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ON

Analysis of the Bioavailable Fluoride Level in Chewable Part of Some Selected Khat Cultivars Commercially Available around Jimma

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Analysis of the Bioavailable Fluoride Level in Chewable Part of Some Selected Khat Cultivars Commercially Available around Jimma

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This is to certify that a thesis submitted by **Sintayehu Gashaw Kebede**: *Analysis of the Bioavailable Fluoride Level in Chewable Part of Some Selected Khat Cultivars Commercially Available around Jimma* submitted in partial fulfillment M. Sc degree in Chemistry (Analytical stream) complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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Abbreviations and Acronyms

EDTA	EthyleneDiamineTetraacetic Acid
FISE	Fluoride Ion Selective Electrode
ISE	Ion Selective Electrode
LOD	Limit of detection
LOQ	Limit of quantification
TISAB	Total Ionic Strength Adjustment Buffer.
WHO	World Health Organization

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Abstract

In this study, the levels of fluoride in selected commercial Khat (Catha edulis Forsk) of Jimma Town, Ethiopia have been investigated using fluoride ion selective electrode. Khat samples were collected from different local Khat shops available in the Town. The collected samples were transported to laboratory and prepared for analysis by taking the chewable parts only, i.e., the youngest leaves and tips of the samples were considered for analysis of both the water soluble and the total fluoride contents. Determination of the water-soluble fluoride content of each sample was carried out by soaking the chopped Khat leaves and tips in distilled water and stirred for five minute. Whereas, determination of the total fluoride content was performed by ashing the dried Khat leaves and tips using muffle furnace. For quantitative determination an external calibration curve which was constructed from seven point fluoride concentration levels was used. Recovery studies were conducted to test the suitability of the method used for the determination of fluoride in studied matrix and was found to be in the range of 88-to-112 %. The content of fluoride in fresh unwashed and washed leaves as well as in fresh unwashed and washed tips samples were found ranging from 0.056 - 0.046 µg /g and 0.041 0.049 µg /g as well as 0.037 - 0.045 µg/g and 0.034 – 0.041 µg/g and in dried leaves and tips samples studied were in the range of 3.35 - 5.30 µg/g and 2.66 - 3.87 µg /g, respectively. Furthermore, the obtained level of fluoride was compared with the reported literature values of other area Khat as well as different guidelines. The study findings, revealed the presence of significant difference in the fluoride level between the Khat leaves and Tips as well as between the unwashed and washed fresh samples. Generally, the observed concentrations of both water soluble and total fluoride in the studied Khat samples were below the WHO guideline for human daily safe intake of fluoride.

Keywords: Khat leaves, Tips, Fluoride ion selective electrode.

1. Introduction

1.1 Background o the study

The element fluorine occurs widely in nature as fluoride ion, either in free or combined forms. It is available in water, soil, minerals, food and in many plant and animal tissues. These days, the level of fluoride in different matrices has drawn the attention of the scholars because of its essentiality and toxicity at trace or lower and at higher concentration levels, respectively. Thus, investigation of the levels of fluoride in food, water and other matrices usually utilized in humans daily consumptions is of great importance since it is known to cause mottling of the teeth, and various bone disorders when ingested higher concentration levels [1, 2]. Fluoride is beneficial for human health if it is taken in concentrations range of 1.5–4.0 mg/day for adults and of course it needs less range for children [3]. According to the world health organization, WHO guideline, the level of fluoride in drinking water should not exceed 1.5 mg/L. In general, the presence of small amount of fluoride in humans daily consumptions could have a positive effect on dental caries [4], whereas long term exposure to its high concentrations could lead to fluorosis of the teeth and bones [5, 6, 7]. Figure 1.1, shows teeth and bone fluorosis.

(a) (b)

Figure 1.1: Photographs of (a) Teeth and (b) skeletal fluorosis
https://www.google.com.et/sekeletal fluorosis images)

All vegetations contain some fluoride, which is absorbed from the soil, air and water [8, 9]. Consequently, our foodstuffs might contain either trace or high levels of fluoride. Thus, the fluoride daily intake level of humans varied depending on the sources their dietary intake, such as water, beverages, and foods. Intake of fluoride at high level, above 6 mg/day for a long time could causes dental, skeletal and non skeletal fluorosis [10]. Moreover, exposure to excessive intake of higher levels of fluoride could also cause acute health problems [11, 12] and in some

cases, it may interferes different metabolic activities as well as influences the DNA [2, 13]. Besides, it may cause various diseases such as liver, brain and kidney damage [2].

The total exposure to fluoride, generally, depends on various products that human beings daily consumption including drinking water, beverages, foods, food supplements such as spices, toothpaste, Khat and so on [14-17]. Khat (Catha edulis Forsk), is evergreen shrub or tree of the family celastraceae [18]. It was named by the Sweden botanist Peter Forskal [19]. It is also spelled and/or named by other names like Catha, Qaat, Qat, Kat, Chat, Quat, Tschat, Kuses-Salahin, Jaad, Kaad, Gat, Jimaa, Herari, Mirra, Tohai, African Tea, Abyssinian tea, Flower of paradise, bushman's tea and African salad in different countries and localities[20, 21]. The commercialized part (cutoff) of Khat is illustrated in Figure 1.2.

Figure 1.2: Cutoff of Khat plant (Khat) commercialized in the market of different cultivars.

