Ee-JRIF

Ethiopian e-Journal
for Research and Innovation Foresight

Vol 2, No 1 (2010) - Health Issue pp (7 - 22)

# Determination of Levels of Cathine in Khat (Catha edulis) Leaves and its Detection in Urine of Khat Chewers: A Preliminary Report

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## Abstract

Analysis of the different parts of the Khat plant revealed that the tender leaves, that are usually preferred by Khat chewers contain the psychoactive alkaloid cathinone, whereas the older and harder leaves contain cathine, also known as d-norpseudoephedrine. In this study we used proton nuclear magnetic resonance method not only to establish presence or absence of these compounds in the leaves, but also to determine their levels. Using this method no cathine was detected in the fresh twigs and young leaves. On the other hand the harder and older leaves from the branches gave upto 1.5% cathine. When the young leaves were dried in the sun and analysed, cathine appeared to be the major component (1.5%), clearly showing the gradual conversion of cathinone to cathine. The same analytical method was also used to establish presence or absence of cathine in urine of Khat chewers.

**Keywords**: Khat; chat; cathine; cathinone, quantitative proton NMR

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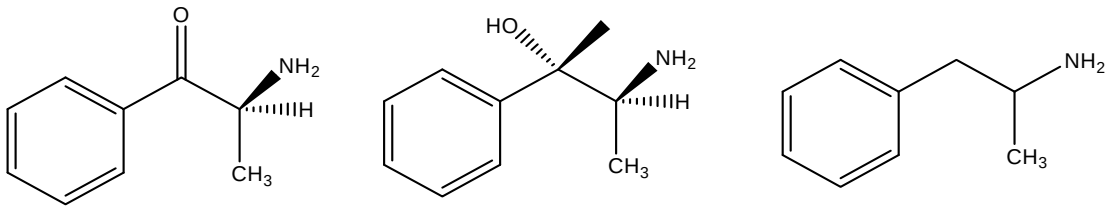
## Introduction

*Catha edulis* Forsk (Celastraceae) is a shrub or tree, 1-25 m high, widely cultivated at altitudes ranging from 1100 to 2100 m in East Africa from Ethiopia to Madagascar and beyond the Red Sea in Yemen and parts of Saudi Arabia. This plant is known by several names, the common ones being: Chat (Amharic), Gat, Khat, Qat (Arabic), Miraa, Mlonge (Swahili), Bushman's tea (South Africa). In most scientific papers the plant is referred to as "Khat". The custom of chewing fresh leaves of Khat is mainly because of its stimulating and euphoric effects. Thus in terms of its effects, its social value to societies that regularly use it, and also its economic significance, Khat may be considered as a rival of coffee (Amare Getahun and Krikorian, 1973).

The first major breakthrough in research on this plant came about in the middle of the 1970's when the simple compound cathinone [α-aminopropiophenone] (**1**) {Scheme 1} was established to be the most important active chemical constituent of the fresh plant (Szendrei, 1980). This compound eluded the attention of chemists of earlier times because they limited their research on dried material, in which the concentration of the active principle is too low for isolation or detection. This finding was consistent with the fact that *Khat* users prefer fresh material to the dried one. It was later confirmed that when the plant dries the active principle cathinone is gradually converted to the less psychoactive compound known as cathine [d-norpseudoephedrine] (**2**) {Scheme 1}.




1 2 3

**Scheme 1:** Structures of the natural products Cathinone (**1)** and Cathine (**2**) and the synthetic compound Amphetamine (**3**)

Pharmacological studies by Kalix (1988) revealed the close similarity between the natural compound cathinone and the well known man-made stimulant drug amphetamine (**3**) {Scheme 1}. In so far as its pharmacological effects are concerned cathinone may be considered as amphetamine-like. This simplifies matters a great deal because much that is already known about the effects of amphetamine on the human body could be extrapolated to cathinone. The reasons for the physical and mental effects observed when chewing Khat can be correlated to similar effects observed in use and abuse of amphetamine.

