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Antimicrobial activity of royal jelly, honey, and their mixture

Žaneta Maželienė[1, 3],

Asta Aleksandravičienė[1, 2*],

Meda Pašvenskaitė[1],

Ingrida Viliušienė[1],

Daiva Šakienė[1],

Evelina Dailidaitė[1]

[1] *Department of Medical Technologies and Dietetics, Faculty of Medicine, Kaunas University of Applied Sciences, Pramonės pr. 22A, Kaunas 50468, Lithuania*

[2] *Department of Biology, Faculty of Natural Sciences, Vytautas Magnus University, K. Donelaičio g. 58, Kaunas 44248, Lithuania*

[3] *Institute of Microbiologyand Virology, Lithuanian University of Health Sciences, A. Mickevičiaus g. 9, Kaunas 44307, Lithuania*

As antimicrobial drugs destroy microorganisms or stop their growth, they are used to treat infections. Due to the increasing resistance of infectious agents to antimicrobial drugs, there is a need to find new natural products with antimicrobial properties. Natural products such as bee products honey, propolis, pollen, bee bread, and royal jelly are important products with numerous different active biological features, antimicrobial and antiviral among them. The aim of this study was to investigate the antimicrobial effect of royal jelly, honey, and the mixture of honey and royal jelly on gram-positive and gram-negative bacteria, spore bacteria, and the fungus *Candida albicans*. Royal jelly and honey were collected in Lithuanian apiaries. The antimicrobial activity of royal jelly, honey, and honey-royal jelly mixture (9% solution) was determined using the 'well' method of diffusion into agar. Reference cultures of gram-positive and gram-negative bacteria, spore bacteria, and the fungus *Candida albicans* were used in the study. Royal jelly was found to be the most effective against *Staphylococcus epidermidis* and *Enterococcus faecalis*. Royal jelly had the strongest antibacterial effect on *Enterococcus faecalis*, honey on *Listeria monocytogenes* and *Staphylococcus aureus*, and the mixture of honey-royal jelly on *S. epidermidis*. Royal jelly, honey, and honey-royal jelly solutions were not antibacterial against *Proteus vulgaris*. Royal jelly, honey, and honey-royal jelly had a weak effect on *Escherichia coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. Royal jelly had no effect on *Bacillus subtilis*, *Bacillus cereus*, and *Candida albicans*, and the antibacterial effect of honey and honey-royal jelly mixture was weak. Royal jelly, honey, honey-royal jelly mixture had the strongest effect on gram-positive bacteria. A weaker antimicrobial effect was observed against gram-negative bacteria, spore bacteria, and *C. albicans*. Royal jelly had no effect on *P. vulgaris*, and honey-royal jelly mixture had similar antimicrobial activity to honey.

Keywords: royal jelly, honey, antimicrobial activity, microorganisms

* Corresponding author. Email: asta.aleksandraviciene@vdu.lt

INTRODUCTION

Antimicrobial drugs that kill microorganisms or stop their growth are used to treat infections. However, the irresponsible, abundant, and irrational use of antimicrobial drugs accelerates the emergence and spread of strains of microorganisms resistant to these drugs. Therefore, as the problem of antimicrobial resistance of infectious agents increases, alternative antimicrobial agents of plant or animal origin are being sought.

Bee products are used not only in folk medicine, but also in traditional medicine: honey, propolis, pollen, bee bread, royal jelly and bee venom can be used not only as a food product but also as a natural alternative medicine for treating diseases. The above-mentioned bee products have antioxidant and anti-inflammatory effects and protect the nervous system (Samarghandian et al., 2017).

Royal jelly is a highly concentrated secretion of certain glands of bees. It is a complete and biologically active food product containing a particularly large number of useful substances: hormones, vitamins, trace elements, proteins, lipids, sugars, and amino acids, which are necessary for the normal growth of the body (Urcan et al., 2017; Kocot et al., 2018). This product is particularly well digested and the human body is able to absorb up to 80% of all substances contained in royal jelly. The most important fatty acid in royal jelly is 10-HAD (10-hydroxy-2-decenoic acid). This acid is believed to be found only in royal jelly and has various biological activities. Studies have shown that royal jelly is useful in medicine because it has an antimicrobial effect on infectious agents, inhibits allergic reactions, and lowers blood cholesterol levels (Scarselli et al., 2005). Royal jelly compounds 10-HAD, gelins, royalisin, royalactin, and apisimin exhibit antimicrobial activity against various bacteria (Fratini et al., 2016; Šedivá et al., 2018).

