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Original article

Chemical Composition, Cytotoxicity and Larvicidal Activity of Essential Oils of Three Medicinal Plants of Ethiopian Flora Against *Anisakis* L_3 Larvae

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Abstract

Background and objectives: *Uvaria scheffleri* Diels (Annonaceae), *Zanthoxylum chalybeu*m Engl. (Rutaceae), and *Vepris dainelli* (Pichi-Serm.) Kokwaro (Rutaceae) are medicinal plants traditionally used in Ethiopia against pathogenic infections. In the present study, the chemical composition, larvicidal activity, and cytotoxic effect of essential oils were investigated. **Methods**: Hydrodistillation technique was used to extract essential oils. In vitro larvicidal activity against *Anisakis* L_3 larvae was tested in marinated solutions. MTT assay was used to assess the cytotoxicity. **Results**: The yields (v/w) of essential oils obtained from *U. scheffleri* roots, *Z. chalybeum*, and *V. dainelli* fruits were 0.5, 2.7, and 2.0 %, respectively. Gas chromatography-mass spectrometry analysis of essential oils revealed a total of 58, 18, and 20 chemical constituents, representing 97.6, 99.6, and 98.8 % of the oil contents, respectively. Tricyclo [5.3.0.0(3, 9)] decane was identified to be the principal constituent in the essential oils of *Z. chalybeu*m (82.8%) and *V. dainelli* (69.8%), reported herein for the first time. Essential oils of *Z. chalybeum*, *U. scheffleri*, and *V. dainelli* displayed a dose-dependent larvicidal activity with LT_{100} values of 3 h, 5 h, and 5 h for 5% concentrations, respectively. The cytotoxicity study of essential oils on VERO cells showed moderate toxicity with IC_{50} values of 65.46 µg/mL, 83.88 µg/mL, and 96.82 µg/mL, respectively. **Conclusion**: The results obtained revealed that the studied essential oils could serve as larvicidal agents in treating human anisakidosis. The observed weak cytotoxicity at low concentrations points out the possibility of developing effective and safe botanical larvicides.

Keywords: *Anisakis*; cytotoxicity; essential oils; larvicidal activity; marinade solutions

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Introduction

The consumption of raw seafood causes a wide range of health problems in humans. Among them, anisakiasis is one of the essential fish-borne zoonotic diseases. This parasitosis, caused by nematodes belonging to the genus *Anisakis*, can invade humans' stomach or intestinal wall [1]. The transmission is due to consuming certain raw or undercooked sea fish and cephalopods. The paratenic hosts of *Anisakis* and harboring the infective third stage (L_3) larvae [1,2], lead to gastrointestinal disorders and/or allergies in humans [3]. In some European countries, where

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the consumption of raw or undercooked sea products is increasing, human cases are increasingly reported [4].

The effective therapy is larval extirpation by endoscopy when the parasite is located in the digestive tract and is accessible by gastroscopy. Several authors have proposed antibiotics, anticholinergics and/or corticosteroids, and anthelmintic drugs to treat anisakiasis [5], but there is no clinically approved pharmacological treatment yet. Therefore, the response of *Anisakis* larvae to most common food preservation techniques, such as salting, marinating, or mild thermal treatments, needs to be assessed [6]. In recent years, much attention has been given to examining the activity of essential oils against L_3 larvae [7-10]. The flora of Ethiopia is very heterogeneous and diverse, with an estimated number of 6,500 to 7,000 species of higher plants, of which about 12% are endemic [11]. Among these species, *Uvaria scheffleri* Diels (Annonaceae), *Zanthoxylum chalybeum* Engl, and *Vepris dainelli* (Pichi-Serm) (Rutaceae) are among the most referred ones with a broad spectrum of traditional ethnobotanical uses in Ethiopia.

Zanthoxylum chalybeum, "Ga'da" (Sidamigna, Ethiopia) and knob wood (in English), is found in various locations of Ethiopia traditionally used to treat toothache, while its leaves are used to treat breast cancer in livestock [12]. Moreover, an infusion made of its roots is drunk to treat bacterial muscle infections, female sterility, venereal diseases and uterine fibroids [13]. It is also used to manage stomach problems, fever, and diarrhea [14]. Phytochemical studies of genus *Zanthoxylum* revealed that alkaloids, lignins, coumarins, flavonoids, and terpenes are common chemical constituents of the genus [15]. A recent study revealed that the essential oils of the plant leaves showed good antimicrobial activity [14].

