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# Determination Of Iron, Cobalt, Chromium And Copper Metals In Commercially Available Khat (*Catha Edulis Forsk*) In Arba Minch, Ethiopia

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**Abstract**
Khat (Catha sedulous foresk) is a plant that grows in a certain area of east Africa and Arab peninsula. A large number of peoples in Ethiopia chew chat leaves because of its pleasurable and stimulating effects. In the present study the levels of selected trace metals (Cu, Co, Cr and Fe) in two different kinds of Khat sampled from different khat shops in Arba Minch were analyzed.
The discern weights of oven dried khat samples were digested by wet digestion using 3ml of $HNO_3$, 1ml of $HClO_4$ and 1ml of $H_2O_2$ by setting the temperature first to $60^oc$ for 30 min and then increased to $210^oc$ for the next 2h and 30 min. The content of minerals in the digests was analyzed using flame atomic spectrometry. The concentration of the trace metals in dry weight (µg/g) were obtained in the khat samples Co 5µg/g, Cu 4µg/g, Fe 2.9µg/g and Cr 3µg/g. Among the metal content analyzed Co was the most abundant one followed by Cu and Cr whereas Fe was less abundant metal in khat sample that grown in Arba Minch area.
**Key words**: flame atomic spectrometry, khat (*sedulous foresk*) and wet digestion method.

## I. INTRODUCTION

Chat (*Catha sedulous forsk*) is the plant that grows in certain area of east Africa and the Arab peninsula .This chat is one of the types of plant whose leaves and steam trips are chewed for their stimulating effects .The chewing of chat leaves is widely practiced in east Africa and the parts of the middle east .Such as Yemen where it forms deep rooted social and cultural functions [1].This habit has now spread to tribal communities in the rest of the world. Including Britain such as Somali communities in south Wales and London [2].The pleasure derived from Chat chewing is attributed to the euphoric action of its content of cathinone Asympathomimetic amine with properties described as similar to those of Amphetamine. Under the misuse of drug act in 1971 Chat is not controlled and its possession and use are not restricted in the U (2). Among the various compounds in the plant two phenyl alkyl amines. Namely cathine and cathinone seem to account for most of the stimulating effect when ingested users get feeling of well being mental alertness and excitement then after effects are usually in Somalia numbness and lack of concentration (1.2). It can be grown rained fed and irrigated through the later covers less than 20 percent of the total chat area the crop could be planted both in home garden and in the field(8).

The leaves and tender twigs are chewed in certain countries of east Africa and the Arabian Peninsula mainly Yemen for their central nervous system stimulating properties. The habit of chewing khat has been common for many centuries. The earliest records with more factual bases showed the Arabic source indicated the ancient use of khat. Historically khat has been used for medicinal purposes as well as a phrodisial; through it was also used for recreational purposes. It is used for avoiding sleepiness, euphoriatic effects, to boast efficiency of work and to increase sexual performance. Chewing khat is the most common mode of administration; it has been taken as a tea and occasionally smoked .In Ethiopia, processed leaves and roots are used to treat influenza, cough, gonorrhea, asthma and other chest problems. The roots are used for stoma cache and an intrusion made from them is taken to treat boils. Local cultivators of khat were described based on geographical location, growth habit and physical appearance that mean color of the leaf, steam sizes and potency of effect. It has been known for a long time that different kinds of khat have different degrees of pharmacological action. Different types of marker systems have been used for genetic analysis and genotyping including morphological, cytological, biochemical and DNA markers (5).

The most favorable part of the plant is leaves, particularly the young shoots near top of the plant. However leaves and steams at the middle and lower sections are also used. Chat is chewed for its stimulating property. This is the presence of the phenyalalkylamine. In Ethiopia khat is grown in most part of the country. There is an ever growing demand both for domestic consumption and for the export

market. Ethiopia is exporting khat to the neighboring and the Middle East countries and in recent years the market for khat has grown to Europe and America. Now a day chewing khat is a common practice among many individuals of all age levels of the country and its use is socially sanctioned and even prestigious. Depending up on the type of khat, availability on the market and nature of the person, up to 500g of fresh edible portion of the leaf can be chewed per day per individual. The amount of khat chewed may increase in certain occasions (4).

