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INVESTIGATION OF THE ANTIMICROBIAL ACTIVITIES OF *Phyllanthus urinaria* LEAVES BEFORE AND AFTER COMBINED WITH *Pandanus tectorius* FRUIT EXTRACTS

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ABSTRACT
The study was to investigate the antimicrobial activities of *Phyllanthus urinaria* leaves extracts before and after combined with *Pandanus tectorius* fruit extracts. This was accomplished by examining the influence of the total aqueous, chloroform and ethylacetate extracts. The biological tests were done on Candida albicans, Salmonella typhi, Pseudomonas aeruginosa and Staphylococcus aureus. Agar diffusion test was applied to determine the antimicrobial activities. Based on the inhibition zone diameter, the result showed that *Phyllanthus urinaria* aqueous extraction had the inhibition on the Pseudomonas aeruginosa (22.75 ± 0.96mm) and Staphylococcus aureus (25.75 ± 0.96mm). The activities decreased after combined with *Pandanus tectorius* fruit extracts on Pseudomonas aeruginosa (14.75 ± 0.50 mm) and Staphylococcus aureus (15.75 ± 0.50 mm). However, the activities of these fractionated extracts in the combination showed the weaker activities than in the using only *Phyllanthus urinaria* extracts. The project was to investigate of the antimicrobial properties of *Phyllanthus urinaria* leaves extract and the combination of *Phyllanthus urinaria* with *Pandanus tectorius* extracts.

KEYWORD: *Phyllanthus urinaria, Pandanus tectorius,* Antimicrobial activity.

INTRODUCTION
Phyllanthus urinaria (P. urinaria) is one of the herbal plants belonging to the genus Phyllanthus (Euphorbiaceae),[1] is widely distributed in Bandarban and chittagong hill tracts and the southern part of Bangladesh, China, South India. The ethylacetate extract of *P. urinaria* was shown to exhibit anticancer activity by inducing apoptosis through the inhibition of telomerase activity and Bcl-2 expression.[2-4] The water extract prepared from *P. urinaria* has an anticancer effect on Lewis lung carcinoma cells through a similar pathway.[5] One of the most commonly used traditional herbs is *Phyllanthus urinaria*. People usually used this herb to treat many types of diseases as hepatitis, diuretic, jaundice, edema, pimples.[6,7] Sometimes, people combined *Phyllanthus urinaria* with some other medical herbs to treat various kinds of diseases as *Pandanus tectorius* (also called pineapple wood, family of Pandanaceae) which was used to treat cough, hemorrhoids, dysentery.[8,9] *Pandanus tectorius (P. tectorius)* is a large shrub or small tree of immense cultural, health, and economic importance in the Pacific where it can withstand drought, strong winds, and salt spray. Probiotics are living microorganisms, which, when ingested or locally applied in sufficient numbers, provide the consumer with one or more proven health benefits.[10] Additionally the investigation to confirm that if people combined *Phyllanthus urinaria* with *Pandanus tectorious* (fruit extracts), the antimicrobial activities of *Phyllanthus urinaria* extracts with or without combination with *Pandanus tectorius* (fruit extracts) was done.

MATERIALS AND METHODS
Collection and identification of plants materials
Phyllanthus urinaria and was collected during the month of June, 2015 from the Bandarban hill track, Bangladesh and were identified by the experts of Bangladesh National Herbarium (accession number: 41778), Dhaka, Bangladesh where the voucher specimen were retained. *Phyllanthus urinaria* (leaves) of the plant was selected for the study.

Bacterial growth conditions
Four pathogen strains were Salmonella typhi, Candida albicans, Pseudomonas aeruginosa and Staphylococcus aureus. These bacteria have been cultured and maintained in LB (Luria-Bertani) broth and agar.[11]

Total water extraction

Fifty grams of dry *Phyllanthus urinaria* and fifty grams of *Pandanus tectorius* were boiled with water enough (50 mL) for 1 hour to obtain a volume of 20 mL.The extracts were collected, then another water portion (50 mL) was added into this mixture and boiled continuously for 1 hour to obtain 20 mL. The second extract was collected and combined with the former for antimicrobial tests.

