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SHORT COMMUNICATION

Antifungal effect of lavender honey against *Candida albicans*, *Candida krusei* and *Cryptococcus neoformans*

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Abstract Monofloral lavender honey samples (n=30), were analyzed to test antifungal effect against *Candida albicans*, *Candida krusei,* and *Cryptococcus neoformans*. The specific growth rates (μ) showed that all the yeast growths were reduced in the presence of honey. The honey concentration (% w/v) that inhibited 10% of the yeasts growth (X_{min}) ranged from 31.0% (*C. albicans*), 16.8% (*C. krusei)* and 23.0% (*C. neoformans).* A synthetic honey solution was also tested to determine antifungal activity attributable to sugars. The presence of synthetic honey in the C. *krusei* culture medium at concentrations above 58.0% (w/v) was established as X_{min}, while C. *albicans* and C. *neoformans* were more resistant, since X_{min} values were not reached over the ranged tested (10–60%, w/v). What the data suggests is that the component in the lavender honey responsible for the observed antifungal in vitro properties is not sugar based. Honey might be tapped as a natural resource to look for new medicines for the treatment of mycotic infections. This could be very useful, onsidering the increasing resistance of antifungals. It should be noticed that this is the first study concerning the effect of lavender honey on the growth of pathogenic yeasts.

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Introduction

Honey is the natural sweet substance produced by honeybees from the nectar of blossoms, or from the secretion of living parts of plants or excretions of plant sucking insects on the living parts of plants, which honeybees collect, transform, and combine with specific substances of their own, and then store and leave in the honey comb to ripen and mature (Codex 2002). As a source of energy, the beneficial characteristics of honey are its high nutritional value and the fast absorption of its carbohydrates upon consumption (Viuda-Martos et al. 2008). When analyzing and studying the therapeutic properties of honeys, modern science has made it possible to specify their medical significance as bactericidal, bacteriostatic, antiviral, antioxidant, anti-inflammatory, and antitumoral (Molan 2001a; Lusby et al. 2005; Bardy et al. 2008; Estevinho et al. 2008). Many researchers have found honey to be a suitable alternative for healing wounds and burns, and for oral health (Lay-Flurrie 2008; Molan 2001b). Honey is also a highly valuable ingredient in foods (Bath and Singh 2001; Naveena et al. 2007; Kotoki and Deka 2010; Wadikar et al. 2010).

To date, very few attempts have been made to assess the antifungical properties of honey (Theunissen et al. 2001; Irish et al. 2006; Küçük et al. 2007; Boukraa et al. 2008; Koc et al. 2008), especially as compared to the large volume of published literature which has established that honey has significant antibacterial activity. The incidence of fungal infections is increasing in community and hospital environments (Fridkin 2005) and no other mycotic patho-

Table 1 Fungal growth rate in the presence of synthetic honey and lavender honey

Yeast culture	Fungal grow rate (h^{-1})												
	Synthetic honey (%,w/v)						Lavender honey (%,w/v)						
	0	10	20	30	40	50	60	0	10	20	30	40	50	60
C. albicans	0.21±0.05	0.20±0.05	0.21±0.05	0.21±0.09	0.19±0.07	0.19±0.05	0.18±0.08	0.21±0.05	0.20±0.05	0.19±0.04	0.18±0.05	0.15±0.05	0.11±0.03	0.09±0.03
C. krusei	0.19±0.05	0.18±0.08	0.17±0.08	0.18±0.05	0.16±0.07	0.15±0.07	0.13±0.04	0.19±0.05	0.18±0.05	0.15±0.05	0.11±0.05	0.08±0.03	0.05±0.01	0.03±0.01
Cr. neoformans	0.17±0.06	0.17±0.05	0.16±0.05	0.17±0.08	0.16±0.05	0.16±0.06	0.15±0.07	0.17±0.06	0.17±0.06	0.16±0.05	0.13±0.04	0.11±0.05	0.09±0.05	0.07±0.02

C.albicans (Candida albicans); C krusei (Candida krusei); Cr. neoformans(Cryptococcus neoformans);(n=30); All tests were performed in triplicate

gen produces as great a spectrum of opportunistic diseases in humans and animals as *Candida* does (Pappas et al. 2003; Tortorano et al. 2006). Furthermore, the rate of candidaemia caused by non-*albicans* species is increasing. Among these candidiases, *candida krusei*, which is an opportunistic pathogen isolated in some medical centers, can cause serious infections in susceptible patients (Nguyen et al. 2010). Another important encapsulated yeast-like fungus is the *Cryptococcus neoformans*, responsible for infectious diseases in patients with AIDS (Perfect and Casadevall 2002).

The aim of this short study is to assess the in vitro antifungical properties of Portuguese lavender honey against *Candida albicans*, *Candida krusei*, and *Cryptococcus neoformans*.

