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Evaluation of *in vitro* antibacterial activity of extract of semi- aquatic plants growing in the polluted water of Sri Lanka.

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Abstract
This study evaluated *in vitro* antibacterial activity and phytochemical profile of four plants (roots of Ipomoea aquatica, Lasia spinosa, Pistia stratiotes and leaves of Monochoria vaginalis) which grows in the wastewater of Sri Lanka. These plants are used in traditional and folk medicine of Sri Lanka for treatment of various bacteria induced infectious diseases. Antibacterial activity evaluated using 0.9% normal saline extracts against two Gram- positive [Staphylococcus aureus (ATCC 25923) and Bacillus subtilis (ATCC 6633)] and two Gram-negative [Escherichia coli (ATCC 25922); Pseudomonas aeruginosa (ATCC 27853)], clinically important bacterial pathogens. The plant extracts were prepared by homogenization of plant in 0.9% sterile isotonic normal saline. The antibacterial activity of the extracts were evaluated using cylinder plate method. The extracts of the four plants showed antibacterial activity with the zone of inhibition values ranging between 12- 15mm. According to the dose-response study, Pistia stratiotes exhibited the highest antibacterial efficacy and Monochoria vaginalis the lowest antibacterial efficacy against Escherichia coli (ATCC 25922). Statistical analysis showed a significant difference ($P<0.05$) between the efficacy of Pistia stratiotes and Monochoria vaginalis against Escherichia coli. Pistia stratiotes exhibited the highest antibacterial efficacy, while Monochoria vaginalis showed weak antibacterial efficacy against Staphylococcus aureus (ATCC 25923). Statistical analysis proved that the efficacy of the Pistia stratiotes and Monochoria vaginalis against Staphylococcus aureus has a significant difference ($P<0.05$). Lasia spinosa showed the highest antibacterial efficacy, while Ipomoea aquatica showed minimum antibacterial efficacy against Pseudomonas aeruginosa. There is a significant difference ($P<0.05$) between the efficacy of the Pistia stratiotes and Ipomoea aquatica against Pseudomonas aeruginosa. Lasia spinosa showed the highest antibacterial efficacy, while Ipomoea aquatica exhibited the lowest antibacterial efficacy against Bacillus subtilis (ATCC 6633). Statistical analysis showed a significant difference ($P<0.05$) between the efficacy of Lasia spinosa and Ipomoea aquatica against Bacillus subtilis. EC50 values of the dose-response study were used to compare the potency of in-vitro antibacterial activity plant of extracts. Here, Monochoria vaginalis 0.9% saline extract showed the highest antibacterial potency against Escherichia coli with the lowest EC50 value. Lasia spinosa 0.9% saline extract showed the highest potency against Staphylococcus aureus and Ipomoea aquatica shows high antibacterial potency against both Pseudomonas aeruginosa and Bacillus subtilis with low EC50 value. It is concluded that the Ipomoea aquatica and Lasia spinosa plant which grown in polluted water may possess antimicrobial activity and its efficacy and potency can't determine only depend on the size of bacterial inhibition zone. Results of these result further provide the information about the possibility of developing safe, efficacious and potent antibacterial medicines.

Keywords: Ipomoea aquatica, Lasia spinosa, Pistia stratiotes and Monochoria vaginalis; antibacterial activity

Introduction

Infectious diseases are caused by micro-organisms (pathogens) that invade and damage organ function. Pathogens cause diseases in several ways. In the case of human diseases, infections are the most common cause of illnesses and death. Pathogenesis is caused by several mechanisms; Such as pathogen cell contacts and damages to the cell; invasion of the cell and damage to the cell; release of endotoxins nearby cells and damage the cell; induction of systemic inflammatory responses by releasing inflammatory inducing substances that obstruct or damage the blood vessels; induction of local acute inflammation; induction of immune- mediated antibody responses; induction of cell-mediated immune responses; occupying lesion in an organ and induction or promotion of malignant tumours etc. (Levison and Muir, 2008) [15].

Antibiotic treatment kills or halts the growth of major, susceptible portion of the microbial population by altering the above one or two pathogenic mechanisms (Drlica and Perlin, 2011) [5]. By definition, antibiotics are antibacterial substances produced by various species of micro- organisms (bacteria fungi and actinomycetes) that suppress the growth of other micro- organisms (Goodman *et al*., 2006) [8]. Also, in common usage, antibiotics include synthetic antimicrobial agents such as sulfonamides and quinolones. Each antibiotic is physical, chemically, and pharmacologically different in properties from each other. Because of that, antimicrobial agents classified into six major classes based on the chemical and proposed mechanisms of action (Goodman *et al*., 2006) [8].

