Bull. Pharm. Sci., Assiut University
Vol 10. Part 1, pp 37 - 49 (1987)

ESSENTIAL OIL OF PULICARIA UNDULATA L. GROWING IN EGYPT AND ITS EFFECT ON ANIMAL BEHAVIOUR.

A.A.Ali[1], M.A.Makboul[1], M.H.Assaf[1], and R.Anton[2].

1- Pharmacognosy Dept., Faculty of Pharmacy, Assiut University Assiut Egypt.
2- Pharmacognosy Dept., Faculty of Pharmacy, University of Louis Pasteur, Strasbourg, France.

ABSTRACT

By combination of GLC and GC/MS, it was possible to analyse the composition of the essential oil obtained by steam distillation from the total aerial parts of Pulicaria undulata L. growing in Egypt. Nineteen components were identified, the main one being carvotanacetone, linalool, benzyl acetate and piperitone. The oil has a marked sedative action on animal behaviour.

INTRODUCTION

Pulicaria undulata L. is an aromatic herb. The genus Pulicaria is one of the richest genera of Family Asteraceae in its volatile oil content[1-3]. The oil of P. undulata L. was analysed by GLC which revealed the identification of thirteen peaks from nineteen peaks appearing in the chromatogram[4]. The oil was found to possess an antibacterial activity on both S. aureus and E. coli organism[5].

The natives of eastern destert of Egypt used a decoction from the herb as a substitute for tea[4]. Because of the incomplete identification of all the constituents found in the oil of P.undulata L.and the incomplete separation that was obtained by the use of SE-30 column, it was deemed of value to carry out GLC analysis using Carbowax capillary column and GC/MS to reach the complete identification of all the constituents in this oil.

The herb is used as a substitute for tea; so our interest was directed to study the effect of both the aqueous extract of the herb and the essential oil of the aerial parts of P.undulata L., on animal behaviour.

EXPERIMENTAL

The plant material was collected from the Red Sea Coastal Region and Aswan region in April 1983.

Authentication of the plant was kindly done by Prof. Dr.N.El-Hadidy Botany Dept., Faculty of Science, Cairo University. A voucher specimen was kept in the Pharmacognosy Dept., Faculty of Pharmacy, Assiut University.

Distillation:-

The air-dried aerial parts of the plant (100g.) was submitted to steam distillation for about four hours, according to Egyptian Pharmacopoeia method[6], giving 1% (on dry basis) of dark yellow essential oil, specific gravity 0.925, refractive index 1.5620, optical rotation $[\alpha]_D^{20}$=65 (in n-hexane).

Gas Chromatography:-

Temperature programmed gas chromatography was carried out with a Carlo-Erba 4200 gas chromatograph. A glass capillary column carbowax (PEG mesh) 20 M, 25m X 0.32mm internal diameter, e=0.25 u was employed.

Carrier gas "N_2", flow rate:60 ml/min., Hydrogen pressure:0.8Kg/cm^2.
Temperature:injector C$^\circ$,F.I.D.220 C$^\circ$,column 70 C$^\circ$,programmed to 180 C$^\circ$

Essential Oil of Pulicaria Undulata L. Growing in Egypt and its Effect on Animal Behaviour.

Carrier gas "N_2", flow rate:60 ml/min., Hydrogen Pressure:0.(Kg/cm^2.

Temperature:injector 228 C^o,F.I.D.220 C^o,column 70 C^o,programmed to 180 C^o as follows:-

2 C^o/min,from 70-100 C^o and hold at 100 $C^o$for 5 minutes

5 C^o/min,from 100-140 C^o and hold at 140 C^o for 20 minutes

5 C^o/min,from 140-180 C^o and hold at 180 C^o for 15 minutes.

Injected volume 1 ul of 1% oil in n-hexane.

GC/MS:-

GC/MS was carried out on mass spectrometer LKB 9000 s., with a Digital equipment computer PDP 11 with an interface LKB 2130. The separation was made on a spirasil spirawax column (made by Spiral Co., Dijon,France) 2m X 0.32mm internal diameter, temperature programing 55-200 C^c (3 C^o/min). Helium pressure:0.8 B,injector temperature 240 C^o. The electron impaction source was 70 ev, 60 A at 250 C^o.

