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IN VITRO SCREENING FOR PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF POISONOUS PLANT FICUS TSEILA ROXB

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ABSTRACT

Leaves of *Ficus tsiela* were extracted with different solvents and evaluated the phytochemical analysis and antimicrobial activity. The presence of biological constituents namely terpenoids, tannins, steroids, anthraquinones, flavonoids, saponins, cardiac glycosides and phlobatannin were confirmed through preliminary phytochemical analysis. Alkaloid was absent in all the extracts treated with different solvents investigated. The presence of these bioactive constituents was associated with the antimicrobial activity of the plants. Agar well diffusion method revealed all the solvent extracts exhibited antimicrobial activity. Similarly, no antimicrobial activity was observed with the aqueous extracts, although high activity was exhibited with the ethanol, moderate activity with methanol and mild activity with chloroform extracts. The Gram-negative bacteria appeared to be more susceptible to the antimicrobial effect of the extracts than the Gram-positive organisms. The results confirmed that *F. tsiela* could be used as source of drugs to fight infections caused by susceptible bacteria. To the best of our knowledge, this may be the first report on antimicrobial activity that has not been previously reported and phytochemical screening is also observed for the first time from the extracts of *F. tsiela*.

KEY WORDS

Ficus tsiela, phytochemical, antimicrobial activity, ethanol extract, methanol extract, chloroform extracts.

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INTRODUCTION

Drug discovery from the medicinal plants has played significant role in the treatment of various diseases and indeed, most new clinical applications of plants secondary metabolites and their derivatives over the last century. In developing countries, traditional medicine is widely used to treat many of non-infectious and infectious ailments and it is estimated that approximately a total of 80% of the world's population use traditional medicine[1]. According to one estimate, 25% of the commonly used medicines contain compounds isolated from plants[2]. Medicinal plants are recognized as important sources of novel biomolecules[3-4], which can theoretically be used in treating multiple life threatening illness such as malaria[5], diabetes[6], hepatitis B virus[7-8], mycobacterial infection[9], HIV[10], Cancer[11]. Thus, it is important to document their therapeutic uses because such information could help in obtaining maximum benefits from the natural resources and increase the possibility of their safe and efficient use in future for various ailments.

Ficus tsiela Roxb is a plant which has not been extensively studied and is of present interest in terms of its phytochemical aspect and pharmacological properties. *F. tsiela* is a plant belonging to the family Moraceae, occurring in the Peninsular India (Central provinces and southwards), Sri Lanka and Maldives Island. Traditionally the plant has been utilized during dysentery and leucorrhoea. The plant content one of the ingredients of medicated oil known as Pancha Valkaladi Tailkum, which is used for external application during skin disease, caused by vitiated blood, such as eczema, leprosy, rheumatism etc. Leaves of young shoots are applied to treat cracked feet. Dried bark powder is used by hakims to treat diseases concerning female reproductive organs. Other traditional uses include treatment of aphthous sores in children and curing pain in ears. The plant is also used as cattle feed but findings indicate nervous disorders[12] and poisoning[13] in calves that are fed with the leaves of this plant. Extensive investigation has been carried out on the digestibility and nutritive value as well as the behaviour of male rats treated with extracts of *F. tsiela*[14-15].

The traditional uses on human beings and its indication as a poisonous plant make it an interesting candidate for phytochemical and pharmacological studies. *F. tsiela* plant has also been listed as poisonous one in the International Poisonous Plants Checklist[16]. However, poisonous plants may contain active compounds with useful biological activities[17]. With the current emphasis on research and development of phyto-medicines for infectious ailments, it is imperative to be aware of and have some information at hand regarding the more common plant poisonings occurring in man and livestock. Therefore, we have taken up poisonous plant *F. tsiela*.

MATERIALS AND METHODS

Plant Material

Leaves of *Ficus tsiela* Roxb were collected from Experimental field station, Bhabha Atomic Research Centre, Mumbai, India.

Processing of Plant Materials

The collected plant materials were washed thoroughly. Leaves were sun dried for 2 consecutive days for 8 hr. thereafter leaves were allowed to dry in oven at 55˚C for 16 hr. The dried plant materials were pulverized by Wiely Mill (Model No. 4276, Thomas Scientific, USA). The dried powder was stored in dark at 25^0C before experiments.

Extraction of Plant Materials

Accurately 25 g of the dried powdered material was weighed and tied in a muslin cloth. The material was then put in a soxhlet apparatus and 150 ml of solvent was added. Extraction was carried out for 8 hours, after which the extract was collected and concentrated using rotary evaporator to remove the solvent. The residue was collected and kept at room temperature for 16 hours for complete evaporation of solvent. The extract was stored in cold room at a temperature of 4˚C for further use.

