

**Daniel Bisrat
Solomon Abate
Wossen Kebede**

Technical Manual 23

Laboratory Manual for Plant Products Analysis

ELAR, 2009

.vrh.'- 2001

* ebsite: <http://www.eiar.gov.et>

- !:+251-11-6462633

Fix: +251-11-6461294

P O.Box: 2003

i dis Ababa, Ethiopia

: BN 978-99944-53-42-9

pyediting: Abebe Kirub

::age *Design* Abebe Kirub

nting: Abesolom Kassa

Binding and collation: Absolom kasa, Miftah Argeta

htribution Akilu Wube, Solomon Tsega

Bibliographic input to WAICENT (FAO): Tigist Beshir

Contents

| | |
|---|-----------|
| Preface | I |
| Safety rules | 2 |
| Part I: laboratory experimental procedures | 3 |
| Aromatic plants | 4 |
| Moisture content determination | 4 |
| Hydro-distillation | 5 |
| Steam distillation | 7 |
| Cold pressing | 8 |
| Solvent extraction | 9 |
| Essential oils analysis | 10 |
| Separation of hydrocarbon and oxygenated fraction using CC | 10 |
| Chemical profiles of essential oils | 11 |
| Essential oils analysis with GC/MS | 12 |
| Specific gravity/ Relative density | 14 |
| Specific optical rotation value | 14 |
| Refractive index value | 15 |
| Oilseeds | 16 |
| Moisture content determination | 16 |
| Oil content determination using soxhlet method | 17 |
| Characterization of fatty acids | 19 |
| Identification of fatty acids methyl ester with GC | 21 |
| Bio diesel production | 22 |
| Spices | 23 |
| Pepper/Paprika | 23 |
| Paprika oleoresin extraction using soxhlet method | 23 |
| Determination of color value-paprika oleoresin | 24 |
| Ginger | 25 |
| Ginger oil | 25 |
| Ginger oleoresin | 25 |
| Preparation of silica gel-coated plat | 26 |
| Determination of total glycerol content with preparative TLC method | 27 |
| Determination of volatile oil in ginger oleoresin | 27 |
| Vanilla | 28 |
| Vanilla extract | 28 |
| Part II: Instrumental operation | 30 |
| Varian 3900 model GC Operation | 30 |
| UV/VIS spectrophotometer operation | 32 |
| Polarimeter (P20) | 33 |
| Refractometer | 34 |
| <i>Pycnometer</i> | 35 |
| Bibliographies | 36 |
| Glossary | 37 |
| Annex 1: Sample code and labeling | 40 |
| Acronyms | 42 |

Preface

Aromatic, Medicinal and Bioenergy Research team is one of the six national research case teams in crop research process that coordinates and leads research of aromatic, medicinal and bio energy issues in the National Agricultural Research System (NARS). The purpose of the Aromatic, Medicinal and Bioenergy Research team project is to develop, in collaboration with our partners, methods, tools and institutional models that facilitate the design and execution of successful projects that integrate market opportunities. The idea of having a laboratory manual for AMBRC is because there is hardly any laboratory manual on plant products analysis at Ethiopian Institute of Agricultural Research, relevant to the aromatic, medicinal and spice analysis. The focus of this manual is to present an easy-to-use methodology for plant products analysis with the instruments available at hand, and their related aspects are worthy of due emphasis.

A common plant analysis manual is also fundamental for success of AMBRC project. To fill the gap that existed, a comprehensive manual on all sorts of needed plant analyses is developed. This manual is designed intentionally in a simple format, for the ease of laboratory technicians. The laboratory is designed in a way to give service for those who need it, in addition to the research conduct by the case team. Analytical services should be linked with advisory services and maintain a functional relationship with the universities, research institutes, etc. The kind of service for such analyses depends on the type of institution it serves, the nature of client, and the volume of samples to be analyzed. Nevertheless, the laboratory, regardless of the size, is designed in a manner to facilitate operational efficiencies, and produce reliable and repeatable results. On completion, the manual draft will be reviewed for endorsement by peer reviewers.

We encourage all the users of this manual to continue provide feedback regarding its contents, and indicate errors, if any.

Daniel Bisrat
Head, Laboratory

Safety rules

The vast majority of injuries in the laboratory are preventable with normal precautions. The following list of laboratory safety rules is to be strictly adhered to. By observing these rules and using common sense, you will protect yourself and your research colleagues from many problems.

Safety rules

- Approved safety goggles (protective eyewear) and gloves are to be worn at all, times when you are in the laboratory.
- No open toed shoes are to be worn in the laboratory.
- Proper laboratory attire is expected. Full-length pants are to be worn in lab. Dresses are strongly discouraged. If you choose to wear a dress, you are also required to wear a full-length laboratory coat. At all times, a laboratory coat or apron is encouraged.
- There are to be no food or drinks in the laboratory at any time.
- Pay close attention to safety notes in your lab manual concerning the chemicals you are using.
- Note the location of all important safety items in your laboratory. You will then be prepared in the event an accident does occur. These items include:
 - o Eye wash stations
 - o Safety showers
 - o Fire extinguishers
 - o Fire blankets
 - o First aid kits
- Follow all the instructions contained in your laboratory manual, contained in handouts, and safety precautions given by your laboratory instructor.
- Use hoods whenever using noxious or fuming chemicals.
- Use pipette bulbs whenever pipetting. Never pipette by mouth!
- Always maintain a clean and organized work area. This will help avoid confusion, which can lead to accidents, and it will save time in lab. Also, help keep common work areas clean. These include the weighing room, the drying ovens, hoods, reagent benches, and sinks.
- Always use common sense. If you have any questions or concerns about what you are doing, first consult your laboratory instructor.

Safety goggles

Fire extinguishers

Laboratory coat

>

Never pipette by mouth

Plant Products Overview

Natural products offer a vast and virtually unlimited source of new agents for cosmetic, agrochemical and pharmaceutical industries. The country Ethiopia is endowed with rich flora and fauna due to its physical and climatic diversity. The diversity of total number of vascular plants in the country is estimated to be about 6500 species, of which an estimated 10% of the species are endemic and more than 14% are aromatic and medicinal plants, which can be exploited for different agro-industrial development purposes. Plants have always been a rich source of lead compounds (e.g. morphine, cocaine, digitalis, quinine, tubocurarine, nicotine, and muscarine). Many of these lead compounds are useful drugs in themselves (e.g. morphine and quinine), and others have been the basis for synthetic drugs (e.g. local anesthetics developed from cocaine). Clinically useful drugs which have been recently isolated from plants include the anticancer agent paclitaxel (Taxol) from the yew tree, and the antimalarial agent artemisinin from *Artemisia annua*.

Sampling

While much attention is given to laboratory procedures, the process of obtaining plant samples for analyses, plant sampling, is often ignored or poorly considered. If the sample is not representative or is incorrectly taken, the resulting analytical data would be meaningless, or at best, difficult to interpret.

Samples should be taken according to international standard and meet the following requirements.

Samples must be put in properly labeled plastic bags, having small holes around it for transpiration; this reduces the possibility of rotting.

A minimum of 250 g of sample is required for analyses

Free from foreign elements such as dusts, dead insects, moulds etc

A plant sample should be composed of several sub-samples representing a seemingly uniform area

Uniformity of the samples with respect to type, age (maturity) and location is essential

Sample should be kept in cool and dark storage room prior to analysis (not exceed three months in storage for spices and one month for aromatic plants), but mold formation need to be controlled

Samples should be chopped into pieces prior to extraction. In most cases, the moisture content of the material should not exceed 12 % unless the fresh plant parts need to be analyzed.

All information about samples with respect to location, treatments, time of harvesting etc is recorded and is given a laboratory code as indicated above (GSLB).

Note: Sample should be labeled by writing initials of 1st genus 2nd species

3rd location See annex1. For example, EG-Addis means *Eucalyptus globulus* from Addis Ababa

Plastic bag with small holes for aeration

Chopped grasses prior to extraction

Flower drying

Aromatic Plants

Aromatic plants are the sources of essential oils, resin, turpentine, flavors and fragrances, which can be used in the preparation of traditional medicines as well as in cosmetic industries. Essential oils are the subtle, aromatic and volatile liquids extracted from the flowers, seeds, leaves, stems, bark and roots of herbs, bushes, shrubs, and trees, through distillation. Some of the important essential oils used in medicine are mint oil (*Mentha arvensis*), peppermint oil (*Mentha piperita*), eucalyptus oil (*Eucalyptus spp.*), citranella oil (*Cymbopogon nardus*) and cinnamon leaf oil (*Cinnamomum zeylanicum*).

Moisture content determination

Principle:

Plant material consists of organic matter, moisture, insoluble matter, fiber etc. Moisture measurement is an important factor affecting the physical and chemical properties related to storage, processing and quality control in respect of products. Moisture content of aromatic plant is one of the most important characteristics for determining quality. It is important in determining the proper time for harvest and the potential for safe storage. It is also an important factor in determining market price, because the dry matter of the sample has more value than the water it contains and because costs of drying for safe storage must be taken into account. There are two ways of reporting laboratory results:

- On wet plant weight basis,
- On dry plant weight basis.

The moisture content approach is used for weighing plant samples, and expressing the analytical results on dry plant weight basis.

Apparatus:

Oven, Aluminum tray, Grinder, Desiccator, Balance

Procedure:

- Chop the plant parts into small pieces or in the case of bark and seed (crush or ground into powder first)
- Weigh the sample (W_{sample} : 3-5g) on an empty aluminum tray
- Place them in oven for 1-2 hr at 105 ± 1°C until the weight difference between two successive measurements gets below 0.5 mg.
- Remove the sample from oven and place them in desiccator until it gets cool
- Weigh the dried amount (W_{dried})

Oven

Spatula

Moisture content is expressed as shown below

$$\text{Moisture content (\%, w/w)} = \frac{W_{\text{sample}} - W_{\text{dried}}}{W_{\text{sample}}} \times 100$$

Desiccator

Aluminum tray

There are different techniques to extract essential oils from aromatic plants. Essential oils are defined as any of various volatile liquids, such as rose oil or lavender oils that have odor and are produced by plants. Essential oils are composed primarily of terpenes and a small amount of alcohols, esters, aldehydes, phenols and other class of compounds imparts particular odors and flavors. They used to make perfumes, soaps, flavorings, and other products. The basic principle of various techniques of extractions of essential oils is the same but it is carried out in different ways depending on the botanical material (plant species, plant parts, oil content) and the

condition of the material (thermal stability). Essential Oils are highly aromatic and therefore, many of the benefits can be obtained by simply inhaling them. This can be done by breathing in the fragrance from the bottle, or they can be diffused into the room. Some of the most common extraction techniques are listed below.

