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Evaluation of *Ex-Vivo* Anti-inflammatory and total phenolic content of fruits of *Parmentiera cereifera seem*

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Abstract: In the present studies, of the crude methanolic (ME) extract, Hexane (HXSF) soluble fraction, carbon tetrachloride (CTCSF) soluble fraction, the Aqueous (AQSF) and chloroform (CSF) soluble fraction fruits of *P. cereifera* demonstrated strong membrane stabilizing activity. The bark extracts and fruits extracts were demonstrated significant membrane stabilizing activity ranging from (38.49% to 28.84%) compared with Acetyl salicylic acid (ASA). Whereas mild total phenolic content was demonstrated ranging from (3-5.4) gm of GAE/100 gm of dried extract.

Keywords: *Parmentiera cereifera*, Membrane Stabilizing Activity, Total Phenolic Content.

1. Introduction

Parmanteira cereifera (commonly- Candle tree) is a small tree with rough bark belonging to the family Bignoniaceae. The leaves are acuminate and oblong. The flowers are white, slightly fragrant, cauliflorous, nocturnal, and calyx spathaceous. The fruits and seeds of this tree are candle-like, berry pale yellow, pendentsmooth, edible and used as fodder source. It is native to Panama and cultivated for ornamental uses in many tropical countries [1,2].

As a part of our continuing studies on medicinal plants of Bangladesh, the organic soluble materials of leaf of *P. cereifera* were evaluated for antioxidant activity in terms of free radical scavenging activity using DPPH and poly phenolic composition, membrane stabilization, thrombolysis, cyotoxicity and antimicrobial activities as well as for determining phytoconstituents for the first time. The leaves are oblong and acuminate. The flowers are cauliflorous, nocturnal, white, slightly fragrant and spathaceous. The fruit and seeds of this tree are berry plate yellow, pendent, candel-like, smooth, edible and used as fodder source. The tree is native to panama and cultivated for ornamental uses in many tropical countries [3].

2. Materials and Methods

2.1. Plant Materials

The leaf of *P. cereifera* was collected from Botanical garden, Mirpur, Dhaka, Bangladesh, in November 2011. A voucher specimen for this plant has been maintained in Bangladesh National Herbarium, Dhaka, Bangladesh (Accession no.36569). The sun dried and powdered leaf (500 gm) of *P. cereifera* was dissolve in 2.5 L of methanol for 7 days and then filtered through a cotton plug followed by Whatman filter paper (number 1). The extract was concentrated at low temperature (40-45 ºC) by rotary

evaporator and reduced pressure. The concentrated methanolic extract (ME) was partitioned by modified Kupchan method [4] and the following partitionates obtained i.e., pet-ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF), and aqueous (AQSF) soluble fractions were used for the experimental processes.

2.2. Anti-inflammatory Activity

Anti-inflammatory activity was conducted by membrane stabilizing method. The membrane stabilizing activity of the extractives was assessed by using hypotonic solution-induced and heat-induced hemolysis of mice erythrocyte by the method developed by [5] and modified by [6] describe by method [7]. In hypotonic solution-induced method, the test sample consisted of stock erythrocyte (RBC) suspension (0.50 mL) is mixed with 5 mL of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing either the extracts (1.0 mg/mL) or acetyl salicylic acid (0.1 g/mL). 0.5 mL of RBCs mixed with hypotonic-buffered saline alone for prepared control sample. The mixture sample was incubated for 10 min at room temperature and centrifuged for 10 min at 3000 g as well as taken the absorbance of the supernatant at 540 nm. The percentage inhibition of either membrane stabilization or haemolysis was determined using the following equation –

% inhibition of haemolysis = 100 x (OD1-OD2/OD1)

Where, OD1=optical density of hypotonic - buffered saline solution alone (control) and OD2= optical density of test sample in hypotonic solution.

In heat-induced haemolysis, isotonic buffer containing aliquots (5 ml) of the different extracts were put into two duplicate sets of centrifuge tubes. Two tubes were prepared with same amount of vehicle and another tube as control. Erythrocyte suspension (30 µL) was added to each tube and mixed gently by inversion. One pair was maintained at (0-5) o C in an ice bath while the other pair of the tubes was incubated at 54 o C for 20 min in a water bath. The vehicle and Erythrocyte suspension containing mixture was centrifuged for 3 min at 1300 g and the absorbance of the supernatant was determinate at 540 nm. The acceleration of hemolysis or percentage inhibition tests was calculated according to the equation:

% Inhibition of hemolysis = 100 x [1-(OD2-OD1/OD3-OD1)]

Where, OD1= optical density of unheated test sample, OD2= optical density of heated test sample and OD3=optical density of heated control sample.

2.3. Total Phenolics Analysis

Total phenolic content of leaves of *P. cereifera* extractives was measured employing the method as described by [8] with Folin-Ciocalteu reagent as oxidizing agent and gallic acid as standard [3]. In brief, 0.5 ml of extract solution (2 mg/ml) in water, 2.5 ml of Folin -Ciocalteu reagent and 2.0 ml of sodium carbonate (7.5 % w/v) solution were added and gently mixed up with each other. After 20 minutes of incubation at room temperature the absorbance was taken at 760 nm. Total phenolics were quantified by (gallic acid) calibration curve and were expressed as mg of GAE (gallic acid equivalent) / gm of the dried extract.

2.4. Drugs and Chemicals

The drugs such as Acetyl salicylic acid were collected from Opsonin Pharmaceuticals Ltd, Dhaka, Bangladesh as gift sample. Other required chemicals were obtained from Merck limited.

