Storage Duration and Temperature Effects of *Strychnos potatorum* Stock Solutions on its Coagulation Efficiency

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Abstract

This study presents the effects of storage duration and temperature of *Strychnos potatorum* stock solution on its coagulation efficiency. Coagulation efficiency of the seed extracts on water samples depended on the initial turbidity of the water sample. The stock solutions could clarify only highly turbid solutions. The optimum dosage of the stock solutions was 5% and optimal time required was 50 minutes. *S. potatorum* stock solutions, which were kept at room temperature (28^0C), had a shelf life of only five days and were able to remove turbidity from high and low turbidity water samples and no coagulation activity was observed for medium turbidity. The highest turbidity removals were observed for stock solutions, which were kept for three days. For stock solutions which were stored in refrigerator, shelf life was extended upto seven days, and the turbidity removal efficiencies improved from 45.9% to 63.8% for low and 43.7% to 64.9 % for high turbidity water samples, respectively.

Keywords: Strychnos potatorum, natural coagulant, storage duration, storage temperature, turbidity removal

1. Introduction

Groundwater is the preferred source for drinking water in rural areas of developing countries and it generally requires no or minimal treatment. In the event that no suitable aquifers are available, relatively clean waters from lakes or streams are preferred. However, only simple, practical technologies such as gravity chemical feed with solutions, hydraulic rapid mixing and flocculation, horizontal-flow sedimentation, and manually operated filters should be used for treatment of such waters (Schulz and Okun, 1984). The use of natural material of plant origin to clarify turbid surface waters is not a new idea. The use of herbal materials to reduce turbidity, or muddiness in the water and to remove the harmful biological material that can lead to illness is an age-old concept (Joshua and Vasu, 2013). Natural coagulants have been used for domestic household for centuries in traditional water treatment in rural areas. Sanskrit writings in India dating from several centuries BC make reference to seeds of the tree *Strychnos potatorum* as a clarifier (Bhishagratna, 1991), Peruvian texts from the 16th and 17th centuries detail the use by sailors of powdered, and roasted grains of *Zea mays* as a means of settling impurities. More recently, Chilean folklore texts from the 19th century refer to water clarification using the sap from the *'tuna' cactus* scientifically known as *Opuntia fiscus indica* (Sutherland et al., 1990). However, of all the plant materials that have been investigated over the years,

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the seeds from *M. oleifera* have been shown to be one of the most effective as a primary coagulant for water treatment (David, 2004) of turbid water (Schulz and Okun, 1984).

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 polyelectrolytes. Even under such conditions, a few plant seeds make effective coagulants (Jahn, 1988), for example, seeds of the plant species of the family Loganiaceae (*Strychnos potatorum*), and Moringaceae (*Moringa oleifera* and *Moringa stenopetala*). *S. potatorum* is a small tree occurring abundantly in central and southern India. In laboratory and field studies, seeds of *S. potatorum* have shown promise as coagulant in the clarification of turbid water (Sen and Bulusu, 1962; Dhekane et al., 1970; Tripathi et al., 1976; Jahn, 1988; Sutherland et al., 1990, 1994; Folkard et al., 1995; Al-Khalili et al., 1997; Folkard and Sutherland, 2002).

Studies using the species have focused on quality of water treated by coagulation using the seed. In laboratory tests, direct filtration with *S. potatorum* seed as coagulant appeared effective in clarifying low turbidity water (Abu-Ghararah, 1983). Rodrigo (2011) conducted a study aimed at investigating the effectiveness of *Strychnos potatorum* seed powder coagulant for the removal of turbidity, water hardness causing cations (Mg^{2+}, Ca^{2+}), heavy metal cations (Pb^{2+}, Cr^{3+}, Cd^{2+}), Fluoride and Chemical Oxygen Demand (COD) of treated water. Muthukumaran et al. (2013) used different types of coagulants like *Moringa oleifera*, *Phaseolus vulgaris* and *Strychnos potatorum* for clarifying water. However, storage studies on the seed material have not been attempted. Since home treatments demand the use of powder or paste, the goal of the present study was to assess suitability of efficient method for home water treatment in rural areas of developing countries using stock solutions of *S. potatorum* and its possible shelf life. Since, systematic studies on the effects of storage duration and condition on its performance have not yet been carried out, this study aimed to investigate the same.