Some oral traditions claim that chat originated from Yemen. However the literature indicates that chat originated from Ethiopia, Yemen and other parts of the world (6). Although khat is relatively low risk drug, it associated with an increase for variety of medical completion include dental disease and mouth cancers, heart problems, liver disease, sexual issues, constipation, sleep problems and reduced appetite. It is unclear whether the health risks associated with khat use are directly to the drug cathinone to consumption of catteinated drinks that intensity the high or if they are partially related to inhalation of second hand smoke in poorly ventilated chewing houses. Some problems may be the result of toxic pesticides which users do not wash off prior to chewing because they believes washing the leaves will reduced their potency. Mental health problem associated with khat use including psychosis symptoms are documented but not will understand. Khat use has also been associated with symptoms of depression mood, swings and violent behavior and it can lead to relationship and social problems especially when khat use takes men away from their families for extended periods and when large amount of money are spent on the drug.

## II. LITERATURE REVIEW

### 2.1. Plant Origin, History and Geographic Distribution

According to existing tradition the use of chat was first discovered by a herder who noticed the effect of the plant on his goats and who tried it and experienced wakefulness and added strength. The distribution of chat in tropical Africa extends from north Arabia to South Africa. In Africa it is well established in Ethiopia, Eritrea, Somalia, etc deposit efforts' of the respective government to discourage its cultivation. In east Africa it growth in the range of 15000-25000 meter above sea level, Outside Africa it is planted in the Arabian Peninsula, Yemen, and Afghanistan etc for consumption and in the USA, UK and France for experimental purposes (11).

### 2.2. The plant

Khat is an evergreen perennial shrub plants belong to the celastraceous family. They are several names for the plants depend on its origin chat Ethiopia qat, Yemen. The dried leaves of chat are known as Abyssinian tea or the Arabian tea(11).Khat usually grows up to 7 meters but occasionally reaches as a high as 15 to 25 meters. The khat plant is polymorphic and the branches have either opposite or alternate leaves. The leaves are 2-5 cm wider and 5-10 cm long. The shape of the leaves euiptical and have started edges. Old leaves are leathery in texture highly polished on their upper surface and deep green in color (7).

The leaf peduncle is around 3-7mm long (12). The leaf odor is faintly aromatic and the taste is astringent and slightly sweet (3).The buds and leaves contain an alkaloid and chewed in a fresh or dried condition as a stimulant. Flowers are small and white. The fruit is smooth and narrow splitting to release narrowly winged reddish seeds when matured. The steam is straight and slender. The bark has different colors depending on the variety and age of the steam and branches. The young branches are smooth and green to pinkish but grey and sometimes rougher and darker on older branches and seams. The root systems can grow as deep and as long as 3-5m (9).

### 2.3. Distribution in Ethiopia

The total area of land under chat cultivation in Ethiopia in the year 1997/98 was estimated at 78,570 hectare(13) oromia, mainly east and west harerghe zones is the most important center of khat production (east harerghe zone alone contributes 53.4 percent of the total production area) in Ethiopia. Harerghe is considered to be the most important product of the quality chat in the world (14).Despite silent support and objection against the crop by development institutions. Khat is cultivated and expanding in different parts of Ethiopia. It can be grown rain fed and irrigated through the cater covers less than 20 percent of the total khat area. The crop could be planted both in home garden and in the field (8).

Now a day khat farmers are practicing use of paste chemicals and fertilizers to protect the plant from pastes and to increase the yield of their product in addition to this, they sometimes use traditional pastes control mechanisms. There is an extensive literature about khat, providing information about its history, chemistry and pharmacology and exploring the social, economical psychological and oral aspect of its use. Despite this literature studies that have investigated its mineral nutrients are much less than one may expect. Thus khat is becoming more popular all over the country and other parts of the world. Having detail documents focusing on minerals is very important to evaluate the total mineral intake of the individual who is using this plant regularly. The mild-stimulant plant commonly known as khat has a history of at least seven centuries in Ethiopia. Its chemical, medical and pharmacological aspects are emphasized in the global literature where as studies

