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EXTRACTION PHYTOCHEMICAL SCREENING AND IDENTIFICATION OF NATURAL PIGMENTS FROM MEDICINALPLANTS BY THIN LAYER CHROMATOGRAPHY

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ABSTRACT

Preliminary qualitative phytochemical analysis made for the leaves of Coriandrum Sativum and Rhizome of Curcuma Longa revealed that presence of glycosides, saponins, fats and oils, sterols and flavonoids. So, these species are expected to have many medical uses. TLC offers several benefits over the other chromatographic separation. The biggest advantage offered to the chromatography is time saving when compared to other chromatographic techniques. The non-volatile compounds can be separated by this method, where in gas chromatography and other chromatographic techniques only volatile compounds can be separated. All components of UV light are achieved to visualize microlite 'quantity of samplecan also be separated in TLC. TLC proved to be successful method of identifying unknown compounds and determining thepolarity of the substance. The rf value helps to identify the phytochemical constituents presentin the chloroform extraction of Coriandrum Sativum leaves and Curcuma Longa rhizome. The present study was designed to extract, identify the phytochemically active constituents inrhizome of Curcuma Longa and leaves of Coriandrum Sativum.

KEYWORDS: Extraction, Maceration, Thin layer chromatography, Curcuma, Coriandrum Sativum.

1. INTRODUCTION

1.1 Pharmaceutical analysis: The pharmaceutical analysis is a branch of chemistry, which involves the series of process for the identification, determination, quantitation, and purification. this is mainly used for the separation of the components from the mixture and for the determination of the structure of the compounds.[1]

Based upon the determination type, there are mainly two types of analytical methods. Theyare as follows:

Types

There are mainly two types of Analysis 1.Quantitative Analysis[estimation] 2.Qualitative Analysis [Identification]

Qualitative analysis: It is performed is establish composition of natural/synthetic substances. These tests are performed to indicate whether the substances or compound is present in the sample or not. Various qualitative test or detection of evolved gas, formation of precipitates, limit test, colour change reactions, melting point and boiling point test etc.

Quantitative analysis: Quantitative Analytical techniques are mainly used to quantify anycompound or substance in the sample. These techniques are based in (a) quantitative performance of suitable chemical reaction and either measuring the amount of reagent added to complete the reaction or measuring the amount of reaction product obtained, (b) the characteristic moment of a substance through a defined medium under controlled conditions, (c) electrical measurement (d) measurement of some spectroscopic properties of the compound.

1.2 Anaytical techniques

Analytical chemistry is the study of the separation identification and quantification of the chemical components of natural and artificial materials. Qualitative analysis gives an indication of the identity of the chemical species in the sample, and qualitative analysis determines the number of certain components in the substance. The separation of components is often performed prior to analysis [2].

These analytical methods are classified as instrumental and classical or non- instrumentalmethods.[3]

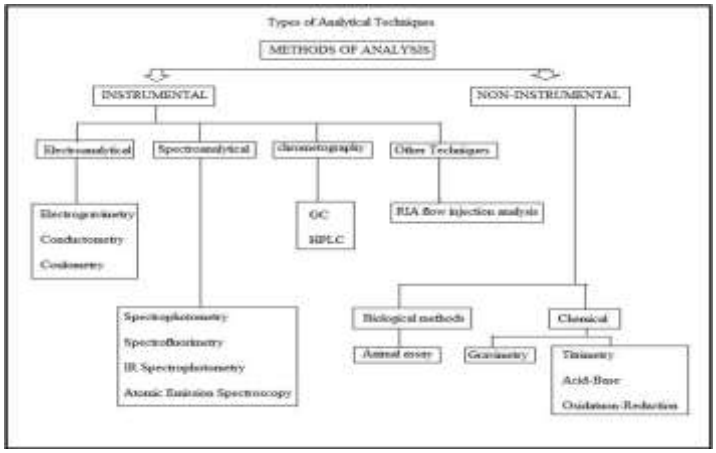


Table 01: Types of analytical techniques.

1.2.1 Importance of analytical method

Quality is important in every product or service, but it is vital in medicine as involves life. Unlike other consumer goods, there can be there is no second quality. Therefore, analytical methods which of a measure of quality of the drug play a very comprehensive role in drug development and follow up activities, to assure that a drug product meets the established standard, is a stable and will continue to meet purported quality throughout its shelf life. Thesemethods should be selective and sensitive to monitor the known and unknown impurities. Haveto be written in a format such that they can be produced over a period of time and from laboratory to laboratory. i.e., these methods should be validated.[4][5]

1.3. Electro analytical method

1.3.1 Poteniometry

- Potentiometry is a technique that is used in analytical chemistry, usually to find the concentration of a solute in solution. In the technique, the potential between two electrodes is measured using a high-impedance voltameter (Wang,2000). Use of a high impedance voltmeter ensures that current flow is negligible. [6]

- One electrode is called the reference electrode and has a constant potential, while the other one is an indicator electrode whose potential changes with the composition of thesample. the difference of potential between the two electrodes gives an assessment of the composition of the sample. Both electrodes are connected to the potentiometer and are immersed in solution the potential difference between the two electrodes is recorded and referred to as the electromotive force. Potentiometry is mainly used to determine the potential or electromotive force of sample solution. The potential id directly proportional to the concentration of the ions.

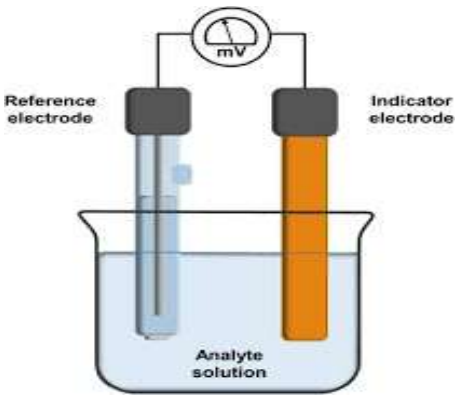


Fig. 1: Potentiometry.

1.3.2 Conductometry

- Conductometry is used to analyse ionic species and to monitor a chemical reaction by studying the electrolytic conductivity of the reacting species or the resultant products. It has notable applications in analytical chemistry. Conductivity measurement can be performed directly by using a conductivity meter or byperforming conductometric titration.
- Conductometry is often applied to determine the total conductance of a solution orto analyse the end point of titrations that include ions. Conductometry is used to determine the water purity.[7]

Fig. 2: Conductometry.

1.4 Spectroscopy

Spectroscopy is the study of the absorption and emission of light and other radiation by matter. It involves the splitting of light (or more precisely electromagnetic radiation) into its constituent wavelengths (a spectrum), which is done in much the same way as a prism splits light into a rainbow of colours. In fact, old style spectroscopy was carried out using a prism and photographic plates. Spectral devices are referred to as spectrometers, spectrophotometers, spectrographs or spectral analysers.[8] The central theory of spectroscopy is that light is made of different wavelengths and that each wavelength corresponds to a different frequency. The importance of spectroscopy is centred on the factthat every different element in the periodic table has a unique light spectrum described by the frequencies of light it emits 5 or absorbs consistently appearing in the same part of the electromagnetic spectrum when that light is diffracted [9].

1.4.1 Ultraviolet-visible spectroscopy

Ultraviolet (UV) spectroscopy is a physical technique of the optical spectroscopy that uses light in the visible, ultraviolet, and near-infrared ranges and it is based on Beer-Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and path length. The method of analysis is based on measuring the absorption of a monochromatic light by colourless compounds in the near ultraviolet path of a spectrum (200-400nm). To determine the "identity, strength, quality and purity" of such compounds.[10]

Principle of UV-Vis Spectroscopy: the absorption of light by a sample in the ultraviolet or visible region is accompanied by a change in the electronic state of the molecules in the sample. The energy supplied by the light will promote electrons from their ground state orbital to higher energy, excited state orbital or anti-bonding orbital. Thus, the following electronic transitions can occur by the absorption of ultraviolet and visible light.[11]

1) σ to σ*
2) n to σ*
3) n to π*
4) π to π*

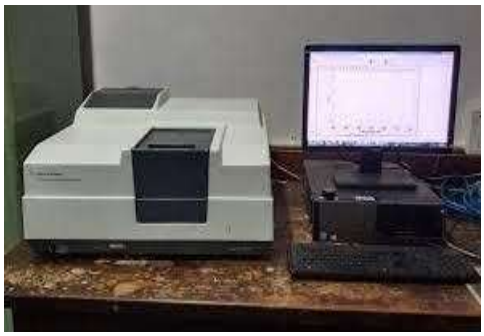


Fig. 3: UV and Visible spectroscopy.

