

# Chemical Constituents from the Leaves of *Euphorbia ammak* Growing in Saudi Arabia

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## ABSTRACT

Investigation of the chloroform extract of *Euphorbia ammak* leaves led to the isolation of three compounds: euphol (1),  $\alpha$ -glutinol (2) and stigmasterol (3). Their structures were elucidated by 1D and 2D NMR, as well as by comparison with the reported data. Compounds 1-3 exhibited cytotoxicity *in vitro* against human cervical adenocarcinoma (Hela), among which, compound 1 showed the best activity.

**Key words:** Cervical adenocarcinoma, euphol, *Euphorbia ammak*

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## INTRODUCTION

The genus *Euphorbia* (family Euphorbiaceae) comprises more than 2000 species. It is widely distributed and considered one of the large genera of Angiosperms.<sup>[1]</sup> Several plants of this genus were traditionally reported to treat cancer.<sup>[1-3]</sup> Terpenes were commonly isolated from *Euphorbia* plants, in addition to other constituents such as steroids, phenolics and flavonoids.<sup>[1,4]</sup> *Euphorbia ammak* grows in Saudi Arabia and Yemen peninsula; the latex of the plant was previously investigated for its cytotoxicity *in vitro* against human cancer cell lines.<sup>[5]</sup> No previous investigation has been reported on the constituents of this plant so the present study aimed to explore its chemical composition and to test the cytotoxic activity of its isolated constituents.

## MATERIALS AND METHODS

### Plant material

*E. ammak* was collected in April 2012 from the western region of Saudi Arabia. The plant material was identified

by members of Plant Taxonomy Department, College of Science, King Abdulaziz University, Saudi Arabia. A voucher specimen is deposited at the Herbarium of the Department of Natural Products and Alternative Medicine, College of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia. The leaves were air-dried in the shade, then ground.

### General experimental procedures

Melting points were uncorrected and measured on a digital melting point apparatus (Buchi melting point B-540), NMR spectra were recorded on Varian Mercury 400 plus spectrometer (<sup>1</sup>H-NMR 400 and <sup>13</sup>C-NMR 100 MHz), using TMS as internal standard and CDCl<sub>3</sub> as the solvent. EIMS were recorded on a Shimadzu PQ-5000 instrument at 70 eV. Column chromatography was carried out on silica gel 60 (70-230 mesh, Merck). TLC analyses were conducted on pre-coated silica gel 60 F<sub>254</sub> plates (0.25 mm thickness, Merck), developed with solvent systems 100% CH<sub>2</sub>Cl<sub>2</sub>. Localization of spots was accomplished by spraying with *p*-anisaldehyde followed by heating at 110°C.

### Extraction and isolation

The air-dried powdered leaves of *E. ammak* (3 kg) were exhaustively extracted with ethanol 70% (4 × 5 L) by percolation. The residue left after the evaporation of the solvent (200 g) was successively fractionated with

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chloroform and ethyl acetate (5 × 500 mL, each). The chloroform extract (8 g) was chromatographed on repeated silica gel column, eluted in increasing polarity with *n*-hexane/CH<sub>2</sub>Cl<sub>2</sub> mixtures (90-10%) followed by 100% CH<sub>2</sub>Cl<sub>2</sub> and CH<sub>2</sub>Cl<sub>2</sub>/MeOH (99-95%) mixtures. Fractions were collected, monitored by TLC and yield compounds 1 (102 mg, *R<sub>f</sub>* = 0.41) from the fraction eluted with 50% *n*-hexane/CH<sub>2</sub>Cl<sub>2</sub>, 2 (68.5 mg, *R<sub>f</sub>* = 0.47) from the fraction eluted with 60% *n*-hexane/CH<sub>2</sub>Cl<sub>2</sub> and 3 (33 mg, *R<sub>f</sub>* = 0.33) from the fraction eluted with 40% *n*-hexane/CH<sub>2</sub>Cl<sub>2</sub>. The isolated compounds were subjected to the determination of their melting points and to structural analysis.

