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In vitro Cytotoxicity of *Euphorbia heterophylla* Against Human Cancer Cell Lines

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ABSTRACT

Euphorbia heterophylla (EH) is a well-known Africa medicinal plant, which has been used in the African traditional medicine for the treatment of various human ailments including tumours and cancers. Previous phytochemical screening of *E. hetrophylla* has detected many flavonoids, terpenoids and sterols (Okeniyi *et al*., 2012: Silva *et al*., 2019). Seven compounds were isolated from the different fractions of EH including three biflavonoids (Cupressuflavone, Quecertin and Quercitrin) and four diterpenes (13-*epi*cupressic acid, imbricatholic acid, 3α-hydroxy sandaracopimaric acid and β− hydroxysandaracopimaric acid). All the compounds were tested for their cytotoxicity against the A549 cell lines and compared with the anticancer standard etoposide. Cupressuflavone and 13-epicupressic acid showed the highest cytotoxicity against human lung cancer cells lines (A549) with IC_{50} values of 58 and 73µM respectively. The current findings support the traditional use of EH for the treatment of tumours and other cancer related diseases. It is suggested that the cytotoxic activity revealed in the polar MeOH fractions was due to the presence of cytotoxic bioflavonoids and the cytotoxicity observed with the non-polar fractions of *E. heterophylla* root were contributed by various cytotoxic terpenes.

Keywords: *Euphorbia heterophylla,* bioflavonoids, terpenoids, Cupressuflavone, 13-*epi*cupressic acid, Cytotoxicity

Introduction

Euphorbia heterophylla is a toxic plant belonging to the family of Euphorbiaceae. (English names: Mexican fire plant, milk weed and Spurge weed). The toxicity of the plant, especially the root and latex is recognized in Africa. The family (*Euphorbiaceae*) is currently more valued for its many ornamental species and is the most important family in tropical and sub-tropical area for phyto medicines of human, veterinary or pesticides nature (Mwine and VanDamme, 2010). *E. heterophylla* has been used in the Nigerian traditional medicine for the treatment of various human ailments including tumours and cancers. *E. heterophylla* is used in folk medicine to treat gonorrhoea, migraine, wart, asthma, malaria, eczema, asthma, pneumonia, rheumatism, tumour gout, diarrhoea, and urinary tract diseases (*Odugbemi, 2008) (Omale and Emmanuel, 2010). (Erden et al., 1999).* Previous phytochemical screening of *E. heterophylla* has detected many alkaloids, glycosides, tannins, phenol, steroids, flavonoids, lignans, terpenoids and sterols (*Adedoyin et al., 2013*). Considering the above medicinal uses, the *E. heterophylla* root extract were chemically examine for their constituents against human cancer cell lines details reported in this paper.

Materials and Methods

***Plant Material*:** The root of *E. heterophylla* was collected from Afaka village, Kaduna and voucher specimen deposited in the Herbarium, Department of Biological Science, Ahmadu Bello University Zaria. Nigeria.

Extraction and Isolation:

Extraction of plant Material: The ground root of *E. heterophylla* (EH) (86.5 g) were extracted by Soxhlet apparatus sequentially, with n-hexane, DCM and MeOH (900 mL each). The extracts were filtered and evaporated to dryness in a rotary evaporator at a temperature not exceeding 45°C and finally stored at 4°C. *Fractionation processes*: The crude extracts of *E. heterophylla* root were separated into various discrete fractions using vacuum liquid chromatography (VLC) on silica gel for the n-hexane and DCM extracts, and solid-phase extraction (SPE) (Strata reversed 20 g Cartridge) on reversed-phase C18 silica for the MeOH extract (Sarker & Nahar., 2012). *Isolation of compounds*: Portions of the MeOH fraction F3 and F4 of (EH) were separated by semi-preparative and preparative reversed-phase HPLC (Agilent) whilst the n-hexane and the DCM fractions were separated by preparative thin layer chromatography (PTLC). The MeOH extract yielded 5 compounds and DCM fractions yielded 3compounds.

