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Phytoconstituents with contact toxicity and antifeedant activities from *Englerodaphne subcordata* (Meisn) Engl. (Thymelaeaceae) crude leaf extracts

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ABSTRACT

Englerodaphne subcordata (Meisn) Engl. (Thymelaeaceae) is a shrub that grows in dry evergreen forests, acacia woodlands and wooded grasslands. Phytochemical evaluation of the ethyl acetate and methanol crude leaf extracts of the plant led to the isolation of seven known compounds; β-amyrin acetate (**1**), 3-hydroxy-11-oxoolean-12-ene (**2**), dihydronitidine (**3**), dihydrochelerythrine (**4**), nagilactone (**5**), quercetin (**6**) and kaempferol-3-*O*-β-galactoside (**7**)**.** The compounds showed moderate contact toxicity and antifeedant activities against *Sitophilus zeamais* and *Prostephanus truncatus*, insect pests of stored maize.

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1. Introduction

Repeated use of synthetic pesticides has led to environmental pollution and toxicity to non-target organisms, besides toxicity to humans (Dubey et al., 2007; Kumar et al., 2007). Knowledge on the negative impacts of indiscriminate use and problems associated with synthetic insecticides have prompted research into development of new substances with insecticidal properties from plants as postharvest storage agents with lower environmental degradation characteristics (Souza et al., 2010; Ladan et al., 2013). Botanical insecticides provide effective control against insect pests that are resistant to other insecticides (Weinzierl, 2000) and they possess low mammalian toxicity, less persistence in the environment, selectivity towards target pests and non-phytotoxicity (Rosenthal, 1986; Isman, 2006).

Englerodaphne is a genus of flowering plants in the family Thymelaeaceae, which is a small family consisting of about 1200 species distributed into 67 genera (Borris et al., 1988; Beaumont, 2009). *Englerodaphne* is the largest genus in the Thymelaeaceae family with 140-160 species growing in form of perennial shrubs, under-shrubs and small trees (Beaumont *et al.*, 2009). Several species of the genus *Englerodaphne* have been used traditionally in treatment of various ailments and for insect pest control (Berhanu et al., 2006; Kosini and Nukunine, 2017).

Englerodaphne subcordata (Meisn) Engl. a species in the genus *Englerodaphne* is a shrub, up to 4 m tall, with slender branches and purple-grey outer bark that is smooth. It grows at 1400-2400 m altitude above mean sea level, in dry evergreen forests, acacia woodlands, wooded grasslands (Brinks, 2009) and is well distributed in the East and South African countries (Olaniyam et al., 2020).

In previous phytochemical analysis, extracts from *E. subcordata* (Meisn) Engl. showed antibacterial activities

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(Kim et al., 2000) while compounds from the plant were found to posses antileukemic activities (Kupchan et al., 1976; He et al., 2002). This prompted the search for compounds with insecticidal activities from *E. subcordata* (Meisn) Engl. leaves for the control of *S. zeamais* and *P. truncatus* which are serious maize storage insect pests (Win et al., 2013). This study aimed at evaluation of the chemical composition and insecticidal activities of secondary metabolites in crude leaf extracts of *E. subcordata* (Meisn) Engl..

In this communication, phytochemical evaluation of the crude leaf extracts of *E. subcordata*, led to isolation of seven known compounds characterized as; β-amyrin acetate (**1**) (Feleke and Brehane, 2005), 3β-hydroxy-11-oxoolean-12-ene (**2**) (Ali et al., 2014), dihydronitidine (**3**) (Iwasaki et al., 2006), dihydrochelerythrine (**4**) (Feng et al., 2014), nagilactone (**5**) (Davila et al., 2014), quercetin (**6**) (Aisya et al., 2017) and kaempferol-3-*O*-β-galactoside (**7**) (da Liu et al., 2009) (Fig. 1). All the isolated compounds had comparatively high to low contact toxicity and anti-feedant activities against *Sitophilus zeamais* and *Prostephanus truncatus*.

2. Experimental

2.1. Instrumentation, fine chemicals and solvents

Melting points were determined using Gallenkamp melting point apparatus (Manchester, UK). IR data were recorded on a Bruker Tensor 27 FTIR spectrophotometer (Bruker Corporation, Bremen, Germany) as KBr pellets. UV spectra were analyzed using a Shimadzu UV-2401 A spectrophotometer (Shimadzu corporation, Kyoto, Japan). NMR data were measured in $CDCl_3$ and DMSO-d_6 on a JEOL NMR instrument operating at 600 and 150 MHz, for ^1H and ^{13}C, respectively except for compounds **1** and **2** which were done using Brucker AM 300 spectrometer operating at 360 and 90 MHz, for ^1H and ^{13}C, respectively. Tetramethylsilane (TMS) was used as zero reference. The mass spectra were measured on a Hewlett-Packard 5989B mass spectrometer. Silica gel 60 (63-200 µm, Merck, Darmstadt, Germany) was used for gravity column chromatography (CC). TLC was performed on pre-coated DC Alufolien 60 F254 sheets (Merck, Darmstadt, Germany) and detected by spraying with anisaldehyde spraying agent. All solvents used were of analytical grade.