Honey also has a healing effect: it prevents infectious agents from entering the wound (Meo et al., 2017); in burn treatment, honey increases the adhesion of transplanted skin and relieves pain (Maghsoudi and Moradi, 2015). Honey has a strong antimicrobial effect on infectious agents, affecting even those resistant to antimicrobial drugs (Nair et al., 2004).

To enhance the antimicrobial activity of royal jelly and honey, they are often used together or mixed with other bee products. This enhances their biological activity, including the antimicrobial activity. Brudzynski and Sjaarda (2015) found that royal jelly and honey, as well as their mixtures, have different antimicrobial effects on various bacterial cultures. However, it should be noted that in different countries, bee products never have the same quantitative and qualitative composition. Geographical origin, environmental biodiversity, climatic and weather conditions, and various anthropogenic factors affect the chemical composition of these products on which the biological properties of these products depend (antioxidant, antimicrobial, antiviral, anti-inflammatory, antimutagenic and cytostatic properties) (Deng et al., 2018; Ota et al., 2019). Therefore, it is important to identify the chemical composition, biological properties, and antimicrobial activity of bee products collected in Lithuania, as this will help to use bee products more appropriately for food and medicine.

The purpose of this study was to investigate the antimicrobial effect of royal jelly, honey, and the mixture of royal jelly and honey on gram-positive and gram-negative bacteria, spore bacteria, and fungus *Candida albicans*.

MATERIALS AND METHODS

The study of the antimicrobial activity of royal jelly, honey, and their mixture was carried out in the microbiology laboratory of the Faculty of Medicine of Kaunas University of Applied Sciences using the agar diffusion method. Mueller-Hinton agar (Mueller-Hinton agar (CM 0337) Oxoid Ltd, Basingstoke, Hampshire, England) was used. The antimicrobial activity of the studied bee products was determined against gram-positive *Staphylococcus aureus* ATCC 25923,

Staphylococcus epidermidis ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 19115 and gram-negative *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853 and *Proteus vulgaris* ATCC 8427, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 11778, and the fungus *Candida albicans* ATCC 10231.

Honey and royal jelly were collected in one apiary in the summer of 2021. Three solutions were prepared from honey and royal jelly in physiological solution: 9% royal jelly, 9% honey, and 9% solution of a mixture of honey and royal jelly. After growing standard cultures of microorganisms in Mueller-Hinton agar, their suspensions were prepared. Microorganism culture suspensions were standardised with McFarland's 0.5 standard.

The dissolved Muller-Hinton agar at a temperature of 45°C was poured into 25 ml Petri dishes with a diameter of 90 cm. After the agar solidified, the suspensions of the microorganisms under study were seeded on the surface of the agar. After that, four 6 mm diameter 'wells' were made in the agar in each Petri dish. In the first 'well', 0.1 ml of 9% royal jelly solution was added, 9% honey solution went in the second 'well', 9% honey and royal jelly solution in the third 'well', and 0.1 ml physiological solution was poured into the fourth 'well' for control. The Petri dishes were cultured in a thermostat for 24 hours at 35°C.

The antimicrobial activity of 9% royal jelly, 9% honey, and 9% honey-royal jelly mixture solutions was evaluated *in vitro* after 24 h cultivation, after measuring the diameter of the transparent zones around the 'wells' in millimetres. If a clear zone did not form around the well, it was concluded that the tested solution did not have an antimicrobial effect on the microorganism culture being tested.

The test was performed three times and the arithmetic mean was derived from the obtained results. Statistical data analysis was performed using MS Excel 2016 (Microsoft, USA) and SPPS 25.00 (IBM, USA) programs.

RESULTS

The results of the antimicrobial activity test of royal jelly, honey, and their mixture are presented in Table.

