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IN VITRO ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF DRUMSTICK (*MORINGA OLIFERA*) LEAF AGAINST SHRIMP PATHOGENIC BACTERIA *VIBRIO PARAHAEMOLYTICUS*

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Abstract

To evaluate the antibacterial effect of ethanolic extract of *Moringa olifera* leaf against shrimp pathogenic bacteria *Vibrio parahaemolyticus*, the bacteria were cultured on TCBS agar media and enumerated. The ethanolic *M. olifera* leaf extract (1 mg/mL) was prepared and its antibacterial activity at different dose (400 µL, 300 µL and 200 µL) was evaluated against *V. parahaemolyticus* by using disk-diffusion method. The bacteria load of 1.75×10^4 CFU/mL, 1.63×10^4 CFU/mL and 1.54×10^4 CFU/mL was employed for each concentration. It was observed that 400 µL of ethanolic extract of *M. olifera* showed the highest inhibition zones (11.97 mm, 13 mm and 15.13 mm) but not equal to the inhibition zones (17.23 mm, 18.20 mm and 19.70 mm) by standard antibiotic (oxytetracycline). The present study revealed that ethanolic extract of moringa leaf (1mg/ml) had significant positive effect (P value = 0.003) on different *V. parahaemolyticus* load at 5% level of significance though effect of different doses with same concentration (1mg/ml) had not significantly differ from each other. Therefore, the ethanolic extract of *M. olifera* might be used as alternative way to prevent diseases which are caused by *V. parahaemolyticus*.

Keyword: *Moringa olifera, Vibrio parahaemolyticus,* leaf extract, shrimp, antibacterial activity, ethanolic extract

Introduction

Acute hepatopancreatic necrosis disease (AHPND) which is also familiar as early mortality syndrome (EMS) causes notable mortalities in penaeid shrimp farming around the world, is mainly caused by *Vibrio parahaemolyticus* (Nguyen *et al.*, 2021). AHPND first appeared in the southwest of China in 2009 and outspread all over the world. Occurrence of this disease first detected in Bangladesh in 2013 and caused significant reduction of national income (Hosain *et al.*, 2021). It was discovered that the virulence's of various strains of this species varied due to several factors and mortality rates rise as virulence power increases (Soto-Rodriguez *et al.*, 2015). Nowadays, a raising attention for the development of new phytochemical base antimicrobial drugs observed due to antibiotics resistance phenomenon. Plants extract have been applied as medicine for treating microbial disease in many countries from ancient period as many of them are rich in antimicrobial and antioxidant properties (Akinmoladun *et al.,* 2007).

Moringa olifera is familiar as the miracle tree because of its health benefits, used as both sustenance and medicinal purpose all over the world (Abdel-Latif *et al.*, 2022). Moringa leaves are enrich in different secondary metabolites which have different pharmacological properties. Extracts of this plant leaves could be a good source of new antimicrobial drug discoveries. Considering the above facts, the present study was attempted to find out the antibacterial activity of ethanolic extracts of *M. olifera* leaf against shrimp pathogenic bacteria *V. parahaemolyticus* and comparing its efficiency with oxytetracycline.

Materials and Methods

Study area and period

The entire research work was conducted in the Fish Pathology and Molecular Biology Laboratory of Fisheries and Marine Resource Technology (FMRT) Discipline; Phytochemistry Laboratory in the Pharmacy Discipline, Life science school; Acharya Prafulla Chandra Ray Central Laboratory, Khulna University from August to October, 2022.

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Preparation of extract

The *M. olifera* leaf was collected from the plants in front of Bangamata Begum Fazilatunnessa Mujib Hall inside Khulna university campus, Khulna. The ethanolic extract of *M. olifera* leaf was prepared by following the method describe by Ibrahim & Kebede (2020). Finally the leaf extract dissolved in ethanol as 1mg/mL ratio and this is the desired extract solution for the determination of the antibacterial activities of ethanolic moringa leaf extract solution by disk- diffusion method.

Bacteria collection and culture

V. parahaemolyticus was collected from Shrimp Research Station, Bangladesh Fisheries Research Institute (BFRI), Bagerhat with the help of gel ice which was isolated from diseased shrimp body. At first, the collected bacteria were cultured in nutrient broth (NB) media at 37°C for 18 to 24 hours. Then a subculture of bacteria on the TCBS agar media was prepare at 37°C for 18 to 24 hours to determine the bacterial load.

