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Full Length Research Paper

Antimicrobial activity of some alimentary and medicinal plants

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***Vicia faba* L., *Vaccinium macrocarpon, Punica granatum, Lavandula officinalis, Artemisia absinthium, Linum capitatum* and *Camellia sinensis* were frequently used in our alimentation. In this study, we have tested the antimicrobial activity of their ethanolic and methanolic extracts on some pathogen bacteria, then their ability to *in vivo* inhibit the growth of *Streptococcus pneumoniae*. The phytochemical screening has given the composition of the most active extracts. According to the obtained results, the ethanolic extract of *Lavendula officinalis* and *A. absinthium* has shown an inhibition of all the tested bacteria. The ethanolic extract of *L. officinalis* has given the highest activity against *S. pneumoniae* followed by the methanolic extracts *of C. sinensis* and *P. granatum.* The phytochemical screening showed that the most active extracts contained mainly phenolic compounds.**

Key words: Plants, extracts, antimicrobial activity, *Streptococcus pneumoniae*, phytochemical screening.

INTRODUCTION

Several plants were used for many generations for their therapeutic virtues, and this was before knowing the exact origin of their benefits. In fact, they were used for treating infections (Bussmann and Sharon, 2006), malaria (Willcox et al., 2005), burns, edema, allergies and prevent several diseases (Bussmann and Sharon, 2006).

These last decades, researchers have began to explain these virtues by the ability of pants to limit infections (Ulanowska et al., 2006; Kuster et al., 2009), prevent lipidic peroxidations (Yamanaka et al., 1996) and their associated diseases (Rein et al., 2000; Martin and Andriantsitohaina, 2002), prevent some cancers (Ames et al., 1995) and cure allergies (Park et al., 2008). Among all these virtues, the anti-infectious activity was considered as one of the most important activities. In this work, we proposed to test the antimicrobial activity of some plants usually used in our alimentation and this in order to establish the benefits that they gave in the protection against some pathogen bacteria and particularly against *Streptococcus pneumoniae*.

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MATERIALS AND METHODS

Plants origin

The plants were picked from different localities of Algeria, in their full bloom because they produce several bioactive compounds during this period (Marston and Hostettmann, 2006). The botanical departments of the Constantine and Jijel Universities (Table 1).

Bacteria origin

The tested bacteria came from the Constantine UHC (University Hospital Center, Constantine, Algeria) and the microbiological and infectious laboratories of the Mohammed Ben-Yahia's hospital (Jijel, Algeria).

Preparation of the ethanolic and methanolic extracts

The ethanolic extracts were obtained by maceration of the plant

Table 1. List of the tested plants.

Common name	Scientific name	Used parts
Broad bean	*Vicia faba L.*	Seed coat
Cranberry	*Vaccinium macrocarpon*	Fruit
Pomegranate	*Punica granatum*	Seed coat
Lavender	*Lavandula officinalis*	Leaves
Wormwood	*Artemisia absinthium*	Leaves
Flax	*Linum capitatum*	Leaves
Tea	*Camellia sinensis*	Leaves

material during 24 h in aqueous ethanol (80%). And the methanolic extracts were prepared using the Soxhlet apparatus with the pure methanol as solvent. These extracts were filtered and evaporated using a rotary evaporator and freeze dryer to give the crude dried extract (Marston and Hostettmann, 2006).

Test of the antibacterial activity of the extracts

Patches of 6 mm in diameter were cut up on the Wattman No. 1 paper and sterilized. The bacteria suspensions were normalized at 10^6 UFC/ml. Petri dishes containing the Muller-Hinton medium were inoculated and the patches containing 20 µl of the tested extract were put in their surface. The incubation was made at 37°C during 18 h (Osato, 2009). The industrial ampicillin was used as a positive control.

Test of the *in vivo* antibacterial activity against *S. pneumoniae*

This test was realized for the extracts which showed a high *in vitro* antibacterial activity against *S. pneumoniae*. The bacteria growth was optimized in the Todd-Hewitt medium by incubation for 24 h at 35°C. Then the microorganisms were separated by centrifugation for 10 min at 2000 x g and recuperated in saline buffered solution.