2.5. Statistical Analysis

The results were expressed as the mean ± standard deviation (SD).

3. Results and Discussion

The various fractionates of fruits of *P. cereifera* at concentration 1.0 mg/mL were tested to know the activity against lysis of human erythrocyte membrane induced by hypotonic solution as well as heat induced, as compared to the standard acetyl salicylic acid (0.10 mg/mL) (Table 1).

In hypotonic solution induced condition the fruits extracts of the carbon tetrachloride (CTCSF) soluble fraction inhibited 38.49%, chloroform soluble fraction (CSF) inhibited 28.67% and aqueous soluble fraction (AQSF) inhibited 21.84% while the crude methanolic (ME) extract inhibited 26.84% Hexane soluble fraction (HXSF) inhibited 18.17% respectively haemolysis of RBC as compared to 71.9 % inhibited by acetyl salicylic acid (0.10 mg/mL).

On the other hand during heat induced condition the fruits extract of different fractions of *P. cereifera* i.e. CSF, AQSF, CTCSF, HXSF and ME showed inhibition about 32.50%, 12.17%, 28.84%, 35.50% and 38.49%, respectively whereas inhibition of ASA was 42.12% (Table 1).

Membrane stabilization results prevention leakage of fluids and serum proteins into the tissues during a period of increased permeability caused by inflammatory mediators. Phytochemicals screening showed that the plant extracts contains flavonoids which indicate potent anti-inflammatory property by inhibitory effect on enzymes, involved in the production of the chemical mediators of inflammation and metabolism of arachidonic acid [9]. Recent investigation isolated two new compounds from this plant which are phenolic acid glycosides, parmentins A (1) and B (2) from the methanolic extract of the leaves and stems of candle tree (Parmentiera cereifera Seem). These compounds were accompanied by a mixture of b-sitosterol and stigmasterol (3), b-sitosterol glucoside (4), isovanillic acid (5), vanillic acid (6), and p-hydroxybenzoic acid (7). The structures of the isolated compounds were determined on the basis of physical and spectroscopic analyses, including 1D and 2D NMR (1H, 13C, COSY, HSQC and HMBC) and mass spectrometry (HR-ESI-MS) [10].

All the partitionates of extract of fruits of *P. cereifera* were tested for total phenolic content. Folin-Ciocalteu reagent was

used as oxidizing agent 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 were expressed as mg of GAE (gallic acid equivalent)/ gm of extractives and are given in (figure 1). Several studies suggested that phenolic compounds exhibited antimicrobial activity, antioxidant effects which may helpful for the treatment and prevention of complicated diseases such as diabetes, atherosclerosis, stroke, cancer and Alzheimer's disease.

Table 1. *Effect of extractives of Fruit (P. cereifera) on hypotonic solution and heat induced haemolysis of erythrocyte membrane.*

Sample code	Concentration	% inhibition of haemolysis	
		Heat induced	Hypotonic solution induced
Hypotonic medium	50 mM	--	--
ME	1 mg/mL	38.49 ± 0.50	26.84 ± 0.76
PESF	1 mg/mL	35.50 ± 0.50	18.17 ± 0.29
CTCSF	1 mg/mL	28.84 ± 0.77	38.50 ± 0.50
AQSF	1 mg/mL	12.17 ± 0.29	21.84 ± 0.76
CSF	1 mg/mL	32.50 ± 0.50	28.67 ± 0.58
Acetyl salicylic acid	0.10 mg/mL	42.12 ± 0.0	71.9 ± 0.0

ME = Methanolic extract; HXSF = Hexane soluble fraction; CTCSF = Carbon tetrachloride soluble fraction; CSF= chloroform soluble fraction; AQSF = Aqueous soluble fraction of the methanolic extract of *P. cereifera*; ASA = Ascorbic acid. All values are expressed as mean ± SEM. of 3 replicates.

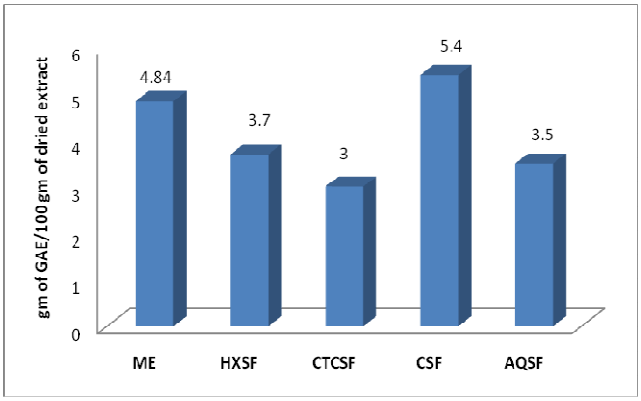

Figure 1. *The total phenolic content of different fractions of fruits (P. cereifera). ME = Methanolic extract; HXSF = Hexane soluble fraction; CTCSF = Carbon tetrachloride soluble fraction; CSF= chloroform soluble fraction; AQSF = Aqueous soluble fraction of the methanolic extract of P. cereifera. All values are expressed as mean ± SEM. of 3 replicates.*

4. Conclusion

In the present investigation, demonstrate significant anti-inflammatory activity. The plant may be safe and easily available source as well as economic of natural agents used in inflammation and total phenolic content. . Future study would be conducted for isolation and identification of lead compounds and purification of the active principles of the plant responsible for the observed biological effects.

Abbreviations

ME= Methanolic extract; PESF= Pet-ether soluble fraction; CTCSF= Carbon tetrachloride soluble fraction; CSF=Chloroform soluble fraction; AQSF =Aqueous soluble fraction of the methanolic extract of *P. cereifera.*

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