

Tannins acid, Ascorbic acid and Fluoride from Khat Chewing Plant

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Abstract

Background/purpose: Khat (*Catha edulis*) is an evergreen shrub belonging to the Celastraceae family. It grows in Yemen and Southern Arabia as well as in certain East African countries such as Ethiopia, Somalia, Djibouti and Kenya. The aim of this study was to quantify tannins acid, ascorbic acid and Fluoride from khat extract.

Materials and methods: Khat leaves were collected and bought from Yemen market. The samples were dried in an oven at 50–52°C. The samples were stored in a cool, dark and dry place. The dried sample (200 g) was ground first to pass a 2 mm screen. Then, tannins acid was detected using Proanthocyanidins technique. Redox titration (Iodometric titration technique) method was used to determine ascorbic acid in khat extracts. Fluoride in khat extracts was detected using a method based on APHA Standard 4500-F-D.

Results: The Average percentage of tannins acid from khat extracts in dry matter was 25.4%. The average percentage of ascorbic acid extracted from khat in sample=25.72%. Fluoride substance was not determined in khat.

Conclusion: It can be concluded that khat contain high amounts of tannins acid, ascorbic acid and fluoride was not found in khat.

Keywords: Khat; Tannins acid; Ascorbic acid; Fluoride

Introduction

Khat (*Catha edulis*) is an ever green shrub that belongs to family Celastraceae. It grows in Yemen and Southern Arabia as well as in certain East African countries, such as Ethiopia, Somalia, Djibouti, and Kenya [1]. Khat grows especially well in moist conditions and is generally cultivated along the mountains lopes at altitudes of 3500 ft to 7000 ft and varies in height from 3 ft to 15 ft. Khat leaves are habitually chewed by inhabitants in these regions because of their psycho stimulant effect, similar to that produced by amphetamine-like substances.

In 1887, Fluckinger and Gerock first found that khat contains alkaloid. The study of Stok man described three different alkaloids, namely, cathine, cathinone, and cathidine, that are present in khat, as reported by Al-Meshal et al. [2] An analysis of 22 khat samples of different origins shows that, on average, a 100 g of fresh khat leaves contains 36 mg of cathinone, 120 mg of cathine, and 8 mg of norephedrine, but the concentration of these constituents varies within wide limits [3].

Chewing khat has been receiving increasing attention because it increases the risk of cardiovascular diseases, such as high blood pressure and acute myocardial infarction, which is caused by the main constituent cathinone [4]. Ayana et al. [5] investigated across-sectional random sample of 1,000 community subjects in Ethiopia and found that 23% of 306 regular (daily) khat chewers are hypertensive. Hassan et al. [6] reported that khat has reinforcing effects that include insomnia, excitement, or even euphoria. It also has a strong pharmacological effect on people, which is manifested through tachycardia, mydriasis, transient

conjunctival and facial congestion, elevated blood pressure, hyperthermia, headache, increased diuresis if taken in considerable volumes, and increased respiration (through counter regulation of hyperthermia, bronchodilation, and central stimulation) [7].

Khat is associated with several oral conditions, such attrition, staining, dental caries, enlargement of salivary gland and inflammation of parotid duct among the chewers [8], enamel demineralization [9], and mouth dryness [10]. Al-Alimi et al. [11] suggested that khat chewer's saliva exhibit slow saliva flow rate, low pH, and unhealthy viscosity. Rosenzweig and Smith [12] documented an exceptionally high rate of period on oral disease in Yemeni who chews khat. Mengel et al. [13] confirmed the previous findings in a large-scale investigation involving 1,001 Yemeni. They found that the community periodontal index of treatment needs, clinical loss of attachment, and calculus index were significantly higher in khat chewers. However, Al-Hebshi et al. [14] found that khat chewing induces antimicrobial profile that is compatible with gingival health.

Materials and Method

Khat leaves were collected and bought from a Yemen market. The samples were dried in an oven at 50°C to 52°C. The samples were stored in a cool, dark, dry place until use. The dried sample (200 g) was initially ground and filtered through a 2 mm screen. All the ground samples, including those parts remaining inside the mill, were obtained and mixed well. Approximately 100 g of sample was ageing round and filtered through a 0.5 mm screen. The ground samples were stored in separate plastic containers for determination of tannins.

