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Isolation of antibacterial peptides from Saudi Arabian honeybees and investigating the antimicrobial properties of natural honey samples

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ABSTRACT

As part of the ongoing search for novel antimicrobial agents and their use in singular or combined drug therapy, a polypeptide fractions of *Mr* about 14.500 and 15.00 KDa were isolated from the lymph fluid of two groups of intact and immunized Saudi Arabian honeybees (*Apis mellifer*a) obtained from Al-Qasim and Hail locations around Riyadh during the honey season 2008 following experimental infection with 1.1 x $l0^6$ viable *Escherichia coli* cells (ATCC 25922). The polypeptide was purified to homogeneity by Reversed Phase High Performance Liquid Chromatography. Antibacterial activity of the isolated polypeptide was evaluated *in vitro* by an agar well diffusion method for *E. coli* strain (ATCC 25922) and *Klebsiella pneumoniae* strain (ATCC 11678) the major Gram negative pathogens causing urinary tract infections, and *Staphylococcus aureus* (ATCC 6538) as Gram positive bacteria. A total of ten honey samples collected from different floral areas around Riyadh were also investigated for their antimicrobial activity against one yeast, *Candida albicans* (ATCC 10231) and four standards bacteria strains, *E. coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *S. aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633) using standard antimicrobial assays. The isolated polypeptide and the different honey samples revealed comparable marked variations in antimicrobial activities and their sensitivity might be depending on their floral source.

Key Words: Antibacterial peptides, Saudi Arabian honeybees

INTRODUCTION

Antibiotic-resistant bacteria continue to be of major health concern world-wide. Since the use of antibiotics became widespread over 50 years ago, bacteria have progressively developed resistance (Hsueh *et al*., 2005). Consequently, scientific efforts have been made to study and develop new compounds to be used beyond conventional antibiotic therapy. Honey has been used since ancient times for the treatment of some respiratory diseases and for the healing of skin wounds. It has been proposed that the healing effect of honey could be due to various physical and chemical properties (Snow and Manley-Harris, 2004). Along with the rapidly increasing interest and research into natural health remedies and supplements, is a resurgence of interest in the therapeutic use of honey. Honey as most natural products, may have a large variance in therapeutic components depending on its origin. Thus, the floral source of honey plays an important role on its biological properties (Molan, 2002). In consequence, it would not be surprising that the provenance of honey could determine its antibacterial properties. Honey produced by honeybees (*Apis mellifera*) is one of the oldest traditional medicines considered to be important in the treatment

of respiratory, gastrointestinal infection and various other diseases due to the absence of sufficient modern health care system, particularly in rural areas.

Often during pathogenic invasion, the first line of defense involves the innate mechanisms of immunity which in turn is followed by acquired immune responses involving the activation of T and B cells against specific antigens (Fearon and Locksley, 1996; Medzhitov and Janeway, 2000). In contrast to these acquired immune mechanisms, endogenous peptides, which are constitutively expressed or induced, provide a fast and effective means of defense against the pathogen. This group of molecules termed 'antimicrobial peptides' (AMPs) constitutes a primitive immune defense mechanism and is found in a wide range of eukaryotic organisms, from humans, plants and insects (Lehrer and Ganz, 1999). AMPs are an important component of the natural defenses of most living organisms against invading pathogens. During the past two decades several AMPs have been isolated from a wide variety of animals, both vertebrates and invertebrates, and plants as well as from bacteria and fungi. These peptides exhibit broad-spectrum activity against a wide range of microorganisms including Gram-positive and Gram-negative bacteria, protozoa, yeast, fungi and viruses, they have potential to overcome bacterial resistance makes them promising candidates for therapeutic drugs (Bals, 2000). AMPs are classified based on the three dimensional structural studies carried out with the help of NMR. Most of these peptides are believed to act by disrupting the plasma membrane leading to the lysis of the cell. AMPs have been found to be excellent candidates for developing novel antimicrobial agents and a few of these peptides show antimicrobial activity against pathogens causing sexually transmitted infection. A few peptides have already entered clinical trials for the treatment of impetigo, diabetic foot ulcers and gastric helicobacter infections (Reddy *et al.,* 2004). One of the most promising among these antimicrobial peptide families are the cell-free immune repertoire of honeybees *(Apis mellifera)* that are induced by bacterial infection provide broad-spectrum antibacterial defense, such as apidaecin, hymenoptaecin, abaecin, and bee defensin. These peptides represent a viable treatment option for the major pathogens in urinary tract infections, that is, *E. coli* and *K. pneumoniae*, causing 90–95% of all urinary tract infections (Czihal *et al*., 2007).

