

DESIGN, DEVELOPMENT AND TO FORMULATE ANTIMICROBIAL GEL OF *TOONA CILIATA* ROEM. LEAVES AND *FICUS BENGALENSIS* LINN. STEM BARK.

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ABSTRACT

The aim of the present study is to investigate the antimicrobial property and to formulate an antimicrobial Gel of *Toona ciliata* Roem. leaves and *Ficus bengalensis* Linn. stem bark. The antimicrobial activity was evaluated by using agar cup plate method and minimum inhibitory concentration against four microorganisms was determined. Soxhlet apparatus was used for successive extraction using solvents - petroleum ether, chloroform, methanol and water. And to formulate polyherbal antimicrobial gel carbopol 940 was used as gelling agent. Petroleum ether extract was found to be the most effective of the three extracts. The antimicrobial activity was observed against the gram positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* and gram negative bacteria *Pseudomonas aeruginosa*; and the fungus *Candida albicans*. The minimum inhibitory concentration (MIC) of *Toona ciliata* petroleum ether extract ranged from 40mg/ml-50 mg/ml and that of *Ficus bengalensis* petroleum ether extract from 10mg/ml-16 mg/ml at which selected organisms showed inhibition. Then using a range of concentrations of carbopol 940 different gel formulations were formulated with petroleum ether extracts of both plants and tested for their antimicrobial potential. Out of these F2 and F6 formulation were combined in different ratios and one with ratio 3:7 was further tested for antimicrobial activity by Agar Cup method. It was found that ratio 3:7 have synergistic effect and possessed considerable antimicrobial activity and may serve as promising antimicrobial gel formulation. From this study, it can be concluded that *Toona ciliata* Roem. leaves and *Ficus bengalensis* Linn. stem bark exhibited antimicrobial activities against selected microorganisms.

Keywords: *Toona ciliata* Roem., *Ficus bengalensis* Linn., Antimicrobial activity, *In-vitro* diffusion study, Agar cup plate method, MIC, Carbopol 940, Hydrogel, Cold method, Topical.

INTRODUCTION

Microorganisms are the causative agents of almost all kinds of acute and chronic diseases. The past three decades have noticed a dramatic increase in microbial resistance to antimicrobial agents that lead to repeated use of antibiotics and insufficient control of the disease. Plants based antimicrobials have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [1]. Tropical applications of gels at pathological sites offer greater advantage at faster release of drug to the site of action, independent of water solubility of drug as compared to creams and ointments. The increasing interest in the use of plant-based formulations is leading to a fast growing market for ayurvedic, nutraceutical and polyherbal formulations [2]. *Toona ciliata* Roem. belongs to the family Meliaceae which is widely distributed in the tropical areas of Asia such as India, Malaysia, Indonesia, and southern China. Traditionally, it is useful in chronic dysentery, ulcer, leprosy, cures fever, headache, blood complaints, cardio tonic, aphrodisiac, anthelmintic etc. Pharmacological study reported the antioxidant, analgesic, antiulcer, antifungal, antimicrobial, antifeedant properties of *Toona ciliata* [3]. *Ficus bengalensis* Linn. the Indian Banyan, is a large and extensive growing tree of the Indian subcontinent. According to Ayurveda, it is astringent to bowels; useful in treatment of biliousness, ulcers, vomiting, vaginal complaints, fever, inflammations, leprosy and also used in skin diseases [4].

Material and methods

Collection and authentication of plant material

The *Toona ciliata* leaves and *Ficus bengalensis* stem bark were collected from Punjab University, Chandigarh in month of August, 2012 and were authenticated and identified by Dr. S. Sidhu, Prof. Department of Botany, Panjab University, Chandigarh. The voucher specimen for *Toona ciliata* (TC/KP/06/2012) and that of *Ficus*

bengalensis (FS/KP/07/2012) has been deposited in Department of Pharmacognosy, ASBASJSM College of Pharmacy, Bela (Ropar), for future reference. Both the plants were shade dried, coarsely powdered and processed for further studies. After collection and authentication both the plants were shade dried and powdered separately. Powder materials were passed through sieve no. 40 and used for extraction. A weighed quantity of powder of both plants was successively extracted using petroleum ether, chloroform, methanol, and aqueous solution in Soxhlet apparatus for 16 h using twice the amount of solvent. The extract was evaporated to dryness at 40°C in rotary vacuum evaporator [5].

