ZANCO Journal of Pure and Applied Sciences
The official scientific journal of Salahaddin University-Erbil
https://zancojournals.su.edu.krd/index.php/JPAS

ISSN (print):2218-0230**, ISSN (online):** 2412-3986**, DOI:** **http://dx.doi.org/10.21271/zjpas**

RESEARCH PAPER

Antimicrobial Activities of Thirteen Insect Species Crude Body Extract Against Some Microbial Pathogens.

Soran H. Ahmed[1], Wand K. Ali[2]

[1]Department of Biology, College of Science, Sulaimani University, Kurdistan Region, Iraq
[2]Department of Biology, College of Education, University of Salahaddin, Erbil, Kurdistan Region, Iraq

A B S T R A C T:

Infectious diseases are ranked as the second cause of death globally. This is mainly caused by the emergence of multi-drug resistant pathogens due to the improper use of antibiotics. This problem has influenced the search for antibiotics, particularly in natural sources from flora and fauna. Insects are about 75% of the fauna and have existed in harsh conditions for millions of years. Antimicrobials are thought to have developed in them for protection against pathogens. Kurdistan is rich in insect biodiversity and there is scarce research on using their body extracts as antimicrobial agents. In this study, thirteen insect species collected around Sulaimani governorate and their crude body extracted by acidified methanol method and tested against some microbial pathogens. Five of the extracts inhibit the growth of multidrug-resistance gram-positive bacteria, *Staphylococcus aureus*, while three of them inhibit the growth of multi-drug-resistant gram-negative bacteria, *Escherichia coli*, while antifungal activities against yeast, *Candida albicans* was not observed. Kurdistan insects can be a source for finding new potential antibiotics. Further studies on the crude body extracts of other species and finding the active compounds as well as the mechanism of action of each compound from active crude body extracts against microbial pathogens are recommended.

KEY WORDS: Insect; Body extract; Antimicrobial activity; Kurdistan
DOI: http://dx.doi.org/10.21271/ZJPAS.35.2.16
ZJPAS (2023) , 35(2);153-159

1. INTRODUCTION:

Infectious diseases are the second leading cause of death worldwide (WHO, 2008) . In addition to the fact that a variety of natural and synthetic antimicrobial agents have been developed, the problems of antimicrobial resistance have drastically increased. It predicts that by 2050, drug-resistant infections will kill ten million people annually, triggering a financial crisis and forcing millions of people into extreme poverty (O'Neill, 2016). The development of multidrug resistant pathogens has become a global issue, posing a serious threat to human health (Maheshwari *et al.*, 2016, Mwangi *et al.*, 2019).
This is mainly due to the indiscriminate use of antibiotics in human and animal medicine and in agricultural practices (Davies and Davies, 2010, Hosain *et al.*, 2021, Obeng-Nkrumah *et al.*, 2013, Van Boeckel *et al.*, 2015), poor sanitation practices, which lead to the introduction of unmetabolized antibiotics into the environment through human and animal waste (Davies and Davies, 2010), and a lack of proper regulation regarding the usage of antibiotics, particularly in developing countries (Ayukekbong *et al.*, 2017).

The rise of multidrug resistance among pathogens has impacted antibiotic research by increasing the expense and time burden of developing new antimicrobial chemotherapy. As a result, there is an urgent need to find new and effective antibiotics. This has inspired a search for antibiotics in natural sources (Mosaheb *et al.*, 2018). Plants and marine algae have long been considered as having antimicrobial properties (Gyawali and Ibrahim, 2014). However, rather than focusing on the flora as a source of non-perishable antimicrobials, it would be interesting to explore the fauna as well. Insects are promising

* **Corresponding Author:**
Soran H. Ahmed
E-mail: soran.ahmed@univsul.edu.iq
Article History:
Received: 03/08/2022
Accepted: 13/10/2022
Published: 20/04 /2023

candidates since they represent about 75% of the fauna (Ratcliffe *et al.*, 2011) and have existed in hazardous environments for millions of years. Antimicrobials are thought to have developed in these organisms to protect them from pathogenic microorganisms (Ratcliffe *et al.*, 2011, Vizioli and Salzet, 2002).

