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Research Article

From Deadly to Life-Saving Effects: Antimicrobial and Antibiofilm Effects of *Tarantula cubensis* Extract on Bacterial and Fungal Pathogens

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Abstract

Every year, many people around the world lose their lives due to antibiotic resistance therapy. These resistances pose serious health threats to both individuals and societies, making even simple services difficult to treat. Antibiotic resistance not only affects medical practices, but also causes a huge economic burden on health systems. In this study, *Tarantula cubensis* spider venom was investigated to produce new antimicrobial agents effective against Gram (+) and Gram (-) bacteria and *Candida* species. Antimicrobial and antibiofilm activity of *T. cubensis* extract against microorganisms were tested using minimum inhibition, resazurin and time killing curve methods. MIC values of *T. cubensis* extract were determined as 14.3-0.45 mg/mL by broth dilution method and 14.3-3.58 mg/mL by resazurin method for the investigated pathogens. Time-kill curve studies confirmed the growth retardant, bacteriostatic/ fungistatic and bactericidal/ fungicidal activity of *T. cubensis* extract. Antibiofilm studies also showed that *T. cubensis* extract significantly inhibited and blocked biofilm formed by pathogens (melittin 8-82%, apamin 1.8-78%). *T. cubensis* was effective in inhibiting biofilm formation (from 99.98% to 75.68%) and eradicating biofilm (from 32.97% to 4.67%) at the highest concentration. *T. cubensis* extract was found to have high antibacterial and antibiofilm properties. Such natural poisons offer a promising and powerful solution to control microbial populations, combat pathogens, and protect human and animal health.

Keywords: *Tarantula cubensis*, Theranekron, Antimicrobial activity, Antibiofilm activity, Time-killing curves

Introduction

Antimicrobial resistance (AMR) has become a persistent global public health issue, with an estimated 10 million deaths annually worldwide by 2050 [1]. When bacteria, fungi, viruses, parasites, and other microbes grow to the point that they eventually develop resistance to the antimicrobial drugs, such antibiotics, that are used to treat such illnesses, it is known as antimicrobial resistance (AMR) [2]. AMR has become one of the biggest worldwide issues of the twenty-first century because of the speed at which the rate of AMR infections is increasing and the lack of new antimicrobial drugs being developed to address this problem [3]. The effects of excessive or improper use of antibiotics in a variety of settings, particularly clinical treatment, agriculture, animal health, and the food chain, may be one of the primary causes of the current problem [4].

AMR is sometimes referred to as the "Silent Pandemic" and is an issue that requires prompt attention, better management, and should not be put off until later [5]. In response to AMR, a number of countries and international health organizations have taken action to solve the issue. The "One Health Approach" was created to make sure that each agency collaborates with other agencies and stays within its area of competence in order to reduce the possible impacts of antimicrobial resistance. The World Organization of Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO) are two organizations that must work together globally to implement this strategy [6]. Furthermore, the World Health Organization (WHO) created the Global Action Plan for controlling AMR (GAP-AMR) and the Global Antimicrobial Resistance and Use Surveillance System (GLASS) in order to fulfill the goals of the GAP-AMR program [7].

Because of the problem of antibiotic resistance, cationic amphipathic peptides, or "antimicrobial peptides," are naturally occurring antibacterial chemicals that are used as one of the preferred antimicrobial groups in the management of infectious illnesses. It has been shown that AMPs have generated attention as additional or alternative antibiotics due to their potency, rapid action, and capacity to eliminate both Gram-positive and Gram-negative bacteria [8]. Theranekron D6 (TD6), sold as an alcoholic extract of the spider *Tarantula cubensis*, is widely used in veterinary medicine for the treatment of animal diseases such as dermatitis, arthritis, inflammatory hoof diseases (panaricium, foot rot), phlegmon, ulcers, abscesses, injuries, purulent lesions and necrosis. In proliferative and necrotic tissues, TD6 demonstrates demarcative, regenerative, antiphlogistic, and resorptive actions. To speed up the healing process, TD6 promotes epithelialization [9]. It is believed to activate the body's defensive mechanisms and naturally reduce inflammation in any proliferative lesion, while its exact mode of action is unknown [10]. According to some reports, it creates a demarcation line around the necrotic tissue as soon as surgery is completed, stops the spread of catabolic enzymes from the necrotic tissue to the surrounding areas and prevents inflammation, all while shielding the ligaments from additional tissue structure degradation [11]. The aim of this study was to investigate the antimicrobial, antibiofilm and biofilm-destroying effects of Theranekron D6 (TD6), an alcoholic extract of *T. cubensis* spider, on pathogens of two Gram-positive bacteria *(Staphylococcus aureus*, and *Enterococcus faecalis),* two Gram-negative bacteria *(Escherichia coli*, and *Proteus vulgaris)* and two *Candida* fungal species *(Candida albicans*, and *Candida parapsilosis).*