The purpose of the present study was therefore to isolate and purified antimicrobial polypeptides and evaluate scientifically the *in vitro* antimicrobial potential of these peptide and ten honey samples produced by honeybees (*Apis mellifera*) against standard microorganisms species among those commonly involved in causing diseases.

MATERIALS AND METHODS

Insects:

Two wild groups of adult honey bees *Apis mellifera*, 1000 each were collected from different natural environmental locations in Saudi Arabia around Riyadh, the floral origin of one group is *Ziziphus spina* (Rhamnaceae), plants from Al- Qasim and the other of *Acacia* spp from Hail. Adult bees were kept in small cages in the laboratory until used for induction by bacteria and isolation of antibacterial peptides.

Microorganisms:

The standard microorganisms used in this study were the yeast *Candida albicans* (ATCC 10231) and five different bacteria strains, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC

11678) as Gram negative and *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633) as Gram positive bacteria.

Immune Induction of Honeybees:

Humoral immunity was induced in adult honeybees by puncturing a leg with the tip of a hypodermic needle dipped in the 1.1 x $l0^6$ viable *E. coli* cells (ATCC 25922), equivalent to 0.5 McFarland tube suspended in 1µl phosphate-buffered saline (PBS, 0.15 M, pH 7.2). intact and induced adult bees were bled and lymph samples (for peptide purification) were taken after 24 hr. post infection, the collected hemolymph was pooled in tubes containing 100µl of 2% trifluoroacetic acid (TFA) to prevent proteolytic degradation of the immunoinduced peptides and to precipitate proteins as described by Casteels *et al*.(1994). The collected hemolymph (2- 4µl) was pooled in ice-cooled Eppendorf tubes containing a few crystals of phenylthiourea to prevent melanization of samples. Hemocytes were centrifuged (10000 g for 10 min.) and the lymph was collected and freeze stored at -70^{0}c till used in the purification experiments.

Purification of antibacterial peptides: (reversed phase- high performance liquid chromatography) RP-HPLC.

The lymph samples were heat-treated (100°C/5 min.). The precipitate was spun down and the clear supernatant was acidified with an equal volume of 0.1% TFA, and fractionated by several rounds of high performance liquid chromatography using reversed-phase column supports, all as described by Casteels *et al*.(1993) and Lauth *et al*. (1998).

Samples (50 µl) aliquots of diluted lymph were taken for RP-HPLC analysis using an ABI 150A system (Applied Biosystems Inc., Ramsey, NJ) with a VYDAC C4 (214 TP54) analytical column (The separations group, Hesperia, CA). Solvent A was 0.1 % TFA (pH 2.0) and solvent B: 70% acetonitrile (MeCN). Fractions were eluted at 1 ml/min. (70 min. total times). UV detection was done at 214 nm. All differential peaks between control and immune lymph, including peaks 1 and 2, were collected and further purified on VYDAC C18 (218TP54). Collected fractions were lyophilized and re-dissolved in Milli Q water, Promega (nuclease free water) before being tested for biological activity against *E. coli* and *K. pneumoniae*. Following this procedure 1.0- 10 µg peptides were routinely purified from a batch of 1000 bees.

Sodium dodecyle sulphate (SDS- PAGE) for Antimicrobial Peptides:

SDS- PAGE of control and purified antimicrobial peptides was carried out by the discontinuous buffer system as described by Laemmili, (1970) with some modifications. Electrophoresis was carried out at a constant voltage of 200 V for 90 min. and 12% polyacrylamide gel, under the denaturing conditions. The gels were calibrated with standard molecular weight proteins (high and low ranges: 200, 97.4, 68, 29, 18.4 and 8.15 kDa). Protein bands were visualized by Commassie Blue dye staining. *Mr* calculations were determined by regression analysis using the manufacturers soft-ware.

