Potent insecticidal activity of Eleocharis dulcis peel extract and its main components against aphids

Jingjing Li
Hebei Agricultural University
Mingsi Wang
Hebei Agricultural University
Yujing Zhao
Hebei Agricultural University
Hualong Yu
Hebei Agricultural University
Xinxin Zhang
Hebei Agricultural University
Yingjian Feng
Hebei Agricultural University
Lili Dong
Hebei Agricultural University
Lihui Zhang
Hebei Agricultural University
Jinggao Dong
Hebei Agricultural University
Yaofa Li
Hebei Academy of Agriculture and Forestry Sciences
Shujie Ma (mashujie89@126.com)
Hebei Agricultural University

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Abstract

Aphids are significant pests of the cash crops and food farm crops. Botanical insecticides are safe for aphid control, especially in organic agriculture. In this study, *Eleocharis dulcis* peel extract (EDPE), a new botanical insecticide, was investigated for its active compositions against several agricultural aphids. The results showed that the EDPE had high insecticidal activity against *Sitobion avenae*, *Aphis gossypii*, *Megoura crassicauda*, and *Acyrthosiphon pisum*, with the half lethal concentration (LC_{50}) values of 95.92, 81.04, 140.31, and 255.73 mg/L after 48 h treatment. In the pot culture assay, the aphicidal effects of 25% EDPE soluble liquid (SL) at a concentration of 0.016% were 68.98%, 79.33%, and 88.82% after the 1st, 3rd, and 7th days of treatment, respectively. Nine compounds were identified by bioactivity-directed fractionation, which was dimethoxy-6, 6-dimethylpyranoisoflavone (**1**), 3-methoxy-4-hydroxylonchocarpin (**2**), 4-hydroxylonchocarpin (**3**), 4-methoxylonchocarpin (**4**), barbigerone (**5**), lonchocarpusone (**6**), 6a, 12a-dehydrodeguelin (**7**), 13-homo-13-oxa-6a,12a-dehydrodeguelin (**8**) and deguelin (**9**). Among them, 4-hydroxylonchocarpin (**3**) showed the highest aphidicidal activity against *M. crassicauda*, *S. avenae*, and *A. pisum*, with the LC_{50} values of 97.24, 140.63, and 112.31 mg/L, respectively. Therefore, EDPE and its major component 4-hydroxylonchocarpin are probably used as new botanical insecticides to control aphids.

1. Introduction

Aphids (Hemiptera: Aphididae) are one of the ‘leaders’ of the most difficult pests to control, which can damage both cash crops and food farm crops in agriculture worldwide (Park et al. 2021). *Sitobion avenae* is an important agricultural pest of cereals, directly sucking sap from the phloem and spreading plant viruses such as barley yellow dwarf virus, causing serious economic losses (Zhang et al. 2017; Zhang et al. 2021). *Aphis gossypii* Glover is the most common type of aphid that directly damages the growth of cotton by sucking up its sap, resulting in reduced yield and quality of cotton (Jiang et al. 2020; Pachu et al. 2021). *Megoura crassicauda* and *Acyrthosiphon pisum* mainly affect legumes, which are the most destructive pests due to their short developmental calendar and rapid population reproduction (Skaljac et al. 2018; Yin et al. 2019). Aphids control is mainly based on chemical pesticides, however, the frequent use of chemical pesticides not only lead to environmental pollution but also makes the aphids to a variety of pesticides with a high level of resistance (Jiang et al. 2018; Ma et al. 2018). Given this situation, the development of new green and safe pesticides has received a recent surge in interest (Campos et al. 2019).

Botanical insecticides have become an active research field due to their environmental friendliness, low-level resistance, and potential safety (Miresmailli and Isman 2014; Pavela et al. 2013). Currently, plant extracts and their active compounds have a wide range of applications in pest control (Isman 2008; Kaleeswaran et al. 2018) *Sophora alopecuroides* alkaloids, including cytisine, matrine, sophocarpine, oxymatrine, etc., are the representative commercial botanical insecticides against *Myzus persicae*, *Macrosiphum rosirvorum* and *Brevicoryne brassicae* (Ma et al. 2020; Ma et al. 2018). *Robinia pseudoacacia* L. seed extract has proven to be an effective aphicide in laboratory and field trials (Jiang et

al. 2018). *Zanthoxylum armatum* leaf extract has tremendous commercial utilization in the management of *Spodoptera litura* (Kaleeswaran et al. 2018). *Phyllostachys pubescens* leaf extract and its major component isoorientin provide new resources for the development of botanical aphicides (Gao et al. 2019).

Eleocharis dulcis (Burm.f.) Trin., belonging to the *Cyperaceae*, is concentrated in tropical and subtropical regions (Aizawa et al. 2010; Li et al. 2016). *E. dulcis* is often used as Chinese medicine to treat pharyngitis, laryngitis, cough, hepatitis, and hypertension and it is also one of the popular aquatic vegetables in China due to its unique flavor (Nie et al. 2019). The peel of *E. dulcis* is often discarded, which is rich in flavonoids and polyphenols, and exhibits strong antioxidant and acrylamide formation activity (Nie et al. 2021; Zhan et al. 2014). It has been reported that *E. dulcis* had antifeedant activity against *Anthonomous grandis* and it can be used as a baiting plant to trap the eggs of *Scirpophaga innotata* in rice fields (Miles et al. 1994; Rajesh et al. 2021). In our past study, we discovered that *E. dulcis* peel extracts (EDPE) had good insecticidal activity against various pests, especially aphids such as *M. crassicauda* and *Aphis citricolavande* (Ma et al. 2021; Yu et al. 2021). Nevertheless, systematic insecticidal activity and the active ingredients of EDPE are still unknown.

Thus, both petri dish assay and pot culture experiment wsa used to evaluate the insecticidal activity of EDPE against *M. crassicauda*, *S. avenae*, *A. gossypii*, and *A. pisum*; and the bioactivity-directed fractionation was also used to identify insecticidal ingredients in this research.

2. Materials And Methods

2.1. Plant materials and Chemicals

The *Eleocharis dulcis* peel was purchased from An Guo Leng Bei Herbs Corporation (Hebei, China) in May 2019. The plant material was authenticated by Prof. Lihui Zhang (Hebei Agricultural University).

The standard compound of matrine (purity, 96%) was obtained from Shaanxi Tengmai Biotechnology Co. LTD (China). The 0.5% matrine AS was purchased from Hebei Zhongbao Green Agricultural Crop Technology Co (China).

