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SHORT COMMUNICATION

A Survey of the *In Vitro* Antifungal Activity of Heather (*Erica* Sp.) Organic Honey

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ABSTRACT Monofloral heather (*Erica* sp.) honey samples ($n = 89$), harvested in Portugal according to European organic beekeeping rules, were analyzed to test their antifungal effect against *Candida albicans*, *Candida krusei*, and *Cryptococcus neoformans*. A synthetic honey solution was also tested to determine antifungal activity attributable to sugars. The specific growth rate (μ) values showed that growth of all the yeasts was reduced in the presence of honey. The honey concentration (% wt/vol) that inhibited 10% of the yeast growth (X_{min}) was 13.5% for *C. albicans*, 20.5% for *C. krusei*, and 17.1% for *C. neoformans*. The respective concentrations of heather honey and synthetic honey in the *C. krusei* culture medium above 60% (wt/vol) that inhibited 90% of the yeast growth (X_{max}) and X_{min}, respectively, were established, whereas *C. albicans* and *C. neoformans* were more resistant because X_{max} values were not reached over the range tested (10–60%, wt/vol). Heather honey might be tapped as a natural resource to look for new medicines for the treatment of mycotic infections. Further studies are now required to demonstrate if this antifungal activity has any clinical application.

KEY WORDS: • ***antifungal effects*** • **Candida albicans** • **Candida krusei** • **Cryptococcus neoformans** • **Erica *species*** • ***honey***

INTRODUCTION

THE FACT THAT HIPPOCRATES, the father of medicine, emphasized the pharmaceutical value of honey is not accidental. 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.[1–3] Very few attempts have been made to date to assess the antifungal properties of honey[4–8] if we compare these findings with the large volume of published literature that has established that honey has significant antibacterial activity.

Although all honeys have a common composition with a high sugar content, low moisture, and acidity that prevents microbial growth, ancient physicians were selective as to which honeys they included in their remedies. In fact, in the last decade, research indicates that honey quality and health properties depend largely on the floral source,[9] together with other factors such as climatic conditions, soil type, and beekeeper activities.[10] In any case, concerns about traces of numerous toxic substances have brought about a certain demand for honey that is certified as being organic.[11] Organic honeys are produced using strict ecological and natural principles that are meant to enhance the good quality of the honey harvested, and they are free from many problems associated with honey from other parts of the world, such as pollution fallout and chemical residues.[12] Honeys to be used for therapeutic purposes should be harvested in areas with no contamination sources.

The incidence of fungal infections is increasing in community and hospital environments,[13] and no other mycotic pathogen produces a spectrum of opportunistic diseases in humans and animals as *Candida* does.[14,15] Furthermore, the rate of candidemia caused by non–*Candida albicans* species is increasing, and among these candidiasis-causing agents, *Candida krusei*, which is an opportunistic pathogen isolated in some medical centers, can cause serious infections in susceptible patients.[16] Those infections are difficult to treat owing to their reduced susceptibility to common antifungal agents. Another important encapsulated yeast-like fungus is *Cryptococcus neoformans*, responsible for infectious diseases in patients with AIDS.[17] As far as we know, there are no studies that report on the action of honey against *C. neoformans* and *C. krusei*.

The detailed characterization of the different honey types existent in Portugal is important because this country is recognized as having nine protected ''denomination of origin'' regions for honey, from a total of 18 in the European Union.[18] Heather honey is characterized by its dark brown color, strong flavor, and a slightly salty taste, which is

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produced in Portugal from *Erica* sp., whereas in Spain and France it comes from either the *Calluna* or *Erica* sp. The Ericaceae family is often used in folk medicine as an alternative therapeutic tool to treat hyperlipidemia, as a diuretic, astringent, or antiseptic, and in the treatment of urinary infections.[19–21]

The aim of this short publication is to assess the *in vitro* antifungal properties of Portuguese organic heather (*Erica* sp.) honey against *C. albicans*, *C. krusei*, and *C. neoformans*.

MATERIALS AND METHODS

Heather honey

Honeys ($n = 89$) were collected in 2008 from various beekeeping organic explorations in the North of Portugal. Even though the beekeepers themselves, according to the best of their knowledge and the location of hives, declared their honey as monofloral heather honey, all the samples were subjected to pollen analysis by the Erdtman acetolysis method; these are described in detail in a previous publication.[22] Results from the quantitative pollen analysis showed that the samples analyzed always had *Erica* sp. as the predominant pollen (at least 45%). Amount per sample varied between 59% and 72%, with the mean value being 69% and having an SD of 4%. Pollen grains of *Lavandulla* sp., *Prunus* sp., and *Echium* sp. were found in all honey samples (100%) with mean values of 14%, 11%, and 6%, respectively.

Control

A synthetic honey solution with a carbohydrate composition similar to that of 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 of sucrose, 7.5 g of maltose, 40.5 g of D-fructose, and 33.5 g of D-glucose in 17 mL of sterile, deionized water. This highly viscous solution was kept refrigerated at 4°C when not in use.

