ADDIS ABABA UNIVERSTIY

COLLEGE OF NATURAL SCIENCES

DEPARTMENT OF CHEMISTRY

STUDIES ON THE LEVELS OF FLUORIDE IN SELECTED SPICES CULTIVATED AND CONSUMED IN ETHIOPIA

BY

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STUDIES ON THE LEVELS OF FLUORIDE IN SELECTED SPICES CULTIVATED AND CONSUMED IN ETHIOPIA

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By

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APPROVAL BY EXAMINING BOARD

As thesis research advisors, we hereby certify that we have read and evaluated this thesis prepared under our guidance by Kassie Nigus Shitaw entitled ‘studies on the levels of fluoride in selected spices cultivated and consumed in Ethiopia’. We recommend that it be submitted as fulfilling the thesis requirement.

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As member of the Board of Examiners of the M.Sc. Thesis Open Defense Examination, we certify that we have read, evaluated the Thesis prepared by Kassie Nigus Shitaw and examined the candidate. We recommended that the Thesis be accepted as fulfilling the Thesis requirement for the Degree of Master of Science in Chemistry (Analytical Chemistry).

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DECLARATION

I, the undersigned, declare that this thesis is my original work and has been submitted in partial fulfillment of the requirements for the degree of masters of Science at Addis Ababa University. All sources of materials used for this thesis have been duly acknowledged. This paper has never been submitted to and/or presented in any other university, college or institution in candidature of any other degree, diploma, or certificate.

Name: Kassie Nigus Shitaw

Signature: _______________

Date: ____________________

DEDICATION

This dissertation is dedicated to Tesfaye Nigus Shitaw who is my brother and to all my friends.

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TABLE OF CONTENTS

List of Tables

List of Figures

Lists of acronyms and abbreviations

FISE	Fluoride Ion Selective Electrode
ANOVA	Analysis of Variance
SNNPR	Southern Nations and Nationalities and Peoples Region
WHO	World Health Organization
MWC	Mental Working Capacity
DNA	Deoxyribo Nuclic Acid
TISAB	Total Ion Strength Adjustment Buffer
EDTA	Ethylene Diamine Tetra Acetic acid
mV	milli Volt
SD	Standard Deviation
RSD	Relative Standard Deviation
WW	Wet Weight
DW	Dry Weight
TFI	Total Fluoride Intake
DIF	Daily Intake of Fluoride
FC	Fluoride Concentration
RDIF	Relative Daily Fluoride Intake
LOD	Limit of Detection
LOQ	Limit of Quantification
SPSS	Statistical Program for Social Sciences
RCDC	Regional Centre for Development Cooperation

ABSTRACT

Fluorosis is a public health problem in certain part of Ethiopia, especially in rift valley areas. Fluoride is widely present throughout the food supply. The general population can be exposed to fluoride through drinking water and food consumption. Foods are the major contributor of fluoride exposure to human being, next to water. Spices are the main food supliments in Ethiopian traditional cucines for the purpose of flavoring, coloring, aroma and sometimes as a preservative agent. Therefore, spices may have their contribution in human daily intake of fluoride. The aim of this study was to determine the levels of fluoride in spices. Spice samples were collected from the market for fluoride analysis and the analysis was done by using FISE. Validation of the procedure was evaluated using spiking method and the percentage recovery confirm the validity of the procedure. One-way ANOVA and t-test were used to check the presence of significance difference at 95% confidence level between mean fluoride levels in different sorts of spice samples. The levels of fluoride in spices found (on dry weight basis) in this study were (in mg/kg dry weight): 2.14±0.04 (coriander seed), 2.53±0.05 (basil leaves), 2.81±0.13 (ginger), 3.1±0.17 (red pepper), 3.18±0.03 (fenugreek seed), 3.27±0.13 (garlic), 3.29±0.07 (turmeric), 4.51±0.12 (nutmeg seed), 5.13±0.23 (cinnamon), 8.14±0.15 (black cumin seed), and 8.57±0.11 (thyme leaves). Analysis of variance showed that there was statistically significant difference ($p < 0.05$) at 95% confidence level in the mean levels of fluoride among the spice samples. It was also found that the daily intake of fluoride from red chili pepper, cinnamon bark, turmeric rhizome, ginger rhizome, and garlic granules for an adult (60 kg body weight) who consume the estimated amounts of these spices per day was 0.082 mg/kg or 2.74% out of the recommended fluoride level (3 mg F^-). This shows that spices play a significant role in daily intake of fluoride for adults.

Key words: Spices; Food; Fluoride; Ethiopia

1. INTRODUCTION

1.1. Background

Spices are defined as dried seed, fruit, root, bark, leaf or vegetative substance used in nutritionally insignificant quantities as a food additive for the purpose of flavoring, coloring, and aroma and sometimes as a preservative by killing or preventing the growth of harmful bacteria. Many of spices are also used for other purposes, such as medicine, cosmetics, perfume, and eaten as vegetables [1, 2].

Different spices have their own pungent feeling due to their content of essential oils. Some of pungent feelings of spices are hot, sharp, biting, or sulfury. For instance, ginger contributes bite aroma because of a higher content of volatile oils. Garlic has sulfury aroma whereas red pepper does not contain much aroma since it has very little essential oils. The tastes of spices such as sweetness, sour, salty, and pungency are due to their different chemical components such as esters, phenols, acids, alcohols, chlorides, alkaloids, and sugars. Sweetness of spices is due to their ester and sugar components; and their sourness is resulted from organic acids such as citric acid, malic acid, acetic acid, or lactic acid while saltiness of spices is due to the presence of cations, chlorides, and citrates. The presence of phenols and tannins make spices astringency; and alkaloids such as caffeine and glycosides cause the bitterness of spices. The pungency of spices is due to acid-amides, carbonyls, thioethers, and isothiocyanates. Spices are used in foods for their antioxidant components that protect cells from free radicals those damage the human cells and limit their ability to fight off cancer, aging, and memory loss [3].

Spices are used in small amount. But, when people have taken spices regularly at high amount, the normality of their health would be affected. Some symptoms such as rapid heart rate, vomiting, agitation, confusion, and hallucination are commonly observed on regular abusers of spices. Using of spices regularly also raise blood pressure, reduced blood supply to the heart (myocardial ischemia), and in a few cases it has been associated with heart attacks [4].

In Ethiopia, the cultivation of spices for centuries is predominantly stayed traditional by small scale land holding farmers. Recently, the average land covered by spices has been 222,700 hectare and the production reached 244,000 ton/year [5]. Ethiopia is the home land of many spices. Among these are red chili pepper (*Capsicum annum*), garlic bulb (*Allium sativum*), cinnamon bark (*Cinnamomum zeylanicum*), turmeric rhizome (*Curcuma longa*), ginger rhizome (*Zingiber officinale* Roscoe), fenugreek seed (*Trigonella fenum graecum*), coriander seed (*Coriandrum sativum* L.), nutmeg seed (*Myristica fragrans* Houtt.), black cumin seed (*Cuminum cyminum* Linn.), thyme leaves (*Thymus vulgaris*), and basil leaves (*Ocimum basilicum*) [2]. Pictures of selected spices used in this study are shown in Figure 1.

Figure 1. Pictures of selected spices used in this study.

The major use of spices in Ethiopia is in the preparation of a highly spiced stew known as 'Wot' which together with 'Injera' is consumed by a large proportion of the population everyday as their main food. In addition, spices are also used by the numerous ethnic groups in the country to flavor bread, meat, soups, different vegetables, and as medicines and perfumes [6].

Fluoride ion which is found everywhere in the environment is beneficial substance for human and other organisms to prevent tooth decay and increase bone strength if it is taken at an optimum level. But, intake of fluoride at high level for a long time results dental, skeletal, and non-skeletal fluorosis. Fluorosis is a public health problem in certain part of Ethiopia, especially in rift valley areas. The major sources of exposure to fluoride for people are drinking water and food [7].

Total exposure of human to fluoride depends on contributions from different sources, such as drinking water, water based beverages, foods, food supplements, toothpaste, and air [8-10]. There are several studies reported in the literature about the dietary intake of fluoride [11-19]. Among these are water, water based beverages, and foods. All vegetation contains some fluoride, which is absorbed from soil and water [20]. As a result, spices cultivated through the world may accommodate significant levels of fluoride and make contributions to raise the fluoride content of spicy human diets. The present study was undertaken to evaluate the levels of fluoride in the most common spices used as food additives in many cuisines.

1.2. Objectives

1.2.1. Main objective

The main objective of this study was to determine fluoride levels in selected spices: red chili pepper (*Capsicum annum*), garlic granules (*Allium sativum*), cinnamon bark (*Cinnamomum zeylanicum*), turmeric rhizome (*Curcuma longa*), ginger rhizome (*Zingiber officinale* Roscoe), fenugreek seeds (*Trigonella foenum graecum*), coriander seeds (*Coriandrum sativum* L.), nutmeg seeds (*Myristica fragrans* Houtt.), black cumin seeds (*Cuminum cyminum* Linn.), thyme leaves *(Thymus vulgaris*), and basil leaves (*Ocimum basilicum*) most widely cultivated and consumed in Ethiopia.

1.2.2. Specific objectives

i. To determine fluoride levels in selected spices (red chili pepper, garlic granules, cinnamon bark, turmeric rhizome, ginger rhizome, fenugreek seed, coriander seed, nutmeg seed, black cumin seed, thyme leaves, basil leaves);

ii. To calculate total daily fluoride intake of an adult person based on fluoride concentrations of spices;

iii. To compare the level of fluoride selected spices analyzed in this study;

iv. To compare the level of fluoride in selected spices cultivated and consumed in Ethiopia with that in the other parts of the world.

1.2.3. Significance of the study

Since all Ethiopian foods are prepared with spices and there is no report in literatures to correlate the fluoride level in spices cultivated and consumed in Ethiopia, this study, therefore, becomes important to give new information on the levels of fluoride in spices and gives clue for further studies. It is also important in determining the exposure of human body to fluoride through the selected spices.

