Research article

Bioassay-guided isolation of dehydrocostus lactone from *Echinops kebericho* as a leishmanicidal drug

Bekri Melka Abdo [a,*], Bizuayehu Tesfaye Asfaw [b], M. Iqbal Choudhary [c], Sammer Yousuf [c], Wendawek Abebe Mengesha [d], Solomon Abate Mekonnen [e]

[a] *Wendo Genet Natural Product Research Laboratory, Ethiopian Institute of Agricultural Research, Addis Ababa, 2003, Ethiopia*
[b] *Plant and Horticultural Science Department, Hawassa University, Hawassa, Ethiopia*
[c] *International Center for Chemical and Biological Science, University of Karachi, Karachi, 75270, Pakistan*
[d] *Department of Molecular, Cellular, and Microbial Biology, Addis Ababa University, Addis Ababa, Ethiopia*
[e] *Food Science and Nutrition Research Process, Ethiopian Institute of Agricultural Research, Addis Ababa 2003, Ethiopia*

ARTICLE INFO

ABSTRACT

Several strains of *Leishmania* parasite are involved in the occurrence of leishmaniasis infections, which makes its prevention and treatment very challenging. Currently, all forms of leishmaniasis are being treated with chemical drugs, which have limitations and adverse effects. Discovering antileishmanial agents from natural sources can lead to novel drugs against this dreadful disease. The essential oils and nonpolar solvent extracts of the roots of *E. kebericho* exhibit antileishmanial activity. Thus, the isolation of the leishmanicidal compounds from the roots of *E. kebericho* through a bioassay-guided technique was carried out in this study. The present finding showed that the essential oil and hexane fraction of crude extract from the roots of *E. kebericho* possessed significant leishmanicidal activity against *L. major* and *L. tropica*. Dehydrocostus lactone (**1**), one of the major constituents of the essential oil and hexane fraction, was more active than the standard drug miltefosine against *L. major* and *L. tropica* promastigotes. The presence of α-methylene, γ-lactone is the responsible moiety of dehydrocostus lactone towards the leishmanicidal activity against the tested *Leishmania* species. The MTT assay of dehydrocostus lactone showed inactive toxicity against the human cervical carcinoma HeLa cells. In addition, dehydrocostus lactone exhibits a broad spectrum of antibiotic activities. Based on this interesting finding, dehydrocostus lactone was identified as a potential lead for treating infections caused by *Leishmania*.

1. Introduction

Leishmaniasis infection is a major health problem in tropical regions. It is caused by more than 20 species of the protozoa genus *Leishmania*. Over 90 species of sandflies serve as transmitting vectors, and more than 70 animal species including humans are zoonotic hosts [1]. The involvement of several strains of *Leishmania* makes their diseases difficult to control and treat various forms of leishmaniasis. Worldwide, 50,000 to 90,000 and 600,000 to 1 million cases of visceral and cutaneous forms of leishmaniasis, respectively, are reported annually [1]. Cutaneous leishmaniasis is the most common form in Ethiopia, with an estimated 20,000 to 50,000 cases

---

* Corresponding author.
E-mail addresses: bekrimelka2003@yahoo.com, bekri.melka@eiar.gov.et (B.M. Abdo).

annually, and *L. aethiopica*, *L. tropica*, and *L. major* are the causative agents [2].

Affordable, safe, and short-course treatments are required for the successful treatment of leishmaniasis, as per the World Health Organization [3]. Currently, pentavalent antimonial, paromomycin, amphotericin B, liposomal amphotericin, pentamidine, and miltefosine are generally used. These drugs have numerous limitations such as drug resistance, high cost, low availability, toxicity, and painful routes of administration [4–6]. Therefore, it is important to search for new leishmanicidal compounds with good efficacy and safety. Medicinal plants are excellent sources of secondary metabolites with diverse biological activities [7]. *Echinops kebericho* Mesfin is an endemic and endangered Ethiopian medicinal plant belonging to the genus *Echinops* and family Asteraceae. Its roots are traditionally used for the treatment of various ailments, such as dispelling nightmares in children, constipation, headache, heart pain, stomachache, typhus, as a fumigant after childbirth, intestinal pains, lung TB, leprosy, syphilis, cough, ward off the evil eye, toothache, and vomiting [8–14]. Additionally, it is used as a snake and mosquito repellant [15,16]. The organic solvent extracts from the roots of *Echinops kebericho* have been identified as antimalarial, antimicrobial, antileishmanial, antischistosomal, antidiarrheal, and spasmolytic active agents [17–22].

The essential oil, extracted from the roots of *E. kebericho*, possesses constituents, such as eudesm-7 (11)-en-4-ol, caryophyllene oxide, τ-cadinol, β-cubebene, β-patchoulene, longifolene, cyperene, dehydrocostus lactone, β-phellandrene, germacrene B, α-selinene, isoshyobunone, modephene, α-pinene, and β-pinene [23,24]. Moreover, modephene, lappaconitine, pyrocurzerenone, dehydrocostus lactone, dihydrodeydrocostus lactone, 3aR,6aR,9aR,9bR)-decahydro-4 hydroxy3,6,9-trimethyleneazuleno[4,5-b] furan-2(9bH)-one, β-sitosterol, stigmasterol, campesterol, β-amyrene, lupeol, and ursolic acid, together with a series of fatty acids are obtained from the solvent extract of *E. kebericho* roots [25–28].

The essential oil, petroleum ether, and chloroform extracts obtained from the roots of *E. kebericho* possess activity against several *Leishmania* species [22]. The biological activities of the isolated ingredients from the roots of *E. kebericho* are not adequately studied. The current study aimed to evaluate the leishmanicidal activity of the crude fractions and isolated compounds against the promastigote stage of *L. major* and *L. tropica* strains. The in vitro leishmanicidal and cytotoxic activities were evaluated through a 96-well plate assay with microscope detection and 3- [4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide assay, respectively [29–31].

2. Materials and methods

2.1. Plant collection and processing

The roots of *Echinops kebericho* Mesfin were collected from the experimental field of Wendo Genet Agricultural Research Center, Ethiopia (1819 m a.s.l., 07°05′664″ N, 038°38′897″ E). All the steps, such as extraction, isolation, structure elucidation, and biological activities, were conducted at the H.E.J. Research Institute (ICCBS), Pakistan.

2.2. Preparation of plant sample

The matured (12 months old) *E. kebericho* roots (500 g) were harvested. The roots were washed with tap water, then chopped and dried in the shade. The dried roots were crushed with mortar and pestle. The powdered sample was stored in a plastic bag until extraction.

