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Fungicidal activity of honey originating from different phytogeographic regions against *Aspergillus niger* and *Penicillium chrysogenum*

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Abstract
Different honey brands from Pakistan, Germany and Arabian countries were examined to test their antifungal potential against *Aspergillus niger* and *Penicillium chrysogenum.* The *in-vitro* antifungal activity with different concentrations (Undiluted, 10, 20, 30% v/v) of honey was compared with commercial antifungal drug fluconazole. The diameters of zone of the inhibition of various concentrations of honey ranged from 11-22 mm for *A. niger* and 13-24 mm for *P. chrysogenum*, respectively. Lenganese honey brand (LH) showed highest activity (62-68%) in undiluted honey sample (100%) while honey samples from Pakistani origin exhibited 60-65.71% activity against tested fungal strains. Multi-floral honey samples inhibited the fungal growth more efficiently than the unifloral honey. The antifungal activities of Pakistani honey brands were comparable with international honey brands and commercial antifungal drug fluconazole and might serve to protect health against these human pathogenic fungi.

Keywords: Antifungal activity, Honey brands, *Aspergillus niger*, *Penicillium chrysogenum*

1. Introduction
The conventional treatment of fungal disease is limited due to the narrow spectrum of available commercial antifungal drugs and their prolonged therapy. Thus, nowadays research is focused on the medicinal properties of natural compounds [10]. Honey has broad spectrum *In vitro* antibacterial and antifungal potential which increased its use in modern medicine [11, 14] and makes it a candidate for application as a safe antifungal medicine [2].
Honey has erratic composition, due to the differences in plant varieties, environmental and climatic conditions [13]. Several factors may influence the antifungal activity of honey. Honeys from different phytogeographic regions vary in their ability to inhibit the growth of fungi, 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 which affect their antifungal properties [5, 17]. Biological activity of honey is mainly attributed to the phenolic compounds particularly flavonoids which are well known for their antimicrobial action, ability to denature proteins and generally classified as surface active agents [8]. The fungal growth inhibition of honey is not related to the presence of sugar in the culture medium but their presence in honey leads to the high osmolality that enhance antimicrobial activity [1]. Flavonoid profiles were found to vary with phytogeographical origin and not related to entomological origin [3].
Apiculture is the practice of keeping honey bees (*Apis indica*, *Apis mellifera*) for its pollination support tool and its products such as honey, wax, propolis, pollens. Honey from both multiflower/wild and apiculture sources from both monoflowers (*Acacia*, citrus, Garanda, beactor, is farmed and used in Pakistan. The studied Pakistani honeys were unifloal (*Acacia*) and multifloral/wild (several mountain flowers) origin, while Arabian and German honeys were multifloral respectively. In order to advise honey as antifungal drug, there is need to evaluate the antifungal activity of honey from different phytogeographic regions around the world and their comparison with commercial antifungal drugs.

2. Materials and methods

2.1 Collection of samples

Popular brands of Pakistani as well as international honey were collected from registered supper store of Islamabad and Peshawar cities of Pakistan. All the samples were purchased in sterile containers from retailers and coded as MH, SH, LH and AH (Marhaba Honey, Sulman Honey, Lenganese Honey and Arabain Honey, respectively). The samples were stored immediately in the refrigerator at about 4 °C until proceed for analysis and studies were performed during 2015 for six months. The microorganisms used were clinical isolates obtained from the laboratory of the Department of Biotechnology, COMSATS institute of information technology, Abbottabad. The microorganisms were *A. niger* and *P. chrysogenum*. All the isolates were sub-cultured on fresh sterile Sabouraud dextrose agar (SDA) slants and stored in refrigeration. Sabouraud dextrose agar was purchased from Merck. The antifungal drug Fluconazole was purchased from market.

2.2 Sample preparation

The purity of honey samples were checked by streaking on blood agar plates after filtration through a sterile mesh. Un-contaminated samples were used for the study. 10, 20, 30% (v/v) of selected honey samples were prepared by dilution with sterile distilled water (SDW).

2.3 Preparation of media and inoculum

Sabouraud dextrose agar (SDA) was prepared by dissolving 65 gm of agar in 1 L of SDW and its pH was adjusted in the range of 5.4 to 5.6. The 5 to 10 days old test moulds were first washed with sterile normal saline (0.9%) and then filtered through sterile cotton wool to obtain the spore suspension. Each test organism was then standardized to 10^6 Cfu (0.5 McFarland unit) spores per ml spectrophotometry. The viability of each isolate was confirmed by inoculation on SDA plates and counting the number of cfu/ml.

2.4 Estimation of antifungal activity

The antifungal activity was determined by agar well diffusion method [4]. 15 ml sterile SDA was dispensed into sterile petri dishes and allowed to solidify. 100 µl of the inoculum was poured onto the agar plate by micropipette and spread with glass rod spreader under sterile conditions. Wells (6 mm diameter) were cut into the agar using sterile cork borer and 0.1 ml of different concentrations of honey was applied in each well. Similarly, for control plates, wells were filled with SDW as negative while Fluconazole was used as positive control.

2.5 Statistical Analysis

Comparison between means was conducted by using Analysis Variance (ANOVA), Minitab Software.

3. Results

The antifungal effect of the four different honey brands was evaluated against two fungal strains i.e. *A. niger* and *P. chrysogenum*. Amongst these four brands best and highly significant activity was shown by undiluted (100%) concentrations of all samples but the most active honey brand was LH against both fungal strains. The results of each brand at different concentration levels against both *A. niger* (AN) and *P. chrysogenum* (PC) is given in details in table 1. SH brand of honey was found the lowest active honey brand in comparison with other three brands against both tested fungal strains.

