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# Research Article
# *Apis mellifera* Venom Inhibits Bacterial and Fungal Pathogens *in vitro*

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## Abstract

**Background and Objective:** Bacterial and fungal infections are major public health problems. Emerging of drug-resistant microbial strains urges the need for the development of alternative untraditional antimicrobial agents. Bee venom is a rich source of secondary metabolites and antimicrobial agents. In this study, the antimicrobial and antifungal potential of *Apis mellifera* BV (*Am*BV) against some medically important bacterial and fungal pathogens was investigated. **Materials and Methods:** Broth microdilution method and Colony Forming Unit (CFU) assay were used to screen the antibacterial potential of *Am*BV. Similarly, the antifungal activity of *Am*BV was evaluated using the agar-well diffusion assay. Moreover, the minimum inhibitory concentration (MIC) values of *Am*BV against tested microorganisms were determined. **Results:** *Am*BV significantly inhibited bacterial and fungal growth. The MIC values of *Am*BV were 15.625, 31.25, 7.8, 7.8 μg $mL^{-1}$ against *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538P, *Serratia marcescens* AUH 98 and *Streptococcus mutans* ATCC 25175, respectively. Similarly, *Am*BV at concentrations of 300 and 600 μg $mL^{-1}$ significantly inhibited the growth of *Aspergillus niger* ATCC 16404, *Alternaria alternata* MLBM09, *Fusarium oxysporum* MLBM212 and *Aspergillus flavus*. **Conclusion:** These results indicated that *Am*BV could be used in future preclinical and clinical studies to develop cost-effective and efficient antibacterial and antifungal agents. Moreover, this study presents *Am*BV as an efficient alternative antimicrobial agent against medically important pathogens.

Key words: Bee's venom, *Apis mellifera*, antibacterial, antifungal, MIC

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Bee's venom (BV) has been used in traditional medicine applications to treat a variety of diseases[1]. There are many pharmacologically and biologically active components in BV, including there are many pharmacologically and biologically active components in BV including melittin, adolapin, apamin, hyaluronidase, phospholipase A2, histamine, epinephrine, lipids, carbohydrates and minerals[2,3]. The BV is released by the poison glands of honeybee workers as a defence mechanism[4].

Natural products such as BV are bioactive compound-rich products and have been shown to mediate a wide range of effects against several diseases[5,6]. Although BV is poisonous to bee predators, it has been developed as a medicinal tool by humans through time. The use of BV for therapeutic purposes dates back to Ancient Egypt (4000 BC), Hippocrates, Aristotle and Galen used it during the Greek and Roman periods[7,8]. Inflammatory disorders such as rheumatoid arthritis, tendinitis, fibrosis, lupus and multiple sclerosis were treated with BV in traditional Chinese medicine and other historical traditions[8,9]. Melittin is the main active polypeptide in BV and has anti-cancer, anti-inflammatory, antibacterial and antiviral properties[10,11]. In their study, Yu *et al.*[12] demonstrated that BV has potent antifungal properties against *Trichophyton mentagrophytes* and *Trichophyton rubrum*, which are far more potent than fluconazole, a commercial antifungal used to treat superficial and systemic fungal infections.

Multidrug-resistant bacteria (MDRB) like *Escherichia coli* (*E. coli*) are becoming more common globally, this in part owing to the expansion of bacterial mobile resistant genetic components like plasmids[13]. Similarly, MDRB strains of *S. marcescens* cause a wide spectrum of clinical features including pneumonia, meningitis, conjunctivitis, sepsis, urinary tract infections and surgical wound infections[14]. *S. aureus* is one of the major opportunistic human pathogens. *S. aureus* evades the immune system and causes a variety of human illnesses ranging from minor skin irritations to life-threatening sepsis[15,16]. *Streptococcus mutans* (*S. mutans*) colonize the oral cavity and are responsible for dental caries and periodontal diseases. The antimicrobial resistance of many bacterial and fungal strains has limited the efficacy of available commercial antimicrobial agents. Thus, new untraditional antimicrobial agents are highly in need to control infection of MDRB. The current study aimed to evaluate the antimicrobial activity of *Apis mellifera* BV (*Am*BV) against some important bacterial and fungal pathogens.

## MATERIALS AND METHODS

**Study area:** This study was conducted in 2021 at the Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Assiut Governorate, Egypt.

**Microbial strains:** For this study, the American Type Culture Collection (ATCC) reference strains (*Streptococcus mutans* ATCC 25175 (*S. mutans* ATCC 25175), *Staphylococcus aureus* ATCC 6538P (*S. aureus* ATCC 6538P), *Escherichia coli* ATCC 8739 (*E. coli* ATCC8739) and *Aspergillus niger* ATCC 16404 (*A. niger* ATCC 16404) and clinical isolates (*Serratia marcescens* AUH 98 (*S. marcescens* AUH 98), *Alternaria alternata* MLBM09 (*A. alternata* MLBM09), *Fusarium oxysporum* MLBM212 (*F. oxysporum* MLBM212) and *Aspergillus flavus* (*A. flavus*)) were used[17]. Bacterial strains were grown on Mueller Hinton Broth (MHB) at 37°C, while the fungal strains were grown in potato dextrose agar (PDA) medium and incubated at 28°C. Bacterial suspensions were freshly prepared before each experiment and adjusted to the turbidity of 0.5 McFarland standards (OD630 ~ 0.08 in MHB). Bacterial suspensions were used within 30 min of preparation.

