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Original Article

IN VITRO ANTHELMINTIC AND ANTI-AMYLASE PROPERTIES OF *GARCINIA PEDUNCULATA* ROXB. ETHANOLIC EXTRACT

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ABSTRACT

Objective: The present study aims to investigate *in vitro* anthelmintic and anti-amylase properties of the ethanolic fruit extract of *Garcinia pedunculata*.

Methods: For the study, mature fruits of *G. pedunculata* were collected from local markets during April-May. Fleshy pericarps of fruits were chopped into small pieces, dried and extracted by using a Soxhlet apparatus. Ethanol extract of *G. pedunculata* was used for evaluation of *in vitro* anthelmintic and anti-amylase activities. *In vitro* anthelmintic activity was evaluated in animal models, *Pheretima posthuma*, an earthworm species. *In vitro* anti-amylase activity was evaluated by using zymographic, achromic point analysis (Starch-Iodine method) and spectrophotometric method [Di Nitro Salicylic acid (DNS)–Maltose method].

Results: Ethanolic extract of *G. pedunculata* showed anthelmintic activity at a concentration of 75 mg/ml, paralysis and death timing was reported at 0.62±0.26 min and 1.42±0.07 min, respectively. The reference standard (Albendazole) showed paralysis time: 2.13±0.28 min and death time: 5.12±0.29 min. In the anti-amylase study, a zymographic density analysis of *G. pedunculata* showed significant variation in band intensity as compared to Starch–Iodine achromic point analysis and DNS–Maltose method. A concentration of 1.5 mg/ml of extract showed inhibition of amylase: 67.65±1.53 % as compared to other concentrations and control sets.

Conclusion: It could be concluded that ethanolic extract of *G. pedunculata* has biological properties which could be utilised in medicine by characterising its bioactive components.

Keywords: Anti-amylase activity, Anthelmintic activity, *Garcinia pedunculata*

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INTRODUCTION

In North East India, 9 species of the genus *Garcinia* (Thekera tenga) have been reported in 1934 which are mainly used in food by different communities [1, 2]. Out of the 9 species, 8 species alone have been reported from the Sonitpur district of Assam [2]. The genus belongs to the family Clusiaceae, the members of this genus are mainly evergreen trees or shrubs with greenish gum resins. They are used in many health problems by different communities like, Kau (*G. cowa* Roxb.) and Kuji (*G. morella* Desr.) thekera tenga are used in the treatment of dysentery by Assamese and Bodo people [3]. Bor thekera tenga (*G. pedunculata* Roxb.) has been used for Diabetes mellitus (DM) by Rabha, Karbi and Missing communities [4]. Local traditional healers suggest that, if a patient takes one teaspoon of juice of the *G. pedunculata* every morning for one week, then the patient would have a normal blood sugar level. But, regular use of this dried peel for a longer time is not suggested because it might cause stomach and constipation problems.

Scientifically, *G. pedunculata* has been reported to have a good amount of Hydroxycitric acid (HCA) among all *Garcinia* sp. One of the isomeric forms of HCA has been reported from this plant, might play a role in reducing obesity [5]. With the traditional knowledge of its use in DM, a hypothesis was designed to evaluate its *in vitro* anti-amylase and anthelmintic properties.

Starch, Sodium hydroxide (NaOH), DNS, Sodium sulphite (Na_2SO_3), Sodium acetate, methanol, ethanol and glacial acetic acid, acrylamide, bis-acrylamide, N,N,N,N-tetramethylene ethylenediamine (TEMED), ammonium persulphate, 0.1 N iodine solution were purchased from Merck, Mumbai, India. All the chemicals were of analytical grade.

