

The Antimicrobial Action of Honey

1. Effect of Temperature on Antibacterial Activity of Honey

Nongluksna Sriubolmas M.Sc. (Pharm)*

Areerat Laorpaksa M.Sc. (Pharm)*

Saree Virunhaphol M.Sc. (Pharm)*

Abstract

Ten samples of pure honey and one sample of control solution containing 80% of glucose and fructose in the ratio of 1:1 were heated at the temperature of 63°, 80°, 100° and 121°C for 30 minutes. Each sample was then determined for viscosity and also for antibacterial activity using modified disc susceptibility test. Although the viscosity of the untreated honey samples varied from 80.43 cps to 7,507.77 cps, the diameters of the inhibition zone of most untreated honey samples were not different. The antibacterial activity of honey was affected by temperatures particularly at 100° and 121°C. This might result from the heat degradation of some antibacterial substances in honey. Viscosity of honey sample increased when heated them at various temperatures. However, the viscosity did not correlate to antibacterial activity of honey.

เรื่องย่อ

ฤทธิ์ต้านจุลชีพของน้ำผึ้ง: 1. ผลของอุณหภูมิต่อฤทธิ์ต้านแบคทีเรียของน้ำผึ้ง
 นงลักษณ์ ศรีอุบลมาศ ภ.ม.,* อารีรัตน์ ลออปักษา ภ.ม.,* สารี วิรุณหพผล ภ.ม.*
 *ภาควิชาจุลชีววิทยา คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
 วารสารโรคติดเชื้อและยาด้านจุลชีพ 2535; 9:1-5.

น้ำผึ้ง 10 ตัวอย่างและกลุ่มควบคุมซึ่งประกอบด้วยน้ำตาลร้อยละ 80 โดยมีกลูโคสและฟรักโทสในอัตราส่วน 1:1 ทำให้อุ่นที่อุณหภูมิ 63°, 80°, 100° และ 121°ซ. เป็นเวลา 30 นาที วัดความหนืดและหาฤทธิ์ต้านแบคทีเรียโดยวิธีที่ดัดแปลงจาก disc susceptibility test ตัวอย่างน้ำผึ้งที่ไม่ได้ทำให้อุ่นมีความหนืดแตกต่างกันมากคือ ระหว่าง 80.43-7,507.77 cps แต่เส้นผ่าศูนย์กลางของโซนใสที่เชื้อไม่ขึ้นของน้ำผึ้งส่วนใหญ่ไม่แตกต่างกัน ความร้อนมีผลต่อฤทธิ์ต้านแบคทีเรียของน้ำผึ้ง โดยเฉพาะความร้อนที่อุณหภูมิ 100° และ 121°ซ. ผลนี้อาจเกิดจากการที่ความร้อนทำลายสารต้านแบคทีเรียบางชนิดในน้ำผึ้ง ความร้อนทำให้น้ำผึ้งมีความหนืดมากขึ้น อย่างไรก็ตาม ความหนืดไม่มีความสัมพันธ์กับฤทธิ์ต้านแบคทีเรียของน้ำผึ้ง

*Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University

INTRODUCTION

Purified honey is used officially in pharmaceutical preparations as demulcent and sweetening agent.¹ It also has antimicrobial activity²⁻⁴ and has been used in enhancing the healing of wounds by topical application.^{4,5} Several mechanisms concerning the antimicrobial activity of honey have been suggested. For example, sugar in honey causes high osmotic pressure at the wound surface and induces an unfavorable low water activity there by inhibiting microbial growth. In addition, the fermentation of honey also produces alcohol in-situ which may be toxic to the microorganism.⁶ However, it was found that honey contained a bactericidal substance called inihbine which was thermolabile and could be destroyed by direct sunlight. It also contained another group of antibacterial substances which was light sensitive but relatively heat-stable. These substances were destroyed by heating at 80°C.²

In this study, we have studied the effect of temperature on the antibacterial activity of honey and also on the viscosity of honey which might be correlated to the antibacterial activity of it. The possible antibacterial mechanism of honey would be discussed.

MATERIALS AND METHODS

1. Honey Nine samples of locally obtained honey from various provinces in Thailand and one sample of imported honey for commercial consumption from U.S.A. (Table 1), all of which passed the test for invert sugar substitute by the method specified in the Pharmaceutical Codex 11th ed,⁷ were used. The samples were heated at 63°, 80°, 100° and 121°C for 30 minutes.

