Dissertation for PhD

Estrogenic and/or antiestrogenic study of the Bangladeshi medicinal plants

Laboratory of Pharmacognosy
Graduate School of Pharmaceutical Sciences
University of Shizuoka, Japan

Md. Abdul Muhit

August, 2016

Abbreviations

HRFABMS	: High resolution fast atom bombardment mass spectroscopy
^{1}H NMR	: Proton nuclear magnetic resonance
^{13}C NMR	: Carbon nuclear magnetic resonance
COSY	: Chemical shift correlation spectroscopy
HMQC	: Heteronuclear multiple quantum coherence
HMBC	: Heteronuclear multiple bond connectivity
NOESY	: Nuclear Overhauser effect spectroscopy
ROESY	: Rotating-frame nuclear Overhauser effect correlation spectroscopy
SiCC	: Silica-gel column chromatography
HPLC	: High-performance liquid chromatography
TLC	: Thin layer chromatography
RP-TLC	: Reverse phase thin layer chromatography
ODS	: Octadecylsilyl silica gel
ECD	: Electronic circular dichroism
UV	: Ultraviolet spectroscopy
DCC	: Dextran-coated charcoal
FBS	: Fetal bovine serum
MEM	: Minimum essential medium
RPMI	: Roswell park memorial institute

Hz (hertz); MHz (megahertz); *J* (coupling constant); s (singlet); br. s (broad singlet); d (doublet); br. d (broad doublet); dd (doublet of doublets); ddd (doublet of doublets of doublets); t (triplet); dt (doublet of triplets); tq (triplet of quartets)

glc (glucose); RI (refractive index); $[\alpha]_D$ (specific optical rotation); Fr. (fraction)

g (gram); mg (milligram); µg (microgram); µM (micromolar); pM (picomolar); mL (milliliter); µL (microliter); nM (nanomolar); nm (nanometer)

t_R (retention time); min (minute); h (hour)

EtOAc (Ethyl acetate); MeCN (acetonitrile); MeOH (methanol); H_2O (water); $CHCl_3$ (chloroform)

Content

Introduction		1
Chapter 1:	Chemical constituents from *Terminalia citrina* (Combretaceae)	2
	1.1 Introduction	2
	1.2 Extraction and isolation	2
	1.3 Identification and structure determination of new compounds	7
	1.4 Identification and structure determination of known compounds	41
	1.5 Summary	51
Chapter 2:	Chemical constituents from *Pothos scandens* (Araceae)	52
	2.1 Introduction	52
	2.2 Extraction and isolation	52
	2.3 Identification and structure determination of new compounds	56
	2.4 Identification and structure determination of known compounds	60
	2.5 Summary	79
Chapter 3:	Estrogenic and/or antiestrogenic activities of isolates	80
	3.1 Introduction	80
	3.2 Dose-response curves	80
	3.3 Estrogenic/antiestrogenic activity of isolates from *T. citrina*	82
	3.4 Estrogenic/antiestrogenic activity of isolates from *P. scandens*	82
	3.5 Discussion	86
Conclusion		88
Experimental		89
Acknowledgements		104
References		105

Introduction

17β-Estradiol (E_2), one of the primary circulating ovarian steroids, is predominantly responsible for the development and regulations of reproductive organs and secondary sex characteristics in female.[1] The development of breast epithelium is mainly regulated by 17β-Estradiol (E_2) but excessive level of it may cause genesis of breast cancer.[2] During the menopause, the level of E_2 is markedly reduced. In response, aromatization of circulating androgens is facilitated causing excessive E_2 production, which increases the risk of breast cancers.[3] The incidence of breast cancer is increasing in developing countries like Bangladesh, due to the lack of proper information, inappropriate sexual life, poor government funding in maternity health, poor birth control, improper lifestyles and lack of facilities for early detection.[4] Previous clinical study suggested that breast cancer and uteri cervical cancers are the most prevalent for last five years among the women in Bangladesh.[5]

17β-Estradiol (E_2) has also physiological role in lactating mother. Generally the level of 17β-Estradiol (E_2) is significantly decreased during breastfeeding while the level of prolactin hormone is increased for stimulating the mammary glands for milk secretion. High level of estrogen inhibits the milk production in lactating mother. [6] In search of potential leads those having estrogen and/or antiestrogenic characteristics, several initiatives has been taken by Laboratory of Pharmacognosy, University of Shizuoka and has reported some isolates like khainaoside A, syringerasinol, principin with antiestrogenic activity. Some isolates such as biochanin, tectorigenin, genistein, dalparvin B, C etc also reported to stimulate cell proliferation of estrogen-responsive breast cancer cells. [7-9]

As the majority of the Bangladeshi people are living under the poverty line, many of them tend to afford traditional medicines because of their relative availability, cheaper price, and little or no side effects. Among the 6,000 plant species which are enlisted in national encyclopedia of Bangladesh, near about 1,000 plant species are medicinally useful and have been documented. [10, 11] So far few attempts have been taken for the scientific evidence of Bangladeshi medicinal plants (*Asparagus racemosus, Withania somnifera, Nigella sativa, Emblica officinalis, Trigonella foenum-graceum, Ferula assa-foetida, Moringa oleifera, Nymphaea alba* etc.) those having estrogenic and/or antiestrogenic properties.[12] In this study, two other Bangladeshi medicinal plants *Terminalia citrina* (Combretaceae) and *Pothos scandens* (Araceae), have been taken into consideration for evaluating these properties. P. *scandens* is known to induce conception in women in certain part of India while fruit and bark extract of *T. citrina* is used to reduce menstrual pain. [12] Thus, it is important to reveal scientific basis of estrogen-like properties and to identify the responsible active ingredients of these medicinal plants.

The present study deals with the extraction and isolation of bioactive secondary metabolites through several spectroscopic methods. Estrogenic and/or antiestrogenic properties were also investigated using two different cell lines MCF-7 and T47D.

Chapter 1

Chemical constituents of *Terminalia citrina* (Combretaceae)

1.1 Introduction

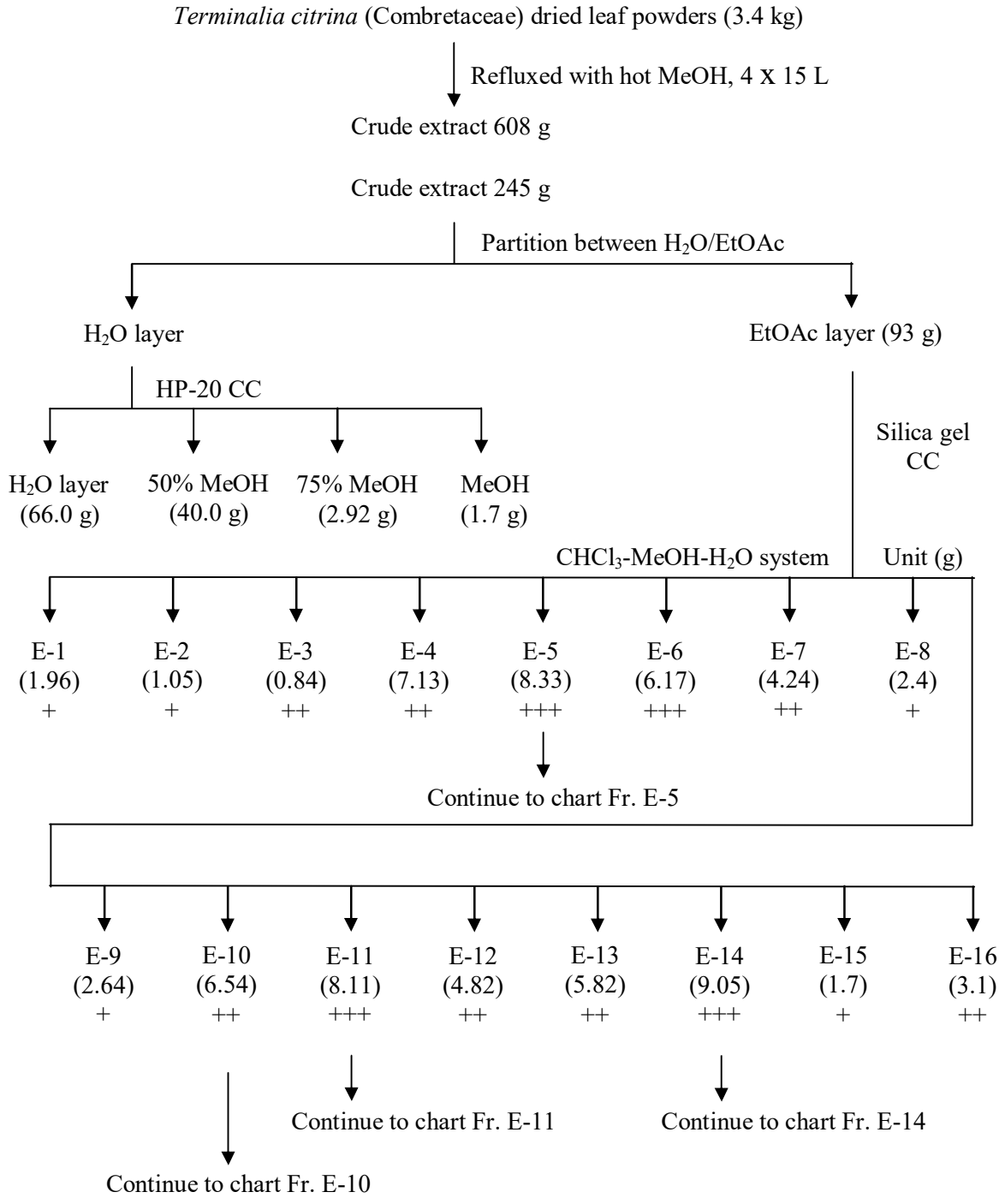
Terminalia plants (Combretaceae) have been exploited to be a potential source of variety of secondary bioactive metabolites such as saponins,[13, 14] lignans,[15, 16] flavonoids,[17] terpenoids,[18] xanthones,[19] tannins,[20, 21] and other phenolic constituents[22] in south-east Asia and Africa. Some of these constituents have shown antiproliferative,[13, 14, 19] anti-HIV-1, antimalarial,[15] antifungal,[21] antimicrobial,[20] and antioxidant[22] properties *in vitro* or *in vivo*.

Terminalia citrina (Gaertn.) Roxb. is commonly found in Bangladesh, Myanmar and India. The plant is traditionally known as Haritaki in Bangladesh and various parts of the plant have been used for the treatment of menstrual pain, bleeding piles, heart diseases, dysentery and constipation.[23] The extract of seed of the plant showed the highest antioxidant property and inhibited the formation of heinz body with *in vitro* model.[24] The leaves part of *T. citrina* was revealed its significant anthelmintic activity by one of our collaborative research groups using *Pheretima posthuma* as animal model.[25] Phytochemical study on the fruit identified tannins along with remarkable antimicrobial properties.[26] Meanwhile, none of the studies has revealed estrogenic properties of any of *Terminalia* plants. In quest for lead compounds having estrogenic and/or anti-estrogenic properties, extracts of the leaves of *T. citrina* were investigated.

In this study, reported are the isolation, structure elucidation of several new chemical constituent along with their estrogenic and/or antiestrogenic activity using estrogen responsive breast cancer cell lines (MCF-7, T47D).

1.2 Extraction and Isolation

The air-dried powdered leaves of the plant (3.4 kg approx.) were extracted four times with hot methanol (4 x 15 L) by refluxing for 3 h each to afford a viscous mass of 608 g. The crude extract was then suspended in 2 L of water and partitioned with EtOAc (2 L x 3). Both EtOAc and H_2O soluble fractions suppressed 80% and 40% of the estradiol (E_2)-enhanced proliferation of breast cancer cells, respectively, at a concentration of 0.2 μg/mL. However, the EtOAc-soluble fraction was also exerted its cytotoxicity at higher concentrations. The EtOAc-soluble fraction was subjected to silica gel column chromatography eluting with $CHCl_3$-MeOH gradient solvent system and HPLC with reversed phase columns, and 46 constituents were obtained as amorphous powder which were summarized in chart 1.

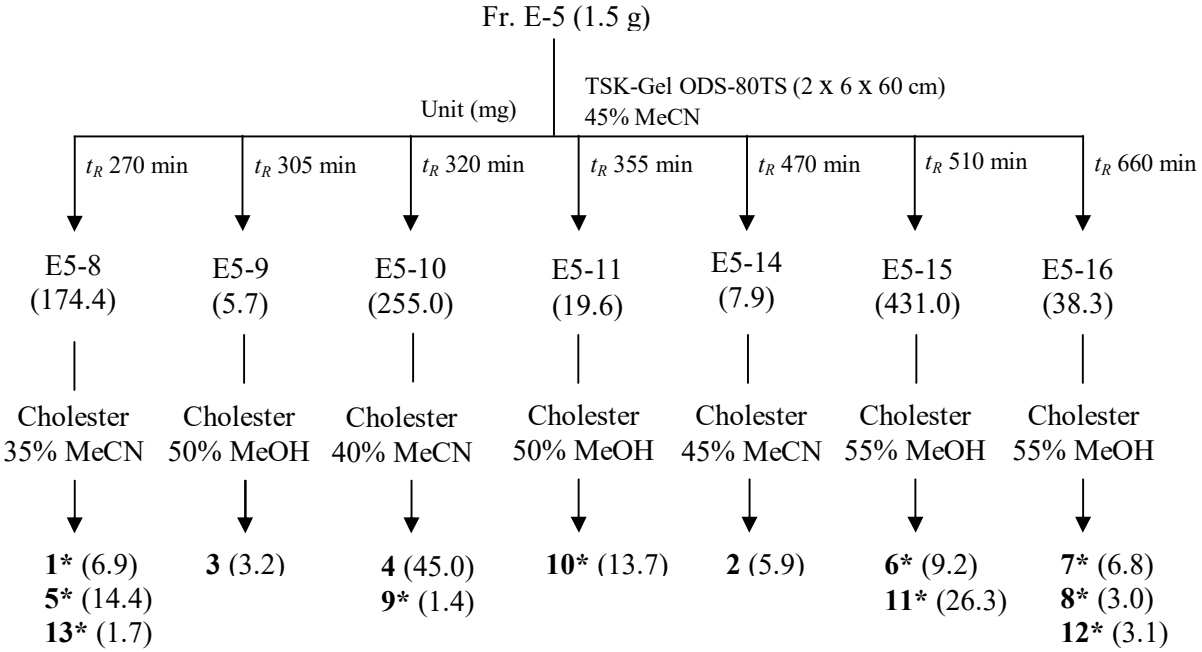


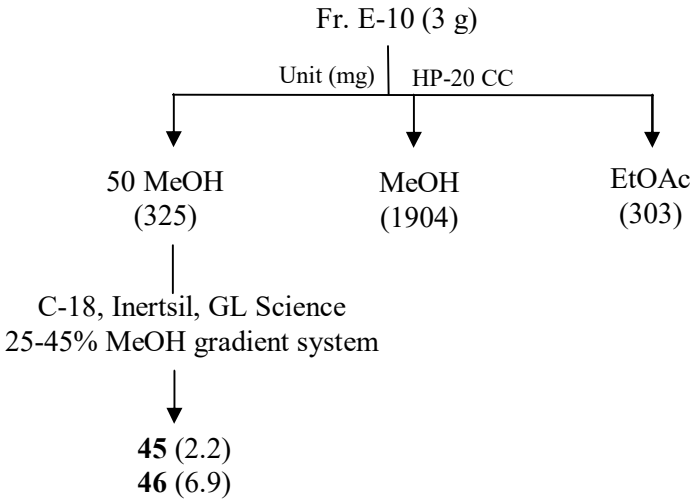
+++ 99% inhibition of Estradiol (E_2)-induced cell proliferation (T47D) at a concentration of <0.2 µg/mL

++ 90% inhibition of Estradiol (E_2)-induced cell proliferation (T47D) at a concentration of <0.2 µg/mL

+ 50% inhibition of Estradiol (E_2)-induced cell proliferation (T47D) at a concentration of <0.2 µg/mL

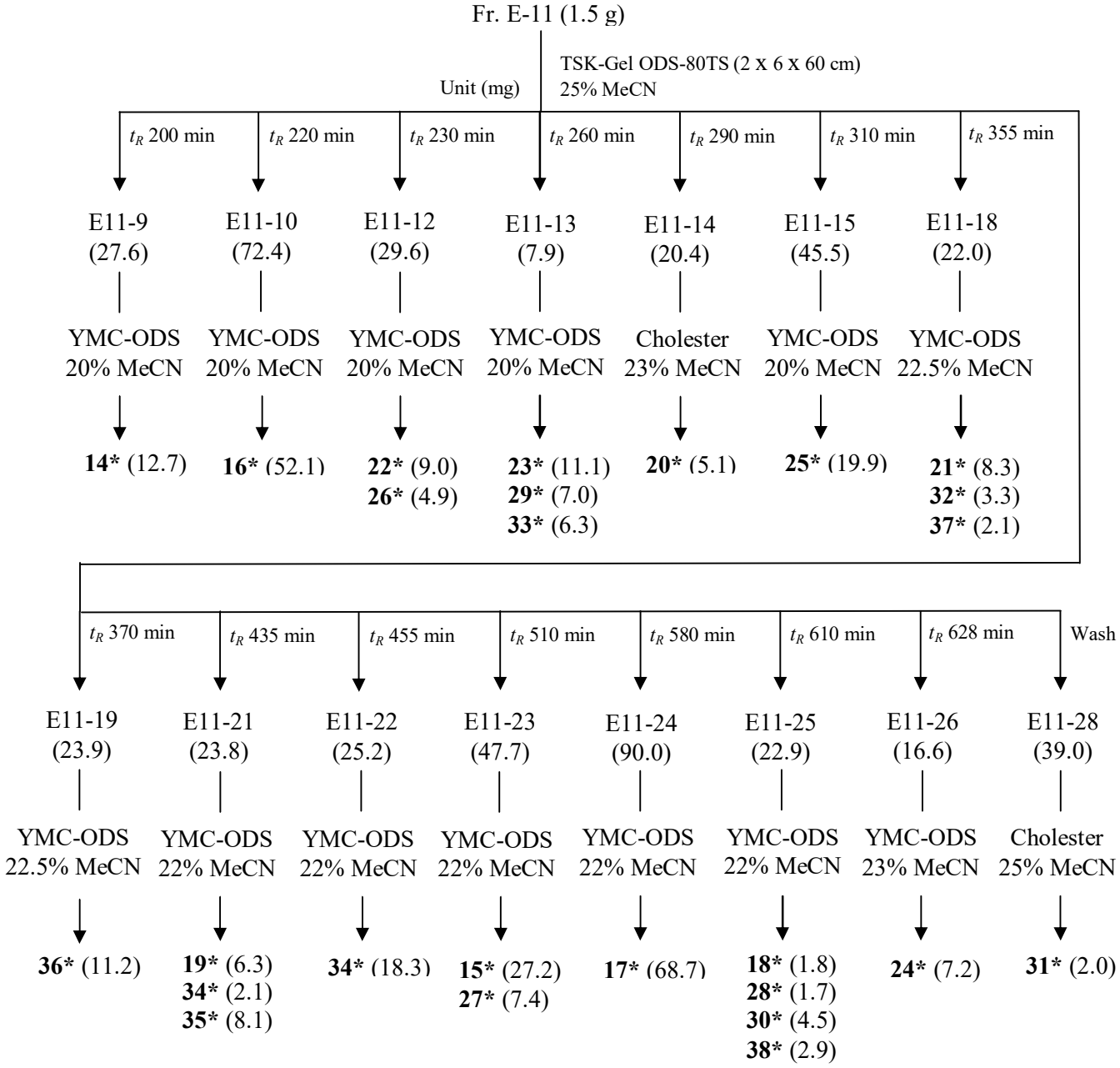
Chart 1. Extraction and isolation of constituents from *Terminalia citrina* (Combretaceae) continued…





Remarks. * new compound

Chart 1. Extraction and isolation of constituents from *Terminalia citrina* (Combretaceae) continued…



Remarks. * new compound

Chart 1. Extraction and isolation of constituents from *Terminalia citrina* (Combretaceae) continued...

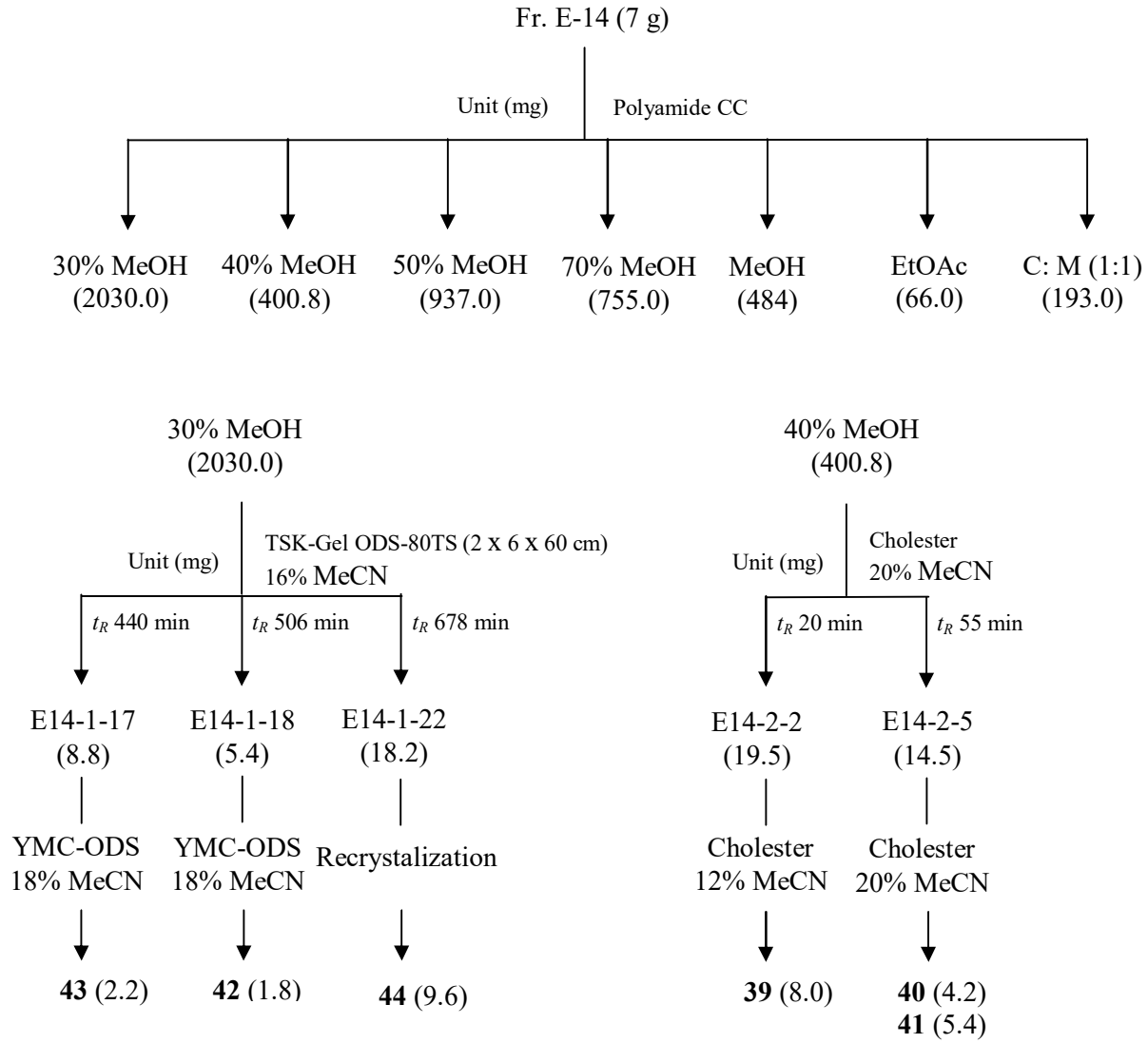


Chart 1. Extraction and isolation of constituents from *Terminalia citrina* (Combretaceae)

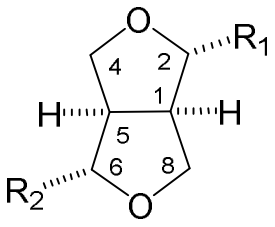
1.3. Identification and structure determination of new compounds

1.3.1. Furofuran lignans

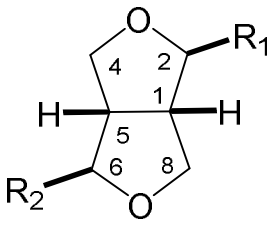
Compound **1** was obtained as a colorless amorphous solid and the molecular formula was assigned as $C_{22}H_{24}O_8$, based on the protonated molecular ion $[M + H]^+$ at *m/z* 417.1537 (calcd 417.1549) in the HRFABMS, which was supported by its ^1H and ^{13}C NMR spectra. The UV spectrum revealed the presence of an aromatic ring (284 and 222 nm). The ^1H NMR of **1** showed two oxygenated methines [δ_H 5.02 (1H, d, J = 5.0 Hz, H-2) and 4.68 (1H, d, J = 5.0 Hz, H-6)], two pair of oxygenated methylenes [δ_H 4.30 (1H, dd, J = 9.0, 7.0 Hz, H-4_a) and 3.90 (1H, dd, J = 9.0, 4.0 Hz, H-4_b); 4.24 (1H, dd, J = 9.0, 7.0 Hz, H-8_a) and 4.08 (1H, dd, J = 9.0, 4.0 Hz, H-8_b)], two methine protons [δ_H 3.04 (1H, overlapped, H-1) and 3.02 (1H, overlapped, H-5)]. One of the methine protons (δ_H 3.04, H-1) was found to be coupled with one oxygenated methine proton (δ_H 5.02, H-2) and with a pair of oxygenated methylene protons (δ_H 4.24, 4.08, H-8) in the COSY spectrum. Accordingly, another methine proton (δ_H 3.02, H-5) coupled with a further oxygenated methine (δ_H 4.68, H-6) and with a pair of oxygenated methylene protons (δ_H 4.30, 3.90, H-4). The above arrangements are attributed to two partial structures of $–CH_2$ (O)-CH-CH (O)- corresponding to a furofuran type lignan. [27] The ^1H NMR spectrum also revealed the presence of an ABX-type aromatic proton signals [δ_H 6.87 (1H, d, J = 1.5 Hz, H-2″), 6.84 (1H, dd, J = 8.0, 1.5 Hz, H-6″) and 6.77 (1H, d, J = 8.0 Hz, H-5″)], a singlet aromatic proton signal [δ_H 6.87 (1H, s, H-6′)], a methylenedioxy signal [δ_H 5.91 (2H, s)], and three methoxy group signals [δ_H 3.84, 3.83, 3.78 (each 3H, s)]. As the ^{13}C NMR spectra showed 12 carbons of aromatic resonances (Table 1), all of these were found to be similar to an asymmetrically substituted furofuran lignan containing a 2,6-diaryl-3,7-dioxabicyclo[3.3.0]octane ring.[28]

Meticulous observations of the HMBC spectra revealed the connectivity of five partial structures: i) one oxymethine proton signal (δ_H 5.02, H-2) to two aromatic carbons [δ_C 142.7 (C-2′) and 106.3 (C-6′)] in ring A; ii) another oxymethine proton signal (δ_H 4.68, H-6) to two aromatic carbons [δ_C 107.5 (C-2″) and 120.7 (C-6″)] in ring B; iii) methylenedioxy protons (δ_H 5.91) to two oxygenated aromatic carbons [δ_C 149.4 (C-3″) and 148.4 (C-4″)] in ring B; iv) a singlet aromatic proton signal (δ_H 6.73, H-6′) to two oxygenated aromatic carbons [δ_C 142.7 (C-2′) and 143.3 (C-4′)] and to a benzylic oxymethine carbon signal (δ_C 83.8, C-2); v) a *meta*-coupled proton signal to an oxymethine carbon signal (δ_C 86.8, C-6) and to an oxygenated aromatic carbon (δ_C 148.4, C-4″). On the other hand, the attachment positions of the three methoxy groups (δ_C 61.6, 61.3 and 57.4) were found to be with C-3′, C-4′ and C-5′ in the ring A. According to the HMBC spectra, the oxymethine proton (δ_H 5.02, H-2) showed no correlation to the oxygenated aromatic carbons that contain methoxy groups [δ_H 3.84 (MeO) / δ_C 142.6 (C-3′), δ_H 3.83 (MeO) / δ_C 143.3 (C-4′) and δ_H 3.78 (MeO) / δ_C 147.4 (C-5′)].

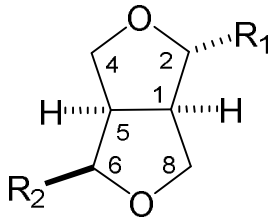
The majority of furofuran lignans have their 2,6-diaryl groups on the *exo* face of the bicyclic core, although many compounds with *endo*, *exo*-aryl substitution and a few compounds with *endo*, *endo* substitution have been reported.[29] In the present study, the absolute configurations at two methines (C-1, C-5) of four asymmetric carbons in these compounds were deduced from their ECD spectra, and those of others (C-2, C-6) were determined from the coupling constants in the ^{1}H NMR spectrum. Based on the coupling constants of the oxymethine protons [δ_H 5.02 (1H, d, J = 5.0 Hz, H-2) and 4.68 (1H, d, J = 5.0 Hz, H-6)] of **1**, two sets of protons (H-2/H-1 and H-6/H-5) were indicated as being *trans* oriented.[30] The NOESY correlations between H-1 and H-6′ and between H-2 and H-6″also confirmed the above arrangement. The ECD spectrum of **1** showed a positive Cotton effect at 230 nm (Δε +1.34) which were identical to (1*R*,2*S*,5*R*,6*S*)-sesamin.[31] Therefore, **1** was identified as (1*R*,2*S*,5*R*,6*S*)-2-(2′-hydroxy-3′,4′,5′-trimethoxyphenyl)-6-(3″,4″-methylenedioxyphenyl)-3,7-dioxabicyclo[3.3.0]octane and was trivially named as terminin A.



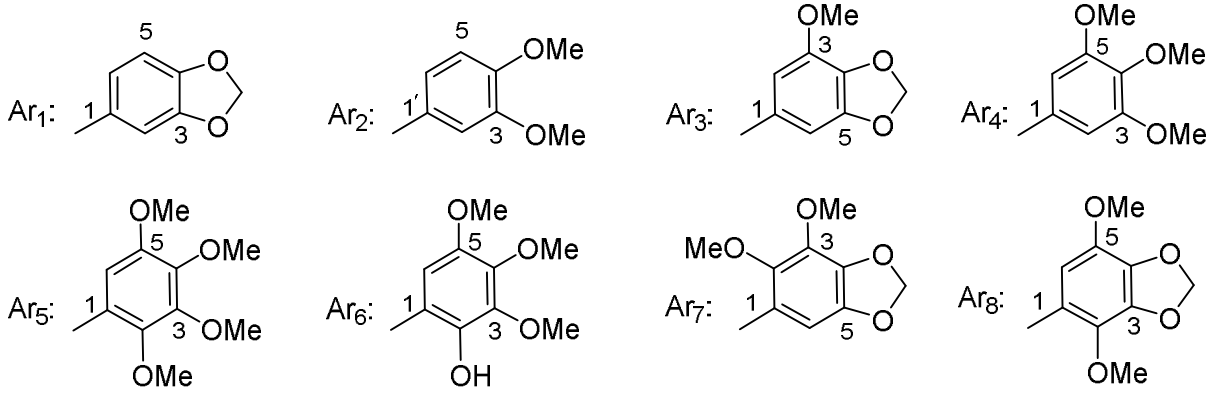
	R_1	R_2
1	Ar_6	Ar_1
5	Ar_6	Ar_3
6	Ar_5	Ar_3
7	Ar_5	Ar_7
10	Ar_5	Ar_4



	R_1	R_2
8	Ar_5	Ar_8
9	Ar_5	Ar_2
11	Ar_5	Ar_5



	R_1	R_2
12	Ar_5	Ar_5



Compound **5** was obtained as a pale yellowish amorphous solid and was assigned the molecular formula as $C_{23}H_{26}O_9$, based on the molecular ion $[M]^+$ at *m/z* 446.1604 (calcd 446.1576) in the HRFABMS, suggesting one carbon, one oxygen and two hydrogen atoms more than that of **1**. The ^1H and ^{13}C NMR spectral data were similar with those of **1** and was supposed to be a furofuran type lignan. However, a pair of meta-coupled protons [δ_H 6.59 (1H, d, J = 1.5 Hz, H-2″) and 6.54 (1H, d, J = 1.5 Hz, H-6″)] were observed in **5**, while ABX-type aromatic proton signals were observed in **1**. As a result, ring B was found to be a tetrasubstituted phenyl ring and the attachment position of ancillary methoxy group [δ_H 3.87 (3H, s), δ_C 57.4] was confirmed at C-3″ from the correlation with δ_C 145.0 in the HMBC spectra. Thus, the structure of **5** was established as (1*R*,2*S*,5*R*,6*S*)-2-(2′-hydroxy-3′,4′,5′-trimethoxyphenyl)-6-(3″-methoxy,4″,5″-methylenedioxyphenyl)-3,7-dioxabicyclo[3.3.0]octane and was named as terminin B.

Table 1. NMR data for **1** and **5** (^1H NMR; 500 MHz and ^{13}C NMR; 125 MHz, δ; ppm, recorded in MeOH-d_4)

Position	**1**		**5**	
	δ_H (J in Hz)	δ_C [a]	δ_H (J in Hz)	δ_C [a]
1	3.04, overlapped	55.4 [b]	3.04, overlapped	55.3
2	5.02, d (5.0)	83.8	5.0, d (5.0)	83.8
4_a	4.30, dd (9.0, 7.0)	72.8	4.30, dd (9.0, 7.0)	72.8
4_b	3.90, dd (9.0, 4.0)		3.90, dd (9.0, 4.0)	
5	3.02, overlapped	55.7 [b]	3.01, overlapped	55.8
6	4.68, d (5.0)	86.8	4.66, d (5.0)	86.8
8_a	4.24, dd (9.0, 7.0)	73.8	4.25, dd (9.0, 7.0)	73.9
8_b	4.08, dd (9.0, 4.0)		4.09, dd (9.0, 4.0)	
1′		124.6		124.6
2′		142.7 [c]		142.7 [c]
3′		142.6 [c]		142.6 [c]
4′		143.3		143.3
5′		147.4		147.4
6′	6.73, s	106.3	6.73, s	106.3
1″		136.6		137.4
2″	6.87, d (1.5)	107.5	6.59, d (1.5)	107.6
3″		149.4		145.0
4″		148.4		136.1
5″	6.77, d (8.0)	109.0		150.6
6″	6.84, dd (8.0, 1.5)	120.7	6.54, d (1.5)	101.1
OCH_2O	5.91, s	102.4	5.89, s	102.6
OMe	3.84, s	61.6	3.87, s	61.6
	3.83, s	61.3	3.84, s	61.3
	3.78, s	57.4	3.82, s	57.4
			3.77, s	57.3

[a] Assignments were based on HMQC and HMBC experiments

[b, c] Signals are interchangeable

Terminin C (**6**) was assigned as the molecular formula of $C_{24}H_{28}O_9$, based on the protonated molecular ion $[M + H]^+$ at *m/z* 461.1819 (calcd 461.1811) in the HRFABMS, representing one carbon and two hydrogen atoms more than those of **5**. The ^1H and ^{13}C NMR spectral features (Table 2) such as furofuran and 3″-methoxy, 4″, 5″-methylenedioxyphenyl rings were similar with terminin B (**5**). However, one additional methoxy group appeared [δ_H 3.81 (3H, s), δ_C 61.4], which was suggestive of methylation of hydroxyl group in ring A. The HMBC spectrum indicated that the methoxy group is attached to C-2′, from the correlation with the singlet aromatic proton signal [δ_H 6.75 (1H, s, H-6′)] to δ_C 145.8. Hence, the structure of **6** (terminin C) was deduced as (1*R*,2*S*,5*R*,6*S*)-2-(2′,3′,4′,5′-tetramethoxyphenyl)-6-(3″-methoxy-4″,5″-methylenedioxyphenyl)-3,7-dioxabicyclo[3.3.0]octane.

Compounds **7** and **8** were isolated as pale yellow amorphous powders, and their molecular formulas were determined to be $C_{25}H_{30}O_{10}$ and $C_{25}H_{30}O_{10}$, based on their protonated molecular ion peaks at *m/z* 491.1906 (calcd 491.1917) and 491.1932 (calcd 491.1917) in the HRFABMS, respectively. Both share the common ^1H and ^{13}C NMR spectral features (Table 2) of furofuran and tetramethoxyphenyl aromatic ring in their structures, supposed to be a congener of **6**. However, a singlet aromatic proton signal appeared [δ_H 6.55 (1H, s, H-6″) in **7**; δ_H 6.59 (1H, s, H-6″) in **8**], while a pair of meta-coupled protons were observed in **6**. In addition to methylenedioxy group proton signals [δ_H 5.89 (1H, d, J = 1.0 Hz), 5.88 (1H, d, J = 1.0 Hz) in **7**; δ_H 5.93 (1H, d, J = 1.5 Hz), 5.92 (1H, d, J = 1.5 Hz) in **8**], a total six methoxy groups [δ_H 3.98, 3.88, 3.83, 3.82, 3.80, 3.80 (each 3H, s) in **7**: 3.92, 3.88, 3.84, 3.82, 3.82, 3.81 (each 3H, s) in **8**] were observed, indicating that the aromatic ring B is pentasubstituted. The only difference in these two compounds is the position of the methoxy groups, which has been implied by six different aromatic carbon resonances of the ring B. Two of these methoxy groups were observed in δ 56-57 ppm in **8**, whereas only one methoxy group was observed in this region in **7**, indicates that these two methoxy groups (δ_H 3.82 /δ_C 57.8, 3.81 / 56.9) are placed beside the aromatic protons in **8**. The above arrangement was also confirmed by the HMBC spectra through the correlation from aromatic proton signal (δ_H 6.59, H-6″) to two aromatic carbon C-2″ and C-5″ in **8**. According to ECD spectra, a positive Cotton effect was observed in **7** at 221 nm (Δε +7.89) whereas a negative cotton effect at 235 nm (Δε -2.53) was observed in **8**.

Accordingly, **7** (terminin D) was determined to be (1*R*,2*S*,5*R*,6*S*)-2-(2′,3′,4′,5′-tetramethoxyphenyl)-6-(2″,3″-dimethoxy-4″,5″-methylenedioxyphenyl)-3,7-dioxabicyclo[3.3.0]octane and **8** (terminin E) was established as (1*S*,2*R*,5*S*,6*R*)-2-(2′,3′,4′,5′-tetramethoxyphenyl)-6-(2″,5″-dimethoxy-3″,4″-methylenedioxyphenyl)-3,7-dioxabicyclo[3.3.0]octane.

Table 2. NMR data for **6-9** (^{1}H NMR; 500 MHz and ^{13}C NMR; 125 MHz, δ; ppm, recorded in MeOH-d_4)

Position	**6**		**7**		**8**		**9**	
	δ_H (J in Hz)	δ_C [a]	δ_H (J in Hz)	δ_C [a]	δ_H (J in Hz)	δ_C [a]	δ_H (J in Hz)	δ_C [a]
1	2.98, m	55.8 [b]	2.97, overlapped	55.9 [b]	2.97, overlapped	55.9 [b]	3.03, m	55.9
2	4.98, d (5.0)	83.5	4.99, d (4.5)	83.0	4.99, d (4.5)	82.9	5.01, d (5.5)	83.6
4_a	4.28, dd (9.0, 7.0)	72.9	4.30, dd (9.0, 7.0)	73.6 [c]	4.32, overlapped	73.5	4.31, dd (9.5, 7.0)	73.6
4_b	3.89, dd (9.0, 4.0)		4.01, dd (9.0, 4.0)		4.03, overlapped		3.93, dd (9.5, 4.0)	
5	3.03, m	55.9 [b]	2.95, overlapped	56.0 [b]	2.96, overlapped	56.0 [b]	3.10, m	55.6
6	4.68, d (5.0)	86.7	4.97, d (5.0)	82.7	4.97, d (4.5)	83.2	4.73, overlapped	87.3
8_a	4.25, dd (9.5, 7.0)	73.6	4.27, dd (9.0, 7.0)	73.7 [c]	4.28, overlapped	73.8	4.28, dd (9.5, 7.0)	72.9
8_b	4.01, dd (9.5, 4.0)		4.03, dd (9.0, 4.5)		4.06, overlapped		4.04, dd (9.5, 5.0)	
1′		131.3		131.3		131.3		131.4
2′		145.8		145.9		145.9		145.8
3′		148.2		148.2		148.2		148.2
4′		143.7		143.7		143.7		143.7
5′		150.9		150.9		150.9		150.9
6′	6.75, s	105.6	6.75, s	105.6	6.76, s	105.6	6.78, s	105.6
1″		137.4		129.3		128.6		135.3
2″	6.59, d (1.5)	107.6		145.2		136.9	6.99, s	111.4
3″		145.0		138.9		139.8		150.8
4″		136.1		138.4		137.8		150.2
5″		150.7		146.4		140.3	6.93, overlapped	113.1
6″	6.54, d (1.5)	101.1	6.55, s	100.2	6.59, s	106.7	6.93, overlapped	119.9
OCH_2O	5.89, s	102.7	5.89, d (1.0)	102.7	5.93, d (1.5)	102.9		
			5.88, d (1.0)		5.92, d (1.5)			
OMe	3.87, s	61.5	3.98, s	61.6	3.92, s	61.5	3.89, s	61.5
	3.86, s	61.5	3.88, s	61.5	3.88, s	61.5	3.85, s	61.5
	3.82, s	61.4	3.83, s	61.5	3.84, s	61.4	3.85, s	61.4
	3.81, s	57.4	3.82, s	61.4	3.82, s	60.3	3.83, s	56.9
	3.80, s	56.9	3.80, s	60.4	3.82, s	57.8	3.83, s	56.6
			3.80, s	56.9	3.81, s	56.9	3.82, s	56.6

[a] Assignments were based on HMQC and HMBC experiments

[b,c] Signals are interchangeable

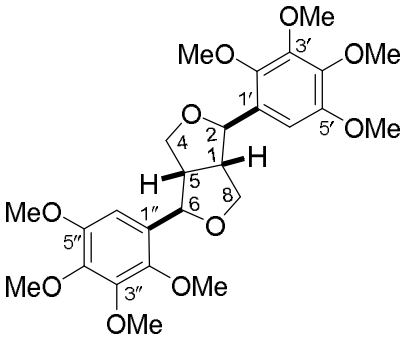
Compound **9** was obtained as a pale yellow amorphous powder and was determined to have the molecular formula of $C_{24}H_{30}O_8$, based on the protonated molecular ion $[M + H]^+$ at *m/z* 447.2041 (calcd 447.2018) in the HRFABMS. Investigation of the ^1H and ^{13}C NMR spectra revealed a close similarity to **6** that has a furofuran ring and a 2′,3′,4′,5′-tetramethoxyphenyl group in its structure (Table 2). Apart from the aromatic proton signal at δ_H 6.78 (1H, s, H-6′), the ^1H NMR spectra also displayed three aromatic proton signals [δ_H 6.99 (1H, s, H-2″), 6.93 (1H, s, overlapped, H-5″) and 6.93 (1H, s, overlapped, H-6″)] which are considered to be a part of a trisubstituted aromatic ring. A total of six methoxy group proton signals were observed, among which four groups [δ_H 3.89, 3.85, 3.85, 3.83, (each 3H, s)] were being part of 2′,3′,4′,5′-tetramethoxyphenyl ring. Another two methoxy groups (δ_H 3.83 / δ_C 56.6, 3.82 / 56.6) were determined to be a part of ring B by observing the correlation of δ_H 3.83 to δ_C 150.8 (C-3″) and from δ_H 3.82 to δ_C 150.2 (C-4″) in the HMBC spectra. A negative Cotton effect at 235 nm (Δε -0.42) was observed in ECD spectra. Thus, the structure of **9** was established as (1*S*,2*R*,5*S*,6*R*)-2-(2′,3′,4′,5′-tetramethoxyphenyl)-6-(3″,4″-dimethoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane.

