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(±)-Schefflone: a trimeric monoterpenoid from the root bark of *Uvaria scheffleri*

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Abstract

The trimeric monoterpene and mildly mosquito larvicidal agent, (±)-schefflone, that is an apparent derivative of the antiparasitic aromatic monoterpene espintanol, was isolated from the antimalarial extracts of the root bark of *Uvaria scheffleri,* together with espintanol. Structural determination of (±)-schefflone was achieved from spectroscopic data and confirmed by single-crystal X-ray diffraction analysis. (±)-Schefflone can be considered a product of a non-enzymatic Diels–Alder-type cycloaddition reaction of the quinonemethide derivative of espintanol as the diene and dienophile.

Keywords: *Uvaria scheffleri*; Annonaceae; Trimeric monoterpene; (±)-Schefflone; Espintanol; Mosquitocides

1. Introduction

Of the 16 *Uvaria* species occurring in Tanzania, nine, including *Uvaria scheffleri* Diels, possess antimalarial activity (Nkunya et al., 1991a; Verdcourt, 1971; Vollesen, 1980). *U. scheffleri* is used traditionally for treating fevers (Kokwaro, 1993) and previous phytochemical investigations of the stem barks yielded 3-farnesylindole as the antimalarial constituent, the condensed chalcones (±)-schefflerin (**1**) and (±)-isoschefflerin (**2**) and their apparent precursor 2′,6′-dihydroxy-3′,4′-dimethoxychalcone, 2′-hydroxy-3′,4′,6′-trimethoxychalcone and D:B-friedoolean-5-en-3β-ol (Nkunya et al., 1990). (±)-Schefflerin and (±)-isoschefflerin were considered to be products of Diels–Alder-type cycloaddition reactions involving 2′,6′-dihydroxy-3′,4′-dimethoxychalcone and the monoterpene β-ocemene as the dienophile and diene, respectively (Nkunya et al., 1990). As part of our investigations for novel and biologically active natural products from Tanzanian Annonaceae species (Makangara et al., 2002; Nkunya et al., 2000), several antifungal and antibacterial flavonoids were recently isolated from the stem barks of *U. scheffleri* (Innocent, 2002). We have now analysed the antimalarial extract of the root barks and obtained the trimeric monoterpenoid (±)-schefflone (**3**) and its apparent precursor espintanol (**4**) that was first obtained as the trypanosomal and leishmanicidal agent of the spruce *Oxandra espintana* (Hocquemiller et al., 1991). The isolation and structural determination of (±)-schefflone are hereby reported. (±)-Schefflone was mildly active against *Anopheles gambiae* mosquito larvae and inactive in the antimalarial tests.

2. Results and discussion

Repeated chromatography of the pet ether extract of the root barks yielded the aromatic monoterpene espintanol (**4**) (Hocquemiller et al., 1991) and the trimeric monoterpenoid (±)-schefflone (**3**) (m.p. 210 °C and MS, $[M]^+$ at m/z 624.3309, $C_{36}H_{48}O_9$) whose structure was established on the basis of its spectroscopic properties and then confirmed by single-crystal X-ray diffraction analysis, which also revealed that the compound was racemic. Thus, the ^1H and ^{13}C NMR spectra (Tables 1 and 2) displayed signals due to a carbonyl function, six methoxy, and three isopropyl groups. However, instead

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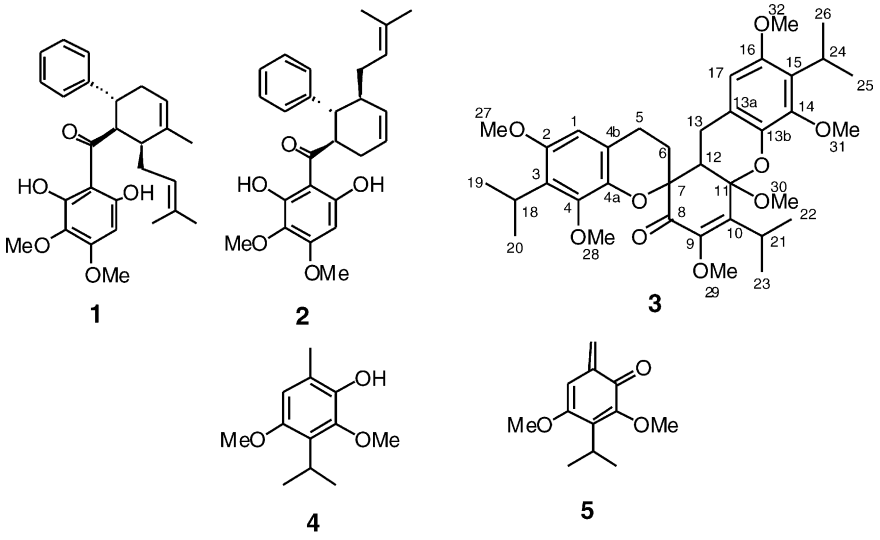


