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Research Article

Antimicrobial evaluation of selected south Indian medicinal plants against *Streptococcus pneumonia*

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Abstract

In India, most people particularly in rural areas use medicinal plants to treat diseases. In order to find new antipneumococcal extracts, an ethno botanical survey has been conducted in different Places of South India. Four plants commonly reproduce by traditional herbalists (*Bauhina purpurea, Bougainvillaea spectabilis, Marsilea quadrifolia, Launaea nudicaulis*) are tested against *streptococcus pneumoniae* responsible for pharyngitis, rhitis, and otitis and sinusitis infections. Aqueous and methanol extracts have been prepared and tested on *S.pneumoniae* collected in four region of India. A significant activity has been observed with methanol extracts of three plants; *Bougainvillaea spectabilis, Marsilea quadrifolia, Launaea nudicaulis,* (MIC=248µg/ml).

Keywords: *Streptococcus pneumoniae,* Phytochemical Screening, Medicinal Plants, Antimicrobial Activity.

Introduction

Pneumococcal disease is an infection caused by streptococcus pneumoniae bacteria (pneumococcus). These bacteria can cause many types of illnesses. *S.pneumoniae* is responsible for disease including bacteraemia, meningitis, pharyngitis, rhitis, otitis, sinusitis, arthritis and pneumonia [1]. Although most *S.pheumoniae* organisms remain susceptible to penicillin, resistant strain has been recognized since 1967, when a resistant strain was identified in Australia [2]. Since then *S.pneumoniae* strains with reduced susceptibility to penicillin have been reported worldwide [3]. Traditional medicine had been a rare and discordant. Medicinal plants have been used for centuries as remedy for human diseases because they contain the compounds of therapeutic values [4]. Infectious diseases are the leading cause of death worldwide. Many Infectious diseases have been known to be treated with herbal remedies throughout the history of mankind.

Materials and Methods

Test organism

The *S.pneumoniae* organism was isolated from child aged 3 to 9 years in three different hospitals in Thanjavur and trichy district, Tamil Nadu, India. The nasophaynx of each subject was swabbed using a sterile swab, which was inserted nasally. The swabs were immediately plated on blood agar plates (tryptic soy agar with 5% sheep blood). An optochin disc (ethyhydrocupreeine hydrochloride) was then applied to the first zone of each streaked plate. The inoculated plate was placed in an incubator at 37 ^{o}C and 5% of CO_2 for 18 to 24 hrs. The alpha- hemolytic colonies

from each plate showing optochin susceptibility were subcultured on another agar plate to confirm optochin inhibition. Those strains with zones of inhibition ≥14 mm in pure culture were considered to be *S.pneumoniae* [5].

Plant materials

An ethno botanical survey has been carried out during the period March -May 2014 in South India to identify plants used in traditional medicine against ORL infections. During this survey, 43 traditional herbalists were interviewed. This investigation brought out four plants according to them conclusion (Table 1). The plants used for the study; *Bauhina purpurea, Bougainvillaea spectabilis, Launaea nudicaulis, Marsilea quadrifolia* have been collected from herbarium in Jun 2014 in south India.

Table 1 Useful parts and medicinal properties of the plants

Scientific name	Local name	Family name	Part used	Mode of Use
Bauhina purpurea	Mandarai	Leguminosae	Whole plant	Antibacterial,Anticancer,Antimalarial
Bougainvillaea spectabilis	Kaaghithapoo	Nyctaginaceae	Whole plant	Jaundice, dysentery
Launaea nudicaulis	Ezhuthanipoondu	Asteraceae	Whole plant	Anti-inflamatory
Marsilea quadrifolia	Aalaikkeerai	Marsileaceae	Whole plant	Cough,Cold

Extraction and phytochemical screening

The dried powder aerial parts of each plant were extracted with water decoction and methanol. The solution was evaporated in vacuo and crude extracts are freeze-drying and stored at 4°c until further use. Phytochemical screening (Table 2) was carried out to highlight the existing groups in the studied plants, in order to have an idea of the chemical nature of the active ingredients responsible for their antibacterial effects [6].

