Search for Biologically Active Compounds from Sri Lankan Plants

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Abstract: In a continuation of our studies towards the discovery of biologically active compounds from Sri Lankan plants, recently we have chemically investigated the various parts of *Diploclisia glaucescens* (Menispermaceae), *Filicium decipiens*, *Pometia eximia* (Sapindaceae), *Artocarpus nobilis* (Moraceae) and *Bridelia retusa* (Euphorbiaceae). These work led to the isolation of a number of ecdysones, triterpenes, saponins, chalcones, stilbenes, flavonoids including over twenty-five new natural products. Some of these compounds showed high molluscicidal, insecticidal, antifungal activities and radical scavenging properties towards DPPH.

INTRODUCTION

The flora of Sri Lanka comprises about 3500 flowering plants of which about 850 species are endemic to the island [1]. Among these, approximately 750 species are claimed to have use in the indigenous system of medicine [2]. In a continuation of our studies towards the discovery of biologically active substances from Sri Lankan plants, recently we have chemically investigated the various parts of *Diploclisia glaucescens* (Menispermaceae), *Filicium decipiens*, *Pometia eximia* (Sapindaceae), *Artocarpus nobilis* (Moraceae) and *Bridelia retusa* (Euphorbiaceae). These works led to the isolation of a number of ecdysteroids, triterpenes, saponins, chalcones, stilbenes, flavonoids including over twenty-five new natural products. Some of these compounds showed high molluscicidal, insecticidal, antifungal and antioxidant activities.

Diploclisia glaucescens (Bl.) Diels (=*Cocculus macrocarpus* W. & A.) is a liana of the family Menispermaceae, growing in the mid-country regions of South India and Sri Lanka. The leaves have been used in the treatment of biliousness and venereal diseases [3]. Earlier studies on the seeds of this plant described the isolation of a principle ecdysteroid, 20-hydroxyecdysone (ecdysterone) (**1**) (0.46% from seeds) along with four additional ecdysteroids, which were active to larvae of the European corn borer, *Ostrinia nubialis* [4]. 20-Hydroxyecdysone (0.5% from roots) was isolated also from the roots of *D. glaucescens* [5]. Our previous studies on the stem of this plant furnished 20-hydroxyecdysone in 3% yield, which is the highest recorded for the isolation of **1** from plants [6], together with a proaporphine alkaloid stepharine [7] and triterpenoids serjanic acid (**2**), phytolaccagenic acid (**3**) and a series of their glycosides [8-11].

Recently we have carried out detailed analysis on the constituents of the fruits and leaves of *D. glaucescens*. Chromatographic separation of the methanol extract of the fruits of *D.*

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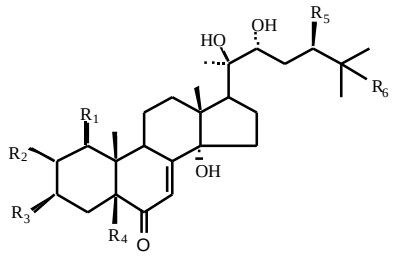
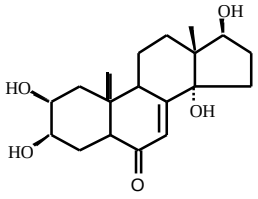
glaucescens furnished a new ecdysteroid, 2-deoxy-5β,20-dihydroxyecdysone (**4**) together with 3-deoxy-1β,20-dihydroxyecdysone (**5**), 2-deoxy-20-hydroxyecdysone (**6**), 24-ethyl-20-hydroxyecdysone (makisterone C) (**7**) [12], bidesmosidic triterpenoidal saponins, 3-*O*-β-D-glucopyranosylphytolaccagenic acid 28-*O*-β-D-glucopyranosyl ester (**8**), 3-O-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl]phytolaccagenic acid 28-*O*-β-D-glucopyranosyl ester (**9**), 3 - *O* - [α - L - rhamnopyranosyl - (1 → 2) - β - D - glucopyranosyl - (1→2) - β-D-glucopyranosyl]phytolaccagenic acid 28-*O*-β-D-glucopyranosyl ester (**10**), 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]serjanic acid 28-*O*-β-D-glucopyranosyl ester (**11)** [13], 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]serjanic acid 28-*O*-β-D-glucopyranosyl ester (**12**), 3-*O*-[β-D-xylopyranosy1-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]serjanic acid 28-*O*-β-D-glucopyranosyl ester (**13**), 3-*O*-β-D-glucopyranosyl-20-hydroxyecdysone (**14**) [14], and phenyl glycosides 4-[β-D-xylopyranosyl-(1→6)-β-D-glucopyranosyloxy]benzonitrile (**15**) and 4-(2-nitroethyl)phenyl β-D-xylopyranosyl -(1→6)-β-D-glucopyranoside (**16**) [15]. Chromatographic separation of the methanol extract of the leaves of *D. glaucescens* furnished a new ecdysteroid 3-deoxy-1β,20-dihydroxyecdysone (**5**) [16] together with makisterone A (**17**), dihydrorubrosterone (**18**), *epi*-pterosterone (**19**) [17] and four oleanane glycosides 3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl]oleanolic acid 28-*O*-β-D-glucopyranosyl ester (**20**), 3-*O*-[β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl]oleanolic acid 28-*O*-β-D-glucopyranosyl ester (**21**) [18], 3-*O*-{β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl}oleanolic acid 28-*O*-β-D-glucopyranosyl ester (**22**) and 3-*O*-{β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-β-D-glucopyranosyl} oleanolic acid 28-*O*-β-D-glucopyranosyl ester (**23**) [17]. The new ecdysteroid (**5**) showed 40% potency of 20-hydroxyecdysone in the spiracle index assay using the fourth instar larvae of the silkworm *Bombyx mori* [16]. Chromatographic separation of the non-quaternary alkaloidal fraction of the methanol extract of *D. glaucescens* furnished a novel pyridine ring-containing ecdysteroid, named diploclidine (**24**) [19].