Extraction of tannins from khat extracts

The samples were extracted to quantitatively diffuse the phenolic present in the materials to liquid phase. Aqueous acetone (70%) was used in the extraction process. Each of the dried sample (200 mg) was placed in a glass beaker of approximately 25 ml capacity. Ten milliliters of aqueous acetone (70%) was added into the sample, and the beaker was suspended in an ultrasonic water bath (Branson 3210, USA) and subjected to ultrasonic treatment for 20 min at room temperature. The contents of the beaker were then transferred into tubes and subjected to centrifugation at approximately $3000 \times g$ at 4°C for 10 min in a refrigerated centrifuge (Kaida). The supernatant was collected and maintained on ice. The pellet left in the tube was transferred into a beaker using two portions of 5 ml each of 70% aqueous acetone and again subjected the contents to ultrasonic treatment for 20 min. The supernatant was collected as previously described.

Determination of tannin acids in khat extracts

The proanthocyanidin method described by Porter et al. [15] was used for the determination of tannin acids in the extracts. In brief, butanol-HCl reagent (butanol-HCl 95:5 v/v) was prepared by mixing 950 mL of n-butanol with 50 mL concentrated HCl (37%). Ferric reagent (2% ferric ammonium sulfate in 2 N HCl) was prepared by dissolving 2.0 g of ferric ammonium sulfate in 2 N HCl (16.6 mL of concentrated HCl was made upto 100 ml with distilled water to make 2 N HCl). The reagents were stored in dark bottles. In a 100 mm \times 12 mm glass test tube, 0.5 mL of tannin extract diluted with 70% acetone was pipetted. The quantity of acetone was large enough to prevent the absorbance (550 nm) in the assay from exceeding 0.6. Three milliliters of butanol-HCl reagent and 0.1 mL of ferric reagent were added to the tubes. The tubes capped with glass marbles were shaken using a Vortex and then placed on a heating block adjusted at 97°C to 100°C for 60 minutes. After cooling the tubes, absorbance was recorded at 550 nm. The absorbance of the unheated mixture (considered as a suitable blank) was subtracted from the absorbance of the heated mixture, which was the actual reading at 550 nm to be used for calculation of tannin acids. Development of pink color without heating the sample indicates the presence of flavan-4-ols. If this development occurs, one heated blank for each sample, comprising 0.5 mL of the extract, 3 mL of butanol, and 0.1 mL of the ferric reagent, was used. In three different volumetric flasks (25 mL), a different aliquot of tannin extract (previously prepared) was diluted with 70% acetone to 25 mL. Tannin acids (% in dry matter) as leucocyanidin in equivalent were calculated using the following formula:

$$(A_{550 \text{ nm}} \times 78.26 \times \text{dilution factor}) / (\% \text{ dry matter})$$

This formula assumes that the effective E 1%, 1 cm, 550 nm of leucocyanidin is 460. In this study, the dilution factor was equal to 1 if no 70% acetone was added, and the extract was made from 200 mg of sample in 10 mL of solvent. If 70% acetone is added (for example to prevent the absorbance from exceeding 0.6), the dilution factor was as follows: 0.5 mL/(volume of extract taken). In this study, the dilution factor was 1 as 0.5 mL extract was taken.

Determination of dry matter of tannin acids in khat extracts

Sample (5 g) was placed in a hot air oven maintained at 100°C to 105°C . The sample was dried to a constant weight. The weight of the dried sample was recorded after cooling the sample to room temperature in a desiccator. Dry matter content was calculated by the following formula:

$$\text{Dry matter (\%)} = W_2/W_1 \times 100$$

Where W_2 is the mass (g) of the sample before drying and W_1 is the mass (g) of the sample after drying.

