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Larvicidal Potential of Essential Oils from Ethiopian Medicinal Plants against Anisakis L3 Larvae: Chemical Composition and Cytotoxic Evaluation

Aiko Tanaka[1], Yusuke Mori[1], Haruto Sakamoto[2]*, Keiko Fujii[2]

[1]Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Kobe University, Kobe, Japan.
[2]Department of Biotechnology, Faculty of Life Sciences, Waseda University, Tokyo, Japan.

***E-mail** ✉ haruto.sakamoto@gmail.com

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ABSTRACT

Medicinal plants used in Ethiopian traditional practices are an important source of bioactive compounds with potential antiparasitic properties. Uvaria scheffleri (Annonaceae), Zanthoxylum chalybeum, and Vepris dainelli (Rutaceae) have long been employed to manage infectious conditions; however, their essential oils have not been previously examined for activity against Anisakis larvae. Essential oils were extracted from the roots of U. scheffleri and the fruits of Z. chalybeum and V. dainelli by hydrodistillation. Chemical profiling was performed using gas chromatography coupled with mass spectrometry. Larvicidal efficacy against third-stage (L3) Anisakis larvae was evaluated in vitro using marinated systems, while cytotoxic effects were determined on VERO cell lines via the MTT colorimetric assay. The extraction process yielded 0.5% essential oil from U. scheffleri, whereas higher yields were obtained from Z. chalybeum (2.7%) and V. dainelli (2.0%). GC–MS analysis identified 58, 18, and 20 compounds in the respective oils, accounting for over 97% of their total compositions. Tricyclo[5.3.0.0(3,9)]decane was the predominant constituent in the oils of Z. chalybeum and V. dainelli, comprising 82.8% and 69.8%, respectively, and is reported here for the first time. All tested oils induced rapid, dose-related mortality in Anisakis L3 larvae, with complete lethality observed within 3 h for Z. chalybeum and within 5 h for U. scheffleri and V. dainelli at a concentration of 5%. Cytotoxicity assays revealed moderate effects on VERO cells, with IC_{50} values of 65.46, 83.88, and 96.82 µg/mL, respectively. The findings demonstrate that the essential oils of these Ethiopian medicinal plants possess strong larvicidal activity against Anisakis L3 larvae while exhibiting relatively low cytotoxicity. These properties support their potential development as safe, plant-derived larvicidal agents for the prevention and control of human anisakidosis.

Keywords: Cytotoxicity, Anisakis, Larvicidal activity, Marinade solutions, Essential oils

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Introduction

Dietary habits involving the consumption of uncooked or insufficiently processed marine products pose notable risks to human health. Among the diseases associated with this practice, anisakiasis represents a major fish-transmitted zoonotic infection. The condition arises from infection by parasitic nematodes of the genus Anisakis, which are capable of penetrating the gastric or intestinal tissues of humans after ingestion [1].

Human infection occurs following the intake of raw or lightly cooked marine fish and cephalopods that function as paratenic hosts, carrying infective third-stage (L3) larvae of Anisakis [1, 2]. Once introduced into the human digestive system, these larvae may trigger a range of clinical manifestations, including gastrointestinal disturbances and allergic responses [3]. The incidence of anisakiasis has increased notably in several European regions, coinciding with the growing popularity of raw or minimally processed seafood products [4].

At present, the primary therapeutic intervention involves the mechanical removal of larvae by endoscopy when parasites are accessible within the gastrointestinal tract. Although pharmacological agents such as antibiotics,

anticholinergics, corticosteroids, and anthelmintics have been proposed as alternative treatments [5], none have received formal clinical approval for anisakiasis management. This limitation has prompted increased investigation into the susceptibility of Anisakis larvae to food processing and preservation strategies, including marination, salting, and mild heat exposure [6]. Concurrently, essential oils derived from plant sources have gained attention due to their reported efficacy against Anisakis L3 larvae in recent studies [7–10].

Ethiopia is recognized as one of the most botanically diverse countries in Africa, hosting approximately 6,500–7,000 higher plant species, with an estimated 12% endemism [11]. Within this rich flora, Uvaria scheffleri Diels (Annonaceae), Zanthoxylum chalybeum Engl., and Vepris dainelli (Pichi-Serm.) Kokwaro (Rutaceae) are frequently cited in ethnomedicinal practices and are traditionally employed to manage a wide range of ailments.

