# ANTIMICROBIAL PROPERTIES SLOVAK HONEYS IN COMPARASION WITH MANUKA HONEY AGAINST PATHOGENIC MICROORGANISMS

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## ABSTRACT

The aim of this work was tested and compared antimicrobial activity three Slovak honeys namely honeydew, flower and acacia against pathogenic microorganisms *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica* and *Listeria monocytogenes* with manuka honey with UMF 14+. The antibacterial activity of honey was determined using a microdilution broth method in Mueller Hinton Broth medium. The concentration of honey used in the study was within the range of 3.125 % to 50 %. The growth of microorganisms was determined spectrophotometrically and the MIC (Minimum inhibitory concentration) was determined as the lowest dilution that resulted in 80 % reduction in growth. For control the osmotic effect of sugars in honey was artificial honey used. Honeydew honey had lower MICs than manuka honey against *Yersinia enterocolitica* and *Staphylococcus aureus* and similar MICs against *Pseudomonas aeruginosa*, *Escherichia coli* and *Listeria monocytogenes*. Flower honey had lower MICs than manuka honey against *Staphylococcus aureus* and similar *Yersinia enterocolitica, Pseudomonas aeruginosa*, *Escherichia coli* and *Listeria monocytogenes.* Acacia honey had higher MICs than manuka honey.

**Keywords**: antibacterial activity, dilution method, honey, MIC

## INTRODUCTION

Honey has been recognized for its medicinal properties since antiqiuty (Namias, 2003). Honey is an important and unique food product containing bioactive compounds derived from bees and plants. Numerous studies demonstrate that honey possesses antimicrobial activity **(Allen et al., 1991; Molan et al., 1992; Molan et al., 1992; Molan, 1998; Cooper and Molan, 1999; Weston et al., 1999; Nzeako and Hamdi, 2000)**; it destroys and/or inhibits the growth of some pathogenic vegetative microorganisms **(Nzeako and Hamdi, 2000)**. The broad spectrum antibacterial activity of honey is multifactorial in nature. Hydrogen peroxide and high osmolarity honey consists of 80 % (w/v) of sugars are the only well characterized antibacterial factors in honey **(Molan, 1992)** and bee defensin-1 **(Kwakman et al., 2010)** also act as antibacterial substances. In addition, phenolic compounds found in dark honeys are partially responsible for antibacterial activity **(Wahdan, 1998; Aljadi and Yusoff, 2003; Estevinho et al., 2008)**. Recently, high concentrations of the antibacterial compound methylglyoxal (MGO) were found specifically in Manuka honey, derived from the Manuka tree (*Leptospermum scoparium*) **(Adams et al., 2008; Mavric et al., 2008)**. Until now, no honey has ever been fully characterized, which hampers clinical application of honey. It has been shown to be active against a diverse range of microorganisms including gram-positive and gram-negative organisms, aerobic and anaerobic bacteria **(Zaghloul et al., 2001; Ndip et al., 2007),** and *Candida albicans* as well as inhibiting the germination of the spores of *Bacillus cereus* **(El-Toun and Yagoub, 2007).** Flavonoids, phenolic and organic acids in honey are known to scavenge for free superoxide and other reactive oxygen metabolites liberated during respiratory burst in H. pylori-induced mucosal damage **(Li et al., 2001)**. Honeys from different countries and regions have a wide variability in their antimicrobial activity as a result of different vegetative flowers and plant species blooming in different seasons **(Ndip et al., 2007; Basson and Grobler, 2008)**. *E. coli* are a model organism for bacteria **(Peter et al., 1998)** and extremely sensitive to antibiotics such as streptomycin or gentamycin but rapidly changing and acquiring drug resistance **(Chapman et al., 2002)** due to overuse of antibiotics in humans **(Johnson et al., 2006).** Management of *E. coli* infections has been increasingly complicated by the emergence of resistance to most firstline antimicrobial agents including fluoroquinolone **(Karlowsky et al., 2001).** Thus, they have been relied on for the treatment of *E. coli* infections as emerging resistence has progressively eclipsed the utility of alternative antimicrobial agents **(Gupta et al., 2001).** However, the prevalence of fluoroquinolone-resistant *E. coli* has reached alarming levels in many parts of the world, jeopardizing their usefulness **(Raz et al., 2002).** The use of fluoroquinolones in food animals has been implicated in the development of fluoroquinolone resistance in zoonotic gram-negative bacilli such as *Campylobacter* and *Salmonella* species, with the subsequent occurrence of drug-resistant infections in humans **(Smith et al., 1999; Chiu et al., 2002).** *E. coli* that are resistant to quinolones and fluoroquinolones contaminate many retail meat products, particularly poultry, corresponding with the use of fluoroquinolones in food animals, particularly chickens and turkeys **(Johnson et al., 2003; Johnson et al., 2005).** However, whether such drug-resistant organisms pose a threat to human health is unknown **(Rahman et al., 2010)**.

