INVESTIGATION OF VOLATILE OIL OF CLAUSENA ANISATA GROWING IN KENYA.

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DEDICATION

To my parents, brothers and sisters who between them provided the stimulus for me to learn and helped to resolve the many problems I inevitably encountered.

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TABLE OF CONTENTS

ABSTRACTS

From Clausena anisata (Hook f.) leaves (family RUTACEAE), collected from Karura Forest Nairobi, the volatile oil was isolated by steam distillation method. Both physical and some chemical properties of the oil were determined. The yield of the oil was 3.1 per cent calculated on moisture free basis. The physical and some chemical properties of the oil were as follows:-

TABLE 1

Density	$0.9^{a}33$
Optical rotation	0^{0}
Refractive index	1.5642

TLC and GLC studies showed anethole occured in an amount of 67 percent.

INTRODUCTION

Volatile oils are volatile in steam. They differ in chemical and physical properties from fixed oils. With the exception of oils such as oil of bitter almonds produced by hydrolysis of glycosides, these oils are contained as such in plants. They are secreted in oil cells, in secretion ducts or in cavities or in glandular hairs. Volatile oils are frequently associated with other substances such as gums or resins and themselves tend to resinify on exposure to air. Volatile oils are used for their therapeutic action (oil of eucalyptus) for flavouring (oil of lemon) or in perfuming (oil of rose).

Volatile oils are frequently mixtures of hydrocarbons and oxygenated compounds derived from these compounds. In some oils (eg oil of turpentine.) the hydrocarbons predorminate while there is a limited amount of oxygenated constituents. The odour and taste of volatile oils is mainly determined by these oxygenated constituents which are appreciably soluble in water but more soluble in alcohol. Many volatile oils are terpenoid in origin, a smaller number such as those of clove contain principally aromatic derivatives mixed with terpenes. A few compounds such as thymol though aromatic in structure are terpenoid in origin. A number of monoterpenes occur in plants in glycosidic form, thus geraniol and citronellol occur as glycosides in petals of Rosa dilecta and thymol and carvarcrol as glycosides and galactosides

in Thymus vulgaris.

According to Guenther (1) the oil distilled from leaves of clausena anisata had the following properties:-

Density at 20°c	0.9611
Optical rotation at 20°c	- 0 .4
Refractive index at 20°c	1.5613
Anethole	82.5 percent.

Guenther also reported anisaldehyde as one of the components of Clausena anisata oil.

Brooks BT. (2) found the following constants for the oil distilled from Clausena anisata.

Density at 20°c	0.963
Optical rotation at 20°C	Inactive
Refractive index at 20°C	1.5235
Saponification number	3.6

Brooks BT also reported methyl chavicol as being the major constituent of the oil.

S. Talalaj (3) found the following constants for the oil distilled from seeds of Clausena anisata

Phenol content	8.2-11.8 per cent
Ester number before acetylation	14.2 - 16.0
Ester number after acetylation	73.2 - 84.0

Anethole is a white crystalline substance of intense anise like odour and flavour. It melts at 22.5°c to a colourless optically inactive but strongly refractive liquid. It is usually present in plants as transanethole but occurs in small quantities as the more toxic cis-anethole (4).

Anethole is practically insoluble in water, miscible with ether, chloroform. Its soluble in benzene Ethylacetate, acetone carbon disulfide and petroleum ether. Anethole is used for the manufacture of anisaldenyde, flavouring agent, in perfumery particularly for soap, sensitizer in bleaching colours in photography and as an embedding material in microscopy. In veterinary anethole is used as a carminative and flavouring agent.

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BOTANY, DESCRIPTION AND DISTRIBUTION

FAMILY: RUTACEAE. Clausena anisata (hook. f) is a tree or shrub about 2 -3.5 metres high. The shrub grows in Philipine Islands. Java, Tanzania, Zaire and other tropical countries including Kenya (5).

