PHARMACOGNOSTICAL STUDY OF CLOVE BUDS (SYZYGIUM AROMATICUM) FROM KENYAN MARKET

BY

C. A. OMECH

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Department of Pharmacy,
Faculty of Medicine,
University of Nairobi.
(Nairobi)

D E D I C A T E D:

To my beloved Parents,

Brothers and Sister.

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

The completion of this dissertation would have been unattainable without the generous assistance and encouragement of many persons. I would like to record my deep appreciation to the following:-

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A B S T R A C T.

From clove buds Syzigium aromaticum (family Myrtaceae) obtained from a Nairobi market, the volatile oil was isolated and evaluated by steam distillation method. The yield of the oil was 18.0 per cent on moisture - free basis.

The physico-chemical properties of the oil were the following:-

Density (d^{20°)	1.0533
Optical rotation ($\alpha_d^{20^\circ}$)	-0°.65'
Refractive Index ($n_d^{24^\circ}$)	1.5310
Solubility in 90% alcohol	1:1
Solubility in 70% alcohol	1:2
Phenolic content	94.9%
Acid value	5.2

Thin layer and gas-liquid chromatographic studies showed the oil contained 80.34% of eugenol which is the major component of the oil.

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

Cloves are the hand-picked, air or sun dried flower buds of an evergreen tree Eugenia caryophyllus (sprangel) (Eugenia caryophyllata Thunberg (1) Caryophyllus aromaticus L., Syzygium aromaticum), of the family Myrtaceae (2). According to Englers classifications (2) the plant is a dicotyledon member of the group Arichlamydeae, order Mytiflorae to which 17 families belong.

The family Myrtaceae consists of about 100 genera and 3,000 species of evergreen shrubs and trees; well represented in Australia, the East Indies and tropical America. The family is subdivided into two sub-families one of which is Myrtoideae, (fruit a berry or drupe) and Leptospermoideae, (fruit a loculicidal capsule). The main genera of the sub-family Myrtoideae are Myrtus (100 spp.) Psidium (140 spp.) Pimenta (18 spp.) Eugenia (1000 spp.) Pseudocaryophyllus and Syzigium (Jambosa). Sub-family Leptospermoideae includes the genera Eucalyptus (over 500 spp.) Leptospermum (50 spp.) and Malaleuca (about 100 spp.).

The noteworthy anatomical features of the plant are the schizolysigenous oil glands found in young stems, leaves, flowers and fruits; and the presence of bicollateral vascular bundles. Many species provide important volatile oils and spices like clove and its oil, eucalyptus oil, cajaput oil, and pimento psidium guajava gives edible fruit guava. Constituents other than volatile oils are leucoanthocynins, cyclitols, tannins, phenolic acid and esters. Cyanogenctic glycosides and alkaloids are rare.

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Cloves were known in China as early as 266 BC. and by the fourth century were known in Europe (3) The exact origin of clove is unknown but it is probable that it originated from the warm humid tropical climate of Asia. (4) It was first encountered by the Portuguese in the Island of Amboyna. Trade was initiated which was disrupted by sea battles between Portuguese, Spanish, Hollanders, French and England man-of-war, the merchants being captured, plantations burnt and seeds stolen. The Molluccas Island was held by the Portuguese until 1605 when Hollanders took over until 1765, thereby monopolising trade. In 1770, Poivre succeeded in transporting clove to the Island of Bourbon and Mauritius. Shortly afterwards, the enterprising British East India introduced it on Penang (Malaysia) off the coast of Mollucca. In 1803, it was introduced in Sumatra and in 1818, the Arabs introduced it to Africa - Zanzibar and Pemba, which later became the major producer. Currently, the principal producers of cloves are Madagascar, Tanzania (Zanzibar and Pemba,) Brazil and Penang, which produces the finest clove (5). Smaller plantations are found in Molluca, Sumatra, Amboyna, Seychelles, Bourbon, Mauritius, West Indies, and Cayenne (3). Madagascar and Indonesia principally supply clove leaf oil while Tanzania clove stem oil.

Production of cloves in the years, 1960-1972 in Zanzibar, Madagascar, and Indonesia; and exports from Tanzania have been estimated as follows: (6).

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TABLE:1. CLOVES PRODUCTION IN TANZANIA, MADAGASCAR: AND INDONESIA (IN THOUSAND TONNES)

YEARS	TANZANIA (ZANZIBAR)	MADAGASCAR	INDONESIA.
1960-61	6.5	5.2	7.3
1961-62	15.2	...	7.1
1962-63	5.9	...	6.6
1963-64	20.2	...	7.9
1964-65	3.9	3.9	12.6
1965-66	15.6	...	13.7
1966-67	1.5	5.0	17.2
1967-68	14.2	7.0	3.0
1968-69	15.0	12.0	3.0
1969-70	....	2.5	11.0
1970-71	5.1	....	11.0
1971-72	3.6	3.5	8.0

TABLE 2. CLOVES (including stems): EXPORTS FROM TANZANIA.

YEAR	TONNES	£'000
1961	8,535	2,414
1962	7,749	2,080
1963	11,395	3,052
1964	8,159	2,191
1965	8,575	2,297
1966	13,727	3,687
1967	17,710	.....
1968	11,716	3,478
1969	7,341	8,869
1970	4,769	6,370
1971	9,034	10,327
1972	11,758	13,468

TABLE 3: CLOVE OIL: EXPORT FROM TANZANIA.

YEAR	TONNES	£.000
1961	157	122
1962	122	98
1963	151	118
1964	164	128
1965	109	83
1966	135	70
1967	87	67
1968	204	221
1969	61	104
1970	42	204
1971	23	109
1972	28	106

The major markets are United Kingdom, United States, Canada, Japan, the Federal Republic of Germany, France, Italy, Netherlands and Switzerland.

The clove plant is columnar shaped, evergreen tree attaining a height of 30 ft. to 70 ft., that grows best in clearings or open slopes but not under shades of other trees (7) It requires a warm, moist equable climate with fairly well distributed rainfalls and prefers low altitudes. The soil should be well drained, sand or loam with proper moisture retaining capacity. The plantings should not be too far from the sea. In East Africa, growth is more vigourous in Pemba than Zanzibar due to more rainfall and superior soil and topographic conditions.

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The plants are produced in nuseries or by underplanting technique whereby seedlings are planted under shelter of old trees when they are soon to die (8). The seedlings are planted on a wet day, and after one year in the nursery resetting is done in the coolest and the rainiest period of the year.