2.3. Essential oil extraction

The powdered roots (200 g) were distilled through hydro distillation by using a Clevenger-type apparatus for 4 h. The distillate was collected using a measuring pipette and stored in cleaned amber vials after drying with anhydrous Na_2SO_4. The essential oil yield was calculated according to the formula described in Equation (1). The essential oil was subjected to the antileishmanial activity test and chemical composition analysis (Fig. 1).
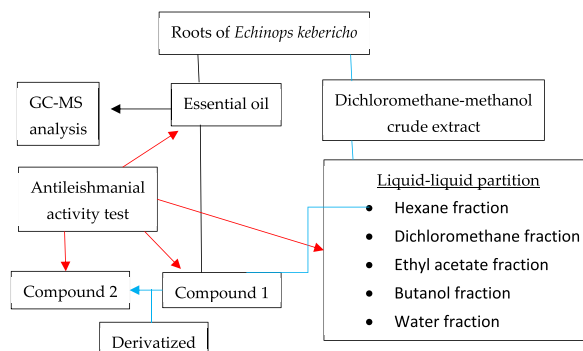
Fig. 1. Diagram showing the workflow of extraction, compound isolation (blue line), and antileishmanial activity tested samples (red arrow). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

$$\text{Essential oil yield} \left(\frac{w}{w}(\%)\right) = \frac{\text{Amount of distilled oil (g)}}{\text{Amount of the root sample (g)}} \times 100 \tag{1}$$

2.4. Chemical composition analysis of essential oil

A GC-MS (Agilent model 7890 A, Agilent, Santa Clara, CA, USA) equipped with GC sampler 120 was used to determine the chemical profile of the essential oil. The instrument was conditioned with a split/splitless injector mode, MS detector (7000 Triple Quad, Agilent, Santa Clara, CA, USA), and Zebron ZB-5 capillary column (320 μm internal diameter × 30 m length × 0.25 μm film thickness, Phenomenex, USA). The injector was operated in a split ratio of 1:20 with an injection volume of 1.2 μL. The injector and detector temperature were set at 250 °C. The MSD was operated on scan mode in the m/z 40–700 range, and the interface temperature was set to 260 °C. Helium was used as a carrier gas in a controlled constant flow mode at a linear velocity of 44.64 cm/s. The oven was programmed to start at 50 °C and held for 5 min; then, the temperature was ramped at 3 °C/min to 200 °C and held for 15 min; subsequently, the temperature was ramped at 10 °C/min to 300 °C and held for 20 min. The solvent delay time was 5 min and took 100 min for the total run.

2.5. Crude extraction and fractionation

The powdered sample of *E. kebericho* roots (892.1 g) was macerated and divided into two 2000 mL volume round bottom flasks using a mixture of dichloromethane and methanol (1:1) solvents (1600 mL for each flask) for 48 h with periodical shaking. The extracted solution was filtered using a muslin cloth, and the residue was repeatedly extracted with 800 mL maceration solvent. All the extract solutions were collected and filtered with filter paper and finally concentrated using a rotary evaporator. The crude extract was added into a 2000 mL separatory flask containing 500 mL distilled water for further partitioning with liquid–liquid extraction using different organic solvents, such as hexane, dichloromethane, ethyl acetate, and n-butanol, starting from a low to high polarity gradient. The extraction was achieved with 1000 mL solvent in the separatory flask having crude extract in distilled water. The extraction was repeated four times with 1000 mL organic solvent. Hexane, dichloromethane, ethyl acetate, and n-butanol solvent extracts were obtained. For dichloromethane solvent, the lower layers were collected and concentrated. Finally, the remaining water extract was concentrated using a freeze drier. The extract yield was calculated according to the formula described in Equation (2).

$$\text{Extract yield} \left(\frac{w}{w}(\%)\right) = \frac{\text{Amount of extract (g)}}{\text{Amount of the root sample (g)}} \times 100 \tag{2}$$

2.6. Isolation of compounds

The major compounds were isolated by using column chromatography (column diameter of 4 cm, silica gel of 70–230 pore size (Merck, Darmstadt, Germany), and a 40 cm pack length). Hexane was used to prepare the slurry of silica gel for column packing. The hexane fraction (5 g) was loaded into the column. Gradient elution of a mobile phase was used starting from 200 mL hexane and continued with 1000 mL hexane–ethyl acetate (95:5); 1000 mL hexane–ethyl acetate (90:10) and 1000 mL hexane–ethyl acetate (86:14). An eluate of 100 mL volume was collected as one fraction and transferred to vials (20 mL) after being concentrated. The purity of the isolated fraction was checked with TLC through a hexane–ethyl acetate (86:14) solvent system. The spots on the TLC were detected with UV light at 254 nm and 366 nm wavelengths for UV-active compounds. The non-UV-active compounds were stained by using a vanillin–sulfuric acid reagent. Finally, the purified compounds were submitted to spectral analysis of EI-MS (JEOL JSM 600H-1, Jeol, Japan) and ^{1}H NMR (Avance NEO, 500 MHz, Bruker, Switzerland).

2.7. Derivatization of dehydrocostus lactone

Michael addition was performed to derivatize the α-β unsaturation of dehydrocostus lactone (**1**) [32]. Dehydrocostus lactone (140 mg) was dissolved in hexane (HPLC grade) and mixed through a vortex shaker. The solution was boiled in a water bath for 2 min, and 140 μL of 2N potassium hydroxide in methanol (HPLC grade) was added. The solution was mixed with a vortex shaker for 30 s and centrifuged. The supernatant hexane solution was separated, and the derivatized compound was checked with TLC through a hexane–ethyl acetate (86:14) solvent system. The derivatized compound was purified using column chromatography (column diameter of 10 mm, a silica gel (flash, 20 g), (Merck, Darmstadt, Germany)). Finally, the purified derivative compound was submitted to spectral analysis of EI- MS (JEOL JSM 600H-1, Jeol, Japan) and NMR (^{1}H, ^{13}C, DEPT (135° and 90°), HSQC, and HMBC) (Avance NEO, 500 MHz, Bruker, Switzerland).

2.8. Antileishmanial activity test

An in vitro test with a 96-well plate assay described by Bouyahya et al. (2018) [33] was used for antileishmanial activity against *Leishmania major* 50155 (ATCC) and *Leishmania tropica* 50129 (ATCC). *Leishmania* promastigotes were grown in RPMI-1640 media with 10 % fetal bovine serum. The parasites at the log phase were centrifuged at 200 rpm for 10 min, and the supernatant was discarded. Fresh media was added to dilute the pallet material till the final density of 10^6 cells/mL. The media (100 μL) was added in all well tissue culture plates except the first column, which added 180 μL. The last two rows were used for negative (5 % DMSO in media)

and positive control (Amphotericin B, Pentamidine, and Miltefosine). The test samples (1 mg) (fractioned extract, essential oil, and isolated compounds) (Fig. 1) were dissolved in 50 μL DMSO and diluted with 950 μL of RPMI-1640 media. The tested solution (20 μL) was added to the first well plate and mixed. A serial dilution of the next well plate was followed. The plates were incubated in the dark at 23 °C for 72 h. After 72 h, the antileishmanial activities of the fractioned extract, essential oil, isolated compounds, and standard drugs were assessed microscopically using an improved Neubauer chamber.

