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Comparative study on the antimicrobial activities of three Indian medicinal plants

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Abstract

Aqueous, methanol and chloroform extracts from the leaves of *Ficus religiosa*, *Thespesia populnea* and *Hibiscus tiliaceus* were completely screened for antibacterial and antifungal activity. The chloroform extract of *F. religiosa* possessed a broad spectrum of antibacterial activity with a zone of inhibition of 10 to 21 mm at very low MIC values. The methanolic extracts possessed moderate antibacterial activity against a few bacterial strains. There was less antibacterial activity or none at all using aqueous extracts. The extracts of *F. religiosa* were found to be active against *Aspergillus niger* and *Penicillium notatum*. The extracts from the leaves exhibited considerable and variable inhibitory effects against most of the microorganisms tested.

Keywords: *Ficus religiosa*, *Hibiscus tiliaceus*, *Thespesia populnea*, antibacterial, antifungal

Introduction

Natural products from plants have been the basis of many treatments of human diseases that date back to the start of human civilization. Plants and algae have been found to be promising sources of new structurally novel natural products with antimicrobial, antineoplastic activities and also hopefully new modes of action (Raja, 2003; Raja *et al*. 2007). Ayurvedha is one of the traditional Indian medicinal systems which has a very important role in indicating sources of future medicine and therapeutics. In addition to the major systems, there are around 7,500 plants used in local health traditions in the rural and tribal villages of India (Nadkarni, 1976).

Three medicinal plants were chosen for study, *Ficus religiosa*, *Thespesia populnea* and *Hibiscus tiliaceus*; their medicinal values have been well documented in traditional and folklore medicine. Many *Ficus* species have long been used in folk medicine as astringents, carminatives, stomachics, vermicides, hypotensives, anthelmintics and anti-dysentery drugs (Trivedi *et al*. 1969). It is believed that some *Ficus* species can be used as a remedy for visceral obstructive disorders, diabetes, leprosy, respiratory disorders and certain skin diseases (Chopra *et al*. 1950), and as an absorbent for inflammatory swellings and burns (Bhattacharjee, 1998). Bark of five different species (*F. religiosa, F. bengalensis, F. glomerata, F. infectora* and *Abizzia lebec*) are used in the treatment of female genital tract infections (Palep *et al.* 2003).

Thespesia populnea Soland ex. Correa (Malvaceae) is a large tree found in tropical regions and coastal forests of India. The bark, leaves, flowers and fruits are useful in cutaneous infections and the decoction of the bark is commonly used for the treatment of skin and liver diseases. The bark, root and fruit were used against dysentery, cholera and hemorrhoids (Ilavarasan *et al*. 2003a) Gossypol was found to be the major component of *T. populnea*, and naturally occurring quinines (thespone, mansonone-d, mansonone-H, thespone and thespesone) have been extracted from the heartwood (Johnson *et al*. 1999). The fruits of the plant are used in Ayurveda for the control of diabetes (Sathyanarayana *et al*. 2004). The barks and flowers possess astringent, hepatoprotective and antioxidant activities in rats (Shirwaikar *et al*. 1995; Ilavarasan *et al*. 2003b). The plant is also used in the treatment of skin ailments such as scabies, psoriasis, wounds and ulcers (Warrier *et al.* 1994) and inhibit tumor formation (Milbrodt *et al.* 1997).

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Hibiscus tiliaceus L. is a typical plant of tropical climates found in the regions of mangroves in significant quantities (Rosa *et al*. 2006). An aqueous extract of wood and fresh flowers is a registered treatment for skin diseases (Whistler, 1985; Melecchi *et al*. 2006). Recently it was shown that methanolic flower extract exerts an antioxidant effect on the yeast *Saccharomyces cerevisiae*, protecting against hydrogen peroxide (H_2O_2) and tert-butyl-hydroperoxide (t-BHP) cytotoxicities. In addition, the extract was not mutagenic in *Salmonella typhimurium* or *S. cerevisiae*, and showed a significant antimutagenic action against oxidative mutagens in *S. cerevisiae* (Rosa *et al*. 2006). It is also reported traditionally, where the leaves are used to treat fevers and soothe coughs, the bark to treat dysentery, and the flowers aid in treating ear infections and abscesses (Petard 1986). The present study was aimed at comparing the antibacterial activities of the leaves of three medicinal plants.

Materials & Methods

The fresh leaves of *F. religiosa*, *T. populnea* and *H. tiliaceus* were collected during March and April 2007 from Coimbatore, Tamil Nadu (South India). The spectrum of each plant was authenticated and a voucher specimen of each plant (MO:1, MO:2, MA:1 respectively) was deposited at the Botanical Survey of India, South Circle, Coimbatore, India.

