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Research Article

Cytotoxic terpenes from the stems of bark of *Echinaceae angustifolia DC* collected from Girei, Adamawa state Nigeria

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Abstract

From the Stems of Bark of *Echinaceae angustifolia* DC three known triterpenes 3a,5,5b,8,8,11a-hexamethyl-1-(prop-1-en-2-yl)icosahydro-1H-cyclopenta[a]chrysene-9-yl acetate (lupeol acetate), 4,4,6a,6b,8a,10,11,14b,octamethyl1,1,2,3,4,4a,5,6,6a,6b,7,8,8a, 9,10, 11,12,12a,14,14a,14b-icosahydropicen-3-yl acetate (derivative of β-amyrin and 9-hydroxy-1-isopropenyl-5a,5b,8,8,11a-pentamethyl-icosahydro-cyclopenta[a]chrysene-3a-carboxylic acid (betulinic acid), labelled as Ea-7-38, Ea-9-10 and Ea-12-85) were isolated and characterized. All isolates were tested for their cytotoxicities against *Artemia salina* (brine shrimp larvae). Compound Ea-12-85 exhibited potent cytotoxic activity against the *Artemia salina*, Ea-7-38, Ea-9-10 were found to be non-toxic in the cytotoxicity test. The result of the study has justified the claim of the traditional medicine practitioners in Girei for the treatment of complicated malaria disease using the stem bark of *E. angustifolia* DC.

1. Introduction

Echinacea angustifolia is a herbaceous, drought-tolerant perennial plant growing up to 140 cm or 4 feet, in height. It grows from a taproot and has an erect unbranched stem that is unbranched. Both the basal and cauline (stem) leaves are arranged alternately. The leaves are normally hairy with a rough texture, having uniseriate trichomes (1–4 rings of cells) but sometimes they lack hairs. The basal leaves and the lower stem leaves have petioles, and as the leaves progress up the stem the petioles often decrease in length. The leaf blades in different species may have one, three or five nerves.

Bioactivity Studies of Echinacea Species revealed promising results as reported that aerial extract of *E. purpurea* alters the clinical course of influenza infection in mice and has antiviral activity [1]. Wu et al., reported that the concentrations of standardized purified dry extract from *Echinacea angustifolia* showed positive effect on proliferation and interferon gamma secretion of peripheral blood mononuclear cells in dairy heifers [2]. It was reported that *Echinacea angustifolia* extract can stimulate mammary epithelial cell physiology and may be considered a candidate to support mammary gland activity during a mamm-

ogenetic and lactogenetic state [3]. Also, *Echinacea angustifolia* has an immunomodulatory action on sheep neutrophils [4]. The study revealed that *E. angustifolia* extract significantly inhibited adhesion and superoxide production induced by Phorbol Myristate Acetate (PMA) that is known to increase the exposure of active b2-integrins on cell surface and to activate ROS generation by NADPH oxidase. Spelman *et al*., demonstrated that undeca-2E-ene-8,10-diynoic acid isobutylamide an Echinacea angustifolia-derived alkylamide, inhibits IL-2 secretion in Jurkat T cells through Peroxisome Proliferator-Activated Receptor gamma (PPARY) activity at low micromolar concentrations (330 ng/mL) [5]. Moreover, Nyalambisa reported that the root and leaf of Echinacea species contain volatile oils which varied in their yield and chemical compositions [6]. The essential root oil is non-toxic orally and it demonstrated significant anti-inflammatory and analgesic activities in laboratory animals. The aim of this study is to investigate the bioactive compounds responsible for the observed bioactivity of the stem bark of *E angustifolia* DC. The specific objectives of the study are to; i) isolate and characterize the pure compounds from the stem bark extracts and ii) investigate the cytotoxicity of the pure compounds.

2. Materials and methods

2.1 Chemicals/reagents

Good quality reagents and solvents of analytical grade were purchased, redistilled and used throughout the laboratory work. Precoated plates (PK6F silica Gel 60A°) with fluorescent indicator size 20cm by 20cm (1000µm thickness) were used for preparatory thin-layer chromatography. Sephadex LH-20 from Pharmacia 17-00-0-01, 25-100 micron were used for some highly Polar fractions.