Uvaria scheffleri, "Boyyiniya" (Wolaitigna, Ethiopia) is widely distributed in East Africa, growing in the coastal evergreen and mountain forests [16]. In Ethiopia, it grows in the highland area of the southern part of the country and is traditionally used for the treatment of skin infections. The fruit is edible [17]. In Kenya, *U. scheffleri* is used for the treatment of cough, tuberculosis, asthma, and sore throat [18]. In Tanzania, it is traditionally used for the treatment of fever [16].

Vepris dainelli, "Chawula" (Goffigna, Ethiopia), is a medium-sized endemic tree that grows in Ethiopia [19]. Its seeds are used to treat abdominal cramps, whereas its bark and fruit are used to treat intestinal worms, skin diseases and tooth pain [20].

To the best of our knowledge, antiparasitic activity against *Anisakis* larvae and cytotoxicity study of essential oils from the aforementioned plant species have not been explored yet. Thus, the present study presented chemical compositions, larvicidal activity against L_3 larvae of *Anisakis* type I, and cytotoxicity of the essential oils of *U. scheffleri* roots and the fruits of *Z. chalybeum* and *V. dainelli*.

Materials and Methods

Ethical considerations

The research and ethical committee board approved the study at Adama Science and Technology University, Ethiopia. No: ASTU, SoNS/2259298/2018. Date: 12/10/2018. Code: PGR031/18

Plant material

Roots of *Uvaria sheffleri*, fruits of *Zanthoxylum chalybeum*, and *Vepris dainelli* were collected from Wolaita, Sidama, and Gofa zones of Southern Nations, Nationalities, and Peoples' Region (SNNPR), Ethiopia in November 2018, respectively. Collected plant specimens were confirmed by a Botanist, Shambel Alemu, at the National Herbarium of Ethiopia, Addis Ababa University, Ethiopia (Voucher codes: MAUs-001/11, MAZc-002/11 and MAVd-003/11, respectively).

Freshly gathered fruits and roots were transported to the Organic Chemistry Laboratory of Wolaita Sodo University, Ethiopia using plastic bags and then washed in distilled water to remove unwanted foreign materials. The cleaned plant material was ground into powder using a mechanical grinder.

Extraction of essential oils

Powdered roots of *U. sheflerri*, fruits of *Z. chalybeum*, and *V. dainelli* (400 g of each) were soaked separately in 5 L distilled water, placed in a round bottom flask and subjected to hydrodistillation using a Clevenger apparatus for 5 h. The condensate (mixture of essential oil and water) was collected in a 100 mL separatory funnel. The essential oil was consecutively

separated from the aqueous layer, dried using anhydrous magnesium sulphate, transferred into small vials, and refrigerated (4 °C) until further analysis.

Chemical composition of essential oils

Essential oil samples were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) (7890A, Agilent-Technologies, USA) with an HP-5MS column (30 m in length, 250 μm in diameter, 0.25 μm in film thickness) which was coated with 5% phenyl and 95% methylpolysiloxane as stationary phase. The syringe was washed with 8 μL of chloroform, and then 2 μL of the essential oil solution in chloroform was injected through an autosampler and analyzed with the HP-5MS column. The column temperature was adjusted from 50 to 120 °C at 20 °C/min, 120 to 150 °C at 4 °C/min, 150 to 250 °C at 20 °C/min (with 10 min hold time) and 3.5 min solvent delay. The temperature of the injector was fixed at 325 °C and that of the detector (5977E MSD) at 350 °C. Helium (1 mL/min) was used as the carrier gas with 69.8 KPa and a split ratio of 100:1. The interface temperature was 280 °C. The mass spectra were recorded in electron impact mode at 70 eV, scanning from 40 to 600 m/z at 0.5 s and ion source temperature at 230 °C. The volatile components were identified by mass spectral comparison with the spectra of reference compounds at the National Institute of Standards and Technology (NIST) mass spectral library. The quantification of each component of essential oils was done by comparing their peak area to the total identified peaks by running GC analysis.

