# Evaluation of Antibacterial Activity of Different Extract of Ipomoea aquatic Leaves against Staphylococcus aureus and Streptococcus pyogenes

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Abstract: The skin is the largest organ in the body and can be vulnerable to various microbial infection. Although antibiotics are clinically proven to be useful in the treatment of bacterial skin infections, they are largely subjected to antibiotic resistance and adverse effects. This has led to the screening of several medicinal plants for their potential antimicrobial activity since they are less expensive, has reduced occurrence of adverse effects and widespread availability. The aim of this research will focus on evaluating the antibacterial activity of different extracts of Ipomoea aquatica leaves against Staphylococcus aureus and Streptococcus pyogenes that causes skin infections. Leaves were extracted separately with 95% methanol and 95% ethanol using maceration process. Phytochemical screening was done for each extract and the minimum inhibitory concentration (MIC) was determined for each extract against both bacteria using 10 different concentrations ranging from 10mg/ml up to 100mg/ml via disc diffusion method in triplicates. Two concentrations above the MIC from each extract were selected and antibacterial assay of the different extracts against the two bacteria respectively was performed using disc diffusion method in triplicates. MIC for methanolic extract against both bacteria was 10mg/ml, while MIC for ethanolic extract was 10mg/ml against Staphylococcus aureus and 30mg/ml against Streptococcus pyogenes. Methanolic extract of the plant at a concentration of 90mg/ml and 100mg/ml was statistically significant against Streptococcus pyogenes with a significance value of 0.00 ($p<0.05$), with 100mg/ml having larger mean inhibition zone of 17.00mm ± 0.00mm than 90mg/ml (14.33mm ± 0.58mm). Statistical analysis was performed using one-way ANOVA (Tukey's Test). Both methanolic and ethanolic extract of the leaves has positive antibacterial activity against both Staphylococcus aureus and Streptococcus pyogenes at different concentrations.

## 1 INTRODUCTION

The skin is the largest organ in the body and acts as a static, stationary or inert wrapping for the body. Large numbers of microorganisms are present in the various components of the skin. For example, the number of bacteria on the skin surface can range from 1000 organisms per square centimetre to more than 10 million. The principal members of the normal skin flora are *Diphtheroids, Staphylococci* and Fungi (Hall, 2001). Skin infections are clinical entities comprising of many etiology, manifestations and severity that involves microbial invasion of the layers of the skin (Ki and Rotstein, 2008). Many types of bacteria can cause infection to the skin, in which the most common ones are *Staphylococcus* and *Streptococcus*.

According to CDC, *Staphylococcus aureus* (*S. aureus*) is a type of bacteria that about 30% of people carry in their noses and often found on the surface of the skin. It is usually harmless, but invasive staphylococcus infections can lead to life threatening medical complications in as little as 12 hours (Dr. . Tom Frieden, MD, 2013). On the other hand, *Streptococcus pyogenes* (*S. pyogenes*) can cause a variety of diseases in immunocompetent individuals, from pharyngo-tonsillitis to life-threatening invasive diseases, such as streptococcal



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toxic shock syndrome, and rapidly progressing deep-tissue infections, such as necrotizing fasciitis. (Johansson *et al.*, 2010).

Although antibiotics have been clinically proven to be useful in the treatment of bacterial skin infections, they are largely subjected to limitations such as antibiotic resistance and adverse effects. The progressing failure of chemotherapeutics and resistance to antibiotics has led to the screening of several medicinal plants for their potential antimicrobial activity (Oyewole and Kalejaiye, 2012). Unlike conventional medicines or treatments, herbal treatments have several advantages in that they are less expensive, more effective in certain chronic conditions, has reduced occurrence of adverse effects as well as widespread availability.

In this regard, one of the plant which is being evaluated for its therapeutic efficacies is *Ipomoea aquatica* (*I. aquatica*). In the ancient science of Indian medicine and homeopathy, extracts of *I. aquatica* leaves are administered orally to alleviate antioxidant related disorders.

The plant is also used effectively against nosebleed and high blood pressure. Furthermore, its leaf extract can be used to reduce blood sugar levels and as an antibiotic against *Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis*. The floral buds are used as an anthelmintic (Prasad *et al.*, 2005).

Water spinach is a perennial herb found throughout India, Sri Lanka, Tropical Asian countries, Africa and Australia. It is grown as weed in India and USA, while in Malaysia, China, Singapore and Hong Kong, it is grown commercially (Mbatchou and Dawda, 2012). It is also known with its common name which is swamp morning glory or 'kangkung' in Malaysia. *I. aquatica*, a green leafy vegetable which is a rich source of amino acids and vitamins, has been explored for the isolation and identification of its bioactive compounds that provides many health benefits. The leaves of *I. aquatica* contains 90% moisture, 4.3% carbohydrates, 3% protein, 2% mineral matter, 0.9% fibre, 0.4% fat, 0.6mg/100g of nicotinic acid, 120mg/100g of riboflavin, 137mg/100g of Vitamin C and 11mg/100g of Vitamin E (Mbatchou and Dawda, 2012).

