Qualitative Analysis Of

Khat

(Catha edulis)

Detection of Active Constituents and its metabolites

PHG 414 Practical course

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INTRODUCTION

A number of plants are known to contain drugs which are subject to control. Of the less common seizures that a forensic scientist might encounter are included products of *Catha edulis* Forsk. (Celastraceae), which have the street names 'Khat', 'Cat', 'Qat', 'Jeff and 'Mulka'.

Genus Catha includes 4 species: *C. edulis* Forsk., *C. spinosa* Forsk., *C. abbotii* Van Wyk & Prins and *C. transvaalensis* Codd (*c. cassino* ides N.K.B. Robson) of which *C. edulis* is the only species known to produce cathinone.

Description

Khat is a slow-growing shrub or tree that grows to between 1.5 meters and 20 metres tall, depending on region and rainfall, with evergreen leaves 5–10 cm long and 1–4 cm broad. The flowers are produced on short axillary cymes 4–8 cm long, each flower small, with five white petals. The leaves have an aromatic odour. The taste is astringent and slightly sweet. The plant is seedless and hardy.

- Khat is growing in a variety of climates and soils. Khat can be grown in droughts where other crops have failed and also at high altitudes.

- Tender leaves from the tip of the branches and those of young shoots are the most potent. leaves.

Preparation

- Khat is usually collected in early morning from the shrubs or trees of more than 5-8 years old.
- The tender twigs and young shoots are collected and made into bundles of about 20-50 g. The bundles are then wrapped in false banana leaves, damp papers or plastic sheets to preserve its moisture content-loss of 'activity' is observed after 48 h if the plant material dries out.
- Khat is generally accepted as fresh for up to 4 days of collection.

Administration

- Khat fresh leaves and tops are chewed or, less frequently, dried and consumed as tea. It is habitually chewed by many peoples in Yemen, East Africa (Ethiopia, Somalia, Kenya, Tanzania, Madagascar) and South Africa for its amphetamine-like stimulating effects.

- Khat leaves are thoroughly masticated one by one for about 10 minutes followed by swallowing the juice with the saliva. The leaves residue is usually stored in the cheek, for further extraction, making a characteristic: bulge in the cheek of chewier. The residue is then spit out as in Yemen or swallowed with a large amount of water as in Ethiopia.

- Khat may be used by farmers and laborers for reducing physical fatigue or hunger and by drivers and students for improving attention.

- One reason for cultivating khat in Yemen so widely is the high income it provides for farmers. Some studies done in 2001 estimated that the income from cultivating khat was about 2.5 million Yemeni riyals per hectare, while it was only 0.57 million riyals per hectare if fruits were cultivated. This is a strong reason farmers prefer to cultivate khat over coffee and fruits. It is estimated that between 1970 and 2000, the area on which khat was cultivated grew from 8,000 hectares to 103,000 hectares.

In 1980 the World Health Organization classified khat as a drug of abuse that can produce mild to moderate psychological dependence.

However, the parent plant material is not controlled in the United Kingdom, *although it is controlled in some European countries (Norway and Sweden)*, the USA and Canada.

Constituents of Fresh Khat Leaves

Fresh khat leaves contains:

◈ Alkaloids of the phenylalkylamine type (basic fraction) known as **khatamines** They are responsible for the CNS-stimulating effect of khat.

◈ More than 14 alkaloids of the sesquiterpene polyester type (weakly basic fairly lipophilic fraction) known as **cathedulins**. The activity of these alkaloids was not yet studied due to their extremely poor water solubility and lack of sufficient amounts of pure material.

◈ Large amount **polyphenols**, including tannins and flavonoid glycosides of -

kaempferol, quercetin and myricetin.
◈ Volatile oil, triterpenes, sterols, amino acids, ascorbic acid and sugar alcohols.

◈ Khatamines

They are the alkaloids of the phenylalkylamine group isolated from fresh khat leaves and considered as the main carriers of CNS activity.

