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Libyan spring honey as a natural anti-fungal *Candida albicans*

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ABSTRACT

Although Candida is a kind of a normal flora of the mucous membranes in the respiratory, gastrointestinal and female genital tracts, its scope of infections is increasing all over the world as a result of their resistance. It is known that certain honey types have significant antifungal activity against range of Candida species; therefore this study has focused on the evaluation of the antimicrobial activity of the Libyan spring honey against Candida species. Concentrations of 50 % and 25 % of Libyan spring honey were prepared and tested for antimicrobial activity against Candida albicans samples that were collected as random samples of human mouth. It was concluded that 25 % and 50 % concentration of Libyan spring honey has no effect on the growth of C. albicans. On the other hand it was clearly observed that the Libyan spring honey was very affective in inhibiting the candial adherence to buccal epithelial cells (BEC) in vitro.

عسل الربيع الليبي كمضاد طبيعي لفطريات *Candida albicans*

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الكلمات المفتاحية:
الفلورا الطبيعية
أنواع *Candida*
Candida albicans
BEC

الملخص

على الرغم من أن Candida هي نوع من الفلورا الطبيعية للأغشية المخاطية في الجهاز التنفسي والجهاز الهضمي والأعضاء التناسلية الأنثوية ، إلا أن نطاق العدوى بها يتزايد في جميع أنحاء العالم نتيجة لمقاومتها. من المعروف أن بعض أنواع العسل لها نشاط مضاد للفطريات ضد مجموعة من أنواع Candida. لذلك ركزت هذه الدراسة على تقييم النشاط المضاد للميكروبات لعسل الربيع الليبي ضد أنواع Candida. تم تحضير تراكيز 50٪ و 25٪ من عسل الربيع الليبي واختبار نشاطها المضاد للميكروبات ضد عينات Candida albicans التي جمعت كعينات عشوائية من فم الإنسان. استنتج أن تركيز 25٪ و 50٪ من عسل الربيع الليبي ليس له أي تأثير على نمو C. albicans. من ناحية أخرى لوحظ أن عسل الربيع الليبي كان له تأثير كبير في تثبيط الالتصاق الشمعي بالخلايا الظهارية الشدقية (BEC) في المختبر.

Introduction

Thousands of years ago, honey as an organic compound has been used as a medicine, and found to be very affective antimicrobial agent (1). The efficiency of the honey comes from the production of hydrogen peroxide from glucose and oxygen by glucose oxidase, a bee derived enzyme (2) and its antimicrobial properties were extensively reviewed (3.4). Honey has a potent antibacterial activity and is very effective in clearing infection in wounds and protecting them from becoming infected specially that comes from certain species of native to Australia and New Zealand contains additional phytochemical components that further enhance its antibacterial activity (5,6,7) The precise nature of these components is yet to be identified.(8,9)

As mentioned by Dustman 1979 (9), the first study on the antimicrobial effects of honey was reported by Ruhnke M (10). A few years later, several researchers have published their results on this subject (11, 12). Most of these researches concentrate on identifying the antimicrobial agents in different honey types and the range of organisms susceptible to this antimicrobial action (13, 12). Although *C. albicans* are present in the oral cavity of up to 75% of the population (14), this colonization generally remains benign

