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PHYTOCHEMICAL EVALUATION AND ANTIBACTERIAL POTENTIAL OF *TRICHOSANTHES TRICUSPIDATA* ROOT EXTRACTS AGAINST HUMAN PATHOGENIC BACTERIA

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ABSTRACT

Trichosanthes tricuspidata (family: Cucurbitaceae) commonly known as Lal indrayan or shvetpushpi and found at an elevation of 1200 to 2300 m. It grows as a large climber, often attaining a height of 9-10 meters. *T. tricuspidata* has been widely used for curing asthma, migraine, fever, diabetic carbuncles and other maladies. The present study was aimed to do phytochemical screening and antibacterial activity of different extracts of *T. tricuspidata* roots. The antibacterial activity of root extracts was tested against Gram positive and Gram negative pathogens viz. *Pseudomonas aeruginosa* (MTCC 1036), *Klebisilla pneumonia* (MTCC 432), *Staphylococcus aureus* (MTCC 96) and *Streptococcus mutans* (MTCC 497). The ethanol, 50%ethanol and water extracts from mature roots of plant were screened for their chemical constituents and antibacterial activity. Ethanolic extract showed the presence of alkaloids, carbohydrates, glycosides, saponins and triterpinoids and maximum antibacterial activity against *Klebisilla pneumonia* (MTCC 432) at 1mg/100µl concentration. The antibacterial potential of the plant was then compared with standard gentamycin.

Key words : *Trichosanthes tricuspidata,* root extracts, Phytochemicals, antibacterial activity.

INTRODUCTION

Man has always been dependent on the plants for food, fuel, medicines and other necessities of life since ages. According to World Health Organization (WHO), more than 80% of the world's population relies on traditional medicines for their primary health care needs (WHO.2002). Plants used for traditional medicine to contain a wide range of substances that can be used to treat chronic as well as infectious diseases (Samy, R.P. and Ignacimuthu, S. 2002). It has now been established that the plants which naturally synthesis and accumulate some phytoconstituents such as flavonoids, alkaloids, tannins and triterpenoids are rich source of medicine and stimulating opportunity for the expansion of modern chemotherapies against wide range of microorganisms (Lutterodt, G.D,1999; Marjori, MC. 1999). Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. Their role is two fold in the development of new drugs: (1) they may become the base for the development of a medicine, a natural blue print for the development of new drugs or; (2) a phyto-medicine to be used for the treatment of diseases (Iwu, M. 1993). In today's common usage, the term antibiotic is used to refer to almost any drug that attempts to rid your body of a bacterial infection. Each species of pathogen has a characteristic spectrum of interactions with its hosts. The less availability and unaffordable cost of new generation antibiotics initiated to look for alternative phytomedicine to discover plant derived constituents with claimed antimicrobial activity. Nowadays, an increasing number of infectious agents are becoming

more resistant to commercial antimicrobial compounds (Hancock et al. 2012). The necessity to develop new drugs requires varied strategies like the bioprospection of secondary metabolites produced by medicinal plants (Dionisi, *et. al*. 2012). *Trichosanthes tricuspidata* (family: Cucurbitaceae) is a vine which is found at an elevation of 1200 to 2300 m ranges from the Eastern Himalayas in India and southern China through southern Japan, Malaysia, and tropical Australia. *T. ricuspidata* is considered to be medicinally important in several traditional systems (Snehlata *et. al*. 2008). It has been widely used for curing asthma, migraine, fever, diabetic carbuncles and other maladies. A number of pharmacologically important phytochemicals, such as cucurbitacins, trichotetrol and octanorcucurbitane glycosides have been isolated from the plant (Kanchanapoom *et. al*. 2002). Tetrahydroxy pentacyclic triterpene "trichotetrol" isolated from the root extract. The root contains methyl palmitate, palmitic acid, suberic acid, spinasterol, stigmast-7-en-3-beta-ol, spinasterol, 3-obeta-D-glucopyranoside, stigmast-7-en-3-beta-ol-3-O-beta-D-glucopyranoside, glyceryl 1-palmitate, glyceryl 1-stearate, bryonolic acid, cucurbitacin B, isocucurbitacin B, 3-epiisocucurbitacinB, 23,24-dihydrocucurbitacin D, isocucurbitacin D and D-glucose. The roots of *T. tricuspidata* contain more than 6 times more cucurbitacin than the roots of *T. kirilowii* Maxim. Var. *japonicum* Kitam (Mohamed, P.A. *et. al*. 1974). The roots of the plant are used to treat lung diseases in cattle and for the treatment of diabetic carbuncles and headache[11]. The oil extracted from the roots is used as a pain killer (Brijesh, K. *et. al*. 2012). The aim of investigation was to evaluate the phytoconstituent and antibacterial activity of *T. tricuspidata* against some Gram positive and Gram negative bacteria *in- vitro* conditions.