Quantification of ascorbic acid in khat extracts

The redox titration (iodometric titration technique) method was used to determine ascorbic acid in khat extracts. A total of 500 mg of khat extracts from the sample was weighed in a beaker, and 500 mL of distilled water was added. Approximately 0.001 g/mL was obtained, and three concentrations were prepared.

Determination of ascorbic acid in khat extracts

Iodine solution preparation: To prepare 0.005 ml^{-1} , 2 g of potassium iodide and 1.3 g of iodine were dissolved in distilled water and made up to 1 L mark using a volumetric flask. A starch indicator solution (1.0%) was prepared by dissolving 1.00 g of soluble starch in 100 mL of near boiling water. Sample solution was transferred into a 500 mL conical flask, and then 5 mL of starch indicator solution was added. The sample solution was titrated with 0.005 molL^{-1} iodine solution. The end point of the titration was identified as the first permanent trace of dark blue-black color because of the starch-iodine complex that lasted for 20 s. Titration was repeated in triplicate for each sample.

Titration and calculation method of ascorbic acid

The sample solution (20 mL) was pipetted into a 250 mL conical flask and added with 150 mL of distilled water, 5 mL of 0.6 molL^{-1} KI, 5 mL of 1 molL^{-1} HCl, and 1 mL of starch indicator solution. The sample was titrated with 0.002 molL^{-1} (PI) potassium iodide solution. The End point of the titration was the first permanent trace of a dark-black color because of the starch - iodine complex. The titration was repeated with further aliquots of sample solution until concordant results were obtained (titers agreeing within 0.1 mL). The average volume of iodate solution used from the concordant titers was calculated. Then, the moles of iodate that reacted forming iodine were calculated. The following equation was used to calculate the moles of iodine formed by the reaction between the iodate ions and iodide ions.

$$\text{Percentage of ascorbic acid in sample} = \frac{\text{Concentration of ascorbic acid}}{\text{Concentration of sample solution}} \times 100\%$$

From the titration equation, the moles of ascorbic acid reaction were determined. The concentration in ml of ascorbic acid in the sample solution was calculated, and then the concentration in mg/100 mL of ascorbic acid in the sample was calculated according to the following equation:

Quantification of fluoride in khat extracts

Sample pre-treatment (distillation)

In a distilling flask containing 400 mL distilled water, 200 ml of concentrated H_2SO_4 was added, stirred, and heated, and then the distillate was discarded (removal of fluoride contamination). The acid mixture was cooled, and 300 ml of the sample (100 mg/L) was added. Distillation procedure was continued, and distillate was retained for analysis.

Determination of fluoride in khat extracts

Method based on APHA Standard 4500-F-D: From standard fluoride solution, 1000 mg F/L of different concentrations was prepared by 0.5, 1.0, 1.5, 2.0, and 2.5 mg F/L based on dilution. The indicator solution (958 mg) of sodium naphthalene disulfonate was dissolved in distilled water and diluted to 500 ml. Then, 133 mg of zirconium chloride was dissolved in 25 mL of distilled water, and 250 mL of concentrated HCl was added and diluted to 500 mL with distilled water.

Acid-zirconium indicator solution: Equal volumes of indicator and zirconium-acid reagent were mixed. For each of the prepared standard fluoride concentrations, 5 mL of zirconium acid indicator was added. The solution was stabilized for an hour before the absorbance was obtained at

570 nm. The same procedure was performed as described. The fluoride concentration of the samples was obtained by extrapolating the value of absorbance from the calibration curve. The fluoride concentration was measured as mg/L.

Results

Quantification of tannin acid in khat extracts

The proanthocyanidin technique was used to detect tannin acid. The percentage of tannin in dry matter % was determined in three volumes of tannin extracts. Table 1 show that the average percentage of tannin acid from khat extracts in dry matter was 25.4%.

Quantification of ascorbic acid (vitaminC) in khat extracts

The redox titration (iodometric titration technique) method was used to evaluate ascorbic acid in khat extracts. This method determines the concentration of ascorbic acid in a solution by a redox titration with potassium iodate in the presence of potassium iodide. All titrate measurement were carried out after deduction of blank titration (0.7 mL). Table 2 shows that the average percentage of ascorbic acid extracted from khat were 25.72%.