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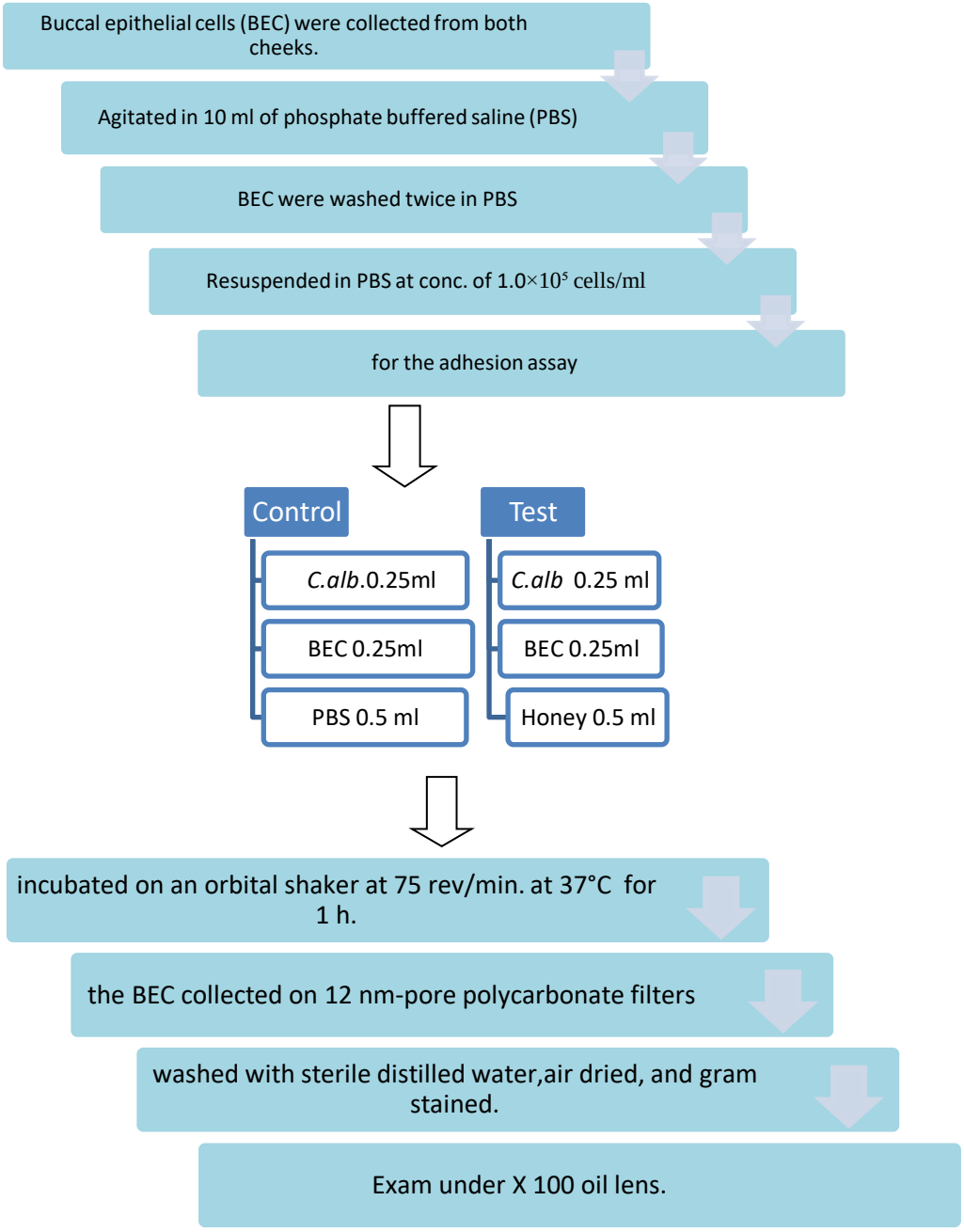
(normal flora) in healthy individuals. However, some Immunocompromised individuals found to be frequently suffering from the oral cavity infections. The infections with Candida species are termed "oral candidacies" (OC) (14).These infections caused by *C. albicans* have the ability to affect the pharynx and/or the esophagus of persons with dysfunctions of the immune system. Other risk factors which can lead to the developments of OC include the wearing of dentures and extremes of age (15). The human mycoses are generally caused by opportunistic fungi (6). This pathogen has the ability to produce illness by taking advantage of debilitated or immune-compromised hosts (7). *C. albicans* can grow on warm and moist surfaces and can easily cause some superficial diseases such as oral and vaginal thrush and chronic mucocutaneous candidacies (16). **The aim** of the present work was to express the antifungal properties of the Libyan spring honeys and the inhibitory effect on Candial adherence to buccal cells.

A diagram 1) samples processing:



Materials and Methods

The honey samples were obtained directly from beekeepers during the harvest. Honey samples were prepared and handled protected from direct sunlight and stored always at 4 °C in the dark until they were used as planned in the next (diagram 1). A honey solution was prepared in nine fractions: 20, 25,30,35,40,45,50,55 and 60 % (mass/ volume). The samples of honey were diluted by using brain heart infusion broth, and incubated at 37 °C for 30 min. before mixing, this was done to facilitate homogenization. The 60 % (mass/ volume) solutions thus prepared were diluted to the other lower concentration consequently. The diluted honey concentrations then exposed to four *C. albicans* strains (A6, A7, A8 and A9). The source of these strains were cervical isolate patient with vaginal candidiasis, oral isolate, oral isolate, and mycological reference lab London, UK, consequently (17).

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RESULTS:

After 18 hour of incubation at 37°C, as high as 75% of candida was inhibited to grow in the presence of 60% honey compared with a free honey control. Subsequently, the other concentrations were simultaneously processed for each strain. The adherence of Candida albicans to human buccal cells was largely reduced from a mean of 192 Candida/100BEC to 80 Candida/100 BEC, in the presence of 25% honey (>50% reduction) as shown in the **Table 1**. All the experiments were repeated twice, including control.

The observation shows that there is no effect of honey could be

observed on the growth of *C. albicans* as in vitro experimentally proved**.**

Table 1: The adherence of *Candida albicans* to human buccal cells was reduced.