***In-Vitro* testing of extracts for antimicrobial activity**

All the strains of microorganism were obtained from MTCC, Institute of Microbial Technology, Chandigarh. The strains of *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* were maintained on Nutrient broth at 37°C and *Candida albicans* were maintained on Sabouraud dextrose agar, incubated at 25°C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in (100 ml) of sterile normal saline and the suspension was stored in refrigerator till used [6].

The agar cup method was adopted for determination of antibacterial activity of the prepared extracts. 1.2 ml of standardized bacterial stock suspensions was thoroughly mixed with 120 ml of sterile Nutrient agar. 40 ml of the inoculated Nutrient agar were distributed into sterile Petri dishes. The agar was left to set and in each of these plates 4 cups, 4 mm in diameter, was cut using a sterile borer and the agar discs were removed. Alternate cups were filled with 0.1 ml of each extracts dissolving in DMSO (20 mg/ml) using microtiter-pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 18 h. After the incubation period formation of zones around the wells, confirms the antibacterial activity of the respective extracts. The same procedure was followed for each strain and extract. Doxycycline was used as standard drug. The same method for fungus was adopted. Instead of nutrient agar, Sabouraud dextrose agar was

used. The inoculated medium was incubated at 25°C for two days for fungus. Fluconazole was used as standard antifungal drug [7].

Minimum Inhibitory Concentration

The Minimum Inhibitory Concentration of extract was determined by broth dilution method. The bacterial strains were grown in nutrient broth and fungal strains were grown in sabouraud media. 0.1 gram (100mg) of dried evaporated extract was dissolved in 100ml of 70% methanol giving final concentration of 1 mg/ml. The microbial work was carried out in aseptic area. Nutrient broth was prepared. The medium was poured in the tubes which were then sterilized by autoclave using 15 lb pressure at 1210C for 15 minutes. Using sterile pipettes 0.5ml amount of extract and 0.5ml of the standardized culture was inoculated to obtain a final volume of 10ml. The tubes were incubated at temperature 370C for 48 hours. The tubes were observed for growth of microorganism by observing the turbidity produced. The test procedure was repeated three times to check the reproducibility of the results. Doxycycline for bacterial strain and Fluconazole for fungal strain was used as reference standard [8].

RESULTS

Table 1 Composition of Various Formulations of *Toona ciliata* Roem. and *Ficus bengalensis* Linn. Hydrogel

Ingredients	Types of Formulation							
	F1	F2	F3	F4	F5	F6	F7	F8
TLPE (%w/w)	5	5	5	5	0	0	0	0
FSPE (%w/w)	0	0	0	0	5	5	5	5
Carbopol 940(%w/v)	0.5	1	1.5	2	0.5	1	1.5	2
Propylene glycol(ml)	10	10	10	10	10	10	10	10
Propyl Paraben(g)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Distilled Water (ml)	100	100	100	100	100	100	100	100
Triethanolamine	qs	qs	Qs	qs	Qs	qs	qs	Qs

F1, F2, F3 are the formulations of the extracts- TLPE *Toona ciliata* leaves petroleum extract and *Ficus bengalensis* stem bark

