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Cytotoxic And Antioxidant Activities Of *Euphorbia Balsamifera* With A New Phenolic Compound.

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ABSTRACT

Cytotoxic and antioxidant activities of the aerial part of Euphorbia balsamifera were evaluated to isolated the most active compounds. The highest antioxidant activity was observed for total ethanol extract and ethyl acetate fraction with IC_{50} of 20 µg/ml and 31.25 µg/ml respectively. The cytotoxic activity for the plant fractions against three human cancer cell lines, HePG2, HCT116 and MCF7 exhibited to significant activity with low cytotoxicity against normal cell. The highest activity was observed for chloroform fraction against HePG2 with LC_{50} of 62.5 µg/ml while hexane fraction exhibited to LC_{50} of 59.7 µg/ml against MCF7 where the latex showed the lowest activity against MCF7 with LC_{50} of 89.6. Isolation and structure determination from ethyl acetate leads to the identification of a new phenolic compound, 4-hydroxyphenethyl 4-hydroxybutanoate (**1**) along with three known secondary metabolites, 3β-cycloartanol (**2**), 1-(2,4-dihydroxy-6-methoxyphenyl) ethanone (**3**), and glutanol (**4**) for the first time from Euphorbia balsamifera. Structure elucidation was accomplished mainly by NMR spectroscopic and mass spectrometric methods.

Keywords: Antioxidant; Cytotoxic; Euphorbia balsamifera; Asir region.

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INTRODUCTION

Natural product compounds showed a broad spectrum of bioactivities as anticancer agents with low side effects [1, 2]. Medicinal plants are rich sources of bioactive compounds [3, 4] with more than 60% of the common anticancer drugs [5]. Natural antioxidant agents have great interest due to their activity to suppress cancers through decreasing oxidative stress and playing a significant role in a number of pathogenic diseases [6, 7]. Euphorbia balsamifera belongs to family Euphorbiaceae which characterized by a broad spectrum of medicinal properties [8, 9]. The latex of Euphorbia balsamifera is effective as a pulpal devitalizing agent [10] and the plant leaves, stems and roots showed antimicrobial activities against some pathogenic microorganisms [11]. Wound healing activity was observed for methanol extract of the plant which is identical with the standard drug Povidone-iodine [12]. Phytochemical screening revealed the presence of different classes of secondary metabolites but no reports on their isolation and structure determination. Due to the south area of Saudi Arabia "Asir" is characterized by a unique habitant with expected new chemical structures and characteristic bioactivities of the plant constituents. Therefore, the present investigation aims to evaluate the cytotoxicity and antioxidant activities of E. balsamifera fractions and identify the major secondary metabolites.

MATERIALS AND METHODS

General Material

NMR spectra (^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H,^{1}H-COSY, HMBC and HSQC) were recorded using a Bruker 850.150 NMR. Operating frequencies were 850.15 MHz for acquiring ^{1}H-NMR and 213.77 MHz for ^{13}C-NMR spectra. Liquid chromatography electrospray ionization mass spectra (LCESIMS) (positive mode) were measured with Bruker's unique UHR-QqTOF (Ultra-High Resolution Qq-Time-Of-Flight) mass spectrometry with > 50,000 Full-Sensitivity Resolution (FSR). Column chromatography (CC): silica gel (40-0.063±0.2 µm, Merck, Darmstadt, Germany). Thin layer chromatography (TLC): silica gel (0.25 and 1 mm precoated plates 60 F254, Merck. Sephadex LH-20 (Sigma-Aldrich, Germany).

Plant Material

The aerial parts Euphorbia balsamifera was collected from Abha, Aseer region in the road of Khamis - Najran, Saudi Arabia, in February 2017. The plant was identified by Biology department, College of Science, King Khalid University. A plant sample had been deposited in the Herbarium of Botany department, college of science under the number of Eb02017.

Extraction and isolation

Dry samples of the aerial parts of Euphorbia balsamifera plant (2.20 kg) placed in a closed container with the extraction solvent at room temperature from about 24 to 48 hours until the soluble matter is dissolved. Then grinding in a powerful mixer to give a homogeneous mass. Plants were extracted firstly with hexane, ethyl acetate, chloroform, methanol/H_2O, respectively. The mixture was filtered by Buchner funnel to provide the total extracts. The total extract was concentrated by Rotavapor- R20 at 40-45ºC to Remove the solvent and obtain the crude extracts.

