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Effect of Herbal Plant Extracts on Inhibition of Pathogenic Bacteria in Nile Tilapia (*Orechromis niloticus*)

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Abstract

Herbal plant extracts could be an alternative treatment tilapia fish for diseases. This research was carried out to evaluate the antimicrobial potential of several plant extracts against fish pathogenic bacteria isolated from diseased or health Nile tilapia. This study selected twenty plant extracts to determine anti-bacterial activity by agar well diffusion method on trypticase soy agar (TSA).Aqueous ethanolic extracts of herbs were tested for activity against three fish pathogenic bacteria, including *Aeromonas hydrophila, Streptococcus agalactiae* and *Edwardsiella ictaluri.* Effective herbal plant extracts was selected to investigate the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values against representative bacterial stains by broth dilution method. The results showed that *Terminalia chebula, Acacia catechu, Glycyrrhiza glabra, Psidium guajava, Houttuynia cordata, Piper sarmentosum* and *Garcinia mangostana* extracts could inhibit all strains of the test bacteria. However, inhibitory zone effects of *A. catechu* extracts showed the best activity against *A.hydrophila, S.agalactiae* and *E.ictaluri* at 22.66±0.29 mm, 24.33±0.09 mm and 21.33±0.34 mm, respectively when compared with other herb extracts, with the zone of inhibition ranging from 8.33±0.84 to 19.33±0.04 mm Generally, MIC and MBC concentration values of *A. catechu* extracts that ranged between 6.5 and 12.5 mg/mL of aqueous plant extracts for all pathogens tested. The results suggest that herbal plant extracts showing several biological activities may be potent inhibitors as a natural anti-bacterial in Nile tilapia.The findings suggest that herbal plant extracts could be a potential alternative to fish disease therapy.

Introduction

In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from herbal plants. Many regions of Thailand have a diverse assortment of plants. Although some indigenous plants are uncommon, their potential bioactivities should not be overlooked

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(Thummajitasakul et al., 2014). Various medicinal properties have been attributed to different components of the plant. Herbs that have been used for centuries to treat bacterial infections were reported to have a high number of phytochemicals with versatile and novel structures. Herbs are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids, steroids and phenolic compounds, shown in vitro to have antibacterial effects (Chahal et al., 2016; Dorman & Deans, 2000). A natural supply for the food business is aquaculture. An increase in demand for aquaculture production for food supply forces the fisheries to increase fish population densities. As a result, poor aquaculture sanitation, a rise in fish populations, and immune system suppression make people more susceptible to bacterial and viral illnesses as well as other diseases (Cabello, 2006). Antibiotics and chemical therapies are currently common treatments for bacterial infection. However, there are restrictions on use, side effects, low efficacy and antibiotic-resistant bacteria with these treatments (Zheng et al., 2000). Utilizing various plant extracts to control pathogen infection in fish is an alternative therapeutic and environmentally friendly method. This is due to the nature of plant-derived extracts which are non-harmful to the environment, water-degradable, ease of preparing procedures, safe in an aquatic ecosystem, low in cost, a good potential source against fish pathogenic bacteria and does not cause a resistant effect on fish, thereby suitable for a sustainable aquaculture (Defoirdt et al., 2011). Due to the fact that many of these promising natural medicinal plants grow in Thailand, the use of plant extract to prevent bacterial infection in fish has a high potential for implementation. Bacterial pathogens are among the emerging diseases and are a major threat to fish production. Some herbs have been reported to have antimicrobial activity against a variety of pathogenic bacteria and have been used as traditional medicines for tilapia fish treatment (Abutbul et al., 2004; Yin et al., 2006).