Instrumentation

Sources of visible radiation: The tungsten filament lamp is commonly employed as a source of visible light. This type of lamp is used in the wavelength range of 350-2500 nm.[12]

Monochromator: A monochromator can use either the phenomenon of optical dispersion in a prism, or that of the diffraction using a diffraction grating, to spatially separate the colours of light. A prism monochromator is a mechanism that emits monochromatic light from a light source.

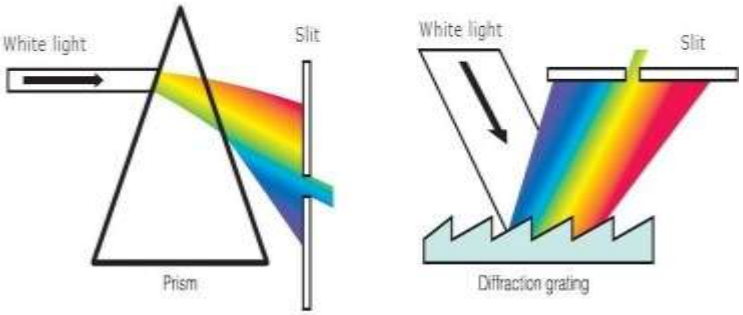


Fig. 4: Monochromator.

Sample cell: The containers for the sample and reference solution must be transparent to the radiation which will pass through them. Quartz or fused silica cuvettes are required foe spectroscopy in the UV region.

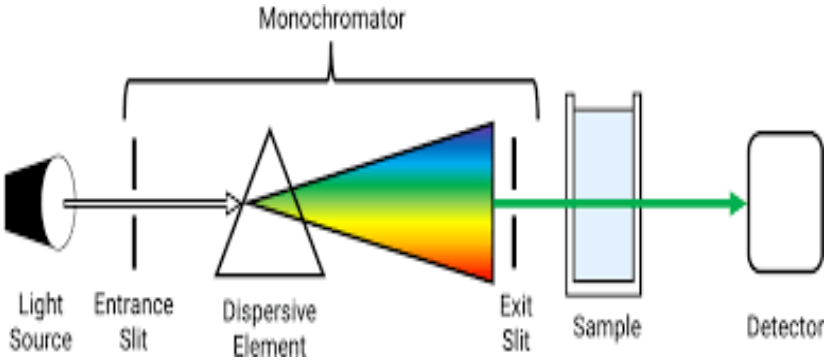


Fig. 5: UV Visible spectroscopy.

Detectors: A detector converts lights signal into an electrical signal. It should give a linear response over a wide range of low noise and high sensitivity.[13]

1. Photomultiplier detector2.Photodiode detector

1.4.2 NMR SPECTROSCOPY

Nuclear magnetic resonance is a spectroscopic technique to observe local magnetic field around atomic nuclei. The sample is placed in a magnetic field and the NMR signal is producedby excitation of the nuclei sample with the radio waves into nuclear magnetic resonance, whichis detected with sensitive radio receivers.[14] The intra molecular magnetic field around an atomin a molecule changes the resonance frequency, thus giving access to details of the electronic structure of the molecules and its functional group.[15]

Instrumentation

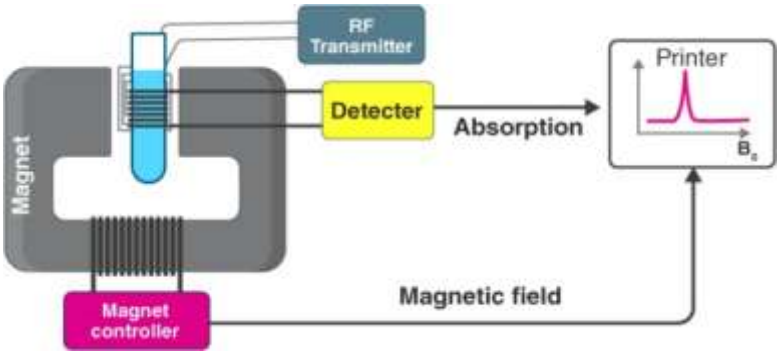


Fig. 6: NMR Spectroscopy.

Sample holder: Glass tube with 8.5 cm long, 0.3 cm in diameter.

Permanent magnet: It provides a homogeneous magnetic field at 60-100 HMZ.

Magnetic coils: These coils induce a magnetic field when current flows through them

Sweep generator: A produce an equal amount of magnetic field pass through the sample

Radio frequency transmitter: A radio transmitter coil transmitter that produce a shortpowerful pulse of radio waves.

Radio frequency receiver: A radio receiver coil that detects radio frequencies emitted as nuclei relax to a lower energy level.

Read out system: A computer that analytes and records the data.

1.4.3 Infrared spectroscopy

Infrared spectroscopy or vibrational spectroscopy is the measurement of the interaction of infrared radiation with matter by absorption, emission, or reflection. IR spectroscopy work onthe principle that molecules absorb specific frequencies that are characteristics of their structure. It is used to study and identify chemical substance or functional group in solid, liquid, or gaseous forms, it can be used to characterize new materials or identify and verify known anunknow sample.[16]

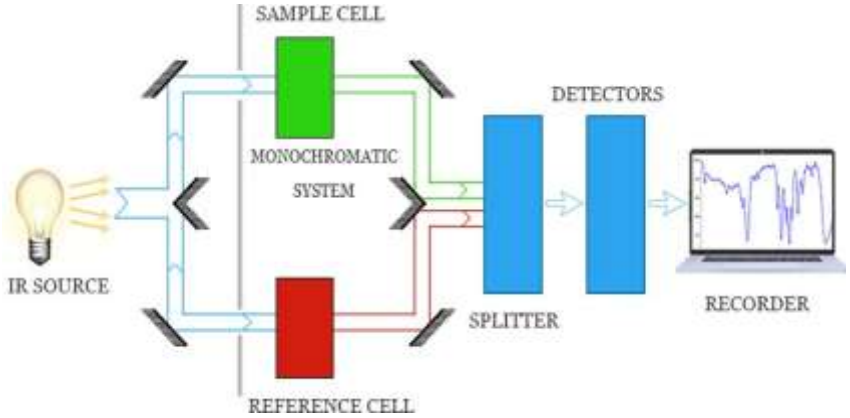


Fig. 7: IR Instrumentation.

1.4.4 Turbidimetry

The measurement of the reduction in light transmission caused by particle formation is termed Turbidimetry. Light transmitted the forward direction id detected. The amount of light absorbed by a suspension of particles depends on the species concentration and on the particle size.[17]

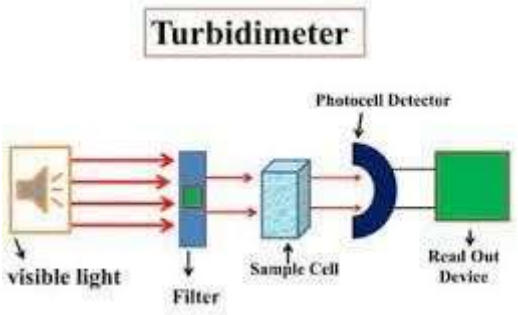


Fig. 8: Turbidimetry.

