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Isolation of Antiulcerogenic Compound (AC-I) from *Ageratum conyzoides* L. Leaves and Effect of Season on Yield of the Compound

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Abstract

A compound (AC-I) was isolated from *Ageratum conyzoides* L. leaves. The compound had anti peptic ulcer activity in ethanol induced gastric ulcers and cysteamine induced duodenal ulcers in albino rats. Seasonal variation in concentration of the compound (AC-I) in *A. conyzoides* L. leaves was studied. Results showed that leaves of *A. conyzoides* L. for the months of July and August yielded maximum amount of the compound.

Keywords: *Ageratum conyzoides* L. leaves, peptic ulcer, ethanol, cysteamine, ranitidine, ulcer index

Introduction

Ageratum conyzoides L. (family, asteraceae) is a plant that grows commonly in the proximity of habitation, thrives in any garden soil and is very common in waste places and on ruined sites [1].The plant is distributed throughout India, lower and middle hill in Sikkim and Darjeeling up to 6000 ft. The plant has erect hairy annual 30 – 90 cm high leaves. Different vernacular names are given to the plant. In Nepali the plant is called as 'Elame'; in Lepcha 'Namyew' and in English the plant is known as 'Goat weed'. Throughout the year the plant gives flower. Purple white flower appears.

A.conyzoides L. is a medicinal plant. The medicinal value of this plant in the treatment of a large number of human ailments is mentioned in Ayurveda, Charaka Samhita and Sushruta Samhita [2]. Leaves, root, stem and flower of *A. conyzoides* L. are widely utilized in traditional medicine. 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 *A. conyzoides* L. [3-6]

Fig. 1 - *Ageratum conyzoides* L.

Modern researchers claimed that *A. conyzoides* L. has antibacterial [7] and wound healing effect [8]. It has neurological [9] and possesses gastro protective effect [10]. The plant acts as analgesic [11] and has effect on circulation [12]. It gives protection against gamma radiation [13]. The plant has anti tumor activity [14] and has allopathic effects [15]. Ita *et al.* [16] demonstrated hepato protective activity of this plant.

Recently we have noted anti ulcerogenic effect of *A. conyzoides* L. leaves in ethanol induced gastric ulcer as well as cysteamine induced duodenal ulcer models in albino rats (unpublished observation). As medicinal values of a plant depend on its chemical compound(s) it was thought worthwhile to isolate the active compound(s) from *A. conyzoides* L. leaves responsible for anti peptic ulcer activity. Further, accumulation of chemical compounds in plants varies with seasons [17]. Therefore, seasonal variation in yield of the active compound in course of isolation from the plant leaves was also studied.

Materials and methods

Plant Material

Ageratum conyzoides L. leaves 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 months of January – February, March – April, May – June, July – August, September – October and November – December 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 *Ageratum conyzoides* L. collected randomly and during the months of January – February, March – April, May – June, July – August, September – October and November – December were separately processed by the following methods to collect active constituent.

***First step*:** Leaves of *Ageratum conyzoides* L. were properly washed, shade dried and powdered. 50g of this powder were extracted with 500 ml of 1:1 (v/v) acetone – ethyl alcohol mixture for 20 min on a rotary shaker. It was then centrifuged. Supernatant was collected and evaporated to dryness. Dry mass was obtained.

Second step: Dry mass was refluxed with 100 ml of 10% hydrochloric acid for 1h on a water bath at 100 degree centigrade. It was cooled and centrifuged. Supernatant was evaporated to dryness.

***Third step*:** Dry mass thus obtained from the supernatant was extracted with 50 ml of ethyl alcohol on a rotary shaker for 10 min. It was then centrifuged. Supernatant was evaporated to dryness.

***Fourth step*:** Dry mass obtained was dissolved in 10 ml ethanol and subjected to column chromatography using alumina as adsorbent. Five bands were separated. Bands were collected in separate beakers. Elution was done by 10% acetone - ethanol mixture. Third band had antiulcerogenic activity against ethanol induced gastric ulceration in albino rats.

***Fifth step*:** Collection of third band was evaporated to dryness. Dry mass obtained. It was dissolved in 10 ml ethanol and subjected to column chromatography using polyamide as adsorbent. Five bands were separated. Bands were collected in separate beakers . Elution was done by 50% acetone - ethanol mixture. Second band had antiulcerogenic activity against ethanol induced gastric ulceration in albino rats.