Successful antimicrobial therapy for an infection ultimately depends on the concentration of antibiotic at the site of infection. This concentration of the antimicrobial agent must be sufficient enough to inhibit the growth of the offending microorganism, which is called as Minimum Inhibitory Concentration (MIC) (Goodman *et al*., 2006) [8]. Currently, many potent antibiotics/chemotherapeutics are available for the treatment of a wide range of infectious diseases caused by bacteria (Fausi *et al*., 2008) [6]. Some of the currently used antibiotics are; amoxicillin, flucloxacillin, chloramphenicol, benzylpenicillin, erythromycin, metronidazole, and ciprofloxacin etc. (Frank and Tacconelli, 2012) [7].

Plants have been used in treating human ailments for thousands of years in traditional medicine in Sri Lanka as well as around the world. There are about 100,000 species of known plants available globally which has important medicinal applications. However, there are many plants activity still unclear and under investigation (Normah, 2013) [18]. Ipomoea aquatica is a semi-aquatic, tropical plant grown as a vegetable for its tender shoots and leaves. Ipomoea aquatica of Convolvulaceae family has been originated in China and distributed throughout India, Sri Lanka, tropical Asia, Australia and native to Africa. It is commonly named as water spinach, kangkong (Harry and Bess, 1969; Kiritkar and Basu, 1952) [9, 13]. In Burma, traditionally use this plant as an emetic agent against poisoning of arsenic and opium (Jayaweera, 2006) [11]. The plant Lasia spinosa is a member of family Araceae, distribution of Lasia spinosa occurs in marshy places in tropical India. Burma, Sri Lanka, Malay Peninsula and China (Jayaweera, 2006) [11]. Pistia stratiotes is a member of the family Araceae. In Sri Lanka, Pistia stratiotes is named as Diya paradel. This plant is generally found in the tropics including Sri Lanka, India, and Philippine islands and it is native to Sri Lanka (Jayaweera, 2006) [11]. Monochoria vaginalis belongs to family Pontederiaceae. This plant is distributed in South East Asia, Iran, Philippines, Indonesia, China, Korea, Japan and North Australia to Sri Lanka (Lansdown, 2013) [14]. This plant is also known as ‘Diya habarala’ in Sri Lanka.

This study evaluated *in vitro* antibacterial activity of roots of Ipomoea aquatica which grows in polluted water of Sri Lanka using 0.9% normal saline extracts against two Gram-positive [Staphylococcus aureus (ATCC® 25923™) and Bacillus subtilis (ATCC® 6633™)] and two Gram-negative [Escherichia coli (ATCC® 25922™); Pseudomonas aeruginosa (ATCC® 27853™)].

2. Methodology

a) Plant Collection & Authentication

Ipomoea aquatica was collected from a canal near the University of vocational technology (6°49'13.0"N 79°53'34.1"E) situated in 7th Lane, Rathmalana, Dehiwala-Mount Lavinia, Colombo, Western Province of Sri Lanka. Lasia spinosa was collected from a canal near the University of vocational technology (6°49'12.3"N 79°53'33.4"E) situated in 7th Lane, Rathmalana, Dehiwala-Mount Lavinia, Colombo, Western Province of Sri Lanka. Pistia stratiotes was collected from Weras ganga (6°48'51.6"N 79°54'07.0"E), near the karadiyana garbage dump, Thumbovila north, Boralesgamuwa, situated in Colombo district, western province of Sri Lanka. Monochoria vaginalis was collected from a canal of the 9th cross street (6°49'02.5"N 79°53'44.2"E), Rathmalana, Dehiwala-Mount Lavinia, Colombo, Western Province of Sri Lanka. Ipomoea aquatica, Lasia spinosa, Pistia stratiotes and Monochoria vaginalis plants were identified and authenticated with the help of National Herbarium, Royal Botanical Garden, Peradeniya, Sri Lanka

b) Preparation of plant extracts

Roots of Ipomoea aquatica, Lasia spinosa, Pistia stratiotes and leaves of Monochoria vaginalis were collected from the mentioned locations followed by immediate washing with running water to remove contaminants and dried on air until a constant weight was reached. 20 g of Plant specimens were cut into small pieces, about 3-6 cm in length and plant parts were ground into a fine powder using a mortar and pestle. The powdered plant materials (20g) were resuspended in 20ml of distilled water and followed by filtration using a muslin cloth in order to remove the debris. The filtrate was collected into the bottles and used for the phytochemical screening.

