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Anticancer potential of four triterpenoids against NCI-60 human tumor cell lines

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Abstract

Background The burden of cancer incidences and mortality is rapidly increasing worldwide resulting in an increased demand for new therapies. Secondary metabolites extracted from medicinal plants have significantly contributed toward discovery of new cancer therapies some of which are in clinical use. In this study, anticancer potential of four triterpenoids, namely oleanonic acid (**EK-2**), 3-*epi*-oleanolic acid (**EK-8**), 1,2,3,22,23-pentahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosatetraene (**EK-4**) and 2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosatetraene (**EK-9**), extracted from *Ekebergia capensis* Sparrm root bark was evaluated.

Results We employed CLC-Pred to initially evaluate cytotoxicity of previously isolated compounds in silico where predictions revealed high probability of bioactivity. The compounds were then submitted to the National Cancer Institute (NCI), Developmental Therapeutics Program, for bioactivity evaluation against NCI-60 human tumor cell lines. The four compounds demonstrated a range of potencies at a concentration of 10 µM. The results revealed that **EK-9** was the most potent with mean growth percent of 32.84 and cases of lethality (negative growth percent) against two leukemia cell lines (HL-60 (TB) and RPMI-8226) and HT29 (colon cancer) and SK-MEL-5 (melanoma). This molecule was further evaluated in a five-dose assay where notable growth inhibition against leukemia cells, HL-60 (TB), RPMI-8226 and K-562 was observed with growth inhibitory activity (GI_{50}) values of 3.10, 3.74 and 5.07 µM, respectively. In addition, total growth inhibition was observed at 11.2 µM and 18.9 µM for HL-60 (TB) and RPMI-8226 cells, respectively, partly accounting for the negative growth percent.

Conclusion The study has demonstrated anticancer properties of the four triterpenoids with compound **EK-9** being the most potent overall having selective bioactivity in leukemia and breast cancer cells. Further studies focusing on elucidating its mechanism of action will be useful in exploration of the therapeutic potential of triterpenoids in general.

Keywords Triterpenoids, Anticancer, NCI-60 panel, *Ekebergia capensis*, CLC-Pred

1 Background

The rapidly surging global burden of cancer necessitates urgent development of new anticancer therapeutics with novel modes of action [13]. Notably, secondary metabolites isolated from medicinal plants have contributed toward discovery of new anticancer therapies by serving as templates of several clinically useful anticancer agents [5]. Our past efforts geared toward discovery of bioactive compounds from *Ekebergia capensis* Sparrm led to the isolation of four triterpenoids from the root bark that included oleanane triterpenoids oleanonic acid (**EK-2**) and *3-epi*-oleanolic acid (**EK-8**); acyclic

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triterpenoids 1,2,3,22,23-pentahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosatetraene (**EK-4**) and 2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosatraene (**EK-9**) [4]. These compounds previously showed varied cell growth inhibition properties against breast cancer cells 4T1 and MDA-MB-231 that had IC_{50} values between 13.3 and 82.0 µM [4]. Their cytotoxicity against VERO and HEp-2 cells was also reported with IC_{50} values between 1.4 and 58.0 µM [4].

Anticancer potential of compounds **EK-2**, **EK-8**, **EK-4** and **EK-9** (Fig. 1) was evaluated where CLC-Pred was used to predict the cytotoxic probability and subsequently submitted them to NCI, Developmental Therapeutics Program (DTP). Following initial molecular structure screening, rationale for in vitro evaluation was built on individual compound contribution to diversity of NCI small molecules library. National Cancer Institute cytotoxicity screen makes use of 60 distinct human tumor cell lines obtained from melanoma, leukemia and cancers of the lung, colon, brain, ovary, breast, prostate and kidney to characterize new molecules with potential to inhibit growth or kill tumor cell lines [12]. In this study, growth inhibition potential at a concentration of 10 µM for the compounds **EK-2**, **EK-8**, **EK-4** and **EK-9** against NCI-60 human tumor cell lines is reported. Additionally, we report anticancer potential of **EK-9** in five-dose assay.

