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Exploring the antibacterial potential of volatile organic compounds (VOCs) from *Chinocossus acronyctoides* larvae

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Abstract
The present study undertook a preliminary assessment of the antimicrobial activity of Volatile Organic Compounds (VOCs) derived from *Chinocossus acronyctoides* moth larvae. Using hexane as a solvent, we extracted the larvae at two developmental stages: young (2nd instar) and old (5th instar). The resulting crude extracts were evaluated for antibacterial activity against two gram-negative bacteria *Escherichia coli*, *Klebsiella pneumoniae* and two gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* by employing the agar diffusion method. The outcomes revealed significant antibacterial activity across all tested bacterial species. Future research should focus on the detailed component analysis of the extracts to identify the active compound/s, thereby validating their safety and efficacy for potential use in medicinal applications as alternative natural antibiotics.

Keywords: Antibacterial, *Chinocossus acronyctoide*s Larvae, VOCs

Introduction
The spread of new strains of antibiotic-resistant pathogenic microorganisms has led to the urgent need to discover and develop new antimicrobial systems as the rapid emergence of antimicrobial resistance in bacterial and fungal pathogens is an open emergency for public health [1, 2]. Novel therapeutics are required to counter resistance, however no modern antimicrobial classes have been clinically affirmed in over three decades [3]. The antibacterial effects of fatty acids have been well-known and recognized since the first experiments of Robert Koch in 1881, and they are now used in diverse fields [4, 5]. Over the last twenty years, there has been a significant surge in research focusing on new insect pheromones, their biosynthesis, modes of action, peripheral olfactory and neural mechanisms, as well as their practical applications in Integrated Pest Management (IPM) ever since the first identification of the silkworm moth sex pheromone in 1959 [6, 7]. Insects have long been recognized as a rich source of bioactive compounds with diverse medicinal properties [8] hence, researchers have increasingly recognized the potential of bioactive compound secretions, example like some volatile organic compounds (VOCs) which are emitted by insects and possess antimicrobial properties [9]. These VOCs are often released as pheromones or defensive secretions by insects [10, 11] and exhibit a wide array of chemical structures and functions, some often possessing distinctive odours [12]. They play crucial roles in plants [13, 14] and insect communication [15]. In insect communication, VOCs helps by serving chemical signals that facilitate various behaviours such as mate attraction [16], host location [17], and territorial marking, and ecological interactions [18].
The antimicrobial potential of insect-derived VOCs has been investigated against a broad spectrum of pathogenic microorganisms both in vitro and *in vivo*, including multidrug-resistant bacteria, enveloped viruses, algae, fungi, and protozoa [19–26]. Studies have demonstrated the ability of these compounds to inhibit microbial growth, disrupt cellular processes, and induce cell death through multiple mechanisms, making them promising candidates for the development of novel antimicrobial agents. Furthermore, the natural origin of insect-derived VOCs offers several advantages over synthetic antimicrobial compounds, including greater biocompatibility, lower toxicity, and reduced environmental impact [27].
Some of the species of Cossid moth larvae are known to possess odour [28]. The moth larvae of *Chinocossus acronyctoides* produces volatile secretion which has a pungent aromatic smell all over its body. Some other studies also reported that the cossid larvae secretions are the volatile organic mixture of alcohol and acetate [28–30]. It was seen that the moth larvae of *Chinocossus acronyctoides* possess substantial concentration of VOCs and so we believed that the moth larvae may have a good therapeutic source of antibacterial agents. Here we explore the preliminary investigation of the moth *Chinocossus acronyctoides* larvae VOCs for antibacterial properties in hopes for finding new avenues for antibacterial agents. The study was assisted by comparing the two different stage of the *Chinocossus acronyctoides* larvae, early stage (2nd instar) and late stage (5th instar).
The identification and development of novel antimicrobial therapeutics represent a promising avenue in response to the growing issue of antibiotic resistance, exacerbated by decades of antibiotic misuse and overuse. The World Health Organization recognizes antibiotic resistance as a major threat to global public health [9]. This resistance crisis necessitates the urgent discovery of alternative therapeutic agents, a complex and demanding task that calls for innovative research into natural sources and identifying novel antibiotic classes. These potential solutions require thorough efficacy, safety evaluations and tackling this critical issue requires sustainable investment in antibiotic research and development, alongside the implementation of judicious practices of antibiotic use.

