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ORIGINAL ARTICLE

New Abietane Diterpenes from *Euphorbia Pseudocactus* Berger (Euphorbiaceae) and Their Antimicrobial Activity

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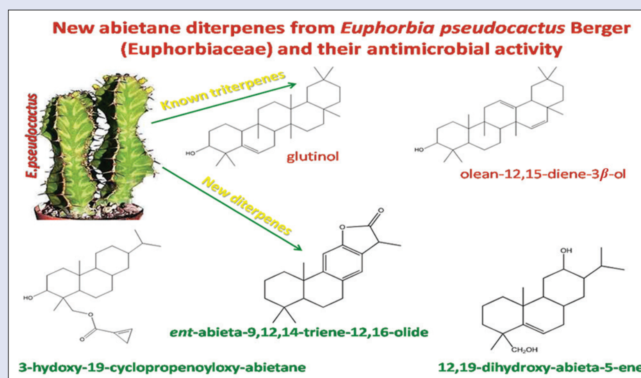
ABSTRACT

Background: *Euphorbia* is the largest genus in *Euphorbiaceae.* Terpenoids were isolated from most species of this genus. **Objective:** Since no previous study was reported about *Euphorbia pseudocactus* Berger, we started here a phytochemical investigation on this species to isolate and identify its terpenoid constituents and to estimate the antimicrobial activity of the isolated compounds. **Materials and Methods:** The *n*-hexane fraction of the ethanolic extract of *E. pseudocactus* Berger was chromatographed on silica gel columns, the structures of the isolated compounds (1–5) were identified based on their MS, 1 D, and 2 D NMR spectral data. The antimicrobial activity of the *n*-hexane fraction and the isolated compounds (1–4) was investigated using diffusion plate method against Gram-positive (*Staphylococcus aureus* [12600] and *Bacillus subtilis* [6051]) and Gram-negative (*Pseudomonas aeruginosa* [10145] and *Escherichia coli* [11775]) bacteria, yeast (*Candida albicans* [7102]), and fungi (*Aspergillus flavus*). **Results:** Two triterpenes (glut-5-en-3 β-ol [1] and olean-12,15-diene-3 β-ol [2]) and three abietane diterpene (3-hydoxy-19-cyclopropenoyloxy-abietane [3], *ent*-abieta-9,12,14-triene-12,16-olide [4], and 12,19-dihydroxy-abieta-5-ene [5]) were isolated. Compound 1 exhibited no antibacterial activity against the tested bacteria, compound 2 and *n*-hexane fraction exhibited weak activity, whereas compounds 3 and 4 showed moderate activity. All samples showed no activity against the tested yeast and fungi. **Discussion and Conclusion:** Five compounds were isolated for the 1st time from *E. pseudocactus* Berger, three of them (3–5) are new natural compounds. As the major isolated compound (1) exhibited no antimicrobial activity, the observed activity of the *n*-hexane fraction is mainly due to its diterpenoid constituents.

Key words: Abietane, antimicrobial, diterpene, *Euphorbia pseudocactus,* triterpene

SUMMARY

- Two known triterpenes and three new diterpenes were isolated from *n*-hexane fraction of *Euphorbia pseudocactus*
- The abietane diterpenoids showed higher antimicrobial activity than *n*-hexane fraction.



Abbreviations used: EtOAc: Ethyl acetate, TLC: Thin layer chromatography.

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INTRODUCTION

Euphorbiaceae is a large family of flowering plants, composed of over 300 genera.[1] Diverse medicinal properties of *Euphorbiaceae* members are associated with their wide distribution which is supported by their survival adaptations such as succulence. Genus *Euphorbia* is the largest genus in this family, comprising about 2000 known species.[2] Traditionally, some species of *Euphorbia* are useful for the treatment of boils, cuts, and wounds.[3] It is useful for cardiovascular complaints, asthma, cough,[4] and spleen disorders.[5] Certain *Euphorbia* species have been reported to possess cytotoxic,[6-10] antimicrobial,[11-15] larvicidal, insecticidal,[16-17] anti-inflammatory, hepatoprotective, and antioxidant[18-20] activities. The diterpenoid constituents, especially those with abietane, tigliane, and ingenane skeletons, are thought to be the main toxicant and bioactive factors.[21-23]

Euphorbia pseudocactus Berger (candelabra spurge) is a multibranched, dwarf-stemmed, candelabra-shaped, succulent herb, 60–120 cm tall. The stems often have distinctive yellow V-shaped markings. It is originating in the subtropical coast of South Africa. It grows in thorny bush-lands and savannah often forming colonies.[24] Herein, we report for the 1st time, the isolation of new abietane terpenoids from this *Euphorbia* member and the antimicrobial activity of the isolated compounds.