Experimental procedures:

The ^{1}H and ^{13}C NMR spectra were recorded in dimethyl sulfoxide (DMSO-d^{6}) on BRUKER NMR AVANCE spectrometer operating at 600 MHz. A volume of 20 µL of tetramethylsilane (TMS) was added as the internal reference. The NMR spectra of ^{13}C were recorded with 2J modulated sequence, and can distinguish peer protons (quaternary and –CH2) from the even down to the odd (CH_3; –CH–). Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) expressed in δ unit. Fourier's transformed infrared (FTIR) transmission Spectra was carried out through a BRUCKER VERTEX 70® spectrometer coupled to a Hyperion® microscope. All samples were scanned using Platinum diamond ATR (Attenuated Total Reflectance) in the wavenumber region between 4000 and 400 cm^{-1} with a resolution of 4 cm^{-1}. At each position 16 scans were averaged. Gas Chromatography-Mass

spectrometry (GC-MS) analyses of the fractions were recorded on a gas chromatograph coupled to the polarized mass spectrometer; the mass spectral data were recorded with electron impact ionization at 70 eV. Gas chromatograph of the type Trace GC Ultra in mode Split, equipped with a flame ionization detector (FID). The temperature of ionization was fixed at 200°C. The column used is a capillary column DB-5 (30m x 0.32 mm ID; 1µm film thickness (Agilent Technologies, J&W Scientific Products, USA connected with a database of NIST6 Main-Mass).

Cytotoxicity assay:

In the present study, the cytotoxicity the isolated compounds of (EH) were evaluated against five human cancer cell lines: EJ138 (human bladder carcinoma), HepG2 (human liver hepatocellular carcinoma), A549 (human lung carcinoma), MCF7 (human breast adenocarcinoma) and PC3 (human prostate carcinoma) using the MTT assay (Mossman, 1983). The cells were washed by phosphate buffer saline (PBS), harvested by trypsinization, then were plated in 96-well plates and incubated under 5% CO_2 at 37ºC for 24 hours. The cells were treated with different concentrations of different extracts, fractions and isolated pure compounds. Dilution of stock solutions was made in culture medium yielding final extracts concentrations with a final DMSO concentration of 0.1%. All concentrations of samples were in triplicates on the same cell batch, growth of cells was quantitated by the ability of living cells to reduce the yellow dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to a blue formazan product. After 24 hours' incubation. The medium in each well was replaced by MTT solution and incubated for 2 hours. MTT reagent was removed and the formazan crystals produced by viable cells were dissolved in iso-propanol and gently shaken. The absorbance was then determined by plate reader at 560 nm.

Statistical analysis

All experiments were carried out in triplicate on separate occasions. Data were expressed as means ± standard error of the mean.

Spectroscopic characterization

IR, ^{1}H NMR, ^{13}C NMR and GCMS spectroscopic methods were used to elucidate the structure of the isolated compounds. The IR spectrum was recorded on FTIR-6890s (Agilent technologies) and the NMR spectra were recorded on a Bruker AVANCE-300 Japan (100 MHz and 400 MHz) in MeOD with TMS as internal standard.

Results and Discussion

Results

The ^{1}H and ^{13}C-NMR data used for the identification of compounds 1-7 are presented in Tables 1and 2. Cytotoxicity effects of the isolated compounds on human cancer cell lines are presented in table 3 respectively. **13 − Epicupressic acid (1).** M.P. 105-106 °C. $CHCl_3$. IR ν max (KBr): 3510, 3090, 1720, 1645, 1250, 1227, 1150, 992, 925 and 877 cm-^{1}. ^{1}H- and ^{13}C-NMR ($CDCl_3$): Table 1. ESIMS: 343 (100, $[M + Na]^+$), $C_{20}H_{32}O_3$**. Imbricatolic acid (2).** IR ν max (kBr): 3500, 3000, 2934, 2873, 1740, 1694, 1645, 1240 and 890 cm-^{1}. ^{1}H- and ^{13}C-NMR ($CDCl_3$): Table 1. ESIMS: 345 (100, $[M + Na]^+$), calc. for $C_{20}H_{34}O_3$. **7 α− Hydroxysandaracopimaric acid (3). M.P. 132-134 °C**. IR ν max (KBr): 3400, 3070, 1720,

