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Fluoride content of Ethiopian khat (*Catha edulis* Forsk) chewing leaves

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The levels of water soluble and total fluoride concentration in 11 different varieties of Ethiopian khat (*Catha edulis* Forsk, an evergreen stimulant plant) commonly consumed in the country and exported to the neighboring countries were determined by fluoride ion-selective electrode. Known amounts of fresh khat samples were suspended in deionized water, stirred, and the supernatants exposed to a chelator that decomplexes fluoride were assayed. The total fluoride concentration in the leaves was also analyzed after the leaves were dried, charred, and ashed. Water soluble and total fluoride concentration in khat varieties varied, ranging from 0.19 to 0.43 $\mu$g/g fresh weight and 3.4 to 7.1 $\mu$g/g dry weight, respectively. The fluoride concentration in matured leaves (12 $\mu$g/g) was higher than that in young leaves (6.5 $\mu$g/g dry weight). Assuming that daily khat leaves chewing for an adult is 100 g, fluoride intake from chewing leaves of the analyzed khat varieties is far below the daily fluoride tolerable upper limit. The safe intake of fluoride is recommended to be 1.5–4.0 mg day$^{-1}$ for adults and less for children. However, chewing khat leaves may contribute a significant amount of fluoride for the total daily intake of an individual and should not be overlooked while estimating the total dietary intake of fluoride for individuals chewing khat leaves regularly.

**Keywords:** khat; *Catha edulis* Forsk; fluoride; Ethiopia

**Introduction**

Fluoride is an essential trace element in humans, and fluoride insufficiency may cause dental problems. However, excessive fluoride may lead to fluorosis, manifested as dental fluorosis and skeletal fluorosis. As for several of the essential elements, there is a suggested range of safe and adequate intake. For fluoride, safe intake is said to be 1.5–4.0 mg day$^{-1}$ for adults and less for children (National Research Council 1989).

Fluorosis is prevalent in Ethiopia for the past many years and the effect of fluoride on human health has been clearly understood by now (WHO 1986; Kloos and Tekle Haimanot 1999; Nemade, Vasudeva Rao, and Alappat 2002; Ayoob and Gupta 2006). The effect of fluoride on human health other than dental and skeletal fluorosis is still under study (Connett et al. 2010).

Although the primary etiological factor for causing fluorosis is mainly due to the consumption of water containing large amounts of fluoride, evidence suggest that certain food items and beverages can add on to the body burden of fluoride (Sanni 1982;...
Pires, Dantas, and Munita 1996; Kubakaddi, Bharati, and Kasturiba 2005). In Ethiopia, the number of people chewing khat (*Catha edulis*) on a regular basis is increasing at an alarming rate.

**Khat** (*C. edulis* Forsk) is an evergreen shrub or tree either growing wild or cultivated in the east of a region extending from Southern Africa to the Arabian Peninsula more specifically in Yemen, Ethiopia, Kenya, Madagascar, Somalia, Tanzania, and others as well (Halbach 1972; Abdulsalam, Jian-Kai, and Xue-Feng 2004).

The most favored part of the plant is leaves, particularly the young shoots near the top of the plant. However, leaves and stems at the middle and lower sections are also used. Khat is chewed for its stimulating property. This is due to the presence of the phenylalkylamines in the plant (Al-Motarreb, Baker, and Broadley 2002).

In Ethiopia, khat is grown in most parts of the country. There is an ever-growing demand both for domestic consumption and for the export market. Most of the exported khat is grown in the eastern part of the country and mainly exported to the neighboring and the Middle East countries and in the recent years, the market for khat has grown to Europe and America (Lemessa 2001; Karlsson 2006). Reports indicate that over 20 million people in the Arabian Peninsula and East Africa chew this plant daily (Al-Motarreb, Baker, and Broadley 2002).

Khat use is widespread and cultivated in most parts of Ethiopia, where its use is socially sanctioned and even prestigious (Belew et al. 2000). To date, it is becoming a common practice among all age levels of the different ethnic groups of the country (Adugna, Jira, and Molla 1994; Gelaw and Haile-Amlak 2004).