The leaves are entire, smooth, coriaceous and glandular puncate, (5) about four inches by two inches on short stalks and are pink when young. After about an average span of six years, (9) buds grows on a main stem in bunches of 10 - 15 buds or more and first appear as tiny pea-green shoots attaining a length of 3/4 inch. The buds are ready for harvesting when the colour turns from green to reddish appearence. Harvesting is done twice a year in Zanzibar and Pemba; Mwaka crop (from July to October) and Mvuli crop (from December to January). Production of crop is however irregular with a bumper crop, half crop and very small crop in three years (10) The yield depends on age, size, form and condition of tree. In the period between 1923 to 1929 the avarage yield was 7Ibs/tree, and a large tree in a favourable year may produce 40Ibs (1). Harvesting involves hand-picking of the buds, removal of stems from buds and sun drying of the buds during which the head changes to light brown and 67% weight of the buds is lost. Five to six days after blooming of the bud the ovary develops and bud opens into tetrameruos, rose coloured flowers arranged in axillary and terminal recemes of cymes. Further on the fruit develops, which is an ovoid berry, and is collected when nearly ripe and sold as "Mother of

Trees on marginal soil or places heavily inter-planted with coconut often suffer from "die-back" disease in which a gradual desication of whole upper part of tree occur, and this is thought to be viral(1)

Clove buds are from 10 to 17.5mm long. Penang and Amboyna varieties are the largest and plumpest and are therefore of the highest quality (13). The Zanzibar variety are smaller and leaner than the Penang and of a blackish brown rather than reddish brown colour, but are of good quality. The buds have a strong fragrance and spicy odour and a pungent aromatic taste. Cloves contain 14-21% volatile oil, 10-13% tannins, various triterpenes, acids and esters, glucosides of sitasterol, stigmasterol and campestrol (14).

The chief commercial product obtained from clove is the volatile oil. It is a colourless or pale yellow becoming darker and thicker with age or exposure to air; and having the characteristic odour and taste of cloves (11). The yield of oil, physicochemical properties and composition depend upon the origin, quality, condition prior to distillation, and type of distillation, whether water or steam distillation. The yield of oil varies between 14-21%. According to Gildemeister and Hoffman, whole clove buds give oils with high eugenol content and specific gravity below 1.06. According to Smith (15) water distillation yield oils for perfumery and flavour purposes containing 85 to 89% eugenol by volume. With dry steam distillation, oils with 91-95% eugenol are obtained.

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Analysis by C. Godstein (17) gave the following:-

	Oil Content	Eugenol	$n_D^{20^\circ}$	Optical Rotation	Specific Gravity
Clove Buds (Whole)	16%	97	1.5368	$-0^\circ.25'$	1.084
Clove Buds (Crashed)	17%	94	1.5378	$-0^\circ.27'$	1.080
Clove stem	7%	95	1.5373	$-0^\circ,30$	1.069

Smith, working in England on imported clove buds reported:

	Water distillation	Steam distillation
Specific Gravity at 15°C	1.048 - 1.055	1.059-1.065
Eugenol Content (by volume)	85 - 89%	91- 95%

Raymond, working on Zanzibar cloves in boiling water (I) and low pressure steam (II) obtained the following physical properties of the oil and its eugenol content.

	I	II
Yield of oil	17.3%	17.35%
Specific Gravity	1.0652	1.0691
Refractive Index	1.5315	1.5319
Eugenol	91.5%	92.5%
Solubility	Soluble in 1: 1 Volume of 70% alcohol	

Gildemeister and Hoffman recorded the following properties of clove bud oil:-

Specific Gravity	1.043 to 1.068
Optical Rotation	Up to 1° 35'
Refractive Index 20°C	1.529. to 1.537

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(Total eugenol content including (eugenol acetate)	78 to 95% seldomup to 98%
Solubility at 20°	Soluble sometimes slight turbidity in 1-2 volumes and more of 70% - alcohol.
	Only freshly distilled oils are soluble in 2.5-3 volume 60% alcohol. And more alcohol,
	except in case of very high % eugenol.

British Pharmacopeia (16) specification for clove oil are as follows:-

Optical Rotation ..... 0. to - 1.5°

Refractive Index .....1.528 to 1.537

Solubility in alcohol..... (20) ..... at 20° in volumes of alcohol 70%

Weight/ml .....1.528 to 1.537

Alkali soluble matter ..... 85 to 90%

As with clove bud oil, the physicochemical properties especially phenol content and specific gravity of the clove stem oil are influenced by conditions of the stem material. Raymond, carrying out tests in Zanzibar found the following properties for the stem oil (18)

Specific Gravity at 15.5°/ 15.6°C 1.0699
Refractive Index at 20°C 1.5393
Eugenol Content 98.0%

Smith (18) recorded the following physicochemical properties for the stem oils distilled in England (I) and Zanzibar (II).

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	I	II
Specific Gravity at 15°C	1.060 to 1.063	1.055 to 1.063
Eugenol content (by volume)	92-94%	90-95%

Gilde meister and Hoffman noted the following limits (18)

Specific gravity 15°C.....1.040 to 1.067

Optical Rotation.....Upto- 1°30'

Refractive Index at 20°C.....1.531 to 1.538

Eugenol content (Determined with 3% NaOH solution)..83 to 95% in exceptional cases higher

Solubility.....soluble in 1 to 2 volumes and more of 70% alcohol. Often Soluble in 2.5 to 3 volumes of 60% alcohol but in many cases opalescent to turbid on further dilution especially with older oils.

Guenther (18) working on 3 samples of Madagascan clove leaf oil recorded the following for the physico-chemical properties of the oil.

	I	II	III
Specific Gravity	1.041	1.040	1.054
Optical Rotation	-1°14'	-1° 40'	-1°20'
Refractive Index at 20°C	1.5329	1.5321	1.5379
Total Phenol content	85.5%	84%	88.5%

Solubility.....Soluble in 1 volume and more of the 70% alcohol.

Smith (18) working on clove leaf oil also recorded the physico-chemical properties:

Specific Gravity at 15°C.....1.043 to 1.055

Eugenol content (by volume).....80 to 88%.

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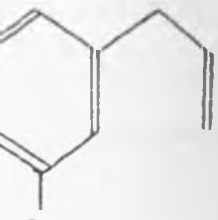
The major constituent of clove oil is eugenol, its content varying with method of distillation. Clove oil is sold according to its eugenol content. Eugenol is a colourless or pale yellow thin liquid with a strongly aromatic odour of clove and a pungent spicy taste which can be prepared from clove oil by shaking with 10% NaOH to form sodium eugenolate which is then washed with ether, decomposed with sulphuric acid and eugenol separated by steam distillation. The British Pharmacopeia gives its physical and chemical constant as follows: (18)



Eugenol

Refractive Index.....1.540 to 1.542

Solubility in alcohol (20°C).....in 2 volumes of alcohol (70%)

Weight/ml.....1.064 to 1.068g.

