



In vitro Antibacterial Activities of Honey Bee Extracts against Bacterial Isolates of Wound Infections

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Authors' contributions

This work was carried out in collaboration among all authors. Author OAA designed the study, managed literature searches and wrote the first draft of the manuscript. Author ATO assisted author OAA in performing statistical analysis. Authors JOA and MKO guided in entire research. All authors read and approved the final manuscript.

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ABSTRACT

Microorganisms most especially bacteria, continue to develop resistance against antimicrobial agents; hence novel sources of antibiotics are urgently needed to reduce this problem. This study was carried out to investigate the antibacterial activities of ethanolic, chloroform and aqueous extracts of *Apis mellifera* (honey bee) on isolates of wound infections. The isolates used in this study were procured from University of Ilorin Teaching Hospital (UIH) and confirmed using morphological and biochemical tests. The isolates used include; *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Proteus vulgaris*. Honey bees were collected from an apitherapist at Sunshine honey and agro foods, Akure, Ondo State Nigeria. The whole insect was used for *in vitro* antibacterial evaluation of the isolates using agar well diffusion method. Ethanolic extract of *A. mellifera* had the highest inhibitory activity with mean zones of inhibition ranging from 7.40 mm to 21.67 mm, chloroform extracts had moderate inhibitory activity ranging from 4.63 mm to 10.03 mm while the aqueous extract had the least activity with zones of inhibition ranging from 3.00 mm to 6.30 mm. However, no antibacterial activity was observed against *P. aeruginosa* for all the extracts. It is concluded that extracts of honey bees most especially the ethanolic extract have antibacterial activity and thus could be a potential antibacterial agent against isolates of wound infections.

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1. INTRODUCTION

The primary function of intact skin is to control microbial populations that live on the skin surface and to prevent underlying tissue from becoming colonized and invaded by potential pathogens [1]. Exposure of subcutaneous tissue following a loss of skin integrity (i.e. wound) provides an environment that is conducive to microbial colonization and proliferation. Indiscriminate use of antibiotics has led to the emergence of multidrug resistant bacterial strains, which over time has become a global public health problem [2,3].

There has been a rising emergence of pathogenic bacteria which are resistant to multiple antimicrobial agents. This has become a significant public health threat as there are fewer, or even sometimes no effective antimicrobial agents available for infections caused by these bacteria [2]. As a result of antibiotic resistance developed by bacterial isolates, alternative sources are required to combat this menace.

The use of insects and their extracts as therapeutic resources in the medical systems of several human societies date back to the early times- a term known as entomotherapy. *Apis mellifera* belongs to the family *Apoidea* and are presently considered a clade, called Anthophila. Its products such as honey, royal jelly, propolis, beeswax and bee venom have been used extensively in medicine [4]. However, there is no documented research on the antibacterial activity of the insect itself. Hence, this study investigated the *in-vitro* antibacterial activity of *Apis mellifera* extracts against bacterial isolates of wound infections.

2. MATERIALS AND METHODS

2.1 Collection of Honey Bees

A total of 75 honey bees were collected in sterile air-free jars from a bee hive with the help of an apitherapist at Sunshine honey and agro industry, Akure, Ondo State, Nigeria and after which they were confirmed not to be adulterated. The bees collected were then transported to the Microbiology Laboratory, School of Sciences, Federal University of Technology, Akure for analysis.

2.2 Preparation of Honey Bee Extracts

The collected honey bees were made inactive by placing them in the freezer for 10- 20mins. The bees were dried in the hot air oven at a temperature of 45°C and crushed into powder in a sterile dry ceramic mortar and pestle. The coarse powders were also made into fine powders, placed in containers and labelled for storage prior to use for the analysis.

2.3 Aqueous Extract Preparation

Ten grams (10 g) of the ground bee powder was placed in a sterile container and 100 ml of distilled water was added. The contents in the container were mixed and capped with a tight fitting lid and allowed to stand undisturbed overnight. The solution obtained was filtered using Whatman No. 1 filter paper to remove solid insect material. The filtrate was then dried in the oven.

