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Chemical and Biological Studies of *Euphorbia Aphylla*

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Abstract

From the aerial part of *Euphorbia aphylla*, nine compounds were isolated (**1**-**9**) and identified by different spectral techniques as well as comparison with authentic samples. The isolated compounds included two triterpenes(*β*-amyrone (**1**) and euphol(**2**)), two sterols (*β*-sitosterol (**3**) and *β*-sitosterolglucoside (**4**)) and five phenolic compounds (gallic acid (**5**), quercetin (**6**), quercetin-3-*O*-(2'',3''-digalloyl)-*α*-L-rhamnoside (**7**), 3,4,3'-*O*-trimethyl ellagic acid 4'-*O*-*ß*-D-glucopyranoside (**8**) and (3,4,3'-tri-*O*-methyl ellagicacid4'-rutinoside)(**9**)).

The anti-inflammatory, antipyretic,and antioxidant and antimicrobial activities were carried out ondifferent plant fractions.

Key words: *Euphorbia aphylla*, ellagic acid derivatives, triterpenes, flavonoids, anti-inflammatory, antioxidant, antimicrobial activity

1. Introduction

The family Euphorbiaceae includes about 8000 species, most of which are characterised by the production of a toxic, skin irritant, milky latex[1–3].

The genus *Euphorbia*comprising about 2000 known species distributed all over the world, more than 750 species are found in Africa and 42 in Egypt, range from annuals to treesgrowing either wild, naturalised, or cultivated [4]. The genus is known to produce various classes of compounds such as diterpenes which are responsible for the skin irritating, tumour promoting, and cytotoxic activities [5–15], phenolics including lactones of an ellagic acid skeleton, triterpenes, flavonoids, and coumarins [16–27].

Euphorbia species have been widely used in folk medicine for treatment of diarrhoea, inflammation, and swellings and is known as a wart remover [28–30]. Some species have been used in treatment of dermatosis, paralysis, and pain of human body as well as poultice for broken bones ulceration, swelling, and haemorroids [31]. A number of interesting biological activity were also reported such as cytotoxic [32,33], hepatoprotective [34–36], antispasmodic [37], pesticide [38], molluscicidal [39–41], larvicidal [42], anti-inflammatory [43], antibacterial [44,45], antifungal [37], anti-mutagenic [46], and antiviral activities [47–50].Latex shows co-carcinogenic [51] and anti-carcinogenic activities [10]. *Euphorbia aphylla*is a perennial herbaceous plant with a milky juice in the aerial parts and roots. To the best of our knowledge,little studies were focusing on the phytochemistry and biological activity of *E. aphylla* [39], and this is the first study describing in details the chemistry of the constituents as well as the potential biological activities of its extracts.

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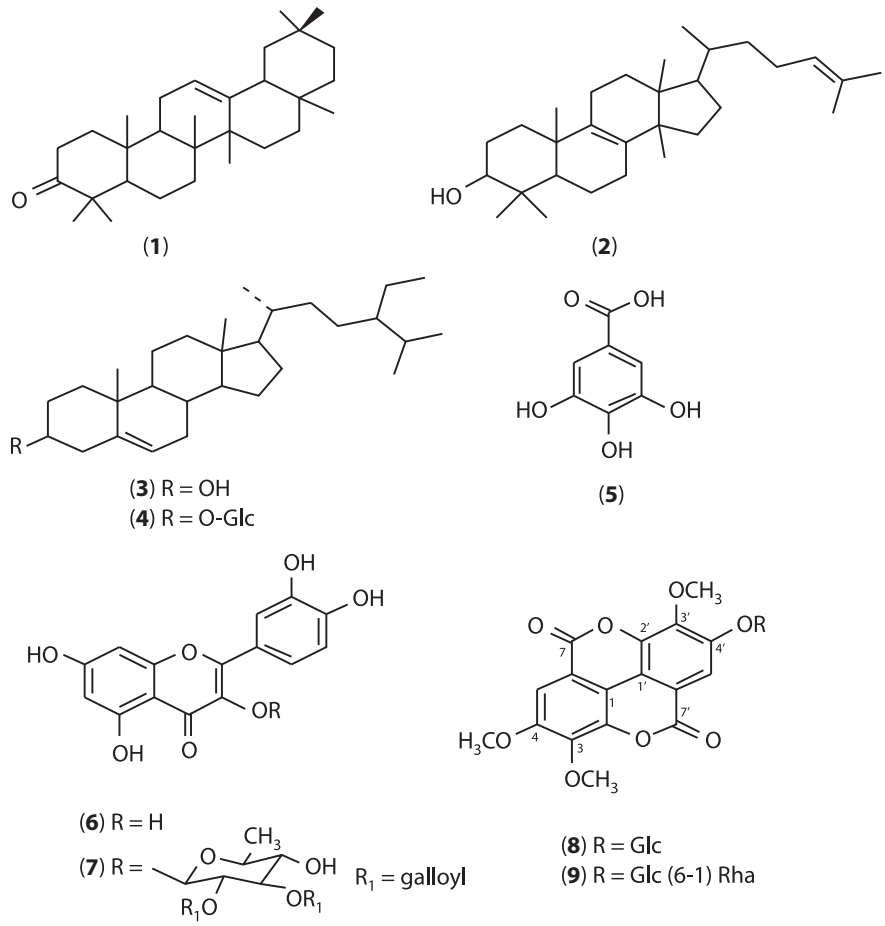