Anisakid nematode collection

The larvae were collected by dissecting the blue whiting specimens (*Micromesis tiuspoutassou*), a much-consumed fish and frequently parasitized by nematodes of the genus *Anisakis* [21]. The fish specimens were bought 1 h before from a local market of Burjassot (Valencia, Spain). All larvae used in the study were collected alive from the viscera of the dissected fish, were morphologically identified as L_3 larvae of *Anisakis* type I. According to morphological features, the worms were washed several times on a sterile solution of 0.9 % NaCl and identified under a light microscope. Only those actively moving helminths and without any injuries were placed in plastic Petri dishes with different concentrations of the test essential oils and then kept at room temperature.

In vitro larvicidal activity of essential oils

In vitro larvicidal activity of the essential oils against L_3 *Anisakis* larvae in marinade solution was tested. The marinade solution usually used to marinate raw fish for consumption was prepared [8], consisting of water/vinegar at a 1:1 ratio, 3% NaCl, and 1% citric acid. The solution was spiked with decreasing concentrations of essential oils: 5%, 1%, 0.5%, and 0.1%. Four larvae were placed on each plate. As a control, larvae were assayed without test compounds under identical experimental conditions in the marinade solution. Larvae were examined under the stereoscopic microscope at hourly intervals of 1 to 7 h, at 12 h, and 24 h to test the anthelmintic effect of the essential oils. During the experimental treatments, at each fixed time interval, the viability was checked [8], assessing the subsequent score: 3 (viable), 2 (reduction of mobility), 1 (mobility only after stimulation), and 0 (death). Larvae were considered dead when no mobility was observed in saline solution (0.9% NaCl) under the stereoscopic microscope. The normalized mean viability according to the previously described method [22], LT_{100} (lethal time required to kill 100% of nematodes), and LT_{50} (lethal time required to kill 50% of nematodes) were calculated. For the in vitro larvicidal activity measurements, the results were presented as mean percentages of three independent experiments, using a total of 104 larvae, 96 tested with essential oils and 8 as controls.

Cytotoxicity assay

Cell lines

In order to investigate the cytotoxic effect of essential oils, VERO cells (kidney normal cell line of monkey) were used. The cell lines were obtained from National Animal Health Diagnostic and Investigation Center (NAHDIC), Ethiopia. The cells were maintained in RPMI-1640 medium supplemented with 10% FBS, glutamine (2 raM), penicillin (100 units/mL), and streptomycin (100 μg/mL). The cells were cultured at 37 °C in a humidified 5% CO_2 incubator.

MTT assay

In vitro cytotoxic activity of essential oils was tested by MTT assay [23]. In MTT cytotoxicity assay, the MTT reduction rate is an indicator of the functional integrity of the mitochondria and hence of cellular viability. The VERO cells were seeded in a 96-well flat-bottom microtiter plate at a density of 2×10^4 cells/well and were allowed to adhere for 24 h at 37 °C in a CO_2 incubator. After 24 h of incubation, the culture medium was replaced with a fresh medium. The cells were then treated with different concentrations of essential oils (100, 50, 25, 12.5, 6.25, 3.125, 1.56, and 0.78 µg/mL) for 24 h at 37 °C in a CO_2 incubator. After 24 h of incubation, the culture medium was replaced with fresh medium. Then, 10 µL of MTT working solution (final concentration of 0.5 mg/mL) was added to each well and the plate was incubated for 4 h at 37 °C in a CO_2 incubator. The medium was then aspirated and the formed formazan crystals were solubilized by adding 50 µL of DMSO per well for 30 min at 37 °C in a CO_2 incubator. Finally, the intensity of the dissolved formazan crystals (purple color) was quantified using the ELISA plate reader at 530 nm and the cell viability was expressed as the percent cytotoxicity (% cytotoxicity). The results are presented as the mean of triplicate experiments.

The cell viability calculated as following equation:

$$\% \text{ cell viability} = (\text{Absorbance of treated}/\text{Absorbance of control}) \times 100$$

The half maximal inhibitory concentration (IC_{50}) values were determined from a concentration-response curve of the percentage of cell viability (Y-axis) versus log concentration (µg/mL) of essential oils (x-axis) by using GraphPad Prism Version 8.0.