Table 1 Sources and locations of honey passing the test for invert sugar substitute.

Sample No.	Sources	Locations (Province/country)
3	Longan	Chiangrai
5	Litchi	Lumpang
7	-	Ubolrajdhani
9	Longan	Lumpang
11	-	Lumpoon
13	-	Lumpang
15	-	U.S.A.
16	-	Chumporn
17	Litchi	Chiangmai
18	Longan	Chiangmai

- = Not specified

2. Control solution Control solution containing 80per cent of glucose and fructose in the ratio of 1:1 was also heated at 63°, 80°, 100° and 121°C as well.

3. Test organisms *Staphylococcus aureus* ATCC 25923, *Micrococcus luteus* ATCC 9341, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 10031.

4. Medium Mueller Hinton agar (Difco).

5. Preparation of inoculum Each organism was grown on Mueller Hinton agar slant at 37°C overnight. The inoculum was prepared in sterile saline and diluted to obtain a turbidity comparable to the 0.5 McFarland turbidity standard.

6. Determination of viscosity of honey The viscosity of honey samples and control solution were measured using Brookfield digital viscometer.

7. Determination of antibacterial activity of honey by using modified disc susceptibility test⁸ Plates with internal diameter of 100 mm containing 25 ml of Mueller Hinton agar were inoculated by streaking method. Six sterile stainless steel cylinders (6 mm internal diameter and 10 mm height) were placed on the inoculated agar surface and filled with honey and control solution. After maintaining at room temperature for 15 minutes, the plates were incubated at 37°C overnight. The results were obtained by measuring the diameters of inhibition zone. The determination were carried out in triplicate.

RESULTS

Honey samples exhibited a wide range of viscosity from 80.43 cps to 7,507.77 cps (Table 2). The lowest viscosity obtained from sample number 16 was still approximately two fold higher than that of the control solution as shown in Table 2. However, the diameters of inhibition zone of most honey samples did not different (Table 2). The control solution had no effect on the test organisms (Table 2). The honey samples could be grouped based on the significant difference of the diameters of inhibition zone (Fisher's Least Significant Difference Multiple Comparison Test at $p < 0.05$), as shown in Table 3. For *S. aureus* the honey samples could be divided into 2 groups, one group contained honey sample No. 3, 6, 9, 11, 13 and 17 while the other group contained honey sample No. 7, 15, 16 and 18. For *M. luteus*, they could be grouped into 3 groups, honey sample No. 3, 6, 9, 11, 13, 15 and 17 for group one, sample No. 7 and 18 for group two and sample No. 16 for group three. For *B. subtilis*, sample No. 3, 6, 7, 9, 11, 13, 15 and 18 could be grouped together and sample No. 16 and 17 could be group together. For *E. coli*, the honey samples could be grouped into only one group including sample No. 3, 6, 9, 11, 13, 16 and 17, the others could not be grouped

Table 2 Viscosity and antibacterial activity of honey and control solution.

Sample No.	Viscosity (cps)	Inhibition Zone Diameter (mm±S.E.M.)					
		<i>S. aureus</i>	<i>M. luteus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>
3	1,634.30	14.96±1.10	11.58±0.44	12.82±0.68	12.24±0.61	14.18±0.40	13.00±1.56
6	2,863.10	13.28±0.80	11.30±0.08	11.42±0.72	12.60±0.94	12.38±0.97	11.96±0.65
7	914.19	10.84±0.25	9.5±0.41	11.52±0.68	10.24±0.18	11.30±0.54	11.28±0.36
9	2,027.52	14.78±1.07	10.98±0.29	13.14±0.86	12.70±0.95	15.00±0.25	12.04±1.03
11	7,507.97	15.42±0.61	11.62±0.30	12.88±0.31	13.66±0.74	14.72±0.56	13.60±0.82
13	2,813.95	13.54±1.11	11.64±0.25	12.10±0.35	13.18±0.60	13.32±0.17	12.30±0.33
15	4,632.58	11.06±0.35	10.64±0.37	12.04±0.84	11.02±0.32	13.28±0.55	13.78±0.82
16	80.43	10.76±0.26	17.96±0.30	15.84±0.32	13.30±0.90	12.68±0.42	12.66±0.43
17	2,125.82	14.38±1.06	11.10±0.62	14.44±0.71	12.14±0.47	13.20±0.87	13.62±1.42
18	2,531.33	11.22±0.19	9.04±0.49	11.74±0.52	11.40±0.94	13.88±1.15	13.44±0.36
C	42.88	0	0	0	0	0	0