**Collection of *Apis mellifera* Bee Venom (*Am*BV):** The venom of *Apis mellifera* forager honeybees descended from naturally mated queens was used to study its antimicrobial activity as previously described by Surendra *et al.*[18]. These bees were derived from a colony pool and collected from the Department of Bees, The Institute of Plant Protection, the Center for Agriculture and the Ministry of Agriculture Egypt. They were rendered immobile by rapid freezing at -20°C. The stinging apparatuses of honeybees were dissected at 4°C and the venom reservoirs were extracted and stored at -20°C till used in subsequent assays. Venom sacs were resuspended in Milli-Q water and whole bee venom (WBV) was extracted by disrupting the reservoir using a glass rod under rapid defrosting and light pressure. Extracted venom samples were centrifuged at 10,000 g for 5 minutes at 4°C and the supernatants were used as protein and enzyme sources before being lyophilized. Lyophilized Bee venom was dissolved in Milli-Q water and filtered through a 0.22 m syringe filter before using it in further experiments.

### Determination of the antibacterial activity of *Am*BV

**Broth microdilution method:** Serially diluted *Am*BV was transferred into wells of 96-well plate in 90 µL volume. The final concentrations of *Am*BV in wells were 7.6-4000 µg $mL^{-1}$. Next, 10 µL of bacterial suspension was added to each well. The plate was incubated at 37°C for 24 hrs before reading the

optical density (OD) at 630 nm using a microplate reader[19]. Untreated (UT) bacterial suspension was used as a growth control.

**Colony Forming Unit (CFU) assay:** Antibacterial activity of *Am*BV was assessed by counting Colony Forming Units (CFUs)[20-22]. Briefly, 1 mL of serially diluted *Am*BV (250 to 4000 μg $mL^{-1}$) was inoculated with 10 μL of bacterial suspension and incubated at 37°C for 6 hrs. Next, 10-fold serial dilutions were prepared from each treatment and 10 to 100 μL of each dilution was streaked on Muller-Hinton agar (MHA) plates. Bacterial colonies were counted after overnight incubation at 37°C. The MHA plates inoculated with untreated bacteria were used as growth control.

**Determination of minimum inhibitory concentration (MIC):** A broth microdilution susceptibility test was used to detect MIC as previously described[20,23-25]. Briefly, two-fold serially diluted *Am*BV was transferred into wells of 96-well plate in 90 μL volume. The serially diluted concentrations of *Am*BV that used to determine MIC were 4000, 2000, 1000, 500, 250, 250, 125, 62.5, 31.25, 15.625 and 7.8 μg $mL^{-1}$. Next, 10 μL of bacterial suspension was added to each well and the plate was incubated at 37°C for 24 hrs before reading the optical density (OD) at 630 nm using a microplate reader. The MIC value was determined as the minimum concentration of *Am*BV that significantly decrease the $OD_{630}$ value with no visible bacterial growth.

**Antifungal activities of *Am*BV:** The antifungal activities of *Am*BV were evaluated through the agar-well diffusion assay as previously described[26-28]. The solution has been tested at different concentrations (600, 300 and 150 μg $mL^{-1}$).

**Statistical analysis:** The growth reduction was calculated relative to the growth control. Data were shown as Means±Standard Deviation (SD) of at least three independent experiments. Comparisons between various treatments were performed by t-test and one-way ANOVA. The $p<0.05$ was regarded as statistically significant.

## RESULTS

### Antibacterial activity of *Am*BV

**Broth microdilution methods:** Results from Broth microdilution methods demonstrated that *Am*BV significantly inhibits the growth of the tested bacterial strains in a concentration-dependent manner. *Am*BV was significantly inhibited the growth of *E. coli* ATCC8739 at concentration of 15.625 μg $mL^{-1}$ ($p<0.05$) in Fig. 1a. The growth of *E. coli*

