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In vitro Anti Thiamine Activity of an Isolated Compound from *Murrya koenigii* (linn.) Spreng Wettst Leaves: Effect of Season

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Abstract

By solvent extraction, acid hydrolysis, chromatographic experiments followed by crystallization a compound was isolated from *Murrya koenigii* (Linn.) Spreng Wettst leaves. The compound could exert anti thiamine activity in *in vitro* experiment. It was found out that 1g of the compound inactivated 31.8 mg of thiamine hydrochloride in 1 h. Effect of season on *in vitro* anti thiamine activity of the compound was also studied. Results showed that compound isolated from the leaves of *Murrya koenigii* (Linn.) Spreng Wettst of the period July - August had maximum anti thiamine activity.

***Key words*:** *Murrya koenigii* (Linn.) Spreng Wettst leaves, anti thiamine activity, seasonal variation

Introduction

Murrya koenigii (Linn.) Spreng Wettst (family, Rutaceae) is a medicinal plant. It is commonly known as curry-leaf tree though the tree has different names. In Hindi it is called 'bursunga' and in Nepali it is known as 'meehi saag'. It is a small tree with dark green bark. The tree is native of Sri Lanka, India, and other South Asian countries. It is widely distributed at foothills of Himalayas from Kumaon to Sikkim, Assam, Bengal, middle and lower hill forests up to the height of 5000 ft. The tree is a rich source of carbohydrates, proteins, amino acids, alkaloids as well as vitamin A, vitamin B , minerals etc.[1,2] .Genus Murraya has 14^{th} global species. In India two species are available - *M. koenigii* (L.) Spreng and *M. Paniculata* (L.) Jack [3]. The former species *M. koenigii* (L.) Spreng is very popular for its use as natural flavoring agent and for its large spectrum of medicinal properties [4].

In traditional system of medicine, leaves and roots of *M. koenigii* (L.) Spreng are used as anti helmintic, analgesic etc. It is applied to cure piles, leucoderma and blood disorders. Burk is used to cure eruptions, poisonous animal bites etc. The tree has also stomachic and tonic properties [5,6]. In modern research this tree has been reported to have many medicinal properties. Shah & Juvekar (2006) showed positive inotropic effect of *M. koenigii* (L.) Spreng extract on an isolated perfused frog [7] while Shrinivasan (2005) noted that the leave of the tree is beneficial for diabetes [8]. Other workers demonstrated anti-oxidative, antibacterial, cytotoxic, cholesterol reducing activities and anti ulcer activities of the tree [9-14]. We also noted anti ulcer activity of *M. koenigii* (L.) leaves in ethanol induced gastric ulcer and cysteamine induced duodenal ulcer in albino rats [15-17].

Numerous phytochemicals like koenigine, koenidine, koenine, mahanine, girinimibine ,girinimbiol, koenimbine, O-methyl murrayamine A, O-methyl mahanine, bismahanine, bispyrayafoline, isomahanine, scopotin, murrayanine were isolated from the leaves of *M. koenigii* (L.) Spreng [18-22].

We earlier confirmed anti thiamine activity of *M. koenigii* (L.) leaves in *in vitro* experiment[23].

We, therefore, intended to carry out studies to isolate active compound from the leaves responsible for anti thiamine activity. Studies were also undertaken to know the effect of season on anti thiamine activity of the isolated compound.

Materials and method

Collection of plant material

From the medicinal plants garden of the University of North Bengal, Dist. Darjeeling, West Bengal, India, *M. koenigii* (L.) leaves were collected sometimes in July around 9 in morning. Leaves were authenticated by the experts of the department of Botany of the said University. A voucher specimen was kept in the department of Biochemistry, North Bengal Medical College, Siliguri, India for future reference.

Leaves were also collected during the periods of January – February, March – April, May – June, July – August, September – October and November – December separately for seasonal variation experiments.

Fig.1: *Murrya koenigii* (Linn.) Spreng Wettst leaves

Extraction and Isolation

Extraction and isolation of the active compound from *M. koenigii* (L.) leaves were done following the standard methods of isolation of chemicals from plant sources [24-27]. Steps were as follow:

First step: Leaves of *M. koenigii* (L.) were washed thoroughly, shade dried and powdered. 100g of this powder were extracted with 1000 ml of 50 : 50 (v/v) acetone – ethyl alcohol mixture for 30 min at 60^0C using a soxhlet apparatus. It was then centrifuged. Supernatant was collected and evaporated to dryness. Dry brown mass was obtained.