c) Antibacterial activity screening

Prepared four aqueous extracts of plants were subjected to antibacterial activity using the cylinder plate method (Souzha-Filho *et al*., 2008) [20]. 0.9% sterile normal saline was used as negative control while Gentamycin (10 µg/ µl) was a positive control. The growth medium used in this experiment was Nutrient Agar and the antibacterial activities were determined against two Gram-positive [Staphylococcus aureus (ATCC® 25923™) and Bacillus subtilis (ATCC® 6633™)] and two Gram-negative [Escherichia coli (ATCC® 25922™); Pseudomonas aeruginosa (ATCC® 27853™)]) which were adjusted the concentration compared with 0.5 McFarland as described by Clinical and laboratory standard Institute (CLSI, 2015). The antibacterial activity was examined in duplicate (n=2) for each sample and the diameter of the inhibition zone (in mm) for extract against the above-mentioned bacterial strains were measured and recorded.

d) Statistical analysis.

Data were expressed as mean ± S.E.M. in order to compare the plant extract against bacteria strains and the values were

analyzed for statistically significance using Independence sample
– t-test. Data analysis was performed using IBM SPSS 21.00 statistical package for windows. In all $P<0.05$ were considered as statistically significant. Graph-pad prism 7 software was used to perform the dose-response curve for the relevant obtained data.

3. Results and Discussion

The results of Antibacterial activity screening of four plants extracts are summarized in Table
As shown, extracts of Ipomoea aquatica, Lasia spinosa, Pistia stratiotes and leaves of Monochoria vaginalis exerted antibacterial activity (in terms of the diameter of the zone of inhibition) against four bacterial strains, with a zone of inhibition values ranging between 12- 15mm. four aqueous extracts show antibacterial activity even at 250μg/μl concentration. The positive control, Gentamycin, provoked a marked antibacterial activity against four bacterial strains.

Table 1: Antimicrobial activity of Ipomoea aquatica root extracts by cylinder plate method

Antimicrobial Activity Assays.	Zone of inhibition by cylinder plate method (mm) Ipomoea aquatica					
	1000µg/µl	750µg/µl	500µg/µl	250µg/µl	Positive control (50µg/ml)	Negative control
Escherichia coli	13.81±0.8	11.12±0.3	10.50±0.3	11.05±0.3	16.68±0.4	No inhibition
Staphylococcus aureus	15.06±0.7	14.03±0.6	13.82±0.5	13.48±0.7	16.87±0.2	No inhibition
Pseudomonas aeruginosa	13.33±0.1	13.60±0.5	12.62±0.2	11.51±0	16.48±0.2	No inhibition
Bacillus subtilis	13.51±0	13.08±0	12.74±0.1	11.37±0	15.60±0.1	No inhibition

Antimicrobial Activity Assays.	Zone of inhibition by cylinder plate method (mm) Lasia spinosa					
	1000µg/µl	750µg/µl	500µg/µl	250µg/µl	Positive control (50µg/ml)	Negative control
Escherichia coli	13.92±0.7	12.69±0.3	11.54±0.3	10.74±0.4	16.68±0.4	No inhibition
Staphylococcus aureus	13.76±0.3	13.02±0.2	11.92±0.1	11.58±0.4	16.87±0.2	No inhibition
Pseudomonas aeruginosa	15.39±0	14.01±0.6	13.31±0.3	12.41±0.2	16.48±0.2	No inhibition
Bacillus subtilis	13.11±0	12.57±0	12.13±0	11.42±0.1	15.60±0.1	No inhibition

Antimicrobial Activity Assays.	Zone of inhibition by cylinder plate method (mm) Pistia stratiotes					
	1000µg/µl	750µg/µl	500µg/µl	250µg/µl	Positive control (50µg/ml)	Negative control
Escherichia coli	14.32±0.5	11.97±0.05	11.80±0.4	11.24±0.05	16.68±0.4	No inhibition
Staphylococcus aureus	12.93±0.2	12.28±0	11.78±0.1	11.67±0.2	16.87±0.2	No inhibition
Pseudomonas aeruginosa	15.61±0.1	14.78±0.2	13.98±0.2	12.67±0.1	16.48±0.2	No inhibition
Bacillus subtilis	14.07±0.1	13.40±0.2	12.68±0.1	11.92±0.4	15.60±0.1	No inhibition