2. LITERATURE REVIEW

2.1. Selected spices used in this study

2.1.1. Red chili pepper (*Capsicum annum, C. frutescens*)

Red chili pepper is also known as chili pepper. It is belonging to the family of *Nightshade* and is among the most widely consumed spices throughout the world, due to its quite flavor and pungency. Red pepper is the leading spice crop in Ethiopia and it is locally consumed in various food preparations particularly for the purpose of its flavor and color to local stews. In addition to dietary benefits, chili pepper often provides excellent income-generating opportunities to small farmers.

The productivity of red chili pepper in Ethiopia is low even when compare to that of other developing countries. At farmers' level in Ethiopia, the yield of chili pepper is about 0.4 tons per hectare while in Turkey the average yield exceeds to 14 tons per hectare [21]. The hotness of chili pepper is due to the presence of a substance called capsaicin which is concentrated in the cross walls of the fruit and around the developing seeds. The degree of hotness of peppers has been measured from Scoville Heat Index [22]. Chili pepper grows at altitude ranging from 1400 up to 2100 meters above sea level (masl). Growing pepper requires soil that is well-dried, free from perennial weed and rich in organic matter, as well as 600−650 mm rainfall [23].

The diverse climatic conditions of Ethiopia are suitable for the production of different types of red chili peppers in different areas of the country. It is widely cultivated in Mareko (SNNPR), Alaba (SNNPR), Ziway (East Shewa), Dembi Dollo (West Wellega), Todalle (Jima Route), and Gojam−Gonder Agricultural Development [5]. In Ethiopia, chili peppers are planted in April or May, when the rainy season starts and they have harvested from October to February [2]. Among the chili pepper cultivars widely spread in the Ethiopia, the “Marko types” those are dark red and pungent are the most popular types due to their flavor, pungency, large and long pod size. Therefore, Mareko type peppers dominate the system of pepper production and marketing in Ethiopia. As a result, it is high demand for consumers and spice industries due to its excellent market quality [24].

2.1.2. Garlic bulb (*Allium sativum*)

Garlic belongs to the family *Alliaceae* and has grown as spice and used for flavouring local dishes. It was cultivated in Central Asia originally [25]. Now a day, it is cultivated globally. But, China is the larger producer of garlic with approximately 10.5 million tons grown annually which is over 77% of world output. Garlic is widely cultivated in Ethiopia. Ambo, Debre Werk, Adet, Sinana and many other highland areas cultivate the bulk of garlic [5].

Most of the time, raw garlic is used in cooked form with various foods since it enhances flavor due to its pungency as well as adds nutritional benefits. Garlic has many demonstrated medicinal properties such as antiviral, antibacterial, antifungal, anti-cancer, reduces cholesterol level and antioxidant capacities. It may also lower blood pressure, increase microcirculation which is important in diabetes where microvascular changes increase heart disease and dementia risks and prevent cognitive decline by protecting neurons from neurotoxicity and apoptosis. This is due to its medicinally active components such as sulfur containing compounds (S-alkylcysteine sulfoxides and the γ-glutamyl-S-alkylcysteines), high trace minerals, and enzymes [25].

2.1.3. Cinnamon bark (*Cinnamomum zeylanicum*)

Cinnamon is the inner bark of Cinnamomum plant with buff or dark reddish color and belongs to the family of *Lauraceae*. The cinnamon variety was introduced in Ethiopia in 1975 and grows at wider agro-ecology. It can grow well in almost all soil types under a wide variety of tropical conditions ranging from semi-dry to wet zones especially in Mizan-Tepi (South West of Ethiopia). It requires a warm and wet climate with average temperature of 20−30 ^{o}C and high rainfall [26].

Cinnamon is commonly used in cooking for its aroma, flavor, and taste. The flavor of cinnamon is due to its aromatic essential oil and its pungent taste and scent come from cinnamic aldehyde or cinnamaldehyde component. Since it is very aromatic and sweet, cinnamon powder is added to dishes during the cooking process of sweets, hot drinks, vegetables (carrots, winter squash and sweet potatoes) and for baking to improve flavor and taste [27]. Cinnamon is also used as flavoring agent in numerous alcoholic beverages such as white wine and vodka. Eugenol and cinnamaldehyde are the two major phenolic chemical components in cinnamon those are responsible for cinnamon health benefits. The two components inhibit *Helicobacter pylori* growth at a low pH, showing their efficacy in eliminating the bacteria present in the human stomach [27].

2.1.4. Turmeric rhizome (*Curcuma longa*)

Turmeric is a ground spice belongs to the family of *Zingiberaceae* (ginger). It is yellow-orange color root and commonly used as herb and spice in all over the world. Before 1972, Ethiopia was one of turmeric importing countries but in 1972, turmeric were introduced from abroad for adaptability study from India and China and planted at Jimma, Metu, Bebeka, Tepi, Wenago, Hawasa, Magi and Bako.

Turmeric has grown well on well dried soils which are rich in organic matter. At the present time, because of the suitability of Southern humid regions, turmeric is widely grown in the Southern part of Ethiopia [26]. Turmeric can be grown well in altitudes between 1300 and 1800 meter above sea level with annual rainfall of 1000 mm. Cultivation of turmeric requires frequent hand weeding for maximum and high quality yield [23]. Turmeric is extensively used as a spice for food coloring and flavoring purposes as well as a coloring material in the textile industries and as food preservative in many countries. Turmeric has been also used in traditional medicine as a household remedy for various diseases including biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis. Due to its active ingredient which is called curcumin, turmeric has many biological actions including its anti-inflammatory, antioxidant, anti-carcinogenic, anti-mutagenic, anti-coagulant, anti-fertility, anti-diabetic, anti-bacterial, anti-fungal, anti-protozoal, antiviral, anti-fibrotic, anti-venom, antiulcer, hypotensive and hypocholestere-mic activities [28].

2.1.5. Ginger rhizome (*Zingiber officinale* Roscoe)

Ginger is underground stem, or rhizome of the plant *Zingiber officinale* Roscoe, belongs to the family *Zingiberaceae*. It is a tropical and subtropical spice plant, originally cultivated in south-east Asia and introduced to many countries of the world and has been cultivated for thousands of years for spice and medicinal purposes mainly in India and China. Ginger rhizome is typically consumed as a fresh paste, dried powder, and it acts as an important food preservative in the preparation of syrup and candy.

In addition, ginger has been used for its medicinal value for the treatment of various diseases, especially to treat gastrointestinal disorders (constipation, diarrhea, anorexia, colic, dyspepsia, nausea, vomiting, and motion sickness) and remains an important cooking spice around the world [29].

Ginger is used in all over the world as coloring, flavoring and imparting aroma in the preparation of various foods. The aromatic and flavoring characteristics of ginger is due to the mixture of zingerone, zingerols, and shogaols volatile oils [30]. Ginger is known to have been introduced to Ethiopia as early as in the 13^{th} century. In Ethiopia, it is known as Zingibil. A tea made from ginger is a common folk remedy for colds in Ethiopia [29]. Ginger can grow in a variety of soils. However it requires high humid and hot areas to get high quality ginger. Ginger is grown in altitudes between 1300 and 2000 meter above sea level and requires about 1200 mm annual rainfall, relatively well distributed throughout the year. If the annual rainfall is below 1200 mm, supplementary irrigation is required. The seeding rate varies between 25 and 37 quintal per hectare [23]. Now a day, ginger has been cultivated in many parts of Ethiopia. It is cultivated in south and west Gojam (Bahir Dar, Dejen, Debere Markos, Metekel, and Agew Mider), north and south Omo (Gamo, Galeb and Hamer Bako, and Gofa), and Sidamo zone (Sidama and Arero) [5]. It is an important spice used in every Ethiopian kitchen for the preparation of pepper powder, stew, bread, etc. It has also some use in traditional medicine for the treatment of flu, stomach-ache, and headache.

2.1.6. Fenugreek seed (*Trigonella foenum graecum*)

Fenugreek seed is one of the well known spices in human food which is cultivated worldwide as a semiarid crop. It belongs to the family of *Fabaceae*. India is a major producer and consumer of fenugreek. Fenugreek provides natural food fibre and other nutrients required in human body. It is rich source of soluble dietary fibre content extract plays a role in its ability to moderate metabolism of glucose in the digestive tract and stimulating the appetite as well as modifying food texture. Aromatic and flavourful fenugreek seed is a popular spice and is widely used for well recognized culinary and medicinal purposes. Fenugreek seed is used in physiological utilization for the treatment of antibacterial, anticancer, hypocholesterolemic, hypoglycemic antioxidant, and antidiabetic agent [31].

Fenugreek seed is used in the treatment of diabetes. It is widely used as a galactagogue (milk producing agent) by nursing mothers to increase inadequate breast milk supply. Studies have shown that fenugreek is a potent stimulator of breast milk production. It is also sometimes used as an ingredient in the production of clarified butter [32]. But due to its teratogenic effect, fenugreek seed may affect the development of foetus and induce abortion when pregnant women take it at high amount. Traditionally, 12−59% of women used herbal products during pregnancy for a variety of reasons, including pregnancy related conditions (nausea, vomiting, constipation); to prepare for labor, to induce abortion [33].

2.1.7. Coriander seed (*Coriandrum sativum* L.)

Coriander, commonly called dinbilal in Ethiopia, is an annual spice and aromatic herb that belongs to the family of *Umbelliferae/Apiaceae*. Due to its wide range of climatic, ecological and topographic conditions, Ethiopia has long been known as a centre of origin and diversity for coriander [34]. It is mainly cultivated in Jima, Wellega, Sidamo, South and North Omo, Illubabour, and East and West Gojam [5].

