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ISOLATION OF AN ANTI THIAMINE COMPOUND FROM AMARANTHUS SPINOSUS LEAVES OF SIKKIM HIMALAYA

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ABSTRACT

Recently we have shown that *Amaranthus spinosus (A. spinosus,* Family-*Amaranthaceae)* leaves of the period May – June had maximum in vitro anti thiamine effect. Aim of the present work was, to isolate anti thiamine compound from the leaves of *A. spinosus*. From the local market *A. spinosus* leaves were collected during May – June and identified by the taxonomist. Solvent extraction and acid hydrolysis were done followed by solvent treatment, chromatographic experiments and crystallization. A compound was crystallized. In vitro anti thiamine activity of the isolated compound was checked by standard method. Results revealed that 100 mg of the isolated compound could inactivate 33.5 mg of thiamine hydrochloride in in vitro experiment.

KEYWORDS: *Amaranthus spinosus* leaves, Isolated compound, Anti thiamine activity.

INTRODUCTION

Anti thiamine activity of the plant was established as early as 1944 when Bhagvat *et al.* demonstrated anti thiamine activity in *Linum usitatissimum.* Authors claimed that the anti-thiamine factor present in the plant was thermostable and largely dialyzable.[1] In 1946 anti thiamine activity of *Pteris aquiline* L. was confirmed in experimental animals, bacteria and human.[2] Haag et *al*., showed that the anti thiamine factor of bracken fern was very stable to heat, it was water soluble but was not soluble in petroleum ether, acetone or ethanol. Inactivation of thiamine by bracken fern extract was produced rapidly.[3] Fabriani *et al.*, confirmed anti thiamine activity of *Pteris aquiline.*[4] In 1952 Henderson *et al.* reported that the plant *Pteris aquiline* contains thiaminase which causes thiamine deficiency in horses.[5] In

1956 Sakamoto and coworkers isolated a thiamine-decomposing thermostable factor from sweet potato leaves which was characterized as isoquercitrin.[6]

Chaudhuri confirmed the presence of a heat stable thiamine inactivating-factor in different varieties of rice and rice bran.[7] In 1964 Kundig & Somogyi found that aqueous extract of parsley contained an anti thiamine factor which was unaffected by cooking or by contact with synthetic gastric juice.[8] Rattanapanone and coworkers showed anti thiamine activity of water extract of *Marsilea crenata*. They noted that the plant has enzyme thiaminase and a thermostable factor responsible for anti thiamine activity. These workers also confirmed anti thiamine activity of several other plants including *Athyrium esculentum*, an edible fern (Family-Athyriaceae) found throughout Asia and Oceania.[9] Kositawattanakul and his colleagues found that tea plant has anti thiamine activity which is protected by ascorbic acid (vitamin C) both at acidic and neutral Ph.[10] We have also noted *in vitro* anti thiamine activity (70% inhibition of thiamine was noted in 120 minutes) of *Abrus precatorius* L. leaves.[11]

Recently we have confirmed *in vitro* anti thiamine activity of *A. spinosus* leaves (results are under communication). We report here isolation of the anti thiamine compound from *A. spinosus* leaves.

MATERIAL AND METHODS

Collection of plant material

Fresh and healthy leaves of *A. spinosus* were collected from the local market of Gangtok, Sikkim during May – June (we have seen earlier that *A. spinosus leaves* of May – June has maximum anti thiamine activity) & identified by the taxonomist. Voucher specimen (No. SM-MB-011/21-4**)** was kept in the department of Medical Biotechnology, Sikkim Manipal University for future references.

Preparation of leaves for anti thiamine activity

Leaves of *A. spinosus* were shed dried and powdered. This powder was used to isolate the anti thiamine compound.

***Amaranthus spinous* leaves**

***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 *A. spinosus* leaves by the method of Bhattacharya and Choudhuri.[12]

Main steps were: an intimate mixture of thiamine hydrochloride (100 mg) and isolated compound from *A. spinosus* leaves (100 mg) 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.[13] 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.

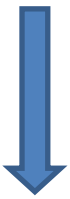
Reagents

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

Isolation of alpha amylase inhibitor from *A. spinosus* leaves

Applying principles of standard isolation procedures of chemical compounds from plant sources,[14,15] isolation was done by the following scheme.

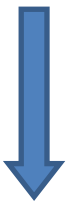
Powdered leaves of *A. spinosus* (100 g)



Solvent extraction

Extracted with 500 ml of methanol for 15 min at 50^0C in a Soxhlet apparatus. It was then centrifuged. Supernatant collected and evaporated to dryness.

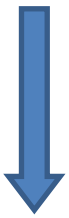
Active brown mass



Acid reflux

Refluxed with 50 ml of 1(N) HCL for 5 min on a water bath at 100 ^{0}C. It was then cooled and centrifuged. Supernatant was evaporated to dryness.

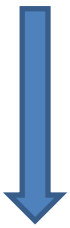
Active brown mas



Treatment with methanol- Chloroform mixture

Treated with 100 ml methanol - chloroform mixture (1:1 v/v) on a rotary shaker for 10 min. It was then centrifuged. Supernatant was evaporated to dryness.

