Proceedings

Anti-Nematodal Essential Oils with Activity against *Anisakis* †

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Abstract: Anisakiasis is a human parasitic infection caused by larvae of the *Anisakis* nematode through the consumption of raw or undercooked seafood, namely fish and cephalopods. To date, no effective drug has been uncovered and common anthelmintic treatments seem to have reduced activity against this parasite. Essential oils (EOs) are an unexplored source of natural products able to counteract *Anisakis*. The present work reviews available literature on EOs tested *in vitro* against *Anisakis* nematodes and compiles the activity and composition of the most active EOs. Over a dozen plant species were used as sources of EOs, mainly from Asteraceae, Lamiaceae, Apiaceae and Myrtaceae families. The lowest half maximal lethal concentrations (LC_{50}) were reported for *Origanum syriacum* and *O. compactum* EOs, both rich in carvacrol (83% and 50%, respectively). The EOs extracted from *Tagetes minuta* and *Nepeta cataria* were reported as the fastest acting, with half maximal lethal times (LT_{50}) under 4 h, and were rich in geraniol (55%) or β-ocimene (36%) and limonene (27%), respectively. Given their complex chemical composition, additive, synergistic and antagonistic interactions between EO compounds can be responsible for EO activity. A deeper analysis of the chemical structures active against *Anisakis*, and the nature of their interactions can be unveiled with further studies on this parasitosis.

Keywords: Anisakiasis; *Anisakis simplex*; Apiaceae; Asteraceae; essential oil; Lamiaceae; Myrtaceae; natural products

1. Introduction

Anisakiasis, a gastrointestinal parasitosis, has become an emerging human health concern due to a rise in worldwide number of cases. This increase was caused by a growing demand for raw or lightly cooked foods and as a result of the improvement in expertise and diagnostic tools. Besides the repercussions of this parasitosis on human health, the negative effect in consumer's confidence and consequent marketability of raw fish products has also high economic impacts. The majority of cases is reported in Japan (more than 90%), as a result of the dietary tradition based on raw fish, however, its occurrence in Europe is rising, particularly in Spain [1]. Anisakiasis is caused by members of the family Anisakidae, the most common being *Anisakis simplex*. The eggs of these gastrointestinal parasitic nematodes hatch freely in the ocean, where the free-living 3rd stage (L3) larvae

are generally consumed by crustaceans. Small crustaceans are the food basis for sea fish, cephalopods and ultimately marine mammals, transmitting the nematode to their gastro-intestinal tract [2]. Although human physiology does not allow the progression of these nematode parasites' life cycles, humans can become accidental hosts and be directly affected by debilitating diseases or by the initiation of a state of immune hypersensitivity [3].

The nematodes enter the human gastrointestinal tract through the ingestion of raw, smoked or undercooked fish contaminated with infective *Anisakis* L3 larvae. Within hours after ingestion, nematode larvae can induce an acute and transient infection (gastric anisakiasis) that may lead to abdominal pain, nausea, vomiting, abdominal distention, diarrhea, blood and mucus in stool, and mild fever. The small intestine is less commonly affected; however, it expresses a more chronic form of the disease. After 1 to 2 weeks of infection, inflammatory mass formation and lumen thickening, severe eosinophilic granulomatous response and interloop ascites characterize intestinal *Anisakis* parasitosis resembling Crohn's disease symptomatology, making the diagnostic difficult. Allergic reactions, such as urticaria and anaphylaxis, are also common, triggering an immune response to the presence of the nematode, with a high production of immunoglobulin E (IgE) [3].

Anisakis larvae can survive most traditional food treatments (i.e. salting, smoking and pickling) but are destroyed by cooking at temperatures above 63 °C or freezing below -20 °C for 7 days (or -35 °C for more than 15 hours). Several anthelmintics have been proposed for *Anisakis* parasitosis therapy, namely mebendazole, thiabendazole and albendazole. Although these anthelmintics are successfully active against other gastrointestinal nematodes, there is no clear evidence of their effectiveness against *Anisakis* L3 larvae [4]. Thus, there is still no effective drug on the market to treat this digestive parasitosis.

