# Antibacterial and antimycotic activities of Slovenian honeys

M. KRALJ KUNČIČ*, D. JAKLIČ*, A. LAPANJE† and N. GUNDE-CIMERMAN*‡

*Department of Biology, Biotechnical Faculty, University of Ljubljana; †Institute of Physical Biology, Toplarniška 19; and ‡Centre of Excellence for Integrated Approaches in Chemistry and Biology of Proteins (CIPKeBiP), Ljubljana, Slovenia*

*Accepted: 9 August 2012*

**ABSTRACT**

In the present study, Slovenian honey samples produced from different floral sources are evaluated for their antibacterial and antifungal properties. The peroxide contribution to antibacterial activity is also determined. Minimum inhibitory concentration (MIC) of the honeys was assessed against four bacterial species (*Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) and against eight fungal species (*Aspergillus niger*, *Aureobasidium pullulans*, *Candida albicans*, *Candida parapsilosis*, *Candida tropicalis*, *Cladosporium cladosporioides*, *Penicillium chrysogenum* and *Rhodotorula mucilaginosa*). Honey at concentrations between 1% and 50% (v/v) were tested. Although all of the bacterial species were inhibited by the different honey samples, the chestnut and pasture honeys showed the highest antibacterial activities. The antifungal activities were concentration-dependent, with five (*Aureobasidium pullulans*, *Candida parapsilosis*, *Candida tropicalis*, *Cladosporium cladosporioides*, *Rhodotorula mucilaginosa*) inhibited only at honey concentrations greater than 50%. The fungi *Aspergillus niger*, *Candida albicans* and *Penicillium chrysogenum* were not inhibited by any of the tested honeys, even at the highest concentrations. The lowest MICs seen were 2.5% (v/v) for the chestnut, fir and forest honeys against *Staphylococcus aureus*, and 10.0% (v/v) for the chestnut and pasture honeys against *Cladosporium cladosporioides*. The non-peroxide action of chestnut honey was tested against *Escherichia coli*. The MIC of the catalase-treated chestnut honey was 50% (v/v). The antibacterial effect of Slovenian honeys is mostly due to peroxide action. These data support the concept that Slovenian honeys are effective antibacterials and antifungals, and can thus be applied for medicinal purposes.

KEY WORDS: Anti-infective agents.
Honey.
Hydrogen peroxide.
Microbial sensitivity tests.

## Introduction

Since ancient times, honey has been used as an effective topical antimicrobial agent for the treatment of burns and other wounds.[1,2] More recently, honey has regained its importance as a natural remedy,[3–6] being particularly appropriate for the treatment of chronic wounds infected with antibiotic-resistant bacteria.[7–9] Due to its antimicrobial properties, honey is at present being more frequently used also as a natural food-preserving agent,[10–12] as well as for the treatment of oral infections.[13]

Studies of the antimicrobial properties of honey have shown that the antimicrobial activity of honey is not only due to hydrogen peroxide, but also to non-peroxide mechanisms of action such as its high sugar content, acidity, and content of phenolics,[14–17] methylglyoxal[18,19] and oligopeptides (e.g., bee defensin-1).[20,21] Hydrogen peroxide activity is generated in diluted honey and contributes to antimicrobial activity; therefore, it can be predicted by hydrogen peroxide levels in the honey.[22,23]

The antimicrobial activity of honey varies greatly according to its botanical and geographic origin and its processing.[17] The honey with antimicrobial properties that has be studied most frequently is manuka honey, which is derived from *Leptospermum* spp.[4,24–26] Comparative studies with different types of honey have revealed that manuka honey, which has been tested against bacteria and fungi, is the honey type with the highest antimicrobial activity.[4,7,27,28]

In contrast to honeys with well-studied antibacterial properties, only a few other non-manuka types have been tested for their antifungal activities, including different floral-source samples of honey from Turkey,[29,30] three samples of South African honey (wasbessie, bluegum and fynbos),[31,32] local honeys from Pakistan,[33] honey from Algeria,[34] rhododendron honey from Turkey,[35] Omani[36–38] and Iranian[39] honey of floral origin. Only a narrow spectrum of fungal species, including opportunistic pathogenic *Candida* spp. and *Trichosporon* spp. that are resistant to conventional chemotherapeutics have been tested.[29,40–43]

