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Antimicrobial Properties of *Pontederia cordata* Extract

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ABSTRACT

The antimicrobial activity of the ethanolic and aqueous extract of Pontederia cordata against selected pathogens were investigated using the Disc Diffusion Assay. The Diameter of Zone of Inhibition, DZOI was used as an indicator of plant extract antimicrobial potency. For the ethanolic extract, the highest DZOI of 36.9 mm was recorded against E.coli with antimicrobial susceptibility following the sequence: E.coli > P.mirabilis > P.aeruginosa > S.aureus.

For the hexane extract, the highest DZOI of 26.5 ± 3.3mm was also obtained against E.coli. The order of antimicrobial susceptibility following the sequence: E.coli > P. mirabilis > P.aeruginosa. Thus, the ethanolic extract was found to be more antimicrobial than the hexane extract. Both the ethanolic and hexane extracts were microbial against C. albicans Compared to the positive control, Ciprofloxacin, the antimicrobial susceptibility for E.coli was registered at 87.86% and 63.1% for the ethanolic and hexane extract. Likewise, the antimicrobial susceptibility for P. mirabilis was registered at 84.5% and 66.71% respectively.

Thus, the ethanolic and hexane extract of Pontederia cordata can be used to treat topically E.coli and P. mirabilis infections.

Keywords

Pontederia cordata, Disc Diffusion Assay, DZOI, *E.coli, P.mirabilis*, *P.aeruginosa.*

Introduction

Research in the design and synthesis of antimicrobials will continue to proliferate on our planet, considering that bacteria and fungi develop resistance to antimicrobials over a period of time [1-5]. This results from indiscriminate use of commercial antimicrobial drugs for the treatment of infectious diseases and the current global antibiotic resistance [1-5]. Antimicrobial resistance is defined as the resistance against pathogenic microorganisms such as bacteria and fungi [6]. Increasing antimicrobial resistance (AMR) presents a major threat to public health because it reduces the effectiveness of antimicrobial treatment, leading to increased morbidity, mortality, and health care expenditure [7]. Bacteria develops antimicrobial resistance via several mechanisms. These include the production of enzymes that will destroy certain prime selective groups in the drug molecule that are necessary for it to act. For example, bacteria produce the enzyme, β-lactamase that will destroy the β-lactam ring of penicillin. The latter is necessary for the antibacterial effect of the drug, penicillin. Other methods bacteria have evolved to protect against the drug, include an increase efflux of the drug across the bacterial membrane, the protection of bacterial ribosomes via processes such as methylation, induced by the enzymes methylase, decrease influx of the drug across bacteria [8].

Many synthetic drugs have been used over the years but have several adverse side effects which are usually irreversible when administered and the cost of synthesizing drugs in most cases is an expensive endeavour [1-5]. Synthetic drugs are also not environmentally friendly. Natural antimicrobials overcome all these problems. Plants have a long therapeutic history over thousands of years and still considered to be promising source of medicine in the traditional health care system [9]. Plants also have a wide variety of secondary metabolites, some of which are antimicrobial [10-12]. Crude plants extracts have also demonstrated antimicrobial activity [13-15].

Antimicrobial resistance is a global health threat. In 2019, it was responsible for 1.27 million deaths worldwide. Persons of all age groups and regions were affected. However, middle and low income countries were mostly affected [16]. Drug shortage which is on the increase globally, can exacerbate the spread of drug resistant pathogens like *E.coli* and methicillin resistant *Staphylococcus aureus* [16]. There is no system in place to monitor, and report priority antimicrobial resistant pathogens in Guyana, although one has been drafted [17].