Young albino male rats for 190 to 210 g were kept at 26 ± 2°C, exhibited to the light for 12 h per day and had an free access to food and water (Miyazaki et al., 2002). The animals were anesthetized with ketamine-xylazine then infected by intra-nasal instillation of 0.5 ml of the bacteria suspension. We divided them in several groups containing each 5 rats. The first group was left without treatment (negative control), the second was infected then treated with amoxicillin (positive control) and the others were treated with different concentrations of the extracts. The treatment administration was made first 20 h after the infection then for 3 days at a rate of tree doses per day spaced by 6 h. The lungs of rats were taken 20 h after the last administration. They were rinsed, ground and homogenized in 5 ml of physiological water. The bacteria count was realized by inoculation of the lungs suspensions on the blood agar medium (Miyazaki et al., 2002). ED_{50} was evaluated with Mann Whitney *U* test.

The phytochemical screening of the extracts

The phytochemical screening was made by a thin layer chromatography (TLC) and the UV-visible spectrum of each separated molecule (Marston and Hostettmann, 2006).

For the ethanolic extracts we have used a TLC on polyamide gel with the solvent: Toluen / Methylethylketon / Ethanol / Petrol ether: 2/1/1/1 (v/v/v/v). And for the methanolic extracts a TLC on silica gel with the solvent: Butanol/Acetic acid/Water: 4/1/5 (v/v/v).

RESULTS

The antibacterial activity of the extracts

Alimentary plants as well as *Camellia sinensis* and *Linum capitaum* had methanolic extracts which were more active than the ethanolic ones. On the contrary, medicinal plants (*Lavendula officinalis* and *Artemisia absinthium*) had ethanolic extracts as the most active ones. They correctly inhibited the Gram positive and the Gram negative bacteria. Methanolic extracts of these plants had an average activity against Gram positive bacteria and a weak activity against Gram negative ones (Table 2).

By comparing the obtained results, we have found that the ethanolic extracts of *L. officinalis* and *A. absinthium* were the most active against all the tested bacteria. Moreover, the ethanolic extract of *L. officinalis*, the methanolic extracts of *Camellia sinensis* and *Punica granatum* were the most active against *S. pneumoniae*.

The *in vivo* antibacterial activity against *S. pneumoniae*

In the untreated group, *S. pneumoniae* caused 100% of mortality after 72 to 96 h of infection. The number of bacteria founded on their lungs was 5.1 x107 ± 0.23 UFC/lung.

All the tested extracts have shown an inhibition of *S. pneumoniae* superior to the one gave by the amoxicillin. The ED_{50} of the ethanolic extract of *L. officinalis* was the most interesting with a value of 9.905 mg/ml (Table 3). This extract has allowed the elimination of all the bacteria with 50 mg/ml administrated on three doses over three days. Methanolic extracts of *C. sinensis* and *P. granatum* gave exactly the same values and this at all the tested doses; also they have a similar ED_{50} of 11.458 mg/ml (Figure 1).

The phytochemical screening of the extracts

The phytochemical screening has shown that the methanolic extract contained an important diversity of the plant secondary compounds while the ethanolic ones contained principally flavonoids and phenolic acids (Table 4). Among the plants which were the most loaded on these compounds we could quote *C. sinensis, L. capitatum* and *L. officinalis.*

DISCUSSION

The obtained results have shown that the ethanolic extract of *L. officinalis* had an important antimicrobial activity. This could be explained by the presence of flavonoids in this extract. Moreover, *L. officinalis* has allowed a considerable *in vivo* inhibition of

Table 2. Results of the antimicrobial activity expressed in minimal inhibitory concentrations (MICs in mg / ml). Ext.: extract; *Sc.s*: *Staphylococcus saprophyticus*; *B.P*: *Bacillus pumilus*; *B.m*: *Bacillus megaterium*; *St.p: Streptococcus pneumoniae; P.l: Pseudomonas luorescens*; *S.t: Salmonella typhi*; *S.p: Salmonella paratyphi*; *Y.p: Yersinia pseudotuberculosis*.