2. Materials and Methods

2.1 Preparation of stock solutions

Strychnos potatorum seeds were collected from Mettupalaym, Coimbatore, India. The seeds were dried in a hot air oven for 24 hours at 50^0C. The seeds were crushed and ground to a medium fine powder with a domestic food blender (Tector). Any traces of fat was removed by mixing the powder in 95% ethanol (5-10% w/v) for 30 minutes and the solids separated by centrifugation and dried at room temperature. 5,000 mg of defatted *S. potatorum* seeds powder was placed in beaker containing 0.1 L of distilled water. The mixture was blended to extract the active ingredient of *Strychnos potatorum*. The suspension was then filtered through a muslin cloth and the filtrate made up to 0.5 L to give a stock solution of 10,000 mg/L. 10,000 mg/L of *S. potatorum* stock solution was used for test trials that were conducted to determine optimum dosages of *S. potatorum* on water samples of varying turbidities.

Turbidity stock solution

Stock kaolin solution was prepared as 1% (w/v) using distilled water, stirred slowly at 20 rpm for 1 hour for uniform dispersion of kaolin particles. The suspension was then allowed to stand for 24 hours to allow for complete hydration of the kaolin. This kaolin suspension was used as the stock solution for the preparation of water samples of varying turbidities for the coagulation tests. Three types of turbidities were carried out namely;

i. Low turbid: less than 50 NTU
ii. Medium turbid: in between 50-200 NTU
iii. High turbid: more than 200 NTU

Storage of seeds stock solution

In order to study the effects of storage temperature, the stock solutions were divided into two groups and stored at two different temperatures namely; room temperature (28^0C) and at 4^0C (refrigerator). The effects of storage duration on *Strychnos potatorum* stock solution were investigated for 0, 1, 3 and 5 days.

2.2 Optimisation of dosage for Strychnos potatorum extracts

Three sets of experiments were carried out with different levels of turbid solutions (Low, Medium and High).

i. Optimization of *Strychnos potatorum* dosage with varying concentration (5-25 %) at room temperature and 4^0C.
ii. Completely randomized experimental runs with varied time durations (0-150 minutes) and varying storage conditions (room temperature and 4^0C).
iii. Completely randomized experiments with varying storage conditions (room temperature and 4^0C) on different days (0, 1, 3 and 5).

The water samples were agitated at the preselected intensity of rapid mixing to obtain samples of varying turbidities. During rapid mixing the crude extract of seeds were added into the samples simultaneously. Subsequently the mixture was left for sedimentation to take place. Following settling, samples were taken from the middle of each beaker using a pipette and placed in a cuvette for turbidity measurement. For determining the optimum dosage, varying stock concentrations were added into the beakers having different turbidity levels.

2.3 Turbidity measurement

Turbidity measurements were made using a Nephelometer (Systronics) and expressed in Nephelometric turbidity units (NTU) in relation to a control sample in a 1 ml cuvette.

2.4 Statistical Analysis

Results, means and standard deviation (S.D.), of the experiments were carried out in all the methods. Statistical significance of the differences between mean values was assessed by ANOVA test.

3. Results and Discussion

3.1 Optimization of dosage of Strychnos potatorum for turbidity removal

Results on optimum dosage of *S. potatorum* to obtain highest turbidity removal on different samples are presented in Table 1. Turbidity removal efficiency of *S. potatorum* on low, medium and high level turbid water at 5% was 37.5, 21.8 and 23.8%, respectively. With increasing initial turbidity of the samples, the turbidity removal efficiency reduced. Further, for optimum dosage of *S. potatorum* seeds extract it was observed that increase in dosage reduced the turbidity removal efficiency. This result revealed that the low turbid water sample showed better performance in terms of turbidity removal in the case of *S. potatorum*.

