

## Estimation of phytochemical components in *Strychnos potatorum* extract from samples collected from different water sources

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World Journal of Biology Pharmacy and Health Sciences, 2025, 21(02), 258-265

Publication history: Received on 19 December 2024; revised on 01 February 2025; accepted on 04 February 2025

Article DOI: <https://doi.org/10.30574/wjbphs.2025.21.2.0116>

### Abstract

Water is an essential drinking source for all life forms, it constitutes about 65% of the body weight in human beings and its quality plays crucial role for maintaining biological functions in human beings and other living organisms. Besides, being essential function for life, it also enhances life in numerous other ways by maintaining cell functions in living beings, particularly as an effective biological solvent and as a reactant molecule in many biological, chemical reactions etc. This interest in drugs of plant origin is due to several reasons, namely, conventional medicine can be inefficient (e.g. side effects and ineffective therapy), abusive or incorrect use of synthetic drugs results in side effects and other problems. Many infectious diseases are associated with faecally contaminated water and are a major cause of morbidity and mortality worldwide. Waterborne diseases are caused by enteric pathogens such as bacteria, viruses and parasites that are transmitted by the faecal oral route. Water borne spread of infection by these pathogenic microorganisms depends on several factors such as: the survival of these microorganisms in the water environment, the infectious dose of the Safe drinking water should be generally free from pathogenic organisms, toxic substances, heavy metals, turbidity, excess of minerals and other organic compounds. It should be colorless, odorless ideally cool and tasteless so as to be enticing and healthy to consumers.

**Keywords:** Waterborne; Diseases; Microorganisms; Science and technology

### 1. Introduction

Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals. Silver nanoparticle-immobilized cloth can find a wide application in various fields. It can be used as an antiseptic bandage material for wound dressing. It has been reported that the bactericidal effect of the silver nanoparticle-immobilized cloth material against Gram -positive, Gram -negative and antibiotic resistant bacteria. Skin irritation tests of the silver nanoparticle immobilized cloth material revealed no side effects. Silver nanoparticles also find application in topical ointments and creams which is used to prevent infection of burns and open wounds. Silver nanoparticles may have a beneficial impact on the treatment of human skin inflammatory conditions. Silver nanoparticles released into the blood mononuclear cells, causing apoptosis and inhibiting the expression level of interleukin-5, interferon and tumour necrosis factor (Prasad et al., 2011). Water is a reactant, a reaction and transport medium, a temperature regulator and an indispensable support for life processes. Globally, 6.5 billion cases of diarrhoea and malaria are reported every year resulting 2.8 million deaths, out of which 75% are children's under five years and in India, the diarrhoea alone accounts 1600 deaths per day (WHO, 2011) {6}. In most popular nations such as India, 80% of the diseases are due to bacterial contamination of drinking water. In India, the majority population still lives in villages and remote areas. These rural or tribal communities do not have access to public water supplies. They consume water from unprotected and polluted resources like open dug wells, small streams and ponds {7}.

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Generally, chemical coagulants such as iron salts, 3 aluminum salts or polymers, should be used to the water treatment process to facilitate bonding among water molecules. The mechanisms associated with different natural coagulants are different as well {12} and it is surprising to point out that a widespread critical analysis of available plant-based coagulants is still Non-existent given the importance of sustainable environmental technology. {13} Our country is rich in a broad variety of seeds and herbs like Nirmali, Sahjana, Khas-khas and elaichi (cardamom), which can provide us pure drinking water directly at home. Even lotus leaves have been discovered to diagnose water-borne diseases like dysentery and diarrhoea every year {14}.