Khat is grown commercially in the mountains of Yemen, Ethiopia, Somalia, and Kenya and naturally in Turkestan, Afghanistan, Tanzania, Uganda, Zambia, South Africa and Madagascar [22-24]. It is also grown in Israel by Jews of Yemeni origin [25]. Khat is usually utilized (chewed) because of its stimulating nature. Khat consumption is a common habit in certain countries of East Africa, and Yemen [26] and Saudi Arabia [27], but beginning of its origin and consumption is inconclusive and speculative [28, 29].

Khat, especially, its youngest leaves and tips, contain "a cathinone", an alkaloid, which is believed to have stimulating effect on the central nervous system and thus, resulting in mood elevation and excitement [30-31]. On the other hand, Khat chewing has also exhibited negative side effects including elevation of blood pressure, tachycardia, hyperthermia, increased sweating, muscular weakness, loss of appetite, spermatorrhea, and gastrointestinal disturbances [32, 33].

Furthermore, regular use of Khat causes tooth discoloration, gum disease, and oral cancer [34]. Nevertheless, these days, Khat is used as a source of income for the cultivars as well as business men. For, instance, in Ethiopia, in addition to, the enormous local or domestic consumptions, nowadays, Khat is used as the sources of foreign exchange revenue [35, 36]. Figure 1.3, demonstrates how Khat is commercialized in Ethiopian local markets.

Figure 1.3: Commercial activities of Khat in local markets of Ethiopia (*https://www.google.com.et/khat images*)

Khat use is widespread, deep-rooted and cultivated in most parts of Ethiopia, where its use is socially condemned and even not impressive. In recent days, Khat consumption is becoming a common practice among all age levels (except children) and different societal groups of the country [37, 38]. For instance, as reported in the literatures 18.4% of Dire Dawa high school students and 37.8 % of Jimma town of the communities [39] as well as 64.9 % of Agaro secondary school students chewed Khat [37]. The reported data indicated that Khat consumption in Jimma Town is high, necesseting the investigation of the concentration levels of various toxic chemicals including fluoride in the product. In some part of Ethiopia, particularly, in Rift Valley Region, the health risk of fluoride, i.e., teeth fluorosis has been seen many years back, because of high accumulation of fluoride in the region [40-43]. Moreover, in addition to the Rift Valley region of the country, high concentration of fluoride was also reported in drinking water in the area around Jimma in the Southwest of the country [44]

Therefore, in this work, the levels of bioavailable fluoride in selected commercially available Khat of Jimma Town of Ethiopia were investigated utilizing fluoride ion selective electrode (FISE). In the study, various Khat samples were collected from different local Khat shops available in the Town and of both the soluble and total fluoride concentrations were determined.

The obtained results were statistically validated utilizing ANOVA. Moreover, the levels of fluoride of the studied Khat samples were also compared with the reported literature values of other area Khat as well as WHO guideline for human daily safe intake of fluoride.

1.2. Problem of the statement

Nowadays, Khat becomes one of the important commercial items in local markets and source of foreign exchange revenue for the country [35, 36]. Despite its economic and business benefit Khat has so many health and social disadvantages. The presence of fluoride in Khat leaves of some areas of Ethiopia, like Hawassa area, Bahir Dar area and Addis Ababa, has been reported [14]. The finding indicated the variation of the fluoride concentrations with Khat cultivars origin and thus, left an assignment for the determination of fluoride content of Khat grown in other parts of the Ethiopia, like Jimma areas, in southwest of the country. Jimma is one of the most commonly Khat consuming [39] and suspected of high level of fluoride [44]. Despite this fact, the fluoride content of Khat cultivated and commercialized in Khat markets of Jimma town and Jimma area woreda towns, in Jimma zone, southwest of the country is not yet investigated. Therefore, this study was conducted to answer the following research questions:

1. What is the level of fluoride in some consumable part of Khat commercially available in Jimma Town?
2. How do the fluoride concentrations of the commercial Khat of Jimma town varied with respect to the reported fluoride contents of Khat in other parts of Ethiopia?
3. Do the fluoride concentrations of commercial Khat of Jimma Towns exceed the daily intake?
4. Does washing of the Khat before consumption beneficial to minimize the risk of fluorosis?

1.3. Objectives of the study

1.3.1. General objectives

To investigate Bioavailable fluoride level in the chewable parts of Khat commercially available in Jimma Town utilizing fluoride ion selective electrode.

1.3.2. Specific objective of the study

1. To determine the bioavailable fluoride concentrations in the consumable part of Khat commercially available in Jimma Town.
2. To evaluate whether washing of Khat prior to consumption is beneficial to minimize the fluoride levels or not

3. To compare the fluoride content of commercially available Khat in Jimma Town with other area of Ethiopia reported Khat fluoride content.
4. To evaluate the commercial Khat daily fluoride contribution on the consumers with the safe fluoride daily intake level.

1.4. Significant of the study

Fluoride even if it is beneficial in small amount it can cause several acute health problems in high level. It's beneficial and toxic level is controlled by managing the daily intake of fluoride from various sources such as food and water. Because of this analyzing the level of fluoride in consumable items are very important to minimize the risk of fluoride related health complications. Therefore, the finding obtained from this study can serve as an indicator for the level of fluoride in Khat, commercially available in Jimma Town. It may also help for other researchers to study the details of the level of fluoride in Khat sampled from the field in line with the soil and water and other related samples.