Honey Samples:

This study was carried out on ten honey samples (1 kg each) collected in Saudi Arabia during the honey season of 2008. The sampling area (Table 1) of different mono- and heteroflora honey collected from the bee hives were marked randomly. Each honey sample was collected in a sterile universal glass container and kept at 2–8°C until tested. Each sample was tested at original concentration 100% and diluted to 30%, 70% of its original concentration using physiological saline PBS pH 7.2 according to the method described by Nzeako and Hamdi, (2000).

Table (1) Examined natural honey samples and their sources

No. of honey sample	Source	Location	Plant cover
1	Hetero flora	Alsomman (plain)	• *Acacia spp* .(Mimosaceae), • *Ziziphus spina* (Rhamnaceae) , • *Peganum harmala* (Zygophyllaceae), • *Rhanterium epapposum* (Asteraceae)
2	Monoflora	Hail (plain)	*Acacia spp*. (Mimosaceae)
3	Monoflora	Al-Qasim (plain)	• *Ziziphus spina* (Rhamnaceae)
4	Hetero flora	Horimalaa	• *Acacia spp*. (Mimosaceae), • *Ziziphus spina* (Rhamnaceae)
5	Hetero flora	Roda (plain)	• *Achillea fragrantissima* (Compositae), • Ziziphus *spina* (Rhamnaceae), • *Neurada procumbens* (Neuradaceae), • *Acacia spp*. (Mimosaceae), • *Peganum harmala* (Zygophyllaceae), • *Rhanterium epapposum* (Asteraceae)
8	Monoflora	Elkharj	• Clover honey
9	Hetero flora	Roda (plain)	• Ziziphus spina (Rhamnaceae), • *Acacia spp*. (Mimosaceae)
10	Hetero flora	Horimalaa	Ziziphus spina (Rhamnaceae), *Acacia spp*. (Mimosaceae)

Antimicrobial activity of honey samples and purified peptides:

An agar well diffusion method was used to assess the antimicrobial activity of the honeys and purified peptides against the selected standard microorganisms (NCCLS, 2003) . Fifty microlitres (50µl) of each honey dilution (undiluted, 70% w/v, and 30% w/v) were used against *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *S. aureus* (ATCC 6538), *B. subtilis* (ATCC 6633) and *C. albicans* (ATCC 10231), separately. While 20µl of two fold serial dilution of purified peptides were tested against *E. coli* (ATCC 25922), *K. pneumoniae* (ATCC 11678) the major Gram negative pathogens causing urinary tract infections and *S. aureus* (ATCC 6538) as G-positive bacteria . Each of the bacterial strains was inoculated into nutrient broth and incubated overnight at 37°C until growth was 0.5 optical density at 640 nm. The honey sample concentrations were added into wells of 5 millimeters (mm) diameter of inoculated Mueller- Hinton agar plates by selected microorganisms, each dilution was done in triplicate. The plates were left at room temperature till the honey seeped into the agar. Zones of growth inhibition were recorded in mm after an overnight incubation at 37^0 C. The end point of antimicrobial activity of each honey was defined as the highest dilution (lowest concentration) producing an inhibition zone with the tested organisms**.** The growth after 24 hr. incubation at 37°C was then compared to a control plate that contained no sample (National Committee for Clinical Laboratory Standards, 1999). All strains were handled under aseptic conditions and the microorganisms were destroyed by autoclave to ensure bio-safety.

Statistical Analysis:

Data analysis were carried out using SPSS for Windows Ver. 17 .0.

