ALKALOIDS OF *CATHA* SPP.

BY

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Submitted in fulfillment of the academic requirements for the degree of

MASTERS OF SCIENCE

in the

DEPARTMENT OF BOTANY
FACULTY OF SCIENCE
UNIVERSITY OF NATAL
PIETERMARITZBURG

AUGUST 2001

I certify that, unless specifically indicated to the contrary in the text, this dissertation is the result of my own work. The investigation was carried out in the Department of Botany, University of Natal, Pietermaritzburg from January 1996 to August 2001 under the supervision of Dr M. T. Smith.

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ABSTRACT

The levels of the psychoactive alkaloids S-(-)-cathinone and its primary metabolite cathine, consisting of the diastereomers (+) – norpseudoephedrine and (-)- norephedrine were determined in *Catha edulis* (Vahl) Forssk. ex Endl., *Catha transvaalensis* Codd and *Catha abbottii* Van Wyk & Prins. Alkaloid levels were investigated in *C. edulis* plants collected from three different localities in South Africa, and one from a Nairobi khat market.

The efficiency of three different methods for the extraction and isolation of cathinone and cathine were investigated, viz. an aqueous acid extraction, an organic solvent extraction and an aqueous acid extraction using the commercially available Extrelut^R procedure. The aqueous acid extraction resulted in the rapid loss of cathinone and yielded variable alkaloid levels in replicate studies. This was also observed when this method was coupled with the Extrelut^R procedure. In contrast, the organic solvent extraction did not result in a loss of cathinone and provided consistent results over a number of replicates; it also proved to be a simple and rapid method for extracting and isolating cathinone and cathine.

A trifluoroacetic acid (TFA) derivatization procedure which has been suggested to produce characteristic diagnostic fragments for gas chromatography / mass spectrometry (GC/MS) identification, was investigated, but failed to produce consistent TFA derivatives of cathinone and cathine. However, underivatized cathinone and cathine were easily identified by GCMS due to their unambiguous mass spectra.

All subsequent studies were undertaken using the organic solvent extraction and isolation method, coupled with GC analysis and GC/MS identification of underivatized cathinone and cathine.

Leaves of *C. edulis* were found to contain cathinone and cathine at levels 100 times higher than those of *C. transvaalensis.* The alkaloids were undetectable in *C. abbottii*. Plants grown from cuttings of *C. edulis* collected from the Durban Botanical Gardens were found to contain cathinone and cathine at levels of 0.410 mg per gram fresh weight and 0.157 mg per gram fresh weight in leaves,

respectively, while these levels in plants derived from different localities decreased in the order: Eastern Cape (0.319 mg/g f.w cathinone and 0.029 mg/g f.w cathine), Mpumalanga (0.139 mg/g f.w. cathinone and 0.171 mg/g f.w. cathine) and Nairobi (0.032 mg/g f.w. cathinone and 0.025 mg/g f.w. cathine).

In an investigation of the cathinone levels in the different plant parts it was found that the highest levels were found in leaves of the shoot tip (0.243 mg/g f.w.) but decreased with the age of the leaf and developmental stage of the plant in the order: juvenile leaves (0.124 mg/g f.w.), mature leaves (0.035 mg/g f.w.), young stem (0.033 mg/g f.w.) and mature stem (0.004 mg/g f.w.).

Concomitantly, cathine levels increased with the age of the leaf: leaves of the shoot tip (0.006 mg/g f.w.), juvenile leaves (0.011 mg/g f.w.), mature leaves (0.019 mg/g f.w.). The cathine level in the young stem material was found to be the highest in the entire plant (0.270 mg/g f.w.) but decreased markedly in the mature stem (0.052 mg/g f.w.).

Both cathinone and cathine levels in the mature root were greater than levels in the mature stem, being 0.012 mg cathinone per gram fresh weight, and 0.063 mg cathine per gram fresh weight. Neither cathinone nor cathine were detectable in young root material.

Acknowledgements

I would like to thank the following people:

Dr Mike Smith for his inspirational guidance, hands-on assistance and his continued interest in and enthusiasm towards my academic growth.

Prof. Van Staden for running a brilliant department which allowed me access to the equipment needed to make this project possible.

Sascha and the girls for their companionship, friendship and willingness to help.

Dr Neil Crouch of the National Botanical Institute for his assistance in collecting plant material.

Mr Martin Watson of the Chemistry Department for his assistance with GC/MS studies.

My mother and father for instilling in me my interest in plants, for making opportunities available to me, and for their financial and moral support.

Leanne for her love and help and for trekking with me around Natal on futile searches for the elusive *Catha edulis*.

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CHAPTER ONE

LITERATURE REVIEW

1.) History of *Catha edulis* (Vahl) Forsk.

1.1) Introduction

Khat is commonly known as the fresh leaves, tender twigs, young shoots or stem tips of *Catha edulis* (Vahl) Forssk. ex Endl. Khat was considered to be a traditional Muslim drug and its origins and use predate European records by many centuries. However, today its popularity transcends many faiths, nationalities, social levels and age groups. The khat habit has become so deeply entrenched in the daily routine of the people of East Africa and Yemen that khat sessions, commencing at noon as the freshly harvested khat becomes available on the market, bring productivity to a standstill.

Although known to Europeans from as early as the 14th century, the earliest scientific report on *Catha edulis* was in the 18th century by the Swedish botanist and physician Peter Forsskal. His description was published posthumously in 1775 in "Flora Aegyptiaco-Arabica" (Krikorian, 1984).

1.2.) Origin of khat

It is not certain whether *C. edulis* was introduced to Ethiopia from Yemen or vice versa; neither is its date of introduction known. Much of what is known about *C. edulis* has been handed down orally. According to one such account, khat originated in Yemen where it was discovered by a goat herd named Awzulkernayien, who noticed the effect of the leaves of the plant on his goats and tried it himself. He experienced wakefulness and added strength, and took some home and consumed a small amount before retiring for the night. He had no sleep that night and was able to stay up to pray and meditate for long hours (Getahun and Krikorian, 1973).

Others believe it was first introduced into Harar City, from whence it spread to other parts of Ethiopia. The legend of the introduction of khat into Harar tells of a group of religious and civic leaders who met to determine a suitable site to establish a new city. They chose four sites. After long argument, a choice was made and Harar was built on the site of the present old world city. The choice was made because of the suitable elevation, splendid landscape and many rivers and streams in the area, but it was soon discovered that the air of the city had a depressing effect on the people and made them tired and very lazy. The council met again to discuss this problem. They agreed that the holy tree of Awzulkernayien was the cure. A mission of merchants was dispatched to Yemen to fetch the khat, and thus the first khat is said to have come to Harar. The first group of trees was said to have been planted in the area where the city of Harar now stands. These original trees were however removed by the Italians in their efforts to landscape and expand the city in the early 1940's.

According to another tradition, reported by Sir Richard Burton in his "First Footsteps in East Africa" (1856), Sheikh Ibrahim Abu Zaharbui, one of the forty-four Hadrami saints who landed at Berbera, introduced khat to Yemen from Abyssinia (cf. Burton, 1856, *et seq*; 1966). However, this account is purely legendary as the historian Ibn Fadl Allah al-'Umari, in his authorative account "Masalik al-Absar" written between 1342 and 1349 A.D., gives an account of its introduction into Yemen in the reign of Al-Mu'ayyard Da'ud, who personally found reasons for disliking it.

Evidence that the use of khat had not spread as far as Uganda by 1853 is found in the observation made by Sir Richard Burton that the people of Uganda used a quid of mud in place of khat as used in Yemen (cf. Burton, 1856, *et seq*; 1966).

Contrary to the popular accounts presented above, most writers believe khat to be of Ethiopian origin. These views are more than likely derived from the fact that khat was first mentioned in "The Wars of 'Amda Syon 1", who reigned A.D. 1314-1344 when the Muslim Sultan of Ifat, Sabrad-Din, bragging of what he would do when he had conquered the Christian Kingdom, said :

> "I will make Ma'ade [the Arab name for Tegeulat] his capital my capital also. And I will plant there plants of cat because the Muslims love that plant, and [it is] a gift which he sent to the king." (Getahun and Krikorian, 1973).

Khat is still considered holy in some areas with Muslim tradition and people offer prayer before they begin to chew it. The mention of the name of Azwulkernayien in this prayer could be taken as direct evidence or testimony to the lore. Additional evidence of khat being considered holy is the method of its harvest and status of its use by the people. It is said that people washed their bodies before harvesting the crop; moreover the harvested crop was placed in clean cloth (Getahun and Krikorian, 1973).

Originally the spread of the khat crop was guarded. A severe penalty was imposed on anyone who gave a khat plant to Kotu (a large ethnic group in Harar who were the lower farming class as opposed to the Adaris who were merchants and owners of khat plantations, and Somalis who were nomads), or Galla farmers to grow. The final breakthrough in the spread of khat to the Gallas was only after the battle of Chellenko in 1887, a battle in which many Adaris lost their lives fighting against emperor Menelik II. Their widowed wives, unable to tend to the khat, took the Kotus as tenants. The Kotus thus gained access to khat; thence began the spread of khat on a wider scale (Getahun and Krikorian, 1973).

1.3.) Khat : Coffee's rival

A review of the literature would not be complete without discussing the history of khat in relation to coffee. Baron Silvestre de Sacy (1826) in his "Chrestomathie Arabe, ou extraits de divers ecrivains Arabes tant en prose qu'en vers", quoted the work of Abd al-Kadir al-Djaziri in which the differences between khat and coffee were clarified :

> "...they made coffee of the vegetable substance called ***cafta*** which is the same as the leaf known under the name Kat, and not of ***bunn*** [the coffee seed] nor the shell [the husks] of ***bunn***. The use of this beverage did not stop and extended in course of time as far as Aden. It was then that the Sheikh Dhabhani advised those who had become his disciples to try the drink made from ***bunn***, which was found to produce the same effect as the kat inducing sleeplessness, and that it was attended with less expense and trouble. The use of coffee has been kept up from time to present." (Krikorian, 1984).

It should be noted here that De Stacy comments that the words ***cafta*** and ***kat***, although appearing similar, are in fact distinguishable in that ***cafta*** refers to the preparation made from the leaves of the tree or ***kat***. (Krikorian, 1984).

As testimony to khat's phenomenal rise in importance, are the reports from travellers at different times. If the use of khat was widespread in Carsten Niebuhr's day, by the time Paul Botta arrived it was even more so. By the time Albert Deflers finally made a comprehensive botanical collection expedition to Yemen in 1887, its use was rife. Deflers observed that although khat was generally ignored in Hedjaj and Jeddah it was being used extensively by inhabitants of Yemen and Hadramaut at vigils for the dead and at feasts and ceremonies celebrating births, circumcisions and marriages. That the use of khat had developed into a highly organised and sophisticated industry is evident from the observation that:

> " Numerous caravans loaded with this merchandise which is nearly as precious as tea arrive daily from the interior to the coast and the sole city of Aden receives each year more than 1000 camel loads. A load consists of certain number of spindle shaped packets, each containing forty leafy branches, tightly compressed and enveloped in a casing of palm leaves pressed with care, to prevent their drying out. The value of these bundles on the coastal markets varies from 0 francs 60 to 0 francs 80" (Krikorian, 1984).

An account published in the "Bulletin de la Societe' Khediviale de Geographie" by Mohammed Moktar, an officer during the Egyptian occupation of Harar gives a clear indication of the establishment of the khat session and the entrenchment of the khat habit in Muslim communities:

> "Towards nine o'clock in the morning, all the guests go to their hosts; there they sit in a circle and begin to read the first chapters of the Quran, and address all sorts of praises to the Prophet. This done, the master of the house gives each a fistful of kat leaves which they chew in eager rivalry, in order to be able to swallow them more easily. If the master of the house is rich, they drink milk; if he is poor they drink water.

After this the same ceremony begins again, reading the Quran, praises to the Prophet, receiving and chewing a fresh fistful of kat, and this goes on until 11 o'clock." (Krikorian, 1984).

Testimony to khat's side effects is given by the historian Ibn Fadl Allah al- Umari, in his "Masalik al-Absar", (The Voyages of the Eyes in Kingdoms of Different Countries) written between 1342 and 1349 A.D. and described khat as one of the trees of Abyssinia which:

"...bears no fruit, but one eats the young tender shoots. It stimulates cleverness and gives happiness; it allows one to almost go without eating, drinking or having sexual intercourse. Everyone eats it, but especially those searching for knowledge, or those who have serious problems, or those who seek to prolong their wakefulness so as to make a journey or carry out work." (Krikorian, 1984).

1.4.) *Catha edulis* in early European literature

The earliest report of khat by a European was made by the French orientalist Barthelemy deHerbelot de Molainville (1625 – 1695). In a work entitled "Bibliothique Orientale". de Herbelot gave a rather confused description of what could only have been khat. Under the heading "Cahuah & Cahveh" (coffee) he says:

"There are three kinds of drinks that bear this name. The first is called Cahuatal Catiat [a corruption of what is presumed to be Khat coffee] of Caftah; the second Cahuat al Caschriat [a beverage from the mesocarp or husks of coffee fruit] and the third Cahuat al Bunniat [coffee from the seed or beans]...The first sort is made with a seed which is unknown to us, which has been forbidden by the doctors of the law in the province of Yemen which is Arabia Felix.... because it is too strong and effects the brain." (Krikorian, 1984).

Carsten Niebuhr in his book "Arabia and other countries of the East" (1778) made the first reference to *Catha* as a taxon, and clarified its status relative to coffee:

> "*Catha* is one of those new genera peculiar to Arabia. This tree which is improved by culture, is commonly grown amongst the coffee shrubs in the hills where these grow." (Krikorian, 1984).

Niebuhr also sheds some light on its widespread use; describing khat as the buds of a certain tree which the Arabs chew constantly:

> "...they are as much addicted to this practice, as the Indians to that of chewing betel. To their kat they ascribe the virtues of assisting digestion, and of fortifying the constitution against infectious distempers. Yet its insipid taste gives no indication of extraordinary virtues. The only effects we felt from the use of those buds were the hindrance and the interruption of our sleep." (Krikorian, 1984).

A great deal of attention seems to have been paid to khat in the European literature immediately after Forsskal or Niebuhr's books were published. It was only about twenty years after Forsskal's death that Martin Vahl, Professor of Botany at the University of Copenhagen, worked on the botanical publications and published them in "Symbolae Botanicae" (1790 – 1794). In his scientific arrangement of the flora, Vahl placed Forsskal's *Catha* in the genus *Celastrus* L. and designated khat as *Celastrus edulis* (Krikorian, 1984).

Paul Emilie Botta, a physician in the service of Muhammad Ali, Viceroy of Egypt, was one of the first to provide more than superficial descriptions of how khat was grown, marketed and used. The method of cultivation of khat has changed little since his description was written:

> "The environs of Hague are extremely well cultivated; the terraces are well maintained, built from stones collected from the soil which they were destined to support, and one irrigates them by means of cisterns which retain the waters from small streams coming down after each rain torrent. One cultivates wheat, barley, maize, fruit trees there but

> mainly kat. This tree comprises the most important cultivation of Jebel Sabir, and it is that plant which attracts all the attention of the inhabitants. One plants it from cuttings, and it remains for three years without care, except for manuring and irrigation of the land when necessary. On the third year, all the leaves are removed except for the buds at the end of each shoot, which the following year will develop into young shoots. They are then cut and sold under the name of "kat mobarreh". This is of inferior quality. The following year, new twigs are induced on the branches thus truncated, and are cut and sold under the designation "kat methani" or the second cutting. This is the most valued and therefore the most expensive. The young leaves and twigs are very tender, and have a taste rather like that of fresh hazel nut [*Corylus avellana*]." (Krikorian, 1984).

Botta also noted that freshly cut material was found to be very intoxicating all be the effects short - lived (Krikorian, 1984).

As already noted there is some controversy as to the country of khat's origin. According to Botta khat had its origin in Ethiopia as he noted :

> "These are the shoots of a tree (*Celastrus edulis*), originally from Abyssinia, as is coffee, and which they grow with great zeal." (Krikorian, 1984).

Achille Richard editing the botanical documentation of a French expedition to Abyssinia in 1839 - 1843, assigned the specimens collected in the modern Tigre and Shoa provinces of Ethiopia to the genus *Catha* and rejected Martin Vahl's contention that the plant should be in the genus *Celastrus*. Richard also notes that the Abyssinians called the plant "Tschut" or "Tschat" or "Tschai", this seems rather confusing as "Tschai" is simply the word for tea (Krikorian, 1984).

In September 1841 the British sent Sir William Cornwallis Harris from India to the ancient Christian highland Kingdom of Shoa. Sir William dwelt considerably on khat's use as a substitute for tea and even likened it to yerba mate' or Paraguay tea (*Ilex paraguariensis* A. st-Hill.); which was only recently introduced from South America to England. It was from this that the designation Abyssinian tea seems to have become ingrained in the European mind (Krikorian, 1984).

Richard Burton, the first European to enter the forbidden city of Harar in Abyssinia, in his "First Footsteps in East Africa" (1856), provides a detailed account. He seems to have tried enough khat to draw conclusions between that grown in Yemen and that grown in Harar. He describes an occasion in Harar where khat was being used:

> "The Grandees were eating kat, or as it is here called Jat. One of the party prepared for the Prime Minister the tenderest twigs of the tree, plucking off the points of even the softest leaves. Another pounded the plant with a little water in a wooden mortar; of this paste, called "Al-Madkuk", a bit was handed to each person, who rolling it into a ball, dropped it into his mouth. All at times, as is the custom, drank cold water from a smoked gourd, and seemed to dwell upon the sweet and pleasant draught. I could not but remark the fine flavour of the plant after the courser quality grown in al Yaman.... [Arabs] declare that, like opium eaters, they cannot live without the excitement. It seems to produce in them a manner of dreamy enjoyment ...The people of Harar eat it every day from 9 a.m. till noon, when they dine and afterwards indulge in something stronger – millet beer and mead." (cf. Burton, 1856, *et seq*; 1966).

2.) History of *Catha edulis* in South Africa

Whether the distribution of *C. edulis* in Southern Africa is as a result of natural or man-induced distribution factors can only be speculated. The earliest record of *C. edulis* in South Africa was in a collection of Burchell in 1814 in which he referred to it as "Bosjemansthee..." (Smith, 1966).

In 1832 Ecklon and Zeyher, while collecting plants in the region of the Zwartkei Rivier, did not make use of its common name in as many words but made unmistakable reference to it in their records under *Methyscophylum glaucum* (*Methyscophylum* is derived from two Greek words meaning intoxicating leaf).

Pappe' whose record is most probably that of Ecklon and Zeyher recorded in 1847 that:

> "The leaves of this shrub are infused as tea, which is of a sweet and pleasant taste, and used in cough, asthma, and other diseases of the chest. It grows about the Zwartkeirivier, where it is a favourite beverage with the Bushmen and others, who also chew it, and call it Bosjemansthee." (Smith, 1966).

Drege, who collected specimens at Windvogelsberg, in the same year also noted its use as a tea and Ernst Meyer named it *Hartogia thea* but never described it (Smith, 1966).

Sonder in " Harvey and Sonder Flora Capensis" gave the following information also under the name of *Methyscophylum glaucum* :

> " This is the Bosjemans'-thee of the colonists. The leaves chewed to excess by the Bosjeman have intoxicating effects; a moderate infusion is said to be as good as tea, and also as a remedy for asthma. The properties, perhaps, are similar to those of the Paraguay tea, a shrub not distantly related to the present." (Harvey and Sonder, 1859 – 1860).

Sir Walter Stanford, then commisioner to the Eastern Cape, noted in 1886 that the San peoples chewed the leaves and young shoots of *C. edulis* at Umnga in the Tsolo district (Macquarrie, 1962), and it was remarked to him that it was done to revive them of hunger and fatigue.

According to Stow, the Bushmen used the leaves as a nourishing food; with a bitter taste when chewed, but with a pleasant flavour, combined with a strong taste of liquorice. The leaves, he added, were said to contain such life-sustaining powers that during the season the Bushmen were not only able to subsist for a long time on the nourishment alone, but, as they termed it, became fat and strong on the invigorating diet (Stow, 1905).

Prins (1994) has noted that during the years of the incorporation of San into Mpondomise culture that some half-starved San would wait in their shelters for the Mpondomise to bring them food in exchange for rain. The San would keep themselves awake, and ease the hunger pains, by chewing the leaves of a stimulant, ***iqwaka*** (*Catha edulis*).

The earliest reference to chewing *C. edulis* was in connection with Madolo, the San chief of Freemanton, recorded by Thomas Baines in 1848. In this Baines writes that from the corners of Madolo's mouth:

> "...oozed the green juice of an intoxicating leaf he chewed incessantly..." (Baines, 1964).

2.1.) Present day use of *Catha edulis* in South Africa

Three species of *Catha* are recognised in South Africa, viz.; *C. edulis, C. transvaalensis* and *C. abbottii*. There exist no records for the past or present utilisation of either *C. transvaalensis* or *C. abbottii*. However, *C. edulis* has long been used as a stimulant in the Eastern Cape and medicinally in Kwazulu-Natal.

In the Bolo River area of the Stutterheim district in the Eastern Cape Hirst (1997) noted that *C. edulis* was used by the local Xhosa inhabitants :

> "There we met the Xhosa maid...she explained that she particularly liked to chew the moist, succulent leaves which imparted ***amandla*** [power or energy] to the user... and that the Xhosa called ***igwaka*** or ***ikambi***..." (Hirst, 1997).

It was also noted that the use of *C. edulis* is very much ingrained in the daily routine of these people in which it was chewed by men and women in the mornings before going out to work. Through its use the labourers were found to be enthusiastic and tireless workers happily tolerating the hot African sun.

Hirst also reported how *C. edulis* is used in the Eastern Cape in a similar fashion to that in East

Africa:

> "The ladies were outside sewing and chatting in the shade of an acacia tree. Resting on an empty plastic packet was a large pile of fresh, yellowy-green and reddy-green *Catha edulis* leaves. They were chewing small amounts of leaves sedately... they invited me to sit down and join them in the shade to chew some leaves... the ladies had a tin beaker of cool water on hand to drink." (Hirst, 1997).

Not all Cape Nguni farm inhabitants, residing in relative proximity to the growing sites, chew ***igwaka / ikambi***. Although ***igwaka / ikambi*** is not traded for cash, it nevertheless remains a valuable gift. An informal network of friends and relatives connects users further afield with those residing in closer proximity to the growing sites (Hirst, 1997).

During periods of drought and the dry winter months, the *C. edulis* trees and shrubs die back completely until resuscitated again by the next good rainfall. The two main seasons in the year for picking material are in early summer and autumn respectively, periods that tend to coincide with rainfall in the area (Hirst, 1997).

Igwaka / ikambi serves as more than just a stimulant in the Xhosa communities; outings for its collection are undertaken by groups involving people from all tiers in the community. These foraging parties allow for social interaction with news and anecdotes being exchanged between participants.

The collection of *C. edulis* serves the community with more than just material on which to chew but also provides them with an adventurous interlude in the routine of daily life; Hirst notes this and comments:

> "Important, too, are the feelings of *communitas* and commensality between the participants, who share tobacco, interesting news and anecdotes and even assist each other through the bush and up the slopes...Indeed, as ***igwaka / ikambi*** is both a means of generating and reinforcing social relations, there is more to simply chewing it."

(Hirst, 1997).

The actual use of ***igqwaka / ikambi*** also lends itself to the fact that amongst regular users it plays an important role as a social and recreational stimulant - it is used to stimulate social interaction and increase the pleasure and enjoyment of conversation (Hirst, 1997).