Plants are potential sources of natural bioactive compounds such as primary and secondary metabolites. Flavonoids are one of the secondary metabolites produced by plants and are present in most plant tissues and often in vacuoles. The basic structures of flavonoid molecules are composed of three rings with various substitutions, including glycosylation, hydrogenation, hydroxylation, methylation and sulfation. Flavonoids are hydroxylated phenolic substances known to be synthetized by plants in response to microbial infection and they have been found to act as antimicrobial agents against a wide array of microorganisms *in vitro*. Their activity is probably due to their ability to complex with extracellular and soluble protein and to complex with bacterial cell wall (Yadav and Agarwala, 2011). The type of flavonoids present in *I. aquatica* leaves are quercetin 3-methyl ether and quercetin 4-methyl ether. Therefore, the aim of this research will focus on evaluating the antibacterial activity of different extracts of *I. aquatica* leaves against *S. aureus* and *S. pyogenes* that causes skin infections.

# 2 METHODOLOGY

## 2.1 Preparation of Plant Extract using Maceration Process

2kg of fresh leaves of *I. aquatica* obtained was first weighed and washed thoroughly using running tap water to remove all adhering foreign materials and soil particles. The leaves were then dried under shade and sun for seven days. The amount of dried leaves was weighed again in order to calculate the percentage of moisture content in the plant. The weight obtained was 101.27g. After that, the dried leaves were coarsely powdered using a mechanical blender. The amount obtained was weighed and equally separated into two portions. One part (50g) was macerated with 95% ethanol and the other part (50g) with 95% methanol.

Both was allowed to stand at room temperature for 7 days with occasional shaking. The final extracts obtained was clarified by filtration using filter papers. The filtrates were then concentrated under vacuum in a rotary evaporator in order to remove the solvent and obtain a solid mass. The solid mass of both methanolic and ethanolic extracts was weighed and the percentage yield of the plant obtained after extraction was calculated.

## 2.2 Phytochemical Screening of Leaf Extracts

Both methanolic and ethanolic extracts of *I. aquatica* leaves were evaluated for qualitative determination of primary and secondary metabolites by preliminary phytochemical screening respectively

(Yadav and Agarwala, 2011). The tests done for the presence primary metabolites were Molisch's Test for the presence of carbohydrates, Millon's Test for the presence of proteins, Ninhydrin Test for the presence of amino acids and Filter Paper Test for the presence of fats and oil. The tests done to detect secondary metabolites were Alkaline Reagent Test for the presence of flavonoids, Liebermann's Test for the presence of glycosides, Mayer's Test for the presence of alkaloids, Foam Test for the presence of saponins, Salkowski's Test for the presence of steroids as well as Ferric Chloride Test for the presence of phenols and tannins.

### 2.3 Determination of Minimum Inhibitory Concentration (MIC) using Disc Diffusion Method

The minimum inhibitory concentration was determined for both methanolic and ethanolic extracts of *I. aquatica* leaves against both *S. aureus* and *S. pyogenes* respectively. This was done by preparing different concentrations of each extract in w/v (100mg/ml, 90mg/ml, 80mg/ml, 70mg/ml, 60mg/ml, 50m/ml, 40mg/ml, 30mg/ml, 20mg/ml and 10mg/ml). Empty sterile discs were impregnated in each concentration of the extracts for a sufficient time and then they were placed on agar plates that has been inoculated with the selected bacteria strains. The control group for this assay was empty sterile discs that has been impregnated in distilled water. The plates were then incubated at $37^0$C for 24 hours in an incubator. After 24 hours, each plate was observed and the zone of inhibition of each sample was measured and recorded. MIC was determined by observing the lowest concentration of plant extract that was able to inhibit the bacteria growth. From this, a suitable concentration of the plant extract was used for Antibacterial Assay using Disc Diffusion method for both methanolic and ethanolic extracts of *I. aquatica* leaves.

### 2.4 Antibacterial Assay using Disc Diffusion Method

**Strains:** Strains of *S. aureus* and *S. pyogenes*
**Medium:** Mueller Hinton Agar (MHA) for *S. aureus*
: Nutrient Agar (NA) for *S. pyogenes*
**Samples:** Concentrations of 30% and 40% of methanolic extract of *I. aquatica* leaves against *S. aureus* and concentrations of 90% and 100% against *S. pyogenes*.
Concentrations of 70% and 90% of ethanolic extract of *I. aquatica* leaves against *S. aureus* and concentrations of 80% and 90% against *S. pyogenes*.
**Positive control (Antibiotic):** Vancomycin disc (30mcg/disc)
**Negative control (Solvent):** Distilled water
The antibacterial activity of *I. aquatica* leaves were tested using Disc Diffusion method. A suitable concentration of both methanolic and ethanolic extracts of *I. aquatica* leaves as stated above were prepared respectively, in which sterile paper discs were impregnated for a sufficient time. The positive control, Vancomycin discs (30mcg/disc) kept in refrigerator was taken out and left to cool to room temperature before use.

