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# Antimicrobial Activity of Bioactive Herbal Extracts Against Streptococcus Agalactiae Biotype 2

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**Abstract:** *Bacterial infection in aquaculture is mainly controlled by the use of antibiotics. However, indiscriminate use of antibiotics is undesirable, as this often leads to the development of drug resistance and therefore to a reduction in the efficacy of drugs. There is urgent need to look for cheaper and more environmentally friendly alternative approaches to improve the immune system of fish than can act as stimulators to control diseases for sustainable aquaculture. In this study, bioactive extracts from five herbs were extracted using aqueous, chloroform and ethanol as solvents. Antimicrobial activity of aqueous extracts of Aeglemarmelos, Emblicaofficinalis&Moringaoleifera, chloroform and ethanol extracts of Azadirachtaindica&Toonasinensis were assessed against S. agalactiae Biotype 2 by using disk diffusion assay. The minimum inhibitory concentrations (MIC) of the herbs were determined by using well diffusion method. Aqueous extract of M. oleifera leaves resulted highest antimicrobial activity with a 13.1 mm inhibition zone and Aeglemarmelos leaves extracts resulted lowest antibacterial activity with a 7.9 mm inhibition zone. Chloroform-extracted bioactive substance from Toonasinenses resulted lowest MIC value (0.15 mg/mL) with high efficacy against S. agalactiaeBiotype 2. M. oleiferaleavescould be a potential source of antimicrobial agents for sustainable aquaculture drug formulation.*

**Keywords:** *Extracts, Streptococcus agalactiaeBiotype 2, Antimicrobial activity, Minimum inhibitory concentration (MIC), Moringaoleifera*

## 1. INTRODUCTION

Nile tilapia industry is facing significant losses due to the disease caused by bacterium *Streptococcus agalactiae*Biotype 2. *S. agalactiae* is a Gram-positive, non-motile, oxidase-negative, catalase-negative coccus. In unusual circumstances, *S. agalactiae* is also closely related groups with disease in human, dogs, cows, horses, and guinea pigs (1).

Antibiotics are used to treat the diseases but indiscriminate use of antibiotics can have impacts on the environment and consumers. Herbs have been mainly utilized traditionally in human and veterinary medicine. Immunostimulants used in fish culture to control disease, as they offer an alternative to the drugs, chemicals and antibiotics. Nowadays herbs play an important role in aquaculture as good alternatives for disease resistance (2). The use of antibiotics and chemotherapeutics, are the most common strategy for prophylaxis and treatment of *Streptococcus*. In order to replace antibiotics, the use of natural products will get good alternatives for chemically synthesized antibiotic and growth promoters (3). Immunostimulants have achieved remarkable success as a more environmental friendly approach to fish disease management.

Plants generally contain certain bioactive chemical compounds which may be applied in nutrition and as pharmacologically active agents (4). *M.oleifera*plants are in high demand for their medicinal value in Asia and Africa. Apart from therapeutic (medicinal) benefits, it was reported to be a good source of vitamins & amino acids (5), & also was claimed to boost immune systems (5, 6 & 7).

This study was designed to investigate the antibacterial activity OF HERBAL EXTRACTS AGAINST *Streptococcusagalactiae* BIOTYPE 2 INFECTION IN TILAPIA (*Oreochromisniloticus*).

## 2. MATERIALS AND METHODS

### 2.1 Herbs collection

Bael, Indian Gooseberry, and *Moringa* were received from All-Season herbs Pvt Ltd, Bangalore, India.Neem and Mahogany herbs were collected from the market in Klong-16, Thailand. For the experiments, herbs were selected based on the bioavailability (Table 1). Herbal leaves were washed separately under running tap water; follow by rinse using sterilized distilled water. Excess of water removed from the plant material by using filter paper, before they were used for extraction.

**Table 1: Botanical classification and parts used of the herbs**

| Scientific Name | Common Name | Extraction Solution | Parts used |
|---|---|---|---|
| Aeglemarmelos | Bael | Aqueous | Leaves |
| Emblicaofficinalis | Indian Gooseberry | Aqueous | Fruits |
| Moringaoleifera | Moringa | Aqueous | Leaves |
| Azadirachtaindica | Neem | Chloroform & Ethanol | Leaves |
| Toonasinensis | Chinese mahogany | Chloroform & Ethanol | Leaves |

### 2.2 Preparation of herbs extract

Herbs extract were prepared according to the method described by Alsaid (2). The Herbs leaves were used to prepare the extracts. Briefly, the herbs were oven dried (50°C for 96 h) and powdered before extracted with 2 different solvents including chloroform & ethanol. 25g quantity of each herb powder was dissolved and extracted with 250 mL of chloroform & ethanol then homogenized by using homogenizer. The extract was centrifuged at 8,000 rpm for 15 min at room temperature and supernatant was collected, filtered and dried by using rotary vacuum at 35°C. The samples of extracts were stored in bottles and refrigerated at 4°C prior to use. Just before use, the extract powder was reconstituted with Dimethyl Sulfoxide (DMSO) and sterilized using 0.2$\mu$ filter device.

