International Journal of Pharma and Bio Sciences

A NOVEL ANTIMICROBIAL AGENTS *TRICHOSANTHES DIOICA*

PRASHANT KUMAR RAI[1], SHIKHA MEHTA[1], RAJESH KUMAR GUPTA[2] AND GEETA WATAL[1*]

[1]Alternative Therapeutics Unit, Drug Discovery & Development Division,. Medicinal Research Lab, Department of Chemistry, University of Allahabad, Allahabad, India

[2]Department of Biotechnology, All India Institute of Medical Sciences, New Delhi, India

[*]***Corresponding author*** geetawatal@rediffmail.com; pksherpuri@gmail.com

ABSTRACT:

It is estimated that one-third of the world's population is infected with tuberculosis (TB). Though a number of drugs have been introduced in past thirty years but still there are unmet needs to develop more effective novel anti-TB drugs. The selection of *Trichosanthes dioica* plant for the present study was based on its previously identified bioactive potential by our research group. This study deals with the *in vitro* assessment of antimicrobial activity of different concentration of extract of different part of *Trichosanthes dioica*. Five clinical isolates of different bacterial strains were used and the disc diffusion method was opted. The results reveal that leaves, fruits and seeds, all three parts of *Trichosanthes dioica* plant, can be used as anti-bacterial agents. Though the leaves extract was active against all five strains and the highest inhibition was observed against *Mycobacterium smegmatis*. Thus the leaves extract could be used for tuberculosis treatment.

KEYWORDS:

Indian medicinal plants; Tuberculosis; Antimycobacterial activity; Ayurveda; *Trichosanthes dioica*

INTRODUCTION:

Tuberculosis (TB), a fatal infectious disease, one of the leading cause of mortality worldwide, infecting about 9 million people kills approximately 2 million people annually[1]. These alarming statistics indicate the devastating nature of the scourge of TB. The reservoir of latent TB cases amounts to 2 billion, approximately a third of the world's population[1]. The organisms responsible for the disease are the tubercle bacilli, *Mycobacterium tuberculosis*, *Mycobacterium tuberculosis complex* including *Mycobacterium bovis, Mycobacterium smegmatis* and *Mycobacterium africanum*[2]. Due to the discovery of effective antimycobacterial agents between 1950 and 1970s, viz., ethambutol, isoniazid, pyrazinamide, rifampicin and streptomycin, and reduction in poverty, there had been a drastic decrease in the number of TB cases, especially in developed countries. However, since 1980s, the number of TB cases throughout the world has been increasing alarmingly due to the emergence of multi-drug resistant *Mycobacterium tuberculosis* (MDR-TB)[3]. MDR – TB was found to be resistant against many front line anti-TB agents. This form of disease is more often fatal and also expensive to treat[4, 5]. What is disturbing is that no anti-TB drug has been introduced in the past 30 years. Hence, the importance of the need to search and develop new, effective and affordable anti-TB drugs cannot be mitigated.

Nature has been a perennial source of medicinal agents for thousands of years and an impressive number of modern drugs which are used in traditional medicine have been isolated from natural sources. Various medicinal plants have been used for years in our daily lives to treat diseases all over the world. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times[6]. The past 20 years, has withessed been a lot of interest in the investigation of natural materials as sources of new antibacterial agents. Different extracts from traditional medicinal plants were tested and some natural products were approved as new antibacterial drugs[7]. However, identification of novel substances, active against pathogens with higher resistance is still warranted[8]. Over 50% of all modern clinical drugs have the origin in natural products[9] and natural products play an important role in drug development programs in the pharmaceutical industry[10]. Lately there has been a revival of interest in herbal medicines and plants have become the basic source of knowledge of modern medicine. The basic molecular and active structures for synthetic fields are provided by rich natural sources. This invasive and burgeoning worldwide interest in medicinal plants reflects a recognition of the validity of many traditional claims regarding the value of natural products in health care. The relatively lower incidence of adverse reactions of plant preparations as compared to modern conventional pharmaceuticals, coupled with their reduced cost, is encouraging to consider plant medicines as alternatives for synthetic drugs.