MATERIALS AND METHODS

Collection of plant material

The roots of *Trichosanthes tricuspidata* were collected from Uttarakhand and authenticated by Systematic Botany Division, Forest Research Institute, Dehradun (Uttarakhand).

Preparation of root extracts

The freshly collected roots (1kg) of *Trichosanthes tricuspidata* were washed with distilled water, shade dried and powdered. The powdered plant material (200 gm) was defatted with petroleum ether (60-80^{0}C). The defatted material was extracted with the solvents of elutropic series viz. ethanol and 50% ethanol/water (1:1, v/v) by using soxhlet apparatus and aqueous extract was prepared by refluxing sequentially. Each time before extracting with the next solvent of high polarity, the powdered roots were dried in air oven below 50° C for 10 minutes. Each extract was concentrated by distilling off the solvent, which was recovered subsequently. The concentrated extracts were evaporated to dryness and their percentages were calculated in terms of initial air dried plant material (Table1). The extracts obtained were subjected to phytochemcial screening and antibacterial activity (Koklate, C 1999).

Phytochemical screening

The successive extracts as mentioned above, were subjected to various qualitative phytochemical tests for the identification of chemical constituents present in the plant material. Phytochemical screening for major constituents done by standard qualitative methods (Sofowora, A 1993; Trease, G et al. 1989; Harborne, J. 1973).

Microorganisms

The antibacterial activity for different extracts (ethanolic, 50% ethanolic and water extract) was determined by Agar Well Diffusion method. Both gram positive and gram-negative bacterial strains were used for the test. The bacterial strains used for the investigation are *Pseudomonas aeruginosa* (MTCC 1036), *Klebsiella pneumonia* (MTCC 432), *Staphylococcus aureus* (MTCC 96) and *Streptococcus mutans* (MTCC 497). The bacterial strains were procured from Institute of Microbial Technology *(IMTECH),* Chandigarh .

Preparation of media

a) **Nutrient broth:** The nutrient broth was prepared by dissolving 39 gm medium in 1000 ml of distilled water. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121^{0}C) for 15 minutes.

b) **Muller Hinton Agar Media:** The dehydrated Muller Hinton Agar (MHA) media was prepared by dissolving 38 gm into 1000 ml of distilled water, boiled and dissolved completely.

It was sterilized by autoclaving at 15 lbs. pressure and 121^{0}C temperature for 15 min. Media poured into sterile Petri dishes and allowed to set. Sterility check was done after the agar was solidified (Atlas, R.M., and Snyder, J.W. 2006).

Antibacterial assay

Antibacterial activity of Ethanol, 50% ethanol and aqueous roots extract of *T. tricuspidata* (roots) were determined by the Agar Well Diffusion method (NCCLS, 1999). The extracts were dissolved in DMSO to evaluate their antibacterial activity. The bacterial cultures were grown at 37^{0}C for 18h in a broth medium and culture was adjusted to 5 x 10^5cfu/ml (Farland Mc. 2000). The 10µl culture was spread on MHA plates and well of 6mm were punched into the agar plates. About 20 (l of the extracts (0.25mg/100 (l, 0.5mg/100 (l, 1mg/100 (l) was delivered into the each of the wells. The plates were incubated at 37^{0}C for 18-24h. Zone of inhibition obtained was a measure of antibacterial activity of the subjected extracts (Mackie, W., and Mc. Cartney, L 1989). The antibiotic Gentamycin at 0.5mg/100µl, 1mg/100µl concentration was used as standard/control. The minimal inhibitory concentration (MIC) was determined only with micro-organisms that displayed highest inhibitory zones.