- Hydro-distillation
- Steam distillation
- Cold Pressing
- Infusion
- Solvent extraction

Hydro-distillation

Principle:

The most important production method for Essential oils is distillation. Raw plant material, consisting of flowers, bark, leaves, roots, seeds, or wood, is put into a distillation apparatus over water. As the water is heated, the steam passes through the plant material, vaporizing the volatile compounds. The vapors flow through a coil where they condense back to liquid in oil separator. Essential oils are obtained either float above the water (such as citriodora oil, grass oils) or sink below the water (Myrrh oil) depending on their density difference. Hydro and steam Distillations are the main methods to extract essential oils from plants. The techniques have two variants:

Distillation at normal atmospheric pressure- during which the distilled substance reaches its normal boiling temperature point; and

Vacuum Distillation during which the pressure in the distillation vessel is far lower than current normal atmospheric pressure and the distilled substance boils at a temperature lower than its normal boiling temperature.

Apparatus:

Clevenger-apparatus, heating mantle, round-bottom flask, condenser, balance, separator funnel, measuring cylinder, flask, rubber tubes, and clamp

Procedure:

Hydro-distillation is done with Clevenger-apparatus as follows

Place the plant parts ($W_{\text{mp}}^{\text{IOG}}$) - such as whole leaves, flowers, stems or in the case of bark (chopped or ground into smaller pieces first) into a 2 lt capacity-round bottom flask.

- * Pour preferably hot water into the flask until the plant parts submersed completely.

Place the round bottom flask on heating mantle, and allows the water and plant parts to boil for 2-4 hrs depending on the plant material. During which the heat of the steam bursts open the membrane of the oil receptacles in the plant and the volatile plant oil molecules are released into the steam.

The steam rises and takes with it hydrophobic and hydrophilic molecules in the clevenger apparatus. As the steam cools through the condenser, mixtures of water and various other molecules of the plant components separate back into liquid depending on their density difference.

Collect the essential oils that float on top or sink below of the separatory funnel

Transfer the oil into the flask and add anhydrous Na_2SO_4 in the flask

Shake the flask for 2 minutes

Filter the oil.

- Weigh the oil (W_{oil}) or measure the volume of the oil (V_{oil})
The distillation water is recovered and is known as a hydrosol or floral water.

Clevenger-

Condenser

Measuring
cylinder

Heating mantle

Round-bottom
flask

$$\text{Essential oil Content (\% w/w Fresh basis)} = \frac{W_{\text{Sample}}}{W_{\text{Sample}} + W_{\text{Water}}} \times 100 \quad \text{Eq-2}$$

$$\text{Essential oil Content (\% v/w Fresh basis)} = \frac{V_{\text{Sample}}}{V_{\text{Sample}} + V_{\text{Water}}} \times 100 \quad \text{Eq-3}$$

Separatory

Rubber lube

Condenser

dam n

Imn stand

Clevenger-apparatus

Round-bottom flask

Heating mantle

Hvdro-distillation set-un

Steam Distillation

Principle:

Steam Distillation is based on the assumption that essential oils are volatile in steam and generally insoluble in water. The distillation water, indirect contact with the raw material, is heated and the volatile constituents of the raw material released into the resulting water's vapor. The vapor is then cool down (condensation) into a liquid containing the raw material's volatile constituents, that is the essential oil. In short, the main purpose of steam distillation is to separate a mixture of several ingredients by taking advantage of their different volatility, or to separate volatile ingredients of a raw material from its nonvolatile parts. Distillation at atmosphere pressure is less costly than vacuum distillation.

Apparatus:

Heating mantle, condenser, round-bottom flask, and rubber tube

Procedure:

Place chopped plant parts (W_{sample}: 100g) into a 1 lt capacity round-bottom flask.

Pour preferably hot water into the first round-bottom flask

Place the round bottom flask on heating mantle, and allows the water to boil for 2-6 hrs depending on the plant material. During which the steam is generated, and goes to the second round-bottom flask where the raw material and steam have an indirect contact.

- The steam rises and takes with it hydrophobic and hydrophilic molecules in the apparatus. As the steam cools through the condenser, mixtures of water and various other molecules of the plant components separate back into liquid depending on their density difference.

Collect the essential oils that float on top or sink below of the separatory funnel

Transfer the oil into the flask and add anhydrous Na₂SO₄ in the flask

Shake the flask for 2 minutes and filter the oil.

Weigh the oil (W_{oil}) or measure the volume of the oil (V_{oil})

The distillation water is recovered and is known as a hydrosol or floral water.

$$\text{Essential oil Content (\% w/w Fresh basis)} = \frac{W_{\text{oil}}}{W_{\text{sample}}} \times 100 \quad \text{Eq-2}$$

$$\text{Essential oil Content (\% v/w Fresh basis)} = \frac{V_{\text{oil}}}{W_{\text{sample}}} \times 100 \quad \text{Eq-3}$$

Round-bottom
flask

Condenser

Heating mantle

Measuring
Cylinder

Separatory

Steam Distillation Set-up

Cold Pressing

Principle:

Cold Pressing is mainly used to prepare citrus fruits' essential oils from orange, lemon, grapefruit and tangerine. Due to the large quantities of oil in citrus peel, most citrus peel oils are usually expressed mechanically, or cold-pressed. The technique uses mechanical squeezer to press the citrus fruit's peels, in which oil receiver is attached to it. The oil released by the cells inside the peels collected and washed with water.

Cold Pressing yields high-grade essential oil if controlled; that is processing temperature kept as low as possible to avoid final product's degradation; raw material (peels of the raw material) are free of external ingredient (residue of pesticides, dust) to avoid contaminating the oil; and working conditions as clean as possible to avoid pollution during extraction.

Apparatus:

Mechanical presser, roller, separatory funnel, and centrifuge

Procedure:

Weigh the peel (W_{sample})

Puncture the oil glands by rolling the peel **over** sharp projections that actually pierce the oil glands.

Press the peel, which removes the oil from **the** glands. It is then washed off with a fine spray of water.

Separate the oil from the water by rotating it at a high-speed using centrifuge or using separatory funnel.

Weigh the oil (W_{oil}) or measure the volume of the oil (V_{oil})

Separatory funnel

$$\text{Oil Content (\% w/w)} = \frac{W_{oil}}{W_{sample}} \times 100 \quad \text{Eq-4}$$

$$\text{Oil Content (\% v/w)} = \frac{V_{oil}}{V_{sample}} \times 100 \quad \text{Eq-5}$$

Infusion

Principle:

Infusion is an old method, which was used in the production of organic perfumes, body cream, and deodorant extracts for perfumery industry. Infusion/cold, enfleurage has the advantage that even the most delicate components of the flower oils are preserved.

The disadvantages of infusion method are; it is not very effective method for total extraction and it is very expensive. For example, flower oils prepared with Infusion method do not contain terpene-hydrocarbons, which indicate that these compounds are not present as such in the flower, but form during distillation.

Apparatus:

Rota evaporator, water bath, thermometer, hot plate, and flask

Reagents:

Fixed oil, fat, wax, ethyl alcohol, and water

Procedure:

Select high quality oil/fat/wax

Note: Oils classified as high grade are:

- « It should be refined, not crude oil
- « Origin of oil is essential to judge the quality (for example, olive oil)

Prepare warm oil by heating the oil/fat over water bath (temperature 45-55 °C)

Rota evaporator

Flower petals (W_{sample}) such as rose or jasmine are layered onto warm oils, cold fat or wax. This process is repeated each day until the base is saturated with the essential oil (no weight difference between two successive measurements as an indication of saturation).

The resulting waxes or pastes usually contain up to 1 percent of essential oil.

Hot plate

- * Extract the essential oil from the wax with a volatile solvent such as ether. Evaporate the solvent at low temperatures and reduced pressure with Rota Evaporator so that the pure essential oil remains as a thick liquid. Weigh the oil (W_{oil})

$$\text{Essential Oil Content (\% w/w)} = \frac{W_{\text{oil}}}{W_{\text{Sample}}} \times 100 \text{ — Eq-5}$$

Water bath

Solvent Extraction

Principle:

Solvent Extraction is adapted to produce essential oils generated by some flowers (rose, violetta, and geranium), gums and resins. The raw material is placed in a glass vessel and soaked with a solvent petroleum, ether for flowers; hexane, acetone for resins and gums). This is a modern method of production of essential oils, which yields high quality essential oils.

Solvent extraction is employed for those plant materials containing essential oils with very low in quantity (less than 0.5%); and material consists of thermally unstable compounds.

Apparatus:

grinder, orbital shaker, flask, rota evaporator, filter paper, and funnel

Reagent:

Hexane, petroleum ether, and diethyl ether

Procedure:

Chop/ground the plant parts (W_{sample}) into small pieces

Chose the extraction solvent such as hexane or ether (it should be non-polar and volatile)

Place the plant parts and the solvent into a flask over a rotary/orbital shaker for 12 hrs at room temperature.

Filter the solution over glass funnel.

Evaporate the solvent at reduced pressure with Rota Evaporator (The result of solvent extraction is a concrete. The solvent is removed from the concrete by vacuum pressure without the use of excess heat to avoid any harmful effect to the oil)

Orbital shaker

Filtration

The concentrated essence resulted is called an absolute. Absolutes are highly concentrated flower products.

Weigh the oil/absolute (W_{oil})

h s .

Funnel

$$\text{Oil Content (\% w/w)} = \frac{W_{\text{oil}}}{W_{\text{Sample}}} \times 100 \text{ — Eq-6}$$

Rota evaporator

Essential oils Analysis

Essential oils are products of secondary metabolism and are secreted by specialized cells located in specified parts of most plants. By virtue of certain characteristic properties, they have wide spread use in perfumery, flavoring and medicine. Essential oils are variable mixtures of terpenoids. Since these oils are produced by plants, composition varies with species and variety of plant besides geography, climate and cultivation practices. Since, chemical components are highly reactive, physical and chemical environment to which oil is subjected to during the processes of its extraction; processing and storage have a great influence on final composition. Entire chain of activities from cultivation, extraction, processing, storage to packing has to be perfectly standardized and regulated to assure product consistency. Being a valuable item of trade, this is important from the point of view of delivering desired activity as well as safety to consumer. Essential oils are odorous products from natural raw materials such as leaves, fruits, roots and wood of many seasonal or perennial plants. An estimated 3,000 essential oils exist of which around 300 are of commercial importance. There are two categories of essential oils are in the trade:

Large volume oils produced from leafy raw materials such as *Lemon Grass*, Citronella, *Eucalyptus*, *roots* (Ginger, Tumeric), some flowers (Lavender, Rose, Jeranium, Violetta); and

Small volume oils produced from fruits, seed (Coriander), buds (Clove), nuts (Almond, Nutmeg), and some flowers (Geranium), wood (Sandalwood).