Bacterial resistance is a growing-problem worldwide[11, 12]. One of the measures to combat the increasing rate of resistance in the long run is to have continuous investigation for new, safe and effective antimicrobials as alternative agents to substitute the non effective ones. There are lot of reports that phytochemical constituents of medicinal plants can be used for the treatment of microbial infections (both topical & systemic applications) as possible alternatives to chemical synthetic drugs to which many infectious microorganisms have become resistant. During the last ten years the pace of development of new antibacterial drugs has slowed down, while the prevalence of resistance (especially multiple) has increased astronomically[13]. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world[14-16]. Much work has been done on ethnomedicinal plants in India[17-19] and thus, the interest in a large number of traditional natural products has increased[20]. It has been suggested that the aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumor and antimicrobial agents[21, 22]. Literature reports and ethno-botanical records suggest that plants the sleeping giants of the pharmaceutical industry[23] are natural sources of antimicrobial drugs that provide novel compounds that may be employed in controlling some infections globally.

Being an extension of our previous work which explored the antidiabetic, hepatoprotective and antioxidant potential of *Trichosanthes dioica*[24-

[27] the present study explore the potential antibacterial activity of *Trichosanthes dioica*.

MATERIALS AND METHODS

(i) Plant material :

Fresh **leaves** of *Trichosanthes dioica* (7 kg) were collected in the month of June from the local area of Sherpur Kalan Ghazipur, (India) and shade dried after authentication. The dried leaves (2 kg) were mechanically crushed and extracted with distilled water at 70℃ using Soxhlet up to 54 h. The extract was filtered and concentrated in rotatory evaporator at 35 ^{o}C ± 5^{o}C under reduced pressure, to obtain semisolid material, which was then lyophilized to get a powder (yield about 11.3% w/w).

Fresh **fruits** (4 kg) of *Trichosanthes dioica* were purchased from the local market of Allahabad (India) and was authenticated. The fruits were cut into small pieces and shade dried. The dried pieces were mechanically crushed and extracted with distilled water at boiling temperature (100 ^{0}C) up to 36 h. The aqueous extract was filtered and concentrated in rotatory evaporator at 55 ± 5 ^{0}C under reduced pressure, to obtain semisolid material, which was then lyophilized to get a powder (yield: 14.9 %w/w).

The fruits were cut and the **seeds** were taken out and shade dried. The dried seeds (2 kg) were mechanically crushed and extracted with distilled water using Soxhlet up to 48 h. The extract was filtered and concentrated in rotatory evaporator at 35 ± 5^{o}C under reduced pressure, to obtain semisolid material, which was then lyophilized to get a powder (yield about 10.5% w/w).

(ii) Bacterial strains, stocks and growth in vitro :

Bacterial strains of *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa* and *Mycobacterium smegmatis* were clinical isolates obtained from the Department of Biotechnology, All India Medical Science, New Delhi (India) and the microbiologist of the department confirmed the identity based on microscopic examination, Gram's character, and biochemical test profile. Bacterial stocks were maintained and stored as 1 ml aliquots at – 80^{o} C in Luria Bertani (LB) broth for *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa* and in Middlebrook 7H9 broth supplemented with 0.1 % Tween 80 and 10 % Albumin Dextrose Complex (ADC) for *Mycobacterium smegmatis* strains. Bacterial stocks were revived from -80 ºC and grown either in Luria Bertani (LB) broth for *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli,* and *Pseudomonas aeruginosa* or in Middlebrook 7H9 medium containing 0.1% Tween-80 and 10% ADC for *Mycobacterium smegmatis*. All cultures were grown at 37º C in a shaker incubator (190-220 rpm) overnight.

Luria Bertani broth (High media), Middlebrook 7H9 (High media.), Luria Bertani Agar (High media) and sterile discs as well as streptomycin discs (High media) were used in antibiotic sensitivity testing.