2.9. Cytotoxicity activity test

Cytotoxic activities of the extracts and isolated compound were evaluated in 96-well flat-bottomed microplates by using the standard MTT (3- [4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) colorimetric assay [34]. HeLa cells (Human Cervical Cancer) were cultured in Minimum Essential Medium Eagle, supplemented with 5 % of fetal bovine serum (FBS), 100 IU/mL of penicillin and 100 μg/mL of streptomycin in 75 cm^2 flasks, and kept in 5 % CO_2 incubator at 37°C. Exponentially growing cells were harvested, counted with a hemocytometer and diluted with a particular medium. Cell culture with a concentration of 6x10^4 cells/mL was prepared and introduced (100 μL/well) into 96-well plates. After overnight incubation, the medium was removed and 200 μL of fresh medium was added with different concentrations of test samples (1–30 μM). After 48 h, 200 μL of MTT (0.5 mg/mL) was added to each well and incubated further for 4 h. Subsequently, 100 μL of DMSO was added to each well. The extent of MTT reduction to formazan within cells was calculated by measuring the absorbance at 570 nm, using a microplate reader (Spectra Max plus, Molecular Devices, CA, USA). The cytotoxicity was expressed as concentration causing 50 % growth inhibition (IC$_{50}$) for HeLa cell. The results of percent of inhibitions were processed by using Soft- Max Pro software (Molecular Device, USA). The percent of inhibition was calculated by using the formula described in Equation (3).

$$Inhibition\ (\%) = 100 - \left(\left(\frac{Absorbance\ of\ test\ sample - Absorbance\ of\ negative\ control}{Absorbance\ of\ positive\ control - Absorbance\ of\ negative\ control} \right) \times 100 \right) \tag{3}$$

2.10. Statistical analysis

The significance variation of the IC$_{50}$ value from leishmanicidal and cytotoxicity activities of the extracts, isolated compounds, and the standard drugs were determined by F test. The means and standard deviations were used for data analysis.

3. Results and discussion

Hydro-distillation of dried roots of *E. kebericho* yielded 0.16 % (*w/w*) of essential oil. The IC$_{50}$ value of the leishmanicidal and cytotoxicity activity test showed that highly significant difference between the tested samples and the standards at p < 0.001 (Table 1). The oil showed in vitro leishmanicidal activity with an IC$_{50}$ value of 38.3 μg/mL against *L. major* and 55.16 μg/mL against *L. tropica* (Table 2). Furthermore, the essential oil profile was characterized, and a total of 43 compounds were identified (Table 4). Dehydrocostus lactone (**1**) (24.95 %), β-guaiene (11.13 %), cis lanceol (5.92 %), Juniperol (4.98 %), (±)-cadinene (3.94 %), δ-neoclovene (3.68 %), aromadendrene oxide (3.29 %), and caryophyllene (3.05 %) were the major constituents of the essential oil.

Dichloromethane–methanol maceration of the dried roots of *E. kebericho* yielded a crude extract of 10.38 % (92.63 g). Different yields of fractions were obtained through liquid-liquid partition, such as hexane (55.61 g), dichloromethane (8.53 g), ethyl acetate (4 g), n-butanol (11.70 g), and water fraction (12.79 g). From the in vitro assay, the hexane fraction showed an antileishmanial activity with IC$_{50}$ values of 33.3 and 36.6 μg/mL against *L. major* and *L. tropica*, respectively. All other crude fractions were not active against the tested *Leishmania* strains. The dichloromethane fraction was not tested due to solubility issues.

The major compound from the hexane fraction was isolated using column chromatography, with an Rf value of 0.57 through elution with the solvent system of hexane–ethyl acetate (86:14). Compound **1** was a colorless crystalline solid. Its structure was identified using EI-MS, which gave a molecular mass ion peak ([M]$^+$) at *m/z* 230.2, having the molecular formula C$_{15}$H$_{18}$O$_2$. The ^{1}H NMR spectra (500 MHz, CDCl$_3$) showed peaks at δ(ppm): 6.19 (1H, d, *J* = 3.5 Hz, H-13a), 5.46 (1H, d, *J* = 3.2 Hz, H-13b), 5.24 (1H, brs, *J* = 2.4 Hz, *J* = 1.5 Hz, H-15a), 5.04 (1H, brs, H-15b), 4.87 (1H, brs, H-14a), 4.79 (1H, brs, H-14b), 3.94 (1H, t, *J* = 9.3 Hz, H-6), 2.93 (1H, m, H-5), 2.89–2.79 (2H, m, H-3a, H-9a), 2.59–2.41 (3H, m, H-1, H-7, H-2a), 2.22 (1H, m, H-9b), 2.13 (1H, m, H-2b), 1.97–1.79 (2H, m, H-8a, H-8b), and 1.39 (1H, m, H-3b). From the spectral analysis and comparisons with the literature [35,36], the structure of compound **1** was characterized as dehydrocostus lactone (**1**), a sesquiterpene lactone of the guaianolides class (Fig. 2).

Compound **2** was a colorless, amorphous solid with an Rf value of 0.43 through elution with the solvent system of hexane–ethyl

Table 1
Descriptive statistics showing the significant variation of IC$_{50}$ value of crude extract, isolated compound, and the standard drugs from leishmanicidal and cytotoxicity activity test.

Activities	Tested organisms	Mean ± SE	Range	F test	CV (%)	LSD (α = 0.05)
Leishmanicidal	*L. major*	21.11 ± 0.12	3.39–38.3	***	1.40	0.79
	L. tropica	23.52 ± 0.15	3.41–55.16	***	1.58	0.88
Cytotoxicity	HeLa cell	9.37 ± 0.14	1.19–15.9	***	2.6	0.65

SE = standard error; *** = highly significant at p < 0.001; F test = fisher test; CV = coefficient of variance; LSD = list significant difference.

Table 2

In vitro antileishmanial activity test of crude extract, essential oil, and isolated compounds from the roots of *E. kebericho*.

| Type of Samples	Tested Samples	*L. major*	*L. tropica*
Crude extract	Hexane fraction	33.3 ± 0.7	36.6 ± 0.5
IC$_{50}$ (µg/mL) $\pm$ SD	Dichloromethane fraction	NT	NT
	Ethyl acetate fraction	>100ND	>100ND
	N-Butanol fraction	>100ND	>100ND
	Water fraction	>100ND	>100ND
	Essential oil	38.3 ± 0.8	55.16 ± 0.9
Compounds	Dehydrocostus lactone	15.3 ± 0.03	14.2 ± 0.2
IC$_{50}$ (µM) $\pm$ SD	13-Methoxy dehydrocostus lactone	>100ND	>100ND
	Amphotericin B	3.39 ± 0.03	3.41 ± 0.02
	Pentamidine	4.56 ± 0.01	4.56 ± 0.01
	Miltefosine	31.8 ± 0.2	27.2 ± 0.6

SD = standard deviation; NT = biological activity not tested; >100 = inactive against the tested biological activity; ND = not determined the statistical analysis.

