Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading doi:10.1016/S2221-1691(12)60018-5

Additive potential of ginger starch on antifungal potency of honey against *Candida albicans*

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ARTICLE INFO

Article history:
Received 15 October 2011
Received in revised form 9 November 2011
Accepted 3 December 2011
Available online 28 April 2012

Keywords:
Honey
Ginger starch
Antifungal potency
Candida albicans

ABSTRACT

Objective: To evaluate the additive action of ginger starch on the antifungal activity of honey against *Candida albicans* (*C. albicans*). **Methods:** *C. albicans* was used to determine the minimum inhibitory concentration (MIC) of four varieties of Algerian honey. Lower concentrations of honey than the MIC were incubated with a set of concentrations of starch and then added to media to determine the minimum additive inhibitory concentration (MAIC). **Results:** The MIC for the four varieties of honey without starch against *C. albicans* ranged between 38% and 42% (v/v). When starch was incubated with honey and then added to media, a MIC drop was noticed with each variety. MAIC of the four varieties ranged between 32% honey (v/v) with 4% starch and 36% honey (v/v) with 2% starch. **Conclusions:** The use of ginger starch allows honey benefit and will constitute an alternative way against the resistance to antifungal agents.

1. Introduction

Fungal diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide[1,2]. *Candida albicans* (*C. albicans*) is the most common species associated with candidiasis and is the most frequently recovered species from hospitalized patients[3-5]. The treatment of mycoses has lagged behind bacterial chemotherapy and fewer antifungal than antibacterial substances are available[6,7]. Therefore, a search for new antifungal drugs is extremely necessary[8-10]. Different natural substances are responsible for antifungal action[11-13]. Among the possible alternatives, products from the hive such as honey, which is considered as natural, non toxic and with a broad spectrum of action, mainly because of its antimicrobial role, are used[14]. This could be promising alternative to substitute synthetic antifungal agent but in several countries the cost of honey is quite expensive which limits its use. Starch is a principal constituent of many foods and it does not only constitute a major energy source, but also is essential to the gross texture or consistency of many food preparations[15]. Therefore, the study was aimed to evaluate the additive action of ginger starch on the antifungal activity of honey against *C. albicans*.

2. Materials and methods

2.1. Honey sample and plant

From the 2009 harvest, four varieties of honeys of different botanical origin, namely: citrus (V1), jujube (V2), orange (V3) and multi floral (V4) were collected from hives located in western Algeria. All honeys were kept in glass vials and protected from light at temperature of 4 ℃.

Rhizome of ginger (*Zingiber officinale*) purchased from local market of Tiaret (western of Algeria) was peeled and crushed using a hammer mill and then diluted in water. Obtained milk was sieved and sediment was separated from the supernatant and washed several times. The deposit obtained was spread out on aluminum foil and dried at 45 ℃ for 48 h. Obtained

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Foundation Project: This work was financially supported by project CNEPRU, Institute of Veterinary Sciences, University Ibn-Khaldoun (TIARET), Algeria (grant No. F023 2009/0009).

product was crushed to get starch powder[16] from which various concentrations were prepared and expressed as percentage (%).

2.2. Fungal strain and inoculums standardization

C. albicans (Institut Pasteur of Algiers) was maintained by subculture in specific media (Sabouraud agar). The inoculum suspension was obtained by taking five colonies (>1 mm diameter) from 24 old cultures grown on Sabouraud agar. The colonies were suspended in 5 mL of sterile saline water (0.85%). The inoculum suspensions were shaken for 15 sec and density was adjusted to the turbidity of a 0.5 McFarland standard (equivalent to $1-5 \times 10^6$ cfu/mL).

2.3. Minimum inhibitory concentration (MIC)

Increased concentrations (10%-50% v/v) were incorporated into media to test their efficiency against *C. albicans*. Each plate with final volume of honey and media of 5 mL was inoculated and incubated at 37 ℃ for 48 h. The MIC was determined by finding the plates with the lowest concentration of honey on which the strain would not grow. All MIC values were expressed in % (v/v).

2.4. Minimum additive inhibitory concentration (MAIC)

To evaluate the effect of starch on the antifungal action of honey, 1% starch solution was prepared using sterile water. Different volumes from the stock solution were added to a range of honey concentrations lower than the MIC. The same volume of starch solution that has given inhibition with honey was added alone to media as control to check whether or not starch alone has an inhibition effect against *C. albicans*. An equivalent volume of water was added to honey instead of starch solution to confirm that additive inhibition is not due to the dilution of honey. The final volume in each plate was 5 mL. Starch content in media ranged between 1% and 8% (w/v). Honey and starch as well as honey and water were incubated for 24 h at 37 ℃ before being incorporated into media. Plates were inoculated and incubated at 37 ℃ for 48 h. All inoculations were carried out in duplicates.

