

Bio-active principles in *Commiphora africana* resin dichloromethane extract and their insecticidal activity against bed bugs

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Research Article

Keywords: *Commiphora africana*, bed bugs, resin, toxicity, repellency

Posted Date: January 17th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-3228063/v2>

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Additional Declarations: The authors declare no competing interests.

Abstract

Bed bugs (*Cimex lectularius* Linnaeus) are ecto-parasite pests that wholly feed on human and domestic animals' blood and can cause anemia to the host on heavy feeding. Bed bug control has proved difficult due to various challenges including; development of insecticide resistance, high associated cost and environmental pollution. Natural herbal-based phytochemicals remain unexploited and we focused on *Commiphora africana* (A. Rich.) Engl. resin traditionally used in bed bug control in the coastal region of Kenya. We previously showed that dichloromethane extract of *C. africana* resin is highly repellent and toxic against bed bugs. In this study, we isolated compounds from the dichloromethane extract using column chromatographic techniques. The isolated compounds were evaluated for repellency and toxicity against bed bugs; and identified using Gas chromatography linked to mass spectrometer (GC-MS), Fourier Transform Infra-red (FTIR), ¹³C- and ¹H Nuclear Magnetic Resonance techniques. Five compounds: taraxasterol, pseudo-taraxasterol, beta-sitosterol, fungisterol and guggusterol were isolated and characterized for the first time in this plant. Fungisterol had the highest repellency (75%) against bed bugs which was not significantly different ($P > .05$) to the positive control (neocidol) (74%) after > one-hour exposure. Fungisterol also elicited highest toxicity against bed bugs with LC₅₀ of 25.73 mg/L after 24 h exposure. Blending fungisterol with other identified active terpenes did not synergize the overall repellent/toxic responses. This study identifies active compounds in *C. africana* resin and therefore lays a solid background in bed bug control using isolated compounds.

Introduction

bed bugs (*Cimex lectularius* Linnaeus) are ecto-parasite pests of significant public health importance that pose a great threat to humans and domesticated animals. They cause anemia upon heavy feeding (blood-sucking) on human or animal host [1]. They also leave physical marks on human skin impacting them negatively on their social life [2]. Several control measures of bed bugs including the use of insecticides have become unsuccessful due to development of resistance to commonly used insecticides that are additionally harmful to the environment [3]. Moreover, these control methods require a heavy capital investment [4]. One of the most promising techniques that have been of success in control of other insects include use of baits (attractants) which lure the insects to traps, use of repellents to keep away the insects from host-feeding, and a combination of attractants and repellents in a 'push-pull' tactic [5]. However, effective compounds/blends are necessary for the success of this technique. There are no reported bed bug attractants and repellents and we herein try to unravel promising candidates.

There is a growing interest in exploitation of natural products as a substitute tool in control and management of bed bugs and other related arthropods. This is due to the fact that, they are believed to be environmentally benign [6]. Several parts and products of selected plant species are used in control and management of various arthropods. For instance, *Commiphora africana* (A. Rich.) Engl. resin in its crude form is locally used in control of bed bugs, lice, ticks and fleas in various parts of Kenyan coastal region. In our previous study on evaluation of aqueous extract, polar organic solvent extracts, and non-polar organic solvent extracts of *C. africana* resin against bed bugs, we found that dichloromethane extract was the most potent repellent and toxic extract against bed bugs [7]. In this follow-up study, it is necessary to isolate pure compounds from the dichloromethane extract that are of biological importance against bed bugs. We hypothesized that; i) the dichloromethane extract of *C. africana* resin contain compounds that are isolable, purifiable and stable ii) the isolated compounds have repellency and toxicity responses against bed bugs and iii) blending of active isolated compounds will lead to a synergistic repellency and toxicity effects against bed bugs.

We extracted crude *C. africana* resin using dichloromethane solvent, isolated and purified compounds from the extract using Column Chromatography techniques, and evaluated their repellency and toxicity alongside with positive control (neocidol) and selected blends against bed bugs under laboratory conditions. This is the first study to be carried out to identify bioactive principles of *C. africana* resin. This study will lay a strong background in the development of an effective and safe bed bug control measures by formulating an eco-friendly insecticide as alternative to commonly used commercial insecticides.

Materials and methods

General Experimental Procedures

Solvents, silica gel 60G (70-230 mesh) and sephadex LH-20 were bought from Sigma-Aldrich (Tauschem, Germany) with percentage purity of > 98%. Instruments used in this study included FTIR, GC-MS and NMR.

Sample collection: *C. africana* resin was harvested from mature trees in Taita Taveta County after identification and confirmation by a botanist from the National Museums of Kenya. The resin of the tree was harvested, packed and transported to KEFRI laboratory as previously reported by Wairagu [7]. *C. africana* resin was collected by making incisions in the stem bark with a sharp knife. The oozed resin was tapped for three hours and transferred into a clean glass container, transported to Kenya Forestry Research Institute (KEFRI) research laboratory and

dried in an oven (Sanyo, model MOV-212f) at 50 °C for 5 days. The aerial parts of the authenticated *C. africana* tree were archived at East African Herbarium in the National Museums of Kenya for referencing (voucher No. NWWairagu 001).

Rearing of bed bugs

Adult bed bugs of mixed sexes were collected, reared and multiplied according to a protocol of Araujo [8] as briefly described by Wairagu [7]. In a glass cage (45 x 45 x 45 cm) partitioned into two sections, five mice were introduced into one, and 100 bed bugs in the other. Various strips of cotton cloths were put across the cage and taped in place to ensure that bed bugs could move freely and feed on the hosts adequately. The cage containing both bed bugs and mice was housed in a carton that was left open on the top side. Laboratory conditions were maintained at $28 \pm 2^\circ\text{C}$ and $75 \pm 2\%$ RH. Pieces of sponge and wood with sufficient cracks were used for bed bug harborage, and also provided sites for rearing of eggs and development into adults. Bellies of mice were shaved to allow bed bugs feed with ease. Adult bed bugs (two months old) of mixed sexes were used for bioassays carried out under laboratory conditions of $28 \pm 2^\circ\text{C}$ and $75 \pm 2\%$ RH.

Solvent extraction of *C. africana* resins

One kilogram (1 kg) of dried *C. africana* resin was extracted with 4 L dichloromethane for 72 h in a 10 L flask. The content was filtered and rinsed through a muslin cloth of pore size 0.7 mm, followed by a Whatman filter paper No 1. The solvent from the filtrate was removed in-vacuo using a rotary evaporator.

