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CONSTITUENTS OF *CATHA EDULIS* (ALKALOIDS, TRITERPENOIDS AND RELATED SUBSTANCES AND SAPONINS)

By

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ABSTRACT

The study of the alkaloids of khat samples from Egypt and Yemen revealed qualitative and quantitative differences. Cathine and the dimer of cathinone occured in both samples, while norephedine was detected only in Yemen samples. Investigation of certain other constituents *viz.* triterpenes, sterols, fatty alcohols, hydrocarbons, fatty acids and saponins resulted in the isolation and identification of several components.

INTRODUCTION

Khat *(Catha edulis* Forsk., Celastraceae) is cultivated in certain parts of Africa and in the southern parts of the Arabian peninsula. The inhabitants of these regions chew khat in order to obtain stimulating effects and the consumption of the plant causes serious problems in some countries. In countries where the use of khat is widespread, the habit has a deep-rooted social and cultural tradition. The desirable effects of khat leaves, as percieved by experienced users, are relief from fatigue, increased alertness and energy levels, feelings of elation, improved ability to communicate, enhanced imaginative ability and capacity to associate ideas, and heightened self-confidences. These effects seem to be more readily percieved by the habitual user (Kalix and Braenden, 1985).

The chemical study of khat goes back to 1887 when Flueckiger and Gerock, searching for caffeine as the possible stimulating principle, found no traces of it but discovered instead an alkaloid, named katin. The chemical composition of khat was later investigated by several investigators and a number of phenyalkylamines has been isolated (Winterfeld and Bernsmann, 1960; Alles *et al.*, 1961; Cais *et al.*,

1975; Szendrei, 1980; Kalix and Braenden, 1985; Al-Meshal *et al.*, 1986). Furthermore, the leaves contain another group of alkaloids called the cathedulins (polyesters or lactones of sesquiterpene polyols), with a molecular weight ranging from 600 to 1200 (Baxter *et al.*, 1976-1979; Crombie *et al.*, 1978). Khat leaves also contain small amounts of volatile oil (Quédan, 1972), sterols and triterpenes (Crombie, 1980), and they have a high tannin content (7-14% in dried material, Luqman and Danowski, 1976). The leaves are also reported to contain about 5% protein (Halbach, 1972) and considerable amounts of ascorbic acid (Mustard, 1952).

EXPERIMENTAL

Plant Material

Upper leaves of *Catha edulis* were collected from Quenater in Egypt. Another sample was obtained from Yemen Arab Republic as a powder. The identity of the latter sample as *C. edulis* was botanically proved.

Thin-Layer Chromatography

Terpenoids and related substances: Silica gel G; solvents: *n*-hexane-ethyl acetate 6:2, toluene-acetone 85:15; spraying reagent: 50% H_2SO_4.

Phenylalkylamines: Silica gel G; solvents: methanol-acetone-ethyl acetate-ammonia 80:20:10:1, *n*-hexane-ethanol-ammonia 40:60:1; spraying reagent: 1% ninhydrin in alcohol.

Saponins: Silica gel G; solvents: choloroform-methanol-water 65:15:10, *n*-hexane-ethyl acetate 6:2; Spraying reagent: *p*-anisaldehyde.

Gas-Liquid Chromatography

Hydrocarbons: Glass column (2 meter x 4 mm i.d.) packed with OV-1, 1% on chromosorb W AW DMCS 80-100 mesh, temp. program. 130-250°C, 4°C/min., inject. temp. 260°C, detector temp. 260°C.

Terpenes: The same conditions of hydrocarbons with a column temp. 245°C.

Fatty Acids: Column 6 feet packed with polyethyleneglycol adipate (10%) on chromosorb W, column temp.: 180°C, inject. temp. 220°C, detector temp. 250°C.

Phenylalkylamines: Column (1 meter x 4 mm i.d.) packed with carbowax (5%) on chromosorb Q (80-100 mesh), column temp. 160°C, inject. temp. 250°C, detector temp. 250°C.

Gas Chromatography-Mass Spectrometry

LKB 9000 instrument was used applying the above conditions.

