Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Document heading doi: 10.12980/JCLM.2.20143D420

Effect of heat treatment on antimycotic activity of Sahara honey

Moussa Ahmed*, Saad Aissat, Noureddine Djebli

Pharmacognosy and Api-Phytotherapy Research Laboratory, Mostaganem University, Mostaganem, Algeria

PEER REVIEW

Peer reviewer

Dr. Abdelkrim Cheriti, Professor, Phytochemistry and Organic Synthesis Laboratory, University of Bechar, Algeria.
Tel/Fax: +213 49 81 52 44
E-mail: karimcheriti@yahoo.com

Comments

This is an interesting and valuable research work in which authors evaluated for the first time the influence of heat on colour, polyphenol contents and antimycotic capacity of honey from Algerian Sahara. This article includes interesting scientific results in the valorization of local honeys as antimicrobial agent.
Details on Page 880

ABSTRACT

Objective: To evaluate the influence of the temperature on honey colour, polyphenol contents and antimycotic capacity and to evaluate the correlation between these parameters.
Methods: Sahara honey were heated up to 25, 50, 75 and 100 °C for 15, 30 and 60 min, and their colour intensity, polyphenol contents and antimycotic capacity. The Folin-Ciocalteu test was used to determine the total polyphenol contents (TPC). The antimycotic activity was evaluated both by agar diffusion method and micro wells dilution method against the *Candida albicans* (*C. albicans*) and *Candida glabrata* (*C. glabrata*).
Results: Initial values for TPC in Sahara honey ranged from 0.55 to 1.14 mg of gallic acid per kg of honey, with the average value of 0.78 mg of gallic acid per kg of honey. The TPC values after heat-treatment were 0.54 to 1.54 with the average value of 1.49 mg. The minimal inhibitory concentrations before heat-treatment of Sahara honey against *C. albicans* and *C. glabrata* ranged from 3.06%-12.5% and 50% respectively. After heat-treatment the minimal inhibitory concentrations between 12.5% and 50% for *C. albicans* and *C. glabrata*, respectively. The diameters of inhibition zones of Sahara honey with 50% concentration varied from (12.67-15.00) mm by *C. albicans* to (14.33-15.67) mm by *C. glabrata*. The diameters of inhibition zones after heat-treatment at 25 and 50 °C for 15.30 and 60 min ranged from (2.00-18.67) mm by *C. albicans* to (8.00-16.67) mm by *C. glabrata*. Statistically significant relations between the TPC and the colour intensity of Sahara honey (r=0.99, P<0.05). Furthermore, the results showed that the TPC and colour is not correlated with the antimycotic capacity.
Conclusions: To our knowledge this is the first report on the antimycotic capacity of Sahara honey.

KEYWORDS

Sahara honey, Antimycotic capacity, Heat treatment

1. Introduction

Yeasts infections have emerged during the past two decades as important pathogens causing formidable morbidity and mortality in an increasingly diverse and progressively expanding population of immunocompromised patients. The therapeutic against yeast infections presents limitations: toxicity, high cost and resistance of fungal. The development of new antifungal agents becomes necessary. Therefore, bees products have been traditionally used as therapeutic agents and about half of the drugs that we use today are derived from natural sources. Honey has been used for its medicinal properties in many cultures since ancient times. The antimicrobial of honey have formed the basis of many applications including pharmaceuticals, alternative medicine and natural therapies[1-3]. Honey has been reported to contain about 600 substances and is considered as an important part of traditional medicine[4]. Several bioactive compounds have been identified in honey which contributed to its antimicrobial action[5]. Honey has antimicrobial effects, which are

*Corresponding author: Dr. Moussa Ahmed, Pharmacognosy and Api-Phytotherapy Research Laboratory, Mostaganem University, Mostaganem, Algeria.
Tel/Fax: +213 65234059
E-mail: moussa7014@yahoo.fr
Foundation Project: Supported by the Algerian Ministry of the Higher Education and Scientific Research, CNEPRU project approved in 2011/2013 (Grant No. F023 2009/0009).