The shrubs occur in Kenya in Samburu, Laikipia, Nakuru and Kiambu Districts. It also occurs in Kakamega, Kajiado, Kilifi and Bungoma Districts (6). Clausena anisata is common in dryish localities Savannah bush and at forest adges from 0 to 2350 metres above sea level. In Philipines alcoholic extracts was used for flavouring. In East Africa the plant was used for its pleasant odouriferous properties especially under beds. Toothbrushes were also made from twigs. The Masai Warrior carried a bunch of leaves when going to battle and he also liked to sleep on the leaves. The Xhosa likened odour of bruised foliage to that of striped field mouse. In areas where shrub grows the leaf is used as a remedy for various childrens complaints

The Xhosa placed the leaves on hot ashes to fumigate the newborn by waving the infant to and fro in the acrid smoke. This was supposed to clear and strengthen the lungs. The Xhosa also hold babies of delicate health over steam of pot water containing some material from Clausena anisata. The leaf was also used as an anthelmintic and Xhosa remedy for sick calf especially if it had taken too much milk (5).

The Zulu use leaf as a parasiticide and purgative and root as antihelmintic. The leaf is one of Zulu remedy for intestinal bettles. The leaf has also been used in febrile conditions and is stated to be diaphoretic and useful in treatment of rheumatic fever. However it has been tried clinically in this condition and found useless.

Here in Kenya, Kikuyu elders who were candidates for the senior grades which enabled them to try cases were given a bunch of leaves well tied with a string at the time of initiation.

The Masai chew the leaves as emetics while in Ashanti it was used for making new shrine. In West Africa, plant was used to keep of revengful Spirits and burned to keep mosquitoes away. It was also used in West Africa as a tooth remedy, sticks were chewed for the treatment of migraine and rheumatism, laxative, as an eye

medicine, and to prevent illness in children.

The Haya used it as a remedy for gastroenteritis and Shambala use root infusion and inhalation of leaf in treatment of furunculosis and swelling of the gums. In Moshi the bruised leaves are mixed with butter and given to a pregnant woman at commencement of labour and also as a post partum. The root of Clausena anisata has also been used as an influenza remedy.

In East Africa some ethnic groups such as the Digo use the plant to make delivery easy. The roots are powdered and put into a soup and the woman drinks it before she goes to bed. She also given some, two days after birth to cleanse the uterus of blood. Roots prepared in a similar way are highly recommended for headache, Malaria, influenza and indigestion. A decoction of roots is taken by women to hasten lactation after birth. A root decoction is drunk three times daily for the treatment of Syphillis (6).

The aim of the project was to determine the volatile oil content and the physical properties of oil in leaves of *Clausena anisata* growing in Kenya.

EXPERIMENTAL

COLLECTION The plants were collected from Karura Forest in Kenya during month of December 1981. The leaves were stored for three days at 4°C before the volatile oil was distilled.

MACROSCOPIC AND MICROSCOPIC EXAMINATION

The size, colour, shape, odour and taste of the leaves was examined. The sections for microscopic examination were made by blade and cleared by warm Chloral hydrate Lignification was revealed by the phloroglucinol reagent.

DETERMINATION OF MOISTURE CONTENT

Moisture content of leaves was determined by gravimetric method as specified in the European Pharmacopia (7). Approximately 2 grammes of accurately weighed finely powdered plant material were dried at 105°c to constant weight.

DETERMINATION OF VOLATILE OIL CONTENT

The determination was carried out according to method described in BP 1973 for oils lighter than water.

A clevenger like apparatus was used. The distillation time was three hours. Three determinations were performed and the average content was calculated on a moisture free basis dried anhydrous sodium sulphate, filtered and stored at low temperature (4°c.) in a tightly closed well filled container.

DETERMINATION OF PHYSICAL PROPERTIES

The colour Odour and taste of isolated oil was examined. Solubility of the oil was determined by method described by Guenther (8).

OPTICAL ROTATION

The Optical rotation was determined in the ATAGO Polarimeter (JAPAN) at 20°c.

SPECIFIC GRAVITY

Specific gravity was determined in the OSVALD Pycnometer of 1ml capacity according to method by Guenther(9).

REFRACTIVE INDEX

Refractive Index was determined in the ABBE refractometer (CENTRAL TRADING CO LTD TOKYO JAPAN) at 20°c according to method described by Guenther (10).

SOLUBILITY

Solubility determination was carried out according to method described by Guenther (11). 70 per cent enthanol was used.