Phytochemical Screening

The major secondary metabolites constituents such as terpenoids, tannins, steroids, anthraquinones, flavonoids, saponins, cardiac Glycosides and phlobatannin were screened using standard phytochemical methods described elsewhere[18-22].

Sources of Bacterial Strains

The Microbial strains *Bacillus pumilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella paratyphi*, *Proteus mirabilis*, *Candida tropicalis*, *Candida albicans* and *Candida glabrata* were obtained from Microbial Type Culture Collection and gene bank (MTCC), Institute of Microbial Technology, Chandigarh, India. The agar-well diffusion method was used to determine the *in vitro* antimicrobial activity of ethanol, methanol, chloroform and aqueous extracts of *F. tsiela*. Pure culture of bacteria organisms were cultured on Nutrient Agar (Himedia Laboratories, Mumbai) and incubated for 24 hr at 37^0C. They were inoculated into nutrient agar slants and stored at 4˚C.

Twenty-four-hour broth culture of the respective bacteria organisms were adjusted to a turbidity of 0.5 McFarland standards. In brief, 0.2 ml broth culture of the respective microbial strain was dispensed into 20 ml sterile nutrient broth and incubated for 24 hr at 37^0C and standardized at 1.5×10^6 CFU/ml by adjusting the optical density to 0.1 at 600 nm and performed on Jasco UV/VIS-spectrophotometer (Model No. V-530, Japan).

In vitro antimicrobial Assay

The agar well diffusion method[23-24] was used. The dried extract was reconstituted with DMSO to obtain a stock solution of 25 mg/ml, 50 mg/ml, 100 mg/ml, 150 mg/ml and 200 mg/ml. Nutrient agar medium (20 ml) supplemented with respective bacteria organisms was transferred into petri-plats (75 mm dia) aseptically. A sterile 6 mm diameter cork borer was used to make wells into the set of inoculated nutrient agar plates. Two wells (6 mm in diameter) were made equidistance in each of the plates using a sterile cork borer. Up to 100 µl of each concentration of the extracts were respectively filled into the wells using sterile automatic pipettes. Petri-plates were allowed to diffuse at room temperature for 1 hour and subsequently the petri-plates were incubated at 37^0C for 24 hr and all the tests were carried out in triplicates. The control antibiotic Kanamycin (2mg/10ml) was used. Diameters of the zone of inhibitions were measured. The antibacterial activities were determined as the mean zone of inhibition diameter (mm) produced by the extracts.

Statistical analysis

Results were depicted as the mean ± SME. Values are mean of three replicates from two experiments. The data were analysed statistically by analysis of variance (ANOVA) and difference between means of the samples was analysed by the least significant difference (LSD) at a probability level of 0.05.

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical screening of crude extracts of leaves of *F. tsiela* using chemical method revealed the presence of bioactive constituents such as terpenoids, tannins, steroids, anthraquinones, flavonoids, saponins, cardiac glycosides and phlobatannin. The test for alkaloid, however, showed negative result (Table 1). Several authors reported that the presence of these bioactive constituents to the antimicrobial, antifungal and antiviral properties of the crude extracts of plant origin[25-27]. El-Mahmood *et al.,*[28] reported that these

compounds were potentially significant application against human pathogens, including those that cause enteric infections. A broad range of the biological properties of terpenoids was described, including cancer chemopreventive effects, antimicrobial, antifungal, antiviral, antihyperglycemic, anti-inflammatory and antiparasitic activities[29].

The presence of glycosides moieties like saponins, cardiac glycosides and flavonoids which are known to anticancer and antimetastatic activities and serve also to protect against gastrointestinal infections are of pharmacognostic importance and give evidence to the use of the plant in ethnomedicine[30-34]. Saponins are widely distributed amongst plants and have a wide range of biological properties. Sparg and co-worker[35] investigated the biological activities of saponins originated from various plants. Cardiac glycosides have been isolated from various medicinal plants and cell cultures[36-39]. Cardiac glycosides compounds are therapeutically relevant for the treatment of heart diseases, such as congestive heart failure and atrial fibrillation[40]. Controversial evidence suggests that C-reactive protein (CRP) may play a causal role in cardiovascular disease. The inhibitory activity of cardiac glycosides on C-reactive protein (CRP) expression may have important implications for the treatment of cardiovascular disease. Cardiac glycosides may be used for CRP synthesis inhibition in the future[41]. Our finding of phytochemical screening noted that ethanol and methanol extracts showed the presence of cardiac glycoside (Table 1). Many tannin rich medicinal plants have been appreciated for their beneficial effects e.g., inhibition of lipid-peroxidation, mutagenicity of carcinogens and tumor promotion, and also host-mediated antitumor activity and antiviral activity without being troubled by any obvious toxicity[42]. Thus, it may be possible to use medicinal plant materials without any fatality. It is important, however, concentration of the biological constituents is known for proper treatment. Preliminary phytochemical analysis of *F. tsiela* shows the presence of secondary metabolites and draws the attention of the researchers towards the isolation of chemotherapeutic agents as well as traditionally used rural herbal remedies.