As in East Africa where the fresh young leaves are preferentially utilised, so too is the case in the Eastern Cape. In order to preserve the freshness of the material a pair of freshly sprouted buds together with a pair of developed leaves are picked, these developed leaves may be discarded later if considered too dry for chewing (Hirst, 1997).

In connection with the folk lore account of a Yemeni goatherd who observed the effects of *C. edulis* on his goats and hence his own discovery of the effects; Hirst relates that:

> "... an Mbalu chief residing near Stutteheim told me that this is how the stimulating effects of ***igwaka*** were first discovered. However, when I repeated this tale in the company of some long-term adult male "***igwaka***" chewers, I was roundly told that the practice actually disseminated from the abaTwa hunter-gatherers, who once occupied the Bolo-Henderson Valley area." (Hirst, 1997).

As for its mode of consumption, the young succulent buds are masticated in the mouth and well sucked before being discarded, the stimulant effects becoming apparent after drinking cold water.

Hirst (1997) also notes that although some of the old Xhosa-speaking men and women brew a tea from *C. edulis* leaves, this is not the regular practice, but is only done occasionally in the dry season when the leaves are too dry and leathery to chew. Most users prefer to chew the fresh material.

3.) Present day use of *Catha edulis* in East Africa

The khat habit has, especially in Yemen, a deep rooted social and cultural function (Weir, 1985) and is an appropriate tool for enhancing social interaction. The khat session also plays an important role at weddings and other family events (Nigg and Siegler, 1992). In East Africa (Ethiopia, Djibouti, and Kenya), the psychosocial benefits of khat consumption are of secondary importance. It is rather the pharmacological action that induces the use of this drug (Kalix, 1988). Khat is frequently used in these countries during work by craftsmen, labourers, taxi and bus drivers, and especially by farmers in order to reduce physical fatigue (Getahun and Krikorian, 1973).

The shoots at the tips of the branches are cut in the early morning, bundled, and then usually wrapped in leaves of banana (*Musa x paradisiaca* L.) or leaves of false banana (*Ensete ventricosum* Cheesm.), damp papers or plastic to avoid drying and wilting.

3.1.) Cultivation

The main areas of commercial cultivation have traditionally been in the Hararge province of Ethiopia, the slopes of Taizz in Yemen, and the highlands in the Meru district in Kenya. While khat is distributed in the wild along the coastal interior of East Africa from Yemen to the Cape it is also cultivated for economic gain in many places, such as the Arabian peninsula, Ethiopia, Zambia and Somalia. In Ethiopia it is grown in several regions and also in parts of Eritrea and Arusi. Hararge is however the principal region of khat cultivation (Getahun and Krikorian, 1973).

Morphologically distinct types of khat are recognised by the farmers. These include, ***Dallota***, which is predominantly white or light green; ***Dimma***, which is red-leaved; ***Hamercot***, which is intermediate between ***Dallota*** and ***Dimma***, but more comparable with ***Dimma***; ***Gohoba***, which is much like ***Dimma*** but with recognisable differences, and ***Mohedella***, green to olive in colour (Getahun and Krikorian, 1973).

In its growth *C. edulis* is quite adaptable to a variety of ecological conditions. It grows well under a wide range of soil types and climatic conditions; terraced plantations being found on fairly moist slopes and hillsides at altitudes of 1500 to 2500 m above sea level. In Ethiopia, sorghum, corn, and sweet potatoes are generally intercropped with khat. Even though the plant flowers and bears fruit, the seeds are not used for propagation. The breeding of khat is done vegetatively from suckers or branches arising near the ground level (Getahun and Krikorian, 1973). The first harvest usually begins 5 to 8 years after planting.

3.2.) Varieties of khat

C. edulis is very polymorphic, especially concerning the shape, size, and colour of its leaves (Krikorian, 1984).

In Ethiopia, three dominant types referred to as "white," "intermediate" or "yellow" and "red" are distinguished (Geisshusler and Brenneisen, 1987; Getahun and Krikorian, 1973). In other regions, additional types may be found (Krikorian, 1984). Usually, these types also differ in quality and psychotropic potency (Geisshusler and Brenneisen, 1987).

It is doubtful that there exist real varietal differences in cultivated khat. People generally distinguish between "red", "yellow" and "white" khat; on the market there are however at least seven types of Khat. These differences being related to cultural practices and to ecological differences.

Although it is not the intention of this review to examine the status of the taxonomic literature of *C. edulis*, it should be noted that most authors and botanists accept the description given by Coates-Palgrave (1977). However, Krikorian (1984) has expressed his concern over the fact that plants may bear alternate or opposite leaves, and that descriptions never include an explanation for the presence of shoots with opposite leaves and shoots with alternate leaves on the same plant.

4.) Indigenous names for *Catha edulis*

Whereas the local names in countries with Muslim tradition are variants of the words ***cat*** or ***kat***, black

Africans have their own designations. The Kikuyu name ***miraa*** or ***muirungi*** is by far the most common. ***Murungu*** is the Kiswahili word for Khat. The Meru people call it ***muraa***, the Kamba ***miungi*** or ***mirungi***, the Masai ***ol-meraa***, the Shamba ***muandama*** or ***mwandama***, the Ndorobo ***tomayot*** (Krikorian, 1984).

In Tanzania, khat is known as ***muhulo*** and in Uganda, as ***musitate*** (Nigg and Siegler, 1992). The plant itself is known by many names depending on the geographical area. The most common term is the Arabic name, ***khat*** sometimes also spelled ***chat***, the way it is pronounced in most of Ethiopia. In some literature, khat is also called Abyssinian, Arabian, or Somali tea (Getahun and Krikorian, 1973), but today ingestion by chewing is more popular.

In Zimbabwe, the Shona call it ***mutsvahari***, and the Ndebele ***inandinandi*** (Gelfand *et al.*, 1985). In Xhosa the abaTwa refer to the fresh leaves of *C. edulis* as ***igwaka*** or ***ikambi*** (Hirst, 1997), while the Zulu name it ***umhlazizi*** or ***umhlazi*** (Hutchings *et al.*, 1996).

5.) Pharmacology

5.1.) Medicinal uses of *Catha edulis*

Medicinal virtues were ascribed to khat in Forsskal's day. In the Harar area of Ethiopia which is traditionally thought of as the centre of the origin of its use, khat is still believed to effect 501 different kinds of "cures"; this equals the numerical value of its Arabic name Ga-a-t (400 + 100 +1). There are Muslim traditions that emphasise that khat is holy and has been held so for a very long time (Getahun and Krikorian, 1973). Leiris described spit from chewed Khat being spat on various parts of individuals who were ill, in Ethiopian magico-religious rituals. A more conventional medical manuscript recommends the use of khat against blennorrhoea (excessive secretion and discharge of mucus) (Krikorian, 1984).

Hutchings *et al.* (1996) in her book "Zulu Medicinal Plants: An Inventory" reports that the Zulu name for *C. edulis* is ***umhlawazizi*** or ***umhlwazi*** and that the Zulu medicinally use bark decoctions as nerve tonics, heart stimulants and also to stimulate the appetite. Bark is boiled in water for ten minutes and doses are restricted to two tablespoonfuls a day. It is also reported to be used for flatulence (Gerstner, 1939).

In Zimbabwe *C. edulis* is used for treating boils, infertility in men and as a lucky charm (Gelfand *et al.*, 1985).

In "Medicinal and Poisonous Plants of Southern and East Africa", Watt and Breyer-Brandwijk (1962) write:

> "In South Africa the leaf and twig have been used chiefly by the Bushman, who making a stimulating beverage from the leaf as well as from chewing the young shoot as a stimulant ....In Arabia and Abyssinia the leaf has been used as a protection against pestilence and in Arabia especially against bubonic plague ....In Tanganyika the leaf and the root are used as an influenza remedy , the root for stomach troubles and the leaf to improve dental health. In South Africa in the early days it has been used for cough, asthma and other diseases of the chest. It has been erroneously suggested that *Catha edulis* may be useful in the treatment of impaired memory. Extracts of the plant have given negative antibiotic tests." (Watt and Breyer-Brandwijk, 1962).

Adult Xhosa speaking users point out that the fresh leaf material can be eaten as a substitute for food without suffering gastric discomfort or constipation, although they mention that this practice must not be kept up for too long as it results in a loss of weight and emaciation. Thus, some overweight Xhosa-speaking woman use it in order to try to lose weight. Some adult male users regard the fresh material as an asexual analeptic, while some adult female uses regard it as a remedy for menstrual pain. Fresh leaf material is also used medicinally to relieve asthma as well as mild fevers associated with influenza and colds. Some users also associate use of fresh material with longevity and as affording protection from snakebite. Apparently, according to local Xhosa oral tradition, the hunter-gatherers also ate the fresh material as a remedy for dysentery. Some adult Xhosa-speaking users point out that an infusion of the roots and/or bark in cold water is an excellent remedy for diarrhoea particularly amongst children (Hirst, 1997).

5.2.) Deleterious effects of chewing khat

The pleasurable as well as the unwanted effects of khat chewing are very similar to those induced by amphetamine consumption, the differences being quantitative rather than qualitative (Eddy *et al.*, 1965; Hodgkinson, 1962; World Health Organization, 1964).

Self-experiments of Alles *et al.* (1961), and Hughes (1973), have shown that the effects of one khat portion were similar to that of 5 to 10 mg amphetamine. The syndrome induced by khat chewing is mainly characterised by stimulating effects on the central nervous system (CNS) and various peripheral sympathomimetic effects. The social environment in which khat is consumed. for example the setting of a typical khat session, plays a certain role in the development and type of the psycho-activity of this drug (Kalix and Braenden, 1985). The CNS action of khat gives, particularly to habitual users, the subjective and euphorigenic feeling of increased energy, well-being, mental alertness, and self-confidence. It improves the ability to communicate and enhances the imaginative ability and capacity to associate ideas (Elmi, 1983; Kalix, 1988; Kalix and Braenden, 1985). Objectively, khat induces a state of mild euphoria and excitement characterised by loquacity and sometimes hyperactivity (Kalix, 1988; Kalix and Braenden, 1985), and hypomania (Margetts, 1967).

Late and unwanted effects of khat consumption are sleeplessness and the subsequent disruption of day-night cycle (Kalix, 1988) as well as nervousness and nightmares (Elmi, 1983; Kennedy, 1987; Pantelis *et al.*, 1989). In exceptional cases, khat may induce toxic psychosis, probably by enhancement of a sub-acute prepsychotic or psychotic condition (Kalix and Braenden, 1985). Several case reports of khat-induced toxic psychosis describe the observed symptoms as manic-like, schizophreniform and paranoia (Carothers, 1945; Critchlow and Seifert, 1987; Dhadphale *et al.*, 1981; Giannini and Castellani, 1982; Gough and Cookson, 1984; Heisch, 1945; McLaren, 1987). Other serious psychic effects are reactive depression, anxiety, and irritation (Elmi, 1983).

Khat induces moderate but often psychic dependence. However, in contrast to amphetamine abuse, no physical dependence or tolerance to the CNS-effects have been observed (Eddy *et al.*, 1965).

Hirst (1997) reports that although most chewers use ***igwaka / ikambi*** on a regular basis, particularly during the seasons when the fresh material is available, it appears to be neither addictive nor associated with any physical withdrawal syndrome. Migrant workers who move to urban centres give up chewing quite readily and report no adverse effects. During the dry seasons most regular users give up chewing, owing to the lack of fresh material, also without suffering physical or psychological side-effects.

No cases of psychosis related to *C. edulis* use could be found by Hirst within his study area, and although investigated in detail, no evidence of behavioural problems associated with the users were reported by the farmers, who generally regard chewing ***igwaka / ikambi*** as a rather harmless practice (Hirst, 1997).

Insomnia is the only side-effect mentioned in connection with the regular use of the fresh leaf material. Nevertheless, it is a common and widely reported side-effect suffered, at one time or another, by most regular uses. Some knowledgeable long-term users point out that insomnia results from chewing ***igwaka / ikambi*** on an empty stomach and can be avoided simply by eating a light meal before chewing. Sedatives, *Cannabis* or alcohol are not generally used as antidotes for insomnia (Hirst, 1997).

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 this drug does not seem to occur. Heavy khat users experience true, but not physical, withdrawal symptoms. They consist of lethargy, mild depression, slight trembling, and recurrent bad dreams (Giannini *et al.*, 1986; Halbach, 1972; Kennedy, 1987; Kennedy *et al.*, 1980; Luqman and Danowski, 1976). Peripheral side effects of khat include cardio-vascular effects of the sympathomimetic type, like arrhythmia, tachycardia (Elmi, 1983), and dose-dependent increase of blood pressure (Halbach, 1972; Kalix and Braenden, 1985; Nencini *et al.*, 1984). 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 (Nencini *et al.*, 1984). Another sympathomimetic effect of khat is mydriasis (Halbach, 1972; Margetts, 1967; Nencini *et al.*, 1984) which, along with a staring look and the brownish staining of the teeth, is characteristic for the khat habit (Luqman and Danowski, 1976).

Side effects of khat on the digestive tract are mainly due to the high tannin content. Inflammations of mouth, gastric disturbances, and constipation often resulting in the high use of laxatives (Pantelis et al., 1989) are common among khat users (Elmi, 1983; Getahun and Krikorian, 1973; Halbach, 1972; Kalix and Braenden, 1985; Kennedy *et al.*, 1983; Luqman and Danowski, 1976). That the polyphenolic tannins increase the probability of oesophageal cancer is not proven (Kalix, 1987; Morton, 1980). Respiratory problems often result from associated heavy smoking during khat sessions (Kennedy *et al.*, 1983). Furthermore, khat consumption may cause spermatorrhoea, impair male sexual function, and after long-term use, lead to impotence (Halbach, 1972; Margetts, 1967). Reduced birth weight of babies (Kalix, 1987) and inhibition of lactation (Luqman and Danowski, 1976) have been observed in khat-chewing pregnant women. As amphetamines, khat also increases the adrenocorticotrophic hormone and the growth hormone (Nencini *et al.*, 1983).

Dry leaf material is widely held to cause headaches, gastric disturbances and efficacy. Thus, its use is contraindicated. It is also widely recognised in the local community that the pharmacological effects of moist *C. edulis* stems and leaves tend to nullify the effects of alcohol (Hirst, 1997) :

> "Far from being hearsay, I have experienced this at first-hand. So, if one enjoys sorghum beer or liquor, it is best to avoid the use of ***igwaka / ikambi***." (Hirst, 1997).

Animal studies indicated that cathinone is the dependence-producing constituent of khat. Cathinone is recognised as the most potent CNS-alkaloid, however, with their considerably higher concentrations in khat compared to cathinone, norpseudoephedrine and norephedrine are mainly responsible for the sympathomimetic side effects of khat on the cardiovascular system. The recently-found khat alkaloids of the phenylpentenylamine type play only a minor role concerning the psychoactive effects of khat.

Kalix *et al.* (1987a,b) have shown with *in vitro* experiments a very low effect on the release of catecholamines from central and peripheral presynaptic storage sites. Other sesquiterpene ester alkaloids with related structures to cathinone have not yet been pharmacologically tested.

6.) Chemical analysis of *Catha edulis*

The chemistry of khat has been an intriguing puzzle to both plant chemists and pharmacologists for more than a hundred years. The plant is alkaloid-containing; but in contrast to the situation with other alkaloid-containing stimulant plants, knowledge of khat's chemistry has progressed slowly and its interpretation has remained fairly controversial until about 20 to 25 years ago. Despite the progress achieved since the first attempt to characterise the active constituents, the actual active principle was either overlooked for various reasons, including poor quality of the starting material, inadequate isolation procedures, and insufficient purity of the final product. Thus, until recently, the characteristic stimulant activity of the fresh plant material could not be fully explained in terms of the then known khat components.

The chemical study of khat dates back to 1887 when Fluckiger and Gerock, searching for caffeine as the possible stimulating principle, found no traces of it but discovered instead an alkaloid they named katin. Mosso in 1891 extracted from the plant a basic fraction with stimulant-like properties and called it celastrine (Nigg and Siegler, 1992). However, the first comprehensive study on khat was carried out at the end of the century by Beitter who obtained crystalline salts of a substance he concluded was identical to both Fluckiger's katin and Mosso's celastrine. The chemical composition of khat was next studied by Stockman in 1912, who described the three distinct alkaloids, cathine, cathinine, and cathidine, without characterising them structurally. Although the cathine he described was in all probability identical to the substance found by former workers, neither cathinine nor cathidine was later identified as an individual substance (Nigg and Siegler, 1992). An important step forward was the contribution of Wolfes in 1930, who, using a technique similar to Beitter's, detected the presence of (+)-norpseudoephedrine in khat and concluded that this substance corresponded to katin. This alkaloid is also referred to as cathine (Kalix and Braenden, 1985; Schorno *et al.*, 1982). He also observed the presence of water-insoluble base which, like Stockman's cathidine, can be regarded as an impure representative of the polyester-type khat alkaloids. In subsequent studies, it was repeatedly stated that (+)-norpseudoephedrine was the main if not the only phenylalkylamine-type constituent present in the plant. Whereas Alles *et al.* (1961) concluded that cathine was the only extractable base present in substantial amounts in the plant, several authors were able to demonstrate the presence of other alkaloid compounds (Nigg and Siegler, 1992). Depending upon the extraction and chromatographic procedures, Paris and Moyse (1958) detected three to six alkaloids and suggested that one of the components might be ephedrine.

Rucker *et al.* (1973), using gas-liquid chromatography/mass spectrometry (GLC/MS), indicated the absence of ephedrine but the presence of seven nitrogen-containing substances in the basic fraction. With the exception of cathine, none were adequately characterised. The assumption that the characteristic stimulating effects of khat could be satisfactorily explained by its cathine content and that the chemistry of khat might be more complex was first questioned by Von Brucke in 1941. Based on simple pharmacological experiments, he felt that the stimulating effect of cathine alone was too small to account for the effect of the fresh plant (Nigg and Siegler, 1992). This suggestion was supported by the fact that consumers show a preference, and pay a higher price, for fresh khat. Most of the earlier chemical and pharmacological studies were performed on dried plant material of varying quality.

The first serious attempt to substantiate the psychoactivity of the fresh dug was undertaken by Friebel and Brilla in 1963, who searched for a specific substance in the fresh plant that might have a greater activity than cathine. Using a combination of chemical and pharmacological methodology, they compared the effect on locomotor activity of synthetic (+)-norpseudoephedrine oxalate with that of the oxalates prepared from freeze-dried and air- and sun-dried khat samples. The three preparations had qualitatively similar effects, but the oxalate from the freeze-dried plant sample showed a stronger effect on the locomotor activity. Differences were also found in the physical and chemical characteristics of the samples, and the authors concluded that the substance isolated from the freeze-dried plant was a cathine-like compound, possibly a labile precursor of cathine, for which no correct structure could be proposed on the basis of the data available (Nigg and Siegler, 1992).

It seems evident now that Stockman's cathidine was the earliest finding of a second class of nitrogen-containing khat constituents: weakly basic, fairly lipophilic polyester alkaloids that were repeatedly overlooked by most investigators because of their non-typical alkaloid nature. One of these compounds, named cathidine D, was isolated in crystalline form and a partial structure proposed for it. It was suggested that this alkaloid, because of its high molecular weight and complicated nature, might be related to alkaloids like evonine and maytoline originating from the same plant family. Unfortunately, no alkaloid structure of this type was known at that time, mainly because of the lack of adequate techniques for structure determination. In 1970 the chemical structures of maytoline and evonine were determined. These structures served as keys for a rapidly increasing number of related structures from various plants of the Celastraceae. On the basis of these structures, Cais *et al.* (1975) proposed two tentative structures for cathidine D. As a result of joint efforts between the Chemistry Department of the Nottingham University (Baxter *et al.*, 1976a,b, 1979a,b; Crombie 1980; Crombie *et al.*, 1979) and Szendrei at the UN Narcotics Laboratory (UN Document, 1976, 1977), a series of new polyester alkaloids named cathedulins were isolated. Until now, no adequate pharmacological testing has been done on these cathedulin-type alkaloids due to their extremely poor water solubility and lack of sufficient amounts of pure material.

Chemical research on khat was taken up again by several groups in the early seventies. The UN Narcotic Laboratory in Geneva, on the recommendation of WHO and the UN Commission on Narcotic Drugs, initiated a research project on the chemical composition of khat involving several major classes of secondary plant products present in the fresh or well-preserved material. A simple thin-layer chromatography (TLC) analysis of the methanolic crude extract clearly showed that, besides varying amounts of (+)-norpseudoephedrine and other minor amine-type components, an unidentified compound was present in large quantities. This was separated and rapidly identified as (-)-K-aminopropiophenone. For this new compound, the trivial name cathinone was proposed (UN Document, 1974, 1975).

Independent from the UN group, research work on khat was started by Schorno. The pharmacognostical and phytochemical thesis, finished in 1979, brought forward important new knowledge about the analysis, stereochemistry, and synthesis of the khatamines (Nigg and Siegler, 1992). The three main khat phenylpropylamines were isolated and identified as S-(-)-K-aminopropiophenone (cathinone), S,S-(+)-norpseudoephedrine (norpseudoephedrine) and R,S-(-)-norephedrine (norephedrine).

Cathinone as a ketoamine base is extremely unstable. Withering, drying, and cleanup of the plant material result in various degradation products or artefacts. Enzymatic reduction transforms cathinone into the less active norpseudoephedrine and norephedrine. Thus, a fresh drug may contain one hundred times more cathinone than dried material, which in turn shows an increased content of norpseudoephedrine and norephedrine. The oxidation product 1-phenyl-1,2- propandione can even be detected in the essential oil from fresh plants.

It is also possible that this diketone is formed by enzymatic desamination or by photolysis. The cathinone dimers 3,6-dimethyl -2,5-diphenylpyrazine and its dihydro derivatives, however, are purely artifacts of the isolation. The instability of the khat principle cathinone explains why nearly 100 years of chemical research were needed to identify this rather simple component and explains why khat users insist on fresh drug material. Due to the easy enolization of the keto group, cathinone racemizes quickly, particularly as a free base and in polar solvents. In contrast, its salts (e.g., oxalates) are stable in solid form.

Three new phenyl-pentenylamines have subsequently been isolated from fresh plant material which was cultivated in the Meru area, North Kenya, sold in Nairobi's khat street market, and identified as S-(+)-merucathinone (merucathinone), S,S-(-)pseudomerucathine (pseudomerucathine), and R,S-(+)-merucathine (merucathine) (Brenneisen and Geisshusler, 1987; Brenneisen *et al.*, 1984; Geisshusler and Brenneisen, 1987). Merucathine, the trans-olefinic cathinone analogue. has already been postulated by Szendrei (1980) as cinnamoylethylamine, based on GLC/MS data. (-)-N-Formylnorephedrine was identified in Saudi Arabian khat (Al-Meshal *et al.*, 1986).

Khat samples from the most important markets of Ethiopia, Kenya, North Yemen, and Madagascar were analysed by HPLC for their alkaloid content (Brenneisen and Geisshusler, 1985; Geisshusler and Brenneisen, 1987). The aim of these studies was not only to screen for psychoactive alkaloids but also to investigate its distribution in the plant tissue and the influence of origin, type, age, and time of harvesting on the variation of the phenylalkylamine profiles. Also a possible correlation between cathinone content and khat quality (related to "psychotropic potency" and hence price) as estimated by dealers and consumers, was investigated. Analyses made on different parts of Kenyan khat trees showed that the absolute content and the percentage (relative to the total khatamine content) of cathinone varied with the age of the respective plant part. The highest concentration of phenylpropylamines was found in young shoots already carrying leaves. In young shoot tops with a high cell differentiation rate, the total khatamine content was lower but the cathinone percentage was significantly higher. In the fully developed leaves the cathinone percentage was, however, reduced. Stem and bark of older branches showed only traces of cathinone, whereas in the bark of the trunk and in the roots, no khatamines could be detected. A considerable variation in the khatamine content, depending on the origin, could be observed. Khat bundles collected from plants cultivated in the Meru region (Kenya) and sold in Nairobi's street market contained, for example, a higher amount of phenylalkylamines and of cathinone than commercial khat samples from Madagascar.

