RSC Advances

PAPER

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Cite this: *RSC Adv.*, 2023, **13**, 29141

Cytotoxicity, anti-diabeticity, and phytocomposition investigation of Vietnamese *Euphorbia tithymaloides* Linn. (Euphorbiaceae)†

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Received 18th August 2023
Accepted 12th September 2023

DOI: 10.1039/d3ra05637f

rsc.li/rsc-advances

In this study, the aerial parts of mature Vietnamese *Euphorbia tithymaloides* plants were put through cytotoxic, anti-diabetic, and phytocompositional evaluations. Specifically, four extracts (petroleum ether (PE), ethyl acetate (EA), methanol (Me), and aqueous (W)) were prepared by maceration at room temperature. All extracts, together with some isolated compounds, were investigated for cytotoxicity against some human normal and cancer cell lines (fibroblasts, HeLa, NCI-H460, HepG2, MCF-7, and Jurkat) using the standardized modified sulforhodamine B (SRB) assay. Additionally, the anti-diabetic activity of extracts and compounds was evaluated *via* their α-glucosidase inhibitory capacity. The obtained results indicated that Vietnamese *E. tithymaloides* extracts exhibited moderate cytotoxic activity, among which the PE extract possessed the highest values, on the NCI-H460 cell line. Second, the aqueous extract was revealed to possess very high α-glucosidase inhibitory activity (IC_{50} = 113.75 ± 14.02 μg ml^{-1}). From the PE extract, three new jatrophane diterpenoids (named tithymal A, tithymal B, and tithymal C) and two known ones were isolated and structurally elucidated using NMR and MS spectroscopies. Noticeably, tithymal A exhibited significantly high inhibitory activity against α-glucosidase (IC_{50} = 10.71 ± 0.52 μg ml^{-1}). These observations have significantly highlighted the medicinal potential of Vietnamese *E. tithymaloides* and expanded its scientific fascination.

Introduction

Euphorbia is one of the most noticeable genera within the Euphorbiaceae family, thanks to its more than 2000 beneficial members.[1–4] Among the top groups of tolerant *Euphorbia* species are latex-bearing Euphorbias (*E. tirucalli*, *E. grantii*, *E. resinifera*, and so on).[5–7] In addition to their very wide range of phytoconstituents, these plants have diverse bioactivities, ranging from cytotoxic, antimicrobial, and antioxidant, to anti-diabetic, anti-inflammatory, and anti-cancer activities.[8,9] In Vietnam, although species of the *Euphorbia* genus are not as diverse as those of others, such as *Aporosa* or *Croton*, most of them are exploited as medicinal plants. *E. tirucalli*, *E. thymifolia*, *E. antiquorum*, and *E. cyathophora* are among the most common members.[10–13]

The Devil's backbone, *Euphorbia tithymaloides*, is among the important medicinal plants in Vietnam, especially for the treatment of dermatological and oral diseases. This is a vertical succulent shrub that grows up to 2.4 m in height, with distinctive green zigzag stems, hence its common name. Opposite along the stems are sessile, glabrescent, acuminate, smooth-edged, simple angiosperm leaves, which are 35–75 mm in length. Each flower is supported by a peduncle in a dichotomous cyme, with bifid egg-shaped floral leaves and bright red, irregularly acuminate, slipper-resembling, involucral bracts. Seed pods are approximately 7.5 mm long and 9 mm wide, and ovoid in shape with truncated ends.[14,15] In Vietnam, *E. tithymaloides* thrives in mountain sides, on sandy, microelement (B, Cu, Fe, Mo, and Zn)-rich terrains. It is also planted in familial medicinal gardens for favourable usages.[10–12]

Regarding the phytocomposition, *E. tithymaloides* is noticeable for its multiform jatrophane diterpenoids and coumarins, with more than ten compounds having been isolated and elucidated in each group.[16–19] Besides, the isolation of other compounds (steroids, flavonoids, organic acids, and esters, for instance) has been reported.[20,21] Bioactivities of this plant have also been demonstrated to be varied, including cytotoxic, antioxidant, antimicrobial, and wound-healing activities.[1,15,21] From this study, the scientific knowledge of phytocomposition and

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† Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d3ra05637f

bioactivities of *E. tithymaloides* will be significantly expanded, assuring its potential for medicinal use.

Experimental

Materials and methods

All extracts were prepared from the dried ground powder of mature plant specimens by maceration at room temperature. They were subsequently investigated for cytotoxicity against human normal and cancer cell lines, using the previously described modified SRB assay.[22,23] Besides, the α-glucosidase inhibitory activity of these extracts was evaluated, based on the procedure described by Luo-sheng *et al.*[24] Isolated compounds obtained in appropriate quantities were also processed in these experiments. For cytotoxicity, fibroblasts were chosen as the representative of normal cells, while HeLa (cervical cancer), HepG2 (liver cancer), Jurkat (blood cancer), MCF-7 (breast cancer), and NCI-H460 (lung cancer) (American Type Culture Collection (Virginia, United States)) were the targeted cancer cell lines. The α-glucosidase inhibitory activity was evaluated *via* the capacity of releasing *p*-nitrophenol from a *p*-nitrophenyl-α-D-glucopyranoside precursor. For phytocompositional investigation, column and thin-layer chromatographies (TLC) were applied, and the obtained compounds were structurally elucidated using NMR and MS spectroscopies. For TLC, silica gel 60 F_{254} aluminum sheets (MilliporeSigma, Darmstadt, Germany) were used, while silica gel 60 (pore size range of 230–400 mesh) (MilliporeSigma, Darmstadt, Germany) was used for column chromatography. Sulfuric acid, ethanol (Xilong Scientific, Guangdong, China), and vanillin (HIMEDIA, Mumbai, India) were used for the preparation of TLC reagents. Other chemicals were of analytical grade and used without further purification. For structure elucidation, NMR spectra were recorded using a Bruker Avance NEO (Bruker, Massachusetts, United States) spectrometer, while a SCIEX X500B QTOF (SCIEX, Toronto, Canada) spectrometer was used for recording MS spectra.

Procedures

Preparation of plant materials and extracts. Fresh aerial parts of mature Vietnamese *E. tithymaloides* plants were collected, processed, and extracted following the procedure described in our previous publication.[25] The obtained extracts were abbreviated as Eth-PE, Eth-EA, Eth-Me, and Eth-W, for petroleum ether, ethyl acetate, methanol, and aqueous extracts respectively.

Cytotoxicity investigation. Cells were cultured in 96-well plates (BioPointe Scientific, California, United States) at 37 °C in a 5% CO_2 atmosphere, at a density of 10^4 cells per well for HeLa, HepG2, MCF-7, and fibroblasts, 7.5×10^3 for NCI-H460; and 5×10^4 for Jurkat, in media of E'MEM (Sigma-Aldrich, Missouri, United States) for MCF-7, NCI-H460, HeLa, HepG2, and fibroblasts, and RPMI 1640 (Sigma-Aldrich, Missouri, United States) for Jurkat. The culture media were supplemented with L-glutamine (2 mM), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (20 mM), amphotericin B (0.025 μg ml^{-1}), penicillin G (100 UI ml^{-1}), streptomycin (100 μg ml^{-1}), and 10% (v/v) fetal bovine serum (FBS) (Sigma-Aldrich, Missouri, United States). After 24 h of incubation, the solutions of extracts or compounds, in 100% dimethyl sulfoxide (DMSO) (MilliporeSigma, Darmstadt, Germany), at assessed concentrations, were added to each well, followed by another 48 h of incubation. Treated cells were fixed with cold 50% (w/v) trichloroacetic acid (MilliporeSigma, Darmstadt, Germany) solution for 1–3 h, washed, and stained with 0.2% (w/v) SRB (Sigma-Aldrich, Missouri, United States) for 20 min. After five washes with 1% acetic acid (MilliporeSigma, Darmstadt, Germany), the protein-bound dye was solubilized in a 10 mM Tris base solution (Sigma-Aldrich, Missouri, United States), and read for optical density values using a Synergy HT 96-well micro-titer plate reader (BioTek Instruments, Vermont, United States) at wavelengths of 492 and 620 nm. Camptothecin (Sigma-Aldrich, Missouri, United States) and neat DMSO were used as the positive and negative controls, respectively. The assay was conducted one time for the cytotoxicity preliminary screening, and in triplicate for detailed investigation. The optical density (OD) values at 492 nm (OD_{492}) and 620 nm (OD_{620}) of each reading were recorded. The cytotoxicity percentage (% *I*) of extracts or compounds was determined for each extract using the following calculations:

$$OD_{492}\ (\text{or } OD_{620})\ \text{of sample/control} = OD_{492/620\ TN/C} - OD_{492/620\ blank} \quad (1)$$

$$OD_{TN/C} = OD_{492\ TN/C} - OD_{620\ TN/C} \quad (2)$$

$$\%\ I = \left(1 - \frac{OD_{TN}}{OD_C}\right) \times 100 \quad (3)$$

where OD_{TN} and OD_C are respectively the optical density of samples (with cells) and control, calculated using formulae (1) and (2). OD_{blank} is the optical density of blank samples (without cells). The results are represented as means ± standard errors (SE) ($n \geq 3$). The Kruskal–Wallis test, followed by Dunn's test, was applied for testing the cytotoxicity on cancer and normal cells (GraphPad Prism software). A p-value of <0.05 was accepted as statistically significantly different. The principal steps of cytotoxic activity evaluation for extracts and compounds of *E. tithymaloides* are shown in Fig. 1.

