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Phytochemical evaluation and antimicrobial properties of *Trichosanthes dioica* root extract

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Abstract

Trichosanthes dioica, belongs to family Cucurbitaceae is a perennial herb and a common vegetable referred to as 'pointed gourd'. It was reported to have several pharmacological properties, however, critical search of literature reviews did not yield any articles that evaluated the phyto-constituents of the aqueous root extract and evaluated its biological properties, therefore, the present investigation was undertaken.

Qualitative analysis of the aqueous extract revealed presence of flavonoids, alkaloids, reducing sugars. The presence of alkaloids and flavonoids were more when compared to other compounds. The UV-VIS profile of root extract revealed peaks at 520nm, 512nm, 505nm, 496nm, 487nm, 483nm, 444nm, 426nm and 222.5nm with the absorption 0.071, 0.083, 0.077, 0.087, 0.090, 0.161, 0.177, 0.268 and 3.996. Further analysis by GC-MS revealed presence of 11 prevailing compounds, such as eicosane 2-methyl (18.16), octadecane (3.68), methoxy acetic acid (0.71), 2 methylhexacosane (16.18), heptacosane (5.85), octacosane (2.53). Antibacterial activity of root extract (water) against one Gram negative bacteria *Proteus mirabilis* and one Gram positive bacteria *Bacillus subtilis* showed a clear zone of inhibition and the zone of inhibition of Gram positive bacteria was slightly greater (13.7 mm) than that of the Gram negative bacteria (12.1mm); against streptomycin, a broad spectrum antibiotic as control which was statistically significant.

Trichosanthes dioica may be considered as an important plant with antimicrobial property due to presence of various phyto-constituents. We would encourage others to confirm and refute our findings.

Keywords: *Trichosanthes dioica*, GC-MS, antimicrobial, phytochemical

1. Introduction

Trichosanthes, a genus of family Cucurbitaceae is a perennial herb distributed in tropical Asia and is a common vegetable. It belongs to: Kingdom: Plantae, Division: Magnoliophyta, Class: Magnoliopsida, Order: Cucurbitales, Genus: *Trichosanthes*, Species: *T. dioica*.

'Pointed gourd' (*Trichosanthes dioica*) is known by the name of parwal, palwal, parmala, patol, potala in different parts of India and Bangladesh and is a prime vegetables of these regions. The fruits and leaves are the edible parts of the plant which are cooked in various ways either alone or in combination with other vegetables. Juice of leaves of *T. dioica* is used as tonic, febrifuge and in treating enlarged liver and spleen. In 'Charaka Samhitha' the leaves and fruits are used for treating alcoholism and jaundice. The leaves of the plants are also used in edema, alopecia and also reported to have antipyretic, diuretic, cardio-tonic and laxative properties. Numerous pharmacological activities were reported by different scientific investigators. Rai *et al.* (2008) reported the glycemic attributes of an aqueous extract of *Trichosanthes dioica* leaves in normal as well as various diabetic models [1]. It has been reported by others that the ethanolic extracts of leaf and fruit of *T. dioica* have free radical scavenging activity [2]. The pastes of leaves are applied by different tribes and communities to relieve from headache and also as a cure for the bald patches of alopecia. Kavitha and Premalakshmi (2012) reported the synergetic effect of *Trichosanthes dioica* and *Clitoria ternatea* leaf extract on the streptozotocin induced diabetic rats [3]. The methanolic extract of *T. dioica* fruits was also reported to have wound healing potential [4]. Some workers also reported that the fruit extracts has cholesterol lowering activity further, scientific literature reveals that alcoholic extract of whole fruit of *T. dioica* lowered the blood sugar, total cholesterol, low density lipoprotein and triglyceride levels, and increased the high density lipoprotein cholesterol, phospholipid and fecal sterol levels [5-6]. Kharbanda *et al.* (2015) reported that ethanolic extract of the aerial parts of *T. dioica* has anti-inflammatory properties while other scientist investigated hepatoprotective activity of aqueous and ethanolic extract of *Trichosanthes dioica* (whole plant) in ferrous sulphate-induced liver injury [7-8].

However, to the best of our knowledge and after critical search of literature reviews [PubMed, Scopus, NCBI] did not yield any articles that evaluated the chemical composition of the root

extracts and its biological properties of *T. dioica* and hence the present investigation was undertaken.

