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Anti-fungal activity analysis of natural honey samples in Golestan Province against some common dermatophyte strains In-vitro

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ABSTRACT

One of the superficial problems of public health is mycotic inflection that its incidence is not correctly known. The most important disease of them is dermatophytosis that comes through by dermatophytes as the major group of keratinophilic fungi. Honey is bee product that has been used as a medicine since ancient times in many cultures, and is still used in folk medicine. This study was designed for the purpose of investigation the antifungal potential of 5 honey samples against various dermatophyte strains from 3 genuses, *Trichophyton*, *Microsporum* and *Epidemophyton* by agar dilution technique and determination of the minimum inhibitory concentrations (MIC). The results showed that honey sample Jahan Nama had the best antidermatophyte effect and Bandargaz had less effect among all 5 honey samples. Also *Trichophyton* strains had shown the most sensitivity in tests with honey. This study shows that honey samples in Golestan province have an antifungal activity against dermatophytes as superficial infective microorganisms, and their static actions are very logical. So that might confirm the medicinal uses of the studied honey samples for the treatment of coutaneus or other various diseases.

1. Introduction

Honey is a useful food product and a valuable elixir considered as one of precious gifts from nature. A matter produced by bees through gathering honey-dew of flowers and exerting needed changes on it and then saved in waxy cells of hives. Essentially, natural honey is a sticky and viscous solution with a content of 80–85% carbohydrate (mainly glucose and fructose), 15–17% water, 0.1–0.4% protein, 0.2% ash and minor quantities of amino acids, enzymes and vitamins as well as other substances like phenolic antioxidants (James et al., 2009). Honey is regarded one of important medicinal resources whose healing properties were taken into consideration for centuries, so that it has been used in conventional medicine by different civilizations throughout the world (Moussa et al., 2012; Olaitan et al., 2007). Physicians and investigators everywhere have reused honey as a medicinal matter in recent

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years so it is being adapted as a natural antimicrobial in healing different wounds and infections. Furthermore, regarding the increasing number of problems new medication has presented in contrast to apparent benefits compared to conventional medicine, also, consequences of immethodical and incorrect use of chemical drugs facing the diseases and new fangled side effects in different societies, significance of natural medications like honey and conventional medicine is more and more cleared up.

Fungus infections were regarded as one of the major health problems all over the globe. Nowadays fungus skin-diseases are raising and they are influenced by different factors like economy of society and poor health conditions. Among them, dermatophyte infections are considered the most common and significant that can contribute to health problems by attacking different parts of body. Numbers of infections caused by these fungi have increased in recent years especially people who have poor immune system account for intense atypical and developed diseases (Babaie Nejad et al., 2007).

Dermatophytes are groups of hemogene keratinolytic fungi that have the ability to attack keratinilized tissues of human and animals and cause dermatophytose infection that is a kind of colonization of fungi on skin. Moreover, this disease is associated with host reaction to their metabolic products. Also, depending on anatomic place, it includes several damaging clinical forms which have been incurred in host body. At first there is an eczema and then an intense allergy and inflation, so that these reactions are associated closely to host immune system and instances responsible for infections; therefore their disease making ability can be seen one after the other or simultaneously (Asticcioli et al., 2008; Vander Straten et al., 2003).

Dermatophytose skin diseases typically react well to anti fungal medications; on the contrary in some cases these medications are useless because of resistant mechanisms against them or some other instances. In recent years important changes in dermatophytose infections incidence have been reported in different countries that is spreading these infections alongside extensive side effects of anti fungal medications and some other factors can be a revolution in public health (Hainer, 2003). Accordingly, global tendency toward conventional medicine in recent decades has caused research activities to concentrate on finding scientific evidences about this branch of medicine. Hence, achieving new medicinal resources and identifying and presenting new and efficient ways to fight these infections especially in instances resistant to drugs would be very vital and significant.

By the same taken and regarding the mentioned necessities this study has been done with the aim of assessing potential anti dermatophytic effect of 5 natural honey samples in Golestan Province, north of Iran against common dermatophyte.

