

In Vitro Anti Thiamine Activity of *Bergenia ciliata* leaves of Sikkim Himalaya: Effect of Solvent, Temperature, pH and Duration of Extraction Process

Tanaya Ghosh¹, Prasanta Kumar Mitra^{1*}

¹Department of Medical Biotechnology, Sikkim Manipal University, Sikkim Manipal Institute of Medical Sciences, Gangtok, Sikkim, India

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*Corresponding author: Prasanta Kumar Mitra

Department of Medical Biotechnology, Sikkim Manipal University, Sikkim Manipal Institute of Medical Sciences, Gangtok, Sikkim, India

Abstract

Effect of solvent, temperature, pH and time on extraction process of anti-thiamine factor present in *Bergenia ciliata* leaves was studied. Results showed that extraction of the leaf-extract with ethanol at 40° C for 15 minutes at pH 3.0 had maximum anti-thiamine activity in *in vitro* experiments.

Keywords: Extraction process, Anti-thiamine factor, *Bergenia ciliata*.

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INTRODUCTION

Bergenia ciliata (*B. ciliata*), family-Saxifragaceae, popularly known as 'Paashanbheda' (meaning 'to dissolve the stone'), is one of the important medicinal plants of Sikkim Himalaya. The plant has spirally arranged of rosette of leaves. Leaves are glabrous or hirsute, suborbicular to orbicular broadly obovate, base cordate or sometimes rounded and apex round in shape. Flowers, produced in cyme, are pink to purplish. Sepals are pink to red. Carpels and styles are green or pinkish. Seeds are elongated about 1 mm long, minutely tuberculate, usually numerous, albuminous. Stamens are inserted with the petals, equaling or double their number. Rhizomes are compact solid, somewhat cylindrical barrel shaped, longitudinally wrinkled, covered with root scars, possess a characteristic, slightly camphoraceous odour and pungent taste. The plant has different vernacular names like patharkuchi in Assamese and Bengali, pashanbheda in Gujrati, pashanbhed in Hindi, kallurvanchi in Malayalam, sirupilai in Tamil, kondapindi in Telegu etc [1].

B. ciliata has many traditional uses. Plant is reported to be used in fever, cough, diarrhea, lungs diseases, asthmatic disorders, vomiting, bruises and boils, digestive disorders, malaria, chronic dysentery, pulmonary disorders, ulcers, dysuria, spleen enlargement, eye diseases, boils, cuts and burn, dissolving kidney stones etc. The plant is also used as

tonic and anthelmintic. Local people of Sikkim use this plant as an anti-tussive for cold and cough [2].

B. ciliata contains many bioactive compounds like gallic acid, methyl gallate, quercetin-3-O-β-D-xylopyranoside, quercetin-3-O-α-L-arabinofuranoside, sitoindoside, eryodictiol-7-O-β-D-glucopyranoside, arbutin, 6'-O-p-hydroxybenzoylarbutin, β-sitosterol bergenin, 4-O-galloylbergenin, 11-O-galloylbergenin, p-hydroxybenzoic acid, gallicin, (-)-3-O-galloylcatechin, β-Sitosterol and many others [3].

B. ciliata has several pharmacological activities like, anti-cancer, antipyretic, anti-diabetic, anti-inflammatory, anti-tussive, antibacterial, antiulcer, antioxidant, anti-malarial, anti-antiurolithic, diuretic, hepatoprotective, antiscorbutic etc [4, 5].

While screening anti thiamine activity of different plants of Sikkim in our laboratory, we have noted that *B. ciliata* leaves possess in vitro anti thiamine activity. In the present paper we report effect of solvent, temperature, pH and duration of extraction process on in vitro anti thiamine activity of *B. ciliata*.

MATERIAL AND METHODS

Collection of plant material

Fresh and healthy leaves of *B. ciliata* were collected from the local market of Gangtok, Sikkim & identified by the taxonomist. Voucher specimen (No.