Phenylpropylamine group:

Three main compounds were isolated and identified as:

1- S-(-)cathinone (cathinone) = [S-(-)-a-aminopropiophenone]
2- S, S-(+)-norpseudoephedrine (norpseudoephedrine) and
3- R, S-(-)norephedrine (norephedrine) = (cathines).

◈ **Cathinone** constitutes about 50% of the total khatamines. It is the main psychoactive alkaloid (CNS-stimulant) of fresh khat leaves and has the same indirect sympathomimetic mechanism of action as amphetamine. Therefore, cathinone may be called, like khat, the "natural amphetamine".
On recommendation of the World Health Organization (WHO) cathinone has been put under international control in 1985 and classified as Schedule I substance (1971 Convention on Psychotropic Substances).
- The diastereomers (+)-norpseudoephedrine and (-)-norephedrine (cathines) are much less active than cathinone.

- Both of khat's major active ingredients -- cathine and cathinone -- are phenylalkylamines, meaning they are in the same class of chemicals as amphetamines. In fact, cathinone and cathine have a very similar molecular structure to amphetamine

- The cathinone content of Yemeni (0.09-0.3 %0) and Madagascarian (0.1 %0) is significantly lower than that of Ethiopian (0.3-1.9%0) or Kenyan (up to 3.3%0) khat.

- Cathinone as a ketoamine base is extremely unstable. Withering, drying and clean-up of the plant material result in various degradation products or artifacts.

- In *in vivo* enzymatic reduction transforms the potent CNS-stimulant cathinone into the less active cathines,

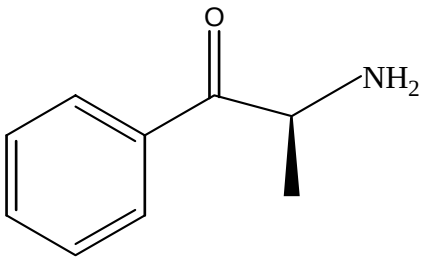
- norpseudoephedrine and
- norephedrine (cathine).

Thus a fresh drug may contain a hundred times more cathinone than dried material, which in turn shows an increased content of cathines.

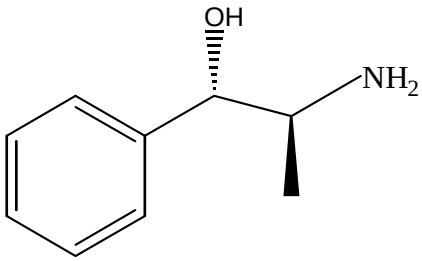
- This instability of the khat principle cathinone explains why about hundred years of chemical research were needed to identify this rather simple component (first identification in 1975) and why khat users insist on chewing fresh khat material.

- R,S-(-)-N-Formylnorephedrine was only reported from Saudi Arabian khat, in addition to S-(-)cathinone and R,S-(-)-norephedrine.

Cathinone

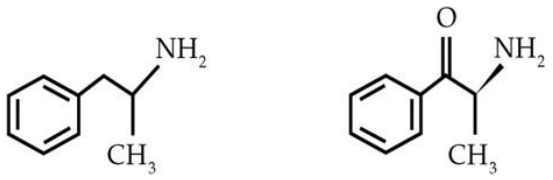


Cathine



Pharmacodynamic Effects

Khat and cathinone have in human marked psycho stimulant and euphorigenic effects, effects which can be expected to reinforce the habit of chewing khat. Cathinone is a potent amphetamine-like compound with indirect sympathomimetic action. It has the same mechanism of action as amphetamine, inducing the release of the neurotransmitters dopamine and noradrenaline from storage sites in the central and peripheral nervous system. It is considered as the dependence-producing constituent of khat. The sympathomimetic side effects of khat include arrhythmia, tachycardia and dose-dependent increase in blood pressure. Changes in pulse rate and blood pressure appear to be less pronounced in chronic users, which would indicate the development of tolerance to the sympathomimetic effects of khat. Another sympathomimetic effect of khat is mydriasis which, along with a staring look and the brownish staining of the teeth, is characteristic for the khat habit.



