Austral Journal of Veterinary Science

Austral J Vet Sci 56, 25-33 (2024)
https://doi.org/10.4206/ajvs.561.04

ORIGINAL ARTICLE

Carvacrol-loaded invasomes biocidal effect against multidrug resistant isolates of Enterobacteriaceae and housefly

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Article History
Received: 08.05.2023
Accepted: 20.10.2023
Published: 17.01.2024

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ABSTRACT. The current study aimed to evaluate the antimicrobial activity of carvacrol-loaded invasomes (CLI) against multidrug-resistant (MDR) Enterobacteriaceae and its mechanical vector, the housefly. CLI were prepared and characterized in the laboratory. *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* enterica subsp. *enterica* serovar Enteritidis, Salmonella *enterica* subsp. *enterica* serovar Typhimurium, and *Klebsiella oxytoca* were among the MDR enterobacteriaceae stains investigated. These strains were first isolated and identified from naturally infected chickens. The antibacterial activity of CLI against the MDR isolates was evaluated using the diffusion method. In addition, the insecticidal activity of CLI against housefly larvae and pupae was tested. The MDR index of all evaluated isolates was greater than 20%, indicating that they were all multidrug-resistant. CLI decreased the growth of all isolates except *S. Typhimurium* and *P. aeruginosa* at a dose of 0.0125%; however, pure carvacrol inhibited the growth of only *Klebsiella* oxytoca. Furthermore, both CLI and pure carvacrol inhibited *Klebsiella oxytoca* growth at different concentrations. CLI inhibited *E. coli* and *S. enteritidis* at lower concentrations than pure carvacrol, even at a doubled concentration. Carvacrol and CLI caused significant larval mortality even at low concentrations, with LC50 reached at concentartions of 2.54 and 2.19 µl/ml, respectively. Furthermore, at a low concentration of 3.125 µl/ml, both elicited a high percentage inhibition rate (PIR) in pupae. In conclusion, CLI demonstrated substantial antibacterial action, particularly against MDR isolates, as well as pesticide activity against houseflies.

Keywords: carvacrol, invasomes, drug resistance, antimicrobial, *Musca domestica*.

INTRODUCTION

Antimicrobial drugs are frequently given to chickens to promote growth and prevent diseases, but prolonged usage or improper use commonly results in bacterial resistance (Fielding *et al*., 2012), a problem that is becoming increasingly prevalent in humans and animals worldwide (Spellberg, 2014). The WHO proposed global programs for both human and animal surveillance in light of this increasing threat. Antimicrobial resistance in chickens is a common problem in developing countries because of the indiscriminate use of antibiotics as feed additives and the prophylactic treatment of infectious diseases. Because there are few or no alternative effective antimicrobial drugs available for the treatment of diseases caused by these bacteria, the evolution of bacterial resistance to antimicrobial treatments has become a serious public health concern (Al Azad *et al*., 2019). Consequently, the chicken industry has recently piqued interest in investigating phytobiotics as an alternative to synthetic antibiotics (Altay *et al*., 2022).

Escherichia coli is a bacterium that has a special place in the microbiological community because it not only causes serious infections in humans and animals but also contributes significantly to the autochthonous microbiota of different species. The potential transmission of virulent and/or resistant *E. coli* from animals to humans via several pathways, including direct contact, contact with animal excretions, and the food chain, is a major source of concern. Additionally, *E. coli* is a significant reservoir of resistance genes, which could be the reason why some treatments in both human and veterinary medicine are ineffective (Agatha *et al*., 2023; Bassi *et al*., 2023). Multidrug resistance in *E. coli* has recently become a global concern (Poirel *et al*., 2018).

Musca domestica, the housefly, is a vector for over 100 human and animal diseases, mainly food-borne pathogens (Kumar *et al*., 2013, 2014). Chemical insecticides are often

used to control houseflies. Long-term use of these chemical insecticides has serious consequences for human and animal health as well as detrimental effects on the environment (Kumar *et al.*, 2012). Given the previously noted increased rate of antibiotic resistance as well as the side effects and disadvantages of insecticides, novel approaches to overcome these obstacles are urgently required. As a result, there is an urgent need to investigate better alternatives to antibiotics to ensure the production of safe and profitable poultry.

