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Larvicidal activity of extracellular secondary metabolites from a *Stereum* species Hill ex Pers. (JO5289) against the dengue fever mosquito, *Aedes aegypti* (Linn) (Diptera: Culicidae)

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The main objective of this investigation was to find mosquito larvicidal secondary metabolites from a basidiomycete – *Stereum* species (JO5289) – against *Aedes aegypti*. The *Stereum* species (JO5289) was collected in July 2005 from undisturbed habitat in Londiani forest in Rift Valley province, Kenya. Extracellular crude extracts from *Stereum* species (JO5289) produced strong activity against *A. aegypti* larvae. Purification of the crude extracts targeting larvicidal activity using chromatography gave three active compounds namely; tyrosol, 3-methoxy-5-methyl-1,2-benzenediol and 2-hydroxy-4-(4-hydroxy-chroman-7yl) but-3-enal. The chemical structures of the compounds were determined using the nuclear magnetic resonance (NMR) spectral data and comparison with literature values. When tested for larvicidal activity, the LC_{50} for the three compounds were 26.7, 17.3 and 14.5 ppm, respectively, while LC_{90} were 85.3, 83.5 and 82.9 ppm, respectively, after 24 h of exposure. These compounds have been produced from cultures of a *Stereum* species and reported to have mosquito larvicidal activity for the first time.

Key words: Basidiomycete, *Stereum* species, *Aedes aegypti*, extracellular metabolites and larvicidal.

INTRODUCTION

Mosquitoes are the major vector for diseases such as malaria, filariasis, dengue fever, Japanese encephalitis and several other diseases globally (Rahuman et al., 2009; Borah et al., 2010). Indeed, the recrudescence of these diseases is due to high number of breeding places and the increasing resistance of mosquitoes to the used commercial insecticides (Chowdhury and Chandra, 2008). The most successful method of minimizing the incidence of mosquito-borne diseases is to eradicate and control the mosquito vectors (Rozendaal, 1997). In the past few decades, synthetic insecticides were used as mosquito control agents but have produced a negative impact on environment, ill effect on non-target organisms and most mosquito species becoming physiologically resistant (Arivoli et al., 2011). Moreover the repeated use of synthetic insecticides has disrupted natural biological systems and led to resurgences in mosquito populations with associated insect resistance (Prabhu et al., 2011).

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Abbreviations: PDA, Potato dextrose agar; **TLC,** thin layer chromatography; **NMR,** nuclear magnetic resonance; **LDL,** low density lipoproteins.

These factors have necessitated a search for eco-friendly, biodegradable and target specific insecticides against the mosquitoes. In recent years, the emphasis to control the mosquito population has shifted steadily from the use of conventional chemicals towards more specific and environmentally friendly materials, which are generally of botanical origin (Neetu et al., 2007; Arivoli et al., 2011).

Fungi are extremely diverse group of heterotrophic organisms that are exploited by humans for various biotechnology applications. The wide range of biologically active secondary metabolites from basidiomycetes has been one of the most attractive groups of natural products studied (Getha et al., 2009). In this study a *Stereum* species belonging to the fungal division of basidiomycetes was studied for larvicidal compounds from its submerged cultures. *Stereum* is type genus of the Stereaceae family of fungi, in the Russulales order. Common names for species of this genus include leaf fungus, wax fungus, and shelf fungus. *Stereum* contains 27 species that have a widespread distribution (Kirk et al., 2008). *Stereum* species are found to live on all kinds of deadwood or hardwood or dead leaves, hence are saprophytic basidiomycetes. Sometimes they are also found on living leaves. *Stereum* species are wood decay fungi that do not have tubes. They are simply small bracket-shaped membranes appearing on dead wood. The underside of the membrane contains spores but no ornament, that is, gills, of any kind. Like most members in the family, *Stereum* lack clamp connection and have amyloid spores.