2. Materials and methods

2.1. Isolation and identification of dermatophyte strains

After sampling from 10 hair dressers and also from infectious wounds on head skin and some parts of feet in 3 visiting patients to a private clinic during the year 2013 in Gorgan, Golestan province, enrichment of samples were done and then they were taken into culture environment. Direct analysis was done using potassium hydroxide 20% and culturing on SCC (Sabouraud Chloramphenicol Cycloheximide) Agar (Merck; Germany) for clinical identification of isolates slide-culture would be used to identify spore producing structure of fungus if colony created and grew. Then, from special cultural environment including Trichophyton agar, environments with urea were used to study existence of urease enzyme and corn-meal agar alongside hair perforation test and also growing in 37°C in addition to basic identification of strains based on fungus spore producing like micro and macro conidia and also colony form to almost certain identify and differentiate kinds of dermatophytes.

2.2. Honey samples

This investigation has been done on 5 honey samples produced in Golestan Province. The samples were poly-floral collected during 7 days late summer 2013 (as autumn honey) from 5 regions including Aq-Emam region around Maraveh Tape Town, Farang Farsian in Galikesh Town, Qale-Maran around Ramian Town, Jahan Nama region around Gorgan Town, and Vatana forests around Bandar Gaz Town. After taking the samples into laboratory,

and passing through special sterile sieves, they were kept out of light; in sterile containers in 20-25°C. The samples were cultured on Blood Agar (Merck; Germany) to prevent any pollution.

2.3. Preparing serial dilutions

First 400 ml of SCC environment was prepared in 4 separate flasks (100 ml in every one) and steriled in 121°C Autoclave with pressure of 15 pound/inch for 20 min. After relative drop in temperature of environment, 5, 2.5, 1.25, 0.625 ml of honey samples were added to every flask respectively. Therefore we had concentrations of 5%, 2.5%, 1.25%, and 0.625% from every sample. Contents of every flask were equally divided into 8 cm sterile plates to culture fungal samples in them. These processes were done for every honey sample separately in three replications. Some plates with only cultural environment without any additives were prepared as control (Ravikumar Patil et al., 2007).

2.4. Culturing fungi

After separation and selection of dermatophyte strains and preparation of environments with different honey concentrations, we studied fungal sensitivity rate. So, polluted environment technique in Agar Dilution was used (Ravikumar Patil et al., 2007). Form *Trichophyton*, *Microsporum* and *Epidermophyton* strains of basic environment gotten from clinical samples were taken equally by sterile inoculation needle next to flame and under hood and inoculated in center of plates containing different honey dilutions and without them.

All in all, they were incubated in 4 different concentrations of studying samples in cultural environment in 25°C.

2.5. Daily control and study the growing rate

All incubated plates were daily studied and after observing growing rate in special time period, fungus colony diameter was measured by callipers and registered separately for every strain and every dilution. The final report and certain results were presented a week after fungal culture.

3. Results

Mycologic investigations in present study have confirmed that isolation and purification of strains including 2 *Microsporum*, that are *Microsporum canis* and *Microsporum gypseum*, 4 *Trichophyton*, containing 2 *Trichophyton verrucosum*, 1 *Trichophyton mentagrophytes* and 1 *Trichophyton rubrum* strains and also 1 *Epidermophyton* and only existed strain that is *flocosom* which in-vitro evaluations were done on all of them.

Based on results, Jahan Nama honey had the most inhibitory rate amongst 5 tested honey samples and Ramian honey was the second after that. While these two samples have shown similar average inhibitory effect on two strains of *Microsporum gypseum* and *Trichophyton rubrum.* After them, Marave Tape honey sample had considerable inhibitory effect on fungal strains, yet it showed less effect against *Trichophyton mentagrophytes* comparing to Galikesh honey. These two samples confirmed similar growing inhibitory effect on two fungal strains of *Trichophyton rubrum* and strain No.2 of *Trichophyton verrucosum.* Thus, Galikesh sample was forth in static effect rate. Bandar Gaz honey sample was the least effective one and showed relatively weak effects on fungi amongst studied honey samples on all dermatophyte strains comparing to basic environment (table 1).

4. Discussion

These data was analysis of results and understanding growing power of every strain without considering their growing rate in basic environment and natural capability of every fungus to reach the maximum growing rate. Accordingly, looking at basic environments, we know that except *Trichophyton mentagrophytes*, other tested dermatophytes had the same growing capability on basic environment with resulted average colony diameters in appropriate concentrations and tested honey samples. So, if honey concentration increases, acceptable decrease of every dermatophyte strains will be observed according to basic environment. Therefore, we cannot evaluate strains' sensitivity just considering growing power of every dermatophyte in honey sample

environments without regarding natural ability of every fungus in basic environment.

