

## International Journal of Research in Pharmaceutical and Nano Sciences

Journal homepage: [www.ijrpns.com](http://www.ijrpns.com)



### STUDIES ON *STRYCHNOS POTATORUM* SEED AND SCREENING THE WATER QUALITY ASSESSMENT OF DRINKING WATER

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#### ABSTRACT

This research was aimed to finding the efficiencies of powdered seeds of *Strychnos potatorum* natural water treatment agents alternative to the use of synthetic chemicals. The optimum dosages and turbidities were observed to the alum and *Strychnos potatorum*. The results obtained the *Strychnos potatorum* prove the plant can be used for the treatment of turbidity in drinking water. Performance of *Strychnos potatorum* seed extract as primary coagulant and compared with the performance of alum. *S. potatorum* seed extract is effective as a prime coagulant compared with alum, it produces water with slightly higher residual turbidity and residual color, but the residual turbidity and residual color are within the WHO drinking water guideline values for turbidity (5 NTU) and color (15 TCU). The effectiveness of *Strychnos potatorum* in the removal of turbidity, total hardness, pH, and total dissolved solids (TDS) has been investigated. Preliminary phytochemical screening were carried out and also IR - Spectrum were analysed. *Strychnos potatorum* seeds column compounds were tested for their antibacterial properties against some pathogenic gram positive and gram negative bacteria. The growth of *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* were significantly inhibited. The maximums zone of inhibition was found in *Pseudomonas aeruginosa*, *Klebsiellapneumoniae* and *Staphylococcus epidermidis*.

#### KEYWORDS

*Strychnos potatorum* Seeds, MPN, Phytochemical, IR - Spectrum and Antibacterial activity.

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#### INTRODUCTION

Water is used for several purposes by humans but the level of purity of the water being consumed is very crucial since it has a direct effect on health. Safe drinking water should generally be free from heavy metals, turbidity, organic compounds and pathogen. Conventional treatments of water often include sedimentation, filtration and disinfection. Among the coagulating agents used in water

treatment, ferric sulphate or alum (aluminium sulphate) is some of the most widely used salts. Aluminium is strongly neurotoxic and may be involved in the development of Alzheimer's disease. It is well known fact that most of the chemical disinfectants used for antibacterial activity generate various unwanted chemicals known as disinfection byproducts (DBPs) in water. These DBPs are associated with harmful effects on humans such as hemolytic anemia, cancer risk, nervous system effect and liver effects. Addressing these problems calls out for a tremendous amount of research to be conducted to identify robust new methods of purifying water at lower cost and with less energy, while at the same time minimizing the use of chemicals impact on the environment. We highlight some of the natural herbals like *Strychnos potatorum*(nirmali) used by humans throughout history in treating drinking water<sup>1</sup>.

*S. potatorum*(nirmali) is a moderate-sized tree found in Southern and central parts of India, Sri Lanka and Burma, used predominantly as a traditional medicinal extract. Seeds of *S. potatorum* are used in dysuria, polyuria, urolithiasis, also in epilepsy. The seeds resemble those of nuxvomica but are non-poisonous. The ripe seeds are used for clearing muddy water. Sanskrit writings from India reported that the seeds were used to clarify turbid surface water over 4000 years ago which indicated that they were the first reported plant-based coagulant used for water treatment. The nuts of this species of *Strychnos* are very largely used in some parts of India for clearing muddy water. The tree, which grows to a very large size, produces a shining, black, one-seeded berry<sup>2</sup>. The present study also used to treat the seed for water quality assessment.

## MATERIALS AND METHODS

### Plant Sample Collection

*Strychnos potatorum* seeds were collected from Velur near Viralimalai, Pudukottai district, Tamil Nadu India. The seed were identified by the Rapinat Herbarium, St. Josephs College, Tiruchirappalli, Tamilnadu, India. The seeds were separated from the

plant and dried under shade. After drying, it was powdered and used for our studies.

### Sampling Sites

The water samples were collected from different areas such as Kallanai, Kollidam, Pulivalam, Thottiyam, Veerampatti, Sathanur, Keeranur, Uyyakondanthirumalai and Kajamalai representing the locations along North, South, West, East, North East, North West, South East, South West directions of the Trichy Taluk. However, the sampling locations include urban as well rural areas.

### Sample Collection

The samples were collected in the month of May 2014 and taken in pre-cleaned polyethylene bottles.

### Physico- Chemical Analysis of Drinking Water

The collected samples were analyzed for different physico - chemical parameters such as pH, alkalinity, Calcium, Magnesium, Total solids, Total dissolved solids and Total suspended solids.

### Preparation of Plant Extract

The *Strychnos potatorum* plant seed were collected and dried at room temperature for 2 - 3 days and further dried at 60° C. The dried seed were extracted with aqueous. The extracts were filtered with the Whatman filter paper and then dried by using rotary evaporator. The filtrate was stored in screw cap bottle at -20° C for further use.

### Colorimeter

A colorimeter is a device used in colorimeter. In scientific fields the word generally refers to the device that measures the absorbance of particular wavelengths of light by a specific solution. This device is most commonly used to determine the concentration of a known solute in a given solution by the application of the Beer-Lambert law, which states that the concentration of a solute is proportional to the absorbance<sup>3</sup>.

### MPN METHODOLOGY

To detect the coli form bacteria from the collected water samples. Coli forms can be identified from the following three methods.

### **Presumptive test**

Three sets of test tubes (each contain three tubes and one is control tube) were taken. 10 ml of double strength lauryl phosphate tryptose broth is added into first set of tubes. In the second and third set of tubes add 10 ml of single strength of lauryl tryptose broth. Durhams tubes were inserted in all tubes without air bubbles inside the Durham's tube. The setup is sterilized. After cooling 10 ml of water sample were added into first set of tubes, 1 ml in second set of tubes and 0.1 ml in third set of tubes. After incubation all the tubes were observed for the presence of gas production. The number of coli forms in the water sample was calculated by comparing the standard chart.