The negative control, distilled water was prepared and empty sterile discs were impregnated in them respectively. *S. aureus* and *S. pyogenes* was inoculated on the agar plates that were prepared and stored earlier, respectively.

The discs impregnated in methanolic extract, ethanolic extract, distilled water as well as Vancomycin discs was then placed on each agar plates with appropriate distance between each disc. All the plates were incubated in an incubator at $37^0$C for 24 hours. After incubation, the agar plates were observed and the diameter of zone of inhibition of each and every agent and disc used was measured. These procedures were performed in triplicates in order to obtain the mean and standard deviation (n=3, mean ± standard deviation) zone of inhibition for each agent used.

### 2.5 Statistical Analysis

The results obtained were statistically analysed using One-Way Analysis of Variance (ANOVA) and Tukey's Test via Statistical Package for the Social Science (SPSS) software. ANOVA was followed by Tukey's Test for control, standard and test group comparisons for statistical evaluation. *p* value less than 0.05 was considered statistically significant.

## 3 RESULTS AND DISCUSSION

The moisture content of *I. aquatica* leaves used in this research was 94.95%, while its percentage yield obtained after extraction with methanol and ethanol was 89.16% and 96.80% respectively. Maceration of the leaves with methanol produced more amount of extract compared to ethanol.

Percentage of Moisture Content (MC) in *I. aquatica* leaves

% Moisture Content = Initial Weight (IW) – Dried Weight (DW) X 100%

Initial Weight (IW)

= 2000g – 100.91g X 100%
2000g

= 94.95%

Percentage of Yield obtained after Extraction of *I. aquatica* leaves

Methanolic Extract of *I. aquatica* leaves

% Yield after Extraction = Initial Weight (IW) – Final Weight (FW) X 100%

Initial Weight (IW)

= 50.0g – 5.42g X 100%
50.0g

= 89.16%

Ethanolic Extract of *I. aquatica* leaves

% Yield after Extraction = Initial Weight (IW) – Final Weight (FW) X 100%

Initial Weight (IW)

= 50.0g – 1.60g X 100%
50.0g

= 96.80%

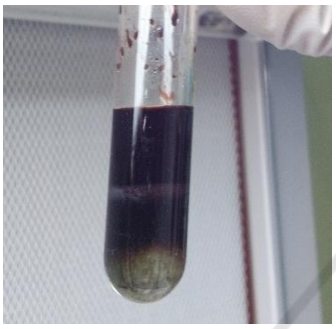
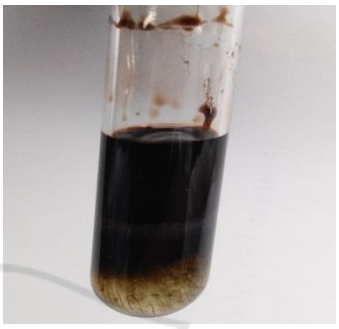
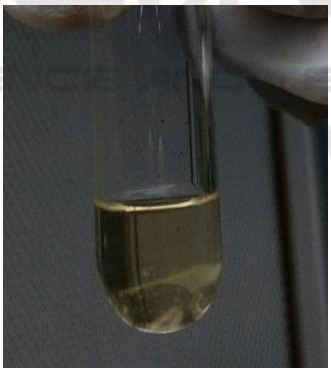
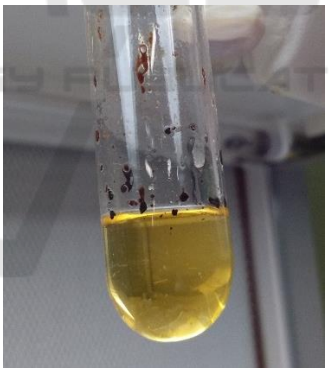
Both methanolic and ethanolic extracts of *I. aquatica* leaves were tested for the presence of primary and secondary metabolites respectively. According to the results, methanolic extract of the leaves contained carbohydrates, amino acids, flavonoids, glycosides, alkaloids, saponins, steroids, phenols as well as tannins. Meanwhile, the ethanolic extract of the leaves showed positive results for the presence of carbohydrates, proteins, amino acids, flavonoids, glycosides, alkaloids, saponins, steroids, phenols and tannins. According to a study conducted in 2011 by Yadav & Agarwala, it was found that flavonoids were responsible for the antimicrobial activity of *I. aquatica* leaves. Flavonoids are hydroxylated phenolic substances known to be synthetized by plants in response to microbial infection and they have been found to act as antimicrobial agents against a wide array of microorganisms *in vitro*. Their activity is probably due to their ability to complex with extracellular and soluble protein and to complex with bacterial cell wall (Yadav and Agarwala, 2011). Therefore, the presence of flavonoids in both methanolic and ethanolic extracts of *I. aquatica* leaves in this study can be said to be accountable for its antibacterial activity.