1640, 1245, 995 and 905 cm-1. ^{1}H- and ^{13}C-NMR (CD_3OD): Table 1. ESIMS: 341 (100, $[M + Na]^+$), 357 (45, $[M + K]^+$), calc. for $C_{20}H_{30}O_3$. **β− Hydroxysandaracopimaric acid (4): M.P. 249-251°C**. IR ν max (KBr): 3495, 3280, 2550, 1696, 1085, 988, 910 and 858 cm^{-1}; ^{1}H- and ^{13}C-NMR ($CDCl_3$): Table 1. ESIMS (m/z 341 $[M + Na]^+$ and 357 (100, $[M + K]^+$), calc. for $C_{20}H_{30}O_3$. **Cupressuflavone (5). M.P. 392** °C UV λmax (MeOH) 329, 276 and 226 nm. IR ν max (KBr): 3440, 1640, 1610, 1580 and 1165 cm-1. ^{1}H- and ^{13}C-NMR (DMSO-d6): Table 2. ESIMS: 539 (100, [M + 1]+), 561 (74, $[M+Na]^{1+}$), 577 (32, $[M+K]^+$), calc. for $C_{30}H_{18}O_{10}$. **5,7,3′,4′-pentahydroxyflavone, quercetin (6) M.P 313-315.** UV λmax (MeOH) 373 nm and 256 nm IR ν max (KBr): 3624, 3473, 3295, 1616, 1458 and 1165 cm^{-1} ^{1}H- and ^{13}C-NMR (DMSO-d6): Table 2. ESI-MS *m/z*: 301.29 $[M-H]^-$. calc. for $C_{15}H_{10}O_7$ δ 5.1. S, 6.20 and 6.40 , 6.89 and 6.91, 7.59 to 7.66 175.92, 164.14, 161.08, 156.80 and 158.30, 147.35 and 146.60, 135.85 , 122.73, 114.82, 114.58 ,103.11, 97.83 and 93.06ppm**. Quercitrin (7) M.P 181-182,** UV λmax (MeOH) 240, 280 and 340nm, IR ν max (KBr): 3417.00 , 2973.92 , 1371.50, 1465.13, 1700, 1415.31, 1298.31 and 1076.82 cm -1^{1}H- and ^{13}C-NMR (DMSO-d6): Table 2. ESI-MS m/z: 447.77 $[M-H]^-$. calc. for $C_{21}H_{20}O_{11.}$ δ 7.57, 7.53, and 6.7, 6.29, 6.10, 5.01, 4.22-3.14 (sugarH), 158.1, 136.0, 179.3,159.1 99.5, 165.8, 94.3, C9,163.0; C10,105.5; C1 122.5 ,′ ; C2 116.2 ,′ ; C3 146.2 ,′ ; C4 149.5 ,′ ; C5 116.7 ,′ ; C6 122.7 103.4, 71.8 to 73.1.

Table 1: ^{1}H and ^{13}C- NMR Chemical Shift Values of Compounds 1-4 Diterpenes

Position	Compound 1		Compound 2		Compound 3		Compound4	
	^{1}H	^{13} C	^{1}H	^{13} C	^{1}H	^{13} C	^{1}H	^{13} C
1	1.10 (brd, 1.50), 1.78 (m)	39.1	1.07 (m), 1.86 (m)	39.2	1.22 (m), 1.77 (m)	39.6	1.05 (m), 1.25 (m)	38.3
2	1.43 (m), 1.76 (m)	19.8	1.53 (m), 1.86 (m)	19.8	1.45 (m), 1.61 (m)	19.4	1.37(m), 1.42 (m)	19.7
3	1.05,(dd; 4.0;12.5) 2.17 (brd;12.8)	37.7	1.06, (dd;5.0;13.0) 2.18 (brd;13.2)	38.2	1.63 (m), 1.92 (m)	38.9	4.04 (dd;4.4, 11.3)	76.3
4		44.4	-	44.4	-	48.2	-	54.8
5	1.25 (m)	57.4	-	56.4	2.55 (dd;2.5,13.32)	43.4	1.52 (m)	51.7
6	1.79 (m), 1.89 (m)	26.3	1.33 (m)	26.2	1.46 (m), 1.71 (m)	33.2	1.00 (m), 1.32 (m)	25.5
7	1.85 (m), 2.51 (brd; 9.5)	38.8	1.91 (m); 1.99 (m)	38.9	4.12 (brs)	73.9	1.85 (m), 2.31 (m)	36.7
8	-	147.7	-	147.5	-	139.7	-	137. 5