Coriander is also cultivated in Bale and Gonder and used for flavoring soups and other foods. Since ancient time, the coriander seed are used to flavor foods and beverages, especially gin and are also used medically to treat various diseases, particularly used as carminative. It is also used in perfumes, soaps, detergents, and pharmaceutical preparations [35]. Coriander seeds are used locally in Ethiopia for flavoring purposes in the preparation of red pepper powder, bread, and sauces. It is boiled in water and drunk on an empty stomach to treat stomach-ache [35].

2.1.8. Nutmeg seed (*Myristica fragrans* Houtt.)

Nutmeg which is commonly called Korerima in Ethiopia is shiny brown seed and usually sold enclosed in the dried fruit. When nutmeg fruit is crushed, it has a distinctive sweet smell and a delicious mild spicy taste. Nutmeg is belonging to the family of *Myristicaceae* and endemic to Ethiopia and perhaps Sudan but it may also present in northern Kenya, western Uganda and

Tanzania. Its use is well known in Ethiopia where it is a very important spice and an essential component of the traditional cuisine [5].

Nutmeg grows naturally at altitudes between 1000 and 2000 meter above sea level. It grows in different parts of Ethiopia such as in Jima, Wellega, Sidamo, Bale, South and North Omo, Illubabour, and East and West Gojam [5]. Nutmeg seed is an important ingredient of berbere, awaze, mitmita, and other Ethiopian spice mixtures and is also used to flavor coffee. Its flavourness is due to the presence of main chemical compounds such as terpinyl acetate, 1,8-cineole and nerolidol in its essential oil. It is also used in Ethiopian as traditional medicine, tonics and carminatives [36].

2.1.9. Black cumin seed (*Cuminum cyminum* Linn.)

Black cumin seed is one of the aromatic seeds within the *Apiaceae* family. Black cumin is known for its antioxidant properties due to its essential oils content. The presences of essential oils make cumin to have flavor.

It is regularly used as a flavoring agent in a number of cultural cuisines. It is used to flavor many dishes (bread, salsa, meat, etc.) due to its natural sweetness [3]. It is commonly used in the preparations of fragrances and medicines (liqueurs, mouthwashes, toothpastes, soaps, and perfumes). Black cumin seed is also used as carminative and appetite stimulating agent. Cumin seeds have been found to possess significant biological activities like; antibacterial, antifungal, anti-carcinogenic, anti-diabetic, anti-thrombotic, and antioxidant properties [37].

2.1.10. Thyme leaves (*Thymus vulgaris*)

Thyme is one of the aromatic and sanitary plants that have been used in curing processes since early. It belongs to the family of *Lamiaceae*. Thyme leaves are considered among the most valuable plants around the world and used in cooking application. It is also used cosmetically and medicinally. Due to its phenolic constituents (thymol and carvacrol), thyme has anti-bacterial and ant-oxidant activities [38].

In the meantime, thyme is used in the treatment of pulmonary infections, liver disorder, catarrh, bronchitis, angina, stomach sore and inflation. Traditionally, it is consumed for remedy of wounds, appetite failure, diarrhea or digestive disorders, whooping cough and other types of coughs [39]. Ethiopian thyme is a good source of iron and is widely used in Ethiopian cuisine such as in tea as medicinal remedy to improve respiratory function and also used for all sauces including pasta sauce [40].

2.1.11. Basil leaves (*Ocimum basilicum*)

Basil which is called besobla in Ethiopia is a popular herb that belongs to the family of *Lamiaceae (mint).* It is an annual spice herb permanently grown in warm conditions, but requires enough soil moisture throughout the growing season for high quality and yields. Basil is cultivated for the purpose of fresh market or for its aromatic leaves which are dried and used as a spice or flavoring agent. It may have originated in India and spread through Asia. Basil is now considered to be native to Africa, Asia, India, Middle East, Caribbean, and South America. It is produced commercially in Egypt, France, Hungary, Israel, Mexico, Indonesia, and United States. The quality of cultivated basil is determined by its appearance (color and absence of decay or insect damage), flavor, moisture content for the fresh market, and volatile oil content and total insoluble ash content for the processing market.

Most commonly, basil leaves are used fresh or dried to add flavor to soups, tomato dishes, fish, poultry, vinegar, vegetables, salads, powdered beef, sausages, candy, gelatins, and ice cream [41]. It is also used in most dairy products to flavor yoghurt and cheese. In addition to these, basil is used to flavor tea and to make berbere [40].

Basil is used medicinally to treat for coughs and fever in Africa. Basil was a traditional treatment for mild nervous diseases and taken as snuff in a dry powder form. Basil leaf tea alleviates vomiting, gas pains, and fevers while basil juice has been used to treat warts, worms, snake bites and insect bites [42]. Commercially, the essential oil of basil is a component of mouthwash, medicine, soaps, shampoos, perfume, and liqueur.

2.2. Fluoride

Fluoride (F^-) is defined as compounds or salts of fluorine and another element(s). Fluoride is not considered to be essential for human growth and development but it is considered to be beneficial in the prevention of dental caries and skeletal fluorosis [43]. Fluoride is the most electronegative and reactive halide. As a result, it exists in the form of compounds in the environment [44]. Fluoride forms both organic and inorganic compounds. Based on quantities released and concentrations present naturally in the environment as well as the effects on living organisms, the most relevant inorganic fluorides are hydrogen fluoride (HF), calcium fluoride or fluorite (CaF_2), sodium fluoride (NaF), sulfur hexafluoride (SF_6), cryolite (Na_3AlF_6) and silico fluorides [8]. Fluoride is a persistent and non-degradable poison that accumulates in soil, plants, wildlife, and humans [45]. Organic fluorides (organofluorine compounds) rarely release fluoride ions under biological conditions and thus are rarely sources of fluoride poisoning. This is because of the strength of the carbon–fluorine bond and its tendency to strengthen as more fluorine atoms are added to a carbon [46].

2.2.1. Sources and occurrence of fluoride in the environment

Fluorides are released into the environment due to human activities and naturally from natural sources. The manufacturing of steel, brick, ceramic, glass, aluminum, copper, nickel, glues, adhesives, and the production of hydrogen fluoride, chlorofluorocarbon and phosphate fertilizer and use of fertilizer released fluoride into the environment (air, water, plants, animals, rocks and soil) [47-50]. Combustion of fluoride impurities containing coals as well as the use of fluoride containing pesticides and controlled fluoridation of drinking water supplies also release fluoride into the environment [8]. Mining activities and deep wells of springs may release a large amount of fluoride into the atmosphere. Fluorides also released into the environment naturally through weathering and dissolution of fluoride-bearing minerals like fluorite, rock phosphate, fluoropatites, and topaz [47, 51]. Fluorides are released into the environment through atmospheric emissions from volcanoes and seawater [8].

2.2.2. Exposure of human to fluoride

Although fluoride is everywhere in the environment, the major environmental sources of population exposure to elevated levels of fluoride are water, food, beverages, air, food supplements, and dental products [10, 52].

2.2.2.1. Water

Fluoride is found in all natural waters at some concentration. Drinking water contains a fluoride concentration (0.5−1.5 mg/L) which is effective for preventing dental caries. This concentration can occur naturally or be reached through water fluoridation [53]. Seawater typically contains about 1 mg/L while rivers and lakes generally exhibit concentrations of less than 0.5 mg/L [54, 55]. The Rift Valley lakes in Ethiopia (Shala and Abijata) have high fluoride concentrations (264 and 202 mg/L F^-) respectively [56]. However, ground water contains low or high concentrations of fluoride depending on the nature of the rocks and the occurrence of fluoride-bearing minerals [54]. High fluoride concentrations may therefore be found in ground waters (0.01−4 mg/L) in non-rift valley areas whereas 4−36 mg/L in rift valley ground water. This is due to the presence of soluble fluoride-bearing minerals like fluorspar, cryolite, fluorapatite, and sellaite in the ground. Fluoride concentrations may also increase in ground water in which cation exchange of sodium for calcium occurs. Fluoride exposure (mg/kg of body weight per day) through drinking-water is determined by the fluoride level in the water and the daily water consumption (liters per day) [54, 55].

High fluoride concentration in drinking water (> 1.5 mg F^-/L) causes the risk of both dental and skeletal fluorosis in many countries. India, Sri Lanka, China, the Rift Valley countries in East Africa including Ethiopia, Turkey, and some parts of South Africa are some countries those exposed to fluorosis [57].

2.2.2.2. Foods and water based beverages

The fluoride content of prepared foods depends on the fluoride content of the food itself and on fluoride concentration and amount of water used and retained in the food during its preparation [12, 58]. The uptake of fluoride by vegetables from cooking water has been reported as proportional to the fluoride content of the water for the concentration range of 1−5 mg/L. The fluoride content of processed foods and beverages prepared from water containing fluoride at 1 mg/L level will contain about 0.5 mg/kg more fluoride than those prepared with non-fluoridated water [59]. Many carbonated drinks and bottled water had fluoride levels close to 1 mg/L [60].

Fluoride levels in meat and fish are (0.2−1.0 mg/kg) and (2−5 mg/kg) relatively. Fluoride intake from fish alone would seldom exceed 0.2 mg F^- per day since fish is consumed in a mixed diet. Human breast milk typically contains low levels of fluoride (0.02 mg/L) and cow's milk contains 0.02−0.05 mg/L fluoride [60, 61]. Tea leaves contain high levels of fluoride (up to 400 mg/kg dry weight). Fluoride exposure due to the ingestion of tea has been reported to range from 0.04 mg to 2.7 mg per person per day [61]. However, some have been observed to ingest large amounts of fluoride (14 mg per day) due to the consumption of brick tea as a beverage. This type of tea is made from older leaves and contains much higher levels of fluoride than standard teas such as black or green tea [62].

