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Defluoridation of water with a coagulant, *Strychnos potatorum* L. seed –agglutinin

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ABSTRACT

The aim of the present study is to assess the suitability of *Strychnos potatorum* L. seed protein for defluoridation of potable water. Protein was isolated from *Strychnos potatorum* L. seed by using Ammonium sulphate precipitation method and coagulation activity was confirmed. Later the extracted protein was used for defluoridation studies of aqueous fluoride solution and real potable water. The effect of three parameters including pH, time and coagulant dosage of protein on the fluoride removal were studied in fluoride aqueous solution. About 52 potable water samples in and around Kodaikanal were collected from tribal and non-tribal areas and water quality was assessed. The exceeding amount of fluoride among 5 water samples was found when compared with BIS standard for drinking water and subjected to defluoridation process with coagulant protein. *Strychnos potatorum* L. seed coagulant protein removed about 75% of fluoride from aqueous fluoride solution (2 ppm) by 2 h treatment time, at 6.6 pH with 0.1 g of coagulant dose, treatment. Potable water defluoridation also showed 75% of fluoride reduction with 0.1 g of coagulant dose treatment. Seed characterization was performed using GC–MS and FT-IR, also proteomic studies carried out using MALDI-TOF. The seed coagulant protein showed, horcolin, a lectin type agglutinin protein (15.1 kDa) with 146 amino acid residues. This study suggests, the horcolin agglutinin protein in seeds is capable to remove fluoride effectively from drinking water samples due to the presence of its metal binding sites.

1. Introduction

Water is lifeblood and elixir of life. Fresh water resources available on hill stations are free from toxic industrial pollutants and require no or less purification. Yet, these drinking water resources acquire fluoride like contaminants

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possibly contributed by granitic rocks or other anthropogenic activities. The people of hill stations mostly depend on open water resources and underground water for all domestic and agriculture purposes. The water with the higher amount of fluoride is unfit for drinking and affects their health causing dental fluorosis and skeletal fluorosis. Phytocoagulants are naturally occurring plant based material which can be used for flocculation–coagulation process of water/wastewater treatment for the reduction of dissolved solids and turbidity. The element fluoride occupies a special place in the daily routine of human life as it encompasses positive impacts such as developing and strengthening of bone, teeth when present in appropriate proportions. Products enhanced with fluoride particles (toothpaste to avoid dental decays) are available in the market to benefit human society because its poor concentration stimulates osteoblasts. Besides, the presence of its higher concentration in any state of matter accessed by human population is unsafe. Regular intake of drinking water with increased fluoride amount builds dental and skeletal disorder in our physique (Biswas et al., 2009). The strong electro negativity potential of ions present in fluoride powerfully associates it to the positively charged calcium ions of our skeletal frame. Also, it obstructs our physiological metabolisms (carbohydrate, protein, mineral and DNA formation) under chronic exposure to high concentrations. The contamination of fluoride and arsenic in drinking water is an essential quality issue. Some natural and anthropogenic activities contribute dramatically to the fluoride recruitment in the ecosystem such as natural decomposition, dissolution and dissociation of rocks which comprises fluoride-bearing minerals like topaz, apatite, amphiboles, cryolite, fluorite and industrial activities (Shahid et al., 2020).

Globally, fluorosis occurrences are reported in Asian countries such as China, Pakistan, Thailand and India. Apart from these nations, Nigeria and South America are also included in this list. Fluorosis has spread its tentacles throughout many countries, including India, Mexico, China, Argentina, and Tanzania. Throughout 220 million individuals around the world are at risk of drinking fluoridated water (Naga Samrat et al., 2017).

Hence, the World Health Organization has set an acceptable guideline limit for fluoride concentration in drinking water (below 1.5 mg/L). According to CGWB reports from 2010, groundwater in 19 Indian states is highly contaminated with fluoride. The first report on the prevalence of excessive fluoride content in groundwater in all of India was published in Andhra Pradesh in 1937 (Short et al., 1937).

Fluorine occurs naturally in most minerals. The entry of fluoride into surface or groundwater occurs by the dissolution or weathering of phosphate rocks, use of phosphate fertilizers for crop cultivation. Fluoride removal in drinking water is performed following several processing techniques such as adsorption, precipitation, ion exchange, membrane filtration, electrodialysis, chemical coagulation and flocculation techniques.

As occurrences of fluoride in fresh drinking water resources is a natural phenomenon, implementation of control methods to maintain the optimal concentration required is the only way to prevent fluorosis caused by fluoride ion in human Shen and Hankins (2017) used a polymer surfactant aggregate process to remove metallic anion from dilute aqueous solutions. Inter-particle bridging was the primary removal mechanism for flocculant. $FeCl_3$, alum, chitosan, Nirmali, as well as Okra, red bean, red maize, *Moringa oleifera*, *Cactus latifera*, and seed powder of *Prosopis juliflora*, were shown to be the most effective at removing trace ion metals from drinking water and wastewater. Electro coagulation method was used to remove the metals. Ramirez et al. (2018) investigated the removal of phosphate from an aqueous solution containing 100 mg P/L using HFeO as an adsorbent material on fixed-bed column systems. According to Kameda et al. (2018), using magnesium oxide to remove fluoride from sodium fluoride aqueous solution involved an adsorption mechanism. The Nalgonda Technique is chosen to remove fluoride at all levels because of its low cost and ease of handling. The Nalgonda Technique has been implemented in Indian villages and tested on a small scale in Kenya, Senegal, and Tanzania (Lagaude et al., 1988). But this technique has some disadvantages like higher pH and residual aluminium. Vendittia et al. (2018) used a synthetic coagulant called Actifluo to remove fluoride from waste water from factories in the south of Italy as well as synthetic wastewaters. The common agents used for fluoride removal are reported using chemical coagulants (aluminium and iron salts) from aqueous solutions. The major delimiting factors applying these synthetic coagulants are large sludge generation and alteration of pH of treated water. Hence, researches and scientists are now shifting and acclimatizing to natural-based materials for the fluoride removal studies. *Strychnos potatorum* L. plant is classified under family Loganiaceae and commonly termed as 'clearing nut tree' and in Tamil language known as 'Tetankottai'. It is a moderate sized tree mostly spread across Asian countries like India, Sri Lanka and Burma. The plant parts and their phytochemical extracts are mainly used in traditional medicines. The matured seeds of the tree are used to treat gonorrhoea, bronchitis, chronic diarrhea, diabetes, dysentery, ulcer, conjunctivitis and eyes related disorders. From ancient times *Strychnos potatorum* L. seed materials are used to purify drinking water due to the presence of alkaloids, colloidal polysaccharides and protein fractions present in it. Studies report *Strychnos potatorum* L. seeds removed lead and fluoride from aqueous solution. Rodrigo (2011) looked into the impact of Strychnos potatorum L. seed powder coagulant for the removal of turbidity, hardness, heavy metals, fluoride, and chemical oxygen demand (COD) in contaminant water and discovered that the seed powder's major clarifying potential was due to the presence of polysaccharide and protein functional groups such as COOH, OH. In terms of fluoride in drinking water, it is a short-term catastrophe because it has both beneficial and detrimental impacts on human health. Consequently, depend on biocoagulants to trim down elemental contaminants present in water (within standard limits) would be an eco-friendly approach. Further, the natural plant-based materials are cheap to afford due to its availability around the hour. Okra and passion fruit seeds, according to Muniz et al. (2020), are viable sources for acquiring coagulant agents that can be utilized to replace metallic coagulants used in agro-industrial waste water treatment.