2.2. Plant material collection and identification

Leaves of *E. subcordata* (Meisn) Engl. were collected from the outskirts of Kitui town, Kitui County (Lat: 1° 22′ 30.2916″ S: Long. 37° 59′ 42.7668″ E), Kenya in April 2014. Identification and authentication was done at the herbarium section, Department of Botany, University of Nairobi by a taxonomist, Mr. P. Mutiso after comparison with authentic specimens and where voucher specimens are deposited (Reference No.GS/MU/2014).

2.3. Plant material preparation and solvent extraction

Dried ground leaves of *E. subcordata* (Meisn) Engl. (2.0 kg) were cold extracted sequentially using ethyl acetate and methanol (4.0 L x 3) each with occasional shaking using an orbital shaker set at 150 revolutions per minute lasting 24 hours. The extracts were filtered then concentrated under *vacuo,* yielding greenish-yellow (45.0 g) of ethyl acetate extract and greenish brown (100 g) of methanol extract.

2.4. Isolation of compounds from EtOAc extract of *E. subcordata* (Meisn) Engl. leaves

Ethyl acetate extract of *E. subcordata* (Meisn) Engl. leaves (35.0 g) was mixed with silica gel (25.0 g) and allowed to dry overnight. The resulting dark green mixture was chromatographed over silica gel column (5.0 x 80.0 cm, SiO_2, 250.0 g, pressure ≈ 1 bar) using solvent gradient *n*-hexane-ethyl acetate mixture (9:1, 4:1, 2:1 and 1:1), ethyl acetate and ethyl acetate-methanol (99:1 and 93:7) to give 200 fractions (each 20.0 mL). Their compositions were monitored by TLC, using solvent systems; *n*-hexane-ethyl acetate (9:1, 4:1, 2:1, 1:1) and ethyl acetate-methanol (99:1 and 97:3). Fractions showing similar TLC profiles were combined resulting into three pools (**I-III**). Pool **I** fractions 25-97 (3.40 g) showed two spots on TLC (Rf values 0.71 and 0.60; solvent system; *n*-hexane: EtOAc, 4:1) which upon further fractionation over silica gel using *n*-hexane-EtOAc (9:1) afforded compounds (**1**) (Rf = 0.71, 20 mg) and (**2**) **(**Rf = 0.60, 15.0 mg). Fractions 102-178 constituted pool II (2.0 g) which exhibited two spots that on further purification using flash chromatography (eluent: *n*-hexane-EtOAc, 4:1) afforded compounds (**3**) (Rf = 0.45, 23.3 mg) and (**4**) (Rf = 0.40, 35.0 mg). Fractions 184-245 constituted pool III (1.5 g) which showed a single spot, gave rise to compound (**5**) (33.5 mg, Rf = 0.27) after crystallization using CH_2Cl_2-MeOH (9:1).

2.5. Isolation of compounds from methanol extract of *E. subcordata* (Meisn) Engl. leaves

MeOH extract of *E. subcordata* (Meisn) Engl. leaves (56.0 g) was made into slurry with silica gel (25 g) in 200 mL MeOH. It was then flash chromatographed (5.0 x 30 cm column, SiO_2 300 g, pressure ≈ 1 bar), eluting with CH_2Cl_2 followed by CH_2Cl_2-MeOH gradient with increasing concentration of the more polar solvent (increment 3%) and elution concluded with MeOH. A total of 255 fractions each 20 mL were sampled and their homogeneity determined by TLC [eluent: CH_2Cl_2-MeOH, 98:2, 97:3, 19:1, 9:1 and 4:1; *n*-BuOH-HOAc-H_2O (BAW): 4:5:1]. Those exhibiting similar profiles were combined into three major pools (**V-VII**). Pool **V** (fractions 30-130, 3 g) mainly eluted using CH_2Cl_2 afforded more of compound **5** (55 mg). Pool **VI** (Fractions 134-183, 8.0 g) showed a major spot Rf = 0.46 (solvent system: BAW, 4:1:5) accompanied by minor impurities. This was further purified by crystallization in 5% aqueous MeOH-H_2O to give compound **6** (Rf = 0.46, 80 mg). Fractions 187-245 (10.5 g) which constituted pool **VII** afforded two spots of Rf values 0.46 and 0.33 (BAW, 4:1:5). Repeated fractionation of this pool (8.3 g, 3.5 x 60 cm, SiO_2 150 g, pressure ≈ 1.0 bar) using BAW (4:1:5, 2.0 L) and collecting 10 mL each gave further compound **6** (43.0 mg) and compound **7** (Rf = 0.33, 76.3 mg).