2.2.2.3. Air

Due to fluoride containing dusts, industrial production of phosphate fertilizers, coal ash from the burning of coal and volcanic activity, fluorides are widely distributed in the atmosphere. However, air is typically responsible for only a small fraction of total fluoride exposure. In areas where fluoride-containing coal is burned or phosphate fertilizers are produced and used, the fluoride concentration in air is elevated leading to increased exposure by the inhalation route [50]. The most prevalent form of atmospheric fluoride is hydrogen fluoride which is rapidly absorbed from the lungs. In spite of this, fluoride exposure from the atmosphere makes only a small contribution to the total daily intake of persons who are not in heavily polluted areas [50, 63]. In heavily industrialized cities, the maximum amount of fluoride inhaled daily ranges between 0.01 and 0.06 mg of fluoride in a day of maximum pollution [50]. In non-industrialized areas, fluoride content in air has been found to be low and is not considered to contribute more than 0.01 mg/day to the total intake [43].

2.2.2.4. Dental products

Dental products like toothpaste, gels and tablets administered by children to reduce dental caries contain fluoride. This includes toothpaste contains 1.0−1.5 g/kg fluoride, fluoride solutions. Brushing with fluoride toothpaste increases the fluoride concentration in saliva 100-1,000 fold; but this concentration returns to baseline levels within 1−2 hours. Gels for topical treatment contain 0.25−24.0 g/kg fluoride. These dental products contribute to the total fluoride exposure in different degrees. WHO estimated that the swallowing of toothpaste by some children may contribute about 0.50 or 0.75 mg fluoride per child per day [54]. Fluorides in gels pose little risk for enamel fluorosis at 3 to 12-month intervals.

2.2.2.5. Other supplements

Dietary fluoride supplements in the form of tablets, lozenges, or liquids (including fluoride-vitamin preparations) have been used throughout the world. Most supplements contain sodium fluoride as the active ingredient. Tablets and lozenges are manufactured with 0.25, 0.5, or1.0 mg fluoride per tablet and they contribute to the total fluoride exposure of human. Fluoridation of drinking water to compensate fluoride-deficient drinking water also influence population fluoride intake. The dosage schedule should require knowledge of the fluoride content of primary drinking water and consideration of other fluoride sources of drinking water [53].

2.2.3. Fluoride metabolism

Fluorides from different sources are entered to the body through the gastrointestinal tract and the ingested fluorides are rapidly absorbed from the stomach and distributed throughout the body via blood. The rate of fluoride absorption is directly related to the acidity of its contents [64]. However; the solubility of the ingested fluoride compound also influences the rate of fluoride absorption. More soluble fluoride compounds such as sodium fluoride and hydrogen fluoride are absorbed very fast whereas less soluble fluoride compounds, like calcium fluoride and magnesium fluoride are absorbed slowly.

Once fluorides are absorbed to plasma, some amounts of fluorides are excreted via the kidneys with urine [65], whereas 90% of fluoride rapidly associated with calcified tissues (bone and teeth) due to the combination of fluorides with calcium ions of teeth and bone and form calcium fluorophosphates (fluorapatite) crystal. This results stiffness of tissues and joints and finally leads to fluorosis in later stage [66].

2.2.4. Fluoride health benefits

2.2.4.1. To prevent and control dental caries

Fluoride is an important nutrient to all age groups of both human and animals to prevent as well as to control tooth decay throughout the life cycle [67, 68]. Fluoride is purposely added to the water supply, toothpaste and sometimes other products to promote dental health [69]. Dental caries (Figure 2) is the most common chronic disease of childhood, with 59% of 12 up to 19 years old. It is the "silent epidemic" that disproportionately affects poor, young, and minority populations.

Figure 2. Dental decay in children.

The process of dental caries requires four components: teeth, bacteria, carbohydrate exposure, and time. Once teeth emerge, they may become colonized with cariogenic bacteria. The bacteria metabolize carbohydrate foods (sugars) in mouth and create acid on tooth surface as a byproduct. The acid dissolves minerals from tooth enamel (demineralization) and over the time with repeated acid attacks; it leads to dental caries [70]. Generally, in the absence of sugars in foods and drinks, dental caries will not be a problem of public health. However in some areas where sugar is consumed highly, dental caries is a major public health problem unless there is appropriate intervention. The main way of dental caries intervention is ingestion of an optimal amount of fluoride [20]. Ingestion of optimum fluoride prevents tooth decay since fluoride promotes tooth remineralization (reformation of tooth mineral). Fluoride inhibits acid creation by interrupting the ability of bacteria to metabolize sugars and able to compensate the mineral losses caused by acid production in the bio-film, by inducing the precipitation of the less soluble mineral phase which is called fluorapatite in the tooth structure [71].

The recommended daily intake of fluoride from all sources (including non-dietary sources) for both children and adults, including pregnant and lactating women is 0.05 mg/kg body weight per day. For pregnant and lactating women, the adequate fluoride intake is based on their body weight before pregnancy and lactation [72, 73].

For adults, fluoride intake in the range of 2 to 4 mg per day prevents tooth decay. In contrast, intake of fluoride more than the optimum value (most of the time above 6 mg/day) integrates within the calcified tissues (tooth and bone) and the calcified tissues changes in structure and composition. This leads to dental and skeletal fluorosis [8, 74].

2.2.4.2. To prevent osteoporosis

Osteoporosis is a progressive bone disease that is characterized by a decrease in bone mass and density which can lead to an increased risk of fracture. Osteoporosis is caused due to calcium deficiency in the body during pregnancy, breastfeeding, and growth of skeletal [75]. In the absence of adequate dietary calcium in the body, the absorbed fluoride may result in secondary hyperparathyroidism, leading to bone loss. So that bone density may include areas of both sclerosis and porosis [76]. However, ingestion of an optimum amount of fluoride has been recommended as a therapeutic agent in the treatment of osteoporosis. Fluoride combination with calcium stimulates osteoblasts and the integration of fluoride into the bone matrix in the form of fluorapatite increases the hardness of bones [77].

2.2.5. Fluoride health risks

Fluoride is a highly toxic and corrosive ion at its high level [9]. It is more poisonous than lead and just slightly less poisonous than arsenic [78]. Acute intoxication caused by fluoride is rare and has occurred mainly as a result of exposure to excess fluoride in accidental poisonings. The symptoms of acute toxicity include severe nausea, vomiting, excess saliva production, abdominal pain, and diarrhea. Severe cases of exposure can result in convulsions, irregular heartbeat, and coma [65].

A long-term intake of higher fluoride amounts could be a reason for chronic intoxication such as fluorosis with typical manifestations. Fluorosis is a result of abnormal deposition of fluoride in hard tissues such as tooth and bones. Fluorosis can be mild, moderate or severe depending on the amount of fluoride ingested during the stages of teeth formation and bone development [66]. There are three typical forms of fluorosis. These are dental fluorosis, skeletal fluorosis and non skeletal fluorosis. The toxicity of fluoride for plants is relatively low. Intoxication could cause changes in plant appearance and/or growth [44].

2.2.5.1. Dental fluorosis

Dental fluorosis (Figure 3.) is alterations in enamel caused by excessive exposure to high concentration of fluoride during tooth development (childhood). The risk of fluoride over exposure occurs between the ages of 3 months and 8 years [8, 79]. In the initial stage of fluorosis, tooth appearance is damaged by discoloration or brown markings in the form of spots or streaks. Depending upon the severity the enamel may be pitted, rough and hard to clean. The spots and stains left by fluorosis are permanent and may darken over time [79, 80]. The severity of dental fluorosis depends on the amount of fluoride exposure, the age of child, individual response, weight of child, degree of physical activity, nutrition, and bone growth [79].

Figure 3. Dental fluorosis.

Dental fluorosis is the main health problem in the world including Ethiopia. Since the fluoride level of ground water in the rift valley areas is high, the risk of fluorosis is mainly observed in the rift valley areas of Ethiopia and spread over different regional states such as Afar, Amhara, Oromia and Southern Nations, Nationalities and People’s Region (SNNPR). The main area with high fluoride levels outside the rift valley is the area around Jimma in the west of the country [81].

2.2.5.2. Skeletal fluorosis

Fluoride is not irreversibly bound to bone. During the skeleton growth phase, a relatively high portion of ingested fluoride will be deposited in the skeleton. With increasing bioaccumulation of fluoride in bone through the advancement of age, there are chances of the appearance of skeletal fluorosis (Figure 4). Such skeletal fluorosis leads to severe and permanent bone and joint deformities.

Figure 4. Skeletal fluorosis.

It causes pain and stiffness in the joints and backbone. Skeletal fluorosis also leads paralysis, severe pain and rigidity in the hip region and cripples a person. Crippling skeletal fluorosis, which is associated with higher levels of exposure to fluoride, can result in osteosclerosis (hardening and calcifying of the bones) [9].

An increased risk of fluoride effects on bone is suggestive at total intake levels above 6 mg fluoride/day whereas skeletal fluorosis and bone fractures are clearly observed at total fluoride intakes of 14 mg/day [8].

2.2.5.3. Non-skeletal fluorosis

Consuming of excessive fluoride can also affect non-calcified tissues besides bone and teeth. The risk of fluoride on soft tissues (aorta, thyroid, lungs, kidneys, heart, pancreas, brain and spleen) is called non skeletal fluorosis [79].

Excessive fluoride stimulates granule formation and oxygen consumption in white blood cells and it reduces the ability of white blood cells to properly destroy foreign agents by the process of phagocytosis. Excessive fluoride inhibits antibody formation in the blood. Since during chronic fluorosis the serum prolactin level decreases, excessive fluoride also inhibits lactation in mammals. It also depresses thyroid activity. Excessive fluoride intake at childhood reduces mental work capacity (MWC) and hair zinc content. Healthy kidneys excrete 50 to 60% of the ingested fluoride. The fluoride content of urine has been suggested as an index of animal exposure and as a diagnostic test for humans in cases of chronic exposure to fluoride [9]. The accumulated fluoride in cells inhibits the DNA repair enzyme, and then inhibits our immune system. As a result, it may cause muscle weakness, stiffness, birth defects and genetic damage as well as cancer disease [78].