THIN LAYER CHROMATOGRAPHIC (TLC) STUDY OF THE OIL

The single development ascending TLC technique was employed using silicagel $_{60}GF_{254}$ (MERCK) as adsorbent. In order to find a suitable mobile solvent and locating agent, preliminary investigations were carried out on microscope slides The mobile phase showing best separation proved to be hexane : Ethyl acetate (95:5). Futher TLC investigation was carried out on larger plates (20 cm by 20 cm). Silicagel $_{60}GF_{254}$ (MERCK) was applied to a thickness of 250 using a Desaga spreader After drying at room temperature, the plates were activated at 105-110°c for half an hour.

The developing tank was allowed to equilibrate with mobile solvent at least 45 minutes before use. A 10% solution of Clausena anisata oil in toluene was used for spotting.

Visualization was achieved by spraying the chromatogram with vanillin sulphuric acid reagent prepared according to stahl (12). Rf valves of the spots obtained were calculated. Subsequent confirmation of major constituent of Clausena anisata oil was carried. Anethole isolated from Anise oil by preparative TLC was used as reference substance. Identification of anethole was done by spot enhancement with reference substance.

PREPARATIVE TLC OF THE ANISE OIL

This was performed in order to obtain reference sample for use in TLC and GLC studies of *Clausena anisata* oil. A commercial sample of Anise oul (with as much as 90% anethole) was separated using silica gel $_{60}$ GF_{254} (MERCK) as adsorbent on 20 cm by 20 cm glass plates. The thickness of the layer was 500 mm. The solvent system used was the same as that used in analytical TLC. After non destructive visualisation (Using short range u.v. light), the layer of major component anethole was scrapped out and extracted with toluene. After concentrating the presence of anethole was confirmed by TLC. The extract which showed only one component was applied as reference in TLC and GLC studies of the oil.

GLC STUDIES OF THE OIL

A Pye Unicam Chromatograph (series 104) fitted with a flame ionisation detector was used for the study of the oil. The following chromatographic conditions were applied:-

Column dimensions - - spiral shaped glass tube 1.5 metres length and 4MM internal diameter

Column parking - - 12 per cent carbowax 20M on 100-200 mesh Diatomite CQ

Temperature - - Programming from 75 to 225°c at 2°C min

Chart speed - - 2 min cm^{-1}

Carrier gas - - Nitrogen

Gas flow rate - - 30 ml min^{-1}

Hydrogen gas pressure 1.4 Kg Cm^{-2}

Air pressure - - 0.6 Kg Cm^{-1}

Volume of oil injected 0.2 ul.

The oil was injected by means of a Hamilton syringe of 10 ml capacity. The results are shown in figures 5 and 6. The same chromatographic conditions were applied for anise oil.

RESULTS

The leaves are small and opposite and obovate or rhomboidally obovate in shape. The length of the leaf varied between 20 and 40 millimetres and width at widest part was between 13 and 18 millimetres. The leaf has a broad apex obtusely with very short petiolules. There are numerous elliptical stomata with 5 or 4 neighbouring cells. The stomata are thus anomocytic.

FIGURE 1

DIAGRAM SHOWING VARIOUS LEAF SHAPES.

Magnification x1.