Other constituents are eugenol acetate, which was found to be 3% by Erdmanu, 7-17% by Sparge and 10-15% by Smith; caryophyllene and caryophyllene oxide. Those occurring in traces in clove oil are methylsalicylate, methylamylketone which is responsible for fruity odour, methylalcohol, furful, β-pinene and methyl benzoate, methyl-n-heptyl ketone and valeraldehyde. Small quantities of furfural, esters and ketones, and vanillin are present.

Further studies have however indicated that caryophyllene is an artefact formed during distillation and not a natural biological product (19). Oleanoleic acid has also been isolated from clove (20). E. Carma.

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and B.Stanchar studied two Madagascar and Zanzibar clove samples by chromatography using á column of carbowax 20M. For all 3 types 99-95.5% of the essential oil was eugenol, eugenol acetate, β- caryophyllene and terpenoid components with retention time relative to that of caryophyllene of 1.32. on carbowax 20M and 1.17 on methyl silicon polymer S.E.30. The relative amounts were as follows:- (21)

	Madagascar I	Madagascar II	Zanzibar
Eugenol	73.74%	73.44%	59.77%
β-Caryophyllene	10.47%	10.14%	12.2%
Unknown component	1.20%	1.11%	1.25%
Eugenol acetate	14.59%	15.31%	26.78%

Extracts of clove have been shown to supress completly the growth of Proteus vulgaris, E.coli, S.aureus. S. haemoly ticus, P.aeruginosa, Bacillus massenterius and Candida albicans in a dilution of 1:1000, the growth of P.aeruginosa being unaffected, (22) thus making it a possible dermatological and stomatological agent. Alcoholic extracts of clove inhibit Clostridium botulim in culture medium. It may be useful as an anticlestridial agent in food (23). Powdered cloves at 0.1g/100 ml decrease Aspergillus flavus mq growth and aflotoxin formation in powered rice corn steep medium by 50 and 13% respectively. At higher concentrations it completely inhibits both growth and aflotoxin formation (24). However, toxicity to Salmonella is inhibited by sulfite compounds- K_2SO_3 K_2SO_4, $NaSO_3$, Na_2SO_4 and $CaCO_3$ (25)

in extraction. The oleoresin is used in flavourings for many kinds of meat products such as sausages and canned stews, in various kinds of pickles and popular table sauces and in certain baked goods and fruit puddings (34). The oil is used for flavouring and in perfumery industries. In medicine, the oil is used as antispasmodic and carminative, and may be used in treatment of flatulence and colic. Externally it is an irritant, rubefacient and slightly analgesic. It has been used in liniment with olive oil. It is used as a remedy for toothache, and when mixed with zinc oxide it is a temporary anodyne dental filling. In 1-3% it is used as a flavouring agent in dentrifices (31). It has preservative properties and is a stimulant and antiseptic. The stem oil produced in Zanzibar and Indonesia is mainly used in flavouring and perfumery. The leaf oil which has somewhat harsh and distinctly different from odour of eugenol, its main constituent, as well as that of clove bud oil is mainly produced in Madagascar and indonesia, and is principally used in isolation of eugenol (14). Eugenol has similar properties as clove oil and is employed in dentistry as a flavouring agent and mild rubifacient in dentrifices; as an obtundent for hypersensitive dentine caries or exposed pulp, and is mixed with zinc oxide as a temporary anodyne dental filling (31). Industrially it is used in perfumery due to its smoother odour than clove oil and was formally used as a starting material for synthesis of vanillin but has been replaced by much cheaper lignin residue, a by-product of the sulfite wood pulping proces.

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MATERIAL AND METHODS.

1. COLLECTION OF PLANT MATERIAL

The dried flower buds were purchased from a Nairobi grocery. The flower buds were said to be imported from Zanzibar but no details of exact origin and time of harvesting could be established.

2. EXAMINATION OF MORPHOLOGICAL AND HISTOLOGICAL CHARACTERS OF THE FLOWER BUDS.

Macroscopical study of the buds involved examination of size, shape, colour, odour, taste of the buds and a study of the longitudinal section. Microscopical study involved a study of the transverse section of the buds.

3. DETERMINATION OF VOLATILE OIL CONTENT.

The determination of volatile oil content was carried out after the method of the British Pharmacopeia (35) for volatile oils heavier than water. 10g of freshly powdered plant material was used in each determination, the steam distillation being done from an electrically heated 2 - Litre round-bottomed flask. The time of distillation was 4 hrs after which no time change in volume of oil was observed. The mixture of oil and xylene were separated from water, dried using anhydrous sodium sulphate, filtered through cotton plug and stored in a closed dried container at low temperature.

Two simultaneous determinations were carried out and the average oil content was calculated on a moisture free basis.

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4. DETERMINATION OF MOISTURE CONTENT.

The gravimetric method specified in the European Pharmacopia (36) was used for determination of moisture content. Aproximately 2 gms of powdered clove buds was accurately weighed in a petridish and spread as a uniform layer. The petridish was placed in the oven at 105°C and loss of weight calculated after drying to a constant weight. Three determinations were simulteneously performed and the avarage results calculated.

5. ISOLATION OF OIL FOR FURTHER INVESTIGATIONS.

In order to obtain sufficient amount of oil for further investigations, larger amounts of freshly powdered plant meterial (100g) was steam distilled using a special type of apparatus for volatile oils heavier than water. The isolated oil was dried over anhydrous sodium sulphate, filtered and stored in a tightly closed container at low temperature. (4°C).

6. DETERMINATION OF PHYSICAL - AND CHEMICAL PROPERTIES OF THE OIL.

(a) PHYSICAL PROPERTIES

Colour, i.e. appearence, odour and flavour of the isolated oil were determined, solubility of the oil in alcohol - 90% and 70% was determined by the method described by GUENTHER (37).

Density was determined in the OSVALD pycnometer of 1ml capacity at 20°C according to the method of GUENTHER (38) The optical rotation was determined in the ATAGO polarimeter (Japan) at 20°C.

Refractive Index was determined in the ABBE refractometer (CENTRAL TRADING CO. LTD. TOKYO, JAPAN) at

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20°C by the method described by GUENTHER (39)

B. CHEMICAL PROPERTIES.

The acid number of the oil was determined by the method of GUENTHER (40). Two determinations were perfomed and the average result calculated.

The test for the presence of phenols was done by addition of alcoholic ferric chloride to a small quantity of oil and the colour change observed.

7. DETERMINATION OF PHENOLIC CONTENT OF THE OIL.

The phenolic content of the oil was determined following the B.P. method (16). 10ml of clove oil was added to 80 ml of aqeous potassium hydroxide in a cassia flask and shaken thoroughly. The volume was made up to 100.ml. mark and the flask shaken and left overnight for the reading of amount of oil left.