2.4 Organic Extracts Preparation

Ten grams (10 g) of the ground powder was mixed and macerated with 100 ml each of absolute ethanol and chloroform in separate containers for 48 hours. The extract was filtrated through Whatman No 1 filter paper. The solvents were removed from the filtrate with the aid of a rotary evaporator to obtain a concentrated oily extract. The extracts were stored in universal bottles prior to usage.

2.5 Sterility Proofing of the Extracts

This was carried out using the method described by Sule and Agbabiaka [5] with some modifications. Briefly, 2 ml of the extract was introduced into 10 ml of Mueller Hinton broth and incubated at 37°C for 24 hours. The absence of turbidity or clearness of the broth after the period of incubation signifies the presence of a sterile extract.

2.6 Test Bacterial Isolates

The test organisms used in this study were clinical isolates of Gram positive and Gram negative bacteria that were preisolated from wound infections. The Gram positive bacterium used was *Staphylococcus aureus* while the Gram negative bacteria include; *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris*.

These clinical bacterial isolates were procured from Unilorin teaching hospital complex (UITH), Kwara State, Nigeria.

2.7 Antibiotic Susceptibility Testing

This test was carried out to determine the resistance and susceptibility of the bacterial isolates to conventional antibiotics. Antibiotic susceptibility of the isolates was determined using the Kirby's Bauer's disc diffusion method on Mueller Hinton agar. Both Gram positive and Gram negative antibiotic discs (MaxiCare, Nigeria) with specified concentrations were used for the assay. Each antibiotic disc contained Septrin (30 µg), Sparfloxacin (10 µg), Ciprofloxacin (30 µg), Amoxicillin (30 µg), Augmentin (10 µg), Gentamycin (30 µg), Pefloxacin (30 µg), Tarivid (10 µg), Streptomycin (30 µg), Chloramphenicol (30 µg) and Erythromycin (10 µg).

The standardized bacteria were swabbed uniformly across Mueller Hinton agar plates, then antibiotic discs were placed on the seeded agar plate. The bacteria were allowed to grow on the agar media, and then observed after 24 hours incubation period at 37°C. The zones of inhibition were measured. The amount of space (zones of inhibition) around each antibiotic disc indicates the lethality of the antibiotic on the bacteria.

Highly effective antibiotics produced a wide ring of no bacterial growth (larger zone of inhibition) while an ineffective antibiotic showed no change in the surrounding bacterial concentration at all. The zones of inhibition were classified into susceptible (17 mm and above), intermediate (13 mm-17 mm), and resistant (0-12 mm) based on the specified standard of zone of inhibition for pathogenic gram negative bacilli [6].

2.8 Antibacterial Assay

The antibacterial screening was carried out using agar well diffusion technique as described by Cheebrough [6]. The bacterial isolates were standardized by introducing 3-5 colonies of the overnight grown cultures into peptone water, incubated for about 2 hours at 37°C. The turbidity of the culture was compared with that of 0.5 MacFarland [7]. Sterile cotton swabs were used to pick the inocula for streaking aseptically across prepared Mueller-Hinton agar plates prepared according to manufacturer's specification.

The cultures were uniformly distributed all over the agar plate with the aid of a sterile glass spreader rotating in three directions at approximately 60°C for even distribution of inocula on the Mueller Hinton agar plates. The inoculated plates were closed and allowed to dry for about 30 minutes. Wells having 6 mm diameters were created in the inoculated Mueller Hinton agar plates with the use of a sterile cork borer.

The wells were sufficiently spaced and labeled accordingly. About 0.1 ml of varying concentrations (25%, 50% and 100%) of the prepared bee extracts were dispensed aseptically into the holes. All plates were then allowed to stand on the bench for 1 hour to ensure proper diffusion of the bee extracts into the medium, after which the plates were incubated at 37°C for 24 hours. After overnight incubations, a clean meter rule was used to measure the diameters of the zones of inhibition around the wells.

The whole experiment was carried out in triplicate and the mean diameters of zones of inhibition were calculated for each bacterium [8].

3. RESULTS AND DISCUSSION

The most effective antibiotics against the bacterial isolates were Chloramphenicol, Streptomycin and Ciprofloxacin as they had a broad-spectrum activity against both Gram positive and Gram negative organisms used in this study.

