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**Original Research Article**

# In Vitro Anti Thiamine Activity of *Bergenia ciliata* leaves of Sikkim Himalaya: Effect of Season

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## Abstract

Effect of season on in vitro anti-thiamine activity of *Bergenia ciliate* (*B. ciliate*, Family- Saxifragacae) leaves was studied. Results showed that leaves of *B. ciliate* of the period July – August had maximum in vitro anti thiamine effect.

**Keywords***: Bergenia ciliate*, Anti-thiamine activity, effect of season.

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## INTRODUCTION

Biological activities of plants vary with seasons of the year. Qinxue *et al.*, studied seasonal variations in the antioxidant activity of ground bamboo *Sasa argenteastriatus* Leaves. They noted that the highest antioxidant activity appeared in December and the lowest was in May [1]. Effect of seasonal variation on the antineoplastic activity of *Alstonias cholaris* R. Br. in HeLa cells was studied by Jagetia and Baliga. Highest cell killing effect was observed by the plant of summer collection [2]. Osadebe *et al.*, worked on seasonal variation for the antidiabetic activity of methanolic extract of *Loranthus micranthus* and noted that the activity is highest at the peak of the rainy season [3]. Ncube *et al.*, studied seasonal variation in antimicrobial activity of frequently used medicinal bulbous plants from South Africa and noted that the activity was higher in spring and winter than in other seasons [4]. Effect of seasonal variation on the anti-inflammatory activity of *Sargassum wightii* was studied by Dar and coworkers. They found that the plant collected during winter was most effective in reducing carrageenan-induced edema in rats [5]. Report from our laboratory showed that *Cassia alata* leaves during the period of May – June had maximum protective effect on anti tubercular drugs induced hepatotoxicity in rats [6].

We also reported that UV absorption property of *Amaranthus spinosus* was maximum during autumn in comparison to other seasons of the year [7].

Recently, we have noted that *B. ciliata* leaves possess in vitro anti thiamine activity. In the present paper we are reporting effect of season on anti-thiamine activity of *B. ciliata* leaves.

## MATERIAL AND METHODS

### Collection of plant material

Fresh and healthy leaves of *B. ciliata* were collected from the local market of Gangtok, Sikkim randomly and during January – February, March – April, May – June, July – August, September – October and November – December 2020 & identified by the taxonomist. Voucher specimen (No. SM-MB-010/21-1-7**)** was kept in the department of Medical Biotechnology, Sikkim Manipal University for future references.

**Figure–1: *Bergenia ciliate* leaves**

**Preparation of leaves for Anti thiamine activity**

Leaves of *B. ciliata* were shed dried and powdered. This powder was used to check the *in vitro* anti thiamine activity.

**Experimental design**

Seven sets of experiment were designed as follow:

1. Incubation of thiamine + Powdered leaves of *B. ciliata* (randomly collected).
2. Incubation of thiamine + Powdered leaves of *B. ciliata* (January – February)
3. Incubation of thiamine + Powdered leaves of *B. ciliata* (March – April)
4. Incubation of thiamine + Powdered leaves of *B. ciliata* (May – June)
5. Incubation of thiamine + Powdered leaves of *B. ciliata* (July – August)
6. Incubation of thiamine + Powdered leaves of *B. ciliata* (September – October) :
7. Incubation of thiamine + Powdered leaves of *B. ciliata* (November – December) :

***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 by the method of Bhattacharya and Choudhuri [8].

Main steps were: an intimate mixture of thiamine hydrochloride (100 mg) and powdered *B. ciliata* leaves (1 g) was incubated at 30 degree centigrade for 1 hour in10 ml M/15 phosphate buffer at pH 6.5. It was then filtered. 2 ml of this filtrate was taken and residual thiamine hydrochloride was estimated by thiochrome method described by Harris and Wang [9]. In short, to 2ml of the filtrate 0.1ml potassium ferricyanide (2.5g/l) and 0.25 ml of sodium hydroxide (150g/l) were added. The solution was mixed thoroughly. 2 ml isobutanol was then added to it. The solution was shaked 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.

**Reagents**

All chemicals used in this study were purchased from Sigma Chemical Company, Mumbai. Chemicals were of analytical grade with high purity

**Statistical analysis**

The values were expressed as mean ± SEM and were analyzed using one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS). Differences between means were tested employing Duncan's multiple comparison test and significance was set at $p < 0.05$.