Plants	Ext.	*Sc.s*	*B.p*	*B.m*	*St.p*	*P.l*	*S.t*	*S.p*	*Y.p*
Camellia sinensis	M	0.065	0.065	0.035	0.010	-	-	0.120	-
	E	-	-	-	0.120	-	-	-	-
Punica granatum	M	0.075	0.100	0.075	0.010	-	0.080	0.120	0.100
	E	0.120	-	-	-	-	-	-	-
Linum capitatum	M	0.085	0.100	0.075	0.080	-	0.120	0.120	-
	E	-	-	-	0.060	-	0.040	0.060	0.060
Lavandula officinalis	M	0.040	0.065	0.050	0.080	0.080	-	0.060	-
	E	0.020	0.015	0.025	0.005	0.040	0.020	0.035	0.025
Artemisia absinthium	M	0.080	0.080	0.060	0.065	0.100	0.060	-	-
	E	0.045	0.020	0.030	0.020	0.070	0.020	0.060	0.035
Vaccinium macrocarpon	M	0.060	0.075	0.040	0.065	-	0.100	0.080	0.100
	E	-	-	-	0.100	-	0.120	0.080	0.130
Vicia faba L.	M	0.065	0.080	0.035	0.060	-	0.085	0.095	0.080
	E	0.075	0.120	0.060	0.085	-	0.080	-	-

Table 3. Diminution of the number of *Streptococcus pneumoniae* depending on the administered doses. M: methanolic extract; E: ethanolic extract.

	UFC / lung							**ED_{50} mg/ml (Confidence limit 95%)**
Dose mg/ml	**10**	**20**	**30**	**40**	**50**	**60**	**70**	
Log dose	**1.000**	**1.301**	**1.477**	**1.602**	**1.699**	**1.778**	**1.845**	
Lavendula officinalis (E)	28000000 ± 1.47	290000 ± 0.56	5730 ± 1.50	88 ± 0.61	0	0	0	9.905 (0.60 – 1.00)
C. sinensis P. granatum (M)	36000000 ± 1.35	5600000 ± 0.78	25000 ± 0.67	6900 ± 0.48	220 ± 1.22	100 ± 0.30	64 ± 0.17	11.458 (1.10 – 1.50)
Amoxicillin	45600000 ± 1.45	9800000 ± 0.54	45600 ± 1.00	29000 ± 0.99	1300 ± 0.39	700 ± 0.54	57 ± 0.97	14.141 (0.60 – 0.90)