### 2.3 Microorganism

For the studies, microorganism (*Streptococcus agalactiae* Biotype 2) was received from National Center of Streptococcus Collection, Department of Microbiology, Faculty of Medical Science, Chulalongkorn University, Thailand. A bacterium was propagated in Brain Heart Infusion (BHI) medium at 35°C overnight. The bacterium (Stock culture) was frozen in 20% glycerol with 0.85% saline solution at -20°C until use. For the infection trials, 100 mL BHI was inoculated with 50µL of the frozen isolate. For the incubation of broth, shaker was used at 35°C for 24 h and then continued by centrifugation at 5000 rpm for 20 min at 10°C.

### 2.4 Screening of antibacterial activity of herbs

Disk diffusion method was used to measure the antibacterial activity according to method described by Aldermon& Smith (8). Briefly, Petri dish containing 20mL of Mueller Hinton agar was inoculated 0.1mL of previously prepared *S. agalactiae* Biotype 2 suspension (adjust to 0.5 McFarland standards, which are equal to 1 x $10^8$ cfu $ml^{-1}$). Sterile Paper disk (6 mm - diameter) was placed in the inoculated agar plate and fill with 25µL of herbal extract. For control, chloroform and ethanol were similarly tested. The plates were kept for incubation at 37°C for 24 h. The test was performed in triplicate. The antibacterial activity was assessed by measuring the diameter of the area in which bacterial growth was inhibited around the well.

### 2.5 Minimum Inhibitory Concentration (MIC)

Agar diffusion method was used to determine the minimum inhibitory concentration (MIC) of the extract according to Valgas (9). For each extract two fold serial dilutions was prepared & eight concentrations of each herbs were prepared (20, 10, 5, 2.5, 1.25 0.6, 0.3 & 0.15 mg $mL^{-1}$). Petri dish containing 20mL of Mueller Hinton agar (MHA) was inoculated 0.1mL of previously prepared *S. agalactiae* Biotype 2 suspension (adjust to 0.5 McFarland standards, which are equal to 1 x $10^8$ cfu $ml^{-1}$) and spread with cotton swabs. 7mm diameter cork borer was used to make wells in the agar plate. 50µLof each prepared concentration was taken to fill the wells and chloroform & ethanol were used as control. The plate was kept for incubation at 35°C for 24 h. The test was performed in triplicate and MIC value was determined by observing the minimum concentration.

### 2.6 Statistical analysis

The Statistical Package for Social Science (SPSS) software was used to analyze the data.Antibacterial activity of herbal extracts was performed by One-way analysis of variance (ANOVA) followed by Duncan post hoc multiple comparison test. Statistical significance was designated as a P value <0.05.

## 3. RESULTS

Thisstudyaimedtofindanatural source,antimicrobial substancetoreplace antibioticsfor thetreatmentof*Streptococcus agalactiae* Biotype 2infectionsinfishaquaculture.Inthisstudy,7herbs were used for screening todeterminetheherbwiththestrongestantimicrobialactivityagainst *Streptococcus agalactiae* Biotype 2. Theherbswereselectedbased on their availabilityand their easilygrown,throughout the world. The inhibition values of the different herbal extracts on the bacterium are shown in Table 2.

Almost all tested extracts displayed antimicrobial activities, with the inhibition zone ranging from 7 to 13 mm. Aqueous extractof *M.oleifera* and Chloroform extract of *A. indica*were significantly (P<0.05) different from chloroform and ethanol extracts of *Toonasinensis* , ethanol extract of *A. indica*, both aqueous extract of *Aeglemarmelos* and *Emblicaofficinalis.* The aqueous extract of *M. oleifera*leaves(13.1 ± 0.23 mm) showed strongest antibacterial activity against *S. agalactiae* Biotype 2 followed by chloroform extract of *A. indica*leaves (12.2 ± 0.20 mm) (Fig. 1).

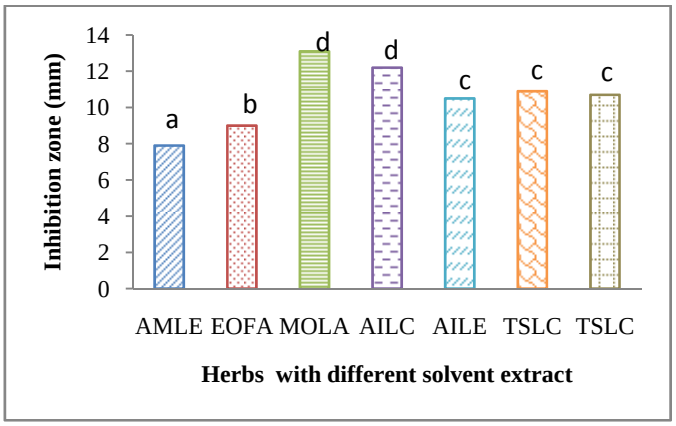


**Fig. 1: Antimicrobial activities of herbal extracts against *S. agalactiae*Biotype 2**

The aqueous extracts of *Aeglemarmelos*leaves(7.9 ± 0.94 mm) showed lowest antibacterial activity against *S. agalactiae* Biotype 2. Both the *A. indica* and *T. sinensis* chloroform leaves extract were found to induce higher inhibitory effect than ethanol extracts. Solubility of active ingredients of *A. indica*& *T. sinensis* were better extracted with chloroform than ethanol.