2. Review of related literature

2.1. Khat and its' chewing habit

Khat (Catha edulis Forsk), is evergreen shrub or tree of the family celastraceae [18]. Khat requires well-drained field with pH range of 6.0–8.2. The optimal altitude and annual rainfall for its growth ranges from 1500 to 2100 m and 1000 to 1500 mm, respectively [35, 45]. Depending upon the geographic areas of cultivation in the country, Khat could be classified into different types. Even in some cases, different varieties of Khat could be grown on the same farmland. Khats are characterized by having different color, size and height of the leaves, and size and height of the plant as a whole [14].

Historical evidences, indicated that the consumption of Khat was began in the southern Red sea area (i.e., in Yemen or Ethiopia) prior to the mid fourteenth century [29]. During the last two decades, Khat chewing has been spread worldwide, particularly to Europe and United State of America, with the advancement of aviation and tourisms industries [46].

Khat chewing habit is a wide spread, deep-rooted in most parts of Ethiopia. These days, it is commonly consumed at all age levels (except children) and different societal groups of the country [37, 38].The most favored part of Khat for consumption is leaves, particularly the soft young shoots near the top of the plant [47]. Some consumers also utilize the top young shoot stems, leaves and stems at the middle and lower part of the plant [14]. Khat is chewed for its stimulating property, because it contains a Cathinone, the important stimulant chemical. In addition, Khat also contains different compounds including alkaloids, terpenoids, flavonoids, sterols, glycosides, tannins, amino acids, vitamins and minerals [32, 48-50]. But, of these components Khat is mostly known due to its phenylalkalyamine(-)-alpha aminopropiophenone named as cathinone [51] and cathine (nor-pseudo-ephedrine) compositions [47].

When the Khat is chewed, a psychoactive substance, cathinone, is extracted and ingested and thus, stimulate the central nervous system, analogous to the stimulation caused by amphetamine [52, 53]. In addition to cathinone and cathine four major alkaloid fractions cathinine, cathidine, eduline, and ephedrine were also isolated [54, 55].

Khat chewing, nowadays, becomes severe problem over east Africa especially in Ethiopia, Somali and southern Arab from the old to young, male and female, urban and rural situation [55]. Though, Khat is used as stimulant, it has also believed to have heath [32, 33]. Reports indicated that long-term Khat chewing causes intrinsic and extrinsic stains on the surface of the teeth [34]. As it is reported, various chemical components in the leaf and some other multiple external sources such as sugar and drinks taken during chewing process cause external and possibly internal teeth stains. The cause of internal staining may be swallowing of the Khat juice due to the fluoride content of the Khat leaves [34].

2.2. Khat and fluoride

Vegetations are expected to contain fluoride, via absorbing from the soil, air and water [8, 9]. The levels of fluoride in the plant varied from place to place depending on its availability in the soil, air and water sources [56-58]. Furthermore, the nature of the soil and its pH also affects the bioavailability of fluoride in the different parts of the plant [57, 59]. Though, in the earlier years, Khat was grown in natural soil, currently, cultivars are utilizing fertilizers to increase its production. Thus, the plants can take up more fluoride due to one or more of the specified reasons [35, 60]. As has been reported, the geographic distribution of fluoride in surface water and groundwater in Ethiopia, indicated that 24.2 % of the total 1438 water samples tested, had fluoride concentrations above 1.5 mg/L, the recommended optimum concentration of fluoride in drinking water set by WHO [61]. Subsequently, Khat grown in such areas may have the possibility to accumulate fluoride and thereby contribute to the daily fluoride intake during its consumption.

Hattab and coworkers, reported Khat leaves contain some amount of fluoride. The study reported on Yemen's Khat samples indicated that the presence of less than 0.02 mg/mL leached fluoride into water or saliva; 0.06 µg/mL in saliva after chewing; 0.93 µg/g in dried leaf; and 2.07 µg /g in ash [18]. Similarly, the study conducted on some Ethiopian Khat leave samples also demonstrated the availability of fluoride in the range of 3.4-7.1 µg /g dry weight (total fluoride) and 0.19–0.43 µg /g fresh weight (i.e., water-extractable content). The report also identified that Khat leave samples from selected Hawassa area districts such Chengie, Yirba and Sike contains more fluoride content than the samples that were collected from other areas of the country including Bahir Dar, Wondo-Kuto, Wondo-Basha, Awadai, Liyu, Gelemso, Belechie, and

Guragie [14]. Ashenef and Engidawork, also reported that similar fluoride concentrations, except the Khat samples collected from home garden in Addis Ababa city, Addis Ababa, Ethiopia, was unexpectedly contained the highest concentration, i.e., 18.53 μg /g, which was justified because of industrial fluoride contamination of the area [62].

2.3. Fluoride and it's human health effect

Trace level of fluoride is beneficial for human health, particularly, for controlling of dental caries [4]. Fluoride for adult in the range of 1.5–4.0 mg/day intake is safe but for children the safe range is less [3]. According to WHO guideline, drinking water should not contains above that 1.5 mg/L of fluoride. However, long term consumption of excessive amounts of fluoride can lead to fluorosis of the teeth and bones [5-7]. Fluoride ion is attracted by positively charged calcium ion in teeth and bones and which results in dental and skeletal fluorosis in high fluoride ingestion, in children as well as in adults. Fluorosis in mild version can be evidenced by mottling of teeth and in high version by embrittlement of bones and neurological damage [63]. In some of the cases, fluoride may even interfere with carbohydrates, proteins, vitamins and mineral metabolism as well as interfere with DNA synthesis [13]. Studies have also shown that, major of the kidney diseases have a great inclination to the toxicity of fluoride, i.e., long term exposure to high concentration of fluoride might exterminate the kidney function [12]. Various diseases such as arthritis, infertility, brain damage, and thyroid disorder might result from excessive intake of fluoride [2]. Exposure to excessively high concentration of fluoride could also cause acute effect on soft tissues like aorta, thyroid, lungs, kidneys, heart, pancreas and brain [28, 29].