Article history:
Received 5 May 2014
Received in revised form 15 May, 2nd revised form 25 May, 3rd revised form 2 Jun 2014
Accepted 27 Jun 2014
Available online undetermined

attributed to the osmotic effect of the substance's sugars, its pH, and particularly its peroxidase activity[6]. Furthermore, heating honey inactivates the glucose oxidates. The antimicrobial effects are also due to the presence of non-peroxidase substances such as phenolic acids, flavonoids, and lysozymes[7-9]. The non-peroxide antimicrobial activity is insensitive to heat and light[10]. Data concerning the effects of honey against different yeasts is limited from different parts of the world[11-13]. To date, no data is available on the antimycotic activity of Sahara honey samples from Algeria. This study has two goals: 1) to investigate the effects of thermal treatment on color intensity and polyphenols content of Sahara honey; 2) to determine antimycotic capacity of unheated and heat-treated honeys.

2. Materials and methods

2.1. Sample collection

Monofloral Sahara honey samples (n=3) from *Apis mellifera* were collected in 2012 from separate apiaries in the south of Algeria. The samples were stored at 4 °C until analysis in dark conditions.

2.2. Thermal treatment

Sahara honey samples were heated at 25, 50, 75 and 100 °C for 15, 30 and 60 min. Then they were cooled down to 4 °C by immediately plunging the tubes in an ice bath and analyzed. The samples were filled into watertight tubes.

2.3. Colour intensity: $ABS_{720-450}$

The net absorbance of the honey was determined by the method of Beretta *et al*[14]. The honeys were diluted to 50% (w/v) with warm (45-50 °C) milli Q water and the solution was filtered through a 0.45 μm filter. There was a complete absence of coarse particles in the honey solutions as all the commercial samples were no crystalline liquid honeys. The absorbance was measured using a spectrophotometer at 450 and 720 nm and the difference in absorbance was expressed as mAU.

2.4. Determination of total phenolic contents (TPC)

TPC was determined spectrophotometrically according to modified Folin-Ciocalteu[15]. A total of 30 μL of honey solution (0.1 g/mL) was mixed with 2.37 mL of milli Q water and 150 μL of 0.2 mol/L Folin-Ciocalteu reagent. The solution was thoroughly mixed by vortexing and incubated for 2 min at ambient temperature. A total of 450 μL of sodium carbonate solution (0.2 g/mL) was added to the reaction mixture and further incubated for 2 h at ambient temperature. The absorbance was measured at 765 nm using a spectrophotometer. The TPC was determined by comparing with a standard curve prepared using gallic acid (0-200 mg/L). The mean of at least three readings was calculated and expressed as mg of gallic acid equivalents (mg GAE)/100 g of honey.

2.5. Strains and growth conditions

Candida glabrata (*C. glabrata*) and *Candida albicans* (ATCC 10231) (*C. albicans*) were cultivated in Sabouraud dextrose agar (bioMerieux, Marcy-l'Etoile, France) and were incubated at 37 °C for 48 h. Colonies from 48 h cultures were suspended in 5 mL of a sterile-saline solution. The count of yeasts was adjusted to yield 1.5×10^8 CFU/mL using the standard McFarland counting method.

2.6. In vitro antimycotic susceptibility assay

2.6.1. Screening of Sahara honey for antimycotic activity

Agar-well diffusion assay was used to evaluate the antifungal activities of the honey based on Ahmed *et al*[16]. Briefly, agar plates (90 mm) containing 20 mL of Sabouraud dextrose agar were inoculated using a swab from a suspension of each organism containing 1×10^8 CFU/mL. An 8-mm diameter well was cut into the agar and 100 μL of 50% and 100% honey solution (w/v,) prepared in sterile distilled water) was aliquoted into the well. The controls were set up with equivalent quantities of water as controls. The plates were incubated at 37 °C for 48 h. Zones of inhibition were measured using a Vernier caliper (Draper). The antifungal potential of test compound was determined on the basis of mean diameter of zone of inhibition around the wells in millimeters. Each assay was performed in duplicate and repeated twice. The diameter of the inhibition zones: <5.5 mm, inactive; 5.5-9.0 mm, very low activity; 9-12 mm, low activity; 12-15 mm, average activity; and >15 mm, high activity.

2.6.2. Broth microdilution assay

The antimycotic activity of Sahara honey was examined by determining the minimal inhibitory concentration (MIC) using the macro dilution broth technique by Eloff[17]. Briefly, serial 2-fold dilutions of honey were inoculated with 10^8 CFU/mL (final concentrations) for each of the microorganisms tested. Yeast suspension of approximately 5×10^8 CFU/mL was inoculated into tubes containing honey

at different dilutions and incubated at 37 °C for 48 h. All MIC values were expressed in percentage (%) (v/v). Bioassay was performed in duplicate and repeated twice.