***Sixth step*:** Eluent of second band was evaporated to dryness. The dry mass was extracted with 15 ml acetone for 10 minutes. It was then filtered. With filtrate silica gel column chromatography was done. Elution was made by ethyl acetone-ethanol mixture (1 : 1 v/v). Three bands were separated. First band had antiulcerogenic activity against ethanol induced gastric ulceration in albino rats.

***Seventh step*:** Eluent of first band was evaporated to dryness. Repeated crystallization was done from ethyl acetate–formic acid (60:40, v/v) mixture. Crystals obtained. The compound was given a trivial name (AC-I).

In each case yield of the compound was noted.

Homogeneity of the active compound

This was ascertained by silica gel- G thin layer chromatography by using the following solvent systems: Ethanol : acetone - 80 : 20; n-butanol : acetic acid : water - 80 : 10 : 10; Chloroform: methanol : water - 60 : 20 : 20

Experimental animals

Wistar strain albino rats (180 - 200 g) of either sex were used for the study. Rats were housed in colony cages (5 rats / cage) and kept for at least a week in the experimental wing of the animal house (room temperature 25 – 28 degree centigrade and humidity 60 – 65% with 12 h light and dark cycle) before experimentation. Animals were fed on laboratory diet with water *ad libitum*. 8 rats were used for each set of experiment. The animal experiment was approved by the ethics committee of the Institute.

Chemicals and Drugs

Ethanol (Baroda Chemical Industries Ltd., Dabhoi) and cysteamine (Sigma Chemical Co., USA) were used in the study, ranitidine (Cipla pharmaceuticals)

Acute toxicity study

Acute toxicity studies were carried out on albino rats by the method of Ghosh [18]. Compound (AC-I) isolated from the leaves of *Ageratum conyzoides* L. collected randomly was given in 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.

Production of peptic ulcer

(a) Ethanol induced gastric ulcer

This was done by the method of Sairam *et al*.[19] Rats were fasted for 18 h when no food but water was supplied *ad libitum*. Gastric ulcers were induced by administering ethanol (95%, 1 mL/200 g body weight) orally. 1 h after administration of ethanol, animals were sacrificed by cervical dislocation and the stomach was taken out and incised along the greater curvature. Stomach was then examined for the presence of bleeding, adhesion, dilatations and ulcers.

(b) Cysteamine induced duodenal ulcer

This was done by the method of Parmar and Desai [20]. To 18 h fasted rats (water was supplied *ad libitum*) cysteamine hydrochloride (400 mg/kg, p.o. in 10% aqueous solution) was administered in two doses at an interval of 4 h to produce duodenal ulcers. After 24 h of the first dose of cysteamine, animals were sacrificed by cervical dislocation and the duodenum was excised carefully and opened along the antimesenteric side. Duodenum was then examined for the presence of ulcers.

Antiulcer Study

Rats were divided into six groups.

1. Control: Rats took normal diet, water and vehicle of the drug.
2. Drug treated : Rats were treated with drug either with ethanol or cysteamine.
3. Drug + AC-I (100 mg/kg) : AC-I in the dose of 100 mg/kg in watery suspension was given to the rats orally through feeding tube 30 minutes prior to administration of drug.
4. Drug + AC-I (200 mg/kg) : AC-I in the dose of 200 mg/kg in watery suspension was given to the rats orally through feeding tube 30 minutes prior to administration of drug.
5. Drug + AC-I (300 mg/kg) : AC-I in the dose of 300 mg/kg in watery suspension was given to the rats orally through feeding tube 30 minutes prior to administration of drug.

6. Drug + Ranitidine : Ranitidine was given in the dose of 50 mg/kg p.o. 30 minutes prior to administration of aspirin. Dose of ranitidine was selected based on report of Khare *et al*. [21].

Evaluation of ulcer index

Evaluation of ulcer index was done by the method of Szelenyi and Thiemer [22]. Gastric /duodenal lesions were counted and the mean ulcerative index was calculated as follows :

I - Presence of edema, hyperemia and single sub mucosal punctiform hemorrhage.

