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Secondary Metabolites from *Euphorbia helioscopia* and Their Vasodepressor Activity

Aslı BARLA[1*], Hüsniye BİRMAN[2], Şükran KÜLTÜR[3] and Sevil ÖKSÜZ[1]
[1] *İstanbul University, Faculty of Pharmacy, Department of Chemistry, 34116 İstanbul-TURKEY*
e-mail:asli_barla@yahoo.com
[2] *İstanbul University, Faculty of Medicine, Department of Physiology, İstanbul-TURKEY*
[3] *İstanbul University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 34116 İstanbul-TURKEY*

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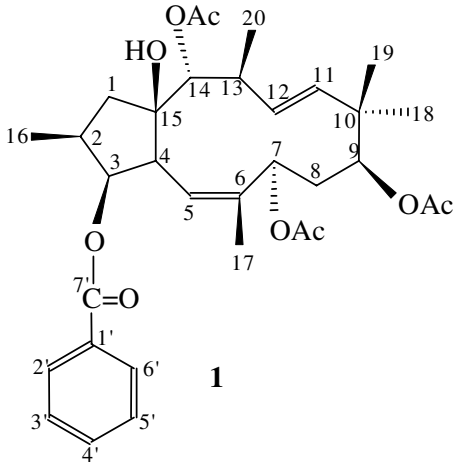
From the aerial parts of *Euphorbia helioscopia* L. (Euphorbiaceae), a jatrophane diterpene ester, 5,11-jatrophadiene-3-benzoyloxy-7,9,14-tri-acetyloxy-15-ol and 2 lupane derivatives, lup-20(29)-ene-3-acetate and lup-20(29)-ene-3-palmitate, together with common triterpenoids of Euphorbiaceae, 24-methylene cycloartanol, 24-methylenecycloart-3-one, cycloartanol, and stigmast-4-ene-3-one were isolated. The last compounds, lup-20(29)-ene-3-acetate, 24-methylene cycloartanol, 24-methylenecycloart-3-one, cycloartanol, and stigmast-4-ene-3-one, were isolated for the first time from *E. helioscopia*. The fractions and the isolates were tested for their vasodepressor effects using Wistar Albino rats, and 5,11-jatrophadiene-3-benzoyloxy-7,9,14-tri-acetyloxy-15-ol, lup-20(29)-ene-3-acetate, and stigmast-4-ene-3-one were found to possess relevant activity. The structures of all of the compounds were identified with high field spectroscopic methods. The detailed spectroscopic data of compound **1** is given in the present study.

Key Words: *Euphorbia helioscopia*, Euphorbiaceae, diterpenoid, triterpenoids, steroid, vasodepressor effect.

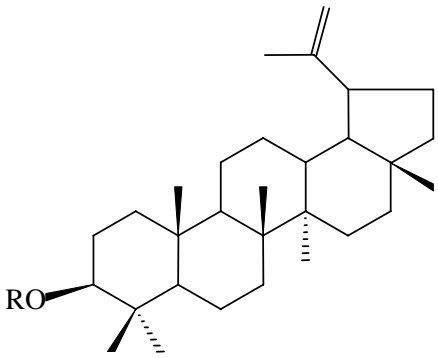
Introduction

The genus *Euphorbia* is the largest genus in the spurge family with over 2000 species and is subdivided into many subgenera and sections[1]. Several species of the genus *Euphorbia* have been studied for their antiviral and anti-tumor effects, based on traditional information[2]. The leaves and the lattices of this genus are used in the ayurvedic system of medicine for bronchitis and rheumatism[3]. *Euphorbia* species have yielded numerous diterpenoids and triterpenoids possessing various biological activities with contraverting biological activities, such as tumor promoting and antitumor[4]. *Euphorbia* species have been used in Turkish folk medicine for rheumatism, swelling, and especially as a wart remover; however, it causes inflammation and diarrhea[5]. In our search for bioactive compounds from *Euphorbia* species, we investigated *Euphorbia helioscopia* L.