Gas Chromatography (GC) equipped with mass spectrometer (MS) or Flame ionization detector (FID) is the best technique for analyzing, separating, identifying and quantifying of individual constituents in essential oils.

Separation of hydrocarbon and oxygenated fraction using column chromatography (CC)

Principle:

Based on their polarities, essential oils are classified into hydrocarbon fraction, and oxygenated fraction. Hydrocarbon fraction is a mixture of non-polar compounds, whereas oxygen

Chemical profiles of essential oils

Identification of major constituents with GC

Principle:

Gas chromatography (GC) is a powerful analytical technique, which is used for separation, identification, and quantitation of chemical compounds. The technique is based on the partitioning equilibrium of compounds in two different phases.

In GC, the two phases are (1) a gas and (2) a solid or viscous liquid.

A volatile analyte, which may be present in a complex mixture, is vaporized into the gaseous phase (mobile phase) which is flowing down a tube. The tube (the "chromatographic column") is coated (or contains beads which are coated) with the solid or liquid phase (stationary phase) into which the analyte partitions. The gas flows at a constant rate and various analytes spend different fractions of time in the mobile and stationary phases. The rate of migration of analytes down the column depends on the fraction of time the analyte spends in the moving (mobile) phase. A chromatogram is thus a plot of the response of the detector at the end of the column as a function of time.

Quantification of analytes is performed by interaction with a detector located at the end of the column. The detector responds to some physical property of the analytes. The response is proportional to the quantity of analyte present and, with the aid of an appropriate calibration curve, allows for quantification of the sample.

Identification of an analyte by GC is also based on calibration. The time required for an analyte to pass the full length of the column (the "retention time") depends on the solubilities of the analyte in the different phases. Retention times must be determined by calibration with a known compound. Thus, qualitative identification of 3-hexanol is obtained by comparison of the retention time of the unknown with the known retention time of 3-hexanol.

Apparatus:

GC, micro syringe, capillary column, nitrogen generator, hydrogen generator, and air generator.

Reagents:

Hexane (analytical grade),

Procedure:

GC analysis of the oil samples is performed on a Varian 3800 Gas Chromatography equipped with FID using the following conditions

Install Non Polar capillary column such as DB-5, Cp-Sil-8 CB (25 m x 0.55 mm, film thickness 0.25 μ m).

Use Nitrogen as carrier gas at 10 psi inlet pressure.

Set column temperature programming from 75 °C (4 min.) to 200 °C at 4 °C/min.

Set both injection and detector temperatures at 250°C.

Prepare the sample for injection by dissolving 1 mg/ml

- Inject the sample (1 μ l) by splitting method with split ratio 1:20. Individual peaks are identified using retention times as well as by peak enhancement.
- Quantitative data is obtained by the external calibration method

GC column

For instance; to quantify thymol content in white cumin species; prepare calibration curve of thymol (standard sample)

Prepare a minimum of five thymol standard solutions by weighing 0.5, 0.75, 1, 1.5 and 2 mg of thymol/1 ml of hexane.

Perform the Gas Chromatography analyses on the five standard samples.

Analyze the peak area for the standard samples

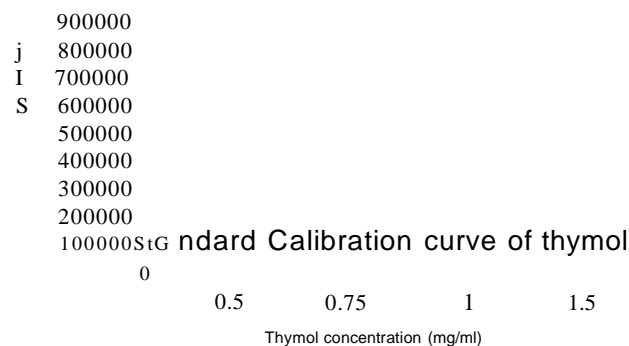
Construct the calibration curve by plotting x-axis as the concentration of the five standard samples and y-axis as their corresponding peak area.

Determine the percentage of thymol in the white cumin oil by comparing its peak area with the calibration curve.

- see below the calibration curve

Nitrogen/air
generator

Micro syringe



Essential oils analysis with GC/MS

Principle:

Mass Spectrometry is one of the fastest growing instrumental techniques. In addition to determining the mass, the fragmentation patterns are used to determine the structure of an unknown compound. Mass spectrometers actually measure the mass to charge ratio (m/z) of an ion. During ionization, many molecules undergo fragmentation. The mass spectrum is produced the different probabilities for the formation of each fragment ion. This provides a structural fingerprint of the molecule.

The GC/MS instrument represents a device that separates chemical mixtures (the GC component) and a very sensitive detector (the MS component) with a data collector (the Chemstation computer). Once the sample solution is introduced into the GC inlet, it is vaporized immediately because of the high temperature (250 degrees C) and swept onto the column by the carrier gas (usually Helium).

The sample flows through the column experiencing the normal separation processes. As the various sample components emerge from the column opening, they flow into the capillary column interface. This device is the connection between the GC column and the MS. Some interfaces are separators and concentrate the sample via removal of the helium carrier. The sample then enters the ionization chamber. The most frequently used is electron impact (EI). Another occasionally used alternative is chemical ionization (CI). For electron impact ionization a collimated beam of electrons, influence the

Essential oils are characterized by their physical and chemical properties. They are commonly distinguished by their characteristic odor or fragrance. They possess high refractive indices and most of them exhibit specific optical activity. They are in general immiscible with water and being lighter, most of them float on surface of water. Chemically they are terpenoids and are soluble in organic solvents like petroleum ether, chloroform, ethyl acetate and alcohol in decreasing order. Extent of miscibility with aqueous alcoholic mixtures of given composition is characteristic of an essential oil.

Each essential oil is characterized by a set of physical properties, which can be used individually, or in combination to ascertain its purity and genuineness. They are:

- Visual evaluation for color, clarity, and foreign materials
- Organoleptic assessment for odor, appearance, taste, etc
- Specific gravity
- Optical rotation
- Refractive index
- Flash point
- Solubility in alcohol/aqueous alcohol

Specific Gravity/Relative density

Principles:

Specific gravity of a substance is the ratio of the substance to the density of water. Adulteration is one of the major problems associated with the production of quality oil. Most common adulteration is addition of cheap vegetable or mineral oils. Most of these materials being not aromatic are usually not detected during odor checking. Since they are non-volatile, they are not detected when analyzed by GC-FID either. However, shift in values of specific gravity value is indicative of such adulterations.

Density is the mass per unit volume of a substance. $\rho = \frac{M}{V}$

Specific Gravity is the ratio of the density of the substance to the density of water. $SG = \frac{\rho}{\rho_w}$

Procedures:

- Add distilled water into Pycnometer (5ml)
- Weigh the distilled water, M_w (be sure there is no bubble or air inside the Pycnometer while weighing)
- Remove the distilled water and dry the Pycnometer
- Add the same volume of oil into Pycnometer
- Weigh the oil (M_o)

Specific gravity/Relative density (at given temperature) $\rho = \frac{M_o}{M_w} \times \rho_w$

Digital balance

Specific optical rotation value

Principles:

Specific optical rotation determines the angle through which the plane of polarization of plane-polarized light is rotated on passing through an optically active substance. Essentially, a polarimeter consists of a light source, a polarizer (e.g. a sheet of Polaroid) for producing plane-polarized light, a transparent cell containing the sample, and an analyzer. The analyzer is a polarizing material that can be rotated. Light from the source is plane-polarized by the polarizer and passes through the sample, then through the analyzer into the eye or onto a light-detector. The angle of polarization is determined

by rotating the analyzer until the maximum transmission of light occurs. The angle of rotation is read off a scale. Simple portable polarimeters are used for estimating the concentrations of sugar solutions in confectionary manufacture.

Apparatus:

Polarimeter and digital balance

Reagents:

Distilled water

Procedure:

- Switch on the polarimeter at least 15 minutes before taking measurements
- Set the p20 polarimeter to the correct scale, as indicated on the right hand side of the digital display (A for angular or K for Z (sugar scale))

Zero setting prior to sample measurement

- Ensure that the sample compartment is empty and the lid is closed
- Turn the drive wheel to adjust the null meter so that the needle is in the center
- Set the digital display read **00.00** using the ZERO SET knob
- The instrument is now ready to measure a sample

Sample measurement

- Ensure that the sample tube and end windows are clean
- Check that end caps are not screwed too tightly; they should be just tight enough to prevent leakage
- Fill the polarimeter tube with solvent
- Rock/shake from end to end to remove any bubbles from the polarimeter tube
- Place the sample tube on the rods in the sample compartment and close the lid
- Set the display to zero using the DRIVE WHEEL and the ZERO SET controls
- Empty the tube; then rinse and fill the sample solution
- Turn the DRIVE WHEEL until the null meter needle is returned to the center
- Read the digital display as optical value, a , when the meter is centered

Specific optical rotation value $[a]$ is expressed as follows

$$[a] = \frac{a}{LXC} \quad \text{--- Eq-10}$$

Where a = optical rotation value; L = Length of the cell in decimeter; C = Concentration in g/ml

Refractive Index Value

Principles:

A *refractometer* measures the extent to which light is *refracted when it moves* from air into a sample and used to determine the index of refraction (refractive index or n) of a liquid sample.

The refractive index is a unit less number, between 1.3000 and 1.7000 for most compounds, and is normally determined to five-digit precision. Since the index of refraction depends on both the temperature of the sample and the wavelength of light used these are both indicated when reporting the refractive index. The refractive index is commonly determined as part of the characterization of liquid samples, in much the same way that melting points are routinely obtained to characterize solid compounds. It is also commonly used to:

- Help identify or confirm the identity of a sample by comparing its refractive index to known values;
- Assess the purity of a sample by comparing its refractive index to the value for the pure substance; and
- Determine the concentration of a solute in a solution by comparing the solution's refractive index to a standard curve.