Natural bioactive compounds derived from plants could be potential alternative candidates (Pavela, 2013; Abdel-Baki *et al.*, 2021). One of these compounds is carvacrol, a phenolic monoterpenoid found in essential oils of different plant species. Carvacrol has been demonstrated to have several biological effects, including antibacterial and antifungal properties (Chavan & Tupe, 2014), antiviral activity (Sánchez *et al.*, 2015), antioxidant properties, immune response regulation (Khazdair *et al.*, 2018), and anti-inflammatory properties (Fitsiou *et al.*, 2016).

According to Di Pasqua *et al.* (2010), carvacrol interacts with the cell membrane via hydrogen bonding, making the membranes and mitochondria more permeable and disintegrating the outer cell membrane. Several *in vitro* studies have revealed that carvacrol and thymol have potent antibacterial activity against pathogenic bacteria, including *E. coli* and *S.* typhimurium (Gholami-Ahangaran *et al.*, 2020). Carvacrol is more effective against gram-positive bacteria than gram-negative bacteria; it damages bacterial membranes, reduces ATP generation, and consequently energy-dependent cell functions (Nostro & Papalia, 2012). However, the application of carvacrol is limited by its high volatility, low water solubility, and low stability (Donsì *et al.*, 2014; Locci *et al.*, 2004).

Carvacrol nanoformulations, such as nanocarriers, are a strategy to overcome these constraints. Nanocarriers have been utilized to increase the chemical and physical stability of essential oils, reduce organoleptic alterations, and promote biological activity (Moraes-Lovison *et al.*, 2017; Ryu *et al.*, 2018; Noori *et al.*, 2018; Chuesiang *et al.*, 2019). Invasomes are one of these nanocarriers that have been used frequently (Kamran *et al.*, 2016). Invasomes are composed of unsaturated phospholipids, water, and trace amounts of ethanol and terpenes, which increase permeability and bioavailability (Aslam *et al.*, 2015; Dwivedi *et al.*, 2016; Kumar *et al.*, 2022).

In the current study, a carvacrol-loaded invasome (CLI) was similarly prepared, with terpenes responsible for the biocidal effect enhancing CLI solubility and penetration of the larval cuticle by disrupting lipid/protein layers and/or removing skin micro-ingredients required for skin barrier maintenance (Sapra *et al.*, 2008; Kumar *et al.*, 2022).

In the present study, the prepared carvacrol-loaded invasome (CLI) was tested against multidrug resistant isolates of *E. coli*, *S. enterica* Enteritidis, *S. enterica* Typhimurium, and *P. aeruginosa*, as well as the mechanical vector of these microorganisms' housefly.

MATERIALS AND METHODS

Preparation and characterization of Carvacrol-Loaded Invasome (CLI)

In our laboratory, a carvacrol-loaded invasome (CLI) was prepared and characterized as described by Gamal *et al.* (2023). In brief, a carvacrol-loaded invasome (CLI) formulation was produced using a thin hydration method. Carvacrol (10 mg), cineole (1% v/v), cholesterol (0.15 %w/w), and phospholipid (3% w/w) were dissolved in 10 mL organic solution of chloroform and methanol (3:1). This solution was evaporated under vacuum using a Stuart rotary evaporator (RE300, UK) at a speed of 100 rpm and temperature of 40 °C. During evaporation, a thin layer of invasomes formed inside the flask. At 40 °C for an hour, isotonic phosphate buffer (IPB, pH 5.5) solution and ethanol (3% v/v) solution were added to hydrate the lipid film. The carvacrol-loaded invasome (CLI) formulation was developed, sonicated, and kept at 4 °C.

Bacterial isolates used in the study

Five types of gram-negative bacteria, including *E. coli*, *P. aeruginosa*, *S.* Enteritidis, *S.* Typhimurium, and *Klebsiella oxytoca*, were found in naturally infected chicken. These chicken isolates affected 10,000 birds aged 1-21 days and were collected from ten broiler flocks between 2020 and 2021. The samples were examined for the presence of pathogenic bacteria that cause conjunctivitis, arthritis, enteritis, and diarrhea. The isolates were identified and serotyped according to Quinn *et al.* (2011). Stock cultures of these bacteria were grown on Mueller-Hinton agar at 37 °C for 24 h for subsequent bioassays. Antimicrobial susceptibility testing and determination of multi-drug resistance index (MDRI).

