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P L E C T R A N T H U S M A R R U B I O I D E S H O C H S T

C U L T I V A T E D I N K E N Y A

B Y

A S A D S E / L A L J I

A dissertation submitted in the partial fulfilment for the award of

the

Degree of Bachelor of Pharmacy of the University of Nairobi.

JUNE, 1984

Department of Pharmacy

Faculty of Medicine

College of Health Sciences

University of Nairobi

(Nairobi, Kenya)

D E D I C A T I O N

This work is dedicated to my parents,

my constant source of

encouragement and inspiration.

A C K N O W L E D G E M E N T S

I am greatly indebted to my supervisor, Mr Julius W Mwangi of the Pharmacognosy Section, Department of Pharmacy of the University of Nairobi, for his advice, guidance and continuous encouragement throughout this study.

My sincere thanks go to Mr G Mwalughu and all the other technical staff for their kind assistance.

My deep appreciation also goes to my sister, Yasmin for typing the manuscript.

C O N T E N T S

A B S T R A C T

Fran the leaves of Plectranthus marrubioides Hochst, obtained frcm a cultivation in Nairobi (at Kabete), the essential (volatile) oil was isolated. Both macroscopic and microscopic examination of the leaves was done. The study of the distilled oil was done by Thin Layer Chromatography and Gas Liquid Chromatography. Cn steam distillation the yield of the oil (calculated on moisture free basis) was 4.25% v/w.

The physical properties of the oil were obtained as follows:-

Specific gravity W24) : 0.9531

Refractive index (np25.4^ ; 1.4810

Optical rotation (»<$_D^{25'4}$) : +4.4°

The oil was found to contain at least 21 components and the major one was identified as camphor and occurred in a quantity of 74.0%.

The volatile oil frcm the stems was also isolated. The yield was 0.43% v/w calculated on a moisture free basis. This oil was compared with the oil obtained from the leaves and was found to contain a slightly lower percentage of camphor (70%).

Finally the volatile oil was isolated from leaves which had not been dried properly and had started rotting to see if it had undergone any changes. GLC studies showed that most of the major consituents remained unchanged.

I N T R O D U C T I O N

Essential or volatile oils are natural substances obtained from plant material. They are composed mainly of hydrocarbons and their oxygenated derivatives eg alcohols, esters, aldehydes, ketones, phenols, ethers, peroxides etc. (1). The odour and taste of the oil depends mainly on the presence of oxygenated compounds.

Plectranthus marrubioides Hochst belongs to the family Labiatae. This is a family of about 200 genera and 3,300 species. It consists of aromatic, annual or perennial herbs or undershrubs. Many members of the family are used as culinary or medicinal herbs, as sources of volatile oils and in some cases for the preparation of constituents of the volatile oils such as menthol and thymol. Also obtained from this family is rosemary, lavender, peppermint and spearmint (1).

The Labiatae family consists of plants whose leaves are opposite, decussate, mainly petiolate; leaf margin nearly always serrate, dentate or crenate. The stem and leaves are characterised by the presence of galandular hairs containing aromatic volatile oil. These hairs consist of a short one-celled stalk and a head (gland) of six or eight cells (2).

Plectranthus marrubioides grows widely in Kenya as found from the following information obtained from the East African Herbarium in Nairobi:

a) Northern Kenya

Habitat: Vibody herb, very aromatic with rough stem

Description: Hairy leaves, pinkish lilac flowers, corolla pale

sky blue, calyx hirsute, stems weak and straggling and leaves sub-succulent.

b) Turkana District

Habitat: Growing on rock plains

Description: Perennial herb, flowers sky blue, growing in rock crevices at 7,000 feet.

c) Rift Valley Province

Habitat: In Naivasha and around Lake Nakuru on rocky slopes. Around Lake Elementeita on the marshy shore of soda lake, and in the dense mature Acacia xanthophloea woodland and on stony grassy slopes. On the Gilgil escarpment on the volcanic rock slope. Cn the Nakuru road near the escarpment.

d) Eastern Province

Habitat: In Machakos district on loam little soil on large rocks.

e) Nyanza Province

Habitat: On the dry rocky hills in South Nyanza District.

f) Goast

Habitat: Near Voi on rocky crevices.

From the above it is seen that E_ marrubioides is a common wild plant in Kenya and grows in a wide range of soil and climatic conditions. Therefore the plant would be very easy to cultivate on a large scale.

The plant is a low pubescent trailing fleshy herb or soft shrub with ovate leaves and is sparsely branched with hairy terminal spike-like racemes of blue or lilac flowers (3).

A literature survey indicated that very little investigation has been done on this plant in particular. Therefore literature on other plants of the same species was also reported.

Plants of the Plectranthus species are used in traditional medicine in East Africa as medicinal plants. The leaf juice is used for treating wounds. The leaves of sylvestris are ground and the liquid extracted is taken as a treatment for abdominal pains. The leaves of P. caninus are chewed to relieve toothache. A decoction of the leaves of cylindraceus is taken together with a similar infusion from Microglossa oblongifolia as a remedy for fever and severe headache. An infusion of pounded leaves of elegans which are strong-smelling is used as a remedy for intestinal worms. A decoction of the root of P. lanuginosus is used for intestinal disorders and the leaves are chewed and believed to make one feel fresh and strong. The pounded roots of E_ laxiflorus are soaked in water and the infusion drunk for the treatment of rheumatism. The boiled roots of albus are used as a purgative. The leaves of P^ amaniensis are macerated in water and the liquid drunk for stomach diseases. A decoction of the root of P. amboinicus is drunk whenever one has pain in the stomach. The leaves of E_ barbatus are crushed and the juice drunk as a remedy for stcmach pains and as a purgative. The Luo use this plant for bathing babies suffering from measles. The leaves are normally pounded and soaked in warm water (4).