Antimicrobial Activity Assays.	Zone of inhibition by cylinder plate method (mm) Monochoria vaginalis					
	1000µg/µl	750µg/µl	500µg/µl	250µg/µl	Positive control (50µg/ml)	Negative control
Escherichia coli	12.41±0.3	11.63±0.3	10.65±0.7	10.37±0.9	16.68±0.4	No inhibition
Staphylococcus aureus	12.97±0.2	11.87±0.2	10.80±0.1	11.21±0.3	16.87±0.2	No inhibition
Pseudomonas aeruginosa	14.26±0.1	13.61±0.1	12.67±0.1	12.04±0.2	16.48±0.2	No inhibition
Bacillus subtilis	14.52±0.2	13.64±0.2	13.39±0.2	12.00±0.2	15.60±0.1	No inhibition

Positive control – gentamycin 50µg/ml Negative control – 0.9% saline
Data expressed hers as mean Inhibition zone diameter ± SEM. (n=3)

Dose-response curves against four bacteria strains showed in figure 1 and figure 2. EC50 and other details of relevant Dose-response graphs are expressed in table 3.

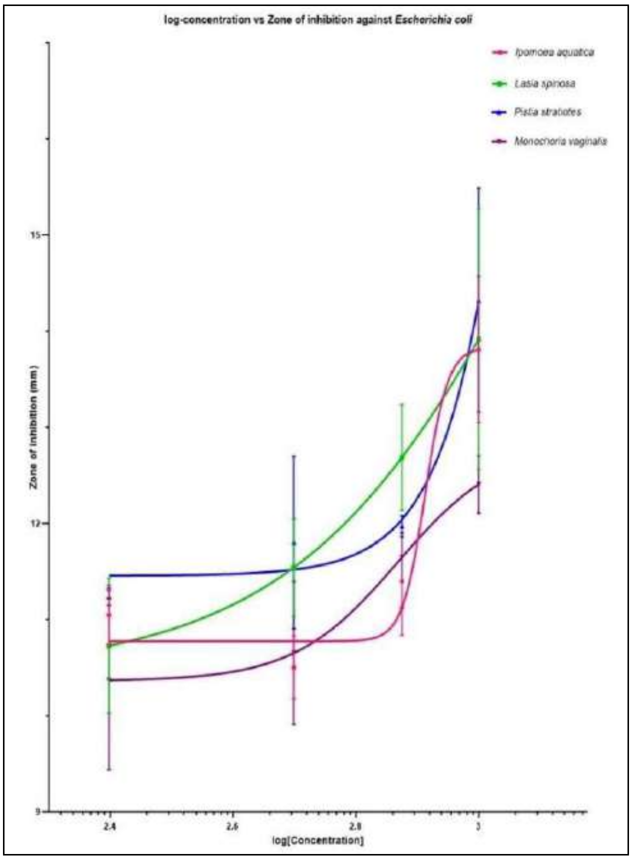

Fig 1: Dose response curves for plant extracts against Escherichia coli

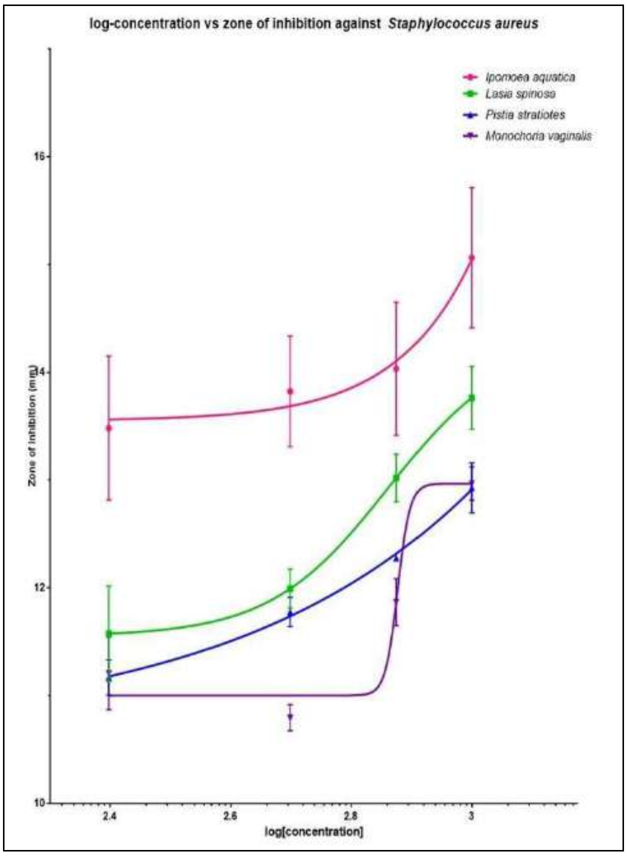

Fig 2: Dose response curves for plant extracts against Staphylococcus aureus

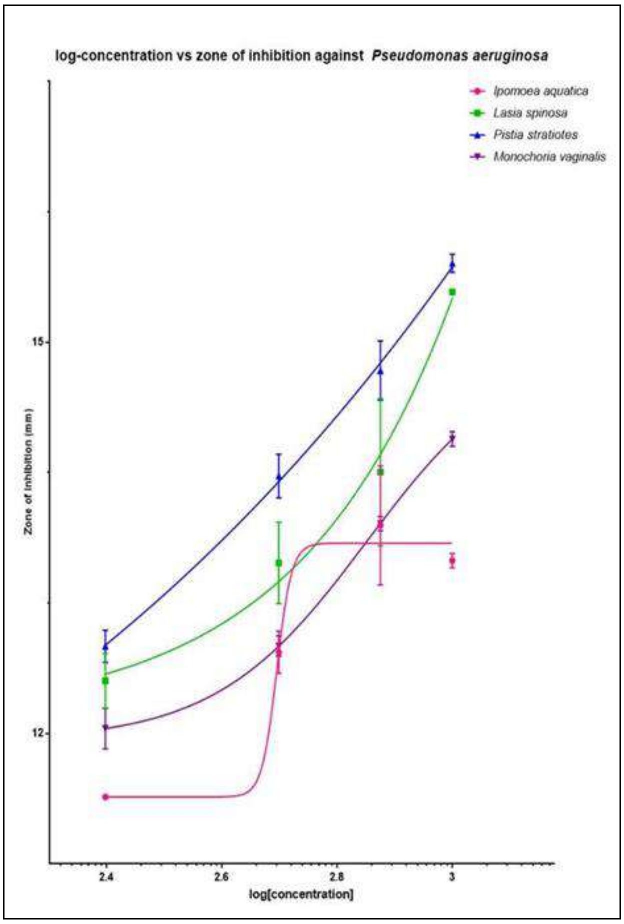

Fig 3: Dose response curves for plant extracts against Pseudomonas aeruginosa

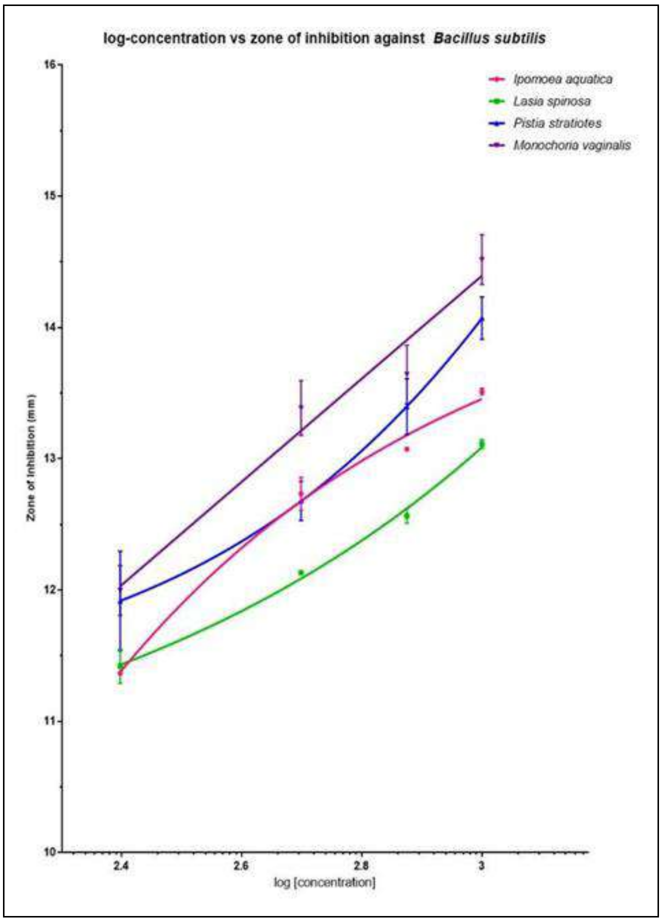

Fig 4: Dose response curves for plant extracts against Bacillus subtilis

Table 2: Dose response curve details for plant extracts against Escherichia coli

Escherichia coli	Ipomoea aquatica	Lasia spinosa	Pistia stratiotes	Monochoria vaginalis
