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Research Article

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Chemical composition and biological activity analysis of semen euphorbiae petroleum ether extracts

Fei-peng Duan[1], Ying-zi Wang[*1], Chao Zhang[*2], Xin-sheng Fang[3], Bin Yan[2] and Cai-xia Li[1]

[1]Department of Chinese medicine, Beijing University of Chinese Medicine, Beijing, China
[2]College of Pharmacy, Shandong University of Traditional Chinese Medicine, Ji'nan, China
[3]College of Agronomy, Shandong Agriculture University, Taian, China

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ABSTRACT

To study the chemical constituents and the effect of petroleum ether extracts on P-gp in Vitro. Compounds were isolated by methods of phase silica gel column chromatography, gel column chromatography, preparation of thin layer chromatography and various chromatographic techniques, and identified by the physicochemical data of spectral analysis. We measured P-glycoprotein(P-gp) mediated ATP hydrolysis to determine the substrates of P-gp. Ten compounds were isolated from the petroleum ether extracts of semen Euphorbia, and their structures were identified as, 5,15-O-diacetyl-3-O-benzoyl-lathyol(I),5,15-O-diacetyl-3,7-O-dibenzoyl-7-hydroxylathyrol(II), 15-O-diacetyl-3-O-phenyl-6(17)-epoxylathyrol(III), Euphorbia factorL8 (IV),5,15,17 -O-triacety-3-O-benzoyl-17 hydroxyisolathyrol(V), daucosterol(VI),esculetin(VII), Glycerol-1-monooleate(VIII), salicylic acid(IX), oleic acid(X). Much ATP was used by EFL2 and EFL3. For the first time, compounds IX is obtained from the plant. The compounds Ⅰ and Ⅱ were first found may be the substrates of P-gp, and they exhibited moderate activity inhibiting the effluxing function of P-gp.

Key words: Semen Euphorbia, petroleum ether extracts, structure identification, ATP-ase, P-gp

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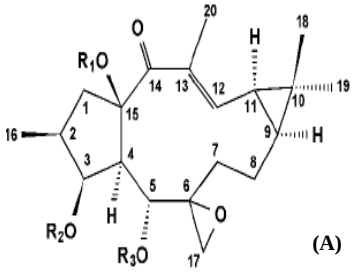
INTRODUCTION

The seeds of Euphorbia lathyris is a traditional Chinese medicine which has been used for the treatment of hydropsy, ascites, anuresis and constipation, amenorrhea, scabies [1]. In recent years, it was reported that the seeds of Euphorbia had a significant effect on Leukemia, Esophageal Carcinoma, and skin cancer[2-5]. The seeds of Euphorbia lathyris is a kind of toxic traditional Chinese medicine, which characterized by pungent, warm and poisonous in drug properties. It shows several side effects such as irritation and inflammation intense on the skin, mouth and gastrointestinal tract irritation, carcinogenic, and so on[6].

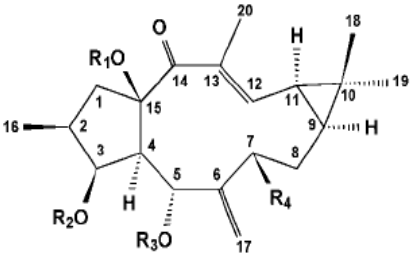
Previous phytochemical studies on this plant revealed the presence of twenty diterpenoid compounds, and a few triterpenoids, steroids, fatty acid esters, coumarins[7]. The diterpenoid[8] accounted 3% -5% of the total fatty oil. They were named as EFL1-L11 according to the discovered successively (Fig.1).

P-glycoprotein(P-gp) is one of the best characterized transporters responsible for the multidrug resistance phenotype exhibited by cancer cells[9]. There is widespread interest in elucidating whether existing drugs are candidate P-gp substrates or inhibitors. Jung SookChoi's[10] research found that Euphorbia Factor L1(III) can reversing the multidrug resistance of MES-SA/Dx5 cell which controlled by P-gp. In order to determine whether have other compounds in Euphorbia lathyris can interacts with P-gp, it is possible to carry out the P-gp-GloAssy Kit to measurement of its effect on the rate of P-gp ATP hydrolysis.