Stereum species are good producers of secondary metabolites in submerged cultures according to various studies that have been reported (Mantle and Mellows, 1973; Mellows et al., 1973; Nair and Anchel, 1975; Ayer and Saeedi-Ghomi, 1981; Sun et al., 2011; Isaka et al., 2012). Several biologically active compounds have also been reported from *Stereum* species. *Stereum hirsutum* is one of several fungi involved in a grapevine disease called esca. From the culture medium of this fungus, four new acetylenic compounds have been isolated and identified with structural elucidation and biological activity reported (Dubin et al., 2000). Omolo et al. (2002) isolated four new pentasubstituted phenolic compounds, named hericenols A, B, C, and D, 6-hydroxymethyl-2,2-dimethylchroman-4-one and the known erinapyrone C from extracts of submerged cultures of a *Stereum* species. Hericenol A showed weak antimicrobial activity while hericenol C was weakly cytotoxic. From the fungus *S. hirsutum*, two new compounds together with two known epidioxysterols have been isolated and identified. Epidioxysterols have been shown to possess a significant activity against *Mycobacterium tuberculosis* (Cateni et al., 2007). Li et al. (2008) isolated five cadinane sesquiterpenoids, named stereumin A, B, C, D and E from the chloroform extract of the culture broth of a *Stereum* species CCTCC AF 207024. The five cadinane sesquiterpenoids showed nematicidal activities against the nematode *Panagrellus redivivus* at 400 mg/l.

From the reported studies on secondary metabolites of *Stereum* species, it is evident that this genus of basidiomycetes is a very rich producer of compounds with interesting biological activities. It is on this basis that we screened *Stereum* species (JO5289) for larvicidal compounds against larvae of *Aedes aegypti*.

MATERIALS AND METHODS

General preparations and analytical procedures

Hands, working benches, blades and the general working environment were sterilized using 70% ethanol while the liquid media was heat sterilized using an autoclave (Danfoss 59407-3 NO. 375). The liquid media was constituted by dissolving 1% malt media, 0.4% of glucose and 0.4% of yeast extract in tap water. The pH of the media was adjusted to 5.5, and then dispensed in 250 ml Erlenmeyer flasks. The flasks were corked with cotton wool plugs and finally wrapped with aluminium foil. The set up was sterilized at a temperature of 121°C and pressure of 1.5 bars for about 15 min. Potato dextrose agar (PDA) solid media were prepared by autoclaving 39.0 g PDA in 1 l distilled water and then cooled to 45°C. This was then dispensed as 15 ml per sterile Petri dishes in sterile lamina flow hood. The preserved mycelia material on agar slants was then transferred onto PDA plates. These solid cultures were allowed to grow for two weeks at IBRL at ambient conditions.

Analytical thin layer chromatography (TLC) was performed with Macherey–Nagel pre-coated silica gel 60 F_{254} plates (ALUGRAM ® SIL G/UV_{254} 0.25 mm). Column chromatography was packed with silica gel 60 (0.063 – 0.2 mm/70-230 mesh). The developed TLC plate was viewed under dual fixed wavelength UV lamp (λ = 254 nm and 365 nm) and the spots visualised by spraying with freshly prepared *p*-anisaldehyde solution, heated to 115°C. The larvicidal experiments were set up in glass beakers. The crude extract and the purified compound were kept under 4°C except when undergoing analysis. The purified compounds were dissolved in deuterated chloroform $CDCl_3$). ^{1}H NMR and ^{13}C NMR spectra were recorded with 300 MHz Bruker AVANCE NMR spectrometers. COSY, HMBC, HSQC and NOESY were acquired using the standard Bruker software.

Sample collection and preservation

Pieces of dead wood colonised by *Stereum* species as evidenced by the fruiting body was collected in July 2005 from a natural habitat in Londiani Forest, Kenya. The collected material was assigned an accession number of JO5259. Upon collection pure culture was prepared by trapping spores from the underside (the hymenium) of a sterile piece of the fruiting body. The culture was preserved as agar slants and the corresponding herbarium material are kept in the Integrated Biotechnology Research Laboratory (IBRL) at Egerton University.

Fermentation of the Stereum species (JO5289)

The sterilized liquid media was used to culture the *Stereum* species (JO5289). Pieces of agar plugs (1 x 1 cm) from a well grown PDA plate were cut and then introduced aseptically into 250 ml liquid media. These were allowed to grow at room temperature and upon assuming steady growth rate were used to inoculate a 20 l fermentor. *Stereum* species (JO5289) was fermented in a 100 l

