

## Phytochemical study of qualitative, quantitative and antioxidant, antibacterial studies of *ipomoea aquatica*

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### Abstract

The ethanolic extract of *Ipomoea aquatica* leaves was used in phytochemical investigations. The leaves of *Ipomoea aquatica* are extracted using a variety of solvents, including water and ethanol. Phytochemicals are studied using this extract. This phytochemical analysis determines the presence of compounds including flavanoids, saponins, phenols, and terpenoids, as well as antioxidant and antibacterial activity.

**Keywords:** *ipomoea aquatica*, phytochemical study, antioxidant, antibacterial

### Introduction

People and plants have had a long and fruitful relationship. Plants are necessary for human survival. We eat them in a number of ways and use them to make medicines, soaps, furniture, fabrics, and tyres, among other things. Plants play a critical role in our lives. We still rely on plants, despite the fact that we now live in a highly industrialised culture. To preserve this long-term relationship, we must be aware of the function that plants play in our lives and ensure that we take care of them [1]. Plants are the best suppliers of chemical components or phytochemical agents for treating a variety of diseases [2]. Herbal remedies have been utilised to treat a wide range of infectious diseases throughout history. As a result of the widespread use of synthetic antibacterial drugs, drug-resistant microbes are on the rise, putting the therapeutic effectiveness of many existing antibiotics in peril [3, 4]. Higher plants could be a source of new antibiotic prototypes, according to the antibacterial activity of plant extracts and plant products [5].

The Convolvulaceae family's *Ipomoea Aquatica* Forsk is a widely produced green leafy vegetable found throughout India. It's also known as water spinach or water convolvulus, and it's a very invasive plant that forms a mat on the surface of water sources like lakes, ponds, canals, and ditches. It's high in vitamins, minerals, proteins, fibres, carotenes, and flavonoids, which all have a variety of health benefits (Manvar and Desai, 2013) [6, 7]. Ayurveda recommends consuming *ipomoea aquatica* Forsk to treat ailments such as jaundice. The efficacy of these plant items based on traditional claims must be shown to aid in the creation of novel drugs to treat these disorders. It has also been shown to reduce inflammation, making it useful in the treatment of fever, jaundice, biliousness, bronchitis, and liver problems [8]. Purgative effects were revealed in the dried juice, whereas cooling properties were discovered in the leaves and stems. It's also been used to treat neurological and general debility, piles, worm infections, leucoderma, leprosy, jaundice, and liver problems for centuries [9, 10]. In China, this plant was used to treat arsenic and opium poisoning [11].



Fig 1: *Ipomoea aquatica* plant and powder of leaves and stems

## Materials and Methods

### Sample Plant Leaves Collection and Identification

A sample of ipomoea aquatica leaves was gathered near the college campus in Villupuram. These plant leaves were identified by the Department of Chemistry at Theivanai Ammal College for Women in Villupuram, Tamil Nadu, India.

### Preparation of Plant Powder

For 10-15 days, the plant leaves were air dried in the shade. The dried material was then ground into a fine powder and kept in airtight bottles using an electrical grinder. The powder was used for further investigation.

### Preparation of the Ethanolic Extract

The ethanolic extract was made using Indian pharmacopoeia methods like Batch extraction of the leaves powder material was done separately and sequentially with 150ml ethanol and 50ml distilled water. Whatmann filter paper was used to filter these extracts. The extract was then stored in an airtight container <sup>[12]</sup>.

### Preliminary Phytochemical Analysis

The crude material was entirely dissolved in ethanol. This solution was used for the phytochemical analysis, which was subsequently followed by the methodology.

### Test for Flavonoids

Place the sample extract and a few drops of weak NaOH solution in a test tube. In the test tube, the fluid turns yellow. When a yellow solution is mixed with dilute acid in a test tube, the yellow solution turns colourless, suggesting that flavonoids are present.

### Test for Saponins

The crude sample was diluted with distilled water. The solution was stirred by hand for a few minutes, resulting in the formation of a foam layer on the test tube. The existence of a foam layer can be used to detect saponins.

### Test for Tannins

The sample extract is diluted with chloroform and almost 1ml of acetic anhydride is added to a test tube. The sulfuric acid is then added to the side of the test tube carefully. The presence of tannins causes the solution to turn green.

Another test for tannin is to add bromine water to the sample. The decolorization of bromine water indicates the presence of tannins.

