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Original Article

Isolation of growth inhibitory factor (CA-I) of albino rats from *Cassia alata* L. Leaves: Effect of season

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ABSTRACT

By solvent extraction, acid hydrolysis, chromatography followed by crystallization, a compound (CA-I) was isolated from the leaves of *Cassia alata* L. The compound could inhibit growth of rats. Growth inhibition was in terms of body weight reduction. Reduction of body weight of the rats by this compound started right from 10th day but significant reduction was observed from 20th day onwards. Effect of seasons on the amount of isolated compound (CA-I) from *C. alata* L. leaves was also studied. Results showed that leaves of *C.alata* L. for the months of July and August yielded maximum amount of the compound.

Keywords: *Cassia alata* L., Growth inhibitory factor, Chromatography, CA-I

INTRODUCTION

Cassia alata L.(family, Caesalpiniaceae) is a medicinal plant of Sikkim and Darjeeling Himalayas of India. It is an erect tropical annual herb with leather compounded leaves. The plant was native to Ghana and Brazil, but it is now widelydistributed throughout the world [1]. Even in India in the state of West Bengal, the plant grows everywhere up to 6 ft tall. *C. alata* L. is widely known in the name of wild senna. It has other names also. Names are: ringworm weed in English, dadmari in Hindi and cakramard in Sanskrit. Therapeutic values of *C. alata* L. as mentioned in Ayurvedic text [2,3] are: 1) leaves are anti parasitic and are used in eczema, bronchitis, asthma, ringworm and in poisonous insect bites 2) bark is used to treat skin diseases 3) extract of aerial parts is CNS depressant , diuretic and has anti inflammatory activity. Modern researchers advocated the use of *C. alata* L. for treatments of blennorrhagia, syphilis, diabetes, haemorrhoids, constipation, inguinal hernia and intestinal parasitosis [4-6]. Traditionally the plant is used as anti helminthic, in infection and in uterus disorder [7,8]. Makinde reported that all parts of *C. alata* L. have one or more medicinal actions especially antimicrobial activities [9]. In 1998 Sakharkar and Patil confirmed antimicrobial activity of *C.alata* L. [10]. We also noted that leaves of *C. alata* L. could inhibit growth of *Staphylococcus aureus* [11-12]. Recently we have noted anti growth activity of *C. alata* L. leaves in albino rats. We have also noted that leaves of *C. alata* L. for the months of July and August had maximum anti growth activity [13]. Tempted on this observation we have undertaken study to isolate the active constituent present in *C.alata* L. leaves responsible for growth inhibition in rats. We also studied effects of different seasons on the amount of isolated compound to justify the maximum growth inhibitory activity during the months of July and August. Results of the experiments are being reported here.

MATERIALS AND METHODS

Plant material: Leaves of *C. alata* L. were collected in morning hours (9 – 10 AM) from the medicinal plants garden of the University of North Bengal, Dist. Darjeeling, west Bengal, India randomly and during the periods of January – February, March – April, May – June, July – August, September – October and November – December of the year 2012. 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, Dist. Darjeeling, West Bengal, India for future reference.

Isolation of the active constituent: Isolation of the active constituent from the leaves of *C. alata* L. collected randomly and during the months of

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January –February, March – April, May – June, July – August, September – October and November – December were separately processed by the following method to collect active constituent.

***First step*:** Leaves of *C. alata* Linn. were properly washed, shade dried and powdered. 50g of this powder were extracted with 500 ml of 10 : 1 (v/v) acetone – ethyl alcohol mixture for 1h on a rotary shaker . 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 1h on a water bath at 100 degree centigrade. It was cooled and centrifuged. Supernatant was evaporated to dryness .

***Third step*:** Dry brown mass thus obtained from the supernatant was extracted with 50 ml of a mixture of water and isobutanol (2 : 1 v/v) on a rotary shaker for 1h.Isobutanol layer was separated from water layer. It was evaporated to dryness.

***Fourth step*:** Brown mass obtained was dissolved in 10 ml methanol and subjected to column chromatography using silica gel G as adsorbent. 5 bands were separated. Bands were collected in separate beakers . Elution was done by 50% methanol – chloroform mixture. Third band had antibacterial activity against *Staphylococcus aureus.*

***Fifth step*:** Eluent of third band was evaporated to dryness. The dry mass was extracted with 15 ml ethyl acetate for 10 minutes. It was then filtered. With filtrate polyamide column chromatography was done. Elution was made by ethyl formate: formic acid mixture (100: 5 v/v). Three bands were separated. Second band showed antibacterial activity against *Staphylococcus aureus.*

***Sixth step*:** Eluent of second band was evaporated to dryness. Repeated crystallization was done from ethyl acetate–cyclohexane (50:50, v/v) mixture. Crystals obtained. The compound was given a trivial name (CA-I). In each case yield of the compound was noted.