RESULTS

A major antimicrobial peptide factor isolated from two groups of intact and immunized Saudi Arabian *Apis mellifer*a obtained from Al- Qasim and Hail locations around Riyadh during the honey season 2008. The peptide was initially fractionated under RP- HPLC by using 70% v/v acetonitrile containing 0.1%v/v trifluoroacetic

acid recovered as two peaks 1 and 2 (Fig. 1) in the final RP-HPLC step. Gel electrophoresis analysis indicated apparent homogeneity and an approximate *Mr* of 14.500 and 15.00 KDa for the isolated peaks 1 and 2 respectively. Differential pattern analysis of described peaks are barely detectable in unchallenged (control) bees but are strongly induced upon infection. All the peptide fractions obtained from chromatography and isolated from honey bees either (intact or bacterial induced) fed on wild *Ziziphus spina* and *Acacia spp.* plants, presented antimicrobial activity against two Gram negative bacteria *E. coli* and *K. pneumoniae*. Fig. 2, represents antibacterial activity of the purified peptide two fractions for each type (immune and intact bees) against the G-negative bacteria *E. coli*. Statistical analysis of the presented data revealed that the isolated peptides have significant moderate activities against *E. coli* while being less active against *K. pneumoniae* for both intact and induced fractions. On the other hand, no antimicrobial activity was observed against *S. aureus* in case of intact fraction while the isolated peptides from immune one have significant activities. The results showed that all the isolated peptides were available to carry out antibacterial activities at very low concentration ranges from 0.0015 to 25 ng. Mean inhibition zone of the induced fraction of *Acacia* bees labeled 3,4 about (23 ± 0.02 mm) was significantly higher than the intact fraction labeled 1, 2 (9 ± 0.001 mm) ($p \leq 0.05$). Comparable significant results were also obtained for purified fractions of *Ziziphus* fed bees. Overall, the activity of purified peptide showed comparable antimicrobial activity in both groups of honey bees tested.