Antibacterial activity assay

Antibacterial activity assay was performed by following disk diffusion method narrated by Ibrahim & Kebede (2020) using previously prepared each petri dish of inoculated bacteria. Here, blank disc (approximately 6 mm) and Oxytetracycline disc (30 µg/disc) were placed into the inoculated bacteria as negative control and positive control respectively. After that, extract of 400 µL, 300 µL and 200 µL of ethanolic extract of leaf solution were tested for the antibacterial activity at 37° C for 18-24 hours. Millimeter scale was used to determine zones of inhibition.

Data collection and analysis

Recorded data were analyzed by using Microsoft excel and IBM SPSS statistics 25 version while two-way ANOVA was performed.

Results

The results showed that ethanolic extract of Moringa leaf (1 mg/mL) effectively inhibit the growth of *V. parahaemolyticus* pathogens of shrimp with varying inhibitory effects as depicted in Figure 1.

The largest inhibitory zones in mm were shown by the standard oxytetracycline (30 µg/disc). For 1.75×10^4 CFU/mL bacterial load, the standard discs showed the average inhibitory zone 17.23 mm; 18.20 mm for 1.63×10^4 CFU/mL bacterial load and the largest zone of inhibition among the standard discs with 19.70 mm when the bacterial load was the lowest, 1.54×10^4 CFU/mL. The most promising results were those for discs with 400 µL extract, producing the inhibitory zones on the average 11.97 mm for 1.75×10^4 CFU/mL of *V. parahaemolyticus* bacterial load, 13 mm for 1.63×10^4 CFU/mL bacterial load and 15.13 mm for 1.54×10^4 CFU/mL bacterial load. After 400 µL extract, 300 µL extract showed the second largest inhibitory zones among extracts on the average 8.20 mm, 8.87 mm and 10.73 mm for 1.75×10^4 CFU/mL, 1.63×10^4 CFU/mL and 1.54×10^4 CFU/mL bacterial loads respectively. The lowest inhibitory zones were found for 200 µL extract per disc which were 6.13 mm, 6.37 mm and 7 mm for 1.75×10^4 CFU/mL, 1.63×10^4 CFU/mL and 1.54×10^4 CFU/mL bacterial loads respectively.

The discs with 400 µL extract showed highest inhibition zone for different bacterial loads than with 300 µL and 200 µL respectively. Though oxytetracycline which used as standard was shown the highest antibacterial activities against tested pathogenic bacteria as compare to all discs.

In a short the present study revealed that ethanolic extract of moringa leaf (1mg/ml) had significant positive effect (P-value= 0.003) on different *V. parahaemolyticus* load at 5% level of significance though effect of different doses with same concentration (1mg/ml) had not significantly differ from each other.