3. Results

The inhibitory action was seen neither in the media containing starch only nor in media with water and starch. All varieties of honey were effective against the tested strain. Without starch of ginger, the MIC of the four varieties ranged between 38% and 42% (v/v). When starch was incubated with honey and added to media, a MIC drop was noticed with each variety and the MAIC of the four varieties ranged between 32% and 36% (v/v) (Table 1).

Table 1
MIC, MAIC and MIC drop of tested honeys.

Honey varieties	MIC values [% (v/v)]			MAIC values [% (v/v)] honey and starch								MIC drop (%)
				Starch solution								
	Honey	Starch	Water and starch	1%	2%	3%	4%	5%	6%	7%	8%	
V1	42	0	0	42	36	36	36	36	36	36	36	14.28
V2	41	0	0	41	41	41	35	35	35	35	35	14.63
V3	38	0	0	38	38	38	32	32	32	32	32	15.78
V4	39	0	0	39	39	39	33	33	33	39	39	15.38

4. Discussion

In humans, fungal infections range from superficial to deeply invasive or disseminated, and have increased dramatically in recent years[6]. The application of honey in medicine has recently been rediscovered and it is gaining acceptance as an antibacterial agent for the treatement of ulcers, wounds, and other surface infection[17].

Using corn starch and four varieties of Algerian honeys[18] has shown an additive effect against *C. albicans* with the MIC values of 38% and 28%, respectively, with a starch concentration of 3.6%. In a previous study, Boukraa *et al*[19] used other five varieties of honey and the same strain of *C. albicans* used in present study, and obtained a MIC value ranging between 30% (starch 2.6%) and 39% (starch 2.4%). Amylases present in honey were expected to split starch chains to randomly produce dextrin and maltose and probably increase the osmotic effect in the media by increasing the amount of sugars and consequently increase the antifungal activity. As a paradox, the variety with the lowest diastase number has shown the highest MIC drop and the variety with the highest diastase number has shown the lowest MIC drop. Resistant starch has received much attention for both its potential health benefits and functional properties. It has properties similar to fiber and shows promising physiological benefits in humans, which may result in disease prevention[20]. Eerlingen *et al*[21] reported that an increase in resistant starch yield was observed with high-amylose corn starch. As the final purpose of our future studies is the use of honey and starch to manage superficial mycoses by an increase in osmotic pressure, the use of resistant starch is not adequate in this case. In previous studies, the same varieties of honey and ginger were used.

We obtained a MIC drop with *Aspergillus niger* ranging

between 10.5% and 11.5%[22]. By using 1% ginger starch solution instead of 10% corn starch used in the above mentioned studies, we obtained better results. But it must be mentioned that the honey varieties used by Boukraa[18] and Boukraa *et al*[19] were different from ours. It then seems that ginger starch for a reason or another is more effective than corn starch, perhaps in regard to its lesser resistance to hydrolysis by amylases. In other hand, Torley *et al*[23] reported that, honeys from different yields were observed with high-amylose corn starch. The experiments showed that the differences in gelatinization temperature, lipid content, and apparent amylose content of the two starches were not the main causes of the different impact of sugars on resistant starch yields. Sources show a varied effect on starch gelatinization with starch viscosity increasing with addition level for some honeys, but decreasing with increasing addition level for other samples. Neither honey nor starch has adverse effects on tissues, so they can be safely used in wounds and inserted in cavities and sinuses to clear infection. A clinical trial would be carried out to validate these findings. The results will enable a systematic study of many varieties of honey on pathogens yeast with increased resistance opposite conventional anticandidiasis.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Authors are thankful to staff of Tiaret University for providing material.

References

[1] Marg KSK. *The wealth of India*. New Delhi: National Institute of Science Communication and Information Resources; 1998, p. 164.

[2] Meena AK, Ramanjeet K, Brijendra S, Yadav AK, Uttam S, Ayushy S, et al. Review on antifungal activities of Ayurvedic medicinal plants. *Drug Invent Today* 2010; **2**(2): 146-148.

[3] Amit KT, Anushree M. Liquid and vapour-phase antifungal activities of selected essential oils against *Candida albicans*: microscopic observations and chemical characterization of *Cymbopogon citratus*. *BMC Complement Altern Med* 2010; **10**: 65.