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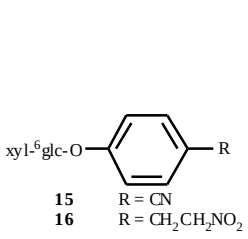
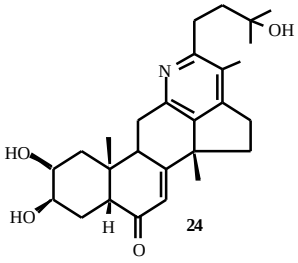
	R_1	R_2	R_3	R_4	R_5	R_6
1	H	OH	OH	H	H	OH
4	H	H	OH	OH	H	OH
5	OH	OH	H	H	H	OH
6	H	H	OH	H	H	OH
7	H	OH	OH	H	C_2H_5	OH
14	H	OH	glc-O	H	H	OH
17	H	OH	OH	H	CH_3	OH
19	H	OH	OH	H	OH	H



15 R = CN
16 R = $CH_2CH_2NO_2$



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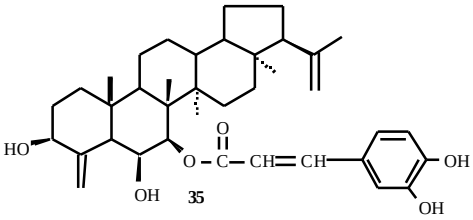

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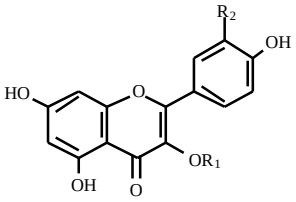
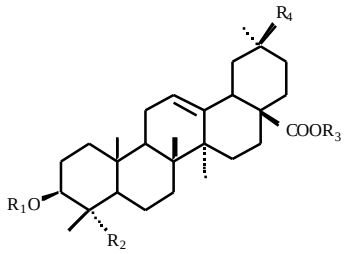


