

Antimicrobial Activity of Bee Pollen Ethanolic and Methanolic Extracts on *Staphylococcus aureus* Bacterial Strain

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Abstract

Bee pollen is consumed for api-therapeutical, nutritional and medicinal properties. Its actions are attributed to its chemical composition and mostly phenolics, carotenoids, fatty acids and vitamins. For this study, bee pollen was freshly harvested and immediately placed at -18°C. Two different extraction solvents were used: ethanol and methanol, in three different dilutions (60, 70, 80%). 5%, 10% and 15% pollen concentrations in each solvent solution were carried out to determine the most efficient variant, possessing the highest antibacterial activity, using the disc diffusion method. Stock culture of *Staphylococcus aureus* bacteria was grown in Nutrient Broth at 37°C for 24h in a shaker. The inhibition zones were different, according to the extraction solvent used and also the pollen concentration. Methanol extract (70%) of 15% pollen has the biggest inhibition diameter. Negative controls (methanol and ethanol) did not show an inhibitory effect on the tested bacteria, while positive control (Streptomycin) has the highest antimicrobial activity.

Keywords: *antimicrobial activity, bee pollen, ethanol extract, methanol extract*

INTRODUCTION

Numerous studies have shown that aromatic and medicinal plants are sources of diverse nutrient and non-nutrient molecules, many of which display antioxidant and antimicrobial properties, which can protect the human body against both cellular oxidation reactions and pathogens (Senguel *et al.*, 2009).

Bee pollen is the result of the agglutination of flower pollens, carried out by worker honey bees, with nectar (and/or honey) and salivary substances, collected at the hive entrance and

used as food for all the developmental stages in the hive (Campos *et al.* 2008).

Bee pollen is known for its therapeutic properties attributed to several compounds, such as minerals, vitamins, essential amino acids, fatty acids, carotenoids, phenolics (Human *et al.*, 2006), and is consumed for its nutritional and medicinal properties (Abouda *et al.*, 2011). The therapeutic action of this hive product has been related to several phenolics compounds, such as flavonol glycoside and hydroxycinnamic acids (Campos *et al.*, 1997) with antioxidant activity.

The bioactive compounds can be extracted using a suitable solvent (Carpes *et al.* 2007).

Methanol extracts used for antimicrobial activity determination on different bacterial strains causing animal and vegetal pathology were studied by Basim *et al.* (2006), Graikou *et al.* (2011), Morais *et al.* (2011) or Pascoal *et al.*, (2014).

Carpes *et al.* (2007) used, for bee pollen extraction, ethanol 40, 50, 60, 70, 80 and 90% and the best antibacterial activity was obtained for bee pollen extract with 70% ethanol.

The aim of this study was the *in vitro* testing of different extraction solvents (methanol, ethanol) for bee pollen, against *Staphylococcus aureus* bacterial strain.

MATERIALS AND METHODS

Bee pollen was freshly harvested and immediately placed at -18°C . Two different extraction solvents were used (ethanol and methanol) in three different dilutions (60, 70, 80%). 5%, 10% and 15% pollen concentration in each solvent solution were carried out to determine the most efficient variant, possessing the highest antibacterial activity.

Extract preparation. Different amounts of bee pollen were milled, homogenized and individually extracted with 10 ml of methanol and ethanol 60, 70 and 80% (v/v) at room temperature for 1h. After sonication (15min), maceration and filtration (Mărghitaş *et al.*, 2009), the resulting solutions were stored at 4°C until antibacterial activity determination.

Microbiological cultures. Gram positive bacterial strain *Staphylococcus aureus* ATCC 6538 P were used for experiments. Stock culture of *Staphylococcus aureus* bacteria was grown in Nutrient Broth at 37°C for 24h in a shaker.

Testing **sensitivity of microorganism** to antibiotics was performed by disc diffusion method (Collins *et al.*, 1995), using Streptomycin (S) H-1141 (10µg/microtablets), Biolab Zrt. Budapest as a positive control. The bacterial suspension (10µl) was inoculated into Mueller-Hinton agar plate (Scharlau Chemie SA Barcelona, Spain). The antibiotic was placed aseptically in the middle of the plate and was incubated at 37°C for 24h. The inhibitory zone diameter was measured in mm.

Antibacterial activity of pollen was studied also by disc diffusion method (Collins *et al.*, 1995). The inoculum suspension was prepared at a concentration of 0.5 MacFarland scale, confirmed by spectrophotometric reading at 580nm. The bacterial suspension (10µl) was inoculated into Mueller-Hinton agar plate. Aliquots of 40 µl bee pollen solutions were applied on paper disk and placed in plates with active culture. The negative control was 40 µl of ethanol and methanol.

RESULTS AND DISCUSSION

The inhibition zones were different, according to the extraction solvent used and also the pollen concentration. Methanol extract (70%) of 15% pollen has the biggest inhibition diameter. Negative controls (methanol and ethanol) did not show an inhibitory effect on the tested bacteria,

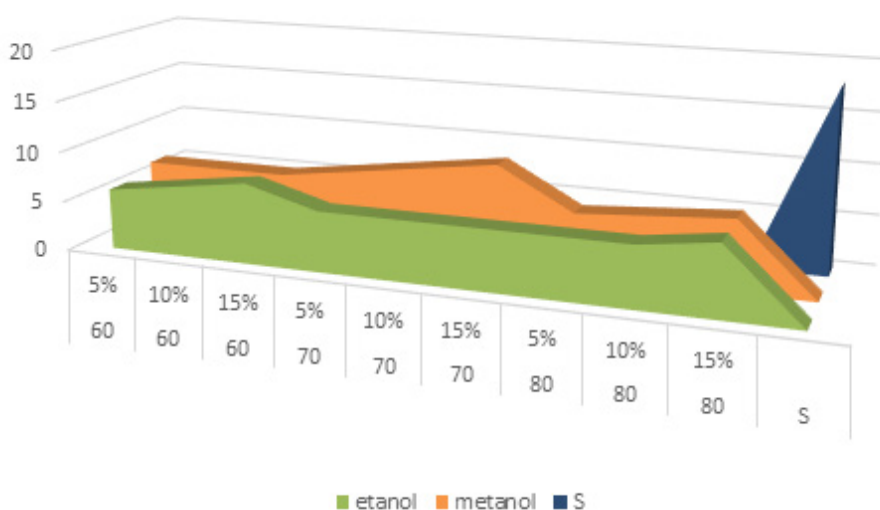


Fig. 1. Inhibition zones (mm) of ethanol, methanol extract and Streptomycin

Staphylococcus aureus, while positive control (Streptomycin) has the highest antimicrobial activity (Fig. 1).

Bee pollen constitutes a good source of healthy compounds, namely phenolics. This product is a potential source of antimicrobial agents since an increasing number of bacteria are developing resistance to antibiotics, extracts of bee pollen hold great promise for novel medicine in our time (Morais *et al.*, 2011).

According to literature reviews, concentration of phenolic compounds does not determine entirely the antibacterial activity, but the nature of phenolic compounds present in extracts (Carpes *et al.*, 2007).

Different extracts exhibited different antioxidant and antibacterial activities. Bee pollen seems to have interesting biological properties and can be considered as a functional food (Carpes *et al.*, 2007).

CONCLUSION

In the present research work, we concluded that the methanol extract 70% with 15% bee pollen concentration presents the highest inhibition zone.

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