Homogeneity of the isolated compound: This was ascertained by silica gel- G thin layer chromatography by using the following solvent systems: Acetone : methanol - 50 : 50;n-butanol : acetic acid : water - 80 : 10 : 10; Chloroform: methanol : water - 60 : 20 : 20.

Acute oral toxicity study: Acute toxicity studies were carried out on Swiss albino mice by the method of Ghosh [14]. Compound isolated from the leaves of *C. alata* L. collected randomly was given at doses of 1, 2, 5, 10 and 30 mg/kg to different groups of mice each group containing six animals. Watery suspension of the test drug was given to the animals orally through a feeding tube. After administering the test drug, the animals were observed for the first three hours for any toxic symptoms followed by observation at regular intervals for 24 hours up to seven days. At the end of the study, the animals were also observed for general organ toxicity, morphological behavior and mortality.

Animals: Male Wister strain rats, body weight between 35 and 40g, were used for this study. Animals were housed individually in polypropylene cages, maintained under standard conditions like 12h light and 12h dark cycle, 20 - 30 degree centigrade, 35 - 60 % humidity. Rats were fed with standard rat pellet diet (Hindustan Lever Ltd., Mumbai, India) and provided water *ad libitum*. The animal experiment was approved by the ethics committee of the Institute.

Experimental design: In first set of experiment, rats were divided into two groups of six each. First group of animals took normal diet while animals of the second group, in addition to normal diet, took compound (CA-I) isolated from randomly collected leaves of *C. alata* L. in the dose of 0.1g/kg body weight daily. Isolated compound (CA-I) in the form of suspension in water was given to the rats orally through a feeding tube. Dose selection of the test drug was as per of our earlier studies [15-17]. Experiment was continued for 40 days.

Growth of rats: Growth of rats was measured on 10^{th}, 20^{th}, 30^{th} and 40^{th} day. Overall behavior of the animals was noted.

Statistical analysis: The 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 AND DISCUSSION

Acute toxicity studies: Acute toxicity studies revealed that the isolated compound (CA-I) from the leaves of *C.alata* L. did not produce any toxic symptoms when administered orally to mice in doses of 1, 2, 5, 10 and 30 mg/kg. Animals were healthy, cheerful and behaved normal throughout the experimental period. No death of animal was

recorded during seven days of experiment.

Homogeneity of the isolated compound: This was ascertained by silica gel- G thin layer chromatography by using three solvent systems as mentioned earlier. In each case single spot was obtained . The isolated compound(CA-I) was thus pure.

Table – 1 shows effect of isolated compound (CA-I) from the leaves of *C. alata* L. (randomly collected) on body weight of rats. It appears from the table that (CA-I)could decrease body weight of rats. For first ten days the decrease was not statistically significant but after that up to 40 days there was significant decrease ($p<0.001$) in body weight in those rats who took CA-I in addition with normal diet. The animals also developed anorexia. Effect of isolated compound on body weight of rats was also shown in figure – 2.

Table – 2 showed seasonal variations in the yield of the isolated compound (CA-I) from the leaves of *Cassia alata* L. Maximum yield of the compound in the leaves of *C. alata* L. was found during the months of July and August and it was 7.2 mg/100g of *C.alata* leave powder. The result was statistically significant at the level of $p<0.001$ when compared to the yield of other season. Seasonal variations in the yield of the isolated compound was also shown in figure – 3.

Fluck and Pharm [18] showed influence of climate on the active principles in medicinal plants. Thereafter, series of experiments were conducted in this direction. Now a days numerous reports are available in literature which suggest that accumulation of chemical compounds in roots, stem and leaves of plants varies with season [19-23]. In the present study we also noted that accumulation of compound (CA-I) in leaves of *C. alata* L. varies with season and was maximum during the period of July to August. We noted earlier [13] that maximum growth inhibition of the rats by the leaves of *C.alata* L. occurred during the period of July to August. It is now clear from the present result that this is due to maximal accumulation of the active compound (CA-I) in the leaves during that period. We are now interested to characterize the compound (CA-I) isolated from *C.alata* L. and to see the underlying mechanism of the growth inhibition of rats by it. Experiments are going on in this direction.

CONCLUSION

An active compound (CA-I) was isolated from the leaves of *Cassia alata* L. The compound could inhibit growth of rats. Seasonal variation in accumulation of the compound in *C. alata* L. leaves was studied. It revealed that leaves contained maximum amount of the compound (CA-I) during the months of July and August.