Materials and Methods
Insect sample collection
Chinocossus acronyctoides larvae were collected from the field site at Kedima-Kohima, Nagaland the GPS position for latitude is 25° 33’33” N and longitude is 94°10’50” E. The 2nd and 5th instar larvae are illustrated in (Fig: 1). Morphological Characters of the larvae is also described in Table 1.

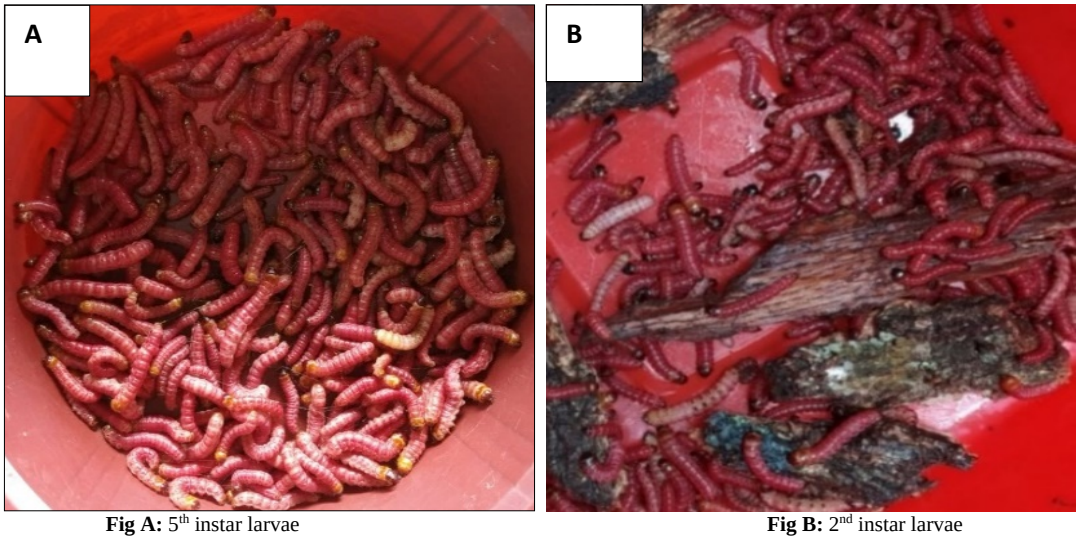


Fig A: 5th instar larvae **Fig B:** 2nd instar larvae

Fig 1: Morphological Characters of the *Chinocossus acronyctoides* larvae

Table 1: Characteristic of the two different stage larvae

Characters	2nd instar larva	5th instar larva
Colour	Red-pink	pink
Odour	Very pungent	pungent
Body segment	11	11

Preparation of the crude VOCs extracts from *Chinocossus acronyctoides* Larvae

Solvent extraction

Solvent extraction was performed by using hexane as the solvent [31]. About 90 g of each larvae sample (older stage/5th instar and younger stage/2nd instar) was used for extraction. Each of the samples were exposed in hexane for 10 min in approximately 100 ml of hexane. Later the extracts were filtered using the Whatman filter paper. The filtrate was concentrates by evaporating the solvent using a slow stream of ultra-high purity nitrogen gas. After concentration the final volume of both the samples were brought down to 15 ml approximately. At the time of extraction both the two different stage larvae were kept enclosed in a sterilised chamber to avoid unnecessary contamination.

Microbial samples used

Bacillus subtilis

B. subtilis bacteria are aerobic, rod-shaped, and sporulating organisms found ubiquitously in nature. Notably, *Bacillus anthracis*, the causative agent of anthrax, is the only obligate pathogen in vertebrates within this genus. Other species, such as Bacillus cereus, can cause food poisoning and various infections, while many *Bacillus* species are harmless saprophytes. *Bacillus* species are crucial in medical, pharmaceutical, agricultural, and industrial applications due to their diverse physiological properties and production of enzymes, antibiotics, and other metabolites. For instance, they contribute to the production of antibiotics like bacitracin and polymyxin, and their spores are used to test and validate sterilization procedures. Despite their beneficial uses, Bacillus spores are resistant to heat, radiation, disinfectants, and desiccation, posing challenges in contamination control in medical and food industries [32].