2.6. Physical and spectral data of compounds **1-7** isolated from EtOAc and methanol leaf extracts of *E. subcordata*(Meisn) Engl.

2.6.1. β-Amyrin acetate (**1**)

White amorphous powder, m.p. 239-241 ºC; Rf = 0.71 (silica gel, TLC, *n*-hexane-EtOAc, 4:1); EI-MS: *m/z* 468.2 $[M]^+$; ^{1}H NMR (360 MHz, $CDCl_3$) δ ppm: 4.64 dd (*J* = 11.0 Hz, 3.0 Hz, H-3), 0.84-0.82 (m, H-5), 5.15 (t, *J* = 3.6 Hz, H-12), 0.88 (s, H-23), 0.84 (s, H-24), 0.92 (s, H-25), 0.98 (s, H-26), 1.12 (s, H-27), 0.81 (s, H-28), 0.80 (s, H-29), 0.83 (s, H-30), 2.08 (s, CH_3CO_2); ^{13}C NMR (90 MHz, $CDCl_3$) δ ppm: 38.2 (C-1), 26.9 (C-2), 80.9 (C-3), 34.7 (C-4), 55.2 (C-5), 18.3 (C-6), 31.1 (C-7), 36.8 (C-8), 47.5 (C-9), 32.5 (C-10), 23.5 (C-11), 121.6 (C-12), 145.2 (C-13), 41.7 (C-14), 26.1 (C-15), 28.0 (C-16), 32.6 (C-17), 55.2 (C-18), 38.2 (C-19), 39.8 (C-20), 28.4 (C-21), 41.7 (C-22), 28.5 (C-23), 15.6 (C-24), 15.6 (C-25), 16.7 (C-26), 23.8 (C-27), 26.9 (C-28), 16.8 (C-29), 21.3 (C-30), 21.3 (CH_3CO), 171.1 (CH_3CO).

2.6.2. 3β-Hydroxy-11-oxoolean-12-ene (**2**)

White amorphous powder, m.p. 184-186 ºC, Rf = 0.60 (silica gel, TLC, *n*-hexane-EtOAc, 4:1); EI-MS: *m/z* 440.1 $[M]^+$; ^{1}H NMR (360 MHz,$CDCl_3$) δ ppm: 4.28 (dd, *J* = 12.0, 4.3 Hz, H-3), 2.54 (s, H-9), 7.29 (s, H-12), 0.93 (s, H-23), 1.11 (s, H-24), 1.20 (s, H-25), 1.08 (s, H-26), 1.17 (s, H-27), 0.81 (s, H-28), 0.85 (s, H-29), 0.87 (s, H-30); ^{13}C NMR (90 MHz, $CDCl_3$): 38.3 (C-1), 27.9 (C-2), 76.7 (C-3), 39.3 (C-4), 58.3 (C-5), 17.9 (C-6), 33.2 (C-7), 43.0 (C-8), 58.3 (C-9), 34.4 (C-10), 213.4 (C-11), 128.8 (C-12), 142.9 (C-13), 41.2 (C-14), 26.5 (C-15), 26.8 (C-16), 33.7 (C-17), 58.3 (C-18), 39.3 (C-19), 39.4 (C-20), 31.1 (C-21), 37.6 (C-22), 28.4 (C-23), 17.5 (C-24), 16.3 (C-25), 18.0 (C-26), 19.7 (C-27), 28.7 (C-28), 33.2 (C-29), 23.0 (C-30).

2.6.3. Dihydronitidine (**3**)

White crystals; m.p. 174-175 ºC (from 25% EtOAc in *n*-hexane) and Rf = 0.45 (eluent: *n*-hexane-EtOAc, 4:1); ESI-MS: *m/z* 350.0 $[M+H]^+$; ^{1}H NMR (600 MHZ, $CDCl_3$) δ ppm: 7.50 (s, H-1), 7.48 (s, H-4), 4.28 (2H, s, H-6), 6.93 (d, *J* = 8.5 Hz, H-7), 7.24 (d, *J* = 8.5 Hz, H-10), 7.69 (d, *J* = 8.4 Hz, H-11), 7.09 (d, *J* = 8.4 Hz, H-12), 3.91 (s, 8-OMe), 3.86 (s, 9-OMe), 2.58 (s, N-Me), 6.03 (s, OCH_2O); ^{13}C NMR (150 MHz, $CDCl_3$): δ ppm: 100.7 (C-1), 152.2 (C-2), 148.0 (C-3), 101.0 (C-4), 126.3 (C-4a), 124.2 (C-4b), 76.7 (C-6), 123.7 (C-6a), 110.9 (C-7), 147.4 (C-8), 166.1 (C-9), 104.3 (C-10), 126.2 (C-10a), 142.7 (C-10b), 118.6 (C-11), 120.1 (C-12), 130.8 (C-12a), 55.8 (8-OMe), 61.0 (9-OMe), 41.3 (N-Me), 104.3 (OCH_2O)