1.4.5 Nephelometry

Nephelometry is defined as the detection of light energy scattered or reflected toward a detector that is not in the direct path of the transmitted light. A nephelometer is an instrument for measuring the concentration of suspended particulate in a liquid or gas colloid.[18]

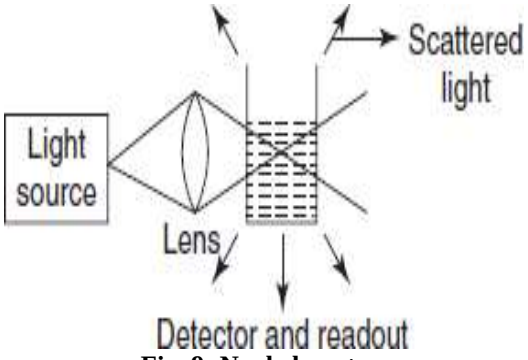


Fig. 9: Nephelometry.

1.5 Chromatography

Chromatography is an important biophysical technique that enables the separation, identification, and purification of the components of a mixture for

qualitative analysis.[19]

Principle of chromatography: Chromatography is based on the principle where molecules in mixture applied on to the surface or into the solid, and fluid stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase. The factor effective on this separation process include molecular related to adsorption (liquid- solid), partition (liquid-solid), and affinity or difference among their molecular weights.[20]

Three components form the basis of the chromatography technique[21]

- Stationary phase: This phase is always composed of a "solid" phase or "a layer of a liquid adsorbed on the surface a solid support".
- Mobile phase: This phase is always composed of "liquid" or a "gaseous component".
- Separated molecules.

1.5.1 Gas chromatography

In gas chromatography, the components of a sample are dissolved in a solvent and vaporized in order to separate the analytes by distributing the sample between two phases: a stationary phase and a mobile phase. The mobile phase is a chemically inert gas such as helium, nitrogen etc. Gas chromatography is one of the unique forms of chromatography that does not need the mobile phase for interacting with the analyte. The stationary phase is either a solid adsorbent, termed gas-solid chromatography (GSC), or a liquid on an inert support, termed gas-liquid chromatography (GLC). The criteria for the components to be analysed in GC is volatility & thermostability.[22][23]

Instrumentation

1) **Sample injection system:** A sample port is necessary for introducing the sample at the head of the column. The vaporization chamber is typically heated 50 C above the lowest boiling point of the sample and subsequently mixed with the carrier gas to transport the sample into the column.
2) **Carrier Gas:** Helium, nitrogen, argon & hydrogen gases are used as carrier gas depending upon the desired performance & detector being used.
3) **Separation column:** Open tubular column or capillary column & packed columns are used in GC.[24]
4) **Column oven or thermostat chambers:** The thermostat oven is there to control the temperature of the column.
5) **Detectors:** Mass spectrometer, flame ionization detector (FID), Electron capture detector (ECD), Thermal conductivity detector (TCD), Atomic emission detector (AED), Photo ionization detector (PID).[25]
6) **Amplification and Recorder system:** These are meant to record the signals that come from the detector. Amplify the signals so as to display in an understandable graphical format that represents several peaks of the constituents of the sample under analysis.

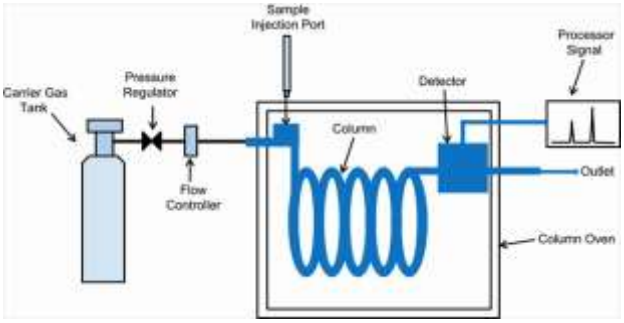


Fig. 10: Gas chromatography.

1.5.2 High performance liquid chromatography

High- performance liquid chromatography (or) High pressure liquid chromatography, (HPLC) is a specific form of column chromatography generally used in biochemistry and analysis to separate, identify, and quantify the active compounds. HPLC mainly utilizes a column that hold packing material (stationary phase), a pump that moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules.[26][27]

Types of HPLC Method

1) **Normal phase chromatography:** NP-HPLC uses a polar stationary phase and a non-polar mobile phase. This, method separates analytes based on polarity.
2) **Reversed phase chromatography:** Reversed phased HPLC (RP-HPLC OR RPC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. RPC operates on the principle of hydrophobic interactions.[28]
3) **Size exclusion chromatography:** Size exclusion chromatography (SEC), also calledas gel permeation chromatography or gel filtration chromatography mainly separatesparticles on the basis of size.
4) **Iron exchange chromatography:** In Ion exchange chromatography, retention is based on the attraction between solute ions and charged sites bound to the stationaryphase

Instrumentation

1) **Injection of the sample:** Septum injection are available; using which sample solution is injection. Sample cab be injection when the mobile phase is flowing or it is stopped. A new advanced rotary valve and loop injector can be used to produce reproducible results.[29]
2) **Detector:** Generally, UV spectroscopy is attached, which detect the specific compounds. Many organic compounds adsorb UV light of various wavelengths. The amount of light absorbed will depend on the amount of a particular compound that is passing through beam at the time.

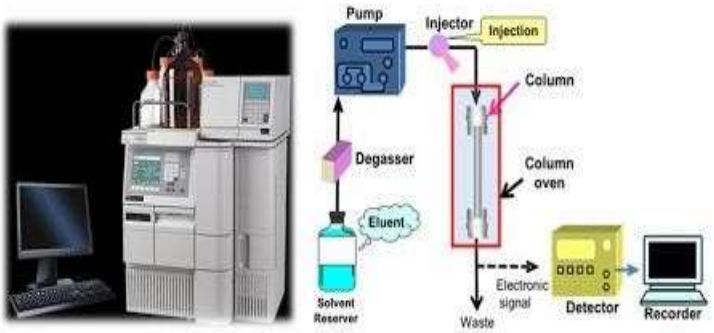


Fig. 11: HPLC spectroscopy.

3) **Interpreting the output from the detector:** The output is recorded as a series of peaks, each one representing a compound in the mixture passing through the detector and absorbing UV light. The area under the peaks is proportional to the amount substance, which is passed through detector, and this area can be calculated automatically by the computer linked to the display.[30]

1.5.3 Thin layer chromatography

Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures. Thin layer chromatography is performed on a sheet of glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide, or cellulose (blotter paper). This layer of adsorbent is known as the stationary phase. After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action.[31]

Principle of tlc

1) Thin layer chromatography uses a thin glass plate coated with either aluminium oxide or silica gel as the solid phase. The mobile phase is a solvent chosen according to the properties of the components in the mixture. The principle of TLC is the distribution of a compound between a solid fixed phase (the thin layer) applied to a glass or plastic plate and a liquid mobile phase (eluting solvent) that is moving over the solid phase.
2) A small amount of a compound or mixture is applied to a starting point just above the bottom of TLC plate.[32]
3) The solvent is drawn up through the particles on the plate through the capillary action, and as the solvent moves over the mixture each compound will either remain with the solid phase or dissolve in the solvent and move up the plate.

Instrumentation[33,34,35]

1) **Glass plate:** Glass plates which are specific dimension like 20cm×20cm, 20cm×10cm, 20cm×5cm can be used. Microscopic slides can also be used for some application can also beused.

Fig. 12: Glass plates (20cm × 20cm).

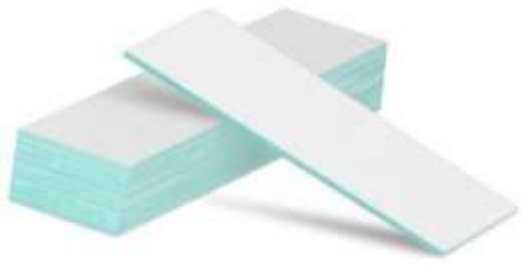


Fig. 13: Glass plates (20cm × 5cm).

2) **Stationary phase:** Several adsorbents are used as a stationary phase. Some of the stationary phase, their composition is mixed with the water or other solvents to form a slurry for preparing thin layer

chromatography.

Table 2: Composition of stationary phase.

