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PESCP, Dept. of Pharmaceutical Analysis

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List of Abbreviations

Abbreviations and Symbols	Full Form
%	Percentage
Ml	Microliters
µ	Micron
Mm	Micro meter
Cm	Centimeter
Nm	Nanometer
Conc.	Concentration
NO	Nitric oxide
MPP+1	1-methyl-4-phenylpyridinium
g	gm
A	Alpha
B	Beta
Ar	aromatic
HPLC	High Performance Liquid Chromatography
UV	Ultra Violet spectroscopy
Sl. No.	Serial Number
TLC	Thin layer chromatography
LC	Liquid Chromatography
WHO	World Health Organization
J	Journal
Res	Research
Ltd	Limited
Pvt	Private
Abs	Absorbance
Mg	Milligram
VIS	Visible

CHAPTER-1:

INTRODUCTION

Chapter-1:INTRODUCTION

The term "**medicinal plant**" include various types of plants used in herbalism ("herbology" or "herbal medicine"). It is the use of plants for medicinal purposes, and the study of such uses.

The word "**herb**" has been derived from the Latin word, *"herba"* and an old French word *"herbe"*. Now a days, herb refers to any part of the plant like fruit, seed, stem, bark, flower, leaf, stigma or a root, as well as a non-woody plant. Earlier, the term "herb" was only applied to non-woody plants, including those that come from trees and shrubs. These medicinal plants are also used as food, flavonoid, medicine or perfume and also in certain spiritual activities.

Recently, WHO (World Health Organization) estimated that 80 percent of people worldwide rely on herbal medicines for some aspect of their primary health care needs .According toWHO,around21,000plantspecieshave the potential for being used as medicinal plants.

Medicinal plants such as *Aloe, Tulsi, Neem, Turmeric* and *Ginger* cure several common ailments. These are considered as home remedies in many parts of the country.

Medicinal plants are considered as a rich resource of ingredients which can be used in drug development either pharmacopeial, non- pharmacopeial or synthetic drugs. A part from that, these plants play a critical role in the development of human cultures around the whole world. Moreover, some plants are considered as important source of nutrition and as a result of that they are recommended for their therapeutic values. Some of these plants include ginger, green tea, walnuts, aloe, pepper and turmeric etc. Now a days medicinal herbs are important sources for pharmaceutical manufacturing.

Recipes for the treatment of common ailments such as diarrhoea, constipation, hypertension, low sperm count, dysentery and weak penile erection, piles, coated tongue, menstrual disorders, bronchial asthma, leucorrhoea and fevers are given by the traditional medicine practitioners very effectively.

1.1.TURMERIC: is a flowering plant, *Curcuma longa*, of the ginger family, Zingiberaceae, the rhizomes of which are used in cooking. The plant is a perennial, rhizomatous, herbaceous plant nativetotheIndiansubcontinentandSoutheastAsiathatrequirestemperaturesbetween20and30°C(68and 86°F) and a considerable amount of annual rainfall to thrive. Plants are gathered each year for their rhizomes, some for propagation in the following season and some for consumption.

Kingdom	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Monocots
Clade:	Commelinids
Order:	Zingiberales
Family:	Zingiberaceae
Genus:	Curcuma
Species:	C. longa

table1:Taxonomical classification

Binomial name : Curcuma longa

Synonyms:Curcuma domestica Valeton

Fig1: botanical view of curcuma longa

fig2: turmeric rhizome and powder

Phytochemistry: Turmeric powder is about 60–70% carbohydrates, 6–13% water, 6–8% protein, 5–10% fat,

3– 7% dietary minerals, 3–7% essential oils, 2–7% dietary fiber, and 1–6% curcuminoids. The golden yellow color of turmeric is due to curcumin.

Phytochemical components of turmeric include diarylheptanoids, a class including numerous curcuminoids, such as curcumin, desmethoxycurcumin, and bisdemethoxycurcumin. Curcumin constitutes up to 3.14% of assayed commercial samples of turmeric powder (the average was 1.51%); curry powder contains much less (an average of 0.29%). Some 34 essential oils are present in turmeric, among which turmerone, germacrone, atlantone,
and zingiberene are major constituents.

1.1.1. PLANT TOXICITY: Toxicity of 200 chemical compounds from turmeric were predicted (includes bacterial mutagenicity, rodent carcinogenicity and human hepatotoxicity). The study shows out of 200 compounds, 184 compounds were predicted as toxigenic, 136 compounds are mutagenic, 153 compounds are carcinogenic and 64 compounds are hepatotoxic. To cross validate our results, we have chosen the popular curcumin and found that curcumin and its derivatives may cause dose dependent hepatotoxicity. The results of these studies indicate that, in contrast to curcumin, few other compounds in turmeric which are non-mutagenic, non-carcinogenic, non- hepatotoxic, and do not have any side-effects. Hence, the cost-effective approach presented in this paper could be used to filter toxic compounds from the drug discovery lifecycle

Studies now numbering in the hundreds have shown that in the spice may curcumin may be helpful for a wide array of health problems. For example, research has shown the turmeric can:

- Support healthy cholesterol levels
- Prevent low-density lipoprotein oxidation
- Inhibit platelet aggregation
- Suppress thrombosis and myocardial infarction
- Suppress symptoms associated with type 2diabetes
- Suppress symptoms of rheumatoid arthritis
- Suppress symptoms of multiple sclerosis
- Protect against radiation-induced damage and heavy metal toxicity
- Inhibit HIV replication
- Suppress tumor formation
- Enhance wound healing
- Protect against liver damage
- Increase bile secretion
- Protect against cataracts
- Protect against pulmonary toxicity and fibrosis
- Protect against dementia and Alzheimer’s disease

1.2.Turmerones: are the principal sesquiterpenes obtained from turmeric. They are categorized as α-turmerone, ar-

2. turmerone, and β-turmerone, and among them ar-turmerone has been found to be more effective against cancer. However, Yue et al. elucidated the immunomodulatory and chemopreventive activities of α-turmerone and unraveled its efficiency to be on par with curcuminoids.

