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## Phytochemical and *in vitro* antioxidant activity of methanolic leaves extract of *Trichosanthes dioica* Roxb.

**Satyendra Deka, Rama Kanta Sharma, Mangala Lahkar**

### Abstract

Plants are an essential component of the universe. Human beings have used plants as medicine from the very beginning of time. *Trichosanthes dioica* is used as a vegetable. Traditionally it is used in India as purgative and as tonic, febrifuge, in treatment of jaundice, anasarca and ascites. This work has investigated its phytochemical properties as well as its antioxidant activity taking quercetin as a standard. Methanolic leaves extract of *Trichosanthes dioica* Roxb is found to contain different phytochemical constituents and significant antioxidant activities in a dose dependent manner. Thus, this traditional vegetable has potential pharmacological activities and proper investigation may give different therapeutic active agent which can used to treat different types of diseases.

**Keywords:** Traditional, leaves, *Trichosanthes dioica*, antioxidant.

### 1. Introduction

Traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their active therapeutic principles. The rich wealth of plant kingdom can represent a novel source of newer compounds with significant therapeutic activity. The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects and low cost.

*Trichosanthes dioica* Roxb. (Cucurbitaceae), called pointed gourd in English, Patol in Bengali and Patola in Sanskrit, is a dioecious climber found wild throughout the plains of North and North-East India from Punjab to Assam and Tripura states of India. It is also grown and commercially cultivated in India, Pakistan, Bangladesh and Sri Lanka for its fruits, a common culinary vegetable in the Indian subcontinent. In India, all parts of this plant have been traditionally used for various medicinal purposes. According to Ayurveda, the traditional system of Indian medicine, its root is used as a strong purgative. The root of *T. dioica* has been traditionally used in India as purgative and as tonic, febrifuge, in treatment of jaundice, anasarca and ascites<sup>[1-4]</sup>. However, reports on the experimental pharmacological studies on its root are comparatively scanty. In earlier studies, anthelmintic effects of leaf and root, antibacterial antimutagenic and antitumor activities of the root of *T. dioica* were reported<sup>[5-9]</sup>.

Many free radicals playing a vital role to produce oxidative stress. Free radicals such as superoxide (O<sub>2</sub><sup>-</sup>), hydroxyl (OH<sup>-</sup>), and peroxy (·OOH, ROO<sup>-</sup>) are the main culprit to generate the different pathological disorders such as aging cancer, coronary heart disease, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, diabetes, and inflammation. Several anti-inflammatory, anti-necrotic, neuroprotective and hepatoprotective drugs have recently been shown to have antioxidant or radical scavenging mechanisms. Due to its side effects, it is more suitable to use the herbal medicine as a remedy. Some natural antioxidants and compounds with radical scavenging activity have been identified over the last few years<sup>[10]</sup>. Our aim of work is to investigate phytochemical and antioxidant activity of methanolic leaves extract of *Trichosanthes dioica* Roxb (MLETD).

### 2. Methods and materials

#### 2.1 Plant material

##### a. Collection and Authentication of Sample

The plant specimen was collected from Nalbari District of Assam. The plant was collected in the month of March, 2012. It was authenticated with the standard Herbarium specimen in the Botany Department of Gauhati University, Assam. (Acct. no: 004328 on dated 27<sup>th</sup> April 2012)

### b. Preparation of extract

100 g of dry Leaves of plant *Trichosanthes dioica* Roxb. were taken in a soxhlet and methanol was added up to 2 siphons that is up to 500 ml. The temperature is set to 70 °C and the extraction was carried out up to 5 hours. Then the extract obtained is filtered and concentrated at 70 C. Dried extracts were kept in a refrigerator and used for further study. The % Yield of methanolic leaves extract of *Trichosanthes dioica* (MLETD) was 17.67% w/w.

## 2.2 Preliminary Phytochemical Test <sup>[11, 12]</sup>

### 2.2.1 Test for Tannin

**a. Ferric Chloride Test:** The test solution was treated ferric chloride solution, dark color appears which shows the presence of Tannin.

**b. Gelatin Test:** The test solution was treated with 1% Gelatin solution containing 10% NaCl, a white p.p.t forms which shows the presence of Tannin.

### 2.2.2 Test for Flavonoid

**a. Ferric Chloride Test:** Treat the test solution with ferric chloride solution, the intense green colour will show the presence of Flavonoids.

**b. Shinoda Test:** Treat the test solution with few fragments of Mg ribbon & conc. HCl, Pink Scarlet Crimson colour occasionally cream to blue colour shows the presence of Flavonoids.

### 2.2.3 Test for Alkaloid

**a. Mayers Test:** Treat the test solution with Mayers reagent, cream color appears which shows the presence of Alkaloid.

**b. Wagners Test:** Treat the test solution some acidic solution & Wagners reagent, brown ppt will show the presence of Alkaloid.

