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A novel γ-lactone and other constituents of a Tanzanian *Antidesma venosum*

Bir Tanzanya *Antidesma venosum*'unun bileşenleri ve yeni bir γ-lactone

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SUMMARY
AIM: This study aimed at investigating the antimicrobial potential of isolated compounds from a Tanzanian *Antidesma venosum*.
METHODS: The pure compounds were isolated from the stem and root barks of *A. venosum* using standard column chromatography procedures with silica gel as a stationary phase and organic solvents of different polarities as mobile phases. Antimicrobial activity was determined using broth microdilution method against different bacteria and fungi.
RESULTS: Repeated column chromatography of the ethanol extract of the root bark of *A. venosum* led to the isolation of a new γ-lactone compound, namely (3R,4R,5S)-4-hydroxy-5-methyl-3-tetradecanyl γ-lactone (1) and *β*-sitosterol, while investigation of the stem bark afforded two known triterpenoids, friedelin (2) and lupeol (3) as well as a mixture of known phytosterols, *β*-sitosterol and stigmasterol. Their structures and absolute configurations were determined by using combined spectroscopic methods. Compound 1 didn't display antimicrobial activity even at the maximum concentration tested (MIC values > 5mg/ml) against all tested strains. Friedelin (2) was the most active compound with MIC of 0.1875 mg/ml against *Staphylococcus aureus* (ATCC 25923) and *Streptococcus pyogenes* (clinical isolate) while lupeol (3) had weak antibacterial activity against *S. aureus* and *S. pyogenes* with MIC of 1.25 mg/ml. No inhibition was observed against other tested strains.
CONCLUSION: These findings indicate that compound 2 is the main contributor for the reported activity in the stem bark, while synergism is suggested to be a causative of high antimicrobial activity reported from root extracts.
Key words: *Antidesma venosum*, isolation, γ-lactone, triterpenoids, antimicrobial activity.

ÖZET
AMAÇ: Bu çalışma, bir Tanzanya *Antidesmo venosum*'undan izole edilen bileşiklerin antimikrobiyal potansiyelini araştırmayı amaçladı.
YÖNTEM: Sabit faz olarak silica jel ve mobil faz olarak farklı polaritelerde organic çözücüler ile standart kolon kromatografisi prosedürleri kullanılarak, *Antidesmo venosum* kök kabuklarından saf bileşiklerin izole edildi. Antimikrobiyal aktivite, farklı bakteri ve mantarlara karşı broth mikrodilüsyon metodu kullanılarak saptandı.
BULGULAR: A. venosum kök kabuklarının etanol ekstrelerinin tekrarlayan kromatografileri, (3R,4R,5S)-4-hydroxy-5-methyl-3-tetradecanyl γ-lactone (1) isimli yeni bir γ-lakton bileşiği ve *β*-sitosterol izolasyonunu sağlarken; gövde kabuklarının araştırılması *β*-fitosteroller olan sitosterol and stigmasterolün bir karışımı yanısıra, bilinen 2 triterponoid olan friedelin (2) ve lupeolü (3) buldu. Bunların yapıları ve mutlak konfigürasyonları, kombine spektroskobik metodlar kullanılarak saptandı. Bileşik 1, test edilen tüm soylara karşı maksimum konsantrasyonlarda (MIC değerleri > 5 mg/ml) bile antimikrobiyal aktivite göstermedi. Friedelin (2), *Staphylococcus aureus* (ATCC 25923) ve *Streptococcus pyogenes*'e (klinik izolasyon) karşı 0,1875 mg/ml MIC değerinde en aktif bileşikken; lupeol (3), *S. aureus* ve *S. pyogenes*'e karşı 1,25 mg/ml MIC değerinde zayıf antibakteriyel aktiviteye sahipti. Test edilen diğer soylara karşı inhibisyon gözlenmedi.
SONUÇ: Bu bulgular, bileşik 2'nin kök kabuklarındaki aktiviteye katkı sağlayan ana bileşik olduğunu gösterirken; gövde ekstrelerinden kaynaklanan yüksek antimikrobiyal aktivitenin bir nedeninin bileşiklerin sinerjizmine bağlı olduğunu öne sürmektedir.
Anahtar kelimeler: *Antidesma venosum,* izolasyon, γ-lakton, triterpenoitler, antimikrobiyal etkinlik.