Khat is openly used, traded, imported and exported in the countries of the region. Ethiopia is most likely the number one grower and exporter of this plant. The livelihood and daily income of millions of people is dependent on it, while nationally it is the second most important foreign exchange earner for the country after coffee. It is also culturally a significant plant as is evident from the proliferation of "Khat Kiosks" and "Khat Chewing Houses" in Addis Ababa and throughout the country. Fresh leaves are readily available and promoted using as brand names the locality where the Khat leaves originate such as Khat from Aweday, Wendo, Belechu, Bahr Dar etc. Fig. 1 shows a typical bundle sold in market. Khat is usually cultivated in the back yard of homesteads in villages and small towns, making it one of the most popular plants grown in home gardens {Figs. 1 and 2}.

Fig. 1: Typical bundle of fresh Khat leaves sold in market

Fig. 2: Khat plant in garden

The Ethiopian Postal Authority issued on September 9, 2008 three Khat stamps {Fig. 3} in order to herald open discussion and debate on the use and abuse of this plant. An article entitled “Chat (Khat) Chewing and Its Consequences“ written by E. Dagne was ciruculated on the day of the launch of these stamps. The full article may be viewed by **clicking here**.


Fig. 3. Three stamps issued by the Ethiopian Postal Service in September 2009

The World Health Organization has classified Khat or cathinone as a "Substance of Abuse" but not as narcotic. However, some European countries prohibit import of Khat, although international law on whether Khat should be legal or not is still highly ambiguous. The effects of long-term use of Khat on humans is becoming more evident from scientific studies conducted in

laboratories and field studies. Islam *et al.* (1994) found that the methanolic extract of khat when administered orally by gavage to rats resulted in reduced food consumption, reduced litter size, several types of malformations, thus confirming khat possesses both embryotoxic as well as teratogenic properties.

Brenneisen *et al.* (1990) showed administration of a single oral dose of cathinone administered to volunteers resulted in increase of blood pressure and heart rate, and these changes could be related to blood plasma levels of cathinone. Furthermore Al-Motarreb *et al.* (2002) showed that increase in blood pressure and heart rate associated with khat chewing may increase incidence of acute myocadial infarction.

Significant association between *Khat* chewing habit and development of hemorrhoid was shown by Al-Hadrani (2000), who found that nearly 62% of chronic *Khat* chewers developed hemorrhoid. Casual and chronic *Khat* chewers should be aware of the fact that *Khat* may interact with certain drugs. Attef *et al.* (1997) showed that *Khat* reduced the absorption of the antibiotics amoxicillin and ampicillin from the gastrointestinal tract. This is similar to the effect of milk on tetracycline absorption where the calcium present in milk binds with the drug and reduces its bioavailability. In the case of *Khat* it is suspected that the effect is due to the binding of tannins with the antibiotics. A study (Belew *et al.* 2000) conducted in the Jimma area of Ethiopia showed prevalence of *Khat* chewing to be 32%, where Muslims more than Christians, males more than females, those between the ages 15 and 34 years more than other age groups, were habitual users.

Kalix (1988) discussed the consequences of long-term Khat use which leads to acute Kha*t* intoxication and psychic dependence, a study later supported by Alem and Shibre (1997), who showed mood disorders, anxiety disorders, complicated psychiatric conditions and even psychosis. Khattab and Amer (1995), using aviation workers as subjects, determined that Khat chewing has adverse effect on perceptual-visual memory and decision-speed. This may also mean that *Khat* chewing habit may not be compatible with safe driving. Khat chewers should therefore be informed of the grave consequences of their habit so that they reduce Khat usage or

abandon it altogether. It is obvious that it is not worth putting at risk ones health for momentary pleasures and stimulation.

**Objective of this study**

The main objective of this study was to develop analytical methods that enable one to assess the distribution of the main metabolites of Khat in the different parts of the palnt. Cathine was chosen as the marker compound in order to follow changes that take place in the leaves. Cathinone is a stronger psychoactive compound than cathine. However as the khat leaf dries the cathinone level declines and eventually disappears altogether because it is gradually converted into cathine. Once in the human body cathinone is also converted to cathine. Hence cathine serves also as a marker compound in order to establish whether a person has chewed khat leaves or not. The potential of such a study in monitoring these and others compounds of abuse is obvious.