Natural products isolated from plants and microbes are known as excellent sources of pharmaceutical drugs, and many are the basis of the most active pharmaceuticals used today. The activity of plant natural products against *Anisakis* is thought to occur given the lower prevalence of the disease in populations that use the aromatic plant *Perilla frutescens* as a condiment in their raw fish diet [5].

Essential oils (EO) are composed of several active phytochemicals that have a wide range of biological activities including anti-microbial, fungicidal, insecticidal/insect repellent, herbicidal, acaricidal and nematicidal [6]. These complex mixtures of volatiles are exclusively obtained from plant material by hydro-, steam- or dry-distillation, or in the case of *Citrus* fruits, mechanically without heating [7]. EOs are generally composed of highly active chemical classes of compounds, namely terpenes (mono-, sesqui-, and diterpenes) and phenylpropanoids, and are usually dominated by one to three major components at relatively high amounts [8]. The study of the activity of EOs against gastrointestinal nematodes has focused mainly on *Haemonchus contortus*, a parasitic nematode of small ruminants (goats and sheep), showing promising results. In the present work, the available literature was reviewed on EOs tested *in vitro* against Anisakis nematodes, and the activity and composition of the most active EOs were compiled.

A comprehensive study of these parameters can contribute for an activity guided screening of natural products active against these parasites and provide a deeper analysis of the most active chemical structures.

2. Available Literature

Research was performed with Web of Science search engine on published works reporting on the activity of EOs against *Anisakis* nematodes, using the topics "*Anisakis*" and "essential oil". Identification of EO source plant, qualitative and quantitative chemical composition and anti-nematode activity was retrieved when available.

Eight publications reported on assays using EOs against *Anisakis* L3 larvae, from 2012 to 2019 [9–16]. These works were published in journals covering mainly areas of parasitology (38%), tropical medicine (38%) and public environmental and occupational health (25%). These reports were cited 135 times (113, excluding those of reports on this list) by

a total of 85 reports (78, excluding those on this list), with an average of 17 citations per work. Citing articles were published by journals publishing on the research areas of food science technology, biochemistry, molecular biology, pharmacology and pharmacy. Cumulative number of citations increased from 2012 to 2018 but has since become stable.

3. Essential Oils and Respective Toxicological Parameters

The reported EOs were extracted from plants that belonged to the Asteraceae, Lamiaceae, Apiaceae and Myrtaceae families. Publications reported on the activity of EOs extracted from *Cuminum cyminum*, *Lavandula angustifolia*, *L. stoechas*, *Matricaria chamomilla*, *Melaleuca alternifolia*, *Nepeta cataria*, *Origanum compactum*, *O. majorana*, *O. syriacum*, *O. vulgare*, *Rosmarinus officinalis*, *Tagetes minuta* and *Thymus vulgaris*. Except for 2 EOs obtained from hydrodistillation, all other EOs were acquired from commercial sources. EO activity was expressed through one of three types of parameters, namely nematode mortality, half maximal lethal concentrations (LC_{50}) and half maximal lethal times (LT_{50}).

3.1. Anisakis Mortality Assays

Mortality percentages were reported for the EOs of *C. cyminum*, *L. angustifolia*, *L. stoechas*, *M. chamomilla*, *O. majorana*, *O. vulgare*, *R. officinalis* and *T. vulgaris*. Only *M. chamomilla* EO showed 100% mortality at 0.125 mg/mL. This EO was composed of bisaboloxide A (49%), α-bisaboloxide B (8%), (−)-α-bisabolol (6%), *trans*-β-farnesene (5%) and chamazulene (2%) (Figure 1).

A previous work, published in 2015 [4], reviewed reports on EOs with activity against Anisakis nematodes, but some references were not currently available. In this work, activities are additionally described for the EOs of *Cymbopogon citratus*, *C. martini*, *C. winterianus*, *Litsea cubeba*, *Mentha piperita*, *Myristica fragans*, *Pelargonium graveolens* and *Piper nigrum*. In these EOs, 100% mortality was obtained at 0.125 mg/mL, except for *M. piperita*, that required a concentration of 0.250 mg/mL to induce complete mortality.