2.7. Data analysis

Each honey was analyzed in triplicate. Results were shown as mean±SD. Correlations were established using Pearson's correlation coefficient (r) in bivariate linear correlations ($P<0.05$). All statistical analyses were performed with the Statistica 7.0 software for windows.

3. Results

3.1. Total phenolic contents

TPC determined by Folin-Ciocalteu method was expressed as GAE mg/100 g of honey. TPC values of Sahara honey before and after heat-treatment were (0.551±0.002) to (1.140±0.000) GAE mg/100 g and (0.590±0.000) to (1.540±0.000) GAE mg/100 g of honey respectively (Figure 1 and Table 1).

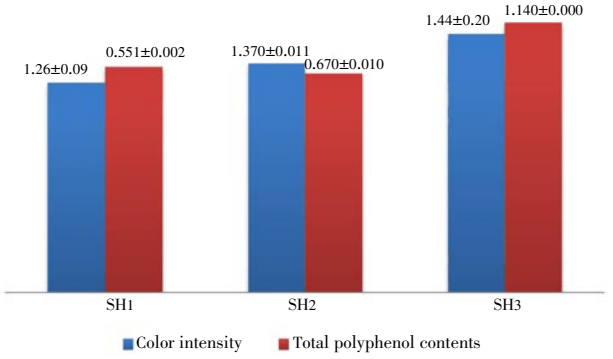

Figure 1. Colour intensity and total polyphenol contents of Sahara honey before heat-treatment.
Data are expressed as mean±SD, n=3.

Table 1
TPC after heat-treatment at different temperatures for different heating times.

Honeys	Time (min)	Total phenolic contents (GAE mg/100 g)			
		25 °C	50 °C	75 °C	100 °C
SH1	15	0.590±0.010	0.66±0.03	0.700±0.010	1.27±0.01
	30	0.850±0.020	0.86±0.03	0.740±0.020	1.38±0.01
	60	0.580±0.020	0.86±0.03	0.660±0.010	1.39±0.02
SH2	15	0.540±0.050	0.66±0.03	0.840±0.040	1.24±0.00
	30	0.710±0.090	0.92±0.03	0.950±0.070	1.54±0.00
	60	0.900±0.020	0.92±0.04	0.730±0.030	1.38±0.01
SH3	15	0.760±0.040	0.76±0.01	0.800±0.000	1.28±0.01
	30	0.770±0.020	0.78±0.01	0.880±0.010	1.50±0.10
	60	0.730±0.016	0.78±0.01	0.860±0.000	1.45±0.02

Data are expressed as mean±SD, n=3.

3.2. Color intensity

The color intensity of the Sahara honey before and after heat-treatment ranged from (1.26±0.09) to (1.44±0.20) and (1.01±0.56) to (1.88±0.45) mAU respectively (Figure 1 and Table 2). Honey color depends on various factors for example: mineral content, ash content and botanical origins. Besides the floral and geographical origin, honey colour can also be affected by heat, time of storage.

Table 2
Colour of Sahara honey (50%, w/v) after heat-treatment at different temperatures for different heating times.

Honeys	Time (min)	Color intensity (mAU)			
		25 °C	50 °C	75 °C	100 °C
SH1	15	1.46±0.20	1.25±0.04	1.23±0.20	1.33±0.18
	30	1.34±0.09	1.24±0.05	1.32±0.14	1.64±0.10
	60	1.01±0.56	1.25±0.07	1.22±0.04	1.81±0.35
SH2	15	1.35±0.03	1.38±0.06	1.34±0.07	1.41±0.18
	30	1.52±0.17	1.27±0.10	1.28±0.20	1.77±0.17
	60	1.31±0.11	1.40±0.04	1.32±0.07	1.88±0.45
SH3	15	1.29±0.15	1.54±0.13	1.51±0.18	1.22±0.09
	30	1.37±0.15	1.45±0.09	1.58±0.19	1.31±0.19
	60	1.38±0.06	1.51±0.16	1.59±0.19	1.43±0.27

Data are expressed as mean±SD, n=3.

3.3. Antimycotic capacity

The antimycotic activity results obtained from the macrodilution assays for Sahara honey samples are presented in Figure 2, Tables 3 and 4.