### **Confirmed test**

A loopful of culture from positive lauryl tryptose broth was transferred to brilliant green lactose bile broth tubes. The tubes were incubated at 37°C for 24 hours. The tubes were examined for the presence of gas in Durham's tube within 48 hours, which constitute a positive confirmed test.

### **Completed test**

A loopful of culture from the positive tubes were transferred to the Eosin methylene Blue agar plates. The EMB agar plates were incubated at 37°C for 24 hours. Following incubation the plates were examined<sup>5</sup>. (Dubey and Maheswari 2006).

### **Enumeration of microbes by spread plate technique**

Prepare plates with suitable nutrient medium place 0.1 ml of diluted sample in the center of the plate. Spread the inoculum over the medium by pushing the glass spreader (L-Rod) backward and forward while rotating the plate. Incubate the plates at suitable temperature<sup>6</sup>.

## **PHYTOCHEMICAL ANALYSIS<sup>7</sup>**

### **Test for Alkaloids**

#### **Mayer's test**

To the little amount of extract were taken and add a few drops of Mayer's reagent formation of precipitate indicate the presence of alkaloids.

### **Mayer's Reagent**

Mercuric chloride (1.358 g) was dissolved in 60 ml of distilled water, potassium iodine (5 g) was dissolved in 10 ml of distilled water. The two solutions were making up to 30 ml of distilled water.

### **Test for Flavonoids**

#### **Ferric Chloride test**

1 ml of extract was taken and a few drops of dilute ferric chloride solution were added. The color changed to pale green or red brown color which indicates the presence of flavonoids.

### **Test for Saponins**

#### **Foam test**

1 ml of extract was diluted separately with distilled water to 20 ml and shaken with graduated cylinder for 15 minutes. Formation of air bubbles indicates the presence of saponins.

### **Test for carbohydrates**

#### **Molisch's test**

Small quantity of extract was dissolved separately in 4 ml of distilled water and filtered, 2 ml of filtrate, 2 drops of alcoholic naphthol solution are added. The mixture is shaken well and 1 ml of concentrated sulphuric acid is added slowly along the sides of the test tube and allowed to stand. Formation of reddish brown ring or violet ring indicates the presence of carbohydrate.

### **Test for tannins**

#### **Lead acetate test**

To 5 ml of extract solution and 1 ml of lead acetate solution was added. Flocculant brown precipitate indicates the presence of tannins.

### **Test for sterols**

#### **Libermannburchard reaction**

A small amount of extract of sample and a few crystal of sodium nitrate were taken in a dry test tube and heated gently for a minute. It was cooled and added 0.5 ml of concentrated sulphuric acid. Orange color indicates the presence of sterols.

### **Test for glycosides**

A portion of the extract was hydrolysed with hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to legal's test to detect the presence of different glycosides.

#### **Legal's test**

1 ml of sodium nitroprusside solution was added and then it was made alkaline with sodium hydroxide solution. If the extract produced pink to red color, which indicates the presence of glycosides.

#### **Test for oil and fats**

Few drops of 0.5N alcoholic potassium hydroxide were added to small quantity of various extract along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soaps or particle neutralization of alkali indicates the presence of fixed oil and fats.

#### **Test for phenolic compounds**

Few drops of extracts were taken separately in water tested for the presence of phenolic compounds with dilute ferric chloride solution (5%) which gives violet color.

#### **Test for protein and Aminoacids**

##### **Biuret test**

A few drops of extract were taken in water and 1 ml of 4% copper sulphate was added to it. Violet or pink colour is conformed proteins are present.

##### **Test for gums and mucilage**

About 10 ml of the extract was added to 25 ml of absolute alcohol with stirring and filtered. The precipitate was dried in air and examined for its swelling properties and for the presence of carbohydrates.

##### **IR – spectrum analysis**

FTIR relies on the fact that the most molecules absorb light in the infra-red region of electromagnetic spectrum. This absorption corresponds specifically to the bonds present in the molecule. The frequency ranges are measured as wave numbers typically over the range 4000 - 400 cm<sup>-1</sup>.

##### **Column chromatography**

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

##### **Preparation of seed extract**

The seed extract was prepared by grinding the mixture in mortar and pestle containing 22 ml of acetone 3 ml of 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 the beaker.

##### **Preparation of Column**

A plug of cotton is placed to the bottom of the column so that silica and soil will not pass through the column. Slurry of silica was prepared and poured into the column carefully. It is allowed to settle and sand is added to the upper portion of the silica.

##### **Loading a 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 zone of colour and a component was eluted from column.

##### **Microbial strains used**

Different microbial strains were used to evaluate the antimicrobial effect of which two were gram positive bacterial strains (i.e) *Staphylococcus aureus*, *staphylococcus epidermidis*, and three strains were gram negative bacterial strains (i.e) *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumonia*. The strains were obtained from Jamal Mohamed College, Trichy, Tamil Nadu, India and maintained agar slants.

##### **Disc Diffusion Method**

Disc diffusion method was carried out for antibacterial susceptibility testing according to the standard method to assess the presence of antibacterial activities of the plant extract (Ravikumar, 2011; Kim, 1999; Nusrat S, 2008)<sup>(8,9)</sup>. Muller Hinton agar (MHA) plates were prepared. Overnight nutrient broth culture of test organisms were seeded over the MHA plates using sterile cotton swab so as to make lawn culture. The discs which had been impregnated with aqueous extracts of leaf were placed on the MHA with the control disc and subjected to antibacterial screening. The plates were then incubated at 37° C for 18 to 24 hours depending on the species of bacteria used in this test. After the incubation, the plates were examined for inhibition zone.

### Chi – Square Test ( $\chi^2$ )

In this study chi - square test ( $\chi^2$ ) was applied. The purpose of chi – square test ( $\chi^2$ ) was to decide whether the set of observed data (Antibiogram of microorganisms) agrees with the standard antimicrobial disc susceptibility test (NCCLS, 2002).

