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Antimicrobial activity of *Coriandrum sativum* and *Pinus* spp. extracts, alone and in combination, against *Streptococcus iniae*

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Abstract

Streptococcus iniae (*S. iniae*) is recognized as one of the most significant bacterial pathogens in aquaculture, responsible for severe infections that lead to substantial economic losses annually. Given the growing concerns surrounding antibiotic resistance and its implications for both aquaculture and public health, there is an urgent need to explore alternative antimicrobial agents. This study was conducted to evaluate the antimicrobial potential of *Coriandrum sativum* (Coriander) and *Pinus* (Pine) extracts, both individually and in combination, against this bacterium. Hydroalcoholic extracts of coriander and pine were obtained using the Soxhlet extraction method. Antibacterial effects were quantified by measuring the minimal concentrations required for inhibition (MIC) and killing (MBC) of bacterial growth. In addition, the combined effect of the extracts was assessed through the determination of the Fractional Inhibitory Concentration (FIC) index. The findings demonstrated that the coriander extract exhibited a significantly stronger inhibitory effect against *S. iniae*, with an MIC value of 15.625 µg/mL, compared to the pine extract, which had an MIC of 250 µg/mL. However, neither extract showed strong bactericidal activity at concentrations below 500 µg/mL. The evaluation of the combined extracts revealed an antagonistic effect with a FIC index of 17. These results confirm the superior efficacy of the coriander extract in inhibiting the growth of *S. iniae*. However, the combination with pine extract not only did not lead to synergistic effects, but also resulted in a lower antimicrobial activity. These results emphasize the need for a more thorough investigation of phytochemical interactions prior to their combined use in therapeutic protocols.

Introduction

The aquaculture industry has seen rapid expansion worldwide in recent decades, with Iran establishing itself as a major contributor through five decades of remarkable progress. Currently, Iran is the second-largest aquaculture producer in the Middle East and ranks nineteenth globally, according to authoritative reports (1). While these achievements emphasize the importance of the sector, they also require increased responsibility

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in addressing critical challenges, particularly in controlling microbial diseases and preventing the transmission of antibiotic resistance from aquatic species to humans.

Among the various pathogenic threats to aquaculture, streptococcal infections stand out due to their serious economic and health impact (2, 3). *Streptococcus iniae* (*S. iniae*), a Gram-positive, catalase-negative, facultative anaerobic cocci, is one of the most virulent pathogens in fish farming. This non-sporulating, non-motile and non-encapsulated bacterium, first isolated from the freshwater dolphin (*Inia geoffrensis*), exhibits haemolytic activity and broad host specificity and infects both farmed and wild fish populations in freshwater and marine environments (4, 5). Clinical infections, which can manifest as meningoencephalitis, septicaemia, or skin lesions, are associated with a mortality rate of up to 80 % and cause significant economic losses (6, 7). Of particular concern is the zoonotic potential of the bacterium, which poses a double threat to the sustainability of aquaculture and public health.

While conventional antibiotic therapies are effective in containing disease outbreaks, they face two major limitations in aquaculture: 1) the emergence of drug-resistant bacterial strains and 2) the risk of antibiotic residues accumulating in seafood intended for human consumption (8). These challenges have intensified the search for sustainable alternatives, with plant-based compounds emerging as promising candidates due to their antimicrobial efficacy and ability to boost host immunity. In particular, coriander (*Coriandrum sativum* L., Apiaceae) and pine (*Pinus spp*., *Pinaceae*) have shown significant potential in this regard.

Coriander, an annual herb grown worldwide, is traditionally utilized for combating microbial infections, with modern studies confirming its broad-spectrum antimicrobial and antifungal properties (9). Pine, evergreen trees widely distributed in Iran and other regions, also provide extracts and essential oils with proven activity against various bacterial and fungal pathogens (10). The antimicrobial mechanisms of coriander and pine extracts are attributed to their bioactive compounds. Coriander contains linalool and geraniol, which disrupt bacterial cell membranes and inhibit protein synthesis. Pine extracts are rich in pinene and terpenoids, known to interfere with microbial enzymatic systems and cell wall integrity (9, 10). These multi-target actions reduce the likelihood of resistance development.