Column separation

A hundred grams (100 g) of powdered crude extract was subjected to separation with a column (100 cm long by 6 cm internal diameter) using Kieselgel silica gel 60G (70 -230 mesh). Silica gel (450 g) was first packed in the separation column as was previously described by Wairagu [7] before introducing the extract on top of silica gel level. The crude extract was first eluted with hexane and followed by a slow gradient of 0, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90 and 100% ethyl acetate in hexane. Fractions with similar Thin Layer Chromatography profiles were pooled together, concentrated in vacuo using a rotary evaporator at 45°C , and further separated and purified in a smaller Sephadex LH-20 column (2 cm diameter and 30 cm long) to afford pure compounds.

Identification of compounds

Compounds with a single spot under TLC were also run with GC to confirm their purity. Those with a single sharp peak were considered pure and further analyzed using FTIR (Bruker, model Alpha - laser class 1), GC-MS (Shimadzu QP 2010-SE, Japan) and NMR spectrometer (Bruker-600, Germany). For FTIR analysis, a mixture of sample and KBr (1:100) was finely ground in a mortar, pelleted and mounted onto the FTIR sample cell for analysis. Data was generated and recorded in absorbance mode. In GC-MS analysis, ultrapure helium was used as the carrier gas at 1 mL/min in a column (A BPX5: 30 m long; 0.25 mm internal diameter; and 0.25 μm film thickness). The GC program was set as: 50°C (1 min); $5^\circ\text{C}/\text{min}$ to 250°C (9 min) with a total run-time of 50 min. ^1H -NMR and ^{13}C -NMR analysis absorption peaks were recorded on an NMR spectrometer using deuterated chloroform (CDCl_3) as the solvent and Trimethylsilane (TMS) as an internal standard.

Bioassay tests

Repellency of bed bugs on treatment with isolated compounds

The isolated compounds from the most active dichloromethane extract, neocidol (positive control), selected blends (active pure compounds mixed in equal proportions as previously described by Wachira [5] and blank (solvent) were evaluated for repellency against bed bugs using the filter paper method of Araujo [8]. In each assay, ten bed bugs were introduced in a line cutting through the middle of a Whatman filter paper. One half was treated with 2 mL test compounds/blends dissolved in acetone (solvent) while the other with 2 mL acetone (control). These assays were carried out in triplicates with different bed bugs in each test. The distribution of bed bugs in the two halves of the filter paper was observed and recorded after five minutes. These assays were carried out at $25 \pm 2^\circ\text{C}$ and $75 \pm 2\%$ RH. Percentage repellency was calculated as $(\text{C}-\text{T})/(\text{C}+\text{T}) \times 100$ (where C and T represent the number of bed bugs in the untreated (control) and treated halves of the filter paper, respectively).

Mortality of bed bug on exposure to isolated compounds

bed bug mortality assays were carried out using the method of Ojjanwuna [9] where circular filter papers (Whatman No. 2) with a diameter of 90 mm were treated by spraying 2 mL of pure compounds at varying concentrations (.0, .25, .5, .75, 1, 1.25 and 1.4 % v/v in acetone solvent). The filter papers were air dried for 30 minutes at 25°C and thereafter placed into a clean petri dish. Ten bed bugs were introduced separately into the dishes with the compounds, blends, solvent and neocidol (positive control) and mortality rates were recorded after 24, 48 and 72 h exposure. Assays were conducted in triplicates at $25 \pm 2^\circ\text{C}$ and $75 \pm 2\%$ RH. The number of dead bed bugs was counted and mortality rates calculated as a ratio of D/T (where; D and T represent the number of dead bed bugs and the total number of bed bugs introduced, respectively), expressed as a percentage.

Data analysis

NMR spectra of isolated compounds were interpreted using MestreNova (spectral data analyzing software); GC-MS and FTIR used fragmentation patterns and absorption bands respectively from a library. Repellency and mortality rate data were rank-transformed and subjected to one-way Analysis of Variance (ANOVA). Means were separated using Student Newman-Keuls' post-hoc analysis test. bed bug mortality data was subjected to dose response analysis to determine LC_{50} of the compounds/blends at a 95 % confidence level. Mean values with $P < .05$ were considered statistically significant. SPSS version 20 (Chicago, IL, USA) software was used in analysis.

Results and discussion

Purification and isolation of compounds

Extraction of *C. africana* resin using dichloromethane solvent obtained 175g (17.5%) of crude extract. Column separation of the crude extract using silica gel (70 - 230 mesh) afforded four major fractions **FR1**, **FR2**, **FR3** and **FR4** which eluted with 10, 15, 20 and 30% ethyl acetate in hexane, respectively. These fractions were obtained by pooling eluants with similar retardation factors (RF) on the TLC profiles. Further individual chromatographic separations using sephadex (LH-20) of **FR1** and **FR2** gave two pure compounds each. Taraxasterol (**1**) and pseudo-taraxasterol (**2**) were obtained from **FR1** while β -sitosterol (**3**) and fungisterol (**4**) from **FR2**. Fraction **FR3** was oily and it was difficult to separate while **FR4** yielded guggulsterol (**5**) and an extra oil fraction. These compounds were isolated from *C. africana* resin for the first time and their identification was based on their physical and spectroscopic characteristics. The yields obtained were 50, 35, 45, 28 and 15 mg for taraxasterol, pseudo-taraxasterol, β -sitosterol, fungisterol and guggulsterol, respectively.

