Potential of herbal extracts to avoid the bacterial infection of *Enterobacter cloacae* in common carp, *Cyprinus carpio* L.

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Abstract. The study shows the possibility of herbal aqueous extracts to avoid the infection caused by *Enterobacter cloacae* in the common carp. The *E. cloacae* vaccinated on agar plates wherever placed to the filter discs, which were intended by filtration of the aqueous extracts (Six herbal species and four concentrations 10%, 15%, 20% and 25%) of *Curcuma longa* (Turmeric), *Panax gingseng* (Ginseng), *Cinnamomum zelianicum* (Cinnamon), *Thymus vulgaris* (Thyme leaves), *Olea europaea* (Olive leaves), *Eugenia caryophyllata* (Clove). The herbal extracts inhibit the growth of *E. cloacae* in the highest concentrations (22 mm inhibition zone in 25%) with *C. longa* which appears to be the most efficient one, whereas the clove was the least efficient. The current study indicates that attainable and inexpensive herbal extracts may be efficient natural agents for preventing infection of *E. cloacae* in fish, and they also have been compared with some antibiotics. Furthermore, a study is required in order to carry out the herbal extracts and to detect the proper practice used in aquaculture.

Keywords: Bacteria, *Enterobacter cloacae*, Medicinal herbal

1. Introduction

The bacterial diseases of fish were implicated in earnest warns to aquaculture systems in Iraq [1][2][3][4]. Enterobacteriaceae (such *E. cloacae*) can be defined as combination waterborne fish infection of microbial bacteria that may commonly inhabit the tissues of obviously healthful fish, and the gastrointestinal tract of animals and humans [5], and may cause the death when the fish are exposed to stress such poor water quality and temperature rise [6]. They were instrumental in the development of obviously diseases as essential pathogenic or acute infections. [7].

The enteric bacterium *E. cloacae*, is deal dispensing in nature. It is instituted in farces of animals and humans, soil, plants, water, insect's plant munitions, and dairy products [8]. It has been recognized as a fundamental pathogen of human so that ability to evolve resistance to antibiotics, it has been reported as a pragmatic pathogen in human [9; 10], and other organisms such as insects and fish, fish live in polluted water bodies was reported to be infected with some species of enteric bacterial [11; 12]. The enteric bacteria (such *E. cloacae*) are not common flora in the intestinal tract of fish and the public in the infective fish may be the result of fish being held in contaminated waters [11]. The infective of fish by the microbes has related to the feeding of fish. Bacterial diseases of catfish (bottom feeding, head of a brown bull) are likely to result in a tradition of human flocculate ingestion containing bacteria. [12]. The current study was planned to determine the execution of six herbal species against *E. cloacae* infection. Performed in a laboratory experiment (in vitro), the efficacy of the aquatic extracts of the herbal to prevent the growth of *E. cloacae*, and compared with antibiotic susceptibility. This would improve our knowledge on the ability to restrict the use of alchemical compounds in aquatic ecosystems.

2. Materials and Methods

The common carp infected with *E. cloacae* (a total of 20 live common carp) were obtain from ponds of Marine Science Centre, Basrah University, and brought to the laboratory during November 2019-January 2020, weighing 40.23±2.14 g and their total length 15.55±1.248 cm.
The bacterial swabs were taken (after the fishes were dissected), sterile using a sterile enteric ring. MacConkey agar medium was used to isolate bacteria. At 37 °C for 24 h, the inoculated plate was incubated. Bacteria and sensitivity to antibiotics were identified using biochemical tests such as oxidase, methyl red, glucose fermentation, citrate, lactose fermentation and were determined by the VITIC 2 system. The antibiotics used in the current study were: amikacin, cefazolin, gentamicin, piperacillin/tazobactan, ceftoxitin, imipenem, ciprofloxam, and trimethoprim/sulfamethoxazolibi.

The plant species were obtained from Iraqi local markets, Curcuma longa (Turmeric), Panax ginseng (Ginseng), Cinnamomum zeilanicum (Cinnamon), Thymus vulgaris (Thyme leaves), Olea europaea (Olive leaves), Eugenia caryophyllata (Clove). The herbal species sensibility test was conducted by disc publishing method on agar of blood at 37°C for 24 h. The execution was based on the combined disc agar publishing method of the NCCLS (National Committee for Clinical Laboratory Standards) for antimicrobial sensitiveness tests [13]. After incubation, the diameter of the zone (inhibition) was determined to assess the susceptibility at four separate concentrations (10, 15, 20, and 25%) of six aqueous extracts of herbal species obtained. Four concentrations (10, 15, 20, and 25%) of herbal aqueous extract, it was prepared according to [14].

