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Level of Fluoride in Khat Leaves and Tips Collected from Local Markets of Jimma Town, Ethiopia

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ABSTRACT

The level of fluoride in selected Khat (Catha edulis Forsk) samples available in Jimma Town local markets, Ethiopia was investigated using Fluoride Ion Selective Electrode (FISE). The samples were collected from different Khat local markets in the Town. Chewable parts, i.e., the youngest leave and tip of the samples were considered for the analysis of both water soluble and total fluoride contents. The fluoride content of fresh unwashed and washed leaves samples were in the range of 0.056 – 0.046 µg/g and 0.041 – 0.049 µg/g, respectively. In fresh unwashed and washed tips samples, the fluoride content were found to be 0.037 – 0.045 µg/g and 0.034 – 0.041 µg/g, respectively. In dried leave and tip samples, the fluoride was in the range of 3.35 – 5.30 µg/g and 2.66 – 3.87 µg/g, respectively. Recovery studies were also performed to test the suitability of the method used and was found in the range of 88 – 112%. Statistical t-test at $p \leq 0.05$ demonstrated the presence of significant differences between the fluoride content of unwashed and washed leaves and as well between leaves and tips. But, no significant difference was observed between unwashed and washed tips in terms of water-exchangeable (soluble) fluoride content. The observed concentrations of both water soluble and total fluoride in the studied Khat samples were below the guideline for human daily safe intake of fluoride. However, should aware that Khat contains some amount of fluoride which may cause problems through long and excessive daily use.
KEYWORDS: Khat, leaves, Tips, Fluoride, Ion Selective Electrode

INTRODUCTION

These days, the level of fluoride in different matrices has drawn the attention of scholars because of its essentiality at lower and toxicity at higher concentration levels. Thus, investigation of the fluoride levels in food, water and other matrices of humans daily consumptions is of great importance since it causes mottling of the teeth and bone disorders when ingested at high concentrations [1, 2]. The presence of small amount of fluoride in humans' daily consumptions could have an effect on dental caries, whereas long term exposure to high fluoride concentration could lead to fluorosis of the teeth and bones [3, 5]. Fluoride is attracted by positively charged calcium in teeth and bones and displaces hydroxide ions from hydroxyapatite, $Ca_5(PO_4)_3OH$, of teeth and bones, resulting in the formation of fluoroapatite, $Ca_5(PO_4)_3F$ [6, 7]. At a small concentration level, fluoride is used to strengthen the enamel, however; prolonged exposure to its high concentration level causes the conversion of large amount of the hydroxyapatite to fluoroapatite, which makes the teeth (enamel) and bones denser, harder and more brittle. In teeth this causes mottling and embrittlement, which is called dental fluorosis [[7, 8]. In some cases, it may also interfere the metabolic activities of carbohydrates, proteins, vitamins and minerals as well as synthesis of DNA [2, 9]. Exposure to excessively high levels of fluoride could also cause acute health problems, such as affecting soft tissues like aorta, thyroid, lungs, kidneys, heart, pancreas and brain [2, 10, 11]. Because of its disaster health effects on human beings, the World Health Organization (WHO) has set the level of fluoride in drinking water not to exceed 1.5 mg/L [12].

Vegetation can accumulate and store fluoride by absorbing from the soil, air and water [13]. Therefore foodstuffs may contain fluoride. The daily fluoride intake of humans is varied depending on its sources such as water, beverages and foods. As it has been indicated in the WHO guideline, fluoride intake at high levels, i.e., above 6 mg/day for a long period could cause dental, skeletal and non-skeletal fluorosis [14].

Khat *(Catha edulis Forsk)*, is evergreen shrub or tree and belongs to the family of celastraceae [15]. It grows in different countries including Yemen, Ethiopia, Somalia, Kenya, Turkestan, Afghanistan, Tanzania, Uganda, Zambia, South Africa, Madagascar and Israel [16 -18] The plant usually grows in well-drained field with soil pH 6.0 – 8.2; altitude 1500 to 2100 m and annual rainfall 1000 to 1500 mm [19, 20]. It has different names in

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different countries and localities [21, 22]. Khat is categorized into different types depending on areas of its origin, the color, size and height of its leaves and/or the plant as a whole [23]. In some cases, different varieties of the plant could grow on the same farmland. Khat is chewed because of its stimulating nature. Khat chewing is a common habit in East Africa countries [24], Yemen [24] and Saudi Arabia [25]. In the last two decades, its consumption has been worldwide, particularly to Europe and the United State of America, with the advancement of aviation and tourisms industries [26].