MATERIALS AND METHODS

General experimental procedures

Mass spectra were measured with Thermo scientific, ISQ single quadrupole mass spectrometer (San Jose, CA, USA). NMR analysis was measured on ^{1}H-NMR (300 MHz), ^{13}C-NMR (75 MHz): Varian

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Mercury-VX-300 spectrophotometer. Thin layer chromatography (TLC) was performed on precoated silica gel plates using solvent systems S_1: *n*-c_6H_{14}: Ethyl acetate (EtOAc) (95:5), S_2: *N*-C_6H_6: EtOAc (9:1), and S_3: *n*-c_6H_{14}: EtOAc (8:2), the spots were detected by spraying with *p*-anisaldehyde-sulfuric acid spray reagent.

Plant material

The whole plant of *E. pseudocactus* Berger was collected from special garden in Abu Rawash-Giza, Egypt, and kindly identified by Dr. Mohamed Elgebaly taxonomy specialist, El-Orman Garden, Giza. A voucher specimen was kept in the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo, Egypt (April 1, 2014).

Extraction and isolation

The fresh whole plant of *E. pseudocactus* Berger (2 kg) was sliced into small pieces then extracted by maceration in 90% ethyl alcohol (5 L × 3). The combined extracts were evaporated under reduced pressure (≤60°C) to give 110 g of green residue. The residue was fractionated into *n*-hexane (12.07 g), chloroform (3.9 g), and *n*-butanol (15.6 g) fractions.

The *n*-hexane fraction (10 g) was chromatographed on a column of silica gel starting with *n*-c_6H_{14} then increasing the polarity by stepwise increments of EtOAc till 20% EtOAc. Fractions, 20 ml each, were collected and monitored by TLC, similar fractions were pooled to afford four main fractions. Combined fractions (46–48) were rechromatographed on a silica gel column using *n*-c_6H_{14}: EtOAc (98:2) as eluent to afford compound 1 (1.5 g). Combined fractions (49–55) were rechromatographed on a silica gel column using *n*-C_6H_{14}: EtOAc (98:2) as eluent to afford compounds 2 (100 mg) and 3 (200 mg). Combined fractions (56–64) were rechromatographed on a silica gel column using *n*-C_6H_{14}: EtOAc (95:5) as eluent to afford compound 4 (80 mg). Combined fractions (68–75) were rechromatographed on a silica gel column using *n*-C_6H_{14}: EtOAc (95:5) as eluent to afford compound 5 (40 mg).

Compound 1

White powder; R_f 0.6 (TLC, S_1); MS m/z: 426 $[M]^+$; ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H 5.62 (1H, *m*, H-6), 3.46 (1H, br *s*, H-3), 1.16 (3H, *s*, H-28), 1.14 (3H, *s*, H-24), 1.10 (3H, *s*, H-27), 1.04 (3H, *s*, H-23), 1.01 (3H, *s*, H-26), 0.99 (3H, *s*, H-29), 0.95 (3H, *s*, H-30), 0.85 (3H, *s*, H-25). ^{13}C-NMR (75 MHz, $CDCl_3$) δ_C: 141.87 (C-5), 122.24 (C-6), 76.53 (C-3), 49.94 (C-10), 47.67 (C-8), 43.31 (C-18), 41.03 (C-4), 39.53 (C-13), 39.18 (C-22), 38.07 (C-14), 36.25 (C-16), 35.31 (C-19), 35.07 (C-9), 34.84 (C-11), 34.74 (C-29), 33.36 (C-21), 32.61 (C-30), 32.31 (C-15), 32.26 (C-28), 30.59 (C-12), 30.31 (C-17), 29.50 (C-24), 28.45 (C-20), 27.84 (C-2), 25.66 (C-23), 23.87 (C-7), 19.83 (C-26), 18.62 (C-27), 18.43 (C-1), 16.41 (C-25).

