**INTRODUCTION**

Water is life for people and for the planet. It is essential to the well-being of humankind, a vital input to economic development, and a basic requirement for the healthy functioning of all the world’s ecosystems. Ironically, more than 125 million children under five years of age live in households without access to an improved drinking-water source. 1

The importance of water was stressed in the Millennium Development Goals adopted by the General Assembly of the United Nations (UN) in 2000. The UN General Assembly also declared the period from 2005 to 2015 as the International Decade for Action, “Water for Life” to achieve the Millennium Development Goal (MDGS) to reduce by half the proportion of the world's population without sustainable access to safe drinking water and sanitation by 2015. 2

Fluoride is one of the very few chemicals that have been shown to cause significant effects in people through drinking-water. Fluoride is often described as a ‘double-edged sword’ as inadequate ingestion is associated with dental caries, where as excessive intake leads to dental, skeletal and soft tissue fluorosis- which has no cure. 3

According to the World Health Organization (WHO), the maximum acceptable concentration of fluoride is 1.5 mg/l. 4 The acceptable and permissible limits in Indian context were 1 mg/l and 1.5 mg/l respectively. **5**

It is estimated that around 260 million people worldwide are drinking water with fluoride content more than 1.0 mg/l. In India alone, endemic fluorosis is thought to affect around one million people and is a major problem in 17 states, especially Rajasthan, Andhra Pradesh, Tamil Nadu, Gujarat and Uttar Pradesh. 6

As fluorosis is an irreversible condition that has no cure, prevention is the only solution for this menace. Hence provision of water with optimal fluoride concentration is the only way to protect future generations against this disease. 7

Various technologies were developed to remove fluoride from drinking water by ion exchange, adsorption, precipitation, electro chemical defluoridation and reverse osmosis etc. But due to high cost involved and by-products generated, such technology is not reasonable for a developing country like India. Therefore there is a great need for environmental friendly and low cost technology for domestic usage. 8

Natural materials have been used in water treatment since ancient times. In recent years there has been a resurgence of interest to use natural materials and usage of algal biomass and plant products for defluoridation has been reported. Thus a study was attempted to evaluate water defluoridating capability of untreated plant materials like tulsi leaves and wheatgrass and in turn compare it with that of Brushite-Calcite combination reported by Larsen and Pearce. 9

**MATERIALS AND METHOD**

**Procurement of test products:**

Tulsi leaves (Ocimum sanctum) and wheatgrass (Triticum aestivum) were obtained from the local market. All plant specimens were identified by a botanist for their authenticity. After washing with distilled water, the plants were dried in a hot air oven at 110°C for 5 hours and micronized in a flour mill. Brushite (CaHPO4.2H2O; Analytical grade; Oxford Laboratory, Mumbai, India), Calcite (CaCO3; Analytical grade; Nice chemicals, Kochi, India) and Sodium fluoride (NaF; Analytical grade; Fischer scientific, Mumbai, India) were purchased from a scientific store. TISAB (Total Ionic Strength Adjustment Buffer) solution required for fluoride analysis in water samples was obtained from Bapuji Institute of Engineering Technology, Davangere.

**Preparation of stock solution:**

The fluoride stock solution of 1000 ppm was prepared by diluting 2.21 g of sodium fluoride salt in one litre of double deionised distilled water at room temperature. The solution was diluted as required to obtain working test solutions of 2 ppm and 5 ppm.