Compound **10** was obtained as pale yellow amorphous powder and its molecular formula was deduced as $C_{25}H_{32}O_9$, based on the molecular ion $[M]^+$ at *m/z* 476.2071 (calcd 476.2046) in the HRFABMS, representing one methoxy group additional to that of **9**. Besides the common ^1H and ^{13}C NMR spectral features of furofuran ring and 2′, 3′, 4′, 5′-tetramethoxyphenyl group, it showed a singlet signal at δ_H 6.68 (2H, s, H-6″) of two magnetically equivalent aromatic protons. One the other hand, ^{13}C NMR displayed four aromatic carbon resonances (δ_C 154.7, 138.8, 138.7, 104.5) for the ring B, which was suggestive of a 1″,3″,4″,5″-tetrasubstituted phenyl ring. Seven methoxy group proton signals were observed, among which two methoxy group proton signals [δ_H 3.84 (6H, s), δ_C 56.7] were equivalent. The methoxy group protons showed the correlation from δ_H 3.84 to δ_C 154.7 (C-3″, 5″) in the HMBC spectra. Therefore, the structure of **10** (terminin G) was identified as (1*R*,2*S*,5*R*,6*S*)-2-(2′,3′,4′,5′-tetramethoxyphenyl)-6-(3″,4″,5″-trimethoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane.

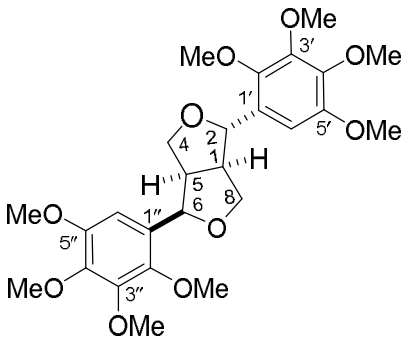
Compound **11** was determined to have the molecular formula of $C_{26}H_{34}O_{10}$, based on the protonated molecular ion $[M + H]^+$ at *m/z* 507.2213 (calcd 507.2230) in the HRFABMS. In contrast to others, the ^1H NMR showed one oxygenated methine [δ_H 5.01 (2H, d, J = 4.5 Hz, H-2, 6)], one pair of oxygenated methylene [δ_H 4.30 (2H, dd, J = 9.0, 7.0 Hz, H-4_a, 8_a) and 4.07 (2H, dd, J = 9.0, 4.5 Hz, H-4_b, 8_b)], one methine proton [δ_H 2.99 (2H, m, H-1, 5)]. On the other hand, ^1H NMR also revealed an aromatic proton signal [δ_H 6.76 (2H, s, H-6′ and 6″)] with four methoxy groups [δ_H 3.88, 3.83, 3.82, 3.80, (each 6H, s)]. The ^{13}C NMR spectra displayed only six aromatic carbon resonances, suggesting that compound **11** could be a symmetrically di-aryl substituted lignan. The ECD spectra revealed a negative Cotton effect at

235 nm (Δε -1.04). Thus, the structure of **11** (terminin H) was established as (1*S*,2*R*,5*S*,6*R*)-2,6-di-(2′,3′,4′,5′-tetramethoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane.

Compound **12** was obtained as a pale yellow amorphous powder and was determined to have the same molecular formula $C_{26}H_{34}O_{10}$ as **11**, based on the protonated molecular ion $[M + H]^+$ at *m/z* 507.2204 (calcd 507.2230) in the HRFABMS. The NMR data of **12** indicated that the compound is an isomer of **11**, due to their spectral similarities. One of the oxymethine signals of the furofuran ring appeared in higher field with larger coupling constant [δ_H 4.95 (1H, d, J = 6.0 Hz, H-6)] whereas one of the methine proton was shifted to lower filed [δ_H 3.45 (1H, m, H-5)] in the ^{1}H NMR spectra. According to ^{13}C NMR spectra, one of the methines and one of the oxymethylene groups were shifted to higher field [δ_C 50.7 (C-5, Δ -5.0 ppm) and [δ_C 70.7 (C-4, Δ -3.0 ppm), respectively, which was supposed to be due to the anisotropic effect of an aromatic ring. In the NOESY spectrum of **12**, correlations were observed between a singlet aromatic proton [δ_H 6.93 (1H, s, H-6″)] and the oxymethylene protons [δ_H 3.78 (1H, overlapped, H-4), 3.25 (1H, overlapped, H-4)]. Because of observing eight methoxy groups in the ^{1}H NMR spectra, both aromatic rings were presumed to be 2′,3′,4′,5′-tetramethoxyphenyl substituted. These data indicated that the structure of **12** is a stereoisomer of **11** at the C-6 position. Accordingly, **12** (6-epiterminin H) was established as (1*R*,2*S*,5*R*,6*R*)-2-(2′,3′,4′,5′-tetramethoxyphenyl)-6-(2′,3′,4′,5′-tetramethoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane.



11



12

Table 3. NMR data for **10-12** (^{1}H NMR; 500 MHz and ^{13}C NMR; 125 MHz, δ; ppm, recorded in MeOH-d_4)

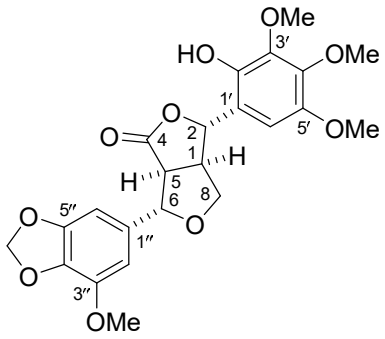
Position	**10**		**11**		**12**	
	δ_H (J in Hz)	δ_C [a]	δ_H (J in Hz)	δ_C [a]	δ_H (J in Hz)	δ_C [a]
1	3.01, m	55.8 [b]	2.99, m	56.0	2.87, m	55.6
2	5.00, d (5.0)	83.5	5.01, d (4.5)	82.9	4.77, d (4.0)	84.5
4_a	4.31, dd (9.0, 7.0)	73.8	4.30, dd (9.0, 7.0)	73.7	3.78, overlapped	70.7
4_b	3.93, dd (9.0, 4.0)		4.07, dd (9.0, 4.5)		3.25, m	
5	3.09, m	55.9 [b]	2.99, m	56.0	3.45, m	50.7
6	4.74, d (5.0)	86.8	5.01, d (4.5)	82.9	4.95, d (6.0)	79.4
8_a	4.29, dd (9.0, 7.0)	72.9	4.30, dd (9.0, 7.0)	73.7	4.29, dd (9.0, 7.0)	72.3
8_b	4.04, dd (9.0, 4.5)		4.07, dd (9.0, 4.5)		3.86, overlapped	
1′		131.3		131.3		131.2
2′		145.8		145.9		145.9
3′		148.2		148.2		148.1
4′		143.7		143.7		143.6
5′		150.9		150.9		151.0
6′	6.77, s	105.6	6.76, s	105.6	6.76, s	105.7
1″		138.8		131.3		128.0
2″	6.68, s	104.5		145.9		144.9
3″		154.7		148.2		147.8
4″		138.7		143.7		143.5
5″		154.7		150.9		150.6
6″	6.68, s	104.5	6.76, s	105.6	6.93, s	106.5
OMe	3.88, s	61.5	3.88, s	61.5	3.89, s	61.5
	3.84, s	61.5	3.88, s	61.5	3.89, s	61.5
	3.84, s	61.4	3.83, s	61.5	3.84, s	61.5
	3.83, s	61.1	3.83, s	61.5	3.84, s	61.5
	3.82, s	56.9	3.82, s	61.4	3.84, s	61.4
	3.81, s	56.7	3.82, s	61.4	3.84, s	61.3
	3.75, s	56.7	3.80, s	56.9	3.82, s	56.9
			3.80, s	56.9	3.79, s	56.8

[a] Assignments were based on HMQC and HMBC experiments

[b] Signals are interchangeable

1.3.2. Furofuranone lignan

Compound **13** was obtained as a pale yellow amorphous solid and the molecular formula was assigned as $C_{23}H_{24}O_{10}$, based on the protonated molecular ion $[M + H]^+$ at *m/z* 461.1427 (calcd 461.1447) in the HRFABMS data, indicating 12 indices of unsaturation. The UV spectrum revealed the absorption band of aromatic rings (283 and 217), which was supported by twelve aromatic carbon resonances in the ^{13}C NMR spectra. The ^1H NMR spectra of **13** showed two oxygenated methines [δ_H 5.47 (1H, d, J = 3.5 Hz, H-2) and 5.12 (1H, d, J = 4.5 Hz, H-6)], two methine protons [δ_H 3.31 (1H, overlapped, H-1) and 3.57 (1H, dd, J = 10.0, 4.5 Hz, H-5), and one oxygenated methylene [δ_H 4.31 (1H, dd, J = 9.5, 7.0 Hz, H-8_a) and 4.07 (1H, dd, J = 9.5, 5.0 Hz, H-8_b)] (Table 4). However, a carbonyl carbon at δ_C 180.1 was found in the ^{13}C NMR spectra, instead of the expected another oxymethylene at C-4. According to the COSY spectra, two sets of partial structures, -O-CH-CH-CH_2- and –O-CH-CH-CO- were assigned to the positions of C-2, 1, 8 and C-6, 5, 4, respectively, which is a characteristic signal of a 4-oxo-3, 7-dioxabicyclo[3.3.0]octane moiety.[32] The ^1H NMR spectra also revealed signal of a singlet aromatic proton [δ_H 6.65 (1H, s, H-6′)] and a methylenedioxy protons signals [δ_H 5.91 (2H, s)], indicating the presence of a 3″-methoxy-4″,5″-methylenedioxyphenyl group in its structure. A pair of *meta*-coupled aromatic protons [δ_H 6.65 (1H, overlapped, H-2″) and 6.58 (1H, d, J = 1.5 Hz, H-5″)] and three methoxy groups [δ_H 3.84, 3.83 and 3.78 (each 3H, s)] indicated the presence of a 2′-hydroxy-3′, 4′, 5′-trimethoxyphenyl group. The later one was found to be attached with C-2 according to the HMBC spectral correlation from singlet aromatic proton (δ_H 6.65, H-6′) to δ_C 85.2 (C-2). Based on the small coupling constants (J = 3.5 Hz) of the oxymethines (H-2 and 6) and the chemical shift of the oxymethylene protons, two sets of protons (H-2/ H-1 and H-6/H-5) were determined as being *trans* oriented. The ECD spectra of **13** revealed a positive Cotton effect at 243 (Δε +0.56). Hence, the structure of **13** was identified as (1*R*,2*S*,5*S*,6*S*)-2-(2′-hydroxy-3′,4′,5′-trimethoxyphenyl)-6-(3″-methoxy-4″,5″-methylenedioxyphenyl)-4-oxo-3,7-dioxabicyclo[3.3.0]octane.



13

Table 4. NMR data for **13** (^{1}H NMR; 500 MHz and ^{13}C NMR; 125 MHz, δ; ppm, recorded in MeOH-d_4)

Position	**13**	
	δ_H (J in Hz)	δ_C [a]
1	3.31, overlapped	50.0
2	5.47, d (3.5)	85.2
4		180.1
5	3.57, dd (10.0, 4.5)	55.6
6	5.12, d (4.5)	84.9
8_a	4.31, dd (9.5, 7.0)	74.9
8_b	4.07, dd (9.5, 5.0)	
1′		122.2
2′		143.0
3′		143.3
4′		144.6
5′		147.5
6′	6.65, s	107.8
1″		136.6
2″	6.65, overlapped	107.3
3″		145.1
4″		136.2
5″		150.7
6″	6.58, d (1.5)	100.9
OCH_2O	5.91,s	102.7
OMe	3.87, s	61.6
	3.84, s	61.3
	3.83, s	57.4
	3.78, s	57.4

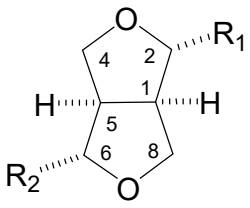
[a] Assignments were based on HMQC and HMBC experiments

1.3.3. Furofuran lignan glucosides

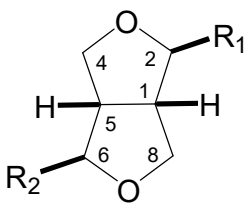
Compound **14**, $[\alpha]_D$ +3.7, was obtained as a pale yellowish solid and was determined to have the molecular formula $C_{27}H_{32}O_{13}$, based on the sodiated molecular ion $[M + Na]^+$ at *m/z* 587.1763 (calcd 587.1741) in the HRFABMS. The ^{1}H NMR spectrum indicated two sets of signal for the partial structure, -CH_2(O)-CH-CH(O), based on the characteristic resonances of the oxygenated methines and methylenes [δ_H 5.33 (1H, d, *J* = 4.5 Hz, H-2), 4.32 (1H, dd, *J* = 9.5, 7.0 Hz, H-8_a), and 3.98 (1H, dd, *J*= 9.5, 5.0 Hz, H-8_b); δ_H 4.66 (1H, d, *J* = 5.5 Hz, H-6), 4.20 (1H, dd, *J* = 8.5, 7.0 Hz, H-4_a), and 3.84 (1H, overlapped, H-4_b)]. The signal of one methine proton (δ_H 3.24, H-1) was found to be coupled with a oxymethine proton (δ_H 5.33, H-2) and a pair of oxymethylene protons (δ_H 4.32, 3.98, H-8) in the COSY spectrum. Additionally, another multiplet methine proton (δ_H 3.05, H-5) coupled with a further oxymethine proton (δ 4.66, H-6) and a pair of oxymethylene protons (δ_H 4.20, 3.84, H-4). The ^{1}H NMR spectrum also showed ABX-type aromatic proton signals [δ_H 6.87 (1H, d, *J* = 2.0 Hz, H-2″), 6.84 (1H, dd, *J*= 8.0, 2.0 Hz, H-6″), and 6.77 (1H, d, *J*= 8.0 Hz, H-5″)], a singlet aromatic proton signal [δ_H 6.63 (1H, s H-6′)], a dioxymethylene signal [δ_H 5.92 (2H, s)], and two methoxy group signals [δ_H 3.88, 3.83 (each 3H, s)], in addition to an anomeric proton signal [δ_H 5.09 (1H, d, *J*= 7.5 Hz, H-1‴)]. These data (Table 5) were found to be similar to a previously reported asymmetrically substituted furofuran lignan containing a 2,6-diaryl *cis*-3,7-dioxabicyclo[3.3.0]octane skeleton.[33] The HMBC spectrum showed the connectivity of four partial structures: (i) dioxymethylene protons (δ_H 5.92) and a *meta*-coupled aromatic proton signal [δ_H 6.87 (H-2″)] to two oxygenated aromatic carbons [δ_C 149.5 (C-3″) and 148.7 (C-4″)] in ring B, (ii) a *meta*-coupled proton signal to an oxymethine carbon signal [δ_C 87.5 (C-6)], (iii) a singlet aromatic proton signal [δ_H 6.63 (H-6′)] and an anomeric proton signal [δ_H 5.09 (H-1‴)] to an oxygenated aromatic carbon [δ_C 141.4 (C-2′)] in ring A, and (iv) an oxymethine proton signal [δ_H 5.33 (H-2)] to two aromatic carbons [δ_C 141.4 (C-2′) and 109.4 (C-6′)]. The ^{13}C NMR data suggested that the attachment positions of the two methoxy groups [δ_C 61.4 and 61.9] were C-3′ and C-4′ in the A-ring, based on their chemical shifts (Table 5).The HMBC spectrum also supported the assignments from the correlated signals [δ_H 3.83 (MeO)/δ_C 147.4 (C-3′) and δ_H 3.88 (MeO)/δ_C 142.4 (C-4′)] and other correlations from a singlet aromatic proton signal to oxygenated aromatic carbon signals [δ_H 6.63 (H-6′)/δ_C 131.6 (C-1′), 141.4 (C-2′), 142.4 (C-4′), 148.2 (C-5′)].

Acid hydrolysis of **14-26** gave a sugar moiety, which was identified as D-glucose by HPLC analysis of the thiazolidine derivative, and the anomeric center of D-glucose was identified to have a β-configuration from the coupling constant of the anomeric proton signal (H-1‴, *J* = 7.5 Hz).

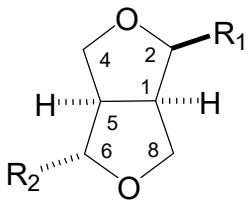
Based on the coupling constants of the oxymethine protons [δ_H 5.33 (1H, d, J = 4.5 Hz, H-2) and 4.66 (1H, d, J = 5.5 Hz, H-6)] of **14**, two sets of protons (H-2/H-1 and H-6/H-5) were indicated as being *trans* oriented.[30] The NOESY correlations between H-1 and H-6′, and between H-5 and H-2″,H-6″ also confirmed the postulated arrangement. In addition, the ECD spectrum of **14** showed positive Cotton effect at 235 nm ($\Delta\varepsilon$ +1.0) and a negative Cotton effect at 285 nm ($\Delta\varepsilon$ -0.6), which were almost identical to those of the *exo*, *exo*-substituted lignan (1*R*,2*S*,5*R*,6*S*)-sesamin.[31] Therefore, **14** was identified as (1*R*,2*S*,5*R*,6*S*)-2-(2′,5′-dihydroxy-3′,4′-dimethoxyphenyl)-6-(3″,4″-methylenedioxyphenyl)-3,7-dioxabicyclo[3.3.0]octane 2′-*O*-β-D-glucopyranoside, and was trivially named as terminaloside A.



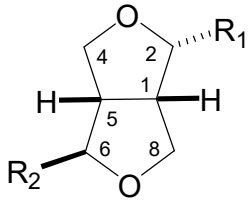
	R_1	R_2
14	Ar_9	Ar_1
15	Ar_{10}	Ar_1
16	Ar_9	Ar_2
17	Ar_{10}	Ar_2
19	Ar_4	Ar_2
20	Ar_9	Ar_5
21	Ar_{10}	Ar_3
25	Ar_{10}	Ar_7



	R_1	R_2
22	Ar_9	Ar_6
23	Ar_{11}	Ar_8
24	Ar_{11}	Ar_6



	R_1	R_2
18	Ar_{10}	Ar_2



	R_1	R_2
26	Ar_{10}	Ar_7

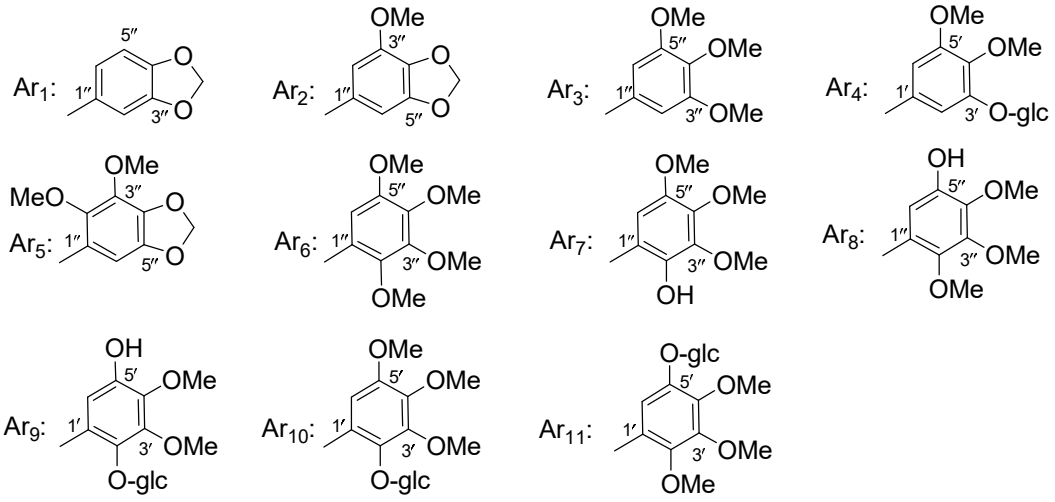


Table 5. NMR data for **14-16** (^{1}H NMR; 500 MHz and ^{13}C NMR; 125 MHz, δ; ppm, recorded in MeOH-d_4)

Position	**14**		**15**		**16**	
	δ_H (J in Hz)	δ_C[a]	δ_H (J in Hz)	δ_C[a]	δ_H (J in Hz)	δ_C[a]
1	3.24, m	54.2	3.25, m	54.5	3.24, m	54.2
2	5.33, d (4.5)	82.2	5.33, d (4.5)	82.5	5.33, d (4.5)	82.2
4_a	4.20, dd (8.5, 7.0)	72.3	4.25, dd (8.5, 6.5)	72.9	4.21, dd (8.5, 7.0)	72.7
4_b	3.84, overlapped		3.88, overlapped		3.88, overlapped	
5	3.05, m	55.7	3.07, m	55.7	3.04, m	55.9
6	4.66, d (5.5)	87.5	4.68, d (5.5)	87.2	4.66, d (5.5)	87.5
8_a	4.32, dd (9.5, 7.0)	74.4	4.30, dd (9.0, 8.0)	74.2	4.32, dd (9.0, 8.0)	74.4
8_b	3.98, dd (9.5, 5.0)		4.03, dd (9.0, 5.0)		3.98, dd (9.0, 4.5)	
1′		131.6		131.3		131.6
2′		141.4		142.5		141.4
3′		147.4		147.4		147.4
4′		142.4		144.1		142.4
5′		148.2		151.2		148.2
6′	6.63, s	109.4	6.75, s	106.4	6.63, s	109.4
1″		136.8		136.7		137.7
2″	6.87, d (2.0)	107.7	6.88, br. s	107.7	6.60, d (2.0)	107.7
3″		149.5		149.5		145.1
4″		148.7		148.7		136.2
5″	6.77, d (8.0)	109.1	6.76, d (8.0)	109.1		150.8
6″	6.84, dd (8.0, 2.0)	120.8	6.84, dd (8.0, 1.0)	120.8	6.56, d (2.0)	101.3
OCH_2O	5.92, s	102.5	5.91, s	102.5	5.91, s	102.7
OMe	3.88, s	61.9	3.88, s	62.1	3.88, s	61.9
	3.83, s	61.4	3.83, s	61.6	3.88, s	61.4
			3.81, s	57.0	3.84, s	57.5
Glc-1‴	5.09, d (7.5)	105.0	5.14, d (7.5)	104.8	5.09, d (7.5)	105.0
2‴	3.35, m	75.9	3.44, m	75.9	3.44, m	75.9
3‴	3.33, m	78.1	3.42, m	78.1	3.42, m	78.1
4‴	3.30, m	71.9	3.36, m	71.8	3.36, m	71.9
5‴	3.26, m	78.6	3.27, m	78.6	3.26, m	78.6
6‴	3.85, overlapped	62.9	3.84, overlapped	62.8	3.85, overlapped	62.9
	3.65, dd (11.5, 6.0)		3.65, dd (12.0, 5.5)		3.67, dd (12.0, 5.0)	

[a,] Assignments were based on HMQC and HMBC experiments

Compound **15**, $[\alpha]_D$ +38.3, was obtained as a pale yellowish solid and its molecular formula was deduced as $C_{28}H_{34}O_{13}$ from the protonated molecular ion $[M + H]^+$ at *m/z* 579.2067 (calcd 579.2077) in the HRFABMS, representing one carbon atom and two hydrogen atoms more than those of **14**. The NMR spectra of **15** indicated the structure to be that of a furofuran lignan like **14**, based on their spectroscopic

similarity, and **15** was observed to have an additional methoxy group [δ_H 3.81 (3H, s), δ_C 57.0]. The HMBC spectrum indicated that the methoxy group is attached to C-5′, from the correlation with the singlet aromatic proton signal, H-6′ [δ_H 6.75 (1H, s)]. Thus, the structure of **15** (terminaloside B) was established as (1*R*,2*S*,5*R*,6*S*)-2-(2′-hydroxy-3′,4′,5′-trimethoxyphenyl)-6-(3″,4″-methylenedioxyphenyl)-3,7-dioxabicyclo[3.3.0]octane 2′-*O*-*β*- D-glucopyranoside.

Compounds **16** and **17** were isolated as pale yellow, amorphous powders and their molecular formulas were determined to be $C_{28}H_{34}O_{14}$ and $C_{29}H_{36}O_{14}$, based on their molecular ion peaks at *m/z* 595.2054 $[M + H]^+$(calcd 595.2027) and 631.2006 $[M + Na]^+$ (calcd 631.2002) in the HRFABMS, respectively. The ^1H and ^{13}C NMR spectroscopic features of the compounds were congruent with **14** and **15**, respectively (Tables 5 and 6). However, a pair of *meta*-coupled aromatic protons [**16**: δ_H 6.60 (1H, d, *J*=2.0 Hz), 6.56 (1H, d, *J*=2.0 Hz); **17**: δ_H 6.61 (1H, d, *J*=2.0 Hz), 6.56 (1H, d, *J*=2.0 Hz)] and signals for an additional methoxy group [**16**: δ_H 3.88 (3H, s), δ_C 57.5; **17**: δ_H 3.87 (3H, s), δ_C 57.5] were appeared, while ABX-type aromatic proton signals were observed in **14** and **15**. The position of the ancillary methoxy group in both compounds was confirmed at C-3″ from the respective HMBC spectrum. Accordingly, **16** (terminaloside C) was determined to be (1*R*,2*S*,5*R*,6*S*)-2-(2′,5′-dihydroxy-3′,4′-dimethoxyphenyl)-6-(3″-methoxy-4″,5″-methylenedioxyphenyl)-3,7-dioxabicyclo[3.3.0]octane 2′-*O*-*β*-D-glucopyranoside, and **17** (terminaloside D) was established as (1*R*,2*S*,5*R*,6*S*)-2-(2′-hydroxy-3′,4′,5′-trimethoxyphenyl)-6-(3″-methoxy-4″,5″-methylenedioxyphenyl)-3,7-dioxabicyclo[3.3.0] octane 2′-*O*-*β*-D-glucopyranoside.

Compound **18** was determined to have the molecular formula, $C_{29}H_{36}O_{14}$, based on its sodiated molecular ion at *m/z* 631.1981 $[M + Na]^+$ (calcd 631.2002) in the HRFABMS, which is same as that of **17**. The NMR data of **18** indicated that the compound is an isomer of **17**, due to their closely similar spectra. However, the two oxymethine signals of the furofuran ring appeared in a higher field with larger coupling constants [δ_H 5.24 (d, *J*= 6.5 Hz, H-2), 4.39 (d, *J*= 7.0 Hz, H-6)] in the ^1H NMR spectrum of **18**, and one of the oxymethylene groups was shifted to higher field [δ_C 71.0 (C-8, Δ -3.3 ppm)], which was presumed to be due to the anisotropic effect of an aromatic ring. In the NOESY spectrum of **5**, correlations were observed between a singlet aromatic proton [δ_H 6.96 (s, H-6′)] and the oxymethylene protons [δ_H 3.18 (overlapped, H-8), 3.83 (overlapped, H-8)], while a *meta*-coupled aromatic proton [δ_H 6.61 (d, *J*= 2.0, H-2″), 6.56 (d, *J*= 2.0, H-6″)] showed correlations with two methine protons [δ_H 2.90 (m, H-5), 4.39 (d, *J*= 7.0, H-6)]. These data indicated that the structure of **18** is a stereoisomer of **17** at the C-2 oxymethine substituent, and the ECD spectrum showed a negative Cotton effect at 281 nm ($\Delta\varepsilon$ - 0.6) and positive Cotton effect at 230 nm ($\Delta\varepsilon$ + 6.6), which is in close agreement with that of the *endo-exo* aryl-substituted lignan, phillyrin.[34] Accordingly, **18** (2-epiterminaloside D) was established as (1*R*,2*R*,5*R*,6*S*)-2-(2′-

hydroxy-3′,4′,5′-trimethoxyphenyl)-6-(3″-methoxy-4″,5″-methylenedioxyphenyl)-3,7-dioxabicyclo[3.3.0]octane 2′-*O*-*β*-D-glucopyranoside.

Table 6. NMR data for **17-19** (^{1}H NMR; 500 MHz and ^{13}C NMR; 125 MHz, δ; ppm, recorded in MeOH-d_4)

Position	**17**		**18**		**19**	
	δ_H (J in Hz)	δ_C [a]	δ_H (J in Hz)	δ_C [a]	δ_H (J in Hz)	δ_C [a]
1	3.23, m	54.5	3.60, m	50.0	3.12, m	55.6
2	5.34, d (5.0)	82.5	5.24, d (6.5)	80.3	4.75, d (5.0)	87.2
4_a	4.26, dd (8.5, 7.0)	72.9	4.11, dd (9.0, 1.0)	71.5	4.25, dd (6.5, 3.5)	73.0
4_b	3.89, overlapped		3.86, overlapped		3.85, overlapped	
5	3.07, m	55.8	2.90, m	56.0	3.06, m	55.7
6	4.68, d (5.0)	87.2	4.39, d (7.0)	89.1	4.67, d (5.5)	87.5
8_a	4.31, dd (9.0, 7.0)	74.3	3.18, overlapped	71.0	4.28, dd (9.5, 3.5)	73.2
8_b	4.03, dd (9.0, 4.5)		3.83, overlapped		3.89, overlapped	
1′		131.3		130.1		139.0
2′		142.5		141.1	6.86, d (2.0)	108.6
3′		147.4		147.5		152.4
4′		144.1		143.4		139.4
5′		151.2		151.4		154.9
6′	6.76, s	106.4	6.96, s	106.6	6.74, d (2.0)	106.0
1″		137.6		137.6		137.5
2″	6.61, d (2.0)	107.7	6.61, d (2.0)	107.8	6.60, d (2.0)	107.7
3″		145.1		145.1		145.1
4″		136.1		136.3		136.2
5″		150.7		150.7		150.8
6″	6.56, d (2.0)	101.3	6.56, d (2.0)	101.3	6.56, d (2.0)	101.3
OCH_2O	5.91, s	102.7	5.91, s	102.7	5.90, s	102.7
OMe	3.88, s	62.1	3.90, s	61.9	3.88, s	61.7
	3.87, s	61.6	3.88, s	61.6	3.85, s	57.5
	3.83, s	57.5	3.85, s	57.5	3.81, s	56.8
	3.81, s	57.0	3.84, s	56.9		
Glc-1‴	5.14, d (7.5)	104.8	4.96, d (8.0)	105.4	4.90, d (7.5)	103.0
2‴	3.45, m	75.9	3.46, m	75.9	3.47, m	75.1
3‴	3.43, m	78.1	3.44, m	78.1	3.45, m	78.2
4‴	3.36, m	71.8	3.19, m	71.7	3.35, m	71.7
5‴	3.26, m	78.6	3.16, m	78.3	3.25, m	78.6
6‴	3.85, overlapped	62.8	3.76, dd (12.0, 2.0)	62.7	3.85, overlapped	62.8
	3.65, dd (12.0, 6.0)		3.65, dd (12.0, 6.0)		3.67, dd (12.5, 6.5)	

[a,] Assignments were based on HMQC and HMBC experiments

Compound **19**, obtained as a pale yellow amorphous powder, was ascribed the molecular formula, $C_{28}H_{34}O_{13}$, based on the positive-mode HRFABMS (*m/z* 579.2096 $[M + H]^+$. When the ^{13}C NMR spectra of **19** were compared with those of the furofuran lignans (**16** and **17**), the data for the sugar moiety and the majority of the aglycone were found to be consistent. The ^1H NMR data of **19** indicated the presence of two *meta*-coupled aryl rings [δ_H 6.86 (d, *J*= 2.0 Hz, H-2′), 6.74 (d, *J*= 2.0 Hz, H-6′), 6.60 (d, *J* = 2.0 Hz, H-2″), 6.56 (d, *J*= 2.0 Hz, H-6″)]. Moreover, signals for two *trans*-coupled hydroxymethine protons [δ_H 4.75 (d, *J*= 5.0 Hz, H-2), 4.67 (d, *J*= 5.5 Hz, H-6)] on the *cis*-3,7-dioxabicyclo[3.3.0]octane skeleton were observed around δ_H 4.7, whereas they were observed at δ_H 5.3 and 4.7 in the case of **16** and **17**, respectively. After considering the spectroscopic data including its ECD data, **19** (terminaloside E) was identified as (1*R*,2*S*,5*R*,6*S*)-2-(3′-hydroxy-4′,5′-dimethoxyphenyl)-6-(3″-methoxy-4″,5″-methylenedioxyphenyl)-3,7-dioxabicyclo[3.3.0]octane 3′-*O*-*β*-D-glucopyranoside.

Compounds **20** and **22** were assigned the molecular formulas $C_{29}H_{36}O_{15}$ and $C_{30}H_{40}O_{15}$ respectively, based on their sodiated molecular ions at *m/z* 647.1933 $[M + Na]^+$ and 663.2281 $[M + Na]^+$ in the HRFABMS. Their spectroscopic features were very similar to one another and shared many features with those of **16**. The ^1H NMR spectra of **20** and **22** showed two singlet aromatic protons [**20**: δ_H 6.58, 6.62; **22**: δ_H 6.77, 6.62] and a pair of oxymethine proton signals [**20**: δ_H 5.33 (d, *J*= 5.0 Hz, H-2), 4.96 (d, *J*= 5.0 Hz, H-6); **22**: δ_H 5.35 (d, *J*= 4.5 Hz, H-2), 4.99 (d, *J*= 5.0 Hz, H-6)], which indicated the presence of a furofuran ring system in their structures (Table 7). The oxymethine signal observed at around δ_H 5.35 suggested that the ring system is attached to a 2′-*O*-glucosyl aryl group. In the HMBC spectrum of **20**, two oxygenated aromatic carbons (δ_C 138.5, 146.5) were recognized as having correlations to both a dioxymethylene signal (δ_H 5.89) and a singlet aromatic proton (δ_H 6.58), which showed a correlation with another oxymethine (δ_H 4.96/ δ_C 83.3). From the above spectroscopic data, the methoxy groups of **20** were assigned as being attached at C-2″, C-3″, C-3′, and C-4′, because these groups were deduced to have substituted groups at their both *ortho* positions based on their chemical shifts [δ_C 61.9, 61.7, 61.4, and 60.5] in the ^{13}C NMR spectrum. In the NMR spectrum of **22**, two additional methoxy signals (δ_H 3.81/ δ_C 57.0, δ_H 3.82/ δ_C 61.6) were observed in place of a dioxymethylene (δ_H 5.89/ δ_C 102.8) when compared with **20**. The attachment positions of the methoxy groups were confirmed from the HMBC spectrum, which showed correlations from the methoxy group protons and the singlet aromatic proton (δ_H 6.77) to oxygenated aromatic carbons (δ_C 143.8 and 151.0). Based on the above evidence, **20** (terminaloside F) was determined as (1*R*,2*S*,5*R*,6*S*)-2-(2′,5′-dihydroxy-3′,4′-dimethoxyphenyl)-6-(2″,3″-dimethoxy-4″,5″-methylenedioxyphenyl)-3,7-dioxabicyclo[3.3.0]octane 2′-*O*-*β*-D-glucopyranoside, and **22** (terminaloside H) was shown to be (1*S*,2*R*,5*S*,6*R*)-2-(2′,5′-dihydroxy-3′,4′-dimethoxyphenyl)-6-(2″,3″,4″,5″-tetramethoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane 2′-*O*-*β*-D-glucopyranoside.

Table 7. NMR data for **20-22** (^{1}H NMR; 500 MHz and ^{13}C NMR; 125 MHz, δ; ppm, recorded in MeOH-d_4)

Position	**20**		**21**		**22**	
	δ_H (J in Hz)	δ_C[a]	δ_H (J in Hz)	δ_C[a]	δ_H (J in Hz)	δ_C[a]
1	3.20, m	54.3	3.25, m	54.5	3.22, m	54.4
2	5.33, d (5.0)	81.6	5.35, d (5.0)	82.5	5.35, d (4.5)	81.5
4_a	4.23, dd (8.5, 6.5)	73.4	4.29, dd (9.5, 7.0)	73.0	4.23, dd (9.0, 6.5)	73.4
4_b	3.97, overlapped		3.92, dd (9.5, 4.0)		4.05, dd (9.0, 5.0)	
5	2.97, m	55.8	3.09, m	55.9	3.00, m	55.9
6	4.96, d (5.0)	83.3	4.76, d (5.5)	87.3	4.99, d (5.0)	83.5
8_a	4.33, dd (8.5, 6.5)	74.3	4.35, dd (9.5, 7.5)	74.4	4.37, dd (9.0, 7.0)	74.4
8_b	4.01, overlapped		4.07, dd (9.5, 4.5)		4.02, dd (9.0, 5.5)	
1′		131.5		131.4		131.6
2′		141.5		142.5		141.5
3′		147.4		147.5		147.4
4′		142.5		144.1		142.5
5′		148.2		151.2		148.2
6′	6.62, s	109.4	6.76, s	106.4	6.62, s	109.4
1″		129.6		139.0		131.5
2″		145.2	6.68, s	104.6		145.9
3″		138.9		154.8		148.2
4″		138.5		138.8		143.8
5″		146.5		154.8		151.0
6″	6.58, s	100.4	6.68, s	104.6	6.77, s	105.8
OCH_2O	5.88, d (1.0)	102.8				
	5.89, d (1.0)					
OMe	3.98, s	61.9	3.88, s	62.1	3.88, s	61.9
	3.88, s	61.7	3.84, s	61.6	3.88, s	61.6
	3.84, s	61.4	3.84, s	61.2	3.84, s	61.6
	3.79, s	60.5	3.83, s	57.0	3.83, s	61.5
			3.81, s	56.8	3.82, s	61.4
			3.75, s	56.8	3.81, s	57.0
Glc-1‴	5.09, d (8.0)	105.0	5.15, d (7.5)	104.8	5.08, d (8.0)	105.1
2‴	3.44, m	75.9	3.45, m	75.9	3.46, m	75.9
3‴	3.42, m	78.1	3.43, m	78.2	3.44, m	78.1
4‴	3.37, m	71.8	3.33, m	71.8	3.37, m	71.8
5‴	3.26, m	78.6	3.26, m	78.6	3.26, m	78.6
6‴	3.83, overlapped	62.9	3.85, overlapped	62.8	3.87, overlapped	62.9
	3.67, dd (12.0, 5.5)		3.65, dd (12.0, 6.0)		3.67, dd (12.0, 5.0)	

[a,] Assignments were based on HMQC and HMBC experiments

Compound **21**, $[\alpha]_D$ +42.3, was obtained as a pale yellow amorphous powder and the molecular formula was assigned to be $C_{30}H_{40}O_{14}$, based on its protonated molecular ion at *m/z* 625.2526 $[M + H]^+$ (calcd 625.2496) in the HRFABMS. The NMR spectra showed similar characteristics to those of **17**, indicating a common partial structure of a furofuran ring system, along with a 2′-*O*-glucosyl-3′,4′,5′-trimethoxyphenyl ring based on the oxymethine signals [δ_H 5.35 (d, *J*= 5.0 Hz, H-2)/δ_C 82.5 (C-2); δ_H 4.76 (d, *J*=5.5 Hz, H-6)/δ_C 87.3 (C-6)], and a singlet aromatic proton (δ_H 6.76), three methoxy groups [δ_C 62.1, 61.6, 57.0], and a sugar anomeric proton and carbon [δ_H 5.15 (d, *J*=7.5 Hz, H-1‴)/ δ_C 104.8]. Moreover, two A_2-type aromatic proton signals (δ_H 6.68) and three methoxy groups (δ_C 61.2, 56.8, 56.8) suggested the presence of a 3″,4″,5″-trimethoxy aromatic ring (Table 7). The oxymethine proton (δ_H 4.76) was deduced to be attached to the A_2-type aromatic ring from the correlation between H-6 (δ_H 4.76) and C-2″, 6″ (δ_C 104.6) in the HMBC spectrum. A positive Cotton effect was observed in the ECD spectrum of **21** as $\Delta\varepsilon_{230}$ +1.8.[35] Accordingly, **21** (terminaloside G) was identified as (1*R*,2*S*,5*R*,6*S*)-2-(2′-hydroxy-3′,4′,5′-trimethoxyphenyl)-6-(3″,4″,5″-trimethoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane 2′-*O*-*β*-D-glucopyranoside.

