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**STUDY OF *STRYCHNOS POTATORAM* AGAINST WATER BORNE PATHOGENS**

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**ABSTRACT**

Water is a necessity for our daily life process. But access to clean drinking water is not available to a major population who live in rural areas. They live on open dug wells, pond water etc. In order to provide a clean drinking water, the water is treated using *Strychnos potatorum*, a seed commonly found in south India. The coagulation activity of the seed is used to coagulate the dirt and also decrease the number of microbes in the water. It also shows activity against *Salmonella* sps, *Klebsiella* sps, *E. coli* and *Enterobacter* sps. It is also proved that the antimicrobial activity more for the compound obtained from column chromatography than the crude extract obtained through soxhlet. The seed paste when added to water shows vast decrease in microbial count at the concentration of 1.0 c.c.

**Keywords: *Strychnos potatorum*, Soxhlet, Column Chromatography**

**INTRODUCTION**

Water is essential for the survival of any form of life. In India, major population drink water from unprotected sources like ponds, wells, lakes and most importantly ground water. These waters are seldom treated before drinking. Nowadays more than 1100 million people live without access to safe drinking water, especially in developing

countries. According to WHO, every year there are 2 million diarrhoeal deaths related to unsafe water, sanitation, and hygiene—the vast majority among children under 5. More than one billion people lack access to an improved water source. Solution to this global problem is aimed to develop simple, effective, low-cost and easy to use technologies able to

reduce organic, inorganic and microbiological water contamination.

Plants contain numerous biologically active compounds, many of which shown to have Antimicrobial activities, coagulation and various other activity. Natural polyelectrolytes of plant origin have been used for many centuries in developing countries for clarifying turbid water [1]. For home water treatment, the materials have to be used in the form of powder or paste, 90% of which consists of substances other than the polyelectrolytes. Even under such conditions, a few plant seeds make effective coagulants.

*Strychnos potatorum* also known as clearing-nut tree is spread throughout the tropical and sub-tropical regions of the world [2]. *Strychnos potatorum* seeds are widely used in ayurvedic and traditional medicine. Apart from its medicinal properties the seed powder is being used for clearing muddy water by the rural community. They are reported to be very effective as natural coagulant aids. This property is attributed because of the presence of polyelectrolyte, proteins, lipids, carbohydrates and alkaloids containing the -COOH and free -OH surface groups in the seed [3].

This property of the seed is used to treat the various types of drinking water in order to detect its potential in antimicrobial activity.

## MATERIALS AND METHODS

### Collection of Seed

The seeds were removed from the fruits collected at Poomaram village in Viralimalai, Tiruchirapalli, Tamil Nadu, India.

### Extraction Procedure

The seeds were shade dried and ground using electrical means. The seed coat were removed from the powder by using 100-mesh (150 micron) screen.

The extract from this powdered seed was obtained with the help of soxhlet extractor using isobutanol.

### Column Chromatography

Column chromatography is used to purify liquids by separating an organic solvent from a mixture of solvents.

### Preparation of Seed Extract

The seed extract was prepared by grinding the mixture in mortar pestle containing 22 ml of acetone, 3 ml petroleum ether and calcium carbonate. The pigments was filtered and mixed with 20 ml petroleum ether and 20 ml of 10% aqueous sodium chloride solution. The separating funnel was shaken carefully and the lower layer was allowed to drain into the beaker.

### Preparation of Column

A plug of cotton to the bottom of the column so that silica and soil won't fall out. A slurry of silica was prepared and poured into the

column carefully. It is allowed to settle and sand is added.

### **Loading of Sample**

The sample was added using a pasture's pipette carefully above the sand. The eluent is added on top of the sand. The mobile phase slowly flows down through the silica gel column by gravity leaving behind zones of colour and two component was eluted from the column.

### **Preparation of Sensitivity Discs**

#### **Bioassay Procedure**

The minimal inhibitory concentration of the seed was observed by swabbing the microorganisms in Muller Hinton agar plate. The discs were saturated with the extract of different concentrations of crude extract and the compound obtained from column chromatography. The discs were placed on to the agar plates. Discs of standard antibiotics were also placed for comparison and the plates were incubated at 37°C for 24 hours.

The diameter of the zone of growth inhibition formed were measured for each concentration of the active fraction using meter rule. Diameter <8.0 mm indicates low sensitivity, while >8.0 mm indicates high sensitivity.