With a very high cathinone content and a very high percentage of cathinone relative to other samples tested, Kenyan khat was the most potent of all samples analysed. Although the percentage of cathinone in Ethiopian khat was low the absolute cathinone content reflected its high potency. Much higher norpseudoephedrine percentages were found than in khat from Kenya so that norpseudoephedrine might participate in the CNS-activity of the drug. The cathinone content of khat originating from north Yemen and Madagascar was significantly lower than that of Kenya or Ethiopia. The quality estimation, especially by Ethiopian and Kenyan consumers, and the price paid for the khat sample correlated with the analytically determined content of the total phenylalkylamines and of cathinone. Although some dealers claim to sell "first quality," real top quality material does not appear on the market as it is consumed by the khat producers themselves. In addition, prices fluctuate in accordance with drug supply, which depends on season, weather conditions, distance from the cultivation area to the market, and efficiency of the transportation facilities.

7.) Chemistry of *Catha transvaalensis*

A preliminary study was made on *C. transvaalensis* by Lehmann *et al.* in 1990 in developing a rapid TLC procedure for the identification of cathinone and norpseudoephedrine, in this study it was found that *C. transvaalensis* was lacking in both cathinone and norpseudoephedrine (Lehmann *et al.*, 1990). It should be noted however that this study was carried out on dried plant material.

8.) Social and economic impact

It has been estimated that each day 2 to 8 million khat portions are chewed world-wide. The average price of a bundle with 50 g fresh, chewable material is about $10 (U.S.) and may climb up to $60 (U.S .) depending on quality as well as supply and demand. The results of the diversion of income are often serious financial problems, followed by neglect of the family needs and disintegration of family structure (Elmi, 1983; Kalix, 1987; Pantelis *et al.*, 1989). Other profound social and economic consequences of khat consumption are due to absenteeism and decreased productivity, frequently leading to unemployment (Kalix and Braenden, 1985). Not all khat users have recreational motives since, particularly among the rural population, the drug is also used for improving work performance and to lessen the feeling of hunger (Kalix and Braenden, 1985). The loss of appetite leads to malnutrition, resulting in acute and chronic diseases, infective and/or deficiency disease (Al-Meshal *et al.*, 1985).

However, khat consumption undoubtedly shows certain beneficial consequences at the social level. It enhances the inter-individual communication and assists structuring social life in rural and urban societies. It plays an important role, not only in family events (celebrations and marriages), but also in business matters and political meetings.

Today, khat circulates freely in most East African countries and Yemen and, in some countries, khat chewing is allowed or tolerated. In others, the use is officially banned, but law enforcement is completely lacking. The prohibition of khat is difficult to enforce and has little effect. Decisions regarding its restriction have largely turned from physiological to psychosocial and, particularly, economic considerations (Krikorian, 1984).

In North Yemen, the khat habit is a socially sanctioned, deep-rooted cultural tradition. Khat is tolerated by the Yemeni religious authorities, while alcohol is not (Kalix, 1987). Fifty percent of male adults are daily khat consumers and attend afternoon khat sessions. Women's sessions are less formal and less frequent (Kalix and Braenden, 1985). About 25% of the daily earnings are spent on purchasing khat (Pantelis *et al.*, 1989). A substantial part of the resources is diverted from back flow money of emigrant workers in Saudi Arabia (Kalix, 1987, 1988). More than 60% of the limited farmland is used for khat cultivation and not for crop production and it has been estimated that the gross agricultural income from khat is about $1 billion (U.S.) (Alhubaishi and Abdel-Kader, 1991).

In Ethiopia, where khat is native, no legal restrictions for khat trade and consumption exist. Khat plantations occupy scarce arable land competing, especially in the Harrar Province, with coffee for well-irrigated terraces as well as with other agro-economical crops like sorghum and corn. In the Harrar district, khat is a high-cash income crop, providing 30 to 50% of the total annual cash income of a family (Getahun and Krikorian, 1973). Khat consumption is practised by high school and university students as well as the general population (Kalix and Braenden, 1985). From the airport at Dire Dawa, khat is exported each day to Djibouti by a special aircraft service, contributing about $20 million (U.S.) per year to the national economy (Kalix and Braenden, 1985).

In Saudi Arabia, khat production, trade, and consumption were banned some 30 years ago by a royal decree, and the ban is still strictly enforced (Kalix and Braenden, 1985). In Somalia, khat trade and use were prohibited in 1983. In Sudan, where khat grows in a small area in the south, cultivation, trade, and use are strictly forbidden. Several other Arab countries, like Kuwait, Egypt, and Morocco, have preventively banned this drug. From Israel, sporadic khat cultivation has been reported since the plant had been introduced by exiles from Yemen (Hes, 1970). In Kenya the British authorities tried, in the early 1930s, to control khat use; but in 1977, President Kenyatta suspended the Miraa Prohibition Act (Krikorian, 1984). Although later the demand for a repressive legislation did not stop, khat is today extensively cultivated around Mount Kenya and sold daily on the important Nairobi khat market in the centre of the city.

In recent years, khat has made its appearance on khat markets in regions far from the areas of cultivation, mainly due to the possibilities of air transportation. Shipments of khat have been observed by custom authorities in France, Italy, Switzerland, Great Britain, and the United States (Kalix and Braenden, 1985). Fresh khat is, for example, sold in London, where a legally non-restricted market and distribution network exists, and New York (Gough and Cookson, 1984, 1987; Kalix *et al.*, 1991; Mayberry *et al.*, 1984; McLaren, 1987).

CHAPTER TWO

SCOPE OF PRESENT INVESTIGATION

1.) Introduction to the *Catha* species of South Africa

Three species of the genus *Catha* are known in South Africa; *Catha edulis* (Vahl) Forssk. ex. Endl. (Figure 1-3), C. *transvaalensis* Codd. (Figure 5) and C. *abbottii* Van Wyk & Prins (Figure 6). The genus *Catha* belongs to the family Celastraceae which contains 80 to 90 other genera and more than 850 species. By far the most well known of the three species is *C. edulis*, whose widespread use, as the drug khat, in East Africa and Yemen has been documented in Chapter One.

1.1.) *Catha transvaalensis* Codd.

In 1965 Robson described a new Celastraceous species, *Lydenbergia cassinoides*, a species currently reported to be confined to a relatively small area in Mpumalanga (Figure 7). He related the new genus to *Catha* and *Cassine* L. (Codd, 1971).

In 1966 Codd considered it preferable to enlarge the circumscription of *Catha* to include *Lydenbergia*, and thus transferred its single species to *Catha* with *C. cassinoides* (N.K.B. Robson) Codd being formed. Unfortunately this name was a later homonym of *C. cassinoides* Webb & Berth. and the new name, *C. transvaalensis* Codd, was proposed; this concept has since been accepted by most authors (Codd, 1971).

C. transvaalensis as described by Coates Palgrave (1977) is a shrub or medium-sized tree up to 9 m in height; occurring in ravines and on rocky hill slopes and mountainsides. The bark is dark brown with the branches at first pale green, later becoming dark reddish brown. The leaves are oblong to elliptic; 25 to 80 x 13 to 50 mm, grey - green above, paler green below with prominent net-veining on the under surface; apex shortly tapering to rounded; base tapering; margin toothed; petiole 12 mm long. The flowers are small, in dense axillary clusters, very similar to those of *C. edulis;* flowering is from April to October. The fruit is a slender three-lobed capsule up to 8 mm long, brownish-green

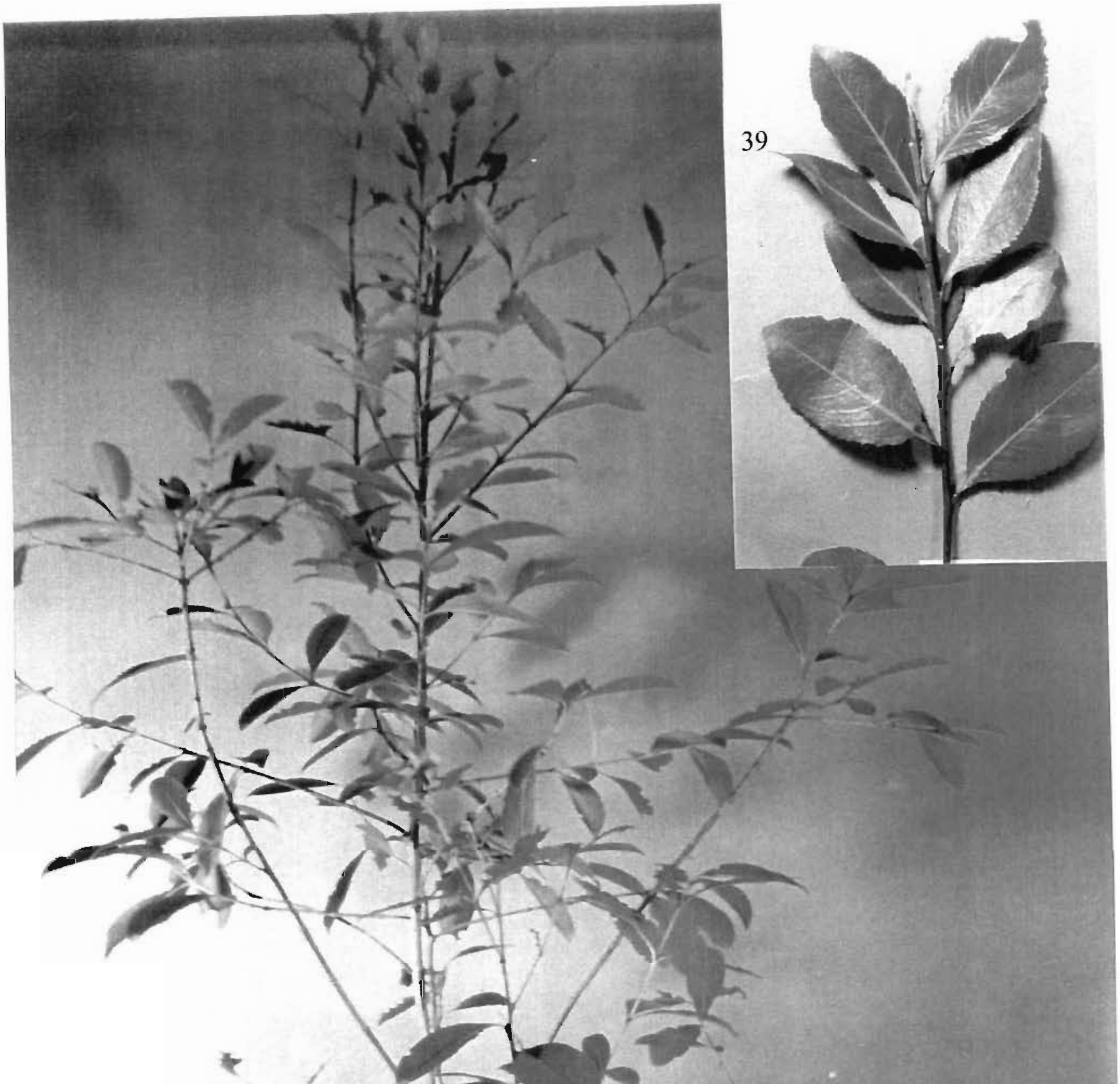


Figure 1: One year old *Catha edulis* sapling grown from a cutting collected from the Durban Botanical Garden. Insert: gross leaf morphology.

Figure 2: One year old *Catha edulis* sapling grown from a cutting collected in Mpumalanga. Insert: gross leaf morphology.

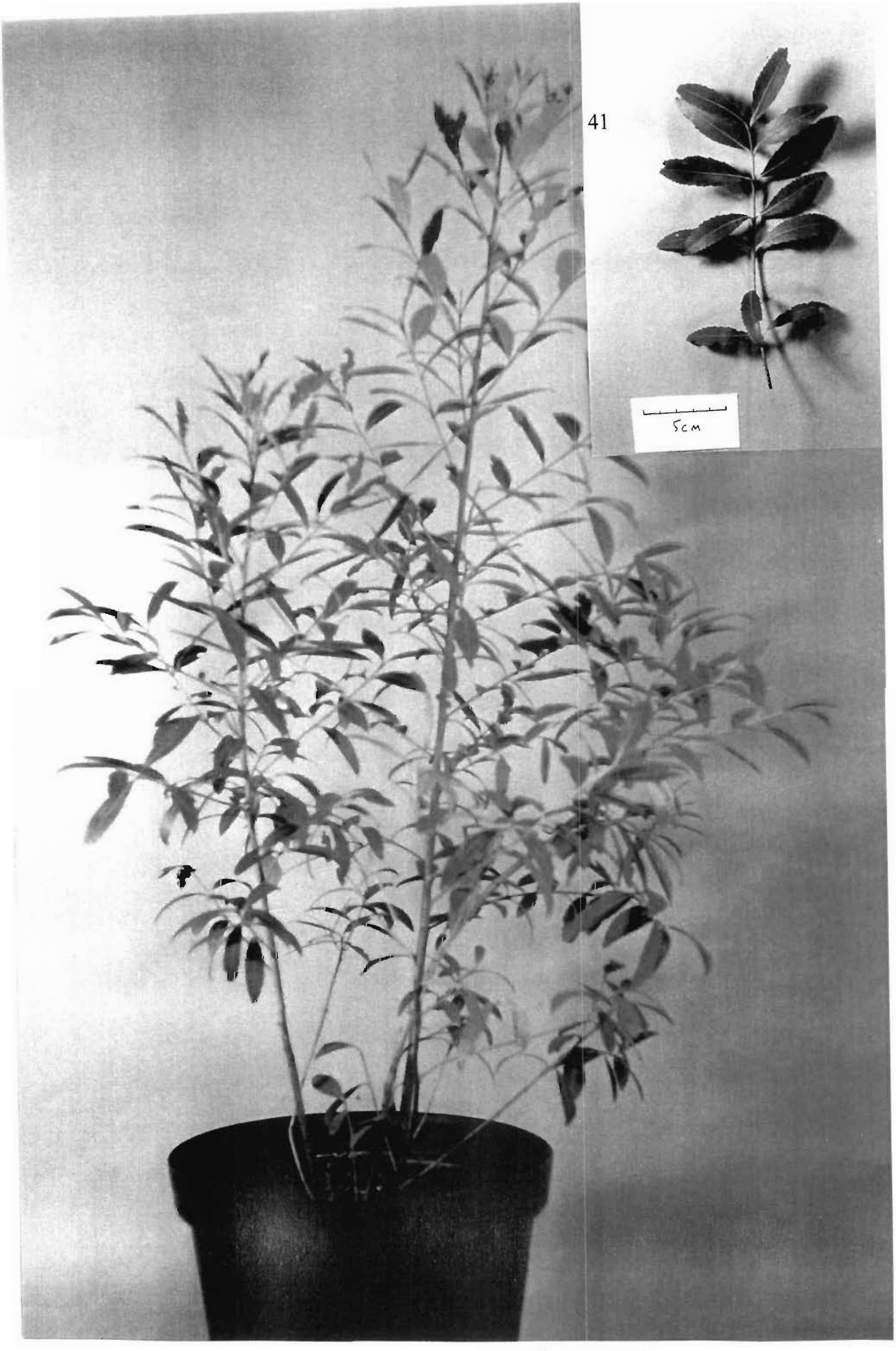


Figure 3: **One year old *Catha edulis* sapling grown from a cutting collected in Eastern Cape. Insert: gross leaf morphology.**

Figure 4: One year old *Catha edulis* sapling grown from a cutting collected at a Nairobi khat market.

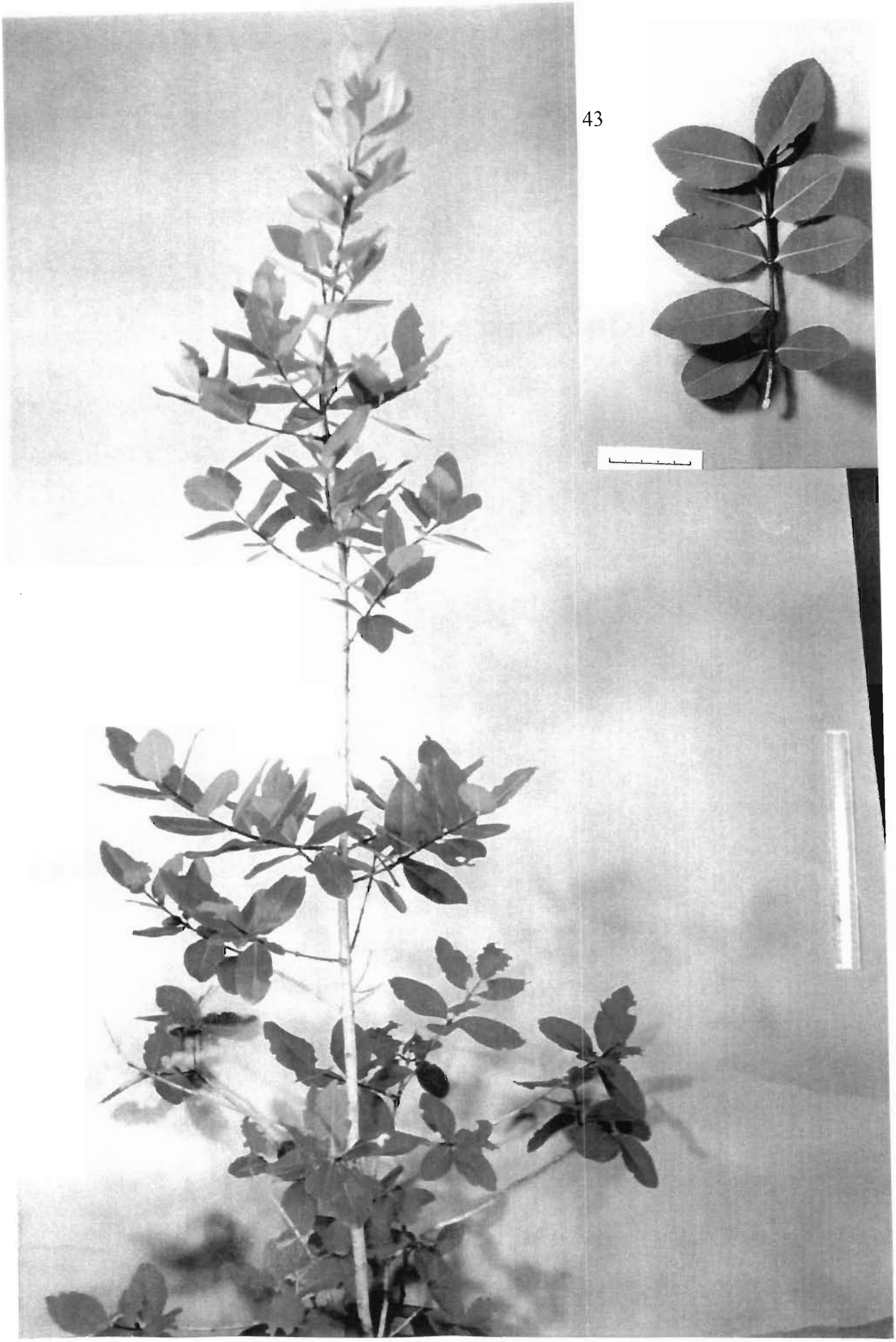


Figure 5: **One year old *Catha transvaalensis* sapling grown from a cutting collected in Mpumalanga. Insert: gross leaf morphology.**

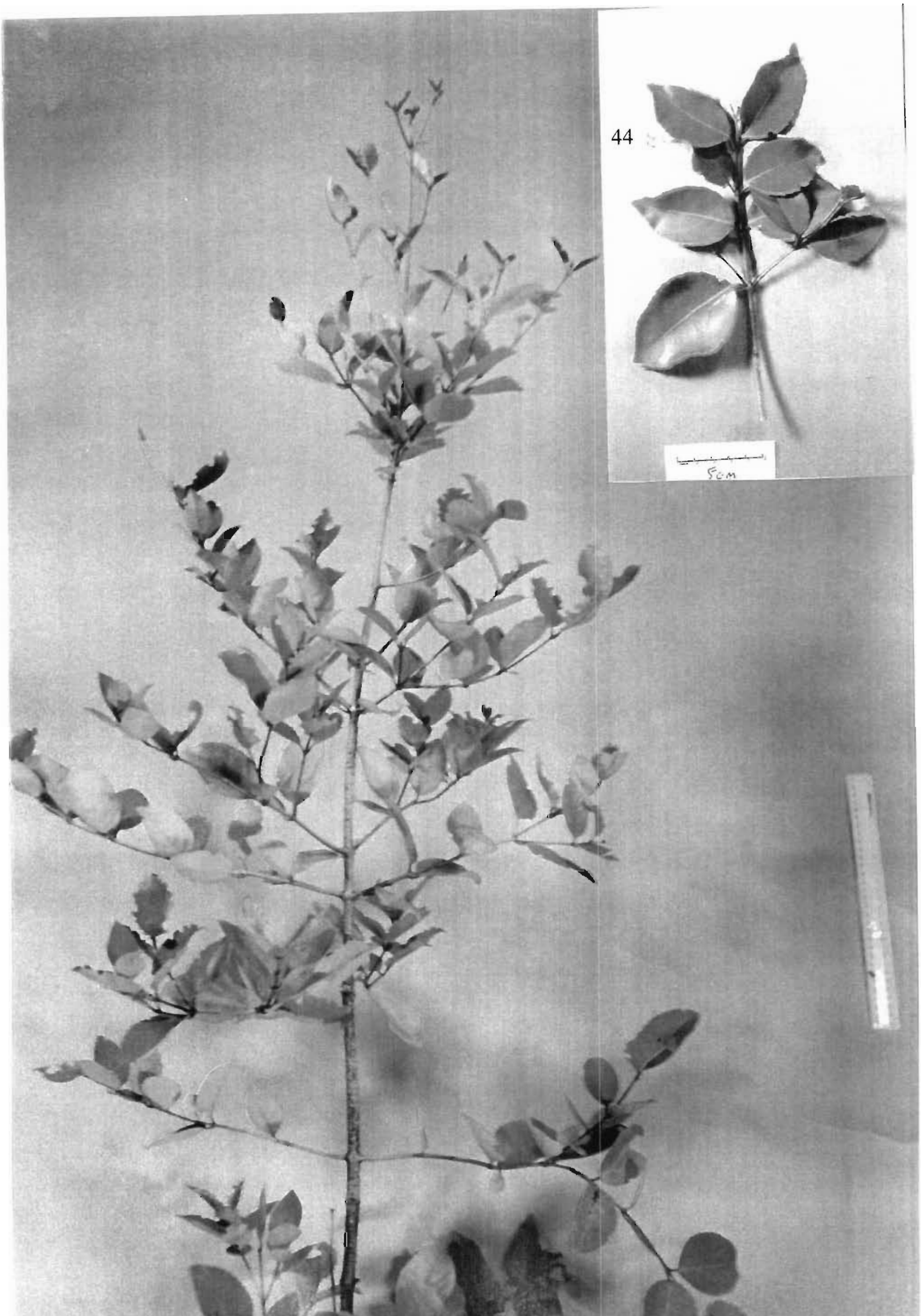


Figure 6: One year old *Catha abbottii* sapling grown from a cutting collected in the Mtumvuna Nature Reserve, Southern Kwa-Zulu Natal. Insert: gross leaf morphology.