2 Methods

2.1 General experimental procedure

The four triterpenoids were previously isolated and characterized by BNI. Their NMR data were previously acquired on Varian 800, 500 and 400 MHz spectrometers. LC–ESI–MS spectra was obtained on a Perkin Elmer PE SCIEX API 150EX instrument equipped with a Turbolon spray ion source and a Gemini 5 mm C-18 110Å HPLC column [4]. One-dose and five-dose assay data were recorded on microplate reader as per NCI, DTP methods.

2.2 Plant Collection

Plant used in this study (*Ekebergia capensis* Sparrm) is already documented in the literature as a useful medicinal plant and therefore is in public domain. Permission to collect the plant was sought from Machakos County local administration.

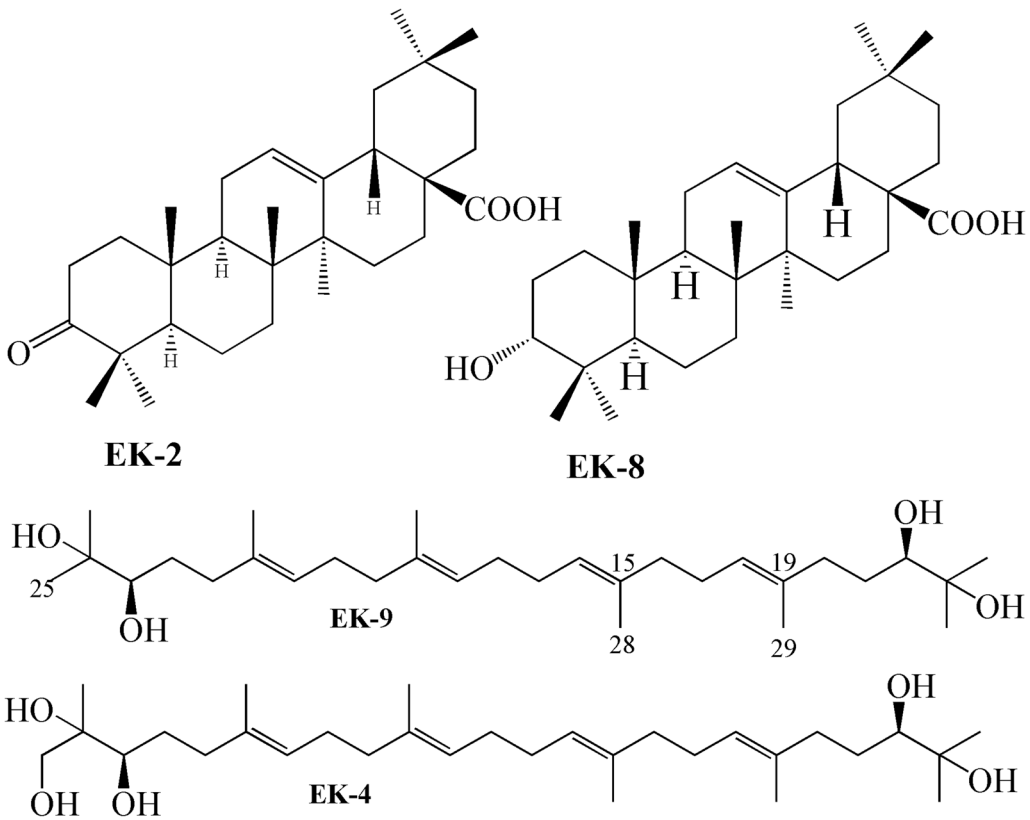

Fig. 1 Structures of triterpenoids isolated from *E. capensis*

2.3 Extraction and isolation of triterpenoids from the root bark of *E. capensis*

Compounds **EK-2** (white crystals), **EK-8** (white amorphous powder) **EK-4** (oil) and **EK-9** (oil) were isolated from the root bark of *E. capensis* as previously described [4].