3.Turmerone is principle flavouring compound of turmeric (Curcuma longa L.). The objective of the research work was to isolate turmerone from turmeric oil and its characterization. Turmerone was extracted from turmeric oil. It was further purified with activated charcoal or preparative TLC. Turmerone shows violet spot at Rf of 0.72 with vanillin-sulfuric acid on heating. A UV spectrum of the isolated compound shows two peaks of almost same intensity at 233.5 nm and 236 nm.

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1.2.1 Study discloses neuroprotective properties of aromatic turmerone and its derivatives:

Scientists from Japan's Kumamoto University discovered that aromatic turmerone (ar-turmerone), a compound extracted from turmeric essential oil, and its derivatives have a direct effect on the dopaminergic nerves to produce a neuroprotective effect on tissue cultures of a Parkinson's disease model. Parkinson's is characterized by immobility, limb tremors, muscle rigidity, and other movement disorders. Right now, it is treated with dopamine supplements; however, there is no means to inhibit dopaminergic neurodegeneration.

Earlier research works found that the inflammatory response produced by the activation of microglia (cells in charge of immune function in the brain) is noticed in the substantia nigra of the midbrain of patients with Parkinson's disease.

Additional experiments to mirror the *in vivo* state of the midbrain (midbrain slice culture) showed that microglial activation activates the selective degeneration of dopaminergic neurons in the substantia nigra, and that nitric oxide (NO) produced from activated microglia was part of the neurodegeneration.

The study outcomes indicate that compounds that have anti-inflammatory effects on microglia may inhibit dopaminergic degeneration.

The team examined aromatic turmerone (ar-turmerone), the main constituent of turmeric essential oil found to exhibit anti-tumor and anti-inflammatory effects on microglia.

Ar- turmerone possesses an asymmetric carbon (S-Tur), hence the scientists created eight analogs and tried to determine the ones with powerful anti-inflammatory effects..The researchers employed the inhibitory effects on the inflammatory response as produced by lipopolysaccharide (LPS)-stimulated activation of BV2 cells as an indicator. The analogs (R)-ar-turmerone (R-Tur), ar-atlantone (Atl), and analog 2 (A2) exhibited higher anti-inflammatory effects when compared to S-Tur.

The formation of NO, released from activated microglia and involved in dopaminergic neurodegeneration, was not inhibited. Additionally, three compounds (S-Tur, Atl, and A2) suppressed dopaminergic degeneration induced by MPP+, a toxin selectively damaging dopaminergic neurons independent of microglial activity. The findings
indicated that S-Tur and its derivatives (Atl and A2) directly impact dopaminergic neurons and show neuroprotective effects. Moreover, analysis with midbrain slice cultures and dopaminergic progenitor cell lines showed that the neuroprotective effects of Atl and A2 are induced by activation of Nrf2, a transcription

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factor that increases the antioxidant potency of cells.

Techniques employed for development of Analytical Method:

1.3. ULTRA VIOLET VISIBLE SPECTROSCOPY (UV Vis SPECTROSCOPY):

Spectroscopy, also known as UV-Visible spectrophotometry, is the study of absorption or reflectance spectra in the visible and ultraviolet portions of the electromagnetic spectrum. This methodology is frequently employed in a variety of practical and theoretical applications since it is reasonably affordable and simple to execute. The sample must only be a chromophore and absorb in the UV-Visible range. Fluorescence spectroscopy is enhanced by absorption spectroscopy. In addition to the measurement wavelength, variables of importance include absorbance (A), transmittance (%T), and reflectance (%R), as well as how they change overtime.

1.3.1. Steps involved in UV Vis Spectroscopy:

- Sample and standard preparation
- Instrumentation
  - Optical cell
  - Thermostatting the samples
  - Stirring the samples
  - Measurements at low temperatures
  - Solvent transparency
  - Parameter to be measured
  - The linear range of a UV-Vis instrument

1.3.2.Sample &standard preparation:

The sample and standard to be estimated by UV-Vis spectroscopy should be in solution form. Hence, the drug or drug derivative is dissolved in a suitable solvent (polar/non polar) and estimated.

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1.3.3.Instrumentation:

Opticalcell:

A standard 10 mm pathlength optical cell. The cell, which can hold about 3.5 ml, contains two parallel optical windows. The opposite sides are typically frosted or grooved to show that they are to be used for handling the cell. The optical windows must be kept as spotless and untouchable as possible.

Thermostatting the samples:

Despite the fact that many samples may be analysed at room temperature, there are specific situations where samples must be heated or cooled such as cooling volatile sample in order to reduce evaporation ,heating viscous samples in order to enhance sample handling or homogeneity and thermostatting the samples susceptible to chemical alterations when heated.

Stirring the samples:

A thermostatted sample should always be stirred in order to maintain homogeneity of the solution and temperature. For viscous samples or to ensure consistently mixed solutions when observing a chemical reaction inside a cuvette, stirring is especially crucial.