2.6.4. Dihydrochelerythrine (**4**)

White crystals, m.p. 166-168 ºC; Rf = 0.40 (eluent: *n*-hexane-EtOAc, 4:1); EI-MS: *m/z* 348 $[M-H]^+$; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm: 7.10 (s, H-1), 7.68 (s, H-4), 4.29 (2H, s, H-6), 6.93 (d, *J* = 8.5 Hz, H-9), 7.51 (d, *J* = 8.5 Hz, H-10), 7.70 (d, *J* = 8.4 Hz, H-11), 7.50 (d, *J* = 8.4 Hz, H-12), 3.92 (s, 7-OMe), 3.87 (s, 8-OMe), 2.59 (s, N-Me), 6.04 (s, OCH_2O), ^{13}C NMR (150 MHZ, $CDCl_3$) δ_{ppm}: 100.7 (C-1), 148.1 (C-2), 147.5 (C-3), 100.9 (C-4), 126.3 (C-4a), 142.7 (C-4b), 48.7 (C-6), 126.4 (C-6a), 146.1(C-7), 152.3 (C-8), 111.0 (C-9), 118.7 (C-10), 126.3 (C-10a), 124.3 (C-10b), 120.1 (C-11), 123.8 (C-12), 130.8 (C-12a), 61.1 (7-OMe), 56.8 (8-OMe), *N*-Me (41.3), 104.3 (OCH_2O).

2.6.5. Nagilactone C (**5**)

White amorphous powder, m.p. 298-301 ºC; Rf = 0.27 (silica gel, TLC, *n*-hexane-EtOAc, 4:1. EI-MS: *m/z* 362 $[M]^+$; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm: 3.62 (d, *J* = 10.2 Hz, H-1), 3.46-3.45 (m, H-2), 3.64 (dd, *J* = 10.0, 4.6 Hz, H-3), 1.86 (d, *J* = 5.4 Hz, H-5), 4.60 (d, *J* = 5.4 Hz, H-6), 4.98 (d *J* = 3.6 Hz, H-7), 7.26 s (H-11), 2.12 (sept, *J* = 6.6 Hz, H-15), 1.21 (d, *J* = 6.4 Hz, H-16), 1.10 (d, *J* = 6.4 Hz, H-17), 1.27 s (H-19), 1.36 s (H-20), 5.95 (d, *J* = 5.4 Hz, 3-OH), 6.01 (d, *J* = 4.8 Hz, 7-OH);^{13}C NMR (150 MHz, $CDCl_3$) δ ppm: 58.6 (C-1), 51.5 (C-2), 63.2 (C-3), 44.1 (C-4), 45.3 (C-5), 73.0 (C-6), 63.2 (C-7), 116.7 (C-8), 163.4 (C-9), 36.3 (C-10), 82.9 (C-11), 158.1 (C-12), 163.4 (C-14), 28.3 (C-15), 21.6 (C-16), 21.2 (C-17), 179.4 (C-18), 26.7 (C-19), 16.4 (C-20).

2.6.6. Quercetin (**6**)

Amorphous yellow powder, Rf = 0.46 (silica gel, TLC, BAW; 4:1:5), 79.0 mg, m.p. 315-317 ºC; UV λ_{max} (MeOH) nm: 354 (band I), 304, 258 (band II), $AlCl_3$: 436 (band I), 316, 270 (band II), $AlCl_3$ + HCl: 402 (band I) 308, 272 (band II), NaOMe: 394 (band I), 322, 270 (band II), NaOAc: 366 (band I), 314, 272 (band II), NaOAc + H_3BO_3: 374 (band I), 300, 260 (band II); EI-MS: *m/z* 302 $[M]^+$, ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm; 6.19 (d, *J* = 1.8 Hz, H-6), 6.41 (d, *J* = 1.8 Hz, H-8), 7.67 (d, *J* = 2.5 Hz, H-2′), 7.53 (d, *J* = 8.4 Hz, H-5′), 6.88 (d, *J* = 8.4 Hz, H-6′), 9.60 s (3-OH), 12.48 (5-OH), 9.30 (3′-OH), 9.34 (4′-OH); ^{13}C NMR (150 MHz, $CDCl_3$) δ ppm: 47.7 (C-2), 135.7 (C-3), 175.8 (C-4), 160.7 (C-5), 98.1 (C-6), 163.8 (C-7), 93.3 (C-8), 156.1 (C-9), 103.0 (C-10), 120.0 (C-1′), 115.0 (C-2′), 145.0 (C-3′), 146.8 (C-4′), 115.6 (C-5′), 121.9 (C-6′).