2.4 CLC-Pred cytotoxicity prediction

To predict in silico cytotoxic probability of **EK-2**, **EK-8**, **EK-4** and **EK-9**, we employed an online Cell-Line Cytotoxicity Predictor at a cutoff Pa > 5; (http://www.way2drug.com/Cell-line/index.php) [7]. This web application is a Prediction of Activity Spectra for Substances (PASS) which allows prediction of cytotoxicity properties of a molecule based on their molecular structure against human cancer and non-tumor cell lines using ChEMBL experimental database of bioactive molecules with drug-like properties. PASS is denoted by a list of activities with probabilities 'to be active' Pa or 'to be inactive' Pi, arranged in descending order. The most probable activity types are placed at the top of the list.

2.5 NCI-60 one-dose and five-dose testing

National Cancer Institute, DTP, initially screens molecules using a single high-dose test (10 μM) in the full NCI-60 cell line panel. Molecules that satisfy NCI predetermined threshold inhibition criteria in a minimum number of cell lines progress to five-dose screen.

The four triterpenoids were tested at a single concentration of 10 μM following published protocol [10]. The objective of this assay is to determine the growth inhibition percent of test compounds against NCI-60 panel. Compounds were added to the culture and incubated for 48 h where endpoint was measured by a sulforhodamine B stain. The results were plotted in a one-dose graph showing growth inhibition percent of the single compound against the 60 cell lines. In this assay, cytotoxicity is growth relative to the no-treatment control and relative to the plated cells number also known as time zero number of cells. One-dose assay results include lethality reported as negative values and growth inhibition reported as positive values between 0 and 100. Data are presented as growth percent. For example, value of 40.52 would mean 59.48% growth inhibition, whereas a value of -100 implies all cells are dead.

Compound **EK-9** displayed notable growth inhibition properties in one-dose assay and was further tested in five doses with concentration ranging from 0.01 to 100 μM. The screening methodology is detailed at https://dtp.cancer.gov/discovery_development/nci-60/methodology.htm. Briefly, the cell lines were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. Cells were inoculated into 96-well microtiter plates at 100 μL at plating densities ranging between 5000 and 40,000 cells/well depending on the doubling time of individual cell lines and incubated for a day. After 1 day, some of the plates were processed to determine a time zero density. Plates with the tested compounds at a five-dose range from 0.01 to 100 μM were incubated for 2 days, then fixed and stained with sulforhodamine B. Growth inhibition was calculated relative to cells without drug treatment and the time zero control [2, 14]. The use of a time zero control allowed the determination of cell kill as well as net growth inhibition. Three dose–response parameters were reported, namely (a) 50% growth inhibition (GI_{50}), (b) total growth inhibition (TGI) and (c) 50% lethal concentration (LC_{50}) [12].

3 Results

3.1 Characterization of triterpenoids isolated *from E. capensis* root bark

Structures of **EK-2** (white crystals), **EK-8** (white amorphous powder), **EK-4** (oil) and **EK-9** (oil) were characterized using ^{13}C NMR, ESI–MS (30 eV) and ^{1}H NMR as previously described [4].

3.2 CLC-Pred cytotoxicity prediction

We used web resource CLC-Pred to predict cytotoxicity at cutoff Pa > 0.5. Oleanane triterpenoids **EK-2** and **EK-8** had predicted cytotoxic probability against 8505C carcinoma cells and acyclic triterpenoids **EK-4** and **EK-9** against SK-MEL-2 melanoma cells among other cell lines as shown in Table 1. Both **EK-2** and **EK-8** had predicted cytotoxic probability against normal cell line, embryonic lung fibroblast cells.