These days, Khat chewing habit is remarkably wide-spread and deep-rooted in most parts of Ethiopia. It is consumed at all age levels (except children and certain religious groups) and in different societal groups of the nation [27, 28]. The most favored part of Khat for consumption is its leaves, particularly the soft young shoots near the top of the plant [29]. Some consumers also utilize the top young shoot stems, leaves and stems at the middle and lower part of the plant [23]. —When the Khat is chewed, psychoactive substances, cathinone and cathine are extracted and ingested and thus, stimulate the central nervous system, analogous to the stimulation caused by amphetamine [29, 30, 31]. Other alkaloid fractions such as cathinine, cathidine, eduline and ephedrine are also isolated [32, 33]. Khat may also contain different compounds including terpenoids, flavonoids, sterols, glycosides, tannins, amino acids, vitamins and minerals [34, 35].

Khat chewing has been spreading because of its stimulating effect on the central nervous system, which causes in mood elevation and excitement [36]. However, it has also negative side effects on consumers including hypertension, tachycardia, hyperthermia, increased sweating, muscular weakness, appetite loss, spermatorrhea and gastrointestinal disturbances [37]. Its regular use can cause tooth discoloration, gum disease and oral cancer [38]. 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, Khat is used as the sources of foreign exchange revenue [17, 19].

The presence of fluoride in Khat leaves in some areas of Ethiopia, such as Hawassa area, Bahir Dar area and Addis Ababa, has been reported [21]. 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 which grows in other parts of Ethiopia, like Jimma areas, in southwest of the country. Jimma is one of the most commonly Khat consuming [39] and suspected area for the high levels of fluoride accumulation in water and other matrices [40]. Despite this fact, the fluoride content of Khat cultivated and commercialized in local Khat markets of Jimma town and the area district (woreda) towns of Jimma zone, is not yet investigated. Therefore, in this study, the fluoride content of the chewable parts (leaves & tips) of Jimma area Khat was investigated using fluoride ion selective electrode (FISE).

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents used in the analysis were analytical grades. 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 glacial acetic acid (CH_3COOH) were purchased from Fisher Chemicals (Leicestershire, England) and Hydrochloric acid (HCl) was obtained from Wardle Chemicals Ltd (Birmingham, England).

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, HI5315 Reference Electrode were also purchased form Hanna instruments, Inc. A digital Bench Top Furnace, obtained from Thermoline Scientific, (Ross Place, Wetherill Park NSW, Australia) was used for ashing of samples.

Preparation of solutions

The total ionic strength adjustment buffer (TISAB) solution was prepared by dissolving 58 g NaCl and 4 g disodium salt of EDTA in distilled water. After adding 57 mL glacial CH_3COOH, pH of resulting solution was adjusted to 5.3 ± 1.0 using 10 M NaOH. Then, distilled water was added to obtain the final volume of 1000 mL. A **s**tock standard solution containing 1000 mg/L fluoride was prepared by dissolving an appropriate amount of anhydrous NaF in distilled water. Intermediate standard solutions were then prepared from the stock solution. A series of standard solutions containing seven concentration levels, ranging from 0.1-30 mg/L were prepared from the intermediate solution and used for the construction of the calibration curve.

Khat sampling

Traditionally, Khat products are catagorized based on the area of cultivations. In Jimma town, various Khat variety are available including Bedabuna, Merewa, Hawari, Koredo, Finicho, Mole, Dedo, Sombo, Aniya, Chora, Sentema, Sedero, Kolasa, Butire and Alemayehu Mecha. In this study, six Khat samples: Mole, Koredo, Sombo, Butire, Bedabuna, and Hawari were collected from four local Khat markets of Jimma town in July, 2016. These varieties were selected based on their availability in the markets. For each cultivar, representative samples were collected from different Khat shops and composite sample of the chewable parts, i.e. the young leaves and tips were used for analysis. Fresh sample was used for investigation the level of water-extractable fluoride. For total fluoride analysis each sample was dried in oven at 80 ºC until constant weight was obtained. The dried samples were ground and kept in plastic containers until analysis.