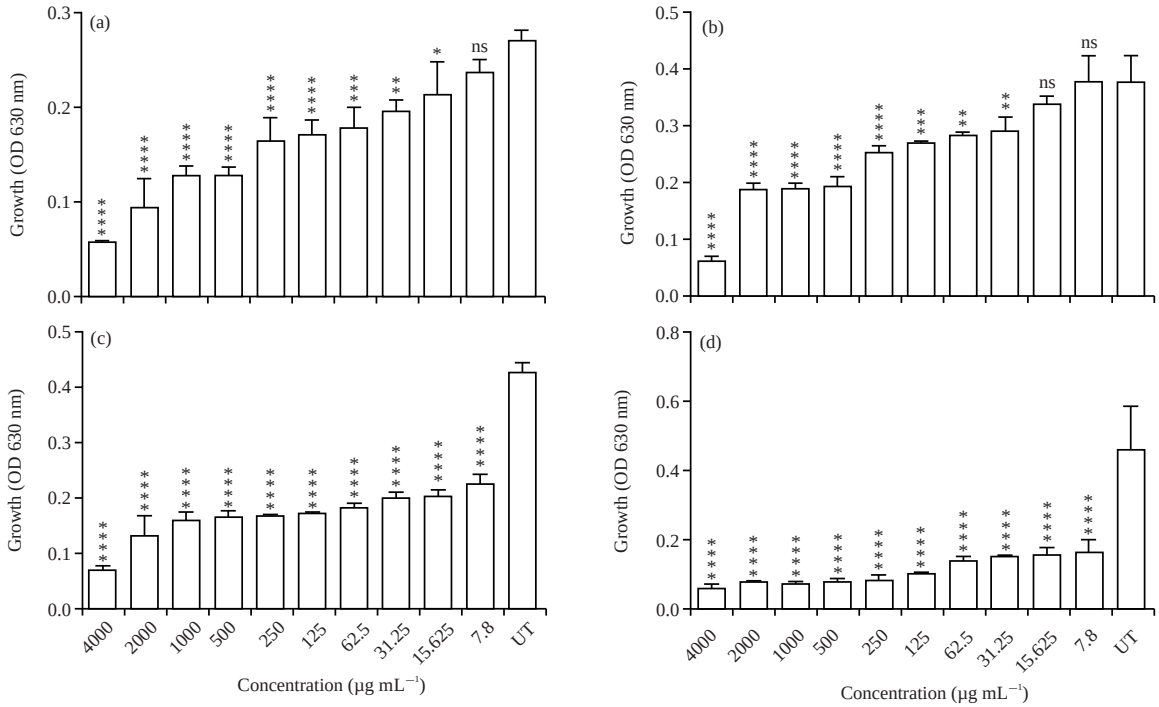


Fig. 1(a-d): Antibacterial activity of different concentrations of *Am*BV against bacterial species, (a) *E. coli* ATCC 8739, (b) *S. aureus* ATCC 6538P, (c) *S. marcescens* AUH 98 and (d) *S. mutans* ATCC 25175

Results are shown as Means±SD of three independent experiments,* $p<0.05$, ** $p<0.01$, *** $p<0.001$ and **** $p<0.0001$ vs. untreated control

Table 1: Antibacterial activity of *Am*BV

| Concentration (μg mL$^{-1}$) | *E. coli* ATCC 8739 | *S. marcescens* AUH 98 | *S. aureus* ATCC 6538P | *S. mutans* ATCC |
|---|---|---|---|---|
| UT | 0.2707±0.009815 | 0.4250±0.01803 | 0.3770±0.04480 | 0.4573±0.1249 |
| 4000 | 0.0580±0.002646 | 0.06767±0.008963 | 0.0630±0.006245 | 0.0620±0.008000 |
| 2000 | 0.09433±0.02977 | 0.1303±0.03661 | 0.1877±0.01102 | 0.07967±0.007638 |
| 1000 | 0.1283±0.008963 | 0.1583±0.01557 | 0.1890±0.008718 | 0.0774±0.006773 |
| 500 | 0.1287±0.007767 | 0.1633±0.01222 | 0.1940±0.01652 | 0.08067±0.008505 |
| 250 | 0.1647±0.02346 | 0.1670±0.001000 | 0.2537±0.01002 | 0.08633±0.01222 |
| 125 | 0.1710±0.01442 | 0.1693±0.002517 | 0.2700±0.004359 | 0.1033±0.007024 |
| 62.5 | 0.1787±0.02065 | 0.1800±0.009165 | 0.2830±0.005000 | 0.1407±0.01172 |
| 31.25 | 0.1967±0.01007 | 0.1990±0.0110 | 0.2910±0.02330 | 0.1523±0.006506 |
| 15.625 | 0.2140±0.03318 | 0.2017±0.01193 | 0.3387±0.01250 | 0.1583±0.01815 |
| 7.8 | 0.2377±0.01185 | 0.2233±0.01893 | 0.3770±0.04480 | 0.1663±0.03213 |

ATCC8739 was further inhibited at *Am*BV concentrations of 31.25, 62.50 and ≥125 μg mL$^{-1}$ ($p<0.05$) (Fig. 1a). The OD value of *E. coli* ATCC 8739 growth was decreased from 0.2707±0.009815 at untreated control to 0.2140±0.03318, 0.1967±0.01007, 0.1787±0.02065, 0.1710 ±0.01442, 0.1647±0.02346, 0.1287±0.007767, 0.1283±0.008963, 0.09433±0.02977, 0.0580±0.002646 when treated with *Am*BV concentrations of 15.625, 31.25, 62.5, 125, 250, 500, 1000, 2000 and 4000 μg mL$^{-1}$, respectively in Fig. 1a and Table 1. Similarly, the growth of *S. aureus* ATCC 6538P was significantly inhibited by *Am*BV at concentrations of 31.25, 62.50, 125 and ≥250 μg mL$^{-1}$ ($p<0.05$) in Fig. 1b. The OD value of bacterial growth was decreased from 0.3770±0.04480 in untreated culture to 0.2910±0.02330, 0.2830±0.005000, 0.2700±0.004359, 0.2537±0.01002, 0.1940±0.01652, 0.1890±0.008718, 0.1877±0.01102, 0.0630±0.006245 when treated with *Am*BV concentrations of 31.25, 62.5, 125, 250, 500, 1000, 2000 and 4000 μg mL$^{-1}$, respectively in Fig.1b and Table 1. Interestingly, the growth of both *S. marcescens* AUH 98 in Fig. 1c and *S. mutans* ATCC 25175 in Fig. 1d were significantly inhibited at all used *Am*BV concentrations (4000-7.8 μg mL$^{-1}$ ($p<0.05$)). The OD value of the growth of *S. marcescens* AUH 98 was decreased from 0.4250±0.01803 at untreated control to 0.2233±0.01893, 0.2017±0.01193, 0.1990±0.0110, 0.1800±0.009165, 0.1693±0.002517, 0.1670±0.001000, 0.1633±0.01222, 0.1583±0.01557, 0.1303±0.03661, 0.06767±0.008963 at *Am*BV concentrations of 7.8, 15.625, 31.25, 62.5, 125, 250, 500, 1000, 2000 and 4000 μg mL$^{-1}$, respectively (Fig.1c and Table 1). While, the OD value in case of *S. mutans* ATCC 25175 was decreased from 0.4573±0.1249 in untreated control to 0.1663 ±0.03213, 0.1583±0.01815, 0.1523±0.006506, 0.1407±0.01172, 0.1033±0.007024, 0.08633±0.01222, 0.08067±0.008505, 0.0774±0.006773, 0.07967±0.007638, 0.0620 ±0.008000 at *Am*BV concentrations of 7.8, 15.625, 31.25, 62.5, 125, 250, 500, 1000, 2000 and 4000 μg mL$^{-1}$, respectively in Fig.1d and Table 1. Bacterial growth inhibition by *Am*BV was concentration-dependent. In case of *E. coli* ATCC 8739, the percentages of growth inhibition were 78.56, 65.26, 52.58, 52.44, 39.26, 36.86, 34.04, 27.35, 21.13 and 16.02% at 4000, 2000, 1000, 500, 250, 125, 62.5, 31.25, 15.625 and 7.8 μg mL$^{-1}$ of *Am*BV, respectively in Fig. 2a. For *S. aureus* ATCC 6538P, the growth inhibition was 82.14, 46.35, 45.90, 44.58, 27.74, 22.94, 19.12, 17.2 and 3.216% at 4000, 2000, 1000, 500, 250, 125, 62.5, 31.25 and 15.625 μg mL$^{-1}$ of *Am*BV, respectively in Fig. 2b. Interestingly, all the used concentrations of *Am*BV inhibited the growth of *S. marcescens* AUH 98 in Fig. 2c. and *S. mutans* ATCC 25175 in Fig. 2d by more than ≥47.4 and ≥60.55%, respectively.