2.2 Insects cultures

Megoura crassicauda, *Sitobion avenae*, *Aphis gossypii*, and *Acyrthosiphon pisum* were provided by Plant Protection Institute, Hebei Academy of Agricultural and Forestry Sciences, China.

S. avenae was raised on wheat (*Triticum aestivum* L) plantlets in a greenhouse at 21 ± 2°C under a 16:8 h (L: D) photocycle and 50 ± 5% RH (Wei et al. 2019).

A. gossypii was reared on the cotton (*Gossypium* spp) seedlings, with the controlled conditions of 23 ± 2°C, 16:8 h (L: D), and 55 ± 5% RH (Wang et al. 2018).

M. crassicauda was fed on *Vicia faba* L seedlings, which were placed at 25 ± 2°C, 55 ± 5% RH, and 16:8 h (L: D) in the greenhouse. *M. crassicauda* was used in both indoor and pot culture assays (Yin et al. 2019).

A. pisum was fed on broad bean seedlings, with the greenhouse conditions of 23 ± 2°C, 16:8 h (L: D), 68 ± 5% RH (Pavela et al. 2013).

2.3. Organic solvent extraction

The air-dried *E. dulcis* peel (10 kg) was powdered by a pulverizer, and then extracted with 95% industrial alcohol three times (3 days per time). The ethanol extract was filtered and concentrated to obtain 682.53 g EDPE. The ethanol extract was further extracted with petroleum ether, ethyl acetate and n-butanol, and the weight of 206.64 g, 60.99 g and 7.99 g were obtained, respectively.

2.4. Petri plate experiments

2.4.1 Topical application method

The aphicidal activities of different extracts, fractions, and partial compounds against *M. crassicauda* and *A. pisum* were determined by the topical application method (Ma et al. 2018). Firstly, The samples were solubilized with acetone and then diluted with 0.1% tween-80 aqueous solution to obtain the concentrations of 62.5, 125, 250, 500 and 1000 mg/L, respectively. Secondly, the 0.03 μL micro dropper was used to drop the sample solution on the pronotum of the *M. crassicauda* and *A. pisum.* Matrine was chosen as a positive control, and the blank control was treated with acetone. Twenty *M. crassicauda* and *A. pisum* were used in every treatment, and three replicates were set for each treatment. After 24 h and 48 h, the number of aphid deaths was checked. Corrected mortality was calculated with the formula: Corrected mortality (%) = $(T_1\text{-}T_0)/(100\text{-}T_0)\times100$. Where T_1 was the mortality of the treated groups, and T_0 refers to the mortality of the control groups.

2.4.2 Leaf-dip method

The toxicity of different extracts and partial compounds to *S. avenae* and *A. gossypii* were determined using a leaf-dip method (Wang et al. 2021). The samples were solubilized with acetone and diluted with 0.1% tween-80 aqueous solution, resulting in the final concentrations of 62.5, 125, 250, 500 and 1000 mg/L, respectively. The wheat leaf containing about sixty *S. avenae* and the cotton leaf containing about sixty *A. gossypii* were dipped into the samples for 10 seconds. The negative control leaves were immersed in 0.1% tween-80 aqueous solution. The treatment leaves were placed on filter paper to dry naturally, and then the petioles were wrapped with absorbent cotton balls. The number of *S. avenae* and *A. gossypii* were recorded and transferred to Petri dishes. The Petri dishes were sealed with plastic wrap, and ventilated with about thirty pinholes. Three replicates were set for each treatment. The formula in 2.4.1 was used to compute mortality after 24 h and 48 h treatment.

2.5. Pot culture assay

EDPE soluble liquid (SL) was prepared with 25% EDPE, 3% Tween-80, 18% N-methyl pyrrolidone, and 54% ethanol for pot culture assay against *M. crassicauda*. The 25% EDPE SL was diluted to the concentrations of 0.004%, 0.008% and 0.016% (w/v), respectively. The broad bean seedlings with some *M. crassicauda* adults were sprinkled with different concentrations of 25% EDPE SL. Commercialized agent 0.5% matrine aqueous solution (AS) (0.006%, w/v) was utilized as a positive control. After spraying, the total number of *M. crassicauda* on each broad bean seedling was assessed on the 1st, 3rd, and 7th days. The following equation was used to calculate the control effect: control effect (%) = $C_1/C_0 \times 100$, where C_1 = the decrease rate of the *M. crassicauda* of a different treatments-the decrease rate of the *M. crassicauda* in the blank control, and C_0 = 100 the decrease rate of *M. crassicauda* of the blank control.

2.6. Bioactivity-directed isolation

The bioassay-guided fractionation was used to isolate active compounds. According to the results of biological activity determination, the active ingredients mainly exist in ethyl acetate extract and petroleum ether extract. According to TLC (Thin-Layer Chromatography) analysis, seven fractions (P1 to P7) were obtained by silica gel column elution of the petroleum ether extract with a mixture of a petroleum ether-ethyl acetate (100:0–0:100).

The highest activity fraction was P4, which was further separated using a gradient mixture of petroleum ether-ethyl acetate (100:1–5:1). And four compounds **1** (127.35 mg), **2** (37.62 mg), **3** (98.31 mg), **4** (4051.32 mg) were obtained. Seven fractions (E1 through E7) were obtained from ethyl acetate extract, and the two active fractions E4 and E5 were continued to be isolated, respectively. E4 was separated with petroleum ether-ethyl acetate (100:1–5:1) to give compound **5** (25.31 mg), compound **6** (105.31 mg) and compound **7** (43.94 mg). E5 was also used with petroleum ether-ethyl acetate (50:1 – 0:1) to obtain compounds **7** (25.27 mg), **8** (32.69 mg) and **9** (8.16 mg). The NMR spectra were measured on a Bruker Avance spectrometer (Bruker, Switzerland) in deuterochloroform (CDCl3). High-resolution mass spectra (HRMS) were obtained on a Q Exactive Focus LC/MS instrument (Thermo Fisher Scientific, USA).

2.7. Data analysis

Microsoft Excel was used to obtain the corrected mortality of aphids after different sample treatments, and LC_{50} and 95% confidence intervals were computed using SPSS 22.0. Differences between treatments were assessed using a one-way ANOVA followed by Tukey HSD test ($P < 0.05$).