3. EXPERIMENTAL

3.1. Sample collection

In this study, spice samples were collected from Merkato and Shola spice markets which are located in Addis Ababa, Ethiopia. The sellers in both spice markets have brought different types of spices from the cultivation areas in the rural parts of Ethiopia and all types of spices were available based on their cultivation areas. Therefore, it was easy to get the representative samples of the selected spices for this study.

All the selected spices (red chili pepper, garlic bulb, cinnamon bark, turmeric rhizome, ginger rhizome, fenugreek seed, coriander seed, nutmeg seed, black cumin seed, thyme leaves, and basil leaves) were collected from Merkato and Shola spice markets in December 10 and 15, 2014, respectively. About 0.25 kg of each spice sample was bought from eight different spice shops (four shops from each market). The total of 88 spice samples (eight samples for each eleven spices) were collected and brought to the laboratory. Then the collected samples for each spice type were mixed together into polyethylene plastic bags to get 2 kg of homogenized or representative spice samples.

3.2. Apparatus, chemicals, reagents and instruments

3.2.1. Apparatus

Polyethylene plastic bags were used for collecting spice samples. An oven (Digitheat, J.P. Selecta, Spain) was used to dry samples to constant weight and an electronic blending device (Geepas electric coffee grinder, Mainland, China) was used for grinding and homogenizing the samples. Electronic balance (Adam Equipment, Model WL 3000, UK), with precision of 0.0001 g was used for weighing of spice samples. Muffle furnace (Audiotronics, Wagtech International Ltd., UK) was used for fusion of samples within nickel crucibles (50 mL). A pH/ISE meter (Orionmodel, EA 940 Expandable Ion Analyzer, USA) equipped with combination fluoride ion selective electrode (Orion Model 96-09, USA) was employed for the determination of fluoride in the samples and standards solutions. A pH meter (HANNA instrument, HI 9025, Singapore) equipped with pH glass electrode was used to measure the pH values of sample solutions. Borosilicate volumetric flasks (250 and 1000 mL) were used for preparation of both 8 M NaOH solution and 1000 mg/L NaF stock solution. Hot plate with magnetic stirrer was used for dissolution of the sample and fusion cake. Measuring cylinders (Duran, Germany), pipettes (Pyrex, USA); micropipettes (Dragonmed, 1−200 µL, Shangai, China) were use during measuring of different volumes of samples solutions and fluoride standard solutions. 50 mL plastic volumetric flasks were used for the storage of sample solutions. Different types of volumetric flasks (50, 100 and 1,000 mL) were used during standard preparation and plastic beakers (50 mL) were used for preparing samples and standards solutions during the determination of fluoride.

3.2.2. Chemicals and reagents

The reagents that were used in the analysis were all of analytical grade. De-ionized water which is chemically pure (with conductivity ≤ 1.5 µs/cm) was used throughout the experiment. Nitric acid (69%, Research Lab Fine Chemical Industries, Mumbai, India) was used for cleaning purpose and sodium fluoride (99%, Analar, NaF, BDH Chemicals Ltd, England) used to prepare standard solutions. The pH standard buffers (pH of 4, 7 and 10) were used for pH calibration purpose. Sodium chloride (Fisher Scientific UK), glacial acetic acid (100%, Sigma-Aldrich Laborchemikalien, Germany), trisodium citrate (BDH Laboratory Supplie poole, England), and EDTA (Scharlau Chemie S.A., Barcelona, Spain) were used to prepare Total Ionic Strength Adjustment Buffer (TISAB) solution. Sodium hydroxide (Scharlau Chemie S.A., Sentmenat, Spain) solution was used to dissolve spice samples homogeneously before alkali fusion and also used to adjust the pH of TISAB solution to pH of 5.3. Hydrochloric acid (36%, Fisher Scientific UK Limited) was used for neutralization of dissolved fusion cake.

TISAB was prepared by dissolving 58 g sodium chloride, 57 mL glacial acetic acid, 7 g of tri sodium citrate and 2 g EDTA in 500 mL de-ionied water into 1000 mL beaker and its pH was adjusted to 5.3 with 5 M sodium hydroxide. The solution was then transferred to 1000 mL volumetric flask and diluted to the mark with de-ionized water [82].

3.2.3. Instrumentation

A pH/ISE meter (Orion model, EA 940 Expandable Ion Analyzer, USA) equipped with combination fluoride ion selective electrode (Orion Model 96−09, USA) was employed for the determination of fluoride ion in standard and sample solutions. The reference electrode which is combined with working electrode was silver/silver chloride (Ag/AgCl) electrode. The pH was measured with pH/ION meter (HANNA instruments HI 9025, Malaysia) using pH glass electrode.

3.3. Sample preparation for fluoride analysis

3.3.1. Fusion of spice samples

The determination of fluoride in spices using fluoride ion selective electrode was done by using the reported method [11]. Spice samples were separately washed first with tap water followed by de-ionized water to reduce contamination. After washing, samples were chopped into small sizes and dried in an oven (Digitheat, J.P. Selecta, Spain) at 70 ^{o}C to constant weight. Then the dried samples were grounded using acid washed electric coffee grinder in the laboratory and sieved through 1 mm sieve. 0.5 g of powdered sample for each sample was weighed accurately and transferred into the 50 mL nickel crucibles. The weighed samples were covered with 5 mL of 8 M sodium hydroxide solution. Then the sample and sodium hydroxide solution were carefully mixed to make the mixture as homogenous as possible. Nine reagent blanks, containing only 5 mL of 8 M sodium hydroxide solution were prepared for fusion for blank determination.

The crucibles were placed on a hot plate at a temperature of 150 ^{o}C for evaporation until the mixture of sodium hydroxide solution and sample was solidified. Then the crucibles were introduced into a muffle furnace and set at a temperature of 200 ^{o}C for 2 h and was increased to 525 ^{o}C for 3 h in order to fuse samples in the crucibles. The crucibles were placed in a hood and allowed to cool at room temperature and 12 mL de-ionized water was added to each crucible and kept on a hot plate at 150 ^{o}C for 2 h in order to aid the dissolution of the fusion cake. The pH meter was calibrated with technical pH buffers of 4.00, 7.00, and 10.00 pH values. Then, 36% hydrochloric acid (about 2.5−3 mL) was added drop wise to adjust the pH of fused samples from 12.0−13.2 to 6.8−7.5 while continuously monitoring with a pH meter. The sample solutions were then transferred to 50 mL plastic beakers. The crucibles was rinsed successively with de-ionized water until the final volume reached 50 mL and all the washings were mixed and filtered with qualitative filter paper (90 mm, diameter) in pre-cleaned and rinsed beakers transferred in to 50 mL plastic volumetric flasks and stored in refrigerator until fluoride measurement.

3.4. Instrument calibration

3.4.1. Calibration of fluoride electrode

Fluoride ion selective electrode was calibrated with five standard solutions prepared from fluoride stock solution of 1000 mg/L. Fluoride stock solution was prepared by dissolving 2.21 g of anhydrous sodium fluoride (99.0% NaF, BDH Chemicals, England) in 500 mL de-ionized water into 1000 mL volumetric flask and diluted to the mark with de-ionized water. The calibration standard solutions of 0.05, 0.5, 1, 5, and 10 mg/L were then prepared by serial dilution from 1000 mg/L fluoride stock solution. Aliquots of 5 mL of TISAB solution were poured into 50 mL plastic beakers containing 5 mL of standard solution (1:1) and the fluoride ion selective electrode was immersed into beakers at room temperature to calibrate fluoride ion selective ion electrode. Aliquot of 5 mL of de-ionized water and 5 mL of TISAB were poured into other 50 mL plastic beaker as standard blank solution for blank correction. The electrode potentials of these standard and blank solutions were then measured by stirring the solutions constantly at room temperature. The calibration curve of five series points (Figure 5) was constructed with electrode potentials versus log of standard fluoride concentrations. The slope of the curve was −57.5 mV/decade which was found in the optimum range (−57 to −60 mV/decade). This value of slope was nearest to the theoretical value (−59.2 mV/decade at room temperature). The calibration curve equation also showed that the correlation coefficient was 0.9999 which indicated very good correlation between electrode potential and concentration of fluoride.

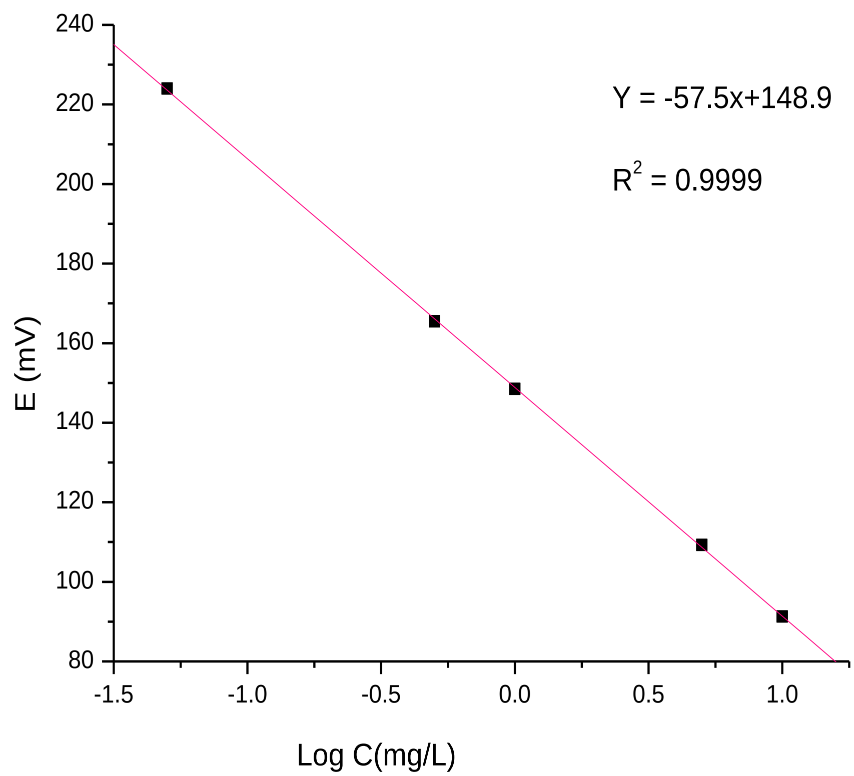

Figure 5. Calibration curve for five standard solutions.