Material and Methods

Ethical Statement

Ethics committee approval is not required for this study.

Tarantula cubensis Venom Extract

Theranekron® (alcoholic extract of *T. cubensis)* was purchased from Richter Pharma AG (Wels, Austria) and obtained from the local veterinary clinic with the permission of the veterinarian (Kütahya, Türkiye). As described from Richter Pharma AG, it is prepared by processing the whole spider and diluting it in alcohol. It is commercially available in 50 mL bottles, contains *T. cubensis* D6 and contains 286 mg in 1 mL of ethanol.

Resazurin Preparation

0.01 g Resazurin was dissolved in 50 mL sterile distilled water and sterilized with a 0.22 µm filter and stored at 4°C for 2 weeks after preparation [12].

Bacterial Strains

S. aureus American Type Culture Collection (ATCC) (29213), *E. faecalis* (ATCC 29212), *E. coli* (ATCC 25922), *P. vulgaris* (ATCC 6386), *C. albicans* (ATCC 10231), and *C. parapsilosis* (ATCC 22019) strains were used in this study. These standard strains stored at -80°C were inoculated on tryptic soy agar (TSA, Neogen®, Lansing, MI, USA) and *Candida* species were inoculated on sabouraud dextrose agar (SDA, Neogen®, Lansing, MI, USA) and Candida species were incubated for 48 h and other pathogens for 24 h at 37°C. All strains used in the experiment were treated with sterile 0.9% NaCl solution to achieve a 0.5 McFarland standard using a DEN-1 densitometer [13].

Antimicrobial Activity of *Tarantula cubensis* Extract on Pathogens

Antimicrobial and antifungal activity of *T. cubensis* extract was determined by disk diffusion, Minimum Inhibitory Concentration (MIC) methods. MIC values were also evaluated by Resazurin method and ELISA method.

Application of the Disk Diffusion Method

Mueller Hinton Agar (MHA, Neogen®, Lansing, MI, USA) medium was used for antimicrobial activity and SDA medium was used for antifungal activity of *T. cubensis* extract by disk diffusion method. 0.5 McFarland standard, *C. albicans* and *C. parapsilosis* were inoculated on SDA medium, *S. aureus*, *E. faecalis*, *E. coli* and P. *vulgaris* suspensions were inoculated on MHA medium and spread all over the medium with a swab and 4 sterile antimicrobial susceptibility test discs (Bioanalyse, Turkey) were placed on the medium. Volumes of 25, 20, 15, and 10 µL of *T. cubensis* extract were added to each disk. The petri dishes were allowed to dry for 10-15 min and incubated at 37°C for 24 h for bacteria and 48 h for *Candida* spp. After incubation, it was checked whether inhibitory zone diameter was formed or not and the zone diameters were measured and recorded [14].

Minimum Inhibitor Concentration (MIC) Determination

RPMI-1640 broth (Becton Dickinson, Heidelberg, Germany) for *C. albicans* and *C. parapsilosis* and 100 µL of cation added Mueller Hinton broth (MHB, Merck, Darmstadt, Germany) for *S. aureus, E. faecalis, E. coli* and *P. vulgaris* were added to all wells of 96 microplates to determine the MIC values of antimicrobial agents by liquid microdilution method. 100 µL of *T. cubensis* extract (286 mg/mL) was added to the first well and serial dilutions were made in duplicate (28.6-0.06 mg/mL). Finally, 100 µL of bacteria/candida suspension at 0.5 McFarland turbidity was added to each of these wells. To control bacterial