Table 1
^{1}H NMR spectral data for (±)-schefflone (**3**)

H	δ	*J* (Hz)	H	δ	*J* (Hz)
1	6.30	*s*	21	3.07	*sept*, 7.0
5α	2.52	*ddd*, 16.4, 10.9, 5.5	22	1.32[a]	*d*, 7.0[a]
5β	2.71	*ddd*, 16.4, 5.3, 4.3	23	1.1	*d*, 7.0
6α	2.79	*ddd*, 13.7, 5.5, 4.3	24	3.50	*sept*, 7.1
6β	2.16	*ddd*, 13.7, 10.9, 5.3	25	1.33[b]	*d*, 7.1
12	2.92	*dd*, 12.2, 4.9	26	1.30[b]	*d*, 7.0
13α	3.28	*dd*, 15.8, 4.9	27	3.75	*s*
13β	2.59	*dd*, 15.8, 12.2	28	3.95	*s*
17	6.35	*s*	29	3.70	*s*
18	3.56	*sept*, 7.1	30	3.43	*s*
19	1.35[a]	*d*, 7.1	31	3.90	*s*
20	1.31[a]	*d*, 7.1	32	3.75	*s*

[a] Assignment resolved by NOE.
[b] Assignments may be interchanged.

Table 2
^{13}C NMR spectral data for (±)-schefflone (**3**)

C	δ	C	δ	C	δ
1	106.55	11	102.28	21	27.50
2	151.84	12	43.16	22	21.44[a]
3	128.80	13α	25.98	23	20.32
4	146.29	13α	120.99	24	25.11
4a	141.10	13b	140.55	25	21.36[a]
4b	118.31	14	146.36	26	21.36[a]
5	21.94	15	128.94	27	55.87
6	28.76	16	152.86	28	61.07
7	81.16	17	105.78	29	58.95
8	194.0	18	25.15	30	49.49
9	148.49	19	21.16[a]	31	61.32
10	147.98	20	21.34[a]	32	55.84

[a] Interchangeable assignments.

of the expected three doublets for the corresponding isopropyl methyl groups, six such signals were observed in the ^{1}H NMR spectrum (Table 1). This indicated that all the isopropyl methyl groups were diastereotopic, because of **3** being a chiral compound. Long-range heteronuclear correlations observed in the HMBC spectrum established the assignment of the methoxy and isopropyl methyl carbon atoms. HMBC correlations also revealed that two methoxy groups flanked each of the isopropyl units, being substituted in a di-*ortho* fashion. Furthermore, NOE interactions showed that the two aromatic CH units ($\delta_H = 6.30$ and 6.35, and $\delta_C = 107$ and 106.2 ppm, respectively, Tables 1 and 2) were *ortho* to one of the methoxy groups, as shown in partial structures A-1 and A-2 (Fig. 1). The absence of additional vinylic proton and unsaturated carbon signals in the ^{1}H NMR spectrum indicated that the remaining di-*ortho* positions relative to the isopropyl groups were substituted in accordance with partial structure B (Fig. 1), whereby the high field position of one of the δ_{OCH_3} signals at 3.43 ppm (Table 1) indicated that the corresponding methoxy group was bonded to a non-aromatic carbon, whose ^{13}C NMR absorption as revealed from HMBC interactions appeared at a particularly low field (δ 102.7), in accordance with its ketalic nature (Breitmaier and Voelter, 1990) (partial structure B, Fig. 1).