(iii) Determination of Zone of Inhibition (ZOI) :

The freshly prepared inoculum was swabbed all over the surface of the LB Agar plates for *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa* and over the surface of Middlebrook 7H11 Agar plate for *Mycobacterium smegmatis* using sterile cotton swab. Three sterile discs of 6 mm diameter were placed on the medium with the help of disc dispenser and were numbered properly. The aqueous extract solutions 25, 50, 75 mg/ml of each *Trichosanthes dioica* Fruits, seeds and leaves were poured on the discs with the help of sterilized micropipette. Discs were left for some time till the extract diffuses in them. The effects were compared with that of the standard antibiotic streptomycin loaded sterile disc at a concentration of 1 mg/ml[28]. Finally incubated with the closed lid at 37℃ for 24 hours. Discs were observed for zone of inhibition by measuring diameter of inhibition zone (DIZ) using scale.

RESULTS AND DISCUSSION

The leaves, fruits and seeds extract of the *Trichosanthes dioica* have been tested for their antimicrobial activities and an interesting antimicrobial profile has been observed against *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa* and *Mycobacterium smegmatis* bacterias at a concentration of 25, 50 and 75 mg/ml of each extract (table 1). Extent of effects of each extract was assessed by measuring diameter of inhibition zone (DIZ). The results were compared with the standard antibiotic streptomycin of 1mg/ml concentration.

Table 1
Zone of inhibition of different bacterias in mm

Plant Used	Zone of inhibition				
	Staphylococcus aureus	*Klebsiella pneumonia*	*Escherichia Coli*	*Pseudomonas aeruginosa*	*Mycobacterium smegmatis*
T. dioica leaves (25 mg/ml)	14 mm	12 mm	10 mm	12 mm	24 mm
T. dioica leaves (50 mg/ml)	16 mm	10 mm	12 mm	10 mm	28 mm
T. dioica leaves (75 mg/ml)	22 mm	12 mm	12 mm	12 mm	30 mm
T. dioica Fruits (25 mg/ml)	18 mm	10 mm	12 mm	10 mm	Nil
T. dioica Fruits (50 mg/ml)	20 mm	12 mm	14 mm	12 mm	Nil
T. dioica Fruits (75 mg/ml)	22 mm	18 mm	10 mm	Nil	Nil
T. dioica seeds (25 mg/ml)	20 mm	Nil	10 mm	Nil	Nil
T. dioica seeds (50 mg/ml)	24 mm	Nil	12 mm	Nil	Nil
T. dioica seeds (75 mg/ml)	25 mm	Nil	12 mm	Nil	Nil
Streptomycin (1 mg/ml)	10 mm	Nil	12 mm	12 mm	Nil

1. Assessment of Antimicrobial activity of leaves extract of Trichosanthes dioica :

All the three different concentrations (25, 50, 75 mg/ml) showed sustained activity against all five bacterias tested (fig 1-5). Though the highest activity observed was against *Mycobacterium smegmatis* having DIZ 24, 28 and 30 mm at concentrations of 25, 50 and 75 mg/ml respectively. However, standard drug streptomycin did not show any activity against *Mycobacterium smegmatis* bacteria (fig 1). Activity was quite reasonable and concentration dependent in *Staphylococcus aureus* bacteria having 14, 16 and 22 DIZ at concentrations of 25, 50 and 75 mg/ml respectively. Whereas, the lowest activity was shown with streptomycin of 10 mm DIZ (fig 2).

Figure 1

Figure 2

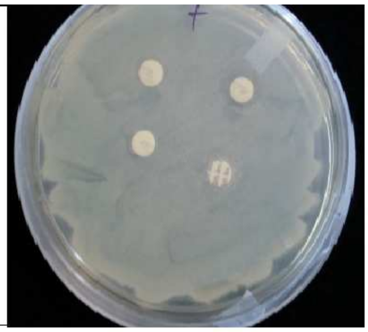

Figure 3

Figure 4

Figure 5

In case of *Escherichia coli,* activity was partly concentration dependent upto 50 mg/ml and then became constant at 75 mg/ml for e.g. DIZ was 10, 12 and 12 mm at concentrations of 25, 50 and 75 mg/ml. Moreover, streptomycin also showed 12 DIZ same as highest DIZ observed at 50 and 75 mg/ml of leaf extract (fig 3). In case of *Klebsiella pneumonia* and *Pseudomonas aeruginosa* both the bacterias showed similar pattern of DIZ i.e. 12, 10 and 12 mm at concentrations of 25, 50 and 75 mm respectively. Though their inhibition response was just opposite in reference of streptomycin, as it was nil incase of *Klebsiella pneumonia* and highest in case of *Pseudomonas aeruginosa* (fig 4 & 5).