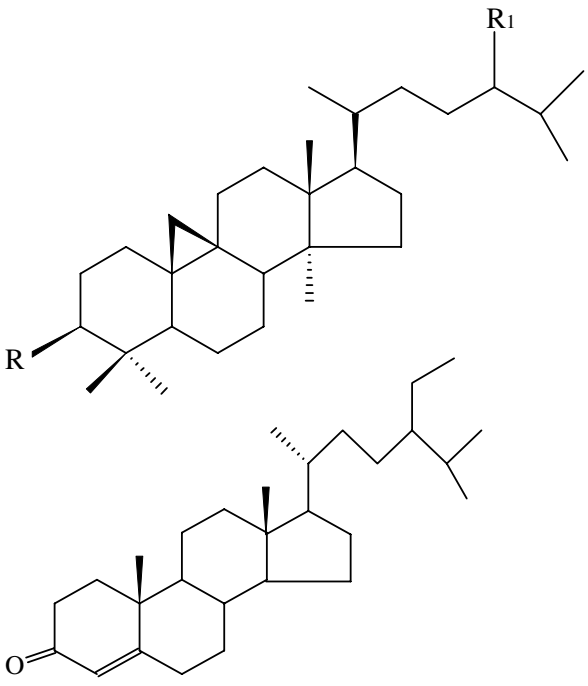
and report herein the isolation and identification of 5,11-jatrophadiene-3-benzoyloxy-7,9,14-tri-acetyloxy-15-ol[6] (**1**), lup-20(29)-ene-3-acetate[7] (**2**), lup-20(29)-ene-3-palmitate[8] (**3**), 24-methylene cycloartanol[9] (**4**), 24-methylenecycloart-3-one[10] (**5**), cycloartanol[11] (**6**), and stigmast-4-ene-3-one[12] (**7**) (Figure). The detailed spectroscopic properties of compound **1** are given. All of the compounds were tested for their vasodepressor effect. **1**, **2**, and **7** showed mild cardiovascular activity, while the others were inactive.



1



2 R= $CH_3(CH_2)_{14}CO$ (Sambuculin A)
3 R= CH_3CO



7

4 R = β-OH, α-H R_1 = H
5 R = O R_1 = CH_2
6 R = β-OH, α-H R_1 = CH_2

Figure

Experimental

General experimental procedures

UV spectra were recorded on a Shimadzu UV-1601 in MeOH, IR spectra on a Perkin Elmer model 983 in $CHCl_3$, NMR spectra on a Bruker Ac-200 (200 MHz for ^1H and 50.32 MHz for ^{13}C) and a Varian Mercury-VX 300 MHz (300 mHz for ^1H and 75.42 MHz for ^{13}C), and Jeol eclipse (for compound **1**, 500 MHz for ^1H and 125.76 MHz for ^{13}C), and FABMS and EIMS were measured on a VG Zabspec (micromass) instrument. For chromatographic separations, silica gel (E. Merck, Art 7734) and ready-made TLC plates (1 mm thick, E. Merck, Art 5554) were used.

Plant material

Euphorbia helioscopia L. (Euphorbiaceae) was collected in May 2001 from Şile (İstanbul), Turkey and identified by Dr. Şükran Kültür. A voucher specimen was deposited in the herbarium of İstanbul University, Faculty of Pharmacy (ISTE 74070).

Extraction and Isolation

Dried and powdered aerial parts of the plant material (2 kg) were macerated 4 times with MeOH. The extraction procedures were carried out as described in our previous work[13]. The crude extract (105 g) was partitioned against petroleum ether and then CH_2Cl_2, successively. The petroleum ether phase (40 g) gave 4 fractions (A-D). These fractions were further submitted to silica gel column chromatography to obtain pure compounds. Final purification was achieved by 0.25-mm thick preparative TLC plates (silica gel) using petroleum ether-CH_2Cl_2 (9:1; 8:2; 5:5; 6:4) and/or CH_2Cl_2-Me_2CO (9:1; 8:2) as developing solvent systems. Fraction A yielded compounds **2** (3.3 mg) and **3** (4.5 mg). Fraction B yielded compounds **4** (7 mg), **5** (7.9 mg), **6** (4.1 mg), and **7** (7.3 mg), and fraction C yielded **1** (12.4 mg).