However, amoxicillin had a narrow spectrum activity as all the Gram negative organisms used were all resistant to it but it inhibited the only Gram positive organism used (*S. aureus*). This could be as a result of the difference in the cell wall composition of both Gram positive and Gram Negative organisms. Peirera [9] reported similar results for susceptibility pattern of bacterial isolates where amoxicillin showed no antibacterial activity against Gram negative isolates.

The antibacterial activity of all the extracts were concentration dependent. At 100% concentration, all the extracts had inhibitory activities on all the isolates used while at other concentrations, no antibacterial activity was observed.

These findings were contrary to Billah [10] who evaluated the brains, exoskeletons and gut of *P. americana* for antibacterial activity using chloroform, water and ethanol as extracts. Results from their study showed that ethanolic extract exhibited antibacterial activity while chloroform and aqueous extracts had no activity against any of the bacterial isolates even at 100% concentration. Observations from their study may be due to the selective use of the body parts (brain, exoskeleton and gut) on the bacteria and not the whole insects as used in this study. Processing and storage techniques (freeze-drying of aqueous extracts and storing in the refrigerator) used by [10] could also contribute to the inactivity of the chloroform and aqueous extracts as *A. mellifera* extracts were used immediately after preparation in this study.

Ethanolic extract had the highest activity on all the wound isolates used followed by chloroform extract and the extract with the least activity was the aqueous extract. The highest zone of inhibition for ethanolic extract and chloroform extract was recorded against *S. aureus* with 21.26 mm and 10.03 mm zone of inhibitions respectively. While the highest zone of inhibition for aqueous extract was observed against *E. coli* (0±0.13 mm).

The highest antibacterial activities observed for ethanolic extract as compared to other extracts used could be as a result of the fact that ethanolic extract had the ability to extract most of the potent antibacterial agent present in the honey bees which other extracts could not.

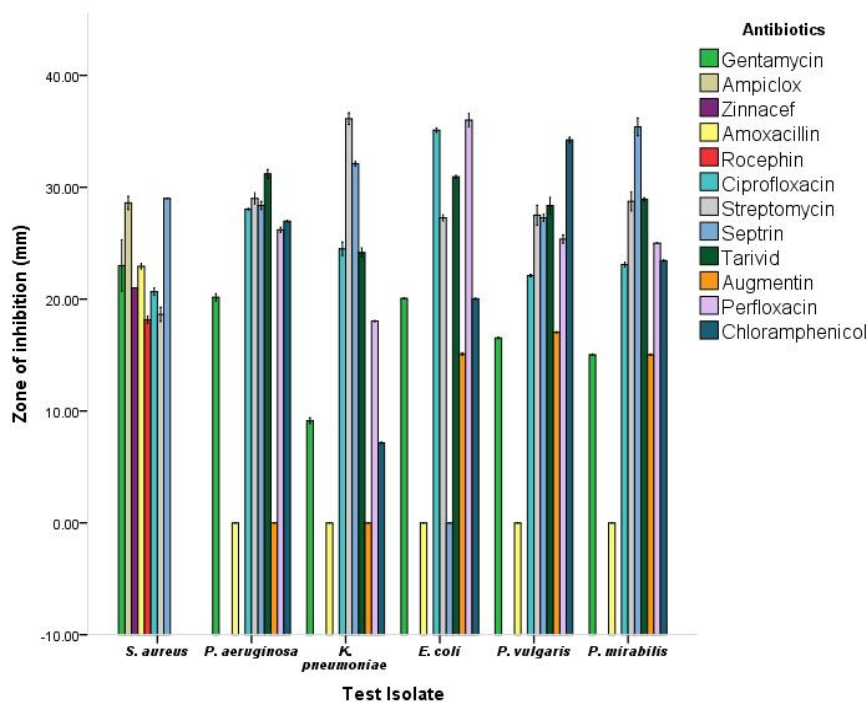


Fig. 1. Antibacterial susceptibility patterns of bacterial isolates

Table 1. Quantitative antibacterial evaluation of *Apis mellifera* extracts at 100% concentration