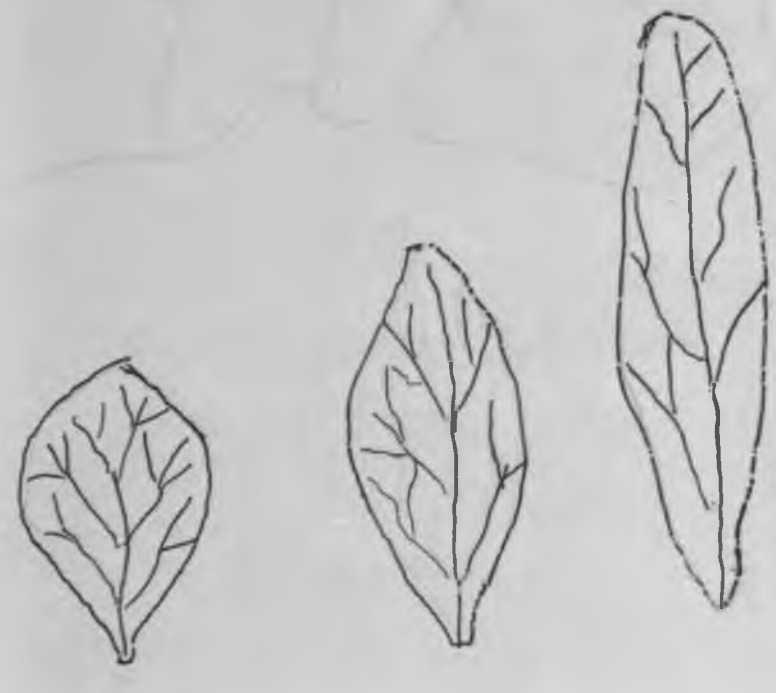


FIG 2

SURFACE SECTION OF EPIDERMIS

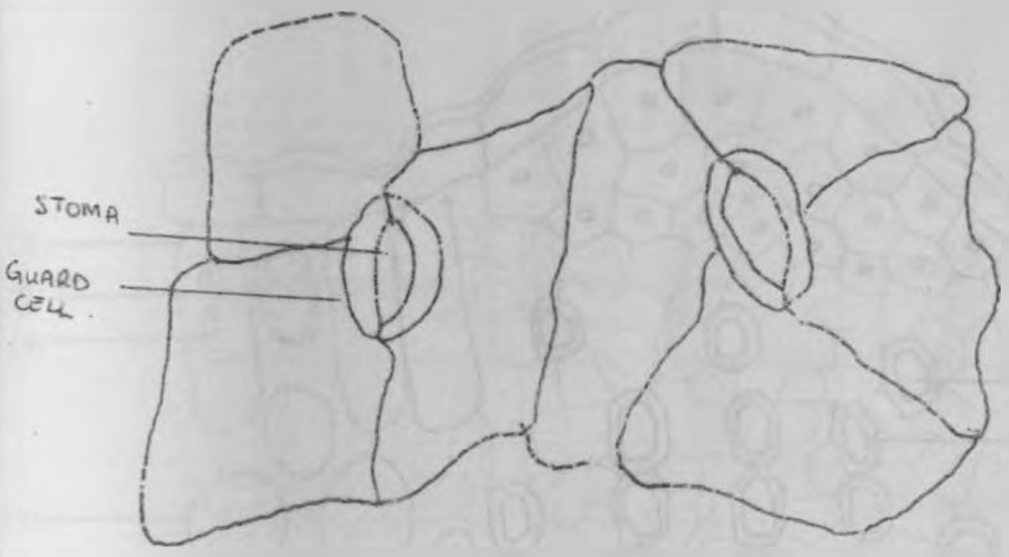
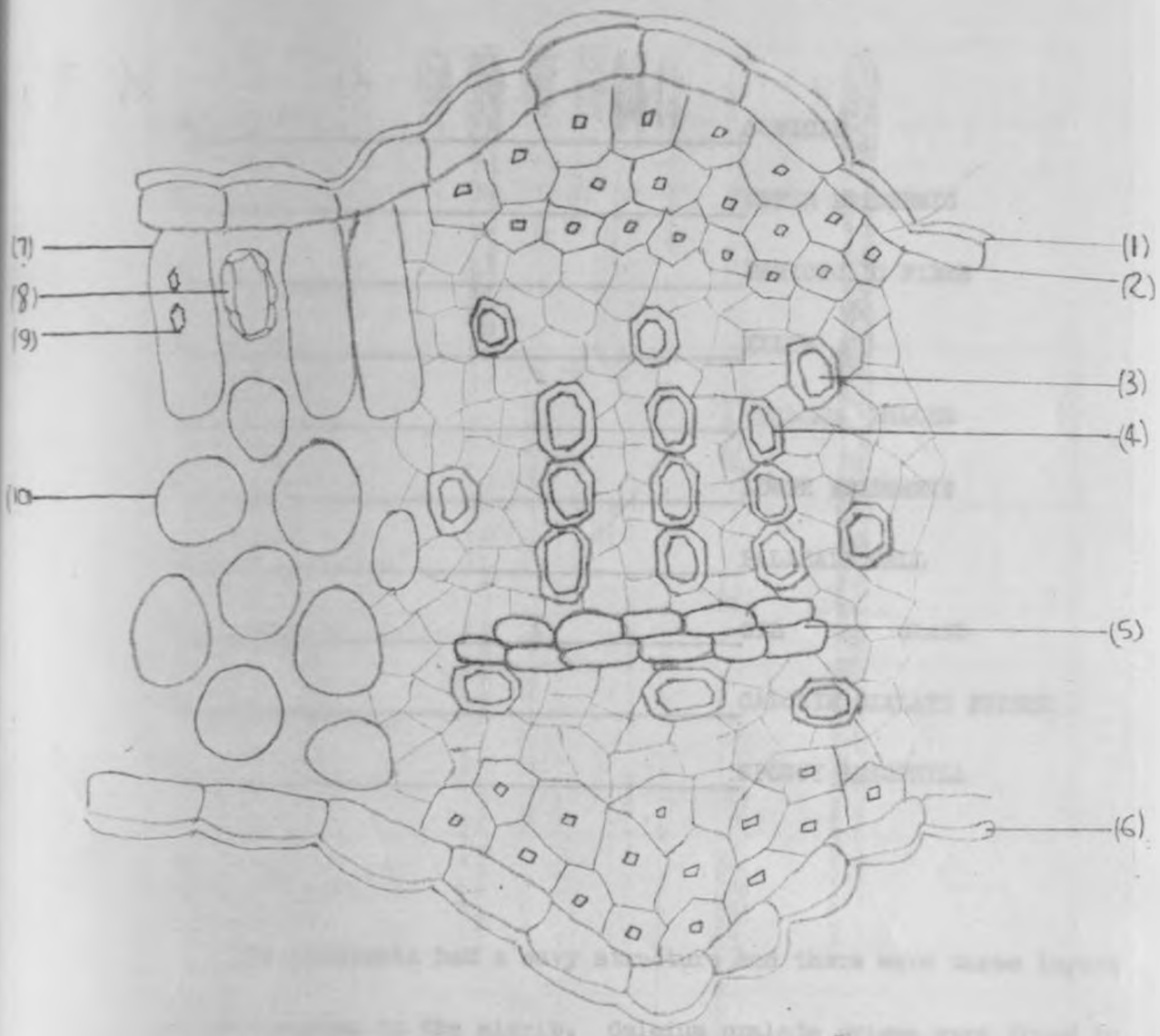