Lup-20(29)-ene-3-acetate (2): UV (MeOH) λ_{max}: 204 nm; IR ($CHCl_3$) ν_{max}: 3445, 3069, 2944, 2872, 2360, 2342, 1731, 1640, 1455, 1379, 1317, 1246, 1216, 1149, 1105, 1027, 979, 944, 883, 757, 667 cm^{-1}, EI-MS (EI, 70 eV) (m/z) 468 [M^+] (calc. for $C_{32}H_{52}O_2$). ^1H NMR (200 MHz, $CDCl_3$): δ_H 4.47(1H, dd, J= 6, 10 Hz, H-3), 0.83 (3H, s, H-23), 0.77 (3H, s, H-24), 0.83 (3H, s, H-25), 1.12 (3H, s, H-26), 0.93 (3H, s, H-27), 0.83 (3H, s, H-28), 4.68 (1H, brs, H-29a), 4.57 (1H, brs, H-29b), 1.68 (3H, s, H-30), 2.05 (3H, s, 3-OAc). ^{13}C NMR (50.32 MHz, $CDCl_3$): δ_C 31.39 (C-1), 23.71 (C-2), 80.98 (C-3), 37.79 (C-4), 55.38 (C-5), 18.21 (C-6), 34.20 (C-7), 40.84 (C-8), 50.34 (C-9), 37.79 (C-10), 20.93 (C-11), 25.09 (C-12), 38.04 (C-13), 42.83 (C-14), 30.63 (C-15), 27.43 (C-16), 41.38 (C-17), 48.28 (C-18), 48.01 (C-19), 150.96 (C-20), 29.83 (C-21), 35.56 (C-22), 27.94 (C-23), 15.97 (C-24), 16.18 (C-25), 16.49 (C-26), 14.50 (C-27), 18.00 (C-28), 110.20 (C-29), 19.28 (C-30), 171.08 (CO), 18.00 (Ac).

Lup-20(29)-ene-3-palmitate (3) : UV (MeOH) λ_{max}: 203 nm; IR ($CHCl_3$) ν_{max}: 3852, 3428, 2922, 2850, 2359, 2342, 1728, 1629, 1464, 1379, 1215, 1114, 1009, 979, 884, 758, 668 cm^{-1}, EI-MS (EI, 70 eV) (m/z) 664 [M^+] (calc. for $C_{46}H_{80}O_2$). ^1H NMR (200 MHz, $CDCl_3$): δ_H 4.45 (1H, dd, J= 6, 10 Hz, H-3), 0.84 (3H, s, H-23), 0.77 (3H, s, H-24), 0.84 (3H, s, H-25), 1.02 (3H, s, H-26), 0.94 (3H, s, H-27), 0.84 (3H, s, H-28), 4.68 (1H, d, J= 1.5, H-29a), 4.56 (1H, brs, H-29b), 1.69 (3H, s, H-30), 0.87 (3H, t, J = 6.5 Hz,

3-OPal). ^{13}C NMR (50.32 MHz, $CDCl_3$): δ_C 40.00 (C-1), 18.20 (C-2), 80.59 (C-3), 39.00 (C-4), 54.00 (C-5), 18.20 (C-6), 34.87 (C-7), 42.00 (C-8), 50.33 (C-9), 37.07 (C-10), 22.69 (C-11), 24.96 (C-12), 39.80 (C-13), 43.90 (C-14), 31.93 (C-15), 34.14 (C-16), 44.00 (C-17), 48.01 (C-18), 51.41 (C-19), 150.06 (C-20), 29.25 (C-21), 37.07 (C-22), 29.00 (C-23), 20.00 (C-24), 16.58 (C-25), 16.58 (C-26), 14.12 (C-27), 18.20 (C-28), 109.34 (C-29), 19.00 (C-30), 173.00 (CO), 29.69($\underline{CH_2}$-CH_3), 21.32 (CH_2-$\underline{CH_3}$).

24-Methylenecycloartanol (4): UV (MeOH) λ_{max}: 203.5 nm; IR ($CHCl_3$) ν_{max}: 3445, 2916, 2848, 2360, 2341, 1653, 1472, 1462, 1061, 1215, 756, 668 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ_H 3.28 (1H, dd, J= 5, 11 Hz, H-3), 0.95 (3H, s, H-18), 0.33 (1H, d, J = 4 Hz, H-19a), 0.55 (1H, d, J = 4 Hz, H-19b), 0.90 (3H, d, J = 6 Hz, H-21), 1.03 (3H, d, J = 7 Hz, H-26), 1.03 (3H, d, J = 7 Hz, H-27), 0.90 (3H, s, H-28), 0.95 (3H, s, H-29), 0.81 (3H, s, H-30), 4.67 (1H, brs, H-31a), 4.72 (1H, brs, H-31b). ^{13}C NMR (50.32 MHz, $CDCl_3$): δ_C 31.99 (C-1), 30.38 (C-2), 78.90 (C-3), 40.54 (C-4), 47.23 (C-5), 21.16 (C-6), 28.19 (C-7), 48.02 (C-8), 19.35 (C-9), 26.50 (C-10), 26.04 (C-11), 32.93 (C-12), 45.41 (C-13), 48.02 (C-14), 29.74 (C-15), 26.58 (C-16), 52.30 (C-17), 18.06 (C-18), 29.74 (C-19), 32.93 (C-20), 18.39 (C-21), 35.15 (C-22), 31.41 (C-23), 159.79 (C-24), 33.89 (C-25), 21.94 (C-26), 19.35 (C-27), 18.09 (C-28), 14.04 (C-29), 25.47 (C-30), 106.10 (C-31).