Zanthoxylum chalybeum, commonly known as knob wood and locally referred to as "Ga'da" in Sidamigna, is widely distributed across Ethiopia. Traditional applications include the use of its bark for alleviating toothache, while its leaves are utilized in ethnoveterinary medicine to treat breast cancer in livestock [12]. Decoctions prepared from its roots are consumed to address bacterial muscle infections, reproductive disorders in women, venereal diseases, and uterine fibroids [13]. The plant is also administered for gastrointestinal complaints, febrile conditions, and diarrhea [14]. Phytochemical investigations of the genus Zanthoxylum have revealed the presence of structurally diverse secondary metabolites, including alkaloids, lignans, coumarins, flavonoids, and terpenoids [15]. Moreover, essential oils extracted from its leaves have demonstrated notable antimicrobial properties [14].

Uvaria scheffleri, locally named "Boyyiniya" in Wolaitigna, is broadly distributed throughout East Africa, particularly in coastal evergreen forests and montane regions [16]. In Ethiopia, the species predominantly occurs in the southern highlands, where it is traditionally applied in the treatment of dermatological infections. Its fruits are edible and consumed locally [17]. Beyond Ethiopia, the plant is employed in Kenya to manage respiratory ailments such as cough, tuberculosis, asthma, and sore throat [18], while traditional healers in Tanzania use it as a remedy for febrile illnesses [16].

Vepris dainelli, known locally as "Chawula" in Goffigna, is an endemic Ethiopian tree species of medium stature [19]. In traditional medicine, its seeds are used to relieve abdominal discomfort, whereas preparations from its bark and fruits are administered to treat intestinal parasitic infections, skin disorders, and dental pain [20].

Despite their extensive traditional use, no scientific studies have yet documented the larvicidal effects of essential oils derived from these species against Anisakis larvae, nor have their cytotoxic properties been systematically evaluated. Accordingly, the present work aimed to characterize the chemical composition of essential oils obtained from the roots of U. scheffleri and the fruits of Z. chalybeum and V. dainelli, and to assess their larvicidal efficacy against Anisakis type I L3 larvae alongside their cytotoxic effects.

Materials and Methods

Ethical considerations

Ethical approval for the study was obtained from the Research and Ethics Committee of Adama Science and Technology University, Ethiopia (Approval No.: ASTU, SoNS/2259298/2018; Code: PGR031/18; Date: 12/10/2018).

Plant material

Plant materials comprising the roots of Uvaria scheffleri and the fruits of Zanthoxylum chalybeum and Vepris dainelli were harvested in November 2018 from the Wolaita, Sidama, and Gofa zones of the Southern Nations, Nationalities, and Peoples' Region (SNNPR), Ethiopia, respectively. Taxonomic authentication was performed by botanist Shambel Alemu at the National Herbarium, Addis Ababa University, where voucher specimens were deposited under the reference numbers MAUs-001/11, MAZc-002/11, and MAVd-003/11.

Following collection, fresh plant samples were transported in plastic containers to the Organic Chemistry Laboratory of Wolaita Sodo University. The materials were thoroughly rinsed with distilled water to remove extraneous debris, air-dried, and subsequently pulverized into fine powder using a mechanical grinder.

Extraction of essential oils

Essential oils were isolated by hydrodistillation using a Clevenger-type apparatus. Briefly, 400 g of powdered plant material from each species was individually submerged in 5 L of distilled water and heated for 5 h. The resulting distillate, consisting of an oil–water mixture, was collected and transferred to a 100 mL separatory

funnel. The essential oil fraction was separated, dried over anhydrous magnesium sulfate, placed into airtight vials, and stored at 4 °C pending analysis.

Chemical composition of essential oils

Gas chromatography–mass spectrometry (GC–MS) analysis was carried out using an Agilent 7890A GC system coupled with a 5977E mass selective detector and fitted with an HP-5MS capillary column (30 m × 250 µm internal diameter, 0.25 µm film thickness) composed of 5% phenyl and 95% methylpolysiloxane. Prior to sample injection, the syringe was rinsed with 8 µL of chloroform, followed by injection of 2 µL of each essential oil solution prepared in chloroform via an autosampler.

The oven temperature program commenced at 50 °C, increased to 120 °C at 20 °C/min, then to 150 °C at 4 °C/min, and finally to 250 °C at 20 °C/min with a 10 min holding period. A solvent delay of 3.5 min was applied. Injector and detector temperatures were maintained at 325 °C and 350 °C, respectively. Helium served as the carrier gas at a constant flow rate of 1 mL/min under a pressure of 69.8 kPa, with a split ratio of 100:1. The interface temperature was set to 280 °C.

