รายงานผลการวิจัย

เรื่อง

ฤทธิ์ต้านจุลชีพของน้ำผึ้ง

The Antimicrobial Action of Honey

โดย

นงลักษณ์ ศรีอุบลมาศ
อารีรัตน์ ลออปีกษา
สารี วิรุฑทพผล

2534

จุฬาลงกรณ์มหาวิทยาลัย
ทุนงบประมาณแผ่นดิน ประจำปี 2531

รายงานผลการวิจัย

เรื่อง
ฤทธิ์ต้านจุลชีพของน้ำผึ้ง

The Antimicrobial Action of Honey

โดย

นงลักษณ์ ศรีอุบลมาศ
อารีรัตน์ ลออปักษา
สารี วิรุฬหผล

2534

ชื่อโครงการวิจัย ฤทธิ์ต้านจุลชีพของน้ำผึ้ง
(1) ผลของอุณหภูมิต่อฤทธิ์ต้านแบคทีเรียของน้ำผึ้ง

ชื่อผู้ทำวิจัย นงลักษณ์ ศรีอุบลมาศ
อารีรัตน์ ลออปักษา
สารี วิรุฬหผล

เดือนและปีที่ทำวิจัยเสร็จ มีนาคม 2534

บทคัดย่อ

น้ำผึ้ง 10 ตัวอย่าง และกลุ่มควบคุมซึ่งประกอบด้วยน้ำตาล 80% โดยมีกลูโคสและฟรักโทสในอัตราส่วน 1:1 ทำให้ร้อนที่อุณหภูมิ 63°, 80°, 100° และ 121°ซ เป็นเวลา 30 นาที วัดความหนืด และหาฤทธิ์ต้านแบคทีเรียโดยวิธีที่ดัดแปลงจาก disc susceptibility test ตัวอย่างน้ำผึ้งที่ไม่ได้ทำให้ร้อนมีความหนืดแตกต่างกันมาก คือ ระหว่าง 80.43 - 7,507.77 cps แต่เส้นผ่าศูนย์กลางของโซนใสที่เชื้อไม่ขึ้นของน้ำผึ้งส่วนใหญ่ไม่แตกต่างกัน ความร้อนมีผลต่อฤทธิ์ต้านแบคทีเรียของน้ำผึ้ง โดยเฉพาะความร้อนที่อุณหภูมิ 100° และ 121°ซ ผลนี้อาจเกิดจากการที่ความร้อนทำลายสารต้านแบคทีเรียบางชนิดในน้ำผึ้ง ความร้อนทำให้น้ำผึ้งมีความหนืดมากขึ้น อย่างไรก็ตามความหนืดไม่มีความสัมพันธ์กับฤทธิ์ต้านแบคทีเรียของน้ำผึ้ง

ชื่อโครงการวิจัย ฤทธิ์ต้านจุลชีพของน้ำผึ้ง
(2) ฤทธิ์ต้านแบคทีเรียของน้ำผึ้ง

ชื่อผู้ทำวิจัย นงลักษณ์ ศรีอุบลมาศ
สารี วิรุฬผล
อารีรัตน์ ลออปักษา

เดือนและปีที่ทำวิจัยเสร็จ มีนาคม 2534

บทคัดย่อ

การศึกษาฤทธิ์ต้านแบคทีเรียของน้ำผึ้ง 10 ตัวอย่าง โดยเปรียบเทียบความแรงกับยาเพนิซิลลิน และเตตราซัยคลิน พบว่า ฤทธิ์ต้านเชื้อ Staphylococcus aureus ของน้ำผึ้งสมมูลกับเพนิซิลลินความแรง 0.675 - 1.45 หน่วย/มิลลิลิตร และฤทธิ์ต้านเชื้อ Escherichia coli ของน้ำผึ้งสมมูลกับเตตราซัยคลินความแรง <14.7-28.1 ไมโครกรัม/มิลลิลิตร เลือกน้ำผึ้งมา 5 ตัวอย่าง ที่มีฤทธิ์สมมูลกับความแรงของเพนิซิลลินในช่วงต่างๆ ต่อเชื้อ Staphylococcus aureus นำมาหาค่าความเข้มข้นต่ำสุดที่ยับยั้งการเจริญของเชื้อ Staphylococcus aureus และ Escherichia coli ชนิดละ 30 สายพันธุ์ รวมทั้งสายพันธุ์มาตรฐาน เชื้อทดสอบส่วนใหญ่เป็นสายพันธุ์ที่ต้านยาปฏิชีวนะ ความเข้มข้นต่ำสุดของน้ำผึ้งที่ยับยั้งการเจริญของเชื้อ Staphylococcus aureus และ Escherichia coli มีค่าอยู่ระหว่าง 0.1 - 0.3 กรัม/มิลลิลิตร และ 0.15 - 0.35 กรัม/มิลลิลิตร ตามลำดับ สำหรับกลุ่มควบคุมซึ่งประกอบด้วยน้ำตาล 80% คือ กลูโคส : ฟรักโทส ในอัตราส่วน 1:1 ไม่สามารถยับยั้งการเจริญของแบคทีเรียได้ จากผลการทดลองแสดงให้เห็นว่าน้ำผึ้งตัวอย่างต่างๆ มีฤทธิ์ต้านแบคทีเรียใกล้เคียงกัน และมีฤทธิ์ต้านแบคทีเรียสายพันธุ์ที่ดื้อยาปฏิชีวนะ