Measurements at low temperatures:

Condensation may develop on the exterior of cuvettes when sampling samples at temperatures below ambient. This can impede the measurement. By blowing a dry, clean gas into the sample chamber of the UV-Vis spectrophotometer, condensation can be avoided.

Solvent transparency:

Solvent transparency must be taken into account while analysing liquid samples or emulsifying solid samples for UV examination. Based on the sample's solubility, stability, required pH, and UV- visible cut- off wavelength, solvents are chosen. Water is a great option for aqueous soluble chemicals since it allows detection across the entire UV spectrum. The spectrum of useful UV wavelengths is really constrained by the usage of organic solvents.

When choosing a solvent, take into account both the sample's solubility in the solvent and the solvent's transparency in the desired wavelength range.

Parameter to bemeasured:

Due to the Beer-Lambert law's description of the linear connection between concentration and absorbance, absorbance (A or Abs) is frequently measured in UV-Vis spectroscopy. The amount of light that is transmitted or absorbed may be more significant for other uses. For particular, it may be more helpful to compare the % transmission or absorbance difference when evaluating a material's optical qualities.

The linear range of a UV-Vis instrument:

The greatest absorbance an instrument will be able to measure at a particular wavelength will be determined by both the instrument design and the measurement parameters utilised. At high absorbance very little light is reaching the detector which decreases the signal to noise ratio (fringe). By being aware of your system's limitations, you can avoid taking samples or performing calibrations that are outside the capabilities of our instrument. For liquid samples, diluting the sample is a way to get the measurement into the linear range of the instrument. Alternatively, a short path length cuvette can be used.

1.3.4.Advantages of UV Vis Spectroscopy:

1. The core advantage is the accuracy of the UV-VIS spectrophotometer
2. The UV-Visible spectrometer is easy to handling and use

3. Provide robust operation
4. UV-Vis spectroscopy is simple to operate
5. Cost effective instrument
6. Cover the entire range of ultraviolet and visible radiation
7. It can be utilized in the qualitative and quantitative analysis
8. The derivative graph can be obtained by UV-Visible spectrophotometer
9. It can be used in the degradation study of drug

1.4. THIN LAYER CHROMATOGRAPHY(TLC): Thin Layer Chromatography is a technique used to isolate non-volatile mixtures. The experiment is conducted on a sheet of aluminum foil, plastic, or glass which is coated with a thin layer of adsorbent material. The material usually used is aluminum oxide, cellulose, or silica gel.

On completion of the separation, each component appears as spots separated vertically. Each spot has a retentionfactor (R_f) expressed as:

R_f = dist. travelled by sample / dist. travelled by solvent

The factors affecting retardation factor are the solvent system, amount of material spotted, adsorbent and temperature. TLC is one of the fastest, least expensive, simplest and easiest chromatography technique.

1.4.1.Thin Layer Chromatography Principle

Like other chromatographic techniques, thin-layer chromatography (TLC) depends on the separation principle. The separation relies on the relative affinity of compounds towards both the phases. The compounds in the mobile phase move over the surface of the stationary phase. The movement occurs in such a way that the compounds which have a higher affinity to the stationary phase move slowly while the other compounds travel fast. Therefore, the separation of the mixture is attained. On completion of the separation process, the individual components from the mixture appear as spots at respective levels on the plates. Their character and nature are identified by suitable detection techniques.

1.4.2.STEPS INVOLVED INTLC:

- Dissolve the sample
- Prepare the TLC chamber and plate

- Spot the TLC plate with sample
- Place the TLC plate in the chamber to elute
- Remove the plate from the chamber

1.4.3.INSTRUMENTATION OF TLC:

Thin Layer Chromatography Plates

Plates used are chemically inert and stable. The stationary phase is applied on its surface in the form of a thin layer and the stationary phase on the plate has a fine particle size and also has a uniform thickness.

Specific dimensions of the glass plate are:

Full plate (20×20cm).

Half-plate (20×10cm).

Quarter plates (20×5cm).

These dimensions are used since the width of the commercially available TLC spreader is 20 cm.

Thin Layer Chromatography Chamber

The chamber is used to develop plates. It is responsible to keep a study environment inside which will help in developing spots also it prevents solvent evaporation and keeps the entire process dust-free.

Thin Layer Chromatography Mobile Phase

The mobile phase is the one that moves and consists of a solvent mixture or a solvent. This phase should be particulate free.

The higher the quality of purity the development of the spot is better.

Thin Layer Chromatography Filter Paper

It has to be placed inside the chamber. It is saturated in the mobile phase. This helps to develop a uniform raise in a mobile phase over the length of the stationary phase.

1.4.4.ADVANTAGES OF TLC :

- TLC is a simple and time-saving way to separate the analytes.
- This is a cost-effective method since it required a minimum sample and solvent.
- TCL is the method used to separate non-volatile compounds.
- Components will separate faster.
- It can possible to identify the unknown analytes by compared with the standard.
- Minimum types of equipment are used compared to other techniques.