## RESULTS

Table-1 showed that *B. ciliata* leaves collected randomly inhibits thiamine. In *in vitro* experiment 1 g of powdered *B. ciliata* leaves (collected randomly) could destroy 19.5 mg thiamine. Initially amount of thiamine was 100 mg. After 1h incubation with 1 g of powdered *B. ciliata* leaves, amount of thiamine came down to 80.5 ± 2.5. Result was statistically significant. Percentage of thiamine destruction was 19.5%.

**Table -1: Showing in vitro anti thiamine effect of *B. ciliata* leaves (Randomly collected)**

| Group | Residual thiamine (mg) | Inhibition (%) |
|---|---|---|
| Control (Thiamine hydrochloride) | 100.0 | -- |
| Thiamine hydrochloride (100 mg) + Powdered *B. ciliata* leaves (1g) collected randomly. | 80.5 ± 2.5* | 19.5 |

Values were mean ± SEM of ten sets of experiment. *p <0.05, **p < 0.001 when compared to control.

Seasonal variation in *in vitro* anti thiamine effect of the leaves of *B. ciliata* is given in Table–2.

**Table-2: Showing seasonal variation on in vitro anti thiamine effect of *B. ciliata* leaves**

| Group | Residual thiamine (mg) | Inhibition (%) |
|---|---|---|
| Control (Thiamine hydrochloride, 100 mg) | 100.0 | -- |
| Thiamine hydrochloride (100mg) + Powdered *B. ciliata* leaves (1g) (January - February) | 97.1 ± 4.09 | 2.90 |
| Thiamine hydrochloride (100mg) + Powdered *B. ciliata* leaves (1g) (March - April) | 92.7 ± 5.27 | 7.30 |
| Thiamine hydrochloride (100mg) + Powdered *B. ciliata* leaves (1g) (May - June) | 81.4 ± 1.81* | 18.6 |
| Thiamine hydrochloride (100mg) + Powdered *B. ciliata* leaves (1g) (July - August) | 71.3 ± 2.2** | 28.7 |
| Thiamine hydrochloride (100mg) + Powdered *B. ciliata* leaves (1g) (September - October) | 79.2 ± 1.0* | 20.8 |
| Thiamine hydrochloride (100mg) + Powdered *B. ciliata* leaves (1g) (November - December) | 91.3 ± 3.11 | 8.7 |

Values were mean ± SEM of ten sets of experiment. *p <0.05, **p < 0.001 when compared to control.

Results showed that *in vitro* anti thiamine effect of the leaves of *B. ciliata* varies with season. Maximum anti thiamine effect was found by the leaves of *B. ciliata* during the period July – August. After 1h incubation with 1 g of powdered *B. ciliata* leaves, amount of thiamine came down to 71.3 ± 2.2. Result was statistically significant up to the level of $p<0.001$. Percentage of thiamine destruction was 28.7%. Powdered leaves of *B. ciliata* during the period May – June and September - October had also *in vitro* anti thiamine effect (amount of residual thiamine were 81.4 ± 1.81 and 79.2 ± 1.0 respectively) but the results were less significant in comparison to that for leaves of *B. ciliata* during July – August.

## DISCUSSION

*Bergenia ciliata* (*B. ciliata*), family-Saxifragacae, is one of the important medicinal plants of Sikkim Himalaya. Popularly it is known as 'Paashanbheda' (meaning 'to dissolve the stone'), Bearing 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 [10].

*B. ciliata* has many traditional uses. The plant is reported to be used in digestive disorders, malaria, chronic dysentery, pulmonary disorders, ulcers, dysuria, spleen enlargement, fever, cough, diarrhea, boils, cuts and burn, dissolving kidney stones lungs diseases, asthmatic disorders, vomiting, bruises and boils, eye diseases etc. The plant is also used as tonic and anthelimintic. Local people of Sikkim use this plant as an anti-tussive for cold and cough [11].

Bioactive compounds like 6′-O-p-hydroxybenzoylarbutin, β-sitosterol bergenin, 4-O-galloylbergenin, 11-O-galloylbergenin, p-hydroxybenzoic acid, gallic acid, methyl gallate, quercetin-3-O-β-D-xylopyranoside, quercetin-3-O-α-L-arabinofuranoside, sitoindoside, eryodictiol-7-O-β-D-glucopyranoside, arbutin, gallicin, (-)-3-0-galloylcatechin, β-Sitosterol and many others are present in *B. ciliata* [12].