### Test for Alkaloids

The substance was warmed and filtered after being treated with HCl. Mayer's reagent (Mercuric chloride + potassium iodide in water) was used to treat this solution. The presence of alkaloids is indicated by the production of a yellow-colored precipitate.

### Test for Phenols

In a test tube, the powdered substance was diluted with distilled water. This solution was heated and filtered before use. When a few drops of Ferric chloride are added, the solution turns blue-black, indicating the presence of phenols.

### Test for Protein

Perform a biuret test in 2mL of test solution. 2 mL sodium hydroxide solution (4%) and a few drops of copper sulphate solution (1%) were added. The violet/pink colour indicates the presence of proteins.

### Test for Carbohydrates

Benedict's interrogation (reducing sugar) 2 mL extract was combined with 2 mL Benedict's reagent and boiled in a boiling water bath for 5 minutes. The appearance of green, yellow, orange, or red to brick red colour proved the presence of reducing sugars.

### *In Vitro* Antioxidant Activity

#### DPPH Radical Scavenging Activity

DPPH (2, 2 – Diphenyl-1-picrylhydrazyl) Method was examined for antioxidant activity for ethanolic extract of Ipomoea Aquatica. To analyze *in vitro* antioxidant activity of a Plant extract, a variety of approaches may be require Standard methods were used to examine the antioxidant potentials of the extract Ipomoea Aquatica. The extract Ipomoea Aquatica concentrations and standard solutions utilized were 100, 50, 25, 12.5, 6.25, 3.125, and 1.56 g mL<sup>-1</sup>. To avoid extract Ipomoea Aquatica cluster, the dilute extract solution was sonicated for 30 minutes at room temperature using a sonicator bath.

The absorbance was compared to the equivalent blank solutions using spectrophotometry <sup>[13]</sup>. The following formula was used to compute the % inhibition:

$$\text{Free radical scavenging activity \%} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100 \quad (1)$$

### Antibacterial Activity

#### Determination of Zone of Inhibition Method

Extract *Ipomoea Aquatica* was tested for antibacterial activity *in vitro*. The agar disc diffusion method was used to test the antibacterial activity of plant component extracts against four pathogenic bacteria (two Gram-positive and two Gram-negative) [13]. Agar cup testing was used to determine antibacterial activity. Purified extracts were dissolved in dimethyl sulfoxide, filtered through a sintered glass filter, and kept at 4°C. To determine the zone of inhibition, pure Gram-positive and Gram-negative bacteria were used as standard antibiotics for comparison. Antibacterial activity of all extracts was tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pyogenes*. In double-distilled water, nutrient agar tubes were used to generate sets of five dilutions (25, 50, 75, and 100 µg/ml) of *Ipomoea Aquatica* extract and standard medicines. Indicator bacterial strains (108 cfu) were planted on Mueller-Hinton sterile agar plates and incubated at 37°C for 3 hours. Control studies were conducted under comparable conditions using ampicillin as a standard antibacterial medication. After 18 to 24 hours of incubation at 37°C for bacteria and 48 to 96 hours at 28°C for fungi, the zones of growth inhibition around the discs were assessed. The diameters of inhibitory zones (including the diameter of the disc) on the agar surface around the microbe species were measured to determine their sensitivity to plant extracts.

### Result and Discussion

**Table 1:** Phytochemical Analysis of *Ipomoea Aquatica*

S.No	Phytochemical Constituents	Ethanol Extract
1.	Alkaloids	+
2.	Saponins	+
3.	Tannins	+
4.	Flavonoids	+
5.	Proteins	+
6.	Phenol	+



**Fig 2:** Phytochemical Test

### Quantitative Phytochemical Analysis

#### Determination of Flavonoids

10g of sample was extracted with 100ml of 80% aqueous ethanol at room temperature. The extract was filtered using whatmann filter paper no.42 the filtrate was transferred into a dish, evaporated to dryness and weighed (okwn and ukanwa, 2007).

$$\% \text{ of flavonoids} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Weight of the sample}} \times 100$$