Control				Test		
Candida	No.CC/100 BEC	% BEC with CC	% Free BEC	No.CC/100 BEC	%BEC with CC	% Free BEC
A6	200	45.5%	54.5%	72	24%	76%
A7	110	64%	36%	32	23.5%	76.5%
A8	134	58.5%	41.5%	45	31%	69%
A9	130	56%	44%	77	38%	62%

*CC: Candida cells

*BEC Buccal epithelial cells

DISCUSSION

Candida species has the ability to infect the host using a range of virulence factors (18). Some of which include the expression of adhesions and invasions on the cell surface, and the morphological transition between yeast and hyphal forms which considered being fatal virulence factors (18, 19), and because of that the incidence of fungal infections is increasing in both the community and hospital environments, with Candida spp. Although *C. albicans* causes oral infections of over 50% of candidaemia cases (20, 21) and more than 90% of vaginal candidiasis (22, 23). Limited observations have found that honey have the ability to inhibit *C. albicans* in vitro (24,25) . In this small scale study the effect of Libyan spring honey on *C, albicans* was tested and the minimum inhibitory concentration (MIC) was also calculated. In this examination the honey was diluted to a concentrations of (20%,25%,30%, 35%, up to 60%, by which we against the use of the pure hone(100%) concentration which was used by (24, 25 and 26),

Although (26, 27) founded that undiluted honey show a high inhibitory effect on bacterial growth comparing to the diluted concentrations. On the other hand (27) concluded that pure honey has negative effect on *C. albicans*, while results of (26) noted no inhibitory effect of the pure hony on *C. albicans*, which was in disagreement to (28), where they found that unprocessed honey can inhibit candida growth. Others like (29), noted that the honey they used in their experiments did not inhibit the growth of *C. albicans* which comes in disagreements with our results along with the results of (30), where all of the strains used were inhibited by honey and the rate of the inhibition depends on the type and concentration of honey.

Looking to what was mentioned above we can say that Libyan spring honey would not be too effective, and insure the need for the adequate concentration of honey that can be used as a MIC. Several studies focus on the optimum MIC (26,30,31 ,32,33) where some of them were to agreements with our results that summarized in the above mentioned **Table 1,** where we found that a 25% Libyan spring honey concentration is the best , Theunissen and Grobler (31) also concluded that the highest concentration tested and founded to be effective is the 25% (w/w), this inhibition was found to be 29.4% and it can play a role on the adherence of *C. albicans*, although at different experimental processing ,Mahdavi and his colleagues (33), has found that different dilution of honey in 20-25% and 15-30% can inhibit candidal growth.

According to literature, the use of pure honey suppress fungus as it was mentioned by Abdullah and Clemencia (26), however; if it was diluted to a concentration of 50% up to 75% as mentioned by Liliana Fernandes etc (28), can act as a good Candida biofilm reducer. Abdel Azeiz, and F. AlGuthami also concluded that the 50% honey concentration (Markh and Manuka) can completely inhibit *C. albicans* growth as well as use of higher concentrations 80% and after incubation (27).

Different concentrations of honey were also tested in order to find the optimum concentration that can affect the growth of *C. albicans*. Ays Nedret and his colleagues (35), illustrated that the greatest inhibition of Candida was seen at concentrations (40% v/v). This was close to a previous study that the amount of honey present in the solution of mixture (50% w/v) inhibited completely the growth of *C. albicans*.

Other researchers added specific compounds (medical and nature) to honey samples or the samples of the organisms under testing and their results shown to be effective when compared with the use of the pure honey. A Mulu (34), shows that susceptibility of the Candida species to fluconazole was tested and the growth of Candida species was inhibited with a minimum fungicidal concentration (MFC) of 35–40% (v/v) honey. While Theun et al (36), have used a combination of medical grade honey and they found that their formulation reduced the growth of some Candida species in a dose-dependent manner. And by increasing the concentration of the honey to 40%, present in an undiluted medical-grade honey formulation, lead to inhibition of all Candida species. On the other hand, Ahmed Moussa et al (37) also added corn starch to the samples tested, the results shown an additive effect against *C. albicans* with the MIC values of 38% and 28%, respectively.

From all above we could say that the additions of specific compounds to the tested honey samples might have promising physiological benefits in humans in a different ways, and that depends on honey effective compositions such as phenolic acids and flavonoids as mentioned by Aminu Shehu et al (38).

To sum up we could say that results of Al-Waili et al (35), is compatible with our results that honey concentration ranging from 30–50% inhibited the growth of several pathogenic microorganisms, including *C. albicans*. Honey may be used to prevent more serious infections and could be incorporated in the therapy of oral and vaginal candidiasis.

CONCLUSION

Libyan spring honey exhibited significant antifungal activities against *C. albicans*.

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