The compounds were identified by comparison of their retention times and their mass spectra with those of authentic samples and/or with data reported in the literature[7].

The quantitative estimation of each constituent was carried out based on peak area measurement by triangulation[8].

Subjects for animal behaviour tests:-

Adult male Swiss mice of about 8-10 weeks old and 35-40g. in weight at the time of testing were used. Prior to experimental training, they were housed five in Makrolon cages (21 X 21 X 14cm) containing a constant supply of food pellets and water and kept on a 12/12 hours light-dark cycle with light on at 1 a.m.[9]

Apparatus:-

It consists of a polyvinyl chloride box (30 X 20 X 20cm) subdivided into six equal square exploratory units and covered with plexiglass. It can be divided lengthwise into two spatially similar halves by three movable slides so that three middle opening can be opened or closed[10].

Method and Studied Parameters[11-16]:-

Mice were randomly divided into five groups, each of ten animals (one as a control and the others as testing groups). Approximately 24 hours before testing, each subject was confined to one half of the exploratory box with the three movable slides separating the two halves in place, thus familiarized with one side of the apparatus (Familiar compartment F). The floor of this half was covered with sawdust and the animal was given unlimited access to food and water.

After 24 hours, each mouse was removed from the box and given an intraperitoneal injection from the prepared solution of the studied essential oil (1% in 3.3 alchol-physiologic solution), and from this solution 0.3 ml/30g for each animal of the testing groups and also for the control group from the same solution (3.3 alcohol-physiologic solution) free from the oil½ hour before the test and then returned to the familiar compartment F.

After 30 min. of treatment, the three movable slides were withdrawn, thus making the previously inaccessible novel half freely available (Novel compartment N), during the 10 min. test,the animal behaviour was observed and the following measures were recorded:-

a) Locomotion (entries):- The number of exploratory units entered in the familiar F or the novel N compartment.
b) Rearing: Standing up on the hind legs.
c) Preference for novelty: The spent by the mice in the novel half.
d) Latent time: The time of the first entery in the novel compartment.
e) Avoidance reaction towards the novel compartment.
f) Self-grooming behaviour.

As the observation were made in real time, data analysis allowed the determination of the frequency of rearing, self-grooming and avoidance reaction the spent minute by minute in the novel half (preference for novelty), the total neotic preference over the 10 min.period, the number of enteries into the familiar or the novel compartment minute by minute (locomotion) and the total latent time.

RESULTS AND DISCUSSION

The gas chromatogram for the oil of Pulicaria undulata L. shows twenty three components which account for about (99%) of the essential oil as shown from Table 1.

From GLC analysis of the oil, we could identify fourteen components by comparison of their retention times with those obtained by authentic samples. From GC/MS analysis we could identify additionally five components, viz:ethyl benzoate, benzyl acetate, carvotanacetone, piperitone and thujyl alcohol (Table 1).

The rest of the non-identified constituents were in minor quantities and represent (0.9%).

Thus we could account for the identification of nineteen components six of which coincide with those identified before by Bishay et.al[5], and the remaining thirteen components now identified for the first time in the essential oil of Pulicaria undulata L.

The carbonyl content of the volatile oil of Pulicaria species is highly flaculated and reached to (54.3%) in the analysed oil of P. undulata L. This percent is much higher than what is reported in P. Salviaefolia Bge. (2%)[17] and lower than that reported in P. mauritanica, (81%)[18].

From the previous study, one can conclude that, the major constituents of P.undulata L. oil are:-carvotanacetone (37.4%), linalool (19.2%), benzylacetate (11.7%) and piperitone (14.1%).

The oxygenated components in the oil amount to 93.4% accounting for the strong warm pleasant smell of the oil. Hence, one can conclude that, the oil obtained by steam distillation

of P. undulata L. herb can be considered as a new and intersting source for cosmetics and perfume industry.

The results obtained from the integrated three parameters adopted in the study of the effect of the essential oil of P. undulata L. on novelty-seeking behaviour by mice, as shown in Table 2 and 3, led to the conclusion that, the essential oil has a sedative effect as can be seen in the following:-

1- Locomotion:- decreased for both familiar F and novel N com partment for mice treated with the oil. This parameter alone is not sufficient to conclude whether the drug has a sedative effect or not.
2- Latent time:- increased in case of mice treated with the oil, indicating that the oil has a sedative effect.
3- Rearing:- depressed by the oil of P. undulata L., where the rearing of mice treated with the oil differed from those treated with the authentic solution free from the oil. This is a parameter of the sedation effect of the oil.