All bacterial isolates were tested for sensitivity to 12 different antimicrobial agents using the Kirby-Bauer disc diffusion method on Mueller-Hinton Agar (Oxoid Ltd., Basingstoke, UK), according to the CLSI guidelines (2018). Resistance to three or more antibiotics from different groups represents multidrug resistance (MDR). Individual isolates' MDR index (MDRI) was calculated by dividing the number of antimicrobials to which the isolate was resistant by the total number of antibiotics to which the isolate had been exposed (Chandran *et al.*, 2008). Isolates with MDRI values greater than 0.2 or 20% were considered highly resistant.

$$\text{MDR index} = \frac{\text{Number of antibiotics resisted}}{\text{Total number of antibiotics used}} \times 100$$

Determination of the effect of CV and CLI on bacterial pathogen growth using the agar diffusion method. Different concentrations of pure carvacrol and CLI (10, 5, 2.5, 1.25, 0.625, 0.313, 0.156, and 0.078 µl/ml) were prepared by diluting with 1% DMSO. The antibacterial activity of the prepared concentrations was tested against MDR isolates as described by Jeff-Agboola *et al.* (2012). Briefly, bacteria were cultivated on tryptone soya agar at 37 °C for 24 h before being suspended in physiological saline (0.9% NaCl) and adjusted to 0.5×108 CFU. Muller-Hinton agar was prepared and autoclaved at 121 °C for 15 min. before being maintained at 55 °C. The tested oils were then combined with TSA according to the tested concentrations. The oil-agar medium (10 ml) was then solidified in sterilized Petri dishes. Equal volumes of bacterial suspensions were inoculated and spread on agar plates. The plates were then incubated at 37 °C for 24-48h. The cells were examined for bacterial colony growth inhibition.

Housefly

Adult houseflies captured in the field using a sweep net were reared in insect-rearing cages on a diet of milk powder and wheat bran, as described by Kumar *et al.* (2011). Hatched larvae were transferred to a 25 × 18.5 cm^2 plastic basin with a larval feed (wheat bran) that was changed daily until the larvae reached the pupal stage.

Larval bioassays. For the larval bioassay, different concentrations (5, 2.5, 1.25, 0.625, 0.312, 0.156, and 0.078%) of carvacrol and CLI were prepared by dilution with acetone. The prepared concentrations were poured on a filter paper disc (in a 9.0 cm Petri plate) together with larval feed (Kumar *et al*., 2011). Acetone-treated filter paper was used as the control. Acetone was evaporated from the treated filter paper by air-drying at ambient temperature for 5 min. Twenty larvae (second instars, with larval diet) were then transferred to treated air-dried filter paper. Petri dishes containing these filter papers were incubated at 28 ± 2 °C and 75 ± 5% relative humidity (RH). The mortality of the treated larvae was recorded daily for four days. This bioassay was performed in triplicates for each concentration.

Pupal bioassays. Different concentrations of carvacrol and CLI (5, 2.5, 1.25, 0.625, 0.312, 0.156, and 0.078%) were prepared for pupal assays by dilution with acetone. The produced concentrations were poured onto a filter paper disc (in a 9.0 cm Petri dish), and the acetone was removed by air drying for 5 min. Twenty pupae (2-3 days old) were placed on the filter papers and monitored for adult emergence for six days. Acetone-treated filter paper was used as a control. This bioassay was performed in triplicates for each concentration. The adult inhibition rate was calculated according to the method described by Kumar *et al.* (2011). Percentage inhibition rate (PIR) was calculated as: $PIR = \frac{Cn-Tn}{Cn} \times 100$ where Cn is the number of newly emerged houseflies in the control and Tn is the number of newly emerged houseflies in the treatment.