Results and Discussion

The yield of essential oils obtained by hydrodistillation were 0.5, 2.7, 2.0 % (v/w) from the roots of *U. scheffleri,* fruits of *Z. chalybeum,* and fruits of *V. dainelli*, respectively. The GC-MS analysis of essential oils from roots of *U. scheffleri* revealed a total of 58 chemical components, accounting for 97.6% of the total compositions, of which sesquiterpenes (51.8%), oxygenated sesquiterpenes (18.9%), oxygenated monoterpenes (17.2%), monoterpenes (6.9%), and benzenoid compounds (5.2%) were identified (Table 1). The major constituents were alloaromadendrene (13.0%), β-maaliene (9.9%), (-)-borneol acetate (9.9%) and (-)-α-gurjunene (5.9%). The remaining constituents ranged from 0.10 to 5.1%. The results are comparable to a previous report by Oguntimein et al. (1989) [24], from leaves and root bark of *U. chamae*, which suggested that thymoquinoldimethyl ether and benzyl benzoate were the major constituents. Despite, previous reports from *Uvaria* essential oils out of Ethiopian flora, our findings on roots of *U. scheffleri* of Ethiopian species suggest that thymoquinoldimethyl ether was absent, whereas benzyl benzoate was found as a minor constituent.

GC and GC-MS analysis of essential oil *of Z. chalybeum* revealed a total of 18 chemical components (Table 2), accounting 99.6 % of the total compositions, of which the major amounts were oxygenated monoterpenes (44.4%), followed by monoterpenes (33.3%), sesquiterpenes (16.7%), and oxygenated sesquiterpenes (5.6%). The principal constituents were tricycle [5.3.0.0(3, 9)] decane (82.8%), followed by 2-tridecanone (2.8%), decanal (2.6%), Phenol, 2, 2'-methylene bis [6-(1, 1-dimethylethyl)-4-methyl- (2.40%), and limonene oxide trans-(2.2%). The remaining compounds ranged from 0.1 to 1.3%, in agreement with previous studies [25, 26] regarding classes of compounds reported from *Zanthoxylum* species.

A recent study showed the major constituents of leaves essential oils of this plant were β-phellanderene and β-myrcene [14]. In contrast, the present study on the fruits of *Z. chalybeum* revealed tricyclo [5.3.0.0(3, 9)] decane (82.8%) as a major constituent, reported herein for the first time from fruit essential oils of *Zanthoxylum* species and could be considered as a marker constituent for Ethiopian species.

GC-MS analysis of essential oils from fruits of *V. dainelli* revealed a total of 20 chemical compositions representing 98.8 % of the total contents. The major compounds were sesquiterpenes (55.0%) of the total composition, followed by monoterpenes (25.0%), oxygenated sesquiterpenes (10.3%) and benezenoid compounds (5%) (Table 3).

The major chemical constituents were tricyclo [5.3.0.0(3, 9)] decane (69.8%), caryophyllene (5.8%), phenol, 2, 2'-methylenebis [6-(1, 1-dimethylethyl)-4-methyl-(4.8%), neoalloocimen (3.5%), and germacrene D (3.20%). Other chemical components were detected in the range of 0.19-2.99%.

