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ROYAL JELLY AND BEE HONEY COMBINATION – A NEW PERSPECTIVE FOR ANTIBACTERIAL THERAPY OF SKIN AND INTESTINAL INFECTIONS

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ABSTRACT

The article discusses data on the specific antibacterial activity of royal jelly and rape honey mixtures against antibiotic-resistant microorganisms. The combination of royal jelly and rape honey in a ratio of 1:100 (w/w) in concentrations of 10% and 30% (v/v) changed the sensitivity of a pathogenic *E. coli* strain – being previously resistant, it has acquired sensitivity after incubation with various antibacterial agents: gentamicin, amoxicillin, chloramphenicol, doxycycline and trimethoprim – sulfamethoxazole. These data could be useful in the future development of effective medicines.

Key words: royal jelly, honey, antibacterial.

Introduction

Nowadays, a number of problems arise regarding the efficacy of therapy of various infectious causative agents, especially intra-hospital infections (Antimicrobial resistance: Global Report on Surveillance. W. H. Organization, 2014).

The role of some bee products as potential alternatives for control of various infectious causative agents (Gethin and Cowman, 2008) has increased over the years.

Antimicrobial activity of honey is determined by the presence of acids, low pH, osmotic activity and hydrogen peroxide production (Bogdanov, 1997).

In the body, this activity is determined by the synergistic relationships of these factors (Mavric et al., 2008).

Manuka honey from New Zealand, originating from the Leptospermum scoparium plant, is used as a therapeutic agent.

Besides these antibacterial factors, the presence of methylglyoxal (MGO)was demonstrated in this type of honey, and it has a unique manuka factor (UMF®), (Willix et al., 1992; Taormina et al., 2001).

Royal jelly is obtained from the hypopharyngeal glands of bees (*Apis mellifera* L.) a, and it is essential for the nutrition of the bee brood and the queen bee (Li et al., 2010).

A risk of allergic reactions, asthma and even fatal anaphylaxis has been established in people taking royal jelly, therefore this product is not recommended for use in many countries (Leung et al., 1997; Lombardi et al., 1998; Takahama and Shimazu, 2006).

On the other hand, many positive effects from the use of royal jelly have been identified: immunostimulant, activating the autonomic nervous system, etc. The main acid in the royal jelly, 10-hydroxy-2-decenoic acid (10-HDA), is known for its antibiotic effect (Blum et al., 1959; Melliou and Chinou, 2005). The presence of a specific antibacterial peptide Royalisin possessing an antibacterial effect against Gram-positive bacteria (Shen et al., 2010) was also found out in royal jelly.

To avoid sour taste and allergic reactions after consumption of royal jelly, some manufacturers recommend taking this product in combination with honey in a ratio of 1:100 % (w/w).

A study published in 2016 presents comparative data on the influence of acacia (*Robinia pseudoacacia* L.), multifloral and honeydew honey against *S. aureus* (ATCC 9144) (Dinkov, 2016).

The purpose of another study was to determine the antibacterial effect of royal jelly, rape honey and their mixtures (1:100 % w/w) against methicillin-resistant *S. aureus* (MRSA) using a microbiological method (Dinkov et al., 2016).

Only a few scientific studies regardng the effects of royal jelly on Gram-negative microorganisms are reported in the literature (Shirzad et al., 2007).

The microorganisms of the family *Enterobacteriaceae* with its about 20 genera cause intestinal intra-hospital infections (Tortorello, 2003).

It is well known that *A. hydrophila* isolated from human gastroenteritis is capable to develop during refrigerator storage of foods, although the latter inhibits the development of other foodborne pathogenic agents (Palumbo et al., 1985; Castro et al., 2008).

It is established that certain types of honey can inhibit the development of *E. coli* and have potential as alternative therapeutic agents (Wilkinson and Cavanagh, 2005).

In 2017, data on the *in vitro* antibacterial activity of mixtures of royal jelly and certain types of honey and the prospects for their use against different microorganisms (Dinkov, 2017) were summarized.

A special attention in the research was paid on parallel changes in antibacterial sensitivity of a pathogenic *E. coli* strain, exposed to mixtures of royal jelly and rape honey to different types of antibacterial agents. The article also discusses data from *in vitro* studies of the antibacterial activity of mixtures of royal jelly and rape honey (1:100 % w/w), against causative agents of intestinal and skin infections – *E. coli*, *A. hydrophila* and *S. aureus*.

