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PROSPECTING ANTIMCROBIAL POTENTIAL OF *Tinospora cordifolia* (Thunb.) Miers. AND ITS PHYTOCHEMICAL EVALUATION

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ABSTRACT *Tinospora cordifolia*., is shrub of the Family Menispermaceae which is used in many traditional medicines. It is rich in many phytochemicals like flavanoids, alkaloids, tanins etc. The bio potential of some parts was not explored. In the present work, we investigate the preliminary phytochemicals of chloroform extract of leaf and stem. The antimicrobial efficacy of these extract against selected microorganisms namely against *E.coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis and Salmonella typhi* were evaluated. Similarly, antifungal activity was also evaluated against selected fungal strains. The leaf and stem extracts were found very significantly inhibiting *E.coli* at the highest concentrations very effectively. The extract also inhibited growth of *Aspergillus* and *Rhizopus sp*. These extracts were efficiently inhibiting the multidrug resistant pathogenic strains. The phytochemical analysis revealed that the extract contained alkaloids, tannins, terpenoids etc. which may be attributed to the antimicrobial effect of the extract. The chloroform extract of the *T.cordifolia* was less explored for its bio-potential and hence the work is a preliminary report with significant findings of its bio-potential.

KEYWORDS : *T.cordifolia*, antimicrobial, antifungal, multidrug resistance, phytochemicals

INTRODUCTION

Plant based drugs have been the part and parcel of Indian medicinal system and it is since times immemorial. Many of these drugs were formulated based on our ancient literature and through indigenous knowledge passed on from ancestors. The emergence of modern medicine has pushed the modern men towards it due to the ease of consumption. However, the challenges like antibiotic resistance in may pathogens made the science community to switch over to the phytomedicines as they do not show such mechanisms.

Tinospora cordifolia, a shrub native to India is widely used as phytomedicines. But the scientific evidences to support its potential is not much available.

Various parts of *T.cordifolia* like root, stems, and leaves are used in Ayurvedic medicine. It is used for, lowering high cholesterol, against allergic rhinitis, diabetes, upset stomach, gout, lymphoma and other cancers, rheumatoid arthritis , hepatitis, peptic ulcer disease, fever, gonorrhea, syphilis, and also to boost the immune system. In the recent days, this plant has been subjected for numerous chemicals, pharmacological, pre-clinical and clinical investigations and many new therapeutic applications have been identified **[1]**. Decoction of stem is administered orally by the people of Jammu and Rajasthan for the treatment of fever and Juice or decoction of leaves is administered orally with honey in fever by the local people of Punjab **[1]**.

The chemical constituents reported from this shrub belong to different classes such as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides. These phyto-constituents are responsible for its various bio-potentials which include 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 **[2]**. The aqueous extract of *Tinospora cordifolia*, was also observed to possess immunomodulatory effect along with antimalarial activity **[3].**

The modern men understand the need for phyto-medicines in their life. Hence, there is a high scale prospecting of new plant based formulations against many diseases. The present research paper focuses on the preliminary study of phytochemical analysis of leave and stem extract of *T. cordifolia* and to evaluate antibacterial and antifungal activity against selected pathogens.

Materials and Methods

Collection of Plant Material

Leaf and stem samples of *Tinospora cordifolia* were collected from Namakkal District of Tamil Nadu, India. The samples were shade dried and powdered for extraction of phytochemicals.

Preparation of extract

Tinospora cordifolia leaves and stems collected were dried, powdered and 10 g of the powder was subjected to Soxhlet extraction. Chloroform extraction was performed overnight to obtain concentrated extract of the leaves and stem. Further the extract was concentrated using a rotary evaporator and was stored at 4°C for further use.

Phytochemical Analysis of Extract

Phytochemical evaluation of the leaf extract of *T. cordifolia* was performed using the standard procedures. Different phytochemicals, namely flavonoids, saponins, steroids, terpenoids, tannins, carbohydrates and glycosides were screened **[4]**.

Antibacterial Activity

The modified agar well diffusion method was employed to evaluate the antibacterial activity. 24hrs old bacterial cultures of *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa and Salmonella typhi* were inoculated onto Muller Hinton agar media (HiMedia, Mumbai) by spread plate technique. 6mm diameter wells were made using sterile gel puncher. Various extracts of *T. cordifolia* leaves and stem were dissolved in DMSO (Dimethyl Sulfoxide) from which 25, 50 75 and 100 µl of extract were added into agar wells. The plates were sealed and incubated at 32 ± 2 °C for 24 hr. The inhibition zone diameter was recorded after the incubation period. All the experiments were conducted in triplicates **[5].**

Antifungal Activity

The modified agar well diffusion method was employed to evaluate the antifungal activity. 1 week old fungal cultures of *Rhizophus sp. Penicillium sp. Aspergillus niger and Aspergillus flavus* were inoculated onto Muller Hinton agar media (HiMedia, Mumbai) by spread plate technique. 6mm diameter wells were made using sterile gel puncher. Various extracts of *T. cordifolia* leaves and stem were dissolved in DMSO (Dimethyl Sulfoxide) from which 25, 50 75 and 100 µl of extract were added into agar wells. The plates were sealed and incubated at 25 ± 2 °C for 24 hr. The inhibition zone diameter was recorded after the incubation period. All the experiments were conducted in triplicates **[5].**

Results and Discussion

Phytochemical Analysis of *T. cordifolia*

Phytochemical analysis of the *T. cordifolia* leaf and stem chloroform extract was performed (Table 1). The results revealed the presence of flavonoids, saponins and terpenoids in both the extracts. Steroids, tannins and glycosides were not detected in both extracts. Terpenoids were detected in both extracts. However, in a previous study, stem extract revealed the presence of phenols, flavonoids, alkaloids, saponins, cardiac glycosides, steroids, carbohydrate and proteins [6].