α-Glucosidase inhibitory activity evaluation

First, 120 μl of each sample and 20 μl of 1 U ml^{-1} α-glucosidase (Sigma-Aldrich, Missouri, United States) were added to each

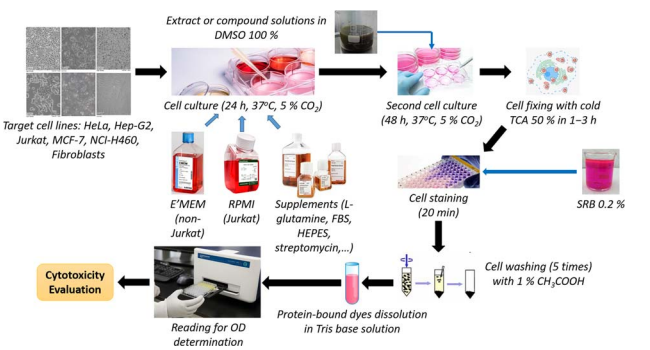

Fig. 1 Principal steps of the applied modified SRB assay.

well of a 96-well plate and incubated at 37 °C within 15 minutes. Then, 20 μl of 5 mM *p*-nitrophenyl-α-D-glucopyranoside (MilliporeSigma, Darmstadt, Germany) was subsequently added to each well, followed by another 15 minute incubation at 37 °C. After that, 80 μl of 0.2 M Na_2CO_3 (MilliporeSigma, Darmstadt, Germany) was added to halt the reaction, and the optical density of the solution was measured at 405 nm (OD_{405}). Acarbose (Sigma-Aldrich, Missouri, United States) was used as the positive control. The α-glucosidase inhibitory percentage (% *I*) was calculated as follows:

$$\% I = \left(1 - \frac{OD_t}{OD_c}\right) \times 100$$

where OD_t and OD_c are respectively the optical density of samples and control (after deducting the optical density of the blank sample without α-glucosidase); the IC_{50} value was determined by measuring the optical density of the sample at different concentrations. The Kruskal–Wallis test, followed by Dunn's test, was applied for the α-glucosidase inhibitory capacity evaluation (GraphPad Prism software), with $R^2 > 0.9$. The principal steps of α-glucosidase inhibitory determination for extracts and compounds of *E. tithymaloides* are shown in Fig. 2.

Phytocomposition investigation. The column chromatographic process started with the mobile phase from 100% (v/v) *n*-hexane to 100% (v/v) ethyl acetate, yielding nine fractions (labeled as **P1** to **P9**, respectively). Fraction **P5**, giving obvious marks after TLC analysis, was chosen for further column chromatography with the mobile phase from *n*-hexane : ethyl acetate 9 : 1 (v/v) to 100% (v/v) ethyl acetate, obtaining 22 sub-fractions (labeled as P5.1 to P5.22, respectively). Precipitation was observed in sub-fractions P5.5, P5.6, and P5.7, leading to the decision to further isolate, purify, and recrystallize it in acetone to obtain 1.3 g of a white crystal, labeled as **P1**. The remaining solution of sub-fraction P5.5 was proceeded to further column chromatography (the mobile phase from 100% (v/v) *n*-hexane to 100% (v/v) ethyl acetate) and purification to yield 2.8 mg of a white powder (**P2**) and 2.7 mg of another white powder (**P3**). The process continued for subfractions P5.10 and P5.11 (the mobile phase from 100% (v/v) *n*-hexane to 100% (v/v) ethyl acetate), yielding 7 fractions for P5.10 and 8 fractions for P5.11. Respective further purification of fractions P5.10.4 and P5.11.4 resulted in 5.0 mg of a white powder (**P4**) and 14.0 mg of another white powder (**P5**). The complete chromatography procedure is summarized in Fig. 3.

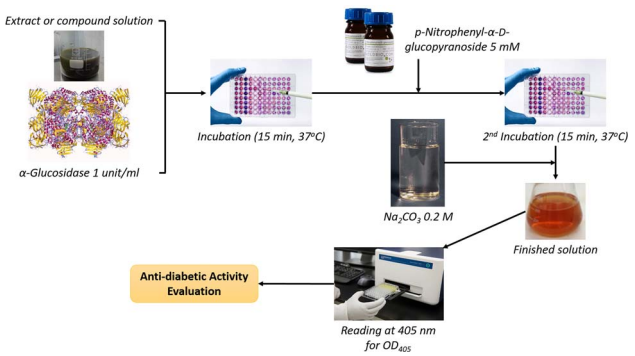

Fig. 2 Principal steps of the applied procedure for α-glucosidase inhibitory activity evaluation.

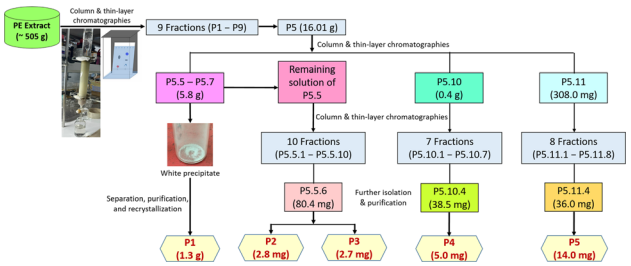

Fig. 3 Compositional investigation for the PE extract.

9β,15β-Diacetoxy-3β,7β-dibenzoyloxy-1α,13β,14α-trihydroxy-jatropha-5,11*E*-diene (P1). White crystal; soluble in acetone; TLC characters: positive reaction with 25% H_2SO_4 reagent (as an almost black mark), $R_f = 0.73$ (*n*-hexane : ethyl acetate 7 : 3, v/v). MS: $[M + Na]^+$ pseudomolecular ion peak at *m*/*z* 701.2924 (calculated for $C_{38}H_{46}O_{11}$, 1.4 mass of difference).

9α,15β-Diacetoxy-1α,3β-dibenzoyloxy-13α,14α-dihydroxy-7β-methoxyjatropha-5,11*E*-diene (tithymal C) (P2). White powder; soluble in chloroform; TLC characters: positive reaction with 25% H_2SO_4 reagent (as a dark brown mark), $R_f = 0.31$ (dichloromethane : ethyl acetate = 95 : 5, v/v). MS: $[M + H_2O]^+$ pseudomolecular ion peak at *m*/*z* 710.3540 (calculated for $C_{39}H_{48}O_{11}$, 0.02 mass of difference).

9α,15β-Diacetoxy-1α,3β,7β-tribenzoyloxy-13β,14α-dihydroxy-jatropha-5,11*E*-diene (P3). White powder; soluble in acetone; TLC characters: positive reaction with 25% H_2SO_4 reagent (as an almost black mark), $R_f = 0.50$ (dichloromethane : ethyl acetate = 8 : 2, v/v). MS: $[M + Na]^+$ pseudomolecular ion peak at *m*/*z* 805.3247 (calculated for $C_{45}H_{50}O_{12}$, 4.7 mass of difference).