The use of these plants in traditional medicine does have some scientific support. For example, an alcoholic tincture of the aerial parts of Plectranthus glaucocalyx was found to have high toxicity to protozoa, to gram positive bacteria and to acid-fast bacteria. The active principle, a plectrin diterpene, was effective against Trichomonas vaginalis, Entamoeba histolytica, Staphylococcus aureus and Mycobacterium tuberculosis (5) .

The essential oil obtained from P^ incanus by steam distillation has been shown to have antimicrobial activity. Seme of the physico-chemical properties of this oil were:- (6)

	d_2i.5	0.8976
	n_D21.5	1.4900
Optical rotation in 95% alcohol	oC.^{2}^*^{5}	-11.71
	Acid No.	4.18
	Saponification No.	41.67

This oil was also shown to have some pharmacological activity. It showed marked inhibition of the heart rate of isolated frog and rabbit hearts. (7)

From the leaves of P. marrubioides chrysosphenentin (4", 5 - dihydroxy - 3, 3', 6, 7 - tetramethoxyflavone) has been isolated. (8)

The literature survey did not show any reported work on the volatile oil of marrubioides although it is a common plant in Kenya with a

strong aroma. It was therefore the intention of this project to find the volatile oil content and the chemical composition of the volatile oil of this plant.

E X P E R I M E N T A L

1. COLLECTION AND PREPARATION OF PLANT MATERIAL

The plant material was collected from a cultivation at Kabete (Nairobi) in October 1983. The plant was identified by the East African Herbarium at Museum Hill, Nairobi. The leaves were separated frcm the stems and laid out to dry at rocm temperature (24 - 25°C).

2. EXAMINATION OF THE MORPHOLGICAL CHARACTERISTICS OF THE LEAVES

The morphological study involved the examination of the size, shape, colour and odour of the leaves. Then a transverse section and a surface preparation of the leaves were examined microscopically.

3. DETERMINATION OF THE VOLATILE OIL CONTENT

This was done by steam distillation using Clevenger-like apparatus according to the method described in the British Pharmacopoea (9) for volatile oils with a lower density than water. 100 gm of the leaves were accurately weighed and placed in a 2 litre round botttomed flask and heated electrically. The time of distillation was 4 hours. After this period, no change in the volume of the oil was observed. TV?o determinations were carried out and the oil content was calculated on a moisture-free basis. The volatile oil frcm the stems was determined similarly.

4. DETERMINATION OF MOISTURE CONTENT

The method described in the United States Pharmacopeia (10) was used.

The moisture content of both the leaves and the stems was determined. Approximately 10 gm of the plant material were accurately weighed. The leaves were reduced in size with a pair of scissors (to approximately 3 mm size cubes) and then dried at 105°C to constant weight. Three determinations were performed simultaneously.

5. ISOLATION OF THE VOLATILE OIL

To obtain a sufficient amount of oil for further investiations, a large amount of the plant material was steam distilled in a round bottomed flask of 10 litre capacity. The isolated oil was dried over anhydrous sodium sulphate, filtered and stored at low temperature (approximately 4°C) in a refrigerator.

6. DETERMINATION OF THE PHYSICAL PROPERTIES OF THE OIL

a) SPECIFIC GRAVITY

A 10 ml pycncmeter was used. The method described by Guenther (11) was applied.

b) REFRACTIVE INDEX

The Abbe Refractcmeter (W. S. R. Tbkyo Serial No. 74006) was used. The method described by Guenther (12) was applied.

C) OPTICAL ROTATION

This was determined using an Atago Polarimeter (Japan No. 75121) . The method described by Guenther (13) was applied.

7. THIN LAYER CHROMATOGRAPHIC STUDY OF THE ISOLATED OIL

Using Kieselgel 60 GF 254 (MERCK) as the adsorbent, single development ascending thin layer chromatographic studies were performed. Preliminary investigations were done using microscopic slides and then 10 cm x 20 cm glass plates to find a suitable mobile solvent. Since partition chromatography was carried out, the layers on the microscopic slides were rehydrated before use by holding the slides over a beaker containing boiling water and then allowing the layers to dry out at rocm temperature. Frctn the preliminary investigations on microscopic slides it was found that the following mobile solvents gave the best separation:-

(i) Benzene : Chloroform (50:50)

(ii) n-Hexane : Ethylacetate (95:5)

(iii) Benzene : Ethylacetate (95:5)

Therefore to obtain the best mobile solvent further investigation was carried out on larger plates which were 10 cm x 20 cm. Kieselgel 60 GF 254 (MERCK) was applied to a thickness of 250 /jm using a Desaga Spreader. The plates were dried in an oven at 105°C for 10 minutes to evaporate the excess water. Before development the chamber was lined with filter paper to ensure complete saturation with the solvent vapour. A solution of the volatile oil in n-hexane was used for spotting. Since only a few of the spots were seen under U. V. light, visualisation was done by spraying the plates with 1% w/v vanillin in concentrated sulphuric acid and then heating the plates at 110°C for a few minutes until the spots attained maximum colour intensity. The best nobile solvent was found to be Benzene : Ethylacetate (95:5).

Scme larger plates (20 cm x 20 cm) were then prepared and used as above. Both the volatile oil and camphor B. P. dissolved in hexane were spotted using a template. However, the camphor solution did not give a large spot which could be identified to be that of camphor. Instead 3 spots, out of which 2 were almost of equal size were obtained. This meant that this camphor could not be used as a reference. Therefore another source of camphor was sought. The camphor crystals from the volatile oil of Qcimum kilimandscharicum (14) were separated and dried on a filter paper. These crystals were used as the reference standard.