Table 1. Phytochemical analysis of leaf extract of *T.cordifolia*

Phytochemicals	Extracts	
	Leaf	Stem
Flavonoids	+	+
Saponins	+	+
Steroids	-	-
Terpenoids	+	+
Tannins	-	-
Carbohydrates	+	-
Glycosides	-	-

Antimicrobial Activity of *T.cordifolia*

Antimicrobial activity of *T.cordifolia* leaf and stem extracts were evaluated using well diffusion assay. Pathogenic cultures namely *E.coli*, *Bacillus subtilis, Salmonella typhi* and *Pseudomonas aeruginosa* were used in the experiment. The spread plated Muller Hinton media were punched and added 2 different concentrations of aqueous bark extract and incubated for 24 hrs. The zone of inhibition was measured following incubation time (Table 2).

The multidrug resistant isolates were used for the study and there was a good inhibition observed in all extracts tested. The activity was compared with standard antibiotics chloramphenicol, ethryomycin and methicillin. All the three were found to be multi-drug resistant isolates. The zone of inhibition was measured in mm and tabulated in Table 2. A higher inhibition was observed in case of *E.coli* at higher concentration of 100 µg tested. The antibacterial activity of *T.cordifolia* leaves and stem were more or less similar. In case of antifungal activity, leaf extract inhibited both *Aspergillus sp*. used while the stem extract inhibited the *Rhizophus sp*. and *Penicillium sp* (Table 3).

The results observed were supported by the results obtained by Mishra et al. [7]. However, they used the the crude ethanol extract of *T. cordifolia* which exhibited activity against tested bacteria except for *E.coli, Proteus vulgaris* and *Pseudomonas aeruginosa*.

Conclusion

This study revealed that the various chloroform extract of leaf and stem of *T.cordifolia* were suitable as an antimicrobial agent against *E.coli*, *Pseudomonas*, *Bacillus* sp. and *S. typhi*. In case of *E.coli,* there was significant inhibition observed at highest concentrations tested. A high antifungal activity was also observed. The phytochemical analysis revealed that the extract possesses flavonoids, tannins, terpenoids etc. which may be attributed to the antimicrobial effect of the extract.

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Table 2. Antimicrobial efficacy of leaf and stem extract of *T.cordifolia* against selected pathogenic bacteria

Microorganisms	Zone of Inhibition (mm)			
	Extract Concentration (µg/mL)			
	25	50	75	100
Leaves				
B.subtilis	14 ± 0.23	16 ± 0.19	18 ± 0.12	22 ± 0.10
E.coli	25 ± 0.18	29 ± 0.26	30 ± 0.21	32 ± 0.18
P.aeruginosa	18 ± 0.06	20 ± 0.13	25 ± 0.20	27 ± 0.27
S.typhii	17 ± 0.15	19 ± 0.19	21 ± 0.27	25 ± 0.25
Stem				
B.subtilis	16 ± 0.26	20 ± 0.24	22 ± 0.19	26 ± 0.21
E.coli	20 ± 0.15	25 ± 0.17	29 ± 0.20	30 ± 0.26
P. aeruginosa	12 ± 0.11	16 ± 0.10	18 ± 0.27	20 ± 0.32
S.typhii	12 ± 0.13	14 ± 0.09	16 ± 0.35	20 ± 0.29

[Values represent Mean ±S.D of triplicate experiments]

Table 3. Antifungal activity of leaf and stem extract of *T.cordifolia* against selected fungus

Microorganisms	Zone of Inhibition (mm)			
	Extract Concentration (µg/mL)			
	25	50	75	100
Leaves				
Rhizopus sp.	15 ± 0.13	18 ± 0.20	21 ± 0.18	26 ± 0.25
Aspergillus flavus	20 ± 0.27	24 ± 0.19	26 ± 0.17	30 ± 0.11
Penicillium sp.	14 ± 0.23	19 ± 0.07	24± 0.08	28 ± 0.19
Aspergillus niger	15 ± 0.21	20 ± 0.24	25 ± 0.11	30 ± 0.12
Stem				
Rhizopus sp.	20 ± 0.28	23 ± 0.05	25 ± 0.16	30 ± 0.10
Aspergillus flavus	17 ± 0.12	19 ± 0.17	22 ± 0.09	28 ± 0.20
Penicillium sp.	15 ± 0.16	18 ± 0.15	26 ± 0.07	31± 0.26
Aspergillus niger	16 ± 0.06	18 ± 0.08	21 ± 0.23	27 ± 0.21

[Values represent Mean ±S.D of triplicate experiments]

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