15α-Acetoxy-3β-benzoyloxy-1α,7α,9β,13α,14β-pentahydroxy-jatropha-5,11*E*-diene (tithymal A) (P4). White powder; soluble in chloroform; TLC characters: positive reaction with 25% H_2SO_4 reagent (as a dark brown mark), $R_f = 0.35$ (dichloromethane : acetone = 8 : 2, v/v). MS: $[M + NH_4]^+$ pseudomolecular ion peak at *m*/*z* 550.3009 (calculated for $C_{29}H_{40}O_9$, 0.7 mass of difference).

15β-Acetoxy-3β-benzoyloxy-1α,9α,13α,14β-tetrahydroxy-jatropha-5,11*E*-diene (tithymal B) (P5). White powder; soluble in chloroform and acetone; TLC characters: positive reaction with 25% H_2SO_4 reagent (as a brown mark), $R_f = 0.42$ (*n*-hexane : ethyl acetate = 5 : 5, v/v). MS: $[M + K]^+$ pseudomolecular ion peak at *m*/*z* 555.2533 (calculated for $C_{29}H_{40}O_8$, 0.1 mass of difference).

All NMR and MS spectra for **P1–P5** were shown in the non-separative ESI† section of this work.

Results and discussion

Cytotoxicity against human normal and cancer cell lines

All isolated compounds from the PE extract of *E. tithymaloides* were elucidated as jatrophane diterpenoids, which have only

recently been studied, and their potential in bioactivity aspect attracted the attraction of scientific community.[26–28] In this work, **P1** and **P4** were priorly chosen for the cytotoxicity and α-glucosidase inhibitory evaluations. Table 1 presents the cytotoxic activity of all extracts, **P1**, and **P4** against human normal and cancer cell lines.

It was observed in Table 1 that both extracts and two selected compounds of *Euphorbia tithymaloides* exhibited low cytotoxic activity against fibroblasts, indicating their potential safety for human administration. Regarding cancer cell lines, only MCF-7 and NCI-H460 were affected by *Euphorbia tithymaloides*' extracts, with the higher cytotoxic activity on NCI-H460. In addition, only the petroleum ether and ethyl acetate extracts expressed noticeable activity against cancer cells, among which higher values belong to the petroleum ether one. For compounds of **P1** and **P4**, none of them showed significant cytotoxic activity against cancer cell lines. Except for NCI-H460, **P1** possessed higher cytotoxic values than those of **P4**. It could be inferred from the obtained results that Vietnamese *Euphorbia tithymaloides* possessed moderate cytotoxicity against human breast and lung cancer cell lines. The composition of extracts, therefore, was essential to be investigated to take deeper steps in studying their bioactivities.

Table 1 Cytotoxicity of extracts and compounds of *E. tithymaloides* ([ ]: extract; [ ]: compound)

Cell line	Extract/compound[a]	Cytotoxicity percentage (%) 1st time	2nd time	3rd time	Average ± SE
HeLa	Eth-PE	33.65	34.62	32.92	33.73 ± 0.85
	Eth-EA	28.40	31.23	32.19	30.61 ± 1.97
	Eth-Me	13.60	16.71	23.10	17.80 ± 4.84
	Eth-W	−8.29	−4.51	−16.31	−9.70 ± 6.03
	P1	21.41	22.30	23.85	22.52 ± 1.23
	P4	5.45	−2.73	−3.34	−0.21 ± 4.91
	Camptothecin (1 μg ml^{-1})	47.97	47.22	49.39	48.19 ± 1.10
	DMSO	−3.20	−5.90	−1.24	−3.45 ± 2.34
HepG2	Eth-PE	7.96	4.70	14.48	9.05 ± 4.98
	Eth-EA	−2.99	−0.83	12.53	2.91 ± 8.41
	Eth-Me	−10.95	−9.39	0.56	−6.59 ± 6.24
	Eth-W	−18.66	−22.38	−13.37	−18.13 ± 4.53
	P1	13.35	10.57	10.77	11.56 ± 1.55
	P4	−2.72	0.26	−3.81	−2.09 ± 2.10
	Camptothecin (0.07 μg ml^{-1})	56.37	55.16	52.82	54.78 ± 1.80
	DMSO	13.17	13.19	7.95	9.05 ± 4.98
Jurkat	Eth-PE	52.36	44.42	48.30	48.36 ± 3.97
	Eth-EA	31.10	40.39	46.75	39.41 ± 7.87
	Eth-Me	−5.27	−0.46	0.00	−1.91 ± 2.92
	Eth-W	4.92	−7.13	−2.16	−1.46 ± 6.05
	P1	18.26	14.26	27.21	19.91 ± 6.63
	P4	5.53	8.46	6.11	6.70 ± 1.55
	Camptothecin (0.005 μg ml^{-1})	60.22	55.53	55.11	56.95 ± 2.84
	DMSO	3.34	1.92	4.81	3.36 ± 1.45
MCF-7	Eth-PE	52.25	43.53	56.45	50.74 ± 6.59
	Eth-EA	53.85	49.58	55.54	52.99 ± 3.07
	Eth-Me	24.01	12.62	16.01	17.55 ± 5.85
	Eth-W	7.82	0.85	−6.97	−0.57 ± 7.40
	P1	16.61	22.96	20.09	19.89 ± 3.18
	P4	−2.60	8.81	3.49	3.23 ± 5.71
	Camptothecin (0.01 μg ml^{-1})	52.02	51.12	53.95	52.36 ± 1.45
	DMSO	−9.12	−15.17	−7.23	−10.51 ± 4.15
NCI-H460	Eth-PE	58.11	58.92	60.48	59.17 ± 1.20
	Eth-EA	52.21	58.07	54.19	54.83 ± 2.98
	Eth-Me	48.23	49.40	52.40	50.01 ± 2.15
	Eth-W	−23.25	−16.07	−7.38	−15.57 ± 7.95
	P1	0.40	4.95	1.13	2.16 ± 2.45
	P4	10.24	7.06	9.97	9.09 ± 1.77
	Camptothecin (0.007 μg ml^{-1})	79.20	79.04	74.95	77.73 ± 2.41
	DMSO	−6.10	−8.50	−5.14	−6.58 ± 1.73
Fibs[b]	Eth-PE	15.10	23.17	15.66	17.98 ± 4.50
	Eth-EA	19.27	29.27	18.67	22.40 ± 5.95
	Eth-Me	11.28	9.15	10.18	10.20 ± 1.06
	Eth-W	1.56	0.61	1.20	1.13 ± 0.48
	P1	−1.71	0.83	2.95	0.69 ± 2.33
	P4	−3.55	3.54	−9.50	−3.17 ± 6.53
	Camptothecin (2.5 μg ml^{-1})	38.54	45.12	38.55	40.74 ± 3.80
	DMSO	0.00	0.00	−2.47	−0.82 ± 1.43

[a] Test concentration of all extracts and compounds was 100 μg ml^{-1}. [b] Abbreviation for human fibroblasts.

α-Glucosidase inhibitory activity

Table 2 presents the α-glucosidase inhibitory activity of all extracts, compounds **P1** and **P4** of *E. tithymaloides*. It was obvious from this table that the aqueous extract of *E. tithymaloides* exhibited the strongest activity. For isolated compounds, both **P1** and **P4** showed very high inhibition of α-glucosidase, with **P1** being as strong as acarbose, and **P4** being significantly stronger. The obtained results contributed considerably to the anti-diabetic activity of *E. tithymaloides*, as well as the bioactivities of different jatrophane-type diterpenoids.

