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Full Length Research Paper

Antibacterial evaluation of selected Moroccan medicinal plants against *Streptococcus pneumoniae*

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In Morocco, most people particularly in rural areas use medicinal plants to treat diseases. In order to find new antipneumococcal extracts, an ethnobotanical survey has been conducted in different regions of Marrakesh (south of Morocco). Four plants often cited by traditional practitioners (*Marrubium vulgare, Thymus pallidus*, *Eryngium ilicifolium* and *Lavandula stoechas*) are tested against *Streptococcus pneumoniae* responsible for pharyngitis, rhinitis, otitis and sinusitis infections. Aqueous and methanol extracts have been prepared and tested on *S. pneumoniae* collected in four regions of Marrakesh. A significant activity has been observed with methanol extracts of three plants; *M. vulgare, T. pallidus* and *L. stoechas* (MIC= 256 µg/ml).

Key words: *Streptococcus pneumoniae,* antibacterial activity, medicinal plants, phytochemical screening.

INTRODUCTION

Streptococcus pneumoniae is responsible for diseases including bacteraemia, meningitis, pharyngitis, rhinitis, otitis, sinusitis, arthritis and pneumonia (Demachy et al., 2001). Although most *S. pneumoniae* organisms remain susceptible to penicillin, resistant strains have been recognized since 1967, when a resistant strain was identified in Aus-tralia (Austrian, 1986). Since then *S. pneumoniae* strains with reduced susceptibility to penicillin have been report-ted worldwide (Simberkoff et al., 1986). This resistance to penicillin is accompanied with other resistance in particu-lar to erythromycin and chloranphenicol (Leclercq, 1999). Data on Moroccan serotypes of *S. pneumoniae* and their sensitivities are rare and disparate. Traditional medicine has been an important source of products for developing countries in treating common infections. Morocco has a long history of traditional herbal medicine and its geogra-phic position in the extreme north-west of Africa has fa-voured a development of a rich flora and biodiversity. Also, Moroccan medicinal plants are frequently used by the population to prevent or cure gastrointestinal disorders, hair and skin care and bronchopulmonary and urinary systems (Bellakhdar et al., 1991). As part of our continuing work on local plants with reputed antimicrobial activties (Larhsini et al., 2001; Markouk et al., 1999) we report in this paper the *in vitro* test of four different plant species against *S. pneumoniae* selected on the basis of interviews with herbalists of popular markets of Marrakesh region (south of Morocco). 82 herbalists have been interviewed in the study and the investigation brought out 4 plants used in the treatment of ORL infections according to the frequency of citation: *Marrubium vulgare, Thymus* pallidus, *Lavandula stoechas* and *Eryngium ilicifolium*.

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MATERIALS AND METHODS

Test organism

The *S. pneumoniae* test organisms used for the bioassay were carriage isolates. The strains were isolated from children aged 2 to 9 years in four different hospitals in Marrakesh (South of Morocco).

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 plates

Table 1. Ethnobotanical investigation (82 herbalists) of the most plants used in traditional medicine against ORL infection (region of Marrakesh).

Scientific name	Local name	Family name	Frequency (%) of citation	Part used	Mode of use
L. stoechas	Lakhzama	Lamiaceae	9 (10)	Whole plant	Decoction in olive oil
M. vulgare	Merruta	Lamiaceae	10 (11.11)	Whole plant	Drops with the fresh ear or decocted in the olive oil
T. pallidus	Z'etra	Lamiaceae	8 (8.89)	Whole plant	Decoction
E. ilicifolium	Zerrigua	Apiaceae	12 (13.33)	Whole plant	Decoction in milk
N. sativa	Chanouj	Renonculaceae	5 (5.55)	Seeds	mixed with honey
E. caryophyllata	Krounfel	Myrtaceae	4 (4.44)	Floral buttons	Decoction
A. herba alba	Chih	Asteraceae	3 (3.33)	Leaves and stems	Fumigation in association with *Marrubium*
R. officinali s	Azir	Lamiaceae	3 (3.33)	Leaves and stems	Decoction

Figures in brackets represent % frequency.