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INTRODUCTION

Antidesma is a homogenous genus in the family *Euphorbiaceae*. It consists of dioecious shrubs and trees mostly native to the old world tropics [1]. There are about 170 different species of *Antidesma* in the world with only six species found in Africa, namely *A. venosum*, *A. laciniatum*, *A. chevalieri*, *A. membranaceum*, *A. madagascariensis* and *A. vogelianum* [2]. Many species from this genus have been used traditionally for medicinal purposes [1]. Furthermore, the methanol extract of *A. ghaesembilla* has been reported to possess antimicrobial, antioxidant and cytotoxic activity [3] while A. madagascariensis, is reported to have significant antimicrobial activity [4-5] and and *A. bornius* is reported to have cytotoxic activity [6]. *In vitro* studies done on the essential oils from *A. laciniatum* revealed considerable antiplasmodial activity against the drug resistant W-2 *Plasimodium falciparum* strain [7-8]. The compounds isolated from different Antidesma species included triterpenoids, cyclopeptide alkaloids, steroids, phenolic acids, megastigmanes, lignans, flavonoids and quinolide-type alkaloids which most of them have reported to have potential medicinal value [9].

A. venosum stem bark extract is used in Namibia for management of HIV/AIDS opportunistic infections like diarrhoea, anaemia and lack of appetite, while the root extract is used for treating tuberculosis and candidiasis [10]. In Tanzania, the root bark is used for the treatment of epilepsy [11], hookworm infestation, tuberculosis and oral candidiasis [12-13]. Recently we reported that the crude extracts of the root and stem barks of *A. venosum* exhibited significant in vitro antimicrobial activities [14]. The compounds responsible for the antyimicrobial activity were not reported.

MATERIAL AND METHODS

Plant materials

Plant materials were collected from Tanga region, northeastern Tanzania, in October 2011. Identification of the plant was done by Mr. Haji Selemani, a botanist from the Department of Botany, University of Dar Es Salaam. Voucher specimen No.HOS 974 is deposited at the Herbarium of the Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences

Chemicals and media

Iodonitrotetrazolium chloride was bought from SIGMA (Sigma- Aldrich, St Louis, USA). The Brine Shrimps eggs were purchased from Aquaculture innovations (Grahamstown 6140, South Africa). Tryptone soya agar and tryptone soya broth were purchased from Himedia Laboratory Pvt. Ltd. (Mumbai, India) while Sabouraud's dextrose agar and Sauboraud's dextrose broth were obtained from Biotec Laboratory Ltd (Ipswich, United Kingdom). Dimethylsulfoxide (DMSO) was purchased from SIGMA (Poole, Dorset, England), cyclophosphamide and gentamicin susceptibility test discs (10 µg) were purchased from Oxoid (Oxiod Basingstoke, Hampshire, England), while fluconazole was purchased from CADILA Pharmaceutical Limited (Dholka, India).

Test organisms

Nine pathogenic microbes were used in this study which included four Gram positive bacteria; *Enterococcus faecalis* (clinical isolate), *Staphylococcus aureus* (ATCC25923), *Bacillus cereus* (clinical isolate), *Streptococcus pyogenes* (clinical isolate), three gram negative bacteria; *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 29953) and *Shigella flexneri* (clinical isolate) and two fungi; *Candida albicans* (ATCC 90028) and *Cryptococcus neoformans* (clinical isolate). All the microbes were obtained from the Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences. Bacteria were sub-cultured on tryptone soya agar while fungi were sub-cultured on sabouraud's dextrose agar

Extraction and preparation of the plant materials

Plant materials were dried under the shade and finely powdered using a milling machine and then extracted using ethanol (100%) for 48 hr and thereafter concentrated under reduced pressure using a rotary evaporator. The extracts were dried, weighed and stored in the refrigerator.