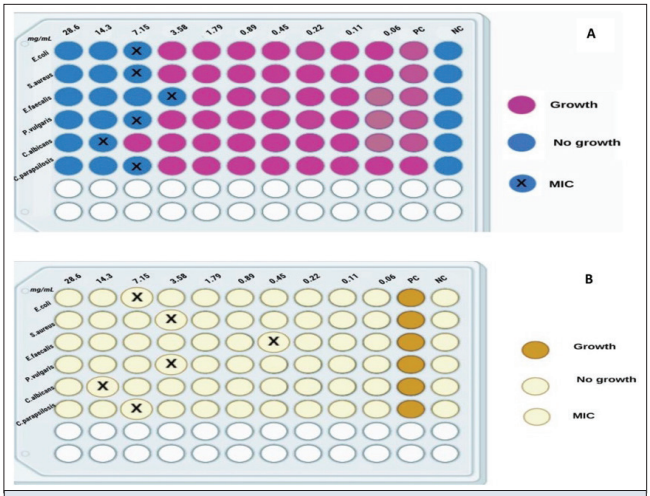

Fig 1. Minimum inhibitor concentration (MIC) determination. **A:** Resasurin method; **B:** Liquid microdilution method

growth, only bacteria (positive control) was added to one last row of the microplate and only medium (negative control) was added to one well. The microplate was incubated in an oven at 37°C for 18-20 h *(Candida* spp. for 48 h). The lowest concentration at which no growth was observed after incubation was considered as the MIC value. After incubation, the absorbance at 600 nm (A600) was determined for each well with a spectrophotometer (Thermo Scientific, Multiskan SkyHigh Microplate Spectrophotometer). Growth inhibition percentages for each microorganism at different *T. cubensis* extract concentrations were calculated as follows: Percent inhibition (%) = [1- (Sample OD/Control OD)] X100

To determine the MIC value by the resazurin method, after incubation, 10 µL of 0.01% resazurin solution was pipetted into all wells and the plates were incubated again at 37°C for 24 h. Any color change from purple to pink was considered positive *(Fig. 1)* [12].

Determination of Time Dependent Kill Curves (Kinetic Kill Curves)

Time-dependent killing level was performed to demonstrate the duration of action of *T. cubensis* extract. *T. cubensis* extract concentrations were prepared at 2X MIC for all microorganisms. To ependorfs, 500 µL of 0.5 McFarland turbid suspension of microorganisms and 500 µL of *T. cubensis* extract concentration were added. Incubated at 37°C. At 0 h, 1 h, 3 h, 6 h, 8 h and 24 h, 50 µL of each tube was added to 450 physiological saline (0.9%) and 100 µL were spread onto TSA/SDA medium. After incubation at 37°C for 24 h (48 h for *Candida* spp.), the colonies formed in the petri dishes were counted and the number of bacteria per mL (cfu/mL) was calculated taking into account the dilution factor. The bactericidal/fungicidal effect's concentration- and time-dependent variation was measured, and its log10 value was examined. Based on the results, a time-death graph was created, with the logarithmic value of the number of bacteria displayed on the "y" axis and time displayed on the "x" axis [15]. After 24 h of incubation, ≥3 $\log_{10}$ and <3 $\log_{10}$ reductions in the total number of CFU/mL relative to the control were considered bactericidal/fungicidal and bacteriostatic/fungistatic activity, respectively. Moreover, after at least 6 h, regrowth was defined as ≥2 $\log_{10}$ rise in viable CFU/mL count [16].

Tarantula cubensis Extract Effect on Biofilm Formation and Eradication

The effect of *T. cubensis* extract on biofilm formation of microorganisms was investigated using microdilution method [17]. RPMI-1640 broth for *C. albicans* and *C. parapsilosis* and 100 µL of Luria Bertani Broth (LB, HiMedia, India) containing 1% glucose for *S. aureus, E. faecalis*, *E. coli* and *P. vulgaris* were added to all wells. Serial dilutions of the prepared *T. cubensis* extract into the first well were prepared from 28.6-0.06 mg/mL to a final volume of 100 µL per well. Each plate was inoculated with 100 uL of 0.5 McFarland bacterial suspension. The positive control with bacteria only and the well with medium only were used as negative controls. After incubation at 37°C for 18-24 h, the medium was carefully aspirated and the biofilms formed were washed three times with 200 µL sterile phosphate buffer saline (PBS) to remove floating bacteria. Staining was then performed with 200 µL of 0.1% crystal violet for 30 min. Excess stain was washed off using PBS and the plates were allowed to dry. The dye bound to the biofilm in each well was dissolved in 200 µL of 33% glacial acetic acid for Gr(+) bacteria and 95% ethanol for Gr(-) bacteria for 10 min and absorbance was checked at 600 nm (Thermo Scientific Multiskan FC). The percentage of biofilm formation was calculated as follows [18]: % Biofilm formation = (OD 600 of treated biofilm/OD 600 of non-treated biofilm) X 100