By SPSS Edition 20 (ANOVA method), statistical calculations of the results were completed to determine the significant differences at 0.05.

3. Results and Discussion
The infection was detected (in the current study) among common carp, although farm managers announced that they use 1% tetracycline in their fish farms. Tetracycline is used closely to treat bacterial infections in aquaculture [15][16][17], and widespread use of antibiotics can lead to the expansion of antibiotics for pathogens resistance, that can contract and infect cultivated fish [18][19].

In the current study 17 antibiotics were tested, and the polymorphism can be said to be ineffective against E. cloacae. Antibiotic sensitivity statistic is a spot, where there was a small area of inhibition insight (Table 1). In proportion to this number, inhibition was only significant by cefazolin, was restraint 40 μg/ ml (> =), ampicillin-sulbactan and Meropenem was restraint 30 μg/ ml (> =) and trimethoprim-sulfamethoxazole was restraint 60 μg/ ml. The antibiotics examined in the current study are not appropriate for pharmaceutical treatment, there are effective agents cheaper and available) [20].

Table 1: Susceptibility test of E. cloacae to antibiotics.

| Antimicrobial         | MIC Interpretation | Antimicrobial | MIC Interpretation |
|-----------------------|--------------------|---------------|--------------------|
| Ampicillin/ Sulbactan | >= 30              | Resistant     | Meropenem          | >= 30              | Resistant     |
| Piperacillin/Tazobactan | 5                | Sensitive     | Amikacin           | 2                  | Intermediate  |
| Cefazolin             | >= 40              | Resistant     | Gentamicin         | <= 0.5             | S            |
| Ceftazidime           | <= 1               | Sensitive     | Tobramycin         | <= 0.5             | S            |
| Ceftriaxone           | <= 0.25            | Sensitive     | Ciprofloxacin      | 2                  | Intermediate  |
| Cefepime              | <= 0.25            | Sensitive     | Levofoxacin        | 0.5                | S            |
| Aztreonam             | <= 0.25            | Sensitive     | Tigecycline        | 2                  | Intermediate  |
| Ertapenem             | <= 0.25            | Sensitive     | Trimethoprim/ Sulfamethoxazole | 60 | Resistant     |
| Imipenem              | 2                  | Intermediate  |                    |                    |              |

Minimum Inhibitory Concentration: MIC (μg/ ml).

Increased bacterial impedance or resistance to antibiotics and the new antibiotics was deficiency introduced at the market, as a result, it is important to follow techniques to combat infections caused by drug-resistant bacteria. [21]. Sundry bioassays like well sawing, disk sawing and dilution of agar or broth are well recognized and ordinarily used methods [22].
While antibiotics offer a beneficial result, they cannot be considered and prescribed due to their backward and other side effects. In aquaculture activities, the development of herbal bio-medicinal products with growth characteristics encourages strength and stimulates the immune or impregnable system, as relish stimulator (that raises levels of physiological) [23]. Antibiotics increase consumption, fast maturation, and antimicrobial ability, and antistrips characteristics, immense use in the culture fin fishes wanting any environmental and critical problems [23][24].

The possibility of plant extracts prohibiting the contagion caused by *E. cloacae*, each plants contagion the growth of *E. cloacae* (p< 0.01) in 25% (the highest concentration), *C. longa* (Turmeric) was the most effective, but Clove (*E. caryophyllata*) tended to be the least efficient. (Fig. 1). The current study indicated that closely available and cheap plant extracts intensity efficacious natural agents to prohibit the *E. cloacae* contagion in fish. Additional experimentation is required in order to achieve the output of plant extracts (in vivo) as well as to detect suitable pursuits in aquaculture.

The herbal extracts product has a possible application in fish culture as an immunostimulant, but the influence is dose subsidiary, always a prospect for overdosing then, dosage optimization is highly potent and recommended [25]. The immunostimulants of plant products in fish culture systems may be of environmental value, to the biodegradability of plant products, due to their recipient attributes [26][27].

**Figure 1.** Inhibition or susceptibility zone (mm) of the six aqueous plants extracted in different conc. on the growth of *E. cloacae*.

4. Conclusion
Herbals are very essential in aquaculture that have growth-promoting ability. This application will decrease the side effects of applying the artificial compounds, and the setback and create it to be eco-friendly. The alternate herbal biomedicines demonstrate to be efficient in aquaculture operations.

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