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# Preliminary Evaluation of Lake Lanao fish *Hypseleotris agilis* Herre for Antimicrobial Activity

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**Abstract**
The search for bioactive compounds from a variety of living organisms as alternative source of cure is a relentless effort of man to fight various diseases. Recently, natural products from freshwater fish have been exploited. Hence this study was conducted primarily to tap the dominating invasive fish species *Hypseleotris agilis* Herre of Lake Lanao, Philippines as possible source of indigenous drugs. The fish is locally known as Katulong belonging to the family Eleotridae which believed to have been accidentally introduced sometime in the 1960s along with tilapia and milkfish fry stocking in the lake for aquaculture. This preliminary study specifically aimed to evaluate the antibacterial and antifungal potentials of the freshwater fish. Crude methanol extract of the whole fish were assayed for antimicrobial activity using the filter paper disc diffusion assay against one fungal strain *Candida albicans*, two Gram-negative bacterial strains *Pseudomonas aeruginosa* and *Escherichia coli* and two Gram-positive strains *Staphylococcus aureus* and *Bacillus subtilis*. As shown in the measured zones of inhibition, the fish extract significantly inhibited the growth of *S. aureus, B. subtilis, and P. aeruginosa* but not the *C. albicans* and *E. coli* and. Results indicated the potential of the fish as source of natural products with antibacterial properties.

**Keywords:** *Hypseleotris agilis*, Eleotridae, Lake Lanao, methanol extract, antibacterial activity

## Introduction

The search for new drugs as source of cure for human infirmities from various forms of life spanning from simple bacteria to complicated organisms of kingdoms Plantae and Animalia is never ending as long as humanity continues to survive. Fish, for instance contribute to a major resource for natural products with bioactivity. Antimicrobial studies on fish epidermis, mucus and gills were reported; the gills and mucus may supply antimicrobial compounds since these parts provide mechanical protective barriers and lubricant to the fish. Mucus and different organs, tissues and fish peptide of snakefish *Channa* spp. had been evaluated and positively

demonstrated properties of pharmaceutical values (Rakers *et al.* 2013). In fact, wound healing creams and ointments have already been formulated from *Channa* sp. (Baie & Sheikh, 2000).

Lake Lanao of Lanao del Sur, Philippines, one of the ancient Lakes in the world, is a home of many freshwater indigenous endemic and invasive fishes. Personal interviews with the local people and market landing surveys revealed that currently the most dominant fish in the lake is the invasive eleotrid *Hypseleotris agilis*. This fish locally known as *katulong* is a freshwater fish belonging to family Eleotridae first described by Herre in 1927 as endemic species of Lake Mainit in Agusan del Norte, Philippines. The species may have been accidentally introduced to Lake Lanao, sometime in the 1960s, along with milkfish and tilapia fry stocking in Lake Lanao (Rosagaron, 2001). This fish are voracious predators that quickly flourished in the lake to the detrimental extinction of the endemic species along with other environmental pressures. The study takes this poignant invasive dominating condition of the fish into an opportune juncture by exploiting its abundance into drug discovery. No studies have been reported to date regarding the antimicrobial potential of the methanol extract of *H. agilis,* hence this study was conducted against selected test microbes using filter paper disc diffusion assay.

## Materials and Methods

### Collection of fish sample and extract preparation

Freshly caught *H. agilis* fish samples from Lake Lanao were collected from Marawi City public market. The body weights ranged from 23-25g and body lengths from 11-13cm (Figure 1). Five individual fish samples (130g), were washed with distilled water (DW), cut into sections and soaked in 100mL 90% methanol for two days. The methanol extract was then filtered and concentrated under vacuum in a rotary evaporator at 40°C and 115rpm. The crude methanol extract was assayed for antimicrobial activities using filter paper disc diffusion method.

**Figure 1.** *Hypseoletris agilis*, Herre

### Microbial cultures and inoculum preparation

One fungal strain *Candida albicans*, two Gram-negative bacterial strains *Pseudomonas aeruginosa* and *Escherichia coli* and two Gram-positive strains *Staphylococcus aureus* and *Bacillus subtilis* were used as test microbial organisms. All strains are clinical isolates obtained from the Department of Biology, College of Natural Sciences and Mathematics, Mindanao State University, Main Campus, Marawi City, Philippines.

