



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd

Document heading

doi: 10.1016/S2222-1808(14)60504-1

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Impact of floral sources and processing on the antimicrobial activities of different unifloral honeys

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PEER REVIEW

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Comments

This is an interesting research in which authors have studied the antioxidant potential of different types of honey. Honey types exhibited antimicrobial activity against different indicator bacteria, wherein the highest antimicrobial activity was recorded for clover honey followed by citrus and cotton honeys, respectively.

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ABSTRACT

Objective: To study *in vitro* antibacterial activity and physicochemical properties of three unifloral honeys (citrus, clover and cotton honeys), and to study the impacts of storage, dilution with water (33%, w/v) and autoclaving (121 °C for 15 min) on honeys characteristics.

Methods: Honey samples from monofloral sources including citrus (*Citrus* spp.), Egyptian clover (*Trifolium alexandrinum*) and cotton (*Gossypium vitifolium*) were obtained during three successive seasons (2010–2012). Physicochemical properties and antimicrobial activities of different honey samples were studied.

Results: In honey samples stored for 12 or 24 month, colour, hydroxymethyl furfural and acidity increased, while refractive index, water activity, total soluble solids, electrical conductivity and pH remained relatively unaffected, but H₂O₂ values decreased. Types of honey exhibited various degrees of antibacterial activity against different indicator bacteria, wherein the highest antibacterial activity was recorded for clover honey followed by citrus and cotton honeys, respectively. Different species of bacteria were differed in their sensitivity to honey, wherein *Salmonella enteritidis* was the most sensitive followed by *Staphylococcus aureus*, *Listeria monocytogenes* and *Escherichia coli*, respectively. Storage up to 24 months at room temperature slightly reduced the antibacterial activity. The reduction levels were about 2.6% and 4.6% after 12 and 24 months, respectively. Diluting honeys with water increased the antibacterial activity by ca. 8.3%, while autoclaving decreased the antibacterial activity by ca. 13.5%. The relative contribution of the peroxide and non-peroxide components in the total antibacterial activity of fresh honeys was investigated. The antibacterial activity of honeys was mainly attributed to non-peroxide antibacterial agents, wherein their contribution was ca. 88%, while the contribution of H₂O₂ was only 12%. The contribution of the thermostable antibacterial components in honey was ca. 86.8%. The antibacterial activity of the fresh clover honey was compared with the effect of 16 antibiotics on indicator bacteria. Clover honey exhibited antibacterial activity comparable to that exhibited by the tested antibiotics. Moreover, antibacterial activity of water diluted fresh clover honey was higher compared to some of tested antibiotics. Clover honey appeared to deserve further investigations, since it may prove to be a promise therapeutic honey.

Conclusions: Honey samples tested in this study exhibited antibacterial activity against tested pathogenic bacteria, and this activity was mainly due to non-peroxide antibacterial factors. Clover honey appeared to deserve further investigations, since it may prove to be a promising therapeutic honey.

KEYWORDS

Honey, Monofloral, Antibacterial activity, Physicochemical properties, Antibiotics

1. Introduction

Honey is nectar collected from plants and produced by

honeybees *Apis mellifera* (*A. mellifera*). Honey has variable sensorial and physicochemical properties due to climatic and environmental conditions as well as the diverse origins

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Foundation Project: Supported by Faculty of Agriculture, Fayoum University (Grant No. 2012).

Article history:

Received 1 May 2014

Received in revised form 9 May, 2nd revised form 16 May, 3rd revised form 28 May 2014

Accepted 12 Jun 2014

Available online 28 Jun 2014

of the plants from which it is harvested^[1–8]. Honey consists of a saturated solution of sugars, of which fructose and glucose are the main contributors, and contains at least 200 bioactive substances including minerals, proteins, free amino acids, enzymes, vitamins, organic acids, flavonoids, phenolic acids, and other phytochemicals^[9–19].

Evidence about honey antioxidant potential, a parameter useful to evaluate antimicrobial activity and possible therapeutic effects, has been reported^[5,6]. Honey is known to be rich in both enzymatic and non-enzymatic antioxidants, including glucose oxidase (GOx), catalase, ascorbic acid, carotenoid derivatives, organic acids, phenolic compounds and Maillard reaction products^[4,20]. Studies indicated that the antioxidant potential of honey varies widely according to its floral source^[3,21]. Honey antioxidant potential appeared to be a result of the combined effect of a range of bioactive compounds. The therapeutic actions of honey include antioxidant and antimicrobial properties, as well as wound healing and anti-inflammatory activities^[11,12,22].