Escherichia coli

E. coli is a gram-negative, rod-shaped bacterium commonly found in the intestines of warm-blooded animals. While most strains are harmless and part of the normal gut flora, some can cause serious food poisoning and infections. Pathogenic strains such as uropathogenic *Escherichia coli* (UPEC) cause urinary tract infections, while others like enteroinvasive *Escherichia coli* (EIEC) lead to intestinal diseases including diarrhoea and colitis [33]. E. coli is a model organism in microbiology due to its well-characterized genetics and ease of manipulation [34]. It is typically sensitive to antibiotics like ciprofloxacin and gentamicin, but misuse of these drugs can lead to antibiotic resistance [35].

Staphylococcus aureus

S. aureus is a gram-positive, round-shaped bacterium that is often found on the skin and in the nasal passages of humans [36]. While it can exist harmlessly as part of the normal flora, it is also a major pathogen capable of causing a wide range of infections, from minor skin infections to life-threatening conditions such as pneumonia, endocarditis, and sepsis [37]. *S. aureus* is known for its ability to develop resistance to antibiotics, notably methicillin-resistant *Staphylococcus aureus* (MRSA), making infections difficult to treat. It produces various virulence factors, including toxins and enzymes that facilitate tissue invasion and immune evasion [37].

Klebsiella pneumoniae

K. pneumoniae is a gram-negative, rod-shaped bacterium commonly found in the environment, including soil, water, and on plants. It is also part of the normal flora of the human intestines but can become pathogenic under certain conditions. *K. pneumoniae* is a major cause of healthcare-associated infections, particularly in immunocompromised individuals, leading to severe conditions such as pneumonia, bloodstream infections, urinary tract infections, and wound infections. This bacterium is notable for its high resistance to multiple antibiotics, including carbapenems, making treatment challenging. It produces a thick, protective capsule that enhances its virulence and ability to evade the host immune system [38].

Sub-culturing of bacterial samples

Pure cultured bacterial strain of *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus* and *Bacillus subtilis*

were obtained from the lab, the pure cultures were sub-cultured onto clean, sterilized Petri plates containing nutrient agar. Each bacterial strain was then streaked onto the nutrient agar plates using the quadrant streaking method. Later a working stock of all the sub-cultured bacteria was also prepared in nutrient broth for the antibacterial assessment.

Assessment of antibacterial assay

The crude extracts of *Chinocossus acronyctoides* larvae were tested for antimicrobial activity using the disc diffusion method (Kirby-Bauer method) [39]. According to the manufacturer's instruction, (28 g of agar per litre) nutrient agar media was prepared by dissolving 2.8% agar in distilled water. On stirring, the media was heated for about a minute to completely dissolve the agar. After dissolving, the media was autoclaved at 121°C for 15 minutes and later cooled to about 50 °C. The cooled agar media was poured into clean sterile petri dishes each about 20 ml. The plates were allowed to cool at room temperature for solidification of the media. After that 200 µl of each sub-cultured bacterial sample were inoculated into their respective petri plates by using sterilised autoclaved, L shaped Glass rod to spread the bacteria uniformly. Two sterile disks each loaded with 20 µl of extracted VOCs from young and old *Chinocossus acronyctoides* Larvae, a positive control disk containing 10 µg dose of ampicillin and a negative control disk containing 20 µl hexane were placed in the petri plates to check and compare the susceptibility of bacteria on the two different extracts.