**Table 2:** Quantitative analysis of Ipomoea Aquatica Leaves

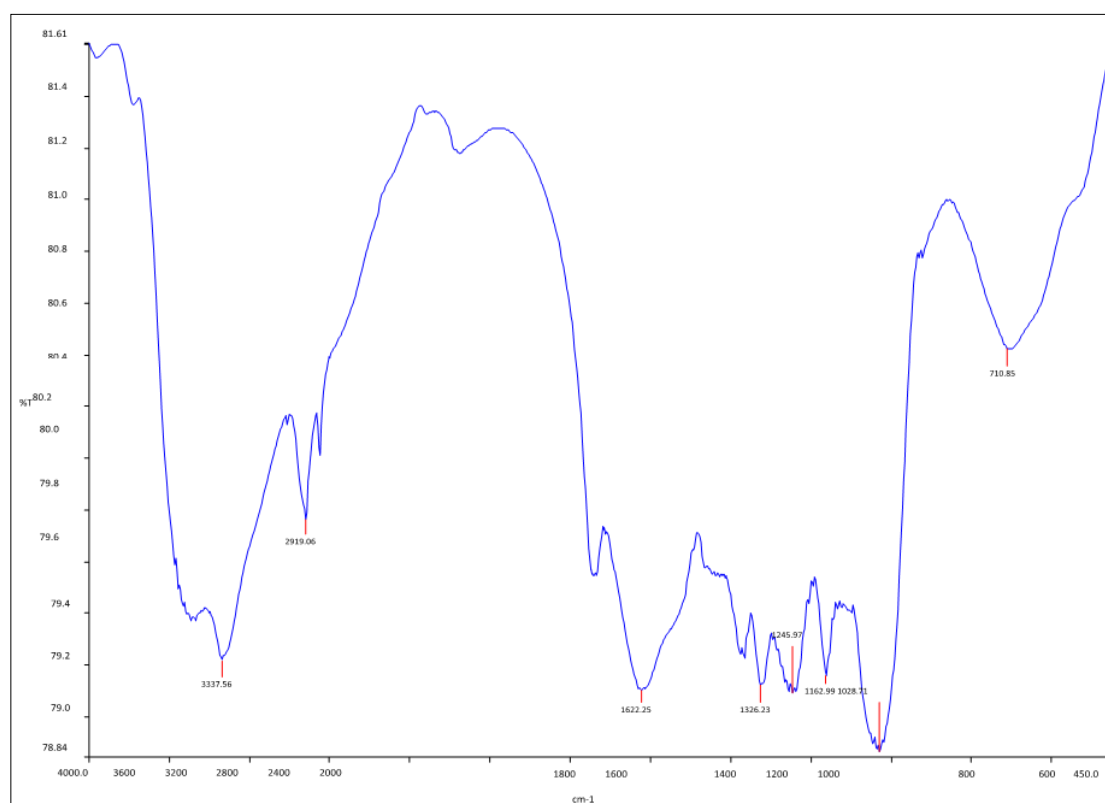
S.NO	Phytochemicals	Ipomoea Aquatica (%)
1	Flavonoids	1.13%

**FT-IR Spectroscopy**

Based on the peak values in the IR radiation band, the FTIR spectrum was utilised to identify the functional group of the active components present in the extract. The functional group of the components was separated based on the peaks ratio when the extract was processed through the FT-IR. The presence of Aliphatic primary amine, Amine salt, Aromatic compound, Sulfone, Amine, Ester, Sulfoxide, and Benzene derivative was confirmed by FT-IR analysis at the peaks of 3337.56 cm<sup>-1</sup>, 2919.06 cm<sup>-1</sup>, 1622.25 cm<sup>-1</sup>, 1326.23 cm<sup>-1</sup>, 1245.97 cm<sup>-1</sup>, 1162.99 cm<sup>-1</sup>, 1028.71 cm<sup>-1</sup>, and 710.85 cm<sup>-1</sup>, respectively. (Table 3).

**Table 3:** FTIR spectral analysis of Ipomoea Aquatica Leaves

Peak value	Group	Interpretation
3337.56	N-H stretching	May be the presence of Aliphatic primary amine
2919.06	N-H stretching	May be the presence of Amine salt
1622.25	C-H bending	May be the presence of Aromatic compound
1326.23	S=O stretching	May be the presence of Sulfone
1245.97	C-N stretching	May be the presence of Amine
1162.99	C-O stretching	May be the presence of Ester
1028.71	S=O stretching	May be the presence of Sulfoxide
710.85	-	May be the presence of Benzene derivative