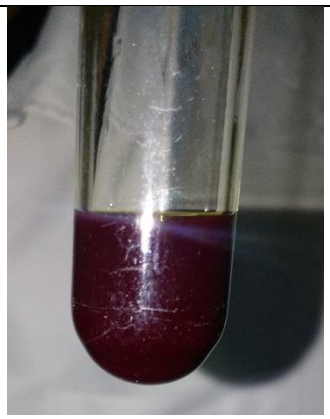
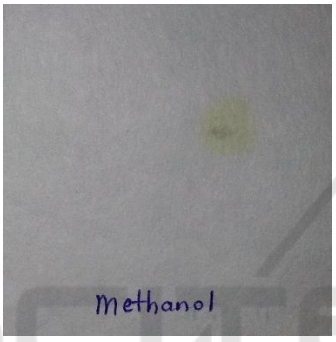
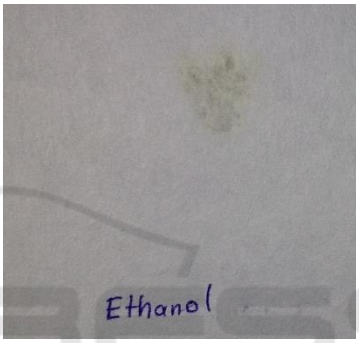
Table 1: Results of phytochemical screening for both methanolic & ethanolic extracts of *I. aquatica* leaves.

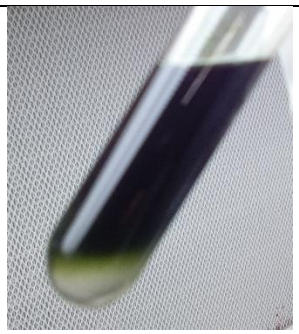
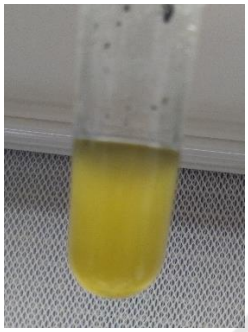
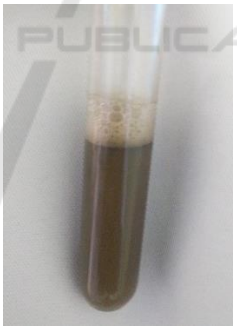
| METABOLITES | TEST | OBSERVATIONS | INTERPRETATION (METHANOLIC EXTRACT) | INTEPRETATION (ETHANOLIC EXTRACT) |
|---|---|---|---|---|
| Carbohydrates | Molisch's Test | Appearance of a violet ring at the interphase. | Present | Present |
| Proteins | Millon's Test | Turning of white precipitate to red upon gentle heating. | Absent | Present |
| Amino Acids | Ninhydrin Test | Appearance of violet colour. | Present | Present |
| Fats & Oil | Filter Paper Test | No permanent staining of the filter paper. | Absent | Absent |
| Flavonoids | Alkaline Reagent Test | Intense yellow changed to colourless. | Present | Present |
| Glycosides | Liebermann's Test | Colour changed from violet to blue / green. | Present | Present |
| Alkaloids | Mayer's Test | Formation of yellow coloured precipitate. | Present | Present |
| Saponins | Foam Test | Formation of stable foam. | Present | Present |
| Phenols & | Ferric Chloride | Appearance of blue- | Present | Present |

| | | | | |
|---|---|---|---|---|
| Tannins | Test | black coloration. | | |
| Steroids | Salkowski's Test | Red colour produced in the lower chloroform layer. | Present | Present |

According to Shamli *et al* 2015, phytochemical analysis of acetone and petroleum ether extract of I.aquatica showed that proteins, carbohydrates, tannins, phenols and terpenoids were present in both exctarct and glycoside and flavonoids were absent in petroleum ether extract. Whereas, Steroids, alkaloids and saponin were absent in both extract (Shamli, Chandra and Nadu, 2015).

