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Original Article

GARDINERIN, A BIOLOGICALLY ACTIVE ACETOGENIN FROM THE SRI LANKAN *GONIOTHALAMUS GARDINERI* HOOK. F. AND THOMSON

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ABSTRACT

Objective: The study was undertaken to isolate biologically active compounds from *Goniothalamus gardineri*, a plant endemic to Sri Lanka.

Methods: Roots and flowers of *Goniothalamus gardineri* were extracted into dichloromethane and methanol. A new acetogenin, gardinerin isolated by column chromatography of the dichloromethane extract was structurally characterized using NMR and Mass spectroscopies. It was found to be mosquito larvicidal (against 2nd instar larvae of *Aedes aegypti*), cytotoxic (in the brine shrimp assay) and antioxidant (DPPH assay).

Results: Gardinerin exhibited potent mosquitolarvicidal activity (LC_{50} = 0.0744±0.37 ppm.), cytotoxicity (LC_{50} = 1.5±0.37 ppm) and antioxidant activity (IC_{50} =10.02±0.01 ppm). The same extract furnished (5*R*)-goniothalamin. The hexane extract of the flowers of *G. gardineri* yielded poriferesterol and stigmast-4, 22-dien-3-one.

Conclusion: The endemic plant *G. gardineri* has yielded an acetogenin possessing highly potent antioxidant, cytotoxic and mosquitolarvicidal activity.

Keywords: Gardinerin, New acetogenin, *Goniothalamus gardineri,* Annonaceae, Antioxidant activity, Cytotoxicity, Mosquitolarvicidal activity.

INTRODUCTION

The variety and the high endemic to non-endemic ratio of its flowering plants makes Sri Lanka a biodiversity hotspot. In total there are 3210 flowering plants recorded, of which 916 are endemic. Among the lower plants such as lichens the recent reports of new species shed light on the tremendous richness of diversity as compared to peninsular India [1, 2]. Screening of Sri Lankan plants for biological activity has revealed promising results [3-6]. In addition, the potential of bioactivity among Sri Lankan higher and lower plants are exemplified by the discovery of aporphine alkaloids [7, 8], D: A-Friedo-oleanane and quinonemethide triterpenoids [9, 10], lichen compounds with siderophore type iron chelating function [11-14], and phenolic acids and ketones [15, 16] possessing a variety of bioactivities.

Significantly, the Annonaceae in Sri Lanka has 15 endemic plant species with a total of 42 species. Worldwide, Annonaceae is an entirely tropical family with about 120 genera and 2300 species. Members of family Annonaceae have been investigated as potential sources of biologically active Annonaceous acetogenins containing five-membered ring lactones, which have exhibited powerful anti-tumor activities [17]. In recent times, the search and synthesis of five membered ring containing natural products have become important [18, 19]. The genus *Goniothalamus* (Annonaceae) consists of over 120 species of shrubs and small to large trees. It is widely distributed in lowland and submontane tropical forests in South-East Asia, with the center of diversity in western Malaysia [20]. It is represented in Sri Lanka by five species, all endemic except *G. thwaitesii* [21]. A number of *Goniothalamus* species are widely used in traditional medicine by the local communities in Malaysia [22]. The most common medicinal usage of plants of this genus is associated with instigating abortions and for undefined post-natal treatments [23]. The endemic plant *Goniothalamus gardneri* Hook, f. and Thomson (Sinhala: katu kera) is confined to the western part of the hill country in Sri Lanka. It is rather common in secondary and disturbed primary rainforests. It is less abundant or rare in undisturbed vegetation, usually at elevations between 300 and 900 m. *G. gardneri* is an erect treelet, 1.5-5 m tall, poorly branched and unbranched when young [21]. During the course of our investigation of the roots of this plant, we have isolated a new acetogenin Gardinerin. The flowers of *G. gardneri* yielded (5*R*)-goniothalamin, poriferasterol and sitgmasta-4, 22-dien-3-one.

MATERIALS AND METHODS

Plant material and preparation of extracts

The root bark (35 g) of *G. gardneri* was collected in January 2002 from the Gannoruwa forest, Central Province, Sri Lanka. The flowers (410 g) of the plant were first collected in August 2002 from the same place. The plant material was identified by Dr. Siril Wijesundara, Department of Royal Botanic Gardens, Sri Lanka. Voucher specimens have been deposited at the National Herbarium, Peradeniya, Sri Lanka.