2. Materials and Methods

2.1 Preparation of the root extract:

The roots of *Trichosanthes dioica* were collected from Midnapore district, West Bengal, India during the months of November 2015 to March 2016 and identified. A voucher specimen has been kept in the Department (V-121/SB/Z). Sundried grounded roots (5g) were extracted in double distilled water (the ratio of plant material to solvent was (1:10 m/v). The extraction was carried out at 50 °C with constant stirring for 28 hours. The extracts obtained were evaporated to dryness and stored at -4 °C until required. The yield of the dried leaves were calculated by following equation: Yield (g/100g of dry [plant material]) = $W1 \times 100 / W2$, where W1 and W2 were the weight of the extract after evaporation of solvent and the weight of the dry plant material.

2.2 Preliminary Qualitative Phytochemical screening

The presence or absence of phytochemical constituents was analyzed by routine procedures.

- **Flavonoids:** Shinodas test: 100g of plant material was extracted with 5 ml ethanol and filtered. To 1 ml of the filtrate, magnesium ribbon and few drops of concentrated HCl was added. Pinkish red colour indicates presence of flavonoids.
- **Alkaloids:** 25g of plant material was boiled in 15 ml of 1% concentrated H₂SO₄ in 50% ethanol and filtered. To the filtrate 5 drops of 5% NH₄OH was added followed by 15 ml chloroform and two layers were separated. The chloroform layer was extracted with 15 ml dilute H₂SO₄. On addition of 5 drops of Mayer's reagent to the extract, a creamy red orange, brownish precipitate indicates presence of alkaloids.
- **Reducing sugars:** 1ml of above filtrate was boiled with 2ml of Fehling's reagent for 3-4 minutes. A brick red precipitate indicates presence of reducing sugars.
- **Test for saponins:** The root extract was shaken vigorously with 5 ml water, persistent of foam even after 15 minutes indicates presence of saponins.
- **Test for steroids:** To about 50mg of root extract equal volume of acetic acid and chloroform was added, followed by addition of few drops of concentrated H₂SO₄. Formation of violet brown ring indicates presence of steroids.

2.3 Ultraviolet visible absorption analysis (UV)

One g of plant powder was kept overnight with 25 ml of distilled water with constant stirring and then filtered. An aliquot of the filtered sample was scanned using UV-visible Spectrophotometer (Shimadzu, UV-1800), at a range of 200 - 800 nm, to detect the characteristic wavelength of the plant extract.

2.4 GC/MS analysis of *Trichosanthes dioica* roots

The chemical composition of *T. dioica* was investigated through Gas Chromatography-Mass Spectrometry/Mass Spectrometry Electron Ionization (GC-MS/EI) mode. The GC-MS/MS is a Scion 436-GC Bruker model coupled with a Triple quadruple mass spectrophotometer with fused silica capillary column BR-5MS (5% Diphenyl/95% Dimethyl polysiloxane) and Length: 30mm; Internal diameter: 0.25 mm; Thickness: 0.25 µm. Helium gas (99.999%) was used as

the carrier gas at a constant flow rate of 1 ml/min and an injection volume of 2 µl was employed (split ratio of 10:1). The column oven temperature program was as follows: 110 °C hold for 3.5 min, Up to 200 °C at the rate of 10 °C/min-No hold, Up to 280 °C at the rate of 5 °C/min-with 9 min hold, Injector temperature 280 °C and total GC running time was 37.5 min. This last increase was to clean the column from any residues. The mass spectrometer was operated in the positive electron ionization (EI) mode with ionization energy of 70eV. The solvent delay was 0-3.0 min. A scan interval of 0.5 seconds and fragments from m/z 50 to 500 Da was programmed. The inlet temperature was set at 290 °C, source temperature 250 °C. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was MS Work station 8 and compared with NIST Version 11.0 library database of National Institute Standard and Technology (NIST) which has more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained. The GC-MS/MS was performed by Food Safety and Quality Testing Laboratory, Thanjavur.

2.5 Study of antibacterial activity

The antibacterial activity of the water extract of *T. dioica* was investigated by standard agar-well diffusion method (Bauer *et al.* 1966; Awhad *et al.* 2013) into one Gram positive (*Bacillus subtilis*) and one Gram negative (*Proteus mirabilis*) bacteria. Briefly, nutrient agar solidified onto petri plates, and the plates containing nutrient medium were evenly inoculated with 100 µg (10⁸ CFU/ml) separately. The wells are prepared on the agar plate with the help of cork borer (0.6 cm diameter). Streptomycin, 5 µg /disc, was used as standard, was placed in the well of each plate. The water extract containing (10 µg /ml) was loaded onto the wells of each plate. The plates were then incubated for 24 hour at 37 °C, and the antibacterial activity was determined by measuring the diameter of the inhibition zone and expressed in millimeter.