The World Health Organization (2012) recommends that water used for drinking purpose should contain at least less than 0.01% of faecal and total coliform counts, in any 1 liter water sample. The complete removal or deactivation treatment process of these groups of disease causing pathogenic microorganisms is the final process in the reduction of microorganisms from drinking water. There is also strong evidence linking aluminium-based coagulants to the development of neurodegenerative illnesses as dementia and with Alzheimer's disease in human beings {9}. It is better to make a progressive replacement of chemical coagulants with alternative coagulants and flocculants preferably from natural and renewable resources for preventing these reasons. Many coagulants and flocculants, {10} which are widely used in conventional water purification processes can be classified into two types as inorganic coagulants (e.g. aluminium and ferric salts) and synthetic organic polymers (e.g. polyacryl amide derivatives and polyethylene amine) {11}. Recent concerns on the usage of the coagulants like detrimental effects on human health, {8} production of large sludge volumes (toxic in some case) and impact on the environment make them questionable and even disregard its usage.

A few recent investigations, reports that natural coagulants from *Strychnos potatorum* (Nirmali) seeds, which has received significant degree of attention for water treatment process and these seeds are indigenous seeds to India {15}. *Strychnos potatorum* Domain : Eukaryota Kingdom : Plantae Subkingdom : Viridaeplantae Phylum : Tracheophyta Subphylum : Euphyllophytina Infraphylum : Radiatopses Class : Spermatopsida Subclass : Asteridae Superorder : Gentiananae Order : Gentianales Family : Loganiaceae Tribe : Strychneae Genus : Strychnos Specific epithet: *potatorum* - L.f. Botanical name: *Strychnos potatorum* L.f. , 4 *Strychnos potatorum* is also known as clearing nut tree is a deciduous tree with medium height up to 40 feet. Its common name is Nirmali and in Tamil it is called as Tethankottai. It belongs to the family Loganiaceae {16}. The tree, which grows to a very large size, produce a shining, black, one-seeded berry. The seeds are described as broadly lenticular, about half an inch in diameter and a quarter an inch in thickness, with a dirty whitish-gray color and covered with a thick coat of delicate hairs. It is commonly referred to as clearing nut tree {17}. It is a medium sized glabrous deciduous tree having height of 6-12 meters. The plant has been described as a common tree for medicinal importance (Ayurveda, Siddha and Unani) in India an also this plant is popular in purifying water for drinking. Biosynthesis of nanoparticles is a kind of bottom up approach {18} where the main reaction occurring is reduction or oxidation. Chemical synthesis method leads to presence of some of the toxic chemical absorbed on the surface that may have adverse effect in the medical applications {19}. This is not an issue when it comes to biosynthesized nanoparticles via green synthesis route. So in the search of cheaper pathways for nanoparticles synthesis, scientist used microbial enzymes and plant extracts (phytochemicals). With their antioxidant or reducing properties they are usually responsible for the reduction of metal compounds into their respective nanoparticles {20}.

Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, {21} temperature and toxic chemicals. Silver nanoparticle-immobilized cloth can find a wide application in various fields. It can used as an antiseptic bandage material for wound dressing {22}. It has been reported that the bactericidal effect of the silver nanoparticle-immobilized cloth material against Gram -positive, Gram -negative and antibiotic resistant bacteria. Skin irritation tests of the silver nanoparticle immobilized cloth material on guinea pigs {23} revealed no side effects. Silver nanoparticles also find application in topical ointments and creams which is used to prevent infection of burns and open wounds. Many infectious diseases are associated with faecally contaminated water and are a major cause of morbidity and mortality worldwide. Waterborne diseases are caused by enteric pathogens such as bacteria, viruses and parasites that are transmitted by the faecal oral route. Water borne spread of infection by these pathogenic microorganisms depends on several factors such as: the survival of these microorganisms in the water environment, the infectious dose of the microorganisms required to cause a disease in susceptible individuals, the microbiological and physico-chemical quality of the water, the presence or absence of water treatment and the season of the year (Ranjana Agrawal, 2010).