9	1.46 (m)	56.6	1.93 (m), 2.45 (dd;2.5, 8.5)	56.7	2.22 (dd; 5.9, 12.3)	47.5	1.62 (m)	51.8
10		41.6	-	41.6	-	39.0	-	38.5
11	1.24 (m), 148 (m)	18.0	-	21.3	1.43 (m), 1.60 (m)	19.2	1.39 (m), 1.44 (m)	27.7
12	1.33 (m), 1.62 (m)	41.5	1.55 (m)	36.5	1.47(m), 1.49(m)	35.5	1.16 (m), 1.25(m)	35.8
13		73.7	-	30.5	-	38.4	-	38.4
14	5.90 (dd;11.1, 17.8)	145.2	-	39.8	5.53(s)	135.0	5.23 (s)	130.3
15	5.06 (dd;1.3,.11.0) 5.23 (dd; 1.1 17.5)	111.9	1.26 (m), 1.53 (m)	61.5	5.80 (dd;10.4, 17.7)	149.3	5.77 (dd;11.2, 18.0)	149.8
16	1.23 (s)	28.3	0.97 (m) 1.46 (m)	19.8	4.93 (d, 10.6) 5.00 (d, 17.5)	11.2	4.63(d, 11. 1) 4.68 (d, 18.1)	110.6
17	4.52(s), 4.84 (s)	107.0	1.54 (m)	106.8	1.08 (s)	26.4	1.05(s)	26.7
18	1.17 (s)	29.0	1.35 (m); 1.66 (m)	29.0	-	182.6	-	182.3
19	-	183.5	3.65(m), 3.370 (m)	183.5	1.18 (s)	17.5	1.15 (s)	11.8
20	0.63 (s)	12.9	0.90 (d; 6.5)	13.0	0.85 (s)	15.2	0.87 (s)	15.8

Table 2 : ^{1}H and ^{13}C- NMR Chemical Shift Values of Compounds 5-7 Flavonoids

Position	**Compound 5**				**Compound 6**		**Compound 7**	
	^{1}H		**^{13} C**		**^{1}H**	**^{13} C**	**^{1}H**	**^{13} C**
1								
2			163.1	163.1		147.26		155.90
3	6.81 (s),	6.81 (s),	103.1	103.1		136.13		135.61
4			182.6	182.6		176.25		178.20

5	13.1 (s) (OH)	13.1 (s) (OH)	161.6	161.6	12.37br.s d(6.8)	160.11	12.66s	161.70
6	6.48 (s)	6.48 (s)	99.2	99.2	6.19 d(2.1)	98.66	6.22 brs	99.20
7	10.9 (s) (OH)	10.9 (s) (OH)	164.3	164.3	10.80 s	164.33		164.66
8			98.7	98.7	6.45 d(2.1)	93.83	6.41br	94.21
9			155.3	155.3		156.50		157.30
10			104.2	104.2		103.44		104.55
1’			121.6	121.6		122.41		121.23
2’	7.51 (dd; 2.0, 8.6)	7.51 (dd; 2.0, 8.6)	128.5	128.5	7.67 d(2.3)	115.45	7.31d(1.5)	115.92
3’	6.77 (dd; 2.0, 8.6)	6.77 (dd; 2.0, 8.6)	116.3	116.3	9.34s	145.46		145.68
4’	10.2 (s) (OH)	10.2 (s) (OH)	161.5	161.5	9.69s	148.13		148.87
5’	6.78 (dd ;2.1, 8.7)	6.78 (dd ;2.1, 8.7)	11.3	11.3	6.88 d(8.4)	116.06	6.86d(8.2)	116.10
6’	7.51 (dd ; 2.1,8.7)	7.51 (dd ; 2.1, 8.7)	128.5	128.5	7.53 dd (8.5, 2.2)	121.50	7.13 d (8.3)	121.34
1’’							5.25s	101.34
2’’							3.89 dd	72.53
3’’							3.43dd	71.05
4’’							3.17dd	70.68
5’’							3.22dd	70.83
6’							0.8d (6.0)	17.89