The collected fresh leaves (18 g) were washed, shade-dried and powdered. For the preparation of aqueous, methanol and chloroform extracts, 5 g of dried powder of the leaves were extracted using a soxhlet apparatus for 3 h at room temperature. The extract was filtered through membrane filters (0.45 µ), with the aid of suction pump. The yield based on dry weight was: aqueous extract 25.98% (w/w), methanol extract 2.74% (w/w), chloroform extract 2.94% (w/w). The filtrate was concentrated under reduced pressure, reconstituted in a minimal volume (less than 2%) of dimethyl sulphoxide (DMSO) and diluted in sterile water to a final concentration of 50 mg/ml. The DMSO solubilized forms of each of the extracts were stored at room temperature and used for the following bioassays.

The crude extracts of the leaves were individually tested against a panel of bacteria, including *Bacillus subtilis* (ATCC 6051), *Escherichia coli* (ATCC 25922), *Shigella flexneri* (ATCC 29508), *Salmonella typhimurium* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 33495), *Staphylococcus aureus* (ATCC 9144), *Pseudomonas aeruginosa* (ATCC 25619), *Salmonella typhi* (ATCC 6539) and *Proteus vulgaris* (ATCC 6380). Bacterial strains were cultured overnight at 37°C in a nutrient broth (Himedia) and used for the determination of antibacterial activities. The fungal species used were *Aspergillus niger* (ATCC 6275), *Penicillium chrysogenum* (ATCC 9480), *Aspergillus terreus* (ATCC 16792), *Rhizopus oryzae* (MTCC 553) and *Fusarium* species, isolated from the soil sample.

The agar-well diffusion method was performed as per the standards of the National Committee for Clinical Laboratory Standards (NCCLS, now Clinical Laboratory Standards International, CLSI) for the determination of antibacterial activity of the extracts (NCCLS, 1999). Antibacterial activities of standard antibiotics like chloramphenicol (30µg/ml) were also determined in parallel experiments as a positive control, and the respective solvents, DMSO as negative controls. All tests were performed in duplicate. Extracts that were active against the tested bacteria (with the zone of inhibition above 6 mm) were taken for the determination of Minimum Inhibitory Concentration (MIC) by the micro-plate method (Sokeman *et al*. 1999). The MIC of each extract was taken as the lowest concentration that inhibited the growth. Antifungal activity of the leaves of the three plants was demonstrated in a radial growth inhibition assay (Schlumbaum *et al*. 1986). Mean values are represented as mean ± SD.

Results

The results from the preliminary screening of antibacterial activity of *F. religiosa*, *T. populnea*, *H. tiliaceus* by the agar-well diffusion assay and the MIC values are given in Tables 1, 2 and 3 respectively. The aqueous extracts of all the plants were found to be active against only a few bacterial strains with a high MIC. The extracts showed a clear zone of 7 to 8 mm, whereas an inhibition zone of more than 10 mm is considered as indicating best antibacterial activity. The chloroform extracts of *F. religiosa* showed a strong inhibitory effect on the growth of most of the infectious *Salmonella* sp. The active dose was lower for *S. typhi* than *S. typhimurium.* Growth of *P. vulgaris* was significantly inhibited by chloroform extract of *F. religiosa* followed by inhibition mediated on *E.coli*. Higher concentrations of the same extract were required for the inhibition of *B. subtilis*, *K. pneumoniae*, *S. aureus* and *P. aeruginosa*. The mean zone of inhibition obtained using the agar-well diffusion assay ranged from 10 mm (for *S. aureus*) to 21 mm (for *S. flexneri*, *P. aeruginosa* and *S. typhi*).

Table 1: Antibacterial activity of *Ficus religiosa.* - = no activity, * = not tested. Chloramphenical is the positive control

Bacteria	Inhibition zone by agar well diffusion (mm)			Minimum Inhibitory Concentration (µg/ml)			Chloramphenicol (mm)
	Aqueous	Methanol	Chloroform	Aqueous	Methanol	Chloroform	
Bacillus subtilis	-	-	18.7±0.1	*	*	625	26.3±1.2
Escherichia coli	-	-	20.7±1.2	*	*	78	20.0±1.5
Shigella flexneri	-	-	22.0±1.0	*	*	2500	25.0±2.0
Salmonella typhimurium	-	-	11.7±0.6	*	*	39	28.7±1.5
Klebsiella pneumoniae	-	8.0±0.0	17.0±1.0	-	2500	625	24.7±1.5
Staphylococcus aureus	-	-	11.0±1.0	*	*	625	22.7±2.1
Pseudomonas aeruginosa	-	7.3±0.6	21.7±1.2	*	625	625	25.0±2.0
Salmonella typhi	7.3±0.6	7.3±0.6	21.7±2.1	5000	39	5	20.7±2.1
Proteus vulgaris	-	10.7±0.6	11.7±0.6	*	2500	20	13.2±0.0