Apparatus:**Refractometer and glass rod****Procedure:**

- Turn the instrument on by just simply plug the cord into the power supply
- The instrument should be switched on at least 30 minutes
- Clean the surface of the prism to prepare it for the test
- Check always the status of the instrument with standard sample prior to actual measurements
- Open the prism box
- Transfer/dischage a few drops of the sample to the prism surface using a pipette
- Close the prism box
- Turn the control knob, with the eye at the field telescope, to a position where the observed field is divided into light and dark portions, the **dark** area below.
- Bring the crosswire into sharp focus by sliding or controlling the wheel
- Read the refractive index value in the scale telescope

Oilseeds

Oilseed crops are those crops that produce seeds containing significant amounts of oil. The oil is extracted from the harvested seed and can be used for several purposes. Oilseed crops are major sources of oils for human nutrition, and an increasing proportion is being utilized for industrial purposes such as Feedstock for biodiesel, and numerous other industrial/commercial products. The functional and nutritional values of different vegetable oils are dependent on the nature of the different fatty acids that are incorporated like building blocks into the oil (triacylglycerols). Although fatty acids can occur in nature in the free (unesterified) state, they are most often found as esters, linked to glycerol, cholesterol or long-chain aliphatic alcohols, and as amides in sphingolipids soybean, brassica, and noug are some of the most common oilseed crops.

Moisture content determination

Principle:

Moisture content is the single most important quality characteristic that determines the safe storage potential for oilseeds. Oilseed that is too high in moisture content is subject to attack by grain storage fungi and stored grain insects. These infections and infestations produce spoilage and loss of value that reach significant proportions every year. Fungi produce toxins such as aflatoxins and fumonisins that are serious health hazards to man and animals that use these products for food and feed. Therefore, better information on the moisture levels of products in maintaining quality and preventing losses and contamination. In addition, the moisture monitoring techniques will provide for better control of processing for value added applications and improved product quality. Accurate and reliable monitoring of moisture content is essential for efficiency in oilseed processing industry.

Apparatus:

Oven, aluminum tray, grinder, desiccators, and balance

Procedure:

Ground air-dried seeds into powdered form.

Transfer (3-5g; W_{sample}) onto an empty aluminum tray

Place them in oven for 1-2 hr at $105 \pm 1^\circ\text{C}$ until the weight difference between two successive measurements gets below 0.5 mg.

Oven

SDatula

Remove the sample from oven and place them in desiccator until it gets cool

Weigh the dried amount (W_{dried})

Desiccator

Aluminum tray

$$\text{Moisture content (\%, w/w)} = \frac{W_{\text{sample}} - W_{\text{dried}}}{W_{\text{sample}}} \times 100 \quad \text{-----Eq-1}$$

Digital balance

Oil content determination using soxhlet method**Principle:**

Oil content can be defined as the maximum amount of material (lipid) that can be removed from the seed by extraction with specific solvents—usually hexane or petroleum ether. Two main types of processes are used to separate oil from an oilseed. The first process is mechanical extrusion, in which the seed is mechanically pressed, allowing the oil to be separated from the meal. The second process is solvent extraction, which is often used hexane as solvent for extraction process. Mechanical/vacuum drier is processed to separate the oil from the solvent. Mechanical extrusion typically recovers about 65% of the oil contained in a seed. Solvent extraction recovers over 95% of the oil contained in a seed. A solvent extraction process is more efficient when the oil content of the oilseed is less than 25%.

Apparatus:

Heating mantle, soxhlet extraction apparatus, thimble, grinder, condenser, and balance

Pv

Reagents:

Hexane, petroleum ether

Procedure**Ground the seeds into powder, and weigh the amount (W_{sample})****Place powdered seeds on a porous cellulose thimble****Place the thimble in extraction chamber, which is suspended above the flask containing hexane.****The flask is then placed on heating mantle, and upon heating, the solvent starts to evaporate and moves up into the condenser where the vapor to liquid conversion occurred****The extraction chamber is designed so that when the solvent surrounding the sample exceeds a certain level, it overflows back down into the boiling flask.****At the end of the extraction process, which lasts 4 hrs, the flask containing solvent and the oil is transferred into round bottom flask.****The solvent in the flask is then evaporated using vacuum evaporator****The remaining oil is then placed in oven at 105°C for 1 hr to remove if in case there is moisture in it.****Weigh the oil (W_{oil})****The percentage of oil content can be calculated as shown below.**

$$\text{Oil Content (\%, w/w)} = \frac{W_{oil}}{W_{sample}} \times 100 \quad \text{-----Eq -II}$$

Soxhlet extraction
glass

Condenser

© o

Heating mantle

Round-bottom
flask

Digital balance

Thimble

Condenser

Thimble

Soxhlet apparatus

Round-bottom flask

Heating mantle

Soxhlet extraction apparatus

Characterization of fatty acids

Fixed oil is the mixture of non-volatile fatty acids of vegetable and animal origin. Any of a large group of monobasic acids, especially those found in animal and vegetable fats and oils, having the general formula CH_2COOH . Characteristically made up of saturated or unsaturated aliphatic compounds with an even number of carbon atoms, this group of acids includes palmitic, stearic, and oleic acids. Therefore, it requires conversion of fatty acid into their corresponding fatty acid methyl ester before it is analyzed with GC.

Conversion of fatty acids into their corresponding form of methyl ester

As fatty acids are non-volatile substance, their composition is determined by a process, which starts with extraction of a portion of the oil and its chemical conversion into individual fatty acids (taking apart the triacylglycerols). The fatty acids are then converted to methyl esters, compounds that can easily be converted to gases. The technique of gas chromatography (GC) revolutionized the study of lipids by making it possible to determine the complete fatty acid composition of a lipid in a very short time. For this purpose, the fatty acid components of lipids are converted to the simplest convenient volatile derivative, usually methyl esters, although other esters may be preferred for specific purposes. The preparation of such esters has therefore become by far the most common type of chemical reaction for lipid analysts. The different fatty acids are then separated and analyzed on a Gas Chromatograph (GC). The method is rapid and accurate. A single sample can be analyzed within 30 minutes on GC. There are two common methods for conversion of fatty acids to methyl esters.

Boron trifluoride in methanol (14% methanolic-BF₃) method

Principle:

The Lewis acid, boron trifluoride, in the form of its coordination complex with methanol is a powerful acidic catalyst for the esterification of fatty acids. For example, esterification of free fatty acids was completed in two minutes with 12 to 14% boron trifluoride in methanol under reflux. This method is often conducted to derivatize fatty acid in small scale/laboratory scale. It is not preferable to do in large-scale esterification.

Apparatus:

Round-bottom* rota evaporator, flask, hot plate, condenser, and separatory funnel

Reagents:

Methanolic-BF₃, heptane, NaCl, NaOH, anhydrous Na₂SO₄, and boiling aid

Procedure:

If the sample consists of fats and oils, the saponification step is necessary before esterification as shown below. On the other hand, the sample is a mixture of pure fatty acid we go directly into esterification.

Rota evaporator

Step 1 Saponification process

Introduce the test sample into the appropriate flask

Prepare 2% methanolic NaOH solution by dissolving 2 g of NaOH pellets in 100 ml of methanol

Add the appropriate amount of 2% methanolic NaOH solution and a boiling aid

Heat the oil to 50 °C, and hold this temperature through out the process

Hot plate

Fit the condenser to the flask

Boil under reflux until the droplets of the oil/fat disappear (usually takes 5 to 10 min, but in certain exception cases, it may take longer, for example, castor oil)

Step 2. Esterification process

Add the appropriate methanolic 12-15% BF₃ solution to the boiling solution through the top of the condenser, and bring to the boil and continue boiling for 2 min.

Add the appropriate amount of heptane to the boiling mixture through the top of condenser, and continue boiling for 1 min.

Round-bottom flask

Stop heating, cool to room temperature and then remove the condenser

Add a small portion of saturated NaCl solution and swirl the flask gently several times

Add more saturated NaCl solution to the flask in order to bring the level of solution into the neck of the flask

Transfer the upper layer (heptane) into a flask and add anhydrous Na₂SO₄ to remove any trace of water

Separatory funnel

Table. 1 An appropriate amount of reagents for conversion of oil/fat into fatty acid methyl ester

| Test sample (mg) | Round-bottom flask (ml) | Methanolic NaOH solution (ml) | BF ₃ solution | Heptane (ml) |
|------------------|-------------------------|-------------------------------|--------------------------|--------------|
| 100-250 | 50 | 4 | 5 | 1-3 |
| 250-500 | 50 | 6 | 7 | 2-5 |
| 500-750 | 100 | 8 | 9 | 4-8 |
| 750-1000 | 100 | 10 | 12 | 7-10 |

Transesterification method with Methanolic sodium hydroxide (2% methanolic NaOH solution)

Principle:

The most useful basic transesterifying agents are sodium or potassium methoxide in anhydrous methanol; prepared by dissolving the sodium or potassium hydroxide pellet in anhydrous methanol (the reaction is strongly exothermic). Sodium methoxide in methanol is therefore a valuable reagent for rapid transesterification of fatty acids linked by ester bonds to alcohols (e.g. cholesterol, glycerol).

Apparatus:

Filter paper, hot plate, round-bottom flask, separatory funnel

Reagents:

NaOH, and methanol

Procedure:

Filter and measure the desired amount of oil/fat into the Reaction flask
Heat the oil/fat to 40-50 °C and hold this temperature through out the process
While the oil is heating, prepare the methoxide by adding 2 g of NaOH pellet in 100 ml of methanol

Introduce the prepared methoxide solution into the oil (25 ml of methoxide solution is added into 100 ml of oil)

- Mix very vigorously at least for 30 min (The more violent in its color, the better indication of the reaction completion)
 - Remove the heat and transfer the mixture into the separatory funnel
 - Allow the mixture to settle for 1 hrs
 - Drain the glycerol (the lower layer) from the separatory funnel
- Wash the biodiesel with distilled water

Separatory funnel

Filtration

\$Li A

Funnel

Rota
evaporator

Identification of fatty acids methyl ester with GC

Principle:

The technique of gas chromatography (GC) revolutionized the study of lipids by making it possible to determine the complete fatty acid composition of a lipid in a very short time. For this purpose, the fatty acid components of lipids are converted to the simplest convenient volatile derivative, usually methyl esters, although other esters may be preferred for specific purposes. The preparation of such esters has therefore become by far the most common type of chemical reaction for lipid analysts. On the other hand, there are other chromatographic techniques, notably high-performance liquid chromatography (HPLC), where alternative derivatives, such as those with UV chromophores, are better.

Apparatus:

Digital balance and micropipette

Reagents:

Heptane and hexane

Procedure:

GC analysis of the oil samples is performed on a Varian 3800 Gas Chromatography equipped with FID using the following conditions

- Install polar Capillary column such as DB-23, CarboWax (25 m x 0.55 mm, Film thickness 0.25Dm).
- Use nitrogen as carrier gas at 10 psi inlet pressure.
- Set column temperature programming from 75 °C (4 min.) to 200 °C at 4 °C/min.
- Set both injection and detector temperatures at 250°C.
- « Samples are injected by splitting method with split ratio 1:20.
- Identify individual peaks using retention" times as well as by peak enhancement.
- Quantitative data is done by the external calibration method.

