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# Phytochemical Profile of *Aloe Barbadensis* and their Proficiency in Defluoridation of Fluoride Contaminated Water

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## ABSTRACT

The quantitative analysis of acetone and ethanol extract of *Aloe barbadensis* were studied, the total flavonoid content was determined as 13 µg/ml in the acetone extracts of gel of the plant whereas in the ethanol extract, it was 30 µg/ml followed by total phenol content which was almost same in the extracts but found higher in acetone extract as 34 µg/ml it followed by ethanol extract as 32 µg/ml. The reducing power assay was determined as 480 µg/ml in the ethanol extracts, followed by, 400 µg/ml in acetone extract. When Carbohydrates were compared, it was found as higher in the ethanol extract of the leaves of about 58 µg/ml, whereas the acetone extract possessed 54 µg/ml of carbohydrates in the *Aloe barbadensis*. Ethanol extract of the gel found to easily absorb the carbohydrates easily compared to acetone. The proteins content of ethanolic extract was about 4.8 mg/ml followed by 4.2 mg/ml in the acetone extracts of leaves. In this test, acetone extract was observed to contain protein less than the ethanol. Unlike other tests, all the extracts of all the plant parts when measured showed more or less similar results of about 10 and 14.2 µg/ml of reducing sugars in the ethanol and acetone extracts of leaves. The DPPH activity in gel extract was observed to be present in both the extracts; higher activity was observed in ethanol 100 µl concentrations and acetone 50 µl concentrations. The *Aloe barbadensis* plant has found having some fluoride removal ability, helped in the fluoride removal process. As 20 ml concentration worked faster and better than the lower concentrations.

**Keywords :** *Aloe barbadensis,* Phytochemicals, Extracts, Flouride

## I. INTRODUCTION

The plants are considered as important source of nutrition and as a result of that they are recommended for their therapeutic values. Some of these plants include ginger, green tea, walnuts, *Aloe barbadensis*, pepper and turmeric etc. [1]. Some plants and their derivatives are considered as important source for active ingredients which are used in aspirin and toothpaste etc. Apart from the medicinal uses, herbs are also used in natural dye, pest control, food, perfume, tea and so on. The *Aloe barbadensis* plant has been known and used for centuries for its health, beauty, medicinal and skin care properties. The name *Aloe barbadensis* derives from the Arabic word "Alloeh" meaning "shining bitter substance," while "vera" in Latin means "true." Today, the *Aloe barbadensis* plant has been used for various purposes in dermatology [2]. There are over several species of *Aloe barbadensis* grown around the world. However, only two species are grown today commercially, with *Aloe barbadensis* and *Aloe aborescens* being the most popular. Concentrated extracts of *Aloe barbadensis* leaves are used as laxative and as a haemorrhoid

treatment because of their phytochemical properties [3]. An *Aloe barbadensis* plant usually produces optimally for five or six years, but may continue to produce at least twice as long. In commercial *Aloe barbadensis* plantations, three leaves of about one kilogram (two pounds) each in weight and 50 – 75 cm (20 – 30 inches) in length can be harvestable for three or four times a year [4]. Apart from antimicrobial activity of *Aloe barbadensis*, it also has the efficiency to remove the pollutant from the polluted environment [5]. With the exponential increase in population and deterioration in the water quality, the dependence on groundwater is increased to meet the water demand in rural as well as urban areas. However, the high exploration rate of groundwater and lowering of groundwater table the dependence on groundwater in deep aquifers is increasing [5]. Fluoride is one of the most abundant constituents occurring in the groundwater in many parts of the world, posing a potential threat to human health. The permissible limit of fluoride concentration in drinking water is 1.5mg/L according to WHO guidelines. Among various methods used for defluoridation of water Adsorption method is relatively simple, economical, and appropriate for drinking water treatment. *Aloe barbadensis* along with $CaCl^2$ has considerable amount of efficiency for the removal of fluorides from water [6, 7].