This study investigated the antibacterial effect of slovak honeys against pathogenic bacterias was compared with the activity of manuka honey.

## MATERIAL AND METHODOLOGY

The antibacterial activity of Slovak honeys against three pathogenic microorganisms *Staphylococcus aureus*,

*Escherichia coli* and *Pseudomonas aeruginosa* were determined by comparision with the commercially available active manuka honey imported from New Zealand (Green Bay™, UMF 14+). Slovak honeys used in this study were acacia, flower and honeydew origin. To distinguish the effect of the antibacterial components of honey from any osmotic effect, artificial honey, a control solution with a sugar content similar to that of natural honey, was also used for comparison. The artificial honey was prepared by dissolving 39 g d-fructose, 31 g d-glucose, 8 g maltose, 3 g sucrose and 19 g distilled water. All honey samples were stored in the dark at 2–5 °C when not in use. A 50 % (w/v) stock solution of each type of honey was prepared by weighing 10 g of honey and bringing the volume up to 10 ml of Mueller Hinton broth (MHB). Further dilutions of stock solution of natural honeys were done to obtain honey concentrations of 25 %, 12.5 %, 6.25 %, and 3.125 %. A dilution range of 50 %, 25 % and 12.5 % of artificial honey was used. In vitro antibacterial activity was determined by the broth microdilution method. The wells were inoculated with a over night bacterial suspension (10 µl) at a density of $10^7$ CFU.ml$^{-1}$, incubated at 37 °C for 18 h, and then observed for the minimum inhibitory concentration (MIC). The growth of microorganisms was determined spectrophotometrically as turbidity at 405 nm. The MIC was determined as the lowest dilution that resulted in 80 % reduction in growth compared with the growth control **(Jorgensen et al., 1999)**. All samples were tested in triplicate.

## RESULTS AND DISCUSION

Honey inhibits the growth of dangerous bacteria such as *Escherichia coli, Staphylococcus aureus, Salmonella, Shigella*, and *Vibrio cholera* **(Zumla and Lulat, 1989)** and is superior to several well-known antibiotics. Honey inhibits the growth of pathogenic organisms isolated in urine samples of patients with urinary tract infections **(Somal et al., 1994).**

Several laboratory studies have evidence to support the use of honey as a wound dressing.6 Honey has been shown to stimulate cytokine production by monocytes, which in turn initiates tissue repair. Honey has broad-spectrum antibacterial activity; however, different honeys vary substantially in the potency of their antibacterial activity. Honey debrides wounds, removes malodor, and its anti-inflammatory activity Aktivity reduces edema and exudates and minimizes scarring. It stimulates the growth of granulation tissue and epithelial tissue and promotes wound healing **(Molan, 2006).** In study of **Mullai and Menon (2005, 2007)**, both locally obtained unprocessed honey and commercially processed therapeutic honey have shown antibacterial activity against *P. aeruginosa*. **Cooper (1999)** has reported that manuka honey had MIC of less than 10 % against 17 strains of *P. aeruginosa* from infected wounds. **Molan (2002)** reported that Manuka honey had a MIC of 6 % against *P. aeruginosa* strains from infected burns. **Nzeako and Hamdi (2000)**, in their study of six commercial honeys, found that *Escherichia coli* and *P. aeruginosa* were inhibited at a concentration of 40 %. In study of **Mullai and Menon (2007)**, the MICs for both manuka honey and heather honey was 20 % MIC. Honey procured from Khadikraft showed better activity with a MIC of 11 %. Honey procured from local beekeepers had a MIC of 20 %, which was quite similar to the other commercially available therapeutic honeys.