Fractionated extraction with chloroform

The water extraction was fractionated with chloroform. 10 mL of total aqueous extract was mixed with 3 mL of chloroform. The mixture of total was shaken well for 30 minutes and the lower layer was collected. This procedure was performed in triplicate. The chloroform extracts were combined for antimicrobial tests.

Fractionated extraction with ethylacetate

After completely extracted with chloroform, this extract was shaken with 3 mL of ethylacetate for 30 minutes. The mixture was separated in three layers. The lowest layer was collected and the extraction with ethylacetate was done for more twice. All the lowest extracts were combined and ready for antimicrobial activity test.

Antimicrobial activity tests

Antimicrobial effects were tested on the pathogens by the agar diffusion method. The tested microorganisms were propagated twice and then grown for 18-24 h in 10 ml of appropriate growth media. Turbidity of the culture broth was compared with McFarland tubes to give an estimate of bacterial population (106 CFU/mL). Supernatant of the cell after expression were collected after centrifugation at 12,000 rpm for 15 min) and the clear supernatant was sterilized by filtration (0.45 ìm), thus yielding cell-free filtrates. The wells (ø 6 mm) were then prepared and filled using 100 ìL of cell-free filtrate. The inoculated plates were incubated for 18-24h at appropriate temperatures, and the diameter of the inhibition zone was measured in millimeters with calipers. The measurements recorded were from the edge of the zone to the edge of the wall.

RESULTS AND DISCUSSION

Table 1: Antimicrobial activity test of the water extract of *Phyllanthus urinaria* according to the inhibition zone diameter (mm).

Pathogens	Inhibition Zone on Diameter (mm) (Phyllanthus)	Inhibition Zone on Diameter(mm) (Phyllanthus combined with Pandanus)
Pseudomonas aeruginosa	22.75 ± 0.96	14.75 ± 0.50
Staphylococus aureus	25.75 ± 0.96	15.75 ± 0.50
Salmonella typhi	0.00 ± 0.00	0.00 ± 0.00
Candida albicans	0.00 ± 0.00	0.00 ± 0.00

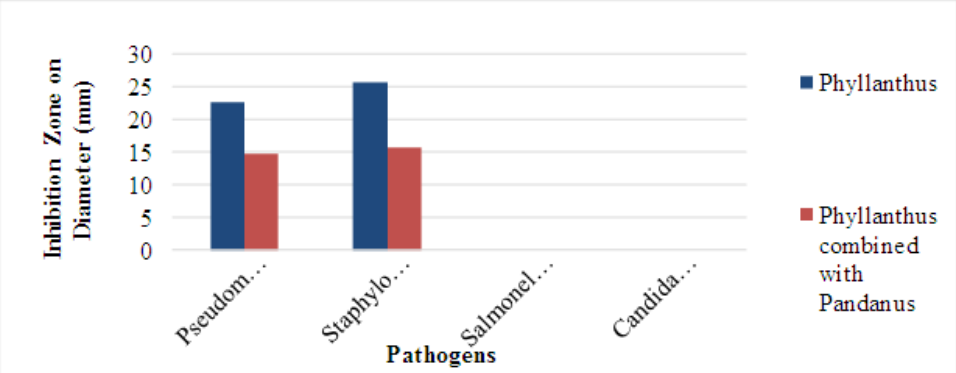

Figure 1: Antimicrobial activity test of the water extract of *Phyllanthus urinaria* according to the inhibition zone diameter (mm).

Table 2: Antimicrobial activity test of the chloroform extract of *Phyllanthus urinaria* according to the inhibition zone diameter (mm).

