

POTENTIAL ANTIFUNGAL ACTIVITY OF DIFFERENT HONEY BRANDS FROM PAKISTAN: A
QUEST FOR NATURAL REMEDY

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Abstract

Background: Besides a wholesome food, honey is known for its therapeutic implications. We investigated the antifungal activity of five honeys of different types from Pakistan against various pathogenic fungal strains. Antifungal properties of processed and non-processed honey were determined.

Materials and Methods: The antifungal assay of honey was carried out against *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigates*, *Alternaria alternata*, *Fusarium solanai*, *Microsporium canis*, *Penicillium funiculosium* and *Rhizopus solanai*. Different dilutions of honey samples were used (20%, 50%, 70%, 90% and 100% w/v so as to find out the minimum effective concentration of each honey type.

Results: It was observed that all of the honey samples were highly active, with percent inhibition range of (3-81%) for *Aspergillus niger*, (2-82%) for *Aspergillus flavus*, (1-76%) for *Aspergillus fumigates*, (1-84%) for *Alternaria alternata*, (2-67%) for *Fusarium solanai*, (1-87%) *Microsporium canis*, (2-78%) *Penicillium funiculosium* and (1-86%) for *Rhizopus solanai*.

Conclusion: The study of Pakistani honey brands reveals that they possess a substantial antifungal nature. Therefore, they may be used in curing fungal infections along with antifungal drugs as a cheaper alternative natural remedy with no side effects.

Key words: Honey, Antifungal activity, Pathogenic fungi, Natural remedy

Introduction

Fungal diseases represent a significant burden on the healthcare of developing and underdeveloped regions. Antibiotics were effectively used in the previous years to treat fungal diseases but with increase in drug resistance to commercially available anti-fungal drugs, the infectious agents are becoming increasingly difficult to treat. The presently available treatments may no longer be effective due to drug resistance (Pfaller, 2012). As a result, there is a need to develop effective and cheap antimicrobials from natural sources. Modern treatment strategies are sometimes accompanied by undesirable side-effects that can increase burden on the already effected person (Khalil et al; 2013). Besides drug resistance, other issues like cost and affordability exist.

Aspergillus is demarcated as a group of conidial fungi. In around 250 species of aspergillus 64% have no known sexual state. Some species of *Aspergillus* are known to cause severe human and animal diseases (Samson et al; 2011). *Aspergillus fumigatus* and *Aspergillus flavus* produces aflatoxin which has both carcinogenic and toxic properties that causes allergic diseases while other species are potential agricultural pathogens (Varga et al; 2007; Handwerk, 2005). *Alternaria alternata* is known as a causative agent for leaf spot, rots, blights any many other diseases in more than 380 plant host species (Wiest et al; 1987). *Fusarium solani* also infrequently causes many fungal infections. *Microsporium canis* is associated with infection of upper, dead layers of skin in humans as well as animals (Kane et al; 1997). *Penicillium*

funiculosum is a plant pathogen which infect pineapples. *Fusarium solani* infects soybeans and occasionally causes Sudden Death Syndrome (SDS)(Das and Ranganathan, 2012).

Due to side effects, lack of efficacy and increasing resistance the quest for new anti-fungal agents has gained pace and scientific community has shown keen interest in honey due to its recently discovered potential anti-fungal effects. Honey is used for healing and nourishing since time immemorial. Now, honey has a global market and produced on massive scales all over the world (Aurongzeb and Azim, 2011). Honey is a brown liquid or pale yellowish, translucent, thick and syrupy which is produced in the combs by *Apis mellifera* (Sheikh et al; 1995). It is a combination of organic acids, carbohydrates, waxes, aromatic acids, enzymes, vitamins and entities with hormonal features (Bogdanov et al. 1999; Chen et al; 2011; Coco et al; 1996).It is investigated that honey possess about 181 substances (Richard, 2009). Laevulose and Dextrose are in elevated amount (White and Doner, 1980) while 30 organic acids are also recovered (Mato et al; 2003). Because of its elevated osmolality, honey possesses a broad spectrum antimicrobial properties (Wahdan 1998; Ceyhan and Ugur, 2000).Wide variety of honey when diluted produces hydrogen peroxide due to activation of glucose oxidase, which is responsible for oxidation of glucose to gluconic acid and hydrogen peroxide. Hydrogen peroxide is regarded as the major contributor to anti-microbial activity of honey. It has been observed that variation in concentration of hydrogen peroxide in honey directly correlates with its antimicrobial activity. Organic acids are other chemicals which are thought to be the cause of antimicrobial nature of honey as the organic acids can be the source of limiting the microbial growth.(Ceyhan and Ugur, 2000). The chemical composition of honey shows variation with changes in climate, environment, bee type and floral origin (Kántor et al; 1999). Therefore, it was imperative to conduct experiments to document the antifungal characteristics of various honey types on regional basis. Therefore, the present research was designed to find the antifungal nature of the processed honey products in market and non-processed honey of Pakistani origin