Amphetamine Cathinone

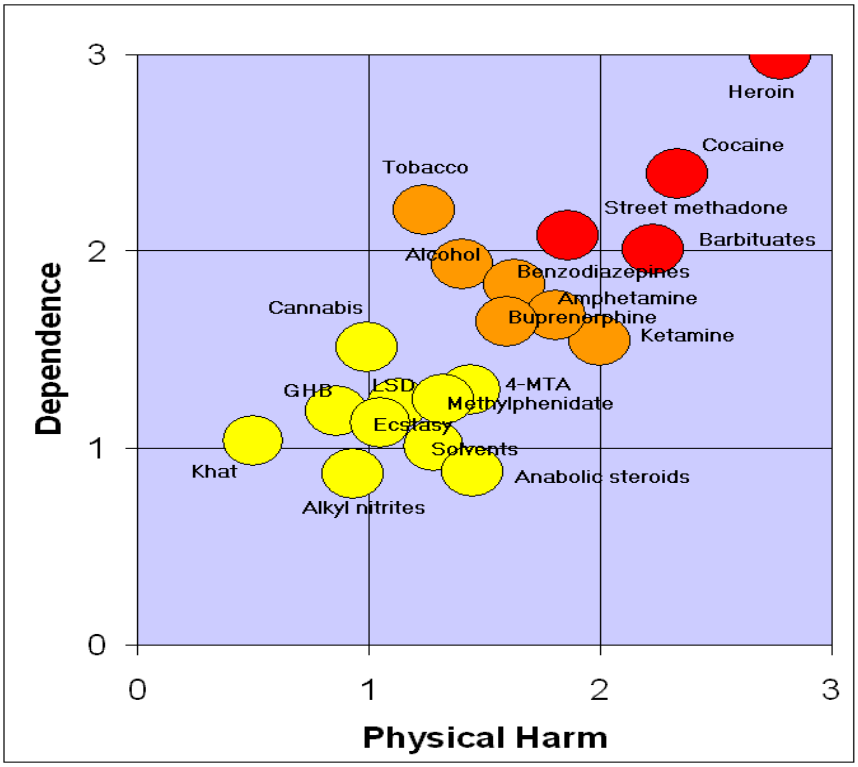
Chemical structures of amphetamine and cathinone.

Dependence, tolerance and withdrawal

Khat induces moderate but often psychic **dependence.** In 1980, the WHO Expert Committee on Drug Dependence classified khat as a dependence-producing drug. Chewing of one bundle of khat of 50 g is equivalent to 5-10 mg amphetamine but, in contrast to amphetamine abuse, no **tolerance** to the CNS-effects. The ingestion and absorption of the active khat alkaloids are limited by the bulkiness of the plant material to be chewed. The physical limits on the amount of khat that can be chewed make an increase of the dose difficult, and this probably explains why tolerance to the CNS effects of the drug does not seem to Occur.

There are conflicting opinions regarding the existence of a withdrawal syndrome. Heavy khat chewers experience true, but not physical, **withdrawal** symptoms. They consist of lethargy, mild depression, slight trembling and recurrent bad dreams.

In one study only 0.6% of khat chewers continued to use in order to prevent withdrawal symptoms.



Comparison of physical harm and dependence regarding various drugs (the British medical journal)

Pharmacokinetics and Detection

The euphoric effect appears shortly after the chewing begins, suggesting absorption from the oral mucosa. The effect of cathinone is maximum after 15–30 minutes. ***Metabolism of cathinone is rapid, occurring mainly during first passage through the liver. Only a small fraction (about 2%) appears unchanged in the urine. Most cathinone is metabolised to norephedrine and is excreted in this form***. The rate of inactivation is about the same as the rate of absorption, which limits the cathinone blood levels attainable by chewing.

Cathine has a slower onset of action, ***with a serum half-life in humans of about 3 hours. It is excreted unchanged in the urine within about 24 hours.*** When taking khat, large amounts of non-alcoholic drinks are consumed. There is pharmacological synergism with drinks containing methylxanthines (e.g. tea and cola), which therefore enhances the effects of khat.

