



Antifungal Effect of Honey on Fungi causing Otomycosis

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ABSTRACT

Otomycosis is a subacute or chronic fungal infection of the external auditory canal. The main fungal agents of Otomycosis are *Aspergillus* species and *Candida* species. Antifungal treatment is used universally but there has been no effective medication used for Otomycosis which opens up new treatment options including the use of the natural product. Honey is a natural product that has been widely used for its therapeutic effects and it has a valued place in traditional medicine since ancient times. The present study highlights the antifungal activities of three varieties of honey: Khadi gram Udyog honey, Apis Himalaya honey, Dabur honey against fungal pathogens such as *Aspergillus flavus*, *Aspergillus tamarii*, and *Candida*. Honey used at different concentrations 20mg, 40mg, 60mg, 80mg, and spore suspension was inoculated in Sabouraud's dextrose broth (SDB). After incubation mycelial growth was harvested and observed microscopically for sporulation. The most effective inhibitory effect of the three types of honey is showing at 80 mg and the less effective inhibitory effect was recorded in a concentration of 20mg. The present study focuses on the three honey varieties that show effective antifungal activity. Honey can be used for the development of natural antifungal drugs for the treatment of Otomycotic pathogens.

Keywords: External auditory canal, *Aspergillus*, Honey, Otomycosis, Antifungal

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INTRODUCTION

Otomycosis is a superficial fungal infection of the external auditory canal [1]. It is a global medical problem, especially in warm, humid, and dusty climates [2]. Several species of fungi are noticed as etiological agents of Otomycosis. The main fungal agents of Otomycosis are *Aspergillus* and *Candida* species [3]. In recent years many scientific studies focus on the natural antifungal product that is more effective and safe for treatment. Antifungal treatment is use universally but there are few effective standardized medications used for Otomycosis and it opens up new treatment options. Recently natural antifungal products such as various types of honey are used as medicine which has great attention within the research [4]. Honey is a natural product that is produced from many floral sources and it is used as a medicine since ancient times [5]. It is a saturated sugar solution that contains 200 substances mainly glucose, fructose, amino acids, vitamins, minerals, and enzymes [6,7]. Honey contains strong antimicrobial properties and is extensively used as traditional remedies to treat various types of clinical problems [8]. Although several *in vitro* studies showed the antibacterial activities of honey have been mentioned in various studies but very less extensive studies on the antifungal action of honey [15]. Antifungal activity of honey is observed against *Aspergillus* and *Candida* species. In India, there are various types of honey available in the market. In this study, the three types of honey are used against Otomycotic fungi Khadi gram Udyog honey, Dabur honey, Apis Himalaya honey.

MATERIAL AND METHODS

Fungal isolates:

During this study, clinical samples were collected at the ENT department of district hospitals Ujjain (M.P.) India. All samples were collected under the aseptic condition from the external auditory canal by using sterile cotton swabs [9]. All samples were inoculated on Sabouraud's dextrose agar (SDA) with chloramphenicol and incubated at 28°C ± 1 for one week. Cultures were examined for growth on alternate days. Fungi were identified based on the cultural, morphological, and molecular, by standard monographs [10,11]. The isolated fungal strains of *Aspergillus niger*, *Aspergillus tamarii*, and *Candida* are used in this study.

Honey samples:

The three honey samples such as Khadi gram udyog honey, Dabur honey, Apis Himalaya honey were collected from the local market shops. All collected honey samples were brought to the laboratory and sterilized by ultraviolet radiation.

Inoculum preparation:

The spore suspension was prepared from 5-days old cultures of all isolated test fungi and inoculated on Sabouraud's dextrose broth (SDB). The three honey varieties i.e. Khadi gram Udyog honey, Dabur honey, Apis Himalayas honey were taken in different concentrations 20mg, 40mg, 60mg, 80mg in SDB media to test antifungal activity against test fungi. A control set of the flask containing each organism without honey was run in duplicate. The flask was incubated at $28^{\circ}\text{C} \pm 1$ for 7 days. Mycelial growth of each flask was harvested on a preweighed Whatman's filter paper no.1 and dried at 80°C temperature. The average dry mycelium weight was recorded.

RESULTS

The data (Table 1) shows that the antifungal effect of Khadi gram udyog honey, Dabur honey, Apis Himalaya honey inhibits the mycelial growth of most test fungi. The effect of the three types of honey is used in four different concentrations i.e. 20mg, 40mg, 60mg, 80mg on *Aspergillus niger*, *Aspergillus tamarii*, and *Candida*. The antifungal activity of Khadi gram Udyog honey was more active in (80 mg) which inhibits the growth and sporulation of *Aspergillus niger* (93.6%), *Aspergillus tamarii* (92.3%), *Candida* (90.2%) as shown in figure-1. The Dabur honey (80 mg) was showing the second maximum inhibition of *Aspergillus niger* (84.6), *Aspergillus tamarii* (87.5), *Candida* (76.0) in figure-2. The Apis Himalaya honey showed the minimum antifungal activity against the three test fungi in figure-3. In all three types of honey, the Khadi gram Udyog honey has the highest antifungal inhibitory effect on all test fungi. The inhibition rate of the mycelial growth and sporulation was recorded by increasing the concentration of honey.