ชื่อโครงการวิจัย ฤทธิ์ต้านจุลชีพของน้ำผึ้ง
(3) ฤทธิ์ต้านเชื้อราของน้ำผึ้ง

ชื่อผู้ทำวิจัย อารีรัตน์ ลออปักษา
สารี วิรุฬหผล
นงลักษณ์ ศรีอุบลมาศ

เดือนและปีที่ทำวิจัยเสร็จ มีนาคม 2534

บทคัดย่อ

น้ำผึ้งทดสอบ 10 ตัวอย่าง มีฤทธิ์ยับยั้งการเจริญเติบโตของ Trichophyton mentagrophytes , Microsporum gypseum , และ Epidermophyton floccosum โดยมีค่าเฉลี่ยเส้นผ่าศูนย์กลางของโซนใสอยู่ในช่วง 19.58 ± 1.05 ถึง 31.00 ± 0.92 , 17.00 ± 0.61 ถึง 29.62 ± 0.29 และ 20.40 ± 1.59 ถึง 46.36 ± 1.06 มิลลิเมตร ตามลำดับ น้ำผึ้งจากต่างประเทศมีฤทธิ์ยับยั้งการเจริญต่ำสุด ในขณะที่น้ำผึ้งตัวอย่างจากจังหวัดชุมพรให้ค่าสูงสุดจากตัวอย่างน้ำผึ้งอื่นๆ อย่างมีนัยสำคัญทางสถิติ ตัวอย่างน้ำผึ้งทั้งหมดไม่มีผลยับยั้งการเจริญเติบโตของ Aspergillus niger และเชื้อยีสต์ 2 ชนิด Saccharomyces cerevisiae และ Candida albicans เมื่อเปรียบเทียบกับกลุ่มควบคุมซึ่งประกอบด้วยกลูโคส : ฟรักโทส ในอัตราส่วน 1:1 พบว่า ไม่มีฤทธิ์ต้านเชื้อทดสอบทุกชนิด ในการหาความเข้มข้นต่ำสุดที่สามารถยับยั้งการเจริญเติบโต (MICs) โดยใช้น้ำผึ้ง 2 ตัวอย่าง ได้ค่า MICs ต่อ Trichophyton mentagrophytes เท่ากับ Microsporum gypseum คือ อยู่ในช่วง 10-30 มิลลิกรัม/มิลลิลิตร ส่วน MIC ต่อเชื้อ Epidermophyton floccosum มีค่า 10-20 มิลลิกรัม/มิลลิลิตร

Project Title The Antimicrobial Action of Honey
(1) Effect of Temperature on Antibacterial Activity of Honey

Name of Investigators Nongluksna Sriubolmas
Areerat Laorpaksa
Saree Virunhaphol

Year March 1991

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.

Project Title	The Antimicrobial Action of Honey (2) Antibacterial Activity of Honey
Name of Investigators	Nongluksna Sriubolmas Saree Virunhaphol Areerat Laorpaksa
Year	March 1991

Abstract

Ten samples of honey were studied for the antibiotic equivalent potency. The antibacterial activity of honey was equivalent to 0.675 - 1.45 units/ml of penicillin against Staphylococcus aureus and to <14.7 - 28.1 mcg/ml of tetracycline against Escherichia coli. Five selected samples, having low to high equivalent potency of penicillin against Staphylococcus aureus, were determined for MICs against 30 strains of Staphylococcus aureus and Escherichia coli including standard strains. Most strains were antibiotic - resistant organisms. The MICs against Staphylococcus aureus and Escherichia coli of honey samples were 0.1 - 0.3 g/ml and 0.15 - 0.35 g/ml, respectively. For control solution containing 80% of glucose and fructose in the ratio of 1:1 , the growth of test organisms was not inhibited. Our study showed that the various samples of honey had approximately the same antibacterial activity. It was also shown that the honey samples were inhibitory against antibiotic - resistant organisms.

Project Title	The Antimicrobial Action of Honey (3) Antifungal Activity of Honey
Name of Investigators	Areerat Laorpaksa Saree Virunhaphol Nongluksna Sriubolmas
Year	March 1991

Abstract

The antifungal activity of ten honey samples against Trichophyton mentagrophytes , Microsporum gypseum and Epidermophyton floccosum was studied. The average inhibition zones were found to be in the range of 19.58 ± 1.05 to 31.00 ± 0.92 , 17.00 ± 0.61 to 29.62 ± 0.29 and 20.40 ± 1.59 to 46.36 ± 1.06 millimeters, respectively. The imported honey provided the lowest antifungal activity whereas the sample from Chumporn provided the highest activity. All samples had no effect on Aspergillus niger and the two yeasts : Saccharomyces cerevisiae and Candida albicans. The control sugar (Glucose : Fructose in ratio 1:1) showed no antifungal effect against all test organisms. The minimal inhibitory concentrations (MICs) of the two selected honey samples against Trichophyton mentagrophytes and Microsporum gypseum were both in the range of 10 - 30 milligrams/milliter while the MICs against Epidermophyton floccosum were in the range of 10 - 20 milligrams/milliliter.