Table 2. Results of phytochemical screening of studied plants.

Phytochemicals	*T. pallidus*	*L. stoechas*	*M. vulgare*	*E. ilicifolium*
Anthocyans	-	+++	-	-
Leucoanthocyans	-	+++	++	++
Flavonoides	+	+++	-	+
Terpenes sterols	+++	+++	+++	+++
Gallic tanins	-	+++	+++	+++
Catechic tanins	+++	+++	-	-
Saponines	-	+	-	+++
Alkaloides	-	-	-	-
Quinones	-	+++	-	+++

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

plates were placed in an incubator at 37°C and 5% of CO_2 for 18 to 24 h. The alpha-hemolytic colonies from each plate showing optochin susceptibility were subcultured on another blood agar plate to confirm optochin inhibition. Those strains with zones of inhibition $\geq$ 14 mm in pure culture were considered to be *S. pneumoniae.*

Plant materials

An ethnobotanical survey has been carried out during the period March-April 2006 in Marrakesh regions to identify plants used in traditional medicine against ORL infections. During this survey, 82 traditional healers and herbalists were interviewed. This investigation brought out four plants according to the frequency of citation. (Table 1).

The plants used for the study; *M. vulgare* L. (Lamiaceae), *T. pallidus* Coss. (Lamiaceae), *L. stoechas* L. (Lamiaceae) and *E. ilicifolium* Lam. (Apiaceae) have been collected in May 2006 in Ourika (region of Marrakesh) and identified in the Laboratory of Biotechnology, Protection and Valorization of Plant resources. Voucher specimens mark 4225, 4223, 4224, and 4226 respectively are deposited at the herbarium of the Faculty of Sciences, Semlalia, Marrakesh.

Extraction and phytochemical screening

The dried powder aerial parts of each plant were extracted with water decoction and methanol. The solutions were evaporated *in vacuo* and crude extracts are lyophilized and stored at 4°C until further use.

Phytochemical screening (Table 2) was carried out to highlight the existing chemical groups in the studied plants, in order to have an idea of the chemical nature of the active ingredients responsible for their antibacterial effects (Bouquet and Paris, 1967; Paris and Nothis, 1969).

Determination of antimicrobial activity

Disc diffusion method: Susceptibility test was carried out using the agar diffusion method (Autore et al., 1984) followed by the dilution method for extracts which gave interesting activities. Petri plates were prepared by pouring 20 ml of Muller Hinton agar (BIO-RAD) supplemented with 5% defibrinated sheep blood. The inoculum was 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. Whatman's filter discs (6 mm) impregnated with extracts in a concentration of 500 µg/disc were deposited on inoculated plates and left at 4°C for 2 h to allow the diffusion of the extract before their incubation for 24 h 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 millimetres. Each test was carried out in triplicates.

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

Origin of *S. pneumoniae*	Diameter of inhibition in (mm)										
	Methanol extracts				Aqueous extracts				Antibiotics		Negative control
	T. pallidus	*L. stoechas*	*M. vulgare*	*E. ilicifolium*	*T. pallidus*	*L. stoechas*	*M. vulgare*	*E. ilicifolium*	Chloramphenicol (30 µg)	Erythromicine (15 µg)	DMSO (1%)
Chichaoua	24 ± 0.38	25 ± 0.42	23 ± 0.47	12 ± 0.69	-	-	-	-	25 ± 0,45	25 ± 0,56	-
Imlil	20 ± 0.58	18 ± 0.93	20 ± 0.76	15 ± 0.76	-	-	-	-	25 ± 0,34	28 ± 0.40	-
Tasseltante	22 ± 0.76	24 ± 0.36	21 ± 0.44	12 ± 0.41	-	-	-	-	17 ± 0,72	15 ± 0,65	-

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

Origin	*T. pallidus*			*M. vulgare*			*L. stoechas*			*E. ilicifolium*			Amoxicilin (10 µg/ml)	DMSO 1%
	Concentrations (µg/ml)													
	512	256	128	512	256	128	512	256	128	512	256	128	-	+
Chichaoua	-	-	±	-	-	±	-	-	±	-	-	±	-	+
Taseltant	-	-	±	-	-	±	-	-	±	-	±	+	-	+
Imlil	-	-	±	-	-	±	-	-	±	-	-	±	-	+