on its economic, social, cultural and ecological aspects have received a little attention. The plant is an important aspect of the agricultural, social and religious lives of many Ethiopians especially the Oromo of Harare and its cultivation has expanded as a result opening up with increasing land shortage and drought. There is already some concern as it has been taking away land previously used for the production of food crops and coffee. Its rejection as a narcotic in some countries may also pose threat to the livelihood of many growers and traders but there has been little concern about this so far. Employing an anthropological perspective, this monograph explores and discusses in detail these aspects of khat in Ethiopia. It is useful to professionals, policy makers and other interested parties in the fields of production, distribution and consumption of khat and affecting programmed social change about the khat culture (10).

**2.4. Soils and topography**

Soil with high clay content is not suitable for chat production. The crop requires well drained dark red brown ,sandy loam with a low percentage of clay and medium to high amounts of total nitrogen organic matter, available phosphorous , potassium and magnesium(15). Khat performs best on soils with a ph of 6 to 8.2.Neverthless once established chat grows well under a wide range of soil types and climatic conditions. The optimal altitude for growing khat ranges from1500-2100m.In Ethiopia khat is extensively grown and thrives best in mid land (1500-2500) m but it can also be grown with irrigation down to 1000m if the area is free of forest. At the early development stage of the plant, water supply is more critical than soil type. The field should be well matured and drained for good crop performance (8).

**2.5. Use**

**Medicinal use:** Processed leaves and roots are used to treat influenza, cough, gonorrhea, asthma and other chest problems. The root is also used for stomach ache and an infusion is taken orally to treat boils.

**Social value:** khat has considerable social value. It is served to welcome and entertain guests, in Mourning, weddings and circumcision ceremonies and collective lab our works. Khat chewing has its own associated ceremonies like smoking of incense, cigarettes and use of drinks (soft drinks, tea and milk. Khat chewing is addictive and has negative physical, economical and social connotations. Although non-users both in rural and urban areas condemn the practice of chewing, the number of people chewing is increasing particularly among the youth. In urban areas, chewing khat is a common leisure activity which, combined with the consumption of it, followed by alcohol is chewing an adverse effect on family life. Students and a number of staff in higher education institutions and high schools are using khat to increase the concentration levels and attention span. Some of the farmers consulted said that khat a great deal of agricultural and other hard work which they say, would otherwise be impossible. Khat chewing enables them to accomplish the work without fatigue. They also cited the additional advantage of a reduced appetite in food shortage periods. It is used for obesity and gastric ulcers, and as stimulant to offset depression and fatigue. Its efficiency for this indication is unproven because of insufficient reliable data.

**Environmental value**: In Ethiopia khat is grown in an intensive production system. It is planted in rows on hillsides along terraces in association with different food crops, mainly annuals, and Oriented against slopes. As such, khat cultivation plays key role in controlling soil erosion, which is a major threat in most area of Ethiopia due to the undulated topography and intensive deforestation for farmland expansion and hence khat culture is considered to be the best Agro forestry system practiced by farmers. Had it not been for the cultivation of khat, the erosion of topsoil would have been severe and possibly disastrous in few area of Ethiopia.

**2.5. Chemical Aspect of Khat**

**2.5.1. The Chemical profile of khat**

The leaves and young shoots of *Catha edulis*, a species of the plant family Celastraceous, are usually referred to as khat (Family: *Celastraceous*, genus: *Catha*, and Species *Catha edulis*).Most Taxonomists consider that the genus Catha consists of the single species Catha edulis. The environment and climate condition determine the chemical profile of khat leaves. In Yemen Arab Republic about 44 different types of khat exists originating from different geographic areas of the country. Khat contains a lot of chemical components that may have different effect on the body system. Such as, alkaloids, terpenoids,flavonoids sterols,glycosides,tannins,more than 10 amino acids including tryptophan, glutamic acid, glycine, alanine and threonine, trace quantities of vitamins including ascorbic acid, riboflavin, niacin, and carotene, negligible amount of Fluoride and Elements including copper, Zinc, and toxic metals like lead and cadmium.