ACKNOWLEDGEMENTS

The authors wish to express their deepest gratitudes 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.

Sincere thanks are expressed to Department of Microbiology, Faculty of Medicine, Chulalongkorn University and Department of Microbiology, Faculty of Medicine, Siriraj Hospital, Mahidol University, for providing some test organisms, to Department of Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for allowing to use the viscometer, and finally, to Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for supporting this work.

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

LIST OF TABLES AND FIGURE

PART I

PART II

PART III

CONTENTS

PART I

EFFECT OF TEMPERATURE ON ANTIBACTERIAL ACTIVITY OF HONEY

INTRODUCTION

Purified honey is used officially in pharmaceutical preparations as demulcent and sweetening agent[1]. It also has antimicrobial activity[2,3,4] 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 thereby 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 inhibine 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.

2. Control solution

Control solution containing 80% 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.

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

Sample No.	Sources	Locations (Province / country)
3	Longan	Chiangrai
6	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

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 test[8]

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 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

Table 2 Viscosity and antibacterial activity of honey and control solution

Sample No.	Viscosity (cps)	Inhibition zone diameter (mm ± S.E.M.)					
		S.aureus	*M.luteus*	*B.subtilis*	*E.coli*	*P.aeruginosa*	*K.pneumoniae*
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±1.08
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	Groups of honey sample		
	1	2	3
S. aureus	3,6,9,11,13,17	7,15,16,18	-
M. luteus	3,6,9,11,13,15, 17	7, 18	16
B. subtilis	3,6,7,9,11,13, 15,18	16, 17[b]	-
E. coli	3,6,9,11,13,16, 17	-	-
P. aeruginosa	3,9,11,13,15,17 18	6[c],7,16[c]	-
K. pneumoniae	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

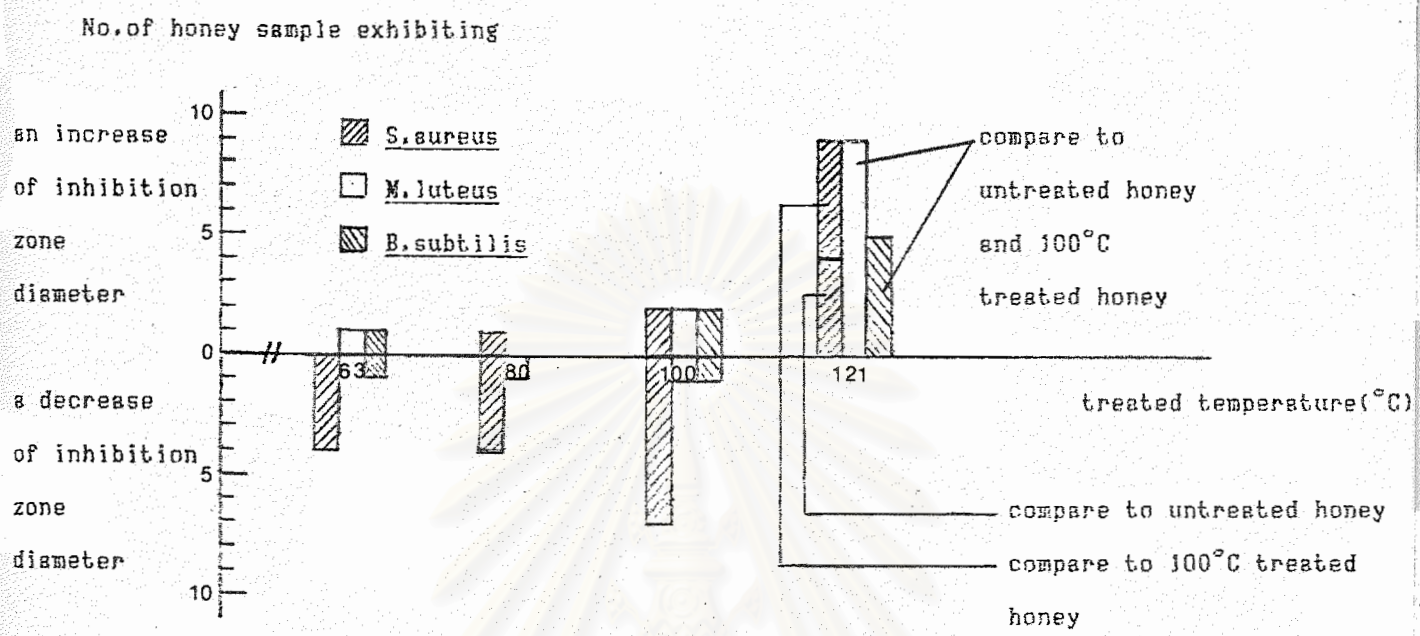
d. Differed significantly from sample No. 11, 15, 17, and 18

in Fig.1. For S. aureus, four honey samples heated at 63°C 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°C 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.