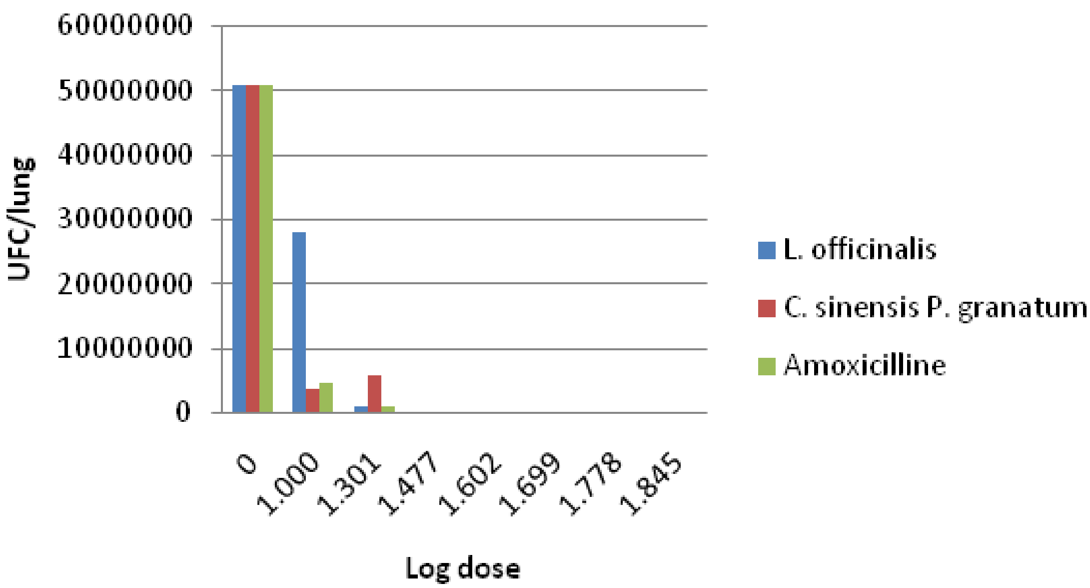

Figure 1. Diminution of *Streptococcus pneumoniae* under the effect of the administered treatments. The ethanolic extract of *L. officinalis* was more active than the methanolic extacts of *C. sinensis* and *P. granatum*. It completely inhibited the bacteria growth after the administration of 50 mg/ml.

Table 4. Results of the phytochemical screening of the extracts; M: methanolic extract; E: ethanolic extract; (number): number of the detected compounds.

Plants	Extract	Detected molecules
Camellia sinensis	M	Tannins (7), alcaloids (5), anthocyans (2)
	E	Phenolic acids (3), anthocyans (2)
Punica granatum	M	Tannins (4), anthocyans (4)
	E	Acides phénoliques (6)
Linum capitatum	M	Flavonoids (5), terpenoids (4), steroids (3), essential oils (3)
	E	Flavonoids (7)
Lavandula officinalis	M	Essential oils (10)
	E	Flavonoids (6)
Artemisia absinthium	M	Essential oils (6)
	E	Flavonoids (5)
Vaccinium macrocarpon	M	Tannins (5), anthocyans (3)
	E	Flavonoids (2)
Vicia faba L.	M	Tannins (5), anthocyans (3)
	E	Phenolic acids (2)

S. pneumoniae. It was interesting to signal that no investigation has found the antimicrobial activity of the ethanolic extract of this plant; this one may be the first.

Also, *A. absinthium* has shown interesting results of the *in vitro* antimicrobial activity and principally against *Bacillus pumilus, S. pneumoniae* and *Salmonella typhi*. Sengul et al. (2011) have affirmed that this plant had an important antimicrobial activity against different bacteria,yeasts and moulds. These researchers have obtained a great inhibition of some bacteria which were near species like *B. subtilis* and this oven if they have used the methanolic and aqueous extracts but not the ethanolic one. The phytochemical screening proved that the activity of this plant maybe depending on its content on phenolic compounds in accordance with the results of Sengul et al. (2011).

For *L. capitatum, Vaccinium macrocarpon* and *Vicia faba* L. we observed that the methanolic extracts have a more important activity than the ethanolic extracts which corresponding with the works of Akroum et al. (2009). Sure enough, the ethanolic extracts of these plants showed high values of MICs and someone a complete absence of the activity. Also, it was important to signal that these plants were the least active ones of this study.

MICs of the methanolic extract of *C. sinensis* were the most interesting against the Gram positive bacteria, but their activity against the Gram negative ones was virtually absent. Nevertheless, this extract has shown an important inhibition of *S. pneumoniae.* Elamparithi and Boominathan (2011) have demonstrated also that the methanolic extract of *C. sinensis* had an important antimicrobial activity against this bacterium. The phytochemical screening of this extract was rich on tannins and alkaloids which could explain the obtained results.

P. granatum has allowed making the same report that the previous plant: an important antimicrobial activity against the Gram positive bacteria, a lower activity against the Gram negative ones and a higher *in vivo* inhibition of *S. pneumoniae*. The phytochemical screening has demonstrated that the tested extract was rich in tannins and anthocyans according to the results given by Reddy et al. (2007).

Some previous studies on this plant have rather shown that its antimicrobial activity was very interesting against the two types of bacteria and also against some yeasts and moulds (Oskay et al., 2009**;** Hayouni et al., 2011), but it had an average activity against *Streptococcus pneumoniae* (Oskay et al., 2009).

So, it was easy to note that plant extracts which shown the most important activities contained principally phenolic compounds: tannins, flavonoids and phenolic acids. These molecules were true enough known as antimicrobials (Greenberg et al., 2008; Akroum et al., 2010; Puupponen-Pimiä et al., 2001; Sengul et al., 2011).

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