24-Methylenecycloart-3-one (5): UV (MeOH) λ_{max}: 204 nm; IR ($CHCl_3$) ν_{max}: 3430, 2951, 2870, 1706, 1629, 1553, 1534, 1464, 1381, 1174, 1112, 755 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 0.95 (3H, s, H-18), 0.57 (1H, d, J = 4 Hz, H-19a), 0.73 (1H, d, J = 4 Hz, H-19b), 0.95 (3H, d, J = 6.6 Hz, H-21), 0.80 (3H, d, J = 6.6 Hz, H-26), 0.80 (3H, d, J = 6.6 Hz, H-27), 1.06 (3H, s, H-28), 0.99 (3H, s, H-29), 0.85 (3H, s, H-30), 4.65 (1H, brs, H-31a), 4.60 (1H, d, J = 1.5 Hz, H-31b). ^{13}C NMR (75.42 MHz, $CDCl_3$): δ_C 33.03 (C-1), 37.50 (C-2), 216.75 (C-3), 50.44 (C-4), 48.97 (C-5), 22.07 (C-6), 26.07 (C-7), 48.09 (C-8), 21.32 (C-9), 26.96 (C-10), 26.21 (C-11), 32.88 (C-12), 45.58 (C-13), 48.66 (C-14), 35.21 (C-15), 28.18 (C-16), 52.50 (C-17), 18.27 (C-18), 29.75 (C-19), 35.78 (C-20), 18.57 (C-21), 31.53 (C-22), 36.32 (C-23), 157.09 (C-24), 33.62 (C-25), 19.52 (C-26), 19.94 (C-27), 22.40 (C-28), 20.97 (C-29), 21.72 (C-30), 106.192 (C-31).

Cycloartanol (6): UV (MeOH) λ_{max}: 204 nm; IR ($CHCl_3$) ν_{max}: 3442, 2935, 2868, 1376, 1214, 1096, 1023, 755 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 3.29 (1H, dd, J= 4.4, 11.5, H-3), 0.94 (3H, s, H-18), 0.33 (1H, d, J = 4 Hz, H-19a), 0.54 (1H, d, J = 4 Hz, H-19b), 0.91 (3H, d, J = 6.6 Hz, H-21), 0.87 (3H, d, J = 6.6 Hz, H-26), 0.87 (3H, d, J = 6.6 Hz, H-27), 0.95 (3H, s, H-28), 0.82 (3H, s, H-29), 0.90 (3H, s, H-30). ^{13}C NMR (75.42 MHz, $CDCl_3$): δ_C 32.18 (C-1), 30.08 (C-2), 79.05 (C-3), 45.51 (C-4), 52.46 (C-5), 22.40 (C-6), 26.32 (C-7), 48.16 (C-8), 21.31 (C-9), 26.68 (C-10), 27.40 (C-11), 30.60 (C-12), 47.32 (C-13), 49.05 (C-14), 33.12 (C-15), 28.40 (C-16), 52.65 (C-17), 18.25 (C-18), 29.20 (C-19), 35.75 (C-20), 19.52 (C-21), 37.25 (C-22), 25.64 (C-23), 40.70 (C-24), 28.25 (C-25), 22.85 (C-26), 23.10 (C-27), 26.20 (C-28), 14.20 (C-29), 20.20 (C-30).