The plant has several pharmacological activities like**,** anti-tussive, antibacterial, antiulcer, antioxidant, anti-malarial, anti-cancer, antipyretic, anti-diabetic, anti-inflammatory, anti-antiurolithic, diuretic, hepatoprotective, antiscorbutic etc [13, 14]. Recently we have noted in vitro anti thiamine activity of *B. ciliate* leaves. Results are under communication.

Since synthesis of bioactive compounds in plants varies with season [1-7], we studied the effect of season on anti thiamine activity of the leaves of *B. ciliata.* Results revealed that leaves of *B. ciliata* of the months July – August had maximum *in vitro* anti thiamine activity in terms of generation of residual thiamine (Figure–2) and percent inhibition of thiamine (Figure–3).

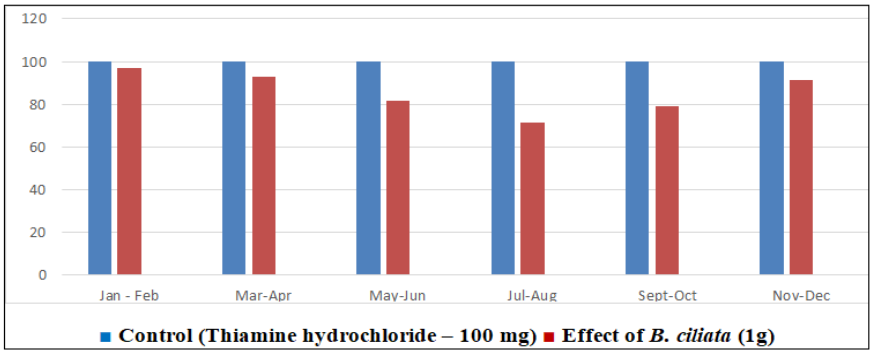


**Fig 2: Residual thiamine (mg) after 1h incubation of thiamine hydrochloride and powdered leaves of *B. ciliata* (1 g) of different seasons**

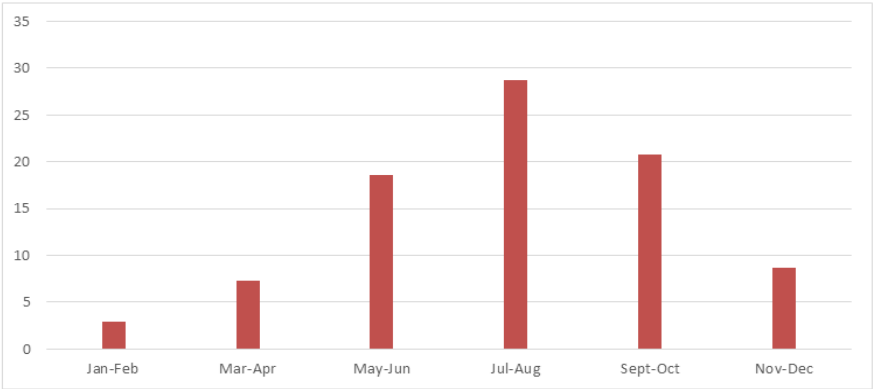


**Fig 3: Inhibition of thiamine (%) after 1h incubation of thiamine hydrochloride (100 mg) and powdered leaves of *B. ciliata* (1 g) of different seasons**

Concept of anti thiamine activity of *B. ciliate* leaves is important as the leaves are being used by several people to get rid of different ailments. Since the present study confirmed anti thiamine activity of *B. ciliate* leaves which varies with season, isolation of the anti thiamine compound from the plant leaves and its characterization are essential. Presently, we are working in this direction.

## CONCLUSION

From this experiment it can be concluded that *B. ciliata* leaves had *in vitro* anti thiamine effect. Results showed that 1 g of powdered leaves of *B. ciliata* Could inactivate 19.5 mg of thiamine hydrochloride *in vitro*. When effect of season on the anti thiamine effect of *B. ciliata* was studied, it was found out that leaves of *B. ciliata* of the period July – August had maximum anti thiamine effect.

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

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