Elimination of biofilm formation was examined using the minimum biofilm elimination concentration assay. RPMI-1640 broth for *C. albicans* and *C. parapsilosis* and 100 uL LB containing 1% glucose for *S. aureus, E. faecalis, E. coli* and *P. vulgaris* were added to all wells. Each plate was inoculated with 100 uL of 0.5 McFarland bacterial suspension. The positive control containing only bacteria and the well containing only medium were used as negative controls. The plates were incubated at 37°C for 18-24 h for biofilm development. The culture was then washed carefully once with sterile PBS. These biofilms were then exposed to 200 µL of *T. cubensis* extract at various concentrations ranging from 28.6-0.06 mg/mL and incubated again at 37°C for 18-24 h. After incubation, adherent cells were washed 3 times with sterile PBS. They were then stained with 200 µL of 0.1% crystal violet for 30

min. Excess stain was washed off using PBS and the plates were allowed to dry. The dye bound to the biofilm in each well was dissolved in 200 µL of 33% glacial acetic acid for Gr (+) bacteria and 95% ethanol for Gr (-) bacteria for 10 min and the absorbance was checked at 600 nm (Thermo Scientific Multiskan FC) and calculated as follows [17]. % Biofilm eradication = [1-(OD 600 of treated biofilm/OD 600 of non-treated biofilm)] X 100. Biofilm inhibition was defined as concentrations indicating 50% and 90% eradication.

Results

Antimicrobial Activity of *Tarantula cubensis* Extract by Disk Diffusion Method

In this study, the antimicrobial and antifungal activities of *T. cubensis* extract were investigated. Antimicrobial and antifungal activities were determined against all test microorganisms with inhibition zones ranging from 24.0-8.0 mm for bacteria and 22.0-8.0 mm for *Candida* spp. *(Table 1)*.

Minimum Inhibitory Concentration of *Tarantula cubensis* Extract

The MIC activities of *T. cubensis* extract on the analyzed microorganisms are presented in *Table 2*. While the MIC values of *T. cubensis* extract were between 14.3-0.45 mg/mL, 14.3-3.58 mg/mL concentrations were determined by the resazurin method. The lowest MIC value was 0.45 mg/mL on *E. faecalis* strain and 3.58 mg/mL *(Table 2)* on *E. faecalis* strain by resazurin method *(Fig. 1)*.

Table 1. *Antimicrobial activity of Tarantula cubensis extract*

Test Microorganisms	Inhibition Zones (mm)*			
	Extract Quantities			
	25 µL	20 µL	15 µL	10 µL
Staphylococcus aureus	18	10	8	8
Enterococcus faecalis	20	19	11	8
Escherichia coli	24	21	18	14
Proteus vulgaris	12	10	9	6**
Candida albicans	16	13	10	8
Candida parapsilosis	22	18	14	10

** Values include disk diameters (6 mm), ** Zones are not observed*

Table 2. *Minimum inhibitory concentration of Tarantula cubensis extract*

Test Microorganisms	MIC (mg/mL)	Resazurin (mg/mL)
Staphylococcus aureus	3.58	7.15
Enterococcus faecalis	0.45	3.58
Escherichia coli	7.15	7.15
Proteus vulgaris	3.58	7.15
Candida albicans	14.3	14.3
Candida parapsilosis	7.15	7.15

