Ur Rahman et al. | J Pure Appl Microbiol | 16(2):1147-1153 | June 2022
Article 7474 | https://doi.org/10.22207/JPAM.16.2.42
Published Online May 31, 2022
Print ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Antifungal Activity of Honey Samples from Khyber Pakhtunkhwa (Pakistan) as affected by Botanical Origin

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Abstract

The present study evaluated the antifungal activity of honey samples collected from different locations at Khyber Pakhtunkhwa (KPK, Pakistan). Disc diffusion method was used to test the antifungal potential of twenty-one (branded, unbranded, and natural comb) honey samples from the different botanical origins at different concentrations (undiluted, 10%, 30%, and 50%, w/v) against *Candida albicans* and Rhodotorula species. Branded, unbranded, and natural comb honey samples generate different inhibition zones (4-13 mm, 5-15 mm, and 8-17 mm) against Rhodotorula species. *Candida albicans* showed resistance for all tested honey samples. Minimum inhibitory concentration (MIC) against *Candida albicans* and Rhodotorula species were 53.33%-88.12% and 1.76%-90.22% for branded, 61.3% - 93.8% and 9.90% - 95.5% for unbranded, and 67.1%-96.8% and 6.39%-98.8% for natural comb honey. In conclusion, natural comb honey from Khyber Pakhtunkhwa may have antifungal therapeutic potential and could be a useful source for generating functional food.

Keywords: Bee products, biological activity, *Candida albicans,* Rhodotorula sp

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(Received: December 9, 2021; accepted: March 31, 2022)

Citation: Ur Rahman K, Ullah I, Ullah K, et al. Antifungal Activity of Honey Samples from Khyber Pakhtunkhwa (Pakistan) as affected by Botanical Origin. *J Pure Appl Microbiol.* 2022;16(2):1147-1153. doi: 10.22207/JPAM.16.2.42

INTRODUCTION

Honey is viscous nectar, collected by the honey bees, combined and converted with a definite substance, then stored to mature and ripen in honeycomb.[1-5] Honey contains 25 different carbohydrates, mainly glucose and fructose.[6,7] Before discovering bacteria, which is a causal agent of infection, honey has been for several wound infections treatment.[4, 8-10]

Antifungal drugs are essential and encourage research on new natural product chemotherapeutic agents.[11-13] Using the agar well diffusion method, different concentrations of honey (10%, 25%, and 50% by mass per volume) showed antifungal activities against Penicillium crustosum, Penicillium expansum, Penicillium griseofulvum, Penicillium raistrickii, and Penicillium verrucosum.[14] Honey inhibitory activity against microorganisms is due to hydrogen peroxide (H_2O_2), released by the oxidase enzyme added by bees to nectar. Rates of H_2O_2 production by glucose oxidase in honey vary greatly and increase disproportionately with different degrees of honey dilution. The rate of H_2O_2 production per milliliter of the honey solution decreased at a higher concentration of honey.[15] Furthermore, the antioxidants activity of honey protects wound tissues from oxygen radicals and may be produced by H_2O_2. [16] Antimicrobial activity of the honey is due to its high acidity, and high osmotic concentration. Honey osmotic effect due to its high sugar content also inhibits microbial growth as the sugar molecules tie up water molecules and induce insufficient water for the microorganism's growth.[2, 4, 17, 18] Data regarding the antifungal activity of honey from various countries demonstrated different results, an aspect of accessibility of pollens or changing of location and types.[12, 13, 19] Nevertheless, up to date, no articles addressed the comparative assessment of antifungal activities of honey found in Khyber Pakhtunkhwa (KPK, Pakistan). The present study aimed to evaluate the antifungal activities of natural comb and farms honey samples from KPK (Pakistan).