Compounds **23**, $[\alpha]_D$ +8.0, and **24**, $[\alpha]_D$ +51.2, were obtained as pale yellow amorphous solids. They were assigned the molecular formulas, $C_{30}H_{40}O_{15}$ and $C_{31}H_{42}O_{15}$, respectively, based on the sodiated molecular ions appearing at *m/z* 663.2250 $[M + Na]^+$ (calcd 663.2264) and *m/z* 677.2399 $[M + Na]^+$ (calcd 677.2421) in the HRFABMS. They shared many common features of furofuran lignans in their NMR spectra (Table 8), namely, two oxymethines with comparable chemical shifts [**23**: δ_H 4.96 (d, *J*= 5.0 Hz, H-2), 5.02 (d, *J*= 5.0 Hz, H-6); **24**:δ_H 5.04 (d, *J*= 5.0 Hz, H-2), 5.00 (d, *J*= 5.0 Hz, H-6)], an anomeric proton signal [**23**: δ_H 4.86 (d, *J*= 7.5 Hz, H-1‴); **24**:δ_H 4.86 (d, *J*= 7.5 Hz, H-1‴)], with highly methoxylated aromatic ring systems from two singlet aromatic protons [**23**:δ_H 6.97 (H-6′), 6.62 (H-6″); **24**:δ_H 6.97 (H-6′), 6.76 (H-6″)], and methoxy signals [**23**: δ_C 62.1, 61.7, 61.6, 61.5, 61.4, 61.4; **24**:δ_C 62.1, 61.7, 61.7, 61.6, 61.6, 61.6, 57.0]. In the HMBC spectra of **23** and **24**, the anomeric proton signal showed a correlation with an aromatic carbon [δ_C 148.4 (C-5′)], which was also correlated with a singlet aromatic proton signal [δ_H 6.97 (H-6′)]. As the methoxy group carbon chemical shifts were all observed in fields lower than 60 ppm, **23** was deduced as having two aromatic ring systems, with one being a 2″,3″,4″-trimethoxy-5″-hydroxy substituent, and the other a 2′,3′,4′-trimethoxy-5′-glucosyl substituent. In turn, the seventh methoxy group (δ_C 57.0) recognized in **24** was assigned as being attached at C-5″ based on its chemical shift. Negative Cotton effects were observed in the ECD spectra of **23** and **24**. Hence, **23** (terminaloside I) was proposed as (1*S*,2*R*,5*S*,6*R*)-2-(5′-hydroxy-2′,3′,4′-trimethoxyphenyl)-6-(5″-hydroxy-2″,3″,4″-trimethoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane 5′-*O*-*β*-D-glucopyranoside, and **24**

(terminaloside J) was identified as (1*S*,2*R*,5*S*,6*R*)-2-(5′-hydroxy-2′,3′,4′-trimethoxyphenyl)-6-(2″,3″,4″,5″-tetramethoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane 5′-*O*-*β*-D-glucopyranoside.

Table 8. NMR data for **23-24** (^{1}H NMR; 500 MHz and ^{13}C NMR; 125 MHz, δ; ppm, recorded in MeOH-d_4)

Position	**23**		**24**	
	δ_H (*J* in Hz)	δ_C [a]	δ_H (*J* in Hz)	δ_C [a]
1	2.98, m	55.8	3.05, m	55.8
2	4.96, d (5.0)	82.8	5.04, d (5.0)	82.6
4_a	4.29, dd (9.5, 6.0)	73.8	4.29, dd (9.0, 6.5)	73.6
4_b	4.04, dd (9.5, 3.5)		4.07, dd (9.0, 4.0)	
5	3.00, m	55.8	3.00, m	56.0
6	5.02, d (5.0)	83.0	5.0, d (5.0)	83.2
8_a	4.26, dd (9.0, 6.5)	73.8	4.33, dd (9.0, 7.0)	74.0
8_b	4.02, dd (9.0, 4.0)		4.02, dd (9.0, 4.5)	
1′		131.7		131.7
2′		147.3		147.4
3′		148.2		148.3
4′		144.5		144.6
5′		148.4		148.4
6′	6.97, s	110.3	6.97, s	110.3
1″		131.7		131.3
2″		144.9		146.0
3″		148.1		148.3
4″		142.1		143.8
5″		147.9		151.0
6″	6.62, s	108.7	6.76, s	105.8
OMe	3.89, s	62.1	3.89, s	62.1
	3.88, s	61.7	3.88, s	61.7
	3.88, s	61.6	3.88, s	61.7
	3.85, s	61.5	3.85, s	61.6
	3.83, s	61.4	3.83, s	61.6
	3.81, s	61.4	3.82, s	61.6
			3.81, s	57.0
Glc-1‴	4.86, d (7.5)	103.2	4.86, d (7.5)	103.3
2‴	3.46, m	75.1	3.46, m	75.1
3‴	3.43, m	78.2	3.43, m	78.3
4‴	3.38, m	71.6	3.38, m	71.7
5‴	3.35, m	78.5	3.35, m	78.5
6‴	3.88, overlapped	62.8	3.85, overlapped	62.8
	3.67, dd (12.0, 6.0)		3.66, dd (12.0, 5.5)	

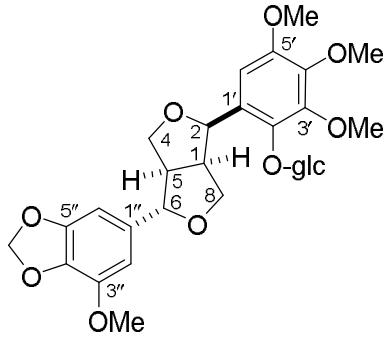
[a] Assignments were based on HMQC and HMBC experiments

Compound **25**, $[\alpha]_D$ +32.3, a yellow solid, was assigned the elemental formula of $C_{30}H_{40}O_{15}$ based on its sodiated molecular ion at *m/z* 663.2263 $[M + Na]^+$ (calcd 663.2264) observed in the HRFABMS. The NMR data of **25** showed a furofuran system with oxymethine signals [δ_H 5.35 (d, *J*= 5.0 Hz, H-2), 5.03 (d, *J*= 5.0 Hz, H-6)], two overlapping aromatic protons [δ_H 6.74 (s, H-6',H-6")], six methoxy signals [δ_C 62.1, 61.7, 61.6, 61.4, 57.5, 57.0], and an anomeric proton signal [δ_H 5.13 (d, *J*= 7.5 Hz, H-1‴)]. The chemical shifts of these oxymethine signals were characteristic of a 2'-glucosyl aromatic substituent and a 2"-oxygenated aromatic substituent. Although an oxymethine proton [δ_H 5.03 (H-6)] was correlated with an oxygenated carbon (δ_C 142.8) in the HMBC spectrum of **25**, no methoxy group protons showed a correlation with this carbon, which suggested the presence of a 2"-hydroxy group. A positive Cotton effect was observed at 243 nm in the ECD spectrum of **25**. Based on its spectroscopic data, **25** (terminaloside K) was assigned as (1*R*,2*S*,5*R*,6*S*)-2-(2′-hydroxy-3′,4′,5′-trimethoxyphenyl)-6-(2″-hydroxy-3″,4″,5″-trimethoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane 2′-*O*-*β*-D-glucopyranoside.

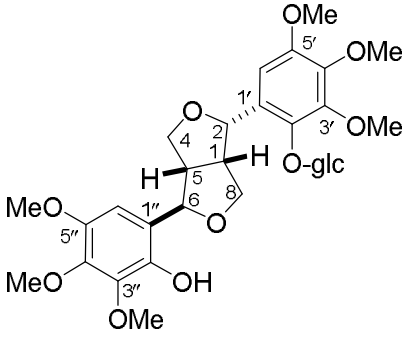
Compound **26**, $[\alpha]_D$ +52.3, obtained as a pale yellow solid, was assigned with the molecular formula, $C_{30}H_{40}O_{15}$, the same as **25**, based on its sodiated molecular ion at *m/z* 663.2238 $[M + Na]^+$ (calcd 663.2264) in the HRFABMS. Its NMR spectra indicated that **26** has the same planar structure as **12**. However, ^{13}C NMR signals for C-4, C-5 and C-6 in the furofuran ring [δ_C 70.9 (Δ - 2.9 ppm, C-4), 50.1 (Δ - 5.4 ppm, C-5), 80.2 (Δ -3.6 ppm, C-6)] were shifted to higher field in comparison to **25**, and an anisotropic effect was inferred by an *endo*-substituted aromatic ring. A singlet aromatic proton signal [δ_H 6.89 (s, H-6")] showed a long-range connectivity with the oxymethine carbon [δ_C 80.2 (C-6)] in the HMBC spectrum. In the NOESY spectrum, the aromatic proton signal [δ_H 6.89 (s, H-6")] showed a correlation with the oxymethylene proton signal [δ_H 3.27 (H-8)] which also showed a cross-peak with an aryl oxymethine proton [δ_H 5.12 (d, *J*= 6.5, H-2)]. Another aryl oxymethine proton [δ_H 4.99 (d, *J*= 6.0, H-6)] exhibited a correlation with a methine proton signal [δ_H 3.54 (H-1)], which showed a correlation with another methine proton [δ_H 3.11 (H-5)]. These data suggested that the structure of **26** is a stereoisomer at the C-6 oxymethine substituent of **25**. The ECD spectrum showed positive Cotton effect at 230 nm ($\Delta\varepsilon$ +5.9). Hence, **13** (6-epiterminaloside K) was established as (1*R*,2*S*,5*R*,6*R*)-2-(2′-hydroxy-3′,4′,5′-trimethoxyphenyl)-6-(2″-hydroxy-3″,4″,5″-trimethoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane 2′-*O*-*β*-D-glucopyranoside.

Twelve of the furofuran lignan glucosides isolates were found to contain rare tetraoxygenated aryl groups and the ^1H and ^{13}C NMR chemical shifts of the oxymethine signals gave useful information about the functional groups on these aryl groups. The oxymethine group, connected to a 2'-glucosyl aryl unit,

was observed at δ_H 5.3/δ_C 82, while a 2'-oxygenated aryl moiety attached to an oxymethine [δ_H 5.0/ δ_C 83] and a 2',6'-nonsubstituted aryl unit bonded to oxymethine [δ_H 4.7/ δ_C 87] were recognized in individually characteristic positions in the case of the 3,7-dioxabicyclo[3.3.0]octane 2,6-di-*exo* substituent's. In addition, the oxymethine groups were recognized at a higher field in the case of *exo*, and *endo* substituents. Thus, a 2'-oxygenated aryl unit in *exo*- [δ_H 5.0/ δ_C 80], a 2'-glucosyl aryl unit in *exo*- [δ_H 4.4/δ_C 89], a 2'-oxygenated aryl unit in *endo*- [δ_H 5.0/ δ_C 84] and a 2'-glucosyl aryl unit in *endo*- [δ_H 5.3/δ_C 82] substituents were found.



18



26

Table 9. NMR data for **25-26** (^{1}H NMR; 500 MHz and ^{13}C NMR; 125 MHz, δ; ppm, recorded in MeOH-d_4)

Position	**25**		**26**	
	δ_H (J in Hz)	δ_C [a]	δ_H (J in Hz)	δ_C [a]
1	3.20, m	54.5	3.54, m	50.1
2	5.35, d (5.0)	81.8	5.12, d (6.5)	83.4
4_a	4.27, dd (9.5, 6.5)	73.8	4.35, dd (9.0, 1.0)	72.9
4_b	4.13, dd (9.5, 4.0)		3.87, overlapped	
5	3.06, m	55.5	3.11, m	53.4
6	5.03, d (5.0)	83.8	4.99, d (6.0)	80.2
8_a	4.36, dd (9.0, 7.5)	74.2	3.79, t (9.0)	70.9
8_b	4.08, dd (9.0, 4.5)		3.27, overlapped	
1′		131.3		131.4
2′		142.6		142.6
3′		147.5		147.3
4′		144.1		144.1
5′		151.2		151.4
6′	6.74, s	106.4	6.78, s	106.6
1″		124.8		121.7
2″		142.8		142.0
3″		142.7		142.4
4″		143.4		143.2
5″		147.5		147.4
6″	6.74, s	106.5	6.89, s	107.4
OMe	3.88, s	62.1	3.88, s	62.1
	3.84, s	61.7	3.86, s	61.7
	3.82, s	61.6	3.85, s	61.6
	3.82, s	61.4	3.83, s	61.5
	3.80, s	57.5	3.82, s	57.5
	3.78, s	57.0	3.82, s	57.0
Glc-1‴	5.13, d (7.5)	104.9	5.15, d (7.5)	104.8
2‴	3.44, m	75.9	3.44, m	75.9
3‴	3.42, m	78.1	3.42, m	78.1
4‴	3.38, m	71.8	3.38, m	71.9
5‴	3.27, m	78.6	3.25, m	78.6
6‴	3.85, overlapped	62.8	3.85, overlapped	62.8
	3.68, dd (12.0, 5.5)		3.68, dd (11.5, 5.0)	

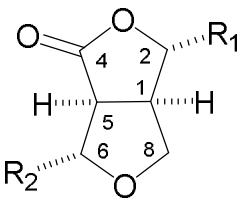
[a,] Assignments were based on HMQC and HMBC experiments

1.3.4. Furofuranone lignan glucosides

Compound **27**, a pale yellowish amorphous solid, had the molecular formula $C_{28}H_{32}O_{14}$ based on the protonated ion peak $[M + H]^+$ at *m/z* 593.1877 (calcd 593.1870) in the HRFABMS data, indicating 13 indices of unsaturation. The UV spectrum revealed the absorption band of aromatic rings (272 and 216 nm) in its structure, which was supported by twelve aromatic carbon resonances in ^{13}C NMR spectra. The ^1H NMR showed the characteristic signals of a furofuran ring, such as two benzylic oxymethine protons [δ_H 5.40 (1H, d, J = 3.5 Hz, H-2) and 5.19 (d, J = 3.5 Hz, H-6)], two methines [δ_H 3.30 (1H, m, H-1) and 3.60 (1H, dd, J = 9.5, 3.5 Hz, H-5)], and one oxymethylene [δ_H H 4.31 (1H, dd, J = 9.5, 7.0 Hz, H-8_a) and 4.05 (1H, dd, J = 9.5, 4.5 Hz, H-8_b)] (Table 10). However, a carbonyl carbon at δ_C 179.4 (C-4) was found in the ^{13}C NMR spectra, instead of the expected oxymethylene. In accordance with the ^1H-^1H COSY spectra, two sets of partial structures, -O-CH-CH-CH_2- and -O-CH-CH-CO-, were assigned to the positions C-2, 1, 8 and C-6, 5, 4, respectively. In the HMBC spectra, two oxymethines (H-2 and 6) showed clear correlations to the carbonyl group (δ_C 179.4), which indicated the partial structure of a 4-oxo-3,7-dioxabicyclo[3.3.0]octane moiety.[32] Two sets of *meta*- coupled aromatic proton resonances [δ_H 6.84 (1H, d, J = 1.5, H-2′) and 6.75 (1H, d, J = 1.5 Hz, H-6′); δ_H 6.64 (1H, d, J = 1.5, H-2″) and 6.59 (1H, d, J = 1.5 Hz, H-6″)] indicated the presence of 1′,3′,4′,5′-tetrasubstituted aromatic moieties. The HMBC spectrum showed the connectivity of four partial structures: (i) dioxymethylene protons (δ_H 5.91) and one of the *meta*-coupled aromatic proton signals [δ_H 6.59 (H-6″)] to two oxygenated aromatic carbons [δ_C 136.3 (C-4″) and 150.8 (C-5″)] in ring B, (ii) the same *meta*-coupled proton signal (H-6″) to an oxymethine carbon signal [δ_C 85.0 (C-6)], (iii) another doublet signal of second aromatic ring [δ_H 6.84 (H-2′)] and an anomeric proton signal [δ_H 4.91 (H-1‴)] to an oxygenated aromatic carbon [δ_C 152.7 (C-3′)] in ring A, and (iv) the second doublet proton signal (H-2′) to another oxymethine carbon signal [δ_C 86.4 (C-2)]. Ring B was recognized to be attached at C-6 based on the HMBC correlation of characteristic methine resonance [δ_H 3.60 (1H, dd, J = 9.5, 3.5 Hz, H-5)], which was observed in a series of isolates, **27** - **31**. The HMBC spectrum also suggested that the attachment positions of the three methoxy groups based on the correlated signals [δ_H 3.82 (MeO)/ δ_C 140.2 (C-4′), δ_H 3.86 (MeO)/ δ_C 155.2 (C-5′), and δ_H 3.88 (MeO)/ δ_C 145.2 (C-3″)]. Based on the small coupling constants (J = 3.5 Hz) of the oxymethines (H-2 and 6) and the chemical shift of the oxymethylene protons, two sets of protons (H-2/H-1 and H-6/H-5) were indicated as being *trans* oriented. The ECD spectra showed positive Cotton effects at 245 ($\Delta\varepsilon$ + 4.0) nm, which was the opposite of (-)-styraxlignolide B.[36] Hence, the absolute configuration of the 4-oxofurofuran nucleus was determined as 1*R*, 2*S*, 5*S*, and 6*S*. Acid hydrolysis of **27**-**31** afforded a sugar moiety, which was identified as D-glucose by comparing it with an authentic sample by HPLC. On the basis of the coupling constant of the anomeric proton resonance [δ_H 4.91 (d, J = 7.5 Hz, H-1‴), the β-configuration of the glucose unit was confirmed. As part of the characterization of furofuran lignan glycosides from *T. citrina*, **27** (terminaloside L) was identified as (1*R*,2*S*,5*S*,6*S*)-2-(3′-hydroxy-4′,5′-

dimethoxyphenyl)-6-(3″-methoxy-4″,5″-methylenedioxyphenyl)-4-oxo-3,7-dioxabicyclo[3.3.0]octane 3′-*O*-*β*-D-glucopyranoside.

Compound **28**, $[\alpha]_D$ +52.4, was obtained as a colorless solid and molecular formula was assigned as $C_{29}H_{34}O_{15}$ based on the sodiated ion peak $[M + Na]^+$ 645.1813 (calcd 645.1795) that appeared in the HRFABMS data. The NMR spectra of **28** were very similar to those of **27**, and exhibited the characteristic resonances of a 4-oxofurofuran, a glucose moiety, and a 1″, 3″, 4″, 5″-substituted phenyl moiety as partial structures (Table 10). In the HMBC spectrum of **28**, a *meta*-coupled aromatic proton signal [δ_H 6.60 (1H, d, J = 1.5 Hz, H-6″)] showed a correlation to an oxymethine carbon [δ_C 85.0 (C-6)], which also shared correlations to two oxygenated carbons [δ_C 136.5 (C-4″) and 150.9 (C-5″)] with a dioxymethylene proton signal (δ_H 5.92). The paired *meta*-coupled aromatic proton signal [δ_H 6.66 (1H, d, J = 1.5 Hz, H-2″)] presented a correlation to the oxygenated carbon [δ_C 145.2 (C-3″)] in common with an oxymethyl proton [δ_H 3.89 (MeO-3″)]. Meanwhile, a singlet aromatic proton resonance [δ_H 6.73 (H-6′)] exhibited correlations to three oxygenated carbons [δ_C 146.3 (C-2′), 145.0 (C-4′) and 151.2 (C-5′)] and an oxymethine carbon [δ_C 85.2 (C-2)]. The attachment position of the sugar moiety was also confirmed from the HMBC spectrum, which showed a correlation from the anomeric proton [δ_H 5.10 (1H, d, J = 7.5 Hz, H-1‴)] to the oxygenated aromatic carbon [δ_C 144.8 (C-3′)]. Thus, this aryl unit was identified as a 3′-glucosyloxy-2′, 4′, 5′-trimethoxyphenyl moiety. The ECD spectrum of **28** displayed positive Cotton effects at 240 (Δε + 4.3) nm. Hence, **28** (terminaloside M) was distinguished as (1*R*,2*S*,5*S*,6*S*)-2-(3′-hydroxy-2′,4′,5′-trimethoxyphenyl)-6-(3″-methoxy-4″,5″-methylenedioxyphenyl)-4-oxo-3,7-dioxabicyclo[3.3.0]octane 3′-*O*-*β*-D-glucopyranoside.



	R_1	R_2
27	Ar_2	Ar_1
28	Ar_3	Ar_1
29	Ar_4	Ar_1
30	Ar_5	Ar_1
31	Ar_6	Ar_1

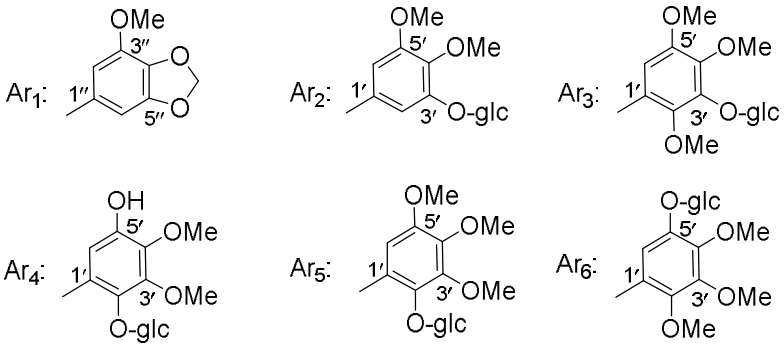


Table 10. NMR data for **27-28** (^{1}H NMR; 500 MHz and ^{13}C NMR; 125 MHz, δ; ppm, recorded in MeOH-d_4)

Position	**27**		**28**	
	δ_H (J in Hz)	δ_C[a]	δ_H (J in Hz)	δ_C[a]
1	3.30, m	51.0	3.30, m	50.7
2	5.40, d (3.5)	86.4	5.48, d, (3.5)	85.2
4		179.4		179.7
5	3.60, dd (9.5, 3.5)	54.4	3.58, dd (10.0, 4.5)	55.4
6	5.19, d (3.5)	85.0	5.16, d (4.5)	85.0
8_a	4.31, dd (9.5, 7.0)	74.1	4.31, dd (10.0, 7.5)	74.8
8_b	4.05, dd (9.5, 4.5)		4.06, dd (10.0, 4.5)	
1′		137.2		129.2
2′	6.84, d (1.5)	108.1		146.3
3′		152.7		144.8
4′		140.2		145.0
5′		155.2		151.2
6′	6.75, d (1.5)	105.8	6.73, s	107.8
1″		136.7		136.6
2″	6.64, d (1.5)	107.3	6.66, d (1.5)	107.4
3″		145.2		145.2
4″		136.3		136.5
5″		150.8		150.9
6″	6.59, d (1.5)	100.9	6.60, d (1.5)	100.9
OCH_2O	5.91, s	102.8	5.92, s	102.8
OMe	3.88, s	61.7	3.89, s	62.6
	3.86, s	57.5	3.89, s	61.9
	3.82, s	56.9	3.88, s	57.5
			3.84, s	57.0
Glc-1‴	4.91, d (7.5)	102.8	5.10, d (7.5)	104.5
2‴	3.52, m	75.1	3.48, m	75.8
3‴	3.44, m	78.2	3.42, m	78.1
4‴	3.35, m	71.6	3.36, m	71.6
5‴	3.40, m	78.5	3.22, m	78.5
6‴	3.87, overlapped	62.7	3.75, dd (12.0, 2.0)	62.7
	3.65, dd (12.0, 6.0)		3.65, dd (12.0, 5.0)	

[a,] Assignments were based on HMQC and HMBC experiments

Compounds **29** and **30** were isolated as pale yellow amorphous powders. Their molecular formula were determined to be $C_{28}H_{32}O_{15}$ and $C_{29}H_{34}O_{15}$ for **29** and **30** based on their respective sodiated ion peaks at *m/z* 631.1635 $[M + Na]^+$ (calcd 631.1639) and 645.1791 $[M + Na]^+$ (calcd 645.1795) in the HRFABMS data. Their spectroscopic features were very similar to one another and shared many features with those of **28**. The ^1H NMR spectra of **29** and **30** showed a pair of oxymethine proton signals [**29**: δ_H 5.79 (1H, d, J = 2.5 Hz, H-2), 5.16 (1H, d, J = 3.5 Hz, H-6); **30**: δ_H 5.82 (1H, d, J = 3.0 Hz, H-2), 5.18 (1H, d, J = 3.0 Hz, H-6)], which indicated the presence of a furofuran ring system in their structures. However, both were lower field shifted by approximately 0.3 ppm when compared with **28** and showed common correlations to the oxygenated carbon signal [**29**: δ_C 140.7 (C-2′); **30**: 142.1 (C-2′)] with the anomeric proton signal [**29**: δ_H 5.09 (1H, d, J = 7.5 Hz, H-1‴); **30**: δ_H 5.15 (1H, d, J = 8.0 Hz, H-1‴)] in their HMBC spectra. In the HMBC spectra, two oxygenated aromatic carbons [**29**: δ_C 136.4 (C-4″) and 150.8 (C-5″); **30**: 136.4 (C-4″) and 150.8 (C-5″)] were recognized as having correlations to both a dioxymethylene signal [**29**: δ_H 5.92 (s); **30**: δ_H 5.92 (s)] and a *meta*-coupled aromatic proton signal [**29**: δ_H 6.59 (1H, d, J = 1.5 Hz, H-6″); **30**: δ_H 6.60 (1H, d, J = 2.0 Hz, H-6″)], which also showed a correlation to another oxymethine [**29**: δ_H 5.16/ δ_C 85.4; **30**: δ_H 5.18/ δ_C 85.4]. The shared correlation between the paired *meta*-coupled aromatic proton signals [**29**: δ_H 6.64 (1H, d, J = 1.5 Hz, H-2″); **30**: δ_H 6.64 (1H, d, J = 2.0 Hz, H-2″)] and the oxymethyl proton to an oxygenated carbon [**29**: δ_C 145.2 (C-3″); **30**: 145.2 (C-3″)] suggested that these compounds have the same aromatic moiety as **27** and **28**. From the above spectroscopic data, the methoxy groups of **29** were assigned as being attached at C-3′ and C-4′, as these groups were deduced to have substituted groups at both of their ortho positions based on their chemical shifts [δ_C 61.4 and 62.6] in the ^{13}C NMR spectrum. In the NMR spectrum of **30**, an additional methoxy signal (δ_H 3.81/ δ_C 57.1) was observed when compared with **29**. The attachment positions of the methoxy groups were indicated by their chemical shifts and were further confirmed from the HMBC spectrum. Based on their ECD spectra, **29** (terminaloside N) was identified as (1*R*,2*S*,5*S*,6*S*)-2-(2′,5′-dihydroxy-3′,4′-dimethoxyphenyl)-6-(3″-methoxy-4″,5″-methylenedioxyphenyl)-4-oxo-3,7-dioxabicyclo[3.3.0]octane 2′-*O*-*β*- D- glucopyranoside, and **30** (terminaloside O) was shown to be (1*R*,2*S*,5*S*,6*S*)-2-(2′-hydroxy-3′,4′,5′-trimethoxyphenyl)-6-(3″-methoxy-4″,5″-methylenedioxyphenyl)-4-oxo-3,7-dioxabicyclo[3.3.0]octane 2′-*O*-*β*-D-glucopyranoside.

Compound **31**, a pale yellow amorphous powder, was assigned the identical molecular formula as **28** and **30**, $C_{29}H_{34}O_{15}$, based on the sodiated ion peak observed at *m/z* 645.1819 $[M + Na]^+$ (calcd 645.1795). The NMR spectra of **31** exhibited similar characteristics to those of **28** and **30**, indicating the presence of a 4-oxofurofuran ring system, a glucose moiety, and ring B in the form of a 3″-methoxy-4″,5″-methylenedioxyphenyl. The NMR data also revealed the same number of methoxy groups presenting on ring A, which possessed a glucose unit. Based on the chemical shifts of the methoxy groups of **31** in the ^{13}C NMR spectrum, these groups were assigned as being attached at C-2′, C-3′, and C-4′. This assignment

is further supported by the glycosylation shifts of the aromatic proton/carbon signals located at α and β positions [Δ +0.3 ppm at H-6′; Δ - 3 ppm at C-5′ and Δ + 5 ppm at C-6′] (Table 11). On the basis of the ECD data, **31** (terminaloside P) was identified as (1*R*,2*S*,5*S*,6*S*)-2-(5′-hydroxy-2′,3′,4′-trimethoxyphenyl)-6-(3″-methoxy-4″,5″-methylenedioxyphenyl)-4-oxo-3,7-dioxabicyclo[3.3.0]octane 5′-*O*-*β*-D-glucopyranoside.

Table 11. NMR data for **29-31** (^{1}H NMR; 500 MHz and ^{13}C NMR; 125 MHz, δ; ppm, recorded in MeOH-d_4)

Position	**29**		**30**		**31**	
	δ_H (*J* in Hz)	δ_C[a]	δ_H (*J* in Hz)	δ_C[a]	δ_H (*J* in Hz)	δ_C[a]
1	3.47, m	49.9	3.50, m	49.8	3.25, m	50.5
2	5.79, d (2.5)	82.6	5.82, d (3.0)	83.0	5.50, d (3.5)	84.1
4		180.3		180.3		179.6
5	3.54, dd (9.0, 3.5)	54.3	3.62, dd (9.0, 3.0)	54.5	3.57, dd (9.0, 4.0)	55.0
6	5.16, d (3.5)	85.4	5.18, d (3.0)	85.4	5.14, d (4.0)	85.0
8_a	4.39, dd (9.0, 7.5)	75.2	4.36, dd (10.0, 7.5)	75.2	4.29, dd (9.0, 7.5)	74.6
8_b	4.03, dd (9.0, 5.0)		4.08, dd (10.0, 5.0)		4.05, dd (9.0, 5.5)	
1′		130.3		129.8		129.0
2′		140.7		142.1		147.3
3′		147.7		147.8		148.4
4′		143.3		145.0		145.7
5′		148.8		151.8		148.5
6′	6.50, s	108.7	6.63, s	106.0	6.89, s	111.1
1″		136.7		136.7		136.5
2″	6.64, d (1.5)	107.5	6.64, d (2.0)	107.5	6.64, d (2.0)	107.3
3″		145.2		145.2		145.1
4″		136.4		136.4		136.4
5″		150.8		150.8		150.7
6″	6.59, d (1.5)	101.1	6.60, d (2.0)	101.1	6.59, d (2.0)	100.9
OCH_2O	5.92, s	102.8	5.92, s	102.8	5.91, s	102.7
OMe	3.89, s	62.0	3.89, s	62.2	3.89, s	62.0
	3.88, s	61.4	3.88, s	61.6	3.88, s	61.7
	3.85, s	57.5	3.85, s	57.5	3.88, s	61.6
			3.82, s	57.1	3.85, s	57.4
Glc-1‴	5.09, d (7.5)	104.8	5.15, d (8.0)	104.7	4.84, d (8.0)	103.1
2‴	3.44, m	75.8	3.44, m	75.8	3.40, m	75.0
3‴	3.41, m	78.1	3.40, m	78.1	3.38, m	78.2
4‴	3.34, m	71.8	3.34, m	71.8	3.35, m	71.5
5‴	3.24, m	78.7	3.23, m	78.7	3.27, m	78.4
6‴	3.79, dd (12.0, 2.0)	62.9	3.80, dd (12.0, 2.5)	62.7	3.86, overlapped	62.6
	3.61, dd (12.0, 6.0)		3.60, dd (12.0, 6.0)		3.65, dd (12.0, 5.5)	

[a,] Assignments were based on HMQC and HMBC experiments

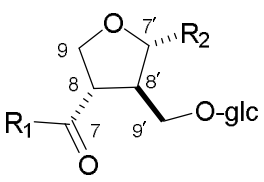
1.3.5. Tetrahydrofuran lignan glucosides

Compound **32**, $[\alpha]_D$ +15.9, was obtained as yellowish white amorphous powder and molecular formula was assigned as $C_{28}H_{34}O_{13}$ based on the protonated ion peak $[M + H]^+$ 579.2060 (calcd 579.2078) that appeared in the HRFABMS data, indicating 12 indices of unsaturation. The UV spectrum revealed the presence of carbonyl group conjugated with aromatic rings (227, 272 and 306 nm) in its structure, which was in accordance with the presence of a carbonyl group at δ_C 200.0 (C-7) in ^{13}C NMR. The ^1H NMR showed the characteristic signals of a furanoid lignan named sylvone,[37] such as a benzylic oxymethine proton [δ_H 4.81 (1H, d, J = 8.5 Hz, H-7′)], two methines [δ_H 4.27 (1H, overlapped, H-8) and 2.74 (1H, m, H-8′)], and two pair of oxymethylenes [δ_H 4.22 (1H, dd, J = 10.0, 8.0 Hz, H-9) and 4.18 (1H, dd, J = 8.0, 5.0 Hz, H-9); 4.06 (1H, dd, J = 10.5, 4.5 Hz, H-9′) and 3.66 (1H, overlapped, H-9′)] (Table 12). The ^1H NMR spectra of **32** displayed a characteristic signal of ABX-type aromatic proton signals [δ_H 7.48 (1H, d, J = 2.0 Hz, H-2), 7.70 (1H, dd, J = 8.0, 2.0 Hz, H-6), and 6.96 (1H, d, J= 8.0 Hz, H-5)] and one methylenedioxy protons signal at δ_H 6.06 (2H, s), suggesting the presence of a 3,4-methylenedioxyphenyl moiety. It was confirmed in HMBC spectra where methylenedioxy protons showed clear correlation to two aromatic carbon resonances at δ_C 150.0 (C-3) and 153.9 (C-4). In addition to an anomeric proton signal [δ_H 4.28 (1H, d, J = 8.0 Hz, H-1")], the ^1H NMR spectra of **32** also displayed a singlet aromatic proton [δ_H 6.78 (2H, H-2′ and 6′)] along with two methoxy group signals at δ_H 3.86 (6H, s) and 3.75 (3H, s), indicating an equivalent ring system of 3′,4′,5′-trimethoxyphenyl moiety.

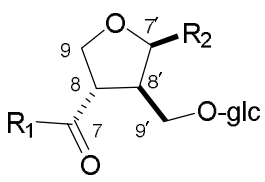
From the above data, the structure of **32** was proposed as a 7′,8,8′-trisubstituted tetrahydrofuranoketone-type lignan, having a glucopyranosyl moiety attached with one of the oxymethylene group. Such kind of tetrahydrofuranoketone lignan glucoside (aketrilignoside B) was also reported from *Akebia trifoliata*.[38] The above arrangement was confirmed by three different HMBC spectral correlations: i) two *meta*-coupled protons [δ_H 7.48 (H-2) and 7.70 (H-6)] to δ_C 200.0 (C-7), ii) singlet aromatic proton signal where two equivalent protons overlapped [δ_H 6.78 (H-2′ and 6′)] to benzylic oxymethine δ_C 85.2 (C-7′) and iii) anomeric proton [δ_H 4.28 (H-1")] and benzylic oxymethine [δ_H 4.81 (H-7′)] proton signal to oxymethylene carbon at δ_C 69.2 (C-9′). Acid hydrolysis of **32-38** gave a sugar moiety, which was identified as D-glucose by HPLC analysis, and the anomeric center of D-glucose was identified to have a β-configuration from the coupling constant of the anomeric proton signal (H-1", J = 7.5 Hz).

The relative configurations of C-7′, 8 and 8′ were determined from the proton chemical shifts, coupling constants (J) of H-7′/H-8′ and the NOESY spectrum analysis. A literature survey revealed that

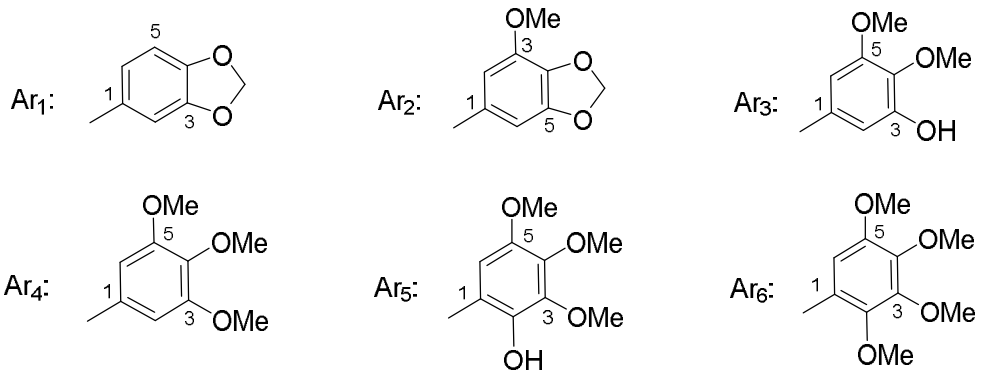
signal of H-7′ would arise at δ 5.5 and 4.7 ppm for *cis* and *trans* orientation of substituent's at C-7′ and C-8′, respectively.[39-43] The coupling constants (*J*) analysis revealed that *trans* and *cis* orientation at C-7′ would give 7.5-9.5 Hz and 6.0-7.0 Hz, respectively.[37, 44, 45] The H-7′ signal of **32** (δ 4.81) and coupling constants (J = 8.5 Hz) agreed well with the *trans* configuration at C-7′. Besides no NOESY correlation was observed in between H-8′ and H-8 protons. These led to the assignment of both *trans* at H-7′/H-8′ and H-8′/H-8, respectively. The benzylic oxymethine proton [δ_H 4.81 (H-7′)] showed clear correlation to oxymethylene protons at H-9′ in NOESY spectra too. The ECD spectra analysis revealed a positive Cotton effect at 276 ($\Delta\varepsilon$ +2.61) and negative Cotton effect at 322 ($\Delta\varepsilon$ -1.3) nm which are found similar to those of wikstrone and (7′*S*,8*S*,8′*R*)-4,4′-dihydroxy-3,3′,5,5′-tetramethoxy-7′,9-epoxylignan-9′-ol-7-one lignans in previous reports.[46-48] Therefore, the structure of **32** (terminaloside Q) was deduced as (+)-(7′*S*,8*S*,8′*R*)-3,4-methylenedioxy-3′,4′,5′-trimethoxy-7′,9-epoxylignan-9′-ol-7-one 9′-*O*-*β*-D-glucopyranoside.



	R_1	R_2
32	Ar_1	Ar_4
34	Ar_2	Ar_4
36	Ar_4	Ar_4
37	Ar_4	Ar_5
38	Ar_4	Ar_2



	R_1	R_2
33	Ar_2	Ar_3
35	Ar_2	Ar_4



Compound **33**, $[\alpha]_D$ +57.6, was assigned as the elemental formula of $C_{28}H_{34}O_{14}$ based on the protonated ion peak $[M + H]^+$ 595.2029 (calcd 595.2027) that appeared in the HRFABMS data. The NMR spectra of **33** indicated the presence of a tetrahydrofuranoketone skeleton with glycosidic linkages like **32**. However, the ^{1}H NMR spectra revealed two pair of *meta*-coupled aromatic protons [δ_H 7.31 (1H, d, J = 1.5 Hz, H-2) and 7.19 (1H, d, J = 1.5 Hz, H-6); δ_H 6.59 (1H, d, J = 1.5 Hz, H-2′) and 6.58 (1H, d, J = 1.5 Hz, H-6′)], the previous one was downfield shifted because of the presence of a carbonyl group δ_C 199.8 (C-7). A methlyenedioxy protons signal [δ_H 6.04 (2H)] and three methoxy groups signal [δ_H 3.92, 3.85, and 3.78 (each 3H, s)] also appeared in the ^{1}H NMR spectra, indicating the presence of a 3-methoxy-4,5-methylendioxyphenyl moiety in its structure. Meticulous investigations of the chemical shifts of methoxy groups revealed that methoxy groups with lack of neighboring aromatic protons would appear at downfield (δ 60-62) in the ^{13}C NMR spectra. Thus, another aromatic moiety was characterized as 3′-hydroxy-4′,5′-dimethoxyphenyl. Coupling constants of H-7′ proton was calculated to be 6.5 Hz, suggesting a *cis* orientation at H-7′/ H-8′, which was confirmed in the NOESY relations where no correlations were observed from H-7′ to H-9′. On the other hand, the oxymethylene protons [δ_H 3.84 (1H, dd, J = 10.0, 5.0 Hz, H-9′) and 3.54 (1H, dd, J = 10.0, 7.0 Hz, H-9′)] were shifted upfield due to the anisotropic effect of the neighboring 3′-hydroxy-4′,5′-dimethoxyphenyl moiety. The ECD spectra displayed positive Cotton effects at 290 ($\Delta\varepsilon$ +3.03) and 330 ($\Delta\varepsilon$ +1.87) nm. Therefore, **33 (**terminaloside R) was identified as (+)-(7′*R*,8*S*,8′*R*)-3′-hydroxy-4,5-methylenedioxy-3,4′,5′-trimethoxy-7′,9-epoxylignan-9′-ol-7-one 9′-*O*-*β*-D-glucopyranoside.

Compound **34** was assigned to have the molecular formula of $C_{29}H_{36}O_{14}$, based on the protonated ion peak $[M + H]^+$ 609.2191 (calcd 609.2183) that appeared in the HRFABMS data, indicating one carbon and two hydrogen atoms more than that of **33**. The NMR spectra of **34** were similar to that of **33**. However, the ^{1}H NMR spectra revealed one singlet signal [δ_H 6.78 (2H, s, H-2′ and 6′) with two equivalent aromatic protons instead of one of the *meta*-coupled aromatic moieties in **33**. In addition to one methoxy signal, the ^{13}C NMR spectra also revealed four aromatic carbons for this ring, suggesting the presence of the 3′,4′,5′-trimethoxyphenyl moiety (Table 13), which was confirmed in the HMBC spectra. Based on the ECD spectra, chemical shift of H-7′ (δ_H 4.81) and the coupling constant ($J_{H\,7'\text{-}8'}$ = 8.5 Hz), compound **34 (**terminaloside S) was identified as (+)-(7′*S*,8*S*,8′*R*)-4,5-methylenedioxy-3,3′,4′,5′-tetramethoxy-7′,9-epoxylignan-9′-ol-7-one 9′-*O*-*β*-D-glucopyranoside.