Mature fruits of *G. pedunculata* were collected from local markets during April-May. This fruit is available in the market only in this period of a year, and it was identified by the traditional farmer expert. It is a tall, erect tree which flowers in mid of January. Herbarium of it was prepared in this period by standard procedure [6]. It was deposited for identification in the Botanical Survey of India (BSI), Shillong, Meghalaya and the herbarium was also deposited in the Herbaria Repository of Defence Research Laboratory, Tezpur, Assam. The mature fruits were washed to remove dirt. Fleshy pericarps of the fruits were chopped into small pieces, shade dried and stored in an airtight container. The extraction was carried out by the percolation method using a Soxhlet apparatus, Optics Technology, Delhi, India. The solvent was used to be ethanol. About 100 g of dried pieces were extracted with 500 ml of ethanol. The extract was concentrated to dryness under a controlled temperature of 40-45 °C. The percentage yield was found to be 15.43 %. The extract was preserved in the refrigerator till further use.

A starch solution (1 % w/v) was prepared in 100 ml of 16 mmol of sodium acetate buffer. The enzyme solution was prepared by mixing 1 ml saliva in 19 ml of distilled water. The saliva mix was then filtered by using Whatman filter paper No. 1. The colorimetric reagent DNS was prepared by dissolving 1 g of DNS in 1 % NaOH solution containing 200 mg crystalline phenol and 50 mg of Na_2SO_3. The test tubes were labelled as a zero min control (Blank), plant extract treated and positive control (untreated amylase). 1 ml of distilled water was added to blank and positive control tubes. Other test tubes contain 1 ml of the extracts with a concentration of 0.5, 1, 1.5 and 2 mg/ml. 1 ml starch solution was added to all the tubes, followed by 1 ml of saliva and incubated for 15 min at 25 °C. In the case of the blank, the reaction was stopped by adding 2 ml of DNS solution in zero min. But, in other sets, the reaction was stopped after 15 min by adding 2 ml DNS solution to each tube. The reaction mixture shows a reduction of DNS to 3-amino-5-nitrosalicylic acid when incubated in boiling water for 5 min. The absorbance of the colour change was detected at 540 nm by using UV-Visible Spectrophotometer (Systronics, India) [7].

The amylase activity can also be detected by running PAGE with 7.5 % gel under basic native conditions. Five lanes were used, named as positive control (saliva only) lane, 0.5, 1.0, 1.5 and 2.0 mg/ml plant extracts containing lanes with saliva in each. Sample mix of 50 µl saliva and 50 µl of plant extract was made for loading in the wells, but in the positive control, 50 µl of distilled water in place of the extract was added to make equal volume as per the other treatment lanes and labelled as control lane. For zymogram, after electrophoresis, the gel was incubated in gelatinized starch (2 % prepared in 0.1 M sodium acetate buffer, pH 5) for 1 h. Subsequently, the gel was stained with iodine reagent (3 %) for 5 min. The gel was destained by dipping it in destaining solution (5 ml glacial acetic acid, 45 ml of methanol and 50 ml distilled water) for overnight and observed on the next day for better band detection [8]. Image J software (National Institute of Health, USA) would be used to analyse the percentage inhibition of the enzyme in the zymogram.

The achromic point of salivary amylase helps in determining the activity of the enzyme by taking starch as a substrate which reacts with 1 % iodine solution. 1 ml of an extract of different concentrations (0.5, 1.0, 1.5 and 2.0 mg/ml) was taken on labelled test tubes and 1 ml of saliva with 1:20 dilution was added. The reaction was started by the addition of 1 ml starch and incubated for 15 min at 25 °C. The control test tube was prepared by stopping the reaction with 2–3 drops of iodine solution to it. Positive control of salivary amylase was prepared by incubating the test tube for 15 min containing untreated saliva with 1 ml of distilled water. The reaction was stopped by adding 2–3 drops of iodine solution [9]. The colour change was observed, and Image J software (NIH, USA) would be used for analysis of percentage inhibition of the enzyme.