3. Results

3.1. Aphicidal activity of EDPE

In the petri plate experiments, the *E. dulcis* peel ethanol extract showed high aphicidal activity against *M. crassicauda*, *S. avenae, A. gossypii*, and *A. pisum* with LC_{50} values of 277.86, 245.73, 214.66, and 651.37 mg/L after 24 h treatment, respectively. The LC_{50} values after 48 h of treatment were 140.31, 95.92, 81.04, and 255.00 mg/L, respectively (Table 1).

Table 1

Aphicidal activity of *E. dulcis* peel ethanol extract against *M. Crassicauda*, *S. avenae*, *A. gossypii* and *A. pisum*

Insects	Method	Treatment time (h)	Toxicity regression equation (y =)	LC_{50} (95% CL) (mg/L)	χ^2
S. avenae	Leaf-dip	24	-1.39+0.58*x*	245.73 (149.18-401.24)	0.26
		48	-2.35+1.19*x*	95.92 (66.43-125.00)	1.16
A. gossypii	Leaf-dip	24	-2.20+0.94*x*	214.66 (158.33-284.07)	1.12
		48	-2.78+1.46*x*	81.04 (58.52-102.93)	0.80
M. Crassicauda	Topical application	24	-3.91+1.60*x*	277.86 (233.87-332.22)	4.47
		48	-3.22+1.50*x*	140.31 (112.02-169.68)	1.87
A. pisum	Topical application	24	-3.54+1.26*x*	651.37 (506.12-918.326)	1.01
		48	-3.22+1.34*x*	255.00 (208.05-3131.38)	4.01

LC_{50} value was determined by log-probit analysis. $\chi^2_{0.05\,(3)}$ =7.81, χ^2 values less than 7.81 were considered as significant. The same for Table 3 and 5.

In the pot culture assay, 25% EDPE SL exhibited a significant control effect against *M. crassicauda*, which was shown in Table 2. The effects of 25% EDPE SL at a concentration of 0.016% were 68.98%, 79.33% and 88.82% after 1 d, 3 d, and 7 d of sprinkled, respectively. At the concentration of 0.008% of 25% EDPE SL, the control efficacy was still above 50%. The efficacies of 25% EDPE SL at a concentration of 0.016% and 0.008% were all significantly higher than those of 0.5% matrine AS (0.006%). The broad bean seedlings were not affected.

Table 2

The control effect of *E. dulcis* peel extract (25% SL) against *M. Crassicauda* in greenhouse

Treatment	Concentration (w/v %)	Control effect (%)		
		1 d	3 d	7 d
EDPE (25% SL)	0.016	68.98± 4.60a	79.33 ± 6.76a	88.82 ± 3.18a
	0.008	57.55±3.56b	65.78±5.71b	79.04 ± 3.88b
	0.004	32.37 ± 4.72d	38.20 ± 4.47d	42.96 ± 4.99d
Matrine (0.5% AS)	0.006	41.62±6.80c	51.89±10.53c	59.62±10.99c

EDPE, *Eleocharis dulcis* peel extract. Data in the table are the average of three replications and are represented as mean ± standard deviation. Values followed by different small letters in the same column are significantly different at $P = 0.05$. The same for Table 4.

3.2. Aphicidal activity of crude organic extracts and fractions

The aphicidal activity of different organic extracts against *M. crassicauda* was showed in Fig. 1. The petroleum ether extract had the highest contact activity with the corrected mortality rates of 85.96% after 24 h treatment against *M. crassicauda*. After 48 h treatment, the ethyl acetate and petroleum ether extracts showed high aphicidal activity, with the result of 82.46% and 92.98%, respectively.

The contact toxicity of petroleum ether and ethyl acetate extracts was further tested, and the results showed that the LC_{50} values of the ethyl acetate and petroleum ether extracts against *M. crassicauda* were 346.37 and 265.20 mg/L after 24 h treatment, and 151.02 and 173.70 mg/L after treatment 48 h, respectively. Interestingly, their contact toxicity was all higher than that of commercialized botanical insecticide matrine (Table 3).

Table 3

Contact toxicity of petroleum ether extract and ethyl acetate extract from *E. dulcis* peel against *M. crassicauda*

Crude extracts	Treatment Time (h)	Toxicity regression equation (y =)	LC_{50} (95% CL) (mg/L)	χ^2
Petroleum ether	24	-3.86+1.60*x*	265.20 (222.99-316.73)	5.28
	48	-3.72+1.71*x*	151.02 (124.92-178.56)	3.26
Ethyl acetate	24	-3.28+1.29*x*	346.37 (281.38-437.83)	2.55
	48	-3.04+1.36*x*	173.70 (138.48-212.08)	1.40
Matrine	24	-3.65+1.17*x*	1314.40 (904.87-2388.11)	0.25
	48	-3.42+1.22*x*	656.06 (505.47-939.76)	0.24

Among the seven fractions of the petroleum ether extract, fraction 4 (P4) showed the highest insecticidal activity with a 24-h corrected mortality rate of 85.71% and a 48-h corrected mortality rate of 92.73%, respectively. The highest insecticidal activity of fraction 4 (E4) and fraction 5 (E5) of the ethyl acetate extract was 81.81% and 78.18% after treatment 48 h, respectively (Fig. 2.).

3.3. Identification of active compounds from *E. dulcis* peel

A total of nine compounds were isolated from *E. dulcis* peel and the structures were also identified (Fig. 3). Four compounds were isolated from the crude extract of petroleum ether, and five compounds were isolated from ethyl acetate extract. They were soluble in chloroform.

4',5'-Dimethoxy-6,6-dimethylpyranoisoflavone (1): $C_{22}H_{20}O_5$, obtained as white crystal. EI-MS m/z (%): 364.40$[M]^+$. ^{1}H NMR (Chloroform-*d*, 600 MHz) δ 8.07 (2H, d, *J* = 8.8 Hz, H-2), 7.97 (2H, s, H-5), 7.21 (2H, d, *J* = 2.0 Hz, H-6'), 7.05 (2H, dd, *J* = 8.2, 2.0 Hz, H-2'), 6.93 (2H, d, *J* = 8.2 Hz, H-3'), 6.87 (2H, d, *J* = 8.6 Hz, H-4''), 6.81 (2H, d, *J* = 10.0 Hz, H-6), 5.73 (2H, d, *J* = 10.0 Hz, H-3''), 3.92 (3H, s, OCH_3), 3.72 (3H, s, OCH_3), 1.50 (3H, s, CH_3), 1.25 (3H, s, CH_3). ^{13}C NMR (Chloroform-*d*, 151 MHz) δ 175.95 (CO), 157.34 (C-8a), 152.35 (C-2), 149.14 (C-5'), 148.81 (C-4'), 130.33 (C-1'), 126.70 (C-3''), 124.76 (C-5), 124.70 (C-3), 121.06 (C-2'), 118.35 (C-4''), 115.28 (C-4a), 114.93 (C-9), 112.60 (C-6'), 111.21 (C-3'), 109.20 (C-8), 77.75 (C-2'), 58.47 (C-7), 55.99 (OCH_3), 55.97 (OCH_3), 39.34 (C-4'') 29.70 (CH_3), 29.37 (CH_3). The above data are consistent with literature report (Ye et al. 2008).