Structure elucidation for isolated compounds from the PE extract

The ^{1}H-NMR spectra of compound **P1** indicated that it possessed a jatrophane skeleton, with signals of three olefin protons at δ_H (ppm) 5.89 (1H, d, 9.0, H-5); 5.63 (1H, d, 15.6, H-

Table 2 α-Glucosidase inhibitory activity of extracts and compounds of *E. tithymaloides* ([ ]: extract; [ ]: compound)

Extract/compound	Concentration (μg ml^{-1})	Inhibitory percentage (%) 1st time	2nd time	3rd time	Average ± SE
Eth-PE	100	−13.11	−18.12	−13.17	−14.80 ± 2.87
	250	10.29	3.88	11.04	8.40 ± 3.94
	500	12.50	−0.41	11.38	7.82 ± 7.15
	1000	10.75	6.16	6.71	7.87 ± 2.50
Eth-EA	100	5.44	−6.25	−5.57	−2.13 ± 6.56
	250	−9.13	−12.04	−7.14	−9.44 ± 2.47
	500	5.72	5.84	−1.50	3.50 ± 3.94
	1000	0.23	−5.25	−5.14	−3.39 ± 3.13
Eth-Me	100	−12.82	−23.40	−10.99	−15.74 ± 6.70
	250	−5.72	1.87	−4.19	−2.68 ± 4.01
	500	7.47	12.82	1.00	7.10 ± 5.92
	1000	13.24	19.53	12.09	14.95 ± 4.00
Eth-W	100	31.95	32.49	49.24	37.89 ± 9.83
	250	94.18	91.38	90.52	92.03 ± 1.92
	500	98.30	98.39	98.66	98.45 ± 0.19
	1000	99.46	99.43	99.20	99.37 ± 0.14
	IC_{50} (μg ml^{-1})	121.8	121.9	97.56	113.75 ± 14.02
P1	63	−4.33	0.00	0.60	−1.24 ± 2.69
	125	8.73	3.55	12.81	8.36 ± 4.64
	250	14.80	15.56	19.60	16.65 ± 2.58
	500	65.07	58.25	56.18	59.83 ± 4.65
	IC_{50} (mg ml^{-1})	0.41	0.45	0.45	0.44 ± 0.02
P4	15.6	77.79	73.37	77.31	76.16 ± 2.42
	31.3	91.52	90.02	91.97	91.17 ± 1.02
	62.5	98.48	98.43	98.53	98.48 ± 0.05
	125	99.15	99.19	99.15	99.16 ± 0.03
	IC_{50} (μg ml^{-1})	10.76	11.21	10.17	10.71 ± 0.52
Acarbose	1000	79.34	71.88	78.44	76.56 ± 4.07
	IC_{50} (mg ml^{-1})	0.50	0.44	0.42	0.45 ± 0.04

11); 5.45 (1H, d, 15.6, H-12); fifteen protons of five methyl groups at δ_H (ppm) 0.91 (3H, d, 6.6, CH_3-16); 1.85 (3H, s, CH_3-17); 1.00 (3H, s, CH_3-18); 0.96 (3H, s, CH_3-19); 1.30 (3H, s, CH_3-20); six protons of two acetoxy groups at δ_H (ppm) 1.67 (3H, s, AcO-9); 2.41 (3H, s, AcO-15); aromatic protons of two benzoyloxy groups at δ_H (ppm) 7.72 (2H, dd, 7.2, 1.2, H-2′ and H-6′); 7.19 (2H, dd, 8.2, 7.2, H-3′ and H-5′); 7.38 (1H, ddd, 8.2, 7.2, 1.2, H-4′); 7.59 (2H, dd, 7.2, 1.2, H-2″ and H-6″); 7.05 (2H, dd, 8.1, 7.2, H-3″ and H-5″); 7.34 (1H, ddd, 8.1, 7.2, 1.2, H-4″). **P1** was revealed from those signals to be possibly an acetylbenzoyloxymethylated jatrophadiene. Such considerations on the structure of **P1** were reinforced by the ^{13}C- and DEPT-NMR spectra, with signals of seven C–OR groups at δ_C (ppm) 87.7 (C-1); 78.6 (C-3); 75.1 (C-7); 74.7 (C-9); 75.2 (C-13); 73.3 (C-14); 92.1 (C-15); five methyl carbons at δ_C (ppm) 12.1 (C-16); 16.7 (C-17); 23.5 (C-18); 21.0 (C-19); 31.5 (C-20); carbons of two acetoxy groups at δ_C (ppm) 32.8 and 173.6 (AcO-9); 39.3 and 170.3 (AcO-15); carbons of two benzoyloxy groups at δ_C (ppm) 165.6 (BzO-3); 165.3 (BzO-7); four olefin carbons at δ_C (ppm) 120.8 (C-5); 134.6 (C-6); 132.4 (C-11); 132.0 (C-12). From the HSQC spectra of **P1**, a signal of each proton of its jatrophane skeleton was defined. Third, the COSY spectra of **P1** showed correlations between H-2/H-1, OH-1/H-1, H-4/H-3 and H-5, OH-14/H-14; while correlations between H-

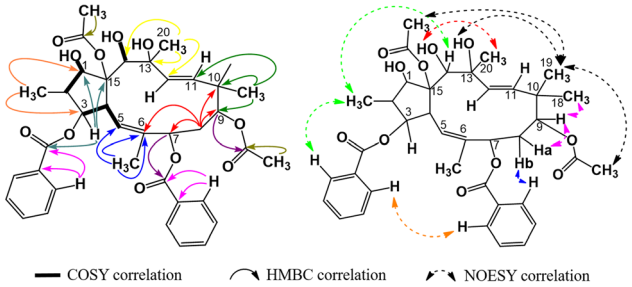

Fig. 4 Major COSY, HMBC, and NOESY correlations in **P1**.

Table 3 Spectral data comparison for **P1** and 9β,15β-diacetoxy-3β,7β-dibenzoyloxy-1α,13β,14α-trihydroxy-jatropha-5,11*E*-diene

	P1 (acetone-d_6)		9β,15β-Diacetoxy-3β,7β-dibenzoyloxy-1α,13β,14α-trihydroxy-jatropha-5,11*E*-diene ($CDCl_3$)[16]	
Position	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
1	4.25 (1H, dd, 15.0, 2.4)	87.7	4.12 (1H, dd, 17.0, 2.5)	86.7
2	2.32 (1H, m)	44.3	2.30 (1H, m)	42.3
3	5.40 (1H, s)	78.6	5.99 (1H, s)	78.0
4	4.23 (1H, dd, 9.0, 4.8)	42.0	4.42 (1H, dd, 10.0, 5.5)	42.8
5	5.89 (1H, d, 9.0)	120.8	5.74 (1H, d, 9.5)	118.2
6	—	134.6	—	134.4
7	5.25 (1H, d, 9.0)	75.1	5.51 (1H, d, 8.5)	75.0
8	2.18 (1H, m)	33.1	1.87 (1H, m)	34.8
	2.06 (1H, m)		1.99 (1H, s)	
9	5.24 (1H, t, 6.6)	74.7	4.78 (1H, t, 6.5)	74.0
10	—	40.3	—	39.2
11	5.63 (1H, d, 15.6)	132.4	5.49 (1H, s)	132.4
12	5.45 (1H, d, 15.6)	132.0	5.53 (1H, m)	129.4
13	—	75.2	—	75.8
14	4.41 (1H, m)	73.3	4.09 (1H, t, 10.5)	72.1
15	—	92.1	—	91.4
16	0.91 (3H, d, 6.6)	12.1	0.84 (3H, s)	11.7
17	1.85 (3H, s)	16.7	1.75 (3H, s)	16.5
18	1.00 (3H, s)	23.5	1.01 (3H, s)	23.0
19	0.96 (3H, s)	21.0	0.98 (3H, s)	20.7
20	1.30 (3H, s)	31.5	1.22 (3H, s)	31.6
AcO-9	1.67 (3H, s)	20.9	2.04 (3H, s)	21.3
		173.6		171.1
AcO-15	2.41 (3H, s)	22.4	2.33 (3H, s)	22.3
		170.3		172.9
BzO-3	—	165.6	—	165.1
1′	—	130.6	—	130.1
2′,6′	7.72 (2H, dd, 7.2, 1.2)	129.1	8.10 (1H, s)	128.6
			8.12 (1H, s)	
3′,5′	7.19 (2H, dd, 8.2, 7.2)	129.7	7.53 (2H, m)	129.0
4′	7.38 (1H, ddd, 8.2, 7.2, 1.2)	133.5	7.61 (1H, m)	133.3
BzO-7	—	165.3	—	166.2
1″	—	130.6	—	130.0
2″,6″	7.59 (2H, dd, 7.2, 1.2)	128.7	7.43 (2H, d, 8.0)	128.7
3″,5″	7.05 (2H, dd, 8.1, 7.2)	130.0	7.92 (1H, d, 8.0)	129.7
			7.94 (1H, s)	
4″	7.34 (1H, ddd, 8.1, 7.2, 1.2)	133.1	7.53 (1H, m)	133.0

16/C-1 and C-3; H-17/C-5, C-6, and C-7; H-18 and H-19/C-9, C-10, and C-11; H-20/C-12, C-13, and C-14 were observed in the HMBC spectra, indicating that **P1** was methylated at the 2, 6, 10, and 20 positions in its structure. In addition, the correlations between H-3, H-7 and benzoyloxy carbons allowed for the determination of benzoyloxylated positions for **P1**. Similar elucidations were made for **P1**'s acetoxy groups (Fig. 4). Finally, the NOESY spectra of **P1** resulted in its stereochemical structure determination (Fig. 9). Based on the spectral similarities to previous publication[16] (Table 3), compound **P1** was revealed to be 9β,15β-diacetoxy-3β,7β-dibenzoyloxy-1α,13β,14α-trihydroxy-jatropha-5,11*E*-diene.