Despite extensive research on the antimicrobial activity of these plants against various microorganisms, no study has yet investigated their individual or combined efficacy against *S. iniae*. To fill this knowledge gap, the present study investigated the antibacterial activity of hydroalcoholic extracts of coriander and pine leaves, both individually and in combination, against *S. iniae*.

Materials and Methods

Plant Materials and Extraction Methods

The plant materials were identified based on their widely recognized botanical characteristics in accordance with traditional ethnobotanical knowledge. Coriander leaves were obtained from local markets where this species is commercially cultivated and unambiguously identified by local producers. Pine leaves were collected from mature trees located on the campus of Islamic Azad University, Shabestar Branch, which have been morphologically confirmed as pine by the university's landscape management records. While formal botanical authentication was not performed, these species represent commonly used plants with distinct morphological features that preclude misidentification in practical applications. For hydroalcoholic extraction, the plant materials were shade-dried and ground into fine powder using an electric mill. The resulting powder was then subjected to Soxhlet extraction. To prepare the hydroalcoholic extract, 50 mL of 70% ethyl alcohol was added to each 10 grams of plant powder. The solution was subsequently placed in a 40°C oven for 24 hours to obtain a dry powdered extract. This powder was used to prepare stock dilutions. The stock solution of hydroalcoholic extract was prepared by dissolving the extract powder in dimethyl sulfoxide (DMSO; Sigma-

Aldrich, USA). Based on previous study results, the stock concentration for both plant extracts was prepared at 1 mg/mL in DMSO [with a final concentration of 10% (v/v)].

Bacterial Strain

For analysis of microbial growth inhibition by hydroalcoholic extracts from coriander and pine, the standard strain *S. iniae* PTCC 1887 was utilized. This strain was obtained from the Iranian Regional Collection of Industrial Fungi and Bacteria (PTCC).

Quantitative Antibacterial Efficacy Analysis

Measurement of Bacterial Growth Suppression Limits

The antimicrobial activity of hydroalcoholic extracts from coriander and pine was evaluated against *S. iniae* PTCC 1887 through assessment of the lowest growth-inhibiting concentrations (MIC values) via standardized broth dilution assays. The experimental procedure was conducted according to established protocols with minor modifications (11, 12). In brief, 100 μL of Mueller-Hinton broth (Himedia, India) was dispensed into each well of a 96-well microplate, followed by the addition of plant extracts dissolved in 10% DMSO at an initial concentration of 1 mg/mL. Two-fold serial dilutions were then performed across the microplate wells. Bacterial suspension standardized to 0.5 McFarland turbidity (OD600 = 0.8) was inoculated into each well at a volume of 100 μL. The microplates were covered with parafilm and incubated at 37°C for 24 hours under aerobic conditions. Following the first incubation phase, each well received 0.01% resazurin dye (Fluka, Switzerland) solution (30 μL) to monitor metabolic activity, with subsequent incubation at 37°C for an additional 2-hour period. MIC values were defined as the lowest plant extract dilutions that prevented observable microbial proliferation, confirmed by maintained blue coloration in the resazurin assay. Control wells included antibiotic (ciprofloxacin) positive controls, solvent (DMSO) negative controls, extract sterility controls, and bacterial growth controls. All experiments were performed in triplicate.

Measurement of Microbial Killing Efficiency

The minimum bactricidal concentration (MBC) represented the threshold plant extract concentration needed to eliminate ≥99.9% of bacterial cells following 24-hour incubation at 37°C. For MBC assessment, 100 μL samples from MIC wells and subsequent higher concentrations (demonstrating complete growth inhibition) were transferred to Mueller-Hinton agar (Himedia, India) and incubated at 37°C for 24 hours. The MBC endpoint was identified as the most dilute extract concentration yielding sterile agar plates. Triplicate measurements were conducted for all determinations (13).