To date, no report has appeared on the antimicrobial properties of honeys from the Slovenian geographical region. Thus, this study aims to evaluate the antimicrobial properties of Slovenian honeys derived from different floral sources, using manuka honey as a reference. The focus is to investigate their antimicrobial activities against four clinically relevant bacterial species, and to determine their antifungal activities against a broad spectrum of opportunistic pathogenic fungal species. In addition, the peroxide content of the honey is assessed, as peroxide

*Correspondence to: Dr Nina Gunde-Cimerman*
*Department of Biology, Biotechnical Faculty*
*University of Ljubljana, Večna pot 111*
*SI-1000 Ljubljana, Slovenia*
*Email: nina.gunde-cimerman@bf.uni-lj.si*

**Table 1.** Minimum inhibitory concentration (MIC) of the Slovenian honeys and manuka honey (UMF 10) used in the present study.

| | MIC (%) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Acacia honey | | Chestnut honey | | Fir honey | | Forest honey | | Lime honey | | Pasture honey | | Manuka honey | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| *Escherichia coli* | 25.0 | 0.0 | 12.5 | 0.0 | 12.5 | 0.0 | 11.7 | 1.4 | 11.7 | 1.4 | 25.0 | 0.0 | 10.0 | 0.0 |
| *Enterococcus faecalis* | 10.0 | 0.0 | 10.0 | 0.0 | 10.0 | 0.0 | 10.0 | 0.0 | 16.7 | 7.2 | 50.0 | 0.0 | 12.5 | 0.0 |
| *Pseudomonas aeruginosa* | 8.3 | 2.9 | 5.0 | 0.0 | 10.0 | 0.0 | 6.7 | 2.9 | 10.0 | 0.0 | 10.0 | 0.0 | 12.5 | 0.0 |
| *Staphylococcus aureus* | 6. 7 | 2.9 | 2.5 | 0.0 | 2.5 | 0.0 | 2.5 | 0.0 | 5.0 | 0.0 | 5.0 | 0.0 | 5.0 | 0.0 |
| *Aspergillus niger* | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | ND | ND |
| *Aureobasidium pullulans* | NA | NA | 50.0 | 0.0 | NA | NA | NA | NA | NA | NA | 50.0 | 0.0 | ND | ND |
| *Candida albicans* | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | ND | ND |
| *Candida parapsilosis* | NA | NA | 50.0 | 0.0 | NA | NA | NA | NA | NA | NA | 50.0 | 0.0 | ND | ND |
| *Candida tropicalis* | 50.0 | 0.0 | 50.0 | 0.0 | 50.0 | 0.0 | 50.0 | 0.0 | 50.0 | 0.0 | NA | NA | ND | ND |
| *Cladosporium cladosporioides* | 10.8 | 1.4 | 10.0 | 0.0 | 25.0 | 0.0 | 12.5 | 0.0 | 10.8 | 1.4 | 10.0 | 0.0 | ND | ND |
| *Penicillium chrysogenum* | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | ND | ND |
| *Rhodotorula mucilaginosa* | 50.0 | 0.0 | 50.0 | 0.0 | NA | NA | 50.0 | 0.0 | 50.0 | 0.0 | NA | NA | ND | ND |

SD: standard deviation; NA: not active against tested microorganism; ND: not determined.

activity has an important contribution to the antimicrobial action of honey.

## Materials and methods

### *The honeys*

Six honey samples used in this study were obtained from commercial Slovenian producers or directly from local beekeepers. The type of honey was determined in all samples by means of sensory analysis[44] and melissopalynology.[45] A botanical classification was considered to be achieved when the pollen spectrum contained >90% of the corresponding dominant pollen in chestnut and lime honey. Classification by the sensory method was applied by at least three experts.

Honey samples were selected to represent a range of different floral sources: pasture (mixed sources), chestnut (*Castanea sativa*), forest (mixed sources), fir (*Abies alba*), lime (*Tilia platyphyllos*) and acacia (*Robinia pseudoacacia*). The measurement of hydroximethylfurfural was performed to ensure that honey samples were not heated during harvesting.