The spectral data of compound **P2** indicated that it was also an acetylbenzoyloxymethylated jatrophadiene similar to **P1**, with signals of three olefin protons and carbons at δ_H (ppm) 5.78 (1H, d, 9.9, H-5), 5.46 (1H, d, 15.6, H-11), 5.27 (1H, d, 15.6, H-12); δ_C (ppm) 118.5 (C-5); 133.9 (C-6); 132.4 (C-11); 129.3 (C-12); protons and carbons of five methyl groups at δ_H (ppm) 1.04 (3H, d, 6.8, CH_3-16), 1.70 (3H, s, CH_3-17), 0.93 (3H, s, CH_3-18), 0.94 (3H, s, CH_3-19), 1.40 (3H, s, CH_3-20); δ_C (ppm) 11.5 (C-16); 16.6 (C-17); 20.7 (C-18); 22.8 (C-19); 31.5 (C-20); protons and carbons of two acetoxy groups at δ_H (ppm) 2.00 (3H, s, AcO-9), 2.35 (3H, s, AcO-15); δ_C (ppm) 21.4 and 169.6 (AcO-9), 22.1 and 171.1 (AcO-15); aromatic protons and carbons of two benzoyloxy groups: BzO-1: δ_H (ppm) 8.05 (2H, dd, 7.2, 1.1, H-2′ and H-6′), 7.53 (2H, dd, 7.7, 7.2, H-3′ and H-5′), 7.64 (1H, ddd, 7.7, 7.2, 1.1, H-4′); δ_C (ppm) 166.2, 130.2 (C-1′), 129.4 (C-2′ and C-6′), 129.6 (C-3′ and C-5′), 133.9 (C-4′); BzO-3: δ_H (ppm) 8.02 (2H, dd, 7.2, 1.0, H-2″ and H-6″), 7.49 (2H, dd, 7.7, 7.2, H-3″ and H-5″), 7.58 (1H, ddd, 7.7, 7.2, 1.0, H-4″); δ_C (ppm) 165.4, 130.0 (C-1″), 128.6 (C-2″ and C-6″), 128.8 (C-3″ and C-5″), 133.2 (C-4″). Notably, the signal of a methoxy group was observed in the spectra of **P2** (δ_H (ppm) 2.68 (3H, s); δ_C (ppm) 55.7 (OCH_3-7)). The HSQC spectra of **P2** allowed for the signal definition for its protons, while from the COSY and HMBC spectra, **P2** was determined to be 2,6,10,13-methylated, 9,15-acetoxylated, 1,3-benzoyloxylated, and 7-methoxylated (Fig. 5). Finally, the stereochemical structure of **P2** was elucidated from its NOESY spectra as shown in Fig. 9 and was named 9α,15β-diacetoxy-1α,3β-dibenzoyloxy-13α,14α-dihydroxy-7β-methoxyjatropha-5,11*E*-diene.

As no referential publications were found for direct spectral data comparisons, **P2** was compared with 9α,15β-diacetoxy-1α,3β,7β-tribenzoyloxy-13α,14α-trihydroxy-jatropha-5,11*E*-diene, which had the highest structural resemblance to that of **P2** (Table 4). From this table, it was obvious that **P2** had significant spectral similarities to those of 9α,15β-diacetoxy-1α,3β,7β-tribenzoyloxy-13α,14α-trihydroxy-jatropha-5,11*E*-diene, except for the replacement of the 7-benzoyloxy group with the 7-methoxy group. A reference on the SciFinder database indicated that the structure of **P2** was new to science and this compound was named tithymal C.

Compound **P3** showed significant similarities in spectral characters to those of **P1**, with a diacetylated jatrophadiene skeleton (δ_H (ppm) 0.86 (3H, d, 6.6, H-16), 1.89 (3H, s, H-17), 0.98 (3H, s, H-18), 0.96 (3H, s, H-19), 1.35 (3H, s, H-20), 5.98 (1H, d, 10.2, H-5), 5.64 (1H, d, 15.6, H-11), 5.45 (1H, d, 15.6, H-12), 1.68 (3H, s, AcO-9); 2.34 (3H, s, AcO-15); δ_C (ppm) 86.9 (C-1), 78.3 (C-3), 75.2 (C-7), 74.5 (C-9), 75.4 (C-13), 73.2 (C-14), 91.4 (C-15), 11.8 (C-16), 16.7 (C-17), 23.4 (C-18), 21.1 (C-19), 31.4 (C-20), 121.2 (C-5), 134.8 (C-6), 132.3 (C-11), 132.2 (C-12), 20.9 and 170.2 (AcO-9), 22.6 and 172.8 (AcO-15)). However, signals of three benzoyloxy groups were observed in the spectra of **P3**, instead of two groups in **P1**, indicating that the structure of **P3** was tribenzoyloxylated (complex signals of fifteen aromatic protons at δ_H (ppm) 8.21–7.08 and signals of oxygen-bonding aromatic carbons at δ_C (ppm) 166.5, (BzO-1); 165.7 (BzO-3); 165.3 (BzO-7)). The HSQC spectra of **P3** allowed for the signal definition for its protons, while the COSY and HMBC spectra of **P3** indicated that it was 2,6,10,13-methylated, 9,15-acetoxylated, and 1,3,7-benzoyloxylated (Fig. 6). Finally, the stereochemical structure of **P3** was determined from its NOESY spectra as shown in Fig. 9. Comparison with a previous publication[17] (Table 5) resulted in our conclusion for **P3** to be 9α,15β-diacetoxy-1α,3β,7β-tribenzoyloxy-13β,14α-dihydroxy-jatropha-5,11*E*-diene.

The spectral data of **P4** showed similarities to those of **P1**, indicating that **P4** was also acetylbenzoyloxymethyljatrophadiene. Specifically, from the ^{1}H-NMR and ^{13}C-NMR spectra, the signals of a jatrophane skeleton were observed: fifteen methyl protons (–CH_3) at δ_H (ppm) 1.07 (3H, d, 6.5), 1.69 (3H, s), 1.11 (3H, s), 0.94 (3H, s), 1.33 (3H, s); three olefin protons at δ_H (ppm) 5.57 (1H, d, 10.0, H-5), 5.56 (1H, d, 15.5, H-11), 5.15 (1H, d, 15.5, H-12); three protons of an acetoxy group (AcO-15) at δ_H (ppm) 2.25 (3H, s); aromatic protons at δ_H (ppm) 7.94 (2H, dd, 7.5, 1.5, H-2′ and H-6′), 7.46 (2H, dd, 7.5, 7.5, H-3′ and H-5′), 7.59 (1H, ddd, 7.5, 7.5, 1.5, H-4′); seven C–OR carbons at δ_C (ppm) 87.2 (C-1), 78.8 (C-3), 72.6 (C-7), 72.5 (C-9), 74.6 (C-13), 72.4 (C-14), 91.5 (C-15); five methyl carbons at δ_C (ppm) 11.8 (C-16), 16.6 (C-17), 19.5 (C-18), 23.4 (C-19), 31.5 (C-20); the signal of carbons from an acetoxy group at δ_C (ppm) 22.6, 172.8 (AcO-15) and benzoyloxy group at δ_C (ppm) 165.9 (BzO-3); aromatic carbons at δ_C (ppm) 130.0 (C-1′), 128.7 (C-2′, C- 6′), 129.3 (C-3′, C-5′), 133.2 (C-4′); four olefin carbons at δ_C (ppm) 117.4 (C-5), 139.2 (C-6), 134.0 (C-11), 128.2 (C-1). Notably, the obtained ^{1}H-NMR and ^{13}C-NMR spectra of **P4** showed that this compound possessed one acetoxy group and one benzoyloxy group less than **P1**. From the HSQC spectra of **P4**, the signal of each proton of its jatrophane skeleton was defined. The correlations between H-2/H-1, H-4/H-3 and H-5, OH-7/H-7 and H-17, OH-9/H-19, OH-14/H-14, OH-1/H-1, and H(AcO-15)/H-1 were

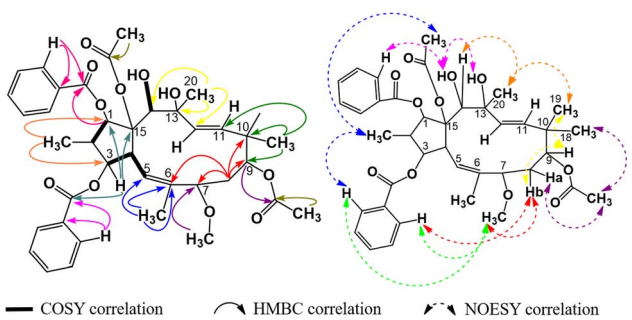

Fig. 5 Major COSY, HMBC, and NOESY correlations in **P2**.