Paper Chromatography

Sugars: Whatman No. 1; Solvent: *n*-butanol-acetic acid-water 4:1:5; Spraying reagent: aniline phthalate.

I- Lipid Fractions

The powdered leaves (500 gm) were extracted with petroleum ether, (40-60°C). Evaporation of the purified extract yielded 12 gm. The residue was dissolved in boiling acetone (50 ml), filtered, left overnight and the preciptated amorphous substance (alcohol fraction) was separated. The acetone-soluble fraction (5.5. gm) was chromatographed on aluminium oxide (Table 1).

Table 1

Column chromatographic fractionation of the Lipid Fraction

Solvents	Fractions	R_f*	
Petroleum ether (40-60°C)	1-57	0.98	Hydrocarbons
Petroleum ether-benzene (80:20)	58-102	0.86	Friedelin
(70:30)	103-128	0.61	Alcohols
	129-198	0.62	Friedelane
(50:50)	199-286	0.51	β -Sitosterol
Benzene	287-412	0.40	

* Solvent: *n*-hexane-ethyl acetate (6:2)

Hydrocarbon Fraction: Crystallization of the residue obtained from fractions 1-57 afforded a white substance. IR showed typical absorption bands of *n*-alkanes. GLC and MS showed that it is a mixture (table 2).

Alcohol Fraction: Both alcohol fractions (obtained from the acetone-insoluble fraction and from column chromatography) were found to possess the same R_f value and melting point. MS showed that it is a mixture of *n*-tetracosanol ($C_{24}H_{50}$, *m/e* 354), *n*-hexacosanol ($C_{26}H_{54}$, *m/e* 382) and *n*-octacosanol ($C_{28}H_{58}$, *m/e* 410).

Friedelin: Fractions 58-102 (Table 1) gave fridelin (TLC, m.m.p., MS).

Friedelane: Fractions 129-198 (Table 1) gave upon crystallization from chloroform-methanol, friedelane (TLC, GLC, m.p. 250°C), reported 248-250°C (Betancor *et al.*, 1980). MS showed M^- at 426 (due to loss of methyl group) and *m/e* at 218 (characterstic of triterpenes).

Sterol Fraction: The sterol obtained from fractions 199-286 (Table 1) melted at 133-134°C and possessed the same R_f as the authentic β-sitosterol. MS showed M^+ at *m/e* 414 as the major component.

Fatty acids: The fatty acids, obtained by saponification of the lipids in the usual manner, were subjected to GLC after conversion to their methyl esters (Table 3).

II. **Phenylalkylamines**

About 1 kg of the powdered leaves was perculated with methanol. An equal volume of water was added to the concentrated extract (500 ml) and the solution was defatted with benzene. The solution was further concentrated to 500 ml, an equal volume of water was added, rendered alkaline with ammonia (pH 9), and the phenylalkylamines were extracted with chloroform. For further purification, the chloroform extract was concentrated to about 500 ml and extracted with 0.5 N sulphuric acid. The acidic solution was rendered alkaline with ammonia and the alkaloids were then extracted with chloroform. The phenylalkylamines fraction was applied to TLC and GLC analyses.

III. **Saponins**

The mother liquor, after extraction of the phenylalkylamines, was shaked with successive portions of *n*-butanol (previously saturated with water). Five saponins were detected by TLC. Hydrolysis of the saponin mixture was carried out by 4 N H_2SO_4 for six hours, and the sapogenins were extracted with chloroform. Fractionation of the sapogenins was carried out by preparative TLC.

RESULTS AND DISCUSSION

Fractionation of the purified petroleum ether extract by column chromatography resulted in the isolation of two terpenoids, a hydrocarbon, an alcohol and a sterol fractions. The results obtained from the GLC and MS of the hydrocarbon fraction showed that it is a mixture of *n*-hexadecane ($C_{16}H_{34}$) to *n*-hentriacontane ($C_{31}H_{64}$); of these *n*-nonacosane ($C_{29}H_{60}$) represents the major constiuent (44.7%.) (Table 2).