To identify camphor, the enhancement technique was also used where both the volatile oil solution and the reference camphor solution were applied together at the same point.

After development and visualisation the hRf values of all the spots were calculated. The spots initially visible under short U. V. light were also noted.

8. PREPARATIVE THIN LAYER CHROMATOGRAPHY

Preparative TLC was carried out to extract some of the best separated spots of the volatile oil. The method used was as above except that thicker layers of the adsorbent (750 ;um) were prepared. Using a capillary tube the volatile oil was applied as a band. After development the bands were visualised by the edge-spraying technique. The bands corresponding to the spots nos. 1, 2, 5, and 6 (Figure 9) were scrapped off and extracted with absolute ethanol. These solutions were then stored at low temperature until further investigations such as Infra Red Spectrophotometry could be carried

out. However, this was not possible since the solvent used was absolute ethanol and the solutions were too dilute to extract and dissolve the compounds obtained frcm the bands in another more suitable solvent for I. R. Spectrophotometry. In all the Thin Layer Chromatographic studies the methods described by Stahl (15) were applied.

9. GAS LIQUID CHROMATOGRAPHY

The volatile oil was further investigated using GLC. The GLC apparatus used was PYE Unicam, Series 104 with Flame lonisation Detector. The following were the conditions applied:

Column: Spiral glass 2.0 m long, 4 mm internal diameter

Stationary phase: Carbowax 20 m (12%)

Adsorbent (Solid support): Chrcmosorb W (Acid Washed HP) 100 - 120 mesh

Temperature programming: 75°C to 225°C at 2°C per minute

Carrier gas: Nitrogen

Flow rate: 30 ml/min

Backing off range: x 100

Oven temperature: 250°C

Chart speed: 120 sec/cm

Injection syringe: Hamilton syringe (lOjul)

Attenuation: 50×10^4

Hydrogen pressure: 1.17 kg/cm2

Air pressure: 0.57 kg/cm2

a) First the volatile oil from the leaves was run.

b) Identification of camphor was performed by enhancement of the peak by running together the volatile oil and the reference camphor solution prepared previously.

c) The volatile oil from the stems was run to compare it with that obtained from the leaves.

d) Finally the volatile oil obtained from the rotting leaves was run to see if there were any major changes in the oil.

The quantitative evaluation was performed by the triangulation method as described by Birchfield and Storrs (16) . Also the retention volume (Ry) of each peak was calculated.

R E S U L T S

MORPHOLOGICAL CHARACTERISTICS OF THE LEAVES

The leaves were mostly 40 mm to 80 mm long and 30 mm to 60 mm wide. They were dark green in colour and had a crenate margin. The apex was sub-acute. Both the surfaces were hairy and the veins were very prominent on the lower surface. The main veins left the midrib at an acute angle. The leaves had a strong non-irritating odour (Figure 1).

Microscopic examination showed that the stcmata were of the diacytic type. There were numerous covering trichomes and glandular secreting trichcmes. The mesophyll cells structure was bifacial (Figure 2 and Figure 3).

THE VOLATILE OIL CONTENT

The average volatile oil content of the leaves was found to be 4.25% v/w calculated on a moisture free basis.

The isolated oil had a greenish-yellow colour (which turned yellow on storage) and had a strong arcmatic odour.

The average volatile oil content of the stems was found to be 0.43% v/w calculated on a moisture free basis.

THE PHYSICAL PROPERTIES OF THE OIL

These were obtained as follows:

Refractive Index	(n_D25.4)	:	1.4810
Optical Rotation	(e<$_D$25.4)	:	+4.4°
Specific Gravity	(d24)	:	0.9531

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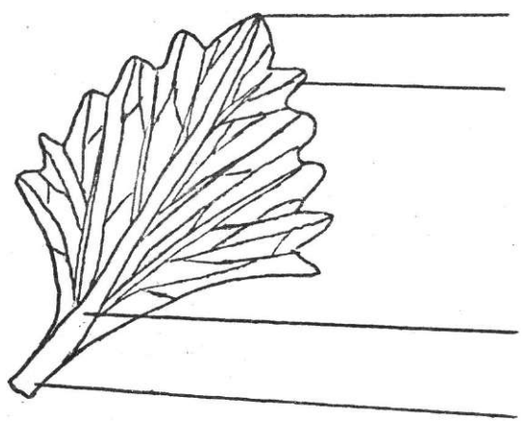

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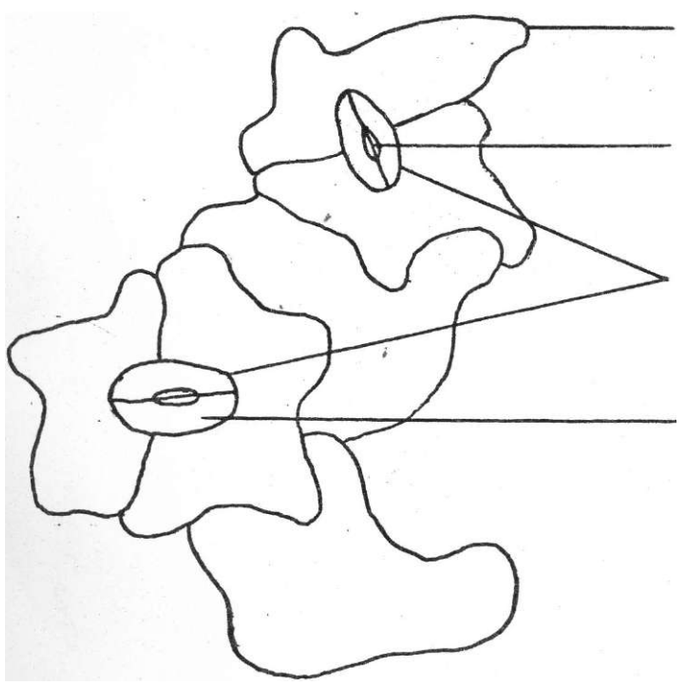

