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The Antibacterial Activity of Moroccan Bee Bread and Bee-Pollen (Fresh and Dried) against Pathogenic Bacteria

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ABSTRACT

Samples of natural bee-bread and bee-pollen from different aromatic and medicinal plants were studied for their antimicrobial activities on antibio-resistant bacterial strains isolated from human pathology. Four samples of bee-bread, two samples of fresh bee-pollen and two samples of dried bee-pollen were collected from different regions in Morocco. Dilutions of bee-bread and bee-pollen from 1/2, 1/4, 1/8 and 1/16 were tested by the agar well diffusion method on various strains of bacteria including *E. coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa*. Results revealed that most of strains were inhibited by the dilution 1/2 and 1/4. The Gram positive bacteria were more sensitive to bee-bread and bee-pollen than Gram negative bacteria. All the samples showed strong antimicrobial activities on the bacterial strains, which were first tested for their resistance to antibiotics. The results showed that bee-bread and bee-pollen samples were inhibitory than dried bee-pollen.

Key words: Antibacterial activity, fresh pollen, dried pollen, bee bread, pathogenic bacteria

INTRODUCTION

In the last years, a change in food habits of consumers is taking place, principally in the developed countries, where people aspire to have more healthy and nutritious diets. The apicultural products have been widely used in diet complements as well as in phytotherapy.

Bee-pollen is a hive derived product of great commercial interest owing to its high nutritional quality and can be considered as potential source of energy and proteins for human consumption (Kroyer and Hegedus, 2001; Campos *et al.*, 2003).

Pollen is a fine, powder-like material produced by flowering plants and gathered by worker bees. Bees use pollen as their nutritional source of proteins (25-30%), carbohydrates (30-55%), lipids, including fatty acids and sterols (1-20%), vitamins and minerals. Bee pollen and beebread (stored pollen) are consumed for api-therapeutical purposes for their nutritional and medicinal properties. In its composition it presents valuable nutrients such as free aminoacids, minerals and oligo-elements and for this reason it is used in the human diets providing a well-being sensation and contributing to functional and harmonious balance of the body (Stanciu *et al.*, 2009). These

components were also rich in carotenoids, flavonoids, phytosterols, polyphenols and other healthy compounds (Serra Bonvehi *et al.*, 2001; Baltrusaityte *et al.*, 2007; Moreira *et al.*, 2008). During storage the pollen undergone chemical changes (Roulston, 2005). Also, stored pollen is commonly associated with microorganisms that supposedly play a role in conversion of pollen into a more digestible food for the bees (Human and Nicolson, 2003). This association may contribute to the pollen's acquisition of certain organoleptic properties, which are specific for each bee species (Fernandes da Silva and Serrao, 2000).

Bee bread will apply more and more as health food and medicine due to its functional properties such as antioxidative ability and scavenging activities of reactive oxygen species and its benefit in various diseases such as cancer, cardiovascular diseases, diabetes and hypertension (Nagai *et al.*, 2004).

Bee pollen is widely used for its therapeutic properties, thus extracts of bee pollen have been used in chronic prostatitis for their presumed anti-inflammatory (Wagenlehner *et al.*, 2009), anti-androgenic effects (Shoskes, 2002; Shoskes and Manickam, 2003), anti-tumor activity (Yang *et al.*, 2007) and used for oral desensitization and allergies (Medeiros *et al.*, 2008). Also, bee pollen has antimicrobial effects (Basim *et al.*, 2006).

Other authors have demonstrated immuno-stimulation activity and an antioxidant role for bee pollen (Moreira *et al.*, 2008). The German Federal Board of Health has officially recognized bee pollen as a medicine (Ishikawa *et al.*, 2008).

Among bee pollen components, the flavonoids profile and their biological activity have been the subject of several investigations in order to establish quality parameters of bee pollen (Serra Bonvehi *et al.*, 2001) to characterize them in terms of botanical origins (Tomas-Barberan *et al.*, 2001) and to evaluate their nutritional and biological properties (Kroyer and Hegedus, 2001; Campos *et al.*, 2003).