Fluoride extraction procedure

Extraction of water-extractable fluoride

Both washed and unwashed Khat samples were used for the analysis of water-exchangeable fluoride content using the earlier reported Atlabachew and coworkers procedure [23]. Washed samples were obtained by washing the portion fresh samples using distilled water. To investigate fluoride content, 4 g fresh chewable parts: leaves and tips of both washed and unwashed samples, were separately taken in plastic beakers and then ground with pestle. Thereafter, addition of 24 mL distilled water, the content was stirred with magnetic stirrer for 5 min. Finally, the aqueous extract was taken for the quantitative analysis. All sample preparation and determinations were made in duplicates and the pestle was also rinsed using distilled water and dried prior to subsequent use to avoid cross contamination.

Extraction of total fluoride content

Extraction of the total fluoride contents from Khat samples was performed by slightly modifying the procedure of Atlabachew and coworkers [22] in terms of sample weight and volume of NaOH solution to insure complete ashing of Khat samples. Accordingly, 0.5 g of dried and ground Khat sample was taken into nickel crucible. Subsequently, after adding 3 mL of 8 M NaOH, the content was placed on the hotplate at 150 ºC for about 1.5 h, until NaOH was solidified. The resulting composition was then placed in a muffle furnace for a total of 4 h; at 200 ºC and 525 ºC for 1.5 h and 2.5 h, respectively, so as to fuse the sample. Eventually, the fused (ashed) sample was cooled to room temperature and then, after adding 10 mL distilled water; the content was again placed on hotplate to facilitate the dissolution. Then, about 4 mL conc. HCl was slowly added to adjust the pH of the solution in between 8 and 9. The resulting solution was then transferred to 50 mL plastic beaker. To ensure the complete transfer of the analyte, the crucible was repeatedly rinsed with distilled water and added to the extract until the final volume was reached 50 mL. The resulting solution was then filtered using Whatman filter paper prior to its determination for the total fluoride content.

Calibration curve

The instrument was calibrated by measuring the potentials of 0.1-30 mg/L standard solutions of fluoride at seven concentration levels. TISAB solution, 1:1 *(v/v)*, was added to each standard solution. 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 (Figure 1). It has demonstrated good linearity with coefficient of determination of 0.998.

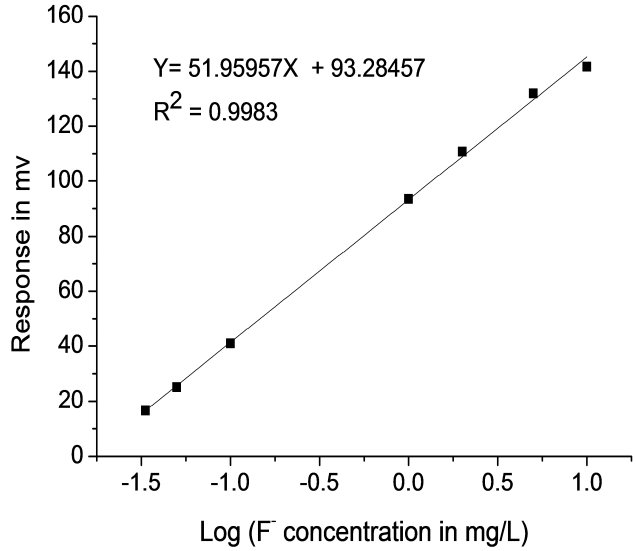

Figure 1*: Calibration curve used for the analysis of the water-soluble and total fluoride content of Khat samples.*

Method Validation

The efficiency of the method used was evaluated using recovery studies. Recovery studies were performed by spiking the fresh and dried Khat (leaves and tips) samples with known concentration of the standard fluoride solution. The experiments were carried out in triplicate.

Statistical Analysis

Statistical student t-test ($p \leq 0.05$) was used to investigate whether the water-soluble and total fluoride concentrations in leaves and tips of each cultivars exhibit significant differences or not. Similarly, one-way ANOVA ($p \leq 0.05$) was employed to evaluate whether the studied Khat samples collected from six cultivars have significant differences in fluoride concentrations or not.