In Ethiopia, the plant is marketed under different names: Awadai, Kuto, Gelemso, Guragie, Wendo, Esoye, Bekela, Kebbele, Gerba, Haik, Sebeta, Bahir Dar, Liyu, Chengie, Berdaye, Afnerara, Colombia, etc. Of these, only some of them are commonly available in the capital city, Addis Ababa, and exported to the neighboring countries while the remaining is chewed by the local people around. Most names of khat are derived from the name of the place where the plant is growing. For example, Bahir Dar khat is cultivated in Bahir Dar.

Depending upon the geographical location, the variety of khat is enormous. Even within the same locality, there are different varieties of khat. They differ in color, size and height of the leaves, and size and height of the plant as a whole.

Khat requires well-drained field with pH range 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 (Lemessa 2001; Al-Motarreb, Baker, and Broadley 2002).

During khat chewing, the bolus is kept in the mouth for 3 h or longer. Saliva/leaf slurry is usually swallowed and may partly be expectorated (Hill and Gibson 1987).

Several investigations were made with regard to phytochemical and pharmacological studies as well as the social effects of khat. Identified constituents are alkaloids, terpenoids, flavonoids, sterols, glycosides, tannins, amino acids, vitamins, and minerals (Halbach 1972; Szendrei 1980; Widler et al. 1994; Cox and Rampes 2003; Matloob 2003; Toennes et al. 2003; Abdulsalam, Jian-Kai, and Xue-Feng 2004).

Cathinone, found in khat leaves, is probably the alkaloid that has stimulating effects upon the central nervous system resulting in mood elevation and euphoria (Ashri and Gazi 1990; Halket, Karasu, and Murray-Lyon 1995). Side effects that are believed to be related to the chewing of khat include elevation of blood pressure, tachycardia, hyperthermia, increased sweating, muscular weakness, loss of appetite, spermatorrhoea, and some gastrointestinal disturbances (Halbach 1972; Nencini, Ahmed, and Elmi 1986).
Furthermore, regular use of khat causes tooth discoloration, gum disease, and oral cancer (Hill and Gibson 1987; Hattab, Qudeimat, and Al-Rimawi 1999; Hailu et al. 2006).

Reports indicate that long-term khat chewing causes intrinsic and extrinsic stains on the surface of the teeth (Hailu et al. 2006; Hassan, Gunaid, and Murray-Lyon 2007). 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 possible internal teeth stains. The cause of internal staining may be swallowing of the khat juice due to the fluoride content of the khat leaves (Hailu et al. 2006).

It is believed that khat may contain some amount of fluoride ($F^-$) in its leaves. Khat samples from Yemen were analyzed and found to posses negligible amounts of $F^-$ in the leaves, $<0.18 \mu g F^{-1} g^{-1}$ fresh weight basis, $0.93 \mu g$ total $F^{-1} g^{-1}$ in dried leaf, and $2.1 \mu g$ total $F^{-1} g^{-1}$ in ash (Hattab and Angmar-Mansson 2000).

It is known that accumulation of fluoride in the plant varies from place to place depending on its availability in the soil and water for irrigation (Häni 1978; Elrashidi and Lindsay 1985; Singh et al. 1995; Fung et al. 1999). Furthermore, the nature of the soil and its pH affects the bioavailability of fluoride in the different parts of the plant (Fung et al. 1999; Miller, Shupe, and Vedina 1999). In the past, khat was grown in natural soil but nowadays fertilizers are used to boost production. It is also known that plants can take up more fluoride when the mineral fertilized soil is used (Hillman, Bolenbaugh, and Convey 1979; Lemessa 2001).

Some parts of Ethiopia are in the rift valley region where fluoride predominantly exists in the soil and water (Malde et al. 1997; Kloos and Tekle Haimanot 1999). Some of these areas are best known for khat production. Thus, khat grown in these areas may conceivably accumulate fluoride and thereby contribute to the daily fluoride intake.

The aim of this study was to measure the fluoride concentration of various khat varieties grown in the country and commonly chewed by most individuals in the region and exported to the neighboring countries. This study, therefore, becomes important in determining the exposure of human body to fluoride through this item. This also stresses the need of inclusion of fluoride level of this item in determining the total dietary intake of fluoride for chewers of such stimulant. The result of this study will also fill the gap and/or controversy that existed in studies on dental effects of khat due to fluoride concentration in the plant.

Materials and methods

Equipment

A pH/ISE meter (Orion model, EA 940 Expandable Ion Analyzer, USA) equipped with combination fluoride selective electrode (Orion Model 96-09, USA) was employed for the determination of fluoride in the samples and standard solutions. The pH was measured with pH/ION meter (WTW Inolab pH/ION Level 2, Germany) using pH glass electrode.