2.2 Collection and preparation of plant materials

The stem-bark of the plants under investigation was collected from Girei Local Government Area of Adamawa State. Botanist in the Department of Biological Sciences Federal University of Kashere identified the plants, the plants specimens and voucher number (F.H.I 56535) were kept in the Herbarium. The plant samples were washed with distilled water, air-dried, ground to fine powder and weighed.

2.3 Extraction

Soxhlet extraction method was employed for the initial extraction to obtain the plant extracts for phytochemical screening. For the large scale extraction powdered sample of *Echinaceae angusifolia DC (1.9Kg)* was successively extracted with dichloromethane DCM, then methanol using Soxhlet apparatus and concentrated using Rotary film evaporator. The yield of the extracts was recorded.

2.4 Phytochemical screening

Standard method described by Usman *et al.* [20] was used to test for the presence of phytochemical compounds (saponins, sterols, terpenoids, glycosides, phlobatannins, resins, flavonoids, phenols, alkaloids, and carbohydrates) in the extracts.

2.5 Brine shrimp lethality assay of isolated compounds

Artemia salina eggs were added into a hatching chamber three quarter filled with ocean seawater. The chamber was kept in an open space for 24 h, after which the eggs hatched into *Artemia salina* larvae. Five concentrations (1000, 500, 250, 125, and 62.5) in µg/ml were prepared in test vials for each compound in triplicate. To each sample vial, a drop of DMSO solvent was added followed by 4ml ocean water. Two control groups were prepared, a positive control vial contained 4ml of methanol and a negative control vial contained 4mL of distilled water. Ten (10) larvae were introduced into each vial using a Pasteur pipette and allowed to stand for 24 h, the number of survivals were counted against a lighted background and recorded. Nauplii (larvae) were considered dead if they were lying immobile at the bottom of the vials, and the percentage of deaths at each dose and in the control were determined. Microsoft Excel spreadsheet application was used to formulate the regression equations from the data of mean results of percentage mortality of the brine shrimp versus the log of concentrations to base ten. These equations were later used to calculate LC_{50} values for the compounds tested noting that value greater than 1000 µg/mL suggests a nontoxic compound [7].

2.6 Column chromatography of the extract

Fractions obtained from the VLC were considered for isolation in the column chromatography. Silica gel (500g) was washed several times with n-hexane and packed into a glass column (38" length 2" diameter) in slurry of n-hexane. It was then removed from the

column after all solvent had drained and was allowed to dry. VLC was first carried to further fractionate the crude extract and ease the Column chromatography. Some quantity (20.0g) of celite was also washed in the same way using a Whatman filter paper and allowed to dry. Fraction from the VLC (25.0g) was mixed properly with silica gel and celite to form a homogenous mixture. Washed silica gel was packed onto a column (86cm length 2cm diameter) and the mixture was carefully loaded on it. An additional portion of silica gel was packed to form a protective layer on top of the adsorbent. Column was then eluted with n-hexane (1.5L), n-hexane: chloroform (1:1 1.0L), chloroform (1.5L), chloroform: ethyl acetate (1:1 1.0L), ethyl acetate (1.5L), ethyl acetate: methanol (1:1 1.0L), and methanol (1.5L). Fractions (50ml) were collected from the column, spotted on thin layer chromatographic (TLC) plates (TLC pre-coated plates (MERCK 20cm by 20cm), developed with various ratios of organic solvents (hexane, dichloromethane, chloroform, ethyl acetate, ethanol) taking into consideration of their polarities. Solvent system that separated the components to a high degree of resolution was considered. Plates were visualized under visible and UV-light (366nm and 254nm) and were sprayed with dodecamolybdophosphoric acid (TLC spray) to find the fraction that gave better separation of the compounds as single spots. Fractions with the same R_f values were pooled together and concentrated. Small columns were assembled for fractions of low mass and few spots, while preparatory thin layer chromatography (Prep-TLC) was carried out for fractions with 2 or three spots well-spaced from each other [8].