In the UK, a commercially available biochemical test to detect khat constituents in the urine can now be used for confirmation of a suspected khat-induced state. Initially, a rapid screen by immunoassay detects amphetamine-related compounds. Then gas chromatography mass spectrometry is performed. ***This cannot detect cathinone directly, but a positive result indicates the presence of***

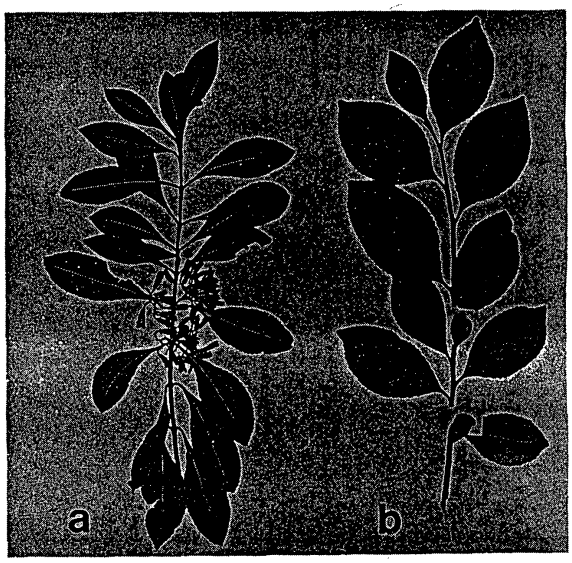
norephedrine, a cathinone metabolite. The test will give a positive result for up to about 48 hours after consumption of khat, although this is dependent on many factors, for example chronic consumption as opposed to a single episode of use, the quantity taken, the user's metabolism, and dilution of urine following consumption of fluids. The test is highly sensitive, but not highly specific, and there are some cross-reactions with other metabolites (Lehmann et al, 1990). However, in the context of the clinical presentation together with the history of khat consumption, the urinalysis is a useful additional test. In many areas urine testing is not available, and an accurate history of (increased) khat consumption prior to the onset of clinical symptoms is equally important, if not more so.

Identification and Quantification of Khat

Morphologic:

Khat has very few morphological and anatomical features that would serve as a basis for the purpose of identification. It is therefore strongly recommended that authentic samples of khat should be available for comparative purposes and that identification should be based on more than one method.

The leaves are opposite or alternate in arrangement (figure I). Occasionally, both arrangements are found on the same twig. The shoots sometimes show heterophylly, one or more of the ordinary oval-lanceolate leaves being replaced by smaller, obcordate leaves (figure I *b*) that may resemble the leaves of *Cassine capensis* L.



figure

Microscopic

No characteristic macro- and microscopic features could serve alone for the identification of khat. However, certain characters in conjunction with chemical data can assist in identifying the plant.

The dry powder can be identified under the microscope, after clarifying with chloral or potassium, by the following elements: many strands isolated or in groups, colourless, tapering, with narrow lumen (Fig. II (4); remains of ducts, streaked or dotted; small, calcium oxalate twin crystals isolated or in series within uniserial cells; pieces of lower epidermis with stomata and slightly sinuate cells (Fig. II (2)) and, in particular, fragments of upper epidermis whose extremely sinuate walls are scalloped in appearance (Fig. II (1)). The absence of sclerites and hairs eliminate tea, and in mate the stomata are surrounded with cells with striped cuticles.