1.5. DRUG PROFILE OF TURMERONE:

1.5.1.Structure:

Fig. 1: Structure of TURMERONE:

Ar Turmerone

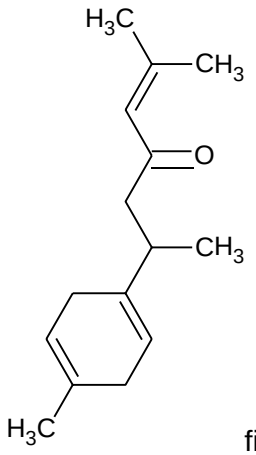

fig:3

- **Formula:** $C_{15}H_{20}O$
- **Molecular weight:** 216.3187
- **IUPAC Standard InChI:** InChI=1S/C15H20O/c1-11(2)9-15(16)10-13(4)14-7-5-12(3)6-8-14/h5-9,13H,10H2,1-4H3
- **CAS Registry Number:** 532-65-0

CHAPTER - 2:

LITRATURE REVIEW

Chapter-2: LITERATURE REVIEW

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2.www.sciencedirect.com › topics › neuroscienceTurmerone - an overview | ScienceDirect Topics

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5.http://www.slideshare.net/ssusera9ba27/spice-as-medicine-turmeric-curcuma-longa?from_m_app=android

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8.pubchem.ncbi.nlm.nih.gov › compound › ar-Turmeronear-Turmerone | C15H20O - PubChem

9. www.researchgate.net › publication › 255995314Extraction and isolation of turmerone from turmeric
22/08/2013 · Turmerone is principle flavouring compound of turmeric

10. www.nature.com › articles › s41598/022/19901-2Molecular insights on ar-turmerone as a structural ...
16/09/2022 · Phytochemicals of such as curcumin, demethoxycurcumin, other curcuminoids and ar-turmerone of c.longa

11. www.researchgate.net › publication › 244516604[PDF] Structures of α- and β-Turmerone - ResearchGate
01/01/1992 · Based on GC-MS and NMR spectroscopy, the major components in CF-3 were identified as -

12.bmccomplementmedtherapies.biomedcentral.comAntidepressant-like activity of turmerone in behavioral ...

01/11/2013 · Turmerone, an active constituent of C. longa, has been shown to possess powerful antioxidant, anti-inflammatory, anti-tumor, and anti-proliferative activities

13.life-enhancement.com › pages › turmerone-inducesTurmerone Induces Neural Stem Cell Proliferation

in a 2005 study, the curcuminoids and ar-turmerone were found to have hypoglycemic effects (the ability to reduce blood sugar) by activating ppar-gamma* as one of the mechanisms

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26/09/2014 · Aromatic (ar-) turmerone is a major bioactive compound of the herb Curcuma longa. It has been suggested that ar-turmerone inhibits microglia activation

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19.www.biocrick.com › ar-Turmerone-BCN7516ar-Turmerone | CAS:532-65-0 | Sesquiterpenoids | High Purity

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08/12/2022 · According to research, turmeric includes several organic compounds that prevent beta-amyloid, the material that slowly impairs cerebral function in Alzheimer's disease,

30. cogentoa.tandfonline.com › doi › absSynthesis of Curcumone and ar-Turmerone via β-Oxoalkylation ...

05/12/2006 · In 1974 I outlined a synthesis of ar-turmerone (1)1 by controlled chain extension from the acetyl terminus of mesityl oxide,

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- Biomaterials, 2011 - ncbi.nlm.nih.gov Curcumin, a hydrophobic polyphenol, is an extract of turmeric root with antioxidant, anti-inflammatory and anti-tumorigenic properties

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OVER 50 PUBLISHED STUDIES - This patented, enhanced absorption curcumin with turmeric essential oil containing ar-turmerone

35. HPTLC Method for the Quantitative Determination of ar-Turmerone and Turmerone in Lipid Soluble Fraction from Curcuma longa Vikas Jaina , Vure Prasad, Satwayan Singha and Raghwendra Pala,* a Pharmaceutics Division, Central Drug Research Institute, Luckonow-226001, India vikasjan@gmail.com

36.personal-care.evonik.com › en › tego-turmerone-theTEGO® Turmerone - The Golden Spice of India -

Evonik

37.cosmetics.specialchem.com › product › i-evonik-tegoTEGO Turmerone - Evonik - SpecialChem
05/08/2021 · Curcuma Longa (Turmeric) Root Extract. TEGO® Turmerone by Evonik has a significant antioxidant activity.

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The effects of α- and aromatic turmerones on curcumin transport as well as P-gp activities were shown here for the first time. The presence of turmerones did affect the absorption of curcumin

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Aromatic-Turmerone Attenuates LPS-Induced Neuroinflammation and Consequent Memory Impairment by Targeting TLR4-Dependent Signaling Pathway

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Augmented with turmerones & ginger extracts to complement curcumin’s health benefits Helps inhibit inflammatory factors to promote both joint & organ health Fights oxidative stress

CHAPTER – 3 :

MATERIALS & METHODS

Chapter-3: MATERIALS ANDMETHODS

3.1:Instrument Picture:

3.1.1.Fig. 4: UV-Vis Spectrophotometer of SHIMADZU(UV-1900i)

UV-Vis Spectrophotometer	: UV-1900i, SHIMADZU
Cuvette	: LARK CIENTIFICO synthetic quartz glass cuvettes
Software	: UV Probe 2.70
Digital weighing balance	: SHIMADZUAUX220

3.1.2.Fig. 5: Digital Weighing Balance of SHIMADZU(AUX220)

3.2.Chemicals and reagents :

:

Table :2

Sl no.	Name of chemicals	Name of the supplier
1	• Petroleum ether	Merck specialities private limited Shiv sagar Estate 'A' Dr. Annie Besant Road, Worli, Mumbi - 400018
2	• Methanol • Vanilline • Sulphuric acid • Ethanol • Toluene • Ethyl acetate • Activated Charcoal	SDFCL S d fine-chem limited 1502,Marathonlcon,Lowerparel, Mumbai - 400013

3.3.Method:

Dehydrate the turmeric until the pieces are completely dry.