Antioxidant activity and colour of the honey samples were previously reported in the study by Bertoncelj *et al*.[46] Additionally, manuka honey (Comvita, UMF 10) was used for comparison in the non-peroxide action assays. The honey solutions used for the antimicrobial tests were prepared by diluting honey with either NB liquid medium (5.0 g NaCl, 8.0 g nutrient broth, to 1 L with distilled water; Biolife, Italy) for the antibacterial activity, or malt extract (ME; Biolife, Italy) liquid medium for the antimycotic activity. The following honey concentrations were tested: 1.0%, 1.25%, 2.5%, 5.0%, 10.0%, 12.5%, 25% and 50% (v/v).

### *The microorganisms*

The bacterial strains selected were *Escherichia coli* EXB S7, *Enterococcus faecalis* EXB V53, *Pseudomonas aeruginosa* EXB V28 and *Staphylococcus aureus* EXB V102, all of which were originally isolated from infected chronic wounds. The fungal strains selected were: *Aspergillus niger* EXF 311, *Aureobasidium pullulans* EXF 3375, *Candida albicans* EXF 525, *Candida parapsilosis* EXF 517, *Candida tropicalis* EXF 526, *Cladosporium cladosporioides* EXF 2246, *Penicillium chrysogenum* EXF 1784 and *Rhodotorula mucilaginosa* EXF 3790. The *Candida albicans* and *Candida tropicalis* originated from human clinical material, while *Candida tropicalis* and *Penicillium chrysogenum* were isolated from hypersaline water. *Aureobasidium pullulans* was isolated from fruit, and *Rhodotorula mucilaginosa* was isolated from a microbial mat in saltpans; all are maintained in the Ex Culture Collection of the Biotechnical Faculty, University of Ljubljana, Slovenia.

### *Assessment of antimicrobial activity*

Assessment of the antimicrobial activity of the selected honeys was followed by minimum inhibitory concentration (MIC) determination.[40] Bacterial cultures were grown in NB liquid nutrient medium at 37°C and diluted 1–2x10$^5$ cells/mL for the final inoculum, and added to the test tubes containing the different concentrations of honey. Owing to the high density of the honey samples, absorbance measurements of honey dilutions were made. For the negative control, only NB liquid medium was used. Following overnight incubation of the samples at 37°C, the MIC values were determined by measuring absorbance (600 nm; Lambda Bio, Perkin Elmer, Waltham, USA). Additionally, 0.1 mL each sample was spread on NB agar plates. The resultant bacterial colonies were counted after 24-h incubation at 37°C to confirm the antibacterial effect.

All fungal strains were maintained on ME medium (MEA) slants. The yeasts were incubated for two days at 30°C, while the filamentous fungi were incubated at 30°C for up to seven days, or until sporulation was observed. Yeast cells and spore suspensions were prepared directly from slants using saline solution (0.9% NaCl). The number of fungal cells was counted in a haemocytometer under light microscopy (x400 magnification). The final inoculum contained 1–2x10$^5$ cells/mL. All antimicrobial assays were performed in triplicate for each honey concentration.

Non-peroxide antibacterial activity of the Slovenian honeys was determined and compared to manuka honey. Catalase (Sigma Aldrich, 1000–2500 U/mL) was used to degrade hydrogen peroxide by inoculation into test tubes containing different dilutions of the honey samples and incubated for 10 min. A glucose oxidase assay kit (Megazyme International, Wicklow, Ireland) was used to test the reduction in hydrogen peroxide (measurement taken 10 min after addition of catalase). The catalase-treated honeys were then tested for antibacterial activity against *Escherichia coli*, as described above.

## Results

Minimum inhibitory concentration of the Slovenian honey samples and manuka honey are shown in Table 1.

Bacterial growth of all species tested was inhibited by the Slovenian honeys. The most sensitive bacterial species was *Staphylococcus aureus*, which was inhibited at an MIC of 2.5% (v/v) by the chestnut, fir and forest honeys. The most resistant bacterial species were *Escherichia coli* and *Enterococcus faecalis*, with MICs ranging from 10% to 50% (v/v). The manuka honey (UMF 10) showed the strongest antibacterial activity against *Staphylococcus aureus* (MIC 2.5%) and *Escherichia coli* (MIC 10.0%).