### RESULTS

The present study deals with the treatment of drinking water by using plant *Strychnos potatorum* seed powder. Drinking water contains the coli form bacteria it was observed by using MPN method, Eosin Methylene Blue agar plate confirmed the coli forms (Figure No.5). Seed powder and chemical substance alum were added with the drinking water separately and mixed well, turbidity level will be observed in colorimeter. Initial and after adding (seed powder and alum) the OD values are noted down, after that colonies were observed by using Spread plate method. Initial level too much of colonies were present in the petriplates (Figure No.6), alum will inhibit the colonies within 30 minutes (Figure No.7) and seed powder will inhibit the organisms within 1 hour (Figure No.8). To analyze the Physical and chemical parameters of drinking water before and after treating with *Strychnos potatorum* seed (Table No.2 and 3).

The present study shows the presence of *Strychnos potatorum* using the organic solvent extraction technique the bioactive constituents have been extracted and subjected to preliminary colour test to identify the nature of the compounds such as Alkaloids, Reducing sugar, Phytosterol, phenolic compounds, tannins, protein and amino acids, absence of gums and mucilage, fixed oil and fats, were confirmed by suitable chemical test (Table No.4), Seed crude powder and column separated compounds having the functional groups, it was identified through IR – Spectrum (Table No.5 and 6) (Figure No.1 and 2).

The result of the antibacterial activity of *Strychnos potatorum* seed crude extract 64  $\mu\text{g}$  concentration is given in (Table No.7). Best zone of inhibition was produced against *Pseudomonas aeruginosa* (23mm), and better zone of inhibition against *Klebsiella sp.*, (18 mm), *Bacillus subtilis* (14 mm), *Proteus vulgaris* (14 mm), *Escherichia coli* (15 mm), and least was produced against *Staphylococcus aureus* (13 mm), *Staphylococcus epidermidis* (12 mm), Maximum zone of inhibition were observed in *Pseudomonas sp.*, and *Klebsiella sp.*, and least zone of inhibition observed in *Staphylococcus aureus* and *Staphylococcus epidermidis* (Figure No.11).

Seed column compound crystals were confirmed in polarizing microscope (Figure No.10), The result of the antibacterial activity of column separated compounds 64  $\mu\text{g}$  concentration is given in (Table No.8). Best zone of inhibition was produced against *Pseudomonas aeruginosa* (32mm), *Escherichia coli* (24 mm), *Klebsiella sp.*, (24 mm), and better zone of inhibition against *Proteus vulgaris* (16 mm), *Bacillus subtilis* (16 mm), *Staphylococcus aureus* (18 mm), and least was produced against *Staphylococcus epidermidis* (14 mm), Maximum zone of inhibition were observed in *Pseudomonas sp.*, and *Klebsiella sp.*, *E.coli* and least zone of inhibition observed in *Staphylococcus epidermidis* (Figure No. 12). Seed compound gives the maximum zone of inhibition, when compared with the seed crude extract.

### DISCUSSION

In earlier studies Rajendran et al., (2013) shows the Seeds of *Strychnos potatorum* (*S. potatorum*) and *Moringa oleifera* (*M. oleifera*) have shown promising result as the source of natural coagulant in the clarification of turbid water. Direct filtration with *S. potatorum* seeds as coagulant appeared effective in clarifying turbid water. This property is attributed due to the presence of polyelectrolytes, proteins, lipids, carbohydrates and alkaloids containing the  $-\text{COOH}$  and free  $-\text{OH}$

surface groups in the seed. Among the other plant materials investigated, seeds of *M. oleifera* were found to be one of the most effective sources of primary coagulant for water treatment<sup>1</sup>.

In previous Vijayaraghavan and Sivakumar *et al.*, studied the application of plant based coagulants for waste water treatment. Natural coagulants function by means of adsorption mechanism followed by charge neutralization or polymeric bridging effect. Frequently studied plant-based coagulants include Nirmali seeds (*Strychnos potatorum*), *Moringaoleifera*, *Tannin* and *Cactus*. Utilization of these coagulants represents important progresss in sustainable environmental technology as they are renewable resources and their application is directly related to the improvement of quality of life for under developed communities<sup>10</sup>.

The earlier study Mallikharjuna*et al.*,(2007) studied the phytochemical screening of *Strychnos potatorum* seed sample and described along with physical and chemical compound such as Alkaloids, Reducing sugar, Phytosterol, Fixed oil and fats, phenolic compounds, and tannins<sup>(11)</sup>.In earlier studies Venkatesh *et al* (2011) a phyto – pharmacological review on *Strychnos potatorum*linn, this review will be helpful to create interest towards *Strychnos potatorum* and may be useful in developing new formulations with more therapeutic and economical value<sup>12</sup>.

The previous study was showed by Srikanth Kagithoju. *et al.*,(2013) to investigate the

Pharmacognostic and phytochemical characteristics of leaves of an endangered medicinally important tree species *Strychnos potatorum*<sup>13</sup>. In previous study Packialakshmi *et al.*, (2014) deals with the phytochemical characteristics of leaves and bark of an endangered medicinally important tree species of *strychnos potatorum*. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, sapiens, carbohydrates, tannins, sterols, glycosides, oil and fats, phenolic compounds, protein and amino acids, gums and mucilage. The functional groups were identified through IR – spectrum<sup>14</sup>.

In earlier studies Mallikharjuna *et al.*, (2009) alkaloid fractions isolated from *Strychnos potatorum* seed were tested for their antimicrobial properties against some pathogenic gram positive, gram negative and acid – fast bacteria and fungi. These fractions have shown considerable antimicrobial activity against both bacteria and fungi at the tested concentrations (100 and 200 µg / ml)<sup>15</sup>. In previous study the Packialakshmi*et al.*, (2013) *Strychnos potatorum* against some water borne pathogens, 62 µg concentration the zone of inhibition occurred in *Salmonella sp.*, *Klebsiella sp.*, *Enterobacter sp.*, *E.coli*<sup>16</sup>.

