Global Journal of Pharmacology 4 (3): 122-126, 2010
ISSN 1992-0075

Antibacterial Activity of *Trichosanthes dioica* Root

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Abstract: *Trichosanthes dioica* Roxb. (Cucurbitaceae), called pointed gourd in English is a dioecious climber grown in India and used traditionally for various medicinal purposes. The present study aimed to evaluate *in vitro* antibacterial effect of *n*-hexane (HXTD), dichloromethane (DCTD), methanol (METD) and aqueous (AQTD) extracts of *T. dioica* root against five Gram-positive bacteria including *Staphylococcus aureus, Streptococcus faecalis, Bacillus cereus, Sarcina lutea, Bacillus subtilis* and two Gram-negative bacteria, *Klebsiella pneumoniae* and *Escherichia coli*; using disk diffusion method followed by determination of minimum inhibitory concentrations (MIC) by broth dilution method, against sensitive bacteria. All extracts, at higher concentrations showed varying degrees of inhibitory activity against all bacteria; except the HXTD, which was inactive against *S. lutea* and *B. subtilis*. The highest activity was exhibited by the DCTD against *B. cereus*, while the lowest activity was exhibited by HXTD against *K. pneumoniae*; at the highest (40 mg/ml) concentration. The DCTD was the most active, showing maximum concentration dependent antibacterial effects followed by the METD, AQTD and HXTD which was the least active. Gram-positive bacteria were more sensitive to the extracts, except aqueous extract of stem bark. Therefore, *T. dioica* root possessed broad spectrum antibacterial efficacy.

Key words: Antibacterial · Minimum inhibitory Concentration (MIC) · Root · Zone of inhibition.

INTRODUCTION

The use of higher plants and their preparations to treat infectious diseases is an age-old practice and in the past possibly the only method available. However, the systematic study of higher plants for detecting antimicrobial activity is of comparatively recent origin [1]. These investigations have been triggered by the emergence and spread of antibiotic resistant microorganisms causing the effective life-span of existing antibiotics limited [2]. Hence, the plant kingdom is being screened for newer and effective chemotherapeutic agents. Higher plants can serve both as potential antimicrobial crude drugs as well as a source of newer anti-infective agents [3].

Trichosanthes dioica Roxb. (Cucurbitaceae), called pointed gourd in English, *Potol* in Bengali, *Palval* in Hindi 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. It is also cultivated, particularly in Uttar Pradesh, Bihar, West Bengal and Assam states of India, for its fruits, a common culinary vegetable in India. In India, all parts of this plant have been used traditionally for several medicinal purposes. According to Ayurveda, the traditional system of Indian medicine, its root is a drastic purgative. The root has traditionally been used as a hydrogouge cathartic, tonic and febrifuge and in the treatment of jaundice, anasarca and ascites [4-7]. However, there are no reports on the pharmacological studies on its root. In our earlier studies, we have reported on nematocidal effect of *T. dioica* leaf and root [8, 9]. The present study attempted to evaluate the broad spectrum *in vitro* antibacterial efficacy of different solvent extracts from *T. dioica* root.

MATERIALS AND METHODS

Plant Material: The mature tuberous roots of *T. dioica* were collected during December 2008 from Majdia, Nadia district, West Bengal, India. The species was identified at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India and a voucher specimen (SB-02) was deposited at the Pharmacognosy Research Laboratory, Bengal School of Technology

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(A College of Pharmacy), Hooghly, India. Just after collection, the plant material was washed thoroughly with running tap water and shade dried at room temperature (24-26°C) and ground mechanically into a coarse powder.

Chemicals: All the chemicals used were of analytical grade, obtained from Merck. The culture media were obtained from Himedia.