**CFU assay:** *Am*BV significantly reduced the CFUs of the treated bacteria. At concentrations of 250-4000 μg mL$^{-1}$ of *Am*BV, CFUs of *E. coli* ATCC 8739 were significantly decreased ($p<0.0001$) compared to untreated control in Fig. 3a. The CFUs of *E. coli* ATCC 8739 were 453 CFU mL$^{-1}$ at untreated control while at *Am*BV concentrations of 250, 500, 1000, 2000 and 4000 μg mL$^{-1}$, CFUs of *E. coli* ATCC 8739 were decreased to 353, 308, 219, 165 and 145 CFU mL$^{-1}$, respectively (Fig. 3a). Similarly, *Am*BV concentrations of 250-4000 μg mL$^{-1}$ were significantly decreased the growth of *S. aureus* ATCC 6538P ($p<0.0001$) in Fig. 3b. The CFUs of *S. aureus* ATCC 6538P were decreased from 505 at untreated control to 360-265, 155, 48 and 30 CFU mL$^{-1}$ at *Am*BV concentrations of 250, 500, 1000, 2000 and 4000 μg mL$^{-1}$, respectively (Fig. 3b). The CFUs of *S. marcescens* AUH 98 were significantly reduced at *Am*BV concentrations of 250 μg mL$^{-1}$ (510 CFU mL$^{-1}$) ($p<0.0001$), 500 μg mL$^{-1}$ (389 CFU mL$^{-1}$) ($p<0.0001$), 1000 μg mL$^{-1}$ (362 CFU mL$^{-1}$) ($p<0.0001$), 2000 μg mL$^{-1}$ (306 CFU mL$^{-1}$) ($p<0.0001$) and 4000 μg mL$^{-1}$ (205 CFU mL$^{-1}$) ($p<0.0001$) compared to untreated control in Fig. 3c. Similarly, the CFUs of *S. mutans* ATCC 25175 were significantly reduced from 750 CFU mL$^{-1}$ at untreated control to 535, 260, 210, 163 and 19 CFU mL$^{-1}$ when treated with *Am*BV concentrations of 250, 500, 1000, 2000 and 4000 μg mL$^{-1}$, respectively in Fig. 3d.

