

Antimicrobial Potentials of Extracts from Ants (*Pheidole megacephala*), Cockroaches (*Periplaneta americana*), and Termites (*Macrotermes bellicosus*) on Bacterial and Fungal Isolates

Robinson, V. K¹, Wilcox, I. M^{*2}, Azike, B. O³ and Woko, P⁴.

^{1, 3 & 4}Department of Microbiology, Faculty of Sciences, Rivers State University, Nkpolu Oroworukwo, Port Harcourt, Nigeria. ²Department of Microbiology, Faculty of Natural and Applied Science, Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt, Nigeria.

***Corresponding Author:** inyingierefagha.wilcox@iaue.edu.ng

ABSTRACT

The upsurge in antimicrobial resistance has spurred the search for new antimicrobial substances. Insects are promising sources of antimicrobials, and their antimicrobial activity is attributed to their ability to disrupt microbial membranes. This study evaluated the antimicrobial potential of extracts from Ants (*Pheidole megacephala*), cockroaches (*Periplaneta americana*), and termites (*Macrotermes bellicosus*) obtained from the Rivers State University school farm against bacterial and fungal isolates. *Shigella* spp., *Escherichia coli*, *Salmonella* spp., *Staphylococcus* spp., *Candida* sp., *Aspergillus* sp and *Mucor* sp. were obtained from the clinical laboratory, Rivers State University. The insect extracts were prepared using standard methods, and antimicrobial susceptibility was determined using the agar disc diffusion method on freshly prepared Mueller-Hinton agar. Results showed that antimicrobial activities of the insects were dependent on the dose and the insect type. The antibacterial activity of the ant, cockroach and termite at 100% concentration on *Shigella* sp. was 20.0±0.6, 20.0±0.7 and 15.0±0.5 mm, respectively. For *Salmonella* sp. at 100% concentration, the zone of inhibition was 0.0±0.0, 32.0±0.7 and 19.0±0.5 mm for the ant, cockroach and termite. The zone diameter of the ant, cockroach and termite extract on *Staphylococcus* sp. at 100% was 12.0±0.5, 19.0±0.6 and 10.0±0.5 mm. The zone diameter of the ant, cockroach and termite extract on *E. coli* was 35.0±0.7, 16.0±0.6 and 15.0±0.5 mm. The zone diameter of the ant, cockroach and termite extract on *Candida* sp at 100% was 0.0±0.00, 14.0±0.58 and 7.0±0.50 mm. The zone diameter of the ant, cockroach and termite extracts on *Aspergillus* sp. and *Mucor* sp. was (0.0±0.00, 0.0±0.00 and 8.0±0.58 mm) and (0.0±0.00, 9.0±0.2 and 6.0±0.50 mm), respectively. Although the antimicrobial activity of the insect extracts varied, cockroach extracts exhibited better antibacterial and antifungal activity than the ant and termite extracts.

Keywords: Insect Extract, Antimicrobial Activity, Cockroach Extract, Termite, Ant, Antimicrobial Resistance, Pathogens.

Introduction

The growing global problem of antimicrobial resistance (AMR) is one of the biggest risks to contemporary medicine and public health. In 2019, bacterial AMR was linked to about 4.95 million deaths globally, and estimates suggest that this number could increase to 10 million deaths annually by 2050 if new treatment approaches are not developed (Murray *et al.*, 2022; Abbasi, 2026). Multidrug-resistant pathogens, such as clinically significant *Escherichia coli*, *Salmonella*, *Shigella*; and *Staphylococcus*, and opportunistic fungi like *Candida*, *Aspergillus*, and

Mucor, are becoming more resistant to conventional antimicrobials (Abbasi, 2026). As a result, the hunt for alternative antimicrobial agents from unexplored biological sources has become more intense (Murray *et al.*, 2022; Abbasi, 2026). One especially promising source of new antimicrobials is insects. Insects, the most diverse group of animals, live in environments rich in microbes and depend on a strong innate immune system that includes antimicrobial peptides (AMPs), which are small cationic molecules with a low propensity to induce resistance that can disrupt microbial membranes and inhibit intracellular targets (Dastidar *et al.*, 2025).