We therefore set out to develop a quick and efficient method of determining levels of cathine in Khat leaves and its detection in urine samples taken from Khat chewers. Another objective of this study was to find out the variation in cathine content in the different types of khat leaves. The young leaves and twigs at branch tips are normally preferred by chewers rather than the older and hardy leaves.

**Methods of Analysis**

$^{1}H$ Nuclear magnetic resonance (NMR) spectroscopy is probably one of the most versatile analytical tools available. Recently this method also proved to be a highly suitable method for the simultaneous selective recognition and quantitative determination of metabolites in complex biological matrixes. The method now known as quantitative proton NMR (qHNMR) finds wide application in the analysis of foods, pharmaceuticals and many other types of natural products (Pauli et al. 2005). However, the other organic NMR nucleus ($^{13}C$) is less sensitive (1.6% of $^{1}H$ sensitivity for an equal number of nuclei) and low natural abundance (1.1%), making it difficult to obtain quantitative information by $^{13}C$ NMR technique. On the other hand qHNMR provides quantitative information about a sample because the intensity (or the area) of a peak is directly

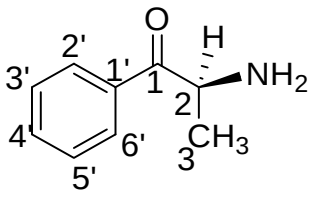
proportional to the number of nuclei producing the signal. The precision of the integrals determines the accuracy of quantification, which depends on the noise level of the spectrum, the line shape, quality of shimming, phase baseline and drift corrections.

For simple compositional analysis, integration of the spectrum or selected spectral region is performed, followed by adjustment of the integrated intensities to reflect the number of protons giving rise to the integrated signals. The individual integrated intensities are summed and then expressed as a percent of summed integrations, from which the molar composition (mole %) of an ananlyte in a mixture is deduced. This procedure normally involves taking a weighed (mg) sample of a crude mixture, then adding a precise quantity of a known internal standard such as caffeine, followed by measuring the $^1$H NMR spectrum. Comparing the signal intensities of a selected signal from the analyte to that of a signal of a proton in the internal standard, allows one to calculate the actual weight of the analyte present in the crude mixture.

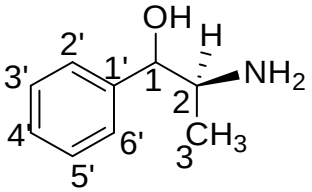
The main advantage of using NMR for quantitative analysis is that it is simple and straight forward. In most applications, the sample only has to be dissolved in a deuterated solvent followed by measurement of the NMR spectrum. Ideally, every signal in the NMR spectrum has the same response factor concerning the number of magnetically equivalent nuclei that constitute the signal, which make the calculation simple. Other advantages of qHNMR is its non-destructive nature and selectivity.

**Results and Discussion**

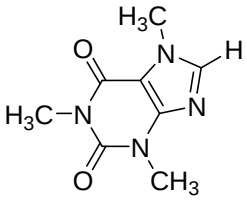
The main bioactive compound cathinone (**1**) was readily isolated from fresh khat leaves, by soaking in 0.1 N HCl, sonication followed by filteration and extraction using diethyl ether. The aqueous acid layer was basified with 10% NaOH, and final extraction with diethyl ether. To the diethyl ether extract saturated oxalic acid solution was added drop wise, from which upon cooling cathinone oxalate precipitated out: mp 172-174$^o$C (Lit. 173-175$^o$C, Dictionary of Natural Products, DNP), $[\alpha]_D$ -39$^0$ (Lit. -40 (DNP), $R_f$ 0.45 (EtOAc:MeOH:$NH_4OH$ (8.5:1:0.5); spraying the TLC plate with ninhydrin solution gave a pink spot (Szendrei, 1980). The $^1$H NMR spectrum (see Fig. 4) gave the characteristic signals for the aliphatic and aromatic protons.