The ethyl acetate fraction (14.0 g) was applied to column chromatography on silica gel and eluted with hexane/ethyl acetate, chloroform, chloroform/methanol. The eluted fractions were monitored with TLC using different solvent systems: hexane/ethyl acetate (9:1),(8:2),(7:3) and (1:1), chloroform, chloroform/methanol (9:1), (8:2) and (7:3). TLC plates were sprayed with 5% sulphuric acid/methanol. The fractions eluted with hexane/ethyl acetate (9:1) afforded two sub-fractions **I** (159.5 mg) &**II** (324.5 mg). Fraction **I**showed major compounds which further purified on SPLH-20 column eluted with chloroform/methanol (1:1) to afford semi-pure compound **4** (12.2 mg). Fraction **II** showed two major compounds which further purified on a silica column eluted with hexane/ethyl acetate (9:1) to afford compounds **1** and **2** in mixture (10.4 mg) and pure compound **4** (16.4 mg). The fractions eluted with hexane/ethyl acetate (7:3) afforded two subfraction. The first fraction (389.7 mg) showed two major compounds which further purified onSPLH-20 and eluted with chloroform/methanol (1:1) to afford pure compound **3** (19.0 mg).Figure **1** showed the structure of isolated compounds from E. balsamifera.

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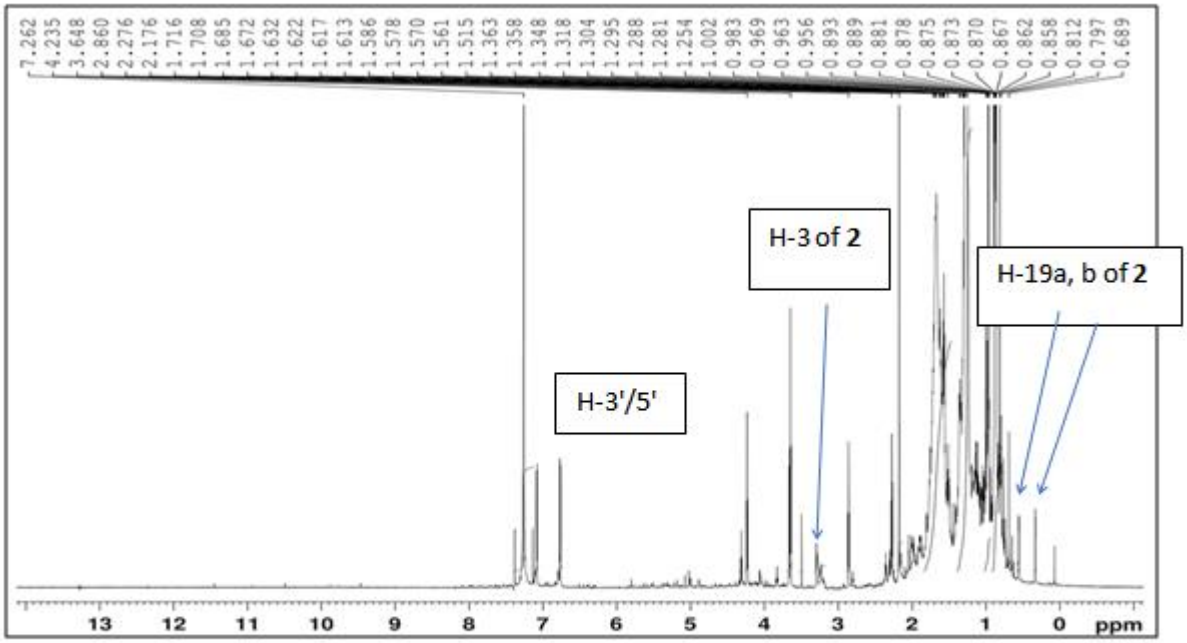
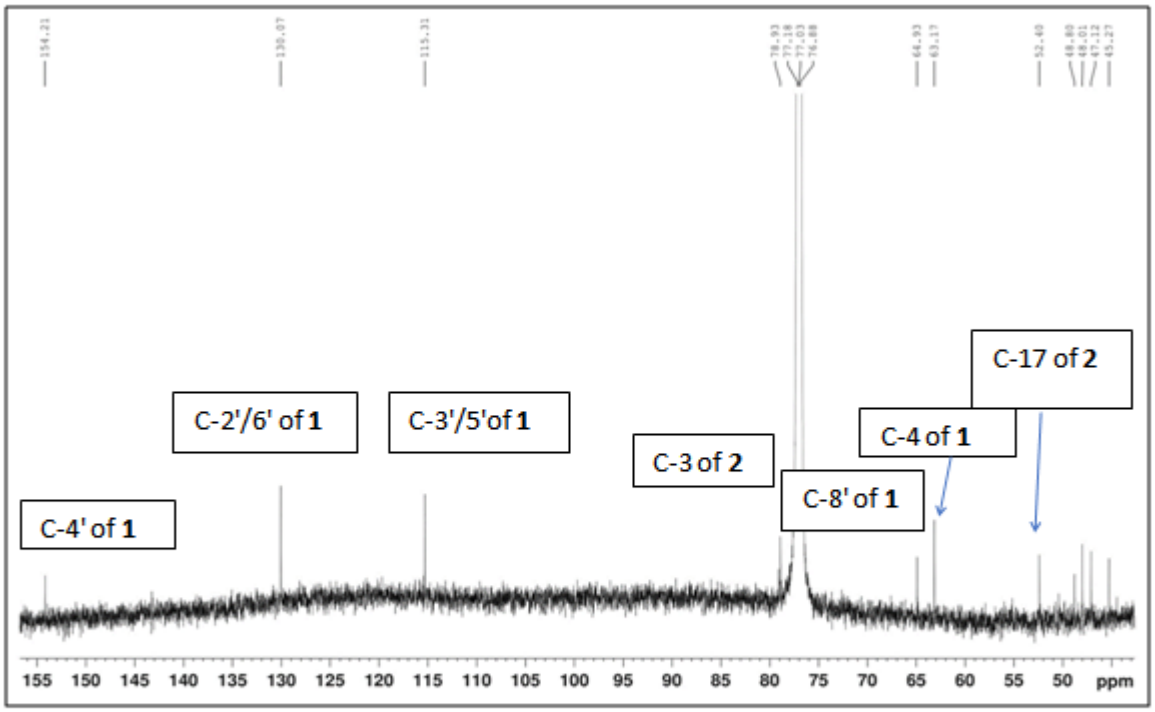