2.6 Statistical analysis

All the qualitative test/analysis was performed in triplicate. The significance of difference between data of the different groups was calculated by Students't-test. ANOVA (SPSS 10.0 Software) was used to compare multiple groups and differences within the groups.

3. Results

The yield of the extract of root was 22.2% and analysis of the water extract of root of *T. dioica* reveals presence of flavonoids, alkaloids, reducing sugars (Table-1). The presence of alkaloids and flavonoids were more when compared to other compounds where the colouring intensity was denoted by +.

The UV-VIS profile of plant extract was taken at 190 to 550nm wavelength due to the sharpness of the peaks and proper baseline. The profile showed the peaks at 520nm, 512nm, 505nm, 496nm, 487nm, 483nm, 444nm, 426nm and 222.5nm with the absorption 0.071, 0.083, 0.077, 0.087, 0.090, 0.161, 0.177, 0.268 and 3.996. (Figure 1).

Eleven compounds were identified from the aqueous root extract of *T. dioica* by GC-MS. The active principles with

their retention time (RT), molecular formula, molecular weight (MW) are presented in (Table 2). The prevailing compounds were Eicosane 2-methyl (18.16), Octadecane (3.68), Methoxyacetic acid (0.71), 2 methylhexacosane (16.18), Heptacosane (5.85), Octacosane. Antibacterial activity of root extract (water) of one Gram negative bacteria *Proteus mirabilis* and one Gram positive bacteria *Bacillus subtilis* (ATCC-11774) is shown in Figure 2 and 3 respectively. In both cases, a clear inhibition zone appeared. It was also observed that the zone of inhibition of Gram positive bacteria is slightly greater (7.1 mm) than that of the Gram negative bacteria (4 mm) which is statistically

significant ($p < 0.01$), further both the zones of inhibition were also statistically low when compared with a broad spectrum antibiotic streptomycin.

Table 1: Preliminary phytochemical screening of root extract of *Trichosanthes dioica*

Chemical compounds	Root extracts of TD
Flavonoids	++
Alkaloids	++++
Reducing sugars	++++
Saponins	+
Steroids	++

Table 2: Compounds identified from the aqueous root extract of *T. dioica*

No	RT	Name of the compound	Mol. Formula	Mol. wt	Peak area%
1.	14.28	Phthalic acid, butyl undecyl ester	C ₂₃ H ₃₆ O ₄	376	0.69
2.	15.50	Dibutyl phthalate	C ₁₆ H ₂₂ O ₄	278	0.03
3.	17.50	Methoxyacetic acid, 4-tridecyl ester	C ₁₆ H ₃₂ O ₃	272	0.71
4.	18.95	Octadecane, 2methyl-	C ₁₉ H ₄₀	268	3.68
5.	20.43	Sulfurous acid, pentadecyl pentyl ester	C ₂₀ H ₄₂ O ₃ S	362	10.55
6.	21.9	Eicosane, 2-methyl	C ₂₁ H ₄₄	296	18.16
7.	23.38	2-methyltetracosane	C ₂₅ H ₅₂	352	20.11
8.	24.83	Eicosane, 7-hexyl	C ₂₆ H ₅₄	366	20.81
9.	26.23	2-methylhexacosane	C ₂₇ H ₅₆	380	16.87
10.	27.61	Heptacosane	C ₂₇ H ₅₆	380	5.85
11.	28.96	Octacosane	C ₂₈ H ₅₈	394	2.53

Table 3: The percentage activity index of root extract of *T. dioica* when compared to standard antibiotics.

S. No.	Microorganism	Inhibition Zone (mm)	Activity Index (%)	Standard antibiotics (mm)
1.	<i>Proteus mirabilis</i>	4.21±0.025*	17.39	23.28±0.31
2.	<i>Bacillus subtilis</i>	7.13±0.121**	28.0	23.16±0.18

Table 4: Pharmacological property of some phyto-constituents present in the aqueous root extract of *T. dioica* obtained from online Dr. Duke's phytochemical and ethnobotanical databases

S. No.	Compound Name	Biological activity
1.	Octadecane, 2methyl	Antioxidant, antibacterial
2.	Heptacosane	Antibacterial
3.	Eicosane, 7-hexyl	Anti-androgenic, aldose reductase inhibitor
4.	Eicosane, 2-methyl	Hemolytic activity, 5 α reductase inhibitor, cytotoxic, antitumor
5.	2 methyltetracosane	Anticancer, antioxidant, antifungal, antibacterial
6.	2-methylhexacosane	Antimicrobial, decrease blood cholesterol

