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Evaluation of anthelmintic properties of *Trichosanthes diocia*

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Abstract
One of the most nutrient-dense cucurbit vegetables is the pointed gourd (*Trichosanthes dioica* Roxb.), which enjoys a prized spot in the Indian market during the warm and wet months. Fruits are easily digested and diuretic in nature, and they have some medical benefits due to their high protein and vitamin A content. Many reports are also available on their usefulness in decreasing blood sugar and serum triglycerides. The goal of the current study is to examine and assess the anthelmintic efficacy of a methanolic extract of *Trichosanthes diocia* leaf tissue. Different extracts and powdered material were subjected to chemical testing, which revealed the presence of proteins, amino acids, phytosterol and steroid, alkaloids, and flavonoids. Using albendazole as a reference, the various extracts were examined for their anthelmintic action at a concentration of 1,2,5, and it was discovered that methanol extracts had the greatest impact.

Keywords: *Trichosanthes diocia*, anthelmintic activity, biochemical test, medicinal plants

Introduction
Medicinal plants are of great value in the field of treatment and cure of disease. Over the years, scientific research has expanded our knowledge of the chemical effect and composition of the active constituents, which determine the medicinal properties of plant [1]. In modern era, herbals are seen as potential medicine for a variety of disease often viewed to supersede the pharmacological efficacy of allopathic drugs. There has been a striking increase in use of herbal in both developing and the developed countries due to their natural origin and minimal or no side effect. World over, the pharmaceutical companies and research organization are focusing on the vast untapped potential of herbal as potential drug.
Pointed gourd (*Trichosanthes dioica* Roxb.) is one of the most nutritive cucurbit vegetables, and it holds a coveted position in the Indian market during the summer and rainy seasons. It is a perennial crop, highly accepted due to its availability for eight months in a year (February–September). Being very rich in protein and vitamin A, it has certain medicinal properties, and many reports are available regarding its role in lowering of blood sugar and serum triglycerides [2]. Fruits are easily digestible and diuretic in nature. They are also known to have antiulcer effects [3]. It grows as a vegetable all over India. It is prescribed to improve appetite and digestion [4]. The decoction of *Trichosanthes dioica* is useful as a valuable alternative tonic, and as a febrifuge, which is given for boils and other skin diseases [5].
The present study has been under taken to investigate and evaluate the anthelmintic activity of methanolic extract of leaf of *Trichosanthes diocia*.

Methedology
In this course of study Pharmacogenetic Study, Phytochemical Investigation, Chromatographic Separation, Pharmacological evaluation of the extracts of *Trichosanthes dioica* were performed.

Collection and authentication Plant
The plant specimens for the proposed study were collected from Nuapada and authenticated with relevant local flora.

1. Phytochemical Investigation of The Leaves of *Trichosanthes dioica* Roxb
1.1 Drying and Pulverization
The collected plant material (Leaf) was shade dried at room temperature, then they are pulverized in mixer grinder to coarsely powdered drug and passed through mesh size 40 sieve.

1.2 Preparation of Extracts by Solvent Extraction
The leaves were dried in shade and powdered to get a coarse powder. About 800gm of dry coarse powder was extracted with methanol by continuous hot percolation using Soxhlet apparatus. The extraction was continued for 8 hours. The methanolic extract was filtered and concentrated to a dry mass by using vacuum distillation.

2. Qualitative Chemical Evaluation

2.1 Detection of Carbohydrates

Small quantities of different extracts were dissolved in distilled water separately and filtered. The filtrates were taken for various tests to detect the presence of carbohydrates. Tests like Molisch's Test,) Fehling's Test, Benedict's Test, Barfoed's Test, test performed. Iodine test carried out for starch test.

2.2 Test for Gums and Mucilages

The extracts were treated with absolute alcohol stirred and filtered. The filtrate was dried and examined for its swelling properties. None of the extract answered for the presence of gums and mucilages.

2.3 Test for Proteins and Amino Acids

Small quantities of different extracts were dissolved in few ml of distilled water and subjected to Ninhydrin, Biuret, Millon, Xanthoproteic test, test with tannic acid and heavy metals.

2.4 Test for Fixed Oils and Fats

For detection of fats and oils Spot Test, Saponification Test performed.

2.5 Test for Alkaloids

Small amount of solvent free various extracts were separately stirred with a few ml of dilute hydrochloric acid and filtered. The filtrates were tested with various alkaloidal reagents such as Mayer's, Dragendroff's, Wagner's and Hager's reagent.

2.6 Test for Glycosides

A small amount of the different extracts were hydrolyzed with hydrochloric acid for one hour on a water bath and hydrolysate was subjected to Legal's, Baljet's, Borntrager's, Keller-Killiani's tests and for the presence of Cyanogenetic glycosides.

2.7 Test for Phytosterols

All the extracts were refluxed with 0.5N alcoholic potassium hydroxide until the saponification was complete. The saponification mixture was diluted with distilled water and extracted with petroleum ether. The ethereal extract was evaporated and unsaponification matter was subjected to Liebermann's, Liebermann – Burchard's and Salkowski's test.

