Antifungal and Anti-bacterial Synergistic Effects of Mixture of Honey and Herbal Extracts

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Abstract

Background: By increasing resistance of several bacterial and fungal species to many kinds of antibiotics, applications of natural base compounds e.g. honey and medicinal herbs have been more attractive. The aim of present research is evaluation of anti-bacterial and anticandida effects of three kinds of honey of Iran together with alcoholic extract of mint and zataria, as well as extract and starch of ginger on Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Candida albicans, as pathogens of human body.

Materials and Methods: Minimum inhibitory concentration (MIC) and minimum additive inhibitory concentration (MAIC) were determined by the agar diffusion method by dilution method in Sabouraud agar. By inoculation of 10 μL from suspension and appearance of colorless vesicles, MIC was determined. Growth inhibition was calculated by ANOVA, Mann-Whitney U and t-student tests. All experiments were conducted three times. MIC for three variety of honey on Staphylococcus aureus (32, 30, 29% v/v), Pseudomonas aeruginosa, (70, 67, 71% v/v), Escherichia coli (40, 35, 39% v/v) and Candida albicans (45, 48, 50% v/v) are reported. While, the MAIC for the growth inhibition of honeys together with extract of mint and zataria, as well as extract and starch of ginger were reduced.

Results: The results show that ginger extract has more significant impact on microorganism growth with respect to others. Pseudomonas aeruginosa was the most susceptible microorganisms to ginger extract. Growth inhibitory effect of ginger extract was more significant than ginger starch.

Conclusion: Addition of herbal extract increases antibacterial and anticandida properties of honey thus letting hope for a honey benefit and would constitute an alternative way against the resistance to bacteria.

Introduction

Due to increase in resistance of Candida species to antifungal medicines, studies on antifungal compounds with natural base have been raised. Candida is an opportunistic fungi which is able to cause acute and chronic infections in the moth, lung and digestive tract. The body reaction ranges from slight into acute inflammation and granulomatous. Candida genus normally is harmless and endosymbionts of hosts but under some conditions they can cause candidiasis or create disease transmitters [1]. Candida genus is almost universal on normal flora of the mouth and gastrointestinal and its generation is through an asymmetric division process or budding of yeast. This specie can cause death in human with impaired immune system such as those with HIV, cancer, hematopoietic stem cell or other transplantation. In recent years there are many reports which show increase in some species resistant to antifungal agents due to vast antifungal misuse.

Honey is a compound known for its anti bacterial and anticandida effect. As a consequence of increase in resistance of Candida species to antifungal medicines in patients with candidiasis, studies on natural antifungal compounds have been largely increased [2, 3]. These natural compounds not only have a proper curing effect, but also show little or no side effects. The results of in-vitro and in-vivo tests of using them showed a good positive effect on the growth inhibition of many fungi species including Candida [5-7]. Some of researchers have been indicated that honey with high concentration has anti-microbial effect and caused inhibition in growth of some bacteria and fungi [8, 9]. These effects depend on chemical factors of honey such as acidity [9], high osmolarity [10, 11] and also hydrogen peroxide [12].
Honey as a natural and non toxic compound which has a vast anti-fungi function has been vastly raised the interest. It can be a good alternative for synthetic antifungal medicines but its relatively high cost in some countries caused limitations for its use. Honey contains few numbers of enzymes such as diastase, invertase, glucose oxidase, catalase, and phosphatase. Amylase in honey hydrolyses the starch chain and thus increases its antibacterial effect [13-15].

Generally, fungi are more resistant to high osmotic property than bacteria. The presence of some plant derivatives such as flavonoids and stimulation of immune system by cytogenese stimulation through activation of neutrophils types B and T can be factors that cause antibacterial function in honey [16-18]. Chyun et al, and also Nelson et al. have been investigated the antibacterial effect of onion and ginger on the Escherchia coli, Bacillus subtilis and Salmonella typhimurium [19, 20]. Ginger is the rhizome of a plant with scientific name Zingiber officinale. It contains some chemical compounds namely flavonoids which show antibacterial activity among growth of micro organisms in vitro [21]. Momeni et al., studied the antibacterial effect of extract, aqueous and methanolic extracts of onion and ginger, against Staphilococcus aureus, Pseudomonase aeroginosa, Eschershia coli and candida albicans isolated from urine. The results showed the methanolic extract of ginger prevent growth of tested organisms more effectively than other extracts. Pseudomonas aeroginosa appeared to be more susceptible to onion and ginger extracts. These antibacterial effects could only be observed on gram negative bacteria, and there were no effect on gram positives. According to their report, ginger extracts showed a more significant inhibition impact in comparison with onion extract [21].

Mahdavi Omran et al. studied the effect of honey taken from north of Iran on Candida albicans. Their results showed that honey can be used as an empirical remedy to treat infections due to its inhibition effect specially honey with no temperature treatment (kept at 37°C)- on the growth of candida species particularly Candida dubliens [22].

Heretofore, there have been so much studies on the joint effect of honey and medicinal plants and their possible synergistic effect. In this study, the antacandida and anti bacterial effects of different batches of honey taken from different parts of Iran, with or without plant extracts were investigated. The objective of this study is to investigate the synergistic effect of three varieties of Iranian honey with ethanolic extract of mint, zataria and also ginger extract and starch, against Staphylococcus aureus, Pseudomonas aeroginosa, Eschershia coli and Candida albicans as pathogens.