Table - 1: Effect of isolated compound (CA-I)from randomly collected leaves of *Cassia alata* L. on growth of rats (Changes of body weight in gram)

Group	Treatment	10^{th} day	20^{th} day	30^{th} day	40^{th} day
1	Normal	41.0 ± 1.4	57.3 ± 1.5	61.1 ± 1.8	72.5 ± 2.1
2	Isolated compound (CA-I) from *Cassia alata* L. leaves	38.3 ± 0.7	47.2 ± 1.8*	43.5 ± 1.0**	35.3 ± 1.1**

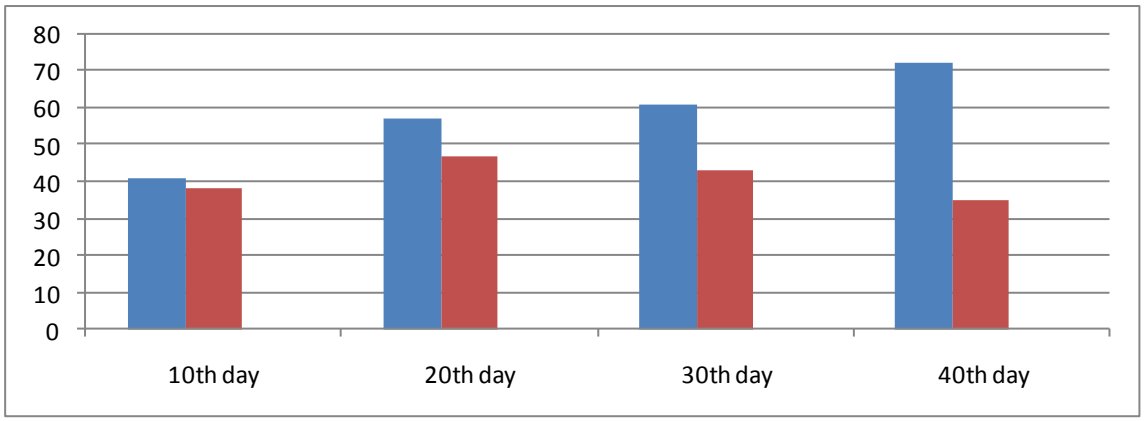
*p<0.001, *C. alata* L. : 1 g / kg, . * p< 0.05,** p < 0.001.

Table - 2: Seasonal variations in the yield of the isolated compound (CA-I) from the leaves of *Cassia alata* L.

Season	Yield of the compound (CA-I) (mg/100g of *Cassia alata* leave powder)
January –February	1.2± 0.01
March – April	3.6± 0.05
May – June	4.8± 0.07
July – August	7.2± 0.09**
September – October	3.9± 0.06
November - December	2.2± 0.05

Results are mean of six sets of experiments. ** p<0.001

Fig – 1 : *Cassia alata* L.



■Normal ■ (CA-I) isolated from *Cassia alata* L. leaves

Fig 2 : Effect of (CA-I) isolated from leaves of *Cassia alata* L. (randomly collected) on growth of rats (Changes of body weight in gram)

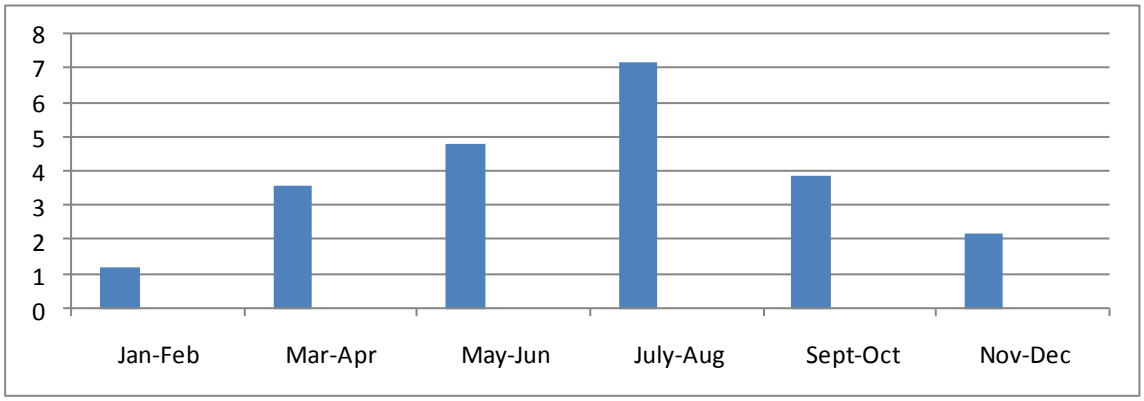


Fig 3 : Seasonal variation in the amount of (CA-I) isolated from *Cassia alata* L. leaves. The amount was in terms of mg/100g of *C. alata* leave powder.

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