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Antibacterial Activity of Different Egyptian Honeys in Relation to Some Bee Products

A.G. Hegazi, Nagia Z. Moharram*, Fayrouz Abd Allah, M. S. Nour* and A.M.Khair

National Research Center and Cairo University, Cairo, Egypt.*

THE ANTIBACTERIAL activities of different types of honey in relation to some bee products were investigated against 5 species of pathogenic bacteria belonging to Gram positive and Gram negative bacteria. The honey types were Citrus, Cotton Sesame and Clover. The bee products were royal jelly and bee venom. The bacteria were *Staphylococcus aureus, Streptococcus faecalis, Corynebacteria pseudotuberculosis, Pseudomonas aeruginosa* and *E.coli.* The influence of the storage was studied in case of clover honey from season of 1965 and 1995. Honey was used in different concentration.

The results revealed that the different Egyptian honey, royal jelly and bee venom were effective antibacterial against different pathogenic bacteria. It was clear that the different types of honey as well royal jelly and bee venom were less effective against *E. coli* than other bacteria.

Hony has a valuable role in traditional medicine for centuries . It was described in many cultures since ancient times (Molan, 1992). The use of honey as therapeutic substance has been rediscovered by medical proficinals in more recent times and has been accepted as antibacterial agent for treatment of ulcers, bed sore and surface wound infection and surface infections resulting from wounds (Tousson *et al.*, 1997). Also honey has been found to be effective in treating bacterial gastroenteritis in infants (Haffeejee, 1985) and liver disease (Yoirish, 1977).

The antibacterial activity of honey referred to the presence of inhibin (Nour, 1988) which acts as antibacterial factor other than H_2O_2 (Molan and Russell,

1988). The antibacterial activity of different types of honey was studied by many authors as Hodgeson (1989); Molan (1992); Elbagauiry and Rasmy (1993); Molan *et al.* (1994) and Hegazi *et al.* (1997). Royal jelly and bee venom have some antibacterial activity to some bacteria as determined against royal jelly (Yatsunami & Echigo, 1984 and 1985) and bee venom (Kondo, 1986 and Mulfinger, 1990). Thus the aim of the present study was to investigate the antibacterial activity of different Egyptian honey and some bee products against Gram positive and negative bacteria in correlation to effect of the storage specially on clover honey

Material and Methods

Bacteria

Five bacterial species included Gram positive and Gram negative were used. The Gram positive bacteria were *Staphylococcus aureus, Streptococcus faecalis* , and *Corynebacteria pseudotuberculosis.* Where the Gram negative bacteria were *Pseudomonas aeruginosia* and *E coli.*

Honey

Fresh sample of four Egyptian types of honey were collected from aprifarm and stored in dark in tan containers at 4°C until being used. The honey samples were citrus honey, cotton honey, sesame honey and clover honey. Beside these honey samples there was a clover honey stored from 1965 to study the effect of storage on antibacterial activity of honey.

Under aseptic condition to different dilutions were prepared for each type of honey using sterile distilled water. Evaluations of the antibacterial activity of different honey dilutions were performed according to Nour (1988) and Moussa (1997). The results of antibacterial activity against different examined bacteria were performed.

Royal Jelly

Twenty samples of royal jelly were used in this study. They were kindly supplied from Faculty of Agriculture, Cairo University, Apiary farm.These samples were immediately transferred to laboratory in ice bath. Under aseptic condition, royal jelly was prepared for each samples using sterile distilled water. Evaluation of the antibacterial activity of royal jelly was performed according to

Yatsunami and Echigo (1984 and 1985). The results of antibacterial activity against different examined bacteria were determined .

Bee venom

One thousand bees were kindly supplied from the Faculty of Agriculture, Cairo University, Apiary farm. Bee venom was collected through the stinging apparatus of the bees where the venom was extracted by absolute ethanol. The bee venom was dried by evaporation of ethanol then de-solved in sterile distilled water then determined the antibacterial activity as Kondo (1986) and Mulfinger (1990) .

Results

The results of antibacterial activity against Gram positive and negative bacteria were illustrated in Tables 1-5. The antibacterial activity of different honey types against *Staphylococcus aureus; Streptococcus faecalis; Corynebacteria pseudotuberculosis; Pseudomonas aeruginosa* and *E. coli* were recorded in Tables 1-5. It was obvious that the inhibition zone of *Staphylococcus aureus; Streptococcus faecalis; Corynebacteria pseudotuberculosis; Pseudomonas* aeruginosa and *E. coli* were increased with the honey concentration. The highest concentration (citrus, cotton, sesame and clover) of honey (21.30 %) gave the highest inhibition zones.