When done drying they will easily crack and snap in half, rather then blending.
Use a blender or coffee grinder to churn the dried turmeric into powder. Pour the Turmeric powder through a fine mesh strainer poised over a bowl

3.4.Extraction and isolation of turmerone :

1kg of turmeric powder (Curcuma longa L.) was extracted with 4 l of petroleum ether (b.p.60-80o C) using soxhlet assembly for 12 h. The extract obtained was then concentrated by using distillation. The solid material from the soxhlet was extracted twice more with petroleum ether and the concentrated filtrates or extracts were combined. This extract was nothing but turmeric oil. Turmeric oil was then fractioned between petroleum ether and methanol using separating funnel. Repeated fractionation was carried out with methanol. Petroleum ether fraction was then treated with activated charcoal to remove any impurities present. This fraction was further purified by preparative TLC. Thus, repeated fractionation and charcoal treatment resulted in isolation of pure turmerone.

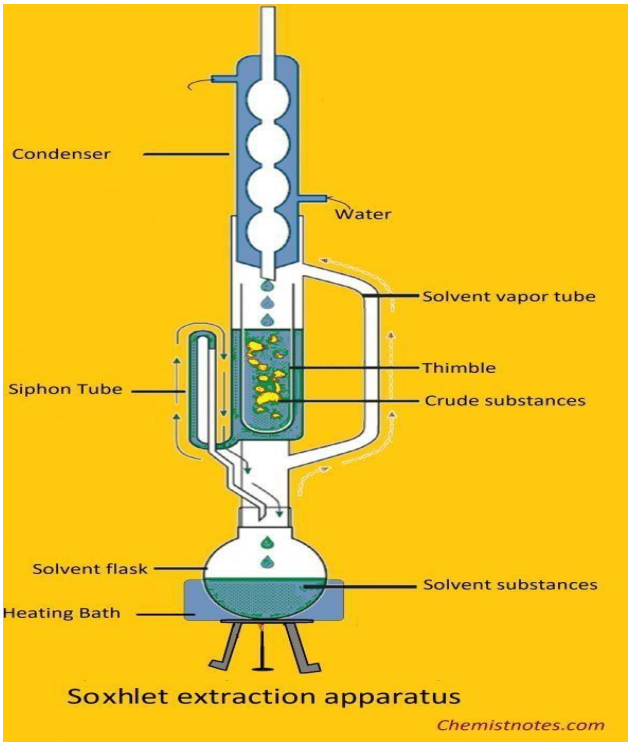


FIGURE- 6

3.4.1.Procedure of Soxhlet extraction:

Purified turmeric powder was place in filter paper cylinder and was placed in the body of the soxhlet extracter. The solvent ,ethyle acetate was placed in the flask. Then the apparatus was fitted accordingly. Ethyle acetate was allowed to boil for few minutes, for converting it into the vapour state. The vapours entered into the

condenser through side tube and got condensed into hot liquid, which fell on to the column of turmeric powder. The level of siphon tube rised up to its top, when the extracter was filled with the solvent. This solvent containig the Active constituents in siphon tube ran in to the flask, there by emptying the body of the extractor. This process Continued, till the turmeric powder got exhausted. The soluble active constituents remained in the flask. The Extract was concentrated under reduced pressure.

3.5. THIN LAYER CHROMATOGRAPHY:

3.5.1.STEPS INVOLVED IN TLC

1) PREPARATION OF THIN LAYERS ON PLATES

• **Pouring of Layers :**

To obtain layers of equal thickness, a measured amount of Turmeric oil was placed on given-size plate that is rested on an absolutely labelled surface. Then the plate is subsequently tipped backward and forward to permit the slurry (or suspension) to spread uniformly on the surface of the plate.

• **Dipping :**

In this technique ,two plates at a time back-to-back are dipped together with turmeric oil in toulene and ethyl acetate in ratio of 93:7 .

• **Spraying :**

In this technique 1 gm of vanilline with 2ml of sulphuric acid and made the volume upto 100ml with useof small paint-sprayer for the distribution of turmeric oil onto the surface of the glass-plate.

• **Spreading :**

In this , the turmeric oil is put in an ‘applicator’, which was subsequently moved over the stationary glass-plate, it is held stationary while the glass plate is pulled or pushed through. This technique termed as ‘spreading’ usually yields uniform thin layers on the glass plates