II – Presence of sub mucosal hemorrhagic lesions with small erosions.

III – Presence of deep ulcer with erosions and invasive lesions.

Ulcer index = (number of lesion I) x1 + (number of lesion II) x2 + (number of lesion III) x 3.

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) 20^{th} versions. 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 (AC-I) from the leaves of *A. conyzoides* L. did not produce any toxic symptoms when administered orally to rats 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 (AC-I) was

thus pure.

Anti gastric ulcer activity of the isolated compound AC-1.

Anti gastric ulcer activity of the compound (AC-I) isolated from the leaves of *A. conyzoides* L. in ethanol induced gastric ulcer in albino rats was shown in Table - 1.

Table - 1 : Showing anti gastric ulcer activity of the compound (AC-I) isolated from the leaves of *Ageratum conyzoides* L. (randomly collected) in ethanol induced gastric ulcer in rats.

Group	Ulcer index (mean ± SEM)	% Ulcer protection
Control	Nil	--
Ethanol	30.2 ± 1.12	--
Ethanol + (AC-I) (100mg/kg)	21.5 ± 1.11*	28.80
Ethanol + (AC-I) (200mg/kg)	13.1± 1.10**	54.63
Ethanol + (AC-I) (300mg/kg)	11.1 ± 1.11**	63.24
Ethanol + Ranitidine(50mg/kg)	8.8 ± 1.01**	70.86

Results were in mean ± SEM, Each group had eight rats, *p<0.05, ** p<0.001

Ethanol produced massive gastric ulcers in all rats. Ulcers were mostly superficial. There was bleeding in the stomach which was associated with adhesion and dilatation. Ulcer index came 30.2 ± 1.12. AC-I reduced ulcer index in dose dependent manner. Maximum anti gastric ulcer activity was noted with the dose of 300 mg/kg of AC-I. Ulcer index came down to 11.1 ± 1.11 with ulcer protection 63.24% . This was comparable to that of ranitidine (50 mg/kg) Ulcer index in this group was 8.8 ± 1.01 with ulcer protection 70.86% .

Anti duodenal ulcer activity of the isolated compound AC-1.

The result was given in Table – 2.

Results showed that cyateamine produced massive duodenal ulcers in all rats. Ulcers were mostly superficial. There was bleeding in the duodenum which was associated with adhesion and dilatation. Ulcer index came 28.2 ± 1.11. AC-I reduced ulcer index in dose dependent manner. Maximum anti duodenal ulcer activity was noted with the dose of 300 mg/kg of AC-I. Ulcer index came down to 11.1 ± 1.02 with ulcer protection 60.64% . This was comparable to that of ranitidine (50 mg/kg) group where ulcer index came 8.5 ± 1.13 with ulcer protection 69.86% .

Table - 2 : Showing anti peptic ulcer activity of the compound (AC-I) isolated from the leaves of *Ageratum conyzoides* L. (randomly collected) in cysteamine induced duodenal ulcers in rats.

Group	Ulcer index (mean ± SEM)	% Ulcer protection
Control	Nil	--
Cysteamine	28.2 ± 1.11	--
Cysteamine + (AC-I) (100mg/kg)	21.5 ± 1.02*	23.75
Cysteamine + (AC-I) (200mg/kg)	13.1± 1.01**	53.54
Cysteamine + (AC-I) (300mg/kg)	11.1 ± 1.02**	60.64
Cysteamine + Ranitidine(50mg/kg)	8.5 ± 1.13**	69.86

Results were in mean ± SEM, Each group had eight rats, *p<0.05, ** p<0.001

Seasonal effect in yield of AC-I

Seasonal effect in yield of AC-I isolated from the leaves of *A. conyzoides* L. was shown in Table – 3.

Table - 3: Seasonal variations in the yield of the isolated compound (AC-I) from the leaves of *Ageratum conyzoides* L.

Season	Yield of the compound (AC-I) (mg/100g of *Ageratum conyzoides* L. leave powder)
January – February	1.5 ± 0.01
March – April	3.2 ± 0.03
May – June	5.4 ± 0.05
July – August	10.2 ± 0.13**
September – October	4.9 ± 0.04
November - December	2.6 ± 0.02

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

Table showed that leaves of *A. conyzoides* L. during the months of July and August yielded AC-I in maximum amount. The value was 10.2 ± 0.13 mg/100g of *A. conyzoides* L. leave powder which was statistically significant up to the level of p<0.001 when compared to other values of

yield during different seasons.

