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19951

DP/ID/SER.A/1611
23 November 1992
ORIGINAL: ENGLISH

ASSISTANCE TO ESSENTIAL OIL INDUSTRY

DP/URT/86/026/11-54

UNITED REPUBLIC OF TANZANIA

Technical report: Maximising the capacity of the clove distillery at Chake-Chake, Pemba, Second mission of the CCC*

Prepared for the Government of the United Republic of Tanzania
by the United Nations Industrial Development Organization,
acting as executing agency for the United Nations Development Programme

Based on the work of M.L. Maheshwari, quality control chemist

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* This document has not been edited.

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ABBREVIATIONS & ACRONYMS

B P	=	British Pharmacopoea
B P C	=	British Pharmaceutical Codex
cm	=	Centimeter
C S D	=	Clove Stem Distillery
C T A	=	Chief Technical Advisor
dcm	=	decimeter
D W B	=	Dry Weight Basis
E O A	=	Essential Oils Association of U.S.A.
Ex.	=	Example
F I D	=	Flame Ionization Detector
F W B	=	Fresh Weight Basis
g	=	Gram
G C	=	Gas Chromatography
G F-254	=	Green fluorescin mixed with adsorbent to absorb UV light at 254 nm wave length
I S O	=	International Standard Organization
Kg	=	Kilogram
KIVUMBASI	=	Swahili name of *Ocimum canum*
l	=	litre
m	=	Metre
ml	=	Millilitre
MREHANI	=	Swahili name of *O. basilicum*
MTULE	=	Swahili name of *O. sauve*
N F T	=	French standard

nm	=	Nanometers
Q C C	=	Quality Control Chemist
Q C L	=	Quality Control laboratory
Rt.	=	Retention time
TCD	=	Thermal Conductivity Detector
T L C	=	Thin Layer Chromatography
tr.	=	Traces
U V	=	Ultra-Violet
V/V	=	Volume by Volume
V/W	=	Volume by Weight
W/W	=	Weight by Weight
ZSTC	=	Zanzibar State Trading Corporation

ABSTRACT

Dr. Mohan Lal Maheshwari, Quality Control Chemist in the project "Maximising the capacity of the clove distillery at Chake-Chake, Pemba, United Republic of Tanzania, No. DP/URT/86/026/11-54J 13422" took up his second phase of assignment for two months (22 April, 1992-21 June, 1992). He was briefed by C T A and had discussions with General Manager ZSTC, National Project Director, Production Manager and Chemist about the Project.

Expert furnished the Quality Control Laboratory, installed the equipments, checked their functionality and gave demonstrations on their applications in essential oils and aroma chemicals. Standard test procedures and specifications were developed for essential oils produced in distillery and being developed in laboratory.

Principles of Gas Chromatography were explained to trainees and various parameters were developed to analyse all essential oils and to monitor production. Expert also demonstrated up grading of quality of products, making use of byeproducts and prepared few value added products such as - eugenol, eugenyl acetate, iso-eugenol, vetiveryl acetate etc.

All the operational parameters, instructions, chromatograms and details of experiments are available on the site. In addition to this expert has brought various standard specifications of different countries (I S O, E O A, French, Russian, Indian, BP & B P C), 135 - reference samples, library of 138 - chromatograms, relevant literature and left at site.

Trainees have learnt techniques of using equipments and their application and now they can handle by them selves. Products prepared in the programme are of good quality and can be compared with good commercial samples. Certain suggestions are made in the recommendations, which will further improve lab. efficiency. Essential oils, their isolates and preparation of related aroma chemicals have great potential in Tanzania for export.

RECOMMENDATIONS

In order to make best use of the project following recommendations are made from quality control and product development point of view :

1. For improvement and regular maintenance in lab. following may be provided:

   (i) Chemicals, glass wares, equipments, GC spares and miscellaneous items (Annexure - 19), Costing about U S $ 4,300

   (ii) For future maintenance an annual provision of U S $ 1,000.

   (iii) Hydrogen generator for G C may be replaced/repaired by Shimadzu, as it arrived in damaged condition.

   (iv) Injection port on channel - 1 (left) on G C is defective, this may be replaced by Shimadzu.

2. **Training**

   (i) Chemist may be given further exposure to well known Institutions like - Medicinal Plants Institute, Poznan, Polland & Pollena Aroma, Warsaw, Polland.

   (ii) Production Engineer with back ground of electronics can be trained with Shimadzu for keeping G C in trouble free condition.

3. **Books**

   Books and journals as drawn in the list (Annexure - 20) may be provided.

4. **Quality of products**

   (i) **Clove bud & stem oil :** Production, specially for bud oil may be monitored by quality control to get desired specifications. If needed, last cut (1 % of oil percentage, i.e. about 6 % of total oil) rich in caryophyllene (16 to 60 %) may not be mixed with main lot of bud oil. Economics may be worked out to get this

fraction out in terms of cost in-put for an additional distillation of 10 hrs.

(ii) **Black oil in distillation storage tank :** About 1200 to 1500 kg oil is collected annually. This is good source of eugenol (91%), which can be marketed after purification and can also be converted into value added products-eugenyl acetate, methyl eugenol, eugenyl benzoate, iso-eugenol, methyl - iso-eugenol and iso- eugenyl acetate.

(iii) **Black clove bud oil from purification tank :** About 40 - 50 kg black oil obtained annualy during removal of last traces of moisture, is very rich in fruity odour (low boilers 5.5 %). This on slight rectification can give product for high class perfumes.

(iv) **Caryophyllene** - a bye-product which may be obtained in following ways:

(a) Last fraction from clove bud & stem distillation.

(b) Non - eugenolic portion during preparation of eugenol.

(c) As a fraction during rectification of black oil from purification tank.

Caryophyllene thus obtained may be attempted to convert it into esters or alcoholic compounds by way of chemical reactions. Such compounds can find use in perfume formulations.

A provision has been made in the glass/equipment list (Annexure-19) for reactions, workup & fractional distillation.

5. For diversification and maximising the capacity of distillery following can serve as a source of raw materials :

Lemon grass - Its oil has good aroma & flavour and is low in sesquiterpenes, which are preferred; cinnamon leaf; bitter orange leaf; basil (Mrehani) and vetiver.

Economics of ylang-ylang flower & *Ocimum sauve* oils may be checked and opinion of perfumer may be btained for use of oils from *Artemisia camphorata, O. canum* and *Pogostemon plectranthoides.*

6. A pilot plant of 200 litres still capacity may be provided for distillation of new oils on experimental basis.

7. An appoinment of agronomist will be highly beneficial for programme of new crops.

LIST OF ANNEXURES

LIST OF ANNEXURES contd.

INTRODUCTION

The Project "Maximising the capacity of the Clove Stem Distillery at Chake-Chake, Pemba, United Republic of Tanzania, No. DP/URT/86/ 026/ 11-54" was joined by Dr. Mohan L. Maheshwari, Quality Control Chemist (Q C C) on 22 April, 1992 as second phase of his mission (first phase of 2 - weeks, 3 August, 1989 - 16 August, 1989). Q C C had a meeting with Mr. A. Krassiakov, UNIDO country Director and Ms. A.. Kostian J.P.O. at UNIDO/UNDP office in Dar-es-Salaam after his arrival on 23 April, 1992. Briefing was done by Dr. Baldev C. Gulati, C T A in Zanzibar on 27 April, 1992. Q C C had also discussions with Mr. A. R. Rashid, General Manager, Z.S.T.C, Mr. S.J. Jongo, Marketing Manager, Mr. Shaib M. Mossi, Board Secretary & Adviser, Mr. Hamad Khamis Hamad, Dy. G.M. (ZSTC) and Mr. Nasib S. Omar, NPD and Factory Manager (C S D) on 28 April, 1992 in Zanzibar.

Detailed discussion were held with C T A and Mr. Ramadhani K. Feruzi, Production Manager (C S D) on 29 April, 92 in Chake-Chake, Pemba. Q C C was taken around C S D and quality control laboratory (QCL) was shown to him. The project at Pemba needed development of a good quality control laboratory to evaluate the oils produced at present and for those which are planned for near future in this distillery.

DUTIES :

The expert was expected to work under the direct supervision of the C T A and N P D as shown in Job Description (Annexure - 1) for total period of 2.5 man months. First phase of assignment was for 2 - weeks (3 Aug., 1989 to 16 Aug., 1989), in which he prepared design and layout of quality control laboratory, lists of equipments, chemicals, glass wares and miscellaneous items; manpower requirements and training needs. He had also examined the preliminary aroma pattern of oils distilled in distillery and those obtained in labortory from locally available plants.

In the second phase (22 April, 92 to 21 June, 1992) of his asignment for 2 months, Q C C has now carried out work based on remaining items of his Job Description (Annexure - 1A). List of trainees, who worked with expert is given in Annexure - 2.

As requested by site, expert brought standard documents on desired essential oils and their isolates. These included 47 - International Standards (Annexure -3), 43 - Essential Oil Association of U.S.A Specifications (Annexure -4), 30 - Indian Standards (Annexure -5), 1- French Standard & 1- Russian standard (Annexure - 6), 13- British Pharmacopoea specifications (Annexure - 7), 15 - British Pharmaceutical Codex specifications (Annexure - 8), 3- Indian Pharmacopoea specifications (Annexure - 9). He also brought 135 - reference standard samples of essential oils, isolates and various aromachemicals for identification and analysis of essential oils in the project (Annexure - 10). Library of Gas chromatograms of 138 samples of essential oils, isolates and various aroma chemicals prepared in Expert's laboratory, were brought to the site (Annexure -11). He also brought relevant standard Gas Chromatograms of essential oils available in the literature (Annexure - 12). Beside this Q C C also brought several tables of variations, conversions, dilutions and other relevant literature used in day to day work in quality control laboratory. All these materials are kept at project site. All the assigned work was completed by expert. Salient features of the work done during the mission of the expert are given in the following pages.

I. DESIGN, LAYOUT & FURNISHING OF QUALITY CONTROL LABORATORY

The distillery has created Quality Control Laboratory as per design and layout plan prepared by expert in his first phase of assignment. This is situated by the side of distillery, connected to it and consists of following rooms:

1.	Main laboratory	7.21 m x 7.49 m
2.	Instrumentation room	4.27 m x 4.11 m
3.	Balance Room	4.27m x 1.52 m
4.	Store Room	5.84m x 2.95 m

All furnitures - Lab. benches, cupboards, shelves, drawers, water and electric connections have been provided according to design. Instrumentation Room has been air conditioned.

Expert checked lists of equipments, glasswares, chemicals and miscellaneous items procured for the project. Almost all the items suggested by expert have been received at the site according to required specifications except a few. All the equipments have now been installed in their respective places, glass wares, chemicals and miscellaneous items of daily use have been arranged in the lab. and remaining major items have been properly kept in store room with proper entry.

II. INSTALLATION, START UP & OPERATION OF INSTRUMENTS AND COMPILATION OF MANUALS FOR THEIR OPERATION & MAINTENANCE

A. Gas Chromatograph

Gas Chromatograph provided for the project is Shimadzu Model 14 APTF (S.No. 264182 S A), equiped with Thermal Conductivity Detector & Flame Ionization Detector and certain S.S. columns of 0.5 m; 1.0 m & 10 m lengths and packing materials. Along with main G C machine, a microprocessor controlled Recorder-cum-Integrator-Shimadzu model C - R 6 A Chromatopac (S. No. - 27 D 369 SA), an air compressor - Hitachi - Super Oil free Bebicon (S.NO. 6685-89) and a Hydrogen generator - Shimadzu O P G U - 1500 S (SNO. 1743514) have been procured. Two Nitrogen cylinders have been procured locally. G C instrument was installed by Shimadzu engineers in Feb., 92 and demonstrated only its operation with Thermal Conductivity Detector with solvent. Demonstration with Flame Ionization Detector could not be given by them as Hydrogen Generator did not function due to damaged components in it. This could not be repaired by Shimadzu so far.

Expert checked all connections of G C instrument and other accessories and installed two S.S. columns (lenght -3m., diameter 2 mm):

S E - 30 (10 %) and Carbowax 20 M (10 %) AT 1000 brought by him in the project for proper analysis of essential oils and left at the site. He operated G C Instrument with Thermal Conductivity Detector (TCD) to check functioning of main machine and Integrator and analysed some essential oils. Since T C D is known to have poor sensitivity for compounds having more than 10 carbon atoms, and its erratic behaviour at higher temperatures, specially in temperature programming, it did not give reproducible results even with standard materials brought by the expert.

It is FID detector, which gives comparable results. Hence expert advised to procure hydrogen cylinder to check/use Flame

Ionization Detector (FID) for analysis of essential oils. A hydrogen cylinder was procured from Dar-es-Salaam, hydrogen gas and compressed air connections were made, leak checked and performance of Flame Ionization Detector was tested. Finally, G C instrument was put into operation for demonstration and analysis of essential oils and their isolates.

Q C C explained principles of G C to local counterpart (Chemist). He was also instructed about maintenance of instrument, column packing, column installation, checking leakage of gases, isothermal/temperature programming and analysis of essential oils/ isolates. Practicals were given to trainee. Efficiency of columns was tested by using standard oils/reference compounds. Instruction manual for daily operation of instrument was prepared (Annexure - 13). G C analysis of various essential oils of Pemba as well as of standard reference compounds and isolates were carried out using SE- 30 & Carbowax - 20 M columns. Different sets of parameters were developed for different oils, iso-thermally and with temperature programming. All these chromatograms as shown in Annexure -14 are kept at the project site for comparison in future.

B. Refractometer

Abbe- Refractometer- Bellingham + Stanley Model 60/70 (S.No. A 89102) was installed, calibration was checked and training was given on its use by carrying out determination on several essential oils. Operation manual for its use and maintenance has been prepared (Annexure - 15).

C. Polarimeter

A polarimeter - Bellingham + Stanley Model D (S.No. R 88060) was installed. Sodium vapour lamp assembly was put to gether and checked its performance and put it in alignment with the axis of polarimeter tube. Instrument is provided with two - 100 mm and two - 50 mm tubes, spare side glass windows and a spare sodium vapour lamp assembly. Trainees have been given training for obtaining optical rotations of essential oils and maintenance of equipment. An operation manual has been prepared (Annexure - 16).