Proteins having the capability of coagulation, agglutination, and flocculation, plays important role in the search of natural water purification methodologies, traditionally the seeds from *Moringa oleifera* Lam. and *Strychnos potatorum* L.

were used to remove micro dust particles and hardness from the water. The ~15KDa lectin like protein extract from the *Strychnos potatorum* exhibit water purification properties, the MALDI analysis of this protein shows similar amino acid sequence with horcolin, a mannose specific lectin present in *Hordeum vulgare subsp. Vulgare (domestic barley)*. The three-dimensional structure of horcolin was modelled using homology modelling technique, to explain the fluoride removal properties of horcolin the modelled structure and the sequence were analysed with other lectins having capabilities of binding to fluoride salts in water. The presence of fluoride salts such as magnesium fluoride, calcium fluoride, and sodium fluoride in hillside water rendered it unfit for human consumption. The lectins such as Concanavalin A, chitin binding lectins were considered as metal dependent lectins where their sugar binding affinity was enhanced or inhibited in occurrence of metal ions such as magnesium, calcium, manganese and zinc. The literature review on the effect of the salts on lectins shows change in structure on metal binding and decrease in sugar binding affinity in presence of chloride salts in mulberry seed lectins. The structure analysis of the metal binding lectins with beta fold shows the metals binds in the region of loop between the beta strands. The modelled structure of horcolin confirms the horcolin will binds to metal, in the presence metal fluoride the horcolin participates in binding of metal bonded with fluoride which leads to decrease in fluoride content of water (Abhilash et al., 2013).

Tribal and non-tribal people of Kodaikanal villages mostly depend on the available freshwater zones from the hills for all sorts of domestic and agricultural purposes. But the presently accessible hill water sources may contain several salts and mineral components emerged due to anthropogenic and natural environmental accomplishment. Hence, the study was aimed for defluoridation of potable water using *Strychnos potatorum* L. seed coagulant protein.

2. Materials and methods

2.1. Collection of potable water samples

Table S1 shows the 52 sampling locations in and around Kodaikanal were collected from tribal and non-tribal areas, where fresh drinking water samples were collected in pre-cleaned and sanitized bottles for this investigation during summer season from May, 2017. The obtained water samples were properly labelled and transferred to the laboratory in a safe manner. To avoid physico-chemical changes, all the water samples were kept at 4 degrees Celsius and used in subsequent experiments.

2.2. Physico-chemical characterization of potable water samples

As per standard protocol, all drinking water samples were tested for pH, electrical conductivity, total solids, total dissolved solids, acidity, alkalinity, dissolved oxygen, calcium, chloride, hardness, fluoride, phosphate, and sulphate, among other physical and chemical parameters (APHA, 2012). Merck and Sigma Aldrich provided all the chemicals utilized in this investigation. Throughout the experiment, double distilled and deionized water was used. Except for pH and EC, all of the parameters studied are expressed in milligrammes per litre. All of the water samples' drinking water characteristic values were compared to the BIS drinking water standard.

2.3. Extraction of coagulant protein from Strychnos potatorum L. seeds

Strychnos potatorum L. seeds shown in graphical abstract were gathered from Mathur, Pudukkottai (Dt), Tamil Nadu, India. To eliminate dirt particles stuck to the seed surface, the mature and healthy seeds were washed with running tap water and then rinsed thoroughly with distilled water. The seeds were air dried in the shade and ground using rice mill equipment. The seed powder was sieved (0.075 mm) and then precipitated in a 60 percent ammonium sulphate solution for protein molecule analysis. The precipitated protein was collected and evaporated to dryness. The isolated seed protein has a fine, coarse, and smooth texture. Later, the dried protein was stored in an airtight container for defluoridation tests in drinking water.

2.4. Seed coagulation activity test for the isolated protein fraction from Strychnos potatorum L

The isolated protein fraction from *Strychnos potatorum* L. seed powder for its coagulation efficiency was tested against 1% kaolin/synthetic clay solution using tap water. This experiment was carried out as illustrated by Ghebremichael et al. (2005).

2.5. Studies on fluoride ion removal from aqueous solution using Strychnos potatorum L. seed coagulant protein

Aqueous fluoride stock solution was prepared with sodium fluoride salt in deionized water. The stock solution was used to make 100 mL of working fluoride solutions with different concentrations such as 1, 2, 3, 4, and 5 ppm. Seed coagulant protein (0.05, 0.1, 0.15, 0.2, 0.25, and 0.3g) was added to each conical flask to investigate the elimination of fluoride ions from aqueous solutions. The added seed powder was initially mixed well and the mixture was left undisturbed for 2 h. The aqueous solution was filtered with Whatmann No.1 filter paper and was examined for fluoride removal assay.