The coupling patterns of the ^{1}H NMR signals in the range δ 2.1–3.3 showed the presence of two spin systems in which the protons, some of them being diastereotopic, experienced effects of a restricted rotation that would be caused by configurations of ring systems, such as in partial structures C and D (Fig. 1).

HMBC and NOESY interactions (Figs. 2 and 3) confirmed the assembly of the substructures A–D and the relative stereochemical configuration of **3**, in which H-6, C-12, and C-30 are on the same side of the central cyclohexenone ring. The methoxy-substituted ketalic C-

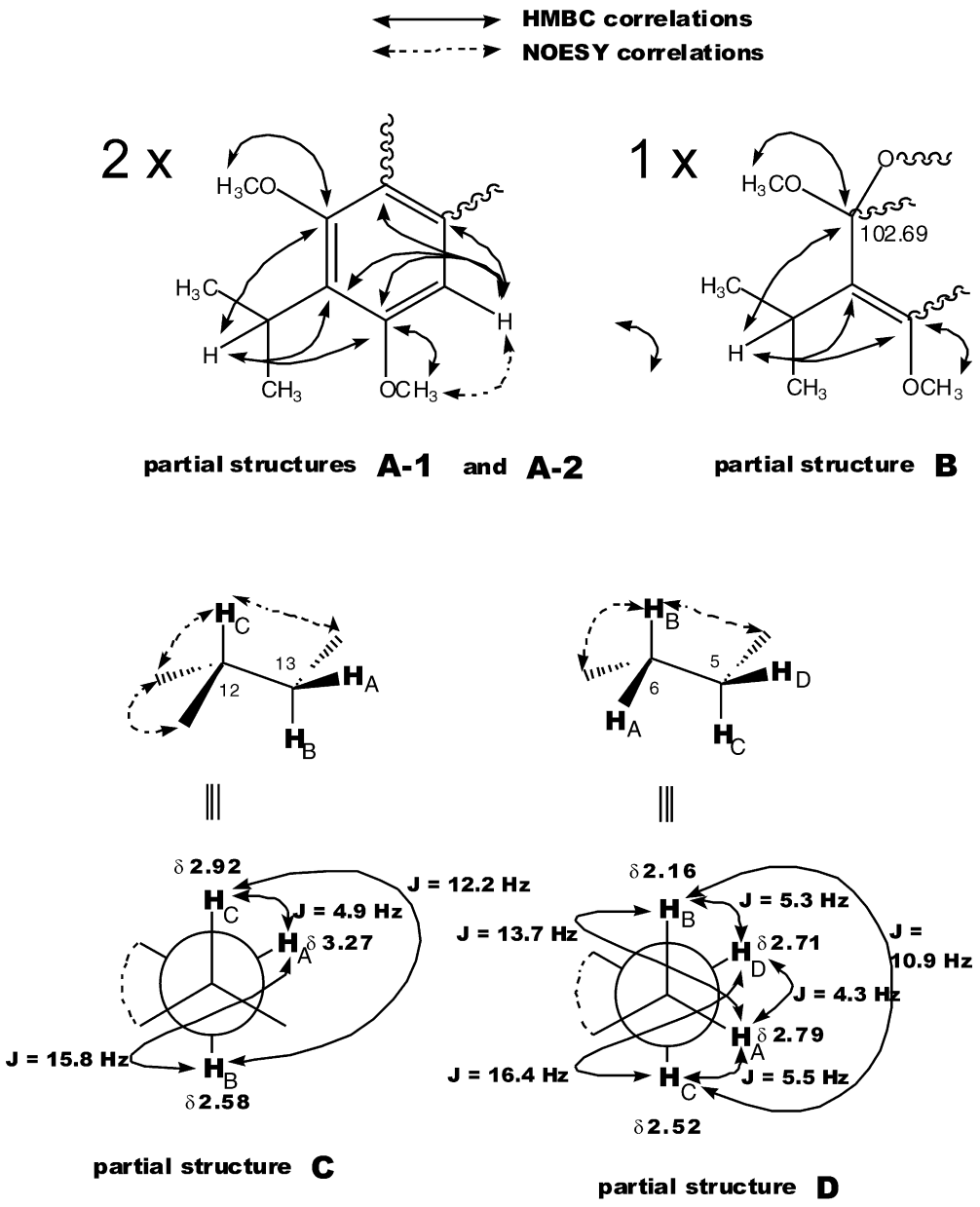
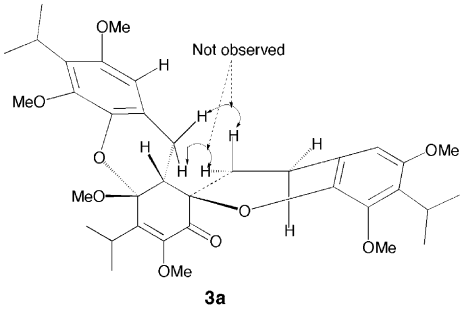