Fig. 1. Structures of the isolated compound

I –Hexane-EtOAc(8:2 v/v)
II –$CHCl_3$-MeOH (9.5:0.5 v/v)
III –$CHCl_3$-MeOH (9:1 v/v)
IV –$CHCl_3$-MeOH (8.5:1.5 v/v)
V – $CHCl_3$-MeOH-H_2O (8:2:0.2 v/v)
VI –*n*-butanol-AcOH-H_2O (4:1:5 v/v)

In the course of our ongoing research activities towards the isolation of biologically active compounds from plants growing in Egypt either wild or cultivated, in particular the species of diverse chemical constituents with various reported biological activity, we had the opportunity to work on the aerial part of *E.aphylla*to investigate its chemical constituents and potential biological activities.

In the present study, we report the isolation of and structural elucidation of nine compounds from *E. aphylla*for the first time in addition to biological evaluation of the different fractions of the plant extract.

2. Materials and Methods

2.1 General

The UV absorbance was measured on Ultrospec 1000, UV/visible spectrometer, Pharmacia Biotech (Cambridge, England). EI-MS was measured on JEOL JMS 600 Hz (Japan). 1D and 2D NMR were measured on Varian mercury 400 MHz NMR Spectrometer (Oxford) using TMS as internal standard. HPLC separations were carried out using a Phenomenex RP column (C18, 250 × 10 mm, 5 µm) and an Agilent 1200 series gradient pump monitored using a DAD G1315B variable-wavelength UV detector. Column chromatography (CC) was performed using a silica gel (Kieselgel 60 Å, 40–63 µm mesh size, Fluorochem, UK) sephadex LH-20 (25–100 mm mesh size, SIGMA, Germany). TLC was carried on pre-coated silica gel plates $G_{60}F_{254}$ and RP-18 each (0.25 mm, ALUGRAM® SIL G/UV_{254}, Macherey-Nagel,

Germany). The plates were examined under UV light (365 and 254 nm) and visualised by spraying with 20% v/v H_2SO_4 in EtOH;they were allowed to dry at room temperature followed by heating at 110–140°C for 1–2 min. The following solvent systems were used for TLC:Authentic reference materials *ß*-sitosterol, *ß*-sitosterolglucoside, and quercetin were obtained from the Pharmacognosy Department, Faculty of Pharmacy, Assiut University, Assiut, Egypt. Authentic sugars D-glucose and L-rhamnose were provided by El-Naser Pharmaceutical and Chemical Co., Egypt (ADWIC).

2.2 Plant Material

In July 2009, the whole plants of *E. aphylla* were collected from the garden of Faculty of Agriculture, Assiut University, Assiut, Egypt. It was identified by Prof. DrMoamenZarea, Faculty of Science, Assiut University, Assiut, Egypt. A voucher specimen was deposited in the Herbarium of the Faculty of Pharmacy, Assiut University (No. EUA-1).

2.3 Extraction and Isolation

Fresh aerial parts of *E.aphylla* (1 kg) were extracted by soxhlet with hexane, $CHCl_3$, EtOAc, and finally EtOH, respectively. Each fraction was concentrated under reduced pressure till constant weight to yield hexane fraction (10 g), $CHCl_3$ (15 g), EtOAc (17 g), and EtOH (15 g) fractions (A-D), respectively. The hexane fraction (10 g) was subjected to alumina CC (300 g). Elution was started with *n*-hexane followed by *n*-hexane:EtOAcgradiently. Fractions of 100 ml each were collected and monitored using TLC and 20% v/v H_2SO_4 in EtOH as spraying reagent; similar fractions were pooled together where three groups were obtained. Group I, fractions eluted with *n*-hexane:EtOAc (97:3) were chromatographed over silica gel CC, which afforded compounds (**1**) (40 mg). Group III, fractions eluted with *n*-hexane:EtOAc (90:10) afforded compounds (**2**) (100 mg) and (**3**) (70 mg) after silica gel CC.