Oreochromis niloticus also known as Nile tilapia is one of the most significant species in freshwater aquaculture. Due to its benefits of easy breeding, specific disease resistance, rapid development and high marketability, it has become the world's second-most extensively farmed freshwater fish species (Etyemez & Balcazar, 2016). Although Tilapia aquaculture has grown rapidly, it also encounters considerable difficulties due to diseases brought on by *Streptococcus agalactiae, Edwardsiella ictaluri, Aeromonas hydrophila, Vibrio* sp., *Pseudomonas* sp. and *Flavobacterium* sp. Bacterial infection in particular can lead to major issues for the fish, such as septicemia, exophthalmia, corneal opacity and various swimming abnormalities, causing mortality and economic losses in aquaculture (Guo et al., 2019). *A. hydrophila* and *E. ictaluri* are short, gram-negative bacteria found to infect freshwater fish. Fish infected with *A. hydrophila* bacteria resemble having ulcers in their external organs under stress conditions, bacteremia, enteritis, respiratory tract failure, dysentery and motile Aeromonas septicemia (MAS). MAS is stress-related and conditions such as poor water quality, overcrowding and rough handling make fish more susceptible to the bacteria. Fish with MAS infection usually have bulging eyes, swollen abdomens and exhibit small pinpoint hemorrhages at the base of the fins or on the skin. Internal signs include abdominal fluid, enlarged liver and spleen, and dilated and fluid-filled intestines (Yu et al., 2007). The infected fish *E. ictaluri* may exhibit internal organs with excess water and a severe chronic inflammatory infiltrate is observed in the spleen, head kidney and liver, with multifocal areas of necrosis and granuloma formation (Soto et al., 2012). *S. agalactiae* is a gram-positive prominent and ubiquitous pathogen in aquaculture, resulting in annually world-wide significant morbidity (generally 20–30%) and mortality (over 95% of diseased fish), particularly in the tilapia aquaculture business (Su et al., 2017). Disease outbreaks have a negative impact on mortality rates and productivity efficiency, resulting in significant economic losses for fish producers (Hatha et al., 2005). Antibiotics are still used as current treatments for bacterial infections in Asian aquaculture. However, considering the inherent negative effects of antibiotics, other alternative antimicrobials from plant origins are increasingly used in aquaculture. Temu kunci (*Boesenbergia pandurata*), terong asam (*Solanum ferox*) and lempuyang (*Zingiber zerumbet*) extracts also have antibacterial properties that might inhibit the development of *A. hydrophila* and *Pseudomonas* sp. bacteria, which are the Nile tilapia's common pathogens (Hardi et al., 2017). Furthermore, the extract of *Rosmarinus officinalis* have been reported to treat the bacteria infection caused by *Streptococcus iniae* in Nile tilapia (Abutbul et al., 2004; Zilberg, et al., 2010). According to Yin et al. (2006), investigated the results of two Chinese herbs (*Astragalus radix* and *Scutellaria radix*) were also found to have therapeutic effect on diseased tilapia. Meanwhile, application of solo garlic (*Allium sativum*), garlic chive (*Allium tuberosum*)

and betel leaves (*Piper betle*) extract on bacterial pathogens in aquaculture and a challenge of Nile tilapia, *O. niloticus* with *S. agalactiae* were presented (Ataguba et al., 2018). Many substances with antibacterial activity can be synthesized by a variety of plant species. These qualities have been attributed to extracts of several Thailand-found plants. Many herb extracts were investigated on their antimicrobial activity against some fish pathogenic bacteria. Conversely, there haven't been any prior studies analyzing this characteristic for several of the plant species included in this present research. Agar diffusion techniques are used widely to assay plant extracts for antimicrobial activity. The zone of inhibition, as assessed by the agar well diffusion technique, differed depending on the plant extracts. The findings from the agar diffusion assay plates were in fair correlation with those from the MIC testing. Therefore, considering their antibacterial qualities, accessibility and diminished concerns about antimicrobial resistance and antibiotic residues in aquaculture products, the use of herbal additives seems to be a sensible and financially advantageous strategy for aquaculture producers (Anka et al., 2013).

The present work investigated the in vitro antimicrobial effects of ethanolic extract of twenty herbal plants against three fish pathogenic bacteria strains, such as *A.hydrophila, S. agalactiae* and *E. ictaluri* which are incriminated in different diseases of Nile tilapia, one of Thailand's most widely cultivated fish species and may serve as a substitute for synthetic antibiotics in fish raised in farms.

Materials and methods

1. Bacterial preparation

Pathogenic bacteria used in the treatment were two types of Gram-negative rod (*A. hydrophila* and *E. ictaluri*) and one type of Gram-positive coccus (*S. agalactiae*). They were obtained and well identified from Charoen Pokphand Foods (CPF) Public Company Limited, Mueang Samut Sakhon District, Samut Sakhon Province, Thailand. All the cultures were maintained and sub-cultured on Trypticase Soy Agar, TSA, (DIFCO®) slants at 37°C overnight. Bacteria were cultured in Trypticase Soy Broth, TSB, (DIFCO®) media at 37°C, for 24 hr until they reached a log-phase in their growth. The cultures were then centrifuged for 10 min at 1000xg. A phosphate-buffered saline (PBS) solution was used to wash the pellet three times while removing the supernatant. For challenge test, the bacteria suspensions were diluted using sterilized distilled water and standardized using spectrophotometer at 600 nm to an optical density (OD) of 0.1, corresponding to 10^7 CFU/ml (Hardi et al., 2017).