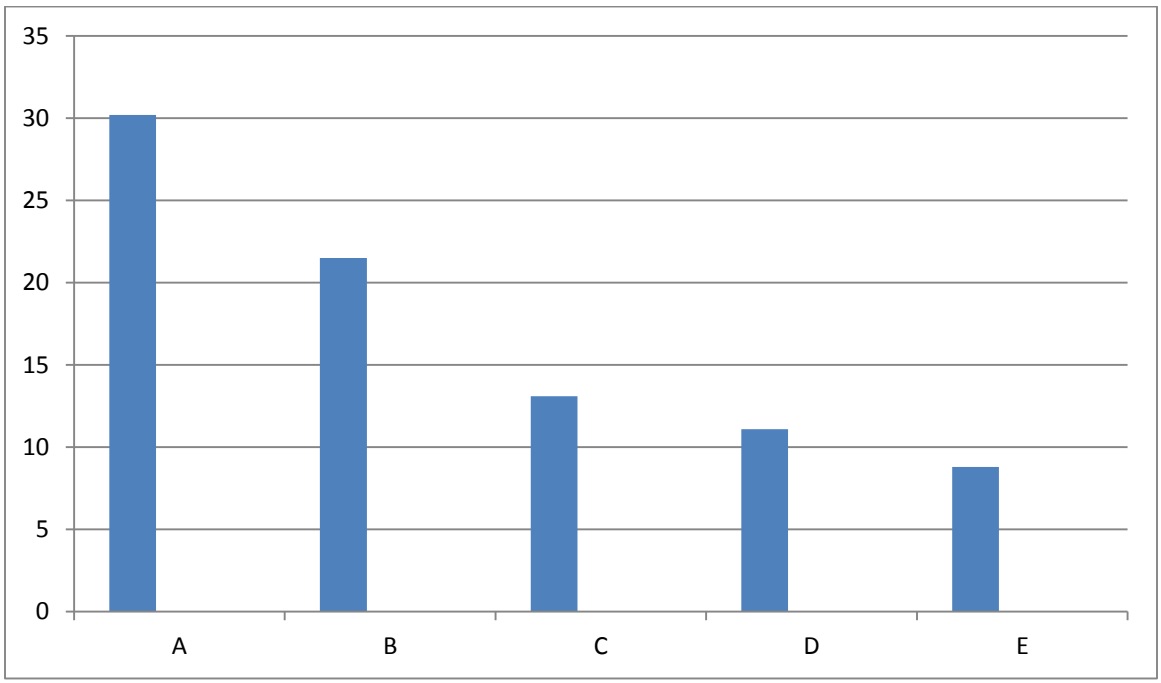
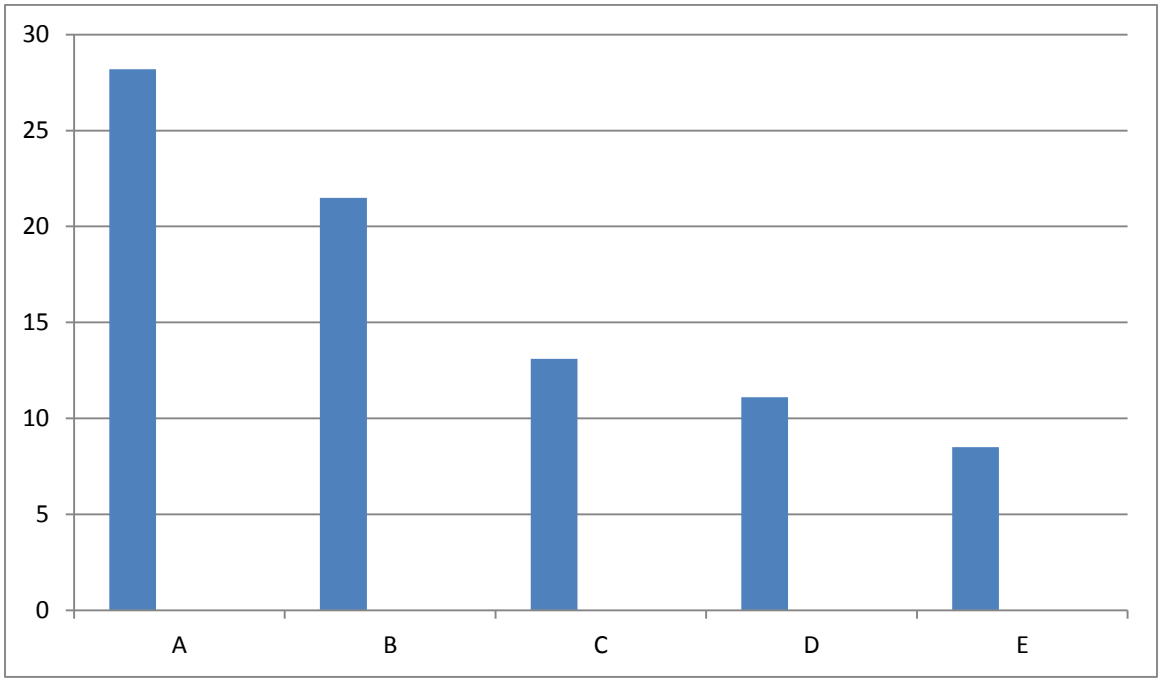