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 , two, three, six and five samples of 63°C, 80°C, 100°C and 121°C heated honey respectively, showed



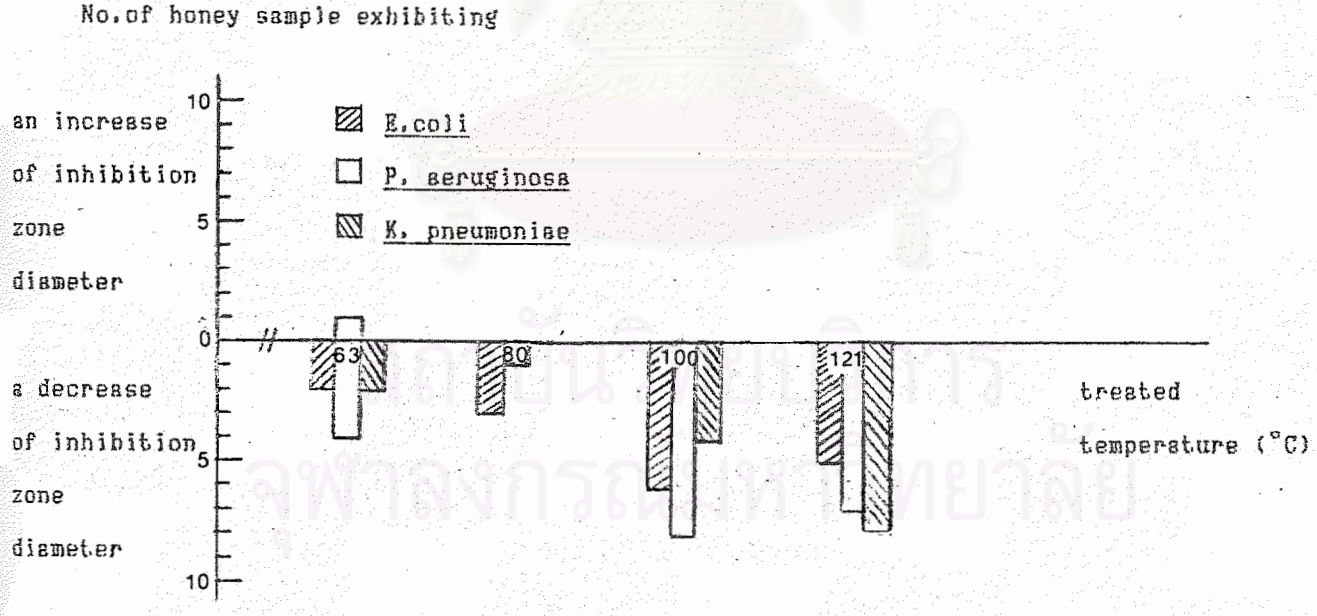


Figure 1 Effect of temperature on antibacterial activity of honey

a decrease in inhibition zone diameters.

For P. aeruginosa, one sample of the 63°C heated honey showed an increase in inhibition zone diameter and four samples showed a decrease. For 80°C heated honey, only one sample showed a decrease in the inhibition zone size, while for 100°C and 121°C heated honey, eight and seven samples, respectively, showed a decrease in the inhibition zone size.

For K. pneumoniae, two samples of 63°C heated honey, four samples of the 100°C heated honey and eight 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°C , 80°C, 100°C 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°C 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°C, 80°C 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.

REFERENCES

1. British Pharmacopoeia (1988), Vol I, 287, London Her Majesty's Stationery Office, London.
2. Crane, E. (1977) Honey a Comprehensive Survey. 260-261 Heinemann, London.
3. Jeddar, A., Kharsany, A., Ramsaroop, U.G., Bhamjee, A., Haffejee, J.E. and Moosa, A. (1985) The antibacterial action of honey, an in vitro study. S. Afr. Med. J. 67, 257-258.
4. Cavanagh, D., Beazley, J. and Ostapowicz, F. (1970) Radical operation for carcinoma of the vulva, a new approach to wound healing. J.Obstet. Gynaecol. Br. Cwlth. 77, 1037-1040.
5. Bergman, A., Yanai, J., Weiss, J., Bell , D. and David, M.P. (1983) Acceleration of wound healing by topical application of honey, an animal model. Am. J.Surg. 145, 374-376.
6. Obaseiki-Ebor, E.E., Afonya, T.C.A. and Onyekweli, A.O. (1983) Preliminary report on the antimicrobial activity of honey distillate. J. Pharm. Pharmacol. 35, 748-749.
7. The Pharmaceutical Codex (1979), 11th ed, 415-416 The Pharmaceutical Press, London.
8. Lorian, V. (1980) Antibiotics in Laboratory Medicine. 29-31 Williams & Wilkins, Baltimore/London.
9. Taylor, R.B., Jappy, B.M. and Neil, J.M. (1971) Kinetics of dextrose degradation under autoclaving conditions. J. Pharm. Pharmacol. 23, 121-129.

