Antibacterial Activities of *Terminalia catappa* Leaves Againsts *Streptococcus sp.* in Tilapia Disease

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ABSTRACT

This study was conducted to evaluate the antibacterial activity of Terminalia catappa leaves extract against Streptococcus sp. on Tilapia fish disease. The leaves extracts used were hexane, chloroform, methanol and aqueous extracts at different concentration of 50 mg/ml, 100mg/ml, 500mg/ml, 1000mg/ml and 1500mg/ml. The disc diffusion method used to screening the antibacterial activity against Streptococcus sp. The inhibition zone used to measure the antibacterial activity. Result found that all the extracts showed the antibacterial activity. However, the extracts (hexane, chloroform and aqueous) at 50 mg/ml do not showed any inhibition. T. catappa methanol extract showed to be the best antibacterial agent against Streptococcus sp. with inhibition zone measured 22.67mm. Based on the finding it can be concluded that Terminalia catappa leaves extract have antibacterial activities against Streptococcus sp. on Tilapia fish disease.

Keywords: *antibacterial activity, Terminalia catappa, Streptococcus sp., tilapia, inhibition zone*

Introduction

An antibacterial is a compound that kills or slows down the growth of the bacteria (Bayarski, 2011). With the advances in medicinal chemistry, many chemical antibacterial has been introduced. In fish disease management, the used of synthetics chemical such as malachite green, formalin, terramycin, potassium permanganate and Romet 30 has been practices for the last 30 years. The extreme use of synthetic antibacterial allowing certain bacteria to survive, change, and become resistant (Manzur et al, 2011) and also endangered to the human health (Bayarski, 2011). It is also resulted pollution to the water sources and very toxic to other living organisms.

Tilapias are the fish species clssified under Cichlidae family and it represents a large number of freshwater fish (Amal et al, 2011). The value of tilapia as a food fish has lead them being deliberately introduced to a large amount of waters around the world. Tilapia is a very hardy fish that can adapt to various environmental conditions and food sources as long as the water is warm enough (Abdel-Fattah, 2006). Tilapias are vulnerably to wide variety of bacterial disease like skin rot or fin rot disease, ulcer, hemorrhagic disease and blood streak disease. Ulcer disease was caused by the ubiquitous Gram negative bacteria *Aeromonas hydrophila* (Bhuvaneswari and Valasundaram, 2009). The most significant disease in tilapia is *Streptococcis* caused by *Streptococcus sp.* (Abdel-Fattah, 2006). The clinical sign for *Streptococcus* include erratic spiral swimming motion, a curved body and abdominal distention. One of the major concerns for these bacteria is that it can transmit to human. *Streptococcus sp.* infect human who cleaned and injured their hand during fish cleaning and human who ate the fish.

Terminalia catappa Linn (*T. catappa*) is a large tropical tree and usually used as shade tree due to its shaped (Gunasena, n.d). In Taiwan, *T. catappa's* leaves boiled in water and used as drink (Chyau et al, 2002) for liver related diseases. *T. catappa* leaves also used by the Siamese breeder to their fighting fish to heal the wound but proper dosage used still not be fixed yet (Chansue and Assawawongkasem, 2008). Study on antioxidant properties conducted by Chyau (2002) anticipated that leaves extracts of *T. catappa* acted as antioxidants at low concentrations. *T. catappa* also reported having many pharmacological activities of its

different part and its major component such as antibacterial, antiparasitic, antifungal, hepatoprotective, hypoglycemic, antioxidant, anti-inflammatory and chemopreventive effect (Mohale et al, 2009).

Nowadays, the fish health and disease control review in integration with environment protection, pollution control and human health. To keep the safety of the ecosystem it is advisable to use natural product as an antibacterial agent to control any bacterial diseases. Although the result using natural product such as herb plant is time consuming but it help in prolong the safety of our ecosystem. The application of natural sources to control disease infection really important since it was ecofriendly, biodegradable and little side effect (Bhuvaneswari and Valasundaram, 2009).

Materials and Methods

Isolation of Streptococcus sp.