3.3 Anticancer potential of *E. capensis* triterpenoids against NCI-60 panel of human tumor cell lines

The four triterpenoids displayed a range of potencies on different cell panels at 10 μM as presented in Table 2 and Additional file 1 (supporting information). Compound **EK-9** was the most potent in limiting cell growth of NCI-60 panel of tumor cell lines with mean growth percent of 32.84 followed by **EK-8, EK-2** and **EK-4** at 54.68, 78.45 and 88.43, respectively. Notably, **EK-9** had four cases of lethality (negative growth percent) against HL-60 (TB) (leukemia), RPMI-8226 (leukemia), HT29 (colon cancer) and SK-MEL-5 (melanoma). Compound **EK-9** was selected for further test in a five-dose screen having fulfilled the set NCI inhibition thresholds. The data were presented in three dose parameters, namely; (a) GI_{50} which is the concentration of compound that causes reduction in net cell growth by 50%; (b) cytostatic activity (TGI); concentration of the compound that totally inhibits cell growth and (c) cytotoxicity

Table 1 CLC-Pred cytotoxicity results (Pa > 0.5)

	Pa	Pi	Cell line	Cell-line name	Tissue/organ
EK-2	0.518	0.004	8505C	Thyroid gland undifferentiated (anaplastic) carcinoma	Thyroid
	0.675	0.006	MRC5	Embryonic lung fibroblast (normal cell line)	Lung
EK-8	0.592	0.002	MKN-7	Gastric carcinoma	Stomach
	0.556	0.004	8505C	Thyroid gland undifferentiated (anaplastic) carcinoma	Thyroid
	0.727	0.005	MRC5	Embryonic lung fibroblast (normal cell line)	Lung
EK-4	0.771	0.005	SK-MEL-2	Melanoma	Skin
	0.726	0.017	MCF7	Breast carcinoma	Breast
	0.613	0.013	MDA-MB-231	Breast adenocarcinoma	Breast
	0.519	0.009	A2058	Melanoma	Skin
EK-9	0.850	0.004	SK-MEL-2	Melanoma	Skin
	0.630	0.010	SK-OV-3	Ovarian carcinoma	Ovarian
	0.547	0.016	HCT-15	Colon adenocarcinoma	Colon
	0.514	0.010	A2058	Melanoma	Skin

Table 2 Cytotoxic activities (growth percent) for compounds EK-2, EK-8, EK-4 and EK-9, against selected cell lines and GI_{50}, TGI and LC_{50} for EK-9

Panel name	Cell name	Growth percent				EK-9		
		EK-8	EK-2	EK-4	EK-9	GI_{50} (μM)	TGI (μM)	LC_{50} (μM)
Leukemia	HL-60 (TB)	30.21	42.39	70.71	−49.27	3.10	11.2	> 100
	K-562	31.69	49.01	66.37	10.38	5.07	> 100	> 100
	MOLT-4	30.69	70.56	75.00	0.46	14.40	44.60	> 100
	RPMI-8226	20.01	58.91	65.89	−11.40	3.74	18.90	> 100
NSCLC	NCI-H226	28.49	80.05	80.84	38.85	12.60	27.30	49.30
Colon cancer	HT29	51.95	89.84	96.21	−66.99	12.0	27.40	62.60
Melanoma	SK-MEL-5	19.18	34.32	81.25	−16.30	5.06	17.40	42.30
Prostate cancer	PC-3	22.66	42.29	83.82	7.84	5.16	20.10	60.30
Breast cancer	MCF7	50.38	77.01	89.16	13.77	6.70	20.00	46.70
	BT-549	40.68	91.81	63.57	8.56	5.14	17.40	45.30
	T-47D	42.92	65.44	72.97	6.38	7.75	25.00	69.90
	MDA-MB-468	21.64	51.37	68.87	0.84	7.97	21.10	48.00

(LC_{50}) which demonstrates lethal dose of the compound that causes death of initial cells by 50%.

The five-dose data are shown in Table 2 and Additional file 2 (supporting information) where the molecule demonstrated varied bioactivities with GI_{50} values ranging from 3.10 μM (HL-60 (TB), leukemia) to 18.5 μM (SR, leukemia). The LC_{50} values ranged from 42.30 to 90.60 μM with the exception of HL-60 (TB), K-562, MOLT-4, RPMI-8226, SR (all leukemia) and U251 (CNS) whose value exceed 100 μM. Remarkable activity against leukemia cell lines was observed with HL-60 (TB) being the most sensitive at GI_{50} of 3.10 μM. With regard to TGI, HL-60 (TB) (11.2 μM), SK-MEL-5 (17.40 μM) and BT-549 (17.40 μM) were most sensitive. **EK-9** was cytotoxic to majority (54/60) of the tested cells with LC_{50} values ranging from 42.3 to 90.6 μM with the exception of SR, MOLT-4, U251, RPMI-8226, K-562 and HL-60 (TB) whose values exceed 100 μM.