Table 1. Chemical composition of essential oil from roots of *Uvaria scheffleri*

No	Chemical compositions	Calculated KI	Reported KI	%
1	α-Pinene	929	932	0.406
2	Camphene	944	946	3.004
3	D-Limonene	1028	1030	0.435
4	δ-Terpinene	1043	1050	0.981
5	Eucalyptol	1050	1059	0.244
6	Bicyclo[2.2.1]heptane, 2-methoxy-1,7,7-trimethyl-	1101	1105	0.300
7	Neoalloocimene	1123	1130	0.238
8	(-)-Trans-myrtanyl acatate	1130	1137	0.139
9	Camphene hydrate	1141	1148	0.108
10	(-)-Borneol acetate	1140	1150	9.901
11	Endo-borneol	1148	1157	0.172
12	Fenchyl acetate	1201	1208	0.282
13	2-Pentanone, 5-(2- methylenecyclohexyl)-, stereoisomer	1240	1244	0.127
14	Bicyclo[2.2.1]heptane-2-carboxylic acid, 3,3-dimethyl-, methyl ester	1243	1252	0.432
15	Isobornyl acetate	1280	1286	0.486
16	α-Terpinyl propionate	1325	1332	0.733
17	(+)-Cyclosativene	1360	1368	1.581
18	Isoledene	1380	1385	0.416
19	Isolongifolenone	1382	1390	0.450
20	(-)-α-Gurjunene	1401	1405	5.933
21	α-Gurjunene	1401	1406	1.843
22	2,5-Dimethoxy-p-cymene	1412	1415	0.387
23	α-Copaene	1420	1426	1.135
24	ButylatedHydroxytoluene	1432	1438	0.073
25	α.-Guaiene	1440	1442	0.439
26	Guaia-9,11-diene	1442	1448	0.336
27	Clovene	1449	1454	0.213
28	11-Isopropylidenetricyclo [4.3.1.1(2,5)] undec-3-en-10-one	1450	1454	1.100
29	Neoisolongifolene, 8,9-dehydro-	1451	1458	5.185
30	γ-Amorphene	1465	1470	0.253
31	(+)-γ-Gurjunene	1466	1469	0.128
32	Naphthalene,1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	1469	1472	0.286
33	δ-Guaiene	1470	1476	2.1745
34	- (+)-β-Selinene	1480	1482	2.111
35	Rotundene	1481	1485	1.922
36	4-Isopropyl-1,6-dimethyl-1,2,3,4-tetrahydronaphthalene	1483	1487	0.117
37	1,1,7,7a-Tetramethyl-1a,2,6,7,7a,7b-hexahydro-1H-cyclopropa[a]naphthalene	1508	1513	4.584
38	7-Epi-α-selinene	1510	1517	0.232
39	1,5-Cadinadiene	1515	1524	0.254
40	(+)-δ-Cadinene	1519	1521	2.592
41	α -Maaliene	1520	1528	3.1539
42	β-Maaliene	1523	1530	9.983
43	Aristolediene	1535	1540	0.445
44	(-)-Gleenol	1568	1574	0.160
45	Neoisolongifolene	1570	1576	1.478
46	Aromadendrene, dehydro-	1610	1620	0.800
47	Guaiol	2082	2091	5.130
48	Epicubenol	1610	1617	0.567
49	Aromadendrene, dehydro-	1613	1620	0.822
50	Alloaromadendrene	1640	1649	12.905
51	Bulnesol	1650	1656	2.269
52	β-Guaiene	1660	1667	0.218
53	11,13-Dimethyl-12-tetradecen-1-ol acetate	1701	1708	0.1238
54	Benzyl Benzoate	1756	1760	4.101
55	α-Cadinene	1770	1779	3.164
56	3-Azepan-1-yl-benzo[d]isothiazole 1,1-dioxide	1782	1789	0.109
57	4-(p-Methoxyphenylazo)-m-phenylenediamine	1864	1870	0.264
58	Acetonitrile,2-(2-aminophenylamino)-	1890	1899	0.203

KI: Kovats index

Table 2. Chemical compositions of essential oil from fruits of *Zanthoxylum chalybeum*

No	Chemical compositions	Calculated KI	Reported KI	%
1	β-Thujene	935	941	0.50
2	Sabinene	973	980	1.13
3	Tricyclo[5.3.0.0(3,9)]decane	980	982	82.40
4	Octanal	981	988	0.83
5	β-Myrcene	983	985	1.03
6	β-Ocimene	1035	1041	0.60
7	Limonene oxide, trans-	1074	1079	2.17
8	Linalool	1084	1092	0.25
9	(±)-Dihydrocarvone	1187	1193	0.19
10	Decanal	1205	1212	2.62
11	Cis-Carveol	1206	1212	0.23
12	Trans-2-Dodecen-1-ol	1253	1255	0.25
13	2-Undecanone	1274	1280	1.30
14	β-Copaene	1426	1438	0.47
15	Germacrene D	1476	1479	0.29
16	2-Tridecanone	1497	1501	2.78
17	(-)-Isogermacrene D	1570	1580	0.11
18	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	1543	1550	2.40

Table 3. Chemical compositions of essential oils from fruits of *Vepris dainelli*