## II. METHODS AND MATERIAL

### Sample collection

The *Aloe barbadensis*, was identified and collected from Research and Development division of Genewin Biotech, Hosur, Krishnagiri. The water sample was collected as per the standard protocol from Hosur lake for the study of deflouridisation using *Aloe barbadensis*.

### Preparation of acetone and ethanol plant extracts

The collected *Aloe barbadensis* samples was crushed to lotion form and it was soaked with 50 ml of acetone and ethanol solvents separately. The whole mixture was extracted at room temperature for 48 hr. After the extraction period, the extracts were filtered with whatman filter paper and were stored at 4°C for further use.

### Qualitative Phytochemicals Analysis

The total qualitative phytochemicals properties of acetone and ethanol extracts of *Aloe barbadensis* were analyzed by following the protocol of Ekwenye and Elegalam [8] and Olasupo *et al.,* [9].

### Test for Alkaloids

Wagner's test protocol was followed to quantify the total alkaloids of acetone and ethanol extracts of *Aloe barbadensis.* It was like 1 ml of acetone and ethanol extracts 2 ml of Wagner's reagent (iodine in potassium iodide) was added and the formation of reddish brown precipitate indicates the presence of alkaloids.

### Test for Saponins

Small quantity of alcoholic and aqueous extracts was separately taken and 20 ml of distilled water was added and agitated well in a graduated cylinder for 15 minutes. Formation of layer of foam indicates the presence of saponins.

### Test for Tannins

To 1ml of the extract, 0.5ml ferric chloride solution was added; formation of a dark blue or greenish black color product shows the presence of tannins. The little quantity of the extract is treated with potassium ferric cyanide and ammonia solution. A deep red color indicates the presence of tannins.

**Molisch's test**

To assess the existence of carbohydrate on both extracts of *Aloe barbadensis* were performed by taken 2ml of the extract, 1ml of a-napthol solution, and 0.5ml concentrated sulphuric acid were added gently through the side of the test tube. Purple or reddish violet color at the junction of the two liquids reveals the presence of carbohydrates.

**Test for Flavonoids**

Shinoda's Test protocol was followed as 0.5gm of each extract was treated with sodium hydroxide and the formation of yellow color indicates the presence of flavones. Another method was performed as for conformation, the extract was treated with concentrated $H_2SO_4$, formation of yellow or orange color indicates flavones.

**Quantitative analysis:**

**Determination of Carbohydrate**

100 mg of sample was hydrolysed in a boiling tube with 5 ml of 2.5 N HCl in a boiling water bath for a period of 3 hours. It was cooled to room temperature and solid sodium carbonate was added until effervescence ceases. The contents were centrifuged and the supernatant was made to 100 ml using distilled water. From this 0.2 ml of sample was pipetted out and made up the volume to 1 ml with distilled water. Then 1.0 ml of phenol reagent was added followed by 5.0 ml of sulphuric acid. The tubes were kept at 25-30˚C for 20 min. The absorbance were read at 490 nm [10].

**Determination of Protein**

The extracts were stirring with 50 ml of 50% methanol (1:5 w/v) at 25 °C for 24 h and centrifuged at 7,000 rpm for 10 min .0.2 ml of extract was pipetted out and the volume was made to 1.0 ml with distilled water. 5.0 ml of alkaline copper reagent was added to all the tubes and allowed it to stand for 10 min. Then 0.5 ml of Folin's Ciocalteau reagent was added and incubated in dark for 30 min. The intensity of the colour developed was read at 660 nm.

**Determination of Total Phenolics**

The total phenolic content was determined according to the method described by Cavallini *et al.,* [11] as 10µl aliquots of the extracts (2 mg/2 ml) was taken in test tubes and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu phenol reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as tannic acid equivalents [11].