The *in vitro* antibacterial activities of Turkish pollen extracts were investigated against 13 different species of agricultural bacterial pathogens, the least active concentrations towards the tested bacteria were 1/100 of the pollen extract (Basim *et al.*, 2006).

The aim of this study was to investigate the antibacterial activity of bee bread and bee-pollen (fresh and dried) extracts against some pathogenic bacteria. To our knowledge, there are few studies of bee bread and dried pollen extract against pathogenic bacteria.

MATERIALS AND METHODS

Sample collection: Samples used in this study were collected in different region of Morocco. Two dry bee pollen samples (1 and 2) were provided by Moroccan bee-keepers, harvested during the periods of floral production in March, 2006. They were collected in the province of Azilal and in the province of Khmissat.

Four samples of bee bread, were obtained from different region of Morocco (Khmissat, Sidi-Sliman and Kénitra) and were collected by beekeepers in April 2006. All samples were kept at 4°C.

Two fresh bee pollen samples loads were collected in period of floral production in 2006, in the Bensliman area, from the beginning of February to the end of April by 20 honey bee colonies (*Apis mellifera*) settled in hives with bottom-fitted pollen traps. The fresh bee pollen was stored at -18°C until analysed.

Preparation of bee pollen and bee bread solutions: Solutions of bee pollen were prepared by 50% (v/v). Each sample was diluted in DMSO (10%) to give concentration of 6.25; 12.5; 25 and 50%. The same concentrations were undertaken as controls test by diluted water DMSO.

Microbial cultures: Forteen isolates of anti-bioresistant gram-ve and gram+ve pathogenic bacteria were used: *E. coli* (3 strains), *Salmonella enteritidis* (1 strain), *Pseudomonas aeruginosa* (3 strains), *Staphylococcus aureus* (3 strains), *Streptococcus* (3 strains), *Bacillus cereus* (1 strain).

Antibiotic sensitivity of microorganisms: Antibiotic sensitivity of bacterial isolates was performed by the disc diffusion method (Collins *et al.*, 1995). The antibiotics (Safoni Diagnostics Pasteur, France) used were, C: Chloramphenicol 30 µg, ST Sulfathiazol 0.25 mg; AMX: Amoxycillin 10 µg; DA: Clindamycin 2 mg; AM: Ampicillin 10 µg. The bacterial suspension (100 µL) was inoculated onto Muller Hinton Agar plates (Biocar, France), and allowed for 15 to 20 min to solidify. The antibiotic discs were placed aseptically in the plates (5 discs/plate). These plates were than incubated at 37°C for 24 h. Diameter of the inhibitory zone around the discs was measured and recorded in millimeters.

Antibacterial activity: The antibacterial activity was studied by the agar well diffusion method (Collins *et al.*, 1995). The inoculum suspension of each strain of bacteria was prepared to give a concentration of 10^7 to 10^8 bacteria mL^{-1} . The bacterial strains were grown on Broth Health infusion for 24 h at 37°C. 100 µL of this culture was added to 9,9 mL of natural saline water (85 g L^{-1}) and 100 µL from this suspension was transferred to sterile petri dishes. Fifteen milliliter of Muller Hinton Agar (Biocar, France) was poured aseptically and the plates were kept for 15-20 min at room temperature to allow agar to solidify. Wells of 4mm height and 6mm in diameter were then made in the solid medium with a metallic device and filled with the different concentration of bee-pollen or bee bread solutions (100 µL well^{-1}). The plates containing bacteria were incubated at 37°C for 24 h. After incubation, the diameter of inhibitory zones was measured in mm.

RESULTS

Most of the strains used in this study were resistant to antibiotics (Table 1) and they were isolated from human samples and may have been involved in some diseases that would imply the use of antibiotics. All the strains showed a normal growth on the medium and no inhibition was observed around the culture for almost all the antibiotics. There were only some strains which were sensitive to some antibiotics in every case. All the strains were resistant to AMX10, DA2 and AM10. There was only isolate of *Pseudomonas aeruginosa* ATCC29733 which was sensitive to ST25 and one isolate of *Staphylococcus aureus*, *Streptococcus* and *Bacillus* which were sensitive to C30 and ST25.