Reagents and chemicals

The reagents that were used in the analysis were all of analytical grade. Anhydrous sodium fluoride (99.0% NaF, BDH Chemicals, England) was used to prepare the fluoride stock and calibration standard solutions. Glacial acetic acid (Techno Pharmchem, Delhi, India), sodium chloride (Oxford Laboratory, Mumbai, India), sodium citrate (BDH Chemicals, England), ethylenediaminetetraacetic acid (EDTA) (Scharlau Chemie S.A., Barcelona,
Spain), and sodium hydroxide (Scharlau Chemie S.A., Sentmenat, Spain) were used to prepare the total ionic strength adjustment buffer (TISAB). The TISAB II was prepared by mixing 57 mL of glacial acetic acid, 58 g of sodium chloride, 7 g of sodium citrate, and 2 g of EDTA in volumetric flask and made to 500 mL with distilled–deionized water, and then the pH was adjusted to 5.3 with 6 M sodium hydroxide, and then made up to 1000 mL in a volumetric flask with distilled–deionized water. Distilled–deionized water was used throughout the experiment for sample preparation, dilution, and rinsing apparatus prior to analysis.

**Prevention of contamination**

To minimize the risk of contamination, all glassware used for the analytical methods were washed with tap water followed by chromic acid and distilled–deionized water. Sterile disposable powder-free plastic gloves were worn when handling the khat sample during the sampling and analyses stages. The extracted solutions in plastic volumetric flasks were kept in the refrigerator until analysis.

**Collection and preparation of khat samples**

As stated above, there are various types of khat plants that grow in Ethiopia and brought to different cities of the country including the capital city, Addis Ababa, and sold in kiosks of different parts of the city. Table 1 summarizes the types of khat analyzed with their corresponding area of cultivation. For this study, samples were selected on the basis of their popularity in consumption in the country and export to the neighboring countries. As a result, Bahir Dar, Wondo-Basha, Wondo-Kuto, Belechie, Awadai, Liyu, Gelemso and Guragie type khat samples were collected from different kiosks of Addis Ababa. While, Sike, Yirba and Chengie types were collected from different kiosks of Awassa (the capital city of Southern Nations, Nationalities, and Peoples Region of Ethiopia, SNNPR).

For Awadai, Liyu, Sike, Yirba, and Chengie types of khat, 8 kiosks were randomly selected while 14 kiosks were selected for collecting the remaining khat types. Around 50 g of the edible portion of the leaves were collected from each kiosk. After mixing the leaves of similar varieties of khat samples from the different sampling sites, 11 bulk samples were collected from different kiosks of different parts of the city. Table 1 summarizes the types of khat analyzed with their corresponding area of cultivation. For this study, samples were selected on the basis of their popularity in consumption in the country and export to the neighboring countries. As a result, Bahir Dar, Wondo-Basha, Wondo-Kuto, Belechie, Awadai, Liyu, Gelemso and Guragie type khat samples were collected from different kiosks of Addis Ababa. While, Sike, Yirba and Chengie types were collected from different kiosks of Awassa (the capital city of Southern Nations, Nationalities, and Peoples Region of Ethiopia, SNNPR).

<table>
<thead>
<tr>
<th>Khat type (cultivars)</th>
<th>Location (area of cultivation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bahir Dar</td>
<td>Bahir Dar</td>
</tr>
<tr>
<td>Wondo-Kuto</td>
<td>Wondo Genet</td>
</tr>
<tr>
<td>Wondo-Basha</td>
<td>Wondo Basha</td>
</tr>
<tr>
<td>Belechie</td>
<td>Wondo Genet</td>
</tr>
<tr>
<td>Guragie</td>
<td>Woliso</td>
</tr>
<tr>
<td>Liyu</td>
<td>Harari district</td>
</tr>
<tr>
<td>Awadai</td>
<td>Awadai</td>
</tr>
<tr>
<td>Gelemso</td>
<td>Gelemso</td>
</tr>
<tr>
<td>Yirba</td>
<td>Yirba</td>
</tr>
<tr>
<td>Sike</td>
<td>Chuko</td>
</tr>
<tr>
<td>Chengie</td>
<td>Chengie</td>
</tr>
</tbody>
</table>
were obtained and a portion of each sample was taken for extraction of the fresh leaves following the optimized procedure for the analysis of water-extractable fluoride. The remaining portion of each sample was oven dried at 70°C for 72 h (until constant weight) and ground in a blender to homogenize and reduce the size of particles. Finally, 11 bulk samples were prepared for total fluoride analysis. Three 1 g aliquots from each sample were taken for ashing and final analysis.