### Spectroscopic data

#### *Eupha-8,24-dien-3β-ol (Euphol) C<sub>30</sub>H<sub>50</sub>O (1)*

White needles, m.p. 118–120°C, EI ± MS *m/z* (rel. int.): 426 [M]<sup>+</sup> (0.5), 419 (34), 388 (21), 258 (24), 149 (21), 135 (24), 133 (19), 123 (26), 121 (28), 119 (26), 109 (48), 107 (28), 105 (23), 97 (19), 95 (49), 93 (25), 91 (19), 83 (31), 81 (40), 79 (18), 71 (22), 69 (100), 67 (25), 57 (36), 55 (70), 43 (63), 41 (69). <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ<sub>H</sub> (ppm): 5.10 (*brt*, *J* = 6.3 Hz, H-24), 3.24 (*dd*, *J* = 4.7, 11.6 Hz, H-3α), 1.69 (*s*, 3H-27), 1.61 (*s*, 3H-26), 1.00 (*s*, 3H-19), 0.96 (*s*, 3H-29), 0.88 (*s*, 3H-30), 0.86 (*d*, *J* = 6.3 Hz, 3H-21), 0.81 (*s*, 3H-28), 0.76 (*s*, 3H-18). <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>) δ<sub>C</sub> (ppm): 134.02 (C-8), 133.54 (C-9), 130.81 (C-25), 125.20 (C-24), 78.98 (C-3), 50.98 (C-5), 50.03 (C-14), 49.66 (C-17), 44.13 (C-13), 38.94 (C-4), 37.28 (C-10), 35.86 (C-20), 35.44 (C-22), 35.27 (C-1), 30.86 (C-12), 29.80 (C-15), 28.12 (C-16), 28.06 (C-28), 27.95 (C-2), 27.68 (C-7), 25.62 (C-27), 24.78 (C-23), 24.46 (C-30), 21.48 (C-11), 20.15 (C-19), 18.96 (C-21), 18.91 (C-6), 17.65 (C-26), 15.63 (C-18), 15.52 (C-29).

#### *Glutin-5-en-3α-ol (α-Glutinol) C<sub>30</sub>H<sub>50</sub>O (2)*

White powder, m.p. 201–203°C, EI ± MS *m/z* (rel. int.): 426 [M]<sup>+</sup> (0.5), 272 (28), 258 (24), 206 (22), 204 (28), 190 (18), 163 (18), 150 (19), 149 (20), 147 (21), 135 (36), 133 (20), 123 (40), 121 (47), 119 (27), 109 (67), 108 (26), 107 (61), 105 (30), 95 (100), 93 (47), 91 (30), 82 (25), 79 (37), 71 (22), 69 (91), 67 (48), 55 (64), 43 (33), 41 (71). <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ<sub>H</sub> (ppm): 5.63 (*m*, H-6), 3.46 (*brs*, H-3α), 1.16 (*s*, 3H-28), 1.14 (*s*, 3H-24), 1.09 (*s*, 3H-26), 1.04 (*s*, 3H-23), 1.01 (*s*, 3H-27), 0.99 (*s*, 3H-30), 0.95 (*s*, 3H-29), 0.85 (*s*, 3H-25). <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>) δ<sub>C</sub> (ppm): 141.81 (C-5), 122.29 (C-6), 76.56 (C-3), 49.90 (C-10), 47.64 (C-8), 43.27 (C-18), 41.05 (C-4), 39.51 (C-13), 39.17 (C-22), 38.05 (C-14), 36.25 (C-16), 35.29 (C-19), 35.06 (C-9), 34.82 (C-11), 34.75 (C-29), 33.33 (C-21), 32.60 (C-30), 32.29 (C-15), 32.26 (C-28), 30.57 (C-12), 30.31 (C-17), 29.17 (C-23), 28.47 (C-20), 28.04 (C-2), 25.68 (C-24), 23.86 (C-7), 19.85 (C-26), 18.65 (C-27), 18.44 (C-1), 16.44 (C-25).