2.7 Preparation of sample for spectroscopic analysis

Infrared (IR) spectroscopy of samples was run on Fourier Transform FT-IR machine. Samples were first dissolved in Dimethylsulfoxide (DMSO) and a small pipette was used to place drop from the sample onto the sample spot provided on the machine. The machine was run and results were displayed on the computer monitor connected to the FT-IR machine. NMR spectroscopy was run on BRUKER NMR-spectrophotometer (700MHz), NMR sample tube (5mm) for 700MHz magnetic field was cleaned and dried in the oven. Deuterated chloroform ($CDCl_3$, 99.96 atom % D) in sealed glass ampoule was used to dissolve the sample (20mg) and transferred into the dried NMR sample tube. This was properly coded, labeled and submitted to the Central Analytical and Applied Research Unit (ISO 9001-2008 Certified) of Sultan Qaboos University Oman for analyses. NMR spectrometer, Bruker Avance DRX 700 MHz was used to run the experiments (^{13}C and ^{1}H) at 298K. Spectrum calibration was performed by using deuterated solvent as internal reference ($CDCl_3$: 7.26ppm for ^{1}H NMR and 77.16ppm for ^{13}C-NMR).

2.8 Spectroscopic measurement

Spectra in the 2D NMR analysis obtained include Correlation Spectroscopy (^{1}H-^{1}H COSY), Heteronuclear Single Quantum Correlation (HSQC), Heteronuclear Multiple Bond Correlation (HMBC), Nuclear Overhauser Effect Spectroscopy (NOESY), Rotational Frame Overhauser Effect Spectroscopy (ROESY) and ^{13}C DEPT (90, 45, and 135) for the pure samples.

3 Results and discussion

3.1 Characterization of compounds

The phytochemical screening of the dichloromethane and methanol extracts of *Echinaceae angusifolia DC (Compositae)* showed the presence of some secondary metabolites with the methanol extracts indicating the presence of most of the secondary metabolites (Table 1). Secondary metabolites have been shown to exhibit therapeutic activities, biological functions and pharmacological properties as supported by [9]. Alkaloids were reported to show antiplasmodial properties, analgesic, cytotoxic and anti-inflammatory activities [10].

Table 1. Phytochemical Screening Result of Solvent Extracts of *Echinaceae angustifolia DC*

Phytochemical Constituents	DCM	MeOH
Indole alkaloid	+	+
Tropane alkaloids	-	+
Quinoline alkaloids	+	+
Morphine alkaloids	-	-
Steroids	++	+++
Flavonoids	+++	++
Saponins	+++	+++
Tannins	+	-
Terpenes	++	+++
Phenols	++	+
Carbohydrates	+++	+++
Anthraquinones	-	++
Resin	-	-
Cardiac glycosides	+	+

Key: - = absent, + = faintly present, ++ = Present, +++ = Highly present

Table 2. Physical Properties of Isolated Compounds

Compounds	Solvent ratio	Ordinary light	UV light	Dodeca-molybdophosphoric acid (MPA) Spray	M P(°C)	R_f value	MW (g/mol)
Ea-7-38	Hex/ DCM 7:3	White	Green	Reddish brown	367-368	0.42	468
Ea-9-10	Hex/ DCM 7:3	White	Green	Reddish brown	693-694	0.51	468
Ea-12-85	DCM/ $CHCl_3$ 4:1	White	Green	Brown	520-521	0.50	456

Table 3. ^{13}C and ^{1}H NMR data (Chloroform-d_1 700 MHz for 1H and 176 MHz ^{13}C) for Compound Ea-7-38

C	δC[a]	δH/ Multiplicity	J value (Hz)
1	39.99 CH_2	1.64 t	2.8
2	38.03 CH_2	1.03, q	3.8
3	80.98 CH	46, dd	3.3, 4.3
4	42.99 C	-	-
5	48.00 CH	2.39, ddd	6.9,7.5, 7.9,
6	35.56 CH_2	1.58, q	5.6
7	34.20 CH_2	1.6, t	5.3
8	37.08 C	-	-
9	48.68 CH	1.06, t	7.2
10	42.81 C	-	-
11	29.82 CH_2	1.07, q	1.4
12	27.92 CH_2	1.37, q	8.5
13	27.94 CH	1.04, q	3.7
14	21.33 CH	2.07, t	1.3
15	21.31CH	2.09, q	9.4
16	25.08 CH_2	1.37, d	8.5
17	21.30CH	-	-
18	21.29CH	1.06, t	8.9
19	48.28 CH	1.61, q	4.8
20	150.96 C	-	-
21	23.71 CH_2	1.69, q	5.6
22	21.32 CH_2	1.66, t	6.4
23	19.28 CH_3	1.69, s	-
24	17.99 CH_3	0.79, s	-
25	16.49 CH_3	0.79, s	-
26	16.35 CH_3	1.03, s	-
27	16.17 CH_3	0.79, s	-
28	15.97 CH_3	1.03, s	-
29	109.35 CH_2	4.57, dd	3.9, 4.2
30	55.37 CH_3	0.79, s	-
1′	O	-	-
2′	171.02 C	-	-
3′	57.53CH_3	0.78, s	-

Tannins have been reported to show anti-oxidant activities and inhibit the growth of microorganisms [11].