Taraxasterol isolated as white crystals with melting point of 233 - 236 °C and was soluble in organic solvents such as dichloromethane and ethyl acetate (Table 1). The IR spectrum (Figure 1A-supplementary) showed characteristic peaks of O-H stretching ($3449 - 3316\text{ cm}^{-1}$), C=C vibrations (1598 cm^{-1}), intense $-\text{CH}_3$ stretches and vibrations (2937 cm^{-1}), $-\text{CH}_2$ vibrations and rocking (890 cm^{-1}), C-C vibrations (995 cm^{-1}) and C-H vibrations (701 cm^{-1}). These absorption bands are characteristic of triterpenes derived from the *Betulaceae birch* Linn. bark species by Cîntă-Pînzaru [10]. $^1\text{H-NMR}$ (500 MHz, CDCl_3) spectrum (Figure 1B-supplementary) confirmed the presence of seven methyl groups, of which six were singlets (δ 1.01 (2x), 0.90, 0.88, 0.93 and 0.89) and one appeared as a doublet at δ 0.971 ($J = 6.93\text{ Hz}$). Moreover, the existence of an axial hydroxymethine (δ 3.732, dd , $J = 3.95$ and 2.09) and two exomethylene protons δ 4.875 ($J = 1.31$) and 4.785 ($J = 1.31$) were observed. These chemical shifts agreed with those of taraxasterol isolated from aerial parts of *Onopordum acanthium* Linn (Khalilov et al., 2003). The $^{13}\text{C-NMR}$ (500 MHz, CDCl_3) spectrum (Figure 1C-supplementary) indicated the presence of thirty carbon signals where six are quaternary carbons (one olefinic at δ 106.2), six ($-\text{CH}$) methines (one oxygen bearing carbon at δ 78.8), eleven methylenes ($-\text{CH}_2$, one sp^2 hybridised showing at δ 106.2), and seven methyl ($-\text{CH}_3$) groups. These chemical shifts correspond to a compound with molecular formula $\text{C}_{30}\text{H}_{50}\text{O}$ with six degrees of unsaturation. These signals agree with those of taraxasterol (a pentacyclic triterpene alcohol with a terminal double bond) isolated from flower receptacles of *Onopordum acanthium* as previously reported by Khalilov [11]. Total ion chromatogram (TIC) from the GC showed a single sharp peak at 36.34 min RT (Table 1). HR-MS spectrum (Figure 1D-supplementary) gave an $[\text{E}^+]$: m/z ratio for this compound as 426.7 while the m/z ratio calculated for molecular ion, $\text{C}_{30}\text{H}_{50}\text{O}$ $[\text{M}^+]$ was 426.428.

The spectroscopic data from FTIR, GC, HR-MS, $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ confirmed the compound to be taraxasterol (**1**). Taraxasterol was also confirmed by co-injection of authentic standard compound with the isolated pure compound obtained from Sigma Aldrich (Tauschem, Germany) where there was a match of the single peaks.

Pseudo-taraxasterol isolated as white crystals with a melting point of 216 - 218 °C was soluble in organic solvents (Table 1). The IR spectrum (Figure 2A-supplementary) showed characteristic absorption peaks at $3608.8 - 3329.3\text{ cm}^{-1}$ (O-H stretch), 2939.3 cm^{-1} (C-H stretch of CH_3), 1589.6 cm^{-1} (cyclic C=C stretch), 1422.6 cm^{-1} (cyclic methylene groups $(\text{CH}_2)_n$ vibrations), and 1067.33 cm^{-1} (C-OH stretch vibrations of

secondary alcohols). These absorption peaks are characteristic of triterpenes isolated from *Anthemis mirheydari* Iranshahr as described by Jassbi [12]. ¹H-NMR (500 MHz, CDCl₃) spectrum (Figure 2B-supplementary) showed unique peaks at δ 5.30 (*dd*, *J* = 9.93 and 9.2 Hz) and δ 3.51 (*dd*, *J* = 10.1 and 3.5 Hz) attributed to alkenyl and to carbinolic hydrogens, respectively. The spectrum confirmed presence of 8 methyl groups, of which seven were singlets [δ 1.01, 1.01, 0.90, 0.89, 1.56, 0.88, 0.90 and 0.93] and one doublet at δ 1.01 (*J* = 6.93 Hz). The presence of an axial hydroxymethine (δ 3.51, *dd*, *J* = 10.06 and 3.49) and an exomethylene proton δ 5.30, *dd* (*J* = 9.93 and 9.20) were also observed. These chemical shifts agreed with those of pseudo-taraxasterol isolated from *Anthemis mirheydari* as described by Jassbi [12]. ¹³C-NMR (500 MHz, CDCl₃) spectrum (Figure 2C-supplementary) gave 30 carbon signals with six quaternary δ 38.7, 41.6, 42.4, 34.5, 37.0 and 140.6 (alkenyl carbon), seven (-CH) methines (one oxygen bearing carbon at δ 78.0), 9 methylenes (-CH₂), and 8 methyl (δ 23.0, 23.0, 16.8, 16.8, 16.2, 17.7, 22.0 and 15.9) groups. These chemical shifts agree with those of pseudo-taraxasterol as reported by Abreu [13]. TIC of GC showed a single peak at RT of 37.10 min (Table 1). The HR-MS (Figure 2D-supplementary) gave an [EI (+)]: *m/z* ratio for this compound as 426.386 while the *m/z* ratio calculated for molecular ion, C₃₀H₅₀O [M⁺] was 426. The spectroscopic data from FTIR, GC, HR-MS, ¹H-NMR and ¹³C-NMR confirmed the compound to be pseudo-taraxasterol(2). Pseudo-taraxasterol was also confirmed by co-injection of authentic standard compound with the isolated pure compound.

Beta-sitosterol isolated as white crystals with a melting point of 137 - 139 °C was soluble in organic solvents (Table 1). The IR spectrum (Figure 3A-supplementary) indicated presence of characteristic absorptions peaks at 3440.38cm⁻¹ (O-H stretch), 2946.82 cm⁻¹ (C-H stretch of CH₃), 1596.70 cm⁻¹ (C=C stretch), 1400.38 cm⁻¹ (cyclic methylene group vibrations), 1219.52 cm⁻¹ (C-O stretch) and 1067.33 cm⁻¹ (C-OH vibration of secondary alcohols). These absorption peaks are similar to those of β -sitosterol as previously reported by Pretsch and Affolter [14]. ¹H-NMR (500 MHz, CDCl₃) spectrum (Figure 3B-supplementary) presented three regions namely aliphatic, hydroxylated and allylic on the spectrum strongly suggesting a triterpenoid skeleton. There were characteristic chemical shifts at δ 0.70 and 1.03 suggesting the presence of two CH₃ attached to quaternary carbons. A signal at δ 5.33 (1H, d, *J* = 7.2 Hz) indicated a double bond at a quaternary carbon atom. A multiplet centered at δ 3.55, characteristic of proton germinal to a hydroxyl group in terpenoids was also observed. Six signals representing the methyl groups were observed at δ 1.03 (3H, s), 1.00 (3H, d, *J* = 8.4 Hz), 0.86 (9H, m) and 0.70 (3H, s) which is characteristic of a modified triterpenoid [15]. ¹³C NMR (500 MHz, CDCl₃) spectrum (Figure 3C-supplementary) showed signals at δ 140.76 and 121.94 involving the double bonds with the former representing a quaternary carbon atom. A hydroxylated carbon atom showed a signal at δ 71.82. The methyl groups were represented by signals at δ 11.87, 11.99, 18.79, 19.03, 19.42 and 20.09. The chemical shifts of the compound agreed to those of β -sitosterol, isolated from *Rubus suavisissimus* leaves [16]. TIC of GC showed a single peak at RT of 38.50 min. (Table 1). HR-MS (Figure 3D-supplementary) gave an [EI (+)]: *m/z* ratio for this compound as 414.436 while the *m/z* ratio calculated for molecular ion, C₂₉H₅₀O [M⁺] was 414.718 [16]. The spectroscopic data from FTIR, GC, HR-MS, ¹H-NMR and ¹³C-NMR confirmed the compound to be b-sitosterol (3). b-sitosterol was also confirmed by co-injection of authentic standard compound with the isolated pure compound.