2.6.7. Kaempferol-3-*O*-β-galactoside (**7**)

Amorphous yellow powder, m.p. ≈ 250 ºC; Rf = 0.33 (silica gel, TLC, BAW, 4:1:5); UV λ_{max} (MeOH) nm: 354 (band I), 302, 262 (band II), $AlCl_3$: 406 (band I), 272 (band II), $AlCl_3$ + HCl: 400 (band I), 352, 300, 272 (band II), NaOMe: 396 (band I), 324, 272 (band II), NaOAc: 362 (band I), 264 (band II), NaOAc + H_3BO_3: 378 (band I), 262 (band II): IR ν_{max} (KBr) cm^{-1}, 3888, 2947, 1721, 1631, 1567, 1458, 1376, 1255, 1216, 1182, 1034, 987, 910; EI-MS: *m/z* (rel. int.) 287.6 $[M+H\text{-galactose}]^+$; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm: 6.20 (d, *J* = 1.8 Hz, H-6), 6.41 (d, *J* = 2.1 Hz, H-8), 7.57 (d, *J* = 8.5 Hz, H- 2′), 6.86 (d, *J* = 9.0 Hz, H- 3′), 6.86 (d, *J* = 8.5 Hz, H- 5′), 7.57 (d, *J* = 8.5 Hz, H- 6′), 5.48 (d, *J* = 7.2 Hz, H- 1″), 4.97-3.09 m, H-2″, 4.97-3.09 m, H-3″, 4.97-3.09 m, H-4″, 4.97-3.09 m, H-5″, 4.97-3.09 m, H-6″; ^{13}C NMR (150 MHz, $CDCl_3$) δ ppm: 156.4 (C-2), 134.0 (C-3), 180.9 (C-4), 158.6 (C-5), 99.4 (C-6), 166.0 (C-7), 95.4 (C-8), 156.5 (C-9), 107.0 (C-10), 116.1 (C-1′), 130.4 (C-2′), 107.3 (C-3′), 162.3 (C-4′), 107.0 (C-5′), 130.4 (C-6′), 102.1 (C-1″), 71.1 (C-2″), 78.4 (C-3″), 62.2 (C-4″), 74.7 (C-5″), 62.4 (C-6″).

2.7. Contact toxicity assay

The contact toxicity activities of *E. subcordata* (Meisn) Engl. leaf extract compounds were monitored according to the method of (Obeng-Ofori and Reichmuth, 1997). The experiment tested the hypothesis that applied solutions of the isolated compounds exhibit contact toxicity activities against *S. zeamais* and *P. truncatus*. Solutions of the compounds were prepared by dissolving in acetone to obtain solutions of concentrations 10, 30, 50 and 100. Three to seven day old *S. zeamais* or *P. truncatus* of mixed sex were first transferred into glass petridishes lined with moist filter paper and chilled for three minutes to reduce their activity and enable topical treatment to be carried out. The treatments were laid out in a completely randomized design replicated 3 times per treatment. The immobilized insects were picked individually for treatment and 0.5 µL of the solution was applied to the dorsal surface of the thorax of each insect using Hamilton's syringe (700 series, Micro liter TM Hamilton Company, USA). In control treatment, the insects were treated with 0.5 µL of acetone as negative control and 0.5 µL of deltamethrine as the positive control. After treatment, the insects were transferred into 11.0 cm diameter glass Petri dishes (10 insects per Petri dish), containing maize kernels and maintained at 27 ± 2 °C and 60 ± 5% relative humidity on a 12.0 (L:D) photo cycle for 48 hours, after which mortalities were recorded. Corrected percent mortalities were calculated as per equation 1 (Eqn. 1) (Abbot, 1925):

$$\% \text{ Mortality (Adjusted)} = \frac{(\%DT - \% DC)}{(100 - \% DC)} \quad \text{(Eqn. 1)}$$

Where DT = dead insects in test and DC = dead insects in control. Probit analysis was then performed to calculate the lethal concentration for 50% (LC_{50}) insect mortality (Finney, 1971).