Fig 2 : : Effect of (AC-I) isolated from leaves of *A. conyzoides* L. (randomly collected) on ulcer index during ethanol induced gastric ulcer in rats.

Quincke [23] was probably the first to use the term 'Peptic ulcer'. Because of its frequency and worldwide distribution, peptic ulcer continues to be a subject of numerous investigations, both experimental and clinico pathological. In this respect peptic ulcer occupies a place secondary to carcinoma in the field of gastroenterology.

There are medicines to treat peptic ulcer [24]. These medicines, no doubt, have brought about remarkable changes in peptic ulcer therapy, but the efficacy of these drugs is still debatable. Reports on clinical evaluation of these drugs show that there are incidences of relapses and adverse effects and danger of drug interactions during ulcer therapy [25].

Hence, search for an ideal anti – ulcer drug continues and has also been extended to medicinal plants / herbs in search for new and novel molecules, which afford better protection and decrease the incidence of relapse.



Ulcer index A : Cysteamine B: Cysteamine + AC-I (100mg/kg) C : Cysteamine + AC-I (200mg/kg) D : Cysteamine + AC-I (300mg/kg) E : Cysteamine + Ranitidine (50mg/kg)

Fig 3 : Effect of (AC-I) isolated from leaves of *A. conyzoides* L. (randomly collected) on cysteamine induced duodenal ulcer in rats.

Numerous medicinal plants showed anti gastric ulcer activity. Sanyal *et al.* [26] found that vegetable banana is efficacious not only for experimentally induced gastric ulcers in albino rats, guinea pigs etc. but also for human being suffering from gastric ulcers. Akah *et al.*[27] demonstrated anti gastric ulcer activity of the herb *Cassampelos mucronata*. Likewise Shetty *et al.* [28] , Sairam *et al.* [29] , Maity *et al.* [30, 31] and Dharmani and Palit [32] confirmed anti gastric ulcer activities of *Ginkgo biloba*, *Convolvulus pluricaulis Chois*, tea root extract and *Vernonia lasiopus* respectively. We also reported anti gastric ulcer activities of few medicinal plants in different experimental ulcer models [33 – 39].

