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Antibacterial Studies on Toona Ciliata. Roemer.

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Abstract: *The use of higher plants and their extracts to treat infectious is an age old practice in traditional Indian medicine. The aromatic and medicinal plants represent enormous reservoir of potential microbicidal compounds that could be useful alternative to synthetic microbicides and are being used to develop drugs. In the present study Toona ciliata Roemer. leaf, stem and root powder extracts were tested against ten different randomly selected bacteria by disc diffusion method. It was found the methanol extract strongly effective against all the chosen bacteria. The Minimum inhibitory concentration value ranges from 10mg/ml to 35mg/ml.*
Key words*: Antibacterial study, Minimum Inhibitory Concentration*

I. Introduction

Medicinal plants and herbal plants has assumed greater importance in recent days, due to the tremendous potential they offer in formulating new drugs against many disease and illness that affect the human kind. Plants have been used in the traditional health care system from time immemorial, particularly among tribal communities. In view of the rich diversity of Indian medicinal plants it is expected that screening and scientific evaluation of plant extract for their antimicrobial substance may prove beneficial for the mankind. Further, synergistic interaction among crude extracts or phytoconstituents *in vitro* may be useful in the preparation of improved polyherbal or drugs formulations. (**Pradeep - Parihar *et al.*, 2003**).Thus, keeping this view in mind medical communities are now trying to seek the solution of above said problems from plants based on medicines in allopathy. Thus, keeping this view in mind medical communities are now trying to seek the solution of above said problems from plants based on medicines in allopathy.

II. Materials and Methods

Plant parts of *Toona ciliata* Roemer. Were collected from hills of Western Ghats. The Taxonomic identities of plants were confirmed by the 'Flora of Presidency and Flora of Carnatic (Gamble **1**: 186 (133). **1915**) for the Botanical verification and authenticating the plant material. Fresh plant materials were shade dried and homogenized to a fine powder.

Extracts were made from air dried samples. 60g of the leaf stem and root (powdered test materials) was extracted successively with of 400ml of petroleum ether ($60\text{-}80^{o}$c), benzene, chloroform and methanol. This sequence of solvents allows for leaching all compounds based on their polarity. The individual fractions were collected and concentrated to obtain crude extracts. The above solvents were diluted and the final concentration was 5-10 mg/ml of solvents used for bacterial bio-assay. Filter paper disc of 6mm diameter of Whatman filter paper No.1 were soaked in solution for an hour and dried at room temperature.

The antibacterial activity was tested against ten randomly selected bacterial strains such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Salmonella typhii, Serratia marcescens, Klebsiella pneumonia, Enterobacter aerogenes, proteus vulgoris* and *Bacillus subtilis.* The selected bacterial strains were obtained fromDepartemnt of Microbiology, Sri Paramakalyani College, Alwarkuruchi.

The media used for antibacterial tests were Muller Hinton (MH) agar (Himedia Laborotories Pvt. Ltd, India). Each organism was maintained in a respective culture medium and was recovered for testing by sub culturing on a fresh media.

Antibacterial assay was demonstrated by a modification of the method described by Bauer *et al.*, (1966). 0.5ml of the dilute microbial culture was spread on sterile Muller Hinton Agar plates. The presoaked and dried discs were placed on the seeded plates and gently pressed down to assure contact.

Streptomycin 10 mg/ml was used as positive control and the respective solvents which were used to dissolve the crude extracts served as negative control. The plates were incubated at room temperature for 24hrs. After the incubation period the inhibition zone around the discs were measured and recorded. Three replicates for each concentration were maintained.

MINIMAL INHIBITORY CONCENTRATION (MIC)

Antibiotic sensitivity test was performed by commonly used agar diffusion method (Ruckmani *et. al., (*1996) which is designed to determine the smallest amount of the antibiotic needed to inhibit growth of

microorganisms. The resulting value is called the minimum inhibitory concentration (MIC). The test for minimum inhibitory concentration was carried out by using methanol extract against selected gram positive and gram negative organisms. To determine potential of extract, crude extract was diluted up to 100mg /ml, 35mg/ml 20mg / ml, 10mg /ml and 5mg/ ml. concentration by dissolving in methanol. Assay plates seed with bacterial cultures were incubated at room temperature for 12 hours. After incubation MIC was determined by measuring the zone of inhibition.