As chestnut honey showed the most effective antibacterial activity, its non-peroxide action was tested on *Escherichia coli*. As with the manuka honey, the chestnut honey was first treated with catalase to eliminate hydrogen peroxide, and then glucose oxidase activity was determined. After treatment with catalase, chestnut honey inhibited *Escherichia coli* at 50% (v/v), while the MIC of manuka honey was 10% (v/v).

The three fungal species *Candida parapsilosis*, *Candida tropicalis* and *Rhodotorula mucilaginosa* were inhibited at 50% honey (v/v), while *Aspergillus niger*, *Candida albicans* and *Penicillium chrysogenum* were not inhibited by any of the tested honeys. *Cladosporium cladosporoides* was inhibited at 10% (v/v). *Aureobasidium pullulans* and *Candida parapsilosis* were inhibited only by chestnut and pasture honey samples.

## Discussion

In the present study, six different Slovenian honeys and manuka honey were investigated for their activities against selected bacterial and fungal species. The MIC method, which was used to determine antimicrobial activity, showed the lowest concentration of honey that prevented visible growth of bacteria or fungi. The MIC dilution assay revealed that the microbial inhibition of growth depended on the floral type, concentration and the pathogen tested.

All tested bacterial species were sensitive to Slovenian honeys. The greatest antibacterial activity was showed by the chestnut, fir and forest honeys, which had the lowest MIC (2.5% [v/v]) for *Staphylococcus aureus*. This was expected as a previous report showed excellent activity of manuka honey against this microorganism (MIC: 2–3%).[4] *Escherichia coli* and *Enterococcus faecalis* were more resistant, with the lowest MICs against these species found with pasture honey. In summary, the Slovenian honeys with the highest antibacterial activities were chestnut, fir and forest honeys, in descending order of efficacy. Similarly, a previous study[30] showed that Anatolian chestnut honey was the most effective, particularly against *Staphylococcus aureus*, but also against *Helicobacter pylori*, *Enterococcus faecalis* and *Bacillus subtilis*.

A recent study of the antioxidant activities of Slovenian honeys also demonstrated that dark honeys (e.g., chestnut, fir and forest) had higher antioxidant activities compared to lighter honeys (e.g., acacia and lime).[46] The data present here also indicate a strong correlation between the antibacterial and antioxidant activities of these Slovenian honeys, as has been shown for honeys in previous studies.[12,47]

The contribution of hydrogen peroxide activity to antibacterial activity was also measured for the chestnut and manuka honey samples. As indicated previously, glucose oxidase can activate hydrogen peroxide only in diluted honeys,[48] and in both cases the diluted honeys showed higher activities than the undiluted honeys (data not shown). After treatment with catalase, the antibacterial activity of chestnut honey decreased (from 12.5% to 50% [v/v]), while the MIC of manuka honey remained unchanged (10% [v/v]). It can be concluded, therefore, that the antibacterial action of this Slovenian chestnut honey is mainly due to its peroxide properties, while the manuka honey had an antibacterial activity that is due to other, non-peroxide, synergistic effects.

In general, antimicrobial activity in various honeys depends to a great extent on endogenous hydrogen peroxide content,[16] which is determined by the respective levels of glucose oxidase and catalase.[49] Despite the characteristics of reactive oxygen species, hydrogen peroxide plays an important role in promoting wound repair in human endothelial cells.[50] It is also known that honeys have additional antioxidant capacity, which may regulate potential free radical production, and this can impact on the wound healing process.[47]

The number of fungi involved in human opportunistic infections has dramatically increased over the past decade.[51–53] Thus, in the present study, the antifungal activity of the Slovenian honeys was also addressed. An overview of the literature shows that only a few types of honey, including *Leptospermum* honey and some floral honeys from Turkey, have been tested for their antifungal properties. Irish *et al*.[42] described significant activities of unprocessed jarrah and manuka honeys against *Candida* species, where their MICs were between 18.5% and 43.1% (w/v), while with Iranian honeys a *Candida* sp. was inhibited at 56% (v/v).[39]

