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Short Communication

Antibacterial Effect of the Extracts of *Piper betel* Linn. and *Syzigium aromaticum* (Linn.) Merrill and Perry to Control the Growth of Fish Pathogenic Bacteria *Pseduomonas aeruginosa* PKB 113 Strain

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Bacteria are one of the major factors causing infections to the edible fishes. The bacterial diseases of fishes have become a growing threat to aquaculture. Thus, necessity of the use of chemicals, drugs and antibiotics in health management has increased to protect the fishes against the bacteria. Although these measures produced enhanced productivity but their continual uses have some deleterious effects on the environment and sustained productivity. Some of the insecticides and biocides used in aquaculture are known to accumulate and concentrate in aquatic organisms. Nowadays, use of commercial antibiotics is no longer effective in aquaculture, due to increased incidence of appearance of antibiotic resistant strains. Antibiotics may also induce resistance in pathogens through mutagenesis and plasmid mediated gene transfers [1]. Furthermore, many countries have banned antibiotics in aquaculture due to public health concerns and environmental hazards. The use of natural compounds is considered as a promising approach and has been proposed since 1990s for different microorganisms [2-3]. The uses of medicinal plants as therapeutics have much lower side effects than the antibacterial drugs. Plant phytochemicals having wide variety of secondary metabolites such as tannins, alkaloids, flavonoids, triterpinoids, saponins, fatty acids and many more, have antimicrobial properties that can be used as potential alternative medicine. It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoral and antimicrobial agents [4-6]. Lubis *et al*. [7] reported antimicrobial activities of *Piper betel* leaf extracts to prevent bacterial conjunctivitis in human. Hamad *et al*. [8], Wankhede [9], Mittal *et al*. [10], Kumari *et al*. [11] reported about antimicrobial activities of *Syzigium aromaticum.* In the present study antimicrobial activity of *Piper betel* Linn. (Paan) [Family: Piperaceae] and *Syzigium aromaticum* (Linn.) Merrill and Perry (Labanga) [Family: Myrtaceae] were tested against *Pseudomonas aeruginosa* strain PKB113 isolated from the freshwater edible fishes.

Collection of plant materials

Fresh leaves of *Piper betel* (Paan) and fruits of *Syzigium aromaticum* (Labanga) were collected from local area. The plants were identified based on their physical characteristics by the experts of the Department of Botany, University of Kalyani, Kalyani, who are dealing with plant taxonomy.

Procedure

1) The leaves of *Piper betel* (Paan) were separated, washed several times in running tap water and once rinsed in distilled water.
2) The leaves were dried in dark using blotting paper for about a month.
3) The dried leaves of *Piper betel* (Paan) and fruits of *Syzigium aromaticum* (Labanga) were powdered in mixer grinder.
4) Both the powders were extracted in methanol. 10gm of powder was soaked in 100ml of methanol and macerated at room temperature for forty-eight hours.
5) The extract was then filtered with Whatman filter paper No. 1. The filtrate was then concentrated with a rotary evaporator under reduced pressure at 60°C to afford crude methanol extract. The dried extract was kept at 4 °C until use.

Antibacterial activity test

The antimicrobial susceptibilities were tested by the disc diffusion method for determining the minimum inhibitory concentration (MIC) according to the guidelines recommended by the Clinical and Laboratory Standards Institute [12].

Disc diffusion method

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1. Disc Diffusion test was performed using sterile 6 mm-diameter filter paper antibiotic discs (Whatman, Maidstone, UK).
2. All discs were dried at room temperature overnight.
3. An isolated colony from previous cultured fish pathogen, *Pseudomonas aeruginosa* strain PKB113 was touched with a wire loop and the growth transferred to a tube containing 5 mL of nutrient broth (Hi Media). The broth culture was incubated at 37 °C for overnight until the turbidity of the 0.5 McFarland standards, this resulted in a suspension containing approximately 1 to 2 X 10^8 cfu/mL.
4. The 0.5 McFarland suspension was transferred to nutrient agar (Hi Media) by spread plate technique, the final inoculum on the agar would be approximately 10^6 CFU/mL.
5. The discs were placed on the surface of the inoculum nutrient agar and incubated at 37 °C for eighteen to twenty-four hours.
6. The Disc Diffusion test was determined by measuring the diameter of the inhibition zone.
7. Experiments were performed in triplicate and the mean of the diameters of the inhibition zones calculated.