Pathogens	Inhibition Zone on Diameter(mm) (Phyllanthus)	Inhibition Zone on Diameter(mm) (Phyllanthus combined with Pandanus)
Staphylococus aureus	19.25 ± 0.50	14.25 ± 0.50
Pseudomonas aeruginosa	22.75 ± 0.96	11.25 ± 0.50

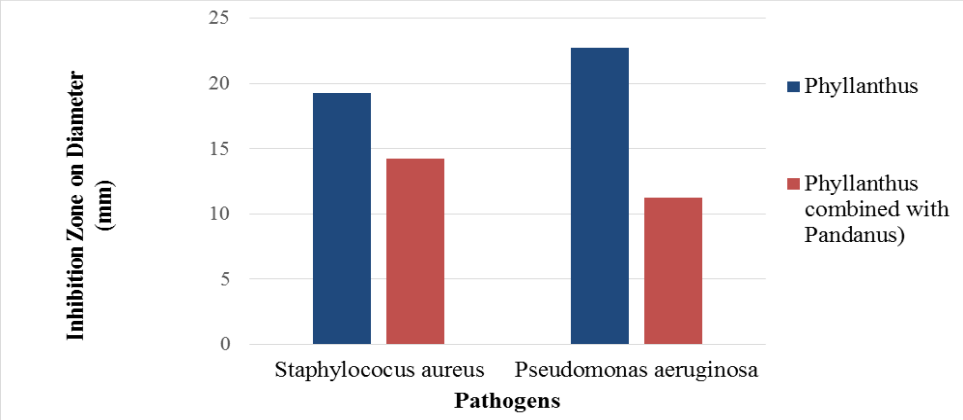

Figure 2: Antimicrobial activity test of the chloroform extract of *Phyllanthus urinaria* according to the inhibition zone diameter (mm).

Table 3: Antimicrobial activity test of the ethylacetate extract of *Phyllanthus urinaria* according to the inhibition zone diameter (mm).

Pathogens	Inhibition Zone on Diameter (mm) (Phyllanthus)	Inhibition Zone on Diameter(mm) (Phyllanthus combined with Pandanus)
Staphylococus aureus	10.5 ± 0.58	12.5 ± 0.58
Pseudomonas aeruginosa	16.50 ± 0.50	18.25 ± 0.50

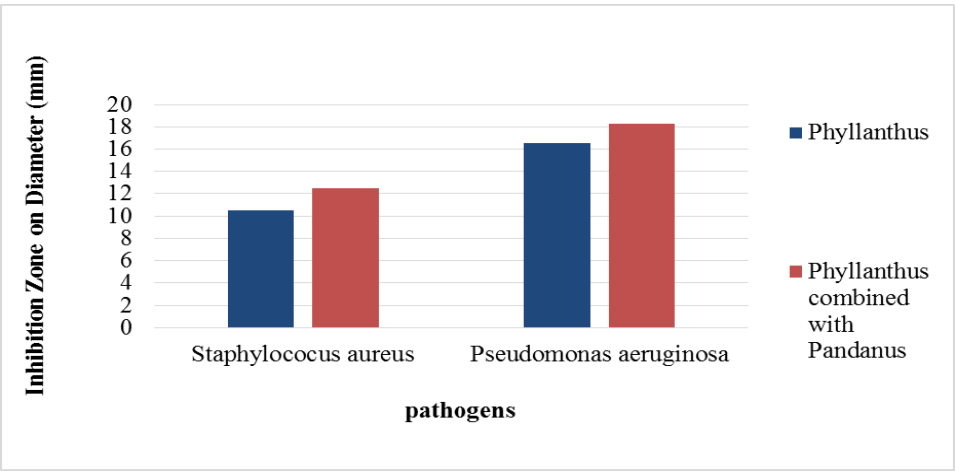

Figure 3: Antimicrobial activity test of the ethylacetate extract of *Phyllanthus urinaria* according to the inhibition zone diameter (mm).