Quantification of fluoride in khat extracts

Fluoride in khat extracts was detected using a method based on APHA Standard 4500-F-D. This colorimetric method was based on the reaction between fluoride and a zirconium-dye lake. Table 3 shows the absence of fluoride in khat using different concentrations.

Discussion and Conclusion

Dried khat leaves contain considerable amounts of tannin. Tannin belongs to phenolic compound, which is commonly found in plant. Tannin is commonly referred to as tannin acid and founding rains, fruits, herbs, and beverages derived from plants. The laboratory finding in this

study showed that tannin acid in dry matter was 25.5%, but other studies reported 12% to 14% [16,7]. Previous studies mentioned that tannin concentration in khat leaves ranges from 3.5 g/100 g to 9.7 g/100 g [17]. The finding of the current study suggested a high concentration of tannin acids, which is in disagreement with that of previous studies. This high concentration of tannins may beat tribute to teeth staining. Brownish discoloration of the mouth is a sign of chronic khat use [18]. Furthermore, Al-Sharabi [4] observed teeth staining among khat chewers.

Khat is associated with a stringency sensation [8]. Higher concentrations of particular saliva proteins and a higher flow rate of saliva generally reduce the sensation of stringency [19]. Interactions between tannin and oral epithelial proteins occur [20] or with taste receptors, particularly bitter receptors in the case of small tannin acids [21], as well as change in saliva Viscosity [22]. The main mechanism behind the loss of saliva lubricity is attributed to the interaction of astringent agents, such as tannins, with salivary proteins and glycos amino glycans (mucopolysaccharides). Furthermore, Al-Alimiet al. [11] found that khat chewers have unhealthy saliva viscosity.

The subsequent aggregation and precipitation of the protein-tannin complexes reduce the lubricity of saliva by increasing friction in the oral cavity [23]. This process causes a drying and grainy sensation in the mouth, which differs based on size and concentration, as well as hardness or softness, of the precipitate [24].

Ascorbic acid concentration from khat extracts in this study was 25.72%. Every 100 g of khat contains 257.20 mg of ascorbic acid. This study is the first to analyze the ascorbic acid from khat extracts since 50 years ago. The ascorbic acid (vitamin C) content of khat was reported to be high; a 100 g of fresh leaves contains 325 mg of ascorbic acid [25]. However, the analysis method was not described. The differences in results may be due to the different types of khat used.

Khat types vary from one country to another and from one region to another. Despite the varied concentrations, khat still contains high

Table 1: Quantification of tannins acid from khat extract

Sample	Volume of tannin extract (ml)	No.	Absorbance (550 nm)	Average Absorbance	Dilution Factor	Percentage of tannins acid (%)
1	0.05	Blank	0.000	-	500	24.9
		(1)	0.066	0.074		
		(2)	0.080			
		(3)	0.076			
2	0.1	Blank	0.000	-	250	25.9
		(1)	0.149	0.154		
		(2)	0.158			
		(3)	0.155			
3	0.3	Blank	0.000	-	83.33	25.5
		(1)	0.450	0.455		
		(2)	0.457			
		(3)	0.457			

Table 2: Ascorbic acid percentage in three different concentrations of khat

Sample	Volume of titrant (I ₂) (ml)	Conc. of ascorbic acid (mg/ml)	Conc. of sample solution (dilution from stock) (mg/ml)	Ascorbic acid (%)	Average Percentage of ascorbic acid (%)
A	3.8	0.06688	0.2	33.44	28.16
	3.3	0.05808		29.04	
	2.5	0.044		22	
B	6.3	0.1109	0.4	27.72	25.52
	5.8	0.1021		25.52	
	5.3	0.0933		23.32	
C	12.4	0.2182	0.1	21.82	23.47
	14.3	0.2517		25.17	
	13.3	0.2341		23.41	

Table 3: Flouride sample analysis in four different concentrations of khat extracts

Sample N	Volume (ml)	Dilution Factor	Absorbance (420 nm)	Average Absorbance	Concentration of Flouride (M)
1	0.5	50	-0.002	-ve	Not determined
			-0.004		
			-0.001		
2	5	5	-0.002	-ve	Not determined
			-0.003		
			-0.002		
3	10	2.5	0.001	-ve	Not determined
			-0.003		
			-0.002		
4	20	1.25	-0.001	-ve	Not determined
			-0.003		
			-0.001		

concentration of ascorbic acid. Furthermore, chewable vitamin C tablets, used daily, have been reported to lead to severe erosion of dental enamel because of the acidity and abrasiveness of these products [26].