3-Methoxy-4-hydroxylonchocarpin (2): $C_{21}H_{20}O_5$, obtained as pale-yellow needle crystal. EI-MS m/z (%): 352.13$[M]^+$. ^{1}H NMR (Chloroform-*d*, 600 MHz) δ 7.80 (1H, d, *J* = 15.4 Hz, H-α), 7.71 (1H, d, *J* = 8.9 Hz, H-2'), 7.42 (1H, d, *J* = 15.3 Hz, H-β), 7.28 (1H, d, *J* = 2.0 Hz, H-2), 7.14 (1H, dd, *J* = 8.4, 2.1 Hz, H-6), 6.88 (1H, d, *J*

= 8.3 Hz, H-5), 6.76 (1H, d, *J* = 10.0 Hz, H-4″), 6.38 (1H, d, *J* = 8.8 Hz, H-3′), 5.59 (1H, d, *J* = 10.0 Hz, H-5′), 3.95 (OCH_3), 1.27 (CH_3×2).^{13}C NMR (Chloroform-*d*, 151 MHz) δ 191.98 (C-β′), 160.95 (C-4′), 159.73 (C-6′), 148.90 (C-3), 146.01 (C-4), 144.15 (C-α), 130.59 (C-2′), 128.62 (C-1), 128.05 (C-5′), 122.79 (C-6), 118.58 (C-β), 115.95 (C-4″), 114.14 (C-5), 113.09 (C-1′), 110.63 (C-2), 109.43 (C-3′), 108.23 (C-5′), 77.76 (C-6″), 56.05 (OCH_3), 29.67 (CH_3), 28.36 (CH_3). The above data are consistent with literature report (Fang and Casida 1999).

4-Hydroxylonchocarpin (3): $C_{20}H_{18}O_4$, obtained as yellow needles. EI-MS m/z (%): 322.12 $[M]^+$. ^{1}H NMR (Chloroform-*d*, 600 MHz) δ 7.78 (1H, d, *J* = 7.5 Hz, H-β), 7.71 (1H, d, *J* = 15.4 Hz, H-6), 7.65 (1H, d, *J* = 15.4 Hz, H-2′, 6′), 7.44 (1H, d, *J* = 15.2 Hz, H-α), 6.88 (2H, d, *J* = 8.6 Hz, H-3′, 5′), 6.63 (1H, d, *J* = 10.1 Hz, H-4″), 6.08 (1H, d, *J* = 7.5 Hz, H-5), 5.62 (1H, d, *J* = 10.1, 1.0 Hz, H-5″), 1.46 (6H, s, CH_3×2). ^{13}C NMR (Chloroform-*d*, 151 MHz) δ 192.18 (CO), 160.92 (C-4), 159.67 (C-2), 159.31 (C-4′), 144.50 (C-β), 130.61 (C-2′,6′), 128.12 (C-6), 128.57 (C-5″), 128.33 (C-1′), 117.38 (C-α), 116.11 (C-4″), 115.97 (C-1), 114.18 (C-3), 109.48 (C-1), 108.22 (C-5), 77.81 (C-6″), 28.37 (CH_3 × 2). The above data are consistent with literature report (Lee et al. 2005).

4-Methoxylonchocarpin (4): $C_{22}H_{22}O_5$, obtained as yellow needles. EI-MS m/z (%): 336.18 $[M]^+$. ^{1}H NMR (Chloroform-*d*, 600 MHz) δ 13.78 (1H, br s,-OH), 7.85 (1H, d, *J* = 15.3 Hz, H-α), 7.72 (1H, d, *J* = 8.9 Hz, H-2), 7.61 (2H, d, *J* = 8.8 Hz, H-2), 7.45 (1H, d, *J* = 15.3 Hz, H-β), 6.94 (2H, d, *J* = 8.8 Hz, H-3), 6.76 (1H, d, *J* = 10.7 Hz, H-4), 6.38 (1H, d, *J* = 9.5 Hz, H-3′), 5.59 (1H, d, *J* = 10.1 Hz, H-3″), 3.86 (3H, s, -OCH3), 1.47 (6H, s, CH_3× 2). ^{13}C NMR (Chloroform-*d*, 151 MHz) δ 192.03 (CO), 161.81 (C-4′), 160.96 (C-2′), 159.70 (C-4), 144.13 (C-19), 130.58 (C-14), 130.34 (C-12,14), 128.09 (C-13), 127.64 (C-3), 117.92 (C-β), 115.97 (C-11), 114.49 (C-3, 5), 114.15 (C-1), 109.46 (C-3), 108.21 (C-5), 77.79 (C-3), 55.45 (OCH_3), 28.39 (CH_3 × 2). The above data are consistent with literature report (Su et al. 2012).

Barbigerone (5): $C_{23}H_{22}O_6$, obtained as white powder. EI-MS m/z (%): 394.42 $[M]^+$. ^{1}H NMR (Chloroform-*d*, 600 MHz) δ 8.05 (1H, d, *J* = 8.7 Hz, H-2), 7.97 (1H, s, H-5), 7.26 (3H, s, H-6′), 6.95 (1H, s, H-4″), 6.84 (2H, dd, *J* = 20.6Hz, H-6), 6.63 (1H, s,H-3′), 5.72 (1H, d, *J* = 10.0 Hz, H-3″), 3.72 (3H, s, OCH_3) 1.27 (6H, s, CH_3×2).^{13}C NMR (Chloroform-*d*, 151 MHz) δ 175.87 (C-4), 157.19 (C-8a), 153.98 (C-2), 152.39 (C-2′), 151.92 (C-4′), 143.09 (C-5′), 130.23 (C-3″), 126.73 (C-5), 121.55 (C-3), 121.25 (C-4″), 118.46 (C-4a), 115.41 (C-6′), 115.08 (C-6), 112.29 (C-1′), 109.27 (C-8), 98.39 (C-3′), 77.65 (C-2″), 56.94 (OCH_3), 56.59 (OCH_3), 56.20 (OCH_3), 29.70 (CH_3), 28.12 (CH_3). The above data are consistent with literature report (Dagne and Bekele 1990).