DISCUSSION

The objective of this project was to find out the antimicrobial activities of *P.urinaria* and *P.urinaria* combined with *P.tectorius* when extract with water. The result showed that From Table- 1, the inhibition of the total extract of *P.urinaria* on, S.aureus(25.75 ± 0.96 mm) was significantly stronger than P.aeruginosa (22.75 ± 0.96 mm) based on the inhibition zone diameter [12]. The total extract of the combination between *P.urinaria* and *P.tectorius* showed the weaker activities than the total extract of the *P.urinaria*. S. aureus(15.75 ± 0.50 mm) was bigger than P. aeruginosa (14.75 ±0.50mm).[13] The Table- 2 and Table- 3 showed the activities of the extracts fractionated with chloroform and ethylacetate. The activities decreased after the chloroform and ethylacetate extracts of *P.urinaria* in the combination with *P.tectorius*. From Table- 2, the inhibition of the chloroform extract of *P.urinaria* on, P.aeruginosa (22.75 ± 0.50mm) was significantly stronger S.aureus (19.25 ± 0.50mm) than based on the inhibition zone diameter.[14] The combination of the chloroform extract of *P.urinaria* and *P.tectorius* showed the weaker activities on S.aureus (14.25 ± 0.50mm) and P.aeruginosa (11.25 ± 0.50 mm). From Table 3, the inhibition of the ether extract of *P. urinaria* on P.aeruginosa (16.50 ± 0.50mm) was significantly stronger than S.aureus (10.5 ± 0.58 mm) based on the inhibition zone diameter.[15] The combination of the ether extract of *P.urinaria* and *P.tectorius* showed the weaker activities on S.aureus (12.50 ± 0.58 mm) and P.aeruginosa (18.25 ± 0.50 mm). The inhibition of the ether extract of *P.urinaria* on S.aureus and P. aeruginosa was insignificantly different from the inhibition of the chloroform extract.[16] So, the results give that the water fraction and the chloroform fraction of *Phyllanthus urinaria* was significantly

showed the antimicrobial properties than the combination of the extract of *P.urinaria* and *P.tectorius*.

CONCLUSION

The study was to investigate the antimicrobial activities of *Phyllanthus urinaria* leaves extracts before and after combined with *Pandanus tectorius* fruit extracts. The water extract show the inhibition of the total extract of *P.urinaria* on, on S.aureus(25.75 ± 0.96 mm) was significantly stronger than P.aeruginosa (22.75 ± 0.96 mm) based on the inhibition zone diameter. The total extract of the combination between *P.urinaria* and *P.tectorius* showed the weaker activities than the total extract of the *P.urinaria*. on S. aureus (15.75 ± 0.50 mm) was bigger than P. aeruginosa (14.75 ±0.50mm). The inhibition of the chloroform extract of *P.urinaria* on P.aeruginosa (22.75 ± 0.50mm) was significantly stronger S.aureus (19.25 ± 0.50mm) than based on the inhibition zone diameter. The combination of the chloroform extract of *P.urinaria* and *P.tectorius* showed the weaker activities on S.aureus (14.25 ± 0.50mm) and P.aeruginosa (11.25 ± 0.50 mm). However, the inhibition of the ether extract of *P.urinaria* on P.aeruginosa (16.50 ± 0.50mm) was significantly stronger than S.aureus (10.5 ± 0.58 mm) based on the inhibition zone diameter. The combination of the ether extract of *P.urinaria* and *P.tectorius* showed the weaker activities on S.aureus (12.50 ± 0.58 mm) and P.aeruginosa (18.25 ± 0.50 mm). So the study represent that *Phyllanthus urinaria* leaves extracts shows significant inhibition at the water and the chloroform fractions than the combination of the extract of *P.urinaria* and *P.tectorius.* However, the ethylacetate extract of *P. urinaria* on S.aureus and P. aeruginosa was insignificantly different from the inhibition of the chloroform and water extracts.

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