RESULTS AND DISCUSSION

Recovery studies

To investigate the efficiency of the method used, recovery studies were performed by spiking Badabuna and Koreda Khat samples with known amount of fluoride standard solution. For each Khat sample, two samples were spiked and the obtained extract of each spiked sample was analyzed in duplicates. The observed recoveries for both water-exchangeable and total fluoride analysis are presented in Table 1. As can be seen, the obtained recoveries are ranging from 88 - 110 % and 90 -112 % for fresh and dried samples, respectively, indicating the reliability of the employed method for the analysis of both water exchangeable and total fluoride in Khat samples.

Table 1: Average recovery (%R ± SD, n = 4) of the water-soluble and total fluoride

Khat type	Water soluble fluoride				Total fluoride	
	Unwashed		Washed		Fused	
	Leave	Tip	Leave	Tip	Leave	Tips
Bedabuna	107 ± 2.52	102 ± 1.53	93 ± 5.57	99 ± 3.06	95 ± 6.43	98 ± 6.51
Koredo	99 ± 5.57	100 ± 5.03	98 ± 3.61	100 ± 3.06	107 ± 4.16	110 ± 2.00

SD: standard deviation

Levels of water soluble fluoride

The fluoride levels of washed and unwashed chewable parts of the Khat samples (i.e., the leaves and tips) were investigated and the results are shown in Table 2. The levels of fluoride in unwashed and washed leave samples were ranging from 0.056 – 0.046 µg/g and 0.041– 0.049 µg/g, respectively. Similarly, the fluoride content of unwashed and washed tip samples were ranging from 0.037 – 0.045 µg/g and 0.034 – 0.041 µg/g, respectively.

Table 2: Concentrations (Mean ± SD, n = 4) of water extractable fluoride in fresh Khat samples

Khat type	Chewable parts	Unwashed*	Washed*
Mole	Leaves	0.056 ± 0.002	0.049 ± 0.001
	Tips	0.045 ± 0.002	0.041 ± 0.002
Koredo	Leaves	0.055 ± 0.002	0.050 ± 0.002
	Tips	0.045 ± 0.002	0.041 ± 0.001
Bedabuna	Leaves	0.046 ± 0.002	0.041 ± 0.002
	Tips	0.037 ± 0.002	0.034 ± 0.002
Sombo	Leaves	0.049 ± 0.002	0.044 ± 0.0012
	Tips	0.038 ± 0.002	0.034 ± 0.001
Butire	Leaves	0.047 ± 0.002	0.041 ± 0.002
	Tips	0.037 ± 0.002	0.034 ± 0.001
Hawari	Leaves	0.048 ± 0.002	0.043 ± 0.001
	Tips	0.039 ± 0.002	0.035 ± 0.001

* *Fluoride concentration in µg/g on fresh weight base.*

The statistical t-test ($p \leq 0.05$), has demonstrated the presence of significant differences in terms of water-exchangeable fluoride concentrations between washed and unwashed as well as leaves and tips of the studied Khat samples. As can be observed from Table 2, unwashed samples contain relatively higher fluoride concentrations than washed samples, indicating that washing may remove some water soluble fluorides from Khat samples. Jacobson and coworkers [41] also reported that the content of fluoride in tomato leaves significantly reduced after washing. They also demonstrated that fluoride is available in plant leaves in a soluble form and thus, can be easily removed during washing, indicating the absence of irreversible binding to cellular components [41]. The results also showed that Khat leaves have relatively higher water soluble fluoride concentrations than the tips. The study reported on tea samples also revealed as the leaves contain higher concentration of water soluble fluoride than buds or tips [42, 43]. The presence of high level of water soluble fluoride in leaves might be due to its greater susceptibility to the accumulation of airborne fluoride [44]. On the other hand, the one way ANOVA test ($p \leq 0.05$) showed that the level of water-exchangeable fluoride among the studied Khat cultivars is not significantly different. This may indicate that Khat plants in the studied areas are grown in similar environmental conditions and agricultural practices.

Generally, Khat samples contain significant amount of water-exchangeable fluoride. For instance, 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 and 0.06 µg/mL in saliva after chewing [15].—Likewise, Atlabachew and coworkers also reported the presence of water-extractable fluoride levels ranging from 0.19 - 0.43 µg/g in selected Khat samples collected from local Khat markets of Addis Ababa city and Hawassa town, Ethiopia [23].

Total fluoride concentrations of dry Khat samples

The total fluoride levels of the studied Khat samples are shown in Table 3.