8. THIN LAYER CHROMATOGRAPHIC STUDY OF THE OIL.

The single development ascending thin layer chromatographic technique was employed using kieselgel 60 GF 254 (MERCK) as the adsorbent.

Preliminary TLC studies were carried out on microscope slides inorder to choose a suitable mobile solvent system and a locating reagent. For partition chromatography, the layers were rehydrated before use by holding the slide over a beaker containing boiling water and then allowing the layer to dry out at room temperature. Various solvent were tried. eg. Benzene, Benzene: Ethylacetate (95:5), Hexane: Methanol (7:3), Benzene:

.../18.

Chloroform (1:1), Chloroform, Benzene: Ethanol (97:3), Benzene : Acetone (97.5: 2.5). The best separation was achieved with Benzene: Ethylacetate (95:5) as recommended by Stahl (41).

Further TLC investigations were carried out on larger plate (20 cm by 20 cm). The layers were prepared by spreading a slurry of 30 gms kieselgel GF 245 (MERCK) in 60 mls of water with a Desaga spreader to a thickness of 250μm. After drying at room temperature the plates were activated by drying in the oven at 110°C for 1 hr and stored in a dessicator over anhydrous self - Indicating gel before spotting. The mobile solvent was placed in a developing chamber of size 21 x 21 x 6cm. The tank was allowed to equilibrate for at least an hour. A 10% V/V solution of clove oil in toluene was used for spotting, visualization was performed using Ultraviolet light followed by spraying with vanillin - sulphuric acid reagent prepared after Stahl (42). The plate was heated at 120°C until spots attained maximum colour intensity.

The hRf values of all the spots obtained were calculated. Pure eugenol, the major component of the oil was not available for reference, therefore isolation of eugenol by preparative TLC was performed, and the eugenol used as reference substance.

9. PREPARATIVE THIN LAYER CHROMATOGRAPHY OF THE OIL

Preparative TLC was carried by the same method as used for separation of clove oil. Thicker layers of 750μm were prepared using Kieselgel 60 GF 254 (MERCK).

The same mobile solvent - Benzene and Ethylacetate (95:5) was used. Spotting was performed using a micropipette;

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many spots were applied very close to one another so that a band of oil was formed. After development, the edge of the plate was sprayed with vanillin-sulphuric acid reagent, covering the rest of the plate with glass plate, and by UV examination the band of the layer containing eugenol was scraped out and extracted with ethylacetate. After evaporation of solvent the isolated eugenol was used as reference substance for further chromatographic studies. The last spot in TLC was also scraped out and isolated for use as a reference in further chromatographic studies.

10. GAS LIQUID CHROMATOGRAPHIC STUDY OF THE OIL.

This technique was applied for quantitative estimation of eugenol in the oil.

GLC was performed in PYE - UNICAM chromatograph (Series 104) with flame ionization detector. A glass column of length 1.5m and diameter 4 mm was used. The stationary phase was 12% carbowax 20M . Nitrogen was used as a carrier gas. The rate of flow was 30 mls/min. Temperature of the column was programmed from 75°C to 225°C. Chart speed was 0.5cm/Min. 1 μl of 33% of clove oil in Hexane was injected by means of a Hamilton syringe. Identification of eugenol was performed by enhancement of the peak with more eugenol. The last spot in TLC isolated and extracted from preparative TLC was also injected. From the chromatograph obtained.

(i) Retention volume of each peak was calculated.

(ii) By triangulation (43), the amount of eugenol was quantitatively estimated.

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RESULTS

MORPHOLOGICAL CHARACTERS OF CLOVE

The dried clove buds were brown in colour, measuring from 10mm to 17mm long. The 'head' consisted of four slightly projecting calyx (figure1) and four membranous imbricated petals. The hypanthium was rough on the surface. The longitudinal section (figure 2) showed incurved stamens surrounding a large style in the 'head' and above the hypanthium an ovary containing ovules attached to the axile placenta.

Microscopic examination of the hypanthium in the region below the ovary showed heavy cuticularized epidermis in which stomata occurred (figure 3). Within this was a zone of parenchyma containing numerous schizolysigenous oil glands and cluster crystals of calcium oxalate occurring in many parenchymatous cells. Within the oil gland layer was a zone of cells embedding a ring of bicollateral vascular bundles; within which there was a zone of aerenchyma. The ground tissue of collumella was parenchymatous and rich in calcium oxalate clusters; and consisted of ring of some seventeen small vascular bundles on the outer part.

(Figure 4) Shows part of the detailed transversesection of the hypanthium in the region below the ovary. The petals and sepals showed simplified leaf structures; the mesophyll parenchyma showed clusters of calcium oxalate and numerous oil glands. The stamen (figure 5) consisted of filament, connective and anther, the ground mass of the parenchyma embedding numerous oil glands and a single

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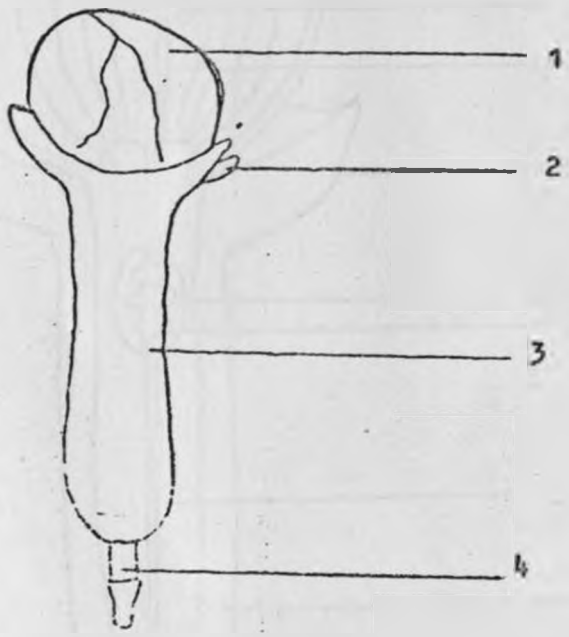
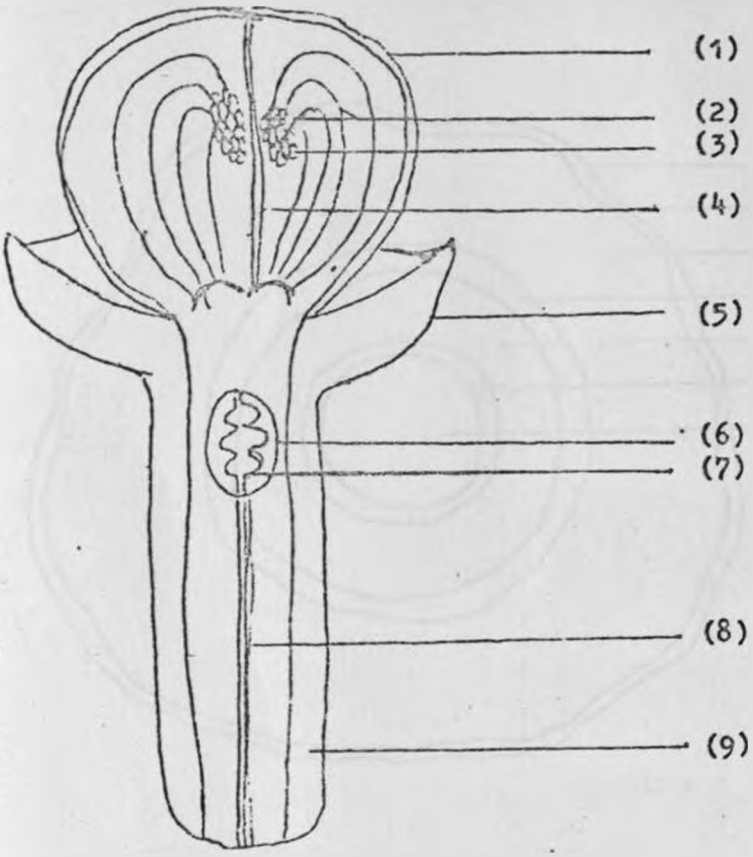
vascular bundle. The vascular bundle was continuous into the connective which terminated with an oil gland. The style and stigma showed similar characteristics to those of filament.

