ANTIBACTERIAL POTENTIAL OF HONEYDEW HONEY IN COMBINATION WITH NATURAL OILS

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Abstract. Currently, the antibacterial properties of honeydew honey are increasingly valued, being regarded as superior to blossom honeys. Over the years, the antibacterial activity of propolis has been highly appreciated, due to the flavonoids and phenolic acids present in its composition. Moreover, essential thyme oil and sea buckthorn oil have been recognized as valuable resources of natural antimicrobial compounds. The present research aimed to evaluate the antibacterial potential of two natural mixtures, consisting of honeydew honey and sea buckthorn oil and honeydew honey, propolis soft extract and essential thyme oil respectively. The tested bacterial strains were represented by Staphylococcus aureus ATCC 6538P, Bacillus cereus ATCC 11778, Escherichia coli ATCC 25922, Salmonella enteritidis ATCC 13076, Pseudomonas aeruginosa ATCC 27853 and Enterococcus faecalis ATCC 29212. Our results showed that the investigated products recorded good antibacterial activity and the Gram-positive bacterial strains were more sensitive than the Gram-negative ones.

Keywords: antibacterial activity, honeydew honey, propolis, sea buckthorn oil, thyme oil.

INTRODUCTION

Nowadays, honeydew honey, produced by bees from sugar-rich secretions of trees and plants or exudates of plant-sucking insects is increasingly treasured for its sensorial characteristics and multiple medical benefits, including antimicrobial activity (Grego *et al.*, 2016).

Generally, the main components responsible for the antibacterial activity of honey are considered to be the hydrogen peroxide (H_2O_2), the bee-derived glucose oxidase (GOX) enzyme and the bee-derived antibacterial peptide defensin-1 (Def-1) (Kwakman *et al.*, 2011; Pita-Calvo and Vazquez, 2017).

Several studies emphasized that GOX contributes significantly to the production of H_2O_2, by GOX-mediated conversion of glucose to gluconic acid under aerobic conditions in diluted honey (White *et al*., 1963; Bucekova *et al*., 2014). H_2O_2 plays a crucial role in the antimicrobial activity of honey, being capable of interfacing with bacterial cell proliferative signals and therefore, affecting bacterial growth even at increased dilutions of honey (Brudzynski *et al*., 2011).

Other investigations have shown that honeydew honey produces higher amounts of H_2O_2 compared with blossom honeys (Bucekova *et al*., 2014). In addition

to this, the content of hydrogen peroxide in honey is primarily influenced by the type of honey without regard to the botanical and geographical origin (Brudzynski *et al*., 2011). Honeydew honey is also recognized for an increased content of polyphenols that are thought to be involved in the production of large amounts of H_2O_2 (Akagawa *et al*., 2003; Long *et al*., 2010).

The antimicrobial properties of propolis have been widely reported throughout time, due to its complex composition and broad spectrum of activities. According to literature, the mechanisms of propolis allow to infer its effect on the permeability of the cellular membrane of the microorganism, disruption of membrane potential and adenosine triphosphate (ATP) production as well as decreasing bacterial mobility (Sforcin, 2016).

The interest toward natural antibacterial products has revived due to endlessly increasing antibiotic resistance. For instance, thymol and carvacrol, present in essential thyme oil are known for their strong antibacterial effects (Hoferl, 2009; Fournomiti *et al*., 2015). In addition, sea buckthorn oil proved to exert antimicrobial activity against a wide array of microorganisms, due to its ability to inactivate microbial adhesins, enzymes and cell envelope transport proteins (Alam, 2015).

The present research aimed to evaluate the antimicrobial activity of two natural products, consisting of honeydew honey and sea buckthorn oil (product no. 1) and honeydew honey, propolis soft extract and essential thyme oil (product no. 2). The antibacterial activity was investigated against the following bacterial strains: *Staphylococcus aureus* ATCC 6538P, *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 10536, *Salmonella enteritidis* 13076, *Pseudomonas aeruginosa* ATCC 27853 and *Enterococcus faecalis* ATCC 29212.

MATERIAL AND METHODS

The experiment was carried out on natural products, namely honeydew honey and sea buckthorn oil (product no. 1) and honeydew honey, propolis soft extract and essential thyme oil (product no. 2), which were provided by Apilife, Sibiu, Romania. Moreover, it is important to mention that the antibacterial activity of honeydew honey present in the two products was separately tested in order to obtain conclusive results.