In order to achieve a more comprehensive view of the potential antifungal effect of Slovenian honeys, in addition to the usually tested *Candida albicans*, two additional species of the same genus (*Candida tropicalis* and *Candida parapsilosis*) were added, along with other widespread opportunistic pathogenic fungi (*Aureobasidium pullulans* and *Cladosporium cladosporoides*, *Aspergillus niger*, *Penicillium chrysogenum* and *Rhodotorula mucilaginosa*) (Table 1). The observed antifungal effects were strongly concentration-dependent, with little antifungal activity at honey concentrations below 50%.

The most resistant fungal species were *Aspergillus niger*, *Candida albicans* and *Penicillium chrysogenum*, which grew in all concentrations of the tested honeys. With *Candida parapsilosis*, *Candida tropicalis* and *Rhodotorula mucilaginosa*, there was no growth inhibition up to 50% honey (v/v), while *Cladosporium cladosporoides* was inhibited at an MIC of

10% (v/v). The chestnut and pasture honeys were the only types to inhibit the growth of *Aureobasidium pullulans*. With *Penicillium chrysogenum*, *Candida tropicalis*, and *Rhodotorula mucilaginosa*, which were isolated from environments with high osmotic potential, higher resistance was expected.[54] Two fungal species, *Candida tropicalis* and *Rhodotorula mucilaginosa*, were sensitive to high honey concentration, which indicates the contribution of mechanisms of action other than high sugar content. Despite these generally low antifungal activities, the chestnut and pasture honeys were the most effective, which is supported by Koc *et al*.,[29] who reported that multifloral honey has a higher antifungal activity than eucalyptus and orange honeys.

In summary, chestnut honey showed the greatest antibacterial and antifungal activity, which appears to be due mainly to its high peroxide content. Undiluted Slovenian chestnut honey therefore represents a complex, natural substance with the potential for medicinal application such as the topical treatment of skin and wound infections. □

*The authors are grateful to the Ministry of Higher Education, Science and Technology of the Republic of Slovenia for its support of this study through Young Researcher grants to MKK and DJ.*

## References


1 Sipos P, Gyory H, Hagymasi K, Ondrejka P, Blazovics A. Special wound-healing methods used in ancient Egypt and the mythological background. *World J Surg* 2004; **28** (2): 211–6.

2 Zumla A, Lulat A. Honey – a remedy rediscovered. *J R Soc Med* 1989; **82** (7): 384–5.

3 Ahmed AK, Hoekstra MJ, Hage JJ, Karim RB. Honey-medicated dressing: transformation of an ancient remedy into modern therapy. *Ann Plast Surg* 2003; **50** (2): 143–7.

4 Cooper RA, Molan PC, Harding KG. Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds. *J R Soc Med* 1999; **92** (6): 283–5.

5 Cooper RA, Molan PC, Krishnamoorthy L, Harding KG. Manuka honey used to heal a recalcitrant surgical wound. *Eur J Clin Microbiol Infect Dis* 2001; **20** (10): 758–9.

6 Natarajan S, Williamson D, Grey J, Harding KG, Cooper RA. Healing of an MRSA-colonized, hydroxyurea-induced leg ulcer with honey. *J Dermatolog Treat* 2001; **12** (1): 33–6.

7 Lusby PE, Coombes AL, Wilkinson JM. Bactericidal activity of different honeys against pathogenic bacteria. *Arch Med Res* 2005; **36** (5): 464–7.

8 Molan PC. The role of honey in the management of wounds. *J Wound Care* 1999; **8** (8): 415–8.

9 Moore OA, Smith LA, Campbell F, Seers K, Mcquay HJ, Moore RA. Systematic review of the use of honey as a wound dressing. *BMC Complement Altern Med* 2001; **1**: 2.

10 Krushna NS, Kowsalya A, Radha S, Narayanan RB. Honey as a natural preservative of milk. *Indian J Exp Biol* 2007; **45** (5): 459–64.

11 Mundo MA, Padilla-Zakour OI, Worobo RW. Growth inhibition of foodborne pathogens and food spoilage organisms by select raw honeys. *Int J Food Microbiol* 2004; **97** (1): 1–8.