[log concentrations] vs. zone of inhibition -- Variable slope (four plants)	Ambiguous		Ambiguous	
Bottom (mm)	10.77	10.45	11.46	10.36
Hillslope (mm)	~ 23.49	2.099	~ 5.54	5.16
Top (mm)	~ 13.83	20.76	~ 388.9	12.83
EC50 (µg/µl)	~ 818.3	1383	~ 2411	738.5
logEC50	~ 2.913	3.141	~ 3.382	2.868
Span	~ 3.062	10.31	~ 377.4	2.475

Table 3: Dose response curve details for plant extracts against Staphylococcus aureus

Staphylococcus aureus	Ipomoea aquatica	Lasia spinosa	Pistia stratiotes	Monochoria vaginalis
[log concentrations] vs. zone of inhibition -- Variable slope (four plants)	Ambiguous		Ambiguous	Ambiguous
Bottom (mm)	13.55	11.55	10.67	11
Hillslope (mm)	3.476	4.41	~ 1.074	~ 42.12
Top (mm)	~ 157.2	14.31	~ 883.6	~ 12.97
EC50 (µg/µl)	~ 3705	727.8	~ 257559	~ 754.3
logEC50	~ 3.569	2.862	~ 5.411	~ 2.878
Span	~ 143.6	2.757	~ 872.9	~ 1.966

Table 4: Dose response curve details for plant extracts against Pseudomonas aeruginosa

Pseudomonas aeruginosa	Ipomoea aquatica	Lasia spinosa	Pistia stratiotes	Monochoria vaginalis
[log concentrations] vs. zone of inhibition -- Variable slope (four plants)	Ambiguous	Ambiguous	Ambiguous	
Bottom (mm)	11.51	12.11	~ 8.972	11.95
Hillslope (mm)	~ 33.67	~ 1.616	~ 0.441	3.328
Top (mm)	13.46	~ 1307	~ 102.3	15
EC50 (µg/µl)	~ 495.8	~ 40743	~ 342876	710.1
logEC50	~ 2.695	~ 4.61	~ 5.535	2.851
Span	[illegible].948	~ 1295	~ 93.33	3.051

Table 5: Dose response curve details for plant extracts against Bacillus subtilis

Bacillus subtilis	Ipomoea aquatica	Lasia spinosa	Pistia stratiotes	Monochoria vaginalis