Table 3: Cell growth inhibitory activities of the isolated compounds against cancer cells *

IC_{50} (µg/mL)					
Compounds µg/mL	**EJ138**	**HepG2**	**A549**	**MCF7**	**PC3**
1	>200	>200	157.4± 3.24	139.1 ± 2.14	>200
2	>200	>200	>200	173.5 ± 4.34	>200
3	173.3± 2.37	>200	>200	>250	>250

4	182.2± 1.18	>200	>200	>200	>250
5	>200	>200	67.4± 2.45	111.7 ± 3.75	>200
6	>200	>250	135.8± 7.41	117.4± 3.71	>200
7	>250	>200	138.1 ± 4.62	105. 3 ± 6.19	>200

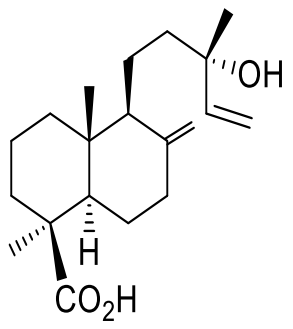
* Data are represented as mean ± SEM (n = 3); IC_{50} = Sample concentration responsible for cell growth inhibition,

Discussion

Owing to the high medicinal values of *Euphorbia heterophylla* plant, intensive investigations lead to isolation of some classes of secondary metabolites such as flavonoids and diterpenes. In this study, seven compounds were isolated from the different fractions of EH, The compounds were identified as 13-epicupressic acid **(1)** imbricatholic acid **(2)** α-hydroxy sandaracopimaric acid **(3),** β-hydroxy sandaracopimaric acid **(4)**, 13-epicupressic acid **(5),** **.**5,7,3′,4′-pentahydroxyflavone, quercetin **(6) and** quercitrin **(7)** respectively. The compounds include three flavonoids**:** Cupressoflavone, **(5)** quercetin (**6) and** quercitrin **(7)** and four diterpenes: 13-epicupressic acid **(1),** imbricatholic acid **(2)** α-hydroxy sandaracopimaric acid **(3),** β-hydroxy sandaracopimaric acid **(4),.** The biflavonoid Cupressoflavone, **(5) and diterpenes** and 13-epicupressic acid **(1)** were isolated for the first time from EH (Fiqure 1).

The ESIMS of compound **1** and **2** showed $[M+Na]^+$ at 343 and 345 m/z consistent with molecular formulae $C_{20}H_{32}O_3$ and $C_{20}H_{34}O_3$ respectively. Both ^{1}H- and ^{13}C-NMR (Table 1) of **1** and **2** showed two proton singlets at δH 4.50, 4.85 and 4.51, 4.85 respectively assigned for H-17. Both compounds showed an 18-CH_3 singlet (δH 1.16, δC 29.0) (δH 1.25 , δC 29.0); 19-COOH (dC 183.5)(dC 183.4); 20-CH3 singlet (dH 0.51, dC 12.7) (δH 0.61, δC 12.8) respectively. These data are typical for laban-8(17)-ene skeleton (Su et al., 1994). ^{1}H- and ^{13}C-NMR (tables 1, 2) of 1 showed another monosubstituted double bond (CH at dH 5.92, dd, J= 11.0, 17.5 Hz and dC 145.0; CH_2 at dH 5.08 dd, J= 1.0, 11.0 Hz, dH 5.22 dd, J= 1.0, 17.5 Hz and dC 111.71) assigned for C-14 and C-15. The methyl singlet (δH 1.20, δC 28.0) was assigned to C-16 on an oxygenated fully substituted C-13 at δC 73.7 as indicated from HMBC experiments. The data of 1 was identical with those reported for **13-epicupressic acid** (Su *et al.*,1994 : Alqasoumi *et al.*, 2013 : Al –groshi *et al.*, 2018).