**Table No.1: Water samples collection from different places of Tiruchirappalli District**

S.No	Places of water samples collected	Types of water sample
1	Kallanai - Vennaru	River
2	Kallanai - Kollidam	River
3	Thottiyam	River
4	UyyakondanThirumalai	Well
5	Kajamalai	Well
6	Pulivalam	Well
7	Kuruvikarankulam	well
8	Gundur	pond
9	Sathanur	Pond
10	Veerampatti	Pond

**Table No.2: Physical and chemical parameters of water samples before treating with *Strychnos potatorum* seed**

S.No	pH	EC (dsm <sup>-1</sup> )	CO <sub>3</sub> mg/L	HCO <sub>3</sub> mg/L	Cl mg/L	SO <sub>4</sub> mg/L	Ca mg/L	Mg mg/L	Na mg/L	K mg/L	Na <sub>2</sub> CO <sub>3</sub> mg/L
1	7.5	0.67	-	2.4	4.3	-	2.0	1.7	2.7	0.3	-
2	7.9	0.61	1.0	3.3	1.8	-	1.9	1.3	2.6	0.3	1.1
3	7.5	0.72	-	4.3	2.9	-	1.8	1.9	3.2	0.3	0.6
4	7.7	0.54	1.2	2.3	1.9	-	1.3	1.5	2.4	0.2	0.7
5	7.4	1.44	0.4	8.7	5.3	-	4.0	3.2	6.9	0.3	2.6
6	8.4	0.83	2.2	4.4	1.7	-	1.9	0.8	5.3	0.3	3.9
7	8.1	2.4	4.0	11.9	8.1	-	4.8	9.1	9.8	0.3	2.0
8	7.9	0.74	-	4.9	2.5	-	1.9	1.8	3.4	0.3	1.2
9	7.7	0.57	-	3.3	2.4	-	1.1	1.5	2.8	0.3	0.7
10	7.1	0.11	-	0.4	0.7	-	0.4	0.6	0.01	0.13	-

**Table No.3: Physical and chemical parameters of water samples after treating with *Strychnos potatorum* seed**

S.No	pH	EC (dsm <sup>-1</sup> )	CO <sub>3</sub> mg/L	HCO <sub>3</sub> mg/L	Cl mg/L	SO <sub>4</sub> mg/L	Ca mg/L	Mg mg/L	Na mg/L	K mg/L	Na <sub>2</sub> CO <sub>3</sub> mg/L
1	5.1	0.63	-	4.6	1.7	-	2.2	3.0	0.72	0.40	-
2	4.7	1.17	-	8.0	3.7	-	2.9	3.4	4.3	1.11	1.7
3	5.2	0.90	-	5.1	4.0	-	1.9	2.4	3.91	0.85	0.8
4	4.8	0.64	-	4.2	2.3	-	1.5	1.9	2.51	0.52	0.8
5	5.1	1.52	-	10.1	5.2	-	4.1	3.4	6.88	0.85	2.6
6	4.9	0.91	-	7.2	2.0	-	1.8	1.4	5.59	0.39	4.0
7	5.7	1.45	-	8.8	5.7	-	3.4	2.4	8.01	0.66	3.0
8	4.5	0.78	-	5.2	2.6	-	1.8	1.4	4.24	0.42	2.0
9	4.7	0.75	-	2.6	4.9	-	1.6	1.3	4.18	0.34	0.3
10	5.0	0.36	-	2.1	1.5	-	0.9	1.8	0.71	0.21	0.6

**Table No.4: Phytochemical analysis of *Strychnos potatorum* seed**

S.No	Phytochemical Constituents	Seed Extract
1	Alkaloids	Positive
2	Flavonoids	Positive
3	Saponins	Positive
4	Carbohydrates	Positive
5	Tannins	Positive
6	Sterols	Positive
7	Glycosides	Positive
8	Oil and Fats	Negative
9	Phenolic Compounds	Positive
10	Protein and Amino acids	Positive
11	Gums and mucilage	Negative

**Table No.5: Infrared spectrum analysis by *Strychnos potatorum* seed crude powder**

S.No	Peak value	Stretching	Interpretation
1	673.16	-	Benzene
2	1089.78	C-C Stretching	Secondary alcohols
3	1440.83	C-H Bonding	CH <sub>2</sub> Symmetric
4	1462.04	C-H Bonding	Alkyl group
5	1525.69	C=C Stretching	Alkenes
6	1558.48	C=C Stretching	Alkenes
7	1639.49	C=O Stretching	Amino acid
8	1651.00	C=O Stretching	Amino acid
9	1687.71	C=O Stretching	$\alpha$ , $\beta$ - unsaturated, 6 membered ring
10	1705.07	C=O Stretching	Aryl group
11	2854.65	C-H Stretching	Alkyl group
12	2924.09	C-H Stretching	Methyl group
13	3437.15	O-H Stretching	Alcohol group



**Table No.6: Infrared spectrum analysis by *Strychnos potatorum* of seeds column separated compounds**

S.No	Peak value	Stretching	Interpretation
1	601.79	C-Cl Stretching	Halogen
2	655.80	C-Cl Stretching	Halogen
3	923.90	C-H Def	Alkenes
4	1074.35	C-O Stretching	Ether
5	1276.88	N=O Stretching	Nitro compounds
6	1344.38	N=O Stretching	Nitro compounds
7	1413.82	C=C Stretching	Aromatic compounds
8	1635.64	N=O Stretching	Nitro compounds
9	2266.36	N-H Stretching	Amino acids
10	2382.09	N-H Stretching	Amino acids
11	2927.94	O-H Stretching	Alcohols
12	3232.70	N-H Stretching	Amide
13	3772.76	N-H Rocking	Amines