Isolation of compounds from root extract of *A. venosum*

The crude ethanol extract was fractionated by Vacuum Liquid Chromatography (VLC) using petroleum ether (100%), dichloromethane (100%) and methanol (100%). After concentration on the rotary evaporator, the extract yields were 8.2 g, 32 g and 36.4 g for PE, DCM and MeOH, respectively. Analysis of the three extracts on TLC paper indicated the presence of long chain fatty acids in PE extract; thesewere not followed up. From the DCM extract, both UV positive and negative spots were observed, while in the methanol extract, a series of sugar

compounds were observed and that were not follow up. Later, 30 g of the dichloromethane extract was adsorbed onto silica gel and subjected to column root chromatography (CC) separation eluting with solvent systems of DCM:PE (3:7 v/v) to get 0.187 mg [F-1], DCM:PE (1:1 v/v) to yield 3.121 g [F-2] and DCM:PE (4:1 v/v) to get 1.410 g [F-3]. F-2 indicated settling crystals and was crystallized in methanol to yield off-white amorphous crystals of compound 1 (126 mg). On a small CC over silica gel, F-1 was subjected to isolation process eluting with 4:1 (DCM: PE) to afford compounds 4. 4g of the stem bark DCM extracts was also subjected to CC separation eluting with different ratio of DCM/PE to afford compounds 2, 3 and 4.

Determination of minimum inhibitory concentration (MIC)

MIC was determined by the broth microdilution technique using sterile flat bottomed 96 well polystyrene microtiter plates. Bacterial suspensions equivalent to 0.5 McFarland concentrations were prepared by suspending microbes' inocular in sterile distilled water and adjusted to get the right turbidity (equivalent to 0.5 McFarland concentrations). Test solution were prepared by dissolving 10 mg of pure compounds in 0.1 ml of DMSO and diluted with 0.9 ml of broth to make a concentration of 10 mg/ml. The stock solution (50 µl) was pipetted and added into the first well of each row of plates pre- loaded with 50 µl of broths. Then serial dilution was performed by transferring the test sample from first row wells to wells of the next rows, down to the last rows. The 50 µl from the last row wells were discarded. This was followed by addition of 50 µl of solution containing the test organisms (0.5 McFarland dilutions) to each of the wells. Wells in two columns were used as growth controls, where no drugs were added, while two were used as the positive controls in which gentamycin and fluconazole were used for bacteria and fungi, respectively. The microtitre plates were incubated at 37°C for 24 hours.

After the incubation period 20 µl of a 0.2% p-iodonitrotetrazolium chloride (INT) were added to the wells followed by incubation at 37°C for 0.5 hr. Presence of microbial growth was indicated by change of INT colour to pink, while absence of growth was indicated by absence of colour change. The lowest concentration at which there was no growth was taken as the minimum inhibitory concentration (MIC).

RESULTS

Antimicrobial activity

Compound 1 and 4 didn't demonstrate any significant activity against the tested bacteria and fungi. Compound 3 exhibited weak antibacterial activity against *S. aureus* and *S. pyogenes* displaying MIC of 1.25 mg/ml but nor inhibition was observed against other tested strains. Compound 2 was the most active inhibiting all the tested strains with lowest MIC of 0.1875 mg/ml observed against S. aureus and S. pyogenes. MIC of 0.375 mg/ml was observed against *E. coli* and *S. flexneri* and MIC of 0.75 mg/ml for *P. aeruginosa***.**

Identification of compounds 1-4

Four compounds were isolated from the plant under investigation.

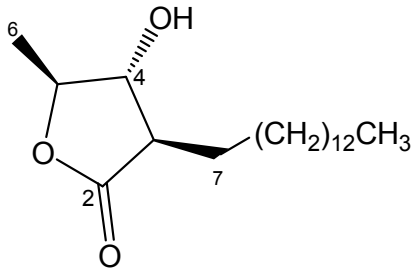
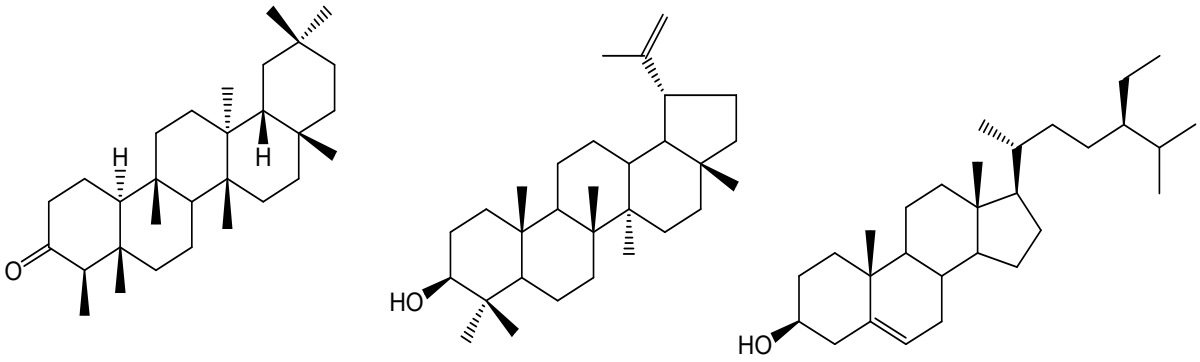


Figure 1. Structure of compound 1.