**Determination of Total Flavonoid Content**

The flavonoid content was determined by the use of a slightly modified colorimetry method of Cavallini *et al.,* [11], as 0.5 ml aliquot of appropriately (2 mg/2 ml) diluted sample solution was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5 % $NaNO_2$ solution. After 6 min, 0.15 ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 min, and then 2 ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5 ml, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm versus water blank. The analysis was performed in triplicate and the results were expressed as routine equivalent [11].

**DPPH Free Radical Scavenging Assay**

The radical scavenging activity of selected root extracts was determined by following the method of Heggers *et al.,* [12]. As 4.3mg of DPPH (2, 2-Diphenyl -1- picrylhydrazyl) was dissolved in methanol (6.6ml) to prepare 0.3mM DPPH solution and it was

protected from light by covering the test tubes with aluminium foil. DPPH (150µl) was added to 3ml of methanol and absorbance was noticed immediately at 516nm for control reading. Different concentrations of test samples i.e 25 µl, 50 µl, 100 µl, 150 µl, 200 µl and 250 µl were taken and each of the samples was diluted with methanol up to 3ml, to it 150 µl DPPH was added. The samples were kept in dark for 15 min after which the optical density was observed at 516nm using methanol as blank.

$$\%\text{Antioxidant activity} = \frac{(\text{Control Absorbance} - \text{Sample Absorbance})}{\text{Control Absorbance}} \times 100$$

**Determination of Total Reducing Power**

The reducing power was determined according to the method of Heggers *et al.,* [12]. As 1ml of the extract (1mg/ml) was mixed with 1ml of 200mM of sodium phosphate buffer (pH- 6.6) and1ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min and 1ml of 10% trichloroacetic acid (w/v) was added. The mixture was centrifuged at 2000rpm for 10min. The upper layer solution (2.5ml) was mixed with 2.5ml of double deionised water and 1ml of fresh ferric chloride solution (0.1%). The absorbance was measured at 700nm. A higher absorbance indicates a higher reducing power.

**Water Analysis – Regular Interval of Time**

The defluoridation efficiency of acetone and ethanol extracts of *Aloe barbadensis* were analyzed on fluoride enriched water. It was collected from Hosur lake. The collected samples were immediately transferred to lab and refrigerated for further analysis and standard protocol was used for all analysis.

**Water Hardness**

The hardness of the water sample was analysed by following the standard protocol. As 20 ml of sample was taken in a conical flask and added 2 mL of ammonia buffer solution (so that pH will be maintained b/w 9 &10), subsequently few drops of EBT indicator was added and the sample turns into wine red in color. Rinsed the burette with few ml of EDTA before titrating and filled the burette with 0.02 M EDTA solution. Then the sample was titrated against the EDTA solution till the appearance of blue color.

**Sulfate Determination in Water**

50 mL of Filtered (Whatman No. 1) water sample was taken in an Erlenmeyer flask and added 20 ml of buffer solution and mix in stirring apparatus. While stirring, 0.15 g of barium chloride was added to the sample and stirs the sample with the help of magnetic stirrer for about an hour. After the incubation period the absorbance of test sample was analyzed against standard solution/distilled water (without barium chloride) at 420 nm using spectrophotometer. The obtained OD values are applied for graph with by using concentration on X-axis and absorbance at 420 nm on Y-axis.

**Chloride Determination in Water**

10 ml of the test sample was taken in conical flask and added 0.05 ml of iodine reagent. Then titrated the contents quickly against sodium thiosulphate (0.2482 g in 100 ml) until the colour turns straw yellow. Later, 1 ml of starch (1%) was added to the conical flask, it turns the turns straw yellow colour to blue. Continued the titration until the sample turns colourless. Then calculate the residual chlorine from titre value.

**Chemical Oxygen Demand (COD) Electrical Conductivity (EC) &Total Dissolved Solids (TDS) & pH**

The Chemical Oxygen Demand, electrical conductivity, TDS and pH of the water was determined in a quick and inexpensive way by using standard commercial portable meters [11].