The potential developed between the FISE and reference electrode, E (mV) has linear relationship with the logarithm of concentration (activity) of the fluoride ions in a solution. This relationship is expressed by the Nernst equation.

$E = E^o - (2.303RT/ZF)\log[F^-]$.

Where: R is the gas constant (8.314 Joules/degree/mole); T is the absolute temperature (K); Z is the charge of fluoride ion in the solution; E is measured electrode potential which is varied with the concentration of fluoride ion in a solution; E^o is constant potential of reference electrode; the number 2.303 is the conversion factor from natural to base 10 logarithm; F is the Faraday constant (96,500 coulombs/mole); and $[F^-]$ is the concentration of fluoride ion in a particular sample solution.

The term, $-2.303RT/ZF$ is the slope of electrode response to ions which shows the plot of E (mV) against logarithm of fluoride ion concentration (Log C_F) is a straight line within the concentration range of the electrode response. At 25 ^oC, the slope is −0.05916 V or −59.2 mV. The negative sign of the slope is for anion analytes in a solution. The measured electrode potential is indirectly proportional with the concentration of anions but, directly related with that of cations in a solution. This is an important diagnostic characteristic of an ion selective electrode [82].

3.5. Total fluoride determination

Fluoride ion in solutions can be measured by using different methods. Potentiometric and chromatographic methods are the main fluoride ion measuring methods. Potentiometric analysis by using fluoride ion selective electrode is the most commonly used measuring method since it is easy to use, relatively cheap, sufficiently sensitive, selective, accurate and very small concentrations of fluoride ions (to 10^{-6} M) can be determined by fluoride selective electrode [11, 51]. The levels of fluoride in samples were measured by fluoride ion selective electrode by using direct measurement technique rather than incremental techniques (standard or known addition, sample addition, and sample subtraction), and potentiometric titration techniques. Because, direct measurement technique is a simple and fast technique for measuring large number of samples and measurements can be made over a wide range of concentration [83].

Addition of total ionic strength adjustment buffer (TISAB) provides constant ionic strength and adjusts pH values of solutions to pH of 5.2−5.5. This pH adjustment helps to avoid the interferences of cations with fluoride ions since the complexing agent called ethylene di-amine tetra acetic acid (EDTA) which is the component of TISAB make complexs with cations (Al, Ca, Fe, Mg, etc.) to generate free fluoride ion in solutions [82]. Adjustment of pH also avoids the interferences of hydroxide ion (OH^-) with fluoride electrode in basic solutions and that of hydronium (H^+) ion with fluoride ion in acidic solutions [84].

In this study, aliquot of 5 mL of each of sample solution was poured into 50 mL plastic beakers and 5 mL of TISAB was mixed to adjust the pH of standards and samples solutions to 5.2−5.5 which is the optimal pH for fluoride determination. Reagent blank solutions were prepared for blank correction by taking 5 mL of blank solution and 5 mL of TISAB into 50 mL plastic beakers. Sample and blank solutions which were prepared for fluoride analysis were stirred while measurement is being made by using magnetic stirrer in order to homogenize the solution and minimize the electrode response time. All the analyses were made in triplicate at room temperature and the concentration of fluoride was recorded in the unit of mg/L directly from the instrument reading for each solution.

3.6. Method validation

3.6.1. Detection limits

The limit of detection (LOD) of fluoride ion selective electrode and limit of quantification (LOQ) of the method was tested by measuring the fluoride levels in nine blank solutions. Detection limit is the lowest amount of fluoride in a sample that can be determined with a certain confidence and it can be calculated as 3 times the average standard deviation of blank solutions or background signal. The limit of quantification is the lowest concentration level at which a measurement is quantitatively meaningful. It was calculated as 10 times the average standard deviation of blank solutions [82]. As a result, the lower detection limit of fluoride ion selective electrode used for this study was found to be 0.02 mg/L. while the method limit of quantification was 0.04 mg/L.

3.6.2. Precision and accuracy

Precision is defined as the closeness or agreement among several determinations of the same quantity. The precision of the analytical method was evaluated under conditions of repeatability, using samples having different fluoride concentrations 2.14 (coriander seed) to 8.57 mg/kg (thyme leaves). The precision was expressed as standard deviation (SD) of the fluoride levels in the spices. Accuracy of an analytical method is the degree of agreement between experimental values with an accepted value. Accuracy is expressed as a percent difference.

3.6.3. Validation of analytical procedure

The validation of fluoride measuring method can be done in different ways such as by proofing recovery test and/or by using certified reference materials. The simple and most widely used way of method validation is recovery test. The procedure for measuring of fluoride level was validated by analyzing fluoride concentration of spice sample solutions prepared by alkali fusion and that of spiked sample solutions. In this study, validation of fluoride measuring method was checked by performing recovery tests for all spice samples. The recovery is often given as a proof for the reliability of a method. A satisfactory recovery tells us that there are no interfering agents and that the fluoride added has been detected [85].

For recovery study, the spiked samples were prepared by adding known amounts of fluoride from 10 mg/L fluoride standard solution. The added fluoride contents were equivalent to 50%, 100%, and 200% of the fluoride concentration in the original (unspiked) spice samples. 0.5 g of red chili pepper, garlic granules, cinnamon bark, turmeric rhizome, ginger rhizome, fenugreek seed, coriander seed, nutmeg seed, black cumin seed, thyme leaves, and basil leaves were spiked in triplicate with 77.3, 154.5, 309 µL; 81.8, 163.5, 327 µL; 128.5, 256.5, 513 µL; 82 .3, 164.5, 329 µL; 70.3, 140.5, 281 µL; 159, 318, 636 µL; 53.5, 107, 214 µL; 112.8, 225.5, 451 µL ; 203.5, 407, 814 µL; 214.3, 428.5, 857 µL; 63.3, 126.5, 253 µL of 10 mg/L standard fluoride solution, respectively. Fluoride levels of the spiked samples and that of unspiked spice samples were analyzed in triplicate and percent recovery was obtained by comparing the results between the fluoride found in unspiked samples and that of in spiked samples as follows [86]:

$$\% \text{ recovery} = C/C_{ref} \times 100$$

Where: C is the concentration of fluoride which is recovered and C_{ref} is reference concentration or fluoride concentration of spiked sample.

4. RESULTS AND DISCUSSION

4.1. Recovery test results of fluoride determination

The recovery test was done for all the samples since all the samples have different matrix and percentage recovery (% R) was calculated by spiking known amounts of fluoride into 0.5 g spice samples. The results of percentage recovery (Table 1) were found in the range of 88−111% (Table 1) which confirms that the analytical method of total fluoride measurement used in this study is reliable. The percentage relative standard deviations (% RSD) were < 5% which showed that the method of analysis was precise.

Table 1. Recovery test results of fluoride determination in Ethiopian spice samples (n = 9).

Spice Type	F^- in un-spiked Samples (mg/kg)	Amount of F^- added in un-spiked Samples (mg/kg)	F^- in spiked Samples (mg/kg)	% Recovery
Red chili pepper	3.1 ± 0.07	1.55	4.7 ± 0.05	102 ±6
	3.1 ± 0.07	3.1	6.34 ± 0.1	105 ± 5
	3.1 ± 0.07	6.2	9.42 ± 0.08	102 ± 2
Garlic granules	3.27 ± 0.13	1.64	5.1 ± 0.9	111 ± 8
	3.27 ± 0.13	3.27	6.25 ± 0.63	97 ± 7
	3.27 ± 0.13	6.54	9.45 ± 0.49	95 ± 7
Cinnamon bark	5.13± 0.23	2.57	7.66 ± 0.31	105 ± 8
	5.13± 0.23	5.13	9.8 ± 1.15	95 ± 9
	5.13± 0.23	10.26	15.1 ± 0.85	96 ± 6
Turmeric rhizome	3.29 ± 0.07	1.65	4.98 ± 0.14	106 ± 7
	3.29 ± 0.07	3.29	6.2 ± 0.46	88 ± 15
	3.29 ± 0.07	6.58	9.54 ± 0.47	95 ± 7

Ginger rhizome	2.81 ± 0.13	1.41	4.37 ± 0.04	109 ± 7
	2.81 ± 0.13	2.81	5.64 ± 0.59	96 ± 6
	2.81 ± 0.13	5.52	8.56 ± 0.28	104 ± 6
Fenugreek seed	3.18 ± 0.13	1.59	4.83 ± 0.07	103 ± 6
	3.18 ± 0.13	3.18	5.1 ± 1.27	105 ± 1
	3.18 ± 0.13	6.3	9.58 ± 0.07	101 ± 2
Coriander seed	2.14 ± 0.04	1.07	3.27 ± 0.44	103 ± 3
	2.14 ± 0.04	2.14	4.35 ± 0.06	102 ± 8
	2.14 ± 0.04	4.28	6.55 ± 0.03	103 ± 3
Nutmeg seed	4.51 ± 0.12	2.26	6.73 ± 0.06	102 ± 1
	4.51 ± 0.12	4.51	9.5 ± 0.5	98 ± 4
	4.51 ± 0.12	9.02	13.5 ± 0.06	111 ± 10
Black cumin seed	8.14 ± 0.15	4.07	12.7 ± 0.46	104 ±8
	8.14 ± 0.15	8.14	15.9 ± 0.16	96 ± 3
	8.14 ± 0.15	16.3	23.9 ± 0.47	97± 2
Thyme leaves	8.57 ± 0.11	4.29	12.6 ± 1.2	98 ± 8
	8.57 ± 0.11	8.57	18 ± 0.75	108 ± 8
	8.57 ± 0.11	17.1	25.2 ± 0.42	97 ± 2
Basil leaves	2.53 ± 0.05	1.27	3.9 ± 0.28	96 ± 10
	2.53 ± 0.05	2.53	4.99 ± 0.39	101 ± 9
	2.53 ± 0.05	5.06	7.74 ± 0.18	103 ± 3

As the recovery results demonstrated, the amount of fluoride added for the purpose of recovery test was detected. This tells us that there were no interfering agents in the samples and it also showed the reliability of the method.