Fig. 1. Partial structures of (±)-schefflone (**3**) also indicating ^{1}H NMR spin systems at δ 2.1–3.3).

11 in partial structure B forms the bridgehead between partial structures A-1, B, and C, therefore confirming that partial structure C is part of a rigid ring system. Long-range heteronuclear correlations between H-12 and H-6β and the carbonyl carbon [$\delta_C = 194.0$ ppm, ν_{max} (>C=O) = 1688 cm^{-1}] conformed to the latter carbon forming linkage between partial structures B and D. The relative stereochemical configuration at C-7 as determined from NOESY interactions between H-6β and H-12 ruled out the other possible stereochemical structure **3a** for (±)-schefflone.



3a

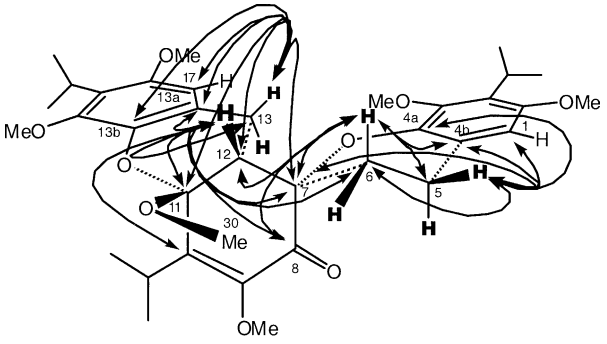


Fig. 2. Important long range H/C correlations observed in the HMBC plot for (±)-schefflone (**3**).

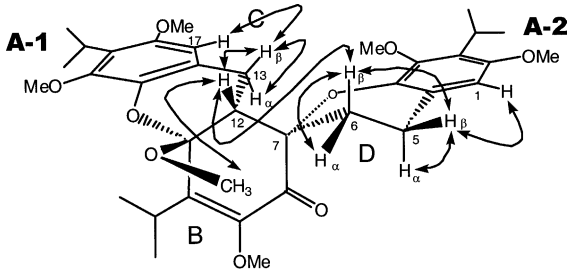


Fig. 3. NOE interactions observed in the NOESY spectrum of (±)-schefflone (**3**) showing the assembly of partial structures A–D.

The presence of even mass fragment ion peaks in the MS at *m/z* 416 ($C_{24}H_{32}O_6$ = [M−**5**]$^+$·) and 208 ($C_{12}H_{16}O_3$ = [**5**]$^+$·) indicated a retro-Diels–Alder fragmentation process expected for compound **3** having taken place, while the other even mass fragment ion peak at *m/z* 592 ($C_{36}H_{48}O_7$) suggested the subsequent extrusion of oxygen from the molecular ion, thereby indicating the presence of a heterocyclic oxygen atom.

The stereostructure **3**, as deduced from NOE interactions, was further confirmed by single-crystal X-ray diffraction analysis. The crystals had the space group $P2_1/c$, indicating that the compound is racemic. Fig. 4 shows the PLUTON (Spek, 2001) drawing of the two configurations of (±)-schefflone as determined by X-ray crystallographic analysis.