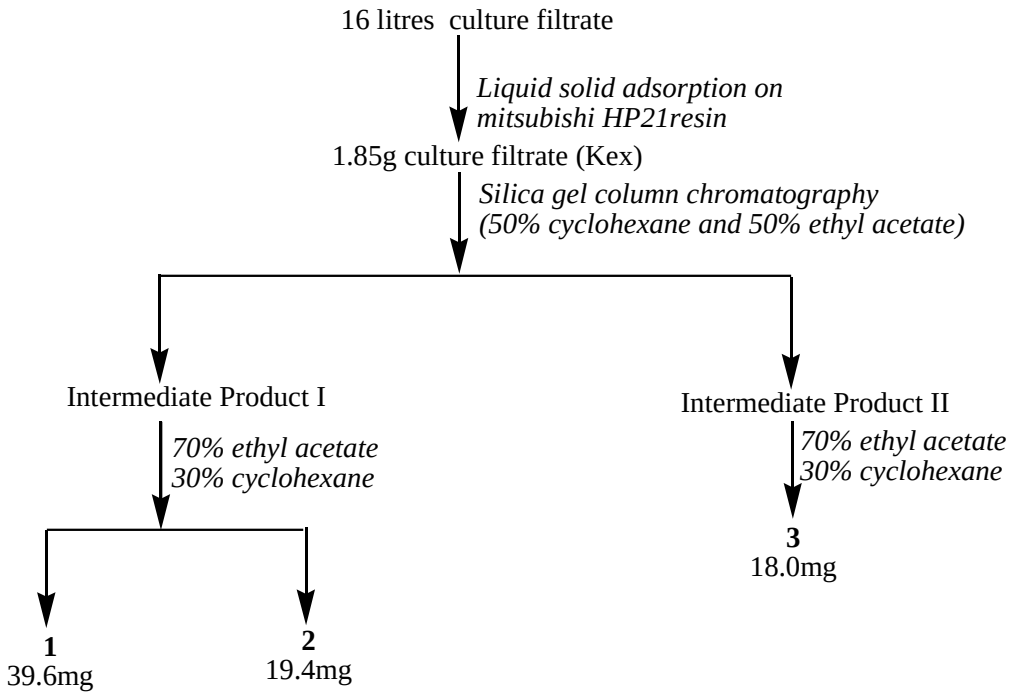

Figure 1. Purification scheme for compounds 1, 2 and 3 from *Stereum* species (JO5289).

fermentor using fermentor type U100 (Braun, Melsungen) with online system control Micro-MFCS 3.2 monitoring O_2-uptake, CO_2-production and oxygen partial pressure in the medium. The medium was aerated at 15 l per minute and stirred at 120 rpm and at 24°C for 6 days.

The pH value of the culture broth during the fermentation period was determined. This was done by withdrawing samples at regular intervals and pH measured using a pH meter (CG 825, Schott, Hofheim). The growth was also closely monitored and evaluated daily to check for glucose depletion using glucose testing stripes. Immediately glucose levels in the culture were depleted, the growth was stopped and the mycelia were separated from culture filtrate by vacuum filtration. From culture filtrate, crude extracts for extracellular secondary metabolites was prepared.

Preparation of crude extracts from culture filtrate

The culture filtrate was passed through a resin (Mitsubishi HP21 DIAION resin) packed in a column thrice. Once all the culture filtrate passed through the resin, the trapped secondary metabolites were eluted with 3 l acetone followed by 2 l of methanol. The eluents were collected and concentrated into a residual aqueous remain, which was further extracted five times with ethyl acetate. The combined ethyl acetate solution extract was dried using anhydrous sodium sulphate and concentrated using rotary evaporator under reduced pressure. The dried crude extract was weighed and kept at 4°C awaiting further analysis.

Purification and identification of larvicidal compounds

The crude extract was tested for larvicidal activity against *A. aegypti* before fractionation and purification of the active compounds using repeated silica gel column chromatography. The solvents used were cyclohexane, ethyl acetate and methanol in ratios of increasing polarity. The eluents from the column were pooled into five intermediate fractions, concentrated under reduced pressure and tested for larvicidal activity to track the active compounds. The active intermediate fractions were further subjected to repeated column chromatography until pure compounds were obtained (Figure 1).

Structures of purified larvicidal compounds (Figure 2) were elucidated using nuclear magnetic resonance (NMR) spectroscopic experiments. NMR experiments were performed on 300 MHz Bruker AVANCE NMR spectrometer. The spectra were recorded in deuterated chloroform ($CDCl_3$) and the chemical shifts were recorded in parts per million (ppm) relative to the solvent signals. The deuterated chloroform was referenced according to the central line at δ 7.260 in the ^1H NMR spectrum and at δ 77.23 in the ^{13}C NMR. The purified compounds were dissolved in deuterated chloroform ($CDCl_3$) in a clean vial. The solution was then transferred to an NMR tube and was placed in the probe for analysis. The same sample was used to obtain the spectra (^1H, ^{13}C, DEPT, NOESY, HMBC and COSY) data for the compound. Data was acquired from the NMR machine as computer print out. From the spectroscopy experiments, structures were proposed based on the interpretation of the spectra and reported literature values.