	R1	R2
37	glc	H
38	glc	OH
39	rha-2glc	H

Pometia eximia Hook f. of the family Sapindaceae is a tree of moderate size growing in Sri Lanka. Preliminary studies of the methanol extract of the stem of the plant showed strong molluscicidal and larvicidal activities. At a minimum concentration of 15 ppm, the methanol extract caused 100% mortality of *Biomphalaria glabrata* snails, one of the intermediate hosts of *Schistosoma* parasite. At a 300 ppm, the methanol extract caused 84% mortality of *Aedes albopictus* larvae within 24 hours. No antileukaemic activity was observed against L-1210 cells *in vitro*. The methanol extract also showed highly positive froth test indicating the presence of saponins. Chromatographic separation of the methanol extract furnished hederagenin (**25**) and nine hederagenin glycosides: 3-*O*-α-L-arabinopyranosyl (**26**), 3-*O*-β-D-xylopyranosyl-(1→3)-α-L-arabinopyranosyl (**27**), 28-*O*-β-D-apiosyl-(1→2)-β-D-glucopyranosyl (**28**), 3-*O*-α-L-arabinofuranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-β-D-xylopyranosyl (**29**), 3-*O*-β-D-apiosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl (**30**), 3-*O*-α-L-arabinofuranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-β-L-arabinopyranosyl (**31**), 3-*O*-β-D-xylopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl (**32**), 3-*O*-β-D-xylopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl (**33**), 3-*O*-β-D-galactopyranosyl-(1→3)-[α-L-rhamno pyranosyl-(1→2)]-β-D-glucopyranosyl (**34**). Saponins **28** – **34** are new natural products [20]. The saponins **26**, **27**, **29**, **31** and **32** which contain arabinose showed strong molluscicidal activity at a concentration of 40 ppm, 40 ppm, 10 ppm, 2.5 ppm and 5 ppm, respectively, whereas the saponins **28**, **30**, **33** and **34** which contain glucose did not show any molluscicidal activity [21]. However, the presence of arabinose moieties and the absence of the glucose does not seems to be a prerequisite for molluscicidal activity, since certain saponins of *D. glaucescens* containing glucose showed activity against the same snails *B. glabrata* [10]. The saponin **31** showed strong insecticidal activity against the brown rice planthopper *Nilaparvata lugens* [22].



	R_1	R_2	R_3	R_4
2	H	CH_3	H	$COOCH_3$
3	H	CH_2OH	H	$COOCH_3$
8	glc	CH_2OH	glc	$COOCH_3$
9	glc-^{3}glc-	CH_2OH	glc	$COOCH_3$
10	rha-^{2}glc-^{2}glc-	CH_2OH	glc	$COOCH_3$
11	rha-^{2}glc-^{2}glc-	CH_3	glc	$COOCH_3$
12	glc-^{2}glc-	CH_3	glc	$COOCH_3$
13	xyl-^{2}glc-^{2}glc-	CH_3	glc	$COOCH_3$
20	glc-^{3}glc-	CH_3	glc	CH_3
21	xyl-^{2}glc-	CH_3	glc	CH_3
22	glc-^{3}glc$_2$- | glc	CH_3	glc	CH_3
23	glc-^{3}glc$_2$- | xyl	CH_3	glc	CH_3
25	H	CH_2OH	H	CH_3
26	ara-	CH_2OH	H	CH_3
27	xyl-^{3}ara-	CH_2OH	H	CH_3
28	H	CH_2OH	api-^{2}glc-	CH_3
29	rha-^{2}xyl$_3$- | ara(*f*)	CH_2OH	H	CH_3
30	rha-^{2}glc$_3$- | api	CH_2OH	H	CH_3
31	rha-^{2}ara$_3$*- | ara(*f*)	CH_2OH	H	CH_3

Table Contd….

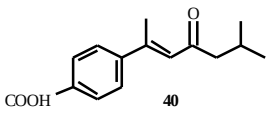
32	rha-^{2}ara$_{3}$- | xyl	CH_2OH	H	CH_3
33	rha-^{2}glc$_{3}$- | xyl	CH_2OH	H	CH_3
34	rha-^{2}glc$_{3}$- | gal	CH_2OH	H	CH_3

api = β-D-apiofuranosyl ara = α-L-arabinopyranosyl
ara* = β -L-arabinopyranosyl ara(f) = α -L-arabinofuranosyl
gal = β -D-galactopyranosyl glc = β -D-glucopyranosyl
rha = α -L-rhamnopyranosyl xyl = β -D-xylopyranosyl

Filicium decipiens (Wight et Arn.) Thw. of the family Sapindaceae is a tree of moderate size growing in wet and intermediate zones of Sri Lanka. Preliminary investigations of the dichloromethane, methanol and n-butanol fraction of the methanol extract from the leaves and the stem showed a variety of biological activities, *e.g.*, antifungal, antibacterial and molluscicidal activities [23]. Chromatographic separation of the dichloromethane extract of the stem furnished a new natural product 24-norneohopa-4(23),22(29)-diene-3β,6β,7β-triol 7-caffeate (**35**) [24] and the *n*-butanol extract from the methanol extract of the leaves furnished sitosterol β-D-glucoside (**36**), 3-*O*-β-D-glucopyranosylkaempferol (**37**), 3-*O*-β-D-glucopyranosylquercetin (**38**) and 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl] kaempferol (**39**) [25].