Ground plant root (125 g) was subjected to sequential extraction with hexane followed by CH_2Cl_2 and MeOH. Plant extracts were concentrated *in vacuo* to yield 3.2 g of brown CH_2Cl_2 extract and 1.71g of brown MeOH extract. Extraction of flowers (32 g) under conditions similar to above gave 10 g of dark green CH_2Cl_2 extract and 22 g of dark green MeOH extract.

Isolation of compounds

The CH_2Cl_2 extract of roots (3.2 g) was made into a slurry with silica gel (3.2 g) and subjected to MPLC on silica gel (5 g) using the solvent gradient hexane to 50% MeOH/CH_2Cl_2 to give late eluting mosquitolarvicidal fractions. These active fractions were combined and further fractioned on a gravity column on silica gel (3 g) using the solvent gradient 2.5% MeOH/CH_2Cl_2 to 20% MeOH/CH_2Cl_2 to yield pure gardinerin as a colorless wax (18 mg); secondly it gave (5*R*)-goniothalamin (white crystals, 42 mg, recrystalized in CH_2Cl_2/hexane mixture to obtain colorless crystals). m. p. 81–82 °C, [23];^{1}H and ^{13}C data were identical to that reported in the literature for (5*R*)-goniothalamin [24, 25].

Air-dried flowers (410 g) of *G. gardneri* were subjected to sequential solvent extraction with hexane followed by CH_2Cl_2 and MeOH. The hexane extract when subjected to MPLC on silica gel (53 g) using the

solvent gradient, 5% MeOH/CH_2Cl_2 gave poriferesterol (white crystalline solid, 9 mg; m. p. 148-149 °C (Lit. m. p. 155-156 °C [26] $[\alpha]_D$[25]-50° (*c* 0.02, CH_2Cl_2); ^1H and ^{13}C data were identical to those reported in the literature) followed by stigmast-4,22-dien-3-one (40 mg, colorless semi-solid; ^1H and ^{13}C data were identical to those reported in the literature) [27].

Bioassays

Mosquitolarvicidal assay was carried out for gardinerin using the second instar larvae of *Aedes aegypti* [28].

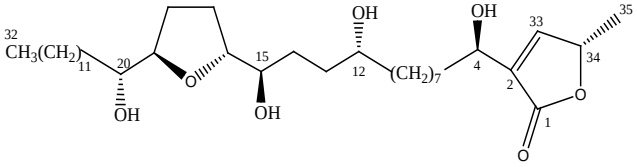
The antioxidant activity of the plant extracts was determined using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method [19]. The final concentration of DPPH in the test mixture was maintained at 1×10^{-4} mol dm^{-3} in methanol, and α-tocopherol was used as a positive control. The IC_{50} values were determined in triplicate using solutions of 1, 5, 10, 15, 20, 40, 60, 80 and 100 ppm. The absorbance of the test solutions was measured at 517 nm after 30 min using a UV spectrophotometer (Shimadzu, UV-1800). The antioxidant activity was calculated using the formula: % antioxidant activity = [(Ai-Af)/Ai] × 100, where Ai is the initial absorbance of the test mixture following the addition of DPPH and Af is the absorbance after 30 min. IC_{50} values stated as mean±SD, in ppm, were based on different concentrations and three replicates.

Cytotoxic activity was determined using brine shrimp assay [29]. A concentration series of the test compound was prepared ranging from 2000, 750, 200, 75.0, 20.0, 7.5, 2.0 and 0.75 ppm in DMSO (1% v/v) in sea water. A final volume of 5.0 ml was maintained for each test sample and 10 one day old shrimp larvae were added to each concentration level. After 24 h, the viable of shrimps was counted for each concentration level and the percent lethality was determined using Probit analysis. The LC_{50} values were determined with 95% confidence intervals using software Minitab®16. (4*S*)-4-Methyl-2-(11-dodecynyl)-2-butenolide [21] and DMSO (1.0% v/v) in sea water were used as a positive control and a negative control, respectively. All tests were carried out in triplicate. The data were statistically analyzed and LC_{50} values, based on eight concentrations and three replicates, expressed as mean±SD.