Insects are one of the major sources of antimicrobial peptides (AMPs), over 150 insect AMPs have been purified or identified. Most insect AMPs are small and cationic, and they show activities against bacteria and/or fungi, as well as some parasites and viruses (Tonk and Vilcinskas, 2017, Wu *et al.*, 2018). Several studies have discovered that crude body and brain extract from some insects has potential antimicrobial activity against multidrug-resistant pathogens, which could be a new source of novel antibiotics (Ali *et al.*, 2017, HASSAN *et al.*, 2015, Ma *et al.*, 2019, Mosaheb *et al.*, 2018, Sagar and JayaPrada, 2015). Kurdistan and Iraq have a rich biodiversity of insects without sufficient work on them regarding extracting active antimicrobial compounds, which will be possible to discover new types and sources of antibiotics. In this study, different species of insects were collected around Sulaimani governorate and their crude body extracts were tested against different clinical isolates of pathogens to investigate their antimicrobial activities.

2. MATERIALS AND METHODS

2.1. Collection of Insects

A total of 13 terrestrial insect species were collected around Sulaimani governorate. All the insects were procured from their natural habitat to ensure their innate ability to counter disease-causing agents. The species identification was carried out by expert zoologists at the University of Baghdad/ Iraq National History Research Center and Museum/ Department of Entomology and Invertebrates (number, 43 on 19, June, 2022), (table 1).

Table 1. list of insects used in this study.

N	Common name	Scientific name	Order
1	Oriental hornet	*Vespa orientalis* Linnaeus, 1761	Hymenoptera
2	Honeybee worker	*Apis mellifera* Linnaeus, 1758	Hymenoptera
3	Ant	*Camponotus xerxes* Forel, 1904	Hymenoptera
4	Ladybird	*Coccinella septempunctata* Linnaeus, 1758	Coleoptera
5	Flower chafer	*Tropinota sp.*	Coleoptera
6	Darkling beetle	*Adesmia fougieri* Koch, 1937	Coleoptera
7	Striped shield bug	*Graphosoma semipunctatum* (Fabricius, 1775)	Hemiptera
8	Egyptian grasshopper	*Anacridium aegyptium* (Linnaeus, 1764)	Orthoptera
9	Short-horned grasshopper	*Truxalis sp.*	Orthoptera
10	Locust	*Locasta sp.*	Orthoptera
11	Bush crickets	*Saga ephippigera* Fischer von Waldheim, 1846	Orthoptera
12	American cockroach	*Periplaneta americana* (Linnaeus, 1758)	Blattodea
13	Plain tiger butterfly	*Danaus chrysippus* (Linnaeus, 1758)	Lepidoptera

2.2 Extraction

The whole bodies of the insects were used for the extraction of antimicrobial compounds. Insects were washed three times with sterile distilled water to remove dirt and disinfected with 70% ethanol. The excess ethanol was dried with paper towel, and the insects were dried for 24 hours at 37 °C. Eight grams of each dried insect was grinded into powder, then it was soaked in 10 ml of acidified methanol (methanol/water/acetic acid; 90/9/1: v/v/v) in a sterilized glass container and shaken vigorously in the shaker incubator for 48 hours to allow for better extraction.

After that, a sterile muslin cloth was used to filter the slurry. The obtained extract was homogenized and centrifuged for 5 minutes at 5000 rpm, and the supernatant was filtered by Millipore filter paper. The acidified methanol was removed from the extract by evaporation through air drying, and the dried extract was stored at 4 °C until required (Abdu-Allah *et al.,* 2019, Castro *et al.,* 2009).

2.3 Test Isolates

Multi-drug resistant bacterial and fungal clinical isolates of gram-positive *Staphylococcus aureus*, gram-negative *Escherichia coli*, and *Candida albicans* were obtained from patients at Shar hospital in Sulaimani. The isolates were cultured on selective media (Mannitol Salt Agar, Eosin Methylene Blue Agar and CHROMagar) and identified by using conventional methods (microscopic analysis, biochemical analysis and the Vitek 2 system).