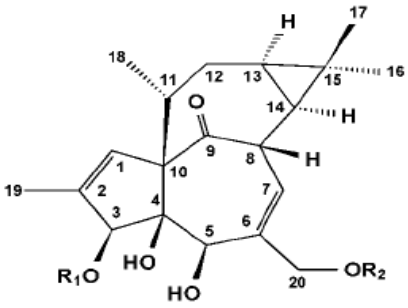
	R_1	R_2	R_3
Epoxylathyrol	H	H	H
Factor L_1	$OCCH_3$	$OCCH_2C_6H_5$	$OCCH_3$

(A)



	R_1	R_2	R_3	R_4
Lathyrol	H	H	H	H
Factor L_2	$OCCH_3$	OCC_6H_5	$OCCH_3$	OCC_6H_5
Factor L_3	$OCCH_3$	OCC_6H_5	$OCCH_3$	H
Factor L_8	$OCCH_3$	OCC_5H_4N	$OCCH_3$	H
Factor L_9	$OCCH_3$	OCC_6H_5	$OCCH_3$	OCC_5H_4N

(B)



	R_1	R_2
Ingenol	H	H
Factor L_4	H	$OCC_{15}H_{31}$
Factor L_5	$OCC_{15}H_{31}$	H
Factor L_6	$OCC_{14}H_{17}$	H

(C

EXPERIMENTAL SECTION

2.1 Materials and Reagents

Stuart smp3 Melting Point Apparatus; Varia INOVA 600 Nuclear Magnetic Resonance; Agilent Trap VL mass spectrometer; Silica gel (Chromatography grade, Qingdao Haiyang Chemical Co., Ltd, Shandong, China); other reagents are analytical; Euphorbia seeds was purchased from Huqiao Chinese Herbal Pieces Works (Anhui, China); Pgp-Glo^{TM} assay systems Kit(USA);

2.2 Detection Method

2.2.1 Extraction and isolation

Powder of *Euphorbia lathyris* seeds (4 kg) was refluxed with 95% alcohol to extract 3 times and 3 hours each time, whose ethanolic extract was concentrated till no alcohol smell and suspended in H_2O and partitioned successively with petroleum ether, ethyl acetate, n-butanol to afford corresponding extracts.

The petroleum ether extracts was separated by silica gel (petroleum ether-ethyl acetate, petroleum ether acetone) and Sephadex LH-20 column chromategraphy (chloroform-methanol, methanol) as well as preparative thin-layer chromatography, which afforded compound I (1100 mg), II (320 mg), III (1120 mg), IV (40 mg), V (20 mg), VI (39 mg), VII (10 mg), VIII (11 mg), IX (10 mg), X (14 mg). In addition, large number of yellow oil-like compounds with low polarity was obtained while not isolated, but afforded 55 compounds utilizing gas chromatography-mass spectrometry analysis, this part will be reported on another paper[11].

2.2.2 P-gp ATP-ase activities assay

Changes of ATPase activity were estimated by Pgp-Glo^{TM} assay systems. Verapamil(Ver) and Sodium orthovanadate (Na_3VO_4) was used as the Pgp ATPase inhibitor. Concentrations of 100μM/L Euphorbia Factor L1(EFL1), 100μM/L

Euphorbia Factor L2(EFL2), 100μM/L Euphorbia Factor L3(EFL3) diluted by assay buffer were incubated in solution containing 5mmol/LMg^{2+}ATP, and 25 mg recombinant human P-gp membranes at 37℃ for 40min. Luminescence was initiated by ATP detection buffer. After incubated at room temperature for 40min to allow luminescent signal to develop, the untreated(NT) white opaque 96-well plate was read on luminometer. The changes of relative light units (ΔRLU) was calculated in relation to non-treated control(NT), ΔRLU_{test}were determined by comparing Na_3VO_4-treated samples with verapamil-treated samples and EFL1, EFL2, EFL3-treated samples to NT. Results are presented as the average of three independent experiments. It was calculated using the following formula that fits the NT control to zero for easier interpretation: ΔRLU = RLUtest -RLU_{NT}.

RESULTS AND DISCUSSION

3.1 Structure Identification

Compound(I), colorless solid (Recrystallized in ethyl acetate), mp152~154°C. ESI-MS (m/z): 545[M+Na]+.1H-NMR(600MHz,CDCl3)δ:3.53(1H,dd,J=14.4,8.4Hz,H-1),5.82(1H,d,J=3Hz,H-3),2.9(1H,dd,J=J=10 .2,3Hz,H-4),6.21(1H,d,J=10.2Hz,H-5),6.54(1H,d,J=11Hz,H-12),0.94(3H,J=6.6Hz,H-16),5.01(1H,s,H-17),4.78(1H,s, H-17),7.46(2H,t,J=7.8Hz,OBz-3),7.59(1H,t,J=7.2Hz,OBz-3),8.03(2H,d,J=7.2Hz,OBz-3),1.83(3H,s,OAc-5),2.21(3H, s,OAc-15).The above data were consistent with that of the reported compound[12], so that the compound(I) was identified as 5,5-O -diacetyl-3-O-benzoyl-lathyol(EFL3).