3.2 Effect of storage on optimal dosage of Strychnos potatorum for turbidity removal

Results on the effect of storage on the optimum dosage of *S. potatorum* to obtain highest turbidity removal on different samples are presented in Table 2. Results showed that highest turbidity removal was observed with medium turbid samples. It was observed that in the case of *Strychnos* unlike in *Moringa*, the turbidity removal efficiency of the extracts improved with storage. Following storage of extract, the turbidity removal efficiency was improved by 26, 63 and 54% for low, medium and high turbidity samples. It was observed that increase in dosage did not improve turbidity removal. However, in the low turbid samples, dosage did not show any significant reduction in efficiency. This suggests that *S. potatorum* seed extract would be effective for low turbid samples following storage of the extract under cold conditions. Turbidity removal efficiency of *S. potatorum* on low, medium and high level turbid water at 5% was 50.7, 60 and 52.2%, respectively. This result revealed that although the same dosage of *Moringa oleifera* seed extract applied on the three types of water samples, the medium turbid water sample showed better performance in terms of turbidity removal.

Table 1: Optimization of dosage of *S. potatorum* extracts on different samples.

Strychnos potatorum Extract	Turbidity Measurements (NTU)		
	Low	Medium	High
Control	54.7	174	281
5 %	34.2	136	214*
10 %	36.8	142	229
15 %	39.3	152	244
20 %	43.5	151	256
25 %	48.0	165	271
Std. Deviation	9.99	6.32	7.91
Test Value t	5.87	5.04	3.85
Sig. (2-tailed)	0.004	0.007	0.018
Lower limit	13.83	6.39	3.78
Upper limit	38.65	22.09	23.42

* Significant variation ($p \leq 0.05$)

Table 2: Effect of storage on optimal dosage of *S. potatorum* extracts on different samples.

Strychnos potatorum Extract	Turbidity Measurements (NTU)		
	Low	Medium	High
Control	56	185	312
5 %	27.6	74	149*
10 %	29.2	80	257
15 %	28.2	86	293
20 %	31.6	98	294
25 %	33.2	113	295
Std. Deviation	2.80	8.41	20.09
Test Value t	38.54	13.62	1.94
Sig. (2-tailed)	0	0	0.12
Lower limit	44.80	40.80	37.51
Upper limit	51.76	61.68	42.39

* Significant variation ($p \leq 0.05$)

3.3 Effect of time duration on performance of Strychnos potatorum extracts

The effect of time period to obtain highest turbidity removal on different samples using *S. potatorum* is presented in Tables 3 and 4 and Figure 1. The extended duration was up to 150 minutes. Results showed that higher turbidity removal was observed as initial turbidity of water samples was increased. Though initially there was no significant variation in turbidity removal in the low and high turbid samples, following the extended duration, the variations observed was significant suggesting that increase in time period could improve turbidity removal. Medium turbid samples could be effectively coagulated in 50 minutes indicating that *S. potatorum* seed extract would be effective for medium turbid samples in shorter periods of time. With extended durations, turbidity removal efficiency increased for low and high turbid samples. However, it was observed that for medium and high turbid samples, with increasing time period, the residual turbidity was found to increase suggesting that longer durations may not be able to effectively bring in turbidity removal. The time period for significant turbidity removal efficiency of *S. potatorum* extracts on low, medium and high level turbid water was 90, 50 and 90 minutes, respectively. In this experiment, medium turbid water sample showed quicker turbidity removal.

Table 3: Effect of varying settling time on performance of *S. potatorum* extracts.

Time in minutes	Turbidity Measurements (NTU)		
	Low	Medium	High
0	48.4	182	252
10	48.1	115	209
20	44.4	102	234
30	46.7	85	193
40	47.8	86	193
50	44.8	82*	192
60	45.1	73*	184

* Significant variation ($p \leq 0.05$)

Table 4: Effect of extended durations of settling on performance of *S. potatorum* extracts.