Second step: Dry brown mass was refluxed with 100 ml of 1(N) HCL for 15 min on a water bath at 100^oC. It was cooled and centrifuged. Supernatant was evaporated to dryness .

Third step: Dry brown mass thus obtained was extracted with 100 ml of isobutanol on a rotary shaker for 30 min. It was then centrifuged. Supernatant was collected and evaporated to dryness. Dry brown mass was obtained.

Fourth step: Brown mass was extracted with 10 ml ethanol and subjected to column chromatography using silica gel G as adsorbent. Seven bands were separated. Bands were collected in separate beakers . Elution was done by ethanol – chloroform mixture (1:1 v/v) . Second band had *in vitro* anti thiamine activity.

Fifth step: Eluent of second band was evaporated to dryness. The dry mass was extracted with 20 ml ethyl acetate for 15 minutes. It was then filtered. With filtrate polyamide column chromatography was done. Elution was made by ethyl formate : formic acid mixture (10 : 1 v/v). four bands were separated. Third band showed *in vitro* anti thiamine activity.

Sixth step: Eluent of third band was evaporated to dryness. Repeated crystallizations were done from ethyl acetate–cyclohexane (1:1, v/v) mixture. Crystals obtained.

Homogeneity of the active compound

Homogeneity of the active compound was ascertained by silica gel- G thin layer chromatography by using the following solvent systems: Chloroform : methanol : water - 3 : 1 : 1; Acetone : methanol - 1 : 1; n-butanol : acetic acid : water - 8 : 1 : 1

In vitro anti thiamine activity

This was done by the method of Bhattacharya and Choudhuri [28].

Anti thiamine activity was determined by estimating the residual thiamine present in a system containing known amount of thiamine hydrochloride and compound collected from *M. koenigii* (L.) leaves. Main steps were: an intimate mixture of thiamine hydrochloride (100 mg) and compound isolaed from *M. koenigii* leaves (1 g) was incubated at 30^0C for 1 h 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 [29]. In short, to 2ml of the filtrate 0.1 ml 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 min. 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.

Chemicals

All chemicals were of analytical grade with high purity. They were purchased from Sigma Chemical Company, Mumbai.

Experimental design for seasonal variation study

Experiments were conducted as follow:

Incubation of

(1) Thiamine + Compound isolated from leaves of *M. koenigii* L. *(January – February)*

(2) Thiamine + Compound isolated from leaves of *M. koenigii* L. *(March – April)*

(3) Thiamine + Compound isolated from leaves of *M. koenigii* L. *(May – June)*

(4) Thiamine + Compound isolated from leaves of *M. koenigii* L. *(July – August)*

(5) Thiamine + Compound isolated from leaves of *M. koenigii* L. *(September – October)* :

(6) Thiamine + Compound isolated from leaves of *M. koenigii* L. *(November – December)* :

Statistical analysis

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

In vitro anti thiamine activity of the compound isolated from *M. koenigii* L. leaves collected randomly is shown in the following table.

Table - 1 : Showing in vitro anti thiamine activity of the compound isolated from *M. koenigii* L. leaves

Group	Residual thiamine (mg)	Inhibition (%)
Control (Thiamine hydrochloride – 100 mg)	100.0 ± 0.01	--
Thiamine hydrochloride (100mg) + compound (1g) isolated from *M. koenigii* L. leaves collected randomly.	68.2 ± 2.1*	31.8

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

Table -1 showed that compound isolated from *M. koenigii* L. leaves collected randomly inhibited thiamine. 1 g of the isolated compound could destroy 31.8 mg thiamine hydrochloride *in vitro*. Initially amount of thiamine hydrochloride taken was 100 mg . After 1h incubation with 1 g of the compound isolated from *M. koenigii* leaves, amount of thiamine came down to 68.2 mg. Result was statistically significant. Percentage of thiamine inhibition was 31.8%.

Table - 2 : Showing seasonal effect on in vitro anti thiamine activity of the compounds isolated from of *M. koenigii* L. leaves.