**Fluoride determination**

The fluoride level of water sample was anaysed by following the method of Massoud Amanlou, et al., [13]. As 10.0 mL sample was taken and mixed with 10.0 ml of TISAB II; after that, fluoride concentrations of test samples were determined in duplicate using fluoride ion selective electrode. One batch number (out of six) for each of the 18 bottled drinking waters was randomly selected and the samples re-analyzed to assess the reliability of the method [13].

**Defluoridization**

The defluoridization experiment was performed by following the protocol of Sneha Jagtap, et al., [14]. Various doses as 5, 10, 15 and 20 ml of Aloe barbadensis gel were added on 100ml fluoride contaminated water. The gel applied water was kept for 15 days of treatment period. The effect of gel on water sample was assessed by analyses the various parameters such as fluoride, pH, EC, TDS, Hardness, COD and sulfate during the pre and post of the treatment in intermittence of 0, 7 and 15 days of treatment period. Obtained results were compared with Indian standard permissible limits.

## III. RESULTS AND DISCUSSION

**Results**

**Phytochemical analysis**

The phytochemical analysis was carried out to qualify either the presence or absence of alkaloids, saponins, carbohydrates, tannins and flavonoids from acetone and ethanol extracts of *Aloe barbadensis* and the results were tabulated table 1.

At the end of phytochemical qualitative analysis, the results showed that the ethanol extract contains almost all the tested ingredients except fats and oils. The acetone extracts shows the all the components except fats and oils.

**Table 1 :** Phytochemical analysis of acetone and ethanol extracts of *Aloe barbadensis*

| S.No. | Phytochemicals | Acetone | Ethanol |
|---|---|---|---|
| 1 | Saponins | + | + |
| 2 | Alkaloids<br>Wagner's Test | + | + |
| 3 | Fats and oils | - | - |
| 4 | Carbohydrates<br>Molisch's Test | + | + |
| 5 | Tannins<br>Lead acetate<br>Ferric chloride | <br>+<br>+ | <br>+<br>+ |
| 6 | Flavonoids<br>Shinoda's Test<br>($H_2SO_4$) | + | + |

The results of the flavonoid content of the acetone and ethanol extracts were found as follows. The 13 µg/ml of flavonoid was recorded in the acetone extracts of gel of the *Aloe barbadensis* plant. Whereas, in the ethanol extract of *Aloe barbadensis*, was double fold quantity of flavonoids as 30 µg/ml. According to the result obtained, leaves of ethanol extract showed have higher Flavonoids content than acetone (Fig. 1).

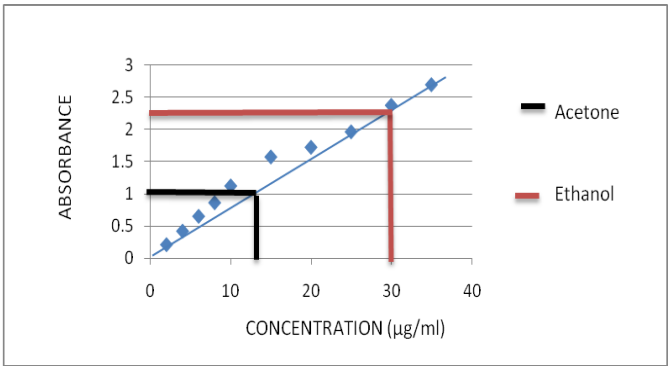


**Figure 1 :** Total flavonoids content of acetone and ethanol extracts

The phenolics content of acetone and ethanol extracts were determined at 725 nm using Gallic acid as standard and the quantity of phenolics represented as figure 2.