Fungisterol isolated as a white powder with a melting point of 132 - 134°C was soluble in dichloromethane and ethyl acetate (Table 1). The IR spectrum (Figure 4A-supplementary) indicated a broad absorption peak 3496 - 3312 cm⁻¹ (O-H stretch), 2948.61 cm⁻¹ (C-H stretch), 1596.77 cm⁻¹ (C-C stretch vibrations), 1406.25 (O-H bending vibrations) and 1115.10 cm⁻¹ (C-O vibrations) as previously reported by Sen [17]. ¹H-NMR (500 MHz, CDCl₃) indicated the presence of six methylene groups at δ 0.52, 0.77, 0.78, 0.79, 0.83 and 0.90 (Figure 4B-supplementary). The two protons appearing at δ 3.61 and 5.15 for carbon number 2 and 7 represented protons of a hydroxyl group and a double bond proton respectively. These chemical shifts are characteristic to a modified triterpenoid as earlier reported by Nazifi [18]. ¹³C-NMR (500 MHz, CDCl₃) spectrum (Figure 4C-supplementary) showed 28 carbon peaks showing presence of a double bond at δ 117.4 and 139.6 suggesting that the possible structure of the compound is fungisterol. This compound was previously identified by Nazifi [18] from the bulbs of *Ornithogalum cuspidatum* Bertol. through GC-MS. TIC of GC showed a single peak at RT of 26.80 min. (Table 1). HR-MS spectrum (Figure 4D-supplementary) gave an [EI (+)]: *m/z* ratio for this compound as 400.371 while the *m/z* ratio calculated for molecular ion, C₂₈H₄₈O [M⁺] was 400.68 [18]. The spectroscopic data from FTIR, GC, HR-MS, ¹H-NMR and ¹³C-NMR confirmed the compound to be fungisterol (4). Fungisterol was also confirmed by co-injection of authentic standard compound with the isolated pure compound.

Guggulsterol isolated as a white powder with a melting point of 165 - 169°C was soluble in dichloromethane and ethyl acetate (Table 1). The IR spectrum (Figure 5A-supplementary) showed a major broad absorption peak between 3691.7 and 3206.4cm⁻¹ (OH stretching vibrations), 2923.1 cm⁻¹ (C-H stretch for CH₃), 1595.7 cm⁻¹ (C=C stretch), 1426.8 cm⁻¹ (-CH₂ vibrations), and 1076.7 cm⁻¹ (C-OH vibrations of secondary alcohols) (Sultana and Jahan, 2005). These absorption bands were characteristic of triterpenes similar to those derived from the gum-resin of *Commiphora mukul* (Hook. ex Stocks) Engl [19]. ¹H-NMR (500 MHz, CDCl₃) indicated two double bond protons at δ 4.30 and 5.3ppm, multiple hydroxyl protons at δ 3.57, 3.60, 3.69 and 3.74 ppm, methyl protons at δ 0.89, 1.31, 1.45, 1.52, 2.02 and 2.20 ppm (Figure 5B-supplementary). These chemical shifts are characteristic of guggulsterol isolated from the gum-resin of *Commiphora mukul* [19]. ¹³C-NMR

(500 MHz, CDCl₃) spectrum indicated 27 carbon peaks with four olefinic carbon atoms whose chemical shifts were δ 129.9, 171.6, 172.3 and 174.4 ppm (Figure 5C-supplementary). These chemical shifts agreed with those of guggulsterol isolated from the gum-resin of *Commiphora mukul* [19]. TIC of GC showed a single peak at RT 27.90 min (Table 1) that corresponded to guggulsterol [19]. HR-MS spectrum (Figure 5D-supplementary) gave an [EI⁺]: m/z ratio for this compound as 418.34 while the m/z ratio calculated for molecular ion, C₂₇H₄₆O₃ [M⁺] was 418.66 (Sultana and Jahan, 2005). The fragmentation pattern was similar that of guggulsterol isolated from *Commiphora mukul* by Sultana [19]. The spectroscopic data from FTIR, GC, HR-MS, ¹H-NMR and ¹³C-NMR confirmed the compound to be guggulsterol (**5**). Guggulsterol was also confirmed by co-injection of authentic standard compound with the isolated pure compound.

Mean repellency of isolated pure compounds against bed bugs

The repellency of bed bugs on treatment with isolated compounds were evaluated separately at varying concentrations of 0.20, 0.50, 1.00, 1.25 and 1.50 mg/l in acetone and the results are summarized in table 2. Generally, the mean percentage repellencies of bed bugs on treatment with taraxasterol, pseudo-taraxasterol, β -sitosterol, fungisterol and guggulsterol were significantly ($P < .05$) higher at all concentrations than that of acetone solvent. The mean repellencies of bed bugs on treatment with the test compounds significantly ($P < .05$) increased with increase in concentration (Table 2). For instance, fungisterol had mean repellencies of 71.0 % and 89.1 % at 0.20 and 1.25 mg/l, respectively. Fungisterol showed significantly higher ($P < .05$) mean repellency against bed bugs than all the other tested compounds (Table 2) in all the concentrations. The repellencies of bed bugs on treatment with the test compounds increased with an increase concentration due to the saturation of the bed bug odor receptors with the test compounds [20]. In all the concentration levels, the higher mean repellency of fungisterol was not significantly ($P > .05$) different from that of neocidol (Table 2). For instance, the repellency of bed bugs on treatment with 1.25mg/l fungisterol and 1.25mg/l neocidol showed mean repellencies of 89.1 % and 91.3 %, respectively.