D. Balances

Two mettler balances - P M - 4000 (S N R - K. 10519) and AE - 100 (S N R - K 10518) have been assembled, installed, leveled and calibrated; stability detector and integration time have been adjusted. P M - 4000 balance is open top for faster weighing up to 4100 g. and accuracy up to 0.01 g. AE - 100 is an analytical balance of high precision weighing up to 109 g. with reproducibility of 0.1 mg. Trainees have been trained to operate and make adjustments/ calibration etc. Procedures have been hanged near the balances for convenience of user.

E. Thin Layer Chromatography Equipment

A complete kit for Thin Layer Chromatography (TLC) - CAMAG brand (S.No. 950629) has been provided. This is constituted of - Plate coater, U V- cabinet with 366 & 254 nm wavelength sources, spotter template, developing chambers, reagent spray bottle, precoated and uncoated glass plates (20 x 20 cm), sandwitch coverplate, plate box, several adsorbents - silica gel. aluminium oxide, cellulose, kieselgel etc. with or without fluorescen- GF. 254, disposable micropipettes & microcapillaries, test dye mixture, saturation pads etc. These components have been checked, installed, performance demonstrated. Separation of components of essential oils was practiced by trainees - e. g. clove bud, clove stem, cinnamon, basils, citrus leaf, lemongrass oils etc.

Separation of components like eugenol, eugenyl acetate and caryophyllene in clove & cinnamon oils; methyl chavicol, anethole, linalool, linayl acetate in basils and petitgrain (Citrus leaf) oils, citral in lemongrass oil and camphor in *Ocimum canum* and *Artemisia camphorata* oils has been practiced by trainees. This way active component in essential oil can be detected qualitatively.

An operational manual for TLC has been prepared (Annexure - 17).

F. Melting Point Apparatus

A meling point apparatus - Buchi 530 (S. No. 890 728) with spares like - mineral oil, melting point tubes, boiling point tubes, thermometer etc. has been provided. The equipment was assembled, practical demonstations were given to trainees. This is a very good quality equipment giving highly accurate results.

G. Other equipments

Besides above several other equipments of common use like - Clevenger apparatus (for determination of essential oil content), weighing scale (up to 25 Kg), water baths, oven with thrmostatic control and blower, hot drying rack, heating mantles of varying sizes (50 ml - 12l) with energy regulators, referigerator cum freezer, dehumidifier have been provided in QCL. Most of them are installed and now being used.

III. DEVELOPMENT OF STANDARD TEST PROCEDURES & SPECIFICATIONS FOR ESSENTIAL OILS PRODUCED IN DISTILLERY AND DEVELOPED IN LABORATORY

C S D is producing mainly clove stem and clove bud oils; sometimes lemongrass oil and basil oil (*Ocimum basilicum*- MREHANI) are produced on trial basis. Beside this experiments are being carried out on cultivation of vetiver (*Vetiveria zizanioides* Linn. Nash) and cinnamon (*Cinnamomum zeylanicum).* Vetiver roots, cinnamon leaves, basil leaves (3-types) and bitter orange leaves were distilled by expert. Earlier C T A had distilled cinnamon leaves, basil leaves, Ylang - Ylang flowers, bitter orange leaf & fruit, vetiver roots, *Ocimum sauve, O. canum, Artemisia camphorata,* cardamom, *Pogostemon plectranthoides* etc. All the obove essential oils have now been evaluated for their quality, following International, E. O. A., and well known standards.

These oils were evaluated for following physico - chemical constants, where ever quantity permitted :

1. Appearance	2. Colour
3. Odour	4. Relative density
5. Refractive index	6. Optical rotation
7. Solubility in alcohol	8. Acid value
9. Ester/saponification value	10. Eser value after acetylation
11. Carbonyl content	12. Phenol content etc.

Though G C analysis is not mandatory in specifications of most of the essential oils but standards for some of the oil give G C profile for information. G C profiles for all the essential oils of the project programme were developed to look into their real chemical composition. Trainees carried out the determinations after practicing standard procedures. Salient features of their specifications are as follows:

A. Clove Stem Oil

Samples from two lots were examined for their physico - chemical constants and compared with those of I S O and E O A standards as shown in table - 1. The oils met these specifications and colour, apppearance & solubility wise these are much better than other market samples. G C profiles were also developed as given in table - 1. There is a French suggestion to I S O to give G C profile for information. It is not mandatory for quantitative purposes. These oils met eugenol requirements (83 - 92 %) very well but gave little higher percentage of Beta - caryophyllene (10 - 11 %) than mentioned in French suggestion (4 - 8%) to I S O. Their suggestion for eugenyl acetate of 8-15 % does not seem to be right as no clove stem oil is found so far to have such a higher percentage of eugenyl acetate, perhaps there is printing error in their suggestion.

B. Clove Bud Oil

Again samples from 2 lots ('91 & '92) were examined for their properties and they compared very well with the specifications of I S O and B P as shown in table - 2. Both the samples gave similar results. According to G C profiles these samples showed lower percentage (7 %) of eugenyl acetate than French suggestion of 8 - 15 % and higher amount of Beta - caryophyllene (10 %) than suggested values of 2-7 %. This problem of lesser amount of eugenyl acetate was examined in detail and discussed in chapter - V.

C. Lemon Grass Oil

Lemon gras oil produced in distillery was *Cymbopogon citratus* Stapf and known as West Indian lemon grass oil in commerce. Its colour is lighter than other lemon grass oils and odour is more like fresh lemon than the intense odour of East Indian lemon grass oil. The oil meets all specifications of I S O and E O A including citrals content as described in table - 3. Its total citral content was determined by bisulphite, hydroxyl ammonium hydrochloride and G C methods (83, 82, 80 % respectively), values were higher than minimum requirement of 75. Its G C composition was found as shown below :

TABLE 1. SPECIFICATIONS OF CLOVE STEM OIL

S.No.	Physico-chemical constants and chemical composition	Pemba (ZSTC) Lot No. 60, March 92	Pemba (ZSTC) Lot of May 92	ISO 3143	EOA 178
1.	Appearance	Clear mobile liquid	Clear mobile liquid	Clear mobile liquid	-
2.	Colour	Light yellow	Light yellow	Yellow to brown; in contact with iron, the oil becomes dark purple brown	Yellow to light brown liquid. In contact with iron the oil acquires a purplish dark brown shade
3.	Odour	Spicy and characteristic of eugenol	Spicy and characteristic of eugenol	Spicy and characteristic of eugenol	Characteristic clove spice odour but less pleasant than the oil from the buds
4.	Relative density*	1.0482 (25°C)	1.0463 (25°C)	Min. 1.041 (20°C) Max. 1.059	Min. 1.048 (25°C) Max. 1.056
5.	Refractive index**	1.5330 (24°C)	1.5329 (24°C)	Min. 1.5310 (20°C) Max. 1.5360	Min. 1.5340 (20°C) Max. 1.5380
6.	Solubility in 70 % (V/V) ethanol	1 vol. clear soln.	1 vol. clear soln.	1:2 clear solution	1:2 + more volumes
7.	Optical rotation	- 1.03°	- 0.69°	-	± 0° to -1°30"
8.	Phenols content % (chemically V/V)	90	89	Min. 85 % Max. 95 %	Min. 89 % Max. 95 %
9.	Chemical components (%) (Gas chromatography profile)			French suggestion to ISO :	
	i. Eugenol	86.72	85.42	83 to 92	
	ii. Beta-caryophyllene	9.95	11.08	4 to 8	-
	iii. Eugenyl acetate	0.87	0.86	8 to 15 (?)	

* Temperature correction factor is 0.0006 per °C.
** Temperature correction factor is 0.0004 per °C.

TABLE 2. SPECIFICATIONS OF CLOVE BUD OIL

S.No.	Physico-chemical constants and chemical composition	Pemba Lot No. 8 'A', Dec. 7, 91	Pemba Lot No. 8 'B', March 3, 92	ISO 3142	B.P.
1.	Appearance	Clear mobile liquid	Clear mobile liquid	Clear mobile liquid sometimes slightly viscous	Liquid
2.	Colour	Light brown	Light brown	From yellow to clear brown; in contact with iron the oil becomes dark purple brown	Colourless or pale yellow, darkens with age or on exposure to air
3.	Odour	Spicy characteristic of eugenol and fruity	Spicy characteristic of eugenol and fruity	Spicy and characteristic of eugenol	Odour and taste of clove
4.	Relative density*	1.0478 (25°C)	1.0474 (25°C)	Min. 1.044 (20°C) Max. 1.057	Min. 1.041 Max. 1.054
5.	Refractive index**	1.5312 (24°C)	1.5311 (24°C)	Min. 1.5280 (20°C) Max. 1.5380	Min. 1.528 Max. 1.537
6.	Optical rotation	-0.68°	-0.58°	-1.50 to 0° (20°C)	-1.50 to 0°
7.	Solubility in 70 % (V/V) ethanol	1 vol. clear soln.	1 vol. clear soln.	1:2 volume (20°C)	1:2 volumes (20°C)
8.	Phenols content % (Chemically V/V)	90	89	Min. 85 % Max. 93 %	Min. 85 Max. 90
9.	Chemical components (%) (Gas chromatography profile)			French suggestion to ISO :	
	i. Eugenol	80.05	80.49	75 to 85	
	ii. Beta-caryophyllene	10.46	10.36	2 to 7	—
	iii. Eugenyl acetate	7.33	6.68	8 to 15	

* Temperature correction factor is 0.0006 per °C.
** Temperature correction factor is 0.0004 per °C.

TABLE 8. SPECIFICATIONS OF LEMON GRASS OIL (*Cymbopogon citratus* - Stapf)

S.No.	Physico-chemical constants and chemical composition	Pemba Lemongrass oil 06-10/3/92	ISO : 3217	EOA : 7 For West Indian Oil
1.	Appearance	Clear mobile liquid	Clear mobile liquid	—
2.	Colour	Pale yellow	Pale yellow to orange yellow	Light yellow to light brown or orange
3.	Odour	Refreshing lemon like with typical note of citral	Characteristic with strong note of citral	Lemon like but of lighter character than the East Indian oil
4.	Relative density*	0.8864 (25°C)	Min. 0.872 (20°C) Max. 0.897	Min. 0.869 (25°C) Max. 0.894
5.	Refractive index**	1.4850 (24°C)	Min. 1.4830 (20°C) Max. 1.4890	Min. 1.4830 (20°C) Max. 1.4890
6.	Optical rotation	-0.10°	-3° to + 1°	-3° to + 1°
7.	Carbonyl compounds content - as citral (%)			
	(i) **Chemically**			
	(a) Bisulphite method	83	-	Min. 75
	(b) Hydroxyl ammonium hydrochloride method	82	Min. 75	-
	(ii) **Gas chromatography**	80	-	-
8.	Solubility in 70 % (V/V) ethanol	Cloudy solutions in 70-95 % ethanol	Fresh oils soluble, solubility diminishes on storage, oil may become insoluble in 90 % ethanol	Cloudy solutions in 70, 80, 90 and 95 % ethanol

* Temperature correction factor is 0.0006 per °C.
** Temperature correction factor is 0.0004 per °C.

	%		%
Low boilers	7.59	Beta-caryophyllene	2.25
Methyl heptenone	0.74	*Citral 'b'*	**32.98**
Citronellal	0.62	*Citral 'a'*	**46.96**
Linalool	0.26	Neryl acetate	0.40
Linalyl acetate	0.20	Geranyl acetate	0.54
Isopulegol	1.53	Nerol	0.14
Unidentified	2.27	Geraniol	2.38
Beta-elemene	0.71	High boilers	0.20

Composition of this oil was compared with those of commercially available oils and newer varieties being developed elsewhere. East Indian lemon grass oil has limonene (2.9 %) while it is present in traces (0.20 %) in this oil. Geranyl acetate and Beta - caryophyllene are present in higher amounts (4.63 & 2.75 %) in East Indian oil.

A good quality lemon grass oil requires, higher amounts of citral a + b and lesser amounts of low boilers (monoterpenes), beta - caryophyllene, beta - elemene, geraniol and its esters.

The oil from Pemba meets all requirements and is clean in colour and odour (no bye odours) and has good potential, specially in flavour use.

D. *Ocimum basilicum* oil (MREHANI)

This is a domesticated plant used in folk medicine. It was attempted to cultivate it, as being a good source of methyl chavicol, used in perfume/flavour industry and its conversion into anethole. Oils were examined from two large scale distillations in 1991 & 1992. Oil has lavender top note followed by spicy anise note. Its physico - chemical constants were compared with those of given in French standard - Methyl chavicol type and E O A (Reunion type) as given in table - 4.

TABLE 4. SPECIFICATIONS OF OCIMUM BASILICUM OIL (MREHANI)

S.No.	Physico-chemical constants and chemical composition	Pemba oil 06/9/91	Pemba oil 5/92	NFT 75-357 [French] (Methyl chavicol type)	E O A 120 (Reunion)
1.	Appearance	Clear mobile liquid	Clear mobile liquid	Liquid	Liquid
2.	Colour	Light yellow	Light yellow	Light pale to light brown	Light yellow
3.	Odour	Characteristic spicy with anise note	Characteristic spicy with anise note	Characteristic spicy with anise note	Spicy, camphoraceous reminiscent of estragon
4.	Relative density*	0.9162 (25°C)	0.9182 (25°C)	Min. 0.948 (20°C) Max. 0.970	Min. 0.952 (25°C) Max. 0.973
5.	Refractive index**	1.4925 (24°C)	1.4932 (24°C)	Min. 1.5100 (20°C) Max. 1.5200	Min. 1.5120 (20°C) Max. 1.5190
6.	Optical rotation	-5.4°	-5.3°	-1° to +2°	0° to +2°
7.	Acid value	0.98	0.64	—	Not more than 1
8.	Saponification value	8	3	—	4-10
9.	Ester value after acetylation	113	112	—	25 - 45
10.	Solubility in 80 % (V/V) ethanol	1 volume	1 volume	7 volumes	4 volumes
11.	Gas chromatography profile (%)				
	i. Trans-Beta ocimene	2.23	2.63	0.9 - 2.8	
	ii. Linalool	33.69	32.65	0.5 - 3.0	
	iii. Methyl chavicol	51.57	53.70	75 - 85	—
	iv. Methyl eugenol	1.88	1.36	0.3 - 2.5	
	v. Eugenol	0.30	0.34	0.3 - 0.5	

* Temperature correction factor is 0.0006 per °C.
** Temperature correction factor is 0.0004 per °C.