2. Preparation of plant extracts

In the investigation, the whole plant of *Acacia catechu* (wood/bark), *Acanthus ebracteatus* (root/stem/leaf), *Allium ascalonicum* (bulb), *Artemisia annua* (root/stem/leaf), *Artemisia lactiflora* (stem/leaf), *Bauhinia malabarica* (leaf), *Cassia fistula* (fruit; seed aril), *Clinacanthus nutans* (root/stem/leaf), *Eclipta prostrata* (root/stem/leaf), *Garcinia mangostana* (peel), *Glycyrrhiza glabra* (root), *Gynostemma pentaphyllum* (root/stem/leaf), *Houttuynia cordata* (stem/leaf), *Piper retrofractum* (fruit), *Piper ribesiodes* (stem), *Piper sarmentosum* (root), *Psidium guajava* (leaf), *Rhinacanthus nasutus* (root/stem/leaf), *Terminalia chebula* (fruit) and *Tinospora crispa* (stem) were obtained from a local market and grown naturally in Thailand. The gathered plants were taxonomically recognized and verified. Each herbal plant was cleaned with distillated water, dried for 48 hr at 40°C and grinded into powder. To the extraction, 300g of dried powdered plant sample was suspended in 600 mL of ethanol 14.5% at 80°C for 24 hr with constant stirring. After heating, the extract was filtered by 5-micron paper filter and evaporated solvent at 80°C into concentrated liquid. Dried in trey dryer under 50°C with constant air flow to obtain extract powder. After complete solvent evaporation, extracts were dissolved in 10% dimethyl sulphoxide (DMSO) to a final concentration of 100 mg/mL and stored at 5°C in labelled sterile screwcapped bottles for further use (Castro et al. 2008).

3. Antibacterial activity assay

The standard agar well diffusion technique was used to evaluate the antibacterial activity of solvent extracts (Valgas et al., 2007). Inoculum containing 10^7 CFU/mL of each bacterial culture to be tested was swabbed on trypticase soy agar (TSA) plates, subsequently wells of 6 mm diameter were punched using sterile cork borer into the agar medium and filled with 50 µL (100 mg/mL) of plant extract and allowed to diffuse at room temperature for 2 hr. The plates were then incubated for 24 hr at 37°C in the upright position. As negative controls, wells containing the same amount of DMSO (10%) were used. After incubation, the diameters of the growth inhibition zones were measured in millimeter (mm). Three replicates were carried out for each extract against each of the test organism and the average values were

recorded. Data were expressed as mean±standard deviation. Four classifications were made based on the zone of inhibition: weak (<5 mm), moderate (5-10 mm), strong (10-20 mm) and very strong (>20 mm) (Dewi & Mardhiyani, 2021).

4. Bacteriostatic and bactericidal activity

The bacteriostatic or MIC is generally defined as the lowest concentration of a given antimicrobial that prevents growth of a microorganism after a specified incubation period. Based on the preliminary screening, ethanol extracts with high antibacterial activity were examined and further tested to determine the MIC for each bacterial sample. By using a broth dilution approach, the MIC of these extracts was determined. The stocks of 100 mg/mL of the extracts were resuspended in 10% DMSO to produce serial twofold dilutions in the range of 0.1-100 mg/mL. The final concentration of each plant extract was 50, 25, 12.5, 6.5, 3.1, 1.6, 0.8, 0.4, 0.2 and 0.1 mg/mL. Briefly, 1 mL of TSB and 1 mL of graded doses of crude extract were added to each test tube. After that, 1 mL of suspended bacterial suspension (1×10^5 CFU/mL) was inoculated to these test tubes followed by incubation at 37°C for 24 hr. After incubation, a spectrophotometer set to 600 nm was used to measure turbidity as the lowest concentration of plant extracts that prevented the bacterial isolates in the test tubes from growing. The MIC value was established and recorded as being the lowest concentration at which no turbidity was detected. Two test tubes including the extract with no bacteria and bacteria with no extract were used as negative control and positive control, respectively. All samples were tested in triplicates. To evaluate the Minimum bactericidal concentration (MBC), the tubes that did not exhibit any apparent growth (clear solution) were collected from each tube using an inoculation loop and re-cultured on TSA. The lowest concentration at which there was no apparent bacterial growth was recorded as the MBC values after incubation. The assays were done in triplicate (Elisha et al.,2017; Aiyegoro et al., 2009).