Biodiesel production

Biodiesel production is the process of making biodiesel, a liquid fuel source largely compatible with petroleum based diesel fuel. Vegetable oils and their derivatives (especially methyl esters), commonly referred to as “biodiesel,” are prominent candidates as alternative diesel fuels. The following steps can be performed in a small, home based biodiesel processor, or in large industrial facilities. The process is similar in either case.

- **Oil preparation**
- **Production methods**
- **Steps in the process**
- **Reaction and mechanism**

Oil Preparation

There are two methods of extracting oil from oilseed crops

- Mechanical oil expeller machine
- Solvent extraction method

Production methods

There are three basic routes to biodiesel production from biolipids (biological oils and fats):

- Base catalyzed transesterification of the biolipid
- Direct acid catalyzed transesterification of the biolipid.
- Almost all biodiesel is produced using base catalyzed transesterification, as it is the most economical process requiring only low temperature[^] and pressures and producing a 98% conversion yield. For this reason, onh this process will be described below.
- Transesterification is crucial for producing biodiesel from biolipids. The conversion of the biolipid to its fatty acids and then to biodiesel; transesterification process is the reaction of a triglyceride (fat/oil) with an methanol/ethanol in the presence of base catalyst (NaOH)

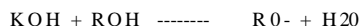
Castor and Jatropha biodiesel

Steps in the process

- Care must be taken to monitor the amount of water and free fatty acids in the incoming biolipid (oil or fat). If the free fatty acid level or water level is too high it may cause problems with soap formation (saponification) and the separation of the glycerin by-product downstream.
- Prepare methoxide solution by dissolving 2 g NaOH pellet (catalyst) in 100 ml methanol using a standard agitator or mixer
- Heat the oil/fat to 40-50 °C and hold this temperature through out the process
- Introduce the prepared methoxide solution into the warm oil (25 ml of methoxide solution is added into 100 ml of oil)
- Mix the reaction mixture very vigorously at least for 30 min
- Cool the reaction mixture at room temperature
- Separate biodiesel from glycerin with separatory funnel (The glycerin phase is much denser than biodiesel phase and the two can be gravity separated with glycerin simply drawn off the bottom of the separatory funnel. In some cases, a centrifuge is used to separate the two materials faster)
- Remove excess alcohol with Rota evaporator
- Wash the biodiesel gently with warm water to remove residual catahst or soaps.

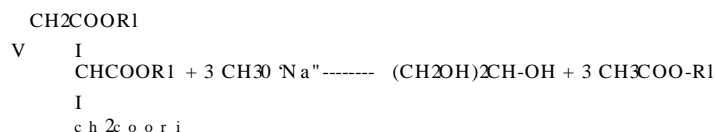
Reaction and Mechanism

Step 1. Mix the base (KOH, NaOH) with the alcohol to make a reactive anion (RO⁻)



KOH and NaOH are strong bases, so the reaction equilibrium is far to the right. The ROH needs to be very dry. Any water in the alcohol will reduce the *amount of* RO⁻ that gets formed.

Step 2. The S_N2 reaction that follows replaces the alkyl group on the triglyceride in a series of reactions. The carbon on the ester of the triglyceride has a slight positive charge, and the oxygen have a slight negative charge, most of which is located on the oxygen in the double bond. This charge is what attracts the RO⁻ to the reaction site



Spices

Spices are any of a class of pungent or aromatic substances of vegetable origin, as pepper, cinnamon, or cloves, used as seasoning, preservatives, etc. Spices not only add flavor to food, but also contain many beneficial nutrients. While today herbs and spices are still used as natural remedies, a review of scientific literature, published in The Medical Journal of Australia, has found that “culinary herbs and spices *contain high concentrations of* antioxidants and phytonutrients (plant-derived chemical compounds important to human health) and may provide long-term health benefits that even outweigh their short term taste sensations”.

Pepper/Paprika

Principle:

Paprika is a red-orange coloring material extracted from the common sweet red pepper, *Capsicum annum* L. The plant is grown mostly in temperate climates. Paprika oleoresin is obtained by either solvent extraction or super critical fluid extraction of paprika, which consists of the ground fruit pods, with or without the seeds, of *Capsicum annum*. It contains the major flavoring and coloring principles of this spice; the major flavoring principle is capsaicin; the major coloring principles are capsanthin and capsorubin; a wide variety of other colored compounds is known to be present. Only the following solvents may be used: trichloroethylene, acetone, propan-2-ol, methanol, ethanol, and hexane. The solvent is subsequently removed. For the case of super critical fluid extraction, we use liquefied carbon dioxide.

Paprika oleoresin extraction using Soxhlet method**Apparatus:**

Heating mantle, soxhlet extraction apparatus, thimble, grinder, condenser, and digital balance

Reagents:

Hexane and petroleum ether

Procedure:

Dry the sample until the moisture gets below 10%

Ground the pods into powder form (W_{sample})

Place the powdered sample on a porous cellulose thimble. The thimble is placed in extraction chamber, which is suspended above the flask containing acetone.

Heat the flask (50-60°C) and the solvent is evaporated and moved up into the condenser where the vapor to liquid conversion occurred. The extraction chamber is designed so that when the solvent surrounding the sample exceeds a certain level it overflows back down into the flask.

At the end of the extraction process, which lasts 4 hrs. the flask containing solvent and the oleoresin is transferred into round bottom flask.

The solvent in the flask is then evaporated and the mass of the remaining oleoresin is measured ($W_{\text{oleoresin}}$). The percentage of oleoresin in the initial sample can then be calculated.

Soxhlet
extraction flask

Condenser

Heating mantle

Round-bottom
flask

$$\text{Paprika Oleoresin Content (\%, w/w)} = \frac{W_{\text{oleoresin}}}{W_{\text{sample}}} \times 100 \quad \text{---Eq. 12}$$

Digital balance

Thimble

Determination of color value-paprika oleoresin

Principle:

Color value of paprika oleoresin is determined using spectrophotometric method with adjustable wavelength at 462nm. Instrumental analysis shall be employed to make the estimation completely objective and the color of a specified dilution shall be estimated at 462nm

Reagents:

During the analysis, use only reagents of recognized analytical grade (hexane, acetone) and distilled water of equivalent purity

Apparatus:

100-ml volumetric flasks, pipette, water bath, Spectrophotometer with adjustable wavelength

Procedure:

Weigh 0.1 gm of oleoresin into a 100 ml of volumetric flask

Dilute with acetone until it shall be made up to 100 ml volume mark

Using a tungsten lamp source and acetone as the blank, take the absorbance of the 0.1 percent solution of oleoresin at 462 nm.

Multiply this reading by the factor 33,000 to obtain color value

Volumetric flask

Color value of oleoresin shall be calculated as follows:

$$\text{Color Value Unit (CU)} = \frac{\text{Absorbance} \times 33,000 \times \text{If}}{\text{Mass of Oleoresin}} \quad \text{---Eq. 13}$$

Where If- Instrumental correction factor

Digital balance

Ginger

Ginger oil

Principle:

Ginger oil can be produced from fresh or dried rhizomes. Oil from the dried rhizomes will contain fewer of the low boiling point volatile compounds (the compounds that give ginger its flavor and aroma) as these will evaporate during the drying process. The best ginger oil is obtained from whole rhizomes that are unpeeled. The yield of oil from dried ginger rhizomes is between 1.5 to 3.0%, and the principal component is zingiberene. The remaining rhizome powder contains 50% starch, which can be used for animal feed. It is sometimes dried and ground to make an inferior spice.

Apparatus:

Clevenger apparatus, condenser, round-bottom flask, and heating mantle

Procedure:

Ground dried rhizomes into powder form

Load powdered rhizomes into a round-bottom flask.

Add water into round-bottom flask until the sample is completely immersed

While boiling, steam is passed through the powder, which extracts the volatile oil components.

The steam is then condensed with tap water.

As the steam condenses, the oils separate out of the steam water and can be collected.

Clevenger-apparatus

Weigh the oil (W_{oil})/ measure the volume (V_{oil})

$$\text{Ginger Oil Content (\% w/w Fresh basis)} = \frac{W_r}{r_{\text{Sample}}} \times 100 \quad \text{--- Ea-2}$$

$$\text{Ginger Oil Content (\% v/w Fresh basis)} = \frac{V_r}{V_{\text{Sample}}} \times 100$$

Heating mantle

Round-bottom flask

Ginger oleoresin

Principle:

Gingerols (6-, 8-, and 10-gingerol) are the compounds responsible for ginger pungency; however, because they are readily decomposed to the less pungent shogaols and zingerones upon heating, oleoresins obtained by solvent extraction are preferred when pungency is desired. Commercial solvents include ethanol, acetone, trichloroethane or dichloroethane, although the latter two are known carcinogenic and ethyl acetate or hexane is preferred. Dried powdered rhizomes are extracted by percolation, and the extract is then cold distilled at 45-55 °C to remove all the solvent, while assuring integrity of gingerols by not overheating. Hydrophilic solvents such as ethanol and acetone also extract water-soluble gums, which may need to be further separated by centrifugation. However, water-soluble solvents may be preferred to prepare extractive to be *used by the beverage industry to* assure water solubility. Supercritical fluid extraction uses carbon dioxide (CO₂) under high pressure and cold temperature. This extraction technique is preferred for higher quality extracts because there is no thermal degradation, and the aromatic profile is therefore closer to the profile in the plant.

Apparatus:

Heating mantle, soxhlet extraction apparatus, thimble, grinder, condenser, and balance

Reagents:

Ethanol

Procedure:

Dried powdered rhizomes are extracted by a typical reflux apparatus, placing it on a water bath as shown below.

Ground the rhizomes (W_p)

Soak the powdered rhizomes in a round-bottom flask, containing ethanol solvent.

Soxhlet extraction glass

Condenser

Heat constantly the flask for 4hrs to facilitate the extraction process.

Control the temperature of the water bath not to exceed 55 °C.

The round-bottom flask is connected to a condenser, such that any vapors given off are cooled back to liquid, and fall back into the round-bottom flask.

Filter the extract, once the extraction is over.

Evaporate the solvent with Rota Evaporator to get dark viscous oleoresin.