Group	Residual thiamine (mg)	Inhibition (%)
Control (Thiamine hydrochloride – 100 mg)	100.0 ± 0.02	--
Thiamine hydrochloride (100mg) +Compound isolated from *M. koenigii* L. leaves (1g) (January - February)	98.0 ± 3.12	2.00
Thiamine hydrochloride (100mg) + Compound isolated from *M. koenigii* L. leaves (1g) (March - April)	95.2 ± 3.08	4.80
Thiamine hydrochloride (100mg) + Compound isolated from *M. koenigii* L. leaves (1g) (May - June)	82.7 ± 1.51*	17.3
Thiamine hydrochloride (100mg) + Compound isolated from *M. koenigii* L. leaves (1g) (July - August)	68.9 ± 1.43**	31.1
Thiamine hydrochloride (100mg) + Compound isolated from *M. koenigii* L. leaves (1g) (September - October)	79.1 ± 1.62*	20.9
Thiamine hydrochloride (100mg) + Compound isolated from *M. koenigii* L. leaves (1g) (November - December)	95.3 ± 3.14	4.7

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

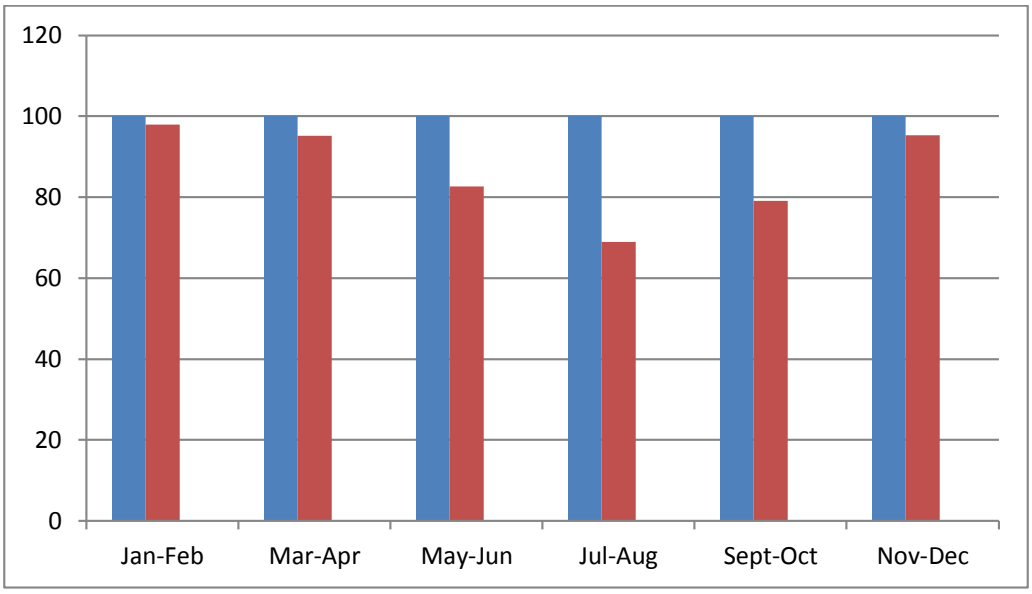
Seasonal effect on *in vitro* anti thiamine activity of the compound isolated from the leaves of *M. koenigii* L. is given in Table – 2. Results showed that anti thiamine activity of the compound varied with season. Maximum anti thiamine activity was found during July and August. After 1 h incubation between 100 mg of thiamine hydrochloride and 1 g of the compound isolated from *M. koenigii* L. leaves, amount of thiamine came down to 68.9 mg. Result was statistically significant up to the level of $p<0.001$. Percentage of thiamine inhibition was 31.1%. Compound isolated from the leaves of *M. koenigii* L. during the period May – June and September - October had also *in vitro* anti thiamine activity (amount of residual thiamine were 82.7 mg and 79.1 mg respectively) but the results were less significant in comparison to that of the thiamine inhibition activity of the compound isolated from the leaves of *M. koenigii* L. leaves during July - August.

Discussion

Several plants showed anti thiamine activity. *Athyrium esculentum* is an edible fern (Family- Athyriaceae) found throughout Asia and Oceania. In 1971 Rattanapanone *et al.,* found that the fern has anti thiamine activity [30]. Anti thiamine activity of *Pteris aquiline* L. was confirmed in experimental animals, bacteria and human by Weswig *et al.* [31]. Somogyi *et al.* in 1949 observed that fern extracts were composed of two thermostable antithiamine factors. They are hydrolysates I and hydrolysates II [32]. *Marsilea drummondii* (family- Marsileaceae), a common and widespread fern of wetland areas across inland Australia, could induce thiamine deficiency in sheep [33]. Sarkar *et al*. (1976) noted anti thiamine activity of *Bombax malabaricum* [34]. Chaudhuri (1962) confirmed the presence of a heat stable thiamine inactivating-factor in different varieties of rice and rice bran[35]. Anti thiamine activity of the plant *Triticum aestivum* (family, poaceae), was due to presence of toxic substances which caused nervous disorders [36]. Bhagvat and Devi detected presence of an anti-thiamine factor in ragi [37]. *Brassica napus* (family, Brassicaceae) has anti thiamine activity [38]. Bhattacharya *et al.* showed anti thiamine activity of *Brassica juncea* [39]. We also found anti thiamine activity of the leaves of *Abrus precatorius* L.[40] as well as of *M. koenigii* (L.) leaves in *in vitro* experiments [23].