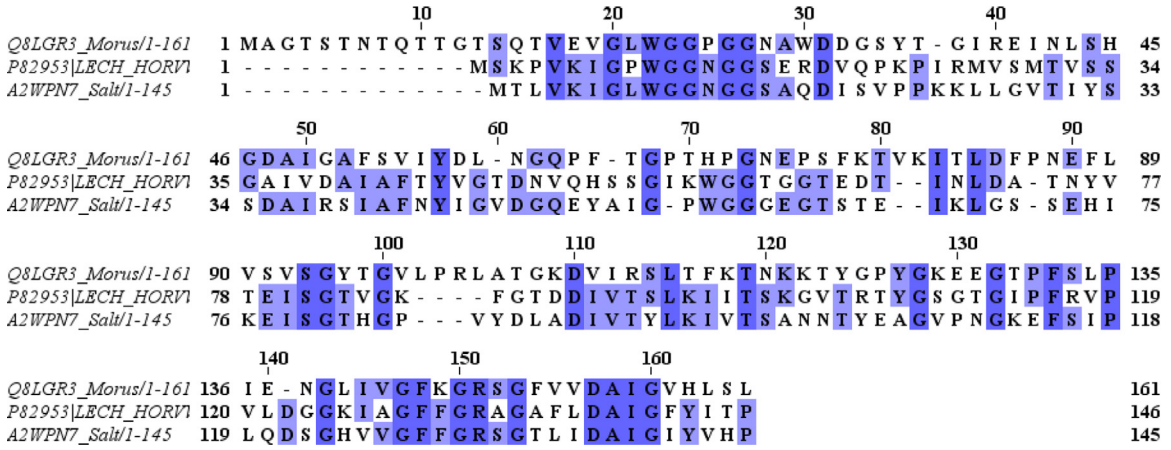

Fig. 1. Sequence alignment of horcolin (Uniprot Id-P82953), mulberry seed lectin (Uniprot Id-Q8LGR3) and salt stress induced carbohydrate binding protein (Uniprot Id-A2WPN7). The dark blue region shows high similar amino acid and pale blue regions shows less similar amino acid regions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Similar experiment was carried out for different pH (6.2, 6.4, 6.6, 6.8, 7, and 7.2) and for various time periods (30–180 mts) to find the optimum pH and optimum time respectively. A control conical flask was maintained for each aqueous fluoride concentrations. The percentage of fluoride removal was noted from each concentration and the experiment was performed numerous times to determine the best seed coagulant dose for each aqueous fluoride concentration.

2.6. Studies on fluoride removal from the potable water samples using Strychnos potatorum L. seed coagulant protein

A 100 ml of each fluoride contaminated drinking water samples (Keelpoomi, Naidupuram, Villpatti, Pallangi and Perumal malai) were treated with 0.1 g of *Strychnos potatorum* L. seed coagulant protein, which was screened and confirmed from aqueous fluoride removal studies. The seed coagulant was added into conical flasks filled with each water samples, mixed thoroughly and left undisturbed for 2 h. The treated water samples were filtered using Whatmann No. 1 filter paper before being tested for fluoride removal %. All the treatment experiments were repeated thrice to confirm the percentage of fluoride removal.

2.7. Characterization of Strychnos potatorum L. seed

2.7.1. GC–MS analysis of the Strychnos potatorum L. seed

The phytocompounds (1 μl) extracted from *Strychnos potatorum* L. seed powder with methanol were employed for GC–MS analysis. Clarus 500 GC The experiment was performed using a Perkin Elmer system that included an AOC-20i auto sampler and a gas chromatograph connected to a mass spectrometer (GC/MS). The mass spectrum created in Fig. 1 was analysed using the National Institute of Standards and Technology (NIST) database, which contains hundreds of patterns. The name, molecular weight, and structure of plant seed components were determined using the NIST Ver. 2.1 MS data library.

2.7.2. FTIR characterization of Strychnos potatorum L. seed material before and after the studies on the treatment of drinking water

Strychnos potatorum L. seed active functional groups matter participated in the fluoride removal from drinking water samples were identified through FTIR spectral analysis (before and after treatment). The characterization of the dried seed powder used for treatment studies was done by using FTIR instrument (Model: Spectrum Perkin RXT) by directly placing the Potassium Bromide crystals over the sample. The mass spectrum obtained in the mid IR region of 450–4000 cm^{-1} was recorded using ATR (Attenuated Reflectance Technique) in transmittance mode.

2.7.3. Protein profiling of Strychnos potatorum L. seed

The protein molecules of *Strychnos potatorum* L. seed were quantified using Bradford assay. The 10% SDS-PAGE gel was used to identify the seed protein's molecular mass as per Lamelli method. A low molecular mass protein of 15.1 kDa appeared on the agarose gel was excised, trypsin digested and subjected for identification using MALDI-TOF mass spectrometry (ms) in linear mode with a Voyager spectrometer (Applied Biosystems, USA). The spectrum peptides were compared to reference peptides in the NCBInr protein database and submitted to the Mascot software (http://www.matrixscience.com) for analysis. Before MALDI-TOF studies, the coagulation property of 15.1 kDa protein band and control (plain gel) was tested as per 2.4.

Table 1
Physico-chemical characterization of drinking water samples.

Parameters	Range of V (mg/L)	BIS std (mg/L)
pH	6 ± 0.2-8–8 ± 0.27	6.5–7.5
EC	0.102 ± 0.17–0.408±0.37 μmho/cm	–
TS	200 ± 0.16–600 ± 0.26	500–1000
TDS	200 ± 0.27–400 ± 0.72	500–1000
Acidity	12.5 ± 0.21–50 ± 0.32	–
Alkalinity	50 ± 0.12–230 ± 0.32	
Ca	80 ± 0.32–200 ± 0.28	75–200
HR	80 ± 0.14–120 ± 0.28	200–600
Mg	12 ± 0.19–30 ± 0.31	30
Cl^-	20 ± 0.27–160 ± 0.24	250–1000
DO	6.5 ± 0.44–9 ± 0.19	9
PO_4^{3-}	0.02 ± 0.48–0.1 ± 0.18	0.2
SO_4^{2-}	70 ± 0.1–150 ± 0.44	200–400
F^-	0.1 ± 0.19–1.97 ± 0.22	1–1.5

The data represent the mean ± standard deviation of three replicates.