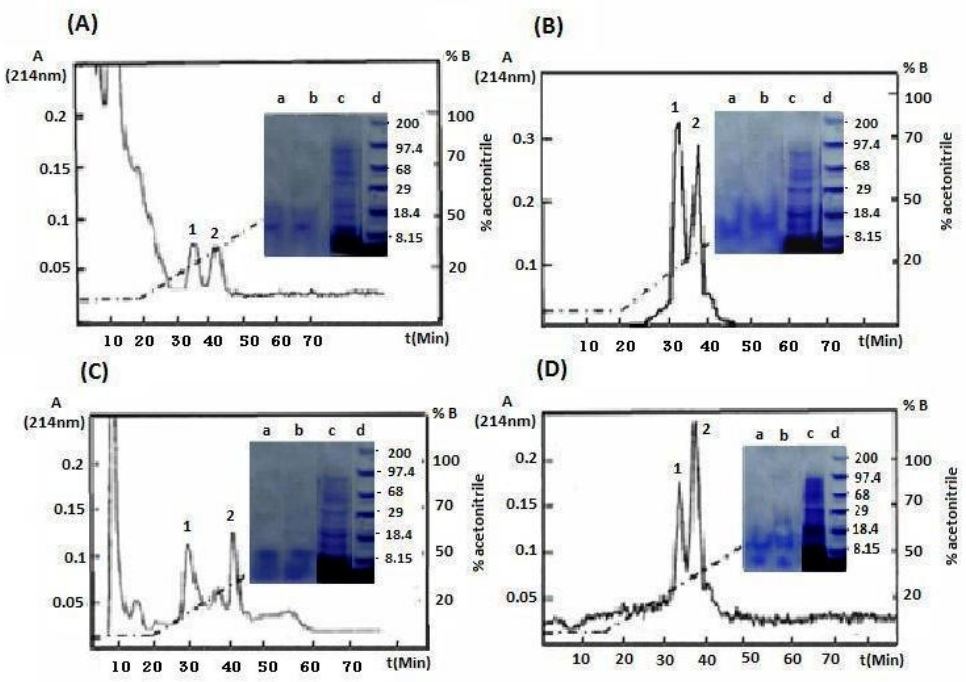

Fig. (1) RP-HPLC purification and SDS-PAGE of antimicrobial polypeptide from *Apis mellifera*. Heat treated lymph from intact and immunized *Apis mellifer*a obtained from Al -Qasim and Hail locations were fractionated, separately, on a VYDAC® C4 and finally C18 columns, respectively. Fractions 1 and 2 developed at a flow rate of 1 ml/min and a gradient of 30-70 % B/70 min. Panels A and B show the HPLC patterns of the isolated polypeptide fractions from, respectively, intact (non-injected) and *E. coli* induced *A. mellifera* (Al Qasim). Panels C and D, respectively, intact and induced bees (Hail). These two fractions isolated from both groups of bees were studied for antibacterial activities. The dashed line represents the percentage of solvent B (70% MeCN in 0.1 % trifluroacetic acid). Electrophoretic analysis of pure fractions (lanes a and b) and crude adult bee lymph (lane c) were shown in the center of the figure. The *Mr* of molecular weight standards are shown in x 10^{-3} lane d.

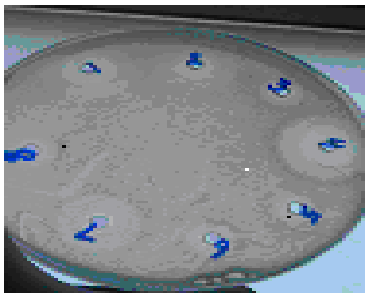

Fig. (2.) Represents the inhibition zones of purified fractions (two fractions) isolated from intact *Apis millefera* lymph of Hail labeled 1, 2; while 3, 4 represent the induced peptide fractions (increase in diameter of inhibition zone observed by induced peptide in label 4). The labels 5, 6 and 7 , 8 indicates the activity of the intact and induced fractions isolated from fed bee lymph from Al- Qasim, respectively increase in diameter of inhibition zone observed by induced peptide in label 7).

Antimicrobial activity of honeys:

The results of the assays of antibacterial activity of the ten honey samples with three concentrations 30% v\v, 70% v\v and undiluted used in this study are shown in Figure (3). The growth of all five standard microorganisms *S. aureus, B. subtilis, P. aeruginosa, E. coli* and *C. albicans* were inhibited by using the agar-well diffusion method (Jorgensen and Ferraro, 1998). Antimicrobial activity was evaluated by measuring the zone of inhibition (mm) against the tested microorganisms. At higher concentrations of honey there was a progressive increase in growth inhibition of the microorganisms. It was observed that *S. aureus* was the most inhibited bacterial strain by all honey samples. The average diameter of the inhibition zones produced by the undiluted honeys samples was approximately (35 ± 0.1) mm. Our data show that all honey samples tested have some antibacterial action at 30%, 70% and undiluted concentrations. In general, all the five tested microorganisms were variably sensitive to honey up to 30% concentration.