#### *Stigmasterol C<sub>29</sub>H<sub>48</sub>O (3)*

White powder, m.p. 165–167°C, EI ± MS *m/z* (rel. int.): 412 [M]<sup>+</sup> (0.2), 409 (21), 254 (20), 161 (20), 159 (26), 147 (22), 145 (28), 135 (19), 133 (25), 123 (19), 121 (26), 119 (28), 109 (23), 107 (34), 105 (37), 97 (30), 95 (50), 93 (37), 91 (32), 85 (22), 83 (53), 81 (72), 79 (34), 71 (27), 69 (57), 67 (34), 63 (30), 57 (55), 55 (100), 43 (89), 41 (53), 29 (24), 28 (41), 19 (67). <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ<sub>H</sub> (ppm): 5.35 (*m*, H-6), 5.16 (*m*, H-22), 4.99 (*m*, H-23), 3.51 (*m*, H-3α), 1.02 (*s*, 3H-19), 0.91 (*d*, *J* = 6.3, 3H-21), 0.85 (*t*, 3H-29), 0.81 (*d*, *J* = 6.5, 3H-26), 0.81 (*d*, *J* = 6.5, 3H-27), 0.68 (*s*, 3H-18). <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>) δ<sub>C</sub> (ppm): 140.97 (C-5), 138.54 (C-20), 129.47 (C-21), 121.90 (C-6), 72.02 (C-3), 56.98 (C-14), 56.16 (C-17), 50.35 (C-9), 46.04 (C-22), 42.52 (C-4), 42.52 (C-13), 40.01 (C-18), 39.99 (C-12), 37.47 (C-1), 36.37 (C-10), 32.12 (C-7), 32.12 (C-8), 31.88 (C-2), 29.35 (C-25), 29.15 (C-16), 26.27 (C-23), 25.64 (C-15), 23.28 (C-19), 21.30 (C-11), 20.05 (C-27), 19.63 (C-26), 19.00 (C-28), 12.27 (C-24), 12.08 (C-29).

### *In vitro* cytotoxic assessment

#### *Chemicals and drugs*

Sulforhodamine-B dye (Sigma Chemical Co.; St. Louis, MO). RPMI-1640 media, fetal bovine serum, trypsin and other cell culture materials (Gibco Invitrogen; Carlsbad, CA, USA). Other reagents were of the highest analytical grade.

#### *Cell culture*

Cervical adenocarcinoma cell line, Hela, was obtained from the Vaccera (Giza, Egypt). Cells were maintained in RPMI-1640 supplemented with 100 μg/mL streptomycin, 100 units/mL penicillin and 10% heat-inactivated fetal bovine serum in a humidified, 5% (v/v) CO<sub>2</sub> atmosphere at 37°C. The cells were maintained as “monolayer culture” by serial subculturing.

#### *SRB cytotoxicity assay*

Cytotoxicity was determined using SRB method as previously described by Skehan.<sup>[6]</sup> Exponentially growing cells were collected using 0.25% Trypsin-EDTA and seeded in 96-well plates at 1000-2000 cells/well in RPMI-1640 supplemented medium. After 24 h, cells were incubated for 72 h with various concentrations of the tested compounds. Following 72 h treatment, the cells will be fixed with 10% trichloroacetic acid for 1 h at 4°C. Wells were stained for 10 min at room temperature with 0.4% SRB dissolved in 1% acetic acid. The plates were air dried for 24 h and the dye was solubilized with Tris-HCl for 5 min on a shaker at 1600 rpm. The optical density (OD) of each well was

measured spectrophotometrically at 564 nm with an ELISA microplate reader (ChroMate-4300, FL, USA).