S.E.M. = Standard error of mean

Table 3 Honey samples grouped according to the diameter of inhibition zone.^a

Test Organisms	Group of Honey Sample		
	1	2	3
<i>S. aureus</i>	3, 6, 9, 11, 13, 17	7, 15, 16, 18	-
<i>M. luteus</i>	3, 6, 9, 11, 13, 15, 17	7, 18	16
<i>B. subtilis</i>	3, 6, 7, 9, 11, 13, 15, 18	16, 17 ^b	-
<i>E. coli</i>	3, 6, 9, 11, 13, 16, 17	-	-
<i>P. aeruginosa</i>	3, 9, 11, 13, 15, 17, 18	6 ^c , 7, 16 ^c	-
<i>K. pneumoniae</i>	3, 6, 9, 11, 13, 15, 16, 17, 18	7 ^d	-

a = The diameters of inhibition zone differed statistically significant ($p < 0.05$) between groups but had no difference within group.**b** = Did not differ from sample No. 3, 9 and 11.**c** = Only differed significantly from sample No. 9 and 11.**d** = Differed significantly from sample No. 11, 15, 17 and 18.

together. For *P. aeruginosa*, sample No. 3, 9, 11, 13, 15, 17 and 18 could be grouped together and sample No. 6, 7 and 16 could be grouped together. For *K. pneumoniae*, all samples exhibited no significant difference in inhibition zone diameter except that of sample No. 7 which differed from those of sample No. 11, 15, 17 and 18.

After heating honey at various temperatures, the antibacterial activity against gram positive and gram negative test organisms were exhibited in different pattern as shown in Fig. 1. For *S. aureus*, four honey samples heated at 63° and 80°C showed a decrease in inhibition zone diameter as compared to the untreated honey, but one sample of 80°C heated honey showed an increase. For 100°C heated honey samples, two pattern of inhibition zone diameters were observed, decrease in seven samples and increase in two samples. For 121°C

treated honey, nine samples showed an increase in inhibition zone diameters comparing to the 100°C heated honey. Moreover, four of these nine samples also showed an increase comparing to the unheated honey. In addition, one sample that did not show different inhibition zone diameter from the 100°C heated honey also showed an increase comparing to the unheated honey.

For *M. luteus*, one sample of the 63° and 80°C heated honey showed an increase and a decrease in inhibition zone diameters, respectively. For 100°C heated honey, the inhibition zone diameters of two samples showed an increase and that of one sample showed a decrease. For 121°C heated honey, the inhibition zone diameters of nine samples showed an increase as compared to those of the 100°C heated honey and unheated honey.