[4] Henry-Staney MJ, Garni RM, Johnson MA, Bendel CM, Wells CL. Comparative abilities of *Candida glabrata* and *Candida albicans* to colonize and translocate from the intestinal tract of antibiotic-treated mice. *Microb Ecol Health Dis* 2005; **17**: 129-137.

[5] Sobel JD, Fisher JF, Kauffman CA, Newman CA. *Candida* urinary tract infections-epidemiology. *Clin Infect Dis* 2011; **52**(Suppl 6): S433-S436.

[6] Duraipandiyan V, Ignacimuthu S. Antifungal activity of traditional medicinal plants from Tamil Nadu, India. *Asian Pac J Trop Biomed* 2011; **1**(Suppl 2): S204-S215.

[7] Duraipandiyan V, Ignacimuthu S. Antifungal activity of rhein isolated from *Cassia fistula* L. flower. *Pharmacology* 2010; **1**(9): WMC00687.

[8] Fortes TO, Alviano DS, Tupinamba G, Padron TS, Antoniolli AR, Alviano CS, et al. Production of an antimicrobial substance against *Cryptococcus neoformans* by *Paenibacillus brasilensis* Sa3 isolated from the rhizosphere of *Kalanchoe brasiliensis*. *Microbiol Res* 2008; **163**: 200-207.

[9] Basma AA, Zuraini Z, Sasidharan S. A transmission electron microscopy study of the diversity of *Candida albicans* cells induced by *Euphorbia hirta* L. leaf extract *in vitro*. *Asian Pac J Trop Biomed* 2011; **1**: 20-22.

[10] Sharma KK, Saikia R, Kotoky J, Kalita JC, Devi R. Antifungal activity of *Solanum melongena* L, *Lawsonia inermis* L. and *Justicia gendarussa* B. against dermatophytes. *Int J PharmTech Res* 2011; **3**(3): 1635-1640.

[11] Koç AN, Silici S, Kasap F, HÖrmet-Oz HT, Mavus-Buldu H, Ercal BD. Antifungal activity of the honeybee products against *Candida* spp. and *Trichosporon* spp. *J Med Food* 2011; **14**(1-2): 128-134.

[12] Manila C, Barbara C, Giuseppe D, Manuela B, Augusto A, Elena P. Honey flavonoids, natural antifungal agents against *Candida albicans*. *Int J Food Prop* 2011; **14**: 799-808.

[13] Supreetha S, Sharadadevi M, Simon SP, Jain J, Tikare S, Amit M. Antifungal activity of ginger extract on *Candida albicans*: an *in-vitro* study. *J Dent Sci Res* 2011; **2**(2): 1-5.

[14] Muhrbeck P, Svensson E. Annealing properties of potato starch with different degrees of phosphorylation. *Carbohydr Polym* 1996; **31**: 263-267.

[15] Kevate BN, Chavan UD, Kadam SS, Chavan JK, Amarowicz R. Isolation and characterization of starch from moth bean. *Afr J Food Sci Technol* 2010; **1**(3): 68-70.

[16] Amani NG, Aboua F, Gnakri D, Kamenan A. Study of physico-chemical properties of cocoyam starch (*Xanthosoma sagittifolium*). *Ind Aliment Agric* 1993; **110**(3): 136-142.

[17] Adewumi AA, Ogunjinmi AA. The healing potential of honey and propolis lotion on septic wounds. *Asian Pac J Trop Biomed* 2011; **1**(Suppl 1): S55-S57.

[18] Boukraa L. Additive action of honey and starch against *Candida albicans* and *Aspergillus niger*. *Rev Iberoam Micol* 2007; **24**: 309-311.

[19] Boukraa L, Hama B, Ahmed M. Synergitic action of starch and honey against *Candida albicans* in correlation with diastase number. *Braz J Microbiol* 2008; **39**: 40-43.

[20] Sajilata MG, Rekha SS, Pushpa RK. Resistant starch-a review. *Compr Rev Food Sci Food Saf* 2006; **5**(1): 1-17.

[21] Eerlingen RC, Van den Broeck I, Delcour JA, Levine H. Enzyme resistant starch. Influence of sugars on resistant starch formation. *Cereal Chem* 1994; **6**(70): 345.

[22] Ahmed M, Djebli N, Aissat S, Aggad H, Boucif A. Antifungal activity of a combination of Algeria honey and starch of ginger against *Aspergillus niger*. *Int J Microbiol Res* 2011; **2**(3): 263-266.

[23] Torley PJ, Rutgersb RPG, D'Arcya B, Bhandaria BR. Effect of honey types and concentration on starch gelatinization. *LWT-Food Sci Technol* 2004; **37**: 161-170.