Human enteric viruses associated with waterborne diseases include Adenoviruses, Caliciviruses, Enteroviruses, Hepatitis A virus and Rotaviruses (Shilpi Saxena and Saloni Gangal, 2011). Although excreted in high numbers in faeces by infected individuals, these viruses may be present in low numbers in environmental samples due to dilution. The detection of specific human enteric viruses can be used to confirm the presence of human faecal pollution . Since the

detection of viruses is mostly based on molecular techniques, it is not a cost-effective method to include in routine monitoring of water. Viability of viruses can also not be indicated by molecular techniques and additional cell culture techniques should be included, thereby further increasing the cost and labour.

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## 2. Material and methods

### 2.1. Plant sample collection

*Strychnos potatorum* leaf, bark and seeds were collected from the botanical garden and near agriculture college, Rewa, Madhya Pradesh, India. The leaf, bark and seeds were separated from the plant and dried under shade{25}. After drying, it was powdered and used for our research purpose. All the glass wares (Borosil or Corning) were immersed in cleaning solution for a few hours. Then the glasswares were washed thoroughly with tap water, followed by detergent solution and finally rinsed with distilled water. The cleaned glassware's were dried in hot air oven and stored.

### 2.2. Preparation of plant extract

The water samples were collected from different areas of Rewa district. However, the sampling locations include urban as well rural areas. The samples were collected and taken in pre-cleaned polyethylene bottles. Four Gram of different plant parts like leaf, bark and seeds powder samples of *Strychnos potatorum* were taken separately and mixed with 100 ml of glass distilled water. This mixture was kept at 55 °C for 15 minutes in a water bath and cooled to room temperature and filtered through Whatman No 1 filter paper. This aqueous extract was refrigerated and used for further experiments {26}.

### 2.3. MPN Methodology

To detect the coliform bacteria from the collected water samples. Coliforms can be identified from the following three methods {28}.

#### 2.3.1. Presumptive test

Three sets of tubes (each set containing three tubes and one 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, 10 ml of single strength lauryl tryptose broth is added. Durhams tubes was carefully inserted in all tubes to avoid air bubbles. The set-up is then sterilized. After cooling, 10 ml of water sample was 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{29}. The number of coliforms in the water sample was calculated by comparing the standard chart.

#### 2.3.2. Confirmed test

A loopful of culture from positive lauryl tryptose broth was transferred to brilliant green lactose bile roth tubes. The tubes were incubated at 37°C for 24 hours and examined for the presence of gas in Durham's tube within 48 hours, which constitute a positive confirmed test{30}.

#### 2.3.3. Completed test

A loopful of culture from the positive tubes was 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 {31}.

### 2.4. Identification of the microorganism

The collected samples of microorganisms were grown in nutrient medium. The medium is defined as the substrate in which microorganisms can grow and multiply. For the present study, synthetic medium was used for culturing, conducting biochemical test and antibiotic sensitivity purpose, {32} due to its chemically defined nature and they are also used to study the specific nutritional requirements of the microbes and following tests were conducted to identify the organisms.

### 2.5. Culture examination

Once the growth was found in primary plate, then the identification was carried out by the following systematic method, for examining the types of colony, colour change in the medium, morphology of the cells under stained and unstained conditions{33} and biochemical tests were done by using Bergey's manual.

## 2.6. Antibacterial activity test

### 2.6.1. Preparation of plant extract

The *Strychnos potatorum* plant such as seed were collected and dried at room temperature for 2 – 3 days and further dried at 60 °C. The dried bark, leaves were extracted with solvents such as Aqueous, Ethanol, Chloroform and Petroleum ether separately and incubated at room temperature for 48 hours with stirring at regular interval. The extracts were filtered with the Whatman filter paper and then dried by using rotary evaporator{34}. The filtrate was stored in screw cap bottle at -20 °C for further use.

### 2.6.2. Microbial strains used

Different microbial strains were used to evaluate the antimicrobial effect of which two were Gram positive bacterial strains *Escherichia coli*, *Proteus vulgaris*, *Psuedomonas aeruginosa*. The strains were obtained from water samples and maintained agar slants for further studies.