Honey has antimicrobial activity towards approximately 60 bacterial species, including aerobes and anaerobes, as well as Gram-positive and Gram-negative bacteria^[23]. The factors responsible for the antimicrobial activity include high osmolarity, acidity, H₂O₂ and non-peroxide factors. The non-peroxide antimicrobial factors of honeys include lysozyme, phenolic acids and flavonoids^[23]. The non-peroxide antimicrobial activity is insensitive to heat, light and remains intact after storage of honey for longer periods^[24]. Feás *et al.* suggested that the non-peroxide antimicrobial activity of honey is mainly affected by honeybee origin^[25]. Nonetheless, honeys from different floral sources showed various levels of antimicrobial activity.

Previous studies indicated that the floral sources used in gathering nectar and pollen affected not only the sensory and physicochemical characteristics of the honey, but also the antimicrobial activity of honey^[3,4,26–28]. Thus, the objectives of this work were to assess *in vitro* antimicrobial activity and physicochemical properties of different unifloral honeys, to investigate the contribution of peroxide and non-peroxide components in the total antimicrobial activity of fresh honeys, and to compare antimicrobial activity of the clover honey, which showed in our study the highest antimicrobial activity, with antimicrobial activity of some antibiotics.

2. Materials and methods

2.1. Honey samples

Nine honey samples from monofloral sources; citrus (*Citrus* spp.), Egyptian clover (*Trifolium alexandrinum*) and cotton (*Gossypium vitifolium*) were obtained by ordinary beekeeping practices during three successive seasons (2010–2012). Citrus honeys were obtained from an apiary situated in Banha (Egypt), while clover and cotton honeys were obtained from apiaries situated in Fayoum (Egypt). Bee colonies in these apiaries were situated in Langstroth's standard bee hives and headed with local hybrid Carniolan, *A. mellifera carnica*,

queens. Honey samples were collected in sterile screwed brown bottles. Samples of the first and second seasons were stored for 24 and 12 months at room temperature (25±5) °C in the dark until analysis. Samples of the third season were used as fresh honeys.

2.2. Indicator bacterial strains and antibiotics

Two Gram-negative bacteria [*Escherichia coli* ATCC 25922 (*E. coli*) and *Salmonella enteritidis* ATCC 13076 (*S. enteritidis*)] and two Gram-positive bacteria [*Listeria monocytogenes* ATCC 15313 (*L. monocytogenes*) and *Staphylococcus aureus* ATCC 8095 (*S. aureus*)] were used as indicator bacteria for determination of antimicrobial activity. The strains were obtained from the culture collection of Agricultural Microbiology Department, Faculty of Agriculture, Fayoum University (Egypt). *In vitro* diagnostics discs (Pasteur Lab, Egypt) of 16 antibiotics were used to compare their antimicrobial activity with that of fresh clover honey.

2.3. Physicochemical properties of honeys

Moisture content in honeys was determined using a refractometer. Ash content, total acidity, pH, refractive index, color, total soluble solid and electrical conductivity were determined according to Association of Official Analytical Chemists^[29]. Hydroxymethyl furfural (HMF) content was determined according to the method of White^[30]. Screening for peroxide accumulation as an indicator of GOx activity was carried out according to López-Sabater *et al.*^[31]. Water activity was measured according to Gleiter *et al.*^[32].

2.4. Preparation of bacterial inocula

An isolated pure colony of an overnight grown bacterial strain was picked carefully using a sterile transfer loop, inoculated to Lysogeny broth (LB) in an Erlenmeyer flask and grown overnight at the optimum temperature for each bacterial strain. About 50 µL of the culture were inoculated to 20 mL of LB broth and grown further for about 3–4 h until an optical density of 0.6 (564 nm) was achieved. The suspension was then diluted to 1:50 with LB broth in order to obtain the standard inoculum.