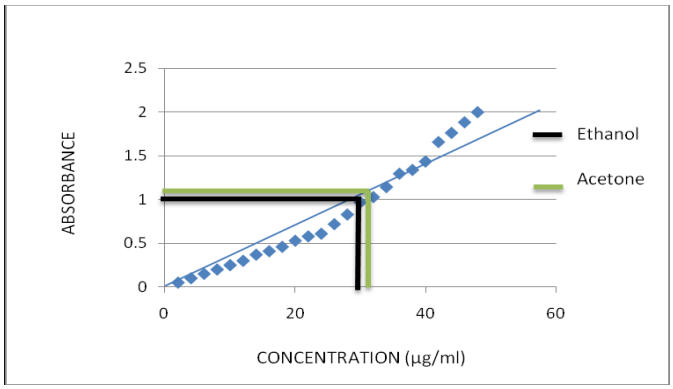


**Figure 2 :** Total Phenolic content of acetone and ethanol extracts

From the standard graph, total Phenol content was almost same in the both extracts but found higher in acetone extract as 34 µg/ml, it followed by ethanol extract as 32 µg/ml of phenolic content was observed.

The reducing sugar of the both extracts was reported as 480 µg/ml in the ethanol extract of leaves gel followed by 400 µg/ml in acetone extract of the leaves gel. The results compared with each solvent by drawn picture as follows (Fig. 3)

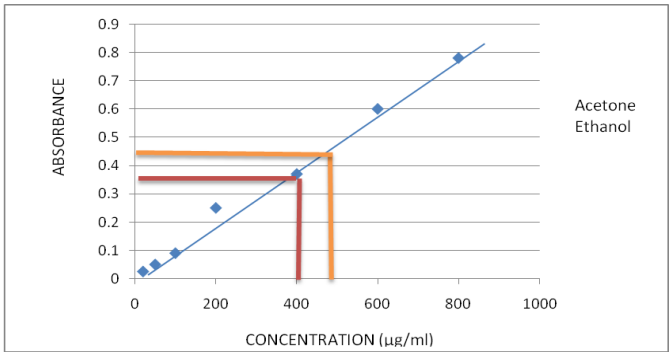


**Figure 3 :** Reducing power assay of acetone and ethanol extracts

In the carbohydrate analysis, the obtained reports are stated that the carbohydrates were found as higher in the ethanol extract about 58 µg/ml whereas, the acetone extract was observed to possess 54 µg/ml of carbohydrates in the leaves. Ethanol extract of the gel found to easily absorb the carbohydrates (Fig. 4).

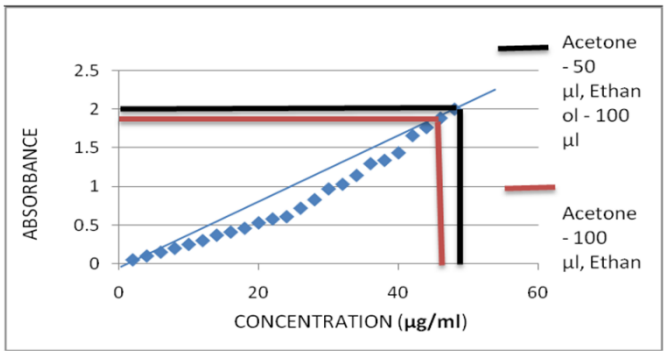


**Figure 4** : Carbohydrate content of acetone and ethanol extracts

Proteins are compared in the collected sample extracts to know the amount of proteins present in the *Aloe barbadensis* along with BSA used as standard.