[log concentrations] vs. zone of inhibition -- Variable slope (four plants)	Ambiguous	Ambiguous	Ambiguous	Ambiguous
Bottom (mm)	~ -476.9	~ 10.19	11.2	~ -24.5
Hillslope (mm)	~ 0.7497	~ 0.6177	~ 1.12	~ 0.09445
Top (mm)	~ 14.6	~ 235.4	~ 25.71	~ 47.93
EC50 (µg/µl)	~ 0.3094	~ 1125359	~ 3487	~ 208.1
logEC50	~ -0.5094	~ 6.051	~ 3.542	~ 2.318
Span	~ 491.5	~ 225.2	~ 14.52	~ 72.43

A limited number of research publications are available on antibacterial activity of medicinal plants in Sri Lanka. In this study we investigated in-vitro antibacterial activity and phytochemical analysis of 0.9% saline extracts of Ipomoea aquatica, which is growing in polluted water of Rathmalana, Sri Lanka, against two Gram-negative [Pseudomonas aeruginosa (ATCC® 27853™); Escherichia coli (ATCC® 25922™)] and two Gram-positive [Staphylococcus aureus (ATCC® 25923™); Bacillus subtilis (ATCC® 6633™)] clinically important bacterial pathogens using cylinder plate method. This plant is growing in polluted water, but they survive in it as they have resistance to bacterial pathogenesis. All the plants have their own immune system called the innate immune system (Jonathan *et al*., 2006) [13]. Which may consist of physical barriers and chemical barriers against the bacterial pathogenesis (Ade and Inness, 2007) [1]. In this study, those chemical substances which act as the chemical barriers are extracted to 0.9% sterile normal saline and tested for their action against bacteria.