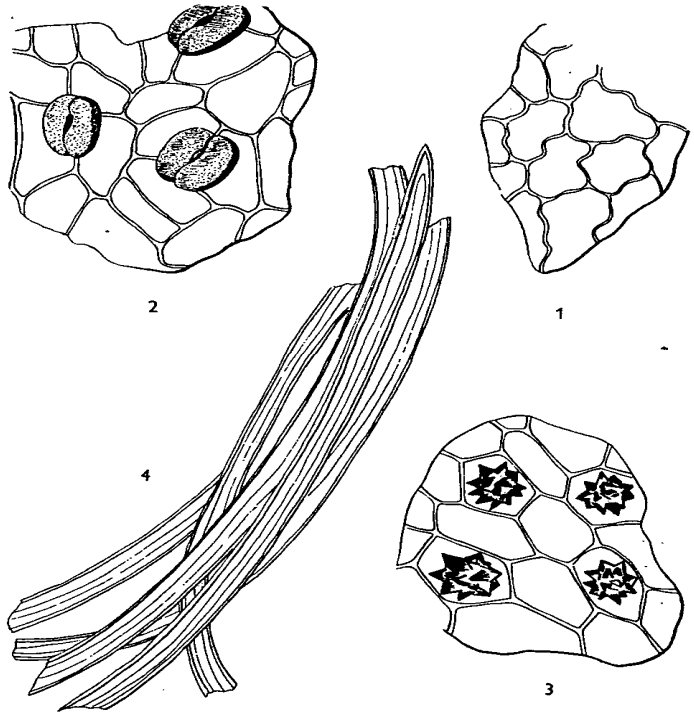


figure II

1. Upper epidermis, front view

2. Lower epidermis, front view .

3. Oxiliferous cells

4. Pericyclic strand

❖ *What details should be reported during a full physical description of a Khat sample?*

The form of the drug, and its size, weight, dimensions and smell should all be recorded. If the sample includes plant material, a botanical description might also be useful. For the analysis of such material, leaf and bark (twig) samples should be analyzed separately.

Having obtained and described a suitable sample, the next stage is to analyze the material for the presence of cathinone and cathine.

NOTE : In terms of identification of the material as Khat, from C. edulis. it is necessary to demonstrate the presence of Cathinone, since this is not found in other members of the genus.

Presumptive Color Tests

Typical drug identifications based on chemical analysis depend upon presumptive (color) test techniques. However. a slightly different approach is required for the analysis of Khat because the spot tests traditionally used for some drugs of abuse (e.g. the Marquis, Simons and Mandelin tests) do not work for Cathinone. Therefore the commercially available kits are not suitable for khat samples or even cathinone.

Chromatographic Analysis:

TLC, GC, GC/MS and HPLC are used for analyzing khat samples. The TLC method was originally proposed as rapid qualitative screening test, e.g. for the presumptive identification of confiscated khat samples basing on the fact that cathinone has only been found in Catha edulis (khat). GC and GC/MS require a careful clean up and derivatization of the phenylalkylamine extracts and the incidence of artifacts is higher than with HPLC. GC/MS is an excellent method for identification of forensic khat samples, but less suited for the acquisition of quantitative profiles of complex Khat extracts. The HPLC method used for the quantization of khat samples, based on a normal phase system, required a very time-consuming extraction procedure of large sample amounts and was not sensitive enough for the detection of low concentrations of norpseudoephedrine/norephedrine (NPE/NE). Recently a specific and

sensitive method for the analysis of confiscated samples, using HPLC with photodiod-array detector (HPLC-DAD),was established.

- In order for chromatographic investigations to be carried out, a representative extract must be obtained which is suitable for the analysis of the drugs. A number of different methods have been employed for extraction of the drug components. All of these are based on the conversion of the drugs to their water-soluble salt forms (for example, as the hydrochlorides) by triturating of the freeze-dried (*) plant material in dilute acid (for example, 1 M HCl), basification of the extracts, back-extraction of the drug free bases into an organic solvent which is immiscible with water, drying of the solvent, concentration of the extracts, and then finally analysis of the drug components

*(*) **Freeze drying** (also known as **lyophilization**) is a dehydration process typically used to preserve a perishable material or make the material more convenient for transport. Freeze drying works by freezing the material and then reducing the surrounding pressure to allow the frozen water in the material to sublime directly from the solid phase to gas. The greatly reduced water content that results inhibits the action of microorganisms and enzymes that would normally spoil or degrade the substance.*