Bacterial growth curve analysis

Another test of antimicrobial susceptibilities was done by introducing the crude extracts into nutrient broth medium where bacteria were cultured. The retardation bacterial growth was studied and growth curve of normal and control bacteria culture were prepared.

Statistical analysis

Data was analyzed by simple arithmetic means of the different extracts and standard error was compared to the control. No other statistical test was applied to show significance since the extracts were either positive or negative for the antibacterial studies.

The in vitro potency of the crude extracts of the plant as antibacterial were assessed against *Pseudomonas aeruginosa* strain PKB113 by measuring the diameter of the clear zone around the discs placed on the Petri plates. The inhibitory zone around the antibiotic discs indicated absence of bacterial growth and it was reported as positive and absence of zone as negative. The diameters of the zones were measured using diameter measurement scale. The minimum inhibition concentration i.e., the minimum concentration at which clear zone of inhibition develops was also determined. The average zone of inhibition was measured ranging between 3mm – 15mm. The antibiotic tetracyclin (100µg/ml) has been used as positive control which produces highly significant result (30mm).

In the present study two minimum inhibitory concentrations of methanolic extracts of the two plants were taken. The antibacterial activities of these plant extracts (mg/ml) and antibiotic (100µg/ml) against *Pseudomonas aeruginosa* strain PKB113 were compared in the (Table 1) in relation to their zone of inhibition (Fig 1).

Table 1 Measurement of zone of inhibition in respect to plant extracts

Name of the plant	Minimum inhibitory concentration zone of inhibition (Diameter in mm)			
	100mg/ml	50mg/ml	Control	Tetracyclin (100µg/ml)
Piper betel (Paan)	8 ± 0.2	3 ± 0.4	-	30 ± 0.5
Syzigiumaromaticum (Labang)	15 ± 0.2	10 ± 0.5	-	30 ± 0.5

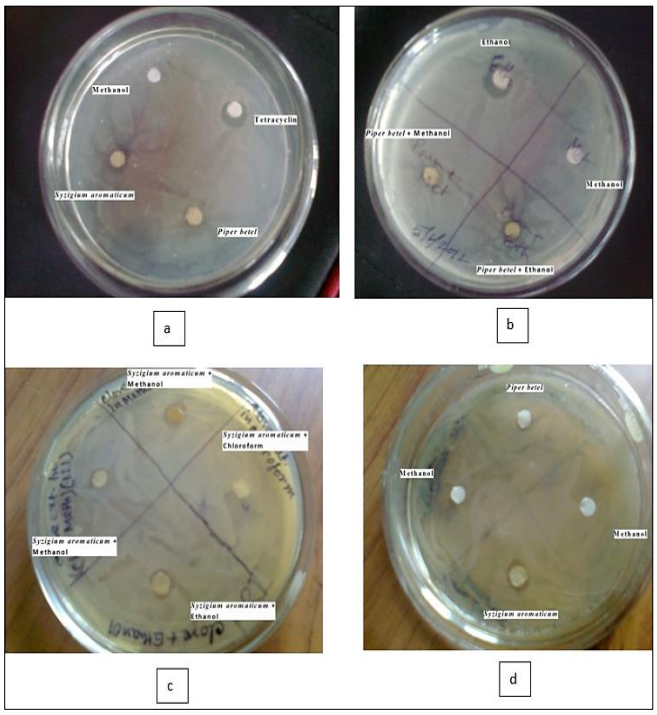

Fig 1 Photographs showing antimicrobial activities of *Piper betel* and *Syzigium aromaticum* using disc diffusion techniques

In the present study the growth curve has also been analyzed regarding the antibacterial effects of the two plants along with a control sample where bacteria grow normally with untreated condition. From the growth curve it was also found that methanolic extracts of *Syzigium aromaticum* (Labanga) is more effective antibacterial component than the methanolic extracts of *Piper betel* (Paan) (Fig 2).