Mosquito larvicidal assay

Eggs of the *A. aegypti* mosquitoes were hatched by submerging them in de-chlorinated tap water at a temperature in the range 25–27°C, as described by the standard WHO protocol (1973). Both the crude extracts and the purified samples were subjected to mosquito larvicidal activity test against late 3rd and early 4th in star larvae of *A. aegypti*. The larvicidal tests were carried out in the Integrated Biotechnology Research Laboratory (IBRL), Egerton University. *A. aegypti* was used as a serotome laboratory specimen given the ease of rearing and the stability of its larval stage. The data generated can be extended to other species of mosquito when the iden-

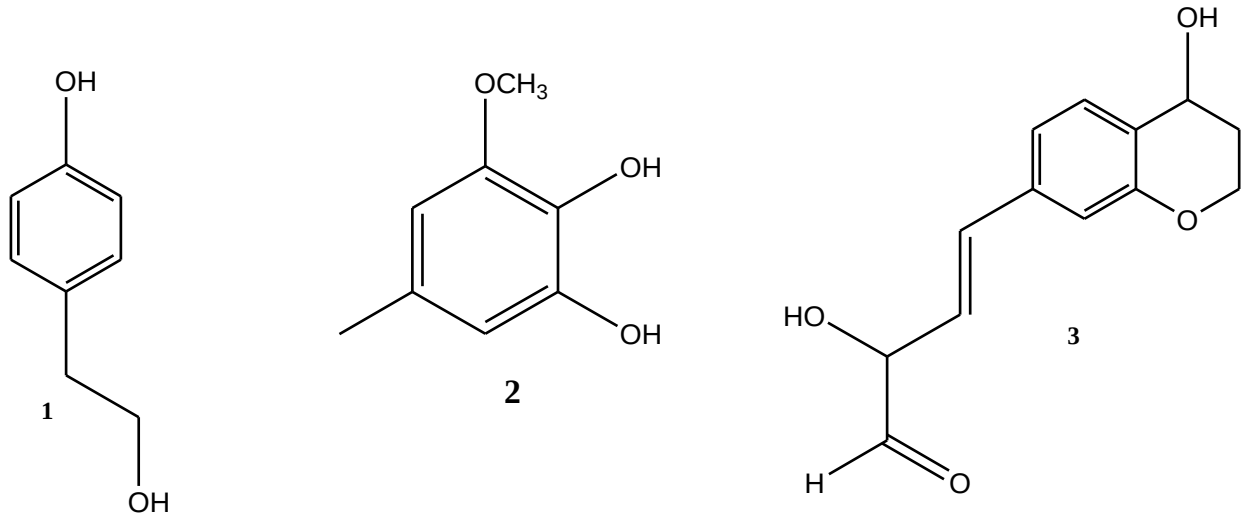

Figure 2. Chemical structures for purified compounds 1, 2 and 3.

tified compounds are to be developed for field larvicidal assays.

Standard methods for assaying larvicidal activity were conducted according to the WHO manual (WHO, 2005). Bioassays were carried out in five replicates using 10 larvae for each assay. Varying range of concentrations (2, 5, 10, 20, 50 and 100 ppm) of the crude extracts, fractions and purified compounds were tested against the late third and/or early fourth instar larvae of *A. aegypti*. Aqueous solutions of methanol were employed as the positive control experiments. The larvae were placed in test plastic pots containing 4 ml of the test solution. The number of dead larvae was determined after 2, 4, 8, and 24 h to monitor the larval mortality.

Data analysis

The analysis of larvicidal assay data was carried out using regression analysis. The LC_{50} and LC_{90} values which were the concentration values for killing 50 and 90% larvae at 8 and 24 h, respectively, were calculated. Statistical analysis of the data was performed according to the method of Lentner et al. (1982). LC_{50} and LC_{90} were calculated using multiple linear regression and data are expressed as mean±standard deviation (Finney, 1971).