The analysis of preference for novelty showed a significant decrease between the tested groups and the control one ($P<0.05$, significant) and this gives an indication that, the drug has a sedative effect on C.N.S.

Thus the essential oil of P. undulata L. herb, in a small dose, has a marked sedative property on the novelty-seeking behaviour of mice, this appears clearly from the statistical analysis of the previous results, shown in Table 2 and 3, obtained from the three integrated parameters that measure the sedative property of the tested oil.

Essential Oil of Pulicaria Undulata L. Growing in Egypt and its Effect on Animal Behaviour.

ACKNOWLEDGEMENT:-

The authors wish to record their thanks to Dr.R.Misslin, Laboratory of Psychophysiology, University of Louis Pasteur, Strasbourg, France, for his kind help in carrying out the tests on animal behaviour.

Table 1:-Constituents of the essential oil of *Pulicaria undulate* L.as revealed by GC/MS.

Peak No.	Constituent	%	tr	$[M^+]$	m/z of significant peaks for identification[7,19,20]
1	α-pinene*	0.1	1.4	136	93,79,77,44,39
2	camphene*	0.7	2.2	136	136,93,91,79,77,45,41
3	unknown	0.1	5.2	----	-- -- -- -- --
4	p-cymene	0.1	7.0	134	134,119,117,103,91,77
5	α,β-thujene	3.3	14.8	136	136,121,93,91,79,43,41
6	menthone*	0.3	16.8	154	154,139,112,97,73,70,69
7	ethyl benzoate	0.4	17.6	150	150,122,105,79,77,51
9	linalool*	19.2	21.2	154	121,93,83,71,69,55,39
10	unknown	0.8	22.4	---	--- -- -- -- -- -- --
11	terpinen-4-01	0.6	24.8	154	154,111,93,86,71,69,43,41
12	unknown	0.3	26.0	---	--- -- -- -- -- -- -- --
13	gegeranial	1.7	28.2	152	152,123,94,84,69,41,39
14	carvotanacetone	37.4	28.6	152	152,108,82,81,54,39,38
15	piperitone	14.1	29.8	152	152,109,95,82,81,69,54,41
16	unknown		30.2	---	--- -- -- -- -- -- -- --
17	unknown ketone	traces	30.4	152	136,121,110,95,93,55,43,41
18	neral	0.1	30.8	152	109,94,84,69,41,39
19	δ-terpineol	0.6	31.2	154	136,110,95,93,81,59,41
20	thujyl alcohol	1.9	32.2	154	136,121,119,93,91,71,43
21	nerol*	2.5	34.6	154	120,93,80,69,68,67,41
22	eugenol*	0.5	43.4	164	164,149,122,110,91,81,43
23	cis-jasmone	2.6	44.6	164	164,110,95,79,67,55,41

*Components reported before in the essential oil of *Pulicaria undulata* L.herb.

tr.:retention times.

Table 2:-Results of analysis for animal behaviour tests on the control group.

Measure	Mean ± S.E	L_1-L_2 =95%
1-Locomotion (F)	27.2 ± 6.55	(12.4 -- 42)
2-Locomotion (N)	42.4 ± 8.58	(23 --- 62)
3-Preference for novelty	345.7 ± 57.63*	(216 -- 476)
4-Avoidance	9.5 ± 3.61	(1.4 -- 17.6)
5-Latent time	141.0 ± 53.48	(20.1 -- 26.8)
6-Rearing (F)	5.3 ± 1.50	(2 -- 8.7)
7-Rearing (N)	24.2 ± 6.8	(8.8 -- 39.7)
8-Self-grooming behaviour	0.4 ± 0.16	(0.04 -- 0.76)

S.E.:Standard error of 10 observation.
L_1-L_2:Confidence limits.
*Significant result at $P < 0.05$.
(F):Familiar compartment.
(N):Novel compartment.

Table 3.Results of analysis for animal behaviour tests on the tested groups.