Compound Ea-7-38 was obtained as white solid which appeared green under UV light, Reddish brown when sprayed with MPA and has a melting point of 368°C and Rf value of 0.42 in a hexane/DCM 9:1 solvent ratio. Compound Ea-9-10 was also obtained as white solid which appeared green under UV light, Reddish brown when sprayed with MPA and has a melting point of 694°C and Rf value of 0.51 in a hexane/DCM 8:2 solvent ratio. Compound Ea-12-85 was obtained as white solid which appeared green under UV light, brown when sprayed with MPA and has a melting point of 521°C and Rf value of 0.50 in a hexane/DCM 7:3 solvent ratio (Table 2).

Compound Ea-7-38 was obtained as white solid with molecular formula $C_{32}H_{52}O_2$ from HR-ESIMS m/z 491.2273 $[M+Na]^+$ (calculated for $C_{32}H_{52}O_2Na$ 491.2273.

Table 4. ^{13}C and ^{1}H NMR data (Chloroform-d_1 700 MHz for 1H and 176 MHz ^{13}C) for Compound Ea-9-10

C Position	δC Ea-9-10 isolated	δH/ Multiplicity	J value (Hz)
1	38.85 CH_2	1.007d	13.0
2	27.39 CH_2	1.645, q	13.3
3	80.98 CH	3.229 dd	11.5,5.8
4	38.83 C	-	-
5	55.33 CH	0.739, ddd	10.9,10.0,9.9
6	18.41 CH_2	1.544 q	13.0
7	34.20 CH_2	1.417, t	13.5
8	41.03 C	-	-
9	49.80 CH	1.446 t	13.1
10	37.22 C	-	-
11	22.23 CH_2	1.523, q	4.2
12	24.92 CH_2	1.885 q	5.8
13	135.41 C	-	-
14	44.98C	-	-
15	26.39CH2	1.046 q	3.4
16	38.89 CH_2	1.459, d	14.5
17	34.30C	-	-
18	136.38C	-	-
19	38.28 CH	2.61, q	7.5
20	34.97CH	1.679s	6.9
21	23.71 CH_2	1.131, q	13.7
22	36.32 CH_2	1.38, t	13.2
23	28.28 CH_3	0.99 s	
24	15.99 CH_3	0.77, s	
25	16.49 CH_3	0.87 s	
26	17.35 CH_3	0.92 s	
27	21.17 CH_3	1.12s	
28	28.27 CH_3	1.10 s	
29	23.15 CH_2	1.09, dd	7.6,7.0

Table 5. ^{13}C and ^{1}H NMR data (Chloroform-d_1 700 MHz for ^{1}H and 176 MHz for ^{13}C) for Compound Ea-12-85

C Position	$^{\delta}C$ Ea-12-85 Isolated Cpd	$^{\delta}H$/ Multiplicity	J value (Hz)
1	38.85 CH_2	1.007d	13.0
2	27.39 CH_2	1.645, q	13.3
3	80.98 CH	3.229 dd	11.5,5.8
4	38.83 C	-	-
5	55.33 CH	0.739, ddd	11.9,10.5,9.9
6	18.41 CH_2	1.544 q	13.0
7	34.20 CH_2	1.417, t	13.5
8	41.03 C	-	-
9	49.80 CH	1.446 t	13.1
10	37.22 C	-	-
11	22.23 CH_2	1.523, q	4.2
12	24.92 CH_2	1.885 q	5.8
13	135.41 C	-	-
14	44.98C	-	-
15	26.39CH2	1.046 q	3.4
16	38.89 CH_2	1.459, d	14.5
17	34.30C	-	-
18	136.38C	-	-
19	38.28 CH	2.61, q	7.5
20	34.97CH	1.679	6.9
21	23.71 CH_2	1.131, q	13.7
22	36.32 CH_2	1.38, t	13.2,
23	28.28 CH_3	0.99 s	0.4
24	15.99 CH_3	0.77, s	0.4
25	16.49 CH_3	0.87 s	1.0
26	17.35 CH_3	0.92 s	0.6
27	21.17 CH_3	1.12s	1.0
28	28.27 CH_3	1.10 s	0.7
29	23.15 CH_2	1.09, dd	7.6,8.0
30	179.8 CO	-	-
	OH	1.26	6.0
	OH	1.26	-

