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FLUORIDE CONTENT OF WIDELY USED TRADITIONAL TOOTHBRUSHES FROM SELECTED AREAS OF ETHIOPIA

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ABSTRACT: People around the world use chewing sticks to clean their tooth. These traditional toothbrushes are prepared from a branch of trees or their roots. The main objective of this study was to determine the fluoride content of six widely used traditional toothbrushes in Ethiopia. These are obtained from six different plants namely *Phoenix reclinata* (known as Zembaba), *Ligustrum vulgare* (known as Mefaqia), *(Olea africana)* (known as Weira), *Sida cuneifolia* (known as Chifrig), *Salix subserrata* (known as Akeya) and *Clausena anisata* (known as Limitch). A total of 18 chewing sticks (three different plants of each of the six types of plants) was collected directly from their growing area. Fluoride content of the traditional toothbrushes was determined using alkali fusion – fluoride ion selective electrode method. The levels of fluoride in the six types of widely used traditional toothbrushes were found in the range 16.0–28.1 mg/kg. The lowest level of fluoride was found in *Sida cuneifolia* collected from Chancho while the highest level of fluoride was found in *Olea africana* collected from Sebeta. The statistical analysis, ANOVA showed significant difference between mean fluoride contents of traditional toothbrushes at $p < 0.05$ confidence level. This might be due to different factors such as pH of the soil, type and age of the plant, environmental conditions and geological origin.

Keywords: Fluoride; Traditional toothbrushes; *Phoenix reclinata*; *Ligustrum vulgare*; *Olea Africana*; *Sida cuneifolia*; *Salix subserrata*; *Clausena anisata*; Ethiopia

INTRODUCTION

Oral health is one of the essential and most important activities done for healthy life. Keeping oral hygiene using plant material is one of the oldest methods practiced by people. According to World Health Organization oral disease is one of the top five causes for loss of life in the world.[1] People around the world use tooth cleaning sticks in order to clean their teeth and these traditional toothbrush sticks can be prepared from a branch of trees or their roots. The traditional oral hygiene tools are used widely in Middle East and most African countries as well as in India, Pakistan and most of Arabian countries. People use a chewing stick because it is easily and freely available, its unique chemical composition and religious beliefs.[1] The World Health Organization has encouraged the use of chewing sticks as an alternative source of oral hygiene in poor countries where many people cannot afford commercial cleaning and whitening dental products.

Chewing sticks have been used in Africa to maintain oral hygiene for centuries. The name chewing stick was given to traditional toothbrushes because the users chew the stick prior to use as a brush for tooth. The chewing stick is used in most part of the world like Africa, Greek, Romans, Jewish and Islamic Empires (Almas, 2001).[2,3]

Majority of people use traditional toothbrush because of different reasons. Among the main reasons people use a traditional toothbrush is that it is easily available everywhere and less costly than commercially prepared toothbrush and toothpaste.[4,5] The other reason is that chewing sticks can be used everywhere because it can be

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easily handled. People use the chewing stick on the way to their work place, at their work place, in office, in cafes, and so on. Another reason is religious influence for the use of the chewing sticks by many people after eating and drinking to remove the remaining parts from their tooth. A traditional toothbrush is prepared from a chewing stick that can be converted into a brush when chewed. Part of the plants that can be used for this purpose is the branch of the trees or their roots. People live in the rural area cut the tree and prepare for use directly and it is used freshly without buying, but people live in the urban area buy the transported toothbrushes from nearby. To transport the toothbrushes for sale first the plant is cut from its growing area and the leaves and some unimportant parts are removed before cutting in to pieces to be used as a chewing stick. The thickness and length of the chewing stick depends on the interest of the user.[4,5]

Commercially prepared toothbrushes and toothpastes are available in the supermarkets and pharmacies but most people in Ethiopia prefer to use the traditional toothbrushes because it is natural and easily available, and less costly. This study was based on six types of widely used traditional toothbrushes in Ethiopia.[4,5] These are known as Zembaba obtained from *Phoenix reclinata*, Mefaqia obtained from *Ligustrum vulgare*, Weira obtained from *Olea africana*, Chifrig obtained from *Sida cuneifolia*, Akeya obtained from *Salix subserrata,* and Limitch obtained from *Clausena anisata*.