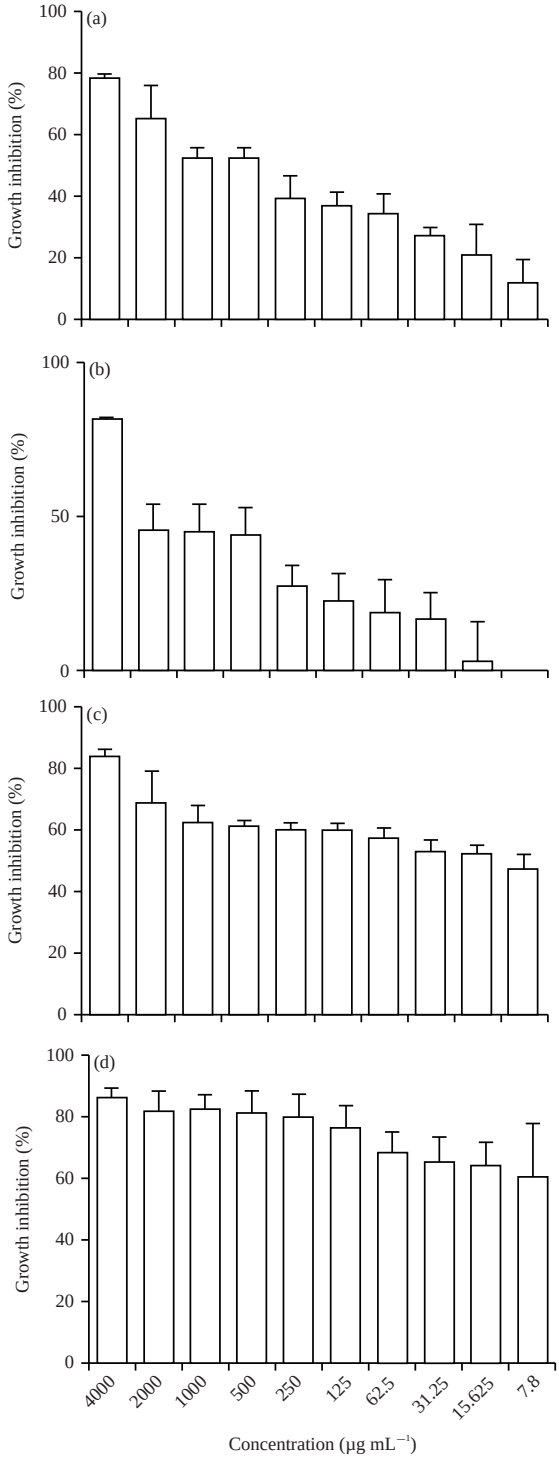


Fig. 2(a-d): Percentage of bacterial inhibition by different concentrations of *Am*BV against bacterial species (a) *E. coli* ATCC 8739, (b) *S. aureus* ATCC 6538P, (c) *S. marcescens* AUH 98 and (d) *S. mutans* ATCC 25175

Results are shown as Means±SD of three independent experiments

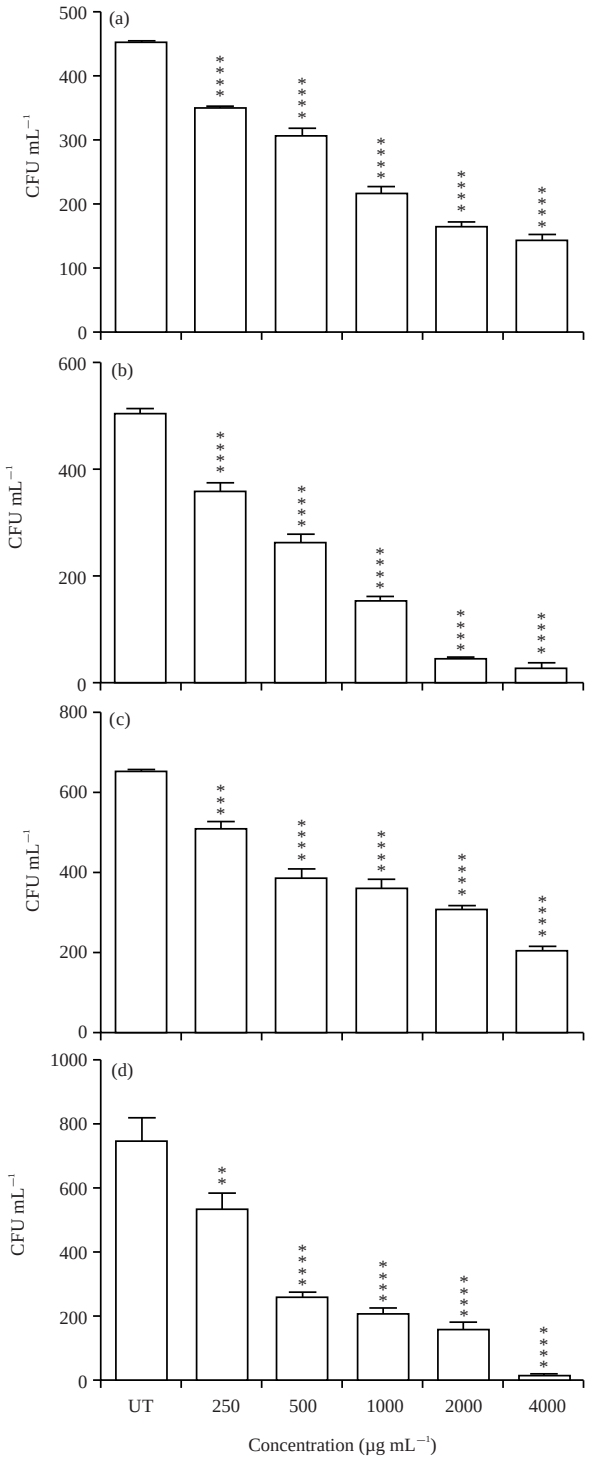


Fig. 3(a-d): Effect of different concentrations of *Am*BV on CFUs of bacterial species, (a) *E. coli* ATCC 8739, (b) *S. aureus* ATCC 6538P, (c) *S. marcescens* AUH 98 and (d) *S. mutans* ATCC 25175

Results are shown as Means±SD of three independent experiments,* p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 vs. untreated control