Table 1: Antifungal effect of Khadi gram Udyog honey, Dabur honey, Apis Himalaya honey on Otomycotic Fungi

Honey	Con. (mg)	<i>Aspergillus niger</i>			<i>Aspergillus tamarii</i>			<i>Candida</i>		
		Dry wt/SE	Inh%	Sporulation	Dry wt/SE	Inh%	Sporulation	Dry wt/SE	Inh%	Sporulation
Khadi gram udyog	20mg	198±0.007	27.7	+++	208±0.007	21.8	+++	115±0.007	16.6	+++
	40mg	164±0.007	40.14	++	171±0.01	35.71	+++	99±0.007	28.2	+++
	60mg	114±0.007	58.39	+	109±0.01	59	++	79±0.004	42.7	++
	80mg	19±0.007	93.6	-	26±0.003	92.3	-	21±0.007	90.2	-
Dabur honey	20mg	209±0.01	23.7	+++	206±0.007	22.5	+++	118±0.004	14.4	+++
	40mg	173±0.01	36.8	+++	181±0.01	31.9	+++	103±0.01	25.3	+++
	60mg	129±0.01	52.9	++	158±0.01	40.6	++	82±0.007	40.5	++
	80mg	42±0.01	84.6	+	33±0.01	87.5	-	33±0.007	76	+
Apis himalaya honey	20mg	232±0.007	15.3	+++	220±0.02	17.29	+++	122±0.01	11.59	+++
	40mg	208±0.007	24.8	+++	192±0.01	27.81	+++	106±0.007	23.18	+++
	60mg	145±0.01	47.08	++	178±0.01	33.08	+++	89±0.004	35.5	+++
	80mg	64±0.01	76.64	+	68±0.007	74.43	+	52±0.01	62.3	+
Control	0	274		+++	266		+++	138		+++

Dry wt.: Each value in table is an average mean of duplicates \pm Standard error;

Grading of Sporulation: No sporulation = (-), Poor = (+), Fair = (++)

Good = (+++); Inh% = Percentage of Inhibition

Figure 1: Effect of different concentration of Khadi gram udyog honey on *Aspergillus niger*, *Aspergillus tamarii* and *Candida*

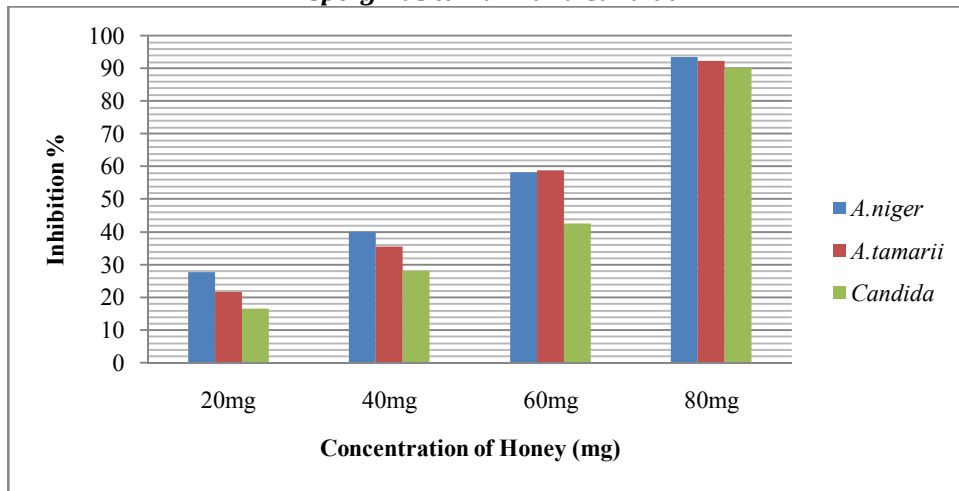


Figure 2: Effect of different concentration of Dabur honey on *Aspergillus niger*, *Aspergillus tamarii* and *Candida*