Table 2 shows results of bee bread (4 samples). All the strains were inhibited by the dilution 1/2 and most were inhibited by the dilution 1/4. Gram positive strains were more sensitive than gram negative bacteria.

Differences in the inhibition between the samples were not so important for both Gram negative and Gram positive bacteria regarding the dilution, but for the diameter of the inhibition zone, differences were observed for all the strains.

Table 1: Antimicrobial sensitivity of the strains used in the antimicrobial activity of bee-bread and bee-pollen

Strains	C 30	AMX 10	ST 25	DA 2	AM10
Gram-ve bacteria					
<i>E. coli</i> 1	R	R	R	R	R
<i>E. coli</i> 2	R	R	R	R	R
<i>E. coli</i> ATCC25921	R	R	R	R	R
<i>P. aeruginosa</i> 1	R	R	R	R	R
<i>P. aeruginosa</i> 2	R	R	R	R	R
<i>P. aeruginosa</i> ATCC 29733	R	R	S:18	R	R
<i>Salmonella enteritidis</i>	R	R	R	R	R
Gram+ve bacteria					
<i>S. aureus</i> 1	R	R	R	R	R
<i>S. aureus</i> 2	R	R	R	R	R
<i>S. aureus</i> ATCC 25923	S:20	R	S:12	R	R
<i>Streptococcus</i> 1	S:22	R	S:12	R	R
<i>Streptococcus</i> 2	R	R	R	R	R
<i>Streptococcus</i> 3	R	R	R	R	R
<i>Bacillus cereus</i>	S: 28	R	S: 22	R	R

R = Resistance, S = Sensitive

Table 2: Effect of different concentrations of bee-bread on different bacterial isolates

Strains	Bee bread 1				Bee bread 2				Bee bread 3				Bee bread 4			
	1/2	1/4	1/8	1/16	1/2	1/4	1/8	1/16	1/2	1/4	1/8	1/16	1/2	1/4	1/8	1/16
Gram-ve bacteria																
<i>E. coli</i> 2	10	9	-	-	14	-	-	-	17	-	-	-	20	14	-	-
<i>E. coli</i> 3	13	10	-	-	16	11	-	-	27	24	19	18	14	11	9	-
<i>E. coli</i> ATCC25921	12	11	-	-	14	12	10	9	18	11	-	-	14	10	-	-
<i>P. aeruginosa</i> 1	15	12	-	-	20	14	12	-	18	11	-	-	14	10	-	-
<i>P. aeruginosa</i> 2	19	18	15	10	20	14	12	-	17	15	11	-	9	9	-	-
<i>P. aeruginosa</i> ATCC 29733	13	-	-	-	16	14	12	10	16	14	10	-	16	12	10	9
<i>Salmonella enteritidis</i>	26	20	10	-	16	13	10	-	12	9	-	-	27	22	17	-
Gram+ve bacteria																
<i>S. aureus</i> 1	13	12	9	-	15	10	-	-	18	16	-	-	14	12	11	-
<i>S. aureus</i> 2	16	12	10	-	14	10	9	-	29	20	18	14	28	25	19	18
<i>S. aureus</i> ATCC25923	10	9	-	-	16	13	10	-	24	19	15	12	12	10	9	-
<i>Streptococcus</i> 1	11	9	-	-	24	20	15	10	25	18	15	11	20	14	11	-
<i>Streptococcus</i> 2	13	10	8	-	14	11	-	-	14	11	-	-	14	10	-	-
<i>Streptococcus</i> 3	11	9	-	-	12	11	-	-	14	12	11	9	12	10	-	-
<i>Bacillus cereus</i>	12	-	-	-	20	18	15	13	16	13	9	-	18	15	13	10

Table 3 shows results of the antimicrobial activity of fresh bee pollen samples. All the strains were inhibited by the dilution 1/2 and most were inhibited by the dilution 1/4, but the sample 2 was more inhibitory for most the strains than sample 1.