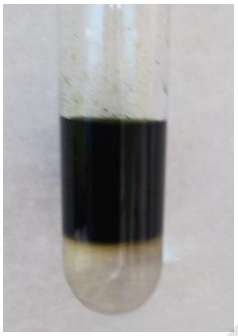
| **Methanolic Extract of *I. aquatica* Leaves** | **Ethanolic Extract of *I. aquatica* Leaves** |
|---|---|
|  Molisch's Test for Carbohydrate |  Molisch's Test for Carbohydrate |
|  Millon's Test for Protein |  Millon's Test for Protein |
| | |

| | |
|---|---|
|  <br>Ninhydrin Test for Amino Acids |  <br>Ninhydrin Test for Amino Acids |
|  <br>Filter Paper Test for Fats & Oil |  <br>Filter Paper Test for Fats & Oil |
|  <br>Alkaline Reagent Test for Flavonoids |  <br>Alkaline Reagent Test for Flavonoids |
| | |

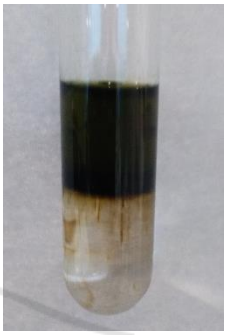
| | |
|---|---|
|  <br>Liebermann's Test for Glycosides |  <br>Liebermann's Test for Glycosides |
|  <br>Mayer's Test for Alkaloids |  <br>Mayer's Test for Alkaloids |
|  <br>Foam Test for Saponins |  <br>Foam Test for Saponins |
| | |

Ferric Chloride Test for Phenols &Tannins

Ferric Chloride Test for Phenols &Tannins



Salkowski's Test for Steroids



Salkowski's Test for Steroids

Figure 1: Results of Phytochemical Screening

The determination of MIC for both methanolic and ethanolic extracts of *I. aquatica* leaves were determined using ten different concentrations of both extracts, ranging from 10mg/ml up to 100mg/ml via Disc Diffusion method, which was done in triplicates (Table 2).

As for methanolic extract of the leaves, the lowest concentration that showed and were able to inhibit the growth of *S. aureus* was 10mg/ml with a mean zone of inhibition of 6.00mm (n=3, mean), while for *S. pyogenes*, the MIC was also 10mg/ml, but with a mean zone of inhibition of 4.00mm (n=3, mean).

On the other hand, as for ethanolic extract of the leaves, the lowest concentration that showed and were able to inhibit the growth of *S. aureus* was 10mg/ml with a mean zone of inhibition of 3.33mm (n=3, mean), while for *S. pyogenes*, the MIC was 30mg/ml, with a mean zone of inhibition of 4.00mm (n=3, mean). After the determination of MIC for both methanolic and ethanolic extracts of the leaves, two concentrations above the MIC with the highest mean value were chosen to be tested for antibacterial activity against *S. aureus* and *S. pyogenes* respectively.

Table 2: Minimum Inhibitory Concentration (MIC) for methanolic and ethanolic extracts of *I. aquatica* leaves after tested against *S. aureus* and *S. pyogenes* respectively.

| Concentration (mg/ml) | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | (-) Control |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Extract / Bacteria | Mean Zone of Inhibition (mm) | | | | | | | | | | |
| Methanolic Extract | | | | | | | | | | | |
| *S. aureus* | 6.00 | 4.67 | 6.33 | 6.33 | 5.67 | 5.67 | 0.00 | 3.33 | 0.00 | 0.00 | 0.00 |
| *S. pyogenes* | 4.00 | 5.33 | 6.00 | 6.00 | 6.67 | 5.33 | 6.33 | 3.00 | 9.33 | 9.00 | 0.00 |

| Ethanolic Extract | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *S. aureus* | 3.33 | 2.00 | 6.33 | 6.67 | 6.00 | 6.00 | 7.67 | 5.67 | 6.67 | 5.33 | 0.00 |
| *S. pyogenes* | 0.00 | 0.00 | 4.00 | 7.33 | 5.00 | 7.00 | 5.67 | 9.67 | 10.67 | 9.33 | 0.00 |

From the determination of MIC for methanolic extract of *I. aquatica* leaves, concentrations of 30mg/ml and 40mg/ml of the extract were observed to have the highest mean values for the zone of inhibition of the growth of *S. aureus*, and therefore they were chosen to perform antibacterial testing against *S. aureus* using disc diffusion method. The results obtained showed that both concentrations had antibacterial activity against *S. aureus* with a mean and standard deviation of 7.33mm ± 0.58mm (n=3, mean ± standard deviation). According to one-way ANOVA test, both the concentrations did not show significant effects against *S. aureus* since the *p* value was 1.00 ($p > 0.05$) when compared to the positive control, Vancomycin (30mcg/disc) which had a mean and standard deviation of 23.67mm ± 1.15mm (n=3, mean ± standard deviation), with a *p* value of 0.00 ($p < 0.05$) (Table 4).