Table 4 Spectral data comparison for **P2** and 9α,15β-diacetoxy-1α,3β,7β-tribenzoyloxy-13α,14α-trihydroxy-jatropha-5,11*E*-diene

	P2 ($CDCl_3$)		9α,15β-Diacetoxy-1α,3β,7β-tribenzoyloxy-13α,14α-trihydroxy-jatropha-5,11*E*-diene ($CDCl_3$)[17]	
Position	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
1	5.89 (1H, d, 11.7)	87.2	5.88 (1H, d, 11.8)	87.1
2	2.57 (1H, m)	44.2	2.52 (1H, m)	44.2
3	5.58 (1H, m)	77.2	5.54 (1H, dd, 5.3, 4.3)	77.2
4	4.13 (1H, dd, 9.8, 5.1)	41.9	4.11 (1H, dd, 9.7, 5.1)	41.9
5	5.78 (1H, d, 9.9)	118.5	5.89 (1H, d, 9.7)	119.3
6	—	133.9	—	134.4
7	2.68 (3H, s)	55.7	5.30 (1H, s)	74.2
8	1.87 (2H, m)	33.7	2.08 (2H, m)	32.4
9	4.97 (1H, t, 3.3)	73.7	5.15 (1H, dd, 3.5, 2.9)	73.9
10	—	39.5	—	39.6
11	5.46 (1H, d, 15.6)	132.4	5.54 (1H, d, 15.5)	132.1
12	5.27 (1H, d, 15.6)	129.3	5.33 (1H, d, 15.5)	129.9
13	—	74.8	—	74.7
14	4.86 (1H, m)	72.3	4.86 (1H, d, 5.6)	72.3
15	—	90.0	—	90.1
16	1.04 (3H, d, 6.8)	11.5	0.93 (3H, d, 6.7)	11.4
17	1.70 (3H, s)	16.6	1.87 (3H, s)	16.5
18	0.93 (3H, s)	20.7	0.94 (3H, s)	20.6
19	0.94 (3H, s)	22.8	0.95 (3H, s)	22.8
20	1.40 (3H, s)	31.5	1.39 (3H, s)	31.5
AcO-9	2.00 (3H, s)	21.4	2.04 (3H, s)	21.3
		169.6		171.1
AcO-15	2.35 (3H, s)	22.1	2.33 (3H, s)	22.3
		171.1		172.9
BzO-1		166.2	—	165.4
1′		130.2	—	130.1
2′,6′	8.05 (2H, dd, 7.2, 1.1)	129.4	8.02 (2H, dd, 7.3, 1.2)	128.7
3′,5′	7.53 (2H, dd, 7.7, 7.2)	129.6	7.50 (2H, dd, 7.7, 7.3)	129.3
4′	7.64 (1H, ddd, 7.7, 7.2, 1.1)	133.9	7.61 (1H, ddd, 7.7, 7.3, 1.2)	133.2
BzO-3	—	165.4	—	165.3
1″	—	130.0	—	129.5
2″,6″	8.02 (2H, dd, 7.2, 1.0)	128.6	7.60 (2H, dd, 7.3, 1.1)	128.2
3″,5″	7.49 (2H, dd, 7.7, 7.2)	128.8	7.13 (2H, dd, 7.8, 7.3)	129.1
4″	7.58 (1H, ddd, 7.7, 7.2, 1.0)	133.2	7.31 (1H, ddd, 7.8, 7.3, 1.1)	132.7
BzO-7	—	—	—	165.0
1‴	—	—	—	129.3
2‴, 6‴	—	—	7.58 (2H, dd, 7.2, 1.0)	127.8
3‴, 5‴	—	—	7.00 (2H, dd, 7.8, 7.2)	129.3
4‴	—	—	7.28 (1H, ddd, 7.8, 7.2, 1.0)	132.2

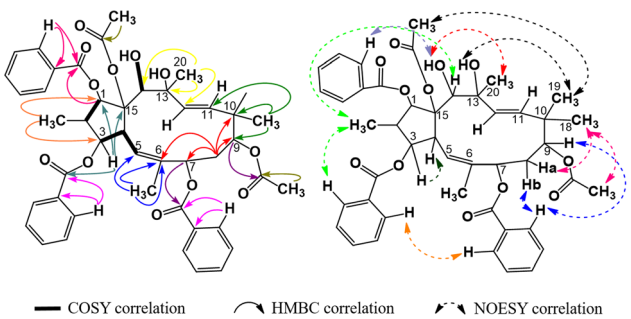

Fig. 6 Major COSY, HMBC, and NOESY correlations in **P3**.

observed in the COSY spectra of **P4**, while in its HMBC spectra, the correlations between H-16/C-1 and C-3; H-17/C-5, C-6, and C-7; H-18 and H-19/C-9, C-10, and C-11; H-20/C-12, C-13, and C-14 were observed (Fig. 7). Such spectral information indicated that **P4** was methylated at the 16, 17, 18, 19, and 20 positions. However, H-3 correlated with the carboxyl carbon atom of the benzoyloxy group, indicating the presence of a 3-benzoyloxy moiety in the structure of **P4**. From all NMR spectral data above, **P4** was considered to be 15-acetoxy-3-benzoyloxy-1,7,9,13,14-pentahydroxy-jatropha-5,11-diene. Finally, **P4**'s NOESY spectra expressed the correlations between OH-13/H-8b, H-16/H-14 và OH-7, OH-14/H-20, H-19/OAc-15, H-9/H-19 (Fig. 7). Compound **P4** was concluded to be 15α-acetoxy-3β-benzoyloxy-1α,7α,9β,13α,14β-pentahydroxy-jatropha-5,11*E*-diene (Fig. 9).

As no referential data were found for **P4**, this compound was compared with pedititin G, to which **P4** had the most similar structure. The comparative data are expressed in Table 6. It was obvious from this table that the structure of **P4** and pedititin G were similar in the 15-acetoxy-3-benzoyloxyjatropha-5,11*E*-diene

Table 5 Spectral data comparison for **P3** and 9β,15β-diacetoxy-3β,7β-dibenzoyloxy-1α,13β,14α-trihydroxy-jatropha-5,11*E*-diene