Nutrient broth was prepared by dissolving 1.04g of Scharlau powder in 80mL DW; 10 ml was dispensed into test tubes and autoclaved at 121 °C at 15 psi for 15 minutes. A loopful of

each bacterial strain from agar slants was inoculated separately into broth tubes and incubated at 37°C for 24 hours. For fungal subculture, Saboraud's broth was prepared by dissolving 0.26g of Saboraud's broth powder in 20 ml DW; 10 ml was also dispensed into tubes and sterilized. A loopful of fungal strain was then inoculated aseptically into the broth tubes and incubated at 37°C for 48 hours.

**Filter paper disc diffusion assay (FPDDA)**

Agar plates were prepared by pouring 5 ml nutrient agar (Scharlau, 23 g dissolved in 1000 ml DW then autoclaved) aseptically into sterile petri dishes and allowed to solidify to serve as the base. Seeding of the agar plates was done following the agar top method. In preparing the sterile top, 50 mL of melted nutrient agar was dispensed into flasks, inoculated respectively with a loopful of test microorganism from each of the previously prepared broth tubes. Using a syringe, 3 ml of inoculated melted agar was dispensed on top of the petri plates with agar base, swirled for even distribution of the sterile top and allowed to solidify.

Filter paper discs (6 mm) were cut using a paper puncher; discs were wrapped with aluminum foil and sterilized. Sterile discs were then impregnated with crude methanol extract of *H. agilis*, allowed to dry (as methanol evaporated) and were placed at the respective bacterial and fungal plates. For negative control, discs were dipped in methanol then dried; and for positive controls Amoxicillin (500 mg dissolved in 10 ml) and Nystatin (100,000 units/ml suspension) were used for bacteria and fungi, respectively. All the tests were performed in triplicates. The zones of inhibition were measured from all plates after incubating 12 hours for bacteria and 72 hours for fungi at 37°C.

**Statistical Analysis**

Analysis of variance (ANOVA) both the one-way and two-way tests as well as the Tukey Multiple Comparison test were the statistical tools used to analyze the results.

**Results and Discussion**

The measured zones of inhibition (Figure 2) exerted by the extract (E), positive control (+) and negative control (-) against the test bacteria including the ANOVA and Tukey's range tests are shown in Tables 1-3. Data revealed that the fish extract significantly inhibited the growth of bacterial strains *B. subtilis, S. aureus* and *P. aeruginosa* but not against the bacteria *E. coli* and fungus *C. albicans* (negative results were not shown).

Table 1 shows the zones of inhibition elicited by the treatments against *B. subtilis*; ANOVA confirmed that the differences caused by the treatments were highly significant. Moreover,

