



Original Research Article

Antimicrobial evaluation of selected south indian medicinal plants against *Streptococcus pneumoniae*

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ABSTRACT

Keywords

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Antimicrobial
Activity.

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 (*Bauhinia purpurea*, *Bougainvillea spectabilis*, *Marsilea quadrifolia*, *Launaea nudicaulis*) are tested against *streptococcus pneumoniae* responsible for pharyngitis, rthritis, 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; *Bougainvillea spectabilis*, *Marsilea quadrifolia*, *Launaea nudicaulis*, (MIC=248µg/ml)

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, rthritis, otitis, sinusitis, arthritis and pneumonia [1]. Although most *S.pneumoniae* 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 Organims

The *S.pneumoniae* organism was isolated from child aged 3 to 9 years in three

different hospitals in thanjavur and trichy district, tamilnadu, India. The nasopharynx 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 (ethyhydrocupreine hydrochloride) was then applied to the first zone of each streaked plate. The inoculated plate was placed in an incubator at 37 °C and 5% of CO₂ 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 their conclusion (Table 1).

The plants used for the study; *Bauhinia purpurea*, *Bougainvillea spectabilis*, *Launaea nudicaulis*, *Marsilea quadrifolia* have been collected from herbarium in Jun 2014 in south India.

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 °C. 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.

Result and Discussion

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 *Bauhinia purpurea*, *Bougainvillea spectabilis* and *Launaea nudicaulis*, *Marsilea quadrifolia*.

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. *Bougainvillea 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 *Bauhinia 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.

Dilution Method

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

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 *Bougainvillea spectabilis* & *Launaea nudicaulis* and *Marsilea quadrifolia* presented antibacterial effects (table 3). Thus, the methanol extract of *Bauhinia purpurea*, showed complete inhibition of *Streptococcus 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 diterpenoids were isolated from the genus *Marrubium* ^[11].

The methanol extract of *Bauhinia 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 many 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*.

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.pneumoniae* 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.

Table 1 Useful parts and medicinal properties of the plants

Scientific name	Local name	Family name	Part used	Mode of Use
<i>Bauhina purpurea</i>	Mandarai	Leguminosae	Whole plant	Antibacterial, Anticancer, Antimalarial
<i>Bougainvillaea spectabilis</i>	Kaagithapoo	Nyctaginaceae	Whole plant	Jaundice, dysentery
<i>Launaea nudicaulis</i>	Ezhuthanipoond u	Asteraceae	Whole plant	Anti-inflammatory
<i>Marsilea quadrifolia</i>	Aalaikkeerai	Marsileaceae	Whole plant	Cough, Cold

Table.2 Results of phytochemical screening of selected plants

Sl.No	Phytochemicals	<i>Bauhina purpurea</i>	<i>Bougainvillaea spectabilis</i>	<i>Launaea nudicaulis</i>	<i>Marsilea quadrifolia</i>
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.

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

Places of <i>S.pneumoniae</i>	Diameter of inhibition in (mm)										
	Aqueous Extracts				Methanol Extracts				Antibiotics		Negative Control
	<i>B.purpurea</i>	<i>B.spectabilis</i>	<i>L.nudicaulis</i>	<i>M.quadrifolia</i>	<i>B.purpurea</i>	<i>B.spectabilis</i>	<i>L.nudicaulis</i>	<i>M.quadrifolia</i>	Erythromycin (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	-

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

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

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

References

- Demachy MC, Faibis F, Hacquard B, Rabenja T, Galanti MJ, Artigou A (2001). Enquete epidemiologique sur la resistance aux antibiotiques de streptococcus pneumoniae observatoire regional de Brie: resultat 1999- evolution 1997/1999. Medecine et Maladies infectieuses.
- Austrian R (1986). Some aspects of the pneumococcal carrier state. J. Antimicrob. Chemother.18, suppl. A, 35-45.
- Simberkoff MS, Lukaszewski M, Cross A, Al-Ibrahim M, Baltch Al, Smith RP, Geiseler PI, Nadler J, Richmond AS (1986). Antibiotic resistant isolates of Streptococcus pneumoniae from clinical specimens: a cluster of serotypes 19A organisms in Brooklyn, New York.J.Infect.Dis. 153:78-82.
- Nair NC, Henry AN, Dictionary of Flowering Plants in India. Council of Scientific and Industrial Research, New Delhi, 1973.
- Warda .K , M.Markouk, K.Bekkouche, M.Larhsini nA.Abbad, A. Romane and M. Bouskraoui. Anti Bacterial Evalu of Selected Moroccan medicinal plants against S.Pneumoniae. African Journal of Pharmacy and Pharmacology Vol.3(3).pp.101-104,March,2009.

6. Bouquet A, Paris R (1967). Note sur le *Dionchorophyllum tholonii* Baill. *Plantes Medicinales et phytotherapie*. tome 1, N°4, 214-220.
7. Autore G, Capasso F, Defusco R, Fasulo MP, Lembo M, Mascolo N, Menaghini A (1984). Antipyretic and antimicrobial action of *Teucrium Polium*. *Pharmacol. Res Commun.* 16, 21-29.
8. Mitscher LA, Lev R, Bathala MS, Wu W, Beal JL (1972). Antimicrobial agents from higher plants. Introduction, rational and methodology. *Lloydia*. 315:157-166.
9. Schemper V, Ribas A, Nicolau M, Cechinel-Filho V (1986). Antispasmodic effects of hydroalcoholic extract of *Marrubium vulgare* on isolated tissues. *Phytomedicine*. 3:211-216.
10. Meyre-silva C, Yunes RA, Schlemper V, campos-Buzzi F, Cechinel-Filho V (2005) Analgesic potential of Marrubin derivatives, a bioactive diterpen present in *Marrubium vulgare* (lamiaceae).II *Farmaco*.60:321-326.
11. Rigano D, Grassia A, Bruno M, Rosselli S, Piozzi F, Formisano C, Arnold NA, Senatore F (2006). Labdane diterpenoids from *Marrubium vulgare*: *J.Nat.Prod.*69 (5): 836-838.
12. Cavanagh HMA, Wilkinson JM (2002). Biological activities of Lavender essential oils. *Phytother.Res.*16:301-308.
13. Rojas G, Levaro J, Tortoriello J, Navarro V(2001). Antimicrobial evaluation of certain plants used Mexican traditional medicine for the treatment of respiratory diseases. *J.Ethnopharmacol.*74,97-101.
14. Singh M, Srivastava S, Rawat AKS (2007). Antimicrobial activities of Indian *berberis* species. *Fitoterapia*.78:574-576.
15. Murugan M and Mohan V.R.(2011) Evaluation of phytochemical analysis and antibacterial activity of *Bauhinia purpurea* L. and *Hiptage benghalensis* L. Kurz. *Journal of Applied Pharmaceutical Science* 01 (09); 2011: 157-160.
16. Kumara Swamy M*1, Phytochemical screening and in vitro antimicrobial activity of *Bougainvillea spectabilis* flower extracts. *International Journal of Phytomedicine* 4 (2012) 375-379.