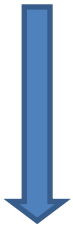
Active brown mass



Alumina column chromatography

Extracted with 20 ml of methanol for 10 min. It was then filtered. With filtrate alumina column chromatography was performed. Elution was done by methanol, chloroform mixture (50:50 v/v).

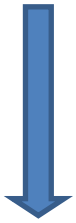
Second band was found active



Polyamide column chromatography

Eluent of active second band was evaporated to dryness. The dry mass was extracted with 20 ml methanol for 10 min. It was then filtered. With filtrate polyamide column chromatography was performed. Elution was done by methanol, chloroform mixture (50:50 v/v).

Third band was active

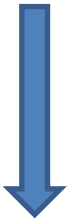


Silica gel g column chromatography

Eluent of active third band was evaporated to dryness. The dry mass was extracted with 20 ml methanol 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 methanol, chloroform mixture (50:50 v/v).

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 Chloroform: Benzene (50:50, v/v) mixture.

Crystals obtained (5.5 mg)

RESULTS

Isolation of compound

One compound was isolated from leaves of *A. spinosus.*

Anti thiamine activity of the isolated compound

Results are summarized in Table -1.

Table 1: Showing in vitro anti thiamine effect of the isolated compound from *A. spinosus* leaves.

Group	Residual thiamine (mg)	Inhibition (%)
Control (Thiamine hydrochloride)	100.0	--
Thiamine hydrochloride (100 mg) + Isolated compound from *A. spinosus* leaves (100 mg)	66.5 ± 2.9*	33.5

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

In *in vitro* experiment 100 mg of the isolated compound could destroy 33.5 mg thiamine.

Initially amount of thiamine was 100 mg. After 1h incubation with 100 mg of the isolated compound, amount of thiamine came down to 66.5 ± 2.9. Result was statistically significant. Percentage of thiamine destruction was 33.5%.

DISCUSSION

The concept of anti-thiamine factor was introduced in literature by Green.[16] Evans *et al.* (1942) classified anti-thiamine compounds broadly into two categories, namely synthetic and natural. Synthetic anti-thiamine compounds are structural analogues or antimetabolites e.g. pyrithiamine type, oxythiamine type, amproleum type, deoxy and ethyl deoxy thiamine,O-

benzoyl thiamine and its derivatives, butyl thiamine, phenyl triazinothiamine, imiodazolethiamine and benzoyl imidazole thiamine etc.; while natural anti thiamine compounds arenon-structural analogues and mostly present in different food-stuffs, plants etc. Natural anti thiamine compounds are further classified into two groups *viz*. large molecule natural anti-thiamine compounds e.g. thiaminase I and thiaminase II, mainly isolated from raw fishes and small molecule natural anti-thiamine compounds e.g. caffeic acid, chlorogenic acid, methyl sinapate, 3-5 dimethoxy salicylic acid etc.[17]

Plants are known having anti thiamine activity and many anti thiamine compounds have been isolated from plants. Reddi in 1950 isolated an active principle from mustard seed which destroyed thiamine. The factor is soluble in water and alcohol, sparingly soluble in chloroform but insoluble in ether and acetone. It was found out that 65% of the added thiamine was destroyed by the anti thiamine factor even within the first minute. The destruction of thiamine was independent of pH 2.5 and 7.5. The reaction was not enzymatic.[18]

In 1971 Hilker and co -workers made some extensive studies on thiamine-inactivating factors present in various types of tea such as jasmine, black tea, etc. Authors demonstrated that thiamine-inactivating factors of tea appeared to be related to the tannin content.[19] Further, it was demonstrated that ortho-catechol such as methylsinapate, found in rapeseed, has antithiamine activity.[20]

Bhattacharya and coworkers in 1974 confirmed that mustard seed contains methyl ester of 3, 5-dimethoxy-4-hydroxy cinnamic acid (methyl sinapate) which acts as anti thiamine factor. It was found out that 1 mg of methyl sinapate inactivated 45 µg of thiamine hydrochloride at pH 6.5 at 30°C. Under similar conditions 1 mg of caffeic acid (3, 4-dihydroxy cinnamic acid) inactivated 135 µg of thiamine hydrochloride. Maximum inactivation of thiamine by methyl sinapate was recorded within 30 min.[12]

The antithiamine factor, isolated from cotton seed, was a light yellow amorphous substance. The antithiamine factor was characterized as 3, 5-dimethoxy salicylic acid.[21]

In 1980 Wills *et al.* studied anti thiamine activity of tea and isolated a fraction from tea which had very strong anti thiamine effect.[22]

In the present study we have isolated an anti thiamine compound from *A. spinosus* leaves, 100 mg of which could inactivate 33.5 mg of thiamine hydrochloride. The isolated compound should need characterization. Work in this direction is now in progress in our laboratory.

CONCLUSION

Thiamine is the coenzyme of both pyruvate dehydrogenase and transketolase enzymes. Activity of these enzymes will decrease in case of presence of anti thiamine compound in edible stuff. Due to this, energy production as well as synthesis of nucleic acid will be hampered. Anti thiamine activity of *A. spinosus* leaves, therefore, should be known to the patients who are taking *A. spinosus* leaves to get rid of various ailments.

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

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