MS, ^{1}H- and ^{13}C-NMR data of compound **2** indicated the lack of the second double bond. The appearance of the C-16 methyl as doublet at δH 0.92 (J=6.5 Hz) and the appearance of CH_2OH (δH 3.64−3.72 (m), δC 61.3) indicated a C-15 hydroxyl in **2**. The data of 2 was consistent with those reported for imbricatolic acid (Wenkert and Buckwalter, 1972 :).



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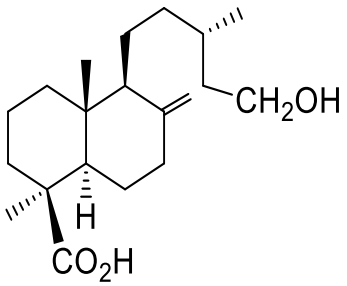

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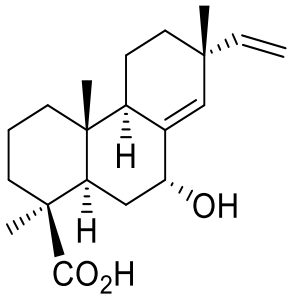

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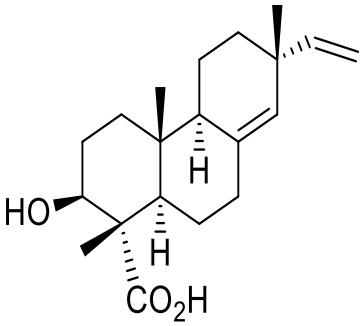

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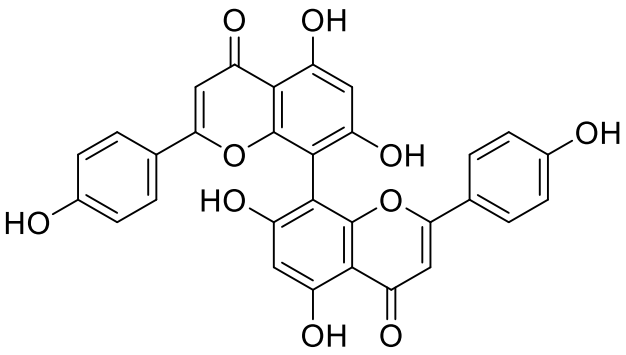

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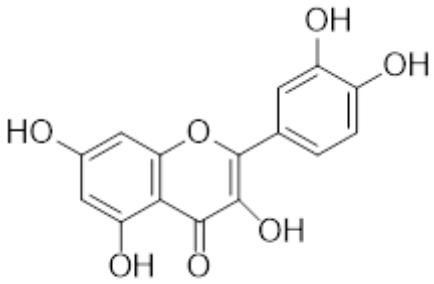

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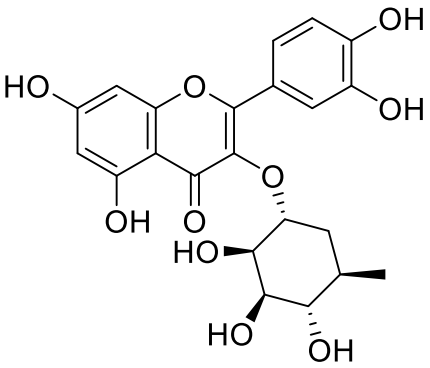

7

1 13-epicupressic acid **2** Imbricatholic acid **3** 7-α-hydroxy sandaracopimaric acid **4** 3β-hydroxy sandaracopimaric acid **5** Cupressoflavone **6** 5,7,3′,4′-pentahydroxyflavone, Quercetin **7** Quercitrin