PART II

ANTIBACTERIAL ACTIVITY OF HONEY

INTRODUCTION

A number of study reported the *in vitro* antibacterial effect of pure honey[1,2,3]. It was found that many bacteria such as *Staphylococcus aureus*, *Proteus mirabilis* , and *Escherichia coli* failed to grow in undiluted and 50% diluted honey. Therefore, honey was used in the postoperative management of patient undergoing radical vulvectomy for vulva carcinoma, and used in the treatment of infantile gastroenteritis[3,4]

To compare the antibacterial activity of honey with some commonly used antibiotics, we would determine the equivalent potency of antibiotics and minimal inhibitory concentrations (MICs) of honey against clinical isolates.

MATERIALS AND METHODS

1. Honey

Nine out of eighteen honey samples from various provinces in Thailand and one out of two samples of imported honey for commercial consumption from U.S.A., passing the test for invert sugar substitute by the method specified in the Pharmaceutical Codex 11th ed$^{(5)}$, were determined for the equivalent potency of antibiotics. Five selected samples, having low to high equivalent potency of penicillin on S. aureus , were determined for minimal inhibitory concentrations.

2. Control solution

Control solution containing 80% of glucose and fructose in the ratio of 1:1 was used in this study.

3. Antibiotic working standard

Penicillin potency	1,664.0	units/mg
Tetracycline potency	100.0	%

4. Antibiotic sensitivity discs

Ampicillin	10	mcg	(BBL, Lot. No.908636)
Cefotaxime	30	mcg	(BBL, lot. No.812534)
Colistin	10	mcg	(BBL, Lot. No.806517)
Erythromycin	15	mcg	(BBL, Lot. No.907532)
Neomycin	30	mcg	(BBL, Lot. No.812519)
Penicillin	10	u	(BBL, Lot. No.903617)
Tetracycline	30	mcg	(BBL, Lot. No.904556)
Trimethoprim - sulfamethoxazole	1.25/23.75	mcg	(BBL, Lot. No.909579)

5. **Test organisms**

Staphylococcus aureus ATCC 25923

Escherichia coli ATCC 25922

Staphylococcus aureus : 30 clinical isolates from Department of Microbiology, Faculty of Medicine, Siriraj Hospital, Mahidol University

Escherichia coli : 30 clinical isolates from Department of Microbiology, Faculty of Medicine, Chulalongkorn University

6. **Medium**

Mueller Hinton agar (Difco)

7. **Preparations of inoculum**

7.1 For determination of the equivalent potency of antibiotics

Each standard organism was grown on Mueller Hinton agar slant at 37°C overnight. The inoculum was obtained from the surface growth of each organism in sterile saline and diluted to give 50% light transmission in 1-cm layer cuvette at a wavelength of 650 nm.

7.2 For determination of disc susceptibility

The culture suspension was prepared as described in 7.1 , except that it was diluted to obtain a turbidity comparable to the 0.5 McFarland turbidity standard.

7.3 For determination of MICs

The culture suspension was prepared as described in 7.2. In addition, a 1:20 dilution of the culture suspension was prepared in sterile saline for inoculation.

8. Determination of the equivalent potency of antibiotic by cylinder-plate method[6]

8.1 1% potassium phosphate buffer pH6 was used to dissolve and dilute penicillin to obtain concentrations of 0.51, 0.64, 0.8, 1.0 and 1.25 units/ml. For tetracycline, 0.1 M potassium phosphate buffer pH 4.5 was used to dissolve and dilute the antibiotic to obtain concentrations of 12.8, 16, 20, 25 and 31.2 mcg/ml.

8.2 The assay plates were prepared using petri dishes 100x20 mm. Twenty one ml of Mueller Hinton agar was placed in each plates which then were allowed to harden. Four ml of seed layer containing 1% of inoculum in Mueller Hinton agar were added and was allowed to harden. Twelve plates were used for the establishment of the standard curve, and three more plates were used for the assay of each sample of honey.

8.3 Plates containing S. aureus ATCC 25923, or E. coli ATCC 25922 were used for penicillin and tetracycline assay, respectively.

8.4 In order to derive the standard curve, 6 stainless steel cylinders (6 mm internal diameter and 10 mm height) were placed on the surface of each of 3 inoculated plates. They were then alternately filled with the medium antibiotic dilution and each of the remaining cylinders with one out of the other four antibiotic concentrations. Repeated the process for the remaining three antibiotic concentrations.

8.5 To determine the antibiotic equivalent potency of honey, the alternate cylinders were filled, on each of 3 plates, with the median antibiotic solution, and the remaining 9 cylinders with one sample of honey. After maintaining at room temperature for 1 hr, the plates were incubated at 37°C overnight. The diameters of inhibition zone were measured.

9. Determination of antibiotic disc susceptibility

9.1 The inoculated plates were prepared by inoculating each test organism on the surface of plates (100 mm diameter) containing 25 ml of Mueller Hinton agar by streak method[7].