Name	Composition
Silica gel H	Silica gel without binder
Silica gel G	Silica gel +$CaSO_4$
Silica gel GF	Silica gel + binder +fluorescent indicator
Alumina	Al_2O_3 without binder
Al_2O_3G	Al_2O_3+Binder
Powdered cellulose	Cellulose without binder
Kieselguhr G	Diatomaceous earth +Binder
Polyamide powder	polyamide
Fullers earth	Hydrous magnesium alumina
Magnesol	Magnesium silicate
Sephadex	Cross linked dextran

3) **Mobile phase:** For silica gel chromatography, the mobile phase is an organic solvent or mixture of organic solvents. As the mobile phase moves pass the surface of the silica gel it transports the analyte pass the particles of the stationary phase. Thus, the fraction of the time that the analyte is bound to the surface of the silica gel relative to the time it spends in solution determines the retention factor of the analyte.[36]

Table 3: Mobile phase.

Component of mobile phase	Ratio	Rf value
Methanol: Chloroform: Acetic acid	8:10:0.2	0.87
Methanol: Chloroform: Acetic acid	3:7:2 drops	0.85
Dichloromethane: Methanol: Acetic acid	4:1:2 drops	0.86
Dichloromethane: Methanol	2:3	0.78
Dichloromethane: Methanol	4:1	0.78
Dichloromethane	1	0.88
Chloroform	1	0.30
Chloroform: Methanol	9.9:0.1	0.55

4) **Preparation and activation of TLC plates:** They are prepared by mixing the absorbent, such as silica gel, with a small amount of inert binder like calcium sulphate (gypsum) and water. This mixture is spread as thick slurry on an unreactive carrier sheet, usually glass, think aluminium foil, or plastic. The resultant plate is dried and activated b heating in an oven for thirty minutes at 110 C. The thickness of the absorbent layer is typically around 0.1-0.23mm for analytical purpose and around 0.5-2.0mm for preparative TLC.[37]

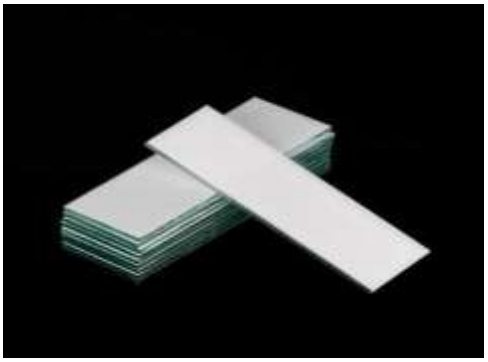


Fig. 14: TLC plates with silica gel.

5) **Methods of preparation of TLC of plates**[38]

- **Pouring method:** The slurry is prepared and poured on to a glass plate which is levelled on a glass plate the slurry is spread uniformly on the surface of the glass plate. After sitting, the plates are dried in oven is used for spotting the samples.

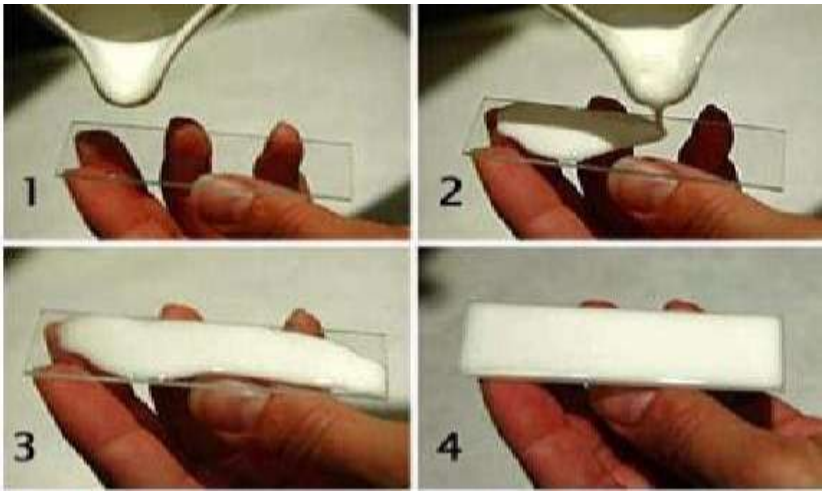


Fig. 15: Pouring method.

- **Dipping method:** This method by making use of a paint sprayer for the distribution ofthe suspension or slurry on to a surface of the glass plates.
- **Spraying method:** This method by making use of a small paint sprayer for the distribution of the suspension or slurry onto a surface of the glass plates.

Fig. 16: Spraying method.

- **Spreading method:** It is best technique where a TLC spreader is used. The specific dimension is stacked on a base plate. After preparation of the slurry is poured inside thereservoir of TLC spreader. The thickness of the adsorbent layer is adjusted by using a knob in the spreader. The plates are allowed for setting (air drying). After setting, the plates are activated by keeping in an oven at 100c 120c for 1 hour.

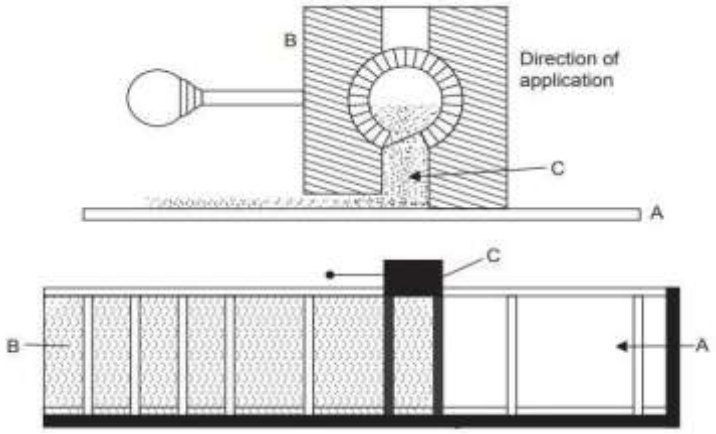


Fig. 17: Spreading method.

6) **Capillary spotters:** Place a melting point capillary and in the dark blue part of the Bunsen burner flame. Hold it there until it softens and starts to sag. Allow the capillary to cool down, and then break it in the middle. Make sure to break off the closed end on one of them.

Fig. 18: Capillary spotting.

7) **Spotting the plate:** The thin end of the spotter is placed in the dilute solution; the solution will rise up in the capillary (capillary forces). Touch the plate briefly at the start line. Allow thesolvent to evaporate and spot at the same place again. Try to avoid spotting too much material, because this will deteriorate the quality of the separation considerably (‘tailing’). Location of spots: The position of various solutes separated by TLC can be located by various methods. Coloured substances can be seen directly when viewed against stationary phase.

8) **Developing methods**

1) **Vertical development or ascending chromatography**. In this common TLC development method, the plate is placed in a suitable TLC developing chamber such that the solvent wets the TLC layer below the starting line. Due to capillary forces, the solvent rises up the layer, transporting the sample mixture. Once the solvent front has reached the predetermined height (10-15 cm for TLC and 3-7 cm for HPTLC), the plate is removed from the chamber, the solvent front is marked with a pencil or spatula, and the plate is dried.

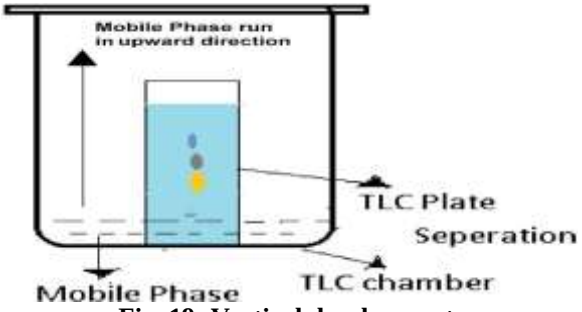


Fig. 19: Vertical development.

2) **Horizontal Development or descending chromatography**

In this TLC developing method, the plate is positioned horizontally inside the chamber, and the solvent is applied using a wick or capillary slit. Development can be performed from one or both sides of the TLC plate.