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In addition to endogenous AMPs, symbiotic and related microbiota (particularly actinobacteria associated with termites, ants, and other social insects) produce structurally distinct secondary metabolites, such as polyketides and non-ribosomal peptides, which have strong antimicrobial activity against pathogens resistant to multiple drugs (Abbasi, 2026). Particular potential has been shown by certain groups of insects. Antibacterial activity against *Staphylococcus aureus*, *E. coli*, and other pathogens has been demonstrated by extracts and compounds derived from termites of the genus *Macrotermes*, including *M. bellicosus* and related species, supporting traditional medicinal uses in parts of Africa and demonstrating environmental influences on efficacy (Hammoud Mahdi *et al.*, 2020). Also, haemolymph and tissue extracts containing bioactive molecules with activity against both Gram-positive and Gram-negative bacteria as well as fungi are produced by cockroaches like *Periplaneta americana*, which flourish in environments contaminated by pathogens (Mutmainnah *et al.*, 2025). Similar promising substances, such as peptides that are effective against resistant strains, have been found in ant-associated microbes and venoms (Abbasi, 2026). Despite these developments, many areas, including the Niger Delta region of Nigeria, still lack systematic testing of crude extracts from widely distributed insect species against a wide range of bacterial and fungal pathogens.

Therefore, the current study evaluates the antimicrobial potential of methanol-based extracts made from three common insects; Ants (*Pheidole megacephala*), Cockroaches (*Periplaneta americana*), and Termites (*Macrotermes bellicosus*) that were collected in Port Harcourt, Rivers State. The study would add to the increasing amount of data that supports insects as potential sources of new antimicrobials in the face of growing antimicrobial resistance.

Material and Methods

Study Area

The research was carried out in the Laboratory of the Department of Microbiology, Faculty of Science, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria.

Insect Collection and Identification

Ants (*Pheidole megacephala*), cockroaches (*Periplaneta americana*), and termites (*Macrotermes bellicosus*) were hand-picked with the aid of a hand net into plastic containers. The insects were collected from the Rivers State University school farm in Nkpolu-Oroworukwo, Port Harcourt, Nigeria. The insects were identified morphologically in the farm using standard entomological keys of Gibb and Oseto (2019).

Further confirmation was done by an entomologist at the Department of Animal and Environmental Biology, Rivers State University. The collected specimens were placed in sterile, ventilated containers and transported immediately to the Microbiology Research Laboratory for processing immediately after collection to preserve their biological integrity.

Processing of Insect Samples

The insects were first euthanised by freezing at -20°C for 30 minutes to ensure humane treatment and preserve cellular structures. Subsequently, they were thoroughly washed three times with sterile distilled water to remove external debris, soil particles, and transient contaminants. Following this, surface disinfection was performed by immersion in a 10% (v/v) ethanol solution for 2 minutes to eliminate surface-associated microorganisms while preserving endogenous bioactive compounds (Hammoud Mahdi *et al.*, 2020; Aiello *et al.*, 2023).

The disinfected insects were blotted dry using a sterile white handkerchief and further dried in a hot air oven at 37°C for 24 hours to remove residual moisture completely without denaturing heat-sensitive compounds. The different types of dried insects were separately pulverized into a fine, homogeneous powder using a sterile electric blender operated at high speed for 5 minutes per batch. Each insect type was placed in sterile labelled containers.

Extraction of Bioactive Compounds

The extraction was performed using a methanol-based solvent system optimised for polar and semi-polar compounds (Hossain *et al.*, 2022; Kebede and Shibeshi, 2022).

In this method, 25g of the powdered insect was quantitatively transferred into a 250mL sterile conical flask and soaked in 80mL of cold acidified methanol solvent (prepared with HPLC-grade methanol, sterile distilled water, and analytical-grade glacial acetic acid in a ratio of 90:9:1 v/v). The mixture was sealed with Parafilm to prevent solvent evaporation and agitated vigorously using an orbital shaker at 150rpm for 45 hours at room temperature ($25\pm 2^{\circ}\text{C}$) to facilitate efficient extraction of bioactive compounds through continuous mechanical mixing.

