Research Article *ISSN: 2277-8713*
Khan JA, IJPRBS, 2012; Volume 1(6): 291-298 *IJPRBS*

ISSN: 2277-8713
IJPRBS
A Path for horizing your innovative work
INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

A STUDY ON ANTIBACTERIAL PROPERTIES OF *TINOSPORA CORDIFOLIA* LEAF, STEM, ROOT EXTRACTS

KHAN JA[1], SRIVASTAVA R[2]

1. R & D Division, MRD Life Sciences (P) LTD, Lucknow, Uttar Pradesh, India.
2. Jacob School of Biotechnology and Bioengineering, SHIATS, Allahabad.

IJPRBS-QR CODE *PAPER-QR CODE*

Accepted Date:

24/11/2012

Publish Date:

27/12/2012

Keywords

Tinospora cordifolia,

Zone of Inhibition,

Agar well Diffusion,

Solvent Extraction

Corresponding Author

Mr. Khan JA

R & D Division, MRD LifeSciences (P) LTD, Lucknow, Uttar Pradesh, India.

Abstract

Water (at room temperature and at elevated temperature), 70% ethanol, 80 % methanol, acetone, ethyl acetate and chloroform extracts of *Tinospora cordifolia* (Giloy) in a final concentration of 100 mg/ml were evaluated for their antibacterial properties against bacterial pathogens such as *Escherichia coli, staphylococcus aureus* and *Pseudomonas aeruginosa* using agar well diffusion method. Water (at room temperature and at elevated temperature), ethanolic and ethyl acetate extracts of *Tinospora* leaves showed an average inhibitory zone of 14mm, 14.5mm, 17mm and 12.5mm respectively which indicates that ethanolic extract shows best result having zone of inhibition more than that of tetracycline (12mm against all pathogens) while extracts of methanol, acetone and chloroform didn't show any result. In case of *Tinospora* stem and root best result was shown by hot aqueous extract with a zone of inhibition of 16mm and 17mm respectively. The ethanolic extract of root also gave a zone of 17mm.

INTRODUCTION

Tinospora cordifolia (Guduchi) a member of family *Menispermaceae* is a large, glabrous, perennial, deciduous, climbing shrub of weak and fleshy stem[1]. It is found throughout tropical part of India and also in China, Bangladesh, Myanmar and Srilanka. Plant refers wide range of soil, acid to alkaline and it needs moderate moisture level[2].

Uddin *et al*., 2011[3] used *Tinospora* for isolation of its secondary metabolites and evaluation of biological activities with special emphasis to the antimicrobial screening and cytotoxic study**.** The chemical constituents have been reported from this shrub belong to different classes, such as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides[1].

The notable medicinal properties have been reported including anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory and anti-neoplastic activities[4]**.**

Rose Mf, *et al.*, 2007[5] studied the antibacterial activities of the hot and cold methanol extracts of the roots of *Tinospora cordifolia* (Willd) Miers on bacterial strains like *Staphylococcus aureus*, *Shigella dysenteriae*, *Escherichia coli* and *Pseudomonas aeroginosa*

Natural/Herbal antimicrobials provide an alternative to conventional antibiotics medicines which are problematic for three main reasons: a) The use and over use of synthetic antibiotic has led to an alarming increase in drug resistant bacteria. b) Prescription antibiotics tend to wipeout good bacteria as well as bad this not only retards the body ability to fight back, it also makes room for drug resistant antibiotic to gain a toehold in the body and c) Herbal antibiotics are smart in a way that typically antibiotics are not.

Looking at the significance of herbal antimicrobial components and previous research interests [3, 5, 6, 7, 8] on antibacterial properties of *Tinospora cordifolia,* the present study was designed with an objective to study the antibacterial properties of *Tinospora cordifolia*.

MATERIALS AND METHODS

Plant Material

Fresh and healthy plant parts (leaves, stem and root) were collected from road side of Gomtinagar Vibhuti Khand, Lucknow, India after proper identification. They were identified according to their taxonomical classification and herbarium specimen. The *Tinospora cordifolia* plant parts were washed with the help of tap water followed by sterilized distilled water, dried and ground into fine powder using electric grinder and used throughout the study.

Bacterial Strain and Culture Preparation

The pathogenic strains used in the study were *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli.* All of them were available at MRD Life Sciences, Lucknow. Bacterial cultures were maintained on nutrient agar plates. They were sub-cultured weekly and subsequently stored at 4°C. The strains were inoculated in the nutrient broth (pH 7.0) and incubated at 37°C for 24 hours at 120 rpm.