Table 1: Antifungal effect of honey samples against *A. niger* (AN) and *P. chrysogenum* (PC)

Fungal strain		**MH**	**SH**	**LH**	**AH**
Aspergillus niger (AN)	**Percentage concentration (w/v)**	Diameter of zone of inhibition (mm)			
	10	12	11	14	15
	20	14	13	17	17
	30	17	17	21	20
	100	21	20	22	21
Penicillium chrysogenum (PC)	**Percentage concentration (w/v)**	**MH**	**SH**	**LH**	**AH**
	10	15	13	18	17
	20	16	15	20	19
	30	20	19	23	22
	100	23	21	24	23

The results obtained from stranded drug i.e fluconazole and the 4 honey brands are compared in figure 1. It is confirmed that the best activity was found against PC amongst all the series of assays by each brand of honey. While the effect of fluconazole is almost similar against PC and AN strains.

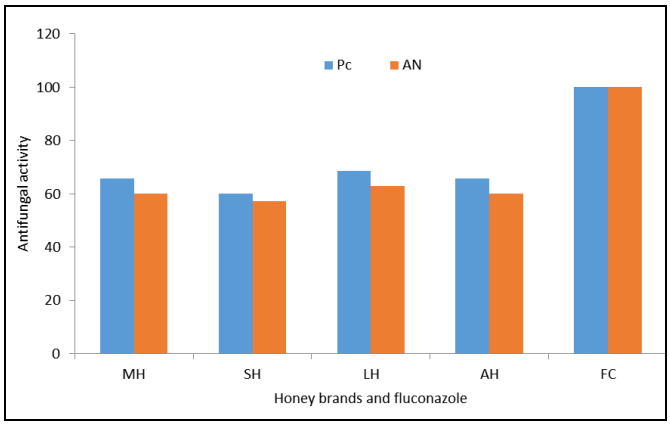

Fig 1: Comparison of antifungal activity of different brands of honey with fluconazole

4. Discussion

The honey samples used in this study showed different levels of antimycotic activity against the tested fungal isolates, namely, *A. niger, P. chrysogenum*. The susceptibility of some of these fungi to honey is of significance, as most of the fungi have been implicated in cases of immuno-compromised patients who frequently develop opportunistic infections[18].The antifungal activity of different brand of honey originating from different floral sources of Pakistan, Germany and Arabian countries against the antimycotic fungal stain *A. niger (*AN) *and P. chrysogenum* (PC) were reported in (Table 1) and compared with commercial antifungal drug fluconazole (Figure 1).The selected fungal species are common disease causing pathogen in Pakistan [18].

Among the four tested honey brands, LH honey from Germen origin showed the highest antifungal activity of 69 and 63% against the two tested fungal isolates *PC* and *AN* as compared to fluconazole. While honey brands from Pakistan origin, MH and SH exhibited 60-65.7% and 57.1- 60% against *PC and AN* respectively. The selected honey samples show greater growth inhibition against *P. chrysogenum* as compared to *A. niger*. The results indicate that zone of inhibition depends on the type and test organism. Honey has already been inhibited the growth of another *Aspergillus sp. (Aspergillus flavus)* by reducing the aflatoxin B1 and B2 levels [16]. The antifungal activity of honey was due to plant-derived antifungal substances (Phenolic acid, flavonoids and other antifungal compounds), hydrogen peroxide and due to high osmolarity [6, 19]. Honey flavonoids show high antifungal activity due to their relative lipophilic properties, they may reach a possible intracellular site of action without compromising membrane-associated functions.

Antimicrobial activity and antifungal activities from different floral sources of honey were different and can be compared to the study of Lee *et al.,* who isolated the phenolic compound form honey and screened for antimicrobial activity so means that honey is antimicrobial due to these phenolic compound [15].

Multifloral honeys have generally more inhibitory effect on fungal growth than single plant origin like *Acacia* honey. The average zone of inhibition of multifloral honey is significantly larger than Acacia honey. This is in agreement with the findings of [12]. In fact antimicrobial activity of honey depends on its osmolarity, acidity, H_2O_2 content, nectar, pollen and propolis [20]. Irish *et al*, reported that hydrogen peroxide content in honey greater antifungal effect [9]. While Estevinho *et al* [7], described that antifungal properties of the honey are not due to sugar content and it might be attributed to the components of plant origin [7]. Our findings provide evidence for the contribution of floral origin to antifungal activity. In addition, the honey samples appeared to deserve further investigation of to their individual active components, which may be use as medicinal ingredients.

Different concentrations of honey showed significant variation in the average inhibition zone diameters produced by different fungal stains at $P < 0.05$. The honey brand LH show 68.57% and 62.8% susceptibility in undiluted honey sample (100 %) while 51.4% and 40% susceptibility at 10% dilution of honey sample against *Aspergillus niger (*AN) *and Penicillium chrysogenum* (PC) respectively. The mean antifungal activity of four tested honey brands deceased 1.5 and 1.6 fold with dilution against the *PC* and *AN*. Little antifungal activity was observed at <2% honey dilution. This variability in the antifungal effect of honey samples was also observed by several other authors [2, 7, 11]. The significant antifungal activity in diluted honey samples was also due to hydrogen peroxide produced by enzymatic activity. Further in-vivo studies required to confirm this antifungal activity for clinical trial.

5. Conclusion

Honey has shown potent antifungal activity against tested fungal strains. The data suggest that the components in the honey samples are responsible for the observed *in vitro* antifungal properties. Thus, the Germany and Arabian honey has the potential to prevent the growth of a wide range of possible human pathogens which include some species of moulds and yeasts. Further research is needed to assess the efficacy of honey as an inhibitor of candidal growth in clinical trials, especially in the treatment of patients with candidiasis.

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