MIC: minimum inhibitory concentration

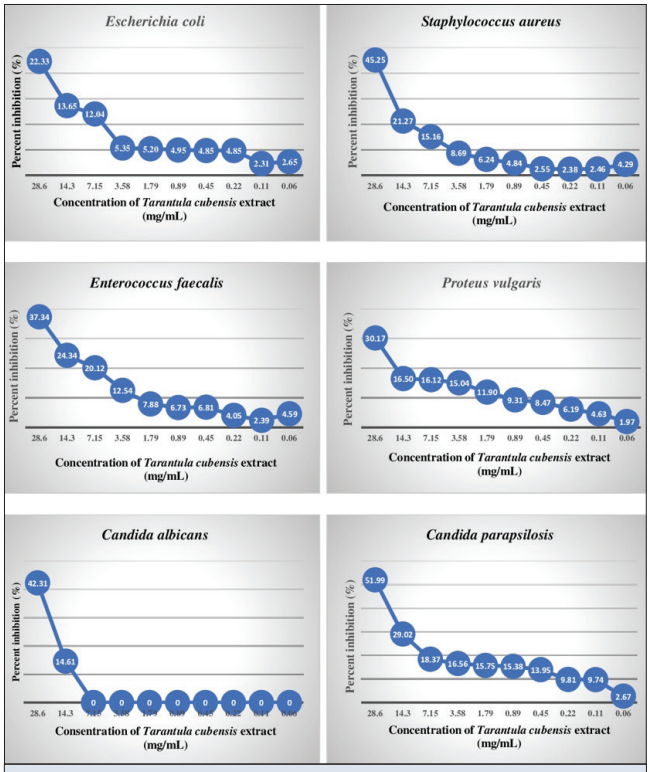

Fig 2. Growth inhibition percentages for each microorganism at different *T. cubensis* extract concentrations

Percent Inhibition of *T. cubensis* Extract

In order to evaluate the effect of *T. cubensis* extract on the inhibition of bacteria, variable amounts (28.6-0.06 mg/mL) were evaluated. The results reveal that the inhibition of bacterial cells is directly linked to the amount of *T. cubensis* extract consumed. It was noticed that the percentage of bacterial inhibition ranged from 1.97% to 51.99% for the variable concentration of *T. cubensis* extract in the range of 28.6-0.06 mg/mL. In general, we can say that the inhibition efficiency is directly proportional to the respective concentrations of *T. cubensis* extract *(Fig. 2)*.

Results for Time-Dependent Kill Curves

According to the analysis of time-dependent killing curves, *T. cubensis* extract was found to be bactericidal/fungicidal against all pathogens we examined. For *E. coli*, *E. faecalis*, and *P. vulgaris*, the positive control and *T. cubensis* group have a similar baseline level at 0 h (~6 log CFU/mL), while the negative control shows no growth. At 1 h, the *T. cubensis* group slightly suppresses growth compared to the positive control, but the difference is not very pronounced. Between 3 and 24 h, *T. cubensis*

Table 3. *Number of viable colonies in the time dependent killing test of the examined pathogens during the evaluation hours of Tarantula cubensis extract (log_{10})*

Test Microorganisms	log_{10} Number of Live Colonies (CFU/mL)					
	Hour					
	0	1	3	6	8	24
Staphylococcus aureus	4.95*	4.93*	4.87*	4.77*	4.47*	**4.59****
Positive control	5.95	6.08	6.19	6.26	6.69	7.81
Enterococcus faecalis	4.7*	4.81*	4.9*	4.89*	4.8*	4.97*
Positive control	5.6	5.85	5.89	6.06	6.54	7.19
Escherichia coli	4.82*	4.79*	4.79*	4.66*	4.22*	5.16*
Positive control	6.08	6.13	6.24	6.39	6.66	7.46
Proteus vulgaris	4.58*	4.85*	4.66*	4.3*	**4.18****	5.01*
Positive control	6.19	6.56	6.77	6.8	7	7.37
Candida albicans	4.25*	4.64*	4.6*	4.43*	4.39*	**4.25****
Positive control	6.03	6.15	6.56	6.6	6.89	7.53
Candida parapsilosis	4.39*	4.51*	4.48*	4.12*	3.97*	**3.6****
Positive control	5.98	6.27	6.4	6.61	5.71	7.01
Negative control	0	0	0	0	0	0