	P3 (acetone-d_6)		9α,15β-Diacetoxy-1α,3β,7β-tribenzoyloxy-13β,14α-dihydroxy-jatropha-5,11*E*-diene ($CDCl_3$)[17]	
Position	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
1	5.87 (1H, d, 11.4)	86.9	5.88 (1H, d, 11.8)	87.1
2	2.34 (1H, m)	44.2	2.52 (1H, m)	44.2
3	5.51 (1H, dd, 5.4, 3.6)	78.3	5.54 (1H, dd, 5.3, 4.3)	77.2
4	4.25 (1H, dd, 9.6, 5.4)	42.4	4.11 (1H, dd, 9.7, 5.1)	41.9
5	5.98 (1H, d, 10.2)	121.2	5.89 (1H, d, 9.7)	119.3
6	—	134.8	—	134.4
7	5.27 (1H, s)	75.2	5.30 (1H, s)	74.2
8	2.21 (2H, m)	33.3	2.08 (2H, m)	32.4
9	5.22 (1H, dd, 3.6, 2.8)	74.5	5.15 (1H, dd, 3.5, 2.9)	73.9
10	—	40.3	—	39.6
11	5.64 (1H, d, 15.6)	132.3	5.54 (1H, d, 15.5)	132.1
12	5.45 (1H, d, 15.6)	132.2	5.33 (1H, d, 15.5)	129.9
13	—	75.4	—	74.7
14	4.67 (1H, d, 6.4)	73.2	4.86 (1H, d, 5.6)	72.3
15	—	91.4	—	90.1
16	0.86 (3H, d, 6.6)	11.8	0.93 (3H, d, 6.7)	11.4
17	1.89 (3H, s)	16.7	1.87 (3H, s)	16.5
18	0.98 (3H, s)	23.4	0.94 (3H, s)	20.6
19	0.96 (3H, s)	21.1	0.95 (3H, s)	22.8
20	1.35 (3H, s)	31.4	1.39 (3H, s)	31.5
AcO-9	1.68 (3H, s)	20.9	1.67 (3H, s)	20.8
		170.2		170.0
AcO-15	2.34 (3H, s)	22.5	2.38 (3H, s)	22.1
		171.1		170.9
BzO-1	—	166.5	—	165.4
1‴	—	134.6	—	133.2
2‴, 6‴	8.17 (2H, dd, 7.3, 1.2)	133.1	8.02 (2H, dd, 7.3, 1.2)	129.3
3‴, 5‴	7.58 (2H, dd, 8.4, 7.3)	131.8	7.50 (2H, dd, 7.7, 7.3)	128.7
4‴	7.70 (1H, ddd, 8.4, 7.3, 1.2)	133.6	7.61 (1H, ddd, 7.7, 7.3, 1.2)	130.1
BzO-3	—	165.7	—	165.3
1′	—	133.7	—	132.7
2′,6′	7.72 (2H, dd, 7.2, 1.1)	130.1	7.60 (2H, dd, 7.3, 1.1)	129.1
3′,5′	7.19 (2H, dd, 8.0, 7.2)	130.0	7.13 (2H, dd, 7.8, 7.3 Hz)	128.2
4′	7.38 (1H, ddd, 8.0, 7.2, 1.1)	130.8	7.31 (1H, ddd, 7.8, 7.3, 1.1)	129.5
BzO-7	—	165.3	—	165.0
1″	—	132.3	—	132.2
2″,6″	7.59 (2H, dd, 7.2, 1.0)	130.7	7.58 (2H, dd, 7.2, 1.0)	129.3
3″,5″	7.05 (2H, dd, 7.8, 1.0)	129.2	7.00 (2H, dd, 7.8, 7.2)	127.8
4″	7.34 (1H, ddd, 7.8, 7.2, 1.0)	130.0	7.28 (1H, ddd, 7.8, 7.2, 1.0)	129.3

moiety, while the 9-acetoxy and 14-benzoyloxy groups of peditithin G were absent in **P4**. In addition, from the MS data, the molecular formula of **P4** was revealed to be $C_{29}H_{40}O_9$. This compound, therefore, was concluded to be 15α-acetoxy-3β-benzoyloxy-1α,7α,9β,13α,14β-pentahydroxy-jatropha-5,11*E*-diene. A reference on the SciFinder database indicated that the structure of **P4** was new to science and this compound was named tithymal A.

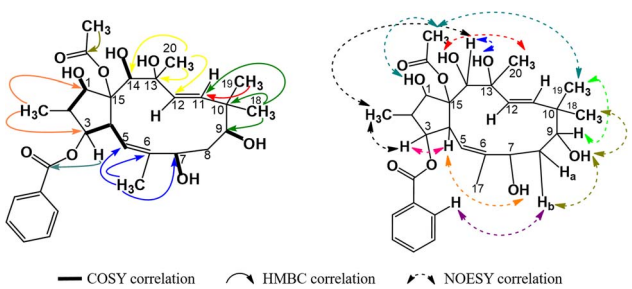

Fig. 7 Major COSY, HMBC, and NOESY correlations in **P4**.

Compound **P5** had significant spectral similarities to **P4**, indicating their structural resemblance. Specifically, **P5** had an acetylbenzoyloxymethyljatrophadiene skeleton, with the signals of olefin protons and carbons at δ_H (ppm) 5.55 (1H, d, 9.6, H-5), 5.42 (1H, d, 15.6, H-11), 5.11 (1H, d, 15.6, H-12); δ_C (ppm) 117.4 (C-5), 139.2 (C 6), 134.0 (C-11), 128.2 (C-12); the protons and carbons of five methyl groups at δ_H (ppm) 1.66 (3H, s, CH_3-17), 1.31 (3H, s, CH_3-20), 1.04 (3H, d, 6.6, CH_3-16), 1.09 (3H, s, CH_3-19), 0.96 (3H, s, CH_3-18); δ_C (ppm) 11.8 (C-16), 16.3 (C-17), 19.5 (C 18), 23.4 (C-19), 31.5 (C-20); the protons and carbons of an acetoxy group at δ_H (ppm) 2.23 (3H, d, 1.8); δ_C (ppm) 22.6, 172.8 (AcO-15); aromatic protons and carbons at δ_H (ppm) 7.91 (2H, dd, 7.2, 1.2, H-2′ and H-6′), 7.44 (2H, dd, 7.2, 7.2, H-3′ and H-5′), 7.54 (1H, ddd, 7.2, 7.2, 1.2, H-4′); δ_C (ppm) 130.0 (C-1′), 129.3 (C-

Table 6 Spectral data comparison for **P4** and pedithithin G

	P4 (acetone-d_6)		Pedithithin G ($CDCl_3$)[17]	
Position	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
1	4.25 (1H, d, 12.0)	87.2	4.12 (1H, d, 12.0)	86.6
2	2.31 (1H, m)	43.1	2.30 (1H, m)	42.2
3	5.43 (1H, dd, 4.0, 4.0)	78.8	5.49 (1H, dd, 4.5, 4.5)	78.0
4	4.06 (1H, dd, 10.0, 4.5)	41.4	4.43 (1H, dd, 9.6, 4.5)	42.7
5	5.57 (1H, d, 10.0)	117.4	5.73 (1H, d, 9.6)	118.1
6	—	139.2	—	138.3
7	4.22 (1H, s)	72.6	4.09 (1H, s)	72.0
8	1.96 (H_a, m)	36.7	2.00 (H_a, m)	34.7
	1.74 (H_b, m)		1.86 (H_b, m)	
9	3.49 (1H, dd, 3.5, 3.5)	72.5	4.77 (1H, dd, 3.2, 3.2)	74.0
10	—	39.3	—	39.2
11	5.56 (1H, d, 15.5)	134.0	5.51 (1H, d, 15.6)	132.2
12	5.15 (1H, d, 15.5)	128.2	5.31 (1H, d, 15.6)	129.0
13	—	74.6	—	75.7
14	5.46 (1H, s)	72.4	5.99 (1H, s)	72.9
15	—	91.5	—	91.3
16	1.07 (3H, d, 6.5)	11.8	0.84 (3H, d, 6.4)	11.7
17	1.69 (3H, s)	16.6	1.75 (3H, s)	16.4
18	0.94 (3H, s)	19.5	0.98 (3H, s)	20.7
19	1.11 (3H, s)	23.4	1.00 (3H, s)	23.0
20	1.33 (3H, s)	31.5	1.21 (3H, s)	31.5
AcO-9	—	—	2.04 (3H, s)	21.3
				171.1
AcO-15	2.25 (3H, s)	22.6	2.34 (3H, s)	22.3
		172.8		172.9
BzO-3	—	165.9	—	166.2
1′	—	130.0	—	130.0
2′,6′	7.94 (2H, dd, 7.5, 1.5)	128.7	7.93 (2H, dd, 7.8, 1.2)	129.4
3′,5′	7.46 (2H, dd, 7.5, 7.5)	129.3	7.43 (2H, dd, 7.8, 7.8)	128.5
4′	7.59 (1H, ddd, 7.5, 7.5, 1.5)	133.2	7.54 (1H, ddd, 7.8, 7.8, 1.2)	133.0
BzO-14	—	—	—	165.0
1″	—	—	—	129.8
2″,6″	—	—	8.11 (dd, 7.8, 1.2)	129.7
3″,5″	—	—	8.11, dd (7.8, 1.2)	128.7
4″	—	—	7.61, ddd (7.8, 7.2, 1.2)	133.3