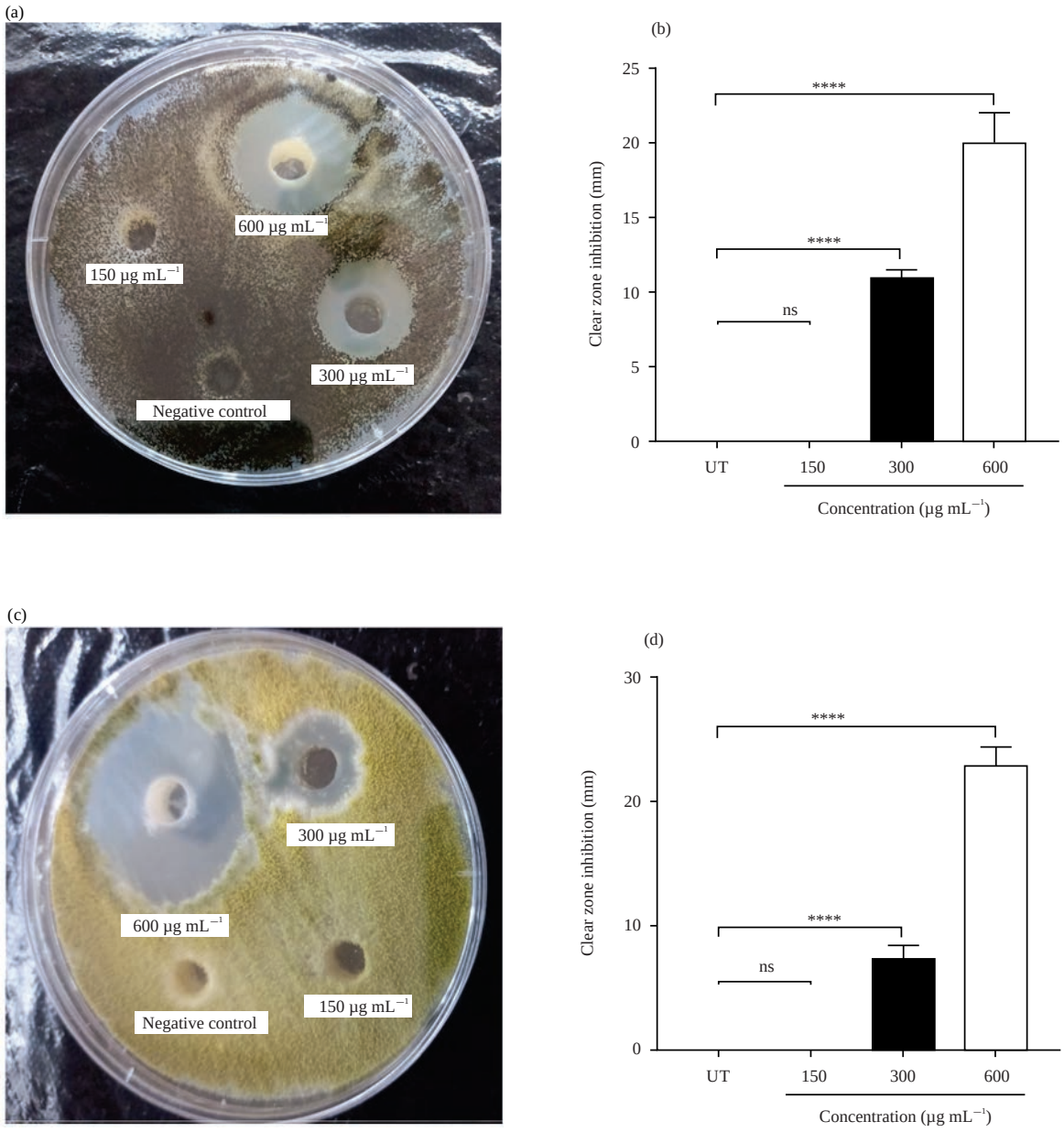


Fig. 4(a-d): Antifungal activity of different concentrations of *Am*BV against bacterial species, (a) Representative photographs of antifungal assays of *A. niger* ATCC 16404, (b) *A. niger* ATCC 16404 diameter of clear zone inhibition in millimeter (mm), (c) Representative photographs of antifungal assays of *A. flavus* and (d) *A. flavus* diameter of clear zone inhibition in millimeter (mm)

Results are shown as Means ±SD of three independent experiments and p-value: ****p<0.0001 vs. negative control

**MIC values of *Am*BV:** There were variations in MIC values of *Am*BV between treated bacterial strains. MIC values of *Am*BV were 15.625 µg mL$^{-1}$ (OD: 0.2140±0.03318), 31.25 µg mL$^{-1}$ (OD: 0.2910±0.02330), 7.8 µg mL$^{-1}$ (OD: 0.2233±0.01893) and 7.8 µg mL$^{-1}$ (OD: 0.1663±0.03213) against *E. coli* ATCC 8739, *S. aureus* ATCC 6538P, *S. marcescens* AUH 98 and *S. mutans* ATCC 25175, respectively in Table 2.

**Antifungal activity of *Am*BV:** *Am*BV exhibited a remarkable antifungal activity at concentrations of 300 and 600 µg mL$^{-1}$. Concentrations of 300 and 600 µg mL$^{-1}$ significantly ($p<0.0001$) inhibited the growth of *A. niger* ATCC 16404 in Fig. 4a. The diameter of clear zone inhibition of *A. niger* ATCC 16404 in the agar-well diffusion assay was 19.97±2.040 and 10.97±0.5033 mm at concentrations of