Results and discussion

In this study, we investigated multiple herbs for antibacterial efficacy against bacterial infections in warm water fish such as tilapia (*Oreochromis niloticus*). In Table 1, the outcomes of the agar diffusion assay used to screen for the presence of antimicrobials are displayed. According to the findings, the test microorganisms' growth was inhibited to varied degrees by plant extracts from plants at a concentration of 5 mg. Twenty ethanolic extracts were shown to have antibacterial action against at least one strain tested. The organism *A. hydrophila* was the most vulnerable and 14 plant extracts inhibited it. *S.agalactiae* and *E. ictaluri* were inhibited by eleven and eight plant extracts, respectively. The efficacy of the antimicrobial agents in the ethanolic extract of plants was assessed using the MIC and MBC assays. In diagnostic laboratories, determining the MIC and MBC is essential because it assists with identifying if a microbe is resistant to an antimicrobial agent and it keeps track of the activity of new antimicrobial agents. The results range between 6.5 and 50 mg/mL for the MIC and MBC of the extract against all pathogenic bacteria tested and the results are presented in Table 2. There was a wide range of MIC and MBC values depending on the specific microbial strains being tested.

Table 1 Bacterial inhibition zone (mm) of plant extracts (5 mg) in agar diffusion assay

No.	Species	Inhibition zones (mm)		
		A. hydrophila	*S. agalactiae*	*E. ictaluri*
1	*Acacia catechu*	22.66±0.29	24.33±0.09	21.33±0.34
2	*Acanthus ebracteatus*	11.00±0.14	-	10.00±0.28
3	*Allium ascalonicum*	-	15.00±0.08	-
4	*Artemisia annua*	12.33±0.24	14.33±0.14	-
5	*Artemisia lactiflora*	13.33±0.81	13.00±0.08	-
6	*Bauhinia malabarica*	8.33±0.02	13.66±0.04	-
7	*Cassia fistula*	11.00±0.14	-	-
8	*Clinacanthus nutans*	10.00±0.03	-	15.33±1.12
9	*Eclipta prostrata*	-	-	-
10	*Garcinia mangostana*	9.33±0.09	9.33±0.09	10.00±0.03
11	*Glycyrrhiza glabra*	19.00±0.29	16.33±0.09	15.33±0.05
12	*Gynostemma pentaphyllum*	14.66±1.14	-	15.00±0.32
13	*Houttuynia cordata*	10.00±0.04	15.00±0.23	10.00±0.46
14	*Piper retrofractum*	-	-	-
15	*Piper ribesiodes*	-	-	-
16	*Piper sarmentosum*	11.00±0.14	12.00±0.17	10.00±0.87
17	*Psidium guajava*	8.33±0.84	9.00±0.14	15.00±0.09
18	*Rhinacanthus nasutus*	-	-	-
19	*Terminalia chebula*	12.00±0.28	12.00±0.54	19.33±0.04
20	*Tinospora crispa*	-	-	-

Remark: (-) Inhibition zone not observed

The ethanolic extract of four plants, as shown in Fig. 1, have antibacterial activity utilizing the agar well diffusion technique against three fish pathogenic bacteria. The results showed that *A. catechu, G. glabra, T. chebula, P. guajava, H.cordata, P. sarmentosum* and *G. mangostana* formed inhibition zones against all bacterial strains. Inhibitory effects of *A. catechu* extracts showed the best activity against *A. hydrophila, S. agalactiae* and *E. ictaluri* with inhibition zone at

Table 2 MIC and MBC of plant ethanolic extracts to selected fish bacterial pathogens