Time in minutes	Turbidity Measurements (NTU)		
	Low	Medium	High
0	59.9	182	238
90	40.7*	145	168*
120	40.6*	140	162*
150	39.6*	149	191
Std. Deviation	14.30	16.34	7.36
Test Value t	2.94	7.41	9.18
Sig. (2-tailed)	0.02	0.00	0.00
Lower limit	3.01	27.79	16.87
Upper limit	24.99	52.90	28.19

* Significant variation ($p \leq 0.05$)

3.4 Effect of storage on time duration to assess performance of Strychnos potatorum extracts

The effect of storage on the time required to obtain highest turbidity removal using *Strychnos potatorum* on different samples are presented in Tables 5 and 6 and Figure 2. Highest turbidity removal was observed with low turbid samples. It was observed that the time duration for turbidity removal reduced with storage. The medium turbid samples showed the fastest removal. This further reiterates the results in Table 2 that *Strychnos potatorum* seed extract would be effective for turbid samples following storage of the extract under cold conditions.

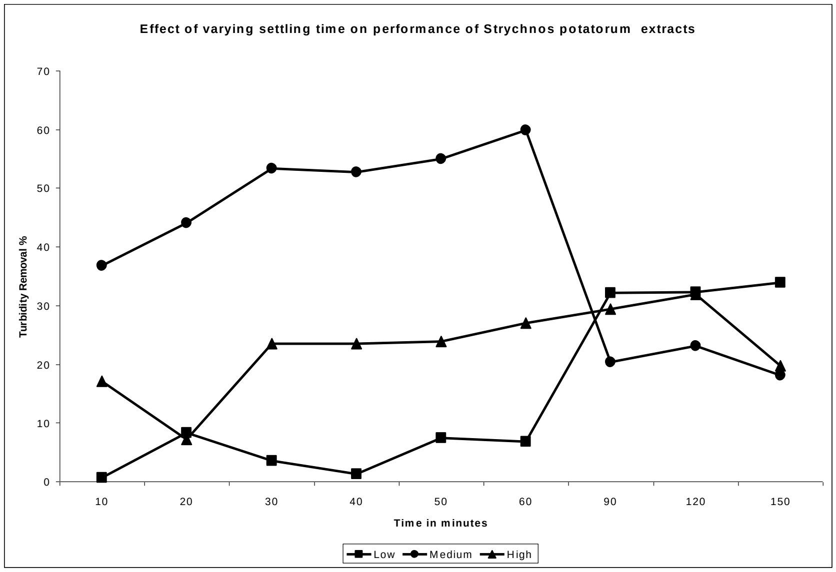

Figure. 1: Effect of varying settling time on performance of Strychnos potatorum extracts.

The low turbid samples could be coagulated in 60 minutes, the medium in 50 minutes and the high at 90 minutes. The results obtained following storage, low turbid samples could be clarified faster while the medium and high turbid samples were on par with the performance of extracts stored at room temperature. However, it was observed that for all the samples, with increasing time period, the residual turbidity was found to increase, suggesting that longer durations may not be able to effectively bring in turbidity removal. In this experiment also, medium turbid water sample showed quicker turbidity removal.

Table 5: Effect of storage on turbidity removal of *S. potatorum* extracts on different samples at varying time durations.

Time in minutes	Turbidity Measurements (NTU)		
	Low	Medium	High
0	62.1	191	246
10	47.1	105	219
20	42.2	86	202
30	42.2	84	195
40	41.9	84	190
50	39.4	79*	179
60	38.2*	79*	174

* Significant variation ($p \leq 0.05$)

Table 6: Effect of storage on turbidity removal of *S. potatorum* extracts on different samples at extended time durations.