SM-MB-010/21 -1) was kept in the department of Medical Biotechnology, Sikkim Manipal University for future references.



Bergenia ciliata leaves

Preparation of leaves for Anti-thiamine activity

Leaves of *B. ciliata* were shed dried and powdered. 100 grams of leaf-powder was separately extracted with 500 ml of different solvents (water, ethanol, methanol, chloroform, acetone, petroleum ether) at different temperatures, pH and duration on a temperature controlled rotary shaker. The extract was filtered and the solvent was evaporated to dryness *in vacuo* with rotary evaporator at 40 – 50° C. A brownish mass was obtained. This mass was stored to test the anti-thiamine activity.

In vitro anti-thiamine activity

The anti-thiamine activity was determined by estimating the residual thiamine present in a system containing known amount of thiamine hydrochloride and test material collected from *B. ciliata* leaves following the method of Bhattacharya & Choudhuri [6]. Main steps were: an intimate mixture of thiamine hydrochloride (100 mg) and test material collected from *B. ciliata* leaves after extraction with different solvents (100 mg) was incubated at 30° C for 1 hour in 10 ml M/15 phosphate buffer at pH 6.5. It was then filtered. 2 ml of the filtrate was taken and residual thiamine hydrochloride was estimated by thiochrome method described by Harris & Wang [7]. In short, to 2 ml of the

filtrate 0.1 ml of potassium ferricyanide (2.5g/l) and 0.25 ml of sodium hydroxide (150g/l) were added. The solution was mixed thoroughly. 2 ml of iso butanol was then added to it. The solution was shaken for 1 minute. Fluorescence of the supernatant was noted by a fluorimeter at 435 nm using excitation at 365 nm. Tubes for standard thiamine solution (400 µg/l) and for blank were run simultaneously.

Effect of solvents on extraction process

Water, ethanol, methanol, chloroform, acetone and petroleum ether were used separately in extraction process.

Effect of time on extraction process

Extraction processes were done separately for 10, 15, 20 and 25 minutes.

Effect of temperature on extraction process

In separate experiments extraction processes were done at 30, 40, 50 and 60° C temperature.

Effect of pH on extraction process

In separate experiments extraction processes were done at pH 3.0, 5.0, 7.0, 10.0 and 14.0. Acidic and alkaline pH was maintained by adding 1N hydrochloric acid and 1N sodium hydroxide respectively.

Reagents

All reagents required for the experiment were procured from Merck, USA.

RESULTS

Table 1 shows effect of solvents on extraction process for isolation of anti-thiamine compound from the leaves of *B.ciliata*. It was found that ethanol extract produced maximum anti-thiamine activity with 65 % inhibition of added thiamine in the *in vitro* experiment. Anti-thiamine activity in terms of percent inhibition of thiamine for different solvent systems were as follow: water - 30%, ethanol - 65%, methanol - 50%, chloroform – 40%, acetone - 25%, and with petroleum ether -15%.

Table 1: Effect of solvents on extraction process of the anti-thiamine factor present in *B. ciliata* leaves

Solvent (Extraction for 1 hour)	Amount of mass in mg (After extraction)	Anti-thiamine activity (% inhibition)
Water	100	30
Ethanol	100	65
Methanol	100	50
Chloroform	100	40
Acetone	100	25
Petroleum ether	100	15

Effect of duration of extraction process for isolation of anti-thiamine compound from *B. ciliata* leaves is shown in Table 2. Time given for extraction in

separate experiments was 10 minutes, 15 minutes, 20 minutes and 25 minutes. It appears from the table that anti-thiamine activity in terms of percent inhibition of

exogenous thiamine was maximum (65%) for 15 minutes extraction time. For 10 minutes, 20 minutes and 25 minutes of extraction time anti-thiamine activity

in terms of percent inhibition of thiamine were determined as 40%, 62% and 61% respectively.