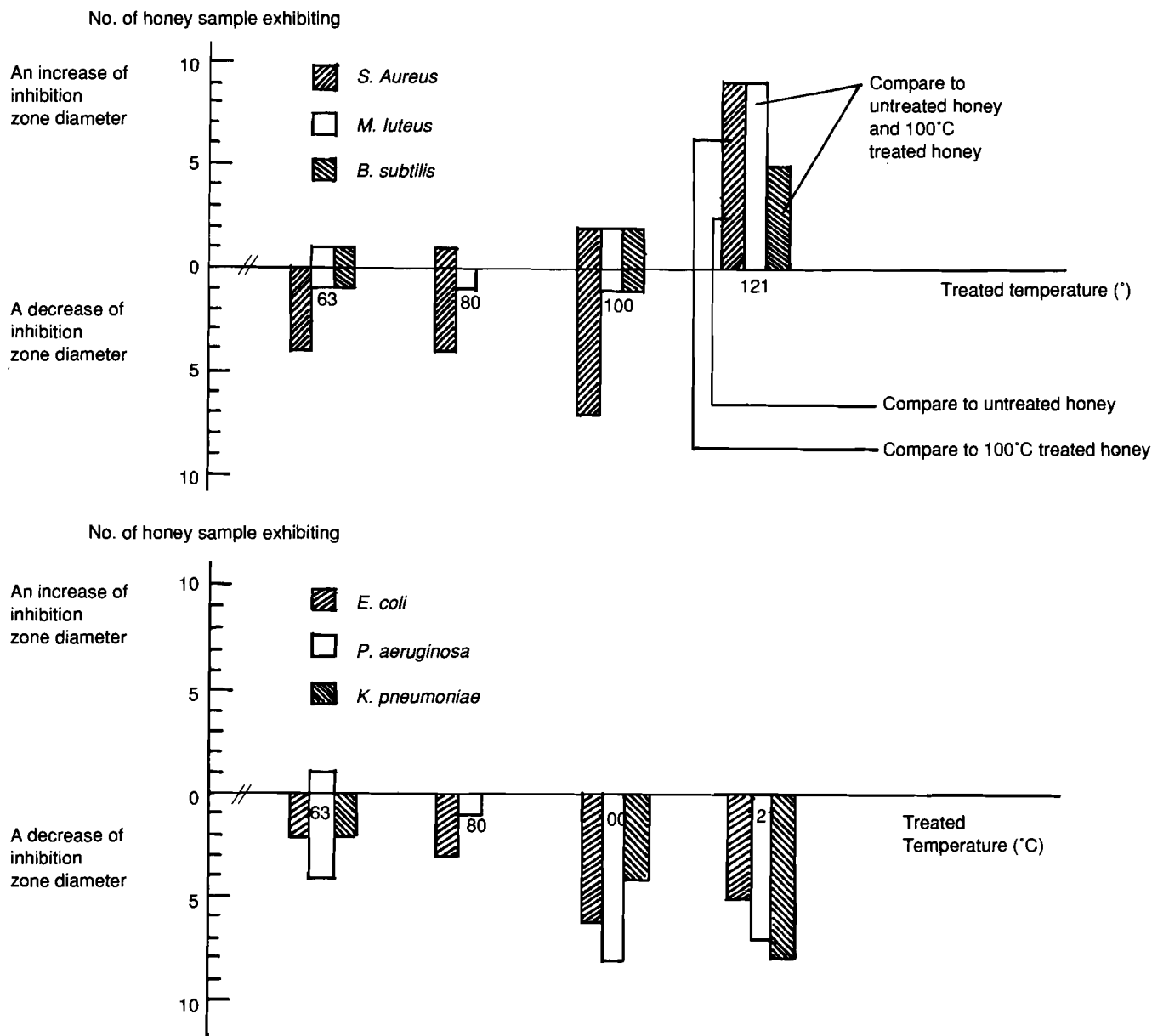


Fig. 1 Effect of temperature on antibacterial activity of honey.

For *B. subtilis*, the inhibition zone diameters of 63°C heated honey increased in one sample and decreased in another one. For 100°C heated honey, the inhibition zone diameters of two samples showed an increase and that of another sample showed a decrease. For 121°C heated honey, the inhibition zone diameters of five samples showed an increase comparing to those of the 100°C heated honey and to those of the unheated honey.

For *E. coli*, 2, 3, 6 and 5 samples of 63°, 80°, 100° and 121°C heated honey respectively, showed a decrease in inhibition zone diameters.

For *P. aeruginosa*, 1 samples of the 63°C heated honey showed an increase in inhibition zone diameter and 4 samples

showed a decrease. For 80°C heated honey, only 1 sample showed a decrease in the inhibition zone size, while for 100° and 121°C heated honey, 8 and 7 samples, respectively, showed a decrease in the inhibition zone size.

For *K. pneumoniae*, 2 samples of 63°C heated honey, 4 samples of the 100°C heated honey and 8 samples of the 121°C heated honey showed a decrease in the inhibition zone size.

No inhibition zone against the test organisms was observed in the heated control solution except for the 121°C heated solution which exhibited the inhibition zone diameters of 11.7 ± 0.21 , 9.88 ± 0.38 , 10.96 ± 0.16 , 8.70 ± 0.25 and 8.80 ± 0.35 mm on *S. aureus*, *M. luteus*, *B. subtilis*, *P. aeruginosa* and *K. pneumoniae*, respectively.