9.2 The antibiotic susceptibility discs were placed on the surface of inoculated plates. The discs tested with S. aureus were cefotaxime, erythromycin, penicillin, tetracycline and trimethoprim-sulfamethoxazole.

The discs tested with E. coli were ampicillin, colistin, neomycin, tetracycline and trimethoprim - sulfamethoxazole.

9.3 After maintaining at room temperature for 15 minutes, the plates were incubated at 37°C overnight. The diameters of inhibition zone were measured. Each determination was carried out in duplicate.

10. Determination of MICs of honey by agar dilution method[8]

10.1 In order to prepare plates containing honey, each sample of honey was added to melted Mueller Hinton agar to give final honey concentration of 50, 45, 40, 35, 30, 25, 20, 15, 10 and 5%. Twenty five ml of each medium containing honey was pipetted into plate (100 mm diameter) and then allowed to harden.

10.2 The test organisms were inoculated onto the plates containing honey, control solution and control plate (no honey and no control solution) by using the inoculum replicating device which delivered 1 µl of inoculum, containing approximately $1x10^4$ viable cells per spot, onto the agar surface. The inoculum plates were allowed to stand undisturbed until the spots of inoculum were absorbed completely. The plates were then incubated at 37°C overnight

and observed for growth of the organisms. Each determination was carried out in triplicate.

RESULTS

The equivalent potency

The antibacterial activities of honey were equivalent to Ø.675-1.45 units/ml of penicillin against S. aureus and to <14.7-28.1 mcg/ml of tetracycline against E. coli

Antibiotic disc susceptibility

The standard strains of S. aureus and E. coli were susceptible to all test antibiotics. The strains of S. aureus and E. coli isolated from clinical specimens could be grouped into 6 and 12 groups according to the patterns of susceptibility as shown in Table 1 and 2, respectively.

Minimal inhibitory concentrations (MICs)

The MICs of honey samples for S. aureus and E. coli were Ø.1-Ø.3 g/ml and Ø.15-Ø.35 g/ml, respectively. For some strains of S. aureus , the MICs of some samples were below Ø.1 g/ml. However, most of them were Ø.1 g/ml. There was only one sample with the MIC of Ø.3 g/ml and this sample also had the highest MIC for E. coli. For plates containing control solution, the growth of test organisms was not inhibited.

Table 1 Susceptibility of clinical isolated strains of S. aureus

No.of strains	Pattern of susceptibility				
	P	T	E	Cef	T-S
1	+	+	+	+	+
16	-	+	+	+	+
10	-	-	+	+	+
1	-	±	+	+	+
1	-	-	-	-	+
1	-	-	-	-	-

P = Penicillin , T = Tetracycline ,

E = Erythromycin , Cef = Cefotaxime

T-S = Trimethoprim + Sulfamethoxazole

\+ = Susceptible

\- = Resistant

± = Intermediate

Table 2 Susceptibility of clinical isolated strains of *E. coli*

No. of strain	Pattern of susceptibility				
	A	Co	N	T	T-S
8	-	+	-	-	-
6	-	+	±	-	-
3	-	+	±	-	+
3	+	+	±	-	+
2	-	+	+	-	-
2	+	+	±	+	+
1	-	+	-	±	-
1	-	+	±	±	-
1	-	+	-	+	-
1	+	+	+	-	-
1	-	+	-	+	+
1	+	+	+	-	+

A = Ampicillin , Co = Colistin

N = Neomycin , T = Tetracycline

T-S = Trimethoprim - sulfamethoxazole

\+ = Susceptible

\- = Resistant

± = Intermediate

DISCUSSIONS AND CONCLUSIONS

The antibiotic equivalent potencies of various honey samples differed about two fold. Most strains of both test organisms were susceptible to the same concentration of honey. The results from our study showed that the antibacterial activity of various samples of honey were similar. Therefore, the honey susceptibility of bacteria did not correlate to antibiotic susceptibility of those test organisms.

REFERENCES

1. Crane, E. (1977) Honey a Comprehensive Survey. 260-261 Heinemann, London.
2. Jeddar, A., Kharsany, A., Ramsaroop, U.G., Bhamjee, A., Haffejee, I.E. and Moosa, A. (1985) The antibacterial action of honey, an in vitro study. S. Afr. Med. J. 67, 257-258.
3. Cavanagh, D., Beazley, J. and Ostapowicz, F. (1970) Radical operation for carcinoma of the vulva, a new approach to wound healing. J. Obstet. Gynaecol. Br. Cwlth. 77, 1037 - 1040.
4. Haffejee, I.E. and Moosa, A. (1985) Honey in the treatment of infantile gastroenteritis. Br. Med. J. 290, 1866-1887.
5. The Pharmaceutical Codex (1979), 11th ed, 415-416 The Pharmaceutical Press, London.
6. Thai Pharmacopoeia (1987), Vol I, 436-437 Department of Medical Sciences, Ministry of Public Health, Bangkok.
7. Lorian, V. (1980) Antibiotics in Laboratory Medicine. 29-31 Williams & Wilkins, Baltimore/London.
8. Lorian, V. (1980) Antibiotics in Laboratory Medicine. 6-8 Williams & Wilkins, Baltimore/London.

