

## Full Length Research Paper

# Investigation of *in vitro* antifungal activity of honey

Anyanwu, C.U

Department of Microbiology, University of Nigeria, Nsukka, Nigeria. E-mail: [chudzizoma@yahoo.com](mailto:chudzizoma@yahoo.com).  
Tel: +2348037740104.

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The study was aimed at determining the antifungal activities of some honey samples obtained from different geographical locations in Nigeria against some fungal isolates by the method of agar well diffusion. The honey samples were examined for antifungal activity against *Aspergillus niger*, *Aspergillus flavus*, *Penicillium chrysogenum*, *Microsporium gypseum*, *Candida albicans*, and *Saccharomyces* sp. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the honeys were also determined. Results obtained reveal that the honey samples showed varying levels of inhibitory activity at various concentrations against the fungi tested with zones of inhibition increasing with increasing honey concentration. *M. gypseum* was the most sensitive of all the fungal isolates studied, while *C. albicans* was the least sensitive. The MIC and MFC values for the honeys ranged between 12.5 and 50% (v/v). The samples of honey used in the study showed broad spectrum and promising antifungal activity. Nigerian honey can serve as sources of antifungal substances for possible development of antifungal drugs for the treatment of fungal infections.

**Key words:** Honey, antifungal activity, moulds, yeasts, Nigeria.

## INTRODUCTION

Honey is a natural sweet substance produced by honeybees from the nectar of blossoms or from the secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which honeybees collect, transform, and combine with specific substances of their own, store, and leave in the honeycomb to ripen and mature (Alimentarius, 2001). It has been reported that honey contains carbohydrates, mainly fructose and glucose in addition to about 25 different oligosaccharides (Bogdanov et al., 2008).

Honey possesses powerful antimicrobial properties that can be utilized at low cost and at no risk (Fessenden, 2008). Various studies have reported the antimicrobial activity of honey (Agbaje et al., 2006; Patten et al., 2006; Vilma et al., 2007). Honey has been shown by Mekky (2007) to inhibit the growth of *Aspergillus flavus* and reduce aflatoxin B<sub>1</sub> and B<sub>2</sub> levels. The intrinsic properties of honey have been reported to affect the growth and survival of microorganisms by bacteriostatic or bactericidal actions (Iurlina and Fritz, 2005).

Although several *in vitro* studies have demonstrated the antibacterial properties of honey (Lusby et al., 2005; Kwakman, 2008; Hassanain et al., 2010), few have examined the action against fungi. The incidence of

fungal infections is increasing in both the community and hospital environments with several causative agents including yeasts with *Candida* spp., among the leading organisms (Abi-Said et al., 1997; Pfaller and Diekema, 2002) and filamentous fungi. Antifungal action of honey has been observed against the yeast *Candida albicans* and most species of *Aspergillus baumannii* and *Penicillium chrysogenum* (Willix et al., 1992) as well as all the common dermatophytes (Brady et al., 1997). The production and type of honey produced by honeybees is dependent on the natural vegetative flowers blooming in different seasons. Thus, the flowers from which bees gather nectar to produce honey may contribute to the difference in the antimicrobial activities. However, large variations in the *in vitro* antibacterial activity of various types of honey have been reported and thus hampered its acceptance in modern medicine (Kwakman, 2008).

Honey is farmed and used in several parts of Nigeria. Initially, local farmers harvested the honey from the wild but today, apiculture is being practiced in many parts of the country. Whereas there are large volumes of data on the biological activities of honeys from different parts of the world, including North America, Europe, Asia, Australia, and South Africa, there is a dearth of data on

the biological activities of Nigerian honeys. Although some researchers (Adesunikanmi and Oyelami, 1994; Omafuvbe and Akanbi, 2009) have reported the antibacterial activity of honey collected from Nigeria, information about the antifungal activity of Nigerian honey are still scarce. The aim of this study is to investigate the antifungal activity of honey samples from different parts of Nigeria.

## MATERIALS AND METHODS

### Collection of samples

Samples of commercial honey were used in this study. The samples, obtained from four different locations in Nigeria, namely, Nsukka (Enugu State), Owerri (Imo State), Anyigba (Kogi State) and Jos (Plateau State), were purchased in sterile containers from retailers and coded as NS, OW, AN, and JS, respectively. The samples were kept in the refrigerator at about 4°C until they were analysed within 48 h of collection.

### Honey preparation

Each sample was first filtered with a sterile mesh to remove debris. The samples were checked for purity by streaking on blood agar plates, and incubated overnight. Samples that showed uncontamination were used for the study. The honey sample was diluted with sterile distilled water to (% v/v): 20, 40, 60 and 80 and undiluted honey (100.0%).