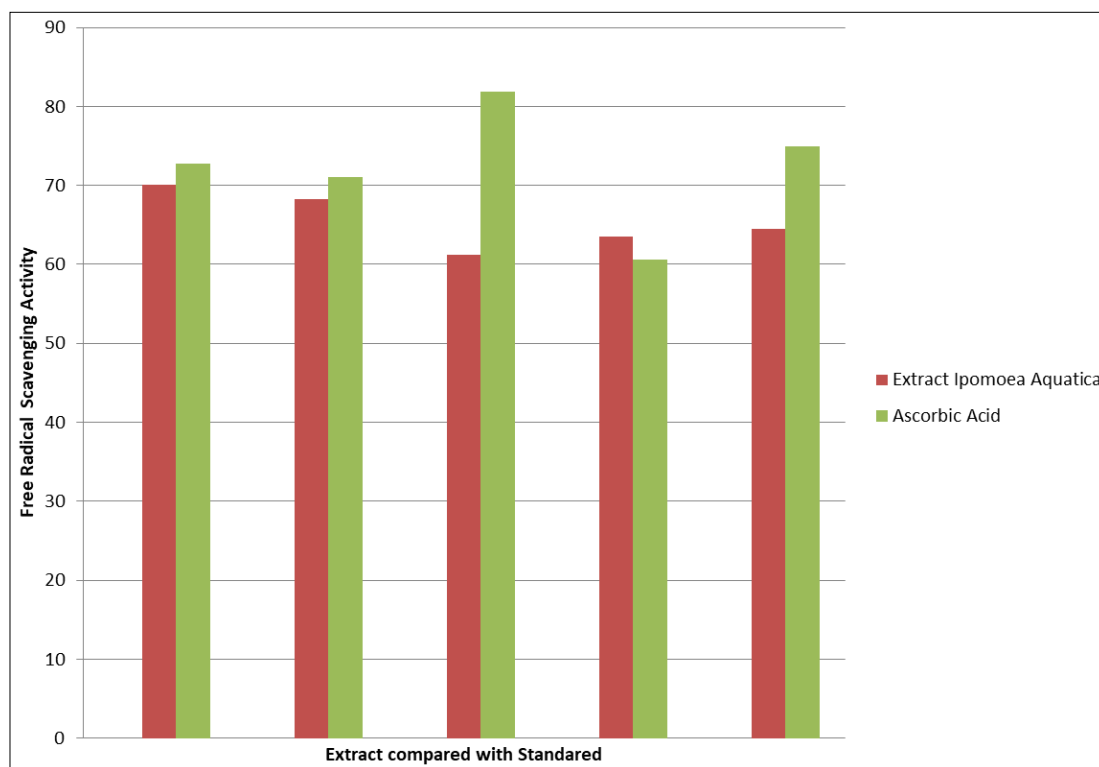
**Fig 3:** IR-Spectroscopy of Ipomoea Aquatica**Antioxidant Activity**

*In vitro* antioxidant activity of the extract Ipomoea Aquatica was estimated DPPH, H<sub>2</sub>O<sub>2</sub>, O<sup>2-</sup>, NO, OH free radical scavenging assays shown in Table 4. The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay is a relatively new one that uses a more powerful, chemically created free radical to screen complex antioxidant mixtures including plant extracts, drinks, and biological fluids. The solubility in both organic and aqueous environments, as well as its stability over a wide pH range, sparked interest in using ABTS<sup>•+</sup> to estimate antioxidant activity. The DPPH-free radical is reduced to a corresponding hydrazine when it reacts with hydrogen donors.

The colour of the DPPH radical is purple and when it reacts with hydrogen donors, it changes to yellow in colour. It's a discoloration test that involves adding the antioxidant to a DPPH solution in ethanol or methanol and measuring the decrease in absorbance at 490 nm <sup>[14]</sup>. Free radical participation, particularly increased generation, appears to be a hallmark of most human diseases, including cardiovascular and cancer diseases. The synthesis of the red dye formazan is inhibited by a superoxide scavenger that can react <sup>[12]</sup> Hydrogen peroxide is produced *in vivo* by several oxidase enzymes. There is mounting evidence that hydrogen peroxide causes serious harm to biological systems, either directly or indirectly through its reduction product, the hydroxyl radical (OH•). Due to the presence of phytochemicals including alkaloids, carbohydrates, flavonoids, gums and mucilage, phenolic compounds, saponins, tannins, and terpenoids the antioxidant activity is higher in the extract of Ipomoea Aquatica. The extract Ipomoea Aquatica chemical method shows the least antioxidant activity with 60%. Based on the above antioxidant results, all the extract Ipomoea Aquatica show significant antioxidant activities compared to standard antioxidants ascorbic acid. The order of the antioxidant activity remains same for all the tested methods.