Filtration

After soaking, the crude extract was first filtered through a double layer of sterile muslin cloth to remove coarse particulate matter. The filtrate was then passed through Whatman No. 1 filter paper under vacuum to ensure clarity; a clarification step consistent with standard crude-extract filtration procedures (Kebede and Shibeshi, 2022). The filtered solution was transferred to sterile 50 mL centrifuge tubes and centrifuged at 5000 rpm for 5 minutes at 4°C with the aid of a centrifuge to sediment any remaining fine particles.

The supernatant was carefully decanted into pre-weighed, sterile glass Petri dishes and subjected to solvent evaporation in a fume hood at room temperature ($25\pm 2^{\circ}\text{C}$) until constant weight was achieved, yielding a concentrated, viscous crude extract. The resulting residue was designated as the 100% stock solution of the extract.

Preparation of Test Concentrations

The 100% stock solution was reconstituted in sterile acidified methanol (90:9:1v/v methanol: water: acetic acid) to ensure homogeneity. Serial dilutions were performed under aseptic conditions using sterile glassware to obtain the 50% and 25% (v/v) concentrations used for the antimicrobial assay (Hossain et al., 2022).

Each dilution was vortexed for 2 minutes to ensure uniform distribution of compounds. The extracts were stored at 4°C in sterile, airtight bottles and labelled accordingly. All procedures were performed in triplicate to ensure reproducibility

Test Microorganisms

The antimicrobial activity of the insect extracts was assessed against bacterial and fungal isolates obtained from the clinical laboratory, Department of Microbiology, Rivers State University. The bacterial isolates included *Shigella* spp., *Escherichia coli*, *Salmonella* spp, and *Staphylococcus* spp., while the fungal isolates comprised *Candida* sp., *Aspergillus* sp and *Mucor* sp. The bacterial isolates were maintained on Nutrient Agar slants, while the fungi were preserved on Sabouraud Dextrose Agar slants, both stored at 4°C and subcultured periodically to maintain purity and viability. Before antimicrobial assays, each strain was reactivated by inoculating into appropriate broth media and incubating under optimal growth conditions to obtain fresh, metabolically active cultures for testing (CLSI, 2023).

Antimicrobial Susceptibility Testing of the Insect Extracts

Testing against Bacterial Isolates

The agar well diffusion method, as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2023), was employed to evaluate the antibacterial activity of the insect extracts (Hossain et al., 2022; Kebede and Shibeshi, 2022). Mueller-Hinton Agar (MHA) plates were used as the culture medium for all bacterial strains due to its optimal diffusion properties and standardized composition. Bacterial suspensions were prepared from fresh 18–24-hour cultures grown in Mueller-Hinton Broth at 37°C . The turbidity of each suspension was adjusted to a 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) using sterile physiological saline (0.85% NaCl), with verification using a spectrophotometer at 625 nm wavelength, in line with standardized inoculum-preparation procedures for susceptibility testing (CLSI, 2023).

The surfaces of the MHA plates were uniformly inoculated by swabbing horizontally and vertically using sterile cotton swabs dipped in the standardized bacterial suspensions. Using a sterile cork borer (6 mm in diameter), three equidistant wells were punched into the agar medium. Then, 100 μL of each extract concentration (100%, 50%, and 25%) was carefully pipetted into the respective wells, ensuring no spillage.

The plates were allowed to stand for 30 minutes at room temperature for pre-diffusion of the extracts and then incubated at 37°C for 18–24 hours in an inverted position to prevent condensation from interfering with zone formation.

Testing against Fungal Strains

The agar well diffusion method was employed to evaluate the antifungal activity of the insect extracts against *Candida spp.*, *Aspergillus spp.*, and *Mucor spp.*, consistent with CLSI (2023) recommendations for antifungal susceptibility testing and with well diffusion approaches used in comparable natural-extract screening studies (Kebede and Shibeshi, 2022). Sabouraud Dextrose Agar (SDA) plates were used as the culture medium to support robust fungal growth. For *Candida spp.*, suspensions were prepared from 24-hour cultures grown on SDA at 30°C, and turbidity was adjusted to a 0.5 McFarland standard using sterile saline. For *Aspergillus spp.* and *Mucor spp.*, spore suspensions were harvested from 5- to 7-day cultures using sterile saline containing 0.1% Tween 80, and turbidity was similarly standardized to 0.5 McFarland.