It is reported that the lower part of the leaf next to the young shoot contains lower cathinone but more cathine content. It is also harder and difficult for chewing. As a result, chewers do not prefer it for consumption. But in some areas, they dry and use it after moistening it with tea or cola drinks in the periods where shortage of khat is observed, particularly in the summer season. It is also taken as tea infusion (Halbach 1972). As a result, determination of fluoride content in such part of the leaf is also of interest because fluoride accumulation varies with the positions of the leaves (Fung et al. 1999) in the same plant. As a result, the lower parts of the leaf next to the young shoot were collected for the Sike-type khat and dried in the oven following the same procedure as for the above.

**Optimization of sample preparation procedures**

To select an optimum procedure (ashing temperature, extraction time, and reagent volume) for extracting fluoride from the fresh and the dried leaves, previously reported methods were referenced and evaluated for this study (Malde et al. 1997; Hattab and Angmar-Mansson 2000; Malde, Bjorvatn, and Julshamn 2001; Yadav et al. 2007). Slight modification was made on the reported procedures for this study.

**Preparation of the samples for total fluoride content analysis**

By slightly modifying reported methods (Malde et al. 1997; Malde, Bjorvatn, and Julshamn 2001), the following method was employed for total fluoride content analysis; 1.0 g of khat leaf powder was transferred to nickel crucible. A volume of 6 mL of 8 M NaOH was added and the crucible was placed in an oven (150°C) for 1.5 h until NaOH was solidified. The crucible was placed in a muffle furnace and set at 200°C for 1.5 h after which the temperature was increased to 525°C and kept there for 2.5 h in order to fuse the sample in the crucible. The crucible was allowed to cool and 10 mL distilled water was added. Then, 37% HCl solution (about 4 mL) was added slowly to adjust the pH to 8–9. The sample solution was transferred to a plastic beaker. The crucible was rinsed successively with 5 mL water until the final volume reached 50 mL. All the washings were mixed and filtered with Whatman filter paper (110 mm, diameter) and transferred to a 50 mL plastic volumetric flask. The filtrate was used for the fluoride content determination. A filtrate was prepared in parallel from a nickel crucible containing no sample to serve as a control. A portion of it was taken, mixed with an equal amount of TISAB II, and fluoride concentration was determined by F⁻-selective electrode following double standard addition method. All the determinations were done in triplicate.

**Preparation of the samples for water-extractable fluoride**

The extraction time for the extraction of water-extractable fluoride was optimized by varying the extraction time from 0.5 to 10 min on two different khat leaf samples. An extraction time of 5 min was found to be optimum for the extraction of fluoride from the khat leaves. The results are given in Table 2. It should also be noted that our aim was
to evaluate the amount of fluoride that is extracted in the mouth during the khat chewing session. We tried to associate the extraction time with the time required to keep the bolus in the mouth during khat chewing. Usually, most khat chewers keep the bolus inside their mouth for less than 5 min at a time before the next intake. This was also the reason for selecting 5 min as an optimum extraction time. Following optimized procedure, 4 g of fresh khat leaves were taken in to plastic beaker and crushed with pestle. Then, 24 mL of distilled–deionized water was added and vigorously stirred with magnetic stirrer for 5 min. A portion of it was taken and analyzed for fluoride concentration following the above procedure. All the determinations were made in triplicates.

### Evaluation of analytical method

The efficiency of the method for total fluoride and water-soluble fluoride analysis was evaluated using recovery experiment, i.e. by adding known concentration of sodium fluoride solution to 4 g of the fresh leaf sample and to 1 g of dry khat leaf sample. The procedure was as follows: each of a 4 g of fresh khat leaves and 1 g of dried khat leaf samples were spiked with sodium fluoride solution of which the fluoride content was equivalent to 50%, 100%, or 200% of the fluoride content of the original (unspiked) khat leaf samples. After extraction, following the procedure, determination was made in triplicates. The results of the measurements are presented in Table 3. As shown in Table 3, the percentage recoveries were found between 93% and 103% from spiked Belechie samples.