MATERIALS AND METHODS

Samples collection

A total of twenty-one samples of honey, including natural honey beera (ziziphus), bekkarr (justice), granda (carissaopaca), palosa (acacia), sperkay (trachyspermum), big bees honey, and small bees honey, were directly collected from the honeycomb. In addition, branded honey samples (Al-Hayat, langnese, marhaba, paksalman, qarshi, versatile, young's) and unbranded honey samples such as sperkay (trachyspermum), bekkarr (justice), beera, (ziziphus), granda (Carissa opaca), palosa (acacia), big and small bees honey were purchased from the local market at KPK. The samples were kept at 4°C for further analysis.

Honey solution preparation

Sample solutions were prepared at different concentrations in distilled water (10%, 30%, and 50%, w/v), incubated at 37°C for 30 min using a shaking water bath in the absence of light.

Tested organisms and yeast strains

Candida albicans (American Type Culture Collection, ATCC Code 90028) and Rhodotorula sp. (PCSIR 001) were obtained from Food Microbiology Laboratory, PCSIR Laboratories Complex, Peshawar, Pakistan.

Preparation of inoculum suspension

Candida albicans and Rhodotorula sp. were maintained on Sabouraud dextrose agar (SDA; BioMerieux, Marcy 1Etoile, France) at 4°C. Subcultures of each species were achieved in the same media for 48 h at 35°C before each experiment. The stock of inoculum suspension was prepared in 5 mL of sterile saline water (0.85%). The suspension was accustomed to 0.5 McFarland turbidity standards. Dilution of the suspension was further sub-cultured on SDA to measure the quantity of cfu/mL. The adjusted inoculums were 1 x 107 cfu/mL

Antifungal activity

The antifungal activity of honey samples was evaluated using an agar disc diffusion method against tested organisms[20]. Fresh culture suspension (100 µL) of the tested microorganisms was spread on respective media Sabouraud dextrose agar (SDA) plates. The concentration of culture was 1×10^7cfu/mL. For screening, sterile filter paper discs (5 mm diameter) were impregnated with 10 µL of honey equivalent to 0.1 mg of honey after being placed on the surface of inoculated media agar plates. The plate was placed at 4°C for 2 h before being incubated under a favorable condition at 37°C for 24 h. Around the disc, a clear inhibition zone (diameter in mm)

indicates the antifungal activity of the honey. An equivalent amount of water was set up as controls. Honey samples were inoculated separately on standards nutrients media with no test organisms to evaluate the possible contamination. The results of all the samples were determined in triplicate with a calculated standard deviation.

Statistical Analysis

Means and standard deviations were calculated for three independent determinations for each variable using the SPSS program.

Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) determination was made by incorporating various honey dilutions (10%, 30%, 50%, w/v) into the nutrient broth to examine their competence against Candida albicans and Rhodotorula sp. Up to 0.2 mL of the cell suspension was inoculated into 4 mL volume of honey concentration in a test tube, while inoculation of 4 mL volume of nutrient broth with 0.2 mL of the cell suspension served as control. The optical density was determined and recorded in a spectrophotometer at 620 nm before incubation (T0), after which the cultures were incubated for 24 h in the dark at 37 °C with constant shaking to prevent adherence and clumping. After 24 h of incubation, the optical densities were again determined and recorded (T24). The optical density for each replicate at T0 was subtracted at determined using the formula: Percentage inhibition = 1 - (OD test/OD control) x 100.

Where the resulting measurement recorded a negative inhibition value (growth promotion), this was reported as stimulation using the formula:

Percentage inhibition = (OD test/OD control) x 100

RESULTS

Antifungal activities of the branded, unbranded, and natural honey samples from Khyber Pakhtunkhwa (Pakistan) were studied. Undiluted and diluted honey samples (10%, 30%, and 50%, w/v) showed statistically significant antifungal activities against Rhodotorula sp. compared to Candida albicans (Table 1). The zone of inhibition of growth for Rhodotorula sp. surrounding branded honey samples (50%) was significantly larger (13 mm) for versatile honey

Table 1. Antifungal activities of branded honey samples against *C. albicans* and Rhodotorula sp