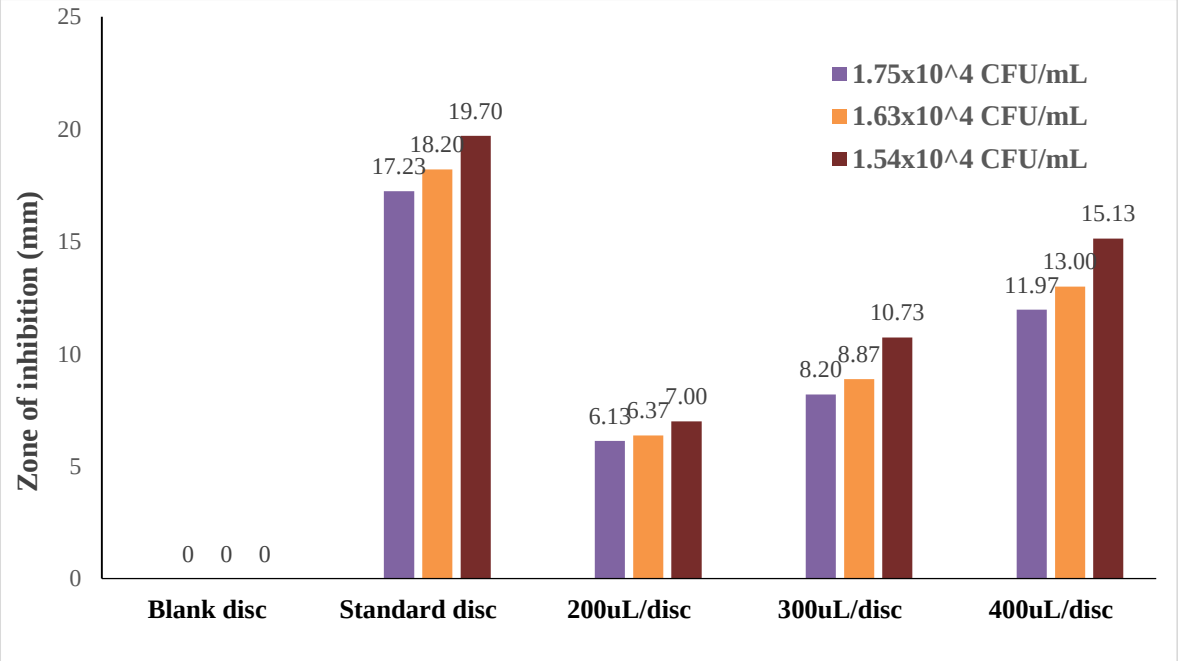

Figure 1. Antibacterial activity of several ethanolic *M. olifera* leaf extracts in comparison to standard antibiotic (oxytetracycline).

Discussion

Shrimp are widely available and plentiful rich source of protein as well as vitamins and minerals. They are a significant food source and perform essential roles in the food chain. But nowadays this species encounter high economic losses due to diseases, EMS or AHPND is also one of them. For remediation various plant extracts are applied made with different solvents.

Hieu *et al* in (2022) demonstrated that *M. olifera* ethanolic extract had high antibacterial activity to *salmonella* spp, *Shigella* spp, *Vibrio* spp and *Escheria coli*.

Present study was attempted to identify the antibacterial activity of ethanolic extracts of *M. olifera* leaf against *V. parahaemolyticus* bacteria by measuring the inhibition zones. According to previous findings, antibacterial activity of methanolic extract of *M. olifera* leaf at 0.4 g/mL concentration showed 10-15 mm inhibition zone against *V. parahaemolyticus* (Dalukdeniya *et al*., 2016).

Nurafifah *et al* in (2021) found highest zone of inhabitation (15,03 ± 0,55 mm; 11,00 ± 1,32 mm; 7,03 ± 0,90 mm) in case of 75%, 50% and 25% of ethanolic extract of *M. olifera* seeds against *E. coli*..

In another study, Peixoto *et al* (2011) investigated that ethanolic extracts of *M. olifera* leaf produced 17.8 mm inhibitory zone for the discs soaked with 400 µL, 17.5 mm for 300 µL and 16.7 mm for 200 µL against *V. parahaemolyticus*, which was larger than the average inhibitory zones 11.97 mm, 13 mm and 15.13 mm respectively for different bacterial loads with discs soaked with 400 µL extract. Again findings had larger zone of inhibition for 300 µL extract's average values 8.20 mm, 8.87 mm and 10.73 mm respectively according to the present study. In case of 200 µL, finding recorded 16.7 mm inhibitory zone which was much higher than present study record of 6.13 mm, 6.37 mm and 7 mm of zone of inhibition respectively.

In this study, it was investigated that with a concentration of 1 mg/mL of *M. olifera* leaf ethanolic extract can inhibit the growth of *V. parahaemolyticus* bacteria at different doses such as at 200 µL, 300 µL and 400 µL. But promising zone of inhibition was shown by 400 µL dose.

In the present study revealed that ethanolic extract of moringa leaf (1mg/ml) had significant positive effect on different *V. parahaemolyticus* load though same concentration (1mg/ml) of different doses had no effect on different *V. parahaemolyticus* load.

Conclusion

In conclusion, the results of the present study confirm the use of *M. oleifera* plant leaf extracts in food preservation as well as new antimicrobial drug discoveries which it might be a very eco-friendly solution in the field of aquatic health management.

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Conflict of Interest

The authors declare no conflict of interest.

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