S1: ^{1}H-NMR (850.15 MHz, $CDCl_3$) Spectrum of Compound 1& 2



S2: Selected ^{13}C-NMR (213.76 MHz, $CDCl_3$) Spectrum of Compound 1 & 2

Spectroscopic data of a new compound

4-hydroxyphenethyl 4-hydroxybutanoate (**1**),HRESIMS m/z: 225.1121, ($[M+H]^+$,$C_{12}H_{16}O_4$, calcd. 225.1172) ; ^{1}H and ^{13}C NMR (Table 1).

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Table 1: ^{1}H NMR and ^{13}C-NMR data for compound 1 (at 800 MHz in $CDCl_3$, δ in ppm, *J* in Hz).

Position	δ_H(*J* in Hz)	δ_C
1		173.5
2	2.28 (2H, *t*, *J* = 6.0)	34.3
3	1.59 (2H, *m*)	24.9
4	3.64 (2H,*t*, *J* = 6.0)	63.2
1′		130.8
2′	7.08 (1H,*d*, *J* = 7.7)	130.1
3′	6.76 (1H,*d*, *J* = 7.7)	115.3
4′		154.6
5′	6.76 (1H,*d*, *J* = 7.7)	115.3
6′	7.08 (1H,*d*, *J* = 7.7)	130.1
7′	2.86 (2H,*t*, *J* = 6.8)	34.7
8′	4.23 (2H, *t*, *J* = 6.8)	65.0

Determination of DPPH Free Radical Scavenging Activity

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of each fraction was evaluated. 1 ml of methanolic solution of varying concentration samples (25, 50 and 100 µg/ml) were added to 1 ml of methanol solution of DPPH (60µM). The prepared solutions were mixed and left for 30 min at room temperature. The optical density was measured at 520 nm using a spectrophotometer (UV-1650PC Shimadzu, Japan). The percent scavenging effect was determined by comparing the absorbance of solution containing the test sample to that of control solution without the test sample taking the corresponding blanks. Mean of three measurements for each compound was calculated according to the method of Matsushige et al. [13].