2.7. Antifeedant assay

The leaf disc no choice bioassay method of (Arivoli and Tennyson, 2013) was used in the study of antifeedant activities of compounds from *E. subcordata* (Meisn) Engl. leaf extract, with some modification. The experiment tested the hypothesis that adult insect feeding is deterred by compounds incorporated in maize flour leaf discs. Compound concentrations at 10, 20, 50 and 100 µg/mL dissolved in acetone were used in the tests. The negative control treatment, made use of pure acetone added to the flour leaf discs, while for the positive control azadirachtin was added. In each petridish, the dried flour disk was placed and then a single pre-starved adult *S. zeamais* or *P. truncatus* was introduced and allowed to feed on the treated flour disk, versus an equal amount of acetone treated and azadirachtin treated flour leaf disc. The insects were incubated at 25 ± 2 °C for 24 hrs and on a 12 (L:D) photo-cycle and fed on treated and untreated maize flour leaf discs for twenty-four hours. At the end of the experiment, unconsumed area of the flour leaf disc was measured with the leaf area meter. Each experiment was replicated three times. The antifeedant index was calculated as per equation 2 (Eqn. 2) (Zhang et al., 2018).

$$\text{Antifeedant index (AI)\%} = \frac{(C - T)}{C} \times 100\% \quad \text{(Eqn. 2)}$$

Where C is the leaf disk consumed in the blank control and T is the leaf disk consumed in the treated groups. The AFI_{50}, which is the effective concentration for 50% antifeedant activity of a substance relative to the control (Huang et al., 2008) was calculated by subjecting the data to probit analysis (Finney, 1971).

3. Results and Discussion

3.1. Chemical structures of compounds isolated from *E. subcordata* (Meisn) Engl.

Chemical structures of compounds isolated from the crude leaf extracts of *E. subcordata* (Meisn) Engl. (SUPPL. S37) have been shown in Fig. 1.

3.2. Contact toxicity

Evaluation of compounds isolated from *E. subcordata* (Meisn) Engl. against *S. zeamais* and *P. truncatus* revealed that all were active. Nagilactone (**5**) (LC_{50} 25.391 and 28.483 µg/mL and quercetin (**6**) (LC_{50} 22.652 and 25.376 µg/mL) exhibited high contact toxicity activities against *S. zeamais* and *P. truncatus*, respectively. The activities were however lower and significantly different, ($p \leq 0.05$, LSD = 15.72) from the activities of deltamethrine (10.425 and 10.742 µg/mL) against *S. zeamais* and *P. truncatus*, respectively. β-Amyrin acetate and kaempferol-3-*O*-β-galactoside (**7**) exhibited moderate contact toxicity activities while 3-hydroxy-11-oxo-olean-12-ene (**2**) had the lowest activities (LC_{50} 88.163 and LC_{50} 97.98 µg/mL) against *S. zeamais* and *P. truncatus*, respectively (Fig. 2).

3.3. Antifeedant activities of *E. subcordata* (Meisn) Engl. Compounds

The compounds exhibited various levels of antifeedant activities against the two insects which were concentration dependent, increasing with increase in concentration. Among the compounds, quercetin (**6**) had the highest activities against the two insects; AFI_{50} 18.858 and 21.576 µg/mL which were not significantly different from the activities of the positive control at AFI_{50} 12.146 and 12.432 µg/mL ($p \geq 0.05$, LSD = 17.44) for *S. zeamais* and *P. truncatus*, respectively. High antifeedant activity of quercetin was also reported by (Adeyemi et al., 2010) on testing it against *Tribolium castaneum*. The high antifeedant activity of quercetin may be due to the presence of a pyran ring and more so the presence of a carbonyl group on C-4 of the pyran ring in its structure (Ohmura et al., 2000). Nagilactone (**5**) and kaempferol-3-*O*-β-galactoside (**7**) exhibited moderate activities against *S. zeamais* and *P. truncatus* while β-amyrin (**1**) acetate and 3-hydroxy-11-oxoolaen-12-ene (**2**) had low antifeedant activities against the two insect (Fig. 3).