Table 2: Antibacterial activity of *Thespesia populnea*. . - = no activity, * = not tested. Chloramphenical is the positive control

Bacteria	Inhibition zone by agar well diffusion (mm)			Minimum Inhibitory Concentration (µg/ml)			Chloramphenicol (mm)
	Aqueous	Methanol	Chloroform	Aqueous	Methanol	Chloroform	
Bacillus subtilis	-	-	-	*	*	*	26.3±1.2
Escherichia coli	-	8.3±0.6	9±1	*	313	313	20.0±1.5
Shigella flexneri	-	-	-	*	*	*	25.0±2.0
Salmonella typhimurium	7.7±1.2	8.0±0.0	8±1	5000	156	1250	28.7±1.5
Klebsiella pneumoniae	-	-	-	*	*	*	24.7±1.5
Staphylococcus aureus	-	-	-	*	*	*	22.7±2.1
Pseudomonas aeruginosa	8.3±0.6	9.0±1.0	8±1	5000	625	2500	25.0±2.0
Salmonella typhi	-	8.0±1.0	-	*	156	*	20.7±2.1
Proteus vulgaris	-	10.0±1.0	-	*	156	*	13.2±0.0

Table 3: Antibacterial activity of *Hibiscus tiliaceus.* - = no activity, * = not tested. Chloramphenical is the positive control

Bacteria	Inhibition zone by agar well diffusion (mm)			Minimum Inhibitory Concentration (µg/ml)			Chloramphenicol (mm)
	Aqueous	Methanol	Chloroform	Aqueous	Methanol	Chloroform	
Bacillus subtilis	-	-		*	2500	625	26.3±1.2
Escherichia coli	7.7±0.6	-	9.0±1.0	5000	-	5000	20.0±1.5
Shigella flexneri	-	-	12.0±1.0	*	*	1250	25.0±2.0
Salmonella typhimurium	-	-	11.7±0.6	*	*	625	28.7±1.5
Klebsiella pneumoniae	-	-	8.0±1.0	*	*	313	24.7±1.5
Staphylococcus aureus	-	8±0	7.7±1.2	*	1250	156	22.7±2.1
Pseudomonas aeruginosa	-	7.7±1.2	10.0±1.0	*	2500	313	25.0±2.0
Salmonella typhi	7.3±0.6	9±1.0	11.7±0.6	5000	313	1250	20.7±2.1
Proteus vulgaris	-	8±1.0	11.7±0.6	*	1250	625	13.2±0.0

The methanolic extracts of the three plants were less potent against most of the pathogens tested, with MIC values of more than 100 µg/ml and inhibition zones less than 10 mm. *S. typhi* was inhibited by the methanolic extract of *F. religiosa* with a low MIC, but with a reduced zone of inhibition (7 mm). Negative controls with respective solvents and DMSO did not inhibit any of the bacteria tested (Figure 1).

Figure 1: Effect of solvents on the bacteria and fungi tested (negative controls). Bars represent the MIC and the line represents the Zone of inhibition