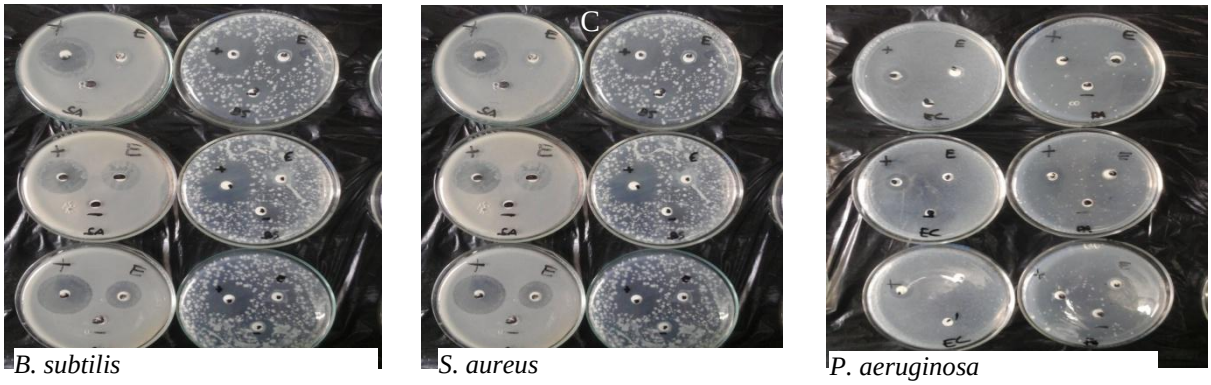


**Figure 2**. Antimicrobial activity of the methanol extract (E) of *H. agilis* fish against *B. subtilis*, *S. aureus*, and *P. aeruginosa* showing zones of inhibition; (+) positive control and (-) negative control.

Tukey's test revealed that wider zone exerted by the extract against the bacteria was significantly higher than the negative control; though it was significantly lower than the positive control. Similar results were also observed in *S. aureus* and *P. aeruginosa* (Tables 2-3). Wider zones were consistently produced by the extract, though they were statistically lower than the positive control (Table 4). These results implied that the effects of the extract in inhibiting bacterial growth was moderately active compared with amoxicillin which was highly active.

The 2-way ANOVA (Table 5) further confirmed that both the treatments and the test microorganisms significantly caused variations, i.e. the treatments (extract and controls) have different effects on the test organisms; and the 5 test microorganisms have different susceptibility to the treatments, particularly to the fish extract. Table 6 compares the susceptibility of the microbial strains against the fish extract wherein *S. aureus* was the most susceptible though it was not significantly different from *B. bacillus;* both are Gram-positive bacteria and were more susceptible than the Gram-positive *P. aeruginosa*. *E. coli* and *C. albicans* were not affected at all by the extract.

Methanol extracts from Boal Fish *Wallago attu* (Sattar et al. 2006), mucus extracts from fish *Channa striatus*, Indian carps and Chinese carps were reported earlier to have antibacterial activities (Wei et al. 2010, Islam 2014). Antibacterial effects were also obtained in the present study using eleotrid fish methanol extract. The bioactivity observed in the current study could probably be attributed to glycoproteins, proteins and peptides present in the fish extracts as mentioned and explained in the above studies. Antimicrobial proteins are important component of the innate immune system which may confer microbial resistance through metal ion chelating mechanism, enzyme inhibition or hydrolytic enzymatic action. Incidence of bacterial presence in the fish habitat and their interaction may stimulate the fish to produce compounds with antimicrobial activities. In the case of the negative activity of the extract against the fungi, Malarvizhi et al. (2012) explained that fishes are rarely in contact with fungi and its mediated infection. The rare presence of fungus in the fish habitat may account for meager antifungal effect.

**Table 1.** Diameter of zones of inhibition (mm) elicited by the *H. agilis* methanol extract, positive and negative controls against the *B. subtilis* observed 12 hours after treatment with ANOVA and Tukey's test

| Test Bacteria | Treatments | Replicates | | | Sum | Means | TUKEY* | ANOVA |
|---|---|---|---|---|---|---|---|---|
| | | R1 | R2 | R3 | | | | |
| *Bacillus subtilis* | Positive control (amoxicillin) | 35.5 | 29.5 | 28.5 | 93.5 | 31.167 | a | P-value: 0.00** F-crit: 6.94 F comp: 75.115 |
| | *H. agilis* fish (extract) | 17.5 | 25.5 | 21 | 64 | 21.333 | b | |
| | Negative control (methanol) | 0 | 0 | 0 | 0 | -1.096E-15 | c | |

*means with different letters are significantly different at $\alpha \leq 0.05$

** if p-value < 0.01, it is highly significant

**Table 2.** Diameter of zones of inhibition (mm) elicited by the *H. agilis* methanol extract, positive and negative controls against the *S. aureus* observed 12 hours after treatment with ANOVA and Tukey's test

| Test Bacteria | Treatments | Replicates | | | Sum | Means | TUKEY* | ANOVA |
|---|---|---|---|---|---|---|---|---|
| | | R1 | R2 | R3 | | | | |
| *Staphylococcus aureus* | Positive control (amoxicillin) | 35 | 36 | 32 | 103 | 34.333 | a | P-value: 0.00** F-crit: 6.94 F-comp: 83.39 |
| | *H. agilis* fish (extract) | 23 | 15 | 25 | 63 | 21.000 | b | |
| | Negative control (methanol) | 0 | 0 | 0 | 0 | -3.067E-15 | c | |