Adult earthworms (*P. posthuma*) were used to evaluate *in vitro* anthelmintic activity (worms were identified by veterinary practitioners). Earthworms were collected from cow dung dumping ground near Panikhaiti, Guwahati, Assam. The average size of an earthworm was found to be 6–8 cm. *In vitro* anthelmintic assay was carried out as per the method [10]. The extract concentrations taken were 25, 50 and 75 mg/ml in distilled water. Each test samples were subjected for triplicate study in 9 cm Petri dish containing 25 ml of it. Albendazole (25 mg/ml) was used as the reference standard, and normal saline was used as a control. The effect was marked by the time taken for paralysis when no movement of any sort could be observed, except under vigorous shaking. Time taken for death was also observed and recorded when no movement was observed on vigorous shaking and also when dipped in warm water at 50 °C. All, the results have been expressed as mean±SD.

Management of DM is a major challenge for the medical community. Herbal sources of treatment show easier and economical for every sect of society. Thus, researches are carried out for finding the different herbal source of treatment of DM. Traditionally in Assam, *G. morella* [2] and *G. pedunculata* [4] are reported to treat DM. But, no scientific evaluation has been done. Thus, in the present investigation, *G. pedunculata* was extracted with ethanol to evaluate *in vitro* anti-amylase activity. The ethanolic extract of *G. pedunculata* showed the presence of flavonoid, polyphenols, carbohydrates and alkaloids in good amount.

α-amylase (EC 3.2.1.1) is one of the enzymes which helps in managing the postprandial blood glucose level in type II Diabetes [11, 12]. Thus, α-amylase of saliva was taken for the study, which would directly suggest the *in vitro* activity of the extract. A comparative analysis of the activities of the enzyme showed variation in the three methods (zymographic, achromic point analysis and DNS-Maltose spectrophotometric method). Zymogram of the enzyme (fig. 1) showed quite a significant variation in band intensity on treating with different concentrations of extract. 1.5 mg/ml showed 67.65±1.53 % of anti-amylase activity as compared to the concentrations of 0.5 mg/ml (0 %), 1.0 mg/ml (16.17±0.78 %) and 2.0 mg/ml (14.70±0.98 %). But, when the starch–iodine method was conducted, no significant variation was observed in anti-amylase activities of al the concentrations (fig. 2). The activities were found to be 77.49±2.31 % (1.0 mg/ml), 90.05±1.36 % (1.5 mg/ml) and 84.18±2.11 % (2.0 mg/ml) in this method. But, in DNS-Maltose method, the result was not found to be constant in its replicates. It has been reviewed that, many researchers have used DNS-Maltose method for anti-amylase activity, for example, Ginger [13], *Evolvulus alsinoides* [14], *Psidium guajava* [15]. But, this plant did not show similar observation with it.

