



## Effect of Lime and Farmyard manure on the Concentration of Cadmium in *Ipomoea aquatica* along with its Antimicrobial and Phytochemical analysis

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Received : November 2017

Accepted : March 2018

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**Abstract :** *Water spinach* is considered native to Africa, Asia, and the southwestern Pacific Islands. Phytochemical analysis on *Ipomoea aquatica* was carried out using standard methods. The result revealed the presence of alkaloid, flavonoid, glycosides, phenol, protein, steroid, saponin, tannin in the leaf extract of the plant. Antibacterial assay was carried out for the ethanol extract by disc diffusion method. In disc diffusion method maximum zone of inhibition was noticed for *Escherichia coli* and less for *Staphylococcus aureus*. Application of lime and farmyard manure reduces the

concentration of cadmium uptake by plant grown in cadmium contaminated soil.

**Keywords :** *Ipomoea aquatica*, antibacterial, phytochemical.

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### Introduction :

*Ipomoea aquatica* has many medicinally and economically significant phytochemicals. The plant being a source of nutrition also consist of cadmium in traces (Austin 2007) . In this work objective was to analyse the presence of alkaloid, flavonoid, glycosides, phenol, protein, tannin and steroid phytochemicals. It has been reported to have antibacterial activities against two stains of bacteria, which were *Escherichia coli* (gram –ve) and *staphylococcus aureus* (gram +ve). The antibacterial activity was observed by disc diffusion method. The study was made on the ethanolic extract of plant. The antibacterial property is due to phenolic compound (Chandra and Shamli, 2015).

*Ipomoea aquatica* grows on contaminated soil and so, has cadmium traces in it. Along with some useful elements like Fe, K, Mg, Mn, (Umar et al 2007) toxic heavy metal cadmium is also present in the plant in certain concentration. Heavy metal is found in it as the plant grows in polluted water. The heavy metal leads to environmental contamination and may be hazardous to human. The toxic heavy metal may lead to malfunctioning of kidney, respiratory system, eyes, reproductive system and even the brain in human. The uptake of heavy metal by plant and subsequent accumulation along the food chain is a potential threat to animal and human health.

The objective of the work along with the antibacterial and phytochemical analysis is also to lower down the cadmium concentration in the plant by treating it with two different combination of lime and FYM. Two types of soil were prepared using the combination of FYM and lime with sandy loamy soil, clay, silt, calcium carbonate and cadmium octahydrate.

pH is a measure of Hydrogen ion concentration of a solution. Loamy soil of basic nature is best suitable for its growth.

### Materials and Methods:

The present research work was conducted in the department of Botany, Patna Women's College, Patna during the period of July to October, 2017. To get the desired result, methods are needed for separation, purification and identification of different constituents present in the plants. Thus phytochemical analysis is very useful to detect the presence of the important phytochemical content present in the plant. The presence of many chemical and compounds in the plant makes them resistive to certain microorganisms like bacteria. The antibacterial assay of a plant reveals its antimicrobial properties which lead to the formation of certain medicines. Lowering down of cadmium in

plant reduces the risk of cadmium to the living organism which includes the plant as a part of their food chain.

The fresh leaves of water spinach (*Ipomoea aquatica*) were collected from (R.P.S More, Danapur, Bihar, India) during August 2017. The plant was identified and authenticated by Vibhishan Pandit (Taxonomist, Patna Science College).

### Mayer's test

1 ml of leaf extract (*Ipomoea aquatica*) was taken in a test tube with 1 ml of Mayer's reagent was added to it. Precipitate indicates the presence of alkaloid in the sample.

A few drops of conc. HCl were added to a small amount of an alcoholic extract of the leaves, immediate development of a red colour indicates the presence of flavonoid.

1 ml of extract was taken with 1 ml of distilled water in a test tube. A few drops of aq. NaOH were added to the solution. Yellowish-green colour shows the presence of glycoside.

2 ml of extract were treated with few drops of concentrated nitric acid. Formation of greenish yellow solution indicates the presence of protein..