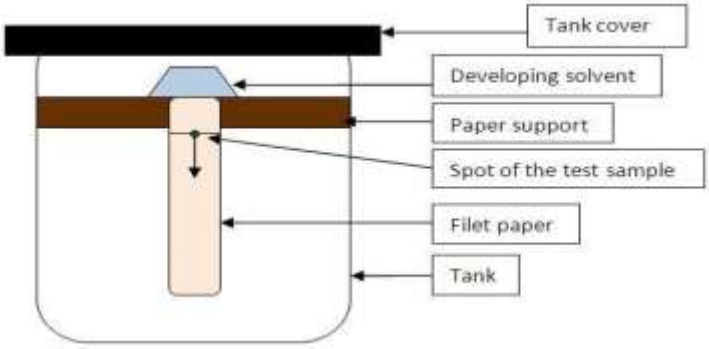


Fig. 20: Horizontal development3)Two-Dimensional Development.

In two-dimensional TLC development, the sample is applied to a starting point in a corner of the TLC plate. The plate is placed in a normal chamber and developed oncefrom bottom to top. After drying, the plate is turned 90° and placed in another chamber with a different solvent and developed again. The chromatogram track fromthe first development is used as the starting line for the second development. Two- dimensional TLC offers the advantage of running a standard with either development.However, the standard cannot be developed in two dimensions on the same chromatogram, since it would mix with the sample.

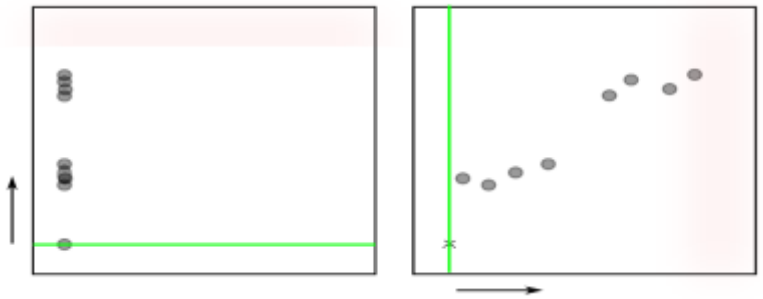


Fig. 21: two-dimensional development.

3) Multiple Development

In this method, the TLC plate undergoes multiple developments with drying between each cycle. The solvent travels repeatedly through the layer, reconcentrating and deforming the spots, often producing elliptical shapes or narrow bands. This significantly improves resolution for substances with Rf values below 0.5. Multiple development can be performed over different separation distances, using the same solvent or different solvents of varying polarity.

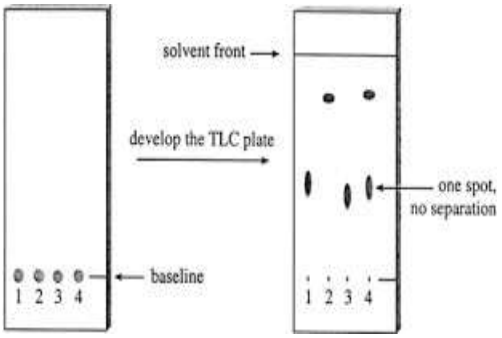


Fig. 22: Multiple dimension.

9) Developing chamber[39]

- Non-polar solvent will force non-polar compounds to the top of the plate, because the compounds dissolve well and do not interact with the polar stationary phase,
- Allow the solvent to travel up the plate until ~1 cm from the top.
- Take the plate out and mark the solvent front immediately. Do not allow the solvent torun over the edge of the plate. Next, let the solvent evaporate completely.
- A TLC plate can be developed in a beaker or closed jar. Place a small amount of solvent(mobile phase) in the container.
- A small spot of solution containing the sample is applied to a plate, about 1cm from thebase.
- The plate is then dipped into to a suitable solvent, such as hexane or ethyl acetate, and placed in a sealed container.
- The solvent moves up the plate by capillary action through the vertical, horizontal, twodimensional and multiple development meets the sample mixture, which is dissolved and is carried up the plate by the solvent.
- Different compounds in the sample mixture travel at different rates due to the differences in their attraction to the stationary phase, and because of differences in solubility in the solvent.
- By changing the solvent, or perhaps using a mixture, the separation of components (measured by the Rf value) can be adjusted.
- The lower edge of the plate is then dipped in a solvent. The solvent (eluent) travels upthe matrix by capillarity, moving the components of the samples at various rates because of their different degrees of interaction with the matrix (stationary phase) and solubility in the developing solvent.

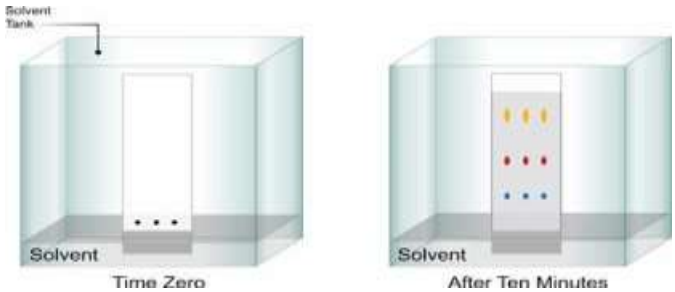


Fig. 23: TLC chromatography.

10) Visualization: When the solvent front has moved to within about 1 cm of the top end of the adsorbent (after 15 to 45 minutes), the plate should be removed from the developing chamber, the position of the solvent front marked, and the solvent allowed to evaporate. If the components of the sample are coloured, they can be observed directly. They can sometimes be visualized by shining ultraviolet light on the plate or allowing the plate to stand for a few minutes in a closed container in which the atmosphere is saturated with iodine vapour.[40]

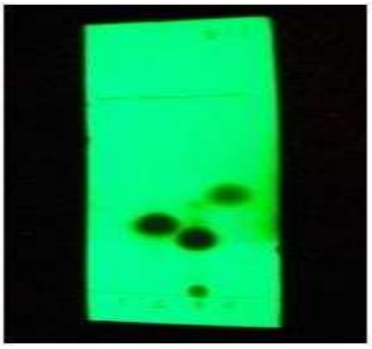


Fig. 24: Visualizing of components.

11) Analysis

The components, visible as separated spots, are identified by comparing the distance they have travelled with those of the known reference materials. Measure the distance of the start line to the solvent front. Then measure the distance of centre of the start line. Divide the distance the solvent moved by the distance the individual spot moved. The resulting ratio is called Rf- value.[41]

RF VALUE

Rf is the "retardation factor", which is the ratio of the distance travelled by a compound in a mobile phase compared with the distance travelled by the front of the mobile phase itself. It is always greater than or equal to zero, and less than equal to 1.

$$Rf = \frac{distance\ travelled\ by\ the\ compound}{distance\ travelled\ by\ the\ solvent\ front}$$

Application

1) Analysis of drug residues and antibiotic in food and environment samples.
2) Identification and qualification of natural product like volatile oil, fixed oil, waves, alkaloids, glycosides, steroids etc.
3) Quality control and purity testing of pharmaceutical formulation.
4) TLC can be used in forensic studies where body fluids, such as urine and blood can be tested for the presence of drugs.
5) Separation of inorganic ions such as cations, anions, purely covalent species.

Advantages of tlc

1) Thin- layer chromatography (TLC) has many advantages over other chromatographic methods, the most important including the simplicity of the equipment, low costs, small requirement as regard the training of personnel and, in many instances, a fairly short time needed for obtaining the results.
2) The separation is done in a very short time as the components elute rapidly. All components of UV light are achievable to visualize. The non-volatile compounds can be separated by this method. The components of complex mixtures easily separate and recover.[42]

1.6 Extraction methods

1.6.1 Maceration

In this process, the whole or coarsely powdered crude drug is placed in a stoppered Container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration decantation after standing.[43]

Fig. 25: Maceration.

1.6.2 Infusion

Fresh infusions are prepared by macerating the crude drug for a short period of time withcold or boiling water. These are dilute solutions of the readily soluble constituents of crude durg.[44]

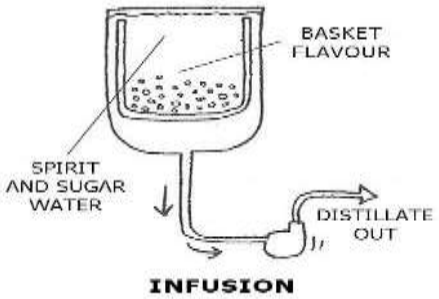


Fig. 26: Infusion.