4. Concluding remarks

The present study evaluated for the first time the

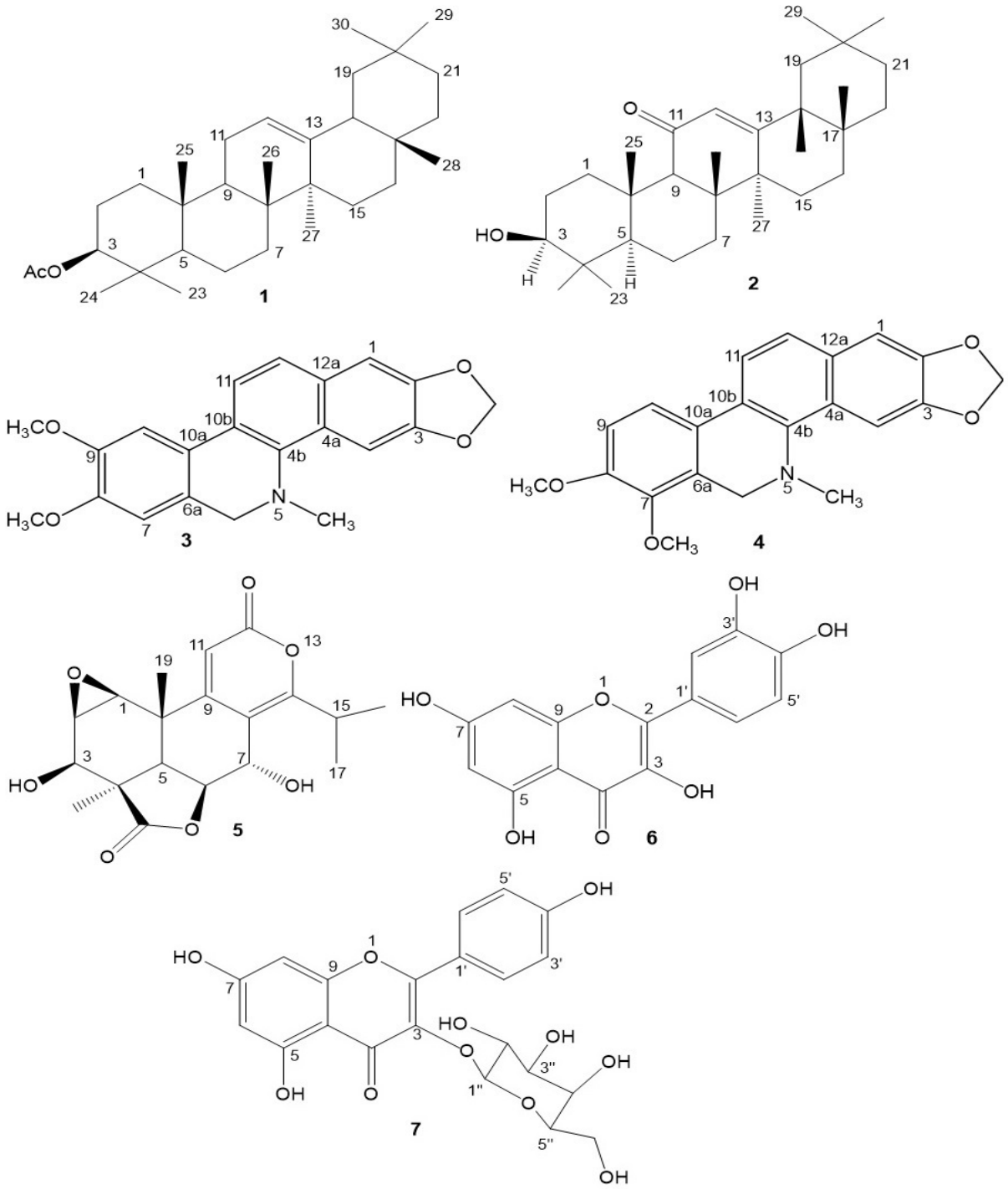

Fig. 1. Structures of compounds **1-7**.

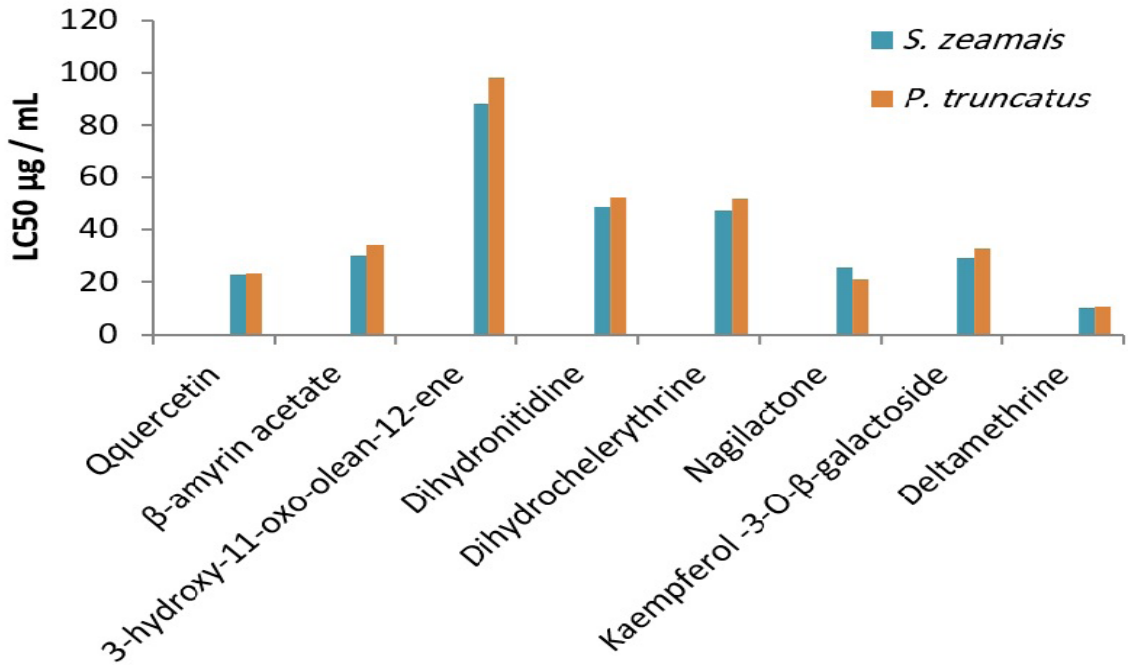

Fig. 2. Contact toxicity activities (LC_{50} µg/mL) of *E. subcordata*(Meisn) Engl. compound against *S. zeamais* and *P. truncatus*.