RESULTS

Taxonomy

The culture grew in the PDA solid medium as moist brown mycelium that covered a diameter of 5 cm in days. The hyphal strands were found to be septate but with no pronounced clamp connections. It was a resupinate fungus with encrusted hyphae (2.5 µm wide) and quite characteristic closely packed septate cystidia. It also had 4-spored basidia (5 µm wide and 12-15 µm long) producing abundant pin-head basidiospores which were non-amyloid and globose to subglobose (2.5-3 µm wide by 5-6 µm). The observations fitted descriptions for *Stereum* species by Reid (1965).

Growth and crude extract yield

The *Stereum* species (JO5289) was grown in defined liquid nutrient media (10 g/L malt extract, 0.4 g/L yeast extract and 0.4 g/L glucose) and produced extra cellular secondary metabolites that were harvested as culture filtrate. The yield from 20 l fermentation led to production 16 l of culture filtrate, which further led to 1.85 g of brown semi-solid crude extract after concentration using a rotary evaporator. This translates to 0.09 g per litre of culture filtrate. The crude extract from culture filtrate showed a stronger larvicidal activity than the mycelia crude extract. Hence the culture filtrate crude extract was investigated further while the mycelia crude extract was discarded because it did not have any larvicidal activity in an initial preliminary screening. The larvicidal activity of the culture filtrate crude extract is given in Table 1 as percent mortality when the assay was evaluated after 2, 4, 8 and 24 h.

Purification of larvicidal compounds

Glass column of 2.5 × 25 cm was packed with 40 g of silica gel (60–120 mesh, Macharey Nagel, Germany) as a slurry using cyclohexane. After testing the crude extract, the remaining amount (1.5 g) was prepared into slurry with 1 g of silica gel. It was loaded in the column and eluted with increasing polarities of organic solvents: cyclohexane:ethyl acetate (9:1), cyclohexane:ethyl acetate (7:3), cyclohexane:ethyl acetate (1:1), cyclohexane:ethyl acetate (3:7) and ethyl acetate (100%) and five fractions were collected, respectively. The total solvent mixture volume was 500 ml and the eluent fraction collected was approximately 500 ml. The fractions were concentrated and tested for larvicidal activity and third fraction that eluted with 1:1 solvent ratio showed the strongest

Table 1. Larvicidal activities (% mortality) and lethal concentrations (ppm) of the culture filtrate crude extract (Kex).

Concentration (ppm)	2 h	4 h	8 h	24 h
0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
10	0.0±0.0	0.0±0.0	0.0±0.0	20.2±0.5
20	0.0±0.0	0.0±0.0	21.0±1.3	80.0±1.0
50	0.0±0.0	20.2±1.4	41.3±1.5	100.0±0.0
100	0.0±0.0	31.0±1.5	51.6±1.5	100.0±0.0
Lethal concentrations (ppm)				
LC_{50}	-	-	100.0±1.2	35.9±0.7
LC_{90}	-	-	185.5±1.6	87.2±0.9

Table 2. Larvicidal activities (% mortality) for tyrosol (1) during the assay period.

Concentration (ppm)	2 h	4 h	8 h	24 h
0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
2	0.0±0.0	0.0±0.0	0.0±0.0	20.0±1.2
5	0.0±0.0	0.0±0.0	0.0±0.0	40.7±1.2
10	0.0±0.0	0.0±0.0	0.0±0.0	52.0±1.0
20	0.0±0.0	0.0±0.0	21.0±0.9	59.5±0.9
50	0.0±0.0	10.0±1.3	30.0±1.1	80.0±1.1
100	0.0±0.0	10.0±1.3	40.1±1.2	100±0.0

activity and it was further subjected to silica gel chromatography. The repeated silica gel was eluted with the same solvent system which led to the larvicidal compounds eluting with the solvent ration 30% cyclohexane and 70% ethyl acetate to afford two intermediate fractions I and II (Figure 1). From intermediate fraction I, two pure compounds were purified tyrosol (1) (39.6 mg) and 3-methoxy-5-methyl-1,2-benzenediol (2) (19.4 mg) while intermediate fraction II afforded one compound identified as 2-hydroxy-4-(4-hydroxy-chroman-7yl) but-3-enal (3) (18.0 mg). The compound was identified by interpretation of the ^{1}H and ^{13}C NMR spectral data and comparison with reported literature values.