Table. **The antimicrobial activity of royal jelly, honey, and their mixture against microorganisms**

Microorganisms		Diameter of the zone of inhibition of the growth of microorganisms, mm			*p*
		Royal jelly	Honey	Royal jelly with honey	
Gram-positive bacteria	*Staphylococcus aureus* ATCC 25923	12.0	19.0	15.3	**0.027***
	Staphylococcus epidermidis ATCC 12228	23.5	18.5	25.0	**0.024***
	Enterococcus faecalis ATCC 29212	12.0	7.5	10.0	0.619
	Listeria monocytogenes ATCC 19115	7.0	12.0	9.8	**0.032***
Gram-negative bacteria	*Escherichia coli* ATCC 25922	7.0	11.0	10.0	**0.044***
	Klebsiella pneumoniae ATCC 13883	7.0	8.5	8.2	0.086
	Pseudomonas aeruginosa ATCC 27853	8.7	9.9	10.0	0.729
	Proteus vulgaris ATCC 8427	0.0	0.0	0.0	1.000
Spore bacteria	*Bacillus subtilis* ATCC 6633	0.0	7.4	7.2	0.619
	Bacillus cereus ATCC 11778	0.0	8.1	7.5	**0.021***
Fungus	*Candida albicans* ATCC 10231	0.0	7.0	7.1	0.721

*p** – a statistically reliable difference with respect to royal jelly or honey.

The bacteria analysed were unicellular prokaryotic microorganisms. When studying the antibacterial effect on gram-positive bacteria, it was found that royal jelly had the most pronounced effect on *S. epidermidis* and *E. faecalis*. The antimicrobial effect of royal jelly on *S. aureus* and *L. monocytogenes* was 1.3 and 1.7 times weaker compared to other tested gram-positive bacteria. Compared to other gram-positive bacteria tested, the honey-royal jelly mixture had a stronger effect only on *S. epidermidis*. Royal jelly had the strongest antibacterial effect on *E. faecalis*, honey on *L. monocytogenes* and *S. aureus*, and honey-royal jelly mixture on *S. epidermidis*.

The study of the antibacterial activity of gram-negative bacteria shows that 9% solutions of royal jelly, honey, and honey-royal jelly mixture were not antibacterial against P. *vulgaris*. Royal jelly had the weakest antibacterial effect on *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. Honey and honey-royal jelly mixture had the strongest antibacterial activity against *E. coli*, *K. pneumoniae* and *P. aeruginosa*.

Royal jelly had no antibacterial effect on *B. subtilis* and *B. cereus* spore bacteria, and the antibacterial effect of honey and honey-royal jelly mixture was weak.

The results of the study show that *C. albicans* is not sensitive to the effects of royal jelly, and the antifungal effect of honey and honey-royal jelly is weak.

DISCUSSION

Royal jelly is a white or yellowish secretion with a strong pungent smell and taste (Kocot et al., 2018). The chemical composition of royal jelly produced by different families and breeds of bees is similar. For centuries, royal jelly has been used as a natural source of energy, and in recent times, many studies have been conducted to support the medical use of royal jelly.

Environmental conditions significantly influence the chemical composition of royal jelly (Sabatini et al., 2009). The highest amount of water and carbohydrates is reached in the rainy season, the amount of lipids is the highest in the dry season. The amount of protein changes slightly during the year, while the number of mineral substances and the pH value remain constant (Wongchai and Ratanavalachai, 2015).

Antimicrobial peptides in royal jelly, such as gelins, royalisin, royalactin, and apisimin, can be an alternative to antibiotics (Zasloff, 2019). The studies of Romanelli et al. (2011) into the antimicrobial activity of royal jelly proved that royal jelly and its main compounds were the most effective on gram-positive bacteria. *B. subtilis* and *B. cereus* are gram-positive spores forming bacteria. In the last stage of their development, these bacteria form a spore that protects the bacterium from adverse environmental conditions. These bacteria are the microbiota of the soil; when they enter the human body, they can cause infectious processes.

The antimicrobial effect on gram-negative bacteria is negligible. Our study confirmed the results of the study by Romanelli and colleagues. Royal jelly was most effective against gram-positive bacteria *S. aureus*, *S. epidermidis*, and *E. faecalis*, which are part of the human microbiota. These are the bacteria that cause serious infectious processes. Therefore, royal jelly can be a component of medicines for the treatment of these processes.

A recent study found that royal jelly inhibits the formation of biofilms in the body and *in vitro* in the food industry. In food, biofilms often contain *L. monocytogenes*, which caused 2549 infections in Europe in 2018. This study confirms the possibility of using royal jelly as a preparation inhibiting the formation of biofilms and reducing the risk of *L. monocytogenes* infection (Altuntas et al., 2020). In our study, royal jelly and honey were found to be effective against *L. monocytogenes*, the effect against listeria was enhanced by the combination of royal jelly and honey.