3.2. Half Maximal Lethal Concentration (LC_{50})

LC_{50} values are a measure of toxicity for a given substance and correspond to the concentration required to kill half of a tested population, after a specified duration. LC_{50} values were reported for the EOs of *M. alternifolia*, *O. compactum* and *O. syriacum* (Table 1).

Table 1. Half maximal lethal concentrations (LC_{50}) reported for the essential oils (EOs) of *Melaleuca alternifolia*, *Origanum compactum* and *O. syriacum* against *Anisakis simplex* after 24 or 48 h of exposure. EO main composition is reported for compounds above 5 %.

EOs	Time of exposure (h)	LC_{50}	Main composition (≥5 %)
M. alternifolia	24 48	4.53[1] 4.27[1]	Terpinene-4-ol, 47 % γ-Terpinene, 23 % α-Terpinene, 10 % *p*-Cymene, 6 %
O. syriacum	24 48	0.087[2] 0.067[2]	Carvacrol, 83 % γ-Terpinene, 65 %
O. compactum	24 48	0.429[2] 0.344[2]	Carvacrol, 50 % Thymol, 15 % γ-Terpinene, 14 % *p*-Cymene, 8 %

[1] µL/mL; [2] mg/mL.

The lowest values were obtained for the EO of *O. syriacum*, that showed a very high relative amount of carvacrol (Figure 1). In the EO of *O. compactum* activities lowered exponentially, probably due to its lower relative amount of carvacrol.

*3.3. Half Maximal Lethal Times (LT$_{50}$)*

LT$_{50}$ values correspond to the time required to kill half of a tested population, after exposure to a specified substance concentration. LT$_{50}$ values were reported for the EOs of *N. cataria* and *T. minuta* (Table 2).

Table 2. Half maximal lethal times (LT$_{50}$) reported for the essential oils (EOs) of *Nepeta cataria* and *Tagetes minuta* against *Anisakis* L3 larvae after exposure to 10, 5, 1, 0.5 and 0.1 % of EO. EO main composition is reported for compounds above 5 %.

EOs	Concentration (%)[1]	LT$_{50}$ (h)	Main composition (≥5 %)
N. cataria	10	3.9	Geraniol, 55 % 2,6-Dimethylocta-2,6-diene, 20 % β-Citronellol, 6 % *trans*-β-Caryophyllene, 6 %
	5	6.6	
	1	14.9	
	0.5	17.7	
	0.1	20.2	
T. minuta	5	1.0	β-Ocimene, 36 % Limonene, 27 % *cis*-Tagetone, 17 % allo-Ocimene, 6 % *trans*-β-Caryophyllene, 5 %
	1	1.0	
	0.5	2.3	
	0.1	11.2	

[1] % of EO in a saline solution of 0.9 % NaCl.

The EO of *T. minuta* showed the highest nematicidal efficacy, killing half of the test population in one hour, in concentrations as low as 1%. This EO was mainly composed (80%) of the hydrocarbon monoterpenes β-ocimene and limonene and the monoterpene ketone *cis*-tagetone (Figure 1).

Bisaboloxide A   Carvacrol   β-Ocimene

Limonene   *cis*-Tagetone   Albendazole
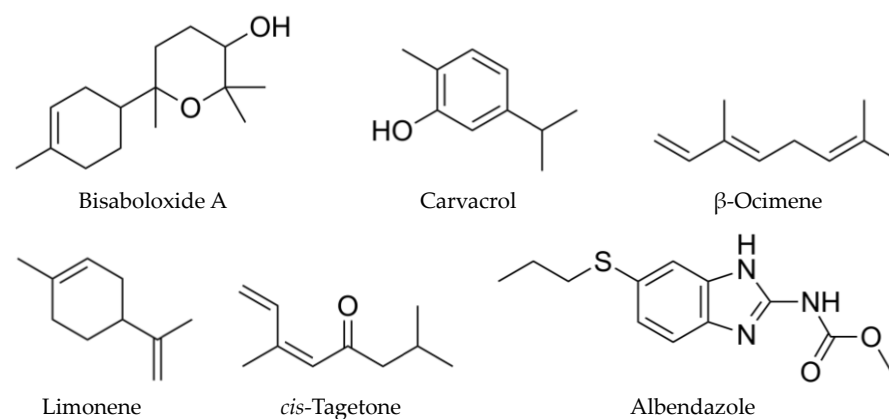
Figure 1. Chemical structure of the main compounds of the most effective essential oils and of the commonly used anthelmintic albendazole.