2-3 ml of extract were treated with 3-4 drops of ferric chloride solution. Formation of bluish colour indicates the presence of phenols.

2 ml of extract was vigorously shaken with 5 ml of distilled water in a test tube then allowed to stand for a while to a room temperature. Persistent frothing indicates the presence of saponin (Adekunle et al 2003).

1 ml solution of chloroform extract was taken and then added 1 ml sulphuric acid. Red colour indicates the presence of steroid.

1 ml extract was taken in a test tube, 0.5 ml of 2 % lead acetate solution was added to it.

Formation of precipitate shows the presence of tannin.

The two bacterial strains used in the antibacterial study were arranged from Patna Medical college and Hospital (PMCH). Bacteria used were, *Staphylococcus aureus* (gram +ve cocci) and *Escherichia coli* (gram –ve bacilli).

The effect of the plant extract on the bacterial strains were assayed by both disc diffusion and well diffusion method. Disc diffusion method showed better result as compared to the well diffusion method.

All the chemicals were purchased from three different companies – Merck Ltd, Nice Ltd and Qualigens Ltd. Extracts and dilution were prepared in sterile water. Antibiotics were purchased from medical stores.

Leaf of *Ipomoea aquatica* were collected from the Danapur local market, Patna. The ethanol extract were prepared according to the method of (Aneja 2010).

The test was carried out on NA media by disc diffusion method and well diffusion method in sterilized condition. The antibacterial test was carried out on the ethanol extract of the plant. Serial dilution up to  $10^{-5}$  suspension were prepared , of both the bacteria. After incubating the bacteria inoculated NA plate for 48 hrs , the zone of inhibition measurement was taken .

Two plastic pots of 1 L capacity will be taken, 300 gm air-dried soil will be placed in each pot. Two treatments one controlled , one label of lime, one label of farmyard manure their combination will be given to the pots. .

The pots were organized in a completely randomized design. In each pot of cadmium was added as  $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$  in solution form at the rate of 5mg/kg. Cadmium was added once prior to the

experiment. The plants were left to grow for 45 days and harvested .

The plant leaves were dried in an oven at  $65^\circ\text{C}$  for 72 h and dry mass was recorded.

Leaf sample was digested with conc. Nitric acid (25ml) and conc. Sulphuric acid (10ml).

Cadmium concentration in the digest was measured by AAS (Jaakkola 1977).

The pH measurement of the soil samples were taken through the pH measuring machine.

## Results and Discussion:

Phytochemical test revealed the presence of alkaloid , flavonoid, glycosides, phenol, protein , saponin and tannin (Table 1). The tannin, as an anti-diabetic, may contribute to the effects such as antibacterial. Tannin as astringent properties, hastens the healing of wounds and inflamed mucous membrane. Chloroform extract of plant shows the presence of steroid.

Table 1. Phytochemical test

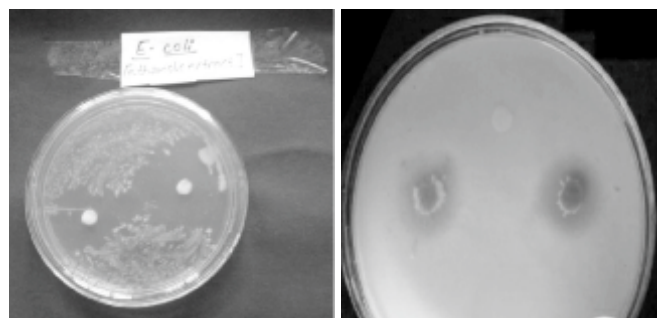
TEST	Aqueous	Ethanol	Methanol
Alkaloid	+ ve	+ve	+ ve
Flavonoid	+ ve	- ve	- ve
Glycosides	+ ve	- ve	+ ve
Phenol	+ ve	- ve	+ ve
Protein	+ ve	- ve	+ ve
Saponin	+ve	+ ve	+ve
Tannin	+ ve	+ ve	+ ve