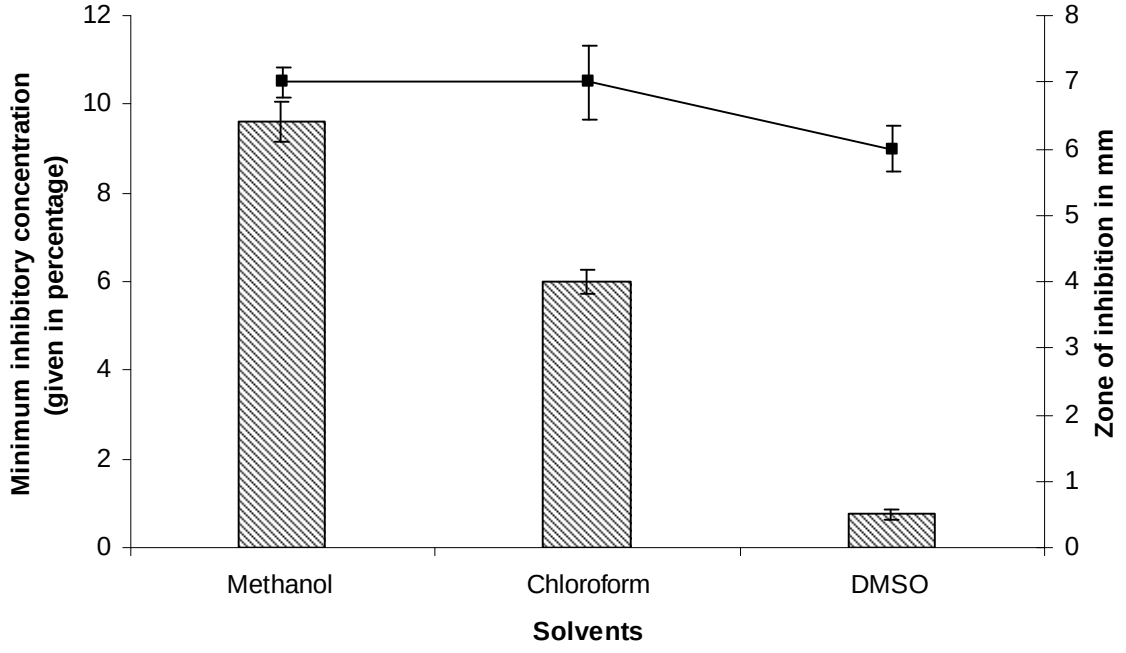

Table 4: Antifungal activity of the three Indian medicinal plants[a]

Fungi	1	2	3	4	5	6	7	8	9	10
Aspergillus niger	+	+	++	-	-	-	+++	-	+	+++
Penicillium chrysogenum	+	+++	+	-	+++	-	-	-	+	+++
Aspergillus terreus	-	-	-	-	-	-	-	+	+++	+++
Rhizopus oryzae	-	-	-	+	++	++	-	+	-	+++
Fusarium sp.	-	-	-	-	-	-	-	-	-	+++

[a]Fungal growth inhibition +++ = 100%; ++ = 50%; + = <50%; - = no inhibition.
1, 2, 3 - Aqueous, methanol, chloroform extracts of *Ficus religiosa* respectively.
4, 5, 6 - Aqueous, methanol, chloroform extracts of *Thespesia populnea* respectively.
7, 8, 9 - Aqueous, methanol, chloroform extracts of *Hibiscus tiliaceus* respectively.
10 – Nystatin

Table 4 shows the growth inhibition produced by the leaf extracts of *F. religiosa*, *T. populnea* and *H. tiliaceus* toward five species of fungus. Growth of *A. niger* and *P. chrysogenum* were inhibited by *F. religiosa*, but not *A. terreus*, *R. oryzae* and *Fusarium* sp. There was a variable degree of inhibition by the different extracts against the fungi. In separate experiments, the leaf extracts of *F. religiosa* were found to possess antitumor activity in the potato-disc tumor assay, as the percentage inhibition was more than 20% (results not shown). Chloroform extracts were found to highly active and therefore could be the best source of effective phytochemicals.

Discussion

The present study indicates strong antibacterial activity of the leaf extracts of *F. religiosa.* With the zone of inhibition more than 10 mm against eight of the nine bacterial strains tested, the chloroform extract of *F. religiosa* clearly possesses a strong and broad spectrum of antibacterial activity. The antibacterial activity against both Gram-positive and Gram-negative bacteria was in the order of chloroform > methanol > aqueous extract of *F. religiosa*.

Previous experiments showed that fruit extracts of *F. religiosa* demonstrated significant antibacterial activity at a concentration of 0.5 mg per disc, but they did not show any antifungal activity (Mousa *et al*. 1994). DMSO has been widely reported to affect the activity of extracts when used as a solvent. The choice of DMSO as a solvent is due to its solvency for a wide range of chemicals, its low antibacterial activity at concentrations less than 2% and its low toxicity to mammals (Brown *et al*. 1963; Weiss & Orzel, 1967; Hill *et al*. 1997).

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الملخص العربي

دراسة مقارنة على الأنشطة المضادة للميكروبات لثلاثة من النباتات الطبية الهندية

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تم خلال هذا البحث دراسة تأثير المستخلصات النباتية لثلاثة أنواع من النباتات وهى: فيكس ريليجوسا – ثيسبثسيا بوبومنا – هيبيسيكس تيمياشيوس من خلال إستخدام المحلول الجاف – الميثانول – الكلوروفورم ودراسة التأثير المضاد للبكتريا وللفطريات لتلك المستخلصات. أثبت المستخلص النباتى المعزول بإستخدام الكلوروفورم كفاءة واسعة المدى ضد أنواع عديدة من البكتريا. أيضا وضح وجود أقل التأثيرات أو عدم وجود تأثيرات على الإطلاق لتلك المستخلصات عند إستخدام المحلول الجاف. وجد أيضا أن مستخلص نبات الفيكس كان له تأثير فعال ضد أسبيرجيميس نيجر و بنسيليم نوتاتم، أيضا أوضحت المستخلصات الورقية تأثيرات مثبطة متباينة ومعتدلة ضد الأنواع المختلفة من الكائنات الدقيقة الممرضة.