Compound 1 (Fig 1) was isolated as an optically active white amorphous powder with an $[\alpha]^{25}_{D}$ = -7.18 (c 0.6, $CHCl_3$). Identification of this compound was achieved using different spectroscopic techniques. The high resolution mass spectrum (HRMS) of compound 1 indicated a molecular ion [M^+] peak at *m/z* 312.2674 and other broad peaks at m/z 116 and 129. The upfield region of ^{1}H NMR spectrum showed the presence of signals at δ_H 1.34, 0.80 and other congested peaks between δ_H 1.15 to 1.75. The downfield region indicates the presence of three signals resonating at δ_H 4.08, 3.73 and 2.44. ^{13}C NMR indicate the presence of one highly deshielded carbon peak at δ_C 176.2, two peak at δ_C 80.2 and 79.1, one peak at δ_C 48.8 and other 15 peaks resonating between δ_C 14.1 to 32.2 (Table 1). Compounds 2, 3, and 4 were isolated from the dichloromethane extracts of the stem bark. Their structures were established based on available spectroscopic data as well as literature data. These compounds were identified as friedelin, lupeol and *β*-sitosterol respectively (Fig. 2).



2 **3** **4**

Figure 2. Structures of compound 2, 3 and 4.

Table 1. NMR data and correlations for compound 1.

C	^{13}C NMR	^{1}H NMR δ_H (*m*, *J* in Hz)	COSY	NOESY	HMBC
2	176.2				
3	48.8	2.44 (*m*)	H-4, H-7	H-5	C-2, C-4, C-7
4	79.1	3.73 (*t*, 7.2)	H-3, H-5	H-7	C-2, C-7, C-6
5	80.2	4.08 (*p*, 6.6)	H-4, H-6	H-3, H-6	C-3, C-4
6	18.2	1.34 (*d*, 6.6)	H-5	H-5	C-4, C-5
7	28.7	1.49, 1.73 (*m*)		H-4	C-2C-3, C-4, C-8
8	27.0	1.37 (*m*)			
9	29.9	1.17-1.21 (*m*)			
10	29.9	1.17-1.21 (*m*)			
11	29.9	1.17-1.21 (*m*)			
12	29.9	1.17-1.21 (*m*)			
13	29.9	1.17-1.21 (*m*)			
14	29.8	1.17-1.21 (*m*)			
15	29.8	1.17-1.21 (*m*)			
16	29.6	1.17-1.21 (*m*)			
17	29.6	1.17-1.21 (*m*)			
18	29.7	1.17-1.21 (*m*)			
19	32.2	1.17-1.21 (*m*)			
20	14.1	0.80 (*t*, 7.2)	H-19		C-19

Table 2. Minimum Inhibitory Concentration (mg/ml).

Microbes	1	2	3	4	Gent	Fluc.
S. aureus	>2.50	0.1875	1.25	>2.50	0.00078	NT
S. pyogenes	>2.50	0.1875	1.25	>2.50	0.00039	NT
E. faecalis	>2.50	N.T	>2.50	>2.50	0.00063	NT
B. cerius	>2.50	N.T	>2.50	>2.50	0.00125	NT
E. coli	>2.50	0.375	>2.50	>2.50	0.00063	NT
P. aeruginosa	>2.50	0.75	>2.50	>2.50	0.00125	NT
S. flexneri	>2.50	0.375	>2.50	>2.50	0.00125	NT
C. albicans	>2.50	N.T	>2.50	>2.50	NT	0.00625
C. neoformans	>2.50	N.T	>2.50	>2.50	NT	0.00313

DISCUSSION

Antimicrobial activity

The antimicrobial results (Table 2) suggest that compound 2 contributes to the antimicrobial activity of the stem bark extract as reported before [15]. The literature indicates that compound 2 possess a broad spectrum activity against both bacteria and fungi [16,17]. Both compound 1 and 4 didn't show antimicrobial activity suggesting that they do not significantly account for the antimicrobial of the crude extract [15]. It is possible the most active compound/s have not yet being identified.