acetate (86:14). The EI-MS gave a molecular mass ion peak ([M]$^{+}$) at *m/z* 262.16, having the molecular formula $C_{16}H_{22}O_3$. The ^{1}H NMR spectrum (500 MHz, CDCl$_3$) showed peaks at δ (ppm) ^{1}H: 5.17 (1H, brs, $J = 2.4$, $J = 0.8$ Hz, H-15a), 5.02 (1H, brs, $J = 2.3$, $J = 0.9$ Hz, H-15b), 4.85 (1H, brs, $J = 1.0$ Hz, H-14a), 4.75 (1H, brs, $J = 1.3$ Hz, H-14b), 3.91 (1H, t, $J = 9.3$ Hz, H-6), 3.68 (1H, d, $J = 9.8$, 4.4 Hz, H-13a), 3.62 (1H, d, $J = 9.8$, 3.3 Hz, H-13b), 3.35 (3H, s, OCH$_3$), 2.92–2.85 (1H m, H-5), 2.85–2.77 (1H, m, H-11), 2.57–2.31 (5H, m, H-3a, 9a, 2a, 1, 7), 2.21–2.12 (1H, m, H-9b), 2.05 (1H,m, H-2b), 1.98–1.87 (1H, m, H-8a), 1.83 (1H, m, H-8b) and 1.30 (1H, m, H-3b). The ^{13}C NMR (201 MHz, CDCl$_3$) δ 175.99 (=C=O, C-12), (=C = , C-4), 150.01 (=C = , C-10), 109.16 (=CH$_2$, H-15), 111.77 (=CH$_2$, C-14), 85.56 (O-CH, C-6), 69.11 (CH$_2$, C-13), 59.35 (O-CH$_3$), 51.82 (CH, C-11), 48.10 (CH, C-7), 47.04 (CH, C-5), 44.22 (CH, C-1), 37.75 (CH$_2$, C-2), 32.65 (CH$_2$, C-9), 32.58 (CH$_2$, C-3), and 30.2 (CH$_2$, C-8). The DEPT-135° ^{13}C NMR spectrum showed a total number of six CH$_3$ and CH carbons and seven CH$_2$ carbons. The DEPT-90° ^{13}C NMR spectrum showed a total of five CH carbons. The ^{1}H - ^{13}C correlation was confirmed by HSQC and HMBC spectra, respectively. From the spectral analysis results, compound **2** was characterized as 13-methoxy dehydrocostus lactone (**2**) (Fig. 2).

In vitro, a leishmanicidal test result of dehydrocostus lactone (**1**) showed IC$_{50}$ values of 15.3 µM for *L. major* and 14.2 µM for *L. tropica*. 13-Methoxy dehydrocostus lactone (**2**) was found inactive against the tested *Leishmania* species. The standard drugs showed IC$_{50}$ values of 3.39 and 3.41 µM (Amphotericin B), 4.56 µM (Pentamidine), and 31.8 and 27.2 µM (Miltefosine) against *L. major* and *L. tropica*, respectively.

The antileishmanial assay showed that the essential oil and hexane fraction of crude extract from the roots of *E. kebericho* have significant activity against *L. major* and *L. tropica*. Dehydrocostus lactone (**1**) is the most abundant constituent of the essential oil and hexane fraction of the crude extract from the roots of *E. kebericho*, which showed potent antileishmanial activity against *L. major* and *L. tropica* promastigotes. Similar results of the chemical composition of essential oils of the roots of *E. kebericho* were reported by Tariku et al. (2011) [24], which included dehydrocostus lactone (41.83 %), β-phellandrene (10.84 %), germacrene B (5.38 %), α-selinene (4.13 %), α-pinene (3.63 %), and β-pinene (3.62 %). A supportive result has been reported as the essential oils of *E. kebericho* showed a concentration-dependent growth inhibitory effects against promastigote forms of *L. donovani* (MIC = 0.0765 µL/mL) and *L. aethiopica* (MIC = 0.0097 µL/mL) [23].

Dehydrocostus lactone (**1**) is twice more active than the standard drug miltefosine against the tested *Leishmania* species. Amphotricin B is the most active standard drug against the tested *Leishmania* species. Some compounds, such as physalin B, 5β, 6β-epoxyphysalin B, and physalin H, have reported comparable antileishmanial activity with amphotericin B against *L. major* promastigotes with an IC$_{50}$ value (3.04–3.76 µM) [37]. Similarly, Azadbakht et al. (2020) [38] reported that compounds isolated from *Colchicum kurdicum* have antileishmanial activities against *L. major* promastigotes with an IC$_{50}$ value of colchicoside (0.22 µg/mL), colchicine (0.39 µg/mL), and the standard drug amphotricin B (0.12 µg/mL). Moreover, physalin B, 5β, 6β-epoxyphysalin B, and
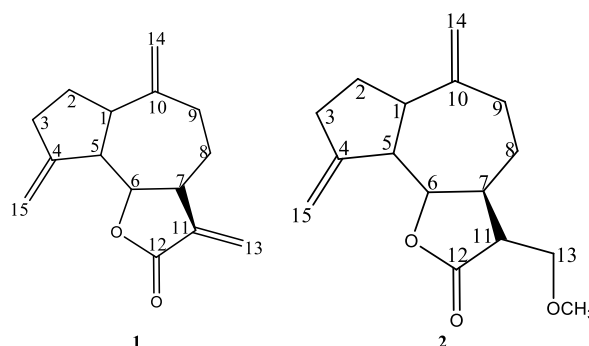
Fig. 2. Chemical structure of isolated compounds from the roots of *E. kebericho*; (**1**) Dehydrocostus lactone, (**2**) 13-Methoxy dehydrocostus lactone.

physalin (IC$_{50}$ = 9.59–18.53 μM) have comparable activities with dehydrocostus lactone (**1**) against *L. tropica* promastigotes [37].

The 13-methoxy-derivative of dehydrocostus lactone (**2**) is not active against the tested *Leishmania* species. From the structure-activity relationship (SAR) of dehydrocostus lactone (**1**) and 13-methoxy dehydrocostus lactone (**2**), the presence of α-methylene, γ-lactone is responsible for leishmanicidal activity against the tested *Leishmania* species. Similar results of the structure–activity relationship have been reported on the antitrypanosomal effects and cytotoxicity of dehydrocostus lactone [39]. However, the 13-amino derivative of dehydrocostus lactone has shown better anticancer activity and reduced cytotoxicity than dehydrocostus lactone [40].

The in vitro cytotoxicity activity test was determined with a cut of value of 50 % inhibition. The Tested samples having inhibition value of below 50 % was considered as inactive toxicity against the human cervical carcinoma HeLa cells. The hexane fraction and essential oil showed significant toxicity against the human cervical carcinoma HeLa cells with IC$_{50}$ value of 15.9 μg/mL and 11.03 μg/mL, respectively (Table 3). The reference standard doxorubicin was scored an IC$_{50}$ value of 1.19 μM. Dehydrocostus lactone (**1**) was non-toxic to the human cervical carcinoma HeLa cells.