It was clear that the storage of clover honey showed a decrease of antibacterial activity against *Staphylococcus aureus; Streptococcus faecalis; Corynebacteria pseudotuberculosis; Pseudomonas aeruginosa* and *E. coli* in particular all concentrations used.

The result of antibacterial activity of different honey types against *Staphylococous aureus* was illustrated in Table 1. Cotton honey at concentrations 8.9 : 13.1 were significantly less active if compared with other types of honey, while sesame honey was increased significantly if compared with cotton honey at concentration 4.5; 8.9 and 13.1 %. On the other hand, activity of sesame honey decreased than citrus honey.

TABLE 1. Antimicrobial activity of different Honey types against *Staphylococcus aureus*.

concentration	Citrus	Cotton	Sesame	Clover 95	Clover 65
4.50%	4.7± 0.129	3.4±[A] 0.540	4.3±[AB] 0.182	4.745± 0.045	3.625± 0.032
8.90%	5.15± 0.193	3.925±[A] 0.275	5.2±[B] 0.483	4.975± 0.096	3.8± 0.06
13.10%	5.25± 0.06	4.7±[A] 0.754	5.61±[B] 0.112	5.225± 0.676	3.901± 0.021
17.30%	5.425± 0.032	5.525± 0.228	5.95±[A] 0.232	5.7± 0.362	4.525± 0.032
21.30%	6.022± 0.048	6.04± 0.087	6.01± 0.025	6.00± 0.052	4.975± 0.075

A= Significant difference to Citrus honey B= Significant difference to Cotton honey

Table 2 showed antibacterial activity of different honey types against *Streprococcus faecalis*. It was clear that cotton honey was less active if compared with other types of honey especially at concentration 8.9 and 13.1 %, while sesame honey showed the highest activity (5.9 ± 0.1). *Corynebacteria pseudotuberculosis* was significantly inhibited by sesame honey at 8.9 % concentration also cotton; sesame and clover honey at concentration 13.1 % gave a significant inhibition zones. While citrus honey was less effective if compared with other honey types . On the other hand, concentration of 17.3 % gave more inhibitory, activity if compared with citrus honey (Table 3).

TABLE 2. Antimicrobial activity of different Honey types against *Streptococcus faecalis*.

concentration	Citrus	Cotton	Sesame	Clover 95	Clover 65
4.50%	4.5± 0.267	3.85± 0.730	4.55± 0.838	4.95± 0.607	3.275± 0.495
8.90%	5.225± 0.271	4.175±[A] 0.625	5.15± 0.505	5.475± 0.525	4.3± 0.5
13.10%	5.775± 0.165	4.875±[A] 0.652	5.9±[B] 0.1	5.625± 0.375	4.525± 0.375
17.30%	5.9± 0.1	5.9± 0.129	5.95± 0.064	5.75± 0.25	4.7± 0.1
21.30%	6.021± 0.070	6.00± 0.057	6.01± 0.08	5.975± 0.047	5.1± 0.01

A= Significant difference to Citrus honey B= Significant difference to Cotton honey

TABLE 3. Antimicrobial activity of different Honey types against *Corynebacteria pseudotuberculosis* .

concentration	Citrus	Cotton	Sesame	Clover 95	Clover 65
4.50%	4.2± 0.129	3.925± 0.566	4.3± 0.204	4.625± 0.943	3.21± 0.064
8.90%	4.4± 0.025	4.55± 0.239	5.425±^{AB} 0.193	4.725± 0.032	3.62± 0.161
13.10%	4.65± 0.35	5.2±^{AB} 0.021	5.625±^{AB} 0.375	5.85±^{AB} 0.332	4.2± 0.082
17.30%	5.35± 0.15	5.9±^{A} 0.016	5.99±^{A} 0.138	5.95±^{A} 0.05	4.8± 0.120
21.30%	6.00± 0.042	6.020± 0.050	6.011± 0.040	6.013± 0.012	5.11± 0.116

A= Significant difference to Citrus honey B= Significant difference to Cotton honey

The antibacterial activity of different honey types against *Pseudomonas aeruginosa* was evaluated in Table 4 . The results revealed that the highest activity of honey at concentration 4.5 was sesame and the lowest activity was clover. But the activity of sesame and clover were significantly increased compared with citrus and cotton honey at concentration 8.9 and 13.1 % while at concentration 17.30 % a significant decrease in inhibition zones in case of citrus honey. The antibacterial activity against *E. coli* to different honey types was demonstrated in Table 5 . It was observed that cotton honey induces the lowest antibacterial activity at concentration 4.5 and 8.9 % if compared with other tested honey.