FIGURE 1: CLOVE BUD.



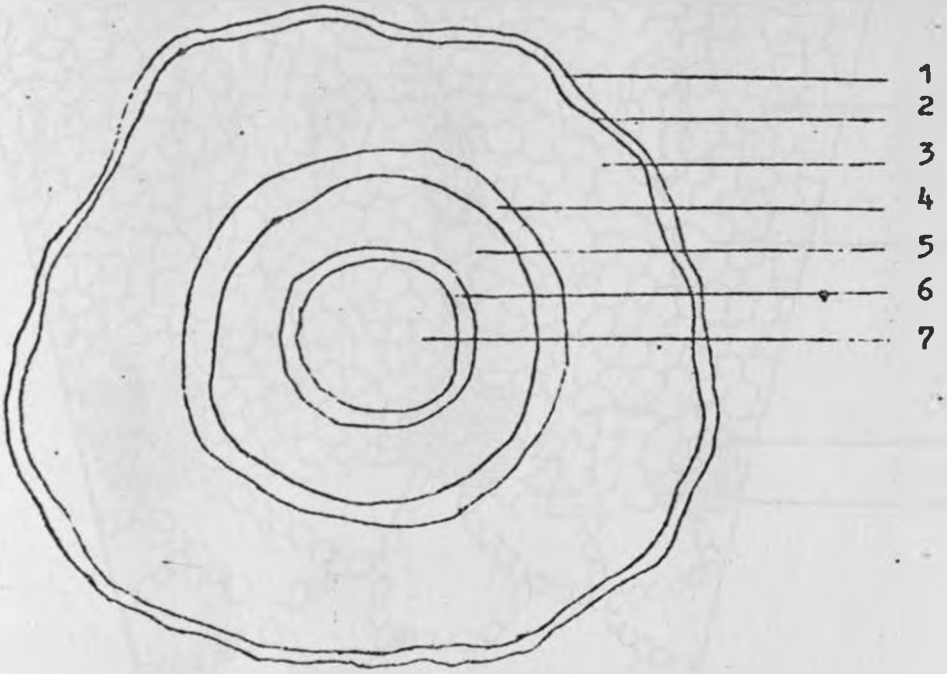
1. PETALS
2. CALYX
3. HYPANTHIUM
4. STALK

FIGURE 2: LONGITUDINAL SECTION OF CLOVE BUD.



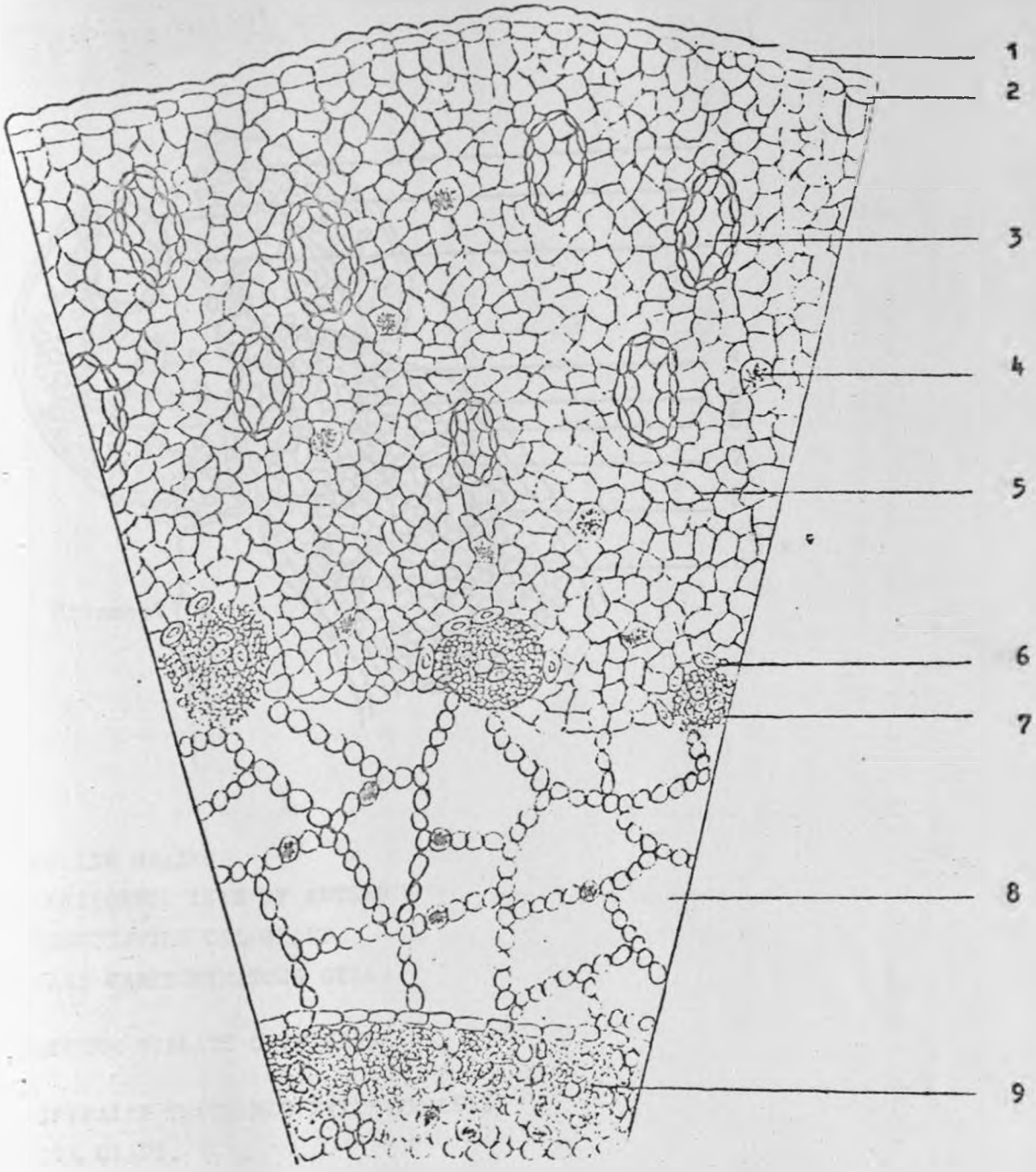
1. PETALS
2. FILAMENTS
3. ANTHERS
4. STYLE
5. CALYX
6. OVARY
7. OVULES
8. HYPANTHIUM
9. REGION CONTAINING OIL GLANDS.