Mosquito larvicidal results

The larvicidal activity of the crude extract and the pure compounds at different concentrations (2, 5, 10, 20, 50 and 100 ppm) was evaluated against the late 3rd instar and early 4th instar larvae of *A. aegypti* after 2, 4, 8 and 24 h of exposure. The results are given in Tables 1 to 4 while the corresponding LC_{50} and LC_{90} values are presented in Table 5. The results of the regression revealed that the mortality rate was positively correlated with the concentration range used, at 8 and 24 h of exposure for both the crude extracts and purified compounds. The regression coefficients in all the cases were close to unity (1.0).

The crude extract gave significant activity at 24 h exposure (Table 1), which showed LC_{50} and LC_{90} of 35.9 and 87.2 ppm, respectively (Table 5). These values of mortality rates at 24 h were more pronounced than at 8 h exposure when the LC_{50} and LC_{90} were 100.0 and 185 ppm, respectively. The results were significant at $p < 0.05$. However, at 2 and 4 h of exposure, there was no significant mortality rate observed for the crude extracts and the pure compounds. Therefore the results were not regressed and there were no corresponding LC_{50} and LC_{90} values. The pure compounds showed varied larvicidal efficacy with all of them showing significant activity at 8 and 24 h of exposure. Mosquito larvicidal efficacies of the purified compounds were relatively higher than that for the crude extract. This suggested that the purification process produced more active compounds, an indicator that bioactivity guided fractionation and purification led to the active chemical compounds responsible for the larvicidal activity that was in the extract.

Tyrosol (1) showed a strong larvicidal activity at 24 h for the concentration ranges tested (Table 2) with LC_{50} and LC_{90} of 26.7 and 85.3, respectively (Table 5). At 8 h of exposure, compound (2) showed relatively weaker larvi-

Table 3. Larvicidal activities (% mortality) for 3-methoxy-5-methyl-1,2-benzenediol (2) during the assay.

Concentration (ppm)	2 h	4 h	8 h	24 h
0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
2	0.0±0.0	0.0±0.0	0.0±0.0	20.0±0.8
5	0.0±0.0	0.0±0.0	10.0±0.9	30.0±0.9
10	0.0±0.0	0.0±0.0	20.0±0.8	50.0±1.1
20	0.0±0.0	20.0±1.1	50.0±1.0	90.0±1.2
50	10±	40.0±1.0	60.0±1.2	100.0±0.0
100	10±	50.0±1.3	70.0±1.2	100.0±0.0

Table 4. Larvicidal activities (% mortality) for 2-hydroxy-4-(4-hydroxy-chroman-7yl) but-3-enal (3) during the assay.

Concentration (ppm)	2 h	4 h	8 h	24 h
0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
2	0.0±0.0	0.0±0.0	0.0±0.0	20.0±1.5
5	0.0±0.0	0.0±0.0	20.0±0.2	40.0±1.2
10	0.0±0.0	0.0±0.0	30.0±1.2	60.0±0.9
20	0.0±0.0	20.0±0.8	60.0±0.4	80.0±1.1
50	10.0±0.0	40.0±0.9	70.0±0.4	90.0±1.4
100	10.0±0.0	50.0±1.2	90.0±0.9	100±0.0

Table 5. Lethal concentrations: LC_{50} and LC_{90} (ppm) for the purified compounds 1, 2 and 3 after 8 and 24 h.

Compound name	LC_{50} (ppm)		LC_{90} (ppm)	
	8 h	24 h	8 h	24 h
Tyrosol (1)	127.0±1.4	26.7±0.2	235.0±1.8	85.3±0.6
3-Methoxy-5-methyl-1,2-benzenediol (2)	63.4±0.6	17.3±0.2	136.4±1.6	83.5±0.7
2-Hydroxy-4-(4-hydroxy-chroman-7yl) but-3-enal (3)	43.0±0.2	14.5±0.2	100.0±1.3	82.9±0.6

cidal activity, with LC_{50} and LC_{90} values of 235.0 and 127.0 ppm. Compound (2) (3-methoxy-5-methyl-1,2-benzenediol) showed larvicidal activity at 8 and 24 h of exposure that could the correlated to the concentration range used (Table 3). This gave LC_{50} and LC_{90} for (2) of 136.4 and 63.4 ppm, respectively, at 8 h of exposure. While at 24 h of exposure, it had LC_{50} and LC_{90} of 83.5 and 17.3 ppm, respectively. Compound (3) (2-hydroxy-4-(4-hydroxy-chroman-7yl) but-3-enal) also showed larvicidal activity at 8 and 24 h of exposure that correlated with the concentration ranges used in the bioassay (Table 3). The LC_{50} and LC_{90} for (3) were 100.0 and 43.0 ppm at 8 h of exposure, respectively while at 24 h of exposure the values were 82.9 and 14.5 ppm, respectively. At 24 hours of exposure it was clear that the order of activity was 3 > 2 > 1 > crude extract. This is evident when the LC_{50} and LC_{90} values are compared, but at 8 h of exposure the crude extract appear to be stronger than tyrosol (1).