A part of the chloroformic fraction (10 g) was chromatographed on silica gel CC (300g). Elution was started with $CHCl_3$ followed by $CHCl_3$-MeOH gradients (fractions 100 ml each were collected), where three groups were obtained. Group III, fractions eluted with $CHCl_3$:MeOH (90:10) were re-chromatographed over silica gel CC to afford compound (**4**) (55 mg). The EtOAc fraction was subjected to Diaion-HP20 CC using H_2O, H_2O-MeOH, and finally MeOH (each 2 l). The methanolic elute was concentrated under reduced pressure to yield a fraction (7 g); part of the methanolic fraction (5 g) was subjected to silica gel CC (150 g) followed by Sephadex LH-20 column with $CHCl_3$-MeOH (1:1) and finally HPLC (RP18) to yield five compounds, such as (**5**) (35 mg), (**6**) (24 mg), (**7**) (13mg), (**8**) (14 mg), and (**9**) (17 mg).

2.4 Hydrolysis of Isolated Glycosides

Acidic and alkaline hydrolysis of isolated glycosides (**7, 8, 9**) was done as described in Harborne and Mabry, 1982 [52].

2.4.1 Partial acid hydrolysis of glycosides

About 3 mg of each glycoside (**7, 8, 9**) was dissolved in 5 ml methanol, to which 10 ml of 2% aqueous HCl was added and refluxed on a boiling water bath for 2 h. A sample of the hydrolysate was withdrawn with a micropipette every 5 min within 2 h. The samples taken were spotted on Whatman No. 1 sheets, and the chromatogram was developed with system VI [52].

2.4.2 Complete acid hydrolysis

About 4 mg of the glycoside (**7**, **8**,**9**)was dissolved in 10 ml methanol, to which an equal volume of 10% sulphuric acid was added. The mixture was refluxed on a boiling water bath for 3 h, after which, samples were withdrawn and tested chromatographically to ensure complete hydrolysis [52].

2.4.3 Alkaline hydrolysis

About 0.5 mg of the glycoside (**7**) was hydrolysed with 1% aqueous KOH (0.5 ml) for 1 h at room temperature. The reaction mixture was adjusted to pH 6 with dilute 1% HCl and then extracted with EtOAc (3 × 0.5 ml). Samples were withdrawn and tested chromatographically to ensure complete hydrolysis [52].

2.5 Chemicals for Biological Assays

Ascorbic acid and quercetin as an antioxidant standard were obtained from Sigma-Aldrich Chemicals Co., Germany. 2,2-Diphenyl-1-picryl hydrazyl (DPPH) was obtained from Sigma-Aldrich Chemicals Co., Germany.

Indomethacin was obtained from El-Nile Company for Pharmaceutical and Chemical Industries, Cairo, A.R.E. Other chemicals used were of high analytical grade and were obtained from Sigma-Aldrich and Merck companies.

2.6 Animals

Albino rats (each 100–120 g) of either sex were bred and housed under standardised environmental conditions in the pre-clinical animal house, Pharmacology Department, Faculty of Medicine, Assiut University. The animals were fed with standard diet and free access to water; they were kept for one week to acclimatise to the environmental conditions. The animals were handled only at the time of experiments and during cage cleaning. All conditions were made to minimise animal suffering.

2.7 DPPH RadicalScavenging Assay

DPPH• radical scavenging activity was measured by spectrophotometric method [53,54].Around 1 ml of the different fractions of *E.aphylla* of various concentrations (10–500 µg/ml) was mixed with 1 ml of ethanolic solution of DPPH• (200 µM). Similarly, 1 ml ethanolic solutions of ascorbic acid and quercetin of various concentrations (10–500 µg/ml) were mixed with 1 ml of DPPH• solution. A mixture of 1 ml of ethanol and 1 ml of ethanolic solution of DPPH• (200 µM) served as control. After mixing, all the solutions were incubated in dark for 30 min and then the absorbance was measured at 517 nm. The experiments were performed in triplicate using ascorbic acid and quercetin as a positive control standards and % scavenging activity was calculated by using the formula [55,56]:

$$Q\ (\%\text{Inhibition}) = [(A_B - A_A)/A_B] \times 100,$$

where A_B – absorption of blank sample (t=0 min),

A_A – absorption of tested extract solution (t=30 min).