1.6.3 Digestion

This is a form of maceration in which gentle heat is used during the process of extraction. It is used when moderately elevated temperature is not objectionable. The solvent efficiency of the menstruum is thereby increased.[45]

1.6.4 Decoction

In this process, the crude drug is boiled in a specified volume of water for a defined time;it is then cooled and strained or filtered. This procedure is suitable for extracting water- soluble, heats table constituents. This process is typically used in preparation of Ayurvedic extracts called "quath" or "kawath". The starting ratio of crude drug to water is fixed,the volume is then brought down to one-fourth its original volume by boiling during the extraction procedure. Then, the concentrated extract is filtered and used as such or processed further.[46]

1.6.5 Percolation

The apparatus used in this process is called percolator. It is a narrow-cone-shaped glass vessel with opening at both ends. A dried, grinded, and finely powdered plant material is moistened with the solvent of extraction in a clean container. More quantity of solvent is added,and the mixture is kept for a period of 4h. Subsequently, the content is then transferred into percolator with the lower end closed and allow to stand for a period of 24h. The solvent of extraction is then poured from the top until the drug material is completely saturated. The lower part of the percolator is then opened, and the liquid allowed to drip slowly. Some quantity of solvent was added continuously, and the extraction taken place by gravitational force, pushing the solvent through the drug material downward. The addition of solvent stopped when the volume of solvent added reached 75% of the intended quantity of the entire preparations. Theextract is separated by filtration followed by decantation. The marc is then expressed and finalamount of solvent added to get required volume.[47]

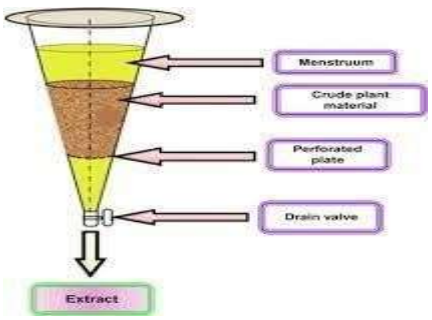


Fig. 27: percolation.

1.6.6 Hot Continuous Extraction (Soxhlet)

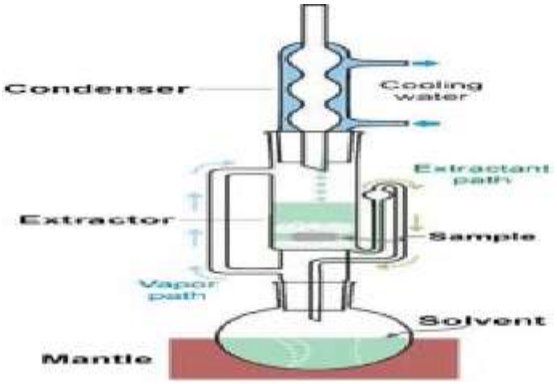


Fig. 28: Soxhlet apparatus.

In this method, the finely ground crude drug is placed in a porous bag or "thimble" made of strong filter paper, which is placed in chamber E of the Soxhlet apparatus. The extracting solvent in flask A is heated, and its vapors condense in condenser D. The condensed extractant drips into the thimble containing the crude drug, and extracts it by contact. Whenthe level of liquid in chamber E rises to the top of siphon tube C, the liquid contents of chamber E siphon into flask A. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated. The advantage of this method, compared to previously described methods, is that large amounts of drug can be extracted with a much smaller quantity of solvent. This effects tremendous economy in terms of time, energy and consequently financial inputs. At small scale, it is employed as a batch process only, but it becomes much more economical and viable when converted into a continuous extraction procedure on medium or large scale [48].

1.7 Phytochemical analysis[56,57]

1.7.1 Test for Alkaloids

a. Mayer's test

To a few ml of plant sample extract, two drops of Mayer's reagent are added along the sides of test tube. Appearance of white creamy precipitate indicates the presence of alkaloids.

b. Wagner's test

A few drops of Wagner's reagent are added to few ml of plant extract along the sides of test tube. A reddish-Brown precipitate confirms the test as positive.

1.7.2 Test for amino acids

The extract (100 mg) is dissolved in 10 ml of distilled water and filtered through WhatmanNo. 1 filter paper and the filtrate is subjected to test for Amino acids.

a. Ninhydrin test

Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) are added to2 ml of aqueous filtrate. Appearance of purple color indicates the presence of amino acids.

1.7.3 Test for carbohydrates

a. Molisch' s test

To 2 ml of plant sample extract, two drops of alcoholic solution of α- naphthol are added.The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.

b. Benedict's test

To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic-colored precipitate indicates the presenceof sugar.

1.7.4 Test for fixed Oils and Fats

a. Spot test

A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

b. Saponification test

A few drops of 0.5 N alcoholic potassium hydroxide solution is added to a small quantityof extract along with a drop of phenolphthalein. The mixture is heated on a water bath for 2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixedoils and fats.

1.7.5 Test for glycosides

For 50 mg of extract is hydrolyzed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests

a. Bontrager's test

To 2 ml of filtered hydrolysate, 3 ml of chloroform is added and shaken, chloroform layeris separated and 10% ammonia solution is added to it. Pink color indicates presence of glycosides.

b. Legal's test

50 mg of extract is dissolved in pyridine, sodium nitroprusside solution is added and made alkaline using 10% NaOH. Presence of glycoside is indicated by pink color.

1.7.6 Test for Phenolic Compounds and Tannins

a. Ferric Chloride test

The extract (50 mg) is dissolved in 5 ml of distilled water. To these few drops of neutral 5% ferric chloride solution are added. A dark green color indicates the presence of phenolic compound.

b. Gelatin test

The extract (50 mg) is dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds.

c. Lead acetate test

The extract (50 mg) is dissolved in of distilled water and to this 3 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

d. Alkaline reagent test

An aqueous solution of the extract is treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

e. Magnesium and Hydrochloric acid reduction

The extract (50 mg) is dissolved in 5 ml of alcohol and few fragments of magnesium ribbon and concentrated hydrochloric acid (drop wise) are added. If any pink to crimson color develops, presence of flavanol glucosides is inferred.

1.7.7 Test for phytosterols

a. Liberman-Burchard's test

The extract (50 mg) is dissolved in of 2 ml acetic anhydride. To this, 1 or 2 drops of concentrated sulphuric acid are added slowly along the sides of the test tube. An array ofcolor change shows the presence of phytosterols.

1.7.8 Test for proteins

The extract (100 mg) is dissolved in 10 ml of distilled water and filtered through Whatman No. 1 filter paper and the filtrate is subjected to test for proteins.

a. Million's test

To 2 ml of filtrate few drops of Million's reagent are added. A white precipitate indicates the presence of proteins.

b. Biuret test

2 ml of filtrate is treated with 1 drop of 2% copper sulphate solution. To this 1 ml of ethanol(95%) is added, followed by excess of potassium hydroxide pellets. Pink colour ethanolic layer indicates the presence of protein.

1.7.9 Test for saponins

The extract (50 mg) is diluted with distilled water and made up to 20 ml. The suspensionis shaken in a graduated cylinder for 15 minutes. A two cm layer of foam indicates the presence of saponins.

1.7.10 Test for Gum and Mucilage's

The extract (100 mg) is dissolved in 10 ml of distilled water and to this 2 ml of absolute alcohol is added with constant stirring. White or cloudy precipitate indicates the presenceof Gums and Mucilage.

1.8 Plant description

1.8.1 Coriandrum sativum[55]

Fig. 29: Coriandrum sativum.

Biological source: Coriander spice is derived from the dried fruits of Coriandrum sativum L. (Apiaceae). It was one of the earliest spices used my mankind.

Geoghraphical source: Cultivated in central and Eastern Europe particularly in Russia, Africa and India. In India it is cultivated in Maharashtra, UP, Rajasthan, Jammu and Kashmir.

