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Research Article

IN VITRO ANTIBACTERIAL ACTIVITY OF CULINARY SPICES ANISEED, STAR ANISE AND CINNAMON AGAINST BACTERIAL PATHOGENS OF FISH

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ABSTRACT

Antibacterial activity of crude extracts of culinary spices like Aniseed (*Pimpinella anisum*), Star anise (*Illicum verum* Hook fruit) and Cinnamon (*Cinnamomum zeylanicum)* against some of the common fish bacterial pathogens like *Aeromonas hydrophila, Aeromonas salmonicida* and *Edwardsiella tarda* were explored by finding minimum inhibitory concentration (MIC). A twofold serial dilution of compounds (100 µl) in sterile normal saline was prepared in 96-well microtitre plate and 50 µl overnight fresh bacterial cultures of one McFarland unit were added to each well. The plates were incubated overnight at 37^0C and bacterial growth was detected by adding 20 µl of *p*-iodonitrotetrazolium violet (INT) to each well and incubating for 30 min. The well that remained color less when there is no bacterial growth and that concentration was taken as MIC. A negative control was also maintained in which all wells with bacterial growth were found in red color. MIC values (mg/ml) for *Pimpinella aisum* against *Aeromonas hydrophila, Aeromonas salmonicida* and *Edwardsiella tarda* were 0.625, 0.15625 and 0.625; *Illicum verum* Hook fruit 0.15625, 0.078125 and 0.15625; *Cinnamomum zeylanicum* 0.3125, 0.078125 and 0.078125, respectively.

Keywords: Culinary spices aniseed, Star anise, Bacterial pathogens of fish.

INTRODUCTION

Fishes are susceptible to several bacterial infections, mainly in culture and wild fishes when reared in high density conditions. Disease outbreaks cause high economic losses to the fish farmers due to elevated mortality rates and decreased productivity[1]. However, microorganisms which are naturally occurring as opportunistic pathogens invade the tissue of a host and render them susceptible to infection[2]. Bacterial infections of fish and fish products may influence human health either directly by inducing diseases or indirectly through the antimicrobial residues deposited following use of antibiotics to treat such infections[3].

The most common fish pathogens like *Aeromonas hydrophila, Aeromonas salmonicida* and *Edwardsiella tarda* are gram-negative bacteria. *Aeromonas hydrophila* is recognized as the most common bacterial pathogen in freshwater fish that was proved to be etiological agent of several distinct pathological conditions including tail/fin rot and hemorrhagic septicemia, especially in freshwater and ornamental fish[4]. However, *Aeromonas salmonicida,* is the etiological agent of furunculosis that infects a broad range of salmonid group and is responsible for substantial grow-out and post-release mortality in anadromous and resident stocks[5]. Lastly, *Edwardsiella tarda*, belonging to *Enterobacteriaceae*, is the etiological agent for Edwardsiellosis, a devastating fish disease prevailing worldwide in aquaculture industries and accounting for severe economic losses[5, 6]. This organism commonly affects more than 20 species of freshwater and marine fishes including carp, tilapia, eel, catfish, mullet, salmon, trout, turbot and flounder, causing systemic hemorrhagic septicemia and emphysematous putrefactive disease with swelling skin lesions, as well as ulcer and necrosis in internal organs such as liver, kidney, spleen, and musculature[5]. Besides piscine species, *E. tarda* also inhabits and infects a broad range of cold or warm -blooded hosts such as reptiles, amphibians, birds, mammals and even humans[6], raising a concern about *Edwardsiella tarda* being a significant zoonotic pathogen.

Spices have been added to foods since ancient times, not only as flavoring agents, but also as folk medicine and food preservatives[7]. Several spices particularly Aniseed (*Pimpinella aisum*) Star anise (*Illicum verum* Hook fruit) and Cinnamon (*Cinnamomum zeylanicum)* are used extensively in the Indian diet and Indian medicine because these are easily absorbed and do not have any adverse effects.

The oil of star anise is stimulant, stomachic, carminative, mildly expectorant and diuretic. It is used in cough lozenges and important for other pharmacological activities. Cinnamon is a popular flavoring ingredient, widely used in food products. It has exhibited beneficial properties to health, such as antimicrobial activity, for controlling glucose intolerance and diabetes, inhibiting the proliferation of various cancer cell lines, and for treating the common cold[8, 9]. As a medicinal plant, *Pimpinella anisum* has been used as a stimulating effect of digestion and antiparasitic, antifungal and antipyretic. Additionally, essential oil of the plant, been shown to have anticonvulsant effects and has been used for the treatment of seizures and epilepsy. Furthermore, it has been used for the treatment of constipation and it also possesses muscle relaxant effect. Recently its oil has been reported to be used as antibiotic substitute in broiler ration[10].

