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Original Article

The effect of Bergamot essential oils against Vibrio anguillarum: a pathogenic fish species

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Abstract

The present study is focused to evaluate the effects of Bergamot Essential Oil and its terpene against fish pathogen species. The antimicrobial activity was investigated *in vitro* against *Vibrio anguillarum* strains. The chemical composition was also analysed. Results showed that both essential oils were effective against this species, reducing bacterial growth for 3 days. The results suggest that bergamot oils may be used as an effective antimicrobial system to reduce the microbial growth and constitute an useful alternative in aquaculture farms to prevent bacterial fish diseases.

Keywords

citrus essential oils,; antimicrobial properties ;*Vibrio anguillarum*

Introduction

Aquaculture is emerging all over the world because of the increasing demand for food fish consumption. The intensive culture if may respond to the request of foods as nobile fish proteins, on the other hand, has led to outbreaks of various bacterial diseases [1-5], resulting in annual economic losses to the aquaculture industry estimated at billions of dollars worldwide [6]. Among bacterial diseases Vibriosis is one of the most important and may affect any marine fish species [7-11]. The vibriosis can be a significant problem in striped bass reared in salt water both plant or cages as well as wild fish [12]. The main etiological agent of Vibriosis is *Vibrio anguillarum,* a Gram negative curved-rod shape bacteria that cause typical hemorrhagic septicemia. *V. anguillarum* is divided in 23 O serotypes (O1–O23), with different pathogenicity and host specificity. Serotypes 01 and 02 of *V. anguillarum* are the main strains responsible for epizootic outbreaks [13,14], the infection by *V. anguillarum* may occur through the skin or gills, or via oral intake of the pathogen in water or food [15]. Antibiotics and chemicals have been applied in farms for traditional treatment and prevention of Vibriosis [16]. In fact, to control the Vibriosis in aquaculture, infected fish are fed with antibiotic-medicated food [17] and the application of antibiotics via the oral route to groups of fish that share tanks or cages are common practices [18]. Unfortunately, over the time, these approaches may be ineffective mainly due to the development of resistance to antibiotics. Moreover,

the unrestricted use of antibiotics causes the presence of residual antibiotics in aquaculture fish and has led to allergy and toxicity in humans [19].

The use of natural substances could be a good solution as alternatives to antibiotics to prevent the most common bacterial diseases in aquaculture. Essential oils (EOs) and their components, have largely used as a flavouring agents in cosmetic industry as well as in foods and beverages, and due to their versatile content of antimicrobial compounds, they possess potential as natural agents for food preservation (Giarratana et al. 2016). However, the effect of EOs on the behaviour of fish pathogens bacteria has not yet been reported.

In this context the authors decided to study the antimicrobial properties of a *Citrus* species.

Citrus bergamia (Risso), commonly named Bergamot, is a fragrant citrus fruit, with the size of an orange, with a yellow or green colour similar to a lime, depending on ripeness. Bergamot essential oil (BO) is used in many industrial sectors such as food field up to perfumery [20]. The bergamot essential oil as well as the other citrus essential oils are reported to be one of the rich sources of bioactive compounds namely coumarins, flavonoids, carotenes, terpenes and linalool [21] and with antimicrobial properties [22].

The aim of this paper was to study the properties of Bergamot oil and its terpene against a fish pathogenic bacterial species: *Vibrio anguillarum*.

Methods and Materials

The citrus fruits came from local producers of southern Italy (Calabria). The Bergamot EOs tested in this paper was extracted at cold with extractor Peeler and kindly furnished by a local Company Simone Gatto s.r.l. (San Pier Niceto - Messina). Two oils were tested, Bergamot oil (BO) and its Terpenes (BT). The essential oils were stored at 4°C in the dark until the laboratory use. All the analysis was carried out at the CNR laboratories.

Vibrio anguillarum, a pathogenic strain isolated from farmed fish, was furnished by Dr. Amedeo Manfrin (IZS of Venice, Padova- Italy). The antimicrobial activity of essential oils was analysed *in vitro* using the disc diffusion method on agar plates. In order to obtain freshly cultured microbial suspensions *Vibrio anguillarum* was spread on Marine Agar (MA, Microbial diagnostic) and incubated at 25°C for 18-24 h, then the strain was suspended in sterile saline solution at a concentration of 10^6 cell/ml (Mc Farland standards) and 1 ml of this suspension was spread on MA plates. Subsequently, sterile paper disks, 9 mm in diameter (Dominique Dutscher), soaked with 30 µL of essential oils (concentration of BO and BT were 26.28mg and 25.39 mg,