Relative density, refractive index & optical rotation are lower while ester values are higher, which are supported by G C (Table - 4), showing about 32 % linalool. *Trans*- beta - ocimene, methyl eugenol and eugenol are comparable with values given by French standard for information. Methyl chavicol is lower (53 %) than suggested value of 75 - 85 %. This oil on fractionation can be of source for linalool and methyl chavicol. Variation in plant types has been discused in chapter - V.

E. Cinnamon Leaf Oil

One fresh leaf sample was distilled to give 1% oil yield (F W B, 1.8 % D W B) during stay of Q C C. This oil and one older sample meet most of the I S O specifications as shown in table - 5. Oil colour is lighter than most commercial samples. Its cinnamaldehyde content is from 1 to 2 % while maximum value in oils from different countries varies from 4 - 7 % analysed chemically. G C values of cinnamaldehyde in these oils are infact much lower. G C profiles of these two samples were compared with oils of different origin. Chemical composition of fresh sample was found as given below:

	%		%
Low boilers	5.11	Cinnamyl acetate	3.26
Cinnamaldehyde	1.32	Caryophyllene	0.98
Cinnamic alcohol	0.11	Eugenyl acetate	7.85
Eugenol	81.16	High boilers	Nil

This oil can find use in commerce.

F. Vetiver Root Oil

Vetiver roots, freshly dug from C S D farm were light brown in colour and 50-90 cm in length. These roots on hydro-distillation in lab. gave 0.6 % W/W (FWB) greenish yellow viscous oil. Physico chemical properties of oil were determined and given in table - 6.

These were compared with those of oils from Reunion & Haiti as specified in I S O : 4716 and Vetiver oil in E. O. A. Its properties

TABLE 5. SPECIFICATIONS OF CINNAMON LEAF OIL (*Cinnamomum zeylanicum* Blume)

S.No.	Physico-chemical constants	Pemba oil (1989)	Pemba oil (1992) 3-Years plants	I S O : 3524
1.	Appearance	Clear mobile liquid	Clear mobile liquid	Clear mobile liquid
2.	Colour	Yellowish brown	Light yellow	Reddish brown to dark brown
3.	Odour	Characteristic spicy, refreshing note of cinnamaldehyde	Characteristic spicy recalling that of cinnamaldehyde	Characteristic, spicy, recalling that of cinnamaldehyde
4.	Relative density (20/20°C)*	1.0426 (25°C)	1.0491 (25°C)	**Seychelles** : 1.042 - 1.059 **Srilanka** : 1.037 - 1.053 **South India** : 1.037 - 1.053 **Madagascar and Comores** : 1.035 - 1.055
5.	Refractive index (20°C)**	1.5358 (25°C)	1.5340 (25°C)	1.5300 to 1.5400
6.	Optical rotation (20°C)	- 0.15°	+ 0.14°	-2.5° to + 2°
7.	Solubility in 70 % (V/V) ethanol at 20°C	Soluble in 1 vol No opalescence in dilution	Soluble in 1 vol No opalescence on dilution	1:2 gives clear solution sometimes opalescence on dilution
8.	Phenols content (% V,V)	90	90	**Seychelles** : 85 - 95 **Srilanka** : 75 - 85 **South India** : 80 - 95 **Madagascar & Comores** : 70 - 95
9.	Carbonyl compounds content expressed as cinnamaldehyde (**Max %**)	2	2	**Seychelles** : 7 **Srilinka** : 5 **South India** : 4 **Madagascar & Comores** : 7

* Temperature correction factor is 0.0006 per °C.
** Temperature correction factor is 0.0004 per °C.

TABLE 6. SPECIFICATIONS FOR VETIVER OIL (*Vetiveria zizanioides* Linn. Nash)

S.No.	Physico-chemical constants	Pemba Oil (May, 1992)	I S O : 4716	E O A : 24
1.	Appearance	Viscous liquid	Viscous liquid	Viscous liquid
2.	Colour	Greenish yellow	Brown to reddish brown	Brown to reddish brown
3.	Odour	Characteristic, woody, earthy	Characteristic	Aromatic somewhat woody
4.	Relative density* (20/20°C)	1.0094 (25°C)	**Reunion** : 0.990-1.015 **Haiti** : 0.986-0.998	0.984-1.035 (25°C)
5.	Refractive index** (20°C)	1.5200 (24°C)	**Reunion** : 1.5220-1.5300 **Haiti** : 1.5210 1.5260	1.5200-1.5280
6,	Optical rotation	+ 25°***	**Reunion** : +19° to +30° **Haiti** : +22° to +38°	+15° to +45°
7.	Miscibility in 80 % (V/V) ethanol	1 vol No opalescence on dilution	Not more than 2 vols. slightly opalescence may sometimes appear	1 to 3 vols; sometimes slight turbidity upon further dilution
8.	Acid value (Max.)	18	**Reunion** : 35 **Haiti** : 14	-
9.	Ester value (Saponification value)	8 (26)	**Min** : 5 **Max.** 16	Saponification value 14 - 45
10.	Ester value after acetylation	155	**Reunion** : 115-145 **Haiti** : 120-165	110 - 165
11.	Carbonyl value (Molar. mass = 218)	37 (14 % ketones)	**Reunion** : 44-68 (17-26.5 % ketones) **Haiti** : 23-59 (9-23 % ketones)	-
12.	Primary and secondary free alcohol content (Molar. mass = 220)	65 %	**Reunion** : 21-50 % **Haiti** : 40 - 60 %	-

* Temperature correction factor is 0.00054 per °C.
** Temperature correction factor is 0.0004 per °C.
*** Specific rotation of 5 % ethanolic solution.

matched more with those of Haiti oil; i. e. lower acid, ester and carbonyl values and higher free alcohol (65 %) contents. Free alcohols are termed as vetiverol in commerce and its higher amount is preferred. Pemba vetiver root oil has more earthy & balsamic, woody odour but has slightly peppery top note. It has a longer lasting woody note.

Gas Chromatograms were run and compared with those of Indonesia, Haiti, Reunion, cultivated in south India and wild in north India. Percantages of components is given below in the table.

Rt (min)	%	Rt. (min)	%
3.74	0.22	**17.69**	**12.45**
6.74	0.10	**18.33** Khusinol (?)	**11.98**
9.54	0.51	19.48	3.23
11.13	3.22	20.68	5.56
12.13	1.06	**21.67** Khusimol	**16.27**
12.64	1.06	**23.76** Khusol	**12.30**
13.98	3.63	**25.11**	**8.38**
15.18	2.77	**26.02**	**6.72**
16.51	**7.05**	28.97	1.24

Vetiver oil is one of the most complicated oil and gives a complex chromatogram even on capillary columms. On comparison it was observed that G C of Pemba vetiver oil compares up to quite an extent with those of Haiti and cultivated Indian (South) vetiver oils. Main differnce is, that Pemba oil has clear distinct (Strong) additional peak at Rt. 18.33, which is absent in all other oils (Indonesian, Reunion, Haiti, Indian cultivated material), except in oil from wild vetiver materials in northern India. This may be checked by perfumer by its odour.

G. Ylang - Ylang flower oil

Three samples - whole oil (1990), Fraction - I & fraction II (1991) of ylang - ylang flower oils were examined and compared with I S O - 3063 specifications for oils from Madagascar and Comores as given in table - 7 (Annexure - 18). Whole oil of 1990 fits with the specifications of first fraction of madagascar oil. In the case of Fraction - I & II of 1991, their densities and refractive indices are higher but optical rotation & ester number of fraction I match with the specifications of extra & first grade materials of ISO specifications.

These three samples were further subjected to G C analysis and results were compared with the commercial samples of ylang-ylang oil. Four grades (Fractions) are available in Ylang - Ylang oil - extra, first, second and third as shown in table - 7 (Annexure - 18). For the sake of brevity G C composition of only fraction -I (1991) is depicted below:

	%		%
Low boilers	0.38	Methyl eugenol	1.26
Benzyl alcohol	1.29	Iso- eugenol-*cis*	4.74
Aldehyde C - 9	2.09	Iso-eugenol-*trans*/	
Linalool	13.64	beta-caryophyllene	5.22
Benzyl acetate	23.25	Methyl iso - eugenol	3.89
Anethole-*trans*	0.32	Eugenyl acetate	14.37
Thymol	1.50	Iso-eugenyl acetate	1.88
Eugenol	0.26	High boilers	23.29

This fraction has all the components of commercial sample, but quantity of peak isoeugenol/beta - caryophyllene is 50 % in commercial sample in comparison to 5.22 % in fraction - I. This component is not in large amount even in fraction II as well as whole oil. This fraction has good flowery and carnation odour.

H. Bitter orange oil

Bitter orange trees are quite common on Pemba Island. Earlier C T A distilled two materials ('89 & '91), now Q C C distilled one leaf sample (0.60% oil, FWB) and one fruit skin (0.4 %, F W B). Their physico-chemical constants are given in table - 8 and compared with values given in I S O : 8901. Fruit skin oil was altogether different (orange oil type). These samples were analysed on G C for their composition. These compared well with G C profiles provided in I S O : 8901 for Italian and Spanish bitter orange petitgrain oils. 1989 sample ranked first followed by 1992 and 1991 samples. Composition of 1989 sample was as given below in table:

	%		%
Alpha-pinene	0.04	Unidentified	0.35
Beta-pinene	0.34	*Linalool*	**28.55**
Myrcene	0.79	*Linalyl acetate*	**40.71**
Limonene	0.29	Terpinen -4- ol	tr.
1:8- cineole	0.74	*Trans*- beta - terpeneol	0.73
Gamma-terpinene/			
Beta-ocimene	0.28	*Cis*-beta terpeneol	0.17
Aldehyde C- 8	0.03	Alpha-terpeneol/	
		terpenyl acetate	9.56
Cis - 3- hexenol	0.04	Neryl acetate	2.80
Aldehyde C- 9	0.09	Geranyl acetate	5.43
Unidentified	0.36	Nerol	2.32
		Geraniol	5.94
		High boilers	0.28

TABLE 8. SPECIFICATIONS OF BITTER ORANGE PETITGRAIN OIL

S.No.	Physico-chemical constants	Pemba 19-19a/89	Pemba 28/91 Fruiting and flowering stage	Pemba leaf oil 5/92	Pemba fruit skin oil 11/5/92	I S O : 8901 (*Citrus aurantium* Linn. spp. *aurantium*)
1.	Appearance	Clear liquid	Clear liquid	Clear liquid	Clear liquid	Clear liquid
2.	Colour	Light yellow	Light yellow	Colourless	Colourless	Pale yellow to amber-yellow with a slight blue fluorescence
3.	Odour	Typical of petitgrain	Citrusy petitgrain	Petitgrain type	Orange style	Characteristic, ethereal and pleasant
4.	Relative density* (20/20°C)	0.8944 (25°C)	0.8778 (25°C)	0.8768 (25°C)	—	0.888 to 0.898
5.	Refractive index** (20°C)	1.4604 (24°C)	1.4622 (24°C)	1.4605 (24°C)	1.4692 (24°C)	1.4560 to 1.4720
6.	Optical rotation (20°C)	-	+ 2.84°	- 1.6°	-	-6° to +1°
7.	Miscibility with 70% (V/V) ethanol (20°C)	1.5 vols	2 vols	2 vols	Cloudy even upto 9 vols	Not more than 1.5 to 5
8.	Acid value	3.78	0.63	0.38	0.74	Max. 2
9.	Ester value	152	95	115	25	Min. 140; Max. 217
10.	Chromatographic profile (GC)	Very similar to ISO profiles	Somewhat similar to ISO profiles	Similar to ISO profiles	Quite different	It does not form an integral part of standard. GC prof. of Italian and Spanish oil

* Temperature correction factor is 0.0006 per °C.
** Temperature correction factor is 0.0004 per °C.

Top note of petitgrain oil is due to linalool and linalyl acetate, later is preferred more. Above sample of 1989 is comprised of 40.7% linalyl acetate and 28.55% linalool while that of 1991 shows 21.36 & 56.27 and 1992 shows 28.23 & 35.06% respectively. These oils, Italian and Spanish oils differ from petitgrain oil of Paraguay, the later has mostly similar components except an additional component Aldehyde- C- 9 from 9 to 22%.

I. *Ocimum sauve* (MTULE)

Ocimum sauve grows wild on Pemba Island and locally known as MTULE, was distilled earlier to give 0.4 to 1.25% (FWB) oil. This was examined as shown in table -9. Its physico - chemical constants are very similar to those of *O. gratissimum*- heavy oil (Russian Standard G O T - 9361-60). Its high content of phenol were supported by its G C composition showing eugenol 79 % & eugenyl acetate 4.25 %. It can serve a good source of eugenol and beta - ocimenes (8 %).

J. *Ocimum canum* (KIVUMBASI)

Ocimum canum plant is locally called as KIVUMBASI. Its oil was subjected to physico - chemical determinations (Table -10) showing high camphor content (40%). This was further confirmed by its G C compostition given in following table:

	%		%
Alpha-pinene	0.36	*Methyl chavicol*	**9.21**
Camphene	0.55	Anethole cis + trans	3.60
Beta - pinene	0.70	*Eugenol*	**4.67**
Myrcene	1.36	Beta-elemene	1.43
Cineole (1:8)	**18.71**	*Beta-caryophyllene*	**6.36**
Aldehyde C- 9	1.23	Humulene	1.34
Linalool	1.33	*Eugenyl acetate*	**3.95**
Camphor	**35.52**	High boilers	8.05
Terpenen-4-ol	1.00		

TABLE - 9

CHARACTERISTICS OF OIL FROM *OCIMIM SAUVE* (MTULE)

S. No.	Physico-Chemical constants	Characteristics	
1.	Appearance	Clear mobile liquid	
2.	Colour	light brown	
3.	Odour	Characteristic odour of eugenol in *Ocimum gratissimum* with top note of ocimenes	
4.	Refractive index at 24°C	1.5290	
5.	Optical rotation	-8°	
6.	Relative density (25°/25°C)	1.0211	
7.	Phenol contents (%) (V/V)	82	
8.	Solubility in 70% (V/V) ethanol	1 vol.	
9.	Gas Chromatographic profile		%
		Beta-ocimenes	**7.91**
		Linalool	0.44
		Terpinen-4-ol	0.12
		Methyl chavicol	0.57
		Eugenol	**79.08**
		Beta-elemene	1.46
		Beta-caryophyllene	1.71
		Eugenyl acetate	**4.25**
		High boilers	2.01

TABLE - 10

CHARACTERISTICS OF OIL FROM *OCIMUM CANUM* (KIVUMBASI)

S.No.	Physico-chemical constants	Characteristics
1.	Appearance	Clear mobile liquid
2.	Colour	Light brown
3.	Odour	Long lasting camphoraceous with top note of cineole and body of herbal note.
4.	Refractive index at 24°C	1.4810
5.	Optical rotation $[\alpha]^{25}_{D}$	+ 4.39° (C,1.14% ethanol)
6.	Solubility in 80% (V/V) ethanol	1 volume
7.	Carbonyl content calc. as camphor (Molar. wt. = 152)	40

Its major components are camphor, 1:8 - cineole, methyl chavicol, eugenol, beta-caryophyllene and eugenyl acetate. It has a long lasting camphoraceous and herbal note and can find use in perfumery formulations and cosmetics.