**Table No.7: Antibacterial activity of *Strychnos potatorum* seed crude extract (Zone of inhibition in mm)**

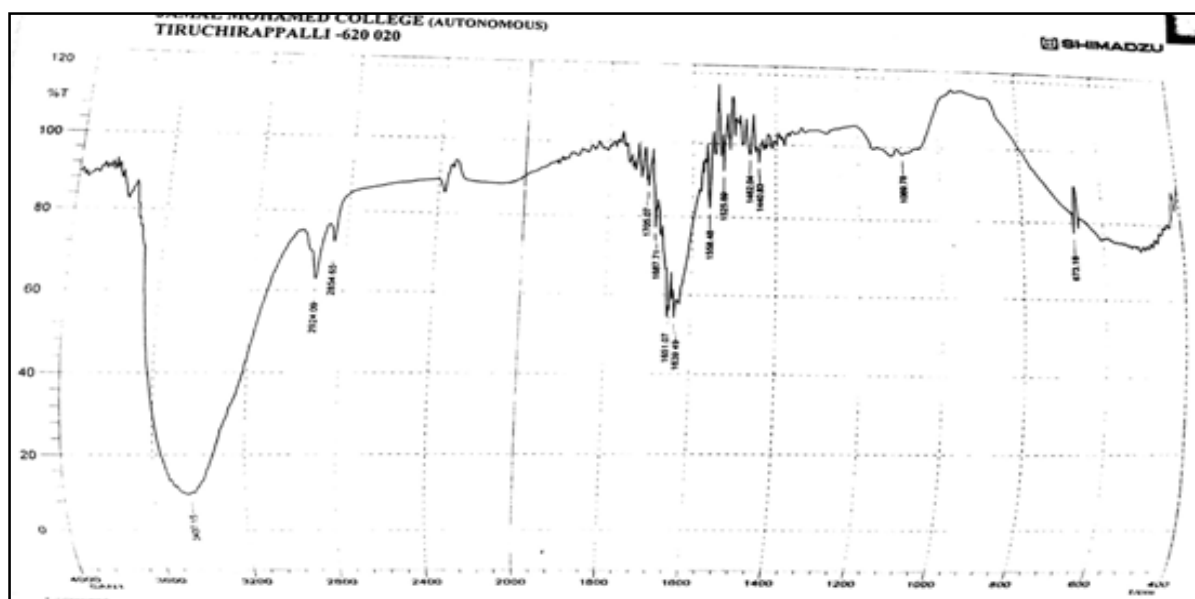
S.No	Sample	µg/ml	Bacterial strains used	Seed crude extract		$\chi^2 = \Sigma[(O-E)^2/E]$
				Standard value	Observed value	
1	<i>Strychnos potatorum</i> seed	64µg	<i>E. coli</i>	21	15	1.714
2			<i>Klebsiella sp.</i> ,	21	18	0.727
3			<i>P. vulgaris</i>	21	14	2.909
4			<i>P. aeruginosa</i>	21	23	0.190
5			<i>S. aureus</i>	21	13	3.047
6			<i>S. epidermidis</i>	21	12	3.857
7			<i>Bacillus subtilis</i>	21	14	2.909

Table value  $\chi^2 (0.05) = 3.841$ , Chi – square value significance at 5% level

**Table No.8: Antibacterial activity of *Strychnos potatorum* seed column extract (Zone of inhibition in mm)**

S.No	Sample	µg/ml	Bacterial strains used	Seed column extract		$\chi^2 = \sum[(O-E)^2/E]$
				Standard value	Observed value	
1	<i>Strychnos potatorum</i> column compound	64µg	<i>E. coli</i>	22	24	0.181
2			<i>Klebsiella sp.,</i>	22	24	0.181
3			<i>P. vulgaris</i>	22	16	1.636
4			<i>P. aeruginosa</i>	22	32	4.545
5			<i>S. aureus</i>	22	18	0.727
6			<i>S. epidermidis</i>	22	14	2.909
7			<i>Bacillus subtilis</i>	22	16	1.636