2.4. Analytical methods to determine fluoride

Different analytical methods have been applied to determine the fluoride content of various samples. Analytical methods, such as potentiometry utilizing fluoride ion selective electrode (FISE) [14, 62, 64], spectrophotometry [15, 16, 65] and ion chromatographic [66] have been widely used for fluoride determinations. Of these techniques, FISE, most abundantly employed than spectrophotometry and ion chromatographic because of its non destructiveness, simplicity, and accessibility [67-69]. For instance, FISE was utilized for determination level of fluoride Khat [14, 62], tea [62, 64, 68], vegetables [69] and toothpaste [15]. In this study, FISE was also used to measure level of fluoride in Khat samples.

3. Materials and methods

3.1. Chemicals and reagents

The chemicals and reagents used in the analysis were all of analytical grade. Anhydrous sodium fluoride (NaF, 99.0 %) and Glacial acetic acid obtained from BDH Chemicals Ltd (Poole, England). Sodium chloride (NaCl) was purchased from Oxford Laboratory Ltd (Mumbai, India), sodium hydroxide (NaOH), disodium salt of ethylene diaminetetraacetic acid (EDTA), and acetone (CH_3OH) were purchased from Fisher Chemicals (England) and Hydrochloric acid (HCl) was obtained from Wardle Chemicals Ltd (Birmingham, England).

3.2. Instruments

Hanna, pH 211 microprocessor-based pH/mV/°C Bench-top Meter and glass pH electrode were obtained from Hanna instruments, Inc (Póvoa de Varzim, Portugal). Fluoride ion selective electrode (FISE)-HI4010 and silver silver-chloride, HI 5315 Reference Electrode for ISE half-cells reference electrode were also purchased form Hanna instruments, (Woonsocket, Rhode Island, USA). An oven-Genlab, MINO/6/SS which was used for Khat samples drying was from Wolf Laboratories Limited (Colenso House, UK). A digital Bench Top Furnace which was used for ashing of samples was obtained from Thermoline Scientific, (10-12 Ross Place, Wetherill Park NSW 2164, Australia), Stuart CB162 Stirrer Hotplate from Keison International Ltd (Chelmsford CM1 3UP, UK) also utilized in the study.

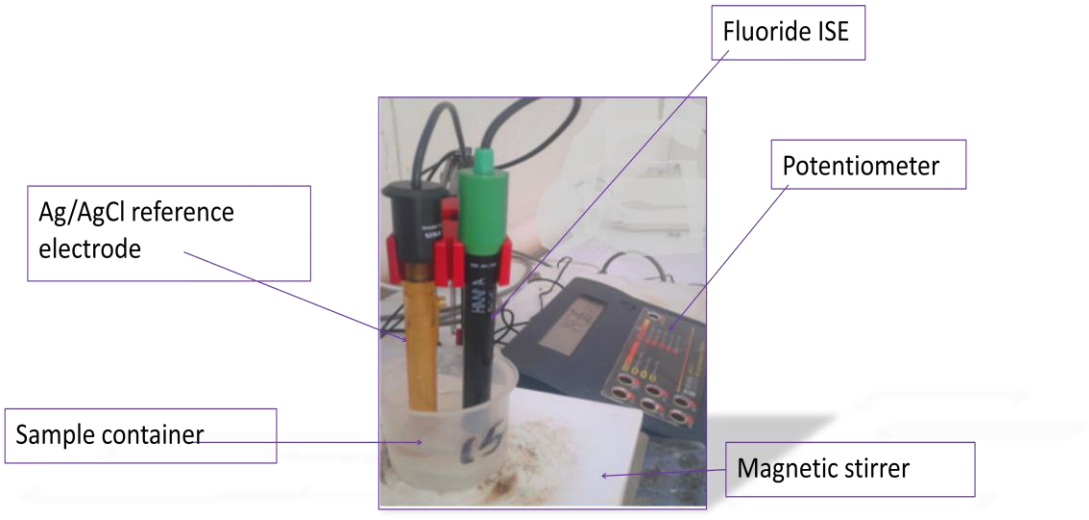


Figure 3.1 Set up of potentiometer for determination of fluoride

3.3. Preparation of solutions

To prepare TISAB solution, firstly, 58 g sodium chloride and 4 g disodium salt of EDTA was dissolved in approximately 350 mL distilled water separately and mixed together. Then, after adding 57 mL glacial acetic acid, the resulting solution volume was adjusted to 800 mL by addition of more distilled water. Subsequently, after adjusting its pH to 5.4, utilizing 10 M sodium hydroxide, distilled water was added until the total volume attain 1000 mL. Buffer solutions of pH 4, 7, and 10 were also used to calibrate the pH-meter in order to adjust the pH of TISAB solution at pH 5.4 and the pH of the ashed sample solutions between 8 and 9. Distilled water was used throughout the experiment for sample preparation, dilution, and rinsing apparatus prior to analysis.

