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A new and 23 known cardenolide glycosides from *Thevetia neriifolia* seeds and their cytotoxic activities against human oral carcinoma cell lines

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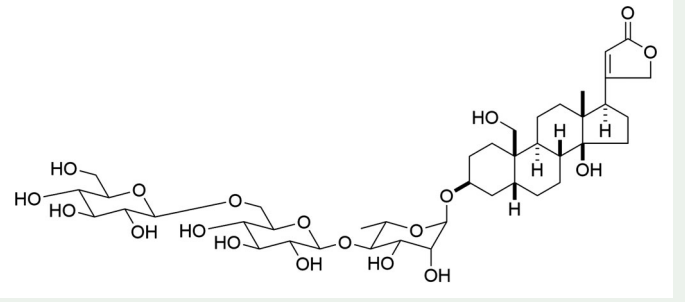
ABSTRACT

Phytochemical analysis of *Thevetia neriifolia* seeds resulted in the isolation of one new (**1**) and 23 known (**2**—**24**) cardenolide glycosides. The structure of **1** was determined based on one- and two-dimensional NMR spectroscopic analysis and acid hydrolytic cleavage reaction. The effect of the cytotoxic activity of **1**—**24** on three human oral carcinoma cell lines was assessed. The cell lines included Ca9-22 human gingival carcinoma cells, HSC-2 human mouth carcinoma cells, HSC-4 human tongue carcinoma cells, and HGF human gingival fibroblast cells. The isolated compounds had a cytotoxic effect on the carcinoma cells with IC_{50} values ranging from 0.004 μM to 64.9 μM. The structure-activity relationship is also discussed.



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Thevetia neriifolia; Apocynaceae; seed; cardenolide glycoside; cytotoxic activity; oral carcinoma cell

1. Introduction

Thevetia neriifolia Juss. ex Steud. is a small ornamental tree belonging to the Apocynaceae family (Siddiqui et al. 1992). The plant is distributed from tropical America to the West Indies and has been used as a natural remedy for treating skin

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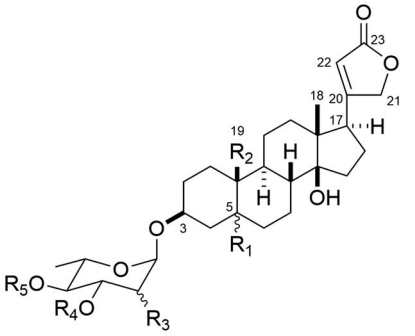
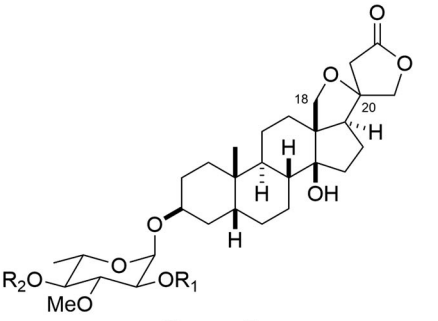
diseases and fever as a purgative and emetic (Siddiqui et al. 1992; Gulati et al. 2000). Phytochemical analyses of *T. neriifolia* leaves were extensively conducted in the past, and various cardenolide glycosides have been reported (Siddiqui et al. 1992; Abe et al. 1992a, 1992b; Begum et al. 1993; Abe et al. 1994). However, a literature survey disclosed that no systematic phytochemical examination of *T. neriifolia* seeds has been performed. Therefore, detailed chemical analysis with a focus on cardenolide glycosides was carried out on *T. neriifolia* seeds. In this study, the structural characterization of one new (**1**) and 23 (**2**−**24**) known isolated cardenolide glycosides from *T. neriifolia* seeds is reported. The isolated compounds were evaluated for the effect of the cytotoxic activities on three human oral carcinoma cell lines, Ca9-22 human gingival carcinoma cells, HSC-2 human mouth carcinoma cells, and HSC-4 human tongue carcinoma cells, along with HGF human gingival fibroblast cells. The structure-activity relationship is also discussed.