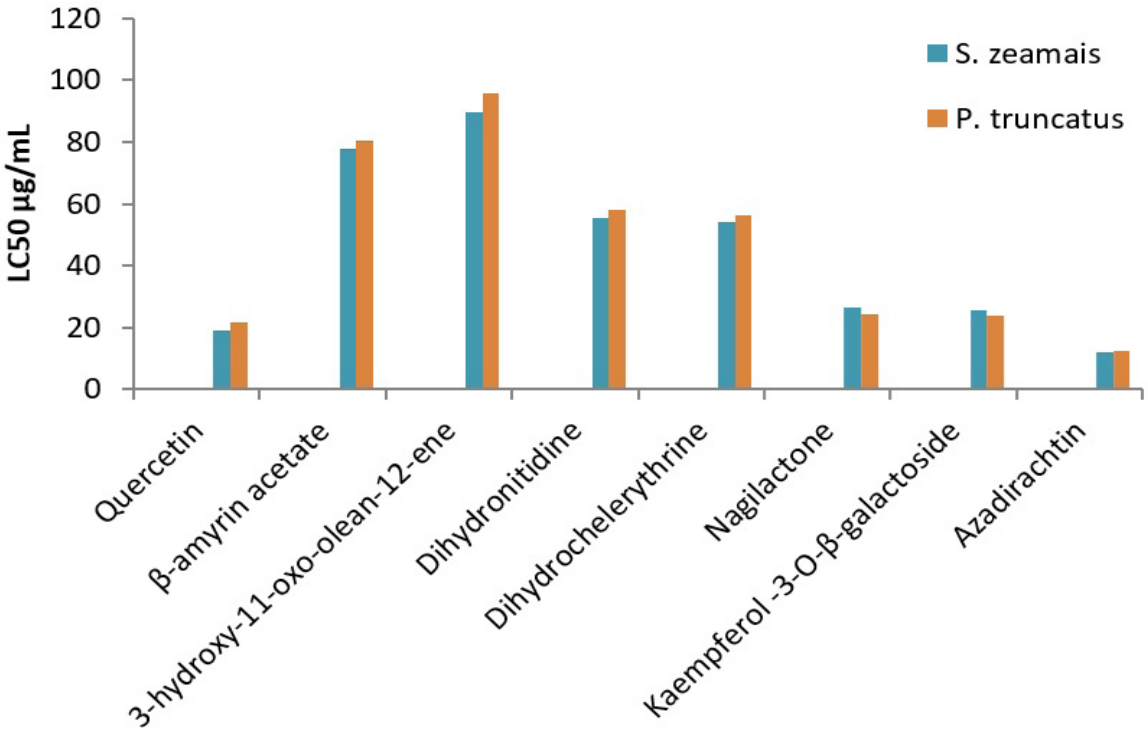

Fig. 3. Antifeedant activities (LC_{50} µg/mL) of compounds isolated from *E. subcordata* (Meisn) Engl. against *S. zeamais* and *P. truncatus*.

insecticidal potential of compounds isolated from crude leaf extracts of *E. subcordata* (Meisn) Engl. against *S. zeamais* and *P. truncatus,* insect pests of stored maize. The genus *Englerodaphne* is a good source of natural compounds with insecticidal properties among them terpenoids, coumarins, flavonoids and lignans being prevalent in the various species of *Englerodaphne*. Seven compounds characterized as β-amyrin acetate (**1**), 3β-hydroxy-11-oxoolean-12-ene (**2**), dihydronitidine (**3**), dihydrochelerythrine (**4**) nagilactone (**5**), quercetin (**6**) and kaempferol-3-*O*-β-galactoside were isolated from *E. subcordata* (Meisn) Engl. leaves and characterized. Quercetin exhibited the highest antifeedant and contact toxicity activities against *S. zeamais* and *P. truncatus*, respectively. The remaining six compounds also showed promising contact toxicity and antifeedant activities against the two insects. Results from this study validated the traditional use of the plant in the control of *S. zeamais* and *P. truncatus.* The most promising compounds may be tested for insecticidal activities using other bioassay methods or against other species of insects.

Author contribution statement

Conceptualization and literature search were performed by Albert Mulianga Makenzi. The first draft of the manuscript was prepared by Albert Mulianga Makenzi while Lawrence Onyango Arot Manguro and Philip Okinda Owuor both critically analyzed and gave suggestions to finalize the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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