Fluoride stock standard solution of 1000 mg /L was prepared by exactly weighing 2.21 g of an oven dried anhydrous NaF and quantitatively dissolving it in distilled water to the final volume of 1000 mL in volumetric flak. Then, intermediate solutions of fluoride were prepared from the stock solution. Thereafter, the working standard solutions containing seven concentration levels, ranging from 0.1-30 mg/L were prepared from the intermediate solution and then used for the construction of the calibration curve.

3.4. Sampling of Khat samples

There are various types of Khat cultivars commercially available in Jimma Town, which are commonly named based on cultivation areas. These verities include Bedabuna, Merewa, Hawari, Koredo, Finicho, Mole, Dedo, Sombo, Aniya, Chora, Sentema, Sedero, Kolasa, Butire and Alemayehu Mecha.

Therefore, in this study Mole, Koredo, Sombo, Butire, Bedabuna, and Hawari Khat verities were selected for the analysis of the levels of bioavailable fluoride. The Six varieties of Khat samples were collected from four Khat local markets of the Town. The studied Khat varieties were selected based on their availability in the market places. Figure 3.1, shows the collected Khat samples.

Figure 3.2 Cultivars of Khat samples analyzed

From different local Khat shop in the Town similar sample cultivars were collected. Composite samples of each cultivar chewable part of 100 g were prepared for both water-exchangeable and total fluoride contents. The chewable parts, i.e. the young leaves and tips, were taken

Picking of the youngest leaves and tips Picked leaves Picked tips

Figure 3.3 Chewable parts of the Khat samples prepared for analysis

Thereafter, a portion of each samples fresh leave was taken for investigation the level of water-extractable fluoride. The remaining portions of the samples were oven dried at 80 ^{o}C for about 12 h, until constant weight was obtained. The dried samples were ground and kept in tightly closed plastic containers until time of analysis.

3.5. Extractions of fluoride from the Khat leaves

3.5.1. Preparation of the samples for water-extractable fluoride

A sample preparation procedure earlier reported by Atlabachew and coworkers [14] was employed for extraction of fluoride from the Khat samples. Washed and unwashed samples were used for the analysis of water-exchangeable fluoride content of each sample in this study. Washed samples were obtained by washing the portion of the earlier prepared samples utilizing distilled water. To investigate fluoride content, 4 g of the fresh chewable part; leaves and tips of both washed and unwashed samples, was separately taken in plastic beakers and then chopped with pestle. Then, after adding 24 mL distilled water the mixture was vigorously stirred with magnetic stirrer for 5 min. Finally, a portion of the extract was taken for the quantitative analysis. Sample preparations procedure for water-soluble fluoride analysis is demonstrated in Figure 3.4. All sample preparation and determinations were made in duplicates. To prevent cross contamination, the chopping pestle was rinsed prior to subsequent use.

(a) Leaves and tips of the Khat sample

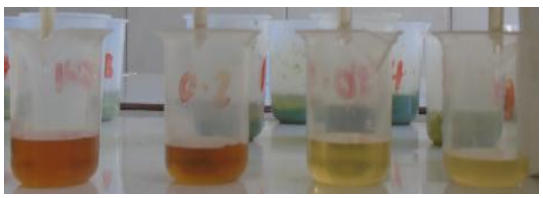
(b) Weighted samples

(c) Chopping of the sample

(d) Chopped leaves

(e) Chopped tips

(f) After 24 mL distilled water added



(g) Water soluble extract of the chopped samples

Figure 3.4: Sample preparations procedure for water-soluble fluoride analysis

3.5.2 Preparation of the samples for total fluoride content analysis

For the total fluoride contents determination, procedure earlier reported by Atlabachew and coworkers [14] was also utilized with sample weight and volume of NaOH solution slightly adjustment to ash the Khat samples. Accordingly, 0.5 g of each earlier dried and ground Khat sample was taken into 30 mL nickel crucible. Then, after adding 3 mL of 8 M NaOH the content was placed on a Hotplate heater at 150 ^{o}C for about1.5 h, until NaOH was solidified. Then, the resulting composition was placed in a muffle furnace at 200 ^{o}C, and 525 ^{o}C for 1.5 h and 2.5 h, respectively, in order to fuse the sample. Eventually, the fused (ashed) sample was allowed to cool to room temperature and then, 10 mL distilled water was added and heated on hotplate to facilitate the dissolution of the content. Then, about 4 mL conc. HCl was slowly added in to the content to adjust the pH between 8 and 9. Figure 3.5, illustrates sample preparations steps for the analysis of total fluoride. The obtained solution was transferred to plastic beaker. The crucibles were rinsed successively with 5 mL distilled water until the final volume reached 50 mL. All washings were mixed and filtered with Whatman filter paper 110 mm and the filtrate was transferred to 50 mL plastic bottles and then, the portions of the filtrate was used for total fluoride content determination [14].All measurements were taken, after mixing a portion of the filtrate with an equal volume of the TISAB solution.

(a) Oven dried sample (b) Powder of dried sample (c) Sample fused with NaOH

(d) Ashed samples (e) dissolution of the ashed sample in water (f) Filtered sample solutions

Figure 3.5 Sample preparations procedure for the analysis of total fluorides

3.6. Calibration and measurements

The instrument was calibrated by measuring the potentials of 0.1-30 mg/L standard solutions of fluoride at seven points. The total ionic strength adjustment buffer (TISAB) solution was added to each of the standard solution in 1:1 volume by volume ratio. The calibration curve was constructed using the potential in mV as a function of the negative logarithm of the concentration of fluoride in the standard solutions.