** Bacteriostatic/fungistatic activity; ** Bactericidal/fungicidal activity*

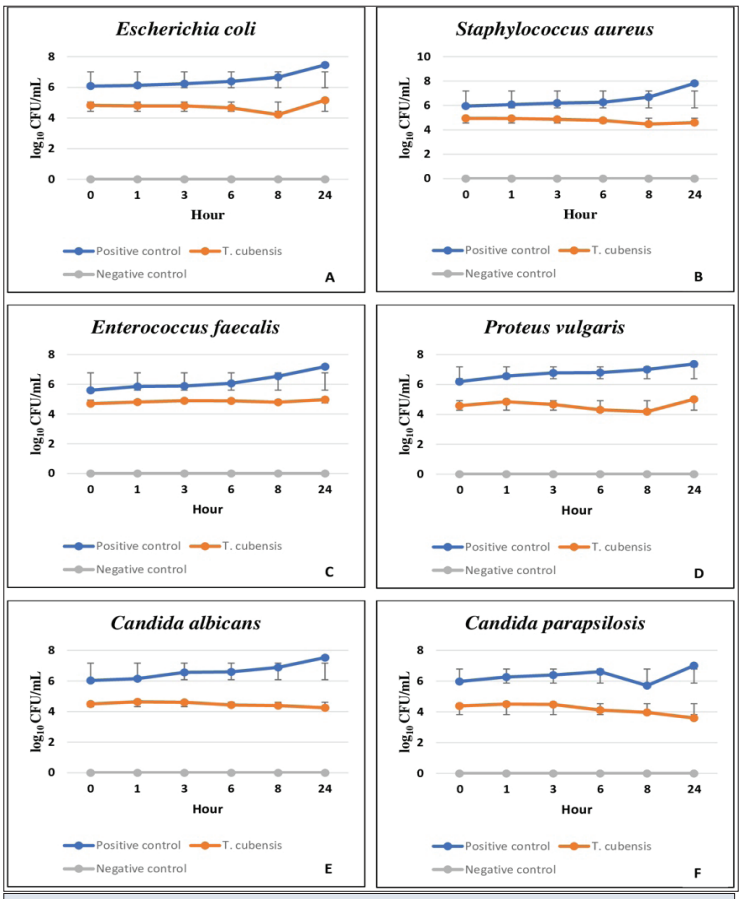

Fig 3. A: *Escherichia coli,* **B:** *Staphylococcus aureus,* **C:** *Enterococcus faecalis,* **D:** *Proteus vulgaris,* **E:** *Candida albicans,* **F:** *Candida parapsilosis.* X-axis represents time to kill and Y-axis represents logarithmic time(log) growth phase. Error bars indicate standard deviation

Table 4. *Effect of different concentrations of T. cubensis extract on biofilm formation and eradication of pathogens (%)*

Microorganisms	Concentrations (mg/mL)																	
	28.6		14.3		7.15		3.58		1.79		0.89		0.45		0.22		0.11		0.06	
	F	E	F	E	F	E	F	E	F	E	F	E	F	E	F	E	F	E	F	E
Staphylococcus aureus	82.41	22.61	81.66	13.94	79.47	11.01	76.83	6.55	76.41	7.11	76.41	5.57	75.65	0	61.86	0	60.49	0	54.0	0
Enterococcus faecium	93.48	29.65	87.62	19.21	59.83	18.85	55.79	5.12	55.43	4.01	54.83	0	54.08	0	50.44	0	50.44	0	42.93	0
Escherichia coli	75.68	32.97	72.45	28.95	68.83	28.52	68.62	22.97	68.60	20.63	66.11	21.20	60.35	17.04	66.11	11.51	57.22	7.13	55.12	0
Proteus vulgaris	99.98	4.67	87.62	19.21	59.83	18.85	99.73	0	99.68	0	99.60	0	99.27	0	99.11	0	97.60	0	88.06	0
Candida albicans	99.96	7.13	99.95	0	92.81	0	83.20	0	82.25	0	79.33	0	73.84	0	72.47	0	64.53	0	54.03	0
Candida parapsilosis	99.96	21.70	99.68	8.45	99.49	3.28	87.08	0	86.59	0	82.14	0	79.89	0	74.87	0	56.42	0	54.58	0