Preparation of Extracts: The powdered plant material (750 g) was initially macerated with *n*-hexane (1 L) overnight and the air-dried marc was macerated separately with dichloromethane (DCM), methanol (MeOH) and distilled water (450 ml each) at room temperature (24-26°C), with frequent shaking for 4 days, followed by re-maceration with the solvents, similarly for 3 days. All four solvent macerates were combined, filtered and evaporated to dryness *in vacuo* (at 35°C and 0.8 Mpa) to yield *n*-hexane (1.38 %), DCM (3.72 %), MeOH (7.22 %) and aqueous extracts (11.05 %), which were denoted as HXTD, DCTD, METD and AQTD, respectively. All the dry extracts were kept in a vacuum desiccator until use. Preliminary phytochemical analysis revealed the presence of fats and steroids in HXTD; flavonoids, triterpenoids and steroids in the DCTD. The METD revealed the presence of flavonoids, triterpenoids and steroids, saponins, amino acids, carbohydrates and reducing sugars, whereas the AQTD indicated the presence of flavonoids, saponins, carbohydrates, reducing sugars and amino acids [10].

Bacteria: The bacteria used in the study included five Gram-positive bacteria, *Staphylococcus aureus, Streptococcus faecalis, Bacillus cereus, Sarcina lutea, Bacillus subtilis* and two Gram-negative bacteria, *Klebsiella pneumoniae* and *Escherichia coli.* All bacterial cultures were obtained from the Central Drug Laboratory, Kolkata, India and maintained in usual laboratory conditions.

Test Samples: Test samples for *in vitro* antibacterial bioassay were prepared freshly from the dry extracts. All the test extract samples were prepared in similar concentrations of 5, 10, 20, 40 mg/ml by dissolving the dry extracts in sterile double distilled water supplemented with 4% v/v dimethyl sulfoxide (DMSO) immediately before use.

Antibacterial Activity

Disk Diffusion Method: The antibacterial activities of the test extracts were evaluated by disk diffusion method as reported by previous workers [11, 12], with minor modifications. The turbidity of bacterial cultures in broth media was adjusted with sterile saline (0.9% w/v) according to 0.5 McFarland turbidity standard, for preparation of the inoculum. Mueller-Hinton agar medium previously prepared and sterilized was cooled down to approximately 45-50°C. 20-25 ml of this media were poured into 9 cm sterile glass Petri dishes previously marked suitably at the bottom surface, to a depth of approximately 4 mm. The inoculum was added to the molten agar media in the Petri dishes and the plates were swirled gently to disperse the microorganisms homogeneously. The plates were then allowed to solidify. Whatman no. 1 filter paper disks (0.5 cm), previously sterilized were impregnated with each test extracts at four different concentrations (40, 20, 10 and 5 mg/ml) and placed on the solidified surface of the media seeded with respective microorganisms. Similarly, filter paper disks impregnated with the vehicle (25% DMSO) were used as control. No standard antimicrobial agent was employed. Then the Petri dishes were incubated in inverted position at 37°C for 24 h. After incubation the zones of inhibition around the disks were measured by means of a transparent ruler in mm.

Broth Dilution Method: The minimum inhibitory concentrations (MIC) of the extracts were determined for the sensitive bacteria (in disk diffusion test) by broth dilution method [13, 14]. All test extracts were serially (two fold) diluted from 40 mg/ml to 0.0781 mg/ml (10 dilutions). To 9 ml of sterile Mueller-Hinton broth in test tubes, 1 ml of varying concentrations of the extracts were added and then a loopful (approximately 0.01 ml) of the bacterial suspensions previously adjusted with sterile saline (0.9% w/v) according to 0.5 McFarland turbidity standard, were introduced to the tubes. Streptomycin sulphate was used as the reference antimicrobial agent, which was also serially (two fold) diluted from 32 μg/ml to 1 μg/ml (6 dilutions) and tested similarly. In each test set, tube containing only medium and inoculum was used as control. Tubes were then incubated at 37°C for 24 h. After incubation the lowest concentration at which no visible growth was observed (i.e. no turbidity) was regarded as minimum inhibitory concentration.