Phoenix reclinata is the wild date palm with evergreen that can grow naturally almost all over the world.[6] It is native to Arabian Peninsula, Comoro Island and tropical Africa.[7] *Phoenix reclinata* grows almost in all parts of Ethiopia naturally and cultivated in urban areas to make the environment green. People use the plant for different purposes such as making mats, hats, baskets and as toothbrushes. Its natural habitat is river banks, swamps and occasionally in grass land if the water level is high (Ellison and Ellison, 2001).[6] Both urban and rural people in the country use the branch of the plant to clean their tooth because of its easily available and can be made into a brush-like shape when chewed.

Ligustrum vulgare is commonly known as common privet. It is a semi evergreen shrub. *Ligustrum vulgare* grows in North Africa, South Asia, Ireland, west Sweden, central and south Europe, south Morocco, east Poland, and north western Iran. *Ligustrum vulgare* (common privet) is a fast growing, bushy, deciduous shrub with lance-shaped dark green leaves and is popular choice as ornamental shrubs and for hedgerows. *Ligustrum vulgare* is cultivated in most urban parts of Ethiopia for evergreen purpose in addition to use as toothbrush. Leaves of *Ligustrum vulgare* have been used for treatment of oropharyngeal inflammations or as an anti-rheumatic, diuretic, and hypotensive agents in folk medicine in southern Europe.[8]

Olea africana is commonly known as African wild olive and it belongs to a family of Oleaceae.[9] *Olea africana* is among the strong plants that can resist wind and drought. The wood material of *Olea africana* is used to make beautiful household furniture. *Olea africana* is widespread in Africa including Ethiopia.[10] In Asia, *Olea africana* is found in Afghanistan, northern India and Pakistan. Nowadays *Olea african* are cultivated in different places like rocky mountain slopes, riverbanks, forest, bush and grass fields. In Ethiopia people use *Olea africana* for different purposes such as having good smell with its smoke and the stem is commonly used as

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toothbrush by most people in rural and urban area. The leaves of *Olea african* were reported to be used as a medicine for the treatment of malaria.[11] The oil of the seed is taken orally as a laxative and also applied externally as a balm for inflammation.[12] Decoctions of dried leaves and fruit are used orally to treat diarrhea, respiratory and urinary tract infections, stomach and intestinal diseases, and as mouth cleanser.[13] Continuous application of olive oil is also useful to prevent hair loss. In East-Africa the infusion of the bark of the olive tree is taken for tapeworm infection.[14]

Sida cuneifolia grows throughout the world, mostly in tropical and subtropical regions. Ethiopian *S. cunefolia* is divided in to two taxonomies without intermediate collection. The study of east African *S. cuneifolia* indicated that Ethiopian taxonomy were widespread in northern Tanzania and Kenya. For many years, *Sida* species are used as folk remedies in Africa, Brazil and India.[15] The roots and leaves of *Sida cuneifolia* is used as folk medicine in treating wounds, skin infection, stomach, anti-septic, respiratory disorder, conjunctivitis, fever, cancer and leukemia. *S. cuneifolia* growth in most parts of Ethiopia and people use the leaves to clean their homes. The root of the plant is one of the most commonly used traditional toothbrushes in Ethiopia.[4]

Salix subserrata is a wild deciduous shrub belonging to a family of Salicaceae. It is a woody stems with dark red-brown or black color. *Salix subserrata* grows usually in moist soil around the bank of streams, rivers, lakes and other surface water. The plant is widespread in African countries like Ethiopia, Egypt, Libya, Sudan, Gambia, Zambia and Zimbabawe.[16] *Salix subserrata* is used as traditional medicine to treat headaches, constipation, stomach ache, rabies and to relieve fever.[17] In Ethiopia *S. subserrata* grows naturally around river banks, lakes, surface water. Nowadays it is cultivated for ornamental purposes in urban areas. The stem of the plant is one of the most commonly used traditional toothbrushes by rural and urban people of the country for many years.