Mass spectral data were acquired in electron impact mode at 70 eV, scanning from 40 to 600 m/z with a scan time of 0.5 s, and an ion source temperature of 230 °C. Identification of volatile constituents was achieved by matching obtained spectra with entries in the National Institute of Standards and Technology (NIST) mass spectral database. Relative quantification of each component was calculated based on peak area normalization relative to the total area of all identified compounds.

Anisakid nematode collection

Live anisakid larvae were obtained from blue whiting (Micromesistius poutassou), a commercially important marine fish species known for its high prevalence of Anisakis infection [21]. Fresh fish specimens were purchased from a retail fish market in Burjassot (Valencia, Spain) and processed within one hour of acquisition. Following dissection, larvae were carefully recovered from the visceral organs and immediately examined.

Recovered nematodes were morphologically classified as third-stage (L3) larvae of Anisakis type I. Prior to identification, larvae were repeatedly rinsed in sterile 0.9% sodium chloride solution and observed under a light microscope to confirm diagnostic morphological characteristics. Only specimens displaying spontaneous motility and intact morphology were selected. These larvae were subsequently transferred to plastic Petri dishes containing test solutions with graded concentrations of essential oils and maintained under ambient laboratory conditions.

In vitro larvicidal activity of essential oils

The nematicidal efficacy of the essential oils was evaluated using an in vitro assay designed to simulate fish marination conditions. A standardized marinade medium was prepared following established procedures [8], composed of a water–vinegar mixture (1:1, v/v) supplemented with sodium chloride (3%) and citric acid (1%). This medium was fortified with essential oils at concentrations of 5%, 1%, 0.5%, and 0.1%.

Each experimental unit consisted of four Anisakis L3 larvae placed in Petri dishes containing the test solutions. Control groups were exposed to the marinade solution alone under identical conditions. Larval behavior and survival were assessed using a stereoscopic microscope at hourly intervals during the first 7 h, followed by additional observations at 12 h and 24 h.

At each time point, larval viability was graded according to a predefined scoring system [8]: fully active larvae (score 3), reduced motility (score 2), movement only upon stimulation (score 1), and absence of movement (score 0). Larvae were considered dead when complete immobility persisted in isotonic saline (0.9% NaCl) under microscopic observation. Mean normalized viability values were calculated as described previously [22]. Lethal time values corresponding to 50% (LT50) and 100% (LT100) mortality were also determined.

All larvicidal experiments were conducted in triplicate. A total of 104 larvae were used, including 96 individuals exposed to essential oils and 8 larvae serving as untreated controls. Results are presented as mean percentage values.

Cytotoxicity assay

Cell lines

Assessment of cytotoxic effects was carried out using VERO cells, a non-tumorigenic monkey kidney epithelial cell line. Cells were supplied by the National Animal Health Diagnostic and Investigation Center (NAHDIC),

Ethiopia. Cultures were maintained in RPMI-1640 medium supplemented with fetal bovine serum (10%), glutamine (2 mM), penicillin (100 units/mL), and streptomycin (100 µg/mL). Incubation was performed at 37 °C under a humidified atmosphere containing 5% CO_2.

MTT assay

The cytotoxic profile of the essential oils was evaluated using the MTT colorimetric assay [23], which measures mitochondrial metabolic activity as an indicator of cell viability. VERO cells were seeded into 96-well microplates at a density of 2×10^4 cells per well and allowed to attach for 24 h.

Following attachment, the growth medium was replaced, and cells were exposed to serial dilutions of essential oils (100 to 0.78 µg/mL) for 24 h under standard incubation conditions. After treatment, fresh medium was added, followed by the addition of MTT solution (0.5 mg/mL final concentration). Plates were incubated for 4 h to allow for formazan crystal formation.

The supernatant was then removed, and intracellular formazan crystals were dissolved using dimethyl sulfoxide (DMSO). Absorbance was measured at 530 nm using a microplate reader. Cell viability was expressed as a percentage relative to untreated controls. All measurements were performed in triplicate and reported as mean values.

Cell viability was calculated using the equation:

$$\% \text{ cell viability} = (\text{Absorbance of treated} / \text{Absorbance of control}) \times 100 \quad (1)$$

Half-maximal inhibitory concentration (IC_{50}) values were obtained from dose–response curves plotting percentage viability against the logarithm of essential oil concentration using GraphPad Prism software (Version 8.0).