Infections in variety of forms and by different microorganisms associate with some problems in patients. In addition, resistant infectious microorganisms have intensified the problem and collapsed treatment processes. Fungal infections are major disorders and abnormalities in a society, they are not considered very important. Yet nowadays increasing this kind of infection in affected people with serious diseases or patients with immune system deficiency is regarded as the main reason of their fatality so, its significance is an open and shut case (Moussa et al., 2012). Spreading diseases such as Aides or different kinds of cancers make infections like fungal disease especially dermatitis appear due to weak immune system because of either the disease itself or using immunosuppressive drugs and increase dramatically (Rashid Achterman and White, 2012; Seebacher et al., 2008).

Since most known anti-fungal medications have broad side effects especially in chronic use, due to similar structure of fungus and host cells that is to say both are eukaryotic (Klepser et al., 1997), thus, using effective anti-fungal material and compounds with least side effects against different fungal strains would be significant in treating affected patients. Furthermore, one of important aspects of fungal infections especially dermatophyte is that they can result in other infections of microorganisms. They straighten the way of rushing other damaging microorganisms like bacteria through making different kinds of wounds and injuries and by necrosis and collapsed tissues provide an appropriate nutrient environment for them to reproduce vastly (Lyscova, 2007).

These instances make selecting an appropriate treating protocol with regard to the most efficiency and least side effects in special time period an inevitable case; therefore, investigators are interested in developing modern treating methods with least damaging consequences and finding effective methods with high natural and anti-microbial compounds and using more natural and conventional medications. So, it might be a contemporary way in medical investigations to fight against infections and drug resistances or an appropriate alternative for present less or non-effective medication. Also, global tendency toward conventional medication in recent years has focused investigations on finding evidences and results of this branch of science.

Honey has been used with healing properties in lots of cultures for thousands of years. Historical evidences have proven that Egyptians, Greeks, and Persians were ancient people who had used honey alongside other herbal teas to treat diseases (Tovey, 2000; Forrest, 1982; Zumla and Lulat, 1989). Honey is full of nutrients and it has got different physicochemical properties and can naturally provide most requirements of our bodies. It also has remarkable effect on growth and human health. It has most elements such as minerals and vitamins even though they are very little. Elements which are produced inside flowers and in nature and turned into honey by bees. All these factors make honey have lots of advantages over other food products.

With a detailed and careful attitude, we can claim that Microsporum strains grow very fast in culture considered as one of remarkable properties of this kind (Shadzi, 2004). *Trichophyton verrucosum* grew very slowly observed in different honey sample environments with diverse concentrations comparing to basic environment. As a result, we cannot present a correct analysis of every dermatophyte strains' power just observing the data and results without regarding natural properties of every strain and their growing capabilities. With relating these properties for every fungus and also resulted growth average, it is assumed that every *Trichophyton verrucosum* strain was the most sensitive dermatophyte against honey samples according to results of primary data and analyzing theories. In the same way, strain No.1 with approximate average of 5.3 and strain No.2 with 3.7 stopped growing in tested honey samples. After them was *Trichophyton mentagrophytes* which abilities of honey samples to inhibit its growth were evaluated three times more than basic environment. Also, honey samples' growth inhibitory effect on *Trichophyton rubrum* was two times more comparing basic environment (table 2).

To sum up, *Trichophyton* was the most sensitive one amongst three studied strains in different concentrations of honey samples and it showed the highest rate of growth negative fluctuations so it had the most growth descent rate.

El-Kady et al. studied anti-dermatophyte effect of vegetable oil extracts and they concluded that *Trichophyton mentagrophytes* had more sensitivity against extracts comparing with *Microsporum canis* (El-Kady et al., 1993).

If tested *mentagrophytes* strain by El-Kady et al. was representative of *Trichophytones* and *Microsporum canis* as representative of *Microsporums* as well, their conclusions would be homogenous with resulted data. Since in both investigations *Trichophyton* strains were considered more sensitive despite different used anti fungal substances.

Also Sheikh et al. reported that *Trichophyton mentagrophytes* was the most sensitive strain amongst other dermatophyte strains that has stopped growing in 4 honey samples out of 10 tested samples (Sheikh et al., 1995).

After *Trichophyton* strains, honey samples influenced most *Microsporum gypseum* strains and *Epidermophyton floccosum* experienced 1.8-fold descent in growing comparing to basic environment.

Laorpaska et al. studied anti fungal effect of several honey samples on 3 dermatophyte strains in which *Trichophyton* strain showed more sensitivity than *Microsporum* that was homogenous with results of two previous investigations. On the other hand, *Epidermophyton* was regarded as the most sensitive strain that was in opposite of the studying results (Laorpaksa et al., 1992).