2. Results and discussion

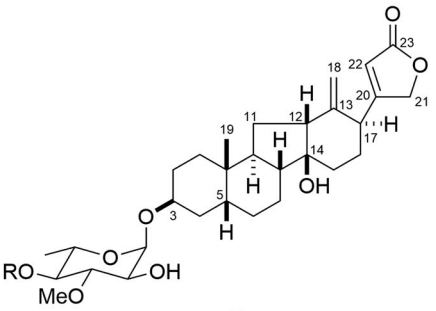
T. neriifolia seeds (2.0 kg) were extracted with hot MeOH (20 L) and concentrated using an evaporator. The MeOH extract (250 g) was loaded on a porous polymer polystyrene resin Diaion HP-20 column and successively eluted with MeOH/H_2O (1:4), EtOH, and EtOAc. The EtOH eluted portion was separated by silica gel column chromatography (CC), octadecylsilanized (ODS) silica gel CC, and preparative HPLC to collect 24 compounds (**1**−**24**). Compounds **2**−**24** were identified as the following cardenolide glycosides: digitoxigenin 3-*O*-α-L-thevetopyranoside (neriifolin) (**2**) (Yamauchi et al. 1987a; Abe et al. 1992b), uzarigenin 3-*O*-α-L-thevetopyranoside (thevefolin) (**3**) (Miyagawa et al. 2009), digitoxigenin 3-*O*-(2-*O*-acetyl-α-L-thevetopyranoside) (cerberin) (**4**) (Zhang et al. 2010), digitoxigenin 3-*O*-β-D-glucopyranosyl-(1→4)-α-L-thevetopyranoside (**5**) (Abe et al. 1992a), uzarigenin 3-*O*-β-D-glucopyranosyl-(1→4)-α-L-thevetopyranoside (**6**) (Abe et al. 1992a), cannogenin 3-*O*-β-D-glucopyranosyl-(1→4)-α-L-thevetopyranoside (**7**) (Tian et al. 2016), cannogenol 3-*O*-β-D-glucopyranosyl-(1→4)-α-L-thevetopyranoside (**8**) (Tian et al. 2016), digitoxigenin 3-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→4)-α-L-thevetopyranoside (thevetin B) (**9**) (Yamauchi et al. 1987b; Kohls et al. 2012), digitoxigenin 3-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-α-L-acofrioside (**10**) (Abe et al. 1992b), uzarigenin 3-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→4)-α-L-thevetopyranoside (**11**) (Abe et al. 1992a), uzarigenin 3-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-α-L-acofrioside (**12**) (Abe et al. 1992a), digitoxigenin 3-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→4)-2-*O*-acetyl-α-L-thevetopyranoside (acetylthevetin B) (**13**) (Endo et al. 1997; Kohls et al. 2012; Tian et al. 2016), cannogenin 3-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→4)-α-L-thevetopyranoside (thevetin A) (**14**) (Abe et al. 1994; Kohls et al. 2012), cannogenin 3-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→4)-2-*O*-acetyl-α-L-thevetopyranoside (acetylthevetin A) (**15**) (Kohls et al. 2012), cannogenol 3-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→4)-α-L-thevetopyranoside (**16**) (Tian et al. 2016), 19-nor-10-hydroxydigitoxigenin 3-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→4)-α-L-thevetopyranoside (**17**) (Tian et al. 2016), (20*R*,*S*)-18,20-epoxydigitoxigenin 3-*O*-α-L-thevetopyranoside (**18**) (Abe et al. 1992a), (20*R*,*S*)-18,20-epoxydigitoxigenin 3-*O*-(2-*O*-acetyl-α-L-thevetopyranoside)



	R_1	R_2	R_3	R4	R_5
1	β-H	CH_2OH	OH_{ax}	H	-Glc^6-Glc
2	β-H	Me	OH_{eq}	Me	H
3	α-H	Me	OH_{eq}	Me	H
4	β-H	Me	O_{eq}-Ac	Me	H
5	β-H	Me	OH_{eq}	Me	Glc
6	α-H	Me	OH_{eq}	Me	Glc
7	β-H	CHO	OH_{eq}	Me	Glc
8	β-H	CH_2OH	OH_{eq}	Me	Glc
9	β-H	Me	OH_{eq}	Me	-Glc^6-Glc
10	β-H	Me	OH_{ax}	Me	-Glc^6-Glc
11	α-H	Me	OH_{eq}	Me	-Glc^6-Glc
12	α-H	Me	OH_{ax}	Me	-Glc^6-Glc
13	β-H	Me	O_{eq}-Ac	Me	-Glc^6-Glc
14	β-H	CHO	OH_{eq}	Me	-Glc^6-Glc
15	β-H	CHO	O_{eq}-Ac	Me	-Glc^6-Glc
16	β-H	CH_2OH	OH_{eq}	Me	-Glc^6-Glc
17	β-H	OH	OH_{eq}	Me	-Glc^6-Glc