2.8 Anti-inflammatory Activity (Yeast-induced Paw Oedema Method)

Different fractions of *E.aphylla* were evaluated for their anti-inflammatory activity [57]. Rats were randomly divided into six groups (five rats per group). Group 1 (negative control) was administered the vehicle (2% tween 80 solution) orally. Groups 3–6 were administered 400 mg/kg of fractions A-D, respectively, suspended in the vehicle orally. Animals of group 2 (positive control) were administered indomethacin (15 mg/kg) as the reference drug in vehicle orally.

The tested fractions and indomethacin were administered orally just one hour after the inflammation was induced by subcutaneous injection of an equal volume of yeast aqueous suspension in 2% tween 80 in the left hind paw of each rat under the sub-plantar region. The increase in linear paw circumference was taken as a measure of oedema.

$$(\%\text{Inhibition}) = [(V_o - V_t)/\ V_o] \times 100,$$

where V_o – the average paw thickness of control group,

V_t – the average paw thickness of the treated group.

2.9 Antipyretic Activity

For screening of the antipyretic activity, the same grouping of animals and their respective treatment were followed where group 2 was received indomethacin as a positive control at a dose of 8 mg/kg. The other groups were separately injected intraperitoneally with the different fractions at a dose of 400 mg/kg body weight. Experimental pyrexia induced with 15% suspension of brewer's yeast in 2% tween 80 was given 0.25 ml/100 g dose as the method described by Bhalla et al. (1971) [58]. The rectal temperature before and after treatment which was recorded with the help of digital clinical thermometer at every hour up to four hours was compared with control.

2.10 Statistical Analysis

Data were analysed by comparing values for different treatment groups with the values for individual controls. Results are expressed as mean±SE (n=5 animals). The significant differences among values were analysed using analysis of variance (one-way ANOVA) followed by Dunnett's"t" test as for comparison between different groups. Therefore,p<0.05 was considered as significant and$p$<0.01 was considered as very significant. Graph Pad Prism was used for statistical calculations (version 3.02 for Windows).

2.11 Antimicrobial Activity

2.11.1 Test Organisms

Bacterial strains used in this study were as follows: *Escherichiacoli* (AUMC No.B-53),

Pseudomonasaeruginosa (AUMCNo.B-739), and *Serratiamarcescens* (AUMC No.B-55) as gram-negative bacteria and *Staphylococcusaureus* (AUMC No.B-59), *Bacilluscereus* (AUMCNo.B-52), and *Micrococusluteus* (AUMC No.B-112) as gram-positive bacteria. *Candidaalbicans* (AUMC No.418), *Geotrichumcandidum* (AUMC No.226), *Fusariumaxysporum* (AUMC No.5119), *Scopulariopsisbrevicaulis* (AUMC No.729), *Trichophytonrubrum* (AUMC No.1804), and *Aspergillusflavus* (AUMC No.1276) were used for determination of antifungal activity. All strains were clinical isolates obtained from the Mycology Unit, Assiut University, Assiut, Egypt.

2.11.2 Antibacterial Activity

The inoculum size of each test strain was standardised according to the Committee for Clinical Laboratory Standards (CLSI/NCCLS) methods [59]. The test bacterial strain was inoculated into Mueller Hinton broth(MHB)from medium Oxoidand incubated for 3–6 h at 35 °C in a shaker water bath until the culture attained a turbidity of 0.5 McFarland unit. The final inoculum was adjusted to 5×10^5cfu/ml.

Antibacterial screening was done by a modified agar-well diffusion method [60]. A 1.0 ml volume of the standard suspension (5×10^5cfu/ml) of each test bacterial strain was spread evenly on MHA plates using sterile glass rod spreader and the plates allowed to dry at room temperature. Subsequently, 6 mm-diameter wells were bored in the agar and a 100 ml volume of each plant fractions (A-D) reconstituted in 50% DMSO to a concentration of 100 mg/ml was pipetted into triplicate wells. After holding the plates at room temperature for 1 h to allow diffusion of extract into the agar, they were incubated at 37 °C for 24 h and the (bacterial growth) inhibition zone diameter (IZD) was measured to the nearest mm. Chloramphenicol, used at concentrations of 8 µg/ml, was included as positive control while DMSO (50% concentration) served as the negative control.