12 Taormina PJ, Niemira BA, Beuchat LR. Inhibitory activity of honey against foodborne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power. *Int J Food Microbiol* 2001; **69** (3): 217–25.

13 Biswal BM, Zakaria A, Ahmad NM. Topical application of honey in the management of radiation mucositis: a preliminary study. *Support Care Cancer* 2003; **11** (4): 242–8.

14 Bogdanov S. Functional and biological propertiews of the bee products: a review (www.bee-hexagon.net).

15 Bogdanov S. Nature and origin of the antibacterial substances in honey. *LWT Food Sci Technol* 1997; **30**: 748–53.

16 Molan PC. The antibacterial activity of honey. 1. The nature of the antibacterial activity. *Bee World* 1992; **73**: 5–28.

17 Molan PC. The antibacterial properties of honey. *Chemistry in New Zealand* 1995; **59**: 10–4.

18 Mavric E, Wittmann S, Barth G, Henle T. Identification and quantification of methylglyoxal as the dominant antibacterial constituent of manuka (*Leptospermum scoparium*) honeys from New Zealand. *Mol Nutr Food Res* 2008; **52** (4): 483–9.

19 Atrott J, Henle T. Methylglyoxal in manuka honey – correlation with antibacterial properties. *Czech J Food Sci* 2009; **27**: S163–5.

20 Kwakman PH, te Velde AA, de Boer L, Speijer D, Vandenbroucke-Grauls CM, Zaat SA. How honey kills bacteria. *FASEB J* 2010; **24** (7): 2576–82.

21 Kwakman PH, te Velde AA, de Boer L, Vandenbroucke-Grauls CM, Zaat SA. Two major medicinal honeys have different mechanisms of bactericidal activity. *PLoS One* 2011; **6** (3): e17709.

22 Brudzynski K. Effect of hydrogen peroxide on antibacterial activities of Canadian honeys. *Can J Microbiol* 2006; **52** (12): 1228–37.

23 Molan PC. The antibacterial activity of honey. 2. Variation in the potency of the antibacterial activity. *Bee World* 1992; **73**: 59–76.

24 Cooper RA, Molan PC, Harding KG. The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds. *J Appl Microbiol* 2002; **93** (5): 857–63.

25 Molan PC. The evidence supporting the use of honey as a wound dressing. *Int J Low Extrem Wounds* 2006; **5** (1): 40–54.

26 Visavadia BG, Honeysett J, Danford MH. Manuka honey dressing: an effective treatment for chronic wound infections. *Br J Oral Maxillofac Surg* 2008; **46** (8): 696–7.

27 Basualdo C, Sgroy V, Finola MS, Marioli JM. Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. *Vet Microbiol* 2007; **124** (3–4): 375–81.

28 Willix DJ, Molan PC, Harfoot CG. A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of manuka honey and other honey. *J Appl Bacteriol* 1992; **73** (5): 388–94.

29 Koc AN, Silici S, Ercal BD, Kasap F, Hormet-Oz HT, Mavus-Buldu H. Antifungal activity of Turkish honey against *Candida* spp. and *Trichosporon* spp: an *in vitro* evaluation. *Med Mycol* 2009; **47** (7): 707–12.

30 Küçük M, Kolayli S, Karaoglu S, Ulusoy E, Baltaci C, Candan F. Biological activities and chemical composition of three honeys of different types from Anatolia. *Food Chem* 2007; **100**: 526–34.

31 Theunissen F, Grobler S, Gedalia I. The antifungal action of three South African honeys on *Candida albicans*. *Apidologie* 2001; **32**: 371–9.

32 Basson N, Grobler S. Antimicrobial activity of two South African honeys produced from indigenous *Leucospermum cordifolium* and *Erica* species on selected micro-organisms. *BMC Complement Altern Med* 2008; **8**: 41.

33 Gulfraz M, Iftikhar F, Raja S *et al*. Quality assessment and antimicrobial activity of various honey types of Pakistan. *Afr J Biotechnol* 2010; **9**: 6902–6.