2.4 Antimicrobial Assay

In vitro antimicrobial tests were conducted to screen the antibacterial activity of the crude extractants against clinical isolates; *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* using the agar well diffusion method. The inocula of microorganisms were prepared from 24-hour agar cultures and suspensions were adjusted to 1-2×10^8 Colony Forming Unit (CFU) per ml for bacterial and 1-5× 10^6 CFU per ml for fungi by Densicheck and also a spectrophotometer at 625 nm, (normal saline as a diluent). From crude insect body extracts, a concentration of 200 mg/ml was prepared by dissolving extracts in sterilized distilled water. For each pathogenic bacterium, 100 µl of standardized inoculum by spectrophotometer (1 -2×10^8 CFU/ ml) was first spread on the Mueller Hinton Agar medium and allowed to dry. For yeast, 100 µl of standardized inoculum by spectrophotometer (1 -5× 10^6 CFU/ml) was spread on the Mueller Hinton Agar medium supplemented with 2% glucose and 0.5 mg/mL methylene blue and allowed to dry. Then wells were made using autoclaved cork borer 6mm, and an amount (100 µL) of the prepared extract solution was poured into the well. They were kept in a refrigerator to allow the extracts to diffuse into the agar for 30 minutes, and further incubated in an incubator at 37°C for 18-24 hours for bacteria and 20-24 hours for yeast. The antimicrobial activity was evaluated by measuring the diameter of the inhibition zone in millimeters (mm) (Abdu-Allah *et al.,* 2019, Balouiri *et al.,* 2016). Also, Gentamycin 10 mg/ml disk was preferred for comparisons, and the data compared using one way ANOVA test depending on the GraphPad Prism program software.

3. RESULTS

Data recorded that the oriental hornet, honeybee worker, ladybird, locust and American cockroach showed variable antimicrobial potentiality against *Staphylococcus aureus*, *Escherichia coli* without activity against *Candida albicans*. Data showed that antimicrobial activity against test isolates was not observed in the crude body extracts of the ants, flower chafers, darkling beetle, striped shield bug, Egyptian grasshopper, short-horned grasshopper, bush crickets and plain tiger butterfly (table 2). Among all insect extracts which showed activities against test isolates, there was a significant difference between the antibacterial activities of insect crude body extracts and gentamycin except for the effect of oriental hornet body extract against *Staphylococcus aureus*, which showed no significant difference with gentamycin (table 2).

Table 2. The effect of insect crude body extracts on inhibition zone (mean ±SD) of the tested pathogen isolates.

insects	*Staphylococcus aureus*	*Escherichia coli*	*Candida albicans*
Oriental hornet	24.67 ± 0.51 P value = 0.943	15 ±0.89 P value = 0.0001	NO
Honeybee worker	16.33 ± 0.51 P value = 0.0001	NO	NO
Ant	NO	NO	NO
Ladybird	21.67 ± 0.51 P value = 0.0001	14.67 ± 1.03 P value = 0.0003	NO
Flower chafers	NO	NO	NO
Darkling beetle	NO	NO	NO
True bug	NO	NO	NO
Egyptian grasshopper	NO	NO	NO
Locust	20.17 ± 0.75 P value = 0.0001	NO	NO
Short-horned grasshopper	NO	NO	NO
Bush cricket	NO	NO	NO
American cockroach	15.83 ± 0.75 P value = 0.0001	10.17 ± 0.75 P value = 0.014	NO
Monarch butterfly	NO	NO	NO
Gentamycin 10 mg/ml disk	25 ± 0.81	12± 0.89	-

NO = inhibition zone NOT Observed.
SD = Standard Deviation

3.1. Antibacterial activity against Gram-positive

Data showed that the highest effect on *Staphylococcus aureus*, was attained by the oriental hornet crude body extract, in which inhibition zone reached recorded 24.67 mm ± 0.51, followed by the ladybird, 21.67 mm ± 0.51, the locust, 20.17 mm ± 0.75, the honeybee, 16.33 mm ± 0.51 and the American cockroach, 15.83 mm ± 0.75. While antimicrobial activity was not observed in the crude body extracts of the ant, flower chafer, darkling beetle, striped shield bug, Egyptian grasshopper, short-horned grasshopper, bush crickets, and plain tiger butterfly (table 2).