Figure 3

Transverse section of the midrib



KEY FOR GIFURE 3

1_____CUTICLE

2_____UPPER EPIDERMIS

3_____PERICYCLIC FIBRE

4_____XYLEM

5_____PHLOEM

6_____LOWER EPIDERMIS

7_____PALISADE CELL

8_____OIL GLAND

9_____CALCUIM OXALATE PRISMS

10_____SPONGY MESOPHYLL

The epidermis had a wavy structure and there were three layers of collenchyma in the miarib. Calcium oxalate prisms were found in some palisade cells and occasionally there was an oil gland between 2 palisade cells. The vascular bundles were surrounded by thick walled fibres. The vascular bundles were bicollatered and the vessels were radially arranged.

The average volatile oil content was 3.1 per cent calculated on moisture free basis. The isolated oil was colourless and had a strong characteristic anise like odour and taste. The physical properties of the oil are given below:-

Specific gravity (at 20°c)	0.9 933
Optical rotation at 28 c.	0°
Refractive index (at 20°c)	1.5642
Anethole content (Percent)	67
Solubility in 70 percent ethanol	Soluble with haziness 1 in 5 -6 volumes of ethanol

TLC of the oil showed 4 components Confirmation of the anethole was by use of pure anethole as a reference substance as well as comparison of hR_f values obtained. These results are given in figure 4.