Test species	Code	Concentration	Zone Diameter Inhibition (mm) at different dilution of honey							
			Marhaba	Qarshi	Versatile	Al-Hayat	Young's	Paksalman	Langness	
C. albicans	ATCC 90028	Undiluted	-	-	-	-	-	-	-	
		10%	-	-	-	-	-	-	-	
		30%	-	-	-	-	-	-	-	
		50%	-	-	-	-	-	-	-	
Rhodotorula	PCSIR 001	Undiluted	* 08±0.01	06±0.02	07±0.01	09±0.10	07±0.02	05±0.04	06±0.02	
		10%	07±0.02	06±0.02	08±0.02	05±0.03	04±0.01	06±0.04	04±0.01	
		30%	08±0.02	07±0.04	09±0.02	06±0.01	06±0.03	07±0.07	05±0.03	
		50%	11±0.07	12±0.10	09±0.03	13±0.13	08±0.05	11±0.12	07±0.03	

(-): not observed; * Results of the average of three replicates ± Standard Deviation.

Table 2. Antifungal activities of unbranded honey samples against *C. albicans* and Rhodotorula sp

			Zone Diameter Inhibition (mm) at different dilution of honey						
Test species	Code	Concen.	Big bees honey	Small bees honey	Beera (Ziziphus)	Palosa (Acacia)	Sperkay (Trachyspermum)	Bekerr (Justicia)	Granda (Carissa opaca)
C. albicans	ATCC 90028	Undiluted	-	-	-	-	-	-	-
		10%	-	-	-	-	-	-	-
		30%	-	-	-	-	-	-	-
		50%	-	-	-	-	-	-	-
Rhodotorula	PCSIR 001	Undiluted	*10±0.01	12±0.10	09±0.13	11±0.14	08±0.01	06±0.02	09±0.11
		10%	07±0.06	08±0.04	07±0.07	08±0.10	06±0.01	07±0.05	07±0.06
		30%	09±0.10	08±0.03	10±0.09	06±0.12	06±0.03	05±0.01	06±0.01
		50%	15±0.14	14±0.18	11±0.04	15±0.18	9±0.07	13±0.13	10±0.08

(-): not observed; * Results of the average of three replicates ± Standard Deviation.

Table 3. Antifungal activities of natural honey samples against *C.albicans* and Rhodotorula sp.

			Zone Diameter Inhibition (mm) at different dilution of honey						
Test species	Code	Concen.	Big bees honey	Small bees honey	Beera (Ziziphus)	Palosa (Acacia)	Sperkay (Trachyspermum)	Bekerr (Justicia)	Granda (Carissa opaca)
C. albicans	ATCC 90028	Undiluted	-	-	-	-	-	-	-
		10%	-	-	-	-	-	-	-
		30%	-	-	-	-	-	-	-
		50%	-	-	-	-	-	-	-
Rhodotrula	PCSIR 001	Undiluted	*12±0.08	10±0.04	10±0.03	13±0.08	11±0.01	14±0.13	12±0.02
		10%	09±0.06	10±0.07	09±0.09	11±0.15	08±0.04	10±0.10	09±0.07
		30%	08±0.11	09±0.04	12±0.02	13±0.01	10±0.16	ND	09±0.10
		50%	17±0.12	13±0.10	14±0.14	15±0.03	12±0.11	13±0.09	16±0.12

(-): not observed; * Results of the average of three replicates ± Standard Deviation.