No.	Species	*A. hydrophila*		*S. agalactiae*		*E. ictaluri*	
		MIC values (mg/mL)	MBC values (mg/mL)	MIC values (mg/mL)	MBC values (mg/mL)	MIC values (mg/mL)	MBC values (mg/mL)
1	Acacia catechu	6.5	6.5	6.5	6.5	12.5	12.5
2	Acanthus ebracteatus	6.5	6.5	-	-	-	-
3	Allium ascalonicum	-	-	12.5	12.5	-	-
4	Artemisia annua	12.5	25.0	25.0	50.0	-	-
5	Artemisia lactiflora	25.0	50.0	25.0	50.0	-	-
6	Bauhinia malabarica	12.5	12.5	12.5	12.5	-	-
7	Cassia fistula	50.0	50.0	-	-	-	-
8	Clinacanthus nutans	12.5	25.0	-	-	-	-
9	Eclipta prostrata	-	-	-	-	-	-
10	Garcinia mangostana	25.0	50.0	6.5	6.5	12.5	12.5
11	Glycyrrhiza glabra	12.5	12.5	12.5	12.5	25.0	25.0
12	Gynostemma pentaphyllum	50.0	50.0	-	-	12.5	12.5
13	Houttuynia cordata	25.0	50.0	12.5	25.0	12.5	25.0
14	Piper retrofractum	-	-	-	-	-	-
15	Piper ribesiodes	-	-	-	-	-	-
16	Piper sarmentosum	12.5	12.5	12.5	12.5	12.5	12.5
17	Psidium guajava	25.0	25.0	25.0	25.0	12.5	12.5
18	Rhinacanthus nasutus	-	-	-	-	-	-
19	Terminalia chebula	12.5	12.5	6.5	6.5	6.5	6.5
20	Tinospora crispa	-	-	-	-	-	-

Remark: (-) MIC/MBC assay were excluded due to no inhibition somes observed (see table 1)

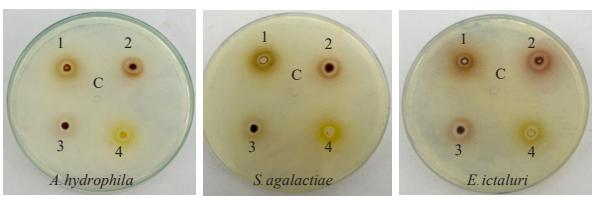

Fig 1. The inhibition zone (mm) of extracts of *T. chebula* (1), A. catechu (2), *P. guajava* (3) and *G. glabra* (4) against *A. hydrophila, S. agalactiae* and *E. ictaluri* at concentration of 5 mg (C) represent the negative control, 10% v/v DMSO

22.66±0.29 mm, 24.33±0.09 mm and 21.33±0.34 mm, respectively. *A. catechu*, commonly known as catechu, cachou and black cutch is a valuable medicinal plant and a crucial forest tree from an economic standpoint (Negi & Dave, 2010). This extract proved to be equally effective against both gram-positive and gram-negative bacteria. All the bacterial strains tested was found most susceptible with maximum inhibition by ethanolic extract producing zone of inhibition >20 mm (> 20 as very strong activity). MIC and MBC of this extract was 6.5 mg/mL against *A.hydrophila* and *S. agalactiae* while it was 12.5 mg/mL for *E. Ictalurid*, respectively. The results of the chemical research on *A. catechu* revealed that different parts had extremely high concentrations of flavonoids, tannins and phenolic substances. More specifically, this plant contains large amounts of catechin which serves as an antioxidant and antimicrobial agent (Singh et al., 2005). The extracts of the roots of *G. glabra* has shown magnificent antibacterial effect. Species of *G. glabra* (licorice or liquorice) also showed the capacity of inhibit the growth of *A. hydrophila, S. agalactiae* and *E. ictaluri* at 19.00±0.29 mm, 16.33±0.09 mm and 15.33±0.05 mm, presenting MIC and MBC values of 12.5 mg/mL, 12.5 mg/mL and 25.0 mg/mL respectively. The primary biomolecules of the roots include flavonoids, glycyrrhizinic acid (glycyrrhizin), glabridin, liqueurite and liqueurizine, which have potential antimicrobial (Mamedov & Egamberdieva, 2019). Recent research has discovered that consuming licorice roots can increase the non-specific immune system in Nile tilapia (Abdel-Tawwab & El-Araby, 2021). *T. chebula* showed inhibition zone of 12.00±0.28 mm against *A. hydrophila* and *S. agalactiae.* The highest mean diameter of inhibition zone of 19.33±0.04 mm against *E. ictaluri.,* MIC and MBC concentration values that ranged between 6.5 and 12.5 mg/mL for all pathogens tested. *T. chebula* fruit extracts have potent antibacterial activity against a number of gram-positive and gram-negative pathogenic bacteria. It is well recognized that this plant is a significant source of secondary metabolites, with hydrolysable tannins accounting for 33% of total phytoconstituents (which can range between 20-50%) and is responsible for antibacterial activity (Sharma et al., 2012). The inhibition zone diameters 8.33±0.84, 9.00±0.14, and 15.00±0.09 mm were obtained from the extract of *P. guajava* leaf against *A. hydrophila, S. agalactiae* and *E. ictalurid*, respectively. This extract had the lowest MIC and MBC (12.5 mg/mL) of *E. ictalurid*, while the highest value (25 mg/mL) of *A. hydrophila* and *S. agalactiae.* Guava is used to isolate flavonoids and saponins from leaves. These leaf extracts are known to have antimicrobial (Metwally et al., 2010). Extract from *G. mangostana* (mangosteen) pericarp was effective against *A. hydrophila, S. agalactiae* and *E. ictaluri* at inhibition zone diameter 9.33±0.09, 9.33±0.09 and 10.00±0.03 mm, respectively. The MIC and MBC for *A. hydrophila* and *S. agalactiae* were 25 mg/mL while it was 12.5 mg/mL for *E. ictalurid* respectively. According to phytochemical investigations, its active components belong to a group of xanthone derivatives such as α-, β- and γ-mangostin, gartinin, 1- and 3-isomangostin, etc. Among these, α-mangostin has the most potent antibacterial activity against both gram-positive and gram-negative bacteria. In our results

