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Note

An iridoid with anticancer activity from the sepals of *Mussaenda 'dona aurora'*

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The sepals of *Mussaenda 'dona aurora*' afford three iridoid glycosides and four flavonoids. Among them, Sanshiside-D, a new iridoid glycoside has been found to suppress the growth of vero cell lines (IC_{50} -1.99 μ*M*/mL). Sanshiside methyl ester and lamalbide are inactive towards vero cell lines.

Keywords: *Mussaenda 'dona aurora'*, Rubiaceae, Sanshiside-D, Vero cell lines

Mussaendas (Rubiacaeae) are increasingly popular for their showy colour. They are native to the old world tropics, from West Africa through the Indian sub continent, South East Asia and into Southern China. Many species of *Mussaenda* have been found to be biologically active[1-3]. Iridoids and flavonoids are more common in the Mussaendas[4-6]. The chemical profile of *Mussaenda phillipica* cultivars has been least explored. In our previous study, the pink variety of *Mussaenda phillipica* cultivar has been found to be cytotoxic[7]. In the present study, we report the isolation and characterization of a new iridoid along with two new flavonoids from the white variety of *Mussaenda phillipica* cultivar, *Mussaenda 'dona aurora'* L.C. Rich. (MD). *Mussaenda 'dona aurora'* is a large shrub with medium sized oval leaves. It has large velvety sepals surrounding small star shaped leaves[8]. The isolated compounds have been screened for cytotoxicity.

Results and Discussion

Compound **3** called Sanshiside-D (**Figure 1**) obtained as a white amorphous powder by preparative HPLC at Rt 9.652, had the molecular composition $C_{39}H_{48}O_{22}$ established on the basis of ESI-MS ($M+Na^+$ - 888) **(Figure 2)**, elemental analysis and ^{13}C NMR (**Table I**). The IR spectrum indicated the presence of a hydroxyl group (3436.38 cm^{-1}), ester group (1697.0 cm^{-1}) and double bond (1634.72 cm^{-1}).

The ^1H NMR spectrum showed NMR signals typical of an iridoid skeleton[9-11]. The sequential assignments of protons and carbon atoms were made with the help of of HOMOCOSY and HSQC experiments. Acetal methine proton at δ_H 5.5 (H-1, d, J = 1Hz) was assigned to position 1 (δ_C 91.81). The iridoid moiety was further confirmed by the correlation of H-9 and H-1′ (δ_H 2.67 and δ_H 4.47) signals with C-1 in the HMBC spectrum. The presence of an anomeric proton (δ_H 4.47, d, J = 7.5Hz) indicated a sugar moiety in the molecule. The chemical shifts of proton and carbon atoms were compared to the values reported in the literature which indicated the sugar to be β-D-Glucose. The structure of the trans-caffeoyl moiety was established by ^1H NMR data showing two vicinal olefinic protons [δ_H 6.13 and δ_H 7.47 (J_{AX} = 16.0 Hz)] and aromatic protons at δ_H 6.75, δ_H 6.96 and δ_H 7.37 (ref. 12). The intense peak at *m/z* 529 (100%) in the ESI-MS spectrum was due to loss of glucose and caffeoyl unit. A typical fragmentation of iridoids i.e., scission at C-9 and C-7 has been observed.

The connectivity's of the three established partial structures (iridoid, glucose, trans-caffeoyl) was established by HMBC experiment. The acetylation of hydroxyl group at C-7 was concluded from the downfield shift to H-7 (δ_H-4.8, d, J = 5Hz). It was further confirmed from the long range coupling of H-7 with -CO (δ_C 169.60) and -CH_3 (δ_C 21.02).