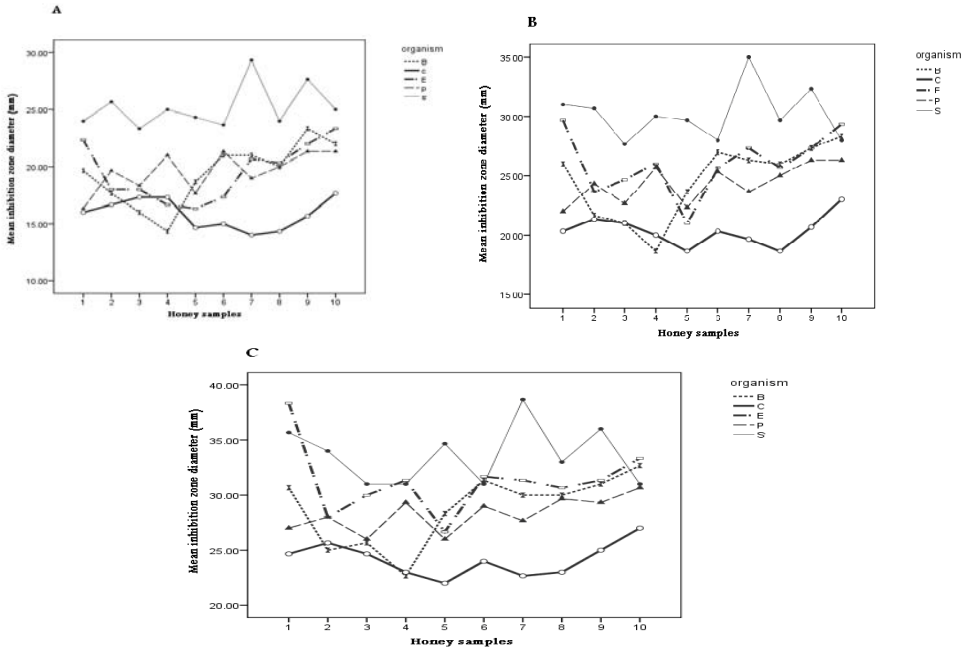

Fig. (3) Mean of bacterial inhibition growth by different concentrations (mm) of ten honey samples (30%, 70% and undiluted A,B, C, respectively) against five standard microorganisms (B refers to *Bacillus subtilius*, C, refers to *Candida albicans*, E refers to *Escherichia coli*, P refers to *Pseudomonas aeruginosa* and S refer to *Stapylococcus aureus).*

DISCUSSION

In recent years there was a dramatically increase in bacteria strains resistance to one or even several antibiotics. Thus, the development of antimicrobial compounds with novel modes of action is a major focus of current pharmaceutical research. A very interesting and promising approach relies on antibacterial peptides, because bacteria do not develop any resistance to these antimicrobial peptide families. One of the most promising among these families are the short, proline-rich antibacterial peptides originally isolated from insects, such as apidaecin, drosocin, formaecin, and pyrrhocoricin. These peptides represent a viable treatment option for the major pathogens in urinary tract infections, that is, *E. coli* and *K. pneumoniae*, causing 90–95% of all urinary tract infections (Czihal *et al*., 2007).

The exact mechanism of action of AMPs remains a matter of controversy, there is a consensus that these peptides selectively disrupt the cell membranes and the amphipathic structural arrangement of the peptides is believed to play an important role in this mechanism.The phospholipids head group charge on cell membranes and peptide charge distribution appears to play an important role in the peptide membrane interactions (Oren and Shai, 1998; Cudic and Otvos, 2002). There is accumulating evidence suggesting that the antibacterial or self defense peptides which are usually highly basic, recognize the acidic phospholipids exposed on the surface of the bacterial membrane (Tytler *et al*., 1995). In the case of microbes, the anionic lipids are present on the outer surface of the membrane whereas for mammalian cells, anionic lipids are present along the cytoplasmic side of the membrane. This feature might account for their preferential activity against bacteria but not against mammalian cells.

Several structure function studies on AMPs have been published (Hanke and Schlue, 1997; Wieprecht *et al*., 1997; Mor, 2000). It is well documented that biophysical properties such as secondary structure, overall charge and hydrophobicity influence the interaction of AMPs with model membranes and biological cells

An ubiquitous polypeptide was purified from the Saudi Arabian honeybee *Apis millefera*. The isolated polypeptide is naturally detected in the adult bees hemolymph, presenting moderate spectrum of antimicrobial activity against *E. coli* and *K.pneumoniae* bacteria the major Gram negative pathogens causing urinary tract infections to human while did not show any activity against G-positive *S. aureus* bacteria. Apparently an immune induction of the bees increased the polypeptide production as appeared from the purification peaks (RP-HPLC, Fig. 1) and more inhibition to the growth of G-negative bacteria (Fig. 2) during evaluate their antimicrobial activity. On the other hand isolated peptides showed significant inhibition to *S. aureus* growth with induced fractions and no significant with intact one. Similar results was obtained by Casteels *et al.*(1993) for the apidaecin as an increase in the transcript level occurred 4 hr. after experimental infection and very high concentrations were sustained throughout the entire 36 hr. post infection. This suggests that there is mounting evidence that activation of insect peptide antibiotic gene is the end- point of a signal pathway that has bacteria, or more specifically lipopolysaccharide (LPS), as initiating agent (Girardin *et al.*,2002).