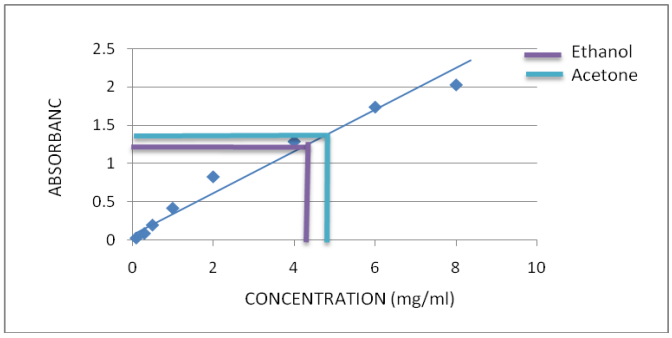


**Figure 5:** Protein content of acetone and ethanol extracts

The proteins were measured higher in the ethanol extract of *Aloe barbadensis* about 4.8 mg/ml followed by 4.2 mg/ml in the acetone extracts (Fig. 5). Reducing sugars in *Aloe barbadensis* samples were measured at 575 nm and glucose was used as standard. The result was noted that all the extracts of *Aloe barbadensis* showed more or less similar results of about 10 and 14.2 µg/ml of reducing sugars in the ethanol and acetone extracts respectively (Fig. 6).

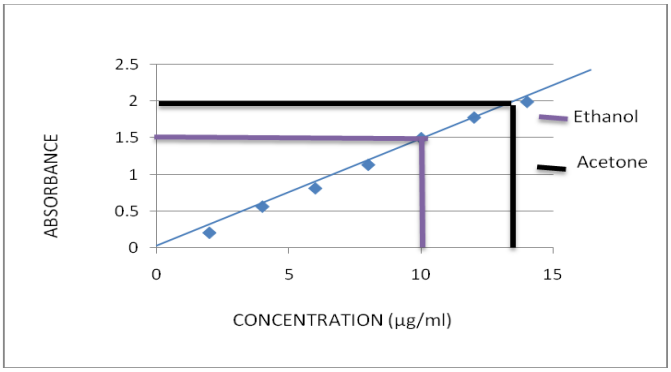


**Figure 6 :** Reducing sugar of acetone and ethanol extracts

## DPPH Radical Scavenging activity

The DPPH activity of both extracts were reported as, the higher activity was observed in ethanol 100 µl concentration and it followed by acetone 50 µl concentration (Fig. 7).

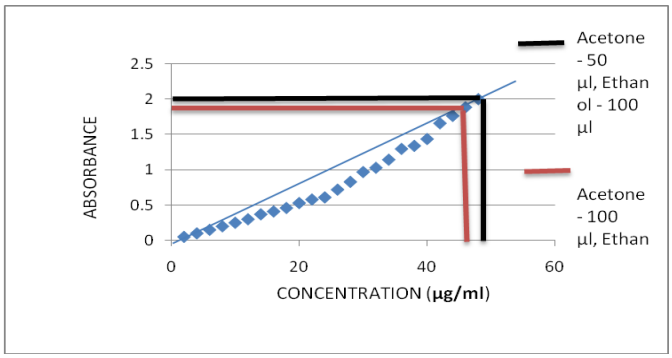


**Figure 7 :** DPPH radical scavenging activity of acetone and ethanol extracts

## Defluoridation by gel of *Aloe barbadensis*

The defluoridation of fluoride contaminated water by gel of *Aloe barbadensis* was determined and tabulated in table 2. The analysis table clearly proves the efficiency of *Aloe barbadensis* gel in fluoride reduction, as we precede the experiment for a 15 days treatment, the fluoride becomes reduced as on 7$^{th}$ days fluoride was 4.4mg/ml on 20 ml concentration and on 15$^{th}$ day it reduced as 3.2mg/ml on same 20ml dosage of *Aloe barbadensis* gel. It conforms that the gel of *Aloe barbadensis* has the efficiency to the defluoridation process. The remaining parameters such as EC, TDS, hardness, COD and quantity of sulfates are also reduced during the 15 days treatment period.