4. Active Essential Oil Components

The activity of isolated EO components was also determined in some reports. The activities of carvacrol and thymol, two oxygen-containing isomers, were higher than *O. compactum* EO, where they can be found in high amounts [10]. A higher efficiency was reported for carvacrol (LD$_{50}$ values of 0.176 mg/mL at 24 h and 0.178 mg/mL at 48 h) than for thymol (LD$_{50}$ values of 0.291 mg/mL at 24 h and 0.214 mg/mL at 48 h), which indicates

the specificity of molecule isomerism in the activity against *Anisakis* nematode. Nematicidal activity was further linked to the inhibition of the enzyme acetylcholinesterase. The EO of *M. chamomilla* and one of its main components α-bisabolol showed high activities, leading to changes in the cuticle and digestive tract of *Anisakis* L3 larvae [16]. Chamazulene, another *M. chamomilla* EO main component, showed no activity, which suggests that oxygen-containing molecules may be more successful in controlling this parasite. Nevertheless, given their complex composition, EO activity cannot be solely attributed to its main components. While the EO of *M. alternifolia* induced mortality in *Anisakis* L3 larvae and inhibited acetylcholinesterase, terpinene-4-ol, that composed 47% of this EO, showed no activity [11]. Suggesting that the activity can be caused by lesser dominant compounds or by the presence of synergistic compound relations.

Monoterpenoids seem to be a particularly successful source of active EO components against Anisakis L3 larvae. Promising *in vitro* and *in vivo* activities have been attributed to the aldehyde citral, a mixture of the enantiomers geranial and neral, and the alcohol citronellol but also to the hydrocarbons α-pinene and ocimene capable of decreasing in 80% disease symptomatology [5,17]. Nematicidal activity is associated to damages to the L3 larvae cuticle and intestinal walls [5,15].

5. Conclusion

Plant extracts and EOs seem to be successful in killing *Anisakis* nematode larvae. The use of EOs and their active components can provide an alternative treatment to this parasitosis or can function as possible additives in raw food preparation for prophylaxis against Anisakiosis. Further studies and screening of additional EOs and active compounds can lead to the discovery of chemical structures with improved activity and security to be used in food products.