One-way ANOVA using Post-hoc Tukey's test was performed in order to evaluate the significance of antibacterial activity of the concentrations obtained from both methanolic and ethanolic extracts of *I. aquatica* leaves against the test bacteria (*S. aureus* and *S. pyogenes*). Based on the interpretation from ANOVA test, *p* values lower than 0.005 ($p<0.05$) were considered significant for antibacterial activity and vice versa.

On the other hand, concentrations of 90mg/ml and 100mg/ml of methanolic extract of *I. aquatica* leaves were prepared to test its antibacterial activity against *S. pyogenes*. The results obtained showed that the extract of 100mg/ml had larger zone of inhibition, with a mean and standard deviation of 17.00mm ± 0.00mm (n=3, mean ± SD) than 90mg/ml of extract.

At the same time, comparison between each group using ANOVA test (Table 5) showed that each group had significant effects against *S. pyogenes*, since the significance value was 0.00 ($p < 0.05$), in which it can be said that 100mg/ml of the extract has higher significant antibacterial effect than 90mg/ml of the extract since 100mg/ml has higher mean zone of inhibition against *S. pyogenes*.

For ethanolic extract of *I. aquatica* leaves, the concentrations of the extract used were 70mg/ml and 90mg/ml in order to test the susceptibility of *S. aureus* towards the extract. The higher concentration (90mg/ml) of the extract had larger zone of inhibition compared to 70mg/ml of the extract. However, there were no significant difference between the two concentrations against *S. aureus* according to ANOVA test (Table 6). This is because the *p* value for each concentration when compared to one another was 0.754 ($p > 0.05$).

Meanwhile, the concentrations of 80mg/ml and 90mg/ml of ethanolic extract was used to test its antibacterial activity against *S. pyogenes*. Clearly, the higher concentration of the extract had bigger zone of inhibition with a mean and standard deviation of 7.67mm ± 0.58mm (n=3, mean ± standard deviation) compared to 80mg/ml of the extract. Nonetheless, ANOVA test showed that there was no significant difference in the antibacterial activity between the two concentrations against *S. pyogenes* since the *p* value was 0.754 ($p > 0.05$) when both concentrations were compared to each other (Table 7).

When we compare the antibacterial activity between methanolic and ethanolic extract of *I. aquatica* leaves against *S. aureus* and *S. pyogenes*, it can be said that both extract require different concentrations in order to inhibit the bacteria's growth (Figure 1 and 2). However, when we compare the antibacterial activity of methanolic and ethanolic extract of *I. auatica* to the standard positive control (Vancomycin); both methanolic and ethanolic extract of *I. aquatica* got low antibacterial activity than vancomycin.

Table 3: Mean and SD of zone of inhibition for methanolic and ethanolic extracts of *I. aquatica* leaves and controls after tested against *S. aureus* and *S. pyogenes* respectively.

| Bacteria / Concentration of Extract (mg/ml) | Mean Zone of Inhibition (mm) ± Standard Deviation (SD)(mm) | | |
|---|---|---|---|
| | Sample Extract | (-) Control | (+) Control (Vancomycin 30mcg/disc) |
| Methanolic Extract | | | |
| *S. aureus* / 30 | 7.3 ± 0.6 | 0.0 ± 0.0 | 23.7 ± 1.2 |
| *S. aureus* / 40 | 7.3 ± 0.6 | 0.0 ± 0.0 | 23.7 ± 1.2 |
| *S. pyogenes* / 90 | 14.3 ± 0.6 | 0.0 ± 0.0 | 35.0 ± 0.0 |
| *S. pyogenes* / 100 | 17.0 ± 0.0 | 0.0 ± 0.0 | 35.0 ± 0.0 |
| Ethanolic Extract | | | |
| *S. aureus* / 70 | 5.0 ± 0.0 | 0.0 ± 0.0 | 24.3 ± 1.2 |
| *S. aureus* / 90 | 5.7 ± 1.2 | 0.0 ± 0.0 | 24.3 ± 1.2 |
| *S. pyogenes* / 80 | 7.3 ± 0.6 | 0.0 ± 0.0 | 30.0 ± 0.0 |
| *S. pyogenes* / 90 | 7.7 ± 0.6 | 0.0 ± 0.0 | 30.0 ± 0.0 |

Table 4: Significance of each methanolic extract of *I. aquatica* Leaves compared to one another against *S. aureus* according to their zone of inhibition.