Bridelia retusa (L.) Spreng. of the family Euphorbiaceae is a tree of moderate size growing in Sri Lanka. Roots and stem bark of this plant used in the indigenous system of medicine for the treatment of rheumatism and as an astringent [26]. Antifungal activity guided fractionation of dichloromethane, ethyl acetate and methanol extracts of the stem bark of *B. retusa* furnished new bisabolane sesquiterpenes, (*E*)-4-(1,5-dimethyl-3-oxo-1-hexenyl)benzoic acid (**40**), (*E*)-4-(1,5-dimethyl-3-oxo-1,4-hexadienyl)benzoic acid (**41**), (*R*)-4-(1,5-dimethyl-3-oxo-4-hexenyl)benzoic acid (**42**) and (-)-isochaminic acid (**44**), together with the known (*R*)-4-(1,5-dimethyl-3-oxohexyl)benzoic acid (ar-todomatuic acid) (**43**), 5-allyl-1,2,3-trimethoxybenzene (elemicin) (**45**), (+)-sesamin (**46**) and 4-isopropyl-benzoic acid (cumic acid) (**47**) [27]. Antifungal bioassay on TLC bioautography method [28] revealed that the minimum amount of compounds needed to inhibit the growth of *Cladosporium cladosporioides* were **40** (50 µg/spot), **41** (25 µg/spot), **42** (5 µg/spot), **43** (25 µg/spot), **44** (10 µg/spot), **45** (No activity), **46** (25 µg/spot), **47** (10 µg/spot). Compound **42** showed the most potent antifungal activity [27].

Artocarpus nobilis Thw. is an endemic tree of the family Moraceae growing in mid country regions of Sri Lanka. This is the only endemic species of the genus *Artocarpus* in Sri Lanka. Several pyranodihydrobenzoxanthones, chromenoflavonoids, triterpenes have been reported from the bark of the plant [29]. Antifungal activity-guided fractionation of the *n*-butanol extract from the methanol extract of the stem bark of *A. nobilis* with a combination of chromatographic separation furnished two stilbene derivatives, *(E)*-4-isopentenyl-3,5,2',4'-tetrahydroxystilbene (**48**) and *(E)*-4-(3-methyl-*E*-but-1-enyl)3,5,2',4'-tetrahydroxystilbene (**49**). Both compounds showed strong antifungal activity at 10 µg/spot against *C. cladosporioides* when assayed by TLC bio-autography method. Antioxidant properties of **48** and **49** were evaluated against the DPPH radical by TLC bio-autography method, in which both compounds were active at 1 µg/spot [30].



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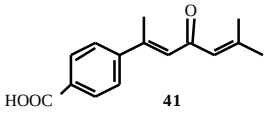

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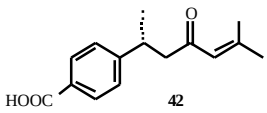

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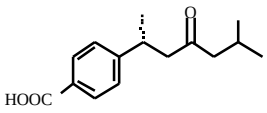

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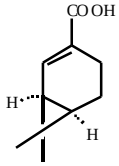

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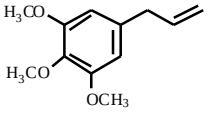

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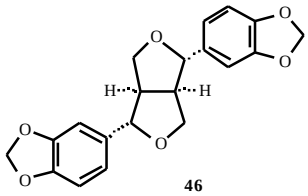

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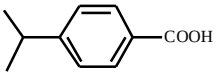

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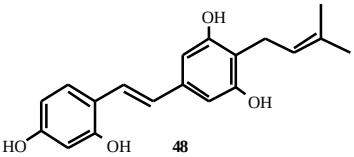

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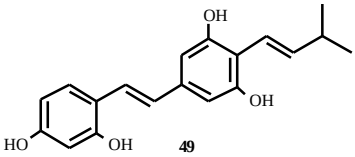

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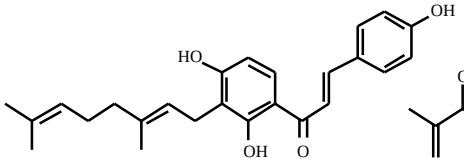