3.7 Method Validation

The efficiency of the method for water-soluble and total fluoride analysis was evaluated using recovery experiment, i.e. by adding known concentration of sodium fluoride solution to 4 g of the fresh Khat leaves and tips of samples in both wash and unwashed conditions and also for total fluoride analysis 0.5 g of dry khat leaves and tips of the samples. The procedure was as follows: each 4 g of fresh khat leaves and tips of the samples were spiked with 16 µL of 1000 mg fluoride/L solution and also 0.5 g of dried Khat leaves and tips of each the samples were spiked with 20 µL of 1000 mg fluoride/L solution and all the ashing process for total fluoride analysis were followed. After extraction, following the procedure, to check the method efficiency the determination was made in triplicates.

Statistical analysis of data was made to verify whether there was a significant difference in water soluble and total fluoride concentration between a pair wise comparative investigation of the six cultivars of khat samples analyzed. For this study, the significance of variation within sample and between samples has been studied using one-way analysis of variance (ANOVA) calculations were made using *Microcal Origin-6.0* software.

4. Results and Discussion

4.1 The calibration curve

The calibration of the instrument is demonstrated in the Figure 4.1. The constructed calibration curve was demonstrating good linearity with coefficient of determination of 0.9983. All the sample solutions were measured following the calibrations of the instrument.

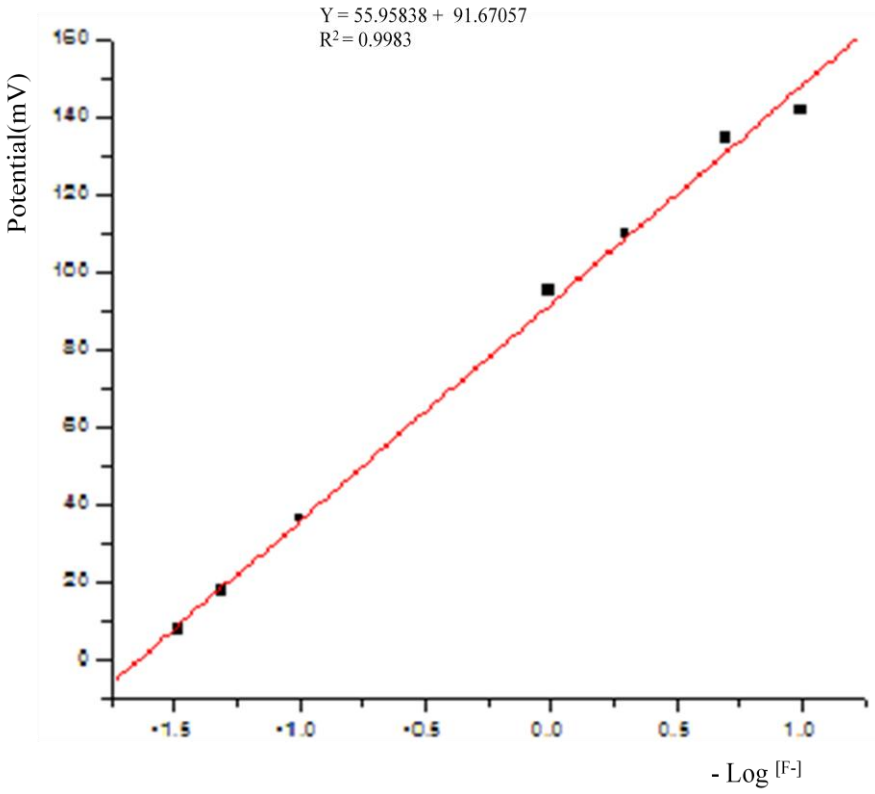


Figure 4.1 Calibration curves of the analysis

4.2 Recovery analysis

To investigate the accuracy of the utilized method recovery studies were performed by spiking the Khat samples. The observed recoveries for both water-exchangeable and total fluoride analysis are presented in Table 4.1. As can be seen, the obtained recoveries were found to be in the range of 88 - 110 % and 90 -112 % for fresh and dried samples, indicating the reliability of the utilized method for the analysis of both water exchangeable and total fluoride in Khat samples. Generally, as shown in Table 4.1, the percentage recoveries were found between 88 to 112 % for all spiked samples, which are within the acceptable range.

Table 4.1 Recovery test results (mean, n=9). **(**a) For water soluble fluoride content analysis and (b) For total fluoride content analysis.

(a)

Cultivar: **Mole**	Sample fluoride amount in µg/g	Spiked fluoride amount in mg/L	Spiked sample fluoride amount in µg/g	% Recovery	Cultivar: **Koredo**	Sample fluoride amount in (µg/g)	Spiked fluoride amount in mg/L	Spiked sample fluoride amount in µg/g	% Recovery
Unwashed leaves	0.056	0.400	0.483	107	Unwashed leaves	0.055	0.400	0.453	100
	0.055	0.400	0.475	105		0.054	0.400	0.470	104
	0.055	0.400	0.497	110		0.055	0.400	0.428	93
Washed leaves	0.049	0.400	0.418	92	Washed leaves	0.051	0.400	0.458	102
	0.049	0.400	0.399	88		0.049	0.400	0.428	95
	0.048	0.400	0.443	99		0.049	0.400	0.435	97
Unwashed tips	0.046	0.400	0.453	102	Unwashed tips	0.045	0.400	0.425	95
	0.043	0.400	0.448	101		0.044	0.400	0.446	101
	0.046	0.400	0.462	104		0.045	0.400	0.465	105
Washed tips	0.041	0.400	0.442	100	Washed tips	0.042	0.400	0.436	99
	0.040	0.400	0.447	102		0.040	0.400	0.453	103
	0.043	0.400	0.427	96		0.041	0.400	0.427	97