Clausena anisata is a small, fast-growing, deciduous tree or a shrub.[4] The bark is thin, grey-green and smooth, becoming brownish with age. *Clausena anisata* is widespread in sub-Saharan Africa, where it is found only in high rainfall regions, in riverine forest, savanna, thicket, secondary forest and disturbed areas. *Clausena anisata* occurs from Guinea and Sierra Leone eastwards to Ethiopia, Sudan and southward to South Africa, where it occurs in the Eastern and Western Cape, Free State, KwaZulu-Natal, and Limpopo. It is also growing in India, Sri Lanka and South East Asia, extending all the way to northeastern Australia and some islands in the Pacific.[18]

Fluorine is among the most toxic elements that can affect the health of animals and environment. Fluoride ion is not considered as essential for the growth and development of human including the development of tooth and bones and it is potentially toxic substance at higher levels.[19] Exposure of fluoride at higher levels more than 6 mg/day for a long period of time can cause skeletal, dental and non-skeletal fluorosis.[20-22] Fluoride can cause diverse health problems like genetic damage and cancer, and impairment of the lungs, kidneys, aorta, thyroid, heart, pancreas, and brain.[23,24]

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The different sources that can expose fluorides to human are drinking water, beverages, foods, toothpaste and air.[25-34] Many countries have fluoride in their drinking water with a higher concentration than recommended by World Health Organization (WHO) which is 1.5 mg/L.[21]

Fluoride is not an essential nutrient for plants.[35] Fluoride is transferred to plant from air, soil and water.[36] Bioaccumulation of fluoride in different parts of the plant vary depending on its transfer in soil and plant type. The fluoride can enter to the plant by absorption through their root. The fluoride found in plants can be affected by type of plant, nature of the soil, amount of fluoride found in the area, and type of fluoride compound.[37,38] The concentration of fluoride ion in soil depends on the solubility of fluoride compound, the acidity of the soil, and presence of water.[19]

The fluoride in plants is less than the expected amount when compared to amount of fluoride ion in the soil because most fluoride containing compounds are insoluble. Fluoride concentration in plants can be increased when there are highly soluble fluoride compounds in soil, low pH value, clay soil and/or organic matter which make available fluoride to be taken by plants.[36] Plants absorb fluoride at maximum pH of 5.5 since fluoride in alkaline soil at pH of above 6.5 is completely fixed in soil as calcium fluoride, where sufficient calcium carbonate is found.[19]

The fluoride levels of some substances like tea,[25,26] spices,[30,31] cereals,[27,28] legumes,[29] alcoholic beverages,[32] cabbage,[33] and soft drinks[34] in Ethiopia were determined. However, there is no literature report on the fluoride levels of traditional toothbrushes. Hence this study was aimed to determine the levels of fluoride in six types of widely used traditional toothbrushes collected from three selected areas of Ethiopia. The main objective of the study was to determine the levels of fluoride in widely used traditional toothbrushes obtained from *Phoenix reclinata*, *Ligustrum vulgare*, *Olea Africana*, *Sida cunefolia*, *Salix Subserrata* and *Clausena anisata* cultivated/grown in three selected areas of Ethiopia. Specific objectives were: (i) to determine the fluoride content in widely used traditional toothbrushes obtained from six plants: *Phoenix reclinata*, *Ligustrum vulgare*, *Olea africana*, *Sida cunefolia*, *Salix subserrata*, and *Clausena anisata* selected from three areas of Ethiopia; (ii) to compare levels of fluoride in the six traditional toothbrushes collected from three different areas; (iii) to compare fluoride levels in traditional toothbrushes of the same plant from different area; and (iv) to compare the fluoride levels of the traditional toothbrushes with that of commercial toothpastes.

MATERIALS AND METHODS

Apparatus: A knife was used to cut the samples in to small pieces. A mortar and pestle were used to ground the samples. An electronic grinder (coffee grinder) was used to ground the samples in to fine powder. An oven (Digitheat, J.P. Selecta, Spain) was used to dry the toothbrushes samples. An electronic balance was used to measure sample amount for the experiment. A muffle furnace (Crison GLP 2^2 Spain) was used for the fusion of sample within nickel crucible (50 mL). A pH/ISE meter (Orion model, EA940 Expandable ion analyzer, USA) equipped with combination of fluoride ion selective electrode was used to measure concentration of fluoride in a sample. Plastic beakers and volumetric flasks were used for the preparation, storage and as container during the measurement of fluoride.