### Table 2. Evaluation of extraction time for the extraction of fluoride from khat leaves.

<table>
<thead>
<tr>
<th>Sample variety</th>
<th>Extraction time (min)</th>
<th>F⁻ (µg g⁻¹)</th>
<th>Sample variety</th>
<th>Extraction time (min)</th>
<th>F⁻ (µg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wondo-Kuto</td>
<td>0.5</td>
<td>–</td>
<td>Yirba</td>
<td>0.5</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.19</td>
<td></td>
<td>1</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.21</td>
<td></td>
<td>2</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.24</td>
<td></td>
<td>3</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.25</td>
<td></td>
<td>4</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.25</td>
<td></td>
<td>5</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.25</td>
<td></td>
<td>10</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Notes: Khat leaf sample (4 g) in 24 mL of water.

### Table 3. Recovery test results.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration in sample³ (µg g⁻¹)</th>
<th>Amount added (µg g⁻¹)</th>
<th>Concentration in spiked sample⁵ (µg g⁻¹)</th>
<th>Recovery⁶ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wondo-Basha</td>
<td>0.24</td>
<td>0.12</td>
<td>0.37 ± 0.01</td>
<td>103 ± 3</td>
</tr>
<tr>
<td></td>
<td>0.24</td>
<td>0.24</td>
<td>0.47 ± 0.02</td>
<td>98 ± 4</td>
</tr>
<tr>
<td></td>
<td>0.24</td>
<td>0.48</td>
<td>0.74 ± 0.03</td>
<td>103 ± 4</td>
</tr>
<tr>
<td>Belechie</td>
<td>4.2</td>
<td>2.0</td>
<td>6.1 ± 0.2</td>
<td>98 ± 3</td>
</tr>
<tr>
<td></td>
<td>4.2</td>
<td>4.0</td>
<td>8.0 ± 0.2</td>
<td>97 ± 3</td>
</tr>
<tr>
<td></td>
<td>4.2</td>
<td>8.0</td>
<td>12 ± 0.3</td>
<td>98 ± 2</td>
</tr>
</tbody>
</table>

Notes: ³Average value of triplicate analyses (µg g⁻¹); ⁵values are mean ± SD of triplicate analyses; ⁶fresh sample; and ⁷dry leaf sample.
and Wondo-Basha khat leaf samples, which are within the acceptable range. For all the measurements, coefficient of variation (CV) was less than 10% which confirms the reproducibility of the measurement.

**Conversion factor determination**

To report the results obtained on dry weight basis in terms of fresh weight basis, a conversion factor was calculated by taking the weight of a known quantity of fresh edible part of the plant before and after drying (at 70°C) to a constant weight. This procedure was repeated for all the 11 types of khat samples collected from 11 different areas. Thus, 3.73 (the mean value) were taken as conversion factors because 3.7 g of the wet sample (on the average) yielded 1 g of dry sample.

**Results and discussion**

Three aliquots of khat leaf samples were taken from each bulk sample and the data on the different aliquots were combined ($n = 9$) to give one mean value. Total fluoride contents in the dried leaf samples and water-extractable fluoride in the fresh leaves of 11 varieties of chewing khat grown in different parts of the country were determined and the results are shown in Table 4. Table 4 also shows percentage of total fluoride that is extracted with water from the fresh leaves. Thus, even though it is not possible to tell how much of the relative standard deviation (RSD) in the results (Table 4) is from sample inhomogeneity and how much result from analytical error, the overall error (resulting from sample inhomogeneity and from analytical error) is within the acceptable range ($\text{RSD} \leq 10\%$).

Since the fresh part of the plant is consumed, the result in terms of the dry weight basis is converted to fresh weight basis so as to correlate the intake of total fluoride with the amount of khat chewed using a conversion factor 3.7. The mean concentration of total fluoride in fresh khat leaves in terms of wet weight basis is given in Table 4.