Although, dehydrocostus lactone (**1**) showed inactive toxicity, a significant cytotoxicity of hexane fraction and essential oil were observed in this study. Previous studies reported that the essential oil of *E. kebericho* showed no mortality of rats in acute oral doses of up to 2000 mg/kg body weight and was well tolerated in 200 mg/kg sub-acute toxicity with repeated dose exposure [23]. Additionally, the decoction of *E. kebericho* root showed LD$_{50}$ greater than 5000 mg/kg in acute toxicity test and was well tolerated up to a dose of 600 mg/kg in sub-acute toxicity [41]. Moreover, an in vivo cytotoxicity test of dehydrocostus lactone (**1**) against laryngeal carcinoma showed inhibition of the growth of the Hep-2 nude mouse xenograft model with no significant signs of toxicity in the organs of nude mice [42]. The pharmacokinetics profile of dehydrocostus lactone in rat plasma after oral administration and intravenous administration have been reported as peak concentration (Cmax) (493.00 ng/mL and 5.79 μg/mL) and area under the plasma drug concentration-time curve (AUC) (7884.51 ng h/mL and1.87 μg h/mL), respectively [43,44].

Several researchers reported that dehydrocostus lactone (**1**) exhibits a range of biological activities such as anticancer activity against different cancer cells [45–48], and it has antibacterial [49], antifungal [50], anti-inflammatory [51,52], anti-allergy [53], and anti-depressant properties [54]. Ancklandia and berberine tablets are clinically available as dehydrocostus lactone-containing drugs, which have been used for the treatment of digestive tract diseases with its anti-inflammatory and anti-microbial activities [47]. Overall, the broad-spectrum antibiotic activities of dehydrocostus lactone could be a potential drug lead for co-infection caused by *Leishmania* and other pathogens.

Dehydrocostus lactone (**1**) transduces different mechanism of actions for various biological activities. Dehydrocostus lactone as an anticancer agent through inhibition of the NF-κB/COX-2 signaling pathway, causing cell cycle arrest, inducing apoptosis and differentiation, promoting the aggregation of microtubule protein, inhibiting the activity of telomerase, inhibiting metastasis, and invasion, reversing multidrug resistance, restraining angiogenesis [55–58]. Dehydrocostus lactone treats the inflammation through the inhibition of pro-inflammatory agents (IL1β, IL6, and TNFα), phosphorylation of p38 MAPK, degradation of IκBα, and the activation and nuclear translocation of NF-κB p65 [59,60]. Regarding dehydrocostus lactone (**1**) mechanism of action as antileishmanial activity has not yet been reported.

4. Conclusions

Dehydrocostus lactone (**1**) is the major constituent of essential oil and hexane fraction of the crude extract from the roots of *E. kebericho*. The leishmanicidal evaluation indicated that the roots of *E. kebericho* possess potent activity, most likely due to the presence of sesquiterpene lactones. Particularly, dehydrocostus lactone has shown more potent leishmaniacidal activity than the antileishmanial standard drug miltefosine. The present in vitro cytotoxicity activity test and previous study report showed that dehydrocostus lactone is non-toxic to normal cells and the human cervical carcinoma HeLa cells. Therefore, dehydrocostus lactone is expected to have a high value of selectivity and safety as a leishmanicidal agent. The presence of α-methylene, γ-lactone is the responsible moiety of dehydrocostus lactone towards the leishmanicidal activity against the tested *Leishmania* species. In addition, the broad-spectrum antibiotic activities of dehydrocostus lactone make this a potential drug lead against co-infection caused by *Leishmania* and other pathogens. The diverse range of antileishmanial activities of dehydrocostus lactone needs to be validated against the different *Leishmania* species both in vivo and in vitro tests. Further, the mechanism of action and potential side effects of dehydrocostus lactone should be tested.

Table 3
In vitro cytotoxicity activity test of crude extract, essential oil, and isolated compound from the roots of *E. kebericho*.

Tested Samples	Test Conc.	Inhibition (%)	IC$_{50}$± SD
Hexane fraction	30 μg/mL	92.91	15.90 ± 0.15 (μg/mL)
Dichloromethane fraction	NT	NT	NT
Ethyl acetate fraction	30 μg/mL	8.88	Inactive
N-Butanol fraction	30 μg/mL	15.77	Inactive
Water fraction	30 μg/mL	12.95	Inactive
Essential oil	30 μg/mL	91.66	11.03 ± 0.54 (μg/mL)
Dehydrocostus lactone	30 μM	46.67	Inactive
Doxorubicin	30 μM	98.7	1.19 ± 0.04 (μM)

Conc. = concentration.

Table 4
Chemical composition of essential oil from the roots of *E. kebericho*.

No.	RT (Minutes)	MW (g/mole)	MF	Name	Relative Concentration (%)
1	18.1	152	$C_{10}H_{16}O$	(S)-Cis-Verbenol	0.21
2	19.1	154	$C_{10}H_{18}O$	Borneol	0.18
3	23.5	154	$C_{10}H_{18}O$	p-Menth-2-en-7-ol, trans-	0.28
4	24.4	196	$C_{12}H_{20}O_2$	Borneol, acetate	2.08
5	26.7	204	$C_{15}H_{24}$	(±)-Cadinene	3.94
6	27.1	204	$C_{15}H_{24}$	δ-Neoclovene	3.68
7	27.5	204	$C_{15}H_{24}$	β-Elemene	2.67
8	28.4	204	$C_{15}H_{24}$	α-Guaiene	2.36
9	28.7	204	$C_{15}H_{24}$	Caryophyllene	3.05
10	30.5	204	$C_{15}H_{24}$	α-Humulene/α-Caryophyllene	1.36
11	30.8	204	$C_{15}H_{24}$	(−)-Alloaromadendrene	1.07
12	31.7	204	$C_{15}H_{24}$	γ-Muurolene	0.15
13	32.1	204	$C_{15}H_{24}$	β-Eudesmene/β-Selinene	0.11
14	32.4	204	$C_{15}H_{24}$	α-Selinene	0.43
15	33.5	204	$C_{15}H_{24}$	γ-Cadinene	2.19
16	33.7	204	$C_{15}H_{24}$	δ-Cadinene	0.33
17	34.1	204	$C_{15}H_{24}$	β-Guaiene	11.13
18	35.6	222	$C_{15}H_{26}O$	±-Trans-Nerolidol	0.48
19	37.7	220	$C_{15}H_{24}O$	Caryophyllene oxide	2.33
20	37.8	220	$C_{15}H_{24}O$	Aromadendrene oxide-(2)	0.74
21	38.2	220	$C_{15}H_{24}O$	Juniperol	4.98
22	38.7	220	$C_{15}H_{24}O$	Diepi-α-cedrene epoxide	0.37
23	38.9	222	$C_{15}H_{26}O$	Germacrene D-4-ol	1.84
24	39.1	222	$C_{15}H_{26}O$	Cubenol	0.18
25	39.8	222	$C_{15}H_{26}O$	τ-Cadinol	0.33
26	39.9	222	$C_{15}H_{26}O$	τ-Muurolol	1.32
27	40.1	222	$C_{15}H_{26}O$	δ-Cadinol, (−)-	0.08
28	40.3	222	$C_{15}H_{26}O$	α-Cadinol	1.10
29	40.5	220	$C_{15}H_{24}O$	γ-Gurjunenepoxide-(2)	0.90
30	40.8	204	$C_{15}H_{24}$	Globulol	0.33
31	41.0	220	$C_{15}H_{24}O$	Aromadendrene oxide-(1)	3.29
32	42.0	232	$C_{16}H_{24}O$	9-Methoxycalamenene	1.43
33	42.6	220	$C_{15}H_{24}O$	Cedren-13-ol, 8-	0.09
34	42.8	220	$C_{15}H_{24}O$	Ledene oxide-(II)	0.43
35	44.0	220	$C_{15}H_{24}O$	Lanceol, cis	5.92
36	44.6	220	$C_{15}H_{24}O$	Santalol, cis, α-	0.80
37	45.5	220	$C_{15}H_{24}O$	2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol	1.95
38	46.2	236	$C_{15}H_{24}O_2$	Bicyclo[4.4.0]dec-5-ene, 1,5-dimethyl-3-hydroxy-8-(1-methylene-2-hydroxyethyl-1)-	0.39
39	46.6	220	$C_{15}H_{24}O$	α-Copaen-11-ol	0.08
40	47.5	236	$C_{15}H_{24}O_2$	Bicyclo[4.4.0]dec-2-ene-4-ol, 2-methyl-9-(prop-1-en-3-ol-2-yl)-	0.18
41	49.0	230	$C_{16}H_{22}O$	Cycloisolongifolene, 8,9-dehydro-9-formyl-	0.20
42	50.5	202	$C_{15}H_{22}$	Aromadendrene, dehydro-	0.19
43	56.9	230	$C_{15}H_{18}O_2$	Dehydrocostus lactone	24.95