**Multiple Comparisons**

Dependent Variable: zon

Tukey HSD

| (I) anti | (J) anti | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| 30 | 40 | .000 | .577 | 1.000 | -1.85 | 1.85 |
| | control | -16.333* | .577 | .000 | -18.18 | -14.48 |
| | H20 | 7.333* | .577 | .000 | 5.48 | 9.18 |
| 40 | 30 | .000 | .577 | 1.000 | -1.85 | 1.85 |
| | control | -16.333* | .577 | .000 | -18.18 | -14.48 |
| | H20 | 7.333* | .577 | .000 | 5.48 | 9.18 |
| control | 30 | 16.333* | .577 | .000 | 14.48 | 18.18 |
| | 40 | 16.333* | .577 | .000 | 14.48 | 18.18 |
| | H20 | 23.667* | .577 | .000 | 21.82 | 25.52 |
| H20 | 30 | -7.333* | .577 | .000 | -9.18 | -5.48 |
| | 40 | -7.333* | .577 | .000 | -9.18 | -5.48 |
| | control | -23.667* | .577 | .000 | -25.52 | -21.82 |

*. The mean difference is significant at the 0.05 level.

Table 5: Significance of each methanolic extract of I. aquatica Leaves compared to one another against S. pyogenes

Dependent Variable: zon

Tukey HSD

| (I) anti | (J) anti | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| 90 | 100 | -2.667* | .236 | .000 | -3.42 | -1.91 |
| | control | -20.667* | .236 | .000 | -21.42 | -19.91 |
| | H20 | 14.333* | .236 | .000 | 13.58 | 15.09 |
| 100 | 90 | 2.667* | .236 | .000 | 1.91 | 3.42 |
| | control | -18.000* | .236 | .000 | -18.75 | -17.25 |
| | H20 | 17.000* | .236 | .000 | 16.25 | 17.75 |
| control | 90 | 20.667* | .236 | .000 | 19.91 | 21.42 |
| | 100 | 18.000* | .236 | .000 | 17.25 | 18.75 |
| | H20 | 35.000* | .236 | .000 | 34.25 | 35.75 |
| H20 | 90 | -14.333* | .236 | .000 | -15.09 | -13.58 |
| | 100 | -17.000* | .236 | .000 | -17.75 | -16.25 |
| | control | -35.000* | .236 | .000 | -35.75 | -34.25 |

*. The mean difference is significant at the 0.05 level.

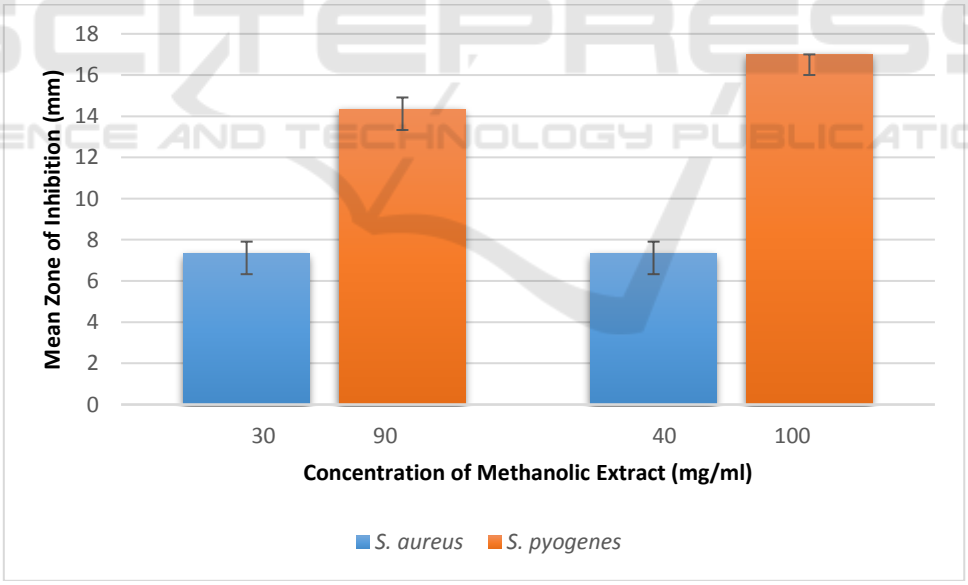



Figure 2: Evaluation of the mean zone of inhibition exhibited by different concentrations of methanolic extract of *I. aquatica* leaves against both *S. aureus* and *S. pyogenes.*

Table 6: Significance of each Ethanolic extract of *I. aquatica* Leaves compared to one another against *S. aureus* according to their zone of inhibition.

Dependent Variable: zon

Tukey HSD

| (I) anti | (J) anti | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| 70 | 90 | -.667 | .667 | .754 | -2.80 | 1.47 |
| | control | -19.333* | .667 | .000 | -21.47 | -17.20 |
| | H20 | 5.000* | .667 | .000 | 2.87 | 7.13 |
| 90 | 70 | .667 | .667 | .754 | -1.47 | 2.80 |
| | control | -18.667* | .667 | .000 | -20.80 | -16.53 |
| | H20 | 5.667* | .667 | .000 | 3.53 | 7.80 |
| control | 70 | 19.333* | .667 | .000 | 17.20 | 21.47 |
| | 90 | 18.667* | .667 | .000 | 16.53 | 20.80 |
| | H20 | 24.333* | .667 | .000 | 22.20 | 26.47 |
| H20 | 70 | -5.000* | .667 | .000 | -7.13 | -2.87 |
| | 90 | -5.667* | .667 | .000 | -7.80 | -3.53 |
| | control | -24.333* | .667 | .000 | -26.47 | -22.20 |

*. The mean difference is significant at the 0.05 level.