Statistics

The results of the different treatments were statistically analyzed using IBM SPSS for Windows version 22 (IBM, Armonk, NY, USA). Analysis of variance (ANOVA) was used to investigate the differences between treatments, and Duncan's test was used to estimate the mean differences ($\alpha = 0.05$). The lethal concentrations as well as the 50% and 90% mortality rates were calculated using SPSS v.22.

RESULTS

Antimicrobial Susceptibility Profiles

All the tested isolates were multidrug resistant, as their MDR indices were more than 20%. *S*. Enteritidis, *S*. Typhimurium exhibited resistance for 9/12 antibiotics (Table 1). However, *E. coli* was sensitive to imipenem and highly resistant to other antibiotics (Table 1). *K. oxytoka* was highly sensitive to chloramphenicol, nalidixic acid, and ampicillin. Also *P. aeruginosa* showed high resistance against all used antimicrobials except impenem (Table 1).

Table 1.
Antimicrobial susceptibility profiles of the study isolates.

	S. Entritidis	*S*. Typhimurium	*E. coli*	*K. oxytoca*	*P. aeruginosa*
	Diameter of inhibition haloes for each bacterial strain against antimicrobial agent				
ATM	R 14mm	R 0mm	R 4mm	R 12mm	R 0mm
IPM	I 20mm	R 0mm	S 28mm	I 20mm	S 22mm
CTX	R 0mm	R 0mm	R 0mm	R 0mm	R 0
AM	R 0mm	S 18mm	R 0mm	S 22mm	I 12
OT	R 0mm	R 0mm	R 0mm	R 6mm	R 6

Table 1 continuation

	S. Entritidis	*S.* Typhimurium	*E. coli*	*K. oxytoca*	*P. aeruginosa*
	Diameter of inhibition haloes for each bacterial strain against antimicrobial agent				
DO	R 2mm	R 6mm	R 2mm	R 0mm	R 4
C	S 24mm	R 0mm	R 0mm	S 42mm	R 9
S	R 2mm	R 0mm	R 4mm	R 2mm	R 6
K	R 8mm	R 10mm	R 10mm	R 8mm	I 14
NA	I 12mm	R 0mm	R 10mm	S 26mm	R 8mm
OF	R 6mm	R 0mm	R 6mm	R 2mm	R 10mm
CIP	R 10mm	R 0mm	R 10mm	R 6mm	R 8mm

ATM (Aztreonam), IPM (Impenem), CTX (Cefotaxime), AM (Ampicillin), OT (Oxytetracycline), DO (Doxycycline), C (Chloramphenicol), S (Streptomycin), K (Kanamycin), NA (Nalidixic acid), OF (Ofloxacin) CIP (Ciprofloxacin). All the bacterial isolates were evaluated for antimicrobial sensitivity to 12 different antimicrobial agents using the Kirby-Bauer disc diffusion method on Mueller-Hinton Agar (Oxoid Ltd., Basingstoke, UK) according to the guidelines of CLSI (2017).

Activity of CLI against multidrug resistance bacterial certain isolates

The antimicrobial activity of carvacrol and CLI against *E. coli*, *S.* Enteritidis, *S.* Typhimurium, *P. aeruginosa*, and *K. oxytoka* was evaluated by determining the inhibitory activity using the agar dilution method. The high concentrations (10, 5, 2.5, 1.25, and 0.625 µl/ml) both of carvacrol and CLI inhibited the growth of all tested organisms. At a concentration of 0.156 µl/ml, CLI inhibited the growth of all isolates, except *S. Typhimurium* and P. aerogenosa. However, carvacrol inhibited only the growth of *K. oxytoka*, whereas the other isolates grew. Meanwhile, at a low concentration (0.078 µl/ml), all isolates grew (Table 2). *K. oxytoka* was similarly inhibited by CLI and carvacrol at various concentrations. In addition, even at low concentrations, CLI inhibited *E. coli* and S. Enteritidis when compared with free carvacrol at doubled concentrations (Table 2).

Larvicidal effects of CLI against housefly larvae

CLI exhibited significant toxicity to house fly larvae, with 100% mortality attained at a concentration of 3.82 µl/ml while pure carvacrol achieved the same result at a concentration of 4.28 µl/ml. Also, the LC_{50} for pure carvacrol was 2.54 µl/ml and 2.19 µl/ml for CLI. (Table 3).