No	Chemical compositions	Calculated KI	Reported KI	%
1	(+)-α-Pinene	940	948	1.08
2	Tricyclo[5.3.0.0(3,9)]decane	980	983	69.86
3	D-Limonene	1011	1018	0.97
4	Cosmene	1020	1023	0.30
5	Neoalloocimene	1360	1367	3.50
6	β-Copaene	1371	1375	2.92
7	δ-Elemene	1370	1377	0.72
8	β-Elemene	1390	1398	0.90
9	Caryophyllene	1400	1405	5.85
10	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	1461	1464	0.44
11	ε-Muurolene	1471	1473	0.85
12	Germacrene D	1472	1476	3.21
13	2-Tridecanone	1475	1477	0.33
14	(Z)-γ-Bisabolene	1511	1512	1.38
15	β-Cubebene	1543	1545	0.61
16	τ-Cadinol	1650	1658	0.19
17	α-Cadinol	1652	1653	0.24
18	Cadina-3,5-diene	1660	1665	0.28
19	Phenol,2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	1677	1682	4.80
20	δ-Cadinene	1750	1758	0.39

The previous studies on leaves essential oils compositions of *Vepris* species suggested α-pinene (60.5%) [27] and terpinolene (49.7%) [28] as major constituents. On the contrary, the present study on the fruits of *V. dainelli* revealed that tricyclo [5.3.0.0(3, 9)] decane (69.9%) as major constituent reported herein for the first time in a *Vepris* species, suggesting this compound could be considered as marker constituent for Ethiopian *Vepris* species.

The larvicidal activity of the essential oils was evaluated in vitro against *Anisakis* type I. The observed larvicidal activity displayed a dose-dependent activity pattern for all studied essential oils. (Table 4, Figure 1).

The presented study revealed that essential oils from fruits of *Z. chalybeum, U. schefflerri*, and *V. dainelli* displayed potential larvicidal activity with LT_{100} values of 3 h, 5 h and 5 h for 5% concentrations, respectively. The larvicidal activity of essential oils might be related to the synergistic effects of the terpenes such as monoterpenes and sesquiterpenes identified in the essential oils, in agreement with previous reports [7,10], and the high activity could be attributed to the damage caused to the cuticle and digestive apparatus of the parasite.

Future studies should also include the mechanisms responsible for this larvicidal activity. The increase of worldwide prevalence of

anisakiasis and the lack of effective pharmacological treatments as well as the effectiveness of tested essential oils against *Anisakis* larvae suggest that the essential oils could be applied in the sea food marinating processes to prevent human anisakiasis. This initiative has interesting applications since marinated sea food products are always kept in oil after the marination process. The potential use of marinade solution enriched with essential oils of *U. scheffleri*, *Z. chalybeum*, and *V. dainelli* could represent, according to our results, an alternative method for the inactivation of Anisakidae larvae, but these results have to be backed up by in vivo studies.

Cell viability assay results on the VERO cell line revealed the level of cytotoxicity increased with the increasing concentrations or cytotoxic effects of each other in a concentration-dependent manner. According to Döll-Boscardin et al. (2012) [29], report the cytotoxic level of essential oils with the IC_{50} values between 10–50 μg/mL represent a strong cytotoxic activity, between 50–100, 100-200, and 200-300 μg/mL indicate moderate, weak, and very weak cytotoxic properties, respectively. Furthermore, IC_{50} values higher than 300 μg/mL represent no cytotoxicity. Considering this comparison, the essential oils of *U. sheffleri*, *Z. Chelybeum*, and *V. dainelli* demonstrated moderate cytotoxicty on VERO cell line, IC_{50} Values of 65.46 ± 0.48, 83.88±2.30, and 96.82±5.95 µg/mL, respectively.

Table 4. LT_{100} and LT_{50} of *Uvaria scheffleri*, *Zanthoxylum chalybeum*, Vepris dainelli essential oils

Plant time	Concentration	LT_{50} (h)	LT_{100} (h)
Uvaria scheflerri	5%	-	5
	1%	5	7
	0.5%	-	7
	0.1%	-	12
	Control	-	-
Zanthoxylum chalybeum	5%	-	3
	1%	5	7
	0.5%	5	7
	0.1%	5	12
	Control	-	-
Vepris dainelli	5%	-	5
	1%	-	7
	0.5%	-	12
	0.1%	-	24
	Control	-	-

(LT_{100}: lethal time required to kill 100% of nematodes; LT_{50}: lethal time required to kill 50% of nematodes