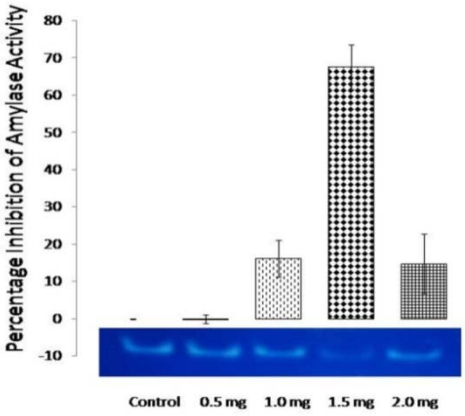

Fig. 1

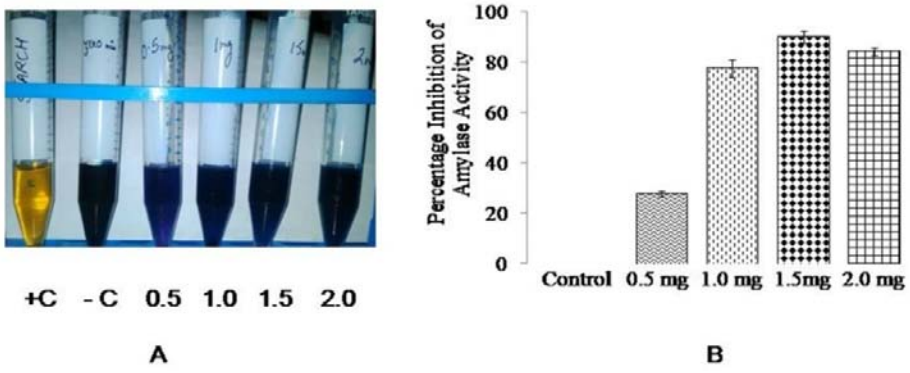

Fig. 2: Zymogram and densitometric analysis of the anti-amylase activity of different concentration of ethanolic extract of *G. pedunculata*. (Lane1: Saliva+distilled water, Lane 2: Saliva+0.5 mg/ml extract, Lane 3; Saliva+1.0 mg/ml extract, Lane 4: Saliva+1.5 mg/ml extract and Lane 5: Saliva+2.0 mg/ml extract). The experiment was conducted in triplicates. The each histogram is expressed with±SD

Fig. 2. Starch–Iodine analysis of amylase activity of the different concentrations of ethanolic extract of *G. pedunculata*. A) Tubes showing the variation in colour intensity, B) Percentage inhibition of the enzyme activity. [Tube (+C): active saliva, Test tube (–C): inactive saliva, Tube 0.5: Active saliva+0.5 mg/ml extract, Tube 1.0: Active saliva+1.0 mg/ml extract, Tube 1.5: Active saliva+1.5 mg/ml, Tube 2.0: Active saliva+2.0 mg/ml extract]. The experiment was conducted in triplicates. The each histogram is expressed with±SD.

For anthelmintic activity, the three animal models that could be used are *P. posthuma*, *Ascaridia galli* and *Raillietina spiralis* [16, 17]. In the study, *P. posthuma* has been used as the animal model because of its anatomical and physiological resemblance to intestinal roundworm [16, 18]. *G. pedunculata* showed anthelmintic activity in a dose-dependent manner, giving shortest time for paralysis and death at 75 mg/ml (table 1). The time required for paralysis and death was recorded to be less than the standard (table 1). The anthelmintic activity of ethanolic extract of *Evolvulus alsinoides* was reported at 50 mg/ml concentration in *P. posthuma* [17], whereas the methanolic extract of 20 mg/ml showed the activity in *Mentha piperita* and *Lantana camara* [19]. Thus, it can be concluded that the concentrations were optimised for the anthelmintic activity study in *G. pedunculata*.

Table 1: Effect of ethanol extract of *G. pedunculata* on *P. posthuma* in anthelmintic activity study

Test substance	Concentration (mg/ml)	Time taken for paralysis (min) (mean±SD)	Time taken for death (min) (mean±SD)
Ethanolic extract	25	1.16±0.10	2.80±1.41
	50	1.16±0.02	1.88±0.41
	75	0.62±0.26	1.42±0.07
Albendazole	25	2.13±0.28	5.12±0.29
Control (Normal saline)	-	Not affected	Not affected

* Values are expressed in mean±SD of three samples for each group. Three concentrations (25, 50 and 75 mg/ml) of ethanolic extract of *G. pedunculata* were used in the study. Reference standard used was Albendazole and normal saline was used as a control group.

G. pedunculata which is an endemic and the critically endangered tree of North East India reported with a good amount of HCA by some researcher. Proper cultivation of it would give a good herbal source for reducing hyperglycemic condition in type II Diabetes. Further scientific elucidation is required to claim its exact dose for reducing DM. In the present study, it can be concluded that zymogram or densitometric study of electrophoretic gel might be a good method for determining the anti-amylase activity of the plant which would help further in the study of the bioactive principles for treatment of type II Diabetes. The extract also shows strong *in vitro* anthelmintic activity in earthworm which could be further evaluated in other anthelmintic animal models.

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CONFLICT OF INTERESTS

Declared none

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