Results relative to the inhibitory activity of dried bee pollen are reported in Table 3. These results were different compared to those obtained from bee bread and fresh pollen. Both dried bee pollen samples were not inhibitory to one isolated of *E. coli* ATCC25921 and tow isolates of *Pseudomonas aeruginosa*. Furthermore, one isolated of *Streptococcus* 2 and one isolated of *E. coli* 1 were resistant to sample 1 and sample 2, respectively.

Table 3: Effect of different concentrations of fresh and dried bee-pollen on different bacterial isolates

	Fresh bee pollen 1				Fresh bee pollen 2				Dry bee pollen 1				Dry bee pollen 2			
	1/2	1/4	1/8	1/16	1/2	1/4	1/8	1/16	1/2	1/4	1/8	1/16	1/2	1/4	1/8	1/16
Gram-ve bacteria																
<i>E. coli</i> 1	25	20	10	-	14	11	9	-	23	20	10	-	-	-	-	-
<i>E. coli</i> 2	31	28	22	14	13	11	10	8	35	24	19	14	19	9	-	-
<i>E. coli</i> ATCC 25921	12	-	-	-	14	12	10	-	-	-	-	-	-	-	-	-
<i>P. aeruginosa</i> 1	12	-	-	-	13	10	8	-	-	-	-	-	-	-	-	-
<i>P. aeruginosa</i> 2	10	9	-	-	15	12	10	-	-	-	-	-	-	-	-	-
<i>P. aeruginosa</i> ATCC 29733	12	8	-	-	25	20	18	14	15	13	-	-	10	9	-	-
<i>Salmonella enteritidis</i>	33	29	21	-	14	12	11	8	19	14	-	-	9	-	-	-
Gram+ve bacteria																
<i>S. aureus</i> 1	13	10	9	-	12	11	8	-	13	11	-	-	12	10	-	-
<i>S. aureus</i> 2	15	10	-	-	16	12	10	-	12	-	-	-	11	10	-	-
<i>S. aureus</i> ATCC 25923	15	12	11	-	18	14	11	8	12	11	9	-	13	11	-	-
<i>Streptococcus</i> 1	14	10	9	-	20	19	13	10	28	20	9	-	21	18	10	-
<i>Streptococcus</i> 2	9	8	-	-	14	12	11	9	-	-	-	-	9	-	-	-
<i>Streptococcus</i> 3	11	9	-	-	15	12	11	10	9	-	-	-	10	-	-	-
<i>Bacillus cereus</i>	16	12	10	-	15	11	9	8	12	10	9	-	12	10	9	-

DISCUSSION

Bee bread and bee pollen samples showed a potential activity against the growth of both gram positive and gram negative bacteria which was resistant to antibiotics. This would be a very interesting approach to control more dangerous species of micro-organism in medical sciences. Because of the development of resistance by the microorganisms to common antibiotics, it has become necessary to search for an alternative approach dealing with this situation. It had been suggested that natural products are preferable to synthetic ones.

In this optic, pollen, increased attention for its therapeutic properties, as antimicrobial (Garcia *et al.*, 2001; Proestos *et al.*, 2005; Basim *et al.*, 2006), antifungicidal (Garcia *et al.*, 2001), anti-caryogenic (Almas *et al.*, 2001), immunomodulatory (Gebara *et al.*, 2002) effects and antioxidant activity (Saric *et al.*, 2009).

Several authors have found phenolic and flavonoid compounds in pollen and bee bread (Nagai *et al.*, 2004; Proestos *et al.*, 2005; Almeida-Muradian *et al.*, 2005). A study on total polyphenols in pollen collected by native bees of the region of Vienna in Austria indicated that the pollen collected by bees generally shows characteristic amounts of total polyphenols with some variations due to its botanical origin (Teresinha *et al.*, 2007).