Most of our present knowledge on the constituents of khat is derived from studies in the late 1970s and 1980s following recommendation by the UN Commission on Narcotic Drugs. The Phenylalkylamines and the cathedulins are the major alkaloids. Szendrei (1980) at the UN Narcotics Laboratory, together with Schorno and Steinegger at the University of Berne, Switzerland, isolated and

identified the phenylalkylamine, aminopropiophenene, later Named as cathinone as a major active constituent in fresh khat. The plant contains the enantiomer of cathinone only which has the same absolute configuration as Amphetamine. During maturation, cathinone is enzymatically converted to cathine [norpseudoephedrine] and \ norephedrine. Sunlight-induced or heat-induced Degradation to cathine and norephedrine also occurs during extraction of cathinone in the laboratory. Indeed, to slow down the degradation process, the chat leaves are usually wrapped in banana leaves immediately after picking to retain their moisture.

## III. MATERIALS AND METHODS

### 3.1. Sample size and Sampling

The samples were gathered from Gamo Goffa zone of Arba Minch city, secha and siklla because most of the khat sold in Arba Minch was distributed form these places. Two samples of different khat varieties (Gidolie and conso) were collected by using the plastic bags from the chat shop. Then the two samples were mixed and stored in a pre-cleaned polyethene bags. The collected samples were washed thoroughly with distilled water before analysis. The consumable part of the khat plant (as judged by consumers) was collected.

### 3.2. Materials and Apparatus

Ceramic pestle and mortar were used to ground and homogenized the khat sample. A drying oven was used to dry chat samples. A digital analytical balance with 0.0001g precision was used to weigh chat samples. A100ml round bottom flasks fitted with reflux condensers were used in Kjeldahl apparatus hot plate to digest the dried and powdered khat samples. Flame Atomic absorption spectrophotometer was used for the determination of all the metals in the samples.

#### 3.2.1. Apparatus and instruments

Apparatus such as volumetric flasks, measuring cylinder and digestion flask were washed with detergents and tap water, rinse with deionized water soaked in 8% nitric acid for 24 hr , rinsed with deionzed water five times, dried in oven and keep in dust free place until analysis begins. FAAS used to detect the metals in the samples.

#### 3.2.2. Chemical and Reagents used

Reagents that were used in the analysis were all analytical grade. (72%) $HNO_3$, (70 %) $HClO_4$ and (30%) $H_2O_2$ were used for the digestion of chat samples. Stock standard solutions containing1000mg/L of the metals Co, Cr, Cu and Fe were used for the preparation of serious calibration standards and in the spiking experiments. Deionized water was used throughout the experiment for sample preparation, dilution and rinsing apparatus prior to analysis.

#### 3.2.3 Preparation of reagents

Working solutions are prepared by further dilution of all the reagents and solvents of analytical reagent graded. There are two stock standard solutions prepared for the determination of metal analysis from the samples. These are primary and secondary stock solutions. The stock solutions are prepared for each metal determination by the dilution of the metal piece and its salt in deionized water and nitric acid. When making mixed calibration standards there may be convenient to prepare secondary stock standard solutions containing 1000mg/ml of the element of interest.

### 3.3. Experimental Procedure (Method)

#### 3.3.1 Sample preparation

Two types of chat vegetables were randomly purchased from different shops. The markets were located at Arba Minch .The chat were washed with distilled water to remove dust particles and then cut to separate the root, stems and leaves using knife .The sample was dried in an oven at a temperature between 60-70$^o$c until the constant weight was achieved. Then it was crushed in mortar and pestle and sieved through a 2 mm sieve. The sieved sample was transferred to plastic container until they were used for digestion. The dried chat sample was protected from sun light and weighted.