	R_1	R_2
18	H	H
19	Ac	H
20	H	-Glc^6-Glc
21	Ac	-Glc^6-Glc



	R
22	H
23	Glc
24	-Glc^6-Glc

Ac: acetyl
Glc: β-D-glucopyranosyl

Figure 1. Structures of **1**–**24.**

(**19**) (Cruz et al. 1979), (20*R*,*S*)-18,20-epoxydigitoxigenin 3-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→4)-α-L-thevetopyranoside (thevetin C) (**20**) (Kohls et al. 2012), (20*R*,*S*)-18,20-epoxydigitoxigenin 3-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→4)-2-*O*-acetyl-α-L-thevetopyranoside (acetylthevetin C) (**21**) (Kohls et al. 2012), thevetiogenin 3-*O*-α-L-thevetopyranoside (thevetioside A) (**22**) (Abe et al. 1992b), thevetiogenin 3-*O*-β-D-glucopyranosyl-(1→4)-α-L-thevetopyranoside (thevetioside C) (**23**) (Abe et al. 1992b), and thevetiogenin 3-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→4)-α-L-thevetopyranoside (thevetioside F) (**24**) (Abe et al. 1992b) (Figure 1).

Compound **1** was obtained as an amorphous powder. The molecular formula was identified as $C_{41}H_{64}O_{19}$, based on the high resolution-electrospray ionization-time of flight-mass spectroscopy (HR-ESI-TOF-MS; *m/z*: 883.3914 $[M+Na]^+$, calculated for $C_{41}H_{64}NaO_{19}$: 883.3940) and ^{13}C-NMR data. The IR spectrum of **1** showed absorption bands for hydroxy groups at 3363 cm^{-1} and the carbonyl group of an α,β-unsaturated γ-lactone moiety at 1737 cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra of **1** exhibited signals for a tertiary methyl group [δ_H 0.88 (s, Me-18); δ_C 16.4 (C-18)], an oxygenated methine

group [δ_H 3.93 (m, H-3); δ_C 73.4 (C-3)], an oxygenated methylene group [δ_H 3.84 (d, $J = 11.5$ Hz, H-19a) and 3.39 (d, $J = 11.5$ Hz, H-19b); δ_C 65.7 (C-19)], an α,β-unsaturated γ-lactone group [δ_H 5.90 (br s, H-22), 5.03 (br d, $J = 18.4$ Hz, H-21a), and 4.91 (br d, $J = 18.4$ Hz, H-21b); δ_C 178.4 (C-20), 177.2 (C-23), 117.8 (C-22), and 75.3 (C-21)], and three anomeric protons and carbons [δ_H 4.78 (br s), 4.60 (d, $J = 7.9$ Hz), and 4.38 (d, $J = 7.8$ Hz); δ_C 105.3, 104.9, and 99.7]. The above spectroscopic data suggest that **1** is a cardenolide glycoside. The ^{1}H- and ^{13}C-NMR spectra of **1** were related to those of **16**, except for the signals arising from the sugar moiety. Acid hydrolysis of **1** with 1 M HCl (dioxane/H_2O, 1:1) exposed D-glucose and L-rhamnose as the carbohydrate moieties. The monosaccharides and their absolute configurations were identified by direct HPLC analysis of the hydrolysate. Analysis of the ^{1}H-^{1}H COSY and HSQC spectra of **1** disclosed that the sugar moiety comprised a 4-substituted α-L-rhamnopyranosyl unit [Rha: δ_H 4.78 (br s, H-1′); δ_C 99.7, 72.8, 72.5, 83.3, 68.5, and 18.3 (C-1′ − 6′)], a 6-substituted β-D-glucopyranosyl unit [Glc (I): δ_H 4.60 (d, $J = 7.9$ Hz, H-1″); δ_C 104.9, 76.0, 78.1, 71.6, 77.2, and 70.2 (C-1″ − 6″)], and a terminal β-D-glucopyranosyl unit [Glc (II): δ_H 4.38 (d, $J = 7.8$ Hz, H-1‴); δ_C 105.3, 75.2, 77.9, 71.6, 78.0, and 62.8 (C-1‴ − 6‴)]. The large $^1J_{C\text{-}1,H\text{-}1}$ value (166 Hz) and intense $^3J_{C,H}$ correlations between H-1′ and C-3′/C-5′ implied that the anomeric configuration of Rha was α (Bock et al. 1973; Jia et al. 1998). The β-orientation of the anomeric centers of Glc (I) and Glc (II) were confirmed by the relatively large $^3J_{H\text{-}1,H\text{-}2}$ values [Glc (I): 7.9 Hz; Glc (II): 7.8 Hz]. In the HMBC spectrum of **1**, long-range correlations were observed between H-1‴ of Glc (II) (δ_H 4.38) and C-6″ of Glc (I) (δ_C 70.2), H-1″ of Glc (I) (δ_H 4.60) and C-4′ of Rha (δ_C 83.3), and between H-1′ of Rha (δ_H 4.78) and C-3 of the aglycone (δ_C 73.4). Compound **1** was defined as cannogenol 3-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside.