The proton (^{1}H) NMR, ^{13}C NMR (Table 3) and IR suggested that Ea-7-38 might be a triterpene with the cluster of methylene and methyl protons at δ 2.0 – 0.1 values range. A terminal C=CH_2 protons were conspicuous at δ value 4.69 and 4.68, a δ value of 4.57 corresponds to O-CH protons. A δ value of 2.3 corresponds to C=C-CH_3 protons, a singlet signal of high intensity at δ value of 2.0 reveal the OCH_3 protons. The carbon-13 NMR revealed a 32-carbon compound with a carbonyl carbon at 171ppm, two peaks at δ150ppm and δ109ppm corresponding to H_2C=CR_2, a moderate peak at δ81ppm revealed an O-CH ring carbon. The peak at δ77ppm with high intensity is the solvent (deuterated chloroform). The 1D carbon-13 NMR, 2D DEPT 90, 45 and 135 (Supplementary Fig S1-S10) revealed 11 CH_2, 8 CH_3, and 6 CH groups, IR spectra revealed a carbonyl (C=O) absorption band at 1732.629cm^{-1} C-O stretch at 1245cm^{-1} indicating an ester, C-H stretch (broad) at 2942.273cm^{-1}, C=C-H stretch at 3074.489cm^{-1} typical of esters. From the relevant literature, compound Ea-7-38 was identified as a pentacyclic triterpene 3a,5,5b,8,8,11a-hexamethyl-1-(prop-1-en-2-yl) icosahydro-1H-cyclopenta[a]chrysene -9-yl acetate (Lupeol acetate).

Compound Ea-9-10 was obtained as white solid with molecular formula $C_{32}H_{52}O_2$ from HR-ESIMS m/z 390.2273 $[M+Na]^+$ (calculated for $C_{32}H_{52}O_2Na$ 390.2273). The proton (^{1}H) NMR, ^{13}C NMR (Table 4), and IR suggested that Ea-7-38 is a triterpene with the cluster of methylene and methyl protons at δ 2.0 – 0.5 values range. A ring (internal alkene) HC=CH protons were conspicuous at δ value 4.69 and 4.68, a δ value of 4.57 corresponds to O-CH protons. A δ value of 2.3 corresponds to >C=C-CH_3 protons, a singlet signal of high intensity at δ value of 2.0 reveal the OCH_3 protons. The carbon-13 NMR revealed a 32-carbon atoms compound with a carbonyl carbon at δ171ppm, two peaks at δ144ppm and δ139ppm corresponding to C=C isolated ring alkene, a moderate peak at δ81ppm revealed a O-CH ring carbon. The peak at δ77ppm with high intensity is the solvent (deuterated chloroform). The 1D carbon-13 NMR, 2D DEPT 90, 45 and 135 revealed 11 CH_2, 8CH_3, and 6CH groups. The IR spectrum revealed a carbonyl (C=O) absorption band at 1732.629cm^{-1}, C-O stretch at 1245cm^{-1}, C-H stretch (broad) at 2942.273cm^{-1}, >C=C-H stretch at 3074.489cm^{-1} typical of an ester. Table 4 provided the data (carbons position, ^{1}H multiplicity, coupling constant, ^{1}H-^{1}H COSY) for Ea-9-10 while Table 4. 24 avails the comparative data from standard (MestReNova), Literature and isolated compound Ea-9-10. From these data and the library data (Table 6) obtained from both ChemBioDraw Ultra 3.0 and MestReNova application software, compound Ea-9-10 was identified as a triterpene 4,4,6a,6b,8a,10,11,14b, octamethyl 1,1,2,3,4,4a,5,6,6a,6b, 7,8,8a,9,10,11,12,12a, 14,14a,14b icosahydropicen- 3-yl acetate (derivative of β-Amyrin).