2. Assessment of Antimicrobial activity of fruits extract of Trichosanthes dioica :

Figure (6-9) clearly demonstrate the antimicrobial activity of fruit extract of varied concentrations as well as streptomycin. In contrast of leaves extract the fruits extract of Trichosanthes dioica did not show any sustain activity against *Mycobacterium smegmatis*. Since, the DIZ was nil in all the three concentrations as well as streptomycin. The highest activity observed in case of *Staphylococcus aureus* (fig 6) having DIZ 18, 20 1nd 22 mm at concentrations of 25, 50 and 75 mg/ml respectively. Whereas, streptomycin was found to be least active with DIZ 10 mm. The inhibition response of fruit extract was concentration dependent too. In Addition to *Staphylococcus aureus* the other bacteria *Klebsiella pneumonia,* also showed concentration dependent inhibition of 10, 12 and 18 mm at concentrations of 25, 50 and 75 mg/ml respectively. But the low activity of streptomycin observed with *Staphylococcus aureus* becomes nil (fig 7) in case of *Klebsiella pneumonia*.

Figure 6

Figure 7

Figure 8

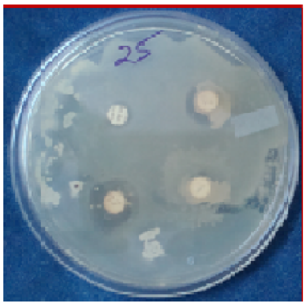

Figure 9

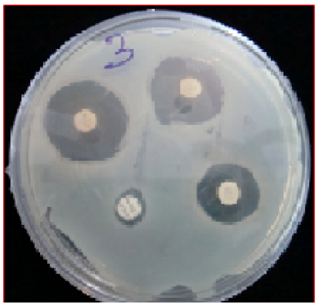

Figure 10

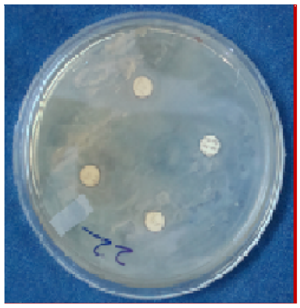

Figure 11

3. Assessment of Antimicrobial activity of seeds extract of Trichosanthes dioica :

Like fruits extract seeds extract also did not show any activity against *Mycobacterium smegmatis* and DIZ was found nil in all the concentrations tested. Surprisingly, seeds extract was found inactive against *Pseudomonas aeruginosa* and *Klebsiella pneumonia* bacterias too at all concentrations used in addition to *Mycobacterium smegmatis*. However, reasonable activity, same as in leaves and fruits extract, was observed in streptomycin against *Pseudomonas aeruginosa* with DIZ 12 mm. The highest activity was observed in *Staphylococcus aureus* bacteria showing concentration dependent action having DIZ 20, 24 and 25 mm at concentrations of 25, 50 and 75 mg/ml respectively (fig 10). Whereas, pretty reasonable DIZ of 10, 12 and 12 mm was observed in case of *Escherichia coli* at concentrations of 25, 50 and 75 mg/ml respectively (fig 11). Moreover, the activity of streptomycin in terms of DIZ was more in *Escherichia coli* (12 mm) than *Staphylococcus aureus* (10 mm).

However, inhibition responses observed for of *Escherichia coli* and *Pseudomonas aeruginosa* were concentration dependent upto certain level and then get decreased at further higher concentration for e.g. DIZ of *Escherichia coli* was 12 and 14 mm (fig 8) and *Pseudomonas aeruginosa* was 10 and 12 mm (fig 9) at concentrations of 25 and 50 mg/ml respectively, indicating thereby a concentration dependent antimicrobial activity. This increased DIZ at higher concentration of 50 mg/ml gets decreased at further higher concentration of 75 mg/ml and DIZ comes down from 14 to 10 mm in case of *Escherichia coli* and from 12 mm to nil in case *Pseudomonas aeruginosa*. The DIZ observed (12 mm) in streptomycin was identical for both *Escherichia coli* and *Pseudomonas aeruginosa*.