2.7.4. Homology modelling of horcolin protein

Horcolin sequences were retrieved from the Uniprot sequence database with Uniprot Id P82953, and the structure of the horcolin was simulated using the SWISSMODEL modelling service (Waterhouse et al., 2018). The sequence of horcolin were used to search the template for model building in SWISSMODEL where the salt stress induced protein from *Oryza sativa* (Uniprot Id: A2WPN7, PDB ID: 5GVY) (Sharma et al., 2017) shows maximum sequence similarity of 43.36% with horcolin. The structure alignment of modelled structure of horcolin, salt stress induced protein and mulberry lectin were carried out using PyMol protein visualization software. The horcolin, salt stress induced protein, and mulberry seed lectin sequences were aligned using the clustal omega multiple sequence alignment server, and the result file was analysed and sequence alignment was prepared using JALVIEW (Waterhouse et al., 2018) multiple sequence alignment visualization software.

2.7.5. Statistical analysis

The treatment data reported in the present study are the means of triplicate samples (n = 3) and statistical analysed for the calculation of standard errors.

3. Results and discussions

3.1. Physical and chemical characteristics of potable water samples

Table 1 summarizes the physical and chemical characteristics of all 52 drinking water samples. The obtained results compared with the BIS guideline for drinking water quality assessment were found within the standard limits except for fluoride concentration among 5 samples. It was also discovered that the quality of the water samples gathered differed from one sampling point to the next.

All of the drinking water samples tested had a pH of 6 ± 0.2-8-8 ± 0.27, which was judged to be within the BIS limit. The pH of water determines how polluted it is and how productive it is. It represents the negative logarithm of the concentration of hydrogen ions in water and thus determines its acidity and alkalinity scale that is produced from the interactions of several mineral salts and their organic components present within the water molecules. The presence of slight acidity in few of the water samples (S5, S6, S7, S8, S9, S10, S11, S12, S13, S15, S16, S17, S18, S19, S29, S30, S31, S32, S33, S34, and S35) might be due to the carbonic acid formation in water samples from the dissolution of carbon dioxide with water, moreover the increased concentration of organic acids such as humic acid and fulvic acid produced by the decaying plant materials. This low pH condition in water releases toxic metal substances like zinc, lead, cadmium and copper into the water and also creates corrosion of metal pipelines. The alkaline pH of water produces salty and bitter taste. A few of the drinking water samples (S23, S47 and S51) showed minor alkalinity which might be formed by the reaction of carbon dioxide gas with rain water and later its percolation into groundwater through the soil medium (Tiwari et al., 2015). The results are clearly evident that the pH of water samples analysed was near neutral. Kumaran et al. (2015) reported 6.9 to 8.1 pH values in drinking water samples collected from Tirunelveli district, Tamil Nadu, India. Popoola et al. (2019) found similar results in water samples taken in Lagos.

All of the water samples tested had EC values between 0.102 ± 0.17- 0.408 ± 0.37 μmho/cm. The values of EC in water are produced by the amount of ionic or dissolved charged particles or the total dissolved solids. Elevated ion concentration in water produces higher conductivity potential due to the charged electrolyte dispersed into the water. EC values beyond 3000 μ mho/cm affect crop regeneration capacity and reduce its yield. Udhayakumar et al. (2016) reported similar range of higher EC values in drinking water samples collected from Villupuram district. Meride and Ayenew (2016) has also reported similar results in drinking water of Ethiopia.

The amount of TDS in the water samples was between 200 ± 0.27 - 400 ± 0.72 mg/L and also found within the standard limit. Water is a universal solvent and consequently capably liquefies most of the organic and inorganic form of minerals and their salts are calcium, magnesium, potassium, carbonates, bicarbonates, chlorides and even more according to Bruvold and Ongerth (1969). This dissolved element alters the taste and appearance of potable water and forms scales on the cooking utensils. These solids are therefore mostly in-filterable and rarely filterable. Few of the water samples (S1, S2, S20, S34 and S48) had higher TDS values and the reason behind this consequence may be dissolution of larger amount of mineral salts into the water samples. Elevated amount of TDS in water is harmful for patients suffering from heart and kidney problems and it also produces gastrointestinal disorder in normal people (Soylak et al., 2002). Sasikaran et al. (2012) has reported disorder such as laxative or constipation effect in human when consumed water with higher TDS values. Udhayakumar et al. (2016) found similar results in drinking water samples from Villupuram.

The acidity levels in the water samples ranged from 12.5 ± 0.21 - 50 ± 0.32 mg/L. The alkalinity of the samples ranged from 50 to 230 mg/L. The species such as OH, CO_3, HCO_3 ions causes the alkaline condition of water. Water's total alkalinity is defined as the capacity of the water to neutralize acids. High amount of alkaline in water causes eye irritation for human and chlorosis in plants. Both bicarbonates and dissolved carbonates released from the limestones, sedimentary rock particles, domestic solid waste, cleaning waste together results in severe alkalinity of water (Sarala and Uma, 2013). Gupta et al. (2013) reported the level of Alkalinity (50–230 mg/L) in the Yamuna River. Umamageswari et al. (2019) found similar results in groundwater samples from Batlagundu block in Dindigul district.

Total hardness, calcium, and magnesium concentrations were 80 ± 0.14 - 120±0.28 mg/L, 80 ± 0.32 - 200±0.28 mg/L, and 12±0.19 - 30 ± 0.31 mg/L, respectively, in all 52 study samples. Hardness in drinking water is caused by the combination of elements such as calcium and magnesium. Rocks and sewage give calcium to drinking water. Sedimentary rocks, seepage, and runoff from soil are the principal sources of these ions, which are directly proportional to the total hardness of water. Presence of higher level of total hardness in water inhibits lather production in soap; moreover it also raises the temperature required for boiling water. Health disorders such as atherosclerosis, changes in blood lipid level and blood sugars, myocardial infarction, stone deposition in kidney, hypertension, type II diabetes, psychiatric problem in adult and premenstrual syndromes are reported to occur in humans due to the deficiency of the magnesium salts (Destexhe and Terrence, 2003). Hardness levels ranged from 156 milligramme/litre to 476 mg/L in water samples studied by Udhayakumar et al. (2016) in the Villupuram district of Tamil Nadu.