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## 3. Result and observation

*Strychnos potatorum* also known as clearing nut tree is a deciduous tree with medium height up to 40 feet. Its common name is Nirmali. It belongs to the family Loganiaceae. The tree, which grows to a very large size, produce a shining, black, one-seeded berry{35}. The different water samples such as River, Pond, Bore and Well from different areas of District Rewa, Madhya Pradesh. However, the sampling locations include urban as well rural areas in Rewa. *Strychnos potatorum* leaves, barks and seeds were collected Agriculture college Rewa, Jyanti college Rewa, Madhya Pradesh, India. The seeds are described as broadly lenticular, about half an inch in diameter and a quarter inch in thickness, with a dirty whitish-gray color and it is covered with a thick coat of delicate hairs. It is commonly referred to as clearing nut tree. It is a medium sized glabrous deciduous tree having a height of 6-12 meters. *Strychnos potatorum* leaves, barks and seeds were collected Agriculture College Rewa, Jyanti college Rewa, Madhya Pradesh, India. *Strychnos potatorum* also known as clearing nut tree is a deciduous tree with medium height up to 40 feet. Its common name is Nirmali. It belongs to the family Loganiaceae. The tree, which grows to a very large size, produce a shining, black, one-seeded berry. The seeds are described as broadly lenticular, about half an inch in diameter and a quarter inch in thickness, with a dirty whitish-gray color and it is covered with a thick coat of delicate hairs. It is commonly referred to as clearing nut tree. It is a medium sized glabrous deciduous tree having a height of 6-12 meters. The plant has been described as a common tree for medicinal importance in India and its plant is popular for purifying drinking water.

### 3.1. Identification of the microorganism

The isolated water samples coliform bacteria were grown in nutrient medium for sub culturing and the identification were carried out by examining the types of colony, colour change in the medium, morphology of the cells under stained and unstained conditions. The biochemical tests were done by using Bergey's manual, through this biochemical test following pathogens *E. coli*, and *P. aeruginosa* were identified. That maximum coliform contaminated samples was pond region (> 1100 cells) followed by river (>1100 cells), well (>1100 cells) and least coliform contaminated sample was bore water (240 cells).

### 3.2. Antibacterial Activity

The *Strychnos potatorum* plant seed were subjected for extraction by using different solvents like Ethanol, Chloroform, Petroleum ether and Aqueous separately and the extract was tested for antibacterial activity against Gram positive and Gram negative human pathogen. Well diffusion method was carried out for antibacterial susceptibility testing according to the standard method to assess the presence of antibacterial activities of the plant seed extract. The study result of the antibacterial activity of seed extracts of the *Strychnos potatorum* using solvents were given {38}. The solvents were prepared in different concentrations. Compared with all the concentration. 128 µg/ml concentration gave the maximum zone of inhibition for all the extracts. Polar extracts like aqueous and ethanol are the best solvent for extraction of antibacterial compounds from the *Strychnos potatorum* plants. Best zone of inhibition was produced by the ethanol seed extract *E. coli* (30 mm) and least was produced against *Klebsiella sp.*, (16 mm). The aqueous extract revealed that maximum zone of inhibition was produced by *E. coli* (35 mm) and least was produced against *Klebsiella sp.*, (17 mm). Non-polar extracts like chloroform and petroleum ether were found to be the best solvent for extraction of antibacterial compounds from the plants. Best zone of inhibition was by petroleum ether seed extract *Pseudomonas aeruginosa* (33 mm) and least was produced against *Klebsiella sp.*, (18 mm). The chloroform seed extract revealed that maximum zone of inhibition was produced by *Pseudomonas aeruginosa* (32 mm) and least was produced against *Klebsiella sp.*, (16 mm).