The isolated alcohol fraction was found to be a mixture of octacosanol, hexacosanol and tetracosanol. The sterol fraction consisted mainly of β-sitosterol. Two triterpenoids were isolated and identified as friedelin and friedelane.

Table 2

The percentages of *n*-alkanes in the hydrocarbon fraction

Alkane	Percentage
n- Hexadecane ($C_{16}H_{34}$; M^+ 226)	0.884
n- Heptadecane ($C_{17}H_{36}$; M^+ 240	0.968
n- Octadecane ($C_{18}H_{38}$, M^+ 254)	0.753
n- Nonadecane ($C_{19}H_{40}$, M^+ 268)	2,040
n- Eicosane ($C_{20}H_{42}$, M^+ 282)	1.680
n- Heneiacosane ($C_{21}H_{44}$, M^+ 296)	1.130
n- Docosane ($C_{22}H_{46}$, M^+ 310)	5.280
n- Triacosane ($C_{23}H_{48}$, M^+ 324)	0.920
n- Tetracosane ($C_{24}H_{50}$, M^+ 338)	1.107
n- Pentacosane ($C_{25}H_{52}$, M^+ 352)	2.348
n- Hexacosane ($C_{26}H_{54}$, M^+ 366_)	1.540
n- Heptacosane ($C_{27}H_{56}$, M^+ 380)	17.10
n- Octacosane ($C_{28}H_{58}$, M^+ 394)	4.89
n- Nonacosane ($C_{29}H_{60}$, M^+ 408)	44.70
n- Triacontane ($C_{30}H_{62}$, M^+ 422)	2.76
n- Hentriacontane ($C_{31}H_{64}$, M^+ 436)	11.90

The percentages of the fatty acids (as determined by GLC) are shown in Table 3).

Table 3

The fatty acids of *Catha edulis*

Fatty Acids	Percentage	Fatty Acids	Percentage
Capric	0.7	Oleic	11.7
Lauric	2.5	Linoleic	0.6
Myristic	2.2	Arachidic	4.3
Palmitic	47.2	Behenic	17.0
Stearic	13.4		

Previous investigation of the phenylalkylamines revealed contradicting results. The present study showed the presence of cathine (norpseudoephedrine), norephedrine and the dimer of cathinone (TLC, GLC, MS). GC-MS of both pseudonorephedrine

and norephedrine were identical with those of the authentic samples. MS of cathine showed M^+ at *m/e* 136 (M^+-CH_3), 133 (M^+ - H_2O+H), 118, 107, 105 and 44 (base peak). The dimer of cathinone (3,6-dimethyl-2,5-diphenyl-hydrazine) showed M^+ at *m/e* 260 and fragments at *m/e* 259 (M^+ - H), 245 (M^+-CH_3), 218, 156, 116 and 77 resulting from the fission of the heteroaromatic ring. Though ephedrine was early reported to occur in khat (Ristic and Thomas, 1962; Karawya *et al.*, 1968), it was not detected in any of the two samples collected from Egypt and Yemen. Cathine (norpseudoephedine) and the dimer of cathinone occurred in both samples, while norephedrine was found in only sample from Yemen. Moreover, cathine occurred in relatively higher percentage in the sample collected from Egypt (Figs. 1 & 2).

