

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

Kianoush Khosravi-Darani,*¹ Ramin Khaksar,¹ Saeideh Esmaeili,¹ Fatemeh Seyed-Reihani,² Alaleh Zoghi,³ Saeede Shahbazzadeh⁴

1. Department of Food Technology Research, National Nutrition and Food Technology Research Institute, Faculty of Nutrition and Food Technology, Shahid Beheshti University of Medical Science, Tehran, Iran
2. Faculty of Industrial Technology, University Science Malaysia, Pulau Penang, Malaysia
3. Department of Chemical Engineering, Faculty of Engineering, Science and Research Branch, Islamic Azad University, Tehran, Iran
4. Department of Food Science and Technology, Shahr-e-Qhods Branch, Islamic Azad University, Tehran, Iran

Article information	Abstract
<p>Article history: Received: 15 Feb 2012 Accepted: 10 June 2012 Available online: 30 Dec 2012 ZJRMS 2013; 15(8): 30-33</p> <p>Keywords: Synergism Honey Ginger Mint Zataria Antibacterial Anticandida</p> <p>*Corresponding author at: Department of Hematology, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran. E-mail: kiankh@yahoo.com</p>	<p>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 <i>Staphylococcus aureus</i>, <i>Pseudomonas aeruginosa</i>, <i>Escherichia coli</i> and <i>Candida albicans</i>, as pathogens of human body.</p> <p>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 <i>t</i>-student tests. All experiments were conducted three times. MIC for three variety of honey on <i>Staphylococcus aureus</i> (32, 30, 29% v/v), <i>Pseudomonas aeruginosa</i>, (70, 67, 71% v/v), <i>Escherichia coli</i> (40, 35, 39% v/v) and <i>Candida albicans</i> (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.</p> <p>Results: The results show that ginger extract has more significant impact on microorganism growth with respect to others. <i>Pseudomonas aeruginosa</i> was the most susceptible microorganisms to ginger extract. Growth inhibitory effect of ginger extract was more significant than ginger starch.</p> <p>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.</p> <p>Copyright © 2013 Zahedan University of Medical Sciences. All rights reserved.</p>

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 mouth, 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 contents 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 contents some chemical compounds namely flavonoids which show antibacterial activity against 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 *Staphylococcus aureus*, *Pseudomonase aeruginosa*, *Escherchia 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 aroginosa* 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 dubliensis* [22].

Heretofore, there have been no much studies on the joint effect of honey and medicinal plants and their possible synergistic effect. In this study, the anticandida 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 aeruginosa*, *Escherchia 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*, *Escherchia 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 inoculum 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 micro-liter 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-candidia 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*, *Escherchia 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.

Table 1. Minimum inhibitory concentration of honey (MIC) and minimum additive inhibitory concentration (MAIC) for growth inhibition^{1,2}