Materials and methods

1. Test substances

The rape honey used in the studies was obtained from apiaries with 50 to 210 bee families located in different parts of Stara Zagora region (Bulgaria). The centrifugation of honey was carried out in June. In the period of honey harvesting, bee families were not fed carbohydrate solutions and antimicrobials. Until the analyses, the samples were stored under refrigerated conditions (0–4°C).

Water content (refractometrically), free acidity (titration with 0.1 N NaOH), pH and conductivity (Consort C 532 with electroconductivity and pH meters), diastase and invertase activities and amount of hydroxymethylfurfural (HMF) (Spectrophotometer SP-870 plus, Meterteh), as well as specific optical activity (Optech Polarymeter Model PL1LED), were determined according to the harmonised methods of the European Honey Commission (Bogdanov et al., 1997). The botanical origin of honey was determined by its malissopalynological, organoleptic, physical and chemical characteristics (von der Ohe et al., 2004).

All data on the physico-chemical parameters of honey were statistically processed using Student's t-test and presented as mean values and standard deviations (Dinkov et al., 2014).

The royal jelly used in the experiments was collected directly from the queen bee's cell cups. Its quality characteristics: (fructose, glucose, sucrose by HPLC by the Sesta method (2006); proteins (Folin-Ciocalteu reagent) were determined. The water content was analysed refractometrically, dry matter was determined by subtracting the percentage of water from 100%, pH – by pH meter model Mi 150 (1% aqueous solution of royal jelly); total acidity by titration by 0.1 N NaOH (ON 2576693-

84, 1984); conductivity of 1% aqueous solution of royal jelly – by conductometry (Bogdanov et al., 1997).

According to literary data, royal jelly freezing immediately after its collection protects its biologically active protein structures against decomposition (Li et al., 2007). Therefore, the royal jelly used in the experiments was stored frozen (-20°C).

Immediately before microbiological studies, all test substances were brought to 40°C in a water bath with regard to preparation of dilutions in solutions of honey, royal jelly and honey (1:100 % w/w) and royal jelly only (Dinkov et al., 2014),. Solutions containing 10, 20, 30, 40 and 45 % (v/v) of the test substances were prepared in sterile Tryptic Soy Broth (TSB). In order to prevent the breakdown of glucose oxidase associated with antimicrobial activity of honey (Bogdanov, 1997), all samples of honey and royal jelly and rape honey mixtures were prepared immediately before the analyses (Sherlock et al., 2010).

2. Microbiological studies

For the studies, a pathogenic *E. coli* strain of having induced septicaemia in ducks was used. The isolate was resistant to various antibacterial agents: amoxicillin, chloramphenicol, doxycycline, enrofloxacin, trimethoprim – sulfamethoxazole (Dinkov et al., 2014).

Each of the pre-prepared solutions of royal jelly, royal jelly and rape honey mixtures (1:100 % w/w) and rape honey only (all with concentrations of 10, 20, 30, 40 and 45 % v/v) was contaminated with a bacterial suspension of the *E. coli* strain according to the concentration of the microorganism in the medium recommended by other authors (Patton et al., 2006).

In order to determine the actual antibacterial activity of the strain, a bacterial suspension with an optical density corresponding to 0.5 McFarland turbidity standard was prepared. In this case, 3–4 colonies of the microorganism grown on blood agar were dissolved in 0.85% sterile saline. The resulting bacterial suspension had a bacterial concentration of about $1.5x10^8$ CFU/ml. To determine the exact number of microorganisms, 1 ml of dilutions were inoculated on ChromoCult ® TBX Agar (Merck) and incubated at 37°C for 24 h. Microbiological studies were conducted twice up to 30 min, 24 h and 48 h post incubation of the test substances contaminated with *E. coli* in TBS at 37°C. In order to determine comparatively the reduction rate, the initial (up to 30 min) and -24 h post incubation microbial counts in TBS (Dinkov et al., 2014) was accepted as baseline.

In parallel with *in vitro* testing of specific antimicrobial activity (Dinkov et al., 2014), the sensitivity of the test *E. coli* strain against six antibiotics and trimethoprim – sulfamethoxazole was also determined by the Kirby-Bauer method (CLSI, 2008).

Table 1: Antibiotics used and Trimetoprim – Sulfamethoxazole (load in µg/ disc), sensitivity zones (S), inter mediality (I) and resistance (R)

Antibiotics used and Trimethoprim – Sulfamethoxazole	S	I	R
Gentamicin (10 µg)	≥ 16	13-15	≤ 12
Colistin (10 µg)	≥ 11	9-10	≤ 8
Enrofloxacin (5 µg)	≥ 23	17-22	≤ 16
Amoxicillin (10 µg)	≥ 14	12-13	≤ 11
Chloramphenicol (30 µg)	≥ 18	13-17	≤ 12
Doxycycline (30 µg)	≥ 16	13-15	≤ 12
Trimethoprim – Sulfamethoxazole (1,25 / 23,75 µg)	≥ 16	11-15	≤ 10

All experiments to determine the antibiotic sensitivity of the *E. coli* strain were conducted in triplicate, and the mean values from the inhibition zones were presented.