**Experimental Procedure:**

1 litre each of 2 ppm water solution was taken in thirty plastic containers, ten designated to each of the three test products. One gram of tulsi powder was added to each of 10 containers in group I, one gram of wheatgrass powder to each of ten containers in group II and one gram of a combination of Brushite and calcite (0.5 g each) to ten containers in group III. All the containers were shaken vigorously for 1 minute to allow uniform dispersion of the test products. Five containers in each group were left undisturbed. Water in the remaining five containers was boiled separately in a domestic electric kettle for 1 minute and transferred back. The electric kettle was cleaned between each run with 0.1 mol/l of acetic acid followed by 5 rinses with deionised water. Samples were drawn from the supernatant after 90 minutes and 24 hours from all the thirty containers. Entire procedure was repeated again using 5 ppm water. Fluoride concentration in each sample was determined by using an ion electrode (Hach-Sension MM 374, Hach Company, USA) after addition of TISAB solution and denoted as fluoride in parts per million (mg/ml). Each sample was analysed in duplicate and average reading was taken.

**Statistical Analysis:**

All the data were entered in a Microsoft excel sheet 2010 and subjected to statistical analysis using SPSS version 20. 10 For intergroup comparisons between boiled and non-boiled samples, unpaired t-test was used and for inter-group comparisons between three test products, One-way ANOVA followed by Tukey’s post Hoc analysis was done.

**RESULTS**

Tables 1 and 2 show fluoride concentration in a litre of 2 ppm and 5 ppm water following the addition of the test products. Addition of test products to distilled water of 2 ppm followed by boiling for 1 minute had shown a reduction in fluoride concentration to 1.38 ppm, 1.81 ppm and 0.49 ppm in groups I, II and III respectively at the end of 30 minutes. Higher reduction of 75.5% was seen with Brushite-Calcite when compared to tulsi (31%) and wheatgrass (9.5%) and this difference was statistically highly significant (p=0.001). Even at the end of 24 hours, Brushite-Calcite combination had shown superior defluoridating potential than tulsi and wheatgrass (p=0.001). Increasing the fluoride concentration to 5 ppm also did not affect the defluoridating capability of Brushite-Calcite combination which had shown a reduction of 82.8% and 86.6% at the end of 30 minutes and 24 hours respectively after boiling. When 2 ppm water was not boiled following addition of test products, a reduction in fluoride concentration to 1.47 ppm, 1.96 ppm and 1.99 ppm was seen in groups I, II and III respectively at the end of 30 minutes and to 1.44 ppm, 1.94 ppm and 1.97 ppm at the end of 24 hours. Tulsi had shown higher reduction rates of about 26.5% at the end of 30 minutes and 28% at the end of 24 hours when compared with other test products and the difference was statistically highly significant (p=0.001). Similar results were also observed in 5 ppm water samples after addition of test products at various time intervals (p=0.001).

**DISCUSSION**

Adsorption of fluoride ions onto the surface of an active agent is a popular method for defluoridation. Among various available materials, a combination of Brushite and calcite developed by Larsen and Pearce serves as a satisfactory and economical domestic defluoridating agent. 9

Plants have been used for water treatment since centuries. Plant materials like seeds, leaves, bark, roots and fruits are known to possess defluoridating capabilities. 11 In the present study, locally available plants like Tulsi and Wheatgrass were selected on the basis of local availability and edibility in addition to medicinal value.

In the present study, when water was boiled following addition of test products, Brushite-calcite combination had shown a superior defluoridating capacity (ranging from 75.5% to 86.6%) than the plant products. On the contrary, this efficacy was totally lost in un-boiled samples. Boiling converts the two salts rapidly into apatite crystals which in turn adsorb the fluoride ions present in the water causing a pronounced decrease in the fluoride concentration. When left undisturbed, sedimentation of adsorbed fluoride in the form of fluoroapatite, hydroxyapatite and unutilized calcite occurs which needs to be separated before consumption. Also boiling drinking water before consumption alters the taste of the water. These two serve as shortcomings of this technique. 9 The defluoridating capability of Brushite-calcite combination in the present study was similar to that shown in studies by Larsen MJ et al and Lakshminarayan L et al. 12