Preparation of Antimicrobial Extracts By Solvent Extraction

Various solvents were used for the extraction of antibacterial components namely water, 70% ethanol, 80% methanol, ethyl acetate, acetone and chloroform. Extraction was carried out at room temperature and in some cases at an elevated temperature below the boiling point of the solvent. For extraction by hot water, 4g of the powdered plant part was soaked in 40ml of hot water and heated at 80°C for two hours and filtered with the help of What man's filter paper no. 1, in a weighed petriplate, and kept for drying in hot air oven. For rest extractions at room temperature, 4g powdered samples were soaked in 40ml of the solvent and kept in dark for 2-3 days. Solution was filtered and filtrate was collected in weighed petriplates, covered with foil having small pores and kept in hot air oven for 50°C for drying. After the evaporation the amount of antimicrobial extract was calculated by subtracting the weight of empty petriplate from the weight of the petriplate after evaporation. Antimicrobial obtained was dissolved in double volume of the DMSO (dimethyl sulphoxide). Thus giving the concentration of antimicrobial to be 500mg/ml. This was further diluted and used in a concentration of 100 mg/ml throughout the study.

Antibacterial Screening

In order to determine the antimicrobial spectrum, the antimicrobial activity was performed by agar well diffusion assay, also called cup plate method (Kirby Bauer method). Sterile NA media was prepared and then poured into sterile petriplates and allowed to solidify. 20µl of pathogen culture was spread on respective plates labeled earlier as *P. aeruginosa, S. aureus* and *E.coli*. Three or four wells of 8mm diameter were bored using a sterile borer. 50µl of tetracycline (50µg/ml, crude antimicrobial extract and DMSO/sterile distilled water were loaded in the respective wells and the plates were incubated at 37°C overnight. The antibacterial activity of each extract was expressed in terms of the mean of diameter of zone of inhibition (in mm) produced by each extract at the end of incubation period. All the tests were performed in triplicates.

RESULTS

Table 1-3 below show the results of antibacterial screening of various extracts of *Tinosporia cordifolia.*

DISCUSSION

Among the three plant parts of *Tinospora cordifolia* most effective plant part were leaves extract. This suggests that leaves contain active ingredients which qualify them for medicinal use. Hot aqueous, cold aqueous, extracts leaves showed an average inhibitory zone of 14mm and 14.5mm respectively which indicates that aqueous extract of *Tinospora cordifolia* leaves have some medicinal properties with a zone of inhibition about similar to that of tetracycline but there was no any zone of inhibition found in case of *P. aeruginosa* either by the plant extract or by the tetracycline. In case of *Tinospora cordifolia* stem and root best result was shown by hot aqueous extract with a zone of inhibition of 16mm and 17mm respectively. Earlier [9], investigated the allopathic effect of aqueous extracts of different parts of *Tinospora cordifolia* (Willd.) Miers on some weed plants and concluded that the aqueous extracts of *Tinospora* from leaf and stem parts at 2 and 4% concentration levels exhibited significant ($P < 0.05$) inhibition on germination and seedling growth.

The ethanolic and ethyl acetate extract of leaves showed an average zone of inhibition

of 17mm and 12.5mm respectively which can further be purified and modified for medicinal use. The ethanolic extract of stem did not show any activity against any of the pathogen while that of root gave a zone of 17mm. Ethyl acetate extract of stem didn't showed any activity against any pathogen while that of root gave a zone of inhibition of 13mm. No inhibitory zone was seen in case of chloroform and acetone extracts. According to [10] ethanolic, chloroform and ethyl acetate did not show any activity up to 20 µg/ml and was found to be comparable to the control. This suggests that it may be possible that by increasing the concentration of extracts its activity can be increased further.

Methanolic extract of root also showed an inhibitory zone of 11mm against *E. coli* and *S. aureus* which proves that the methanolic extract of *Tinospora cordifolia* roots can be used for medicinal purposes.[6] Revealed that *Tinospora cordifolia* is an excellent drug, which could be a good remedy for various ailments of animals as well as human beings yet the safety and potential indications in human beings and animals have to be established using modern techniques.

CONCLUSION

Based on the above research work it can be concluded that *Tinospora cordifolia* can be a good source for herbal drug and specially the solvents water, ethanol and ethyl acetate can be explored further for the extraction of antimicrobials by more sophisticated procedures for extartion in order to increase the yield.

Future work also includes the further pharmacological investigation of the solvent extracts and also the investigation of phytochemicals responsible for antimicrobial activity.

ACKNOWLEDGEMENT

Authors are thankful to the Director Mr. Manoj Verma for providing the facilities available at **MRD Life Sciences (P) Ltd., Lucknow**. We are also thankful to all the members of **MRD Life Sciences (P) Ltd., Lucknow** for their kind support. We also can't forget to acknowledge the almighty without whose consent nothing is possible.