Materials and Methods

In three different varieties of honey taken from beehives in Chaharmahal Bakhtiari (a), Tehran (b) and mountains in North of Iran (c) were collected and kept in glass vials in dark at 4°C. Ginger (Zingiber officinal) was purchased from Research Institute of Forests and Rangelands (RIFR), Iran national herbarium. Ginger rhizomes were washed with distilled water and were dried by exposing to the air for one hour. Then they were peeled, washed and grinded by using hammer miller. Thereafter, by adding water an aqueous solution obtained which were filtrated and the residue was separated, washed and kept in an oven at 45°C for 4 hours to get dry. The dried residue was grinded to obtain ginger starch powder. This powder was used to prepare different concentration of starch solution expressed by percentage.

Preparation of ethanolic extract: According previous reports ethanolic extract showed more effectiveness in comparison with the plain or aqueous extract [21]. To prepare the ethanolic extract, 200 gram of fresh ginger rhizome were added into 100 ml ethanol (95%) and kept for 24 hours. All extracts were kept at 4°C before use. Zataria and mint extracts were purchased from Soha plantation Industries and Herbal medicine Processing Company.

Cultivation of Fungal Strain and Inoculum Development: Staphylococcus aureus, Pseudomonas aeruginosa, Eschershia coli and Candida albicans were obtained from microbial laboratory of department of food technology research, National Nutrition and Food Technology Research Institute (NNFTRI). Pure cultures were inoculated in slants of Nutrient Agar and kept at 4°C before use. Candida albicans was maintained by subculture in specific media sabouraud agar. The inoculums suspensions was obtained by taking five colonies (>1 mm diameter) from 24 old cultures grown on sabouraud agar. The colonies were suspended in 5 mL of sterile saline water (0.85%). The inoculums suspensions were shaken for 15 s and density adjusted to the turbidity of a 0.5 McFarland standard (equivalent to 1-5.106 cfu/mL). Direct enumeration was conducted to control similarity microorganism number in inoculums. 10 microliter of water extract was added to medium and incubated for 24 h at 37°C. The results were reported according to minimum inhibitory concentration for extracts after 24 and 48 h. To determine anti-candida effect of honey, it was added to medium at 10-50% v/v. All treatments have done triplicate and statistical analysis were conducted using ANOVA, t-student and Mann-Whitney U tests.

Results

The results of this study showed that honeys from different parts of Iran have different effects on growth inhibition of Staphylococcus aureus, Pseudomonas aeruginosa, Eschershia coli and Candida albicans due to different sources which lead to having different compounds. According to our observations, the rate of inhibition impact depends on the honey variety, microbial type and concentration of medicinal plant (% w/v). All three different varieties of honey were shown having inhibition effect on microbial growth. In addition, increase in the percentage of medicinal plants (extract or starch), caused increase in the inhibition effect.
After letting the mixture of honey and medicinal plants interact with microorganisms for a certain time, results were obtained and shown in Table 1. Few numbers of samples show no reaction to injected extract, or the zones of inhibition were small. However, in most of the samples the inhibition zones were vast.

**Discussion**

The inhibition impacts of zataria extract on the bacteria were more than those on Candida. Also, ginger starch showed significantly more synergistic effect than that of other extracts on inhibition of bacterial growth. Honey varieties from North of Iran (sample a and c) showed more anti-bacterial effect in comparison with honey variety from Tehran (sample b). Bukra et al. showed in their report that honey and ginger extract have a significant impact on the growth inhibition of Candida albicans and Aspergillus, and Minimum inhibitory concentration of honey on the two bacteria were reported 42 and 46 (% v/v). Their report also indicated that after adding ginger extract to honey, minimum inhibitory concentration on Candida albicans and Aspergillus were decreased to 28 and 38 respectively. Increase in osmotic property of honey can be the factor which is responsible for its anticandida impact [23-25]. This can supports the carbohydrates role and their oxidation in anti-bacterial impact of honey. In Al-Walli et al. study, the minimum inhibitory concentration to inhibit the Candida growth was reported 70%. Addition of 80% honey 2-6 hour after inoculation could completely inhibit the Candida growth. In some studies it has been proved that the time of inoculation of honey into culture media is also effective in its inhibition impact [27]. Mercan et al. have been reported difference in inhibition impact of different varieties of honey from Turkey on Escherchia coli and Staphylococcus aureus. This can be due to difference in honey sources; in other words, difference in variety of plants which honey is made of. Therefore, their biological impacts will differ.

According to obtained results, honey can significantly inhibit the growth of Candida while having no effect on Lactobacillus. This is important because Lactobacillus is a part of normal vaginal flora. Future studies can be conducted to evaluate the effect of honey on Candida albicans and Lactobacillus isolated from human body.

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![Table 1](image-url)

**Table 1. Minimum inhibitory concentration of honey (MIC) and minimum additive inhibitory concentration (MAIC) for growth inhibition**

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<th>Honey and mint extract (%v/v)</th>
<th>Honey and ginger extract (%v/v)</th>
<th>Honey and Zataria extract (%v/v)</th>
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*All data have been calculated in triplicates.

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*Honey varieties; (a): Honey taken from Chaharmahal Bakhtiar, (b) from Tehran, (c) from North of Iran
conducted to evaluate the effect of honey on Candida albicans and Lactobacillus isolated from human body.

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**References**

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**Conflict of Interest**
The authors declare no conflict of interest.

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