Results

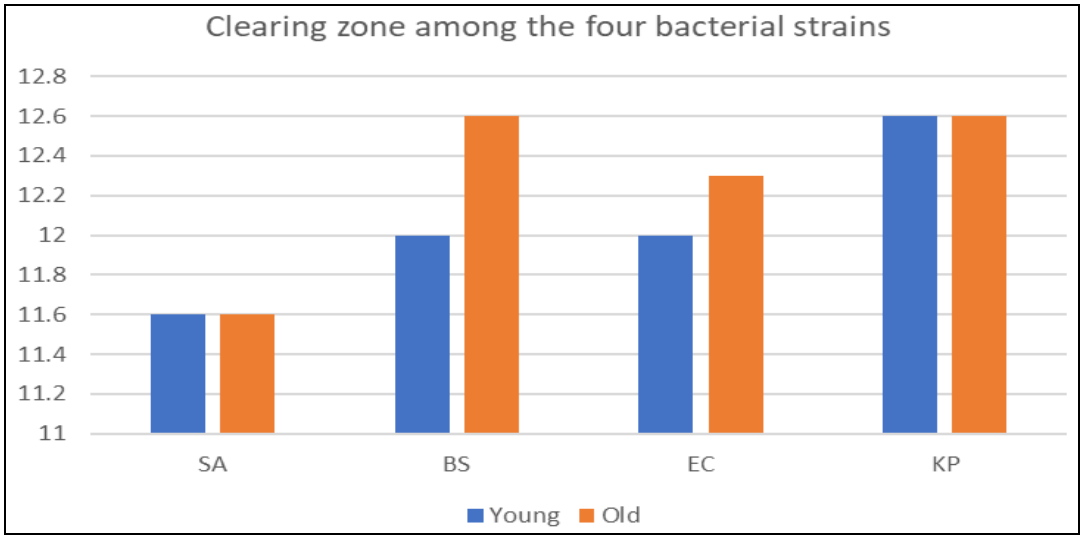
Antimicrobial Activity of VOCs from *Chinocossus acronyctoides* Larvae

Table 2 shows the clearing zone activity of the various bacterial species, while Graph 2 shows the graphical representation of antimicrobial activity of *Chinocossus acronyctoides* larvae. The hexane crude extract of the larvae at two different stages showed various degree of inhibition against the four bacterial strains. Antibacterial potency of the larvae sample was quantitatively confirmed by the absence or presence of an inhibition zone in all over the disc loaded. *In vitro* test for the antibacterial activity of the larvae extracts was found to be highly active in both the gram-negative and gram-positive bacteria. The experimental test revealed that hexane extract from *Chinocossus acronyctoides* larvae inhibited most of the growth of all the bacterial strains. Among the four bacterial strains tested, extracts from larvae at both stages were most effective against *Klebsiella pneumoniae*, producing an inhibition zone of 12.6 ± 0.3 mm in both the samples. The extract from older larvae was also particularly effective against *Bacillus subtilis*, with the same inhibition zone of 12.6 ± 0.3 mm. For Staphylococcus aureus, both the young and old larvae extracts demonstrated good antibacterial activity, each with an inhibition zone of 11.6 ± 0.3 mm. Similarly, Escherichia coli was inhibited by both extracts, showing inhibition zones of 12 ± 1 mm for the old extract and 12.3 ± 0.3 mm for the young extract.

Table 2: Clearing zone of C*hinocossus acronyctoides* larval VOCs extract (Young and Old) against the four bacterial samples

Extract samples	Tested organism	Zone of inhibition (mm)		
Young	*Staphylococcus aureus*	Extract	+ve	-ve
		11.6 ± 0.3	10	0
Old	*Staphylococcus aureus*	11.6 ± 0.3	10	0
Young	*Bacillus subtilis*	12 ± 1	10	0
Old	*Bacillus subtilis*	12.6 ± 0.3	10	0
Young	*Escherichia coli*	12 ± 1	10	0
Old	*Escherichia coli*	12.3 ± 0.3	10	0
Young	*Klebsiella pneumonia*	12.6 ± 0.3	10	0
Old	*Klebsiella pneumonia*	12.6 ± 0.3	10	0

The antimicrobial assessment was carried out in triplicate, here n=3. Basing on this experimental observation, antibacterial activity was most shown in *Klebsiella pneumonia* and the least was shown by *Staphylococcus aureus*.



Graph 1: Graphical representation of antimicrobial activity of VOCs of *Chinocossus acronyctoides* larvae. SA=*Staphylococcus aureus*, BS=*Bacillus subtilis*, EC=*Escherichia coli*, KP=*Klebsiella pneumoniae*

Discussion

According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs [40]. In Kohima-Nagaland, India, the moth larvae of *Chinocossus acronyctoides* are not traditionally used for treating wounds or microbial inhibition but are consumed as a food source. These larvae are highly valued, selling for around ₹10, 000 per kilo thus providing significant socio-economic benefits to local sellers. The indigenous population also attributes therapeutic benefits to the larvae.