All samples were kept in the refrigerator, in glass containers, hermetically sealed and before running any tests, they were properly homogenized. The samples were prepared by dissolving 1 g of each test piece in 1000 µl ultra-pure water by using a magnetic stirrer. Subsequently, 100 µl of the previous mixture were transferred into clean Eppendorf tubes. After sonication and filtration, the obtained solution was stored at 4˚C until antibacterial activity determination. The resulting solution was diluted with the aim of determining the minimal inhibitory concentration and 5 different concentrations were obtained, as follows: 50%, 40%, 30%, 20%, 10%.

The antibacterial activity of the samples was tested against different standard bacterial strains: *Staphylococcus aureus* ATCC 6538P, *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 10536, *Salmonella enteritidis* 13076, *Pseudomonas aeruginosa* ATCC 27853 and *Enterococcus faecalis* ATCC 29212, which were obtained from the Bacteria Collection of the Department of Microbiology and

Immunology, Faculty of Animal Science and Biotechnology, University of Agricultural Science and Veterinary Medicine Cluj-Napoca.

The bacterial strains were initially cultivated at 37°C on agar plates from cultures stored at −80°C. Only one colony was selected in order to produce a streak plate, which was stored at 4°C and it was used as inoculum for overnight in liquid media cultures. The preparation of the nutrient broth consisted of 10 g/L meat extract, 10 g/L peptone and 5 g/L sodium chloride. 10 g/L agar agar were added regarding the agar plates. The medium was autoclaved after preparation (Urcan *et al*., 2018). Bacterial growth assays were conducted according to the 96-well plate protocol implemented by Erler *et al.* (2014), which enables the monitoring of bacterial growth phase and leads to the determination of the lag phase length and of the slope during the logarithmic phase. The inhibitory activity of each sample was ascertained on liquid growth medium by using the protocol for determination of the inhibitory activity upon microorganisms. Concisely, 100 µl of sterile nutrient broth and 100 µl of each tested sample were added into each well. Afterwards, 10 µl of the bacterial suspension, with a density of 0.5 on the McFarland scale were pipetted into the wells that had been previously prepared.The positive control was represented by the culture medium, namely the nutrient broth (200 µl) plus the bacterial suspension (10 µl). The nutritional broth plus the product extract were used as a negative control. Following seeding, the microplates were thermostated at 37°C and the absorbance was read every 15 minutes for 24 hours. The optical density was read at a wavelength of 600 nm. The implemented protocol involved the dilutions technique which was performed in growth media followed by seeding with an equal microbial culture quantity.

Melissopalynological analysis is used to determine the botanical origin of honey, to assess its geographical origin and to provide valuable information related to bee ecology (Werner Von der Ohe *et al*., 2004). The melissopalynological analysis of the honey sample was realised in the Cellular Analysis Laboratory of the Institute of Advanced Horticultural Research of Transylvania, Cluj-Napoca, Romania, by the method described by Louveaux *et al*. (1978) and Werner von Der Ohe *et al*. (2004). Microscopic examination was performed with an Olympus BX 51 optical light microscope, by using the 40X objective for identification. Furthermore, electrical conductivity represents a valuable criterion for the determination of honeydew honey. The electrical conductivity was measured at 20 °C in a solution of honey sample (20.0 g dry matter of honey in volume solution in 100 ml distilled water) by using a Hanna conductometer.

RESULTS AND DISCUSSIONS

Our results regarding the melissopalynological analysis revealed the presence of specific elements of honeydew honey. Furthermore, electrical conductivity represents a valuable criterion for the determination of honeydew honey. The electrical conductivity of our honey sample registered a value of 940 µS, indicating a high quality honeydew honey, in accordance with the European legislation (EU Directive110/2001), which states that honeydew honeys must possess an electrical conductivity ≥ 800 µS.

To the best of our knowledge this is the first time that the antibacterial activity of natural products consisting of honydew honey and sea buckthorn oil (product no. 1) and honeydew honey, soft propolis extract and thyme oil (product no. 2) were investigated in the same formula. Therefore, we evaluated the results obtained by other researchers in the field regarding the antibacterial potential of the compounds that make up our final products. The honeydew honey sample from our study proved to possess good antimicrobial activity in concentrations greater than 20%, as shown by the graphical representation (Fig. 1).