Compounds **1** − **24** were evaluated for the effect of the cytotoxic activities on three oral carcinoma cell lines including Ca9-22, HSC-2, and HSC-4 cells, and HGF oral normal cells using a modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay method (Table 1) (Sakagami et al. 2015; Sakagami et al. 2017). Etoposide was used as a positive control. Compounds **2** − **4** exhibited a particularly potent cytotoxic activity toward three carcinoma cell lines, with IC_{50} values ranging from 0.004 μM to 0.066 μM. The average IC_{50} values of **18** and **19** for the three carcinoma cell lines was ten times lower than for HGF normal cells, indicating that **18** and **19** were selectively cytotoxic to the carcinoma cell lines. Comparison of the IC_{50} values of **2**, **4**, **9**, and **13** with those of **18**, **19**, **20**, and **21**, respectively, revealed that the formation of an ether linkage between C-18 and C-20 of the aglycone decrease the cytotoxic effect. Furthermore, **22**, **23**, and **24** are the corresponding C-nor-D-homo type steroids of **2**, **5**, and **9**, respectively, and this structural rearrangement significantly lowered the cytotoxic effect of **2**, **5**, and **9**. The following structure-activity relationships corresponded to previously reported results (Tian et al. 2016): 1) the cardenolide glycosides with the A/B *cis* ring fusion (**2**, **5**, **9**, and **10**) exhibited stronger cytotoxic activities than those with the A/B *trans* ring fusion (**3**, **6**, **11**, and **12**); 2) comparison of the cytotoxic activities of **9**, **14**, and **16** implied that the oxidation of Me-19 of the aglycone to hydroxymethyl and aldehyde decreased the cytotoxic activity; 3) comparison of the cytotoxic activities of **2**, **5**, and **9**, or **3**, **6**, and **11** revealed that an increase in

Table 1. Cytotoxic activity of **1**–**24** and etoposide toward Ca9-22, HSC-2, HSC-4, and HFG cells

Compounds	Ca9-22 IC_{50} (μM)	HSC-2 IC_{50} (μM)	HSC-4 IC_{50} (μM)	HGF IC_{50} (μM)
1	12.1 ± 4.2	6.5 ± 0.51	7.6 ± 0.47	25.7 ± 4.6
2	0.011 ± 0.0023	0.0077 ± 0.00055	0.0095 ± 0.0021	0.030 ± 0.045
3	0.066 ± 0.0045	0.043 ± 0.015	0.022 ± 0.011	0.021 ± 0.0055
4	0.004 ± 0.0002	0.0073 ± 0.0012	0.0061 ± 0.00014	0.0029 ± 0.0002
5	0.40 ± 0.05	0.12 ± 0.01	0.13 ± 0.02	0.89 ± 0.06
6	3.4 ± 0.29	1.3 ± 0.08	1.3 ± 0.08	4.2 ± 0.21
7	0.58 ± 0.13	0.21 ± 0.09	0.24 ± 0.03	1.1 ± 0.06
8	2.8 ± 0.14	1.1 ± 0.11	1.1 ± 0.04	10.7 ± 0.14
9	5.1 ± 1.6	1.1 ± 0.06	0.85 ± 0.20	14.0 ± 10.0
10	8.7 ± 0.77	1.6 ± 0.40	1.7 ± 0.37	7.2 ± 2.6
11	39.9 ± 12.5	6.5 ± 0.71	6.4 ± 0.77	51.6 ± 25.9
12	30.1 ± 0.88	7.9 ± 0.41	7.3 ± 1.2	36.8 ± 18.1
13	8.4 ± 0.46	3.8 ± 0.87	3.1 ± 0.06	28.5 ± 2.3
14	5.6 ± 0.50	2.3 ± 0.28	2.6 ± 0.30	14.9 ± 3.9
15	9.4 ± 1.1	3.6 ± 0.43	4.7 ± 1.3	19.6 ± 4.4
16	19.3 ± 1.3	7.6 ± 2.3	6.4 ± 1.6	47.5 ± 10.3
17	64.9 ± 8.7	23.4 ± 0.65	18.5 ± 3.6	96.2 ± 15.2
18	0.80 ± 0.26	0.28 ± 0.14	0.37 ± 0.04	7.7 ± 4.4
19	0.99 ± 0.20	0.35 ± 0.03	0.49 ± 0.03	7.0 ± 0.86
20	> 100	31.9 ± 3.2	32.7 ± 2.8	> 100
21	30.2 ± 1.6	6.9 ± 2.1	7.8 ± 1.1	74.2 ± 28.6
22	> 100	52.8 ± 1.0	> 100	> 100
23	1.9 ± 0.76	0.95 ± 0.06	1.2 ± 0.03	11.5 ± 2.6
24	26.8 ± 0.07	12.1 ± 3.6	9.7 ± 0.46	70.1 ± 7.6
Etoposide	7.0 ± 2.8	1.6 ± 0.94	2.3 ± 0.69	> 100