In our preliminary study assessing the antibacterial properties of these larvae, we tested the crude extracts against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli,* and *Klebsiella pneumoniae*. The results showed promising antimicrobial activity across both gram-positive and gram-negative strains. *Klebsiella pneumoniae* exhibited highest sensitivity to the extracts compared to the other three bacterial strains. Interestingly, older larvae sample showed strong activity against *Bacillus subtilis* and *Klebsiella pneumoniae*. Younger sample also had the best activity on *Klebsiella pneumoniae*. In contrast, *Staphylococcus aureus* showed less sensitivity to both the larvae samples, this likely may be due to its higher resistance rate to antibiotics [41, 42]. It was also observed that the younger larvae emitted a more pungent and aromatic smell compared to older larvae, which might be related to the progression of the larvae development as it matures. This differences in the chemical composition of the larvae also prompted the comparative antibacterial study. The extraction of a mere 3 grams of organic matter from 90 grams of larvae highlights the difficulties insects face in synthesizing essential compounds for cellular integrity, metabolism, and reproduction. Unlike plants, insects lack efficient biosynthetic pathways, relying instead on dietary sources [43]. This reliance may have them susceptible to fluctuations in food availability and environmental conditions. These findings suggest a need for further research to explore the full potential and mechanisms of the antibacterial properties of *Chinocossus acronyctoides* larvae. Additionally, understanding the metabolic constraints of synthesising essential compounds in insects can provide insights into their nutritional ecology and potential applications in medicine and biotechnology.

The investigation into the antimicrobial activity of VOCs extracted from *Chinocossus acronyctoides* larvae presents compelling evidence of their potential as effective natural antibiotics. Building on historical recognition of antimicrobial properties from insect derived VOCs, this study reaffirms their efficacy against a spectrum of pathogenic bacteria, including both gram-negative and gram-positive strains. The natural origin of these VOCs offers advantages such as biocompatibility, low toxicity, and environmental sustainability, aligning with the current imperative to develop alternatives to conventional antibiotics. Notably, the study reveals variability in antimicrobial activity between larvae at different developmental stages, underscores the importance of further research into the underlying factors influencing efficacy.

Moving forward, elucidating the specific bioactive organic volatile compounds within the crude extracts from *Chinocossus acronyctoides* larvae and understanding their mechanisms of action will be crucial for validating their safety and efficacy for medicinal applications. Moreover, exploring potential synergies with existing antibiotics and investigating scalable production methods are essential steps towards translating these findings into clinically viable antimicrobial therapies. In addressing the urgent global challenge of antibiotic resistance, this study contributes valuable insights into harnessing insect-derived volatile organic compounds as a rich source of novel antimicrobial agents.

References

1. Brown ED, Wright GD. Antibacterial drug discovery in the resistance era. Nature,2016:529:336–43. doi:10.1038/nature17042.
2. Sprenger M, Fukuda K. New mechanisms, new worries. Science (1979),2016:351:1263–4. doi:10.1126/science.aad9450.
3. Newman DJ, Cragg GM. Natural Products as Sources of New Drugs from 1981 to 2014. J Nat Prod,2016:79:629–61. doi:10.1021/acs.jnatprod.5b01055.
4. Thormar H. Antibacterial Effects of Lipids: Historical Review (1881 to 1960). Lipids and Essential Oils as Antimicrobial Agents. Wiley: 2011. pp,25–45. doi:10.1002/9780470976623.ch2.
5. Desbois AP, Smith VJ. Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. Appl Microbiol Biotechnol,2010:85:1629–42. doi:10.1007/s00253-009-2355-3.
6. Karlson P, Lüscher M. 'Pheromones': A New Term for a Class of Biologically Active Substances. Nature,1959:183:55–6. doi:10.1038/183055a0.
7. Butenandt VA, Beckmann R, Stamm D, Hecker E. Über den Sexual-Lockstoff des Seidenspinners Bombyx mori. Reindarstellung und Konstitution. Z Naturforschung B,1959:14:283–4.
8. Siddiqui SA, Li C, Aidoo OF, Fernando I, Haddad MA, Pereira JAM, *et al*. Unravelling the potential of insects for medicinal purposes-A comprehensive review. Heliyon, 2023, 9. doi:10.1016/j.heliyon.2023.e15938.
9. Manniello MD, Moretta A, Salvia R, Scieuzo C, Lucchetti D, Vogel H, *et al*. Insect antimicrobial peptides: potential weapons to counteract the antibiotic resistance. Cell Mol Life Sci,2021:78:4259–82. doi:10.1007/s00018-021-03784-z.
10. Zweerus NL, Caton LJ, de Jeu L, Groot AT. More to legs than meets the eye: Presence and function of pheromone compounds on heliothine moth legs. J Evol Biol,2023:36:780–94. doi:10.1111/jeb.14173.
11. Zhang X, Miao Q, Xu X, Ji B, Qu L, Wei Y. Developments in Fatty Acid-Derived Insect Pheromone Production Using Engineered Yeasts. Front Microbiol,2021:12. doi:10.3389/fmicb.2021.759975.
12. Besis A, Georgiadou E, Samara C. Odor-active volatile organic compounds along the seafront of Thessaloniki, Greece. Implications for sources of nuisance odor. Sci Total Environ,2021:799:149388. doi:10.1016/j.scitotenv.2021.149388.
13. Bouwmeester H, Schuurink RC, Bleeker PM, Schiestl F. The role of volatiles in plant communication. Plant J,2019:100:892–907. doi:10.1111/tpj.14496.
14. Abbas F, O'Neill Rothenberg D, Zhou Y, Ke Y, Wang H. Volatile organic compounds as mediators of plant communication and adaptation to climate change. Physiol Plant,2022:174. doi:10.1111/ppl.13840.