2.11.3 Antifungal Activity

The antifungal activity of the prepared fractions was evaluated by using the potato dextrose agar at 28°C for 48 h as the growth medium. Stock solutions of the tested fractions and the reference standard antifungal drugClotrimazole (discs) were prepared at initial concentration of 10,000 µg/ml of DMSO. Serial 2-fold concentrations (0.025–100 µg/ml) were incorporated into the growth medium and the plates were poured.

Compound (**1**)(*ß*-amyrone) was obtained as colourless fine needles (methanol), m.p. 177–179°C, R_f=0.64 (system I); IR (KBr) ν_{max} cm^{-1}: 1695 (C=O) and 2925 (C–H).

Compound (**2**) (euphol) was obtained as white powder; IR (KBr) ν_{max} 3410, 1650 $cm^{-1}$and 3340, 2994, 1455, 1347, 1216, 1094, 1023 cm^{-1}.^{1}H-NMR spectral data ($CDCl_3$, 400 MHz) δ_H : 0.74 (3H, s, H_3-18), 0.78 (3H, s, H_3-29), 0.81 (3H, s, *J*=6.6 Hz, H_3-21), 0.87 (3H, s, H_3-30), 0.91 (3H, s, H_3-19), 0.99 (3H, s, H_3-28), 1.49 (2H, m, H-2), 1.62 (3H, s, H_3-27), 1.70 (3H, s, H_3-26), 3.22 (1H, m, H-3), 5.00 (1H, m, H-24). ^{13}C-NMR spectral data ($CHCl_3$, 100 MHz) δ_C: 15.5 (C-18), 15.6 (C-30), 17.7 (C-26), 18.9 (C-21), 18.9 (C-6), 20.1 (C-19), 21.5 (C-11), 24.5 (C-28), 24.7 (C-23), 25.7 (C-27), 27.6 (C-2), 27.9 (C-7), 28.0 (C-29), 28.1 (C-15), 29.7 (C-16), 30.9 (C-12), 35.2 (C-1), 35.4 (C-22), 35.9 (C-20), 37.2 (C-10), 38.9 (C-4), 44.1 (C-13), 49.6 (C-17), 50.0 (C-14), 50.9 (C-5), 79.0 (C-3), 125.1 (C-24), 130.9 (C-25), 133.5 (C-8), 134.0 (C-9).

Compound (**3**) (*ß*-sitosterol)wasobtained as white amorphous powder (methanol), m.p. 134–136°C, R_f=0.33 (system I); IR ν_{max} (KBr) cm^{-1}: 3440 (OH), 2930 (C–H), and 1645 (C=C).

Compound (**4**) (*ß*-sitosterol-3-*O*-*ß*-glucoside) wasobtained as white granular powder (methanol), R_f=0.36 (system IV); IR ν_{max} (KBr) cm^{-1}: 3415 (OH), 2960 (C–H), and 1636 (C=C).

Compound (**5**) (gallic acid) was obtained as a yellowish white crystals from MeOHm.p. 250–252°C, R_f=0.39 (system, IV);UV: λ_{max} (EtOH): 220, 271 nm. EI-MS showed peak at *m/z* 170 $[M]^+$. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H: 7.37 (2H, brs, H-2, and H6) and^{13}C-NMR spectral data (DMSO-d_6, 100 MHz) δ_C: 108.7 (C-2 and C-6), 121.0 (C-1), 142.0 (C-4), 147.7 (C-3 and C-5),and 168.9 (COOH).

Compound (**6**) (quercetin)wasobtained as a yellow powder from MeOH, R_f=0.40 (system, III). EI-MS showed peak at *m/z* 303 $[M+H]^+$. ^{1}H-NMR spectral data (DMSO-d_6, 400 MHz) δ_H: 6.18 (1H, d, *J*=1.5, H-6), 6.40 (1H, d, *J* =1.5, H-8), 6.86 (1H, d, *J*=8.5, H-5'), 7.54 (1H,dd, *J*=8.5, 2, H-6'), 7.67 (1H, d, *J*=2, H-2'), 12.18 (1H

brs, 5-OH) and ^{13}C-NMR spectral data (DMSO-d_6, 100 MHz) δ_C: 93.8 (C-8), 98.7 (C-6), 103.4 (C-10), 115.5 (C-2'), 116.1 (C-5'), 120.8 (C-6'), 122.4 (C-1'), 136.2 (C-3), 145.5 (C-3'), 147.2 (C-2), 148.1 (C-4'), 156.6 (C-5), 161.2 (C-9), 164.4 (C-7), 176.3 (C-4).