Guyana flora is richly biodiversified and it's organic and aqueous extracts have been shown to possess potent and selective antimicrobial activity to date, compared with standard synthetic antibiotics: penicillin, nystatin and ampicillin [18-22]. The objectives of this research were: To prepare the hexane and ethanolic extracts of *Pontederia cordata* for antimicrobial testing against *Staphylococcus aureus,* Methicillin-Resistant *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa, Proteus mirabilis,* and *Candida albicans* and to compare the potency of *Pontederia cordata* hexane extract against 95% ethanol extract against existing antibiotics (Ciprofloxacin and Ketoconazole)

Pontederia cordata, is commonly called pickerelweed (USA) or pickerel weed (UK). It is a monocotyledonous aquatic plant native to the Americas. It grows in a variety of wetlands, including pond and lake margins across an extremely large range from eastern Canada south to Argentina. It also grow profusely in the trenches in Guyana. It is a resilient plant that thrives in shallow freshwater shorelines and is a valuable addition to gardens that provide consistent moisture and sunlight. *Pontederia cordata*, has been recognized for its medicinal properties. Its used in medicine to support the body systems. It has been used to make a contraceptive. The plant's seeds and leaves are edible and can be consumed raw, cooked, or dried. The seeds are edible raw, and can be ground into grain [23-25]. *Pontederia cordata*, has been recognized for its medicinal properties. Its used in medicine to support the body systems. The plant's seeds and leaves are edible and can be consumed raw, cooked, or dried. Pickerelweed is a resilient plant that thrives in shallow freshwater shorelines and is a valuable addition to gardens that provide consistent moisture and sunlight [26,27]. Figure 1 is a morphological display of the plant.

Although little or no research has not been conducted on the antimicrobial properties of *Pontederia cordata*, another plant in its family, *Pontederia (Eichhornia) crassipes*, has been extensively studied [28-33]. It was proven that *Eichhornia crassipes* contains a range of bioactive components which are useful as antimicrobial agents. The benefits of this plant also extend past pharmacological uses, as *E. crassipes* has been exploited due to its high availability for the production of possible biofuels and animal feed. This research made use of the disc diffusion technique and serial tube dilution for leaf, root, stem and flower extracts, and it has proven that *P. crassipes* is effective against the bacteria *S. faecalis, E.coli and S.aureus* [28].

Figure 1: Morphological display of ***Pontederia cordata*.**

The antimicrobial properties of *Pontederia (Eichhornia). crassipes* was tested against a range of bacteria: *Escherichia coli, Bacillus subtilis, Bacillus cereus, Lactobacillus casei, Pseudomonas aeruginosa, C. gloeosporioides, Microcystis aeruginosa, Streptococcus faecalis, Escherichia coli*, and *Staphylococcus aureus*, as well as the algae *Chlorella vulgaris* and the fungi *Aspergillus flavus, A. niger, Alternaria alternata, Colletotrichum gloeosporioides, Candida albicans*, and *Fusarium solani*. Ethanol, water, acetone, n-butyl alcohol and methanol were used as the extraction solvents, and the latter two were proven to be the best solvents for extraction. In most cases, the positive control: Streptomycin and Fluconazole were used. The Minimum Inhibitory Concentration (MIC) for the bacteria in most cases range from 18-63µg mL. The Disc diffusion and the serial tube dilution technique were used to assay the antimicrobial properties of *P. crassipes* in most cases [28-33]. The methanolic extracts of the sample were shown to have better antibacterial activity, while the water sample had better results against fungi [31]. The highest DZOI of 15.3mm was reported for the methanolic extract against *E. coli*.

Materials and Apparatus

Method

The plant material was provided by Prof. R. Jagessar who collected it from the Turkeyen drain.All microbial procedures were conducted at the University of Guyana's College of Medical Science, Medical Laboratory. All phytochemical screening procedures were conducted at the University of Guyana's Faculty of Natural Sciences, Natural Products Laboratory.