### Microorganisms used

The microorganisms used were clinical isolates obtained from the laboratory of the Department of Microbiology, University of Nigeria, Nsukka, Nigeria. The microorganisms were *Aspergillus niger*, *A. flavus*, *P. chrysogenum*, *Microsporium gypseum*, *C. albicans*, and *Saccharomyces* sp. All the isolates were subcultured unto fresh sterile Sabouraud dextrose agar (SDA) slants and stored at refrigeration temperature.

### Preparation of inoculum

The cultures of each of 5 to 10 days' old test moulds and 24 to 48 h old yeasts were used. The test moulds were washed out with sterile normal saline (0.9%) and filtered through sterile cotton wool to obtain the spore suspension. Each test organism was then standardized to  $10^5$  spores or cells/ml by serial dilution. The viability of each isolate was confirmed by inoculation into SDA plates and counting the number of cfu/ml, where applicable.

### Determination of antifungal activity

The agar well diffusion method (NCCLS, 2002) on SDA was used for the fungi. 15 ml sterile SDA (LAB M) was dispensed into sterile Petri dishes and allowed to solidify. A micropipette was used to introduce 100 µl of the inoculum onto the agar plate, 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 in sterile distilled water (20, 40, 60, 80 and 100%, v/v) was applied in each well. Similarly, for control plates, wells were filled with sterile distilled water.

Three replicates were produced for each concentration for each fungus. Culture plates containing *C. albicans* and *Saccharomyces* sp. were incubated at 37°C for 24h while other culture plates containing the moulds were incubated at room temperature (30±2°C) for 72h. The assessment of antifungal activity was based on measurement of the diameter (mm) of the inhibition zone formed around the well after the incubation period.

### Determination of minimum inhibitory concentration (MIC)

The estimation of MIC of the honey samples was carried out using the modified method of Akinpelu and Kolawole (2004). A suspension of each of the organisms was adjusted to  $1.5 \times 10^5$  spores/ml or cfu/ml in Sabouraud dextrose broth. The honey samples were diluted with Sabouraud dextrose broth to give different final concentration regimes of 50, 40, 25, 20, 12.5, 10 and 6.25% (v/v). Honey-free media were used as negative controls. The diluted medium and honey mixture was then poured into sterile Petri dishes and allowed to set. The surface of the medium was allowed to dry before streaking with fungal inoculum. The experiment was carried out in triplicates. The plates were later incubated at room temperature for up to 72 h for the moulds and 37°C for 24 h for the yeasts, after which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration of honey that prevented the growth of the test microorganisms compared with that of the control. All MIC values are expressed in % (v/v).

### Determination of minimum fungicidal concentration (MFC)

The MFC of the honey samples was determined by the modification of the method previously described by Woods and Washington (1995). The MFC was determined by further subculturing from the plates which showed no visible growth in the MIC assay onto fresh sterile Sabouraud dextrose agar plates. The plates were incubated at room temperature until growth was seen in the growth control subculture. The MFC was, therefore, taken as the lowest concentration or highest dilution of honey that did not show any visible growth on the new set of SDA plates.

### Statistical analyses

Statistical analyses of data was carried out using analysis of variance. Statistically significant treatment differences were considered at  $P < 0.05$ .

## RESULTS AND DISCUSSION

The honey samples used in this study showed different levels of antimycotic activity against the tested fungal isolates, namely, *A. niger*, *A. flavus*, *P. chrysogenum*, *M. gypseum*, *C. albicans*, and *Saccharomyces* sp. (Table 1). 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 (Portillo et al., 2001). As a general rule, an antimicrobial is considered active against both bacteria and fungi, if the zone of inhibition was greater than 6 mm (Muhammad and Muhammad, 2005). In the present study, the honey sample designated NS generally showed the highest activity against all the

**Table 1.** Antifungal effect of honey samples.

Test organism	Honey sample																			
	NS					OW					AN					JS				
	Percentage concentration (w/v)																			
	20	40	60	80	100	20	40	60	80	100	20	40	60	80	100	20	40	60	80	100
	Diameter of zone of inhibition (mm)																			
<i>Aspergillus niger</i>	0	3	4	7	10	0	3	3	7	10	0	3	4	6	8	0	3	4	6	10
<i>Aspergillus flavus</i>	0	3	4	6	9	0	4	6	7	9	0	3	3	6	9	0	3	4	7	10
<i>P. chrysogenum</i>	0	3	3	7	12	0	3	4	6	9	0	3	3	7	10	0	3	4	7	9
<i>M. gypseum</i>	0	3	8	12	15	0	4	4	7	10	0	3	4	6	10	0	3	4	6	10
<i>Candida albicans</i>	0	0	4	6	10	0	3	4	6	8	0	3	3	5	7	0	3	3	5	8
<i>Saccharomyces</i> sp.	0	0	3	5	9	0	3	3	6	9	0	3	4	6	8	0	3	3	6	8

fungus isolates. This was followed by the sample designated OW and the least was observed in the AN sample. The ability of the honey samples to inhibit the growth of several fungal species is an indication of the broad spectrum antifungal potential of the honey which makes it a candidate for application as an antifungal agent.