Table 2

Spot no of oil	hR_f	Identified
1	18	Unknown
2	33	unknown
3	57.5	Anethole
4	74	unknown

Fig 4

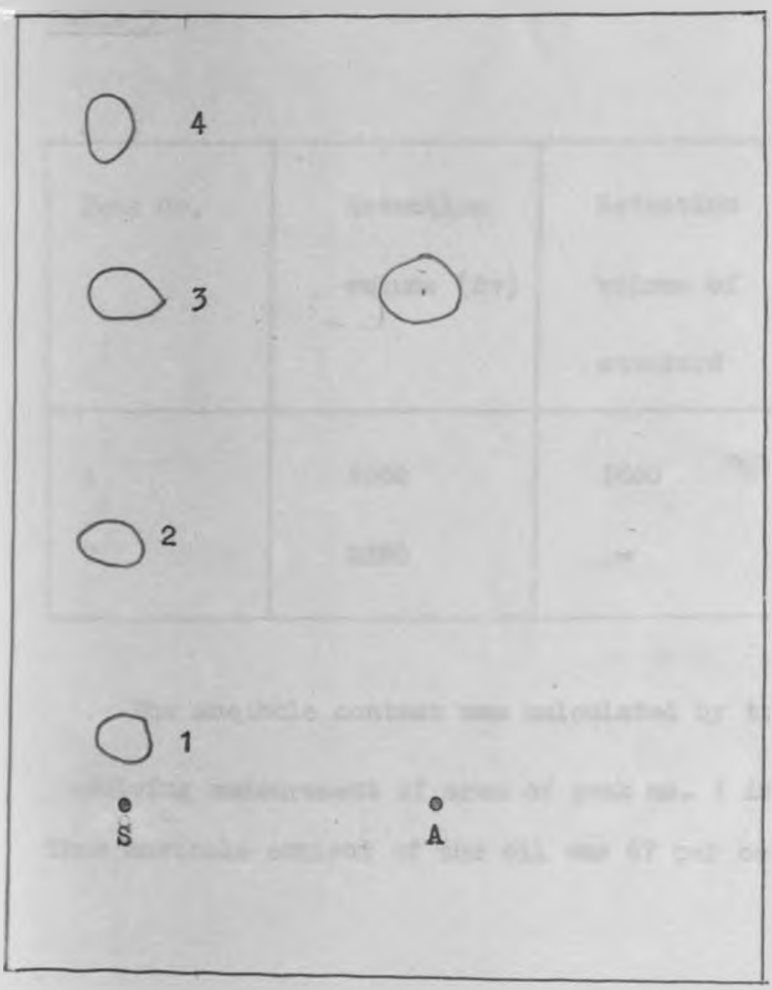
TLC EXAMINATION OF THE OIL

Solvent System - - n hexane : ethylacetate (95:5).

Length of the run - 14 cm.

A - Anethole

S - Oil



GLC separated the oil into two major peaks. By comparing the Rv of the peak obtained with that of anethole and peak enhancement the peak number 1 was confirmed as being anethole. Table 3 gives the Rv values calculated for the peak obtained for oil and for anethole.

Table 3

Peak no.	Retention volume (Rv)	Retention volume of standard	Identified
1	1860	1860	Anethole
2	2280	-	Unknown

The anethole content was calculated by triangulation method involving measurement of area of peak no. 1 in chromatogram. Thus anethole content of the oil was 67 per cent.