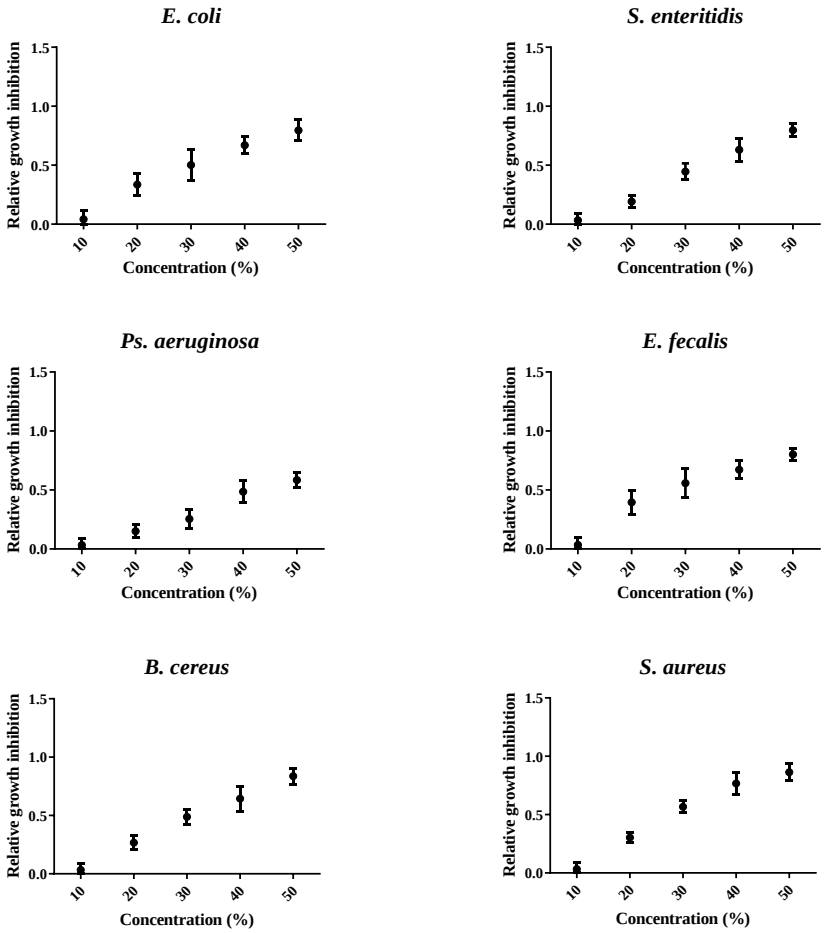

Fig. 1. Honeydew honey sample- Relative inhibition of bacterial growth depending on the concentration of the mixture (mean ± standard deviation). Growth inhibition was determined by using the slope of bacterial growth curves.

Our results indicated a good antimicrobial activity of the honeydew honey sample against all the tested bacterial strains, but the best effect was observed against Gram positive bacteria, especially against *S. aureus*. This is in agreement with the findings of Sagdic *et al*. (2013), who evaluated the antimicrobial activity of different

types of Turkish honeys, including honeydew honey, against 12 bacterial strains and 2 yeasts. The concentration of honeys at 5, 10 and 25% had no inhibitory effect on the tested microorganisms, whereas at 75% concentration, the honey samples demonstrated an increased antimicrobial activity against *S. aureus, E. coli* O157:H7, *S. typhimurium, L.monocytogenes* and *P. mirabilis*. Generally, the antimicrobial effect of honey is particularly attributed to polyphenolic substances. Phenolic compounds can affect the growth and metabolism of bacteria, but they can have either an inhibitory effect or a stimulatory effect, depending on the concentration and type of phenolic compound (Valachova *et al*., 2016).

An improvement in the antimicrobial action of our honeydew honey sample was observed following the addition of sea buckthorn oil (Fig. 2), as well as thyme essential oil and soft propolis extract (Fig. 3), regarding both Gram-negative and Gram-positive bacteria.