#### 3.3.2 Digestion of the sample

0.5g of dried and homogenized khat samples were transferred into a 150mL round bottomed flask. 5mL of a mixture of $HNO_3$ (72%) $HClO_4$ (70%) and $H_2O_2$ (30%) were added to the flask with a volume ratio of 3:1:1 and the mixture was digested on a micro Kjeldahl digested apparatus by setting the temperature first to $60^0$c for 30 min and then increased to 210 $^0$c for the next 2 h and 30 min. Then digested solution was allowed for 5 min without dismantling the condenser from the flask to cool and for 10 min after removing the condenser. To the cooled solution and dissolve the precipitate formed 8 mL of deionized water was added to cooled and minimize dissolution of filter paper by the digest residue. Then the solution was filtered with Whatman filter paper. The round bottom flask was renised with 8mL deionized water and the solution was filled with deionized water to the mark (50ml). Three separated digestions of the samples were carried out. The digested samples were kept in the refrigerator, until the level of all the metals in the sample solution were determined by FAAS. The blank solutions were prepared followed by the same digestion procedure as the sample.

**3.3.3 Determination of the concentration of metals in the sample solution**

Secondary standard solutions containing 10 mg/L were prepared from standard stock solutions that contained 1000 mg/L. These secondary standards were diluted with deionized water to obtain four working standards for each metal of interest. Cu, Co, Cr and Fe were analyzed with FAAS (BUCK SCIENTIFIC MODEL 210GP) equipped with deuterium arc background corrector and standard air-acetylene flame system using external calibration curve after the parameters (burner and lamp alignment, slit width and wavelength adjustment) were optimized for maximum signal intensity of the instrument. Three replicate determinations were carried out on each sample. Hallow cathode lamp for each metal operated at the manufacturer's recommended conditions were used at its respective primary source line. The acetylene and air flow rates were managed to ensure suitable flame conditions. The four elements were determined by absorption/concentration mode. The same analytical procedure was employed for the determination of elements in six-digested blank solutions. The operating conditions for FAAS employed for each analyte are given in Table 1.

**Table 1.** The Instrumental operating condition for the determination of metals in Khat Samples using flame atomic absorption spectrophotometery.

| Element | Wavelength (nm) | Slit width (nm) | Lamp current (mA) | Energy |
|---|---|---|---|---|
| Cu | 324.7 | 0.7 | 1.5 | 3.775 |
| Fe | 248.3 | 0.2 | 5.0 | 3.321 |
| Cr | 357.9 | 0.7 | 2.0 | 3.623 |
| Co | 240.7 | 0.2 | 4.5 | 3.338 |

## IV. Results and Discussion

**4.1 Instrument calibration**

The qualities of results obtained for Cu, Fe, Co and Cr metals analysis using FAAS are seriously affected by the calibration and standard solution preparations procedures. The instrument was calibrated using four series of working standards. Concentrations of the intermediate standards, working standards and value of correlation coefficient of the calibration graph for each of the metals are listed in Table 3. The calibration graph of each of metals of interest is shown in Fig 3.

**Table 2** Working standards and correlation coefficients of the calibration curves for determinations of metals using FAAS.

| S. No. | Metal | Concentration of intermediate standard (mg/L) | Concentration of standards, in mg/L | Correlation coefficient of calibration curves |
|---|---|---|---|---|
| 1 | Cu | 10 | 0.1, 0.2, 0.8,1.2 | 0.947 |
| 2 | Fe | 10 | 0.1, 0.2, 1.0,1.2 | 0.950 |
| 3 | Co | 10 | 0.1, 0.5, 1.0,1.2 | 0.986 |
| 4 | Cr | 10 | 0. 1, 0.6,0.9, 1.2 | 0.993 |

The calibration graph of the four trace metals standard solution were drown by using the experimental and standard solution data and the unknown concentration of each metals determined using the slope equation from the calibration graph.