At the highest concentration (100%), of the honeys, sample designated NS provided the largest average inhibition zone diameter of 15 mm against *M. gypseum*, while honey sample AN, also at 100% concentration, provided the lowest average inhibition zone diameter against *C. albicans* which was 7 mm (Table 1). The inhibition zone diameters shown by the fungal isolates indicated that the fungi had intermediate sensitivity to the honey samples as was reported by El-Shawaf and Gomma (2000).

There was significant difference ( $P < 0.05$ ) in the average inhibition zone diameters provided by different concentrations, especially at 100%, of each of the honey samples against the test fungal isolates. *C. albicans* was found to show the least sensitivity to the honey samples. Previous studies with different types of honey tested at

several concentrations ranging from 0.1 to 20% (Lusby et al., 2005) and from 25 to 100% (Omafuvbe and Akanbi, 2009) revealed that the growth of *C. albicans* was not inhibited by the honeys. On the other hand, Khosravi et al. (2008) reported that all honeys tested were able to produce complete inhibition of candidal growth with minimum fungicidal concentrations ranging from 29 to 56 %. The authors also observed varying sensitivities of six species of *Candida* to the anti-candidal properties of different honeys, thus emphasizing the variability in the antifungal effect of honey samples. Moreover, several reports (Al-Waili, 2005; Boukraâ and Bouchehrane, 2007; Estevinho et al., 2011) indicated variable sensitivity of *Candida albicans* to different honey samples.

Table 2 shows the results of the determination of minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) of the honey samples on the fungal isolates. Results showed that MIC and MFC values for the honey samples ranged between 12.5 and 50% with the least MIC value of 12.5% demonstrated against *M. gypseum* and least MFC value of 20% against

*A. flavus*. The highest MIC value of 40% was shown against *Saccharomyces* sp. while the highest MFC value of 50% was shown against *C. albicans*. Boukraâ and Bouchehrane (2007) had shown that two varieties of honey were effective against tested fungal strains with MIC of variety A being 42% (v/v) and for variety B, 46% (v/v) against *C. albicans*, while for *A. niger*, the MIC of variety A of honey was 51% (v/v) and for variety B, it was 59% (v/v).

The data obtained in this study suggest that all the honeys did not show the same level of activity against the fungi tested. The low susceptibility of some of the test organisms to the honey samples could be due to the emergence of resistant strains. In addition, several factors may influence the antifungal activity of honey. These factors include its physico-chemical properties, botanical origin and entomological origin. For example, DeMera and Angert (2004) reported that honey from different phyto-geographic regions varied in their ability to inhibit the growth of bacteria and yeasts, suggesting that botanical origin plays an important role in influencing the antimicrobial activity. In addition, there are a great variety of

**Table 2.** Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) (% v/v) of the honey samples against test fungi.

Test organism	Honey sample							
	NS		OW		AN		JS	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
<i>Aspergillus niger</i>	20	40	20	20	25	40	20	25
<i>Aspergillus flavus</i> .	20	40	20	25	20	40	20	20
<i>P. chrysogenum</i>	20	25	20	40	20	25	20	25
<i>M. gypseum</i>	12.5	25	12.5	40	20	40	20	40
<i>Candida albicans</i>	25	40	20	40	20	50	20	25
<i>Saccharomyces</i> sp.	20	25	20	40	40	40	20	40

components, including phenolic acids, flavonoids and other biomolecules, in different honeys. Biological activity of honey is mainly attributed to the phenolic compounds (Estevinho et al., 2008). The antimicrobial action of phenolics has been related to their ability to denature proteins, being generally classified as surface active agents.

Although this study demonstrates the antifungal effect of honey *in vitro*, the results have some practical considerations for its use *in vivo*. Honey can be used prophylactically in topical treatment to stop the colonization or infection of external sites which is a leading risk factor for bloodstream infection (Eggimann et al., 2003). It has been shown by Quadri and Huraib (1999) and Johnson et al. (2005) that whole honey placed directly around catheters was found to be effective in preventing exit site infection. English et al. (2004) found a significant reduction in mean plaque scores and bleeding sites in patients given a chewable 'honey leather'; this same technique could be applied for the treatment of oral candidiasis.

## Conclusion

The antifungal effect of different Nigerian honey samples was evaluated in culture media containing different concentrations of honey. The data suggest that the components in the honey samples are responsible for the observed *in vitro* antifungal properties. Thus, the Nigerian 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 studies are, therefore, required to demonstrate if this antifungal activity has any clinical application. Nevertheless, identification of the bioactive agents in honey, their clinical evaluation, and pharmacological standardization are crucial. 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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