Table 1

Antimicrobial Susceptibility Assay of *Tinospora cordifolia* Leaf Extract

Sr. No.	Extracts	Diameter of ZOI Against *E. coli* (in mm)	Diameter of ZOI Against *S. aureus* (in mm)	Diameter of ZOI Against *P. aeruginosa* (in mm)
1	COLD AQUEOUS	14.5	14.5	0
2	HOT AQUEOUS	12.5	14	0
3	ETHANOLIC	13	17	0
4	METHANOLIC	0	0	0
5	ACETONE	0	0	0
6	CHLOROFORM	0	0	0
7	ETHYL ACETATE	12.5	0	0

Table 2

Antimicrobial Susceptibility Assay of *Tinospora cordifolia* Stem Extract

Sr. No.	Extracts	Diameter Of ZOI Against *E. coli* (in mm)	Diameter Of ZOI Against *S. aureus* (in mm)	Diameter Of ZOI Against *P. aeruginosa* (in mm)
1	COLD AQUEOUS	10.5	13.5	0
2	HOT AQUEOUS	10.5	15.5	0
3	ETHANOLIC	0	0	0
4	METHANOLIC	0	0	0
5	ACETONE	0	0	0
6	CHLOROFORM	0	0	0
7	ETHYL ACETATE	0	13	0

Table 3

Antimicrobial Susceptibility Assay of *Tinospora cordifolia* Root Extract

Sr. No.	Extracts	Diameter Of ZOI Against *E. coli* (in mm)	Diameter Of ZOI Against *S. aureus* (in mm)	Diameter Of ZOI Against *P. aeruginosa* (in mm)
1	COLD AQUEOUS	13	12.5	0
2	HOT AQUEOUS	0	17	0
3	ETHANOLIC	13	17	0
4	METHANOLIC	11	11	0
5	ACETONE	0	0	0
6	CHLOROFORM	0	0	0
7	ETHYL ACETATE	0	0	0

Note: well Diameter= 8mm, ZOI = Zone of Inhibition; ZOI by Tetracycline = 12 mm against all The Pathogens

REFERENCES

1. Upadhyay AK, Kumar A and Mishra HS: *Tinospora cordifolia* (Willd.) Hook. F. and Thoms. *(Guduchi)* – Validation of the Ayurvedic pharmacology through experimental and clinical studies. Int J Ayurveda Res. 2010; 1(2): 112–121.

2. Sharma A, Gupta A, Singh S and Batra A: *Tinospora cordifolia* (Willd.) Hook. F. & Thomson - A plant with immense economic potential. J. Chem. Pharm. Res. 2010; 2(5): 327-333.

3. Uddin MH, Hossain MA, and Kawsar MH: Antimicrobial and cytotoxic activities of *Tinospora cordifolia* (FAM: Menispermaceae), IJPSR. 2011; 2(3): 656-658.

4. Singh SS, Pandey SC, Srivastava S, Gupta VS, Patro B and Ghosh AC: Chemistry and medicinal properties of *Tinospora cordifolia* (Guduchi). Indian Journal of Pharmacology. 2002; 35: 83-91.

5. Rose MF, Noorulla KM, Asma M, Kalaichelvi R, Thangabalan KVB and Sinha BN: *Invitro* antibacterial activity of methanolic root extract of *Tinospora cordifolia* (willd). IJPRD/2010/PUB/ARTI/VOV-2. 2007.

6. Sankhala LN, Saini RK and Saini BS: A review on chemical and biological properties on *Tinospora cordifolia*. Int. J. Med. Arom Plants, 2012.

7. Jeyachandran R, Xavier TF and Anand SP: Antibacterial activity of stem extracts of *Tinospora cordifolia* (willd) hook. F & Thomson. Ancient Science of Life. 2003; XXIII (1).

8. Aher VD and Wahi AK. 2010. Pharmacological Study of *Tinospora Cordifolia* as an Immunomodulator, International Journal of Current Pharmaceutical Research. 2010; 2(4).

9. Raoof KMA and Siddiqui MB: Allelopathic effect of aqueous extracts of different parts of *Tinospora cordifolia* (Willd.) Miers on some weed plants. Journal of Agricultural Extension and Rural Development. 2012; 4(6), 115-119.

10. Ranjith MS, Ranjitsingh AJA, Shankar SG, Vijayalaksmi GS, Deepa K and Sidhu HS: Enhanced Phagocytosis and Antibody Production by *Tinospora cordifolia* - A new dimension in Immunomodulation. African Journal of Biotechnology. 2007; 7 (2), 081-085.