Lonchocarpusone (6): $C_{23}H_{22}O_6$, obtained as white powder. EI-MS m/z (%): 394.42 $[M]^+$. ^{1}H NMR (Chloroform-*d*, 600 MHz) δ 8.04 (1H, s, H-2), 7.97 (1H, d, *J* = 8.2 Hz, H-5), 7.55 (1H, s, H-2′), 6.74(1H, d, *J* = 8.8Hz, H-6), 6.71(1H, d, *J* = 8.8Hz, H-4″), 6.64 (1H, s, H-5′), 5.59 (1H, d, *J* = 10.1Hz, H-3″), 3.72 (3H, s, OCH3), 1.47 (6H, s, CH_3×2).^{13}C NMR (Chloroform-*d*, 151 MHz) δ 175.85 (C-4), 157.20 (C-7), 153.97 (C-9), 152.40 (C-2), 151.94 (C-6′), 149.83 (C-4′), 143.14 (C-3′), 130.23 (C-3″), 126.75 (C-5), 121.56 (C-3), 118.49 (C-1″), 115.47 (C-2′), 115.08 (C-6), 112.35 (C-1′), 109.28 (C-8), 98.47 (C-5′), 76.81 (C-2″), 56.95 (OCH_3), 56.61

(OCH_3), 56.21 (OCH_3), 28.13 (CH_3 × 2). The above data are consistent with literature report (Kaouadji et al. 1986).

6a,12a-Dehydrodeguelin (7): $C_{23}H_{20}O_6$, obtained as white powder. EI-MS m/z (%): 322.12 $[M]^+$.^{1}H NMR (Chloroform-*d*, 600 MHz) δ 8.46 (1H, s, H-1), 8.05 (1H, d, *J* = 8.8 Hz, H-11), 6.88 (1H, d, *J* = 8.7 Hz, H-10), 6.78 (1H, d, *J* = 10.0 Hz, H-4'), 6.57 (1H, s, H-4), 5.74 (1H, d, *J* = 10.1 Hz, H-5'), 5.03 (2H, s, H-6), 3.96 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 1.50 (6H, s, CH3). ^{13}C NMR (Chloroform-*d*, 151 MHz) δ 182.35 (C-12), 157.29 (C-9), 156.17 (C-6a), 149.14 (C-4a), 144.26 (C-2), 130.60 (C-5'), 126.58 (C-11), 115.44 (C-12a), 114.74 (C-4'), 114.48(C-10), 112.36 (C-12a),111.86 (C-1a), 109.14 (C-8), 100.56 (C-4), 76.84 (C-6'),64.92 (C-6), 56.39 OCH_3), 56.00 (OCH_3), 29.71 (CH_3), 28.18 (CH_3). The above data are consistent with literature report (Ye et al. 2008).

13-Homo-13-Oxa-6a,12a-dehydrodeguelin (8): $C_{23}H_{20}O_{7,}$ obtained as white powder. EI-MS m/z (%): 408.41 [M]+. ^{1}H NMR (Chloroform-*d*, 600 MHz) δ 8.05 (2H, d, *J* = 8.7 Hz, H-11), 7.97 (2H, s, H-4'), 6.95 (1H, s, H-10), 6.84 (4H, dd, *J* = 20.3, 9.3 Hz, H-1), 6.63 (1H, s, H-4), 5.72 (1H, d, *J* = 9.9 Hz, H-5'), 3.87 (3H, s, OCH3), 1.27 (6H, s, CH_3). ^{13}C NMR (Chloroform-*d*, 151 MHz) δ 171.86 (C-12), 157.54 (C-9), 150.97 (C-7a), 150.52 (C-1a), 145.94 (C-3), 145.65 (C-2), 142.54 (C-4a), 142.07 (C-6a), 140.88 (C-12a),130.44 (C-5'), 126.59 (C-11), 115.26 (C-4'), 114.66 (C-10), 108.91(C-8), 117.46 (C-1), 104.81 (C-4), 77.86(C-6'), 69.63 (C-6), 56.39 (OCH_3), 56.34 (OCH_3), 29.70 (CH_3), 28.14 (CH_3). The above data are consistent with literature report (Fang and Casida 1997).

Deguelin (9): $C_{23}H_{24}O_{6,}$ obtained as white powder. EI-MS m/z (%): 396.16$[M]^+$. ^{1}H NMR (Chloroform-*d*, 600 MHz) δ 8.07 (1H, d, *J* = 8.8 Hz, H-11), 7.97 (1H, s, H-1), 7.21 (1H, s, H-4'), 7.05 (1H, d, *J* = 8.2 Hz, H-10), 6.93 (1H, d, *J* = 8.4 Hz, H-4), 6.87 (1H, d, *J* = 8.8 Hz, H-5'), 6.81 (1H, d, *J* = 10.0 Hz, H-6a), 5.72 (1H, d, *J* = 10.1 Hz, H-6),4.17 (1H, d, *J* = 12.0 Hz, 12a), 3.81 (6H, d, *J* = 9.8 Hz, OCH_3×2), 1.50 (6H, s, CH_3×2). ^{13}C NMR (Chloroform-*d*, 151 MHz) δ 175.91 (C-12), 157.37 (C-9), 152.39 (C-7a), 151.90 (C-4a), 149.25 (C-3), 148.93 (C-2), 130.34 (C-5'), 126.75 (C-11), 118.42 (C-4'), 115.26(C-11a), 114.85(C-1), 112.79 (C-10), 111.38 (C-8), 105.70(C-1a), 100.87 (C-4), 77.53 (C-6a), 56.04 (OCH_3), 56.02 (OCH_3), 28.18(CH_3 × 2). The above data are consistent with literature report (Ye et al. 2008).

3.4. Aphicidal activity of compounds

As shown in Table 4, all nine compounds showed aphicidal activity. Among them, 4-hydroxylonchocarpin had the highest activity against *M. crassicauda*, *S. avenae*, and *A. pisum*, with the corrected mortality of 88.27%, 84.06%, and 80.25% after 48 h treatment, and its effectiveness is higher than matrine.