Taraxasterol, pseudo-taraxasterol, β -sitosterol, fungisterol and guggulsterol indicated that they are potent repellents against bed bugs. Some *Commiphora* species such as *Commiphora holtziana* Engl. have previously been identified to have larvicidal properties, lowering oviposition and repellent activity against arthropods species such as mosquitoes [21]. *Commiphora swynnertonii* Burt. extract has been shown to possess anti-ectoparasitic and repellency activities against lice, mosquito, ticks, fleas, trypanosome and mites [22, 23]. Its stem bark exudate extract has high acaricidal activity against the brown ear tick (*Rhipicephalus appendiculatus* Neumann) and can be used in tick control [22]. The biological effects are due to the presence of triterpenes in greater amount which are responsible for repellency and toxicity activities [24].

Mean mortality of isolated pure compounds against bed bugs

The mortality of bed bugs on exposure to the pure isolated compounds was evaluated at varying concentrations [25] and their LC₅₀ values summarized in table 3. Generally, all the tested compounds showed significantly ($P < .05$) lower mortality than neocidol indicated by their higher LC₅₀ values than that of neocidol (Table 3). Higher LC₅₀ values indicate lower mortality, the vice versa is true. Generally, the LC₅₀ of the tested compounds significantly ($P < .05$) decreased with increase in exposure time. Fungisterol had significantly ($P < .05$) lower LC₅₀ values (indicating higher mortality) than all the other tested compounds (Table 3). The LC₅₀ values of fungisterol was not significantly different ($P > .05$) to that of neocidol (Table 3).

The optimal concentration of fungisterol (most lethal compound) that killed all the bed bugs was 1.25mg/l which was not significantly ($P > .05$) different from that of the positive control (neocidol). The mortality of bed bugs on exposure to 1.25 mg/l of isolated compounds and neocidol in acetone solvent was evaluated at varying exposure times. All bed bugs (100%) were killed by neocidol after 24 h exposure, which was not significantly ($P > .05$) different to that of fungisterol (97.2%) (Figure 1). This was considered as the optimal exposure time for neocidol and fungisterol. The exposure time of taraxasterol, pseudo-taraxasterol, β -sitosterol and guggulsterol with optimal mortality against bed bugs was 48 h (Figure 1). The different optimal exposure times for mortality of bed bugs is attributed to the nature of the active molecule.

Taraxasterol, pseudo-taraxasterol, β -sitosterol, fungisterol and guggulsterol were isolated for the first time from *C. africana* resin. However, previous studies have reported wide range of medicinal properties other than insecticidal activity. For instance, Sharma and Zafar [26] reported that taraxasterol possesses anti-tumor, anti-allergy, anti-oxidant and anti-inflammatory actions and also acts as a control against snake venom. Pseudo-taraxasterol on the other hand was shown to exhibit anti-inflammatory and anti-nociceptive activity in a combination with other triterpenoids [27]. Beta-sitosterol isolated from *Cestrum diurnum* Linn. leaves were reported to exhibit toxicity against larval forms of *Aedes aegyptica* Linn. and *Anopheles stephensi* Theobald [28]. A study by Mabrouk [28] screened activity of fungisterol from *Penicillium brevicompactum* Lindley and was confirmed to have antibacterial activity against gram-positive and gram-negative bacterial pathogens. It also demonstrated anticancer activity against breast and cervix carcinoma cell [28].

Blending studies

The most potent repellent/toxic compounds were blended in equal proportions [5] and evaluated for repellency and toxicity against bed bugs. The results of the selected blends are summarised in table 4. The resultant mean percentage repellency of bed bugs on exposure to a two- or three- constituent blend of fungisterol (most repellent and toxic compound) with other active compounds (β -sitosterol and guggusterol), was not significantly different ($P>.05$) to that of fungisterol as an individual compound (Table 4). On the other hand, there was also no significance difference ($P>.05$) in LC_{50} values of fungisterol (9.93 mg/L) and its blend of either β -sitosterol or guggusterol or both (Table 4).

These three- or two- constituent blends are in part behaviorally redundant, since repellency and mortality of bed bugs to a 2- or 3-component blend of fungisterol, β -sitosterol and guggusterol was similar to that of fungisterol as individual compound. A similar pattern was previously observed by Tasin [29] where attraction of grapevine moth to a 3-component blend of b-caryophyllene, (E)-b-farnesene and (E)-4,8-dimethyl-1,3,7-nonatriene was not significantly different from a 10-component blend. There was no synergistic repellent or toxic effect of bed bugs observed after exposure to blends of fungisterol with β -sitosterol or/and guggusterol. This was attributed to all the compounds which belonged to one homologous class of compounds (terpenoids) competing for the same receptors [30] where their modes of action leading to mortality is the similar.

Conclusion

Compounds in *C. africana* resin responsible for bed bug repellency and mortality have been identified and includes taraxasterol, pseudo-taraxasterol, β -sitosterol, fungisterol and guggulsterol. Although these compounds have been previously reported in other plant species, they have been isolated from *C. africana* resin for the first time. Fungisterol was the most potent repellent and toxic compound and can be applied in bed bug control upon further research on toxicology to humans and the environment. There was no synergistic repellent or toxic effects upon exposure of bed bugs to blends of fungisterol with β -sitosterol or/and guggulsterol since they compete for the same receptor neurons and associated genes in bed bug odor stimuli pathway. We anticipate that blending active terpenoids with other active compounds from other classes such as flavonoids, fatty acids, steroids and esters will lead to a synergistic/additive effect. Since bed bugs have developed a mechanism in resisting the commonly used insecticides and are, therefore, resurgent, a bio-pesticide product from fungisterol can be developed and used as a substitute in control of bed bugs and thereafter near eradication.

Declarations

Data availability: All data in this study have been provided.

Conflict of interest: The authors declared no conflict of interest

Compliance with Ethical Standards: The study followed the guidelines of animal care and husbandry as stated by IUCAC committee of Kenyatta University.

Funding: NWW, National Forest Products Research Programme, Kenya Forestry Research Institute (KEFRI), 2018/2019.

Acknowledgments

We appreciate the support received from Prof. Lugenwa F. Nelson (University of Nairobi) in Spectroscopic analyzes and compound elucidation; and Mr. Richard Odera from the Department of Plant Sciences of Kenyatta University for technical support in bed bug rearing.