Pupicidal inhibition activity of CLI against housefly pupae

CLI and the pure carvacrol caused 100% inhibition rate at the concentration of 50 µl/ml against pupae with LC_{50} attained at concentrations of 12.10 µl/ml and 13.20 µl/ml, respectively (Table 4).

DISCUSSION

Bacterial infections commonly cause morbidity and mortality in humans and animals globally (WHO, 2010), and can even cause food deterioration (Srinivasa & Tharanathan, 2007). The main strategy for managing these bacterial infections is the use of antibiotics (Zaffiri *et al.*, 2012). However, improper use of these chemicals has contributed to the development and spread of multidrug resistant pathogens (English & Gaur, 2010). As a result, antibiotic-resistant organisms pose a significant threat to animal and public health. Therefore, it is critical to conduct research and develop novel chemicals with antibacterial properties that do not harm animal or human cells (Ling *et al.*, 2015). Essential oils and their constituent small molecules offer a good alternative therapeutic option for microbial diseases (Basri *et al.*, 2014; Raut & Karuppayil, 2014).

Musca domestica is a mechanical vector of more than 100 pathogens (bacteria, fungi, viruses, and parasites), some of which cause severe diseases in humans and domestic animals. Chemical pesticides, particularly pyrethroids, are frequently used to control houseflies (Shah *et al.*, 2015). Unfortunately, resistant pyrethroid houseflies have emerged. Furthermore, chemical insecticides are hazardous to the environment, domestic animals, and humans (Scott *et al.*, 2013). Therefore, it is necessary to develop new materials that use biodegradable and target-specific insecticides to control flies in a manner that is safe for animals, humans, and the environment.

Several studies have shown that essential oils are effective in controlling houseflies (Chauhan *et al.*, 2016; Benelli *et al.*, 2018; Pavela *et al.*, 2018). The antibacterial and an-

Table 2.
Antibacterial activity of carvacrol and carvacrol-loaded invasomes (CLI).

Isolates Concentrations	*S.* Entritidis	*S.* Typhimurium	*E. coli*	*K. oxytoca*	*P. aeruginosa*
			Inhibition of bacterial growth		
Carvacrol 10 µl/ml	+	+	+	+	+
CLI 10 µl/ml	+	+	+	+	+
Carvacrol 5 µl/ml	+	+	+	+	+
CLI 5 µl/ml	+	+	+	+	+
Carvacrol 2.5 µl/ml	+	+	+	+	+
CLI 2.5 µl/ml	+	+	+	+	+
Carvacrol 1.25 µl/ml	+	+	+	+	+
CLI 1.25 µl/ml	+	+	+	+	+
Carvacrol 0.625 µl/ml	+	+	+	+	+
CLI 0.625 µl/ml	+	+	+	+	+
Carvacrol 0.313 µl/ml	-	+	-	+	-
CLI 0.313 µl/ml	+	+	+	+	+
Carvacrol 0.156 µl/ml	-	-	_	+	-
CLI 0.156 µl/ml	+	-	+	+	-
Carvacrol 0.078 µl/ml	-	-	-	-	-
CLI 0.078 µl/ml	-	-	-	-	-

+ means inhibition of bacterial growth
- means growth of bacteria

Table 3.
Larvicidal activity of pure carvacrol and Carvacrol-loaded invasomes against house fly larvae.

Concentrations µl/ml	Carvacrol Larval Mortality percentage	Carvacrol-loaded invasomes Larval Mortality percentage
50 µl/ml	100 ± 0.00*	100 ± 0.00*
25 µl/ml	100 ± 0.00*	100 ± 0.00*
12.50 µl/ml	100 ± 0.00*	100 ± 0.00*
6.25 µl/ml	100 ± 0.00*	100 ± 0.00*
3.125 µl/ml	63.0 ± 5.70*	74.0 ± 4.18*
1.56 µl/ml	26.0 ± 4.18*	33.0 ± 2.74*
0.78 µl/ml	14.0 ± 4.18	19.0 ± 4.18*
LC_{50}	2.54 µl/ml	2.19 µl/ml
LC_{90}	4.28 µl/ml	3.82 µl/ml
Acetone (Negative control)	2.00 ± 2.74	2.00 ± 2.74
Deltamethrin 2 ml/l	12.0 ± 2.74	12.0 ± 2.74

(*) Significant for negative control

Table 4.
Pupicidal activity of pure carvacrol and Carvacrol-loaded invasomes against house fly pupae.