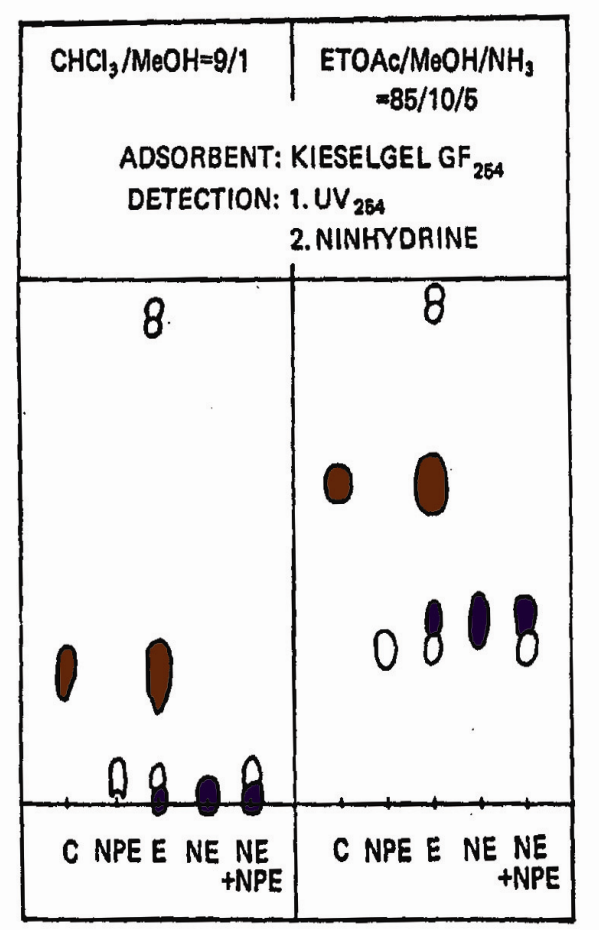
LAB WORK

- Morphological & Microscopically Identification:
  As mentioned before.
- Thin Layer Chromatography (TLC) :

Procedure :

1. Cut the few fresh leaves of khat into small pieces, add suitable volume of methanol or $CHCl_3$ (using a mortar)
2. Triturate the cut leaves with methanol and filter, receiving the methanol in porcelain dish.
3. Repeat step 2.
4. Concentrate the filtrate, preferably under vacuum at low temperature, to about 0.5 ml.
5. Apply for TLC according to the following condition.
   Condition :
   - Stationary phase : Silica gel 60 F_{254} (5X10)
   - Mobile phase : ethylacetate : MeOH : ammonia (17 : 2 : 1)
     or $CHCl_3$: MeOH (9 : 1)
   - Detection :
     1. by UV at 254 nm
     2. spray with Ninhydrine (0.1%) reagent then heat at 110^oC
   - References : Cathinone and cathine in MeOH .
   - Result : Cathinone has high R_f value with brownish color while cathine gives violet color at low R_f value.

Thin-layer chromatogram of the chloroform fraction of khat



C = CATHINONE

NE = NOREPHEDRINE (cathine)

NPE = NORPSEUDOEPHEDRINE

E = EXTRACT

HPLC of Alkaloids from Khat

HPLC can be used to identify the drugs present in this controlled substance, with resolution of cathinone, norpseudoephedrine and norephedrine being achieved on silica gel (as the stationary phase), with a mobile phase consisting of 1,2-dichloroethane/methanol/acetic acid/diethylamine/water (800:200:10:5:5, by volume), employing UV detection at 257 nm. This method has also been used to quantify the drug components present in these samples. However, retention time data were not provided in reference paper.

GC-MS of Alkaloids from Khat

Another approach to identifying the drug is to use GC-MS. Two such methods have been described recently. One of these involves components analysis of the underivatized alkaloids on a 30 m HP-5 column (i.d. 0.25 mm; d_f 0.25 µm) under the following conditions: split ratio of 50:1; carrier gas flow rate of 0.87 $mlmin^{-1}$; temperature program. starting at 40^oC, rising to 95^oC at a rate of 25$^oCmin^{-1}$, holding for 18 mm, then rising to 270^oC at a rate of 20^oC min^{-1}, and holding for 10 min. The retention times of cathinone and cathine were 18 and 19.5 mm, respectively. However, it was found in this case that the mass spectra of the two compounds were very similar.