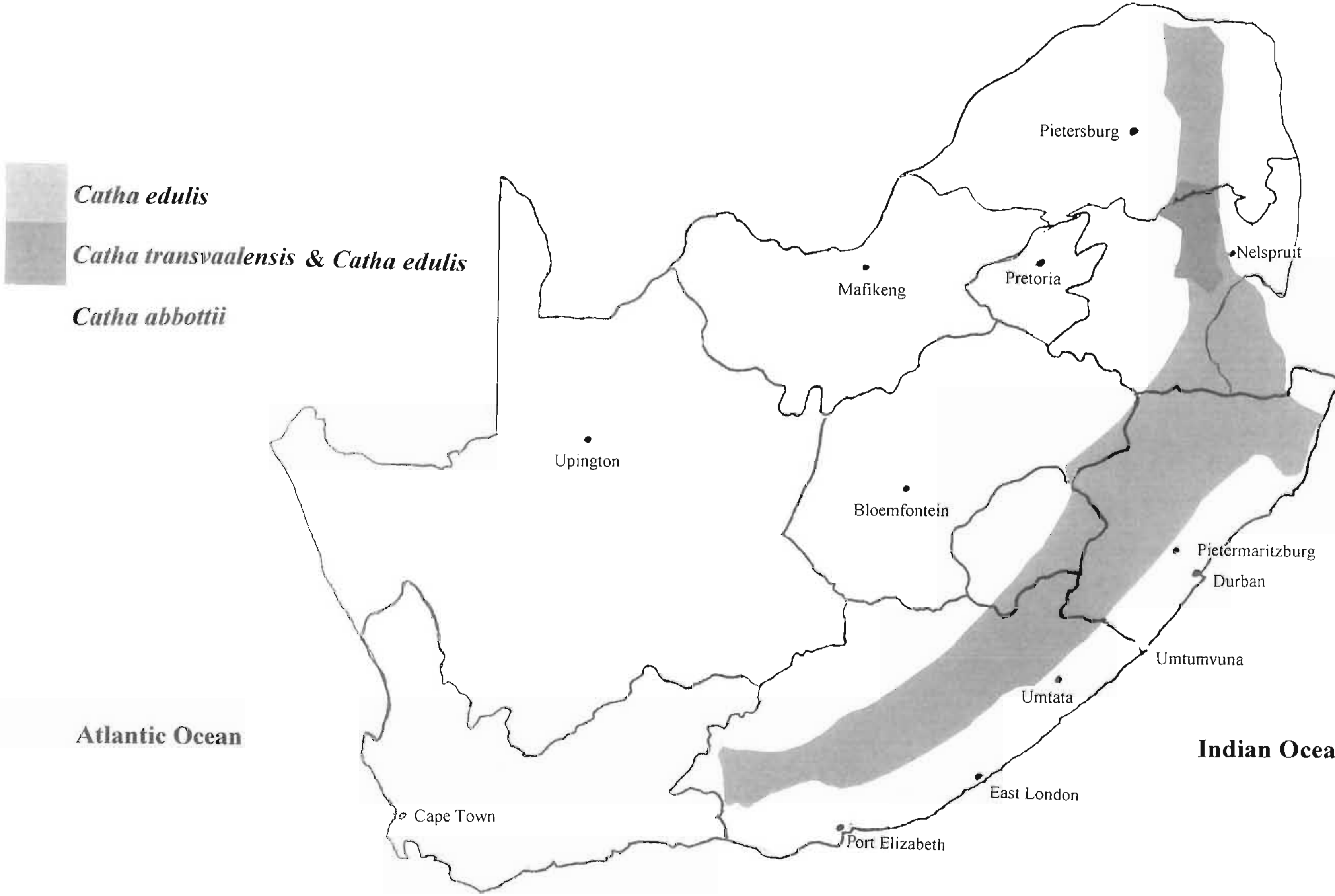


Figure 7: Distribution of *Catha* species in South Africa.

when mature, splitting to release the wingless, three-angled seeds, each of which has a small white aril at the base; fruits from June to January.

1.2.) *Catha abbottii* Van Wyk & Prins

Recently Van Wyk and Prins (1987) described a new species of *Catha* that was discovered by Mr A. T. D. Abbot in the Umtumvuna Nature Reserve during a floristic survey of the southern Natal/Pondoland sandstone region.

C. abbottii is described as an evergreen tree up to 30 m in height, unarmed and glabrous. The bark is greyish with pockets or layers of a powdery yellow pigment in the rhytidome, the surface is fairly smooth and exfoliates in rather thick, irregular scales, often tending to be dippled-scaly; young growth is usually reddish brown. The leaves are opposite and elliptic to broadly elliptic, rarely ovate to rotund; 50 to 70 mm x 25 to 40 mm, base attenuate, apex acute, margin glandular crenate, crenations usually 8 to 15 on each side; dark green and shiny above, whitish-green and dull below, petiole 6-10 mm long; bracts minute, usually caduceus. The flowers are pentamerous, 5mm in diameter. The petals are white, 3 x 1.75 mm, when open the flowers are almost erect-spreading with the margin slightly dorsally recurved and sweetly scented. The fruit is capsular, oblong and roughly trigonous with apex truncate and slightly concave, usually tipped with the persistent remains of the stigma, 6 x 3.5 mm, dehiscing loculi dally to the base. The seeds are usually 3 to 4 mm x 2 to 2.5 mm, exarillate and wingless with an oily endosperm. Flowers in spring and fruits in summer and winter (Van Wyk and Prins, 1987).

C. abbottii is currently known from only a few localities in a very limited area of southern KwaZulu-Natal and Pondoland. Several trees are scattered in climax ravine forest and in the open along the Sikuba River. Subsequent exploration lead to the discovery of others in the Engonyama River and in the forests of the Umtumvuna Nature Reserve in southern KwaZulu-Natal (Figure 7). Plants grow in rocky places with shallow acidic and sandy soil derived from sandstone (Van Wyk and Prins 1987).

1.3.) *Catha edulis* (Vahl) Forssk. ex Endl.

C. edulis is a shrub 1 to 2 m in height. to a medium - to - large tree, 12 to 15m in height; occurring in medium to high altitude evergreen forest, in open woodland and on rocky hillsides. The bark is grey and smoothish in young specimens, becoming dark brown and rough in large trees. Leaves are elliptic to oblong, 55 to 110 mm x 15 to 45 mm, pendulous, glossy bright green above, paler green below, thick rather leathery; close net-veining is visible, especially on the under surface; tapering to apex and base; margin even toothed; petiole up to 10 mm long. The flowers are lemon yellow or white with greenish throat, produced in small dense clusters up to 20 mm in diameter; flowering is from January to October but mainly April to June. The fruit is a 3-lobed capsule, about 10 mm long and comparatively narrow, becoming reddish brown to brown by maturity, splitting to release narrowly winged seeds which lack arils; fruits from March to October but mainly June to August (Coates Palgrave, 1977).

C. edulis in its natural state has a wide ecological adaptation ranging from 5000 - 8000 feet and more in elevation. This is also the range under cultivation. Its distribution extends from the Arabian Peninsula (Yemen) to East Africa (Ethiopia, Somalia, Kenya and Tanzania) to South Africa and the Cape. It was introduced into northern Madagascar and occurs in Afghanistan and Turkestan (Krikorian, 1984).

Historically the distribution of *C. edulis* in South Africa spreads from south of the Limpopo River spreading along the Eastern Transvaal highveld through Swaziland and into Northern Natal, where it stretches to the coast. It then follows an interior distribution in a south westerly direction along the escarpment as far south as Port Elizabeth (Figure 7).

In the Eastern Cape the distribution of *C. edulis* is given by Hirst (1997) as being along the Zwarte Kei, Thomas and Qwanti rivers in the Cathcart district whence it extends across the north-westerly boundary of the Bolo Reserve into The Deeps and The Bushes. From here the distribution spreads intermittently in a wide arc along the Kei River down to Garland and Flosdale in the south-east of Bolo. Thus, in Bolo Reserve, the distribution of *C. edulis* extends across an area of about 400 km through which the Great Kei River meanders for about 25 km. *C. edulis* also occurs in the Amathole Mountains in the Kologha and Price forests, near Stutterheim and King William's Town respectively. It also occurs near Kei Road and at Macleantown near East London (Hirst, 1997).

2.) Primary psychoactive compounds of *Catha edulis*

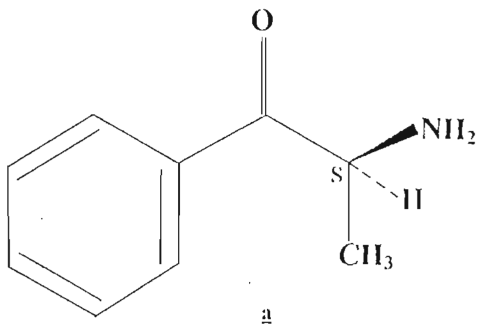
Extensive chemical analysis of khat samples has shown the two alkaloids primarily responsible for the central nervous system activity of khat are S-(-)-cathinone [S-(-)-K-aminopropiophenone] (Figure 8a) and cathine; consisting of the diastereomers S,S-(+)-norpseudoephedrine (Figure 8c) and R,S-(-)-norephedrine (Figure 8d).

Cathinone as a ketoamine base is extremely unstable. Withering, drying and cleanup of the plant material result in various degradation products or artifacts. Enzymatic reduction transforms cathinone into the less active cathine. Thus, a fresh drug may contain up to one hundred times more cathinone than dried material. Due to the easy enolization of the keto group, cathinone racemizes quickly, particularly as the free base and in polar solvents to form R-(+)-cathinone (Figure 8b); this in turn can be reduced to form R,R-(-)-norpseudoephedrine (Figure 8e) and S,R-(+)-norephedrine (Figure 8f). In contrast, its salts are stable in solid form.

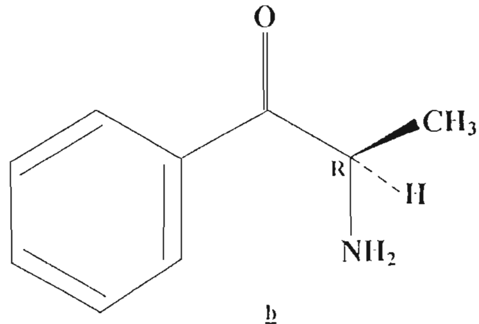
Due to the physical and chemical similarities between S,S-(+)-norpseudoephedrine and its diastereomer R,S-(-)-norephedrine they are not separated by GC analysis and instead appear as a single peak on the chromatogram. This also applies to the enanteomers of S-(-)-cathinone and R-(+)-cathinone.

3.) Studies of the *Catha* species in South Africa

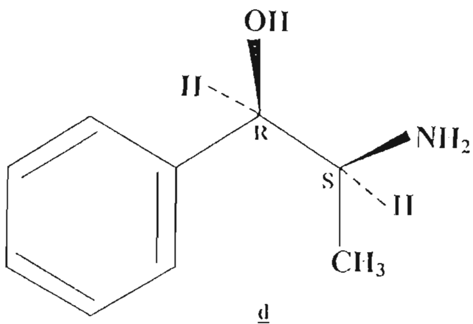
In contrast to the extensive studies which have been carried out on *C. edulis* from East Africa and Yemen, botanical information and chemical analysis of the three *Catha* species found in South Africa is limited. Although observations were made by the early European explorers in South Africa of *C. edulis* use by the San (hence the name Boesman's Tee or Bushman's Tea) and lately by Hirst



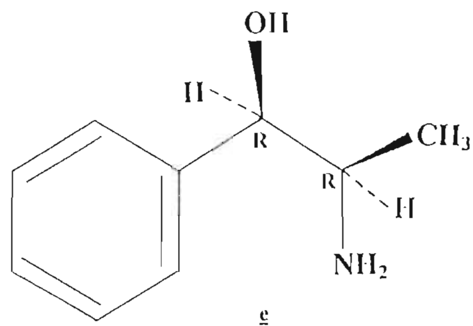
a
S-(-)-cathinone



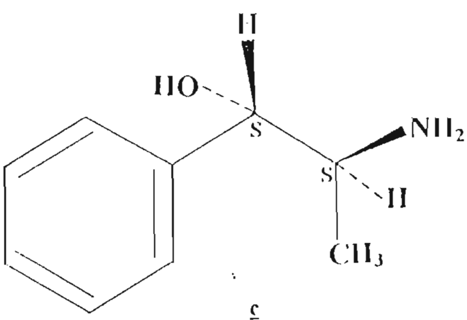
b
R-(+)-cathinone



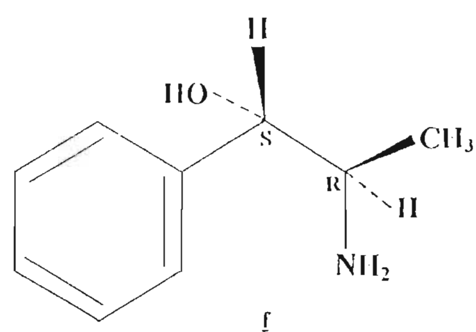
d
R,S-(-)-norephedrine



e
R,R-(-)-norpseudoephedrine



c
S,S-(+)-norpseudoephedrine



f
R,S-(+)-norephedrine

Figure 8: Structures of the primary psychoactive compounds of *Catha edulis*.

(1997) and Hutchings *et al.* (1996), very little chemical analysis has been carried out on *C. edulis* in South Africa. With the exception of TLC analysis by Lehmann *et al.* (1990) no investigations into the chemical nature of either *C. transvaalensis* or *C. abbottii* have been carried out.

4.) Objectives of the present study

The utilization of *C. edulis* in South Africa for its psychoactive properties by some Xhosa farm laborers in the Bolo District of the Eastern Cape and its use as a ***muti*** (medicine) by Zulu ***inyangas*** (herbalists) has received little attention. This study was undertaken in order to bring together information on the *Catha* species found in South Africa, and to investigate the levels of the psychoactive compounds cathinone and cathine in the three species. As a necessary prelude to such an investigation a number of published methods were first examined for their efficiency in extracting, isolating and analyzing cathinone and cathine. From the results of this investigation the method of analysis deemed to be most suitable and efficient would be selected and employed for analyses of other samples in this study.

A comparison of the levels of cathinone and cathine in the different *Catha* species and some specimens of *C. edulis* of different origin would be undertaken. These would be compared with published levels of alkaloids reported for East African khat. In further investigations the distribution of cathinone and cathine within *C. edulis* was also to be determined by examination of leaves of different ages, and other plant parts.

CHAPTER THREE

DEVELOPMENT OF AN ANALYTICAL TECHNIQUE

1.) Introduction

Before undertaking a comparative study of the alkaloid levels in the *Catha* species of South Africa, it was necessary to arrive at a simple, reliable and reproducible method for the extraction, isolation and analysis of cathinone and cathine. A review of the literature revealed two commonly used methods for the extraction and isolation. Along with these, a method incorporating the Extrelut^R column procedure was also investigated.

To extract and isolate the alkaloids of *C. edulis* Brenneisen and co-workers extracted plant material with a strong aqueous acid (2M HCl). After filtering, the filtrate was basified with a strong base (4M NaOH) and the free base alkaloids re-extracted with diethyl ether (Brenneisen *et al.*, 1984, 1986; Geisshusler and Brenneisen, 1987).

Other investigators (Lehmann *et al.*, 1990; El - Domiaty *et al.*, 1994; El - Hag and Mossa, 1995; Lee, 1995) began with a methanol extract of the plant material. Thereafter, a dilute acid (0.02N H_2SO_4) was used by Lee (1995) to extract the alkaloids from which non-alkaloidal constituents were removed using chloroform partitioning. After basifying with a weak base (saturated $NaHCO_3$) the alkaloids were re-extracted into dichloromethane.

The Extrelut^R procedure has previously been successfully employed in the isolation of extracted alkaloids from the Mesembryanthemaceae (Smith *et al.*, 1997). This procedure involves the application of an aqueous acid extract of the plant material onto an Extrelut^R column; elution with an organic solvent removes non-alkaloidal constituents from the column, and finally basification with ammonia vapor allows the free base alkaloids to be recovered from the column by elution with an organic solvent.

Three methods are commonly used for analysis of the isolated alkaloids. HPLC was described as the

"...method of choice..." to determine the qualitative and quantitative composition of khat alkaloid extracts (Geisshusler and Brenneisen, 1987) and has been used successfully in a number of studies (Brenneisen *et al.*, 1986; 1987; 1991; Mathys and Brenneisen, 1992; El - Domiaty *et al.*, 1994; El - Hag and Mossa, 1995). The production of the trifluoroacetate (TFA) derivatives of cathinone and cathine by Brenneisen *et al.*(1984; 1986) allowed for their analysis and identification by GC/MS. Analysis of the underivatized free bases was successfully carried out on GC and GC/MS by Lee (1995). For simple rapid qualitative analysis of the alkaloids a TLC method was developed by Lehmann *et al.* (1990) in which the researchers applied underivatized extracts directly onto TLC plates for developing.

2.) Materials and Methods

2.1.) Extraction and isolation

All extraction and isolation methods were investigated using five 5 g replicates of the top four leaves of *C. edulis* plants grown from cuttings under shade cloth at the Botany Department, University of Natal, Pietermaritzburg. Care was taken when harvesting that the leaves were of uniform size, condition and age.

2.1.1.) Method 1 : Acid extraction method

The plant material was ground with an Ultraturrax for 1 minute in 100 ml 1M HCl, and extracted for 90 minutes at room temperature with occasional stirring. After filtering the aqueous acid extract through glass wool, the filtrate was concentrated almost to dryness under vacuum at 40 °C, and resuspended in 10 ml distilled water. The aqueous acid phase was extracted five times with 100 ml diethyl ether and adjusted to pH 10 with 4M NaOH. This basic aqueous phase was extracted four times with 125 ml dichloromethane and the combined extracts reduced under vacuum at 30 °C to 2 ml.

2.1.2.) Method 2 : Organic solvent extraction method

The plant material was ground with an Ultraturrax for 1 minute in 100 ml 100% methanol, and extracted for 90 minutes at room temperature with occasional stirring. After filtering through glass wool the methanolic extract was concentrated to dryness under vacuum at 40 °C, and resuspended in 15 ml 0.05N H_2SO_4. This aqueous acid phase was extracted five times with 100 ml diethyl ether and adjusted to pH 8 with a saturated aqueous $NaHCO_3$ solution. This basic aqueous phase was extracted four times with 125 ml dichloromethane and the combined extracts reduced under vacuum at 30 °C to 2 ml.

2.1.3.) Method 3 : Extrelut^R method

The plant material was ground with an Ultraturrax for 1 minute in 100 ml 1M HCL, and extracted for 90 minutes at room temperature with occasional stirring. After filtering the aqueous acid extract through glass wool the filtrate was concentrated almost to dryness under vacuum at 40 °C, and resuspended in 20 ml water. This aqueous acid phase was placed onto a column packed with 70 ml Extrelut^R (E. Merck, Darmstadt) and allowed to equilibrate for 30 minutes. The column was then eluted with 40 ml 85 : 15 dichloromethane : isopropanol to remove contaminants. Thereafter the column was basified with a stream of ammonia vapour and the free base alkaloids were recovered from the column by elution with 40 ml dichloromethane : isopropanol (85:15); this eluant was reduced under vacuum at 30 °C to 2 ml.

2.1.4.) Comparison of extraction and isolation methods

The three extraction and isolation methods were compared by injection of 1 :l of the underivatized sample directly onto the GC. Good chromatographic separation and consistency of the chromatographic profile (represented by the standard deviation as a percentage of the mean) were used as criteria for evaluating the best extraction method.

2.2.) Analytical methods

2.2.1.) Analysis of derivatized alkaloids

The TFA derivatives of cathinone and cathine were prepared by taking the 2 ml dichloromethane extracts from the extraction and isolation methods (described above) to dryness, and resuspending them in 2 ml benzene. Following the addition of 100 :l 0.05N triethylamine in benzene, and 10 :l trifluoroacetic acid anhydride, the reaction mixture was heated at 50 °C for 30 minutes. After cooling 1 ml of water was added and the solution shaken for 1 minute; thereafter 1 ml of 5% NaOH was added. The reaction mixture was shaken for a further 5 minutes and then centrifuged. 1 :l of the upper benzene phase was used for GC and GC/MS analysis.

2.2.2.) Analysis of underivatized alkaloids

For analysis of the underivatized alkaloids 1 :l of the dichloromethane extract was injected directly onto the GC and GC/MS.

2.2.3.) Gas Chromatography

GC analysis was carried out using a Varian 3300 instrument fitted with a Nitrogen - Phosphorous specific Detector (NPD) and a 25 m. BP-5 bonded column (SGE, Australia) of 0.53 mm internal diameter and 1 :m film thickness. The injector temperature was set at 200 °C and the detector at 220 °C. The attenuation of the detector was set at 4 x 10^{-10} mV/V. Integration of the detector information was by a Hewlett Packard 3395 integrator.

The temperature program for analysis of derivatized samples was : 100 °C to 170 ° C at 3.5 °C/min. The temperature program for analysis of underivatized samples was : 80 ° C to 90 °C at 1 °C/min; 90 °C to 95 °C at 0.5 °C/min; and finally 95 °C to 220 °C at 20 °C/min.

In order to test the precision or reproducibility of the GC, five injections of each sample in the dilution series were made onto the GC. Confidence limits were calculated for the means of the peak area counts for each dilution series at a 99% confidence level and expressed as percentages of the means. The mean percentage value was used to express the overall confidence limit for the reproducibility of the GC.

2.2.4.) Gas Chromatography / Mass Spectrometry

GC/MS identification of cathinone and cathine was carried out using a Finnigan MAT ITS40 instrument fitted with a 30 metre fused silica capillary column DB-5 of 0.25 mm internal diameter (J and W Scientific, Inc.). The injector temperature was set at 200 °C.

The temperature program for analysis was : 80 °C to 90 °C at 1 °C/min; 90 °C to 95 °C at 0.5 °C/min; and finally 95 °C to 220 °C at 20 °C/min.

2.3.) Identification of cathinone and cathine

2.3.1.) Preparation of standard

A cathine standard was produced using a commercially prepared diet tablet (Thinz ^{R}. Lennon) containing 50 mg (+) - norpseudoephedrine hydrochloride. The tablet was ground in 10 ml distilled water and the solution basified using a saturated solution of $NaHCO_3$ and extracted four times with 100 ml dichloromethane. The resulting extracts were combined and reduced under vacuum at 30 °C to 1 ml. 50 mg cathinone hydrochloride was obtained from the Sigma Chemical Company and treated in the same way to produce the cathinone standard as the free base.

To identify cathinone and cathine on the GC on the basis of retention times, the cathinone and cathine standards were first injected onto the GC/MS and identified on the basis of their relative retention times and respective mass spectra. These standards were then injected on the GC and the retention times noted for reference purposes. Based on this information it was possible to identify cathinone and cathine by GC analysis of many samples without having to carry out GC/MS analysis for each sample.

2.3.2.) Standard curve

A standard curve of detector response vs concentration was prepared for a cathine dilution series prepared from a commercial diet tablet containing 50 mg (+) - norpseudoephedrine hydrochloride. Five replicates of each concentration in the dilution series were made and analyzed by injection of 1 :l onto the GC. The mean peak area counts were calculated for each dilution. A standard curve of mean peak area counts (representing detector response) was plotted against mean concentration of (+) – norpseudoephedrine (Figure 9). The curve was subjected to linear regression analysis and the gradient and regression coefficient reported. The presence of several impurities in the cathinone hydrochloride standard presented problems with regards to the production of an accurate dilution series and thus the standard curve for cathinone. As it was likely that some of the impurities identified would have been breakdown products of cathinone the actual cathinone concentration in the standard would have been lower than that calculated; thus giving an inaccurate dilution series. Therefore the standard curve derived for (+)-norpseudoephedrine was also used for the quantitative determination of cathinone. This was possible due to the structural similarities between the two compounds in that they contain equal amounts of nitrogen and the fact that the detector response was based on the presence of nitrogen.