Table value x 2 (0.05) = 3.841, Chi – square value significance at 5% level



**Figure No.1: Infrared spectrum analysis by *Strychnos potatorum* seed crude powder**

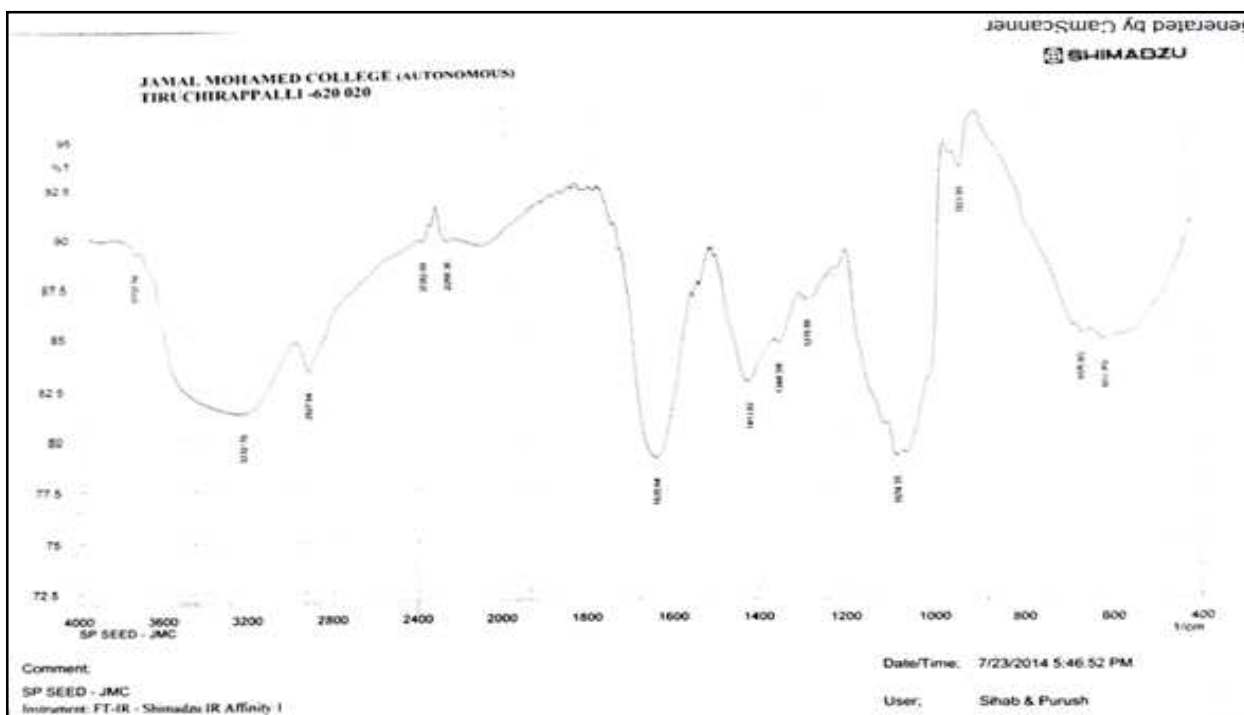


Figure No.2: Infrared spectrum analysis by *Strychnos potatorum* seed column separated compound



