

Ex-Vivo Cardioprotective and Cytotoxic Screening of Fruits of *Parmentiera cereifera* Seem

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Abstract

In this present investigation, the fruits extract of *Parmentiera cereifera* were subjected to a comparative evaluation of the cardio-protective activities and Cytotoxic to brine shrimps (*Artemia salina*) as compared with standard streptokinase (SK) and Vincristine sulphate (VS). 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* were subjected to these studies. In thrombolytic study, the fruits extract crude methanolic (ME) extract and Hexane (HXSF) soluble fraction were demonstrated mild to moderate (2.5 to 21.50%) thrombolytic activity in human blood specimen compared with standard streptokinase (SK) 66.77%. The fruits extracts were demonstrated (1.78 to 79.90) significant Cytotoxic to *A. salina* with LC₅₀ values ranging from 6.07 to 7.83 µg/mL compared with standard Vincristine sulphate (VS, LC₅₀ value 0.45 µg/mL).

Keywords

Parmentiera cereifera; Fruits extract; Cardio-protective activities; Cytotoxic screening

Introduction

Parmanteira cereifera (commonly- Candle tree) is a small tree with rough bark belonging to the family Bignoniaceae. The leaf is oblong and acuminate. The flowers are caulioflorous, 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 pnama and cultivated for ornamental uses in many tropical countries. Previous studies demonstrate significant anti-inflammatory, cardioprotective, antimicrobial activity of leaves of *P. cereifera*. Several potential phytochemicals present in *P. cereifera* such as flavonoids, saponins, tannins, triterpenoids, steroids of leaves fraction [1]. Barks fraction contains flavonoids, saponins, triterpenoids, terpenoids [2]. Fruits fraction consists of flavonoids, saponins, tannins, triterpenoids, terpenoids, steroids [3]. As a part of our continuing studies on medicinal plants of Bangladesh, the organic soluble materials of fruits of *P. cereifera* were evaluated for thrombolysis, Cytotoxicity activity for the first time.

Materials and Methods

Plant materials

The fruits of *P. cereifera* were 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 fruits (500 gm) of *P. cereifera* 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. The extracts were concentrated with a rotary evaporator at low temperature (40-45°C) and reduced pressure. The concentrated methanolic extracts (ME) were partitioned by modified Kupchan method [4] and the resultant partitionates which are pet-ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF), and aqueous (AQSF) soluble fractions were used for the experimental processes.

Thrombolytic activity

The thrombolytic activity was conducted for evaluation of cardio-protective drugs. The thrombolytic activity of all extracts was evaluated by the method developed by Dagainwala et al., [5] and modified by Kawsar et al., [6] using streptokinase (SK) as the standard.

In short, the plant extract (100 mg) suspended in 10 mL of distilled water was kept overnight. The soluble supernatant was decanted and filtered by using a 0.22 µ syringe filter. For clot lysis, venous blood (500 µL) drawn from human healthy volunteers was distributed in different sterile pre weighed microcentrifuge tube and incubated at 37°C for 45 minutes for clot formation.

After clot formation, the serum was completely removed without disturbing the clot and the clot weight was determined. 100 µL aqueous solutions of different partitionates and crude extract were added separately to each microcentrifuge tube with the pre-weighed clot. Then, 100 µL (30,000 I.U) of commercial streptokinase (SK) and 100mg of distilled water were separately added to the control tube as positive and negative controls, respectively.