Compound **35** was assigned the same molecular formula $C_{29}H_{36}O_{14}$ as **34**, based on the protonated ion peak $[M + H]^+$ 609.2196 (calcd 609.2183) that appeared in the HRFABMS data. The NMR spectra were very similar as **34**, indicating the same planar structure. However, coupling constants of H-7′ was calculated to be 6.5 Hz, presumed to be *cis* orientation at H-7′/ H-8′ as like in **33**. The above arrangement was also confirmed by the high field shifted oxymethylene protons [δ_H 3.85 (1H, overlapped, H-9′) and 3.54 (1H, dd, J = 10.0, 6.5 Hz, H-9′)]. Anomeric proton of sugar moiety was also observed high field

shifted [**33**: δ_H 4.04 (1H, d, J = 7.5 Hz, H-1″), **35**: 4.05 (1H, d, J = 7.5 Hz, H-1″)] due to *cis* configuration at H-7′/ H-8′. Therefore, **35** was deduced as stereochemical isomer of **34** at C-7′ position. Based on the ECD data as well as the above evidences, terminaloside T (**35**) was identified as (+)-(7′*R*,8*S*,8′*R*)-4,5-methylenedioxy-3,3′,4′,5′-tetramethoxy-7′,9-epoxylignan-9′-ol-7-one 9′-*O*-*β*-D-glucopyranoside.

Table 12. NMR data for **32** and **33** (^{1}H NMR; 500 MHz and ^{13}C NMR; 125 MHz, δ; ppm, recorded in MeOH-d_4)

Position	**32**		**33**	
	δ_H (J in Hz)	δ_C[a]	δ_H (J in Hz)	δ_C[a]
1		131.7		133.7
2	7.48, d (2.0)	109.2	7.31, d (1.5)	111.3
3		150.0		145.1
4		153.9		141.8
5	6.96, d (8.0)	109.2		150.9
6	7.70, dd (8.0, 2.0)	126.7	7.19, d (1.5)	103.9
7		200.0		199.8
8	4.27, overlapped	50.8	4.34, q (6.5)	48.8
9	4.22, dd (10.0, 8.0)	71.7	4.29, dd (10.5, 8.0)	71.3
	4.18, dd (8.0, 5.0)		4.24, dd (8.0, 6.5)	
1′		138.8		139.2
2′	6.78, s	105.4	6.59, d (1.5)	108.0
3′		154.7		151.7
4′		138.4		137.3
5′		154.7		154.8
6′	6.78, s	105.4	6.58, d (1.5)	103.3
7′	4.81, d (8.5)	85.2	4.90, d (6.5)	84.9
8′	2.74, m	53.0	2.88, quintet (6.5)	53.2
9′	4.06, dd (10.5, 4.5)	69.2	3.84, dd (10.0, 5.0)	68.3
	3.66, overlapped		3.54, dd (10.0, 7.0)	
OCH_2O	6.06, s	103.6	6.04, s	103.9
OMe	3.86, s	61.2	3.92, s	61.1
	3.75, s	56.8	3.85, s	57.5
			3.78, s	56.7
Glc-1″	4.28, d (8.0)	104.9	4.04, d (7.5)	104.5
2″	3.23, m	75.3	2.98, t (7.5)	75.1
3″	3.37, m	78.2	3.14, m	78.0
4″	3.32, m	71.8	3.18, m	71.8
5″	3.22, m	78.3	3.24, m	78.0
6″	3.82, dd (12.0, 2.5)	62.9	3.80, overlapped	62.9
	3.63, dd (12.0, 5.5)		3.60, dd (11.5, 5.5)	

[a,] Assignments were based on HMQC and HMBC experiments

Table 13. NMR data for **34** and **35** (^{1}H-NMR; 500 MHz and ^{13}C NMR; 125 MHz, δ; ppm, recorded in MeOH-d_4)

Position	**34**		**35**	
	δ_H (J in Hz)	δ_C[a]	δ_H (J in Hz)	δ_C[a]
1		132.8		133.7
2	7.35, d (2.0)	111.7	7.32, d (2.0)	111.3
3		145.1		145.1
4		141.8		141.8
5		150.9		150.9
6	7.24, d (2.0)	104.2	7.19, d (2.0)	103.9
7		200.0		199.8
8	4.27, q (6.0)	50.9	4.34, overlapped	48.5
9	4.22, dd (11.0, 8.0)	71.7	4.32, overlapped	71.4
	4.18, dd (8.0, 5.5)		4.26, dd (8.0, 6.5)	
1′		138.9		139.4
2′	6.78, s	105.4	6.75, s	104.7
3′		154.7		154.7
4′		138.3		138.7
5′		154.7		154.7
6′	6.78, s	105.4	6.75, s	104.7
7′	4.81, d (8.5)	85.2	4.96, d (6.5)	85.1
8′	2.75, m	53.1	2.90, quintet (6.5)	53.2
9′	4.07, dd (10.0, 4.5)	69.3	3.85, overlapped	68.4
	3.65, overlapped		3.54, dd (10.0, 6.5)	
OCH_2O	6.06, s	103.9	6.04, s	104.0
OMe	3.95, s	61.2	3.92, s	61.2
	3.86, s	57.6	3.86, s	57.5
	3.76, s	56.8	3.76, s	56.9
Glc-1″	4.29, d (7.5)	105.0	4.05, d (7.5)	104.4
2″	3.21, t (7.5)	75.3	2.98, t (7.5)	75.1
3″	3.34, m	78.2	3.14, m	77.9
4″	3.28, m	71.7	3.17, m	71.8
5″	3.26, m	78.3	3.24, m	78.1
6″	3.82, dd (12.0, 2.0)	62.9	3.80, dd (11.5, 2.0)	62.9
	3.62, overlapped		3.60, dd (11.5, 5.5)	

[a,] Assignments were based on HMQC and HMBC experiments

Compound **36**, was obtained as yellowish white amorphous powder and was assigned the molecular formula as $C_{30}H_{40}O_{14}$, based on the protonated ion peak $[M + H]^+$ 625.2486 (calcd 625.2496) that appeared in the HRFABMS. The NMR data revealed the presence of a tetrahydrofuranoketone ring with glycosidic linkage as same as compound **32**. However, the NMR spectra displayed two individual

singlet aromatic proton signal and each integrated two equivalent protons [δ_H 6.78 (2H, s, H-2′ and 6′), 7.32 (2H, s, H-2 and 6)] along with 8 aromatic carbon resonances (Table 14), suggesting the presence of two 3,4,5-trimethoxyphenyl moieties in its structure. Due to conjugation with a carbonyl group [δ_C 200.7 (C-7)], one of the singlet aromatic proton signals (δ_H 7.32) was downfield shifted. The ^{1}H NMR spectra also showed four methoxy proton signals [δ_H 3.92, 3.86, 3.85, 3.76] in which first two were integrated six proton signals each. Considering the ECD data, chemical shift of H-7′ proton (δ_H 4.85) and the coupling constants ($J_{H\ 7'\text{-}8'}$ = 8.0 Hz), the structure of **36** (terminaloside U) was determined to be (+)-(7′*S*,8*S*,8′*R*)-3,3′,4,4′,5,5′-hexamethoxy-7′,9-epoxylignan-9′-ol-7-one 9′-*O*-*β*-D-glucopyranoside.

Compound **37**, was obtained as a yellowish white amorphous powder and the molecular formula was assigned as $C_{30}H_{41}O_{15}$, based on the protonated ion peak $[M + H]^+$ 641.2462 (calcd 641.2445) that appeared in the HRFABMS, suggesting the addition of one oxygen atom with the structure of **36**. Similar NMR spectra of tetrahydrofuranoketone skeleton, anomeric proton [δ_H 4.35 (1H, d, *J* = 7.5 Hz, H-1″) and a 3,4,5-trimethoxyphenyl moiety were observed in **36**, indicating similar series of compound. However, the NMR spectra showed a singlet aromatic proton signal [δ_H 6.80 (1H, s, H-6′) along with six aromatic carbon resonances (Table 14), possessing a pentasubstituted aromatic moiety in its structure which is unlike others of this series of compound. The ^{1}H NMR spectra displayed six signals of methoxy groups (δ_H 3.91, 3.91 3.84, 3.83, 3.825, 3.82) among which three (δ_H 3.91, 3.91 3.84) were a part of 3,4,5-trimethoxyphenyl moiety. Among others, one methoxy group was appeared in δ_C 57.3, possessing a neighboring aromatic proton. The above arrangement was confirmed in the HMBC and NOESY spectra. Therefore, compound **37** (terminaloside V) was established as (+)-(7′*S*,8*S*,8′*R*)-2′-hydroxy-3,3′,4,4′,5,5′-hexamethoxy-7′,9-epoxylignan-9′-ol-7-one 9′-*O*-*β*-D-glucopyranoside.

Compound **38** was assigned to have the same molecular formula $C_{30}H_{41}O_{15}$ as **34**, based on the sodiated ion peak $[M + Na]^+$ 631.1982 (calcd 631.2002) that appeared in the HRFABMS. Presence of a tetrahydrofuranketone skeleton, a sugar moiety, a 3,4,5-trimethoxybenzoyl and a 3′-methoxy-4′,5′-methylenedioxyphenyl moieties were found similarly as like others. However, the connectivity of two aromatic rings was different from **34**. The singlet and overlapped two equivalent aromatic protons [δ_H 7.32 (2H, s, H-2 and 6)] showed correlations to the conjugated carbonyl group [δ_C 200.7 (C-7)] whereas correlations were observed from a pair of *meta*-coupled protons [δ_H 6.64 (1H, d, *J* = 1.5 Hz, H-2′) and 6.70 (1H, d, *J* = 1.5 Hz, H-6′)] to δ_C 85.0 (C-7′). Based on the ECD data, chemical shift of H-7′ proton (δ_H 4.81) and the coupling constant ($J_{H\ 7'\text{-}8'}$ = 8.0 Hz), the structure of **38** (terminaloside W) was determined to be (+)-(7′*S*,8*S*,8′*R*)-4′,5′-methylenedioxy-3,3′,4,5,-tetramethoxy-7′,9-epoxylignan-9′-ol-7-one 9′-*O*-*β*-D-glucopyranoside.

Table 14. NMR data for **36, 37** and **38** (^{1}H NMR; 500 MHz and ^{13}C NMR; 125 MHz, δ; ppm, recorded in MeOH-d_4)

Position	**36**		**37**		**38**	
	δ_H (J in Hz)	δ_C[a]	δ_H (J in Hz)	δ_C[a]	δ_H (J in Hz)	δ_C[a]
1		133.4		133.5		133.5
2	7.32, s	107.8	7.31, s	107.8	7.32, s	107.8
3		154.7		154.7		154.7
4		144.4		144.2		144.4
5		154.7		154.7		154.7
6	7.32, s	107.8	7.31, s	107.8	7.32, s	107.8
7		200.7		201.2		200.7
8	4.36, q (6.5)	50.7	4.36, overlapped	50.9	4.35, q (6.0)	50.6
9	4.25, overlapped	71.6	4.33, overlapped	71.2	4.24, t (8.5)	71.6
	4.24, overlapped		4.24, t (8.0)		4.20, dd (8.5, 5.5)	
1′		138.3		123.2		136.9
2′	6.78, s	105.4		142.7	6.64, d (1.5)	102.0
3′		154.7		143.4		145.1
4′		138.8		143.6		136.4
5′		154.7		147.7		150.6
6′	6.78, s	105.4	6.80, s	107.8	6.70, d (1.5)	108.4
7′	4.85, d (8.0)	85.1	5.14, d (7.0)	80.0	4.81, d (8.0)	85.0
8′	2.77, m	53.2	2.87, quintet (6.5)	52.8	2.73, m	53.2
9′	4.09, dd (10.0, 4.5)	69.4	4.13, dd (10.0, 4.0)	69.9	4.07, dd (10.0, 4.5)	69.1
	3.65, dd (10.0, 5.5)		3.75, dd (10.0, 6.0)		3.61, overlapped	
OCH_2O					5.91, s	102.7
OMe	3.92, s	61.3	3.91, s	61.6	3.92, s	61.3
	3.86, s	61.2	3.84, s	61.4	3.89, s	57.5
	3.85, s	57.1	3.83, s	61.3	3.85, s	57.1
	3.76, s	56.9	3.825, s	57.3		
			3.82, s	57.1		
Glc-1″	4.29, d (7.5)	105.2	4.35, d (7.5)	105.1	4.29, d (8.0)	105.1
2″	3.21, t (7.5)	75.3	3.15, t (7.5)	75.4	3.20, t (8.0)	75.3
3″	3.37, m	78.2	3.35, m	78.1	3.33, m	78.2
4″	3.26, m	71.8	3.30, m	71.8	3.25, m	71.8
5″	3.25, m	78.3	3.25, m	78.2	3.24, m	78.3
6″	3.82, dd (12.0, 2.0)	62.9	3.85, dd (11.5, 2.0)	62.9	3.82, dd (12.0, 2.0)	62.9
	3.61, overlapped		3.63, dd (11.5, 5.5)		3.61, dd (12.0, 5.5)	

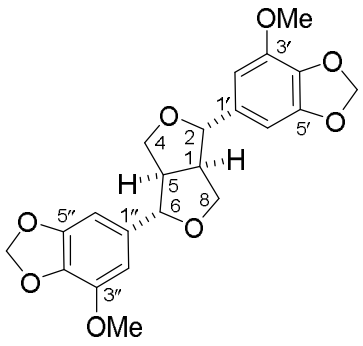
[a,] Assignments were based on HMQC and HMBC experiments

1.4. Identification and structure determination of known compounds

Eleven known compounds were isolated and identified as (+)-excelsin (**2**),[49] (1*R*,5*R*,2*S*,6*S*)-2-(3′,4′-dimethoxyphenyl)-6-(3″-methoxy-4″,5″-methylenedioxyphenyl)-3,7-dioxabicyclo[3.3.0]octane (**3**),[50] sesartemin (**4**),[51, 52] isoorientin (**39**),[53] nicotiflorine (**40**),[53, 54] isorhamnetin-3-*O*-rutinoside (**41**),[55] *threo*-secoisolariciresinol-9′-*O*-*β*-D-glucopyranoside (**42**),[56, 57] *erythro*-secoisolariciresinol-9′-*O*-*β*-D-glucopyranoside (**43**),[58] caprolactam (**44**),[59] *p*-hydroxybenzoic acid (**45**),[60] and blumenol A (**46**),[61] by comparing their spectroscopic and MS data with the reported literatures.

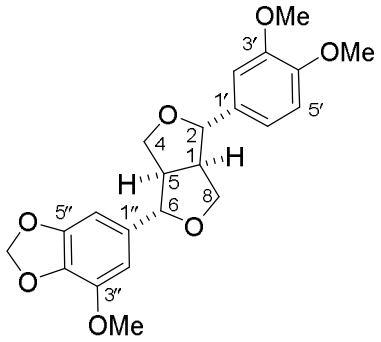
1.4.1. Furofuran lignans

Compound **2** was obtained as colorless amorphous powder and the molecular weight was recognized to be 414 from the MS data. The ^{1}H-NMR spectra showed one oxygenated methine [δ_H 4.68 (1H, d, J = 4.5 Hz, H-2 and 6), a pair of oxygenated methylenes [δ_H 4.24 (2H, dd, J = 9.0, 7.0 Hz, H-4 and 8) and 3.86 (1H, dd, J = 9.0, 4.0 Hz, H-4 and 8)], one methine proton [δ_H 3.07 (2H, m, overlapped, H-1 and 5), suggesting a symmetrically substituted furofuran lignan containing a 2,6-diaryl *cis*-3, 7-dioxabicyclo[3.3.0] octane ring (Table-15).[28] A pair of meta-coupled aromatic protons [δ_H 6.60 (2H, d, J = 1.5 Hz, H-2′ and 2″) and 6.55 (2H, d, J = 1.5 Hz, H-6′ and 6″)], a methoxy group protons [δ_H 3.87 (3H, s)] and a methylenedioxy protons signal [δ_H 5.91 (2H, s)], suggested the presence of 3-methoxy-4,5-methylenedioxyphenyl moiety in its structure. Therefore, after comparing the data with the published literature, **2** was identified as (+)-excelsin.[49] It was first reported from the leaves of *Macropiper excelsum*.



2

Compound **3** was obtained as colorless viscous oil and its molecular weight was determined to be 400 from the MS data. The ^{1}H NMR of **3** showed two oxygenated methines [δ_H 4.71 (1H, d, J = 5.0 Hz, H-2) and 4.73 (1H, d, J = 5.0 Hz, H-6)], two pair of oxygenated methylenes [δ_H 4.26 (1H, dd, J = 9.0, 7.0 Hz, H-4$_a$) and 3.86 (1H, dd, J = 9.0, 4.0 Hz, H-4$_b$); 4.24 (1H, dd, J = 9.0, 7.0 Hz, H-8$_a$) and 3.84 (1H, dd, overlapped, H-8$_b$)], and two methine protons [δ_H 3.10 (1H, overlapped, H-1) and 3.1 (1H, m, overlapped, H-5)]. One of the methine protons (δ_H 3.10, H-1) was found to be coupled with one oxygenated methine proton (δ_H 4.71, H-2) and with a pair of oxygenated methylene protons in the COSY spectrum. Accordingly, another methine proton ([δ_H 3.09 (H-5)] was coupled with a further oxygenated methine [δ_H 4.73, (H-6)] and with a pair of oxygenated methylene protons. The above arrangements are attributed to two partial structures of –CH_2 (O)-CH-CH (O)- corresponding to a furofuran type lignan. [28] The ^{1}H NMR spectra also displayed a pair of *meta*-coupled aromatic protons [δ_H 6.60 (1H, d, J = 1.5 Hz, H-2″) and 6.55 (1H, d, J = 1.5 Hz, H-6″)], a methoxy group protons [δ_H 3.87 (3H, s)] and a methylenedioxy protons signal [δ_H 5.91 (2H, s)], suggesting the presence of 3-methoxy-4,5-methylenedioxyphenyl moiety in its structure. On the other hand, a singlet aromatic signal [δ_H 6.97 (1H, s, H-2′), two aromatic protons overlapping around at δ_H 6.92 along with two other methoxy group protons signal [δ_H 3.82 and 3.81 (each 3H, s)] might come from a 3,4-dimethoxyphenyl moiety as ring A. Thus, the structure of **3** was found as to be (1*R*,5*R*,2*S*,6*S*)-2-(3′,4′-dimethoxyphenyl)-6-(3″-methoxy-4″,5″-methylenedioxyphenyl)-3,7-dioxabicyclo[3.3.0]octane by comparing with the published values. The compound was first synthesized as a methylation product of the naturally occurred methoxypiperitol in *Nectandra turbacensis*. [50]



3

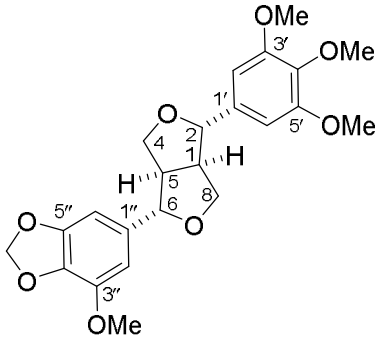
Table 15. NMR data for **2-4** (^{1}H NMR; 500 MHz and ^{13}C NMR; 125 MHz, δ; ppm, recorded in MeOH-d_4)

Position	**2**		**3**		**4**	
	δ_H (J in Hz)	δ_C [a]	δ_H (J in Hz)	δ_C [a]	δ_H (J in Hz)	δ_C [a]
1	3.07, m	55.6	3.10, overlapped	55.4	3.08, overlapped	55.6
2	4.68, d (4.5)	87.3	4.71, d (5.0)	87.2	4.73, d (4.5)	87.3
4_a	4.24, dd (9.0, 7.0)	72.8	4.26, dd (9.0, 7.0)	72.8	4.27, overlapped	72.9 [b]
4_b	3.86, dd (9.0, 4.0)		3.86, dd (9.0, 4.0)		3.90, overlapped	
5	3.07, m	55.6	3.09, overlapped	55.7	3.07, overlapped	55.6
6	4.68, d (4.5)	87.3	4.73, d (5.0)	87.3	4.68, d (4.5)	87.3
8_a	4.24, dd (9.0, 7.0)	72.8	4.24, dd (9.0, 7.0)	72.8	4.25, overlapped	72.8 [b]
8_b	3.86, dd (9.0, 4.0)		3.84, overlapped		3.89, overlapped	
1′		137.4		135.4		138.8
2′	6.60, d (1.5)	107.6	6.97, s	111.4	6.66, s	104.4
3′		145.0		150.8		154.7
4′		136.1		150.3		138.7
5′		150.7	6.92, overlapped	113.1		154.7
6′	6.55, d (1.5)	101.1	6.92, overlapped	119.9	6.66, s	104.4
1″		137.4		137.4		137.4
2″	6.60, d (1.5)	107.6	6.60, d (1.5)	107.6	6.59, d (1.5)	107.5
3″		145.0		145.1		145.0
4″		136.1		136.1		136.0
5″		150.7		150.7		150.6
6″	6.55, d (1.5)	101.1	6.55, d (1.5)	101.1	6.54, d (1.5)	101.1
OCH_2O	5.91, s	102.7	5.91, s	102.6	5.89, s	102.6
OMe	3.87, s	57.4	3.87, s	57.4	3.87, s	61.1
			3.82, s	56.6	3.83, s	57.4
			3.81, s	56.6	3.83, s	56.7
					3.74, s	56.7

[a,] Assignments were based on HMQC and HMBC experiments

[b,] Signals are interchangeable

Compound **4**, $[\alpha]_D$ +52.0, was obtained as yellow oil and was determined molecular weight to be 440 from the MS data in literature. The ^{1}H NMR data showed the characteristic dioxabicyclooctane skeleton signal [δ_C 55.6 (C-1 and 5), 87.3 (C-2 and 6) and 72.9 (C-4 and 8)] in its structure. It also displayed a singlet aromatic proton signal [δ_H 6.66 (2H, s, H-2′ and 6′)] and two equivalent aromatic protons along with 3″-methoxy-4″,5″-methylenedioxyphenyl moiety signals in the ^{1}H NMR spectra. By comparing the published values, the structure of **4** was identified as sesartemin. [51, 52]



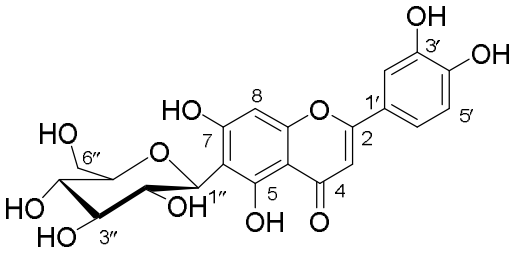
4

1.4.2. Flavonoid glycosides

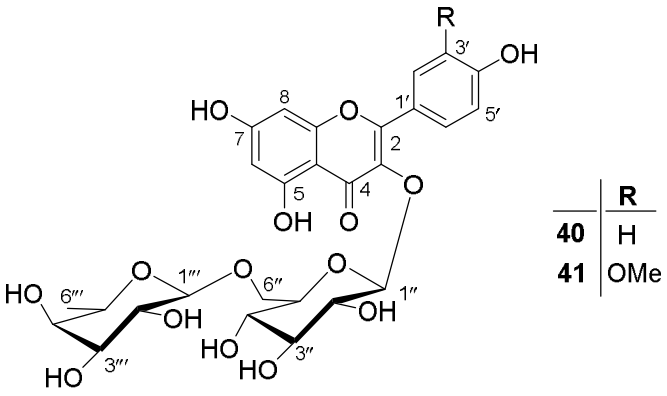
Compound **39** was obtained as yellow amorphous powder. It showed ABX-type aromatic proton signals [δ_H 7.36 (1H, overlapped, H-2′), 6.90 (1H, d, J = 8.0, H-5′) and 7.38 (1H, dd, J = 8.0, 1.5 H-6′)], two singlet aromatic protons at δ_H 6.49 and 6.54 in ^{1}H NMR along with fifteen aromatic carbon resonances, depicting a flavonoid skeleton (luteolin like) structures. The ^{1}H NMR spectra also displayed an anomeric proton [δ_H 4.90 (1H, d, J = 10.0, H-1″) along with 6 oxygenated carbon resonances of a sugar moiety. By comparing these data with published literatures, **39** was identified as isoorientin. [53]

Compounds **40** and **41** were obtained as yellow amorphous powder. Compound **40** showed A_2B_2-type aromatic protons signals [δ_H 8.05 (1H, J = 9.0, H-2′ and 6′), 6.89 (1H, J = 9.0, H-3′ and 5′)], a pair of *meta*-coupled aromatic protons [δ_H 6.21 (1H, J = 2.0, H-6), 6.41 (1H, J = 2.0, H-8)] along with fifteen carbon resonances, suggesting a kaempferol type structures. The ^{1}H NMR spectra also displayed two anomeric protons [δ_H 5.12 (1H, d, J = 7.5, H-1″, Glc-1) and 4.52 (1H, d, J = 1.5, H-1‴, Rha-1)] along with twelve sugar carbon resonances. Their HMBC spectra gave information on the connectivity of

partial structures such as i) δ_H 5.12 (Glc-1) to δ_C 135.5 (C-3) and ii) δ_H 4.52 (Rha-1) to δ_C 68.6 (Glc-6). By comparing these data coupled with published literatures, **40** was identified as kaempferol-3-*O*-rutinoside or nicotiflorine. [53, 54] Compound **41** showed ABX-type aromatic proton signals [δ_H 7.93 (1H, *J* = 1.5, H-2′), 6.92 (1H, d, *J* = 8.0, H-5′) and 7.64 (1H, dd, *J* = 8.0, 1.5, H-6′)] instead of A_2B_2-type protons in **40**. The NMR spectra also showed a methoxy group protons signal [δ_H 3.94 (3H, s)/ δ_C 56.8] suggesting a isorhamnetin skeleton in its structure. Therefore, **41** was identified as isorhamnetin-3-*O*-rutinoside.[55]



39



	R
40	H
41	OMe

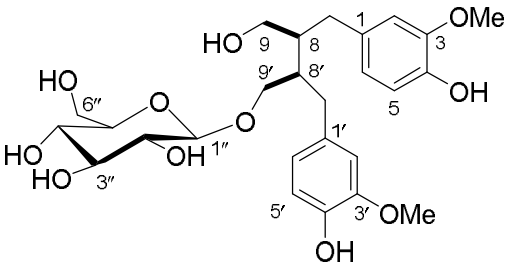
Table 16. NMR data for **39, 40** and **41** (^{1}H NMR; 500 MHz and ^{13}C NMR; 125 MHz, δ; ppm, recorded in MeOH-d_4)

Position	**39**		**40**		**41**	
	δ_H (J in Hz)	δ_C[a]	δ_H (J in Hz)	δ_C[a]	δ_H (J in Hz)	δ_C[a]
2		166.2		161.5		158.9
3	6.54, s	103.9		135.5		135.5
4		184.0		179.5		179.4
5		161.9		163.0		163.0
6		109.1	6.21, d (2.0)	100.0	6.22,d (2.0)	100.0
7		164.8		166.2		166.1
8	6.49, s	95.3	6.41, d (2.0)	95.0	6.41,d (2.0)	95.0
9		158.7		158.6		158.5
10		105.2		105.7		105.7
1′		123.6		122.8		123.1
2′	7.36, overlapped	114.2	8.05, d (9.0)	132.5	7.93, d (1.5)	114.7
3′		147.0	6.89, d (9.0)	116.2		148.4
4′		151.0		159.4		150.9
5′	6.90, d (8.0)	116.8	6.89, d (9.0)	116.2	6.92, d (8.0)	116.1
6′	7.38, dd (8.0, 1.5)	120.3	8.05, d (9.0)	132.5	7.64, dd (8.0, 1.5)	124.1
OMe-3′					3.94, s	56.8
Glc-1″	4.90, d (10.0)	75.4	5.12, d (7.5)	104.6	5.21, d (7.5)	104.5
2″	4.14, m	72.7	3.45, overlapped	75.8	3.44, overlapped	75.9
3″	3.47, m	80.1	3.40, overlapped	78.2	3.39, overlapped	78.2
4″	3.48, m	71.8	3.25, overlapped	71.5	3.24, overlapped	71.7
5″	3.42, m	82.6	3.33, overlapped	77.3	3.34, overlapped	77.4
6″	3.87, dd (12.0, 2.0)	62.9	3.80, dd (12.0, 2.0)	68.6	3.81, dd (11.0, 1.0)	68.6
	3.75, dd (12.0, 5.5)		3.36, overlapped		3.36, overlapped	
Rha-1‴			4.52, d (1.5)	102.4	4.53, d (1.5)	102.5
2‴			3.62, dd (3.5, 1.5)	72.1	3.60, dd (3.5, 1.5)	72.1
3‴			3.51, dd (10.0, 3.5)	72.4	3.50, dd (10.0, 3.5)	72.3
4‴			3.27, m	74.0	3.25, m	73.9
5‴			3.45, m	69.8	3.43, m	69.8
6‴			1.11, d (6.0)	17.9	1.10, d (6.5)	17.9

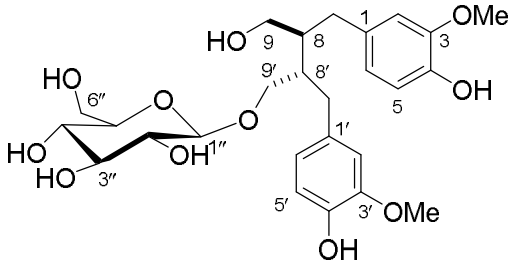
[a] Assignments were based on HMQC and HMBC experiments

1.4.3. lignan glycosides

Compounds **42** and **43** were obtained as colorless amorphous powders and were determined to have a molecular weight 524, based on MS data in literature. Both compounds showed an ABX-type aromatic protons signal [**42**: δ_H 6.63 (1H, d, J = 2.0, H-2 and 2′), 6.67 (1H, d, J = 8.0, H-5 and 5′) and 6.58 (1H, dd, J = 8.0, 2.0, H-6 and 6′); **43**: δ_H 6.62 (1H, d, J = 2.0, H-2), 6.66 (1H, d, J = 8.0, H-5) and 6.57 (1H, dd, J = 8.0, 2.0, H-6)] along with an anomeric proton signal [**42**: δ_H 4.20 (1H, d, J = 7.5, Glc-1‴); **43**: δ_H 4.23 (1H, d, J = 8.0, Glc-1‴)] in its structure. The above spectra were similar with the secoisolariciresinol-9′-*O*-*β*-D-glucopyranoside isolated from various plants. However, compound **43** showed small downfield shifted compared with another ABX-type aromatic proton signals [**43**: δ_H 6.60 (1H, d, J = 2.0, H- 2′), 6.66 (1H, d, J = 8.0, H-5′) and 6.56 (1H, dd, J = 8.0, 2.0, H-6′)], indicating an asymmetrical *E*-configuration in C-8/C-8′ positions. Thus, comparing the NMR spectra with published values, **42** was identified as *threo*-secoisolariciresinol-9′-*O*-*β*-D-glucopyranoside, [56, 57] and **43** was found to be *erythro*-secoisolariciresinol-9′-*O*-*β*-D-glucopyranoside.[58]



42



43

Table 17. NMR data for **42** and **43** (^{1}H NMR; 500 MHz and ^{13}C NMR; 125 MHz, δ; ppm, recorded in MeOH-d_4)

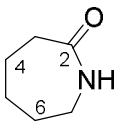
Position	**42**		**43**	
	δ_H (J in Hz)	δ_C [a]	δ_H (J in Hz)	δ_C [a]
1		134.1		134.1 [b]
2	6.63, d (2.0)	113.6	6.62, d (2.0)	113.7
3		148.8		148.8
4		145.5		145.5
5	6.67, d (8.0)	115.8	6.66, d (8.0)	115.8
6	6.58, dd (8.0, 2.0)	122.8	6.57, dd (8.0, 2.0)	122.9
7	2.70, m	35.6	2.67, m	35.6
8	2.10, m	44.1	2.11, m	44.0
9	3.64, dd, (12.0, 6.0)	62.9	3.64, dd, (12.0, 6.0)	62.9
	3.57, dd, (12.0, 4.5)		3.57, dd, (12.0, 4.5)	
1′		134.1		133.9 [b]
2′	6.63, d (2.0)	113.8	6.60, d (2.0)	113.7
3′		148.8		148.8
4′		145.5		145.5
5′	6.67, d (8.0)	115.8	6.66, d (8.0)	115.8
6′	6.58, dd (8.0, 2.0)	122.8	6.56, dd (8.0, 2.0)	122.9
7′	2.63, m	35.6	2.61, m	36.0
8′	2.00, m	41.7	1.96, m	41.5
9′	3.90, dd, (12.0, 6.0)	70.5		71.1
	3.54, dd, (12.0, 4.5)			
OMe		56.4		56.3
Glc-1″	4.20, d (7.5)	104.7	4.23, d (8.0)	104.7
2″	3.43, m	75.3	3.45, m	75.3
3″	3.39, m	78.0	3.41, m	78.0
4″	3.25, m	71.8	3.25, m	71.8
5″	3.37, m	78.3	3.38, m	78.3
6″	3.87, dd (12.0, 2.0)	62.9	3.86, dd (12.0, 2.0)	62.9
	3.68, dd (12.0, 5.0)		3.68, dd (12.0, 5.5)	

[a,] Assignments were based on HMQC and HMBC experiments

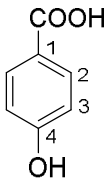
[b,] Signals are interchangeable

1.4.4. Miscellaneous

Compound **44** was obtained as colorless powder. It showed six carbon resonances among which one was appeared at δ_C 176.1, suggesting a carbonyl group in a lactam ring. The ^{1}H NMR spectra showed five signals of methylene protons in the range of δ 1.3-3.1 ppm. By comparing the NMR spectra with published values, **44** was identified as caprolactam.[59] Compound **45** was obtained as crystalline solid and the ^{1}H NMR spectra showed only A_2B_2-type aromatic protons signal [δ_H 7.87 (1H, d, J = 9.0, H- 2 and 6) and δ_H 6.80 (1H, d, J = 9.0, H- 3 and 5). The ^{13}C NMR spectra showed five carbon resonances among which one appeared at δ_C 169.2, suggesting the presence of carboxyl group. Thus, by comparing the NMR spectra with published values, **45** was identified as *p*-hydroxybenzoic acid.[60]



44



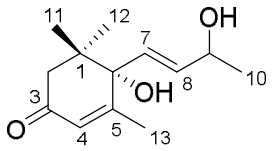
45

Table 18. NMR data for **44** and **45** (^{1}H NMR; 500 MHz and ^{13}C NMR; 125 MHz, δ; ppm, recorded in MeOH-d_4)

Position	**44**		**45**	
	δ_H (J in Hz)	δ_C [a]	δ_H (J in Hz)	δ_C [a]
1				122.9
2		176.1	7.87, d (9.0)	132.9
3	2.19, t (7.0)	37.1	6.80, d (9.0)	116.0
4	1.52, q (7.0)	26.8		163.2
5	1.64, q (7.0)	30.2	6.80, d (9.0)	116.0
6	1.35, m	27.6	7.87, d (9.0)	132.9
7	3.17, t (7.0)	40.3		169.2

[a,] Assignments were based on HMQC and HMBC experiments

Compound **46** was obtained as colorless plates and the molecular formula was found to be 224 in the HRFABMS data in literature. The ^{13}C NMR spectra showed thirteen carbon resonances, which included as four methyls, one methylene, one methine, two quarternary, two pairs of double-bonded SP^2 hybridized carbon along with one carbonyl group, suggesting a dihydroxy enone structure. The above arrangement was also supported by appearing three SP^2- hybridized protons signal [δ_H 5.86 (1H, s, H-4), 5.77 (1H, d, J = 16.0 Hz, H-7) and 5.81(1H, dd, J = 16.0, 6.0 Hz, H-7) in the ^1H NMR spectra. Based on the data in the published literature, **46** was identified as blumenol A. [61]



46

Table 19. NMR data for **46** (^1H NMR; 500 MHz and ^{13}C NMR; 125 MHz, δ; ppm, recorded in MeOH-d_4)

Position	**46**	
	δ_H (J in Hz)	δ_C [a]
1		42.4
2	2.48, d (17.0)	50.8
	2.17, d (17.0)	
3		201.2
4	5.86, br. s	127.1
5		167.4
6		79.9
7	5.77, d (16.0)	137.0
8	5.81, dd (16.0, 6.0)	167.4
9	4.31, q (6.0)	68.6
10	1.23, d (7.0)	24.5
Me-11	1.00, s	23.8
Me-12	1.03, s	23.5
Me-13	1.92, s	19.5

[a,] Assignments were based on HMQC and HMBC experiments

1.5. Summary

Extracts from the leaves of *Terminalia citrina* (Combretaceae) were purified to give forty six compounds, among which thirty five were identified as new constituents and eleven other known constituents were characterized using various spectroscopic techniques. Among the new compounds, nine furofuran lignans (**1**, **5-12**), one furofuranone lignan (**13**), thirteen furofuran ligan glucosides (**14-26**), five furofuranone lignan glucosides (**27-31**), and seven tetrahydrofuran lignan glucosides (**32-38**) were reported. Almost all the lignans and the glycosides had rare tetraoxygenated aryl groups in their partial structures, and ^{1}H and ^{13}C NMR chemical shifts of oxymethine signals in furofuran ring provided a pragmatic approach to evaluate their stereochemistry. However, optical rotation and electronic circular dichroism spectroscopic data were also informative to determining the absolute configuration of furofuran and furofuranone lignan series. The absolute configuration of the sugar moiety was confirmed by acid hydrolysis followed by detection in HPLC compared to authentic samples.

Chapter 2

Chemical constituents of *Pothos scandens* (Araceae)

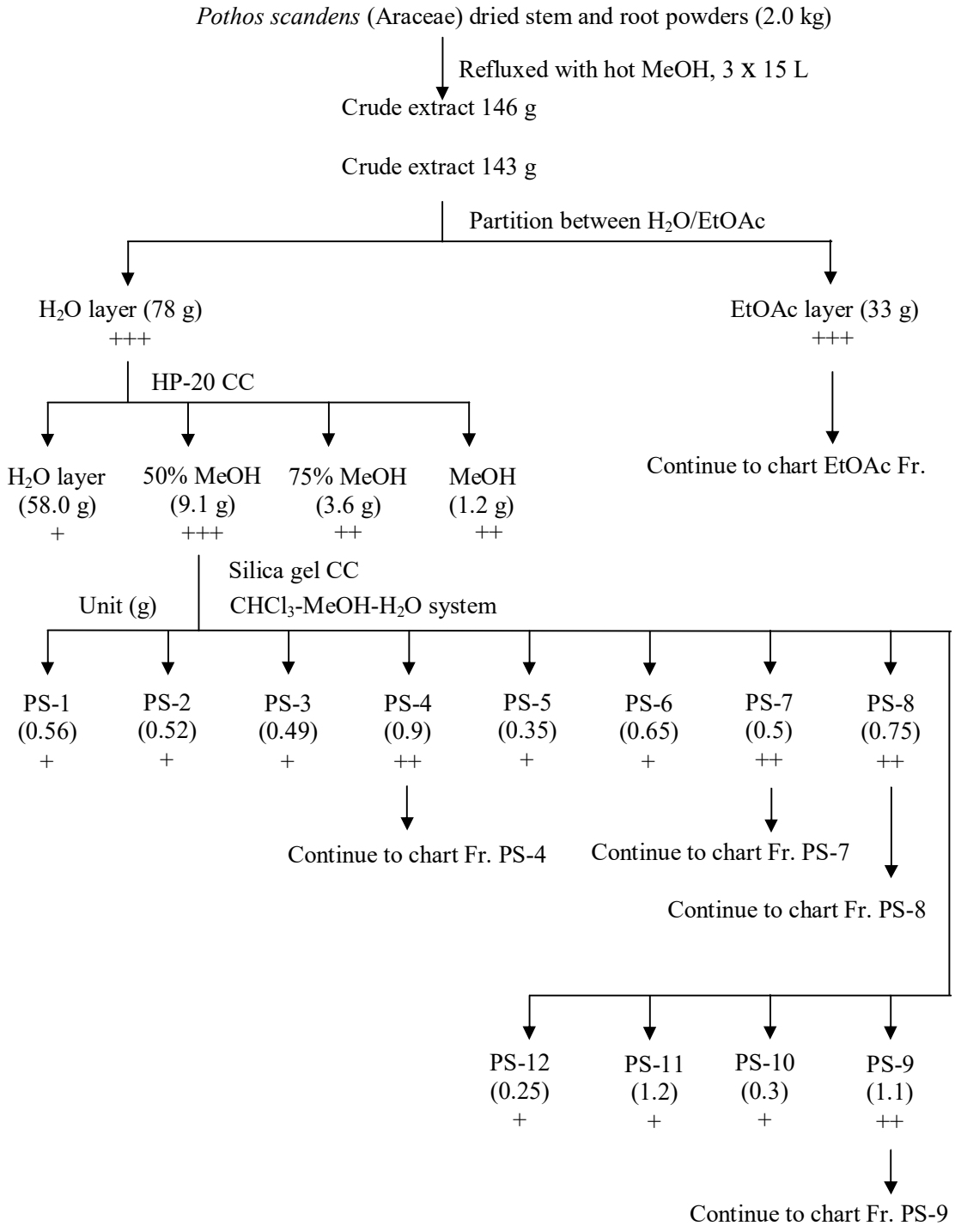
2.1 Introduction

Pothos scandens L. (Araceae) is a medicinal aroid commonly known as 'Batilata' among the tribal peoples of the hill regions of Bangladesh. The whole plant is used for treating skin disorders, asthma, snake bite, diarrhea, cancer, small pox, sprains, epilepsy, convulsions, and wounds.[62-67] The root of the plant is also cut and fried in oil to promote the curing process of abscesses. In certain parts of India, such as Tamil Nadu, leaves of the plant are used to reduce body heat and induce conception.[68] This correlates with a study where a root extract showed potent antipyretic activity.[69] The aqueous ethanolic extract showed dose-dependent inhibition of mast cell-derived immediate type allergic reactions.[70] It significantly decreased the concentration of different inflammatory mediators in an asthmatic mice model.[71] Previous phytochemical studies led to the isolation of dodecanoic acid, tetradecanoic acid, phytol, methyl pothoscandensate, *N-trans*-cinnamoyltyramine, *N-trans*-feruloyltyramine, serotobenine and syringaresinol, among other compounds.[72, 73]

In this study, reported are the isolation, structure elucidation of several new chemical constituent along with other known constituents and evaluated their estrogenic and/or antiestrogenic activity using estrogen responsive breast cancer cell lines (MCF-7, T47D).