**Table 4. Mean concentration ($X \pm SD$, $n = 9$, $\mu g \cdot g^{-1}$) and RSD (%) of water extractable and total fluoride in khat samples.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total fluoride content (dry weight basis) $\pm SD$</th>
<th>RSD (%)</th>
<th>Total fluoride content* (wet weight basis) $\pm SD$</th>
<th>Water-extractable fluoride (fresh weight basis) $\pm SD$</th>
<th>RSD (%)</th>
<th>$F^-$ extracted* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bahir Dar</td>
<td>3.4 ± 0.2</td>
<td>5.9</td>
<td>0.91</td>
<td>0.19 ± 0.01</td>
<td>3.3</td>
<td>21</td>
</tr>
<tr>
<td>Wondo-Kuto</td>
<td>3.9 ± 0.3</td>
<td>7.7</td>
<td>1.0</td>
<td>0.25 ± 0.02</td>
<td>8.0</td>
<td>24</td>
</tr>
<tr>
<td>Wondo-Basha</td>
<td>3.8 ± 0.3</td>
<td>7.9</td>
<td>1.0</td>
<td>0.24 ± 0.02</td>
<td>8.3</td>
<td>23</td>
</tr>
<tr>
<td>Guragie</td>
<td>4.0 ± 0.3</td>
<td>8.0</td>
<td>1.1</td>
<td>0.23 ± 0.01</td>
<td>4.3</td>
<td>21</td>
</tr>
<tr>
<td>Belechie</td>
<td>4.2 ± 0.3</td>
<td>7.2</td>
<td>1.1</td>
<td>0.26 ± 0.02</td>
<td>7.7</td>
<td>24</td>
</tr>
<tr>
<td>Liyu</td>
<td>4.7 ± 0.4</td>
<td>8.5</td>
<td>1.3</td>
<td>0.25 ± 0.02</td>
<td>8.0</td>
<td>20</td>
</tr>
<tr>
<td>Awadi</td>
<td>4.8 ± 0.3</td>
<td>6.3</td>
<td>1.3</td>
<td>0.26 ± 0.02</td>
<td>7.7</td>
<td>20</td>
</tr>
<tr>
<td>Gelemso</td>
<td>4.6 ± 0.4</td>
<td>8.7</td>
<td>1.2</td>
<td>0.28 ± 0.02</td>
<td>7.1</td>
<td>22</td>
</tr>
<tr>
<td>Yirba</td>
<td>5.8 ± 0.3</td>
<td>5.2</td>
<td>1.6</td>
<td>0.32 ± 0.01</td>
<td>3.1</td>
<td>21</td>
</tr>
<tr>
<td>Sike</td>
<td>6.5 ± 0.2</td>
<td>3.1</td>
<td>1.8</td>
<td>0.31 ± 0.02</td>
<td>6.5</td>
<td>18</td>
</tr>
<tr>
<td>Chengie</td>
<td>7.1 ± 0.7</td>
<td>9.8</td>
<td>1.9</td>
<td>0.43 ± 0.04</td>
<td>9.3</td>
<td>23</td>
</tr>
</tbody>
</table>

Notes: RSD, relative standard deviation.  
*Calculation was made after converting the dry weight basis of total fluoride into fresh weight basis using a conversion factor of 3.7.
As can be seen from Table 4, water-extractable fluoride concentration varies from 0.19 to 0.43 μg g⁻¹ fresh weight and was the highest in Chengie-type khat variety, while the lowest concentration was found in Bahir Dar khat. The table also shows that total fluoride concentration in different khat varieties ranges between 3.4 and 7.1 μg g⁻¹ dry weight. Chengie-type khat contains the highest total fluoride than the rest of analyzed khat varieties. The variations in the fluoride content in the different varieties of khat analyzed are not very large, except for the last three, and it could have been caused by the wide fluctuations of fluoride concentration in the water and soils in which the plant was grown (Fung et al. 1999; Miller, Shupe, and Vedina 1999). Whether variation is significant or not, it is discussed at the end of this section.

Plants do not require F⁻, and tissue concentrations from uncontaminated soils rarely exceed 30 mg kg⁻¹ dry mass (Kabata-Pendias 2001). Plant F⁻ uptake is dependent on solution F⁻ activity, pH (physical property of the soil), application of fertilizers and pesticides, substrate composition, and the plant species (Häni 1978; Elrashidi and Lindsay 1985; Singh et al. 1995; Fung et al. 1999; McLaughlin et al. 2001).

Generally, the natural source is the major source of fluoride pollution. But increase in population and industrialization also result in pollution to water, air, and soil, which in turn causes an unexpected concentration 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 (Miller, Shupe, and Vedina 1999; McLaughlin et al. 2001).