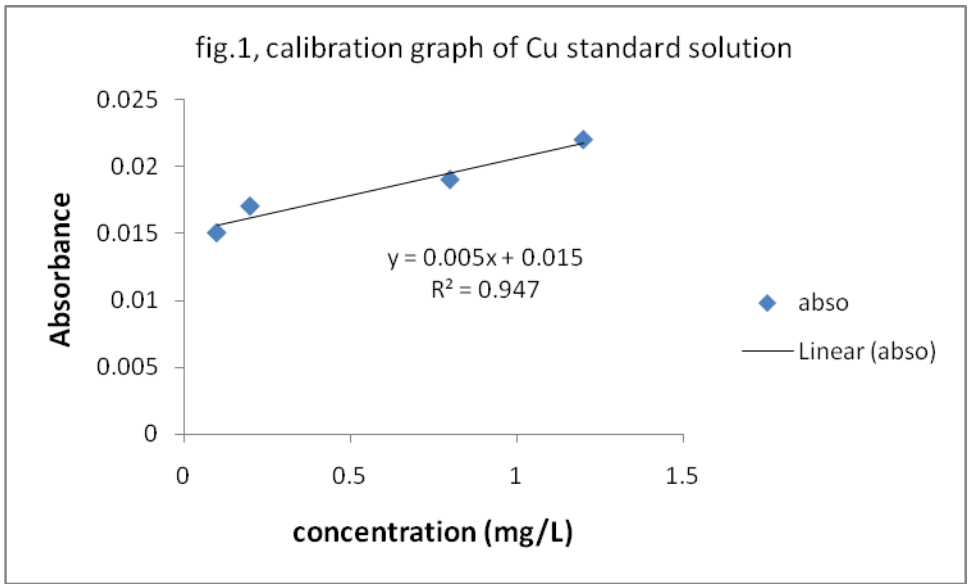


fig.1, calibration graph of Cu standard solution
Absorbance
0.025
0.02
0.015
0.01
0.005
0
y = 0.005x + 0.015
R² = 0.947
abso
Linear (abso)
0
0.5
1
1.5
concentration (mg/L)