Cathinone (**1**)




Cathine (**2**)




Caffeine **(4)**

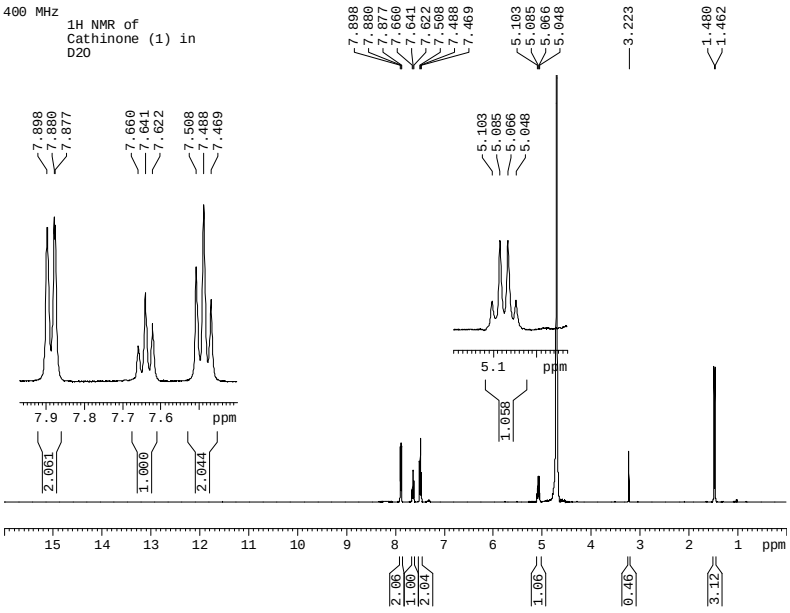



**Fig. 4:** $^{1}$H NMR spectrum of cathinone oxalate run in $D_2O$ (See Experimental Section for signal assignments)

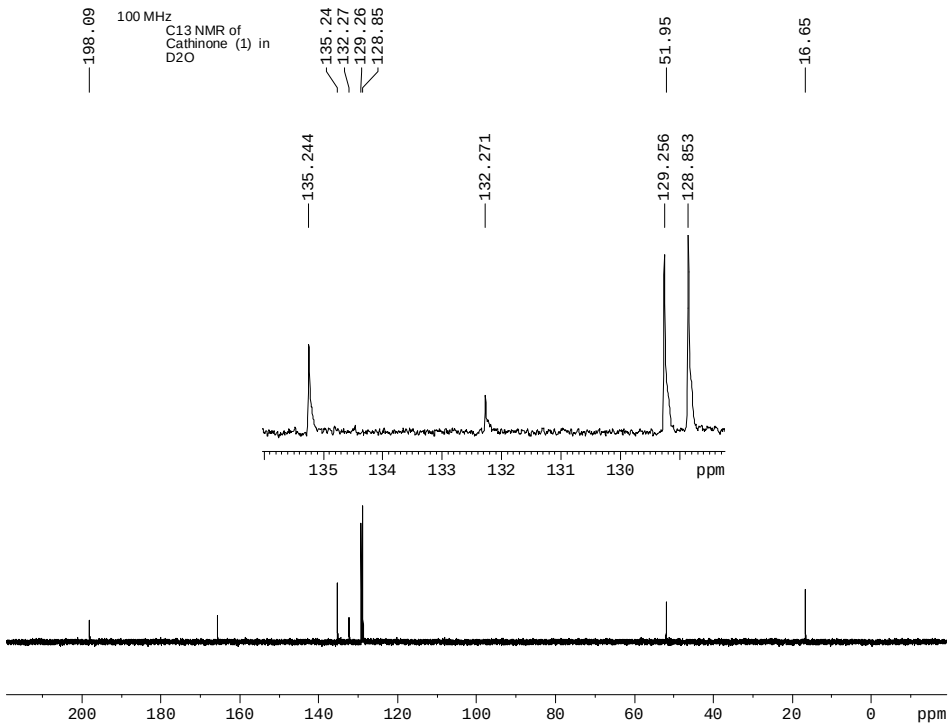



Fig. 5: $^{13}C$ NMR spectrum of pure cathinone (See experimental for full assignment

Cathine (2) could also be readily isolated by using similar procedure as for cathinone. Its $^{1}H$ NMR is shown in Fig. 6.