ANOVA test demonstrated that there was no significant difference between the effect of the Oriental hornet extract and gentamycin, (24.67 mm ± 0.51 and 25 mm ± 0.81, respectively), p value = 0.943, while both of them showed significant differences with the ladybird, locust, honeybee and American cockroach body extract, (21.67 mm ± 0.51, 20.17 mm ± 0.75, 16.33 mm ± 0.51 and 15.83 mm ± 0.75, respectively) p value = 0.0001 (figure 1). Lady bird body extract, (21.67 mm ± 0.51), shows significant difference with the locust body extract, (20.17 mm ± 0.75), p value = 0.0057, it also showed significant difference with the honeybee and American cockroach body extract, (16.33 mm ± 0.51 and 15.83 mm ± 0.75, respectively), p value = 0.0001, (figure 1). Locust body extract, (20.17± 0.75), showed significant difference with honeybee and American cockroach, (16.33 ± 0.51 and 15.83 ± 0.75, respectively), p value = 0.0001, (figure 1). Significant difference was not observed between the honeybee and American cockroach crude body extract, (16.33 ± 0.51 and 15.83 ± 0.75, respectively), p value = 0.78, (figure 1).

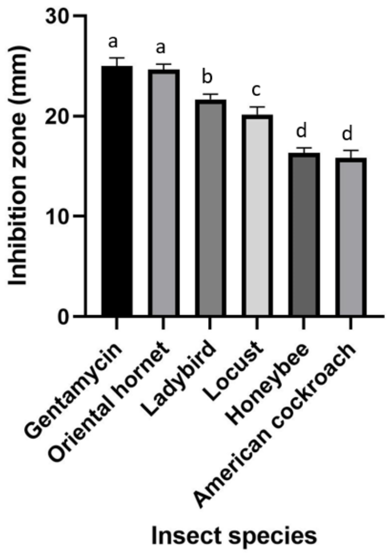

Figure 1. Antibacterial activities of insect crude body extracts and gentamycin 10mg/ml disk on *Staphylococcus aureus*. The values are expressed as mean ± S.D (n=6), (P ≤ 0.05). Different letters mean significant differences between means.

3.2. Antibacterial activity against Gram-negative

Data showed that the highest effect on *Escherichia coli* was attained by the oriental hornet crude body extract, which was reached 15 mm ± 0.89, followed by the ladybird, 14.67 mm ± 1.03, and the American cockroach, 10.17 mm ± 0.75. While antimicrobial activity was not observed in the crude body extracts of the locust, honeybee worker, ant, flower chafers, darkling beetle, striped shield bug, Egyptian grasshopper, short-horned grasshopper, bush crickets and the plain tiger butterfly (table 2).

ANOVA test clarify that there was a significant difference between gentamycin and the oriental hornet crude body extract, (12 mm ± 0.89 and 15 mm +0.81, respectively), p value = 0.0001, Gentamycin and the ladybird crude body extract, (12 mm ± 0.89, and 14.66 mm ± 0.94, respectively), p value = 0.0003 and gentamycin with the American cockroach crude body extract, (12 mm ± 0.89 and 10.16 mm ± 0.47, respectively), p value = 0.01, (figure 2). Significant difference between the oriental hornet and the ladybird crude body extract was not observed, (15 mm ± 0.81 and 14.66 mm ± 0.94, respectively), p value = 0.917, while both of them showed significant difference with the American cockroach crude body extract, (15 mm ± 0.81, 14.66 mm ± 0.94 and 10.16 mm ± 0.47, respectively), p value = 0.0001, (figure 2).