Table 2 Antimicrobial activity of *Toona ciliata* leaf extract

Extract	<i>Pseudomonas aeruginosa</i>							
	U1 20mg/ml	U2 50mg/ml	U3 75mg/ml	U4 100mg/ml	S1 20µg/ml	S2 50µg/ml	S3 75 µg/ml	S4 100µg/ml
TLPE	9.2±1.24	12.3±0.47	15.6±1.72	18.3±1.19	10.3±0.47	15±0.8	19.3±0.14	21.6±0.47
TLCE	7.3±0.43	10±0.81	13.3±0.23	16.3±1.24	9.66±0.47	13.6±0.47	16.3±0.47	20±0.81
<i>Staphylococcus aureus</i>								
TLPE	8.6±1.5	10.6±0.57	13.6±0.81	17±2.00	10.3±0.57	15.6±2.08	17.3±0.47	19±0.81
TLCE	7.3±0.47	9.33±0.59	11.6±1.15	14.6±0.47	9.33±0.47	13.3±0.47	16.3±1.11	18.6±0.47
<i>Bacillus subtilis</i>								
TLPE	9.6±1.15	11.3±1.15	12.6±1.67	14.3±1.52	9.6±1.25	16.3±1.47	19.3±1.25	22.6±0.35
TLCE	8.3±2.00	11.3±1.52	12.3±1.55	13.3±2.05	9.3±0.47	16.3±0.47	19±2.00	22±0.57
<i>Candida albicans</i>								
TLPE	7.3±1.29	9.3±1.24	11.3±0.81	13.3±0.45	--	--	12.6±1.24	15.6±1.94
TLCE	6.5±1.24	8.2±0.47	9.6±1.15	10.3±0.47	7.3±0.97	9.6±0.47	11.6±1.47	14.2±1.47

U₁, U₂, U₃are the concentrations of the extracts -TLPE *Toona ciliata* leaves petroleum extract and TLCE *Toona ciliata* leaves chloroform extract *Ficus bengalensis* stem bark

Table 3 Antimicrobial activity of *Ficus bengalensis* stem bark extracts.

Extract	<i>Pseudomonas aeruginosa</i>							
	U1 20 mg/ml	U2 50 mg/ml	U3 75 mg/ml	U4 100mg/ml	S1 20 µg/ml	S2 50 µg/ml	S3 75µg/ml	S4 100 µg/ml
FSPE	8.6±0.47	10.3±0.47	12.6±0.94	16±0.81	--	--	15±0.81	19.6±1.24
FSCE	7.3±0.43	9.6±0.81	11.3±0.23	16.3±1.24	9.66±0.47	13.6±0.47	16.3±0.47	20±0.81
<i>Staphylococcus aureus</i>								
FSPE	9.6±0.47	12.3±0.47	15±0.81	18±0.81	11.2±0.47	14.3±0.47	17.3±0.47	20±0.81
FSCE	7.3±0.47	10.6±0.47	13.6±0.47	15.6±0.47	9.33±0.47	13.3±0.47	15.3±0.47	18.6±0.47
<i>Bacillus subtilis</i>								
FSPE	9.6±0.57	11.6±0.57	15.3±0.57	16±1	12.3±1.15	13.6±0.57	18.3±1.15	19.6±1.52
FSCE	7.3±1.24	9.3±0.47	12.3±0.94	15.3±2.05	11.3±0.47	13.3±0.47	17±0.81	22±0.81
<i>Candida albicans</i>								
FSPE	9.3±0.47	11.3±0.94	13.3±0.47	15.3±2.05	12±0.81	15.3±0.47	17.6±0.94	20.6±0.94
FSCE	8.6±1.24	10.3±0.47	12.6±0.47	11.3±0.47	10.3±0.47	14.6±0.47	16.6±0.47	18.2±0.47

U1, U2, U3 and U4 were concentrations of extracts and S1, S2, S3 and S4 were concentrations of Standards. Values are mean inhibition zone (mm) \pm SD of three replicates, (-) No zone of inhibition; Inhibition zone including 4 mm bore diameter.