2.5. Assessment of antibacterial activity

2.5.1. Plate count assay

The antimicrobial activity of honey samples was screened using standard plate count method. Before applying, 100 µL of inoculums, containing a known initial counts of each tested microorganism, were thoroughly mixed with 1 mL of crude honey, honey diluted with water, diluted honey with water treated by catalase, 10 µL mL⁻¹ (329 300 IU/mL) of bovine liver catalase (Fluka), and raw honey autoclaved at 121 °C for 15 min (ADH). All treatments incubated at room temperature for 45–230 min depending on honey type and storage period. The percentage of survived viable counts of tested microorganism were determined by applying 1 mL of each

honey treatment onto sterile Petri dish, followed by mixing with agar media. The plates were leaved to solidify at room temperature for 1 h, the plates were incubated for 48 h at the optimum temperature for each bacterial strain. The inhibition was expressed as decreasing percentage of initial counts.

2.5.2. Well–diffusion bioassay^[33]

Sterilized LB agar medium was cooled to 48 °C, then 5 mL of each standard inoculum were mixed with one litter LB agar medium and 20 mL of each inoculum poured into sterile Petri dishes. When the agar was solidified, three holes of 8 mm diameter were bored per plate. Each hole was then filled with 200 µL honey and the plates were placed in refrigerator for 2 h, giving the honey enough time to diffuse. Finally, the plates were incubated for 24 h at the optimum temperature for each bacterial strain. This method was used to assess the antimicrobial activity of honey when compared with antibiotics. The antimicrobial activity was assessed by measuring the zone of inhibition (mm) against the indicator bacteria.

2.5.3. Disc diffusion assay^[34]

LB agar plates were inoculated by swabbing overnight cultures onto the surface of agar plates which allowed standing at room temperature for 3 h before antibiotic discs were applied. The plates were incubated for 24 h at the optimum temperature for each bacterial strain. The antimicrobial activity of antibiotics was assessed by measuring the zone of inhibition (mm) against the indicator bacteria.

3. Results

Honey is the naturally sweet substance produced by the honey bee *A. mellifera* from the nectar of blossoms or exudates of trees and plants giving nectar honeys or honeydews, respectively. Honeys of different floral origin have been studied for their physicochemical properties and the results are shown in Table 1. Generally, properties of fresh honeys were within the international honey standards^[35]. Regarding fresh honey samples, except values of HMF content, values of ash, electrical conductivity and the acidity were found to be the highest in cotton honey compared to clover and citrus honeys. Clover honey had higher HMF content than that of citrus and cotton honeys. However, values of HMF content in the honeys were much lower than the upper limit (40 mg/kg), which indicated the freshness of the studied honey samples. Some European bee federations marketed a part of their honey as quality honey, having a maximum level of HMF of 15 mg/kg^[35]. In samples of honeys stored for 12 or 24 month, color, HMF and acidity increased, while refractive index, water activity, total soluble solid, electrical conductivity and pH remained almost unaffected, wherein H₂O₂ values decreased. In literature, studies on correlation between the antimicrobial activity of honey and their physicochemical properties were

rare. Vorlova *et al.* reported that the higher conductivity of honey, the higher its antimicrobial activity^[36]. They also found that the pH values did not have a significant influence on antimicrobial activity. Our results showed opposite trend which may be due to number of honeys used compared to the 20 honey types used in the study of Vorlova *et al.*^[36].

Table 1

Physicochemical characteristics of different unifloral honeys produced during three successive seasons.

Properties	Citrus			Clover			Cotton			IHS
	Fresh	1 year	2 year	Fresh	1 year	2 year	Fresh	1 year	2 year	
Refractive index	1.50	1.50	1.50	1.50	1.50	1.50	1.49	1.49	1.50	–
Colour	0.04	0.05	0.09	0.04	0.06	0.11	0.11	0.15	0.22	–
Water activity	0.56	0.56	0.52	0.56	0.56	0.52	0.55	0.55	0.52	–
TSS (%)	82.00	82.00	84.00	84.00	82.00	84.00	82.00	82.00	84.00	–
Ash (%)	0.13	0.18	0.15	0.16	0.16	0.20	0.42	0.41	0.44	<0.6
HMF (mg/kg)	10.40	73.80	186.00	12.10	47.60	73.60	6.12	65.00	135.00	<40.0
EC (mScm ⁻¹)	0.40	0.44	0.34	0.42	0.41	0.50	0.88	0.85	0.91	<0.8
Acidity (mEq/kg)	10.80	14.60	15.20	11.00	11.50	11.60	28.06	33.70	37.70	<50.0
pH	4.47	4.50	4.60	4.64	4.76	4.73	4.66	4.70	4.74	–
H ₂ O ₂ (mg/L) *	2.23	1.98	0.92	2.40	2.16	1.25	3.98	3.04	1.94	–

TSS: total soluble solids, HMF: hydroxy methyl furfural, EC: electrical conductivity, IHS: international honey standard values. *: In water diluted honey (33%, w/v).