The dissolved oxygen range was between 6.5 ± 0.44 - 9±0.19 mg/L for all the 52 water samples. Fresh water samples gain good percentage of dissolved oxygen by direct diffusion of atmospheric oxygen gas into the aquatic system along with brighter and prolonged sunlight during day time. Aquatic plants' photosynthetic activity also contributes a significant amount of dissolved oxygen to water by releasing a large amount of O_2. At last results showed all water samples can be used for drinking and irrigation purpose, due to the availability of good amount of dissolved oxygen. Umamageswari et al. (2019) has reported 5.0 to 8.1 mg/L of in water samples analysed from Batlagundu district. Similar results are reported by Lewis (2000) in drinking water samples.

Chloride level for all the drinking water samples was between 20 ± 0.27 - 160±0.24 mg/L. The concentration of chloride element in the measured water could be caused by several types of salts such as calcium, sodium, and potassium. The affinity of chloride toward sodium is very high Chlorides tend to remain in dissolved state in solutions. Chloride levels in water can also be used as a metric for calculating the water pollution index (Chanda, 1999). Shyamala et al. (2008) has reported similar chloride values water samples of Coimbatore district.

The amount of phosphate was between 0.02 ± 0.48 - 0.1 ± 0.18 mg/L for all the study samples. Presence of phosphate element in their water may be caused by the pesticides and fertilizers regularly used for agriculture practice in fields in these locations. This phosphate on entering into our digestive tract creates phosphine gas interacting with our gastric juice. The sulphate levels in all of the water samples ranged from 30 to 120 mg/L. It is a primary cation found in natural water, and larger concentrations cause the water to smell like rotten eggs. None of the water samples had such an odour, and the analysed water samples very little amount of sulphate. Dev et al. (2015) has reported 10–41 mg/L of phosphate and 19 to 62 mg/L of sulphate in Dhankuta municipality potable water. Similar results are reported by Aravindkumar (1995).

Fluoride concentrations ranged from 0.1 ± 0.19 - 1.97 ± 0.22 mg/L in all of the samples tested. Fluoride levels were high in five samples, including Naidupuram S5 (1.71 mg/L), Pallangi S7 (1.97 mg/L), Villpatti S9 (1.79 mg/L), Perumal malai S17 (1.90 mg/L), and Keelpoomi S4 (1.81 mg/L), all of which exceeded the BIS limits. Fluoride levels in drinking water that is too high. According to the BIS standard, the maximum permitted concentration of fluoride is 1–1.5 mg/L. The source of fluoride in drinking water (pool,river,stream) is from soil and rock. The main reason for higher level of fluoride in these water samples is due to leaching, weathering and erosion of fluorine mineral from rock of the hills. Hence, such higher level of fluoride obtained from the drinking water samples. The various other forms of minerals including fluorapatite, mica and fluorite on interaction with the water form fluoride ion. People with higher fluoride intake (beyond 1.0 mg/L) suffer from decreased blood haemoglobin proportion, distorted red blood cells, disintegration of muscle fibre, headache, gastrointestinal disorders, skin rashes, abdominal pain, nausea, poor immunity, infertility in male (Saxena and Ahmed, 2001). Hence, removal of fluoride and limiting within standards could benefit human health. There is several fluoride removal techniques proposed and practised globally. The most widely followed method for the water defluoridation is reported as Nalgonda technique. This was developed by NEERI (National Environmental Engineering Research Institute,Nagpur,India). In this method some of the chemical agents like aluminium sulphate, lime

Table 2
Effect of SP L. seed protein on the removal of F^- from aqueous solutions.

F^- con (ppm)	Seed powder (g)					
	0.05	0.1	0.15	0.2	0.25	0.3
	F^- removal (%)					
1	24 ± 0.16	55 ± 0.02	38 ± 0.15	33 ± 0.15	47 ± 0.23	42 ± 0.24
2	25 ± 0.18	57 ± 0.05	34 ± 0.13	30 ± 0.12	30 ± 0.18	38 ± 0.29
3	20 ± 0.11	30 ± 0.09	21 ± 0.17	23 ± 0.09	25 ± 0.25	26 ± 0.32
4	19 ± 0.10	32 ± 0.10	25 ± 0.15	22 ± 0.07	30 ± 0.33	28 ± 0.14
5	17 ± 0.08	30 ± 0.12	23 ± 0.11	21 ± 0.05	33 ± 0.31	27 ± 0.23

The data represent the mean ± standard deviation of three replicates.

and bleaching powder are added to the water and mixed thoroughly and maintained for 3 h for defluoridation process to take place. Though widely practised method it has reported to carry major drawback too. The treated water acquires higher concentration of aluminium (2–7 mg/L) exceeding the BIS standard (0.03–0.2 mg/L) limits which could in turn cause Alzheimer's disease; majority of the fluoride ion present in the water is converted into soluble aluminium fluoride ion complexes, the sulphate ion from alum chemical dissolves in water and increased tremendously in treated water; the sludge produced through this treatment method is huge with increased concentration of fluoride in it and it is a secondary problem with environmental disposal (Thivya et al., 2015). Therefore alternative treatment methods have to be identified to overcome these problems. Natural coagulant agents such as *Strychnos potatorum* L. seed are harmless, safer to use and environmental friendly. These bioagents produce little sludge, do not require pH variation, and do not change the pH of treated water. In water samples from the Villupuram district of Tamil Nadu, Udhayakumar et al. (2016) found fluoride levels ranging from 0.21 mg/L to 1.2 mg/L.