15. Zhou S, Jander G. Molecular ecology of plant volatiles in interactions with insect herbivores. J Exp Bot,2022:73:449–62. doi:10.1093/jxb/erab413.
16. Böttinger LC, Hüftlein F, Stökl J. Mate attraction, chemical defense, and competition avoidance in the parasitoid wasp Leptopilina pacifica. Chemoecology,2021:31:101–14. doi:10.1007/s00049-020-00331-3.
17. Bruce TJA, Wadhams LJ, Woodcock CM. Insect host location: a volatile situation. Trends Plant Sci,2005:10:269–74. doi:10.1016/j.tplants.2005.04.003.
18. Burger BV, Viviers MZ, Bekker JPI, le Roux M, Fish N, Fourie WB, *et al*. Chemical Characterization of Territorial Marking Fluid of Male Bengal Tiger, Panthera tigris. J Chem Ecol,2008:34:659–71. doi:10.1007/s10886-008-9462-y.
19. Hilmarsson H, Traustason BS, Kristmundsdóttir T, Thormar H. Virucidal activities of medium- and long-chain fatty alcohols and lipids against respiratory syncytial virus and parainfluenza virus type 2: comparison at different pH levels. Arch Virol,2007:152:2225–35. doi:10.1007/s00705-007-1063-5.
20. Welch JL, Xiang J, Okeoma CM, Schlievert PM, Stapleton JT. Glycerol Monolaurate, an Analogue to a Factor Secreted by Lactobacillus, Is Virucidal against Enveloped Viruses, Including HIV-1. mBio,2020:11. doi:10.1128/mBio.00686-20.
21. Herdiyati Y, Astrid Y, Shadrina AAN, Wiani I, Satari MH, Kurnia D. Potential Fatty Acid as Antibacterial Agent Against Oral Bacteria of Streptococcus mutans and Streptococcus sanguinis from Basil (Ocimum americanum): In vitro and In silico Studies. Curr Drug Discov Technol,2021:18:532–41. doi:10.2174/1570163817666200712171652.
22. Zhou Z, Huang J, Hao H, Wei H, Zhou Y, Peng J. Applications of new functions for inducing host defense peptides and synergy sterilization of medium chain fatty acids in substituting in-feed antibiotics. J Funct Foods,2019:52:348–59. doi:10.1016/j.jff.2018.11.028.
23. Yoon B, Jackman J, Valle-González E, Cho N-J. Antibacterial Free Fatty Acids and Monoglycerides: Biological Activities, Experimental Testing, and Therapeutic Applications. Int J Mol Sci,2018:19:1114. doi:10.3390/ijms19041114.
24. Bergsson G, Arnfinnsson J, Steingrimsson O, Thormar H. In Vitro Killing of Candida albicans by Fatty Acids and Monoglycerides. Antimicrob Agents Chemother,2001:45:3209–12. doi:10.1128/AAC.45.11.3209-3212.2001.
25. Marusich E, Mohamed H, Afanasev Y, Leonov S. Fatty Acids from Hermetia illucens Larvae Fat Inhibit the Proliferation and Growth of Actual Phytopathogens. Microorganisms,2020:8:1423. doi:10.3390/microorganisms8091423.
26. Mudalungu CM, Mokaya HO, Tanga CM. Beneficial sterols in selected edible insects and their associated antibacterial activities. Sci Rep,2023:13:10786. doi:10.1038/s41598-023-37905-4.