3.) Results and discussion

3.1.) Extraction and isolation

3.1.1.) Method 1 : Acid extraction method

Application of this method produced inconsistent results. Examination of the chromatograms for the five replicates (Figure 10 a – e) indicates that not only did the absolute peak area counts for cathinone and cathine vary but also the ratio of these putative alkaloids. In one of the replicates this method appeared to result in the modification of the cathine peak as represented by the change in profile of the peak at R_t =12.0 min. The nature of this modification was not determined. The standard

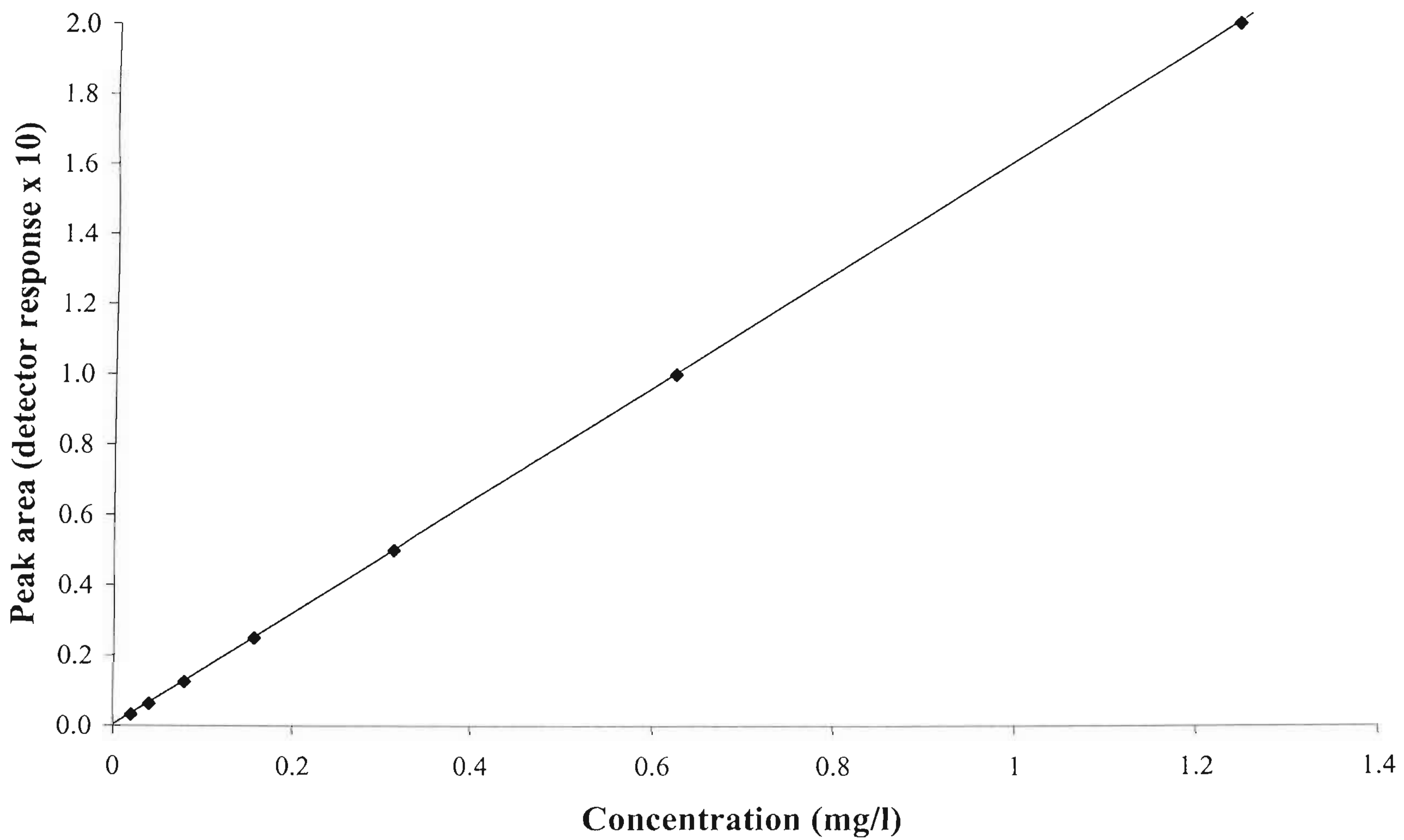


Figure 9: **Standard curve for (+)-norpseudoephedrine dilution series.**

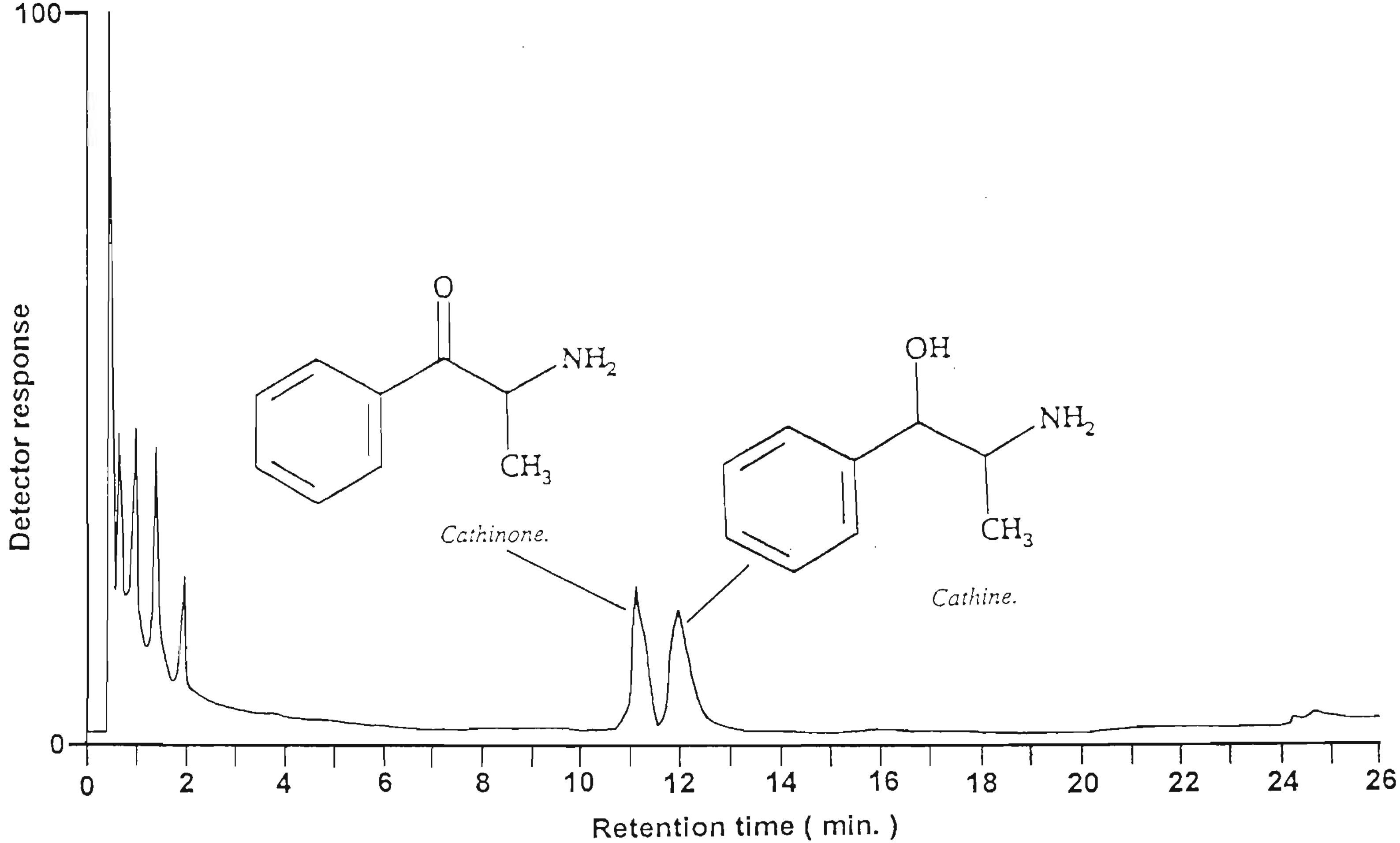


Figure 10a: Chromatogram obtained with Method 1 (acid extraction method).

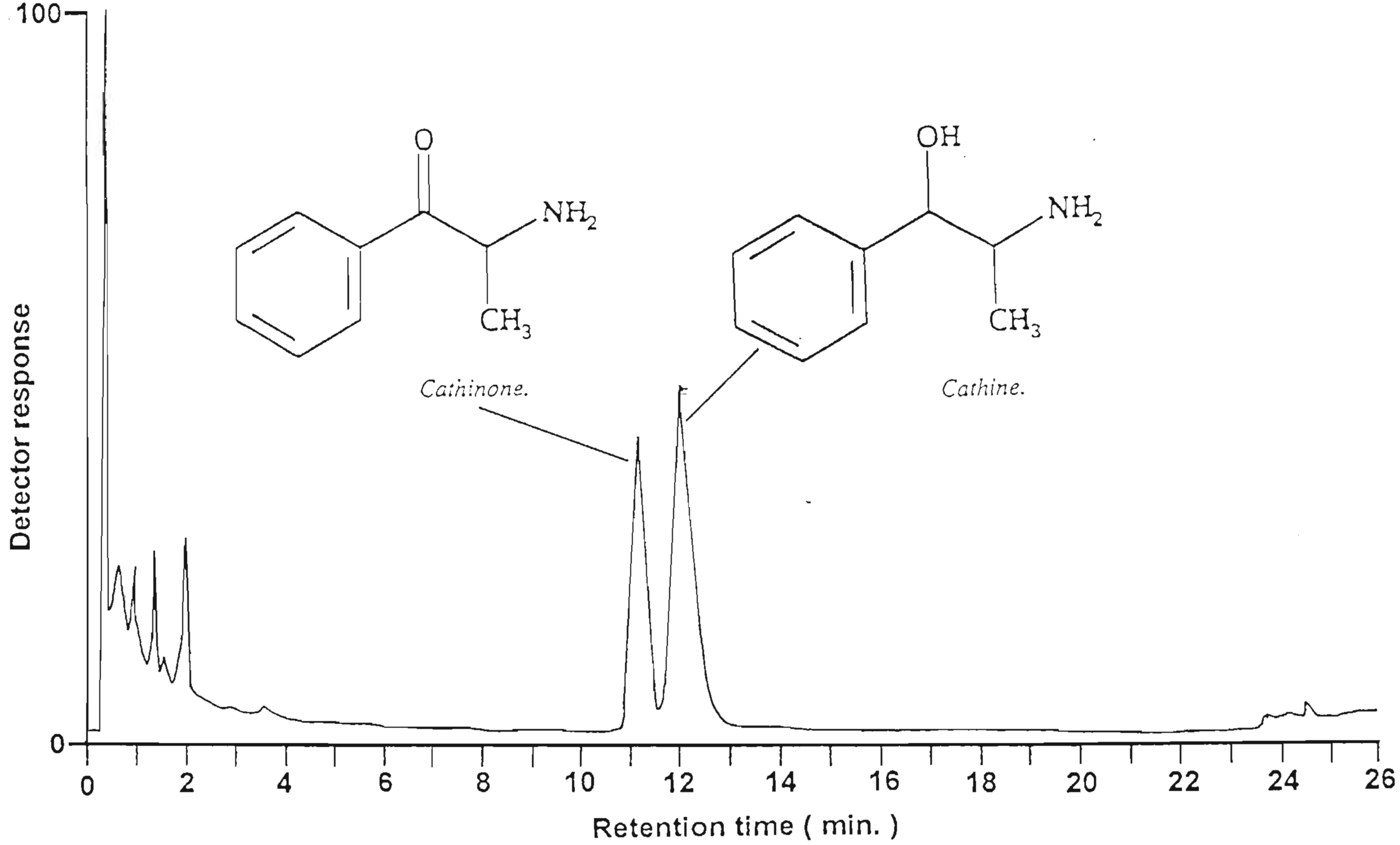


Figure 10b: Chromatogram obtained with Method 1 (acid extraction method).

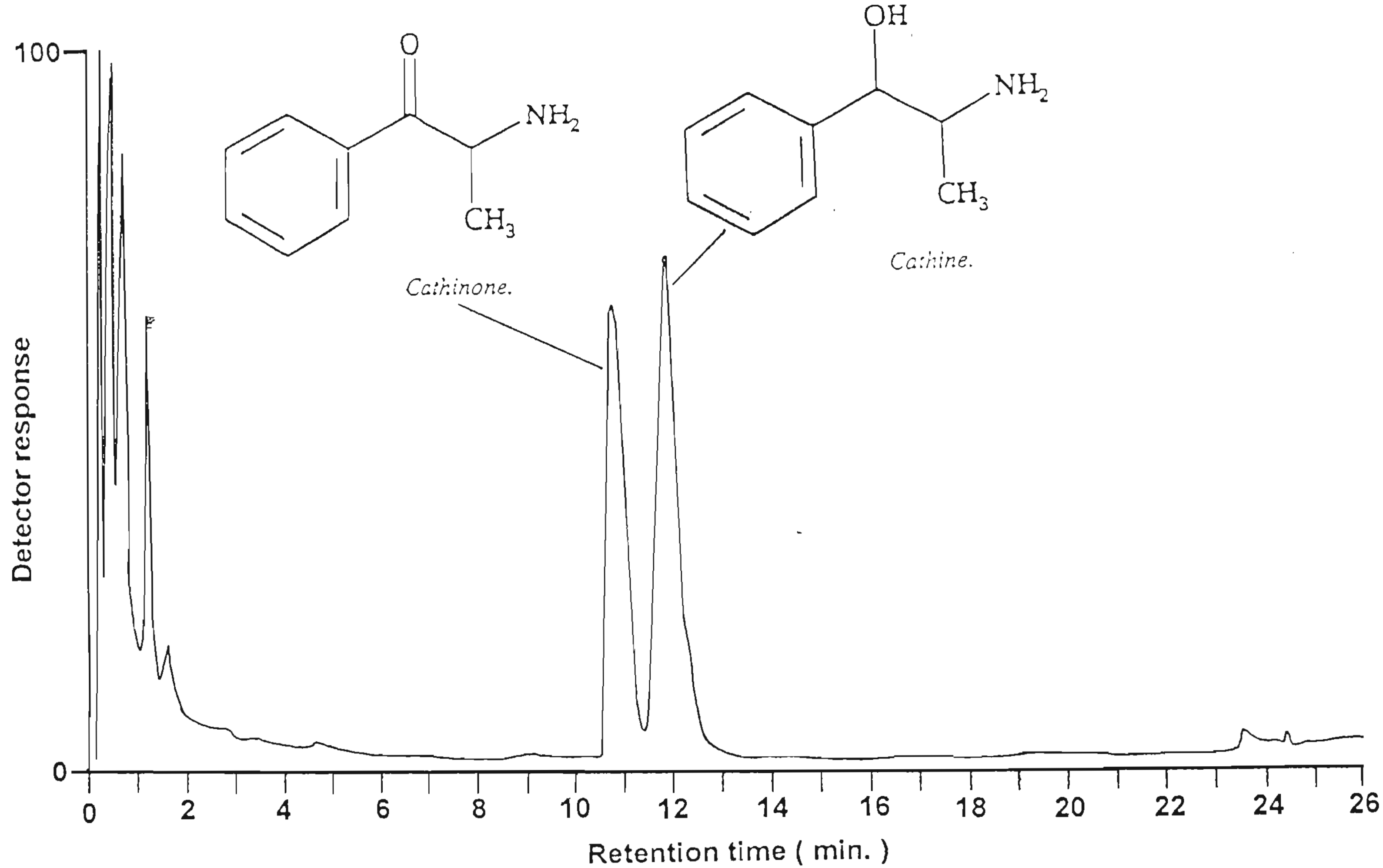


Figure 10c: Chromatogram obtained with Method 1 (acid extraction method).

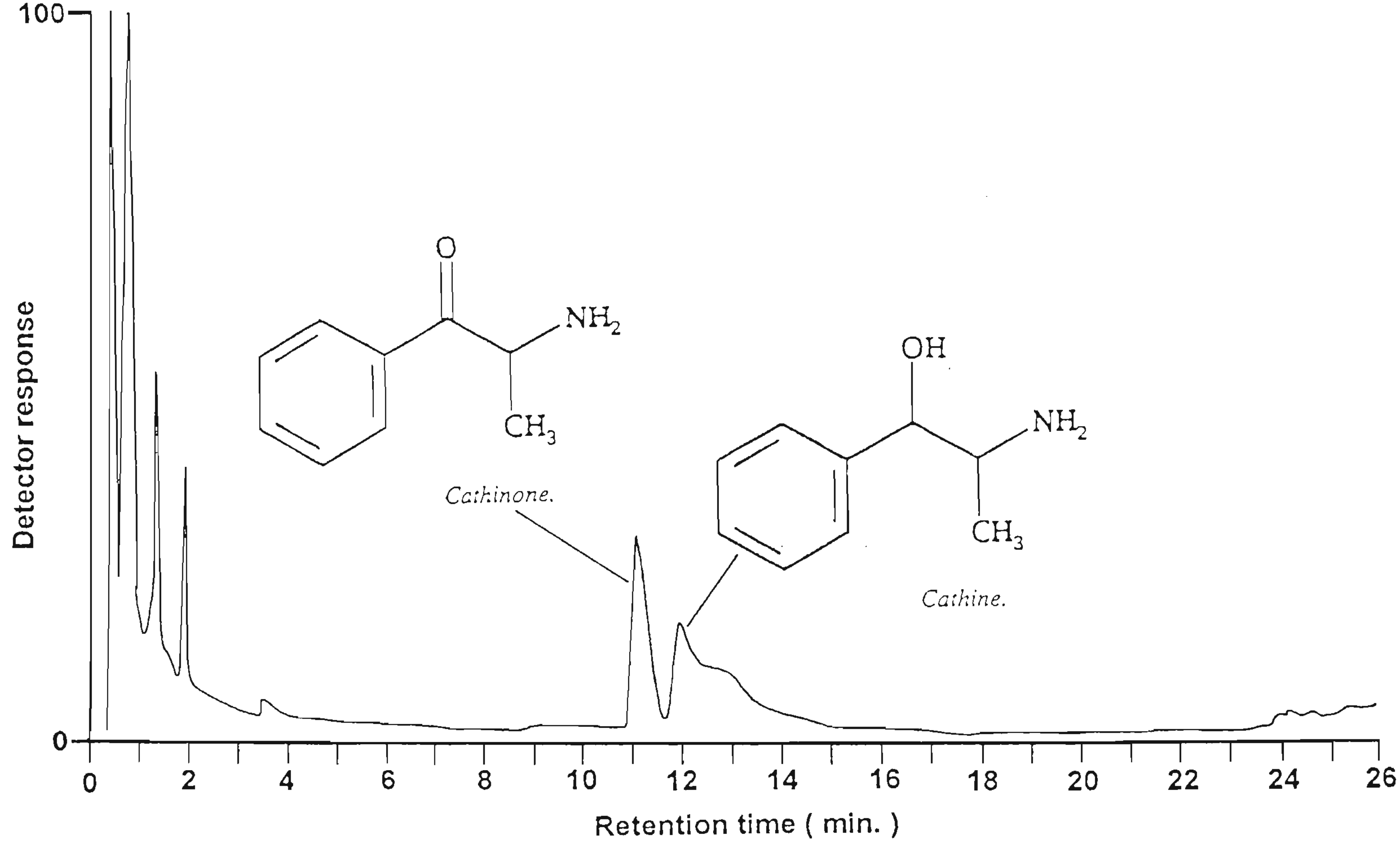


Figure 10d: Chromatogram obtained with Method 1 (acid extraction method).

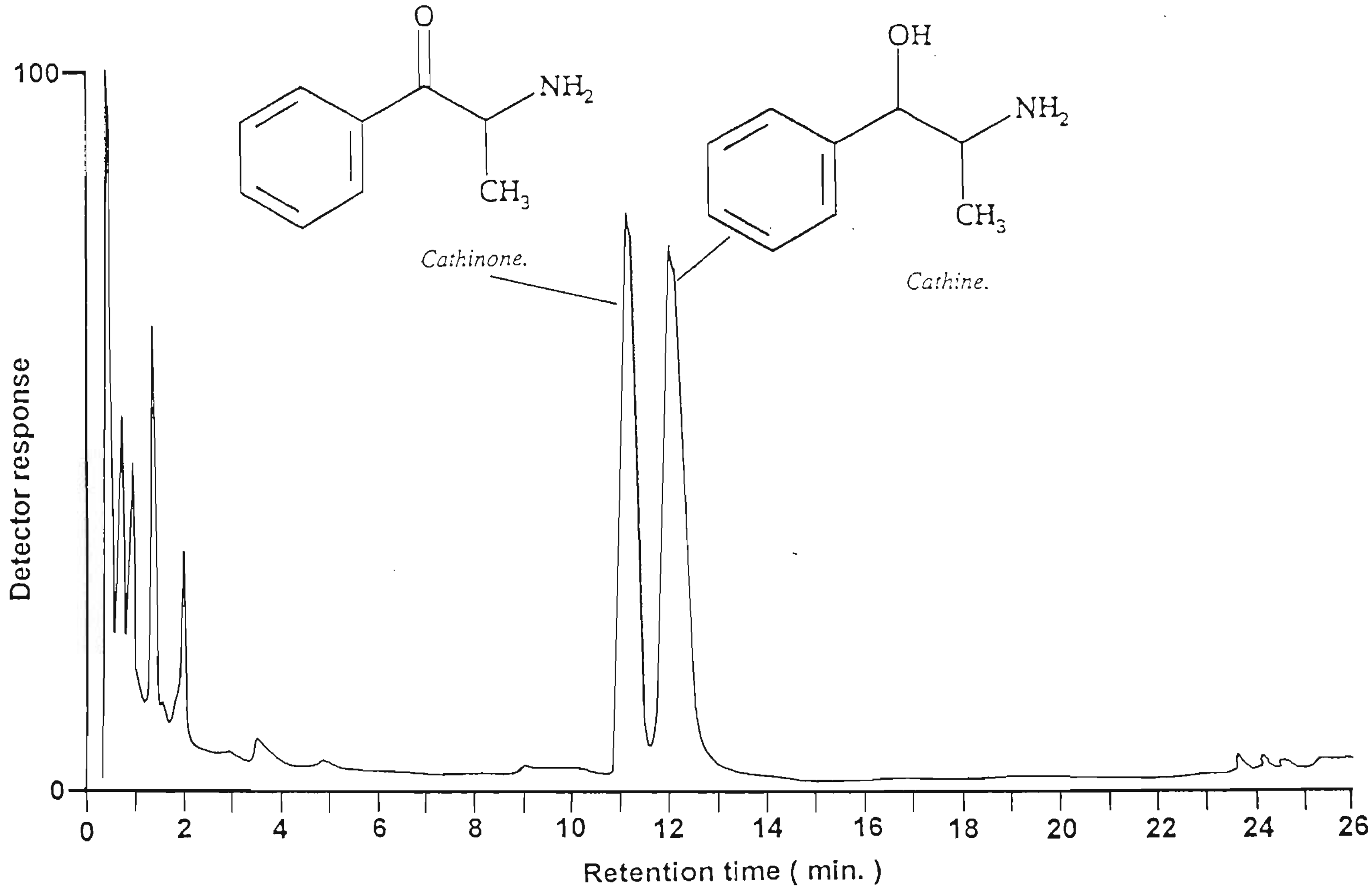


Figure 10e: Chromatogram obtained with Method 1 (acid extraction method).

deviation as a percentage of the mean was calculated to be 39.39% and 23.92% for the cathinone and cathine peaks respectively.