RESULTS AND DISCUSSION

HRFABMS of gardinerin (m. p. 55-57 °C; $[\alpha]_D$[25]+6° (*c* 0.01, CH_2Cl_2) showed a self-protonated molecular ion at *m/z* 597.4752 $[MH]^+$(calcd. 597.4730) indicating a molecular formula of $C_{35}H_{64}O_7$. The ^1H, ^{13}C and DEPT NMR spectrums obtained for gardinerin showed 35 carbon atoms with 64 attached hydrogen atoms. The ^{13}C NMR clearly indicated seven carbon atoms bearing oxygen atoms and one ester carbonyl. Gardinerin had a UV absorption at 228 nm (λ_{max}, CH_2Cl_2) indicating that it had an α, β-unsaturated methyl–γ-lactone moiety. ^1H and ^{13C} spectral data confirmed the presence of an α, β-unsaturated methyl–γ-lactone moiety bearing a secondary methyl group (δ_H 1.42, H-35; δ_C 19.1, C-35; δ_H 5.05, H-34; δ_C 78.1; δ_H 7.20, H-33; δ_C 152.0, C-33; 131.2, C-2; 174.6, C-1) (table 1), a common feature in Annonaceous acetogenins [29].

A signal at δ 3.0 in ^1H NMR representing four hydrogens disappeared upon the addition of D_2O, a prominent IR OH absorption at 3400 cm^{-1} and four successive losses of H_2O (*m/z* 18) from the $[MH^+]$ in the FABMS suggested the presence of four hydroxyl functions (fig. 1). ^1H and ^{13}C signals further indicated the presence of a tetrahydrofuran (THF) ring with two flanking hydroxyl groups (δ_H 3.42, H-15; δ_C 74.4, C-15; δ_H 3.82, H-16; δ_C 82.7, C-16; δ_H 3.82, H-19; δ_C 82.7, C-19; δ_H 3.42, H-20; δ_C 74.1, C-20). FABMS of gardinerin showed this moiety to be located at C-15-C-20; this placement was further corroborated by the HRFABMS in which the fragment appearing at *m/z* 299.2241 (calcd. 299.2238) corresponding to an elemental composition of $C_{18}H_{35}O_3$. In the FABMS the major fragment ion responsible for the peak at *m/z* 397 was attributed to that formed by the cleavage at C-19/C-20. The ion responsible for the peak at *m/z* 299 was formed by the C-14/C-15.



Gardinerin

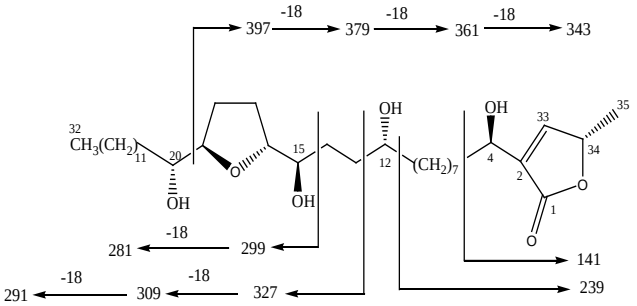


Fig. 1: Mass spectral fragmentation profile of gardinerin

Table 1: ^1H and ^{13}C NMR data for gardinerin

Position[a]	δ_C	δ_H (*J* in Hz)
1	174.6	
2	131.2	
3		33.3 3.45 m
4		69.9 3.82 m
5-11	22.7-38.3	1.25-1.60 m
12	71.8	3.59 m
13-14	33.9-34.2	1.41-1.48 m
15	74.4	3.42 m
16	82.7	3.82 m
17	28.1	1.72 m, 1.94 m
18	28.9	1.64 m, 1.96 m
19	82.7	3.82 m,
20	74.1	3.42 m
21-31	22.7-38.3	1.25-1.60 m
32	14.1	0.88 t (6.9)
33	152.0	7.20 s
34	78.1	5.05 qd (6.6, 1.5)
35	19.1	1.42. d (6.6)

[a]Spectra collected in C_6D_6 at 400 MHz

The ions at *m/z* 327 formed by the C-12/C-13 cleavage. Furthermore, a fragment ion appearing at *m/z* 141 was due to the cleavage at C-4/C-5 further proving the presence of an α, β-unsaturated methyl–γ-lactone moiety. Based on the above data, it was evident that gardinerin

were a mono-THF acetogenin containing hydroxyl groups at C-4, 12, 15 and 20. The relative stereochemistry across the THF ring and the flanking hydroxyls were assigned *threo/trans/threo* based on ^{1}H and ^{13}C data of gardinerin which were consistent with those of model acetogenins [30, 31].

Gardinerin showed highly potent larvicidal activity with an LC_{50} = 0.0744 ppm, cytotoxicity (LC_{50} = 1.5±0.37 ppm) and antioxidant activity (IC_{50} =10.02±0.01 ppm). The same extract furnished (5*R*)-goniothalamin. The hexane extract of the flowers of *G. gardineri* yielded poriferesterol and stigmast-4, 22-dien-3-one.

CONFLICT OF INTERESTS

Declared None.

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