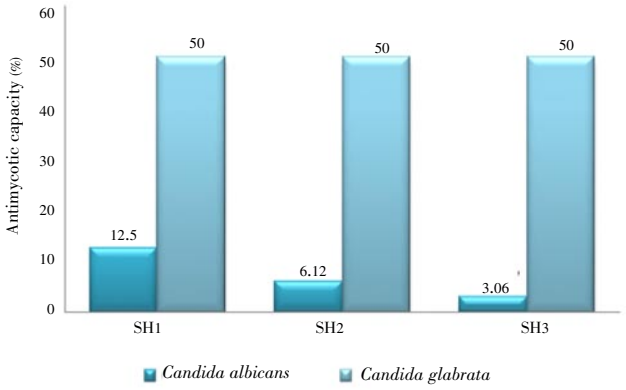

Figure 2. Antimycotic capacity of unheated Sahara honey.

Table 3
Antimycotic capacity of heat-treated honeys at different temperatures for different heating times against *C. albicans*.

Honeys	Time (min)	Temperatures			
		25 °C	50 °C	75 °C	100 °C
SH1	15	50	50	50	ND
	30	50	50	50	ND
	60	50	50	50	ND
SH2	15	50	50	50	ND
	30	50	50	50	ND
	60	50	50	50	ND
SH3	15	50	50	50	ND
	30	50	50	50	ND
	60	50	50	50	ND

MIC was used to determine the lowest concentration of

honey-in-water solution (w/v) at which the percentage inhibition is almost 100%[18,19]. The MIC before heat-treatment of Sahara honey at 25 and 50 °C for 15, 30, and 60 min against *C. albicans* and *C. glabrata* ranged from (3.06% to 12.5%) and 50% respectively. After heat-treatment the MIC ranging from 12.5% and 50% for *C. albicans* and *C. glabrata*, respectively. No inhibition of *C. albicans* growth was observed when honey at 75 and 100 °C, and no inhibition of *C. glabrata* growth was observed when honey at 100 °C.

Table 4

Antimycotic capacity of heat-treated honeys at different temperatures for different heating times against *C. glabrata*.

Honeys	Time (min)	Temperatures			
		25 °C	50 °C	75 °C	100 °C
SH1	15	12.5	12.5	12.5	ND
	30	12.5	12.5	12.5	ND
	60	12.5	12.5	12.5	ND
SH2	15	12.5	12.5	12.5	ND
	30	12.5	12.5	12.5	ND
	60	12.5	12.5	12.5	ND
SH3	15	12.5	12.5	12.5	ND
	30	12.5	12.5	12.5	ND
	60	12.5	12.5	12.5	ND

Using agar well-diffusion assay, Sahara honey were tested for their ability to inhibit growth of *C. albicans* and *C. glabrata*. The results of the *in vitro* antimycotic capacity of Sahara honey determined by diameters of inhibition zones (DIZ) are presented in Figure 3. The DIZ produced by the undiluted honeys against *C. albicans* and *C. glabrata* ranged from (16.67±0.00) to (23.00±0.00) and 0 mm respectively. The DIZ with 50% concentration varied from (12.67-15.00) mm by *C. albicans* to (14.33-15.67) mm by *C. glabrata*.

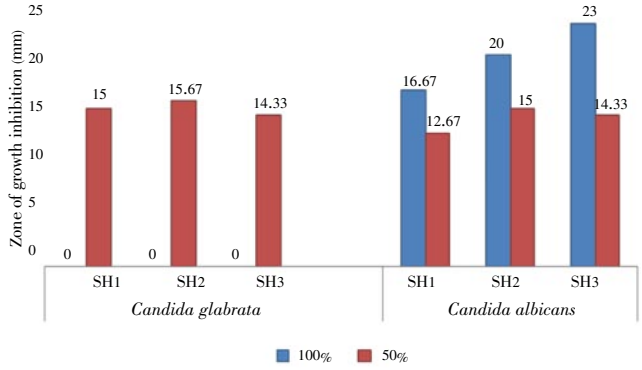

Figure 3. Means of zone of growth inhibition of Sahara honey before heat-treatment.

After heat-treatment (at 25, 50 and 75 °C for 15, 30, and 60 min), the DIZ ranged from (2.00-18.67) mm by *C. albicans* to (7.33-16.67) mm by *C. glabrata* (Tables 5 and 6).

Table 5

Means of zone of growth inhibition (mm) of Sahara at different temperatures for different heating times, using agar-well diffusion assays against *C. albicans*.