Compound(II), colourless needle crystal(Recrystallized in ethyl acetate), mp200~202°C, ESI-MSm/z:665[M+Na]+.1H-NMR(600MHz,CDCl3)δ:1.77(1H,dd,J=14.4,12Hz,H-1),3.41(1H,dd,J=14.4,8.4Hz,H-1'),2.93(1H,dd,J=8.4,3.6Hz,H-4),6.38(1H,d,J=7.8Hz,H-5),1.34(1H,m,H-9),1.50(1H,dd,J=10.8,8.4Hz,H-11),6.51(1H ,d,J=11.4Hz,H-12),0.94(3H,d,J=6.6Hz,H-16),1.19(3H,s,H-18),1.26(3H,s,H-19),1.80(3H,s,H-20),7.46(2H,t,J=7.8Hz, OBz-3),8.05(2H,d,J=8.4Hz,OBz-3),7.59(1H,t,J=7.2Hz,OBz-3),1.28(3H,s,OAc-5),7.36(2H,t,J=8.4Hz,OBz-7),7.50(1 H,t,J=7.52Hz,OBz-7),7.93(2H,d,J=8.4Hz,OBz-7),2.22(3H,s,OAc-15).The above data was consistent with that of the reported compound[12], so that the compound(II) was identified as 5,15-O-diacetyl-3,7-O-dibenzoyl -7-hydroxylathyrol (EFL2).

Compound(III), colourless needle crystal(Recrystallized in ethyl acetate), mp198~200°C, ESI-MSm/z:575[M+Na]+.551.0[M-H]-.1H-NMR(600MHz,CDCl3)δ3.33(1H,dd,J=14.4,8.4Hz,H-1),1.37(1H,dd,J=1 4.4,12.6Hz,H-1'),2.10(1H,M,H-2),1.87(1H,dd,J=9,3Hz,H-4),6.25(1H,d,J=9Hz),0.93(1H,t,J=13.2Hz,H-7),1.73(1H,m ,H-8),0.66(3H,d,J=7.2Hz,H-16),2.49(1H,d,J=3Hz,H-17),1.21(3H,s,H-18),1.22(3H,s,H-19),1.84(3H,s,H-20). The above data was consistent with that of the reported compound [12], so that the compound(III) was identified as5,15-O-diacetyl-3 -O-phenyl-6(17)- epoxylathyrol (EFL1).

Compound (IV), colourless solitary crystal (Recrystallized in ethylacetate), ESI-MS m/z:546[M+Na]+,524[M+H]+.1H-NMR(600MHz,pyridine)δ:3.60(1H,dd,J=13.2,7.2Hz,H-1),2.16(1H,m,H-7),1.74(1 H,m,H-8),6.83(1H,d,J=10.2Hz,H-12),1.21(3H,d,J=7.2Hz,H-16),1.31(3H,s,H-17),1.35(3H,s,H-19),9.20(1H,t,J=1.2H z,COC5H4N-3),7.74(1H,dd,J=7.8Hz,COC5H4N-3),13C-NMR(150MHz,pyridine)δ:170.1(s,OAc-5),21.1(q,OAc-5), 170.1(s,OAc-15),22.0(q,OAc-15),165.4(s,ONic-3),151.5(d,ONic-3),126.6(s,ONic-3),137.4(d,ONic-3),123.4(d,ONic -3),154.2(d,ONic-3).The above data was consistent with that of the reported compound[12], so that the compound(IV) was identified as(2S*,3S*,4R*,5R*,9S*,11S*,15R*)-5,15-Diacetoxy-3-nicotinoyloxy-14-oxolathyra -6(17), (12E)-diene (Euphorbia Factor L8).

Compound(V), colourless needle crystal (Recrystallized in ethyl acetate),mp134~136°C, ESI-MSm/z:603[M+Na]+.1H-NMR(600MHz,CDCl3)δ:6.39(1H,d,J=9Hz,H-5),6.68(1H,d,J=12Hz,H-12),0.95(3H,d, J=6.6Hz,H-16),4.48(2H,s,H-17),1.34(3H,s,H-18),1.18(3H,s,H-19),1.78(3H,s,H-20),2.23(3H,s,OAc-17),8.02(2H,d,J =7.2Hz,OBz-3),2.04(3H,s,OAc-5),1.68(3H,s,OAc-15).The above data was consistent with that of the reported compound[13], so that the compound(V) was identified as 5,15,17-O- triacety-3-O-benzoyl-17hydr-oxyisolathyrol.