References

1. Bao, M.; Pierce, G.J.; Pascual, S.; González-Munõz, M.; Mattiucci, S.; Mladineo, I.; Cipriani, P.; Bušelić, I.; Strachan, N.J.C. Assessing the risk of an emerging zoonosis of worldwide concern: Anisakiasis. *Sci. Rep.* **2017**, *7*, 43699, doi:10.1038/srep43699.
2. Audicana, M.T.; Ansotegui, I.J.; De Corres, L.F.; Kennedy, M.W. Anisakis simplex: Dangerous—Dead and alive? *Trends Parasitol.* 2002, *18*, 20–25.
3. Audicana, M.T.; Kennedy, M.W. Anisakis simplex: from Obscure Infectious Worm to Inducer of Immune Hypersensitivity. *Clin. Microbiol. Rev.* **2008**, *21*, 360–379, doi:10.1128/CMR.00012-07.
4. Valero, A.; Romero, M.C.; Gómez-Mateos, M.; Hierro, I.; Navarro, M.C. Natural products: Perspectives in the pharmacological treatment of gastrointestinal anisakiasis. *Asian Pac. J. Trop. Med.* **2015**, *8*, 612–617, doi:10.1016/j.apjtm.2015.07.017.
5. Navarro, M.C.; Noguera, M.A.; Romero, M.C.; Montilla, M.P.; González de Selgas, J.M.; Valero, A. Anisakis simplex s.l.: Larvicidal activity of various monoterpenic derivatives of natural origin against L3 larvae in vitro and in vivo. *Exp. Parasitol.* **2008**, *120*, 295–299, doi:10.1016/j.exppara.2008.07.014.
6. Batish, D.R.; Singh, H.P.; Kohli, R.K.; Kaur, S. Eucalyptus essential oil as a natural pesticide. *For. Ecol. Manage.* **2008**, *256*, 2166–2174, doi:10.1016/j.foreco.2008.08.008.
7. Figueiredo, A.C.; Barroso, J.G.; Pedro, L.G.; Scheffer, J.J.C. Factors affecting secondary metabolite production in plants: Volatile components and essential oils. *Flavour Fragr. J.* **2008**, *23*, 213–226, doi:10.1002/ffj.1875.
8. Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M. Biological effects of essential oils - A review. *Food Chem. Toxicol.* **2008**, *46*, 446–475, doi:10.1016/j.fct.2007.09.106.
9. López, V.; Pavela, R.; Gómez-Rincón, C.; Les, F.; Bartolucci, F.; Galiffa, V.; Petrelli, R.; Cappellacci, L.; Maggi, F.; Canale, A.; et al. Efficacy of origanum syriacum essential oil against the mosquito vector culex quinquefasciatus and the gastrointestinal parasite anisakis simplex, with insights on acetylcholinesterase inhibition. *Molecules* **2019**, *24*, doi:10.3390/molecules24142563.
10. López, V.; Cascella, M.; Benelli, G.; Maggi, F.; Gómez-Rincón, C. Green drugs in the fight against Anisakis simplex—larvicidal

activity and acetylcholinesterase inhibition of Origanum compactum essential oil. *Parasitol. Res.* **2018**, *117*, 861–867, doi:10.1007/s00436-018-5764-3.

11. Gómez-Rincón, C.; Langa, E.; Murillo, P.; Valero, M.S.; Berzosa, C.; López, V. Activity of tea tree (Melaleuca alternifolia) essential oil against L3 larvae of Anisakis simplex. *Biomed Res. Int.* **2014**, *2014*, doi:10.1155/2014/549510.

12. Giarratana, F.; Muscolino, D.; Ziino, G.; Giuffrida, A.; Marotta, S.M.; Lo Presti, V.; Chiofalo, V.; Panebianco, A. Activity of Tagetes minuta Linnaeus (Asteraceae) essential oil against L3 Anisakis larvae type 1. *Asian Pac. J. Trop. Med.* **2017**, *10*, 461–465, doi:10.1016/j.apjtm.2017.05.005.

13. Giarratana, F.; Muscolino, D.; Ziino, G.; Lo Presti, V.; Rao, R.; Chiofalo, V.; Giuffrida, A.; Panebianco, A. Activity of catmint (Nepeta cataria) essential oil against anisakis larvae. *Trop. Biomed.* **2017**, *34*, 22–31.

14. Pérez, M.G.M.; Moll, C.N.; Espinosa, G.M.; López, A.V. Evaluation of different mediterranean essential oils as prophylactic agents in anisakidosis. *Pharm. Biol.* **2016**, *55*, 456–461, doi:10.1080/13880209.2016.1247880.

15. Giarratana, F.; Muscolino, D.; Beninati, C.; Giuffrida, A.; Panebianco, A. Activity of Thymus vulgaris essential oil against Anisakis larvae. *Exp. Parasitol.* **2014**, *142*, 7–10, doi:10.1016/j.exppara.2014.03.028.

16. Romero, M.D.C.; Valero, A.; Martín-Sánchez, J.; Navarro-Moll, M.C. Activity of Matricaria chamomilla essential oil against anisakiasis. *Phytomedicine* **2012**, *19*, 520–523, doi:10.1016/j.phymed.2012.02.005.

17. Hierro, I.; Valero, A.; Navarro, M.C. In vivo larvicidal activity of monoterpenic derivatives from aromatic plants against L3 larvae of Anisakis simplex s.l. *Phytomedicine* **2006**, *13*, 527–531, doi:10.1016/j.phymed.2005.05.001.