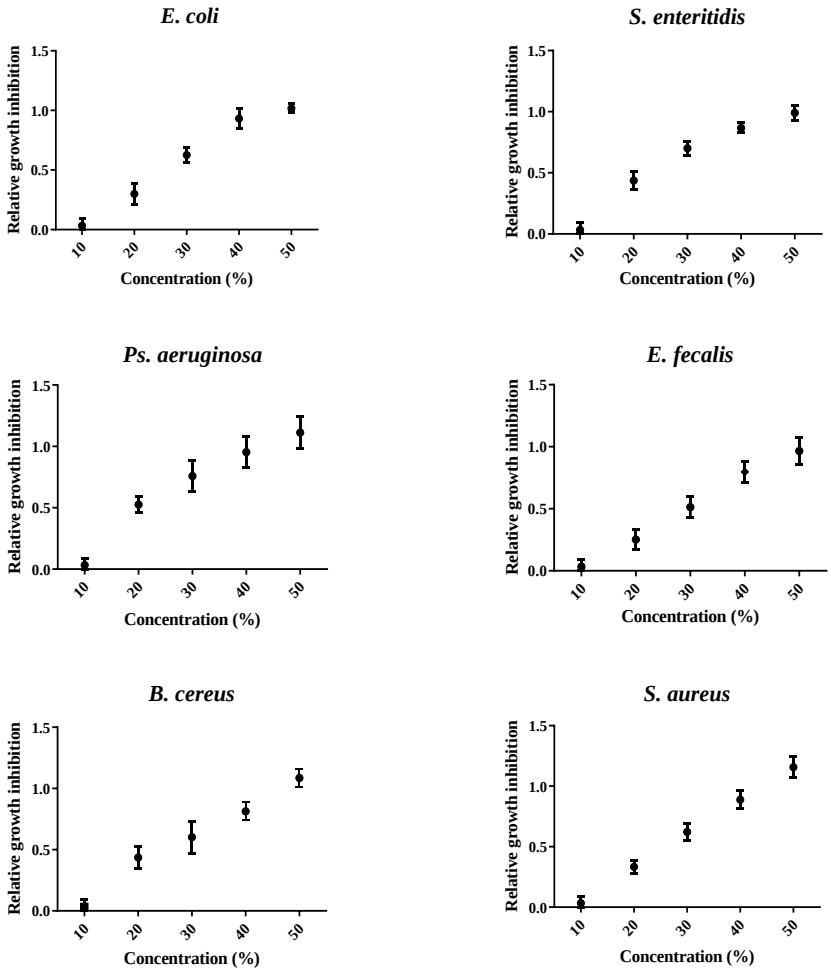

Fig. 2. Honeydew honey and sea buckthorn oil - Relative inhibition of bacterial growth depending on the concentration of the mixture (mean ± standard deviation). Growth inhibition was determined by using the slope of bacterial growth curves.

In the study performed by Yue *et al*. (2017), the antibacterial activity of sea buckthorn oil, obtained from different parts of the plant (pulp, seed, leaf), was tested against five bacteria: *B. subtilis, B. cereus, E. coli, S. aureus* and *B. coagulans*. The minimum inhibitory concentration (MIC) was determined by using the microdilution method. The results of the inhibition effect of the sea buckthorn oil (from pulp, seed, leaf) revealed an equal MIC value against *S. aureus* (12.20 mg/mL). Concerning the obtained MIC values, the pulp oil proved to be the most effective against *B. subtilis* (0.19 mg/mL), *B. cereus (*3.05 mg/mL) and *B. coagulans* (0.10 mg/mL). Regarding the *E. coli* bacterial strain, the seed oil displayed twice an inhibitory effect that of leaf or pulp oil (6.10 mg/mL).