Table 4. Percent of growth inhibition (MIC) of *C. albicans* and Rhodotorula sp. by branded honey samples

Honey sample	*Candida albicans*				Rhodotorula sp.			
	Concentration of sample				Concentration of sample			
	Undiluted	50% (v/v)	30% (v/v)	10% (v/v)	Undiluted	50%(v/v)	30%(v/v)	10%(v/v)
Marhaba	88.12	73.07	≥100	≥100	90.22	86.33	45.43	≥100
Qarshi	84.03	75.33	≥100	≥100	88.26	85.16	36.62	≥100
Versatile	81.44	77.42	≥100	≥100	83.21	80.17	27.13	≥100
Al-hayat	76.34	72.31	≥100	≥100	81.47	76.21	22.55	≥100
Young's honey	73.32	68.51	≥100	≥100	77.35	62.41	19.66	≥100
Pak-salman	70.21	64.55	≥100	≥100	72.61	65.10	16.22	≥100
Langness	69.42	53.33	≥100	≥100	69.46	56.44	11.76	≥100

MIC: Minimum Inhibitory Concentration.

Table 5. Percent of growth inhibition (MIC) of *C. albicans* and Rhodotorula sp. by unbranded honey samples

Honey sample	*Candida albicans*				Rhodotorula sp.			
	Concentration of sample				Concentration of sample			
	Undiluted	50% (v/v)	30% (v/v)	10% (v/v)	Undiluted	50%(v/v)	30%(v/v)	10%(v/v)
Big bees honey	93.88	89.45	≥100	≥100	95.55	93.08	54.46	≥100
Small bees honey	90.74	85.22	≥100	≥100	94.87	95.55	44.22	≥100
Beera	86.33	82.42	≥100	≥100	90.21	88.23	37.99	≥100
Palosa	83.75	77.58	≥100	≥100	85.44	87.64	25.58	≥100
Sperkay	78.90	70.69	≥100	≥100	81.73	78.37	19.21	≥100
Bekerr	75.43	67.60	≥100	≥100	73.42	67.74	15.66	≥100
Granda	73.47	61.39	≥100	≥100	70.77	69.33	9.90	≥100

MIC: Minimum Inhibitory Concentration.

while significantly lower (4mm) for young's honey at 10% dilution (Table 1). All other branded samples showed moderate values against the Rhodotorula sp. In unbranded honey, the zone of inhibition at 50% dilution for big bee honey was the highest (15 mm) against Rhodotorula sp., while Bekerr honey (30%) exhibited the lowest zone of inhibition (5 mm). The remaining honey samples showed moderate antifungal activities (Table 2). In natural comb honey, big bee's honey (50% dilution) displayed the maximum zone of inhibition (17 mm), while sperkay honey (10% dilution) showed the minimum (8 mm) zone of inhibition against Rhodotorula sp. (Table 3). In contrast, Candida albicans showed significant resistance against all tested honey samples.

The MIC of the tested branded honey sample was 53.33%-88.12%, and 11.76%-90.22% against Candida albicans and Rhodotorula sp., respectively (Table 4). MIC of the unbranded honey was 61.39%-93.88% for Candida albicans, and 9.90%- 95.55% against Rhodotorula sp. (Table 5). Natural comb honey exhibiting 67.19%-96.83% and 6.39%-98.87% MIC range against the two species of fungus (Candida albicans and Rhodotorula sp.), correspondingly (Table 6).

DISCUSSION

The incidence of fungal infections is growing worldwide.[12, 13] The severe nature of the infections is due to their drug resistance efficiency. Due to lack of efficacy, side effect, and/or resistance related to the existing drugs, hive products such as honey have been rediscovered for antimicrobial actions. We evaluated various honeyfrom KPK (Pakistan) efficacy against clinically isolated Candida albicans and Rhodotorula sp. The result showed considerable variations. Our study showed that the inhibitory actions of branded, unbranded, and natural comb big bee's honey samples (50%

Table 6. Percent of growth inhibition (MIC) of *C. albicans* and Rhodotorula sp. by natural honey samples