SDA plates were uniformly inoculated with the standardized fungal suspensions using sterile cotton swabs. Wells (6 mm diameter) were aseptically punched into the agar, and 100 µL of each extract concentration (100%, 50%, and 25%) was added to the respective wells. The plates were allowed to stand for 30 minutes at room temperature for pre-diffusion and then incubated at 25–30°C for 24–48 hours. After incubation, the diameters of the zones of inhibition were measured in millimetres (mm) using a calibrated

ruler, with all assays performed in triplicate to ensure reproducibility and statistical reliability.

Data Analysis

The mean and standard deviation of the resulting zone diameter were calculated using the statistical package for social science (SPSS v 27). The analysis of variance (ANOVA) was the tool used for separation of means at a confidence interval of 95% ($P = 0.05$).

Results

The results in Table 1 demonstrate significant variation in efficacy among the insect extracts. Results of the antimicrobial activity of ants, cockroaches and termites are presented in Table 1. Results showed that at 100% concentration, the zones of inhibition recorded by the ant extract on *Shigella*, *Staphylococcus spp* and *E. coli* were 20.0 ± 0.6 , 12.0 ± 0.5 and 35.0 ± 0.7 mm, respectively. There was no antimicrobial activity observed for *Salmonella sp.*, *Candida*, *Aspergillus* and *Mucor spp.* The zones of inhibition recorded by the cockroach extract at 100% concentration on *Shigella*, *Salmonella*, *Staphylococcus*, *E. coli*, *Candida*, *Aspergillus* and *Mucor spp* were 20.0 ± 0.7 , 32.0 ± 0.7 , 19.0 ± 0.3 , 16.0 ± 0.6 , 14.0 ± 0.58 , 0.0 ± 0.00 and 9.0 ± 0.2 mm, respectively. While the zones of inhibition recorded by the termite extract at 100% concentration on *Shigella*, *Salmonella*, *Staphylococcus*, *E. coli*, *Candida*, *Aspergillus* and *Mucor spp.*, were 15.0 ± 0.5 , 19.0 ± 0.5 , 15.0 ± 0.5 , 7.0 ± 0.50 , 8.0 ± 0.5 and 6.0 ± 0.50 mm, respectively.

Table 1: Antibacterial Activity of Insect Extracts against Test bacterial and Fungal Isolates

	Concentration of Insect Extract/Zone of Inhibition (mm)								
	100% Conc.			50% Conc.			25% Conc.		
	Ant	Cockroach	Termite	Ant	Cockroach	Termite	Ant	Cockroach	Termite
Bacterium									
<i>Shigella</i>	20.0 ± 0.6	20.0 ± 0.7	15.0 ± 0.5	12.0 ± 0.5	19.0 ± 0.6	12.0 ± 0.6	12.0 ± 0.4	7.0 ± 0.5	12 ± 0.5
<i>Salmonella</i>	0	32.0 ± 0.7	19.0 ± 0.5	0.0 ± 0.0	20.0 ± 0.6	11.0 ± 0.6	0	18.0 ± 0.5	8.0 ± 0.7
<i>Staphylococcus</i>	12.0 ± 0.5	19.0 ± 0.3	0	8.0 ± 0.6	13.0 ± 0.5		3.0 ± 0.5	7.0 ± 0.1	
<i>E. coli</i>	35.0 ± 0.7	16.0 ± 0.6	15.0 ± 0.5	31.0 ± 0.6	10.0 ± 0.5	10.0 ± 0.6	20.0 ± 0.5	9.0 ± 0.6	7.0 ± 0.5
Fungus									
<i>Candida</i>	0	14.0 ± 0.58	7.0 ± 0.50	0	11.0 ± 0.50	5.0 ± 0.50	0	9.0 ± 0.58	3.0 ± 0.5
<i>Aspergillus</i>	0	0	8.0 ± 0.5	0	0	6.0 ± 0.50	0	0	4.0 ± 0.58
<i>Mucor</i>	0	9.0 ± 0.2	6.0 ± 0.50	0	5.0 ± 0.5	3.0 ± 0.50	0	2.0 ± 0.58	1.0 ± 0.5