Figure No.3: *Strychnos potatorum* plant



Figure No.4: *Strychnos potatorum* seed and seed powder

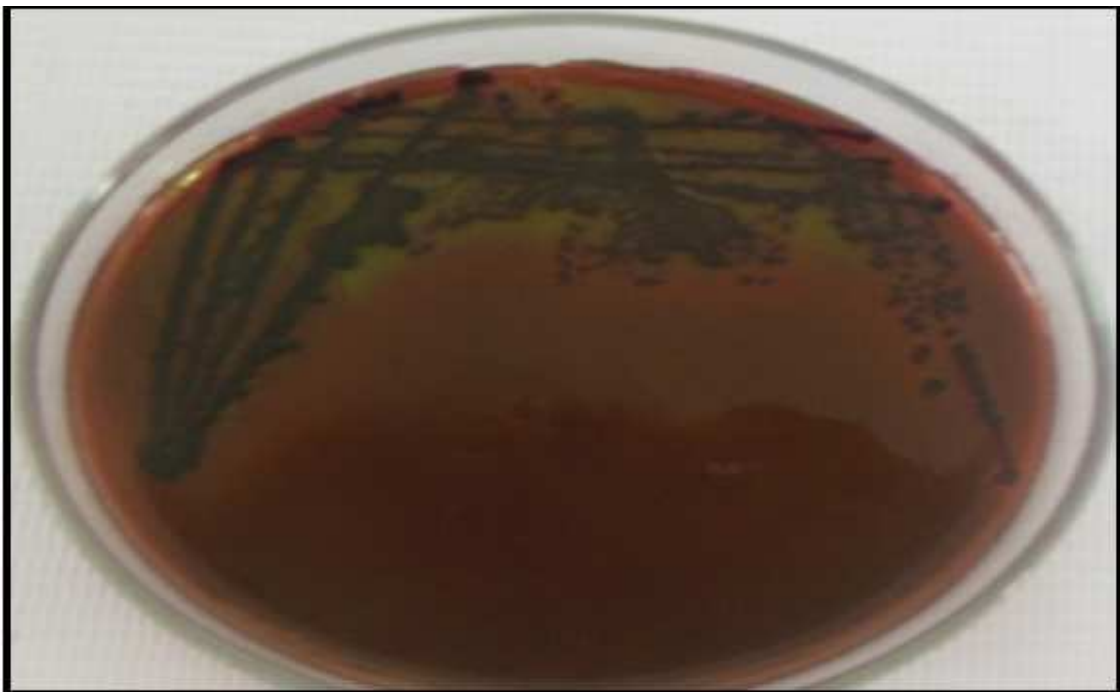


Figure No.5: Eosin Methylene Blue agar plate confirm the *coli form* bacteria

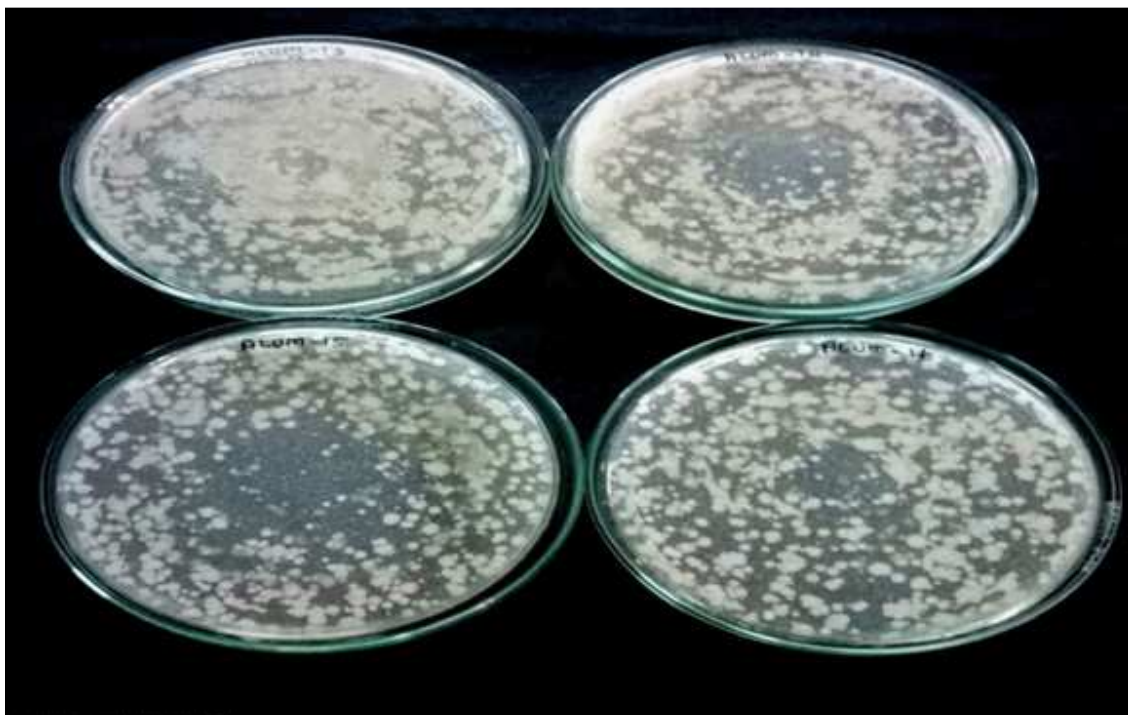


Figure No.6: Water sample initial stage using spread plate method



Figure No.7: Water samples treated with *S.potatorum* seed powder

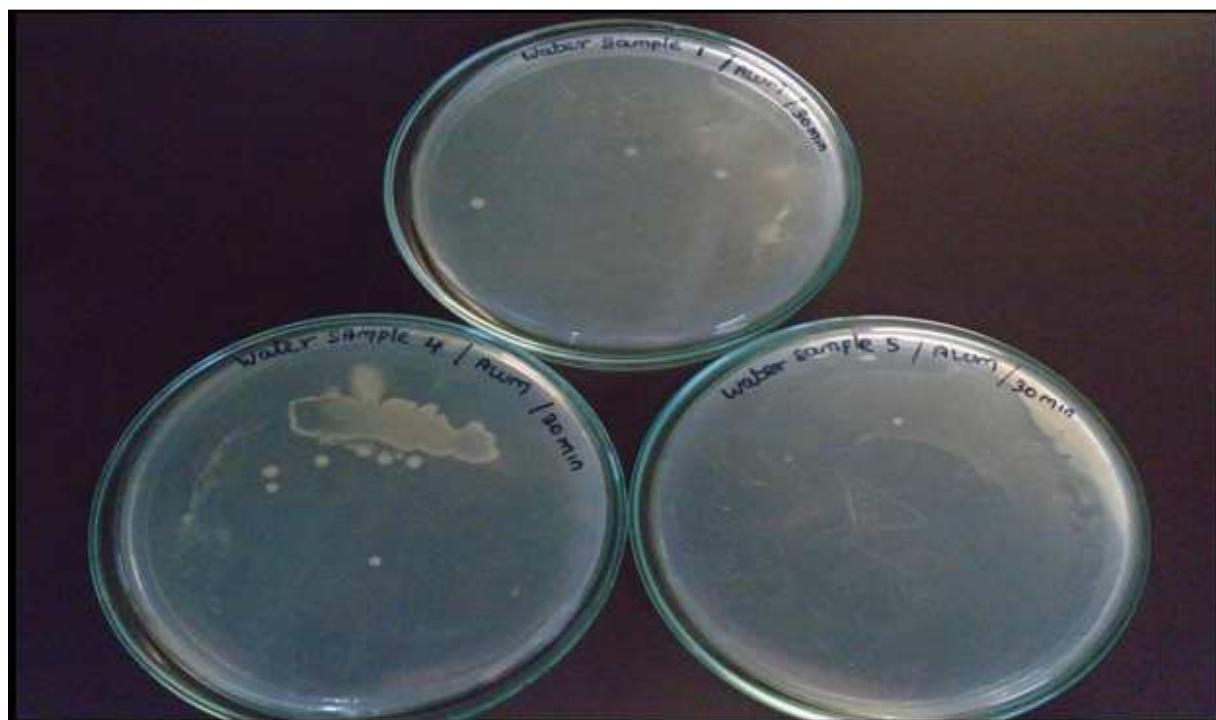


Figure No.8: Water samples treated with alum

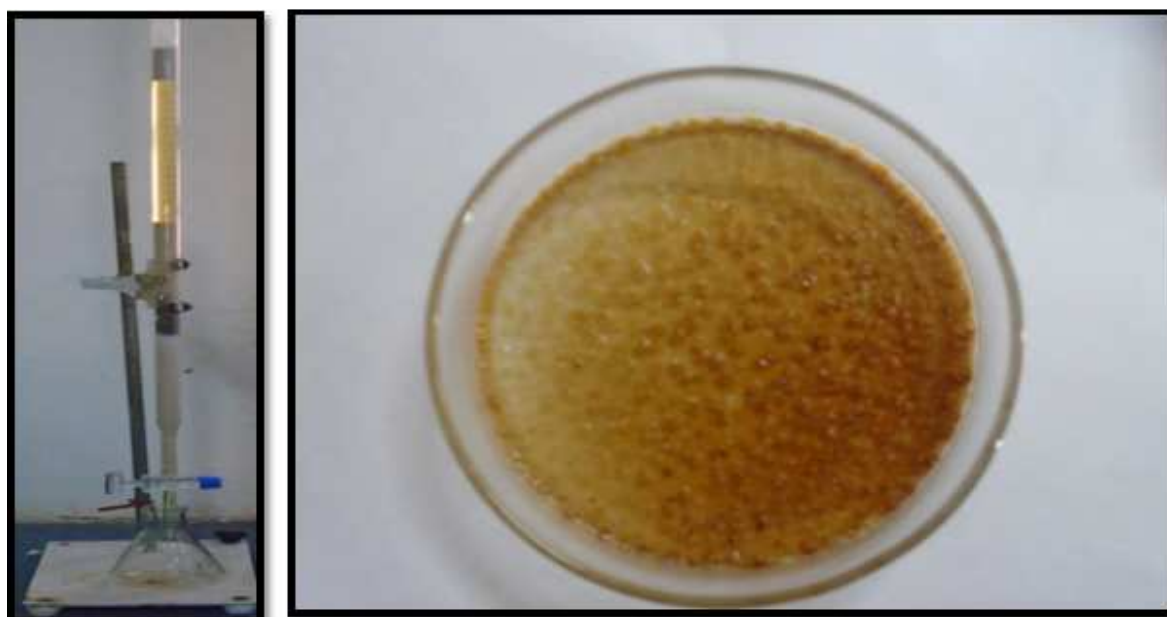


Figure No.9: *Strychnos potatorum* seed Column chromatography and *S. potatorum* seed dried crystals