Honey sample	*Candida albicans*				Rhodotorula sp.			
	Concentration of sample				Concentration of sample			
	Undiluted	50% (v/v)	30% (v/v)	10% (v/v)	Undiluted	50%(v/v)	30%(v/v)	10%(v/v)
Big bees honey	96.83	92.43	≥100	≥100	98.87	91.21	57.76	≥100
Small bees honey	91.44	88.57	≥100	≥100	95.43	89.55	48.33	≥100
Beera	89.31	85.22	≥100	≥100	92.79	86.99	42.27	≥100
Palosa	85.56	79.95	≥100	≥100	88.36	82.44	37.76	≥100
Sperkay	83.78	74.39	≥100	≥100	83.43	77.21	31.65	≥100
Bekerr	77.48	70.28	≥100	≥100	76.27	71.55	25.44	≥100
Granda	75.66	67.19	≥100	≥100	72.77	64.45	6.39	≥100

dilution) against Rhodotorula sp. was 13 mm (Alhayat), 15 mm (Palosa), and 17 mm (Big bees honey). Honey from different phytogeographic regions varies to inhibit yeast growth, suggesting the importance of botanical origin in displaying antifungal activity.[21] In different kinds of honey, biological activity was attributed to their phenolic compounds, as their ability to denature proteins.[12, 13, 22] High sugar concentration in honey leads to high osmolarity and thus produces antimicrobial potential. Previous data reported that undiluted honey might inhibit the growth of many species of Rhodotorula sp., but there was no effect against C. albicans.[19] The MIC for branded, unbranded, and natural honey samples were comparatively assessed, and the result revealed thatMIC for natural honey was significantly lower (67.19%-96.83%) and (6.39%-98.87%) against C. albicans and Rhodotrula sp., respectively.

CONCLUSION

It could be concluded that the alterations in the antifungal activity of honey samples are directly related to their floral origin. The current study highlighted that natural honey might significantly inhibit the growth of a fungus and possibly be established as a topical antifungal agent. However, further research is needed to isolate and identify the active compounds from different honey samples of KPK and standardize their product for healthy living.

ACKNOWLEDGMENTS

The authors would like to thank Food Technology Lab, PCSIR, Peshawar, Pakistan for providing the research facilities.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

KUR and KU designed the study, collected the data and wrote the manuscript. AR, AH, SU and ZG analyzed the data. IU and MFR revised the study. All others read and approved the final manuscript.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

REFERENCES

1. Pohl P. Determination of metal content in honey by atomic absorption and emission spectrometries. *TrAC Trends in Analytical Chemistry*, 2009;28:117-118.
2. Sforcin JM, Bankova V, Kuropatnicki AK. Medical Benefits of Honeybee Products. Evidence-Based Complementary and Alternative Medicine, 2017; Article ID 2702106. doi: 10.1155/2017/2702106
3. Sateriale D, Scioscia E, Colicchio R, et al. Italian acacia honey exhibits lytic effects against the crayfish pathogens Aphanomycesastaci and Fusarium avenaceum. *Lett Appl Microbiol,* 2019;68: 64-72. doi:10.1111/lam.13085
4. Bouacha M, Benbouzid H. Antibacterial Properties of Honey from Different Algerian Regions against Staphylococcus aureus Strains from Wounds,