Determination of antimicrobial activity

Disc diffusion method

Susceptibility test was carried out using the agar diffusion method [7] followed by the dilution method for extracts which gave interesting activities. Petri plates were prepared by pouring 20 ml of Muller Hinton agar supplemented with 5% defibrinated sheep blood. The inoculums were prepared by transferring colonies from an overnight culture and the turbidity was corrected by adding sterile saline until a Mc Ferland turbidity standard of 0.5. What man's filter paper (6mm) impregnated with extracts in a concentration of 500 µg/disc were deposited on inoculated plates and left at 4°c for 2hr to allow the diffusion of the extract before their incubation for 24hr at 37 °c. Negative control (DMSO 1%) and positive control (chloramphenicol 30 µg and erythromycin 15 µg) were also used. The inhibition zones formed around the discs were evaluated in millimeters. Each test was carried out in triplicates.

Dilution Method

Minimum Inhibitory Concentration (MIC) was carried out by agar dilution method [8]**.** The methanol and water extracts were dissolved in 1% dimethylsulfoxide (DMSO) and added to a melted agar culture medium in Petri dishes at the following final concentration: 496, 248 and 124 g /ml .The antimicrobial assay was carried out on Muller- hinton's agar with sheep blood (5%) for 24 hr at 37 °. Negative control containing DMSO 1% and positive control amoxicillin clavunate (10 g/ml) were also maintained. Observations were performed in duplicate and results (MIC) expressed as the lowest concentration of plant extract that produced a complete suppression of colony growth.

Results

Phytochemical screening

As reported in Table 2, high doses of terpenes and sterols were noticed in all plants. In general, flavonoids and sponines have been found in high doses respectively in extracts of *Bauhina purpurea, Bougainvillaea spectabilis* and *Launaea nudicaulis, Marsilea quadrifolia.*

Table 2. Results of phytochemical screening of selected plants

Sl.No	Phytochemicals	*Bauhina purpurea*	*Bougainvillaea spectabilis*	*Launaea nudicaulis*	*Marsilea quadrifolia*
1.	Alkaloids	+	-	+++	-
2.	Flavonoids	+	+	+	-
3.	Saponins	+	+	++	+++
4.	Phenols	+	-	-	++
5.	Terpenoids	++	+++	-	+++
6.	tannins	+++	-	-	-
7.	Cardinolides	+++	-	-	-
8.	Anthraquinones	+	-	-	-
9.	Xanthoprotein	+	-	-	-
10.	Sugar	+++	-	-	-

+: present; ++: present in average quantity; +++; present in high quantity; - : absence.

Antimicrobial activity

Disc diffusion method

The results of disc diffusion assay are presented in Table 3. From the results it was concluded that methanol extracts of three plants are effective on *S.pneumoniae* whereas aqueous extracts do not show any effect on the bacterium tested. *Bougainvillaea spectabilis* and *Launaea nudicaulis, Marsilea quadrifolia* methanol extracts have strong activities with diameter of inhibition varying from 17 to 23 mm. Methanol extract obtained from *Bauhina purpurea* showed weak antimicrobial activity as assessed by the diffusion method. Methanol extract of *Marsilea quadrifolia* showed highest diameter of inhibition against Streptococcus pneumoniae strain isolated from thiruvarur (diameter = 23 mm). This value is similar to that obtained for standard antibiotics, erythromycin and chloramphenicol.

Table 3. Antibacterial effects of the four plant extracts on *Streptococcus pneumoniae* by agar diffusion method.
diffusion method.