The antimicrobial activity of honey is one of the characteristics that make it beneficial to human health, but some factors can affect of this character. Phenolic compounds (flavonoids and phenolic acids), as well as non–phenolics (ascorbic acid, carotenoid–like substances, organic and amino acids, and proteins including certain enzymes such as GOx and catalase) can contribute to honey antimicrobial activity^[21]. The reasons for the antimicrobial activity of honey are so far controversial. There are two sorts of antibacterial agents or so called “inhabines”. One of them is heat–and light–sensitive and has its origin in the H₂O₂, produced by honey GOx^[3]. Some workers believe that H₂O₂ is the main antimicrobial agent^[37,38]. Others find that the non–peroxide activity is the more important one. The argument of the latter is that in ripe honey the GOx is inactive and honey contains only a small peroxide amount, not sufficient to inhibit bacterial growth. The non–peroxide antimicrobial activity is insensitive to heat and light and remains intact after storage of honey for longer periods^[24]. It has been documented that honey has a bacteriostatic and bacteriocidal effect against various species of Gram–positive and Gram–negative bacteria, as well as an antifungal effect^[39]. Extensive review on the antimicrobial activity of honey showed that, pure honey has bactericidal activity against many enteropathogenic organisms, including those of the *Salmonella* sp., *Shigella* sp., *E. coli* and was also found to be more effective as an antibacterial agent against several *Pseudomonas*, *Mycobacterium*, and *Staphylococcus* strains^[3,40]. In the present study, the antimicrobial activity of honeys of different floral origin, fresh or stored, crude or treated was *in vitro* assessed. The results in Table 2 revealed that all indicator bacteria, regardless of Gram reaction, were sensitive to tested honeys. Honeys exhibited various degrees of antimicrobial activity against different indicator bacteria as indicated by reduction percentage in initial bacterial count. However, no honey exhibited a complete inhibition

of bacterial growth at any the honey treatments tested. The exception was clover honey when diluted with water against both *S. enteritidis* and *S. aureus*.

Table 2

Antibacterial activity (%) of honeys against pathogenic bacteria as affected by storage period, heating, dilution and catalase treatment.

Treatment	Fresh year				1 year				2 year				
	Ec	Se	Lm	Sa	Ec	Se	Lm	Sa	Ec	Se	Lm	Sa	
Citrus	CH	87.5	90.1	85.4	89.1	86.7	88.0	85.0	87.3	85.1	86.6	84.8	88.3
	DH	93.9	96.3	93.0	95.9	92.8	93.5	92.0	93.8	89.4	91.9	91.1	94.8
	CDH	82.3	89.7	79.2	86.9	82.6	84.0	78.0	83.5	78.0	81.2	76.2	82.0
	ADH	81.5	89.5	78.3	86.6	80.1	83.6	77.3	82.2	77.4	80.3	75.3	81.1
Clover	CH	94.2	99.7	95.4	95.2	89.4	97.8	93.2	92.1	87.9	97.8	93.0	91.0
	DH	98.0	100.0	98.5	100.0	92.6	98.0	95.0	95.6	91.0	97.9	94.2	95.0
	CDH	87.5	98.7	92.0	90.7	81.9	97.6	89.2	87.3	80.0	97.5	88.4	83.3
	ADH	86.2	98.4	91.3	89.9	80.3	97.5	89.0	86.6	78.7	97.2	88.0	82.1
Cotton	CH	78.0	82.2	80.0	78.1	79.4	82.7	77.1	77.6	77.1	82.1	74.6	79.0
	DH	94.9	94.0	96.4	93.6	92.3	93.0	92.3	91.0	89.4	91.1	87.5	88.5
	CDH	76.2	78.3	79.4	75.1	78.2	77.5	74.2	73.1	73.1	75.2	70.9	71.9
	ADH	75.1	76.6	79.0	73.0	78.0	76.2	72.7	72.0	71.6	73.9	70.0	70.4

CH: crude honey, DH: water diluted honey (33%, w/v), CDH: catalase-treated, diluted honey, ADH: Autoclaved, diluted honey. Ec: *E. coli*, Se: *S. enteritidis*, Lm: *L. monocytogenes*, Sa: *S. aureus*.