FIGURE 3: TRANSVERSE SECTION OF THE HYPANTHIUM OF CLOVE BUD.



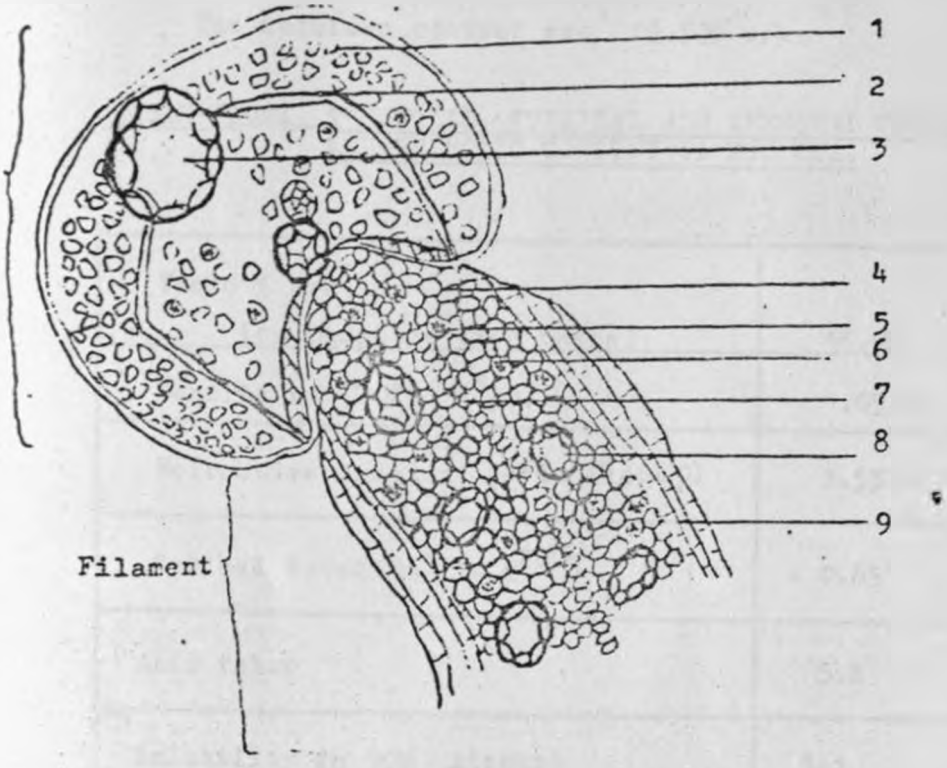
1. THICK CUEICLE
2. EPIDERMIS
3. PARENCHYMA CONTAINING SCHIZOLYSIGENOUS OIL GLANDS
4. REGION CONTAINING VASCULAR BUNDLES
5. COLLUMELLA
6. REGION CONTAINING VASCULAR BUNDLES
7. AERENCHYMA.

FIGURE 4: TRANSVERSE SECTION OF CLOVE BUD.



1. CUTICLE
2. EPIDERMIS
3. OIL GLAND
4. ROSETTE OF CALCIUM OXALATE
5. PARENCHYMA
6. FIBRE
7. FIBROVASCULAR BUNDLE
8. AERENCHYMA
9. FIBROVASCULAR BUNDLE WITH INTERNAL PHLOEM.

FIGURE 5: STAMEN OF CLOVE BUD.



1. POLLEN GRAINS
2. DEHISCENCE LINE OF ANTHER
3. TERMINATING OIL GLAND
4. MASS PARENCHYMATOUS CELL
5. } CALCIUM OXALATE CRYSTALS.
6. }
7. SPIRALLY THICKENED XYLEM VESSELS
8. OIL GLAND.
9. EPIDERMIS.

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THE YIELD AND PHYSICO - CHEMICAL CHARACTERISTICS.

The isolated oil was clear, pale yellow. It had a strong aromatic odour, a flavour resembling clove buds and a bitter taste.

The moisture content was 19.53% w/w.

TABLE FOUR: THE YIELD, PHYSICAL AND CHEMICAL PROPERTIES, AND PHENOLIC CONTENT OF THE OIL.

Yield % v/w (on moisture free basis)	18.0%
Density at 20°C ($^{d}20$)	1.0533
Refractive Index at 24°C ($\eta_{d}24°C$)	1.5310
Optical Rotation ($\alpha d^{20°C}$)	- 0.65°
Acid Value	5.2
Solubility in 90% Alcohol	1:1
Solubility in 70% Alcohol	1:2.
Phenolic content of clove oil by the B.P. Method.	94.9%

The test for phenolics with alcoholic ferric chloride gave dark blue colouration, indicating that phenols were present in the oil.

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THIN LAYER CHROMATOGRAPHY:

Thin layer chromatographic examination of clove oil indicated the presence of six components, only one being the major constituent. The colour of the spots obtained were brownish- violet with vanillin-sulphuric acid spray reagent. Visualization with UV was also done. Only two spots - 4 and 5 could be visualised under UV.

TABLE 5. THIN LAYER CHROMATOGRAM OF CLOVE OIL (FIGURE 6)

Spot No.	hRf	identified
1	14.28	
2	19.88	
3	29.19	
4	42.24	Eugenol
5	53.61	
6	72.05	

..../29

THIN LAYER CHROMATOGRAPHY OF CLOVE OIL.