Concentrations µl/ml	Percentage inhibition rate (PIR) of carvacrol	Percentage inhibition rate (PIR) of carvacrol-loaded invasomes
50 µl/ml	100 ± 0.00*	100 ± 0.00*
25 µl/ml	82.5 ± 4.92*	90.3 ± 4.30*
12.50 µl/ml	54.3 ± 6.34*	53.2 ± 5.25*
6.25 µl/ml	40.2 ± 5.69*	40.2 ± 4.12*
3.125 µl/ml	22.8 ± 2.80*	23.9 ± 2.94*
1.56 µl/ml	2.16 ± 2.96	3.33 ± 3.03
0.78 µl/ml	0.00 ± 0.00	0.00 ± 0.00
LC_{50}	12.2	12.1
LC_{90}	24.9	22.3
Acetone (Negative control)	0.00 ± 0.00	0.00 ± 0.00
Deltamethrin 2 ml/l	0.00 ± 0.00	0.00 ± 0.00

(*) Significant for negative control

tioxidant properties of monoterpenoids, such as carvacrol, make them an excellent alternative to commercially available chemical compounds for pest control and regular bactericides (Didry *et al.*, 1994; Undeger *et al.*, 2009). Carvacrol has been shown to be acutely toxic to a variety of invertebrate pests, including insects, flies, and mosquitoes (Cetin *et al.*, 2009; Dolan *et al.*, 2009; Lei *et al.*, 2010), anti-parasitic (Force *et al.*, 2000), and to cause little harm to mammals, fish, and other non-target organisms, while also biodegrading or disintegrating efficiently in the environment (Aboelhadid *et al.*, 2013; Sinthusiri & Soonwera, 2014).

In the current study, carvacrol-loaded invasomes (CLI) were prepared and compared to pure carvacrol against isolates of *E. coli*, *S. enterica* Enteritidis, *S. enterica* Typhimurium, and *P. aeruginosa* that were multidrug resistant, as well as against housefly resistant to insecticides.

The results showed that at a concentration of 0.625 µl/ml and more carvacrol and CLI, stopped the growth of all organisms examined. CLI inhibited the growth of only *E. coli* and *S. enterica* Enteritidis at a dose of 0.156 µl/ml or less, but pure carvacrol had no impact even at a two-fold concentration. Similarly, Bnyan *et al.* (2014) determined the antibacterial activity of carvacrol against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Enterobacter* spp., and *Serratia* spp., and the results revealed that all examined bacterial isolates showed significant growth inhibition at different concentrations, except *Pseudomonas aeruginosa*. Also, Burt *et al.* (2007) found that carvacrol is efficient at inhibiting the growth of *S. enterica* serotype Enteritidis on agar as well as eliminating these bacteria from raw chicken surface.

Kamimura *et al.* (2014) found that carvacrol inclusion complexes with hydroxypropyl-beta-cyclodextrin (HPB-CD) had stronger antibacterial activity against *Escherichia coli* K12 and *Salmonella enterica* serovar Typhimurium LT2 than pure carvacrol. In addition, Persico *et al.* (2009) discovered significant antibacterial activity of nanocomposite films based on low-density polyethylene containing carvacrol compared to pure carvacrol. Similarly, Liu *et al.* (2022) prepared a carvacrol nanoemulsion to overcome the instability and water insolubility of commercial carvacrol and then tested its antibacterial activity.

These findings suggest that CLI disrupted the structure and permeability of bacterial cells, allowing them to permeate the contents of the bacterial cells and, as a result, greatly outperform commercial carvacrol in terms of antibacterial activity. The antibacterial properties of carvacrol have been attributed to its ability to alter bacterial membrane permeability and trigger the leakage of potassium, phosphate, and protons (Lambert *et al.*, 2001).