Vitamin C possesses a positive function in oral health, but may be a predisposing factor for caries progress among khat chewers because of its high acidity. High acidity of ascorbic acid in khat may damage the hard tissues, particularly enamel. Moreover, the pH of khat extracts is 5.3 [9], which may beat tribute to the high amount of ascorbic acid. The findings of this study suggest that the acidity of khat may be detrimental to the enamel surface. However, in the oral environment, the buffering capacity of saliva may increase the pH of khat. Jensen [27] reported that neutralization of plaque acids by the alkaline buffer system in saliva may take as long as two or more hours.

The presence of fluoride in khat is mentioned in several studies [28,29]. This substance is mainly related to enamel remineralization. Several methods are available to detect fluoride. SPADNS spectrophotometric method is among these methods [30-32], which his based on American Public Health Association Standard 4500-F-D [30-32]. This colorimetric method is based on their action between fluoride and a zirconium-dye lake. Fluoride reacts with the dye lake, dissociating a portion of it into a colorless complex anion (ZrF6²⁻) and the dye. As the amount of fluoride increases, the color produced becomes progressively lighter. This method is considered accurate, specific, and sensitive compared with other techniques, but time consuming. Other techniques are rapid, but limited because of the response to interfering ion and may not provide accurate data. The interesting finding in this study was that fluoride was not determined in khat extracts. The current finding was in disagreement with that of previous studies by Hill and Gibson [28] and Hattab [29]. However, Hattab [29] reported that khat contains negligible amount of fluoride. Furthermore, negative result may be also due to fluoride deterioration in sample because the amount present is in part per million quantities. Further investigation is necessary to confirm this finding. This study revealed that khat contained high concentration of tannin acids and ascorbic acid, but fluoride substance was not found.

We conclude that khat contained high concentration of condensed tannin (25.4%) based on the chemical analysis of extracts. Every 1 g of khat extracts contained 254 mg of condensed tannins. High concentration of ascorbic acid (vitamin C) was also found in khat extracts (25.72%). Every 1 g of khat contained 257.20 mg of vitamin C, and khat did not contain fluoride.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