Although the polar solvents used in this method for the extraction of cathinone and cathine may have resulted in the enolization of the ketone group of S-(-)-cathinone to form its enanteomer R-(+)-cathinone it is unlikely that this would result in the distortion of the cathine peak, as these two enanteomers will co-elute on the GC. More likely is the creation of a compound, due to nucleophilic addition to cathine, whose chemical polarity is slightly different to cathine and as such its retention time overlaps that of cathine hence causing the distortion.

3.1.2.) Method 2 : Methanol extraction method

Application of this method resulted in higher recoveries of cathinone and cathine and produced consistent and reproducible chromatograms (Figure 11). The standard deviation as a percentage of the mean was calculated to be 3.44% and 13.84% for the cathinone and cathine peaks respectively.

3.1.3.) Method 3 : Extrelut^{R} method

Application of this method failed to extract any compounds from four of the samples with only one distorted chromatogram (Figure 12) being produced. It can be speculated that either cathinone and/or cathine bonded to, or possibly degraded on, the Extrelut^{R} column packing material to form a compound with sufficiently different polarity to the target compounds and thereby causing distorted peaks.

3.2.) Analytical method

3.2.1.) Analysis of derivatized alkaloids

Although mass spectral patterns produced by injection of the TFA derivatives onto GC/MS were unambiguous and allowed for their positive identification (Figure 13a – b) the application of the

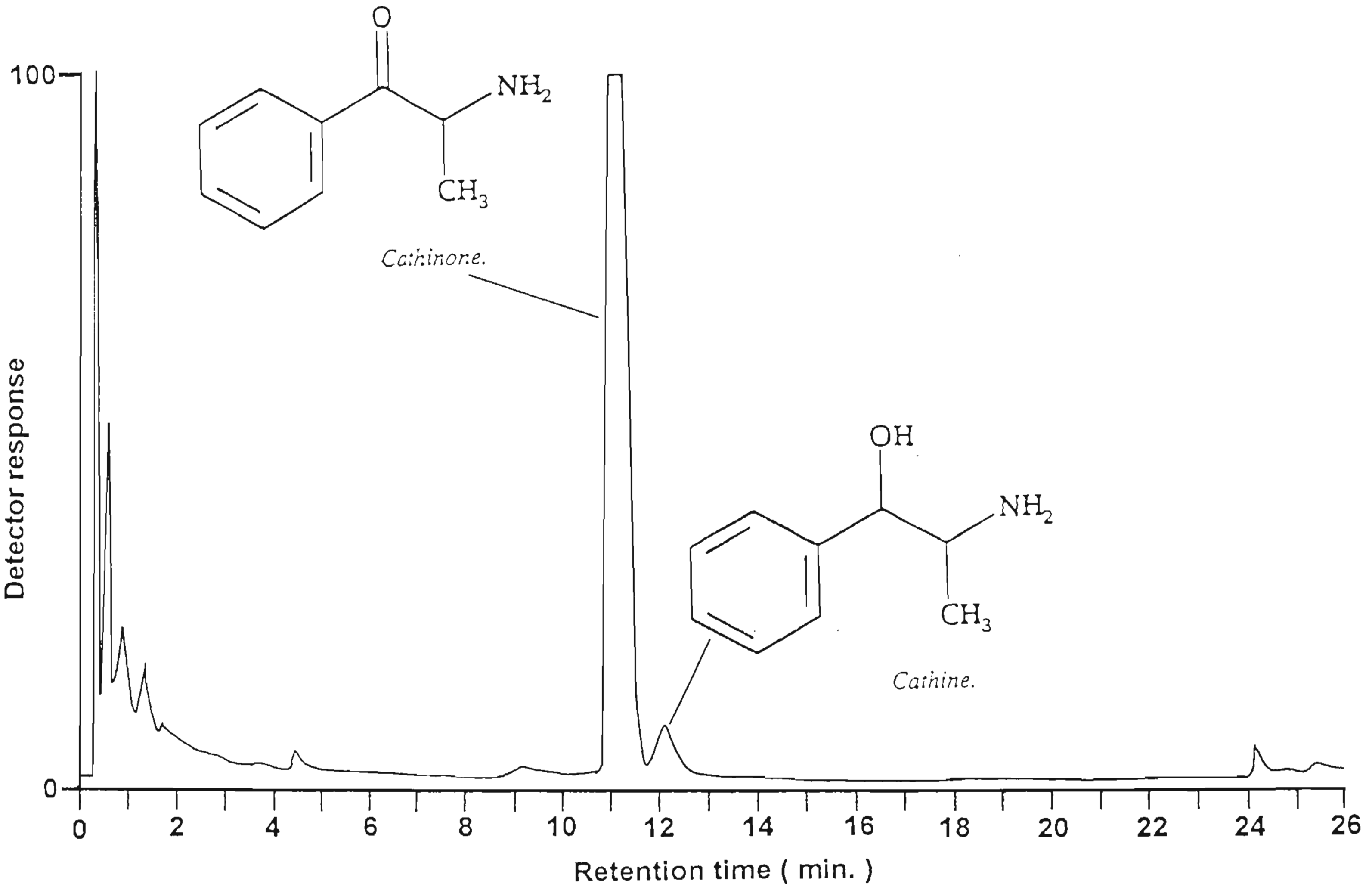


Figure 11: Chromatogram obtained with Method 2 (methanol extraction method).

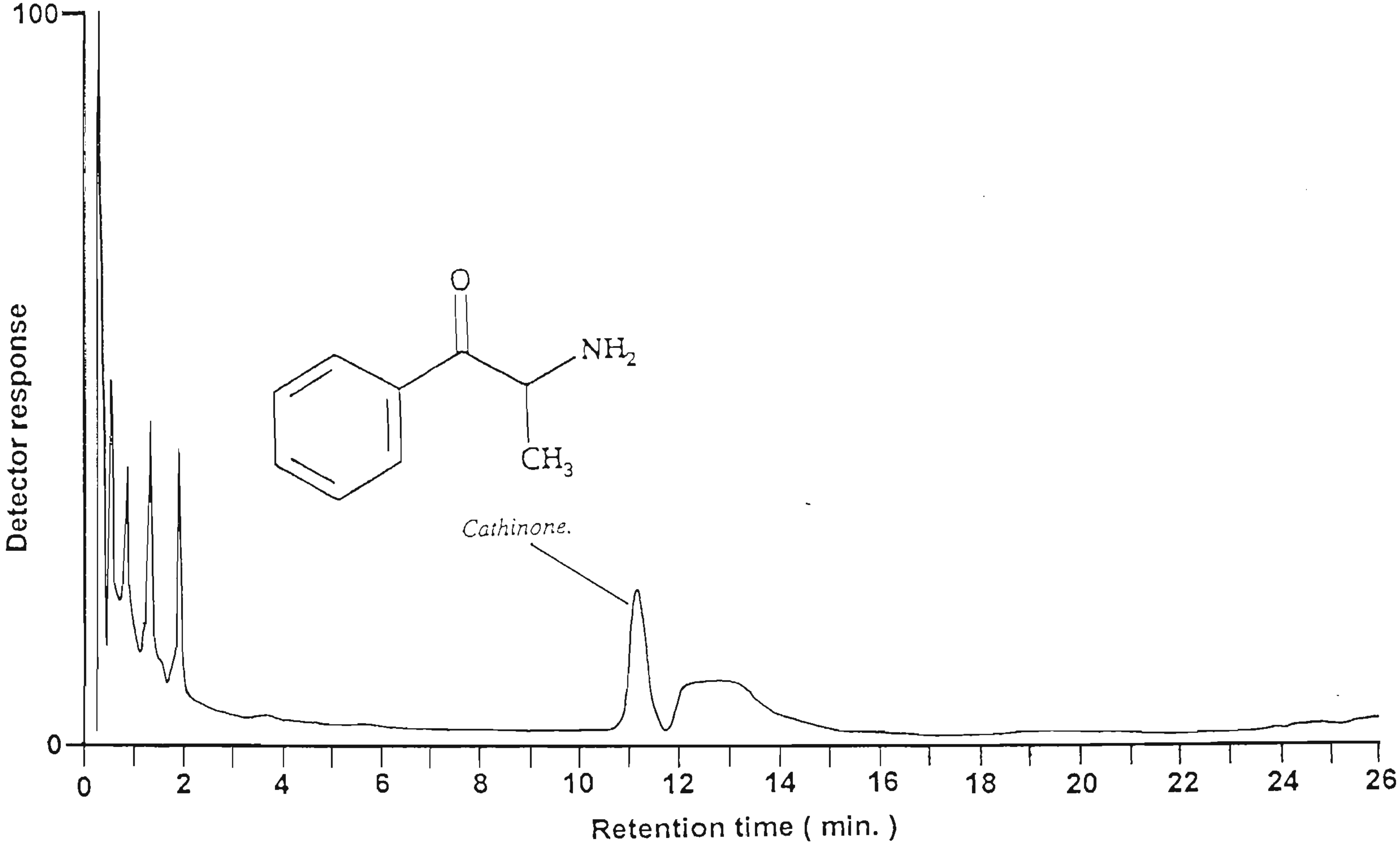


Figure 12: **Chromatogram obtained with Method 3 (Extrelut[R] technique).**

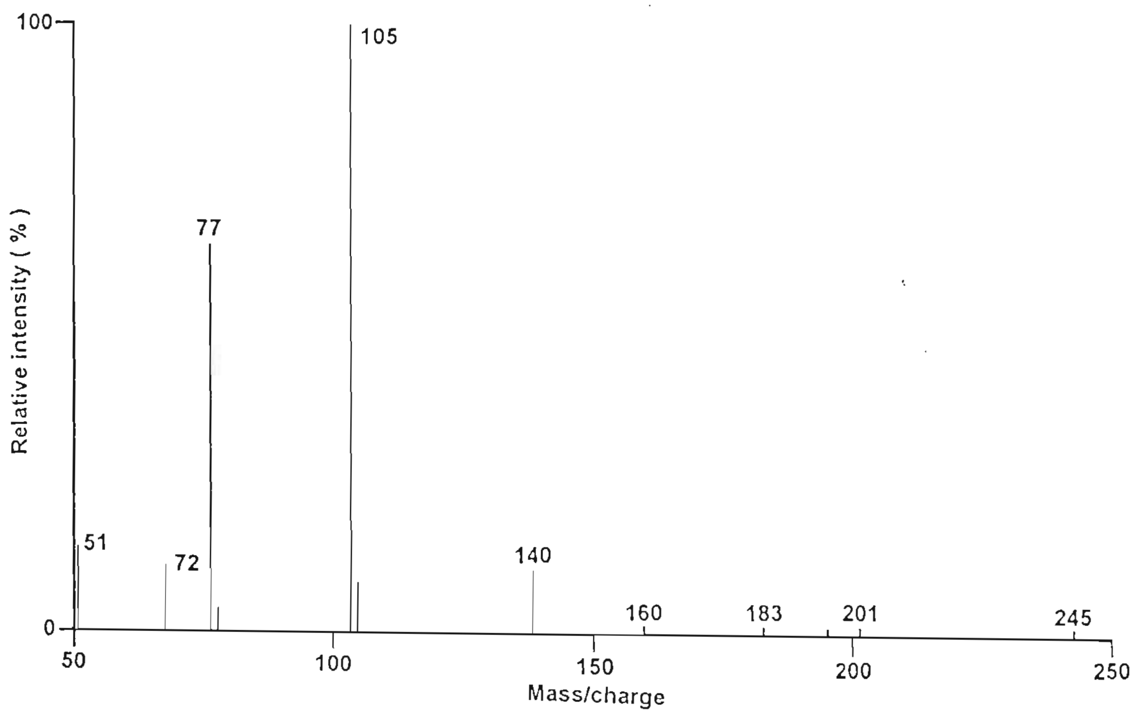


Figure 13a: Mass spectrum for cathinone – TFA derivative.

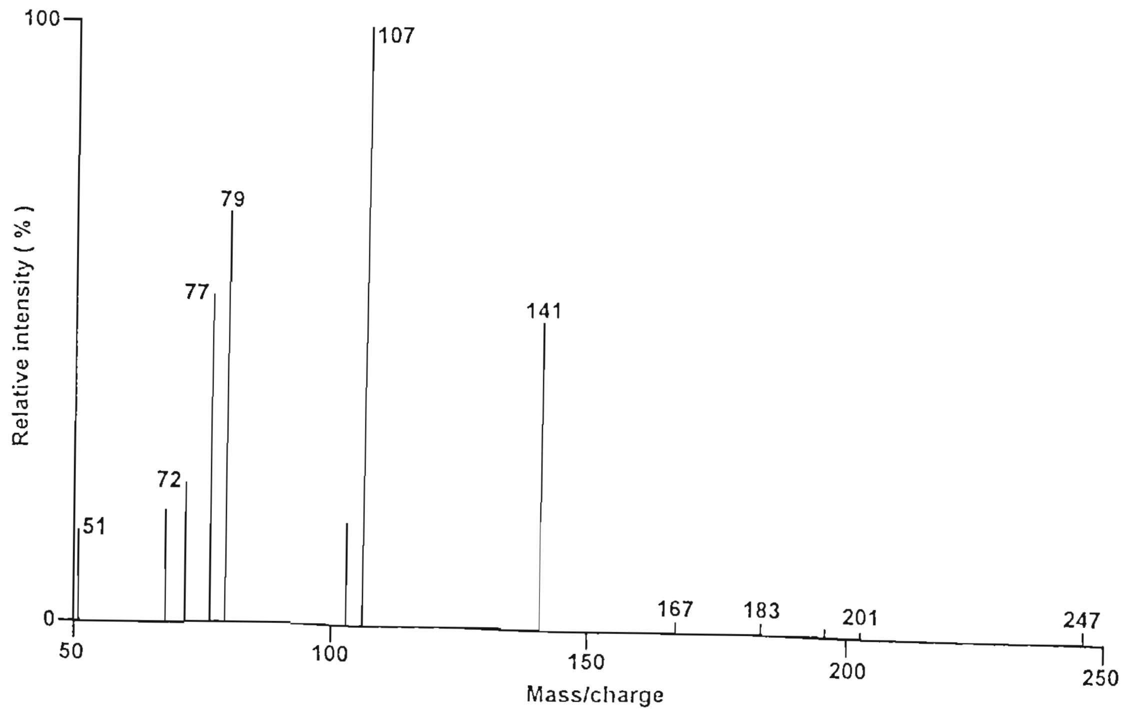


Figure 13b: Mass spectrum for cathine – TFA derivative.

derivatization method to obtain the TFA derivatives of cathinone and cathine resulted in inconsistent chromatographic profiles. Of the five samples subjected to the derivatization method only two yielded peaks representative of cathinone and cathine, however, the peak area counts of the two chromatograms varied considerably. It was thought that aspects of the derivatization reaction, and the sensitivity of the reactants to slight changes in moisture may have produced this inconsistency.

3.2.2.) Analysis of underivatized alkaloids

When the free base alkaloids were injected directly onto the GC and GC/MS consistent chromatographic profiles and unambiguous mass spectral patterns, from which the compounds could be easily identified, were produced (Figure 14 a – b). The GC analysis of the underivatized compounds was found to be an accurate and precise means, as judged by the confidence limits calculated for the mean by which to carry out quantitative and qualitative analysis of the samples (Figure 11).

The confidence limits for the GC reproducibility were calculated, using a 99% confidence level, to be 2.1% of the mean of the peak area counts obtained from the injection of the dilution series.

3.2.3.) Identification of cathinone and cathine

Confirmation of the alkaloids in *C. edulis* was made by GC/MS analysis of an extracted plant sample. Cathinone and cathine were identified on the basis of their mass spectra (Figure 14 a-b) and the characteristic mass fragments *m/e*. 44, 51, 77, 105 and 149 for cathinone and *m/e* 44, 51, 77, 105 and 151 for cathine. On the basis of this identification cathinone and cathine were found to have retention times of R_t=11.2 min. and R_t=12.0 min respectively on the GC with good baseline-to-baseline separation being achieved (Figure 11).

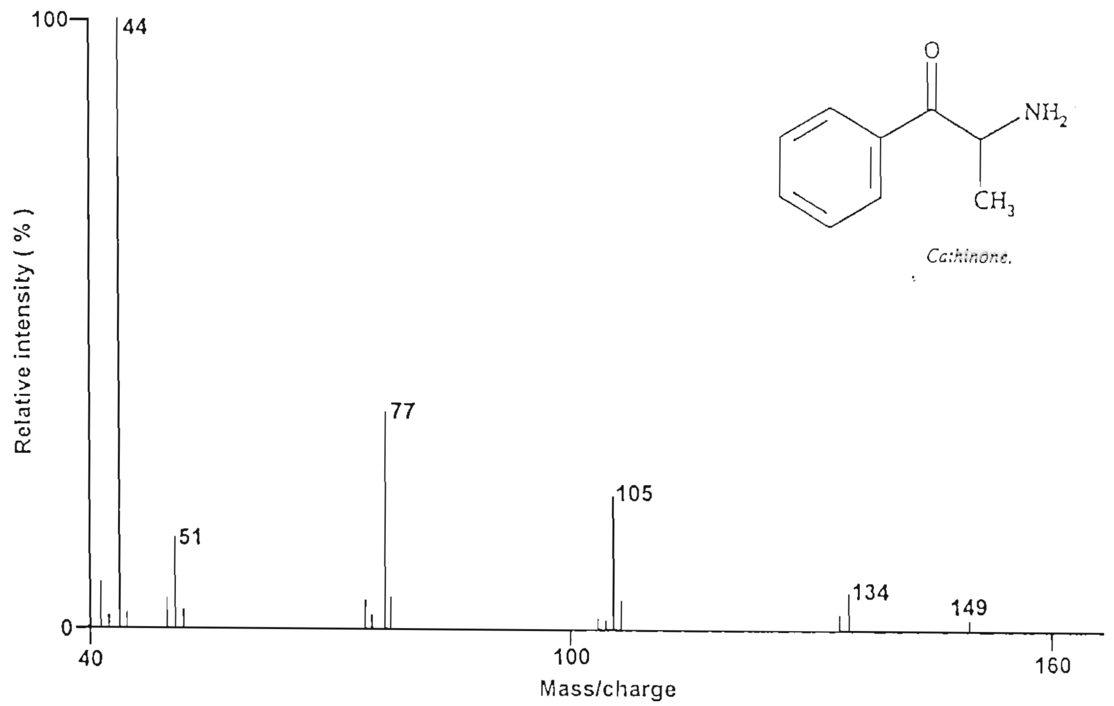


Figure 14a: Mass spectrum for cathinone.

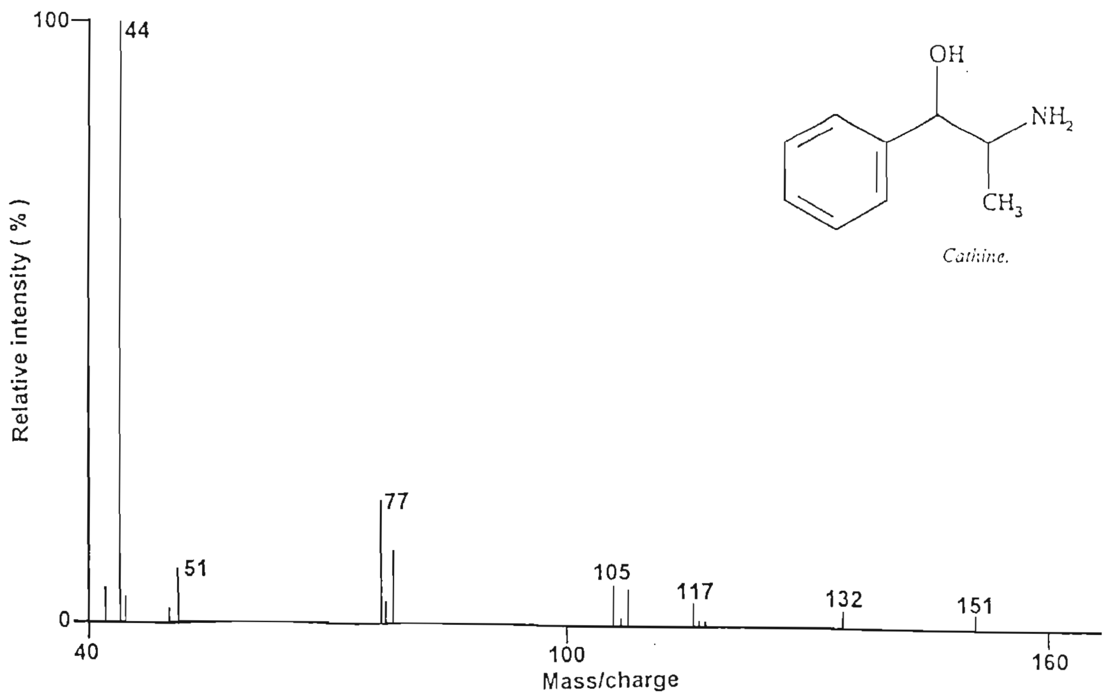


Figure 14b: Mass spectrum for cathine.

4.) Chemical changes in cathinone and cathine

Cathine exists as the diastereomers S,S-(+)-norpseudoephedrine and R,S-(-)-norephedrine, with S,S-(+)-norpseudoephedrine being the predominant epimer. In the course of developing an analytical technique for this study a (+)-norpseudoephedrine standard was produced by extracting a commercial diet tablet. When a 1 :l sample of this standard suspended in acetone was injected onto the GC it was found that the chromatographic profile typical for cathine, as obtained when suspended in dichloromethane (Figure 15a), was altered in that the acetone sample yielded two peaks; one with R_t=12.0 min. and one with R_t=13.2 min. (Figure 15b). A time course study was undertaken to examine the production of this second peak (Figure 15b-d). Whereas the dichloromethane sample was found to be stable over a number of days, it was noted that in the acetone sample the dominant peak, with R_t=12.0 min., slowly degraded, with the subsequent production of the peak with R_t=13.2 min. The chemical structure of the compound responsible for this peak was not determined, however, it is most likely that the (+)-norpseudoephedrine underwent nucleophilic addition from the acetone forming a new compound with sufficiently different chemical polarity to result in its separation from the original cathine peak. The relatively slow rate of this reaction (judged by the number of days for total alterations in relative peak sizes) is typical of such a nucleophilic addition.