2.2 Extraction and Isolation

The air-dried powdered stem and root of the plant (2.0 kg approx.) were extracted four times with hot methanol (3 X 15 L) by refluxing for 3 h each. The extracts were then combined, and the solvent was evaporated at reduced pressure at 45°C to yield a viscous mass (146 g). The crude extracts were then suspended in 1.5 L of water and partitioned with EtOAc (1.5 L x 3). Both the EtOAc soluble fraction and the H_2O soluble fraction suppressed 80% and 40% of the estradiol (E_2)-enhanced proliferation of breast cancer cells, respectively, at a concentration of 0.2 μg/mL. However, the EtOAc soluble fraction was also recognized by its cytotoxicity at higher concentrations. The H_2O soluble fraction was subjected to HP-20 column chromatography by elution with H_2O, H_2O-MeOH (1:1), and MeOH, and the H_2O-MeOH (1:1) derived fraction was applied to repeated silica gel chromatography and high-performance liquid chromatography (HPLC) to yield 28 compounds in total, including four new compounds which were summarized in chart 2.

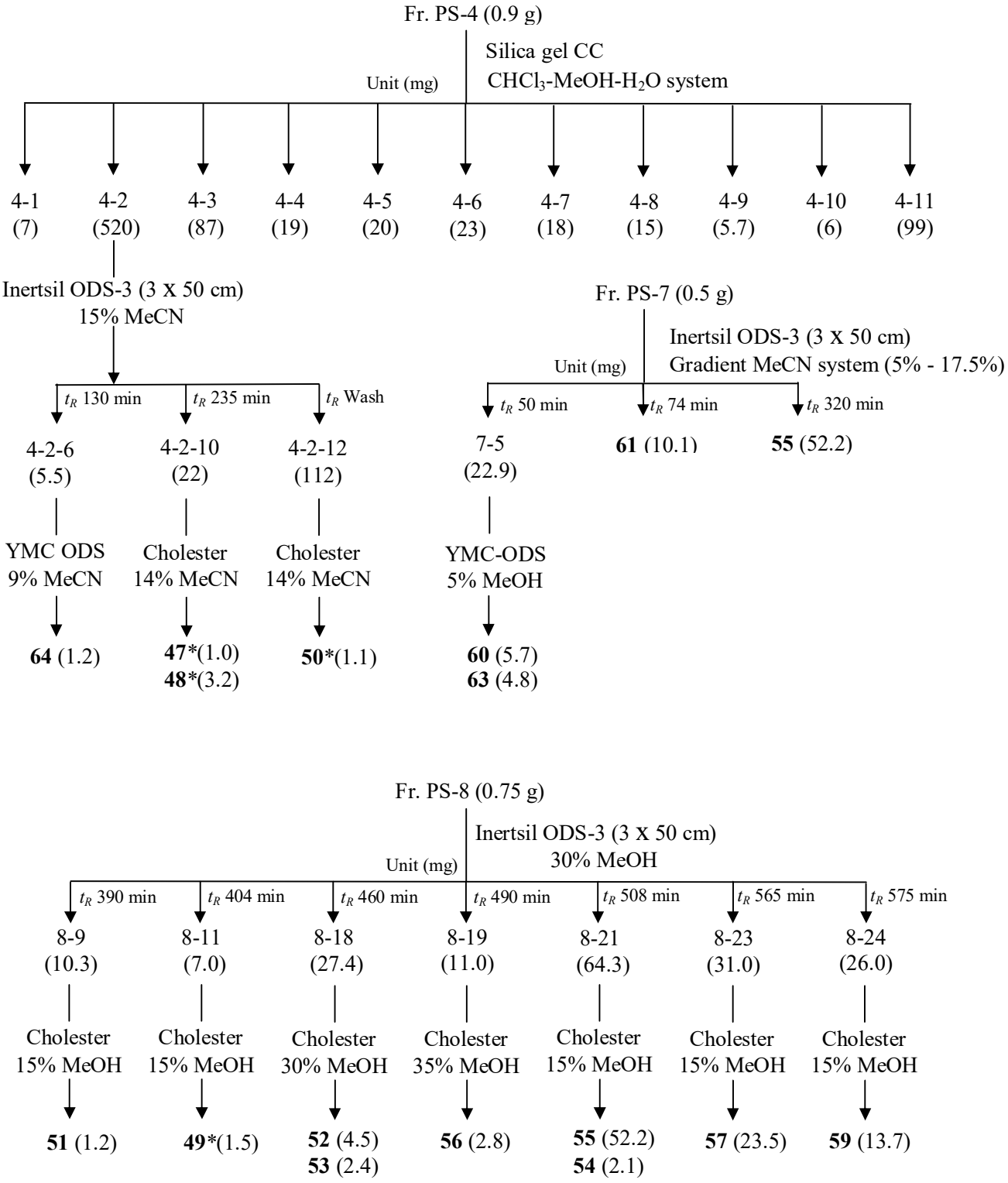


+++ 99% inhibition of Estradiol (E_2)-induced cell proliferation (T47D) at a concentration of <0.2 µg/mL

++ 90% inhibition of Estradiol (E_2)-induced cell proliferation (T47D) at a concentration of <0.2 µg/mL

+ 50% inhibition of Estradiol (E_2)-induced cell proliferation (T47D) at a concentration of <0.2 µg/mL

Chart 2. Extraction and isolation of constituents from *Pothos scandens* (Araceae) continued….



Remarks. * new compound

Chart 2. Extraction and isolation of constituents from *Pothos scandens* (Araceae) continued….

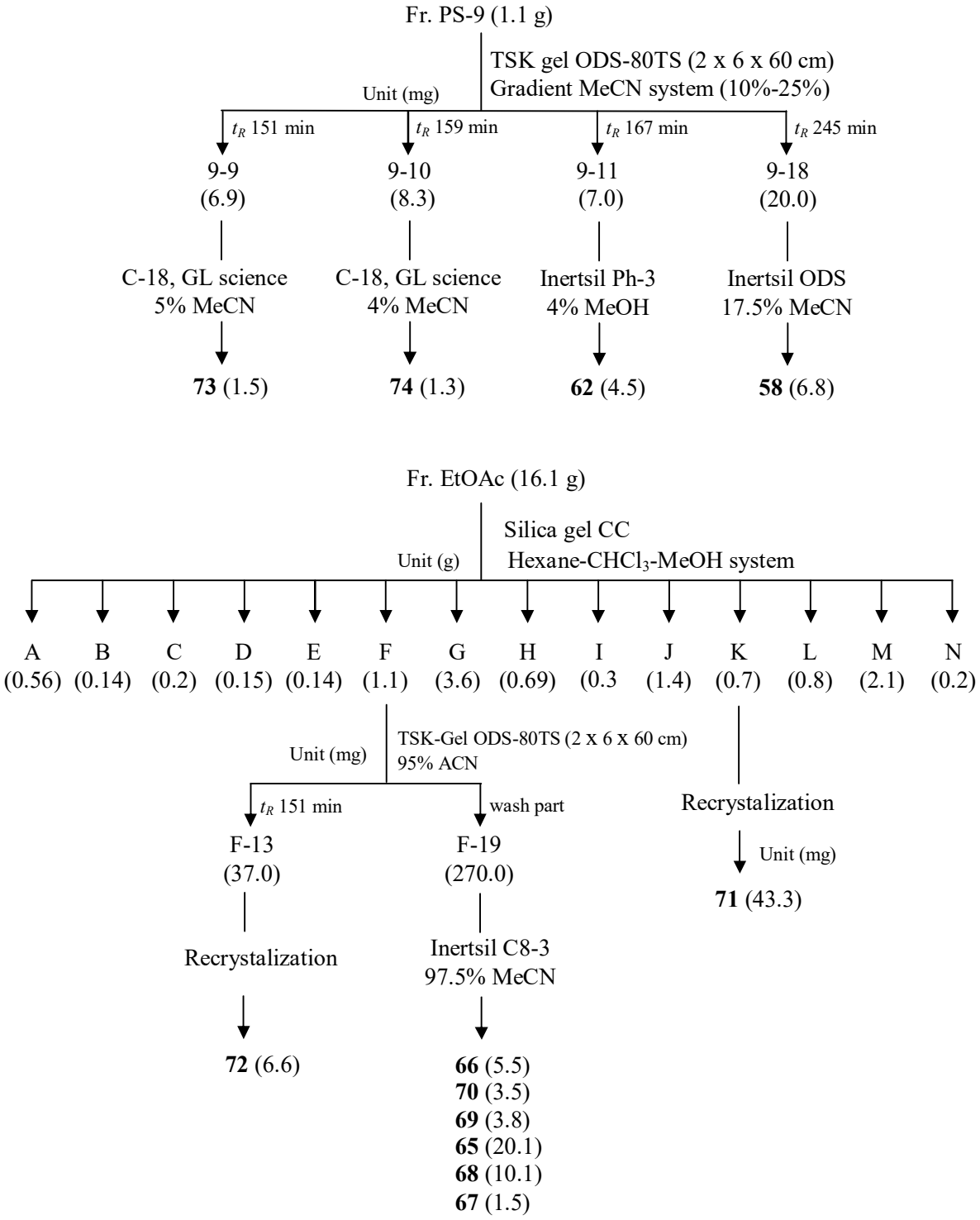


Chart 2. Extraction and isolation of constituents from *Pothos scandens* (Araceae)

2.3. Identification and structure determination of new compounds

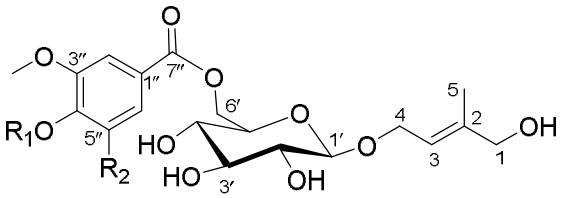
2.3.1. Hemiterpene glucoside aromatic esters

Compound **47**, $[\alpha]^{25}_{D}$ –30.9, was obtained as a colorless amorphous powder, and the molecular formula $C_{19}H_{26}O_{10}$ was assigned from its quasimolecular ion $[M + Na]^+$ peak at *m/z* 437.1451 in the positive HRFABMS. According to the UV spectrum, the presence of an aromatic ring was apparent from the absorption band at 259 nm. The ^{1}H NMR spectrum showed characteristic signals of oxygenated methylenes [δ_H 4.30, 4.26 (each 1H, dd, J = 12.0, 7.0 Hz, H-4) and 3.92 (2H, br s, H-1)], a vinyl methyl group [δ_H 1.60 (3H, br. s, H-5)] and an olefinic proton [δ_H 5.60 (1H, tq, J = 7.0, 1.5 Hz, H-3)]. These signals indicated a partial structure of (2*E*)-methyl-but-2-ene-1,4-diol. The chemical shifts of the vinyl methyl group (δ_H 1.60) and the oxygenated singlet methylene signal (δ_H 3.92) were informative to identify the geometric isomerism and were coincident with reported values in published data.[74] The ^{1}H NMR spectrum also showed an anomeric proton signal at δ_H 4.33 (1H, d, J = 7.5 Hz, H-1′) and the presence of a tri-substituted aromatic ring system from signals at δ_H 6.84 (1H, d, J = 8.5 Hz, H-5″), 7.57 (1H, d, J = 2.0, H-2″) and 7.59 (1H, dd, J = 8.5, 2.0 Hz, H-6″). The ^{13}C NMR spectrum supported the presence of an aromatic ring system, an ester carbonyl, and a glucose in addition to the (2*E*)-methyl-but-2-ene-1,4-diol. In the difference NOE spectrum of **47**, an NOE was observed for H-2" (δ_H 7.57) by irradiation at δ_H 3.89 (OCH_3-3"), which indicated a 3-methoxy-4-hydroxyphenyl moiety. The HMBC spectrum indicated that the carbonyl group is attached to C-1" from the correlations across three bonds from both H-6" and the oxymethylene proton signals [δ_H 4.42 (1H, dd, J = 12, 7), 4.65 (1H, dd, J = 12.0, 3.0)] of the glucose to C-7" (δ_C 168.0). Oxymethylene protons [δ_H 4.26 (1H, dd, J = 12.0, 7.0), 4.30 (1H, dd, J = 12.0, 7.0)] of (2*E*)-methyl-but-2-ene-1,4-diol also exhibited long-range connectivity with C-1′ (δ_C 102.8) of the glucopyranose unit. Meanwhile, another oxymethylene signal [δ_H 3.92 (2H, br.s)] showed a correlation with the vinyl methyl group (δ_C 13.8). Accordingly, the structure of **47** was confirmed as (2*E*)-1-hydroxy-2-methyl-but-2-ene-[6'-(3"-methoxy-4"-hydroxybenzoyl)]-4-*O*-*β*-D-glucopyranoside and was named as pothobanoside A (**47**).

Compound **48**, $[\alpha]^{25}_{D}$ –25.0, and compound **49**, $[\alpha]^{25}_{D}$ –22.3, were isolated as colorless amorphous powders and were assigned the molecular formulae $C_{20}H_{28}O_{11}$ and $C_{26}H_{38}O_{16}$, respectively, as determined from their molecular ion peaks at *m/z* 444.1626 $[M]^+$ and 629.2053 $[M+Na]^+$ in the HRFABMS, respectively. The spectroscopic features of these compounds were very similar to one another and shared many features with those of pothobanoside A (**47**). The ^{1}H NMR spectrum of **48** showed two oxymethylene proton signals [δ_H 4.26 (dd, J = 12.0, 5.0 Hz), 4.30 (dd, J = 12.0, 4.0 Hz), and 3.90 (2H, br. s)], an olefinic proton signal [δ_H 5.60 (tq, J = 5.0, 1.5 Hz)] and a vinyl methyl group [δ_H 1.59 (3H, br. s)] of the (2*E*)-methyl-but-2-ene-1,4-diol moiety, aromatic proton signals from a pyrogallol moiety [δ_H 7.35 (2H, s)], oxymethyl proton resonances [δ_H 3.89 (6H, s)], and an anomeric proton signal

[δ_H4.33 (d, J = 8.0 Hz)]. Analysis of the ^{13}C NMR spectrum indicated the presence of an ester carbon (δ_C 168.0). The HMBC spectrum showed connectivities of four partial structures, namely, (i) δ_H 4.26, 4.30 (H-4) to δ_C 102.9 (C-1'); (ii) δ_H 4.42, 4.64 (H-6') to δ_C 168.0 (C-7"); (iii) δ_H 7.35 (H-2′,6') to δ_C 168.0 (C-7"); and (iv) δ_H 3.89 (O-CH_3) to δ_C 149.0 (C-3",5"). On the basis of the above spectroscopic evidence, pothobanoside B (**48**) was determined to be a new compound with the structure (2*E*)-1-hydroxy-2-methyl-but-2-ene-[6'-(3",5"-dimethoxy-4"-hydroxybenzoyl)]-4-*O*-*β*-D-glucopyranoside. In the ^{1}H-NMR spectrum of **49**, a second anomeric proton signal [δ_H 5.08 (d, J = 8.0 Hz)] was additionally observed, and the ^{13}C NMR spectrum suggested the presence of one more glucose unit in the structure. The HMBC spectrum of **49** indicated the bonding position of the sugar with the syringoyl moiety from the H-C long range connectivity of H-1'" [δ_H 5.08 (d, J = 8.0 Hz)] to C-4" (δ_C 140.5). On the basis of this spectroscopic evidence, pothobanoside C (**49**) was determined to be (2*E*)-1-hydroxy-2-methyl-but-2-ene-[6'-(3",5"-dimethoxy-4"-*O*-β-D-glucopyrano-benzoyl)]-4-*O*-*β*-D-glucopyranoside.

(2*E*)-Methyl-but-2-ene-1,4-diol (MBDO) is known to be biosynthesized via the 1-deoxy-D-xylulose 5-phosphate (DXP) pathway to isoprenoids.[75] In the metabolomic analysis of *Arabidopsis*, its glycosides were identified in nitrate-deficient conditions, and also their levels were induced after conversion of exogenously fed DXP.[76] This report suggested that esterification can occur with novel isolates (**47**-**49**) after MBDO glycoside formation.



	R_1	R_2
47	H	H
48	H	OMe
49	glc	OMe

Table 20. NMR data for **47-49** (^{1}H NMR; 400 MHz and ^{13}C NMR; 100 MHz, δ; ppm, recorded in MeOH-d_4)

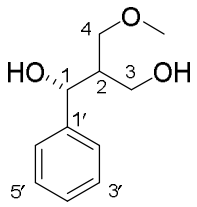
Position	**47**		**48**		**49**	
	δ_H (J in Hz)	δ_C [a]	δ_H (J in Hz)	δ_C [a]	δ_H (J in Hz)	δ_C [a]
1	3.92, br. s	68.0	3.90, br. s	68.0	3.90, br. s	68.0
2		141.3		141.3		141.3
3	5.60, tq (7.0, 1.5)	121.5	5.60, tq (5.0, 1.5)	121.4	5.63, tq (5.0, 1.5)	121.4
4	4.30, dd (12.0, 7.0)	65.9	4.30, dd (12.0, 4.0)	65.9	4.30, dd (12.0, 5.0)	65.9
	4.26, dd (12.0, 7.0)		4.26, dd (12.0, 5.0)		4.26, dd (12.0, 5.0)	
5	1.60, s	13.8	1.59, s	13.8	1.60, s	13.8
Glc-1′	4.33, d (7.5)	102.8	4.35, d (8.0)	102.8	4.35, d (7.5)	102.9
2′	3.22, t (7.5)	75.1	3.20, t (8.0)	75.1	3.22, overlapped	75.1
3′	3.39, overlapped	78.1	3.40, overlapped	78.1	3.42, overlapped	78.1
4′	3.37, overlapped	72.1	3.38, overlapped	72.1	3.39, overlapped	72.1
5′	3.57, overlapped	75.6	3.62, overlapped	75.6	3.62, overlapped	75.6
6′	4.65, dd (12.0, 3.0)	65.1	4.64, dd (12.0, 3.0)	65.2	4.67, dd (11.0, 3.0)	65.2
	4.42, dd (12.0, 7.0)		4.42, dd (12.0, 6.0)		4.48, dd (11.0, 6.0)	
1″		122.6		121.4		127.2
2″	7.57, d (2.0)	116.0	7.35, s	108.5	7.39, s	108.8
3″		148.8		149.0		154.2
4″		153.1		142.3		140.5
5″	6.84, d (8.5)	113.9	7.35, s	149.0	7.39, s	154.2
6″	7.59, dd (8.5, 2.0)	125.2		108.5		108.8
7″		168.0		168.0		167.4
Glc-1‴					5.08, d (8.0)	104.5
2‴					3.52, overlapped	75.8
3‴					3.28, overlapped	78.5
4‴					3.45, overlapped	71.4
5‴					3.41, overlapped	77.9
6‴					3.77, dd (12.5, 2.0)	62.6
					3.66, dd (12.5, 5.0)	
OMe	3.89, s	56.5	3.89, s	57.0	3.89, s	57.2

[a,] Assignments were based on HMQC and HMBC experiments

2.3.2. Phenylisobutanoid

Compound **50** was assigned to have a molecular formula of $C_{11}H_{16}O_3$, as determined from its molecular ion $[M + H]^+$ peak at *m/z* 197.1168 in the HRFABMS. The ^{1}H NMR spectra indicated a mono-substituted aromatic ring system [δ_H 7.31 (2H, dd, J = 8.0, 2.0 Hz, H-2',6'), 7.33 (2H, t, J = 8.0 Hz, H-3',5'), and 7.25 (1H, tt, J = 8.0, 2.0 Hz, H-4')], an oxymethyl group [δ_H 3.23 (3H, s)], and a highly

oxygenated aliphatic moiety. The carbinol proton signal [δ_H 4.78 (1H, d, J = 7.0 Hz)] showed a HMQC correlation with C-1 [δ_C 74.3], where the aromatic ring protons [δ_H 7.31 (2H, dd, J = 8.0, 2.0 Hz, H-2', 6')] also showed a HMBC correlation. Two sets of oxymethylene signals [δ_H 3.75 (2H, d, J = 5.0 Hz, H-3′), 3.28 (1H, dd, J = 9.0, 5.0 Hz, H-4′$_a$), and 3.20 (2H, dd, J = 9.0, 6.0 Hz, H-4′$_b$)] were recognized from their correlations with a methine proton [δ_H 2.00 (1H, m)] in the COSY spectrum, which also correlated with the H-1 carbinol proton. An oxymethyl proton signal at δ_H 3.23 was observed from its long-range coupling with the C-4′ oxymethylene carbon (δ_C 72.0) in the HMBC spectra. Relative configurations of two chiral centers were deduced from the vicinal coupling constant $J_{H\text{-}1,2}$ value of H-1 (d, J = 7.0 Hz) to be *threo* or 1*R**, 2*S**.[77] In the HPLC analysis of **50** using a chiral column, two peaks with equal intensity were recognized at t_Rs 17 and 19 minutes. The chromatogram suggested **50** as being a racemic mixture; however, they were not separated because of small amount of sample. Nevertheless, the structure was confirmed as *threo*-(1-phenyl-2-methoxymethyl)- propane 1,3- diol and named as pothobanol (**50**).



50

Table 21. NMR data for **50** (^{1}H NMR; 400 MHz and ^{13}C NMR; 100 MHz, δ; ppm, recorded in MeOH-d_4)

Position	**50**	
	δ_H (J in Hz)	δ_C[a]
1	4.78, d (7.0)	74.3
2	2.00, m	49.5
3	3.75, d (5.0)	61.4
4	3.28, dd (9.0, 5.0) 3.20, dd (9.0, 6.0)	72.0
1′		144.9
2′	7.31, dd (8.0, 2.0)	127.5
3′	7.33, t (8.0)	129.2
4′	7.25, dd (8.0, 2.0)	128.3
5′	7.33, t (8.0)	129.2
6′	7.31, dd (8.0, 2.0)	127.5
OMe	3.23, s	59.1

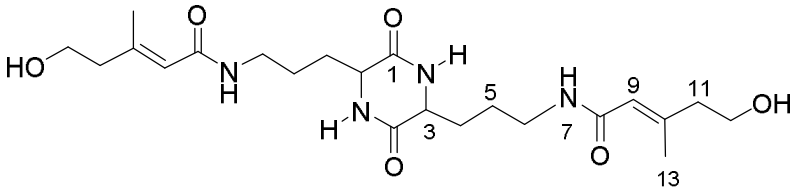
[a,] Assignments were based on HMQC and HMBC experiments

2.4. Identification and structure determination of known compounds

Twenty four known compounds containing a variety of structural skeletons were isolated from *P. scandens* and they were identified as eleutherazine B (**51**),[78] isoschaftoside (**52**), [79] vicenin-2 (**53**), [80] neoschaftoside (**54**), [81] vitexin 2″-*O*-xyloside (**55**), [82] scoparin 2″-*O*-xyloside (**56**), [82] kaempferol-3-*O*-gentiobioside (**57**), [83] quercetin-3-*O*-gentiobioside (**58**), [84] isorhamnetin-3-*O*-gentiobioside (**59**), [85] canthoside B (**60**), [86] zizybeoside I (**61**), [87] (3*S*)1,2,3,4-tetrahydro-3-carboxy-2-carboline (**62**), [88] markhamioside F (**63**), [89] canthoside A (**64**), [86] stigmast-4-en-3-one (**65**), [90, 91] stigmast-4, 22-dien-3-one (**66**), [91, 92] 24-methylenecycloartanol (**67**), [93] 24-methylenecycloartenone (**68**), [94, 95] 24-en-cycloartenone (**69**), [96] 24-methylenecycloartanyl ferulate (**70**), [97] *β*-sitosterol glucoside (**71**), [98] tetradecanoic acid (**72**), [99] L-phenyl alanine (**73**) [100] and L-tryptophan (**74**), [100] by comparing their spectroscopic and MS data with the reported literatures.

2.4.1. Diketopiperazine

Compound **51**, was obtained as a colorless amorphous powder. The ^{13}C NMR spectra of **51** showed eleven carbon resonances that has a completely symmetrical system. The NMR spectra of **51** revealed the typical ^{13}C chemical shifts of two –CONH groups (δ_C 167.8, 165.9) and ^{1}H NMR shift protons of the two α-methine residues (δ_H 3.79), suggesting the presence of a diketopiperazine unit. The presence of two spin coupling units, namely –CONH-CH-CH_2-CH_2-CH_2-NHCO- and –CH_2-CH_2-OH were confirmed through ^{1}H-^{1}H-COSY spectra. Thus, **51** was identified as eleutherazine B by comparing with published values.[78]



51

Table 22. NMR data for **51** (^{1}H NMR; 400 MHz and ^{13}C NMR; 100 MHz, δ; ppm, recorded in DMSO-d_6)

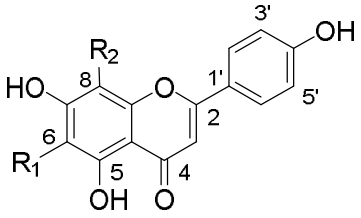
Position	**51**	
	δ_H (J in Hz)	δ_C [a]
1		167.8
2	8.06, d (1.0)	
3	3.79, br. t	53.8
4	1.59, m	30.8
5	1.44, m	24.8
6	3.03, m	37.9
7	7.69, t (6.0)	
8		165.9
9	5.61, s	119.8
10		149.1
11	2.16, t (7.0)	43.5
12	3.50, q (7.5)	59.1
13	2.05, s	17.8

[a,] Assignments were based on HMQC and HMBC experiments

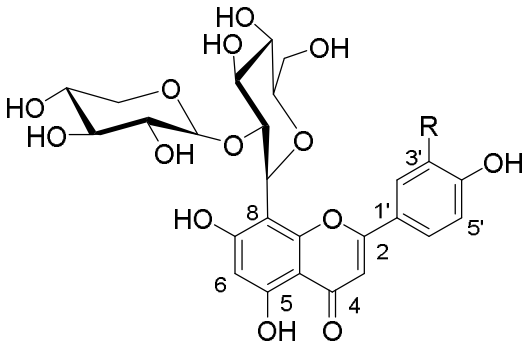
2.4.2. Flavone *C*-glycosides

Compound **52-54** were suggested to be a diglycoside of apigenin based on the ^{1}H-NMR spectra [**52**: δ_H 6.61 (1H, s, H-3), 6.93 (2H, d, J = 8.0 Hz, H-3′ and 5′) and 7.97 (2H, d, J = 8.0 Hz, H-2′ and 6′); **53**: δ_H 6.64 (1H, s, H-3), 6.95 (2H, d, J = 8.5 Hz, H-3′ and 5′) and 7.99 (2H, d, J = 8.5 Hz, H-2′ and 6′); **54**: δ_H 6.62 (1H, s, H-3), 6.93 (2H, d, J = 8.5 Hz, H-3′ and 5′) and 7.95 (2H, d, J = 8.5 Hz, H-2′ and 6′)] and two anomeric protons [**52**: δ_H 5.03 (1H, d, J = 7.5 Hz, Glc-1″) and 4.87 (1H, d, J = 8.0 Hz, Ara-1‴); **53**: δ_H 5.02 (1H, d, J = 8.0 Hz,, Glc-1″) and 4.88 (1H, d, J = 8.0 Hz, Glc-1‴); **54**: δ_H 4.89 (1H, d, J = 8.0 Hz, Glc-1″) and 5.70 (1H, br. s, Ara-1‴)] (Table 23). The ^{13}C NMR as well as COSY spectrum strongly suggested the presence of glucopyranose and arabinopyranose units that were directly linked through *C*-atom to the apigenin moiety. The differences were observed in the connectivity of these sugar moieties to the apigenin center, which were confirmed by HMBC spectra [**52**: δ_H 5.03 (Glc-1″) to δ_C 105.8 (C-8) and 4.87 (Ara-1‴) to δ_C 108.4 (C-6); **54**: δ_H 4.89 (Glc-1″) to δ_C 109.7 (C-6) and 5.7 (Ara-1‴) to δ_C 103.2 (C-8)]. Thus, the NMR data of compound **52** was found to be apigenin-6-*C*-*α*-L-arabinopyranosyl-8-*C*-*β*-D-glucopyranoside (isoschaftoside), **53** was found to be apigenin-6,8-di-*C*-*β*-D-glucopyranoside (vicenin-2) and **54** was apigenin-6-*C*-*β*-D-glucopyranosyl-8-*C*-*β*-L-arabinopyranoside (neoschaftoside) by comparing with the published values in literatures.[79- 81]

Compound **55** was obtained as a yellow amorphous powder and showed the characteristic signal of apigenin skeleton, whereas **56** displayed an ABX-type aromatic protons signal [δ_H 7.39 (1H, d. J = 1.5 Hz, H-2′), 6.94 (1H, d, J = 8.0 Hz, H-5′) and 7.55 (1H, dd, J = 8.0, 1.5 Hz, H-6′)] instead of A_2B_2-type aromatic protons signal of apigenin center in the ^{1}H NMR spectra (Table 24). Both compounds showed two anomeric protons signal [**55**: δ_H 5.02 (1H, d, J = 8.5 Hz, Glc-1″) and 4.15 (1H, d, J = 8.0 Hz, Xyl-1‴); **56**: δ_H 5.03 (1H, d. J = 8.0 Hz, Glc-1″) and 4.13 (1H, d, J = 8.0 Hz, Xyl-1‴)]. The HMBC spectra showed connectivity of xylose unit to the C-2″ position of the glucose unit. The attachment of the two sugar moieties were supposed to be *C*-glycosylation at C-8 positions because of observing H-6 proton signal in the ^{1}H NMR [**55**: δ_H 6.24 (1H, s, H-6); **56**: δ_H 6.23 (1H, s, H-6)]. The presence of a methoxy group was found in **56** (δ_H 3.96, s). Taking all these spectral data into consideration, **55** was found similar with vitexin-2″-*O*-xyloside and **56** was identified as scoparin-2″-*O*-xyloside.[82]



	R_1	R_2
52	ara	glc
53	glc	glc
54	glc	ara



	R
55	H
56	OMe

Table 23. NMR data for **52-54** (^{1}H NMR; 400 MHz and ^{13}C NMR; 100 MHz, δ; ppm, recorded in MeOH-d_4)

Position	**52**		**53**		**54**	
	δ_H (J in Hz)	δ_C [a]	δ_H (J in Hz)	δ_C [a]	δ_H (J in Hz)	δ_C [a]
2		166.7		166.5		165.7
3	6.61, s	103.9	6.64, s	103.8	6.62, s	103.6
4		184.3		184.2		184.2
5		160.4		161.8		161.8
6		108.4		108.5		109.7
7		162.8		167.7		164.9
8		105.8		107.0		103.2
9		157.5		154.6		154.8
10		105.6		104.5		104.9
1′		123.5		123.5		122.9
2′	7.97, d (8.0)	130.2	7.99, d (8.5)	130.2	7.95, d (8.5)	129.7
3′	6.93, d (8.0)	117.0	6.95, d (8.5)	117.1	6.93, d (8.5)	117.2
4′		162.8		162.8		162.9
5′	6.93, d (8.0)	117.0	6.95, d (8.5)	117.1	6.93, d (8.5)	117.2
6′	7.97, d (8.0)	130.2	7.99, d (8.5)	130.2	7.95, d (8.5)	129.7
1″	5.03, d (7.5)	75.1	5.02, d (8.0)	75.1	4.89, d (8.0)	74.8
2″	4.09, m	73.2	4.04, m	73.1	4.12, m	72.1
3″	3.53, m	80.3	3.43, m	80.2	3.40, m	80.4
4″	3.64, m	72.5	3.54, m	72.4	3.61, m	71.9
5″	3.48, m	83.0	3.49, m	82.9	3.36, m	82.6
6″	3.95, dd (11.0, 2.0)	63.1	3.93, dd (12.0, 1.5)	63.0	3.95, dd (11.0, 1.5)	63.2
	3.77, dd (11.0, 5.0)		3.75, dd (12.0, 5.0)		3.77, dd (11.0, 4.0)	
1‴	4.87, d (8.0)	76.6	4.88, d (8.0)	75.8	5.70, br. s	74.0
2‴	4.02, m	75.3	4.00, m	73.4	3.83, m	74.2
3‴	3.75, m	72.0	3.40, m	80.4	3.90, m	71.3
4‴	3.98, m	71.3	3.50, m	72.6	4.10, m	64.8
5‴	4.04, dd (12.0, 2.5)	70.4	3.37, m	83.0	3.71, overlapped	68.4
	3.65, overlapped				3.62, overlapped	
6‴			3.90, dd (12.0, 1.5)	62.9		
			3.70, dd (12.0, 5.0)			

[a,] Assignments were based on HMQC and HMBC experiments

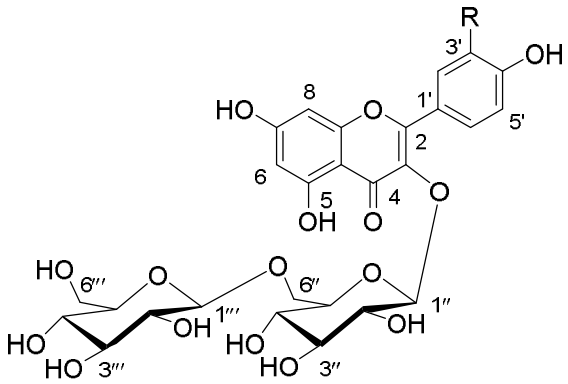
Table 24. NMR data for **55** and **56** (^{1}H NMR; 400 MHz and ^{13}C NMR; 100 MHz, δ; ppm, recorded in MeOH-d_4)

Position	**55**		**56**	
	δ_H (J in Hz)	δ_C[a]	δ_H (J in Hz)	δ_C[a]
2		166.5		166.6
3	6.61, s	103.6	6.54, s	103.7
4		184.3		181.9
5		160.3		162.6
6	6.24, s	99.3	6.23, s	99.3
7		162.6		164.9
8		107.0		107.0
9		158.7		158.5
10		104.5		104.3
1′		123.6		120.9
2′	7.98, d (8.0)	130.0	7.39, d (1.5)	114.9
3′	6.96, d (8.0)	117.1		150.9
4′		162.7		147.0
5′	6.96, d (8.0)	117.1	6.94, d (8.0)	116.9
6′	7.98, d (8.0)	130.0	7.55, dd (8.0, 1.5)	124.1
1″	5.02, d (8.5)	73.7	5.03, d (8.5)	73.7
2″	4.13, m	82.9	4.10, m	82.9
3″	3.52, m	80.2	3.56, m	80.3
4″	3.68, m	72.0	3.62, m	72.1
5″	3.48, m	77.7	3.43, m	77.4
6″	3.92, overlapped	62.9	3.90, overlapped	63.1
	3.75, overlapped		3.78, overlapped	
1‴	4.15, d (8.0)	106.3	4.13, d (8.0)	106.3
2‴	3.80, m	75.2	3.75, m	75.2
3‴	2.97, m	75.8	2.98, m	77.4
4‴	4.20, m	70.9	4.15, m	71.0
5‴	3.96, overlapped	66.6	3.92, overlapped	66.7
	3.68, overlapped		3.66, overlapped	
OMe			3.96, s	56.9

[a,] Assignments were based on HMQC and HMBC experiments

2.4.3. Flavonol di-*O*-glycosides

Compounds **57-59** were obtained as yellow amorphous powders and were recognized as flavonol from their NMR data. All of them commonly displayed meta-coupled aromatic proton [**57**: δ_H 6.21 (1H, d, J = 2.0 Hz, H-6), 6.41 (1H, d, J = 2.0 Hz, H-8); **58**: δ_H 6.21 (1H, d, J = 1.5 Hz, H-6), 6.41 (1H, d, J = 1.5 Hz, H-8); **59**: δ_H 6.21 (1H, d, J = 1.5 Hz, H-6), 6.42 (1H, d, J = 1.5 Hz, H-8)]. A set of ABX-type aromatic protons [**58**: δ_H 7.70 (1H, d, J = 2.0 Hz, H-2′), 6.87 (1H, d, J = 8.0 Hz, H-5′) and 7.66 (1H, dd, J = 8.0, 2.0 Hz, H-6′); **59**: δ_H 8.01 (1H, d, J = 2.0 Hz, H-2′), 6.90 (1H, d, J = 8.5 Hz, H-5′) and 7.63 (1H, dd, J = 8.5, 2.0 Hz, H-6′)] were observed in both **58** and **59,** whereas a A_2B_2-type aromatic protons [**57**: δ_H 6.90 (2H, d, J = 8.0 Hz, H-3′ and 5′) and 8.10 (2H, d, J = 8.0 Hz, H-2′ and 6′)] were observed in **57**. All of the compounds showed two anomeric protons [**57**: δ_H 5.24 (1H, d. J = 7.5 Hz, Glc-1″) and 4.15 (1H, d, J = 7.5 Hz, Glc-1‴); **58**: δ_H 5.23 (1H, d, J = 7.5 Hz, Glc-1″) and 4.16 (1H, d, J = 7.5 Hz, Glc-1‴) **59**: δ_H 5.36 (1H, d, J = 7.5 Hz, Glc-1″) and 4.16 (1H, d, J = 7.5 Hz, Glc-1‴)] (Table 25). The ^{13}C NMR as well as the COSY spectrum strongly suggested the connectivity of these sugar moieties to the C-3 position of flavones moiety. Additionally, a methoxy group proton signal appeared in **59**. Consequently, the structures of **57-59** were identified as kaempferol-3-*O*-gentiobioside (**57**), quercetin-3-*O*-gentiobioside (**58**), and isorhamnetin-3-*O*-gentiobioside (**59**), respectively. [83-85]



	R
57	H
58	OH
59	OMe

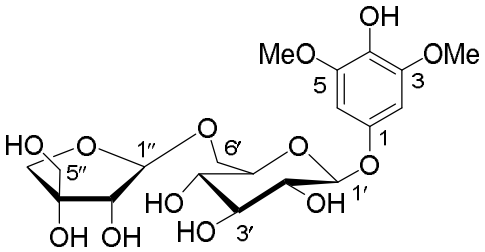
Table 25. NMR data for **57-59** (^{1}H NMR; 400 MHz and ^{13}C NMR; 100 MHz, δ; ppm, recorded in MeOH-d_4)

Position	**57**		**58**		**59**	
	δ_H (J in Hz)	δ_C [a]	δ_H (J in Hz)	δ_C [a]	δ_H (J in Hz)	δ_C [a]
2		158.5		158.9		158.5
3		135.6		135.6		135.3
4		179.5		179.4		179.4
5		163.0		163.0		163.0
6	6.21, d (2.0)	99.9	6.21, d (1.5)	99.9	6.21, d (1.5)	99.9
7		166.0		166.1		166.0
8	6.41, d (2.0)	94.9	6.41, d (1.5)	94.9	6.42, d (1.5)	94.9
9		159.0		158.5		158.4
10		105.8		105.8		105.9
1′		122.8		123.2		123.0
2′	8.10, d (8.0)	132.4	7.70, d (2.0)	117.6	8.01, d (2.0)	114.6
3′	6.90, d (8.0)	116.2		149.8		148.4
4′		161.5		145.9		150.9
5′	6.90, d (8.0)	116.2	6.87, d (8.0)	116.1	6.90, d (8.5)	116.2
6′	8.10, d (8.0)	132.4	7.66, dd (8.0, 2.0)	123.5	7.63, dd (8.5, 2.0)	123.8
Glc-1″	5.24, d (7.5)	104.6	5.23, d (7.5)	104.6	5.36, d (7.5)	104.5
2″	3.17, t (7.5)	75.1	3.17, t (7.5)	75.1	3.18, t (7.5)	75.1
3″	3.48, m	78.0	3.48, m	78.0	3.47, m	78.0
4″	3.35, m	71.4	3.35, m	71.4	3.35, m	71.4
5″	3.02, m	77.9	3.02, m	77.9	3.02, m	77.9
6″	3.98, dd (12.0, 2.0)	69.7	3.98, dd (12.0, 2.0)	69.7	3.99, dd (12.0, 2.0)	69.4
	3.65, dd (12.0, 5.0)		3.65, dd (12.0, 5.0)		3.64, dd (12.0, 5.0)	
Glc-1‴	4.15, d (7.5)	104.1	4.16, d (7.5)	104.0	4.16, d (7.5)	104.0
2‴	3.06, t (7.5)	75.8	3.06, t (7.5)	75.8	3.06, t (7.5)	75.8
3‴	3.45, m	78.0	3.45, m	78.0	3.45, m	77.7
4‴	3.35, m	71.3	3.35, m	71.4	3.35, m	71.3
5‴	3.23, t (7.5)	77.8	3.23, t (7.5)	77.6	3.23, t (7.5)	77.6
6‴	3.75, dd (12.0, 2.0)	62.6	3.75, dd (12.0, 2.0)	62.6	3.74, dd (12.0, 2.0)	62.7
	3.55, dd (12.0, 5.0)		3.55, dd (12.0, 5.0)		3.54, dd (12.0, 5.0)	
OMe					3.96, s	57.0

[a,] Assignments were based on HMQC and HMBC experiments

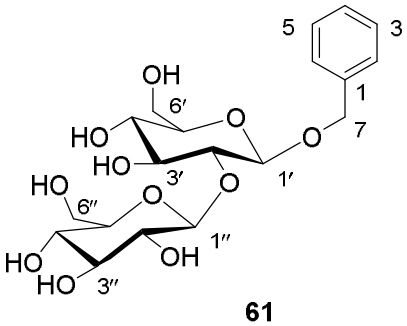
2.4.4. Phenolic glycosides (60, 61, 63, 64)

Compound **60** was obtained as colorless amorphous powder and the NMR spectra revealed the presence of a tetrasubstituted symmetrical aromatic ring, because of two equivalent aromatic protons signal at δ_H 6.48 and two equivalent methoxy groups proton signal at δ_H 3.82. The NMR spectra also showed two anomeric protons signal [δ_H 4.74 (1H, d, J = 8.0 Hz, Glc-1′) and 4.96 (1H, d, J = 2.5 Hz, Api-1″), suggesting the presence of a *β*-D-apiofuranosyl-(1→6)-*O*-*β*-D-glucopyranosyl unit in its structure. Thus, by comparing with the published values, the structure of **60** was identified as canthoside B.[86]



