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EFFECT OF SOLVENT, TEMPERATURE, pH AND TIME ON EXTRACTION PROCESS OF ANTI THIAMINE FACTOR PRESENT IN LEAVES OF *Abrus precatorius* Linn.

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ABSTRACT

Effect of solvent, temperature, pH and time on extraction process of anti thiamine factor present in *Abrusprecatorius* Linn.leaveswas studied. Results showed that extraction of leaves of *Abrusprecatorius* Linnwith 50% acetone –water mixture at 40^0C for one hour at pH 3.0 had maximum anti thiamine activity in *in vitro* experiments.

Keywords: Extraction process, Anti thiamine activity, *Abrus precatorius* Linn.

INTRODUCTION

Abrus precatorius Linn. has been used in Hindu medicines from very early times, aswell as in china and other ancient cultures [1]. The plant was consideredbeneficial for the hair and the seeds extract isused in the treatment of ulcer and skin affection [2]. Seeds of the plant are very much attractive, used in ornaments, but are highly poisonous. Seeds are reported to have anti diabetic property [3], may induce abortion [4], have anti oxidative property [5] as well as anti– inflammatory analgesic activity [6]. Saganuwan and Gulumbe[7,8]reported antimicrobial activity of the aqueous extract of *Abrus precatorius* Linn. Against *Salmonella typhimurium, Escherichia c*oli, *Klebsiellapneumoniae, Streptococcus pyogenes* and *Streptococcus pneumoniae.* Karamoko*et al*. also showed antibacterial activities of the aqueous extract of the plant [9]. Other uses of theplant are observed in cancer [10] and in malaria[11] .

Phytochemical components of the plant are abricin,abrin, abrisin, abrine, abraline, abrasine, abrusgenic acid-methylester, abruslectone,abrussic acid, anthocyanins etc. [12,13]. Recently

we observed anti thiamine activity of the leaves of *Abrusprecatorius* Linn. in*in vitro* experiments. Tempted by this observation we undertook studies for isolation of the bio active compound present in*Abrusprecatorius* Linn.responsible for anti thiamine activity. In this communication we report effect of solvent, temperature, pH and time on extraction process of anti thiamine compound from the leaves of *Abrusprecatorius*Linn.

MATERIALS AND METHODS

Collection of plant material

Leaves of*Abrusprecatorius*Linn.were collected from the medicinal plant garden of the University of North Bengal and identified by the taxonomists of the department of Botany of the said University. A voucher specimen of the leaf was kept in the department for future references.

***Abrusprecatorius*Linn.**

Preparation of leaves for Anti thiamine activity

Leaves of *Abrusprecatorius* Linn.were shed dried and powdered. 50 grams of this powder was separately extracted with 500 ml of different solvents at different temperatures, pH and time on a temperature controlled rotary shocker. The extract was filtered and the solvent was evaporated to dryness *in vacuo* with rotary evaporator at 40 – 50 degree centigrade. A brownish mass was obtained. This mass was kept to check the anti thiamine activity.

In vitro anti thiamine activity

The anti thiamine activity was determined by estimating the residual thiamine present in a system containingknown amount of thiamine hydrochloride and test material collected from *Abrusprecatorius* Linn. leavesby the method of Bhattacharya and Choudhuri[14]. Main steps were: an intimate mixture of thiamine hydrochloride (100 mg) and test material collected from *Abrusprecatorius* Linn. 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 [15]. 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.

Effect of solvents in extraction process

Distilled water as well as 50% (v/v) of chloroform, ethanol, methanol, acetone and petroleum ether were used separately in extraction process.

Effect of time in extraction process

Extraction processes were done separately for 30, 60, 90 and 120 minutes.

Effect of temperature in extraction process

In separate experiments extraction processes were done at 30, 40, 50 and 60 degree centigrade.

Effect of pH in extraction process

In separate experiments extraction processes were done at 3.0, 5.0, 7.0, 10.0 and 14.0 pH. Acidic and alkaline pH was maintained by adding 1(N) hydrochloric acid and 1(N) 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 *Abrusprecatorius* Linn.It was found out that acetone (50%, v/v) extract of the leaves of *Abrusprecatorius* Linn. had maximum anti thiamine activity. 70% 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 – 50%, ethanol (50%, v/v) – 35%, methanol (50%, v/v) – 28%, chloroform (50%, v/v) – 20%, petroleum ether (50%, v/v) – 15%.

Effect of time on extraction process for isolation of anti thiamine compound from the leaves of *Abrusprecatorius* Linn.is shown in Table – 2.Time given for extraction in separate experiments were 30 minutes, 60 minutes, 90 minutes and 120 minutes. It appears from the table that antithiamine activity in terms of percent inhibition of exogenous thiamine was maximum(70%) 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 came 40%, 70% and 70% respectively.

Table – 3 shows effect of temperature on extraction process for isolation of anti thiamine compound from the of leaves of *Abrusprecatorius* Linn.. Increase in temperature during extraction elevated anti thiamine activity. When extraction was done at thirty degree centigrade 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 forty degree centigrade. After that even increasing the temperature of extraction process, anti thiamine activity was not elevated. For fifty and sixty degree centigrade temperature of extraction process, anti thiamine activity in terms of percent inhibition of thiamine were 68% and 64% respectively. Results thus showed that maximum anti thiamine activity came when the temperature of extraction process was forty degree centigrade.