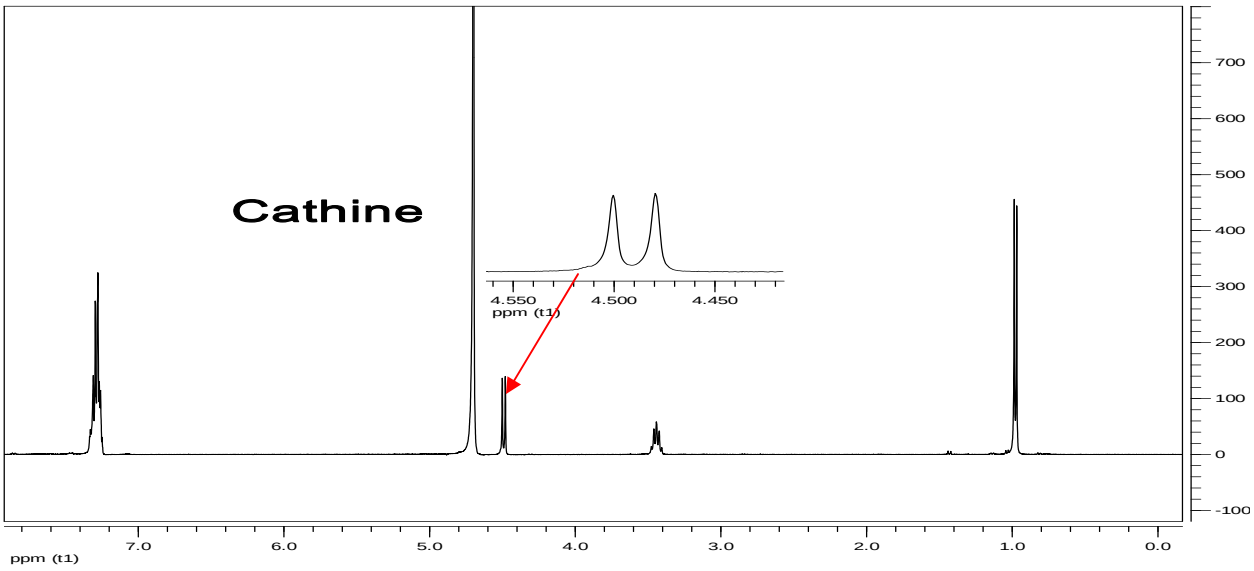



**Fig. 6:** $^{1}$H NMR spectrum of pure cathine: δ 7.19 (m, H2'-H5'), 4.53 (d, H-2), 3.5 (m, H-2) and 1.0 (d, Me)

**Determination of cathine level in khat leaves:** The level of cathine in the khat leaves was determined using the qHNMR method of analysis by adding reference caffeine solution in Khat extract as explained in the Experimental section. The peaks used for integration were selected at 7.5 - 7.4, 4.6 - 4.4 ppm for caffeine and cathine, respectively as shown in Fig. 7. The percentage of cathine in the extract was calculated by taking the integral ratio of cathine to caffeine. Using this method no cathine was detected in the fresh twigs and young leaves that are normally preferred by khat chewers. The hard and old leaves from the lower part of the branches gave 1.5% cathine. Cathinone could not be detected in sun dried young leaves, while the cathine level was 1.5%.