(b)

Cultivar: **Bedabuna**	Sample fluoride amount in µg/g	Spiked fluoride amount in mg/L	Spiked sample fluoride amount in µg/g	% Recovery	Cultivar: **Sombo**	Sample fluoride amount in (µg/g)	Spiked fluoride amount in mg/L	Spiked sample fluoride amount in µg/g	% Recovery
Leaves	3.460	0.400	3.830	92	Leaves	4.370	0.400	4.810	110
	3.500	0.400	3.910	102		4.420	0.400	4.830	102
	3.510	0.400	3.870	90		4.460	0.400	4.890	108
Tips	2.940	0.400	3.310	92	Tips	2.660	0.400	3.100	110
	3.000	0.400	3.420	105		2.800	0.400	3.230	108
	2.990	0.400	3.380	98		2.860	0.400	3.310	112

4.3 Water soluble fluoride level of washed and unwashed fresh Khat samples

The fluoride levels of washed and unwashed chewable parts of Khat; the leaves and tips were investigated for the collected samples. The water soluble fluoride contents of unwashed leaves, unwashed tips, washed leaves and washed tips of the Khat samples, are shown in Table 4.2

Table 4.2: Mean concentrations ($\overline{x}$ ± SD, n = 4) of water extractable fluoride in fresh Khat samples

Khat type	Chewable parts	Unwashed (*)	% RSD	Washed (*)	% RSD
Mole	leaves	0.056 ± 0.002	4.410	0.049 ± 0.001	1.960
	tips	0.045 ± 0.002	3.920	0.041 ± 0.002	3.640
Koredo	leaves	0.055 ± 0.002	4.200	0.050 ± 0.002	3.640
	tips	0.045 ± 0.002	3.920	0.041 ± 0.001	3.360
Bedabuna	leaves	0.046 ± 0.002	3.500	0.041 ± 0.002	3.780
	tips	0.037 ± 0.002	4.940	0.034 ± 0.002	4.940
Sombo	leaves	0.049 ± 0.002	4.070	0.044 ± 0.0012	4.360
	tips	0.038 ± 0.002	4.940	0.034 ± 0.001	2.910
Butire	leaves	0.047 ± 0.002	4.590	0.041 ± 0.002	3.730
	tips	0.037 ± 0.002	4.310	0.034 ± 0.001	3.730
Hawari	leaves	0.048 ± 0.002	4.880	0.043 ± 0.001	2.300
	tips	0.039 ± 0.002	4.310	0.035 ± 0.001	3.440

* Content of fluoride in µg /g on fresh weight base

As can be seen from Table 4.2 it was observed that the levels of fluoride in unwashed and washed leave samples as well as in unwashed and washed tip samples were found ranging from 0.056 - 0.046 µg /g and 0.041 0.049 µg /g as well as 0.037 -0.045 µg/g and 0.034 – 0.041 µg/g, respectively. The statistical t-test studies at $p \leq 0.05$ indicated that the presence of significant difference in water-exchangeable concentrations of fluoride in washed and unwashed leaves samples. The study demonstrated that washing significantly removes fluoride from the leave samples. The statistical t-test studies at $p \leq 0.05$ also revealed the existence of significant differences between leaves and tips samples. The finding showed that relatively higher fluoride

concentrations in leaves than that of the tips. Though, there was no reported justification on the variation of the water exchangeable fluoride content in leaves and tips Khat samples, the relatively larger size of the leaves than the tips might be attributed to accumulate the variations. A report on tea samples also indicated that as the leaves samples contain more fluoride than the buds or tips [62]. However, the one way ANOVA test at $p \leq 0.05$ showed that there were no significant differences in the concentration of water-exchangeable fluoride, among the studied Khat cultivar samples, indicating Khat plants in the studied areas are grown in similar agricultural practices and environmental conditions.

The study conducted on Yemeni Khat, showed that the presence of about 0.06 (µg/mL) fluoride in saliva after chewing Khat [18]. Atlabachew and coworkers also reported, the existence of water-extractable fluoride in the range of 0.19-to-0.43 µg/g in selected Khat samples collected from other parts of Ethiopia [14] In general, the obtained results demonstrated that the studied commercial Khat samples in Jimma town have significantly lower water-exchangeable fluoride levels than similar studies reported in other areas of Ethiopia [14].

On the other hand, according to the United States Department of Agriculture, USDA, the national fluoride database selected beverages and foods, most fresh fruits, fresh vegetables, and surface water, contain very low level of fluoride ranging from 0.01 - 0.1 mg/L. But, the level of pesticides in such products may increase when agrochemicals, such as pesticides, containing fluoride are sprayed on the product [70].

Generally, fluoride is available in soil, water and air. But, increase in population and industrialization also resulted in pollution to water, air, and soil, which in turn increases the content of fluoride in the plant. Particularly, agricultural activities such as use of fertilizers, pesticides, and irrigation with contaminated sewage are the major source of contaminations [71, 72]. Naturally, water in some areas contain higher amount of fluoride [41, 44, 61]. Therefore, plants grow on these areas get higher fluoride content than other areas. Agricultural products grown in areas where soils have high amounts of fluoride or where phosphate fertilizers are used may have higher levels of fluoride [60, 73]. Thus, the reported water-exchangeable fluoride content obtained result 0.19–0.43 µg/g, is significantly higher than that of this study and may have some sources for higher fluoride exposure as aforementioned factors. On the other hand the harvesting season of the Khat may contribute for the lower content of the water soluble fluoride

in this study due to the rain wash some amount of surface fluoride from the leaves on the plant in the field.

