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ISOLATION OF ANTI THIAMINE COMPOUND FROM THE LEAVES OF *ABRUS PRECATORIUS* LINNAEUS: EFFECT OF SEASON ON YIELD OF THE COMPOUND

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ABSTRACT
Abrus precatorius Linnaeus (*A. precatorius* L.), a medicinal plant popularly known as gunja, rosary pea, jequirity bean etc. has been used for therapeutic purpose since Vedic period. Recently we observed anti thiamine activity of *A. precatorius* L. leaves. Objective of the present work was to isolate the anti thiamine compound present in the plant leaves. As amount of secondary metabolite in plants varies with season, effect of season on yield of the anti thiamine compound was also studied. *A. precatorius* L leaves were collected in different seasons, identified by taxonomist, washed thoroughly, shade dried and powdered. Isolation of the anti thiamine compound from the powdered leaves was carried out maintaining principles of standard isolation procedures of chemical compounds from plant sources. A compound was isolated from *A. precatorius* L. leaves . 1 mg of the isolated compound could inactivate 24.9 µg of thiamine hydrochloride in 1 h in *in vitro* experiment. Yield of the anti thiamine compound was maximum during the months of May – June. Isolation of anti thiamine compound from *A. precatorius* L. leaves suitably compliments the anti thiamine activity of the plant leaves.

KEYWORDS: *Abrus Precatorius* linnaeus leaves, anti thiamine activity, seasonal variation.

1. INTRODUCTION
Several plants showed anti thiamine activity. As early as 1944 Bhagvat & Devi observed that certain foodstuffs and oil seeds could inactivate thiamine.[1] In 1946 Weswig *et al*.[2] confirmed anti thiamine activity of plant materials. Somogyi and Muralt in 1949 noted presence of two thermostable anti thiamine factors in fern extracts They were hydrolysates I and hydrolysates II.[3] Chaudhuri (1962) confirmed presence of a heat stable thiamine inactivating-factor in different varieties of rice and rice bran.[4] In 1971 Rattanapanone *et al.* found presence of anti thiamine factor in raw vegetables of northern part of Thialand.[5] Bhattacharya and Chaudhuri (1974) showed anti thiamine activity of *Brassica juncea*[6] and in 1976 Sarkar and Chaudhuri found anti thiamine activity of *Bombax malabaricum*.[7] McCleary and Chick (1977) purified Thiaminase I enzyme from *Marsilea drummondii* which was found responsible for inactivation of thiamine.[8] Anti thiamine activity of *Abrus precatorius* L.,[9] *Ageratum conyzoides* L.[10] and *Murrya koenigii* (linn.) Spreng Wettst leaves[11] was reported by us. In one screening program we have recently found anti thiamine activity of few other medicinal plants of North East Himalayas of Indian Subcontinent.[12]

A. precatorius L. (family leguminosae, Fabaceae). has been used for therapeutic purpose since Vedic period.[13] The plant is popularly known as gunja, rosary pea, jequirity bean etc. The plant is found through out India in hedges and bushes in exposed areas. Roots, seeds and leaves of *A. precatorius* L. are used in traditional Medicine. The plant is mainly used in treatment of ulcer and skin infection.[14] Besides, the plant has antimicrobial activity,[15] anti-diabetic property,[16] anti– inflammatory analgesic activity,[17] anti oxidative activity [18] etc. The plant is also found efficacious in malaria[19] and in cancer.[20] *A. precatorius* L. has several bad effects too. The plant has post-testicular antifertility effects[21] and teratogenic activity.[22] Seeds of the plant is toxic and induce abortion.[23] We have shown that *A. precatorius* L leaves could cause body weight loss in normal healthy growing albino rats.[24,25]

As *A. precatorius* L. leaves showed anti thiamine activity,[9] the aim of present work was to isolate the anti thiamine compound from the plant leaves. Effect of season on yield of the compound was also studied.

2. METHODOLOGY
2.1 Plant materials used
Leaves of *A. precatorius* L. were collected in morning hours (9 – 10 AM) from the medicinal plants garden of

the University of North Bengal, Siliguri ($26^0 41' 30.9984"$ N, $88^0 27' 4.5756"$ E, elevation, 410 ft), Dist. Darjeeling, West Bengal, India during the months of January - February, March – April, May – June, July – August, September – October and November – December of the year 2016. Leaves were authenticated by the experts of the department of Botany of the said University. A voucher specimen was kept in the department of Medical Biotechnology, Sikkim Manipal Institute of Medical Sciences of Sikkim Manipal University, Gangtok, Sikkim, India for future references.