There are few reports on fish pathogens pertaining to antibacterial evaluation of *Illicum verum, Cinnamomum zeylanicum and pimpinella anisum.* Considering their therapeutic potential, the present investigation was undertaken to evaluate antibacterial activity of acetone extracts of *Illicum verum, Cinnamomum zeylanicum and pimpinella anisum* against three major bacterial fish pathogens viz. *Aeromonas hydrophila, Aeromonas salmonicida* and *Edwardsiella tarda.*

MATERIALS AND METHODS

The culinary spices of Aniseed, Star anise and Cinnamon were purchased from well known market with ISI label, and they were identified and authenticated. A voucher specimen (b4397) was deposited. The dried raw material were washed with sterile distilled water and dried using laminar air flow, ground into fine powder using a blender and stored in air tight container for further analysis.

Extraction procedure

Ten grams of fine powder from three culinary spices were weighed into three separate 250 ml conical flasks and each component was extracted with 100 ml of acetone. The mixture was kept for 24 hours on rotary shaker at 190-220 rpm. The mixtures were filtered using Whatman No.1 filter papers. The precipitates were discarded and the filtrate was collected. Each extract was concentrated using rotary evaporator. Stock solution of each extract was prepared by weighing 10 mg of each dried solvent extract dissolved in 1 ml of dimethyl sulphoxide (DMSO) giving a final concentration of 10,000 µg/ml. The stock solution was kept in screw capped bottles for subsequent use.

Test Organisms

Three reference strains of *Aeromonas hydrophila* MTCC646, *Aeromonas salmonicida* MTCC1522 and *Edwardsiella tarda* MTCC2400 were purchased from the Microbial Type Culture Collection and Gene Bank (MTCC) Chandigarh.

Antibacterial Screening

Determination of Minimal Inhibitory Concentration (MIC)

Quantitative antibacterial activity assay was carried for acetone extracts of three culinary spices against *Aeromonas hydrophila, Aeromonas salmonicida* and *Edwardsiella tarda* by finding MIC[11, 12]. A twofold serial dilution of each compound (100 µl) in sterile normal saline was prepared in 96-well microtitre plates and 50 µl overnight fresh bacterial cultures of one McFarland unit were added to each well. The antibiotic enrofloxacin and normal saline were included as standard reference in each assay. The plates were incubated overnight at 37^{0}C. As an indicator of bacterial growth, 40 µl of *p*-iodonitrotetrazolium violet (INT) was added to each well and incubated at 37^{0}C for 30 min. MIC values are recorded as the lowest concentration of the extract that completely inhibited bacterial growth that is clear well. The colorless tetrazolidium salt acts as an electron accepter and is reduced to a red colored formazan product by biologically active organisms[11]. Where bacterial growth was inhibited, the solution in the well remained clear after incubation with INT.

RESULT AND DISCUSSION

The antibacterial activity of acetone extracts of the three culinary spices, namely *Illicum verum, Cinnamomum zeylanicum and pimpinella anisum* investigated quantitatively by the MIC showed good inhibitory activity against three major bacterial fish pathogens Table 1, Fig. 1, 2 & 3. Among the three culinary spices tested, Star anise and Cinnamon showed the best antibacterial activity than the aniseed against three major bacterial fish pathogens. Star anise and Cinnamon showed good antibacterial activity at very lowest concentration of MIC i.e. 0.078125 mg/ml. Aniseed was found to be most active against *Aeromonas salmonicida at* MIC of 0.15625 mg/ml.

Table 1: MIC values of acetone extract of culinary spices against bacterial pathogens of fish

Compound	*A.Hydrophila*	*A.salmonicida*	*E.tarda*
Aniseed (10mg/ml)	0.625	0.15625	0.625
Star anise (10mg/ml)	0.15625	0.078125	0.15625
Cinnamon (10mg/ml)	0.3125	0.078125	0.078125
Enrofloxacin (250µg/ml)	0.1220704	0.2441407	0.2441407