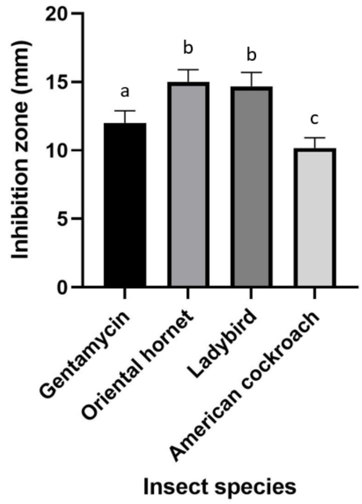

Figure 2. Antibacterial activities of insect crude body extracts and gentamycin 10mg/ml disk on *Escherichia coli*. The values are expressed as mean ± S.D (n=6), (P ≤ 0.05). Different letters mean significant differences between means.

3.3. Antifungal Activity

Data showed that all experimented insect species crude body extracts were not active against *Candida albicans* (Table 2).

4. DISCUSSION

The antimicrobial potential of insect species is an untapped source that ought to be explored fully. Despite the fact that Kurdistan is rich in insect biodiversity, there is a scarcity of literature on them in terms of extracting active antimicrobial compounds. In this study, we tested the antimicrobial activities of Thirteen insect crude body extracts by acidified methanol method against microbial pathogens: *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. Antibacterial activity was observed in five of the selected insect species, and antifungal activity was not observed in any of the selected insect species against *Candida albicans*. This result was supported by other studies, as undertaken by Ma *et al*. (2019) eleven insect species body extracts were tested against different microbial pathogens, three of them had antibacterial activities, while none of them had antifungal activities (Ma *et al.*, 2019) and this supported our results for not observing any antifungal activity. Another study observed that crude body extracts by methanol of three insect species had antibacterial activities but no antifungal activity against *Candida albicans* (HASSAN *et al.*, 2015).

Oriental hornet crude body extracted by methanol observed to has antibacterial activities against both *Staphylococcus aureus* and *Escherichia coli* (HASSAN *et al.,* 2015), same research observed antimicrobial activity of locust crude body extract against *Staphylococcus aureus*, while there was no antibacterial activity against *Escherichia coli*, as recorded in the present study. Abdu-Allah *et al*., (2019) observed antimicrobial activities of honeybee worker crude body extracts against *Staphylococcus aureus* and *Escherichia coli* however, in our study, antibacterial activity against *Escherichia coli* was not observed, same research observed antibacterial activities of ladybird crude body extract against both *Staphylococcus aureus* and *Escherichia coli*, as was recorded in our results. Antibacterial activities of American cockroach crude body extract against both *Staphylococcus aureus* and *Escherichia coli* were observed, it is in agreement with some studies which recorded antibacterial activities against *Staphylococcus aureus* and *Escherichia coli* in the crude brain extract *(Ali et al.,* 2017, Ali *et al.,* 2021) and the hemolymph (Hussein Al-Hindera and Hamoudi, 2020) of the American cockroach. Our results clarify that *Candida albicans* was not inhibited by crude body extracts of all selected insect species; this result was inconsistent with the effect of crude body extracts of several insect species against *Candida albicans* (Cytryńska *et al.,* 2007, HASSAN *et al.,* 2015, Ma *et al.,* 2019). However, it disagreed with some studies which found that some insect species' body extracts have antifungal activities against *Candida albicans* (Leem *et al.,* 1999, Yamada and Natori, 1994).

Our results on the sensitivity of the tested pathogens to different insect body extracts revealed that bacterial strains were more susceptible in comparison with fungal strains, it also demonstrated that gram-positive, *Staphylococcus aureus* was more susceptible than both gram-negative bacterial, *Escherichia coli* and fungus strain, *Candida albicans*. Our results are consistent with a number of previous research on the antimicrobial activity of several insect crude body extracts against gram-positive bacteria, gram-negative bacteria and yeast (HASSAN *et al.*, 2015, Ma *et al.,* 2019, Mohtar *et al.,* 2014). These microorganisms' cell wall structure may be consistent with this response. The fungal cell wall is mostly made of chitin and other polysaccharides (Hudler, 2019), whereas the cell wall of bacterial is primarily peptidoglycan in composition (Heijenoort, 2001). In addition, gram-negative bacteria are generally more resistant to antimicrobials in comparison to gram-positive, because they have an extra lipopolysaccharide membrane that is hydrophilic and it creates a substantial barrier that inhibits some pharmaceuticals and antibiotics from entering the cells (Delcour, 2009).