Cytotoxicity Assays

Cytotoxicity of the plant extracts was assayed against three human cancer cell lines namely HCT116 [Colon cell line], HePG-2 [Human hepatocellular carcinoma cell line], MCF7 [Human Caucasian breast adenocarcinoma], beside one normal epithelium cell line namely RPE1[normal retina cell line] by using MTT assay. (Cell viability was assessed by the mitochondrial-dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan. The cultivation of the cell lines and the cytotoxicity assays were performed as described by Mosmann et al. [14]. This cytotoxic activity test (In vitro bioassay on human tumor cell lines) was conducted and determined by the Bioassay-Cell Culture Laboratory, National Research Centre, El-Tahrir St., Dokki, Cairo 12622, Egypt.

RESULTS AND DISCUSSIONS

DPPH radical scavenging assay of extracts

Radical scavenging activity of plant extracts against stable DPPH was determined spectrophotometrically(Figure 2)at 517 nm by Matsushige et al. [13]. Higher level of antioxidant activity was observed in the ethanol and ethyl acetate when compared with other tested fractions where total ethanol extract showed IC_{50} of 20 µg/ml while ethyl acetate showed IC_{50} of 31.25 µg/ml**.**

Cytotoxic activities

The plant fractions were evaluated in vitro cytotoxic activity against three human cancer cell lines HepG2, HCT116 and MCF7. Interesting result was observer for chloroform fraction against HepG2, HCT116 and MCF7 with LC_{50} of 62.5 µg/ml, 66.1 µg/ml and 69.3 µg/ml respectively while its cytotoxicity against normal retina cell line RPE1was 21% at concentration of 100 ppm. While hexane fraction showed LC_{50} of 73.7 µg/ml, 93.1 µg/ml, 59.7 µg/ml with less value against the normal retina cell line, 18.6% at 100 ppm. The latex showed the lowest activity on MCF7 only with LC_{50} of 89.6(Table 2).No significant activities for other plant fractions. These findings could be important for evaluating the mode of action of hexane and chloroform fractions and their compounds in vivo which is proceeding for the next study.

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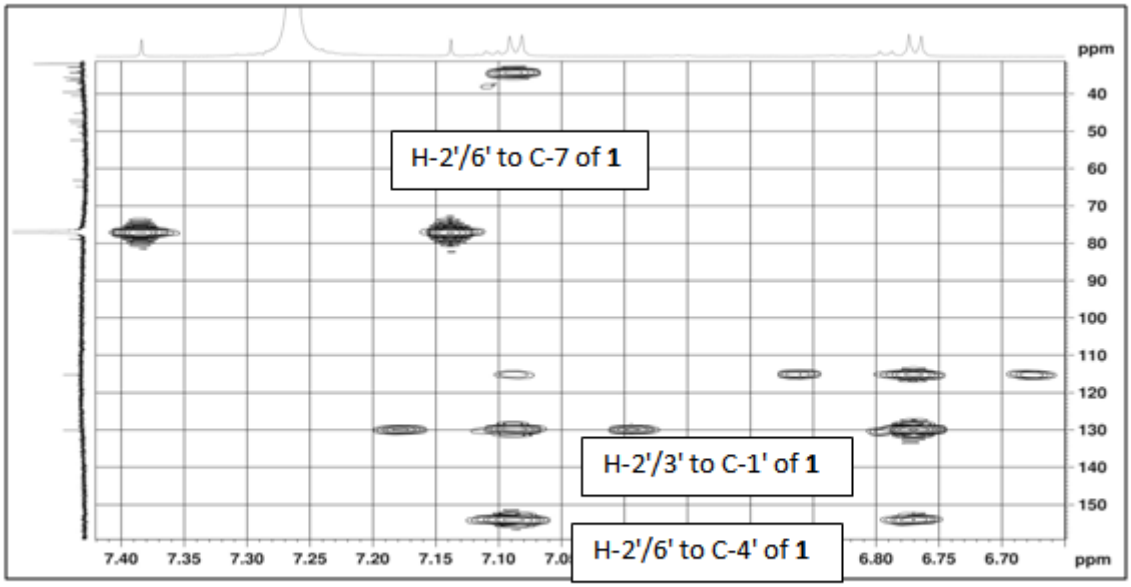
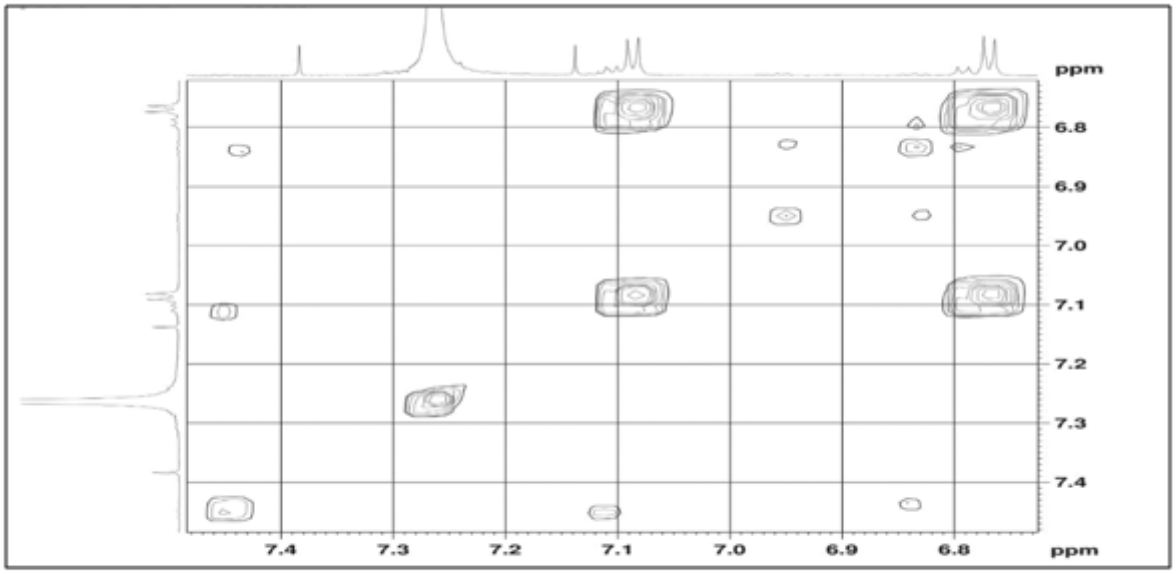