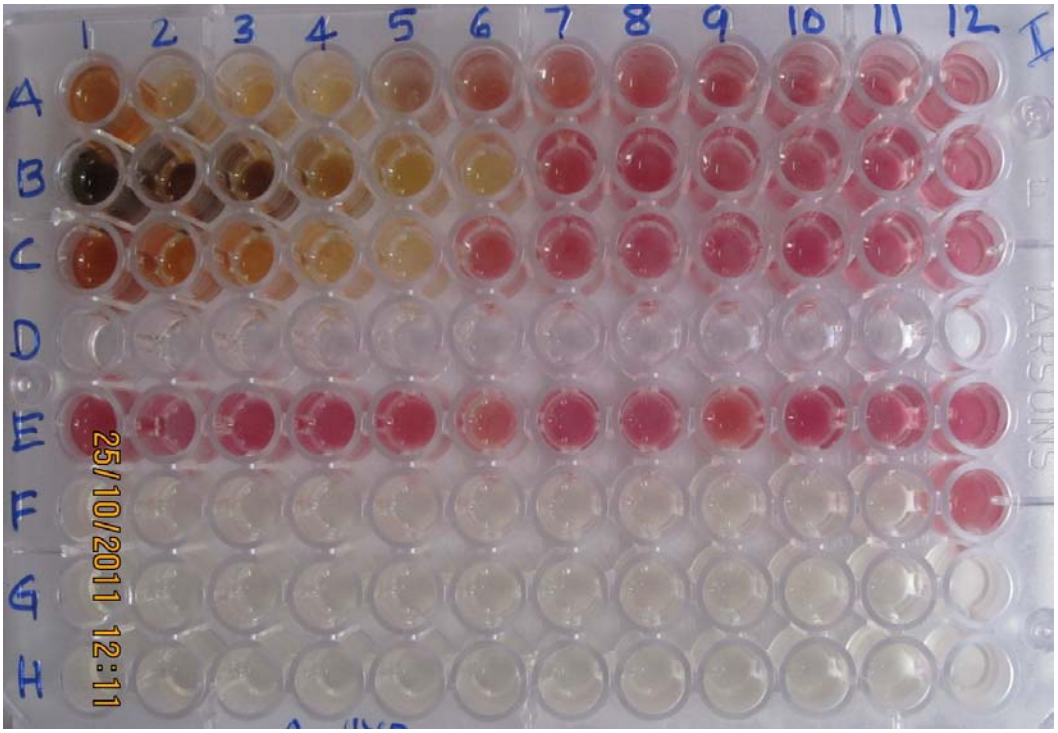

Fig. 1: Minimum inhibitory concentration (MIC) of acetone extracts of selected culinary spices against *Aeromonas hydrophila* (pink colour indicates growth)

Rows A: Acetone extract of Aniseed (compound in serial dilution+broth culture+saline+ indicator)

Rows B: Acetone extract of Star anise (compound in serial dilution+broth culture+saline+ indicator).

Rows C: Acetone extract of Cinnamon (compound in serial dilution + broth culture + saline + indicator)

Row D: Normal saline+Indicator

Row E: Normal saline+broth culture+Indicator

Row F: Enrofloxacin+Normal saline+broth culture+Indicator

Row G&H: only Normal saline

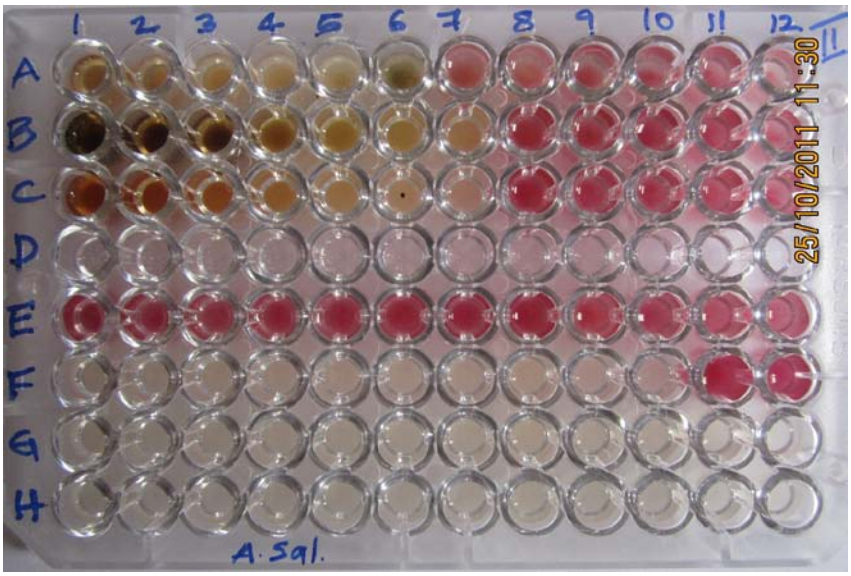

Fig. 2: Minimum inhibitory concentration (MIC) of acetone extracts of selected culinary spices against *Aeromonas salmonicida* (pink colour indicates growth)