Compound (**7**) (quercetin-3-*O*-(2",3"-digalloyl)-*α*-L-rhamnoside) was obtained as yellowish white powder; UV: λ_{max} (MeOH): 268, 355 nm. FAB-MS at *m/z* 753 $[M+H]^+$. ^{1}H-NMR spectral data (DMSO-d_6, 400 MHz) δ_H: 0.85 (3H, d, *J*=6.9, H-6"), 3.14 (1H, m, H-5"), 3.20 (1H, m, H-4"), 5.22 (1H, brs, H-1"), 5.23 (1H, m, H-3"), 5.71 (1H, m, H-2"), 6.20 (1H, d, *J*=1.5, H-6), 6.40 (1H, d, *J*=1.5, H-8), 6.86 (1H, d, *J*=8.2, H-5'), 6.92 (2H, s, H-2"", 6""), 6.94 (2H, s, H-2"",6""), 7.24 (1H, dd, *J*=8.2, 1.9, H-2'), 7.31 (1H, d, *J*=1.9, H-6') and ^{13}C-NMR spectral data (DMSO-d_6, 100 MHz) δ_C:17.8 (C-6"), 69.7 (C-5"), 70.3 (C-2"), 70.7 (C-3"), 72.8 (C-4"), 93.7 (C-8), 98.8 (C-6), 99.5 (C-1"), 104.2 (C-10), 108. 9 (C-2"", 6"", 2"", 6""), 115.7 (C-2'), 116.4 (C-5'), 119.4 (C-1""), 120.5 (C-1""), 121.5 (C-6'), 122.4 (C-1'), 134.3 (C-3), 138.1 (C-4""), 138.5 (C-4""), 145.3 (C-3'), 145.5 (C-3"", 5"", 3"", 5""), 149.0 (C-4'), 156.5 (C-2), 157.4 (C-9), 161.3 (C-5), 164.3 (C-7), 167,5 (C-7"", 7""), 177.8 (C-4).

Compound (**8**) (3,4,3'-*O*-trimethyl ellagic acid 4'-*O*-*ß*-D-glucopyranoside) was obtained as white powder; UV: λ_{max}(MeOH): 255, 354 nm. FAB-MS at *m/z* 507 $[M+H]^+$.^{1}H-NMR spectral data (DMSO-d_6, 400 MHz)δ_H:3.34 (1H, m, H-6"b), 3.69 1H, m, H-6"a), 3.17-3.72 (4H, m, H-2", 3", 4", 5"), 4.01 (3H, s, OCH_3), 4.06 (3H, s, OCH_3), 4.10 (3H, s, OCH_3), 5.12 (1H, d, *J*=7.1, H-1"), 7.47 (1H, s, H-5), 7.67 (1H, s, H-5') and ^{13}C-NMR spectral data (DMSO-d_6, 100 MHz)δ_C:57.2 (OCH_3), 61.4 (OCH_3), 61.7 (OCH_3), 62.6 (C-6"), 70.0 (C-4"), 73.8 (C-2"), 76.9 (C-5"), 77.7 (C-3"), 101.8 (C-1"), 108.2 (C-5), 112.5 (C-6), 112.5 (C-5'), 112.7 (C-1), 113.1 (C-6'), 113.4 (C-1'), 141.7 (C-2, C-2`), 141.7 (C-3, C-3`), 151.6 (C-4'), 154.8 (C-4), 158.3 (C=O), 158.5 (C=O).

Compound (**9**) (3,4,3'-tri-*O*-methyl ellagic acid 4'-rutinoside) was obtained as white powder; UV: λ_{max}(MeOH): 255, 354 nm. FAB-MS at *m/z* 653 $[M+H]^+$. ^{1}H-NMR spectral data (DMSO-d_6, 400 MHz) δ_H:0.90 (3H, d, *J*=6.0), 3.46 (1H, m, H-6"b), 3.09-3.60 (m, other sugar protons), 3.83 (1H, m, H-6"a), 4.01 (3H, s, OCH_3), 4.05 (3H, s, OCH_3), 4.10 (3H, s, OCH_3), 4.50 (1H, brs, H-1""), 5.20 (1H, d, *J*=7.2, H-1"), 7.66 (1H, s, H-5), 7.81 (1H, s, H-5'),and^{13}C-NMR spectral data (DMSO-d_6, 100 MHz) δ_C:17.7 (C-6""), 56.7 (OCH_3), 61.3 (OCH_3), 61.7 (OCH_3), 62.5 (C-6"), 68.2 (C-5""), 69.7 (C-4"), 70.1 (C-2""), 70.6 (C-3""), 71.9 (C-4""), 73.2 (C-2"), 75.8 (C-5"), 76.3 (C-3"),100.5 (C-1""), 101.5 (C-1"), 107.6 (C-5), 112.5 (C-6), 112.5 (C-6'), 112.7 (C-5'), 112.9 (C-1), 113.8 (C-1'), 140.9 (C-2), 141.2 (C-3), 141.2 (C-2'),141.9 (C-3'), 151.7 (C-4'), 154.3 (C-4), 158.1 (C=O), 158.5 (C=O).