**Table 1** Collection of Water samples from various regions of Rewa District

Number of Water Samples	Places of Water Samples Collected	Types of Water Sample
1	Beehar River Site - 1	River water samples
2	Beehar River Site - 2	River water samples
3	Beehar River Site - 3	River water samples
4	Beehar River Site - 4	River water samples
5	Beehar River Site - 5	River water samples

**Table 2** MPN Analysis

Samples	Presumptive test				Confirmed test	Completed test
	10ml(D.S)	1ml(S.S)	0.1ml(S.S)	100ml		
Sample 1	+	+	+	>1100	Metallic sheen colony in EMB plate	Gram negative rods grown in nutrient agar
Sample 2	+	+	-	240	No Metallic sheen colony in EMB plate	Gram negative rods grown in nutrient agar
Sample 3	+	+	+	>1100	Metallic sheen colony in EMB plate	Gram negative rods grown in nutrient agar
Sample 4	+	+	+	>1100	Metallic sheen colony in EMB plate	Gram negative rods grown in nutrient agar
Sample 5	+	+	+	>1100	No Metallic sheen colony in EMB plate	Gram negative rods grown in nutrient agar

**Table 3** Isolation and Identification of Pathogens

S.No	Test	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>
1	Gram 'sstaining	Gram Negative	Gram positive	Gram negative
2	Motility	Non Motile	Non motile	Motile
3	Spore formation	Non sporing	Non sporing	Non sporing
4	Colony appearance	Round and convex	Round and convex	Rod shape
5	Indole	Positive	negative	negative
6	Methyl red	Positive	positive	negative
7	Voges Proskauer	negative	positive	negative
8	Citrate	Positive	positive	positive
9	Urease	negative	positive	positive
10	Oxidase	Positive	negative	positive
11	Catalase	negative	positive	positive
12	TSI	negative	Acidic fermentation	No fermentation

**Table 4** Phytochemical Analysis of *Strychnos potatorum* leaves, bark and seed

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

#### 4. Discussion

The present study deals with the treatment of drinking water by using plant *Strychnos potatorum* seed powder. Different types of water samples were collected from the Rewa and Sidhi district. The present observation *Strychnos potatorum* seed powder showed positive result for the presence of fluorescence character under UV light and ordinary day light. The results showed the characteristic fluorescence when treated with different reagents which supported results of phytochemical studies. Seed powder showed the presence of alkaloids, flavonoids, saponins, carbohydrates, tannins, sterols, glycosides, phenolic compounds, protein and amino acids and absence of gums, mucilage, oil and fats. In earlier study Mallikh arjuna *et al.* (2007) revealed that the seed, leaves, stem and bark of *Strychnos potatorum* have the phyto constituents such as alkaloids, flavonoids, glycosides, lignin, phenols, saponins, sterols and tannins. Previous research studies leaves of *Strychnos potatorum* showed the presence of alkaloids, glycosides, tannins, flavonoids, sterols, triterphenoids, phenols, quinones, saponins and absence of fats and oils. In the present study the jar test method to treat the water samples to Aluminium sulphate and *Strychnos potatorum* seed powder. The result concluded, with in 30 minutes all the samples effective in the clarification and sedimentation of inorganic and organic matter in raw water. The alum treated water gave the clear appearance compared to all the two samples but the microbial and coliform counts were reduced all the two, so we conclude *Strychnos potatorum* seed powder are natural coagulant for water purification.

#### 5. Conclusion

Our study concludes that leaves, bark and seed were powdered and the aqueous extracts were screened for Preliminary phytochemical investigations. The results showed the characteristic fluorescence when treated with different reagents which supported results of phytochemical studies. Preliminary phytochemical investigations in leaves powder showed the presence of alkaloids, flavonoids, tannins, sterols, glycosides, oil and fats, phenolic compounds, protein, amino acids, gums and mucilage and absence of saponins and carbohydrates. Bark powder showed the presence of alkaloids, flavonoids, carbohydrates, sterols, glycosides, oil and fats, protein and amino acids, gums and mucilage and absence of saponins, phenolic compounds and tannins.

#### Compliance with ethical standards

##### *Disclosure of conflict of interest*

There is no conflict of interest between the authors and research centre.

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