Structure elucidation

The crude extract was closely analysed using TLC and further purified using column chromatography. From these only three compounds tyrosol (1), 3-methoxy-5-methyl-1,2-benzenediol (2) and 2-hydroxy-4-(4-hydroxy-chroman-7yl) but-3-enal (3). The data obtained from NMR was correlated to the literature and the structures determined.

DISCUSSION

Stereum species like most basidiomycetes produce biologically active secondary metabolites when grown in laboratory defined submerged nutrient cultures (Lorenzen and Anke, 1998). Such compounds have been shown to have mosquito larvicidal activities from submerged cultures of the mushroom *Cyptotrama asprata* (Njogu et al., 2009). Here extra-cellular secondary metabolites from

cultures of *C. asprata* led to the purification of a strongly larvicidal compound; (oxiran-2-yl)methylpentanoate. This compound showed strong larvicidal activity against the *A. aegypti* larvae with LC_{50} of 1.50 ppm and an LC_{90} of 1.90 ppm after 24 h. The findings were found to mirror the results reported in this paper, where the extra-cellular secondary metabolites were in the culture filtrate of *Stereum* species (JO5289). Like in the case of *C. asprata*, the crude extracts from *Stereum* species (JO5289) were purified using column chromatography to give three compounds; tyrosol (1), 3-methoxy-5-methyl-1,2-benzenediol (2) and 2-hydroxy-4-(4-hydroxy-chroman-7yl) but-3-enal (3). These compounds were found to have larvicidal activity against *A. aegypti*.

From literature reports tyrosol (1) had been obtained from mycelia of an ascomycetes fungus *Cordyceps ophioglossoides* as an estrogenic substance (Kawagishi et al., 2004). It has also been obtained from phytopathogenic fungus *Ceratocystis adipose* as an antioxidant (Guzman-Lopez et al., 2007). Its production had been reported in other *Ceratocystis* species before, including *Ceratocystis fimbriatacoffea* (Gremand and Tabacchi, 1996), *Ceratocystis clarigera, Ceratocystis ips* and *Ceratocystis huntii* strains (Ayer et al., 1986).

Tyrosol has been reported to have pharmacological activity particularly antioxidant activity. It has also been used in atherosclerosis treatment, protecting low density lipoproteins (LDL) from oxidation which play a role in the initiation and progress of cardiovascular diseases (Guzman-Lopez et al., 2007). Phytotoxic activity of tyrosol has been observed in lettuce leaves and certain toxicities in mice (Ayer et al., 1986). However, to our knowledge this was the first time the larvicidal activity was reported for tyrosol.

Compound 3 is a chromanone derivative and such compounds have been isolated from fungi and plants (Lee et al., 2007). Among the known naturally occurring chromanones, nearly all have alkyl substituent at the C2 or C3 position. They have also been synthesised by chemical methods for their extensive bio-activities such as antifungal, antibacterial, antitumour and antiviral activities (Li et al., 2007). The three compounds (1, 2 and 3) belong to two classes of compounds, the phenols and chromans (a derivative of flavonoids). The observed LC_{50} and LC_{90} values were significant and were within the range of those previously isolated larvicidal compounds reported from the literature. Ocimenone, a monoterpenoid isolated from *Tagetes minuta* oil exhibited LC_{50} value of 40 ppm and a triterpene from *Azadiracta indica* showed an LC_{50} value of 21.0 ppm (Geris et al., 2008). The observed LC_{50} and LC_{90} values for compounds 1, 2 and 3 were significant since they were within the range of those previously isolated larvicidal compounds reported from the literature. We wish to state that these com-pounds have no mammalian toxicities reported in the literature.

In conclusion the findings from this study reveal that cultures of *Stereum* species (JO5289) have larvicidal compounds that can be used to control mosquitoes and by extension control of spread of diseases by the vectors.

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