Discussion

This study investigated the efficacy of different insect extracts against selected pathogens. According to the concentration-dependent pattern seen against *Shigella* spp, the antibacterial activity found in the Ant and Cockroach extracts is dose-sensitive. This is in line with the behaviour of cationic antimicrobial peptides (AMPs) and small bioactive metabolites, which need a threshold concentration to damage bacterial membranes or obstruct vital metabolic processes (Dho *et al.*, 2023). A higher local density or greater diversity of active compounds in cockroach-derived extracts is suggested by the ability of the extract to retain susceptibility down to 50% concentration, as opposed to the more pronounced decline in the ant extract. This is generally in line with reports that the whole-body and organ lysates of *Periplaneta americana* contain several co-eluting bioactive fractions that can create sizable inhibition zones against both Gram-positive and Gram-negative bacteria, including *Enterobacteriaceae* species (Chen *et al.*, 2020; Amin *et al.*, 2022).

Also, the findings that termite antimicrobial potency is strongly influenced by extraction conditions and the ecological environment from which the insects are sourced, with habitat-associated variation in gut microbiota and cuticular chemistry driving differences in potency, are reflected in the moderate, concentration-sensitive activity of the termite extract (Oluoch *et al.*, 2025). All of these findings suggest that insect-derived extracts, especially those from cockroaches, have potential as sources of antidysenteric agents at a time when multidrug-resistant *Shigella* strains are becoming more clinically concerning. However, the significant loss of activity at lower concentrations highlights the necessity of concentrating and purifying the active fractions before any translational application can be contemplated.

Furthermore, in this study, against *Salmonella* spp., the consistent, dose-dependent susceptibility at all tested concentrations of cockroach extract confirmed that it is the most dependable antibacterial source of the three insect extracts studied. It is interesting to note that this result differs from that of Amin *et al.* (2022), who claimed that ethanol and cyclohexane extracts of *P. americana* had no inhibitory effect on *Salmonella enterica*.

This discrepancy is most likely due to variations in the extraction solvent, cockroach species, tissue source, and potentially strain-specific resistance mechanisms. It also showed how methodological variation can significantly affect apparent antibacterial spectra even within the same insect group. Also, the non-linear, biphasic response of termite extract, which is active at 100% and 25% but only intermediate at 50%, is uncommon. It is comparable to the intricate, non-monotonic dose-response relationships previously reported for termite-derived antibacterial metabolites, where interactions among several bioactive constituents can result in partial antagonism at intermediate dilutions before synergy reemerges at greater dilution (Afolayan *et al.*, 2019). The current conclusion that termite extracts have real, if inconsistent, activity against this pathogen is directly supported by Afolayan *et al.* (2019), who specifically showed anti-*Salmonella* activity in metabolites derived from the African soldier termite *Macrotermes bellicosus*. The total resistance of Ant extract indicated that its chemical defenses, which are probably dominated by formic acid and low-molecular-weight alkaloids rather than membrane-active peptides, are not well suited to piercing the lipopolysaccharide-rich outer membrane of *Salmonella*. This pattern is consistent with the relatively narrow antibacterial spectra reported for extracts derived from ants and ant plants, which typically act more consistently against *Escherichia coli* and Gram-positive organisms than against *Salmonellae* (Srisawat *et al.*, 2021; Lisnanti *et al.*, 2024).

Additionally, in this study, the strong and consistent susceptibility of *Staphylococcus* spp. to the Cockroach extract is supported by a significant amount of research which has identified cockroaches as a particularly rich source of anti-staphylococcal, including anti-MRSA. While Chen *et al.* (2020) isolated actinomycin X2 and collismycin A from actinomycete symbionts living in the gut of cockroaches, Amin *et al.* (2022), in their study against methicillin-resistant *S. aureus* using cyclohexane extracts of *P. americana*, reported some of the largest inhibition zones. Ant-derived and ant-plant-derived preparations have consistently shown moderate but repeatable activity against Gram-positive cocci, which is consistent with the stable, intermediate inhibition produced by the Ant extract across all concentrations.

This activity is probably mediated by phenolic and flavonoid constituents rather than strong membrane-lytic peptides (Srisawat *et al.*, 2021).

Also, in this study, termite antimicrobial activity against *S. aureus* was significantly habitat- and preparation-dependent, with only specific environmental sources or extraction protocols yielding potent, concentration-stable effects. Notably, the rapid decline in termite efficacy with dilution suggests either a lower baseline concentration of active antistaphylococcal compounds or a requirement for a higher threshold dose to achieve membrane disruption (Oluoch *et al.*, 2025). When combined, these findings make cockroach extract the most promising option for additional bioprospecting against this clinically significant Gram-positive pathogen.