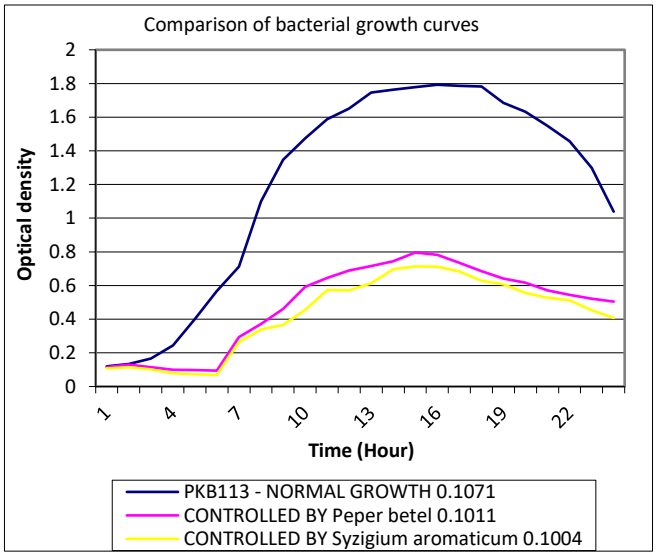

Fig 2 Graphical comparison of bacterial growth curves in normal and controlled condition

The present study shows the potential antibacterial effect of these locally available plant extracts against the *Pseudomonas* strain. Methanolic extracts of plants generally possess terpines and phenolics, which are reported by different workers as antimicrobial compounds [13-15]. The presence of phytosterols as antibacterial agent in *Piper betel* [16-17]. Zhang *et al.* [18] studied the effect of eugenol from essential o ils of *Syzigium*

aromaticum over other bacteria also supported our study. The present study also showed that methanolic extracts of *Piper betel* (Paan) has significant antibacterial effect against the test bacterium *Pseudomonas* PKB 113 but showed less effective than *Syzigium aromaticum* (Labanga).

SUMMARY

Bacteria are one of the major factors causing haemorrhagic septicemia to the edible fishes and create a growing threat to aquaculture. Thus, necessity of use of chemical compounds with antibacterial activities has increased to protect the fishes against the bacteria. Nowadays, use of commercial antibiotics is no longer effective in aquaculture, due to increased incidence of appearance of antibiotic resistant strains. The uses of medicinal plants as therapeutics have no side effects or very less side effects than the antibacterial drugs. In the present study antibacterial activity of *Piper betel* (Paan) and *Syzigium aromaticum* (Labanga) were tested against *Pseudomonas aeruginosa* strain PKB113 (accession number JX426137 in the GenBank database) which was isolated from the septicemic lesions of the freshwater edible fishes. The in vitro antibacterial activity test of both the plants was done by means of disc diffusion method as well as bacterial growth curve analysis. The present study also showed that methanolic extracts of both *Piper betel* (Paan) and *Syzigium aromaticum* (Labanga) have significant antibacterial effect against the test bacterium *Pseudomonas* PKB 113 but *Syzigium aromaticum* (Labanga) was more effective than *Piper betel* (Paan). From the present study, it can also be commented that the use of medical herbs may be an alternative treatment for the remedy of disease as to replace antibiotics, which is preferable for health of animals and humans' beings. It is also an option to reduce cost of treatment, and to promote the use of available natural sources within the country.

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Conflict of Interest

Both the authors don't have any conflict of interest from this research work.

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