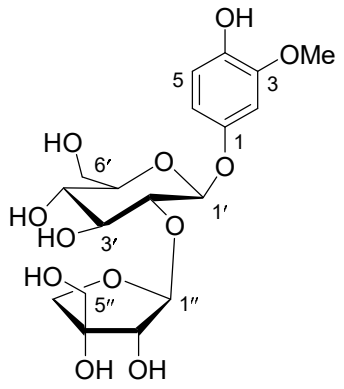
60

Compound **61** was obtained as colorless needles and the molecular weight found to be 432 in the literature. The NMR spectra revealed the presence of a benzyloxy aromatic ring and two anomeric protons signal [δ_H 4.52 (1H, d, J = 7.5 Hz, Glc-1′) and 4.64 (1H, d, J = 7.5 Hz, Glc-1″), suggesting the presence of a *β*-D-glucopyranosyl-(1→2)-*O*-*β*-D-glucopyranosyl unit in its structure. Thus, by comparing with the published values, the structure of **61** was identified as zizybeoside I.[87]



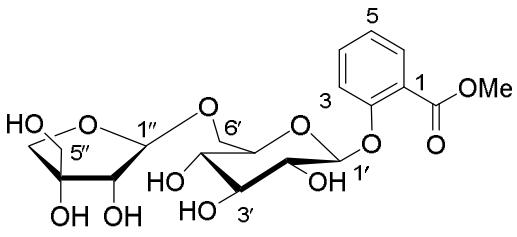
61

Compound **63** was also obtained as colorless needles and the molecular weight was found to be 434 in the published literature. The NMR spectra revealed the presence of a set of ABX-type aromatic protons [δ_H 6.77 (1H, d. J = 2.5 Hz, H-2), 6.69 (1H, d, J = 8.0 Hz, H-5) and 6.56 (1H, dd, J = 8.0, 2.5 Hz, H-6)], a methoxy group protons at δ_H 3.83, along with two anomeric protons [δ_H 4.80 (1H, d, J = 8.0 Hz, Glc-1′) and 5.45 (1H, d, J = 1.5 Hz, Api-1″) in its structure. Based on the published values in literature, the structure was confirmed as markhamioside F.[89]



63

Compound **64** was also obtained as colorless amorphous powder and the NMR spectra revealed the presence of a 1, 2-disubstituted aromatic ring, with one carbomethoxyl group in addition to a *β*-D-apiofuranosyl-(1→6)-*O*-*β*-D-glucopyranosyl unit in its structure. Thus, the structure of **64** was confirmed as canthoside A and it was first reported from C*anthium berberidifolium*.[86]



64

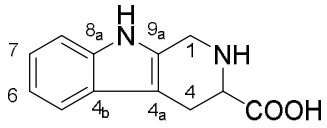
Table 26. NMR data for **60, 63** and **64** (^{1}H NMR; 400 MHz and ^{13}C NMR; 100 MHz, δ; ppm, recorded in MeOH-d_4)

Position	**60**		**63**		**64**	
	δ_H (J in Hz)	δ_C[a]	δ_H (J in Hz)	δ_C[a]	δ_H (J in Hz)	δ_C[a]
1		152.3		152.8		122.5
2	6.48, s	97.2	6.77, d (2.5)	103.7		158.7
3		149.4		149.4	7.38, dd (8.5, 1.0)	119.2
4		132.4		142.9	7.56, ddd (8.5, 7.0 1.0)	135.2
5		149.4	6.69, d (8.0)	116.1	7.12, ddd (7.5, 7.0 1.0)	123.7
6	6.48, s	97.2	6.56, dd (8.0, 2.5)	109.9	7.76, dd (8.0, 2.0)	132.1
1-COOMe						168.5
COOMe					3.89, s	52.8
2-OMe						
3-OMe	3.82, s	56.9	3.83, s	56.5		
5-OMe	3.82, s	56.9				
Glc-1′	4.74, d (8.0)	103.9	4.80, d (8.0)	102.5	4.84, d (8.0)	104.1
2′	3.34, m	74.9	3.37, m	78.9	3.36, m	75.0
3′	3.56, m	78.0	3.67, m	78.1	3.52, m	78.1
4′	3.44, m	71.6	3.54, m	71.7	3.47, m	71.6
5′	3.58, m	77.0	3.59, m	77.7	3.59, m	77.4
6′	4.02, dd (11.0, 2.0)	68.7	3.88, dd (11.0, 2.0)	62.6	4.03, dd (11.5, 2.0)	68.8
	3.63, dd (11.0, 5.0)		3.67, dd (11.0, 4.5)		3.65, dd (11.5, 5.0)	
Api-1″	4.96, d (2.5)	110.9	5.45, d (1.5)	110.8	5.0, d (2.5)	111.1
2″	3.87, d (2.5)	77.9	3.96, d (1.5)	78.0	3.91, d (2.5)	77.6
3″		80.5		80.7		80.5
4″	3.94, d (10.0)	74.9	4.10, d (11.0)	75.5	3.97, d (10.0)	75.0
	3.73, d (10.0)		3.79, d (11.0)		3.75, d (10.0)	
5″	3.55, s	65.5	3.57, s	66.1	3.58, s	65.6

[a,] Assignments were based on HMQC and HMBC experiments

2.4.5. Carboline derivative (62)

Compound **62** was also obtained as colorless amorphous powder and the NMR spectra revealed the presence of a 1, 2-disubstituted indole ring, with one carboxyl group, a methine proton and two sets of methylene protons in its structure. The structure of **62** was found as (*3S*)-1,2,3,4-tetrahydro-*β*-carboline-3-carboxylic acid.[88]



62

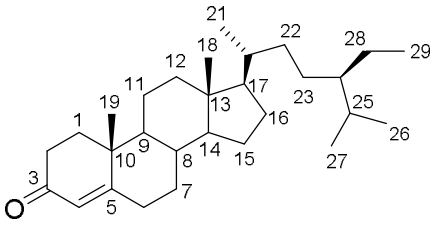
Table 27. NMR data for **61** and **62** (^{1}H NMR; 400 MHz and ^{13}C NMR; 100 MHz, *δ*; ppm, recorded in MeOH-d_4)

Position	**61**		Position	**62**	
	δ_H (*J* in Hz)	δ_C [a]		δ_H (*J* in Hz)	δ_C [a]
1		139.1	1	4.18, d (16.0)	40.5
2	7.44, d (7.5)	128.8		4.16, d (16.0)	
3	7.30, t (7.5)	129.2	2		
4	7.25, t (7.5)	128.6	3	3.56, m	56.6
5	7.30, t (7.5)	129.2	4	3.14, dd (16.0, 5.0)	23.0
6	7.44, d (7.5)	128.8		4.16, dd (16.0, 5.0)	
7	4.97, d (12.0)	71.9	4_a		106.6
	4.75, d (12.0)		4_b		126.2
			5	7.35, d (7.5)	117.6
Glc-1′	4.52, d (7.5)	102.3	6	7.10, td (7.5, 1.5)	118.7
2′	3.35, overlapped	83.1	7	6.95, td (7.5, 1.5)	121.1
3′	3.59, t (7.5)	77.9	8	7.45, d (7.5)	111.1
4′	3.27, overlapped	71.5	8_a		136.2
5′	3.21, m	78.2	9		
6′	3.91, dd (12.0, 2.5)	62.8	9_a		128.0
	3.65, dd (12.0, 5.5)		COO		175.0
Glc-1″	4.64, d (7.5)	105.2			
2″	3.37, m	76.1			
3″	3.39, m	77.8			
4″	3.57, t (7.5)	71.4			
5″	3.22, m	78.0			
6″	3.76, dd (12.0, 2.0)	62.6			
	3.70, dd (12.0, 5.0)				

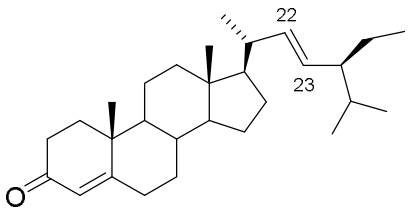
[a,] Assignments were based on HMQC and HMBC experiments

2.4.6. Stigmastane triterpenoids (65-66)

Compounds **65** and **66** were obtained as colorless needles. The ^{1}H NMR spectra of both compounds revealed the presence of one olefinic proton [δ_H 5.72 (1H, s, H-4)] and six methoxy groups among which four appeared as doublet [**65**: δ_H 0.71 (3H, s, Me-18), 0.85 (3H, d, J = 7.0 Hz, Me-26), 0.84 (3H, d, J = 7.5 Hz, Me-29), 0.83 (3H, d, J = 7.0 Hz, Me-27), 0.92 (3H, d, J = 7.5 Hz, Me-21), 1.18 (3H, s, Me-19); **66**: δ_H 0.73 (3H, s, Me-18), 1.01 (3H, d, J = 7.5 Hz, Me-26), 0.85 (3H, d, J = 7.5 Hz, Me-29), 0.80 (3H, d, J = 7.5 Hz, Me-27), 0.83 (3H, d, J = 7.5 Hz, Me-21), 1.19 (3H, s, Me-19)]. Compound **66** showed additionally two olefinic protons signals [δ_H 5.16 (1H, dd, J = 15.5, 8.0 Hz, H-22) and 5.02 (1H, dd, J = 15.5, 8.0 Hz, H-23)] in its ^{1}H NMR data. The ^{13}C NMR spectra of both compounds displayed 29 carbon resonances along with one carbonyl group at δ_C 199.7 (C-3), indicating to have a stigmastane type skeleton in their structures. Compound **65** showed a pair of olefinic carbon signals at δ_C 123.9 (C-4) and 171.8 (C-5) ppm while **66** displayed two pair of olefinic signals [δ_C 124.0 (C-4), 171.7 (C-5) and 138.3 (C-22), 129.8 (C-23) in ^{13}C NMR spectra. The above NMR data were completely matched with published values of stigmast-4-en-3-one (**65**) and stigmast-4, 22-dien-3-one (**66**) in literatures.[90-92]



65



66

Table 28. NMR data for **65** and **66** (^{1}H NMR; 500 MHz and ^{13}C NMR; 125 MHz, δ; ppm, recorded in $CDCl_3$)

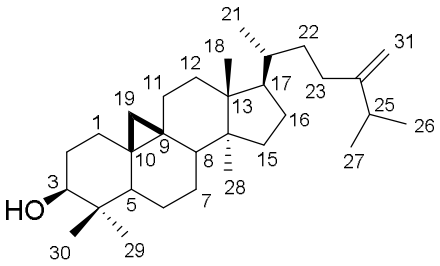
Position	**65**		**66**	
	δ_H (J in Hz)	δ_C [a]	δ_H (J in Hz)	δ_C [a]
1	2.02, m	35.9	2.02, m	36.0
	1.70, m		1.70, m	
2	2.41, m	34.2	2.41, m	34.2
	1.30, m		1.30, m	
3		199.7		199.7
4	5.72, s	123.9	5.72, s	124.0
5		171.8		171.7
6	2.25, tq (5.0)	33.2	2.25, tq (5.0)	33.2
7	1.84, m	32.3	1.84, m	32.3
	1.10, m		1.10, m	
8	1.51, m	35.9	1.51, m	36.0
9	0.91, m	54.1	0.91, m	54.1
10		38.8		38.9
11	1.53, m	21.3	1.53, m	21.3
	1.43, m		1.43, m	
12	2.35, m	39.9	2.35, m	39.8
	1.15, m		1.15, m	
13		42.7		42.5
14	1.01, m	56.2	1.01, m	56.2
15	1.66, m	24.4	1.66, m	24.5
	1.61, m		1.61, m	
16	1.85, m	28.4	1.85, m	29.0
	1.25, m		1.25, m	
17	1.13, m	56.3	1.13, m	56.3
18	0.71, s	12.2	0.73, s	12.4
19	1.18, s	17.6	1.19, s	17.6
20	1.20, m	36.3	2.04, m	40.6
21	0.92, d (7.5)	18.9	0.83, d (7.5)	19.2
22	1.37, m	34.2	5.16, dd (15.5, 8.0)	138.3
	1.30, m			
23	1.15, m	26.4	5.02, dd (15.5, 8.0)	129.8
	1.15, m			
24	0.94, m	46.1	1.57, m	51.5
25	1.68, m	29.3	1.70, m	29.0
26	0.85, d (7.0)	20.0	1.01, d (7.5)	21.4
27	0.83, d (7.0)	19.3	0.80, d (7.5)	21.3
28	1.23, m	23.3	1.55, m	25.6
	1.23, m		1.10, m	
29	0.84, d (7.5)	12.2	0.85, d (7.5)	12.3

[a,] Assignments were based on HMQC and HMBC experiments

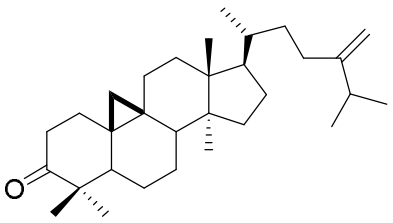
2.4.7. Cycloartane triterpenoids (67-70)

Compound **67** was obtained as white amorphous powder. The NMR spectra showed seven methyl groups, among which three appeared as doublet [δ_H 0.81 (3H, s, Me-30), 0.91 (3H, s, Me-28), 0.97 (6H, s, Me-18 and 29), 0.90 (3H, d, J = 5.5 Hz, Me-21), 1.02 (3H, d, J = 5.0 Hz, Me-26), 1.03 (3H, d, J = 5.0 Hz, Me-27)] along with 31 carbon resonances. The ^{1}H NMR spectra also displayed a characteristic cycloartane type methylene signal [δ_H 0.57 (1H, d, J = 4.0 Hz, H-19) and 0.33 (1H, d, J = 4.0 Hz, H-19)] and two olefinic methlene protons at δ_H 4.73 and 4.68 in addition to an oxymethine signal at δ_H 3.29 (H-3). Considering the ^{1}H-^{1}H COSY and HMBC spectra, **67** was found to be 24-methylenecycloartanol.[93]

Compound **68**, obtained as a white amorphous powder, showed very much similar NMR spectra with **67**. However, instead of an oxymethine signal at C-3 position in **67**, a carbonyl group resonance at δ_C 216.6 appeared in ^{13}C NMR spectra. Thus, the structure of **68** was identified as 24-methylenecycloartenone by comparing with published values in literatures.[94-95]



67



68

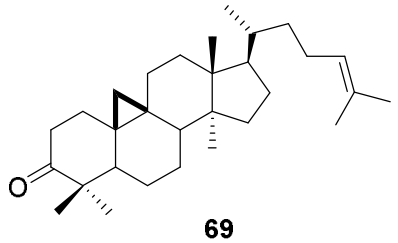
Table 29. NMR data for **67, 68** and **69** (^{1}H NMR; 500 MHz and ^{13}C NMR; 125 MHz, δ; ppm, recorded in $CDCl_3$)

Position	**67**		**68**		**69**	
	δ_H (J in Hz)	δ_C [a]	δ_H (J in Hz)	δ_C [a]	δ_H (J in Hz)	δ_C [a]
1	1.62, m	32.1	1.86, td (14.0, 5.5)	33.5	1.87, m	33.6
	1.29, m		1.54, m		1.53, m	
2	2.32, m	30.5	2.71, td (14.0, 5.5)	37.5	2.71, td (14.0, 5.5)	37.7
	2.24, m		2.31, m		2.31, m	
3	3.29, dd (12.0, 4.5)	78.9		216.6		216.6
4		40.6		50.3		50.5
5	1.55, m	47.2	1.72, dd (12.0, 4.0)	48.5	1.71, m	48.7
6	1.51, m	21.2	1.55, m	21.6	1.54, m	21.7
7	1.92, m	26.1	1.92, m	28.2	1.90, m	28.4
	1.31, m		1.31, m		1.33, m	
8	1.59, m	48.0	1.59, m	47.9	1.60, m	48.1
9		20.1		21.2		21.4
10		26.2		26.9		26.3
11	1.40, m	26.6	1.40, m	25.9	1.39, m	26.1
	1.10, m		1.10, m		1.10, m	
12	1.32, m	33.0	1.32, m	32.9	1.32, m	33.1
	1.32, m		1.32, m		1.32, m	
13		45.4		45.5		45.6
14		48.9		48.8		49.0
15	2.00, m	35.7	1.67, m	35.7	1.67, m	35.8
	1.67, m		1.67, m		1.67, m	
16	2.01, m	28.2	2.05, m	26.1	2.05, m	27.0
	1.14, m		1.14, m		1.14, m	
17	1.64, m	52.4	1.64, m	52.4	1.70, m	52.6
18	0.97, s	18.1	1.00, s	18.4	0.99, s	19.5
19	0.57, d (4.0)	29.9	0.79, d (4.5)	29.6	0.79, d (4.5)	29.7
	0.33, d (4.0)		0.57, d (4.5)		0.56, d (4.5)	
20	1.55, m	36.2	1.41, m	36.2	1.67, m	36.1
21	0.90, d (5.5)	18.4	0.90, d (6.0)	18.1	0.88, d (6.0)	18.3
22	1.28, m	35.1	1.13, m	35.1	1.13, m	36.6
	1.28, m		1.13, m		1.13, m	
23	2.09, m	31.5	2.13, m	31.4	2.13, m	25.2
	1.89, m		1.89, m		1.89, m	
24		157.0		156.9	5.10, td (5.5, 1.0)	125.5
25	2.25, m	33.9	2.24, m	33.9		131.1
26	1.02, d (5.0)	22.1	1.02, d (5.0)	22.1	0.90, s	17.8
27	1.03, d (5.0)	21.9	1.03, d (5.0)	21.9	0.92, s	25.9
28	0.91, s	25.5	0.91, s	19.4	0.91, s	18.5
29	0.97, s	14.2	1.05, s	22.9	1.05, s	21.0
30	0.81, s	19.4	1.10, s	20.8	1.10, s	22.4
31	4.73, br. s	106.0	4.72, br. s	106.1		
	4.68, br. s		4.67, br. s			

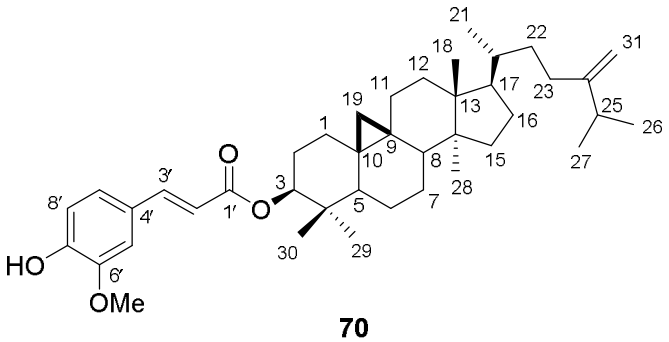
[a,] Assignments were based on HMQC and HMBC experiments

Compound **69** was also obtained as a white amorphous powder. The NMR spectra showed very much similarity with **68**. Instead of olefinic methylene group protons signal in **68**, an olefinic proton signal [δ_H 5.10 (1H, td, J = 5.5, 1.0 Hz, H-24) appeared in **69**, suggesting the difference was occurred in position C-24. In accordance with the published literatures, **69** was identified as 24-en-cycloartenone. [96]

Compound **70** was obtained as white amorphous powder and showed characteristic 24-methylenecycloartane type triterpenoids structural resonances in its NMR spectra. However, a ferulate moiety was observed in **70**, which was configured on the basis of an ABX-type aromatic protons signal [δ_H 6.98 (1H, d, J = 2.0 Hz, H-5′), 6.86 (1H, d, J = 8.0 Hz, H-8′) and 7.02 (1H, dd, J = 8.0, 2.5 Hz, H-9′)], a pair of olefinic SP^2-hybridized proton [δ_H 6.24 (1H, d, J = 16.0 Hz, H-2′) and 7.54 (1H, d, J = 16.0 Hz, H-3′)] and an esterified carboxyl group at δ_C 167.1. Hence, the structure of **70** was identified as 24-methylenecycloartanyl ferulate.[97]



69



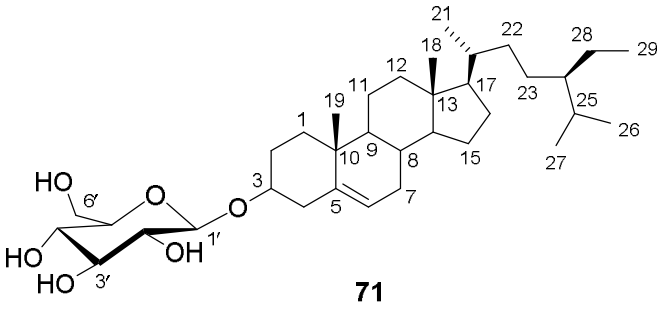
70

2.4.8. Phytosterol (71)

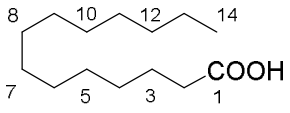
Compound **71** was obtained as a white amorphous powder and it showed similar NMR spectra for stigmastane type skeleton. The ^{1}H NMR spectra showed an additional anomeric proton signal [δ_H 4.21 (1H, d. J = 7.5 Hz, Glc-1′), suggesting a glycosidic linkage at position C-3. The HMBC spectra of **71** showed the correlation between anomeric proton of glucopyranosyl unit and δ_C 76.9 (C-3) of β-sitosterol structure. Considering the ^{1}H-^{1}H COSY, HSQC and HMBC spectra, **71** was found to be β-sitosterol-3-*O*-glucopyranoside.[98]

2.4.9. Miscellaneous (72-74)

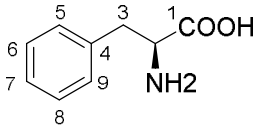
Compound **72**, obtained as white amorphous powder, showed similar NMR spectra for a primary metabolite called, tetradecanoic acid.[99] Compounds **73** and **74** were also showed same NMR spectra as two amino acids L-phenylalanine and L-tryptophan, respectively.[100]



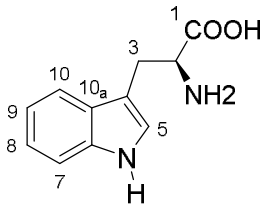
71



72



73



74

Table 30. NMR data for **70** (^{1}H NMR; 500 MHz and ^{13}C NMR; 125 MHz, δ; ppm, recorded in $CDCl_3$) and **71** (^{1}H NMR; 400 MHz and ^{13}C NMR; 100 MHz, δ; ppm, recorded in $CDCl_3$)

Position	**70**		Position	**71**	
	δ_H (J in Hz)	δ_C [a]		δ_H (J in Hz)	δ_C [a]
1	1.66, m & 1.28, m	31.8	1	1.79, m & 0.98, m	36.8
2	1.84, m & 1.68, m	27.1	2	1.83, m & 1.46, m	29.2
3	4.66, dd (12.0, 4.5)	80.7	3	3.46, m	76.9
4		39.9	4	2.36, m & 2.12, m	38.3
5	1.40, dd (12.0, 4.0)	47.4	5		140.4
6	1.58, m & 0.77, m	21.1	6	5.32, d (5.0)	121.1
7	1.90, m & 1.20, m	28.3	7	1.93, m & 1.50, m	31.3
8	1.50, dd (12.0, 4.0)	48.0	8	1.40, m	31.4
9		20.3	9	0.88, m	49.6
10		26.2	10		36.2
11	1.28, m & 1.04, m	25.9	11	1.47, m & 1.0, m	20.5
12	1.26, m	35.7	12	1.96, m & 1.14, m	39.1
13		45.5	13		41.8
14		49.0	14	0.98, m	56.1
15	1.56, m	33.0	15	1.54, m & 1.03, m	23.8
16	1.94, m & 1.11, m	26.7	16	1.80, m & 1.25, m	27.7
17	1.59, m	52.4	17	1.09, m	55.4
18	0.92, s	18.1	18	0.64, s	11.6
19	0.56, d (4.0) & 0.31, d (4.0)	29.9	19	0.95, s	19.0
20	1.26, m	36.3	20	1.34, m	35.4
21	0.85, d (6.0)	18.4	21	0.89, d (6.5)	16.8
22	1.54, m & 1.25, m	35.2	22	1.30, m & 1.01, m	33.3
23	2.06, m & 1.87, m	31.3	23	1.15, m	25.5
24		157.0	24	0.91, m	45.1
25	2.19, septet	34.0	25	1.63, m	28.7
26	0.98, d (3.0)	22.0	26	0.81, d (7.5)	18.9
27	0.97, d (3.0)	22.1	27	0.81, d (7.5)	19.6
28	0.86, s	19.4	28	1.25, m & 1.19, m	22.6
29	0.84, s	25.6	29	0.82, d (7.5)	11.7
30	0.92, s	15.5			
31	4.67, s & 4.61, s	106.1	Glc-1′	4.21, d (7.5)	100.7
1′		167.1	2′	2.88, overlapped	73.4
2′	6.24, d (16.0)	116.5	3′	3.11, overlapped	76.6
3′	7.54, d (16.0)	144.4	4′	3.01, overlapped	70.0
4′		127.4	5′	3.05, overlapped	76.7
5′	6.98, d (2.0)	109.5	6′	3.63, overlapped	61.2
6′		146.9		3.39, overlapped	
7′		148.0			
8′	6.86, d (8.0)	114.8			
9′	7.02, dd (8.0, 2.0)	123.1			
OMe	3.87, s	56.1			

[a,] Assignments were based on HMQC and HMBC experiments

Table 31. NMR data for **72** (^{1}H NMR; 500 MHz and ^{13}C NMR; 125 MHz, δ; ppm, recorded in $CDCl_3$)

Position	**72**	
	δ_H (J in Hz)	δ_C [a]
1		178.9
2	2.34, t (7.5)	34.0
3	1.30, overlapped	24.9
4	1.30, overlapped	29.3
5	1.30, overlapped	29.6
6	1.30, overlapped	29.9
7	1.30, overlapped	29.9
8	1.30, overlapped	29.9
9	1.30, overlapped	29.8
10	1.30, overlapped	29.5
11	1.30, overlapped	29.4
12	1.30, overlapped	32.1
13	1.65, pentet (7.5)	22.9
14	0.88, t (7.0)	14.3

Table 32. NMR data for **73** and **74** (^{1}H NMR; 400 MHz and ^{13}C NMR; 100 MHz, δ; ppm, recorded in MeOH-d_4)

Position	**73**		Position	**74**	
	δ_H (J in Hz)	δ_C [a]		δ_H (J in Hz)	δ_C [a]
1		173.6	1		174.3
2	3.79, dd (9.0, 4.0)	57.6	2	3.86, dd (9.0, 4.0)	56.8
3	3.30, overlapped	38.3	3	3.51, dd (16.0, 4.0)	28.4
	2.99, dd (15.0, 8.0)			3.16, dd (16.0, 7.0)	
4		137.4	4		109.6
5	7.30, d (8.0)	130.0	5	7.19, br. s	125.1
6	7.37, d (8.0)	130.4	6		
7	7.34, d (7.0)	128.4	6a		138.4
8	7.37, d (8.0)	130.4	7	7.69, d (8.0)	112.4
9	7.30, d (8.0)	130.4	8	7.11, td (8.0, 1.0)	122.7
			9	7.02, td (8.0, 1.0)	120.1
			10	7.25, d (8.0)	119.3
			10a		128.5

[a,] Assignments were based on HMQC and HMBC experiments

2.5. Summary

Crude methanolic extracts from the stem and root parts of *Pothos scandens* (Araceae) were isolated, and twenty eight compounds among which four were identified as new constituents and twenty four known constituents were characterized using various spectroscopic techniques. Among the new compounds, three hemiterpene glucoside aromatic esters, pothobanosides A (**47**), B (**48**), and C (**49**), and a new phenylisobutanoid, pothobanol (**50**) were characterized. Among the known constituents, flavonoids and triterpenoids were isolated as major portion from this plant. The absolute configuration of the sugar moiety in the new constituent's was confirmed by acid hydrolysis followed by detection in HPLC technique and compared to authentic samples.

Chapter 3

Estrogenic/antiestrogenic activities of isolates from *T. citrina* and *P. scandens*

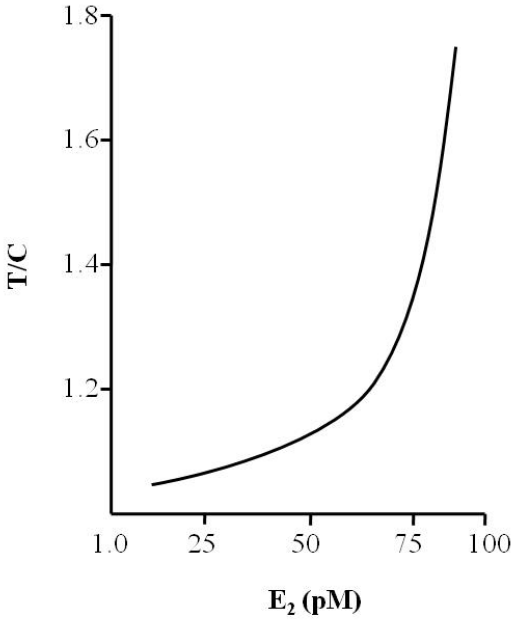
3.1. Introduction

In vitro cell-based assay technique using Alamar blue as fluorescence compounds was used for the determination of estrogenic and/or antiestrogenic activities of the isolates. In this respect, two American type culture collection (ATCC) cancer cells such as MCF-7 (ATCC® HTB-22) and T47D (ATCC® HTB-133) were used because of their exquisite hormone sensitivity through expression of estrogen receptor. Both the cells are epithelial phenotype and classified as luminal A ($ER^+/PR^{+/-}/Her2^-$). MCF-7 cells are known to have oncogene p53 wild-type whereas T47D has mutant p53. The doubling time of the cultured cells of MCF-7 and T47D are 29 and 32 h, respectively. Certain natural compounds may show cell specific activity therefore two different cell lines were used. All the cells were co-cultured with total 74 isolates from *T. citrina* and *P. scandens* using four different concentrations (10, 1, 0.1 and 0.01 μM). The cells were treated with or without 100 pM of estradiol (E_2) to evaluated their estrogenic and/or antiestrogenic property. A standard curve was prepared by using positive control estradiol (E_2) (100, 10, and 1 pM). The estrogenic activities of the isolates were shown in concentration equivalent to estradiol. Antiestrogenic activity were also shown in concentration inhibition equivalent to estradiol and also compared with positive control tamoxifen.

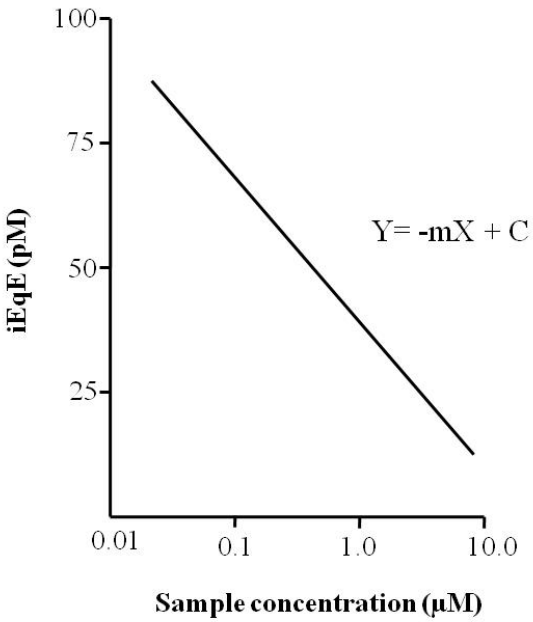
3.2. Dose-response curves

The relationship between the concentration of the tested sample and the extent of cell proliferation was analyzed from the dose-response curves. First three columns of the 96 well plates were treated with three different concentrations (1, 10 and 100 pM) of estradiol (E_2) and another column was treated only with EtOH. Standard curve was prepared with the best fit line slope using the concentration of estradiol vs times of average cell count (T/C) compared to EtOH only (Curve A). As none of the isolates showed any discernable cell proliferation activity when the cells were co-treated, estrogenic activity was not calculated. Proliferation of ertain number of cells in the 96 well plates were induced with 100 pM of estradiol (E_2) and those were co-treated with tested samples to observe the antiestrogenic like activity. Inhibition of 100 pM of estradiol (E_2)-induced cell proliferation was observed with all the tested samples. As a result, antiestrogenic activity was calculated on the basis of cell proliferation respective to the standard curve estradiol (E_2) concentrations and iEqE values of each sample ($iEqE_{50}$, $iEqE_{10}$, and $iEqE_1$) were determined. The sample concentration that is required to inhibit the E_2 effect by 50% (reduce the induced cell population count 50%), is referred as $iEqE_{50}$, 90% inhibition of induced cell proliferation was recognized as $iEqE_{10}$ and finally 99% inhibition of induced cell proliferation was recognized as $iEqE_1$ values. When all the tested concentration reduced the cell proliferation with a concentration dependent manner, the curve followed linear regression (Curve B). Some of the isolates showed more than 50%

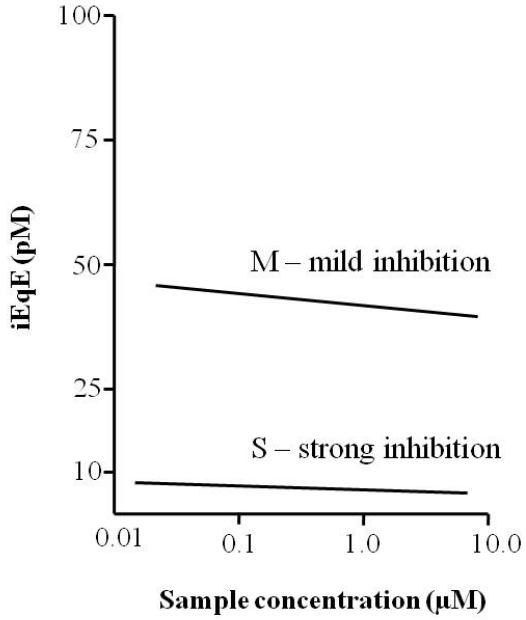
inhibition constantly of induced cell proliferation along the tested concentrations, were referred as mild inhibition and those who showed more than 90% of inhibition constantly of the induced cell proliferation, were referred as strong inhibition and followed non-liner regression (Curve C).



A. Standard curve of estradiol (E_2)



B. Linear regression analysis of iEqE (µM)



C. Non-linear regression analysis of iEqE (µM)

3.3. Estrogenic/antiestrogenic activity of isolates from *T. citrina*

Crude methanolic, EtOAc and H_2O soluble fractions were evaluated for their estrogenic and/ or antiestrogenic properties in both cell lines. The EtOAc and H_2O soluble fractions suppressed 80% and 40% of the estradiol (E_2)-enhanced proliferation of cancer cells, respectively, at a concentration of 20 µg/mL. However, the EtOAc fraction also exhibited cytotoxicity at higher concentration of 200 µg/mL. Based on the preliminary assay results, the EtOAc soluble fraction was subjected for further partitioning to afford 16 combined fractions. Among these fractions, fraction no. 5, 6, 11 and 14 displayed 99% inhibition of estradiol (E_2)-enhanced proliferation of T47D cells at a concentration of <0.2 µg/mL, hence selected for further isolation process (chart 1). Among the fractions those inhibited 90% of estradiol (E_2)-enhanced proliferation, fraction 10 was chosen for isolating flavonoid constituents.

From the EtOAc layer, nine new furofuran lignans (**1**, **5-12**), one furofuranone lignan (**13**), thirteen furofuran ligan glucosides (**14-26**), five furofuranone lignan glucosides (**27-31**), and seven tetrahydrofuran lignan glucosides (**32-38**) together with three know furofuran lignans (**2-4**), three flavonoid glycosides (**39-41**), two lignan glycosides (**42-43**) and three other miscellaneous constituents (**44-46**) were isolated. All of them were evaluated for their biological activity at four different concentrations. None of them showed any discernible cell proliferation activity at highest tested concentrations (data not shown). Almost all of them showed cell proliferation inhibitory activity in both cell lines, which are listed in tables 33 and 34.

3.4. Estrogenic/antiestrogenic activity of isolates from *P. scandens*

The EtOAc and H_2O soluble fractions of *P. scandens* suppressed 80% and 40% of the estradiol (E_2)-enhanced proliferation of T47D cells, respectively, at a concentration of 20 µg/mL. However, the EtOAc fraction also exhibited cytotoxicity at higher concentration of 200 µg/mL. Based on the preliminary assay results, the EtOAc soluble fraction was subjected for further partitioning to isolate the active constituents.

From the H_2O layer, three new hemiterpene glucoside aromatic esters, pothobanosides A (**47**), B (**48**), and C (**49**), and a new phenylisobutanoid, pothobanol (**50**) together with one known diketopiperazine (**51**), eight flavonoid glycosides (**52-59**), four phenolic glycosides (**60-61, 63-64**), and four other miscellaneous constituents (**62, 73-74**) were isolated. From the EtOAc layer, seven triterpenoids (**65-71**) and one primary metabolite (**72**) were isolated. All of them were evaluated for their biological activity at four different concentrations which are listed in table 35.

Table 33. Inhibitory activities of new constituents (**1, 5-38)** from *T. citrina*.

Cpds	**MCF-7** (iEqE in µM)				**T47D** (iEqE in µM)			
	$iEqE_{50}$ [a]	$iEqE_{10}$ [a]	$iEqE_1$ [a]	IL [b]	$iEqE_{50}$ [a]	$iEqE_{10}$ [a]	$iEqE_1$ [a]	IL [b]
1	< 0.01	<0.01	-	**S**	< 0.01	<0.01	-	**S**
5	< 0.01	< 0.01	-	**S**	< 0.01	5.2	9.3	M
6	< 0.01	<0.01	< 0.01		< 0.01	<0.01	< 0.01	
7	< 0.01	<0.01	< 0.01		< 0.01	<0.01	< 0.01	
8	< 0.01	<0.01	-	**S**	< 0.01	<0.01	-	**S**
9	< 0.01	< 0.01	-	**S**	< 0.01	< 0.01	10.0	**S**
10	< 0.01	9.7	-	**M**	0.5	8.0	9.7	M
11	< 0.01	< 0.01	-	**S**	< 0.01	< 0.01	10.0	**S**
12	< 0.01	< 0.01	< 0.01		< 0.01	< 0.01	< 0.01	
13	< 0.01	< 0.01	< 0.01		< 0.01	< 0.01	< 0.01	
14	< 0.01	-	-		< 0.01	-	-	
15	< 0.01	< 0.01	3.5	**S**	< 0.01	< 0.01	-	**S**
16	< 0.01	< 0.1	-	**M**	10.0	-	-	
17	< 0.01	-	-		-	-	-	
18	< 0.01	0.6	-	**M**	5.6			
19	< 0.01	-	-		< 0.01	< 0.01	-	**S**
20	< 0.01	< 0.01	-	**S**	< 0.01			
21	< 0.01	9.8		**M**	< 0.01	< 0.01	-	**S**
22	< 0.01	-	-		< 0.01	-	-	
23	< 0.01	< 0.1	-	**M**	< 0.01	-	-	
24	< 0.01	-	-		< 0.01	< 0.1	-	**M**
25	< 0.01	-	-		< 0.01	-	-	
26	< 0.01	< 0.01	-	**S**	< 0.1	-	-	
27	< 0.01	-	-		< 0.01	< 0.01	-	**S**
28	< 0.01	< 0.01	-	**S**	< 0.01	-	-	
29	< 0.1	10.0	-		8.4	-	-	
30	0.5	1.8	-		8.6	-	-	
31	< 0.01	1.0	-	**M**	< 0.01	< 0.01	-	**S**
32	< 0.01	-	-		< 0.01	-	-	
33	< 0.1	-	-		< 0.01	-	-	
34	< 0.01	< 0.1	-	**M**	< 0.01	< 0.01	-	
35	< 0.01	-	-		< 0.01	-	-	
36	< 0.01	-	-		< 0.01	< 0.01	-	**S**
37	< 0.01	-	-		< 0.01	-	-	
38	< 0.01	-	-		< 0.01	-	-	
Tam	0.1	0.5	5.0		0.1	0.8	9.0	

[a] $iEqE_{50}$, $iEqE_{10}$, $iEqE_1$ represent the concentrations of the compounds (µM) that decrease the cell proliferation (enhanced by 100 pM of E_2) to equivalent levels of those induced by 50 pM, 10 pM, and 1 pM of E_2 treatment, respectively. The values were calculated by linear regression analysis using four different concentrations. [b] IL inhibitory level of the compound. Mild inhibition (M), more than 50% inhibition through the concentrations were tested. Strong inhibition (S), more than 90% inhibition through the concentrations were tested. Tamoxifen (Tam).

Table 34. Inhibitory activities of known constituents (**2-4** and **39-46)** from *T. citrina*.

Cpds	**MCF-7** (iEqE in µM)				**T47D** (iEqE in µM)			
	$iEqE_{50}$ [a]	$iEqE_{10}$ [a]	$iEqE_1$ [a]	IL [b]	$iEqE_{50}$ [a]	$iEqE_{10}$ [a]	$iEqE_1$ [a]	IL [b]
2	< 0.1	1.0	-	**M**	< 0.01	10.0	-	**M**
3	< 0.01	< 0.1	-	**M**	< 0.01	5.6	-	**M**
4	< 0.01	< 0.01	-	**S**	3.2	8.7	-	
39	< 0.01	< 0.01	-	**S**	< 0.01	5.2	9.3	**M**
40	< 0.01	<0.01	< 0.01		< 0.01	<0.01	< 0.01	
41	< 0.01	<0.01	< 0.01		< 0.01	<0.01	< 0.01	
42	< 0.01	<0.01	-	**S**	< 0.01	<0.01	-	**S**
43	< 0.01	< 0.01	-	**S**	< 0.01	< 0.01	10.0	**S**
44	< 0.01	9.7	-	**M**	0.5	8.0	9.7	
45	< 0.01	< 0.01	-	**S**	< 0.01	< 0.01	10.0	**S**
46	< 0.01	< 0.01	< 0.01		< 0.01	< 0.01	< 0.01	
Tam	0.1	0.5	5.0		0.1	0.8	9.0	

[a] $iEqE_{50}$, $iEqE_{10}$, $iEqE_1$ represent the concentrations of the compounds (µM) that decrease the cell proliferation (enhanced by 100 pM of E_2) to equivalent levels of those induced by 50 pM, 10 pM, and 1 pM of E_2 treatment, respectively. The values were calculated by linear regression analysis using four different concentrations. [b] IL inhibitory level of the compound. Mild inhibition (M), more than 50% inhibition through the concentrations were tested. Strong inhibition (S), more than 90% inhibition through the concentrations were tested. Tamoxifen (Tam).