Heating mantle

Round-bottom flask

Weigh the oleoresin ($W_{oleoresin}$)

$$\text{Ginger Oleoresin Content (\%, w/w)} = \frac{W_{oleoresin}}{OWI} \times 100 \quad \text{—EG-11}$$

Digital balance

Thimble

Preparation of silica gel-coated Plate (PTLC)**Principle:**

Preparative Thin layer chromatography (PTLC) is a form of liquid/solid chromatography (LSC) where the stationary phase, instead of taking the form of a packing in an open tube, is formed as a thin layer on the surface of a suitable plate. The mobile phase is allowed to flow over the surface (normally driven by surface tension forces) eluting the solutes along the plate in the process. Although in preparative thin layer chromatography the distribution systems (the phase systems) are basically similar to those used in liquid chromatography, the necessary apparatus is much simpler and, as a consequence, a great deal less expensive.

Apparatus:

PTLC-maker, flask, and oven

Reagents:

Silica gel, distilled water

Procedure:

Add 65 ml distilled water into a flask containing 30 g of powder silica gel.

Prepare the slurry by shaking the mixture for 3-5 min.

Spread the slurry on 20 cm x 20 cm glass plate by hand.

Allow the silica gel-coated plates to dry overnight after tapping the plate to make it uniform.

Put the plate in an oven at 100 °C for 1 hr in order to activate the plates

PTLC Jar

Flask

Homemade
PTLC

PTLC- maker

Determination of total gingerols content with Preparative thin-layer chromatography (TLC) method

Principle:

A sensitive and accurate High/Performance TLC (HPTLC) method has been developed to determine the quantity of total gingerol in rhizomes of *Zingiber officinale* commonly known as ginger. Total gingerols comprises of 6, 8 and 10-gingerol. Ethanol extracts of rhizomes are developed on PTLC, using hexane, and ethyl acetate (40:10 v/v) as the mobile phase. The R_f of total gingerol was found to be 0.50-0.80. The method permits reliable quantification of total gingerol and good resolution and separation of total gingerols from other constituents of ginger. This PTLC method for quantitative monitoring of total gingerols in ginger can be used for routine quality testing of ginger extracts.

Apparatus:

PTLC, PTLC chamber/jar, spatula, funnel, filter paper

Reagents:

Hexane and ethyl acetate

Procedure:

Steps for Quantification/isolation of total gingerols

- * Apply a few milligram of ginger oleoresin ($W_{oleoresin}$) on PTLC in the form of band using pipette.
Place the plates into a jar containing Hexane/Ethyl acetate (4:1) as a developing solvent system.
Take out the plates when the solvent front reached at the top (takes about half an hour).
Scratch three yellow bands with R_r value b/n 0.5-0.8 from plates, representing total gingerols.
Collect the scratched sample in the flask and add 25 ml of acetone
Filter the solution to recover the total gingerols from the silica gel.
Weigh the total gingerols (WTG)

Filtration

$$\text{Total Gingerol (\%, w/w)} = \frac{W}{W_{oleoresin}} \times 100 \quad \text{--- Eq-14}$$

Digital balance

Determination of volatile oil in ginger oleoresin

Principle:

Major components in ginger essential oil are zingiberene (20-37%), curcumene (5-20%), farnesene, bisabolene and sesquiphellandrene. The volatile oil content in ginger oleoresin, along with total gingerols content, defines the quality of ginger oleoresin. Ginger oil is used largely as a flavoring agent in various alcoholic and non-alcoholic beverages. It is also used in confectionery, bakery, and perfumery. It is occasionally mixed with ginger oleoresin to restore an ideal balance between aroma and pungency.

Apparatus:

Hydro-distillation set-up, heating mantle, and round bottom flask

Procedure:**Quantification of volatile oil in ginger oleoresin is done using Clevenger-apparatus as follows**Place a few gram of ginger oleoresin ($W_{oleoresin}$) into a round-bottom flask.

Pour hot water into the flask until the sample submersed completely.

Place round-bottom flask containing the sample on heating mantle,

Allow the water and the oleoresin to boil for 4 hrs, and the volatile oil molecules are released into the steam.

The steam cools through the condenser, mixtures of water and various other molecules of the plant components separate back into liquid in the oil separator.

Collect the essential oils that float on top of the oil separator (W_{oil}). The distillation water is recovered too and is known as a hydrosol or floral water.

Clevenger-apparatus

Condenser

$$\text{Volatile Oil Content (\%, w/w)} = \frac{W_{oil}}{W_{oleoresin}} \times 100 \quad \text{—Eq-15}$$

Heating mantle

Round-bottom
flask**Vanilla****Vanilla extract****Principle:**

Vanilla has a long history of use as a food flavoring and fragrance. Madagascar produces approximately 80% of the world's supply. The quality of the vanilla bean is not dependent on the vanillin content even though vanillin is associated with the characteristic fragrance of the plant. Numerous other constituents characterize the flavor and quality of vanilla and its extracts. Vanilla extracts are prepared by percolating ground vanilla bean with an alcohol/water mixture. Vanilla has been reported to contain up to approximately 3% vanillin, the major flavoring component. However, more than 150 other minor components contribute to the full-bodied fragrance of natural vanilla. Because synthetically produced vanillin can be obtained inexpensively, it is often used as a substitute or adulterant for natural vanilla extract. Unfortunately, there is no simple method to distinguish if a vanilla extract is authentic, although sophisticated chromatographic method, it can assist in defining the quality of an extract.

Apparatus:

Flask, funnel, filter paper, and rota evaporator

Reagents:

Ethanol

Procedure:**Vanilla extracts are prepared as follow**

- Ground the vanilla pods
Soak grounded vanilla pods ($W_{m\ i l}$) in ethanol for 12 hours at room temperature.
- Filter the solution
Wash the pod residue with an extra a few ml ethanol
Combine the extracts, and the solvent is evaporated at reduced pressure to get vanilla extract
- Weigh the vanilla extract (W_{extran}).

Orbital shaker

Filtration

i i r

$$\text{Vanilla Extract Content (\%, w/w)} = \frac{W_{extran}}{W_{Manila}} \times 100 \quad \text{—Eq-16}$$

Funnel

Rota evaporator

Procedure:

Vanillin content is analyzed with GC as described below.

GC analysis of the extracts is carried out by using a Varian 3800 GC equipped with a capillary column CPSil-8 (30 m *0.25 mm and 0.25 μ m thickness).

Nitrogen is used as a carrier gas at 10 psi flow rate, and detector is flame ionization detector (FID).

1 μ l of sample in dichloromethane is injected into column

Set column temperature program at 120°C (hold 2 min) to 200°C at the rate of 4°C/min with a hold time of 10 min at 200 °C.

Set both detector and injector temperatures at 250 °C.

Vanillin and moisture content in Vanilla pod (Source: Girma/Tepi)

| Sample code | Vanilla ethanol extract from pod (%, w/w) | Vanillin content in the vanilla pod (%, w/w) | Moisture content (%, w/w) |
|--------------------|---|--|------------------------------|
| Teti vanilla Rep.1 | 32.6% | 2.49% | 3.7% |
| Tepi vanilla Rep.2 | 33.2% | 2.53% | 3.8% |

Varian 3800 model GC operation

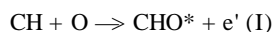
Principle

Gas chromatography (GC) is a powerful analytical technique, which is used for the separation, identification, and quantification of chemical compounds. The technique is based on the partitioning equilibrium of compounds in two different phases. In GC, the two phases are (1) a gas and (2) a solid or viscous liquid.

Not all samples may be analyzed directly by GC. The sample must be

- Volatile at the GC temperature
- Thermally stable at the GC temperature and
- Not irreversibly, interact with the stationary phase (1) and (2) simply require the analyte to partition into the gaseous phase (3) Requires that the analyte not stick to the column forever.

There are varieties of different detectors for GC. Common detectors include thermal conductivity (TCD), flame ionization (FID), electron capture (ECD), electrochemical (EC), and massspectrometric (MS) detectors. A flame ionization detector consists of igniter, electrode, and a H₂/air valves. When organic compounds pass into the H₂/air flame, CH radicals are formed which react with oxygen atoms as in reaction 1.



The current is measured. The response of the detector is dependent on the analyte and the detector is insensitive to many common inorganic compounds including O₂, CO₂, and NH₃. Thus, a calibration curve for detector response must be generated for each analyte to be studied.

Procedure

Analysis of a mixture

Select one of the following samples or pick one of your own choosing.

- Essential oils
- Fatty acid methyl ester

Obtain a gas chromatogram for your sample.

Next, you will need to identify the compounds corresponding to each significant peak in your chromatogram. To calibrate the instrument, you will need to run pure standards for each compound you suspect to be in your sample. Comparison of these standards with your sample chromatogram should allow you to identify the components of your sample.

Finally, you need to quantify the major organic compound in your sample using an external standard or other method you choose.

Steps for GC operation _____

Step1. Hydrogen generator

Hydrogen generator operates with double distilled and deionized water.

- * Water should be replaced once every two weeks
High purity of hydrogen gas produced as soon as the generator on

Step 2. Nitrogen and Air generator

Wait 30 minutes after switch on the generator to get high quality of nitrogen

Step 3. GC operation

- Turn the switch control on the top panel of the instrument to ON position
- Turn the computer on and load the software that control/monitor the GC
Check on the instrument status on the program
First set your appropriate GC method, and load the method, and follow the set and actual value of the instrument on the computer
The instrument is ready for operation when the Green light READY signal appear o computer

Sample preparation

weigh 10 mg of essential oil

Dissolve in 1 ml of hexane/heptane/diethylether

- * Inject 1-2 μ l of sample for GC injection

GC software operation

Inject a single sample from System Control by using the *Inject Single Sample* dialog box.

Display the *Inject Single Sample* dialog by selecting it from the Inject menu or by clicking on the Inject Single Sample button on the toolbar.

Specifies the number of injections of this sample.

Enter notes about the sample.

Select the Method to use for the run.

How to create method

- Click on the FILE menu and set new method file
- Instrument parameter will appear on the screen
- Set values of GC operational parameters, and save it as a file
- Change the location and name of the Data Files
- Click Inject to start the run.
- The status of the run can be monitored in the instrument window

UV/VIS spectrophotometer operation

Principle:

UV-Visible spectrophotometer is a very common technique employed by chemists for qualitative and quantitative analysis. Qualitative identification is through measurement of the absorbance of a solution as a function of wavelength while quantitative analysis is through the application of the Beer-Lambert law.

Procedure:

Prepare a standard solution in an appropriate solvent (like hexane). Make a series of dilutions based upon the standard. Obtain a UV-Vis spectrum for one of the solutions in the middle of the concentration range. If the absorbance is too high, use a more dilute solution. Identify the origin of each peak in the spectrum. Next, measure the absorbance of all of your samples at the wavelength of maximum absorbance. Plot a calibration curve and determine the molar absorptivity for your compound.