2′ and 6′), 128.7 (C-3′ and 5′), 133.2 (C-4′), together with an oxygen-bearing benzoyl carbon at δ_C (ppm) 165.9 (BzO-3) and six other oxygen-bearing ones at δ_C (ppm) 87.2 (C-1), 78.8 (C-3), 72.5 (C-9), 74.6 (C-13), 72.4 (C-14), 91.5 (C-15). From the COSY spectra of **P5**, the correlations of H-2/H-1, H-4/H-3 and H-5, OH-9/H-8, OH-14/H-14, OH-1/H-1, H(CH_3COO-15)/H-1 were observed, while those between H-16 and C-1, C-3; H-17 and C-5, C-6; H-18, H-19 and C-9, C-10, C-11; H-20 and C-12, C-13, C-14 were observed in the HMBC spectra (Fig. 8). Such data led to the consideration for **P5**'s structure to be 15-acetoxy-3-benzoyloxy-1,9,13,14-tetrahydroxyjatropha-5,11*E*-diene. Finally, from the NOESY spectra of **P5**, the correlations of H-18/OH-9, H-8a/

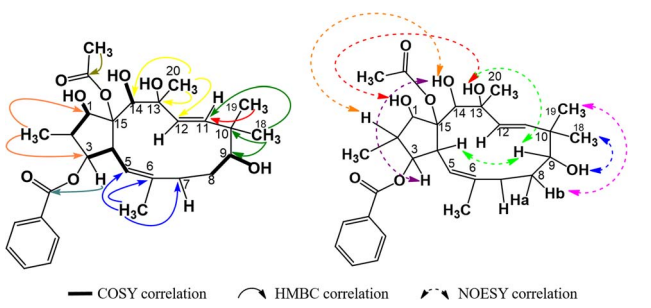

Fig. 8 Major COSY, HMBC, and NOESY correlations in **P5**.

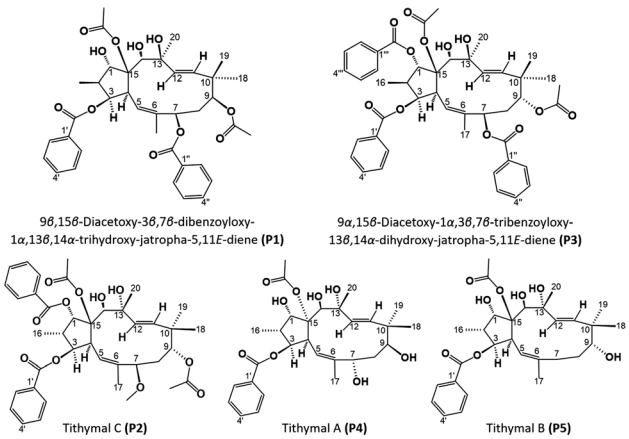

Fig. 9 Molecular structures of compounds **P1**–**P5**.

Table 7 Spectral data comparison for **P4** and **P5**

	P4 (acetone-d_6)		**P5** ($CDCl_3$)	
Position	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
1	4.25 (1H, d, 12.0)	87.2	4.22 (1H, d, 11.4)	87.2
2	2.31 (1H, m)	43.1	2.29 (1H, m)	43.1
3	5.43 (1H, dd, 4.0, 4.0)	78.8	5.49 (1H, dd, 3.8, 3.8)	78.8
4	4.06 (1H, dd, 10.0, 4.5)	41.4	4.04 (1H, m)	41.4
5	5.57 (1H, d, 10.0)	117.4	5.55 (1H, d, 9.6)	117.4
6	—	139.2	—	139.2
7	4.22 (1H, s)	72.6	2.17 (2H, d, 1.8)	30.9
8	1.96 (H_a, m)	36.7	1.72 (H_a, m)	36.8
	1.74 (H_b, m)		1.94 (H_b, m)	
9	3.49 (1H, dd, 3.5, 3.5)	72.5	4.77 (1H, dd, 3.0)	72.5
10	—	39.3	—	39.3
11	5.56 (1H, d, 15.5)	134.0	5.42 (1H, d, 15.6)	134.0
12	5.15 (1H, d, 15.5)	128.2	5.11 (1H, d, 15.6)	128.2
13	—	74.6	—	74.6
14	5.46 (1H, s)	72.4	4.28 (1H, s)	72.4
15	—	91.5	—	91.5
16	1.07 (3H, d, 6.5)	11.8	1.04 (3H, d, 6.6)	11.8
17	1.69 (3H, s)	16.6	1.66 (3H, s)	16.3
18	0.94 (3H, s)	19.5	0.96 (3H, s)	19.5
19	1.11 (3H, s)	23.4	1.09 (3H, s)	23.4
20	1.33 (3H, s)	31.5	1.31 (3H, s)	31.5
AcO-15	2.25 (3H, s)	22.6	2.23 (3H, s)	22.6
		172.8		172.8
BzO-3	—	165.9	—	165.9
1′	—	130.0	—	130.0
2′,6′	7.94 (2H, dd, 7.5, 1.5)	128.7	7.91 (2H, dd, 7.2, 1.2)	129.3
3′,5′	7.46 (2H, dd, 7.5, 7.5)	129.3	7.44 (2H, dd, 7.2, 7.2)	128.7
4′	7.59 (1H, ddd, 7.5, 7.5, 1.5)	133.2	7.54 (1H, ddd, 7.2, 7.2, 1.2)	133.2

CH_3COO-15, H-19/H-8b/H-3, OH-13/OH-1, OH-13/H-9/H-4, OH-14/H-2/H-3 were observed (Fig. 8), allowing for the stereochemical determination for this compound, as shown in Fig. 9, and **P5** was revealed to be 15β-acetoxy-3β-benzoyloxy-1α,9α,13α,14β-tetrahydroxyjatropha-5,11*E*-diene.

As no referential data were found for **P5**, this compound was compared with **P4** to evaluate the spectral and structural similarities and differences. The comparative data are expressed in Table 7. It was obvious from this table that the structure of **P5** is very similar to that of **P4**, except only for the absence of 7-OH group. However, from the MS data, the molecular formula of **P5** was revealed to be $C_{29}H_{40}O_8$. This compound, therefore, was concluded to be 15β-acetoxy-3β-benzoyloxy-1α,9α,13α,14β-tetrahydroxyjatropha-5,11*E*-diene.
A reference on the SciFinder database indicated that the structure of **P5** was new to science and this compound was named tithymal B.

Conclusions

In this study, the cytotoxicity and anti-diabetic activity of extracts and some isolated compounds of Vietnamese *Euphorbia tithymaloides* against human normal (fibroblasts) and cancer cell lines (HeLa, HepG2, Jurkat, MCF-7, and NCI-H460) were investigated. The obtained results indicated that this *Euphorbia* plant exhibited moderate toxicity to MCF-7 and NCI-H460, with the petroleum ether extract having the highest values. In addition, all extracts of the plant and tested isolated jatrophanes were non-toxic to normal cells. The aqueous extract of the plant exhibited the highest α-glucosidase inhibitory activity. From the petroleum ether extract, five jatrophane diterpenoids were isolated, three of which were new to science and respectively named tithymal A, tithymal B, and tithymal C. Interestingly, tithymal A exhibited significantly high α-glucosidase inhibitory activity. The obtained results contributed significantly to scientific knowledge on Vietnamese *Euphorbia tithymaloides*, reinforcing its potential to be exploited medicinally. In future studies, deeper steps will be taken on the remaining fractions of the petroleum ether extract, other extracts, as well as their other bioactivities and isolated compounds.

Author contributions

Nguyen Vu Duy Khang: methodology; validation; formal analysis; data curation; investigation; writing – original draft; visualization. Dinh Thi Hong Dao: methodology; data curation; investigation. Nguyen Thi Thanh Mai: conceptualization; methodology; validation. Tran Le Quan: conceptualization; methodology; supervision. Nguyen Thi Y. Nhi: conceptualization; methodology; validation; supervision; writing – review & editing.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This research is funded by Vietnam National University, Ho Chi Minh City (VNU-HCM) under grant number NCM2020-18-01.

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