Table 35. Inhibitory activities of all constituents (**47-74)** from *P. scandens*

Cpds	**MCF-7** (iEqE in µM)				**T47D** (iEqE in µM)			
	$iEqE_{50}$ [a]	$iEqE_{10}$ [a]	$iEqE_1$ [a]	IL [b]	$iEqE_{50}$ [a]	$iEqE_{10}$ [a]	$iEqE_1$ [a]	IL [b]
47	4.2	9.8	-		5.7	-	-	
48	< 0.01	< 0.01	-	**S**	< 0.01	8.9	-	**M**
49	< 0.01	5.0	-	**M**	< 0.01	8.2	-	**M**
50	-	-	-		< 0.01	-	-	
51	-	-	-		-	-	-	
52	-	-	-		-	-	-	
53	-	-			1.0	-	-	
54	-	-	-		-	-	-	
55	0.1	-	-		-	-	-	
56	-	-	-		-	-	-	
57	< 0.1	0.1	-	**M**	0.1	-	-	
58	-	-	-		< 0.01	-	-	
59	3.0	-	-		0.1	-	-	
60	2.1	8.6	10.0		< 0.01	6.7	9.3	**M**
61	0.8	-	-		-	-	-	
62	-	-	-		5.8	-	-	
63	-	-	-		-	-	-	
64	-	-	-		-	-	-	
65	< 0.01	10.0	-	**M**	< 0.01	-	-	
66	< 0.01	-	-		< 0.01	< 0.01	-	**S**
67	< 0.01	< 0.01	-	**S**	< 0.01	< 0.01	< 0.01	
58	< 0.01	-	-		< 0.01	-	-	
69	< 0.01	-	-		< 0.01	< 0.1	-	**M**
70	< 0.01	10.0	-	**M**	< 0.01	< 0.01	< 0.01	**S**
71	< 0.01	-	-		< 0.01	-	-	
72	< 0.01	-	-		< 0.01	-	-	
73	< 0.01	< 0.01	-	**S**	< 0.01	-	-	
74	-	-	-		< 0.01	-	-	
Tam	0.1	0.5	5.0		0.1	0.8	9.0	

[a] $iEqE_{50}$, $iEqE_{10}$, $iEqE_1$ represent the concentrations of the compounds (µM) that decrease the cell proliferation (enhanced by 100 pM of E_2) to equivalent levels of those induced by 50 pM, 10 pM, and 1 pM of E_2 treatment, respectively. The values were calculated by linear regression analysis using four different concentrations. [b] IL inhibitory level of the compound. Mild inhibition (M), more than 50% inhibition through the concentrations were tested. Strong inhibition (S), more than 90% inhibition through the concentrations were tested. Tamoxifen (Tam).

3.5 Discussion

Extracts from the two Bangladeshi medicinal plants, leaves of *Terminalia citrina* (Combretaceae) and aerial parts of *Pothos scandens* (Araceae) were isolated, and thirty nine new compounds of different structures along with thirty five other known constituents were characterized using various spectroscopic techniques. Among the new compounds, nine new furofuran lignans (**1**, **5-12**), one furofuranone lignan (**13**), thirteen furofuran ligan glucosides (**14-26**), five furofuranone lignan glucosides (**27-31**), and seven tetrahydrofuran lignan glucosides (**32-38**) were reported from *T. citrina* whereas three hemiterpene glucoside aromatic esters (**47-49**) and one phenylisobutanoid alocohol (**50**) were isolated from *P. scandens*.

Lignans and lignan type compounds were the major constituents of *T. citrina* plant whereas flavonoid and triterpenoids comprised the major portion of *P. scandens*. The isolates were also tested for their estrogenic/anti-estrogenic activity using the estrogen-responsive human breast cancer cell lines MCF-7 and T47D. Furofuran ring containing lignans (**1-26**) exhibited significant inhibitory effects on the estrogen-induced cell proliferation in the both cell lines compared to other series of lignans. Glycosylation of lignans reduces the antiestrogenic property significantly such as furofuran lignan glucoside **17** showed highest 50% cell inhibition of MCF-7 cells at a concentration 0.01 μM whereas respective aglycone lignan **5** inhibited 90% cell proliferation in both cells. While glycoside linkages decrease the inhibitory efficacy to a greater extent, very few of them (**15, 21,** and **31**) showed even 90 %-suppression of estrogen-induced cell proliferation at the concentrations lower than 10 nM.

Polyalkoxylation of aromatic rings in furofuran lignan increases the inhibitory activity significantly. Such as, eight methoxylated group containing **12** inhibited 99% cell proliferation at the lowest tested concentration of 10 nM during the experiment. All lignan glycosides showed mild to moderate antiestrogenic activity. The above findings are very similar to those of a previous study in which a furofuran lignan glycoside showed antiestrogenic activity on MCF-7 and T47D cell lines.[7] Few glycosides showed cell specific activity such as **19** showed inhibitory activity against T47D cells, whereas **16**, **20**, **23** and **26** showed antiestrogenic activity against MCF-7 cells. In case of furofuranone lignan glucosides, **27** showed 90% cell inhibition in T47D cells whereas 90% cell proliferation was inhibited in MCF-7 cells by **28**. The furofuranone lignan glycosides showed less potency than the corresponding furofuran lignan glycosides but more potent than tetrahydrofuran lignan glycosides. One the other hand, furofuran lignan glycosides with ditetraoxygenated aryl groups showed less potency when compared to the other compounds.

Plant-derived lignans are considered to be phytoestrogens that may exert estrogenic or antiestrogenic effects in the body. [101, 102] The majority of lignans are conjugated with sugar moieties and are commonly found as glycosides in nature. However, these lignans are converted by intestinal microflora into mammalian lignan metabolites that have estrogenic activity, such as enterodiol and

enterolactone. [103] Several studies have shown that dietary sesamin[104] and sesaminol triglucoside [105] can be biotransformed into enterolactone. In addition, tumor cell apoptosis and the suppression of E_2-enhanced MCF-7 cell proliferation were observed in enterolactone- and enterodioltreated nude mice. [106] A cross-sectional study of healthy postmenopausal women using urinary biomarkers has suggested that dietary lignans stimulate sex-hormone-binding globulin (SHBG) levels while lowering testosterone levels by inhibiting the catalytic conversion of androstenedione to testosterone. [107] Thus, the use of plant lignans to promote increased binding of free estradiol to SHBG may reduce the risk of breast cancer.

Pyrogallol derivatives (**48**, **49**, **60**) showed strong inhibition against both cell lines at low concentrations for their $iEqE_{10}$ values (**48,** <0.01 and 8.9 μM; **49**, 5.0 and 8.2 μM; **60,** 8.6 and 6.7 μM for MCF-7 and T47D, respectively). In particular, **60** exhibited the highest activity among the isolates from this plant, which antagonized 99% of E_2-induced cell proliferation with $iEqE_1$ values of 10 and 9.3 μM against MCF-7 and T47D cells, respectively. Their less oxygenated analogs (**47**, **61**) were almost inactive. Although the estrogenic/anti-estrogenic activity of gallic acid was predicted from possible hydrogen-bonding of its hydroxy groups with estrogen receptors (ERs) at His524 and Arg394–Glu353 in ER-α (His 475 and Arg 346–Glu305 of ER-β),[108] a non-estrogenic activity and cytotoxicity at high concentrations were reported so far.[109, 110] This is the first report on anti-estrogenic activity in naturally occurring syringoyl derivatives in vitro.

Flavonoid diglycosides such as apigenin *C*-glycosides (**52-55**), luteolin *C*-glycoside (**56**), kaempferol *O*-glycoside (**57**), and quercetin *O*-glycosides (**58, 59**) were isolated as abundant constituents of *P. scandens* in this study. Although apigenin and luteolin were reported to have anti-estrogenic activity via a binding-independent manner and through binding with ERs, respectively,[111] their *C*-glycosides did not show any notable activity. Flavonols (kaempferol and quercetin) were reported to have less affinity for ERs than flavones (apigenin and luteolin). [112] However, these flavonols have also been shown to inhibit growth factor signaling, such as EGF receptor tyrosine kinase, which plays an important role in breast cancer cell proliferation. [113] Because these *O*-glycosides (**57-59**) can be hydrolyzed to afford aglycones, they are supposed to have potential to act as antiestrogenic substances *in vivo*. Among the cycloartane type triterpenoids, inhibitory activity of enol form (**67**) was prominent.

However, the exact pathway how these isolates exerted antagonistic activity to estradiol and their estrogen receptor binding affinity was also not carried out due to lack of facility. As estrogen receptors such as ERα and ERβ are the main substrate for estradiol actions, immunoblotting assay can be carried out to identify the effect on the expression of these proteins in future. Besides, flow cytometric measurement of cell proliferation and immunofluroescence staining of estrogen receptors can provide useful information about the cell cycle arrest pathway. Detailed mechanistic pathway as well as animal trial also can be performed to establish their antiestrogenic efficacy in human.

Conclusion

The following study was conducted to investigate two Bangladeshi medicinal plants, *Terminalia citrina* (Combretaceae) and *Pothos scandens* (Araceae) because of their traditional uses in various ailments. The bio-assay guided isolation with several chromatographic techniques was used to isolate the constituents. These were identified by different NMR study and spectrophotometric analyses. All the isolates were investigated for their estrogenic and/or antiestrogenic activities in two different cell lines (MCF-7 and T47D).

The dissertation was separated into three chapters. In the chapter 1, author explained the isolation and structure elucidation of the novel furofuran lignans, furofuranone lignan, furofuran lignan glycosides, furofuranone lignan glycosides, tetrahydrofuran lignan glycosides from the leaves of *T. citrina*. Lignans seem to be the major constituents in this plant. Moreover, author also reported some known furofuran lignans, flavonoid glycosides, lignan glycosides along with miscellaneous constituents.

In the chapter 2, the isolation and structure elucidation of novel hemiterpene aromatic glucoside esters and a phenylisobutanoid were described. Consequently, a variety of known constituents such as flavone glycosides, flavonol glycosides, triterpenoids, phenolic glycosides and few primary metabolites were reported.

In the chapter, 3, author described the cell-based assay procedures for estrogenic and antiestrogenic activity of all isolates and presented their data. None of the isolates reported estrogenic activity whereas antiestrogenic potentiality of lignans, triterpenoids and flavonoid di-glycosides were prominent.

EXPERIMENTAL

General experimental procedures

A JASCO DIP-360 digital polarimeter was used to determine the optical rotations. The UV spectra were recorded on a Hitachi U2010 spectrophotometer. The ECD spectra were recorded using two different spectrophotometers, named as JASCO J-720WI and JASCO J-20A. 1D and 2D NMR spectra were measured on JEOL ECX-500 instrument (operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C) and JEOL JNM-α 400 instrument (operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C). Chemical shifts are expressed in a δ (ppm) scale with tetramethylsilane and/or residual solvents as internal standard and coupling constants (*J*) are in hertz. Mass spectra were recorded on a JEOL JMS 700 spectrometer using an *m*-nitrobenzyl alcohol matrix for HRFABMS measurements. Column chromatography was carried out with powdered silica gel (Kieselgel 60, 230-400 mesh, Merck KGaA, Dermstadt, Germany) and styrene-divinylbenzene (Diaion HP-20, 250-800 μm particle size, Mitsubishi Chemical Co., Ltd.). Precoated glass plates of silica gel (Kieselgel 60, F_{254}, Merck Co., Ltd., Japan) and RP-18 ($F_{254}S$, Merck KGaA) were used for TLC analysis. The TLC spots were investigated under UV light at 254 nm wavelength and spraying with dil. H_2SO_4 followed by heating. Repeated HPLC was carried out mainly with a JASCO model 887-PU pump and isolates were detected by an 875-UV variable-wavelength detector. Reversed-phase HPLC columns for preparative separations (Tosoh TSK gel ODS-80Ts, 5 μm, 6 x 60 x 2 cm, Nomura Chemical Co. Ltd., Tokyo, Japan, at flow rate 45 mL/min with detection at 205 nm; Inertsil ODS-3, 10 μm, 3 x 50 cm, GL science Co. Ltd., Tokyo, Japan, at flow rate 10 mL/min with detection at 205 nm) and semipreparative separations (Cosmosil Cholester, 5 μm, 2 x 25 cm, Nacalai Co. Ltd, Kyoto, Japan; YMC-Pack R&D ODS, 5 μm, 2 x 25 cm, YMC Co. Ltd.; Inetsil ODS, 5 μm, 2 x 25 cm; Inertsil Ph-3, 5 μm, 2 x 25 cm, GL science Co. Ltd., Tokyo, Japan) were used mainly for effective separations.

Chemicals

Fetal bovine serum (FBS) was purchased from Gibco (Grand Island, NY, USA). Eagle's minimum essential medium (EMEM) and Roswell park memorial institute medium (RPMI-1640) were purchased from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan). Penicillin and streptomycin were purchased from Meiji Seika Kaisha Ltd. (Tokyo, Japan). L-Glutamine, *D*- and *L*-glucose were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). 17β-Estradiol and dextran-coated-charcoal (DCC) were purchased from Sigma Chemicals (St. Louis, MO).

Plant materials

The leaves of *Terminalia citrina* were collected from Rangamati district in the hill tracts region of Bangladesh in May 2013, with prior official permission. The leaves were identified by Mr. Sardar Nasir Uddin, Senior Scientific Officer, National Herbarium, Mirpur, Dhaka and a voucher specimen has been

deposited in this herbarium (DACB accession no. 38094). Whole plant, *Pothos scandens* was collected from the National Botanical Garden, Dhaka, Bangladesh in September, 2013. The leaves were separated from other parts immediately. The plant was identified at the National Herbarium, Dhaka and a voucher specimen was deposited in the herbarium for future references (DACB accession no. 38578).

Extraction and Isolation

1) Extraction and isolation of *Terminalia citrina*

The air-dried powdered leaves of the plant (3.4 kg) were extracted four times with hot MeOH (4 x 15 L) by refluxing for 3 h each to afford a viscous mass of 608 g. The crude extract was then suspended in 2 L of water and partitioned with EtOAc (2 L x 3). The EtOAc extract (93 g) was subjected to silica gel column chromatograpy using a glass column and was eluted with a $CHCl_3$-MeOH gradient solvent system (100:0, 99: 1, 98:2, 95:5, 90:10, 67:33, 50:50). Individual fractions were collected and pooled by analyzing their TLC profiles to afford 16 combined fractions.

Among the combined fractions, fraction 5 [1.5 g: eluted with $CHCl_3$-MeOH (98:2)] was subjected to preparative HPLC with Tosoh TSK gel ODS-80Ts column (6 x 60 x 2 cm) using mobile phase MeCN-H_2O (45: 55) system and successive several semi-preparative HPLC's to afford **1** [6.9 mg; t_R 92 min, Cosmosil Cholester column with MeCN-H_2O (35:65), flow rate 9 mL/min], **13** [1.7 mg; t_R 102 min, Cosmosil Cholester column with MeOH-H_2O (50:50), flow rate 9 mL/min], **5** [14.4 mg; t_R 95 min, Cosmosil Cholester column with MeCN-H_2O (35:65), flow rate 9 mL/min], methoxy piperitol (**3**) [3.2 mg; t_R 126 min, Cosmosil Cholester column with MeOH-H_2O (50:50), flow rate 9 mL/min], **9** [1.4 mg; t_R 55 min, Cosmosil Cholester column with MeCN-H_2O (40:60), flow rate 9 mL/min], sesartemin (**4**) [45.0 mg; t_R 59 min, Cosmosil Cholester column with MeCN-H_2O (40:60), flow rate 9 mL/min], **10** [13.7 mg; t_R 112 min, Cosmosil Cholester column with MeOH-H_2O (50:50), flow rate 9 mL/min], excelsin (**2**) [5.9 mg; t_R 67 min, Cosmosil Cholester column with MeCN-H_2O (45:55)], **11** [26.3 mg; t_R 110 min, Cosmosil Cholester column with MeOH-H_2O (55:45), flow rate 9 mL/min with recycle mode], **6** [9.2 mg; t_R 155 min, Cosmosil Cholester column with MeOH-H_2O (55:45), flow rate 9 mL/min with recycle mode], **12** [3.1 mg; t_R 158 min, Cosmosil Cholester column with MeOH-H_2O (55:45), flow rate 9 mL/min], **8** [3.0 mg; t_R 208 min, Cosmosil Cholester column with MeOH-H_2O (55:45), flow rate 9 mL/min], and **7** [6.8 mg; t_R 215 min, Cosmosil Cholester column with MeOH-H_2O (55:45), flow rate 9 mL/min].

From the combined fractions, fraction 11 [1.5 g: eluted with $CHCl_3$-MeOH (90:10)] was subjected to preparative HPLC with Tosoh TSK gel ODS-80Ts column (6 x 60 x 2 cm) using MeCN-H_2O (25:75) system as the mobile phase, followed by semi-preparative HPLC to afford furofuran lignan series compounds such as **14** [13.7 mg; t_R56 min, YMC ODS column with MeCN-H_2O (20:80), flow rate 9

mL/min], **16** [52.1 mg; t_R65 min, YMC ODS with MeCN-H_2O (20:80), flow rate 9 mL/min], **22** [9.0 mg; t_R 107 min,YMC ODS column with MeCN-H_2O (20:80), flow rate 7 mL/min], **23** [11.1 mg; t_R 135 min, YMC ODS column with MeCN-H_2O (20:80), flow rate 7.5 mL/min with recycle mode], **26** [4.9 mg; t_R 123 min, YMC ODS column with MeCN-H_2O (20:80), flow rate 7 mL/min], **25** [19.9 mg; t_R 140 min, YMC ODS column with MeCN-H_2O (20:80), flow rate 9.0 mL/min], **15** [27.2 mg; t_R 144 min, YMC ODS column with MeCN-H_2O (22:78), flow rate 9.0 mL/min with recycle mode], **17** [68.7 mg; t_R 160 min, YMC ODS column with MeCN-H_2O (22:78), flow rate 9.0 mL/min with recycle mode], **19** [6.3 mg; t_R 116 min, YMC ODS column with MeCN-H_2O (22:78), flow rate 9.0 mL/min with recycle mode], **24** [7.2 mg; t_R 168 min, YMC ODS column with MeCN-H_2O (23:77), flow rate 9.0 mL/min with recycle mode], **18** [1.8 mg; t_R 202 min, CosmosilCholester column with MeCN-H_2O (22:78), flow rate 9.0 mL/min with recycle mode], **20** [5.1 mg; t_R 98 min, Cosmosil Cholester column with MeCN-H_2O (23:77), flow rate 9.0 mL/min with recycle mode], and **21** [8.3 mg; t_R 144 min, Cosmosil Cholester column with MeCN-H_2O (20:80), flow rate 7.5 mL/min with recycle mode], respectively.

Five Furofuranone and seven tetrahydrofuran series lignan glycosides were also afforded from preparative separations of fraction 11 using MeCN-H_2O (25:75) system as mobile phase which are **29** [7.0 mg; t_R 93 min, YMC ODS column with MeCN-H_2O (20:80), flow rate 7.5 mL/min with recycle mode], **33** [6.3 mg; t_R 121 min, YMC ODS column with MeCN-H_2O (20:80), flow rate 7.5 mL/min with recycle mode], **32** [3.3 mg; t_R 110 min, YMC ODS column with MeCN-H_2O (22.5:77.5), flow rate 6.5 mL/min with recycle mode], **37** [2.1 mg; t_R 203 min, Cosmosil Cholester column with MeCN-H_2O (20:80), flow rate 7.5 mL/min with recycle mode], **36** [11.2 mg; t_R 90 min, YMC ODS column with MeCN-H_2O (22.5:77.5), flow rate 9.0 mL/min with recycle mode], **34** [20.4 mg; t_R 127 min, YMC ODS column with MeCN-H_2O (22:78), flow rate 9.0 mL/min with recycle mode], **35** [8.1 mg; t_R 124 min, YMC ODS column with MeCN-H_2O (22:78), flow rate 9.0 mL/min with recycle mode], **27** [7.4 mg; t_R 130 min, YMC ODS column with MeCN-H_2O (22:78), flow rate 9.0 mL/min with recycle mode], **28** [1.7 mg; t_R 160 min, YMC ODS column with MeCN-H_2O (22:78), flow rate 9.0 mL/min with recycle mode], **30** [4.5 mg; t_R 152 min, YMC ODS column with MeCN-H_2O (22:78), flow rate 9.0 mL/min with recycle mode], **38** [2.9 mg; t_R 220 min, Cosmosil Cholester column with MeCN-H_2O (22:78), flow rate 9.0 mL/min with recycle mode], and **31** [2.0 mg; t_R 120 min, Cosmosil Cholester column with MeCN-H_2O (25:75), flow rate 9.0 mL/min].

From the combined fractions, fraction 10 [3.0 g: eluted with $CHCl_3$-MeOH (90:10)] was subjected into HP-20 column chromatography and eluted with MeOH-H_2O (50:50), MeOH and EtOAc (each 2 L). Sub-fraction 10-1 [325.0 mg: eluted with MeOH- H_2O (50:50)] was subjected into preparative HPLC with Inertsil ODS-3 column (3 x 50 cm) using MeOH-H_2O gradient solvent systems (started with 25% MeOH and reached 45% within 10 hrs) to afford 29 fractions. Sub-fraction 10-1-10 (8.4 mg; t_R 270 min) was again subjected to semi-preparative HPLC to afford *p*-hydroxybenzoic acid (**45**) [2.2 mg; t_R 60 min,

Cosmosil Cholester column with MeOH-H_2O (20:80), flow rate 6 mL/min with recycle mode]. Sub-fraction 10-1-14 (14.0 mg; t_R 520 min) was again subjected to semi-preparative HPLC to afford blumenol A (**46**) [6.9 mg; t_R 73 min, Cosmosil Cholester column with MeOH-H_2O (20:80), flow rate 7 mL/min with recycle mode].

Among the combined fractions, fraction 14 [7.0 g: eluted with $CHCl_3$-MeOH (2:1)] was subjected into polyamide column chromatography and eluted with decreasing the polarities of the MeOH-H_2O gradient solvent systems. Fractions were collected and pooled by TLC analysis to afford 7 fractions.

From the polyamide column eluted fractions, fraction no. 14-1 [2.0 g: eluted with MeOH- H_2O (30:70)] was subjected to preparative HPLC with Inertsil ODS-3 column (3 x 50 cm) using MeCN-H_2O (16:84) system as the mobile phase to afford 27 fractions. From these, sub-fraction 14-1-17 (8.8 mg; t_R 440 min) was subjected to semi-preparative HPLC to afford *erytrho*-secoisolariciresinol-9′-*O*-glucoside (**43**) [2.2 mg; t_R 34 min, YMC ODS column with MeCN-H_2O (18:82), flow rate 8 mL/min with recycle mode]. Sub-fraction 14-1-18 (5.4 mg; t_R 506 min) was subjected to semi-preparative HPLC to afford *threo*-secoisolariciresinol-9′-*O*-glucoside (**42**) [1.8 mg; t_R 44 min, YMC ODS column with MeCN-H_2O (18:82), flow rate 7 mL/min with recycle mode]. Sub-fraction 22 [18.2 mg; t_R 678 min] was subjected to dissolve in MeOH for further purification and caprolactam (**44**) (9.6 mg) was precipitated which was purified through filtration.

From these fractions, fraction no. 14-2 [400.0 mg: eluted with MeOH-H_2O (40:70)] was subjected to semi-preparative HPLC with Cosmosil Cholester column (8 times injected) using MeCN-H_2O (12:84) system as the mobile phase to afford 7 fractions. From these, sub-fraction 2 (19.5 mg; t_R 20 min) was again subjected to semi-preparative HPLC to afford isoorientin (**39**) [8.0 mg; t_R 116 min, Cosmosil Cholester column with MeCN-H_2O (12:88), flow rate 9 mL/min with recycle mode]. Sub-fraction 5 (14.5 mg; t_R 55 min) was again subjected to semi-preparative HPLC to afford nicotiflorine (**40**) [4.2 mg; t_R 52 min, Cosmosil Cholester column with MeCN-H_2O (20:80), flow rate 9 mL/min with recycle mode] and isorhamnetin-3-*O*-rutinoside (**41**) [5.4 mg; t_R 56 min, Cosmosil Cholester column with MeCN-H_2O (20:80), flow rate 9 mL/min with recycle mode].

1) Extraction and isolation of *Pothos scandens*

Dried powdered of stem and root parts (2.0 kg approx.) were extracted three times with hot MeOH (3 x 15 L) by until reflux conditions were achieved, this being maintained for 3 h. The extracts were then combined and solvent was evaporated at reduced pressure at 45^0C to yield a viscous mass of 146 g. The concentrated extracts were suspended in water (1.5 L) and partitioned with EtOAc (3 x 1.5 L) to yield dried EtOAc fraction 33 g and H_2O-soluble fraction 78 g. The H_2O-soluble fraction was subjected to HP-20 column chromatography using H_2O, 50% MeOH, 75% MeOH and MeOH as eluted solvents (5 L

each) to yield dried H_2O (58 g) , 50% MeOH (9.1 g), 75% MeOH (3.6 g) and MeOH (1.2 g) fractionates, respectively. The 50% MeOH soluble fraction (9.1 g) was subjected to silica gel column chromatography using glass column (6 x 50 cm) and fractionated (150 mL for each fraction) using a $CHCl_3$-MeOH-H_2O gradient solvent system (90:10:0, 85:15:0, 80:20:2, 65:35:10, 50:50:0, 3 L each). All the fractions were collected and pooled by TLC analysis to afford 12 combined fractions.

From these combined fractions, fraction 4 [0.9 g: eluted with $CHCl_3$-MeOH-H_2O gradient solvent system (85:15:0)] was chromatographed again on silica gel glass column (2 x 50 cm) to be fractionated into 11 fractions using a $CHCl_3$-MeOH-H_2O gradient solvent system (90:10:0, 85:15:0, 80:20:2, 65:35:10, 50:50:0, 500 mL each). Among these, fraction 4-2 [520 mg: eluted with $CHCl_3$-MeOH (90:10)] was subjected to preparative HPLC with Inertsil ODS-3 column (3 x 50 cm) using MeCN-H_2O (15:85) system as the mobile phase to afford 12 fractions. Among the fractions, fraction 4-2-6 (5.5 mg; t_R 130 min) and 4-2-12 (112.0 mg; wash part) were again subjected to semi-preparative HPLC to yield compound canthoside A (**64)** [1.2 mg; t_R 106 min, YMC ODS column with MeCN-H_2O (9:91), flow rate 9 mL/min] and pothobanol (**50)** [1.1 mg; t_R 78 min, Cosmosil Cholester column with solvent MeCN-H_2O (14:86)], respectively. Fraction 4-2-10 (22.0 mg; t_R 235 min) was subjected to semi-prepartive HPLC [Cosmosil Cholester column with solvent MeCN-H_2O (14:86), flow rate 9 mL/min with recycle mode] to afford pothobanoside A (**47)** [1.0 mg; t_R 50 min] and pothobanoside B (**48)** [3.2 mg; t_R 54 min].

From the combined fractions, fraction 8 [0.75 g: eluted with $CHCl_3$-MeOH-H_2O gradient solvent system (80:20:2)] was subjected to preparative HPLC with Inertsil ODS-3 column (3 x 50 cm) using MeOH-H_2O (30:70) system as the mobile phase to afford 26 fractions. Among these fractions, fraction 8-9 (10.3 mg; t_R 390 min), 8-11 (7.0 mg; t_R 404 min), 8-18 (27.4 mg; t_R 460 min), 8-19 (11.0 mg; t_R 490 min), 8-21 (64.3 mg; t_R 508 min), 8-23 (31.0 mg; t_R 565 min) and 8-24 (26.0 mg; t_R 575 min) were subjected to semi-preparative HPLC with Cosmosil Cholester column to give compound eleutherazine B (**51)** [1.2 mg; t_R 94 min with solvent MeOH-H_2O (15:85)], pothobanoside C (**49)** [1.5 mg; t_R 190 min with solvent MeOH-H_2O (15:85)], isoschaftoside (**52)** [4.5 mg; t_R 92 min with solvent MeOH-H_2O (30:70)] along with vicenin-2 (**53)** [2.4 mg; t_R 102 min with solvent MeOH-H_2O (30:70)], scoparin 2″-*O*-xyloside (**56)** [2.8 mg; t_R 44 min with solvent MeOH-H_2O (35:65)], vitexin 2″-*O*-xyloside (**55)** [52.2 mg; t_R 74 min with solvent MeOH-H_2O (35:65)] together with neoschaftoside (**54)** [2.1 mg; t_R 89 min with solvent MeOH-H_2O (35:65)], kaempferol-3-*O*-gentiobioside (**57)** [23.5 mg; t_R 128 min with solvent MeOH-H_2O (35:65)] and isorhamnetin-3-*O*-gentiobioside **(59)** [13.7 mg; t_R 178 min with solvent MeOH-H_2O (35:65)], respectively. All known compounds were identified by comparison with the reported data.

From the combined fractions, fraction 7 [0.5 g: eluted with $CHCl_3$-MeOH-H_2O gradient solvent system (80:20:2)] was subjected to preparative HPLC with inertsil ODS-3 column using MeCN-H_2O

gradient system as mobile phase (started with 5% and reached to 17.5% within 10 hrs) to afford 22 fractions. Among these fractions, fraction 7-5 (22.9 mg; t_R 50 min) was again subjected to semi-preparative HPLC to yield compound canthoside B **(60)** [5.7 mg; t_R 78 min, YMC ODS column with MeOH- H_2O (5:95), flow rate 9 mL/min] and markhamioside F (**63)** [4.8 mg; t_R 106 min, YMC ODS column with MeOH- H_2O (5:95), flow rate 9 mL/min]. Sub-fraction 7-11 and 7-20 were identified as zizybeoside I (**61**) (10.5 mg; t_R 74 min) and vitexin 2″-*O*-xyloside (**55)** (52.2 mg; t_R 320 min), respectively.

The fraction 9 [1.1 g: eluted with $CHCl_3$-MeOH-H_2O gradient solvent system (65:35:10)] was subjected to preparative HPLC with Tosoh TSK gel ODS-80Ts column (6 x 60 x 2 cm) using MeCN:H_2O gradient solvent system [starting MeCN:H_2O (10:90) with increased the concentration of MeCN at 2.5% in every hour; flow rate at 45 mL/min] to afford 24 fractions in which most of the compounds were identified as same occurred in other fractions. The HPLC eluted fraction 9-9 (6.9 mg; t_R 151 min), 9-10 (8.3 mg; t_R 159 min), 9-11 (7.0 mg; t_R 167 min) and 9-18 (20.0 mg; t_R 245 min) were purified again in semi-preparative HPLC to afford L-phenylalanine (**73**) [1.6 mg; t_R 21 min, C-18 GL science column with solvent MeCN-H_2O (5: 95), flow rate 9 mL/min with recycle mode], L-tryptophan (**74**) [1.3 mg; t_R 37 min, C-18 GL science column with solvent MeCN-H_2O (4: 96), flow rate 9 mL/min with recycle mode], (3*S*)1,2,3,4-tetrahydro-3-carboxy-2-carboline (**62)** [1.7 mg; t_R 48 min, Inertsil ph-3 column with solvent MeCN-H_2O (4: 96), flow rate 9 mL/min] and quercetin-3-*O*-gentiobioside (**58)** [6.8 mg; t_R 39 min, Inertsil ODS column with solvent MeCN-H_2O (17.5: 82.5), flow rate 9 mL/min].

The EtOAc soluble fraction (16.1 g) was subjected to silica gel column chromatography using glass column (6 x 50 cm) and fractionated (150 mL for each fraction) using a hexane: $CHCl_3$-MeOH (95:5) gradient solvent system [4:1, 2:1, 1:1, 0:1, $CHCl_3$-MeOH (9:1, 1:1), 3 L each]. All fractions were collected and pooled by TLC analysis to afford 14 combined fractions.

Among these fractions, fraction F [1.1 g: eluted with hexane: $CHCl_3$-MeOH (95:5) gradient solvent system (2:1)] was subjected to preparative HPLC with Tosoh TSK gel ODS-80Ts column (6 x 60 x 2 cm) using MeCN-H_2O (95:5) solvent system as mobile phase with flow rate at 16 mL/min to afford 19 fractions. From these fractions, fraction F-13 (37 mg; t_R 430 min) was dissolved in MeOH. As a result, tetradecanoic acid (**72**) (6.6 mg; white powders) was precipitated which was purified through filtration. Fraction F-19 (270 mg; wash part) was subjected to semi-preparative HPLC with Inertsil C8-3 column (five times injected) using MeCN-H_2O (97.5: 2.5) solvent as mobile phase to afford 7 fractions. From these sub-fractions, fraction F-19-3 (19.6 mg; t_R 28 min) and F-19-6 (28.8 mg; t_R 52 min) were again subjected to semi-preparative HPLC to afford stigmast-4, 22-dien-3-one (**66**) [5.5 mg; t_R 142 min, YMC ODS column with solvent MeCN-H_2O (95: 5), flow rate 9 mL/min] and 24-methylenecycloartanyl

ferulate (**70**) [3.5 mg; t_R 188 min, YMC ODS column with solvent MeCN-H_2O (97.5: 2.5), flow rate 9 mL/min], respectively. Fraction F-19-4 (44.8 mg; t_R 37 min) was again subjected to semi-preparative HPLC with YMC ODS column using MeCN-H_2O (97.5: 2.5) solvent as mobile phase to afford 24-en-cycloartenone (**69**) (3.8 mg; t_R 128 min), stigmast-4, 22-dien-3-one (**66**) (1.8 mg; t_R 140 min) and stigmast-4-en-3-one (**65**) (20.1 mg; t_R 155 min). Fraction F-19-5 (27.5 mg; t_R 46 min) was again subjected to semi-preparative HPLC with YMC ODS column using MeCN-H_2O (97.5: 2.5) solvent as mobile phase to afford 24-methylenecycloartenone (**68**) (10.1 mg; t_R 145 min) and 24-methylenecycloartanol (**67**) (1.5 mg; t_R 178 min).

From these fractions, fraction K [0.7 g: eluted with chloroform-MeOH (9:1) gradient solvent system] was dissolved in MeOH and white precipitation of *β*-sitosterol glucoside (**71**) (43.3 mg; white crystals) appeared which was purified through recrystallization.

Acid hydrolysis and sugar identification of glycosides (14-38, 47-49)

The absolute configurations of the sugar moieties were identified according to the procedure described in a previous report, with a slight modification.[114] All of the isolates (0.5-1.0 mg) were hydrolyzed in the presence of 50% TFA (50 μL) in a hot water bath at 100 °C for 1 h. The reaction mixtures were air-dried, diluted with H_2O, and extracted with EtOAc. The H_2O layers were concentrated under a vacuum evaporator. The residues were stirred with *L*-cysteine methyl ester hydrochloride in pyridine (20 mg/mL, 50 μL) at 60 °C for 1 h. *o*-Tolylisothiocyanate (5 μL) was added to the mixtures and heated at 60 °C for 1 h. The reaction mixtures were then air-dried and concentrated under a vacuum evaporator. A few drops of MeOH were added to each sample before HPLC analysis [column: YMC-pack R&D ODS, 4.6 x 300 mm, MeCN-H_2O (22:78) used as mobile phase, flow rate 1 mL/min, UV detection at 205 nm] and D-glucose (t_R 35 min) and L-glucose (t_R 32.5 min) were identified by comparison with standard samples.

New compounds from *Terminalia citrina*

Terminin A (**1**): Colorless, amorphous powder; $[\alpha]^{25}_D$ +38.75 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 222.5 (3.83), 284 (3.72) nm; ECD (*c* 0.27 mM, MeCN) 210 (*Δ*ε +10.4), 230 (*Δ*ε +1.34), 290 (*Δ*ε -0.25) nm; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; HRFABMS *m/z* 417.1537 $[M + H]^+$ (calcd for $C_{22}H_{25}O_8$, 417.1549).

Terminin B (**5**): Pale yellow, amorphous powder; $[\alpha]^{25}_D$ +21.8 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 222.5 (3.98), 282.5 (3.65) nm; ECD (*c* 0.2 mM, MeCN) 210 (*Δ*ε +14.06), 238 (*Δ*ε +1.89), 280 (*Δ*ε

+0.5) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; HRFABMS *m/z* 446.1604 $[M]^{+}$ (calcd for $C_{23}H_{26}O_9$, 446.1576).

Terminin C (**6**): Pale yellow, amorphous powder; $[\alpha]^{25}_{D}$+43.3 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 228 (3.95), 278.5 (3.5) nm; ECD (*c* 0.25 mM, MeCN) 212 (*Δ*ε +14.8), 245 (*Δ*ε +0.5), 275 (*Δ*ε +0.45) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 2; HRFABMS *m/z* 461.1819 $[M + H]^{+}$ (calcd for $C_{24}H_{29}O_9$, 461.1811).

Terminin D (**7**): Pale yellow, amorphous powder; $[\alpha]^{25}_{D}$ +17.82 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 219.5 (3.94), 281.5 (3.4) nm; ECD (*c* 0.22 mM, MeCN) 208 (*Δ*ε +15.6), 221 (*Δ*ε +7.89), 290 (*Δ*ε +0.52) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 2; HRFABMS *m/z* 491.1906 $[M + H]^{+}$ (calcd for $C_{25}H_{31}O_{10}$, 491.1917).

Terminin E (**8**): Pale yellow, amorphous powder; $[\alpha]^{25}_{D}$ +9.72 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.03), 279.5 (3.15) nm; ECD (*c* 0.17 mM, MeCN) 207 (*Δ*ε +12.5), 235 (*Δ*ε -2.53), 290 (*Δ*ε -0.12) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 2; HRFABMS *m/z* 491.1932 $[M + H]^{+}$ (calcd for $C_{25}H_{31}O_{10}$, 491.1917).

Terminin F (**9**): Pale yellow, amorphous powder; $[\alpha]^{25}_{D}$ +93.75 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 209 (3.91), 277.5 (3.17) nm; ECD (*c* 0.12 mM, MeCN) 210 (*Δ*ε +2.7), 235 (*Δ*ε -0.42), 275 (*Δ*ε +0.2) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 2; HRFABMS *m/z* 447.2041 $[M + H]^{+}$ (calcd for $C_{24}H_{31}O_8$, 447.2018).

Terminin G (**10**): Pale yellow, amorphous powder; $[\alpha]^{25}_{D}$ +28.1 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 225 (4.1), 277 (3.44) nm; ECD (*c* 0.2 mM, MeCN) 212 (*Δ*ε +15.6), 230 (*Δ*ε +2.02), 280 (*Δ*ε +0.9) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 3; HRFABMS *m/z* 476.2071 $[M]^{+}$ (calcd for $C_{25}H_{32}O_9$, 476.2046).

Terminin H (**11**): Pale yellow, amorphous powder; $[\alpha]^{25}_{D}$ +22.1 (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ε) 227 (4.08), 280 (3.69) nm; ECD (*c* 0.3 mM, MeCN) 215 (*Δ*ε +10.4), 235 (*Δ*ε -1.04), 275 (*Δ*ε +0.52) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 3; HRFABMS *m/z* 507.2213 $[M + H]^{+}$ (calcd for $C_{26}H_{35}O_{10}$, 507.2230).

6-Epiterminin H (**12**): Pale yellow, amorphous powder; $[\alpha]^{25}_{D}$ +49.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 217 (3.99), 279.5 (3.4) nm; ECD (*c* 0.13 mM, MeCN) 210 (*Δ*ε +14.7), 233 (*Δ*ε +3.6), 277 (*Δ*ε +0.1) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 3; HRFABMS *m/z* 507.2204 $[M + H]^{+}$ (calcd for $C_{26}H_{35}O_{10}$, 507.2230).

Terminin I (**13**): Pale yellow, amorphous powder; $[\alpha]^{25}_{D}$ +24.3 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 217.5 (4.02), 283.5 (3.45) nm; ECD (*c* 0.2 mM, MeCN) 217 (*Δ*ε +10.9), 243 (*Δ*ε +0.56), 290 (*Δ*ε +0.22) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 4; HRFABMS *m/z* 461.1427 $[M + H]^{+}$ (calcd for $C_{23}H_{25}O_{10}$, 461.1447).

Terminaloside A (**14**): Pale yellow, amorphous powder; $[\alpha]^{25}_{D}$ +3.7 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 212 (4.17), 283 (3.82) nm; ECD (*c* 0.2 mM, MeOH) 208 (*Δ*ε +5.5), 235 (*Δ*ε +1.0), 285 (*Δ*ε -0.6) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 5; HRFABMS *m/z* 587.1763 $[M + Na]^{+}$ (calcd for $C_{27}H_{32}O_{13}Na$, 587.1741).

Terminaloside B (**15**): Pale yellow, amorphous powder; $[\alpha]^{25}_{D}$ +38.3 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 226 (4.08), 282 (3.84) nm; ECD (*c* 0.2 mM, MeOH) 210 (*Δ*ε+10.9), 230 (*Δ*ε +2.1), 285 (Δε -0.4) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 5; HRFABMS *m/z* 579.2067 $[M + H]^{+}$ (calcd for $C_{28}H_{35}O_{13}$, 579.2077).

Terminaloside C (**16**): Yellow, amorphous powder; $[\alpha]^{25}_{D}$ +28.1 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.21), 281 (3.67) nm; ECD (*c* 0.2 mM, MeOH) 215 (*Δ*ε +6.8), 235 (*Δ*ε +1.2), 288 (*Δ*ε -0.9) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 5; HRFABMS *m/z*595.2054 $[M + H]^{+}$ (calcd for $C_{28}H_{35}O_{14}$, 595.2027).