Measure	Mean ± S.E	L_1-L_2= 95%
1-Locomotion (F)	51.3 ± 4.96	(40.1 -- 91.4)
2-Locomotion (N)	59.1 ± 5.51	(46.6 -- 71.55)
3-Preference for novelty	359.5 ± 28.73*	(294.6 -- 424.4)
4-Avoidance	9.5 ± 2.27	(4.4 -- 14.6)
5-Latent time	111.6 ± 19.80	(77 -- 156.3)
6-Rearing (F)	9.8 ± 2.22	(4.8 -- 14.8)
7-Rearing (N)	42.0 ± 5.95	(28.6 -- 55.4)
8-Self-grooming behaviour	0.7 + 0.26	(o.11 -- 1.29)

S.E.:Standard error of 10 observation.

L_1-L_2:Confidence limits.

*Significant results at $P < 0.05$.

(F):Familiar compartment.

(N):Novel compartment.

Essential Oil of Pulicaria Undulata L. Growing in Egypt and its Effect on Arimal Behaviour.

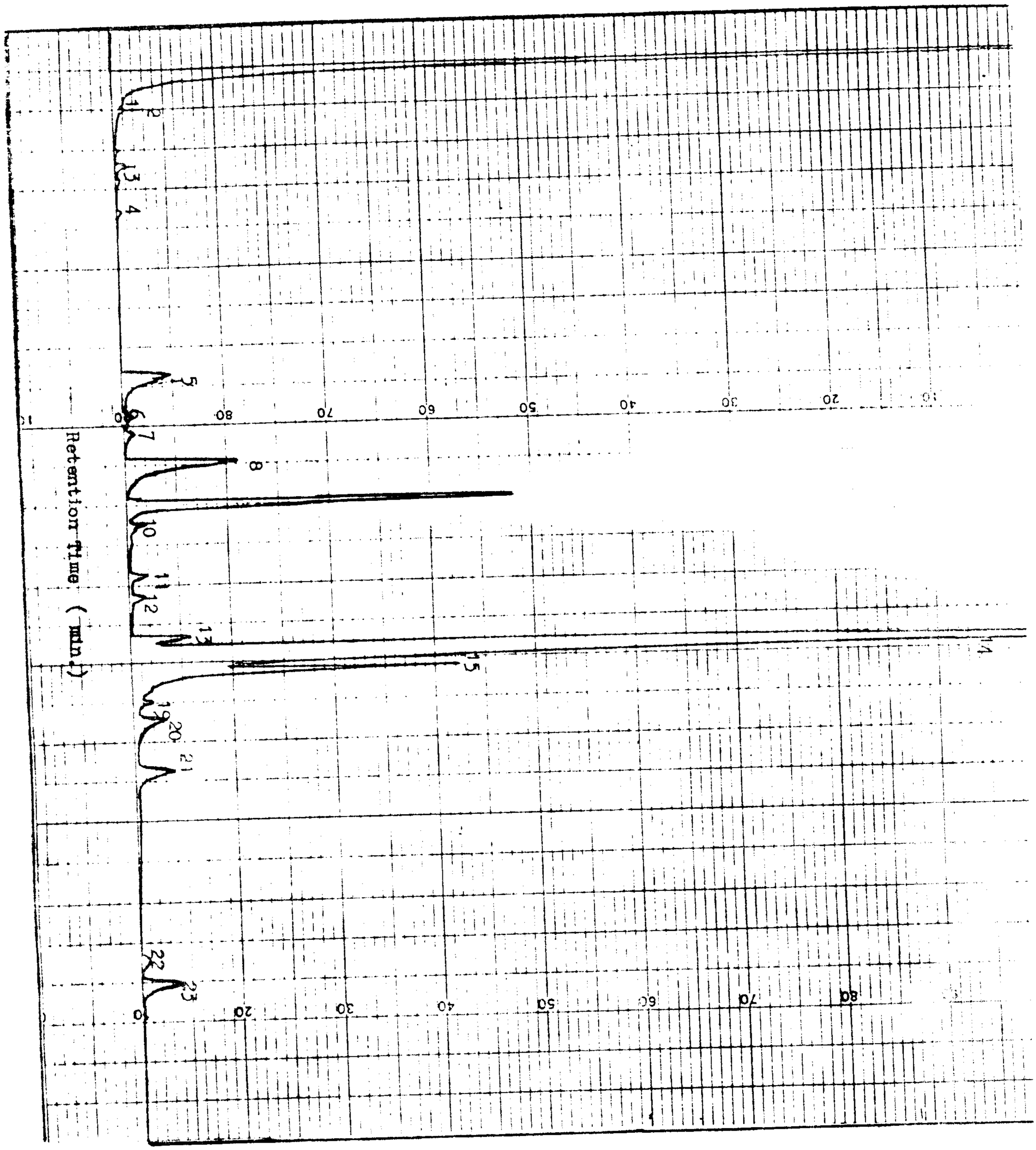

Fig. 1 : G L C Chromatogram Of The Essential Oil Of Pulicaria undulata(L.) Herb.

REFERENCES

1) J.Gattefosse and G.Igolen, Ind. Perfum., 4,110, (1949).

2) S.Kudryashev, Perfums de France, 12,98,(1932).

3) E.K.Schulte, G.Ruecker and F.Mueller, Arch. Pharm., (Weinheim), 301,(2) 115, (1968).

4) D.W.Bishay, C.S.Gomaa and M.A.Saleh, Second Inter-African Symposium on traditional Pharmacopoeia and African Medicinal Plants, Cairo, Egypt-July, (1975),

5) D.W.Bishay, S.H.Ahmed, C.S.Gomaa and M.H.Assaf, Bull. Pharm. Sci., Assiut University, Egypt, 4, 46, (1981).

6) Egyptian Pharmacopoeia, El-Ameria Press, Cairo-Egypt, 14, (1972).

7) E.Stenhagen, S.Abrahamsson and F.W.Mclafferty, Registry of Mass Spectral Data, John Wiley & Sons, Vol.I,II,422, (1974).

8) D.W.Grant, Gas Liquid Chromatography, C.Tinling & Co. LTD., London and Prescot, 19, (1871).