Here, we selected the roots of this plant as they are the part which is directly contacted with polluted water and assumed that the roots may have the highest antibacterial activity. Further, there are reported studies showing the antibacterial activity of plants using water extracts for the leaves of the above-mentioned plant (Bhakta *et al*., 2008) [2]. The cylinder plate method for antibiotic analysis was performed with the help of State Pharmaceutical Manufacturing Corporation of Sri Lanka. Gentamycin is a broad - spectrum aminoglycoside produced by Micromonospora echinospora (Ni *et al.*, 2016) [17]. It is a bactericidal agent against many aerobic Gram-negative bacteria but has lower activity against most Gram-positive organisms with the exception of staphylococci. (Harvick, *et al*, 1973) [10] Because of its Broad- spectrum of activity, we selected the gentamycin as the positive control in this study.

Escherichia coli is known to cause gastrointestinal, meningeal, urinary tract and wound infections and also peritonitis, septic wounds, bedsores and cholecystitis (Mackie and McCartney, 1989) [16]. Secondary pneumonia, endocarditis, osteomyelitis and septic arthritis, scalded skin syndrome and toxic shock syndrome are the major cause of the Staphylococcus aureus (Sherris, 1984) [19]. Pseudomonas aeruginosa can attack healthy people and also to people who have a disease with immune compromised. Pseudomonas aeruginosa is a responsible microorganism for a wide range of infections in the human body. These are urinary, surgical site, respiratory, ocular, ear, skin infection, and ventilator-associated pneumonia (Trautmann *et al*., 2008) [21]. Bacillus subtilis is known to cause food poisoning with several gastrointestinal symptoms such as nausea, vomiting, epigastric pain or diarrhea. Bacillus subtilis causes a number of systemic and local infections in both immunologically compromised and fatal pneumonia, septicemia, in addition to food poisoning and infection of a necrotic axillary tumor in breast cancer and breast prosthesis (De Vos. *et al.*, 2011) [4].

The results of our study show for the first time, that the 0.9% normal saline extract of roots of Ipomoea aquatica, showed an antibacterial activity with the zone of inhibition against four bacterial strains which we tested. However, the present study does not pinpoint whether the antibacterial activity of those plants mediated via bactericidal and/or bacteriostatic mechanisms.

Among the available antibiotic research, many have been conducted just to find out the zone of inhibition against bacterial pathogens (Jelodarian, 2013) [12]. But, in this study, we performed the Dose-response studies and found out the EC50 for comparison of antibiotic potency of plant extracts against four pathogenic bacteria which we tested. Dose-response studies are performed to investigate the potency and efficacy of a compound and also the EC50 is the concentration of the compound that gives half maximum response. Dose-response data are typically evaluated by using log concentration versus activity and that includes EC50 as one of the model parameters.