Time in minutes	Turbidity Measurements (NTU)		
	Low	Medium	High
0	59.9	182	238
90	36.2*	139	147*
120	39	133	165
150	35.8*	131	151*
Std. Deviation	5.04	14.94	8.84
Test Value t	20.54	9.10	8.84
Sig. (2-tailed)	0.00	0.00	0.00
Lower limit	30.61	33.82	19.25
Upper limit	38.35	56.78	32.84

* Significant variation ($p \leq 0.05$)

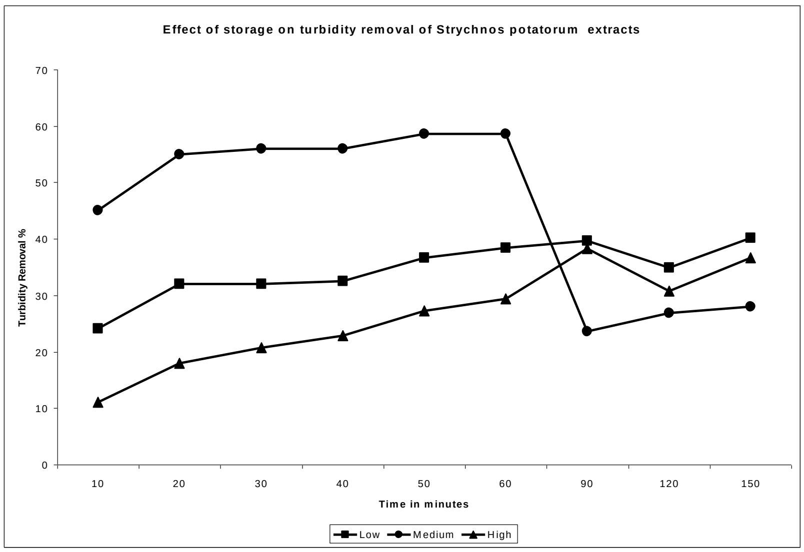

Figure. 2: Effect of storage on turbidity removal of *S. potatorum* extracts.

3.5 Effects of storage duration of Strychnos potatorum extracts kept at room temperature (28^0C)

Table 7 shows the results of turbidity removal using *Strychnos potatorum* stock solutions, which were kept for 1, 3 and 5 days at room temperature. The results showed that *Strychnos potatorum* kept under this condition were able to remove turbidity from low and high turbidity water samples. However, it was observed that in the medium turbid samples, the residual turbidity was found higher than of the initial turbidity levels. The experiment was not conducted beyond day 5 as the extract began to decay. For the low and high initial turbidity value water samples, the highest turbidity removals were observed for stock solutions which were kept for five days. The turbidity removal efficiencies improved from 31.5 to 52.4 for low and 26.1 to 29.4% for high turbidity water samples, respectively.

Table 7: Effects of storage duration of *S. potatorum* extracts at room temperature.

Days	Turbidity Measurements (NTU)		
	Low	Medium	High
Day 0	69.9	136	245
Day 1	47.9	94	181
Day 3	46.7	199	189
Day 5	33.3	165	173
Std. Deviation	11.87	.	3.25
Test Value t	0.03	.	0.01
Sig. (2-tailed)	38.70	.	26.13
Lower limit	9.21	.	18.06
Upper limit	68.19	.	34.21

* Significant variation ($p \leq 0.05$)

3.6 Effects of storage duration of Strychnos potatorum extracts kept under refrigeration (4^0C)

Table 8 shows the results of turbidity removal using *Strychnos potatorum* stock solutions**,** which were kept for 1, 3 and 5 days under refrigerated conditions. The results showed that *Strychnos potatorum* extract kept under this condition was able to remove turbidity from low, medium and high turbidity water samples. However, it was observed that except in the high turbid samples, the medium and low turbid samples did not show a linear increase in turbidity removal. In these cases, the residual turbidity after coagulation on the third day was found higher than the first day, which further reduced on day 5, beyond which the experiment was not conducted.