Oleanane triterpenoid, **EK-2** and **EK-8**, demonstrated highest growth inhibition against SK-MEL-5 (melanoma) at 34.32 and 19.18 growth percent, respectively. Other notable growth inhibition effects (growth percent < 30) for cells treated with **EK-8** were observed in RPMI-8226 (leukemia), NCI-H226 (non-small cell lung cancer), PC-3 (prostate cancer) and MDA-MB-468 (breast cancer) cell lines. Compound **EK-4** demonstrated the lowest growth inhibition potential against the NCI-60 cells (S1) with growth percent between 63.57 (BT-549 breast cancer) and 122.85 (COLO 205 colon).

4 Discussion

This study used in silico and in vitro methods to evaluate cytotoxicity of four triterpenoids. The in silico data at Pa > 0.5 suggested varied cytotoxicity profiles against thyroid, lung, stomach, skin, breast, colon and ovarian cancer cell lines inspiring us to pursue in vitro cytotoxicity evaluation. The potential of the four triterpenoids to inhibit growth of NCI-60 cancer cell lines at a concentration of 10 μM was determined while **EK-9** was further evaluated in a five-dose assay determining GI_{50}, TGI and LC_{50}.

Compound **EK-9** was the most potent displaying varied inhibitory effects with growth percent ranging from − 66.99 to 98.05. In the one-dose assay, **EK-9** was the most cytotoxic to leukemia cell panel with lethality (negative growth percent) against HL-60 (TB) and RPMI-8226. In the five-dose assay, decreased proliferation was observed for HL-60 (TB), RPMI-8226 and K-562 having GI_{50} values at 3.10, 3.74 and 5.07 μM, respectively, in line with one-dose data. Total growth inhibition for HL-60 (TB) and RPMI-8226 cells was observed at 11.20 and 18.90 μM, respectively, partly accounting for the reported negative growth percent in one-dose assay. Additionally, GI_{50} values of < 10 μM were observed in four out of six breast cancer cells panel. A study by Yazdani et al. [15] reported weak cytotoxic activity of **EK-9** (IC_{50} > 25 μM) against COLO 205 (colon cancer) corroborating our findings where a growth percent of 41.91 was observed [15]. Additionally, Yazdani et al. [15] reported that **EK-9** demonstrates P-gp inhibitory activity and acts in synergism when combined with doxorubicin against COLO 320 (adenocarcinoma cells).

On the contrary, compound **EK-4** which is structurally similar to **EK-9** differing at C-1 (presence of a hydroxyl group in **EK-4**) displayed very minimal inhibitory effects with growth percent ranging from 63.57 to 122.85. We postulate that the additional hydroxyl group located at C-1 in **EK-4** reduced its cytotoxic effects in all NCI-60 panel cell with growth percent > 60. A study by Lim et al. corroborates our findings where a compound similar to **EK-4** with an additional hydroxyl group at C-5 did not inhibit the growth of J774 cell lines (mouse macrophage cell line) [8].

In silico data suggested cytotoxicity of **EK-4** at Pa > 5 against SK-MEL-2 (melanoma), MCF7 (breast cancer) and MDA-MB-231 (breast cancer) cell lines. However, the in vitro data displayed very minimal inhibition against these cell lines with growth percent of 85.70, 89.16 and 88.06, respectively. The varied cytotoxicity results provided by CLC-Pred 2.0 prediction and NCI-60 one-dose data on similar cell lines further emphasize the need for experimental assays in drug discovery studies.