TABLE 4. Antimicrobial activity of different Honey types against *Pseudomonas aeruginosa* .

concentration	Citrus	Cotton	Sesame	Clover 95	Clover 65
4.50%	4.775± 0.225	4.65± 0.064	4.8±^{B} 0.086	4.35±^{AC} 0.064	2.8± 0.258
8.90%	4.8± 0.129	4.8± 0.258	4.8± 0.042	4.75± 0.024	3.6± 0.129
13.10%	4.9± 0.120	5.05± 0.064	5.35±^{AB} 0.064	5.2±^{A} 0.129	3.8± 0.035
17.30%	4.975± 0.032	5.825±^{A} 0.096	5.925±^{A} 0.225	5.6±^{A} 0.129	3.95± 0.064
21.30%	6.001± 0.040	6.01± 0.032	6.02± 0.070	6.00± 0.043	4.25± 0.052

A= Significant difference to Citrus honey B= Significant difference to Cotton honey

TABLE 5. Antibacterial activity of different Honey types against *E. coli*.

concentration	Citrus	Cotton	Sesame	Clover 95	Clover 65
4.50%	2.425± 0.165	1.5± [A] 0.288	2.9± [B] 0.604	2.45± [B] 0.202	1.875± 0.426
8.90%	2.825± 0.340	2.00± [A] 0.408	3.325± 0.962	2.65± 0.805	2.25± 0.25
13.10%	3.15± 0.589	2.475± 0.841	3.4± [BC] 0.025	2.9± 0.501	3.1875± 0.571
17.30%	3.6± 0.675	3.125± 0.826	3.75± 0.314	3.525± 0.861	3.25± 0.478
21.30%	3.875± 0.327	3.5± 0.288	3.875± 0.264	3.875± 0.160	3.775± 0.131

A= Significant difference to Citrus honey B= Significant difference to Cotton honey

The antibacterial activity of royal jelly and bee venom against different pathogenic bacteria was evaluated in Table 6 . The results revealed that the highest activity of royal jelly was 5.00 ± 0.02 against *Corynebacteria pseudotuberculosis* and the lowest activity was 1.32±0.23 against *E. coli* but the antibacterial activity of bee venom was higher against *Staphylococcus aureus* (5.60±0.03) and the lowest activity was 2.90+0.21 against *E. coli.* The antibacterial activity against different pathogenic bacteria varied according to the type of pathogens in both royal jelly and bee venom.

TABLE 6. Antibacterial activity of Royal jelly and bee venom .

Bacteria	Royal jelly	Bee venom
Staphylococcus aureus	4.35±0.23	5.60± 0.03
Streptococcus faecalis	4.17±0.08	4.82±0.11
Corynebacteria pseudotuberculosis	5.00±0.02	4.86±0.01
Pseudomonas aeruginosa	3.55±0.17	3.72±0.08
E.coli	1.32±0.23	2.90±0.21

Discussion

Regarding to the results of antibacterial activity of different honey types against *Staphylococcus aureus; Streptococcus faecalis; Corynebacteria pseulotuberculosis; Pseudomonas aeruginosa* and *E. coli,* it was obvious that the inhibition zones of *Staphylococcus aureus; Streptococcus faecalis; Corynebacteria pseudotuberculosis; Pseudomonas aeruginosa* and *E. coli* were increased with the honey concentration. The highest concentration (citrus,

cotton, sesame and clover) of honey (21.30 %) gave the highest inhibition zones. This inhibition due to the osmotic effect of honey (Listrer, 1975; Chirief *et al.*, 1982 and Molan, 1992), acidity of honey (pH range from 3.2-4.5) or activity of glucose oxidase in the ripening of nectar (Roth *et al.*, 1983). The presence of hydrogen peroxide (Dustmann, 1987 and Effem, 1988), non-peroxide substances (Bogdanov *et al.*, 1984 and Radwan, 1984), propolis which contain flavonoid (Bogdanov *et al.*, 1984 and Hegazi *et al.*,1996) and volatile antibacterial substances (Christov, 1961).

The storage of clover honey showed a decrease of antibacterial activity in all tested honey samples against *Staphylococcus aureus; Streptococcus faecalis; Coynebacteria pseudotuberculosis; Pseudomonas aeruginosa* and *E. coli* in particular all concentrations used. These results attributed to the loss of volatile antibacterial substances, lowering of activity of hydrogen peroxide, inactivation of glucose oxidase. Such results agree with Molan *et al.* (1991) who examined the antibacterial activity of 345 samples of honey through New Zeland against *Staphylococcus aureus* in an agar diffusion assay and found neither the age of honey samples nor whether they had been processed by apiarist was associated with lower activity.