Data are represent the mean value ± S.E.M. of three experiments performed in triplicate.

the number of sugar units attached to C-3 of the aglycone lowered the cytotoxic activity.

3. Experimental

3.1. Compound 1

Amorphous powder; $[\alpha]_D^{25}$ −151.9 (*c* 0.049, MeOH); IR (film) ν_{max}: 3363 (OH), 2928 (CH), 1737 (C = O) cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 270 (3.43), 218 (3.80); ^{1}H-NMR spectral data of the aglycone moiety (500 MHz, CD_3OD) δ_H: 5.90 (1 H, br s, H-22), 5.03 (1 H, br d, *J* = 18.4 Hz, H-21a), 4.91 (1 H, br d, *J* = 18.4 Hz, H-21b), 3.93 (1 H, m H-3), 3.84 (1 H, d, *J* = 11.5 Hz, H-19a), 3.39 (1 H, d, *J* = 11.5 Hz, H-19b), 0.88 (3 H, s, Me-18); ^{13}C-NMR spectral data of the aglycone moiety (125 MHz, CD_3OD) δ_C: 24.9, 27.3, 73.4, 30.8, 30.1, 27.5, 22.4, 42.5, 36.5, 40.4, 22.2, 41.3, 51.0, 86.5, 33.2, 28.0, 52.1, 16.4, 65.7, 178.4, 75.3, 117.8, 177.2 (C-1 − 23); ^{1}H-NMR spectral data of the sugar moiety (500 MHz, CD_3OD) δ_H: 4.78 (1 H, br s, H-1′), 4.60 (1 H, d, *J* = 7.9 Hz, H-1″), 4.38 (1 H, d, *J* = 7.8 Hz, H-1‴), 4.13 (1 H, dd, *J* = 11.7, 1.7 Hz, H-6″a), 3.95 (1 H, dd, *J* = 9.5, 3.2 Hz, H-3′), 3.87 (1 H, dd, *J* = 11.5, 1.8 Hz, H-6‴a), 3.81 (1 H, br d, *J* = 3.2 Hz, H-2′), 3.78 (1 H, dd, *J* = 11.7, 5.8 Hz, H-6″b), 3.75 (1 H, m, H-5′), 3.68 (1 H, dd, *J* = 11.5, 5.2 Hz, H-6‴b), 3.64 (1 H, dd, *J* = 9.5, 9.5 Hz, H-4′), 3.47 (1 H, m, H-5″), 3.36 (1 H, m, H-3″), 3.35 (1 H, m, H-3‴), 3.33 (1 H, m, H-4″), 3.29 (1 H, m, H-4‴), 3.27 (1 H, m, H-5‴), 3.21 (1 H, dd, *J* = 9.0, 7.9 Hz, H-2″), 3.20 (1 H, dd, *J* = 9.2, 7.8 Hz, H-2‴), 1.31 (3 H, d, *J* = 6.1 Hz, H-6′); ^{13}C-NMR spectral data of the sugar moiety (125 MHz, CD_3OD) δ_C: 99.7, 72.8, 72.5, 83.3, 68.5, 18.3 (C-1′ −

6′), 104.9, 76.0, 78.1, 71.6, 77.2, 70.2 (C-1″ − 6″), 105.3, 75.2, 77.9, 71.6, 78.0, 62.8 (C-1‴ − 6‴). HR-ESI-TOF-MS *m/z*: 883.3914 $[M + Na]^+$ (calcd for $C_{41}H_{64}NaO_{19}$: 883.3940).