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THIN LAYER CHROMATOGRAPHIC STUDY

(a) In the preliminary investigations to find the best mobile solvent TLC on microscopic slides was done. The following mobile solvent systems were tried out:-

LI	Chloroform	
L2	Benzene	
L3	Benzene : Chloroform	(50:50)
L4	Benzene : Methanol	(75:25)
L5	Benzene : Ethanol	(95:5)
L6	N-Hexane: Ethylacetate	(95:5)
L7	Benzene : Acetone	(97.5:2.5)
L8	Benzene : Ethylacetate	(95:5)

L3, L6 and L8 gave the best separations as seen in Figure 4. These solvent systems were then investigated on larger plates (10 cm x 20cm).

The best separation of the volatile oil was achieved by the solvent system L8, Benzene: Ethylacetate (95:5) which was then used as the mobile solvent system in all further TLC investigations (See Figures 5, 6 and 7).

TLC on 20 cm x 20 cm plates using camphor B. P. as the reference did not give good results since the reference camphor gave several spots of the same size (Figure 8).

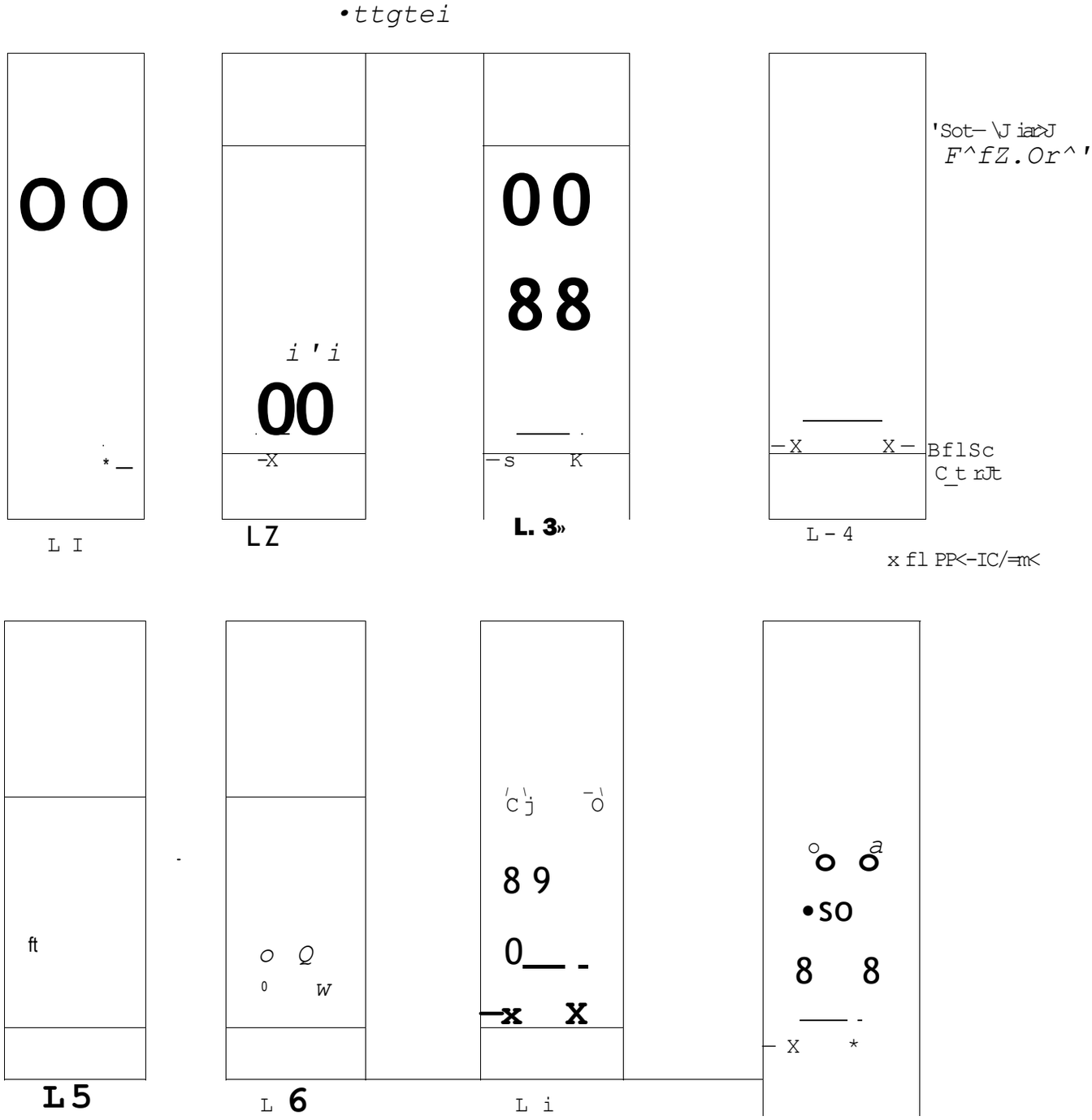
TI£ on 20 cm x 20 cm plates using camphor crystals of the volatile oil of Ocimun ki 1 imandscharicum gave good results. The largest spot after separation of the Ocimum kilimandscharicun crystals was that of

camphor (14) and the other spots probably represented traces of the other oil constituents in the crystals. This spot had the same hRf value as one of the spots of the marrubioides leaf oil and that spot was confirmed as being of camphor by the TLC enhancement technigue (Figure 9).

TABLE 1 ; THIN LAYER CHROMATOGRAM OF PLECTRANTHUS MARRUBIOIDES OIL .