The viscosity of heated honey increased variably, the ranges were 106.84-7,950.34 cps, 144.29-7,729.15 cps, 207.53-7,680 cps and 200.78-7,360.51 cps for 63°, 80°, 100° and 121°C heated honey, respectively. For heated control solution, the viscosity did not differ from the viscosity of unheated control solution.

DISCUSSIONS AND CONCLUSIONS

The antibacterial activity of most honey samples against test organisms regarded to the inhibition zone diameter were not different. The control solution containing approximately the same amount of sugar in honey has no antibacterial activity. This result supported the earlier study which mentioned that honey contained some antibacterial substances.²

After heating honey at various temperatures, we found that temperature played some roles on the antibacterial activity of honey against gram positive and gram negative bacteria in different manners. Antibacterial activity against *S. aureus* of 7 in 10 samples of the 100°C heated honey was affected by temperature which cause a decrease in the activity. In contrary, the antibacterial activity against gram positive bacteria of most of 121°C heated honey samples showed an increase. Against gram negative bacteria, the antibacterial activity of most honey samples was shown to be decreased when the samples were heated both at 100° and 121°C. The antibacterial activity of 121°C heated control solution against all test organisms except for *E. coli* was shown to be increased.

Therefore, it may also be suggested that the antibacterial activity of honey was resulted from some antibacterial substances containing in honey. Among these substances, the one which was inhibitory against *S. aureus* and various gram negative bacteria seemed to be heat labile whereas the substance against *M. luteus* and *B. subtilis* seemed to be heat stable. The heat stability of these substances may be influenced by other factor such as the pH of the sample. This may be the explanation of the variation in the results obtained from different samples. The increase of antibacterial activity against gram positive bacteria of some of the 63°, 80° and 100°C heated honey sample, most of 121°C heated honey samples and 121°C heated control solution may be resulted from the degradation products of sugars. On sterilization, glucose degrades to a product which subsequently degrades to 5-hydroxy-methylfurfural and finally to formic acid and laevulinic acid.⁹ The degradation products may have little or no effect on gram negative bacteria. Our results suggested that in the determination of minimal inhibitory concentration of honey, it should be added honey into culture medium after sterilization.

We found that viscosity of honey samples increased when heated them at various temperature, but the viscosity did not correlate to antibacterial activity of honey.

ACKNOWLEDGEMENT

The authors wish to express their deep thanks to Professor Dr. Samuel Halevy, Department of Pharmaceutical Chemistry, School of Pharmacy, Hebrew University, Jerusalem, for his valuable suggestion concerning this work, to Assistant Professor Dr. Pintip Pongpech, Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for her comments and her computer program for statistical analysis, to Department of Microbiology, Faculty of Medicine, Siriraj Hospital, Mahidol University, for the culture of *P. aeruginosa* ATCC 27853, and to Department of Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University for the viscometer.

This study is supported by the grant from the National Research Council of Thailand.

REFERENCES

1. British Pharmacopoeia. Vol. 1. London: London Her Majesty's Stationery Office, 1988:287.
2. Crane E. Honey a comprehensive survey. London: Heinemann, 1977:260-1.
3. Jeddar A, Kharsany A, Ramsaroop UG, Bhamjee A, Haffejee JE, Moosa A. The antibacterial action of honey, an *in vitro* study. S Afr Med J 1985; 67:257-8.
4. Cavanagh D, Beazley J, Ostapowicz F. Radical operation for carcinoma of the vulva, a new approach to wound healing. J Obstet Gynaecol Br Cwlth 1970; 77:1037-40.
5. Bergman A, Yanai J, Weiss J, Bell D, David MP. Acceleration of wound healing by topical application of honey, an animal model. Am J Surg 1983; 145:374-6.
6. Obaseiki-Ebor EE, Afonya TCA, Onyekweli AO. Preliminary report on the antimicrobial activity of honey distillate. J Pharm Pharmacol 1983; 35:748-9.
7. The Pharmaceutical Codex. 11th ed. London: The Pharmaceutical Press, 1979:415-6.
8. Lorian V. Antibiotics in Laboratory Medicine. Baltimore, London: Williams & Wilkins, 1980:29-31.
9. Taylor RB, Jappy BM, Neil JM. Kinetics of dextrose degradation under autoclaving conditions. J Pharm Pharmacol 1971; 23:121-9.