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# Effect of solvent, temperature, pH and duration on extraction process of anti-thiamine factor present in *Ageratum conyzoides* Linnaeus (Asteraceae) leaves

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**Abstract**

Effect of solvent, temperature, pH and time on extraction process of anti-thiamine factor present in *Ageratum conyzoides* Linnaeus (Asteraceae) leaves was studied. Results showed that the leaf-extract with 10 % chloroform –water mixture at 40º C for one hour at pH 3.0 had maximum anti-thiamine activity in *in vitro* experiments .

***Key words****:* Extraction process, Anti-thiamine factor, *Ageratum conyzoides*

## INTRODUCTION

*Ageratum conyzoides* Linnaeus of Asteraceae is a medicinal plant (Gurung 2002). this pantropical weed is distributed throughout India, including Sikkim and Darjeeling up to 2100 m *amsl*. In Nepali the plant is called as '*Elame'*; in Lepcha '*Namyew'* and in English it is known as 'Goat weed'. The plant flowers throughout the year (Das & Chanda 1987; Handa *et al.* 2006).

*Ageratum conyzoides* is used in the treatment of a large number of human ailments as mentioned in Ayurveda, Charaka Samhita and Sushruta Samhita (Vaidyaratnam 2002). Leaves, root, stem and flower of *Ageratum conyzoides* are widely utilized in traditional medicine. (Gurung 2002). Leaves are styptic effective in healing of wounds, used in boils and prevent tetanus. Leaf juice is also used as eye lotion. The root juice has antibiotic property. The plant is boiled with oil and applied externally in rheumatism. Phenol, essential oil, friedolin, sitosterol, stigmasterol and unidentified esters are active components of *Ageratum conyzoides* (Chopra *et al*. 1958; Gurung 2002; Okunade 2002; Kong *et al*. 2002; Tailor & Goyal 2012).

Recently we have observed anti-thiamine activity of the leaves of *Ageratum conyzoides* (unpublished observation). Tempted by this observation we undertook studies for isolation of the bio-active compound present in the species which is responsible for anti-thiamine activity. In this communication we report the effects of solvent, temperature, pH and duration on extraction process of anti-thiamine factor from the leaves of *Ageratum conyzoides* Linnaeus.

## METHODOLOGY

### Collection of plant material

Fresh and healthy leaves of *Ageratum conyzoides* Linnaeus (Asteraceae) were collected from the Garden of Medicinal Plants, University of North Bengal and identified by matching in NBU Herbarium. A voucher specimen was kept in the department of Biochemistry, North Bengal Medical College for future references.

### Preparation of leaves for Anti-thiamine activity

Leaves of *Ageratum conyzoides* were shed dried and powdered. 50 grams of leaf-powder was separately extracted with 500 ml of different solvents 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 *Ageratum conyzoides* leaves following the method of Bhattacharya & Choudhuri (1974). Main steps were: an intimate mixture of thiamine hydrochloride (100 mg) and test material collected from *Ageratum conyzoides* leaves (100 mg) was incubated at 30° C for 1 hour in10 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 (1941). 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 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.

### Effect of solvents on extraction process

Distilled water as well as 10 % (v/v) of chloroform, ethanol, methanol, acetone and petroleum ether were used separately in extraction process.

### Effect of time on extraction process

Extraction processes were done separately for 30, 60, 90 and 120 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 the effect of solvents on extraction process for isolation of anti-thiamine compound from the leaves of *Ageratum conyzoides*. It was found chloroform (10 % v/v) extract produced maximum anti-thiamine activity with 65 % inhibition of added thiamine was noted in the *in vitro* experiment. Anti-thiamine activity in terms of percent inhibition of thiamine for different solvent systems were as follow: water 40%, ethanol (10 %, v/v) 35%, methanol (10 %, v/v) 30 %, acetone (10 %, v/v) 28 %, and with petroleum ether (10 %, v/v) 15%.

**Table 1.** Effect of solvents on extraction process of the anti-thiamine factor present in *Ageratum conyzoides* Linnaeus leaves.

| **Solvent (1 hr extraction)** | **Amount of mass in mg (after extraction)** | **Anti-thiamine activity (% inhibition)** |
|---|---|---|
| Water | 100 | 40 |
| Chloroform (10 %, v/v) | 100 | 65 |
| Ethanol (10 %, v/v) | 100 | 35 |
| Methanol (10 %, v/v) | 100 | 30 |
| Acetone (10 %, v/v) | 100 | 28 |
| Petroleum ether (10 %, v/v) | 100 | 15 |