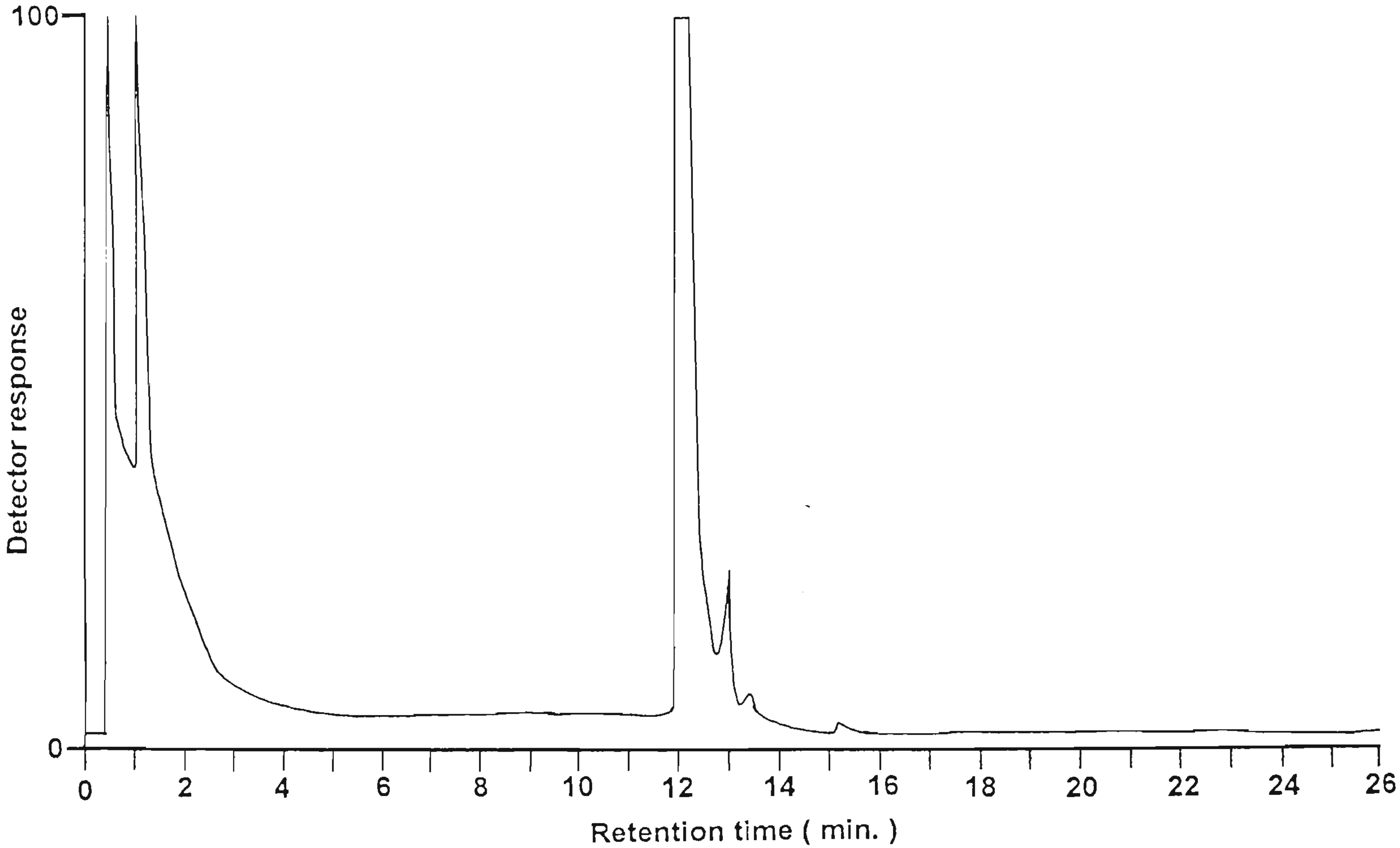


Figure 15a: **Chromatogram for a (+)-norspeudoephedrine standard in dichloromethane.**

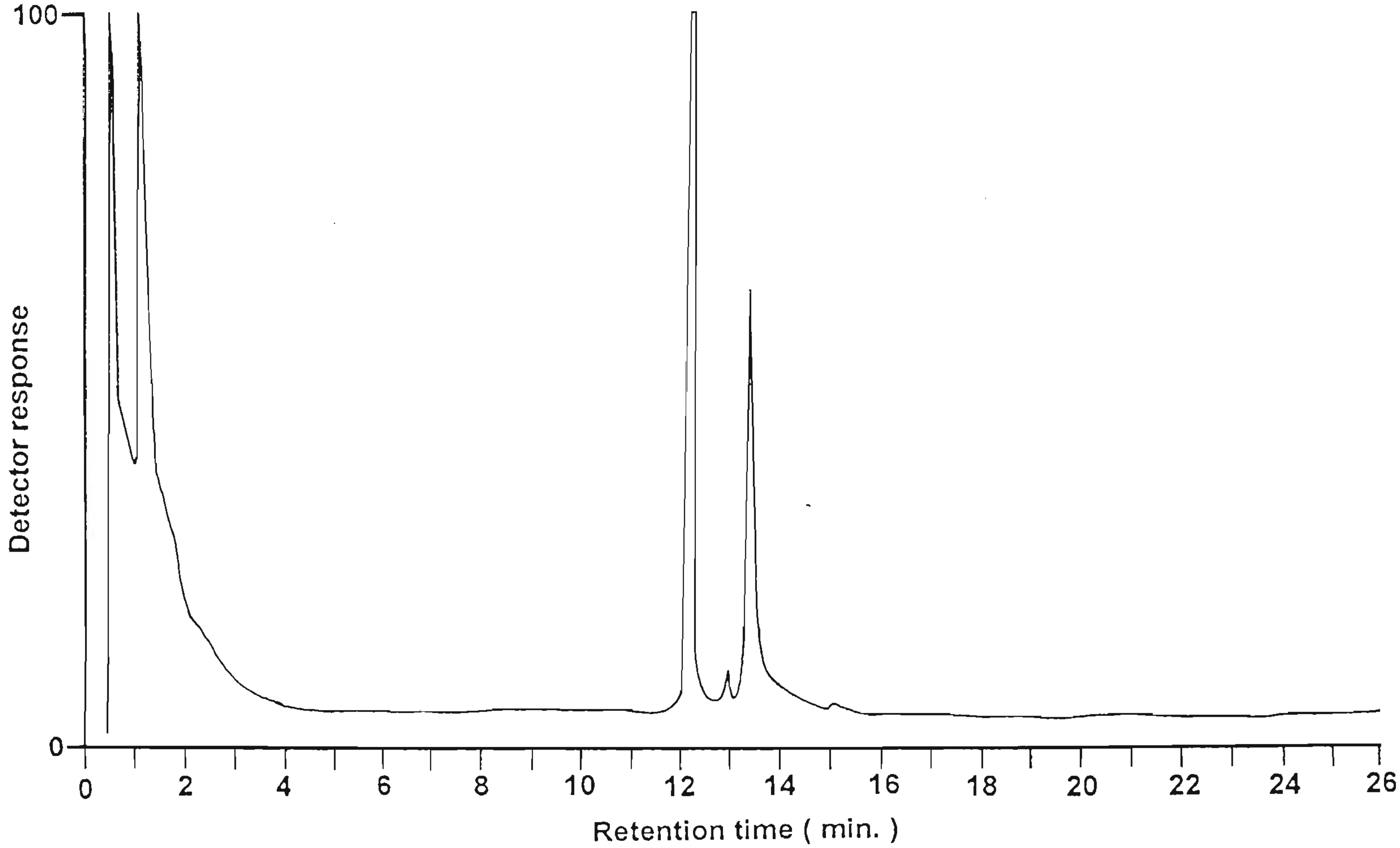


Figure 15b: **Chromatogram for a (+)-norspeudoephedrine standard in acetone at 6 hours.**

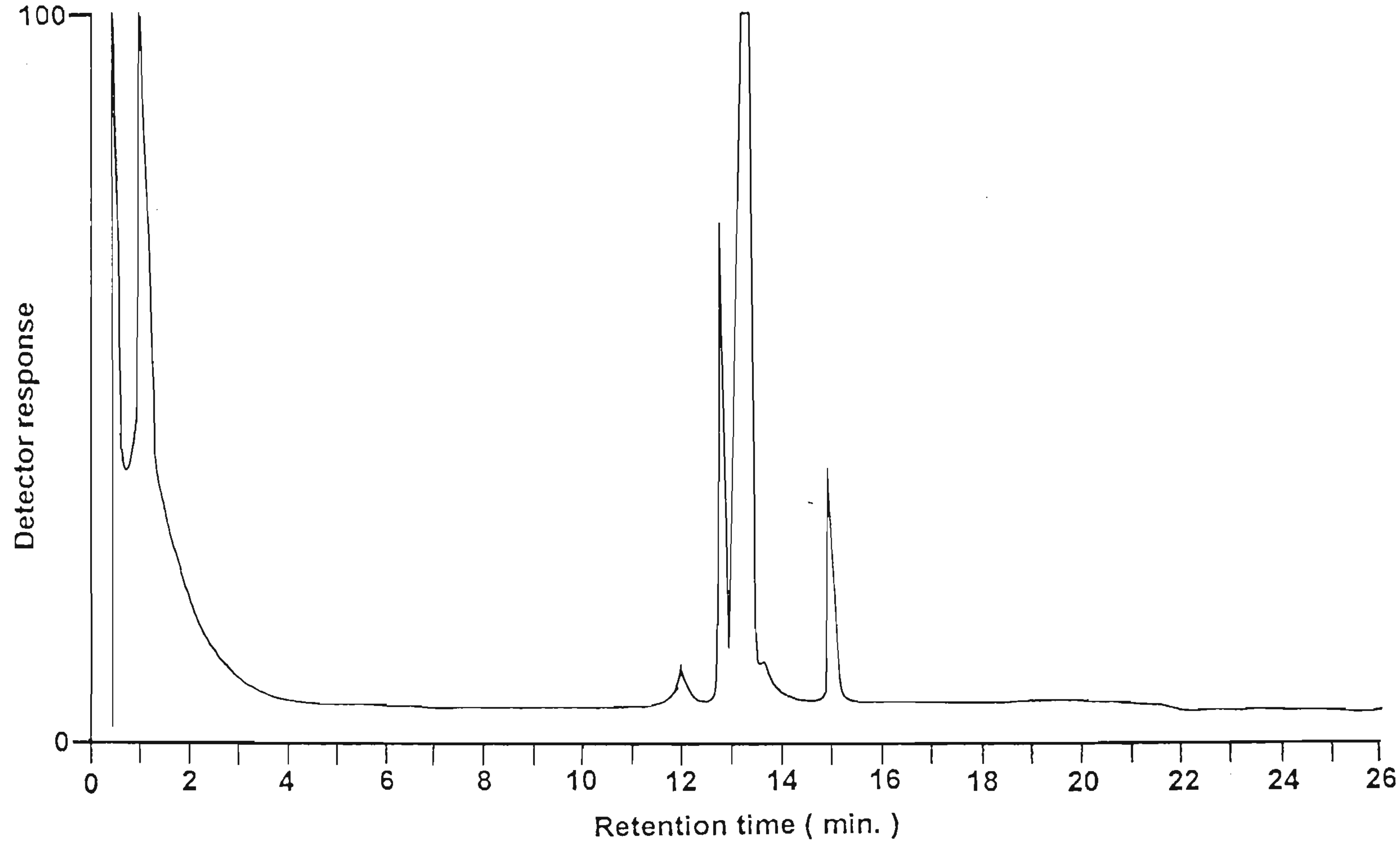


Figure 15c: Chromatogram for a (+)-norspeudoephedrine standard in acetone at 12 hours.

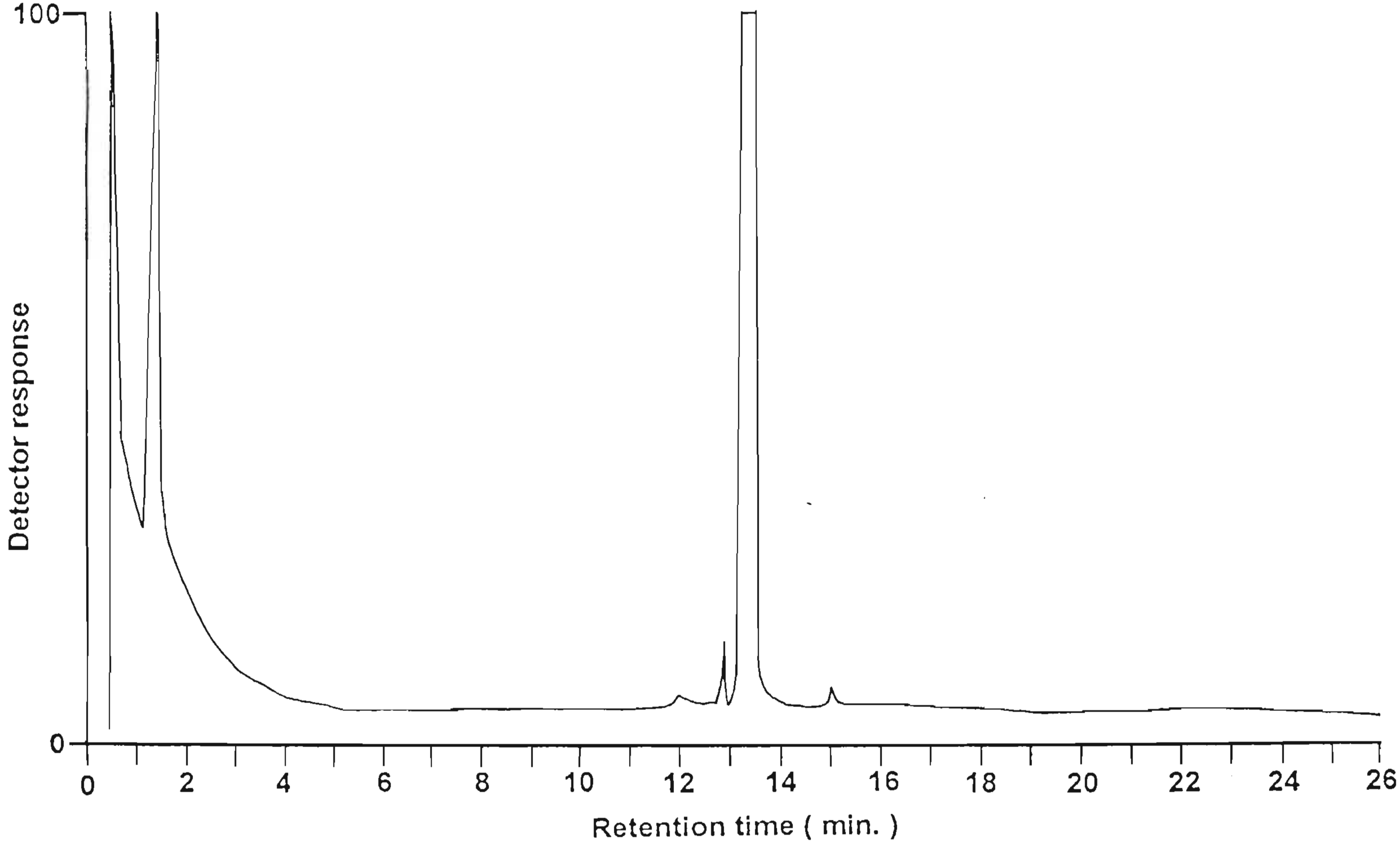


Figure 15d: Chromatogram for a (+)-norspeudoephedrine standard in acetone at 18 hours.

CHAPTER FOUR

ANALYSIS OF ALKALOID LEVELS IN DIFFERENT SPECIES OF *CATHA* AND *C. edulis* PLANTS OF DIFFERENT ORIGIN

1.) Introduction

The only study which has investigated differences in the levels of cathinone and cathine in leaves of *C. edulis* of different origin was carried out by Geisshusler and Brenneisen (1987). In this study khat samples were purchased at markets in Addis Ababa and Awedia/Harrar (Ethiopia), Nairobi and Mombasa (Kenya), Sanaa (North Yemen) and Anivorano (Madagascar). The samples were deep frozen within 24 hours and transported by air in a cooler to the laboratory in Switzerland for analysis. No leaf material from South Africa was analyzed. The only analysis reported on the South African species, *C. edulis* and *C. transvaalensis,* was by Lehmann *et al.* (1990) who analyzed dried samples from the Lowveld Botanic Garden and the Johannesburg Botanic Garden on a TLC system.

Other than the above-mentioned studies no complete analysis of cathinone and cathine has been carried out for different species of *Catha* existing in South Africa.

2.) Materials and methods

2.1.) Plant material

Cuttings of *C. edulis* were obtained from the Bolo Reserve in the Eastern Cape, the Durban Botanic Gardens (Natal), the Lowveld Botanic Gardens (Mpumalanga) and from a Nairobi khat market; these will be abbreviated as : Eastern Cape, Durban, Mpumalanga and Nairobi. Cuttings of *C. transvaalensis* were collected from the Lydenburg area (Mpumalanga). The cuttings were treated with a commercial rooting medium and planted out in the mist house for six weeks. After roots were initiated the plants were transferred from Vermiculite to a sand / soil mix in pots and planted out under shade cloth where they were watered regularly. A sapling of *C. abbottii* was collected from the

Umtumvuna Nature Reserve and similarly treated.

All plants were between 12 and 18 months of age at the time of harvesting. Only the top four leaves were harvested for analysis and care was taken to ensure that they were of uniform size, age and condition. Due to the limited availability of *C. abbottii* leaf material it was only possible to undertake a single analysis.

2.2.) Method

Five 5 g replicates of the plant material were extracted using Method 2 and 1 :1 samples of this extract were injected directly onto a GC for analysis as outlined in Chapter 3. The absolute values ($mg.g^{-1}$ fresh weight) of cathinone and cathine were determined by extrapolating the respective peak area counts of the two compounds from the standard curve for cathine. The correlation coefficient and gradient for the standard curve were calculated to be 0.97 and1472496.69 peak area/concentration respectively.

3.) Results and discussion

Leaves from the Durban sample contained the highest levels of cathinone with levels in the leaves from the other samples decreasing in the order Eastern Cape > Mpumalanga > Nairobi. Cathine levels were highest in leaves from the Mpumalanga sample with the levels in the leaves from the other samples decreasing in the order Durban > Nairobi > Eastern Cape. The total alkaloid content decreased in the order Durban > Mpumalanga > Eastern Cape > Nairobi (Table 1).

Analyses made of twenty two different khat samples from Kenya, Ethiopia, Madagascar and North Yemen revealed that the cathinone level varied from 0.09 to 3.30 $mg.g^{-1}$ dried plant material with the average cathinone content being 1.09 $mg.g^{-1}$ dried plant material (Geisshusler and Brenneisen, 1987). In order to relate these values (recorded in $mg.g^{-1}$ dried plant material) to the values obtained in this study (recorded in $mg.g^{-1}$ fresh weight) the percentage of water constituting the fresh plant material was determined by drying samples for three days in an oven at 100 °C and was found to be 80 %;

Table 1: Cathinone and cathine levels in *C. edulis* plants collected from four localities. (f.w. = fresh weight).

Source of *C. edulis*	Cathinone conc. ($mg.g^{-1}$ f.w.)	cathine conc. ($mg.g^{-1}$ f.w.)
Durban	**0.410**	**0.157**
Mpumalanga	**0.139**	**0.171**
Eastern Cape	**0.319**	**0.029**
Nairobi	**0.032**	**0.025**

taking this into account the published values of Geisshusler and Brenneisen (1987) equate to 0.02 to 0.66 $mg.g^{-1}$ fresh weight with a mean of 0.22 $mg.g^{-1}$ fresh weight. Thus, the greatest level of cathinone found in a khat sample from the Meru district (one the largest khat growing areas in the world) was 56% greater than that found in the leaves of the Durban sample, with the levels of cathinone in this sample being only slightly above the mean for samples tested from East Africa (Table 2).

The same study by Geisshusler and Brenneisen (1987) indicated that the cathine levels of the twenty two samples varied from 0.061 to 7.67 $mg.g^{-1}$ dried plant material with a mean of 3.8 $mg.g^{-1}$ dried plant material. This is equivalent to 0.01 to 1.53 $mg.g^{-1}$ fresh weight and a mean of 0.77 $mg.g^{-1}$ fresh weight (Table 2).

From Table 1 it is evident that the leaves from both the Durban and Eastern Cape samples contain higher levels of cathinone than cathine while the leaves from the Mpumalanga sample contain comparable levels of cathinone and cathine. All khat samples analyzed by Geisshusler and Brenneisen (1987) consistently showed that cathine was present at higher levels than cathinone (Table 2). This apparent difference in relative levels of cathinone to cathine can be explained in terms of changes bought about by post-harvest physiology the samples. Geisshusler and Brenneisen (1987) analyzed khat samples purchased at a market which were then deep frozen and transported to the laboratory for analysis. It is thus probable that a degree of enzymatic reduction of cathinone to cathine would have occurred between the time of harvest and the time of purchase, and to a lesser degree from the time of it being deep frozen to the time of its analysis in the laboratory. In this investigation leaf material was extracted within 15 min. of harvesting.

Table 2: **Results of Geishusler and Brenneisen (1987) for cathinone and cathine levels in 22 samples of khat purchased from khat markets in East Africa and Yemen. (Represented as $mg.g^{-1}$ fresh weight).**

Market where purchased	Quality	Origin of *C. edulis*	cathinone conc. ($mg.g^{-1}$ f.w.)	cathine conc. ($mg.g^{-1}$ f.w.)
Awedia/Harrar (Ethiopia)	First	Awedi/Harrar	0.35	1.39
Awedia/Harrar (Ethiopia)	Second	Awedi/Harrar	0.11	0.78
Awedia/Harrar (Ethiopia)	Third	Awedi/Harrar	0.09	0.83
Awedia/Harrar (Ethiopia)	First	Awedi/Harrar	0.12	0.70
Awedia/Harrar (Ethiopia)	Second	Awedi/Harrar	0.13	0.98
Awedia/Harrar (Ethiopia)	Third	Awedi/Harrar	0.06	0.59
Awedia/Harrar (Ethiopia)	Second	Awedi/Harrar	0.21	0.67
Awedia/Harrar (Ethiopia)	Third	Awedi/Harrar	0.10	0.72
Addis Ababa (Ethiopia)	Second	Wollene-Village	0.37	1.16
Addis Ababa (Ethiopia)	Second	Wollene-Village	0.37	1.53
Addis Ababa (Ethiopia)	Third	Wollene-Village	0.34	0.73
Addis Ababa (Ethiopia)	First	Wollene-Village	0.35	1.21
Addis Ababa (Ethiopia)	First	Hosana-South	0.13	1.51
Addis Ababa (Ethiopia)	First	?	0.24	0.80
Addis Ababa (Ethiopia)	First	?	0.15	0.71
Gondar (Ethiopia)	?	Tiki Dengay	0.13	0.47
Mombasa (Kenya)	First	Meru	0.66	0.35
Mombasa (Kenya)	First	Meru	0.42	0.36
Nairobi (Kenya)	First	Meru	0.34	0.48
Sanaa (North Yemen)	First	?	0.07	0.54
Sanaa (North Yemen)	Second	?	0.02	0.32
Anivorano (Madagascar)	?	Anivorano	0.02	0.01

Although the degree to which enzymatic reduction affects the levels of cathinone and cathine is not known, it is known that a decrease in the levels of cathinone would result in a concomitant elevation of cathine levels. From this it can be assumed that the levels of cathinone in the intact plants studied by Geisshusler and Brenneisen (1987) would have been considerably higher than those levels found for the samples analyzed in this investigation with the levels of cathine being correspondingly lower.

The higher levels of cathinone and cathine in khat samples analyzed by Geisshusler and Brenneisen (1987) may be explained in terms of inherent genetic differences between the natural *C. edulis* populations of East Africa and South Africa. The geographical differences between their distributions and subsequently the different natural environmental pressures may have resulted in the natural selection of populations that differ in their genetically controlled ability to biosynthesize cathinone and consequently cathine.

However, the extensive history of interaction between *C.edulis* and man in East Africa and Yemen may be responsible for the higher levels found for khat samples by Geisshusler and Brenneisen (1987). Through this extensive history *C.edulis* use evolved into highly organized and sophisticated systems of cultivation, harvesting, trade and consumption.

In areas of East Africa climatically suited to its growth *C.edulis* is cultivated as a cash crop. From these areas khat is harvested and transported by truck or airplane to major centers where khat markets serve local populations from mid-morning, whence upon acquiring their daily portion, the "khat session" begins. At markets buyers can be very particular and differentiate between a number of varieties.