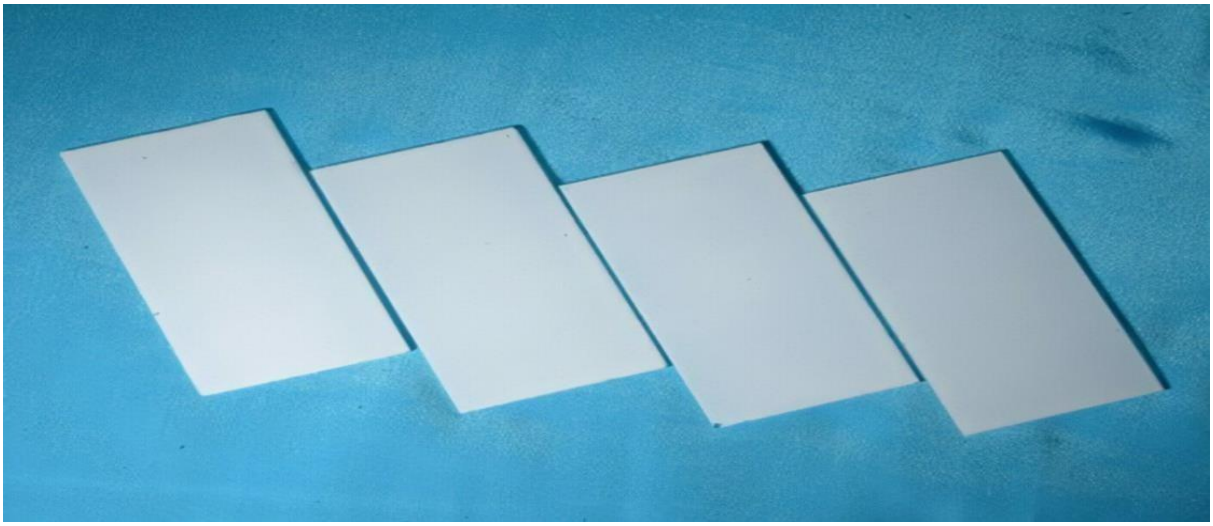


Fig 7:

THIN LAYER CHROMATOGRAPHY PLATES

2) ACTIVATION OF TLC PLATE

It is extremely important to eliminate as completely as possible the solvent imbedded into the thin layer of coated adsorbent. It is achieved conveniently first by air-drying the TLC plates for a duration of 30 minutes and then in a hot-air oven maintained at 110 °C for another 30 minutes and subsequently cooling them in a dessicator. This drying process helps a great extent in rendering the adsorbent layer active. In order to achieve very active layers, silica gel and alumina coated plates may be heated upto 150 °C for a duration of 4 hours and colling them in a dessicator.

3) APPLICATION OF SAMPLE ON TLC PLATE

- Wipe any excess adsorbent from the back and edges of the plate.

•Sample should normally be applied about 5mm (for small plates) to 10mm (larger plates) from the edge of the plate.

•However, care should be taken not to immerse the spot in the solvent pool in the development chamber.

•The spots should be separated from each other by at least 10mm for larger plates.

•Sample application is performed by spotting or streaking the thin layer.

- Analytical plates are usually spotted while preparative plates are streaked.

3.5.2. DEVELOPMENT TECHNIQUES

1) ONE DIMENSIONAL DEVELOPMENT

In this technique,the plates are kept verticle and the solvent flows against gravity Because of capillary action.Most seperations done practically are of this type only

2) 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 once from 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 from the 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.

3) HORIZONTAL TLC DEVELOPMENT

Here,thetlc plate is placed in a horizontal plane for development and suitable horizontal development tank is chosen for development of chromatogram

4) MULTIPLE DEVELOPMENT

This technique, is widely used for separation of very complex mixture and is similar to that of two dimensional technique but here the plate is rotated through 90 degree several times to develop in different Axis multiple times

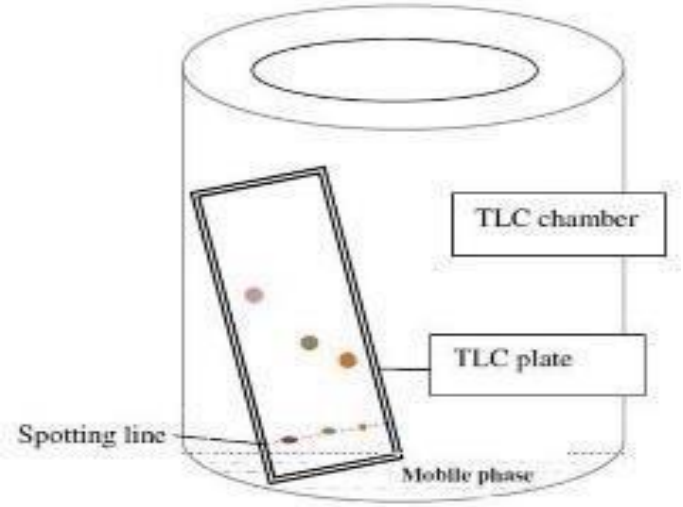


Fig 8:

DEVELOPMENT OF TLC

3.5.3.PROCEDURE OF TLC

Thin Layer Chromatography of volatile oil of turmeric and isolated compounds was performed by the Ascending technique. The process parameters includes Silica gel 60F254 pre-coated TLC plate (Merck) as Adsorbent, Toluene: Ethyl acetate (93:7) as Chromatography solvent. Vanillin sulphuric acid was used as spraying agent after the length 5.0 cm leng

3.5.4.DETECTION OF COMPONENTS

After development of TLC plates, the next important step is to detect the separated components so as to determine their respective Rf values.

Example :

(i) **Coloured Substances :** *e.g.*, Xanthophylls, Chlorophylls, Carotenes, etc., may be located visually.

(ii) **Colourless Substances :** *e.g.*, alkaloids, steroids, amino acids and the like may be detected under short-wave UV-light or a long-wave UV-light. These substances may also be detected as brown/dark brown spots when exposed to I_2-vapours in a closed dessicator.

Specific Detecting Reagents : A few specific detecting reagents are normally used for a particular class of compounds *e.g.*,

Aniline-phthalate reagent :for carbohydrates ;

Ninhydrin reagent : for amino-acids, and

Dragendorff's reagent :for alkaloids

(iv) Chromic acid/conc. H2SO4 : These corrosive reagents usually char the organic TLC plates and may be seen as dark brown spots.