No	MIC Honey (%v/v)	plan extract Volume percent honey mixed with MAIC																MIC control including 2 (%v/v)			
		Honey and mint extract (%v/v)				Honey and ginger extract (%v/v)				Honey and Zataria extract (%v/v)				Honey and ginger starch (%v/v)				Mint	Ginger	Zataria	Ginger starch
Escherhia Coli																					
a*	40	2 f2	4 ef2	6 de1	8 de1	2 de1	4 bcd1	6 ab2	8 a	2 de1	4 cd1	6 abc2	8 ab1	2 f2	4 ef1	6 de1	8 de1	0	0	0	0
b*	35	d1	d1	d1	cd1	cd1	bc1	ab12	ab	d1	cd1	a1	ab1	d1	d1	d1	d1	0	0	0	0
c*	39	f12	ef2	def1	de1	cd1	b1	a1	a	cd1	bc1	b12	b1	f12	ef1	de1	de1	0	0	0	0
Staphylococcus Aureus																					
a*	30	ef1	cd12	cd1	cd1	cd1	bc1	ab12	a1	f1	f1	ef1	ef	ef1	cd12	cd1	cd1	0	0	0	0
b*	32	def1	cde1	cd1	cd1	c1	b1	a1	a1	f1	ef1	ef1	def	def1	cde2	cd1	cd1	0	0	0	0
c*	29	de1	bc1	bc1	bc1	bc1	b1	a1	a1	e1	e1	d1e	cd	de1	bc1	bc1	bc1	0	0	0	0
Pseudomonas Aeruginosa																					
a*	70	f1	ef1	de1	cde2	de1	c1	b1	a1	ef12	de2	cd2	c12	f1	ef1	de1	cde2	0	0	0	0
b*	67	d1	cd1	c1	bc12	bc1	bc1	ab15	a1	cd16	bc1	ab1	ab1	d1	cd1	c1	bc12	0	0	0	0
c*	71	efg1	defg1	ab1	a1	cdef1	bcde2	bc2	bc2	g2	fg3	bcd2	ab2	efg1	defg1	ab1	a1	0	0	0	0
Candida Albicans																					
a*	45	d1	cd2	ab12	ab2	ab1	a1b	ab1	ab1	d1	bc1	ab1	a2	d1	cd2	ab12	ab2	0	0	0	0
b*	48	f1	ef2	bcd2	bcd2	bcd1	abc13	ab1	a1	f1	de1	cde2	bcd3	f1	ef2	bcd2	bcd2	0	0	0	0
c*	50	f1	cd1	bc1	ab1	f2	c1	cd1	bc1	f1	de1	c1	a1	f1	cd1	bc1	ab1	0	0	0	0

¹All data have been calculated in triplicates.²Standard deviation were obtained about 0.67

* Honey varieties; (a): Honey taken from Chaharmahal Bakhtiari, (b) from Tehran, (c) from North of Iran

After letting the mixture of honey and medicinal plants interact with micro organisms for a certain time, results were obtained and shown 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 impact in comparison with honey variety from Tehran (sample b). Bukrra 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 *Escherhia 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.

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.

Acknowledgements

The authors wish to thank the National Nutrition and Food Technology Research Institute (NNFTRI) for technical support

References

- Rashidi N, Arash D. [Cutaneous *Candida albicans* infection in diabetic patients] Persian. *J Ardebil Univ Med Sci* 2008; 8(3): 250-5.
- Citak S, Ozcelik B, Cesur S and Abbasoglu U. In vitro susceptibility of *Candida* species isolated from blood culture to some antifungal agents. *Jpn J Infect Dis* 2005; 58(1): 44-6.
- Walsh TJ, Finberg RW, Arndt C, et al. Liposomal amphotericin B for empirical therapy in patients with persistent fever and neutropenia. National Institute of Allergy and Infectious Diseases Mycoses Study Group. *N Engl J Med* 1999; 340(10): 764-71.
- Ribeiro MA, Paula CR, John R, et al. Phenotypic and genotypic evaluation of fluconazole resistance in vaginal *Candida* strains isolated from HIV-infected women from Brazil. *Med Mycol* 2005; 43(7): 647-50.
- Devkatte AN, Zore GB, Karuppaiyl SM. Potential of plant oils as inhibitors of *Candida albicans* growth. *FEMS Yeast Res* 2005; 5(9): 867-73.
- Lopez A, Hudson JB, Towers GH. Antiviral and antimicrobial activities of Colombian medicinal plants. *J Ethnopharmacol* 2001; 77(2-3): 189-96.
- Motsei ML, Lindsey KL, Van Staden J and Jager AK. Screening of traditionally used south African plants for antifungal activity against *Candida albicans*. *J Ethnopharmacol* 2003; 86(2-3): 235-41.
- Alnaqdy A, Al-Jabri A, Al Mahroogi Z, et al. Inhibition effect of honey on the adherence of *Salmonella* to intestinal epithelial cells in vitro. *Int J Food Microbiol* 2005; 103(3): 347-51.
- Wahdan HA. Causes of the antimicrobial activity of honey. *Infection* 1998; 26(1): 26-31.
- Theunissen F, Grobler S, Gedalia I. The antifungal action of three south African honeys on *Candida albicans*. *Apidologie* 2001; 32(4): 371-9.
- Namias N. Honey in the management of infections. *Surg Infect* 2003; 4(2): 219-26.
- Allen KL, Molan PC, Reid GM. A survey of the antibacterial activity of some New Zealand honey. *J Pharm Pharmacol* 1991; 43(12): 817-22.
- Moussa A, Saad A, Nouredine D, et al. The influence of starch of ginger on the antibacterial activity of honey of different types from Algeria against *Escherichia coli* and *Staphylococcus aureus*. *Int J Microbiol Res* 2011; 2(3): 258-262.
- Oddo LP, Piazza MG, Pulcini P. Invertase activity in honey. *Apidologie* 1999; 30: 57-65.

Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

Funding/Support

Shahid Beheshti University of Medical Science, Tehran.

- Boukraa L, Amara K. Synergistic action of starch on the antibacterial activity of honey. *J Med Food* 2008; 11(1): 195-198.
- Theunissen F, Grobler S, Gedalia I. The antifungal action of three South African honeys on *Candida albicans*. *Apidologie* 2001; 32(4): 371-79.
- Lusby PE, Coombes AL, Wilkinson JM. Bactericidal activity of different honeys against pathogenic bacteria. *Arch Med Res* 2005; 36(5): 464-7.
- Kucuk M, Kolayh S, Karaogla S, et al. Biological activities and chemical composition of three honeys of different types from Anatolia. *Food Chem* 2004; 100(2): 526-34.
- Chyun JC, Huang L. Ginger and its bioactive component inhibit enterotoxigenic *Escherichia coli* heat-labile enterotoxin-induced diarrhea in mice. *J Agric Food Chem* 2007; 55(21): 8390-7.
- Nelson C, Regiland A. Antimicrobial properties of extracts of *Allium cepa* and *Zingiber officinale* (ginger) on *Escherichia coli*, *Salmonella typhi* and *Bacillus subtilis*. *Int J Trop Med* 2007; 3(2): 1540-470.
- Momeni L, Zamanzad B. [Evaluation of antimicrobial effects of onion and ginger extracts on some bacteria and *Candida albicans* isolated infected urinary systems] Persian. *J Shahr-e-Kord Univ Med Sci* 2010; 11(4): 81-87.
- Mahdavi-Omran S, Maliji Gh, Sefidgar SA, et al. [effect of honey from north of Iran on *Candida albicans*] Persian. *J Babol Univ Med Sci* 2008-2009; 10(5): 15-22.
- Boukraa L, Benbarek H, Moussa A. Synergistic action of starch and honey against *Candida albicans* in correlation with diastase number. *Braz J Microbiol* 2008; 39(1): 40-43.
- Boukraa L, Bouchehrane S. Additive action of honey and starch against *Candida albicans* and *Aspergillus niger*. *Rev Iberoam Micol* 2007; 24(4): 309-11.
- Al-Waili NS, Akmal M, Al-Waili FS, et al. The antimicrobial potential of honey from United Arab Emirates on some microbial isolates. *Med Sci Monit* 2005; 11(12): 433-8.
- Mercan N, Guvensen A, Celik A and Katircioglu H. Antimicrobial activity and pollen composition of honey samples collected from different provinces in Turkey. *Nat Prod Res* 2007; 21(3): 187-95.
- Al-Waili NS. Mixture of honey, beeswax and olive oil inhibits growth of *Staphylococcus aureus* and *Candida albicans*. *Arch Med Res* 2005; 36(1): 10-3.

Please cite this article as Khosravi-Darani K, Khaksar R, Smaeili S, Seyed-Reihani F, Zoghi A, Shahbazadeh S. Antifungal and antibacterial synergistic effects of mixture of honey and herbal extracts. *Zahedan J Res Med Sci (ZJRMS)* 2013; 15(8): 30-33.