Thus, the extent of antimicrobial activity against different bacterial strains was as follows:

Table 2

Extent of Antimicrobial activity of *Trichosanthes dioica* against certain pathogens

Leaves extract: *M. smegmatis* >*S. aureus* > *E. coli* >*K. pneumonia* & *P. aeruginosa*
Fruits extract: *S. aureus* > *K. pneumonia* > *E. coli, P. aeruginosa* & *M. smegmatis* (Nil)
Seeds extract: *S. aureus* > *E. coli* > *K. pneumonia, P. aeruginosa* & *M. smegmatis* (Nil)
Streptomycin: *E. coli* & *P. aeruginosa* > *S. aureus* > *K. pneumonia* & *M. smegmatis* (Nil)

The data also reveals that the leaves and seeds extract showed identical patterns of zone of inhibition of *Escherichia coli* bacteria whereas, their patterns of inhibition against *Mycobacterium smegmatis* were just opposite. Hence the results suggest that Trichosanthes dioica plant is very promising plant not only for its antidiabetic and antioxidant potential but also for its antimicrobial potential with special reference of anti-infective and anti-tuberculosis. Though, the extent of antibacterial activity of Trichosanthes dioica extract was of the following order: leaves> fruits> seeds. Therefore, the most important finding is that its fruits and to some extent its seeds can be used as anti-infective whereas its leaves could be used for anti- tuberculosis treatment.

It is important to mention that lots of medicinal plants have been found to have antimicrobial activity, out of which some of them are part of our daily food for e.g. garlic and onion have considerable antimicrobial activity[29-31]. In the same way *Trichosanthes dioica*, used as vegetable in our daily food, was also found to possess antimicrobial activity. Since most of the plants rich in flavonoids possess antimicrobial activity[32] therefore, the flavonoids present in *Trichosanthes dioica* must be responsible for its antimicrobial activity.

ACKNOWLEDGEMENTS

The authors are grateful to Indian council of medical research (ICMR), New Delhi, India, for providing the financial assistance. The first author PKR is thankful to ICMR for the award of SRF.

REFERENCES

1. World Health Organization, Global Tuberculosis Report. WHO Report, 2004; Geneva, Switzerland
2. P. Sensi and G.G. Grassi. Chemotherapeutic agents. In: Abraham, D.J. (Ed.), Burger's Medicinal Chemistry and Drug Discovery, vol. 5, John Wiley & Sons Inc., 2003, pp. 821–824.
3. Chan ED, Iseman MD, Current medical treatment for tuberculosis. Brit Med J, 325: 1282–1286, (2002).
4. Basso LA, Blanchard JS, Resistance to anti-tubercular drugs. Adv Exp Med Biol, 456: 115–144, (1998).
5. Bastian I, Colebunders R, Treatment and prevention of multidrugresistant tuberculosis. Drugs, 58: 633–666, (1999).
6. Farombi EO, African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. Afr J Biotechnol, 2: 662-671, (2003).
7. Cragg GM, Newman DJ, Snader KM, Natural products in drug discovery and development. J Nat Prod, 60: 52–60, (1997).
8. Malika N, Mohamad F, Chakib EA, Antimicrobial activity of natural honey from aromatic and medicinal plants on antibioresistant strains of bacteria. Int J Agric Biol, 6: 289-93, (2004).

9. Stuffness M, Douros J, Current status of the NCI plant and animal product program. J Nat Prod, 45: 1-14 (1982).

10. Baker JT, Borris RP, Carte B. et al., Natural product drug discovery and development: New perspective on international collaboration. J Nat Prod, 58: 1325-1357, (1995).