Isolates	Aqueous (mm)	Chloroform (mm)	Ethanol (mm)	Chloramphenicol (mm)
<i>Staphylococcus aureus</i>	5.53±0.13	10.03±0.13	21.67±0.17	21.03±0.06
<i>Pseudomonas aeruginosa</i>	0.00±0.13	0.00±0.00	0.00±0.17	28.03±0.06
<i>Klebsiella pneumoniae</i>	3.00±0.13	5.53±0.07	7.40±0.17	24.13±0.06
<i>Escherichia coli</i>	6.30±0.13	7.17±0.07	14.93±0.17	35.03±0.06
<i>Proteus vulgaris</i>	4.20±0.13	4.63±0.07	16.13±0.17	22.00±0.06
<i>Proteus mirabilis</i>	4.17±0.13	4.77±0.07	18.10±0.17	23.17±0.06

Results expressed as mean ± standard deviation

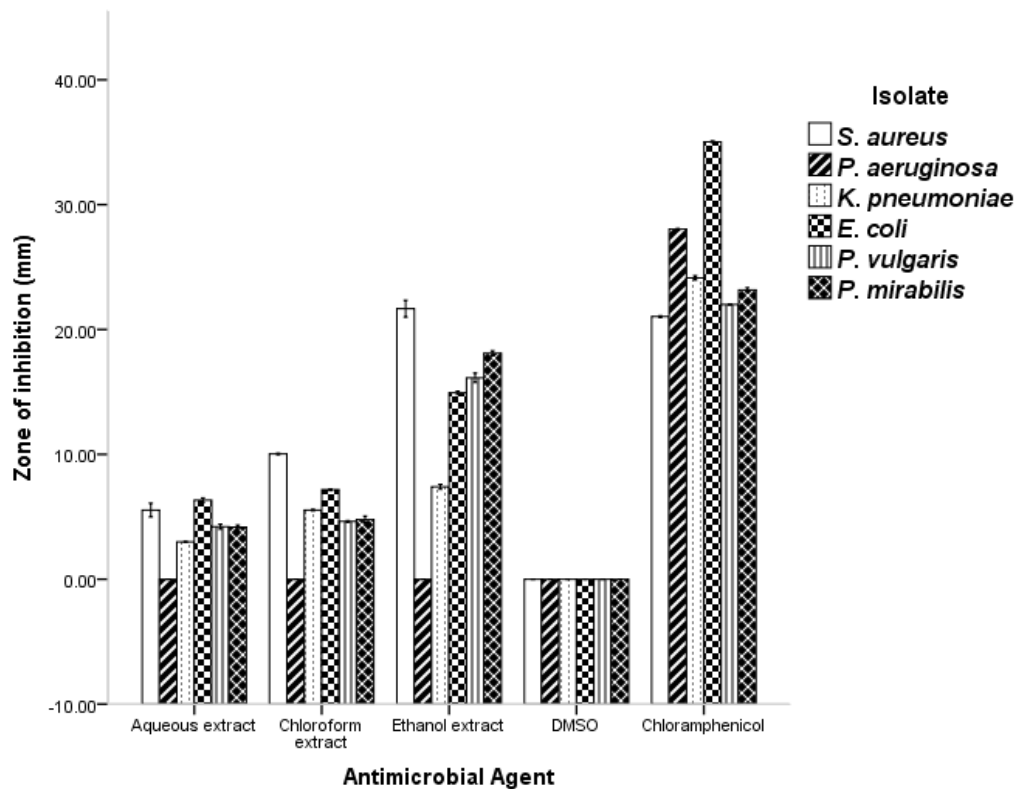


Fig. 2. Comparison of the antibacterial activities of various extracts against bacterial isolates at 100% concentration

4. CONCLUSION

The result of this study revealed the potential of whole insect of *Apis mellifera* as a probable antibacterial agent against isolates of wound infections. It also revealed that ethanol extract of the honey bee had the highest antibacterial activity on all the wound isolates used. Further isolation, identification and purification studies of the individual components of *Apis mellifera* are necessary as these can act as breakthrough in developing novel therapeutics against pathogens of wound infections.

ETHICAL APPROVAL

As per international standard written ethical permission has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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