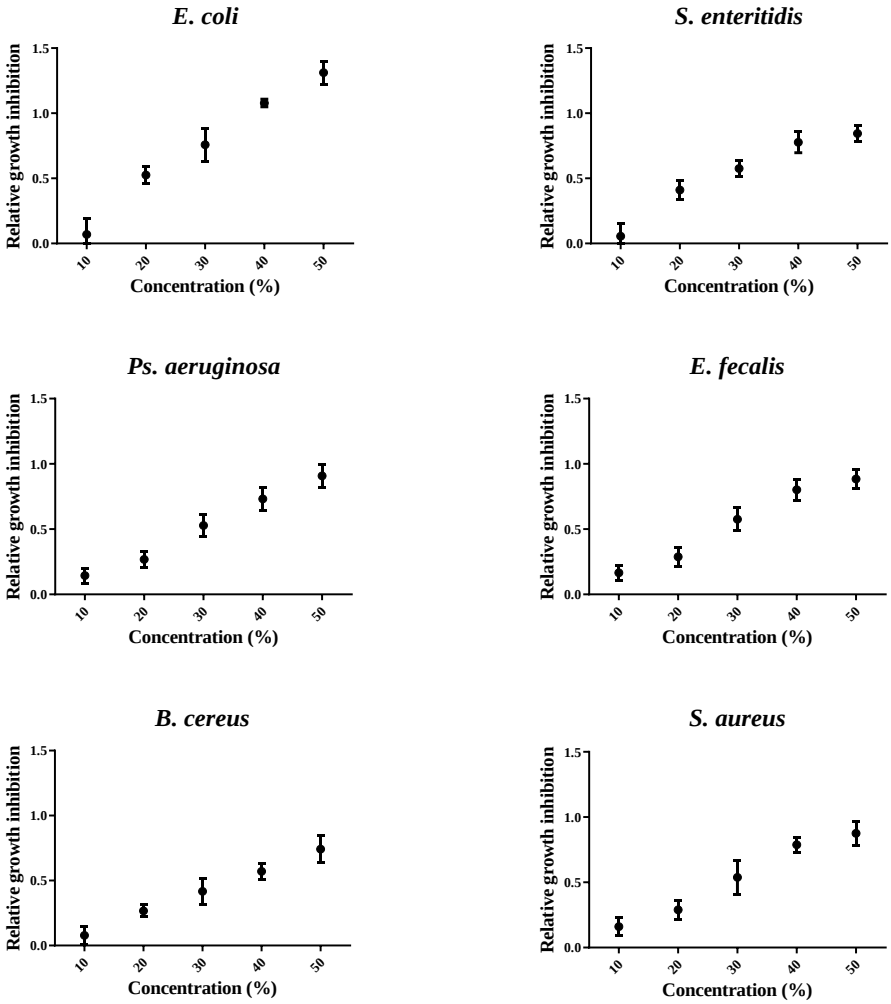

Fig. 3. Honeydew honey, soft propolis extract and thyme essential oil - Relative inhibition of bacterial growth depending on the concentration of the mixture (mean ± standard deviation). Growth inhibition was determined by using the slope of bacterial growth curves.

According to literature, thyme essential oil contains significant amounts of thymol (46%), carvacrol, p-cimen, terpine, compounds that are recognized for their strong antimicrobial effect and for preventing the development of bacterial resistance (Semeniuc *et al*., 2017). It was also observed that the antimicrobial activity of propolis is higher in relation to Gram-positive than Gram-negative pathogens, due to the species-specific structure of the outer membrane of the Gram-negative bacteria and the production of hydrolytic enzymes which break down the active ingredients of propolis (Sforcin, 2016). Regarding the combination of some bee products, Al-Waili *et al*. (2012) confirmed that the combination of propolis and honey enhanced their individual antimicrobial effect against *S. aureus* and *E. coli*. For instance, the MIC of propolis collected from Saudi Arabia, when used alone against *S. aureus* and *E. coli* was 0.15%, whereas combined with honey, it was 0.08 % for both the bacterial strains.

In the present research, the microdilution assay indicated that all the tested products exhibited antibacterial activity against *S. aureus, B. cereus, E. coli, S. enteritidis, Ps. aeruginosa* and *E. faecalis*, but the best effect was recorded against *S. aureus*. Nevertheless, the products demonstrated a low inhibitory effect against *Ps. aeruginosa* compared to *S. aureus*, thus indicating that *Ps. aeruginosa* is less susceptible to the antibacterial activity of the tested products. This finding is in line with the conclusions of previous studies (Boateng *et al*., 2015; Deng *et al*., 2018), possibly reflecting a higher intrinsic resistance of *Ps. aeruginosa* to the antibacterial potency of honey.

Overall, the antimicrobial activity was directly proportional to the concentration and all tested samples registered the best results at concentrations higher than 20%, although there were some variations depending on the bacterial strain, some bacteria being more sensitive than others. In general, Gram-positive bacteria were more sensitive to the action of the investigated products, but good results were also found on Gram-negative bacteria.

CONCLUSIONS

According to the results obtained in the present study, the best antimicrobial activity was presented by the mixture consisting of honeydew honey, essential thyme oil and soft propolis extract, followed by the mixture consisting of honeydew honey and sea buckthorn oil. Our findings revealed that the addition of natural oils in honeydew honey has increased its antibacterial effect, and thus led to obtaining novel products in terms of antibacterial activity. Being natural antibacterial sources, the utility of the elaborated products is yet to be explored in more fields. This research could provide a reference for further investigations and deep processing of as many bee products with addition of natural oils.

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