Results and Discussion

Hydrodistillation of plant materials resulted in variable essential oil yields, with 0.5% obtained from U. scheffleri roots and higher yields of 2.7% and 2.0% from the fruits of Z. chalybeum and V. dainelli, respectively (v/w).

Chemical characterization of U. scheffleri root oil by GC–MS led to the identification of 58 compounds, collectively accounting for 97.6% of the total oil content. The composition was dominated by sesquiterpenes (51.8%), followed by oxygenated sesquiterpenes (18.9%), oxygenated monoterpenes (17.2%), monoterpenes (6.9%), and benzenoid derivatives (5.2%) **(Table 1).** The most abundant constituents included alloaromadendrene, β-maaliene, (–)-borneol acetate, and (–)-α-gurjunene, while minor constituents were detected at concentrations below 5.1%.

Comparison with previous investigations on Uvaria species, including the report by Oguntimein *et al*. (1989) [24] on U. chamae, revealed notable differences. While earlier studies identified thymoquinoldimethyl ether and benzyl benzoate as dominant components, the present analysis showed complete absence of thymoquinoldimethyl ether and only trace amounts of benzyl benzoate, highlighting chemotypic variation within the genus.

The essential oil extracted from Z. chalybeum fruits contained 18 identified constituents, representing 99.6% of the total composition **(Table 2).** Oxygenated monoterpenes constituted the largest fraction, followed by monoterpenes, sesquiterpenes, and oxygenated sesquiterpenes. Tricyclo[5.3.0.0(3,9)]decane was overwhelmingly predominant (82.8%), whereas other constituents such as 2-tridecanone, decanal, phenolic derivatives, and trans-limonene oxide were present in much lower amounts. The chemical classes observed align with previous reports on Zanthoxylum species [25, 26]. However, unlike earlier studies on leaf oils dominated by β-phellandrene and β-myrcene [14], the present findings identify tricyclo[5.3.0.0(3,9)]decane as a novel major component in fruit oils, reported here for the first time and potentially useful as a chemotaxonomic marker for Ethiopian populations.

Analysis of V. dainelli fruit oil revealed 20 constituents accounting for 98.8% of the total oil composition **(Table 3).** Sesquiterpenes formed the principal chemical group, followed by monoterpenes and oxygenated sesquiterpenes. Tricyclo[5.3.0.0(3,9)]decane was again the dominant compound, accompanied by caryophyllene, phenolic derivatives, neoalloocimene, and germacrene D. Remaining components were present at low concentrations.

Table 1. Identified volatile constituents in the essential oil extracted from the roots of *Uvaria scheffleri*

No.	Identified compound	KI (lit.)	KI (exp.)	Relative abundance (%)
1	α-Pinene	932	929	0.406
2	Camphene	946	944	3.004
3	D-Limonene	1030	1028	0.435
4	δ-Terpinene	1050	1043	0.981
5	Eucalyptol	1059	1050	0.244
6	Bicyclo[2.2.1]heptane, 2-methoxy-1,7,7-trimethyl-	1105	1101	0.300
7	Neoalloocimene	1130	1123	0.238
8	(–)-trans-Myrtanyl acetate	1137	1130	0.139
9	Camphene hydrate	1148	1141	0.108
10	(–)-Borneol acetate	1150	1140	9.901
11	Endo-borneol	1157	1148	0.172
12	Fenchyl acetate	1208	1201	0.282
13	2-Pentanone, 5-(2-methylenecyclohexyl)- (stereoisomer)	1244	1240	0.127
14	Bicyclo[2.2.1]heptane-2-carboxylic acid, 3,3-dimethyl-, methyl ester	1252	1243	0.432
15	Isobornyl acetate	1286	1280	0.486
16	α-Terpinyl propionate	1332	1325	0.733
17	(+)-Cyclosativene	1368	1360	1.581
18	Isoledene	1385	1380	0.416
19	Isolongifolenone	1390	1382	0.450
20	(–)-α-Gurjunene	1405	1401	5.933
21	α-Gurjunene	1406	1401	1.843
22	2,5-Dimethoxy-p-cymene	1415	1412	0.387
23	α-Copaene	1426	1420	1.135
24	Butylated hydroxytoluene	1438	1432	0.073
25	α-Guaiene	1442	1440	0.439
26	Guaia-9,11-diene	1448	1442	0.336
27	Clovene	1454	1449	0.213
28	11-Isopropylidenetricyclo[4.3.1.1(2,5)]undec-3-en-10-one	1454	1450	1.100
29	Neoisolongifolene, 8,9-dehydro-	1458	1451	5.185
30	γ-Amorphene	1470	1465	0.253
31	(+)-γ-Gurjunene	1469	1466	0.128
32	Naphthalene, hexahydro-dimethyl-isopropyl derivative	1472	1469	0.286
33	δ-Guaiene	1476	1470	2.175
34	(+)-β-Selinene	1482	1480	2.111
35	Rotundene	1485	1481	1.922
36	Tetrahydronaphthalene derivative	1487	1483	0.117
37	Tetramethyl cyclopropa[a]naphthalene derivative	1513	1508	4.584
38	7-epi-α-Selinene	1517	1510	0.232
39	1,5-Cadinadiene	1524	1515	0.254
40	(+)-δ-Cadinene	1521	1519	2.592
41	α-Maaliene	1528	1520	3.154
42	β-Maaliene	1530	1523	9.983
43	Aristolediene	1540	1535	0.445
44	(–)-Gleenol	1574	1568	0.160
45	Neoisolongifolene	1576	1570	1.478
46	Dehydroaromadendrene	1620	1610	0.800
47	Guaiol	2091	2082	5.130
48	Epicubenol	1617	1610	0.567
49	Dehydroaromadendrene	1620	1613	0.822
50	Alloaromadendrene	1649	1640	12.905
51	Bulnesol	1656	1650	2.269
52	β-Guaiene	1667	1660	0.218