**Table 2 :** Defluoridation by gel of *Aloe barbadensis*

| Various concentration (ml) of *Aloe barbadensis* gel | 1 -1.5 mg/l | Permissible limits | | | | | |
|---|---|---|---|---|---|---|---|
| | | 6.5-8.5 | <1000 | <500 | <60 | <100 | <200 |
| | **Fluoride** | **pH** | **EC** | **TDS** | **Hardness** | **COD** | **Sulfates** |
| 0 | 8.9 | 7.8 | 860 | 480 | 340 | 430 | 215 |
| DAY 7 | | | | | | | |
| 20 | 4.4 | 7 | 780 | 320 | 250 | 360 | 156 |
| 15 | 4.7 | 7.1 | 834 | 480 | 336 | 410 | 167 |
| 10 | 5.3 | 7.2 | 812 | 485 | 343 | 398 | 189 |
| 5 | 7.3 | 7.4 | 854 | 475 | 349 | 393 | 201 |
| DAY 15 | | | | | | | |
| 20 | 3.2 | 6.5 | 630 | 189 | 80 | 128 | 110 |
| 15 | 3.6 | 6.98 | 690 | 270 | 165 | 352 | 115 |
| 10 | 4.9 | 7.12 | 684 | 280 | 173 | 248 | 180 |
| 5 | 6.3 | 7.28 | 754 | 354 | 251 | 352 | 187 |

## Discussion

The Aloe barbadensis is a shrubby, perennial, xerophytic, succulent plant. It has thick and pulpy leaves. It belongs to Liliaceae family. The medicinal importance of Aloe barbadensis plants has been well known for centuries. The whole Aloe plant has been used as a stomachic, antihelmintic and emmenagogue, menstrual suppression and the root for colic pain etc because of their phytochemical components [10]. It have number of nutrients such as sugars, minerals (iron, zinc, sodium, potassium, calcium, magnesium etc.), amino acids, enzymes, fatty acid etc. In this study, Aloe barbadensis gel was aimed at the analysis of phytochemicals using 2 solvents such as Acetone and Ethanol which proved the presence of plant phytochemicals and followed by the antioxidant activity tests which showed the highest activity in both the solvents. Aloe barbadensis gel can be useful richly for topical applications, wide ranges of products are now available on the market; however, simply pure Aloe gel is sufficient to treat several skin disorders. Apart from their medicinal uses because of phytochemical content, they also possess the efficiency to remediation process on contaminated water [15]. The force responsible for precipitation and adsorption of fluoride with Aloe barbadensis and calcium chloride is supposed to be some columbic forces between the positively charged surface and negatively charged fluoride ions. Most probably, the calcium ions in Aloe barbadensis and calcium chloride react with negatively charged fluoride ions from the solution.

The probable responsible ion in this process may be the hydrogen ion. It accelerates the positive charge on calcium which favors the precipitation and adsorption of fluoride. The main mechanism may be through fluoride precipitation and adsorption (CaF2) by Aloe barbadensis and calcium chloride as well as filtered through local available Sikar clay. The optimum fluoride removal (88%) was achieved at pH 7.4±0.1 with 40.0 g Aloe barbadensis, 3.0g calcium chloride in 1000 ml sample and in a contact period of 40 minutes [6].

Many plants and herbs have been studied and analysed for their fluoride removal capacity. The Aloe barbadensis plant has found having some fluoride removal capacity. Local lake water was selected for the confirmation of Aloe barbadensis gel efficiency in fluoride removal which examined for fluoride removal at various concentrations of ale gel and showed sharp reduction of all the parameters such as EC, TDS, hardness, COD and quantity of sulfates in 15 days.

The fluoride becomes reduced as on 7th days the fluoride was 4.4mg/ml on 20 ml concentration and on 15th day it reduced as 3.2mg/ml on same 20ml dosage of Aloe barbadensis gel. It conforms that the gel of Aloe barbadensis has the efficiency to the defluoridisation process. Similar kind of study was performed by Radhey Shyam and Kalwania [6], they have been used the Aloe barbadensis and calcium chloride to precipitate out fluoride ions from drinking water.

## IV. Conclusion

The overall results concluded that the Aloe barbadensis gel has number of phytochemical ingredients. Further it also has water purification properties. The Aloe barbadensis plant has found having some fluoride removal capacity, helped in the fluoride removal process. Since plants are available naturally, the phytoremediation method can be adopted instead of chemical treatment for the benefit of the humans.

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