(Figure 9)

Spot No.	hRf	Identified
1	62.7	
2	54	
3	46.7	
4	42	
5	36.7	
6	31.3	Camphor
7	26	
8	21.3	
9	17.3	
10	13.3	
11	6	



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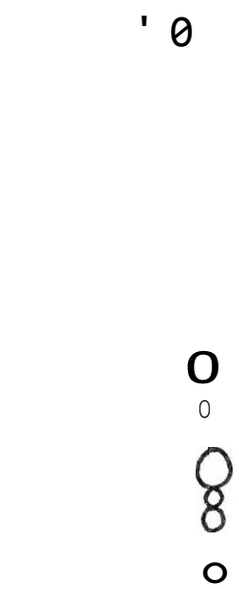

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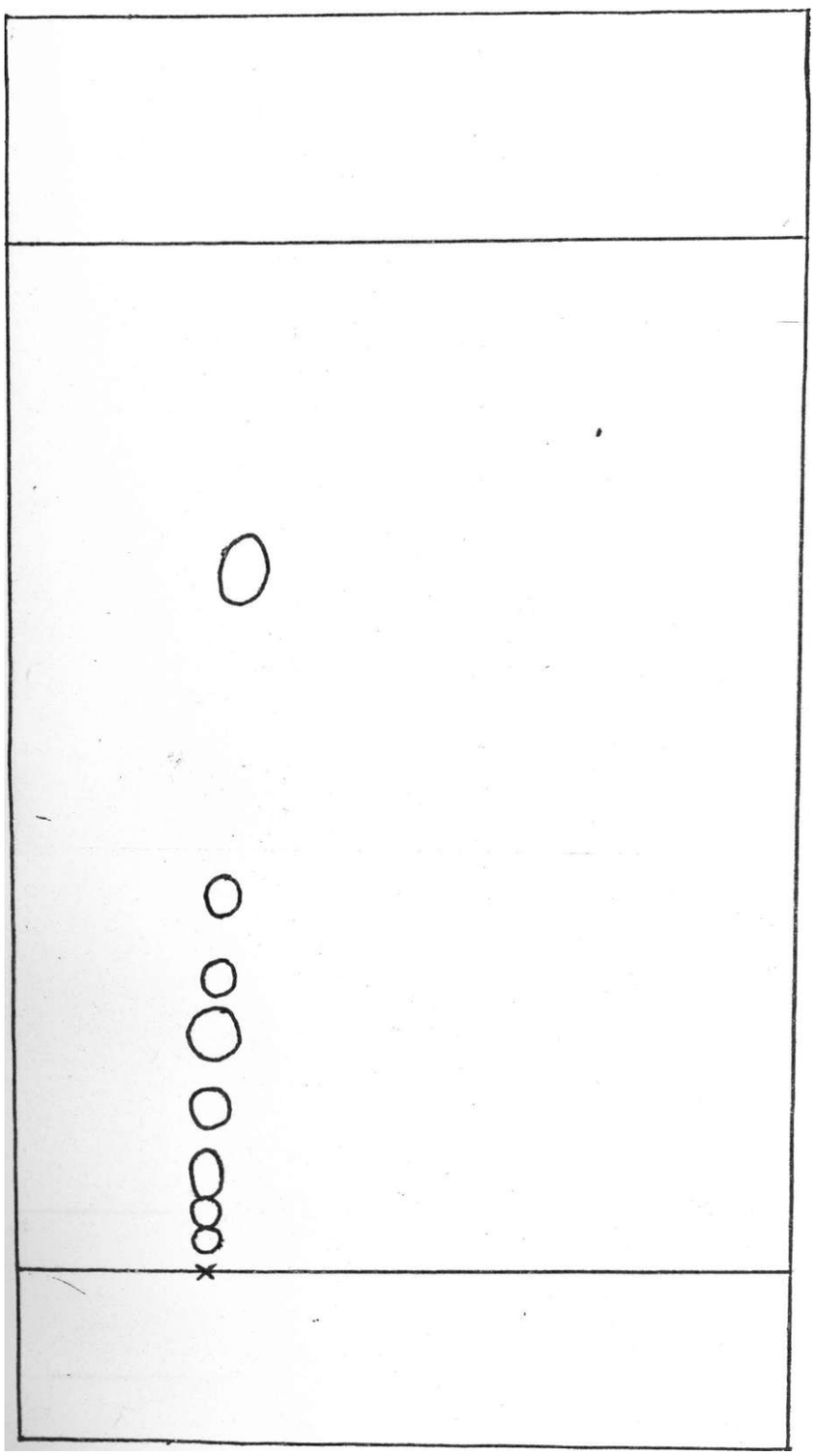