Compound(VI), white powder(Recrystallized in ethyl acetate), mp 300~302°C, Liebermann- Burchard reaction was positive, Chromogenic reaction of 10% Sulfuric acid ethanol solution was purple. Three different solvent system to developed in TLC compound(X) and daucosterol standard was developed in TLC with three different developing agent, as a result their R_f value were the same, and mixed melting point did not fall[14], so that the compound(VI) was identified as daucosterol.

Compound(VII),fine light yellow powder, ESI-MS m/z:177[M- H]-,201[M+ Na]+. 1H-NMR (600 MHz , DMSO)δ:6.13(1H,d,J=9.5Hz,H-3), 7.82 (1H,d, J=9.5Hz,H-4),6.96(1H,s,H-5), 6.73(1H,s,H-8).The above data was consistent with that of the reported compound[15], so that the compound (VII) was identified as esculetin.

Compound(VIII), white powder(Recrystallized in ethyl acetate),without ultravioletabsorption, 1H-NMR(600MHz,DMSO)δ:0.870(3H,t,6.6Hz,H-18),5.303(1H,dt,6Hz,H-9),2.30(2H,t,7.2Hz,H-2),1.25-1.27(20H,m ,H-4,H-5,H-6,H-7,H-12,H-13,H-14,H-15,H-16,H-17),4.06(1H,dd,4.8,9Hz,H-1').13C-NMR(150MHz,pyridine)δ:173 .1(C-1),33.8(C-2),24.8(C-3),27.0(C-4),29.1(C-6),29.6(C-8).29.4(C-7),129.8(C-10),130.0(C-9),31.8(C-11),29.6(C-12),22.6(C-17),14.1(C-18),69.7(C-2'). The above data was consistent with that of the reported compound[16], so that the compound (VIII) was identified as Glycerol-1-monooleate.

Compound(IX), colourless needle crystal,mp158~160°C.1H-NMR(600MHz, DMSO)δ: 7.76(1H,dd,H-3),7.41(1H,dt,H-5),6.85(1H,dd,H-6),6.69(1H,dt,H-4),13C-NMR(150MHz,DMSO)δ:161.9(C-3),134.9 (C-7),130(C-5),118(C-6),117(C-2),110(C-4).The above data was consistent with that of the reported compound[17], so that the compound (IX) was identified as salicylic acid.

Compound(X), white paste, 1H-NMR(600MHz, DMSO) δ:0.84(3H,t), 5.32(2H,t,H-9), 2.17 (2H, t,H-2),1.28 (H of aliphatic chain). Compound (X) and oleic acid standard was developed in TLC with three different developing agents, as a result their R_f value were the same, so that the compound(X) was identified as Oleic acid.

3.2 EFL1, EFL2 and EFL3 stimulated the ATP-ase activity of P-gp

MDR of the cancer cell is due to the drug-efflux function of P-gp. And this function is linked to ATP hydrolysis and ATP consumption reflecting ATP-ase activities[18]. We measured P-gp-mediated ATP hydrolysis with 100μm/L of EFL1(Ⅲ), EFL2(Ⅱ) and EFL3(Ⅰ) to assay the effects of EFL1(Ⅲ), EFL2(Ⅱ)and EFL3(Ⅰ) on the ATPase activity of P-gp. Based on the results(Figure 2) ,we found that in addition to EFL1(Ⅲ), EFL2(Ⅱ)and EFL3(Ⅰ) are the possible substrates of P-gp.

