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In vitro Antimicrobial Activity of Tinospora cordifolia and its Phytochemical screening

Nagaprashanthi.Ch*, Rafi khan.P, Gopi chand.K, Aleemuddin MA, Rajiya Begum.G.

Department of Pharmacy,PRIST University,Thanjavur, Tamil Nadu.,India.

***Corres.author: shanthi.chiluvuru@gmail.com**
Phone no.:07200242668.

Abstract: The present investigation was carried out to evaluate the *in vitro* antifungal and antibacterial activity of hydro alcoholic extract of *Tinospora cordifolia* creeped on *Azadirachta indica* Tree (TC_1) in comparison with that of *Tinospora cordifolia* (TC_2) creeped on fencing. Hydroalcoholic extract of *T.cordifolia* stem was prepared by maceration technique. The microorganisms used as antibacterial and antifungal were *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pseudomonas sp, Aspergillus niger, Aspergillus fumigates, mucor sp* and *Pencillium*. The extract of TC_1 exhibit effective antimicrobial activity against all the organisms, while the extract of TC2 exhibits inhibition zone on limited species such like *Staphylococcus aureus* (12 mm), *Klebsiella pneumonia* (10 mm), *Pseudomonas sp* (8 mm), *Aspergillus niger* (6 mm), *Aspergillus fumigates* (8 mm) *and mucor sp* (12 mm). The results suggest that *T.cordifolia* creped on neem tree having the potential antimicrobial activity similar to *Azadirachta indica.* This can explain that the host plants (T.cordifolia) will encorporate the medicinal virtue when they survive on neem plants. It is expected that using *T.cordifolia* as therapeutic agents for treating infections in traditional medicine.
Key words: Antifungal, Antimicrobial, Hydroalcoholic extract, *Tinospora Cordifolia, Azadirachta indica.*

INTRODUCTION

In developing countries, bacterial infections are extensive due to reduced sanitation and unhygienic conditions. Infectious diseases are the world's primary roots for premature deaths, killing almost 50,000 people every day. Human infections, predominantly those linking with skin and mucosal surfaces constitute a serious problem, particularly in tropical and subtropical developing countries[1]. The hunt for new effective antimicrobial agents is necessary to resist bacterial and fungal pathogens due occurrence of fatal opportunistic infections associated with AIDS, antineoplasic chemotherapy and transplants.

Medicinal plants are an important medicinal aid for various diseases. Scientific experiments on the antimicrobial properties of plant components were first documented in the 19th century[2].Ethnobotanical data is helpful in providing new antimicrobial therapy[3]. The use of current microbiological techniques demonstrates that medicinal plants normally exhibit significant strength against human bacterial and fungal pathogens [4,5,6]. In India, medicinal plants are extensively used by ancient people as folk remedies but in this recent year these herbal drugs are widely using in the pharmaceutical

preparations of modern medicines[7]. According to National Health Experts, 2000 different plants are used for medicinal preparations for both internal and external use in India alone [8]. Up to now, only 10 % of higher plants were chemically investigated and about 80% of the world population is dependent herbal drugs. Plants contain numerous biologically active constituents, many of which have been exposed to have antimicrobial properties. Medicinal plants are of important therapeutic aid for various human ailments. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century. The screening of plant extracts and natural products for antimicrobial activity has shown that higher plants represent a potential source of new anti-infective agents.

The plant *Tinospora Cordifolia* (Willd.) is commonly known as Guduchi and it belongs to family Menispermaceae[9]. It is large, deciduous extensively climbing. During last two decades, the drug has been subjected to extensive Phytochemical, pharmacological and clinical investigations and many interesting findings in the areas of immunomodulation[10], anticancer activity[11], liver disorders[12], antidiarrhoeal[13], anti-oxidant activity[14,15,16], aphrodisiac activity[17], anthelmintic activity[18,19], antipsychotic activity[20] and hypoglycemic [21] are reported.

Guduchi plant growing on Neem tree (*Azadirachta indica*) is bitterer and more efficacious and is said to encorporate the medicinal virtue of Neem also [22]. However, in vitro antifungal and antimicrobial activity of hydro alcoholic extract of *Tinospora cordifolia* creeped on Neem tree in comparison with that of creped on a fencing has not so far been scientifically proved, so the present study was carried out to assess the antifungal and antimicrobial activity of *Tinospora Cordifolia* on *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pseudomonas sp, Aspergillus niger, Aspergillus fumigates, mucor sp* and *Pencillium.*

MATERIALS AND METHODS

Plant collection and authentification:
Tinospora Cordifolia (Menispermaceae), matured stems was collected locally at Thanjavur, Tamilnadu, India and were identified and authenticated (BSI/SRC/5/23/2011-12/Tech-1055) by Dr. G.V.S.Murthy, Scientist 'F' & Head of Botanical Survey of India, Coimbatore, India.

Preparation of hydro alcoholic extract:
The plant material (matured stems) was collected and shade dried. These dried stems then crushed and powdered by using electrical grinder. The powdered material was extracted by hydroalcoholic (2:8) using maceration method [23]. The coarsely powdered plant material was placed in a stoppered container with the solvent and was allowed to stand at room temperature for 14 days with frequent agitation until the soluble matter was dissolved. The mixture was strained, the marc (the damp solid material) is pressed, and the extract was concentrated using distilled evaporator.