Table 4

Crude extracts	Treatment Time (h)	Toxicity regression equation (y =)	LC_{50} (95% CL) (mg/L)	χ^2
Petroleum ether	24	-3.86 + 1.60*x*	265.20 (222.99-316.73)	5.28
	48	-3.72 + 1.71*x*	151.02 (124.92-178.56)	3.26
Ethyl acetate	24	-3.28 + 1.29*x*	346.37 (281.38-437.83)	2.55
	48	-3.04 + 1.36*x*	173.70 (138.48-212.08)	1.40
Matrine	24	-3.65 + 1.17*x*	1314.40 (904.87-2388.11)	0.25
	48	-3.42 + 1.22*x*	656.06 (505.47-939.76)	0.24

Aphicidal activity of compounds from *E. dulcis* peel extract against *M. crassicauda*, *S. avenae* and *A. pisum*

The compound deguelin also showed effective aphicidal activity, similar to that of matrine. The corrected mortalities of the other seven compounds against *M. crassicauda* were in the range of 26.32–50.87%, lower than that of the control.

Contact toxicity of the 4-hydroxylonchocarpin, the highest insecticidal active compound, was further tested and the results were shown in Table 5. 4-Hydroxylonchocarpin had high contact toxicity against *M. crassicauda*, *S. avenae*, and *A. pisum*, with the LC_{50} values of 97.24, 140.63, and 112.31 mg/L after 48 h treatment, respectively.

Table 5

No.	Compounds	Corrected mortality (%)					
		M. crassicauda		*S. avenae*		*A. pisum*	
		24 h	48 h	24 h	48 h	24 h	48 h
1	dimethoxy-6, 6-dimethylpyranoisoflavone	30.51 ± 6.24bcde	38.60 ± 4.71b	16.95 ± 2.36bc	40.35 ± 2.36bc	23.73 ± 4.08bc	31.58 ± 4.08d
2	3-methoxy-4-hydroxylonchocarpin	28.81 ± 4.08bcde	49.12 ± 10.27b	16.07 ± 2.36bc	33.33 ± 2.36bc	15.25 ± 2.36c	38.60 ± 6.24bcd
3	4-hydroxylonchocarpin	82.47 ± 5.67a	88.27 ± 1.91a	73.99 ± 4.26a	84.06 ± 5.17a	76.19 ± 6.64a	80.25 ± 5.61a
4	4-methoxylonchocarpin	18.64 ± 4.08e	33.33 ± 4.71b	8.47 ± 7.07d	14.03 ± 6.24d	23.73 ± 4.08bc	35.09 ± 2.36cd
5	barbigerone	38.98 ± 4.08b	47.37 ± 4.08b	38.98 ± 4.08bc	47.37 ± 4.08cd	13.56 ± 4.08c	26.32 ± 4.08d
6	lonchocarpusone	20.34 ± 4.71de	26.32 ± 10.80b	8.47 ± 4.08d	13.04 ± 6.24d	22.03 ± 6.24bc	24.56 ± 8.50e
7	6a,12a-dehydrodeguelin	23.73 ± 4.08bcde	35.09 ± 6.24b	27.12 ± 6.24b	38.60 ± 4.71bc	29.51 ± 8.16bc	40.35 ± 4.71bcd
8	13-homo-13-Oxa-6a, 12a-dehydrodeguelin	22.03 ± 4.71bc	50.87 ± 9.42b	18.64 ± 4.08bc	36.84 ± 4.08bc	18.33 ± 4.71c	28.07 ± 2.36d
9	deguelin	35.59 ± 6.24bc	54.39 ± 6.24ab	27.12 ± 6.23b	47.37 ± 4.08b	27.45 ± 6.24b	38.10 ± 4.71b
10	matrine	37.29 ± 4.71bc	59.65 ± 2.36ab	22.03 ± 6.24bc	31.58 ± 7.07cd	30.51 ± 6.24bc	52.63 ± 4.08bc

Aphicidal activity of 4-hydroxylonchocarpin from *E. dulcis* peel extract against *M. crassicauda, S. avenae* and *A. pisum*

4. Discussion

The EDPE showed potent aphidicidal activities against *S. avenae*, *A. gossypii*, *M. Crassicauda*, and *A. pisum* both in petri plate and pot culture experiments. Surprisingly, EDPE exhibited stronger or the same

level of insecticidal activity against aphids compared to other botanical aphidicides. For example, *Parthenium hysterophorus* extract showed certain insecticidal activity against *Aphis craccivora* Koch (LC_{50} = 839 mg/L) (Reddy et al. 2017); the LC_{50} value of *Ungernia severtzovii* bulb extract against *Schizaphis graminum* was 2350 mg/L (Chermenskaya et al. 2012); *Angelica archangelica* L. extract exhibited high toxic for the aphids and the LC_{50} was about 1100 mg/L (Pavela et al. 2013). In this study, the LC_{50} value of EDPE against *M. Crassicauda* was 140.31 mg/L, the effects of 25% EDPE SL at a concentration of 0.016% was 88.82%. Therefore, EDPE is expected to be exploited as a new botanical insecticide for aphid control.

During isolation of active compounds of EDPE, nine insecticidal compounds (4', 5' -dimethoxy-6, 6-dimethylpyranoisoflavone, 3-methoxy-4-hydroxylonchocarpin, 4-hydroxylonchocarpin, 4-methoxylonchocarpin, barbigerone, lonchocarpusone, 13-homo-13-Oxa-6a,12a-dehydrodeguelin, 6a, 12a-dehydrodeguelin and deguelin) were identified. There are numerous reports on the medicinal activity of these compounds, for example, 3-methoxy-4-hydroxylonchocarpin showed significant anti-inflammatory activity (Jeon et al. 2012; Peng et al. 2012); 4-hydroxylonchocarpin have various pharmacological effects, such as antibacterial, antifungal, antitubercular and antimalarial activities (Kuete et al. 2013; Mbaveng et al. 2008); 4-methoxylonchocarpin has the potential to attenuate inflammatory diseases including colitis (Jang et al. 2017); barbigerone has antioxidant activity, antiplasmodial activity, and apoptosis-inducing effect (Li et al. 2009; Wangensteen et al. 2006); deguelin has been reported as a potential therapeutic agent of lung cancer (Chun et al. 2003; Clarissa et al. 1997). However, these compounds have been less reported for agricultural activity studies. Only deguelin has insecticidal activity, with the LC_{50} value was about 10 mg/L against *Callosobruchus maculatus* (Belmain et al. 2012; Zhang et al. 2020). In the present study, deguelin and 4-hydroxylonchocarpin were found to have high aphicidal activity against *S. avenae*, *M. Crassicauda*, and *A. pisum.* Considering the structural specificity and high activity of 4-hydroxylonchocarpin, it has the potential to be used as a lead compound for insecticides.