### 2.2.4 Test for Steroid:

**a. Salkowski Test:** Treat the test solution with few drops of Conc. H<sub>2</sub>SO<sub>4</sub>, shake, allowed to stand, lower layer turns Red, indicates the presence the Steroid.

### 2.2.5 Test for glycosides

#### a. Keller Killiani Test

Test solution was treated with few drops of glacial acetic acid and Ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides.

#### b. Bromine water test

Test solution was dissolved in bromine water and observed for the formation of yellow precipitate to show a positive result for the presence of glycosides

### 2.2.6 Test for carbohydrates

#### a. Benedict's test

Test solution was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate.

## 2.3 *In vitro* antioxidant activity <sup>[13, 14, 15, 16, 17]</sup>

In this study free radical scavenging activity of MLETD was determined by *in vitro* assay models such as DPPH free radical, reducing ability. Quercetin was used as reference standard.

### 2.3.1 DPPH radical scavenging activity

#### Principle

Antioxidants scavenged DPPH radical by through the donation of proton forming the reduced DPPH. After reduction, the colour is changing from purple to yellow, which can be quantified by its decrease of absorbance at wavelength 517 nm. Radical scavenging activity increased with increasing percentage of the free radical inhibition. The degree of discoloration indicates the free radical scavenging potentials of the sample/antioxidant by their hydrogen donating ability. The electrons become paired off and solution loses colour stoichiometrically depending on the number of electrons taken up.

#### Procedure

DPPH radical scavenging activity was measured using the method of Kiranmai *et al.*; with some modifications. 2 ml of reaction mixture containing 1 ml of DPPH (100 µM in methanol) 1 ml of test solution, at various concentrations of the extract fractions was incubated at 37 °C for 30 min absorbance of the resulting solution was measured at 517 nm using Beckman model DU-40 spectrophotometer. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with extract) using the following equation;

Percentage inhibition =  $(1 - \text{absorbance of test} / \text{absorbance of control}) \times 100$

### 2.3.2 Reducing Ability

#### Principle

The reducing power increased with increasing amount of the extract. When potassium ferricyanide react with ferric chloride in the present of antioxidant, potassium ferrocyanide and ferrous chloride are found as a product. Presence of reducers causes the conversion of the Fe<sup>3+</sup>/ferricyanide complex used in this method to the ferrous form.

#### Procedure

1 ml of different concentrations (25 to 900 µg/ml) of the extract fractions were mixed with potassium ferricyanide (2.5 ml, 1%) 2.5 ml of phosphate buffer (pH 6.6). The mixture was incubated at 50 °C for 20 min. 2.5 ml TCA (10%) was added to it and centrifuged at 3000 rpm for 10 min. 2.5 ml of supernatant was taken and 2.5 ml water and 0.5 ml FeCl<sub>3</sub> (0.1%) were added to it. The absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated higher reducing power.

## 3. Result and conclusion

*Trichosanthes dioica* Roxb. is an easily available plant. The fruit being an integral part of an average Indian diet consumed as a vegetable. The plant belongs to family Cucurbitaceae. In the present study, we have investigated the phytochemical constituents and antioxidant activities of methanolic leaves extract of *Trichosanthes dioica* Roxb. In the phytochemical investigation, we have found that, the extract contains carbohydrates, alkaloids, glycosides, flavonoids, steroids and tannins as constituents. These results expose that the chemical

constituents, which may be responsible for the many pharmacological actions. From different research work it was found that many herbal plants showing antioxidant activity. Different phytochemical constituents were found in the extract. The result of phytochemical study is listed in table; 1. The extract was checked for its antioxidant activity in two model systems i.e. in DPPH method and reductive ability. Methanolic leaves extract of *Trichosanthes dioica* Roxb. showed *in vitro* antioxidant activity in Dose dependant manner.

**Table 1:** Phytochemical test result of the sample

Sl. No.	Constituents	Results
1	Tannin	+
2	Alkaloids	+
3	Carbohydrate	+
4	Flavonoids	+
5	Glycosides	+
6	Steroid	+

“+” means presence and “-” means absent.

**Table 2:** Reducing ability of MLETD With respect to standard Quercetin at 700 nm

Sl. No	Concentration (µg/ml)	Absorbance at 700 nm	
		<i>Trichosanthes dioica</i>	Quercetin
1	400	0.736	2.421
2	600	1.069	2.548
3	1000	1.562	2.855
4	2000	2.078	2.917

**Table 3:** DPPH Radical Scavenging Activity of MLETD

Sl. No.	Concentration (µg/ml)	Absorbance at 517 nm	
		MLETD	Quercetin
1	22.08	1.861	2.968
2	44.16	1.312	2.392
3	55.20	1.009	1.911
4	77.28	0.565	1.252
5	110.40	0.373	0.965

**Table 4:** Percentage inhibition of MLETD

Sl. No	Concentration (µg/ml)	Percentage inhibition of MLETD
1	22.08	37.21
2	44.16	45.15
3	55.20	47.20
4	77.28	54.87
5	110.40	61.34

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