The seasonal aviation on the fluoride content of tea indicates that the tea leaves harvested in the season of early summer are superior in quality to those collected in later seasons. i.e., the harvesting season, that is the rain, may have impact on the fluoride content of the tea [74]

4.4 Total fluoride level of dry Khat samples

The total fluoride level of Khat leaves and tips were investigated and the obtained results are shown in Table 4.3.

Table 4.3: Mean concentration ($\bar{x}$ ± SD, n =4) of total fluoride content in dry Khat samples

Khat type	Chewable parts	Content (*)	% RSD
Mole	leaves	3.45 ± 0.15	4.42
	tips	3.32 ± 0.04	1.18
Koredo	leaves	3.35 ± 0.09	2.65
	tips	3.09 ± 0.07	2.36
Bedabuna	leaves	3.40 ± 0.08	2.36
	tips	2.91 ± 0.06	2.06
Sombo	leaves	4.37 ± 0.05	1.18
	tips	2.66 ± 0.05	1.77
Butire	leaves	5.30 ± 0.08	1.47
	tips	3.64 ± 0.06	1.77
Hawari	leaves	4.96 ± 0.10	2.06
	tips	3.87 ± 0.09	2.36

* Content of fluoride in µg /g on dry weight base

The observed results, Table 4.3, demonstrated that the total fluoride contents of the chewable leaves and tips of the Khat samples studied were in the range of 3.35 - 5.30 µg/g and 2.66 - 3.87 µg /g, respectively. The statistical t-test study at $p \leq 0.05$ exhibited the existence of significant difference between the chewable leaves and tips Khat samples collected from the same cultivar. Similar to the water exchangeable fluoride content studies, leaves samples have relatively higher concentrations of total fluoride than the tip samples. Moreover, like the water-extractable

fluoride content studies, the ANOVA test at $p \leq 0.05$ revealed that there is no significant differences in terms of the total fluoride concentrations of the commercial Khat samples collected from the town, evidencing the selected Khat samples are grown in the same agricultural practices and environmental conditions. On the other hand, the studied Khat samples have relatively lower total fluoride content than the reported findings of other areas of Ethiopian Khat samples [14]. As mentioned earlier, the variation observed may be attributed to the difference of the sampling seasons, i.e., in the present study the samples were collected during the heavy rain season, which has expected to have dilution effect on the fluoride content in the soil and other sources. In general, similar to other reported evidences [14, 18], the obtained result demonstrated that the chewable part of Khat is not a significant contributor of fluoride. In all cases the findings were below the health risk threshold set by the WHO and other similar guide lines [10, 3].

However, the problem of fluoride arises from frequency and amount of exposures, toxicity from high level or long term accumulation. Since the daily fluoride intake is the sum from each contributor all possible sources such as fluoride-containing toothpaste, various beverages, spices, food additives, fish, tea and the like should be considered. The cumulative effect of fluoride in the Khat, that is the amount and frequency of consumption, may contribute health problems but not the Khat. Even if, the concentration of fluoride in Khat is far below the daily allowable limit, it may sum up for the daily intake. Therefore, it is better the chewers of Khat should try to minimize excessive use of it and try to use after washing the Khat. Also they should avoid use of matured leaves since they have higher content than the young leaves (young leaves, 6.5 μg/g; matured leaves 12 μg/g) [14]

5. Conclusion and Recommendation

5.1 Conclusion

In the present study, the level of fluoride in six different Khat cultivars, which are cultivated in different areas and commercially available in Jimma Town, was thoroughly investigated. The investigation involved analysis of the level of the water-soluble fluoride and total fluoride, in fresh and dried chewable parts; leaves and tips of the collected Khat samples. The analysis of water-soluble fluoride was done for both unwashed and washed samples. The obtained results demonstrated the existence of significant difference between unwashed and washed leaves as well as between leaves and tips of the Khat. The result of the study also indicated that the leaves of Khat have higher fluoride than the tips. However, the statistical test at $p \leq 0.05$ demonstrated the absence of significant difference between unwashed and washed tips as well as between cultivars water-exchangeable fluoride content.

Analysis of the total fluoride level was also done on leaves and tips of the collected samples. The findings revealed that similar to the water-exchangeable fluoride, leaves and tips of Khat samples have exhibited a significant difference at $p \leq 0.05$. On the other hand, ANOVA test at $p \leq 0.05$ indicated that the absence of significant differences in both water-soluble and total fluoride levels among the various Khat samples collected from the Jimma Town. In general, the observed, water-soluble and total fluoride content of the collected Khat samples were relatively lower than other areas Khat of Ethiopia and also below the recommended values of fluoride daily intake.

8.2 Recommendation

Based on observed results, the researcher would like to forward the following recommendations.

- In order to obtain details of the level of fluoride in the Khat commercially available in the town further study should be conducted by correlating soil, water and the Khat samples from the field.
- Further study has to be conducted on seasonal variation of the fluoride content of the Khat.

- Awareness has to be created by the consumers that Khat contains some trace amount of fluoride which may bring problem through long and excessive daily use.
- It is advisable that washing may aid to minimize the level of fluoride in Khat.

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