F: formation; E: eradication

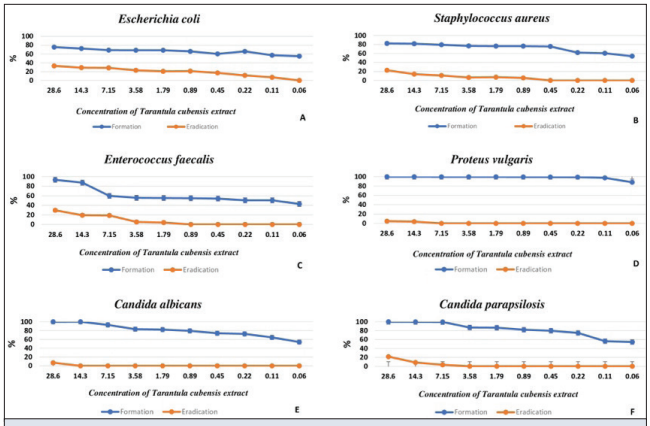

Fig 4. A: Effect of different concentrations of *T. cubensis* extract on biofilm formation and eradication of pathogens (%). *Escherichia coli,* **B:** *Staphylococcus aureus,* **C:** *Enterococcus faecalis,* **D:** *Proteus vulgaris,* **E:** *Candida albicans,* **F:** *Candida parapsilosis*

continues to suppress growth compared to the positive control, but not completely. The growth rate slows down, but does not increase as much as in the positive control. For *S. aureus*, *C. albicans*, and *C. parapsilosis*, the positive control and *T. cubensis* group have a similar baseline level (~6-6.5 log CFU/mL) at 0 h, while the negative control shows no growth. At 1 h, *T. cubensis* starts to suppress growth earlier and significantly compared to the positive control. Between 3 and 24 h, *T. cubensis* significantly suppresses growth compared to the positive control, and the difference persists. The effect of *T. cubensis* is stronger in these species. The change in colony number as a result of the time-dependent killing test is shown in *Table 3* and the time-dependent killing graph of the isolate studied is shown in *Fig. 3*.

Antibiofilm Activities

T. cubensis extract was found to significantly affect biofilm formation and eradication on the tested bacteria. *T. cubensis* extract was found to be more effective on biofilm formation from 99.98% to 75.68% at the highest concentration *(Table 4)*. Although a significant reduction in biofilm formation was detected, it gave a low percentage (32.97% to 4.67%) in biofilm eradication. Significant reduction in biofilm formation was observed at all concentrations, while biofilm eradication was not observed at all concentrations *(Fig 4, Table 4)*. *T. cubensis*, extract showed the greatest effect on *P. vulgaris* in biofilm formation and on *E. coli* in biofilm inhibition *(Fig 4, Table 4)*.

Discussion

Infectious diseases continue to be a major global health problem, contributing 41% of the disease burden as defined by Disability-Adjusted Life Years worldwide [21]. One of the primary reasons of this issue is the widespread occurrence of acquired bacterial resistance to antibiotics, which poses a major danger to public health worldwide

today in the form of pandemics and epidemics of antibiotic resistance. Due to the problem of resistance to antibiotics, there is an urgent need to develop new, potent antimicrobial strategies based on natural agents with different mechanisms of action, which serve as sources of antibacterial and antifungal activity [22]. The antimicrobial and antibiofilm effects of Theranekron D6, an alcoholic extract of the *T. cubensis* spider were investigated.

Nature is known to offer many bioactive compounds in the form of animal venoms, algae and plant extracts that have been used in traditional medicine for decades. Animal venoms and secretions are a pharmaceutically promising wealth of molecules [23]. Studies have observed that many animal poisons and secretions such as frogs, bees, scorpions and ants show antimicrobial effects [15,24,25].

Many studies have shown the antimicrobial activity of many antimicrobial peptides obtained from spider species, which are venomous animals. Al-Kalifawi et al.[26] investigated Al-Ankabut's home (spider web) extract as an antimicrobial biomaterial against multidrug resistance bacteria and found an inhibition zone of 12 mm for *Enterobacter* spp. 8 mm for *E. coli*, 10 mm for *Proteus* spp., and 16 mm for *S. aureus*. The venom of *Lycosa coelestis* has also been reported to have antimicrobial activity on *S. aureus* and *E. coli* [27]. In animal studies of *T. cubensis* extract, it has been reported that it has a therapeutic effect on oral lesions [28], and clinically accelerates uterine involution and completes uterine involution by supporting the resolution and elimination of infection in the uterus [29]. By disk diffusion method, we detected a zone of 24.0-8.0 mm for bacteria and 22.0-8.0 mm for *Candida* spp. The MIC concentration was 14.3-0.45 mg/mL by liquid dilution method and 14.3-3.58 mg/mL by resazurin method.