Table 1
Results of phytochemical screening of extracts leaves of Ficus tsiela

Phytochemical Constituents	Chloroform extract	Methanol extract	Ethanol extract	Aqueous extract
Terpenoids	-	+	+	-
Tannins	-	+	+	-
Steroids	+	+	+	-
Anthraquinones	-	-	+	-
Flavonoids	-	+	+	-
Saponins	+	+	+	-
Cardiac Glycosides	-	+	+	-
Phlobatannins	-	-	+	-
Alkaloids	-	-	-	-

Antimicrobial Activity

For all the different solvent extracts tested, the result indicated meaningful zones of growth inhibition on the test microorganisms by the ethanol extracts of *F. tsiela* compared to the methanol and chloroform extracts. This is in direct conformity with the analysis of phytochemicals earlier carried out. In contrast, earlier studies showed that ethanol extract of some medicinal plants were not a good solvents for extraction for antimicrobial substances[43-44] due to lack of antibacterial activity[45]. Our finding shows significant results that ethanol extracts exhibited remarkable antibacterial activity against test microorganisms. In addition, antimicrobial activity exhibited that gram positive bacteria were not as susceptible as gram negative

bacteria and the results are presented as shown in Table 2 and Table 3. The present results show that the ethanol extracts exhibited relatively highest antimicrobial activity against *S. paratyphi* compared to other tested microorganisms. Rodrigues and co-workers[46] reported that *S. paratyphi* emerged as an important cause of enteric fever in South Asia during the past decade. Each bacterial strain has a different intrinsic growth rate and susceptibility of bacteria to plant extracts and therefore on the basis of inhibition zone diameters they vary according to strains, species and crude extract concentrations. All the crude extracts from different solvent and different concentrations ranging from low to high (25 to 200 mg/ml) were susceptible to *P. mirabilis, S. paratyphi*, *B. cereus* and *B. megaterium,* whereas aqueous extracts exhibited no antimicrobial activity against the tested bacteria. The highest zone of growth of inhibition was shown by ethanol extracts (200 mg/ml) against at gram negative bacteria *S. paratyphi* and *P. mirabilis* (Table 2) and gram positive bacteria *B. megaterium* and *B. cereus* (Table 3). The growth of inhibition was moderate activity against gram negative bacteria *P. aeruginosa, K. pneumonia* and gram positive bacteria *B. subtilis.* Chloroform extracts (25 mg/ml) inhibited gram negative *P. mirabilis and S. paratyphi* but did not show any considerable activity against *E. coli, K. pneumonia* and *P. aeruginosa*. Similarly, at the same concentrations also inhibited gram positive bacteria *B. cereus* and *B. megaterium* and no activity was recorded with *B. pumilis, B. subtilis,* and *S. aureus*.

Table 2
Antibacterial activity of different solvent extracts of Ficus tsiela leaf

Sr. No	Name of the Extracts	Concentration of Extracts (mg/ml)	Diameters of zones of Inhibition (mm)				
			Gram Negative Bacteria				
			E.c	*K.p*	*P.m*	*P.a*	*S.p*
1	**Aqueous**	25	--	--	--	--	--
		50	--	--	--	--	--
		100	--	--	--	--	--
		150	--	--	--	--	--
		200	--	--	--	--	--
2	**Ethanol**	25	14.38±0.09	16.06±0.32	19.72±0.62	17.03±0.09	21.86±0.25
		50	14.99±0.22	17.11±0.09	23.14±0.21	19.12±0.31	23.73±0.25
		100	16.01±0.05	17.97±0.31	25.62±0.60	21.95±0.23	26.59±0.32
		150	17.54±0.31	19.85±0.27	27.28±0.02	23.08±0.33	28.75±0.10
		200	18.83±0.21	23.78±0.21	28.14±0.06	25.62±0.08	30.32±0.57
3	**Methanol**	25	10.88±0.17	12.76±0.09	19.01±0.40	15.34±0.21	19.51±0.33
		50	12.88±0.36	14.01±0.34	21.62±0.28	18.02±0.04	23.23±0.52
		100	13.99±0.41	17.89±0.29	22.56±0.15	19.76±0.41	26.79±0.62
		150	16.92±0.05	19.66±0.05	24.05±0.44	21.78±0.51	27.65±0.34
		200	17.66±0.23	23.01±0.07	25.90±0.28	23.87±0.16	28.64±0.42
4	**Chloroform**	25	--	--	7.70±0.08	--	8.62±0.12
		50	8.67±0.36	8.48±0.19	14.80±0.11	11.65±0.11	17.05±0.81
		100	9.01±0.06	10.32±0.42	18.71±0.36	15.38±0.38	19.11±0.63