See supplemental material for others.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Abe F, Yamauchi T, Nohara T. 1992b. C-nor-D-homo-cardenolide glycosides from *Thevetia neriifolia*. Phytochemistry. 31(1):251–254.

Abe F, Yamauchi T, Wan A. 1992a. Cardiac glycosides from the leaves of *Thevetia neriifolia*. Phytochemistry. 31(9):3189–3193.

Abe F, Yamauchi T, Yahara S, Nohara T. 1994. Glycosides of 19-formylthevetiogenin and 5α-thevetiogenin from *Thevetia neriifolia*. Phytochemistry. 37(5):1429–1432.

Begum S, Adil Q, Siddiqui SB, Siddiqui S. 1993. Constituents of the leaves of *Thevetia neriifolia*. J Nat Prod. 56(4):613–617.

Bock K, Lundt I, Pedersen C. 1973. Assignment of anomeric structure to carbohydrates through geminal ^{13}C-H coupling constants. Tetrahedron Lett. 13:1037–1040.

Cruz A, Guzmán A, Iriarte J, Medina R, Muchowski MJ, Maddox LM. 1979. 18,20-oxido-20,22-dihydroneriifolin, an unusual oxygenated cardenolide. J Org Chem. 44(20):3511–3515.

Endo H, Warashina T, Noro T, Castro HV, Mora AG, Poveda JL, Sanchez EP. 1997. Cardenolide glycosides from *Thevetia ahouai* (LINN.) A.DC. Chem Pharm Bull. 45(9):1536–1538.

Gulati A, Jain SK, Srivastava PS. 2000. Experimental studies on *Thevetia neriifolia* Juss-A review. Indian J Chem. 39B:808–812.

Jia Z, Koike K, Nikaido T. 1998. Major triterpenoid saponins from *Saponaria officinalis*. J Nat Prod. 61(11):1368–1373.

Kohls S, Scholz-Böttcher MB, Teske J, Zark P, Rullkötter J. 2012. Cardiac glycosides from Yellow Oleander (*Thevetia peruviana*) seeds. Phytochemistry. 75:114–127.

Miyagawa T, Ohtsuki T, Koyano T, Kowithayakorn T, Ishibashi M. 2009. Cardenolide glycosides of *Thevetia peruviana* and triterpenoid saponins of *Sapindus emarginatus* as TRAIL resistance-overcoming compounds. J Nat Prod. 72(8):1507–1511.

Sakagami H, Okudaira N, Masuda Y, Amano O, Yokose S, Kanda Y, Suguro M, Natori T, Oizumi H, Oizumi T. 2017. Induction of apoptosis in human oral keratinocyte by doxorubicin. Anticancer Res. 37(3):1023–1030.

Sakagami H, Uesawa Y, Ishihara M, Kagaya H, Kanamoto T, Terakubo S, Nakashima H, Takao K, Sugita Y. 2015. Quantitative structure-cytotoxicity relationship of oleoylamides. Anticancer Res. 35(10):5341–5355.

Siddiqui S, Siddiqui SB, Adil Q, Begum S. 1992. Cardenolides and triterpenoids of the leaves of *Thevetia neriifolia*. Phytochemistry. 31(10):3541–3546.

Tian DM, Cheng HY, Jiang MM, Shen WZ, Tang JS, Yao XS. 2016. Cardiac glycosides from the seeds of *Thevetia peruviana*. J Nat Prod. 79(1):38–50.

Yamauchi T, Abe F, Wan A. 1987a. Cardenolide monoglycosides from the leaves of *Cerbera odollam* and *Cerbera manghas* (*Cerbera*. III). Chem Pharm Bull. 35(7):2744–2749.

Yamauchi T, Abe F, Wan A. 1987b. Studies on Cerbera. IV. Polar cardenolide glycosides from the leaves of *Cerbera odollam* and *Cerbera manghas*. Chem Pharm Bull. 35(12):4813–4818.

Zhang XP, Pei YH, Liu MS, Kang SL, Zhang JQ. 2010. Chemical constituents from the leaves of *Cerbera manghas*. Asian Pac J Trop Med. 3(2):109–111.