The overall values in Table 2 indicated that honeys exhibited differing antimicrobial activity against pathogenic bacteria. Clover honey showed the highest antimicrobial activity followed by citrus and cotton honeys, respectively. This result highlights the findings that the floral origin of honey may have contributed to differences in their antimicrobial activity[41]. Results also showed that different species of bacteria differ in their sensitivity to honey wherein *S. enteritidis* was the most sensitive followed by *S. aureus*, *L. monocytogenes* and *E. coli*, respectively.

Assessment the antimicrobial activity of tested honeys as affected by storage up to 24 months revealed that these storage conditions slightly reduced the antimicrobial activity and the reduction was increased by increasing the storage period (Table 2). The average reduction percentages were 2.6% and 4.6%, after 12 and 24 months, respectively. This result is in agreement with that of Bogdanov who found that the non-peroxide antimicrobial activity of honey was only slightly affected by storage at room temperature for 15 months[42].

Regarding the effect of different processing or treatments on the antimicrobial activity of honeys, the results indicated that antimicrobial activity was slightly increased by diluting honeys with water (33%, w/v) (Table 2). These effects were varied according the floral origin of honey, and it was the highest for cotton honey and the lowest for clover honey (ca. 8.3%). The effect of dilution with water is expected as honey when diluted with water; the bee-derived GOx present in honey is activated and catalyzed slow generation of H₂O₂[27]. On the other hand, when the diluted honey treated with catalase, antimicrobial activity was reduced which indicates that H₂O₂ contributes to antimicrobial activity of the tested honeys.

Concerning the effect of autoclaving, the antimicrobial activity of these honeys were relatively decreased. The

decreasing percentage was the highest for cotton honey and the lowest for clover honey (ca. 13.5%). In this respect, White and Subers found that heating of honey at 70 °C had no or very little effect on the non-peroxide antimicrobial activity[43], whereas the peroxide accumulation capacity is severely damaged. This finding may explains the results in Table 3 which show that the tested honeys retain most of their antimicrobial activity (ca. 87%) after heating.

Table 3

Relative contribution of peroxide and non-peroxide antibacterial factors in the total antimicrobial activity of different unifloral honeys.

Honeys	Total AA ¹		Peroxide AA ²		Non-peroxide ³		Thermolabile AA ⁴		Thermostable AA ⁵	
	AA	%	AA	% ⁶	AA	%	AA	%	AA	%
Citrus	94.8	100	10.0	10.5	84.8	89.5	11.3	11.9	83.5	88.1
Clover	99.1	100	6.9	7.0	92.2	93.0	7.6	7.7	91.5	92.3
Cotton	94.7	100	17.4	18.4	77.3	81.6	18.8	19.9	75.9	80.1
Average		100		12.0		88.0		13.2		86.8

AA: antimicrobial activity, DH: water diluted honey (33%, w/v), CDH: catalase-treated, diluted honey, ADH: Autoclaved, diluted honey. 1: AA of DH, 2: AA of DH-AA of CDH, 3: AA of CDH, 4: AA of DH-AA of ADH, 5: AA of ADH, 6: related to total AA.

It was reported that antimicrobial activity of most honeys depends mainly on the enzymatic generation of H₂O₂, and the phytochemical (non-peroxide) components make only a minor contribution to the honey antimicrobial activity[26]. It was also reported that for few honeys, unidentified non-peroxide components make a major contribution[27]. The Manuka honey from the plant *Leptospermum scoparium* grown in New Zealand had antimicrobial activity being of phytochemical origin and it was suggested that this honey had specific antimicrobial activity due to non-peroxide agents[27,44,45].