3.5.5.SPRAY PREPARATION

1gm of vanillin was dissolved in 2ml of sulphuric acid and make up the volume with ethanol upto to 100ml

3.5.6.CALCULATION

$$RF = \frac{\text{Distance travelled by sample from origin}}{\text{Distance travelled by solvent from origin}}$$

RF =

0.6/5.5=0.15

3.6. ULTRA VIOLET VISIBLE SPECTROSCOPY

UV-Visible spectroscopy or UV-Visible spectrophotometry is a form of absorption spectroscopy. The UV- Visible spectrophotometry is concerned with the ultraviolet and visible regions of electromagnetic Spectrum which ranges between 200-780nm.

3.6.1.PROCEDURE INVOLVED IN THE VU-VISIBLE SPECTROSCOPY FOR TURMERONE

- Prepare a sample solution of turmeric oil in ethanol
- Switch on visible spectrophotometer and allow it worm for about 15min
- Adjust the instrument to 100% transmittent or zero absorbance (using distilled water and blank)
- Put prepared sample solution in the cuvette and measure the absorbance value in the wavelength Of 450-600nm at a band width of fine nanometer . Adjust the instrument 100% transmittance or zero absorbance after each change in wavelength plot the graph by taking the absorbance value

3.7.DETERMINATION OF DISPERSABILITY

DEFINATION OF DISPERSIBILITY:

The quality or state of being dispersible

3.7.1.PROCEDURE FOR DISPERSIBILITY:

Dispersibility was determined by method of Kulkarni etal

The 10g of turmeric sample was weighed into100ml measuring cylinder and distilled water was added to volume of 100 ml

The set up was stirred vigorously and allowed to settle for three hours (3 hours)

The volume of settled sediment was recorded as shown on measuring cylinder and value substracted from 100 . The difference was determined

3.7.2.CALCULATION OF DISPERSIBILITY:

Dispersibility of turmeric powder after stirred vigorously in 100ml distilled water = 8.5

Dispersibility of turmeric powder = 100 – 8.5

= 91.5

Dispersibility of turmeric powder = 91.5

3.8.DETERMINATION OF RELATIVE BULK DENSITY:

DEFINATION OF RELATIVE BULK DENSITY:

Density is determined by multiplying the relative density (specific gravity) of the aggregate times the density of water. Bulk Density of Aggregate The bulk density or unit weight of an aggregate is the mass or weight of the aggregate that required to fill a container of a specified unit volume. Bulk Density = Mass / volume

3.9.DEFINATION OF TAPPED DENSITY:

Tapped density of a powder is the ratio of the mass of the powder to the volume occupied by the powder after it has been tapped for a defined period of time. The tapped density of a powder represents its random dense packing. Tapped density can be calculated using Eq

, where *M*=mass in grams

V_f=the tapped volume in milliliters**.**

EQUATION:

Tapped density(g/ml)=M/Vf

DEFINATION OF BULK DENSITY:

Bulk density, also called apparent density or volumetric density, is a property of powders, granules, and other "divided" solids, especially used in reference to mineral components (soil, gravel), chemical substances, (pharmaceutical) ingredients, foodstuff, or any other masses of corpuscular or particulate matter (particles).

PROCEDURE FOR RELATIVE BULK DENSITY:

- The sample was poured into a calibrated sample tube
- The sample was added till it gets to 5mal mark on tube and then weight was taken
- The tube with samples was tapped while more samples were added
- This continued till sample is steady at 5ml mark of tube and then final

CALCULATION OF RELATIVE BULK DENSITY:

$$\text{Bulk density} = \frac{\text{Sample weight after tapping} - \text{sample weight before tapping x100}}{\text{5 ml of centrifuge tube}}$$

$$= \frac{2.70 - 2.28 \text{ x } 100}{5}$$

Bulk density = 8.4%

3.10. DETERMINATION OF MOISTURE CONTENT:

DEFINITION

The natural water content also called the natural moisture content is the ratio of the weight of water to the weight of the solids in a given mass of soil. This ratio is usually expressed as percentage.

3.10.1.PROCEDURE

1. Clean the container with lid dry it and weigh it (W1).
2. Take the sample in the container and weigh with lid (W2).
3. Keep the container in the oven with lid removed. Dry the specimen to constant weight

maintaining the temperature for 105 degree for 30 minutes

4. Record the final constant weight (W3) of the container with dried turmeric sample

3.10.2.OBSERVATIONS AND RECORDING

Data and observation sheet for water content determination

Table2:

S.No.	Sample No.	1	2
1	Weight of container with lid W_1 gm	15.5	15.5
2	Weight of container with lid +wet turmeric powder W_2 gm	60.00	60.01
3	Weight of container with lid +dry turmeric powder W_3 gm	58.01	58.05

3.10.3.CALCULATION :

1) Weight of container with lid +wet turmeric (W2) = 60.00

Weight of container with lid +dry turmeric (W3) =58.01

Formula =

$$\frac{\text{Weight of wet turmeric powder – weight of dry turmeric powder}}{\text{Weight of wet turmeric powder}}$$

$$\frac{60.00-58.01}{60.00} \times 100 = 3.31\%$$

2) weight of wet turmeric powder(W2)=60.06

3) Weight of dry turmeric powder(W3) =58.05

$$\frac{\text{Weight of wet turmeric powder – weight of dry turmeric powder}}{\text{Weight of wet turmeric powder}}$$

$$= \frac{60.06 - 58.05}{60.06} \times 100$$

$$= 3.34\%$$

Fig:9 EXTRACTIN AND ISOLATON OF TURMERON USING SOXHLET APPARATUS

Fig:10 SEPARATION OF TURMERIC OIL USING SEPARATION FUNNELS

CHAPTER – 4 :

RESULT AND DISCUSSION

Chapter-4: RESULTS AND DISCUSSION

4.1: RESULTS AND DISCUSSION

4.1.1. Physicochemical properties of turmeric powder : The turmeric powder before isolation of bioactive compound was subjected to physico-chemical analysis. The obtained result are depited in table-1 . The results revealed that power was sufficiently dried with the lower moisture content of 4.37 per cent .