EDPE and its main constituent are safe for humans and other non-target organisms. *E. dulcis* is one of the most popular aquatic vegetables in China and the peel is often discarded when consumed, but previous studies have demonstrated that *E. dulcis* peel exhibits good antioxidant activity and has potential use in food preservation as a natural food additive (Nie et al. 2021; Zhan et al. 2014). The active substance 4-hydroxytryptamine has pharmacological activity and may be a potential antibacterial drug against tuberculosis and gonorrhea (Kuete et al. 2013; Mbaveng et al. 2008). This indicates that extracts from *E. dulcis* peel may be non-toxic or only slightly toxic to human and non-target species. Thus, EDPE and 4-hydroxylonchocarpin may contribute to developing environmental safety insecticides to protect crops from various pests.

5. Conclusion

EDPE has high aphicidal activity against *M. Crassicauda*, *S. avenae*, *A. gossypii*, and *A. pisum* both in petri plate and pot culture experiments. Nine ingredients were characterized as 4', 5'-dimethoxy-6, 6-dimethylpyranoisoflavone, 3-methoxy-4-hydroxylonchocarpin, 4-hydroxylonchocarpin, 4-

methoxylonchocarpin, barbigerone, lonchocarpusone, N-pentylaniline, 6a, 12a-dehydrodeguelin, and deguelin. 4-hydroxylonchocarpin and deguelin are the active components of *E. dulcis* peel. EDPE and its main active constituents could be regarded as a prospective source of botanical insecticides for aphid control.

AUTHOR INFORMATION

Declarations

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Competing interests

The authors declare that they have no competing financial interests.

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Figures

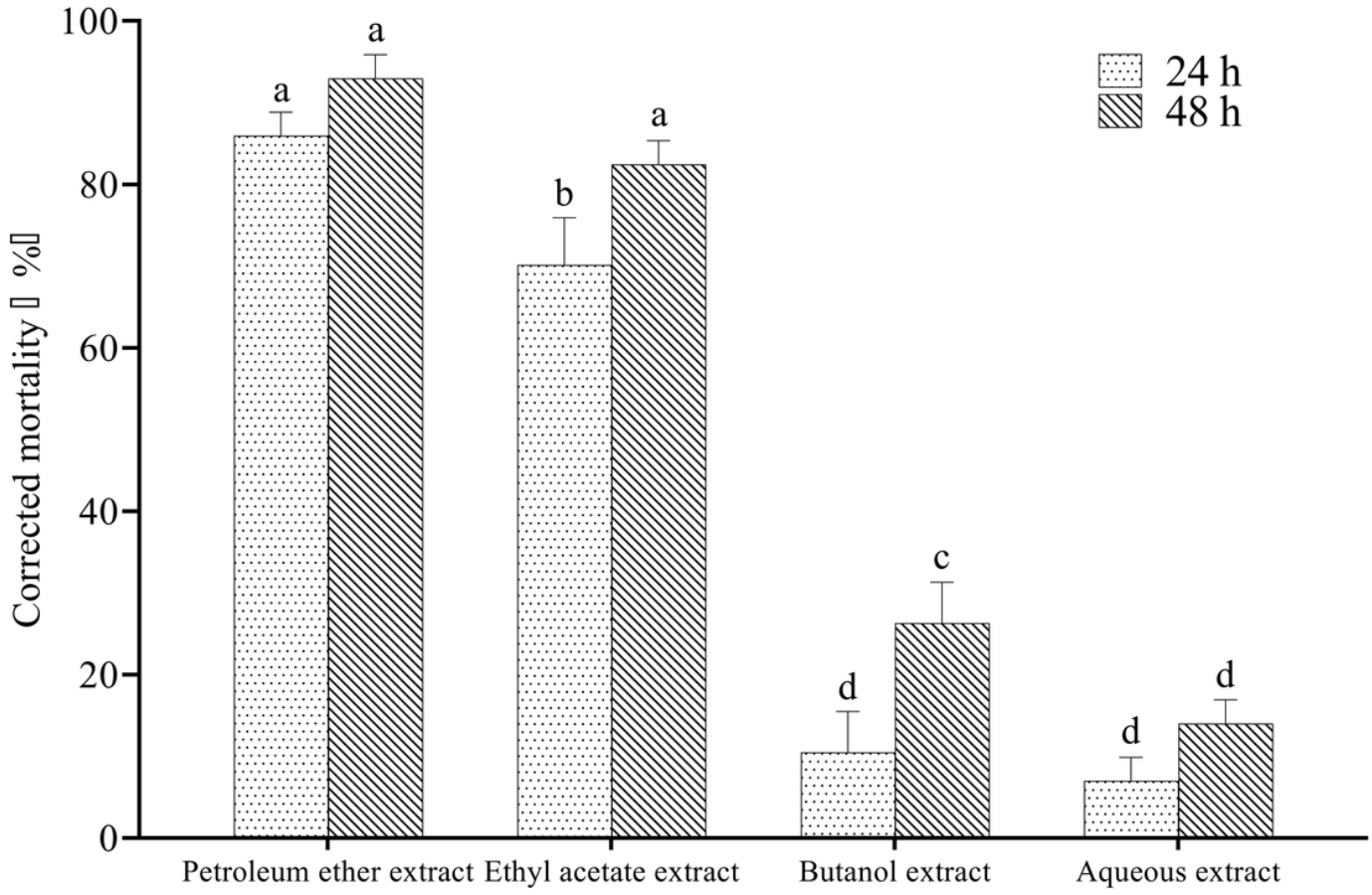

Figure 1

Aphicidal activity of different organic extracts of *E. dulcis* against *M. Crassicauda*