3. Results

From the aerial parts of *E. aphylla,* nine compounds were isolated using different chromatographic techniques and identified by different physical, chemical, and spectroscopical methods.

Compounds (**1**-**4**) were obtained from the hexane and chloroformic fractions and gave positive test with Salkowski's and Liebermann-Burchard's test indicating theirtriterpenoidal and/or steroidal nature. From 1D (^{1}H and^{13}C) NMR data, mass spectroscopy, and co-chromatography, the compounds were identified as *ß*-amyrone(**1**) [61], euphol (**2**) [62], *ß*-sitosterol (**3**) [63], *ß*-sitosterol-3-*O*-*ß*-glucoside (**4**)[64].

From the ethyl acetate fraction, five compounds (**5**-**9**) were isolated and identified as gallic acid (**5**)[21], quercetin (**6**)[21], quercetin-3-*O*-(2",3"-digalloyl)-*α*-L-rhamnoside (**7**)[65,66], 3,4,3'-tri-*O*-methyl ellagicacid4'-*O*-*ß*-D-glucopyranoside (**8**)[67,68], and 3,4,3'-tri-*O*-methyl ellagicacid4'-rutinoside (**9**)[68,69].

Different biological studies were carried out to evaluate the activity of fractions, such as antioxidant activity, anti-inflammatory, antipyretic, as well as antimicrobial activity. For antioxidant activity, the direct measurement of radical scavenging activity was determined using DPPH$^•$ [70]. The different fractions of *E. aphylla* exhibited different radical quenching activity against DPPH$^•$ radical (Table 1). Results indicated strongradical scavenging activity for ethyl acetate fraction towards DPPH$^•$ in comparison with ascorbic acid and quercetin (positive controls), while other fractions showed no scavenging activity at the same concentration. Alcohol fraction gives good scavenging activity starting from concentration of 500µg/ml.

For anti-inflammatory activity, different fractions of *E. aphylla* were evaluated using yeast-induced paw oedema method (Table 2). The hexane fraction exhibited a significant anti-inflammatory activityat

Table 1: Antioxidant activity of the different fractions of *Euphobiaaphylla*

Fraction/Compound	Concentrations (µg/ml)					
	10	25	50	100	250	500
	%Inhibition±SE					
Ascorbic acid	47.1±2.03%	66.3±1.79%	86.9±3.12%	98.8±1.54%	99.6±3.10%	N.T
Quercetin	45.0±2.95%	65.0±2.88%	85.0±3.62%	97.3±0.91%	99.1±3.22%	N.T
Hexane fraction	–	–	–	–	–	–
Chloroform fraction	–	–	–	–	–	–
Ethyl acetate fraction	27.7±1.32%	46.6±1.52%	71.7±1.43%	79.3±3.00%	89.4±1.76%	N.T.
Alcohol fraction	–	–	–	–	–	77.7±1.88%

N.T.=not tested, - = inactive

Table 2: Inhibitory effects of the different fractions of *Euphobiaaphyllaon* yeast-induced oedema in rats

Fraction/Compound	Dose (mg/kg)	Percentage of inhibition				
		1/2 h	1 h	2 h	3 h	4 h
Control (negative)	–	–	–	–	–	–
Indomethacin	15	0.9	1.4	9.0	12.6	14.8
Hexane fraction	400	5.0	12.8	24.1	40.0	41.7
Chloroform fraction	400	5.0	3.4	4.6	8.3	11.7
Ethyl acetate fraction	400	3.3	5.1	7.9	10.9	11.7
Alcohol fraction	400	5.0	6.9	7.9	8.3	10.0

Table 3: Antipyretic activity of the different fractions of *Euphobiaaphyllaon* yeast-induced pyrexia in rats