Number	Extraction	Number	
1.	Blender	19	Metre ruler
2.	Dark brown jar	20	Glass vial
3.	Ethanol (95%)	21	Gloves
4.	Hexane	22	Test tubes
5	Blender	23	Measuring cylinder
	Antimicrobial Studies	24	Beakers
6	Petri dishes	25	Hot plate
7	Tweezers	26	Perforated discs
8	Balance	27	Stationary
9	Cotton wool	28	Distilled water
10	Foil paper	29	Mueller Hinton powder
11	Guaze	30	Ciprofloxacin
12	Spirit lamp	31	Ketoconazole
13	Autoclave		
14	Inoculating loops		
15	Conical flask		
16	Incubator		
17	Samples of each microbe		
18	Water bath		

Experimental Design

The Completely Randomised Block (CRB) design method was used. Three blocks, control, positive, and experimental, were repeated in triplicate. The 6 organisms used represented the treatments. A total of 48 plates were investigated.

Collection and Treatment of Plant Material

The leaves and flowers of the plant were collected and identified at the Centre for Biological Diversity of the University of Guyana. The leaves were picked, washed and left to air dry for 24 hours. The following day, the leaves were pulverised using a blender into small pieces. where it was weighed and portioned off into 2 clean jars. A total of 1002.3g of leaves were used. To each jar, 2 L of hexane were added, and the jars were sealed. The plant sample was left to extract in hexane for a total of 3 weeks. The hexane extract was filtered and dried over Na_2SO_4. After this, solvents were removed in *vacuo*. The remaining plant material was then further extracted in 95% ethanol for three (3) weeks. After this, the ethanol extract was filtered, dried over anhydrous sodium sulphate. The extract was then filtered and solvents removed in *vacuo* to leave a black viscous liquid.

Test for Antimicrobial Activity [34-37]

Preparation of Media

53.2 grams of Mueller Hinton Agar (MHA) was added to 1400 ml of distilled water and mixed in a conical flask. The mixture was then heated on a hot plate and swirled constantly until all of the agar powder had dissolved uniformly. A cotton wool and foil stopper was then used to plug the flask as it was placed in the autoclave for 20 minutes at 121ºC. The sterilised media was then poured into sterile petri dishes, to a depth of 3.9-4.1mm. The plates were cooled and placed in the fridge for use the next day.

Method for Preparation of Control Solutions & Test Extracts

5% hexane and ethanolic extract were prepared. Two (2) tablets of Ciprofloxacin were crushed and 0.5g was weighed and dissolved in 10ml ethanol. Two (2) tablets of Ketoconazole were crushed and 0.5g was weighed and dissolved in 10ml of 95% ethanol.

Method for Preparing Test Solutions

Perforated discs were made from Whatman filter paper and were autoclaved in a glass petri dish for 20 minutes at 121 ºC. The disks were consequently soaked in beakers containing the hexane and ethanolic extract, ciprofloxacin solution, ketoconazole solution, and ethanol solution for two and a half hours. They were then applied to the agar medium streak with microorganisms.

Method for Preparing Microorganisms & Broth

50 plates of Mueller-Hinton agar were made up, with two being monitored as quality control standards. *Staphylococcus aureus,* Methicillin-resistant *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli,* and *Candida albicans* were collected from the Georgetown Public Hospital, GPHC. All microbes wer subcultured onto Mueller-Hinton Agar and incubated for 24 hours before use. After this, the microbes were inoculated into saline solution and compared against a McFarland standard of 0.5 to ensure that the correct approximate colony size ($1.5*10^8$ bacteria) was achieved before plate inoculation. Once the appropriate turbidity was achieved, the microbes were inoculated onto the prepared petri dishes, and they were labelled appropriately.

Agar Disc Diffusion Method (48 plates)

The soaked perforated disks were transferred to the respective petri dishes using aseptic techniques and were placed equidistant from each other. Four disks were used per plate. The plates were then placed in the incubator for 24 hrs and 48hrs at 37ºC for the bacteria and fungal species respectively. Finally, the diameters of the zones of inhibition were read off and recorded.

Results

Table 1: Antimicrobial activity of the Ethanolic Extract.