***Abrus precatorius* Linnaeus**

2.2 Test drug

Leaves of *A. precatorius* L. were washed thoroughly, shade dried and powered. The powder was used as test drug.

2.3 In vitro anti thiamine activity

Anti thiamine activity was determined by the method of Bhattacharya and Choudhuri.[6] by estimating the residual thiamine present in a system containing known amount of thiamine hydrochloride and compound isolated from *A. precatorius* L. leaves. Main steps were as follow:

An intimate mixture of thiamine hydrochloride (100 µg) and compound isolated from *A. precatorius* L. leaves (1 mg) was incubated at 30^0C for 1 h in 10 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.[26] According to this method, 0.1 ml potassium ferricyanide (2.5g/l) and 0.25 ml of sodium hydroxide (150g/l) were added to 2ml of the filtrate. The solution was mixed thoroughly. 2 ml isobutanol was then added to it. The solution was shook for 1 min. Fluorescence of the supernatant was noted by a fluorimeter (FluoroLog-3, Horiba Scientific) at 435 nm using excitation at 365 nm. Tubes for standard thiamine solution (400 µg/l) and for blank were run simultaneously.

2.4 Chemicals

Required chemicals(analytical grade) were purchased from Sigma Chemical Company, Mumbai. *Isolation of anti thiamine compound*

2.5 Isolation of anti thiamine compound

Isolation of anti thiamine compound from the leaves of *A. precatorius* L. was processed as per following scheme. Principles of standard isolation procedures of chemical compounds from plant sources[27,30] were followed.

3. RESULTS

3.1 Isolation of anti thiamine compound from A. precatorius L. leaves

By solvent extraction, acid hydrolysis, chromatographic experiments followed by crystallization a compound has been isolated from the leaves of *A. precatorius L.* Diagrammatic scheme of isolation procedure is given.

3.2 *In vitro* anti thiamine activity of the isolated compound

In vitro anti thiamine activity of the compound isolated from *A. precatorius L.* leaves. This is shown in table 1. Results showed that the compound isolated from *A. precatorius* L. leaves could inactivate thiamine in *in vitro* experiment. 100 µg thiamine hydrochloride was incubated with 1 mg of the compound isolated from *A. precatorius* L. leaves.

After 1h of incubation amount of thiamine came down to 75.1 µg. Rate of inactivation was 24.9%. Ten sets of experiment were performed.

Diagrammatic scheme for isolation of anti thiamine compound from leaves of *A. precatorius* L.

Powdered leaves of *A. precatorius* L. (100 g)

SOLVENT EXTRACTION

Extracted with 1000 ml of 1 : 1 (v/v) Ethyl acetate, methyl alcohol mixture for 20 min at 37^0C in a soxhlet apparatus. It was then centrifuged. Supernatant collected and evaporated to dryness.

Active brown mass

ACID REFLUX

Refluxed with 150 ml of 1(N) HCL for 10 min on a water bath at 100 ^{0}C. It was then cooled and centrifuged. Supernatant was evaporated to dryness.

Active brown mass

TREATMENT WITH N-HEXANE

Extracted with 50 ml n-hexane on a rotary shaker for 20 min. It was then centrifuged. Supernatant was evaporated to dryness.

Active brown mass

ALUMINA COLUMN CHROMATOGRAPHY

Extracted with 10 ml n-butanol, methanol mixture (1:1) for 10 min. It was then filtered. With filtrate alumina column chromatography was performed. Elution was done by 50% chloroform – methanol mixture.

Second band was found active

POLYAMIDE COLUMN CHROMATOGRAPHY

Eluent of active second band was evaporated to dryness. The dry mass was extracted with 10 ml ethyl acetate for 10 min. It was then filtered. With filtrate polyamide column chromatography was done. Elution was made by ethyl formate: formic acid mixture (80:20 v/v).

Third band was active

SILICA GEL G COLUMN CHROMATOGRAPHY

Eluent of active third band was evaporated to dryness. The dry mass was extracted with 10 ml acetone for 10 min. It was then filtered and the filtrate was subjected to silica gel column chromatography using silica gel G as adsorbent. Elution was done by 50% acetone–chloroform mixture.

First band was found active

CRYSTALLIZATION

Eluent of the active first band obtained from the above step was evaporated to dryness. Repeated crystallization was done from ethyl acetate–formic acid (50:50, v/v) mixture.