Effect of pH on extraction process for isolation of anti thiamine compound from the leaves of *Abrusprecatorius* Linn.is shown in Table – 4. Different pH was maintained in separate extraction process. 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 came 73%, 70%, 60% and 50% respectively.

Table – 1.Anti thiamine activity of extract of *Abrusprecatorius* Linn. leaves.
Effect of solvent on extraction process.

Solvent (Extraction was for 1h)	Dose of mass (After extraction)	Anti thiamine activity (% inhibition)
Water	100 mg	50%
Acetone (50%, v/v)	100 mg	70%
Ethanol (50%, v/v)	100 mg	35%
Methanol (50%,v/v)	100 mg	28%
Chloroform (50%,v/v)	100 mg	20%
Petrolium ether (50%,v/v)	100 mg	15%

Table – 2. .Anti thiamine activity of extract of *Abrusprecatorius* Linn.leaves.

Effect of time on extraction process.

Solvent	Time (minutes)	Anti thiamine activity (% inhibition)
Acetone (50%, v/v)	30	40%
	60	70%
	90	70%
	120	70%

Table – 3.Anti thiamine activity of extract of *Abrusprecatorius* Linn.leaves.

Effect of temperature on extraction process.

Solvent	Degree centigrade	Anti thiamine activity (% inhibition)
Acetone (50%, v/v)	30	60%
	40	72%
	50	68%
	60	64%

Table – 4.Anti thiamine activity of extract of *Abrusprecatorius* Linn.leaves.

Effect of pH on extraction process.

Solvent	pH	Anti thiamine activity (% inhibition)
Acetone (50%, v/v)	3.0	80%
	5.0	73%
	7.0	70%
	10.0	60%
	14.0	50%

Results were mean value of five sets of experiment.

In all experiments of measuring anti thiamine activity 100 mg of extracted mass from the leaves of *Abrusprecatorius* Linn. was used. Thiamine added, 100 mg.

DISCUSSION

The concept ofanti thiamine factor was introduced in literature by Green[16,17], Evans *et al*. [18] and other workers [19, 20]. De *et al.* [21] classified anti thiamine compounds broadly into two categories such as 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 anti thiamine compounds are further classified into two groups viz. natural anti thiamine compounds of large molecule e.g. thiaminase I and thiaminase II mainly isolated from raw fish and natural anti thiamine compounds of small molecule e.g. caffeic acid, chlorogenic acid, methyl sinapate, 3-5 dimethoxy salicylic acid etc.

Plants also showed anti thiamine activity. Few such plants are blue berries [22], coffee [23], *Brassicajuncea*[14], *Bombexmelabericum*[24]etc. Recently we observed anti thiamine activity of the leaves of *Abrus precatorius* Linn. We undertook isolation studies to get the bio active compound present in the leaves of*Abrusprecatorius*Linn.Extraction process is a part of isolation work. Different solvents yield different extracts and extract compositions [25]. Therefore, a suitable extracting solvent should be selected for extraction of the active compound for its maximum activity [26]. We thus used distilled water, chloroform, ethanol, methanol, acetone and petrolium ether separately as solvents for the extraction of the active compound from leaves of*Abrusprecatorius* Linn. Results showed that acetone (50%, v/v) extract had maximum anti thiamine activity in *in vitro* experiment. This was followed by water extract. Other solvents used in extraction process like ethanol, methanol, acetone and petrolium ether had little effect on anti thiamine activity.

Extraction time is important to extract active compound in maximum amount[27,28]. We allowed extraction time as half an hour, one hour, one and half hours as well as two hours in separate experiment. It was found out that one hour extraction time produced the extract from leaves of *Abrusprecatorius* Linn.which had maximum *in vitro* anti thiamine activity. Even after prolonging the time of extraction after one hour anti thiamine activity of the extract was not increased.

The extraction temperature is another important factor influencing the recovery of the bio reactive compound from the source [26]. In separate experiments we conducted extraction at temperatures like thirty, forty, fifty and sixty degree centigrade. Results showed that extraction of the leaves of *Abrusprecatorius* Linn. at forty degree centigrade had maximum anti thiamine activity.

Extraction pH is also important to obtain more bioactive compound from the source as most of the compounds are present in complex form with bio molecules [29]. We thus maintained

acidic, neutral and alkaline environment by keeping pH 3, 5, 7, 10 and 14 in extraction process. It was revealed that at pH 3 of extraction process the obtained extract had maximum anti thiamine activity. This is perhaps due to release of bio active compound from complex by acid.

CONCLUSION

In conclusion it may be stated that maximum anti thiamine activity of the leaves of *Abrus precatorius* Linn. was noted when conditions like 50% (v/v) acetone as solvent system, one hour time, forty degree centigrade temperature and pH 3.0 were maintained during extraction process. By maintaining these conditions we are now interested to isolate the bio active compound present in *Abrus precatorius* Linn. leaves responsible for *in vitro* anti thiamine activity by undertaking several chromatographic experiments. Work is progressing in this direction.

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