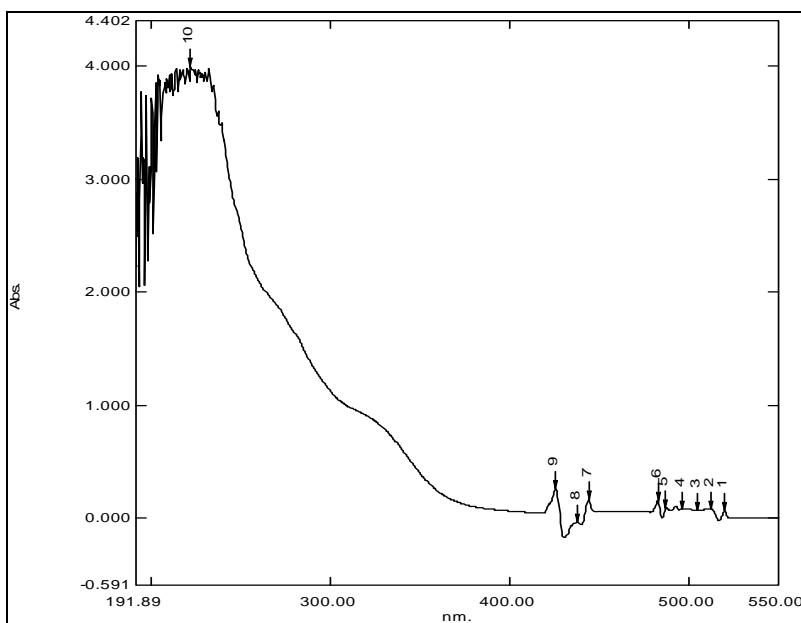


Fig 1: UV-vis spectra of aqueous root extract of *T. dioica*

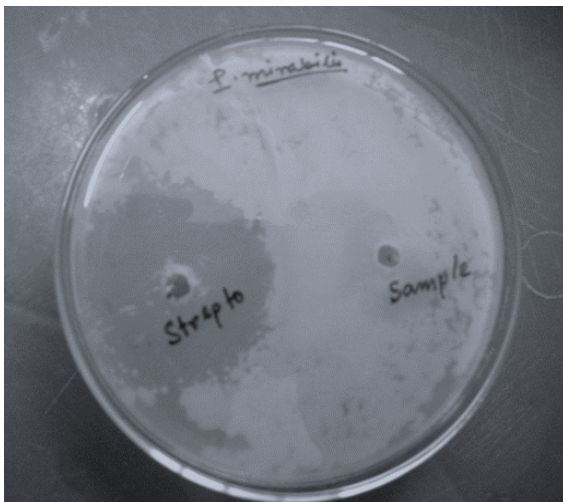


Fig 2: Zone of inhibition by *T. dioica* extract against *P. mirabilis* with regard to streptomycin control



Fig 3: Zone of inhibition by *T. dioica* root extract against *B. subtilis* with regard to streptomycin control

4. Discussion and conclusion

Spectroscopic technique has become a powerful and analytical tool for the qualitative and quantitative analysis of pharmaceutical and biological materials. The flavonoids spectra typically consist of two absorption maxima in the ranges 220-285 nm and 300-350 nm. The high peak at 222.5nm is an indicative of presence of flavonoids.

Bhattacharya and Haldar (2011) reported that *Trichosanthes dioica* root extract induces tumor proliferation and attenuation of anti-oxidant system in albino mice bearing Ehrlich ascites carcinoma [9]. Further the same group reported acute and sub-chronic toxicity of *Trichosanthes dioica* root in mice and also showed *in vitro* cytotoxic effects [10]. Antibacterial activity of extract indicates that it comprises of compounds which would probably inhibit broad spectrum of microorganisms. This study reveals that, in addition of being toxic to livestock as reported by other workers it may also be considered as an important medicinal plant. In fact, a high percentage of these compounds may possess desirable/modulatory biological activities. Table 3 shows activity of some phyto-constituents of root identified in the water extract of *T. dioica*. Hence the results suggest that *Trichosanthes dioica* plant is very promising not only for its anti-diabetic and antioxidant potential but also for its antimicrobial properties. A similar result was also obtained by other workers (Rai *et al* 2010) but they carried the experiments by leaf and seed extract [11]. As mentioned earlier this is probably the first report of antibacterial activity of the root extract and identification of the compounds present there in. The investigation concluded that the extraction produced number of active constituents which are responsible for the biological activities. Further studies are crucial towards isolation, identification and characterization of bioactive compounds which might have some importance and societal benefit.

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