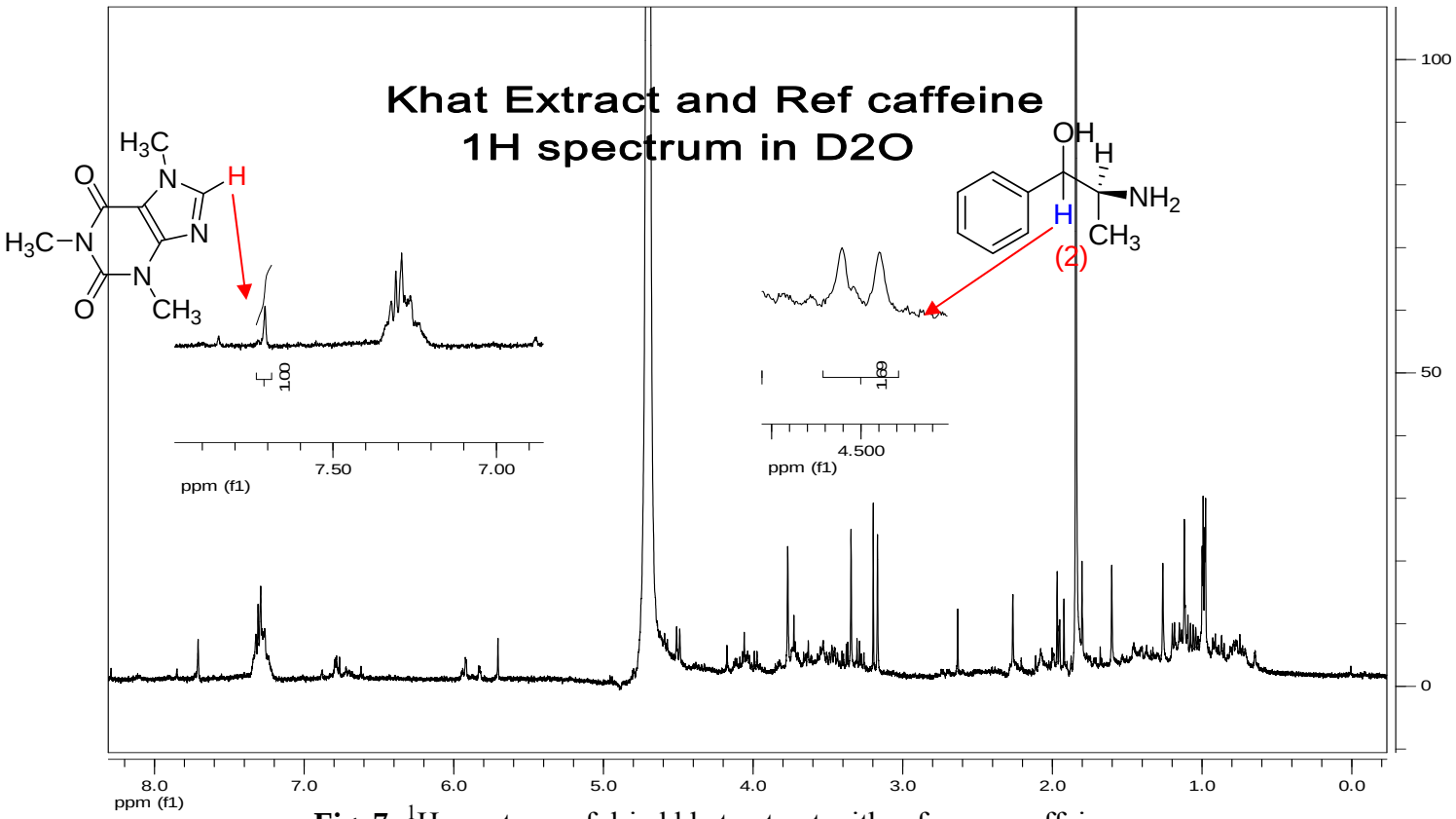



**Fig. 7:** $^{1}$H spectrum of dried khat extract with reference caffeine

**Detection of cathine in urine sample of khat chewers:** Urine samples taken from three volunteer chewers were compared to three volunteer non-chewers. These were extracted as described in the Experimental Section. The $^{1}$H NMR spectra were measured, appearance of the key signals indicates presence of cathine as shown in Fig. 7. In the case of one volunteer who chewed once, cathine could be detected in the urine after day 2, 3 and 4.