(±)-Schefflone can be envisaged to be a trimer of 3-isopropyl-2,4-dimethoxy-6-methylene-cyclohexa-2,4-dienone (**5**) having resulted from Diels–Alder-type cycloaddition reactions of **5**, a quinonemethide derivative of espintanol (**4**). The fact that (±)-schefflone is racemic is an indication of its formation having involved a concerted cycloaddition reaction, with or without enzymatic catalysis. It is unreasonable to consider (±)-schefflone being an artifact, since the relatively unstable **5** has never been isolated as a natural product. Furthermore, compound **5** cannot be formed ex situ from the more stable aromatic compound **4**. Thus, no traces of (±)-schefflone were detected even when either a crude fraction containing espintanol (**4**), or purified espintanol were left to stand at room

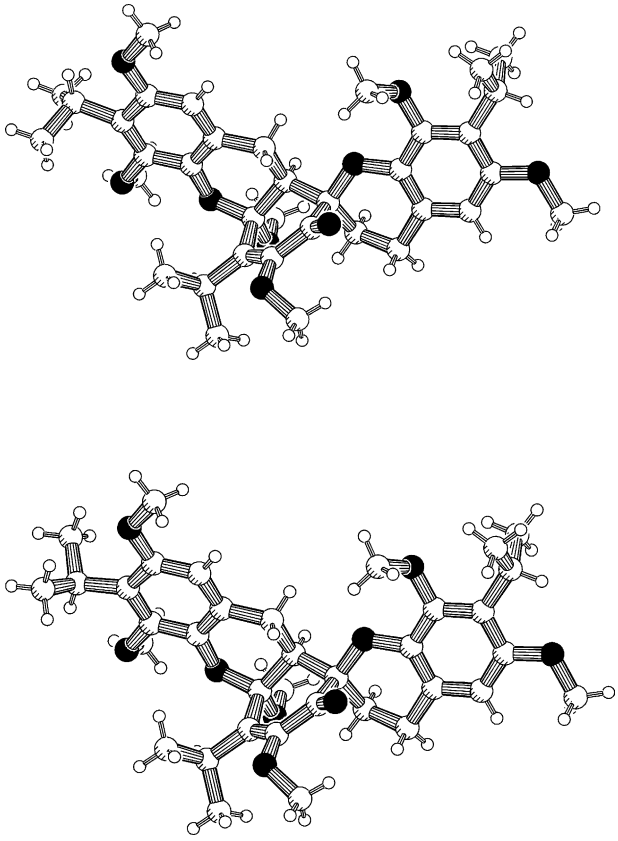


Fig. 4. PLUTON (Spek, 2001) drawing of the two conformations of (±)-schefflone (**3**) as determined by X-ray crystallographic analysis.

temperature for several days. This and the fact that (±)-schefflone was detected even in freshly obtained crude extracts further indicated that the compound is a true natural product.

The condensed chalcones (±)-schefflerin (**1**) and (±)-isoschefflerin (**2**), and other natural products that were previously isolated from the stem bark of *U. scheffleri* (Nkunya et al., 1990) were not detected in the root bark. Similarly, (±)-schefflone (**3**) and espintanol (**4**) have neither been detected in the stem bark nor in the leaf extracts of this plant species (Innocent, 2002).

It is still debatable as to whether the biosynthesis of racemic natural products proceeds enzymatically or not. In fact several natural products that would be formed through Diels–Alder-type cycloaddition reactions, like artonins C and D (Hano et al., 1990) or tanzanene (Nkunya et al., 1991b), have been obtained in optically pure forms, thus indicating an enzymatic biosynthetic pathway for these compounds. It is interesting to note that optically impure, non-racemic natural products also occur in plants, like 2′,3′-epoxyasteranthine that was recently obtained from *Asteranthe asterias* (Annonaceae) (Nkunya et al., 1996) and later from *A. lutea*. Indeed racemic plant natural products that would be considered to be products of Diels–Alder-type cycloaddition reactions have been known for a long time now

(e.g. Stipanovic et al., 1978; Pancharoen et al., 1987; Nkunya et al., 1990). Hence, there is now even circumstantial evidence on the existence of *dielsalderase* enzymes that could catalyze Diels–Alder cycloaddition reactions in nature (Sanz-Gervera et al., 1993). The fact that other Diels–Alder-type cycloaddition products [(±)-schefflerin (**1**) and (±)-isoschefflerin (**2**)] occur in *U. scheffleri* (Nkunya et al., 1990) is an indication of the possibility of this plant species possessing *dielsalderase* type enzyme systems that would be responsible for biosynthetic processes towards the racemic secondary metabolites.

(±)-Schefflone showed mild activity against *A. gambiae* mosquito larvae, displaying LC_{50} values of 0.9305, 0.0183 and 0.0005 mg/ml after 48, 72 and 96 h respectively. However, these results are just preliminary, as the activity was not compared with any standard reference compound.