**Table 2.** Effect of duration of extraction process for testing the anti-thiamine factor present in *Ageratum conyzoides* Linnaeus leaves.

| **Solvent** | **Duration (minutes)** | **Anti-thiamine activity (% inhibition)** |
|---|---|---|
| Chloroform (10 %, v/v) | 30 | 45 |
| | 60 | 65 |
| | 90 | 63 |
| | 120 | 62 |

**Table 3.** Effect of temperature on the extraction process of anti-thiamine factor present in *Ageratum conyzoides* Linnaeus leaves.

| **Solvent** | **Temperature in °C** | **Anti-thiamine activity (% inhibition)** |
|---|---|---|
| Chloroform (10 %, v/v) | 30 | 60 |
| | 40 | 72 |
| | 50 | 70 |
| | 60 | 68 |

Effect of duration of extraction process for isolation of anti-thiamine compound from the leaves of *Ageratum conyzoides* is shown in Table 2. Time given for extraction in separate experiments was 30 minutes, 60 minutes, 90 minutes and 120 minutes. It appears from the

table that anti-thiamine activity in terms of percent inhibition of exogenous thiamine was maximum (65 %) for 60 minutes extraction time. For 30 minutes, 90 minutes and 120 minutes of extraction time anti-thiamine activity in terms of percent inhibition of thiamine were determined as 45 %, 63 % and 62 % respectively.

**Table 4.** Effect of pH on the extraction process of anti-thiamine factor present in *Ageratum conyzoides* Linnaeus leaves.

| Solvent | pH | Anti-thiamine activity (% inhibition) |
|---|---|---|
| Chloroform (10%, v/v) | 3.0 | 85 |
| | 5.0 | 70 |
| | 7.0 | 65 |
| | 10.0 | 63 |
| | 14.0 | 61 |

Table 3 shows the effect of temperature on extraction process for isolation of anti-thiamine compound from the leaves of *Ageratum conyzoides*. 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 60 % but the same value was 72 % when the extraction temperature was raised to 40° C. Increase of temperature for extraction above this has not elevated anti-thiamine activity. Results thus showed that for maximum anti-thiamine activity the extraction should be done at 40° C.

Effect of pH on the extraction process for isolation of anti-thiamine compound from the leaves of *Ageratum conyzoides* 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 (85 %) 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.

Results were mean value of five sets of experiment.

## DISCUSSION

The concept of anti-thiamine factor was introduced in literature by Green (1936, 1937), Evans *et al*. (1942) and other workers (Spitzer *et al*. 1941; Sealok & Goodland 1944). De *et al*. (1974) 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, imiodazole thiamine and benzoyl imidazole thiamine etc.; while natural anti thiamine compounds are non-structural analogues and mostly present in different food-stuffs, plants etc. Natural antithiamine 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.

Plants also showed anti-thiamine activity. Few such plants include blue berries (*Vaccinium* spp.; Hilker 1968), coffee (*Coffea arabica* Linnaeus; Bonicks & Georg 1969), *Brassica juncea* (Linnaeus) Czernajew (Bhattacharya & Chaudhury 1974), *Bombax ceiba* Linnaeus (Sarkar & Chaudhury 1976) etc.

Present study exposed the anti-thiamine activity of the leaves of *Ageratum conyzoides* Linnaeus. Extraction process was a part of the work to isolate the bio-active compounds present in the leaves of this species. Extracts with different solvents generally show different composition of bio-active molecules (Zarnowski & Suzuki 2004). Therefore, a suitable extracting solvent is needed to be selected for extraction of the active compound with maximum activity (Wang & Weeler 2006). Use of different solvents (distilled water, chloroform, ethanol, methanol, acetone and petroleum ether) has led to the selection of chloroform (10%, v/v) for this purpose for this species. This was followed by water. Other solvents used in extraction process [ethanol, methanol, acetone and petroleum ether] showed only little anti-thiamine activity.

Extraction time is very important to extract active compounds in maximum amount (Cannell 1998; Huie 2002) and the present investigation selected one hour extraction duration.

The extraction temperature is another important factor influencing the recovery of the bio-reactive compound from the sources (Wang & Weller 2006) and the 40º C has been determined as the most suitable temperature for this in *Ageratum conyzoides*.

Extraction pH is also important to obtain more bioactive compound from the source as most of the compounds are present in complex form with many other biomolecules (Sasidharan *et al*. 2011) and for *Ageratum conyzoides* pH 3 was the most suitable during the present set of experiments.

By maintaining these selected or determined parameters new sets of experiments are now being designed to isolate the bioactive compound present in *Ageratum conyzoides* leaves responsible for *in vitro* anti-thiamine activity.

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