53	11,13-Dimethyl-12-tetradecen-1-ol acetate	1708	1701	0.124
54	Benzyl benzoate	1760	1756	4.101
55	α-Cadinene	1779	1770	3.164
56	3-Azepan-1-yl-benzo[d]isothiazole 1,1-dioxide	1789	1782	0.109
57	4-(p-Methoxyphenylazo)-m-phenylenediamine	1870	1864	0.264
58	Acetonitrile, 2-(2-aminophenylamino)-	1899	1890	0.203

KI: Kovats index

Table 2. Volatile constituents identified in the fruit-derived essential oil of *Zanthoxylum chalybeum*

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

Table 3. GC–MS-identified volatile constituents of the fruit essential oil obtained from *Vepris dainelli*

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

Earlier investigations focusing on the essential oils obtained from the leaves of Vepris species reported α-pinene (60.5%) [27] and terpinolene (49.7%) [28] as the predominant constituents. In contrast, the present investigation

of the fruit essential oil of V. dainelli demonstrated that tricyclo[5.3.0.0(3,9)]decane (69.9%) was the dominant compound. This constituent is reported here for the first time in a Vepris species, indicating that it may serve as a chemotaxonomic marker for Ethiopian Vepris taxa.

The anthelmintic potential of the extracted essential oils was assessed in vitro against Anisakis type I larvae. All tested oils exhibited concentration-dependent larvicidal effects **(Table 4, Figure 1).** The findings showed that essential oils derived from the fruits of Z. chalybeum, U. scheffleri, and V. dainelli achieved complete larval mortality (LT100) within 3 h, 5 h, and 5 h, respectively, at a concentration of 5%.

The larvicidal efficacy observed may be attributed to the combined or synergistic action of terpene constituents, particularly monoterpenes and sesquiterpenes, which are known for their bioactive properties. This explanation is consistent with previously published studies [7, 10]. The pronounced activity may also be associated with structural damage to the larval cuticle and impairment of the digestive system, leading to parasite death.

Further investigations are required to clarify the mechanistic basis underlying the larvicidal action of these essential oils. Given the increasing global incidence of anisakiasis, the absence of approved pharmacological therapies, and the demonstrated effectiveness of these oils against Anisakis larvae, their application in seafood marination processes could represent a promising preventive strategy. This approach is particularly attractive since marinated seafood products are typically stored in oil following processing. Based on the present results, the incorporation of essential oils from U. scheffleri, Z. chalybeum, and V. dainelli into marinade formulations may offer an alternative method for inactivating Anisakidae larvae. Nevertheless, these findings should be validated through in vivo studies before practical application.

Evaluation of cytotoxic effects using the VERO cell line demonstrated that cell viability decreased progressively with increasing essential oil concentrations, indicating a concentration-dependent cytotoxic response. According to the classification proposed by Döll-Boscardin *et al*. (2012) [29], essential oils with IC50 values between 10–50 µg/mL are considered strongly cytotoxic, while values ranging from 50–100 µg/mL, 100–200 µg/mL, and 200–300 µg/mL correspond to moderate, weak, and very weak cytotoxicity, respectively. IC50 values exceeding 300 µg/mL indicate absence of cytotoxic effects. Based on this framework, the essential oils of U. scheffleri, Z. chalybeum, and V. dainelli exhibited moderate cytotoxicity toward VERO cells, with IC50 values of 65.46 ± 0.48 µg/mL, 83.88 ± 2.30 µg/mL, and 96.82 ± 5.95 µg/mL, respectively.