Monofloral lavender (*Lavandula stoechas*) honey samples (n=30), from *Apis mellifera iberica*, were collected in 2007 from separate apiaries in the North of Portugal. A synthetic honey solution with a carbohydrate composition similar to natural honey was used to determine whether inhibitory effects were due to the sugar content of the honey samples—100 g was prepared by dissolving 1.5 g sucrose, 7.5 g maltose, 40.5 g D-fructose, and 33.5 g D-glucose in 17 ml of sterile, deionized water.

The Coleción Española de Microorganismos Tipo (CECT) microorganisms were obtained from the (CECT) of Valencia University, while Escola Superior Agrária (ESA) microorganisms were strains that were clinically isolated in the *Centro Hospitalario do Nordeste* E.P.E. of Bragança, and identified in the Microbiology Laboratory of the Polytechnic Institute of Bragança. The fungi strains used were *Candida albicans* (CECT 1394), *Candida krusei* (ESA 11) and *Cryptococcus neoformans* (ESA 3). Microorganisms were cultured aerobically at 30 °C on sterile Yeast Peptone Dextrose (YPD) medium containing 2% (w/v) glucose, 1% (w/v) peptone, 1.5% (w/v) agar and 0.5% (w/v) yeast extract. Before the test assays for antifungal activity, the honeys samples were tyndallized according to Becker et al. (1996). Erlenmeyer flasks (150 ml) with 50 ml of YPD medium were inoculated with the yeast suspension (108 CFU/ml) and each concentration of honey over a range of 0% to 60% (w/v) to be tested was

Table 2 X_{min} (%) in the presence of synthetic honey and lavender honey

Yeast culture	Synthetic honey	Lavender honey
Candida albicans	>60[a]	31±0.05[b]
Candida krusei	58±0.08[a]	16.8±0.04[b]
Cryptococcus neoformans	>60[a]	23±0.05[b]

The letters (a, b) represent which X_{min} are different by Tukey test with significance of α=0.05

added. Incubation was carried out for 2 days at 37 °C in a rotary shaker at 150 rpm (Stuart Scientific SI50 model, United Kingdom). The specific growth rates (μ) of yeast cultures were monitored by measuring optical density at 640 nm in a spectrophotometer UV–visible (Varian Cary 50 Scan model, United States of America) and were calculated by least-squares fitting to the linear part of the semilog growth plot. Xmin (concentration that inhibited 10% of the yeasts growth) was determined by linear regression analysis (Calhelha et al. 2006).

The differences between Xmin were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with α=0.05. This treatment was carried out using SPSS 18.0 for Windows®.

The specific growth rate (μ) for fungi was determined and results are presented in Table 1. The results showed that the increase of lavender honey concentrations caused a decreased in μ of all organisms studied. Xmin values ranged from 31.0% (C. albicans), 16.8% (*C. krusei)* and 23.0% (*C. neoformans); C. krusei was* the most susceptible to honey since growth inhibition is reached at the minor level. The presence of synthetic honey in the C. *krusei* culture medium at concentrations above 58% (w/v) was established as X_{min}, while C. *albicans* and C. *neoformans* were more resistant, since X_{min} values were not reached over the range tested (10–60%, w/v). Antifungal activity against C. *krusei*, in particular, is noteworthy given the acquired and intrinsic resistance of this species to fluconazole (Nguyen et al. 2010). As far as we know, there are no studies that report on honey action against *C. neoformans* and C. *krusei*.

Our data suggest that the honey mechanism for fungal growth inhibition is not related to the osmotic shock derived from the presence of sugar in culture medium. In fact, using the Tukey test (P<0.05), we verified significant differences between X_{min} determinate in synthetic honey and lavender one. This was observed in all microorganisms tested (Table 2). In the same way, previous reports demonstrated that increased honey concentrations resulted in reduced growth of *C. albicans*, namely 29.4% inhibition on the growth was verified in the presence of wasbessie honey at concentrations of 25% (Theunissen et al. 2001). The minimum inhibitory concentration of honeys against isolates of *Candida* species (*C. albicans C. glabrata* and *C. dubliniensis*) would be achievable in a clinical setting (Irish et al. 2006); *C. dublinensis* was more susceptible to the osmotic effect of all honeys, and to the antifungal effects of Jarrah honey. However, on the other hand, previous studies with different types of honey tested at several concentrations ranging from 0.1 to 20% (Lusby et al. 2005) and from 25 to 100% (Omafuvbe and Akanbi 2009) revealed that the growth of *C. albicans* was not inhibited by the honeys.

Several factors may influence the antifungal activity of honey. For example, DeMera and Angert (2004) reported that honey from different phytogeographic regions varied in their ability to inhibit the growth of yeasts, suggesting that botanical origin plays an important role in influencing the antifungal activity. In addition, there are a great variety of components, including phenolic acids, flavonoids and other biomolecules, in different honeys. Biological activity of honey is mainly attributed to the phenolic compounds (Estevinho et al. 2008). In fact, the antimicrobial action of phenolics is well known and it is related to their ability to denature proteins, being generally classified as surface-active agents.