K. *Artemisia camphorata*

This domestic plant having light green lobed leaves, with high fragrance has been identified as named above. On distillation it gave 0.8 % blue oil. Its physico - chemical constants were compared with those of *Artemisia absynthium* (table - 11). Blue colour of oil is due to azulene, but it is some what different than chamazulene of Chamomilla oil. It gave following G C compostion :

	%		%
Thujene	1.06	Neral	0.58
Alpha-pinene	6.54	Geranial	0.30
Camphene	3.54	Thymol	2.90
Sabinene	1.98	Eugenol	0.71
Myrcene	1.47	Methyl eugenol	1.19
Cineole/Ocimene	1.30	Beta-caryophyllene	2.00
Aldehyde C- 9	5.21	Eugenyl acetate	1.25
Linalool/Thujone/			
Iso-thujone	4.83	Iso-eugenyl acetate	12.49
Camphor	31.69	Farnesol	2.52
Terpenen-4-ol	6.30	High boilers	4.42

Camphor is major component followed by eugenyl derivatives, aldehyde C- 9 and terpenen-4-ol. Later component may add to its antibacterial properties and can find use in place of chamomila oil in cosmetics and worm wood oil in flavour use.

TABLE - 11

CHARACTERISTICS OF *ARTEMISIA CAMPHORATA* OIL

(BLUE OIL)

S.No.	Physico-chemical constants	Characteristics	
		A. Camphorata oil	*A. absynthium* oil E.O.A. 114
1.	Appearance	Mobile liquid	Mobile liquid
2.	Colour	blue sometimes greenish blue	Dark brownish green, green or greenish blue
3.	Odour	Camphoraceous & herbal	Strong herbaceous
4.	Sp. gravity (25°C)*	—	0.908 to 0.939
5.	Refractive index (20°C)**	1.4762 (24°C)	1.4610 to 1.4770
6.	Ketone content (calc. as thujone)	30 %	34 to 71 %
7.	Solubility in 80 % (V/V) ethanol	1 vol, no cloudiness with additional ethanol	1 to 2 vols; cloudy with additional solvent

* Temp. correction factor is 0.0006 per °C.

** Temp. correction factor is 0.0004 per °C.

L. *Pogostemon plectranthoides*

Pogostemon plectranthoides plant grows all over the Island. It has purple flowers and leaves give sticky substance on rubbing. Its yellowish brown oil was distilled earlier from leaves + infloresence (0.11 % F W B & 0.48 % D W B) as well as from leaves (0.06 % F W B & 0.20 % D W B). Its physico-chemical constants were determined (table - 12). The oil has herbal, woody, slightly sweet fatty note.

It reminds slightsimiliarity withthe patchouli oil (*Pogostemon patchouly*). Its G C composition was as follows:

	%		%
Beta - pinene	1.99	Beta- elemene	1.29
Sabinene	4.64	Beta - patchoulene	0.71
Alpha-terpinene	1.15	*Beta - caryophyllene* +	
Limonene + cineole	5.41	*Alpha - guaiene*	**12.25**
Aldehyde C - 9	1.65	Seychellene +	
Linalool	2.28	Alpha - patchoulene	2.98
Camphor	4.05	Bulnesene	2.62
Aldehyde C - 10	**10.75**	*Delta - cadinene:*	**18.72**
Copaene	1.86	Pogostol	2.23
Eugenol	1.28	*Patchouli alcohol:*	**7.66**
		Unidentified	2.82
		Farnesol	4.68
		High boilers	3.51

This oil has all the basic components of patchouli oil but it has large proportion of low boilers (monoterpenic hydrocarbons),

TABLE - 12

CHARACTERISTICS OF OIL

FROM

POGOSTEMON PLECTRANTHOIDES

S.No.	Physico-Chemical constants	Characteristics
1.	Appearance	Clear mobile liquid
2.	Colour	Yellowish brown
3.	Odour	Herbal, earthy followed by heavy note, slightly sweet, oriental
4.	Refractive index at 24°C	1.5000
5.	Optical rotation $[\alpha]_D^{25}$	-3.88° (C,1.805 % ethanol)
6.	Solubility in 80 % (V/V) ethanol	1 Volume

delta-cadinene and very low proportion of patchouli alcohol (should not be less than 32 % in good patchouli oil). It lacks typical camphoraceous note of patchouli. It may find use in cheaper patchouli and oriental formulations.

M. Cardamom oil

Large amount of cardamom is grown on the Island. Oil was distilled earlier, now its G C analysis has been carried out and compared with high quality cardamom oil as given below:

Component	Pemba oil %	Standard oil %
Alpha-pinene	0.02	**1.29**
Camphene	0.005	0.33
Beta - pinene	0.16	**2.57**
Myrcene	0.05	0.74
Limonene	0.89	**2.10**
Cineole 1:8	**15.44**	**31.93**
Para - cymene	0.17	0.81
Unidentified	0.22	0.11
Unidentified	0.35	0.03
Unidentified	1.04	0.18
Linalool	**2.40**	**2.53**
Linalyl acetate	**2.01**	**4.37**
Beta-terpeneol	2.01	2.33
Unidentified	0.58	0.41
Terpenyl acetate	**65.43**	**45.25**
Neryl acetate	2.20	1.28
Geranyl acetate	1.21	0.39
Geraniol	3.76	2.80
High boilers	0.69	0.39

Components are similar in both the oils,main difference is that Pemba oil has lower concentration of alpha-pinene, beta-pinene, limonene, cineole, and linanyl-acetate; due to which top note is subdued. As the other components are less, naturally terpenyl acetate concentration has considerably increased. This needs further improvement in distillation.

N. Orange oil

Two oil samples from orange skin-one old & one fresh were analysed on G.C. These were rich in limonene (92. 61 & 89.40 %), Linalool (2.04 & 2.37 %), and beta-pinene (2.13 & 2.23 %), other components like aldehydes, esters were low. These samples were not appealing for flavour, cold expressed oils will be preferred.

IV. TRAINING OF THE COUNTERPART STAFF IN THE OPERATION OF INSTRUMENTS AND THEIR USE

Two trainees (Annexure-2) were given training in evaluation of quality of essential oils and their isolates in a newly developed QCL by UNIDO assistance. Mr. Slim Rashid Juma (Chemist) was given very intensive training covering all aspects of quality of essential oils and their isolates, while Mr. Abdalla Suleiman Haji (Lab. Technician) received training in general maintenance of lab, determination of essential oil content and their physico-chemical constants.

An intensive programme was made for installation of equipments and their starting. Principles of instruments and their functioning were explained to trainees. Manuals for operation were prepared for them. Ready guide line charts were hanged near instruments. After certain demonstrations trainees were asked to evaluate samples of oils produced in distillery and lab. (results are given in the text of this report). A highly specialized use oriented programme was developed for GC operation, development of parameters for different analysis on polar and non-polar columns and maintenance of GC equipment and allied accessories.

Trainees received training enthusiastically and they can now operate instruments like Refractometer, Polarimeter, Electronic balances, Thin Layer Chromatography and microprocessor controlled Gas Liquid Chromatograph by themselves. They are ranked as follows:

Mr. Slim Rashid Juma - First (85 out of 100 marks)

Mr. Abdalla Suleiman Haji - Second (40 out of 100 marks)

V. IMPROVEMENT OF THE QUALITY OF PRODUCTS AND PREPARATION OF VALUE ADDED PRODUCTS

A. Clove bud oil

It has been indicated in chapter III that while beta-caryophyllene content is high and eugenyl acetate is lower in clove bud oil samples (table - 2 p. 21). It has been observed by checking some of the old samples that some times samples are better and marginally come in the range of suggested limits of ISO. This problem was looked into and an experiment was designed in laboratory. Observations made are recorded in table - 13.

Fractions were removed after certain time intervals and oil % was recorded and GC composition was determined. Eugenol % was highest (92.4 %) in first fraction and lowest (10.9 %) in sixth fraction, while caryophyllene content increased considerabley from first (2.1 %) to sixth fraction (63 %). Eugenyl acetate also increased from first fraction (3.99 %) to fifth fraction (27.4 %) then sharply decreased in sixth fraction (9.9 %). Based on this experiment, aroma pattern of all the six fractions and French suggestion to I S O for G C profile, a formulation strategy was developed to make two combinations, i.e I - 1 to 5 fractions and II - 2 to 5 fractions were combined in the same ratio as they were collected from distilla tion. These two formulations I & II gave following G C profile:

Components	Formulation-I	Formulation-II
Low boilers	0.295	0.279
Eugenol	**79.664**	**76.028**
Beta-elemene	tr.	tr.
Beta-caryophyllene	**8.029**	**7.543**
Humulene	0.643	1.143
Eugenyl acetate	**13.098**	**14.746**
High boilers	0.270	0.262

TABLE 13. FRACTIONS OF CLOVE BUD OIL DISTILLATION IN LAB.

Clove buds used : 100 g.

Fraction	Time (Hrs.)	Oil %	G C COMPOSITION						
			Low boilers	Eugenol	Beta-elemene	Beta-caryophyllene	Humulene	Eugenyl acetate	High boilers
1.	2.5	3.2	0.8884	92.4904	-	2.1392	0.2652	3.9932	0.2234
2.	4.5	3.2	0.3381	89.8870	-	3.0586	0.3927	6.0600	0.2638
3.	7.0	3.6	0.2304	85.5360	-	3.8477	0.4006	9.7753	0.2100
4.	10.5	3.2	0.2391	77.4909	-	5.0377	0.5520	16.3966	0.2838
5.	22.5	3.6	0.3699	53.5560	0.0728	15.9281	2.2409	27.4104	0.4218
6.	32.0	1.2	4.4273	10.9333	1.0732	63.0368	8.1197	9.9289	2.4805
Total oil %		18							

The formulation-I is perferred over II, as having better fruity aroma and economy reason and discard of last fraction (6th) is only 6.5 % of total oil. The formulation-I is much better and fits very well for the requirement of I S O.

Earlier C T A had made cuts from clove bud oil distillation in distillery and these cuts have now been examined to give results as shown in table - 14. The composition of cuts compares well with those in table -13. Here again by excluding cut -4 (6 %) the quality of bud oil will improve considerably incomparison to present bulk sample, which is just within margin of I S O and often caryophyllene content is high which is undesirable.

B. Clove Stem oil

As this oil also has higher amounts of beta- caryophyllene (more than 8 %), which can be lowered by above procedure as in case of bud oil This will improve quality according to sugestion to I S O for stem oil (Table - 1)

C. Bud oil from different stages of bud growth

Oils distilled earlier by C T A from green bud, normal bud and Khokar (mature) bud, were examined for their composition (table -15). Two oils from green bud had variations in % of their composition. Normal bud oil again had higher caryophyllene content of 10.52 %. Khokar clove bud oil matches with I S O specifications with high % of eugenyl acetate (15.48 %), having mature clove odour, but lacking fruity odour of normal bud and green bud oils.

D. Black clove bud oil from final purification:

In the end of distillation during separation of oil, last traces of moisture are removed by steam under vacuum. During this process some portion of oil goes with steam water, this gets accumulated and becomes black in colour. Its composition (table - 15) shows that it has high content of low boiling compounds (5.57 %), caryophyllene (45.26 %) and low contents of eugenol (42.79 %) and eugenyl acetate (0.67 %). It has very strong fruity odour of clove bud. This portion on purification can give product useful in perfumery.

TABLE 14. CUTS FROM CLOVE BUD OIL DISTILLATION IN DISTILLERY

S.No.	Sample	Oil recovered distillation		Temp. of	Phenols	% (G C)			
		%	Hrs.	Still (°C)	Total	Eugenol	Caryo-phyllene	Humulene	Eug. acetate
1.	CB-1A	4.42	6	110	88	79.45	10.26	1.19	7.67
2.	CB-1B	4.42	11	100	92	84.01	7.63	0.91	6.77
3.	CB-1C	4.30	22	100	94	77.91	5.50	0.42	15.69
4.	CB-1-End	~1.0	27	100	85	45.70	16.22	2.09	34.63
5.	CB-1-Bulk	—	—	—	—	80.07	7.00	0.57	11.77

Total oil % 14.14

TABLE 15. G C COMPOSITIONS OF BUD OILS FROM DIFFERENT STAGES OF ITS GROWTH AND BYE-PRODUCTS OF DISTILLERY

S.No.	Product	Low boilers	Eugenol	Beta-elemene	Beta-caryophyllene	Humulene	Eugenyl-acetate	High boilers
1.	Clove bud oil normal	0.75	78.79	0.08	**10.52**	1.25	8.44	0.16
2.	Green clove bud oil-brown	**1.12**	83.25	0.06	7.78	0.95	6.76	0.08
3.	Green clove bud oil-light color	0.79	72.80	Tr.	3.09	0.41	**22.90**	0.01
4.	Khokar clove bud oil	0.46	75.42	0.07	7.89	0.65	**15.48**	0.03
5.	Clove bud oil (black) from purification tank	**5.57**	42.79	1.61	**45.26**	3.90	0.67	0.19
6.	Clove stem oil (black) from water storage tank	0.65	91.53	0.05	6.58	0.74	0.44	0.02

E. Black clove stem oil from water storage tank

After separation of oil, water distillate goes to a storage tank, where further oil gets accumulated but black in colour, so it cannot be mixed with main stem oil. This oil was examined for its G C composition (table -15) giving high percentage of eugenol (91.53 %). This can serve as a source of eugenol or be mixed with stem oil on purification.