For the low and high initial turbidity water samples, highest turbidity removals were observed for stock solutions which were kept for five days. The turbidity removal efficiencies improved from 45.9 to 63.8 for low and 43.7 to 64.9% for high turbidity water samples, respectively. Another interesting observation made through these experiments was that the turbidity removal efficiency of *S. potatorum* stock solutions increased following storage and with increase in the number of days stored. The maximum was observed in the stock stored for 5 days under refrigerated conditions (64.9%).

Table 8: Effects of storage duration of *S. potatorum* extracts kept at 4^0C.

Days	Turbidity Measurements (NTU)		
	Low	Medium	High
Day 0	69.9	136	245
Day 1	37.8	153	138
Day 3	39.6	85	88
Day 5	25.3	131	86
Std. Deviation	11.13	23.90	12.02
Test Value t	7.94	1.22	8.30
Sig. (2-tailed)	0.02	0.44	0.01
Lower limit	23.39	-194.13	27.72
Upper limit	78.67	235.33	87.41

* Significant variation ($p \leq 0.05$)

Figure 3 gives an overall view of the effects of temperature on the turbidity removal efficiency of *S. potatorum* stock solutions.

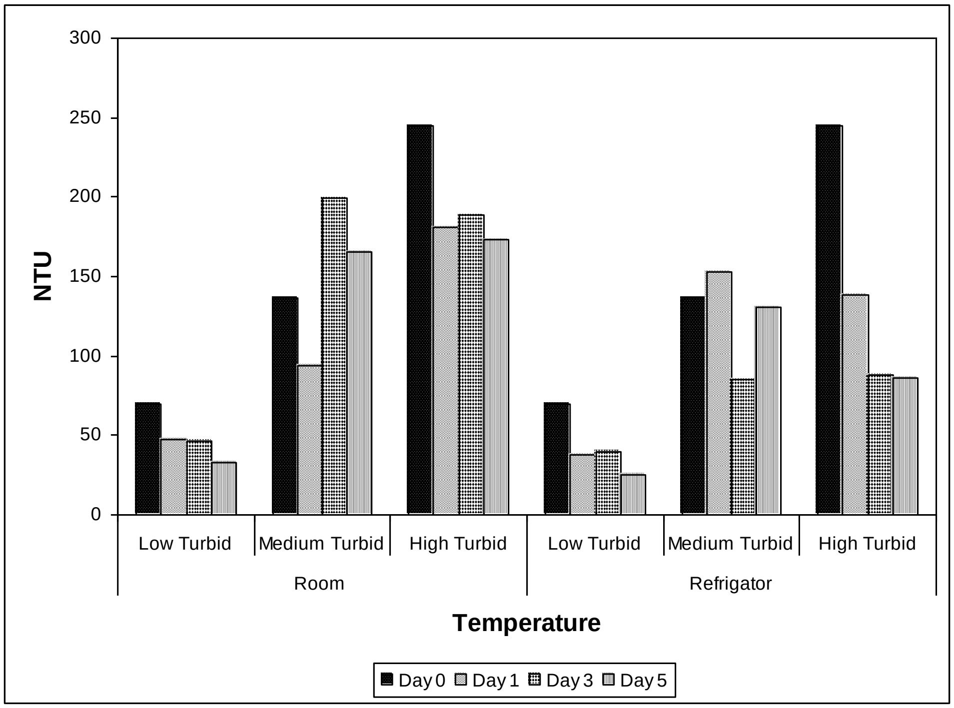

Figure 3: Effects of temperature on the storage duration of *S. potatorum* extracts.

