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Full Length Research Paper

Antifungal activity of honey on fungi isolated from contaminated wound

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Studies on the antifungal activity of honey on fungi isolated from wound at different concentration of honey was carried out, wound sample were collected from Specialist hospital Sokoto and isolated in the laboratory. The antifungal activity of honey were examine against *Candida trophicalis, Cryptococcus neoformans, Aspergillus fumigatus, Aspergillus flavus* and *Aspergillus nidulans*at concentration of 1, 2, 4, 6 and 10(v|v). *Candida trophicalis* (21mm, 17mm, 13mm,11mm and 12mm),*Cryptococcus neoformans*(26mm, 20mm, 16mm, 13mm and 11mm), *Aspergillus fumigatus* (47mm, 43mm,34mm,37mm and 29mm), *Aspergillus flavus* (47mm, 43mm, 38mm, 41mm and 28mm), *Aspergillus nidulan* (47mm,44mm, 41mm and 36mm) with respect to above mentioned concentration (v|v).The result showed that the higher the concentration of honey, the higher the zone diameter of inhibition. Statistical tool indicates that there is no significant difference in the reduced concentrations of honey on the isolates at 5% level of significance. This indicates that honey at all level of dilutions shown significant effect on preventing fungal growth.

Key words: Antifungal, *Candida trophicalis, Cryptococcus neoformans, Aspergillus species*.

INTRODUCTION

Honey is a sweet food made by bees using nectar from flower (French *et al*., 2001). Honey is also produce by bumble bees, stingless bees, and other hymenoptera insect such as honey wax, though the quantity is generally lower and they have slightly different properties compare to honey produce by the genus Apis (Broosk *et al*., 2004). Honey bees convert nectar in to honey by a process of regurgitation. They stored it as primary food source in wax honey combs inside the beehive (Leddar, *et al*., 2013).Honey get its sweetness from the monosaccharide, fructose and glucose, and having the same relative sweetness as granulated sugar (Allen *et al*.,2000).

It has attractive chemical properties for baking and distinctive flavor that makes some people to prefer it over sugar. During foraging this assessed part of the nectar collected supports the metabolic activities of light muscle by hydrolysis of sucrose to glucose and then fructose within the majority of the collected nectar been distinct for regurgitation digestion and storage (Molan *et al.,* 2015). Most type of honey generates hydrogen peroxide when diluted because of the activation of the enzyme glucose oxidase which oxidizes glucose to glucoronic acid and hydrogen peroxide (Kingsley, 2001). Hydrogen peroxide is the major contributor to the antimicrobial activity of honey and the different honey results in their varying antifungal activity (Radians *et al.,* 2009).

The increase in the resistance of antifungal drugs in use has attracted the attention of the scientific community. Thus in recent years there has been an increasing research for new antifungal compounds, due to the lack of efficacy, side effect and resistance associated with some of the existing drugs, it become necessary to evaluate the antifungal activity of honey at varying concentration on fungi that are isolated from wound (Brudzynski,2006).

Only a couple of centuries ago man come to know that honey comes from the belly of the bee. This fact was

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Table 1: **Fungi Isolated From Wound Sample**

Samples	ORGANISMS ISOLATED
A	*Aspergillus nidulan, Aspergillus flavus,* and *Cryptococcus neoformans.*
B	*Aspergillus nidulan* and *Aspergillus fumigatus.*
C	*Aspergillus fumigates* and *Candida tropicalis.*
D	*Candida trophicalis, Aspergillus nidulan*and *Aspergillusflavus*
E	*Cryptococcus neoformans* and *Candida tropicalis.*
F	*Aspergillus flavus* and *Aspergillus nidulan.*
G	*Aspergillus fumigatus.*
H	*Aspergillus flavus* and *Cryptococcus neoformans. Candida tropicalis.*
I	*Candida tropicalis.*
J	*Cryptococcus neoformans* and *Aspergillus nidulan.*
K	*Cryptococcus neoformans,*
L	*Aspergillus nidulan* and *Candida tropicalis*
M	*Aspergillus flavus*
N	*Aspergillus nidulan*

mentioned in the QUR'AN 1,400 years ago in the following verse'' And thy lord taught the bee to build its cells in hills, on trees, and in (men's)habitations, there issues from within their bodies a drink of varying colours, wherein is healing for men (ALQUR'AN 16:68-69).Most microorganisms do not grow in honey because of its lower water activity of 0.6.

However, honey sometimes contains dormant end spores of the bacterium *Clostridium botulinum,* which can be dangerous to infants, as the endospores can transform in to toxin producing bacteria in the infant's immature intestinal tract, leading to illness and even death (Molan, 1992). The study of pollens and spores in raw honey (Melissopalynology) determines floral sources of honey. As the bees carry an electrostatic charge, and can attract other particles, the same techniques of melissopalynology can be use in area like environmental studies of radioactive particles, dust or particular solution.

Other factors, such as low protein content, high carbon to nitrogen ratio, low redox potential due to the high content of reducing sugars, viscosity anaerobic environment and other chemical agents phytochemicals are also likely to play some role in defining anti fungal activity of honey. Furthermore, honey has been employed to shorten the duration of diarrhea in patients with bactericidal gastroenteritis due to bacterial infection. However, honey has important beneficial characteristics that are less influenced by storage condition (Cooper, 2002).

Honey was being used for its antimicrobial properties in treating wounds. But with the advent of penicillin and other antibiotic drugs in the twentieth century, honey's medicinal qualities have taken a back seat. Treatment with honey is called Apitherapy, which includes replenishing energy, enhancing physical stamina and improving immune systems (Mossel, 2009).