Fractional Inhibitory Concentration (FIC)

The combined effect of coriander and pine extracts against *S. iniae* was evaluated using the FIC method. After determining the MIC of each extract individually, concentrations of 50%, 25%, and 12.5% of the extracts were used to determine the FIC. In this method, the final volume in each microplate well was 200 μL, prepared as follows: 100 μL of Mueller-Hinton broth was added to each well, followed by 50 μL of coriander extract and 50 μL of pine extract in the first row, with serial dilutions performed. Finally, 100 μL of the bacterial suspension, previously adjusted using a spectrophotometer, was added to each well. The same procedure was applied for the 25% and 12.5% concentrations in the remaining wells. The microplate was subjected to 30 seconds of agitation prior to a 24-hour culture period at 37°C. Subsequently, each well received 0.01% resazurin solution (30 μL), and plates were maintained at 37°C for a further 2-hour period before FIC determination (14).

The FIC index was calculated as:

FICindex = (MICcombA/MICaloneA) + (MICcombB/MICaloneB)

Where MICcomb represents the MIC in combination and MICalone represents the individual MIC value. Interpretation followed established criteria: synergy (FIC ≤0.5), addition (0.5<FIC ≤1), indifference (1<FIC ≤4), or antagonism (FIC >4) (15).

Results

Antimicrobial Susceptibility Testing

The outcomes established that the hydroalcoholic extract of coriander exhibited an MIC value of 15.625 µg/mL against *S. iniae*, while the hydroalcoholic extract of pine showed an MIC of 250 µg/mL against the same bacterial strain (Table 1). The MBC assay demonstrated that both coriander and pine hydroalcoholic extracts exhibited bactericidal concentrations exceeding 500 µg/mL against *S. iniae* (Table 1). The positive control (ciprofloxacin) showed MIC and MBC values of 3.906 µg/mL and 15.625 µg/mL, respectively, confirming the sensitivity of *S. iniae* to standard antibiotics.

Table 1. MIC and MBC of hydroalcoholic extracts from *Coriandrum sativum* and *Pinus* against *S. iniae*

Extract source	MIC (µg/mL)	MBC (µg/mL)
Coriander	15.625	>500
Pine	250	>500
Ciprofloxacin (control)	3.906	15.625

FIC Index

The FIC index evaluation was performed based on the 50% concentration of each extract, as this was the only effective concentration in our combination studies. Table 2 presents the results of the combined antibacterial activity assessment of coriander and pine hydroalcoholic extracts against *S. iniae* using the microdilution method. As demonstrated in the table, the calculated FIC index value of 17 indicates a clear antagonistic interaction (FIC >4) between the two hydroalcoholic extracts.

Table 2. FIC index for combined hydroalcoholic extracts of *Coriandrum sativum* and *Pinus* against *S. iniae*

Combination	Component	FIC	FIC Index	Interaction
Coriander + Pine	Coriander	16	17	Antagonistic
	pine	1		

Discussion

Medicinal plants have demonstrated significant therapeutic and prophylactic value in disease management, with their application in both traditional and modern medicine dating back centuries (16). Therefore, our study focused on assessing the microbial growth inhibition by hydroalcoholic extracts from coriander and pine against *S. iniae*, both individually and in combination.

In this study, the coriander extract showed stronger inhibitory activity against S. iniae with an MIC of 15.625 µg/mL compared to the pine extract (MIC = 250 µg/mL). This finding is consistent with previous studies reporting the antimicrobial effects of coriander against multiple bacterial species across Gram classifications. Omeidi Myrzai *et al*. (2020) reported significant antibacterial activity of coriander seed extract against *Salmonella* Typhi, *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Escherichia coli* (17), while Tabatabaei Yazdi *et al*. (2016) confirmed similar effects against *Staphylococcus aureus*, *E. coli*, and *P. aeruginosa* (18).