#### Data analysis

The dose response curve of compounds was analyzed using  $E_{\max}$  model.

$$\% \text{Cell viability} = (100 - R) \times \left( 1 - \frac{[D]^m}{K_d^m + [D]^m} \right) + R$$

where  $R$  is the residual unaffected fraction (the resistance fraction),  $[D]$  is the drug concentration used,  $K_d$  is the drug concentration that produces a 50% reduction of the maximum inhibition rate and  $m$  is a Hill-type coefficient.  $IC_{50}$  was defined as the drug concentration required to reduce fluorescence to 50% of that of the control (i.e.  $K_d = IC_{50}$  when  $R = 0$  and  $E_{\max} = 100 - R$ ).

## RESULTS AND DISCUSSION

Chromatographic separation of the chloroform extract of leaves resulted in the isolation of three compounds [Figure 1] which were identified by physical and spectral means. The NMR spectra of compounds 1-3 showed close similarity. Compounds 1 and 2 showed triterpenoidal skeleton, while compound 3 showed a steroidal one. Compound 1 was obtained as white needles,  $^1\text{H-NMR}$  spectrum showed seven singlet signals assigned for the protons of tertiary methyl groups ( $\delta_{\text{H}}$  0.76-1.69), a sharp doublet signal ( $\delta_{\text{H}}$  0.86,  $J = 6.3$  Hz) for a secondary methyl group, a carbinol proton ( $\delta_{\text{H}}$  3.24,  $dd$ ,  $J = 4.7, 11.6$  Hz) at H-3 and an olefinic proton ( $\delta_{\text{H}}$  5.10,  $brt$ ,  $J = 6.3$  Hz) at H-24. Compound 2 was obtained as a white powder,  $^1\text{H-NMR}$  spectrum displayed singlet signals corresponding to eight methyl groups ( $\delta_{\text{H}}$  0.85-1.16), a carbinol proton ( $\delta_{\text{H}}$  3.46) at H-3 and an olefinic proton ( $\delta_{\text{H}}$  5.63) at H-6. From the