Table 7: Significance of each Ethanolic extract of *I. aquatica* Leaves compared to one another against *S. pyogenes* according to their zone of inhibition.

Dependent Variable: zon

Tukey HSD

| (I) anti | (J) anti | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| 80 | 90 | -.333 | .333 | .754 | -1.40 | .73 |
| | control | -22.667* | .333 | .000 | -23.73 | -21.60 |
| | H20 | 7.333* | .333 | .000 | 6.27 | 8.40 |
| 90 | 80 | .333 | .333 | .754 | -.73 | 1.40 |
| | control | -22.333* | .333 | .000 | -23.40 | -21.27 |
| | H20 | 7.667* | .333 | .000 | 6.60 | 8.73 |
| control | 80 | 22.667* | .333 | .000 | 21.60 | 23.73 |
| | 90 | 22.333* | .333 | .000 | 21.27 | 23.40 |
| | H20 | 30.000* | .333 | .000 | 28.93 | 31.07 |
| H20 | 80 | -7.333* | .333 | .000 | -8.40 | -6.27 |
| | 90 | -7.667* | .333 | .000 | -8.73 | -6.60 |
| | control | -30.000* | .333 | .000 | -31.07 | -28.93 |

*. The mean difference is significant at the 0.05 level.

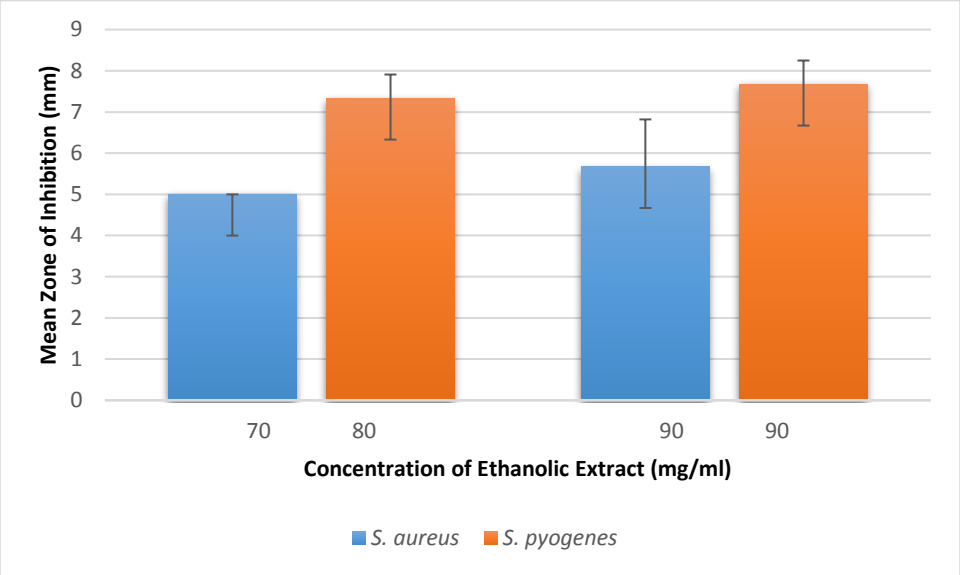



Figure 3: Evaluation of the mean zone of inhibition exhibited by different concentrations of ethanolic extract of *I. aquatica* leaves against both *S. aureus* and *S. pyogenes*.

## 4 CONCLUSION

Based on the conducted study, it can be concluded that both methanolic and ethanolic extracts of *I. aquatica* leaves contain flavonoids that is thought to be responsible for its antimicrobial properties. At the same time, both type of the extracts showed positive antibacterial activity and were effective against both notorious *S. aureus* and *S. pyogenes* that are known to cause skin infections. However, each extract require different concentrations in order to inhibit the bacteria's growth. Further studies such as determination of the total flavonoid content of *I. aquatica* leaves and fractionation of the extract of *I. aquatica* leaves can be carried out in order to isolate the leaves' constituents as well as improving its antibacterial properties. Moreover, future research can be done on developing a formulation using ethanolic extract of *I. aquatica* leaves in order to heal and fight infection on the skin caused by *S. pyogenes*, since in this research, ethanolic extract of the leaves showed a significant effect against the mentioned bacteria.

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## CONFLICT OF INTEREST

The author confirms that there is no conflict of interests.

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