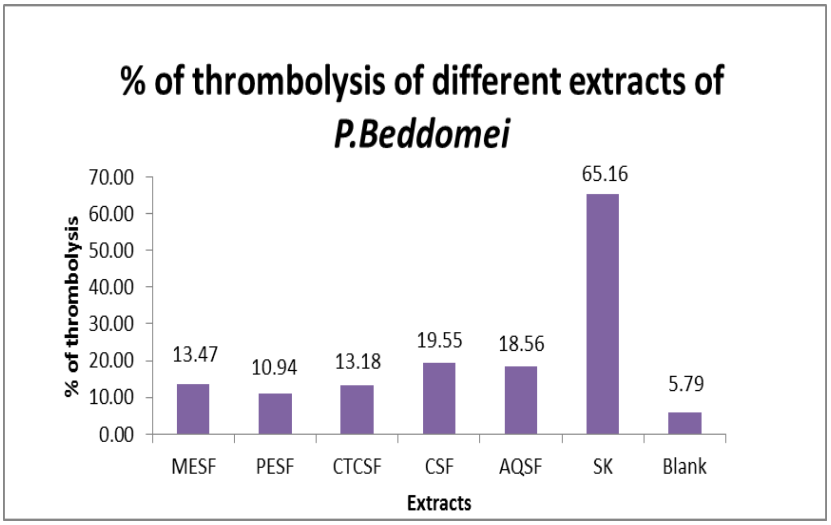

Figure-1: Thrombolytic activity of leaves of *P. Beddomei*

In investigating cardio protective drugs from natural sources the extracts obtained from *P. Beddomei* were assessed for thrombolytic activity and the results are presented in Table 9.1. After addition of 100 µl SK, a positive control (30,000 I.U.), to the clots, the system was incubated for 90 minutes at 37° C which exhibited 66.77% lysis of clot. On the other hand, distilled water was applied as negative control which exhibited a negligible percentage of lysis of clot (8.51%). The mean difference in clot lysis percentage between positive and negative control was found very significant. In this study, the carbontetrachloride soluble fraction (CSF) of methanol extract of *P. Beddomei* demonstrated highest thrombolytic activity (19.55%). However, the other partitionates of methanol extract of *P. Beddomei* i.e. methanol extract (ME), petroleum ether (PESF), chloroform (CTCSF) and aqueous (AQSF)

soluble fractions showed mild thrombolytic activity. The comparisons of thrombolytic activities of different extractives are shown in figure-1.

CONCLUSION

The methanolic extract and its different partitionates of leaf of *Parathelypteris Beddomei* were subjected to biological investigations cardiopretective 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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