S3: Selected HMBC Spectrum of Compound 1&2



S4: Selected COSY Spectrum of Compound 1&2

Table 2: Cytotoxic activities of the plant fractions against three cancer cell lines, LC_{50}, LC_{90} (µg/ml)

	HePG2			HCT116			MCF7		
	LC_{50}	LC_{90}	100ppm	LC_{50}	LC_{90}	100ppm	LC_{50}	LC_{90}	100ppm
Hexane	73.7	110.7	78.3	93.1	146.8	50.6	59.7	108.5	76.3
Chloroform	62.5	94.4	90.5	66.1	109.2	75.3	69.3	117.9	71.2
Latex			25.4			45.3	89.6	145	53.4
Ethanol			36.5			31.2			18.7

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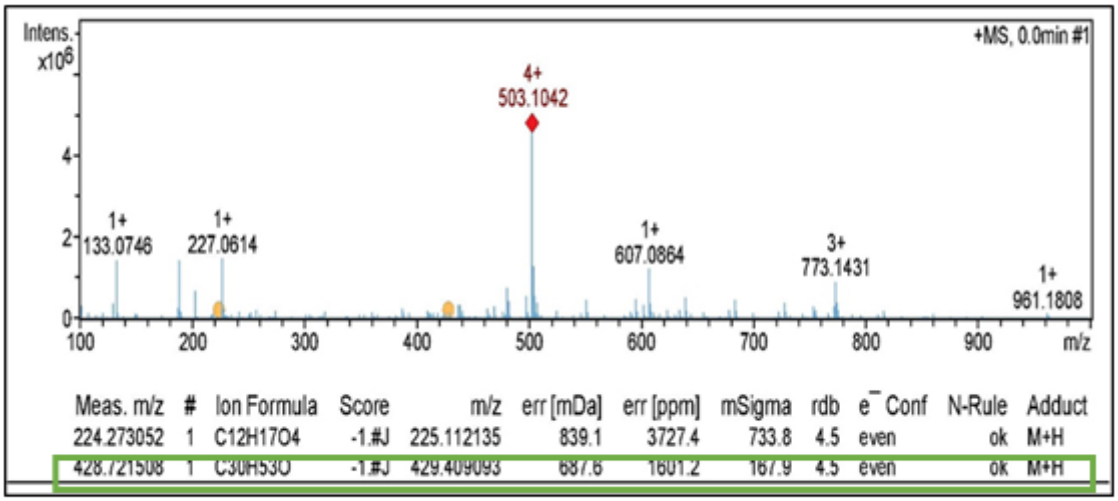
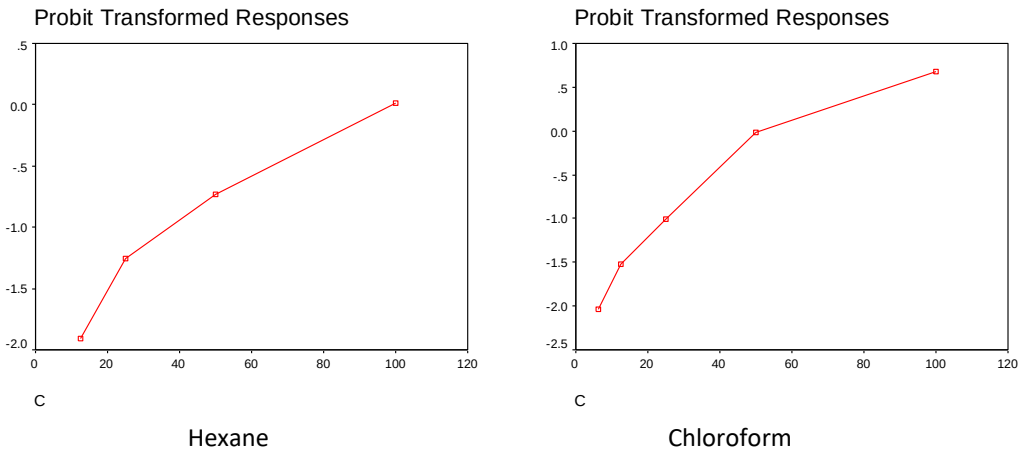
Methanol			**20.1**			**22.4**			**12.4**
Ethylacetate			**71.3**			**51.8**			**21.8**