27. Rizvi SAH, George J, Reddy GVP, Zeng X, Guerrero A. Latest Developments in Insect Sex Pheromone Research and Its Application in Agricultural Pest Management. Insects,2021:12:484. doi:10.3390/insects12060484.
28. Schoorl JW. A phylogenetic study on Cossidae (Lepidoptera: Ditrysia) based on external adult morphology. Zoologische Verhandelingen,1990:263:1–295.
29. Garanti L, Marchesini A, Um P, Trave R. Synthesis of (5z)-5, 13-tetradecadien-1-yl acetate and (3e, 5z)-3, 5, 13-tetradecatrien-1-yl acetate (the secretion of the larvae of the lepidopterus cossus cossus) and of their geometrical isomers,1976.
30. Bergmann J, Lopez K, Buono-Core G. Identification and synthesis of some fatty acid derivatives from larvae of Chilecomadia valdiviana (Lepidoptera: Cossidae). Nat Prod Res,2007:21:473–80. doi:10.1080/14786410601129986.
31. Sheppard S. Methods in Chemical Ecology. Volume 1-Chemical Methods. J Environ Qual,1999:28:2032–3. doi:10.2134/JEQ1999.00472425002800060048X.
32. Turnbull PCB. Bacillus. 4th ed. Samuel Baron, editor. Galveston: Medical Microbiology: 1996.
33. Mueller M, Tainter CR. Escherichia coli Infection,2024.
34. Tuttle AR, Trahan ND, Son MS. Growth and Maintenance of Escherichia coli Laboratory Strains. Curr Protoc,2021:1. doi:10.1002/cpz1.20.
35. Pépin J, Plamondon M, Lacroix C, Alarie I. Emergence of and Risk Factors for Ciprofloxacin-Gentamicin-Resistant Escherichia coli Urinary Tract Infections in a Region of Quebec. Can J Infect Dis Med Microbiol,2009:20–8. doi:10.1155/2009/971624.
36. Lowy FD. Staphylococcus aureus Infections. N Engl J Med,1998:339:520–32. doi:10.1056/NEJM199808203390806.
37. Ali Alghamdi B, Al-Johani I, Al-Shamrani JM, Musamed Alshamrani H, Al-Otaibi BG, Almazmomi K, *et al*. Antimicrobial resistance in methicillin-resistant staphylococcus aureus. Saudi J Biol Sci,2023:30:103604. doi:10.1016/j.sjbs.2023.103604.
38. Paczosa MK, Mecsas J. Klebsiella pneumoniae: Going on the Offense with a Strong Defense. Microbiol Mol Biol Rev,2016:80:629–61. doi:10.1128/MMBR.00078-15.
39. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol,1966:45:493–6.
40. Arullappan S, Zakaria Z, Basri DF. Preliminary Screening of Antibacterial Activity Using Crude Extracts of Hibiscus rosa sinensis. Trop Life Sci Res,2009:20:109–18.
41. Foster TJ. Antibiotic resistance in Staphylococcus aureus. Current status and future prospects. FEMS Microbiol Rev,2017:41:430–49. doi:10.1093/femsre/fux007.
42. Vestergaard M, Frees D, Ingmer H. Antibiotic Resistance and the MRSA Problem. Microbiol Spectr,2019:7. doi:10.1128/microbiolspec.GPP3-0057-2018.
43. Franco A, Scieuzo C, Salvia R, Pucciarelli V, Borrelli L, Addeo NF, *et al*. Antimicrobial activity of lipids extracted from Hermetia illucens reared on different substrates. Appl Microbiol Biotechnol,2024:108:167. doi:10.1007/s00253-024-13005-9.