Compound 2

White powder; R_f 0.5 (TLC, S_1); MS m/z: 424 $[M]^+$; ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H 5.21 (1H, *t*, *J* = Hz, H-12), 4.83 (1H, br *s*, H-16), 4.62 (1H, br *s*, H-15), 3.20 (1H, *m*, H-3), 1.14 (3H, *s*, H-28), 1.01 (3 H, s, H-24), 0.98 (3H, *s*, H-27), 0.96 (3H, *s*, H-26), 0.94 (3H, *s*, H-25), 0.89 (3H, *s*, H-30), 0.88 (3H, *s*, H-29), 0.80 (3H, *s*, H-23). ^{13}C-NMR (75 MHz, $CDCl_3$) δ_C: 142.96 (C-13), 129.24 (C-15), 121.97 (C-12), 109.56 (C-16), 79.56 (C-3), 55.43 (C-5), 51.45 (C-9), 47.47 (C-19), 47.34 (C-14), 47.07 (C-18), 39.18 (C-8), 39.00 (C-4), 38.83 (C-1), 37.61 (C-10), 37.04 (C-22), 35.28 (C-21), 33.40 (C-7), 33.55 (C-29), 32.90 (C-20), 28.19 (C-23), 27.75 (C-2), 24.92 (C-27), 23.92 (C-11), 23.76 (C-30), 18.61 (C-6), 18.50 (C-28), 18.22 (C-26), 16.90 (C-24), 16.32 (C-24), 15.62 (C-25).

Compound 3

Yellow oily liquid; R_f 0.4 (TLC, S_1); MS m/z: 374 $[M]^+$; ^{1}H-NMR (300 MHz, DMSO): δ_H 7.69 (1 H, br *s*, H-3\`), 4.64 (1 H, *d*, *J* = 4.5 Hz, H-19a), 4.12 (1 H, *d*, *J* = 4.5 Hz, H-19b), 2.83 (1 H, br *s*, H-3), 0.97 (3 H, *s*, H-20), 0.90 (3 H, *s*, H-18), 0.85 (6 H, *d*, *J* = 7.2 Hz, H-16 and 17). $^{13}$C-NMR (75 MHz, DMSO) $\delta_C$: 166.91 (C-1\`), 131.51 (C-3\`), 128.59 (C-2\`), 74.38 (C-3), 67.37 (C-19), 50.00 (C-9), 49.38 (C-5), 46.79 (C-13), 42.56 (C-4), 38.07 (C-1), 35.85 (C-10), 34.65 (C-8), 34.46 (C-15), 33.05 (C-14), 31.01 (C-7), 30.30 (C-12), 28.33 (C-2), 24.14 (C-11), 23.22 (C-20), 22.34 (C-16, 17), 20.41 (C-6), 13.82 (C-4\`), 10.74 (C-18).

Compound 4

Yellow oily liquid; R_f 0.3 (TLC, S_2); MS m/z: 298 $[M]^+$; ^{1}H-NMR (300 MHz, DMSO): δ_H 7.24 (1 H, *s*, H-11), 7.12 (1 H, *m*, H-14), 3.89 (1 H, *d*, *J* = 2.4 Hz, H-15), 1.16 (3 H, *s*, CH_3-20), 0.83 (6 H, *s*, CH_3-18 and 19), 0.72 (3 H, *d*, *J* = 6.3 Hz, CH_3-17). ^{13}C-NMR (75 MHz, DMSO): δ_C 174.25 (C-16), 161.98 (C-12), 147.97 (C-9), 135.60 (C-8), 128.11 (C-14), 127.32 (C-11), 125.68 (C-13), 52.37 (C-15), 50.50 (C-5), 45.12 (C-3), 38.68 (C-1), 36.02 (C-10), 33.62 (C-4), 31.26 (C-7), 29.29 (C-18), 24.46 (17), 21.55 (C-20), 18.34 (C-2 and 6), 13.86 (C-19).

Compound 5

Yellow oily liquid; R_f 0.6 (TLC, S_3); MS m/z: 306 $[M]^+$; ^{1}H-NMR (300 MHz, DMSO): δ_H 5.42 (1H, broad *s*, H-6), 1.15 (3H, *s*, H-19), 0.96 (3H, *s*, H-20), 0.84 (6 H, *m*, H-16 and 17). ^{13}C-NMR (75 MHz, DMSO): δ_C 140.74 (C-5), 120.51 (C-6), 74.43 (C-12), 68.77 (C-19), 48.33 (C-13), 47.36 (C-9), 44.74 (C-8), 44.09 (C-4), 42.99 (C-10), 38.08 (C-1), 34.80 (C-11), 31.48 (C-7), 30.18 (C-3), 29.26 (C-14), 29.01 (C-20), 25.82 (C-15), 25.54 (C-17), 18.97 (C-2), 18.02 (C-16), 14.20 (C-18).