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Chemicals: All the reagents used in this study were of analytical grade. A standard fluoride stock solution was prepared by dissolving 2.21 g of sodium fluoride (99% NaF, BDH, England) in to 1000 mL of de-ionized water to make 1000 mg/L of solution. Total ionic strength adjustment buffer (TISAB) was prepared from the following chemicals: Glacial acetic acid (100% sigma-Aldrich Labrochemicalin, Germany), sodium hydroxide (NaOH) (Scharlau Chemie S.A., Spain), sodium chloride (Oxford Laboratory, Mumbai, India), ethylene diamine tetra acetic acid (EDTA) (Scharlau Chemie, S.A., Barcelona, Spain), and trisodium citrate (BDH Chemicals, England). The amount of each chemicals used in the preparation of total ionic strength adjustment buffer (TISAB) was as follow: 58 g of sodium chloride was dissolved in to 57 mL of glacial acetic acid and then added 7 g of tri sodium citrate and 2 g of ethylene diamine tetra acetic acid in to 500 mL of de-ionized water. pH of the solution was adjusted to 5.0-5.5 with 9 M sodium hydroxide and diluted the solution to 1000 mL in volumetric flask with de-ionized water.

Study area: For this study six types of widely used traditional toothbrushes obtained from *Phoenix reclinata*, *Ligustrum vulgare*, *Olea africana*, *Sida cuneifolia*, *Salix subserrata*, and *Clausena anisata* were collected from three selected areas of Ethiopia. The three sites selected for this study was from Oromia regional state of Ethiopia. All the samples were collected directly from their growth area. Six samples from Sebeta, six samples from Burayu and six samples from Chancho which gave a total of 18 samples were collected. The selection of study area of the traditional toothbrushes was based on availability of plants, widespread use by people, easiness to handle and transport. For the study of fluoride level in traditional toothbrushes three areas were selected. The areas are Sebeta (South West Shoa), Burayu (South Shoa), Chancho (North Shoa).

Sebeta is the town found in a south west of Addis Ababa, a capital city of Ethiopia, around 30 km. It has an altitude and longitude of 8º55"N 38º37"E with an elevation of 2356 m above sea level. In this area many types of plants grow. Those plants selected for this study are used widely as traditional toothbrushes in the area and surrounding.

Burayu is a town found in Oromia regional state of Ethiopia. It is directly adjacent to national capital city of the country. It has a latitude and longitude of 9º07"N 38º67"E with an altitude of 2591 m. Burayu has an average rain fall of 1188 mm and temperature of 25 ºC. Many types of plant grow in a good climatic condition of Burayu and all the selected traditional toothbrush are found in the area and most commonly used by the people as traditional toothbrushes.

Chancho is located at 40 km to the north of Addis Ababa on the road to Gojam. Chancho is one of the high land area in Ethiopia with an altitude of 2800 m above sea level. The annual rain fall of Chancho is 1440 mm and the mean minimum and maximum temperature is 10 ºC and 14.9 ºC, respectively. All the selected plants for this study are found in the area and most commonly used by the people as traditional toothbrushes.

Sample collection: Six different types of widely used traditional toothbrushes were collected directly from their growing area of three different places. The selection of place for the traditional toothbrushes was random. The branch or root of the selected chewing sticks were collected from matured trees of three different places separately. When the sample were collected from the growing area the leaves and unimportant parts of the plant were removed. The chewing stick used for traditional toothbrushes were collected directly from their grown area in to polyethylene bags and transported to the laboratory for further preparation and treatment to give a total of 18 samples. The selected ones are *Phoenix reclinata*, *Ligustrum vulgare*, *Olea africana*, *Sida cunefolia*, *Salix substrata*, and *Clausena anisata*. The samples were washed by running water and then by distilled water to minimize contamination. The washed samples were cut in to pieces using cutter and knife and placed in laboratory in order to dry the sample for one month.

Sample preparation for analysis: Six types of widely used traditional toothbrushes were collected from three different places to give total of 18 samples. The collected samples were washed thoroughly using tap water to minimize contamination and rinsed with de-ionized water. All the samples were cut in to small pieces using cutter and placed in laboratory to dry for one month at room temperature. The dried samples were ground using pestle and mortar. The powder were collected in to plastic bottle and stored until fusion with alkali.

