EFFECT OF COLCHICINE ON THE ACTIVE PRINCIPALS OF TRICHOSANTHES ANGUINA L.

Madan Mohan Gorai* and Shanker Kumar Pandey**
*Research Scholar, PG Dept. of Botany, Sido Kanhu Murmu University,
Jharkhand-814101. India,
**Assistant Professor, Department of Botany, Jamtara College, Jamtara,
Jharkhand- 815351. India,

Abstract

Trichosanthes anguina is an underutilized plant, the fruit of which is mainly consumed as a vegetable by rural dwellers most especially in western part of Africa. It is an annual climber belonging to the family Cucurbitaceae. It is commonly called snake gourd, viper gourd, snake tomato or long tomato . The endocarp pulp when fully mature is sweet tasting, aromatic and deep red which does not go sour as quickly as the paste of S. lycopersicum, this account for the reason why it is employed as an alternative to the Solanaceous tomato. The plant is richly constituted with a series of secondary metabolites like flavonoids, carotenoids, alkaloids, phenolic acids which makes the plant pharmacologically and therapeutically active. The preliminary phytochemical screening of leaf, stem, fruit wall and seed extracts of different solvents viz., aqueous, benzene, chloroform, methanol and petroleum ether of *T. anguina* have been carried out for the identification and analysis of biologically active phytochemicals by using standard procedures. The amount of total alkaloid in healthy leaves was found 0.84 mg/g and 0.67 mg/g in treated one. The percentage decrease in total sugar, biomass and alkaloids was found to the tune of 21.3%, 25% and 28.9% respectively under treatment. The biomass was reduced upto 16.6% and the total alkaloid was decreased upto 20.3%. The growth of a plant or its particular organ is an important endogenous process which results in variation in the concentration of plant metabolites. The percent decrease in treated leaves might be due to the alteration of organic and inorganic components by the treatment of colchicines.

Key Words: Effect, Colchicine, Active principals, Photochemical Screening, T. anguina.

Introduction: Snake gourd (*Tricosanthes anguina)* is a well known plant, the fruit of which is mainly consumed as a vegetable. It is an annual climber belonging to the family Cucurbitaceae. It is commonly called as snake gourd, viper gourd, snake tomato or long tomato. The fruit is usually consumed as a vegetable due to it is good nutritional value. The fruit is a good source of Vitamin A, Vitamin B and Vitamin C. It improves the appetite and acts as a tonic and stomachic and cures biliousness. The wild bitter forms are used in many ayurvedic preparations. The fruits of cultivated forms also have medicinal uses and are useful for people suffering from blood pressure, heart diseases, rheumatism and psoriasis. It has a prominent place in alternative systems of medicine like Ayurveda and Siddha due to its various pharmacological activities like antidiabetic, hepatoprotective, cytotoxic, anti-inflammatory, larvicidal effects. The fruits become inedible upon ripening, they taste bitter and developed hardened fibro vascular bundles. Fruits of the wild forms are very bitter and non edible. They are used in traditional medicine as a purgative. The pulp of ripe fruits is used as a substitute of tomato paste. There are limited reports on the chemical composition and nutritive value of snake gourd seed. Information on its functional properties is lacking. Essential in determining potential uses for snake gourd seed flour, is the identification and improvement of its functional properties.

Review of Literature: Carbohydrates or sugars are the chief photosynthetic products of the plants. As early as in 1972 Vidyashekharan and Kandaswamy showed severe reduction in both starch and sugar contents of infected tissue of *Phaseolus aureus*. All living plant cells requires an abundance of water and an adequate amount of organic and inorganic nutrients in order to live and to carry out their physiological function. On the other hand, nearly all organic nutrients of plants are produced in the leaf cells, following photosynthesis and are located downward and distributed to all the living plant cells. Photosynthesis is reduced or stopped and few or no nutrients are available to move to the roots, which in turn become starved and treated and may die or resulting in the loss of biomass (Singh and Agarwal 1973; Prasad *et al*., 1995). The term Alkaloid has been proposed by pharmacists as basic nitrogen containing compounds from the plants and

other natural resources in which at least one nitrogen atom forms a part of the cyclic system. The further work carried out by Mishra & Kumar (2000), Vander *et al.*(2004), Shukla,(2005) suggested that some proteins, amino acids, nicotinic acid and anthranilic acid may act as precursor for the biosynthesis of alkaloids.

Materials and Methods:

Estimation of Total Sugar (Dubois *et al.,* 1956**):** To 200 mg of each sample, 25 ml hot 80% ethanol was added and stirred thoroughly. After 5 minutes, it was centrifuged and supernatant was decanted. Extraction was repeated by adding 30ml. of hot 80% ethanol. The extracts were mixed and ethanol was evaporated to dryness in an evaporating disc. The residue left at the bottom was dissolved in 5ml. of glass-distilled water and centrifuged again. To 2ml. of the supernatant 0.14 ml. of 80% aqueous phenol was added and then with fast flowing pipette 5 ml. of concentrated H_2SO_4 was mixed and shaken gently. The tubes were allowed to stand for ten minutes and then placed in a water bath at 25°C for 10-20 minutes. The optical density was recorded against the blank at 490nm. The percent amount of the total sugar was determined by comparing the readings with that of standard curve of glucose.