11. Cohen ML, Changing patterns of infectious disease. Nature, 406: 762–767, (2002).

12. World Health Organization *Global Strategy for Containment of Antimicrobial Resistance*. Availabe on Internet 2001; at *www.who.int/emcdocuments. antimicrobial_resistance/docs/EGlobal_Start.pdf*

13. Hugo WB, Russel AD, *Pharmaceutical Microbiology*, 3rd edition, Blackwell Science Publications, pp179–200, (1984).

14. Reddy PS, Jamil K, Madhusudhan P, Anjani G, Das B, Antibacterial activity of isolates from Piper longum and Taxus baccata. Pharm Biol, 39: 236-238 (2001).

15. Erdourul OT, Antibacterial activities of some plant extracts used in folk medicine. Pharm Biol, 40: 269-273, (2002).

16. Ate DA, Erdourul OT, Antimicrobial activities of various medicinal and commercial plant extracts. Turk J Biol, 27: 157-162, (2003).

17. Maheshwari JK, Singh KK, Saha S, Ethnobotany of tribals of Mirzapur District, Uttar pradesh, *Economic Botany Information Service*, NBRI, Lucknow, 1986, pp 17.

18. Rai MK, Ethnomedicinal studies of Chhindwara district (M.P.): Plants used in stomach disorders. Indian Med, 1: 1-5, (1989).

19. Negi KS, Tiwari JK, Gaur RD, Pant KC, Notes on ethnobotany of five districts of Garhwal Himalaya, Uttar pradesh, India. Ethnobotany, 5: 73-81, (1993).

20. Taylor RSL, Manandhar NP, Hudson JB, Tower JHN, Antiviral activities of Nepalese medicinal plants. J Ethnopharmacol, 52: 157-163, (1996).

21. Chung TH, Kim JC, Kim MK, Choi SC, Kim SL, Chung JM, Lee IS, Kim SH, Hahn KS, Lee IP, Investigation of Korean plant extracts for potential phytotherapeutic agents against B-virus Hepatitis. Phytotherapy Research, 9: 429-434, (1995).

22. Vlietinck AJ, Van Hoof L, Totte J, Lasure A, Berghe DV, Rwangabo PC, Mvukiyumwani J, Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. J Ethnopharmacol, 46: 31-47, (1995).

23. Hostettmann K, Hamburger M, Antimicrobial effect of some native plants. Phytochem, 30: 3864–74, (1991).

24. Rai PK, Jaiswal D, Diwakar S, Watal G, Antihyperglycemic Profile of *Trichosanthes dioica* Seeds in Experimental Models Pharm. Biol, 46: 360-365, (2008).

25. Rai PK, Jaiswal D, Singh RK, Gupta RK, Watal G. Glycemic Properties of *Trichosanthes dioica* Leaves. *Pharm Biol*, 46: 894-899, (2008).

26. Rai PK, Jaiswal D, Rai DK, Sharma B, Watal G, Effect of water extract of *Trichosanthes dioica* fruits in STZ induced severe diabetes in rats. Indian J Clin Biochem, 23: 387-390, (2008).

27. Rai PK, Jaiswal D, Rai DK, Rai NK, Rai AK, Sharma B, Watal G, LIBS Based Detection of Antioxidant Elements: A New Strategy. Adv Prot Oxi Stress. 2009 (accepted).

28. Khan MR, Omotoso AD, Antimicrobial activities of medicinal plants used in folklore remedies in south-western. Fitoterapia, 74: 695-698, (2003).

29. Tsao SM, Yin MC, *In vitro* antimicrobial activity of four diallyl sulphides occurring naturally in garlic and Chinese leekoils. J Med Microbiol, 50: 646–649, (2001).

30. Benkeblia N, Antimicrobial activity of essential oil extracts of various onions

(*Allium cepa*) and garlic (*Allium sativum*). Leb Wiss Technol, 37: 263–268, (2004).

31. Lee CF, Han CK, Tsau JL, *In vitro* inhibitory activity of Chinese leek extract against *Campylobacter* species. Int J Food Microbiol, 94: 169–74, (2004).

32. Cavallito CJ, Bailey JH, Allicin, the antibacterial principle of (*Allium sativum* L.) Isolation, physical properties and antibacterial action. J Amer Chem Soc, 66: 195–200, (1944).