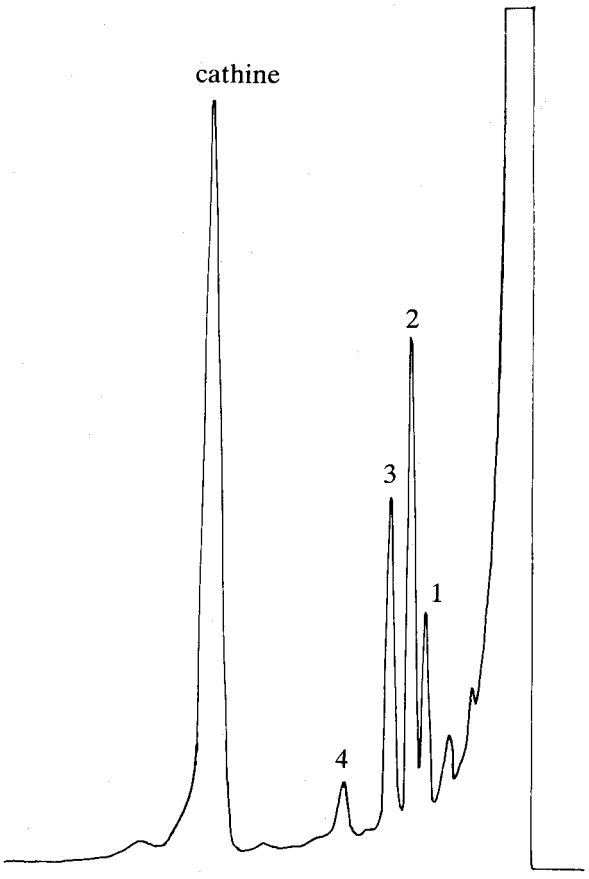

Fig. 1: Gas Chromatogram of the Phenylalkylamine Fraction of khat sample growing in Egypt

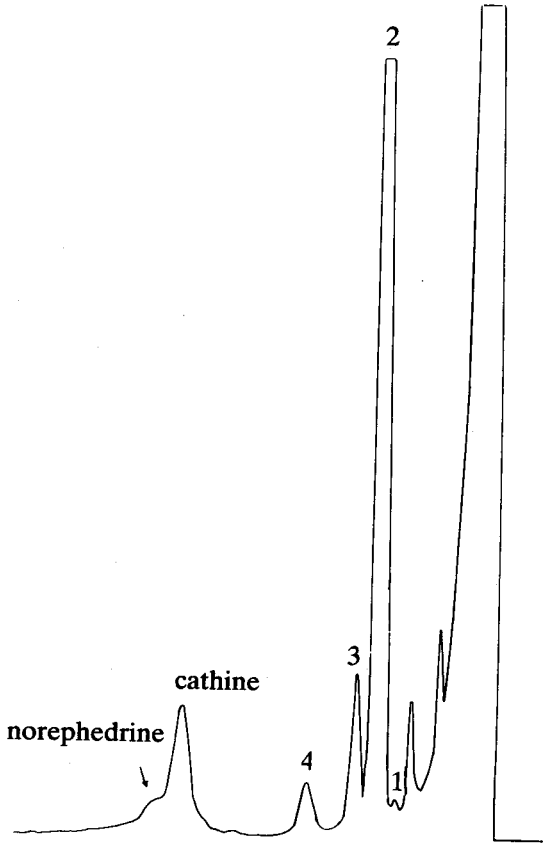

Fig. 2: Gas Chromatogram of the Phenylalkylamine Fraction of khat sample growing in Yemen.

Investigation of the saponins was carried out by extraction with *n*-butanol, followed by hydrolysis and fractionation of the sapogenins by preparative TLC. Of the four triterpenoid sapogenins detected, only one was found in relatively higher percentage and was tentatively identified by MS as a hydroxyfriedelin. Betancor *et al.* (1980) reported the isolation of several triterpenes including 29-hydroxyfriedelan-3-one and 30-hydroxyfriedelan-3-one from *Catha cassinoides.* Paper chromatography of the sugar moiety of the saponins revealed the presence of glucose and rhamnose.

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REFERENCES

Alles, G., D. Faircild and **M. Jensen. 1961.** Chemical pharmacology of *Catha edulis.* J. Med. Pharm. Chem., 3:323-352.

Al-Meshal, I.A., M. Nasir and **F.S. El-Feraly. 1986.** (—) N-Formyl-ephedrine from *Catha edulis.* Phytochem., 25:2241-2242.

Baxter, R., L. Crombie, D. Simmonds and **D. Whiting. 1976.** Extractives of *Catha edulis:* occurrence of celastraceous alkaloids having mono- and bis-macrolide bridges. J. Chem. Soc., Chem. Commun.; 463-465.

Baxter, R., L. Crombie, D. Simmonds and **D. Whiting. 1976.** Structures of cathedulin-2 and cathedulin-8, new sesquiterpene alkaloids from *Catha edulis.* J. Chem Soc., Chem. Commun.; 465-466.