Table 6. BST Assay Results of Compound **1**

Conc. (µg/mL)	Survivals			Deaths			Mortality (%)	Log_{10} Conc.
	V1	V2	V3	V1	V2	V3		
1000	10	9	9	0	1	1	6.67	3
500	9	10	8	1	0	2	10	2.7
250	8	10	9	2	0	1	10	2.4
125	9	9	9	1	1	1	10	2.1
62.5	10	10	10	0	0	0	0.00	1.8
Ctrl(+)	0	0	0	10	10	10	100	
Ctrl(-)	10	10	10	0	0	0	0.00	

LC_{50}(µg/mL) 1336.5

Table 7: BST Assay Results of Compound **2**

Conc. (µg/mL)	Survivals			Deaths			Mortality (%)	Log_{10} Conc
	V1	V2	V3	V1	V2	V3		
1000	8	9	7	2	1	3	20.0	3
500	9	10	8	1	0	2	10	2.7
250	8	9	8	2	1	1	16.67	2.4
125	9	10	9	1	0	1	6.67	2.1
62.5	10	10	10	0	0	0	0.00	1.8
Ctrl(+)	0	0	0	10	10	10	100	
Ctrl(-)	10	10	10	0	0	0	0.00	

LC_{50}(µg/mL) 1036.5

Table 8. BST Assay Results of Compound **3**

Conc. (µg/mL)	Survivals			Deaths			Mortality (%)	Log_{10} Conc
	V1	V2	V3	V1	V2	V3		
1000	3	2	2	7	8	8	76.67	3
500	5	1	8	5	9	2	53.33	2.7
250	8	7	8	2	3	2	26.67	2.4
125	9	10	6	1	0	4	16.67	2.1
62.5	10	10	10	0	0	0	0.00	1.8
Ctrl(+)	0	0	0	10	10	10	100	
Ctrl(-)	10	10	10	0	0	0	0.00	

LC_{50}(µg/mL) 136.5

Compound Ea-12-85 was obtained as white solid and molecular formula proposed to be $C_{30}H_{49}O$ from HR-ESIMS m/z 390.2273 $[M+Na]^+$(calculated for $C_{30}H_{49}ONa$, 337). The proton (^1H) NMR, ^{13}C NMR (Table 5) and IR suggest that Ea-12-85 might be a triterpene with the cluster of methylene and methyl protons at δ 2.0 – 0.1 values range. A ring (internal alkene) HC=CH protons were conspicuous at δ value 4.69 and 4.68, a δ value of 4.57 corresponds to O-CH protons. A δ value of 2.3 corresponds to C=C-CH_3 protons, a singlet signal of high intensity at δ value of 2.0 reveal the OCH_3 protons. The carbon-13 NMR revealed a 32-carbon compound with a carbonyl carbon (acid) at δ179ppm, two peaks at δ144ppm and δ139ppm corresponding to C=C isolated ring alkene, a moderate peak at 81ppm revealed a O-CH ring carbon. The peak at δ77ppm with high intensity is typical of the solvent (deuterated chloroform). The 1D carbon-13, 2D DEPT90, 45 and 135 revealed 11 CH_2, 8 CH_3, and 6 CH groups. The IR spectrum revealed a carbonyl (C=O) absorption band at 1732.629cm^{-1}, C-O stretch at 1245cm^{-1} revealing an ester, O-H stretch (broad) at 3242.273cm^{-1} revealing an alcohol, C=C-H stretch at 3074.489cm^{-1} indicating an alkene. From the relevant literature compound Ea-12-85 was identified as betulinic acid. The earlier work reported by Oliver *et al.*, 2015 on the isolation of triterpene from plants was in agreement and supported this submission with comparative data.