Table 4 Minimum inhibitory concentration MIC(mg/ml)

S.No	Test microbe	TLPE	TLCE	FSPE	FSCE
1	P. aeruginosa	50	30	14	14
2	B. subtilis	40	20	16	16
3	S. aureus	40	25	12	10
4	C. albicans	45	35	14	14

MIC determined by broth dilution method.

Table 5 Evaluation of prepared hydrogel

S.No.	Parameter	Formulation Code							
		F1	F2	F3	F4	F5	F6	F7	F8
1	Homogeneity	+	+	+	+	+	+	+	+
2	Grittiness	-	-	-	-	-	-	-	-
3	Skin Irritation	-	-	-	-	-	-	-	-
4	Spreadability (cm)	5.9	5.2	4.6	4	5.6	5.1	4.5	4.2
5	Ph	9.02	8.87	8.62	839	9.06	8.58	8.17	7.98
6	Viscosity (CPs)	42640	43100	43990	44500	42700	43450	43900	44500

(+) represent good; (-) represent absent. F1, F2, F3, F4 were formulations of *Toona ciliata* and F5, F6, F7, F8 were formulations of *Ficus bengalensis*.

Table 6 Antimicrobial activity of polyherbal gel formulation (3:7)

S.No.	Microorganisms	Polyherbal Gel Formulation	Standard
1	<i>S. aureus</i>	10.33+0.47	16.33+0.94
2	<i>B. subtilis</i>	12.33+0.47	18.66+1.24
3	<i>P. aeruginosa</i>	12.0+1.41	16.33+1.24
4	<i>C. albicans</i>	11.66+1.24	19.33+0.47

Values are mean inhibition zone (mm) \pm SD of three replicates, Inhibition zone including 4 mm bore diameter.

Antimicrobial Activity by Agar Cup Method

Most of the extracts of *Toona ciliata* leave and *Ficus bengalensis* stem bark have considerable antibacterial and antifungal activity against all microbial strains but petroleum ether extract of both plants showed maximum antimicrobial activity against tested species. Petroleum ether extract of both the plants showed higher activity than chloroform extracts. Results of petroleum ether and chloroform extract were shown in Table 2 and Table 3.

Minimum Inhibitory Concentration

The minimum inhibitory concentration of *Toona ciliata* petroleum ether extract ranges from 40mg/ml-50 mg/ml at which all the micro organism show inhibition and minimum inhibitory concentration of *Ficus bengalensis* petroleum ether extract ranges from 10mg/ml-16 mg/ml at which all organisms show inhibition. Result of minimum inhibitory concentration of both the plants is given in Table 4.

Evaluation of hydrogel

Results are shown in following Table 5

In-vitro Diffusion Study

All the gel formulations were subjected to in-vitro diffusion Study. Study was carried out using Keshary-Chen diffusion cell using cellophane membrane. In all formulations the TLPE concentration was kept constant (5% w/w) and the concentration of carbopol 940 was varied. At 37°C, F1 formulation was stable but the consistency was not too good and F3, F4 were too viscous but F2 formulation was having optimum viscosity and consistency. The release of F1 formulation was 89.62% whereas for F2 was 78.28%, F3 was 67.77% and F4 was 54.53%. So the F2 formulation was selected for further antimicrobial activity.

In the next four formulations of FSPE, extract concentration was kept constant (5% w/w) and the concentration of carbopol 940 was varied. At 37°C, F5 formulation was stable but the consistency was not too good and F7, F8 were too viscous but F6 formulation was having optimum viscosity and consistency. The release of F5 formulation was 67.26% whereas for F6 was 56.19%, F7 was

50.83% and F8 was 45.72%. So the F6 formulation was selected for further antimicrobial activity.