The antibacterial activity of different honey types against *Staphylococcus aureus* showed that, cotton honey at concentrations 8.9; 13.1 were significantly less active if compared with other types of honey, while sesame honey was increased significantly if compared with cotton honey at concentration 4.5: 8.9 and 13.1 %. On the other hand, activity of sesame honey decreased than citrus honey. It was clear that cotton honey was less active if compared with other types of honey especially at concentration 8.9 and 13.1 %, while sesame honey reached 5.9 ± 0.1 against *Streptococcus faecalis. Corynebacteria pseudotuberculosis* was significantly inhibited by sesame honey at 8.9 % concentration also cotton; sesame and clover honey at concentration 13.1 % gave significant inhibition zones. While citrus honey was less affective if compared with other honey types. On the other hand, concentration of 17.3 % gave more inhibitory activity if compared with citrus honey. The antibacterial activity of different honey types against *Pseudomonas aeruginosa* revealed that the highest activity of honey at concentration 4.5 was sesame and the lowest activity was clover. But the activity of sesame and clover was significantly

increased if compared with citrus and cotton honey at concentration 8.9 and 13.1 % , while at concentration 17.30 % a significant decrease in inhibition zones in case of citrus honey. It was observed that cotton honey induces the lowest antibacterial activity at concentration 4.5 and 8.9% if compared with other tested honey against *E. coli.*

Many authors studied the antibacterial activity of honey as Hodgeson (1989) who compared the antibacterial effect of Manuka honey with ling heather honey. He found that whereas *Staphylococcus aureus* and *Pseudomonas aeruginosa* were inhibited by both honeys, inhibition of *E. coli, Proteus mirabilis* and *Streptococcus fecalis* was not seen with ling heather honey, yet Manuka honey inhibited all these species. Also, Jeddar *et al.* (1985) evaluated the growth of various gram positive and gram negative bacteria in media containing various concentrations of honey and they found that most pathogenic bacteria failed to grow in honey at a concentration of 40 % or above. Where Molan *et al.* (1994) examined the sensitivity of *helicobacter pylori* to honey using five isolates from biopsies of gastric ulcers and found all five isolates were sensitive to 5 % solution of Manuka honey incorporated in the agar media.

The variations of the activity of different honey was attributed to the previously mentioned factors which influenced the antibacterial activity as osmotic properties of honey (Listner 1975; Chirief *et al.*,1982 and Molan 1992); honey pH or activity of glucose oxidase (Roth *et al.*,1983); hydrogen peroxide (Dustmann, 1987 and Effem, 1988), non peroxide substances (Bogdanov *et al.*, 1984 and Radwan, 1984), presence of propolis which contain flavonoids (Bogdanov *et al.*, 1984 and Hegazi *et al.*, 1996) and volatile antibacterial substances (Christov,1961).

The results of antibacterial activity of royal jelly and bee venom against different pathogenic bacteria revealed that the highest activity of royal jelly was 5.00 ± 0.02 against *Corynebacteria pseudotuberculosis* and the lowest activity was 1.32 ± 0.23 against *E. coli.* These results were due to the damage caused by bacterial DNA caused by royal jelly (Tamura *et al.*, 1985) or the presence of 10- hydroxy-2-decenoic acid and 10- hydroxydecanoic which both showed antibacterial propoerties (Yatsunami and Echigo, 1984 and 1985) .

The antibacterial activity of bee venom revealed that the highest antibacterial activity was against *Staphylococcus aureus* (5.60 ± 0.03) and the lowest activity was 2.90±0.21 against *E. coli.* These results were due to the effect of melittin from bee venom which have antibacterial activity (Kondo 1986, Kondo and Kanai ,1986 and Mulfinger, 1990)

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الكفاءة المضادة للبكتريا للأنواع المختلفة للعسل المصرى

احمد جعفر حجازى , ناجية محرم* ، فيروز عبد الله ، محمود السيد نور* و عمر محمد خير

المركز القومى للبحوث - جامعة القاهرة* - القاهرة - مصر .

درست الكفاءة المضادة للبكتيريا للأنواع المختلفة للعسل المصرى ضد خمسة أنواع من البكتيريا الممرضة إيجابية وسالبية الجرام شملت أنواع العسل المصرى : عسل الموالح والقطن والسمسم والبرسيم . كما كانت البكتيريا المستخدمة : الميكروب العنقودى المكور الذهبى والميكروب السبحى - والكرينى بكتيريا و الإسيدوموناس والميكروب القولونى . كما درس تأثير التخزين على الكفاءة المضادة للبكتيريا على عسل البرسيم من موسمى ١٩٦٥ و ١٩٩٥ .

ولقد أسفرت النتائج من الأنواع المختلفة للعسل المصرى كانت فعالة ضد الأنواع المختلفة من البكتريا الممرضه كما ظهرت النتائج أن الأنواع المختلفة للعسل لها تأثير أقل على الميكروب القولونى دون سائر الأنواع البكتيرية الأخرى .