Fraction/compound	Dose (mg/kg)	Average rectal temperature (°C) ± S.E., n=5				
		1/2 h	1 h	2 h	3 h	4 h
Control (negative)	–	37.81 ± 0.0091	37.80 ± 0.0118	37.88 ± 0.0143	37.81 ± 0.0176	37.89 ± 0.0116
Indomethacin	8	37.40 ± 0.134*	37.08 ± 0.122***	37.00 ± 0.143***	37.00 ± 0.146***	36.98 ± 0.092***
Hexane fraction	400	37.97 ± 0.013	37.97 ±* 0.0177	37.20 ±** 0.0150	37.45 *± 0.0153	37.45 *± 0.012
Chloroform fraction	400	37.80 ± 0.0165	38.13 ± 0.0238	37.60* ± 0.0120	37.80 *± 0.0165	37.90 ± 0.0114
Ethyl acetate fraction	400	37.90 ± 0.0168	38.05 ± 0.0129	37.85* ± 0.0125	38.20 ± 0.0163	38.33 ± 0.0130
Alcohol fraction	400	37.87 ± 0.0163	37.87 ± 0.0188	37.75* ± 0.0114	38.30 ± 0.0153	38.17 ± 0.0124

SE: standard error, n=number of animals

Differences with respect to the control group were evaluated using the Student's *t*-test (*p<0.05, **p<0.01, ***p<0.001)

dose (400 mg/kg) which significantly reducedthe yeast-induced hind paw oedema in rats compared with indomethacinat dose 15mg/kg. Other fractions (chloroform, ethyl acetate, and alcohol) exhibited moderate to weak activity (Table 2).

For antipyretic activity, only hexane fraction showed moderate antipyretic activity after 2 h from pyrexia induction using yeast compared with indomethacin as positive control (8 mg/kg) (Table 3), while other fractions are inactive.

Table 4: Antimicrobial activity of the different fractions of *Euphobiaaphylla*

Organisms	Inhibition zone diameter IZD(mm/sample)					
	Hexane fraction	Chloroform fraction	EtOAcfraction	Alcoholfraction	Choramphenicol	Clotrimazole
Bacteria						
E.coli	12	0	14	16	27	–
Pseudomonasaeruginosa	9	0	13	12	14	–
Serratia marcescens	0	0	0	0	26	–
Staphylococcusaureus	10	8	16	0	23	–
Bacilluscereus	0	0	12	0	28	–
Micrococusluteus	0	0	13	13	22	–
Fungi						
Candidaalbicans	0	0	0	0	–	28
Geotrichumcandidum	0	0	13	0	–	22
Fusariumaxysporum	0	0	0	0	–	18
Scopulariopsisbrevicaulis	0	0	0	0	–	28
Trichophytonrubrum	0	0	0	0	–	34
Aspergillusflavus	0	0	0	0	–	26

- = Not determined

Antimicrobial activity for the different fractions was tested against gram +ve and –ve bacteria as well as fungi showing that the ethyl acetate fraction was the most active fraction, followed by alcohol,then hexane fractions as shown in Table 4. All fractions showed no activity against all of the tested fungal strains. The hexane fraction showed moderate activity against *Escherichia coli*, *Pseudomonas aeruginosa,* and *Staphylococcus aureus*, whilst the chloroform fraction only showed activity against *Staphylococcus aureus*. The ethyl acetate fraction showed good activity against *Pseudomonas aeruginosa* which is nearly similar to the chloramphenicol (antibacterial standard). All fractions showed no activity against *Serratiamarcescens*. The alcohol fraction is the only one showingno activity against *Staphylococcus aureus* (Table 4).

4. Discussion

Nine known compounds including sterols, triterpenoids, flavonoids, and tannins were isolated from the ethanolic extraction of the aerial part of *E.aphylla*. This study is considered as the first report of these compounds from *E. aphylla* which could be helpful and can contribute in the chemotaxonomic analysis of this complex genus. The different biological assays for the different fractions exhibited that the ethyl acetate fraction showed strong antioxidant activity with moderate anti-inflammatory effect, while the strong anti-inflammatory activity was observed with the hexane fraction.The antioxidant activity of ethyl acetate fraction may be attributed to the presence of flavonoids (quercetin derivatives) as well as gallic and ellagic acid derivatives [33,71–73]. The observed anti-inflammatory activity of the hexane fraction could be attributed to the presence of triterpenes. Euphol, the most predominant triterpene alcohol constituent, is exhibiting strong anti-inflammatory activity [28]. The moderate antipyretic activity of hexane fraction may be attributed to its strong anti-inflammatory effect.

The antimicrobial activity for all fraction showed that the ethyl acetate was the most active fraction against gram +ve and gram –ve bacteria, followed by alcohol, then hexane fraction. None of the tested fractions showed activity against fungi.The antimicrobial activity of fractions is attributed to phenolics and terpens contents [74].

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