Conclusion

In conclusion, the antifungal effect of lavender honey was evaluated in culture media containing different concentrations of honey. What the data suggests is that the component in the lavender honey responsible for the observed antifungal in vitro properties is not sugar. The use of novel and powerful high-throughput techniques that currently are used in drug development will be of value to ascertain the medicinal properties of honey. Once the compound's structure is known, the chemical can serve as a prototype or "lead compound" for designing more effective therapeutic agents of similar chemical structure. Further studies are now required to demonstrate if this antifungal activity has any clinical application.

References

Bath PK, Singh N (2001) Effect of microwave heating on hydroxymethylfurfural formation and browning in Helianthus annuus and Eucalyptus lanceolatus honey. J Food Sci Technol 38:366–368

Bardy J, Slevin NJ, Mais KL, Molassiotis A (2008) A systematic review of honey uses and its potential value within oncology care. J Clin Nurs 17:2604–2623

Becker JM, Caldwell GA, Zachgo EA (1996) Sterilization methods, biotechnology, 2nd edn. San Diego, Academic Press, pp 210–211

Boukraa L, Benbarek H, Moussa A (2008) Synergistic action of starch and honey against *Candida Albicans* in correlation with diastase number. Braz J Microbiol 39:40–43

Calhelha RC, Andrade JV, Ferreira IC, Esteviño LM (2006) Toxicity effects of fungicide residues on the wine-producing process. Food Microbiol 23:393–398

Codex A (2002) Codex standard 12– revised codex standard for honey. Stan Methods 11:1–8

DeMera JH, Angert ER (2004) Comparison of the antimicrobial activity of honey produced by Tetragonisca angustula (Meliponinae) and Apis mellifera from different phytogeographic regions of Costa Rica. Apidologie 35:411–417

Estevinho L, Pereira AP, Moreira L, Dias LG, Pereira E (2008) Antioxidant and antimicrobial effects of phenolic compounds

extracts of Northeast Portugal honey. Food Chem Toxicol 46:3774–3779

Fridkin SK (2005) The changing face of fungal infections in health care settings. Clin Infect Dis 41:1455–1460

Irish J, Carter DA, Shokohi T, Blair S (2006) Honey has an antifungal effect against Candida species. Med Mycol 44:289–291

Koc AN, Silici S, Kasap F, Hörmet-Oz HT, Mavus-Buldu H (2008) Antifungal activity of Turkish honey against Candida spp: and Trichosporon spp an in vitro evaluation. Med Mycol 2:1–6

Kotoki D, Deka SC (2010) Baking loss of bread with special emphasis on increasing water holding capacity. J Food Sci Technol 47:128–131

Küçük M, Kolaılı S, Karaoğlu Ş, Ulusoy E, Baltacı C, Candan F (2007) Biological activities and chemical composition of three honeys of different types from Anatolia. Food Chem 100:526–534

Lay-Flurrie K (2008) Honey in wound care: effects, clinical application and patient benefit. Br J Nurs 17:32–36

Lusby PE, Coombes AL, Wilkinson JM (2005) Bactericidal activity of different honeys against pathogenic bacteria. Arch Med Res 36:464–467

Molan P (2001a) Why honey is effective as a medicine. Bee World 82:22–40

Molan PC (2001b) Potential of honey in the treatment of wounds and burns. Am J Clin Dermatol 2:13–19

Naveena BM, Sen AR, Vaithiyanathan S, Muthukumar M, Babji Y (2007) Effect of honey and vitamin C on quality of microwave cooked chicken patties. J Food Sci Technol 44:505–508

Nguyen KT, Ta P, Hoang BT, Cheng S, Hao B, Nguyen MH, Clancy CJ (2010) Characterising the post-antifungal effects of micafungin against *Candida albicans*, *Candida glabrata*, *Candida parapsilosis* and *Candida krusei* isolates. Int J Antimicrob Agents 35:80–84

Omafuvbe BO, Akanbi OO (2009) Microbiological and physicochemical properties of some commercial Nigerian honey. Afr J Microbiol Res 3:891–896

Pappas PG, Rex JH, Lee J, Hamill RJ, Larsen RA, Powderly W et al (2003) A prospective observational study of candidemia: epidemiology, therapy, and influences on mortality in hospitalized adult and pediatric patients. Clin Infect Dis 37:634–643

Perfect JR, Casadevall A (2002) Cryptococcosis. Infect Dis Clin North Am 16:837–874

Theunissen F, Grobler S, Gedalia I (2001) The antifungal action of three South African honeys on Candida albicans. Apidologie 32:371–379

Tortorano AM, Kibbler C, Peman J, Bernhardt H, Klingspor L, Grillot R (2006) Candidaemia in Europe: epidemiology and resistance. Int J Antimicrob Agents 27:359–366

Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Álvarez JA (2008) Functional properties of honey, propolis, and royal jelly. J Food Sci 73:117–124

Wadikar D, Nanjappa C, Premavalli KS, Bawa AS (2010) Development of ready-to-eat appetisers based on pepper and their quality evaluation. J Food Sci Technol 47:638–643