*means with different letters are significantly different at $\alpha \leq 0.05$
** if p-value < 0.01, it is highly significant

**Table 3.** Diameter of zones of inhibition (mm) elicited by the *H. agilis* methanol extract, positive and negative controls against the *S. aureus* observed 12 hours after treatment with ANOVA and Tukey's test

| Test Bacteria | Treatments | Replicates | | | Sum | Means | TUKEY* | ANOVA |
|---|---|---|---|---|---|---|---|---|
| | | R1 | R2 | R3 | | | | |
| *Pseudomonas aeruginosa* | Positive control (amoxicillin) | 16 | 20 | 19 | 55 | 18.333 | a | P-value: 0.00** F-crit: 6.94 Fcomp: 176.846 |
| | *H. agilis* fish (extract) | 11 | 11 | 11 | 33 | 11.000 | b | |
| | Negative control (methanol) | 0 | 0 | 0 | 0 | -1.203E-15 | c | |

*means with different letters are significantly different at $\alpha \leq 0.05$
** if p-value < 0.01, it is highly significant

**Table 4**. Tukey's test comparing the effects of the treatments in causing zones of inhibition

| Treatments | Mean | Tukey |
|---|---|---|
| Positive control (amoxicillin) | 24.067 | A |
| *H. agilis* fish (extract) | 10.667 | B |
| Negative control (methanol) | 0 | C |

*means with different letters are significantly different at $\alpha \leq 0.05$

**Table 5.** Two-way ANOVA on the interaction between treatments and the test microorganisms

| **Source of Variations** | **DF** | **Sum of Squares** | **Mean Square** | **F Value** | **Pr > F** |
|---|---|---|---|---|---|
| Treatments | 2 | 4362.711 | 2181.356 | 325.575 | .000* |
| Test microorganisms | 4 | 1317.589 | 329.397 | 49.164 | .000* |
| Treatments*microorganisms | 8 | 811.178 | 101.397 | 15.134 | .000* |
| Error | 30 | 201.000 | 6.700 | | |
| Total | 44 | 6692.478 | | | |

*Significant at 0.05 level of significance

**Table 6**. Tukey's test comparing the susceptibility of the test microorganisms against the fish extract

| **Test Microorganisms** | **Mean** | **Tukey*** |
|---|---|---|
| *Staphylococcus aureus* | 18.444 | A |
| *Bacillus subtilis* | 17.500 | A |
| *Pseudomonas aeruginosa* | 9.778 | B |
| *Escherichia coli* | 0 | C |
| *Candida albicans* | 0 | C |

*means with different letters are significantly different at $\alpha \leq 0.05$

In this preliminary study, though the potential of the bioactive compounds in *H. agilis* fish was not broad-spectrum as some test microbes in the study were not affected nevertheless the effect of fish extract was potent enough to bring about the antibacterial activity against the two Gram-positive test pathogens and one of the Gram-negative test bacteria hence the results can't be readily discarded. Narrow-spectrum results were also obtained from previous works on crude fish extracts as mentioned by Mat Jais et al. (2008) in his work with fish *Channus striatus*. Other extraction procedure as well as purification of the crude methanol extract may enhance its

antimicrobial potential. The future direction therefore of this research is towards the analysis of possible active compounds present in the fish extract and the compound purification and isolation responsible for the antibacterial activity.

## Conclusion

By using filter paper disc diffusion assay, antimicrobial property was found exhibited by the methanol extract of the freshwater fish *H. agilis* against bacterial test organisms *S. aureus* and *B. subtilis* and *P. aeruginosa* but not against *E. coli* and fungus *C. albicans*. The results indicated the potential of fish *H. agilis* as alternative source of natural products with antibacterial activity. Further studies must be conducted to exploit this potential.

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