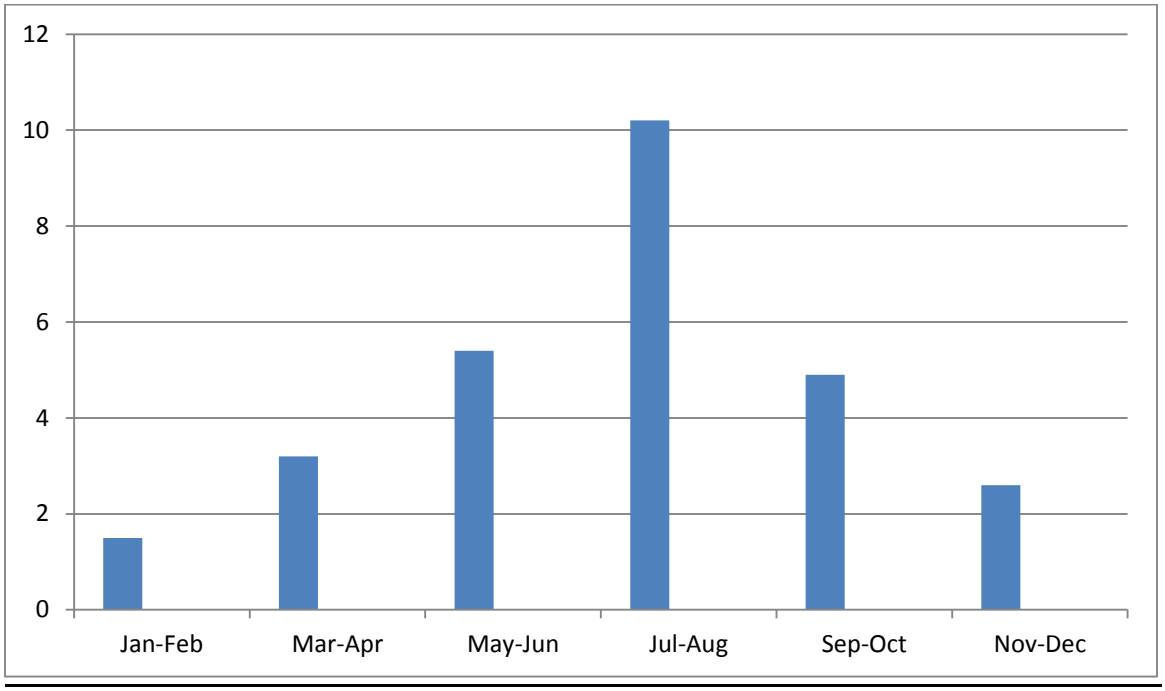


Fig 4 : Seasonal variation in the yield of (AC-I) isolated from *A. conyzoides* L. leaves. The amount was in terms of mg/100g of *A. conyzoides* L. leave powder.

A.Conyzoides L., a plant of Eastern Himalaya, was known to possess gastro protective effect [10]. Recently we have noted anti ulcerogenic effect of *A. conyzoides* L. leaves in ethanol induced gastric ulcer as well as cysteamine induced duodenal ulcer models in albino rats (unpublished observation). We were interested to isolate the active compound responsible for anti peptic ulcer activity and by solvent extraction, acid hydrolysis, chromatography followed by crystallization, we have isolated a compound (AC-I) from the leaves of *A. conyzoides* L. It was found out that the compound could exert anti peptic ulcer activity in rats as induced by the above said drugs.

Medicinal values of plants vary with season [40-45]. We also reported seasonal variation of medicinal values of several plants [46-51]. We, therefore, intended to note the seasonal variation, if any, on concentration of the active compound (AC-I) in leaves of *A. conyzoides* L. Results showed that leaves *A. conyzoides* L. during the months of July and August yielded maximum amount of (AC-I).

We are now interested to characterize the compound (AC-I) isolated from *A. conyzoides* L. leaves and to see the underlying mechanism of anti peptic ulcer activity of it. Experiments are in progress in this direction.

Conclusion

A compound (AC-I) was isolated from the leaves of *A. conyzoides* L.. The compound had anti peptic ulcer activity against ethanol induced gastric ulceration and cysteamine induced duodenal ulcerations in rats. The compound was accumulated in maximum quantity in the leaves of *A. conyzoides* L. during the months of July and August.

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Authors Column

Prof. (Dr.) Prasanta Kumar Mitra is a very senior medical teacher and researcher. He has completed thirty seven years in medical teaching and about forty years in research. His research area is 'Medicinal plants of India'. He has four Ph.D.s to his credit and published one hundred nine research papers in national and international journals. Fifteen students did their Ph.D. work under his guidance. He was co-supervisor of the research projects of five MD students.
Prof. Mitra was Editor-in-Chief of the European Journal of Biotechnology and Biosciences. He is now Editor, Associate Editor and Member of Editorial Board of many national and international research journals.
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