Determination of total fluoride: The fluoride content in each traditional toothbrush was determined using alkaline fusion by slightly modifying the reported method.[39] An accurately weighed 0.5 g powdered sample of each traditional toothbrush was placed separately in a 50 mL nickel crucible, 6 mL of 9 M NaOH solution was added and mixed thoroughly. The mixture was subjected to 150 ºC to dry the sample in an oven for 2 hours. After that it was transferred to a furnace for fusion at 570 ºC for 2 hours. The fusion cake was cooled to room temperature and 15 mL of de-ionized water added and the crucible was placed on hot magnetic plate to aid dissolution of fused cake. After dissolving the cake the solution was transferred in to 50 mL plastic beaker. The sample solution was highly basic and to neutralize the solution was first treated using concentrated HCl to decrease the pH of the solution from 13.85 to 8.5 and then with dilute HCl to decrease the pH to 7.0–7.4 with continuous string and pH control. The concentration of fluoride ions was measured by adding 5 mL of sample solution in to 50 mL plastic beaker and to this solution 5 mL of TISAB, i.e. total ionic strength adjustment buffer solution was added and magnetic stirrer was placed in the solution to get homogeneous solution. The fluoride concentration was measured using an Orion ion selective electrode meter combined with an expandable electrode in triplicate for each sample with continuous stirring with a magnetic stirrer.

RESULTS AND DISCUSSION

Fluoride electrode calibration: For the calibration of ion selective electrode instrument standard fluoride solution with concentration of 0.5, 5, 10, and 25 mg/L were prepared by serial dilution from the 1000 mg/L of fluoride stock solution. Then 5 mL of each standard solution was added in to prior prepared plastic beaker for the five serial dilution and 5 mL of total ionic strength adjustment buffer (TISAB) was added and the ion selective electrode was calibrated. From the five values of measurement calibration curve was constructed as electrode potential versus logarithm of standard fluoride concentration. The slope of calibration curve was found to be 58 mV/decade which indicate good precision measurement.

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Method validation: In this study, method validation for determination of fluoride content in the widely used traditional toothbrushes (*Phoenix reclinata*, *Ligustrum vulgare*, *Olea africana*, *Sida cuneifolia*, *Salix subserrata*, and *Clausena anisata*) was done by the spiking experiment. The spiked samples were prepared by adding known concentration of the stock standard solution of sodium fluoride to 0.5 g of the powder samples. The validity of the procedure was then checked by performing recovery test. The selected widely used traditional toothbrushes (*Phoenix reclinata*, *Ligustrum vulgare*, *Olea africana*, *Sida cuneifolia*, *Salix subserrata*, and *Clausena anisata*) were spiked with standard fluoride solution of which fluoride ion content was equivalent to 25%, 50% or 100% of the fluoride content of unspiked (original) samples. Then the fluoride concentration in un-spiked and spiked samples was measured. The results are given in Tables 1-6.

Table 1. Recovery test results of the fluoride ion determination in samples of the traditional toothbrush *Phoenix reclinata* from Sebeta, Burayu and Chancho

Type of sample	Concentration of fluoride in unspiked samples (mg/kg)	Amount of fluoride added (mg/kg)	Concentration of fluoride in spiked samples (mg/kg)	Recovery (%)
Phoenix reclinata from Sebeta	22.7± 2.1	5.60	28.2±1.4	98.0±5
	22.7± 2.1	11.2	34.9±0.9	108±2
	22.7 ±2.1	22.4	45.6±1.2	102±3
Phoenix reclinata from Burayu	26.6±1.9	6.40	32.0±1.2	100±4
	26.6±1.9	12.8	39.5±0.8	101±2
	26.6±1.9	25.4	52.1±1.0	100±1
Phoenix reclinata from Chancho	22.1 ±5.0	5.20	27.3±2.1	100±1
	22.1±5.0	10.4	32.2±1.7	97.0±5
	22.1±5.0	20.8	42.7±1.2	99.0±2

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Table 2. Recovery test results of the fluoride ion determination in samples of the traditional toothbrush *Ligustrum vulgare* from Sebeta, Burayu and Chancho

Type of sample	Concentration of fluoride in unspiked samples (mg/kg)	Amount of fluoride added (mg/kg)	Concentration of fluoride in spiked samples (mg/kg)	Recovery (%)
Ligustrum vulgare from Sebeta	16.4±0.9	4.2	20.7±0.4	102±3
	16.4±0.9	8.4	24.6±0.6	98.0±2
	16.4±0.9	16.7	32.6±0.5	97.0±5
Ligustrum vulgare from Burayu	25.0±2.0	4.9	29.8±1.3	97.9±5
	25.0±2.0	9.8	34.9±0.9	101±7
	25.0±2.0	19.6	44.3±1.4	98.0±6
Ligustrum vulgare from Chancho	18.5±0.5	4.6	23.1±0.1	100±4
	18.5±0.5	9.2	27.1±0.25	93.0±6
	18.5±0.5	18.4	36.9±0.4	100±4