References

1. Goddard J, Deshazo R (2009) bed bugs (*Cimex lectularius*) and clinical consequences of their bites. *JAMA* 301:1358-1366. DOI: [10.1001/jama.2009.405](https://doi.org/10.1001/jama.2009.405)
2. Reinhardt K, Kempke D, Naylor RA, Siva-Jothy MT (2009) Sensitivity to bites by the bed bug, *Cimex lectularius*. *Med Vet Entomol* 23:163-166. doi: [10.1111/j.1365-2915.2008.00793.x](https://doi.org/10.1111/j.1365-2915.2008.00793.x)
3. Dang K, Doggett SL, Veera Singham G, Lee CY (2017) Insecticide resistance and resistance mechanisms in bed bugs, *Cimex* spp. (Hemiptera: Cimicidae). *Parasites vectors* 10:1-31. <https://doi.org/10.1186/s13071-017-2232-3>
4. Rukke BA, Roligheten E, Aak A (2022) Procurement Competence and Framework Agreements for Upgraded Bed Bug Control [*Cimex lectularius* (Hemiptera: Cimicidae)]. *J Econ Entomol* 115:240-249. <https://doi.org/10.1093/jee/toab233>
5. Wachira BM, Kabaka JM, Mireji PO, Okoth SO, Murilla GA, Hassanali A. Blending studies with selected waterbuck odor constituents or analogues in the development of a potent repellent blend against savannah tsetse. *Acta Trop.*2020;211(1):105-135.

6. Gondhalekar AD, Appel AG, Thomas GM, Romero A (2021) A review of alternative management tactics employed for the control of various cockroach species (order: blattodea) in the USA. *Insects* 12:550-551. doi: [10.3390/insects12060550](https://doi.org/10.3390/insects12060550)
7. Wairagu NW, Wachira BM, Githiomi JK, Oduor N, Ng'ang'a, MM. Repellency and Toxicity of Selected Fractions, Identified Compounds and Blends of *Commiphora africana* Resin Against bed bugs. *Nat. Prod. Commun.* 2022;17(6): 1934578X221106898.
8. Araujo RN, Costa FS, Gontijo NF, Gonçalves TC, Pereira MH (2009) The feeding process of *Cimex lectularius* (Linnaeus 1758) and *Cimex hemipterus* (Fabricius 1803) on different blood meal sources. *J Insect Physiol* 55:1151-1157. <https://doi.org/10.1016/j.jinsphys.2009.08.011>
9. Ojianwuna CC, Olisedeme P, Ossai SL (2016) The toxicity and repellency of some plant extracts applied as individual and mixed extracts against termites (*Macrotermes bellicosus*). *J Entomol Zoo* 4:406-418.
10. Cîntă-Pinzaru S, Dehelean CA, Soica C, Culea M, Borcan F (2012) Evaluation and differentiation of the Betulaceae birch bark species and their bioactive triterpene content using analytical FT-vibrational spectroscopy and GC-MS. *Chem Cent J* 6:1-12. [10.1186/1752-153X-6-67](https://doi.org/10.1186/1752-153X-6-67)
11. Khalilov LM, Khalilova AZ, Shakurova ER, Nuriev IF, Kachala VV, Shashkov AS, Dzhemilev UM (2003) PMR and ¹³C NMR spectra of biologically active compounds XII - Taraxasterol and its acetate from the aerial part of *Onopordum acanthium*. *Chem Nat Compd* 39:285-288. <https://doi.org/10.1023/A:1025478720459>
12. Jassbi AR, Firuzi O, Miri R, Salhei S, Zare S, Zare M, Baldwin, IT (2016) Cytotoxic activity and chemical constituents of *Anthemis mirheydari* *Pharm Biol* 54:2044-2049.
13. Abreu VG, Corrêa GM, Lagos IA, Silva RR, Alcântara AF (2013) Pentacyclic triterpenes and steroids from the stem bark of uchi (*Sacoglottis uchi*, Humiriaceae). *Acta Amazon* 43:525-528. <https://doi.org/10.1590/S0044-59672013000400015>
14. Pretsch EB, Affolter A (2000) Structure determination of organic compounds-Table of spectral data. Springer Verlag, Berlin, Heidelberg pp71-150.
15. Aliba, M. O., Ndukwe, I. G., & Ibrahim, H. (2018). Isolation and characterization of B-sitosterol from methanol extracts of the stem bark of large-leaved rock fig (*Ficus abutilifolia* Miq). *Journal of Applied Sciences and Environmental Management*, 22(10), 1639-1642.
16. Chaturvedula V, Prakash I (2012) Isolation of Stigmasterol and beta-sitosterol from the CH₂Cl₂ extract of *Rubus suavissimus*. *Int Curr Pharm J* 1:239-242. [10.3329/icpj.v1i9.11613](https://doi.org/10.3329/icpj.v1i9.11613)
17. Sen A, Dhavan P, Shukla KK, Singh S, Tejovathi G (2012) Analysis of IR, NMR and antimicrobial activity of β-sitosterol isolated from *Momordica charantia*. *Sci Secure J Biotechnol* 1:9-13.
18. Nazifi E, Delazar A, Movafeghi A, Hemmati S, Nazemiyeh H, Nahar L, Sarker, SD (2008) GC-MS Analysis of the Dichloromethane Extract of the Bulbs of *Ornithogalum cuspidatum* Bert (Family: Liliaceae) from Iran. *Rec Nat Prod* 2:1-23.
19. Sultana N and Jahan S (2005) Studies on the constituents of *Commiphora mukul*. *Naturforsch* 60:1202-1206. <https://doi.org/10.1515/znbn-2005-1114>
20. Hansen IA, Rodriguez SD, Drake LL (2014) The odorant receptor co-receptor from the bed bug, (*Cimex lectularius*). *PLoS One* 9:113-120. <https://doi.org/10.1371/journal.pone.0119059>
DOI: [10.3109/13880209.2016.1141220](https://doi.org/10.3109/13880209.2016.1141220)
21. Deepa J, Gokulakrishnan J, Baranitharan M, Dhanasekaran S (2015) Larvicidal activity of Indian medicinal plants on the dengue fever mosquito, *Aedes aegypti* Linnaeus. *Int J Pure Appl Zool* 3:130-136.
22. Edwin D, Erasto P, Temba S, Chacha M (2017) Repellency activity of *Commiphora swynnertonii* exudates against *Rhipicephalus appendiculatus* larvae. *J biodivers environ sci* 11:281-286.
23. Nagagi YP, Silayo RS, Kweka EJ (2016) Trypanocidal activity of ethanolic extracts of *Commiphora swynnertonii* Burt on *Trypanosoma congolense*. *BMC complement Med* 16:1-6. doi: [10.1186/s12906-016-1191-0](https://doi.org/10.1186/s12906-016-1191-0)
24. Birkett M, Al Abassi S, Kröber T, Chamberlain K, Hooper A, Guerin PM, Wadhams L (2008) Antiectoparasitic activity of the gum resin, gum haggat, from the East African plant, *Commiphora holtziana*. *Phytochem* 69:1710-1715. [10.1016/j.phytochem.2008.02.017](https://doi.org/10.1016/j.phytochem.2008.02.017)