	TEST	RESULT
1	Moisture content	4.37 %

4.1.2. Characterization of isolated turmerone by TLC and UV : Characterization of isolated Compound was carried out with the analytical techniques like TLC and UV .

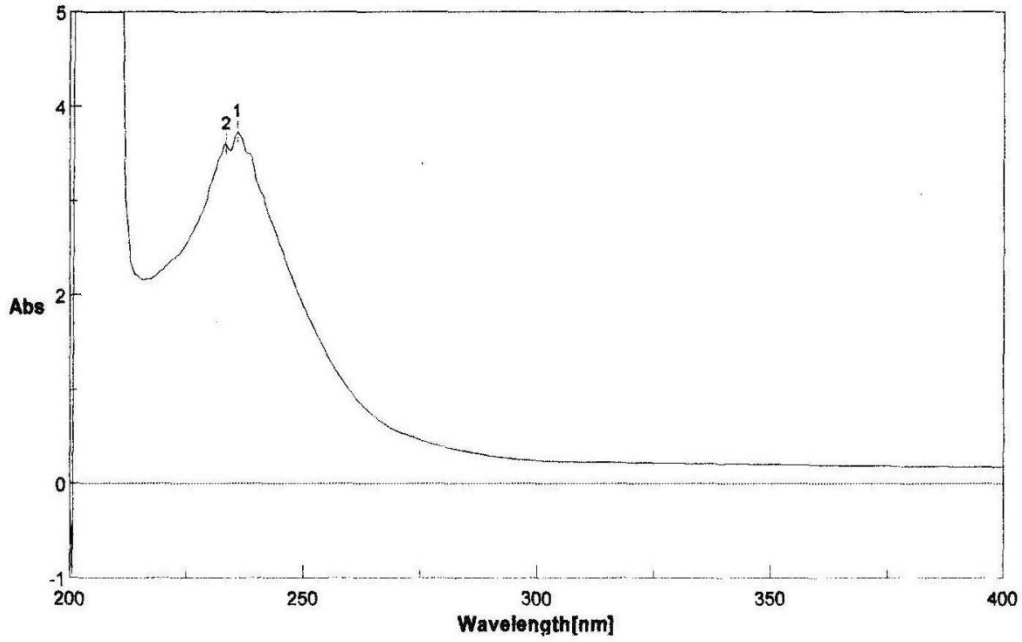
Characterization data of isolated compound

Sr. No.	Parameters	Observation
1	Colour	Light yellow
2	odour	Dry , woody , spicy
3	TLC RF value	0.15
4	UV	233.5 nm and 236 nm

Fig :11 TLC of turmeric oil

The layer chromatography is an important tool for quick identification of required constituents in drug or in extract. The thin layer chromatography (TLC) was preformed for presence of turmerone in turmeric oil. Mobile phase comprising of toluene : ethyl acetate(93:7). Turmerone shows violet spot at Rf of 0.72 with vanillin–sulfuric acid on heating. This spot matched exactly with the reported Rf in same mobile phase.

The UV absorption maxima of the isolated compound were recorded using ethanol as solvent. A UV spectrum of the isolated compound shows two peaks of almost same intensity at 233.5 nm and 236 nm.



File name	turmerone
Model	V-530
Serial No.	B203060512
Band width	2.0 nm
Response	Fast
Measurement range	400 - 200 nm
Data pitch	0.5nm
Scanning speed	400nm/min
Sample ID	1
No. of cycle	1
Sample name	
Operator	a
Comment	

CHAPTRE – 5 :

SUMMERY AND CONCLUSION

5.1 . SUMMARY

Turmeric (Curcuma longa) is a medical plant of the family Zingiberaceae Widely growing throughout India. Turmeric oil is secondary metabolite of turmeric and obtained By steam distillation of its rhizomes. Turmeric fraction from turmeric , exhibits several therapeutic Potentials. Turmeric oil chiefly comprises ar-turmerone and beta-turmeron. During more recent decades broad spectrums of therapeutically interesting pharmacological properties of turmeric and its secondary metabolites have been reported. Recent several efforts made to explore the pharmacological profile and mechanism of action of turmeric revealed exceptionally broad spectrums of pharmacological activity profile of turmeric oil. It is now well recognized that additive or synergistic interactions between divers combinations of phytochemicals are involved in health benefits of vegetarian diets and herbal remedies and that regular consumption of appropriate combination of some such edible phytochemicals with every day meals could as well be used for prevention and cure of different health problems. Critical analysis of available preclinical and clinical information on turmeric oil strongly suggest it is pharmacologically polyvalent and prossrss several pharmacological properties. Aim of this communication is to summarize for more rationally exploiting their therapeutic potential For discovering novel therapeutic leads, or for obtaining pharmacologically better standardized Phyto-pharmaceuticals.

5.2. Conclusion:

Thus based on the test carried out and from spectral studies, the observed data was Found to match well with that of reported data for turmerone and the isolated compound was Identified as turmerone.

CHAPTER-6:

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Chapter-6: REFERENCES

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