Further supporting evidence comes from Dua *et al*. (2014), who documented coriander seed extract's efficacy against *E. coli*, *P. aeruginosa*, *S. aureus*, and *Bacillus pumilus* (19). Mansouri *et al*. (2018) specifically evaluated Algerian coriander essential oil, reporting MICs ranging from 0.6 to 10 μg/mL against various *E. coli* strains (20), a finding consistent with Khalil *et al*. (2018) (21). The active compounds in coriander, including linalool, geraniol, and phenolic compounds, may be responsible for these antimicrobial effects. These compounds likely inhibit bacterial growth by disrupting cell membrane structure and inhibiting the synthesis of essential proteins (22).

On the other hand, although the pine extract required higher concentrations to inhibit bacterial growth, it still showed significant antimicrobial activity. These results align with findings from studies reporting antimicrobial effects of various pine species against pathogenic bacteria. Nozohor *et al*. (2018) demonstrated antimicrobial activity of pine leaf extract against urinary tract pathogens, including *E. coli* and *S. aureus* (23). Earlier work by Assar *et al*. (2005) on Tehran pine extracts revealed inhibitory effects against skin infection-causing bacteria (*E. coli* and *S. aureus*) (24). Kashani *et al*. (2017) further expanded these findings by showing antimicrobial activity of aqueous and ethanolic extracts from Tehran pine fruit against *E. coli*, *S.* Typhi, *S. aureus*, and *B. cereus* (25). Compounds such as pinene, camphene, and resin acids present in pine extract may contribute to these antimicrobial effects (26).

A particularly interesting observation in this study was the antagonistic effect of combining the two extracts, as indicated by an FIC index of 17. This phenomenon may occur for several reasons: First, the active compounds in each extract may neutralize each other or prevent effective absorption. Second, the different mechanisms of action of the two extracts may reduce each other's effects when combined (27, 28). For example, if the coriander extract primarily affects bacterial cell membranes while the pine extract targets metabolic systems, their combination may reduce both effects. Third, the mixing ratios used in this study may not have been optimal, suggesting the need for further investigation in this area.

From a practical perspective, these findings hold particular significance for the aquaculture industry. Given the increasing antibiotic resistance in aquatic pathogenic bacteria and concerns regarding antibiotic residues in aquatic products, the use of plant-derived compounds as natural alternatives to antibiotics could serve as an appropriate strategy. The results of this study indicate that coriander extract alone may be a suitable option for controlling S. iniae infections. However, its combination with pine extract not only proved ineffective but may also reduce its beneficial effects.

These results contrast with some other studies reporting synergistic effects of plant compounds (14). This discrepancy may stem from differences in the types of plant compounds used, extraction methods, or variations in the bacterial strains tested. Therefore, before making any practical recommendations, additional in vivo studies using animal models are necessary.

This study had several limitations, including examination of only one bacterial strain, use of in vitro methods, and lack of precise identification of active compounds. Thus, future studies should investigate these extracts under in vivo conditions, examine synergistic ratios, identify active compounds using advanced methods, and elucidate their molecular mechanisms of action.

In summary, the results of this study demonstrated that while coriander and pine extracts individually exhibited significant antimicrobial effects against *S. iniae*, their combination may lead to the antagonistic effects. This finding underscores the importance of thoroughly examining interactions between plant compounds before their combined use. The use of coriander extract as a natural antimicrobial agent appears promising, but requires further investigation for practical application in the aquaculture industry.

Conclusion

This study demonstrated that both coriander and pine extracts exhibited significant antimicrobial activity against *S. iniae* when used individually, but their combination resulted in reduced efficacy rather than synergistic effects. The coriander extract showed superior potency, inhibiting bacterial growth at a lower concentration (15.625 μg/mL) compared to the pine extract (250 μg/mL). Given the growing concern of antibiotic resistance, these plant extracts represent promising natural alternatives for aquaculture applications. However, further investigation under in vivo conditions and detailed mechanistic studies are required before practical implementation. This research provides a foundation for developing plant-based strategies to control bacterial infections in aquaculture systems.

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Conflict of interest statement
The authors declare no conflicts of interest.

Ethical approval
No ethical approval was required as this study did not involve animal or human experiments.

Artificial Intelligence Statement
The authors declare that no artificial intelligence tools were used in the preparation of this manuscript.

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