Estimation of Reducing Sugar (Paech and Tracy, 1955): 300mg. of each sample was finely crushed and blended with 1.5ml. of glass distilled water in a glass homogenizer. To this 0.2ml. of 0.3N Barium hydroxide solution was added followed by 0.2ml. of 5% $ZnSO_4$ solution and was thoroughly mixed. The total volume was centrifuged. To one ml. of the supernatant, one ml. of alkaline copper reagent (Prepared by dissolving 4 gm. $CuSO_4$, $5H_2O$; 24 gm. Anhydrous Na_2CO_3 and 16 gm. Na-K tartarate in 1 liter of water) was added. To this one ml. of Arsenomolybdate reagent was added and left to stand for few minutes till the effervescence ceased. The blue colour was diluted with glass distilled water up to 10ml. and was read at 510 nm. The optical density was compared with that of the standard fructose solution.

Estimation of Non-reducing Sugar: The amount of non-reducing sugar was calculated by subtracting the value of reducing sugar from total sugar. It is because the total sugar constitutes equally reducing and non-reducing sugars.

Loss in Biomass: The test plant *Trichosanthes anguina* was treated with 0.5% aqueous solution of colchicine. The plant after artificial treatment was left for 15 days for the development in the experimental plot. The healthy and treated leaves were detached separately. Weight was taken after proper washing and kept into oven at 60-80^oC for 48 hours. The biomass was calculated by the following method: -

For healthy leaves: Wt. of healthy fresh leaves – Wt. of oven dried leaves.

For treated leaves: Wt. of colchicines treated leaves – Wt. of oven dried leaves.

Changes in Total Alkaloid (Mukerjee, 1953**)**

20gm of powdered sample was soaked in 28% ammonium hydroxide solution and little dried-up. Subsequently the sample was soxhlated with a mixture of chloroform & ethanol (3: 1 v/v) for 8 hours. 100ml of solvent was extracted and vigorously shaken with 25ml of N/2 H_2SO_4 and the acid extract was collected. The process was repeated thrice for the complete extraction of alkaloids. The combined acid extract was made alkaline with dilute NH_4OH. The alkaloids were extracted from alkaline extract with 20 and 15ml of chloroform. The chloroform extract was distilled on water bath until only few ml was left. The left solvent was completely dried up and left residue was weighed to calculate the total crude alkaloids.

Results and Discussion: Phytochemicals are non-nutritive plant chemicals that are more complex and specific and exert their action by resembling endogenous metabolites. These natural constituents can be derived from any part of the plant such as leaves, flowers, roots, fruits, seeds and bark, etc. As the synthetic drugs are associated with side effects, plant derived antimicrobials have been employed now a day as they possess the therapeutic value to cure infectious diseases. In view of the importance of these phytochemicals, we have undertaken the present investigation to screen the bioactive compounds in *T. anguina*.

Phytochemical analysis of various solvent extracts of leaf of *T. anguina* revealed the presence of alkaloids. The leaf extracts of aqueous, benzene, chloroform and methanol showed rich in alkaloids, whereas in petroleum ether extracts less amount was present. The leaf extract of colchicines treated plant showed a general pattern of decrease in Sugar, Biomass and total alkaloid. The results are depicted in table-1.

Table-1: Showing Changes in Sugar contents, Biomass and Alkaloids of *Trichosanthes anguina* by colchicine treatment.

Experiment / Contents	Control	Colchicine treated plant	% Loss
Total sugar (TS)mg/g	6.1	4.8	21.3
Reducing sugar (RS)mg/g	0.8	0.6	25
Non reducing sugar (NRS)mg/g	5.2	3.7	28.9
Biomass (B)mg/g	9.2	8.6	16.6
Total Alkaloid (TA)	0.84	0.67	20.3

The data in table-1 reveals that there was a general pattern of decrease of all kinds of sugars. 6.1mg/g total sugar, 0.8mg.g reducing sugar and 5.2 mg/g non-reducing sugar in control leaves whereas these were only 4.8mg/g, 0.6mg.g and 3.7mg/g were estimated from colchicines treated leaf samples respectively. The biomass of healthy and treated leaves was recorded 9.2g/50g, and 8.6g/50g respectively. The amount of total alkaloid in healthy leaves was found 0.84 mg/g and 0.67 mg/g in treated one. The percentage decrease in total sugar, biomass and alkaloids was found to the tune of 21.3%, 25% and 28.9% respectively under treatment. The biomass was reduced upto 16.6% and the total alkaloid was decreased upto 20.3%. The growth of a plant or its particular organ is an important endogenous process which results in variation in the concentration of plant metabolites. The percent decrease in treated leaves might be due to the alteration of organic and inorganic components by the treatment of colchicine

Conclusion: The preliminary phytochemical screening of various parts of *T. anguina* indicated that the plant possesses medicinally important phytochemicals hence can be employed as herbal medicine for primary health care. A general pattern of loss in the total sugar, reducing sugar, non reducing sugar, biomass and alkaloid was recorded in the colchicines treated plant after the phytochemical estimation. This may be due to the alteration of metabolic pathway due to the treatment of colchicines in the normal plant.

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