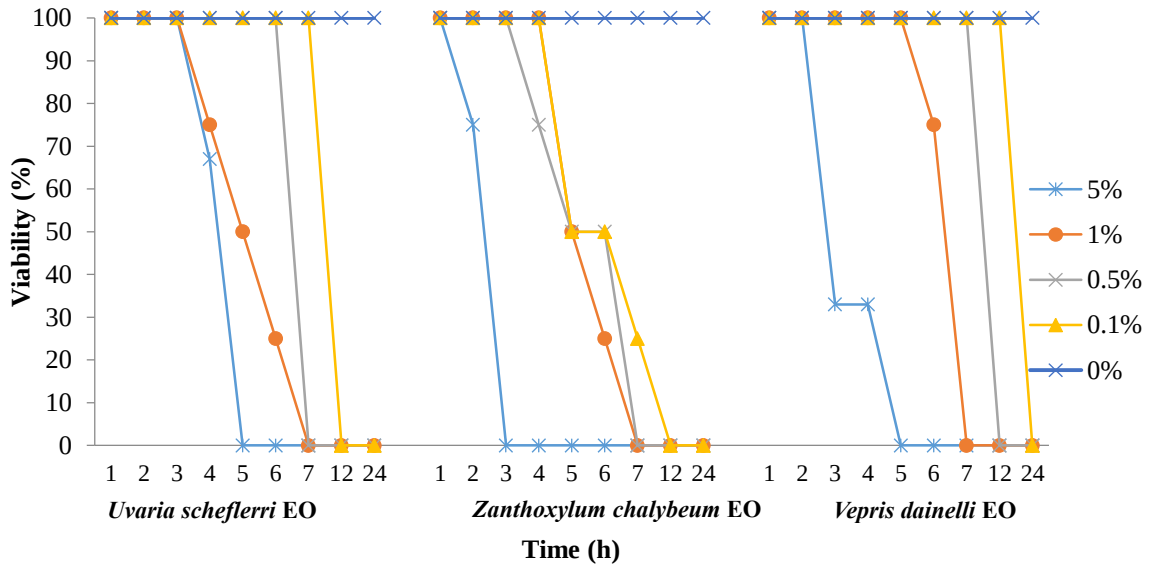

Figure 1. Viability (%) of L_3 larvae of *Anisakis* Type I in marinate solution with 0.1%, 0.5%, 1%, 5% of *Uvaria scheffleri*, *Zanthoxylum chalybeum*, and *Vepris dainell* essential oils (EO), respectively. Control larvae remained alive during the experiment

This result indicated that using the aforementioned EOs as a food preservative in marinating processes might serve as anti-Anisakiasis type I considering their weak cytotoxic effect at lower concentrations that possessed larvicidal properties.

Conclusion

The findings of our study highlighted that the essential oils obtained from three plant species showed promising larvicidal activity against *Anisakis* L_3 larvae and hence could serve as good ingredients in the development of products for the control of anisakiasis. Further studies on the safety (in vivo) and palatability of marinating with essential oils should be carried out. Notably, the observed bioactivity is related to the high content of monoterpenes and sesquiterpenes identified in the essential oils. Tricyclo [5.3.0.0(3, 9)] decane was a principal constituent in essential oils of *Z. chalybeu*m and *V. dainelli*, whereas alloaromadendrene in essential oils of *U. scheffleri*. These major constituents could be considered as chemotaxonomic markers for the identification of Ethiopian species. Essential oils from roots of *U. scheffleri*, fruits of *Z. chalybeum*, and *V. dainelli* displayed a dose-dependent larvicidal activity with LT_{100} values of 3 h, 5 h, and 5 h for 5% concentrations, respectively. The possibility of developing effective, eco-friendly, and safe botanical larvicides with these essential oils is promising, considering their weak cytotoxic effect at low concentrations.

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Author contributions

The authors have made substantive intellectual contributions to this study. Mathewos Anza participated in plant material collection, experimental work, preparation of the manuscript, and proofreading; Milkyas Endale, Luz Cardona, Diego Cortes Nuria Cabedo, Belen Abarca and were involved in preparation of the manuscript and proofreading; Maria Trelis and Màrius Vicent Fuentes participated in the experimental work, preparation of the manuscript, and proofreading

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

MTT: 3-(4, 5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide; DMSO: dimethylsulfoxide; GC-MS: gas chromatography-mass spectrometry; EOs: essential oils