Comparison of the mean inhibition zones of antibacterial activity between the two purified fractions 1 and 2 revealed non significant differences $P \leq 0.05$ indicating that these peptide fractions are functionally identical. These results are also indicative, as revealed from the electrophoretic profile that showed apparent homogeneity and an approximate *Mr* of 14.500 and 15.00 KDa, respectively and this may be attributed to

the difference in its amino acid modification and the cDNA gens coding the different antimicrobial families as confirmed by Xu *et al.* (2009) in the honey bee *Apis cerana*. Originally the presented data showed that the polypeptide fractions proved be non effective as an antibacterial agent at concentrations up to 0.0015 ng in the lymph of the intact bees towards the tested stansrad bacteria. These results are indicative, as in case of the corresponding hymenoptaecin of (Casteels *et al.,* 1993) and defensin of (Chernysh *et al.,* 1996), but unlike apidaecin (Casteels *et al.*,1990). Consequently the isolated polypeptide holds its place some where between the group of peptides that attack G-positive and G-negative bacteria equally well (Boman, 1994) and many other antibacterial peptides that seem to have clear preference for either G-negatives such as apidaecins (Casteels *et al*., 1989) and diptericins (Bulet *et al.*,1995) or G-positives e.g. insect defensin (Lauth *et al*.,1998), lysozyme and royalisin (Fujiwara *et al.*,1990). In addition, as the corresponding results indicates the purified fractions profile proved to be comparably identical for both groups of adult *Apis millefera* collected from different floral origins.

The variation in the antimicrobial potential of honey samples used in this study as compared to the previous similar studies highlights that the source of the nectars may have contributed to the difference in the antimicrobial activities of honey that is, the flowers from which bees gathered nectar to produce the honey, since flora source determines many of the attributes of honey, for example flavor, aroma, color and composition. As being a natural product, the composition of honey is highly variable (NHB, 1994). Antimicrobial activity of honey is not dependent alone on its phytochemical nature i.e. tetracycline derivatives, ascorbic acid, peroxidase or amylases, streptomycin, sulfonamides which are claimed as heat labile. On the other hand, the antimicrobial effect of honey is attributed to its phenolic acid, flavonides, benzyl - alcohol, 2-hydroxy benzoic acid which are heat stable and may be active agents but their concentration in honey appears too low to solely responsible Heerng, 1998).

The obtained antimicrobial data of ten honey samples obtained from different flora were generally consistent with other reports showing that honey has good antibacterial activity (Patricia *et al*., 2005). Also, Ceyhan and Ugar (2001) tested 84 honeys against eight bacteria and two fungi showing that honey has broad-spectrum activity. In addition, these authors found that the antibacterial activity of honey was greater than that which could be attributed to the sugar content of the honey. Nzeako and Hamdi (2000) in their study of six commercial honeys found that inhibition of *S. aureus*, *E. coli* and *P. aeruginosa* did not occur at honey concentrations 40%, in contrast to the current study where all the tested bacteria showed growth inhibition up to 30% of natural honey concentrations and have shown an excellent activity against *S. aureus*. Interestingly, the obtained results of the ten honey samples under investigation revealed that *C. albicans* sensitivity, although the zones of inhibition were small compared with other bacterial organisms tested and these are consistent with the data proved by Obeseiki and Afonya, (1984) and Nzeako and Hamdi (2000).

The results shown by honey samples in relation to *S. aureus* may be important, given that in recent decades there has been a marked increase in difficult to treat skin and underlying tissue infections associated with *S. aureus* (Halco´n and Milkus, 2004). It has been informed that *S. aureus* has developed resistance against several antibiotics and that it is the principal contaminant agent in many clinical infections (Moreno *et al*., 2005). Thus, new strategies to treat wounds infected with *S. aureus* are needed, and the possibility to use honey appears as a convenient and less costly treatment option. Poor activity of the honeys against *S. aureus* was unexpected as

previous reports by Cooper *et al*. (1999). Part of the explanation for the difference in results from other studies may be due to methodological differences between studies because the agar dilution method used by these authors different from an agar well diffusion method that is used in this study. However it is also likely to be due to variation in the natural floral origin of the honey being produced. Our honey samples also exerted antimicrobial activities on *P. aeruginosa*, which were resistant to some antibiotics.