According to the Dose-response curve details (Table 4.9), highest EC50 value is for the Dose-response curve of Pistia stratiotes and lowest EC50 for the Dose-response curve of Monochoria vaginalis. Based on EC50 values and dose-response graph for Escherichia coli (figure 4.43), Monochoria vaginalis has the highest potency and Pistia stratiotes have the highest antibacterial efficacy among these four plant extracts against Escherichia coli. On the other hand, the lowest antibacterial efficacy and lowest potency against Escherichia coli by the Monochoria vaginalis and Pistia stratiotes extracts respectively. Statistical analysis has proven that there is a significant difference between the highest and lowest efficacious plant extracts ($P<0.05$) against Escherichia coli. Pistia stratiotes plant extract has a wide range of antibacterial activity against Escherichia coli. As shown in Table 4.10, highest and lowest EC50 value among these dose-response curves in figure 4.44 are observed by respectively Pistia stratiotes and Lasia spinosa. According to graph details and dose-response graph for Staphylococcus aureus (figure 4.43), Pistia stratiotes has the highest antibiotic efficacy and Lasia spinosa has the highest potency against Staphylococcus aureus. The lowest potency and lowest antibiotic efficacy against Staphylococcus aureus was observed with Pistia stratiotes and Monochoria vaginalis. Statistical analysis has proven, there is a significant difference ($P<0.05$) between highest and lowest efficacious plant extracts, Pistia stratiotes and Monochoria vaginalis respectively against Staphylococcus aureus. Pistia stratiotes plant extract has a wide range of antibacterial activity against Staphylococcus aureus. According to the result shown in Table 4.11, highest and lowest EC50 values have shown by the Pistia stratiotes and Ipomoea aquatica. Depends on EC50 values and Dose-response graph for Pseudomonas aeruginosa (Figure 4.43), the highest antibacterial efficacy against Pseudomonas aeruginosa was observed by Lasia spinosa and the highest potency was observed by the Ipomoea aquatica. In another hand, the lowest antibiotic efficacy and lowest potency are showed by Ipomoea aquatica and Pistia stratiotes. Statistical analysis has shown, there is a significant difference ($P<0.05$) between highest and lowest efficacious plant extracts, Pistia stratiotes and Ipomoea aquatica respectively. Pistia stratiotes and Lasia spinosa plant extracts have a wide range of antibacterial activity against Pseudomonas aeruginosa. As mentioned in Table 4.12, highest EC50 was given by the Lasia spinosa extract and lowest EC50 was given by the Ipomoea aquatica extract. According to the EC50 values and Dose-response graph drawn against Bacillus subtilis (Figure 4.44), the highest antibacterial efficacy was represented by the Lasia spinosa and highest potency was observed with Ipomoea aquatica against Bacillus subtilis. Here, the lowest potency and lowest antibiotic efficacy against Bacillus subtilis were presented by Lasia spinosa and Ipomoea aquatica respectively. According to the Dose- response study details, Statistical analysis has proven, that there is a significant difference ($P<0.05$) between highest and lowest efficacious plant extracts, Lasia spinosa and Ipomoea aquatica respectively.

EC50 values of this dose-response study were used to compare the antibacterial activity of plant extracts against Gram-negative and Gram-positive pathogenic strains. When compare Escherichia coli and Staphylococcus aureus as Gram-negative and Gram-positive bacteria, Pistia stratiotes has the highest antibacterial efficacy against both Gram-negative bacteria and Gram-positive bacteria. In another case of Lasia spinosa extract has significantly high antibacterial efficacy against Gram-positive bacteria than the Gram negatives. Monochoria vaginalis extract wasn't showed an antibacterial efficacy in much different than other extracts against both Gram-negative and Gram-positive bacteria. When compare Pseudomonas aeruginosa and Bacillus subtilis consider as Gram-negative and Gram-positive bacteria, Lasia spinosa showed a significantly higher antibiotic efficacy against both Gram-positive and Gram- negative bacteria. Pistia stratiotes present greater antibiotic efficacy than Lasia spinosa against Pseudomonas aeruginosa. And also, the lowest antibiotic efficacy was shown by Ipomoea aquatica on both Gram-negative and Gram-positive bacteria strains. Monochoria vaginalis has little antibiotic efficacy on both Gram-negative and Gram-positive strains than the Ipomoea aquatica. Pistia stratiotes had the lowest potency on both Gram-negative and positive bacteria stains which we tested except Pseudomonas aeruginosa. Ipomoea aquatica showed much higher potency on all bacteria strains which we were tested except Staphylococcus aureus and Escherichia coli.

These results are novel findings which have implications in medicine and in the food industry. However, the present study of *in vitro* antimicrobial evaluation of this Ipomoea aquatica plant forms a primary platform for further phytochemical and pharmacological studies. As mentioned earlier, the antibacterial activity of Ipomoea aquatica root extract was confirmed to bacterial pathogen against all four bacterial strains that tested in this study. This indicates that four plant extracts may possess as broad-spectrum antibacterial.

4. Conclusions

In conclusion, this study demonstrates, for the first time, potent in-vitro antibacterial activity of 0.9% saline extract of roots of Ipomoea against two Gram-positive pathogens, Staphylococcus aureus (ATCC® 25923™) and Bacillus subtilis (ATCC® 6633™), and two Gram-negative Pseudomonas aeruginosa (ATCC® 27853™) and Escherichia coli (ATCC® 25922™) pathogens which are responsible for wide range of infections in human body and food poisoning. This Ipomoea aquatica plant which is grown in polluted water have significantly the highest antibacterial activity to survive against these bacteria that live in polluted water. It is an advantage for discovering novel antibacterial medicines to refitting to the antibiotic drug resistance in the future.

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