Results

The Kirby-Bauer method identified different areas of incubation with 10 % (v/v) added from a mixture of royal jelly and honey (1:100 w/w) and incubation for 24 hours (Fig. 1 and Tab. 2).

Figure 1: Sensitivity zones of the *E.coli* strain before and after incubation in TSB with 10 % (v/v) of royal jelly and honey (1:100 % w/w) mixture established by the disc diffusion method

Table 2: Antibacterial inhibition zones in the *E. coli* strain before and after incubation in TSB with addition of 10 % (v/v) mixture of royal jelly and honey (1:100 % w/w)

Antibiotics and trimethoprim – sulfa-methoxazole	Before	After
Colistin	12 mm (S)	12 mm (S)
Enrofloxacin	10 mm (R)	11 mm (R)
Gentamicin	15 mm (I)	16 mm (S)
Amoxicillin	6 mm (R)	18 mm (S)
Chloramphenicol	10 mm (R)	25 mm (S)
Doxycycline	6 mm (R)	18 mm (S)
Trimethoprim – Sulfamethoxazole	6 mm (R)	24 mm (S)

E. coli isolated from 30 % (v/v) mixture of royal jelly and rape honey (1:100 % w/w), demonstrated even greater inhibition zones relative to the antibiotics studied. The resistance of the strain to enrofloxacin was not affected.

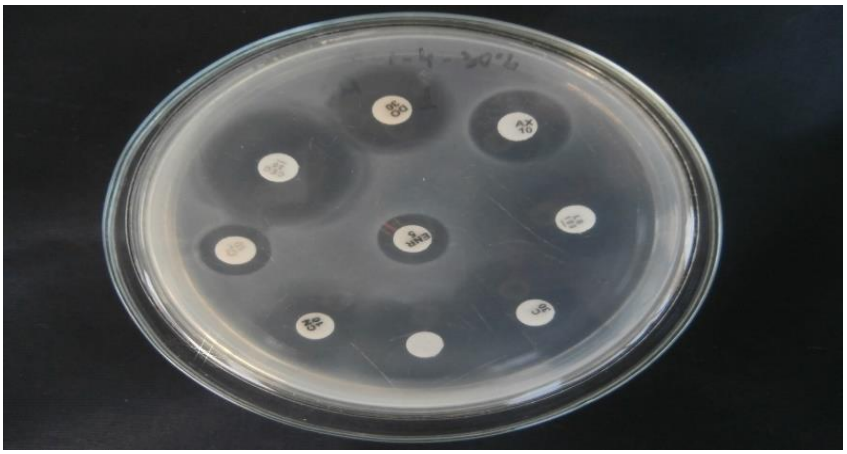

Figure 2: Inhibition zones of the *E. coli* strain after incubation in TSB with 30 % (v/v) mixture of royal jelly and honey (1:100 % w/w)

The parallel testing of the antibiotic sensitivity of strain's colonies established in the 45% honey solution, confirmed their sensitivity. The inhibition zones were identical or very close to those of the strain used. In rape honey, the strain was sensitive to gentamicin and colistin, without change in the behaviouir to other antibiotics and trimethoprim – sulfamethoxazole (Table 3).

Table 3: Susceptibility zones for the E.coli strain after incubation in TSB with 45 % (v/v) of rape honey added

Antibiotics used and trimethoprim – sulfamethoxazole	Before	After
Colistin	12 mm (S)	13 mm (S)
Enrofloxacin	10 mm (R)	11 mm (R)
Gentamicin	15 mm (I)	16 mm (S)
Amoxicillin	6 mm (R)	6 mm (R)
Chloramphenicol	10 mm (R)	10 mm (R)
Doxycycline	8 mm (R)	8 mm (R)
Trimethoprim – sulfamethoxazole	6 mm (R)	6 mm (R)

This shows that the rape honey used in the experiments, even at concentration of 45% (v/v), did not affect the antimicrobial sensitivity of *E. coli*.