Switch the UV Rocker to switch ON position on the front panel

Depending upon the desired spectral region of operation, the UV or VIS spectral region is selected (UV spectral region: 190-350 nm and VIS spectral region: 350 -1000nm)

Follow the following steps to operate on UV spectral region

Turn the UV-VIS control on the front panel clockwise to UV position
Allow minimum 15 min of warm up time
Set the desired wavelength by pressing the SET and the ENTER keys simultaneously and release them (please refer the manual for detailed information)

Absorbance measurement

- Set the wavelength of interest as explained above
- Push the lid of the sample compartment
- Check the dummy(opaque) cuvette is in the 1st position
- Put the blank cuvette in the 2nd position
- Put the cuvette filled with sample in 3rd position
- Set the function switch at % T (percentage Transmission) position
- Bring dummy (opaque) cuvette in the light path by
 - Set both CAL COURSE & FINE controls in their maximum clockwise position
- Rotate 0%T- control in appropriate direction to adjust 00.0 on the data display
- Bring the blank cuvette in light path by pulling the CUV-SEL control of sample compartment in 2nd position
- Adjust COURSE & FINE control to set 100.0 on the readout
- Bring the sample cuvette in the light path by pulling the CUV-SEL control of sample compartment in 3rd position
- * The exact value of % Transmission of sample at the set wavelength will be displayed in the readout
- To obtain the corresponding ABSORBANCE, bring the FUNCTION switch at abs. position

Polarimeter (P20)

A polarimeter used to determine the angle through which the plane of polarization of plane-polarized light is rotated on passing through an optically active substance. When a beam of plane polarized light is passed through a solution of a chiral compound such as (S)-alanine, the plane of polarization of the light that emerges is rotated relative to the original plane. This phenomenon is known as optical activity, and compounds that rotate the plane of polarized light are said to be optically active.

Main components

P20 polarimeter; polarimeter Tube

Operation

- Turn the instrument on using the on/off switch on the rear services panel
- The instrument should be switched on at least 15 minutes before taking accurate measurements
- Set the p20 polarimeter to the correct scale, as indicated on the right hand side of the digital display (A for angular or K for Z (sugar scale))

Zero setting prior to sample measurement

- Ensure that the sample compartment is empty and the lid is closed
- Turn the drive wheel to adjust the null meter so that the needle is in the center
- Set the digital display read 00.00 using the ZERO SET knob
- The instrument is now ready to measure a sample_____

Sample measurement

- Ensure that the sample tube and end windows are clean
- Check that end caps are not screwed too tightly; they should be just tight enough to prevent leakage
- Fill the polarimeter tube with solvent
- Rock/shake from end to end to remove any bubbles from the polarimeter tube
- Place the sample tube on the rods in the sample compartment and close the lid
- Set the display to zero using the **DRIVE WHEEL** and the **ZERO SET** controls
- Empty the tube; then rinse and fill the sample solution
- Turn the **DRIVE WHEEL** until the null meter needle is returned to the center
- Read the digital display as optical value, α , when the meter is centered

Specific optical rotation value $[\alpha]$ is expressed as follows

Where α = optical value
 L = Length of the cell in decimeter
 C = Concentration in g/ml

Refractometer

Principles:

Refractometer employs the critical angle effect marked by a demarcation line between light and dark portions of the telescope field, this demarcation line generally being known as the borderlines.

Main components

Prism box, dispersion drum, field telescope

Operation

- Turn the instrument on by just simply plug the cord into the power supply
- The instrument should be switched on at least 30 minutes
- Clean the surface of the prism to prepare it for the test
- Check always the status of the instrument with standard sample prior to actual measurements
- Open the prism box
- Transfer/discard a few drops of the sample to the prism surface using a pipette
- Close the prism box
- Turn the control knob, with the eye at the field telescope, to a position where the observed field is divided into light and dark portions, the dark area below.
- Bring the crosswires into sharp focus by sliding or controlling the wheel
- Read the refractive index value in the scale telescope

Pycnometer

Principles:

Pycnometer - A standard vessel used in measuring the density or specific gravity of materials.

Relative density of a substance is the ratio of the substance to the density of water.

Density is the mass per unit volume of a substance. M / V

Specific Gravity is the ratio of the density of the substance to the density of water. $SG = \frac{M}{w}$

Procedure

Add distilled water into Pycnometer (5ml)

Weigh the distilled water, M_{water} (be sure there is no bubble or air inside the Pycnometer while weighing)

Remove the distilled water and dry the Pycnometer

Add the same volume of oil into Pycnometer

Weigh the oil (M_o)

Pycnometer

Bibliographies

- Ashurst, P.R., A.R. Firth and O.M. Lewis (1973). A new approach to spice processing. *Tropical Institute Conference papers*, M. 209 -214.
- Ethiopian standard authority, spice and condiments- oleoresins of capsicum and paprika. Reference ES 684:2002.
- FAO (1995a). Flavours and fragrances of plant origin, Non-Wood forest products , Rome
- FRIDGE (2004), Aroma Chemicals derived from Essential Oils, South Africa
- Guise, E. (2004), Refining Oregano Raw Production, Program, Introduction of New Varieties, Drying of Leaf Material, Distillation of the Various Essential Oils and General Improvements of the Entire Program
- Landgrebe, Theory and Practice in the Organic Laboratory
- Lawrence B.M., 1995, *The Isolation of Aromatic Materials from Natural Plant Products*, In Tuley De Silva. I (Ed), A Manual on the Essential Oil Industry, UNIDO, Vienna.
- Narayamam, M. R., Sarwar. M., Khan. M. N. A. Geranium and its Cultivation in India. Central Institute of Medicinal and Aromatic Plants, Farm Bulletin No. 15. Lucknow, India.
- Operating Instructions for ABBE 60 REFRACTOMETER (1991) Bellingham and Stanley Ltd, Tunbridge Wells, Kent TN2 3EY, England
- P20 Polarimeter Operators Manuals (1991) Bellingham and Stanley Ltd, Tunbridge Wells, Kent TN2 3EY, England
- Rai S, Mukherjee K, Mai M, Wahile A, Saha BP and Mukherjee PK. (2006). Determination of 6-gingerol in ginger (*Zingiber officinale*) using high performance thin layer chromatography. *J Sep Sci.* 2006 Oct; 29(15):2292/5.
- Raymond P. W. Scott Raymond P. W. Scott, Thin Layer Chromatography part of the Chrom-Ed Series
- Silverstein and Basler, Spectrometric Identification of Organic Compounds Eaton, Laboratory Investigations in Organic Chemistry
- Virmani, O. P., Singh, K. K., Singh. Pratap. (1979). Java Citronella and its Cultivation in India. Central Institute of Medicinal and Aromatic Plants, Farm Bulletin No. 1. Lucknow, India.
- Vogel, A Textbook of Practical Organic Chemistry
- Yiljep Y., Fumen G. and Ajisegiri E. (2005) The Effects of Peeling, Splitting and Drying on Ginger Quality. *Agricultural Engineering International: the CIGR E Journal*. Manuscript FP 05 009. Vol. VII. December, 2005.

Glossary

Absolute - A concentrated semi-solid aromatic material, usually obtained by alcohol extraction.

Adsorption - The process of interaction between the solute and the surface of an adsorbent. The forces involved can be strong (for example, hydrogen bonds) or weak (van der Waals forces). For silica gel, the silanol group is the driving force for adsorption, and any solute functional group that can interact with this group can be retained by liquid-solid chromatography on silica.

Adulteration - Pure essential oils are changed, cut, diluted or mixed with synthetic fragrance

Aromatherapy- the use of essential oils and hydrosols obtained by distillation (from named botanical species) via inhalation through the sense of smell and through direct application, to treat mental, emotional and physical states.

Calibration curve - Plot of response vs. amount for several standards

Calibration factor- Relation of response to actual amount of standard; depends on compound and detector.

Carrier - Often a vegetable oil, which is used to dilute pure essential oils for application. A common carrier is sweet almond oil.

Carrier gas - The GC mobile phase

Chromatogram - A plot of detector signal output versus time or elution volume during the chromatographic process.

Chromatography - Any of various techniques for the separation of complex mixtures that rely on the differential affinities of substances for a gas or liquid mobile medium and for a stationary adsorbing medium through which they pass, such as paper, gelatin, or magnesia.

Cold Pressed - A natural physical process used to extract vegetable oils and essential oils from citrus fruits. The seeds of the fruit are "pressed" which releases their oil. Since no heat is used in this process, the oils retain their essential fatty acid content.

Column chromatography - A form of partition chromatography in which a liquid phase flows down a column packed with a solid phase.

Concrete - A waxy semi-solid or solid material derived from plant material by solvent.

Distillation- is a one-way process of vaporizing a substance by heat in a still, condensing the fluid by cold in a condenser, and collecting the liquid in a receiver.

Electron Capture Detector (ECD) - Uses electron emitting source to ionize the carrier gas. Any electron-deficient analyte will reduce this level of ionization. The ECD detector is sensitive to any analyte with electronegative functionality (e.g. Cl⁻).

Eluate- Combination of mobile phase and solute coming off a chromatographic column; also called effluent

Eluent- The gas or liquid carrying the eluate/ Mobile phase used to carry out a separation.

Elution Volume (VR) - Refers to the volume of mobile phase required to elute a solute from the column at maximum concentration (apex). $VR = F \times tR$, where F is flow rate in volume/time and tR is the retention time for the peak of interest.

Essential oil - any of a class of volatile oils obtained from plants, possessing the odor and other characteristic properties of the plant, used chiefly in the manufacture of perfumes, flavors, and pharmaceuticals.

Fatty acid - Any of a large group of monobasic acids, especially those found in animal and vegetable fats and oils, having the general formula $CH_2 + COOH$. Characteristically made up of saturated or unsaturated aliphatic compounds with an even number of carbon atoms, this group of acids includes palmitic, stearic, and oleic acids.

Fixative - A material that "fixes" the scent and slows evaporation of the essential oils

Fixed oil - nonvolatile oil, especially a fatty oil of vegetable origin

Flame ionization detector (FID) - is a type of gas detector used in gas chromatography. FID is best for detecting hydrocarbons, and other easily flammable components. They are very sensitive to these components, and response tends to be linear across a wide range of concentrations.