The strain of *Streptococcus sp.* from fresh infectious fish were isolated using serial dilution technique. Adequate 1 gram of fish infectious sample were put into 10 ml dilution tube and shakes for a few minutes. Ten 9ml dilution tubes of sterile water prepared and labeled as 10^{-1} until10^{-10}. Spread plate techniques take place after all the serial dilution process complete. 0.1ml of the dilution in range 10^{-5} to 10^{-8} were transferred to the nutrient agar plates and kept for future used.

Preparation of Plant Extract

For organic solvent extraction, the plant sample powder was extracted with organic solvent (500g plant sample powder/ 1L of solvent) at room temperature for 24 hours. The extract was filtered and concentrated using the rotary evaporator. The obtained *T. catappa* extracts were stored in specimen jar.

For aqueous extraction, *T. catappa* aqueous extract obtained using freeze dry method where 500g of dry *T. catappa* leaves soaked in 1L of water. The extract then were filtered and centrifuged. After centrifuged, the extracts were again filtered and freeze in the freezer before pass through the freeze dry process. The *T. catappa* extract obtained was kept for future used.

Treatment

Four different solvent used for the extraction are hexane, chloroform, methanol and aqueous. Each solvent was prepared with five different concentration (50 mg/ml, 100mg/ml, 500mg/ml, 1000mg/ml and 1500mg/ml) yielding 20 treatments. Gentamycin was used as positive control while blank disc were used as negative control.

Antibacterial assay

The antibacterial assay was carried out using disc diffusion method. The *Streptococcus sp* was inoculated into broth media and the inoculums were uniformly spread over the agar plates. A total of 1.5ul of *T. catappa* leaves extracts were pipetted onto the disc (6mm AA disc) and allowed to dry. The disc were then placed on the top of the agar plate and incubated at 37°C for 24 hour. The diameters of inhibition zone were then recorded.

Statistical analysis

Data were analyzed using SPSS. Data were expressed as mean $\pm$ standard deviation. The data obtained were evaluated using ANOVA test to determine whether there was a significant difference between extracts used.

Results and Discussion

Figure 1 show the antibacterial activity for different concentrations of different *T. catappa* extract against *Streptococcus sp.*