Honeys	Time (min)	25 °C		50 °C		75 °C		100 °C	
		100%	50%	100%	50%	100%	50%	100%	50%
SH1	15	13.00	11.67	15.33	ND	8.33	ND	ND	ND
	30	13.33	10.00	10.00	ND	6.33	ND	ND	ND
	60	13.00	9.67	13.67	ND	9.00	ND	ND	ND
SH2	15	13.33	10.67	17.33	2.00	8.67	ND	ND	ND
	30	18.00	11.00	16.33	ND	6.00	ND	ND	ND
	60	18.00	9.67	12.33	ND	9.33	ND	ND	ND
SH3	15	12.67	ND	17.67	2.33	8.67	ND	ND	ND
	30	15.00	ND	12.33	2.00	7.67	ND	ND	ND
	60	18.67	ND	12.33	ND	8.00	ND	ND	ND

Table 6

Means of zone of growth inhibition (mm) of Sahara at different temperatures for different heating times, using agar-well diffusion assays against *C. glabrata*.

Honeys	Time (min)	25 °C		50 °C		75 °C		100 °C	
		100%	50%	100%	50%	100%	50%	100%	50%
SH1	15	ND	10.00	ND	16.67	ND	12.67	ND	ND
	30	ND	12.67	ND	8.33	ND	11.67	ND	ND
	60	ND	8.00	ND	10.00	ND	11.33	ND	ND
SH2	15	ND	8.67	ND	14.67	ND	12.67	ND	ND
	30	ND	12.33	ND	8.33	ND	11.00	ND	ND
	60	ND	8.00	ND	10.33	ND	12.33	ND	ND
SH3	15	ND	10.00	ND	14.67	ND	11.67	ND	ND
	30	ND	12.00	ND	8.33	ND	7.33	ND	ND
	60	ND	7.67	ND	10.00	ND	9.00	ND	ND

ND=not determined. Diameter of the inhibition zones: <5.5 mm, inactive; 5.5-9 mm, very low activity; 9-12 mm, low activity; 12-15 mm, average activity; and >15 mm, high activity.

4. Discussion

In many countries, some fungal infections have been treated with natural products remedies and also some pathogenic fungi are commonly resistant to many antibiotics. So, interests at antifungal properties of natural products from bee products particularly honey are very important. Several types of honey are produced in Algeria, where honey production is a traditional practice, well implanted in several regions. The Sahara region is located in the south of Algeria, where, due to its edaphoclimatic conditions and flora diversity, sidr and euphorbia are the principal honey types produced. A great number of research studies focused on the pharmacological and biological properties present by honey, including anti-inflammatory, antioxidative, antibacterial and antifungal activity among others[20]. Honey is an effective antifungal activity as shown by several studies. Large number of honeys from different geographical locations and different botanical sources show growth inhibitory action[21,22]. Limited numbers of studies have examined the activity of Algerian honey against fungi. In brief, Ahmed *et al.* found that the MIC of six varieties of honey against *Rhodotorula mucilaginosa* ranged between 28% and 36% (v/v)[23].

In our previous studies, Ahmed *et al.* was the first to report the antifungal effects of heat processing on the antifungal activity of honey (40%-45% *C.albicans* and 45%-50% *Aspergillus niger*[24,25]. Molan reported that honey loses its antimicrobial activity when subjected to heat or exposed to light[26]. Radwan *et al.* showed that heat exposure in a water bath at a temperature that was not clearly specified, but appeared to be 50 °C for 10 min, had an adverse effect on the antibacterial activity of honey[27].

Bogdanov tested the effect of heat onperoxide and non-peroxide antibacterial activity of blossom and honeydew honey against *Staphylococcus aureus*[28]. Heating at 70 °C for 15 min resulted in a decrease of the initial peroxide activity of blossom honey by 92% and of honeydew honey by 22%, whereas the non-peroxide activity of both honey types was decreased only slightly.

Several factors may influence the antifungal activity of honey, which include its botanical origin, aromatic acids, including benzyl cinnamate, methyl cinnamate, caffeic acid, cinnamyl cinnamate, cinnamoylglcine, terpenoids, and polyphenols[20]. In recent years, a number of studies have been reported on the antifungal activity of phenolic compounds from natural sources[29]. Polyphenols in honey considered a potential source of the antimicrobial properties of honey[30]. In the studied Sahara honeys, only very small amounts of polyphenols contents were present.