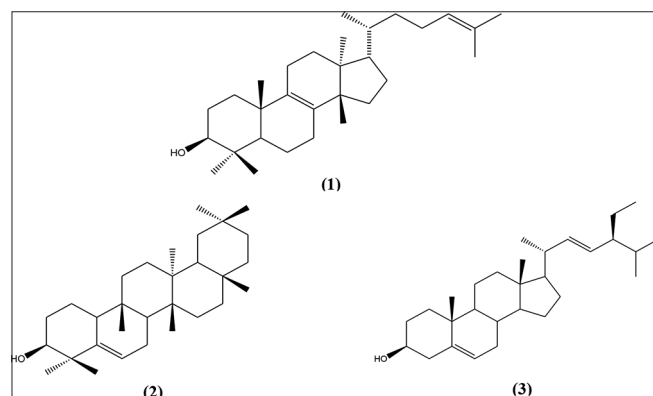


Figure 1: Structures of compounds 1-3

aforementioned results compound 1 and 2 could be identified as euphol (1),  $\alpha$ -glutinol (2). Compound 3 was obtained as a white powder,  $^1\text{H-NMR}$  spectrum revealed the presence of C-24 ethyl sterol nucleus that was confirmed by the appearance of the protons of the six methyl groups at  $\delta_{\text{H}}$  0.68 and 1.02 appearing as two singlet signals corresponding to Me-18 and Me-19, at 0.81 ppm for two equivalent doublets ( $J = 6.5$  Hz) corresponding to Me-26 and Me-27, at 0.91 ppm for the doublet corresponding to Me-21 ( $J = 6.3$  Hz) and a signal at 0.85 ppm appeared as triplet, assigned to the methyl group Me-29. Moreover, four protons multiplet at  $\delta_{\text{H}}$  3.51 corresponding to H-3, at 5.35 ppm to the olefinic proton at C-6, at 5.16 and 4.99 ppm for the olefinic protons at C-22 and C-23, respectively. From the aforementioned data, compound 3 was identified as stigmaterol (3). The full assignments of the structures of compounds 1-3 were deduced from full correlation analysis of its COSY, HMBC, HSQC spectra and confirmed by comparing their data with published ones.<sup>[7-16]</sup> All isolated compounds were tested *in vitro* for their cytotoxic activity against human cervical adenocarcinoma cell line (Hela), Compound 1 is the best one among the tested compounds having low  $IC_{50}$  value 9.25  $\mu\text{g/ml}$  together with low resistant fraction 13% compared to the other tested compounds 2 and 3 which encountered high resistance fraction of about 48 and 45%, respectively [Figure 2]. The result was in accordance with previous reports concerning

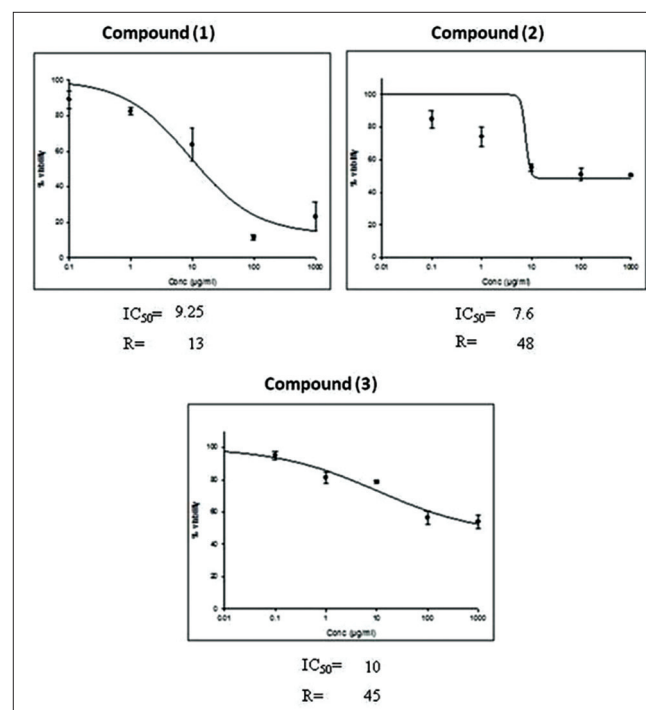


Figure 2: Results of cytotoxicity for compounds 1-3 against human cervical adenocarcinoma

the cytotoxic activity of euphol (1).<sup>[9,17-21]</sup> It is worthy to mention that euphol (1) was the only major compound found in the milky sap of leaves as detected by thin layer chromatography, while  $\alpha$ -glutinol (2) and stigmasterol (3) were absent. This is the first report on the chemical investigation of *E. ammak* growing in Saudi Arabia.