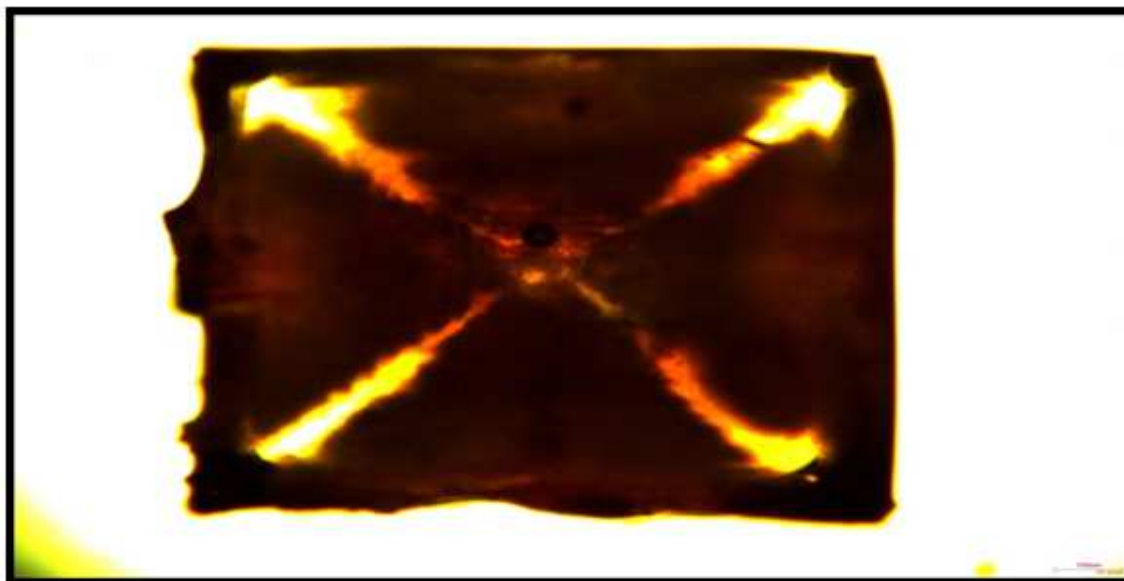


Figure No.10: *Strychnos potatorum* seed separated column compound crystals observed in Polarizing Microscope (Size; 100 $\mu$ m / 161 pixels)

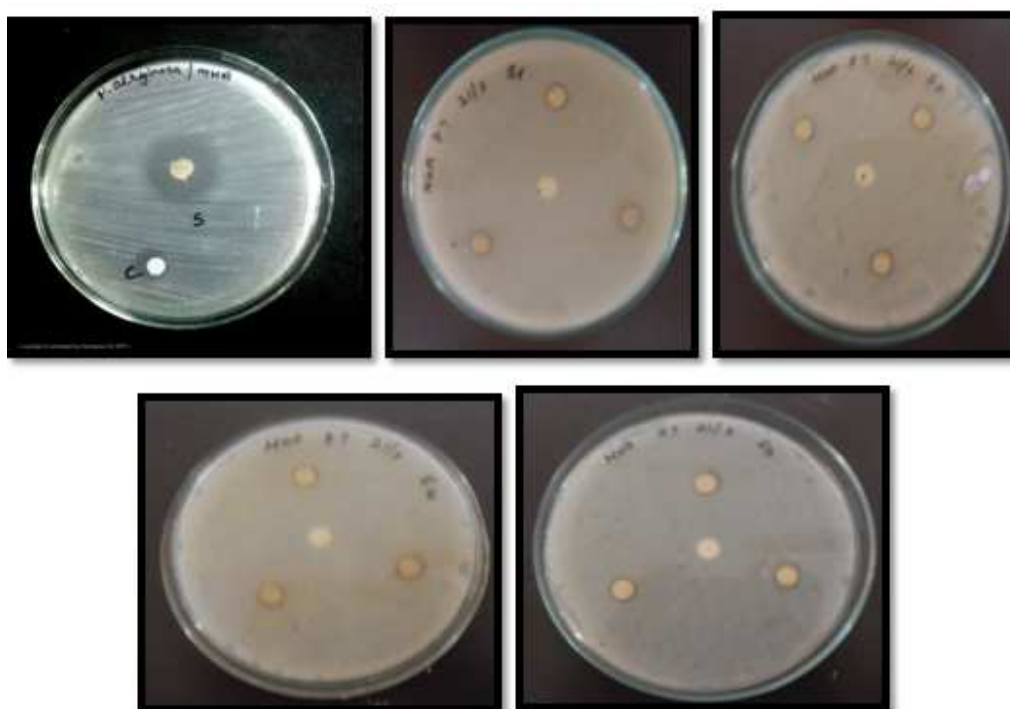


Figure No.11: Zone Inhibition formed by *Strychnos potatorum* seed crude extract



Figure No.12: Zone Inhibition formed by *strychnos potatorum* seed column extract

## CONCLUSION

This research study concludes that the *Strychnos potatorum* seed are very effective for drinking water treatment and also it is very effective against the pathogenic organisms.

## ACKNOWLEDGEMENT

The authors are thankful to the Principal and Management of Jamal Mohamed College (Autonomous) Tiruchirapalli for offering facilities to carry out this research.

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