Bacteria can adapt to different conditions thanks to biofilms, which aid in their survival. They include persister cells, which are well-known for having a high level of antibiotic resistance [19]. When bacteria are in a planktonic stage, they are susceptible to antibiotics, but because of the durability of biofilms, they become resistant to them [20]. Thus, the effectiveness of *T. cubensis* extract against pathogen biofilms was assessed in this work. Tests were conducted on the effects of *T. cubensis* extract on the prevention of biofilm formation as well as the elimination of pre-formed biofilm. The natural peptide isolated from the venom of the spider *Lycosa erythrognatha (L. erythrognatha)* showed a strong reduction of antibiofilm properties (67%) as well as antifungal activity [30]. *L. coelestis* effectively inhibited biofilm formation on *E. coli* and disruption of mature biofilms [27]. *L. erythrognatha* showed remarkable efficacy with >30% inhibition of biofilms on Methicillin-resistant *Staphylococcus aureus* (MRSA) and was reported to be superior to vancomycin in terms of rapid bactericidal and anti-biofilm effects [31]. In our study, we found that *T. cubensis* extract showed inhibition from 99.98% to 75.68% and a significant reduction in biofilm formation from 32.97% to 4.67%.

L. erythrognatha spider toxin has been reported to show high bactericidal activity against MRSA cells and can eliminate a high bacterial load after only 1 h of exposure and completely eliminate the bacterial load within 3 h [31]. It was observed that the number of visible colonies of the Chinese wolf spider Lycosa sinensis on *E. coli* decreased significantly in the first 5 min [32]. In the time-dependent killing method of *Lycosa singoriensis* on *E. coli*, significant microbial reduction was reported in the first 10 min [33]. In our study, we found significant microbial reduction from the beginning of the time-dependent killing method on all pathogens.

In this study, the growth of various microorganisms over time was observed, and bacteriostatic, fungistatic, bactericidal, and fungicidal effects were noted. *E. coli* and *S. aureus* showed clear bactericidal effects at 24 h, while *P. vulgaris* exhibited bactericidal activity at 8 h. *C. parapsilosis* displayed fungicidal activity at 24 h, but no significant reduction was observed for other microorganisms, particularly *E. faecalis* and *C. albicans*, indicating bacteriostatic or fungistatic effects. Overall, antimicrobial and antifungal activities were effective on certain microorganisms at specific time points, with varying results depending on the organism.

The current research reveals a real solution for antimicrobial resistance using natural *T. cubensis* extract. The antimicrobial and antibiofilm effects of *T. cubensis* extract on bacterial and fungal pathogens support the use of this natural compound as a potential therapeutic agent, paving the way for future research and therapeutic applications. These findings suggest that tarantula venom and similar biological resources may play an important role in developing infection control and treatment strategies.

Only standard laboratory strains were used in the study and no comparative evaluation was made with clinical isolates. This is considered as a limitation of the study.

In conclusion, the antimicrobial and antibiofilm effects of *T. cubensis* extract on bacterial and fungal pathogens are remarkable for their ability to inhibit biofilm formation as well as the action of these components against microbes. These properties allow the development of new treatment methods, especially in the fight against antibiotic-resistant microorganisms. Identification of active compounds in *T. cubensis* extract and further investigation of the mechanisms of these compounds may help to take important steps towards clinical applications. In addition, it can be said that these studies have great potential in

terms of reducing the risk of infection and increasing the effectiveness of existing treatment methods, especially in individuals with weak immune systems. Future research should further investigate the pharmacological properties and application potential of tarantula venom.

Declarations

Availability of Data and Materials: Materials and data sets from the study are available upon request from the corresponding author.

Funding Support: No funding was provided for this study.

Competing Interest: The authors declare that they have no conflict of interest.

Ethical Approval: Ethics committee approval is not required for this study.

Declaration of Generative Artificial Intelligence: The author of the current study declare that the article and/or tables and figures were not written/created by AI and AI-assisted technologies.

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