RESULTS AND DISCUSSION

The antibacterial activity of different extracts from *T. dioica* root against seven bacterial strains was initially assessed by disk diffusion method. The results are presented in Table 1.

Table 1: *In vitro* antibacterial activity of *T. dioica* extracts by disk diffusion method.

		Zone of Inhibition (mm)						
Extracts	Conc. (mg/ml)	Sa	Sl	Sf	Bc	Bs	Kp	Ec
DCTD	40	16	10	14	17	15	8.5	11
	20	11	7	10	12.5	9.5	-	7
	10	8.5	-	6	9.5	6.5	-	-
	5	6	-	-	5.5	-	-	-
METD	40	10	7	9	12	16	9	6
	20	6	-	-	7.5	9	5.5	-
	10	-	-	-	5.5	6	-	-
	5	-	-	-	-	-	-	-
AQTD	40	13	9	7	12.5	10	6	10
	20	8	-	-	7	6	-	5.5
	10	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-
HXTD	40	9	-	6	7	-	6	8
	20	5.5	-	-	-	-	-	-
	10	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-

Table 2: The MIC values of *T. dioica* extracts and streptomycin sulphate by broth dilution method.

	Minimum inhibitory concentrations (MIC) (mg/ml)						
Extracts	Sa	Sl	Sf	Bc	Bs	Kp	Ec
DCTD	0.1563	0.625	0.3125	0.0781	0.1563	1.25	0.625
METD	0.625	1.25	1.25	0.3125	0.1563	1.25	2.5
AQTD	0.3125	0.625	1.25	0.3125	0.625	2.5	0.625
HXTD	1.25	ND	2.5	2.5	ND	5.0	1.25
STM	16*	8*	16*	4*	16*	2*	4*

Sa = *Staphylococcus aureus*, Sl = *Sarcina lutea*, Sf = *Streptococcus faecalis*, Bc= *Bacillus cereus*, Bs = *Bacillus subtilis*, Kp = *Klebsiella pneumoniae*, Ec= *Escherichia coli*.

ND = Not determined, because the extract was inactive in the preliminary screening.

STM: Streptomycin sulphate, *Values are in µg/ml.

The extracts showed antibacterial activity against most of the tested bacteria mainly at higher concentrations. The DCTD exhibited moderate to feeble inhibition against all test bacteria with maximum against *B. cereus* (17 mm) and minimum against *K. pneumoniae* (8.5 mm) at highest concentration (40 mg/ml) employed. The activities decreased with decrease in concentration. This extract was found to possess maximum concentration dependent antibacterial effects showing least inhibition even at the lowest concentration of 5 mg/ml against *S. aureus* (6 mm) and *B. cereus* (5.5 mm).

The METD showed comparatively weak inhibitory activity against all test bacteria with maximum against *B. subtilis* (16 mm) and minimum against *E. coli* (6 mm) at 40 mg/ml (highest) concentration. Here also activities diminished with concentration but were not detectable up to the lowest concentration employed.

The AQTD also showed weak activity in comparison with the DCTD, against all bacteria tested with maximum inhibition (13 mm) against *S. aureus* and minimum against *K. pneumoniae* (6 mm). The effects were observed at the highest and next lower concentration (20 mg/ml) of extract against some bacteria.

The HXTD exhibited no inhibitory activity against *S. lutea* and *B. subtilis* and it showed the weakest activity as compared to other three extracts against other five bacteria mostly at the highest (40 mg/ml) concentration.

The results of broth dilution test for MIC of extracts and streptomycin sulfate are shown in Table 2. This method was performed only on bacteria that were found to be sensitive to the extracts in the disk diffusion method. Hence, the HXTD was not tested against *S. lutea* and *B. subtilis* for MIC. The DCTD had the lowest MIC (0.0781 mg/ml) against *B. cereus*, while the highest MIC

was shown by the HXTD against *K. pneumoniae*; indicating the highest and lowest inhibitions respectively. The results of broth dilution test i.e. MIC values were roughly in concert with the results of disk diffusion, thereby confirming differential antibacterial effects of the test extracts.