5. CONCLUSIONS

We concluded that insects' crude body extract by methanol can be used as a new and potential source of antibiotics to deal with multidrug resistant microbes after different species of microbes showed resistance to classic antibiotics. this research on the antimicrobial activity of insect body extracts is limited to only 13 insect species. However, the insect resources of Kurdistan and Iraq are extremely rich and the number is very large, providing a new research resource for further investigation of the antibacterial active substances. Moreover, as five crude extracts had antibacterial activities in this study, further research is needed to find the effect of each compound in the crude extracts as well as understand the mechanism of action of active compounds on tested microbes.

Acknowledgements

We are delighted to express our deepest gratitude to experts at University of Baghdad/ Iraq National History Research Center and Museum/ Department of Entomology and Invertebrates for their help in the identification of the selected insect species that used in this study. Our gratitude extends to anonymous reviewers for their thoughtful comments on the initial manuscript of this paper.

Conflict of Interest

The authors declare no conflicts of interest regarding this article.

References

ABDU-ALLAH, S. N., MZHR, N. N., ALUBADI, A. E. M. & SHANYOOR, G. J. 2019. Effect of Crude Extracts of Natural Compounds from local Iraqi insects of Worker bees and Ladybirds as Antimicrobial Activity on Pathogens. *Journal of Pharmaceutical Sciences and Research,* 11**,** 371-374.

ALI, S. M., SIDDIQUI, R., ONG, S.-K., SHAH, M. R., ANWAR, A., HEARD, P. J. & KHAN, N. A. 2017. Identification and characterization of antibacterial compound (s) of cockroaches (Periplaneta americana). *Applied microbiology and biotechnology,* 101**,** 253-286.

ALI, S. M., SIDDIQUI, R., SAGATHEVAN, K. A. & KHAN, N. A. 2021. Antibacterial activity of selected invertebrate species. *Folia Microbiologica,* 66**,** 285-291.

AYUKEKBONG, J. A., NTEMGWA, M. & ATABE, A. N. 2017. The threat of antimicrobial resistance in developing countries: causes and control strategies. *Antimicrobial Resistance & Infection Control,* 6**,** 1-8.

BALOUIRI, M., SADIKI, M. & IBNSOUDA, S. K. 2016. Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis,* 6**,** 71-79.

CASTRO, M. L., VILELA, W. R., ZAULI, R. C., IKEGAKI, M., REHDER, V. L. G., FOGLIO, M. A., DE ALENCAR, S. M. & ROSALEN, P. L. 2009. Bioassay guided purification of the antimicrobial fraction of a Brazilian propolis from Bahia state. *BMC Complementary and Alternative Medicine,* 9**,** 1-6.

CYTRYŃSKA, M., MAK, P., ZDYBICKA-BARABAS, A., SUDER, P. & JAKUBOWICZ, T. 2007. Purification and characterization of eight peptides from Galleria mellonella immune hemolymph. *peptides,* 28**,** 533-546.

DAVIES, J. & DAVIES, D. 2010. Origins and evolution of antibiotic resistance. *Microbiology and molecular biology reviews,* 74**,** 417-433.

DELCOUR, A. H. 2009. Outer membrane permeability and antibiotic resistance. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics,* 1794**,** 808-816.

GYAWALI, R. & IBRAHIM, S. A. 2014. Natural products as antimicrobial agents. *Food control,* 46**,** 412-429.

HASSAN, M. I., MOHAMED, A. F., AMER, M. S., HAMMAD, K. M. & MAHBOUB, M. T. 2015. ANTIMICROBIAL ACTIVITY OF THREE INSECT SPECIES, CRUDE EXTRACTS AGAINTS CERTAIN MICROBIAL AGENTS. *Al-Azhar Bulletin of Science,* 26**,** 19-24.

HEIJENOORT, J. V. 2001. Formation of the glycan chains in the synthesis of bacterial peptidoglycan. *Glycobiology,* 11**,** 25R-36R.

HOSAIN, M. Z., KABIR, S. L. & KAMAL, M. M. 2021. Antimicrobial uses for livestock production in developing countries. *Veterinary World,* 14**,** 210.