Carvacrol and CLI showed significant larval *Musca* mortality even at low concentrations with LC_{50} reached at concentrations of 2.54 and 2.19 µl/ml, respectively, both also showed a significant PIR at low concentration (3.125 µl/ml). Similarly, Xie *et al.* (2019) assessed the insecticidal ac-

tivity of carvacrol against the housefly (*M. domestica*) and found considerable toxicity with an LC_{50} attained at concentration of 0.03 µl/l for contact toxicity and 2.78 µl/l for fumigation toxicity. Also, carvacrol showed a percentage inhibition rate (PIR) of 29.5% at concentration of 0.025 µl/l and 81.8% at concentration of 1.25 µl/l for the contact toxicity and fumigation assay, respectively (Xie *et al*., 2019).

The insecticidal activity of carvacrol can be explained by a number of mechanisms, including binding to the nicotinic acetylcholine receptor found in the housefly central nervous system (Itier & Bertrand, 2001; Jeschke & Nauen, 2008), targeting the gamma-aminobutyric acid receptor (Garcia *et al*., 2006), an octopamine receptor (Gross, 2010), and a tyramine receptor (Enan, 2005), and inhibition of acetylcholinesterase (Anderson & Coats, 2012). Tong *et al*. (2013) used [14C]-Nicotine binding assays with *M. domestica* nicotinic acetylcholine receptors (nAChRs) to explain the mode of action of carvacrol against *M. domestica* and they discovered that carvacrol binds to housefly nAChRs at a different binding site than nicotine and acetylcholine, which may support the idea that carvacrol's insecticidal effect involves nAChRs as a potential target.

CLI achieved better antibacterial and insecticidal activities than pure carvacrol, which can be attributed to the invasome formulation, which increased the penetration capacity of the loaded carvacrol. This was supported by the HPLC data, which showed that ticks treated with the CLI formulation had a significantly ($p < 0.001$) higher penetration than carvacrol by 3.86 folds (Gamal *et al*., 2023). The combination of ethanol and terpene in the invasomal bilayer breaks down hydrogen bonds between ceramides in the insect cuticle, increasing the space available for medicinal assimilation (Ahmed *et al*., 2019; Ahad *et al*., 2011).

We were limited by the use of invasomes without carvacrol as a control. The invasomes were prepared in a 10 ml organic solution of chloroform and methanol (3:1), in which carvacrol (10 mg), cineole (1% v/v), cholesterol (0.15% w/w), and phospholipid (3% w/w) were dissolved. Except for carvacrol, all ingredients were added at a range of 1%, which had little effect as an insecticide or antibacterial agent. Moo *et al.* (2021) found that 1,8-cineol possessed bactericidal effect against carbapenemase-producing *Klebsiella pneumoniae* (KPC-KP) at 28.83 mg/ml. Furthermore, cineole has been shown to be toxic to *Musca domestica* at concentrations of 4 µl/l (Rossi & Palacios, 2015). The effective concentration of CLI against larvae was 6.25 µl/ml and that against pupae was 50 µl/ml. This implies that the concentration of cineol used to prepare the CLI was higher than that reported by Rossi & Palacios (2015). This indicates that cineol had no discernible impact on the study. As a result, we did not use individual invasome components as controls.

In conclusion, the carvacrol-loaded invesomes demonstrated significant antibacterial activity, particularly against MDR isolates, as well as insecticide activity against their mechanically transmitted vector, the housefly.

DECLARATIONS

Competing interests statement

The authors declare that they have no competing interests.

Ethics statement

Not applied

Author contributions

Conceptualization: SMA, FIAE, AG; Data curation: SAQ, ASA, AOH; Formal analysis: SMI, NH A; Funding acquisition: SAQ, ASA; Investigation: MAY, SMI, AG; Methodology: MAY, FIAE; Supervision: SMA, ASA, KAMS; Validation: SAQ, SMA, AOH; Visualization: MAY, SMI, KAMS; Roles/Writing - original draft: MAY, SMI, FIAE; Writing - review and editing: ASA, SMA.

Funding

This work was supported by the Researcher Supporting Project [RSP-2023/3] of King Saud University.

Acknowledgements

The authors appreciate the help of the veterinarians in the collection of samples for this study.

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