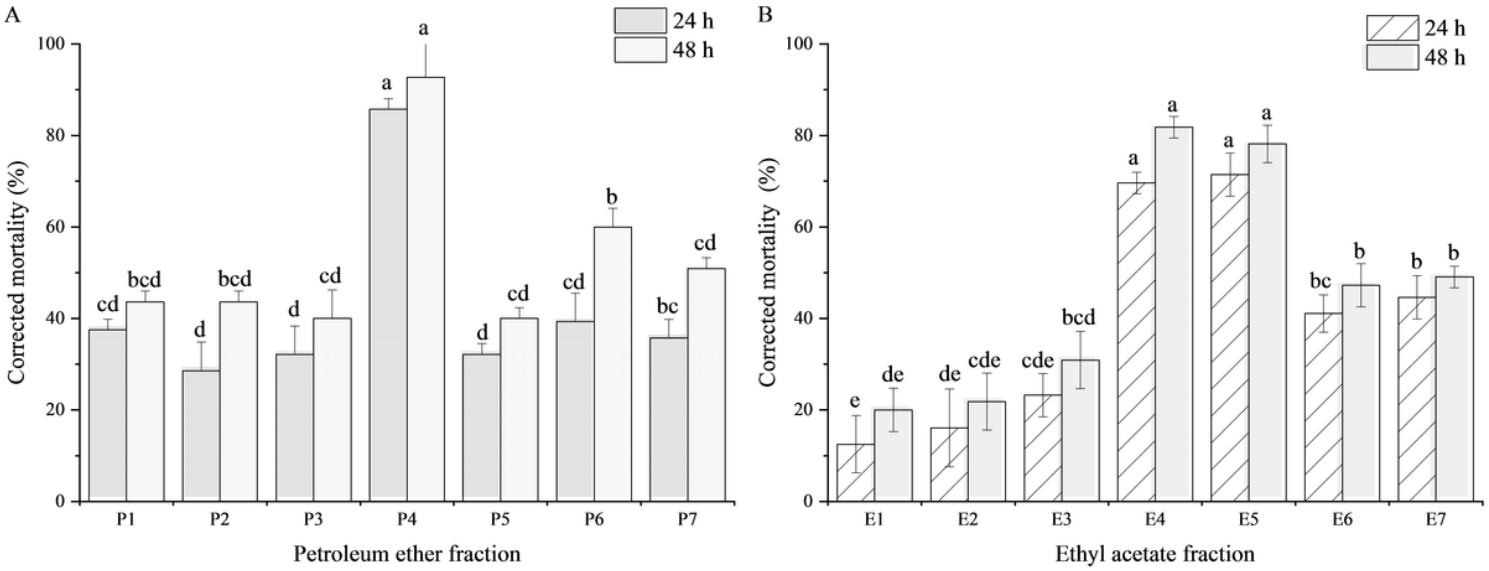

Figure 2

Aphicidal activity of different fractions from petroleum ether extract and ethyl acetate extract against *M. crassicauda*

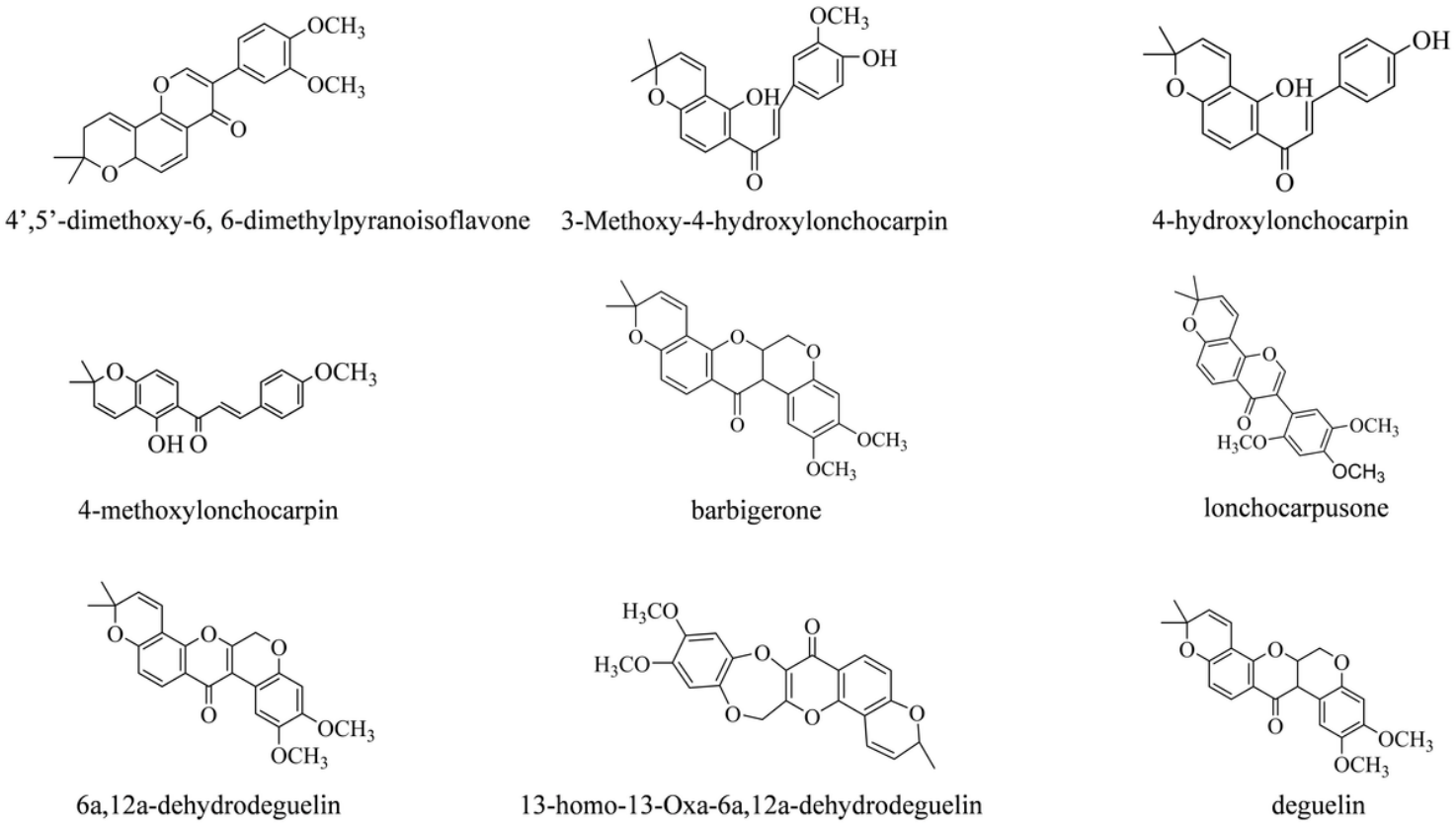

Figure 3

The structure of compounds soluble in chloroform isolated from *E. dulcis*

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