Both compounds 3 and 4 (Table 1) have a pimarandiene skeleton with a C-8, C-14 double bond (δC 139.8, 135.1 and δC 137.6, 130.4 respectively) (Su et al., 1994). They also showed same EISMS $[M+ Na]^+$ at 341 m/z consistent with the molecular formula $C_{20}H_{30}O_3$. In each compound there were an oxygenated CH (δH 4.11 br s, δC 73.8; δH 4.02 dd, J= 4.5, 11.0 Hz, δC 76.4 respectively). The locations of the hydroxyl groups were assigned to C-7 and C-3 in 3 and 4 respectively based on the HMBC correlations. Comparison of their data with the literature identified **3**as 7α−Hydroxysandaracopimaric acid and **4** as

3β−Hydroxysandaracopimaric acid, respectively (Muhammad *et al.,* 1992; Su *et al.,* 1994; Wenkert and Buckwalter, 1972 Alqasoumi *et al*., 2013).

UV data of **5** indicated a 5, 7, 4'-trihydroxy flavone, ^{1}H-NMR (tables 3) showed one singlet for ring A at dH 6.48, one singlet for H-3 at dH 6.80 and two doublets each integrated for two protons at dH 6.76 and 7.51 assigned for 4'-monosubstituted ring B. ^{13}CNMR data (tables 4) indicated that C-8 appeared as fully substituted at dC 98.8 bears non-oxygenated substituent (Mabry *et al.,* 1970; Agrawal, 1989). However, ESIMS showed peaks at m/z 539 $[M + 1]^{+}$, 561 $[M + Na]^{+}$ and 577 $[M + K]^{+}$ calc. for $C_{30}H_{18}O_{10}$ indicating a dimeric structure. Comparison with literature data enable the identification of **5** as cupressuflavone (Markham ***et al.,*** 1987; Kang *et al*., 2005; Williams *et al*.,1987; Chari *et al*., 1977).

The UV data of **6** shows λ max of 373 nm. ^{1}H NMR 6 shows solvent peak observe at δ 5.1. Singlet signal at δ 6.20 and 6.40 indicates H-6 and H-8 proton of flavonol doublet peak at δ 6.89 and 6.91 indicate presence of H-5′ proton of flavonol. Peak at δ 7.59 to 7.66 indicate H-2′ and H-6′ proton of flavonol While ^{13}CNMR Signal at 175.92 ppm indicate carbonyl carbon (C-4) at normal low field position. signal at 164.14, 161.08, 156.80 and 158.30 ppm indicate C-7, C-5, C-4′ and C-3′ position of flavonol carbons. Singlet at 147.35 and 146.60 ppm attributed for C-2′ and C-6′ position of the carbon, signal at 135.85 ppm indicate C-3 oxygenated aromatic carbon. signal at 122.73 ppm indicate C-1 carbon atom of flavonol. Signal at 114.82, 114.58 ppm showed the C-1 and C-1′ position of non-oxygenated carbon atom. Signal at 103.11 ppm showed C-10 carbon position. Another signal at 97.83 and 93.06 ppm indicate C-6 and C-8 position of carbon. Comparison with literature data enable the identification of **6** as Quercetin. (Hassen *et al*, 1997: Selen *et al*, 2017 : Sumit and Prakash, 2018).

Compound **7** was isolated as an amorphous yellow powder with the molecular formula $C_{21}H_{20}O_{11}$, ESI-MS m/z: 447.77 [M-H]. The UV data showed peaks at wavelengths 245, 288 and 325nm with λ max of 340nm, Flavonoids generally show two characteristic bands in these region and their are band 1 is between 300 to 340 nm and band 2 is 18-20 between 240 and 280 nm indicating flavonoid compound (Sankhadip *et al*., 2013). The ^{1}H NMR 7 shows solvent peak observe at δ 5.1. Singlet signal at δ 6.20 and 6.40 indicates H-6 and H-8 proton of flavonol doublet peak at δ 6.89 and 6.91 indicate presence of H-5′ proton of flavonol similar to **6**. δ 7.57 (1H, d, J = 1.9 Hz, H-2'), 7.53 (1H, dd, J = 8.2, 1.9 Hz, H-6'), 6.77 (1H, d, J = 8.2 Hz, H-5'), 6.29 (1H, d, J = 1.8 Hz, H-8), 6.10 (1H,d, J = 1.8 Hz, H-6), 5.01 (1H, d, J = 2.0 Hz, H-1''), 4.22-3.14 (sugarH), 1.02 (3H, d, J = 6.0 Hz, H-6''). 13C-NMR (100 MHz, CD3OD): C2,158.1; C3,136.0; C4,179.3; C5,159.1; C6,99.5; C7,165.8; C8,94.3; C9,163.0; C10,105.5; C1 122.5 ,′ ; C2 116.2 ,′ ; C3 146.2 ,′ ; C4 149.5 ,′ ; C5 116.7 ,′ ; C6 122.7 ,′ ; C1″,103.4; C2″,71.8; C3″,72.0; C4″,73.1; C5″,71.7; C6″,17.5. Comparison of the ^{1}H NMR and ^{13}C NMR spectroscopic data of compound 7 with literature data enable the identification of **7** as Quercitrin. (Sankhadip *et al*., 2013: Selen *et al*, 2017).