4.2. Level of fluoride in spice samples

The fluoride levels in the spices selected for this study was analyzed using fluoride ISE and each spices have their own significant amount of fluoride. Mean values of fluoride were determined from triplicate analysis for each spice sample. That means, the mean values determined were triplicate of triplicate analysis for each spice sample and the results were recorded in terms of mean ± SD, (n = 9). The levels of fluoride (mg/kg dry wt.) in spices are presented in Table 2. As indicated in Table 2, the levels of fluoride in spices were (mg/kg dry wt.): 3.1 ± 0.17 (red chili pepper), 3.27 ± 0.13 (garlic granules), 5.13 ± 0.23 (cinnamon bark), 3.29 ± 0.07 (turmeric rhizome), 2.81 ± 0.13 (ginger rhizome), 8.57 ± 0.11, 3.18 ± 0.03 (fenugreek seed), 2.14 ± 0.04 (coriander seed), 4.51 ± 0.12 (nutmeg seed), 8.14 ± 0.15 (black cumin seed), (thyme leaves), and 2.53 ± 0.05 (basil leaves).

Table 2. Mean concentration (mean ± SD, mg/kg dry weight basis, n = 9) of fluoride in spice samples.

Spice type	Levels of F^- (mg/kg)	Spice type	Levels of F^- (mg/kg)
Red chili pepper	3.1 ± 0.07	Coriander seed	2.14 ± 0.04
Garlic granules	3.27± 0.13	Nutmeg seed	4.51 ± 0.12
Cinnamon bark	5.13 ± 0.23	Black cumin seed	8.14 ± 0.15
Turmeric rhizome	3.29 ± 0.07	Thyme leaves	8.57 ± 0.11
Ginger rhizome	2.81 ± 0.13	Basil leaves	2.53 ± 0.05
Fenugreek seed	3.18 ± 0.03		

4.3. Level of fluoride in fresh (wet) spices

From the selected spices for this study; garlic granules, ginger rhizome, and thyme leaves are commonly consumed both as fresh and dry basis. Therefore, the fluoride level found in these spices can be also reported in fresh weight basis (wet weight basis). In order to convert the dried weight basis fluoride concentration into fresh weight basis concentration, percentage moisture (% moisture) of sample should be determined from the dried and wet weights of the sample as follows [87]:

% moisture = [(ww-dw)/ww] x 100

In this study, 500 g of wet garlic granules was taken to dry in an oven at 70 ^{o}C and after it was dried, it became constant weight (76.9 g) that means 84.6% of wet garlic was moisture (water). It means that in 1 kg wet garlic sample, there was 846 g moisture and 154 g dried mass.

Similarly, about 500 g of fresh (wet) ginger rhizome, thyme leaves, and basil leaves, samples were taken separately to dry in an oven at 70 ^{o}C and after dried; they become to the constant dry weight of 91.6, 83, and 64.5 g, respectively. As a result, the moisture content of garlic granules, ginger rhizome, thyme leaves, and basil leaves were 81.7%, 83.4%, and 87.1%, respectively.

The wet weight basis fluoride levels in garlic granules, ginger rhizome, thyme leaves, and basil leaves were calculated and were reported in Table 3. In general, such conversion is accomplished by using the following formula [88]:

Wet weight result = Dry weight result x (100 − % moisture)/100

Table 3. Mean concentration of fluoride in fresh garlic granules, ginger rhizome, thyme leaves, and basil leaves (mean ± SD, mg/kg wet weight basis, n = 9).

Spice type	Mean ±SD F^- level (mg/kg)
Garlic granules	0.5 ± 0.02
Ginger rhizome	0.51 ± 0.02
Thyme leaves	1.42 ± 0.02
Basil leaves	0.33 ±0.007

4.4. Daily intake of fluoride by an adult person through spices

Total fluoride intake (TFI) is the summation of the daily fluoride intake through entire food sources. Daily intake of fluoride (DIF) from the particular food was calculated by multiplying the fluoride concentration of the respective item with total quantity of the particular item consumed per day. The total fluoride intake (TFI) can be calculated as follows [16]:

$$TFI = \sum (DIF)$$

$$DIF = FC \times QF$$

Where: FC is the fluoride concentration in the food source; and QF is the quantity of the particular food item intake per person per day. The quantity of a particular food item intake per day has been calculated by multiplying the amount of food item taken in a day (kg) with frequency of consumption in a week and divided by the number of days in a week.

QF = [Amount of a particular food item consumed (kg) x Frequency of consumption in a week]/number of days in a week [16].

In this study, daily intake of fluoride from spices was calculated based on those spices which are consumed regularly and did not include other spices which are infrequently consumed. The average amount (g) of a particular spice item consumed by an adult was suggested and daily intake of fluoride by adult person from spices was calculated and presented in Table 4. Most Ethiopian adult people consume red chili pepper, cinnamon bark, and turmeric rhizome in dried form whereas they commonly consume garlic granules and ginger rhizome as fresh or dried. In this study, the daily intake of fluoride from these five spices was calculated based on the dried weight. The wet weight of fresh ginger and garlic which are consumed by people were converted into dried weight. Since people have used dried garlic and ginger as a supplement of shiro and pepper flours, the amount of these spices used in red pepper and shiro flours were considered when the daily intake of fluoride was estimated.

The recommended daily intake of fluoride (RDIF) from all sources for both children and adults, including pregnant and lactating women is 0.05 mg/kg body weight. For pregnant and lactating women, the adequate fluoride intake is based on their body weight before pregnancy and lactation [72, 73].

This value has been extensively used as a reference to estimate the risk of fluorosis from spices or the relative contribution of fluoride intake from spices. For an adult of 60 kg body weight, the recommended daily intake of fluoride (RDIF) is 3 mg. The percent of relative daily intake of fluoride (% RDIF) from a particular spice can be calculated as follows [17]:

$$\% \text{ RDIF} = (\text{DIF}/\text{RDIF}) \times 100$$

In this study, the daily intake of fluoride was estimated if one adult consumed the suggested amount of a particular spice indicated in Table 4. The percent of relative intake of fluoride was also presented for an adult of 60 kg body weight.

Table 4. Daily intake of fluoride for adult person from different spices.

Spice type	Average amount of spices consumed by an adult (g/day)	Frequency of consumption in a week	Average amount of spice consumed (g/day)	Concentration of fluoride in spice (mg/kg)	Fluoride intake through spices (mg/day)	% Relative daily intake of fluoride
Red chili pepper	20	7	20	3.1	0.06	2
Garlic granules*	2.31	6	1.98	3.27	0.006	0.2
Cinnamon bark	1.5	5	1.07	5.13	0.005	0.17
Turmeric rhizome	4.5	4	2.57	3.29	0.008	0.27
Ginger rhizome*	1.83	4	1.05	2.81	0.003	0.1
Total					0.082	2.74

*An adult normally consumes 15 g fresh garlic granules and 10 g fresh ginger rhizome which correspond to 2.31 g and 1.83 g dry wt, respectively. The moisture contents of fresh garlic granules and ginger rhizome were found 84.6% and 81.7%, respectively.

In this study, the daily intake of fluoride from spices for an adult of 60 kg weight was found between 0.003 mg (ginger rhizome) and 0.08 mg (red chili pepper). The contribution of red hot pepper, garlic granules, cinnamon bark, turmeric rhizome, and ginger rhizome in fluoride intake of adults per day were 2, 0.2, 0.17, 0.27, and 0.1%. For an adult who consume all five spices listed in Table 4 daily (which is most commonly the case), he may ingest 0.082 mg or 2.74% of fluoride from spices alone. That means the source of 2.74% of fluoride out of the recommended fluoride level (3 mg F^-/day) was spices used in the food.

4.5. Comparison of results

4.5.1. Comparison of fluoride levels in spice samples found in this study

As indicated in Table 2, fluoride levels in the selected spices were found in the range of 2.14 mg/kg (coriander seed) to 8.57 mg/kg (thyme leaves). The order of fluoride level in spices obtained in this study was thyme leaves > black cumin seed > cinnamon bark > nutmeg seed > turmeric rhizome > garlic granules > fenugreek seed > red hot chili pepper > ginger rhizome > basil leaves > coriander seed. Coriander seed has the lowest fluoride level (2.14 mg/kg) while thyme leaves has the highest fluoride level (8.57 mg/kg). Such variation of fluoride levels among selected spice samples may be due to the heterogeneity of spices; variation of minerals (Al, Ca, Mg, Fe, and F^-) accumulated in soil and irrigated water; variation of agrochemicals (fertilizers, pesticides, and herbicides) used during cultivation [8]. Fluoride levels in spices highly depend on the levels of fluoride in the soil and irrigated water. The use of phosphate fertilizer during cultivation also has high contribution on the fluoride levels in spices. The comparison of fluoride levels in the spices is presented in Figure 6.