Looking at Table 4, fluoride concentration varies with growth area. Chengie contains the highest fluoride concentration followed by Sike Yirba, Awadai, Liyu, Gelemso, Belechie, Guragie, Wondo-Kuto, Wondo-Basha and Bahir Dar types of khat. This variation is attributed to various factors mentioned above. Fluoride concentration in Chengie, Yirba, and Sike varieties of khat which are cultivated around Awassa region in Sidamo district is higher than the rest of khat varieties analyzed. This could be attributed to higher fluoride concentration in the water for irrigation and soil. Research indicates that this district is found in rift valley region of the country, and up to 8.0 mg kg⁻¹ of fluoride has been reported in the different parts of the water body within the district (Malde et al. 1997). The same trend has been observed in vegetables, cereals, and other plants analyzed from the same district (Malde et al. 1997).

Comparison of the F⁻ content in khat with that of other plants such as vegetables, cereals, and tea revealed that F⁻ in khat is close to that of vegetables and cereals (Sanni 1982; Malde et al. 1997), but much less than that found in tea, which ranges from 117 to 682 μg g⁻¹ (Pires, Dantas, and Munita 1996; Yadav et al. 2007). Even compared to chewing tobacco, khat leaves contain lower concentration of fluoride (Yadav et al. 2007).

Nowadays, use of fertilizer and pesticides is a common practice among khat cultivars to increase their yield (Lemessa 2001). This in turn contributes a significant amount of fluoride to the soil and hence to the plants (Hillman, Bolenbaugh, and Convey 1979; McLaughlin et al. 2001).

The result indicates that khat leaves from the same province seemed to have similar total F⁻ contents. Wondo-Kuto and Wondo-Basha are from the same province (Wondo province) and they have a similar distribution of fluoride. The same is true for Awadai, Gelemso, and Liyu types, which are cultivated from Harari province.

For water-extractable fluoride, higher F⁻ contents were released from Chengie and Sike types of khat than those from the rest, due to the original higher total F contents in the khat leaves of these samples. However, lower percentage of fluoride were released from Sike and Liyu types khat (17–19%) than the others (22–24%), due to the fact that the
Sike- and Liyu-type khat leaves had broader, harder, and lower surface area than those with relatively soft and smaller size and hence larger surface area. These small-sized leaves were easily crushed during extraction and therefore were more efficient in releasing $F^-$ ion. Research indicates that more fluoride is extracted in mouth while chewing and in acidic media (Hattab and Angmar-Mansson 2000; Yadav et al. 2007).

One paper has been reported on the fluoride contents of Yemeni khat (Hattab and Angmar-Mansson 2000). Looking at the report by Hattab and Angmar-Mansson (2000), less than 0.18 $\mu$g g$^{-1}$ of water-extractable fluoride has been reported, while 0.19–0.43 $\mu$g g$^{-1}$ has been obtained in this study. Similarly, there is significant variation in the total fluoride content between the reported result (2.1 $\mu$g g$^{-1}$) and this study (3.4–7.1 $\mu$g g$^{-1}$). Only one variety of Ethiopian khat (Bahir Dar khat) has result comparable with Yemeni khat. In general, Ethiopian khat contains higher fluoride than Yemeni khat. This might be due to either of the aforementioned factors. Furthermore, Ethiopian farmers use chemicals and traditional techniques, such as tobacco extract, ash, garbage from house cleaning, etc., to increase soil fertility and control pastes (Lemessa 2001). This might contribute for higher fluoride content in the investigated samples.

Fluoride distributions in different parts of the leaves (young shoot and matured leaves) were investigated for Sike-type khat and fluoride contents accumulated in leaves were found to be proportionally related to the age of leaves (young leaves, 6.5 ± 0.2 $\mu$g g$^{-1}$; matured leaves 12 ± 0.7 $\mu$g g$^{-1}$).