F. Preparation of eugenol

As C S D is working mainly on clove products and some local plants, certain value added items can be prepared from raw materials available. One such compound is eugenol highly uesful in perfumery and flavour industry. Following methods have been developed in laboratory:

1. Eugenol from clove stem oil

Aqueous solution of potassium hydroxide (120 g in 1200 ml water) was added to clove stem oil (328 g) under stirring. This was further stirred for 30 min. at 90°C. Aqueous layer was separated and thoroughly washed with benzene. Aqueous portion then was acidified by dilute hydrochloric acid in cold. Non aqueous layer fromed was washed with water to make it neutral to pH paper, dried over anhydrous sodium sulphate. On G C analysis product was found to be 99.86 % pure. Yield of eugenol (287 g) was 97.22 % based on eugenol content of 90 % in clove stem oil. Non eugenolic portion can also find use.

2. Eugenol from black - stem oil of water storage tank

Above reaction was carried out on 328 g black oil with 112 g of potassium hydroxide to give 291 g of eugenol with a yield of 97 % (based on 91.5 % eugenol in black oil). GC showed its purity of 99.2 %.

3. Eugenol from cinnamon leaf oil

With the similar above method eugenol (4.3 g) was prepared from cinnamon leaf oil (5 g), with an yield of 98 % based on eugenol content of 89 % in cinnamon leaf oil. This eugenol can be used obly in high class colognes etc.

4. Eugenol from *Ocimum sauve* oil

Ocimum sauve oil (5 g) was reacted with KOH (2.2 g) in the same way as in above cases of 1, 2, 3 to give eugenol (3.89 g) with an yield of 91.6 %. Product was 99.83 % pure & clean in odour.

G. Eugenyl acetate from eugenol

Several methods were attempted to convert eugenol into eugenyl acetate. finally it (84 g) was reacted with acetic anhydride (65 g) at room temperature to give an yield of 97 % (99.5 g) of eugenyl acetate. G C showed its purity of 99.4 %. Eugenyl acetate is highly useful material for carnation odour in several perfumes.

H. Iso-eugenol from clove stem oil

Clove stem oil (50 g) was reacted with potassium hydroxide (31 g) at high temperature (slow reflux) for 4 hrs. Reaction product was diluted with water (100 ml). Non aqueous layer containing non-phenolic portion was removed. Aqueous portion was thoroughly extracred with benzene to remove any non phenolic portion. Auqueous portion was acidified as in the case of above 1 or 2 to get phenolic portion. This furnished 39.6 g product (yield 88 % based on 90 % eugenol in clove stem oil). G C showed the product to have 90 % iso-eugenol and 9.6 % eugenol (unreacted). The reaction can be further improved in a proper reaction vessel.

I. Basil Oil

As described in Chapter III - D, *O. basilicum* oil was low in methyl chavicol content (53%) in comparison to Madagascar/ Comores/Reunion type oils, but it has good amount of linalool

(32 %). Therefore on fractionation it can give natural linalool as well as methyl-chavicol, both are useful materials.

In order to search other plant types in natural population of *O. basilicum* following two types were noticed. These were distilled and oil analysed on G C :

Plant Characters	Oil% (F W B)	G. C. %			
		Ocimenes	Linalool	Methyl chavicol	Methyl eugenol
1. Leaf serrated but not wavy like normal material, green stalk	0.20	3.02	1.67	**89.29**	1.12
2. Leaf serrated but not wavy, purple stalk	0.24	0.85	1.32	**85.28**	0.71

These two materials have high content of methyl chavicol and low content of linalool and G Cs compare fairly well with that of Madagascar basil oil. In *Ocimum* natural crossing rate is very high and materials do get changed in nature, therefore better type material be grown vegetatively or away from other types. Of course this is certain that this type of material has very good potential.

J. Vetiver oil and Vetiveryl acetate

It has been described in chapter III - F that vetiver oil obtained from Pemba is unique having combination of Haiti and wild material of northern India, though having positive optical rotation (+ 25^0 unlike wild material having negative rotation).

This may be checked with perfumer, conversant with different types of vetiver oils. This oil shows highest content of free alcohols (65 %) in comparison to other oils of positive optical rotation. This fact was proved by preparation of its acetate and analysing its G C

composition, which showed that larger proportion of material got acetylated.

Acetylated oil has unique odour and this type of acetates are not available normally. Use of this may be further explored.

VI. MANPOWER REQUIREMENTS & TRAINING NEEDS

QCL has two persons (Chemist & Lab. Technician) working at present (Annexure - 2) and this meets the requirements completely. These persons have been given intensive basic training during this short period, right from scratch to G C operation and its use. Now they can handle daily operations by themselves, evaluate quality of distillery products and monitor quality during production.

To improve upon the skill further, chemist may be given exposure in well established laboratories in foreign country, so that he can sharpen the acquired techiques and knowledge. This will also help him to develop work culture in lab.

VII. MANAGEMENT SYSTEM & OPERATIONAL NEED

Q C L is now well equiped with all basic facilities required to give support to Distillery for monitoring quality of its products. Two persons can manage the laboratory. Q C L will require yearly $ 1000 for replenishment of chemicals, gases, broken glass wares, expenses on repair of equipments and for spares of instruments. Beside these, to improve Research & Development facilities for preparing value added products certain facilities like-5 1 reaction flask, product washing unit, fractional distillation unit with high vacuum pump are required. List of these items has been prepared and given as an Annexure-19. Their approximate cost will be around U S $ 4300.

Q C L also needs certain essential books and journals for reference, consultation and to keep abreast with the modern developments in the related area of work. A list has been given in Annexure—20.

ANNEXURE 1

JOB DESCRIPTION
DP/URT/86/026/11-54

Post title	:	Quality Control Chemist
Duration	:	2.5m/m (2 Weeks-I phase)
Date required	:	1989-90
Duty station	:	Chake-Chake, Pemba
Purpose of Project	:	Maximising the capacity of the Clove Distillery in Chake Chake
Duties	:	The expert's main duty will be the establishment of a modern laboratory for evaluation and grading of crops of essential oil bearing plants especially clove buds and stems and establishing methods for quality control, standardisation and certification of manufactured products, such as essential oils.

Specifically the expert will be responsible fo. :

- Design and layout of a small quality control laboratory;
- Istallation, start-up and operation of instruments;
- Compilation of manuals of operating instructions and routines for maintenance for instruments;
- Development of standard test procedures and specifications for products, such as clove oils;
- Estimation of manpower requirements and training needs;
- Development of management systems and estimation of operational need.

After tthe end of the mission the expert will be required to present to UNIDO a fully prepared report embodying his findings and recommendations.

Contd...

ANNEXURE 1A

JOB DESCRIPTION

DP/URT/86/026/11-54

Post Title	:	Quality Control Chemist
Duration	:	2.0 w/m (II Phase)
Date Required	:	April 1992
Duty Station	:	Chake Chake, Pemba
Purpose of Project	:	Maxmising the capacity of the Clove Distillery in Chake Chake
Duties	:	The expert is expected to carry out the following in collaboration with the CTA and the counterpart staff.

The expert's main duty will be the establishment of a modern laboratory for evaluation and grading of crops of essential oil bearing plants especially clove buds and stems and establishing methods for quality control, standardisation and certification of manufactured products, such as essential oils.

Specifically the expert will be responsible for :

— Design and layout of a small quality control laboratory;

— Installation, start-up and operation of instruments;

— Compilation of manuals of operating instructions and routines for maintenance for instruments;

— Development of standard test procedures and specifications for products, such as clove oils;

— Train the counterpart staff in the operation of instruments and their use for quality control;

— Develop methods for the improvement of the quality of products and production of value added products from clove stem and clove bud oils;

— Estimation of manpower requirements and training needs;

— Development of management system and estimation of operational need;

After the end of the mission the expert will be required to present to UNIDO a fully prepared report embodying his findings and recommendations.

ANNEXURE 2

NAMES OF TRAINEES :

1. Mr. Slim Rashid Juma	Chemist
2. Mr. Abdalla Suleiman Haji	Lab. Technician

ANNEXURE 3

INTERNATIONAL STANDARD (ISO)TC 54

S.No.	Reference	Ed.	Pages	Title
1.	ISO 770-1980	1	2	Oil of *Eucalyptus globulus*
2.	ISO 855-1981	1	2	Oil of lemon, Itlay, obtained by expression
3.	ISO 856-1981	1	3	Oil of Peppermint, France, Italy, United Kingdom and USA
4.	ISO 1202-1981	1	3	Essential oils-Determination of 1,8-Cineole content
5.	ISO 1279-1984	2	3	Essential oils-Determination of carbonyl value-Hydroxyl ammonium chloride method.
6.	ISO 3043-1975	1	1	oil of Pimento berry
7.	ISO 3044-1974	1	2	Oil of *Eucalyptus citriodora*
8	ISO 3063-1983	1	2	Oil of Ylang-ylang {*Cananga odorata* (Lamark) J.D. Hooker and Thomson}
9.	ISO 3064-1977	1	2	Oil of Petitgrain, Paraguay.

No.	Reference	Ed.	Pages	Title
10.	ISO 3140-1990	1	2	Oil of Sweet orange {*Citrus sinensis* (Linnaeus) obsbeck} obtained by mechanical treatment.
11.	ISO 3141-1986	2	3	Oil of clove loaf {*Syzygium aromaticum* (Linnaeus) Merrill et Perry Syn. *Eugenia caryophyllus* (*C. sprengel*) Bullock et Harrison}
12.	ISO/CD 3141-1990 Document in circulation	3	4	Oil of clove leaf {*Syzygium aromaticum* (Linnaeus) Merry and Perry Syn. *Eugenia Caryophyllus* (sprengel) Bullock and S.Harrison}
13.	ISO 3142-1974	1	2	Oil of clove bud.
14.	ISO/CD 3142-1990	2	4	Oil of clove bud {*Syzygium aromaticum* (Linnaeus) Merry and Perry syn. *Eugenia caryophyllus* (sprengel) Bullock and S. Harrison}
15.	ISO 3143-1975	1	1	Oil of clove stem
16.	ISO/CD 3143-1990 Document in circulation	2	4	Oil of clove stem

No.	Reference	Ed.	Pages	Title
17.	ISO 3215/1974	1	1	Oils of nutmeg
18.	ISO 3216/1974	1	2	Oils of Cassia
19.	ISO 3217/1974	1	2	Oil of Lemon-grass (*Cymbopogon citratus*)
20.	ISO 3518/1979	1	2	Oil of Sandal wood (*Santalum album* Linnaeus)
21.	ISO 3519-1976	1	2	Oil of lime, obtained by distillation
22.	ISO 3520-1980	1	2	Oil of bergamot, Italy
23.	ISO 3523-1976	1	2	Oil of Cananga
24.	ISO 3524-1977	1	2	Oil of Cinnamon leaf
25.	ISO 3528-1977	1	2	Oil of mandarin, Italy
26.	ISO/CD 3528-1989 Document in circulation	2	5	Oil of mandarin (Italian proposal)
27.	ISO 3757-1978	1	2	Oil of Patchouli
28	ISO 3809-1987	2	5	Oil of Lime, Mexico {*Citrus aurantiifolia* (Christmann) Swingle} obtained by mechanical means.
29.	ISO 3848-1976	1	2	oil of Java citronella
30.	ISO 4716-1987	1	5	Oil of Vetiver {*Vetiveria zizanioides* (Linnaeus) Nash}

S.No.	Reference	Ed.	Pages	Title
31.	ISO 4718-1981	1	2	Oil of Lemon grass. (*Cymbopogon flexuosus*)
32.	ISO 4729-1984	1	2	Oil of Pimento leaf [*Pimenta dioica* (Linnaeus) Merrill]
33.	ISO 4730-1993	1	4	Oil of *Melaleuca* ssp. *Melaleuca alternifolia Melaleuca Linerufolia, Melaleuca dissitiflora*, type terpineol-1-01-4
34.	ISO 4731-1978	1	3	Oil of geranium
35.	ISO 4732-1983	1	2	Rectified oil of *Eucalyptus globulus* Labillardiere, Portugal
36.	ISO 4733-1981	1	2	Oil of Cardamom
37.	ISO 4734-1981	1	2	Oil of mace
38.	ISO 7355-1985	1	4	Oil of Sassafras and nutmeg - Determination of safrole and *cis-* and *trans-* isosafrole content Gas chromatogrphic method on packed columns.
39.	ISO 7356-1985	1	4	Oil of thujone-containing artemisia and oil of sage (*Salvia officinalis* Linnaeus) Determination of α– and β-thujone content Gas chromatographic method on packed columns.

S.No.	Reference	Ed.	Pages	Title
40.	ISO 7359-1985	1	7	Essential oil- Analysis by gas chromatogrphy on packed columns-General method.
41.	ISO 7609-1985	1	7	Essential oils- Analysis by gas chromatogrphy on capillary columns-General method.
42.	ISO 7611-1985	1	5	Oil of lemon and petitgrain citronnier, and oil of lime obtained by a mechanical process-Determination of citral (neral+geranial) Content -Gas chromatographic method on capillary columns.
43.	ISO 8898-1991 Draft in circulation	1	4	Oil of mandarin petitgrain (*Citrus reticulata* Blanco)
44.	ISO 8900-1987	1	3	Oil of bergamot petitgrain [*Citrus aurantium* (Linnaeus) ssp bergamia (Weight et Arnott Engler]
45.	ISO 8901-1987	1	4	Oil of bitter orange petitgrain (*Citrus aurantium* Linnaeus ssp. aurantium)
46.	ISO 9776-1987 Draft proposed	1	8	Oil of *Mentha arvensis*, Partially dementholized (*Mentha arvensis* Linnaeus var. piperascence Malinvand
47.	ISO/CD 11020-1993 Draft in circulation	1	6	Essentiial oil of turpentine (*Pinus pinester* Ait)

ANNEXURE 4

SPECIFICATIONS OF ESSENTIAL OIL ASSOCIATION OF USA (E.O.A.)