3.2 *Brine Shrimp Lethality Test*

The result of the *in vitro* cytotoxicity studies (Tables 6 - 8) revealed that Compounds 1 and 2 (Fig. 1) were non-toxic (LC_{50}(µg/mL) ≥1000) to the nauplii while compound 3 (Fig. 1) was toxic (LC_{50}(µg/mL) = 136.5) to the nauplii. In similar studies terpenes have been reported to exert anti-inflammatory effects by inhibiting various proinflammatory pathways in ear edema, bronchitis, chronic obstructive pulmonary disease, skin inflammation, and osteoarthritis [12-15, 16]. A natural compound linalool found in essential oils of aromatic plants, inhibited cigarette smoke-induced acute lung inflammation [17]. The findings of this study suggest that the plant extracts are reliable natural sources of terpenes terpenoids and their derivatives. A report revealed that structurally related monoterpenes p-Cymene, carvacrol and thymol isolated from essential oil from leaves of *Lippia sidoides* cham. (Verbenaceae) protected mice against elastase-induced emphysema [18]. Also based on the reported antiplasmodial studies carried out on solvent extracts of this plant [19], the use of this

plant by traditional medicine practitioners especially at lower doses to cure jaundice and malaria becomes scientifically valid especially in rural communities where orthodox drugs are unaffordable because of the costs.

The three isolated compounds were found to be nontoxic to the shrimps except one. However, the study revealed that the stem bark extract of this plant contained important secondary metabolites which include but not limited to triterpenes and their derivatives. A report by [12] revealed that pentacyclic lupane-type triterpenes, possess beneficial effects as a therapeutic and preventive agent for a range of disorders which include anti-inflammatory and anti-arthritic activities both in *in vitro* and *in vivo* systems. There has been a tremendous effort by researchers worldwide to develop the use of triterpenes toward the treatment of a variety of disorders especially the mechanism of action of lupeol and suggest that it is a multi-target agent with immense anti-inflammatory potential targeting key molecular pathways which involve nuclear factor kappa B, phosphatidylinositol-3-kinase in a variety of cells. It was reported that lupeol at its effective therapeutic doses exhibits no toxicity to normal cells and tissues. The efficacy of triterpenes as antitumor (carcinogenesis) by the use of either natural or synthetic substances individually or in combination therapy was promising. However, there was no report on the use of triterpenes as remedy to malaria disease.

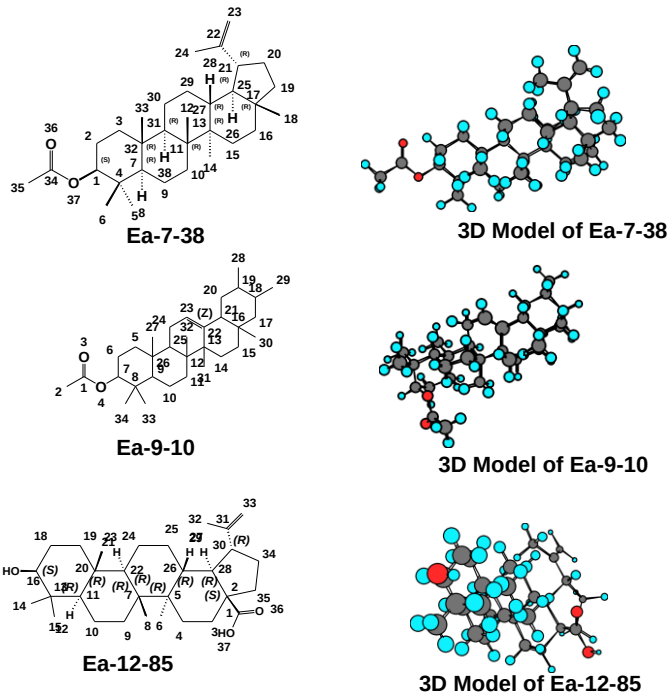

Figure 1. 1D and 3D structures of the Isolated Compounds

4 Conclusions

Our current study revealed that dichloromethane and methanol extracts of *Echinaceae angusifolia DC (Compositae)* contained significant amount of some secondary metabolites with the methanol extracts indicating the presence of most of the secondary metabolites. The Secondary metabolites present includes flavonoids, terpenes, saponins, steroids, alkaloids and tannins. The revealed cytotoxicity activity in this study is due to the triterpenes isolated from the plant extract. Consequently, this scientific information can serve as an important baseline data for the development of safe and effective natural medicine.

Supplementary Data

Supplementary Fig S1-S10

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Authors' contributions

AZ: conducted the research work, SJ: assisted in chromatography and spectral analysis, FMO: Supervision of the research work and helped for the structural elucidation, MHS: assisted in the purification and TLC experiment, AS: assisted in VLC and general extractions.

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Conflicts of interest

The Authors wish to declare that there is no conflict of interest.

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