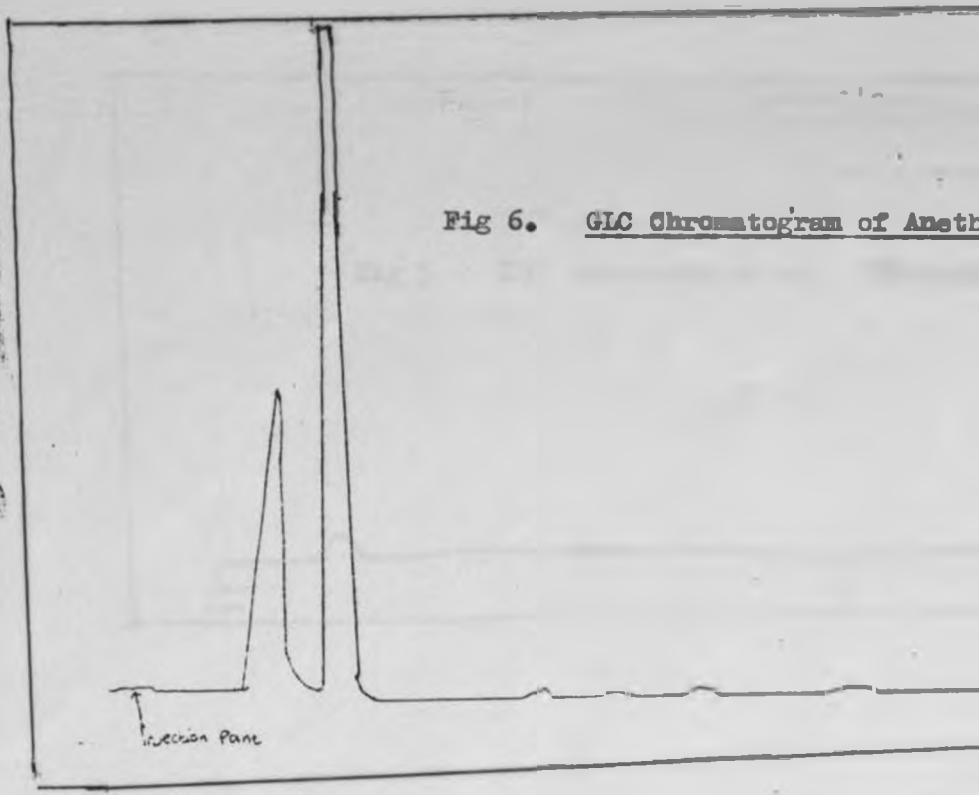
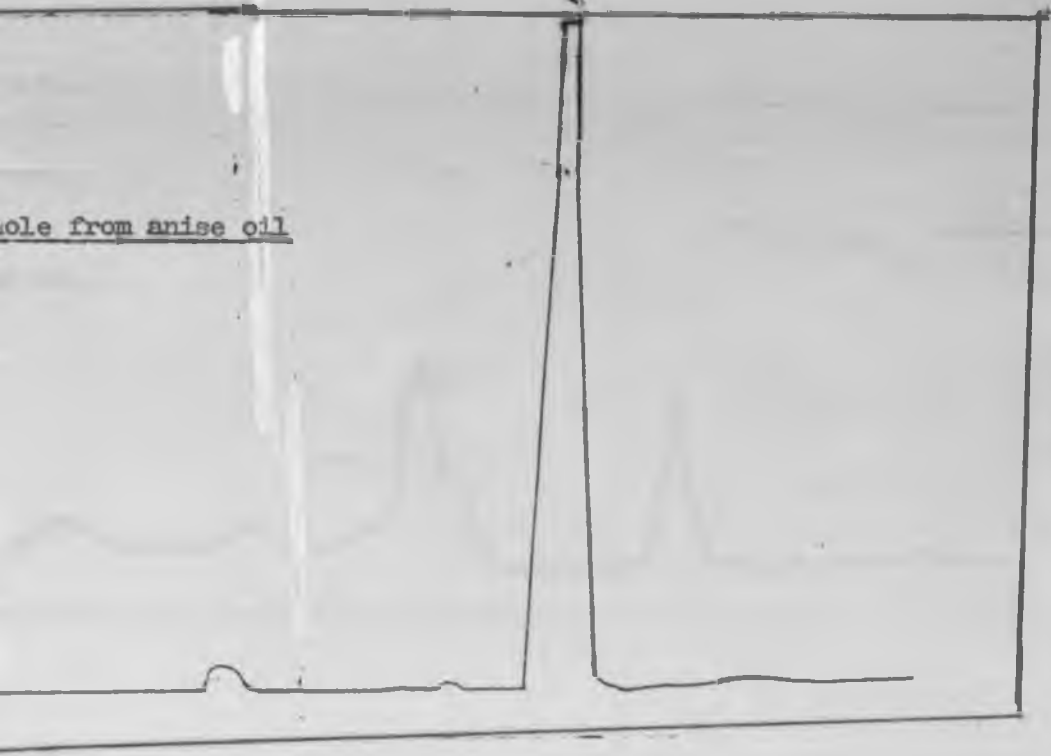