III. Results and discussion

Toona ciliata Roemer is an important medicinal plant which has been used for many centuries throughout the south pacific. It is a small shrub, three to five meters height. Results of antibacterial property of crude extracts and standard antibiotic (Streptomycin) were summarized in Table 1. Petroleum ether, Benzene, Chloroform, Ethanol and Distilled water, as pure solvent was not showed antibacterial activity.

Antibacterial activity of leaf extract: Extract of *Toona ciliata* Romerleaves in petroleum ether solvents was represented anti bacterial activity against four chosen bacteria. The Benzene extracts pronounced antibacterial activity by inhibiting the growth of almost all isolates used except *Streptococcus pyogenes,* and S*almonella typhii*. Methanol extracts exhibited good antibacterial activity inhibited growth of all the isolates used. The aqueous extract was effective against all the bacterial cultures except *Staphylococcus aureus, Enterobactor aerogenes* and *Bacillus subtilis.*

Antibacterial activity of stem extract: Petroleum ether stem extracts had no activity against *Klebsiella pneumoniae.* The Benzene extract was inhibitory in the order *Staphyloccus aureus* < *Pseudomonas aeruginosa* < Streptococcus *pyogenes* < *Bacillus subtilis* < *Serratia marcescens.* The chloroform extract showed inhibitory activity against all bacteria except S*almonella typhi.* The methanol and water extracts showed inhibition zone against all the used bacterial cultures. The maximum inhibitory zone reported against *Klebsiella pneumoniae* in methanol extract while comparing with the other extracts

Antibacterial activity of root extract: Petroleum ether root extract showed antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Salmonella typhi*, *Enterobacter aerogenes* and *Bacillus subtillis.* Benzene extract reported inhibitory zone against all bacteria except S*taphylococcus aureus, Klebsiella pneumoniae* and *Enterobacter aerogenes*. The chloroform extracts was moderately produce inhibition zone against all bacteria except *Streptococcus pyogenes* and *Proteus vulgaris*. All the test organisms were susceptible to methanol, water extracts and control drug.

Thirty-one species in twenty genera of the plant family Meliaceae were assayed for the production of growth-inhibiting phytochemicals. Most species were inhibitory when methanolic extracts were incorporated into artificial diets at concentrations at or below those occurring naturally. (Kraus *et al*., 1978).

The aqueous extract and ethanol extract exhibit a high degree of activity and this seems to confirm the traditional therapeutic claims of three herbs. Among the five tested extracts ethanol in root (3.3 mm), stem (2.4mm) and Leaf (2.8 mm) registered maximum inhibitory effect on *Bacillus subtilis, Klebsiella pneumoniae* and *Streptococcus pyogenes* respectively.

This result is in agreement with the earlier reported of Hiremath *et. al.,* (1997) that the higher plants have alkaloids and flavonoids which control the growth of microbial pathogen. Natural plant protecting agents are Toonacilin, R ═H, and its 6-acetoxy derivatives whose structure has now been elucidated. They occur in the bark of a *Toona ciliata* species; extracts exert a powerful ant feeding effect on the Mexican bean beetle.

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Table 1: Antibacterial activity of leaf, stem and root of *Toona ciliata* ***L.***

S NO	Solvent name	Plant Part	Inhibition zone (m.m)									
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	Petroleum ether (40 -60)	Leaf Stem root	- 0.6 1.3	1.0 0.6 0.7	1.5 0.7 -	- 2.2 0.7	- 1.2 1.2	- 1.4 -	-1.2 - -	- 0.8 0.7	- 1.0 -	2.0 0.7 0.7
2	Benzene (79 – 81)	Leaf Stem root	0.7 - 0.7	0.9 0.7 1.0	1.2 0.6 -	- 0.9 1.2	- - 1.0	0.7 1.4 1.0	1.4 - -	1.4 - -	0.8 - 0.7	- 1.2 1.0