S.No.	(Sec.II) Essential Oils	Specification Number
1.	Oil of petitgrain Paraguay	3
2.	Oil lemon grass	7
3.	Oil ginger	13
4.	Citronella oil	14
5.	Oil patchouli	23
6.	Vetiver oil	24
7.	Oil palmarosa	29
8.	Oil geranium Reunion	49
9.	Oil geranium algerian	53
10.	Oil clove leaf	55
11.	Cinnamon leaf oil	56
12.	Oil pimento leaf	73
13.	Oil cananga	75
14.	Oil limes distilled	78
15.	Oil ylang- ylang extra	81
16.	Oil cinnamon bark Ceylon	87
17.	Oil limes expressed	88
18.	Oil mandarin expressed	95
19.	Oil wormwood (Artemisia)	114
20.	Oil of basil (Reunion)	120
21.	Oil bitter orange	155

ANNEXURE 5

INDIAN STANDARDS

(Natural and Synthetic Perfumery Materials Sectional Committee PCD 18)

S. No.	IS No.	Title	Amendments
1.	326:1968	Methods of sampling and test for natural and synthetic perfumery materials.	
2.	326:1986	**Part II** : Methods of Test for natural and synthetic perfumery materials (Determination of carbonyl value and content of carbonyl compounds)	Revised (Second Revision)
3.	326:1986	**Part V** : Methods of Test for natural and synthetic perfumery materials (Determination of Refractive index)	Second Revision
4.	326:1986	**Part VI** : Methods of Test for natural and synthetic Perfumery materials (Deternination of solubility in ethanol)	Second Revision
5.	326:1980	**Part VII** : Methods of sampling and test for natural and synthetic perfumerly materials (Determination of Acid value)	Second Revision

S. No.	IS No.	Title	Amendments
6.	326:1980	**Part XII** : Methods of sampling and test for natural and synthetic perfumery meterials (Determination of Phenols)	Second Revision
7.	327:1961	Oil of Lemon-grass (East Indian Oil of Lemon-grass)	Second Revision Under revision Draft in print (1992)
8.	328:1957	Oil of *Eucalyptus globulus*	Second Revision Draft in print (1992)
9.	512:1988	Oil of citronella (Java)	
10.	526:1988	Oil of Palmorosa	
11.	587:1988	Oil of Geranium	
12.	761:1988	Oil of Ginger	
13.	1177:1969	Oil of Vetiver	
14.	1799:1981	Citral	
15.	1800:1989	Geraniol	
16.	1801:1988	Citronellol	
17.	1802:1975	Ionones	
18.	3124:1975	Terpeneols	Under Revision 1992.
19.	3180:1984	Linalyl acetate	
20.	3250:1982	Methyl ionone	
21.	3398:1965	Oil of Patchouli	

S. No.	IS No.	Title	Amendments
22.	3925:1980	Eugenol	
23.	6617:1972	Oil of Mandarin Orange, cold pressed	
24.	6698:1972	Oil of clove	
25.	6699:1989	Oil of cinnamon leaf	
26.	9257:1979	Oil of *Eucalyptus citriodora*	
27.	9784:1981	Tejpat Leaf Oil	
28.	13358:1992	**Part I:** Code of practice for cultivation of aromatic plants - Part I : Geranium	Draft in print (1992)
29.	13358:1992	**Part II** : Code of practice for cultivation of aromatic plants - Part II : Lemongrass	Draft in print (1992)
30.	13358:1992	**Part III** : Code of practice for cultivation of aromatic plants - Part III - Citronella (Java)	Draft in print (1992)

ANNEXURE 6

FRENCH STANDARD

Essential Oil	Specification number
Basil Oil-methyl-Chavicol type (*Ocimum basilicum* Linnaeus)	NFT 75-357

RUSSIAN STANDARD

Essential Oil	Specification Number
Ocimum gratissimum, oil	GOST 9361-60

ANNEXURE 7

BRITISH PHARMACOPOEA 1980 VOLUME 1

S.No.	Name of item	Page No.
1.	Cinnamon (Cinnamon bark, Ceylon cinnamon)	113-114
2.	Cinnamon oil	114
3.	Clove oil	119
4.	Eucalyptus oil	189
5.	Eugenol	189-190
6.	Lemon oil	252
7.	Terpeneless lemon oil	253
8.	Dried lemon peel	253
9.	Orange oil	317
10.	Terpeneless orange oil	317
11.	Dried bitter orange peel	317
12.	Terpencol	444
13.	Turpentine oil	469

ANNEXURE 8

BRITISH PHARMACEUTICAL CODEX 1973

S.No.	Name of item	Page No.
1.	Cineole	112-113
2.	Cinnamon	113-114
3.	Cinnamon oil	114
4.	Citronella oil	115
5.	Clove oil	118-119
6.	Eucalyptus oil	193
7.	Eugenol	193
8.	Lemon oil	264
9.	Terpeneless lemon oil	264
10.	Dried lemon peel	265
11.	Orange oil	339
12.	Terpeneless orange oil	339
13.	Dried bitter orange peel	339-340
14.	Terpeneol	494
15.	Turpentine oil	524-525

ANNEXURE 9

INDIAN PHARMACOPOEA 1966

S.No.	Name of item	Page No.
1.	Clove	195
2.	Clove powder	196
3.	Clove oil	196

ANNEXURE 10

LIST OF STANDARD CHEMICALS BROUGHT FOR PROJECT

1. Aldehyde C-8
2. Aldehyde c-9
3. Aldehyde C-10
4. Aldehyde C-12
5. Anethole
6. Benzaldehyde
7. Benzyl acetate
8. Benzyl alcohol
9. Bergamot oil (Italy)
10. Borneol
11. Cajeput oil (Vietnam)
12. Camphor
13. Cannaga oil
14. Cardomom oil
15. Carene delta-3
16. Carvone-L
17. Caryophyllene/β- elemene
18. Cineole-1:8
19. Cinnamic alcohol
20. Cinnamic aldehyde
21. *Cinnamomum cassia* oil (Vietnam)
22. Cinnamon bark oil
23. Cinnamon leaf oil (S.H.K.)
24. Cinnamyl acetate
25. *Cis*-3- hexenol
26. Citral
27. Citronella oil (S.H.K.)
28. Citronellal
29. Citronellol
30. Citronellyl acetate
31. Citronelly butyrate
32. Citronellyl formate
33. Clocimum oil
34. Clove leaf oil (Indonesia)
35. Dipentene
36. Ethyl Phenyl acetate
37. Ethyl Vanillin
38. *Eucalyputs citriodora* oil
39. Eugenol
40. Eugenyl acetate
41. Franesol
42. Geraniol (S.H.K.)
43. Geraniol (ex.Palmarosa oil)
44. Geraniol (ex. Citronella oil)
45. Geranium oil (Hyd.)
46. Geranyl acetate
47. Geranyl butyrate
48. Geranyl caproate
49. Geranyl formate
50. Geranyl iso-valerate
51. Ginger oil

52. Hydroxy citronellal (Pure)
53. Indian basil (*Ocimum basilicum*)
54. Ionone alpha
55. Ionone Beta
56. Iso-borneol
57. Iso- eugenol
58. Iso-eugenyl acetate
59. Iso-pulegol
60. Iso-safrole
61. Jamrosa oil
62. Lemon grass oil (LS-48) *C. flexuosus*
63. Lemon grass oil (CKP-25) Hybrid
64. Lemon grass oil (Vietnam)
65. Lemon grass (S.H.K.)
66. Lemon oil (Italian)
67. Lime oil (distilled)
68. Lime oil (Expressed)
69. Limonene
70. Linalool
71. Linalyl acetate
72. Mace oil
73. Mace oil (GCI)
74. *Mentha arvensis* (Shivalik)
75. Menthofuran
76. Menthol
77. Menthone/Isomenthone
78. Menthyl acetate
79. Methyl amyl ketone
80. Methyl anthranilate
81. Methyl chavicol
82. Methyl cinnamate
83. Methyl eugenol
84. Methyl heptenone
85. Methyl Ionone
86. Methyl iso-eugenol
87. Methyl salicylate
88. Nerol
89. Nerolidol
90. Nonyl alcohol
91. Nutmeg oil
92. Nutmeg oil (GCI)
93. Beta Ocimene *(cis+trans)*
94. *Ocimum basilicum* (Egypt-Linalool type)
95. *Ocimum basilicum* (Madagaskar)-Methyl chavicol type
96. *Ocimum basilicum* (0095, NBPGR, Methyl cinnamate type)
97. *Ocimum basilicum* (EC-174526, NBPGR - Linalool type)
98. ***Ocimum basilicum*** (EC-282721-NBPGR, Eugenol-Methyl chavicol type)

99. *Ocimum basilicum* (EC-291415-Methyl chavicol type)
100. *Ocimum basilicum* (Vietnam)
101. *Ocimum gratissimum* (Vietnam)
102. Orange oil (Brazil)
103. Palmarosa oil
104. Patchouli oil (NBPGR-Indonesian variety)
105. Patchouli oil (S.H.K.)
106. Perilla aldehyde
107. Perilla alcohol
108. Petitgrain oil
109. Petitgrain paraguay oil (GCI)
110. Pimento oil (berry)
111. Pine oil
112. Pinene-Alpha
113. Pinene- Beta
114. Piperitone
115. Safrole
116. Sandal wood oil
117. Sassafras oil
118. Sassafras oil (Vietnam)
119. Turpentine oil
120. Turpentine oil (Vietnam)
121. Terpinene-1-01-4
122. Terpinene-alpha
123. Terpineol-Alpha
124. Terpinyl acetate
125. Thymol
126. Vanillin
127. Vetiver oil (Haiti)
128. Vetiver oil (Indonesia)
129. Vetiver oil (NC 66404 NBPGR)
130. Vertier oil (NC-66416 NBPGR)
131. Vetiver oil (Reunion)
132. Vetiver oil (South Indian)
133. Vetiverol
134. Vetiveryl acetate
135. Ylang- ylang oil

ANNEXURE 11

LIBRARY OF GAS CHROMATOGRAMS OF STANDARD SAMPLES BROUGHT BY EXPERT

COLUMN SE -30 (NON POLAR)

1. Aldehyde C-8
2. Aldehyde C-9
3. Aldehyde C-10
4. Alpha-pinene
5. Anethole extra
6. Anise oil (DAB)
7. Anise oil (NBPGR)
8. Anisic aldehyde
9. *Artemisia camphorata* oil (Blue oil)
10. Benzaldehyde
11. Benzyl acetate
12. Benzyl alcohol
13. Beta- *cis* & *trans*- ocimenes
14. Beta-elemene and Beta-caryophyllene
15. Beta-pinene
16. Borneol
17. Camphor
18. Cananga oil
19. Cinnamic alcohol
20. Cinnamic aldehyde
21. *Cinnamomum cassia* oil (Vt)
22. Cinnamon bark oil (GCI)
23. Cinnamon leaf oil (Pemba)

24. Cinnamon leaf oil (SHK)
25. Cinnamyl acetate
26. Citronellal
27. Clocimum oil
28. Clove bud oil (Pemba-Fresh)
29. Clove bud oil (Pemba- old)
30. Clove bud oil (S.H. Kelkar, India)
31. Clove leaf oil
32. Clove stem oil (Pemba- fresh)
33. Clove stem oil (Pemba- old)
34. Cuminic aldehyde
35. Dipentene
36. Eugenol
37. Eugenyl acetate
38. Hydroxy citronellal
39. Sacred basil (*Ocimum sanctum*)
40. Iso- eugenol
41. Iso- eugenyl acetate
42. Limenene
43. *Litsea cubeba* oil (Vt)
44. Mace oil (G C I)
45. Mace oil (old)
46. *Mentha piperita* oil
47. Methyl eugenol
48. Methyl iso- eugenol
49. Methyl salicylate
50. *O. basilicum* (Egypt)-Linalool type
51. *O. basilicum* (Madagaskar)-Me- Chavicol type
52. *O. basilicum* (Mrehani-Pemba)- Methyl chavicol type

53. *O. basilicum* (NBPGR- EC 282721) Methyl chavicol -eugenol type
54. *O. basilicum* (NBPGR-0095) Methyl cinnamate type
55. *O. basilicum* (NBPGR-EC 174526) Linalool type
56. *O. basilicum* (Vt.)-Methyl chavicol type
57. *O. gratissimum* (Vt.)
58. *Ocimum sauve* (Pemba) (MTULE)
59. Patchouli oil (NBPGR)
60. Patchouli oil (SHK)
61. Pimenta berry oil
62. Safrole
63. Sandal wood oil
64. Sassafras oil
65. Star anise oil (Vt.)
66. Thymol
67. Vetiver oil (Bourbon-Reunion)
68. Vetiver oil (Haiti)
69. Vetiver oil (Indonesia)
70. Vetiver oil (NC 66403)
71. Vetiver oil (NC. 66404)
72. Vetiver oil (SHK)
73. Vetiver oil (South India)
74. Vetiverol
75. Vetiveryl acetate
76. Vanillin
77. Ylang-ylang oil

COLUMN CARBOWAX- 20 M (POLAR)

78. Aldehyde C-8
79. Aldehyde C-9
80. Aldehyde C-10
81. Aldehyde C-12
82. Anise oil

83. Be rgamot oil
84. Borneol
85. Cajeput oil
86. Camphor
87. Cardamom oil
88. Carene -delta-3
89. Citral (Neral + Geranial)
90. Citronellal
91. Citronella oil (Hyd)
92. Citronella oil (Vt.)
93. Citronelol
94. Citronellyl acetate
95. Citronellyl butyrate
96. Citronellyl formate
97. Coriander oil
98. *Cymbopogon jwarancusa* oil
99. *Eucalyptus citriodora* oil
100. Eucalyptus hybrid oil
101. Geraniol
102. Geranium oil
103. Geranyl acetate
104. Geranyl butyrate
105. Geranyl caproate
106. Geranyl formate
107. Ginger oil
108. Hexenol-cis -3
109. Ionone-Alpha
110. Ionone-Beta
111. Iso-bornyl acetate
112. Jamrosa oil

113. Lemon grass oil (C K P-25)
114. Lemon grass oil (Dist. Pemba)
115. Lemon grass oil(Lab.-Pemba)
116. Lemon grass oil (L S-48)
117. Lemon grass oil (SHK)
118. Lemon grass oil (Vt.)
119. Lime oil
120. Lime peel oil
121. Linalool
122. Linalyl acetate
123. *Litsea cubeba* oil
124. Mentha oil
125. *Mentha spicata* oil
126. Mosami 20 fold (*Citrus* sps.)
127. *O. canum* (KIVUMBASI)
128. Orange oil (Brazil)
129. Palmarosa oil
130. Petitgrain oil
131. Pinene-alpha
132. Pinene-beta
133. Pine oil-special (C A P)
134. *Pogostemon plectranthoides*
135. Terpinene- alpha
136. Terpineol-alpha
137. Turpentine oil (C A P)
138. Terpinyl acetate

ANNEXURE 12

List of useful literature from the book "Analysis of Essential Oils by Gas chromatogrpahy and Mass spectrometry by Yoshiro Masada" brought by expert.