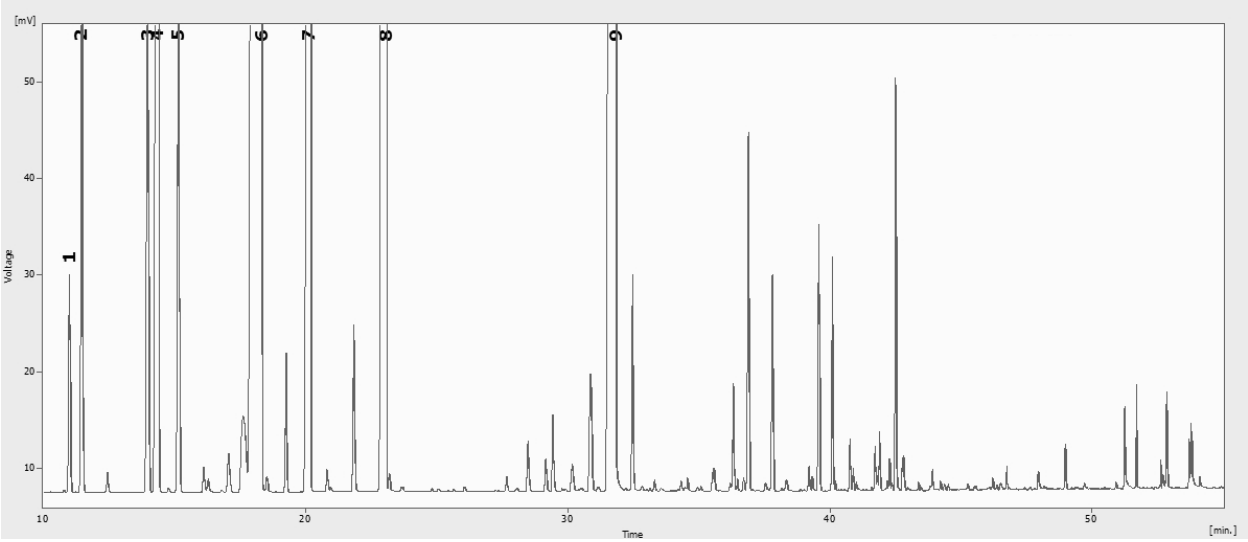

Figure 1: gas cromatografic prophyles of bergamot (a) oils: 1 α-Thujene, 2 α-Pinene, 3 Sabinene, 4 α-Pinene, 5 Myrcene, 6 Limonene, 7 ɣ-Terpinene, 8 Linalool, 9 Linalyl acetate.

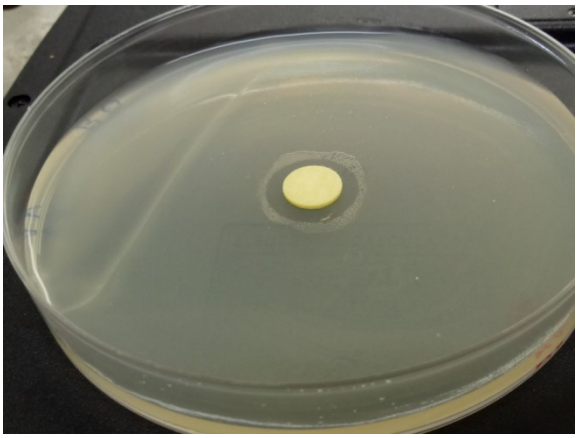

Figure:2: inhibition halo of Bergamot oil against V. anguillarum

respectively), placed on the agar surface and the plates were incubated for 24 -72 h at 25°C. The antimicrobial activity was evaluated by measuring the diameter of the inhibition zone. Each experiment was carried out in triplicate, and the mean diameter of the inhibition zone was recorded. Moreover BO was characterized and analyzed with a gas chromatograph (DANI Instruments Master GC) **(Figure.1).**

Results and discussion

The results represent the net zone of inhibition including the diameter of paper disk (9 mm); the scale of measurement was: area > 30 mm, very strong inhibition; 30-19 mm, strong inhibition; 18-10 mm, moderate inhibition; < 10 mm, non–inhibition zone.

Halos were measured up to 72 h and no differences between 24 and 72 h was observed; 6 days after incubation the halos were disappeared, because there was not further inhibition with time.

Vibrio anguillarum showed a stronger inhibition against BT (26 mm of inhibition halo), than BO (20 mm) (Figure. 2).

In **(Table 1)** the mean and standard deviation of halos are shown. The results revealed that bergamot oils inhibited the growth of fish pathogens for 3 days.

In this paper is highlighted the inhibition of bergamot essential oil and its terpene against *Vibrio anguillarum* strain demonstrating the effectiveness of both against bacteria and suggesting their use for disease prevention as natural bacterial inhibition, instead of antimicrobials.

In Figure 1 the gas cromatografic prophyle of bergamot oil was shown. The chemical composition of BT showed that terpene fraction has a higher concentration of: α-Pinene, β-Pinene (almost double value) than the BO; moreover, the limonene component showed values higher in terpene than in bergamot (almost twice, **Table 2**).

The observed differences in the chemical component of two oils could explain the different antimicrobial effectiveness.

According other Authors, the antibacterial and antiseptic activity of bergamot oil was related to the presence of well-recognized antimicrobial compounds [23]. These compounds are very effective against the bacterial growth as reported for limonene by Giarratana et al. [24] and α-Pinene, β-Pinene [25], therefore the synergistic action of these components gives the bergamot and its terpene the antimicrobial characteristics. The action of the oils is performed against the cytoplasmic membrane of target microorganism cells [26]. The hydrophobicity is also an important characteristic, which enables EOs to accumulate in cell membranes causing an increase of permeability until cell death [27].

Our results revealed that bergamot reduce fish pathogens for 3 days, and suggest the possibility of citrus EOs to be used as alternative to antibiotics, as previously confirmed by Perricone et al. [28].

Conclusions

The results suggested the use of these oils as a prevention of bacterial disease in farmed fish in aquaculture farms, instead of antibiotics that may be residing in food

Table 1: inhibition halos and efficiency percentage

Bergamot terpene	Bergamot essential oil	Efficiency % oil vs strain
26	20	100

Table 2: Chemical composition of Terpene and Bergamot essential oil.

Chimical compound	T	EO
	Bergamot	Bergamot
α-Thujene	0.43	0.30
α-Pinene	1.81	1.17
Sabinene	1.66	1.06
β-Pinene	10.14	5.97
Myrcene	1.54	1.03
Limonene	78.90	41.07
ɣ-Terpinene	1.49	6.71
Σ *(1-7) %*	*95.98*	*57.31*
Linalool	0.21	8.52
Linalyl acetate	0.07	28.06
Σ *(8-9) %*	*0.28*	*36.58*
Σ *(1-9) %*	*96.26*	*93.89*
Total volatile area (mV.s)	31,592	29,180

when fish are carried out to sell in commercial sites.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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