RT: Retention time; MW: molecular weight; MF: molecular formula.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Research.

References

[1] WHO (World Health Organization), Leishmaniasis report, Available online: https://www.who.int/news-room/fact-sheets/detail/leishmaniasis, 2023. (Accessed 8 March 2024).

[2] G.A. Amare, G.G. Mekonnen, M. Kassa, A. Addisu, D.A. Kendie, B. Tegegne, A. Abera, D. Tadesse, S. Getahun, Y.M. Wondmagegn, B. Merdekios, M.S. Asres, J. Van Griensven, G. Van der Auwera, S. van Henten, M. Pareyn, First report of cutaneous leishmaniasis caused by *Leishmania donovani* in Ethiopia, Parasites Vectors 16 (2023) 457, https://doi.org/10.1186/s13071-023-06057-9.

[3] WHO (World Health Organization), Regional strategic framework for accelerating and sustaining elimination of Kala-Azar in the South-East Asia Region 2022-2026. https://iris.who.int/bitstream/handle/10665/361887/9789290209812-eng.pdf?sequence=1, 2022 page 21.

[4] A.A. Hassan, H.E. Khalid, A.H. Abdalla, M.M. Mukhtar, W.J. Osman, T. Efferth, Antileishmanial activities of medicinal herbs and phytochemicals in vitro and in vivo: an Update for the Years 2015 to 2021, Molecules 27 (2022) 7579, https://doi.org/10.3390/molecules27217579.

[5] S. Tabrez, F. Rahman, R. Ali, A.S. Alouffi, B.M. Alshehri, F.A. Alshammari, M.A. Alaidarous, S. Banawas, A.A. Bin Dukhyil, A. Rub, Assessment of the antileishmanial potential of *Cassia fistula* leaf extract, Am. Chem. Soc. Omega 6 (2021) 2318–2327, https://doi.org/10.1021/acsomega.0c05629.

[6] P. Pokharel, R. Ghimire, P. Lamichhane, Efficacy and Safety of Paromomycin for Visceral Leishmaniasis : A Systematic Review, 2021.

[7] H. Lodish, A. Berk, C.A. Kaiser, M. Krieger, A. Bretscher, H. Ploegh, A. Amon, M. Scott, Molecular Cell Biology, seventh ed., W.H. Freeman and Company, New York, NY, USA, 2013, pp. 1119–1120.

[8] B. Abera, Medicinal plants used in traditional medicine by Oromo people, Ghimbi district, southwest Ethiopia, J. Ethnobiol. Ethnomed. 10 (2014) 40.

[9] E. d'Avigdor, H. Wohlmuth, Z. Asfaw, T. Awas, The current status of knowledge of herbal medicine and medicinal plants in Fiche, Ethiopia, J. Ethnobiol. Ethnomed. 10 (2014) 38.

[10] F. Mesfn, T. Seta, A. Assefa, An Ethnobotanical study of medicinal plants in Amaro woreda, Ethiopia, Ethnobot. Res. Appl. 12 (2014) 341–354.

[11] T. Teklehaymanot, M. Giday, Ethnobotanical study of medicinal plants used by people in Zegie Peninsula, northwestern Ethiopia, J. Ethnobiol. Ethnomed. 3 (2007) 12.

[12] T. Teklehaymanot, M. Giday, G. Medhin, Y. Mekonnen, Knowledge and use of medicinal plants by people around Debre Libanos monastery in Ethiopia, J. Ethnopharmacol. 111 (2007) 271–283.

[13] D. Abebe, A. Debella, K. Urga, Echinops kebericho, in: Medicinal Plants and Other Useful Plants of Ethiopia, Camerapix Publishers International, Addis Ababa, Ethiopia, 2003, p. 34.

[14] F. Fullas, Ethiopian Traditional Medicine: Common Medicinal Plants in Perspective, Sioux City first ed., 2001. F. Fullas.

[15] K. Karunamoorthi, T. Hailu, Insect repellent plants traditional usage practices in the Ethiopian malaria epidemic-prone setting: an ethnobotanical survey, J. Ethnobiol. Ethnomed. 10 (2014) 22, https://doi.org/10.1186/1746-4269-10-22.

[16] N. Asfaw, S. Demissew, Echinops kebericho, in: *Aromatic Plants Of Ethiopia*; Shama Books: Addis Ababa, Ethiopia, 2009, p. 77.

[17] B. Abdissa, Z. Ahmed, A. Yonas, Y. Moti, D. Asfaw, U. Geme, De S. Bart, S. Sultan, *Zingiber officinale* roscoe and *Echinops kebericho* Mesfin showed antiplasmodial activities against *Plasmodium Berghei* in a dose-dependent manner in Ethiopia, Ethiop J Health Sci 28 (2018) 655.

[18] Y. Alemu, Z. Mekonnen, A. Zeynudin, M. Yohannes, A. Biruksew, S. Suleman, Anti-schistosomal activities of Echinops kebericho Mesfin root and Hagenia abyssinica (Bruce) J.F Gmel flower part crude extracts in Swiss albino mice, Asian Pac. J. Tropical Med. 11 (2018) 570–575.

[19] G. Ameya, A. Gure, E. Dessalegn, Antimicrobial activity of *Echinops kebericho* against human pathogenic bacteria and fungi, Afr. J. Tradit., Complementary Altern. Med. 13 (2016) 199–203.