- Floral water** - are hydrosols are produced by water or steam distillation of the flower or herb, and contain most of the hydrophilic (water-soluble) molecules from the plant or flower material that did not distil into the essential oil. It is also known as hydrolat, hydrolate, distillate water or floral water.
- Gas chromatography**- chromatography in which the sample mixture is vaporized and injected into a stream of carrier gas (as nitrogen or helium) moving through a column containing a stationary phase composed of a liquid or a particulate solid and is separated into its component compounds according to the affinity of the compounds for the stationary phase.
- Herbs**-are leafy or soft flowering parts of plants used to add flavor and/or aroma to food and beverage. In addition to flavoring food and beverages, herbs are also used as medicine, cosmetics, dyes, air fresheners, disinfectants, insect repellants, decorative materials, herbal drinks and teas.
- Hydrophilic** - "Water loving": refers both to stationary phases that are compatible with water and to water-soluble molecules in general.
- Hydrophobic** -"Water hating": refers both to stationary phases that are not compatible with water and to molecules in general that have little affinity for water. Hydrophobic molecules have few polar functional groups: most are hydrocarbons or have high hydrocarbon content.
- Hydrosol**- a product of the distillation process -- it is the fragrant therapeutic water that collects when plants are steam-distilled to release their essential oil. The term hydrosol is a combination of hydro (water) and sol (solution) —that is, a distilled watery solution that contains some micro-drops of essential oil as well as the water-soluble plant components.
- Infusion** - A remedy prepared by soaking plant material in vegetable oil or water, i.e., placing dried lavender buds into olive oil, letting sit in sunlight for several days, will create a lavender infusion.
- Ion Exchange Chromatography (IEC)** - A mode of chromatography in which ionic substances are separated on cationic or anionic sites of the packing. The sample ion (and usually a counterion) will exchange with ions already on the ionogenic group of the packing. Retention is based on the affinity of different ions for the site and on a number of other solution parameters (pH, ionic strength, counterion type, etc).
- Maceration** - A remedy prepared by soaking plant material in vegetable oil or water. The process of maceration makes the material soft.
- Mobile Phase** - The solvent that moves the solute through the column
- Octadecylsilane (ODS or C18)** -The most popular reversed phase packing in HPLC. Octadecylsilane phases are bonded to silica or polymeric supports. Both monomeric and polymeric phases are available.
- Oilseed**- a seed that contains a significant amount of oil that can be extracted for use in biodiesel production
- Oleoresin** - A naturally occurring mixture of oil and a resin extracted from various plants, such as ginger, turmeric or pepper.
- Polarimeter** - An instrument used to determine the angle through which the plane of polarization of plane-polarized light is rotated on passing through an optically active substance.
- Preparative Thin Layer Chromatography (PTLC)**- in which the stationary phase is a thin layer of an adsorbent such as silica gel coated on a flat plate. It is used for isolation of organic compounds
- Pycnometer** - A standard vessel used in measuring the density or specific gravity of materials
- Refractometer** - An instrument that measures the bending (refraction) of light through a liquid; used to figure out the refractive index of a sample
- Retention Time (tR)** - The time between injection and the appearance of the peak maximum.
- Resolution** - A measure of the separation of two peaks taking into account both the difference in elution time and the peak widths

• **Solid Phase Extraction (SPE)** - A sample preparation technique that uses a solid phase packing contained in a small plastic cartridge. The solid stationary phases are the same as HPLC packing; however, the principle is different from HPLC. The process, as most often practiced, requires four steps: conditioning the sorbent, adding the sample, washing away the impurities, and eluting the sample in as small a volume as possible with a strong solvent.

solute - The dissolved component of a mixture that is to be separated in chromatographic column

spectrophotometer - A method of chemical analysis based on the absorption or attenuation by matter of electromagnetic radiation of a specified wavelength or frequency. The radiation interacts with specific features of the molecular species being determined, such as the vibrational or rotational motions of the chemical bonds. The radiation can also interact with specific atoms or the whole molecule, for example, by causing the molecule to change its electronic energy state.

Split - A GC injector that divides the injected sample and sends only a fraction of it to the column

Splitless - A type of GC injector that sends the entire injected sample to the column

Stationary Phase - The immobile phase involved in the chromatographic process.

Thermal Conductivity Detector (TCD) - Heated elements form the arms of a Wheatstone bridge. Analytes passing through one chamber change the temperature and therefore the resistance, which is monitored. It is a universal, non-destructive detector.

Volatile - Evaporating readily at normal temperatures

Annex-1: Sample code and labeling

| No. | Scientific name | Common name | Local name | Sample code |
|-----|------------------------------|-------------------|---------------------|-------------|
| 1 | <i>Achillea millefolium</i> | Yarrow | - | AM |
| 2 | <i>Ajuga remota</i> | - | Harmagusa (Anamiro) | AR |
| 3 | <i>Aloe vera</i> | Aloes | Eret | AV |
| 4 | <i>Aloysia triphlolia</i> | | Lominat | AT |
| 5 | <i>Amaranthus spp</i> | Amaranthus | | AS |
| 6 | <i>Ananas comosus</i> | Pineapple | Ananas | AC |
| 7 | <i>Artemisia abyssinica</i> | | Chigugn | AA |
| 8 | <i>Artemisia afra</i> | Worm-Wood | | AA |
| 9 | <i>Artemisia annua</i> | Chinese worm wood | | AA |
| 10 | <i>Borago officinalis</i> | Borrage | | BO |
| 11 | <i>Cajanus cajan</i> | Pigeon pea | Yergib Ater | CC |
| 12 | <i>Calendula Officinalis</i> | Marygold | | CO |
| 13 | <i>Camelina sativa</i> | | | CS |
| 14 | <i>Catha edulis</i> | Arabian tea | Cat | CE |
| 15 | <i>Catharanthus albus</i> | Periwinkle | | CA |
| 16 | <i>Catharanthus roseus</i> | Periwinkle | | CR |
| 17 | <i>Chrysanthemum</i> | Pyrethrum | Pyrethrum | C |
| 18 | <i>Cinnamomum zeylanicum</i> | Cinamon | Kerefa | CZ |
| 19 | <i>Citrus Medica</i> | Citron | Tiringo | CM |
| 20 | <i>Coriandrum sativum</i> | Coriander | Dimblal | CS |
| 21 | <i>Crambe abyssinica</i> | Crambe | | CA |
| 22 | <i>Crambe hespanica</i> | Carambe | | CH |
| 23 | <i>Curcuma domestica</i> | Turmeric | Ird | CD |
| 24 | <i>Cymbopogon citratus</i> | Lemon grass | Lemi-sar | CC |
| 25 | <i>Cymbopogon martinii</i> | Palmarosa | Tej-sar | CM |
| 26 | <i>Cymbopogon nardus</i> | Citronella grass | | CN |
| 27 | <i>Datura stramonium</i> | Jimson weed | Etse faris | DS |
| 28 | <i>Echinacea purpurea</i> | Echinacea | | EP |
| 29 | <i>Echinops kebericho</i> | | Kebericho | EK |
| 30 | <i>Euphorbia hirta</i> | Pil palm | | EH |
| 31 | <i>Feoniculum vulgare</i> | Asthma plant | Inslal | FV |
| 32 | <i>Guizotia abyssinica</i> | Fennel | Noug | GA |
| 33 | <i>Hagenia abyssinica</i> | Niger seed | Kosso | HA |
| 34 | <i>Hibiscus mushata</i> | | | HM |
| 35 | <i>Jasminum floriundum</i> | Jasmine | Tembelel | JF |
| 36 | <i>Lantana camara</i> | | Yewef kollo | LC |
| 37 | <i>Lavandula spicata</i> | Lavander | | LS |
| 38 | <i>Lipidium sativum</i> | Cress | Feto | LS |
| 39 | <i>Lippia adonisis</i> | | Kosseret | LA |
| 40 | <i>Majorana hortensis</i> | Marjoram | | MH |
| 41 | <i>Mentha arvensis</i> | Japanese mint | | MA |
| 42 | <i>Mentha longifolia</i> | | | ML |
| 43 | <i>Mentha piperita</i> | Pepper mint | | MP |
| 44 | <i>Mentha spicata</i> | Spear mint | Nana | MS |
| 45 | <i>Nerium oleander</i> | Oleander | | NO |
| 46 | <i>Nigella sativa</i> | Black cumin | Tikur Azmud | NS |

| No. | Scientific name | Common name | Local name | Sample code |
|-----|-------------------------------------|-------------------|------------------|-------------|
| 47 | Ocimum basilicum Var. Thyrstifolium | Basil | Adjuban | OB |
| 48 | Ocimum basilicum | Basil | Besobila | OB |
| 49 | Oenothera biennis | Evening Primerose | | OB |
| 50 | Oenothera lamarckiana | Evening Primerose | | OL |
| 51 | Pelargonium garviolence | Geranium | | PG |
| 52 | Persea americana | Avocado | Abukato | PA |
| 53 | Phytolaca dodecandra | Poke Berry | Indod | PD |
| 54 | Pimpinella anisum | Anis | | PA |
| 55 | Piper longum | | Timiz | PL |
| 56 | Plumbago zylanica | | | PZ |
| 57 | Punicua granatum | Pomegrarate | Roman | PG |
| 58 | Rosmarinus Offcinalis | Rosmary | Te'siga metbesha | RO |
| 59 | Ruta chaopensis | Rue | Tenadam | RC |
| 60 | Salvia officinalis | Sadge | | SO |
| 61 | Salvia schimper | | Ketetina | SS |
| 62 | Salvia sclarea | | | SS |
| 63 | Tanacetum partenium | Fever few | | TP |
| 64 | Thymus schemperi | Thyme | Tosgne | TS |
| 65 | Thmus vulgares | thyme | Tosgne | TV |
| 66 | Verbascum sinaiticum | | Yehaia joero | VS |
| 67 | Vernonia amygdalina | Bitter Leaves | Grawa | VA |
| 68 | Vemonia galamensis | Vernonia | Vemonia | VG |
| 69 | Vetivaria zezanoid | Vetivar | Vetivar | VZ |
| 70 | Vinca minor | Leser Periwinkle | | VM |

Acronyms

| | |
|----------------------|--|
| AMBRC | Aromatic, Medicinal and Bioenergy Research Case |
| b f 3 | Boron trifluoride |
| CC | Column Chromatography |
| ECD | Electron Capture Detector |
| FID | Flame Ionization detector |
| GC | Gas Chromatography |
| GC/MS | Gas Chromatography coupled with Mass Spectrometer |
| GLP | Good laboratory practice |
| HPLC | High-performance liquid chromatography |
| LC | Liquid chromatography |
| LSC | Liquid/solid chromatography |
| MPLC | Medium pressure liquid chromatography |
| PTLC | Preparative thin Layer Chromatography |
| R_f | Retention factor |
| RI | Refractive index |
| RPC | Reversed phase chromatography |
| R_t | Retention time |
| TCD | Thermal Conductivity Detector |
| TLC | Thin-layer chromatography |
| UV/Vis | Ultraviolet/Visible |
| WVO | Waste vegetable oil |