Table 3: Concentration (mean ± SD, n = 4) of total fluoride content in dry Khat samples

Khat type	Chewable parts	Fluoride content**
Mole	Leaves	3.45 ± 0.15
	Tips	3.32 ± 0.04
Koredo	Leaves	3.35 ± 0.09
	Tips	3.09 ± 0.07
Bedabuna	Leaves	3.40 ± 0.08
	Tips	2.91 ± 0.06
Sombo	Leaves	4.37 ± 0.05
	Tips	2.66 ± 0.05
Butire	Leaves	5.30 ± 0.08
	Tips	3.64 ± 0.06
Hawari	Leaves	4.96 ± 0.10
	Tips	3.87 ± 0.09

** *Fluoride concentration in µg/g on dry weight base.*

The observed results demonstrated that the total fluoride contents of chewable leaves and tips of the studied Khat samples were ranging from 3.35 – 5.30 µg/g and 2.66 – 3.87 µg/g, respectively. Similar to the water-soluble fluoride, the statistical t-test study ($p \leq 0.05$) also exhibited the presence of significant difference in the concentrations of total fluoride between the leaves and tips of Khat samples collected from the same cultivar. Besides, leave samples have also relatively higher concentrations of total fluoride than tip samples. However, the ANOVA test ($p \leq 0.05$) demonstrated that there is no significant difference in terms of the total fluoride content of the studied Khat samples.

Fluoride is normally available in soil, water and air. The increase in population and industrialization also resulted in rise of fluoride content in soil, water and air, which in turn increases its content in plants. Agricultural activities such as use of fertilizers, pesticides and irrigation with contaminated sewage are also the major sources of fluoride [45, 46]. Naturally, water in some regions contain high amount of fluoride [39, 47, 48]. Agricultural products grown in areas where soils have high amounts of fluoride or where phosphate fertilizers used, may have higher levels of fluoride [49]. Consequently, plants grow on such areas may contain higher fluoride concentration than other areas, resulting from the passive up take of the fluoride by roots and ease transportation in plant tissues including tips and leaves [44].

Therefore, the Khat fluoride content is expected to be varied based on its areas of cultivation. For instance, earlier report indicated that Yemen's Khat leaf samples contain less than 0.02 g/mL and 2.07 µg/g leached (water soluble) and total fluoride, respectively [15]. Similarly, the study conducted on some Ethiopian Khat leaves also demonstrated that the presence of fluoride in the range of 0.19 – 0.43 µg/g and 3.4 – 7.1 µg/g water-extractable content and total fluoride, respectively [23]. This report also identified that Khat leaves from selected Hawassa area districts such as Chengie, Yirba and Sike contains more fluoride content than Khat leaves collected from other areas of the country including Bahir Dar, Wondo-Kuto, Wondo-Basha, Awadai, Liyu, Gelemso, Belechie, and Guragie. Ashenef and Engidawork, also reported similar fluoride concentrations to that of Atlabachew and coworkers findings, except Khat samples collected from home garden in Addis Ababa city, Ethiopia, was unexpectedly contained high concentration of fluoride, i.e., 18.53 µg/g, which was justified because of industrial fluoride contamination of the area [42]. As compared to the present study, Khat samples collected from other parts of the country contain higher amount of both water-exchangeable and total fluoride. Such variation might be due to the differences in the natural fluoride content of the areas and/or because of some anthropogenic sources. The low fluoride level of khat samples in this study, may also be attributed to the sampling season. Because the studied samples were harvested during the heavy rainy season and thus, the rain could wash off the surface fluoride from the leaves and tips of the plant in the field and/or dilute the fluoride in the soil.

CONCLUSION

In the present study, six Khat samples, representing various cultivation areas, were collected from four Khat local markets in Jimma Town. Then, the water-soluble fluoride and total fluoride contents of were determined from the fresh and dried chewable parts; leaves and tips of the collected Khat samples, respectively. For the analysis of water-soluble fluoride both unwashed and washed samples were considered. The obtained results demonstrated that washing of Khat samples significantly decrease the level of water-soluble fluoride. The findings also indicated that the leaves of Khat contain higher level of fluoride (both water-soluble and total) than the tips. The ANOVA test ($p \leq 0.05$) indicated that the absence of significant difference in both water-soluble and total fluoride levels among the various Khat samples collected from the Jimma Town. The studied Khat samples have also relatively lower water-soluble and total fluoride content than other areas Khat of Ethiopia and also below the recommended values of fluoride daily intake.

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