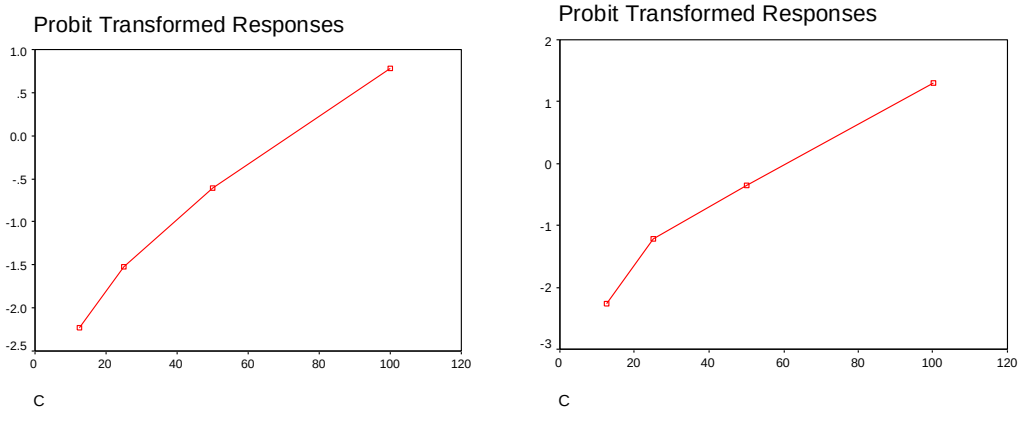
LC_{50}: Lethal concentration of the sample which causes the death of 50% of cells in 48 hrs
LC_{90}: Lethal concentration of the sample which causes the death of 90% of cells in 48 hrs



S5: LCHRESI-MS Spectrum of Compound 1&2



S6: Probit transformed responses of hexane and chloroform fractions againstHCT116