Table 4. Lethal time (LT_{50} and LT_{100}) values of essential oils from Zanthoxylum chalybeum, Uvaria scheffleri, and Vepris dainelli against Anisakis type I larvae

Plant species	Essential oil concentration	LT_{100} (h)	LT_{50} (h)
Uvaria scheffleri	5%	5	–
	1%	7	5
	0.5%	7	–
	0.1%	12	–
	Control	–	–
Zanthoxylum chalybeum	5%	3	–
	1%	7	5
	0.5%	7	5
	0.1%	12	5
	Control	–	–
Vepris dainelli	5%	5	–
	1%	7	–
	0.5%	12	–
	0.1%	24	–
	Control	–	–

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

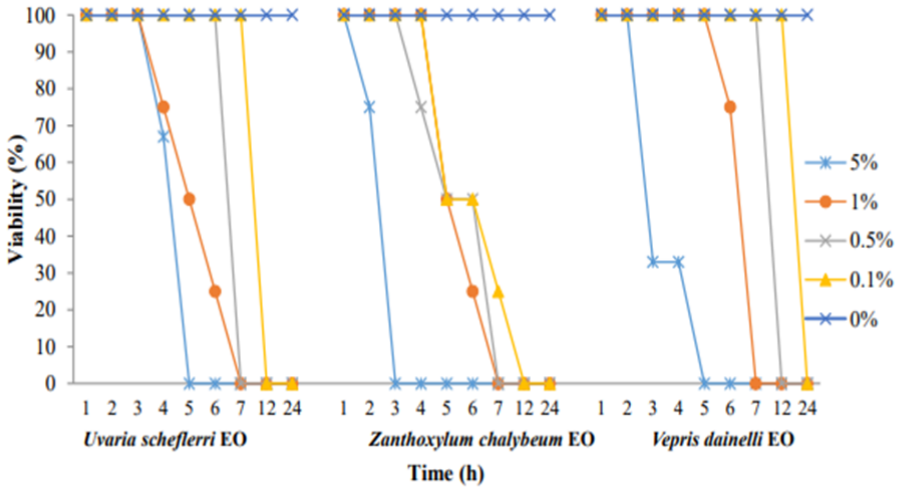

Figure 1. Survival profile of third-stage (L3) Anisakis type I larvae following exposure to marinating media supplemented with 0.1%, 0.5%, 1%, and 5% essential oils (EOs) extracted from Uvaria scheffleri, Zanthoxylum chalybeum, and Vepris dainelli. Larvae maintained in EO-free marinating solution showed no mortality throughout the duration of the experiment.

The results indicate that the tested essential oils may be effectively integrated into seafood preservation systems, particularly marination processes, as a means of reducing the risk of anisakiasis. This potential application is supported by the observation that larvicidal efficacy was achieved at concentrations associated with only limited cytotoxic effects.

Conclusion

This study provides evidence that essential oils isolated from three Ethiopian medicinal plants exhibit substantial anthelmintic activity against Anisakis L3 larvae, highlighting their promise as natural agents for anisakiasis management. Prior to any commercial or dietary application, however, further investigations focusing on in vivo toxicological evaluation and consumer acceptability are essential.

The larvicidal effects observed are likely linked to the chemical richness of the oils, particularly their high proportions of mono- and sesquiterpenoid compounds, which are widely recognized for their biological activity. Among the identified constituents, tricyclo[5.3.0.0(3,9)]decane predominated in the essential oils of Z. chalybeum and V. dainelli, whereas alloaromadendrene was the most abundant compound in U. scheffleri. These dominant metabolites may therefore be useful as chemotaxonomic markers for Ethiopian plant species within these genera. Essential oils obtained from U. scheffleri roots and from the fruits of Z. chalybeum and V. dainelli demonstrated a clear concentration–response relationship, with complete larval mortality (LT_{100}) achieved within 3 h, 5 h, and 5 h, respectively, at a 5% concentration. Given their effectiveness at doses exhibiting minimal cytotoxicity, these plant-derived oils represent promising candidates for the development of environmentally benign and biologically safe larvicidal formulations.

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