The least growth fluctuation was for *Microsporum canis* considered as the most resistant strain against all tested honey samples with growth inhibitory of 1.7 comparing the basic environment.

Avizhgan et al. have done some dermatophyte researches in which they studied anti fungal activity of *Echinophora platyloba*, as an anti mold plant in conventional medication, on three strains of *Trichophyton*, *Microsporum* and *Epidermophyton.* They have concluded that *Trichophyton verrucosum* was the most sensitive strain and so-called plant's extract can be used desirably against it (Avizhgan et al., 2006).

Avizhgan has also reported that extract of *Echinophora platyloba* cannot be used against *Trichophyton rubrum* and *Microsporum gypseum* since they showed relative resistance in their investigations.

Comparing this studying with other investigations despite using different regions and different honey bees, we conclude that the results are all homogenous since sensitivity or resistance rate of strains were almost similar. Different investigations about anti microbial property of honey and also sensitivity or resistance rate of testing fungi have supported it.

Used honey samples in whole investigations have shown substantial anti microbial activity which supports results of our studying about inhibitory ability of honey. It would be worthwhile noting that differences in kind or different honey's effect rate in different parts of the world on different microbes might be as a result of different environmental factors such as climatic or continental circumstances, vegetation type based on blossoming principals, growing rate, fostering techniques of bees, honey producing ways and preserving them. Tested honey samples have very effective antimicrobial substances which the strongest fungi in this studying could not resist against them. To prove the fact that honey can be a natural antimicrobial factor against microorganisms associated with infections especially cutaneous infections dermatophytosis and an appropriate alternative for less or non effective antibiotics, broad and very detailed investigations are needed.

Conclusion

Proving effectiveness of Golestan honey samples on dermatophyte fungi this hope comes alive that we can get a compound with acceptable anti fungal effect and least side effects to treat fungal infections through more investigations and getting the highest biological provision in future, yet broad researches are needed in vitro and in vivo circumstances alike to study effective concentration of honey in natural condition to be able to finally introduce this paradisal honey dew as a suitable alternative for present less or even non effective drugs to modern medical world.

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Table 1. Effect of honey samples on dermatophyte strains (mm)

		Dermatophyte strains						
Honey samples	Concentrations	*E. floccosum*	*M. gypseum*	*M. canis*	*T. verrucosum No. 1*	*T. verrucosum No. 2*	*T. rubrum*	*T. mentagrophytes*
Ramian	5 cc	3.5 *	8	8.5	0	0	0	0
	2.5 cc	6	14	13	0	0	5	0
	1.25 cc	8	20	16	0	0	10	8
	0.625 cc	11	29	18	4	6	15	10
Jahan Nama	5 cc	3	8	8	0	0	0	0
	2.5 cc	5	14	10.5	0	0	5	0
	1.25 cc	8	21	14	0	0	10	6
	0.625 cc	10	28	16	2	5	15	8
Bandar Gaz	5 cc	5	11	10	0	0	4	2
	2.5 cc	7	23	15	3	4	10	4
	1.25 cc	13	29	21	5	11	13	8
	0.625 cc	13	34	21	9	11	15	10
Marave Tape	5 cc	4	9	9	0	0	2	0
	2.5 cc	7	15	13	0	0	6	4
	1.25 cc	9	20	16	0	5	12	8
	0.625 cc	11	30	18	5	9	16	10
Galikesh	5 cc	4	9	9.5	0	0	2	0
	2.5 cc	8	16	14	0	0	6	2
	1.25 cc	10	21	17	3	4	12	8
	0.625 cc	11	30	19	5	10	16	10
-	basic	14	36	25	10	12	18	15

* Data based on colony diameter in culture

Table2. Dermatophyte strains' average sensitivity rate against all honey samples in all dilutions

	E. floccosum	*M. gypseum*	*M. canis*	*T. verrucosum No. 1*	*T. verrucosum No. 2*	*T. rubrum*	*T. mentagrophytes*
Honey sample	7.8	19.4	14.3	1.9	3.2	8.7	4.9
Basic	14	36	25	10	12	18	15
Total	10.9	27.7	19.7	5.9	7.6	13.3	9.9
Ability*	1.8	1.8	1.7	5.3	3.7	2.0	3.0

*honey samples' growth inhibitory capabilities comparing to basic environment

Refereces

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