1. Wormwood oil
2. Basil oil
3. Patchouli oil
4. Clove oil
5. Eucalyptus oil
6. Lemon oil
7. Orange oil
8. Lime oil
9. Petitgrain oil
10. Bergamot oil
11. Mandarin oil
12. Neroli oil
13. Geranium oil
14. Cinnamon oil
15. Sassafras oil Brazilian
16. Nutmeg oil and Mace oil (Myristica oil)
17. Cananga oil
18. Sandalwood oil
19. Cardamom oil
20. Citronella oil
21. Lemon grass oil
22. Vetiver oil

List of useful literature from the book "Essential Oils Analysis by Capillary Gas Chromatography and Carbon-13 NMR sepectroscopy by V. Formacek and K.-H. Kubeczka" brought by expert.

1. Bergamot oil
2. Cinnamon oil
3. Citronella oil
4. Clove oil
5. Dwarf pine oil
6. Eucalyptus oil
7. Geranium oil
8. Lemon oil
9. Lemon grass oil
10. Mandarin oil
11. Mint oil
12. Orange oil sweet
13. Petitgrain oil
14. Sassafras oil

ANNEXURE 13

OPERATION OF SHIMADZU GAS CHROMATOGRAPH MODEL 14 APTF, C-R6A CHROMATOPAC AND THEIR MAINTENANCE

I. OPEN THE CARRIER GAS FROM CYLINDER (N_2) :

1. Open main valve and second stage valve to set required pressure (5 kg/cm²), then open stop cock.
2. Open primary valve, then mass transfer valve and set desired flow rate values for both the channels.

II. SUPPLY HYDROGEN GAS AND AIR:

1. Turn on power switches of voltage stabilizer and stepdown transformer for the air compressor.
2. Open the stop valve of air copressor and set air pressure at 0.5 kg/cm² on a required channel (1/2) on G C machine by pressure regulator.
3. Open the main valve and second stage valve of Hydrogen gas cylinder, then open stop cock valve of hydrogen cylinder.
4. Open hydrogen pressure regulator on G C machine and set at 0.6 kg/ cm² on the channel required (1/2)
5. Ignition:
   - Press ignition button of required channel (1/2)
   - Ignite flame by lighter on F I D
   - Check flame by bringing glass slide to the relevant channel F I D top, look for moisture deposition.

III. SWITCHING ON THE EQUIPMENT:

1. Turn on main switch
2. Turn on servocontrolled voltage stabilizer

3. Turn on power switch on G C -14A

4. Put detector switch on

5. Set analysis programme with the help of keys

6. Turn on HEATER switch on G C- 14A

7. Press start button on G C -14A

8. Turn on Power on Chromatopac C-R 6A

9. Check the controls and peak processing parameters on C-R 6A

IV. INJECTION OF SAMPLES

1. Check two 'Ready' lamps on both G C-14A and C-R 6A

2. Inject sample

3. Press two 'Start' buttons on both G C- 14A and C-R 6A simultaneously on injection.

SETTING OF ANALYSIS PROGRAM ON GC-14A

Column oven temp. program :

- Initial temperature : Ex. 80°C

COL / AUX.I	I. TEMP.	8	0	ENT

- Time of retaining initial temp. Ex. 10 min.

COL / AUX.I	I. TIME	1	0	ENT

- Temp. program rate : Ex. 10°C/min.

COL / AUX.I	P. RATE.	1	0	ENT

- Final temp. of column : Ex. 180°C

COL / AUX.I	F. TEMP.	1	8	0	ENT

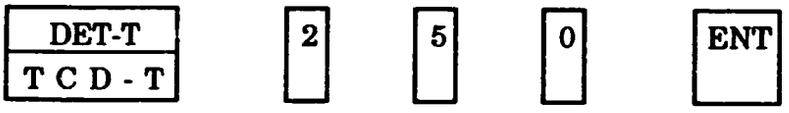
- Time of retaining final temp. : Ex. 30 min.

COL / AUX.I	F. TIME	3	0	ENT

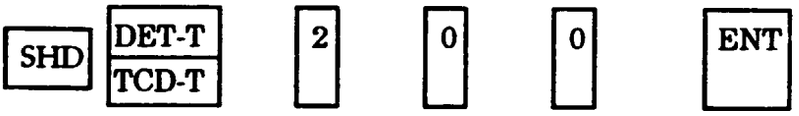
- Injector Temp. : Ex. 250°C

INJ / AUX.2	2	5	0	ENT

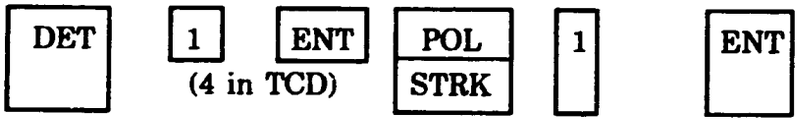
- Detector Temp. (FID) : Ex. 250°C



- Detector temp. (TCD if required) : Ex. 200°C



- Detector polarity : Ex. FID 1



- Range (only in case of FID) : Ex. 2

- Current (only in case of TCD) : Ex. 70

CONFIRMATION OF SET ANALYSIS PROGRAM

Press Key of desired program then press [ENT]

Fig. will be displayed

1. PROGRAM RATE

[COL / AUX.1] [PROG / RATE] [ENT]

2. INJ. TEMP.

[INJ / AUX.2] [ENT]

MONITORING OF ANALYSIS PROGRAM

Press MONIT then key of desired parameter

1. TEMP. MONITOR : Ex. column temp.

[MONIT] [COL / AUX.1]

2. RETENTION TIME MONITOR Ex. Retention time

[MONIT] [SHIFT D] [STW / RET.T]

SETTING OF PEAK PROCESSING PARAMETERS ON C-R6A CHROMATOPAC (PRINTER/INTEGRATOR)

1. **Turn on the power switch** - All lamps light and then flicker, if no defect "READY" lamp lights.

2. **Return of memory to initial values** -

   Keep pressing [SHD] Press [I] [N] [I] then [ENT]

3. **Check base line** -

   [SHD] [PLOT] [ENTER] Plotting begins

   If base line straight

   [SHD] [PLOT] [ENTER] Plotting ends

4. **Check signal level** -

   [PRINT] Keep pressing [CTRL] press [LEVEL] then [ENTER]

   Print out value of signal (μV) should be in the range of - 1000 ~ + 5000.

   If not, adjust by zero control of detector on GC machine (FID/ TCD)

5. [ZERO] [ENT] Putting pen at the origin.

6. **S. TEST** -

   [SHD] [S. TEST] [ENT]

   50 seconds noise test carried out and slope is printed.

   If zero point is out of the range of - 1000 ~ + 5000 (μV)

   "**WARNING LEVEL OUT OF RANGE**" is aprinted, if so, repeat operation of 4 (Zero adjustment).

7. **LEARNING OF SET PARAMETERS -**

[SHD] [LIST] [WIDTH] [ENT]

Following print out comes :

LIST WIDTH (0)

ANALYSIS PARAMETER FILE 0

WIDTH	5	SLOPE	70
DRIFT	0	MIN. AREA	10
T. DBL	0	STOP TM	655
ATTEN	0	SPEED	10
METHOD $	41	FORMAT $	0
SPL. WT.	100	IS. WT.	1

Values of above parameters can be changed according to requirements.

8. INJECT SAMPLE, [START]

9. **ANALYSIS REPORT -**

After chromatogram run is complete press [STOP]

Complete report of analysis is printed.

10. **EXCLUSION OF SOLVENT PEAK FROM DATA -**

[TIME PROG] [ENTER]

NEW FILE ? (Y/N) [SHD] [Y]

LINE PROGRAM - Ex. 0.01 to 3 min.

[0] [.] [0] [1] [SPACE] [SHD] [L] [.] [SPACE] [SHD] [0]

[SHD] [N] [ENT]

[3] [SPACE] [SHD] [L] [.] [SPACE] [SHD] [O] [SDH]

[F] [SHD] [F] [ENT]

[END] [ENT]

CONFIRMATION OF TIME PROGRAM -

[SHD] [LIST] [TIME PRG] [ENT]

TIME	PROGRAM	FILE	0
0.01	L. ON		
3	L.OFF		

Maintenance of G C and Integrator

G C EQUIPMENT

1. Cleanliness of place and instrument is most esssential.
2. Clean connections- tie electrical cables properly to look neat.
3. Keep Instruction charts handy.
4. Keep carrier gas flowing from begining to end in both columns till oven and T C D temp, arrive at room temp.
5. F I D Flame should be ignited immediately after opening hydrogen.
6. Before injecting sample, parameters on machine, flame and ready lamp are to be checked.
7. Check septa of Injectorport periodically as crack may cause leakage of carrier gas.
8. Syringe should be cleaned with solvent few times and to be rinsed several times with material to be injected. No air bubble, Keep syringe straight (necessary for longer life of septa and syringe)
9. Synchronisation of injection with timer (start)
10. After run is over pl. check baseline is straight and column is clean otherwise, keep carrier gas running till baseline is straight.
11. After shutting of machine, please check that hydrogen cylinder main valve is closed and carrier gas (N_2) to flow till oven and T C D temp. arrive at R.T.
12. Periodically columns are to be checked for leak test.
13. Oven to be kept clean without foreign particles or soap solution.
14. Injection port to be checked after certain use for brocken septa as well as sleeve to be cleaned otherwise there will be obstruction in carrier gas flow.

15. While changing column please check that silanised glass wool is in both the ends of column to prevent loss of packing material.

16. Keep column temp. 25° below the recommended maximum temp. of particular column.

17. Detectors (FID) : Not easily contaimnated, easy to clean but chloroform use is corrosive. After prolonged use, clean jet tube and collector with the solvent methanol - acetone and by brush provided, jet nozzle should not get damaged.

INTERGRATOR (Chromatopac C-R 6A)

1. Be sure to ground main body.

2. Once power is turned off, wait at least three seconds before turning on power again.

3. Be sure to load chart paper after the "Ready" lamp lights. Learn about operation and panel keys from Instruction Manual (p.24-27).

4. Before turning off power, be sure to stop everything in operation such as analysis, time program and BASIC program.

ANNEXURE 14

LIST OF GAS CHROMATOGRAMS OF ESSENTIAL OILS AND ISOLATES CARRIED OUT AT C S D

ESSENTIAL OILS:

1. *Artemisia camphorata* Oil
2. Bitter Orange fruit peel Oil
3. Bitter Orange leaf oil
4. Cardamom Oil (2)
5. Cinnamon leaf Oil (3)
6. Cinnamon leaf oil-non eugenolic portion
7. Citronella Oil (India)
8. Clove Bud Oil (6)
9. Clove bud oil (cuts from different stages in distillery)
10. Clove bud oil (cuts from lab.) (6)
11. Clove bud oil (green)
12. Clove bud oil (khokar cloves)
13. Clove bud oil (dark) from purification tank (oil going with steam)
14. Clove stem oil (5)
15. Clove stem oil (dark) from water storage tank (after removal of main oil)
16. Lemon grass oil (5)
17. Lemon grass oil without citral
18. *Ocimum basilicum* (11)
19. *O. canum* (Kivumbasi)
20. *O. sauve* (Mtule)
21. Orange oil
22. Palmorasa oil (India)
23. Patchouli oil (NBPGR)
24. Petitgrain oil (3)
25. Pine oil (India)
26. *Pogostemon Plectranthoides* (2)
27. Vetiver Oil (8)
28. Ylang Ylang (2)
29. Ylang Ylang fractions(2)

ISOLATES/CHEMICALS:

30. Aldehyde C-8
31. Aldehyde C-9
32. Aldehyde C-10
33. Alfa- Terpenecl
34. Beta- Ocimenes-*cis* & *trans*
35. Beta- pinene
36. Borneol
37. Camphor
38. Cineole 1:8
39. Cinnamyl acetate
40. Cis-3 - hexenol
41. Citral
42. Eugenol (5)
43. Eugenyl acetate(5)
44. Farnesol
45. Iso-eugenol
46. Iso-eugenyl acetate
47. Limonene
48. Methyl salicylate
49. Terpenyl acetate
50. Thymol
51. Vetiveryl acetate

ANNEXURE 15

OPERATION OF ABBE REFRACTOMETER
(Bellingham + Stanley Model 60)

* Put on light of source lamp for prism or use window light.
* Put on scale illumination lamp provided in refractometer.
* Open prism box by releasing the toggle on the right hand side and swinging the hinged box to the left.
* Open shutter of lower prism by turning control knob in clockwise direction.
* Sliding shutter infront of hinged prism box should be opened approx. 10 mm from bottom and locked by two clamp screws.
* Transfer test liquid (few drops to the prism surfacing, using a dropper or pipette and not rod/ spatula and close prism box.
* With the eye at the field telescope turn the control knob to a positon where the observed field divided into light and dark portions.
* Remove color by means of the dispersion drum in order to obtain contrasty colourless border line.
* Take reading on illuminated scale.
* Open prism box, clean sample with the help of hexane, dry, close prism box and both shutters.
* Put off both lamps.
* Do not scratch prism surface (Most Important)
* Clean off samples as soon as measurements are concluded.
* Drain off water if used in prism jackets.

* Carry instrument by handle.

* Keep instrument covered.

* Calibration can be made by double distilled water or monobromonaphthalene once in a while. Adjustement to the zero if required can be made by a screw concealed beneath the khurled cap on the right hand side of the instrument.