**Table 4:** Shows the percentage of DPPH radical, Hydrogen peroxide radical, Superoxide radical, Nitric oxide radical and Hydroxyl radical scavenging activity in extract Ipomoea Aquatica extract with standard

Compound	Free radical Scavenging activity (%)				
	DPPH	H <sub>2</sub> O <sub>2</sub>	O <sub>2</sub> -	NO	OH
Extract Ipomoea Aquatica	70.1	68.2	61.2	63.5	64.5
Ascorbic Acid	72.7	71.0	81.9	60.6	74.9



**Fig 4:** *In vitro* antioxidant activity various free radical assay method compared with Standard ascorbic acid

#### H<sub>2</sub>O<sub>2</sub> Radical Scavenging Assay

In this method, when a scavenger is incubated with hydrogen peroxide, the decay or loss of hydrogen peroxide can be measured spectrophotometric ally at 230 nm. The antioxidant activity of extract Ipomoea Aquatica was reinvestigated by H<sub>2</sub>O<sub>2</sub> radical scavenging assay method. Table 4 shows the anti-oxidant activity of extract Ipomoea Aquaticaequal concentrations of 100 NM measured at 230nm with 63% with compared to standard ascorbic acid.

#### Superoxide Radical Scavenging Assay

The antioxidant activity of the Extract Ipomoea Aquaticawas again evaluated by superoxide scavenging assay method. In the present study, superoxide scavenging activity Extract Ipomoea Aquaticaat equal concentration of 100 nM was determined at 560 nm with 61% with compared to standard ascorbic acid. Table 4.

#### Nitric Oxide Radical Scavenging Assay

Nitric oxide radical scavenging assay is yet another method to determine the anti-oxidant behaviors it shows better antioxidant activity with 65% compared to standard ascorbic acid.

### Hydroxyl Radical Scavenging Activity

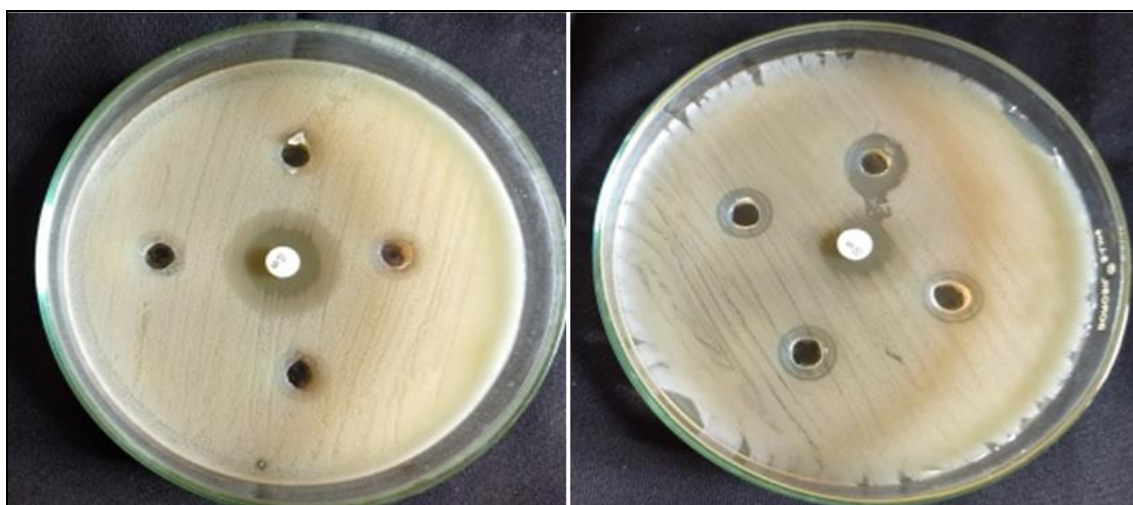
Hydroxyl radical scavenging method is also employed in the present study to determine the antioxidant behavior its shows 65 % free radical scavenging compared with ascorbic acid.