we found that *H. cordata* and *P. sarmentosum* extracts showed inhibition activity against *A. hydrophila, S. agalactiae* and *E. ictaluri.* The shoots of *H. cordata* are edible in China and Malaysia and have been used medicinally throughout Asia. According to reports, *H. cordata* extracts contains many constituents such as essential oil, flavonoids and other polyphenols, alkaloids, organic acid and fatty acid, sterols and microelements and have a variety of pharmacological activities including antibacterial (Kumar et al., 2014). In addition, phytochemical screening of the *P. sarmentosum* crude extract indicated the presence of tannins, flavonoids, alkaloids glycosides and anthraquinone (Fernandez et al., 2012).

The other antimicrobial active herb extracts, including produced zones of growth inhibition against *A. Hydrophila,* ranged between 8.33±0.84 and 11.66±1.14 mm. While *S. agalactiae* was inhibited with a range between 9.33±0.09 and 15.00±0.23 mm. and *E. ictalurid* was inhibited ranging between 10.00±0.03 and 15.33±0.05 mm. Moreover, these herb extracts had MIC values of 6.5 mg/mL (2 extracts), 12.5 mg/mL (6 extracts), 25.0 mg/ml (4 extracts) and 50.0 (2 extracts) against *A. hydrophila,* 6.5 mg/mL (3 extracts), 12.5 mg/mL (5 extracts) and 25.0 mg/mL (3 extracts) against *S. agalactiae.* and 6.5 mg/mL (1 extract), 12.5 mg/mL (4 extracts) and 25.0 mg/mL (1 extracts) against *E. ictaluri.* The outcomes for the MBC values matched those for the MIC values. Those presenting the MBC ranged from 6.5 mg/mL to 50 mg/mL. These herbs and their components, such as levamisole, alkaloids, flavonoids, essential oils, terpenes, organic acids, coumarins and lignans, have been shown to have potential antimicrobial effects in tests both in vitro and in vivo (Hardi et al., 2016). Herbs bring forth antibacterial activities by damaging cell walls, inhibiting nucleic acid and protein synthesis and increasing intracellular osmotic pressure (Liang et al., 2022). In addition, antibacterial chemicals have the ability to quickly damage the bacterial cell wall and cytoplasmic membrane resulting in cytoplasm leakage (Shan et al., 2007). In this research, the aqueous extract of *A. ebracteutus* inhibited the growth of *A. hydrophila.* It is a mangrove plant used in traditional medicine in Malaysia, Thailand and China. *A. ebracteatus* was also found to contain megastigmane, benzoxazinoid glycosides and aliphatic alcohol and it has been tested against several bacteria (Kanchanapoom et al., 2001). An antimicrobial property of shallot (*A. ascalonicum*) can effectively inhibit a wide range of pathogenic bacteria. According to Cowan (1999), the antibacterial mechanism of quercetin in shallot may be through membrane rupture and destruction of bacterial extracellular proteins by generating an irreversible complex. In the present study, *S. agalactiae* was inhibited with Shallot extracts of 15±0.08 mm. inhibition zone. Globally, there are around 500 species of *Artemisia* (Astraceae). The primary objective of this study was to evaluate their antimicrobial activities against fish bacterial strains of *Artemisia* spp. (*A. annua* and *A. lactiflora*). The outcomes showed that certain bacterial strains were successfully inhibited by the crude extract of these plants. Corresponding our data, *A. annua* extracts were previously shown to have strong bactericidal activity against *A. hydrophila* and *S. agalactiae,* suggesting that these plants contain bactericidal compounds beyond artemisinin (Soares et al., 2020). Along with artemisinin, *Artemisia* spp. also contains flavonoids and phenolic compounds like rosmarinic and chlorogenic acids (Gouveia & Castilho, 2013). From this research, the extracts of *B. malabarica* demonstrated promising activity against fish pathogenic bacteria *A. hydrophila* and *S. agalactiae.