[20] A. Toma, S. Deyno, A. Fikru, A. Eyado, A. Beale, *In vivo* antiplasmodial and toxicological effect of crude ethanol extract of *Echinops kebericho* traditionally used in treatment of malaria in Ethiopia, Malar. J. 14 (2015) 196.

[21] F. Shiferie, W. Shibeshi, *In vivo* antidiarrheal and *ex-vivo* spasmolytic activities of the aqueous extract of the roots of Echinops kebericho Mesfin (Asteraceae) in rodents and isolated Guinea-pig ileum, International Journal of Pharmacy and Pharmacognocy 2 (2013) 110–116.

[22] Y. Tariku, In Vitro Efficacy Study of Some Selected Medicinal Plants against *Leishmania spp*. MSc Thesis, Addis Ababa University, Ethiopia, 2008, pp. 1–108.

[23] S. Deyno, M.A. Tola, J. Bazira, E. Makonnen, P.E. Alele, Acute and repeated-dose toxicity of *Echinops kebericho* Mesfin essential oil, Toxicol Rep 8 (2021) 131–138, https://doi.org/10.1016/j.toxrep.2020.12.027.

[24] Y. Tariku, A. Hymete, A. Hailu, J. Rohloff, In vivo evaluation of antileishmanial activity and toxicity of essential oils of *Artemisia absinthium* and *Echinops kebericho*, Chem. Biodivers. 8 (2011) 614–623, https://doi.org/10.1002/cbdv.201000331.

[25] A. Hymete, J. Rohloff, T.H. Iversen, H. Kjøsen, Volatile constituents of the roots of *Echinops kebericho* Mesfin, Flavour Fragrance J. 22 (2006) 35–38.

[26] M. Tadesse, B.M. Abegaz, AETFAT Proceedings, Symposium VI, Mittl. Inst. Allg. Bot. Hamberg, vol. 23, 1990, p. 605.

[27] A. Anbasie, Standardization of the Roots of *Echinops kebericho* Mesfin, vol. 1, Addis Ababa University, Ethiopia, 2019, p. 83. MSc. thesis.

[28] M.M. Woldeamanuel, S. Mohapatra, T.K. Bastia, P. Rath, A.K. Panda, Isolation and structural elucidation of a drug-leading bioactive new lactone from *Echinops Kebericho* MESFIN, J. Pharm. Negat. Results 14 (2023) 1079–1087, https://doi.org/10.47750/pnr.2023.14.02.139.

[29] M.M.Y. Chan, J.C. Bulinski, K.P. Chang, D. Fong, A microplate assay for *Leishmania* amazonensis promastigotes expressing multimeric green fluorescent protein, Parasitol. Res. 89 (2003) 266–271, https://doi.org/10.1007/s00436-002-0706-4.

[30] T. Mosmann, Rapid Colorimetric Assay for Cellular Growth and Survival : Application to Proliferation and Cytotoxicity Assays, vol. 65, 1983, pp. 55–63.

[31] G. Lu, L. Duan, S. Meng, P. Cai, S. Ding, X. Wang, Development of a colorimetric and turn-on fluorescent probe with large Stokes shift for H2S detection and its multiple applications in environmental, food analysis and biological imaging, Dyes Pigments 220 (2023) 111687, https://doi.org/10.1016/j.dyepig.2023.111687.

[32] Z. Liu, Michael addition reaction and its examples, Appl. Comput. Eng. 24 (2023) 1–6, https://doi.org/10.54254/2755-2721/24/20230669.

[33] A. Bouyahya, A. Et-Touys, N. Dakka, H. Fellah, J. Abrini, Y. Bakri, The antileishmanial potential of medicinal plant extracts from the north-west of Morocco, Beni-Suef Univ. J, Basic Appl. Sci. 7 (2018) 50–54, https://doi.org/10.1016/j.bjbas.2017.06.003.

[34] B.S. Thavamani, M. Mathew, S.P. Dhanabal, Plants against HeLa Cell Line, vol. 33, 2013, pp. 81–84, https://doi.org/10.4103/0257-7941.139040.

[35] A. Bhushan, D. Rani, M. Tabassum, S. Kumar, P.N. Gupta, S. Gairola, A.P. Gupta, P. Gupta, HPLC-PDA method for quantification of bioactive compounds in crude extract and fractions of *Aucklandia costus* Falc. and cytotoxicity studies against cancer cells, Molecules 28 (2023) 4815, https://doi.org/10.3390/molecules28124815.

[36] A. Li, A. Sun, R. Liu, Preparative isolation and purification of costunolide and dehydrocostuslactone from *Aucklandia lappa* Decne by high-speed counter-current chromatography, J. Chromatogr. A 1076 (2005) 193–197, https://doi.org/10.1016/j.chroma.2005.04.042.

[37] S. Bano, M. Bibi, S. Farooq, H. Zafar, M. Shaikh, B.K. Khoso, S. Yousuf, M.I. Choudhary, Anti-leishmanial physalins-Phytochemical investigation, in vitro evaluation against clinical and MIL-resistant *L. tropica* strains and in silico studies, PLoS One 17 (2022) e0274543, https://doi.org/10.1371/journal.pone.0274543.

[38] M. Azadbakht, A. Davoodi, S.J. Hosseinimehr, M. Keighobadi, M. Fakhar, R. Valadan, S. Emami, M. Azadbakht, A. Bakhtiyari, Tropolone alkaloids from *Colchicum kurdicum* (Bornm.) Stef. (Colchicaceae) as the potent novel antileishmanial compounds; purification, structure elucidation, antileishmanial activities and molecular docking studies, Exp. Parasitol. 213 (2020) 107902, https://doi.org/10.1016/j.exppara.2020.107902.

[39] S. Zimmermann, G. Fouché, M. De Mieri, Y. Yoshimoto, T. Usuki, R. Nthambeleni, C.J. Parkinson, C. Van Der Westhuyzen, B.M. Kaiser, M. Hamburger, M. Adams, Structure-Activity relationship study of sesquiterpene lactones and their semi-synthetic amino derivatives as potential antitrypanosomal products, Molecules 19 (2014) 3523–3538, https://doi.org/10.3390/molecules19033523.

[40] D. Kemboi, M.K. Langat, X. Siwe-Noundou, T. Tshiwawa, R.W.M. Krause, C. Davison, C.J. Smit, J.A. de la Mare, V.J. Tembu, 13-Amino derivatives of dehydrocostus lactone display greatly enhanced selective toxicity against breast cancer cells and improved binding energies to protein kinases in-silico, PLoS One 17 (2022) e0271389, https://doi.org/10.1371/journal.pone.0271389.

[41] S. Deyno, A. Abebe, M.A. Tola, A. Hymete, J. Bazira, E. Makonnen, P.E. Alele, Acute and sub-acute toxicity of *Echinops kebericho* decoction in rats, BMC Complement. Med. Ther. 20 (2020) 2, https://doi.org/10.1186/s12906-019-2794-z.

[42] R. Zhang, J. Hao, Q. Wu, K. Guo, C. Wang, W.K. Zhang, W. Liu, Q. Wang, X. Yang, Dehydrocostus lactone inhibits cell proliferation and induces apoptosis by PI3K/Akt/Bad and ERS signalling pathway in human laryngeal carcinoma, J. Cell Mol. Med. 24 (2020) 6028–6042, https://doi.org/10.1111/jcmm.15131.