Microorganisms and culture conditions

Microorganisms labeled CECT were obtained from the Spanish Type Culture Collection of Valencia University, Valencia, Spain, whereas microorganisms labeled ESA were strains clinically isolated in the Centro Hospitalario do Nordeste E.P.E. of Bragança, Portugal, and identified in the Microbiology Laboratory of the Polytechnic Institute of Bragança. The fungal strains used were *C. albicans* (CECT 1394), *C. krusei* (ESA 11), and *C. neoformans* (ESA 3). Microorganisms were cultured aerobically at 30°C on sterile yeast peptone dextrose medium containing 2% (w/vol) glucose, 1% (w/vol) peptone, 1.5% (w/vol) agar, and 0.5% (w/vol) yeast extract. Before experimental use, cultures from solid medium were subcultured in liquid medium, incubated, and used as the source of inocula for each experiment.

Test assays for antifungal activity

Before the test assays for antifungal activity, the honey samples were pasteurized according to the technique of Becker *et al.*[23] Erlenmeyer flasks (150 mL) with 50 mL of yeast peptone dextrose medium were inoculated with the yeast suspension (10^8 colony-forming units/mL), and each concentration of honey over a range of 0% to 60% (wt/vol) to be tested was added. Incubation was carried out for 2 days at 37°C in a rotary shaker at 150 rpm.

The specific growth rate (μ) values of yeast cultures were monitored by measuring optical density at 640 nm in a ultraviolet–visible spectrophotometer and were calculated by least-squares fitting to the linear part of the semilog growth plot. The concentration that inhibited 10% of yeast growth (X_{min}) and the concentration that inhibited 90% of yeast growth (X_{max}) were determined by linear regression analysis. A more detailed presentation of this method has been previously reported.[24]

In the analysis involving synthetic honey, the same methodology was followed, replacing the heather honey by the same concentrations of synthetic honey and treating as described above. All heather and synthetic honey samples were analyzed during the same time period by three different analysts to ensure uniform conditions and comparability.

RESULTS AND DISCUSSION

Different concentrations of heather honey (10–60%) were screened for their antifungal activity against *C. albicans*, *C. neoformans*, and *C. krusei.* A synthetic honey solution was also tested to determine activity attributable to sugars. The μ for fungi was determined, and results are presented in Figure 1. The results showed that the increase of heather honey concentrations caused a decrease in μ for all organisms studied. The X_{min} and X_{max} were determined by linear regression analysis and are shown in Table 1. X_{min} values ranged from 20.5% for *C. albicans* to 13.5% for *C. krusei* to 17.1% for *C. neoformans*, with *C. krusei* being the most susceptible to honey because growth inhibition is reached at the minimum level. Moreover, according to the X_{max} obtained, *C. krusei* was the most susceptible to negative effects of the tested solutions. The presence of heather honey in the *C. krusei* culture medium at concentrations above 60% (wt/vol) inhibited 90% of the yeast growth (X_{max}). In contrast, the same concentration of synthetic honey was established as X_{min} for *C. krusei*, whereas *C. albicans* and *C. neoformans* were more resistant. More important is that this is the first report testing the *in vitro* antifungal potential of honey against *C. krusei*. Antifungal activity against *C. krusei*, in particular, is noteworthy given the acquired and intrinsic resistance of this species to fluconazole.[16]

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 the culture medium. In the same way, previous reports have demonstrated that increased honey concentrations resulted in reduced growth of *C. albicans*, namely, 29.4% inhibition of the growth was