PART III

ANTIFUNGAL ACTIVITY OF HONEY

INTRODUCTION

Various biological effects of honey had been described in scientific and medical journals. Honey was effective in killing bacteria[1,2] and treating wounds, sores and burns[3,4]. However, there was only a few reports refered to the antifungal activity of it. Preliminary study of antimicrobial activity of honey distillated showed a broad spectrum antibacterial and antifungal activity and excellent activity against Candida albicans compared with nystatin[5].

This paper presents an in vitro study evaluating the antifungal effect of honey on saprophytic and pathogenic fungi.

MATERIAL AND METHODS

1. Ten honey samples that passed the test for invert sugar substitute[6] were used in this study. The source of each sample were shown in Table 1.

2. Antifungal susceptibility test was performed by agar diffusion method

   2.1 The test organisms

   Mold : Trichophyton mentagrophytes*
   Microsporum gypseum*
   Epidermophyton floccosum*
   Aspergillus niger**

   Yeast : Candida albicans ATCC 10231
   Saccharomyces cerevisiae TIS 5047

   2.2 Medium : Sabouraud dextrose agar (Difco)

   2.3 Preparation of inoculum

   The cultures of each 5-10 days old test mold and 24-48 hours old yeast were used. The test organisms were washed out with sterile normal saline (0.9%) The filamentous fungi were filtered through sterile cotton to obtain the spore suspension. Each test organism was then standardized to 10^7 spores or cells/ml

---

* Department of Microbiology, Faculty of Medicine, Chulalongkorn Hospital

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

Table 1 The honey samples and their sources

Sample	Source	Province / Country
3	Longan	Chiengrai
6	Litchi	Lumpang
7	-	Ubolrajadhani
9	Longan	Lumpang
11	-	Lumpoon
13	-	Lumpang
15	-	U.S.A.
16	-	Chumporn
17	Litchi	Chiangmai
18	Longan	Chiangmai
C	Fructose:glucose(1:1)	-

- = not specified

2.4 Preparation of assay plate

Twenty-five ml of molten prepared Sabouraud dextrose agar was poured into sterile 150 mm petri dishes and allowed to solidified to form base layer while 1 ml of each test organism suspension (2.3) was inoculated into 100 ml of media (45°-50°C) and was mixed to obtain seed media. Ten ml of seed media was then poured evenly over the surface of the base layer.

2.5 Test procedure

Three sterile stainless steel cups (6 mm internal diameter and 10 mm height) were placed on the surface of the seed layer for each test organism. The honey sample was fully filled in each cup and the plates were then incubated at room temperature.

2.6 Control groups

2.6.1 The assay plate was prepared in the same method as in 2.4

2.6.2 The sterile cup was placed on the assay plate and fully filled with solution (C) of fructose : glucose = 1:1

2.6.3 The untreated assay plate was used as growth control.

2.7 Test results

The zones of inhibition were measured in millimeter by vernier caliper after 3-5 days for mold and after 24-48 hours for yeast.

3. The minimal inhibitory concentrations (MICs) by broth dilution

3.1 The test mold

Trichophyton mentagrophytes

Microsporum gypseum

Epidermophyton floccosum

3.2 Medium : Sabouraud dextrose broth (Difco)

3.3 Samples

In order to obtain the MICs of the honey sample that provided the lowest antifungal activity, sample number 15 was used. In addition, sample number 11 was also used in order to observe the MIC of sample which provided intermediate antifungal activity.

3.4 Preparation of inoculum

The procedure was the same as in 2.3 but the standardized suspension contained 10^5 spores/ml.

3.5 Sample dilutions

The samples and solution C were diluted with Sabouraud dextrose broth to 50, 45, 40, 35, 30, 25, 20, 15 10, 5, 0 (growth control) mg/ml.

3.6 Test procedure

One ml of each sample dilution was transfered into sterile 12x75 mm test tube. One-tenth ml of the inoculum (3.4) was added into each tube. The samples were then mixed and incubated at room temperature for 3-6 days or until visible growth was observed in growth control tube. The experiment was carried out in triplicate.

3.7 Interpretation of the test result

According to the growth of the test organism in serial sample dilution, minimal inhibitory concentration (MIC)

was determined from the lowest concentration of honey that inhibited the visible growth.

RESULTS

1. The antifungal susceptibility test by agar diffusion

All ten honey samples inhibited the growth of three test molds : Trichophyton mentagrophytes , Microsporum gypseum , Epidermophyton floccosum whereas there was no effect on Aspergillus niger and two yeasts, Saccharomyces cerevisiae and Candida albicans. (Table 2)

Honey sample number 16 provided the largest average inhibition zone diameter against T. mentagrophytes , M. gypseum and E. floccosum which were 31.00 ± 0.92 , 29.62 ± 0.29 and 46.36 ± 1.06 mm., respectively, while honey sample number 15 provided the lowest average inhibition zone diameters which were 19.58 ± 1.05 , 17.90 ± 0.61 and 20.40 ± 1.59 mm., respectively.