+

Test	Chloroform
Steroid	+ ve

In antibacterial test the ethanol extract of the plant shows greater zone of inhibition against *E.coli* (gram + ve) in compare to zone of inhibition shown against *S.aureus* (gram – ve) (Fig. 1, 2). The antibacterial test leads to the conclusion that the ethanol extract of the plant shows more

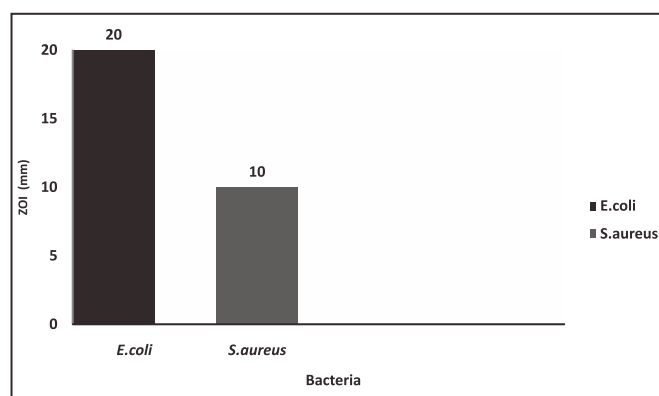
antimicrobial effect against the gram negative bacteria than the gram positive bacteria.



Zone of inhibition  
(*E.coli*) (20mm)

Zone of inhibition  
(*S.aureus*) (10mm)

**Fig. 1. Antibacterial susceptibility test**



**Fig. 2. Zone of inhibition by bacteria**

pH test of the FYM and the soil samples using pH meter at 2.92°C depicts the basic nature of all the three sample (Table 2).

**Table 2. pH test of the samples**

SAMPLE	pHVALUE	NATURE
Farmyard manure	8.4	Basic
soil (10g -lime +FYM)	8.54	Basic
Soil (20g- lime +FYM)	9.0	Basic

AAS test of this plant sample confirmed the presence of nutritious elements Fe and K in it (Table 3). The test confirmed the presence of Cd in the untreated plant sample in high traces. The treated

plant shows comparatively less concentration of Cd in the test (Table 4).

**Table 3. Concentration of iron and potassium in plant**

Element test	Untreated plant	Treated plant (10g-lime+FYM)	Treated plant (20g-lime+FYM)
Iron (Fe)	0.219 ppm	0.277 ppm	0.337 ppm
Potassium (k)	0.212 ppm	0.286 ppm	0.670ppm

**Table 4. AAS test result (Cd)**

Element test	Untreated plant	Treated plant (10 g- lime +FYM)	Treated plant (20 g- lime + FYM)
Cadmium (Cd)	0.203 ppm	0.012 ppm	0.010 ppm

### Conclusion :

Growth of water spinach (*Ipomoea aquatica*) and concentration of cadmium were influenced by lime, FYM and their combinations. It was found that lime was effective in reducing Cd concentration in leaves of water spinach.

The effect of farmyard manure has negligible effect in reducing Cd concentration but it increased biomass production of Water spinach over lime mainly due to nutritional effects of farmyard manure. So of lime could be recommended to be used to reduce Cd uptake by plants grown in Cd – contaminated acidic soil. The phytochemical analysis showed the presence of alkaloid, flavanoid, glucosides, phenol, protein, saponin, steroid and tannin in the plant.

In antibacterial test the ethanol extract of the plant showed greater zone of inhibition against *E.coli* (gram + ve) in comparison to zone of inhibition shown against *S.aureus* (gram – ve). The antibacterial test led to the conclusion that the ethanol extract of the plant showed more antibacterial effect against the gram negative bacteria than the gram positive bacteria.

**Acknowledgement :**

We extend our gratitude to the Former Principal, Dr. Sister Marie Jessie A.C. and Dr. Pinky Prasad, Head of the Botany Department and Co-ordinator Department of Industrial Microbiology, Patna Women's College for their constant support.

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