HUDLER, G. W. 2019. Magical mushrooms, mischievous molds. *Magical Mushrooms, Mischievous Molds.* Princeton University Press.

HUSSEIN AL-HINDERA, A. A. & HAMOUDI, H. N. 2020. Effect of Antimicrobial Agents Extracted from American Cockroach Insect Periplaneta Americana L. on Some Species of Microbes. *Indian Journal of Forensic Medicine & Toxicology,* 14.

LEEM, J. Y., JEONG, I. J., PARK, K. T. & PARK, H. Y. 1999. Isolation of p-hydroxycinnamaldehyde as an antibacterial substance from the saw fly, Acantholyda parki S. *FEBS letters,* 442**,** 53-56.

MA, G., WU, L., SHAO, F., ZHANG, C. & WAN, H. Antimicrobial Activity of 11 Insects Extracts Against Multi Drug Resistant (MDR) Strains of Bacteria and Fungus. IOP Conference Series: Earth and Environmental Science, 2019. IOP Publishing, 022132.

MAHESHWARI, M., AHMAD, I. & ALTHUBIANI, A. S. 2016. Multidrug resistance and transferability of blaCTX-M among extended-spectrum β-lactamase-producing enteric bacteria in biofilm. *Journal of Global Antimicrobial Resistance,* 6**,** 142-149.

MOHTAR, J. A., YUSOF, F. & ALI, N. M. H. 2014. Screening of novel acidified solvents for maximal antimicrobial peptide extraction from Zophobas morio fabricius. *Advances in Environmental Biology,* 8**,** 803-809.

MOSAHEB, M. U.-W. F. Z., KHAN, N. A. & SIDDIQUI, R. 2018. Cockroaches, locusts, and envenomating arthropods: a promising source of antimicrobials. *Iranian journal of basic medical sciences,* 21**,** 873.

MWANGI, J., HAO, X., LAI, R. & ZHANG, Z.-Y. 2019. Antimicrobial peptides: new hope in the war against multidrug resistance. *Zoological research,* 40**,** 488.

O'NEILL, J. 2016. Tackling drug-resistant infections globally: final report and recommendations.

OBENG-NKRUMAH, N., TWUM-DANSO, K., KROGFELT, K. A. & NEWMAN, M. J. 2013. High levels of extended-spectrum beta-lactamases in a major teaching hospital in Ghana: the need for regular monitoring and evaluation of antibiotic resistance. *The American journal of tropical medicine and hygiene,* 89**,** 960.

RATCLIFFE, N. A., MELLO, C. B., GARCIA, E. S., BUTT, T. M. & AZAMBUJA, P. 2011. Insect natural products and processes: new treatments for human disease. *Insect biochemistry and molecular biology,* 41**,** 747-769.

SAGAR, S. & JAYAPRADA, R. C. Periplaneta species brain proteins and their efficacy as antibiotics. International Conference on Advances in Biotechnology (BioTech). Proceedings, 2015. Global Science and Technology Forum, 109.

TONK, M. & VILCINSKAS, A. 2017. The medical potential of antimicrobial peptides from insects. *Current topics in medicinal chemistry,* 17**,** 554-575.

VAN BOECKEL, T. P., BROWER, C., GILBERT, M., GRENFELL, B. T., LEVIN, S. A., ROBINSON, T. P., TEILLANT, A. & LAXMINARAYAN, R. 2015. Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences,* 112**,** 5649-5654.

VIZIOLI, J. & SALZET, M. 2002. Antimicrobial peptides from animals: focus on invertebrates. *Trends in pharmacological sciences,* 23**,** 494-496.

WHO 2008. *The global burden of disease: 2004 update,* World Health Organization.

WU, Q., PATOČKA, J. & KUČA, K. 2018. Insect antimicrobial peptides, a mini review. *Toxins,* 10**,** 461.

YAMADA, K. & NATORI, S. 1994. Characterization of the antimicrobial peptide derived from sapecin B, an antibacterial protein of Sarcophaga peregrina (flesh fly). *Biochemical Journal,* 298**,** 623-628.