* In other research, using crude extracts of Bauhinia parts, promising activities were demonstrated, with substantial potential for antibacterial inhibition of the plant against a variety of bacteria (Neto et al., 2008). For this reason, the crude extracts of *Bauhinia* spp. presented saponins, tannins and alkaloids since this compound is known to have an antimicrobial effect against various microorganisms (Neto et al., 2008). Also considerable effects of *C. fistula* (*Leguminosae*) extracts against some fish pathogenic bacteria was observed. Consequently, the extracts inhibited *A. hydrophila* efficiently. According to Rizvi et al., (2009), this is the outcome of several substances in the aerial parts of *C. fistula*, such as flavonoids and polysaccharides. It can be found that *C. nutans* extracts showed activity against *A. hydrophila.* This medical herb has numerous chemical compounds that possess antibacterial activities such as, β-sitosterol, stigmasterol and flavonoids (Xie et al., 2015). The results of our experiment showed that *G. pentaphyllum* extracts against *A. hydrophila* and *E. ictalurid*. It is an edible plant used as a medicine in oriental countries. The bacterial activity of *G. pentaphyllum* has been reported. For instance, it has been shown that extract substances such as saponin were used to inhibit fungi and bacteria human diseases (Srichana et al., 2011).

The results of this study showed that the six herbs

(*A. ascalonicum, E. prostrata, E. prostrata, P. retrofractum, P. ribesiodes, R. nasutus* and *T. crispa*) did not inhibit *A. hydrophila,* while nine herbs (*A. ebracteatus, C. fistula, C. nutans, E. prostrata G. pentaphyllum P. retrofractum, P. ribesiodes, R. nasutus* and *T. crispa*) did not inhibit *S. agalactiae* and twelve herbs (*A. ebracteatus, A. ascalonicum, A. annua, A. lactiflora, B. malabarica, C. fistula, C. nutans, E. prostrata, P. retrofractum, P. ribesiodes, R. nasutus* and *T. crispa*) did not inhibit *E. ictaluri.* In this investigation, none of the extracts examined shown antibacterial efficacy against all test bacterial strains. Antibiotic-resistant bacterial strains have been observed in aquaculture systems. Possibly, the potentially deleterious impact of the extracts on the bacterial cells should be inhibited by the same mechanism engaged in antibiotic resistance. Nonetheless, some extracts were effective against pathogens, suggesting a potential alternative to fish disease therapy.

Conclusion

According to the study's findings, some herbs may have the ability to act as natural antimicrobial agents to prevent the growth fish pathogenic microorganisms. Of the 20 plants, crude ethanolic, i.e., extracts of seven, *A. catechu, G. glabra, T. chebula, P. guajava, H.cordata, P. Sarmentosum* and *G. mangostana* exhibited an inhibitory effect against all test bacterial strains, including *A. hydrophila, S. agalactiae* and *E. ictaluri.* The *A. catechu* showed high antimicrobial activity against all pathogens tested. No aqueous crude extract was found to inhibit any tested bacteria. The results indicated that different plant types significantly affected antibacterial activities. The extracts may be further investigated to identify and isolate the active components as a complementary therapy for the bacterial fish infections that are currently seen in Thailand fish farming.

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