A number of effective coagulants have been identified of plant origin. Some of the common ones include nirmali (Tripathi et al., 1976), *M. oleifera* (Olsen, 1987; Jahn, 1988), okra (Al-Samawi and Shokrala, 1996), *Cactus latifaira* and *Prosopis juliflora*, tannin from *Valonia* (Özacar and Sengil, 2000), apricot, peach kernel and beans (Jahn, 2001), and maize (Raghuwanshi et al., 2002). Bhole (1995) compared 10 natural coagulants from plant seeds. Since the early 1970's a number of studies have been carried out to determine the effectiveness of the seeds for the treatment of surface water (Olsen, 1987; Jahn 1988; Sutherland et al., 1994; Muyibi and Evison, 1995, 1996; Ndabigengesere and Narasiah, 1998; Ghebremichael et al., 2005) using different extraction methods for the active coagulant from *M. oleifera.* Utilising artificially prepared turbid water and naturally turbid raw waters, laboratory investigations have confirmed the seeds to be highly effective in the removal of suspended solids from waters containing medium to high initial turbidities (Sutherland et al., 1994). A comparative study on electro-coagulation and natural coagulation (*M. oleifera*) methods devised for the treatment of pharmaceutical residues from both, synthetic solutions and real pharmaceutical wastewaters revealed that electro coagulation was a more effective technique than natural process (Dixit and Parmar, 2013).

Muthukumaran et al. (2013) reported that sodium chloride extract of *Moringa* was found to provide a high turbidity removal of >99% compared to NaOH and distilled water extract of the same species. Effective turbidity by *Strychnos* was not effective. However, our studies have proved the efficacy of the water extract of the seeds of *S. potatorum* in claryIng turbid waters to as high as 80%. Rodrigo (2011) showed effectiveness of *S. potatorum* for turbidity removals of up to 92%, 91% and 85% for initial turbidities of 25, 50 and 100 Nephlometric Turbidity Units (NTU) respectively. Being a natural coagulant, the species is non-toxic and an effective coagulant aid useful for removing turbidity from water. Muthukumaran et al. (2013) report that the presence of anionic polyelectrolyte, which contains carboxylic (COO^-) and hydroxyl (OH^-) as main active groups which might play a role in coagulation.

4. Conclusions

Much progress has been made in investigating the coagulation potential of crude seed extracts as well as purified seed proteins from *M. oleifera*, which have been proven to be the main active agent in seed based coagulants. However, the species *S. potatorum*, which is popularly used in rural areas for waste water treatments, especially on a large scale has not been studies in depth. Hence the present study was taken up to investigate the performance of *S. potatorum* as a primary coagulant. Our studies showed that *Strychnos* extract was effective only for high turbid solutions. It was also observed that storage of extracts at 4^0C did not improve its turbidity removal efficiency. Reduction in turbidity in our experiments is also comparable to those achieved by natural coagulant like *M. drouhardii*, *M. stenopetala* and *M. peregrine* seeds as reported by Jahn (1988). These results are also comparable to the result obtained by Aririatu et al. (1999), which indicates a reduction in turbidity using *Moringa* seeds in the range of 72.8-92.4% while *Jatropha curcas* was 75.2-84.7%. Chemical coagulants, like aluminium sulphate reduced the same turbidity of the same effluent in the range of 66.00-84.29% and ferric chloride in the range of 98.29-99.80%. Turbidity removal is very dependent on proper coagulant doses and time but again the fine adjustment is less critical for highly turbid waters, which are thus also easiest to treat in this respect. The chemical composition of the coagulant in *S. potatorum* has been identified as a polysaccharide consisting of a 1:7 mixture of galactomannan and galactan. These findings suggest that such seed extracts may function as a particulate, colloidal and soluble polymeric coagulant as well as a coagulant aid. The presences of other constituents in these seed extracts are uncertain, because the portions of the plant also are used for medicinal purposes. Also, little has been done define, optimize and standardize conditions for their use.

Thus use of easily available indigenous plants might be seriously considered as a temporary solution where water treatment is badly needed and where the people are very motivated to start as soon as possible. Pulses, such as *Lens esculenta, Cajanus indicus* and *Phaseolus roxborghii* have been utilized with good success as coagulant aids. The above results confirmed the use of this natural coagulant as a good clarifying agent and also unveil its potential as material for water clarification through coagulation and flocculation process. These studies have indicated that clarification and pre-purification (partial purification) of raw water and waste water samples can be obtained using *S. potatorum*. This method is simple, quick and does not require any special device, regulatory control and technology and would find applicability for home water treatment in rural areas of developing countries.

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