Also, given that ant venoms and ant-plant preparations have been specifically and repeatedly reported to be more consistently active against *E. coli* than against many other Gram-negative genera, the significantly superior, dose-dependent efficacy of the Ant extract against *E. coli* in this study is a noteworthy and biologically coherent finding. *E. coli* was shown to be inhibited by ethanolic ant-plant tuber extract in a concentration-dependent manner by Srisawat *et al.* (2021), and Lisnanti *et al.* (2024) similarly identified *E. coli* as one of the organisms most consistently suppressed across studies of Myrmecodia (ant-plant) preparations. This convergence with the current findings implies that rather than having a broadly non-specific mode of action, Formicidae-associated extracts may contain compounds with a specific affinity for structural or metabolic targets specific to *E. coli*. According to research, crude cockroach organ extracts inhibit neuropathogenic *E. coli* (Chen *et al.*, 2020) and related Enterobacteriaceae like *Enterobacter cloacae* (Amin *et al.*, 2022). This is consistent with the strong performance of cockroach extract in this study, which only declines at the lowest concentration. Also, termite extract showed moderate, dilution-sensitive activity which is in line with the environment-dependent antimicrobial potency reported for termite extracts, where termites from hilly thicket habitats demonstrated significantly higher anti-*E. coli* activity than those from farmland or grassland habitats (Oluoch *et al.*, 2025). This suggests that ecological sourcing may be just as significant a factor in determining the efficacy of extract concentration.

Given the ongoing prevalence of antimicrobial-resistant Gram-negative infections worldwide, the particularly potent Gram-negative activity of the ant extract has a significant implication, showing that ants may be an underutilized but targeted source of anti-*E. coli* agents (Murray *et al.*, 2022).

The Cockroach extract dose-dependent antifungal activity against *Candida* spp. in this study is widely supported by literature. Amin *et al.* (2022) reported large inhibition zones and a low minimum inhibitory concentration for *P. americana* cyclohexane extracts against *Candida albicans*, and transmission electron microscopy confirmed disruption of fungal cell-wall and membrane integrity after treatment.

The known presence of termicin-type antifungal peptides within termite lineages (a peptide family initially identified in the termite *Pseudacanthotermes spiniger* and recognized for strong, stable activity against yeasts) is consistent with the constant, moderate inhibition generated by the termite extract across all concentrations (Dho *et al.*, 2023).

The total lack of activity in the ant extract, on the other hand, suggests that the defense chemistry of ants, which is primarily composed of small organic acids and alkaloids, is largely ineffective against the ergosterol-containing membranes and chitinous cell walls typical of *Candida* species. This limitation is also reflected in the relatively narrow antifungal spectra reported for extracts derived from ants and ant plants in relation to their antibacterial range (Lisnanti *et al.*, 2024).

These results suggest that of the three insects studied, cockroach extract has the most potential as a source of new antifungal agents against candidiasis, an infection that is becoming more clinically concerning due to the rise in azole-resistant *Candida* isolates.

The specific association between termites and termicin-family antifungal peptides, which are reported to act preferentially on filamentous fungi and were initially identified precisely because of this selective antifungal role within termite immunity (Dho *et al.*, 2023), is consistent with the finding of this study, which showed that only the termite extract demonstrated activity against *Aspergillus* spp. and this activity was stable across all concentrations.

A plausible biological basis for a termite-specific antifungal advantage against filamentous molds has been established by chemical profiling of the fungus-growing termite *Macrotermes bellicosus*, which has confirmed the presence of antimicrobial secondary metabolites linked to this insect's ecological role in cultivating and defending fungal gardens (Mahdi *et al.*, 2020). Also, the efficacy of Cockroach extract against *Aspergillus species* has been demonstrated to be species- and solvent-specific, with cyclohexane extracts of *P. americana* reported to be active against *A. fumigatus* and *A. flavus* but inactive against *A. niger* (Amin *et al.*, 2022). This suggests that cockroach extract antifungal activity against this genus is real but narrower and more strain-dependent than its activity against yeasts or bacteria, and may simply not apply to the *Aspergillus* isolate used in this study. The interpretation that was previously established for *Candida* (that ant-derived chemical defences are generally poorly matched to fungal targets) is further supported by the consistent resistance of Ant extract in this study. All of these findings point to the termite extract as a unique and hitherto unidentified potential source of anti-*Aspergillus* agents, necessitating bioassay-guided fractionation to identify the precise responsible compound or compounds.