Estimation of microbiological activity

The antibacterial and antifungal activities were carried out in the Microbiology Division of Microanalytical Center of Cairo University, using the diffusion plate method.[25-27]

One mL of each sample (100 mg/mL) is placed on a dish (9 cm diameter) containing a solid bacterial medium (nutrient agar broth) or fungal medium (Dox‘s medium) which has been heavily seeded with the spore suspensions of the tested organism. After incubation (24 h for bacteria and 5 days for fungi), the diameter of the clear zone of inhibition surrounding the sample is taken as measure of the inhibitory power of the sample against the particular tested organism. Compounds were tested against Gram+ve [*Bacillus subtilis* (6051) and *Staphylococcus aureus* (12600)], Gram-ve (*Pseudomonas aeruginosa* [10145] and *Escherichia coli* [11775]) bacteria, yeast [*Candida albicans* (7102)] and fungi [*Aspergillus flavus*]. Ampicillin and amphotericin B were used as references for bacteria and fungi, respectively. The results are depicted in Table 1.

RESULTS AND DISCUSSION

Two triterpenes and three diterpenes [Figure 1] were isolated from the *n*-hexane fraction of the ethanolic extract of *E. pseudocactus* Berger by successive fractionation on silica gel columns. Mass and ^{13}C-NMR spectral data of compounds 1 and 2 suggesting a triterpene nucleus with a molecular formula $C_{30}H_{50}O$ and $C_{30}H_{48}O$, respectively. The presence of correlation in the HMBC spectrum of compound 1 between an olefinic quaternary carbon at δ_C 141.87 and carbon of a tertiary alcohol at δ_C 76.53 with two singlet at δ_H 1.14 (CH_3-24) and 1.04 (CH_3-23) confirm the presence of a double bond at C-5. By comparing the chemical shift of compound 1 with the reported data,[28] it was identified as glut-5-en-3 β-ol (glutinol). NMR spectrum of compound 2 showed signals of three olefinic protons, two equivalent protons at δ_H 4.83 (br *s*) and 4.62 (br *s*)

Table 1: Antimicrobial activity of *Euphorbia pseudocactus* Berger (*n*-hexane fraction and isolated compounds)

Tested sample	Inhibition zone diameter (mm)					
	Gram-positive		Gram-negative		Yeast	Fungi
	Staphylococcus aureus	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Candida albicans*	*Aspergillus flavus*
Control (DMSO)	0 -	0 -	0 -	0 -	0 -	0 -
n–hexane	10 +	9 +	9 +	10 +	0 -	0 -
Compound 1	0 -	0 -	0 -	0 -	0 -	0 -
Compound 2	9 +	9 +	10 +	9 +	0 -	0 -
Compound 3	12 ++	11 ++	12 ++	13 ++	0 -	0 -
Compound 4	11 ++	13 ++	12 ++	12 ++	0 -	0 -
Ampicillin	18 +++	20 +++	17 +++	22 +++	0 -	0 -
Amphotericin	0 -	0 -	0 -	0 -	19 ++	17 ++