3	Chloroform (59 -61)	Leaf Stem root	1.0 0.7 0.8	0.8 1.0 0.5	1.2 0.9 1.0	0.9 0.8 -	- - 0.9	1.2 0.7 1.4	0.9 1.1 1.9	- 0.5 1.1	1.2 0.5 -	- 05 1.3
4	ethanol	Leaf Stem root	1.9 2.3 14	1.2 2.1 1.4	1.9 1.5 2.2	2.8 0.7 1.6	1.9 1.7 2.0	2.2 1.6 1.5	1.7 2.4 2.3	1.8 1.5 1.9	2.7 1.4 1.7	1.2 1.4 3.3
5	Distilled water	Leaf Stem root	1.1 0.7 1.9	1.0 1.2 0.8	- 2.2 0.9	0.9 1.3 1.9	1.2 1.0 1.2	1.2 0.9 1.0	1.5 1.2 2.3	- 0.9 1.3	1.8 0.9 -	- 1.0 0.8
6	Positive control	Leaf Stem root	2.5 2.9 0.9	1.4 1.5 3.5	4.8 4.0 2.2	3.0 2.3 2.7	2.6 2.7 2.8	1.7 3.0 3.3	1.9 2.11 2.4	2.5 2.0 2.5	1.9 3.0 1.0	1.6 3.7 2.6

1.Escherichia coli. **2.**Pseudomonas aeruginosa. **3.**Staphylococcus aureus. **4.**Streptococcus pyogenes. **5.**Salmonella typhii. **6.**Serratia marcescens. **7.**Klebsiella pneumonia. **8 .**Enterobacter aerogenes**. 9.** Proteus vulgoris and **10.** Bacillus subtilis.

IV. Minimum Inhibitory Concentration:

The lowest concentration of the herbal extract that inhibited the growth of micro organisms completely was regarded as minimum inhibitory concentration. The test were performed and presented in the table 2

The leaf methanol extract showed the minimal inhibitory concentration 10mg / ml was less to *Staphylococcus aureus, Salmonella typhii, Serrasia marcescens, Klepsiella pneumoniae* and *Bacillus subtillus* when compared to that of other microorganisms tested. It was high to *E.coli* indicating tested that this microorganisms. The minimum inhibitory concentration of stem methanol extract was reported less to *Staphylococcus aureus, Streptococcus pyogenes, Salmonella typhii, Kelbsiella pneumoniae* and *Enterobacter aerogenes* with 100 mg / ml concentration, when compared to those of other microorganisms tested. The root methanol extract was strongly produce inhibitory towards P*seudomonas aureginoase, Ptreptococcus pyogenes, Salmonella typhii, Proteus vulgaris and Bacillus subtilis* in the low concentration 10 mg / ml .

Table 2.Minimum inhibitory concentration for methanol extracts of leaf, stem and root of *Toona ciliata* L. (mg/ml)

S No	Bacteria	Plant part	100 / 1	35/1	20/1	10/1	5/1
1	*E. coli*	Leaf Stem root	1.3 1.0 1.5	- 0.9 1.4	- 0.9 -	- - -	- - -
2	*Pseudomonas aeruginosa*	Leaf Stem root	1.2 1.0 1.1	1.0 0.8 1.0	0.9 - 1.1	- - 0.9	- - -
3	*Staphylococcus aureus*	Leaf Stem root	0.9 0.7 2.1	0.6 0.8 1.5	0.7 0.7 -	0.6 0.7 -	- - -
4	*streptococcus pyogens*	Leaf Stem root	1.3 1.2 1.2	1.0 1.0 1.1	0.9 0.9 1.0	- 0.8 0.9	- - -
5	*Salmonella typhii*	Leaf Stem root	1.4 2.0 1.6	0.9 1.0 1.2	1.0 1.0 1.2	0.8 0.9 1.1	- - -
6	*Serratia Marcescens*	Leaf Stem root	1.4 1.2 1.1	0.8 1.0 0.9	0.6 0.8 0.9	0.6 - -	- - -
7	*Klebsiella pneumoniae*	Leaf Stem root	1.9 2.0 1.5	1.3 1.0 1.1	0.6 1.2 0.9	0.6 0.9 -	- - -
8	*Enterobacter aerogenes*	Leaf Stem root	1.7 2.0 1.2	1.0 1.9 0.9	0.9 0.7 0.9	0.7 0.6 -	- - -

9	*Proteus vulgaris*	Leaf Stem root	1.2 1.0 1.3	1.0 0.8 1.2	- 0.7 1.2	- - 1.1	- - -
10	*Bacillus subtilis*	Leaf Stem root	1.3 1.0 1.3	1.9 0.6 1.2	0.9 - 1.1	0.6 - 1.0	- - -

The results of present study reveal that the employed extracts of *Toona ciliata* exhibited potential antibacterial activity against the tested pathogens. The present study supports the view that several ethnomedical plants might be useful as antimicrobial agents resulting in the development of novel drugs for many centuries through ethanopharmacy. (Heinrich 2000, Heinrich and Simon 2001)

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