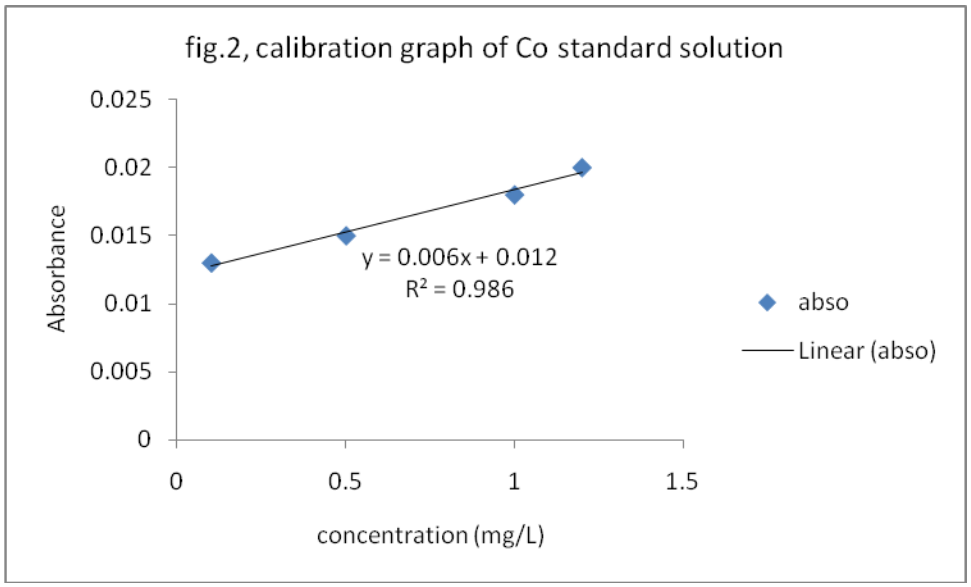
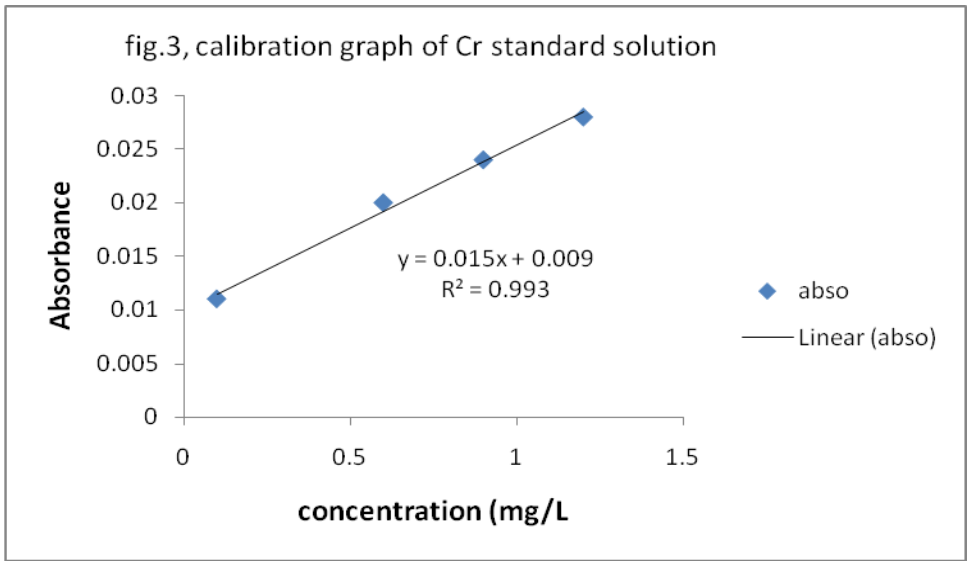
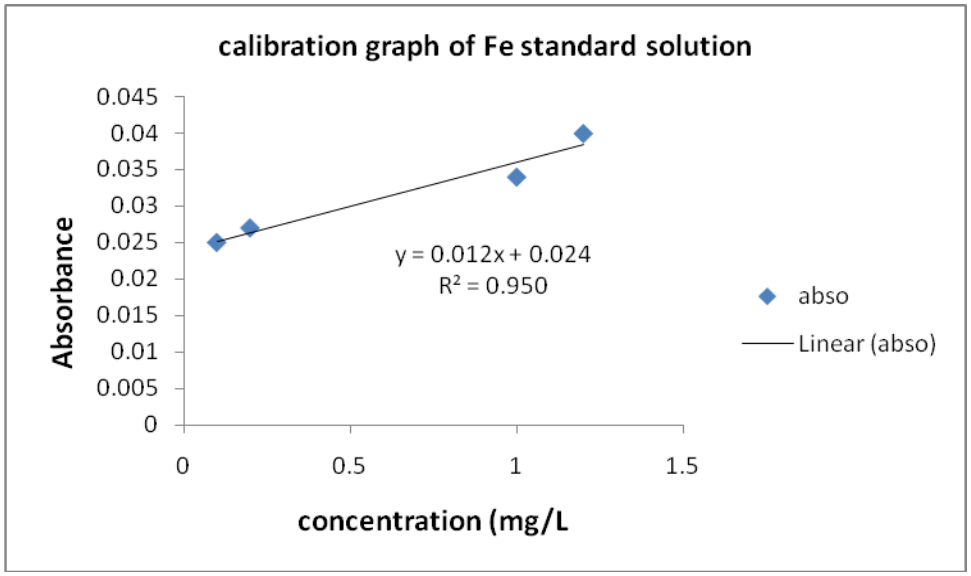


fig.2, calibration graph of Co standard solution
Absorbance
0.025
0.02
0.015
0.01
0.005
0
y = 0.006x + 0.012
R² = 0.986
abso
Linear (abso)
0
0.5
1
1.5
concentration (mg/L)




Those calibration graphs are important to determine the unknown concentration of the metals that found in the plant. The unknown concentration the metals were determined from the graph and the metal cobalt has the highest concentration followed by cupper, chromium and iron in Arba Minch area khat depending up on this determinations using digestion of the leaves. The concentrations of the metals are Co 5µg/g, Cu 4µg/g, Cr 3µg/g and Fe 2.9µg/g that calculated from the graph using the equation of the line.

**4.2. Optimization of Digestion procedure of Khat Samples**

One of the basic requirements for sample preparation for analysis is to get an optimum condition for digestion. The optimum condition is the one which leads: Minimum reagent volume consumption, Minimum digestion time, Minimum residue (clear solution) and Ease of simplicity.