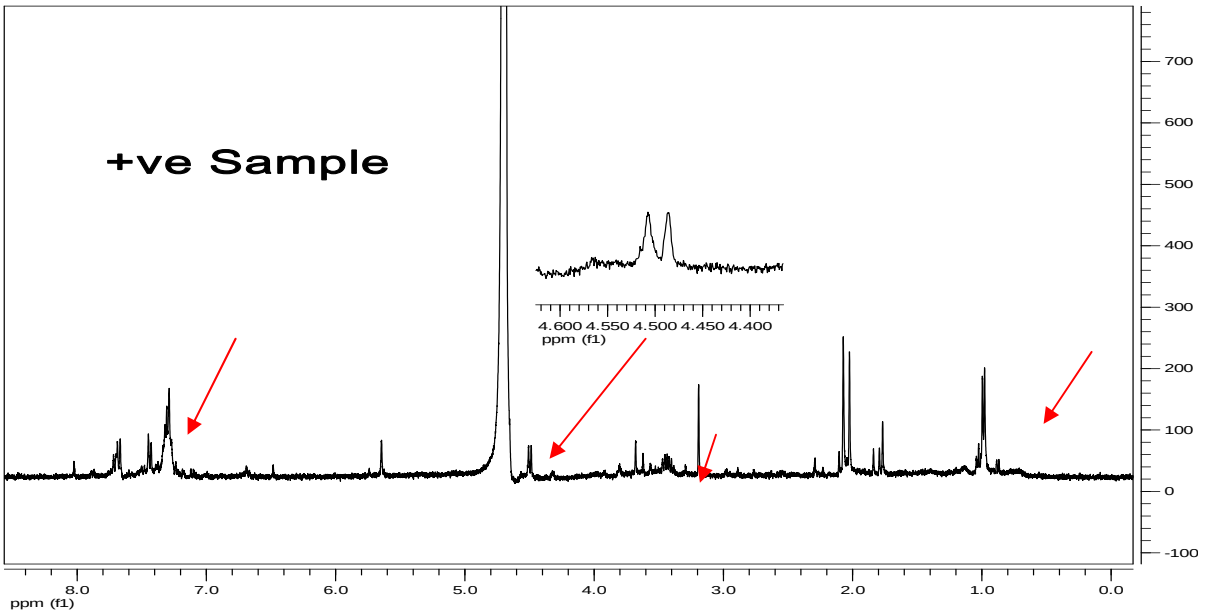



**Fig. 8:** Typical spectrum of a sample +ve for cathine. The arrow indicates the key proton signals of cathine.

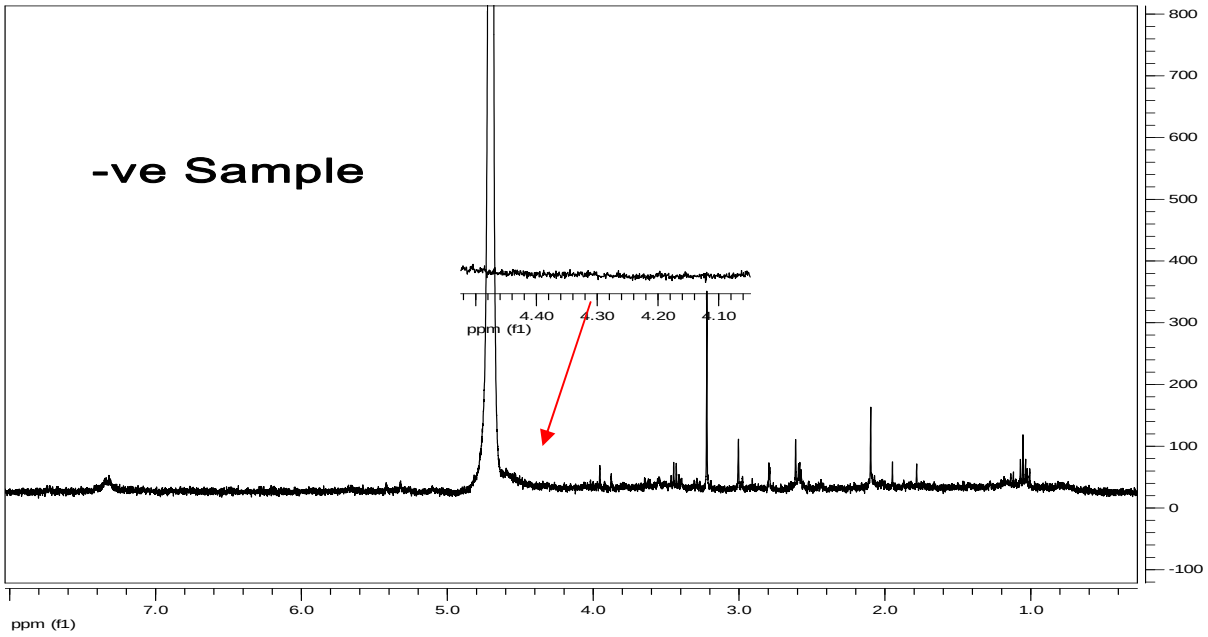



**Fig. 9:** Typical spectrum of urine from a volunteer who did not chew Khat, showing absence of cathine

## Experimental

**Isolation of cathinone:** The extraction method of Krizevski (2007) was modified as follows**.** Fresh leaves (400 g) soaked in 0.1 N HCl, was sonicated for 30 min, filtered by using suction filtration and then extracted with $Et_2O$. The aqueous acidic layer was basified with 10% NaOH and extracted with $Et_2O$ (2x). Oxalic acid (1% in $Et_2O$) was added drop wise to the $Et_2O$ extract and left to stand for 20 h in the refrigerator ($4^oC$) to yield cathinone oxalate (230 mg) as white precipitate.$^1$H NMR (400 MHz $D_2O$): δ 7.89 (2H, dd, J = 1.2, 7.2 Hz), 7.64 (1H, t, J = 7.6 Hz), 7.48 (2H, t, J = 7.6 Hz), 5.07 (1H, *q*, J= 7.2 Hz) ) and 1.48 ppm (3H, *d*, J = 7.2 Hz). $^{13}$C NMR (100 MHz $D_2O$): δ 198.1 (C-1), 51.9 (C-2), 16.6 (C-3), 132.3 (C-1'), 129.3 (C-2',6'), 128.9 (C-3',5'), 135.2 (C-4').

**Isolation of cathine:** Cathine was isolated using similar procedure as above. $^1$H NMR (400 MHz $D_2O$): δ 7.19 (m, 5H), 4.53 (d, 1H), 3.5 (m, 1H) and 1.0 (d, 3H). $^{13}$C NMR ($D_2O$): δ 74.7 (C-1), 52.6 (C-2), 16.5 (C-3), 128.8 (C-1'), 128.9 (C-2',6'), 126.8 (C-3',5') and 128.7 (C-4').

**Quantifying cathine in khat leaves:** Soak powdered leaves (60 g) of khat in 5% HCl, place on shaker for 1 h, filter, add 5% $NH_3$ to pH 10 and extract with EtOAc. Dry the EtOAc extract with anhyd $Na_2SO_4$ , filter, concentrate and determine weight of the residue (fresh young leaves usually yield 200 – 250 mg, and old leaves 100 – 150 mg). The qHNMR experiment is conducted as follows: 10-20 mg of the EtOAc extract is introduced