Scientific classification:

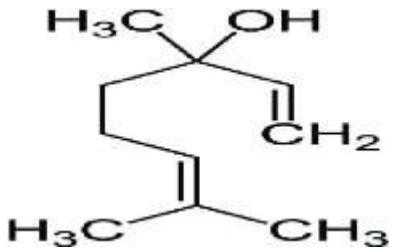
Family	**:** Apiaceae
Ayurvedic name	**:** Dhanyaka (Dhaniya)
Hindi name	**:** Dhania
English	**:** Coriander
Parts used	**:** Fresh leaves and dried seed
Genus	**:** Coriandrum
Species	**:** C. Sativum
Botanical name	**:** Coriandrum sativum

Chemical constitutents:

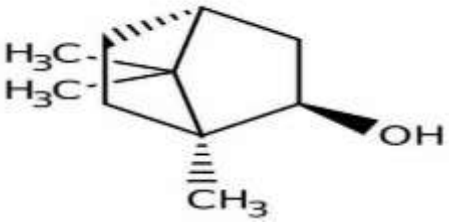
- 0.3-1% of volatile oil
- Volatile components are 90% od D- (+)- Linalool (coriandrol), and coriandryl acetate along with other constituents like, borneol, p-cymene, camphor, geraniol, limonene, and alpha- pinenes.
- The fruits also contain fatty oil and

hydroxycoumarins.

- The fatty oil includes acids of petroselic acid, oleic acid, linolenic acid, whereas the hydroxycoumarins include the umbelliferon and scopoletine.
- Coriander leaves are rich in vitamin A contents.
- Fixed oil 13%, proteins, 20%.



G-Linalool



L-Borneol

Pharmacological activites of corindrum sativum[53]

- Antioxidant.
- Antidiabetic.
- Hepatoprotective.
- Antibacterial.
- Antifungal activities.
- Antimutagenic.
- Anthelmintic
- Sedative- hypnotic.
- Anticonvulsant.
- Diuretic.
- Cholesterol- lowering.
- Antifungal.
- Anticancer.
- Anxiolytic.
- Anti- ulcer active

Medicinal uses: Coriandrum sativum seeds relieve pain, rheumatoid arthritis, prevents from allergies, cure from mouth ulcers. Improves cardiovascular health, maintains blood pressure. Keep anaemia in bay. Regulate menstruation, defend against food poisoning, protectyour nervous system, nourish your eyes, help detoxify heavy metals.

1.8.2 Curcuma longa Linn[45]

Fig. 30: Curcuma longa.

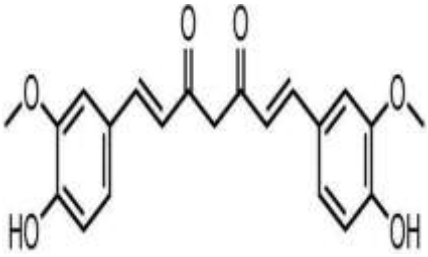
Bological source: Turmeric consist of dried, as well as fresh rhizomes of plant knownas CURCUMA LONGA.
Geographical source: The plant is native to southern Asia and is cultivated extensively in temperate regions. West Pakistan, India, Malaysia, and China.

Scientific classification

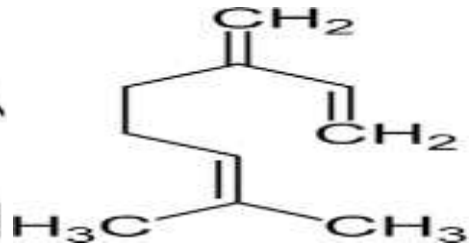
Family : Zingiberaceae
Ayurvedic name : Haldi
English : Turmeric
Hindi name : Haldi
Parts used : Rhizomes
Genus : Curcuma
Species : Longa
Botanical name : Curcuma longa

Chemical constitutents

- Turmeric contents about 5% curfew Nights as a colouring matter. Containscurcumine-1, curcumine-2, curcumine-3.
- Turmeric contains about 5% volatile oil. there were a volatile oil containssesquiterpenes, alcohol, ketone, monoterpene.
- Example: zingiberene, turmerone, AR turmerone, alcohol-p-Toly methyl, carbinol, cineole, borneol, etc.
- It also contains arabinose, fructose, glucose, and starch grains.



Curcumin



Monoterpene

Pharmacological activies

- Anti- inflammatory.
- Antioxidant.
- Anticancer.
- Antimutagenic.
- Antimicrobial.
- Anti-obesity.
- Antidiabetic
- Hypolipidemic.
- Cardioprotective.
- Neuroprotective effect.
- Antifungal activities.
- Anthelmintic
- Sedative- hypnotic.
- Anticonvulsant.

Medicinal uses: Oxidation by free radicals is linked with accelerated aging and virtually every major chronic disease, cataracts, and rheumatoid arthrities. One way to stop this is with anti-oxidants like vitamin C and E and turmeric. Turmeric is stop this is with anti-oxidant capacity, reduce inflammation, treats and prevents cancer, improves immune system, improved brain function. Improves digestion, promotes weight loss, helps in wound healing, improve the skin health, detoxifies the liver, helps control diabetes, it has an anti-inflammatory property[54].

2. MATERIALS AND METHODS

2.1. Plant material collection

The plants were collected from local market and areas of Jagans college oof pharmacy campus.The collected leaves of plants were made thorougly free from any foreign organic matter, driedunder shade, powdered and stored in a well closed conatainer.

2.1.1. Glasswares used

Table 4: Glassware.

S. NO.	Glasswares	Materials
1.	Conical flask	Borosillicate glass
2.	Funnel	Borosillicate glass
3.	Beakers	Borosillicate glass
4.	Tripod stand	Aluminum
5.	Glass rod	Borosillicate glass
6.	Pipette	Borosillicate glass
7.	Measuring cylinder	Polypropylene
8.	Capillary tube	Borosillicate glass
9.	Test tubes	Pyrex glass
10.	Motor and pestle	Ceramic
11.	Glass slides	Borosillicate glass

2.1.2. Chemicals

Table 5: Chemicals.

S NO	Chemicals	Company
1	Ethanol	Simhapuri chemicals
2	Chloroform	Simhapuri chemicals
3	Acetic acid	Simhapuri chemicals
4	Mayers reagent	Simhapuri chemicals
5	Molisch's reagent	Simhapuri chemicals
6	NaoH	Simhapuri chemicals

2.2. Preparation of plant extraction

2.2.1. Maceration method[56]

5gm of the powdered rhizomes of curcuma longa and powdered leaves of coriander were extracted separately with 100 ml of water for 7 days and kept in air tight container until the extracts were obtained. The solvent extracts were subjected to phytochemical screening and Thin layer chromatography technique.

Fig. 31: Maceration method.

2.3 Phytochemical screening

The phytochemical screening of the three plants showed the presence of different primary and secondary bioactive molecules like carbohydrates, proteins, fixed oils, alkaloids, flavanoids, tannins and saponins.[58]

2.3.1 Tests for Alkaloids

- **Dragendorff's test:** To 1 ml of each of the sample solution taken in a test tube few drops of Dragendorff's reagent (potassium bismuth iodide solution) was added. A reddish-brown precipitate was observed indicating the presence of alkaloids.
- **Meyer's test:** To 1ml of each of the sample solution few drops of Meyer's reagent (potassium mercuric chloride solution) was added. A creamish white precipitate was formed indicating the presence of alkaloids. 3) Wagner's test: To few ml of each of the sample solution, Wagner's reagent (Iodine in potassium iodide) was added, which resulted in the formation of reddish-brown precipitate indicating the presence of alkaloids.

2.3.2 Tests for glycosides

- **Concentrated Sulphuric acid test:** Conc.H2SO4 was added to test sample which resulted in appearance of reddish colour.

2.3.3. Tests for Tannins and Phenolic compounds

- **Gelatine test:** When gelatin and water were added to test samples formation of white precipitate was resulted.
- **Alkaline reagent:** When sodium hydroxide solution was added to the sample solution results in the formation of yellow to red precipitate.