By Fisher's Least Significant Difference Multiple Comparison Test ($P<0.05$) , the average inhibition zone diameters provided by sample number 16 was shown to be significantly difference from the other samples and from the control sugar, meanwhile, the average inhibition zone diameters provided by sample number 15 was also shown to be significantly different from the other honey samples except for sample 17.

2. The determination of MICs by broth dilution

The MICs of the sample number 11 against T. mentagrophytes , M. gypseum and E. floccosum were the same (10 mg/ml) as shown in Table 3. The MICs of sample number 15 against T. mentagrophytes and M. gypseum were the same (30 mg/ml) while the MICs of the same sample against E. floccosum was lower (20 mg/ml).

Table 2 Inhibition zone diameters of honey against test organisms

Organisms / Sample number	Average of inhibition zone (mm) ± S.E.M.					
	Trichophyton mentagrophytes	*Microsporum gypseum*	*Epidermophyton floccosum*	*Aspergillus niger*	*Saccharomyces cerevisiae*	*Candida albicans*
3	25.64±0.51	23.34±0.63	28.34±0.54	0	0	0
6	22.68±1.46	20.66±0.55	26.06±0.55	0	0	0
7	23.84±0.85	25.86±0.84	40.58±0.77	0	0	0
9	23.74±1.40	26.04±0.81	34.64±3.02	0	0	0
11	27.12±0.68	26.60±0.66	30.36±1.57	0	0	0
13	25.14±1.37	26.14±0.48	37.28±0.87	0	0	0
15	19.58±1.05	17.90±0.61	20.40±1.59	0	0	0
16	31.00±0.92	29.60±0.29	46.36±1.06	0	0	0
17	20.92±0.71	20.58±0.74	23.26±1.43	0	0	0
18	21.48±0.80	20.86±0.43	27.88±1.37	0	0	0
C	0	0	0	0	0	0

S.E.M. = Standard error of mean

Table 3 The minimal inhibitory concentrations of honey

Organism / Semple number	Highest dilution of no growth (mg/ml)		
	Trichophyton mentagrophytes	*Microsporum gypseum*	*Epidermophyton floccosum*
11	10	10	10
15	30	30	20
C	>50	>50	>50

DISCUSSIONS AND CONCLUSIONS

All tested honey samples provided the antifungal action against dermatophytes : T. mentagrophytes , M. gypseum and E. floccosum. Each honey sample has no antifungal effect on A. niger and two yeasts : S. cerevisiae and C. albicans. Similar activity was shown by honeys from the various sources eventhough there were some variation in the inhibition zone diameters. The results were different from the preliminary report[5,7] that honey distillated (destructively distilled under dry nitrogen) provided the antifungal activity against A. niger , Penicillium spp., and excellent activity against C. albicans. In vitro study of a new approach to wound healing by applications of honey[3] , there was a test of neat honey and dilutions prepared with distilled water (10-50%) was inoculated with variety of microorganisms. It was found that undiluted honey has bactericidal effect and was able to inhibit the growth of many species of Candida such as C. albicans where as there was no effect on Torulopsis glabrata and S. cerevisiae. However, the growth of C. albicans in dilutions of honey from 50% to 10% could also be observed. The control group had no antifungal activity.

The MICs of the selected honey samples, number 11 and number 15, were determined by broth dilution. The MICs of sample number 11 against T. mentagrophytes , M. gypseum and E. floccosum were all the same (10 mg/ml) which were much lower than the MIC of the sample number 15 against the same organisms (30 mg/ml against T. mentagrophytes and M. gypseum ,20 mg/ml against E. floccosum). This result indicated that the MIC value of all the honey samples tested should not be more than the value obtained from sample number 15 which provided the lowest inhibition zone. The MIC of the control groups against all test organisms was much higher than the samples (>50 mg/ml).

REFERENCES

1. Crane. E, (1977) Honey a Comprehensive Survey. 258-266 Heinemann , London.
2. Ibrahim AS. (1981) Antibacterial action of honey. Bull Islamic Med 1, 363-365.
3. Cavanagh D, Beazley, J, Ostapowicz F. (1970) Radical operation for carcinoma of the vulva : a new approach to wound healing. J. Obstet. Gynaecol. Br. Cwlth. 77 , 1037 - 1040.
4. Bergman A., Yanai. J, Weiss. J, Bell.D. and David, M.P. (1983) Acceleration of wound healing by topical of honey. Am. J. Surg. 145, 374-376.
5. Obaseiki-Ebor, E.E., Afonya, T.C.A. and Onyekweli, A.O. (1983) Preliminary study on the antimicrobial activity of honey distillate. J. Pharm. Pharmacol. 35, 748-749.
6. The Pharmaceutical Codex (1979), 11th ed, 415-416 The Pharmaceutical Press, London.
7. Obaseiki-Ebor, E.E., Afonya, T.C.A. (1984) In-vitro. evaluation of the anticandidiasis activity of honey distillate (HY-1) compared with that of some antimycotic agents. J. Pharm. Pharmacol. 36, 283-284.