34 Laìd Boukraâ HBMA. Synergistic action of starch and honey against *Aspergillus niger* in correlation with diastase number. *Mycoses* 2008; **51** (6): 520–2.

35 Erturk O, Karakas FP, Pehlivan D, Nas N. The antibacterial and antifungal effects of rhododendron-derived mad honey and extracts of four rhododendron species. *Turk J Biol* 2009; **33**: 151–8.
36 Al-Jabri AA, Al-Hosni SA, Nzeako BC, Al-Mahrooqi ZH, Nsanze H. Antibacterial activity of Omani honey alone and in combination with gentamicin. *Saudi Med J* 2005; **26** (5): 767–71.
37 Al-Jabri AA, Nzeako B, Al Mahrooqi Z, Al Naqdy A, Nsanze H. *In vitro* antibacterial activity of Omani and African honey. *Br J Biomed Sci* 2003; **60** (1): 1–4.
38 Al-Jabri AA, Al Hosni SA, Nzeako B, Nsanze H. Antistaphylococcal activity of Omani honey in combination with bovine milk. *Br J Biomed Sci* 2005; **62** (2): 92–3.
39 Khosravi AR, Shokri H, Katiraee F, Ziglari T, Forsi M. Fungicidal potential of different Iranian honeys against some pathogenic *Candida* species. *J Apicult Res* 2008; **47**: 256–60.
40 Ceyhan N, Ugur A. Investigation of *in vitro* antimicrobial activity of honey. *Riv Biol* 2001; **94** (2): 363–71.
41 Brady N, Molan P, Bang L. A survey of non-manuka New Zealand honeys for antibacterial and antifungal activities. *J Apicult Res* 2004; **43**: 47–52.
42 Irish J, Carter DA, Shokohi T, Blair SE. Honey has an antifungal effect against *Candida* species. *Med Mycol* 2006; **44** (3): 289–91.
43 Ijaz T, Ranjha FA, Shahzad MK *et al*. Antibacterial and antifungal activity of different honeys. *Int J Infect Dis* 2008; **12**: e403.
44 Piana ML, Persano-Oddo L, Bentabol A, Bruneau E, Bogdanov S, Declerck CG. Sensory analysis applied to honey: state of the art. *Apidologie* 2004; **35**: S26–37.
45 Kropf U, Korošec M, Bertoncelj J *et al*. Determination of the geographical origin of Slovenian black locust, lime and chestnut honey. *Food Chem* 2010; **121**: 839–46.
46 Bertoncelj J, Doberšek U, Jamnik M, Golob T. Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. *Food Chem* 2007; **105**: 822–8.
47 Henriques A, Jackson S, Cooper R, Burton N. Free radical production and quenching in honeys with wound healing potential. *J Antimicrob Chemother* 2006; **58** (4): 773–7.
48 White JW Jr, Subers MH, Schepartz AI. The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system. *Biochim Biophys Acta* 1963; **73**: 57–70.
49 Weston RJ. The contribution of catalase and other natural products to the antibacterial activity of honey: a review. *Food Chem* 2000; **71**: 235–9.
50 Eligini S, Arenaz I, Barbieri SS *et al.* Cyclooxygenase-2 mediates hydrogen peroxide-induced wound repair in human endothelial cells. *Free Radic Biol Med* 2009; **46** (10): 1428–36.
51 Cuenca-Estrella M, Bernal-Martinez L, Buitrago MJ *et al*. Update on the epidemiology and diagnosis of invasive fungal infections. *Int J Antimicrob Agents* 2008; **32** (Suppl 2): S143–7.
52 Tortorano AM, Peman J, Bernhardt H *et al*. Epidemiology of candidaemia in Europe: results of 28-month European Confederation of Medical Mycology (ECMM) hospital-based surveillance study. *Eur J Clin Microbiol Infect Dis* 2004; **23** (4): 317–22.
53 Seebacher C, Bouchara J-P, Mignon B. Updates on the epidemiology of dermatophyte infections. *Mycopathologia* 2008; **166** (5–6): 335–52.
54 Snowdon JA, Cliver DO. Microorganisms in honey. *Int J Food Microbiol* 1996; **31** (1–3): 1–26.