Identification of compound I

The molecular formula of $C_{19}H_{36}O_3$, requiring *m/z* 312.2664 was suggested for the molecular ion [M^+] peak at m/z 312.2674 observed from high resolution mass spectrum (HRMS). The unsaturation index of two was deduced from the formula which corroborates with the proposed structure. Proposed mass fragmentation patterns involved the loss of alkyl chain by α-cleavage and *β*-cleavage at C-7 which accounts for the observed peak at m/z 116 and 129 respectively.

Inspection of 1H NMR spectrum showed the presence of two methyl proton signals at δ_H 1.34 and 0.80 which were assigned to H-6 and H-20 respectively. Furthermore, three methine protons were observed resonating at δ_H 4.08, 3.73 and 2.44 corresponding to H-5, H-4 and H-3 respectively. In the COSY spectrum, H-5 showed correlation with methyl protons at H-6 and H-4 which in return correlated to H-3 and a methylene proton signal at δ_H 1.49 (H-7). In HMBC spectrum H-5 showed correlation with carbon resonances at δC 79.1 and 48.8, assigned to C-4 and C-3 respectively. H-4 showed HMBC correlation to a carbon signals resonating at δ_H 176.2 (C-2), 28.7 (C-7) and 18.2 (C-6) while in the HSQC spectrum, a methine protons at H-5, H-4 and H-3 corresponded to the carbon resonances at δ_C 80.2, 79.1 and 48.8 respectively. Methylene proton resonances at δ_H 1.49 and 1.73 corresponded to the carbon resonance at δ_C 28.7, assigned to C-7 while a methylene proton resonance at δ_H 1.39 corresponds to the carbon resonance at δ_C 27.0 assigned to C-8. The rest of the proton and carbon signals were assigned to the side chain.

The assignment of the relative stereochemistry of compound 1 was achieved using the NOESY spectrum in conjunction with the reported ^{13}C NMR values [18] from which the trans-trans-cis stereochemistry relationship of the lactones moiety was determined. Inspection of ^{13}C NMR spectrum suggest the trans configuration between C-5 methyl (δ_C 18.2) and the hydroxyl group at C-4, when the methyl group is cis to hydroxyl group its ^{13}C NMR chemical shift is around δ_C 13 and it is around δC 18 when it is trans to it [19]. Also CH_2 (δ_C 28.7) at C-3 is trans to hydroxyl group at C-4, if the CH_2 is cis to

hydroxyl group its signal appears at around δ_C 23 and it appear at around δ_C 27 if its trans to it [16]. The actual relative configuration of the γ-lactone moiety was assigned to be 3R, 4R, 5S by comparing the δC values with the literature [18-21].

From the literature search, the average values around 1 ppm ($\Delta\delta_C$-5 – C-4 = 1) indicate a vicinal trans relative configuration between H-5 and H-4, while the lateral side chain and the methyl group are cis with typical values being δ_C 80 (C-5) and 79 (C-4) [18]. In the ^{13}C NMR spectrum of compound 1, the carbon signals for C-5 and C-4 were observed to resonate at δ_C 80.2 and 79.1 respectively giving a difference of 1.1 ppm and confirming the relative configuration of 3R, 4R, 5S of the γ-lactone moiety. To the best of our knowledge, this is the first report of the isolation of γ-lactone compound from the genus *Antidesma*. Thus, the structure of 1 was assigned as a new compound named as (3R, 4R, 5S)-4-hydroxy-5-methyl-3-tetradecanyl γ-lactone (1).