Scientific studies have proven that honey extracts inhibit the growth of gram-positive and gram-negative bacteria and kill fungi. It has been shown to be effective even against microorganisms resistant to antimicrobial drugs (Nair et al., 2004; Ocampo et al., 2014).

The antibacterial effectiveness of honey can be explained by its osmotic effect, a high sugar content, and a low moisture content, as well as gluconic acid, which promotes hydrogen peroxide (H_2O_2) (Almasaudi, 2021). Al-Jabri et al. (2003) studied honey samples from Oman and Africa. They found that honey collected in different regions had different antimicrobial activity against *S. aureus*, *E. coli*, and *P. aeruginosa*. We found that honey collected in Lithuania also had a stronger antimicrobial activity against gram-positive bacteria and weaker against gram-negative bacteria. We found that the antimicrobial effect of the honey-royal jelly mixture was similar to that of honey.

Fungi are eukaryotic microorganisms with a complex cell structure compared to prokaryotes. In our study, royal jelly had no antimicrobial activity against spore bacteria and the fungus *C. albicans*. Honey and the honey-royal jelly mixture had weak antimicrobial activity against the above-mentioned bacteria.

CONCLUSIONS

This study into the antimicrobial activity of 9% solutions of royal jelly, honey, and honey-royal jelly mixture confirmed that bee products have the strongest effect on gram-positive bacteria *S. aureus*, *S. epidermidis*, *E. faecalis*, and *L. monocytogenes*. A weaker antimicrobial effect was found against gram-negative bacteria *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. The weakest antimicrobial effect was found against the spore-forming bacteria *B. subtilis* and *B. cereus* and the fungus *C. albicans*. The antimicrobial activity of honey-royal jelly mixture was similar to that of honey.

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Žaneta Maželienė, Asta Aleksandravičienė, Meda Pašvenskaitė, Ingrida Viliušienė, Daiva Šakienė, Evelina Dailidaitė

BIČIŲ PIENELIO, MEDAUS IR JŲ MIŠINIO ANTIMIKROBINIS VEIKIMAS

Santrauka

Medus šimtmečius užėmė vertingą vietą tradicinėje medicinoje dėl jo antimikrobinių savybių. Medicinoje vartojami bičių produktai – medus, propolis, žiedadulkės, bičių duonelė, bičių pienelis. Darbo tikslas – ištirti bičių pienelio, medaus ir jų mišinio antimikrobinį poveikį gramteigiamoms ir gramneigiamoms bakterijoms, sporinėms bakterijoms ir grybeliui *Candida albicans*.

Bičių pienelis ir medus buvo renkamas Lietuvos bitynuose. Iš surinktų produktų buvo ruošiami 9 % tirpalai ir atliekama analizė panaudojant „šulinėlių“ metodą. Tyrime naudotos etaloninės gramteigiamų ir gramneigiamų bakterijų, sporinių bakterijų ir grybelio *Candida albicans* kultūros.

Nustatyta, kad bičių pienelis antibakteriškai efektyviausiai veikia *Staphylococcus epidermidis* ir *Enterococcus faecalis* bakterijas. Gauti rezultatai rodo, kad *Enterococcus faecalis* bakterijas stipriausiai antibakteriškai veikė bičių pienelis, *Listeria monocytogenes* ir *Staphylococcus aureus* – medus, o *Staphylococcus epidermidis* – medaus ir bičių pienelio mišinys. Bičių pienelio, medaus-bičių pienelio tirpalai silpnai antibakteriškai veikė *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* bakterijas, tačiau jokio poveikio nebuvo *Proteus vulgaris* bakterijoms.

Tyrimai rodo, kad bičių pienelis neturėjo antimikrobinio veikimo prieš *Bacillus subtilis* ir *Bacillus cereus*, o medaus, bičių pienelio-medaus mišinio antibakterinis poveikis silpnas. Bičių pienelis, medus ir jų mišinys turėjo silpną priešgrybelinį poveikį tiriamam grybeliui *Candida albicans*.

Raktažodžiai: bičių pienelis, medus, antimikrobinis veikimas, mikroorganizmai