Table 3. Recovery test results of the fluoride ion determination in samples of the traditional toothbrush *Olea africana* from Sebeta, Burayu and Chancho

Type of sample	Concentration of fluoride in unspiked samples (mg/kg)	Amount of fluoride added (mg/kg)	Concentration of fluoride in spiked samples (mg/kg)	Recovery (%)
Olea africana from Sebeta	28.1±2.0	9	37.4±1.0	103±3
	28.1±2.0	18	46.0±1.3	99±5
	28.1±2.0	26	54.3±1.2	100±4
Olea africana from Burayu	25.7±0.8	6.4	31.8±0.3	96±8
	25.7±0.8	12.8	38.2±0.5	98±7
	25.7±0.8	25.6	49.8±0.4	94±9
Olea africana from Chancho	20.3±1.5	6.2	26.8±0.8	104±3
	20.3±1.5	13.4	33.3±0.8	97±5
	20.3±1.5	26	45.4±1.0	96±7

Table 4. Recovery test results of the fluoride ion determination in samples of the traditional toothbrush *Sida cuneifolia* from Sebeta, Burayu and Chancho

Type of sample	Concentration of fluoride in unspiked samples (mg/kg)	Amount of fluoride added (mg/kg)	Concentration of fluoride in spiked samples (mg/kg)	Recovery (%)
Sida cuneifolia from Sebeta	22.2±1.5	4.8	26.8±0.9	96.0±3
	22.2±1.5	9.6	31.4±1.2	95.0±4
	22.2±1.5	19	41.2±0.8	100±2
Sida cuneifolia from Burayu	19.7±0.7	4.7	24.2±0.4	96.0±3
	19.7±0.7	9.4	28.6±0.5	95.0±5
	19.7±0.7	19	38.6±0.6	99.±2
Sida cuneifolia from Chancho	16.0±1.0	4.0	20.0±0.4	100±4
	16.0±1.0	8.0	24.2±0.6	102±2
	16.0±1.0	16	32.2±0.7	101±3

Table 5. Recovery test results of the fluoride ion determination in samples of the traditional toothbrush *Salix subserrata* from Sebeta, Burayu and Chancho

Type of sample	Concentration of fluoride in unspiked samples (mg/kg)	Amount of fluoride added (mg/kg)	Concentration of fluoride in spiked samples (mg/kg)	Recovery (%)
Salix subserrata from Sebeta	21.4±0.6	5.4	26.8±0.2	100±5
	21.4±0. 6	10.8	32.4± 0.3	102±4
	21.4±0.6	21.5	42.8± 0.2	99.5±5
Salix subserrata from Burayu	19.2±0.8	4.8	23.9±0.3	98.0±6
	19.2±0.8	9.6	28.3±0.4	95.0±7
	19.2±0 .8	19.2	38.2±0.6	99.0±4
Salix subserrata from Chancho	23.9±1.8	12.1	36.0±0.7	100±7
	23.9±1.8	24.2	48.0±1.3	99.5±3
	23.9±1.8	6.05	30.4±1.1	102±4

Table 6. Recovery test results of the fluoride ion determination in samples of the traditional toothbrush *Clausena anisata* from Sebeta, Burayu and Chancho

Type of sample	Concentration of fluoride in unspiked samples (mg/kg)	Amount of fluoride added (mg/kg)	Concentration of fluoride in spiked samples (mg/kg)	Recovery (%)
Clausena anisata from Sebeta	23±5.0	5.20	27.9±1.3	94±5
	23±5.0	10.4	33.4±2.0	100±2
	23±5.0	20.8	42.9±1.8	96±4
Clausena anisata from Burayu	26.5±3.8	6.20	32.7±2.0	100±1
	26.5±3.8	12.4	38.8±1.3	99±3
	26.5±3.8	24.8	49.8±1.9	94±6
Clausena anisata from Chancho	25.5±0.9	6.10	31.7± 1.0	102±3
	25.5±0.9	12.2	37.1±0.4	95±2
	25.5±0.9	24.3	50.2±0.6	102±2