In the present study, the relative contribution of both peroxide and non-peroxide antimicrobial agents to the total antimicrobial activity of the fresh honeys was determined and the results are given in Table 3. These results suggest that the antimicrobial activity of different unifloral honeys is mainly attributed to non-peroxide antibacterial factors which contributed to ca. 88%, while the contribution of H₂O₂ was only 12% to the total antimicrobial activity of honeys. To the best of authors' knowledge, this finding was not reported in the previous studies on honeys. The results also indicate that the contribution of the thermostable antibacterial components in honey was ca. 86.8%. According to Roberts *et al.*[44], honeys with non-peroxide antimicrobial activity are likely to be more effective *in vivo* as compared with honeys with H₂O₂ antimicrobial activity which would be relatively inactivated by the catalase in tissues and blood.

The emergence of multi-antibiotic resistant bacteria created a lot of concern in the medical field; hence, there is a need to find an alternative to counter these multi-antibiotic resistant bacteria. In the present study, the antimicrobial activity of the fresh clover honey, which showed the highest antimicrobial activity, was compared with the antimicrobial activity of 16 different antibiotics. The results in Table 4 show that the clover honey exhibited antimicrobial activity comparable to that exhibited by the tested antibiotics. Moreover, antimicrobial activity of water

diluted honey was generally higher compared to that of the tested antibiotics. However, these results should be regarded as indicative rather than conclusive since two different methods were used to assay antimicrobial activity and different doses were applied. In a study of Farouk *et al.*[46], honey was found to be more effective as antibacterial agent against *Pseudomonas* and *Staphylococcus* strains than the antibiotic gentamicin. Karayil *et al.* also found that honey at concentrations of 30%–50% was superior to cephaloridine and gentamicin in inhibiting growth of nine pathogenic bacterial[47].

Table 4

Antimicrobial activity of fresh clover honey in comparison to 16 antibiotics.

Antibacterial agent	Inhibition zone diameter (mm)			
	Ec	Se	Lm	Sa
Clover honey* Crude	38	31	35	36
Water diluted (33% w/v)	42	38	43	40
Autoclaved	35	28	31	29
Antibiotics** Streptomycin 10 µg	28	30	19	36
Ampicillin 10 µg	28	30	28	38
Erthromycin E 10 µg	20	28	13	–
Neomycin 30 µg	–	28	14	32
Chloramphenicol 30 µg	7	25	22	25
Zinnat cmx 30 µg	20	16	–	26
Germycin 10 µg	24	28	–	32
Augmentin 30 µg	30	35	30	46
Rocephan 30 µg	28	18	7	30
Pyopen 100 µg	36	22	12	22
Rifadn 30 µg	8	24	17	16
Colistin sulfate 10 µg	18	24	15	32
Claforan 30 µg	18	–	–	18
Amiks 30 µg	30	22	–	38
Penicillin 10 µg	–	–	–	20
Negram 30 µg	19	25	–	8

Ec: *E. coli*, Se: *S. enteritidis*, Lm: *L. monocytogenes*, Sa: *S. aureus*. *: well-diffusion assay (8 mm well with 200 µL honey), **: disc diffusion assay.

4. Discussion

The flower type used in the harvesting of nectar and pollen by bees affects the composition of the produced honey, resulting in variation of the biological activities of honeys from different sources[4,48]. The heterogeneous geomorphology of Egypt and the rich floral diversity offer the opportunity for production of a variety of honeys from different floral origins. We compared the antimicrobial activity activities of honeys from different floral sources. The major antibacterial factors in honey are H₂O₂, catalase, and COx. Non-peroxide factors also contribute to the antimicrobial activity of honey, including lysozyme, phenolic acids, and flavonoids[49].

Since honey is produced by bees from floral nectars, it is reasonable to believe that the floral source of the honey could affect its chemical composition[3,4]. Flavonoids and other phenolic components in nectar have antimicrobial activity and inhibit the growth of a wide range of Gram-negative and Gram-positive bacteria. The mechanisms responsible for the antimicrobial activity of polyphenols include membrane disruption, metal ion complexation, and

enzyme inhibition by polyphenols[3,50]. Moreover, several authors have concluded that honey from certain plants has better antimicrobial activity than that of others[28,49].