CONCLUSIONS

Honey and AMPs produced by honeybees (*Apis mellifera*) has antimicrobial activity when tested *in vitro* against standard microorganisms. However, pharmacological standardization and clinical evaluation on the effect of honey are essential before using honey as a preventive and curative measure to common diseases related to the tested bacterial species**.** The isolated polypeptide fractions are further subjected to amino acid characterization and NMR spectrum, to estimate its concentration in the immune bee lymph (Ayaad and Shaker, ongoing research).

In spite of all the positive facts associated with antimicrobial peptides there have been a few problems. Firstly, there are fewer data available on the unknown *in vitro* /*in vivo* toxicities of the peptides. Secondly, the stability of the synthesized compound formulations *in vivo* has not been studied in detail. Lastly, the cost of the production of these peptides on a large scale has been a major obstacle for quite some time.

Hence, further foci would be to identify more of such novel peptides, re-design the existing peptides to get rid of their toxicity and develop novel recombinant protocols to obtain greater yield of peptides at a lower cost.

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ARABIC SUMMARY

عزل ببتيدات مضادة للميكروبات من نحل العسل ودراسة الخصائص الميكروبية لعينات من العسل بالمملكة العربية السعودية

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ساهمة فى مجال الأبحاث الفعالة لايجاد موارد جديدة تؤثر فعل لمقاومة المتزايدة من الإصابة بالميكروبات الممرضة لما أظهرته تلك السلالات الميكروبية من مقاومة للمضادات الحيوية المستخدمة حاليا . جمعت مجموعتين من نحل العسل البرى (*Apis mellifera*) من منطقتى القصيم وحائل بالمملكة العربية السعودية فى موسم العسل لعام 2008م .

تم تجميع بلازما دم الطور البالغ لشغالات النحل و حقن مجموعة من النحل ببكتريا القولون العصوية *E. coli* (ATCC 25922) الحية بتركيز 1.1×10^6 وقد تركت المجموعة الأخرى بدون حقن . تم عزل وتنقية ببتيد من عاملين جزء 1 و2 على التوالى لكل منهم وزن جزئى يتراوح بين 14.5 - 15 كيلو دالتون من بلازما مجموعتى النحل المحفزة وغير المحفزة بالبكتيريا باستخدام جهاز الفصل الكروماتوجرافى (RH-HPLC) .

وقد أثبتت تجارب تقييم فاعلية الببتيد المعزول باستخدام طريقة الانتشارفى اطباق الاجار فى المعمل ضد ميكروبى *E. coli* strain (ATCC 25922) و*Klebsiella pneumoniae* strain (ATCC 11678) التى تعتبر من اكثر انواع البكتريا سالبة الجرام المسببة لالتهابات المسالك البولية أن الببتيد المعزول له فاعلية مؤثرة على كل من الميكروبين على حدة وذلك حتى تركيزات قليلة جدا تتراوح بين 0.0015 to 25 ng وباختباره ضد بكتريا موجبة الجرام *S.aureus* (ATCC 6538) وجد ان له تاثير مثبط فى حالة مجموعة النحل المحفزة و ليس له اى تاثير مثبط فى حالة مجموعة النحل غير المحفزة. تم أيضا إختيار عشرة أنواع من عسل النحل البرى التى جمعت من خلايا النحل من مناطق محيطة بمنطقة الرياض تتميز بتنوع الغطاء النباتى البرى والطبيعة الجغرافية. وقد أظهر التحليل الإحصائى عند تقييم فاعلية تلك الأنواع المختلفة من العسل تجاه مجموعة من الممرضات متمثلة فى البكتيريا القياسية سالبة الجرام مثل *E. coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), والبكتريا القياسية موجبة الجرام مثل *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633) وعينة خميرة *Candida albicans* (ATCC 10231) تواجد فروق معنوية مثبطة ومتباينة بين عينات العسل بإختلاف مصدرها النباتى على نمو الميكروبات المختلفة وذلك حتى تركيز يصل الى 30% . وتلك الاختلافات فى الفاعلية المثبطة لنمو الميكروبات قد يعزى الى تواجد ببتيدات مضادة للميكروبات فى العسل تختلف تبعا لتنوع النبات المغتذى عليه من قبل النحل.