into the NMR tube, then add the reference caffeine solution (0.2 ml equivalent to 0.001 mmole), followed by $D_2O$ (0.4 ml) and its $^1$H NMR spectrum is measured. Determine the integral ratio H-8 of caffeine with H-1 of cathine. This gives the caffeine:cathine mmole ratio, from which the amount of cathine present in the 60 g khat leaves is derived. This will give the % cathine in the analysed leaf.

**Preparation of reference caffeine Solution:** Caffeine (5 mg, 0.0258 mmole) was introduced into 5 ml volumetric flask to give 1 mg/ml reference solution in $D_2O$ .

**Detecting cathine in urine sample:** Urine sample (60 ml) was basified using drops of $NH_3$ solution till the indicator was deep blue. This was extracted with 60 ml of EtOAc using separatory funnel. The EtOAc layer was dried with anhyd $Na_2SO_4$ (300 mg), filtered and

concentrated. Using a total of 0.6 ml of $D_2O$ the whole extract is transferred into the NMR tube and its $^1H$ NMR spectrum measured. Presence or absence of cathine is deduced by overlaying this spectrum with that of reference cathine (See Fig. 6 and 7).

## Conclusion

qHNMR is a quick and efficient tool for the determination of levels of cathinone and cathine, the two most important natural metabolites present in Khat. The advantage of the qHNMR method over HPLC is its simplicity and efficiency. This method can also be applied in the future to find out if there are significant differences among Khat samples originating from different geographic locations.

## Acknowledgements

International Program in Chemical Sciences (IPICS-Sweden) is thanked for research grant. We are grateful to Della Berhanu for bringing to our attention the importance of monitoring cathine in urine of Khat chewers . Hanna Kassaye and Bikila Alemu are thanked for technical assistance.

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