Stigmast-4-ene-3-one (7): UV (MeOH) λ_{max}: 242.5 nm; IR ($CHCl_3$) ν_{max}: 2938, 2365, 1673, 1455, 1373, 1240 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 5.72 (1H, brs, H-4), 0.70 (3H, s, H-18), 1.17 (1H, s, H-19), 0.91 (1H, d, J = 6.6 Hz, H-21), 0.83 (3H, d, J = 6.6 Hz, H-26), 0.81 (3H, d, J = 6.6 Hz, H-27), 0.83 (3H, t, J = 6.6 Hz, H-29). ^{13}C NMR (75.42 MHz, $CDCl_3$): δ_C 35.92 (C-1), 34.19 (C-2), 199.80 (C-3), 123.96 (C-4), 171.84 (C-5), 32.63 (C-6), 29.90 (C-7), 35.86 (C-8), 54.05 (C-9), 39.05 (C-10), 21.25 (C-11), 39.86 (C-12), 42.62 (C-13), 56.11 (C-14), 24.40 (C-15), 33.16 (C-16), 56.25 (C-17), 12.16 (C-18), 17.60 (C-19), 36.33 (C-20), 18.91 (C-21), 34.12 (C-22), 26.35 (C-23), 46.08 (C-24), 28.40 (C-25), 20.01 (C-26), 19.25

(C-27), 23.30 (C-28), 12.18 (C-29).

Vasodepressor activity test

An experimental model was applied as described in our previous work[14]. The compounds were dissolved in the least amount of ethanol possible and the solution was diluted to 25% with saline to 4 mL. Adult male Wistar Albino rats (250-300 g) were used. Rats were purchased from the Research Institute for Experimental Medicine (İstanbul University, İstanbul), and the procedures for using laboratory animals were approved by the local ethics committee of this institute based on the Use and Care of Animals Guidelines (Prot# 45/2003). Each animal was anesthetized intraperitoneally (i.p.) with sodium pentothal 35 mg/kg, and the femoral artery and vein were cannulated separately to monitor the arterial blood pressure and for drug administration. The femoral vein was catheterized for injection of the petroleum ether phase of the methanolic crude extract, fractions A-D, and the isolated compounds, separately. Direct blood pressure was recorded from the cannulated femoral artery with a polygraph (Nihon-Kohden RM 6000, S. Adella)[15]. The experimental study was carried out with 2 groups of animals. The control group (n = 6) received ethanol diluted to 25% with saline, while the test group (n = 6) received 2 mg/kg intravenous doses of single compounds. The dose-response curve was prepared using various doses and 2 mg/kg was found to be the best dose. A mild reduction in direct blood pressure was observed ($P < 0.01$) as seen in Table 2. Data are presented as mean ± SEM. Control and experimental groups of animals were compared using Student's t-test. Propranolol and phentolamine were used as controls.

Results and Discussion

Since the petroleum ether phase of the methanolic extract of *E. helioscopia* showed remarkable cardiovascular activity, we investigated this extract for its chemistry to define the biologically active compound. From the silica gel column, 4 fractions were obtained (A-D). Compound **1,** being one of the most active compounds, was isolated from fraction C. This compound was first isolated from *Euphorbia maddeni* by Bohlmann et al. and named euphornin[6], which was later reported from *Euphorbia helioscopia* by revision of its stereostructure by Yamamura et al.[16]. Since spectral data of **1** were not given in detail, in the present study, we report detailed ^{1}H- and ^{13}C-NMR properties of **1** (Table 1). Full assignments of all of the ^{1}H and ^{13}C data were achieved by ^{1}H-^{1}H-COSY, APT, HETCOR and HMBC spectra.

Compounds **2** and **7** were identified as lup-20(29)-ene-3-palmitate (sambuculin A)[8] and lup-20(29)-ene-3-acetate[12], respectively, and their ^{1}H- and ^{13}C-NMR spectral data are in good agreement with those given in the literature. Lup-20(29)-ene-3-acetate, 24-methylene cycloartanol, 24-methylenecycloart-3-one, cycloartanol, and stigmast-4-ene-3-one were reported for the first time from *Euphorbia helioscopia*.

The petroleum ether extract and its fractions (A-D), and the pure compounds **1-7** were tested for their vasodepressor effect. As seen in Table 2, fraction A, and compounds **1**, **2,** and **7** showed mild reductions in direct blood pressure ($P < 0.01$). Among the compounds, **1** was the most active vasodepressor (42 mmHg). The period for the effective reduction of blood pressure was 45 min. This effect lasted 70 min and did not return to normal during this period. Compound **2** dropped blood pressure by about 34 mmHg; this effect continued for 45 min. Compound **7** had the lowest vasodepressor effect (28 mmHg); however, it returned to normal after 30 min. The vasodepressor effect of these compounds might be due to vasorelaxation activity.