Bogdanov mentioned that factors responsible for the antimicrobial activity of honey are high osmolarity[25], acidity and particularly H₂O₂, which is formed from the oxidation of glucose by COx, during ripening of honey[50]. COx originates from the hypopharyngeal glands of honeybees[23]. When H₂O₂ is removed by adding catalase, some honeys still show significant antimicrobial activity[51] and this activity is referred to as non-peroxide antimicrobial activity. The non-peroxide factors of honeys include lysozyme, phenolic acids and flavonoids[23]. Bogdanov suggested that the main part of the non-peroxide antimicrobial activity might be of honeybee origin, while part may be of plant origin[25]. Wahdan also suggested that flavonoids and phenolic acids might be a part of the antimicrobial activity of honey[28]. The non-peroxide antimicrobial activity is more heat- and light insensitive than the H₂O₂, and remains intact after storage of honey for long periods. Therefore, it was found that the non-peroxide antimicrobial activity is more important than the H₂O₂ in terms of antimicrobial activity[23]. However, the contribution to antibacterial properties of non-peroxide antimicrobial activity may be smaller than that of H₂O₂[52]. Thus, for optimum antimicrobial activity, honey should be stored in a cool, dark place and be consumed when fresh.

An Australian honey from *Leptospermum polygalifolium* has been found to possess a high level of non-peroxide antimicrobial activity[52], though the cause of the non-peroxide antimicrobial activity is still unclear and requires further investigation[10].

In conclusion, different unifloral honeys tested in this study exhibited high antimicrobial activity against tested pathogenic bacteria, and this activity was due mainly to non-peroxide antibacterial factors. The antimicrobial activity of honeys can withstand storage at room temperature for 24 months and autoclaving. Among these honeys, clover honey appeared to deserve further investigations, since it may prove to be a promising natural food preservative and/or a valuable therapeutic honey.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This research was funded by Faculty of Agriculture, Fayoum University (Grant No. 2012).

Comments

Background

The therapeutic actions of honey include antioxidant and antimicrobial properties, as well as wound healing and anti-

inflammatory activities. Evidence about honey antioxidant potential, a parameter useful to evaluate antimicrobial activity and possible therapeutic effects, has been reported. Studies indicated that the antioxidant potential of honey varies widely according to its floral source. Honey antioxidant potential appeared to be a result of the combined effect of a range of bioactive compounds.

Research frontiers

The aims of this work were to assess *in vitro* antimicrobial activity and physicochemical properties of different unifloral honeys, and to investigate the contribution of peroxide and non-peroxide components in the total antimicrobial activity of fresh honeys. Another important goal was to compare antimicrobial activity of the clover honey, with antimicrobial activity of some antibiotics.

Related reports

Honey is known to be rich in both enzymatic and non-enzymatic antioxidants, including glucose oxidase, catalase, ascorbic acid, carotenoid derivatives, organic acids, phenolic compounds and Maillard reaction products. Reports about honey antioxidant potential, a parameter useful to evaluate antimicrobial activity and possible therapeutic effects, have been published.

Innovations & breakthroughs

The results suggest that different unifloral honeys exhibited high antimicrobial activity against different pathogenic bacteria, and this activity was due mainly to non-peroxide antibacterial factors.

Applications

Honey has variable sensorial and physicochemical properties due to climatic and environmental conditions as well as the diverse origins of the plants from which it is harvested. Clover honey appeared to deserve further investigations, since it may prove to be a valuable therapeutic honey.

Peer review

This is an interesting research in which authors have studied the antioxidant potential of different types of honey. Honey types exhibited antimicrobial activity against different indicator bacteria, wherein the highest antimicrobial activity was recorded for clover honey followed by citrus and cotton honeys, respectively. Different species of bacteria were differ in their sensitivity to honey, wherein *S. enteritidis* was the most sensitive followed by *S. aureus*, *L. monocytogenes* and *E. coli*, respectively. Diluting honeys with water increase the antimicrobial activity by ca. 8.3%, while autoclaving decreased the antimicrobial activity by ca. 13.5%. The antimicrobial activity of honeys was mainly attributed to non-peroxide antibacterial agents, wherein their contribution was ca. 88%, while the contribution of H₂O₂ was only 12%. The antimicrobial activity of the fresh clover honey was compared with the effect of 16 antibiotics on indicator bacteria. Clover honey exhibited antimicrobial activity comparable to that exhibited by the tested antibiotics. Clover honey appeared to deserve

further investigations, since it may prove to be a promise therapeutic honey.

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