#### **Graduation Technique**

In the graduation technique, the water samples are added in a series of conical flask and to the samples 0.5 c.c, 1.0 c.c, 1.5 c.c and 2.0 c.c of the paste of *Strychnos potatorum*

seed was added. This setup was kept undisturbed for 24 hours. After 1 hour, the *Strychnos potatorum* paste clear the water by coagulating and forming the sedimentation at the bottom of the flask which can be removed. The property of the clean water was analyzed

### **RESULT AND DISCUSSION**

The present study shows, at 62 µg concentration the zone of inhibition of the crude extract of *Strychnos potatorum* against the *E.coli* is 15mm and the zone of inhibition of compound obtained through column chromatography against the *E.coli* is 24mm. The zone of inhibition of the crude extract of *Strychnos potatorum* against the *Salmonella sp.* is 13mm and the zone of inhibition of compound obtained through column chromatography against the *Salmonella sp.* is 24mm. The zone of inhibition of the crude extract of *Strychnos potatorum* against the *Klebsiella sp.* is 18mm and the zone of inhibition of compound obtained through column chromatography against the *Klebsiella sp.* is 24mm. The zone of inhibition of the crude extract of *Strychnos potatorum* against the *Enterobactea sp.* is 13mm and the zone of inhibition of compound obtained through column chromatography against the *Enterobactea sp.* is 25mm (**Table 1**). The Chi-square values obtained respectively

which was less than the calculated table value.

### **X<sub>2</sub> (0.05) = at 5% Level of Signification**

**Mallikharjun and Seetharam, 2009, [4]**, tested the alkaloid fractions isolated from *Strychnos potatorum* L.f. (Loganiaceae) seed for their antimicrobial properties against some pathogenic gram positive, gram negative and acid-fast bacteria and proved that these plants are effective in treating diseases causing microbes.

In the earlier study **Subbaramiah and Rao, 1936, [5]**, first found the clarification activity of *Strychnos potatorum* powder due to the presence of casein and albumin. Followed by **Tripathy et al., 1976, [3]**, reported that *Strychnos potatorum* seeds powder is a very effective as natural coagulant aids. This property was attributed because of the presence of polyelectrolyte, proteins, lipids, carbohydrates and alkaloids containing the -COOH and free -OH surface groups in the seed. In the present studies the amount required for successfully treating 100 ml water is found to be 1.0cc. (**Table 2**).

### **CONCLUSION**

This experiment conclude that, Both crude extract and compound obtained from column chromatography of *Strychnos potatorum* has antimicrobial activity against various water pathogens like *E. coli*, *Salmonella sp.*,

*Klebsiella sp.* and *Enterobacter Sp.* but the activity of compound obtained through column chromatography is more pronounced than crude extract. The samples added with 1.0 c.c paste of *Strychnos potatorum* seed also shown decrease in microbial growth than 0.5 c.c, 1.5 c.c and 2.0 c.c of *Strychnos potatorum* seed Paste.

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**Table 1: Zone of Inhibition Formed by Crude Extract of *strychnos potatorum* Seed Powder Against Various Microbes**

S. No	Micro organisms	Antimicrobial agent											
		Crude extract of <i>Strychnos potatorum</i>						Compound obtained through Column Chromatography of <i>Strychnos potatorum</i>					
		60 µg		30 µg		15 µg		60 µg		30 µg		15 µg	
		Std (mm)	Spl (mm)	Std (mm)	Spl (mm)	Std (mm)	Spl (mm)	Std (mm)	Spl (mm)	Std (mm)	Spl (mm)	Std (mm)	Spl (mm)
1	<i>E. Coli</i>	21	15	21	14	21	13	21	24	21	23	21	20
	$\frac{\sum X^2 - (\sum E)^2}{E}$	1.714		2.33		3.04		0.428		0.190		0.047	
2	<i>Salmonella sp.</i>	17	13	17	12	17	11	17	24	17	21	17	20
	$\frac{\sum X^2 - (\sum E)^2}{E}$	0.941		1.470		2.117		2.88		0.941		0.529	
3	<i>Klebsiella sp.</i>	23	18	23	16	23	15	23	24	23	21	23	19
	$\frac{\sum X^2 - (\sum E)^2}{E}$	2.782		2.130		1.086		0.043		0.173		0.695	
4	<i>Enterobacter sp.</i>	18	13	18	12	18	11	18	25	18	23	18	21
	$\frac{\sum X^2 - (\sum E)^2}{E}$	1.388		2		2.722		2.722		1.388		0.428	

**Table 2: Colonies Found After Addition of *Strychnos potatorum* Seed Paste**

S. No	Water Samples	0.5 c.c	1.0 c.c	1.5 c.c	2.0 c.c
1.	Sample 1	4	No growth	27	52
2.	Sample 2	3	No growth	33	43
3.	Sample 3	5	No growth	20	35
4.	Sample 4	4	No growth	18	46
5.	Sample 5	4	No growth	25	35
6.	Sample 6	5	No growth	13	48
7.	Sample 7	8	No growth	25	41
8.	Sample 8	7	No growth	31	53
9.	Sample 9	5	No growth	16	29
10.	Sample 10	3	No growth	12	45