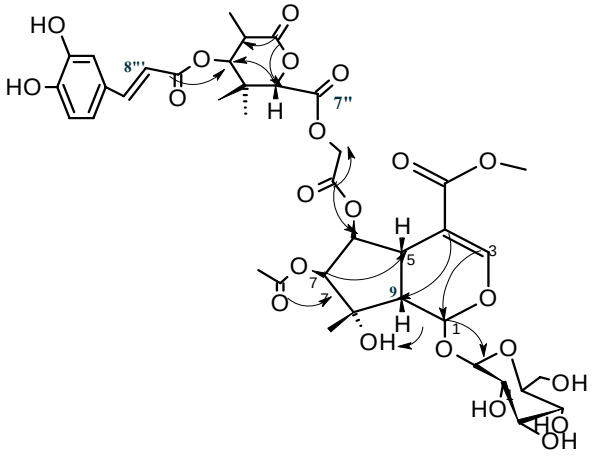

Figure 1 — Sanshiside-D

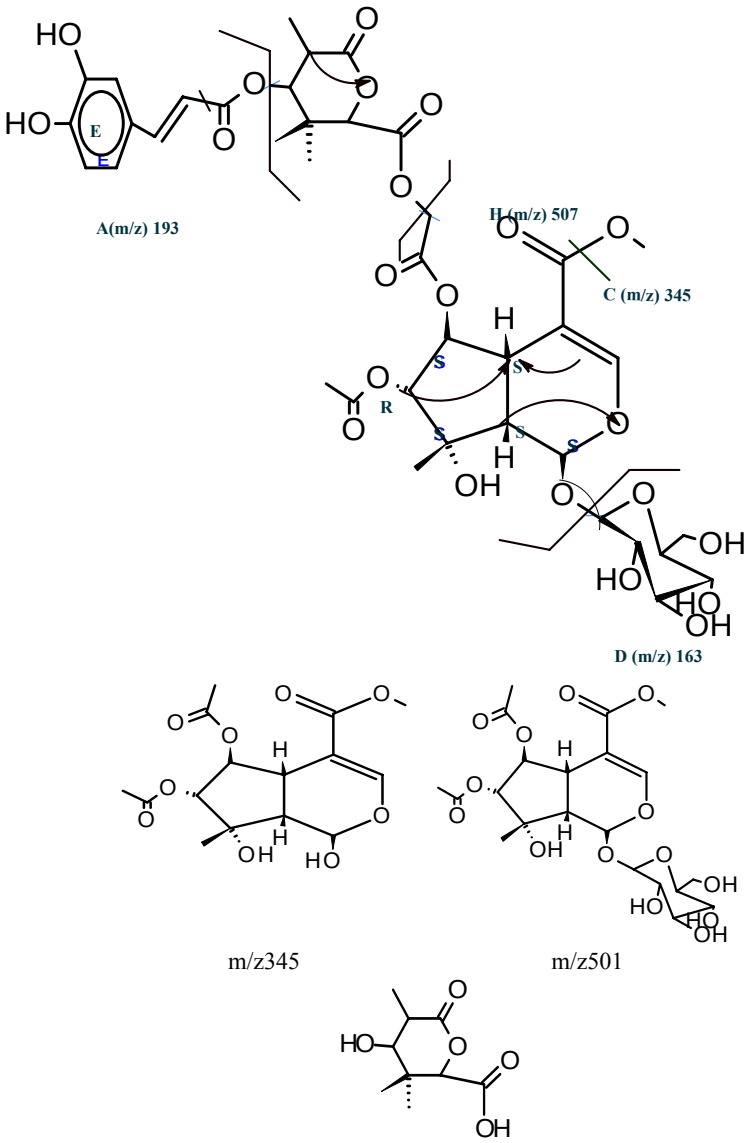

Figure 2 — Fragmentation pattern of sanshiside-D

The caffeoyl unit was linked to 6-OH of the iridoid moiety through a lactone unit. The acetylation of 6-OH was confirmed by the downfield shift of 3.305 to C-6 (δ_C 74.73). The lactone ring was assigned on the basis of HSQC and HOMO-COSY experiment. The appearance of a high field carbonyl at δ_C 174.9 confirms the presence of lactone moiety. The connectivity of lactone unit with iridoid at 6-OH was substantiated by the long range coupling of H-2″ with 6-OCO$\underline{C}H_2$ (δ_H-3.5). Further carbonyl (δ_C-171.96) group attached at C-2″ showed correlation only with protons in the lactone ring and not with protons in the caffeoyl and iridoid moiety which confirms its location. The coupling of H-4″ (δ_H 5.05, d, J = 4Hz) with caffeoyl carbonyl (δ_C 165.69) in the HMBC spectrum establishes the fact caffeoyl moiety was linked to lactone unit. Further, the correlation of H-4″ with δ_H - 4.0 (S) and δ_H -1.8 (m) proves the linkage. The stereochemistry at C-6, C-7 and C-8 was assigned based on literature data[13,14]. The alkaline hydrolysis of the glycoside produces caffeic acid which has been identified by comparing with an authentic sample of caffeic acid by TLC [*n*-butanol:formic acid:H_2O (4:1:5)] and also a change in the UV spectrum of the compound. The glycosylation was further confirmed by enzymatic hydrolysis.