9) R.Misslin and M.Cigrang, Behav. Precesses, 12, 45, (1986).

10) R.Misslin, Biol.Behav., 3, 209, (1982).

11) R.Misslin and P.Ropartz, Psychopharmacology, 75, 39, (1981).

12) R.Misslin, R.Bouchon and P.Ropartz, Physiol. Behav., 17, 767, (1976).

13) R.N.Hughes, Anim. Behav., 13, 30, (1965).

14) R.Misslin and P.Ropartz, Behav. Processes, 6, 329, (1981).

15) R.Hughes, Psychopharmacologia, (Berl.), 24, 462, (1972).

16) R.Misslin, Unpublished Work, (1986).

17) V.Isaev, Perfums de France, 11, 191, (1933).

18) L.Carbalona, Bull. Soc. Chim., France, 845, (1949).

19) Eight Peak Index of Mass Spectra, Vol.I & II, Compiled by mass Spectrometry data centre in collaboration with ICI Ltd, (Organic division) M.S.D.C,UK, (1974).

20) M.Yoshiro, Analysis of essential oil by Gas chromatography and Mass Spectrometry, John Wiley & Sons, Inc,New York, (1976).

دراسة مكونات الزيت الطيار لنبات الشاى الجبلى وتأثيره على تصرف الحيوان

أحمد عبد الرحمن على ـ مقبول أحمد مقبول ـ محمود حافظ عساف وربرت أنطون

ـ قسم العقاقير كلية الصيدلة ـ جامعة أسيوط

،، ـ كلية الصيدلة ـ جامعة لويس باستير ـ ستراسبورج ـ فرنسا

باستخدام كروماتوجرافيا الغاز وكروماتوجرافيا الغاز مع مطياف الكتلة أمكن تحليل مكونات الزيت الطيار لنبات الشاى الجبلى الذى ينمو فى مصر وتم التعرف على تسعة عشر مركبا والمكونات الاساسية كانت كارفوتان اسيتون ألفا لول، اسيتات البنزيل ، والبيبرتون. وتم اجراء تجارب على تأثير الزيت على تصرف الفئران السويسرية ووجد أن له تأثير مسكن وبدا واضحا من تأثيره على العقاقير المختلفة التى استخدمت فى هذة الدراسة .

---

recevied in 3/12/1986 & accepted in 16/8/1987