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

Dilution method: Minimum Inhibitory Concentration (MIC) was carried out by agar dilution method (Mitscher et al., 1972). 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 concentrations: 512, 226 and 128 µg/ml. The antimicrobial assay was carried out on Muller-Hinton's agar with sheep blood (5%) for 24 h at 37°C. Negative control containing DMSO 1% and positive control plates containing amoxicillin (10 µg/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 depicted in Table 2, high doses of terpenes and sterols were detected in all plants. In general, flavonoides and sponines have been found in high doses respectively in extracts of *L. stoechas* and *E. illicifolium*. *T. pallidus* and *M. vulgaris* and were also rich in terpenes, sterols and tannins. It is noteworthy that no alkaloid has been reported in all plants.

Antimicrobial activity

Disc diffusion method: The results of the 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. *L. stoechas*, *T. pallidus* and *M. vulgaris* methanol extracts have strong activities with diameter of inhibition varying from 18 to 25 mm.

Methanol extract obtained from *E. ilicifolium* showed weak antimicrobial activity as assessed by the diffusion method. Methanol extract of *L. stoechas* showed the highest diameter of inhibition against *S. pneumoniae* strain isolated from Chichaoua (diameter = 25 mm). This value is similar to that obtained for standard antibiotics, chloramphenicol and erythromicin.

Dilution method: The minimum inhibitory concentration obtained for methanol extracts of the three plants were as low as 256 µg/ml (Table 4).

DISCUSSION

The traditional healers interrogated in the ethnobotanical survey declared that *M. vulgaris* was the most common drug used for otitis and sinusitis treatments. In general pharyngitis disease was

treated with *E. ilicifolium*.

In our study, the result of phytochemical screening (Table 2) was according to that reported in the literature. Thus, the presence of tannins and sterols in *M. vulgaris* has been also reported by El Bardai et al. (2003) and Sahpaz et al. (2002). The saponins were also detected by Küpeli et al. (2006) in *E. ilicifolium*. Previous phytochemical investigations of *L. stoechas* revealed the presence of acetylated glucoside of luteolin and flavone glucoside in ethylacetate extracts of the aerial parts (Gabrieli and Kokkalou, 2003).

The methanol extracts of *L. stoechas*, *M. vulgaris* and *T. pallidus* presented antibacterial effects (Table 3). Thus, the methanol extract of *M. vulgaris* showed complete inhibition of *S. pneumoniae* with MIC= 256 µg/ml. The antibacterial activity detected for *M. vulgare* is due probably to terpenoids as we found the presence reported for this plant (Schlemper et al., 1996; Mayre-Silva et al., 2005). Marrubiin 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* (Rigano et al., 2006).

The methanol extract of *L. stoechas* was active on the growth of *S. pneumoniae* with MIC= 256 µg/ml. This extract was rich in phenolic compounds (flavonoids and tannins) (Table 2). The activity observed may probably be due to these compounds. 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 (Cavanagh and Wilkinson, 2002).

In general, the minimum inhibitory concentration obtained for methanol extracts of all plants studied were as low as 256 µg/ml. This value is much lower than that observed for example by Rojas et al. (2001) on the same germ which showed an MIC value of 1.2 mg/ml for chloroformic extracts of *Crescentia alata* and *Gnaphalium americanum*, hexanic extract of *Gossypium hirsutum* and methanolic extract of *Gnaphalium oxyphyllum*. However, Singh et al. (2007) working on four Indian *Berberis* spp. have shown lower MIC values on *S. pneumoniae* (MIC= 0.31 µg/ml) especially, the hydro-alcoholic extracts of stems of *Berberis aristata* and *Berberis asiatica*.

Conclusion

The use of medicinal plants in the treatment of ORL infections is a common practice in Moroccan folk medicine. We have found that the activities of methanol extracts obtained from *T. pallidus*, *M. vulgare* and *L. stoechas* 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 principle(s) and evaluating possible synergism of antimicrobial activity among these extracts.

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