Collection of test samples

Five (5) samples of honey (Ume-e-Shifa, Hamdard, Azka, Marhaba and raw honey) were purchased from the local market of Peshawar city, Pakistan. Dilutions (20%, 50%, 70%, 90% and 100% w/v) were made in double deionized water to investigate the minimum effective concentration. The test samples were stored at 4°C.

Collection of fungal strains

Fungal strains were collected from the plant pathology lab Agriculture University Khyber Puktoonkhwa and Centre of Biotechnology and Microbiology, University of Peshawar.

Fungal strains

Sterilized slants Potato Dextrose Agar (PDA) (Code: CM0139, Oxoid) were used for the collection of fungal strains followed by incubation for 6 days at 27°C. Subsequent sub culturing was carried out to maintain the fresh spores. Normal saline was used to dilute the cultures. Master cultures were stored at 4°C. Antifungal activity was determined against. *Curvilaria lunata*, *Penicillium funiculosium* *Aspergillus flavus*, *Aspergillus fumigates*, *Aspergillus niger*, *Candida albicans*, *Alternaria alternata*, *Fusarium solanai*, *Rhizopus solanai* and *Microsporium canis*.

Antifungal assay

To determine the antifungal activity, agar tube dilution assay was used as described by (Mahmood et al. 2012). The experiment was performed in triplicate. Briefly, 100 µl of the test sample was added to the prepared PDA media and was allowed to solidify in a slanting position. Spores from the cultured fungal strains was transferred to a fresh PDA slant with a sterilized nichrome wire loop 4 mm (Master micro) and then incubated (Mettler) for 7days at 27°C. The inoculation was done at the base of the slant. Metronidazole (5 µg/ml) served as positive control while the slant deprived of test sample was used as negative control. Antifungal activity was determined by measuring the percent inhibition as

$$\text{Percent inhibition} = 100 - \frac{\text{growth in sample}}{\text{growth in negative control}} \times 100$$

Results

Impressive antifungal activity of test samples at higher concentrations was observed. The activity of Marhaba and Hamdard showed relatively good activity. Generally, all of the test samples were active, with a percent growth inhibition range of (3-81%) for *Aspergillus niger*, (2-82%) for *Aspergillus flavus*, (1-76%) for *Aspergillus fumigates*, (1-84%) for *Alternaria alternata*, (2-67%) for *Fusarium solanai*, (1-87%).

Table 1: Antifungal activity (Percent growth Inhibition) of honey at 20% (W/V) concentration.

Test fungal strain	Honey Brand (percent inhibition)					
	Marhaba	Azka	Ume Shifa	Hamdard	Crude honey	Positive control MTZ (5 µg/ml)
<i>Aspergillus flavus</i>	-	-	-	-	2%	65%
<i>Aspergillus niger</i>	3%	-	-	4%	-	73%
<i>Aspergillus fumigates</i>	1%	-	2%	2%	-	62%
<i>Alternaria alternata</i>	2%	-	-	-	1%	67%
<i>Candida albicans</i>	-	-	-	-	-	54%
<i>Curvilaria lunata</i>	1%	-	1%	4%	-	44%
<i>Fusarium solanai</i>	4%	2%	3%	5%	-	62%
<i>Microsporium canis</i>	3%	4%	1%	3%	-	64%
<i>Penicillium funiculosium</i>	2%	-	3%	2%	-	65%
<i>Rhizopus solanai</i>	1%	-	-	-	-	78%

Table 2: Antifungal activity (Percent growth Inhibition) of honey at 50% (W/V) concentration.