Crystals obtained (Yield, 7.9 mg)

Table 1: Showing *In vitro* anti thiamine activity of the isolated compound.

Group	Residual thiamine (µg) after 1 h incubation.	Inactivation (%)
Control (Thiamine hydrochloride – 100 µg)	100.0	--
Thiamine hydrochloride (100 µg) + compound (1 mg) isolated from *A. precatorius* L. leaves	75.1	24.9

Values were mean of ten experiments.

3.3 Effect of season on yield of the anti thiamine compound isolated from A. precatorius L. leaves

This is shown in Figure 1. Yield of the anti thiamine compound isolated from 100 g of powdered leaves of *A. precatorius* L. leaves was maximum in the months of May – June (9.9 mg) followed by July – August (6.8 mg) and March – April (6.5 mg). Yield of the compound was, however, low during January – February (0.9 mg), September - October (2.1 mg) and November – December (1.9 mg).

4. DISCUSSION

In the present study an anti thiamine compound has been isolated from *A. precatorius* L. leaves through solvent extraction, acid hydrolysis, chromatographic experiments followed by crystallization. 1 mg of the isolated compound could inactivate 24.9 µg of thiamine hydrochloride in 1h in *in vitro* experiment. Bhagvat and Devi showed that 1 mg of the compound isolated from *Eleusine coracana* L. could inactivate 135 µg of thiamine hydrochloride in 1h.[1] One anti thiamine compound has been isolated by Bhattacharya and Chaudhuri from mustard seed. The investigators noted that 1 mg of the isolated compound destroys 45 µg of thiamine hydrochloride in 1h.[6] Sarkar and Chaudhuri isolated anti thiamine compound from cotton seed. They reported that 1 mg of the compound could inactivate 25.5 µg of thiamine hydrochloride in 1h.[7]

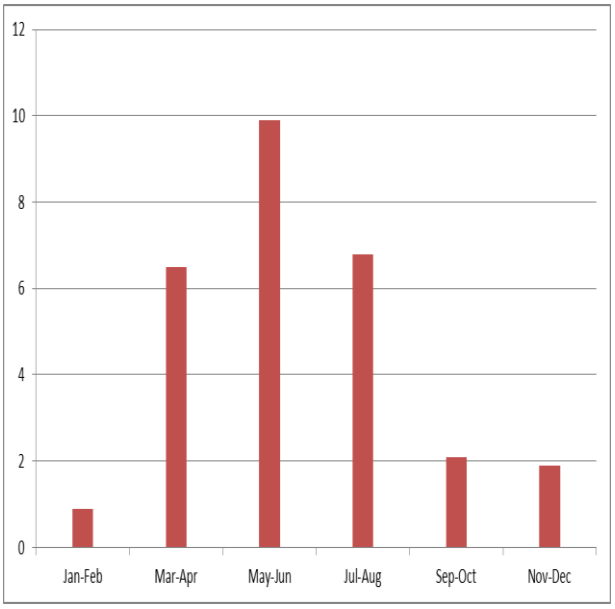

Figure 1: Showing seasonal effect on yield (in mg) of the anti thiamine compound isolated from 100 g of powdered leaves of *A. precatorius* L.

Values were mean of six experiments.

Studies have confirmed that seasonal variation in biological activities of plants is due to variation in amount of secondary metabolite in plants in different season.[31,33] Fernandez De Simon *et al*. (1999) studied evolution of phenolic compounds of Spanish oak. They found that phenolic compounds were more in rainy season.[34] Ganjewala and his co-workers (2000) noted that accumulation of bacoside A in *Bacopa monniera* was maximum in summer.[35] Salminen and his coworkers (2001) observed that amount of hydrolysable tannins in leaves of *Betula pubescens* varies with season and was maximum during summer.[36] In the present study we have noted that amount of the anti thiamine compound in *A. precatorius* L. leaves was maximum during the months of May and June (Figure – 1).

A. precatorius L. leaves have medicinal values. People throughout the world consume these leaves either as such or through traditional medicine to get rid of ailments. As the present study showed that leaves of *A. precatorius* L contain anti thiamine compound, this is the additional information and perhaps may be a caution to those who routinely use *A. precatorius* L. leaves for medicinal purposes.

5. CONCLUSION

Isolation of anti thiamine compound from *A. precatorius* L. leaves and effect of season on yield of the anti thiamine compound suitably compliments the anti thiamine activity of the plant leaves.

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Conflicts of interest

There are no conflicts of interest.

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