Terminaloside D (**17**): Pale yellow, amorphous powder; $[\alpha]^{25}_{D}$ +45.4 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.25), 279 (3.61) nm; ECD (*c* 0.2 mM, MeOH) 215 (*Δ*ε +10.4), 230 (*Δ*ε +1.7), 285 (*Δ*ε -0.4) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 6; HRFABMS *m/z* 631.2006 $[M + Na]^{+}$ (calcd for $C_{29}H_{36}O_{14}$ Na, 631.2002).

2-Epiterminaloside D (**18**): Colorless, amorphous powder; $[\alpha]^{25}_{D}$ +119.7 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.41), 277 (3.66) nm; ECD (*c* 0.1 mM, MeOH) 210(*Δ*ε +7.5), 230 (*Δ*ε +6.6), 281 (*Δ*ε -0.6) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 6; HRFABMS *m/z* 631.1981 $[M + Na]^{+}$ (calcd for $C_{29}H_{36}O_{14}$ Na, 631.2002).

Terminaloside E (**19**): Pale yellow, amorphous powder; $[\alpha]^{25}_{D}$ +38.1 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 217 (4.21), 273 (3.45) nm; ECD (*c* 0.2 mM, MeOH) 212(*Δ*ε +4.2), 238 (*Δ*ε +1.0), 280 (*Δ*ε +0.5) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 6; HRFABMS *m/z*579.2096 $[M + H]^{+}$ (calcd for $C_{28}H_{35}O_{13}$, 579.2078).

Terminaloside F (**20**): Pale yellow, amorphous powder; $[\alpha]^{25}_{D}$ +40.6 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.26), 283 (3.69) nm; ECD (*c* 0.2 mM, MeOH) 210 (*Δ*ε +12.7), 235 (*Δ*ε +3.3), 280 (*Δ*ε -0.4) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 7; HRFABMS *m/z* 647.1933 $[M + Na]^{+}$ (calcd for $C_{29}H_{36}O_{15}$ Na, 647.1951).

Terminaloside G (**21**): Pale yellow, amorphous powder; $[\alpha]^{25}_{D}$ +42.3 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 214 (4.40), 277 (3.52) nm; ECD (*c* 0.2 mM, MeOH) 215 (*Δ*ε +10.9), 230 (*Δ*ε +1.8), 285 (*Δ*ε -0.5) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 7; HRFABMS *m/z*625.2426 $[M + H]^{+}$ (calcd for $C_{30}H_{41}O_{14}$, 625.2496).

Terminaloside H (**22**): Pale yellow, amorphous powder; $[\alpha]^{25}_{D}$ +7.6 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 215 (4.23), 281 (3.63) nm; ECD (*c* 0.1 mM, MeOH) 215 (*Δ*ε +12.6), 235 (*Δ*ε -3.4), 288 (*Δ*ε -

0.7) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 7; HRFABMS *m/z* 663.2281 $[M + Na]^+$ (calcd for $C_{30}H_{40}O_{15}$ Na, 663.2264).

Terminaloside I (**23**): Pale yellow, amorphous powder; $[\alpha]^{25}_{D}$ +8.0 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.2), 280 (3.63) nm; ECD (*c* 0.2 mM, MeOH) 210 (*Δ*ε +11.5), 232 (*Δ*ε -4.0), 286 (*Δ*ε +0.4) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 8; HRFABMS *m/z* 663.2250 $[M + Na]^+$ (calcd for $C_{30}H_{40}O_{15}$ Na, 663.2264).

Terminaloside J (**24**): Yellow, amorphous powder; $[\alpha]^{25}_{D}$ +51.2 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 212 (4.6), 279 (3.77) nm; ECD (*c* 0.1 mM, MeOH) 210 (*Δ*ε +14.5), 235 (*Δ*ε -5.0), 280 (*Δ*ε + 0.3) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 8; HRFABMS *m/z* 677.2399 $[M + Na]^+$ (calcd for $C_{31}H_{42}O_{15}Na$, 677.2421).

Terminaloside K (**25**): Yellow, amorphous powder; $[\alpha]^{25}_{D}$ +32.3 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 214 (4.29), 283 (3.77) nm; ECD (*c* 0.2 mM, MeOH) 210 (*Δ*ε+12.0), 243 (*Δ*ε +0.1), 288 nm (*Δ*ε -1.0); for ^{1}H and ^{13}C NMR spectroscopic data, see Table 9; HRFABMS *m/z* 663.2263 $[M + Na]^+$ (calcd for $C_{30}H_{40}O_{15}$ Na, 663.2264).

6-Epiterminaloside K (**26**): Pale yellow, amorphous powder; $[\alpha]^{25}_{D}$ +52.3 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 214 (4.24), 283 (3.76) nm; ECD (*c* 0.2 mM, MeOH) 205 (*Δ*ε +11.6), 230 (*Δ*ε +5.9), 290 (*Δ*ε +0.5) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 9; HRFABMS *m/z* 663.2238 $[M + Na]^+$ (calcd for $C_{30}H_{40}O_{15}$ Na, 663.2264).

Terminaloside L (**27**): Pale yellow, amorphous powder; $[\alpha]^{25}_{D}$ +42.0 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log *ε*) 216 (4.46), 272 (3.47) nm; ECD (*c* 0.2 mM, MeOH) 215 (Δ*ε* +12.4), 245 (Δ*ε* +4.0), 280 (Δ*ε* +0.8) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 10; HRFABMS *m/z* 593.1877 $[M + H]^+$ (calcd for $C_{28}H_{33}O_{14}$, 593.1870).

Terminaloside M (**28**): Colorless, amorphous powder; $[\alpha]^{25}_{D}$ +52.4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log *ε*) 216 (4.48), 280 (3.65) nm; ECD (*c* 0.1 mM, MeOH) 220 (Δ*ε* +10.2), 240 (Δ*ε* +4.3), 285 (Δ*ε* +0.8) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 10; HRFABMS *m/z* 645.1813 $[M + Na]^+$ (calcd for $C_{29}H_{34}O_{15}$ Na, 645.1795).

Terminaloside N (**29**): Pale yellow, amorphous powder; $[\alpha]^{25}_{D}$ +26.7 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log *ε*) 216 (4.41), 281 (3.66) nm; ECD (*c* 0.2 mM, MeOH) 215 (Δ*ε* +10.5), 245 (Δ*ε* +2.2), 290 (Δ*ε* -0.6) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 11; HRFABMS *m/z* 631.1635 $[M + Na]^+$ (calcd for $C_{28}H_{32}O_{15}$ Na, 631.1639).

Terminaloside O (**30**): Pale yellow, amorphous powder; $[\alpha]^{25}_{D}$ +70.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log *ε*) 216 (4.52), 280 (3.66) nm; ECD (*c* 0.1 mM, MeOH) 215 (Δ*ε* +14.8), 245 (Δ*ε* +3.4), 290 (Δ*ε* -0.4) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 11; HRFABMS *m/z* 645.1791 $[M + Na]^+$ (calcd for $C_{29}H_{34}O_{15}$ Na, 645.1795).

Terminaloside P (**31**): Pale yellow, amorphous powder; $[\alpha]^{25}_{D}$ +12.6 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 225 (4.11), 278 (3.53) nm; ECD (*c* 0.2 mM, MeOH) 215 ($\Delta\varepsilon$ +8.7), 245 ($\Delta\varepsilon$ +2.8), 285 ($\Delta\varepsilon$ +1.4) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 11; HRFABMS *m/z* 645.1819 $[M + Na]^{+}$ (calcd for $C_{29}H_{34}O_{15}$ Na, 645.1795).

Terminaloside Q (**32**): Yellowish white, amorphous powder; $[\alpha]^{25}_{D}$ +15.9 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 212 (4.37), 227 (4.38), 272 (3.94), 306 (3.3.95) nm; ECD (*c* 0.14 mM, MeCN) 220 ($\Delta\varepsilon$ +13.51), 245 ($\Delta\varepsilon$ -1.04), 276 ($\Delta\varepsilon$ +2.61), 322 ($\Delta\varepsilon$ -1.3) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 12; HRFABMS *m/z* 579.2060 $[M + H]^{+}$ (calcd for $C_{28}H_{35}O_{13}$, 579.2078).

Terminaloside R (**33**): Yellowish white, amorphous powder; $[\alpha]^{25}_{D}$ +57.6 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 231 (4.18), 302 (3.93) nm; ECD (*c* 0.2 mM, MeCN) 220 ($\Delta\varepsilon$ +12.23), 245 ($\Delta\varepsilon$ -2.02), 290 ($\Delta\varepsilon$ +3.03), 330 ($\Delta\varepsilon$ +1.87) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 12; HRFABMS *m/z* 595.2029 $[M + H]^{+}$ (calcd for $C_{28}H_{35}O_{14}$, 595.2027).

Terminaloside S (**34**): Yellowish white, amorphous powder; $[\alpha]^{25}_{D}$ +53.7 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.36), 298 (3.99) nm; ECD (*c* 0.16 mM, MeCN) 210 ($\Delta\varepsilon$ -14.56), 235 ($\Delta\varepsilon$ +4.88), 285 ($\Delta\varepsilon$ +1.45), 320 ($\Delta\varepsilon$ -0.48) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 13; HRFABMS *m/z* 609.2191 $[M + H]^{+}$ (calcd for $C_{29}H_{37}O_{14}$, 609.2183).

Terminaloside T (**35**): Yellowish white, amorphous powder; $[\alpha]^{25}_{D}$ +77.6 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.53), 301 (4.12) nm; ECD (*c* 0.11 mM, MeCN) 225 ($\Delta\varepsilon$ +15.8), 245 ($\Delta\varepsilon$ -2.34), 295 ($\Delta\varepsilon$ +3.06), 335 ($\Delta\varepsilon$ +1.8) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 13; HRFABMS *m/z* 609.2196 $[M + H]^{+}$ (calcd for $C_{29}H_{37}O_{14}$, 609.2183).

Terminaloside U (**36**): Yellowish white, amorphous powder; $[\alpha]^{25}_{D}$ +21.5 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 224 (4.26), 279 (4.04) nm; ECD (*c* 0.19 mM, MeCN) 210 ($\Delta\varepsilon$ +11.2), 230 ($\Delta\varepsilon$ +0.6), 250 ($\Delta\varepsilon$ -0.8), 285 ($\Delta\varepsilon$ +0.97), 325 ($\Delta\varepsilon$ -0.44) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 14; HRFABMS *m/z* 625.2486 $[M + H]^{+}$ (calcd for $C_{30}H_{41}O_{14}$, 625.2496).

Terminaloside V (**37**): Colorless, amorphous powder; $[\alpha]^{25}_{D}$ +37.1 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.2), 220 (4.25), 285 (4.02) nm; ECD (*c* 0.2 mM, MeCN) 215 ($\Delta\varepsilon$ +14.1), 230 ($\Delta\varepsilon$ +3.4), 255 ($\Delta\varepsilon$ -0.5), 295 ($\Delta\varepsilon$ +0.45), 330 ($\Delta\varepsilon$ -0.23) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 14; HRFABMS *m/z* 641.2462 $[M + H]^{+}$ (calcd for $C_{30}H_{41}O_{15}$, 641.2445).

Terminaloside W (**38**): Yellowish white, amorphous powder; $[\alpha]^{25}_{D}$ +24.5 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 224 (4.16), 280 (4.05) nm; ECD (*c* 0.21 mM, MeCN) 215 ($\Delta\varepsilon$ +13.8), 230 ($\Delta\varepsilon$ +2.5), 255 ($\Delta\varepsilon$ -0.7), 280 ($\Delta\varepsilon$ +0.8), 325 ($\Delta\varepsilon$ -0.4) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 14; HRFABMS *m/z* 631.1982 $[M + Na]^{+}$ (calcd for $C_{29}H_{36}O_{14}Na$, 631.2002).

New compounds from *Pothos scandens*

Pothobanoside A (**47**): Colorless, amorphous powder; $[\alpha]^{25}_{D}$ – 30.9 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 221 (4.02), 259 (3.94), 288.5 (3.68) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 20; HRFABMS *m/z* 437.1451 $[M + Na]^{+}$ (calcd for $C_{19}H_{26}O_{10}Na$, 437.1924).

Pothobanoside B (**48**): Colorless, amorphous powder; $[\alpha]^{25}_{D}$ – 25.0 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 221 (4.10), 273.5 (3.92) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 20; HRFABMS *m/z* 444.1626 $[M]^{+}$ (calcd for $C_{20}H_{28}O_{11}$, 444.1632).

Pothobanoside C (**49**): Colorless, amorphous powder; $[\alpha]^{25}_{D}$ – 22.3 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 217 (4.04), 260.5 (3.85), 297.5 (3.12) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 20; HRFABMS *m/z* 629.2053 $[M + Na]^{+}$ (calcd for $C_{26}H_{38}O_{16}Na$, 629.2058).

Pothobanol (**50**): Colorless, amorphous powder; $[\alpha]^{25}_{D}$ 0 (*c* 0.2, MeOH); UV(MeOH) λ_{max}(log ε) 214 (3.31), 253.5 (2.57), 297.5 (3.12) nm; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 21; HRFABMS *m/z* 197.1168 $[M + H]^{+}$ (calcd for $C_{11}H_{17}O_{3}$, 197.1178).

Known compounds from *Terminalia citrina*

(+)-Excelsin (**2**): [49] Colorless, amorphous powder; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 15.

(1*R*,5*R*,2*S*,6*S*)-2-(3′,4′-Dimethoxyphenyl)-6-(3″-methoxy-4″,5″-methylenedioxyphenyl)-3,7-dioxabicyclo[3.3.0]octane (**3**): [50] Colorless, viscous oil; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 15.

Sesartemin (**4**): [51, 52] Yellow oil; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 15.

Isoorientin (**39**):[53] Yellow powder; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 16.

Nicotiflorine (**40**): [53, 54] Yellow powder; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 16.

Isorhamnetin-3-*O*-rutinoside (**41**): [55] Yellow powder; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 16;.

Threo-secoisolariciresinol-9′-*O*-β-D-glucopyranoside (**42**): [56, 57] Colorless, amorphous powder; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 17.

Erythro-secoisolariciresinol-9′-*O*-β-D-glucopyranoside (**43**): [58] Colorless, amorphous powder; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 17

Caprolactam (**44**): [59] Colorless, amorphous powder; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 18.

***p*-Hydroxybenzoic acid** (**45**): [60] Crystalline solid; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 18.

Blumenol A (**46**): [61] Colorless, amorphous powder; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 19.

Known compounds from *Pothos scandens*

Eleutherazine B (**51**): [78] Colorless, amorphous powder; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 22.

Isoschaftoside (**52**): [79] Yellow, amorphous powder; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 23.

Vicenin-2 (**53**): [80] Yellow, amorphous powder; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 23.

Neoschaftoside (**54**): [81] Yellow, amorphous powder; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 23.

Vitexin 2″-*O*-xyloside (**55**): [82] Yellow, amorphous powder; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 24.

Scoparin 2″-*O*-xyloside (**56**): [83] Yellow, amorphous powder; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 24.

Kaempferol 3-*O*-gentiobioside (**57**): [83] Yellow, amorphous powder; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 25.

Quercetin 3-*O*-gentiobioside (**58**): [84] Yellow, amorphous powder; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 25.

Isorhamnetin 3-*O*-gentiobioside (**59**): [85] Yellow, amorphous powder; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 25.

Canthoside B (**60**): [86] Colorless, amorphous powder; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 26.

***Zizybeoside* I** (**61**): [87] Colorless needles; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 27.

(3*S*) 1,2,3,4-Tetrahydro-3-carboxy-2-carboline (**62**): [88] Colorless, amorphous powder; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 27.

Markhamioside F (**63**): [89] Colorless needles; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 26.

Canthoside A (**64**): [86] Colorless, amorphous powder; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 26.

Stigmast-4-en-3-one (**65**): [90, 91] Colorless needles; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 28.

Stigmast-4, 22-dien-3-one (**66**): [91, 92] Colorless needles; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 28.

24-Methylenecycloartanol (**67**): [93] White, amorphous powder; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 29.

24-Methylenecycloartenone (**68**): [94, 95] White, amorphous powder; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 29.

24-en-Cycloartenone (69): [96] White, crystalline solid; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 29.

24-Methylenecycloartanyl ferulate (70): [97] White, crystalline solid; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 30.

***β*-Sitosterol glucoside (71)**: [98] White, crystalline solid; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 30.

Tetradecanoic acid (72): [99] White, amorphous powder; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 31.

L-Phenyl alanine (73): [100] White, amorphous powder; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 32.

L-Tryptophan (74): [100] White, amorphous powder; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 32.

Cell cultures

MCF-7 and T47D human breast cancer cells were purchased from the American Type Culture Collection (Manassas, VA, USA) and cultured as described in a previous report.[9] The T47D cells were grown in RPMI-1640 supplemented with 6 ng/mL of insulin, 1 mM of sodium pyruvate, 1 mM of nonessential amino acids, 2 mM of glutamine, 10% Fetal bovine serum, and antibiotics (100 U/mL of penicillin, 100 µg/mL of streptomycin and 50 µg/ mL of kanamysin), under 5% CO_2 generated humidified atmosphere at 37 °C. The MCF-7 cells were grown in Eagles MEM supplemented with 6 ng/mL of insulin, 1 mM of sodium pyruvate, 1 mM of nonessential amino acids, 2 mM of glutamine, 10% Fetal bovine serum, and antibiotics (100 U/mL of penicillin, 100 µg/mL of streptomycin and 50 µg/mL of kanamysin), under 5% CO_2 generated humidified atmosphere at 37^{0}C.

Antiestrogenic assay

The antiestrogenic assay was performed according to the procedure described in a previous report.[9] MCF-7 and T47D cells were seeded at a density of (1.0-1.2) x 10^{4} cells/well in 96-well plates in 90 µL of 5% DCC-treated, FBS-supplemented RPMI phenol red-free medium. After 3 h incubation, 5 µL of each test compound at four different concentrations ranging from 0.01 to 10 µM was added to each well along with 5 µL of estradiol (E_2) at a concentration of 20 nM, making a final volume of 100 µL in each well. Finally, the plates were incubated in a CO_2 incubator for 96 h. To evaluate the cell populations, Alamar blue (10 µL) was added in each well. After incubation under 5% CO_2 humidified atmosphere at 37 °C for 3 h, fluorescence was measured at 590 nm with excitation at 550 nm. Five µL of serially diluted

tamoxifen at concentrations ranging from 0.01 to 10 μM was used as a positive control. The results were calculated from the cell populations, and the iEqE values of each sample ($iEqE_{50}$, $iEqE_{10}$, and $iEqE_1$) were determined based on the concentration required to inhibit the E_2 effect ($iEqE_{50}$, $iEqE_{10}$, and $iEqE_1$: the concentrations suppressing the E_2 effect to the equivalent level of 50, 10, and 1 pM, respectively). If the samples suppressed E_2 activity to a level of less than 10 or 50 pM with the concentrations tested, they were categorized as strong (S) or mild (M), respectively.

Data and Statistical analysis

Statistical differences were determined by analysis of variance followed by Dunnett's multiple comparison tests. Statistical significance was established at the $p < 0.05$ level.

Acknowledgements

There is a strong believe in the mind of Bangladeshi people that Japan is the most honest and credible country of the world. That's why I chose Japan for doing my higher studies with full of confidence. In this regard, I contacted to Professor Hiroshi Noguchi expressing my interest. He was very much kind and chose me to supervise my PhD study. I want to express my deepest gratitude and sincere thanks for his generosity for giving me such opportunity to study here. He was the first person who taught me how to live in Japan as well as interpreting NMR spectra. He gave me infinite guidelines, valuable comments and endless support during his supervising tenure.

I would like to appreciate Professor ABM Faroque, University of Dhaka for his prudent decision to recommend my name to Professor Hiroshi Noguchi under a memorandum of understanding. I also want to acknowledge University of Dhaka for the sanction of leave for the study purpose.

I would like to give my deep appreciation and full respect to Professor Shigeru Itai whom I first met in Bangladesh. At that time, I had a chance to talk with a Japanese professor for the first time and knew about the Japan. He gave me valuable suggestions and infinite support during my study here. Although he has expertise on pharmaceutical engineering, he supervised my administrative works during the last year of my PhD study. I am grateful to him.

Among the people who involved in my PhD study, I want to express my deepest sincere thanks to Dr. Kaoru Umehara. He was beside me in every circumstance like a dearest friend. He is my one and only teacher who taught me how to do NMR, chromatography and biological activities. I published my research outcome in three journals which were seems impossible to me unless the help from Dr. Umehara. He has given me endless support, motivation, guidance, appreciation and overall courage during my living in Japan. It is really appreciable task and I am grateful to him.

I would like to express my full respect and appreciation to Professor Kenji Watanabe for his kind advices and valuable comments in my dissertation.

I would like to thank to Japanese government and University of Shizuoka to select me as a candidate for MONBUKAGAKUSHO (MEXT) scholarship from University recommendation for my PhD study.

I would like to thanks Mr. Philip Hawke, for his kind suggestion regarding English language and teaching.

I would like to thank Mr. Narahari Das and Mr. Abid, Faculty of Pharmacy, DU for their generous support for collecting medicinal plants.

After all, I am grateful to my parents, my beloved wife and child to give me full support for doing PhD study in abroad.

Md. Abdul Muhit

August, 2016

References

1. Nussey, S.; Whitehead, S. *Endocrinology*-an integrated approach; BIOS scientific publishers Ltd. Oxford, UK; 2001.
2. Russo, J.; Russo, I. H. *J. Steroid Biochem. Mol. Bio.*, **2006**, 102, 89-96.
3. Russo, J.; Hu, Y. F.; Russo, I. H. Estrogens and breast cancer in humans, in: M. Metzler (Ed.), Endocrine Disrupters of the Environment, Springer-Verlag, Heidelberg, **2000**, pp. 1–26.
4. Engel, N.; Oppermann, C.; Falodun, A.; Kragl, U. *J. Ethnopharmacol.*, **2011**, 137, 1003-1010
5. Hussain, S. M. A. *South Asian J. Cancer*, **2013**, *2 (4)*, 279-284.
6. Buhimschi, C. S. *Obstet. Gynecol. Clin. N. Am.*, **2004**, 31, 963-979.
7. Luecha, P.; Umehara, K.; Miyase, M.; Noguchi, H. *J. Nat. Prod.*, **2009**, *72*, 1954-1959.
8. Umehara, K.; Nemoto, K.; Matsushita A.; Terada, E.; Monthakantirat, O.; De-Eknamkul, W.; Miyase, T.; Warashina, T.; Degawa, M.; Noguchi, H. *J. Nat. Prod.*, **2009**, *72*, 2163-2168.
9. Umehara, K.; Nemoto, K.; Kimijima, K.; Matsushita, A.; Terada, E.; Monthakantirat, O.; De-Eknamkul, W.; Miyase, T.; Warashina, T.; Degawa, M.; Noguchi, H. *Phytochemistry*, **2008**, *69*, 546-552.
10. *Banglapedia: National Encyclopedia of Bangladesh*, Asiatic Society of Bangladesh. Dhaka, Bangladesh, **2003**
11. Mia, M.M.K.; *Traditional Medicines of Bangladesh*. Asiatic society of Bangladesh, Dhaka, Bangladesh, **1990**
12. Yusuf, M.; Begum, J.; Hoque, M. N.; Chowdhury, J. U. Medicinal Plants of Bangladesh, *Bangladesh Council of Scientific and Industrial Research Laboratories, Chittagong,* **2009**, *2nd Ed.*
13. Cao, S.; Brodie, P. J.; Callmander, M.; Randrianaivo, R.; Rakotobe, E.; Rasaimison, V. E.; Kingston, D. G. I. *Phytochemistry*, **2010**, *71*, 95-99.
14. Ponou, B. K.; Teponno, R. B.; Ricciutelli, M.; Quassinti, L.; Bramucci, M.; Lupidi, G.; Barboni, L. Tapondjou, L. A. *Phytochemistry*, **2010**, *71*, 2108-2115.
15. Valsaraj, R.; Pushpangadan, P.; Smitt, U. W.; Adersen, A.; Christensen, S. B.; Sittie, A.; Nyman U.; Nielsen, C.; Olsen, C. E. *J. Nat. Prod.*, **1997**, *60*, 739-742.
16. Wansi, J. D.; Lallemand, M. C.; Chiozem, D. D.; Toze, F. A. A.; Mbaze, L. M.; Khan, S. N.; Iqbal, M. C.; Tillequin, F.; Wandji, J.; Fomum, Z. T. *Phytochemistry*, **2007**, *68*, 2096-2100.
17. Srivastava, S. K.; Srivastava, S. D.; Chouksey, B. K. *Fitoterapia*, **1999**, *70*, 390-394.
18. Conrad, J.; Vogler, B.; Klaiber, I.; Roos, G.; Walter, U.; Kraus, W. *Phytochemistry*, **1998**, *48*, 647-650.
19. Cao, S.; Brodie, P. J.; Miller, J. S.; Randrianaivo, R.; Ratovoson, F.; Birkinshaw, C.;

Andriantsiferana, R.; Rasamison, V. E.; Kingston, D. G. I.; *J. Nat. Prod.*, **2007**, *70*, 679-681.

20. Conrad, J.; Vogler, B.; Reeb, S.; Klaiber, I.; Papajewski, S.; Roos, G.; Vasquez, E.; Setzer, M. C.; Kraus, W. *J. Nat. Prod.*, **2001**, *64*, 294-299.
21. Liu, M.; Katerere, D. R.; Gray, A. I.; Seidel, V. *Fitoterapia*, **2009**, *80*, 369-373.
22. Pfundstein, B.; Desouky, S. K. E.; Hull, W. E.; Haubner, R.; Erben, G.; Owen, R. W. *Phytochemistry*, **2010**, *71*, 1132-1148.
23. Yusuf, M.; Begum, J.; Hoque, M. N.; Chowdhury, J. U. Medicinal Plants of Bangladesh, *Bangladesh Council of Scientific and Industrial Research Laboratories, Chittagong,* **2009**, *2nd Ed.* pp. 625.
24. Palasuwan, A.; Soogarun, S.; Lertlum, T.; Pradniwat, P.; Wiwanitkit, V. *Asian Pac. J. Can. Prev.*, **2005**, *6*, 458-463.
25. Das, N.; Goshwami, D.; Hasan, M. S.; Raihan, S. Z.; Subedi, N. K. *Asian Pac. J. Trop. Dis.* **2015**, *5*, 166-168.
26. Burapadaja, S.; Bunchoo, A. *Planta Med.*, **1995**, *61*, 365-366.
27. Rojas, S.; Acevedo, L.; Macias, M.; Toscano, R. A.; Bye, R.; Timmermann, B.; Mata, R. *J. Nat. Prod.*, **2003**, *66*, 221-224.
28. Pelter, A.; Ward, R.; Rao, V.; Sastry, K. *Tetrahedron*, **1976**, *32*, 2783-2788.
29. Greger, H. *Biochem. Syst. Ecol.*, **1981**, *9*, 165-169.
30. Takahashi, K.; Nakagawa, T. *Chem. Pharm. Bull.*, **1966**, *14*, 641-647.
31. Hofer, O.; Scholm, R.; *Tetrahedron*, **1981**, *37*, 1181-1186.
32. Duan H.; Takaishi, Y.; Momota, H.; Ohmoto, Y.; Taki, T. *Phytochemistry*, **2002**, *59*, 85-90.
33. Rahman, M. M. A.; Dewick, P. M.; Jackson, D. E.; Lucas, J. A. *Phytochemistry*, **1990**, *29*, 1971-1980.
34. Li, C.; Yao, Z.; Qin, Z.; Zhang, J.; Cao, R.; Dai, Y.; Yao, X. *Fitoterapia*, **2014**, *97*, 92-97.
35. Lu, Y.; Xue, Y.; Liu, J.; Yao, G.; Li, D.; Sun, B.; Zhang, J.; Liu, Y.; Qi, C.; Xiang, M.; Luo, Z.; Du, G.; Zhang, Y. *J. Nat. Prod*., **2015**, *78*, 2205-2214.
36. Min, B. S.; Na, M. K.; Oh, S. R.; Ahn, K. S.; Jeong, G. S.; Li, G.; Lee, S. K.; Joung, H. Lee, H. K. *J. Nat. Prod.*, **2004**, *67*, 1980-1984.
37. Banerji, A.; Sarkar, M. (Chowdhury, N.); Ghosal, T.; Pal, S. C.; Shooley, J. N. *Tetrahedron*, **1984**, *40*, 5047-5052.
38. Guan, S. H.; Xia, J. M.; Lu, Z. Q.; Chen, G. T.; Jiang, B. H.; Liu, Z. Guo, D. A. *Magn. Reson.Chem*., **2008**, *46*, 186-190.
39. Inoue, K.; Inoue, H.; Chen, C. C. *Phytochemistry*, **1981**, *20*, 2271-2276.
40. Macrae, W. D.; Towers G. H. N.; *Phytochemistry*, **1985**, *24*, 561-566.

41. Jakupovic, J.; Pathak, V. P.; Bohlmann, F.; King, R. M.; Robinson, H. *Phytochemistry*, **1987**, *26*, 803-807.
42. Tan, R. X.; Jakupovic, J.; Jia, Z. *Planta Med.*, **1990**, *56*, 475-477.
43. Chen, I. S.; Chen, J. J.; Duh, C. Y.; Tsa, I. L. *Phytochemistry*, **1997**, *45*, 991-996.
44. Jung, K. Y.; Kim, D. S.; Oh, S. R.; Park, S. H. Lee, I. S.; Lee, J. J.; Shin, D. H.; Lee, H. K. *J. Nat. Prod.*, **1998**, *61*, 808-811.
45. Marchand, P. A.; Kato, M. J.; Lewis, N. G. *J. Nat. Prod.*, **1997**, *60*, 1189-1192.
46. Liao, S. G.; Wu, Y.; Yue, J. M. *Helvetica Chimica Acta*, **2006**, *89*, 73-79.
47. Lee, S. Y.; Suh, W. S.; Cha, J. M.; Moon, E.; Ha, S. K.; Kim, S. Y.; Lee, K. R. *Phytochemistry Letters*, **2016**, *17*, 79-84.
48. Xiong, L.; Zhu, C.; Li, Y.; Tian, Y.; Lin, S.; Yuan, S.; Hu, J.; Hou, Q.; Chen, N.; Yang, Y.; Shi, J. *J. Nat. Prod.*, **2011**, *74*, 1188-1200.
49. Russell, G. B. *Phytochemistry*, **1973**, *12*, 1799-1803.
50. De Carvalho, M. G.; Yoshida, M.; Gottlieb, O. R.; Gottlieb, H. R. *Phytochemistry*, **1987**, *26*, 265-267.
51. Greger, H.; Hofer, O. *Tetrahedron*, **1980**, 3*6*, 3551-3558.
52. Ahmed, A. A.; Mahmoud, A. A.; Ali, E. T.; Tzakou, O.; Couladis, M.; Mabry, T. J.; Gati, T.; Toth, G. *Phytochemistry*, **2002**, *59*, 851-856.
53. De Oliviera, D. M.; Siqueira, E. P.; Nunes, Y. R. F.; Cota, B. B. *Brazilian J. Pharmacognosy*, **2013**, *23 (4)*, 614-620.
54. Lee, H. B.; Kim, E. K.; Park, S. G.; Bang, S. G.; Kim, T. G.; Chung, D. W. *J. Agric. Food Chem.*, **2010**, *58*, 4808-4813.
55. Rastrelli, L.; Saturnino, P.; Schettino, O.; Dini, A. *J. Agric. Food Chem.*, **1995**, *43*, 2020-2024.
56. Inoshiri, H.; Sasaki, M.; Kohda, H.; Otsuka, H.; Yamasaki, K. *Phytochemistry*, **1987**, *26*, 2811-2814.
57. Achenbach, H.; Waibel, R.; Addae-Mensah, I. *Phytochemistry*, **1983**, *22*, 749-753.
58. Baderschneider, B.; Winterhalter, P. *J. Agric. Food Chem.*, **2001**, *49*, 2788-2798.
59. Carraher, C. E. *J. Chem. Educ.*, **1978**, *55*, 51-52.
60. Dhakal, R. C.; Rajbhandari, M.; Kalauni, S. K.; Awale, S.; Gewali, M. B. *J. Nepal Chem. Soc.*, **2009**, *23*, 89-92.
61. Li, J.; Kadota, S.; Kawata, Y.; Hattori, M.; Xu, G.; Namba, T. *Chem. Pharm. Bull.*, **1992**, *40 (12)*, 3133-3137.
62. Bhandary, M.J.; Chandrashekar, K.R.; Kaveriappa, K.M. *J. Ethnopharmacol.*, **1995**, *47*, 149-158.

63. Ayyanar, M.; Ignacimuthu, S. *J. Ethnopharmacol*., **2005**, *102*, 246-255.
64. Angkhana, I.; Shengji, P.; Balslev, H.; Wong, P.W.; Trisonthi, C.A. *J. Ethnopharmacol*., **2008**, *116*, 508-517.
65. Sangwoo, L.; Chunjie, X.; Shengji, P. *J. Ethnopharmacol*., **2008**, *117*, 362-377.
66. Yusuf, M.; Begum, J.; Hoque, M. N.; Chowdhury, J. U. Medicinal Plants of Bangladesh, *Bangladesh Council of Scientific and Industrial Research Laboratories, Chittagong,* **2009**, *2nd Ed.* pp. 535-536.
67. Haneefa, M. K.P.; Hanan, S.K.; Saraswathi, R.; Mohanta, G.P.; Nayar, C. *Asian Pac. J. Trop. Med*., **2010**, *3*, 988-992.
68. Lalitharani, S.; Mohan, V.R.; Regini, G.S. *J. Econ. Taxon. Bot.*, **2010**, *34*, 472-477.
69. Sajeesh, T.; Aruachalam, K.; Parimelazhagan, T. *Asia Pac. J. Trop. Med*., **2011**, *4*, 889-899.
70. Gupta, S.; Duraiswamy, B.; Satishkumar, M.N. *Indian J. Pharmacol*., **2013**, *45*, 83-86.
71. Gupta, S.; Basavan, D.; Nataraj, S.K.M.; Raju, K.R.S.; Babu, U.V.; Sharath, K.L.M.; Gupta, R. *Int. Immunopharmacol*., **2014**, *18*, 151-162.
72. Lalitharani, S.; Mohan, V.R.; Regini, G.S.; Kalidass, C. *J. Herb. Med. Toxicol.*, **2009**, *2*, 159-160.
73. Liu, H.X.; Bi, J.L.; Wang, Y.H.; Gu, W.; Su, Y.; Liu, F.; Yang, S.X.; Hu, G.W.; Luo, J.F.; Yin, G.F.; Long, C.L. *Helvetica Chimica Acta*, **2012**, *95*, 1231-1237.
74. Ohta, N.; Mori, N.; Kuwahara, Y.; Nishida, R. *Phytochemistry*, **2006**, *67*, 584-588.
75. Page, J. E.; Hause, G.; Rachke M.; Gao, W.; Schmidt, J.; Zenk, M. H.; Kutchan, T. M. *Plant Physiol*., **2004**, *134*, 1401-1413.
76. Ward, J. L.; Baker, J. M.; Llewellyn, A. M.; Hawkins, N. D.; Beale, M. *Proc. Natl. Acad. Sci*., **2011**, *108*, 10762-10767.
77. Nakata, T.; Tani, Y.; Hatozaki, M.; Oishi, T. *Chem. Pharm. Bull.*, **1984**, *32*, 1411-1415.
78. Li, Z. F.; Xu, N.; Feng, B. M.; Zhang, Q. H.; Pei, Y. H. *J. Asian Nat. Prod. Res.*, **2010**, *12 (1)*, 51-55.
79. Wada, S.; He, P.; Hashimoto, I.; Watanabe, N.; Sugiyama, K. *Biosci. Biotechnol. Biochem.*, **2000**, *64 (10)*, 2262-2265.
80. Islam, M. N.; Ishita I. J.; Jung, H. A.; Choi, J. S. *Food Chem. Toxicol.*, **2014**, *69*, 55-62.
81. Xie, C.; Veitch, N. C.; Houghton, P. J.; Simmonds, M. S. J. *Chem. Pharm. Bull.*, **2003**, *51 (10)*, 1204-1207.
82. Fiasson, K. G.; Jay, M.; Viricel, M. R. *Phytochemistry*, **1989**, *28*, 2471-2475.
83. Iwashina, T.; Lopez-saez, J. A.; Herrero, A.; Kitajima, J.; Matsumoto, S. *Biochem. Syst. Ecol.*, **2000**, *28*, 665-671

84. Liao, H.; Liu, H.; Yuan, K. *Phcog. Mag.*, **2012**, *8*, 12-15.
85. Xinxiao, H.; Wenyuan, G.; Wanshun, Z.; Tiejun, Z.; Jun, X. *China J. Chinese Materia Medica*, **2010**, *35 (22)*, 2994-2998.
86. Kanchanapoom, T.; Kasai, R.; Yamasaki, K. *Phytochemistry*, **2002**, *61*, 461-464.
87. Okamura, N.; Yagi, A.; Nishioka, I. *Chem. Pharm. Bull.*, **1981**, *29 (12)*, 3507-3514.
88. Ai-lian, Z.; Min, Y.; Hong-hua, X.; Jin-ping, S. *China J. Chinese Materia Medica*, **2013**, *38 (6)*, 844-847.
89. Kanchanapoom, T.; Kasai, R.; Yamasaki, K. *Phytochemistry*, **2002**, *59*, 557-563.
90. Ibrahim, S. R. M.; Elkhayat, E. S.; Mohamed, G. A.; Khedr, A. I. M.; Fouad, M. A.; Kotb, M. H. R.; Ross, S. A. *Phytochemistry letters*, **2015**, *14*, 84-90.
91. Lone, S. H.; Bhat, K. A. *Steroids*, **2015**, *96*, 164-168.
92. Sandjo, L. P.; Rincheval, V.; Ngadjui, B. T.; Kirsch, G. *Chem. Nat. Compd.*, **2011**, *47*, 731-734.
93. Lee, S. Y.; Choi, S. U.; Lee, J. H.; Lee, D. U.; Lee, K. R. *Arch. Pharm. Res.*, **2010**, *33*, 515-521.
94. Yonemoto, R.; Shimada, M.; Gunawan-puteri, M. D. P. T.; Kato, E.; Kawabata, J. *J. Agric. Food Chem.*, **2014**, *62*, 8411-8414.
95. Lian-Niang, L.; Hong, X. *Planta Med.*, **1986**, *52*, 492-493.
96. Mao-juan, Z.; Bing, L.; Yan-feng, A.; De-qiang, F.; You-kai, J. *Chinese Traditional and Herbal Drugs*, **2014**, *45 (2)*, 175-180.
97. Liu, M.; Yang, F.; Shi, H.; Akoh, C. C.; Yu, L. *Food Chem.*, **2013**, *139*, 919-924.
98. Jayaprakasha, G. K.; Jadegoud, Y.; Gowda, G. A. N.; Patil, B. S. *J. Agric. Food Chem.*, **2010**, *58*, 180-186.
99. Yang, X.; Yang, Y.; Zhao, L.; Zhou, H.; Xu, L.; Ding, Z. *J. Am. Oil Chem. Soc.*, **2013**, *90*, 159-162.
100. Wang, X.; Liu, R.; Yang, Y.; Zhang, M. *Food Chem.*, **2015**, *187*, 37-43.
101. Dixon, R. A. *Annu. Rev. Plant Biol.*, **2004**, *55*, 225−261.
102. Aehle, E.; Muller, U.; Eklund, P. C.; Willfor, S. M.; Sippl, W.; Drager, B. *Phytochemistr*y, **2011**, *72*, 2396−2405.
103. Axelson, M.; Sjoevall, J.; Gustafsson, B.; Settchell, K. *Nature*, **1982**, *298*, 659−660.
104. Penalva, J. L.; Heinonen, S. M.; Aura, A. M.; Adlercreutz, H. *J. Nutr.*, **2005**, *135*, 1056−1062.
105. Jan, K. C.; Hwang, L. S.; Ho, C. T. *J. Agric. Food Chem*., **2009**, *57*, 6101−6106.
106. Power, K. A.; Saarinen, N. M.; Chen, J. M.; Thompson, L. U. *Int. J. Cancer*, **2006**, *118*, 1316−1320.
107. Low, Y. L.; Dunning, A. M.; Dowsett, M.; Forkerd, E.; Doody, D.; Taylor, J.; Bhaniani, A.; Luben, R.; Khaw, K. T.; Warenham, N. J.; Bingham, S. A. *Cancer Epidemiol., Biomarkers*

Prev., **2007**, *16*, 1009−1016.

108. Hidalgo, M.; Santamaria, S.M.; Recio, I.; Moreno, C.S.; Teresa, B.D.P.; Rimbach, G.; Teresa, S.D.P. *Genes Nutr*., **2012**, *7*, 295-306.
109. Okubo, T., Kano, I. *Yakugaku Zasshi*, **2003**, *123*, 443-452.
110. Fernandes, I., Faria, A., Azevedo, J., Soares, S., Calhau, C., Freitas, V.D.; Mateus, N. *J. Agric. Food Chem*., **2010**, 58, 3785-3792.
111. Collins-Burow, B.M., Burow, M.E., Duong, B.N.; Mclachlan, J.A. *Nutr. Canc*., **2000**, *38*, 229-244.
112. Kuiper, G. G. J. M., Lemmen, J. G., Carlsson, L. B., Corton, J. C., Safe, S. H., Saag, P. T. V. D., Burg, B. V. D.; Gustafsson, J. A. *Endocrinology*, **1998**, *139*, 4252-4263.
113. Maggiolini, M., Vivacqua, A., Fasanella, G., Recchia, A. G., Sisci, D., Pezzi, V., Montanaro, D., Musti, A. M., Picard, D.; Ando, S. *J. Biol. Chem.*, **2004**, *279*, 27008-27016.
114. Myose, M. Warashina, T.; Miyase, T. *Chem. Pharm. Bull.*, **2012**, *60*, 612-623.