Further comparison of the ^{1}H NMR and ^{13}C NMR spectroscopic data of compounds **5, 6** and **7** revealed that they all showed a typical flavonol pattern with a quercetin aglycon (Table 2). Compound **7** was identified as quercetin 3-O-α-L-rhamnoside which was in accordance with the reported data (Lee *et al*., 2004; Sankhadip *et al*., 2013: Selen *et al*, 2017).

All the seven compounds (Table 1 & 2) were tested for their cytotoxicity against five human cancer cell lines (Table 3): EJ138 (human bladder carcinoma), HepG2 (human liver hepatocellular carcinoma), A549 (human lung carcinoma), MCF7 (human breast adenocarcinoma) and PC3 (human prostate carcinoma) and compared with the anticancer standard etoposide. Compound 2- **5** were inactive against HepG2 and PC3 cells, however 5-7 exhibited moderate cytotoxicity against A549 and MCF7 IC_{50} 67.4± 2.45 and 111.7 ± 3.75 µg/mL respectively, compound **1 and 5** (13-epicupressic acid **and** Cupressuflavone)

showed the high cytotoxicity against human lung cancer cells lines (A549) with IC_{50} values of 58 and 73µM respectively, However, compound **5 (**Cupressuflavone) showed the highest cytotoxicity against human lung cancer cells lines (A549) with with IC_{50} values of 73.4± 2.45 µg/mL (Table 3).

The cytotoxicity of compound **5** from EH was revealed for the first time. Findings also indicated that cupressoflavone-induced cell death might involve the plasma membrane damage resulting in the release of LDH enzyme from the necrotic cells (Alqasoumi *et al*, 2013: Al-groshi *et al*., 2018 :) . In literatures, quercetin were reported to have antioxidant, chelation, anti-carcinogenic, cardio protective, bacterio static, and secretory properties, (Lee et al 2004 :Sha, 2017 selen *et al*, 2017)

Conclusions

In this study, seven known compounds were isolated from E. *hetrophylla,* including three biflavonoids (Cupressuflavone, Quecertin and Quercitrin) and four diterpenes (cupressuflavone 13-*epi*cupressic acid, imbricatholic acid, 3α-hydroxy sandaracopimaric acid and β− hydroxysandaracopimaric acid). Five of seven isolated compounds were isolated for the first time from the root extract of this plant: 13-*epi*cupressic acid, imbricatholic acid, 3α-hydroxy sandaracopimaric acid and β− hydroxysandaracopimaric acid). The study also revealed the ability of the compounds to inhibit cancer cell growth, however, two of the seven compounds (Cupressuflavone and 13-epicupressic acid) showed the high cytotoxicity against human lung cancer cells lines.; it competes favourably with standard anticancer agents at certain concentrations. The current findings support the traditional use of EH for the treatment of tumours and other cancer related diseases. It is suggested that the cytotoxic activity revealed in the polar MeOH fractions was due to the presence of cytotoxic bioflavonoids and the cytotoxicity observed with the non-polar fractions of *E. heterophylla* root were contributed by various cytotoxic terpenes.

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Conflict of interest

Authors declared no conflict of interest

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