3.2. Studies on fluoride ion removal from aqueous solution using Strychnos potatorum L. seed coagulant

Coagulation activity test was confirmed the coagulation property of isolated protein. The fluoride removal capacity by *Strychnos potatorum* L. seed coagulant protein at various concentrations of fluoride aqueous solution was evaluated. The maximum fluoride uptake by the seed coagulant was 57% at 0.1 g optimum dosage from 2 ppm aqueous solution. The percentage of fluoride removal efficiency was increased with the addition of seed coagulant. After reached a particular dosage of the coagulant the percentage of fluoride removal was decreased and stabilized. Further addition of the seed coagulant to the aqueous solution after the optimal dosage caused turbidity. Among the various coagulant dosages screened for aqueous fluoride removal estimation in this study, 0.1 g seed coagulant had removed the highest percentage (30%–57%) of fluoride (Table 2). This reaction of fluoride removal may be due to the release of hydroxyl ions from the organic polyelectrolytes and their highly cationic nature present in the seed coagulant proteins (Ndabigengesere et al., 1995). Wendimu et al. (2017) used aluminium iron modified activated bamboo charcoal to remove fluoride from aqueous solution. Inter particle bridging and the presence of active sites in the coagulants were found to be the mechanisms for fluoride removal in the study, which stabilized the colloidal substances, produced flocs, and settled them. When reached the coagulant optimum dose, the active sites of ions might have been locked for the coagulant to react and hence destabilized the colloidal interaction and produced poor or no removal. Various pH values and coagulant contact time length were used to test the performance of the *Strychnos potatorum* L. seed coagulant in removing fluoride ion from aqueous solutions. From different pH (6 ± 0.28, 6.2±0.07, 6.4±0.12, 6.6±0.07, 6.8±0.03 and 7.2 ± 0.12) aqueous solutions the optimum seed coagulant dose removed maximum fluoride ion when the solution pH was 6.6±0.07 (Table 3). The pH of aqueous solution is more significant for the fluoride removal studies, and the overall percent of fluoride ion removal was best in acidic pH conditions. This might be by the neutralization of surface charges of coagulant and aqueous solution. The investigation found that 120 min (2 h) was the best time necessary for the elimination of fluoride from a 2 ppm aqueous solution with 0.1g seed coagulant dosage (Table S3). Increased coagulant contact time in the aqueous solution resulted in increased fluoride removal, possibly due to surface charge neutralization of active sites, and further created stabilized colloids. Beyond the optimum pH and contact time the fluoride removal capacity of the *Strychnos potatorum* L. seed coagulant might have become inaccessible due to saturation of active sites (Yu et al., 2006).

Millar et al. (2017) discovered that aluminium-exchanged chelating resins with imino-diacetate functional groups are efficient at removing fluoride ions from aqueous fluoride solutions. Saif et al. (2012) reported cadmium removal using *Strychnos potatorum* L. seed from cadmium aqueous solution under varying pH condition and contact time.

3.3. Studies on fluoride removal from the drinking water samples using Strychnos potatorum L. seed coagulant

The effectiveness of the optimum dosage (0.1 g) of *Strychnos potatorum* L. seed coagulant in removing fluoride ion from five fluoride-contaminated drinking water samples was investigated (Naidupuram, Villpatti, Pallangi, Perumal malai and Keelpoomi). The defluoridation treatment of the water samples carried for 2 h removed (53%–75%) fluoride ions. The seed coagulant effectively reduced 70%, 75%, 68%, 66% and 53% fluoride ions from Naidupuram, Villpatti, Pallangi,

Table 3
Removal of F^- ion in aqueous solution (2 ppm) using SP L. seed under various pH concentrations.

pH value	S4		S5		S7		S9		S17	
	Reduc. in mg	(%) removal	Reduc. in mg	(%) removal	Reduc. in mg	(%) removal	Reduc. in mg	(%)removal	Reduc. in mg	(%) removal
6 ± 0.28	0.85 ± 0.18	50	0.6 ± 0.19	50	0.95 ± 0.19	50	0.7 ± 0.17	50	0.95 ± 0.22	50
6.2 ± 0.07	0.8 ± 0.35	55	0.7 ± 0.20	55	0.42 ± 0.35	55	0.6 ± 0.15	55	0.42 ± 0.35	50
6.4 ± 0.12	0.6 ± 0.41	60	0.5 ± 0.34	60	0.29 ± 0.3	66	0.3 ± 0.15	70	0.7 ± 0.35	40
6.6 ± 0.07	0.3 ± 0.32	66	0.5 ± 0.23	70	0.2 ± 0.41	68	0.30 ± 0.17	75	0.45 ± 0.30	53
6.8 ± 0.03	0.3 ± 0.30	65	0.6 ± 0.15	65	0.3 ± 0.17	65	0.40 ± 0.25	65	0.4 ± 0.44	50
7 ± 0.12	0.8 ± 0.35	55	0.7 ± 0.20	55	0.42 ± 0.35	55	0.6 ± 0.15	55	0.4 ± 0.42	50

The data represent the mean ± standard deviation of three replicates.

Perumal malai and Keelpoomi samples respectively. The highest fluoride removal (75%) was achieved from Villpatti sample (Table S2). The seed of *Strychnos potatorum* L. has good coagulant protein. The polyelectrolyte and cationic amino acids of protein (15.1 kDa) might have attracted the negatively charge fluoride ions through surface charge neutralization and flocculation followed by coagulation. This reaction might have finally removed the fluoride ions from the contaminated water samples. After 2 h of treatment the fluoride removal percentage was decreased and this might be because the seed coagulants active sites had reached its equilibrium. A research on removal of pollutants from water samples using *Strychnos potatorum* L. seed protein (12 kDa) was reported by Arunkumar et al. (2019). Metal ions have been found to attach to protein molecules that are rich in amino acids like aspartic acid and glutamic acid (Rogers et al. 2000; Clemens, 2006). The presence of positively charge amino acids such a arginine, lysine, and hydroxyl group (-OH) radicals such as threonine, serine, unbalance histidine amino acids (-NH groups), (-S) group of methionine and –COOH groups also might have interacted with fluoride ions and formed bridges of salts or fluro-compounds and thus reduce fluoride through stabilized precipitates and colloids. Rajalakshmi et al. (2019) reported removal of water hardness and chloride contaminants using *Strychnos potatorum* L. seed powder as natural coagulant. Similar results are reported by Rajendran et al. (2013) for fluoride removal studies using *Strychnos potatorum* L. seed material natural coagulant.