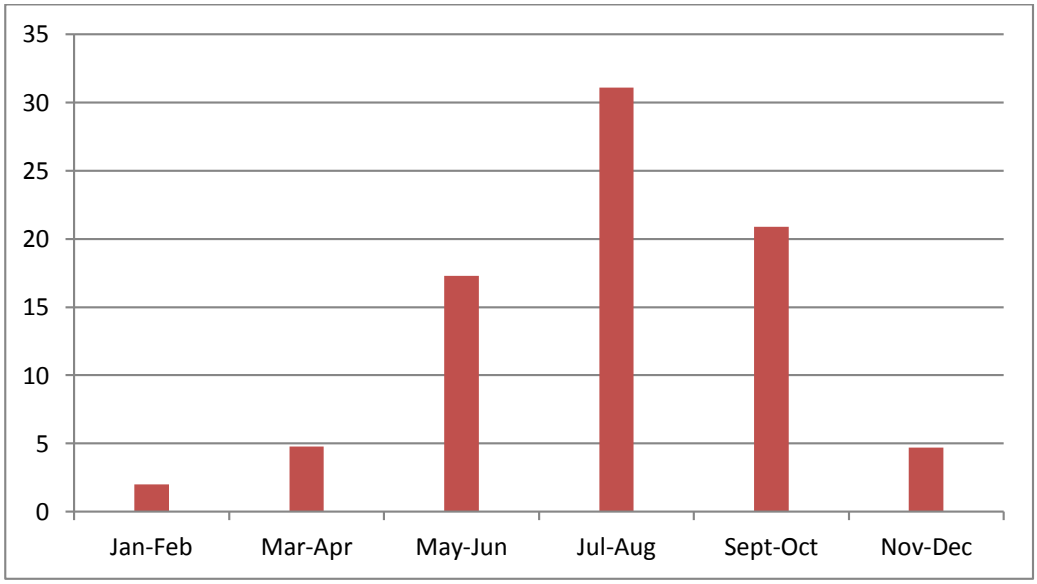
Present work was undertaken to isolate the chemical from *M. koenigii* (L.) leaves responsible

Fig 2 : Residual thiamine (mg) after 1 h incubation of thiamine hydrochloride and compound isolated from the leaves of *M. koenigii* L. of different seasons.



■ **Control (Thiamine hydrochloride – 100 mg)** ■ **Effect of compound (1 g) isolated from *M. koenigii* L. Leaves**

Fig 3 : Inhibition of thiamine (%) after 1h incubation of thiamine hydrochloride (100 mg) and 1 g of compound isolated from leaves of *M. koenigii* L. of different seasons.



for *in vitro* anti thiamine activity. A compound was isolated. 1 g of the compound could inactivate 31.8% of thiamine hydrochloride within 1 h. Anti thiamine compounds were also isolated from different plants by other workers [34, 39].

Influence of climate on active principles in medicinal plants was shown by Fluck and Pharm as early as 1955 [41]. Thereafter, series of experiments were conducted in this direction. Study revealed that amount of oak leaf tannins and nutrients changes with season [42]. Gupta in 1977 observed that amount of active constituents of *Eclipta prostrata* L. varies under different seasonal conditions and was maximum during summer [43] That leaf quality of two northern hardwoods tree species varies with season was the observation of Schultz *et al.* [44] . Vasicine contents and their seasonal variation in *Adhatoda vasica* was found maximum in autumn [45]. We also found seasonal variation in activities of different medicinal plants [46-50].

. In present study we worked on the effect of season on anti thiamine activity of the compound isolated from the leaves of *M. koenigii* L. Results showed that the compound had maximum *in vitro* anti thiamine activity during the months of July and August . (Figures -- 2 & 3). This may be due to production of maximum amount of thiamine inactivating active compound in the leaves of *M. koenigii* L. during July and August. Work is now in progress in this direction.

Conclusion

One compound has been isolated from *M. koenigii* L. Spreng Wettst leaves. The compound showed *in vitro* anti thiamine activity. 1 g of the compound could inactivate 31.8 mg of thiamine hydrochloride in 1 h. Seasonal variation in anti thiamine activity of the compound was studied. It was found out that the compound collected from *M. koenigii* L. leaves of the period July – August had maximum anti thiamine activity.

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