J. Pure Appl. Microbiol, 2020;14(1): 447-453. doi: 10.22207/JPAM.14.1.46

5. Roby MHH, Abdelaliem YF, Esmail AM, Mohdaly AAA, Ramadan MF. Evaluation of Egyptian honeys and their floral origins: phenolic compounds, antioxidant activities, and antimicrobial characteristics. *Environ Sci Pollut Res Int.* 2020;27(17):20748-20756. doi: 10.1007/s11356-020-08586-7
6. Bogdanov S, Jurendic T, Sieber R, Gallmann P. Honey for nutrition and health: A Review. *Journal of the American College of Nutrition,* 2008; 27:677-689.
7. Pajor M, Worobo RW, Milewski S, Szweda P. The Antimicrobial Potential of Bacteria Isolated from Honey Samples Produced in the Apiaries Located in Pomeranian Voivodeship in Northern Poland. *Int. J. Environ. Res. Public Health*, 2018;15: 2002.
8. Allen KP, Molan. The sensitivity of mastitis-causing bacteria to the antibacterial activity of honey. *New Zealand Journal of Agricultural Research,* 1997; 40:537-540.
9. Ahmed S, Sulaiman SA, Baig AA, et al. Honey as a Potential Natural Antioxidant Medicine: An Insight into Its Molecular Mechanisms of Action. *Oxidative Medicine and Cellular Longevity*. 2018;Article ID 8367846. doi: 10.1155/2018/8367846.
10. Baqer LK, Yaseen RT. The effect of whole honey bee venom (Apismellifera) on reducing skin infection of rabbits caused by methicillin resistant Staphylococcus aureus: An In vivo study, *J. Pure Appl. Microbiol.,* 2018; 12(4): 2111-2116. doi: 10.22207/JPAM.12.4.48
11. Zahoor M, Naz S, Sangeen N. Antibacterial, antifungal and antioxidant activities of honey collected from Timergara (Dir, Pakistan). *Pakistan Journal of Pharmaceutical Science,* 2014;27:45-50.
12. Shehu A, Ismail S, Rohin MAK, Harun A, Aziz AA, Haque M. Antifungal Properties of Malaysian Tualang Honey and Stingless Bee Propolis against Candida albicans and Cryptococcus neoformans. *J App Pharm Sci.* 2016;6 (2): 044-050.
13. Kolayli S, Can Z, Yildiz O, Sahin H, Karaoglu SA. A comparative study of the antihyaluronidase, antiurease, antioxidant, antimicrobial and physicochemical properties of different unifloral degrees of chestnut (Castanea sativa Mill.) honeys. *Journal of Enzyme Inhibition and Medicinal Chemistry.* 2016; 31: 96-104. doi: 10.1080/14756366.2016.1209494
14. Kacániová M, Vukobic N, Bobkoba A, et al. Antimicrobial and antiradical activity of Slovakian honeydew honey samples. *The Journal of Microbiology, Biotechnology and Food Sciences,* 2011; 1:354-368.
15. Bang L, Buntting MC, Molan P. The effect of dilution on the rate of hydrogen peroxide production in honey and its implications for wound healing. *The Journal of Alternative & Complementary Medicine*, 2003;9:267-273.
16. Frankel SG, Robinson MB. Antioxidant capacity and correlated characteristics of 14 unifloral honeys. *Journal of Apicultural Research*, 1998;37:27-31.
17. Basualdo C, Sgroy V, Finola MS, Marioli JM. Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. *Veterinary Microbiology*, 2007;124(3-4):375-381. doi: 10.1016/j.vetmic.2007.04.039
18. Pasupuleti VR, Noureddine D, Saad A, Abdelmelek M, Abdelkader B. Honey, Propolis, and Royal Jelly: A Comprehensive Review of Their Biological Actions and Health Benefits. *Oxidative Medicine and Cellular Longevity.* 2017;7:Article ID 1259510. doi: 10.1155/2017/1259510
19. Moussa A, Noureddine D, Saad A, Abdelmelek M, Abdelkader B. Antifungal activity of four honeys of different types from Algeria against pathogenic yeast: Candida albicans and Rhodotorula sp. *Asian Pacific Journal of Tropical Biomedicine*, 2012;2:554-557. doi: 10.1016/S2221-1691(12)60096-3
20. 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. *Journal of Medicinal Food,* 2011;14(1-2):128-134. doi: 10.1089/jmf.2009.0296
21. DeMera JH, Angert ER. Comparison of the antimicrobial activity of honey produced by Tetragoniscaangustula (Meliponinae) and Apismellifera from different phytogeographic regions of Costa Rica. *Apidologie ,* 2004;35(4):411-417. doi: 10.1051/apido:2004033
22. Estevinho ML, Afonso SE, Feás X. Antifungal effect of lavender honey against Candida albicans, Candida krusei and Cryptococcus neoformans. *Journal of Food Science and Technology,* 2011; 48(5):640-643. doi: 10.1007/s13197-011-0243-1