In Vitro Testing of Gel Formulation (F2, F6) for Antimicrobial Activity

Antimicrobial activity of gel formulation (F2, F6) was determined by Agar Cup Method. For this different ratio of F2 and F6 formulations were used as F2:F6 i.e. (5:5, 4:6, 3:7, 2:8, 1:9). F2, F6 formulation were selected because they having optimum viscosity, consistency and drug release. Zone of inhibition were measured in mm (Including bore diameter 4 mm). Antimicrobial activity of ratio 3:7 of F2 and F6 gel formulation was maximum than all other ratio for *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans*, and *P. aeruginosa*. The results are shown in the Table 6.

Therefore it was concluded that polyherbal antimicrobial gel having activity against three strains of bacteria i.e. *S. aureus*, *B. subtilis*, *P. aeruginosa* and one strain of fungus i.e. *C. albicans* and it is also concluded that it could be a broad spectrum antimicrobial polyherbal gel.

DISCUSSION

The knowledge of medicinal property of plants has been accumulated in the course of many centuries. The local inhabitants have inherited rich traditional knowledge on the use of many plants or plant parts for treatment of common disease. Medicinal plants provide accessible and culturally relevant sources of primary health care. The remedies based on these plants often have minimal side effect [11]. The past three decades have noticed a dramatic increase in microbial resistance to antimicrobial agents that lead to repeated use of antibiotics and insufficient control of the disease. New prototype antimicrobial agents are required to overcome this situation. The literature study reveals that plants have been the source of potent antimicrobial agents. The plant *Toona ciliata* leaves and *Ficus bengalensis* stem bark. have been successively used as antimicrobial agent by the traditional communities and quite familiar in Ayurveda, 2007. So, an attempt has been made to develop an antimicrobial broad spectrum antimicrobial gel based on these two traditional plants. In vitro antimicrobial test results shows the positive tests against Gram positive and gram negative bacteria

along with antifungal activity of petroleum ether extract of both plants by agar cup method. After zone of inhibition, minimum organisms completely was regarded as minimum inhibitory concentration. The minimum inhibitory concentration of *Toona ciliata* petroleum ether extract ranges from 40mg/ml-50 mg/ml at which all the micro organism show inhibition is and minimum inhibitory concentration of *Ficus bengalensis* petroleum ether extract ranges from 10mg/ml-16 mg/ml at which all organisms show inhibition. Therefore from this we finalize the dose of the extract that has to be incorporated in the final gel formulation.

Four types of gel formulations (F1, F2, F3 and F4) were prepared for *Toona ciliata* leaves extract and another four types of gel formulations (F5, F6, F7 and F8) were prepared for *Ficus bengalensis* stem bark extract. In all formulations the extract concentration was kept constant (5% w/w) and the concentration of carbopol 940 was varied (0.5%, 1.0%, 1.5%, 2.0%). The various gel characteristics viz. homogeneity, grittiness, pH, viscosity, spreadability, in-vitro release of drug were also studied. It was found that formulation F2 and F6 (5% drug extract in 1% carbopol 940) exhibited more considerable in-vitro drug release as compare to other developed formulation (F1, F3, F4, F5, F7 and F8). Therefore, F2 and F6 formulation were combined in different ratio (F2:F6) and further tested for antimicrobial activity by Agar Cup method. It was found that ratio 3:7 have synergistic effect and possessed considerable antimicrobial activity and may serve as promising antimicrobial gel formulations.

CONCLUSION

It is inferred from result that petroleum ether extracts of both the plants show maximum antimicrobial activity against gram positive bacteria, *Staphylococcus aureus*, *Bacillus subtilis* and gram negative bacteria *Pseudomonas aeruginosa*; and the fungus *Candida albicans*. It also concluded that polyherbal antimicrobial gel of both the plants having activity against three strains of bacteria i.e. *S. aureus*, *B. subtilis*, *P. aeruginosa* and one strain of fungus i.e. *C. albicans* and it is also concluded that it is a broad spectrum antimicrobial polyherbal gel.

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inhibitory concentration was calculated. In this the lowest concentration of herbal extract that inhibited the growth of micro

Conflict of Interest

The authors declare no conflict of interest.

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