Optimizing of the digestion procedure involved some changes of parameters such as reagent volume, digestion temperature and digestion time. Accordingly, thirteen procedures were tested for digestion of khat (Table 4). Based on the above listed criteria, the optimal digestion procedure chosen was the one that fulfilled the selected criteria for complete digestion of 0.5 g of the dry sample powders, with 3 mL of $HNO_3$ ( 62- 79 %), 1 mL $HClO_4$ ( 70 %) and 1 mL $H_2O_2$ (30 %) for a total of 3 hours. The mixture was digested smoothly by setting the temperature first to 60 $^0$C for 30 min and then increased to 210 $^0$C for the next 2 h and 30 min then after the digested solution was allowed to cool for 5 min without dismantling the condenser from the flask and for 10 min after removing the condenser. The procedures that required higher reagent volume longer digestion time and colored digest solution were rejected

**Table 3** Procedures tested during optimization of method for digestion of khat samples

| S.No. | Sample size | Reagent added | aInitial Temp. | Final Temp. | bDigestion time | Nature of the Digest After Filtration |
|---|---|---|---|---|---|---|
| 1 | 0.5g | 3ml $HNO_3$ (72%)<br>1ml $H_2O_2$ (30%)<br>1ml $HClO_4$ (70) | 60 $^0$C | 210 $^0$C | 3h&15 min | Clear and yellowish color |
| 2 | 0.5g | 3ml $HNO_3$ (72%)<br>1ml $H_2O_2$ (30%)<br>1ml $HClO_4$ (70) | 60 $^0$C | 210 $^0$C | 210 $^0$C | Clear and yellowish color |
| 3 | 0.5g | 3ml $HNO_3$ (72%)<br>1ml $H_2O_2$ (30%)<br>1ml $HClO_4$ (70) | 60 $^0$C | 210 $^0$C | 210 $^0$C | Clear and yellowish color |

**4.3 Levels of essentials Metals in chat**

The concentration of four trace elements (Cu, Fe, Co and Cr) in the digested and diluted solutions of khat were identified with flame AAS. The levels of total metal contents of two kinds of khat samples show that khat were a source of nutrients in addition to it's as a stimulant. The concentration of the metals that determined in the analysis was Co 5µg/g, Cu 4µg/g, Cr 3µg/g and Fe 2.9µg/g. The result shows that out of the four analyzed trace metals Co was found in the large amount compared to other with the concentration of 5µg/g, but Fe was the smallest one with the concentration of 2.9µg/g. The amount of the analyzed metals in the khat samples were arranged in an increasing order of their concentration Fe<Cr<Cu<Co.

## V. Conclusion

In this study commercially available Khat in Arba Minch city were analyzed for their contents of Cu, Co, Cr, and Fe. The optimized wet digestion method for Khat analysis was found effective for all of the minerals and as it was analysis using flame atomic absorption spectrometry. Daily metal intake can be minimized by washing the khat properly and reducing the amount of khat digested. This analysis help to know different minerals that found in the khat plant and to identify which one is essential or non essential elements to consider the consumption of the khat. Cobalt has higher concentration compared to other three metals that analyzed in Arba Minch city khat this study. Generally based on the current status, chewing Ethiopian khat in addition to its stimulating property, it contribute appreciable amount of trace metals for the individuals. Particularly khat could be good sources of Co, Cu and Fe, for individuals who are chewing this plant regularly. In edible portion of khat leaves of khat cultivators grown in different area of Ethiopia using FAAS. The results showed the ability of these plants to accumulate relatively higher amounts of Co, Cu and Fe among the determined trace metals respectively.

It is recommended that a better understanding of the health related effect and chemical profile of Ethiopian khat, be developed through a multidisciplinary approach with the full involvement of khat growers. Because most of Ethiopian farmers use fertilizer and pesticides to get good and attractive crop without considering its side effect on consumers since khat is consumed directly after harvesting. The findings derived from such an approach should then be counter balanced with a realistic understanding of the negative health effect of khat use. Generally speaking, in Ethiopia the number of khat chewers is increasing through time to time due to its economic importance and the available reports simply on negative and positive aspect of khat on economic, social, cultural and agricultural value of the country. In relation to the ever increasing human consumption of khat and the benefit from this crop an overall investigation of its chemical profile and disadvantage remains an immediate issue of scholarly attention.

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