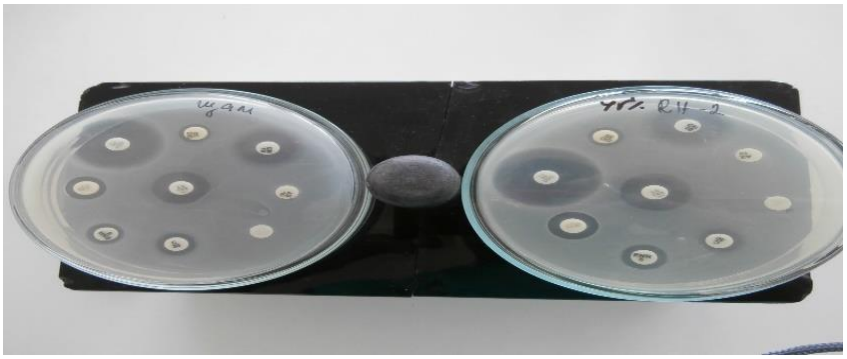

Figure 3: Inhibition zones for the *E. coli* strain after incubation in TSB with addition of 45 % (v/v) rape honey

Discussion

Results from studies conducted on acacia (*Robinia pseudoacacia* L.), multifloral and honeydew honey against S. aureus (ATCC 9144) showed that the antibacterial activity of honeydew and multifloral honey were higher compared to those of acacia honey with the lowest antibacterial activity. The antibacterial activity of honeydew honey relative to S. aureus (ATCC 9144) was established for an extended period of time (Dinkov, 2016).

Another study found out that royal jelly (concentrations 10, 20 and 30 % v/v) showed a total inhibitory effect on *A. hydrophila* (ATCC 7965). In doing so, royal jelly, mixtures of royal jelly and rape honey (1:100 w/w) may be potential alternative therapeutic agents against *A. hydrophila* (Stratev et al., 2015).

In mixtures of royal jelly and rape honey, a change in the sensitivity of the pathogenic *E. coli* strain to certain antibiotics (Tables 2 and 3) was detected after incubation for 24 hours.

These data can be discussed along with the results of parallel *in vitro* determination of the specific antimicrobial activity of the same mixtures (Dinkov et al., 2014). In the latter studies, we found that at almost all concentrations of royal jelly, a total reduction effect against *E. coli* was achieved . Mixtures of royal jelly and rape honey (1:100 % w/w) showed a higher antibacterial effect compared to the independent effects of rape honey. At 40 and 45 % (v/v), a 100 % reduction was established after 24 and 48 h. In rape honey, after the 100% absence of microorganisms detected at the 24th hour, a different logarithmic *E. coli* reduction was demonstrated after 48-hour incubation (88.8–92.22%), (Dinkov et al., 2014).

In rape honey, the strain retained its sensitivity to gentamicin and colistin, but there was no change against other antibiotics and sulfonamides with added trimethoprim (Table 3). It was found that even in concentration of 45% (v/v) rape honey did not affect the antibiotic sensitivity of the examined pathogenic *E. coli* strain.

Summarizing the results of parallel studies, it could be pointed out that the established real antibacterial activity in royal jelly and rape honey mixtures (Dinkov et al., 2014) was also associated with changes in the antimicrobial sensitivity of the pathogenic *E. coli* strain.

Even in 10% (v/v) royal jelly and rape honey mixtures, a significant increase in the inhibition zones relative to the antibacterial agents tested was found out. Thus, with regard to amoxicillin, chloramphenicol, doxycycline and trimethoprim – sulfamethoxazole, a dramatic change was also detected as being resistant, the strain had become sensitive to these antibacterial agents (Table 2). The absence of a change in antibiotic sensitivity in rape honey as well as the changes in the mixtures of this honey with royal jelly indicated that the reason for the change was the presence of royal jelly in the mixture.

These results are consistent with other studies of ours establishing the real bactericidal concentration of royal jelly and rape honey relative to methicillin-resistant *S. aureus* (MRSA), which also proved the potential of the royal jelly and rape honey combination as alternative therapeutic agents (Dinkov et al., 2016), yet additional research, including clinical studies are needed.

Studies on changes in antibiotic sensitivity with different types of microorganisms with proven real bactericidal concentrations to mixtures of royal jelly and honey are envisaged (Dinkov et al., 2014; Stratev et al., 2015, Dinkov et al., 2016).

Conclusion

Even at concentration of 45% (v/v) rape honey did not affect the antibiotic sensitivity of the pathogenic *E. coli* strain.

The combination of royal jelly and honey at a ratio of 1:100 % (w/w) in concentrations of 10% and 30 % (v/v) changed the size of the inhibition zones of the tested pathogenic *E. coli* strain, which acquired sensitivity to gentamicin, amoxicillin, chloramphenicol, doxycycline and trimethoprim – sulfamethoxazole.

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