Additionally, against *Mucor* spp., both the Cockroach and Termite extracts showed intermediate, dose-dependent activity; however, the Cockroach extract consistently produced larger inhibition zones. The Cockroach extract exhibits the strongest overall antifungal potential against yeasts, while the Termite extract maintained a stable, moderate effect due to its peptide-based antifungal defenses. This pattern is consistent with the wider trend established across fungal isolates in the study by Dho *et al.* (2023). There are still few reports in the literature that specifically address insect-derived activity against zygomycete fungi like *Mucor*, which highlights the novelty of the current observation and implies that this genus has been relatively overlooked in insect-derived antimicrobial screening in comparison to *Candida* and *Aspergillus*.

The conclusion that ant-derived extracts, at least as prepared in this study, are essentially non-antifungal and that their pharmacological value lies specifically in the antibacterial, and especially anti-*E. coli*, domain is further supported by the consistent lack of activity

of Ant extract against *Mucor*, mirroring its inactivity against the other two fungal genera tested.

Overall, the Cockroach extract exhibits the most broad-spectrum behaviour across all seven pathogens, maintaining significant antibacterial and antifungal activity across Gram-positive, Gram-negative, and fungal targets alike. This finding is consistent with the chemically diverse, multi-compound composition frequently reported in *Periplaneta americana* lysates (Chen *et al.*, 2020; Amin *et al.*, 2022). The termicin-type antifungal peptide lineage linked to termite immunity is most likely reflected in the termite extract's more selective profile, which combines moderate, stable activity against a number of bacteria with a unique and relatively strong effect against *Aspergillus* (Mahdi *et al.*, 2020; Dho *et al.*, 2023). With strong, dose-dependent anti-*E. coli* activity, virtually no antifungal activity, and inconsistent activity against other Gram-negative bacteria like *Salmonella*, the Ant extract stands out as the most specifically targeted of the three. This pattern is consistent with a chemical defense system based on phenolics and small organic acids rather than broad-spectrum membrane-active peptides (Srisawat *et al.*, 2021; Lisnanti *et al.*, 2024). Also, the threshold-dependent, membrane- and biofilm-disrupting mode of action typical of insect antimicrobial peptides, which usually require a minimum effective concentration to achieve pore formation, membrane permeabilization, or interference with intracellular macromolecular synthesis, is mechanistically consistent with the recurrent concentration dependence seen across almost all extract-pathogen combinations (Dho *et al.*, 2023).

These results encourage further investigation of insect-derived extracts, particularly cockroach and termite extracts, as a source of new antibacterial and antifungal leads, given the growing global burden of antimicrobial resistance, which is thought to have been directly linked to over a million deaths worldwide in a single recent year (Murray *et al.*, 2022). Notably, before any of these extracts are taken into consideration for additional pharmaceutical development, future research should focus on bioassay-guided fractionation, structural characterization of the active principles, determination of precise minimum inhibitory and minimum fungicidal/bactericidal concentrations, and assessment of cytotoxicity toward mammalian cells.

Conclusion

Findings in this study showed significant variation in efficacy of the insect extracts against *Shigella* spp., *Salmonella* spp., *Staphylococcus* spp., *E. coli*, *Candida* spp., *Aspergillus* spp., and *Mucor* spp. Overall, the cockroach extract exhibits the broadest-spectrum activity across all seven pathogens, maintaining significant antibacterial and antifungal activity across Gram-positive, Gram-negative, and fungal targets alike. The activity of termite extract showed a more selective profile, which combines moderate, stable activity against a number of bacteria with a unique and relatively strong effect against *Aspergillus*. With strong, dose-dependent anti- *E. coli* activity, virtually no antifungal activity, and inconsistent activity against other Gram-negative bacteria like *Salmonella*, the Ant extract stands out as the most specifically targeted of the three.

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