Test fungal strain	Honey Brand (percent inhibition)					
	Marhaba	Azka	Ume Shifa	Hamdard	Crude honey	Positive control MTZ(5 µg/ml)
<i>Aspergillus flavus</i>	25%	22%	36%	43%	21%	66%
<i>Aspergillus niger</i>	37%	31%	23%	34%	24%	72%
<i>Aspergillus fumigates</i>	22%	25%	28%	33%	19%	64%
<i>Alternaria alternata</i>	43%	33%	36%	29%	26%	67%
<i>Candida albicans</i>	3%	2%	10%	10%	4%	54%
<i>Curvilaria lunata</i>	3%	1%	4%	8%	2%	44%
<i>Fusarium solanai</i>	6%	3%	4%	9%	5%	62%
<i>Microsporium canis</i>	10%	6%	6%	6%	4%	64%
<i>Penicillium funiculosium</i>	32%	34%	33%	31%	30%	65%
<i>Rhizopus solanai</i>	39%	35%	40%	41%	40%	78%

Table 3: Antifungal activity (Percent growth Inhibition) of honey at 70% W/V concentration.

Test fungal strain	Honey Brand (percent inhibition)					
	Marhaba	Azka	Ume Shifa	Hamdard	Crude honey	Positive control MTZ (5 µg/ml)
<i>Aspergillus flavus</i>	56%	43%	55%	57%	51%	65%
<i>Aspergillus niger</i>	57%	35%	43%	45%	40%	72%
<i>Aspergillus fumigates</i>	53%	47%	52%	61%	48%	64%
<i>Alternaria alternata</i>	64%	45%	56%	55%	50%	66%
<i>Candida albicans</i>	20%	14%	25%	22%	19%	53%
<i>Curvilaria lunata</i>	15%	12%	23%	27%	20%	44%
<i>Fusarium solanai</i>	23%	15%	17%	28%	17%	62%
<i>Microsporium canis</i>	58%	32%	59%	67%	44%	65%
<i>Penicillium funiculosium</i>	43%	57%	57%	51%	49%	65%
<i>Rhizopus solanai</i>	57%	56%	57%	45%	52%	78%

Table 4: Antifungal activity (Percent growth Inhibition) of honey at 90% W/V concentration.

Test fungal strain	Honey Brand (percent inhibition)					
	Marhaba	Azka	Ume Shifa	Hamdard	Crude honey	Positive control MTZ(5 µg/ml)
<i>Aspergillus flavus</i>	68%	62%	65%	74%	64%	66.5%
<i>Aspergillus niger</i>	75%	70%	75%	63%	66%	71.4%
<i>Aspergillus fumigates</i>	71%	69%	81%	76%	72%	64.5%
<i>Alternaria alternata</i>	71%	60%	65%	66%	61%	66.3%
<i>Candida albicans</i>	45%	42%	45%	54%	47%	53%
<i>Curvilaria lunata</i>	53%	43%	69%	74%	54%	45%
<i>Fusarium solanai</i>	64%	54%	64%	78%	58%	63%
<i>Microsporium canis</i>	79%	71%	80%	83%	73%	65%
<i>Penicillium funiculosium</i>	68%	60%	65%	57%	63%	64%
<i>Rhizopus solanai</i>	56%	61%	74%	68%	65%	76%

Table 5: Antifungal activity (Percent growth Inhibition) of pure honey.

Test fungal strain	Honey Brand (percent inhibition)					
	Marhaba	Azka	Ume Shifa	Hamdard	Crude honey	Positive control MTZ (5 µg/ml)
<i>Aspergillus flavus</i>	81%	70%	74%	82%	71%	65%
<i>Aspergillus niger</i>	81%	71%	71%	78%	75%	72%
<i>Aspergillus fumigates</i>	83%	73%	76%	81%	75%	64%
<i>Alternaria alternata</i>	75%	78%	71.5%	84%	73%	66%
<i>Candida albicans</i>	61%	54%	67%	65%	55%	53%
<i>Curvilaria lunata</i>	65%	54%	75%	86%	61%	45%
<i>Fusarium solanai</i>	82%	68%	78%	85%	68%	63%
<i>Microsporium canis</i>	87%	79%	83%	79%	79%	65%
<i>Penicillium funiculosium</i>	78%	70%	69%	76%	71%	65%
<i>Rhizopus solanai</i>	71%	65%	84%	86%	75%	78%