Among the three products, Tulsi is the only material that had shown a consistent defluoridation capacity both with boiling (31% to 42%) and without boiling (24.4% to 28.2%). This reduction could be attributed to coagulant proteins in plant leaves. 11 Addition of tulsi tends to alter the taste of water. However the taste is culturally acceptable in Indian scenario considering the importance the plant has in daily life. In addition, the medicinal properties of tulsi would enrich the water making it safe and healthy for consumption.13 The reduction in fluoride level after addition of tulsi was consistent with an Indian study reported by Patni M et al though the amount of reduction observed was lower in the present study. In that study, water was pre-treated and pH adjusted with chemicals to eliminate interference with ions other than fluoride which would have resulted in higher defluoridation potential. 14 But in the present study, no prior chemical adjustments were done. Our methodology was simple and easy for the public to replicate.

Wheatgrass had shown a lower defluoridating capability on boiling among the three test materials. However it was better than Brushite-calcite combination when water was not boiled following addition. Though it had shown a limited defluoridating potential, it can be tried, as something is better than nothing however a slight alteration in taste is reported.

In the present study, distilled water was used instead of domestic drinking water which usually contains other minerals in addition to fluoride. So, further studies are recommended to confirm these observations and also to consider if these findings hold true with domestic drinking water also.

**CONCLUSION**

Our experiments investigated defluoridating capacity of Brushite-calcite chemical combination in comparison with two herbs – tulsi and wheatgrass. Based on the results obtained, it can be concluded that addition of tulsi leaves appears to be an economical, effective and natural method for domestic defluoridation of water on a small scale. However, further research is warranted with varying fluoride concentrations using different concentrations of tulsi and at different time intervals to evaluate its practical application.

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**TABLES**

**Table 1: Fluoride concentration after addition of different test products in 1litre of 2ppm fluoridated water**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Test Product** | **Fluoride Concentration at the end of 30 minutes** | **t-test** | **Fluoride Concentration at the end of 24 hours** | **t-test** |
| **On Boiling for 1 minute** | **Without Boiling** | **On Boiling for 1 minute** | **Without Boiling** |
| **Tulsi** | 1.38 ± 0.03(31%) | 1.47 ± 0.04(26.5%) | p=0.003 | 1.16 ± 0.06(42%) | 1.44 ± 0.05(28%) | p=0.001 |
| **Wheat grass** | 1.81 ± 0.03(9.5%) | 1.96 ± 0.01(2%) | p=0.001 | 1.82 ± 0.03(9%) | 1.94 ± 0.02(3%) | p=0.001 |
| **Brushite-Calcite** | 0.49 ± 0.01(75.5%) | 1.99 ± 0.01(0.5%) | p=0.001 | 0.37 ± 0.02(81.5%) | 1.97 ± 0.01(1.5%) | p=0.001 |
| **ANOVA** | p=0.001 | p=0.001 |  | p=0.001 | p=0.001 |  |

**Table 2: Fluoride concentration after addition of different test products in 1litre of 5ppm fluoridated water**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Test Product** | **Fluoride Concentration at the end of 30 minutes** | **t-test** | **Fluoride Concentration at the end of 24 hours** | **t-test** |
| **On Boiling for 1 minute** | **Without Boiling** | **On Boiling for 1 minute** | **Without Boiling** |
| **Tulsi** | 3.28 ± 0.04(34.4%) | 3.78 ± 0.03(24.4%) | p=0.001 | 3.10 ± 0.05(38%) | 3.59 ± 0.04(28.2%) | p=0.001 |
| **Wheat grass** | 4.57 ± 0.05(8.6%) | 4.68 ± 0.05(6.4%) | p=0.005 | 4.46 ± 0.04(10.8%) | 4.60 ± 0.03(8%) | p=0.001 |
| **Brushite-Calcite** | 0.86 ± 0.03(82.8%) | 4.90 ± 0.01(2%) | p=0.001 | 0.67 ± 0.01(86.6%) | 4.88 ± 0.45(2.4%) | p=0.001 |
| **ANOVA** | p=0.001 | p=0.001 |  | p=0.001 | p=0.001 |  |