Fig 6. GLC Chromatogram of Anet



ole from anise oil

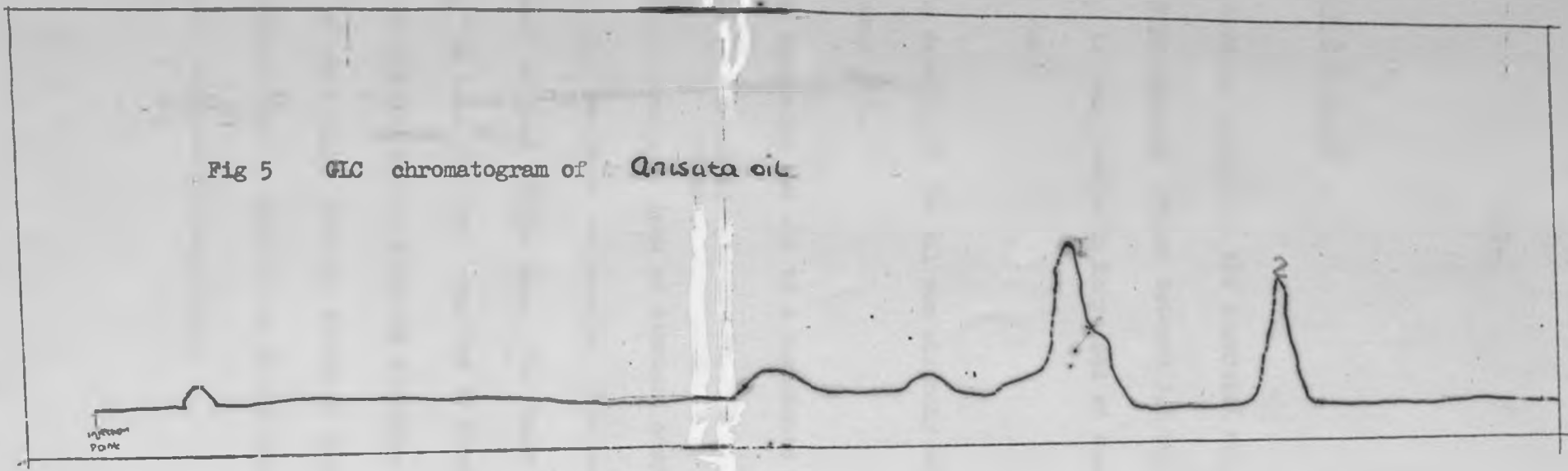


Fig 5 GLC chromatogram of Anisata oil

D I S C U S S I O N

According to Watt(5) the essential oil content of the leaves of *Clausena anisata* varies between 1.2 to 7.1 per cent. *Clausena anisata* leaves growing in Kenya had an essential oil content of 3.1 per cent.

The density of the oil was slightly higher than that reported by Guenther (1).

TLC separated the oil to 4 components and one component was identified from the Rf values. The other components couldn't be identified because of lack of standard compounds. GLC separated the oil into two major components. Peak no.1 was identified by Rv as well as peak enhancement. The amount of anethole in *Clausena anisata* was less than that reported by Guenther. Further research should be carried out to identify the other components and also the effect of soil type, climate, season on yield should be investigated and therfore lead to cultivation to provide a source of anethole in perfumery and cosmetic industries.

REFERENCES

1. Guenther, E 1948
(i) "The essential oils" volume 1
P 250
Van Norstrand company Inc.
New York U.S.A.

2. Brooks, B.T. Phillipines Journal of Science (1911) 6 344-346

3. S. Talalaj - West African Pharmacist (7/4) 44-5(1965) from Chemical Abstracts 63 16123a

4. Naves, Y.R and Tucakov, J 1959
"Presence of Anethole in essential oil of fennel in Yugoslavia".
Compt Rend 248 843-5
From Chemical abstracts 53 1442(h) 1959

5. Watt J M and Breyer-Brandwijk MC (1962)- Medicinal and poisonous plants of Southern and Eastern African 2nd Edition
P 917-918
E & S Livingstone LTD.

6. KOKWARO, J.O - Medicinal Plants of East Africa P 195
East Africa Literature Bureau

7. European Pharmacopiea (1969)
Vol. 1 P93
Maisonneuve France.

8. Guenther, E 1948
"The essential oils" volume 111, Von Norstrand company Inc.
New York U.S.A. P372

9. Ibid : P 239

10. Ibid : P242

11. Ibid : P241

12. Stahi E (1970) - Chromatographic and macroscopic analysis of crude drugs Ed Fischer, Stuttart (polish translation) P 159