Preliminary phytochemical analysis revealed the presence of various compounds in METD, whereas the DCTD mainly contained triterpenoids and steroids. It appears that the presence of triterpenoids and/or steroids was responsible for the enhanced activity of DCTD. In this connection, it is noteworthy to mention here that the METD also contained triterpenoids and steroids, along with several other constituents, but the expected synergistic effect, however, was not observed here, as DCTD was more active than METD. The AQTD exhibited weaker effect, possibly because of the absence of triterpenoids and steroids. Some synergy was obvious, indicating METD to be more active than AQTD, because of the presence of triterpenoids and steroids in METD. Nevertheless, activity in the AQTD indicated that not only triterpenoids and steroids were responsible for the antibacterial activity. Negligible activity in HXTD indicated the minor contribution of steroids in activity. Dereplication strategies based on these findings could be helpful for isolation of the active antibacterial constituents.

Out of the solvents employed for extraction the DCM and MEOH extracts exhibited higher activity against the test organisms. Different solvents have the capacity to extract different antimicrobial constituents from plants. Since nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial choloroform, dichloromethane or methanol extraction [2, 15].

The extracts were effective against most Gram-positive and Gram-negative bacteria tested, thereby indicating a broad spectrum of activity. However, the results revealed that the Gram-positive bacteria were in general, more sensitive to the extracts, except the HXTD. In this investigation, the agar disk diffusion method was employed to serve as initial antibacterial screening procedure. The diameter of zone of inhibition is a function of initial concentration (in the disk), solubility and diffusion rate of the antibacterial compound(s) present in the extract through the agar media and thus not a true measure of effectiveness [16, 17]. Many physical and chemical factors unrelated to antibacterial activity affect the rate of diffusion of compound(s) through bacteria seeded solid media [18]. Therefore, it is not possible to measure the antibacterial activity of a new compound or plant extract in terms of another antibiotic as reference by comparing the diameters of zone of inhibition produced by disk diffusion. Hence, in the present study, no antibiotic reference was employed while disk diffusion method was used to assess antibacterial activity.

More sensitive, quantitative and confirmatory results were obtained with broth dilution test. Here the method employed can be termed as broth two-fold macro dilution where the extracts were diluted serially (two-fold) in a sequence of decreasing concentration in broth by using test tubes and inoculated with the test bacteria. The smallest concentration of the extract that prevented visible growth (turbidity) is called the minimum inhibitory concentration (MIC). Here, a reference broad spectrum antibiotic streptomycin sulphate was employed as reference which was also tested for MIC by serial dilution similarly. It was found that the MIC values of streptomycin sulphate were very much lower (2-16 μg/ml) than that of extracts (0.0781-5 mg/ml) from *T. dioica*. Furthermore, some MIC values obtained for streptomycin sulfate were roughly in agreement with literature values [12, 19]. Therefore, the reference antibiotic was employed not only for comparison but also to ensure the bacteria used in the study and the experimental conditions were appropriate and acceptable [20, 21].

The present study confirmed the *in vitro* antibacterial effect of *T. dioica* root extracts. The results indicated that the METD and DCTD, especially, possess potential antibacterial activity predominantly in Gram positive bacteria, but more positive results are definitely needed in other resistant bacterial and fungal test systems involving *in vivo* animal studies for qualifying the wide chemotherapeutic potential. To the best of our knowledge, the present investigation is the first experimental demonstration of broad spectrum antibacterial efficacy of *T. dioica* root. The antibacterial effects of *T. dioica* root may substantiate its traditional uses in India. Further pharmacological and toxicological studies on *T. dioica* root are presently underway.

ACKNOWLEDGEMENT

The first author (SB) would like to thank the authority of Bengal School of Technology (A College of Pharmacy), Sugandha, Hooghly 712102, West Bengal, India, for necessary facilities.

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