**Table 1 Mimimum inhibitory concentrations in % of different honeys**

| | Type of honey | | | | |
|---|---|---|---|---|---|
| **Microorganisms** | **Manuka** | **Honeydew** | **Flower** | **Acacia** | **Sugar control** |
| *Staphylococcus aureus* | 25 | 12.5 | 12.5 | 25 | 50 |
| *Pseudomonas aeruginosa* | 25 | 25 | 25 | 50 | 50 |
| *Escherichia coli* | 25 | 25 | 25 | 50 | 50 |
| *Yersinia enterocolitica* | 12.5 | 6.25 | 25 | 50 | >50 |
| *Listeria monocytogenes* | 25 | 25 | 25 | 25 | >50 |

Our study clearly shows that honeys produced from Slovakia have antimicrobial activity. Honeydew honey and flower honey were lower MICs than manuka honey against tested pathogenic microorganisms *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Yersinia enterocolitica* and *Listeria monocytogenes*.

The MIC values of the manuka, honeydew, flower, acacia and artificial honey are shown in Table 1. The MICs for honeydew honey ranged from 6.25 % to 25 %, for flower honey ranged from 12.5 % to 25 % and acacia honey ranged from 25 % to 50 % while those for active manuka honey 12.5 %. Artificial honey inhibited the growth of all microorganisms at a concentration higher than 50 %.

The similar results with the testing microorganisms and Slovak honeys reached **Majtán (2009)**. Honey has several well-known properties responsible for its antimicrobial activity. These include a high osmolarity due to the high concentration of sugars (80 % w/v) **(Chirife et al., 1982)**, a low pH (3.2–4.5 for undiluted honey), and the production of hydrogen peroxide, which, after dilution of honey, is produced by glucose oxidase originating from the bees **(Molan, 1992)**. **Kwakman et al. (2010)** found bee defensin-1 in honey, this suggests that after the transition in hypopharyngeal gland function of the worker bees with age, the gland still produces bee defensin-1 and they considers that this peptide therefore, likely contributes to protection of both royal jelly and honey against microbial spoilage. Honey

inhibits the growth of dangerous bacteria such as *E. coli*, *S. aureus*, *Salmonella*, *Shigella*, and *V. cholera* **(Zumla and Lulat, 1989)**. The concentration of honey varied 30 to 50 % was bactericidal to S. shigella, E. coli and v. cholera, making honey an anti-bacterial agent and superior to several well-known and currently prescribed antibiotics. Honey inhibits the growth of pathogenic organisms isolated in urine samples of patients with urinary tract infections as well **(Somal et al., 1994)**.

Accacia honey has very low antimicrobial effect against three pathogenic microorganisms *P. aeruginosa*, *E. coli* and *Y. enterocolitica*. Its effect (MIC 50) was comparable with artificial honey. Unfortunately, large variation in antimicrobial activity exists among honeys collected from different environments **(Allen et al., 1991; Molan and Betts, 2004)** possibly related to spatial and temporal variation in sources of nectar **(Molan, 1992)**.

## CONCLUSION

Assessment of antimicrobial activity of different Slovak honey samples against *S. aureus, P. aeruginosa, E. coli, Y. enterocolitica and L. monocytogenes* showed that inhibitory effects are not inherent to all the selected honey samples. To achieve the inhibition of bacterial growth, the concentration of honey should be sufficiently high, usually higher than 12.5 % (by mass per volume).

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