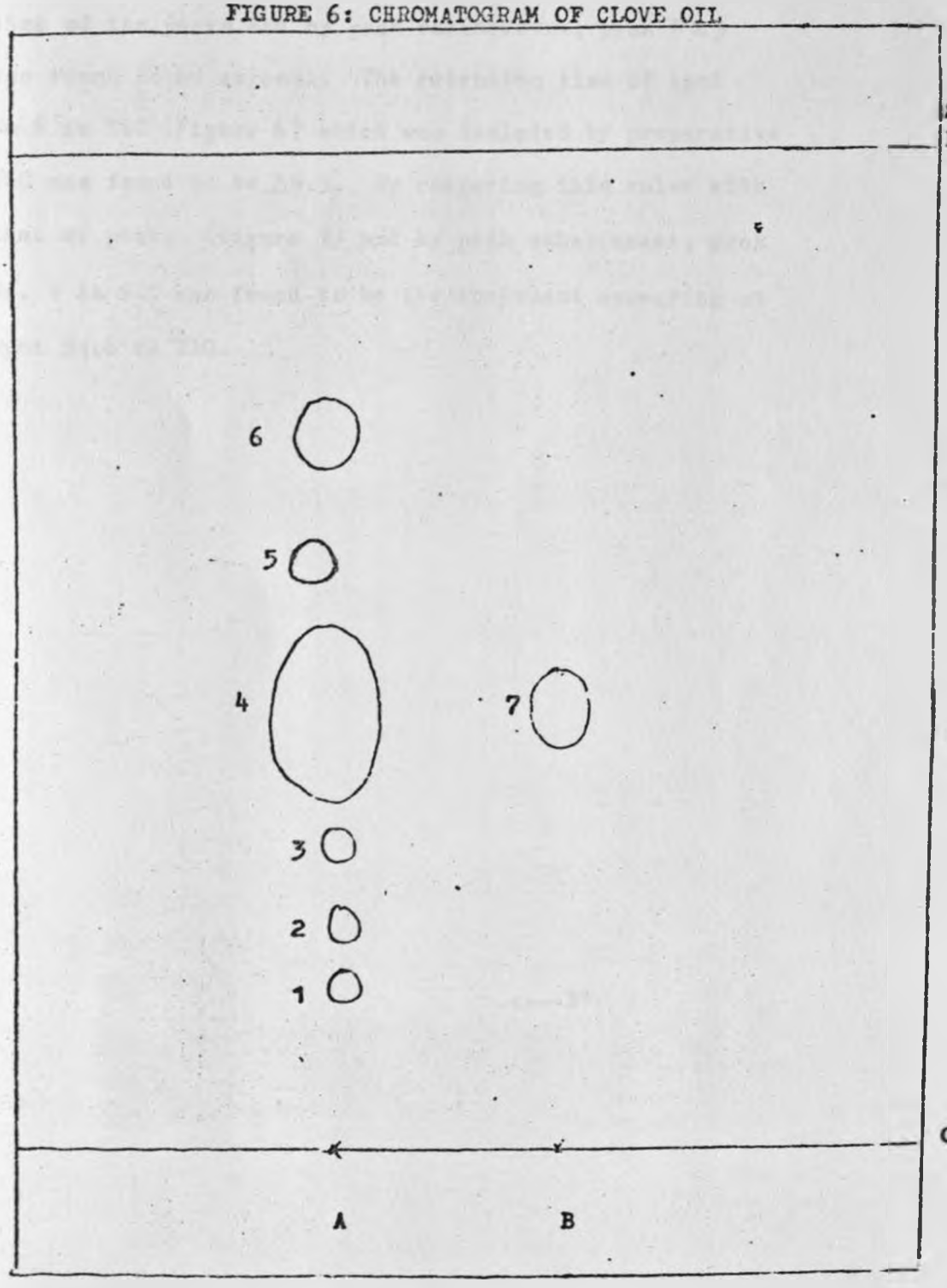
Mobile Solvent : Benzene: Ethylacetate (95:5)

Visualising Reagent: Vanillin- Sulphuric acid reagent.

A. Clove Oil

B. Standard

FIGURE 6: CHROMATOGRAM OF CLOVE OIL



GAS LIQUID CHROMATOGRAPHY.

The results of GLC separation of clove oil showed that it separated into 4 peaks (Figure 7). The retention time of eugenol (Figure 8) was found to be 70.5, and by comparing this value with the retention time of the peaks and by peak enhancement, peak N 3 was found to be eugenol. The retention time of spot No.6 in TLC (Figure 6) which was isolated by preparative TLC was found to be 39.5. By comparing this value with that of peaks (figure 7) and by peak enhancement, peak No. 1 in GLC was found to be the component appearing at spot No.6 in TLC.

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Table 6: GAS LIQUID CHROMATOGRAM OF CLOVE OIL.

Peak No.	Retention Time	% of Total Oil	Substance identified
1	39.5	13.	
2	44.4	1.96	
3	70.6	80.34	Eugenol
4	74.2	5.0	

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FIGURE 7: GAS -LIQUID CHROMATOGRAM OF CLOVE OIL.

CONDITIONS:

COLUMN PACKING:	GLASS COLUMN WITH 12% CARBOWAX 20M.
CARRIER GAS:	NITROGEN: FLOW RATE 30 Ml/Min.
TEMPERATURES:	PROGRAMING FROM 75°C TO 225°C
ATTENUATOR:	20×10^4
CHART SPEED:	0.5cm/Min.
VOLUME INJECTED:	0.4µl.

..../33.

FIGURE 8: GAS-LIQUID CHROMATOGRAM OF EUGENOL OIL.

CONDITION

COLUMN PACKING:	GLASS COLUMN WITH 12% CARBOWAX 20M.
CARRIER GAS:	NITROGEN: FLOW RATE 30Ml/Min.
TEMPERATURES:	PROGRAMMING FROM 75°C TO 225°C
ATTENUATOR:	20×10^4
CHART SPEED:	0.5cm/Min
VOLUME INJECTED:	0.4µl.