- Kalix P (1987) Khat: Scientific knowledge and policy issues. *Br J Addict* 82: 47-52.
- Al-Meshal IA, Ageel AM, Parmar NS, Tariq M (1985) *Catha edulis*: use, abuse and current status of scientific knowledge. *Fitoterapia* 17: 131-150.
- Geissshusler S, Brenneisen R (1987) The content of psychoactive phenylpropyl and phenylpentenyl khatamines in *Catha edulis* forsk of different origin. *J Ethnopharmacol* 19: 269-277.
- Halket JM, Karasu Z, Murray-Lyon IM (1995) Plasma cathinone levels following chewing khat leaves (*Catha edulis* Forsk.). *J Ethnopharmacology* 49: 111-113.
- Ayana AM, Sherief HT, Tekli Y (2002) Effect of khat (*Catha edulis* Forsk) on blood pressure and heart rate, a community based study. *Ethiopian J Health Develop* 16: 325-334.
- Hassan NA, Gunaid AA, El-Khally FM, Al-Noami MY, Murray-Lyon I (2005) Khat chewing and arterial blood pressure. A randomized controlled clinical trial of alpha-1and selective beta-1 adrenoceptor blockade. *Saudi Med J* 26: 537-541.
- Halbach H (1972) Medical aspects of the chewing of khat leaves. *Bull World Health Organ* 47: 21-29.
- Al-Sharabi AK (2002) Oral and para-oral lesion caused by qat chewing. PhD Thesis University Khartoum, Sudan.
- Al-Alimi K (2008) Demineralization potential of qat extarcts on enamel and at restoration interface. MDS Sc Thesis, University of Malaya, Kuala Lumpur, Malaysia.
- Yarom N, Epstein J, Levi H, Porat D, Kaufman E, et al. (2010) Oral manifestations of habitual khat chewing: a case- control study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 109: 60-66.
- Al-Alimi KR, Abdul Razak AA, Saub R (2014) Salivary caries parameters: Comparative study among Yemeni khat chewers and non-chewers. *J Dent Sci* 9: 328-331.
- Rosenzweig KA, Smith P (1966) Periodontal health in various ethnic groups in Israel. *J Periodontal Res* 1: 250-259.
- Mengel R, Eigenbrodt M, Schünemann T, Florès de Jacoby L (1996) Periodontal status of a subject sample of Yemen. *J Clin Periodontol* 23: 437-443.
- Al-Hebshi NN, Nielsen Ø, Skaug N (2005) *In-vitro* effects of crude khat extracts on the growth, colonization, and glucosyltransferases of *Streptococcus mutans*. *Acta Odontol Scand* 63: 136-142.
- Porter LJ, Hrstich LN, Chan BG (1985) The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry* 25: 223-230.
- Kalix P (1988) Khat: a plant with amphetamine effects. *J Subst Abuse Treat* 5: 163-169.
- Dhaifalah I, Santavy J (2004) Khat habit and its health effect. A natural amphetamine. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 148: 11-15.
- Luqman W, Danowski T (1976) The Use of Khat (*Catha edulis*) in Yemen Social and Medical Observations. *Ann Intern Med* 85: 246-249.
- Condelli N, Dinnella C, Cerone A, Monteleone E, Bertuccioli M (2006) Prediction of perceived astringency induced by phenolic compounds II: Criteria for panel selection and preliminary application on wine samples. *Food Qual Prefer* 17: 96-107.
- Payne C, Bowyer PK, Herderich M, Bastian SEP (2009) Interaction of a stringent grape seed procyanidins with oral epithelial cells. *Food Chem* 115: 551-557.

21. Brossaud F, Cheynier V, Noble AC (2001) Bitterness and astringency of grape and wine polyphenols. *Aust J Grape Wine Res* 7: 33-39.
22. Smith AK, June H, Noble AC (1996) Effects of viscosity on the bitterness and astringency of grape seed tannin. *Food Qual Prefer* 7: 161-166.
23. Dinnella C, Recchia A, Fia G, Bertuccioli M, Monteleone E (2009) Saliva characteristics and individual sensitivity to phenolic astringent stimuli. *Chem Senses* 34: 295-304.
24. deWijk RA, Prinz JF (2005) The role of friction in perceived oral texture. *Food Qual Prefer* 16: 121-129.
25. Mustard MJ (1952) Ascorbic acid content of some miscellaneous tropical and subtropical plant and plant products. *J Food Sci* 17: 31-35.
26. Giunta JL (1983) Dental erosion resulting from chewable vitamin C tablets. *J Am Dent Assoc* 107: 253-256.
27. Jensen ME (1986) Responses of interproximal plaque pH to snack foods and effect of chewing sorbitol-containing gum. *J Am Dent Assoc* 113: 262-266.
28. Hill CM, Gibson A (1987) The oral and dental effects of qat chewing. *Oral Surg Oral Med Oral Pathol* 63: 433-436.
29. Hattab FN, Angmar-Månsson B (1999) Fluoride content in khat (*Catha edulis*) chewing leaves. *Arch Oral Biol* 45: 253-255.
30. Jaafari-Ashkavandi Z, Kheirmand M (2013) Effect of home-used water purifier on fluoride concentration of drinking water in southern Iran. *Dent Res J (Isfahan)* 10: 489-492.
31. APHA (1981) Standard methods for determination of water and wastewater. American Public Health Association, Washington DC.
32. Clesceri LS (1998) Standard methods for the examination of water and wastewater. American Public Health Association Washington.