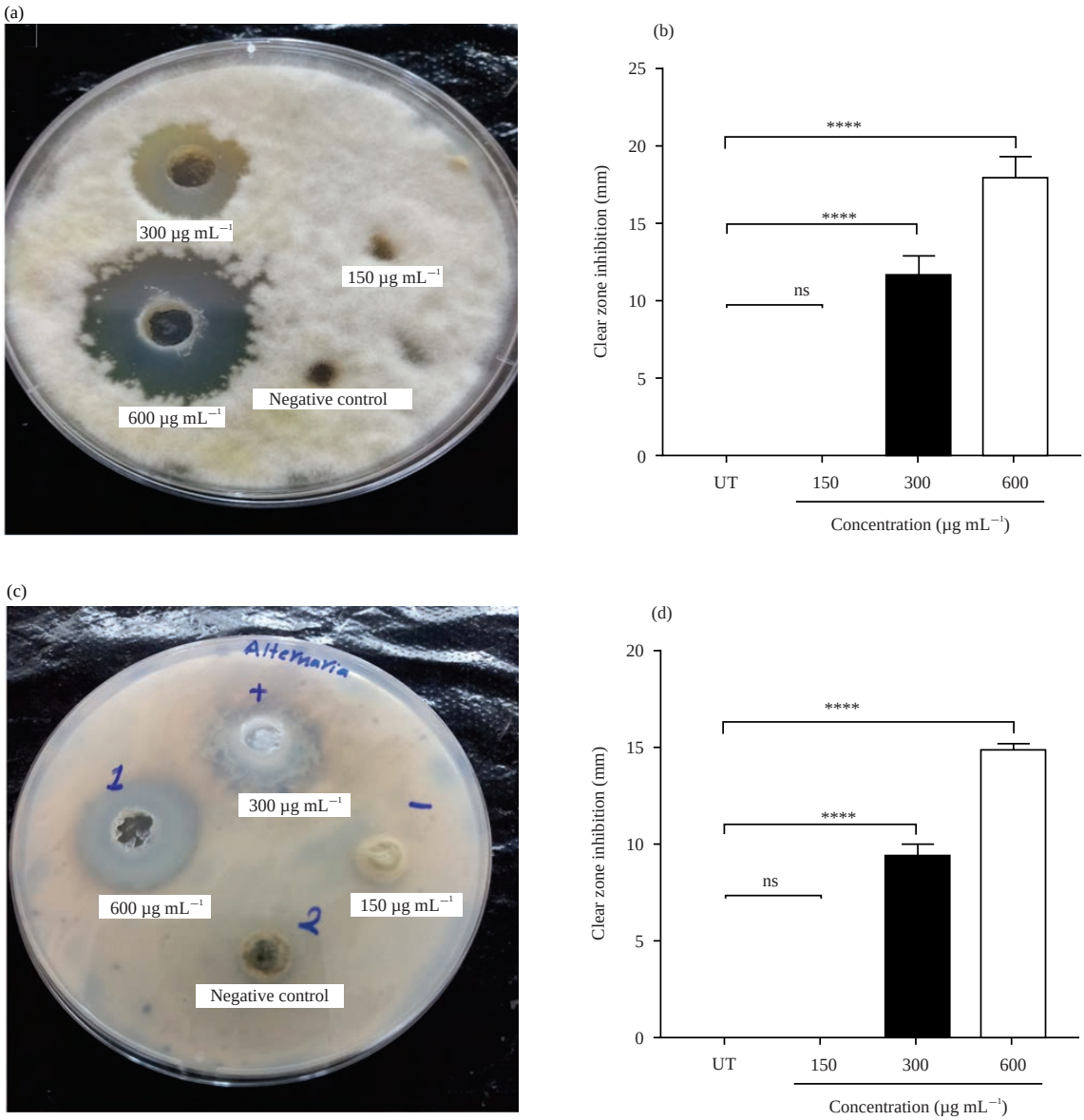


Fig. 5(a-d): Antifungal activity of different concentrations of *Am*BV against bacterial species, (a) Representative photographs of antifungal assays of *F. oxysporum* MLBM212, (b) *F. oxysporum* MLBM212 diameter of clear zone inhibition in millimeter (mm), (c) Representative photographs of antifungal assays of *A. alternata* MLBM09, (d) *A. alternata* MLBM09 diameter of clear zone inhibition in millimeter (mm)

Results are shown as Means±SD of three independent experiments and p-value: ****p<0.0001 vs. negative control

Table 2: MIC Values of *Am*BV

| Bacterial isolates | MIC (µg mL$^{-1}$) |
|---|---|
| *Escherichia coli* ATCC 8739 | 15.625 |
| *Staphylococcus aureus* ATCC 6538P | 31.25 |
| *Serratia marcescens* AUH 98 | 7.8 |
| *Streptococcus mutans* ATCC | 7.8 |

300-600 µg mL$^{-1}$ of *Am*BV, respectively in Fig. 4b. Similarly, *Am*BV significantly decreased the growth of *A. flavus* in Fig. 4c. The clear zone inhibition was 22.83±1.528 mm at a concentration of 300 µg Ml$^{-1}$ of *Am*BV and 7.333±1.041 mm at a concentration of 300 µg mL$^{-1}$ of *Am*BV in Fig. 4d. Moreover, *Am*BV inhibited the growth of *F. oxysporum* MLBM212 in Fig. 5a and *A. alternata* MLBM09 in Fig. 5c. At a concentration of 300 µg mL$^{-1}$ of *Am*BV, the diameters of clear zone inhibition of *F. oxysporum* MLBM212 in Fig. 5b and *Alternaria alternata* MLBM09 in Fig. 5d were 11.67±1.258 and 9.47±0.5508 mm, respectively. Whereas at a concentration of 600 µg mL$^{-1}$ of *Am*BV, the diameters of clear zone inhibition of *F. oxysporum* MLBM212 (Fig. 5b) and *Alternaria alternata* MLBM09 (Fig. 5D) were 18.0±1.323 mm and 14.88±0.34 mm,

respectively. There was no antifungal activity for *Am*BV against all tested fungi at a concentration of 150 µg $mL^{-1}$.

## DISCUSSION

New untraditional antibacterial and antifungal agents are highly required to tackle the problem of microbial resistance to available commercial antimicrobial substances. BV contains a variety of bioactive molecules including amino acids (aa), peptides, proteins, enzymes, sugars, biogenic amines, volatile compounds, phospholipids and pheromones. These biomolecules could be harnessed to develop effective antibacterial and antifungal agents, yet the antimicrobial potential of BV was not fully explored. Thus, in this work, the antimicrobial effect of *Apis mellifera* BV (*Am*BV) against some medically important and common bacterial and fungal pathogens was elucidated.