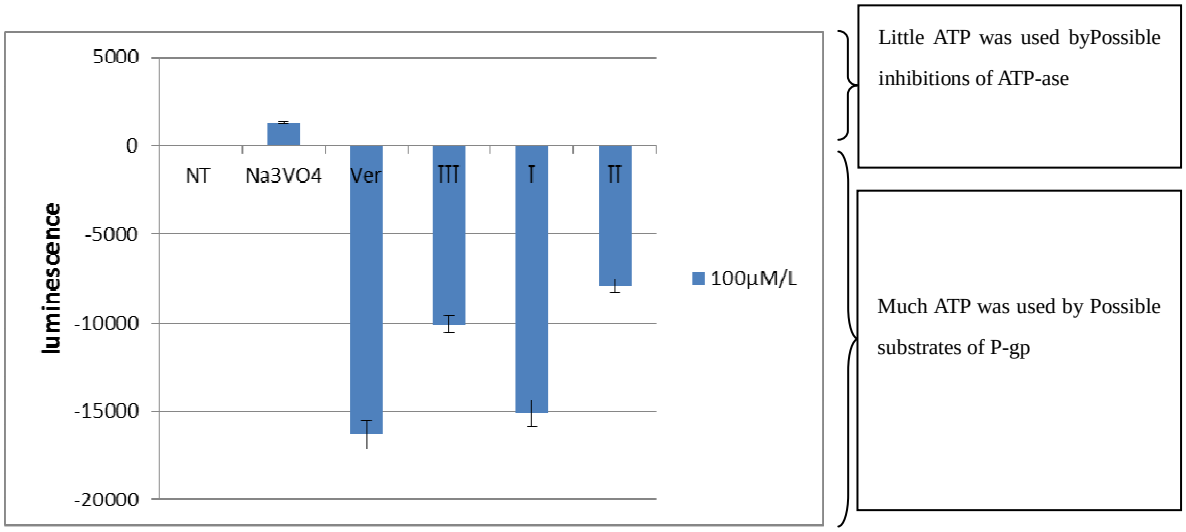


Fig2. The result of ATP-ase Activity of P-gp

DISCUSSION

The ABC transporter (ATP binding cassette) family is one of the largest protein families, including more than 100 membrane transport protein. P-gp as a protein of transmembrane transport has important effects to drugs' ADME process in vivo. The drug-efflux function of P-gp is responsible for the drugs' low bioavailability in gut and the blood brain barrier. Most of this drug has been found it is the substracts of P-gp, and it is the reason why cancer cell's MDR happen. So the screening of P-gp inhibitor has a great practical value and market potential.

In this study, the remaining ATP of I, II, III decreased significantly. And the result suggests that I, II, III may be the possible substracts of P-gp. They inhibited the function of P-gp by consuming ATP competitively. Some recent reports have also discovered III is the substracts of P-gp. It is necessary to presume that there may exist other substracts in semen Euphorbiae, and the assumption turn out to be true in this study.

CONCLUSION

It was analyzed that the chemical components of the seeds of *Euphorbia lathyris*extracted by petroleum ether .And the structures of the compounds were identified. Moreover the compound IX was obtained from the seeds of

Euphorbia lathyris for the first time. The compounds I and II were first found may be the substrates of P-gp. and they exhibited moderate activities inhibited the effluxing function of P-gp.

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REFERENCES

[1] GP Zhao, S Dai, RS Chen,Dictionary Traditional Drugs, 2st Edition, Shanghai science and technology Publishers & Distributors, Shanghai, **1986**,216-217.
[2] XL Chen; LJ Tang; QL Li, *China Pharm.*,**2007**,18, 1124.
[3] 3.CJ Wang, YJ Hsieh, CY Chu, et al. *Cancer Lett.*,**2002**, 183,163-168.
[4] CY Chu, YY Tsai, CJ Wang, et al.*Eur J Pharmaco*., **2001**, 416(1/2),25.
[5] J Yang, SL Wang, GY Fu, et al. *Chin J ClinRehabil*., **2005**, 27,101.
[6] ZH Dou, AW Ding, *The Sixth Academic symposium on Chinese herbal medicine processing*; **2006**,226-232.
[7] W Jiao, L Lu, MC Deng, et al. *China Tradit Herb Drugs*.,**2010**, 41, 181-187.
[8] C Bicchi, G Appendino, C Cordero, et al. *Phytochem Anal*., **2001**, 12, 255–262.
[9] J Wu; JH Duan, *Journal of Chemical and Pharmaceutical Research.*,**2013**, 5(10),415-423.
[10] JS Choi,NS Kang, YK,Min et al.*Phytotherapy Research*., **2010**, 24,1042-1946.
[11] G Uma; V Balasubramaniam, *Journal of Chemical and Pharmaceutical Research.*,**2012**, 4(9),4417-4419.
[12] G Appendino, GC Tron,G Gravotto, et al. *J Nat Prod*., **1999**, 62, 76-79.
[13] W Adolf, I Kohler, E Hecker, *Phytochemistry*.,**1984**, 23, 1461-1463.
[14] A Santoni; S Ibrahim; R Salim, et al. *Journal of Chemical and Pharmaceutical Research.*,**2014**, 6(2),753-757.
[15] W Yao, WY Lin, CX Zhou, et al. *China J chin Mater Med*., **2007**, 32,926.
[16] HQ Yin, XL Qi, HM Hua, et al. *Chinese Journal of Medicinal Chemistry*., **2005**, 15, 217-220.
[17] CJ Chang, *J Org Chem*., **1976**, 41, 1881-1883.
[18] AK Pandurangan; SK AnadhaSadagopan; P Dharmalingam. *Journal of Chemical and Pharmaceutical Research*.,**2013**, 5(6),123-127.