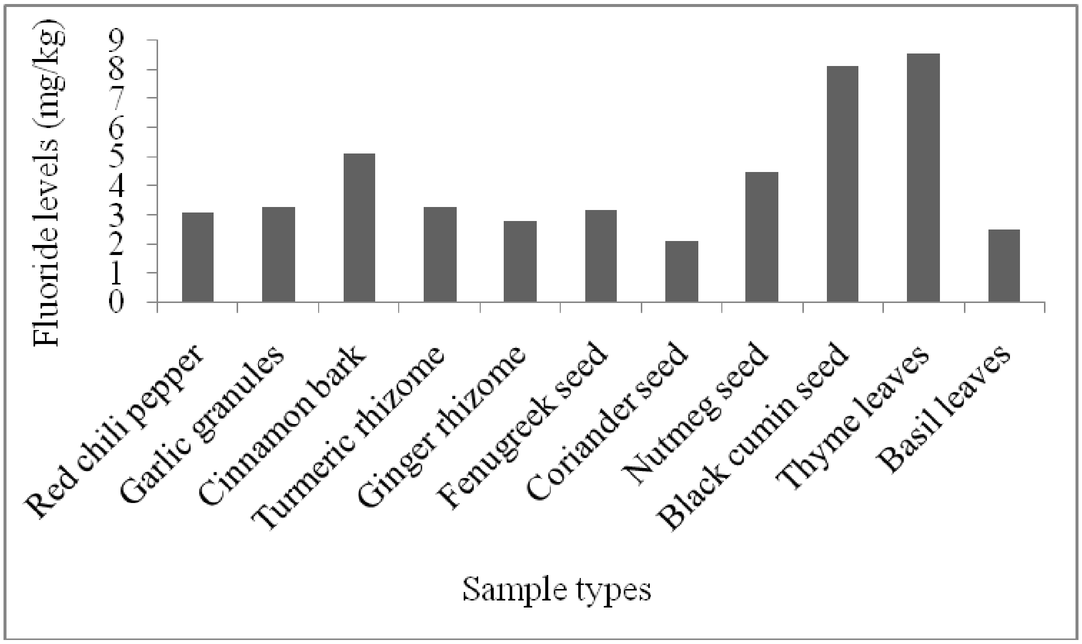

Figure 6. Comparison of mean fluoride levels (mg/kg dry weight basis) in spice samples.

4.5.2. Comparison of fluoride levels of this study with literature values

Some investigators have studied the levels of fluoride in spices cultivated in different countries. The levels of fluoride in the spices determined in this study were compared with reported values in different literatures as presented in Table 5. Šucman *et al*. [44] found that the fluoride levels in red hot pepper, chili, basil leaves, garlic granules, and cinnamon bark cultivated in Spain, China, Egypt, Spain, and Mexico respectively are 7.82 mg/kg, 8.13 mg/kg, 8.85 mg/kg, 8.73 mg/kg, and 6.95 mg/kg, respectively. The reported values are higher than the fluoride levels found in this study (3.1 mg/kg, 2.53 mg/kg, 3.27 mg/kg, and 5.13 mg/kg) respectively. This variation of fluoride levels may be due to the variation of analytical method used, differences in levels of fluoride and other minerals like Al, Ca, Mg, Fe, and F^- in soil, irrigated water, and atmosphere. The variation of agrochemicals like fertilizers, pesticides, and herbicides used during the process of cultivation may also cause the variation of fluoride contents among similar spices cultivated in different countries even among spices cultivated in different areas of the same country. The fluoride containing industrial dusts, mining activities, and burning of coal increase the fluoride levels of spices in industrialized countries. In the other studies in Ethiopia, Dessalenge and Zewge [16] found that the levels of fluoride in pepper flour and green pepper were 18 mg/kg and 4.9 mg/kg, respectively. These reported values are higher than the fluoride level of red chili pepper (3.1 mg/kg) found in this study. This variation of fluoride content may be due to since pepper flour which is collected from the households may be contaminated with other spice flours during the grinding process. Mahapatra [9] reported the fluoride level in garlic granules cultivated in Orissa (India) is 5 mg/L. He also studied the levels of fluoride in turmeric (3.3 mg/kg), ginger (2.0 mg/kg), coriander (2.3 mg/kg), and cumin seeds (1.8 mg/kg). Fluoride levels in turmeric and coriander reported by Mahapatra are comparable with the levels of fluoride found in this study. Fluoride concentration in ginger reported by the author is somewhat comparable with fluoride level in ginger (2.81 mg/kg) found in this study, whereas level of fluoride in cumin seed reported by the same author is lower than the present value (8.14 mg/kg). In the other study, Khandare and Rao [89] found that the level of fluoride in coriander cultivated in India was 3.97 mg/kg which is higher than the present value (2.14 mg/kg). Fluoride levels in fenugreek leaves (18.2 mg/kg) reported by Gautam *et al.* [90] in India is higher than fluoride level in fenugreek seed found in this study (3.18 mg/kg). In addition to the reported reasons for variation of fluoride levels among spices, the variation of fluoride level in fenugreek may be also due to differences in the plant parts since Gautam *et al.* analyzed fluoride in fenugreek leaves whereas in this study fluoride was determined in fenugreek seed. Because all parts of the plant do not accumulate equal amount of fluoride level.

Since there is no any reported fluoride levels in thyme leaves and nutmeg seed, the levels of fluoride found in this study were not compared with other reported values. In general, the levels of fluoride in some spices found in this study are comparable with literature values and the comparison is presented clearly in Table 5.

Table 5. Comparison of fluoride levels in spices with literature values.

Spice type	F^- concentration (mg/kg dry wt.)	Origin	Reference
Pepper flour	18	Ethiopia	[16]
Green pepper chili	4.9	Ethiopia	[16]
Chili	8.13	China	[44]
Red hot pepper	7.82	Spain	[44]
Red chili pepper	3.1	Ethiopia	Present study
garlic granules	8.73	Spain	[44]
Garlic	5	India	[9]
Garlic granules	3.27	Ethiopia	Present study
Cinnamon	6.95	Mexico	[9]
Cinnamon bark	5.13	Ethiopia	Present study
Turmeric	3.3	India	[9]
Turmeric rhizome	3.29	Ethiopia	Present study
Ginger	2	India	[9]
Ginger rhizome	2.81	Ethiopia	Present study
Fenugreek leaves	18.2	India	[90]
Fenugreek seeds	3.18	Ethiopia	Present study
Coriander	3.97	India	[89]
Coriander	2.3	India	[9]
Coriander seeds	2.14	Ethiopia	Present study
Black cumin	1.8	India	[9]
Black cumin seeds	8.14	Ethiopia	Present study
Basil	8.85	Egypt	[44]
Basil leaves	2.53	Ethiopia	Present study

4.6. Statistical analysis

4.6.1. Analyses of variance

All measurements were measured in triplicate and the results were recorded as mean ± standard deviation (SD). The variation between the mean fluoride levels in spice samples was tested. T-tests and analyses of variance (ANOVA) are widely used to check the presence of significance difference at 95% confidence level between mean fluoride levels in spice samples and it tells whether the source for fluoride variation was random error during sampling and measurement or treatments and heterogeneity among the samples. One-way ANOVA was used to compare the means among samples, whereas independent sample t-test was used to compare the means of two groups. The statistic analysis in this study was done by use of SPSS 20.0 Window Evaluation Version program. One-way ANOVA results (Table 6) showed that there exist statistically significant differences ($p < 0.05$) at 95% confidence level among mean fluoride levels (mg/kg dry wt.) of studied spices.

Table 6. Analysis of variance (ANOVA) between and within spice samples at 95% confidence level.

Comparison	Sum of Squares	Mean Square	Df	F_{cal}	F_{crit}	Remark
Between groups	441	44.1	10	1,297	1.94	Significant difference between sample means
Within groups	2.95	0.034	88			

Df = Degree of freedom, F_{cal} = F calculated, F_{crit} = F critical.

The mean fluoride levels of spices were also compared by using t-test and non-significant difference ($p > 0.05$) at 95% confidence interval was observed in fluoride concentration for the following pair-wise analysis: red hot chili pepper, fenugreek seed; red chili pepper, garlic granules; garlic granules, fenugreek seed; turmeric rhizome, garlic granules; and fenugreek seed, turmeric rhizome paired groups, whereas any other pair-wise combinations other than the mentioned groups have showed significant difference ($p < 0.05$) at 95% confidence level in fluoride concentration.

The variation of fluoride levels in spice samples may be due to heterogeneity of spices; mineral variations (Al, Ca, Mg, Fe, F^-) available in soil and irrigated water; variations in agrochemicals like fertilizers, pesticides, herbicides, etc. used during cultivating processes. In the other hand, the cause of non-significant different mean fluoride levels in the mentioned pair-wise spice sample groups may be due to the presence of similarities in climatic conditions, soil type, and water for irrigation.

5. CONCLUSION

In this study the levels of fluoride in 11 most widely consumed traditional Ethiopian spices were determined. The levels of fluoride in studied spices were (in mg/kg dry wt.) found in the order: thyme leaves (8.57±0.11) > black cumin seed (8.14±0.15) > cinnamon bark (5.13±0.23) > nutmeg seed (4.51±0.12) > turmeric rhizome (3.29±0.07) > garlic granules (3.27±0.13) > fenugreek seed (3.18±0.03) > red chili pepper (3.10±0.07) > ginger rhizome (2.81±0.13) > basil leaves (2.53±0.05) > coriander seed (2.14±0.04). Analysis of variance showed that there was statistically significant difference at 95% confidence level in the mean levels of fluoride among the spice samples. It was also found that the daily intake of fluoride from red chili pepper, cinnamon bark, turmeric rhizome, ginger rhizome, and garlic granules for an adult (60 kg body weight) who consume all of these spices per day was 0.082 mg/kg or 2.74% out of the recommended fluoride level (3 mg F^-). This shows that spices play a significant role in daily intake of fluoride for adults.

Fluoride levels in some spices found in this study were comparable with literature values but fluoride in some spices was not comparable with literature values. The variation of fluoride levels in the same spices cultivated in different countries was may be due to physical and chemical characteristics of the soil and irrigated water.

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