	150	10.88±0.31	14.09±0.21	20.65±0.21	18.02±0.02	21.55±0.54
	200	14.07±0.19	17.89±0.09	20.88±0.57	18.97±0.27	23.89±0.39

- No activity, E.c. - Escherichia coli; K.p - Klebsiella pneumoniae; P.m -Proteus mirabilis; P.a - Pseudomonas aeruginosa; S.p - Salmonella paratyphi. The control antibiotic kanamycin was used. Each value represents the mean of triplicate analysis. Standard deviation was 0.5 for the values.

Table 3
Antibacterial activity of different solvent extracts of Ficus tsiela leaf

Sr. No	Name of the Extracts	Concentration of Extracts (mg/ml)	Diameters of zones of Inhibition (mm)				
			Gram Positive Bacteria				
			B. p	*B. s.*	*B. c.*	*B. m.*	*S. a*
1	**Aqueous**	25	--	--	--	--	--
		50	--	--	--	--	--
		100	--	--	--	--	--
		150	--	--	--	--	--
		200	--	--	--	--	--
2	**Ethanol**	25	12.87±0.35	14.06±0.45	15.57±0.21	19.19±0.97	14.50±0.22
		50	12.96±0.19	14.95±0.27	17.28±0.29	21.05±0.70	16.59±0.27
		100	13.76±0.45	16.11±0.52	18.94±0.33	24.57±0.46	18.21±0.13
		150	16.55±0.32	18.72±0.60	21.15±0.80	26.55±0.21	19.44±0.23
		200	17.89±0.65	19.01±0.43	23.43±0.39	26.88±0.51	20.82±0.24
3	**Methanol**	25	-	9.85±0.65	18.34±0.68	18.68±0.36	--
		50	11.20±0.28	12.03±0.18	19.12±0.22	19.84±0.14	16.01±0.11
		100	11.88±0.52	13.33±0.52	20.08±0.39	22.90±0.52	17.62±0.28
		150	12.98±0.13	14.05±0.07	21.21±0.62	22.39±0.26	18.82±0.61
		200	13.88±0.32	14.78±0.45	20.34±0.43	24.54±0.47	19.83±0.27
4	**Chloroform**	25	--	--	11.58±0.15	14.47±0.15	--
		50	8.10±0.18	--	11.49±0.01	15.53±0.10	--
		100	8.76±0.43	9.12±0.15	11.59±0.12	17.64±0.15	13.22±0.24
		150	10.45±0.67	10.11±0.46	12.59±0.51	20.21±0.12	14.69±0.52
		200	13.92±0.26	11.24±0.34	13.25±0.07	21.88±0.31	16.21±0.39

- No activity, B.p.-Bacillus pumilis; B.s - Bacillus subtilis; B.c - Bacillus cereus; B.m - Bacillus megaterium; S.a - Staphylococcus aureus. The control antibiotic kanamycin was used. Each value represents the mean of triplicate analysis. Standard deviation was 0.05 for the values.

Irobi et al.,[47] reported that gram positive bacteria were not as susceptible as gram negative bacteria. In accord to this report our findings also show that gram negative bacteria appeared to be more vulnerable to the plant extracts than the gram positive test microorganisms. In contrast, More et al.,[48] demonstrated that gram negative bacteria were found to be more resistant to the plant extracts than gram positive bacteria. In general, the activity of plant extracts could be depending on the different cell wall structure of bacteria. Gram-negative bacteria possess an outer phospholipidic membrane with structural lipopolysaccharide components and make their cell well impermeable to antimicrobial agents

which is not found in gram-positive bacteria and therefore diffusion and impermeable depend on the composition of cell wall[49]. The large zone size produced by the crude extracts against the test microorganisms, especially the ethanol extracts is an indication of the potency of the bioactive constituents of the *F. tsiela* against test bacteria. The lowest zone of growth inhibition was chloroform. There was no activity in aqueous extracts. The large inhibition of zone sizes produced by the plant extract against the test bacteria, especially the ethanol extracts is an indication of the ethanol solvents has extracted high potency of the bioactive components from the plant leaves. The antimicrobial results of this study confirmed the traditional use of *F. tsiela* in traditional medicine.

CONCLUSION

The present work reports the first phytochemical screening and antimicrobial activity of *F. tsiela.* Medicinal plants could be sources of bioactive constituents which might be useful against bacteria and extended broad spectrum antimicrobial activity. Although the nature and number of active components involved in each extract are not clear, these findings are promising and further studies about the toxicity of plant extracts and the isolation of active compounds are important to propose.

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