[43] J. Zhang, X. Hu, W. Gao, Z. Qu, H. Guo, Z. Liu, C. Liu, Pharmacokinetic study on costunolide and dehydrocostuslactone after oral administration of traditional medicine *Aucklandia lappa* Decne, by LC/MS/MS, J. Ethnopharmacol. 151 (2014) 191–197, https://doi.org/10.1016/j.jep.2013.10.024.

[44] Z. Peng, Y. Wang, X. Gu, X. Guo, C. Yan, Study on the pharmacokinetics and metabolism of costunolide and dehydrocostus lactone in rats by HPLC-UV and UPLC-Q-TOF/MS, Biomed, Chromatogr 28 (2014) 1325–1334, https://doi.org/10.1002/bmc.3167.

[45] F.B. Tessema, Y.H. Gonfa, T.B. Asfaw, M.G. Tadesse, A.J. Bachheti, A.N. Singab, R.K. Bachheti, Dehydrocostus lactone from the root of *Ajuga integrifolia* (Buch.-Ham. Ex D. Don): quantitative determination and in- silico study for anti-breast cancer activity, Plant Sci. Today 11 (2023) 34–44, https://doi.org/10.14719/pst.2344.

[46] Q. Li, Z. Wang, Y. Xie, H. Hu, Antitumor activity and mechanism of costunolide and dehydrocostus lactone: two natural sesquiterpene lactones from the Asteraceae family, Biomed. Pharmacother. 125 (2020) 109955, https://doi.org/10.1016/j.biopha.2020.109955.

[47] J. Wang, Z. Yu, C. Wang, X. Tian, X. Huo, Y. Wang, C. Sun, L. Feng, J. Ma, B. Zhang, Q. Yang, X. Ma, Y. Xu, Dehydrocostus lactone, a natural sesquiterpene lactone, suppresses the biological characteristics of glioma, through inhibition of the NF-κB/COX-2 signaling pathway by targeting IKKβ, Am. J. Cancer Res. 7 (2017) 1270–1284.

[48] Y.L. Hsu, L.Y. Wu, P.L. Kuo, Dehydrocostuslactone, a medicinal plant-derived sesquiterpene lactone, induces apoptosis coupled to endoplasmic reticulum stress in liver cancer cells, J. Pharmacol. Exp. Therapeut. 329 (2009) 808–819, https://doi.org/10.1124/jpet.108.148395.

[49] Y.X. Wu, F.J. Jiang, G. Liu, Y.Y. Wang, Z.Q. Gao, S.H. Jin, Y.J. Nie, D. Chen, J.L. Chen, Q.F. Pang, Dehydrocostus lactone attenuates methicillin-resistant *Staphylococcus aureus*-induced inflammation and acute lung injury via modulating macrophage polarization, Int. J. Mol. Sci. 22 (2021) 9754, https://doi.org/10.3390/ijms22189754.

[50] J. Zhang, J. Sun, Y. Zhang, M. Zhang, X. Liu, L. Yang, Y. Yin, Dehydrocostus lactone inhibits *Candida albicans* growth and biofilm formation, Amb. Express 13 (2023) 82, https://doi.org/10.1186/s13568-023-01587-y.

[51] A. Paço, T. Brás, J.O. Santos, P. Sampaio, A.C. Gomes, M.F. Duarte, Anti-inflammatory and immunoregulatory action of sesquiterpene lactones, Molecules 27 (2022) 1142, https://doi.org/10.3390/molecules27031142.

[52] Q. Zhou, W.X. Zhang, Z.Q. He, B.S. Wu, Z.F. Shen, H.T. Shang, T. Chen, Q. Wang, Y.G. Chen, S.T. Han, The possible anti-inflammatory effect of dehydrocostus lactone on DSS-induced Colitis in Mice, Evid.-Based Complement, Altern. Med. (2020) 5659738, https://doi.org/10.1155/2020/5659738.

[53] C.S. Seo, H.S. Lim, S.J. Jeong, H.K. Shin, Anti-allergic effects of sesquiterpene lactones from the root of *Aucklandia lappa* Decne, Mol. Med. Rep. 12 (2015) 7789–7795, https://doi.org/10.3892/mmr.2015.4342.

[54] T. Abebe, A. Hymete, M. Giday, D. Bisrat, Antidepressant-like activity and molecular docking analysis of a sesquiterpene lactone isolated from the root bark of *Ximenia americana* L, Evid.-Based Complement, Altern. Med. (2024) 6680821, https://doi.org/10.1155/2024/6680821.

[55] R. Zhang, J. Hao, Q. Wu, K. Guo, C. Wang, W.K. Zhang, W. Liu, Q. Wang, X. Yang, Dehydrocostus lactone inhibits cell proliferation and induces apoptosis by PI3K/Akt/Bad and ERS signalling pathway in human laryngeal carcinoma, J. Cell Mol. Med. 24 (2020) 6028–6042, https://doi.org/10.1111/jcmm.15131.

[56] J. Wang, Z. Yu, C. Wang, X. Tian, X. Huo, Y. Wang, C. Sun, L. Feng, J. Ma, B. Zhang, Q. Yang, X. Ma, Y. Xu, Dehydrocostus lactone, a natural sesquiterpene lactone, suppresses the biological characteristics of glioma, through inhibition of the NF-κB/COX-2 signaling pathway by targeting IKKβ, Am. J. Cancer Res. 7 (2017) 1270–1284.

[57] X. Lin, Z. Peng, C. Su, Potential anti-cancer activities and mechanisms of costunolide and dehydrocostuslactone, Int. J. Mol. Sci. 16 (2015) 10888–10906, https://doi.org/10.3390/ijms160510888.

[58] E. Jiang, X. Sun, H. Kang, L. Sun, W. An, Y. Yao, X. Hu, Dehydrocostus lactone inhibits proliferation, antiapoptosis, and invasion of cervical cancer cells through PI3K/Akt signaling pathway, Int. J. Gynecol. Cancer 25 (2015) 1179–1186, https://doi.org/10.1097/IGC.0000000000000474.

[59] Y.X. Wu, F.J. Jiang, G. Liu, Y.Y. Wang, Z.Q. Gao, S.H. Jin, Y.J. Nie, D. Chen, J.L. Chen, Q.F. Pang, Dehydrocostus lactone attenuates methicillin-resistant staphylococcus aureus-induced inflammation and acute lung injury via modulating macrophage polarization, Int. J. Mol. Sci. 22 (2021), https://doi.org/10.3390/ijms22189754.

[60] F. Zhao, Y. Yang, S. Seesregdorj, A. Shiirevnyamba, A. Tovuudorj, A. Gura, Y. Zhang, The potential mechanisms of dehydrocostus lactone in treating the inflammatory processes in lipopolysaccharide-activated microglial cells: a transcriptome analysis, Int. J. Clin. Exp. Med. 13 (2020) 6434–6447.