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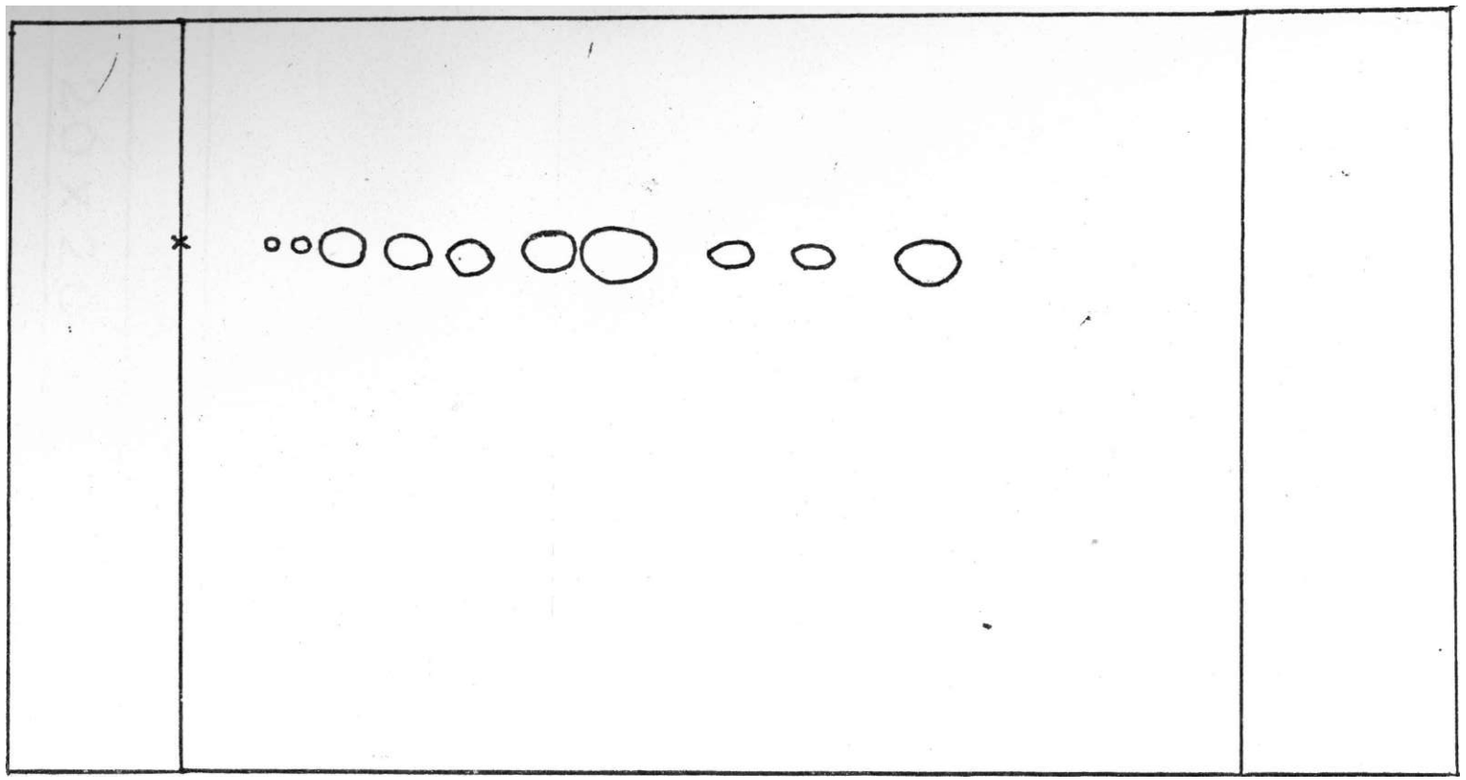

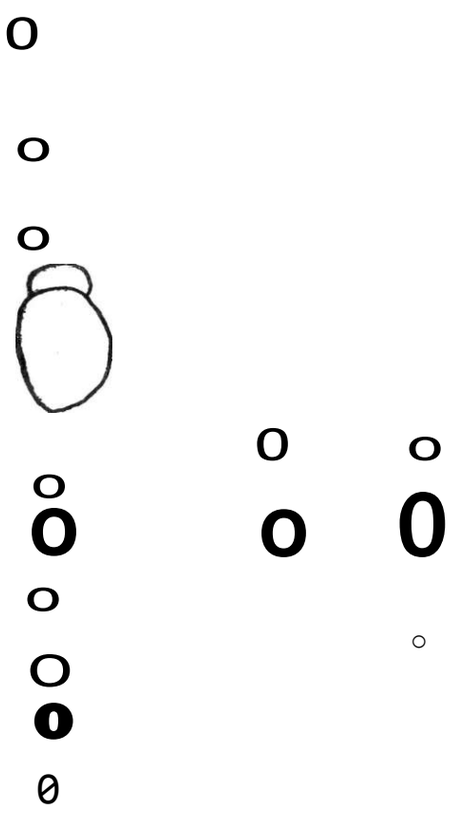

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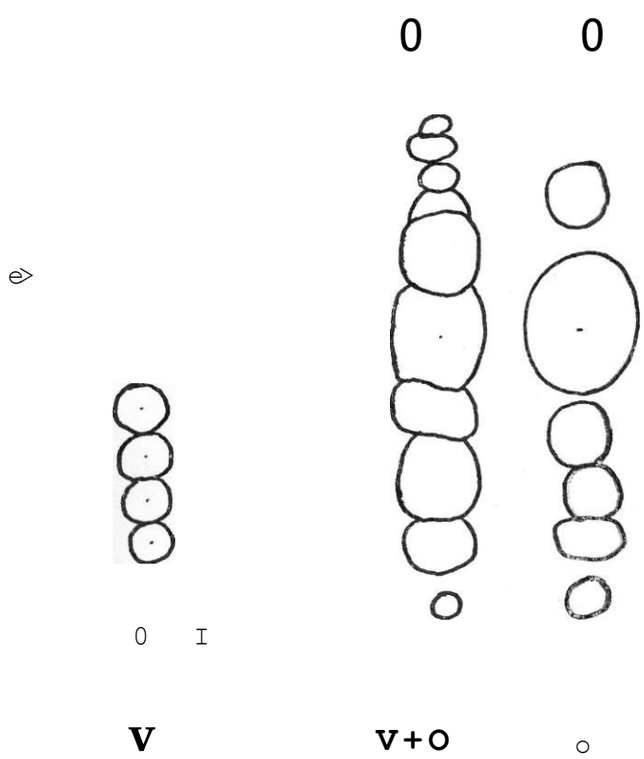

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GAS LIQUID CHROMATOGRAPHY

(a) 0.2 Ail of neat P. marrubioides leaf volatile oil were injected to observe what sort of separation is obtained. At least 21 significantly large peaks were obtained as seen in Figure 10. Their Retention Volume (R_v) values were calculated and are given in Table 2. Then 0.8 /j1 of a dilute solution of the oil (in Hexane) was run to quantitatively estimate seme of the major peaks (Table 2).