3. Experimental

3.1. General experimental procedures

^1H and ^{13}C NMR: 600 and 150 MHz, respectively, in $CDCl_3$, internal standard TMS (^1H NMR) and solvent signal (^{13}C NMR); EI mass spectra at 70 eV with direct injection; FTIR in $CHCl_3$. Unless key ions only ions >20% and m/z >100 are presented. TLC: Kieselgel 60 F_{254} and detection by UV and anisaldehyde/heat (Stahl, 1969); CC: Silica gel 60 [Merck], *n*-hexane/EtOAc (19:1 v/v)]. Melting point is uncorrected.

3.2. Plant materials

The root barks of *U. scheffleri* Diels were collected in December 1999 from Kwamgongo village near Mombo town, besides the road to Lushoto, Tanzania. Mr. L.B. Mwasumbi of the Herbarium of the Department of Botany (University of Dar es Salaam) identified the plant species on site and confirmed it at the above Herbarium, where a voucher specimen is preserved.

3.3. Extraction and isolation

Air-dried and powdered root barks were soaked (2×48 h) in pet ether at room temp (ca. 30 °C). The conc. crude extract was partitioned by vacuum liquid chromatography (VLC) over silica gel (pet ether/EtOAc gradient) and the combined medium polar fractions upon repeated CC and then recrystallization (*n*-hexane/EtOAc) yielded (±)-schefflone (**3**). Repeated CC of the filtrate gave espintanol (**4**) (Hocquemiller et al., 1991) as yellow oil (850 mg) and a mixture of β-sitosterol and stigmasterol (45 mg).

3.4. (±)-Schefflone (3)

Creamy crystals, yield, 200 mg, m.p. 210 °C, $[\alpha]_D = \pm 0.0$ (*c* 0.16, $CHCl_3$); anisaldehyde/heat: brown; IR (ν_{max} cm^{-1}): 1688 (>C=O); MS, m/z (% rel. int.) 624.3309 ($[M]^+\cdot$, 29), 592 ($[M-O_2]^+\cdot$, 100), 416 ($[M-$**5**$]^+\cdot$, 58), 383 ($[416-HO_2]^+$, 39), 208 ([**5**]$^+\cdot$, 37) and 165 ($[193-C{=}O]^+$, 21); ^1H and ^{13}C NMR: Tables 1 and 2.

3.5. X-ray diffraction analysis

A colourless, transparent and regular rod-shaped crystal of **3**, with dimensions 0.44×0.14×0.10 mm, was mounted in air on a glass fiber and intensity data were collected at room temperature. An Enraf–Nonius CAD4 single-crystal diffractometer was used, MoK_α radiation, $\omega-\theta$ scan mode. Semi-empirical absorption correction (ψ-scans) (North et al., 1968) was applied. The structure was solved using the computer program CRUNCH (de Gelder et al., 1993) and was refined with standard methods (refinement against F^2 of all reflections using SHELXL97 (Scheldrick, 1997). Anisotropic parameters for all non-hydrogen atoms were used except for C-24, C-25, and C-26, which were refined isotropically due to static disorder in the corresponding fragment. All hydrogen atoms were placed at calculated positions and were refined riding on the parent atoms. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC 179447. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: (+44) -1223-336-033; e-mail: deposit@ccdc.cam.ac.uk].

3.6. Larvicidal bioassay

An. gambiae mosquito larvae from a laboratory colony of the Department of Behavioural and Chemical Ecology at the International Centre for Insect Physiology and Ecology (ICIPE) in Nairobi, Kenya were used in the assays, the colony having been originally obtained from the Ifakara Research Centre, Morogoro Region in Tanzania. Larvae were exposed in distilled water treated with a series of at least five concentrations of each test sample in acetone, kept in beakers. Twenty late third or young fourth instar larvae were used per beaker with three beakers per concentration at 25±1 °C. For each test three beakers containing distilled water and test larvae but without sample were used as controls. Observations were made at intervals of 24 h and the mortality of the larvae was monitored. The lethal concentration at which 50% of the test larvae were killed (LC_{50}) was derived graphically by plotting percentage mortalities against the logarithms of concentration.

Acknowledgements

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