RESULTS AND DISCUSSION

The prevalence of antibiotic resistant strain of microorganisms is increasing and the identification of safe and effective natural agents for use in these infections is an important issue of researchers. Present study was conducted to investigate antibacterial properties of *T. tricuspidata.* Powdered samples of plant roots were extracted in three different solvents (Ethanol, 50% ethanol with water (1:1) and aqueous). Hence, total three extracts were tested against four different pathogens *Pseudomonas aeruginosa* (MTCC 1036), *Klebsiella pneumonia* (MTCC 432), *Staphylococcus aureus* (MTCC 96) and *Streptococcus mutans* (MTCC 497). The percentage yields after extraction from *T. tricuspidata* ranges from 2.86 % to 4.91% (Table1). Qualitative phytochemical screening of extract shows the presence of alkaloids, carbohydrates, glycosides, saponins, triterpinoids, tannin and phenolic compounds summarized in Table 2. These compounds are known to be biologically active and therefore, aid the antibacterial activities of plants. The various phytochemical compounds detected are known to have beneficial importance in medicinal sciences. Alkaloids were used in treating malaria and in managing heart diseases (Okwu, D.2001). Plant phenolics possess various biological properties like antioxidant, anti-inflamatory and estrogenic activities(Bawa, A et al. 2006). Glycosides and steroids play a critical role in defense mechanisms (Oomah, D.2003). It is revealed that the highest inhibition zone diameter was observed in 1 mg/100µl of ethanolic extract against *Klebisilla pneumonia* (24 mm) and lowest inhibiton zone diameter in 0.25 mg/100µl of aqueous extract against *Pseudomonas aeruginosa* (08 mm).

Table 1

% yield of crude extracts of T.tricuspidata (root) in different solvent

S. No.	Solvent	Weight of extract	Yield (%)
1.	Ethanol	2.08 gm	3.46%
2.	50% (Ethanol:water)	1.72 gm	2.86%
3.	Water	2.97 gm	4.91%

Table 2
Phytochemical analysis of ethanolic extract

S. No.	Name of the test	Ethanolic extract
1.	**Alkaloids Test**	
	a) Hager's Test b) Wagner's test c) Dragendroff's test	+ + +
2.	**Carbohydrate Test**	
	a) Molish's test b) Fehling's test c) Benedict's test	+ + +
3.	**Glycosides Test**	
	a) Legal's test b) Baljet's test c) Borntrager's test	+ + +
4.	**Phenol Test**	
	a) FeCl3 test b) Lead acetate test c) Gelatine test	+ + +
5.	Saponins test	+
6.	Test for fixed oils	-
7.	Triterpenoids test	+
8.	Test for gums and mucilage	-
9.	Tanin	+

Table 3
Antibacterial activity of T .tricuspidata ethanolic extract

Concentration of extract(µl)	**Micro Organism/Zone of Inhibition (mm)**			
	Pseudomonas aureginosa	*Klebsiella pnemonia*	*Staphylococcus aureus*	*Streptococcus mutans*
0.25mg/100µl	13	19	14	13
0.5 mg/100µl	14	23	15	16
1.0 mg/100µl	15	25	18	18
Gentamycin 1mg/100µl	26	25	27	26

Table 4
Antibacterial activity of T.tricuspidata 50% (Ethanol:water) extract

Concentration of extract(µl)	**Micro Organism/Zone of Inhibition (mm)**			
	Pseudomonas aureginosa	*Klebsiella pnemonia*	*Staphylococcus aureus*	*Streptococcus mutans*
0.25mg/100µl	11	16	10	13
0.5 mg/100µl	13	18	11	16
1.0 mg/100µl	15	22	14	18
Gentamycin 1mg/ 100µl	26	25	27	26

Table 5
Antibacterial activity of aqueous extract of T.tricuspidata

Concentration of extract(µl)	Micro Organism/Zone of Inhibition (mm)			
	Pseudomonas aureginosa	*Klebsiella pnemonia*	*Staphylococcus aureus*	*Streptococcus mutans*
0.25mg/100µl	08	11	10	09
0.5 mg/100µl	09	13	10	10
1.0 mg/100µl	09	13	11	10
Gentamycin 1mg/100µl	26	25	27	26

CONCLUSION

Further study of phytochemicals present in these medicinal plants may reveal some new antibacterial. From the results, we conclude that the crude extracts of *T.tricuspidata* exhibited significant antibacterial activity and properties that support their use in folk medicines.

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