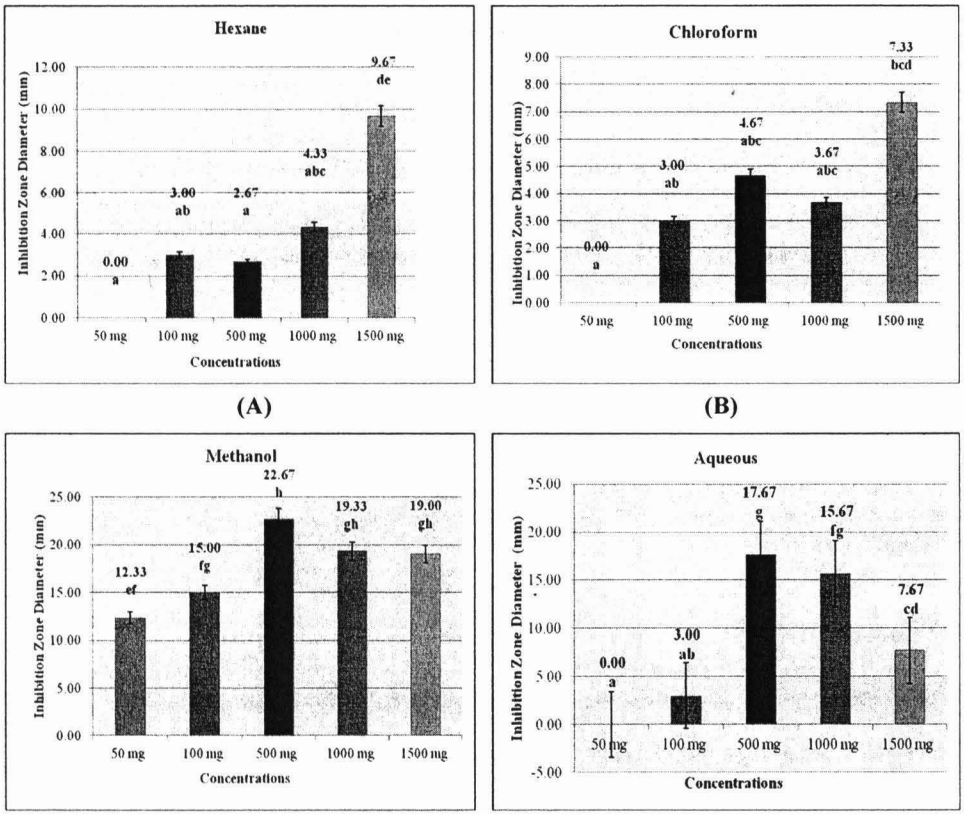

Figure 1 Effect of *T. catappa* leaves extract concentrations using (A) hexane, (B) chloroform, (C) methanol and (D) aqueous against *Streptococcus sp.*

All the extracts except for 50 mg/ml hexane, chloroform and aqueous extracts were found to be active in inhibiting *Streptococcus sp.* The disc diffusion assay of the leaves extracts against *Streptococcus sp.* showed that the 500 mg/ml methanol extract had the most distinct effect in inhibiting *Streptococcus sp.* with 22.67mm zone of inhibition. This was followed by the 1000 mg/ml methanol extract, which inhibited with 19.33mm zone of inhibition and 1500 mg/ml methanol extract with 19.00mm zone of inhibition.

The screening found that 1500 mg/ml hexane extract have significant difference compared to others concentration in hexane extract. While for methanol extract, all the concentration does not show any significant difference. 100 mg/ml and 500 mg/ml methanol extracts show significant difference when compared to each other. However, 100 mg/ml, 1000mg/ml and 1500mg/ml methanol extracts have significant difference. As for aqueous extracts, the inhibition zone for 1000mg/ml is high compared to 1500mg/ml and these two concentration show significant difference when compared to each other.

The antibacterial assay of all the extracts except for 50 mg/ml hexane, chloroform and aqueous extracts against *Streptococcus sp.* gave 2.67mm until 22.67mm of inhibition zone. However, the antibacterial activities were found to be less effective to the positive control (gentamicin, 24 mm). The negative control, blank disc did not show any inhibition zone.

The result shows that all *T. catappa* extracts have potential as antibacterial agents. Pharmacological studies on the *T. catappa* bark and leave extract show that the plant part can possess as anticancer, antioxidant, anti HIV reverse transcriptase, and anti-inflammation (Chansue and Assawawongkasem, 2008). Review of the literature on the phytochemical constituents revealed that saponin and tannin are the major components of *T. catappa* leaves (Babayi et al, 2003). Tannin (punicalagin, punicalin, terflavin A and B, tergallagin,tercatain, chebulagic acid, geranin, granatinB, corillagin) is polyphenolic compounds that commonly found in herbs plant and have antibacterial properties (Chansue and Assawawongkasem, 2008 : Doss et al, 2009).Suitable concentration of antibiotic must be applied in order to kill or inhibit the bacteria growth. Based on World Health Organization, the reason that will drive of antimicrobial resistant are

inappropriate and irrational use of medicines which will provide favourable condition for the microorganism to merge and spread. Nevertheless, the amount of the antibiotic applied, the frequent and duration for how long the antibiotic apply should also been considered (Roy, 2003). Insufficient amount of antibiotic amount will cause the bacteria to build resistant and when the bacteria develop resistant too high concentration used to control the bacteria will not giving any antibacterial activity (Roy, 2003).

Conclusion

Different extracts from the leaves part of *T. catappa* showed antibacterial activity against *Streptococcus sp.* The crude methanol extract also showed effective antibacterial activity, suggesting a potential of *T. catappa* as an antibacterial agent. The antibacterial compounds present in *T. catappa* may serve as an affordable and new source for the treatment of Tilapia fish disease. Moreover, it will help in reducing pollution in food, water, soil and so on.

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