25. Gaire S, Scharf ME, Gondhalekar AD (2019) Toxicity and neurophysiological impacts of plant essential oil components on bed bugs (Cimicidae: Hemiptera). Sci Rep 9:1-12. DOI: [10.1038/s41598-019-40275-5](https://doi.org/10.1038/s41598-019-40275-5)
26. Sharma K, Zafar R (2014) Simultaneous estimation of taraxerol and taraxasterol in root callus cultures of *Taraxacum officinale* Weber. Int J Pharmacogn Phytochem Res 6:540–546.
27. Da-Silva FA, De-Farias FS, Da-Rocha BM, Barros FE, Da-de-Sousa M, De-Sousa RM, Guilhon GM, Müller AH, Borges AC (2017) Antinociceptive and anti-inflammatory effects of triterpenes from *Pluchea quitoc* DC. aerial parts. Pharmacognosy Res 9:123-134. [10.4103/pr.pr_51_17](https://doi.org/10.4103/pr.pr_51_17)
28. Mabrouk AM, Kheiralla ZH, Hamed ER, Youssry AA, Abd E, Aty AA (2011) Physiological Studies on Some Biologically Active Secondary Metabolites from Marine-Derived fungus *Penicillium brevicompactum*. Gate2Biotech 1:1-15. <https://api.semanticscholar.org/CorpusID:53059371>
29. Tasin M, Bäckman AC, Coracini M, Casado D, Ioriatti C, Witzgall P. Synergism and redundancy in a plant volatile blend attracting grapevine moth females. Phytochem. 2007;68(2):203-209.
30. Dambolena JS, Zunino MP, Herrera JM, Pizzolitto RP, Areco VA, Zygadlo JA (2016) Terpenes: Natural products for controlling insects of importance to human health-A structure-activity relationship study. Psyche J Entomol 2016:1-17. <https://doi.org/10.1155/2016/4595823>
31. Zhang, X., Xiong, H., & Liu, L. (2012). Effects of taraxasterol on inflammatory responses in lipopolysaccharide-induced RAW 264.7 macrophages. *Journal of Ethnopharmacology*, 141(1), 206-211.
32. Dehariya, R., & Dixit, A. K. (2015). A Review on Potential Pharmacological Uses of *Carthamus tinctorius* L. *World Journal of Pharmaceutical Sciences*, 1741-1746.
33. Chanioti, S., Katsouli, M., and Tzia, C. (2021). β -Sitosterol as a functional bioactive. In *A century of valuable plant bioactives* (pp. 193-212). Academic Press.
34. Weete, J. D., & Laseter, J. L. (1974). Distribution of sterols in the fungi I. Fungal spores. *Lipids*, 9(8), 575-581.
35. Benvegnu, R., Cimino, G., De Rosa, S., & De Stefano, S. (1982). Guggulsterol-like steroids from the Mediterranean gorgonian *Leptogorgia sarmentosa*. *Experientia*, 38(12), 1443-1444.

Tables

Table 1: Physical and chromatographic properties, pharmacological uses and confirmation of Isolated compounds from *C. africana* resin dichloromethane extract

Isolated Compound	Molecular formula	% composition(w/w)	Melting point (°Celsius)	Physical state	RT (Min.)	Class	Identification	Plants isolated	Other pharmacological uses
Taraxasterol	C ₃₀ H ₅₀ O	0.05	233-236	White crystals	36.34	Phytosterol	Chemical and physical properties, spectroscopy, RI, Inj	<i>C. africana</i> - this study <i>Taraxacum officinale</i> [31] <i>Carthamus lanatus</i> L. <i>Hemistepta lyrate</i>	Anti-inflammatory [31]
Pseudo-taraxasterol	C ₃₀ H ₅₀ O	0.04	216-218	White crystals	37.10	Phytosterol	Chemical and physical properties, spectroscopy, RI, Inj	<i>C. africana</i> - this study <i>Anthemis mirheydari</i> [12] <i>Carthamus tinctorius</i> L. flowers [32]	Antimicrobial [12]
β-Sitosterol	C ₂₉ H ₅₀ O	0.05	137-139	White crystals	38.50	Phytosterol	Chemical and physical properties, spectroscopy, RI, Inj	<i>C. africana</i> - this study Vegetable oils [33]	Anti-inflammatory, antibacterial, antifungal and antioxidant [33]
Fungisterol	C ₂₈ H ₄₈ O	0.03	132-133	White powder	26.80	Phytosterol	Chemical and physical properties, spectroscopy, RI, Inj	<i>C. africana</i> - this study Fungal species e.g. <i>Penicillium claviforme</i> , <i>Linderina pennispora</i> [34] <i>Ornithogalum cuspidatum</i> [18]	Antibacterial, anticancer [28]
Guggusterol	C ₂₇ H ₄₆ O ₃	0.02	165-168	White powder	27.90	Phytosterol	Chemical and physical properties, spectroscopy, RI, Inj	<i>C. africana</i> - this study <i>Commiphora mukul</i> [35]	Anti-rheumatic, hypocholesterolemic, anti-inflammatory [35]

RT: Retention time, Inj: injection of authentic standard compounds

Table 2: Mean percentage repellency of bed bugs on treatment with isolated compounds at varying concentrations in acetone