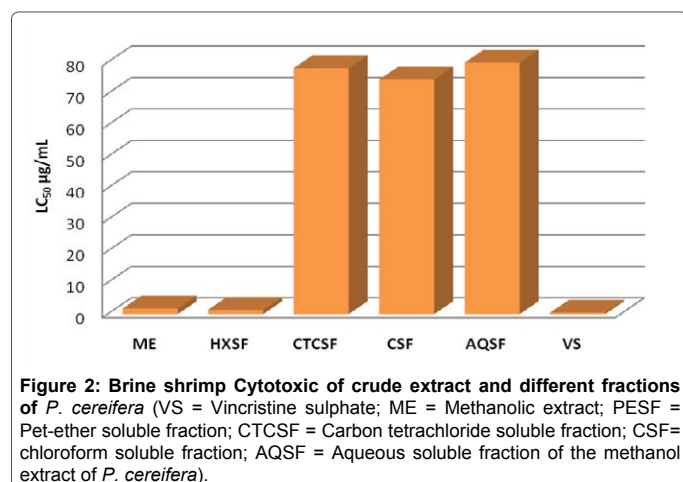
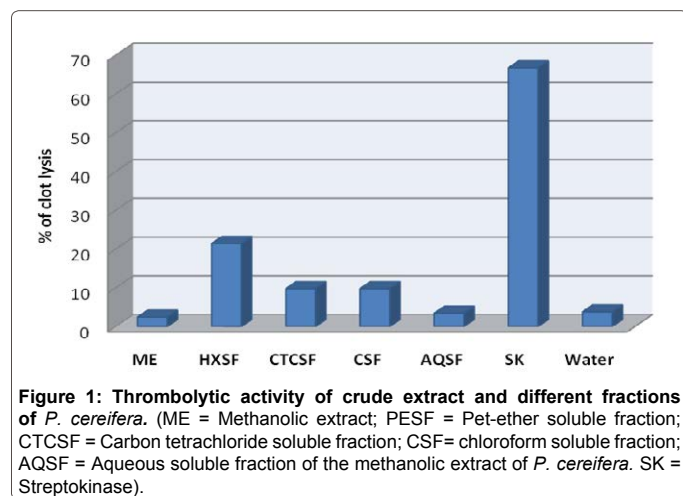
All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, the released fluid was removed and tubes were reweighed to observe the difference in weight after clot disruption. Percentage of clot lysis was determined from below formula.

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$$\% \text{ of clot lysis} = (\text{weight of released clot} / \text{clot weight}) \times 100$$

Cytotoxic screening

The Cytotoxic screening was conducted by Brine shrimp lethality bioassay. This technique was applied for the determination of general toxic property of the DMSO solutions of plant extractives [7,8] against *Artemia salina* in a 1-day in vivo assay. Vincristine sulphate was used as positive control.

For the experiment, different concentrations (400-0.781 µg/mL) of methanol extract and its carbon tetrachloride, petroleum ether, chloroform and aqueous soluble fractions were prepared in DMSO. General toxic property of the plant extract and its different fractions against *Artemia salina* was determined in a 1-day in vivo assay. Vincristine sulphate was used as positive control. The mortality percentage and LC₅₀ (lethal concentration for 50% of the population) were determined using statistical analysis and the graph plotted concentration against percent lethality.

Statistical Analysis

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

Results and Discussion

As a part of discovery of cardio protective drugs from natural resources the extractives of Candle tree (*P. cereifera*) were assessed

for thrombolytic activity and the results are presented in (Figure 1). Addition of 100 µL SK, a positive control (30,000 I.U.), to the clots and subsequent incubation for 90 minutes at 37°C, showed 66.77% lysis of clot. On the other hand, distilled water was treated as negative control (3.87%) of lysis of clot. In this study, the fruits extracts were demonstrated moderate activity ranging from (3.5% to 21.50%). The Hexane (HXSf) soluble fraction exhibited highest thrombolytic activity (21.50%) showed in the figure-1. The thrombolytic activity is demonstrate due to the presence of plant's diverse composition like flavonoids, tannins and terpenoids [9].

The fruits extracts were demonstrated ranging from (1.78-79.90) significant Cytotoxic to *A. salina* with LC₅₀ values ranging from (6.07-7.83) µg/mL as compared to standard Vincristine sulphate (LC₅₀ value 0.45 µg/mL). The results are given in Figure 2.

The brine shrimp test represents a simple, rapid and inexpensive bioassay for testing plant extract lethality which in most cases has a good correlation with cytotoxic and anti-tumour properties [10,11]. Crude extracts resulting in LC₅₀ values of less than 250 µg/mL is usually considered significantly active and potential for further investigation [12]. The result obtained from the brine shrimp lethality bioassay of *P. cereifera* can serve as a guide for the isolation of cytotoxic compounds from the methanol extract and different fractions of the plant by using more specific and more sophisticated bioassays.

Conclusion

Results of the present investigation for the first time indicate that the *P. cereifera* fruits possess significant cardio-protective and cytotoxic activities and suggest that the plant may be a safe, economical and easily available source of natural agents used in cardiovascular disorders involving blood clot and anticancer agents due to mild toxic effect. These activities may occur due to the presence of both types of active lead compounds in different fraction of this plant fruit extracts. Those compounds may responsible for cardio-protective and anti-cancer activities. Further investigation on identification, isolation and purification of active moieties of the plant responsible for these therapeutic properties may lead to new drug development.

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