Table I — ^{13}C NMR (125 MHz, DMSO-d_6) and ^{1}H NMR (500 MHz, DMSO-d_6) data of Sanshiside–D

C-No	^{13}C	^{1}H	C-No	^{13}C	^{1}H
1	91.81	5.5 (d, 1.5Hz)	10″	20.38	2.0(s)
3	151.17	7.4(s)	1‴	125.56	
4	109.06		2‴	121.29	6.9(dd 8.5Hz, 1.5Hz)
5	32.88	2.9(m)	3‴	144.86	7.4(d, 1.5Hz)
6	74.73	5.0(m)	4‴	148.80	
7	77.28	4.8(d, 3Hz)	5‴	114.74	
8	76.47		6‴	115.70	6.7(d,2Hz)
9	46.34	2.67(d, 5Hz)	7‴	114.26	6.27(d,16Hz)
10	21.02	1.9(s)	8‴	145.53	7.43(d, 16Hz)
1′	97.66	4.4(d, 7.5Hz)	9‴	165.69	
2′	73.06	2.9(m)	6- CO$\underline{C}$H$_2$	70.40	5.1(s)
3′	77.75	3.1(t)	6- $\underline{C}$OCH$_2$	169.03	
4′	70.85	3.0(t)	7- OCO$\underline{C}$H$_3$	21.02	
5′	69.99	4.5 (br S)	7- O$\underline{C}$OCH$_3$	169.60	
6′	61.05	3.4(d, 2Hz)	6″ -CO	174.90	
2″	70.73	4.0(br S)	7″ -CO	171.96	
3″	36.28		4 -$\underline{C}$OOCH$_3$	166.20	
4″	68.10	5.05 (d, 4Hz)	4 -COO$\underline{C}$H$_3$	51.04	3.5(s)Overlapped signal
5″	37.18	1.8(m)			
8″	21.6	1.7(s)			
9″	30.66	2 .1(d)			

Chemical shifts are in (δ) ppm, multiplicities and coupling constants in Hz in parentheses

Table II — Cytotoxic effect of compounds **1-7** against various cell lines (IC_{50}, μM/mL)

Sample	Vero	HeLa	SMMC-7721
1	41.02±0.34	25.04±0.51	58.81±7.06
2	33.53± 2.10	12.14±0.15	24.39±4.16
3	1.99±0.53	0.12± 0.9	1.53±0.31
4	> 100	6.29±0.21	16.21±0.13
5	> 100	18.31±0.26	41.82±0.58
6	> 100	> 100	> 100
7	> 100	> 100	> 100
Methrotrexate	3.89± 1.53	0.07 ±0.03	1.37±0.81
Taxol	2.05± 1.13	0.05±0.01	0.74±0.17
5-Fluorouracil	> 50	7.26± 0.31	0.43±0.13

Key to cell lines used: Vero cell (African green monkey), human carcinoma of uterine cervix (HeLa), human hepatoma (SMMC-7721).

The other compounds were also identified by spectral analysis and compared with the literature values whereupon compounds **1**, **2, 6,** and **7** have been designated as quercetin **1**, rhamnetin **2**, isoquercitrin **6** and rutin **7** (refs. 15, 16) and compounds **4** and **5** as sanshizide methyl ester **4** and Lamalbide **5** (ref. 17)**.**

Among the compounds tested for cytotoxicity, the potency of sanshiside-D was by far the largest. It suppressed the growth of vero cell lines with an IC_{50} value of 1.99 μ*M*/mL. (**Table II**). However, sanshizide and sanshizide methyl ester isolated from *Barleria lupina* showed no activity with vero cell lines[18]. The presence of caffeoyl and lactone unit could be accounted for the enhanced activity of sanshiside-D. In order to assess the potency of sanshizide-D as an anticancer drug, it was compared with commonly used drugs (5-Fluorouracil, Methrotrexate and Taxol). Its activity was comparable to that of taxol but found to be greater than methrotrexate. Hence, the iridoid may be considered as a new pharmacophore for the generation of new anticancer drug. The wide availability of the sepals make it a potent target for commercial exploitation.

Experimental Section

Optical rotations were obtained on a Jasco DIP-360 digital polarimeter. NMR spectra was recorded on a Bruker AMX 500 MHz instrument in DMSO-d_6. Mass spectra was recorded on a Q ToF Micromass spectrometer with electronspray ionization. UV

spectra was recorded on a Perkin-Elmer UV spectrophotometer and IR spectrum in Perkin-Elmer IR spectrometer. Chromatography was performed using Silica gel 100-200 mesh, RP C 18 (HPLC) column and Silica gel GF_{254} (TLC plates).