Rows A: Acetone extract of Aniseed (compound in serial dilution+broth culture+saline+ indicator)

Rows B: Acetone extract of Star anise (compound in serial dilution+broth culture+saline+ indicator)

Rows C: Acetone extract of Cinnamon (compound in serial dilution+broth culture+saline+ indicator)

Row D: Normal saline+Indicator

Row E: Normal saline+broth culture+Indicator

Row F: Enrofloxacin+Normal saline+broth culture+Indicator

Row G&H: only Normal saline

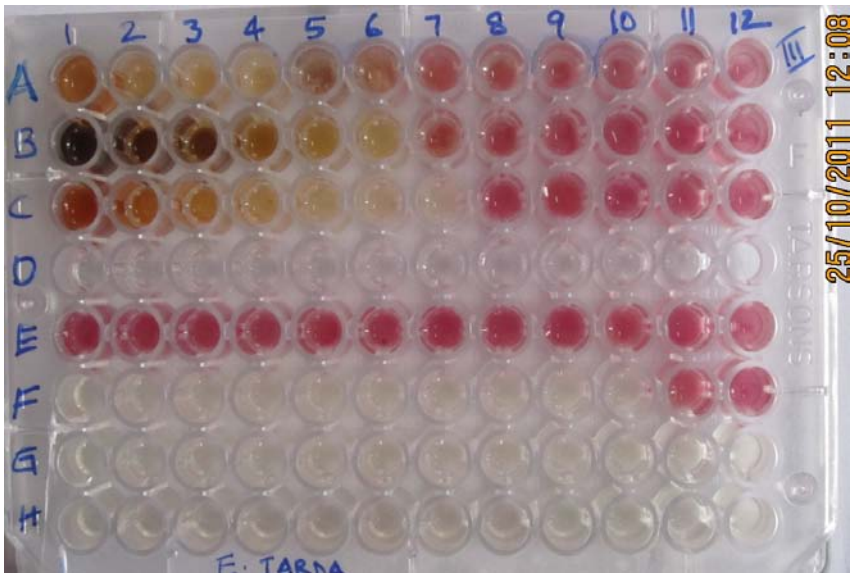

Fig. 3: Minimum inhibitory concentration (MIC) of acetone extracts of selected culinary spices against *Edwardsiella tarda* (pink colour indicates growth)

Rows A: Acetone extract of Aniseed (compound in serial dilution + broth culture + saline + indicator)

Rows B: Acetone extract of Star anise (compound in serial dilution + broth culture + saline + indicator)

Rows C: Acetone extract of Cinnamon (compound in serial dilution + broth culture + saline + indicator)

Row D: Normal saline+Indicator

Row E: Normal saline+broth culture+Indicator

Row F: Enrofloxacin+Normal saline+broth culture+Indicator

Row G&H: only Normal saline.

The effectiveness of inhibitors can be sequenced as follows in descending order against three fish pathogens. *Aeromonas hydrophila* Star anise >Cinnamon > Aniseed; *Aeromonas salmonicida* Star anise > Cinnamon > Aniseed; *Edwardsiella tarda* Cinnamon > Star anise > Aniseed.

Anti microbial activity of Aniseed (*Pimpinella anisum*) and Star anise (*Illicum verum* Hook fruit) could be attributed to Anethole, an active constituent of *Illicum verum* Hook. It has been shown by previous researchers that star anise had antimicrobial activity due to the chemical constituent of Anethole[13]. Anethole has also been shown to possess anti inflammatory and anti fungal activities.

Essential oils of *Cinnamomum* have been shown to possess anti bacterial, antifungal, antiviral, insecticidal and antioxidant properties. Oil contains Eugenol, Cinnamic acid and Cinnamaldehyde. Potent antimicrobial activity of Cinnamon can be attributed to phenolic compounds. Hydrophobicity of essential oils and their compounds enables them to partition the lipids of bacterial cell membrane and mitochondria disturbing the cell structure and rendering them more permeable. Extensive leakages from bacterial cells or exit of critical molecules and ions will lead to death of the microorganisms. It has also been shown that, Eugenol limits the growth of microorganisms by inhibiting the production of certain enzymes needed for growth.

CONCLUSION

Based on the results, it was concluded that Aniseed, Star anise and Cinnamon have significant antibacterial activity against tested fish pathogens. Substituting the commercial antibiotics with crude extracts of these culinary spices or using their combination would have potential benefits and consequently decrease the antibiotic residue load in fish meat. These antibacterial properties would warrant further studies on polyphenolic compounds of the spices and clinical applications in Veterinary or aquaculture field.

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