The flavonoids constitute a large group of secondary plant metabolites. Dietary flavonoids have attracted much interest recently because *in vitro* and *in vivo* studies suggest that they have a variety of beneficial biological properties, which may play an important role in the maintenance of human health.

The samples of bee-pollen and bee-bread were found to inhibit the growth of a wide range of microorganisms that were resistant to antibiotics; they showed varied degrees of antimicrobial activity. This activity was higher against gram+ve than gram-ve bacteria. Our findings agree with those reported by Teresinha *et al.* (2007) who finds that *Pseudomonas aeruginosa* bacteria were inhibited by extracts of pollen at 80 and 90% of ethanol solution whereas, *Staphylococcus aureus* bacteria were inhibited at 50, 60, 70 and 80%.

Phenolic compounds may affect growth and metabolism of bacteria. They could have an activating or inhibiting effect on microbial growth according to their constitution and concentration (Rauha *et al.*, 2000; Reguant *et al.*, 2000; Alberto *et al.*, 2001, 2002; Estevinho *et al.*, 2008; Rodríguez Vaquero *et al.*, 2010).

Fresh bee-pollen and bee-bread samples showed in this study the same potential activity against the growth of both gram +ve and gram -ve bacteria but there is the difference in the diameter of the inhibition zone between samples for all strains. These different patterns of sensitivity are due to different phenolic compounds in pollen (Almeida-Muradian *et al.*, 2005). Each pollen type has its own specificity mainly linked to the floral species or cultivars (Nagai *et al.*, 2004).

In floral pollen mostly flavonoids and their glycosides derivatives of cinnamic acid are present (Markham and Campos, 1996). Apart from common flavonoids (quercetin, kaempferol, luteolin and their derivatives), specific flavonoid glycosides, characteristic of some floral pollen, such as 7- α -8-o-methylherbacetin-3-o-sophorosides (Markham and Campos, 1996) or found in the Myrtaceae family aglycone triacin (Campos *et al.*, 2002) were determined.

From *Cistus ladanifer* bee-pollen 12 flavonoid glycosides and four aglycones have been isolated and identified. They are glycosides of myricetin, quercetin, isorhamnetin and kaempferol (Tomas-Lorente *et al.*, 1992).

Serra and Escola (1995) studied the antimicrobial activity of phenolic constituents from 12 samples of propolis and established a relationship between the flavonoid components and the bacteriostatic activity of propolis. Campos *et al.* (1997) founded that the phenolic composition of pollen has been related to the therapeutic properties (antibiotic, antineoplastic, antidiarrhoeic and antioxidant).

The variation of the antimicrobial activities of the tested fresh and dried bee-pollen extracts may be due to the presence of some constituents degradable by the temperature, light and oxygen.

Numerous studies have evaluated the quality of bee pollen by parameters as: colony growth brood production, development of hypopharyngeal glands and ovaries or radical scavenging activity and they have demonstrated that the age of pollen used to feed honey bees can influence these parameters. Thus, Hagedorn and Moeller (1968) indicated that workers fed dried pollen that is 1-year-old or older have smaller hypopharyngeal glands and lower rates of weight gain than workers fed fresh pollen. Also Haydak (1963) reported that colonies fed diets composed of 2-year-old dried pollen rear less brood than colonies fed freshly-dried pollen. Campos *et al.* (2003) demonstrated that pollen aging over 3 years reduce the free radical scavenging activity by up to 50%.

So, since the age of pollen can affect its quality and its utilization by work Hagedorn and Moeller (1968) the degradation of some constituents in dried pollen may occurred and its antimicrobial activity can be affected. However, more detailed studies such as chemical analysis of dried and fresh bee pollen are necessary to confirm this hypothesis.

CONCLUSION

Given the non-toxic and natural origin of bee bread and bee-pollen and the results obtained on their antimicrobial action, it is concluded that their properties are of great interest in academic situations and food, cosmetic and pharmaceutical industries, since their possible use as natural additives emerged from a growing tendency to replace synthetic preservatives by natural ones.

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