3.4. Characterization of Strychnos potatorum L. seed material

3.4.1. GC/MS analysis of the Strychnos potatorum L. seed material

The biologically important active compounds of *Strychnos potatorum* L. seed material were identified through GC/MS assay. The results showed presence of 11 major and minor peaks for the bio components isolated and extracted with methanol solvents with their respective retention time as presented in Fig.S1. The compounds identified were such as 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- (RT: 5.510); 2-Furancarboxaldehyde, 5-(hydroxymethyl)- (RT: 5.795); 9-Methyl-Z-10-pentadecen-1-ol (RT: 6.050); Phenol, 2-methoxy-4-(1-propenyl)-, (Z)- (RT: 7.535); d-Glycero-d-ido-heptose (RT: 9.299); 3-O-Methyl-d-glucose (RT: 9.406); 4,8,13-Cyclotetradecatriene-1,3-diol,1,5,9-trimethyl-12-(1-methylethyl) (RT: 9.410); 4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z) (RT: 10.005); 1-Cyclohexene-1-methanol, (RT: 10.311). -(1-methylethyl) (RT: 9.410); 4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z) (RT: 10.005); 1-Cyclohexene-1-methanol, (all-Z) (RT: 10.005); 1,7,10,13,16,19-Docosahexaenoic acid, methyl ester, ((RT: 10.311). All the identified compounds showed significant role like antibacterial, antioxidant, antimicrobial and reductase activities which also enhanced the use of nirmali seeds for potable water treatments. Similar results like antioxidant, antifungal and antibacterial properties in the *Strychnos potatorum* L. seed extracts are reported by Lakshmi (2014).

3.4.2. FT-IR analysis of Strychnos potatorum L. seed material before, after the drinking water treatment studies

Characterization of *Strychnos potatorum* L. seed material prior to fluoride contaminated water treatment as shown in Table S4 & S5 and Fig. S.2 & S3 showed 16 absorbance peaks. This included some of functional groups like aliphatic (P-Cl, P-S), alkanes (CH), amide, nitro compounds (NH), amine, and carboxylic acid (OH). The existence of an N-H stretch in the chemical indicates the existence of an amide group and a protein molecule in the seed material. Six peaks were found in the seed materials after fluoride removal. The absence of most of the peaks from the initial assay shows, the compounds with their respective functional groups had participated in the charge neutralization and floc formation of colloidal material through ionic interaction with their active binding sites. Certain peak values such as 1128, 1427, and 1627 are found to obtain a shift from its initial placing. All these essential amino groups might have reacted with the fluoride ion present in the water through electrostatic and ionic interaction and caused its removal during the treatment (Rose et al., 2006). Lakshmi (2014) reported similar such results.

3.4.3. Protein profiling of Strychnos potatorum L. seed

Preliminary coagulation test was confirmed the coagulation property of 15.1 kDa protein band and control/plain gel has not shown in the property. This low molecular protein fractions produced flocs and precipitate when added to synthetic kaolin solution. Proteomic analysis of *Strychnos potatorum* L. seed using SDS-PAGE showed isolated bands corresponding to protein at 15.1 kDa the result predicted in Fig. S4. The Mascot assay followed by MALDI-TOF spectral analysis (Fig. S5) showed horcolin agglutinin protein (LECH_HORVU), belong to Jacalin lectin domain family represented in Fig. 1. The major function of this protein is reported as active site (sugar-mannaose) binding lectin protein. The seed horcolin agglutinin protein amino acid sequences were similar to protein components of Barley seeds (*Hordeum vulgare*). This horcolin lectin