Comparison of fluoride levels of the toothbrushes from the same plant in different areas: The fluoride concentration of the six traditional toothbrushes (*Phoenix reclinata*, *Ligustrum vulgare*, *Olea africana*, *Sida cuneifolia*, *Salix subserrata*, and *Clausena anisata*) collected from the three selected areas of Ethiopia is given in Table 7. The study showed that the highest concentration of fluoride is found in *Olea africana* from Sebeta and the lowest concentration of fluoride is found in *Sida Cuneifolia* from Chancho of the selected six types of traditional toothbrushes used in Ethiopia. The concentration of fluoride in the six types of traditional toothbrushes is different due to different factors such as pH of the soil, type of plant, type of soluble fluoride compound in the area, age of the plant and environmental conditions.

The entire selected traditional toothbrushes contain significant amount of fluoride. The levels of fluoride as it is observed from the study are different for different areas. Generally there is significance difference ($p < 0.05$) in the fluoride levels of the six selected widely used traditional toothbrushes in the three selected areas of Ethiopia. The levels of fluoride found in *Clausena anisata* found in Chancho (25.3 mg/kg) and *Ligustrum vulgare* found in Burayu (25.0 mg/kg) were almost similar and fluoride levels found in *Sida cuneifolia* from Burayu (19.7 mg/kg) and *Salix suserrata* from Burayu (19.2 mg/kg) and fluoride levels found *in Ligustrum vulgare* of Sebeta (16.4)

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and fluoride levels found in *Sida cuneifolia* of Chancho were similar. The fluoride level in the other traditional toothbrushes collected from different areas were significantly different.

The mean concentration of fluoride ion from the same plant collected from three different areas is different for *Phoenix reclinata*, *Ligustrum vulgare*, *Olea africana*, *Sida cuneifolia*, *Salix subserrata* and *Clausena anisata*. This may be due to different factors such as age of the plant, concentration of fluoride in the soil, pH of the soil. This study shows that the levels of fluoride in plant are affected both by type of plant and type of soil.

Table 7. Comparison (mean ±SD, n = 3, mg/kg) of fluoride levels of the same plant in different areas

Sample site	*Phoenix reclinata*	*Ligustrum vulgare*	*Olea africana*	*Sida cuneifolia*	*Salix subserrata*	*Clausena anisata*
Sebeta	22.7 ±2.1	16.4 ±0.9	28.1 ±2.0	22.2 ±1.5	21.4 ±0.6	23.0 ±5.0
Burayu	26.6 ±1.9	25.0 ±1.0	25.7 ±0.8	19.7 ±0.7	19.2 ±0.8	27.4 ±3.8
Chancho	22.1 ±5.0	18.5 ±0.5	20.3 ±1.5	16.0 ±1.0	23.9 ±1.8	25.3 ±0.9

Comparison of levels of fluoride in commercial tooth paste and widely used traditional toothbrushes: The mean concentrations of fluoride ion in the widely used traditional toothbrushes determined in this study were in the range of 16.0–28.1 mg/kg. The mean concentration of fluoride in commercial toothpastes varies from company to company and on the type of toothpaste. The mean concentration of fluoride in commercial toothpaste is from 1000 to 1500 mg/kg. The average daily intake of 1–3 mg/day of fluoride is not harmful but long-term exposure to higher amounts may have hazardous effects on tooth enamel and bone; single doses of 5–10 mg/kg body weight cause acute toxic effects, and death was reported following ingestion of 16 mg/kg.[20,40] The usual lethal concentration range is 70–140 mg/kg.[40] The average fluoride concentration in the toothpastes was 1251 mg/kg, which is higher than the maximum recommended value (1000 mg/kg).[41] The comparison of the fluoride concentration in commercial toothpastes and traditional toothbrushes of this study is given in Table 8.

Table 8. Comparison of fluoride levels in commercial toothpastes and widely used traditional toothbrushes

Type of toothpaste	F- concentration in mg/kg	Origin	Reference
Colgate gel	1350	India	42
Close up deep action	1225	India	42
Anchor	825	India	42
Pepsodent	1000	Indonesia	43
Close up	1351	Indonesia	43
Sensodyne	1350	Bangladesh	Bought from pharmacy
Colgate	1450	China	Bought from pharmacy
Signal	1450	Ethiopia	Bought from pharmacy
Sensodyne	1000	Thailand	Bought from pharmacy
Traditional toothbrushes	16–28	Ethiopia	Present study

Comparison of fluoride levels of different plants in the same area: The levels of fluoride ion in different plants from the same area are given in Table 9. The different types of plants in the same areas have different mean concentration of fluoride ion due to different factors. This difference is mainly due to different age of plants and different part of plant used for this study. Therefore the levels of fluoride in plants are mainly affected by the type of plant as well as type of soil. The concentration of fluoride determined for the six different types of widely used traditional toothbrushes is significantly different at $p = 0.05$.