In most cases, the average quantity of khat chewed by Ethiopians ranges from 100 to 500 g daily. Thus, the consumption of khat contributes 91–190 $\mu$g/100 g of total fluoride and 19–43 $\mu$g/100 g of water-extractable fluoride in wet weight basis per day. This value increases with increasing weight of chewing khat and reaches up to 950 $\mu$g/500 g. Thus chewing khat contributes up to 27% of the recommended daily dose of fluoride depending on the varieties taken. Matured leaves contribute double of the stated concentration in young shoot. In general, the fluoride concentration in khat chewing was found to be low and implies no problem related to dental fluorosis. However, khat chewing is accompanied by smoking, use of sugar, excessive hot drinks such as tea, and excessive amount of water. Some chewers drink more than four cups of tea during the chewing session. Reports indicate that drinking about four cups of tea contribute up to 50% of the recommended daily dose of fluoride (Amanlou et al. 2008). Furthermore, khat chewers drink much more water during chewing because of excessive sweating as a result of excitement. It is also known that drinking fluoridated water contribute more fluoride to the daily intake. Chewers who are living in the rift valley district of the country are highly infected with fluoride problem since up to 8.0 mg L$^{-1}$ of fluoride has been reported in different bodies of water for consumption (Malde et al. 1997). In addition to other possible sources of fluoride, such as fluoride-containing toothpaste, various beverages and fish increase the daily fluoride intake to a level higher than the recommended value. Thus, we conclude that not khat leaves but a cumulative effect may cause dental health problem among khat-leaf chewers. Even if the concentration is low, it contributes a significant amount of fluoride for the daily intake of an individual. Therefore, we recommend that khat leaves chewers should try to minimize excessive use of the above-mentioned additives during khat leaves chewing and should avoid use of matured leaves.

Statistical analysis of data was made to verify whether there was a significant difference in total fluoride concentration between a pairwise comparative investigation of the 11 varieties of khat analyzed. For this study, the significance of variation within sample and between samples has been studied using one-way analysis of variance (ANOVA) and calculations were made using SPSS software.
No significant difference ($p > 0.05$) at 95% confidence interval was observed in fluoride concentration for the following pairwise analysis: Bahir Dar, Wondo-Kuto, Wondo-Basha, Guragie, and Belechie. The same was true for Gelemso, Awadai, Liyu; Yirba, Sike, Chengie; Kuto, Gelemso, Liyu; Guragie, Gelemso, Liyu; and Belechie, Gelemso and Liyu types of khat varieties, whereas any other pairwise combinations other than the mentioned groups have showed significant difference ($p \leq 0.05$) at 95% confidence interval in fluoride concentration.

The absence of significant difference in fluoride concentration in different varieties of khat may indicate the presence of similarities in certain factors or variables such as climatic conditions, soil type, water for irrigation, age of the harvested khat, etc. Similarly, presence of significant difference in fluoride concentration indicates that either of the studied area contains higher concentration of fluoride in the soil or variation in agricultural activities, for example, application of fertilizers, insecticides, irrigation, etc. In addition to these, difference in the age of the harvested khat may result in significance difference in the level of fluoride.

Conclusion

The fluoride concentrations (total and water-soluble fluoride) in selected 11 commercially available Ethiopian khat (C. edulis) (Bahir Dar, Wondo-Kuto, Wondo-Basha, Awadai, Liyu, Gelemso, Belechie, Guragie, Yirba, Sike, and Chengie types of khat varieties) were determined by fluoride selective electrode. The total fluoride contents were determined to be $3.4–7.1\, \mu g\, \text{g}^{-1}$ dry weight in khat leaves and $0.19–43\, \mu g\, \text{g}^{-1}$ fresh weight of water-extractable fluoride which was prepared by extracting fresh khat leaves in water for 5 min. Khat (Chengie, Yirba and Sike) from Awassa district contains more fluoride than khat from other parts of the country. Matured leaves contain higher fluoride concentration which is nearly twice than young shoot. Percent of fluoride extracted from khat leaves depends on the size and age of harvested khat leaves and can be up to 24% of total fluoride contents of khat leaves. Though fluoride content in the khat leaves is low, it should not be overlooked during the estimation of total dietary intake of fluoride for those who chew this stimulant. We also recommend that chewers should avoid using the matured leaves of the plant in any case since it can contribute more fluoride than the young leaves. The ANOVA results suggest that there were significant variations in the level of fluoride concentration among some of khat varieties which could be attributed to different factors such as age of the harvested khat, geographical and climatic variation, difference in physicochemical nature of the soil, and different agricultural practices among khat cultivars, whereas absence of significant variation have been observed in some khat varieties analyzed which might be due to similarity in basic factors mentioned above.

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