The literature data indicate that there is a strong positive correlation between honey color and total phenolic content. In the present study, relationship between phenolic content and colour of Sahara honeys (r=0.99) is possibly due to a highly extended conjugated systems, especially when complexed with minerals. Furthermore, in the present study, no relationship between phenolic content, colour and the antimycotic capacity of Sahara honeys was observed.

Resistance of microorganisms to antifungal drugs is becoming a growing concern worldwide. Natural products have been traditionally used in the treatment of fungal diseases because they are sources of many active compounds. Honey is a honeybee product known for its biological and pharmacological properties for centuries. It has been extensively used in traditional medicine and also in complementary medicine because of its antibacterial and antimycotic activities. This is the first study reporting the results of a representative screening of Sahara honeys for antimycotic capacity, deserving further investigation for clinical applications against fungal infections.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Authors are thankful to staff of Mostaganem University for providing material. This work was supported by the Algerian Ministry of the Higher Education and Scientific Research, CNEPRU project approved in 2011/2013 (Grant No. F023 2009/0009)

Comments

Background

Bees products attracted an increasing interest in many cultures since ancient times because of their medicinal properties. Thus, honey is used for its pharmacological activities, especially as antimicrobial agent. This activity is attributed to the osmotic effect of sugars substances, pH, peroxidase activity, phenolic acids, flavonoids, and lysozymes. The non-peroxide antimicrobial activity is insensitive to heat and light which forced scientists to search the thermal effect on honeys activities.

Research frontiers

In this work, authors evaluate the thermal effect on honey colour, polyphenol contents and antimycotic capacity and to determine the correlation between these parameters.

Related reports

Large number of honeys from different geographical locations and different botanical sources show growth inhibitory action. But, Ahmed *et al.* (2012) are the first to report the heat effects on the antifungal activity of honey. In another hand, the literature data indicate that there is a strong positive correlation between honey color and total phenolic content. Authors of this study indicate that there is no relationship observed between phenolic content, colour and the antimycotic capacity of Sahara honeys.

Innovations & breakthroughs

This study has explored for the first time of the influence of heat on colour, polyphenol contents and antimycotic capacity of honey Algerian Sahara.

Applications

It is very interesting to use honey from Sahara as antibacterial natural agent. The results of the present study is an interesting form of the valorization of local honeys and its possibility use in clinical applications against fungal infections

Peer review

This is an interesting and valuable research work in which authors evaluated for the first time the influence of heat on

colour, polyphenol contents and antimycotic capacity of honey from Algerian Sahara. This article includes interesting scientific results in the valorization of local honeys as antimicrobial agent.

References

[1] Al-Waili N, Al Ghamdi A, Ansari MJ, Al-Attar Y, Osman A, Salom K. Differences in composition of honey samples and their impact on the antimicrobial activities against drug multiresistant bacteria and pathogenic fungi. *Arch Med Res* 2013; **44**(4): 307-316.

[2] Boorn K, Khor Y, Sweetman E, Tan F, Heard TA, Hammer KA. Antimicrobial activity of honey from the stingless bee *Trigona carbonaria* determined by agar diffusion, agar dilution, broth microdilution and time-kill methodology. *J Appl Microbiol* 2010; **108**: 1534-1543.

[3] Khalil MI, Alam N, Moniruzzaman M, Sulaiman SA, Gan SH. Phenolic acid composition and antioxidant properties of Malaysian honeys. *J Food Sci* 2011; **76**: C921-C928.

[4] Bogdanov S, Haldimann M, Luginbühl W, Gallmann P. Minerals in honey: environmental, geographical and botanical aspects. *J Apicult Res* 2007; **46**(4): 269-275.

[5] Brudzynski K, Abubaker K, Miotto D. Unraveling a mechanism of honey antibacterial action: polyphenol/H_2O_2-induced oxidative effect on bacterial cell growth and on DNA degradation. *Food Chem* 2012; **133**: 329-336.

[6] Al-Waili NS, Khelod SK, Butler G, Al Ghamdi AA. Honey and microbial infections: a review supporting the use of honey for microbial control. *J Med Food* 2011; **14**(10): 1079-1096.

[7] Kwakman PH, Zaat SA. Antibacterial components of honey. *IUBMB Life* 2012; **64**: 48-55.

[8] White JW. *Composition of honey. honey: a comprehensive survey*. London: Heinemann; 1979, p. 157-158.