Analysis of variance: Analysis of variance (ANVOA)[44] is a widely used statistical method to compare the difference in groups or within a group whether the source of variation is from sampling or heterogeneity between samples. The variation between mean levels of fluoride ion in samples were tasted using one way ANOVA whether the variation was from random error or treatment that means difference in fluoride concentration of soil, atmosphere and water. The statistical analysis was done using SPSS 15.0 window evaluation version program. There was significance difference ($p < 0.05$) in the mean fluoride concentration between widely used traditional toothbrushes collected from three sites and among plants in the same area. Therefore the variation in mean fluoride content was not due to random error during sampling and analysis but it was due to sample heterogeneity. Such variation is due to different content of fluoride available to plants in the environment and different type of plants. The analysis of ANOVA showed that there is a significance difference at 95% confidence in levels of mean concentration of fluoride in widely used traditional toothbrushes.

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Table 9. Comparison of fluoride levels of six different plants in the same area.

Sample area	Type of sample	Trial 1	Trial 2	Trial 3	Mean±SD (F^- mg/kg)
Sebeta	*Phoenix reclinata*	24.0	21.1	23.1	22.7±1.5
	Ligustrum vulgare	16.5	15.9	16.9	16.4±1.0
	Olea africana	29.6	27.8	27.0	28.1±2.0
	Sida cunefolia	22.7	22.2	21.7	22.2±1.5
	Salix subserrata	21.1	21.4	21.8	21.4±2.3
	Clausena anisata	22.6	23.0	23.5	23.0± 2.3
Burayu	*Phoenix reclinata*	27.7	26.8	25.2	26.6±1.4
	Ligustrum vulgare	23.0	28.0	24.0	25.0±1.4
	Olea africana	25.4	25.0	26.8	25.7±0.8
	Sida cunefolia	19.5	19.3	20.3	19.7±0.7
	Salix subserrata	18.9	18.8	19.8	19.2±0.8
	Clusena anisata	26.8	27.1	28.4	27.4±1.9
Chancho	*Phoenix reclinata*	22.6	21.8	22.0	22.1±2.3
	Ligustrum vulgare	18.2	17.8	19.4	18.5±0.7
	Olea africana	19.8	21.2	20.0	20.3±1.5
	Sida cuneifolia	15.9	15.5	16.5	16.0±1.0
	Salix subserrata	24.5	24.0	23.4	23.9±1.8
	Clausena anisata	24.5	25.5	26.0	25.3±1.0

CONCLUSIONS

In this study, levels of fluoride were determined in the six types of widely used traditional toothbrushes collected from three selected areas of Ethiopia. The levels of fluoride in the traditional toothbrushes were found in the range of 16.0 mg/kg in the *Sida cuneifolia* from Chancho to 28.1 mg/kg in the *Olea africana* from Sebeta. The study showed that traditional toothbrushes contain significant amount of fluoride. The levels of fluoride vary from plant to plant and from place to place. The average mean levels of fluoride from the three places found in *Clausena anisata* was highest among the widely used traditional toothbrushes and the mean average levels of fluoride from the three places found in *Sida cuneifolia* was lowest of all the six types of widely used traditional toothbrushes in the three selected areas of Ethiopia. The mean fluoride level of the six types of widely used traditional toothbrushes in Ethiopia are in the order of *Clausena anisata* (25.2 mg/kg) > *Olea africana* (24.7 mg/kg) > *Phoenix reclinata* (23.8 mg/kg) > *Salix subserrata* (21.5 mg/g) > *Ligustrum vulgare* (20.0 mg/kg) > *Sida cuneifolia* (19.3 mg/kg). The level of fluoride determined in the six types of widely used traditional toothbrushes in three selected areas of Ethiopia was from 25.2 mg/L to 19.3 mg/kg which show that it is safe to use chewing sticks as toothbrushes.

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