Table 1. Spectral Properties of Compound **1**.

H	^{1}H NMR	^{13}C NMR	HMBC
1a	2.01 m	46.2 t	C-13, C-4, C-3, C-15
1b	1.78 m		
2	2.56 m	39.5 d	C-16
3	5.42 t (4.5 Hz)	80.9 d	
4	2.88 dd (4.5; 10 Hz)	47.9 d	C-3, C-5, C-14
5	5.71 d (10 Hz)	120.1 d	C-17, C-7
6		133.9 s	
7	4.94 dd (1; 7.8 Hz)	72.9 d	C-6, C-5, C-9, C-10
8a	1.77 m	32.4 t	
8b	2.05 m	32.4 t	
9	4.77 t (3.5 Hz)	73.5 d	
10		39.6 s	
11	5.06 d (15.6 Hz)	138.3 d	C-18, C-20, C-10, C-9
12	5.63 dd (9.4;15.6 Hz)	128.5 d	C-10
13	2.17 m	36.7 d	C-20
14	4.94 t (3.6 Hz)	80.7 d	C-12
15		83.8 s	
16	0.96 d (6 Hz)	19.4 q	C-1, C-3
17	1.73 s	16.5 q	C-5, C-6, C-7
18	0.89 s	20.2 q	C-9, C-10, C-11
19	1.18 s	19.9 q	
20	0.97 d (6.2 Hz)	22.6 q	C-11, C-12
OBz			
1'		130.2 s	
2'-6'	8.09 dd (1.5; 8 Hz)	129.8 d	
3'-5'	7.44 br t (8.1 Hz)	128.6 d	
4'	7.52 tt (1.5; 8.1)	132.8 d	
7'		165.6 s	
OAc-7	1.95 s	21.0 q	C-9
		169.06 s	
OAc-9	1.95 s	21.1 q	
		169.65 s	
OAc-14	2.22 s	21.1 q	
		171.23 s	

Table 2. Vasodepressive effects of fractions **A-D** and Compounds **1**, **3,** and **7**.

	Control group Blood Pressure (mmHg)	Experimental groups Mean blood Pressure (mmHg)	P
Petroleum extract	135.83 ± 3.85	115.83 ± 3.80	$P < 0.01$
Fraction A (1-24)	135.00 ± 4.89	91.16 ± 4.52	$P < 0.01$
Fraction B (25-34)	104.83 ± 6.51	184.50 ± 5.37	$P < 0.01$
Fraction C (35-48)	130.00 ± 8.10	158.00 ± 7.57	$P < 0.01$
Fraction D (49-64)	104.16 ± 6.72	125.00 ± 15.81	$0.02 > P > 0.05$
Euphornin **(1)**	116.5 ± 5.75	74.67 ± 8.04	$P < 0.01$
lup-20(29)-ene-3acetate **(2)**	109.33 ± 5.89	75.67 ± 9.14	$P < 0.01$
stigmast-4-en-3-one **(7)**	120.00 ± 5.47	92.67 ± 5.23	$P < 0.01$
Propranolol	150.00 ± 32.4	93.20 ± 12.1	$P < 0.05$
Phentolamine	125.12 ± 4.1	104.25 ± 5.7	$P < 0.01$

n = Number of rats for test and control (6 animals were used in each experimental group)

P = Compared with the control

propranolol (β-blocker) and phentolamine (α-blocker) are positive controls.

Conclusion

Seven compounds were isolated from *Euphorbia helioscopia*, and Lup-20(29)-ene-3-acetate, 24-methylene cycloartanol, 24-methylenecycloart-3-one, cycloartanol, and stigmast-4-ene-3-one were reported for the first time from *Euphorbia helioscopia* L. The full spectroscopic assignments of compound **1** are given this study. All of the compounds were tested for their vasodepressor effect. Euphornin (**1**), lup-20(29)-ene-3acetate (**2**), and stigmast-4-en-3-one (**7**) showed significant vasodepressor effect. Dichloromethane extract from the dried methanolic extract of *Euphorbia helioscopia* is still under investigation.

In this study, we investigated the biological activity of *Euphorbia helioscopia*. To date HIV activity[17], and some biological activity tests have been investigated[18,19] in Turkish *Euphorbia* species. In the future, additional biological activities will be determined.

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