Results demonstrated that *Am*BV is highly potent in inhibiting the growth of the tested bacterial and fungal strains. Using broth microdilution method and CFU assays, it was discovered that *Am*BV significantly inhibits the growth of *E. coli* ATCC8739, *S. aureus* ATCC 6538P, *S. marcescens* AUH 98 and *S. mutans* ATCC 25175 in a concentration-dependent manner. Interestingly, in this study MIC values of *Am*BV against all tested bacteria were markedly low compared to the majority of the reported MIC values, which indicated the efficacy of *Am*BV as an antibacterial agent. The MIC value of *Am*BV for *S. aureus* was 31.25 µg $mL^{-1}$ which is markedly lower than the MIC value reported by Samy *et al.*[29] and Al-Ani *et al.*[9]. On the other hand, the MIC values for *S. aureus* were higher than what was demonstrated[30,31]. Similarly, the MIC values of *Am*BV were 15.625 µg $mL^{-1}$ for *E. coli* ATCC8739 and 7.8 µg $mL^{-1}$ for both *S. marcescens* AUH 98 and *S. mutans* ATCC 25175 which was much lower than the previously reported MIC values[9,32-35].

Like the antibacterial activity of *Am*BV, antifungal activity was very prominent in the agar well diffusion assay. *Am*BV significantly inhibited the growth of *A. niger* ATCC 16404, *F. oxysporum* MLBM212, *A. flavus* and *A. alternata* MLBM09. Inhibition of the fungal growth required higher concentrations of *Am*BV compared to concentrations that were required for the inhibition of bacterial strains. Study detected a potent antifungal activity at a concentration of $\geq$300 µg $mL^{-1}$ from *Am*BV. There was a significant increase in the clear zone diameter when fungi were treated with *Am*BV at concentrations of 300 and 600 µg $mL^{-1}$. Similar to this study, the antifungal activity of *Am*BV has been evaluated by many other groups. Yu *et al.*[12], demonstrated that *Am*BV inhibited the growth of *Trichophyton mentagrophytes* and *Trichophyton rubrum* by 92 and 32%, respectively. The antifungal inhibitory concentration reported in their study was 0.63 ppm which was higher than the current study. Similarly, BV has been reported to inhibit the growth of *C. albicans* and *C. krusei* at concentration of 60 to 125 µg $mL^{-1}$ [9,36].

Although, *Am*BV significantly decreased the growth of the tested bacteria and fungi, there were variations in antimicrobial potency and the inhibitory concentrations of *Am*BV between different bacterial and fungal strains. *Am*BV was more efficient in inhibiting the growth of *E. coli* ATCC8739, *S. marcescens* AUH 98 and *S. mutans* ATCC 25175 compared to *S. aureus* ATCC 6538P. As noted in the antibacterial activity of *Am*BV, there were also variations in the antifungal activities of *Am*BV. *A. flavus* and *A. niger* ATCC 16404 were the most sensitive to *Am*BV treatment followed by *F. oxysporum* MLBM212 and finally *A. alternata* MLBM09.

Variations in the antibacterial and antifungal potency of *Am*BV between the tested bacterial and fungal strains could be attributed to the nature of each microbial strain or the mechanism by which *Am*BV inhibits the growth of each microbial strain. *Am*BV contains a wide range of bioactive molecules that could exploit different strategies to inhibit each microbial strain[37,38]. Similarly, melittin which is one of the major components of *Am*BV is more active against gram-positive bacteria than gram-negative ones[39]. Similarly, PLA2 of *Am*BV inhibited the growth of *Lactobacillus casei* at a MIC value of 400 mg $mL^{-1}$, however, it didn't yield any satisfactory antibacterial effect against the growth of *Streptococcus salivarius*, *S. sobrinus*, *S. mutans*, *S. mitis*, *S. sanguinis* and *Enterococcus faecalis*[32]. Taken together variations in the antibacterial and antifungal potency of *Am*BV and its bioactive molecules could be exploited to develop a microbe-specific antimicrobial agent to avoid the side effects associated with the use of broad-spectrum antimicrobial agents on the beneficial normal flora.

## CONCLUSION

Microbial diseases are the most prevalent health challenge particularly in developing countries due to drug resistance, high cost and high risk of synthetic and semisynthetic antibiotics. Therefore, new antimicrobial agents are in need to control microbial diseases. Natural products like BV are considered efficient alternatives to synthetic and semisynthetic antibiotics and a promising source for the discovery of new antimicrobial agents as they contain a variety of antimicrobial bioactive compounds. In this study, the antibacterial and antifungal activities of *Am*BV against

medically important bacterial and fungal strains were demonstrated. *Am*BV significantly inhibited the growth of the bacterial and fungal pathogen, *in vitro*. There were variations in antimicrobial potency of *Am*BV between microbial strains that may use in a future study to invent new antimicrobial agents that have no side effects on the beneficial normal flora. This study provides a perspective on new antibacterial and antifungal therapeutic approaches using *Am*BV. Results from this study may, thus, could be utilized to develop cost-effective and efficient antibacterial and antifungal therapeutic agents.

## SIGNIFICANCE STATEMENT

This study discovered that *Am*BV has strong antibacterial and antifungal activities against many medically important pathogens. This study will help researchers to exploit the antimicrobial activity of *Am*BV in future preclinical and clinical studies to develop cost-effective and nontraditional antibacterial and antifungal agents.

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