-: No activity; +: Weak activity; ++: Moderate activity

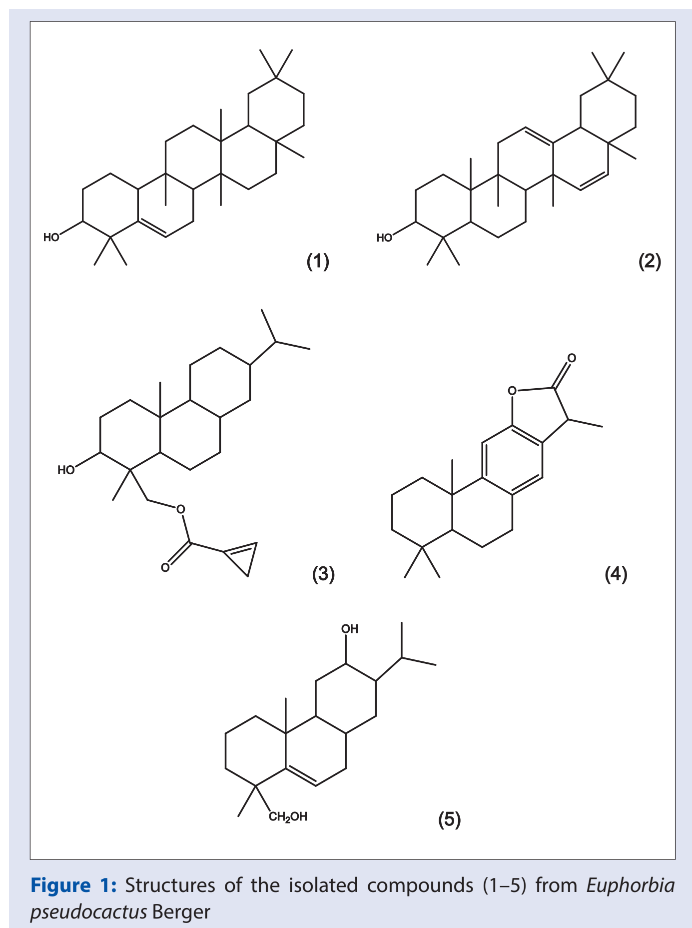

Figure 1: Structures of the isolated compounds (1–5) from *Euphorbia pseudocactus* Berger

corresponding to H-15 and 16, and a third one at δ_H 5.21 (*t*) assigned for H-12. Their corresponding carbons according to HMQC displayed at δ_C 129.24, 109.56, and 121.97, respectively, and another quaternary olefinic carbon appeared at δ_C 142.96. HMBC spectrum showed a correlation between carbon of a tertiary alcohol at δ_C 79.56 and the signals of 23 and 24 CH_3. By comparing our data with the reported one,[28,29] compound 2 was identified as olean-12,15-diene-3 β-ol. Mass and ^{13}C-NMR spectral data of compounds 3, 4, and 5 indicate a diterpene skeleton with a molecular formula $C_{24}H_{38}O_3$, $C_{20}H_{26}O_2$, and $C_{20}H_{34}O_2$, respectively. NMR spectrum of compound 3 showed a signal of an oxygenated carbon at δ_C 67.37 has two protons at δ_H 4.12 and 4.64, these protons showed a correlation with carbonyl carbon at δ_C 166.91. This was assigned for the acyl group at C-19. Two olefinic carbons appeared at δ_C 131.51 and 128.59, and a downfield shifted olefinic proton appeared at δ_H 7.69, indicating the presence of a conjugated olefinic bond with the carbonyl carbon. Another oxygenated carbon appeared at δ_C 74.38, its proton displayed at δ_H 2.83 and showed a correlation with a methyl signal (CH_3-20), this revealed the presence of hydroxyl group at C-3. From the given data, compound 3 was identified as 3-hydoxy-19-cyclopropenoyloxy-abietane. NMR spectrum of compound 4 showed signals of six olefinic carbons at δ_C 161.98, 147.97, 135.60, 128.11, 127.32, and 125.68 and two olefinic protons at δ_H 7.24 and 7.12, which revealed the presence of an aromatic system at ring C. The olefinic carbon at 161.98 showed a correlation with a proton at δ_H 3.89 (*d*, J = 2.4 Hz, H-15), this proton present on carbon displayed at δ_C 52.37 according to HMQC. Furthermore, a signal of carbonyl carbon appeared at δ_C 174.25, this confirmed the presence of C-12,16 olide group. Thus, compound 4 was identified as *ent*-abieta-9,12,14-triene-12,16-olide. NMR spectrum of compound 5 displayed an olefinic proton at δ_H 5.42 (br *s*) and two olefinic carbons at δ_C 140.74 and 120.56. Furthermore, signals of two oxygenated carbons displayed at δ_C 74.43 and 68.77, while their corresponding protons overlapped with the solvent peak. A methyl signal at δ_H 1.15 (*s*) showed a correlation in HMBC with the olefinic carbon at δ_C 140.74 and the oxygenated one at δ_C 68.77, this revealed the presence of an olefinic bond at C-5 and a hydroxyl group at C-19. From the given data and by comparison with similar structures,[21,30] compound 5 was identified as 12,19-dihydroxy-abieta-5-ene.

Compounds 1–4 and the *n*-hexane fraction were tested for their antimicrobial activity, the results showed that compounds 3 and 4 exhibited moderate activity against the tested Gram-positive and Gram negative bacteria while compound 2 and *n*-hexane fraction exhibited weak activity. Compound 1 exhibited no activity against the tested bacteria. All the tested samples showed no activity against the tested yeast and fungi.

CONCLUSION

Two triterpenes (1 and 2) and three abietane diterpenes (3–5) were isolated for the 1st time from *E. pseudocactus* Berger. The three diterpenes were isolated for the 1st time in nature. Compounds 3 and 4 exhibited moderate antibacterial activity, whereas the major isolated compound (1) showed no activity. Thus, the observed antibacterial activity of the *n*-hexane fraction could be attributed mainly to its diterpenoid content.

Acknowledgments

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Nil.

Conflicts of interest

There are no conflicts of interest.

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