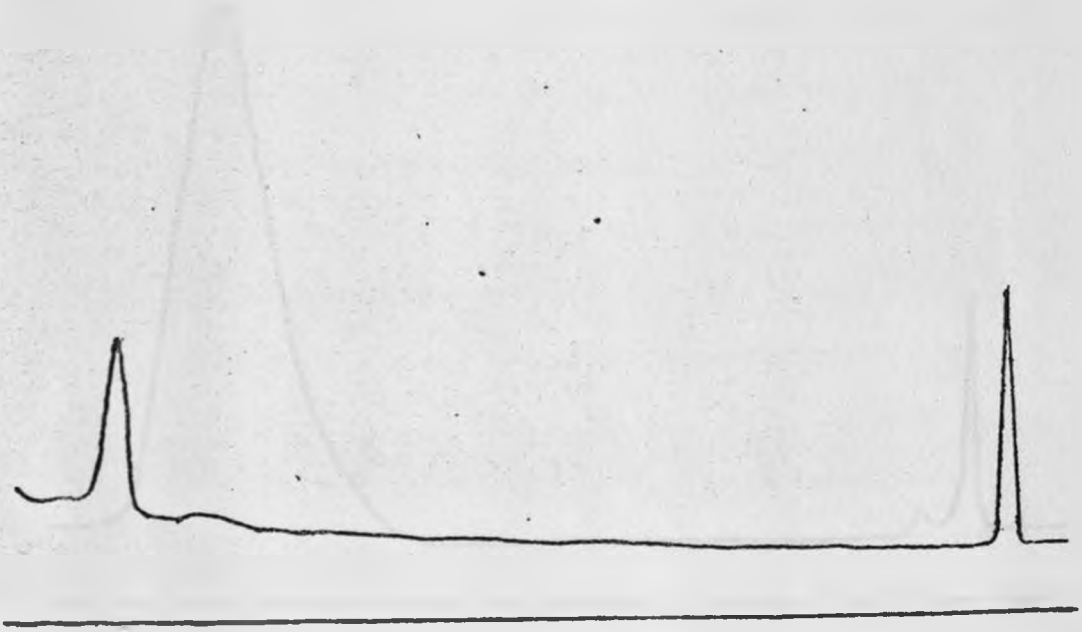

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FIGURE 9: GAS LIQUID CHROMATOGRAM OF PEAK NO 6 ISOLATED BY PREPARATIVE CHROMATOGRAPHY.

CONDITIONS

COLUMN PACKING; GLASS COLUMN WITH 12% CARBOWAX 20M.

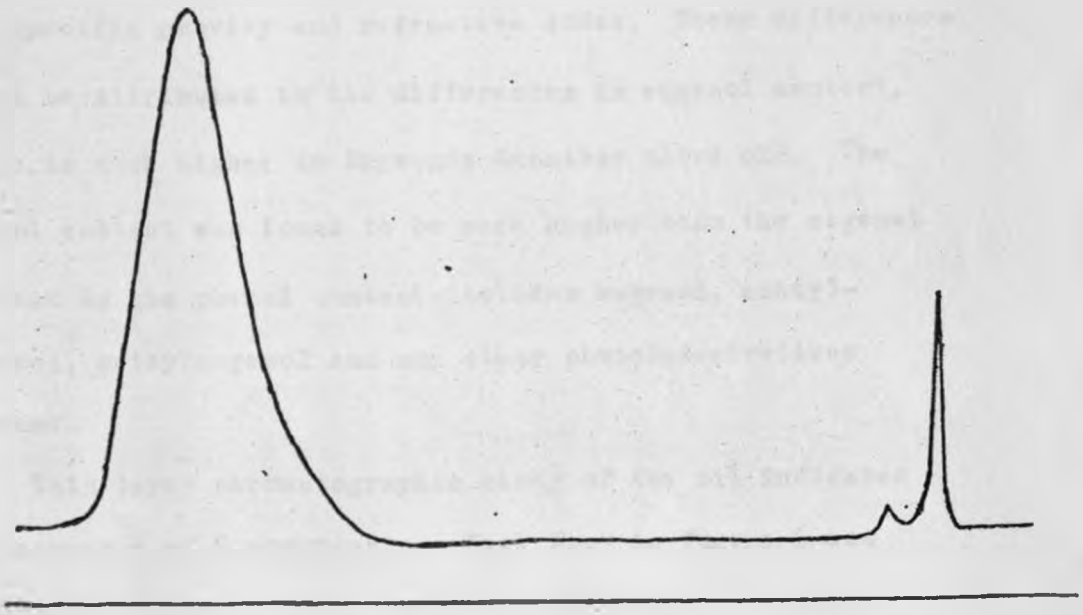
CARRIER GAS: NITROGEN: FLOW RATE 30 ml/Min.

TEMPERATURES: PROGRAMMING FROM 75°C TO 225°C

ATTENUATOR: 20×10^4

CHART SPEED: 0.5cm/Min.

VOLUME INJECTED: 0.4µl.



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DISCUSSION AND CONCLUSION.

The morphological characters of the clove bud compare well with the features described by Evans and Trease (13). The essensial oil content of the clove buds was found to be 18.0% calculated on moisture - free basis. This is higher than values obtained by Raymond (15) on Zanzibar cloves, but falls within the range given by Evans and Trease (14). The yield of oil may be higher than that obtained by Raymond as various factors such as botanical uniformity of plant material used (chemical races), origin, climate, quality, storage, processing procedures and chemical changes which occur during isolation, form in which the distillation takes place and type of distillation. As seen from Table 4, specific gravity, optical rotation, refractive index and solubility compare well with values obtained by Gildemeister and Hoffman (15). The values obtained by Raymond (15) on Zanzibar cloves are much higher for specific gravity and refractive index. These differences could be attributed to the differences in eugenol content, which is much higher in Raymonds Zanzibar clove oil. The phenol content was found to be much higher than the eugenol content as the phenol content includes eugenol, acetyl-eugenol, methyleugenol and any other phenolic derivatives present.

Thin layer chromatographic study of the oil indicated the presence of 6 components. Spot No.4 in fugure 6 was identified as eugenol by comparing hRf values. The best

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solvent system was found to be Benzene: Ethylacetate (95:5) Due to lack of reference substances, other components could not be identified. The minor compunds could have been oxygenated terpenoids.

Gas Liquid chromatographic study showed that peak No. 3 was eugenol by comparing Retention time values and by peak enhancement. The amount of eugenol present was calculated to be 80.34%. However, the value recorded by Gildemeister and Hoffman include eugenol and eugenol acetate. The eugenol content obtained from the extracted oil was higher than that obtained by E.Carma and B.Stanchar who worked on Madagascan cloves using carbowax 20M as stationary phase and found value of 73.74% and 73.44% for two samples and 59.77% for Zanzibar sample. Spot No.6 in TLC was found to be peak No.1 in GLC, by comparison of retention time values and peak enhancement. This could probably be caryophyllene as it appeared before eugenol in GLC. The number of spots obtained in TLC were more than those obtained in GLC. This could have been due to the concentration difference of oil used, and difference in technique of separation and sensitivity of the systems.

In commercial work, the quality of oil is dependent on its eugenol content. Studies on cultivation and other factors influencing yield should be done to give grades of oil with higher eugenol content. The antimicrobial and antifungal activity of the oil could be made use of in dermatology and the anticlostridial and antioxidant action made use of in food.

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clove plant is not grown in Kenya, but the results obtained show that clove sample found on Kenyan market show good yield of oil and physico-chemical properties compare well with those obtained by other authors.

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