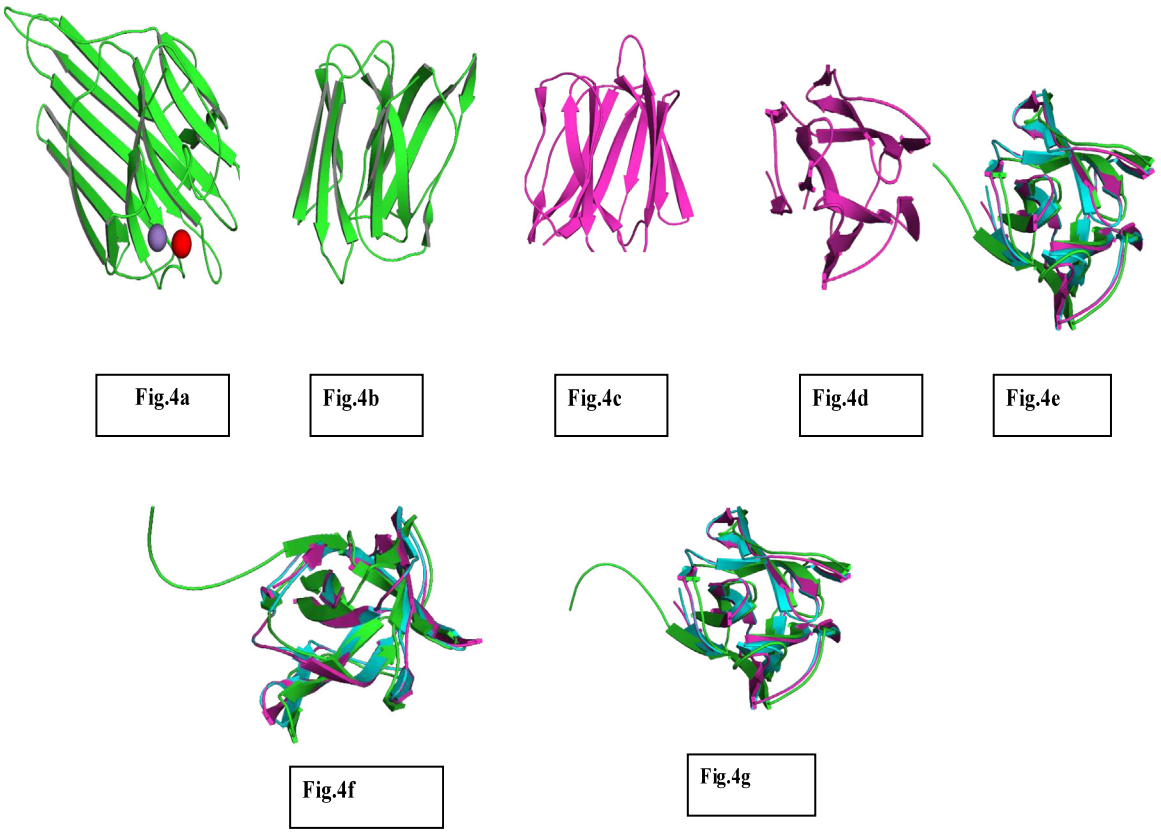

Fig. 2. **(a)** Cartoon representation of concanavalin A lectin (PDB ID-3CNA) from Canavalia ensiformis, where the metal atoms manganese (purple sphere) and calcium (red sphere) binds to loop region of beta strand structure; **(b)** Cartoon representation modelled structure of horcolin using PDB-5GVY of *Oryza sativa* salt stress induced protein; **(c)** Cartoon representation of Chain A of modelled Horcolin structure; **(d)** Cartoon representation top view of modelled horcolin structure showing beta-prism fold; ∈ Cartoon representation of structure alignment of Horcolin (pink cartoon), Salt stress induced protein (PDB ID-5gvy) (cyan cartoon) and Mulberry seed lectin (PDB Id-1xxq) (green cartoon); **(e)** Cartoon representation of structure alignment of Horcolin (pink cartoon), Salt stress induced protein (PDB ID-5gvy) (cyan cartoon) and Mulberry seed lectin (PDB Id-1xxq) (green cartoon); **(f)** Cartoon representation (top view) of structure alignment of Horcolin (pink cartoon), Salt stress induced protein (PDB ID-5gvy) (cyan cartoon) and Mulberry seed lectin (PDB Id-1xxq) (green cartoon); **(g)** Cartoon representation (lower view) of structure alignment of Horcolin (pink cartoon), Salt stress induced protein (PDB ID-5gvy) (cyan cartoon) and Mulberry seed lectin (PDB Id-1xxq) (green cartoon). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(LECH_HORVU) protein has 146 amino acid residues. The amino acid constituent are mostly positively charged due to free radical compounds (-NH, -OH, COOH) in unbalanced state. Hence they possess cationic property. The sequence alignment of agglutinin protein amino acids has conserved residues such as (M, P, W, G, K, T, S, F, D, N, V, Q, E, I, R) in different location of the polypeptide chain. MSA showed amino acids with strong (27 pairs) and weak (26 pairs) properties. Methionine, a sulphur-containing amino acid found in protein molecules, may have had a significant role in fluoride removal from drinking water samples due to its cationic nature and metal binding sites on the side chain, which encourage hydrogen bonding between molecules (Hatakeyama et al., 1986). Arunkumar et al. (2019) extracted 12 kDa proteins from Strychnos potatorum L. seeds and employed them in water treatment experiments. Lefranc et al. reported similar findings (2009).

3.4.4. Homology modelling of horcolin protein

From the literature review the effects of chloride salts such as magnesium chlorides, calcium chlorides in the mulberry seed lectins shows there was a change in carbohydrate binding affinity with the increase in salt concentration. The sequence alignment of horcolin with mulberry seed lectin (Uniprot Id: Q8LGR3, PDB ID: 1XXQ) Rabijns et al. (2005) from *Morus nigra* (Black mulberry) shows 30.50% sequence similarity. The structure of horcolin was modelled using *Oryza sativa* salt stress induced protein (Uniprot Id: A2WPN7, PDB ID: 5GVY) having sequence identity of 43.36% with horcolin and analysing the three structures results, three of them shows beta-prism fold which is common fold present in most of the carbohydrate binding lectins. The structure alignment of modelled horcolin, salt stressed protein (5GVY) and mulberry seed lectin (1XXQ) shows 0.093 Å RMSD value (Fig. 2a–e). From these structure and sequence studies we confirm that the horcolin exhibits binding of fluoride salts such as magnesium fluoride, calcium fluoride and sodium fluoride in the water like mulberry seed lectin and salt stress *i*nduced lectin, which mentioned in the literature (Yeasmin et al., 2007).

The structure analysis of the metal binding lectins with beta fold shows the metals binds in the region of loop between the beta strands. The presence of similar loop in the modelled structure of horcolin confirms the horcolin will binds to metal (Fig. 2a, b), in the presence of metal fluoride the horcolin participates in binding of metal bonded with fluoride which leads to decrease in fluoride content of water.

4. Conclusion

Strychnos potatorum L. seed used in the present study effectively removed fluoride from synthetic aqueous solutions and drinking water samples. The seed protein materials was identified to contain agglutinin molecules and confirmed as horcolin lectin like protein (15.1 kDa). The amino acid residues of the coagulant protein were associated with several metal binding sites and thus actively participated in the water defluoridation process through agglutination/coagulation mechanism. Hence, the seed protein efficiently removed 75% of fluoride from potable water in 120 min/2 h. The characterization of coagulant protein molecules confirmed the presence of novel amino acid components and functional groups favouring defluoridation property. Hence, this study suggests the *Strychnos potatorum* L. seed horcolin protein material could be used for defluoridation of potable water effectively by replacing the chemical agents and their treatment methods to purify drinking water.

CRediT authorship contribution statement

B. Sowmiya Rajalakshmi: Data curation, Writing – original draft, Writing – review & editing. **M. Vasanthy:** Conceptualization, Methodology, Software, Data curation, Writing – original draft, Writing – review & editing. **V. Rajakannan:** Conceptualization, Methodology, Software. **Balasubramani Ravindran:** Conceptualization, Methodology, Software, Supervision. **Soon Woong Chang:** Visualization, Investigation. **Murugesan Chandrasekaran:** Visualization, Investigation. **Mona S. Alwahibi:** Visualization, Investigation, Writing – review & editing. **M. Ajmal Ali:** Visualization, Investigation, Writing – review & editing. **Siham Albuluwi:** Writing – review & editing. **C. Thamarai Selvi:** Data curation, Writing – original draft, Visualization, Investigation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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