[9] Candiracci M, Citterio B, Piatti E. Antifungal activity of the honey flavonoid extract against *Candida albicans*. *Food Chem* 2012; **131**: 493-499.

[10] Bogdanov S. Characterisation of antibacterial substances in honey. *Lebensm Wiss Technol* 1984; **17**: 74-76.

[11] Katiraee F, Mahmodi R, Mardani K, Babaei E. Antifungal activity of Iranian honeybees against *Candida, Aspergillus* species and *Trichophyton rubrum*. *J Food Process Pres* 2013; doi: 10.1111/jfpp.12187.

[12] Borujeni SB, Mobini GR, Pourgheysari B, Validi M. Comparison of the effect of honey and miconazole against *Candida albicans in vitro*. *Adv Biomed Res* 2013; **2**: 57.

[13] Burlando B, Cornara L. Honey in dermatology and skin care: a review. *J Cosmet Dermatol* 2013; **12**(4): 306-313.

[14] Beretta G, Granata P, Ferrero M, Orioli M, Facino RM. Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. *Anal Chim Acta* 2005; **533**: 185-191.

[15] Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol* 1999; **299**: 152-178.

[16] Moussa A, Noureddine D, Saad A, Abdelmelek M, Abdelkader B. Antifungal activity of four honeys of different types from Algeria against pathogenic yeast: *Candida albicans* and *Rhodotorula* sp. *Asian Pac J Trop Biomed* 2012; **2**: 554-557.

[17] Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Med* 1998; **64**(8): 711-713.

[18] Cooper RA, Jenkins L, Henriques AF. Absence of bacterial resistance to medical-grade manuka honey. *Eur J Clin Microbiol Infect Dis* 2010; **29**(10): 1237-1241.

[19] Tan HT, Rahman RA, Gan SH, Halim AS, Hassan SA, Sulaiman SA, et al. The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to manuka honey. *BMC Complement Altern Med* 2009; **9**: 34.

[20] Israili ZH. Antimicrobial properties of honey. *Am J Ther* 2013; doi: 10.1097/MJT.0b013e318293b09b.

[21] Zainol MI, Mohd Yusoff K, Mohd Yusof MY. Antibacterial activity of selected Malaysian honey. *BMC Complement Altern Med* 2013; **13**: 129.

[22] Brudzynski K, Kim L. Storage-induced chemical changes in active components of honey de-regulate its antibacterial activity. *Food Chem* 2011; **126**: 1155-1163.

[23] Ahmed M, Djebli N, Aissat S, Khiati B, Meslem A, Bacha S. *In vitro* activity of natural honey alone and in combination with curcuma starch against *Rhodotorula mucilaginosa* in correlation with bioactive compounds and diastase activity. *Asian Pac J Trop Biomed* 2013; **3**(10): 816-821.

[24] Ahmed M, Djebli N, Aissat S, Meslem A. Anti-*Aspergillus niger* of eucalyptus honey influenced by thermal treatment. Open Access Scientific Reports; 2012. [Online] Available from: http://omicsonline.org/scientific-reports/1948-5948-SR175.pdf [Accessed on 20th February, 2014]

[25] Ahmed M, Djebli N, Aissat S, Meslem A. Influence of temperature on the inhibitory potency of eucalyptus honey against *Candida albicans*. *Asian Pac J Trop Dis* 2012; **2**(Suppl 2): S567-S570.

[26] Molan PC. The antibacterial activity of honey: 2. variation in the potency of the antibacterial activity. *Bee World* 1992; **73**(2): 59-76.

[27] Radwan SS, El-Essaway AA, Sarhan MM. Experimental evidence for the occurrence in honey of specific substances active against microorganisms. *Zentralbl Mikrobiol* 1984; **139**: 249-255.

[28] Bogdanov S. Nature and origin of the antibacterial substances in honey. *LWT-Food Sci Technol* 1997; **30**: 748-753.

[29] Proestos C, Zoumpoulakis P, Sinanoglou VJ. Determination of plant bioactive compounds. antioxidant capacity and antimicrobial screening. *Focusing on Modern Food Industry* 2013; **2**(1): 26-35.

[30] Sagdica O, Silicib S, Ekicic L. Evaluation of the phenolic content, antiradical, antioxidant, and antimicrobial activity of different floral sources of honey. *Int J Food Prop* 2013; **16**(3): 658-666.