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REFERENCES

1. Tchinda AT, Tshome A, Dagne E, Arnold N, Wessjohann LA. Squalene and amentaflavone from *Antidesma laciniatum*. Bull Chem Soc Ethiop. 2006; 20: 325-8.
2. Pax F and Hoffmann P. *Euphorbiaceae-Phyllanthoideae-Phyllanthae*, in Das Pflanzenreich 81, Engler, A. (Ed.), Wilhelm Engelmann: Leipzig; 1922. pp. 1-349.
3. Habib R, Rahman M, Hamid K, Raihan O, Sayeed AM. Phytochemical Screening, Cytotoxicity, Antioxidant Capacity and Antibacterial Potentiality of Methanol Extract of *Antidesma ghaesembilla* Gaertn. Adv in Nat Appl Sci. 2011; 5: 69-74.
4. Fakim AG, Subratty H, Narod, J. Soulange G, and Mahomoodally F. Biological activity from indigenous medicinal plants of Mauritius. Pure Appl Chem. 2005; 77: 41-51.
5. Mohomoodally MF, Fakin AG, and Subratty AH. Antimicrobial activity and chemical profiles of endemic medicinal plants of Mauritius. Pharmaceut Biology. 2005; 43: 237-42.
6. Micor JRL, Deocaris CC and Rico EE. Mojica. Biological Activity of Bignay [*Antidesma bunius* (L.) Spreng] crude extract in *Artemia salina*. J Medic Sci. 2005; 5: 195-8.
7. Titanji VPK, Zofou D and Ngemenya MN. The antimalarial potential of medicinal plants used for the treatment of malaria in Cameroonian folk medicine. Afr J Trad CAM. 2008; 3: 302-21.
8. Boyom FF, Ngouana V, Zollo PH, Menut C, Bessiere JM, Gut J, Rosenthal PJ. Composition and anti-plasmodial activities of essential oils from some Cameroonian medicinal plants. Phytochem. 2003; 64: 1269-75.
9. Buske A, Schimidt J, Porzel A and Adam G. Benzopyrans and ferullic acid derivatives from *A. membranceum*. Phytochem. 1997; 46: 1385-8.
10. Chisembu KC and Hedimbi M. Ethnomedicinal plants and other natural products with anti-HIV active compounds and their putative modes of action. Intern J Biotech Molec Biol Res. 2010; 1: 74-9.
11. Moshi MJ, Kagashe GAB, Mbwambo ZH. Plants used to treat epilepsy by Tanzanian Traditional Healers. J Ethnopharmacol. 2005; 97: 327-39.
12. Kisangau DP, Herrmann TM, Lyaluu VM, Hosea KM, Joseph CC, Mbwambo ZH, et al. Traditional knowledge, use, practice and conservation of medicinal plant for HIV/AIDS care in rural Tanzania. J Ethnob Res. Appl. 2011; 9: 43-57.
13. Kokwaro JO. Medicinal plant of East Africa. East Africa literature bureau, Nairobi, 1976.p.23-6.
14. Mwangomo DT, Moshi MJ, Magadula JJ. Antimicrobial activity and phytochemical screening of Antidesma venosum root and stem bark ethanolic extracts. Int J Phytochem Pharmacol. 2012; 2: 90-5.
15. Mwangomo DT, Moshi MJ, Magadula JJ. Antimicrobial and phytochemical screeninng of *Antidesma venosum* root and stem bark ethanolic extracts. Int J Res Phytcochem Pharmacol. 2012; 2(2): 90-5.
16. Ghosh P, Mandal A, Chakraborty M, Saha A. Triterpenoids from Quercus suber and their antimicrobial and phytotoxic activities. J Chem Pharm Res. 2010; 2: 714-21.
17. Mann A, Ibrahim K, Oyewale AO, Amupitan JO, Fatope MO, Okogun JI. Antimycobacterial Friedelane-terpenoid from the Root Bark of *Terminalia Avicennioides*. A J Chem. 2011; 1: 52-5.
18. Lorenzo M, Brito I, Cueto M, Croz LD, Daras J. ^{13}C NMR-Based Empirical Rules to Determine the Configuration of Fatty Acid Butanolides. Novel γ-Dilactones from *Pterogorgia* spp. Org Lett. 2006; 8: 5001-4.
19. Chaves MH, Roque NF. Acetogenins from *Porcelia macrocarpa*: Stereochemical determination of 2-alkyl-3-hydroxy-4-methyl-γ-lactones by ^{13}C NMR Spectroscopy. Phytochem. 1997; 44: 523-8.
20. Cheng W, Zhu C, Xu W, Fan X, Yang Y, Li Y, et al. Chemical constituents of the bark of *Machilus wangchiana* and their biological activities. J Nat Prod. 2009; 72: 2145-52.
21. Takahashi S, Ogawa N, Sakairi N, Nakata T. Stereoselective synthesis of (2R,3S,4S)-3-hydroxy-4-methyl-2-tetradecyl-4-butanolide starting from 2,5-anhydro-D-mannitol. Tetrahedron. 2005; 61: 6540-5.