S7: Probit transformed responses of hexane and chloroform extracts against HePG2

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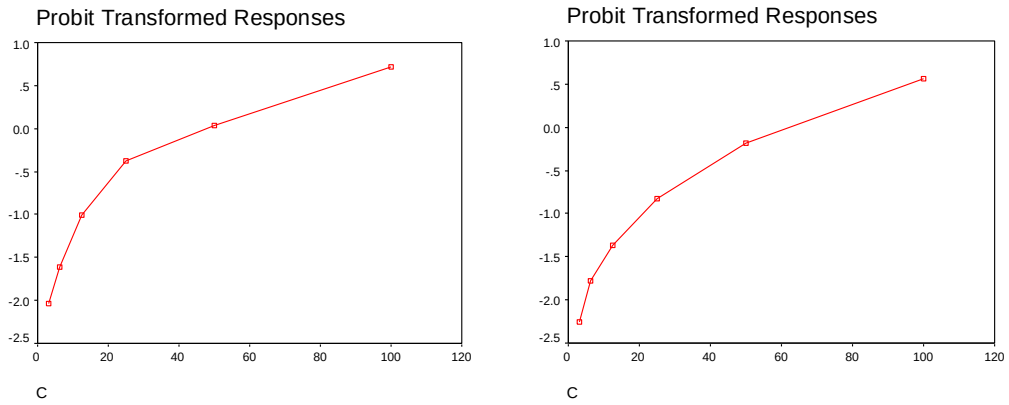
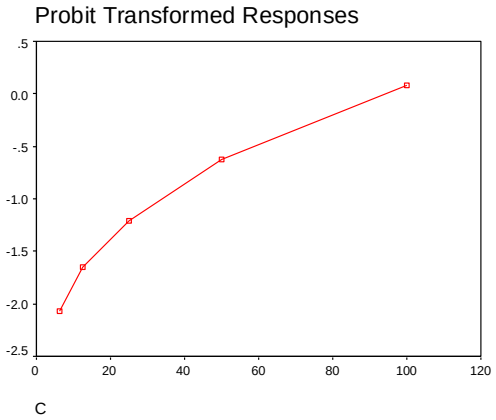


S8: Probit transformed responses of hexane and chloroform extracts against MCF



S9: Probit transformed responses of the latex against MCF

Structure determination

^{1}H-NMR spectrum of **1/2** showed a mixture of phenolic compound **1** and triterpene moiety **2** (1:1) which confirmed from ^{1}H-^{1}H COSY, HSQC and HMBC connectivity. The phenolic compound **1** exhibited to aromatic signals of AA'XX' spin system of a p-substituted phenyl ring at δ 7.08 and 6.76, d, (J = 7.7 Hz) for H-2'/6' and H-3'/5' with corresponding ^{13}C-signals at δ 130.1 and 115.3 respectively. HMBC $^{2}J_{H\text{-}C}$and $^{3}J_{H\text{-}C}$showed a correlation of H-2'/6' to C-7' at δ 34.4, the other correlations were observed between 2H-8' to C-1' and carbonyl carbon, as well as 2H-2, 2H-3 to carbonyl at δ 173.0 (Fig. 3). ^{1}H-^{1}H COSY confirmed the connectivity of methylene protons from 2H-2 to 2H-4 and 2H-7' with 2H-8'. The LCESIMS positive mode exhibited to ion at m/z 225.1121 corresponding to molecular formula of $C_{12}H_{16}O_4$. Based on the above finding, compound **1** was identified as: 4-hydroxyphenethyl 4-hydroxybutanoate which is a new natural product.^{1}H-NMR of triterpene moiety **2** showed two doublet signals at δ 0.33 and 0.55 (J ≈ 4.0 Hz) with corresponding carbon signal at δ 29.7 through HSQC which characteristic for methylene protons of cyclopropane to suppose the presence of 9,19 methylene moiety. A complete assignments of ^{1}H and ^{13}C-NMR chemical shifts by means of ^{1}H,^{1}H-COSY, HSQC and HMBC spectra, the triterpene was identified as cycloartanol. LCESIMS confirmed the supposed structure by ion peak at m/z 429.4050, $[M+H]^{+}$ with corresponding molecular formula of $C_{30}H_{53}O$. Comparison of ^{13}C-data with the published date of cycloartanol showed agreement with the suggested structure [15].

Other compounds were identified by extensive analysis of ^{1}H and ^{13}C-NMR and 2D-NMR (^{1}H-^{1}H COSY, HSQC, HMBC) spectroscopy and mass spectrometry where compound **3** was identified as 1-(2,4-dihydroxy-6-methoxyphenyl)ethanone which was previously isolated from Euphorbia sikkimensis [16] and compound **4** as glutanol [17].

CONCLUSION

Chloroform and n-hexane fractions of Euphorbia balsamifera exhibited to interesting cytotoxic activities against three cancer cell lines, HepG2, HCT116 and MCF7 with low cytotoxicity against normal cell line.Ethanol 80% extract and Ethyl acetate fraction showed the highest antioxidant activities. A new phenolic compound,4-hydroxyphenethyl 4-hydroxybutanoate along with three metabolites were identified which confirm that Asir region of Saudi Arabia is characterized with a new chemical structure.

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