In Ethiopia there are at least seven kinds of khat recognized in the market place, however, this is generally simplified to either "red" or "white" khat (Getahun and Krikorian, 1973). Geisshusler and Brenneisen (1987) report that at Awedai (the most important market in the Hararghe region) and Addis Ababa the "red" type is considered to be the best drug and fetches the highest price, followed by "intermediate" and "white" types. They found on analysis that the "red" type had the highest levels of total khatamines and cathinone. However, no differences were found between the total khatamine and cathinone levels and the "intermediate" and "white" types. Ethiopian producers and dealers also classify khat into first ("**kudda**"), second ("**uretta**") and third quality ("**kerti**") based on size, age and taste; the younger, tender leaves with lower levels of tannins and terpenes being favored over older, leathery leaves. A good correlation between these quality assessments and the cathinone content was found by Geisshusler and Brenneisen (1987).

The evolution of this organized khat industry to a degree where different varieties are recognized by consumers for their quality would undoubtedly have driven selection for wild specimens or populations of *C.edulis*, with higher levels of cathinone. In particular the activities of cultivation and trade from which greater profits can be attained from higher cathinone yielding plants would vigorously drive this selection. Cultivation practices too, may influence the physiology of the plant thus resulting in higher levels of cathinone in the harvested drug.

In South Africa, although utilized for both medicinal and psychoactive properties, no sophisticated systems of cultivation, harvest, trade or use have been developed. In the Bolo Reserve of the Eastern Cape, Xhosa farm laborers harvest **igqwaka** from trees growing in a natural environment and although the young, soft leaves are preferentially harvested, no attempt has been made to identify, select and cultivate higher-yielding specimens. In contrast to the extensive system of trade in khat that exists in East Africa and Yemen, **iqwaka** is not traded, but is considered as a gift or a gesture of friendship. Also, no grades of **iqwaka** are recognized.

Thus by purchasing khat samples at market places Geisshusler and Brenneisen (1987) were probably sampling a population of plants selected for their higher yields of cathinone. In comparison, the samples investigated in this study were harvested from plants grown from specimens growing in the wild which had not been subjected to any human imposed selection criteria.

The lower levels of cathinone and cathine found for samples analyzed in this study may also be explained in terms of the overall physiological state of the plant in relation to its age and the manner in which it is cultivated. Waller and Nowacki (1978), amongst many other researchers, have reported a number of examples in which alkaloid levels in plants fluctuate with the age of the plant and the manner in which it is cultivated.

Botta (Nigg and Siegler, 1992) reported that cuttings were planted and allowed to grow for three years, at which time all the leaves were removed and sold as "kat moubarreh" which was considered to be of inferior quality. It was only the following years harvest "kat methein" that was considered to be of high quality. In terms of resource partitioning this report by Botta may indicate that either *C.edulis* plants apportion a majority of their resources to metabolism which is responsible for the synthesis of compound essential for the rapid growth of plant tissues and organs or, more likely, that with the removal of all the leaves from the plant apical growth is stunted and, as a result, a greater portion of plant resources are partitioned into secondary metabolic processes. This may explain the lower levels of cathinone and cathine in the samples investigated in this study as they were harvested from plants from cuttings between 12 and 18 months of age. In particular it would explain the low levels of the Nairobi sample which was harvested from plants grown from a khat sample purchased at a Nairobi khat market.

It is not possible, however, to explain the higher levels of cathinone and cathine in khat samples from East Africa solely on the basis of human - imposed selection pressures or by inherent genetic differences between populations as there exist a number of factors which can affect alkaloid levels in plants.

Although the level of alkaloid biosynthesis is gene governed, there are remarkable fluctuations in the concentrations and amounts of alkaloids produced per plant due to environmental conditions. Environmental factors such as light, supply of nitrogen, potassium, phosphorous and other minerals, temperature, moisture of the soil, and height above sea levels will all affect the general growth of the plant, and to a greater degree the young actively dividing tissues; the source of synthesis of many alkaloids. Hence a situation can arise that plants from the same species but with different environmental conditions can have different alkaloid levels.

Cathinone is considered to be one-third as potent in its stimulatory effects as amphetamine (Lee, 1995) and roughly ten times more potent than cathine (Geisshusler and Brenneisen, 1987).

When this is taken into account the ratio of cathinone to cathine becomes crucial when considering the psychoactive potency of the fresh drug. In order to compare the *C. edulis* samples from different origins on the basis of their psychoactive potency the term Effective Potency was devised. The Effective Potency was calculated by multiplying the cathinone concentration ($mg.g^{-1}$ f.w.) by ten, and adding this value to the cathine concentration ($mg.g^{-1}$ f.w.). Hence the formula would read:

Cathinone conc. ($mg.g^{-1}$ f.w.) x 10 + cathine($mg.g^{-1}$ f.w.) = Effective Potency

For example the Durban sample would have an Effective Potency calculated as follows:

$$0.410 \times 10 + 0.157 = 4.257$$

In this way the psychoactive potency of leaves from plants of different origin could be compared (Table 3).

Table 3: Cathinone to cathine ratio and Effective Potency of *C. edulis* from different localities.

Source of C. edulis	Total cathinone and Cathine ($mg.g^{-1}$ f.w.)	Cathinone:cathine Ratio	Effective Potency
Durban	**0.567**	**2.6**	**4.3**
Mpumalanga	**0.310**	**0.8**	**1.6**
Eastern Cape	**0.348**	**11.0**	**3.2**
Nairobi	**0.057**	**1.3**	**0.4**

From Table 3 the variations in the ratio of cathinone to cathine can be seen. Whereas leaves from the Eastern Cape sample have a total alkaloid level 12% greater than for those from Mpumalanga, their effective potency is greater by 200%. As such, the leaves from the Eastern Cape sample would be considered to be of better quality, as less of the plant material would have to be chewed in order to elicit the same stimulatory effect. It should be noted however that the although the ratio of cathinone to cathine was lower in the leaves from the Durban sample than that obtained in the leaves from the Eastern Cape the greater total levels resulted in it having a greater Effective Potency value than any of the other samples.

Analysis of the three *Catha* species in South Africa revealed that cathinone and cathine were only present in appreciable levels in *C. edulis*. Analysis of the *C. transvaalensis* sample with the NPD sensitivity set an attenuation 100 times greater than that for the analysis of the *C. edulis* samples, revealed peaks with Rt = 11.4 and Rt = 12.4 minutes (Figure 16). Although slightly different from the Rt for *C. edulis* samples and for the standards, these peaks were further analysed with GC/MS. The peak at Rt = 11.4 showed the characteristic mass spectrum of cathinone, whereas the Rt = 12.4 min. peak did not produce a mass spectrum characteristic of cathine. It would appear from these results that with cathinone being present in *C. transvaalensis* at levels 7000 fold lower than found in *C. edulis*, any cathine present would be below detectable levels.

That cathinone is detectable in *C. transvaalensis* may be taken as evidence for a functional biosynthetic pathway; perhaps operating at a very low rate. However, to use the presence of cathinone as an argument for the taxonomic relatedness between *C. edulis* and *C. transvaalensis* would not be warranted, as numerous examples exist in which unrelated genera produce the same alkaloids.

C. abbottii was found to contain neither cathinone nor cathine, analysis of the sample at elevated NPD sensitivities showed no peaks corresponding to the cathinone and cathine putative peaks.

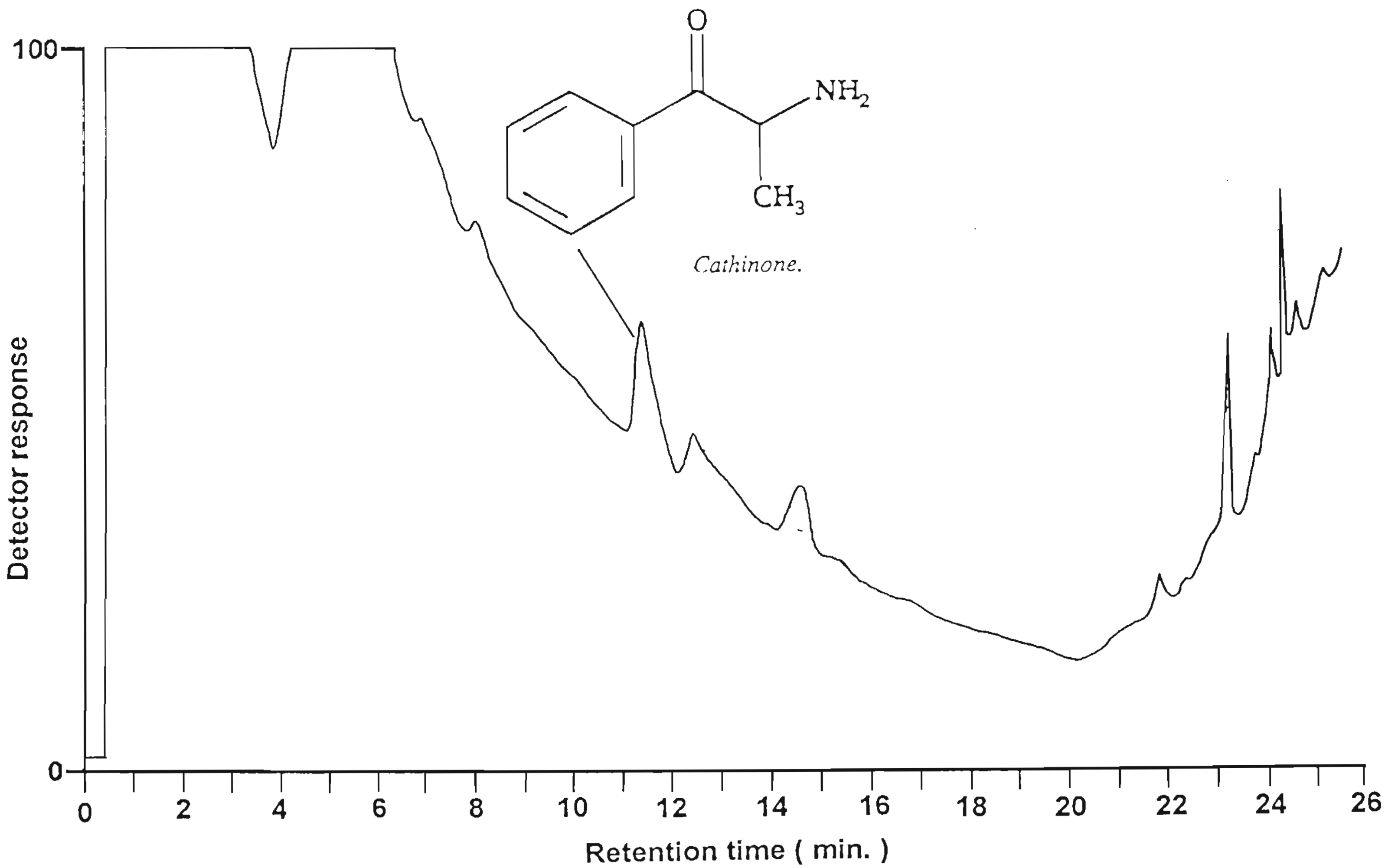


Figure 16 : Chromatogram for *Catha transvaalensis*.

CHAPTER FIVE

LEVELS OF CATHINONE AND CATHINE IN DIFFERENT PARTS OF *C. edulis*

1.) Introduction

While alkaloids in plants are often seen as merely inert products of secondary metabolism, there are some studies which indicate that they participate in the overall metabolism of plants. Evidence for this comes from a multitude of studies examining the fluctuation of alkaloid concentrations between different plant parts (Mothes, 1960). These fluctuations can be observed on a diurnal, circadian, seasonal and over the developmental life span of the plant. Generally the greatest levels are found to coincide with the optimum growth stage of plants and rarely with the onset of senescence, it is generally held that alkaloid content increases and then declines with respect to this physiological state of the plant (Waller and Nowacki, 1978).

For analysis of cathinone and cathine in *C. edulis* most recent studies have relied on the young fresh leaves as these are commonly chewed as the drug khat. As a result, very little is known about the distribution of cathinone and cathine in other plant parts, and how this distribution changes with the development of the plant. The only investigation to have dealt with differences in the levels of cathinone and cathine in *C. edulis* was carried out by Brenneisen and Geisshusler (1985), where samples of the young shoots, fully developed leaves, bark from older branches and the trunk and roots were collected from a mature tree and analysed.

2.) Materials and Methods

2.1.) Plant material

To determine the distribution of cathinone and cathine in different plant parts of *C. edulis* leaves, stems and roots were taken from potted plants and subjected to analysis.

Leaves of three developmental stages were chosen : youngest leaves – young, soft, red leaves (typically the apical pair); juvenile leaves – partially expanded green leaves (typically leaves four to six from the apex); mature leaves – fully expanded green leaves (the sixth pair of leaves from the apex and beyond). Two categories of stems and roots were also chosen : young stems - shoot or stem material red in colour; mature stem – stem material with secondary thickening (typically the lower 10 cm of the stem); young non-woody roots; and mature roots - showing secondary thickening.

2.2.) Method

5 gm of the plant material was extracted using Method 2 and 1 :l of the resultant extract was injected directly onto a GC for analysis as outlined in Chapter Three.

3.) Results and discussion

Cathinone levels were found to be the highest in the youngest leaves with the levels decreasing considerably with the developmental stage of the leaf; the mature leaves containing only 14.4% of that found in the youngest leaves. A 31% increase in the levels of cathine was observed between the youngest leaves and the mature leaves (Table 4).

Analysis of stem material showed that cathinone and cathine levels were considerably lower and higher respectively than those levels for the youngest leaves. Young stem material contained both at higher levels than mature stem material (Table 4).

Young root material showed no detectable levels of cathinone or cathine at increased NPD sensitivity. Mature root material was found to contain both at levels higher than those for mature stem material (Table 4).

Table 4: Levels of cathinone and cathine ($mg.g^{-1}$ fresh weight) for different plant parts.

Developmental stage	Cathinone Conc. ($mg.g^{-1}$ f.w.)	Cathine conc. ($mg.g^{-1}$ f.w.)
Youngest leaves	0.243	0.006
Juvenile leaves	0.124	0.011
Mature leaves	0.035	0.019
Young stem	0.033	0.270
Mature stem	0.004	0.052
Mature root	0.012	0.063
Young root	0.000	0.000

Although nothing is known about factors such as site(s) of synthesis, aspects of translocation and accumulation and modification of alkaloids over time, on the basis of these results and those obtained by Brenneisen and Geishussler (1985) it would appear that some dynamic aspects of alkaloid physiology exist in *C. edulis*.

It was reported that the highest levels of cathinone and cathine in trees were found in young shoots already carrying leaves, with reduced levels in fully developed leaves. Stem and bark of older branches showed only traces of cathinone and the roots contained neither cathinone nor cathine (Brenneisen and Geisshusler, 1985). The greatly elevated levels of cathine in the young stem material observed in this study and the marked decline seen with stem maturation suggest progressive decrease with age to minimal levels in much older stems and branches and suggests an age dependant pattern of alkaloid breakdown or loss. This suggestion is in keeping with the results of Brenneisen and Geisshusler (1987).

It is generally recognised that the most intensively studied alkaloids are those of the genus *Nicotiana*. Nicotine is synthesised predominantly in the youngest parts of roots and then rapidly transported from the roots to the shoots and leaves (Mothes, 1960), where it accumulates to a level higher than any other part of the plant. Once in the leaf, nicotine can be demethylated to nornicotine (Waller and Nowacki, 1978). While nicotine is synthesised in the roots and translocated to the aerial parts of the plant, the opposite is true for the lupine alkaloids which are synthesised and accumulate in the aerial parts of the plant (Waller and Nowacki, 1978). Grafting a stem of *Lupinus angustifolius* (which contains enzymes capable of oxidising alkaloids)onto an entire *Lupinus arbacus* plant, so that both stems exist off the same root stock, resulted in the accumulation of only the oxidised alkaloids.

Ricinus communis shows the ability to synthesise ricinine in all parts of the plant, although it is probable that some organs are more efficient than others. The highest levels of ricinine where found in the mature leaves, but, with the onset of senescence ricinine is translocated from these leaves to younger ones (Waller and Nowacki, 1978).

It seems unlikely that the roots are either a major site of cathinone synthesis or source of translocation to other parts of the plant as Brenneisen and Geisshusler (1985) did not detect any cathinone in the roots of *C. edulis* trees; in the present study on saplings only low levels of cathinone and cathine were detected in the mature roots, with these being absent from the young roots.

Cathinone exhibits an acropetal distribution pattern within the plant; i.e. levels are highest at the apex and lowest in basal leaves. The increase in the cathinone levels from the mature stem material to the mature root material can be explained in terms of the roots acting as a reservoir for the storage of cathinone.

Cathine shows the same distribution pattern as cathinone in the stem. The high levels of cathine in the young stem below the leaves may be explained by the translocation of cathinone to the stems with the subsequent reduction to cathine taking place in the young stems. However, there is no concrete evidence for this translocation and to determine this fact would require further investigation. It is unlikely that cathinone is reduced to cathine in the leaf which in turn is selectively translocated to the young stems; as cathinone and cathine have very similar physical and chemical characteristics and a model describing such a selective translocation system in plants does not exist. This also excludes the possibility of cathinone being synthesised in the young stem and being selectively translocated to the leaves.

It is possible that the high levels of cathinone and cathine in the young developing leaves takes the place of tannins and terpenes as an anti-herbivory mechanism which are lacking in the young leaves of *C. edulis*.

El-Domiaty *et al.* (1994) indicated that undifferentiated callus from various hormonal treatments did not contain any cathinone or cathine, while micro-propagated plants contained cathinone at low levels, indicating that differentiation or organogenesis is needed for cathinone synthesis.

The ethnobotanical relationship between plants and man has in instances revolved around the plant's psychoactive properties; examples abound but some of the more studied include *Erythroxylum cocoa*, *Papaver spp.*, *Cannabis sativum* and *Psilocybe spp.* In each of these cases a specific means of consumption or use has been developed. The relationship between *C. edulis* and man is no exception, and has resulted in the identification of the youngest, freshest leaves as the most potent form of the drug. This selection process by indigenous people need not have taken many thousands of years as we see a parallel subculture of *C. edulis* use evolving seemingly independently in the comparatively young society of the Xhosa farm labourers of the Eastern Cape.

Coupled with the benefits of higher levels of cathinone and cathine in the young shoots and leaves are the added benefits of lower levels of tannins and terpenes which are unpleasant to the taste. Furthermore, younger leaves are easier to masticate thus in turn making the extraction of the constituents by saliva easier. However, in the Bolo Reserve in winter young soft leaves are not readily available and as a result users are known to make a hot infusion of the older leaves (Hirst, 1997); this method of consumption has a considerable history as the early Dutch explorer Pappe (Smith, 1966) stated that *C. edulis* leaves were not only chewed by the Khoisan but that it was also a popular beverage.

Thus, the results show how pharmacological efficacy is selected for by users choosing plant parts with the highest levels of alkaloids. Additionally, the low alkaloid levels in some plant parts make conventional consumption totally impracticable.

CHAPTER SIX

CONCLUSION

It is not certain whether the use of *C. edulis* as a stimulant had its origin in east Africa or Yemen. Originally the young, fresh leaves of *C. edulis*, known as khat, were chewed predominantly by Muslims whose religious book, the Koran, outlawed the use of alcohol. It can be summized that khat acted as the alcohol equivalent in social gatherings and family events. Traditionally, as a Muslim drug, khat's use was limited to male users who would typically gather indoors at midday with their khat portions. While chewing khat and sipping cold water they would give praise and discuss daily events and matters of communal importance. A bastardized form of this "khat session" has since become common place amongst the general populace in areas where khat is available as its use has spread to many cultures, nationalities and religions. Today khat is recognized as a social and economic evil with the daily "khat session" bringing local economies to a standstill each afternoon. Due to this cathinone, the primary psychoactive ingredient of *C. edulis*, was included in Schedule 1 of the United Nations Convention on Psychoactive Substances.

The khat industry in east Africa has evolved into highly organized and sophisticated systems of cultivation. harvest and trade. Although officially banned the trade in khat flourishes in east African countries and Yemen. It is probable that the evolution of these sophisticated systems are responsible for the elevated levels of cathinone and cathine found in khat. By becoming a common practice the "khat session" created a means by which selection criteria were imposed by consumers. As in any industry the end consumer controls the quality of supply by trying to maximize their buying power. In so doing the growers would respond to purchasing trends and thus identify higher yielding (better quality) specimens. It is most likely that this process was imposed unconsciously by consumers and producers. but it offers a unique example of human-imposed selection as apposed to selection in the plant kingdom.

The levels of cathinone and cathine in khat as analyzed by previous researchers were found to be higher than those found for samples of *C. edulis* analyzed in this study. These differences may reflect: 1.) a human – driven selection process resulting in the higher levels of cathinone in plants where there has been a history of khat cultivation, harvest and trade; or 2.) there exists a relationship between the age of the plants and alkaloid levels; or 3.) the environmental conditions in which *C. edulis* grows in the two regions has an effect on the plants ability to synthesise cathinone and cathine.

C. edulis use as a stimulant in South Africa has a relatively short recorded history. Originally used by the San to ward off hunger and fatigue it appears the Xhosa people of the Eastern Cape learnt of its properties from these people and named the young fresh leaves **iqwaka**. **Iqwaka** portions are harvested by groups of pickers from trees growing in the wild. These foraging excursions serve as social events where community relationships are strengthened. **Iqwaka** collected by these parties is not traded but rather given to friends and family. In keeping with the use of khat in East Africa, **iqwaka** is selected as the young, fresh leaves which are chewed but not swallowed. Very often a group of adults will sit in the shade of a tree and chew **iqwaka** and drink cool water akin to the present day "khat session" of east Africa and Yemen.

Limited information exists as to *C. edulis* utilization as a medicinal in South Africa. Zulu herbalists have used the bark of *C. edulis* as a remedy for stomach pains and it may be that through the harvesting of the bark material over many generations that the trees status in the wild has been significantly reduced in kwa-Zulu Natal. The more sustainable usage of *C. edulis* by Xhosa people in the Eastern Cape may explain why the tree is abundant in this area.

Although HPLC has become the "method of choice" employed by most researchers in the analysis of *C. edulis* alkaloids this study shows that accurate and precise results can be achieved by gas chromatography. Investigation of three popular extraction and isolation methods revealed that a methanol extraction; avoiding polar solvents and excesses in temperature, was a rapid, reliable and relatively uncomplicated means by which cathinone and cathine could be extracted and isolated. Application of a derivatization technique was found to be inconsistent and unnecessary as identification of cathinone and cathine via their mass spectra on GCMS was carried out using underivatized samples.

Analysis of saplings grown from cuttings collected from three forms of *C. edulis* growing in different localities in South Africa, and a forth from a khat sample purchased at a Nairobi khat market, revealed levels of cathinone and cathine that varied between localities. The levels from these localities were also found to be markedly lower that those found by other researchers analyzing khat samples from East Africa.

Division of saplings into the different groups of youngest leaves, juvenile leaves, mature leaves, young stem, mature stem, mature roots and young roots showed that the levels of cathinone and cathine varied with a general decline of cathinone with the increase in age of the plant part, while cathine showed an increase from youngest leaves to young stem and then declined with increase in stem age. Cathinone and cathine was not detectable in the young roots. An increase in cathinone and cathine was observed between the mature stems to mature roots. The speculation that roots serve as a source of storage for these compounds requires further more detailed study.

Analysis of *C. transvaalensis* revealed that cathinone was present only at greatly reduced levels and cathine was undetectable, it can be speculated that the biosynthetic pathway for the synthesis is not expressed as greatly as it is in *C. edulis*.

The absence of both cathinone and cathine in *C. abbottii* may indicate the divergent nature of this species due to its geographical isolation.

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