2.8 Test for Flavonoids

The different extracts were separately dissolved in ethanol and then hydrolyzed with 10%sulphuric acid, cooled it extracted with diethyl ether subjected to the tests like Ferric Chloride Test, sodium hydroxide test, Fluorescence Test, Reaction with Ammonia.

2.9 Test for Tannins and Phenolic Compounds

The extracts were dissolved in distilled water and filtered. The filtrates were treated with various reagents. Few ml. of the filtrates was treated with 5% ferric chloride solution. A bluish black color was not observed in any of the extracts indicating the absence of phenolic compounds. Few ml of the filtrates was treated with copper sulphate solution. Precipitates were not produced in any of the extracts indicating the absence of tannins. Few ml of the filtrates was treated with lead acetate solution. White precipitates were not produced in any of the extracts indicating the absence of tannins. Few ml of the filtrates was treated with strong potassium dichromate solution. Precipitate bluish black was produced in all of the extracts showed the presence of tannins. Few ml of the filtrates was treated with potassium ferricyanide followed by ammonia. A deep red color was not observed in any of the extracts indicting the absence of phenolic compounds.

2.10 Test for Saponins

The extracts were diluted with 20ml of distilled water and agitated in a graduated cylinder for 15 minutes. One-centimeter layer of foam was formed in hydroalcoholic extract indicating the presence of saponins.

2.11 Test for Volatile Oil

50gm. of coarsely powdered material was taken in a Cocking- Middleton's apparatus and distilled for 6 hours. No volatile oil was separated out during this period indicating the absence of volatile oil.

3. Chromatographic Seperation

Thin layer chromatography separation was performed with Methanol and Petroleum ether extract [6,7,8]

4. Pharmacolgical Screening

4.1 Anthelmintic Activity of Leaf

The suspensions of various extracts were prepared in Tween 80 (1%) to obtain 1, 2.5 and 5% concentrations. Solutions of similar concentrations of the reference standard drug albendazole were also prepared in distilled

water. Two ml of each concentration of various extracts and standard drug albendazole were diluted to 10 ml separately with normal saline and poured in Petri dishes. The Petri dishes were divided into 5 groups. Group I consist of normal saline, Group II consists of standard drug albendazole and Group III to V consists of three extracts. Each group consists of 1, 2.5 and 5% concentrations and to each concentration equal size of adult earthworms of 6 numbered were released into Petri dishes. Times were recorded at the time of releasing the earthworms to each concentration. Then the time was taken in minutes for the paralysis and death of the earthworms. The anthelmintic activity was evaluated on adult Indian earthworm *Pheritima posthuma* due to its anatomical and physiological resemblance with the intestinal round worm parasites of human beings. Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when the worms lost their mobility followed by fading away of their body color. Petroleum ether, Methanol and extracts of the leaf were screened for anthelmintic activity.

Results

Results of various tests given in following tables.

Table 1: Percentage of Extracts

Sl. No.	Extracts	% Yield
1.	Methanol	12.07%
2.	Petroleum Ether	2.50%

Table 2: Report of Chemical Test on Powder Materials and Various Extracts

Plant constituents test/reagent used	Powdered Drug	Hydro alcoholic extract	Petroleum ether extract	Ethayl acetate extract	Chloform extract
Test for Carbohydrates					
Molisch's Test	-	+	+	-	-
Fehling's Test	-	-	-	-	-
Benedict's Test	-	-	-	-	-
Barfoed's Test	-	-	-	-	-
Test for Starch	-	-	-	-	-
Test for Gums & Mucilage	-	-	-	-	-
Test for protins & amino acids					
Ninhydrin Test	+	-	+	-	-
Biuret Test	-	-	-	-	-
Millon's Test	-	-	+	+	+
Xanthoproteic Test	+	+	+	+	+
Tannic Acid (10% w/v)	+	+	+	+	+
With Heavy Metals	-	-	-	-	-
Spot Test	-	-	-	-	-
Saponification Test	-	-	-	-	-
Test for Phytosterols					
Liebermann-Burchard's Test	+	+	+	+	+
Salkowski's Test	+	+	+	+	+
Test for alkaloids					
Dragendroff's Test	+	+	-	-	-
Mayer's Test	+	–	-	+	-
Wagner's Test	+	+	+	+	+
Hager's Test	+	+	+	+	+
Test for Glycosides					
Legal's Test	-	-	-	-	-
Baljet's Test	-	-	-	-	-
Borntrager's Test.	-	-	-	-	-
Keller-Killiani's Test	+	-	+	-	-
Test for Fixed Oils & Fats					

Table 3: Rf value of different extract

Solvent system	Methanol Extract	Pet Ether Extract
Ethylacetate: Methanol: Water 100:13.5:10	1 spot, Rf:0.8	No spot
Benzene:Pridine: Formic acid 72:18:10	1 spot, Rf:0.8	1 spot, Rf:0.8
n-Butanol: Glacial acetic acid: water 50:10:40	1 spot, Rf:0.8	1 spot, Rf:0.8

Table 4: Anthelmintic Effect of The Leaves of Trichosanthes Dioica Roxb

Group	Concentration of extract in %	Time taken in minutes ± SEM	
		Paralysis	Death
Albendazole	1.0	30.66±0.57	42.00±1.57
	2.5	24.00±0.57	37.00±1.57
	5.0	17.33±1.15	28.66±0.91
Petroleum ether extract	1.0	N/A	N/A
	2.5	N/A	N/A
	5.0	N/A	N/A
Methanol	1.0	N/A	N/A
	2.5	N/A	N/A
	5.0	525.33±1.15	726±0.91

Control worms were alive up to 24 hrs. of observation.
N/A= No Activity shown within 24 hours.