The minimum inhibitory concentrations (MIC) of herbal extracts against *S. agalactiae* Biotype 2 was lowest in chloroform extract of *T. sinensis*leaves (0.15mg $mL^{-1}$) followed by ethanol extract of *T. sinensis*leaves (0.6 mg $mL^{-1}$), aqueous extracts of *E. officinalis*fruits (0.6 mg $mL^{-1}$) & aqueousextracts of *M. oleifera leaves* (0.6 mg $mL^{-1}$) while the chloroform extracts of *A. indica*leaves showed the highest value of 10 mg/ml.

**Table 2: Antimicrobial activities and minimum inhibitory concentration of herbal extracts against *S. agalactiae*Biotype 2; Results with the means of three replicates ± SD;*Duncan Test, mean values withsamecolumn followed by same superscriptsarenotsignificantlydifferent(p<0.05)**

| Herbs | Parts used | Extraction | Inhibition zone (mm)* | MIC(mg/ mL) |
|---|---|---|---|---|
| Aeglemarmelos[AM] | Leaves [LE] | Aqueous [A] | 7.9 ± 0.94a | 5 |
| Emblicaofficinalis[EO] | Fruits[F] | Aqueous [A] | 9 ± 0.11b | 0.6 |
| Moringaoleifera[MO] | Leaves [LE] | Aqueous [A] | 13.1 ± 0.23d | 0.6 |
| Azadirachtaindica[AI] | Leaves[LE] | Chloroform [C] | 12.2 ± 0.20d | 10 |
| | Leaves [LE] | Ethanol [E] | 10.5 ± 0.8c | 1.25 |
| Toonasinensis [TS] | Leaves [LE] | Chloroform [C] | 10.9 ± 0.11c | 0.15 |
| | Leaves [LE] | Ethanol [E] | 10.7 ± 0.11c | 0.6 |

## 4. DISCUSSION AND CONCLUSION

*M. oleifera* possess antibacterial activity against Gram positive and Gram negative bacteria whereas the presence of antibiotic compounds may be in the plant (10, & 11).*Moringa*leaf is a good source of natural antioxidant such as ascorbic acid, phenolics and carotenoids (12). *Moringa* has antibacterial activity due to the presence of phytochemicals in the plant. *Moringa* leaves extract was active at concentration of 200 mg/ml against *Enterobacter*sp, *S. aureus*, *P. aeruginosa*, *S.typhy*& *E.coli* (13).

*A.indica* were found antibacterial activity against *Streptococcus mutans*&*S. faecalis* (14), *S. aureus* (15), *Salmonella typhosa* (16,17) may be due to the presence of triterpenoids, phenolic compounds, tetra-triterpenoidsazadirachtin, ketoes, flavonoids, steroids &caretonoids. Phytochemical extract of *A. indica* plants have potential activity of antiviral, antitumor and antimicrobial agents (18).

*Aeglemarmelos*found low inhibition zone caused of the insolubility of the active components of herbs in water. chloroform& ethanol extracts of phytochemicals of *A. marmelos* leaf were found to be more active against Gram positive and Gram negative bacteria (19). Extract of *A. marmelos* possessed antibacterial activity and were found active against food borne bacterial species such as *Staphylococcus aureus*, *S.epidermidis*, *Proteus vulgaris* (20).

*E. officinalis* has antimicrobial activity against human pathogen (21). *T. sinensis* plant has many phytochemical components with various type of bioactivity such as antioxidant, anti-inflammatory and anticancer activities (22).

Belemtougri(23) reportedthatantimicrobial compounds found in*A. paniculata*leafextracts wereanthocyans, alkaloids, flavonoids,tannins, andterpenoids.Among thesecompounds,onlytheflavonoidswerefoundtohaveabacteriostaticeffect onfishpathogenicbacteriaincluding*A.hydrophila*, *A. salmonicida*subsp.*salmonicida*,*Flavobacteriumcolumnare*, *Lactococcusgarvieae*,*Streptococcusagalactiae*,and*Vibrio salmonicida*(24).

In conclusion, basedon diameter of inhibitionzoneandMICvalueagainstbacterium,theaqueous

extractof*Moringa*leafhadthe strongestantimicrobialactivity.In the present study, *Moringa* leaves extract was chosen for experimental trials based on the extract availability and the antibacterial results obtained.

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