### Antibacterial Activity

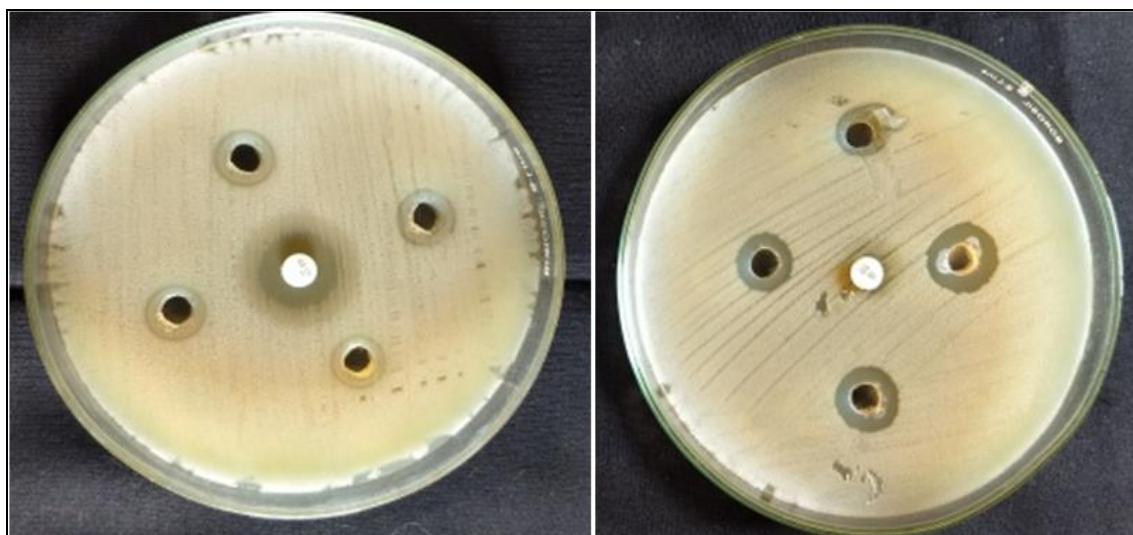
**Table 5:** Zone of inhibition of the extract Ipomoea Aquatica

S. No	Positive and negative Pathogen	Zone of inhibition (diameter in mm)				Standard (ampicillin)
		25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL	
1	<i>Bacillus Subtilis</i>	8	10	12	14	16
2	<i>Staphylococcus aureus</i>	7	9	10	13	15
3	<i>Pseudomonas aeruginosa</i>	9	10	14	16	17
4	<i>Escherichia coli</i>	9	11	14	16	18
5	Control (DMSO)	NI	NI	NI	NI	NI

NI: No Inhibition



**Fig 5:** Antibacterial activity of the Extract Ipomoea Aquatica against positive pathogen *Bacillus Subtilis* and *Staphylococcus aureus*



**Fig 6:** Antibacterial activity of the Extract Ipomoea Aquatica against negative pathogen *Escherichia coli* and *Pseudomonas aeruginosa*.

The antibacterial activity of extracts of Extract Ipomoea Aquatica was investigated in four different concentrations (25, 50, 75, and 100 µg/ml) against four pathogenic bacterial strains, two Gram-positive (*Staphylococcus aureus* MTCC 96, *Streptococcus pyogenes* MTCC 442) and two Gram-negative (*Escherichia coli* MTCC 443, *Pseudomonas* Table 5 shows the results of the antibacterial activity.

The antibacterial activity of the extracts rose linearly as the concentration (g/ml) was raised. The results showed that *Bacillus Subtilis* and *Staphylococcus aureus* were more sensitive to extracts for bacterial activity than *Escherichia coli* and *Pseudomonas aeruginosa* when compared to standard medications. The growth inhibition

zone measured ranged from 7 to 20 mm for all the sensitive bacteria strains [Figures 5, 6]. The findings show that *Ipomoea Aquatica* extracts are more effective against all of the microbes tested.

### Conclusion

The Phytochemical analysis of Extract *Ipomoea Aquatica* were studied. The presence of flavanoids, tannins, saponins, alkaloids, proteins, and phenols in *Ipomoea aquatica* is investigated. The *ipomoea aquatica* were characterized by FT-IR and Aliphatic primary amine, Amine salt, Aromatic compound, Sulfone, Amine, Ester, Sulfoxide, Benzene derivative are identified. Antibacterial activity of *Ipomoea Aquatica* was studied using different concentrations with different pathogens to give zone of inhibition. Antioxidant activity of Extract *Ipomoea Aquatica* was investigated using the DPPH, H<sub>2</sub>O<sub>2</sub>, and O<sup>2-</sup> free radical assay methods because it contains various phytochemicals such as alkaloids, flavonoids, phenolic compounds, and terpenoids, *Ipomoea aquatica* displays exceptional good activities in all biological research.

### Acknowledgement

The authors of this study are appreciative to the Department of Chemistry, Theivanai Ammal College For women (A), Tamilnadu, India, for their tremendous support and for providing all the research facilities. The authors are also grateful for the financial assistance and fund facilities provided by the TNSCT project in Chennai for this project.

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