2.3.4. Tests for Saponins

- **Foam test:** 5ml of extract was shaken vigorously to obtain a stable persistent froth. The froth was then mixed with three drops of olive oil and observed for the formation of an emulsion, which indicated the presence of saponins.

2.3.5. Tests for Fats and Oils

- **Stain test:** Press the small quantity of each extract between two filter papers, the stain on filter papers indicates the presence of the oils.

2.3.6. Tests for flavonoids

- **Alkaline reagent test:** When sodium hydroxide solution was added to the test samples formation of intense yellow colour, which turns to colour less on addition of few drops of dilute acid indicates the presence of flavonoids.

2.3.7. Tests for sterols

- **Libermann-Buchard test:** When samples were treated with few drops of aceticanhydride, boiled and few drops of concentrated sulphuric acid from the sides of the test tube were added, shows a brown ring at the junction of two layers and the upper layer turns green which shows the presence of steroids.

2.3.8. Test for carbohydrates

- **Molisch test:** When alpha naphthol and concentrated H2SO4 were added to testsamples reddish violet ring at junction of two layers was resulted.
- **Benedict's Test:** Add 1 mL of Benedict's reagent to test tube C and heat the mixture to boiling in a water bath for 2 minutes. The formation of an orange red precipitate due to the formation of copper (I) oxide indicates the presence of reducing sugar.

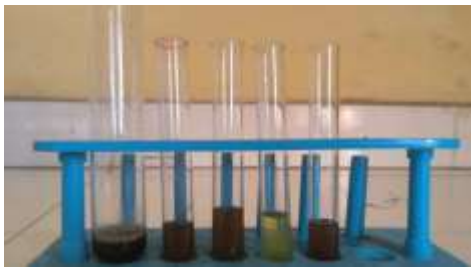


Fig. 32: Phytochemical screening.

2.4. Thin layer chromatography[63]

2.4.1. Stationary phase

These are stable and chemically inert plants, where a thin layer of stationary phase is applied on its whole surface layer. The stationary phase on the plates is uniform thickness and is in fine particle size. Here artificially coated stationary plates were used.

Fig. 33: Artificial TLC plates.

2.4.2. Mobile phase

This comprises a solvent and solvent mixture. The mobile phase used should be particulate- free and of the highest purity for the proper development of TLC spots. The solvents recommended are chemically inert with the sample, A stationary phase.

Table 6: Mobile phase for TLC

S. no.	Plants	Mobile phase	Ratio
1	Curcuma longa	Chloroform: acetic acid: ethanol	7:2:3
2	Coriandrum sativum	Chloroform: acetic acid: ethanol	7:2:3

2.4.3. Capillary spotters: Place a melting point capillary in the dark blue part of the bunsen burner flame. Hold it there until it softens and starts to sag. Allow the capillary to cool down and then break it in the middle. Make sure to break off the closed end on one of them.

2.4.4. Spotting the plate: The thin end of the spotter is placed in the dilute solution; the solution will rise up in the capillary (capillary forces). Touch the plate briefly at the start line.

Fig. 34: Spotting of curcuma.

Fig. 35: spotting of corindrum sativum.

2.4.5. Developing chamber

1) A TLC plate can be developed in a beaker or closed jar. Place a small quantity of solvent(mobile phase) in the container.
2) A small spot of solution containing the sample is applied to a plate, about 1cm from thebase
3) The plate is then dipped into a suitable solvent such as hexane or methyl acetate, and placed in a sealed container.
4) The solvent moves up the plate by capillary action through the ascending order and meets the sample mixture, which is dissolved and is carried up the plate by solvent. **Advantages:** Ascending order requires a very small amount of sample and takes only about a half an hour or so for chromatographic separations
5) By changing the solvent, or perhaps using a mixture, the separation of components (measured by the Rf value) can be adjusted.
6) Non -polar solvents will force non polar compounds to the top of the plate because the compounds dissolve well and don't interact with the polar stationary phase. And don't interact with the polar stationary phase and don't interact with the stationary

polar phase allow the solvent to travel up the plate until 1cm from the top.

7) Take the plate out and mark the solvent front immediately don't allow the solvent to run over the edge of the plate. Next, let the solvent evaporate.

Fig. 36: Ascending order of developing chamber.

2.4.6. Visualization under visible light

The plate should be removed from the developing chamber, the position of the solvent front marked, and the solvent allowed to evaporate. If the components of the sample are coloured, they can be observed directly.

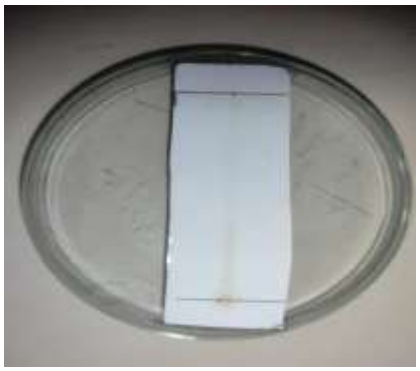


Fig. 37: Coriandrum sativum

Fig. 38: Curcuma longa.

2.4.7. Analysis of TLC Plates

- The components, visible as separated spots, are identified by comparing the distances they have travelled with those of the known reference materials.
- Measure the distance of the start line to the solvent front. Then measure the distance of the centre of the spot to the start line.
- Divide the distance the solvent moved by the distance the individual spot moved.
- The resulting ratio is called Rf-value.

2.4.8. Rf Value

Rf is the "Retardation Factor", which is the ratio of the distance travelled by a compound in a mobile phase compared with the distance travelled by the front of the mobile phase itself. It is always greater than or equal to zero, and less than or equal to one.

$$Rf = \frac{distance\ travelled\ by\ the\ compound}{distance\ travelled\ by\ the\ solvent\ front}$$

3. RESULT AND DISCUSSION

Table 07: Phytochemical analysis of plants.

Bio active groups	Curcuma longa	Coriandrum sativum
Alkaloids	Absent	Absent
Glycosides	Present	Present
Tannins	Absent	Absent
Saponins	Present	Present
Fats and oils	Absent	Present
Sterols	Present	Absent
Flavonoids	Present	Present
Carbohydrates	Absent	Absent

Table 08: Rf value of plants.

Sample id	Plant species	No of spots detected	Rf value
1	Curcuma longa	2 spots	A) 2.3 B) 5.6
2	Corinadrum sativum	2 spots	A) 1.2 B) 4.4

- Bio active compounds of two plant extracts were identified by using phytochemical analysis test like Mayer's test, Molisch's test, Bontrager's test, And Ferric chloride test. Phyto chemicals like alkaloids tannins, glycosides, carbohydrates, amino acids, flavonoids and saponins are identified in these plants. Most extracts that we handled are curcuma longa rhizome and Coriandrum sativum leaves should give a single spot on two TLC plates ideally each compound in a mixture will produce distinct spot as a sample with two extracts will give two different spots and so on. Mixture of components are separated identically in TLC method.
- The Rf value is dependent on both the compound and the solvent used for development if we analyse two compounds and they give the Rf value with the same solvent system.

4. SUMMARY AND CONCLUSION

The present study was designed to extract, identify the phytochemically active constituents in rhizomes of Curcuma and leaves of Coriandrum Sativum by TLC method.

On the basis of the results obtained, the following salient findings have emerged

- Preliminary qualitative phytochemical analysis made for the leaves of Coriandrum sativum and Rhizome of Curcuma longa revealed that the presence of glycosides, flavonoids, fats and oils, saponins and sterols. so, these species are expected to have many medicinal uses.
- TLC offers several benefits over the other chromatographic separation. The biggest advantage offered to the chromatography is time saving when compared to other chromatographic techniques. The non-volatile compounds can be separated by this method, where in gas chromatography and other chromatographic techniques only volatile compounds can be separated. All components of UV Light are achieved to visualise microlite `quantity of sample can also be separated in TLC.
- TLC proved to be successful method of identifying unknown compounds and determining the polarity of the substance. the manual error is accepted in TLC up to 1%but also this provides accurate results where as in other or HPTLC Methods the same error may give different results and also damage the equipment.
- The Rf value helps to identify the different phytochemical constituents present in the extracts of Curcuma longa and Coriandrum sativum.

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