Microbe tested	Ethanol Extract: DZOI + SD	AZOI (mm^2)	Relative % Inhibition of Ethanolic Extract to Positive Control
C. albicans	0.0 ± 0.0	0.0	0.0
MRSA	0.0 ± 0.0	0.0	0.0
S. aureus	21.8 ± 8.8	414.5	59.7
E. coli	36.9 ± 1.0	1070.9	87.9
P. mirabilis	35.5 ± 0.9	990.2	84.6
P. aeruginosa	27.1 ± 2.3	578.8	64.5

Table 2: Antimicrobial activity of the hexane extract.

Microbe tested	Hexane Extract: DZOI + SD	AZOI (mm^2)	Relative % Inhibition of Hexane extract To Positive Control
C. albicans	0.0 ± 0.0	0.0	0.0
MRSA	0.0 ± 0.0	0.0	0.0
S. aureus	0.0 ± 0.0	0.0	0.0
E. coli	26.5 ± 3.3	557.1	63.1
P. mirabilis	23.7 ± 1.5	441.1	66.8
P. aeruginosa	2.3 ± 2.0	96.6	5.5

Table 3: Antimicrobial activity of positive control.

Microbe tested	Positive Control: DZOI + SD	AZOI (mm^2)
C. albicans	0.0 ± 0.0	0.0
MRSA	28.5 ±1.9	637.9
S. aureus	36.5 ± 1.7	1046.3
E. coli	42.0 ± 0.0	1385.4
P. mirabilis	42.0 ± 0.0	1385.4
P. aeruginosa	42.0 ± 0.0	1385.4

Table 4: Antimicrobial activity of negative control.

Microbe tested	Negative Control: DZOI with SD	AZOI (mm^2)
C. albicans	0.0	0.0
MRSA	0.0	0.0
S. aureus	0.0	0.0
E. coli	30.3 ± 2.4	718.7
P. mirabilis	24.8 ± 1.5	481.1
P. aeruginosa	0.0	0.0

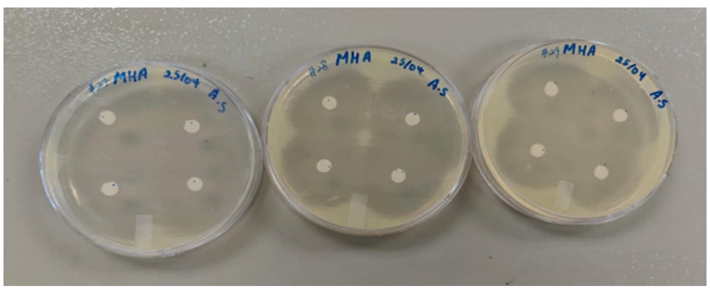


Figure 2: Zones of inhibition recorded against *E. coli* using the ethanolic extracts (Repeated in Triplicate).

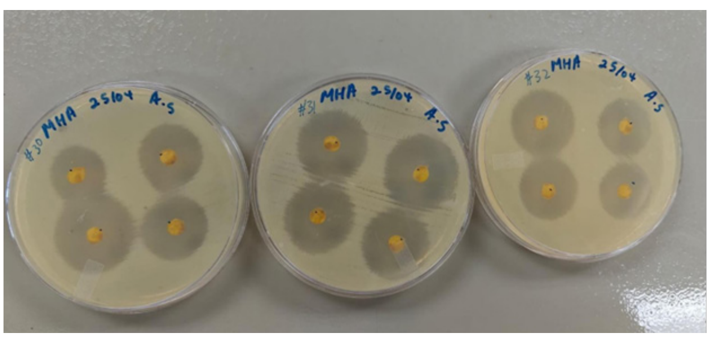


Figure 3: Zones of Inhibition recorded against *E. coli* using the hexane extracts (Repeated In Triplicate).