Compound	Repellency (%) Mean ± SD				
	Concentration (mg/l)				
	0.20	0.50	1.00	1.25	1.50
Taraxasterol	50.0 ± 1.1 ^{Ab}	65.0 ± 1.1 ^{Bc}	70.0 ± 0.5 ^{Cb}	77.0 ± 0.7 ^{Dbc}	79.0 ± 0.1 ^{Dc}
Pseudo-taraxasterol	55.0 ± 1.1 ^{Ac}	61.0 ± 0.8 ^{Bb}	69.0 ± 0.4 ^{Cb}	75.0 ± 0.9 ^{Db}	76.0 ± 0.6 ^{Db}
β-Sitosterol	65.0 ± 0.4 ^{Ad}	70.0 ± 0.8 ^{Bd}	73.0 ± 0.9 ^{Cc}	76.0 ± 1.0 ^{Db}	77.0 ± 0.3 ^{Db}
Fungisterol	71.0 ± 0.8 ^{Ae}	75.0 ± 1.0 ^{Be}	84.4 ± 0.8 ^{Cd}	89.1 ± 0.2 ^{Dd}	90.5 ± 0.2 ^{Dd}
Guggusterol	65.0 ± 1.0 ^{Ad}	70.0 ± 0.3 ^{Bd}	75.0 ± 1.1 ^{Cc}	78.0 ± 0.6 ^{Dc}	78.8 ± 0.2 ^{Dbc}
Neocidol	73.0 ± 0.5 ^{Ae}	74.0 ± 1.0 ^{Ae}	85.0 ± 0.1 ^{Bd}	91.3 ± 0.5 ^{Cd}	92.0 ± 0.2 ^{Cd}
Acetone	0.0 ± 0.0 ^{Aa}	0.0 ± 0.0 ^{Aa}	0.0 ± 0.0 ^{Aa}	0.0 ± 0.0 ^{Aa}	0.0 ± 0.0 ^{Aa}

^f solvent; Means followed by different small and capital letters in a column and row respectively indicate significant difference ($P < .05$)

Table 3: Mean LC₅₀ of bed bugs on exposure to isolated pure compounds of *C. africana* resin CH₂Cl₂ extract

Compound	Mean LC ₅₀ ± SE (mg/L)	Slope	Degree of freedom	χ ²
Taraxasterol	22.48 ± 0.28 ^{Ac}	1.21	4	13.00
Pseudo-taraxasterol	20.33 ± 0.33 ^{Ac}	1.37	4	12.00
β-Sitosterol	27.71 ± 0.20 ^d	1.23	4	14.00
Fungisterol	9.40 ± 0.27 ^{Aa}	1.25	4	12.23
Guggusterol	17.26 ± 0.54 ^{Ab}	1.32	4	11.21
Neocidol	5.38 ± 0.04 ^{Aa}	1.27	4	13.12

Means followed by different small and capital letters in a column and row respectively indicate significant difference ($P < .05$)

Table 4: Mean bed bug repellency and toxicity on treatment with selected blends

Blend	Mean % repellency±SE	LC ₅₀ (mg/L)			
		Mean±SE	Slope	Degree of freedom	c ²
1+2+3	70.2±0.2 ^b	9.69±0.29 ^a	1.34	4	12.51
1+2	69.5±0.3 ^b	9.72±0.09 ^a	1.65	4	14.12
1+3	64.3±0.5 ^a	9.61±0.11 ^a	1.78	4	12.34
2+3	69.0±0.1 ^b	9.54±0.03 ^a	1.89	4	13.12
2	71.0±0.8 ^{bc}	9.93±0.08 ^a	1.34	4	12.76
Neocidol	73.0±0.5 ^c	9.81±0.05 ^a	1.44	4	13.21

Numbers 1, 2 and 3 represents β -sitosterol, fungisterol and guggusterol, respectively. Means followed by different letters in a column indicate significant difference ($P<.05$)

Figures

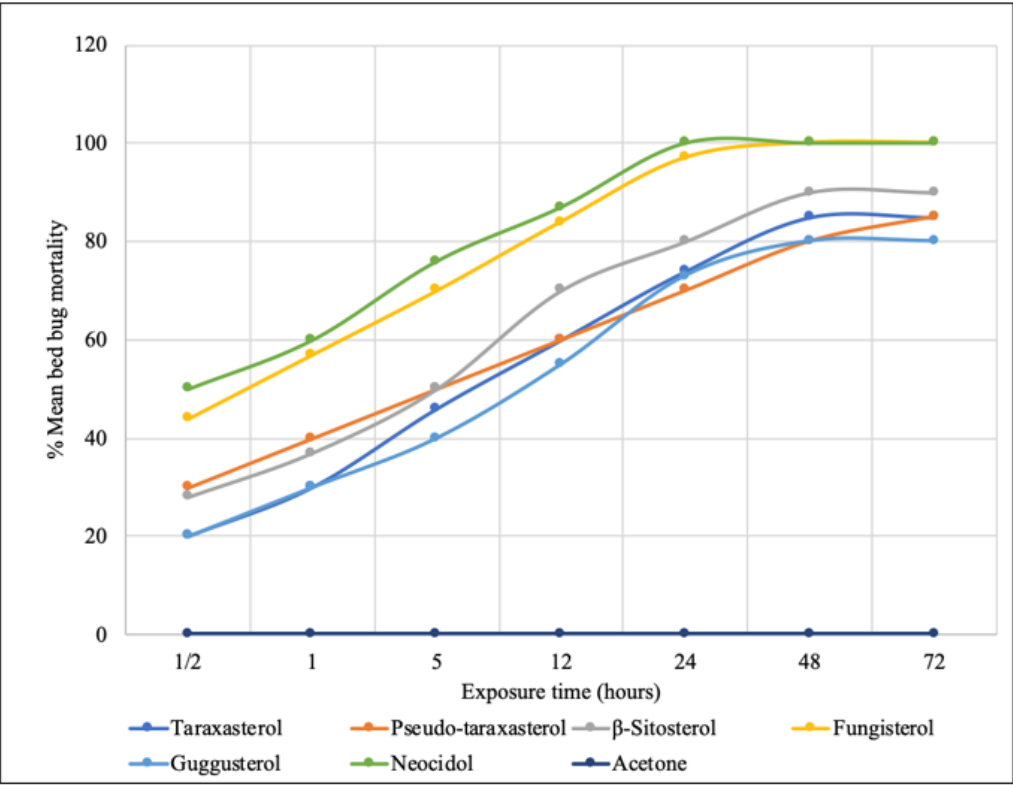


Figure 1

Mean bed bug mortality on exposure to 1.25 mg/l of isolated compounds in acetone at varying exposure times

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