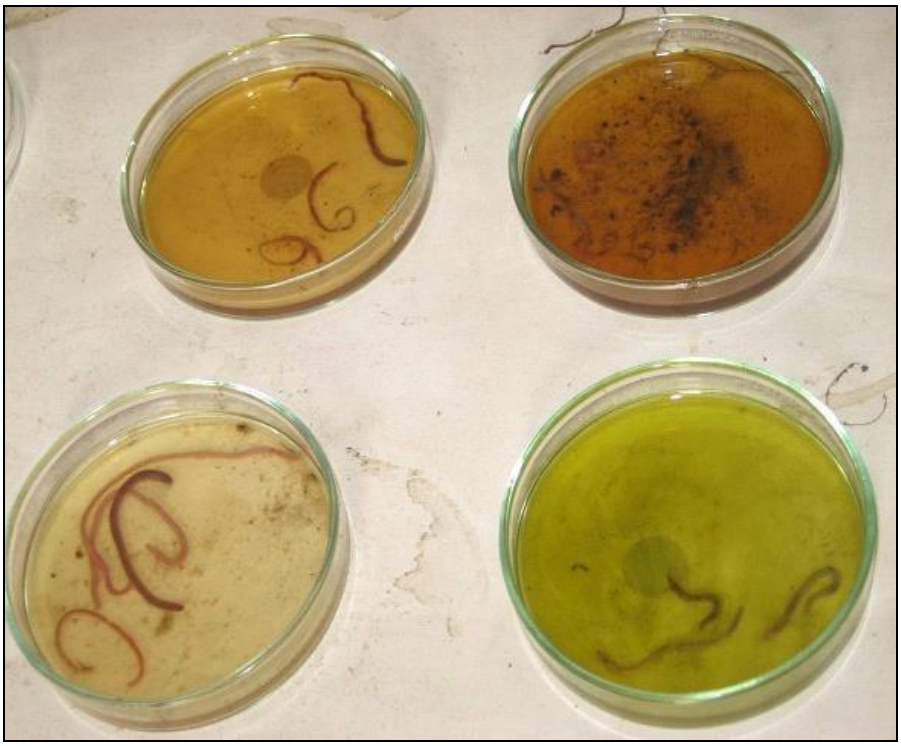

Fig 1: Anthelmintic Effect of The Leaves of Trichosanthes Dioica Roxb

Conclusion

Pointed gourd (Trichosanthes dioica Roxb.) is one of the most nutritive cucurbit vegetables, and it holds a coveted position in the Indian market during the summer and rainy seasons. It is a perennial crop, highly accepted due to its availability for eight months in a year (February–September). Being very rich in protein and vitamin A, it has certain medicinal properties, and many reports are available regarding its role in lowering of blood sugar and serum triglycerides. The physico-chemical characters of powdered drug of leaves of *Trichosanthes dioica* roxb such as total alcohol soluble extractive, water soluble extractive, ash value, acid insoluble ash, water-soluble ash, loss on drying, swelling index, and foreign matter are presented in table 8. The preliminary phytochemical investigation of the methanol and petroleum ether extracts of *Trichosanthes dioica* roxb showed the presence of phytosterols, flavonoids, terpenoid saponins, carbohydrates, tannins, glycosides alkaloids proteins organic acids. Thin layer chromatography (TLC) is particularly valuable for the preliminary separation and determination of plant constituents. The chromatographic profile may serve as a characteristic finger print for qualitative evaluation of leaf. Thin layer chromatography of the methanol and petroleum ether extracts was carried out using Ethyl acetate: Methanol: Water 100:13.5:10, Benzene: Pyridine: Formic acid 72: 18: 10 and n-Butanol: Glacial acetic acid: water 50:10:40 as mobile phase respectively and the Rf values were recorded. The visualizing employed was U.V chamber to effect visualization of the resolved spots. The leaf powders were subjected to successive extraction in Soxhlet apparatus by using hydroalcoholic solvents Than by using the separating funnel other extract in increasing of polarity like petroleum ether, and methanol extracted from the hydroalcoholic extract. Chemical tests on various extracts and powdered material showed the presence of proteins and amino acids, phytosterol & steroid, alkaloids, flavonoids. The different extracts at a conc. Of 1, 2.5, 5% were studied for their anthelmintic effect & found that methanol extracts were showing maximum effect amongst other extracts using albendazole as a standard.

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