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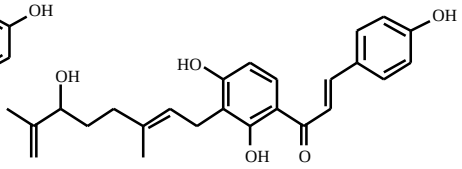

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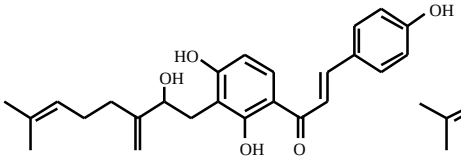

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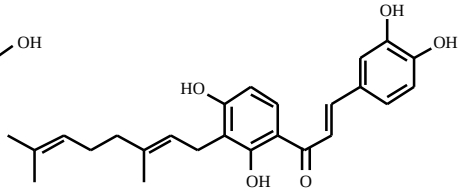

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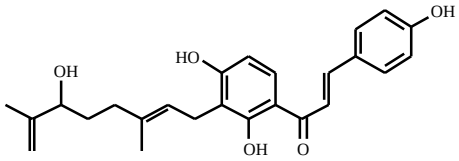

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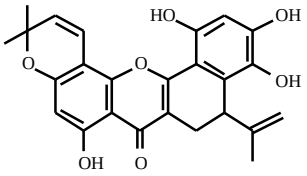

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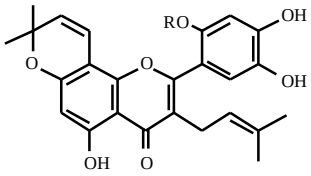
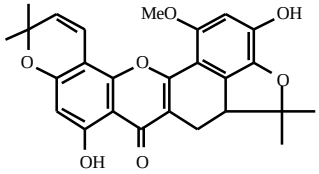


56 R = H
58 R = Me



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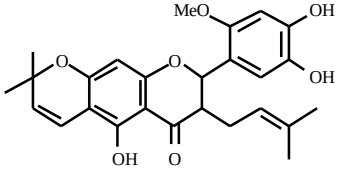

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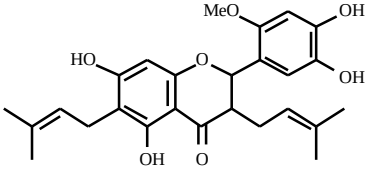

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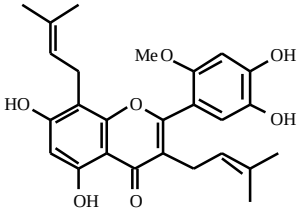

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Antifungal activity guided fractionation of the *n*-butanol extract from the methanol extract of the leaves of *A. nobilis* furnished 2',4',4-trihydroxy-3'-geranylchalcone (**50**), 2',4,4'-trihydroxy-3'-[6-hydroxy-3,7-dimethyl-2(*E*),7-octadienyl]chalcone (**51**), 2',4', 4-trihydroxy-3'-[2-hydroxy-7-methyl-3-methylene-6-octaenyl]chalcone (**52**), 2',3,4,4'-tetrahydroxy-3'-geranylchalcone (**53**), 2',3,4,4'-tetrahydroxy-3'-[6-hydroxy-3,7-dimethyl-2(*E*),7-octadienyl]chalcone (**54**). The chalcones **52** and **54** are new natural products, whereas **50** and **51** are reported for the first time from the family Moraceae. All these compounds **50**-**54** showed fungicidal activity at **50** (5 μg/spot), **51** (5 μg/spot), **52** (5 μg/spot), **53** (2 μg/spot) and **54** (15 μg/spot) against *C. cladosporioides* on TLC bio-autography method. Compounds **50**-**54** exhibited strong radical scavenging property (active at 1 μg/spot) towards DPPH radical when assayed by TLC bio-autography method [31].

Chromatographic separation of the *n*-butanol extract from the methanol extract of the root bark of *A. nobilis* furnished four new prenylated flavonoids, artonine E 2'-methyl ether (**58**), isoarotonine E 2'-methylether (**59**), dihydroisoarotonine E 2'-methyl ether (**60**) and artonin V 2'-methyl ether (**61**), together with known artobiloxanthone (**55**), artonine E (**56**) and cycloartobiloxanthone (**57**). All these compounds showed strong radical scavenging properties towards DPPH radical[32].

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