The presence of camphor was confirmed by spiking or enhancement of the major peak (No. 8 in Figure 10) while the other peaks remain virtually the same.

(b) 0.5 ;ul of a solution of the volatile oil in Hexane frcm the rotting leaves was injected. The chrcmatogram obtained is shown in Figure 11. Comparing this with Figure 10 shows that the major components of the oil remain unchanged and do not seem to have degraded significantly, and the major peak still has R_v of 948 ml.

Therefore it is confirmed that camphor is the major component (occuring in a percentage of approximately 74%) of the volatile oil frcm the leaves and occurs as the major peak with Retention volume of 948 ml.

(c) Finally 1 jul of the volatile oil dissolved in Hexane obtained frcm the stems was run. The largest peak occured at R_v value of 948 ml. (Figure 12). Hence the stem oil also contains camphor as its major constituent.

Using the triangulation method, it was found that the camphor content of the volatile oil frcm the stems is approximately 70%.

FIG. 10 : GLC OF 0·2 μl OF THE NEAT VOLATILE OIL FROM THE LEAVES OF P. MARRUBIOIDES

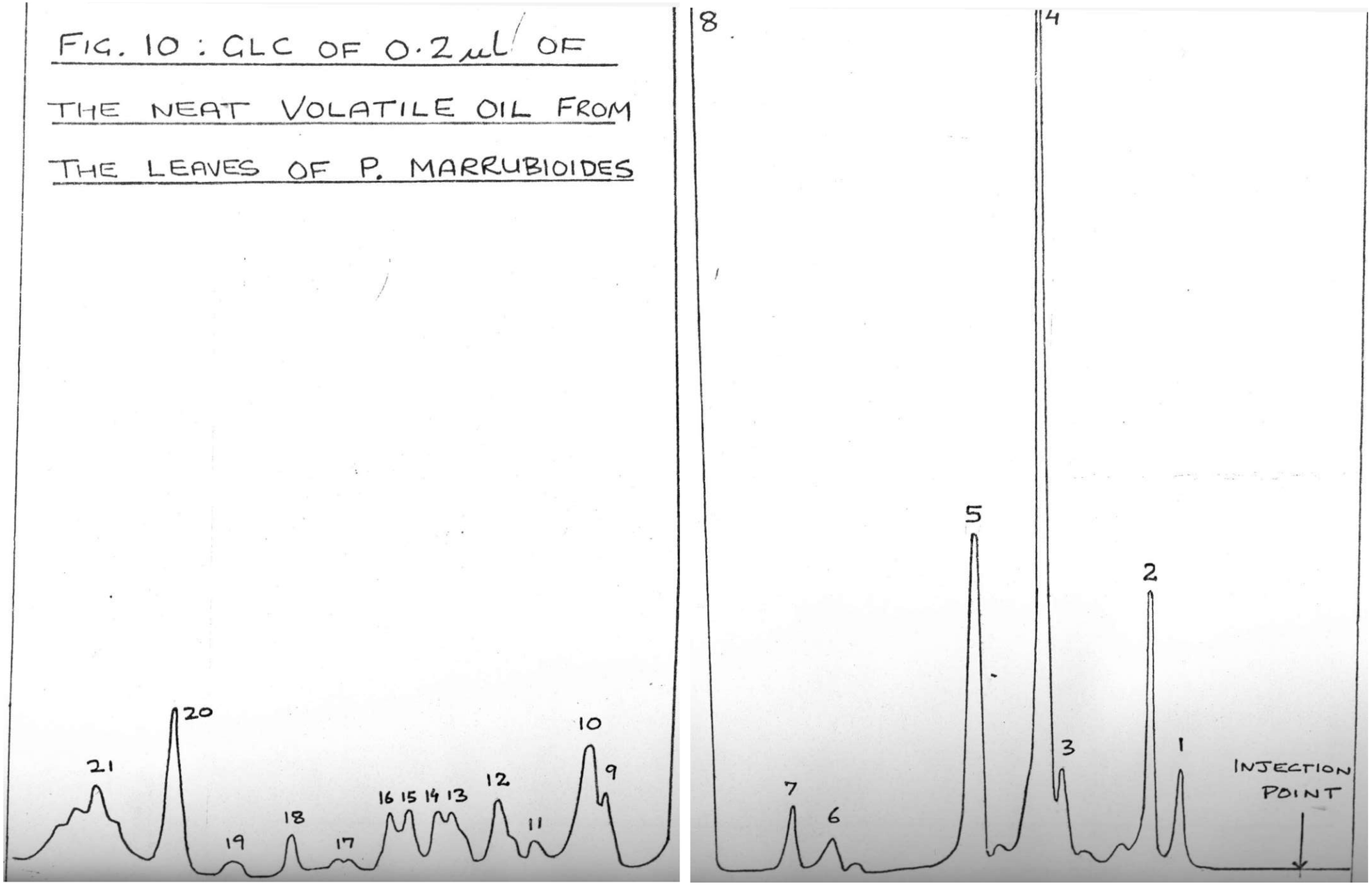
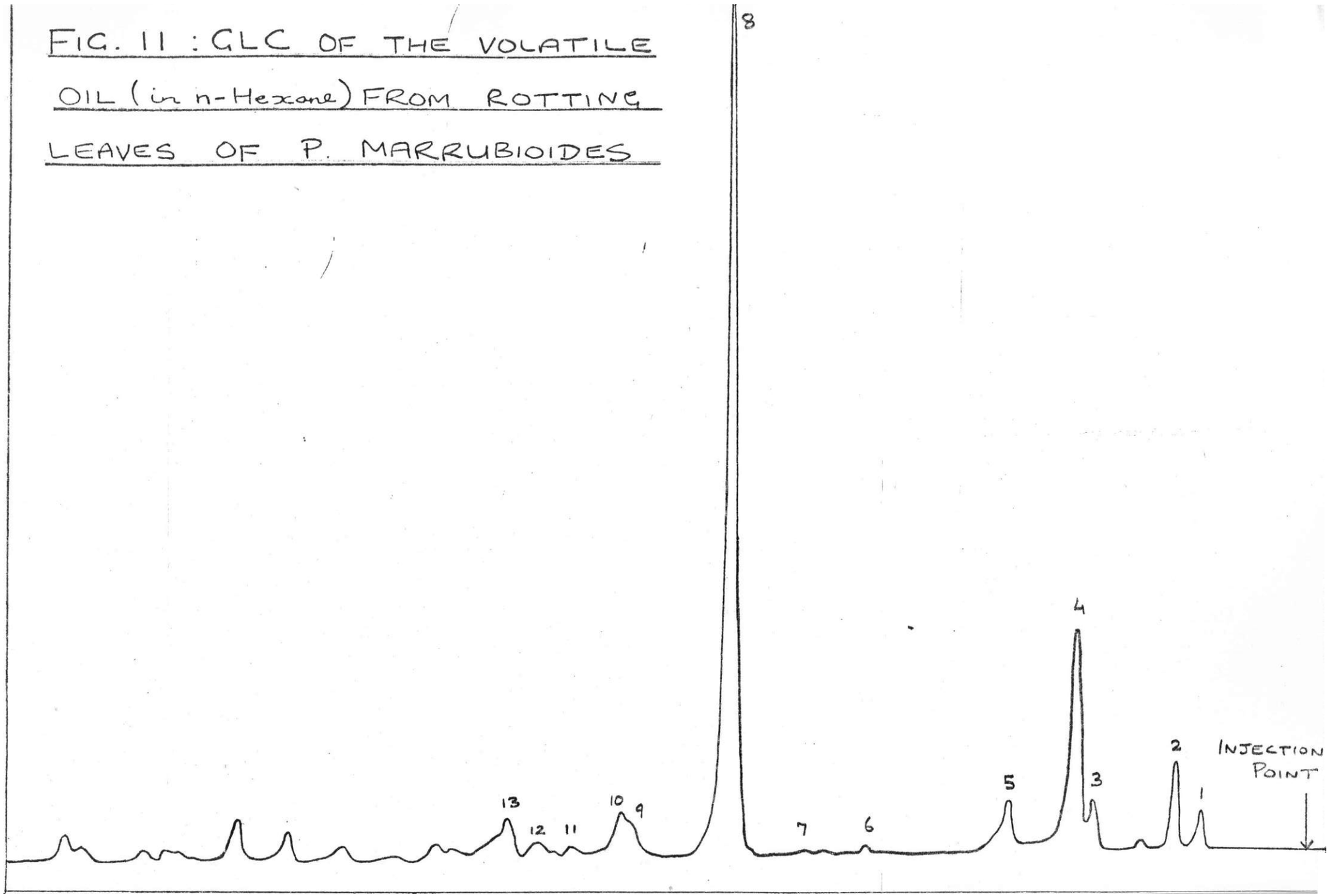