Places of *S.pneumoniae*	**Diameter of inhibition in (mm)**										
	Aqueous Extracts				**Methanol Extracts**				**Antibiotics**		**Neg. Control**
	B.purpurea	***B.spectabilis***	***L.nudicaulis***	***M.quadrifoliа***	***B.purpurea***	***B.spectabilis***	***L.nudicaulis***	***M.quadrifolia***	**Erythromycine (15 g)**	**Chloramphenicol (30g)**	**DMSO (1%)**
Thiruvarur	-	-	-	-	17±0.74	20±0.56	17±0.69	23±0.81	23+ 0.46	23+0.65	-
Thanjavur	-	-	-	-	13±0.58	21±0.46	12±0.76	22±0.62	23+0.38	25+0.42	-
Trichy	-	-	-	-	12±0.43	22±0.76	15±0.45	18±0.91	17+0.68	15+0.63	-

Dilution method

The minimum inhibitory concentration obtained for methanol extracts of two pants were as low as 248 µg/ml (table 4).

Table 4. Minimum inhibitory concentration MIC (µg/ml) of the methanol extracts (Agar dilution Method)

Places of *S.pneumoniae*	***B.purpurea***			***B.spectabilis***			***L.nudicaulis***			***M.quadrifolia***			**Amoxicillin Clavunate (10 µg/ml)**	**DMSO 1%**
	496	**248**	**124**	**496**	**248**	**124**	**496**	**248**	**124**	**496**	**248**	**124**		
Thiruvarur	-	-	±	-	-	±	-	-	±	-	-	±	-	+
Trichy	-	-	±	-	-	±	-	-	±	-	-	±	-	+

-: inhibition of growth: ± average growth; +: no inhibition

Discussion

The traditional herbalists Concluded in the *Marsilea quadrifolia* was the most common drug used cough and cold treatments. In common anti inflammatory was treated with *Launaea nudicaulis.*

The methanol extracts of *Bougainvillaea spectabilis & Launaea nudicaulis* and *Marsilea quadrifolia* presented antibacterial effects (table 3). Thus, the methanol extract of *Bauhina purpurea,* showed complete inhibition of Streptococccus pneumoniae with = 248 µg/ml. The antibacterial activity detected for *M.vulgare* is due probably to terpenoids as we found the presence reported for this plant [9, 10]. Marrubin for example, a furan labdane diterpene has been found to be the main analgesic compound. Several other labdane dierpenoids were isolated from the genus Marrubium [11].

The methanol extract of *Bauhina purpurea* was active on the growth of Streptococcus pneumoniae with MIC= 248µg/ml. This extract was rich in compounds (Cardinolides and tannins) (table 2). Antimicrobial activity of lavandula spp. was conducted mainly on essential oils and has been found to be active against marny species of bacteria and fungi. It has also been suggested that essential oils, including lavender, may be useful in treating bacterial infections that are resistant to antibiotics [12].

In general, the minimum inhibitory concentration obtained for methanol extracts of all plants studied were as low as 248 µg/ml. this value is much lower than that observed for example by [13] on the same germ showed an MIC value of 1.2 mg/ml for chloroformic extracts of *Crescentia alata* and *Gnaphalium americanum,* hexanic extract of *Gnaphalium hirsutum* and methanolic extract of *Gnaphalium oxyphyllum*. However, [14] working on four Indian berberis Spp have shown lower MIC values on *Streptococcus pneumoniae* (MIC = 0.31 µg /ml) especially, the hydro- alcoholic extracts of stems of *Berberis aristata* and *Barberis asiatica.*

In our study, the result of phytochemical screening (table 2) was according to that reported in the literature. Thus, the presence of cardinolides, tannins and Sugar, in *Bauhina purpurea* has been also reported by [15]. The terpenoids were also detected by [16] in *Bougainvillaea spectabilis*. The alkaloids and saponins were detected *Launaea nudicaulis,* saponins and terpenoids were also detected from *Marsilea quadrifolia.*

Conclusion

The use of medicinal plants in the treatment of ORL infections is a common practice in South India folk medicine. We have found that the activities of methanol extracts obtained from *Launaea nudicaulis,*

Marsilea quadrifolia and *Bougainvillaea spectabilis* have promising activity against *S.pnemoniae* and show a correlation between the traditional uses of these plants and the experimental data against *S.pneumoniae*. The activities may be considered sufficient for further studies aimed at isolating and identifying active principles and evaluating possible synergism of antimicrobial activity among these extracts.

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