Preliminary Phytochemical Screening:
Hydroalcoholic extracts of *Tinospora Cordifolia* stem were subjected to preliminary phytochemical screening for the presence or absence of various active metabolites [24,25].

Test microorganisms:
In this present study the test microorganisms used (bacteria: *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumonia*; fungi: *Aspergillus niger, Aspergillus fumigates, mucor sp* and *Penicillum sp* were obtained from the culture collections of Department of Microbiology, PRIST University, Thanjavur, Tamil Nadu, India. The bacterial were first isolated and subcultured in a nutrient broth (Oxoid) and incubated at 37°C for 24 h while the fungal were isolated and subcultured on a Rose bengal agar (RBA) for 72 h at 25°C.

Antibacterial activity:
The antibacterial activity of the hydroalcoholic extracts of *Tinospora cordifolia* was determined by agar-well diffusion method. The isolated bacterial were first cultured in a nutrient broth for 24 h before use. 200 µl of the standardized cell suspensions were spread on a Mueller-Hinton agar (Oxoid). Sterile 4 mm diameter of cork borer was used to bored wells into the MH agar. Approximately 100 µl of 10 mg *T. cordifolia* (TC1 & TC2) extract were introduced into the wells separately, allowed to stand at room temperature for about 2 h and then incubated at 37°C. The plates were observed for zones of inhibition after 24 h and compared with Gentamycin at a concentration of 10 mg/ml.

Antifungal activity:
The fungal organisms were first isolated and allowed to grow on a rose bengal agar (RBA) (Oxoid) at 25°C for 72 h. The fungi were harvested after sporulation by pouring distilled water on the surface

of the plate and later scraped the spores with a sterile glass rod. 100 µl of the standardized fungal spore suspension was spread on the Potassium Dextrose Agar (PDA) using a glass spreader. Sterile 4 mm diameter of cork borer was used to bored wells into the PDA. Approximately 100 µl of 10 mg *T. cordifolia* extract were introduced into the wells and allowed to stand (1h) for proper diffusion of the extract into the media. The plates were observed for zones of inhibition after 72 h at 25°C and compared with ketokanazole at a concentration of 10 mg/ml.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of the extracts of *Tinospora cordifolia* showed the presence of Alkaloids, carbohydrates, flavonoids, terpenoids, sterols and tannins (Table.1). These chemical constituents present in the hydroalcoholic extract of *T.cordifolia* have many therapeutic values. Thus further work can be carried on the isolation procedure for finding out the exact moiety responsible for the biological activity.

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the natural therapies [26] .The antimicrobial assay showed that hydroalcoholic extract of *Tinospora cordifolia* stem exhibited *in-vitro* antibacterial activity against Grampositive and Gram-negative bacteria. Hydroalcoholic extract of TC1 does not show any activity on *Escherichia coli*. The results reveal that extracts of TC1 stem were significantly effective against both Gram-positive and Gram-negative organism when compared to TC2. (Table.2)

Table.1. Preliminary phytochemical screening of Hydroalcoholic stem extracts of *Tinospora cordifolia*.

S.No	Phytochemical Test	Hydroalcoholic extract of *Tinospora cordifolia*
1	Carbohydrates	+
2	Proteins and Amino acids	-
3	Alkaloids	+
4	Flavonoids	+
5	Steroids	+
6	Triterpenoids	-
7	Glycosides	+
8	Tannins	+
9	Cholesterol	-
10	Phenols	+
11	Saponins	+

Table.2 Antibacterial activity of hydro alcoholic stem extract of *Tinospora cordifolia.*

Microorganism	Zone of Inhibition (mm)			
Gram +ve/ Gram –ve	Test samples			
	Control	TC1	TC2	Gentamicin (10 mg/ml)
Staphylococcus aureus	04	14	12	17
Escherichia coli	04	12	-	15
Klebsiella pneumonia	04	11	10	13
Pseudomonas sp	04	12	08	16

The variation in the effectiveness could be due to the present of different active constituents in the plants. Both Gram +ve and Gram –ve bacteria were more sensitive to TC1.

Table.3 Antifungal activity of hydro alcoholic stem extract of *Tinospora cordifolia.*

Microorganism	Zone of Inhibition (mm)			
	Test samples			
	Control	TC1	TC2	Ketoconazole (10 mg/ml)
Aspergillus niger	**04**	**12**	**06**	**17**
Aspergillus fumigates	**04**	**09**	**08**	**19**
Mucor sp	**04**	**17**	**12**	**23**
Pencillium	**04**	**07**	**-**	**18**

The larger zones of inhibition exhibited by TC1 extract may be due to the presence of variety of active compounds. This is well known, since tannins and saponins are important plant metabolites which is responsible for their antimicrobial activity [27]. From the results obtained, the stem extract of TC1 showed antifungal activity among the entire fungal organism when compared to TC2. This suggests that *T.cordifolia* contains more of the active compounds and has high potency.

CONCLUSION

It may be concluded from this study that *Tinospora cordifolia* stem extract creping on *Azadirachta indica* has potential antimicrobial activity similar to that of neem tree when compared to *Tinospora cordifolia* creping on fencing. This can explain that the host plant will gain some of the activities when they survive on medicinal plants. It is essential that research should continue to isolate and purify the active components of this natural herb and use in experimental animals.

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