Microsporium canis, (2-78%) *Penicillium funiculosium* and (1-86%) for *Rhizopus solanai*. Table 1 shows percent inhibition of various honey samples at 20% (w/v) concentration which depicts low activity by all samples. Generally slight to moderate antifungal activity was found at 50% (w/v) (Table 2), while relatively higher activities were recorded at 70% w/v (Table 3). Highest activities were observed at 90% (w/v) and 100% (w/v) concentration as mentioned in Tables 4 and 5 respectively. All the tabulated results are the average of the triplicate experiment.

Discussion

The present study presents the antifungal properties of honey at different concentrations. Good antimicrobial activities of honey is not a matter a surprise as earlier reports indicated good antimicrobial potential for honey (Khalil et al; 2013; Khalil et al; 2014) however less data are available on the antifungal properties of honey. In the study, antifungal properties of honey were determined at different concentration. We found that the antifungal characteristics of honey are enhanced with increase in concentration of honey. At low concentration, 20% (w/v) “Hamdard” test sample was the most effective in terms of reducing the growth of fungal mycelia. Other test samples showed less antifungal properties at low concentration. “Hamdard” was the most effective against *Fusarium solanai* with percent inhibition of 5 %, followed by “Marhaba” with 4 % as indicated in Table 1. At 50 % (w/v), the antifungal features of the test samples have been significantly improved especially against *Aspergillus* species. “Hamdard” showed more effectiveness against *Aspergillus flavus* with percent inhibition of 43 %, while Marhaba also showed percent inhibition of 43% against *Alternaria alternata* (Table 2). At 70% w/v concentration, moderate to high inhibitory activity was observed. *Microsporium canis* was effectively inhibited to 67% at 70% (w/v) concentration, however, *Curvilaria lunata* and *Candida albicans* were among the least inhibited fungi (12%-27%) and (14%-25%) respectively. Highest antifungal activities were recorded at higher concentrations as tabulated in table 4 and 5 significantly high activity was observed at 90% (w/v) concentration and pure honey samples as mentioned in table 4 and 5 respectively due to presence of high concentrations of biomolecules.

Earlier studies have indicated different responses by *Candia albicans* to honey. Some studies indicated that *Candia albicans* was resistant to honey (Moussa et al. 2012) while some reports conclude the inhibition of *Candida albicans* at higher concentrations (Al-Waili 2001). In present study, it was observed that *Candia albicans* is susceptible to honey at higher concentrations.

Honey is used for the treatment of fungal diseases from ancient time. There are many factors which can affect the ability of honey for its anti-fungal activity (Jessup et al; 2000; Gupta et al; 1994). The floral origin of honey plays a very important role in the biochemical components of honey and, therefore, also effects the antimicrobial potential. Honey collected from the different phytogeographic regions indicated change in the inhibitory potential against yeast (DeMera and Angert, 2004). The biological activity of honey is mainly due to phenolic compounds (Estevinho et al; 2008). It is also reported that the mechanism by which honey inhibits fungi have no correlation with the osmotic shock due to sugar presence in media (Diekema et al; 2005).

Variation in these studies can be attributed to the floral origin of honey and other physical and chemical parameters such as temperature, methods of study etc.

Conclusion

The beneficial and healing characteristics of honey are already established from ancient time while it is also mentioned in Holy Scriptures therefore has a prominent status in folklore medicines. To date, the antifungal drugs are relatively less in number and therefore expensive. Current study unveils the potential of honey from Pakistan as source of antifungals which can become a subject of future studies and trials. Moreover, this study also indicates that the processing methods of honey can also have an impact on the antifungal properties. However, further research needs to be carried out to discover the role of different processing methods on the antifungal properties of honey.

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Authors' Contributions

Ali Talha Khalil for carrying out the experimental work of the project, Kafeel Ahmad and Zabta Khan Shinwari for supervising the work, Ramla Somayya, Syed Ali Raza Shah and Faisal Nouruz Khan for writing the paper.

Conflicts of Interest: The authors declare that this research presents no conflicts of interest.

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