Baxter, R., L. Crombie, D. Simmonds, D. Whiting, O. Braenden and **K. Szendrei. 1979.** Alkaloids of *Catha edulis* (khat). Isolation and characterization of eleven new alkaloids with sesquiterpene cores (cathedulins). J. Chem. Soc., Chem. Commun.: 2965-2971.

Baxter, R., M. Crombie, L. Crombie, D. Simmonds. D. Whiting and **K. Szendrei. 1979.** Alkaloids of *Catha edulis* (Khat). Structures of cathedulins E3, E4, E5, E6 and K 12. Novel sesquiterpene alkaloids with mono- and bismacrolide bridges. J. Chem. Soc., Chem. Commun.: 2982-2989.

Betancor, C., R. Freire, A.G. Gonzalez, J.A. Salzer, C. Pascard and **T. Prange. 1980.** Three triterpenes and other terpenoids from *Catha cassinoides.* Phytochem., 19: 1989-1993.

Cais, M., D. Ginsburg, A. Mandelbaum and **R. Smith. 1975.** Constituents of *Catha edulis:* isolation and structure of catheduline D., Tetrahedron, 31: 2727-2731.

Crombie, L. 1980. The cathedulin alkaloids. Bull. Narc. 32: 37-50.

Crombie, L., W.M.L. Crombie and **D.A. Whiting. 1978.** Structure of cathedulin alkaloids from *Catha edulis* (khat) of Kenyan and Ethiopian origin. J. Chem. Soc., Chem. Commun.: 107-108.

Flueckiger, F. and **J. Gerock. 1887.** Contributions to the knowledge of *Catha* leaves. Pharmacol. J. Trans., 18: 221-224.

Gellért, M., K. Szendrei and **J. Reisch. 1981.** Dihydromyricetin 3- *O*-rhamnoside from leaves of *Catha edulis.* Phytochem. 20: 1759-1760.

Halbach, H. 1972. Medical aspects of the chewing khat leaves. Bull. WHO., 47: 21-29.

Kalix, P. and **O. Braenden. 1985.** Pharmacological aspects of the chewing khat leaves. Pharmacol. Rev., 37: 149-164.

Karawya, M.S., M.A. El-Keiy and **M.G. Ghourab. 1968.** A study of the alkaloids of *Catha edulis* Forsk. growing in Egypt. U.A.R. J. Pharm. Sci., 9: 147-157.

Luqman, W. and **T. Danowski. 1976.** The use of khat in Yemen: Social and medical observations. Ann. Intern. Med., 85: 246-249.

Mustard, M. 1952. Ascorbic acid content of some tropical and subtropical plants and plant products. Food Res., 17: 31-35.

Quédan, S. 1972. Ueber das aetherische Oel von *Catha edulis.* Planta Med., 21: 410-419.

Ristic, S. and **A. Thomas. 1962.** Constituents of *Catha edulis.* Arch. Pharm., 295: 524-525.

Szendrei, K. 1980. The chemistry of khat. Bull. Narc., 32: 5-35.

Winterfeld, K. and **G. Bernsmann. 1960.** Zur kenntnis der Inhaltstoffe von *Catha edulis.* Arch Pharm., 63: 991-1000.

مكونـــات نبـــات القـــات
(القلويدات ـ التربينات والمركبات الأخرى المشابهة ـ السابونينات)

عبد الفتـــاح محمـد رزق ـ زيـــن العابـدين مبـــارك
و طـــه الشـــيحي

أسفرت دراسة القلويدات في عينتين من القات المنزرع في مصر واليمن عن وجود بعض الإختلافات (نوعياً وكمياً) ، فقد وجد الكاثين ودايمر للكاثينين في كلا العينتين بينما وجد النورايفدرين فقط في النبات المنزرع في اليمن . كذلك فصلت عدة مركبات أخرى : تربينات ـ ستيرويدات ـ هيدروكربونات ـ كحولات اليفاتيه ـ سابونينات . كما دُرست الأحماض الدهنية الموجودة في النبات .