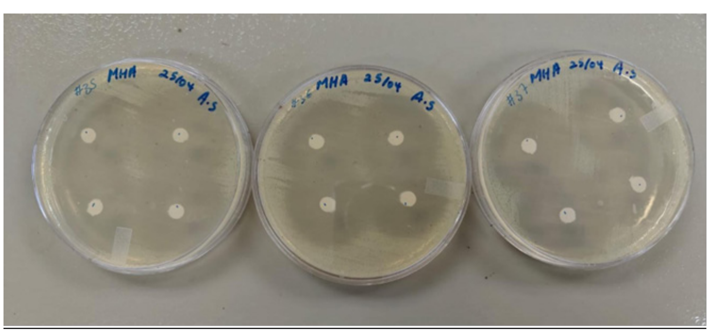


Figure 4: Zones of Inhibition recorded against *P. Mirabilis* using the ethanolic extracts (Repeated In Triplicate).

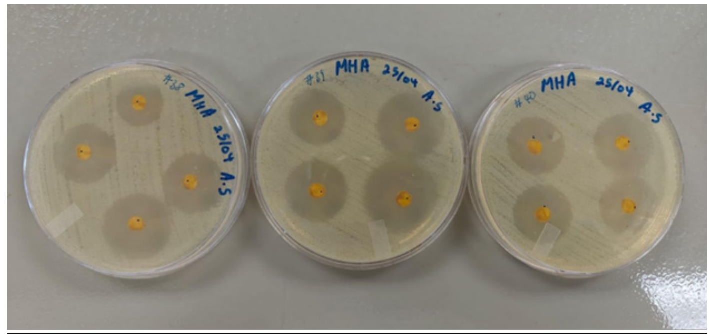
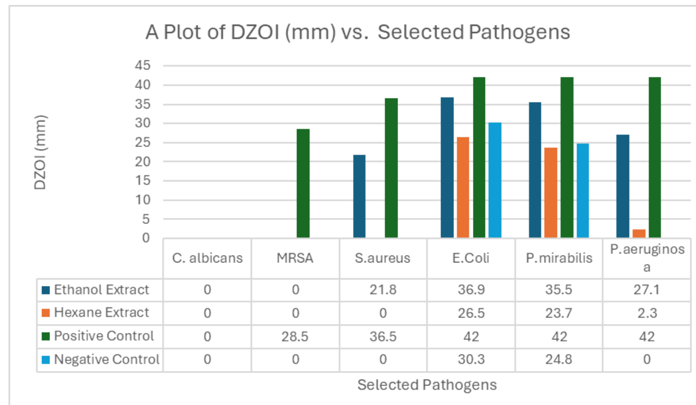


Figure 5: Zones of Inhibition recorded against *P. Mirabilis* using the hexane extracts (Repeated In Triplicate).



ANOVA Analyses

Table 5: Comparison of DZOI of Ethanolic extract vs DZOI of Hexane Extract towards pathogens.

ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Rows	1914.697	5	382.9393	6.978097	0.02626	5.050329
Columns	394.4533	1	394.4533	7.18791	0.043766	6.607891
Error	274.3867	5	54.87733			
Total	2583.537	11				

Table 6: Comparison of DZOI of Ethanolic extract vs DZOI of Positive Control.

ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Rows	1616.844	5	323.3688	2.943226	0.130582	5.050329
Columns	1598.521	1	1598.521	14.54936	0.012445	6.607891
Error	549.3442	5	109.8688			
Total	3764.709	11				

Table 7: Comparison of DZOI of Hexane extract vs DZOI of Positive Control.

ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Rows	2481.098	5	496.2195	9.750282	0.012934	5.050329
Columns	404.8408	1	404.8408	7.954771	0.03709	6.607891
Error	254.4642	5	50.89283			
Total	3140.403	11				

Discussion

Antimicrobial activity of the hexane and ethanolic extract of *Pontederia cordata* were investigated using the disc diffusion assay. Here the DZOI and AZOI were used as indicators of the plant extract's antimicrobial activity. From Table 1, the largest DZOI of 36.9 ± 1.0 mm was induced against *E.coli* and least numerical DZOI of 21.8 ± 8.8 mm against *S. aureus*. Zero DZOI was induced against *C. albicans* & MRSA. Figure 2 and Figure 4, show the Zone of Inhibition, DZOI, induced by the ethanolic and hexane extract of *P. cordata* against *E.coli* and *P. mirabilis* respectively. The order of antimicrobial susceptibility follow the sequence: *E.coli > P.mirabilis > P. aeruginosa > S. aureus > MRSA = C. albicans*. Compared to the positive control, Ciprofloxacin, the antimicrobial potency against *E.coli*, *P. mirabilis* and *P. aeruginosa* showed a relative % inhibition of 87.9, 84.6 and 65% respectively.

Table 2 shows the antimicrobial activity of the hexane extract of *P. cordata* against the selected pathogen. Again, the largest DZOI of 26.5 ± 3.3 mm was induced against *E.coli* and the lowest numerical DZOI of 2.3 ± 2.0 was induced against *P. aeruginosa*. The order of antimicrobial susceptibility follow the sequence: 26.5 ± 3.3 > 23.7± 1.5 > 2.3 ± 2.0. Zero DZOI were induced against *C. albicans, MRSA & S. aureus.* Compared to the positive control, Ciprofloxacin, the antimicrobial potency against *E.coli*, *P. mirabilis* and *P. aeruginosa* showed a relative % inhibition of 63.1, 66.8 and 5.5% respectively. Thus, the antimicrobial potency of the ethanolic extract was greater than that of the hexane extract. The antimicrobial activity of the positive control Ciprofloxacin showed higher DZOI compared to the hexane and ethanolic extract. The relative % inhibition, compared to the ethanolic and hexane extract are shown in Table 1, Table 2, Table 3 and Table 4 show the antimicrobial activity of the positive and negative control. Figure 3 and Figure 5 show the DZOI induced by the hexane extract of *P. cordata* against *E.coli* and *P. mirabilis*.

ANOVA analysis were done to see whether there is any significant difference with regards to:

i. DZOI of ethanolic extract vs. DZOI of hexane extract
ii. DZOI of ethanolic extract vs. Positive control
iii. DZOI of hexane extract vs. Positive control

According to the ANOVA data shown in Table 5, there is significant difference in DZOI, as p value = 0.026 is less than 0.05 and $F_{value} > F_{crital}$. This conforms that the DZOI of ethanolic extract are greater than those of hexane extract and that the ethanol extract is more potent than the hexane extract. Table 6 gives a comparison of the DZOI of ethanolic extract vs. Positive control. As can be seen, the P_{value} = 0.135 is less than 0.05 and F_{value} [2.94] is < $F_{critical}$ (5.05). This confirms that the ethanolic extract is as potent as the positive control. Table 7 shows the DZOI for the hexane extract vs. DZOI of positive control. As can be seen, the P-value = 0.012934 is < 0.05, indicating significance difference and that the hexane extract is not as potent as the ethanolic extract against the pathogens.

Conclusion

The antimicrobial activity of the ethanolic and hexane extract of *Pontederia cordata* against selected pathogens were investigated using the Disc Diffusion Assay. For both ethanolic and hexane extracts, the most susceptible pathogen was *E.coli* and least *P. aeruginosa* and *S. aureus* for ethanolic and hexane extract respectively. Compared to the positive control, Ciprofloxacin, the antimicrobial susceptibility for *E.coli* was registered at 87.86% and 63.1% for the ethanolic and hexane extract respectively. Likewise, the antimicrobial susceptibility for *P. mirabilis* was registered at 84.5% and 66.71% respectively. Thus, the ethanolic and hexane extract of *Pontederia cordata* can be used to treat topically *E.coli* and *P. mirabilis* infections.

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