TABLE 2 ; THE RETENTION VOLUMES AND THE PERCENTAGE OF TOTAL OIL

OF THE CONSTITUENTS OF P. MARRUBIOIDES LEAF OIL

Peak No.	Ry in ml.	Percentage of total oil (%)
1	186	1.0
2	228	2.6
3	372	-
4	396	14.7
5	510	5.7
6	738	-
7	804	-
8	948	74.0
9	1098	-
10	1116	1.4
11	1212	-
12	1272	0.3
13	1344	-
14	1365	-
15	1410	-
16	1434	-
17	1545	-
18	1602	-
19	1686	-
20	1782	-
21	1896	-

FIG. 11 : GLC OF THE VOLATILE OIL (in n-Hexane) FROM ROTTING LEAVES OF P. MARRUBIOIDES



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D I S C U S S I O N

Frcm the results it was found that the leaves of Plectranthus marrubiodes Hochst have a high content of volatile oil (4.25% v/w on moisture free basis). This oil consists mostly of camphor (approximately 74%).

Therefore, this plant which is widely distributed in Kenya, and should therefore be easy to cultivate, is a potential source of natural camphor in Kenya. An advantage is that even if the leaves are not dried well after collection, the chemical composition of the volatile oil does not change significantly and the leaves can be used to extract camphor.

The stems cannot be used economically to yield the oil (even though it contains camphor) since the oil content is very low (0.43% v/w on moisture free basis). However, the stems and the leaves can be distilled together.

Camphor is used extensively in the manufacture of plastics, especially celluloid. It is also used in lacguers, varnishes, explosives and as a moth repellant.

In medicine camphor is applied externally in liniments and as a counter-irritant in fibrositis and neuralgia as it acts as a rubefacient and mild analgesic (17).

Camphor is weakly antiseptic and mildly anaesthetic (antipruritic) when rubbed on the skin. It is also used to reduce the itching due to insect stings, and as a counter-irritant for inflammed joints, sprains and rheumatic and other inflammatory conditions such as colds in the

throat and chest (18).

Therefore this plant can not only be useful in industry but also in medicine. Further work should be done on the plant and more constituents of the volatile oil identified.

This could not be done due to lack of pure reference substances and time.

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