ANNEXURE 16

OPERATION OF POLARIMETER (BELLINGHAM + STANLEY MODEL-D)

1. Put on sodium lamp, wait till illumination is bright yellow.
2. Keep lamp in the axis of instrument and about 12cm from the end.
3. Focus top eye piece to see scale and index line.
4. Observing through the scale telescope, turn control wheel (on Rt. side) until scale reads 180° on the upper scale.
5. Transfer the eye to the field telescope and focus to obtain a circular disc of light.
6. By means of control wheel, move the scale a few degrees about the position of 180°, observe field dibvided into two halves, a position will be found that gives equal intensity. This is balance position used in all measurements.
7. Take clean blank tube (100 mm/ 50 mm) with glass windows and caps and observe blank reading as seen in -6.
8. Fill up with desired esential oil and take reading like-6 or 7.
9. Optical rotation will be difference of two readings. If reading is on lower side of blank reading, then material will be *laevo*-rotatory (-), if it is on higher side, it will be *dextro*-rotatory (+). If reading is taken with 50 mm. tube then multiply by 2.
10. In case of viscous liquids and soilds determinations are made in solution (per cent dilution in a particular solvent). In such cases blank reading is taken with solvent alone then with solution and optical rotion is calulated as specific optical rotation by following formula :

$[\alpha]_D^t$ = $[(100 \times \theta) / (l \times c)]$

$[\alpha]_D^t$ = Specific rotation on sodium D- line & room temp. that time

θ = difference of rotation (blank and with solution)

l = Length of tube in decimeter

c = Number of gms. of substance in 100 ml of solution.

11. Taking reading **Ex. : between 177° and 178°**

First examination shows reading between 177°and 178° upon the angular scale. Turn the micrometer drum (left side of polarimeter) to bring index line coincident with the scale 177° division. The drum then suppose reads 0.75 (on left hand scale). True determination is therefore 177.75°.

12. Filling up of tube

Clean tube and side windows, dry them, on one end put glass window, washer and screw the cap gently. Keep it standing vertically on side of cap, fill the desired solution with the help of long dropper untill a meniscus appears bulging above the tube end. Slide the window from the side over the tube end with one movement and centralise without tilting. The cap then be screwed down gently without breaking windows. Avoid air bubble if there is no bubble trap in the tube.

13. Keep the tube, side windows, washers and screw caps clean and store them in a box so it does not roll down.

14. Keep instrument covered in order to avoid dust on optics.

ANNEXURE 17

OPERATION OF THIN LAYER CHROMATOGRAPHY

1. Preparation of slurry and its application on plates

Mix the adsorbent e.g. silica gel (50 g) and water (110 ml ± 10 %) and shake in a conical flask (500 ml). The slurry neither be too mobile nor too viscous. Place the T.L.C. plates (20 X 20 cm) on the base plate of applicator and place the hoopper on it and fix the gate with the help of a desired gauge blade. Pour the slurry into the hopper. Push the glass plate quickly by the finger in 5 seconds and keep coating other plates till slurry is exhausted.

Put the coated plates on the bench for air drying.

2. **Activation:** Put plates in oven at 110°C for 30 Min. Take out and store in closed chamber or desicator for future use.

3. **Spotting :** Spot the samples dissolved in a solvent by using microcapillary tubes on the base-line (2 cm from bottom) at an interval of 1.5 to 2.0 cm. Put a straight line at 2 cm from top.

4. **Development :** Place the T.L.C. plate in the flat bottom chamber containing suitable solvent system and saturation pads. Check the level of the solvent (1 cm from bottom of chamber). When approaching the front line, take out the plate from solvent chamber.

5. **Detection of Spots :** Following methods can be used:

   (i) U.V. light at 336 or 254 nm (specially when adsorbent used is mixed with G F 254).

   (ii) Iodine vapours in chamber.

   (iii) Suitable reagent spray e.g. vanillin in conc. sulphuric acid, heating the plates in the oven at 100°C or spray of any suitable reagent specific to compounds.

6. Calculation of Rf. values

After drawing the picture, the Rf. values of individual spots are calculated as follows:

$$Rf = \frac{\text{Distance moved by the spot from base line}}{\text{Distance moved by the solvent up to solvent front}}$$

7. Record Rf. values for identification purpose.

ANNEXURE 18

TABLE 7. SPECIFICATIONS OF YLANG-YLANG OIL (*Cananga odorata*)

S.No.	Physico-chemical constants	I Fraction 13.09.91 20/91	II Fraction 13.09.91 20/91	Whole Oil 31/90	ISO : 3063					
					Country	Limits	Fractions Extra	First	Second	Third
1.	Appearance	Liquid	Liquid	Liquid			Liquid	Liquid	Liquid	Liquid
2.	Colour	Slight yellow	tinge of yellow	Slight yellow			—— Pale yellow to Dark yellow ——			
3.	Odour	Flowered, recalling jasmine	Flowered, recalling jasmine	Flowered recalling jasmine			—— Characteristic, flowered and recalling jamine -----			
4.	Relative density (20/20°C)*	0.9845 (25°C)	0.9721 (25°C)	0.9436 (25°C)	Madagascar	Min.	0.950	0.933	0.923	0.906
						Max.	0.965	0.945	0.929	0.921
					Comores	Min.	0.956	0.940	0.926	0.906
						Max.	0.976	0.950	0.936	0.921
5.	Refractive index (20°C)**	1.5102 (24°C)	1.5190 (24°C)	1.5040 (24°C)	Madagascar	Min.	1.5010	1.5000	1.5050	1.5060
						Max.	1.5090	1.5100	1.5110	1.5130
					Comores	Min.	1.4980	1.5000	1.5050	1.5070
						Max.	1.5060	1.5090	1.5100	1.5110
6.	Optical rotation (20°C)	-35.4°	-	-29°	Madagascar		-45° to -36°	-44° to -28°	-55° to -40°	-63° to -49°
					Comores		-40° to -25°	-46° to -38°	-55° to -42°	-63° to -49°
7.	Acid value	1.17	0.87	3	Madagascar Comores			Under 3		
8.	Ester value	134	71	107	Madagascar	Min.	125	90	65	38
						Max.	160	120	80	58
					Comores	Min.	145	110	75	45
						Max.	185	140	100	70

* Temperature correction factor is 0.0006 per °C. ** Temperature correction factor is 0.0004 per °C.

ANNEXURE 19

LIST OF CHEMICALS/ SOLVENTS, GLASS WARES, EQUIPMENTS, SPARES & MISCELLANEOUS ITEMS

A. CHEMICALS

S.No.	Name	Quantity	Price (US $)
1.	Acetic anhydride	10 x 500 g	60.00
2.	Acetone	2 x 2.5 l	25.00
3.	Benzene (commercial grade)	1 x 30 l	140.00
4.	Benzoic acid	2 x 500 g	18.0
5.	Chloroform	1 x 2.5 l	23.00
6.	Diethyl-ether	10 x 500 ml	59.00
7.	Dimethyl aniline	2 x 500 ml	18.00
8.	Dimethyl sulphate	5 x 500 ml	25.00
9.	Ethanol (95%)	4 x 2.5 l	120.00
10.	Ethyl acetate	4 x 500 ml	27.00
11.	Ethylene glycol monomethyl ether	2 x 500 ml	18.00
12.	Ethylene glycol dimethyl ether	2 x 500 ml	20.00
13.	n-Hexane	2 x 2.5 l	100.00
14.	Iodine	1 x 100 g	5.00
15.	Methanol	2 x 2.5 l	20.00
16.	Para- toluene sulphonic acid	1 x 500 g	5.00
17.	Phosphoric acid	1 x 500 g	4.00
18.	Potassium hydroxide pellets	10 x 500 g	40.00
19.	Silver nitrate	1 x 10 g	20.00
20.	Di-sodium di-sulphite	4 x 500 g	14.00
		Total $	761.00

B. GLASS WARES

S.No.	Name	Quantity	Price (US $)
1.	Clevenger-for determination of volatile oil-1000 ml flask with 34/35 socket, oil separatory tube with 34/35 cone and condenser.		
	(a) For lighter than water	2	400.00
	(b) For heavier than water	3	600.00
2.	Divided adapter- two way- cone 24; sockets -24 two on divided arms.	1	11.00
3.	Reaction unit-51, 3-necked with standard joints bottom opening with teflon stopcock, thermometer pocket, air leak, teflon stirrer with padles, glands(without motor).	1	100.00
4.	a. dropping funnel 500 ml with 24 cone & 24 socket and equaliser arm.	1	25.00
	b. ---same--- 100 ml with 19 cone & 19 socket and equaliser arm.	1	20.00
5.	Flask -1000ml -3-necked, Central 29, sides 24 &14 sockets.	1	25.00
6.	a. Guard tubes- 24 cone	2	15.00
	b. ---same-- 19 cone	2	12.00
	c. --same-- 14 cone	2	10.00
7.	Measuring cylinders with stopper		
	(a) - 100 ml	2	30.00
	(b) - same 50 ml	2	20.00
	(c) - same -25 ml	2	10.00

S.No.	Name	Quantity	Price (US $)
8.	Distillation flask - 51, 3- necked standard joints with fractionating columns- two (30 cm & 90 cm long, 5 cm- diam.) filled with helices, a perkin triangle, with fractions receiver and stopcocks to keep under vacuum.	1	200.00
9.	Reduction adapter cone 60, socket 42	2	10.00
10.	vacuum adapters with bent		
	(a) Cone 24, socket 24	2	16.00
	(b) Cone 19, socket 24	1	5.00
	(c) Cone 14 socket 19	1	5.00
11.	Volumetric flask with stoppers		
	a. 10 ml	10	20.00
	b. 5 ml	10	20.00
	c. 2 ml	5	15.00
12.	Washing unit : 10L flask, 3-necked, arrangement for stirring like in Reaction Unit (No.3), with bottom opening by teflon stopcock.	1	100.00
		Total	1669.00

C. EQUIPMENTS

S.No.	Name	Quantity	Price (US $)
1	Energy regulators (electrical) for water baths	3	100.00
2.	Mcleod gauge for vacuum		
	(a) 0 to 1 mm	1	100.00
	(b) 0 to 10 mm	1	100.00
3.	High vacuum pump - two stage for vacuum up to 0.1 mm with a displacement of air 50L per min.	1	500.00
		Total	800.00

D. SPARE PARTS FOR G C

S.No.	Name	Quantity	Price (US $)
1.	Thermal paper Chart rolls for Shimadzu C-R6A Chromatopac (Part 221-25412)	10	200.00
2.	Fuses for chromatopac C-R 6A 0.5 A/250V(P/N 072-016 52-13)	5	5.00
3.	(a) Syringes 1 microlitre	2	50.00
	(b) Syringes 10 microlitre	2	
4.	Thermalhead for C-R 6A (P/N 221-25362-91)	1	60.00
5.	Servo-stabilisers for G C, Chromatopac, hydrogen generator & air compressor 2 KVA, 220 volts/50 cycles, voltage correction 35 volts/second.	2	500.00
6.	Hydrogen generator OPGU- 1500 S Deffective supply- Shimadzu	1	Defective supply
7.	Left Injection Port of G C-14 APTF Shimadzu- defective (Part no. 221-29280-93)	1	Defective Supply
		Total	815.00

E MISCELLANEOUS ITEMS

S.No.	Name	Quantity	Price (US $)
1.	Pressure rubber/latex tubings for high vacuum (10 mm-I.D.)	20m.	80.00
2.	High vacuum silicone grease 50 g	4	40.00
3.	pH (1-14) paper books packets	4	20.00
4.	Glass sample bottles with plastic stopper and screw cap.		
	(a) 3 ml capacity	100	20.00
	(b) 10 ml capacity	100	35.00
	(c) 25 ml capacity	100	50.00
5.	Rubber Vacuo pads to suck solutions in pipette	2	10.00
		Total	255.00
		Grand Total:	**US $ 4300**

ANNEXURE 20

LIST OF BOOKS & JOURNALS SUGGESTED FOR QUALITY CONTROL LABORATORY

Books :

1. Modern Practice of Gas Chromatography by R. L.Grob. Pub.: John Wiley & Sons Inc.

2. Analysis of Essential Oils by Gas Chromatography and Mass spectrometry by Yoshiro Masada. Pub.: Halsted Press Book, J. Wiley & sons, Inc. New york (1976).

3. Essential Oil Analysis by Capillary Gas Chromatography and Carbon- 13 N M R spectroscopy by V. Formacek and K-H. Kubeczka, J. Wiley & Sons, New York (1982).

4. The Essential Oils. vols I- VI by E. Guenther. Pub.: D.van Nostrand & Co., Inc. New york.

5. Essential Oils Vols I- III By B.M. Lawrence. Pub. : Allured Publishing, P.O. Box 318, Wheaton, Illinois 60189 U.S.A.

6. Handbook of Chemistry and Physics. Pub.: Chemical Rubber Co. 18901 Cranwood Parkway, Clevland, Ohio 44128,U.S.A.

7. Merck Index. Pub. : Merck and Co., Inc., Rahway, N.J. 07065, U.S.A.

8. Practical Organic Chemistry by A.I.Vogel.

9. Thin Layer Chromatography by E. Stahl.

JOURNALS :

1. Perfumer and Flavorist (Bimonthly). Allured Pub. Corp. P.O.Box 318, Wheaton, Illinois 60189-0318 U.S.A.

2. Journal of Essential Oil Research. Alllured Publishing 214 W. Willow Av., P.O.Box 318 Wheaton, Ill. 60189-0318 U.S.A.

3. Flavour & Fragrance journal (Quarterly) John Willey & Sons Ltd., Baffins Lane Chichester, Sussex , P.O. 19 1 UD, England.

4. Indian Perfumer (Quarterly). Essential Oils Association of India (Gen. Secretary) Dua Complex 24- Veer Savarkar Block Shakarpur, Vikas Marg, Delhi-110092, INDIA.

T. De Silva/sk
18 November 1992

Backstopping Officer's Technical Comments
based on the work of Mr. M.L. Maheshwari
DP/URT/86/026/11-54

Mr. Maheshwari has prepared a comprehensive report detailing the activities carried out by him during a short mission. He has commissioned all the analytical equipment and trained the counterpart staff on their use. Manuals for operation and maintenance of equipment compiled by him will be of great assistance in protecting the equipment. Standard test procedures and specifications for essential oils produced have been developed. The preparation of some value added products and the improvement of the quality of essential oils have been demonstrated. Specific recommendations have been made about the recovery of valuable by-products from black oil residues, which we hope would be followed up. The recommended spare parts, glassware, chemicals and books are being ordered.

Mr. Maheshwari has gifted 135 reference samples, a library of 138 standard chromatograms and some relevant literature to the distillery. Diversification of activities has been recommended, which would necessarily require the services of an agronomist for initiating systematic cultivation.

The expert has very successfully discharged the duties given in the job description.