## REFERENCES

1. Wu, QC, Tang YP, Ding AW, You FQ, Zhang L, Duan JA. <sup>13</sup>C-NMR data of three important diterpenes isolated from *Euphorbia* species. *Molecules* 2009;14:4454-75.
2. Betancur-Galvis L, Palomares E, Marco JA, Estornell E. Tigliane diterpenes from the latex of *Euphorbia obtusifolia* with inhibitory activity on the mammalian mitochondrial respiratory chain. *J Ethnopharmacol* 2003;85:279-82.
3. Ravikanth V, Niranjan Reddy VL, Prabhakar Rao T, Diwan PV, Ramakrishna S, Venkateswarlu Y. Macrocyclic diterpenes from *Euphorbia nivulia*. *Phytochemistry* 2002;59:331-5.
4. Shi QW, Su XH, Kiyota H. Chemical and pharmacological research of the plants in genus *Euphorbia*. *Chem Rev* 2008;108:4295-327.
5. Almeddar H, Abdallah HM, Osman AM, Abdel-Sattar EA. *In vitro* cytotoxic screening of selected Saudi medicinal plants. *J Nat Med* 2012;66:406-12.
6. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, *et al.* New colorimetric cytotoxicity assay for anticancer-drug screening. *J Natl Cancer Inst* 1990;82:1107-12.
7. Good JL, Akihisa T. One-dimensional and two-dimensional NMR spectroscopy of sterols. In: *Analysis of sterols*. 1<sup>st</sup> ed. London: Chapman and Hall; 1997. p. 197-275.
8. Ferreira MU, Madureira AM, Ascenso JR. A tetracyclic diterpene and triterpenes from *Euphorbia segetalis*. *Phytochemistry* 1998;49:179-83.
9. Mazoir N, Benharref A, Bailén M, Reina M, González-Coloma A. Bioactive triterpene derivatives from latex of two *Euphorbia* species. *Phytochemistry* 2008;69:1328-38.
10. Yayli N, Baltaci C. The sterols of *Cyclamen Coum*. *Tr J of Chemistry* 1996;20:329-34.
11. Wu QX, Su YB, Zhu Y. Triterpenes and steroids from the roots of *Scorzonera austriaca*. *Fitoterapia* 2011;82:493-6.
12. Leong Y, Harrison LJ. (20R,23E)-Eupha-8,23-diene-3 $\beta$ ,25-diol from *Tripetalum cymosum*. *Phytochemistry* 1999;50:849-57.
13. Fraga BM, Reina M, Luis JG, Rodriguez ML. Rhoiptelenol and Rhoiptelenone, Two Pentacyclic Triterpenes from *Sideritis macrostachya*. *Z Naturforsch C* 2003;58:621-5.
14. Gaertner M, Müller, L, Roos JF, Cani G, Santos AR, Niero R, *et al.* Analgesic triterpenes from *Sebastiania schottiana* roots. *Phytomedicine* 1999;6:41-4.
15. El-Domiaty MM, Wink M, Abdel Aal MM, Abou-Hashem MM, Abd-Alla RH. Antihepatotoxic Activity and Chemical Constituents of *Buddleja asiatica* Lour. *Z Naturforsch C* 2009;64:11-9.
16. Huynk NV, Nguyen TH, Nguyen KP, Hansen PE. Structure studies of the chemical constituents of *Tithonia tagetiflora* Desv. (Asteraceae). *Magn Reson Chem* 2013;51:439-43.
17. Dutra RC, Simão da Silva KA, Bento AF, Marcon R, Paszcuk AF, Meotti FC, *et al.* Euphol, a tetracyclic triterpene produces antinociceptive effects in inflammatory and neuropathic pain: The involvement of cannabinoid system. *Neuropharmacology* 2012;63:593-605.
18. Zhang L, Gao L, Li Z, Yan X, Yan Y, Tang Y, *et al.* Bio-guided isolation of the cytotoxic terpenoids from the roots of *Euphorbia kansui* against human normal cell lines. *Int J Mol Sci* 2012;13:11247-59.
19. Wang L, Wang G, Yang D, Guo X, Xu Y, Feng B, *et al.* Euphol arrests breast cancer cells at the G1 phase through the modulation of cyclin D1, p21 and p27 expression. *Mol Med Rep* 2013;8:1279-85.
20. Lin MW, Lin AS, Wu DC, Wang SS, Chang FR, Wu YC, *et al.* Euphol from *Euphorbia tirucalli* selectively inhibits human gastric cancer cell growth through the induction of ERK1/2-mediated apoptosis. *Food Chem Toxicol* 2012;50:4333-9.
21. Bigoniya P, Rana AC. Radioprotective and *in-vitro* cytotoxic sapogenin from *Euphorbia nerifolia* (Euphorbiaceae) leaf. *Trop J Pharm Res* 2009;8:521-30.

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