Honey has been reported to have inhibitory effects on fungi. Pure honey inhibits fungal growth and diluted honey appears capable of inhibiting toxin production (Alwaili, 2005). An antifungal action has also been observed for some yeast and species of *Aspergillus* and *Penicillium*, as well as all the common dermatophytes (El-shawaf andGomaa, 2000.). Candidiasis, caused by *Candida albicans*, may respond to honey. (Estevinho *et al.*, 2008). Cutaneous and superficial mycoses like ringworm and athletes foot are found to be responsive to honey. This responsiveness is partly due to the inhibition of fungal growth and partly to inhibition of bacterial infection. (Bogdanov, 2008). In addition, some studies have reported that topical application of honey was effective in treating seborrhea dermatitis and dandruff. (Estevinho *et al.,* 2011).

MATERIALS AND METHODS

Wound samples were obtained at the male/female surgical ward and male/female casualty ward of Specialist hospital from affected individuals by the use of a sterile swabs sticks and labeled appropriately and then transferred to Microbiology laboratory, Mycology section Usmanu Danfodiyo University Sokoto for further processing and analysis. Honey were obtained from honey vendors within Sokoto Metropolis and transferred to general biology laboratory for identification before taking to Microbiology Laboratory, Usmanu Danfodiyo University, and Sokoto for its processing.

Sample processing and analysis

The wound samples were inoculated into the already prepared Potato Dextrose Agar(PDA) and incubated at the incubating room and growth observed for 5-7 days after which the different colonies obtained were

Table 2: Frequency of Occurrence and Percentage (%) of Occurrence of Fungi Isolates.

Isolated Fungi	Frequency of Occurrence	Percentage of Occurrence (%)
Aspergillus nidulan	7	28
Aspergillusflavus	5	20
Aspergillus fumigatus	3	12
Cryptococcus neoformans	5	20
Candida trophicalis	5	20
Total	25	100

Table 3: Antifungal Activity of Honey on fungi isolates.

Fungi	Zone of Inhibition of Honey at reduced volume concentration (mm)				
	1	2	4	6	10(v/v)
C. trophicalis	21	17	13	11	12
C. neoformans	26	20	16	13	11
A. fumigatus	47	43	34	37	29
A. flavus	47	43	38	41	28
A. nidulan	47	44	41	36	31

subculture in to freshly prepared PDA for the isolation of the pure culture and then incubated for another 5-7 days for the fungal growth. The colonial and morphological characteristics of fungal colonies were observed and noted (Cheesbrough, 2000).

The honey was processed into varying the concentrations by dissolving the honey in different volume of water to reduce its concentration as follows (1ml, 2ml, 4ml, 6ml and 10ml) (Cheesbrough, 2000).

Fungal identification

The fungi were identified based on the slide culture technique. In the identification of these fungi, each of the pure cultured isolates was identified using the slide culture technique as described by (Oyeleke and Manga, 2008).The fungi culture was picked using an inoculating needle and placed in a glass slide containing lacto phenol blue, after which it was covered with a cover slip then viewed under the microscope to check for the aerial and hyphal morphology which was the compared with that of the mycology atlas for confirmation.

DISCUSSION AND CONCLUSION

Honey purchase from honey vendors were evaluated for their antifungal activity against fungi isolated from wound samples these were *Candida trophicalis, Cryptococcus neoformans*, Aspergillus *fumigatus, Aspergillus flavus,* and *Aspergillus nidulan*. The result was agreed with Tasiu *et al*., 2015 who reported similar species in which they isolated *Microsporum canis, Aspergillus nig*er and *Candida albicans. A.nidulan* had the highest frequency of occurrence seven(7) while *A*. *fumigatus* had the lowest frequency of occurrences three(3), A. flavus, C. *neoformas* and *C*. *tropicalis* has the same frequency of occurrence five(5), this is however, in agreement with Molan (2015).Who reported similar frequency of occurrences but with slight variations.

The antifungal activity of honey against *Aspergillus fumigatus, Aspergillus flavus* and *Aspergillus nidulans* showed zone of inhibition of 47mm, 47mm, and 47mm respectively at concentration of one (1ml). *A*. *flavus*, *A*. *fumigatus* and *A*. *nidulan* were the most sensitive to the honey. While the *Candida tropicalis* and *Cryptococcus neoformas* (26mm) and (21mm) showed less susceptibility. Sanaa and Sanaa, (2007) also reported that *Aspergillus* species are susceptible to honey at varying concentrations and that the acidity of honey explained the relatively resistance of *Candida albicans* to honey compared to other fungi.

Thus since the Antifungal resistance of fungi is on the increase, the discovery of alternative therapy is urgently needed and honey possesses such therapeutic potential including wound healing properties and antifungal activity (Molan, 1992). He also explained that the antimicrobial properties of honey might be attributed to factors like high osmotic pressure and low pH.

The statistical analysis showed that the antifungal activity of honey had no significant difference on the result obtained from the antimicrobial properties of honey at $P<0.05$

The research work gives the potential of antifungal activity of honey at different concentration (1ml), (2ml), (4ml), (6ml) and (10ml). On the fungi isolated from wound exudates. These fungi include *Candida tropicalis, Cryptococcus neoformans, Aspergillus nidulans,*

Aspergillus fumigatus and *Aspergillus flavus*. Indicates response to the honey antifungal activity. This could be as a result of presence of hydrogen peroxide, osmotic effect and other component of honey that may be of medicinal importance. However, no statistical difference between the results of the antimicrobial activity. Thus honey is a best use for treatment of wounds.

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