Extraction and isolation

The sepals of *Mussaenda 'dona aurora'* were collected in and around Thanjavur in Dec 2005. The plant has been authenticated by botanists at Rabinad Herbarium, St.Josephs College, Trichy. A voucher specimen (CARISM 0018) has been deposited at the herbarium of CARISM. The fresh sepals (5 kg) were extracted with 85% methanol. After concentrating under reduced pressure, it was dissolved in water and extracted with petroleum ether (500 mL), diethyl ether (3×500 mL) and ethyl acetate (5×2.5 L) successsively. The diethyl ether fraction (283 mg) was separated by silica gel column (2.5 × 50 cm, 60-120 mesh, 175 g) using solvents of increasing polarity (Pet. ether → chloroform → diethyl ether → ethyl acetate) to furnish two flavonols. The diethyl ether: chloroform (8:2; 25 mL) fraction afforded compound **1**. Compound **2** was obtained from diethyl ether:chloroform (6:4; 25 mL) fraction.

Ethyl acetate (EtOAc) extract was evaporated *in vacuo* to dryness at 30-35ºC. The obtained crude mixture was chromatographed by silica gel 100-200 mesh (45 g) following elution with ethyl acetate, 30 fractions were collected (15 mL each). Fractions 1-15 were pooled and purified by preparative HPLC [bondapak RP C-18, 4μm, 21.20 × 250 mm and UV detection at 254 nm) 70-30 (H_2O:methanol, 15 min) → 50-50 (H_2O: methanol, 20 min) → 20-80 (H_2O: methanol, 50 min)] to afford pure **3** (Rt 9.652, 86mg), **4** (Rt 11.531, 27 mg) and **5** (Rt 14.173, 18 mg). At Rt 44.54 min, a flavonol glycoside fraction obtained was purified by PTLC (EMK:EtOAc:HCOOH:water (4:3:1:1)) to give compounds **6** (Rf = 0.46, 18 mg) and **7** (Rf = 0.56, 32 mg).

Sanshiside-D (3): White powder. m.p. 65-67°C**.** $[\alpha]_D^{30}$ -30° (*c* 0.40, MeOH); λ^{MeOH} max (in nm) 283.18, 328.26; (+ NaOMe) 268.55, 375.70; NMR: (**Table I**); QToF MS: *m/z* 529, 345, 327, 313, 285, 267, 253, 225, 211, 207, 193, 163, 149, 145.

Cell viability assay

The cytotoxicity of compounds **1-7** were tested in three cell lines according to an established protocol[19].

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References

1 Vidyalakshmi K S, Charles Dorni A I, Hannah R Vasanthi, Rajamanickam G V & Sukumar D, *J Appl Sci*, 6, **2006,** 2251.
2 Quin G W & Xu R S, *Medical Res Rev*, 18, **1998,** 375.
3 Kim N C, Desjardins A E, Wu C D & Kinghorn A D, *J Nat Prods*, 62, **1999,**1379.
4 Weimin Zhao, Genjin Yang, Rensheng Xu & Guowei Qint, *Phytochemistry,* 41, **1996**, 1553.
5 Ranarivelo Y, Skaltsounis A L, Andriantsiferana M & Tillequin F, *Ann Pharm Fr*, 48, **1990**, 273.
6 Yoshio Takeda, Hiroshi Nishimura & Hiroyuki Inouye, 16, **1977**, 1401.
7 Vidyalakshmi K S, Charles Dorni A I & Hannah R Vasanthi, *J Pharm Toxic,* 2, **2007**, 660.
8 Rauch F D & Weissich P R, *Plants for Tropical landscapes: A gardener's guide*, (Hawaii Press) **2000**, pp 43.
9 Eigtved P, Jensen S R & Nielsen B, *Acta Chem Scand B*, 28, **1974**, 85.
10 Garcia J & Chulia A J, *Planta Med,* 53, **1987**, 101.
11 Garcia J, Lavaitte S & Gey C, *Phytochemistry,* 28, **1989**, 2199.
12 Pýnar Sahin F, Nurten Ezer & Ihsan C Alýs, *Phytochemistry* , 65, **2004**, 2095.
13 Damtoft S, Franzyk H & Jensen S R, *Phytochemistry,* 45, **1997,** 743.
14 Pittaya T, Kanokon Rayanil & Walter C Taylor, *Science Asia,* 29, **2003,** 21.
15 Agrawal P K, *Carbon 13 NMR of Flavonoids*, (Elsevier Science Publishers B.V.) **1989.**
16 Markham K R & Geiger H, *The Flavonoids, Advances in Research since 1986*, edited by J B Harborne (Chapman & Hall, London) **1994**.
17 Boros C A & Stermitz F R, *J Nat Prod,* 53, **1990,** 1055.
18 Sunit Suksamrarn, Kanjana Wongkrajang, Kanyawim Kirtikara and Apichart Suksamrarn, *Planta Med,* 69, **2003,** 87.
19 Mosmannun T, *J Immunol Meth,* 65**, 1983,** 55.