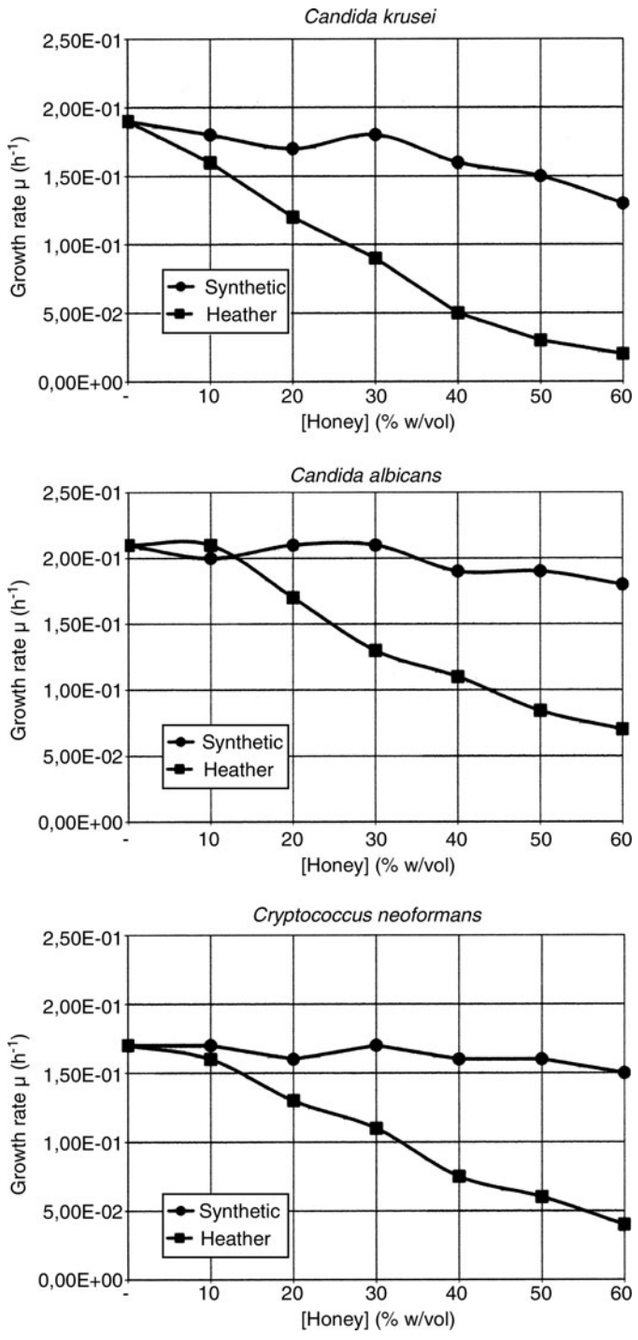

FIG. 1. Fungal growth rate (μ, in h^{-1}) in the presence of heather honey and synthetic honey at different concentrations (wt/vol).

verified in the presence of wasbessie honey at concentrations of 25%.[4] The minimum inhibitory concentration of honeys against isolates of *Candida* species (*C. albicans*, *Candida glabrata*, and *Candida dubliniensis*) would be achievable in a clinical setting,[5] with *C. dubliniensis* being more susceptible to the osmotic effect of all honeys and to the antifungal effects of Jarrah honey. However, in contrast,

Table 1. Heather Honey and Synthetic Honey Concentrations That Inhibited 10% and 90% of Yeast Growth

	Concentration (%, wt/vol)			
	Heather honey		*Synthetic honey*	
Yeast	X_{min}	X_{max}	X_{min}	X_{max}
C. albicans	20.5	>60.0	>60.0	—
C. krusei	13.5	60.0	60.0	—
C. neoformans	17.1	>60.0	>60.0	—

X_{min} and X_{max} represent concentrations that inhibited 10% and 90%, respectively, of yeast growth.

previous studies with different types of honey, tested at several concentrations ranging from 0.1% to 20%[2] and from 25 to 100%,[25] revealed that the growth of *C. albicans* was not inhibited by the honeys.

Several factors may influence the antifungal activity of honey. These factors include its physicochemical properties, botanical origin, entomological origin, and symbioses with beneficial bacteria. The literature reviewed shows us that research on monofloral *Erica* sp. honeys is mainly focused on their physicochemical and palynological features,[26–29] the assessment of the possible markers for their floral origin,[30] the development of an electronic tongue for heather honey classification,[31] and, more recently, the antioxidant and antibacterial properties of the phenolic compounds found in Portuguese heather honey, demonstrated with promising results.[32]

The healing properties of honey depend largely on the floral source that nourishes the honeybees. For example, DeMera and Angert[33] reported that honeys 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. *Erica* sp. contain many active substances such as flavonoids, anthocyanidols, coumarins, and triterpenic compounds, which are expected to be found in heather honey. Moreover, major compounds of *Erica* sp. flowers have been isolated and proved to have antimicrobial[34] and antiulcer[35] activities as well as cytotoxic and anticarcinogenic properties.[36] Analysis of phenolic compounds in heather honey samples showed that about 14 phenolic compounds could be identified (five flavonoids and nine phenolic acids) and that the phenolic pattern of honey contains protocatechic acid, *p*-hydroxybenzoic acid, caffeic acid, chlorogenic acid, vanillic acid, *p*-coumaric acid, benzoic acid, chrysin, and cinnamic acid, as well as the flavonoids naringenin, kaempferol, apigenin, pinocembrin, and ellagic acid.[37] In plants, ellagic acid is present in the form of ellagitannin, which is ellagic acid bound to a sugar molecule. Ellagitannins are hydrolyzed by bee enzymes to yield ellagic acid, the bioactive agent that offers protection. Although recent work shows that there are other compounds present, which could not be identified because of lack of availability of standard compounds,[32] it has been concluded that ellagic acid and

myricetin-30-methylether (which have not been identifying in any of the monofloral honeys investigated so far) seem to be potential markers for the floral origin of heather honey.[32,38,39] Therefore, different honey properties were expected because the composition of active compounds in honey from different locations should be different.

In conclusion, the antifungal effect of organic heather honey was evaluated in culture medium containing different concentrations of honey. What the data suggest is that the component in the heather honey responsible for the observed antifungal *in vitro* properties is not sugar based. Although the therapeutic action of honey has been given some attention by researchers, studies have only been done by screening raw honey samples. The use of novel and powerful high-throughput techniques that currently are used in drug development will be of value to ascertain the medical 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.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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