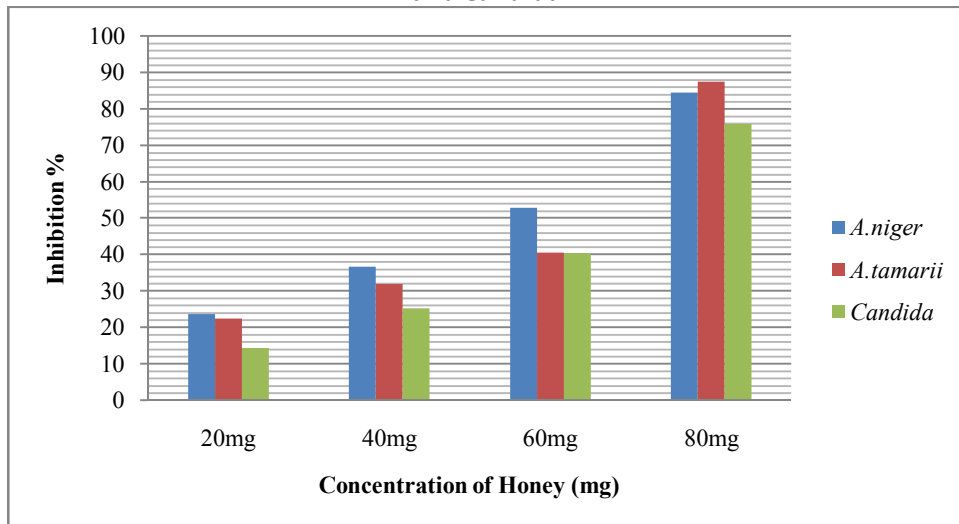
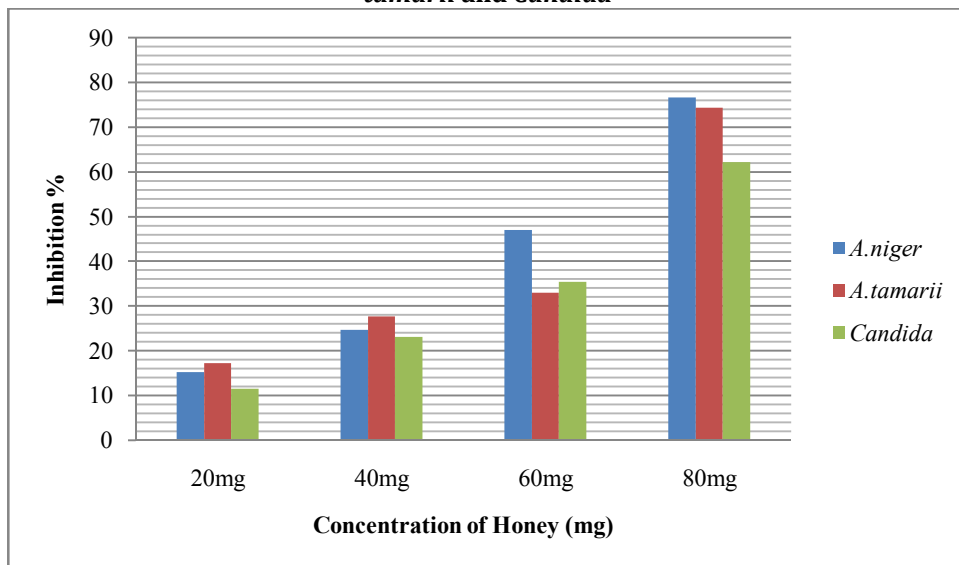


Figure 3: Effect of different concentration of Apis Himalaya honey on *Aspergillus niger*, *Aspergillus tamarii* and *Candida*



DISCUSSION

In the present study the three types of honey, Khadi gram Udyog honey, Dabur honey, Apis Himalaya honey shows antifungal properties against *Aspergillus niger*, *Aspergillus tamarii*, and *Candida*. In recent years several researchers have attention to the remedial properties of natural compounds. Honey is a natural product that is widely used for antifungal activity and it inhibits microbial growth because of the presence of a high amount of sugar content, low water activity, flavonoids, phenolic compounds, and biomolecules [12,13]. The phenolic acids in honey influence the porosity of the cytoplasmic membrane which allows the complete death [14]. Several researchers reported that the effect of gamma irradiation (25 to 50 kilograys) on the samples of five different types of New Zealand honey and South African honey remain unchanged after gamma irradiation [15,16]. In our present study the honey samples are also irradiated with ultraviolet light, the antifungal activities of the honey remain unchanged. Few studies observed that the in vitro antifungal activity of various types of honey like Algeria honey shows effect on *Aspergillus niger* and *Candida albicans* [17,18,19]. In our present study 80mg concentration of honey inhibits the maximum growth of *Aspergillus niger*, *Aspergillus tamarii*, and *Candida*. The honey concentration range about 30% to 50% prohibits the growth of number of microorganisms [20]. The highest concentration of the honey samples inhibits the growth of *M. gypseum* while average inhibition against *Candida albicans* [21].

Some studies reported that the intermediate sensitivity of honey samples on fungal isolates [22]. In vitro studies using three types of Malaysian honey Tualang, Kelulut and Acacia showed inhibitory effect on *Aspergillus niger* and *Candida albicans* [18]. The presence of a high amount of sugar concentration in honey shows the high osmolarity which produces antimicrobial activity. In this study, all three types of Indian origin honey were able to inhibit the growth of Otomycotic fungi [23]. Wadi reported that honey is used to treat various types of infections [5]. The in vivo studies on the efficacy of Acacia honey and Brazilian green propolis extract are used against fungal infections such as (tinea capitis and tinea versicolor) and this treatment is very beneficial in the treatment of superficial mycosis [24]. Few studies suggested that honey can be used in treatment procedures to cure an infection of external sites of the body [25].

CONCLUSION

The present study focuses on the antifungal activity of three different types of honey against Otomycotic fungi. Honey is a natural product that is potent therapeutic medicine for the treatment of Otomycosis as compare to synthetic drugs. Several researchers suggested that honey is a new effective medicine for various types of diseases. The efficacious concentration of honey can be used as natural antifungal drugs against Otomycotic pathogens.

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