Oleanane triterpernoids **EK-2** and **EK-8** differ structurally at C-3 where **EK-2** has an oxo group, whereas **EK-8** contains an α-OH. They both demonstrated selective cytotoxicity toward leukemia cells panel. Our findings on oleanane triterpenoids corroborate with previous reports that have demonstrated varied cytotoxicity properties against cancer cell lines. For example, Kwon et al. reported cytotoxic effects of **EK-2** and **E-K8** against A549, HCT-15, SK-MEL-2 and SK-OV-3 cell lines with IC_{50} values less than 7.0 μg/ml [6]. In our study, we observed growth percent > 50 with **EK-2** at 10 μM in A549, HCT-15, SK-MEL-2 cells, whereas **EK-8** had growth percent < 50 in the same cell types except for SK-OV-3 which was greater than 50%. Huang et al. demonstrated cytotoxic effects of **EK-2** against highly metastatic mouse melanoma cell (B16-BL6) and A549 (NSCLC), with IC_{50}'s of 10.8 μg/ml and 2.8 μg/ml, respectively [3]. Fontana et al. demonstrated cytotoxic activities of oleanolic acid against HL-60 leukemia cell lines with IC_{50} value of 44 μM [1]. Oleanolic acid differs from **EK-8** with hydroxyl group at C-3 being β-oriented. This study did not evaluate whether presence of a hydroxyl group at C-3 in compound **EK-2** enhanced its cytotoxicity making it more than twofold cytotoxic against MOLT- and RPMI-8226 leukemia cell lines. However, this observation has been reported in other studies suggesting that C3-OH is an essential structural element in the activity of oleanane type triterpenes [9, 11]. Notably, mean growth percent of **EK-8** and **EK-2** was 54.67 and 78.45, respectively, further suggesting the role of C3-OH in bioactivity of oleanane triterpenoids.

5 Conclusion

This study has demonstrated anticancer potential of four triterpenoids against NCI-60 panel of human tumor cell lines. Results revealed that **EK-9** was the most potent overall with selective bioactivity in leukemia and breast cancer cells. Notably, **EK-9** was potent against HL-60 (TB), RPMI-8226 and K-562 with GI_{50} values less than 5 μM. In addition, TGI was observed at 11.2 μM and 18.9 μM in HL-60 (TB) and RPMI-8226 cells, respectively, which accounts for the negative growth percent of − 49.27 and − 11.40 in the same order. Of note is the low GI_{50} of ≤ 10 μM observed in breast cancer panel. Further studies geared toward elucidating its mechanism of action would be useful in exploration of therapeutic potential of triterpenoids in general.

Abbreviations

NCI	National Cancer Institute
DTP	Developmental Therapeutics Program
GI_{50}	50% Growth inhibition
TGI	Total growth inhibition
μM	Micromolar
NMR	Nuclear magnetic resonance

PASS	Prediction of activity spectra for substances
LC–ESI–MS	Liquid chromatography–electrospray ionization–mass spectrometry
HPLC	High-performance liquid chromatography
LC_{50}	50% Lethal concentration
^{13}C NMR	Carbon nuclear magnetic resonance
ESI–MS	Electrospray ionization–mass spectrometry
^{1}H NMR	Proton nuclear magnetic resonance
CNS	Central nervous system
NSCLC	Non-small cell lung cancer
IC_{50}	50% Inhibition concentration
µL	Microliter
µg/ml	Microgram per milliliter
P-gp	P-glycoprotein

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43088-024-00507-8.

Additional file 1: NCI-60 single-dose data at 10 µM for compounds **EK-2**, **EK-8**, **EK-4** and **EK-9**.

Additional file 2: NCI five-dose data for compound **EK-9**.

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Author contributions
BNI conceived the study and drafted the manuscript, and MN and FTN analyzed the data and edited the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article [and its Additional files 1 and 2].

Declarations

Ethics approval and consent to participate
This was obtained from Kenya Medical Research Institute, Scientific and Ethics Review Unit (KEMRI/SSC/CTMDR/1824).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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