Table 2: Effect of duration of extraction process (solvent used: ethanol) to test the anti-thiamine factor present in *B. ciliate* leaves

Duration (minutes)	Anti-thiamine Activity (% inhibition)
10	40
15	65
20	62
25	61

Table 3 shows the effect of temperature on extraction process for isolation of anti-thiamine compound from the leaves of *B. ciliate* leaves. Increase in temperature during extraction has elevated the anti-thiamine activity. When extraction was done at 30° C anti-thiamine activity in terms of percent inhibition of

added thiamine was 50 % but the same value was 70 % when the extraction temperature was raised to 40° C. Increase of temperature for extraction above this could not elevate anti-thiamine activity. Results thus showed that the extraction should be done at 40° C to get maximum anti-thiamine activity.

Table 3: Effect of temperature on the extraction process (solvent used: ethanol) of anti-thiamine factor present in *B. ciliate* leaves

Temperature in °C	Anti-thiamine Activity (% inhibition)
30	50
40	70
50	70
60	71

Effect of pH on the extraction process for isolation of anti-thiamine compound from the leaves of *B. ciliate* is shown in Table 4. Different pH was maintained in separate extraction sets. It was noted that anti- thiamine activity in terms of percent inhibition of exogenous thiamine was maximum (80 %) at pH 3.0. For pH 5.0, 7.0, 10.0 and 14.0 of the extraction process, anti-thiamine activity in terms of percent inhibition of thiamine was much less.

selection of ethanol to get maximum anti thiamine activity of *B. ciliate* leaves. This was followed by methanol and chloroform respectively Other solvents used in extraction process like water, acetone and petroleum ether showed little anti-thiamine activity.

Table 4: Effect of pH on the extraction process (solvent used: ethanol) of anti-thiamine factor present in *B. ciliate* leaves

pH	Anti-thiamine activity (% inhibition)
3	80
5	78
7	67
10	50
14	29

Results were mean of five sets of experiment.

DISCUSSION

Extraction process is a part of the work of isolation of bio-active compounds from plant materials. Extracts with different solvents generally show different composition of bio-active molecules [8]. Therefore, a suitable extracting solvent is needed to be selected for extraction of the active compound with maximum activity [9]. In the present experiment use of different extracting solvents (distilled water, chloroform, ethanol, methanol, acetone and petroleum ether) has led to the

Extraction time is very important to extract active compounds in maximum amount [10]. In this experiment we have noted that extracts with ethanol for 15 minutes gave maximum in vitro anti thiamine activity of *B. ciliate* leaves.

Extraction temperature is another important factor influencing the recovery of the bioactive compound from the sources [9]. In this experiment we have seen that extract of *B. ciliate* leave at 40° C had maximum in vitro anti thiamine activity.

Extraction pH is also important to obtain more amount of bioactive compound from the source as most of the compounds are present in complex form with many other biomolecules [11]. In the present experiment it was noted that extract of of *B. ciliate* leaves at pH 3 had maximum in vitro anti thiamine activity.

Many plants have shown in vitro anti thiamine activity. Few are, blue berries, *Coffea arabica* Linnaeus, *Brassica juncea* (Linnaeus) Czernajew, *Bombax ceiba*, *Ageratum conyzoides* Linn. etc [6, 12, 13]. Linnaeus etc. Present study confirmed in vitro anti-thiamine activity of the leaves of *B. ciliate*.

It is known that biological activity of medicinal plants depends on season [13-15]. We are now working on seasonal variation on in vitro anti-thiamine activity of the leaves of *B. ciliata*.

CONCLUSION

B. ciliata leaves have many medicinal properties. People therefore take this leaves to get rid of different ailments. They should be aware of the presence of anti-thiamine compound in *B. ciliata* leaves. Further research is needed to ascertain the amount of anti-thiamine compound in *B. ciliata* leaves.

Conflict of interest: The authors declare that they have no conflict of interest.

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